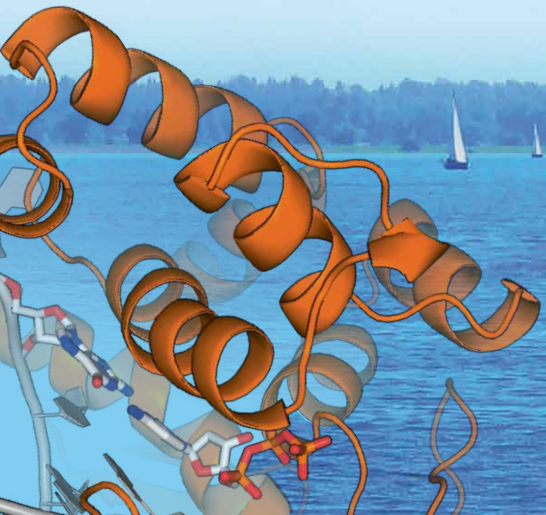
GERMAN BIOPHYSICAL SOCIETY MEETING

September 25–28, 2022

University of Konstanz, Germany

DGfB Deutsche Gesellschaft für Biophysik e.V.

CONFERENCE PROGRAM



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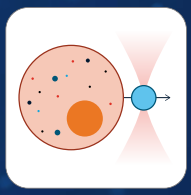
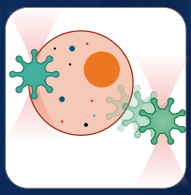
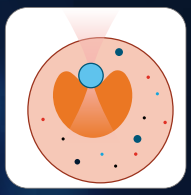


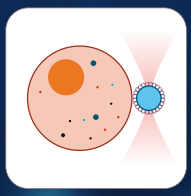
Tether Pulling



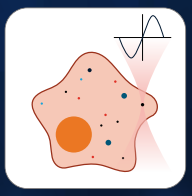
Immune Cells Interactions



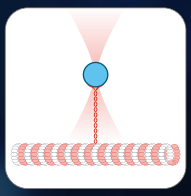
Cell & Nucleus Deformation



Cell-ECM Interactions



Active Micro-Rheology



In Vivo Motor Proteins Activity

Need more information?

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German Biophysical Society
(Deutsche Gesellschaft für Biophysik e. V., DGfB)
www.dgfb.org

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WELCOME NOTE OF THE CONFERENCE CHAIRS

Dear colleagues,

on behalf of the German Biophysical Society (DGfB) it is our great pleasure to welcome you at the University of Konstanz for the DGfB meeting 2022.

The DGfB represents biophysics in Germany and promotes the interdisciplinary exchange of scientists from various research fields and from other associations. Following this spirit, the conference comprises joint sessions, co-organized with the Society for Biochemistry and Molecular Biology (GBM) and the German Physical Society (DPG).

We are delighted to present a diversified program with a large number of lectures and poster presentations. A particular focus of the conference is the support of young researchers. We would like to draw your attention to the Young Investigator Award lecture. The DGfB awards this prize to honor outstanding research achievements of a young scientist. Additionally, the best three poster contributions of the poster sessions will be awarded with poster prizes.

The scientific program will be accompanied by an industry exhibition, giving insights into technical innovations and intensifying the exchange between academia and industry.

We gratefully acknowledge the support of the German Science Foundation (DFG), the Collaborative Research Center (CRC) 969, the University of Konstanz, as well as all the exhibitors and companies sponsoring our meeting.

Many thanks to all conference participants who present their work and contribute to lively discussions. We would also like to thank the scientific committee for supervision of the program. Last, but not least, our special thanks go to Corinna Palz and Marc Jochimsen from the Event and Conference Management of the University of Konstanz for coordinating everything at and around the conference.

We are looking forward to an informative meeting and inspiring discussions!

With best regards,

Karin Hauser

Andreas Zumbusch

Conference Chairs
German Biophysical Society Meeting 2022
University of Konstanz

GENERAL INFORMATION

Venue	University of Konstanz, Universitätsstraße 10, 78464 Konstanz, Germany Building A, levels A5, A6, A7, Audimax The registration desk, all talks and the industry exhibition are located in building A (see campus map on page 11). Poster areas will be indicated on-site.
Registration	Registration opens on Sunday, Sep 25, 15:00 and is possible throughout the duration of the conference. The registration desk is located on level A6.
Certificate of Attendance	If you need a certificate of attendance, please refer to the registration desk.
Name Badge	Participants receive a name badge at the registration desk. Please wear your name badge during all conference events, including the social program. The name badge is required for the welcome reception and lunches in the mensa (included in the conference fee), the boat trip, the conference dinner (both required prior registration) and free bus rides to the campus.
Catering	The welcome reception on Sunday evening, all coffee breaks and lunches are included in the conference fee. On Mon and Tue, Sep 26 + 27, you can go for lunch in the mensa, make your own meal choice and pay with your lunch voucher at the check-out. On Wed, Sep 28, lunch bags will be provided for pick up at the coffee desks.
Internet Access	You can use wireless internet with eduroam. Additionally, a free conference network is provided. You can login into the „conference" network with the Username „Biophys2022" and the password „rcsal5a2".
Wardrobe	Wardrobe may be stored next to the registration.
Photography & Copyright	Taking pictures and recording during lectures and/or at the poster exhibition without the prior written consent of the presenter of the work are prohibited.
Book of Abstracts	The abstracts of all oral presentations and posters can be downloaded as a PDF file from the conference website www.uni.kn/biophys2022

Speakers Information

Please ensure that your presentation works at least 10 min before your session starts. You can either bring your presentation (in ppt or pdf format) on an USB stick to be uploaded on a conference computer or use your own laptop (HDMI connection required). A local volunteer will assist you with the upload or connection of your laptop or any technical difficulties.

Young Investigator Award

The conference hosts a Young Investigator Award competition. The German Biophysical Society (DGfB) awards this prize to honor outstanding achievements of a young investigator in the field of biophysics. The award comes with a prize money of 1000 Euros donated by the DGfB. Award ceremony and lecture will take place on Tuesday, Sep 27, 2022, 17:40-18:10, Audimax.

Poster Prizes

Poster presentations are an important part of the conference. Three poster prizes will be awarded in the closing ceremony on Wednesday, Sep 28, 2022, 12:10-12:30, Audimax. Each poster prize comes with a prize money of 200 Euros donated by the DGfB. All conference attendees can participate in the digital voting over the course of the conference.

DGfB Member Meetings

DGfB Section Meetings: Tuesday, Sep 27, 18:15 – 18:30
Section I Molecular Biophysics: A 702
Section II Membrane Biophysics: A 703
Section III Cellular Biophysics: A 704

DGfB General Assembly:
Tuesday, Sep 27, 18:30 – 19:30, A 701

Public Transportation

We are grateful for the support of the Stadtwerke Konstanz: they sponsor the bus transfer to the boat trip. In addition, conference attendees can use the public buses in Konstanz for free to commute to the university with the provided conference ticket. Bus lines 9A/B/C and 11 stop directly at the university main entrance. Face masks are obligatory in public transportation in Germany.

Corona

In the current pandemic situation, it is recommended that all university members and visitors wear either a medical or FFP2 face mask on the campus (www.uni.kn/coronavirus).

HOW TO GET THERE

By car

Street address for your navigation device:
Universitätsstrasse 10, 78464 Konstanz, Germany

From Stuttgart (180 km): Autobahn A 81 towards Singen.

From Munich (220 km): Autobahn A 96 towards Lindau and Meersburg, take the vehicle ferry to Konstanz.

From Zurich (75 km): Autobahn A7 towards Kreuzlingen/Konstanz

Car parking is available on North parking area (open air car park) and South parking area (parking garage with 2m height restriction).

By train

Train station destination: **Hauptbahnhof Konstanz** (main train station). From the train station you can take buses 9A and 9B directly to the main entrance of the university.

By bus

Public bus transportation within Konstanz is free with your conference name badge in combination with your conference bus ticket.

Bus line 9A, 9B and 9C: Starting from, for example, the main train station.

Bus line 11: Starting from, for example, the bus stop at the Wollmatingen train station.

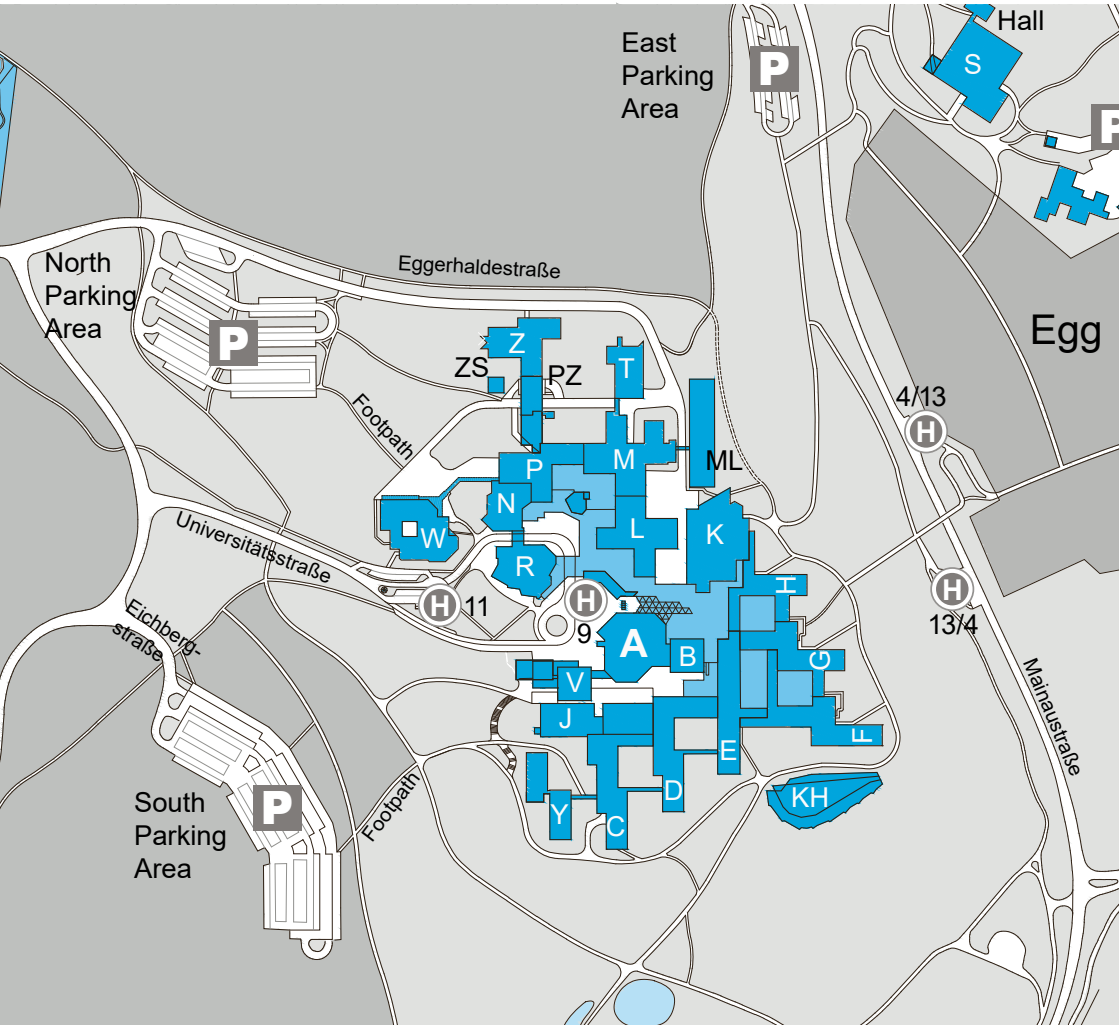
Bus line 4: Starting from, for example, the bus stop at the main train station, exit at the Egg/Universität bus stop. You have to walk 5 minutes uphill to the campus.

Timetable information: www.stadtwerke-konstanz.de/en/

CAMPUS MAP

This University of Konstanz campus map provides you with orientation. The main entrance of the university is located directly at the bus stop #9 in front of the A building. The staff of the Information-Point at the main entrance will be happy to assist you with directions.

The conference is located in the A-building. The registration desk is on floor A6. Just follow the signs. The plenary talks will be held in our Audimax lecture hall, all other talks in the lecture halls on level A7.



SUPPORTERS AND SCIENTIFIC PARTNERS

SOCIETIES

DPG — German Physical Society

GBM — Society for Biochemistry and Molecular Biology

RESEARCH CENTERS

CRC 969 — CRC 969: Chemical and Biological Principles of Cellular Proteostasis

SUPPORTERS

DFG Deutsche Forschungsgemeinschaft — German Research Foundation

VEUK – Der Alumni-Verein der Universität Konstanz — University of Konstanz Alumni Association

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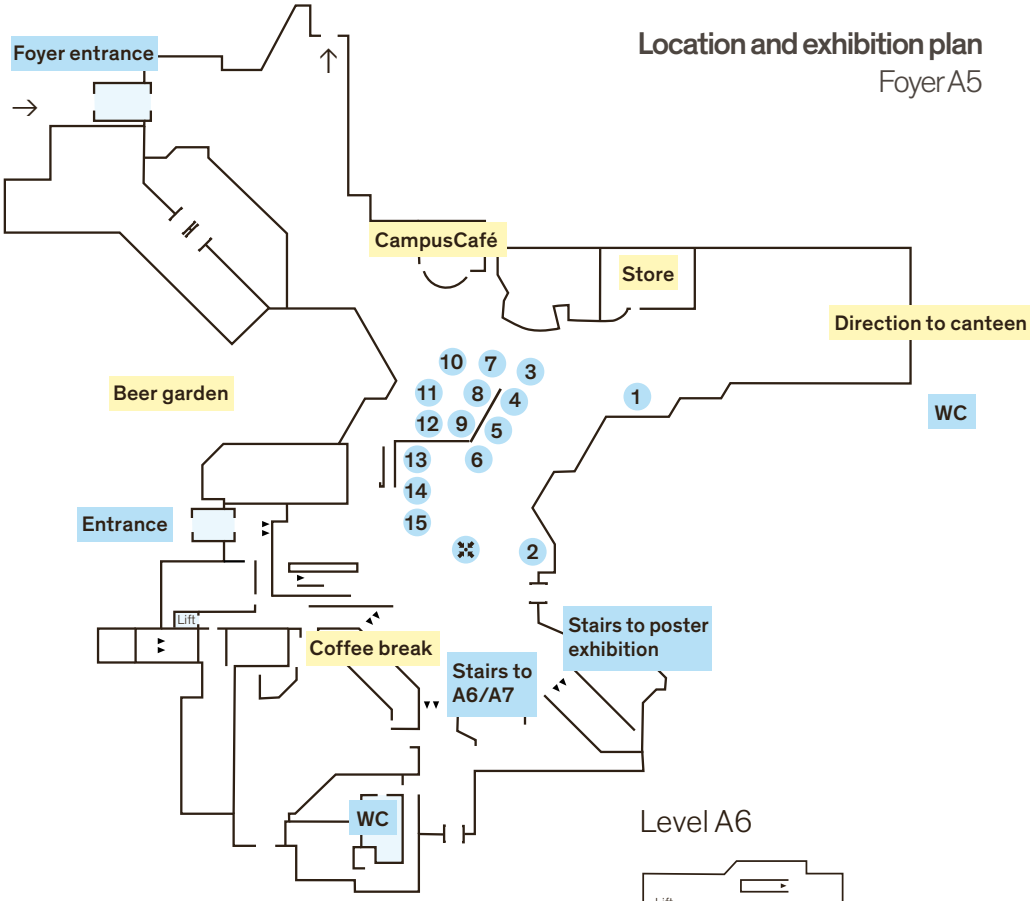
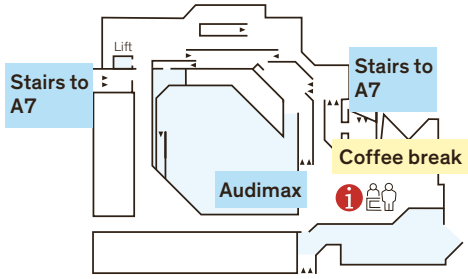
Location and exhibition plan

Foyer A5



Level A6



Foyer A5:

- Meeting point boat trip
- 1 Dynamic Biosensors
- 2 AHF Analysetechnik
- 3 Bruker
- 4 Nanion
- 5 IRsweep
- 6 Rapp Optoelectronic
- 7 Lumicks
- 8 Hamamatsu
- 9 Cube Biontech
- 10 Thorlabs
- 11 Mad City Labs
- 12 MG Optical Solutions
- 13 ACAL Bfi
- 14 Refeyn Ltd.
- 15 Applied Photophysics

Level A6:

- Registration desk

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LOCAL ORGANIZING COMMITTEE

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Andreas Zumbusch, University of Konstanz (Conference Chair)

Malte Drescher, University of Konstanz

Michael Kovermann, University of Konstanz

Christine Peter, University of Konstanz

Marc Jochimsen, University of Konstanz (Event and Conference Management)

Corinna Palz, University of Konstanz (Event and Conference Management)

ADDITIONAL PROGRAM COMMITTEE MEMBERS

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Klaus Gerwert, University of Bochum

Gerhard Gompper, Research Center Jülich (DPG)

Helmut Grubmüller, Max Planck Institute for Multidisciplinary Sciences Göttingen

Thomas Gutsmann, Research Center Borstel

Sandro Keller, University of Graz

Claus Seidel, University of Düsseldorf (GBM)

Claudia Steinem, University of Göttingen

INVITED SPEAKERS

Sophie Brasselet (Marseille, FRA)

Frauke Gräter (Heidelberg, GER)

Marloes Groot (Amsterdam, NL)

Peter Hamm (Zürich, CH)

Mike Heilemann (Frankfurt, GER)

Alf Honigmann (Dresden, GER)

Daniel Huster (Leipzig, GER)

Jan Phillip Junker (Berlin, GER)

Sandro Keller (Graz, AT)

Sarah Köster (Göttingen, GER)

Madhavi Krishnan (Oxford, UK)

Nina Morgner (Frankfurt, GER)

Gary Pielak (Chapel Hill, USA)

Joachim Rädler (München, GER)

Jonas Ries (Heidelberg, GER)

Claudia Steinem (Göttingen, GER)

Florian Stengel (Konstanz, GER)

Emad Tajkhorshid (Urbana, USA)

Philip Tinnefeld (München, GER)

Charlotte Uetrecht (Hamburg, GER)

Stefan Weber (Freiburg, GER)

PROGRAM OVERVIEW

Sunday, September 25th

Registration
Desk Opening
15:00–17:00
Foyer

Welcome
Plenary Session
17:00–19:00
Audimax

Get-Together
19:00
Food & Drinks

Monday, September 26th

Plenary Session (DPG)
08:30–10:30
Audimax

Coffee Break 10:30–11:00

Parallel Sessions
11:00–12:00

1A Membrane Biophysics I	A 701
1B Computational Biophysics I	A 703
1C Biospectroscopy I	A 704

Lunch Break 12:00–13:00 DFG Talk

Plenary Session
13:00–14:30
Audimax

Coffee Break 14:30–15:00

Poster Session 1
15:00–16:30
Industry Talks A 701

Parallel Sessions
16:30–17:15

2A Biomolecular Interactions	A 701
2B Channels & Transporters	A 703
2C Genetic Regulatory Systems	A 704

Boat Trip
Departure 17:30

Conference Dinner Konzil
19:30–22:00

Tuesday, September 27th

Plenary Session (GBM)
08:30–10:30
Audimax

Coffee Break 10:30–11:00

Parallel Sessions
11:00–12:00

3A Imaging & Microscopy I	A 701
3B Computational Biophysics II	A 703
3C Protein. Struct., Dyn., Funct.	A 704

Lunch Break 12:00–13:00

Plenary Session
13:00–14:30
Audimax

Coffee Break 14:30–15:00

Poster Session 2
15:00–16:30
Industry Talks A 701

Parallel Sessions
16:30–17:30

2A Membrane Biophysic II	A 701
2B Cell Biophysics	A 703
2C Protein Fold., Aggr., Disease	A 704

Young Investigator Award
Audimax 17:40–18:10

DGfB Section Meetings 18:15–18:30
DGfB General Assembly 18:30–19:30

Guided City Tours

Wednesday, September 28th

Plenary Session
08:30–10:30
Audimax

Coffee Break 10:30–11:00

Parallel Sessions
11:00–12:00

5A Imaging & Microscopy II	A 701
5B Photobiophysics	A 703
5C Biospectroscopy II	A 704

Poster Awards & Closing

SUNDAY, SEPTEMBER 25TH, 2022

Welcome **Audimax**
17:00–17:30

Helmut Grubmüller, Göttingen, GER
Chair of the German Biophysical Society

Karin Hauser, Konstanz, GER
Conference Chair

Plenary Session 1 **Audimax**
17:30–19:00 **Chair:** Karin Hauser, Konstanz, GER

17:30–18:00 **T1** **Claudia Steinem**, Göttingen, GER
How does the new antibiotic lugdunin kill MRSA? – An in vitro analysis

18:00–18:30 **T2** **Sandro Keller**, Graz, AT
New native nanodiscs for membrane-protein biophysics

18:30–19:00 **T3** **Daniel Huster**, Leipzig, GER
Rhomboid-catalized intramembrane proteolysis requires hydrophobic matching with the surrounding lipid bilayer

19:00–21:00 **Get Together (Food & Drinks)** **Foyer**

MONDAY, SEPTEMBER 26TH, 2022

Plenary Session 2 – DPG Session **Audimax**
08:30–10:30 **Chair:** Andreas Zumbusch, Konstanz, GER

08:30–09:00 **T 4** **Sarah Köster**, Göttingen, GER
Intermediate filaments in the cytoskeleton: safety belt and shock absorber for the cell?

09:00–09:30 **T 5** **Jan Philipp Junker**, Berlin, GER
Simultaneous lineage tracing and cell type identification using CRISPR/Cas9 induced genetic scars

09:30–10:00 **T 6** **Madhavi Krishnan**, Oxford, UK
Bringing electrostatics to light: electrometry probes a new dimension at the molecular scale

10:00–10:30 **T 7** **Joachim Rädler**, München, GER
Mechanisms of LNP-based RNA delivery and models of time-resolved gene expression in single cells

10:30–11:00 **Coffee Break**

Parallel Session 1A – Membrane Biophysics I **A 701**
11:00–12:00 **Chair:** Andreas Janshoff, Göttingen, GER

11:00–11:15 **T 8** **Katia Cosentino,** Osnabrück, GER
Membrane permeabilization in regulated cell death at the single molecule level

11:15-11:30 **T 9** **Maria Hoernke,** Freiburg, GER
Leaky membrane fusion: an ambiguous effect induced by antimicrobial polycations

11:30-11:45 **T 10** **Christoph Westerhausen,** Augsburg, GER
Membrane transport in cell ensembles is modulated by the membrane state

11:45–12:00 **T 11** **Matthias Wilm,** Dublin, IRL
Synthesis of large lipid membranes with integrated membrane proteins from gas phase

Parallel Session 1B – Computational Biophysics I **A 703**
11:00–12:00 **Chair:** Roberto Covino, Frankfurt, GER

11:00–11:15 **T 12** **Jochen Hub,** Saarbrücken, GER
Free energy landscape of pore and stalk formation are controlled by lipid composition and lipidprotein interactions

11:15–11:30 **T 13** **Till Rudack,** Bochum, GER
A scale-spanning integrative modeling strategy to study structure, dynamics, and function of molecular machines

11:30–11:45 **T 14** **Hendrik Jung,** Frankfurt, GER
Artificial intelligence for molecular mechanism discovery

11:45-12:00 **T 15** **Marius F. W. Trollmann,** Erlangen, GER
mRNA lipid nanoparticle phase transition

Parallel Session 1C – Biospectroscopy I **A 704**
11:00–12:00 **Chair:** Jacek Kozuch, Berlin, GER

11:00–11:15 **T 16** **Tilman Kottke,** Bielefeld, GER
In-cell infrared difference spectroscopy on photoreceptors

11:15–11:30 **T 17** **Henrike M. Müller-Werkmeister,** Potsdam, GER
Time-resolved serial crystallography of an enzyme at work: the role of lasers, timing, and spectroscopy

11:30–11:45 **T 18** **Ellen Adams,** Dresden, GER
Key role of the solvent in driving liquid-liquid phase separation

11:45–12:00 **T 19** **Lara Williams,** Konstanz, GER
Site-directed labelling with photoexcitable spin labels for light-induced dipolar spectroscopy

12:00–12:30 **T 20** **Christian Bamann,** DFG **A 701**

DFG-funding opportunities for graduates of Life Sciences

12:00–13:00 **Lunch Break**

Plenary Session 3 **Audimax**
13:00–14:30 **Chair:** Helmut Grubmüller, Göttingen, GER

13:00–13:30 **T 21** **Mike Heilemann,** Frankfurt, GER
Quantification of protein subunits in dense clusters using kinetics-assisted quantitative super-resolution microscopy

13:30–14:00 **T 22** **Frauke Gräter,** Heidelberg, GER
How collagen converts mechanical into chemical stress

14:00–14:30 **T 23** **Emad Tajkhorshid,** Urbana-Champaign, USA
Lipid-mediated organization of prestin in the cochlear membrane and implications in sound amplification

14:30–15:00 **Coffee Break**

Poster Session 1 **Poster Areas**
15:00–16:30

Industry Talks **A 701**
15:00–16:30

Parallel Session 2A – Biomolecular Interactions **A 701**
16:30–17:15 **Chair:** Luuk van Wilderen, Frankfurt, GER

16:30–16:45 **T 24** **Nils-Alexander Lakomek,** Düsseldorf, GER
Structural dynamics of intrinsically disordered proteins at the membrane interface: recent insights into the pre-fusion state of SNARE proteins by NMR spectroscopy

16:45–17:00 **T 25** **Radek Šachl,** Prague, CZ
In-membrane protein oligomerization as a critical step for membrane pore formation

17:00–17:15 **T 26** **Andres Manuel Vera,** München, GER
Heterogeneous assembly of the cohesin-dockerin interaction and its modulation by isomerization of a single proline

Parallel Session 2B – Channels and Transporters **A 703**
16:30–17:15 **Chair:** Jörg Fitter, Aachen, GER

16:30–16:45 **T 27** **Indra Schroeder,** Jena, GER
Role of ion distribution and energy barriers for concerted motion of subunits in selectivity filter gating of a K+ channel

16:45–17:00 **T 28** **Marcus Schewe, Kiel,** GER
An alternative mechanism of Kv channel inhibition: binding of cellular lipids to side-pockets induces C-type inactivation

17:00–17:15 **T 29** **Abhishek Acharya,** Bremen, GER
A mechanistic view of the role of L3 loop conformational dynamics in antibiotic permeation and gating in OmpF

Parallel Session 2C – Genetic Regulatory Systems **A 704**
16:30–17:15 **Chair:** Karim Fahmy, Dresden, GER

16:30–16:45 **T 30** **Hannes Witt,** Amsterdam, NL
The mechanics of mitotic chromosomes

16:45–17:00 **T 31** **Michelle Paulina Rademacher,** Düsseldorf, GER
Time-resolved spectroscopy of an angular psoralen intercalated into DNA

17:00–17:15	**T 32**	**Alice Frederike Rosa Grün,** Hamburg, GER *Structural analysis of the interaction of the herpes simplex virus 1 terminase with secondary DNA structures*
17:30		**Departure for Boat Trip**
19:30–22:00		**Conference Dinner Konzil**

TUESDAY, SEPTEMBER 27TH, 2022

Plenary Session 4 – GBM Session **Audimax**
08:30–10:30 **Chair:** Claus Seidel, Düsseldorf, GER

08:30–09:00	**T 33**	**Philip Tinnefeld,** München, GER *Single-molecule biophysics and biosensing with DNA origami devices and graphene*
09:00–09:30	**T 34**	**Jonas Ries,** Heidelberg, GER *Superresolution microscopy for structural cell biology*
09:30–10:00	**T 35**	**Sophie Brasselet,** Marseille, FRA *Imaging of proteins' organization in 3D using single molecule orientation and localization microscopy (SMOLM)*
10:00–10:30	**T 36**	**Alf Honigmann,** Dresden, GER *Structure and assembly of epithelial tight junctions: super-resolution and reconstitution*
10:30–11:00		**Coffee Break**

Parallel Session 3A – Imaging, Microscopy, Single Molecule Biophysics I
11:00–12:00 **Chair:** Jacob Piehler, Osnabrück, GER **A 701**

11:00–11:15 **T 37** **Iliya Stoev,** Dresden, GER
Highly sensitive force measurements enabled by a new optofluidic particle trap

11:15–11:30 **T 38** **Oleksii Nevskyi,** Göttingen, GER
Super-resolution microscopy with metal-induced energy transfer

11:30–11:45 **T 39** **Charlotta Lorenz,** Göttingen, GER
Mechanical properties of keratin and vimentin intermediate filaments

11:45–12:00 **T 40** **Andrea Pruccoli,** Konstanz, GER
Electronically enhanced Stimulated Raman Scattering microscopy of visible dyes

Parallel Session 3B – Computational Biophysics II **A 703**
11:00–12:00 **Chair:** Rainer Böckmann, Erlangen, GER

11:00–11:15 **T 41** **Roberto Covino,** Frankfurt, GER
Extracting free energy and dynamics from incomplete single molecule measurments with simulation based inference

11:15–11:30 **T 42** **Vania Calandrini,** Jülich, GER
Subdiffusive-Brownian crossover in membrane systems: a generalized Langevin equation-based approach

11:30–11:45 **T 43** **Christoph Allolio,** Prague, CZ
Multiscale modeling of specific interactions on biomembranes

11:45–12:00 **T 44** **Leonie Chatzimagas,** Saarbrücken, GER
Simulation of liquid jet explosions and shock waves induced by X-ray free-electron lasers

Parallel Session 3C – Protein Structure, Dynamics, Function **A 704**
11:00–12:00 **Chair:** Indra Schroeder, Jena, GER

11:00–11:15 **T 45** **Jochen Balbach,** Halle, GER
Macromolecular crowding induces a binding competent transient structure in intrinsically disordered Gab1

11:15–11:30 **T 46** **Olga Mayans,** Konstanz, GER
Stretch-induced unfolding of titin-like kinases as mechanosignalling mechanism in vivo

11:30–11:45 **T 47** **David Scheerer,** Rehovot, ISR
Substrate inhibition of an enzyme: are ultrafast motions affecting catalytic activity?

11:45–12:00 **T 48** **Sven Stripp,** Berlin, GER
Made in the dark – infrared difference spectroscopy for the analysis of gas-processing metalloenzymes

12:00–13:00 **Lunch Break**

Plenary Session 5 **Audimax**
13:00–14:30 **Chair:** Thomas Gutsmann, Borstel, GER

13:00–13:30 **T 49** **Nina Morgner,** Frankfurt, GER
Wanted and unwanted assemblies of biomolecular complexes – what can we learn with native mass spectrometry?

13:30–14:00 **T 50** **Peter Hamm,** Zürich, CH
Using azobenzene photocontrol to set proteins in motion

14:00–14:30 **T 51** **Marloes Groot,** Amsterdam, NL
Translation of higher harmonic generation microscopy into the clinic for tumor tissue assessment

14:30–15:00 **Coffee Break**

Poster Session 2 **Poster Areas**
15:00–16:30

Industry Talks **A 701**
15:00–16:30

Parallel Session 4A – Membrane Biophysics II **A 701**
16:30–17:30 **Chair:** Jochen Hub, Saarbücken, GER

16:30–16:45 **T 52** **Andreas Janshoff,** Göttingen, GER
Cortex mechanics – the impact of actin architecture and the plasmamembrane

16:45–17:00 **T 53** **Daniel Mann,** Jülich, GER
Macromolecular organization of atg18 oligomers

17:00–17:15 **T 54** **Oliva Saldanha,** Konstanz, GER
Calcium induced vesicular interactions studied with ATR- FTIR spectroscopy

17:15–17:30 **T 55** **Larissa Socrier,** Göttingen, GER
Optical manipulation of Gb3 enriched lipid domains: impact on Shiga Toxin B binding

Parallel Session 4B – Cell Biophysics **A 703**
16:30–17:30 **Chair:** Maria Hörnke, Freiburg, GER

16:30–16:45 **T 56** **Andra Schromm,** Borstel, GER
Bacterial outer membrane vesicles (OMVs): Dissecting the delivery process to host cells

16:45–17:00 **T 57** **Malgorzata Lekka,** Krakow, PL
Understanding a link between the biomechanics and invasiveness of bladder cancer cells

17:00–17:15 **T 58** **Johannes Rheinlaender,** Tübingen, GER
Measuring the cortical tension of living cells using the scanning ion conductance microscope

17:15–17:30 **T 59** **Karim Fahmy,** Dresden, GER
Conserved patterns of heat release from cultured microorganisms reveal simple growth-metabolism relations

Parallel Session 4C – Protein Folding, Aggregation, Disease **A 704**
16:30–17:30 **Chair:** Henrike M. Müller-Werkmeister, Potsdam, GER

16:30–16:45 **T 60** **Jörg Fitter,** Aachen, GER
Mapping multiple distances in a multidomain protein for the identification of folding intermediates

16:45–17:00 **T 61** **Miloš Ivanović,** Zürich, CH
Protein dynamics in a biomolecular condensate

17:00–17:15 **T 62** **Kay Saalwächter,** Halle, GER
Gel formation of alpha crystallin solutions

17:15–17:30 **T 63** **Christian Nehls,** Borstel, GER
Visualization and force spectroscopy of mineral desert dust and associated microbes: unraveling a bacterial long-distance propagation strategy

Young Investigator Award **Audimax**
17:40–18:10 **Chair:** Hans-Joachim Galla, Münster, GER

17:40–18:10 **T 64** **Georg Krainer,** University of Cambridge, UK
Next generation microfluidic approaches for protein biophysics

18:15–18:30	**DGfB Section Meetings**	
	Section I – Molecular Biophysics	**A 702**
	Section II – Membrane Biophysics	**A 703**
	Section III – Cellular Biophysics	**A 704**
18:30–19:30	**DGfB General Assembly**	**A 701**
TBA	**Guided City Tours**	

WEDNESDAY, SEPTEMBER 28TH, 2022

Plenary Session 6 **Audimax**
08:30–10:30 **Chair:** Michael Kovermann, Konstanz, GER

08:30–09:00 **T 65** **Gary Pielak,** Chapel Hill, USA
Protein- & protein-complex stability in living cells

09:00–09:30 **T 66** **Stefan Weber,** Freiburg, GER
EPR and NMR studies of paramagnetic intermediates in the primary processes of blue-light photoreceptor proteins

09:30–10:00 **T 67** **Florian Stengel,** Konstanz, GER
Studying proteome organization and cellular compartmentalization: from proteins to functional compartments

10:00–10:30 **T 68** **Charlotte Uetrecht,** Hamburg, GER
Flying viruses – understanding corona- and norovirus lifecycles

10:30–11:00 **Coffee Break**

Parallel Session 5A – Imaging, Microscopy, Single Molecule Biophysics II
11:00–12:00 **Chair:** Don Lamb, München, GER **A 701**

11:00–11:15 **T 69** **Andre C. Stiel,** München, GER
Photoswitching across the scales – photoswitching proteins in super-resolution microscopy and optoacoustic imaging

11:15–11:30 **T 70** **Hauke Winkelmann,** Osnabrück, GER
Quantifying cytokine receptor dimerization dynamics in the plasma membrane by smFRET

11:30–11:45 **T 71** **Nazar Oleksiievets,** Göttingen, GER
Single-molecule fluorescence lifetime imaging using wide-field and confocal-laser scanning microscopy: a comparative analysis

11:45–12:00 **T 72** **Daniel Dornbusch,** Dresden, GER
Anion-specific Sstructure and stability of guanidinium-bound DNA origami

Parallel Session 5B – Photobiophyscis, Electron and Proton Transfer
11:00–12:00 **Chair:** Tilman Kottke, Bielefeld, GER **A 703**

11:00–11:15 **T 73** **Patrycja Kielb,** Bonn, GER
Do Tyr/Trp redox pathways protect O2-reducing C. Coelicolor laccase from oxidative damage?

11:15–11:30 **T 74** **Sarah M. Mäusle,** Berlin, GER
S-state Transitions of Photosystem II from Spinach and T. elongatus – Insight by Time-Resolved Single-Frequency Infrared Spectroscopy

11:30–11:45 **T 75** **Jheng-Liang Chen,** Berlin, GER
Revealing the mechanism of a light-driven inward proton pump, NsXeR, by site-directed mutagenesis and spectroscopic investigations

11:45–12:00 **T 76** **Florian Brünig,** Berlin, GER
Spectral signatures of excess-proton waiting and transfer-path dynamics

Parallel Session 5C – Biospectroscopy II **A 704**
11:00–12:00 **Chair:** Jochen Balbach, Halle, GER

11:00–11:15 **T 77** **Luuk van Wilderen,** Frankfurt, GER
Femtosecond-to-millisecond mid-IR spectroscopy of Photoactive Yellow Protein uncovers structural micro-transitions of the chromophore's protonation mechanism

11:15–11:30 **T 78** **Tiago Mendes Ferreira,** Halle, GER
Towards complex biological lipid bilayers by solid-state NMR spectroscopy

11:30–11:45 **T 79** **Jacek Kozuch,** Berlin, GER
Nitrile infrared intensities characterize electric fields and hydrogen bonding in protic, aprotic, and protein environments

11:45–12:00 **T 80** **Elena Erben,** Dresden, GER
Optofluidic method for highly precise and non-invasive manipulations on the microscale

Poster Awards and Closing **Audimax**
12:10–12:30

MONDAY, SEPTEMBER 26TH, 2022 — A 701

15:00–15:15 **Andreas Hugi**, IR Sweep AG
Mid-infrared QCL dual-comb spectrometer for advanced bio-applications

15:15–15:30 **Anja Huss**, Thorlabs
Large Apperture Raman Spectrometer

15:30–15:45 **Philipp Hanisch**, Cube Biotech
Copolymer vs Detergent – an advanced toolbox for native membrane protein solubilization and stabilisation

15:45–16:00 **Simona Stelea**, Rapp Optoelectronics
FLUCS Micro Flow Photomanipulation for Cell Biology and Micro Fluidics

16:00–16:15 **Michael Kehr**, Hamamatsu Photonics
Igniting Questions, Detecting Answers: Hamamatsu Photonics presents the world's first photon-number-resolving scientific camera

TUESDAY, SEPTEMBER 27TH, 2022 — A 701

15:00–15:15 **Matthias Godejohann**, MG Optical Solutions
Recent achievements in vibrational QCL-IR spectroscopy

15:15–15:30 **Kristina Popova**, Dynamic Biosensors
Helix: The Modular Biosensor for Measuring Interactions from Small Molecules to Cells

15:30–15:45 **Matthias Langhorst**, Refeyn Ltd.
Mass photometry – an analytical technology for biomolecular characterization

15:45–16:00 **Eric Klein**, Bruker Optics
FT-IR and QCL imaging application with Bruker HYPERION II

16:00–16:15 **Philipp Rauch**, LUMICKS
Single molecule biophysics with correlative fluorescence optical tweezers

2024 IUPAB CONGRESS

+ 62nd Annual Meeting of the Biophysical Society of Japan

JUNE 24-28

at the **KYOTO INTERNATIONAL CONFERENCE CENTER**

Program Overview:

10 Plenary Lectures
(including IUPAB lectures),
25-30 Symposia,
500+ Posters.

~ 70 Bursaries available

Research Topics:

- **Structural biology**
- **Membrane/organelle dynamics**
- **Single-molecule biophysics**
- **Bioimaging**
- **Theoretical biophysics**
- ***De novo* enzyme design**
- **Machine learning in biophysics**
- **LLPS/membrane-less organelle**
- **Synthetic biology**
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Biophysics for health and disease

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10th - 14th Oct. 2027

Poster Session 1 (**odd numbers**): Monday, Sep 26, 2022: 15:00-16:30

Poster Session 2 (**even numbers**): Tuesday, Sep 27, 2022: 15:00-16:30

Please check your poster number in the program booklet. Each session will be split in 2 groups, the presenters of group 1 (colored in blue) should be available at the poster for discussion from 15:00-15:45, the presenters of group 2 (colored in orange) from 15:45-16:30.

Posters should be displayed as long as possible, so that there is also ample time for discussions during the breaks. Poster walls are accessible from Sunday afternoon. Pins will be provided on the poster boards.

Plans with all poster sessions will be posted on-site so that you can easily find your poster location.

Please note that all posters should be hanging on Monday morning by 10:00 and need to be taken off on Tuesday by 18:30. Left posters will be removed afterwards.

All posters participate in the poster contest and are rated by the conference participants according to scientific quality and visual appearance. Each participant can give a digital vote until Wednesday morning, Sep 28, 2022, 9:00 by choosing three (different) favorite poster numbers. You can access the voting procedure by scanning the QR Code below. The link will also be provided at the poster session or by visiting our website.

The three best posters will be awarded with poster prizes. Each poster prize comes with a prize money of 200 Euros each donated by the German Biophysical Society. The poster prizes will be awarded in the closing ceremony on Wednesday, Sep 28, 2022, 12:10-12:30, Audimax.

SESSION 1: PROTEIN STRUCTURE, DYNAMICS AND FUNCTION

P001	**Investigation of proton motive force establishment from single cytochrome c oxidase enzymes using a fluorescence assay based on a voltage-sensitive protein** Jens Balke, Berlin, DE
P002	**Effect of O-Glycans on Structure and Friction of the Intrinsically Disordered Synovial Joint Protein Lubricin** Saber Bousheri, Karlsruhe, DE
P003	**Conversion of a Halogenase to a Photoenzyme: Impact of Tryptophans Identified by FTIR, Difference Spectroscopy** Niklas Diepold, Rahden, DE
P004	**Role of Obscurin Dual-Kinase System in Mechanosensing in Active Muscles** Till Dorendorf, Konstanz, DE
P005	**Effect of Multivalent Salts on Protein Diffusion under Crowding Conditions** David Haselberger, Halle (Saale), DE
P006	**How redox-state of the -2 disulfide bond in HLA-B*1501 affects the binding groove opening** Jose Saul Hernandez Fragoso, Jülich, DE
P007	**Structure and Organization of monoclonal antibodies at the air/water interface in the presence of pharmaceutical polymers** Elise Johanna Hingst, Halle, DE
P008	**Influence of pH on Fibrinogen and its Surrounding Ions during Salt-Induced Fiber Assembly** Jana Lierath, Bremen, DE
P009	**Activity boost of Thermomyces lanuginosus lipase by interaction with polymethyl methacrylate** André Lorenz, Krefeld, DE

P010	**Understanding the determinants of complex formation between the Archaeoglobus fulgidus, Ammonium transporter Amt2 and its regulatory partner GlnK2** Fernando Ormeno, Freiburg, DE
P011	**Spin-labelling via metabolic glycoengineering for studying post-translational protein modification by electron paramagnetic resonance spectroscopy** Anna Rubailo, Konstanz, DE
P012	**The energy barrier for the alternating access of an ATP-Binding Cassette (ABC) exporter** Michael Rudolph, Frankfurt am Main, DE
P013	**Active Site and Gates Structure of Chrimson Wild-Type and Mutants and their Absorption Spectra** Katharina Spies, Karlsruhe, DE

SESSION 2: DNA, RNA, GENETIC REGULATORY SYSTEMS

P014	**The chromatin remodeler ISWI transiently bridges phase separated chromatin fibers in an ATPdependent manner** Dieter Kamp, München, DE
P015	**Towards the genetic clearance of living tissues to improve imagin** Susan Wagner, Dresden, DE
P016	**Charge renormalization in DNA oligonucleotides** Xin Zhu, Oxford, GBR

SESSION 3: PHOTOBIOPHYSICS, ELECTRON & PROTON TRANSFER

P017	**Ultrafast Photoinduced Dissociation of Delphinidin-3-Rutinoside** Florian Bartonitz, Berlin, DE

P018	**Proton channel communication in cytochrome c oxidase** Metehan Celebi, Berlin, DE
P019	**Investigating D1-Glu189 variants of Photosystem II by time-resolved O2-polarography** Kalman Christer, Berlin, DE
P020	**Tracking water oxidation through time-resolved FTIR Spectroscopy** Paul Greife, Berlin, DE
P021	**The photoreaction of the proton-pumping rhodopsin 1 from the maize pathogenic Basidiomycete Ustilago maydis** Mariafrancesca La Greca, Berlin, DE
P022	**Structural and dynamic analysis of the third conformational state of T4 Lysozyme by photoinduced electron transfer** Alexander Larbig, Düsseldorf, DE
P023	**Proton collecting antenna residues at the K-channel entrance of the redox-coupled proton pump cytochrome c oxidase** Victor Manuel Loyo Cruz, Berlin, DE
P024	**Simulation of Exciton Transfer in Light-Harvesting Complexes using Machine Learning Techniques** Monja Sokolov, Berlin, DE
P025	**Complex formation of FDH:MBH using Coarse Grained Molecular Dynamics Simulation** Meritxell Wu Lu, Berlin, DE
P026	**Ultrafast Protein response of Bacteriorhodopsin** Clark Zahn, Berlin, DE

SESSION 4: CHANNELS AND TRANSPORTERS

P027	**Heterotrimeric concatamers of ionotropic P2X4 and P2X7 receptors** Malte Berthold, Halle (Saale), DE

P028	**The functional interplay of the ABC transporter PgP with its lipid substrates** Dario De Vecchis, Bochum, DE
P029	**Expression and purification of connexin 43 for electrophysiological studies** Manuel Fink, Göttingen, DE
P030	**Investigation of conformational changes at the K-channel entrance of cytochrome c oxidase using the fluorescent molecular rotor Sulfo-Cy3-maleimide and site-directed labeling** Jacqueline Gottwald, Berlin, DE
P031	**Complex interactions of the antidepressant clomipramine with an ABC transporter** Nadja Hellmann, Mainz, DE
P032	**pH-dependent gating of the human voltage-gated proton channel from molecular dynamics simulations** Christophe Jardin, Nürnberg, DE
P033	**Identification of residues involved in homotrimeric stabilization of the hP2X4R receptor channel by molecular dynamic simulations** Aparna Sai Malisetty, Bremen, DE
P034	**A vibrational spectroscopic Approach to elucidate the molecular Mechanism behind the Channel Activity of Viroporins** Ronja Paschke, Berlin, DE

SESSION 5: PROTEIN AGGREGATION AND DISEASES

P035	**When are Amyloid β Fibrils Most Toxic? Combining two Fluorescence Assays Can Help Answering this Question.** Juliane Adler, Leipzig, DE
P036	**Salt-induced precipitation of fibrinogen: new insights from experiments and simulations** Susan Köppen, Bremen, DE

P037	**C25-modified rifamycin derivatives with improved activity against Mycobacterium abscessus** Laura Paulowski, Borstel, DE
P038	**Aggregation behaviour of beta-lactoglobulin and beta-lactoglobulin fragments** Srdjan Pusara, Eggenstein-Leopoldshafen, DE

SESSION 6: MEMBRANE BIOPHYSICS, MEMBRANE PROTEINS AND PROTEIN-LIPID INTERACTIONS

P039	**Ceramide and Annexin A1 membrane binding; Insights from QCM-D** Lisa Baum, Münster, DE
P040	**Antimicrobial Peptides Induce Membrane Permeabilisation and Lipid Clustering** Katharina Beck, Freiburg, DE
P041	**Electron spin resonance spectroscopic investigation of the lipopolysaccharide exporter** Marina Dajka, Frankfurt am Main, DE
P042	**POPS-doted solid-supported lipid bilayers as a membrane model for the GABAergic post synapse** Lara Dohmen, Göttingen, DE
P043	**Small molecules can modulate phase separation in complex membranes** Oskar Engberg, Leipzig, DE
P044	**Martini Cholesterol gives membranes the chills and what you can do about it** Balázs Fábián, Frankfut am Main, DE
P045	**Lipid phase transitions in cell and synthetic membranes** Nicolas Färber, Augsburg, DE

P046	**A versatile toolbox for constructing nanoscale signaling platforms in live cells** Arthur Felker, Osnabrück, DE
P047	**Reconstituting ATP synthase and monitoring its activity in photoacid-containing vesicles** Hendrik Flegel, Göttingen, DE
P048	**Beta2-adrenergic receptor promotes transmembrane ligand flip-flopping** Christina Gil Herrero, Frankfurt am Main, DE
P049	**Polymer nanodiscs for single-molecule protein spectroscopy** David Glück, Graz, AT
P050	**Azobenzene based lipids and light-induced switching of membrane properties** Justin Hornbogen, Kaiserslautern, DE
P051	**Lipid Membrane Modulation under Hypoxia: Towards Lipid-Based Therapy & Diagnosis in Pancreatic Cancer** Prema Kumari Agarwala, Bombay, Indien
P052	**ATR-FTIR spectroscopy of calcium-dependent lipid-binding proteins** Shane Maguire, Konstanz, DE
P053	**Investigation of the influence of lipid bilayer lateral pressure on Bacteriorhodopsin functionality** Raiza Maja, Berlin, DE
P054	**Unravelling the Molecular Mechanisms of Hepatitis C Virus Assembly** Titas Mandal, Potsdam, DE
P055	**Regulation of JAK activation by the membrane environment** Thomas Meyer, Osnabrück, DE
P056	**Atomic force microscopic experiments to decipher the function of Candidalysin** Simon Pennuttis, Bad Oldesloe, DE

P057	**Interplay of local spontaneous membrane curvature and cholesterol asymmetry** Matthias Pöhnl, Erlangen, DE
P058	**Lipid specificity of Viral Fusion Proteins** Chetan S Poojari, Saarbrücken, DE
P059	**Inside out – The role of mycobacterial ESX secretion systems in phagosome escape** Monika Rangole, Sulfeld, DE
P060	**Membrane interactions and curvature sensing of the autophagic LC3 lipidation machinery** Shanlin Rao, Frankfurt am Main, DE
P061	**Mimicking the minimal neuronal fusion machinery** Merve Sari, Göttingen, DE
P062	**Nanostructured lipid carriers for chronic inflammation in non-healing skin wounds based on promising natural bioactive compounds** Proscila Schilrreff, Berlin, DE
P063	**An Azidolipid Monolayer Transitions, Miscibility, and UV Reactivity studied by Infrared Reflection Absorption Spectroscopy** Christian Schwieger, Halle (Saale), DE
P064	**Are pure-protein bilayers similar to lipid bilayers?** Leonhard Starke, Saarbrücken, DE
P065	**Resolving lipid dynamics in the photocycle of bacteriorhodopsin by mid-IR quantum cascade laser spectroscopy** Paul Stritt, Konstanz, DE
P066	**From cell to substrate – A plasma membrane system for Cav1.3 cluster detection** Nikolas Teiwes, Göttingen, DE
P067	**Two cooperative binding sites sensitize PI(4,5)P2 recognition by the tubby domain** Sebastian Thallmair, Frankfurt am Main, DE

P068	**Designed membrane protein heterodimers and control of their affinity by binding domain and membrane linker properties** Maximilian Ulbrich, Freiburg, DE
P069	**The role of membrane composition in JAK binding** Isabelle Watrinet, Osnabrück, DE
P070	**Lipid membrane dynamics via joint analysis of NMR and MD** Kai Zumpfe, Leipzig, DE

SESSION 7: CELL BIOPHYSICS, INTRACELLULAR TRANSPORT AND SIGNALLING

P071	**Surface acoustic waves stimulate wound healing in vitro** Kathrin Baumgartner, Augsburg, DE
P072	**Sustainability from a Cell's Perspective** Ronald Clarke, Sydney, AUS
P073	**Exploring the Mechanism of Autophosphorylation in the Bacterial Sensory System using QM/MM Studies** Lena Eichinger, Karlsruhe, DE
P074	**Live or let die: Bcl-2 protein transmembrane domain interactions in apoptosis signaling** Thomas Peter Fellmeth, Ostfildern, DE
P075	**Super-resolution microscopy of GSDMD pores in polymer-supported plasma membranes** Shirin Kappelhoff, Osnabrück, DE
P076	**Mechanical and adhesive properties of Pancreatic ductal adenocarcinoma cells** Shruti G Kulkarni, Bremen, DE
P077	**Optically controlled micro-transport at microscale with reduced heating impact** Antonia Minopoli, Dresden, DE

P078	**The effective dynamic elastic modulus of cancer cells as function of the membrane order** Simon Neidingen, Schwabmünchen, DE
P079	**Comparison of healthy versus Dupuytren fibroblasts behavior in 3D-collagen I matrices** Sandra Pérez Dominguez, Bremen, DE
P080	**2D Mechanoresponsive Surfaces for Measuring Cellular Traction Forces** Russel Wilson, Linz, AT

SESSION 8: COMPUTATIONAL BIOPHYSICS

P081	**Experiment-guided molecular simulations reveal the heterogeneous ensemble of the SH2 tandem of SHP2 phosphatase** Massimilano Anselmi, Saarbrücken, DE
P082	**Mechanistic Insight into the Early Events of the Activation of the c-Met Receptor during Listeria Invasion** Maria Serena Arghittu, Frankfurt am Main, DE
P083	**QM/MM Molecular Dynamics Simulation of Thiol-Disulfide Exchange by Glutaredoxin** Julian Böser, Karlsruhe, DE
P084	**Energetics and permeation of photo-resists used for 3D-laser printing across biological lipid bilayers** Lucas Diedrich, Heidelberg, DE
P085	**Simulation-based inference for single-molecule force-spectroscopy experiments** Lars Dingeldein, Frankfurt am Main, DE
P086	**The roles of PIP2 lipids in based signalling of ß2-adrenergic receptor** Wenzel Gaßner, Stuttgart, DE

P087	**Multiscale simulations of proteins** Christoph Globisch, Konstanz, DE
P088	**Investigations into Single Transduction Mechanism in Phytochromes** Oanh Tu Hoang, Berlin, DE
P089	**Constant pH Simulations of the Proton Exit Channel in P. Denitrificans Cytochrome c Oxidase** Jesse William Jones, Berlin, DE
P090	**Free Energy Simulations of Electroporation** Gari Kasparyan, Saarbrücken, DE
P091	**Molecular Transport in Mesoporous Carbon Materials** Sofia Kolin, Stuttgart, DE
P092	**What the Phos? Parametrizing Protein Phosphorylation for the CHARMM36 and Martini Force Fields** Viktoria Korn, Stuttgart, DE
P093	**Artificial intelligence sheds light on protein folding dynamics at the atomic scale** Gianmarco Lazzeri, Frankfurt am Main, DE
P094	**Investigation of Ion Permeations through CNGA1 Channels by Molecular Dynamics Simulations** Haoran Liu, Berlin, DE
P095	**Generalized workflow for automated evaluation of isothermal microcalorimetry traces** Mani Lokamani, Dresden, DE
P096	**QM/MM metadynamics of thiol-disulfide exchange using a neural network correction** Denis Maag, Karlsruhe, DE
P097	**Overcoming hysteresis in ligand binding free energy calculations** Alejandro Martínez-León, Saarbrücken, DE
P098	**Resolution transformation in molecular dynamics: Boosting backmapping via knowledge-driven machine learning** Christian Pfaendner, Stuttgart, DE

P099	**Towards excitonic properties of antenna complexes in Photosystem II** Pooja Sarngadharan, Bremen, DE
P100	**Sublytic gasdermin-D pores captured in atomistic molecular dynamics simulations** Stefan Schäfer, Frankfurt am Main, DE
P101	**Effect of Transmembrane Domains on the Free Energy of Stalk Nucleation during Membrane Fusion** Katharina Scherer, Saarbrücken, DE
P102	**GlycoSHIELD: an online tool to address glycan dynamics and heterogeneity in glycoproteins** Mateusz Sikora, Frankfurt am Main, DE
P103	**Modelling drug permeation using simulated tempering-enhanced umbrella sampling** Carla F. Sousa, Saarbrücken, DE
P104	**Investigating human Ire1 assembly process via multiscale Molecular Dynamics simulations** Elena Spinetti, Frankfurt am Main, DE
P105	**The Role of Interfacial Water in Biomolecular Interactions** Rowan Walker-Gibbons, Oxford, GBR

SESSION 9: BIOMOLECULAR SPECTROSCOPY

P106	**FTIR spectroscopy on the carbonyl region of variations of photosystem I isolated from cyanobacteria** Viktor Eichmann, Berlin, DE
P107	**FTIR and UV-Vis spectroscopic studies of a Rhodopsin Guanylyl Cyclase** Paul Fischer, Berlin, DE

P108	**Signal Progression in a LOV Photoreceptor Studied by Time-resolved In-Cell Infrared Difference Spectroscopy** Lukas Goett-Zink, Bielefeld, DE
P109	**Adsorption of shape-directing agents to differently shaped ZnO particles** Wolfgang Hinze, Konstanz, DE
P110	**Dual-comb-IR-spectroscopy to study temperature-jump dynamics of polyQ model peptides** Lorenz Mattes, Konstanz, DE
P111	**The Photo-physics of 2-Cyanoindole probed by Femtosecond Spectroscopy** Mahbobeh Morshedi, Düsseldorf, DE
P112	**In-cell Infrared Difference Spectroscopy on the Bacterial Chloride Pump NmHR** Sabine Oldemeyer, Berlin, DE
P113	**CASS – A new tool to investigate dynamics in complex samples** Moritz Schlötter, Konstanz, DE
P114	**The global conformation of the kinase Akt1 monitored by DEER spectroscopy and multilateration** Juliane Stehle, konstanz, DE
P115	**Photophysics and photochemistry of a new fluorescent molecular rotor dye: CCVJ boronic acid** Florian Telschow, Blankenfelde-Mahlow, DE
P116	**Structural and functional determinants governing constitutive dimerization of an oncogenic gp130 mutant** Steffen Wolke-Hanenkamp, Osnabrück, DE

SESSION 10: IMAGING, MICROSCOPY, SINGLE MOLECULE BIOPHYSICS

P117	**Surface plasmon resonance microscopy as a platform to assess cell-substrate separation using HEK293 cells as a model** Justus Bednár, Jülich, DE
P118	**Far-field electrostatic signatures of macromolecular 3D conformation** Timothy Bennett, Oxford, GBR
P119	**Controlled mechanochemical coupling in DNA origami arrays** Fiona Cole, München, DE
P120	**Reversibly labeled HaloTags enable live cell long-term high- and superresolution imaging** Michael Holtmannspötter, Osnabrück, DE
P121	**Screening for GHOSTs (Genetically Enhanced Optically uperior Tissues).** Venkat Raghavan Krishnaswamy, Dresden, DE
P122	**Studying biomolecular dynamics and structure with high-speed atomic force microscopy** Florian Kumpfe, Berlin, DE
P123	**An easy and reliable way to perform single molecule FRET measurements** Maria Loidolt-Krüger, Berlin, DE
P124	**Nanomechanics of molecular motor molecules linked by self-assembled DNA building blocks** Inga Melnyk, Saarbrücken, DE
P125	**Dual color subunit counting method using GFP/YFP overlapping spectra** Antara Mukhopadhyay, Freiburg im Breisgau, DE
P126	**Spatiotemporal dynamics of IL-17 family receptor signaling complexes in the plasma membrane** Christoph Pollmann, Osnabrück, DE

P127	**Thermodynamic behaviour of EGFP-Booster-antibody-binding using brightness-gated two color coincidence detection** Benno Rüdiger Schedler, DE
P128	**Molecular Adhesion and Friction on Porous Membrane** Kordula Schellnhuber, Saarbrücken, DE
P129	**Fluorescence Lifetime DNA-PAINT for Multiplexed Super-resolution Imaging of Cells** Roman Tsukanov, Göttingen, DE
P130	**Microgels as carrier systems for enzymes studied with a combination of different super-resolution fluorescence microscopy methods** Dominik Wöll, Aachen, DE
P131	**Brightness-gated two-color coincidence detection for characterization of picomolar affinity bimolecular interactions** Olessya Yukhnovets, Aachen, DE
P132	**Single immune-complex detection by Escape-Time electrometry** Konstantin C. Zouboulis, Oxford, GBR

WELCOME RECEPTION

Sunday, September 25, 2022, 19:00 – 21:00

The welcome reception will take place in in the foyer of the A-building at the University of Konstanz right after the opening plenary session on Sunday. All participants are cordially invited to join this Get-Together with food and drinks subsequent to the conference's kick-off and to spend a pleasant evening with colleagues.

BOAT TRIP AND CONFERENCE DINNER

Monday, September 26, 2022

Boat Trip: Departure **17:30** at the university – Arrival **19:30** at the city harbor of Konstanz
Conference Dinner Konzil: **19:30 – 22:00**

For Monday evening we have organized a boat trip on Lake Constance and the Conference Dinner. For both events prior registration was required, last minute participation is not possible, unfortunately. The meeting point for the boat trip is in the exhibition hall at 17:30. There will be a short bus ride from the university to the boat landing stage. The boat will pass beautiful sights of Lake Constance (prehistoric settlement on the lake shore, Birnau Monastry, Mainau Island). You will enjoy a 1 hour boat trip including a Sundowner. The boat trip ends in the city harbor of Konstanz. The conference dinner will take place in the historic Konzil building at the city harbor which is located close to the city center. You will have the opportunity to chat with colleagues and enjoy a relaxing evening.

Address Konzil: Hafenstraße 2, D-78462 Konstanz, www.konzil-konstanz.de

GUIDED CITY TOURS

Tuesday, September 27, 2022

On Tuesday evening you can join one of our city tours with professional tourist guides in the historic old part of Konstanz. There will be a number of themed tours to choose from (dependent on availability) offered in German and English language. Let us know during the conference at the registration desk if you are interested (no later than 13:00 on Tuesday). Here you can also pay the fee of 5 Euros in cash. Transfer to the city center is by public bus (free with the conference ticket). Leave the bus at station „Marktstätte", walk to the harbor, meeting point is at the harbor clock close to the Konzil building.

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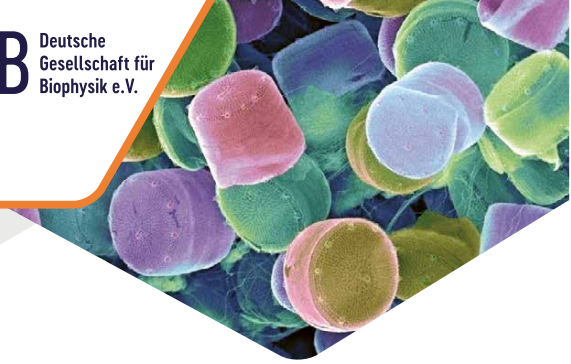
DGfB Deutsche Gesellschaft für Biophysik e.V.



German Biophysical Society e.V.

The DGfB e.V. promotes and disseminates science and research in the field of biophysics, represents its interests and supports the exchange of scientists working in the field of biophysics.
A central task of the DGfB is the promotion of young scientists with the focus on scientific competences, professional career, networking and acquisition of external funds.

Section 1: Molecular Biophysics

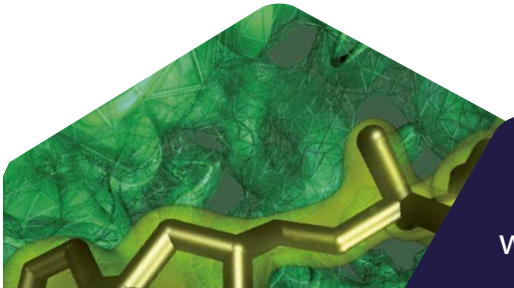
Molecular Biophysics combines experimental approaches (from single molecule imaging to mechanical studies and crystal structures) with theoretical methods to understand the broad spectrum of life processes at the molecular level.

Section 2: Membrane Biophysics

Biomembranes form the universal envelopes of all cells and organelles and, together with integrated proteins, are the basic building blocks for communication between cells and in cellular networks.

Section 3: Cellular Biophysics

Cellular Biophysics analyses life processes at the level of organelles and whole cells. Integrative matching of experiments and models are the basis for building quantitative models.



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DGfB Deutsche Gesellschaft für Biophysik e.V.

Save the date:

German Biophysical Society Meeting
September 22-25, 2024
Leipzig, Germany

UNIVERSITÄT LEIPZIG

Biennial Meeting of the German Biophysical Society

LEIPZIG 24

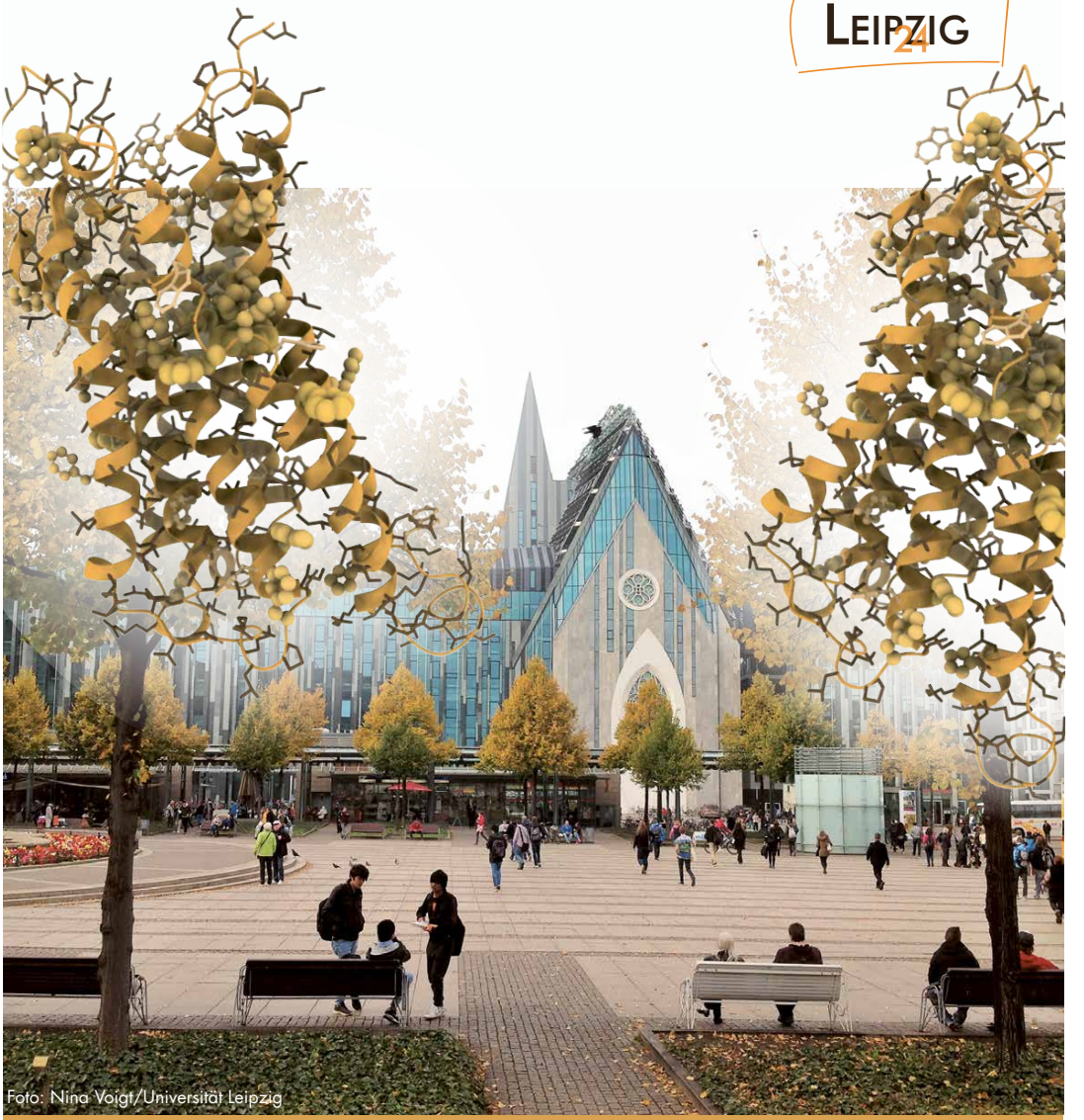


Foto: Nina Voigt/Universität Leipzig

https://www.dgfb.org/de/dgfb-tagungen/german-biophysical-society-2024-leipzig.html

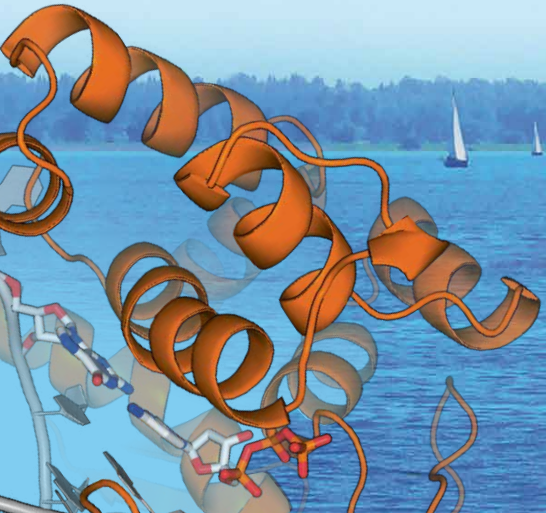
GERMAN BIOPHYSICAL SOCIETY MEETING

September 25–28, 2022

University of Konstanz, Germany

DGfB Deutsche Gesellschaft für Biophysik e.V.

ABSTRACT BOOKLET



www.uni.kn/biophys2022

ABSTRACTS OF THE TALKS

T001 STEINEM, CLAUDIA

Dominik Ruppelt[1]; Sebastian N. Wirtz[2]; Dr. Tom Robinson[3]; Prof. Dr. Stephanie Grond[2]; Prof. Dr. Claudia Steinem[1]

[1] Georg-August Universität Göttingen; [2] Eberhard Karls Universität Tübingen;
[3] Max-Planck-Institut für Kolloid- und Grenzflächenforschung

How does the new antibiotic lugdunin kill MRSA? – An in vitro analysis

The increase in bacterial pathogens resistant to common antibiotics poses a serious and global threat to human health. Efforts to overcome antibiotic resistance have intensified with the success of increased approval rates. However, the success is mainly based on optimization or re-sensitization of established core structures. In 2016, a new structure, a thiazolidine cyclopeptide, was found in an antibiotic screen of human bacterial nasal isolates. This peptide, called lugdunin, shows micromolar activity against MRSA. Using chemically accessible lugdunin and lugdunin derivatives, we were able to study its structure activity relationship in a lipid membrane environment. Using tryptophan fluorescence spectroscopy, we found that the distribution of lugdunin in artificial membranes depends on the lipid composition. Once in the membrane, it makes the bilayer permeable for protons. IR-spectroscopy suggests that lugdunin forms beta-sheet structures leading to stacked rings. Currently, we are addressing the question of whether lugdunin preferentially acts as a carrier or forms a channel-like structure by pursuing a new approach based on giant unilamellar vesicles filled with a pH-sensitive dye captured in a microfluidic device.

T002 KELLER, SANDRO

Prof. Dr. Sandro Keller, University of Graz, Institute of Molecular Biosciences

New Native Nanodiscs for Membrane-Protein Biophysics

Nanodiscs that harbour individual membrane proteins or membrane-protein complexes in a lipid-bilayer environment hold great promise for biophysical investigations under well-controlled yet native-like conditions. Our laboratory focusses on two recent approaches based on the direct ex-traction of proteins from cellular membranes in the absence of conventional detergents. On the one hand, some amphiphilic polymers extract membrane proteins and surrounding lipid molecules to form polymer-encapsulated nanodiscs that are compatible with a broad range of ensemble and single-molecule biophysical techniques.[1,2] On the other hand, we have recently discovered that certain small-molecule amphiphiles carrying a diglucose headgroup are equally capable of forming native-like lipid-bilayer nanodiscs directly from cellular membranes.[3] In this talk, I will present re-cent case studies demonstrating how new, bioinspired polymers as well as small-molecule am-phiphiles can be employed to render membrane proteins amenable to in vitro biophysical investi-gations without ever removing these proteins from a lipid-bilayer environment.

[1] B. Danielczak, M. Rasche, J. Lenz, E. Pérez Patallo, S. Weyrauch, F. Mahler, M.T. Agbadaola, A. Meister, J.O. Babalola, C. Vargas, C. Kolar, S. Keller. A bioinspired glycopolymer for capturing membrane proteins in native-like lipid-bilayer nanodiscs. Nanoscale 2022, 5, 1855

[2] D. Glück, A. Grethen, M. Das, O.P. Mmeka, E. Pérez Patallo, A. Meister, R. Rajender, S. Kins, M. Räschle, J. Victor, C. Chu, M. Etzkorn, Z. Köck, F. Bernhard, J.O. Babalola, C. Vargas, S. Keller. Electroneutral polymer nanodiscs enable interference-free probing of membrane proteins in a lipid-bilayer environment. Small 2022, in revision

[3] F. Mahler, A. Meister, C. Vargas, G. Durand, S. Keller. Self-assembly of protein-containing lipid-bilayer nanodiscs from small-molecule amphiphiles. Small 2021, 17, 2103603

T003 HUSTER, DANIEL

Daniel Huster, University of Leipzig

Rhomboid-Catalized Intramembrane Proteolysis Requires Hydrophobic Matching with the Surrounding Lipid Bilayer

Membrane thinning of the rhomboid GlpG has been proposed to reduce the hydrophobic mismatch between the enzyme and its surrounding lipid environment. Here, we directly show that the membrane environment of the rhomboid influences the velocity of substrate cleavage. We first measure the impact of GlpG on the hydrophobic thickness in phosphatidyl-choline membranes of varying thickness, where the rhomboid only marginally alters the surrounding membrane. However, in an E. coli relevant lipid mix of phosphatidyl-ethanolamine and phosphatidylglycerol, a decrease in hydrophobic thickness of -1.1 Å per leaflet is observed. The cleavage velocity of GlpG is highest in DMPC followed by POPC, POPE/POPG and DLPC, while in the thickest membranes (DPPC/cholesterol) enzyme function is abolished. This suggests that an optimal window of membrane thickness (between ~24 – 26 Å) exists while headgroup specificity does not seem to be decisive for protein function. We infer from these results that the lipid environment can fine-tune GlpG function. By adjusting membrane thickness, for instance through dynamic domain formation, the cell can regulate membrane protein function.

T004 KÖSTER, SARAH

Prof. Dr. Sarah Köster, University of Göttingen

Intermediate filaments in the cytoskeleton: safety belt and shock absorber for the cell?

Mechanical properties of eukaryotic cells are to a great part determined by the cytoskeleton, a composite biopolymer network composed of three filament systems – intermediate filaments, F-actin and microtubules – along with cross-linkers and molecular motors. Among the three filament types, Intermediate filaments are the most flexible and the most extensible ones, with an intriguing non-linear behavior. It has been shown previously that the presence of intermediate filaments in a cell has an influence on cell mechanics. Here we unravel different contributions to network properties and cell mechanics, such as the assembly kinetics and mechanical properties of the individual filaments (in particular at high strains), filament-filament interactions, and network rheology. To explain our experimental results on molecular grounds, we design models that include the strictly hierarchical build-up of the filaments (with multiple alpha-helical domains arranged in parallel) and non-equilibrium transitions between folded and un-folded states. Taken together experiments and modelling indicate that intermediate filaments serve as "safety belts" and shock absorbers" for the cell, thus avoiding damage at high, fast impact while maintaining flexibility (e.g., during cell motility).

T005 JUNKER, PHILIPP

Prof. Dr. Philipp Junker, Max Delbrück Center for Molecular Medicine

Simultaneous lineage tracing and cell type identification using CRISPR/Cas9 induced genetic scars

Every time an egg is fertilized, the cellular diversity of an animal must be built again from scratch. This is achieved by the differentiation of initially pluripotent cells into a multitude of different cell types with distinct gene expression programs. A key goal of developmental biology is to understand how the large variety of different cell types in a fully grown organism is formed. Identification of cell types can be performed in a systematic manner based on single-cell RNA sequencing. However, such snapshot data does not provide information about the lineage history of cells. Here, I present LINNAEUS, a strategy for massively parallel lineage tracing on the single cell level. We exploit the fact that Cas9-induced generation of DNA double-stranded breaks leads to formation of short insertions or deletions ("genetic scars") that are variable in their length and position. We demonstrate that these genetic scars have very high complexity, making them ideal cellular barcodes for lineage analysis. Reading out scar sequences by single-cell RNA sequencing enables us to simultaneously measure lineage relationships and transcriptome profiles in thousands of single cells. We introduce novel computational methods that address the major challenges of CRISPR/Cas9-based lineage tracing, such as preferential creation of specific scar sequences and stochastic dropout events. We use LINNAEUS to reconstruct developmental lineage trees in zebrafish at larval stages, and we demonstrate the potential of our approach for studying organ regeneration upon injury. Specifically, use our method to identify the origin and function of transient fibroblast states that are generated upon heart injury and contribute to organ regeneration.

T006 KRISHNAN, MADHAVI

Prof. Madhavi Krishnan, University of Oxford

Bringing electrostatics to light: Electrometry probes a new dimension at the molecular scale

The desire to "freely suspend the constituents of matter" in order to study their behavior can be traced back over 200 years to the diaries of Lichtenberg. From radio-frequency ion traps to optical tweezing of colloidal particles, methods to trap matter in free space or solution rely on the use of external fields that often strongly perturb the integrity of a macromolecule in solution. We recently introduced the 'electrostatic fluidic trap', an approach that exploits equilibrium thermodynamics to realise stable, non-destructive confinement of a single macromolecule in a room temperature fluid, and represents a paradigm shift in a nearly century-old field. The spatio-temporal dynamics of a trapped molecule reveals important information on its properties, e.g., size and electrical charge. We have demonstrated the ability to measure the electrical charge of a single macromolecule in solution with a precision much better than a single elementary charge. Since the electrical charge of a macromolecule in solution is in turn a strong function of its 3D conformation, our approach enables for the first time precise, general measurements of the relationship between 3D structure and electrical charge of a single macromolecule. I will present our most recent advances in this emerging area of molecular measurement and demonstrate how high-precision interaction energy measurements may be opening up a unique view of molecular-scale matter in solution.

T007 RÄDLER, JOACHIM

Prof. Dr. Joachim Rädler, LMU – Ludwig Maximilian University of Munich

Mechanisms of LNP-Based RNA Delivery and Models of Time-Resolved Gene Expression in Single Cells

Lipid nanoparticles (LNPs) emerged as powerful delivery platform for mRNA based therapies. Yet the physico-chemical mechanisms of assembly, uptake, release and mRNA expression are not fully understood. Here we discuss biophysical aspects of ionizable lipids and LNPs core-shell structure. in particular we study as a model system for LNP bulk phases ionizable lipid/cholesterol/nucleic acid mixtures using small angle X-ray scattering (SAXS). The pH dependence of the mesostructures hint towards possible pH dependent mechanisms of endosomal release. To track it down further we follow the fate of LNPs inside cells taking time courses of single cell fluorescence of mRNA delivery and eGFP expression. Employing automated time-lapse microscopy in combination with micro-patterned surfaces, reporter signals from many individual cells are monitored in high-throughput. The statistics of gene expression traces yields access to delivery delay times, mRNA translation efficiency and mRNA stability. Correlating physico-chemical properties and time resolved activity of LNPs in living cells provides a basis to further improve LNPs for the requirements of next generation of multicomponent nucleic acid delivery.

T008 COSENTINO, KATIA

Prof. Katia Cosentino PhD, University of Osnabrück

Membrane permeabilization in regulated cell death at the single molecule level

Membrane permeabilization is a crucial step in the execution of cell death programs such as apoptosis and pyroptosis, with important physiological and pathological consequences related to infection, immunity, cancer and neurodegeneration. This process is executed by proteins that have the ability to organize into supramolecular complexes and create stable pores into membranes. However, the detailed molecular mechanisms of assembly and membrane pore formation by these proteins is poorly understood.

By combining single-molecule imaging, super-resolution and atomic force microscopy, we have i) revealed the mechanisms of pore formation in apoptosis by the proteins Bax and Bak, by dissecting their stoichiometry in model membranes as well as the real-time assembly of single oligomers in cells. Furthermore, we have provided the first direct evidence that Bax and Bak co-assemble into the same supramolecular complex and elucidated their interconnected, yet different, involvement in the ill-characterized role of apoptosis in inflammation.

In independent studies, we have provided new molecular insights into the nanostructure and mechanism of assembly of Gasdermin D (GSDMD) pores in the plasma membrane of cells undergoing pyroptosis. By implementing correlative nanoscale imaging of GSDMD pores in polymer-supported plasma membrane (PSPMs) in combination with DNA PAINT microscopy we could resolve heterogeneous GSDMD structures at the nanoscale level (about 20 nm in size) and quantify their stoichiometry. Furthermore, we have identified the minimal GSDMD oligomer able to insert into lipid bilayers.

The outcomes of these studies constitute fundamental building blocks in the understanding of the molecular mechanisms of membrane permeabilization in cell death and its relation to downstream inflammatory signaling, with important implications in both physiological and pathological settings.

References:

1. Cosentino K, Hertlein V, Jenner A, et al. Molecular Cell, 2022
2. Danial J, Quintana Y et al. Journal of Physical Chemistry Letters, 2022
3. Jenner A, Shalaby R & Cosentino K. Advances in Biomembranes and Lipid Self-Assembly, 2020.

T009 HOERNKE, MARIA

Shuai Shi; PhD Maria Hoernke, Albert-Ludwigs-Universität Freiburg

Leaky membrane fusion: an ambiguous effect induced by antimicrobial polycations

Both, antimicrobial peptides and their synthetic mimics are potential alternatives to classical antibiotics, as they can induce several membrane perturbations including permeabilization. Especially in model studies, interactions of such polycations with charged vesicles often cause vesicle aggregation and fusion. We show that these side effects cannot be ignored, as they can change the outcome of biophysical studies unnoticed.

We study two antifungal but also antibacterial biomimetic polymers representing two typical groups: either relatively hydrophobic, highly active, but unselective/toxic polycations or highly charged, less active, but highly selective membrane-active polycations.

Our approach involves binding, electrostatic lipid clustering, sophisticated fluorescence lifetime-based leakage and fusion studies, prevention of fusion by sterical shielding and increase of fusion at higher vesicle numbers. Thus, the relation of vesicle aggregation and vesicle fusion with membrane permeabilization was elucidated and a network of membrane perturbation mechanisms was revealed.

At first sight, both polycations bind to negatively charged PG/PE membranes and induce similar permeabilization/leakage. By a combination of methods, we revealed that in one case, asymmetric packing stress is the unselective leakage mechanism, while in the other case, an unexpected mechanism: leaky vesicle fusion causes leakage. Regarding antibacterial activity, leaky vesicle fusion has to be considered an artefact in model studies that is easily overlooked and can result in severe misinterpretations. Leaky fusion might play a role in fungicidal activity and inprove drug delivery.

T010 WESTERHAUSEN, CHRISTOPH

Nicolas Färber MSc; Jonas Reitler; Julian Schäfer; Andrej Kamenac;
Prof. Christoph Westerhausen, Universität Augsburg

Membrane transport in cell ensembles is modulated by the membrane state

We measure the uptake of various fluorescent dyes into adherent HeLa cells and determine simultaneously the degree of membrane lipid chain order on a single cell level. The latter is measured by spectral analysis of the membrane-embedded dye Laurdan and quantified as the generalized polarization GP. First, the mean GP value of single cells varies within a cell population in a range that is equivalent to a temperature variation of 9 K. We exploit this natural variety of lipid membrane chain order to examine the uptake of fluorescent dyes as a function of GP at constant temperature. Using this approach, we show that transport across the cell membrane correlates with the membrane phase state. Specifically, we observed higher transport with increasing lipid chain order. As a result, HeLa cells adapted to lower culture temperatures by reducing lipid membrane chain order reduced transport than the 37°C culture. Moreover, environmental factors influence the transport as well. Local high cell densities increase the lipid order and in turn lead to increased uptake. To demonstrate the physiological relevance of this concept, we analyzed cell phase state and transport at different stages of an in vitro wound healing process. While the uptake within a confluent cell layer is high due to the high degree of lipid chain order it decreases from the wound edge towards the wound centre where the membrane lipid chain order is lowest. Looking ahead we discuss the phenomenon of a non-linear dependence of uptake as function of shear stress at constant temperature.

T011 WILM, MATTHIAS

Prof. Dr. Matthias Wilm PhD, University College Dublin

Synthesis of Large, Lipid Membranes with Integrated Membrane Proteins from Gas Phase

Membrane proteins evolved to gain a large variety of specialised functionality in their biological context. Here, I am presenting a new method to generate large, transportable membranes with integrated membrane proteins. These membranes can be used as an in-vitro system to study membrane protein function, investigate its interaction with lipids or study protein complex assembly in a system whose composition is controlled on a molecular basis. The method lends itself to produce anisotropic membranes in the lipid composition and protein orientation.

Bio-membranes are generated by self-assembly. There are two established methods - self-assembly on a solid support [1] and self-assembly within a lipo-protein ring: nanodiscs [2]. Both methods solve the principal problem of bio-membrane assembly - their lack of stability in a hydrophilic environment - in a different way. However, solid support based membranes can not be moved from their support and nanodiscs can not be made larger than 17 nm in diameter.

Here, large bio-membranes self assemble in a thin sheet of glycerol generated on a liquid surface. I call this layer-based self assembly of bio-membranes. I use a nano-electrospray ion source to generate molecular beams of large molecules. The target area is the liquid surface of a container filled with buffer solution. The procedure consists of several steps:

1. formation of a lipid bi-layer; 2. formation of a thin glycerol layer; 3. formation of lipid mono-layer as template for the membrane assembly; 4. adding detergent solubilised membrane proteins and some more lipid; 5. sealing the layers off by some glycerol; 6. detergent extraction from the surface by SM-2 Biobeads over several days.
   This procedure leads to the formation of a large bio-membrane covering the entire liquid surface which can be transported to an electron microscope (EM) for inspection [3].

1 J. Andersson and I. Köper, "Tethered and Polymer Supported Bilayer Lipid Membranes: Structure and Function.," Membranes, vol. 6, no. 2, pp. 30, 2016

2 I. G. Denisov and S. G. Sligar, "Nanodiscs in Membrane Biochemistry and Biophysics.," Chemical reviews, vol. 117, no. 6, pp. 4669-4713, 2017

3 M. Wilm, "Synthesis of Extended, Self-Assembled Biological Membranes containing Membrane Proteins from Gas Phase," bioRxiv, vol. 23, pp. 661215, 2019

T012 HUB, JOCHEN S

Katharina Scherer; Dr. Chetan S Poojari; Gari Kasparjan; Prof. Dr. Jochen S Hub[1],

[1] Saarland University

Free energy landscape of pore and stalk formation are controlled by lipid composition and lipid-protein interactions

Topological transitions of membranes, such as pore formation or membrane fusion, play key roles biophysical processes including exocytosis, viral infection, intracellular trafficking, proton transport, and many others. In biological cells these transitions are carried out by complex, asymmetric membranes composed of thousands of lipid species and crowded by membrane proteins. How the lipid composition and lipid-protein interactions control the free energy landscape of topological transitions is poorly understood.

We have developed computationally efficient methods for screening the effects of lipids and membrane-associated proteins on the energetics of the formation of membrane pores or membrane stalks at atomic or near-atomic resolution. The simulations reveal that the membrane composition may bias such topological transitions by tens or even hundreds of kilojoule per mole, suggesting that the complexity of membranes may have evolved to control the kinetics of such events. For instance, we found that the inner leaflet of a typical plasma membrane is far more fusogenic than the outer leaflet, which may be an adaptation for allowing efficient exocytosis while resisting viral infection. In addition, our simulation question established theoretical models for electroporation, and they quantify the effects of arginine-rich membrane-active peptides on pore formation.

[1] Poojari, Scherer, Hub, Nature Commun., 12, 6594 (2021)

[2] Verbeek et al., Eur. Biophys. J. 50, 127-142 (2021)

[3] Hub, J. Chem. Theory Comput. 17, 1229-1239 (2021)

[4] Ting et al. Phys. Rev. Lett (2018)

T013 RUDACK, TILL

Dr. Till Rudack, Ruhr University Bochum

A scale spanning integrative modeling strategy to study structure, dynamics, and function of complex molecular machines

Life is motion driven through cellular processes carried out by molecular machines. In order to understand and manipulate such cellular processes, scale-spanning knowledge of structure, dynamics, and function of molecular machines is needed. This knowledge cannot be obtained by one method alone. Therefore, I developed a strategy that combines ab initio structure prediction, molecular dynamics (MD) simulation, and quantum chemical calculations with data from structure resolving experiments to investigate the assembly and functional cycle of molecular machines from the electron up to the molecular level.

First, experimental data is converted into static structural models of different mechanistic states of molecular machines using bioinformatics methods. Second, active sites are refined at sub-Å resolution and intermediate states inaccessible by experiments are identified employing QM/MM simulations. Third, the obtained refined snapshots are merged to map the dynamics of the processes in their native environment using MD simulations. The strategy provides insights into the interplay between local processes like chemical reactions at the active sites and global conformational changes driving cellular function. These insights lead to mechanistic hypotheses and key functional residues that motivate targeted experiments, e.g. mutagenesis studies of the suggested residues, to validate the structural dynamic models.

This generally applicable strategy was applied e.g. to obtain insights into ATP hydrolysis driven protein recycling by the proteasome (Hung et al, Nature Communications 2022) or the assembly of proteins involved in photosynthesis like photosystem II (Zabret et al, Nature Plants 2021) or vesicle inducing protein in plastids Vipp1 (Gupta et al, Cell 2021).

T014 JUNG, HENDRIK

Hendrik Jung[1]; Dr. Roberto Covino PhD[2]; A Arjun[3]; Christian Leitold[4]; Peter G Bolhuis[3]; Christoph Dellago[4]; Gerhard Hummer[1]

[1] Max-Planck-Institut für Biophysik; [2] Frankfurt Institute for Advanced Studies; [3] Van't Hoff Institute for Molecular Sciences, University of Amsterdam; [4] Faculty of Physics, University of Vienna

Artificial Intelligence for Molecular Mechanism Discovery

We develop a machine learning algorithm to extract the mechanism of transitions between metastable states from molecular dynamics simulations. Our algorithm combines transition path sampling (TPS), deep learning, and statistical inference to simulate the dynamics of complex molecular reorganizations. We iteratively train a deep learning model on the outcomes of the shooting moves used in TPS. By learning to predict the transition dynamics, the artificial intelligence (AI) system at the center of the algorithm gradually reveals the underlying mechanism of the transition dynamics, and at the same time increases the efficiency of the rare-event sampling. The AI system can simultaneously learn from and guide multiple TPS simulations running in parallel. In a second step, we then distill the knowledge about the transition mechanism encoded in the deep learning model into a reduced mathematical model. The reduced model concisely represents the key features of the mechanism in an explicit analytical, human-understandable form.

We apply the algorithm to molecular systems ranging from ion dissociation in aqueous solution over gas hydrate formation to the oligomerization of a transmembrane alpha helix involved in membrane sensing. In all cases, the AI system accurately predicts the transition dynamics and extracts reduced mathematical models of the underlying mechanisms.

T015 TROLLMANN, MARIUS F. W.

Marius F. W. Trollmann; Prof. Dr. Rainer A. Böckmann, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen National High Performance Computing Center (NHR@FAU)

mRNA lipid nanoparticle phase transition

mRNA-based vaccines have recently gained attention for their promising therapeutic potential in the prevention of a severe SARS-CoV-2 infection [1]. An important part in their mode of action are lipid nanoparticles (LNPs) which act as a carrier system to deliver the bioactive mRNA into the target cells. The nanoparticles protect the nucleotides against a premature degradation leading to an increased expression of the encoded protein [2]. In addition, the nanoparticles increase the transfection rate of the vaccine through an enhanced interaction with the membrane of the target cells.

Unfortunately, less is known about the organization of the nanoparticles at molecular and atomistic scales. Here, we characterize the spatial composition and the physico-chemical properties of the lipids used in the BioNTech & Pfizer vaccine employing molecular dynamics (MD) simulations of both lipid bilayer systems and full LNPs at atomistic resolution [3]. At physiological pH, the LNP is characterized by an oil-like core that is surrounded by a lipid monolayer formed by DSPC lipids, cholesterol and PEGylated lipids creating an external PEG layer around the LNP. Self-assembly simulations with nucleoside-modified mRNA strands further corroborate the necessity of protonated cationic aminolipids to envelope the negatively charged poly-nucleotides within the core of the LNPs. Such inverted micellar structures stabilize the mRNA within the LNPs and provide a shielding and likely protection from environmental factors. At low pH, in contrast, the lipid composition used in the Comirnaty vaccine spontaneously forms lipid bilayers that display a high degree of elasticity. Thus, a change in pH of the environment as occurring upon LNP transfer to the endosome, likely acts as a trigger for lipid reorganization followed by LNP destabilization and subsequent mRNA cargo release from the LNP core.

References

[1] Polack, F. P. et al., N. Engl. J. Med. 2020, 383, 2603–2615.

[2] Schoenmaker, L. et al., Int. J. Pharm. 2021, 601, 120586

[3] Trollmann, M. F. W., and R. A., Böckmann, 2022, (Submitted)

T016 KOTTKE, TILMAN

Prof. Dr. Tilman Kottke; Dr. Lukas Gött-Zink; Anna Lena Toschke, Bielefeld University

In-Cell Infrared Difference Spectroscopy on Photoreceptors

A range of in-cell spectroscopic techniques has been developed to provide us with information on proteins in the specific environment of a native host cell. This environment includes important effects of molecular crowding and of interaction with other biomolecules on the structure and mechanism of the proteins. We demonstrated that infrared difference spectroscopy can be applied to soluble photoreceptors in living bacterial cells using the transmission and attenuated total reflection (ATR) configuration [1]. In-cell infrared difference (ICIRD) spectra on a light-, oxygen, voltage (LOV) protein did not agree with those recorded in vitro but were successfully emulated by addition of a protein-based molecular crowder. A sensitivity to detect well-resolved difference signals at 300.000 copies per cell was achieved.

We extended our studies to include a receptor that cannot be purified from bacterial cells, as it is degraded upon isolation. The full-length plant cryptochrome photoreceptor was characterized in living bacterial cells. A shift in the signal characteristic for a beta-sheet reorganization was observed upon illumination as compared to a truncated version. This shift was interpreted to gain information on the tertiary structure of the full-length receptor [2].

Moreover, we performed time-resolved ICIRD spectroscopy on a LOV protein in E. coli. Difference signals were detectable at a time resolution of 7.6 ms after the excitation by the laser pulse using the rapid-scan technique. This approach opens up opportunities to study effects of the cellular environment not only on the structure but also on the kinetics of protein reactions. To move on from bacteria to human cell lines, we built an ATR assembly to cultivate and investigate human cell lines and recorded first difference spectra of cryptochrome. In conclusion, the range of target systems for ICIRD spectroscopy is continuously being expanded.

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T017 MÜLLER-WERKMEISTER, HENRIKE

Prof. Dr. Henrike Müller-Werkmeister, University of Potsdam

Time-resolved serial crystallography of an enzyme at work: The role of lasers, timing, and spectroscopy

Observing protein dynamics experimentally with both, highest spatial and temporal resolution is one of the biggest challenges in biophysics. Understanding and resolving structure-dynamics relationships will help to gain a full molecular understanding of biological function. Yet only few experimental methods allow to resolve multiscale dynamics and structural information of proteins in the same experiment.

Time-resolved serial crystallography at XFELs and synchrotrons is a rapidly emerging technique and now provides accesses to fully resolved dynamic protein structures at room temperature [1,2]. We have studied the irreversible enzymatic reaction of fluoroacetate dehalogenase in real-time by TR-SX. The intricate details of the time-resolved, dynamic structures (for 18 independent time points, from ms to seconds), provide not only a detailed insight into the catalytic mechanism, but also revealed a previously unknown mechanism for allostery mediated by transient structures of ordered/disordered interfacial water molecules.

Crucial for the success of the experiment was the use of a photocaged substrate, which was released by a femtosecond pump pulse and the optimized timing scheme, building on the sample delivery by a fixed target.

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T018 ADAMS, ELLEN

Dr. Ellen Adams, Technische Universität Dresden

Key Role of the Solvent in Driving Liquid-Liquid Phase Separation

In recent years the importance of the aqueous solvent in influencing protein structure, function, and dynamics has been recognized. Coupling of water molecules to the protein surface results in an interfacial region in which water molecules within this region have distinctly different properties than bulk water. However, the structure and dynamics within this interfacial region are still not easy to access experimentally. Terahertz (THz) spectroscopy has been shown to be a powerful tool to investigate solvent dynamics in bulk solutions. Radiation in the THz regime is directly sensitive to the low frequency collective intermolecular hydrogen-bonding vibrations of water (0.3-6 THz or 10-200 cm-1), and thus to any changes in the hydrogen-bonding network. Changes in these sub-picosecond collective motions, such as protein-water interactions, result in changes in the measured THz absorption. Individual hydrations shells of proteins have been shown to contribute largely to structure-function relationships and ultimately modulate the binding properties of proteins.

Here the role of solvation dynamics in the liquid-liquid phase separation (LLPS) of the intrinsically disordered protein fused in sarcoma (FUS) is probed. Characterization of the hydrogen bonding network reveals that water solvating hydrophobic groups is stripped away in the membrane-less FUS biomolecular condensates. Additionally, water left inside of the biomolecular condensates is highly constrained, indicative of a population of bound hydration water. These results uncover the vital role of hydration water in LLPS: the entropically favorable release of unfavorable hydration water serves as a driving force for LLPS.

T019 WILLIAMS, LARA

Lara Williams

Site-directed labelling with photoexcitable spin labels for light-induced dipolar spectroscopy

Light-excitable triplet states are a promising addition to the class of spin labels, and their use in distance determinations in the nanometer range has been successfully transferred from model peptides to proteins that incorporate an intrinsic heme chromophore [1]. For light induced dipolar spectroscopy to expand beyond the fraction of biomacromolecules with a native photoexcitable centre, the development of exogenously introducible photolabels is necessary.

Here, we show the feasibility of combining a persistent nitroxide radical and the photoexcitable labels Eosin Y, Rose Bengal and ATTO Thio12 [2]. Time-resolved and pulsed EPR spectroscopy was used to obtain the zero-field splitting parameters and the relaxation times of their triplet state. Then, the nitroxide and the light-excitable marker were site-selectively attached to the oxidoreductase thioredoxin via the orthogonal thiol-maleimide and copper-catalyzed azide-alkyne cycloaddition (CuAAC) reactions. Distance constraints were determined by laser-induced magnetic dipole (LaserIMD) spectroscopy [3].

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T020 BAMANN, CHRISTIAN

Dr. Christian Bamann, German Research Foundation / Deutsche Forschungsgemeinschaft (DFG)

DFG-Funding Opportunities for Graduates of Life Sciences

This talk is aimed at Master and PhD Students as well as early Post-Docs in Life Sciences. It will provide an overview of the diverse funding options with practical examples, problems and tricks as observed by a Programme Officer at the German Research Foundation (DFG). Elaborating on the different career stages linked to the funding programmes, it will provide the opportunity for general questions and answers.

T021 HEILEMANN, MIKE

Prof. Dr. Mike Heilemann, Goethe-Universitaet Frankfurt

Quantification of protein subunits in dense clusters using kinetics-assisted quantitative super-resolution microscopy

Resolving protein subunits within a densely packed protein cluster in a cell remains challenging for current optical super-resolution methods. To address this challenge, we developed an approach for quantitative single-molecule localization microscopy (qSMLM) [1] which retrieves molecule numbers from the analysis of single fluorophore "blinking" kinetics [2]. We validated this approach for various photophysical models [3] and fluorophore labels for different flavors of SMLM [4-5], and applied it to reveal the function-dependent oligomerization of various membrane receptors [6-8].

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T022 GRÄTER, FRAUKE

Prof. Dr. Frauke Gräter, Heidelberg Institute for Theoretical Studies

How collagen converts mechanical into chemical stress

Proteins sense and respond dynamically to mechanical forces. Over the last decades, enormous progress has been made in understanding how proteins undergo conformational changes and eventually unfold under force. That forces can even lead to covalent rupture of a protein molecule has not been considered as a likely scenario.

We recently discovered collagen, the major force-carrying protein material of our body, to undergo bond rupture under physiologically relevant forces, resulting in radicals and oxidative stress molecules [1]. Using hybrid multiscale simulations [2] and electron-paramagnetic resonance spectroscopy, we identified the radical chemistry within stretched collagen and found collagen of our tissues to be equipped with excellent radical scavenging functions. I will also show how we more recently employ graph-based Machine Learning techniques to inform and enhance the simulation's predictive power for capturing the ongoing chemistry of the protein amidst its dynamics.

Our combined simulation and experimental results suggest collagen to act not merely as a passive load-bearing protein material in our body but as a huge and dynamic 'mechano-enzyme'.

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T023 TAJKHORSHID, EMAD

Sepehr Dehghani-Ghahnaviyeh; Dr. Zhiyu Zhao; Prof. Dr. Emad Tajkhorshid PhD,
University of Illinois, Urbana-Champaign, USA

Lipid-mediated organization of prestin in the cochlear membrane and implications in sound amplification

Prestin is a motor protein with a high membrane density in the the outer hair cells (OHCs). The conformational response of prestin to acoustic signals substantially alters the shape of the OHCs, thereby playing a major role in sound amplification by the cochlea. Recent structural studies have captured prestin in active and inactive (inhibited) conformational states. Nevertheless, the mechanism and details of its intimate interactions with the lipid milieu of the membrane, which are central to its function remained unresolved. Here, employing a large set of coarse-grained molecule dynamics (MD) simulation, we both study in detail the nature of lipid-protein interactions of prestin in the membrane, and demonstrate the impact of such strong interactions on the organization of prestin copies in the membrane and their cooperativity in shaping the membrane structure. Beyond local enrichment/depletion of various lipid types, prestin is found to cause a long-range membrane deformation by drastically modulating the organization of its surrounding lipids. The membrane deformation propagates up to ~100 Å away from the protein, raising the probability of communication of prestin copies with each other in the membrane. Using a specifically designed set of simulations, each with two copies of prestin in membrane separated by 200 Å in varying relative orientations, we show that the different patterns of membrane deformation caused by different alignments of the protein result in varying energy levels, suggesting that the proteins might communicate with and affect each other's orientations through the lipids. The bending energy is lowest when the membrane deformations generated by individual prestin copies are synchronized. Prestin-prestin configurations with the lowest membrane bending energy, captured here, approximately captures the prestin packing pattern in the OHCs. This observation was furthered confirmed by long MD simulations in which prestin copies were allowed to rotate freely.

T024 LAKOMEK, NILS-ALEXANDER

Tobias Stief[1]; Mirko Kraus[2]; Prof. Reinhard Jahn[3]; Dr. Angel Perez-Lara[4]; Dr. Nils-Alexander Lakomek[2]

[1] Forschungzentrum Jülich, IBI-7: Structural Biochemistry; [2] Heinrich-Heine-Universität Düsseldorf; [3] Max-Planck Institut für Multidisziplinäre Naturwissenschaften; [4]University of Granada

Structural dynamics of intrinsically disordered proteins at the membrane interface: Recent insights into the pre-fusion state of SNARE proteins by NMR spectroscopy

About 30 to 40% of the human proteome consists of proteins containing large regions with no clearly defined three-dimensional structures. Those intrinsically disordered regions (IDRs) or intrinsically disordered proteins (IDPs) are highly dynamic entities, making them challenging to study by standard techniques in structural biology, such as X-ray crystallography or cryo-EM. However, IDPs are well suited to be examined by NMR spectroscopy.

We develop a combined NMR approach, employing both solution NMR and proton-detected solid-state NMR spectroscopy at very fast MAS (magic angle spinning) frequencies to reveal insights into the structural dynamics of intrinsically disordered proteins and their conformational transitions at the lipid membrane interface. In our approach, IDPs are anchored in lipid-bilayer nanodiscs or interact with the lipid bilayer. Solution NMR is sensitive to the unbound part of the IDP, while solid-state NMR allows access to the lipid-bound conformation of the IDP.

For NMR methods development, we use the SNARE proteins as a model system. SNARE proteins play a crucial role during neuronal exocytosis by eliciting the fusion of the synaptic vesicle membrane with the presynaptic plasma membrane. A fusion pore will open, and the neurotransmitters are released into the synaptic cleft.

In their pre-fusion state, the membrane-anchored SNARE proteins are IDPs. However, the degree and mode of interaction between the SNARE proteins and the lipid membrane and how this leads to membrane fusion are not well understood. Here, NMR spectroscopy can provide novel structural and dynamic insights at an atomic resolution [1].

At the conference, we will present recent (unpublished) insights into the structural dynamics of the SNARE proteins synaptobrevin-2 and SNAP25 at the lipid membrane interface.

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T025 ŠACHL, RADEK

PhD Radek Šachl[1]; MSc. Vandana Singh[1]; Dr. Sabina Cujova[1]; Dr. Petra Riegerova[1]; Prof. Dr. Martin Hof[1]; Dr. Julia Steringer[2]; Walter Nickel[2]

[1] J. Heyrovský Institute of Physical Chemistry; [2] Heidelberg University Biochemistry Center

In-membrane protein oligomerization as a critical step for membrane pore formation

Oligomerization of membrane proteins into multicomponent units is often critical for their function (or dysfunction). To give an example for all, apoptosis, also known as programmed cell death, is induced at the molecular level by a change in the aggregation behavior of proteins, subsequent formation of protein complexes with a broad distribution of oligomerization numbers, and finally opening of functional pores in the mitochondrial membrane. It is, however, often unclear which of these complexes can open the pores, and which of them they cannot.

This motivated us to develop a statistical single molecule and single vesicle assay that determines the brightness of individually diffusing in-membrane oligomers and correlates their oligomerization state with membrane pore formation. We demonstrate the applicability of the method by investigating membrane translocation of Fibroblast Growth Factor 2 (FGF2) that is accompanied by membrane oligomerisation and pore formation. The new approach reveals FGF2 oligomers with about 7 to 8 subunits to represent the functional entities of productive membrane insertion and translocation. Moreover, by monitoring the oligomeric state of FGF2 on the same lipid vesicle over time, we detected sudden increases in the FGF2 oligomeric state and protein surface concentration accompanied by increased membrane permeability. In this way, in-membrane oligomerization of FGF2 was linked directly to the formation of membrane pores in one experiment under physiological conditions. Our findings demonstrate that quantifying oligomeric states alone does not allow for a deep understanding of the structure—function relationship of membrane-inserted oligomers.

T026 VERA, ANDRÉS MANUEL

Dr. Andrés Manuel Vera PhD, Ludwig-Maximilians-Universität Munich

Heterogeneous assembly of the cohesin-dockerin interaction and its modulation by isomerization of a single proline

Cellulosomes are extraordinary complex protein nanomachines with unmatched efficiency in degrading lignocellulose, the most abundant and hydrolysis-resistant biomaterial on earth. Over the last years, single-molecule techniques have unveiled surprising hidden features of these complexes, including remarkable mechanical sturdiness of their building blocks (cohesins) and record-breaking stability of their pivotal assembling interaction (the cohesin-dockerin interaction). Cellulosomes are also excellent model systems to study the self-assembly of protein complexes, as the cohesin-dockerin interactions generate a relatively simple assembly code that results in enormous structural heterogeneity and allows for structural changes to adapt to the substrate over time. Here, we use smFRET and molecular dynamics simulations to uncover two alternative binding modes of the cohesin-dockerin interaction. Using our single-molecule approach, we showed that the isomerization state of a single proline residue determines the population ratio between these binding modes, its assembly kinetics, and the overall stability of the cohesin-dockerin interaction. Furthermore, these processes can be enzymatically modulated by a prolyl-isomerase. We propose that this enzymatic modulation can explain how cellulosomes can dynamically change their cellulase composition despite the extremely high stability of the cohesin-dockerin interaction.

T027 SCHROEDER, INDRA

Dr. Oliver Rauh[1]; Jennifer Opper[1]; Maximilian Sturm[1]; Nils Drexler[2]; Deborah-Désirée Scheub[1]; Prof. Dr. Ulf-Peter Hansen[3]; Prof. Dr. Gerhard Thiel[1]; Prof. Dr. Indra Schroeder[2]

[1] Technische Universität Darmstadt; [2] University Hospital Jena, Friedrich Schiller University Jena; [3] Christian-Albrechts-Universität zu Kiel

Role of ion distribution and energy barriers for concerted motion of subunits in selectivity filter gating of a K+ channel

The two most prevalent gates in potassium channels are found at the cytosolic entrance (inner gate) and in the selectivity filter (filter gate). The function of both gates is in many channels coupled and regulated by various cellular signals. To investigate the molecular mechanisms that determine gating kinetics of the filter gate, we employ the minimal viral channel KcvNTS. This channel is devoid of an inner gate and its gating can therefore be entirely assigned to the filter gate. By a combination of single-channel recordings and gating analysis we find that mutations of S42 in the pore helix strongly stabilize the open state of this filter gate at negative membrane voltages. This effect does not depend on the ability of the critical amino acid to form H-bonds. This observation contrasts with KcsA, where the critical E71 in the equivalent position forms a salt bridge. Therefore, the coupling between selectivity filter and surrounding structures must in KcvNTS rely on different modes of interaction. Quantitative gating analysis of concatemers with different numbers of S42T mutations reveals that each subunit contributes the same amount of ~0.4 kcal/mol to the Eyring barrier for filter closure indicating a concerted action of the subunits. Since the mutations have no influence on the open-channel current and the voltage dependency of the gate, we conclude that the high subunit cooperativity is mediated through conformational changes in the protein rather than via changes in the ion occupation in the selectivity filter.

T028 SCHEWE, MARCUS

Dr. Marcus Schewe PhD[1]; Dr. Aytug K. Kiper PhD[2]; Dr. Wojciech Kopec PhD[3]; Dr. Stefanie Marzian PhD[2]; Dr. Mauricio Bedoya PhD[4]; Dr. David Ramirez PhD[5]; Dr. Elena B. Riel PhD[6]; Dr. Annemarie Köhler PhD[1]; Dr. Susanne Rinne PhD[2]; Prof. Dr. Wendy Gonzalez PhD[4]

[1] Kiel University; [2] University of Marburg; [3] Max Planck Institute for Biophysical Chemistry; [4] Universidad de Talca; [5] Universidad Autónoma de Chile; [6] Weill Cornell Medical College

An Alternative Mechanism of Kv Channel Inhibition: Binding of Cellular Lipids to Side-pockets Induces C-type Inactivation

Voltage-gated potassium (K+) ion channels (Kv channels) have a determining influence on electrical cell signaling through the regulation of fast and selective K+ flow across biological membranes. Activation and inactivation of these K+ channels in the process of voltage gating inevitably involves C-type inactivation, a mechanism that controls K+ flux at the selectivity filter (SF). The inactivation process is defined by conformational changes within the SF and can further be modulated by the natural environment of the channels. Various binding sites of lipids, like polyunsaturated fatty acids (PUFAs) in Kv channels are proposed, however, the mechanisms how these lipids induce C-type inactivation and the underlying structural alterations in the SF are so far elusive. Here we report the `side-pockets´ in Kv channels as universal PUFA and hormone accommodation sites that moreover represent the starting point of an allosteric SF inactivation mechanism cascade accelerated by lipid and hormone binding. This lipid-mediated inactivation-like mechanism is strictly ion-dependent and differs from classically open channel block behavior. In silico electrophysiology simulations reveal a two-step filter alteration that limits K+ permeation through the channel. These results highlight lipid regulation of K+ channel activity through an allosteric SF inactivation process.

T029 ACHARYA, ABHISHEK

Abhishek Acharya; Ishan Ghai; Dr. Claudio Piselli; Prof. Dr. Roland Benz; Prof. Dr. Mathias Winterhalter; Prof. Dr. Ulrich Kleinekathöfer, Jacobs University Bremen gGmbH

A mechanistic view of the role of L3 loop conformational dynamics in antibiotic permeation and gating in OmpF.

The OmpF porin is involved in the translocation of antibiotics into Gram-negative bacteria. Studies have investigated these channels to uncover the molecular factors that influence permeation rates of different bacteria. While the application of empirical rules for permeation have led to improvements in permeation of antibiotics, the structural details of the mechanism of permeation is less understood. Previous work has highlighted the importance of solute properties (size, charge distribution and polarity) and channel properties (pore size, electrostatics, and internal electric field) to the translocation process. Here, we extend the molecular understanding of the antibiotic translocation process by providing a mechanistic picture of permeation wherein the conformational dynamics of the L3 loop plays a central role. We identify the so-called L3-FS segment that is essentially involved in the permeation process. Furthermore, we show that conformational fluctuation in the L3-FS segment is responsible for the observed current fluctuations in electrophysiological studies of OmpF. Preliminary findings show that similar mechanisms could also be operative in OmpF orthologs from Klebsiella pneumoniae and Enterobacter cloacae.

T030 WITT, HANNES

Dr. Hannes Witt[1]; Dr. Anna Meijering[1]; Janni Harju[1]; Christian Nielsen[2]; Ian Hickson[2]; Chase Broedersz[1]; Erwin Peterman[1]; Gijs Wuite[1]

[1] Vrije Universiteit Amsterdam; [2] University of Copenhagen

The mechanics of mitotic chromosomes

To ensure that each daughter cell inherits a full genome during mitotic cell division, the DNA forms a highly condensed structure – the metaphase chromosome. Although the metaphase chromosome is of fundamental relevance for eukaryotic life, its structure and dynamics are not yet fully resolved. We have developed a novel method to study metaphase chromosomes and their mechanical properties by using optical tweezers to hold and manipulate single isolated chromosomes, pushing the boundaries of this already powerful single-molecule technique. To fully characterize the mechanical properties of mitotic chromosomes, we not only performed force-distance measurements, but also used oscillatory experiments similar to active microrheology. We observed a unique stress-stiffening behavior following an unusually weak powerlaw, that we explain with a hierarchical worm like chain model based on the chromosome's heterogeneity. When measuring the complex stiffness of chromosomes over a wide range of frequencies (0.001 – 100 Hz), we observed a remarkable elasticity and robustness. By combining genetic modifications and chemical treatment we unravel the contributions of different histone and non-histone proteins to the elasticity of mitotic chromosomes. We find that in particular Condensin I plays a crucial role to provide stiffness and mechanical stability for the mitotic chromosome. Furthermore, we use mechanical characterization to examine the physiological process of ion-mediated chromosome condensation.

T031 RADEMACHER, MICHELLE PAULINA

Michelle Paulina Rademacher; Robert Heße; Dr. Janina Bertling; Prof. Dr. Peter Gilch, Institute for Physical Chemistry II, Heinrich Heine University Duesseldorf

Time-Resolved Spectroscopy of an Angular Psoralen Intercalated into DNA

Psoralens are natural compounds which are employed in PUVA (psoralen + UV-A irradiation) therapy [1]. With PUVA therapy, skin diseases like psoriasis and atopic dermatitis are treated. In the PUVA approach, psoralens intercalate between the base pairs of the diseased cells' DNA. Upon subsequent irradiation with UV light, psoralens can irreversibly bind to the base thymine in nominal [2+2]-cycloaddition. Through this process, psoralens are covalently bound to the DNA via a cyclobutane ring. This photo-addition can involve the double bond in the furan ring (marked in red in Figure 1b) as well as the one in the pyrone ring (highlighted blue in Figure 1b). Subsequent additions may result in a DNA cross-linking. Cross-links and mono-adducts inhibit the replication of DNA and can result in apoptosis of the diseased cell. With angled derivatives only mono-adducts are formed.

Our group pioneered the characterization of the PUVA process by time-resolved spectroscopy [2,3]. The studies revealed a photo-induced electron transfer (PET) [2] competing with the photo-addition as well as the mechanism of the photo-addition [3]. In the PET process, the photo-excited psoralen is quenched by reductive electron transfer whereby the base guanine acts as donor. Studies with synthetic, guanine-free DNA (AT-only DNA) showed that the photo-addition occurs on the microsecond time scale [4]. It proceeds via the local triplet state of the psoralen as well as a triplet biradical in which the psoralen is bound to the thymine base via a covalent bond. These findings refer to linear psoralens (LP). Angled psoralen (AP) derivatives like angelicin are also PUVA active but have not yet been characterized by time-resolved spectroscopy. Here, first results on their PET behaviour will be represented.

T032 GRÜN, ALICE FREDERIKE ROSA

Alice Frederike Rosa Grün[1]; Jens Bosse[2]; Charlotte Uetrecht[3]

[1] Centre for Structural Systems Biology (CSSB), Leibniz Institute of Virology (LIV); [2] Centre for Structural Systems Biology (CSSB), Leibniz Institute of Virology (LIV), Hannover Medical School (MHH); [3] Centre for Structural Systems Biology (CSSB), Leibniz

Structural analysis of the interaction of the herpes simplex virus 1 terminase with secondary DNA structures

The lifecycle of herpes-simplex-virus-1 (HSV-1) is highly complex. Despite considerable effort, it is still largely unclear how capsid packaging is structurally mediated. During this step, the viral genome is translocated into the procapsid. One crucial protein complex during this step is the terminase that also cuts the genome concatamers after the capsid is filled. The terminase consists of three different proteins: UL15, UL28, and UL33, forming a heterotrimer. Six heterotrimers assemble into a donut-shaped hexamer that binds DNA at the center and then translocates it into the procapsid using ATP. During this step, the terminase binds to specific sequences at the genome termini called pac sequences (containing pac1 and pac2). It is unclear how the terminase recognizes these sequence motifs.

Interestingly pac1 is predicted to form G-quadruplexes, which are a form of secondary structure. These quadruplexes play a vital role in protein-DNA interactions and could be the key to understanding how the terminase binds to the viral DNA. Via CD-spectroscopy we confirmed quadruplex structures in pac1, whereas mutated pac1 and pac2 showed no quadruplex formation. To elucidate the structural mechanism of terminase-quadruplex binding, we are now employing native mass spectrometry (nMS), and we will report the most recent results.

T033 TINNEFELD, PHILIP

Prof. Dr. Philip Tinnefeld, LMU Munich

Single-Molecule Biophysics and Biosensing with DNA Origami Devices and Graphene

Graphene acts as an energy transfer acceptor with an 1/d4 scaling law and an d0-value of 18 nm.1 Thus graphene-on-glass coverslips are a platform for sensitive single-molecule experiments with an unbleachable broadband energy transfer acceptor that does not require labeling. We show several applications of graphene-on-glass coverslips for biosensing, monitoring biomolecular dynamics and for determining the orientation of FRET-pairs with respect to the surface.2 In these examples, the bioassay is placed at a defined distance above graphene by DNA origami nanopositioners. In other applications, we use DNA origami devices for sensing of membrane potentials,3 to apply forces on biomolecules,4 and for increased selectivity in molecular recognition events.

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T034 RIES, JONAS

Dr. Jonas Ries, European Molecular Biology Laboratory (EMBL) Heidelberg

Superresolution microscopy for structural cell biology

Superresolution microscopy, such as single-molecule localization microscopy (SMLM), is becoming a key technique for structural cell biology, ideally complementing electron microscopy.

In my group we are developing technologies to image the structure and dynamics of molecular machines in cells. I will discuss how we a) pushed the 3D resolution in multi-color towards the nanometer scale, b) increased imaging speeds, c) developed reference standards for quality control and counting and d) developed software to extract quantitative information from SMLM data.

These new technologies enabled us to gain mechanistic insights into the machinery that drives clathrin-mediated endocytosis. We developed a high-throughput superresolution microscope to reconstruct the nanoscale structural organization of 23 endocytic proteins in yeast. This allowed us to visualize where individual proteins are localized throughout the endocytic process and resulted in a model of how the force is produced to pull in the membrane and form a vesicle. In mammalian cells, we could address a long-standing question how the clathrin coat is formed during vesicle formation.

I will conclude with first results illustrating the potential of MINFLUX in imaging dynamic structural changes of protein machines in the living cell with nanometer resolution.

T035 BRASSELET, SOPHIE

Dr. Sophie Brasselet, Institut Fresnel

Imaging of proteins' organization in 3D using Single Molecule Orientation and Localization Microscopy (SMOLM)

Imaging molecular orientation at the nanoscale in live cells and tissues is fundamental in the understanding of proteins' organization, which is driven by their structural and conformational properties. Measuring fluorescent molecules' orientation is a way to approach this problem, providing that the label is rigidly attached to the protein of interest. Despite the great progresses in fluorescence imaging down to nanometric scales with Single Molecule Localization Microscopy (SMLM), orientation imaging is still only at its early stage. Measuring single molecules' 3D orientations in addition to their 3D spatial localization is a challenge due to the difficulty to disentangle spatial and orientational parameters in the SM point spread function (PSF) image formation, nevertheless the search for optimal methods to solve this challenge is rapidly progressing. We present examples of polarized fluorescence microscopy methods that are able to report both orientational and spatial information from single molecules in a non-ambiguous way. These methods, based on phase and polarization manipulation in the Fourier plane [1] or polarized splitting imaging [2], give access to orientation parameters in combination with high spatial localization precision. We will present the potential and limits of these approaches for the imaging of the nanoscale organization of proteins in cells.

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T036 HONIGMANN, ALF

Prof. Dr. Alf Honigmann, MPI-CBG / TUD

Structure and assembly of epithelial tight junctions: Super-resolution and Reconstitution

Tight junctions regulate cell adhesion, cortical tension and permeability of epithelial tissues. How these functions are organized by the supra-molecular structure of this complex is still largely unclear. Here we combined STED nanoscopy with genetics to reconstruct the molecular architecture of tight junctions in a 3D tissue culture model (MDCK cysts). Based on co-localization analysis we established the spatial relation between proteins of the apical polarity complex, tight junctions (TJ) and lateral adherens junctions (AJ). In addition, we find a stratification of receptors, adapters and cytoskeletal proteins with respect to the cell membrane. Together, our structural model provides novel insights into how tight junctions are constructed and how they interact with the cell polarity machinery and the force generating actomyosin cortex. In addition, I will discuss how we use biochemical reconstitutions of the main scaffolding proteins of tight junctions to understand the assembly mechanism of junction formation.

T037 STOEV, ILIYA

Dr. Iliya Stoev; Dr. Benjamin Seelbinder PhD; Elena Erben; Dr. Nicola Maghelli PhD; Dr. Moritz Kreysing PhD

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Highly Sensitive Force Measurements Enabled by a New Optofluidic Particle Trap

In recent years there have been increasing attempts by life scientists to find a non-invasive method that generates minimal heating, yet could allow extracting mechanical information from difficult-to-access compartmentalised regions within cells. This may have now become possible through the use of thermoviscous expansion phenomena to optically generate flows of tunable magnitude and thus perform active microrheology. We will first show how a new optofluidic trap characterised by a linear force-extension relationship can be used to sense femtoNewton-range forces without any laser-particle contact. By lifting probe-material constraints and introducing only moderate heating at the trap position, this method offers an appealing alternative to optical tweezers in living systems. Our planned future work builds on this approach by drawing parallels with classic bulk rheology, where a predefined stress is applied to shear a system and extract its mechanics. We anticipate that the establishment of our new framework will present a route towards measuring the mechanics of densely crowded environments, such as the cellular cytoplasm, with minimal external intervention.

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T038 NEVSKYI, OLEKSII

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Super-Resolution Microscopy with Metal-Induced Energy Transfer

Fluorescence lifetime imaging microscopy is an important technique that adds another dimension to intensity and color acquired by a conventional microscopy. Nowadays, single molecule localization microscopy (SMLM) techniques have become one of the most successful and widely applied methods of super-resolution fluorescence microscopy in life science. At the moment, the only super-resolution technique that is capable of recording super-resolved images with lifetime information is stimulated emission depletion microscopy.[1] In contrast, all SMLM techniques which utilize wide-field cameras completely lack the lifetime dimension. Here we demonstrate a combination of fluorescence-lifetime confocal laser-scanning microscopy(CLSM) with SMLM for realizing single-molecule localization-based fluorescence-lifetime super-resolution imaging, which can be used for multiplexing on samples with different labels that differ only by fluorescence lifetime but not by their spectral properties.[2] The technique is straightforward to be implemented on a commercial confocal scanning microscope setup with TCSPC capability and fast laser scanning unit. The method combines all the advantages of CLSM with those of SMLM: axial sectioning, shot-noise limited single-photon detection, pixel-free continuous position data, and fluorescence lifetime information acquired by CLSM with the exceptional spatial resolution and single-molecule identification of SMLM, moreover, method is conceptually free of chromatic aberrations. Additionally, we combine the extreme axial resolution of metal-induced energy transfer (MIET) imaging with the extraordinary lateral resolution of single-molecule localization microscopy utilizing confocal setup. This combination allows us to achieve isotropic three-dimensional super-resolution imaging of sub-cellular structures. [3]

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T039 LORENZ, CHARLOTTA

Dr. Charlotta Lorenz; Johanna Forsting; Stefan Klumpp; Sarah Köster,
University of Goettingen

Mechanical properties of keratin and vimentin intermediate filaments

Different cell types require different cellular mechanics. Prominent examples of different mechanical requirements are migrating versus non-migrating cells. Cells transit from a migrating to a non-migrating state during cancer metastasis, wound-healing and embryogenesis in a process called epithellial-to-mesenchymal transition. Interestingly, the expression of different intermediate filament (IF) proteins highly correlates with this transition, specifically, epithelial-like cells express more keratin IF protein, whereas mesenchymal cells primiarily express vimentin IF protein. Here we compare the mechanical response of single keratin and vimentin IFs, using optical tweezers. We find that both filament types dissipate a large amount of mechanical input energy, which predestines them to act as a cellular shock absorbers. Yet, the two filament types dissipate energy by very different mechanisms: Keratin IFs dissipate energy by internal friction of sliding filament subunits, while in vimentin IFs the nonequilibrium unfolding of alpha helices dissipates energy. The sliding subunits within keratin IFs cause filament elongation upon repeated loading, while vimentin IFs retain their length and soften. Since the alpha helices within keratin IFs do not unfold, keratin IFs retain their stiffness although the filaments elongate. This constant stiffness suggests a similar binding and rebinding mechanism of subunits to the sliding mechanism of atoms in metals under load. We conclude that cells can tune their mechanics by differential use of keratin versus vimentin.

T040 PRUCCOLI, ANDREA

Andrea Pruccoli[1]; Mustafa Kocademir, [1] University of Konstanz

Electronically enhanced Stimulated Raman Scattering microscopy of visible dyes

The goal of this project is the improvement of the sensitivity of Stimulated Raman Scattering (SRS) microscopy. To this end, we employ electronic enhancement of the vibrationally resonant SRS signals. This allows us to achieve sensitivities comparable to those of fluorescence microscopy. The two techniques can be used in parallel on commercial dyes which opens new possibilities for multiplex imaging especially in studies of biological processes. In addition to this approach, the sensitivity can be further enhanced by a combination of the population of vibrational states with electronically resonant SRS and the read-out with fluorescence probing. This Stimulated Raman Excited Fluorescence (SREF) pushes the sensitivities to the single molecule detection limit.

T041 COVINO, ROBERTO

Dr. Roberto Covino PhD[1]; Lars Dingeldein[1]; Pilar Cossio[2]

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Extracting free energy and dynamics from incomplete single molecule measurments with simulation-based inference

Proteins and other biomolecules must often reorganize between alternative structural states to carry out their biological functions. Obtaining an accurate description of this reorganization is instrumental in developing physical theories and facilitates rational engineering. Single-molecule experiments offer an unprecedented way of measuring fundamental quantities that characterize the population of the alternative structural states (free energies) and the interconversion dynamics between these states (rates). However, even though these experiments take measurements at the level of a single molecule, these measurements are often incomplete and indirect. For instance, in a force-spectroscopy experiment, an experimental apparatus keeps a protein under constant tension and monitors its jumps between two states. Ideally, this time series would enable us to estimate free energies and rates directly. In practice, what we measure is a single extension that is the outcome of the coupled dynamics of the molecule and the mesoscopic experimental apparatus, which can lead to artifacts and possible bias in our estimates. We, therefore, face an inverse problem: estimating intrinsic molecular properties from measurements that do not directly report on the molecule's dynamics. We discuss this problem as a Bayesian inference and how a physical description of the process leads to an intractable likelihood, hindering conventional optimization approaches. We illustrate how simulation-based inference provides a powerful solution to this problem. We show that coupling a simulator that encodes the physics of the measuring process with density estimation using neural networks leads to accurate solutions to the inverse problem. We discuss the robustness of these solutions and the possibility of selecting alternative models within the Bayesian framework. In conclusion, simulation-based inference provides a general and powerful way to extract accurate quantitative information from biophysical experiments.

T042 CALANDRINI, VANIA

Dr. Vania Calandrini PhD, Forschungszentrum Jülich GmbH

Subdiffusive-Brownian crossover in membrane systems: a generalized Langevin equation-based approach

Statistical physics approaches are instrumental for the development of mesoscale models able to capture diffusion, encounter, and binding rates of molecular partners involved in subcellular signaling.

With this aim, we developed a Generalized Langevin Equation-based model to reproduce anomalous diffusive processes occurring at the membrane (1). The memory kernel is represented in terms of a viscous (instantaneous) and an elastic (non-instantaneous) component modeled through a Dirac □ function and a three-parameter Mittag-Leffler type function, respectively. The different dynamical regimes, namely ballistic, subdiffusive, and Brownian, as well as the crossover between them, are captured within a unique model. The spectrum of relaxation times underlying the transition from the subdiffusive to the Brownian regime is given as well. The flexibility of the model is tested versus diffusive properties of protein-membrane systems of different composition as obtained from molecular dynamics simulations.

T043 ALLOLIO, CHRISTOPH

Dr. Balazs Fabian[1]; Dr. Mark Dostalik[2]; Dr. Christoph Allolio[2]

[1] Max Planck Institut für Biophysik; [2] Charles University in Prague | CUNI

Multiscale modeling of specific interactions on biomembranes

The continuum description of lipid membranes via the Helfrich functional and its extensions is widely employed in the modeling of membranes at the mesoscopic scale. A major problem in the application of this theory to membrane remodeling by proteins and other adsorbates is the local modification of membrane shape, lipid composition and elastic properties by adsorbates, such as proteins. We address this problem by direct incorporation of specific interactions into the local parametrization of the continuum model. For these purposes, we have developed methods to extract the local parameters from molecular simulations at atomic resolution.[1,2] Bridging the scales in this way we were able, to explain the lipid specificity of fusion of membrane fusion in presence of Ca2+ via a stalk-mechanism. [2,3] In order to generalize this approach to complex systems, we have developed a solver based on a quasi-G1 continuous surface representation.[4,5] This approach is sufficiently flexible to accomodate contact deformations and lipid demixing phenomena and allows us to explore the origins of mesoscopic membrane deformations. We show a first application to specific effects in the endosomal escape of cell penetrating peptides and first examples of protein-membrane coupling.

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T044 CHATZIMAGAS, LEONIE

Leonie Chatzimagas, Saarland University

Simulation of Liquid Jet Explosions and Shock Waves Induced by X-Ray Free-Electron Lasers

X-ray free-electron lasers (XFELs) produce X-ray pulses with very high brilliance and short pulse duration. These properties enable structural investigations biomolecular nanocrystals, and they allow resolving the dynamics of biomolecules down to the femtosecond timescale. To deliver the samples rapidly into the XFEL beam, liquid jets are used. The impact of the X-ray pulse leads to vaporization and explosion of the liquid jet, while the expanding gas triggers the formation of shock wave trains traveling along the jet, which may affect biomolecular samples before they have been probed. Here, we used atomistic molecular dynamics simulations to reveal the structural dynamics of shock waves after an X-ray impact. Analysis of the density in the jet revealed shock waves that form close to the explosion center and travel along the jet. A trailing shock wave formed after the first shock wave, similar to the shock wave trains in experiments. Although using purely classical models in the simulations, the resulting explosion geometry and shock wave dynamics closely resemble experimental findings, and they highlight the importance of atomistic details for modeling shock wave attenuation.

T045 BALBACH, JOCHEN

Dr. Tobias Gruber[1]; Dr. Marc Lewitzky[2]; Lisa Machner[2]; Dr. Ulrich Weininger[1]; Prof. Dr. Stephan M. Feller[2]; Prof. Jochen Balbach[1]

[1] Martin Luther University Halle-Wittenberg; [2] Martin Luther Universität Halle-Wittenberg

Macromolecular Crowding Induces a Binding Competent Transient Structure in Intrinsically Disordered Gab1

Intrinsically disordered proteins (IDPs) are an important class of proteins which lack tertiary structure elements. Their biophysical properties can depend on reversible post-translational modifications and the complex cellular milieu, which provides a crowded environment. Both influence the folding, dynamic, and thermodynamic stability not only of folding globular proteins but also the conformational plasticity of IDPs. Here we investigate the intrinsically disordered C-terminal region (amino acids 613–694) of human Grb2-associated binding protein 1 (Gab1), which binds to the SH2 domain-containing protein tyrosine phosphatase SHP2. This binding is mediated by phosphorylation at Tyr 627 and Tyr 659 in Gab1. We characterized induced structure in Gab1(613–694) and its binding to SHP2 by NMR, CD, and isothermal titration calorimetry (ITC) under non-crowding and crowding conditions, employing chemical and biological crowding agents and compare the results of the non-phosphorylated and tyrosine phosphorylated C-terminal Gab1 fragment.

A residue-by-residue analysis of the NMR relaxation rates revealed, that tyrosine phosphorylation of Gab1(613–694) has no impact on its dynamics and IDP character. However, under biological crowding (BSA and Xenopus egg lysates) conditions pre-structured motifs close to the tyrosine sites are formed in the absence of phosphorylation. These structured regions are identical to the binding regions towards the SHP2 protein as verified by ITC. Therefore, biological crowders could induce some SHP2 binding capacity. Chemical crowders such as Ficoll or PEG showed almost no effects, which indicates that sole excluded volume effects and a general stabilizing effect due to hydrophobic and hydrophilic interactions with the protein backbone or side chains are of minor importance for crowding-induced local compaction of Gab1(613–694). Our results therefore indicate that high concentrations of biological macromolecules stabilize the preformed or excited binding state in the C-terminal Gab1 region and foster the binding to the SH2 tandem motif of SHP2, even in the absence of tyrosine phosphorylation.

T046 MAYANS, OLGA

Prof Olga Mayans[1]; Julius Bogomolovas; Hang Lu; Guy Benian

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Stretch-induced unfolding of titin-like kinases as mechanosignalling mechanism in vivo

The cyclic, force-induced unfolding of titin-like proteins is speculated to be a key mechanism of mechanosensing and transduction in muscle. Yet, little evidence has been gathered of its existence in vivo. Prominently, stretch-unfolding is thought to regulate twitchin/titin sarcomeric kinases, which are autoinhibited by mechanosensory regulatory tails and functionally linked to muscle stress responses. To test stretch-unfolding as mechanism of kinase regulation in living muscle, we generated transgenic C. elegans expressing twitchin containing FRET moieties flanking the kinase domain and used computer vision algorithms to simultaneous measure fluorescence and muscle contraction in freely swimming worms [1]. The data revealed a periodic change in kinase conformation during muscle activity supporting the postulated mechanism. We next investigated the functional outcome of strech-unfolding using human titin kinase (TK) [2]. We found that TK is robustly ubiquitinated by the E3 Ub ligase MuRF1 and that ubiquitination promotes the assembly of the autophagy receptors Nbr1/p62 onto TK in the absence of stretch, potentially linked to sarcomere break-down upon inactivity. Ubiquitination and, thereby, Nbr1/p62 recruitment appears down-regulated by mechanical signals in active muscle, as the unfolding of the regulatory tail decouples TK from the MuRF1-receptor site in titin. This offers a possible molecular rational of how mechanical signals derived from exercise counteract atrophic processes in the muscle tissue and, thereby, regulate sarcomere remodelling.

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T047 SCHEERER, DAVID

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Substrate Inhibition of an Enzyme: Are Ultrafast Motions Affecting Catalytic Activity?

Many proteins harness large-scale motions of domains and subunits to promote their activity. The enzyme adenylate kinase (AK) has served as a playground for testing the potential relation between conformational dynamics and catalytic activity. AK plays a crucial role in maintaining ATP levels in cells by catalyzing the reaction ATP+AMP⍰AD-P+ADP. Efficient catalysis is ensured by a large-scale domain motion that encloses the substrates. Combined with H2MM, a photon-by-photon hidden Markov model analysis, single-molecule FRET has recently revealed that this process occurs within tens of microseconds, two orders of magnitude faster than the enzyme's turnover. This surprising result calls for answers whether and how these ultrafast motions influence activity.

In the present work, we find it is the equilibrium between the open and closed states that dictates turnover, not the explicit rates for interconversion. This is demonstrated by the effect of AMP as a substrate inhibitor. We show that inhibition arises due to allosteric communication between the domains, which manifests in a highly cooperative closure. Mutations are introduced to demonstrate that the level of cooperativity between the binding events is coupled to the strength of inhibition. On that basis, we propose a kinetic model of AK enzyme activity that explains why a more extensive sampling of the closed state in the presence of AMP leads to substrate inhibition. According to this model, initial binding of AMP followed by ATP can result in a closed state that does not allow correct positioning of the ligands for effective phosphate transfer. In contrast, initial binding of ATP followed by AMP leads to a productive reaction.

With experimentally observed opening and closing rates, the above model can effectively reproduce substrate inhibition profiles of the different mutants studied. Our findings suggest that the explicit rates for opening and closing do not determine turnover as long as they are significantly faster than the catalytic step. The role of the ultrafast domain movements is likely to reorient the substrates optimally for catalysis and to tune the equilibrium between differently active substates. In summary, the control of conformational equilibria via very fast domain motions explains substrate-dependent effects in AK. Since ultrafast dynamics have recently been observed in several other enzymes and protein machines, this might be a universal mechanism to regulate activity in proteins.

T048 STRIPP, SVEN

Dr. Sven Stripp, Freie Universitaet Berlin

Made in the Dark – Infrared Difference Spectroscopy for the Analysis of Gas-processing Metalloenzymes

Infrared spectra of proteins are dominated by the intense absorbance of liquid water and the protein backbone, precluding an analysis of small signals at frequencies higher 2800 cm-1 and lower 1800 cm-1. Difference spectroscopy facilitates addressing the full energy regime of the mid-IR by subtraction of spectrum 'A' from 'B' after application of a specific trigger (1). In transmission configuration, where the protein sample is squeezed between two atmospherically sealed, IR-transparent windows, visible light can be used as a trigger, e.g., investigating chromoproteins or exploiting the photolysis of 'caged' substrates.

Analyzing gas-processing metalloenzymes like hydrogenase, nitrogenase, or cytochrome c oxidase (CcO) visible light can be used to destabilize certain cofactor intermediates. However, triggering the catalytic turnover with H2, N2, CO, or O2 is precluded in transmission configuration. To this end, I developed a concept to stabilize hydrated protein films on a silicon crystal that is probed in ATR configuration (attenuated total reflection), making the atmosphere above the sample exchangeable in situ (2), The evanescent field at the interface of ATR crystal and sample does not probe the gas atmosphere, which is enriched with water vapor ('aerosol') to maintain a hydrated protein film.

I will explain how the catalytic H2 turnover of [FeFe]-hydrogenase can be followed via gas titrations (3) and how infrared difference spectroscopy can be used to investigates the catalytic proton transfer in [FeFe]- and [NiFe]-hydrogenase (4). Based on this work, I optimized the setup to minimize any unspecific changes when titrating the gas atmosphere, which opened the path for in situ ATR FTIR difference spectroscopy 'in the dark' (2). I will discuss recent work on nitrogenase (N2 reduction), CcO (O2 reduction), and various enzymes of CO2 conversion like CoA-carboxylase/reductase, formate dehydrogenase, and RuBisCO.

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T049 MORGNER, NINA

Prof. Nina Morgner PhD, Frankfurt University

Wanted and unwanted assemblies of biomolecular complexes – what can we learn with native mass spectrometry?

Assembly processes play an important role in the cellular environment. Large macromolecular complexes such as the ATPase from the respiratory chain, need to self-assemble into the correct complex structure in order to be fully functional. Opposed to these well guided processes there are assembly processes, which lead to less wanted structures, such as Amyloid-ß fibrils, which are correlated to Alzheimer's disease.

We investigate such processes by means of native mass spectrometry and ion mobility. We can reveal underlying mechanisms for the above mentioned processes, including environmental conditions which are prerequisite for assembly of the ATPase into a functional complex, or the weak point in the Amyloid-ß assembly, which allows to disrupt this process.

T050 HAMM, PETER

Prof. Peter Hamm, University of Zurich, Zurich, Switzerland

Using Azobenzene Photocontrol to Set Proteins in Motion

Controlling the activity of proteins with azobenzene photoswitches is a potent tool for manipulating their biological function. With the help of light, one can change e.g. binding affinities, control allostery or temper with complex biological processes. Additionally, due to their intrinsically fast photoisomerisation, azobenzene photoswitches can serve as triggers to initiate out-of-equilibrium processes. Such switching of the activity, therefore, initiates a cascade of conformational events, which can only be accessed with time-resolved methods; in particular transient IR spectroscopy. We will show how combining the potency of azobenzene photoswitching with transient spectroscopic techniques helps to disclose the order of events and provide an experimental observation of biomolecular interactions in real-time, see Fig. 1 [1-5]. This will ultimately help us to understand how proteins accommodate, adapt and readjust their structure to answer an incoming signal and it will complete our knowledge of the dynamical character of proteins.

FIG. 1. Full sequence of events of allosteric propagation inside a protein domain and ultimate ligand unbinding together with their typical timescales.

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1 ps1 ns1 μs1 mstimePhotoswitchingand heat dissipation Protein response to local perturbation and/or allosteric signalLigand unbindingProtein response to ligand unbinding++Local perturbation

T051 GROOT, MARLOES

Prof. Dr. Marloes Groot, Vrije Universität Amsterdam

Translation of higher harmonic generation microscopy into the clinic for tumor tissue assessment

Surgeons ultimately rely on tactile information or visual inspection when they need to decide which tissue to remove. Depending on tumor and type of surgery, tumor positive margins occur in 10–35 % of surgeries. In addition, surgeries may be hampered or lead to loss of function because nerve tissue or other functionally important parts are close to the tumor location and cannot be well recognized. Similarly, when taking biopsies for diagnosis, multiple biopsies are taken to increase the chance for representative tissue, resulting in prolonged procedures, patient discomfort, and increased risk of complications. Clearly, there is a pressing need for a technology that can aid in quick assessment and diagnosis of excised tissue.

Higher harmonic generation (HHG) microscopy is a novel promising imaging technique that meets these requirements. This technique is non-invasive, label-free, and provides 3D images with a high, sub-cellular resolution, within seconds. Before, we demonstrated that HHG microscopy can generate high quality images of freshly excised unprocessed lung and brain tissue, in less than a minute with information content comparable to that of the gold standard, histopathology [1,2].

In our most recent study we brought mobile HHG microscopes into two hospitals to image freshly excised biopsies. The results so far show that HHG microscopy enables real-time 3D imaging of the biopsies and reveals pathological hallmarks which enable making a quick preliminary diagnosis. Here, I will present our latest results on lung, thyroid, pancreas and pediatric tumor tissue.

Finally, automatic image analysis would eliminate the need for a pathologist to be present in the endoscopy suite or operation theatre. I will discuss our progress towards using convoluted neural networks in the assessment of brain and lung tissue.

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T052 JANSHOFF, ANDREAS

Prof. Dr. Andreas Janshoff, University of Goettingen

Cortex mechanics – the impact of actin architecture and the plasmamembrane

Cell cortices are responsible for the resilience and morphological dynamics of cells. Measuring their mechanical properties is impeded by contributions from other filament types, organelles, and the crowded cytoplasm. We established a versatile concept for the precise assessment of cortical viscoelasticity based on force cycle experiments paired with continuum mechanics. Apical cell membranes of confluent MDCK II cells were deposited on porous substrates and locally deformed. Force cycles could be described with a time-dependent area compressibility modulus obeying the same power law as employed for whole cells. The reduced fluidity of apical cell membranes compared to living cells could partially be restored by reactivating myosin motors. A comparison with artificial minimal actin cortices (MACs) reveals lower stiffness and higher fluidity attributed to missing cross-links in MACs.

T053 MANN, DANIEL

Dr. Daniel Mann[1]; Dr. Simon Fromm[2]; Prof. Dr. Carsten Sachse[1]

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Macromolecular organization of atg18 oligomers

Atg18 is a core protein in macroautophagy, a highly conserved eukaryotic pathway critical for the cell's stress response and homeostasis. The precise structural role of Atg18 in the autophagy process remains to be established. Atg18 belongs to the PROPPIN family consisting of seven WD40 repeats that form a beta propeller motif. Atg18 has been shown to bind Atg2 and Atg9 and thereby participates in the elongation of phagophores. Atg18 has a conserved PIP/PIP2-binding motif (FRRGT) that constitutes a specific membrane anchor. We demonstrate how Atg18 can form filaments in low-salt solution by solving the helical structure to near-atomic resolution using electron cryo-microscopy. The oligomerization sites are conserved even under high-salt conditions, as we demonstrate by single particle analysis of this small 55 kDa protein. We reconstituted Atg18 with liposomes and elucidated the macromolecular organization of Atg18 oligomers on cellular membranes using subtomogram averaging. The structure reveals that oligomerization motifs present in helical filaments and in solution are also observed between juxtaposed membranes, demonstrating how Atg18 may function to remodel and extend phagophore membranes in concert with the larger autophagy machinery.

T054 SALDANHA, OLIVA

Dr. Oliva Saldanha PhD

Calcium induced vesicular interactions studied with ATR- FTIR spectroscopy

Calcium plays an essential role in physiological functions such as membrane protrusion, fusion, foreign body engulfment, cell signaling and motility [1]. When the intra and extra cellular concentration levels are not maintained or fluctuate due to biochemical triggers, a eukaryotic cell can experience cell injury or death [2]. But the singular role of calcium on the membrane is too complex to study in a eukaryotic cell and a better understanding can be gained when stripped down to minimal components. Therefore, we generate a minimalistic vesicle model of size ~30-70 μm diameter with electroformation called giant unilamellar vesicles (GUVs) mimicking the cell membrane. The interaction of Ca2+ and DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) is studied with attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy at molecular resolution. In order to understand the calcium interactions from within the cell, firstly CaCl2 is encapsulated within the core of the GUVs during production. Changes occurring in the vibrational modes of the lipid groups show that the Ca2+ predominantly interacts with the phosphate head of the lipid molecule and by extension, the alkyl chain undergoes ordering. As the calcium concentration within the GUV increases, IR intensities change indicating dehydration and furthermore that the phosphate head groups accomplish attractive lateral pressure leading to compression. Secondly, the effect of calcium gradient across the membrane is studied by keeping the CaCl2 concentration inside at 500 μM and varying the concentration from 500 μM – 10 mM outside the GUV. In addition to the effect of compaction from within, vesicle-vesicle interaction occurs as Ca2+ ions can bind to the outer leaflets of several vesicles leading to vesicle clustering and it is found that larger calcium gradients induce stronger interactions. We demonstrate that divalent calcium ions cause local and temporal changes to the lipid packing in a biomimetic cell-like model.

T055 SOCRIER, LARISSA

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Optical manipulation of Gb3 enriched lipid domains: impact on Shiga Toxin B binding

The plasma membrane is a complex assembly of proteins and lipids that can self-assemble in transitory submicroscopic domains, commonly termed "rafts". These dynamic structures are implicated in membrane signaling and trafficking. Artificial membranes composed of certain lipids mixed with cholesterol spontaneously phase-separate in liquid-ordered (lo) and liquid-disordered (ld) domains, which are proposed to have similar properties to those of lipid rafts of cells membranes. Recently, photo-sensitive lipids were introduced to study membrane organization and biophysical properties in a non-invasive manner. Light-triggered isomerization of these compounds modifies the geometry of lipids from a cylindrical shape to a conical one. In vitro studies performed with phase-separated lipid systems have shown that such a change of configuration greatly alters domain organization. These findings raise the questions how does the configuration of photo-lipids impact lipid distribution in membrane domains and protein-lipid interactions.

Here, we used globotriaosylceramide glycolipids (Gb3) bearing a photo-sensitive group to study the influence of the isomerization on membrane domain organization. Using phase-separated lipid bilayers doped with photo-Gb3s, we found that the size of the domains was drastically modified when the photo-sensitive group is deeply embedded in the membrane. Since Gb3 constitute a natural receptor for the bacterial protein Shiga Toxin, we also investigated the impact of isomerization on lipid-protein interactions. Fluorescence microscopy images showed that Shiga toxin exhibited a natural behavior in the presence of photo-Gb3s. As expected, the protein was found in the lo phase of the membrane. Interestingly, isomerization did not alter protein binding but led to the formation of clusters in both lo and ld phases of the membrane. These findings can bring further understanding on the lipid raft concept given that a control of membrane lateral organization, protein-lipid interactions and cluster formation is accessible with this approach.

T056 SCHROMM, ANDRA

Leonard Christian; Prof. Dr. Andra Schromm, Research Center Borstel - Leibniz Lung Center

Bacterial outer membrane vesicles (OMVs): Dissecting the delivery process to host cells

Outer membrane vesicles (OMV) of Gram-negative bacteria are increasingly recognized as important bacterial shuttles with high relevance in host-pathogen interactions. The membrane-derived nanoparticles of 20 – 200 nm size carry bacterial molecules, lipids, toxins, and genetic material between microbes and to host cells. The release of OMVs is a highly conserved mechanism and fundamentally associated with living bacteria. Interaction of OMVs with host cells can induce inflammation and tissue pathology. They can modulate host cell responses and metabolism, thus shaping the stage for pathogen entry and intracellular survival.

Our study is aimed to dissect the target membrane lipid specificity for OMV membrane interaction, fusion and possible subsequent internalization of membrane compartments to provide a better understanding of the mechanisms underlying OMV-mediated delivery of pathogen-factors to host cells.

Förster-resonance-energy-transfer (FRET)-spectroscopy was employed to obtain time-resolved information on OMV host membrane interaction and define the lipid specificity. Polarization-spectroscopy demonstrates membrane rigidization upon OMV integration in liposomes as well as in the cytoplasmic membrane of living cells. Cholesterol has been suggested as a specific target for OMV entry into eukaryotic cells. Using a panel of phospholipids and lipid mixtures without and with cholesterol, we systematically analyzed the role of cholesterol for membrane interaction with liposomes. These experiments did not support a role for cholesterol in OMV membrane fusion. In line with these data, also cholesterol depletion of eukaryotic cells did not reduce the uptake of vesicles into HEK293 cells. Nevertheless, cholesterol is shown to play a role in the membrane distribution of OMVs, as demonstrated by analysis of the preference of OMV for liquid-ordered or liquid-disordered lipid phases in phase-separated giant unilamellar vesicles (GUVs) made from DOPC:SM:Chol by confocal microscopy. In addition, employing complex macrophage membrane mimicry systems we have gained indications for self-promoted uptake of membrane areas after OMV-interaction, presumably a post-fusion effect.

Based on our data, we propose a 2-step model for the interaction of bacterial membrane vesicles with eukaryotic membranes which is orchestrated by anionic phospholipids and cholesterol.

T057 LEKKA, MALGORZATA

Malgorzata Lekka PhD, Institute of Nuclear Physics PAN

Understanding a link between the biomechanics and invasiveness of bladder cancer cells

The bladder is a highly variable mechanical microenvironment expanding from a few to hundreds of kPa due to functional reasons. Despite that, the urothelial cancer cells become more deformable already at the early stages of cancer progression as measured by atomic force microscopy (AFM).

Over two decades of AFM employment in measurements of elastic and rheological properties of living cells unravel the importance of biomechanics in various aspects of cell functioning. Gathered evidence showed that cell mechanics is not only limited to the mechanical properties of cells. Several structural components contribute to the mechanical properties of bladder cancer cells. Primarily, the organization of polymerized actin form (actin filaments) and the overall actin content are related to cell mechanics. By applying the brush model, the mechanical properties of the cells studied under specific cleavage conditions were correlated with the presence of the particular type of the pericellular glycocalyx layer. The actin cytoskeleton is linked with the focal adhesion molecules; thus, altered expressions of related molecules affect cell deformability. The specific interactions with multiple components of the extracellular matrix (ECM), particularly basement membranes (BMs, mainly composed of laminins or type IV collagen), contribute to cancer dissemination. Laminin binding to receptors on a cell surface initiates cell invasion through BMs. Distinct contributions of cell structural elements to cell biomechanics form a question of the leading cause of cell alterations in mechanical properties during cancer progression. Obtained results reveal not only distinct deformability but also variability in the single-molecule interactions. Our findings show that cell morphological, mechanical, and adhesive properties are significant in bladder cancer progression (This work was supported by the Norwegian Financial Mechanism for 2014–2021, National Science Center (Poland), project no. UMO-2019/34/H/ST3/00526 (GRIEG)).

T058 RHEINLAENDER, JOHANNES

Dr. Johannes Rheinlaender; Prof. Dr. Tilman Schäffer, Tübingen University

Measuring the Cortical Tension of Living Cells using the Scanning Ion Conductance Microscope

Surface or interface tension can dominate soft matter systems at the micro- and nanoscale, for example, in surface chemistry and engineering, but also in living systems such as living cells or tissue. However, there are only limited methods to measure surface tension at small scales due to the complex geometries and interactions involved. Here, we present a new method to measure the surface tension at the micrometer scale using the scanning ion conductance microscope (SICM). The SICM is a scanning probe microscope using the ion current through an electrolyte-filled nanopipette to measure the topography of the sample, which was previously extended to also probe the mechanical stiffness of the sample. We now present a procedure to measure the surface tension of interfaces on the micrometer scale in a contact-free manner and verify it on micro-droplets with known surface tension. We then apply the concept to quantitatively measure the (cortical) tension of living cells, which is on the order of few mN/m and directly depends on the cytoskeletal prestress. We found that cortical tension strongly varies with cell type and e.g. differs between healthy and cancerous breast epithelial cells. Hence, the cortical tension appears to be an important parameter in mechanobiology and our new method might be a useful tool in biophysical research.

T059 FAHMY, KARIM

Prof. Dr. Karim Fahmy, Helmholtz-Zentrum Dresden-Rossendorf

Conserved patterns of heat release from cultured microorganisms reveal simple growth-metabolism relations

Quantitative analyses of cell replication address the connection between metabolism and growth. Various growth models approximate time-dependent cell numbers in culture media, but physiological implications of the parametrizations are vague. In contrast, isothermal microcalorimetry (IMC) measures with unprecedented sensitivity the heat (enthalpy) release from chemical turnover in metabolizing cells. Hence, the metabolic activity can be studied independently of modelling the time-dependence of cell numbers. Unexpectedly, IMC traces of various origin exhibit conserved patterns when expressed in the enthalpy-, rather than time-domain as exemplified for cultures of Lactococcus lactis (prokaryote), Trypanosoma congolese (protozoan) and non-growing Brassica napus (plant) cells. The data comply extraordinarily well with a dynamic adsorption-reaction model of nutrient uptake and catalytic turnover resembling a Michaelis-Menten relation generalized here to the non-constancy of catalytic capacity. The proposed formalism reproduces the "life span" of cultured microorganisms from exponential growth to metabolic decline by a succession of distinct metabolic phases following remarkably simple nutrient-metabolism relations. The analysis enables developing advanced enzyme-network models of unbalanced growth, and has fundamental consequences for the derivation of toxicity measures and the transferability of metabolic activity between laboratories.

T060 FITTER, JÖRG

Prof. Jörg Fitter, RWTH Aachen University

Mapping Multiple Distances in a Multidomain Protein for the Identification of Folding Intermediates

The investigation and understanding of the folding mechanism of multi-domain proteins is still a challenge in structural biology. The use of single-molecule Förster resonance energy transfer (smFRET) offers a unique tool to map conformational changes within the protein structure. Here, we present a study following denaturant-induced unfolding transitions of yeast phosphoglycerate kinase (yPGK) by mapping several inter- and intra-domain distances of this two-domain protein, exhibiting a quite heterogeneous behavior [1]. On the one hand, the development of the inter-domain distance during the unfolding transition suggests a classical two-state unfolding behavior. On the other hand, the behavior of some intra-domain distances indicates the formation of a compact and transient molten globule intermediate state. Furthermore, different intra-domain distances measured within the same domain show pronounced differences in their unfolding behavior, underlining the fact that the choice of dye attachment positions within the polypeptide chain has a substantial impact on which unfolding properties are observed by the smFRET measurements. In addition to measurements with millisecond observation times (confocal detection with freely diffusing molecules), we collected also for the inter-domain distance smFRET data by using total internal reflection fluorescence microscopy (TIRFM). This approach allowed for extended observation times (tens of seconds) for single molecules. Under equilibrium conditions at the denaturant half-transition concentration, the corresponding time traces exhibit at least two different constant FRET levels, corresponding to folded and unfolded states, as well as transitions between these states.

Our results suggest that, to fully characterize the complex folding and unfolding mechanism of multi-domain proteins, it is necessary to monitor multiple intra- and inter-domain distances. As shown here, the approach of mapping multiple intermolecular distances seems to be a key to discover hidden intermediates during folding and unfolding transitions.

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T061 IVANOVIĆ, MILOŠ

Dr. Miloš Ivanović[1]; Dr. Nicola Galvanetto[1]; Dr. Aritra Chowdhury[1]; Andrea Sottini[1]; Mark Nüesch[1]; Dr. Daniel Nettels[1]; Dr. Robert Best[2]; Prof. Benjamin Schuler[1]

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Protein Dynamics in a Biomolecular Condensate

A wide range of biomolecules in solution can phase-separate and form membraneless organelles in the cell. These assemblies often have liquid-like properties, and the corresponding dynamics and exchange of molecules with the environment are important for biological function. The dynamics and materials properties of these systems are commonly assessed based on translational diffusion or rheological properties, typically covering timescales of milliseconds and longer. However, information on the structure and dynamics at the molecular level is lacking.

We have studied coacervates of two disordered highly oppositely charged nuclear proteins [1,2] as a prototypical example of heterotypic liquid-liquid phase separation similar to that observed in the cell nucleus. Using single-molecule fluorescence spectroscopy, we show that the proteins in the condensates remain disordered, and that their chain dynamics occur on a sub- microsecond timescale, remarkably close to the dynamics of the 1:1 complex in dilute solution [3], even though the condensate is almostt hree orders of magnitude more viscous than the dilute solution and the translational diffusion of proteins is 50 times slower in the dense than in the dilute phase.

The experimental results are in good agreement with all- atom explicit-solvent molecular dynamics simulations consisting of ~200 proteins and ~4 million atoms in total (Fig. 1), which provide an atomistic picture of protein interactions and dynamics within the condensate and reveal the molecular origin of the rapid dynamics.

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T062 SAALWÄCHTER, KAY

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Gel formation of alpha crystallin solutions

Mammalian eye lenses contain large amounts of □ crystallins, which act both as chaperones and highly refractive structural component. At very high concentrations, the large, roughly spherical and polydisperse oligomers with a radius of around 10 nm form a colloidal suspensions subject to diffusive slowdown and eventually jamming upon increasing the volume fraction. Rotational motions remain weakly affected beyond simple hydrodynamic interactions, owing to the only weak hard-sphere interactions [1]. In previous work concerned with studies of chaperone activity and aggregation using low-resolution NMR [2], we have found that concentrated □ crystallin solutions form nearly transparent gels when heat-treated at 60°C (below their denaturation threshold) for many hours.

In this contribution, I describe our efforts to better understand the gel formation, which may have physiological relevance with regards to cataract formation. We apply a range of techniques including DLS, SAXS and cryo-TEM for size and structure determination, optical transmission measurements, as well as IR and solid-state NMR spectroscopy to characterize the folding state. The picture that emerges is one of stable filamentous gels composed of only weakly modified a crystallin oligomers. We take the known gel formation of BSA as a reference, and compare recombinant human □B crystallins with □A/□B native mixtures extracted from bovine eye lenses, for which we find differences in gel formation propensity and in the aggregation structures. Studies of the mechanical properties of the gels using AFM indentation experiments are ongoing.

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T063 NEHLS, CHRISTIAN

Dr. Christian Nehls[1]; Dr. Khanneh Wadinga Fomba[2]; Prof. Dr. Thomas Gutsmann[1]

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Visualization and force spectroscopy of mineral desert dust and associated microbes: Unraveling a bacterial long-distance propagation strategy

Dust storms can transport mineral dust (MD) from desert soil through the atmosphere to regions far from the dust origin. This impacts not only the quality of life, but also the health of millions of people in the "dust belt" and beyond: 330,000 deaths worldwide were associated with MD exposure in 2010 [1]. The climate change promotes a desertification and thereby an increase in global MD exposure. Respiratory, cardiovascular and allergic diseases can be observed in affected individuals. However, non-pathogenic and pathogenic microbes are also transported with the dust [2]. These not only spread to new regions but may also lead to direct infections.

The "Dust-Risk" consortium takes a highly interdisciplinary approach with direct public health relevance and aims to link the composition and properties of MD and associated microbes with its health risk. MD and health data have been collected in the Cape Verde Islands, which serve as a model region.

In our project part, we use atomic force microscopy and scanning electron microscopy to visualize and characterize the collected MD. With force spectroscopy, we determine the strength and type of molecular bonds between MD and different bacterial species that have been identified on the MD. Samples from different sampling sites are compared to distinguish between local dust and desert dust, and between microbes of local, marine, and desert dust origin.

Our biophysical experiments help to understand how bacteria bind to MD and wrap in it to travel long distances through the atmosphere. This knowledge will be complemented by physicochemical, microbiological, toxicological, modeling, clinical and epidemiological data from further project parts to generate a composition-based dust-health-risk-index. The Cape Verde weather service will use this risk index to provide precise and specific advice and warnings to the population.

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T064 KRAINER, GEORG

Georg Krainer

Next-generation microfluidic approaches for protein biophysics

Due to the central role of proteins throughout biology, the biophysical characterization and exploration of protein behavior has become a crucial element in many areas of modern biomolecular sciences. New and improved experimental approaches enable a deeper understanding of protein function, malfunction, and drug design. In particular, technologies based on the control of fluids at the micron scale, in conjunction with orthogonal methods such as single-molecule technologies, enable key developments in the area of protein biophysics and are transforming our understanding of protein behavior. In my lecture, I will provide insights into our work in this space and touch upon a recently developed single-molecule microfluidic sensing platform for digital protein biomarker detection, termed DigitISA (Digital Immuno-Sensor Assay). DigitISA realizes a fundamentally new design principle combining microchip electrophoretic separation with single-molecule detection and enables calibration-free, in-solution quantification of protein biomarkers in complex sensing scenarios including challenging targets such as amyloid aggregates, exosomes, and biomolecular condensates. Moreover, the digital nature of the assay enables immunochemical and thermodynamic analyses of probe–analyte interactions in terms of binding valency, affinity, and biomarker abundance. Overall, the DigitISA platform constitutes a new experimental paradigm for protein biomarker sensing, providing quantitative information beyond stoichiometric binding interactions, and enables analyses of biomolecular targets that would otherwise be hard or impossible to address by conventional immuno-sensing techniques.

T065 PIELAK, GARY

Prof. Dr. Gary Pielak PhD, University of North Carolina at Chapel Hill

Protein- & protein- complex stability in living cells

Macromolecular crowding is ubiquitous in biology and essential to biotechnology. Cells amass macromolecules to concentrations exceeding 300 g/L1 and industry mimics this environment using empirically-derived formulations crowded with synthetic polymers to protect protein-based drugs and industrial enzymes.2 Despite the crowded nature of cells, until recently biophysical studies were rarely performed at concentrations exceeding 10 g/L. Such studies are now possible. We have learned that proteins, whether crowded in cells or in vitro, behave differently than they do in dilute buffer,3 but the results contradict the seminal model of crowding4, the Asakura–Oosawa depletion interaction. This model posits a net attractive interaction between molecules immersed in a solution of macromolecules that is purely entropic. Specifically, recent experiment-based results shift the emphasis from 'hard' entropic effects to 'soft', enthalpic chemical interactions.3 Here, we extend the Asakura–Oosawa model to include enthalpic, chemical interactions and test our ideas on an extensive set of 19F NMR data that quantifies the effect of polyethylene glycol (PEG) molecular weight and concentration on the temperature dependence of protein stability. The results reveal that PEG drives protein stabilization via polymer interactions that are almost always enthalpic. Most importantly, different size crowders have different modes of actions. Small PEGs stabilize proteins enthalpically by binding the folded state. Larger PEGs stabilize proteins via exclusion from the unfolded state originating from changes in PEG-water interactions at the protein interface. The subsequent release of solution from this interface upon folding is favored enthalpically. These efforts provide both a deeper understanding of life and essential information that will turn formulation into an evidence-based endeavor. Acknowledgement: We thank the US National Science Foundation (MCB 1410854) and the U.S.-Israel Binational Science Foundation (BSF 2017063) for support.

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T066 WEBER, STEFAN

Prof. Dr. Stefan Weber, Albert-Ludwigs-Universität Freiburg im Breisgau

EPR and NMR studies of paramagnetic intermediates in the primary processes of blue-light photoreceptor proteins

Paramagnetic intermediates, such as triplet states or spin-correlated radical pairs, and stable radicals are frequently encountered in the blue-light-induced primary response of photoreceptor proteins containing flavins as cofactors. Such species may be favorably probed by both branches of magnetic resonance spectroscopy: electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR). Time-resolved variants thereof are well suited to characterize reaction intermediates in highly spin-polarized states, i.e., with non-thermally populated electron-spin or nuclear-spin levels, on nanosecond to microsecond (EPR) and microsecond to millisecond (NMR) time scales.

In this contribution, selected examples of how time-resolved EPR [1] and photo-chemically induced dynamic nuclear polarization (photo-CIDNP) NMR [2,3] contribute to unravelling mechanistic details of the primary processes in (i) cryptochrome photoreceptors, a class of flavoproteins that are involved in the circadian rhythms of plants and animals, and possibly also in the sensing of magnetic fields in a number of species [4–6], and (ii) the light-oxygen-voltage-sensing (LOV) domains, which are protein sensors used to control phototropism, chloroplast relocation and stomatal opening in higher plants, will be presented. Particular emphasis will be given on protein variants that have been modified in their amino-acid sequence and/or reconstituted with modified flavins as cofactors [7,8].

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T067 STENGEL, FLORIAN

Florian Stengel

Studying proteome organization and cellular compartmentalization: from proteins to functional compartments

Cells need to control an almost infinite number of processes and biochemical reactions in time and space. The emerging concept of liquid-liquid phase separation is increasingly identified as a mechanism to organize the cellular space into functional units via the formation of biomolecular condensates. Biomolecular condensates offer a confined space where specific protein protein interactions (PPIs) can take place in order to facilitate distinct biochemical reactions. As the formation and maturation of biomolecular condensates appears to be of fundamental relevance to biology and a disease-associated process, it is critical to understand the underlying molecular interactions. During my talk I will describe how mass spectrometry-based proteomics can be used to study PPIs and protein dynamics within molecular condensates, focusing on stress granules and in particularly the role of chaperones in organizing and maintaining these specific molecular condensates.

T068 UETRECHT, CHARLOTTE

Charlotte Uetrecht, Centre for Structural Systems Biology (CSSB),
Deutsches Elektronen-Synchrotron (DESY)

Flying viruses – understanding corona- and norovirus lifecycles

Viruses affect basically all organisms on earth. Some are detrimental to human development as we experience during the ongoing pandemic, whereas those targeting pathogenic bacteria or crop pathogens can be beneficial for us. An integral part of icosahedral viruses is the capsid protein shell protecting the genome. Many copies of the capsid protein often self-assemble into shells of defined size. Low binding affinity of individual subunits allows efficient assembly and gives rise to highly stable particles. These capsids can be studied by native mass spectrometry (MS), a single molecule like approach, in terms of stoichiometry, dynamics, assembly pathways and stability revealing coexisting states. A focus will be on variant specific differences of noroviruses, the main cause of viral gastroenteritis.

Moreover, the highly dynamic replication machinery of coronaviruses (CoV) has been a longstanding interest. Using native MS, processing of the polyproteins into individual subunits and subsequent complex assembly can be monitored simultaneously revealing striking differences between CoV species. Latest newest results on various CoVs including SARS-CoV-2 will be presented. How method development related to native MS enables deeper insights will also be pitched.

T069 STIEL, ANDRE C.

Dr. Andre C. Stiel, Helmholtz Munich

Photoswitching across the scales – photoswitching proteins in super-resolution microscopy and optoacoustic imaging

Optical imaging and genetically encoded tools already allowed us unprecedented insights into cellular and organismal structure and function. However, this is often with low resolution (for cells > 300 nm and for opaque organisms in vivo > 1 2 mm) which precludes two essential insights: 1) truly studying and understanding (patho) physiological and developmental processes in live mammalian systems, and 2) on the subcellular level understand the microdomain and spatio-temporal patterning of small molecules and ions which manifest control and functioning of a cell. While advances in optical imaging now provide methods to tackle those two realms with higher resolution the necessary genetically encoded tools – a prerequisite for life science studies – are still largely amiss. Photoswitching proteins can overcome this limitation. While for fluorescence super-resolution microscopy the use of photoswitching proteins to visualize cellular structures at the nanoscale is already firmly established, we could recently introduce the use of such proteins to optoacoustic imaging, visualizing transgene labeled cells with high resolution in a live mouse. Beyond that, we translated the use of photoswitching to small-molecule and ion sensing. For super-resolution first results show the feasibility of, for example, visualizing Ca2+ distributions on the nanoscale, while for optoacoustics, we could show that it is possible to sense insight a live animal with the resolution and added anatomical contrast inherent to the method. Beyond that, photoswitching is an – almost haptic – reversible and light induced molecular state change entailing exciting structural and spectroscopic research on this molecular switch and its protein matrix. Finally, our work is a prime example of the interdisciplinarity between application driven protein engineering, protein functional studies and imaging to answer the fundamental questions of life.

T070 WINKELMANN, HAUKE

Hauke Winkelmann[1]; Mykhailo Girych[2]; Bakeeran Pathmalolan[1]; Thomas Meyer[1]; Steffen Wolke-Hahnenkamp[1]; Christian P Richter[1]; Dr. Lukas Schrangl[3]; Dr. Rainer Kurre[1]; Prof. Ilpo Vattulainen[2]; Prof. Gerhard J Schütz[3]; Prof. Jacob Piehler[1]

[1] University of Osnabrück; [2] University of Helsinki; [3] TU Wien

Quantifying cytokine receptor dimerization dynamics in the plasma membrane by smFRET

Class I/II cytokine receptors (CR) signal via the JAK/STAT pathway, which is initiated by cytokines simultaneous interacting with two or more receptor subunits. The spatiotemporal organization and dynamics of CR assembly in the plasma membrane (PM) is currently controversially debated. By dual-color single molecule (co-)tracking in living cells, we have identified ligand-induced dimerization as the key switch of signal activation. However, we found weak intrinsic receptor dimerization affinity that can explain dysregulation of receptor dimerization by overexpression and oncogenic mutations. Our studies suggest that both interactions between the transmembrane/juxtamembrane (TM/JM) domains and the JAK pseudokinase (PK) domains, respectively, are responsible for the intrinsic dimerization propensity. To pinpoint the interaction dynamics of transient CR dimers in the PM, we have devised single molecule FRET (smFRET) imaging/tracking in living cells. To this end, we optimized the labeling precision by using the ALFA-tag and engineered nanobodies. Combined with alternating laser excitation (ALEX) and homogenous illumination at a newly designed home-built TIRF microscope detection and tracking of receptor dimers was achieved with minimum photobleaching. Thus, transient ligand-independent dimerization of the thrombopoietin receptor (TpoR) could be unambiguously detected by smALEX-FRET. TpoR dimer lifetimes were strongly stabilized by constitutively activating receptor or JAK mutations, which was in line with predictions from extensive all-atom molecular dynamics simulations. By combining different mutations that alter receptor dimerization, we pinpoint cooperation of TM/JM and JAK PK interaction sites. These studies yield a coherent model of CR dimerization, activation and dysregulation by oncogenic mutations.

T071 OLEKSIIEVETS, NAZAR

Nazar Oleksiievets[1]; Christeena Mathew[2]; Jan Christoph Thiele[1]; José Ignacio Gallea[1]; Oleksii Nevskyi[1]; Ingo Gregor[1]; André Weber[3]; Roman Tsukanov; Jörg Enderlein[1]

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Single-Molecule Fluorescence Lifetime Imaging Using Wide-Field and Confocal-Laser Scanning Microscopy: a Comparative Analysis

A recent addition to the toolbox of super-resolution microscopy methods is fluorescence-lifetime single-molecule localization microscopy (FL-SMLM). The synergy of SMLM and fluorescence-lifetime imaging microscopy (FLIM) combines superior image resolution with lifetime information and can be realized using two complementary experimental approaches: confocal-laser scanning microscopy (CLSM) or wide-field microscopy. Here, we systematically and comprehensively compare these two novel FL-SMLM approaches in different spectral regions. For wide-field FL-SMLM, we use a commercial lifetime camera, and for CLSM-based FL-SMLM, we employ a home-built system equipped with a rapid scan unit and a single-photon detector. We characterize the performances of the two systems in localizing single emitters in 3D by combining FL-SMLM with metal-induced energy transfer (MIET) for localization along the third dimension and in the lifetime-based multiplexed bio-imaging using DNA-PAINT [3]. Finally, we discuss the advantages and disadvantages of wide-field and confocal FL-SMLM and provide practical advice on rational FL-SMLM experiment design.

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T072 DORNBUSCH, DANIEL

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Anion-Specific Structure and Stability of Guanidinium-Bound DNA Origami

The "bottom-up fabrication" of DNA origami is an approach developed by Rothemund in 2006 for the fabrication of highly complex nanostructures by self-assembly, which allows the creation of 2D and 3D objects of arbitrary shape. DNA origami nanostructures are particularly well suited as substrates for the assembly of various functionalities such as proteins, nanoparticles, or specific DNA structures with unprecedented precision that can be assessed by numerous spectroscopic and microscopic techniques. In particular, the immobilization of individual proteins on DNA origamis appears attractive for the observation of conformational changes and folding by single-molecule techniques. Chemical denaturants such as urea and guanidinium chloride are commonly used to trigger such transitions in proteins. The effects of these chaotropic salts are well described for proteins, less so for DNA, and hardly at all for DNA origami nanostructures. Moreover, because of the unique properties of DNA nanostructures, their interaction with denaturants is of fundamental interest.

To reveal the interplay between DNA origami and chaotropic agents, atomic force microscopy (AFM) images of the denaturation process of DNA origami triangles with different guanidinium salts were compared with the corresponding circular dichroism spectra. The AFM data show an early break of the origami, while the hyperchromic shift indicates that the original melting process starts much later. Using principal component analysis, iterative target test factor analysis (ITTFA) and various methods of 2D correlation spectroscopy, the two processes can be correlated and explained with a thermodynamic model by the additional factor of a change in heat capacity. It is found that DNA-origami nanostructures undergo changes in secondary structure leading to breaks at the vertices. The reason for this could be due to the specific interaction of counter anions that shape the properties of the surrounding water structure and thus control the interaction between DNA and guadinium. These findings help to optimally tailor DNA-origami as a substrate for denaturant-induced folding dynamics of individual proteins while improving the fundamental understanding of the effects of water structure on DNA.

T073 KIELB, PATRYCJA

Jun-Prof. Dr. Patrycja Kielb, University of Bonn

Do Tyr/Trp redox pathways protect O2-reducing C. Coelicolor laccase from oxidative damage?

O2 reduction is one of the most important life-sustaining reactions in the biocatalysis field. The kinetically challenging task of reducing O2 to H2O requires the delivery of four electrons and four protons in a well-coordinated manner. The lack of electrons in the instance of 'catalytic action' might lead to the formation of highly reactive oxygen species (ROS), which at elevated levels are toxic to any biological environment. Although there has been substantial focus on the molecular mechanism of O2 reactions at the enzymatic active site, it is necessary to expand the viewpoint to the redox role of the entire protein matrix in the context of protection against oxidative damage. In this respect, Gray and Winkler recently demonstrated the presence of redox-active Tyr and Trp chains in O2- utilizing metalloenzymes, potentially providing conduits for transferring highly oxidizing holes away from protein active sites.

In the presented work, the hypothesis of the protective role of Tyr/Trp chains is explored in the example of S. Coelicolor laccase, in which the trinuclear center-proximal Tyr108 was shown to be involved in O2 reduction by donating an electron during catalytic turnover. Accordingly, the Tyr/Trp redox pathways are identified, and their role in oxygen conversion is studied by means of site-directed mutagenesis, a UV-Vis, and EPR spectroscopy.

Involvement of Tyr/Trp chains in SLAC's catalysis may indicate a regulatory mechanism of the protein.

T074 MÄUSLE, SARAH M.

Sarah M. Mäusle; Dr. Philipp S. Simon; Dr. Rebeca Perez; Dr. Yvonne Zilliges;
Prof. Dr. Holger Dau, Free University Berlin

S-state Transitions of Photosystem II from Spinach and T. elongatus – Insight by Time-Resolved Single-Frequency Infrared Spectroscopy

The Photosystem II (PSII) protein complex of plants and cyanobacteria catalyses the oxidative splitting of two water molecules, thereby yielding molecular oxygen, four protons and the four electrons that are transferred to the PSII acceptor side. The oxidative power required for this reaction is accumulated by a series of four photon absorption events, which each lead to charge separation and removal of one electron from the protein-bound Mn4CaOx cluster. Starting in the most reduced S0 state, every light-driven electron removal oxidizes the system further, until the transient S4 state is reached and dioxygen is formed.

When driving PSII by laser flashes through its S-state cycle, variations in protein-internal H-bond networks and electric fields as well as metal-ion oxidation can be investigated by infrared (IR) difference spectroscopy. Employing tuneable quantum cascade lasers, we have developed a time-resolved single-frequency (TRSF) IR experiment with automated samples exchange that facilitates tracking of electron transfer as well as protonation dynamics at high temporal resolution (ca. 50 ns), which previously was applied to determination of activation energies [1].

PSII membrane particles from spinach were investigated in the mid-IR regime at pH 5.4, 6.2 and 7.0 in H2O and D2O. The transients of the oxygen-evolution transition (S3->S4->S0+O2) revealed a relatively small H/D kinetic isotope effect (KIE) of 1.2-1.4 for the rate-determining 2.8 ms electron transfer (ET) step. For the pre-ET kinetics assignable to proton removal from the oxygen-evolving complex, we observed a larger KIE (2.3-2.6). These results show that the reaction previously detected i.a. by photothermal spectroscopy [2] are 'sensed' by specific carboxylate and amide vibrations.

For the ET step in the S2->S3 transition, a KIE-value of about 2 supports the notion of a proton-coupled ET (PCET) step. For the pre-ET phase of ~100 µs we observed a KIE of 2.3–2.8 and a significant pH dependency, which contrasts previous TRSF-IR reports on PSII core particles from T. elongatus [3]. Performing our own experiments on T. elongatus PSII confirmed surprising species-specific differences.

Aside from future avenues of the TRSF experiment, the relation between our time-resolved IR results and the mechanism of photosynthetic water oxidation is discussed.

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T075 CHEN, JHENG-LIANG

Jheng-Liang Chen; Luiz Schubert; Prof. Dr. Joachim Heberle; Dr. Ramona Schlesinger, Free University Berlin

Revealing the mechanism of a light-driven inward proton pump, NsXeR, by site-directed mutagenesis and spectroscopic investigations.

Xenorhodopsin (XeR), a microbial rhodopsin, was the first identified light-driven inward proton pump, followed by the discovery of schizorhodopsins. Although an x-ray structure for NsXeR from Nanosalina sp, has been solved, the proton translocation mechanism and physiological role of XeRs remains unclear up to date. Pumping in the opposite direction as described for the well-known light-driven outward proton pump, bacteriorhodopsin (HsBR), was identified to energize haloarchaea under harsh conditions by establishing a proton gradient which is used for ATP synthesis. In this study, we would like to understand what the molecular mechanism is for the inward pumping direction in NsXeR by comparison with the outward pump HsBR. Here, we combine site-directed mutagenesis, flash photolysis and FTIR spectroscopy, in order to understand single steps in proton translocation and report on some crucial amino acid residues involved. We show that a proton is released to the cytoplasm during L-state accumulation and is taken up during M-state decay to reprotonate the Schiff base of the chromophore retinal. The role of a single cysteine near the Schiff base of NsXeR will be discussed as it seems to be involved in the pumping mechanism.

T076 BRÜNIG, FLORIAN

Florian Brünig, Freie Universität Berlin

Spectral signatures of excess-proton waiting and transfer-path dynamics

Signatures of solvated excess protons in infrared difference absorption spectra, such as the continuum band between the water bend and stretch bands, have been experimentally known for a long time and have recently been used to analyze protonation dynamics in photoactive proteins [1-3].

However, the theoretical basis for linking spectral signatures with the microscopic proton-transfer mechanism so far relied on normal-mode analysis.

We analyze the excess-proton dynamics in ab initio molecular-dynamics simulations of aqueous hydrochloric acid solutions by trajectorydecomposition techniques [4]. The continuum band in the 2000 cm−1 to 3000 cm−1 range is shown to be due to normal-mode oscillations of temporary H3O+ complexes. An additional prominent peak at 400 cm−1 reports on the coupling of excess-proton motion to the relative vibrations of the two flanking water molecules. The actual proton transfer between two water molecules, which for large water separations involves crossing of a barrier and thus is not a normal mode, is characterized by two time scales: Firstly, the waiting time for transfer to occur in the range of 200 fs to 300 fs, which leads to a broad weak shoulder around 100 cm−1, consistent with our experimental THz spectra.

Secondly, the mean duration of a transfer event of about 14 fs, which produces a rather well-defined spectral contribution around 1200 cm−1 and agrees in location and width with previous experimental mid-infrared spectra.

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T077 VAN WILDEREN, LUUK

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Femtosecond-to-millisecond mid-IR spectroscopy of Photoactive Yellow Protein uncovers structural micro-transitions of the chromophore's protonation mechanism

A long-standing debate in the photochemistry of the important protein model system Photoactive Yellow Protein (PYP) is the origin of the proton that is required to form the biologically-active signalling state. It can either stem from an amino-acid residue that is hydrogen-bonded to the p-coumaric acid chromophore,[1-4] or from a nearby water molecule.[5-6] We present spectroscopic evidence that unmistakably shows that the deprotonation of the closeby amino acid candidate is asynchronous with the actual protonation of the chromophore, suggesting water as the donor.[7]

Furthermore, it is shown that the formation of transient 'stable' intermediates, typically associated with the surpassing of a high activation barrier, is actually more complex for PYP and consists of multiple smaller intermediate steps.

This view is consistent with the current view on protein folding on a rugged energy landscape, rationalizing the seemingly contradicting broad range of time constants reported for PYP's main protonation event. As different experimental methods probe different observables, the measured kinetics may on the one hand reflect the same 'overall' structural transition, but on the other hand only probe a fraction of the actually occurring 'micro transitions' and hence lead to apparently dissimilar time constants.

Our work thus may shift the general view on protein structural changes from simple high barrier transitions between a few intermediates towards a more heterogeneous picture.

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T078 MENDES FERREIRA, TIAGO

Tiago Mendes Ferreira

Towards complex biological lipid bilayers by solid-state NMR spectroscopy

The ultimate aim of studying lipid model membranes, such as two or three-component lipid systems, is to relate the understanding of these simpler systems to complex biological systems, e.g. lung surfactant, myelin or nanotechnology applications such as complex lipid nanoparticles (LNPs). For this endeavour, it is very important that accurate microscopic observables are measured not only from simple models but also from the complex systems, a rather challenging task.

Here, we present a novel solid-state nuclear magnetic resonance (NMR) strategy to investigate the molecular structure of complex membranes which have been inaccessible so far. The new methodology consists of accounting for the inhomogeneity of the transverse magnetic field used in solid-state NMR experiments to simulate the outcome of proton detected local field techniques. This enables to use time domain analysis, bringing a highly significant increase of accuracy of the C-H bond order parameters measured, as well as an increase of the range of systems that may be investigated.

We demonstrate the application of this novel method on a lipid brain extract, for which C-H bond order parameters were not accessible with previous techniques, and discuss potential applications in structural lipid research to be unlocked by the new methodology.

T079 KOZUCH, JACEK

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Nitrile Infrared Intensities Characterize Electric Fields and Hydrogen Bonding in Protic, Aprotic, and Protein Environments

Noncovalent interactions play pivotal roles in chemistry, from microscopic solvation environments to electrode interfaces, homo- and -heterogenous catalysts, biological membranes, and enzyme active sites. Despite their importance, it can be difficult to experimentally quantify the many types of interactions present in the condensed phase. One quantitative metric for these interactions is the electric field, often determined using vibrational frequency shifts of vibrational probes, such as the nitrile (CN), according to the linear vibrational Stark effect (VSE). Unfortunately, the interpretation of CN frequency shifts using the language of the VSE is often complicated by the well-known blueshift in H-bonding environments. This blueshift interferes with the linear VSE and undermines the utility of the CN as a direct quantitative probe of local electric fields. Intriguingly, while studying CN probes within simple solvents and proteins, we observed a straightforward correlation between CN stretch absorption intensity and the electric field acting onto the CN group. This correlation is based on a linear response of the transition dipole moment to electric fields and applies to both H-bonding and non-H-bonding interactions. It can therefore be generally applied to determine electric fields in all molecular environments. To further explore this finding, we introduced the non-canonical amino acid ortho-cyanophenyl alanine at various non-H-bonding and H-bonding positions into the photo-active yellow protein (PYP). Using this model system, we correlate experimental infrared spectroscopic data with electric fields extracted from polarizable molecular dynamics simulations based on the AMOEBA force field. In addition to resolving the complications due to the interfering H-bonding blueshift, the combined analysis of changes in vibrational frequency and intensity of the CN shows potential in providing insight into both local electrostatics and H-bonding geometry.

T080 ERBEN, ELENA

Elena Erben; Antonio Minopoli PhD; Nicola Maghelli PhD; Benjamin Seelbinder PhD; Iliya D. Stoev PhD; Sergei Klykov; Moritz Kreysing PhD

Max Planck Institute of Molecular Cell Biology and Genetics

Optofluidic method for highly precise and non-invasive manipulations on the microscale

Stem cell biology, developmental biology and tissue engineering are fields of active biological research that would greatly benefit from non-invasive and contact-free methods for the precise positioning of cells or even their internal constituents. While optical tweezers are a well-established tool for the precise control and manipulation of microparticles, they are restricted to probes of high refractive index and low absorption and exclude the use of photosensitive samples such as living cells. This necessitates the search for alternative techniques for precise micromanipulation.

Recently, we developed a novel optofluidic method for the manipulation of individual microparticles based on feedback-controlled thermoviscous flows. These flows can be induced optically in a liquid film merely by the repeated scanning of a moderately heating infrared laser beam [1]. We could show that in combination with feedback control thermoviscous flows automatically control the position of a single microparticle with a precision of up to 24 nm [2]. This approach does not require direct exposure of the probe-particle to high-intensity laser light which may enable the positioning of biological samples, such as single cells. Furthermore, it has earlier been shown that thermoviscous flows can be applied inside a living cell [3], opening up the possibility for precise manipulations in-vivo using this technique. Recently, we extended this approach to enable simultaneous control over the position of not only one, but multiple particles, thus generating dynamic micropatterns. In an alternative realisation requiring multiple light-induced flows, our new positioning method can be combined with implicit force sensing [4].

The versatility and non-invasive nature of our approach should render this technique a valuable tool for future research in the life sciences and beyond.

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ABSTRACTS OF THE POSTER SESSIONS

P001 BALKE, JENS

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Investigation of proton motive force establishment from single cytochrome c oxidase enzymes using a fluorescence assay based on a voltage-sensitive protein

Cytochrome c oxidase (CcO) is an important part of the oxidative energy conversion system for many aerobic cells [1], present in both eukaryotes and most aerobic prokaryotes. Functioning as a redox-coupled proton pump, CcO transports protons across the membrane and gains the necessary energy by reducing oxygen to water while oxidizing cytochrome c at the positive side of the membrane. The coupling of these processes increases the electrochemical transmembrane proton gradient that is the driving force for ATP synthesis by ATP synthase. While differences in the protonation state of specific residues and water organization around the binuclear center and the proton entry channels have been investigated [e.g. 2], the exact mechanism of proton uptake on one side and pumping against a gradient to the other side of the membrane is still not fully understood. To understand the impact of changes at specific sites of the protein, investigations into the establishment of the proton motive force by single CcO enzymes is mandatory.

For this we developed a fluorescence-based proteoliposome assay using an electric potential sensitive protein to monitor the changes in membrane potential. A genetically encoded voltage indicator was identified as a possible candidate. Specifically, we used a variant of Archaerhodopsin-3 from Halorubum sodomense, called Archon 1 [3], which is sensitive to membrane voltage changes by altering its dim fluorescence in the NIR wavelength region with submilliseconds response times.

We show the application of this fluorescence-based membrane potential read-out by the direct correlation of the fluorescence changes and the changes in the membrane voltage. Enzymatic turnover of the incorporated CcO variants in single vesicles and voltage read-out was achieved in total internal reflection fluorescence microscopy. This technique allows for a direct time-dependent monitoring of the proton pumping ability of CcO via the build-up of the membrane potential in a native-like environment. Pumping, non-pumping, and pumping-impaired enzymes can be easily distinguished.

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P002 BOUSHEHRI, SABER

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Effect of O-Glycans on Structure and Friction of the Intrinsically Disordered Synovial Joint Protein Lubricin

Lubricin is one of the main compounds responsible for providing excellent lubrication by reducing friction in synovial joints. Lubricin is a highly glycosylated protein, which contains an intrinsically disordered segment and a folded element. The role of glycans on the Lubricin conformation and, more generally, the molecular mechanisms by which Lubricin acts as a lubricant are yet to be fully understood.

Here, we used atomistic molecular dynamics simulations under equilibrium and shearing conditions to elucidate the molecular mechanism behind the Lubricin low friction properties. For this purpose, we generated a set of intrinsically disordered fragments from the Lubricin sequence, which are representative of the main physicochemical properties of this protein, including glycosylation and proline content.

Our simulations demonstrate that the more glycosylation sites are added, the more negatively charged the fragments become, and thereby the more extended the Lubricin's intrinsically disordered fragments are. Accordingly, the size of the fragments appears to follow the same charge-dependency as other intrinsically disordered proteins. Non-equilibrium molecular dynamics simulations are allowing us to determine the effect of Lubricin, and more specifically its glycans, on the shear viscosity of the medium. Our results show how glycans impact locally the conformation of Lubricin a result of relevance at understanding the molecular determinants underlying the low friction properties induced by this intrinsically disordered protein.

P003 DIEPOLD, NIKLAS

Niklas Diepold; Dr. Lea Schröder; Prof. Dr. Tilman Kottke, Bielefeld University

Conversion of a Halogenase to a Photoenzyme: Impact of Tryptophans Identified by FTIR Difference Spectroscopy

The conversion of a native redox active enzyme into a photoenzyme is a promising goal to solve issues with cofactor regeneration. A big challenge is the improvement of the quantum yield of the photoreaction at the cofactor while preserving the natural biophysical coupling of the cofactor conversion with secondary structural changes of the enzyme. Amino acids prone for efficient electron transfer to the cofactor would depopulate the excited state and need to be removed by structure-guided mutations which should not alter the catalytic mechanism.

The halogenase PyrH converts tryptophan to 5-Cl-tryptophan only using oxygen, sodium chloride and the fully reduced form of the cofactor flavin [1]. In vivo, flavin is regenerated to the fully reduced form by an auxiliary enzyme, a flavin reductase. Instead, catalysis can be performed by blue light in the presence of a reducing agent leading to full conversion of tryptophan [2]. The cofactor binding pocket is coupled with the site of enzymatic conversion at a distance of 10 Å.

We applied light-induced Fourier transform infrared difference spectroscopy for studying the influence of mutations. By isotopic labeling of the protein moiety, difference signals were assigned to secondary structural changes in the enzyme upon photoreduction. Two tryptophan residues close to flavin were exchanged by phenylalanine to improve the quantum yield of the cofactor conversion. By exchange of a single tryptophan, a downshift in the flavin modes points to a more hydrophilic binding pocket but secondary structural changes from the coupling are mostly preserved. Additional exchange of a second tryptophan further increases the hydrophilicity around flavin close to that of the bulk and further secondary structural changes are lost. Flash photolysis was employed to determine the relative quantum yields of the mutants. In conclusion, single tryptophan residues have a strong impact on the environment of the cofactor and the coupling to secondary structural elements.

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P004 DORENDORF, TILL

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Role of Obscurin Dual-Kinase System in Mechanosensing in Active Muscles

Mechanical force generated during muscle contraction is sensed in the sarcomere and communicated to cell organelles throughout the myofibril as to regulate metabolic energy supply, calcium levels, protein transcription and cellular turnover processes. These changes are aimed to match tissue performance to mechanical demand. Prime candidates for sarcomere-based mechanosensing are kinases of the family of elastomeric, filamentous titin proteins. Among these, proteins of the obscurin class remain largely uncharacterised. Obscurins most profound features are the extensive variety of isoforms and the existence of a C-terminal dual tandem kinase region (termed Protein Kinase 1 and 2). To date, it remains unknown whether the serial PK1 and PK2 kinases in these proteins act independently, concurrently or are otherwise functionally linked. To investigate this question, we have elucidated the structure of PK1 from the obscurin homolog UNC-89 by X-ray crystallography for two invertebrate species, Drosophila melanogaster and Caenorhabditis elegans, to a resolution of 1.75Å and 1.68Å, respectively. The sequences of these PK1 domains show them to be pseudokinases that deviate from canonical active kinases, and bioinformatic analysis of the elucidated 3D-structure reveal the degree of functional alteration. C. elegans PK1 shows a structurally altered N-lobe and interlobular hinge region, being presumably unable to bind ATP. Conversely, Drosophila PK1 has many active site features that can support phosphor-transfer and the ability to bind ATP, but biochemical assays indicate that it is also an inactive pseudokinase unable to perform phosphorylation. Therefore, we conclude that both kinases should be classified as inactive pseudokinases. We show that Drosophila PK1 can bind its tandem partner PK2, whose sequence suggests it might be an active kinase. Therefore we propose that PK1 and PK2 form a dual kinase system, where the function of the PK1 pseudokinase lies in binding and regulating the active PK2 and that the force induced separation of these two interaction partners acts as a mechanosensing dual-kinase system that signals muscle activity to the cell. As these proteins and their human homolog are important for muscle development and function, this mechanism links muscle integrity with sarcomere activity.

P005 HASELBERGER, DAVID

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Effect of Multivalent Salts on Protein Diffusion under Crowding Conditions

The biophysical properties of biomacromolecules like proteins or RNA change in the presence of high concentrations of other surrounding macromolecules. This phe- nomenon is called macromolecular crowding and can be seen e.g. in cells. However, many protein studies and assays are conducted in dilute conditions. To be more ac- curate in their interpretation a broad understanding of concentration effects on the protein behavior is mandatory. We aim to contribute to this field by analyzing the rotational and translational diffusion of proteins via a variation of the protein-protein- interactions, with a particular focus on anisotropic interactions.

Previous Studies have shown that the concentration effect on protein diffusion is not universal but depends on shape and charge distributions of the proteins. For proteins with a complex charge distribution like hen egg white lysozyme (HEWL) a strong coupling between rotational and translational diffusion and macroviscosity was observed, while proteins with mainly hard-sphere interactions (αB-crystallin) show the opposite. The most abundant protein in bovine blood, bovine serum albumin (BSA), shows an intermediate coupling.[1] We modify the strength of the coupling through the addition of multivalent salts, mainly $LaCl_3$. Such salts are known to induce a rich phase behaviour including a reentrant condensation and UCST-LLPS in acidic proteins like BSA.[2] We analyze the diffusional changes at different points of the phase diagram with with NMR-relaxometry and pulse-field-gradient NMR, probing rotation and translation, respectively.

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P006 HERNANDEZ FRAGOSO, JOSE SAUL

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How redox-state of the α-2 disulfide bond in HLA-B*1501 affects the binding groove opening

Human Leucocyte antigen class I (HLA-I) proteins are in charge of presenting foreign antigens to lymphocytes for pathogens screening and immune system activation. After proteasomal degradation of cytosolic antigens into peptides of 8-12 aminoacids, the latter are recognized and bound to HLA-I in the endoplasmic reticulum.
The peptide-HLA complex (pHLA-I) is then transported to the cellular membrane to be assembled with the T cell receptor during the so-called immunological synapsis. Optimal peptide binding is assisted by chaperones and enzymes such as the protein disulfide bond isomerase (Park et al., 2006), which oxidizes the disulfide bond (C101-C164) located between the alpha2 helix and beta-sheet portions of the peptide-binding groove. In this context, it has been suggested that the redox state of this disulfide bond plays a critical role in regulating the optimal peptide selection and formation of mature pHLA-I complexes (Park et al., 2006). In principle, molecular dynamics (MD) simulations can provide some hints on the underlying molecular mechanisms. So far, all the efforts done in this direction have focused on the oxidized state of HLA-I proteins (Anjanappa, R. et al., 2020), whilst, to the best of our knowledge, the study of the reduced state has not been attempted.

Here, by using MD simulations, we provide a first molecular picture on the effects of the reduced versus oxidized state of HLA-B*1501 on the closing/opening of the binding groove, with and without peptide (Hernández-Fragoso et al, 2022).

P007 HINGST, ELISE JOHANNA

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Structure and Organization of monoclonal antibodies at the air/water interface in the presence of pharmaceutical polymers

Understanding structure and organization of monoclonal antibodies (mAbs) at the air/water interface is important for stability and effectiveness of protein drug formulations used in pharmaceutical industry. To impede the adsorption of mAb, the surfactants Poloxamer 188 (P188), Polysorbate 20 (PS 20) and Polysorbate 80 (PS 80) are used. In this diploma thesis the effect of P188 and PS 20 on mAb adsorption was studied. The aim was to prevent mAb adsorption to the interface or to desorb mAb from the interface by posterior surfactant addition. The experiments were conducted by Langmuir Film Balance measurements, Drop Shape Tensiometry (pendant drop), and Infrared Reflection-Absorption-Spectroscopy (IRRAS).

P188 is known to exist in different phases at the air/water interface depending on its surface concentration. [1] The experiments show, that the phase state has an impact on mAb adsorption. The presence of P188 in a concentration above 0.3 mg/L always inhibited mAb adsorption. Desorption of mAb wasn´t caused by addition of P188, whereas it was by addition of PS 20. However, IRRA-spectra reveal that PS 20 is only able to displace freshly formed interfacial mAb layers.

A 17-hour old mAb layer couldn´t be desorbed by PS 20, whereas a 2 hour old mAb layer was completely displaced from the surface by PS 20. This suggests time dependent reorganization of mAb at the air/water interface which increases its resistence to desorption. It is conceivable that internal hydrophobic parts of the mAb structure are exposed to the air-phase. [2]

The here studied subphase concentrations of mAb P188 and PS 20 are lower than those used in pharmaceutical industry, nevertheless the molar ratios are comparable.

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P008 LIERATH, JANA

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Influence of pH on Fibrinogen and its Surrounding Ions during Salt-Induced Fiber Assembly

Understanding the reaction of proteins to variations in their aqueous environment and the changes induced is essential for the development of new biomedical materials. In recent years, fibrinogen has become highly interesting to be processed into nanofibrous scaffolds, as it is part of the natural wound healing process where nanofibrous blood clots are formed. Therefore, the ability of fibrinogen to form nanofibrous scaffolds through salt-induced self-assembly is very attractive for regenerative medicine [1]. In this process, the scaffold assembly is strongly determined by the hydration shell surrounding the protein, or lack thereof, and the interactions, of the ions with the protein surface [2]. In particular, it has been shown in experiments that the pH value of the buffer significantly influences fiber formation. Acidic solutions repressed a fiber formation while basic solutions promoted the formation of fibrinogen scaffolds (Fig. 1 A-C) [3].

To understand the influence of the pH on fiber formation on a molecular level, atomistic models were established with adjusted pH properties. The description takes place through the distribution of protonation and deprotonation of titratable amino acids in the separated domains of fibrinogen (Fig. 1 D-F) via H++ [4]. The resulting pH-dependent surface charge distribution will be discussed by isopotential surfaces of fibrinogen created by the use of a Poisson-Boltzmann Solver [5]. Based on these findings, the relevant protonated and deprotonated fibrinogen domains and their interactions with Na+ and Cl- ions in solution were simulated with classical molecular dynamics (MD) simulations with the GROMACS code [6].

As a result of the APBS calculations, we obtained a detailed map of the surface charge distribution in different fibrinogen domains in dependence of different pH values. The classical MD simulations reveal regions with a high probability of ion–protein interactions. The protein–ion interaction depending on the pH value will enable us to correlate pH-dependent fibrinogen conformations with process parameters during salt-induced fiber formation of fibrinogen.

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LORENZ, ANDRÉ

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Activity boost of Thermomyces lanuginosus lipase by interaction with polymethyl methacrylate

In view of the steadily growing demand for climate-neutral and resource-saving products, enzyme-catalyzed reactions are becoming increasingly important in academia and industry. In contrast to conventional chemistry, enzymes are able to catalyze very selectively numerous reactions under mild reaction conditions and are inherently biodegradable.

Lipases are one of the most commonly used classes of enzymes in biocatalysis. Lipases not only catalyze the hydrolysis but also the esterification and transesterification of non-natural substrates often in a stereo- or regioselective way. Due to their broad substrate specificity, they are used as catalysts in pharmaceuticals, cosmetics, detergents and food production.

Technical applications of enzymes routinely require non-native environments, as non-neutral pH values, which typically weaken enzyme activity and stability. It has been suggested that an interaction of enzymes with polymers can boost enzyme activity. The present work aims to investigate the cooperative effect of pH and polymer addition on the activity of the lipase from Thermomyces lanuginosus (TLL). By a combined analysis of the activity and the diffusional dynamics, activity boosting polymers are identified and the respective polymer-lipase interaction characterized.

The enzymatic activity is determined by means of fluorescence assay and the reaction of 4-methylumbelliferone butyrate. In addition, the interaction between enzyme and polymer is investigated with Fluorescence Correlation Spectroscopy (FCS). For this purpose, a home built inverted fluorescence microscope is used. While the PMMA is synthesized using ARGET ATRP , the pDMAPS is performed via RAFT.

Already the presence of the small concentrations of PMMA increases the enzymatic activity of the lipase. Michaelis Menten analysis reveals that PMMA increases vmax whereas Km remains nearly unaffected. On the contrary, no effect of the zwitterionic polymer DMAPS on the enzyme activity is observed. Thus, the addition of PMMA has a comparable effect on the lipase as the addition of molecular surfactants, where hyperactivation has been reported.

In order to reveal the mechanism of hyperactivation, the lipase is labelled with the fluorescent dye Atto 488 and subjected to FCS analysis. Here, an increase in the diffusion constant is observed with the addition of PMMA. This indicates that multimer formation is successfully inhibited by the presence of PMMA.

P010 ORMENO, FERNANDO

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Understanding the determinants of complex formation between the Archaeoglobus fulgidus Ammonium transporter Amt2 and its regulatory partner GlnK2

The genome of the hyperthermophile euryarchaeon Archaeoglobus fulgidus (Af) contains three amt genes encoding for Ammonium Transport (Amt) proteins. These are trimeric integral membrane proteins that selectively mediate the uptake of the most reduced form of nitrogen, ammonium (NH4+) for bio-assimilation. As is frequently observed amongst prokaryotes, amt genes are present in an operon along with a glnk gene [1]. GlnK proteins are members of the broad PII protein family and their main function is the regulation of nitrogen metabolism. In particular, upon ADP binding to GlnKs, conformational changes occur in the protein which promotes a specific GlnK:Amt interaction that functions to prevent ammonium uptake into the cytoplasm. Conversely, ATP and 2-oxoglutarate binding to GlnKs are signals for GlnK:Amt complex dissociation and thus transport ammonium into the cell [2]. Structural and functional analysis of these proteins in our group revealed that operon-2 contains the most unusual proteins. Ligand binding to Af-GlnK2 confirmed the expected nucleotide recognition but, most intriguingly, revealed a unique incapacity of this PII protein to recognize 2-oxoglutarate [3]. The characterization of Amt proteins that have evolved from highly selective transporters to ammonium receptors, such as the ammonium sensor histidine kinase Ks-Amt5 from "Candidatus Kuenenia stuttgartiensis" [4] or the Sd-Amt1 from Shewanella denitrificans [5], allowed us to identify, from amino acid sequence comparisons, the presence of two potential binding sites for ammonium in Af-Amt2. Here we describe investigations on the Af-Amt2 and Af-GlnK2 pair to understand what effectors molecules and events affect their interaction. The structure of the complex, solved by cryo-EM, reveals the presence of ADP and we can infer that NH4+ is bound to Af-Amt2. Our results so far validate the particularities of these two very interesting proteins in ammonium transport, sensing and homeostasis in Archaeoglobus fulgidus cells.

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P011 RUBAILO, ANNA

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Spin-labelling via metabolic glycoengineering for studying post-translational protein modification by electron paramagnetic resonance spectroscopy

Post-translational modifications (PTMs) are involved in many cellular processes. The addition of O-linked β-N-acetylglucosamine (O-GlcNAc) is an ubiquitous PTM, essential for mammalian cells.[1] O-GlcNAcylation is a reversible serine/threonine glycosylation for regulating protein activity and availability inside cells.[2] O-GlcNAcylation regulates a myriad of physiological and pathological processes. However, understanding the diverse functions of O-GlcNAcylation is often challenging due to the difficulty of detecting and quantifying the PTM. Thus, robust methods to study O-GlcNAcylation are crucial to elucidate its key roles in the regulation of individual proteins, complex cellular processes, and disease. Here, we demonstrate glycosilation specific spin labeling of post-translationally modified proteins. Metabolic glycosaccharide engineering (MGE) [3] in cooperation with nitroxide spin-labeling (SL) was utilized to enable mapping of O-GlcNAc to specific serine/threonine residues within proteins of interest to facilitate functional studies. The approach exploited the incorporation of azide group of the sugar moieties of the protein of interest followed by site-directed spin labelling technique. We discuss application of the copper-catalyzed azide-alkyne cycloaddition "click" reaction 6 to attach alkyne-containing chemical spin label to GlcNAz and demonstrate how this functionalization of O-GlcNAz-modified proteins can be used to identify PTM of two model proteins (OGT and p53) by electron-paramagnetic resonance (EPR) spectroscopy.

Continuous wave electron paramagnetic resonance (CW-EPR) spectroscopy allows for quantitative detection of the spin labelled PTMs and for spectral simulations. This approach also allows for optimisation of the spin labelling technique, to achieve conditions appropriate for pulse EPR, and thus for a variety of protein structure characterisation methods. For the future extension of this strategy we are going to test further chemical moieties in modified sugars for milder reaction conditions and more efficient spin labelling. The ultimate challenge of this approach is to perform in cell spin labelling and EPR detection.

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P012 RUDOLPH, MICHAEL

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The energy barrier for the alternating access of an ATP-Binding Cassette (ABC) exporter

ATP-binding cassette (ABC) transporters shuttle diverse substrates across biological membranes. They consist of two solvent-exposed nucleotide binding domains (NBDs) connected with two transmembrane domains (TMDs). The NBDs contain two nucleotide binding sites (NBSs). TmrAB, the only ABC exporter in Thermus thermophilus, is a heterodimeric exporter with asymmetric NBSs [1]. Despite the vast amount of biochemical, biophysical, and structural data available, the kinetic and thermodynamic aspects underlying the conformational changes remain elusive. Pulsed electron-electron double resonance (PELDOR or DEER) spectroscopy has been used to explore the conformational heterogeneity of several ABC transporters. Though PELDOR data can provide information on the equilibrium populations, a quantitative interpretation is often difficult for membrane proteins owing to the necessity for sample freezing (taking ~1 s). Here we exploited the thermophilic nature of TmrAB, which increases the transition rates to the seconds range under non-hydrolyzing condition. Earlier, this allowed us to characterize the conformational equilibrium at the TMDs and NBSs in a temperature- or ATP-dependent manner [2, 3].

Here we quantified the conformational equilibrium at the NBSs and TMDs in a time-resolved manner for a broad temperature range (30 – 60 °C). The data were analyzed based on a four-state kinetic model to determine the rates for the change in the conformational equilibrium. The temperature dependence of these rate constants was further investigated using Arrhenius equation to determine the energy barrier for the conformational transition. We show that the IF-to-OF transition of the TMDs in TmrAB is separated by an energy barrier of 75 ± 1 kJ/mol. The activation energy for the closure of the consensus NBS is shown to be 59 ± 2 kJ/mol. Strikingly, the rate and magnitude for the IF-to-OF transition is significantly increased under hydrolyzing conditions (ATP+Mg2+, [4]). These results provide novel insights into the mechanism of an ABC exporter and demonstrate that PELDOR spectroscopy would be a potential tool to determine the thermodynamic and kinetic parameters for large membrane protein complexes.

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P013 SPIES, KATHARINA

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Active Site and Gates Structure of Chrimson Wild-Type and Mutants and their Absorption Spectra

The channelrhodopsin Chrimson is the most red shifted cation channel currently known and thus of great importance in optogenetics studies as red light has low phototoxicity and penetrates deeply into biological tissue. [1-4] Its active site consists of the protonated retinal Schiff base and two counterions (E165 and D295) with an unique protonation state in comparison to other channelrhodopsins since one of them is protonated. [2-4]

We present a computational study to investigate the ground state structure of the active site and the gates of the putative ion pore of Chrimson. Quantum mechanics/molecular mechanics (QM/MM) simulations of the wild type and different mutants were performed and the excitation energies of a large ensemble of QM/MM trajectory snapshots were calculated with long range corrected Density Functional Tight Binding (LC-DFTB3) , long range corrected Density Funtional Theory (LC-DFT: ωB97X functional) and Spectroscopy Oriented Configuration Interaction (SORCI).

The experimental hypothesis that E165 is the protonated counterion [2–4] is verified as irrespective of the starting structure the proton is found in close vicinity to residue E165. In addition, two stable configurations were observed in the active site of Chrimson: The proton of the counterion E165 is either shared by both counterions or the counterions form a hydrogen bond via a water molecule. Furthermore, the protonation states of several titrable amino acids in the putative ion pore were determined. Additionally, the computed absorption spectra of mutants of residues in and near the active site agree well with experimental results.

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P014 KAMP, DIETER

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The chromatin remodeler ISWI transiently bridges phase separated chromatin fibers in an ATP-dependent manner

Liquid-liquid phase separation is important for numerous biological processes like the formation of membraneless organelles or the spatial organization and regulation of the genome within the nucleus. In particular, condensates formed by phase separating chromatin are involved in DNA damage repair, transcription, DNA looping and DNA splicing. Although these processes require high chromatin dynamics, little is known about how chromatin-interacting and -remodeling proteins affect the properties of these condensates.

In our study we focus on the chromatin remodeler ISWI, which is required to restore proper nucleosome positioning during RNA transcription and influences chromosome structure and cell viability.

Employing a model system of reconstituted chromatin arrays, we studied the effect of ISWI on the rheological properties of chromatin condensates using FRAP and optical tweezers.

Our experiments reveal a nucleotide-dependent cycle of ISWI-nucleosome interactions involving transient bridging of nucleosomes by ISWI upon ATP binding that is resolved upon hydrolysis. The transient interactions allow ISWI to rapidly translocate in the dense environment of chromatin condensates. However, the loss of ATPase activity leads to stable bridging of chromatin fibers by the remodeler, resulting in hardened condensates. Corse-grained MD simulations replicate our findings. We conclude that the ATP hydrolysis cycle of ISWI tunes the physical properties of chromatin condensates and influences nucleosome positioning as well as the fluidity of chromatin.

P015 WAGNER, SUSAN

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Towards the genetic clearance of living tissues to improve imaging

The effort in improving optical microscopes led to unprecedented resolution which allows fundamental discoveries at molecular level. Nevertheless, optical access of biological samples by microscopes is usually restricted to the sample's outer most surface owing to tissue-induced light scattering which blurs the view. Light scattering is caused by heterogenous refractive properties of cellular constituents, like organelles, chromatin and lipid droplets.

We aim to understand the optical plasticity of cells, and genetically clear living mammalian tissues for microscopy by specifically targeting responsible genes.

Using directed evolution, we successfully improved the optical properties of cultured mammalian cells and found that evolved transparency frequently goes along with the phenotypic reduction of nuclear granularity (1). As a next step, we are investigating how improved optical properties of individual cells influence the optical properties of 3D cell clusters, such as spheroids. In order to determine imaging quality of spheroids in an unbiased way, we are setting up an imaging strategy based on external fluorescent microspheres interspersed within the tissue models.

To obtain more holistic and enabling view, which genes confer transparency, we are applying a genome wide gene activation screen making use of the CRISPRa technology. Understanding the full potential of a cell's optical plasticity will provide us with a broad toolkit, so that different genetic strategies can be applied depending on the specific nature of the various biological samples.

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P016 ZHU, XIN

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Charge renormalization in DNA oligonucleotides

Charge renormalization of highly charged molecules such as DNA or RNA has been studied in depth for decades. Renormalization refers to a reduction in effective electrical charge compared with the structural electrical charge of a molecular species in solution. Manning condensation theory has been widely used to theoretically interpret an explain the underlying electrostatic mechanism[1]. Although renormalization is ubiquitous in nucleic acids because of their high charge density, the renormalization factor is in fact a function of contour length for fragments of small length[2]. To carefully explore small differences in the renormalization factor between short-length DNAs (e.g., 11ss and 12ss), a measurement technique with sub elementary charge sensitivity is needed, which is in general is quite challenging. In this report, we systematically measure the effective charge of 8ss-12ss DNA with the aid of Escape-Time electrometry (ETe) technology described in detail in our previous work[3]. The effective charge of molecules is extracted through by measuring trapping (escape) times in arrays of electrostatic traps. Trapping time depend exponentially on the effective charge of a molecular species (Fig. 1a). In a given measurement we collect 104 trapping events which gives a measurement precision of ~1%. We measure clearly distinguishable escape times for 8ss-12ss DNAs which permits us to accurately infer their effective charge. As the structural charge of our fluorescently labeled molecules is known a priori, we can further determine the renormalization factor for each 8ss -12ss single-strand DNA (Fig. 1b). In summary, the ability of ETe to discriminate between effective charges of molecular species at the 1% level holds great promise for a new charge-based biomolecular analytical tool operating at the single molecule level.

P017 BARTONITZ, FLORIAN

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Ultrafast Photoinduced Dissociation of Delphinidin-3-Rutinoside

Delphinidin-3-rutinoside (D3R) is found in grapes and berries. It acts as antioxidant and radical scavenger. The antioxidant mechanism involves proton and electron transfer but further details remain largely unknown. The ultrafast photoreaction of protonated D3R in aqueous solution (pH=2) is addressed by a combination of fs broadband Vis-Vis and Vis-IR pump-probe spectroscopies.[1] The IR data reveal a broad band extending from 1600 to more than 1800 cm-1 (see Fig. 1a). This feature is assigned to a proton-continuum band resulting from D3R deprotonation. In addition, Vis-Vis experiments demonstrate rapid changes in the stimulated emission signal (0.4 ps, unresolved in the IR measurements) and a decay of the population back to the ground state within a time constant of 2.6 ps. The latter matches the decay of the IR proton continuum band. Remarkably, a positive absorption at around 600 nm remains on later timescales (Fig. 1b). This absorption is compatible with the neutral form of D3R. Therefore, transient proton release from cationic D3R to the surrounding solvent is concluded. Understanding these very first steps should shed light on the mechanism of how exactly D3R scavenge free radicals.

P018 CELEBI, METEHAN

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Proton channel communication in cytochrome c oxidase

In the aerobic respiratory chain, cytochrome c oxidase (CcO) is an essential membrane protein complex that catalyzes the reduction of molecular oxygen to water by consuming four substrate protons. Additional four protons are taken up from N-side, translocated through the protein and released to the P-side of the membrane thereby establishing a proton gradient. Proton translocation from the N-side takes place via two different channels named D- and K-channel [1]. The K-channel is only active during the reductive phase of CcO. We recently found evidence on how the K-channel becomes activated, how protonation activities, water dynamics and helix-6 conformational dynamics at the channel entrance site are involved in channel opening [2]. Helix-6 harbors the catalytic Tyr280 as well as Glu278 (Paracoccus denitrificans amino acid numbering), and thus helix-6 mobility could play a critical role during proton translocation in both channels. Here, we aim at revealing a possible communication between the D- and K-channels. The effect of the D124N mutation, which blocks the D-channel at its entrance, will be investigated to test whether the D-channel closure influences helix-6 mobility, H-bonding networks and water dynamics at the K-channel surface site [2]. We will use site-specific mutagenesis [3] together with time-resolved fluorescence anisotropy experiments, and pKa measurements by employing a sensor site (K299C) at the interface between helix-6 and loopa6-a7, which doesn't affect the electrostatics of the enzyme [2].

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P019 CHRISTER, KALMAN

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Investigating D1-Glu189 variants of Photosystem II by time-resolved O2-polarography

Photosystem II is a protein complex performing the photocatalysis that transforms water into O2, protons, and reducing equivalents, the latter representing the electron source for oxygenic photosynthesis. Central to the water-splitting catalysis is the oxygen-evolving complex (OEC) consisting of the Mn4Ca-oxo cluster with its nearby water-protein environment. The OEC includes a number of highly conserved residues and water molecules which are well resolved in crystallographic models [Kern et al. (2018) Nature 563, 421-425]. The OEC accumulates oxidation equivalents in a light-driven sequential process, transitioning through the so-called ‚S-states' while undergoing the necessary electronic and conformational changes. The role of individual amino acids in these reactions is still poorly understood. One residue of interest is Glutamate 189 (E189) which is a likely ligand to the Mn4Ca-oxo cluster (< 2Å) and connected to the Tyrosine 161 (Yz) by an H-bonding network. It is involved in calcium binding and has been suggested to be pivotal in shifting of water molecules during different stages of the S-state cycle [Debus et al. (2018) Nature 563, 421-425; Capone et al. (2020) Chem. Phys. Lett.]. In our work, a series of saturating light flashes of ca. 10µs duration triggers the release of O2, which is detected by self-designed centrifugable bare-platinum electrodes with mounted temperature control devices. With this experiment for time-resolved O2 polarography, the kinetics of oxygen release can be detected with better 50µs time resolution. With the help of numerical simulations involving an adapted diffusion model, rate constants for O2 formation in the sub-millisecond and millsecond time domain can be determined [Assunção et al. (2019) Biochim Biophys Acta 1860, 533-540]. Here, we examine the effect of exchanging E189 for lysine (K), arginine (R) and glutamine (Q) and observe little or no effect on the oxygen release kinetics at 25°C. Other mutations, besides leucine (L) and isoleucine (I) replacements, result in complete loss of photoautotrophy [Debus et al. (2000) Biochemistry 39, 6275-6287]. Due to the nature of E189 (charged residue) and its position in the OEC, the lack of effect of the K, R and Q mutants is a suprising; putative explanations are discussed. A promising next steps is to perform temperature dependent measurements to investigate possible effects on activation energies, specifically searching for an enthropy-enthalpy compensation effect.

P020 GREIFE, PAUL

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Tracking water oxidation through time-resolved FTIR Spectroscopy

In the light-driven reaction cycle of Photosystem II (PSII), two 'substrate' water molecules are oxidized resulting in the release of O2 at the oxygen-evolving complex (OEC), which consists of a Mn4Ca-oxo cluster and its water-protein environment. Driven by a sequence of light flashes, the OEC cycles through its five S-states, alternating the release of electrons and protons.

Infrared spectroscopy (IR) offers unique insight into these processes, as even slight structural or environmental changes are impactful. Spectrally extensive Fourier Transform IR (FTIR) investigations of the semi-stable S-state intermediates have been performed under near-native and perturbed conditions [1, 2]. Typically, rapid-scan FTIR results in insufficient time resolution for PSII. Therefore, recent IR works have focused on resolving kinetics at specific bands [3, 4], sacrificing broader spectral context.

To investigate events associated with the PSII photocycle, we heavily modified the sample compartments of two commercial FTIR spectrometers to push the time resolution to their respective limits. Furthermore, optimizations in sample preparation and data analysis allows for the efficient processing of thousands of excitation sequences.

In a step-scan experiment, more than 230,000 excitation cycles of dark-adapted PSII were recorded and combined to resolve processes from 50 µs to 130 ms in the spectral range of 1800-1300 cm-1, for all S-state transitions. Alongside quantum chemical simulations, analysis of spectral features associated with the evolution of dioxygen (~2.5 ms) during the S3->S4->S0 transition identifies a critical transient carboxylate deprotonation preceding the formation of an oxygen radical at the OEC. In another series of experiments, extending the sampled range to 3800 cm-1 while pushing rapid-scan time resolution below 10 ms allows for the investigation of PSII systems with slowed kinetics (mutations, water analogues, etc.).

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P021 LA GRECA, MARIAFRANCESCA

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The photoreaction of the proton-pumping rhodopsin 1 from the maize pathogenic Basidiomycete Ustilago maydis

Microbial rhodopsins have been found widespread in bacteria and archaea exhibiting different functions such as pumps, sensors, and channels. Recently new rhodopsins have been discovered in pathogenic fungi and it has been postulated that they might be involved in the signaling process during infection. Here we report on rhodopsin-1 from the fungal organism Ustilago maydis (UmRh1), responsible for the corn smut disease in maize plants. UmRh1 is an outward proton pump as shown by recent electrophysiological investigations. Here, we describe the spectroscopic characterization of UmRh1, showing a pH dependent photocycle. Using time resolved UV-Vis spectroscopy and steady state FTIR, we have investigated the photocycle of UmRh1 at different pHs and different membrane environments, and compared to the well-known bacteriorhodopsin. Furthermore, we investigated the effect of the plant hormone IAA (indole-3-acetic acid) on UmRh1 photocycle, proving an impact on specific intermediates and the pump activity. We report on preliminary spectroscopic results and discuss the putative role of UmRh1 in pathogenesis.

P022 LARBIG, ALEXANDER

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Structural and dynamic analysis of the third conformational state of T4 Lysozyme by photoinduced electron transfer

In this work, a novel structure elucidation approach based on dynamic fluorescence quenching by photoinduced electron transfer (PET) is used to find a structural solution for the recently discovered third conformational state (C3) of T4 Lysozyme (T4L) [1]. Furthermore, the associated dynamics are resolved by fluorescence correlation spectroscopy (FCS).

It is well known that the enzyme T4L undergoes a dynamical exchange between two states, an open (C1) and a compact closed (C2) state, which arises in the course of substrate binding and conversion. By combining multiparameter fluorescence detection (MFD), EPR spectroscopy as well as other biochemical and biophysical methods, Sanabria, H., Rodnin, D., Hemmen, K. et al. show the existence of an even more compact conformational state, the C3 state, which is thought to play a major role in formation of the enzyme-product complex and the subsequent product release [1].

Twelve possible structures for the C3 state were generated considering the structural information from the previous work [1]. In order to find the most suitable structural model, a new approach for structure elucidation is applied. This approach based on PET between the fluorescence quencher tryptophan and the fluorophore Alexa488 coupled to T4L at specific positions. To find the best structural model, the dynamic quantum yields of selected PET-pairs were simulated using the quenching estimation software QuEST [2] and then compared to the corresponding experimentally obtained dynamic quantum yields.

Based on the single molecule MFD experiments [1], it was shown that the transitions between the C1, C2 and the C3 states obey a reversible sequential three-states kinetic, in which the exchange between C2 and C3 follows a slow dynamic in the range of hundreds of microseconds [1]. In the second part of this work, the dynamic nature of this slow transition is detected using PET-FCS.

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P023 LOYO CRUZ, VICTOR MANUEL

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Proton collecting antenna residues at the K-channel entrance of the redox-coupled proton pump cytochrome c oxidase

Cytochrome c oxidase (CcO) is part of the electron transport chain. As a redox-coupled proton pump, CcO establishes a proton motive force across the membrane. This mechanism employs two proton channels, the D- and the K-channel. The K-channel is only active in the reductive phase and is relatively dry compared to the D-channel. We recently found evidence on how the K-channel becomes activated [1], how changes in H-bond networks, water dynamics and helix 6 conformational dynamics at the channel entrance site are involved in channel opening. Proton uptake through the K-channel has been suggested to proceed via a glutamic acid in subunit II for R. spheroides (E101II) [2]. For the corresponding residue E78II in P. denitrificans contradicting results were found [3], and could may be attributed to a detergent binding site close to the K-channel.

We aim at investigating the contribution of potential proton collecting antennae residues (Asp, Glu, His) for proton uptake through the K-channel by measuring the catalytic activity, H-bond network and structural changes by site-directed mutagenesis and labeling with a sensor dye at specific sites [4]. H73II, H526, and E78II were mutated to alanine; individually and in a combination. Moreover, the effect of the detergent/ligand binding site was tested. Additionally, a fluorescent sensor for pH and conformational dynamics was attached to P301C in combination with the functional mutants. Our results show how a long-range H-bonding network along the surface affects proton uptake, enzyme activity, as well as ligand binding, and how conformational changes of loop structures attribute to the effects.

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P024 SOKOLOV, MONJA

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Simulation of Exciton Transfer in Light-Harvesting Complexes using Machine Learning Techniques

The first step of photosynthesis, the collection and transfer of sunlight, is called light-harvesting (LH). Biological LH complexes operate at extremely high quantum efficiency, meaning the probability of an absorbed photon to reach a reaction center and to drive charge separation is high. To date, artificial appliances lack this highly desirable efficiency. Therefore, biological LH complexes are interesting compounds for an inspiration of a new generation of appliances, e.g. photovoltaic devices.

The dynamics of the excited state, as arising from the coupled motion of electronic and nuclear degrees of freedom, can be simulated with non-adiabatic molecular dynamics (NAMD) methods. Such simulations are challenging due to the size of biological LH complexes. Therefore, we apply a data-driven approach to substitute the computationally costly quantum-chemical calculations with machine learned models.

Here we present simulations of exciton transfer in the Fenna-Matthews-Olson complex (FMO) and the light-harvesting complex II (LH2), which are investigated in terms of their underlying transfer mechanisms, pathways, (de-)localization and time scales. Neural network models are trained for the prediction of transfer Hamiltonian elements on reference data from semi-empirical time-dependent long-range corrected density functional tight binding (TD-LC-DFTB). The models consider the specific environment in terms of the electrostatic potential induced on the atoms of individual Bacteriochlorophyll a pigments for the prediction of excitation energies.

P025 WU LU, MERITXELL

Meritxell Wu Lu[1]; Dr. Jovan Dragelj; Prof. Dr. Maria Andrea Mroginski; Dr. Ingo Zebger; Konstantin Laun; Sagie Katz; Armel Franklin Tadjoung Waffo; Dr. Giorgio Caserta; Stefan Frielingsdorf

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Complex formation of FDH:MBH using Coarse Grained Molecular Dynamics Simulation

Most of the life on earth keeps its internal metabolic balances, which most of them are catalyzed by enzymes.

Enzymes are known as protein molecules which facilitate chemical modification conferred by the combination of functional such as specific cofactors or coenzymes for catalytic performances. Considering the physicochemical roles of enzymes, it is possible to design new artificial enzymes either by modifying the active site or linking enzymes with different functions. This work aims the rational design and optimization of a hybrid Formate Dehydrogenase (FDH) (from Rhodobacter capsulatus) and Hydrogenase complex. Structural insights into their architectures can be greatly supplemented by computational docking techniques, as they provide means for the refinement of experimental data that is often limited.

In our current study, we present the scoring of enzyme-enzyme complexes obtained from the docking service ClusPro. The thermal stability of these protein-complexes was investigated by microsecond- long molecular dynamics simulation at Coarse Grain (CG) resolution. For this purpose, new MARTINI - compatible force field parameters for the CG-description of the metal cofactors were derived and optimized.Thorough analysis of the complex's conformational dynamics contributed to the identification of the most favorable binding modes of the enzymes for efficient electron transfer and, in turn, catalytic coupling.

P026 ZAHN, CLARK

Clark Zahn; Dr. Yang Yang; Dr. Till Stensitzki; Dr. Ramona Schlesinger;
Prof. Dr. Karsten Heyne, FU Berlin

Ultrafast Protein response of Bacteriorhodopsin

Bacteriorhodopsin (bR) serves as a model system for studying the photo- biology of rhodopsins. However, in contrast to the retinal isomerization up to now only little is known about the ultrafast response of the protein. Recently, fs x-ray studies [1] have revealed changes in the hydrogen-bonded water network (HBWN) within the protein, even before the isomerization. Here, we present polarization resolved fs Vis-pump IR-probe data of bR in D2O and H2O, from 1350 cm-1 to 1800 cm-1, tracing both the isomerization and changes of the protonation state. Transient absorption spectra of bR in D2O are shown in Fig 1 a, including assignment of different signal contributions. Most notably, our data show a yet unreported continuum band (CB), appearing directly after excitation and subsequently decays on a sub picosecond timescale. A similar broad CB was observed for the bR M intermediate [2], assigned to changes of the HBWN protonation state at the proton release site (PRS). Therefore, we suppose that the electronic excitation induces ultrafast electric field changes, which lead to ultrafast changes within the HBWN at the PRS and formation of the CB. This is supported by the fs X-ray data [1], showing ultrafast changes in HBWN at the PRS. In addition, transient dynamics of the CB, see Fig 1 b, indicate coherent oscillations of the HBWN, induced by the ultrafast electric field changes affecting the polar groups.

P027 BERTHOLD, MALTE

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Heterotrimeric concatamers of ionotropic P2X4 and P2X7 receptors

P2X4 and P2X7 receptors are cation channels expressed in immune cells. They are activated by binding ATP released from damaged and inflamed tissue and are involved in inflammation and pain sensation. Whether P2X4 and P2X7 receptors assemble exclusively as homotrimers or also as P2X4-P2X7 heterotrimers is controversial.

To clarify this, we cloned concatamers encoding heterotrimers of P2X4 and/or P2X7 subunits in permutated arrangement. We expressed these concatamers in X. laevis oocytes and investigated their function by recording ATP-induced whole cell currents using two-electrode voltage clamp.

We found that heterotrimeric P2X4-P2X7 concatamers have ATP binding site affinities intermediate between those of non-concatenated P2X4 and P2X7 receptors, which have high and low ATP affinity, respectively. The pKd values for ATP depended on the order of the P2X4 and P2X7 subunits in the heterotrimer. The whole-cell currents of non-concatenated P2X4 receptors showed an inactivating phenotype, whereas non-concatenated P2X7 receptors showed slowly increasing currents upon longer ATP applications of several seconds. Heterotrimeric concatamers showed current kinetics in between the P2X4 and P2X7 phenotype, also depending on the P2X4-P2X7 subunit order. The whole cell kinetics remained unchanged during repeated ATP application indicating the existence of stable concatamers.

Furthermore we characterized the binding behavior of the theoretically three possible binding pockets via whole cell current measurement at different ATP concentrations. First, using specific blockers and single point mutations. Second, in general, by modeling the current-response curves via an adapted Hill function.

We conclude that P2X4 and P2X7 heterotrimers are functional, with their ATP concentration dependence and current kinetics depending on the specific order of the individual P2X4-P2X7 subunits.

P028 DE VECCHIS, DARIO

Dr. Dario De Vecchis PhD; Prof. Dr. Lars Schaefer, Ruhr University Bochum

The functional interplay of the ABC transporter PgP with its lipid substrates

The multidrug efflux pump P-glycoprotein (PgP) is an ATP-binding cassette transporter which hydrolyzes ATP to energize the translocation of lipids and hydrophobic compounds through the plasma membrane. The protein is associated with the development of multidrug resistance and is overexpressed in a variety of cancer cells. PgP is expressed in highly specialized membranes that are often rich in cholesterol and sphingolipids. However, the link between this apparently consistent environment and Pgp dynamics and function has been largely overlooked. We have investigated the structure and dynamics of human PgP employing all-atom and coarse-grained molecular dynamics (MD) simulations in asymmetric lipid mixtures that resemble the hepatocyte membrane where PgP is expressed. We explored and compared for the first time the dynamics of two human PgP inward-open structures, which were previously solved in detergent and nanodiscs and greatly differ in their proposed portal helices. The MD simulations visualize preferential interactions of PgP with sphingolipids at the portal. Furthermore, they reveal cholesterol and different lipid species wedging, snorkeling, and in some cases even entering within the main PgP substrate cavity. The volume and dynamics of this cavity largely differ between the two PgP structures, and are modulated by removal of cholesterol and presence or absence of ATP. Moreover, the MD simulations reveal that absence of cholesterol renders the nucleotide binding domains highly dynamic. Our study emphasizes the critical role of the membrane environment for the functional dynamics of PgP, where lipids and sterols are more than mere substrates and play a key role for an overall efficient efflux. Finally, we will briefly discuss similarities and differences concerning protein-lipid interactions in other ABC transporters, such as MsbA.

P029 FINK, MANUEL

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Expression and purification of connexin 43 for electrophysiological studies.

Ion channels perform essential functions in biological membranes. They transport metabolites and ions across the lipid membrane. One important class of channel proteins are connexins. They form cell-cell connections called gap-junctions. These channels connect adjacent cells and allow the communication and exchange of metabolites between them. Six connexin monomers form a hexameric channel called connexon that spans one of the cell membranes. Each gap junction channel consists of two connexons, one from each cell. Due to their importance it is of great interest to investigate the electrophysiological properties of these channels. Connexin 43 (Cx43) is the major gap junction channels in mammalia [1]. Additionally to gap-junction channels Cx43 also forms unopposed hemichannels [2]. The electrophysiological properties of these channels can be detected by voltage clamp experiments. By reconstituting the proteins into an artificial planar lipid bilayer, it is possible to investigate their behavior on the level of a single channel. Proteins can be reconstituted directly from detergent micelles or by first reconstituting the protein into lipid vesicles, then fusing these with the bilayer. The single channel properties can be used to gain deeper understanding of the function of Cx43. In the future the cooperativity of Cx43 hemichannels will be investigated, for which the properties of a single channel are an important baseline. Cx43 was heterologously expressed in Hi-5 insect cells using a recombinant baculovirus as expression vector. The proteins were solubilized from the membranes using detergent and purified by immobilized metal ion chromatography. After purification reconstitution screenings into artificial planar lipid bilayers were performed to test in which conditions the protein can be reconstituted into the model membrane.

P030 GOTTWALD, JACQUELINE

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Investigation of conformational changes at the K-channel entrance of cytochrome c oxidase using the fluorescent molecular rotor Sulfo-Cy3-maleimide and site-directed labeling

As part of the respiratory chain, the redox-coupled proton pump cytochrome c oxidase (CcO), catalyzes the reduction of molecular oxygen to water thereby establishing the transmembrane proton gradient that fuels ATP synthesis. The catalytic cycle is composed of two partial reactions, the reductive and the oxidative phase. A-type CcOs translocate a proton from the N-side to the catalytic center through two uptake pathways, named the D- and the K-channel. The K-channel delivers the first two protons in the reductive phase for the chemical reaction. In contrast to the D-channel the transient opening of the K-channel is a concerted event that involves restructuring of the extended H-bonding network upon electrostatic changes of the binuclear center, and conformational changes propagating to the K-channel entrance [1]. This also involves helix 6 (contains catalytic Y280), which becomes more flexible upon reduction and shows higher water diffusivity at the helix-loop border and around loop 6-7.

To investigate the conformational changes at the K-channel entrance of CcO in more detail, we used the fluorescent molecular rotor (FMR) Sulfo-Cyanin-3-maleimide (Cy3) and site-directed labeling. FMRs are fluorophores that have a fluorescence quantum yield that depends on intermolecular rotation/twisting motion. Thus, the fluorescence lifetime of the FMRs varies as a function of nanoviscosity/nanofriction (i.e. water and conformational changes). High viscosities or steric restriction inhibiting molecular rotation and thus increasing the dyes fluorescence lifetime. Here, we analyzed the Cy3 fluorescence lifetime changes after binding at the K-channel entrance i) in helix 6 (position 295), ii) at the helix6/loop border (position 299), and iii) in the loop 6-7 (position 301)). Site- directed Cy3 binding was achieved by the respective single cysteine mutants [2]. The Cy3 lifetimes were measured in the oxidized and reduced state of the enzyme. The results were compared to data for steric restriction obtained from time-resolved fluorescence depolarization experiments [1]. We interpret the results in the context of a pulsed oxidized form (OH) that fosters the enzymatic reaction.

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P031 HELLMANN, NADJA

Nadja Hellmann; Dr. Christian Kersten; Kristin Oepen; Prof. Dr. Dirk Schneider, Johannes-Gutenberg Universität Mainz

Complex interactions of the antidepressant clomipramine with an ABC transporter

Multidrug resistance (MDR) transporters belong to the family of ABC (ATP binding cassette) transporters that use the energy of ATP hydrolysis to transport substrates across membranes. These transporters are expressed in basically all types of cells, to protect these cells against exogenous and endogenous substances. A well-studied example is the P-glycoprotein (Pgp), which is responsible for the transport of substances across the blood-brain barrier. Tricyclic antidepressants (TCAs) often show poor efficiency, which is partly ascribed to transport out of the brain by Pgp (O'Brien et al, Br J Pharmacol. 2012). On the other hand, the antidepressant clomipramine might inhibit ATPase activity in general, as shown for the sarcoplasmic reticulum Calcium-ATPase (Soler et al, J Bioenerg Biomembr. 2000). In order to investigate the interaction of clomipramine with a MDR transporter in detail, we used the bacterial ABC transporter BmrA as a model. Clomipramine inhibits BmrA-mediated Hoechst transport, plus the ATPase activity of the full-length protein and the isolated nucleotide-binding domain (NBD) is inhibited. Based on isothermal titration calorimetry and fluorescence spectroscopy, two different binding sites appear to exist on BmrA's NBD. Molecular docking studies indicate that a large binding area might exist in the BmrA NBD monomer, which encompasses the ATP-binding site. Furthermore, also in the dimer the ATP binding site appears to be occupied by clomipramine, leading to a non-productive dimer. In agreement with this, the isolated BmrA NBD forms more dimers in solution when clomipramine is present. Thus, competitive binding of clomipramine to the ATP-binding site likely is responsible for the inhibition of the BmrA ATPase activity. Yet, binding of clomipramine to a region, where monomer-monomer contacts are established via the interhelix loop 2, were also identified in the computational analysis of the full-length proteins, and here binding might inhibit the BmrA ATPase activity in a non-competitive manner. This hypothesis is supported by steady-state enzyme kinetics, indicating a competitive and a non-competitive effect of clomipramine on the BmrA ATPase activity. Assuming that the closely related Pgp is affected in a similar manner, clomipramine could inhibit its own transport back into the blood.

P032 JARDIN, CHRISTOPHE

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pH-dependent gating of the human voltage-gated proton channel from molecular dynamics simulations

Gating of the voltage-gated proton channel Hv1 is strongly controlled by pH which evidently involves the sidechains of titratable amino acids. Despite experimental investigations to identify the amino acids involved in pH sensing only few progress has been made, including one histidine that is involved in sensing intracellular pH. We have used constant pH molecular dynamics simulations in symmetrical and asymmetrical pH conditions across the membrane to investigate the pH- and ΔpH-dependent gating of the human Hv1 channel. Therefore, the pKa of every titratable amino acids has been assessed in single simulations. Our simulations captured initial conformational changes between a deactivated and an activated state of the channel induced solely by changes of the pH. The pH-dependent gating is accompanied by an outward displacement of the three S4 voltage sensing arginines that moves the second arginine past the hydrophobic gasket (HG) which separates the inner and outer pores of the channel. Hv1 activation, when outer pH increases, involves amino acids at the extracellular entrance of the channel that extend the network of interactions from the external solution down to the HG. Whereas, amino acids at the cytoplasmic entrance of the channel are involved in activation, when inner pH decreases, and in a network of interactions that extend from the cytoplasm up to the HG.

P033 MALISETTY, APARNA SAI

Aparna Sai Malisetty[1]; Lukas Schaffrath[2]; Saskia Cönen PhD[3]; Dr.med. Jan-Philipp Machtens[3]; Prof. Dr. med Fritz Markwardt[4]; Dr. rer. nat. Susan Köppen[1]; Prof. Dr. med. Günther Schmalzing[2]

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Identification of residues involved in homotrimeric stabilization of the hP2X4R receptor channel by molecular dynamic simulations

P2X receptors (P2XRs) are a family of ATP-gated cation channels with seven members, P2X1-P2X7. P2XRs are involved in numerous physiological and pathophysiological functions and are thus considered as potential drug targets. Functional P2XRs are obligate trimers of three identical (or homologous) subunits, with each subunit having a large ectodomain linked by two membrane-spanning helices, TM1 and TM2. When extracellular adenosine triphosphate (ATP) binds to one or more of the three interfacial ATP binding sites of a P2XR, a conformational change occurs that is transmitted to the trihelical TM2 bundle in such a way that the narrowest part of the TM2 bundle widens and allows the influx of cations into the cell. The trimeric structure has been unraveled biochemically [1,2,3] and confirmed by X-ray crystallography [4]. To date, there is little information about the residues involved in stabilizing the trimeric structure.

We used computer simulations to identify the residues involved in the trimeric stabilization of human P2X4R (hP2X4R) and verified the predictions by biochemical experiments. The whole system with hP2X4 homology model in lipid bilayer was simulated using GROMACS software [5] to determine the presence of non-covalent intermolecular interactions such as salt bridges and hydrogen bonds.

In total, We identified five groups of multiple interactions and five single interactions that contribute to the overall stability of the homotrimeric hP2X4. We verified the computational results experimentally by generating hP2X4 alanine mutants that we expressed in Xenopus laevis oocytes and analyzed biochemically by native polyacrylamide gel electrophoresis (native PAGE) and functionally by two-electrode voltage-clamp (TEVC) electrophysiology. Our biochemical readouts were (i) the hP2X4R homotrimer assembly itself and (ii) the required concentration of the denaturing detergent lithium dodecylsulfate to achieve half-maximal disassembly of the homotrimer. In summary, we found that the majority of residues that stabilize the hP2X4 homotrimer, both computationally and biochemically, belong to group I residues.

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P034 PASCHKE, RONJA

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A vibrational spectroscopic Approach to elucidate the molecular Mechanism behind the Channel Activity of Viroporins

The prevalence of diseases caused by pathogenic viruses and the scarcity of treatment and vaccination options call for extensive research of the molecular mechanisms underlying the function of these viruses. Small hydrophobic viral proteins with ion channel activity, so-called viroporins, are involved in the virus particle entry into and release from the host cell. One of these viroporins, the Influenza A M2 channel, has shown to be the target for anti-viral drugs motivating spectroscopic investigations of other viroporins to understand their relevance as potential anti-viral treatment targets. We used the Influenza A M2 viroporin as a model system to establish a vibrational spectroscopic approach to examine the gating mechanism and proton conductance of this pH-activated proton channel. In this work, we immobilized reconstituted M2 on a self-assembled monolayer bound to a nanostructured gold film that can be used for surface-enhanced infrared absorption (SEIRA) spectroscopy. Employing pH-induced SEIRA difference spectroscopy we monitor protonation events and changes in the α-helix amide I absorption due to a pH-dependent channel opening that leads to proton conductivity. Furthermore, the surface selection rule of SEIRA enabled us to observe the reorientation of the α-helical structures associated with the channel opening in-situ. Combining the spectroscopic data with computational vibrational spectroscopic calculations we model the large-scale reorganization of M2's α-helices, and by this quantify the opening angle of the channel transitioning from closed to the activated state. We aim to utilize this approach to enable a combined structural and functional analysis of viroporins of current relevance.

P035 ADLER, JULIANE

Dr. Juliane Adler, Leipzig University

When are Amyloid β Fibrils Most Toxic? Combining two Fluorescence Assays Can Help Answering this Question.

Despite all research efforts in the last years, amyloid fibrils are still a scientific topic of very high interest. An open question of the fascinating world of amyloids concerns the nature the transient fibrillar pre-stages and their relevance in cellular toxicity. For amyloid β (Aβ), which is associated with Alzheimer´s disease, it is widely accepted that oligomers are most likely the structures with the highest toxicity. These aggregates are formed during the fibrillation process from unfolded monomers to the energetically very stable amyloid fibrils. Since oligomers are not stable and never present as a single structural assembly during fibrillation, their investigation is challenging. In this study, we want to determine the time point during fibrillation when Aβ aggregates are most toxic. One possibility how Aβ toxicity is exerted is lysis of the neuronal cell membranes. Therefore, we investigate carboxyfluorescein (CF) vesicle leakage in the presence of Aβ peptides and correlate it with thioflavin T (ThT) fluorescence simultaneously measured. This provides the opportunity to link membrane disruption directly with the kinetics of fibril formation. Our setup allows measuring both processes simultaneously in the same sample. We use a model membrane system consisting of POPC, POPG and cholesterol in equimolar mixture and produce vesicles filled with CF. We add Aβ to the system under fibrillation conditions and the dye ThT which reports the binding to characteristic cross-β structures of amyloid fibrils. While vesicle-entrapped CF is quenched, it shows fluorescence intensity when pores are formed due to interactions of Aβ oligomers with the membrane followed by dye leakage. Correlating dye leakage as a measure of membrane disruption with ThT fluorescence indicating fibril formation kinetics, we can determine the time point when most toxic species are formed. This, most probably, indicates the time point when the highest amount of oligomers is present. This knowledge could help further investigations of structure, dynamics and other characteristics of these key-stages in amyloid fibrillation.

P036 KÖPPEN, SUSAN

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Salt-induced precipitation of fibrinogen: new insights from experiments and simulations

Fibrinogen nanofibers are very attractive biomaterials to mimic the native blood clot architecture. Recently, we reported that fibrinogen nanofibers were successfully produced by salt-driven self-assembly during drying of concentrated aqueous solutions with monovalent cations [1], whereas divalent cations caused smooth fibrinogen films [2]. Yet, little is known about the underlying mechanism of fiber formation in the presence of varying salts [3].

Therefore, we studied the influence of different mono- and divalent salts on the precipitation of fibrinogen experimentally via FTIR and SEM to get insights into the morphology of the formed fibers as well as the conformations of fibrillar proteins whenever formed. While with divalent cationic species significant changes in the secondary structure of fibrinogen could be observed without the formation of fibers, the fiber formation in the presence of monovalent ions did not go along with any conformational changes. The chemical composition of fibrinogen precipitates was further characterized with XPS and EDX to investigate potential uptake of metal cations by fibrinogen. Upon rinsing, divalent salts were washed away from smooth fibrinogen films while monovalent sodium species were still detectable in fibrinogen fibers.

This motivated us to simulate the ion specific interaction of fibrinogen with classical molecular dynamics simulations. The monovalent ions were found closer to fibrinogen molecules than divalent cations. Furthermore, the hydration shell of monovalent ions was remarkably downsized upon direct adsorption with the protein, whereas adsorbed divalent ions kept their hydration shell complete resulting in indirect adsorption via the first ionic water layer.

In summary, we therefore conclude that self-assembly of fibrinogen into nanofibers can be induced by the presence of monovalent cations in direct contact with the protein.

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P037 PAULOWSKI, LAURA

Laura Paulowski, Research Center Borstel – Leibniz Lung Center

C25-modified rifamycin derivatives with improved activity against Mycobacterium abscessus

The prevalence of infections caused by nontuberculous mycobacteria (NTM) has increased during the last two decades [1]. In Germany, recent estimates conclude that the prevalence of NTM pulmonary disease (NTM-PD) is similar to the prevalence of TB at approximately 5 cases per 100,000 population [2]. Mycobacterium abscessus (Mab), a rapidly growing NTM, can cause severe pulmonary and extrapulmonary infections that are very difficult to treat due to the intrinsic resistance of Mab to most clinically available antimicrobials. The treatment success rate of Mab pulmonary infections is less than 50% under a highly toxic multidrug regimen comprising 4-5 drugs that are administered over 12-18 months. This is partially explained by formation of biofilms and the capacity of Mab to survive inside host phagocytes, two niches that are only insufficiently covered by current therapeutic regimens. In contrast, rifamycins show excellent activity against bacterial pathogens contained in both niches, in particular Mycobacterium tuberculosis. In Mab, all clinically available rifamycins are fully (rifampicin, RMP) or partially (rifabutin, RBT) inactivated by ArrMab, an ADP-ribosyltransferase which modifies the carbon atom at position C23 of the rifamycin scaffold. Here, we show that addition of a carbamate-linked group at the C25 position on the ansa chain of rifamycin SV blocks enzymatic inactivation by ArrMab. We report an in-depth characterization of the activity of 5j, a C25-benzyl piperidine rifamycin derivative with a morpholino-substituted C3 position. Thermal profiling of ArrMab in the presence of 5j, RMP or RBT shows that 5j does not bind to purified ArrMab, resulting in lower minimal inhibitory concentrations against clinical Mab isolates (MIC range between 0.125-2 µg/mL for 5j and >64 µg/mL for RMP, respectively). Moreover, we show that the ArrMab D82 residue is essential for catalytic activity. Lastly, we found that 5j also exerts potent antimicrobial activity against Mab in human macrophages and pellicles and that it shows synergistic activity with amikacin and azithromycin in vitro (log2F-IC50 −0.75 for amikacin and −0.83 for azithromycin, respectively).

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P038 PUSARA, SRDJAN

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Aggregation behaviour of beta-lactoglobulin and beta-lactoglobulin fragments

Beta lactoglobulin (BLG) is the major whey protein found in milk and as such it has a wide importance in the food industry. BLG attains its monomer-dimer equilibrium strongly dependent upon the pH and ionic strength of the solution, where BLG monomers are dominantly present at pH ≤ 3 and at pH > 8, otherwise, dimers and higher oligomers are present.[1] Here, electrostatic interactions play the key role for protein-protein interactions, as many amino acids of BLG have pH-dependent charge states.[2] In addition, it was shown that BLG can form amyloid or amyloid-like aggregates at high temperatures, low pH (< 3.5) and low ionic strengths. At pH 2 BLG is hydrolyzed into peptide fragments, which self-assemble into weakly associated nuclei, followed by the growth phase where further addition of building blocks results in the formation of linear amyloid-like aggregates.[3] BLG amyloids posses several promising applications as biosensors, nanocomposites or catalysts, therefore more studies are needed to better understand factors leading to the formation of various amyloid-like morphologies.

In this combined experimental and theoretical study, we have investigated aggregation behaviour of BLG and BLG peptide fragments which contain fibrilization-prone sequences. In order to investigate dimerization affinity, we have performed umbrella sampling MD simulations with an all-atom force field at pH 3 and pH 7 and NaCl (10 mM and 100 mM). In addition, we have simulated N-terminal peptides with increased fibrillization propensity (1-33 and 1-52aa) at pH 2 and 10 mM NaCl concentration. These MD simulations show that peptides spontaneously assemble into aggregates followed by an increase of beta sheet structures, directly supporting experimental findings.

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P039 BAUM, LISA

Lisa Baum, UKM Münster

Ceramide and Annexin A1 membrane binding; Insights from QCM-D

Annexin proteins bind Ca2+-dependently to cellular membranes containing negatively charged phospholipids, such as phosphatidylserine. Changes in membrane lipid composition are a hallmark of altered physiological cellular conditions, such as stress. Ceramide and Cholesterol are suggested to function in structural membrane reorganizations, including clustering of signalling molecules, membrane trafficking and fusion.

In a Quartz Crystal Microbalance with dissipation (QCM-D)-based approach, we investigate the calcium-dependent binding of AnxA1 to synthetic lipid bilayers, mimicking various cellular membranes, emphasising on the impact of ceramide and cholesterol on Annexin A1 binding to POPS.

P040 BECK, KATHARINA

Katharina Beck; Janina Nandy; Dr. Maria Hoernke, University of Freiburg

Antimicrobial Peptides Induce Membrane Permeabilisation and Lipid Clustering.

Constantly growing antibiotic resistance increases the importance of alternatives to classical antibiotics. We examine two trivalent cyclic hexapeptides with different sequences and antimicrobial activity which interact with lipid membranes in multiple ways. Both peptides induce electrostatic lipid clustering in PG/PE membranes [1]. We further the analysis of their mechanism of action by investigating the effects of the peptides on various binary model membranes containing anionic and zwitterionic phospholipids.

We use the self-quenching dye calcein to monitor the vesicle leakage over a wide range of concentrations and long incubation times. Isothermal titration calorimetry (ITC) measurements were performed to study the binding between the peptides and the respective model membranes.

Both peptides induce leakage, aggregation, and fusion of vesicles. In sterically shielded vesicles containing PEG-lipids, aggregation and fusion are impeded. This is accompanied by a reduced leakage and electrostatic lipid clustering. This indicates that more than one leakage mechanism is at play. In a more detailed mechanistic analysis, both, leakage activity and lipid cluster ability, differ in PG/PE compared to PG/PC mixed membranes. All results combined point towards a plethora of membrane-mediated effects contributing to the antimicrobial activity of the peptides.

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P041 DAJKA, MARINA

Marina Dajka; Dr. Benesh Joseph, Goethe University Frankfurt am Main

Electron spin resonance spectroscopic investigation of the lipopolysaccharide exporter

The outer membrane (OM) of Gram-negative bacteria is an asymmetric bilayer made of phospholipids (PL) and lipopolysaccharides (LPS). The LPS molecule consists of lipid A, inner- and outer core oligosaccharides and the O-antigen. The lipid A-core oligosaccharide and the O antigen, which are synthesized in the cytoplasm are joined at the inner membrane (IM) to form the LPS molecule. In Escherichia coli, seven essential proteins, LptABCDEFG form the LPS transport (Lpt) system to export LPS from the IM to the OM.[1] Using pulsed dipolar electron spin resonance spectroscopy (PELDOR or DEER),[2] we characterized the conformational heterogeneity of the LptB2FG complex, which is the ATP-binding cassette (ABC) exporter responsible for LPS transport from the outer leaflet of the IM to the periplasmic domain of LptC. Our results are in agreement with previous observations that the nucleotide binding domains (NBDs) close upon ATP binding.[3,4] This is coupled to the opening of the lateral gate formed by the transmembrane domain (TMD) helices TM1of LptF (TM1 F) and TM5 of LptG (TM5-G). At the second other lateral gate formed by TM5-F and TM1-G, the short loops attached to these helices exhibits a very dynamic behavior occupying a broad conformational space. Overall, these observations markedly deviate from the general response of many other ABC exporters in which the TMDs undergo a synchronized transition between inward-open and outward-open states. Such an asymmetric behavior of the lateral gates might have an important role for LPS transport towards LptC.

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P042 DOHMEN, LARA

Lara Dohmen[1]; Prof. Dr. Claudia Steinem[2]

[1] Georg-August-Universität Göttingen; [2] Georg-August-Universität Göttingen

POPS-doted solid-supported lipid bilayers as a membrane model for the GABAergic post synapse

The assembly of the GABAergic post-synapse is a key step in synaptogenesis and crucial for the regulation of neuronal communication. Its formation is dependent on a plethora of different proteins. The scaffolding protein gephyrin is one critical component for assembly, since it is essential for clustering of the inhibitory GABAA-receptor. Thereby, collybistin 2 (CB2) is necessary to recruit gephyrin to the postsynaptic membrane. Due to its self-associated conformation, CB2 requires activation by neuroligin 2 in order to change to an active state.[1] Owing to its complexity, the formation of the GABAergic inhibitory post synapse is extremely prone to disfunctions causing diseases, such as anxiety disorders[2], autism spectrum disorders[3] and epilepsy[4]. Thus, it is crucial to understand the mechanism underlying the process of synapse organization.

For a better comprehension, it is essential to investigate single protein interactions in detail without the influence of unknown components. Therefore, we use an in vitro system, which allows us to examine different interaction properties in a bottom-up approach in a highly controlled manner. For that purpose, solid-supported lipid bilayers doped with phosphoinositides (PtdInsPs) have been examined and rated for their performance. PtdInsPs function as receptor lipids for CB2 and are necessary for the attachment of the protein to the membrane.

Previous measurements have been performed with hybrid monolayers. However, it has been shown that the addition of POPS to the lipid composition can induce a homogeneous phosphoinositide distribution between the leaflets of solid supported bilayers.[5] Therefore, we aim at evaluating the potential of such POPS-doped solid-supported lipid bilayers as a suitable model membrane for the investigation of the interaction of CB2 with phosphoinositides in contrast to solid-supported hybrid monolayers. For that purpose, we use reflectometric interference spectroscopy to quantify the binding behavior of CB2 to both membrane types, providing information about phosphoinositide distribution between the leaflets.

P043 ENGBERG, OSKAR

Dr. Oskar Engberg[1]; Guzel Musabirova[2]; Debsankar Saha Roy[3]; Ankur Gupta[3]; Prof. Dr. Sudipta Maiti[3]; Prof. Dr. Daniel Huster[1]

[1] University of Leipzig, Institute for Medical Physics and Biophysics ; [2] Kazan (Volga Region) Federal University; [3] Tata Institute of Fundamental Research

Small molecules can modulate phase separation in complex membranes

Lipid membranes may phase separate when composed of more than one species, especially if they differ in acyl chain length or degrees of saturation as found in complex biological membranes. Small molecules like neurotransmitters, fluorescence probes, anaesthetics, and clinically-relevant drugs can bind membranes with high affinity. Because both endogenous and clinical small molecules affect both physiological and pathophysiological conditions their membrane binding is of high relevance. However, small molecule interaction with membranes has mostly been studied in membranes composed of a single lipid species and therefore their possible effect on lipid phase separation has often been neglected. We have studied a model membrane of the outer plasma membrane leaflet containing 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC)/ N-palmitoyl-D-erythro-sphingosylphosphorylcholine (PSM)/Cholesterol (molar ratio 4:4:2) using 2H NMR. This mixture is prone to phase separate. By deuterating either the palmitoyl chain of the unsaturated POPC or the saturated PSM, we observe the effect of the small molecules on the phase separation and on the chain order of both lipids. For instance, serotonin and serotonergic drugs can modulate the phase behaviour of these complex mixtures. We have interpreted this as serotonin and other molecules increasing the hydrophobic mismatch between the two co-existing phases, leading to domain coalescence to minimize the unfavourable line tension caused by the hydrophobic mismatch. This increased domain size can then clearly be observed as two-component spectra in 2H NMR. We further characterize if other small molecules modulate the domain size in the same model membranes. In summary, small molecules can modulate phase behaviour in complex bilayers, possibly explaining the side effects of drugs.

P044 FÁBIÁN, BALÁZS

Dr. Balázs Fábián[1]; Dr. Sebastian Thallmair[2]; Prof. Dr. Gerhard Hummer[1]

[1] Max Planck Institut für Biophysik; [2] Frankfurt Institute for Advanced Studies

Martini Cholesterol gives membranes the chills – and what you can do about it

Cell membranes contain hundreds of lipid species and exhibit lateral inhomogeneities and domains with specific biophysical properties. The separation of membranes into liquid-ordered (Lo) and liquid-disordered (Ld) phases can be studied using coarse-grained molecular dynamics simulations. These systems contain a ternary mixture of phospholipids with high and low melting temperatures and cholesterol to model the complexity of cell membranes. We recently uncovered the presence of artificial temperature gradients in simulations of phase separating ternary lipid mixtures using the popular Martini force field. We found the origin of the artifact to be treatment of cholesterol in the molecular simulations, whose geometry is maintained by constrained dynamics as implemented in the linear constraint solver algorithm LINCS. Incomplete numerical solutions of the constraint equations for typical integration time steps lead to the artificial cooling of cholesterol, which in turn affects the degree of phase separation and other membrane properties such as the distribution of cholesterol inside the membrane. We use rigid body mechanics to construct equimomental systems of particles to optimize the constrained "scaffold" of cholesterol. Our optimized cholesterol topology reproduces the properties of the original model under strict LINCS settings and/or small simulation time steps, that is, in the absence of temperature gradients. The optimized model does not develop temperature gradients even at less strict LINCS settings and larger time steps. The optimized model is computationally less expensive than the original model combined with strict LINCS settings, and the comparison is even more favorable when other constrained moieties are present that do not require such vigorous constraining.

P045 FÄRBER, NICOLAS

Nicolas Färber MSc; Sophie Mauritz; Prof. Christoph Westerhausen,
University of Augsburg

Lipid phase transitions in cell and synthetic membranes

Employing fluorescence spectroscopy and the membrane-embedded dye Laurdan we show that lipid membrane phase transitions can be characterized optically and that the resulting data are equivalent to calorimetric measurements. Furthermore, our custom-made measurement system enables us to determine melting transitions even far below 0°C in frozen water. Using this technique, we show that linear changes of cell membrane order in the physiological temperature regime are part of broad order-disorder-phase transitions which extend over a temperature range from -40°C to 90°C. Even though these extreme temperatures are usually not object of live science research due to failure of cellular functions, our findings help to understand and predict cell membrane properties such as permeability under physiological conditions as they explain the underlying physics. The phase transitions in membranes of various cell lines, red blood cell ghosts and lipid vesicles are sensitive to cholesterol content, pH and pharmaceutics such as tamoxifen.

P046 FELKER, ARTHUR

Arthur Felker, Department of Biology and Center for Cellular Nanoanalytics, University of Osnabrück

A versatile toolbox for constructing nanoscale signaling platforms in live cells

Nanopatterning approaches for enriching transmembrane receptors with high-density have been used for triggering cell signaling. Such spatially-controlled signaling platforms on bio-chips are powerful tools for exploring the molecular mechanisms of signal transduction. Here, we develop a versatile toolbox for high-contrast nanopatterning of proteins in vitro and in live cells. We devised capillary nanostamping of biofunctional proteins and polymers onto glass substrate with optimized surface chemistry for stable nanostamping. To the end, we obtained close-packed biofunctional nanodot arrays (bNDAs) with ~500nm in diameter and 0.8μm spacing. In combination with engineered covalent and non-covalent binding pairs, orthogonal immobilization and dimerization of proteins into bNDAs with a high contrast was achieved. We used this approach to enrich receptor tyrosine kinases TrkB1 and EGFR in the bNDAs for triggering signalling in live cells. Recruitment of signalling-specific effector Grb2 and the guanine nucleotide exchange factor SOS1 were successfully observed. These results allowed us to spatiotemporally trigger key events of TrkB and EGFR signalling for quantitative analyses. The obtained nanoscale signaling platform therefore paves the way for high-throughput quantification of protein-protein interactions in cell signaling pathways. By combining bNDA approach with metal-/graphene induce energy transfer (MIET/GIET), we aim to resolve the axial conformation and the structural dynamics of signaling complexes at the plasma membrane of live cells.

P047 FLEGEL, HENDRIK

Hendrik Flegel; Prof. Dr. Claudia Steinem, Georg-August-Universität Göttingen

Reconstituting ATP synthase and monitoring its activity in photoacid-containing vesicles

The ATP synthase is one of the most important protein complexes and responsible for synthesizing the energy currency ATP from ADP and phosphate.[1] This transmembrane protein is a molecular motor driven by an electrochemical gradient called proton motive force pmf.[2] Since the pmf is composed of the pH difference ΔpH and the electric potential $\Delta\phi$ across the respective membrane, ATP synthesis can be induced by a proton gradient.[2]

While the proton pathway within the protein during ATP synthesis is rather understood, the question of how the protons reach the protein entrance is still elusive. A new experimental approach enables the control of proton release in time and location. We use photoacid molecules exhibiting different pKa values in the ground and in the first excited state.[3] Hence, upon light excitation the photoacid becomes highly acidic and a proton release follows.[3] Placing these proton sources either on the membrane surface or in bulk solution should allow differentiation between the transfer of interfacial and bulk protons towards the ATP synthase.

For this aim, the reconstitution of FOF1 ATP synthase from thermophilic Bacillus in large unilamellar photoacid-containing vesicles with the right protein orientation is required. In this project, we employ the photoacid 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS). While water soluble HPTS is entrapped in the vesicle lumen, the amphiphilic derivative C12-HPTS can be incorporated within the vesicle membrane. It remains to be elucidated whether it is necessary to prepare vesicles with an asymmetric distribution of photoacid molecules in order to ensure a proton gradient across the membrane. To verify whether the developed reconstitution protocols and the presence of the photoacid itself have any influence on the protein activity, the highly sensitive luciferin-luciferase assay is used. The successful reconstitution of the key structures described above will be the first step towards examination of the influence of proton localization on protein activity.

P048 GIL HERRERO, CRISTINA

Cristina Gil Herrero; Dr. Sebastian Thallmair, Frankfurt Institute for Advanced Studies

Beta2-adrenergic receptor promotes transmembrane ligand flip-flopping

The β2-adrenergic receptor (B2AR) belongs to the family of G protein-coupled receptors, one of the major drug targets. G protein-coupled receptors are integral membrane proteins that convert external signals into intracellular responses. Two already known drugs employed in the treatment of several respiratory diseases are the β2-adrenoceptor agonists salmeterol and salbutamol. They show a high affinity to B2AR, inhibiting the contraction of the airway smooth muscles upon binding, however, their binding pathways have not yet been fully characterized.

Along this project we will shed light on the binding process by means of coarse-grained molecular dynamics simulations using the Martini 3.0 force field. This methodology enables us to study the binding pathway of both drugs in an unbiased way.

First, the new ligands and the target protein were parametrized according to Martini 3.0. The obtained models were in good agreement with all-atom simulations and experimental properties. In addition, the analysis of the ligands' behaviour within different membrane compositions provided fundamental details such as the high membrane affinity of salmeterol as evidence by its longer residence time in the membrane compared to salbutamol. This is in accordance with their pharmacological properties, being salmeterol a long-acting bronchodilator and salbutamol a short-acting one. The placement of the ligands in their known binding site showed residence times expected for their high affinity to B2AR. Afterwards, a system composed of B2AR embedded in a membrane including multiple ligands in the water phase was simulated. In contrast with previous simulations, a flip-flop movement of both ligands was observed. We will discuss the B2AR residues allowing the flip-flopping and the free energy of the process. Based on the observed binding events, we will analyse the ligand hot spots on the B2AR surface as well as their binding pathways and affinities.

P049 GLÜCK, DAVID

David Glück[1]; Dr. Carolyn Vargas[2]; Prof. Dr. Sandro Keller[2]

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Polymer nanodiscs for single-molecule protein spectroscopy

Single-molecule spectroscopy paired with Förster resonance energy transfer (FRET) is a unique tool for the observation of protein dynamics and folding states. FRET can be exploited as a molecular ruler, as it reports on inter-fluorophore distances and thus delivers time-resolved structural information about the conformation of proteins. Currently, the application of single-molecule FRET to membrane proteins in lipid nanoparticles is in its infancy. A recent and promising candidate for single-molecule membrane-protein analysis are polymer-bounded nanodiscs. Nanodiscs encapsulated by polymers self-assemble from lipid membranes and extract membrane proteins with patches of surrounding lipid without the use of traditional detergent.

In this study, we present the use of nanodisc-forming polymers for single-molecule spectroscopy of membrane proteins, using the model protein Outer membrane phospholipase A (OmpLA). A double-cysteine mutant of OmpLA was labelled with two fluorophores for FRET, reconstituted in lipid vesicles, and solubilized by a novel amino-variant of the polymer diisobutylene maleic acid (a-DIBMA). OmpLA nanodiscs were characterized by microfluidic diffusional sizing and immobilized on a glass slide via a biotin modification of the polymer for total internal reflection (TIRF) microscopy, obviating the need for protein- or lipid-bound affinity tags. Finally, we deliver a proof of concept for this polymer-mediated immobilization approach by surface plasmon resonance (SPR) surface binding studies of readily assembled nanodiscs.

P050 HORNBOGEN, JUSTIN

Justin Hornbogen[1]; Emma Resmann[1]; Ritu Rajender[1]; Prof. Stefan Kins[1]; Prof. Dr. Sandro Keller[2]; Prof. Dr. Rolf Diller[1]

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Azobenzene based lipids and light-induced switching of membrane properties

Controlling of physiological membrane properties is an auspicious approach towards the treatment of various illnesses, e.g. Alzheimer disease (AD). It is assumed that the generation of amyloid plaques (Aβ), as a result of γ-secretase cleaving the amyloid precursor protein (APP), is strongly affected by the membrane thickness. Winkler and co-workers (1) showed an increase of aggregation-prone, pathogenic Aβ42/43 in smaller membranes and concluded, that bilayer thickness is a crucial parameter affecting γ-secretase activity and cleavage specificity.

Azobenzene (AB) decorated lipids are a strategy to manipulate membrane properties through the photoinduced AB trans-cis-isomerization.

Here we investigate the optical switching behaviour of 18:0- azo-phosphatidylcholin (trans azo PC) embedded in unilamellar phosphatidylcholine (POPC) LUV's, diisobutylene/maleic acid lipid particles (POPC-(glyco-)DIBMALPs) and in solution. We performed femtosecond UV/Vis/NIR transient absorption spectroscopy measurements to investigate differences in the isomerization process of azo PC in constrained lipid environments and used size determining methods as dynamic light scattering (DLS) and microfluidic diffusional sizing (MDS) for the membrane mimetic systems.

Ongoing experiments on the isolation of beta-/gamma-secretase and the beta-amyloid precursor protein (APP) and their inclusion in nanodiscs together with azo PC will allow to examine the impact of trans-/cis-azo PC isomerization on gamma-secretase activity and beta-amyloid composition.

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P051 KUMARI AGARWALA, PREMA

Prema Kumari Agarwala, IIT Bombay

Lipid Membrane Modulation under Hypoxia: Towards Lipid-Based Therapy & Diagnosis in Pancreatic Cancer

Cancer cells display altered cellular lipid metabolism, including disruption in endogenous lipid synthesis, storage, and exogenous uptake for membrane biogenesis and functions. Consequently, altered lipid metabolism and lipid composition impact cellular function by affecting membrane structure and properties, such as fluidity, rigidity, membrane dynamics, and lateral organization. Hypoxia, a state of oxygen deprivation, is most prevalent in pancreatic cancer and is one of the significant factors implicit in tempering lipid metabolism. Hypoxia-induced alterations in lipid composition, membrane structure, and properties involved in metastasis and invasion remain unknown in pancreatic cancer. In this work, we combine membrane biophysics, cell biology, and lipidomics and show that global lipidome reprogramming occurs in pancreatic cancer under hypoxia. We discuss how this lipidome modulation affects cell membrane biophysical properties such as fluidity, order, hydration, stiffness and their impact on cellular physiological processes such as wound healing, proliferation, and migration. Our results suggest that altered cancer lipidome may provide cues for developing lipid-inspired innovative therapeutic and diagnostic strategies in pancreatic cancer.

P052 MAGUIRE, SHANE

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ATR-FTIR spectroscopy of calcium-dependent lipid-binding proteins

Arabidopsis ALG-2-INTERACTING PROTEIN X (ALIX) is a cytoplasmic protein and is responsible for endocytic degradation of ubiquitinated cargos to the late endosomes. However, recently a novel interactor of ALIX, calcium-dependent lipid-binding protein (CaLB) was identified. CaLB is a C2 domain-containing protein that binds in the presence of calcium to phosphatidylinositol 3-phosphate (PI3P) phospholipid, which may occur at the late endosomes and on autophagosomes. The study of CaLB is therefore of significant relevance as seems that it might be involved in autophagy.2 In this study, attenuated total reflection (ATR)-Fourier-transform infrared (FTIR) spectroscopy was used to study protein-membrane interactions.3 Herein, ATR-FTIR methods provide continuous monitoring of protein-membrane interactions of solid-supported lipid membrane (SSLB) and lipid vesicles of different lipid compositions and sizes. It was seen that there was an increase in binding of CaLB to a POPC/PI3P 99:1 bilayer with increasing calcium concentration over a 2 hours. Furthermore, a lipid-binding specificity of CaLB to POPC/PI3P bilayer in the presence of 20 μM calcium was observed with less binding specificity to a POPC/PI4P bilayer control. This suggests that CaLB binds to specific membranes and that the binding is calcium-dependent.

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P053 MAIA, RAIZA

Raiza Maia[1]; Antreas Vorkas[2]; Aoife Redlich[1]; Constantin Flommersfeld[1]; Ramona Schlesinger[2]; Joachim Heberle[1]

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Investigation of the influence of lipid bilayer lateral pressure on Bacteriorhodopsin functionality.

Biological membranes in nature are responsible for supporting essential biological functions such as transport, signaling and energy production. Membranes are multi-component environments composed of a vast and diverse selection of lipids and proteins, key to the functionality of biomolecular systems. Their functions are controlled by intra- and extracellular stimuli as like light, temperature, pressure and so on.

Conventionally, the optogenetics field makes use of proteins that gain their light sensitivity naturally from chromophores. However, an alternative approach is to control biological activity with the help of synthetic photoswitches, as proven by recent investigation that triggered the opening of a mechanosensitive channel through photoisomerization of azolipids.

Here, we propose combine the photosensitive azobenzene-derived lipid and the well-known Bacteriorhodopsin to investigate the influence of the lipid bilayer lateral pressure in the bR functionality.

For this, bR has been reconstituted in nanodiscs, which provide a native-like environment to the protein and physically limit the expansion of the membrane. The bR-azolipid system was characterized by FTIR, time-resolved UV/vis and time-resolved IR spectroscopy.

The experiments performed try to elucidate the influence the trans and cis states of the azobenzene photolipid on the bR vibrational modes and photocycle, allowing us to describe a concept of the influence of lateral pressure on the functionality and activation of membrane proteins.

P054 MANDAL, TITAS

TITAS MANDAL; Prof. Dr. Salvatore Chiantia PhD, Potsdam Universität

Unravelling the Molecular Mechanisms of Hepatitis C Virus Assembly

Lack of fundamental knowledge of the molecular mechanisms involved in Hepatitis C Virus (HCV) life cycle within the infected host cells hinders the development of specifically aimed therapeutic approaches. Several studies suggest that the interaction between HCV core protein (C) and lipid interfaces is crucial to produce HCV particles in infected cells, but this process was not characterized in detail yet and the lipids which might be involved in the assembly of the HCV remain unknown. To fill this knowledge gap, we are quantitatively characterizing the interaction between C and host cells lipid structures in a in vitro biophysical model system that closely mimics lipid droplet (LD) and endoplasmic reticulum (ER) lipid environments. Using the modern fluorescence-based microscopy methods (including super-resolution microscopy and fluorescence fluctuation analysis) methods, we monitor protein-lipid and protein-protein interactions driving C oligomerization, under well-defined and controlled conditions. We have so far confirmed the intracellular localisation of C and its partition between the lipid monolayer and bilayer in hepatic cells depending on the maturation state. This, in turn, has served as a model for our in vitro localisation studies. We have studied C binding dynamics on model LDs as a function of lipid headgroup, protein concentration and time. The investigation is being further supplemented and extended with studies performed directly in living cell models. We also propose to quantify the interaction of another HCV protein (i.e., NS5A) with C and investigate its effect on core-lipid binding. By combining the information deriving from model and cellular systems, we will be able to clarify the molecular mechanisms driving HCV capsid formation.

P055 MEYER, THOMAS

Thomas Meyer[1]; Hauke Winkelmann[1]; Isabelle Watrinet[1]; Dr. Julie Tucker[2]; Dr. Joni Vuorio[3]; Mykhailo Girych[3]; Prof. Ian Hitchcock[2]; Prof. Ilpo Vattulainen[3]; Prof. Jacob Piehler[1]

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Regulation of JAK activation by the membrane environment

Class I and II cytokine receptor signaling is activated via Janus family tyrosine kinases (JAK) that are non-covalently associated via membrane-proximal binding motifs of the intracellular receptor domains. Dysregulation of JAK activation is involved in multiple diseases including inflammatory and immune disorders as well as various types of cancer. We have recently developed an atomistic model of JAK2 activation that critically depends on interactions between the regulatory pseudokinase (PK) domains. Interestingly, this model suggested a conserved membrane interaction site within the FERM/SH2 (FS) domains of JAKs that is important for orienting JAK within the signaling complex. This project aims to (i) uncover the principles of a JAK - receptor association on basis of a membrane interface and its role in downstream signal activation, (ii) explore the regulation by the different membrane environment encountered during trafficking and (iii) unravel the role of the juxta-membrane amphipathic helix of the receptor. Using mutagenesis and live cell micropatterning, we have identified an anchoring of JAK2 into the membrane via a conserved hydrophobic residue that is supported by positively charged residues in the FERM-SH2 (FS) domain. Strikingly, mutations in the membrane binding site can not only reduce JAK2 recruitment but also lead to either compromised, or enhanced activity. Moreover, fluorescence microscopy revealed a key role of the JAK2 membrane binding site in an efficient recruitment to receptors located in the plasma membrane, while much weaker receptor binding was observed in the ER. This points to a confined JAK activity at the plasma membrane due to an interaction with negatively charged lipids. Interestingly, mutations in the juxta-membrane domains of the cognate receptors, which are located in very close proximity of the JAK-membrane interactions site, also resulted in loss of JAK binding and activity. These results suggest an intricate interplay of protein-protein and protein-membrane interactions regulating cytokine receptor signaling in the context of subcellular membrane environment.

P056 PENNUTTIS, SIMON

Simon Pennuttis; Dr. Christian Nehls; Prof. Thomas Gutsmann, Research Center Borstel – Leibniz Lung Center

Atomic force microscopic experiments to decipher the function of Candidalysin

The opportunistic fungal pathogen Candida albicans can cause severe systemic infections in individuals with a compromised immune system. The mortality rates of these infections go as high as 40%, which make this a serious problem for public health [1]. Candidalysin is a secreted peptide and was identified to play a crucial role in the pathogenicity of C. albicans. It causes a proinflammatory response in host cells has cytolytic potential, by intercalating into membranes and thereby causing the formation of membrane lesions [2,3]. Seemingly, the own membrane of the pathogenic C. albicans is not affected by Candidalysin in the same severe way a host cell membrane is. According to recent result of Moyes et al. (2016), the positively charged c terminal region of the peptide is especially involved in the formation of membrane lesions. This region is modified before secretion by the separation of a c-terminal arginine via the Kex1p protease [3].

The membrane activity of Candidalysin and the propeptide Ece1 III62 93KR was analyzed by using reconstituted membranes as a model system. Lipid specificity was investigated as a possible mechanism by which a non self discrimination of target membranes might occur.

The investigation of peptide intercalation into lipid monolayers was done via a film balance and showed no significant lipid specificity. However, the intercalation of Ece1 III62 93KR was more extensive than the intercalation of Candidalysin. Atomic force microscopic imaging of reconstituted membranes showed a difference in the aggregation of Ece1 III62 93KR and Candidalysin. Ece1 III62 93KR-aggregates were more interconnected or larger than the ones of Candidalysin.

The higher degree of aggregation and membrane intercalation of Ece1 III62 93KR indicates an attenuating effect of the Kex1p mediated processing. This mechanism possibly enables the resistance of C.albicans membranes against Candidalysin and needs to be further investigated to possibly use it as a therapeutic access to Candida infections.

A lipid specific behavior of Candidalysin in regard of peptide aggregation and intercalation into lipid membranes was not confirmed with the conducted experiments.

P057 PÖHNL, MATTHIAS

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Interplay of local spontaneous membrane curvature and cholesterol asymmetry

Biological membranes are mainly composed of various kinds of phospholipids and cholesterol. The cellular interface may undergo local and global reshaping processes, depending on the environment, the lipid composition, and induced by integral and associated proteins. In this respect, the (local) spontaneous curvature is a decisive property of membranes in fusion and fission, or in immune processes. It is affected by the membrane elastic properties and in particular by the asymmetric membrane composition.

Here, we devised a simple albeit powerful lipid bicelle model system that (i) displays similar structural, dynamical and mechanical properties as compared to infinite periodic lipid membrane systems, (ii) allows for the study of asymmetric lipid bilayer systems, and (iii) enables for an approximately unbiased analysis of the local spontaneous curvature induced by lipids or proteins in molecular dynamics simulations [1,2]. In addition, the system is characterized by largely unbiased thermal fluctuations as opposed to standard bilayer systems in simulations.

We show that thermal and protein-induced bending of symmetric phospholipid bilayers acts in concert with a redistribution of cholesterol displaying a strong preference for the compressed leaflet. Our results suggest that a lipid-dependent cholesterol dynamics may explain the experimentally reported increase of the bending modulus upon addition of cholesterol for DPPC lipid bilayers and decrease for DOPC bilayers [3]. Our results underpin the combined influence of membrane tension, thermodynamic driving forces, and of local molecular interactions.

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P058 POOJARI, CHETAN S

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Lipid specificity of Viral Fusion Proteins

Viral fusion proteins drive fusion of viral and host cell membranes in a series of complex structural transition events. Although the structure of several fusion proteins has been solved, the characterization of viral protein-membrane interactions at atomistic resolution is still missing. Membrane interactions of fusion proteins are conserved and occur via fusion peptides (FPs) in class I and fusion loops (FLs) in class II/III proteins. Previously, we had characterized the glycerophospholipid binding in class II fusion protein glycoprotein C (gC) of Rift Valley fever virus (RVFV) [2] and the studies revealed specific binding pocket for PC lipid. Here we aim to understand if specific lipid binding site also exists in class I and III viral fusion proteins and dependence of lipid headgroup type, tail length and degree of lipid tail unsaturation for protein binding. Molecular dynamics (MD) simulations is an excellent technique to understand how proteins associates with lipid membrane at atomistic resolution and here we make use of MD simulations to gain structural insights into lipid contact sites and membrane insertion of FP / FL residues.

P059 RANGOLE, MONIKA

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Inside out – The role of mycobacterial ESX secretion systems in phagosome escape

In this project, we apply different biophysical methods to study how Esx proteins from different bacterial species contribute to the escape mechanism of the respective bacterial species. One quarter of the world population is infected with Mycobacterium tuberculosis, a pathogen that propagates within phagosomes and prevents the phagosome maturation. Phagosome escape depends on the Esx-1 type VII secretion system (T7SS), which has two important immunodominant substrates, ESAT-6 (6 kDa early secretory antigenic target, Esx-A) and CFP-10 (10 kDa culture filtrate protein, Esx-B). However, the function of ESAT-6 and CFP-10 for pathogenicity and intracellular escape is largely unknown. The aim of this project is to study the inter- and intra-species variation of Esx-like T7SS proteins and the molecular interaction of ESAT-6/CFP-10 like T7SS substrates with biological membranes. First species-specific variations in genes belonging to Esx secretion systems have been identified by screening of a genomic database including more than 21,000 genomes of pathogenic and non-pathogenic mycobacteria. The impact of genetic variation may influence protein characteristics. The screening will identify a set of Esx protein candidates suitable for in-depth structural analysis and host-pathogen interaction studies. Based on the screening outcome, approximately 10 Esx protein pairs will be expressed in M. smegmatis and will be purified without detergent. These protein pairs will be identified using single molecule Förster resonance energy transfer (FRET). Further, biophysical techniques such as electrophysiological experiments (tethaPOD, orbit mini, Montal-Mueller setup), surface acoustic wave biosensor (SAW), synchrotron radiation oriented circular dichroism (SROCD) will be used to understand the molecular basis of the Esx-like T7SS interaction with reconstituted membranes as a model system which mimics the phagosome membrane.
In the first phase of the study, we characterized the membrane interaction of ESAT-6 and CFP-10 as monomers and of the ESAT-6/CFP-10 heterodimer of M. tuberculosis at pH4 and pH7. This membrane interaction takes place through binding, intercalation and permeabilization. In SAW measurements, ESAT-6 showed stronger binding at pH4 compared to pH7. In FRET experiments, we found a strong intercalation of ESAT-6 at pH4. In Orbit-mini and tethaPOD measurements, ESAT-6 exhibited stronger permeabilisation at pH4 compared to pH7. Based on OCD measurements, the heterodimer showed surface, inserted, and tilted states depending on the lipid composition. With our experiments we demonstrate that the pH value, protein concentration, transmembrane voltage and lipid compositions influence pore formation.

P060 RAO, SHANLIN

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Membrane interactions and curvature sensing of the autophagic LC3 lipidation machinery

Macroautophagy is an evolutionarily conserved degradative pathway for maintaining cellular homeostasis. Cytosolic cargoes are sequestered by an expanding double-membrane structure, termed the phagophore, that eventually seals and delivers its contents to the lysosome. The process is facilitated by the ubiquitin-like ATG8/LC3 proteins, which are covalently conjugated to phosphatidylethanolamine lipids of the phagophore membrane upon autophagy initiation.

Here we use structural modelling and molecular dynamics simulations to examine the localisation and interactions of the machinery leading to LC3 lipidation. All-atom simulations of the human ATG12–5-16L1 complex, recruited to the membrane by WIPI2 or by direct binding via ATG16L1, capture a configuration poised for promoting the conjugation reaction. Curvature sensitivity analysis on the membrane-inserting amphipathic helical elements in ATG16L1 and WIPI2 shows a preference for positively curved membrane, providing a molecular basis for the experimentally observed curvature-dependent membrane binding of the complex.

Molecular dynamics simulations complement membrane nanotube pulling experiments to reveal how membrane curvature and lipid interactions localise the autophagic machinery on the expanding phagophore.

P061 SARI, MERVE

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Mimicking the minimal neuronal fusion machinery

Fusion of neurotransmitter-containing synaptic vesicles with the planar presynaptic membrane is a fundamental process in signal transduction within neurons. The driving force for this process are soluble N-ethylmaleimide-sensitive-factor attachment protein receptors (SNAREs) enabling the merging of the lipid bilayers and the release of the neurotransmitter into the presynaptic cleft.

Here, we mimic the planar target membrane using pore-spanning membranes (PSMs) which contain target SNAREs. To produce PSMs, giant unilamellar vesicles (GUVs) are spread on porous substrates. On the one side, we use conventional methods such as electroformation to establish a fusion assay based on content release mimicking the neurotransmitter release into the synaptic cleft. Currently, work is done in a protein-free system on closed porous substrates which will be transferred to protein-containing PSMs followed by an investigation of further fusion intermediates and pathways.

On the other side, we use droplet-based microfluidics for high-throughput GUV production to reconstitute the neuronal fusion machinery into droplet-stabilized GUVs (dsGUVs) which are then released as freestanding GUVs and spread to form PSMs. Herein, we showed a charge-depending reconstitution of a transmembrane domain into microfluidics-derived GUVs. Furthermore, we successfully reconstituted the target-SNAREs into dsGUVs and characterized the lipid and protein mobility, so far. Next, it is aimed to release these GUVs with a higher reconstitution efficiency and characterize them as spread proteo-PSMs using confocal laser scanning microscopy. To investigate the influence of the reconstitution efficiency on fusion events a high time resolution method (spinning disk confocal microscopy) will be used.

P062 SCHILRREFF, PRISCILA

Dr. Priscila Schilrreff PhD; Jens Balke Balke; Prof. Dr. Ulrike Alexiev,
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Nanostructured lipid carriers for chronic inflammation in non-healing skin wounds based on promising natural bioactive compounds

Natural compounds with antioxidant, anti-inflammatory, and antibacterial activities could be powerful candidates for chronic wound treatment [1]. Carotenoid pigments (tetraterpenes) are not only known for their industrial application, but carotenes and xanthophylls from natural sources like plants or algal species have recently received more attention in health food application as well as in wound treatments and therapeutic strategies [1]. Carotenoid pigments include, e.g., b-carotenes, astaxanthin, or lutein. However, the use of fat-soluble carotenoids is hampered by the high instability of the active compound and the poor water solubility. To overcome this limit, lipid nanoparticles were developed. In particular the second generation of lipid nanoparticles, nano-structured lipid nanocarriers (NLCs) made from a blend of a solid and liquid lipid, increase drug loading capacity and influence drug distribution within the nanoparticle as well as the release profile [2]. Here, we synthesized NLCs for high-load of the natural compound depending on the liquid/solid lipid, and characterized their physico-chemical properties (size, polydispersity index (PDI), morphology, zeta-potential), encapsulation efficiency, and long-term stability. Antioxidant activity was determined spectroscopically. Under the optimum preparation conditions, the size of the NLCs is 198 ± 26 nm with a PDI of 0.39 ± 0.11. The corresponding zeta potential was -22.2 ± 1.1 mV with the highest encapsulation efficiency of 81.52 %. Moreover, single molecule total internal reflection fluorescence microscopy (smTIRFM) will allow us to gain insight into carotenoid distribution according to published methods in the group [2]. Retention rates of 39% and 73% were obtained after 15 days of storage at 4°C in the stability study and after 72 hs of in vitro dialysis at room temperature, respectively, illustrating how the specific lipid structure could retain the natural active and preserve antioxidant activity.

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P063 SCHWIEGER, CHRISTIAN

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An Azidolipid Monolayer – Transitions, Miscibility, and UV Reactivity studied by Infrared Reflection Absorption Spectroscopy

We developed an azide-modified lipid with the potential to be used in photocrosslinking studies in lipid bilayers [1,2] or monolayers[3] with interaction partners, such as peptides or proteins. The UV-activatable lipid is a phosphatidylcholine (PC) and bears a terminal azide moiety in one of its hydrophobic tails (AzidoPC). Here, we present systematic monolayer studies of pure AzidoPC and its mixtures with the model lipid DPPC. Besides a thorough thermodynamic analysis with the Langmuir film balance technique, we performed infrared reflection-absorption spectroscopy (IRRAS) to get detailed insights in the organization of those (mixed) monolayers. Additionally, we applied high-resolution mass spectrometry (HRMS) to see effects of UV-irradiation on the lipids' chemical structure and organization. Our results suggest that in expanded monolayers of pure AzidoPC the azido terminated chain folds back towards the air-water interface. Conversely, in condensed monolayers, the chains stretch and the azide moiety detaches from the interface. For future applications as UV-activatable dopant, we studied the miscibility of the azide-modified lipid with DPPC and found a sufficient miscibility over all investigated mixing ratios. Finally, we showed photo-dissociation of AzidoPC upon irradiation with UV light at 305 nm, leading to chemical crosslinking with adjacent monolayer lipids. This shows the potential of AzidoPC to be used as crosslinking agent within self-assembled lipid or lipid/protein layers.

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P064 STARKE, LEONHARD

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Are pure-protein bilayers similar to lipid bilayers?

While lipids are the main building blocks of natural occuring membranes, several other types of molecules are studied for manufacturing artificial biomimetic membranes. [1]. One particular outstanding example is a membrane made from the fungal protein HFBI, marking the first time a pure protein membrane has been made from a naturally occuring source [2]. HFBI and the other members of the hydrophobin protein family are characterized by a large exposed hydrophobic region, rendering them highly amphiphilic [3]. Therefore, they self-assemble into monolayers at hydrophobic-hydrophillic interfaces [4], which allows for the preparation of pure proteins membranes and vesicles [2]. Our experiments shows that hydrophobin bilayers exhibit exceptionally low permeability for water and ions, hence providing a biocompatible platform for a range of experiments or biotechnological applications. With the aim to provide a molecular explanation for these properties, we carried out atomistic and coarse grained molecular dynamics (MD) simulations of HFBI bilayers. Our simulations show that HFBI bilayers greatly differ from lipid bilayers. HFBI bilayers are far less fluid than lipid bilayers, and the presence of a hydrophobic core inside the HFBI bilayers is insufficient for explaining the low permeability. Instead, we hypothesize that well-defined stable protein contacts are required to rationalize the bilayer characteristics. Modeling such pure-protein bilayers remains a challenge for future research.

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P065 STRITT, PAUL

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Resolving lipid dynamics in the photocycle of bacteriorhodopsin by mid-IR quantum cascade laser spectroscopy

The function of membrane proteins is highly influenced by their membrane environment. Insights into the impact of different lipid membranes to the photocycle of the light-driven transmembrane proton pump bacteriorhodopsin (BR) were provided by time-resolved Fourier-transform infrared (FTIR) spectroscopy [1]. In the next step of the investigation of photoreceptor-membrane interactions, minor absorbance changes of the lipid membrane dynamics itself must be resolved.

DSPC proteoliposomes serve as a model environment and by reconstituting BR into liposomes with fully deuterated lipid chains (DSPC-d70), the corresponding bands are frequency-shifted into a spectrally silent window (A). Our home-built quantum cascade lasers (QCLs)-based spectrometer (B) enables time-resolved single wavelength measurements of protein [2] as well as lipid vibrational modes during the BR photocycle.

By similar time constants of the analyzed protonation dynamics of BR in purple membrane, DSPC and DSPC-d70, the suitability as a model environment was confirmed. In comparison to step-scan FTIR, the dynamics of these lipids are resolved by QCL measurements (C). Therefore, direct investigation of lipid modes is enabled. Time constants of the resolved lipid transients and the protein dynamics show correlations.

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P066 TEIWES, NIKOLAS

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From cell to substrate – A plasma membrane system for Cav1.3 cluster detection

The native plasma membrane is tremendously overwhelming. Lipid Rafts, Protein Clusters and other emergent membrane properties are due to cellular and cytosolic complexity poorly understood. To investigate the function and dynamics of membrane components and simultaneously adjust the surrounding environment, a new in vitro system is needed. To empower further membrane analysis Giant Plasma Membrane Vesicles (GPMVs) derived from cells are spread on solid supported & porous silicon dioxide (SiO2) substrate, creating solid-supported plasma membrane bilayers (SPMB) & pore-spanning plasma membranes (PSPM).

Amongst other investigations, GPMVs, SPMBs and PSPMs allow for the characterization of phase behavior, diffusion and viscosity of abstracted cellular membrane.

Subsequently, we present besides a proof of principle of these promising bottom-up systems, insights to fundamental properties.

P067 THALLMAIR, SEBASTIAN

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Two cooperative binding sites sensitize PI(4,5)P2 recognition by the tubby domain

Phosphoinositides (PIs) are important signaling lipids multitasking in diverse cellular signaling pathways. They operate by recruiting proteins to the membrane surface by means of PI recognition domains. One of the recognition domains for PI(4,5)P2 lipids, which is the major PI species in the plasma membrane, is the tubby domain. It is conserved in the tubby-like protein (TULP) family and plays an important role in targeting proteins into cilia.

We used coarse-grained (CG) molecular dynamics (MD) simulations with the re-parametrized Martini 3 force field to explore the PI(4,5)P2 affinity of the C-terminal tubby domain (tubbyCT). Our CG MD simulations revealed a novel second binding site consisting of a conserved cationic cluster at the protein-membrane interface. The simulations together with mutation experiments in living cells showed that the second binding site substantially contributes to the fine-tuned PI(4,5)P2 affinity of tubbyCT. Moreover, the two binding sites exhibit cooperativity as shown by the computationally evaluated binding free energies as well as whole-cell voltage-clamped experiments.

We will discuss the computational and experimental characterization of the novel binding site, its importance for the membrane targeting properties of tubbyCT, and for its ability to recognize distinct PI(4,5)P2 pools in the plasma membrane. The two-ligand binding mode may contribute to sharpen the membrane association-dissociation cycle of tubby-like proteins and thus play a crucial role for the delivery of ciliary cargo.

P068 ULBRICH, MAXIMILIAN H

Dr. Chenyang Lan[1]; Dr. Anja Stulz[1]; Dr. Nicolas P F Barthes[1]; Dr. Susan Lauw[1]; Dr. Pavel Salavei[1]; Prof. Manfred Jung[1]; Prof. Heiko Heerklotz[1]; Dr. Maximilian H Ulbrich[2]

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Designed membrane protein heterodimers and control of their affinity by binding domain and membrane linker properties

Many membrane proteins utilize dimerization to transmit signals across the cell membrane via regulation of the lateral binding affinity. The complexity of natural membrane proteins hampers the understanding of this regulation on a biophysical level. We designed simplified membrane proteins from well-defined soluble dimerization domains with tunable affinities, flexible linkers, and an inert membrane anchor. Live-cell single-molecule imaging demonstrates that their dimerization affinity indeed depends on the strength of their binding domains. We confirm that as predicted, the 2-dimensional affinity increases with the 3-dimensional binding affinity of the binding domains and decreases with linker lengths. Models of extended and coiled linkers delineate an expected range of 2-dimensional affinities, and our observations for proteins with medium binding strength agree well with the models. Our work helps in understanding the function of membrane proteins and has important implications for the design of synthetic receptors.

P069 WATRINET, ISABELLE

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[1] University of Osnabrueck; [2] University of Helsinki

The role of membrane composition in JAK binding

Janus kinase (JAK) family proteins are associated to Class I and II cytokine receptors (CR) to mediate ligand induced CR signaling via the JAK/STAT pathway. Malfunction in JAK activation can lead to severe diseases including abnormal immune responses and various types of cancer. By all-atom molecular dynamic simulations of JAK2 and the Thrombopoietin Receptor (TpoR), we found a membrane interacting region in the JAK2 FERM-SH2 domain consisting of a conserved lysine residue and a patch of positively charged residues. To pinpoint the contribution of this interaction between the kinase and lipid environment, we built up a minimal receptor-kinase-complex in vitro. Reconstitution of this complex into polymer supported lipid systems enabled us to investigate the JAK FERM-SH2 (FS) association to CRs. In addition, this approach opens an avenue to study the influence of lipid composition on the stability and conformation of the receptor-kinase-complex. To this end, we purified a truncated version of the TpoR. Together with the purified JAK FS, we reconstituted the truncated TpoR in liposomes to determine the binding properties of JAK FS to the receptor. We developed nanobody binding or chemical coupling to capture the liposomes on polymer-supported surfaces. Surface sensitive label-free and fluorescence detections allowed us to quantify the complex stability of different JAK FS to the TpoR. The lipid composition of the artificial membrane systems was varied with respect to charge and density to assess their influence on the binding stability. As a follow up, surface charge altering mutations will be introduced to the membrane-interacting region of the FS to pinpoint the roles of lipid composition in JAK binding.

P070 ZUMPFE, KAI

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Lipid membrane dynamics via joint analysis of NMR and MD

Investigation of the dynamics of lipid bilayer membranes is challenging due to the limited access to dynamics parameters that can be obtained by spectroscopic methods. We use relaxation rate measurements from NMR for a timescale-specific analysis of reorientational dynamics with detectors to observe different „windows" of correlation times. A comparison with the analysis of all atom MD simulations will allow us to determine the validity of the simulation for each timescale and bond of the system.

However, dynamics and the resulting parameters in NMR are sensitive to small changes of the system, such as membrane composition, pH, and temperature. Different components of total reorientation dynamics will respond differently to adjustment of these parameters, such that varying them will help deconvolute the distribution of correlation times of motion. Furthermore, detailed analysis of MD simulations can aid in separating sources motion. Therefore we investigate the change of the dynamic properties as a function of temperature and membrane composition both with NMR and MD in order to deconvolute the shape of the distribution of motion. Addition of small molecules such as serotonin, which are known to bind to the membrane and affect membrane properties, are investigated as well.

P071 BAUMGARTNER, KATHRIN

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Surface acoustic waves stimulate wound healing in vitro

Over the last years, surface acoustic waves (SAW) have become a powerful tool for biophysical applications like manipulation, sensoring and stimulation of living cells. We stimulate epithelial wound healing assays on LiNbO3 substrates with an interdigital transducer (IDT) that produces propagating Rayleigh waves at f = 160 MHz ($\lambda = 25\mu m$). By keeping the mean energy input constant to minimize heat input and maximize efficiency, we show that the wound healing stimulation efficiency increases with increasing pulse width of the SAW. Following the hypothesis that the cytoskeleton is responsible for faster cell migration in SAW-stimulated wound healing, we measure the orientation and thickness of actin fibers. Furthermore, we quantify the influence of mechanosensitive proteins and membrane proteases like SPPL3 on the stimulation process. By identifying the role of these factors, we hope to unravel the underlying biophysical and biochemical mechanisms of SAW-stimulated wound healing.

P072 CLARKE, RONALD

Prof. Dr. Ronald Clarke, University of Sydney

Sustainability from a Cell's Perspective

Sustainability is a worldwide problem, for cells as it is for human beings and our whole planet. Key to solving the sustainability problem is recycling. In cells, one important recycling process occurs within the lysosome. All biological macromolecules, such as proteins and nucleic acids, which are past their use-by date, are transported into the lysosome, where enzymes degrade them into their monomeric building blocks. These enzymes are only active at the low pH of ~4.7 of the lysosome lumen. After degradation, these building blocks are transported out of the lysosome into the cytoplasm, where they are used for the biosynthesis of new proteins and nucleic acids. Because of the need to encapsulate the degrading enzymes within the lysosome to avoid damage to functionally active proteins and nucleic acids, the lysosome membrane is a powerhouse of solute transport within the cell.

This recycling process is crucial for the survival of cells, particularly neurons, which are no longer capable of cell division. Defects in this process cause many hereditary neurodegenerative diseases. But because of their rarity, pharmaceutical companies are not particularly interested in finding cures. However, the diagnosis of neurodegenerative disease is devastating for the family of any afflicted child. One group of neurodegenerative diseases is termed Batten's disease. While symptoms vary, all are caused by lysosomal dysfunction, resulting in the steady build-up of waste material in the cell. Disease progression is typically characterised by the development of blindness, followed by the onset of seizures and early death, typically in the 20s. Understanding the disease would not only help its sufferers but also provide valuable information on a crucial cell function, i.e., how cells recycle their waste.

A further form of cellular recycling is "apoptosis", or programmed cell death, which allows old cells to be carefully disposed of without the release of toxic cell contents to the surrounding tissue. Both lysosome function and apoptosis rely on the careful control of membrane composition, which directly affects the activity of integral membrane proteins, such as ion pumps. In this seminar I will focus on how biophysical investigations are helping to unravel the lipid-protein interactions crucial to controlling the cell's recycling mechanisms.

P073 EICHINGER, LENA

Lena Eichinger; Mayukh Kansari; Dr. Tomáš Kubař; Prof. Dr. Marcus Elstner

Exploring the Mechanism of Autophosphorylation in the Bacterial Sensory System using QM/MM Studies

Two Component Systems (TCS) are one of the basic signalling transduction pathways in bacteria. They consist of a histidine kinase (HK) that senses changes in the environment and a response regulator that initiates the corresponding cellular response. TCS are not found in the human genome, which makes them a promising target for the development of selective antibacterial drugs.

The membrane-bound HK is a homodimer with each monomer containing a dimerisation histidine phosphotransfer (DHp) domain and an ATP binding domain (catalytic domain, CA). When an extracellular signal is detected, the HK performs an autophosphorylation of a conserved histidine residue in the DHp domain. Depending on the TCS, one of two different autophosphorylation mechanisms occurs: In cis-HKs, the ATP bound to one CA domain phosphorylates its own DHp domain, whereas in trans-HKs, the histidine of the DHp domain of the other monomer is phosphorylated. The autophosphorylation thereby leads to a protonated phosphohistidine intermediate, which is subsequently deprotonated by a nearby base.

As the typical time-scale of biochemical reactions is in the range of several hundred nanoseconds to microseconds, previous computational studies of HK were unable to capture the whole mechanism. While employing semi-empirical DFTB gives similar results to DFT, the computational cost is 2 to 3 orders of magnitude lower. This allows us to investigate this reaction mechanism in a trans-HK in a QM/MM Hybrid Enhanced Sampling (Metadynamics) simulation with different bases, showing the detailed mechanism and its free energy landscape. We simulate and compare different HK systems with different proton acceptors and finally compare the outcomes of the simulation with experimental observations.

P074 FELLMETH, THOMAS PETER

Thomas Peter Fellmeth; Tobias Beigl; PD Dr. Frank Essmann; Dr. Kristyna Pluhackova

Live or let die: Bcl-2 protein transmembrane domain interactions in apoptosis signaling

The intimate interaction network of the Bcl-2 protein family is a key factor for cell survival or death(1). The interactions of pro- and anti-apoptotic members of the family govern mitochondrial outer membrane permeabilization (MOMP), which results in cytochrome C release and ultimately apoptotic cell death(2). The C-terminal □-helices, named transmembrane domains (TMDs), anchor most Bcl-2 proteins to membranes and thus affect their subcellular localisation(3). Moreover, the TMD of BAX has been shown to be involved in MOMP execution by promoting BAX aggregation within the membrane and pore growth(4). Over the recent years, increasing evidence appeared that the TMDs influence interactions among Bcl-2 proteins and therefore apoptosis signalling(5). The Bcl-2 protein BOK –alongside with BAX and BAK- is recognised as a pro-apoptotic effector molecule, which is able to induce MOMP and apoptosis. Although pioneering studies suggest that the homo- and heterodimerisations of TMDs of certain pro- and anti-apoptotic Bcl-2 proteins fine tune apoptotic signalling(6), the molecular mechanisms of BOK function and possible regulation by anti-apoptotic proteins like BCL-2 remain largely in the dark. Here, we unravel the interactions of BOK and BCL-2 TMDs in atomistic detail by high-throughput multiscaling molecular dynamics (MD) simulations and thus shed light on the molecular mechanisms of their function and possible regulation of apoptosis by TMD interaction of pro- and anti-apoptotic Bcl-2 proteins. Moreover, the newly discovered interaction interfaces will form a solid basis for future mutagenesis studies.

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P075 KAPPELHOFF, SHIRIN

Shirin Kappelhoff; Dr. Michael Holtmannspötter; Dr. Rainer Kurre;
Prof. Dr. Jacob Piehler; Jun.-Prof. Dr. Katia Cosentino, Universität Osnabrück

Super-resolution microscopy of GSDMD pores in polymer-supported plasma membranes

The pore-forming protein Gasdermin D (GSDMD) is a main executor of pyroptosis, a highly inflammatory form of regulated cell death characterized by plasma membrane (PM) permeabilization. Upon stimulation, inflammatory caspases cleave cytosolic GSDMD into its active domain, which translocates to and forms pores at the PM allowing the release of inflammatory cytokines and promoting cell lysis. So far, the strong GSDMD cytosolic background and the morphological changes of the PM during pyroptosis prevented resolving the structure of GSDMD pores in their native PM environment. To overcome this issue, we applied a newly developed approach called polymer-supported plasma membranes (PSPMs) on pyroptotic cells. PSPMs are plasma membrane sheets tethered to a polymer-coated surface, which are generated from living cells. This strategy preserves membrane topography and integrity while removing any cytosolic fluorescence contribution, and, notably, allows efficient labelling of intracellular endogenous membrane components. The application of this technique to pyroptotic cells in combination with DNA-PAINT super-resolution microscopy has turned out to be a powerful tool to visualize and resolve heterogeneous GSDMD nanostructures directly in the PM of pyroptotic cells with a nanometer resolution below 20 nm. The extension of this approach to quantitative PAINT additionally allowed us to stoichiometrically characterize GSDMD structures.

P076 KULKARNI, SHRUTI G.

Shruti G. Kulkarni[1]; Prof. Dr. Malgorzata Lekka[2]; Prof. Dr. Manfred Radmacher[1]

[1] University of Bremen; [2] Institute of Nuclear Physics PAN

Mechanical and adhesive properties of Pancreatic ductal adenocarcinoma cells

Pancreatic ductal adenocarcinoma (PDAC) is one of the most common causes of cancer-related mortality, with an overall 5-year survival rate of only 5% among patients. Due to the lack of early symptoms, the disease is usually detected in its late stages, frequently after it has metastasised. Cancer cell deformability and adhesion play a major role in tumour metastasis. In this work, we use Atomic Force Microscopy (AFM) to characterise the mechanical and adhesive properties of PDAC cell lines from the primary tumour sites and from liver and lymph node metastases.

Cells were probed with a pyramidal AFM cantilever, and their response was recorded in the form of AFM force curves. The apparent Young's modulus (E), which is a measure of the stiffness of cells, can be calculated from the approach part of the force curves by applying the Hertz contact mechanics model. Cells were also attached to tipless cantilevers, and then pressed against a layer of cells to create attachment. When the cantilever (with its attached cell) is retracted, its contact with the cell layer is disrupted, and this can also be recorded by and characterised from the retract part of the force curve.

Cell lines from primary tumour site (PANC1 and PL45) are stiffer in a cluster as compared to single cells, albeit to different extents. PANC1 shows more stiffening from single cells (1130 Pa) to cell clusters (2300 Pa) as compared to PL45 (single:1300 Pa, cluster:1750 Pa). Interestingly, preliminary reports show that PANC1 cells have more adhesion between themselves as compared to PL45 cells. Liver metastasis (CFPAC1) cells soften in clusters (single:2310 Pa, cluster:1470 Pa), but also show a large adhesion between themselves. Lymph node metastasis (HS766t) cells show slight stiffening from single cells (2000 Pa) to clusters (2700 Pa).

P077 MINOPOLI, ANTONIO

Dr. Antonio Minopoli PhD; Elena Erben; Dr. Moritz Kreysing PhD
Max Planck Institute of Molecular Cell Biology and Genetics

Optically controlled micro-transport at microscale with reduced heating impact

Recently it was demonstrated that thermoviscous flows can be used to move the cytoplasm of cells and developing embryos.1,2 These flows, the so-called focused-light-induced cytoplasmic streaming (FLUCS), reach velocities comparable with flow velocities occurring during early stages of embryogenesis. The flows are induced by mid-infrared laser scanning of a temperature spot through the cytoplasm. As a side effect, the laser scanning causes temperature gradients across the sample that are on the order of 1-2 kelvins when time-averaged. While this is sufficient to avoid side effects in heterothermic animals, some mammalian cells might require even more stable temperature conditions.

Here, we present that exploiting symmetry relations during laser scanning, we can still generate significant localized net flow fields, while greatly reducing time-averaged temperature gradients (Fig. 1). Importantly, given any arbitrary flow field, additional scanning patterns have to be considered (e.g., specular- and inverted-like) in order to counteract every broken thermoviscous symmetry, which would entail undesired preferential directions or drift in the sample.

The resulting temperature distributions are near homogenous across the region of interest and can therefore be much better compensated for by ambient cooling, ensuring compatibility with heterothermic animals. As an outlook, isothermal FLUCS (Iso-FLUCS) may be used to move the cytoplasm of mammalian cells and developing embryos with highly reduced heating impact.

P078 NEIDINGER, SIMON

Simon Neidinger; Anna Jötten; Prof. Christoph Westerhausen, University of Augsburg

The effective dynamic elastic modulus of cancer cells as function of the membrane order

The precise characterization of the mechanical properties of cells, e.g., by employing atomic force spectroscopy (AFS) is particularly important regarding numerous applications. AFS delivers information about the topography, viscoelasticity of cells and adhesive forces. Here, we systematically compare differences in the results depending on the measurement concept. For A375 melanoma cells we find local differences in the Young's modulus E covering about one order of magnitude and a logarithmic dependence of E on the loading rate as known for binding forces between single proteins. Moreover, we compare different tip geometries and fit models resulting in considerable differences, stressing the limitations of comparability of the data between publications. Comparing AFS data using tipless cantilevers with the results of deformation analysis of cells in microchannels we find good agreement of the obtained effective Young´s moduli. Finally, we employ fluorescence spectroscopy to correlate membrane order and mechanical properties as function of the temperature.

P079 PÉREZ DOMÍNGUEZ, SANDRA

Sandra Pérez Domínguez; Prof. Dr. Manfred Radmacher, University of Bremen

Comparison of healthy versus Dupuytren fibroblasts behavior in 3D-collagen I matrices

Cells are viscoelastic materials combining both elastic and viscous properties and the ratio between them varies depending on cell type and malignancy. Recently, tissue mechanics is gaining importance due to the preservation of cell environment both biochemically and physically in comparison to cell mechanics. Nevertheless, it is not always easy to have access to tissue samples, especially from human. To solve this issue, artificial gels made of the most relevant extracellular matrix (ECM) proteins could be an interesting and well-defined alternative to mitigate the above-mentioned issue.

Dupuytren disease is a fibromatosis of the connective tissue of the palm, which in the worst cases results in one or more fingers bend and remaining immobile. To solve or release contraction in the palm, acupuncture or collagenase injections can be used, but in most cases the contracture often recurs. In advanced stages of the disease, often removal of the affected tissue is done by surgery. We obtained 3 different cell lines from the palm of the same patient with Dupuytren disease (healthy, scar and diseased fibroblasts). To assess the mechanical properties of our samples, Atomic Force Microscope (AFM) was used. We employed large radius tips (5.5 μm) to encompass larger sample area, which helps us to better average heterogeneous samples, like 3D-gels. In an attempt to mimic a natural environment for cells, we have made 3D collagen I matrices from rat-tail, into which the different fibroblasts were inserted. Mechanical properties of these artificial hydrogels were measured at different incubation times (2 days, 1 week and 2 weeks after cells embedding) and a consistent increase of stiffness over time has been seen. Cell staining inside the gels showed that healthy and scar fibroblasts need more time to spread inside the gel, presenting rounded shape, however; diseased fibroblasts displayed an extended shape just after 2 days of incubation. Moreover, diseased cells appear to grow and duplicate faster than the rest, especially, at longer incubation times. These experiments show a promising approach to study tissue mechanics using artificial hydrogels and it will help to better understand diseases and has future prospects for finding a cure.

P080 WILSON, RUSSELL

Dr. Russell Wilson PhD[1]; Dr. Melis Goktas[2]; Zeynep Atris[2]; Prof. Dr. Kerstin Blank[1]

[1] Johannes Kepler University Linz; [2] Max-Planck Institute of Colloids and Interfaces

2D Mechanoresponsive Surfaces for Measuring Cellular Traction Forces

Cells sense mechanical forces in their local microenvironment through specific attachments to the extracellular matrix (ECM). Despite the essentiality of mechanical forces for basic cellular function, very little is known about the forces that act at the cell-ECM interface and how these forces feed into a variety of intracellular mechanosensing cascades. To improve our understanding of biomechanical processes at the cell-ECM interface, we introduce a library of coiled coil-based molecular force sensors. Using atomic force microscope-based (AFM) single-molecule force spectroscopy (SMFS), we have calibrated the rupture force-loading rate dependence of a series of heterodimeric coiled coils with different loading geometries and coiled coil lengths. The mechanically calibrated coiled coils with rupture forces between 20-50 pN have been utilized for the fabrication of mechanoresponsive surfaces that allow for determining the forces that are transmitted across cell integrin-ligand attachments. As a proof of concept, fibroblast cells were allowed to adhere to surfaces containing RGDS-functionalized coiled coil sensors or covalently coupled RGDS (control). We show that all tested force sensors allow initial attachment within 30 min after cell seeding. At time points between 60-120 min, distinct cell spreading behavior is observed for coiled coil sensors with different thermodynamic and mechanical stabilities. These results aid the future design of smart mechanoresponsive 3D materials for investigating cellular mechanosensing processes at the single-molecule level.

P081 ANSELMI, MASSIMILIANO

Dr. Massimiliano Anselmi PhD[1]; Prof. Teresa Carlomagno[2]; Prof. Jochen S. Hub[1]

[1] Saarland University; [2] University of Birmingham

Experiment-guided molecular simulations reveal the heterogeneous ensemble of the SH2 tandem of SHP2 phosphatase

SHP2 plays an important role in upregulating cellular processes, so much that its mutations cause developmental disorders and are found in many cancer types. SHP2 is a multidomain protein, comprising two tandemly arranged SH2 domains and a catalytic PTP domain. SHP2 is activated upon binding of two linked phosphopeptides to its SH2 domains. Experiments showed that peptide orientation and spacing between binding sites are critical for the enzymatic activation. For decades, the tandem SH2 has been extensively studied to disclose the relative orientation of the two SH2 domains that effectively drives to activation. So far, crystallography has provided only contradictory results, while measures in solutions resulted of difficult interpretation. By means of experiment-guided molecular simulations, we have finally revealed the heterogeneous ensemble of the tandem SH2 in solution, in agreement with the available experimental data from small-angle X-ray scattering and NMR residual dipolar couplings.

P082 ARGHITTU, SERENA MARIA

Serena Maria Arghittu[1]; Dr. Roberto Covino[2]; Prof. Dr. Mike Heilemann[3]

[1] Frankfurt Institute for Advanced Studies, International Max Planck Research School for Cellular Biophysics; [2] Frankfurt Institute for Advanced Studies; [3] Institute of Physical and Theoretical Chemistry, Johann Wolfgang Goethe-University

Mechanistic Insight into the Early Events of the Activation of the c-Met Receptor during Listeria Invasion

The human receptor tyrosine kinase Met is a transmembrane glycoprotein placed on the plasma membrane. The Met receptor is crucial in regulating cell migration, replication, and growth. Its dysregulation leads to a spectrum of diseases that feature cancer and bacterial invasion. In particular, the intracellular bacterium Listeria monocytogenes infects cells via a Met-mediated internalization through the invasion protein Internalin-B (InlB). Here, we focused on investigating the activation mechanism of Met's ectodomain triggered upon binding with InlB. We used atomistic molecular dynamics simulations to study the conformational plasticity of the receptor in different scenarios: isolated or in complex with InlB and with different glycosylation patterns. Our simulations reveal the signalling-competent and the inactive conformations of the receptor. In addition, we observed alternating bridging interactions among the glycans that stabilize the inactive conformation of the receptor.

In the future, we will characterize how the complex membrane environment modules MET's activation.

P083 BÖSER, JULIAN

Julian Böser, Karlsruher Insitut für Technologie (KIT)

QM/MM Molecular Dynamics Simulation of Thiol-Disulfide Exchange by Glutaredoxin

In this theoretical study, we performed classical and quantum mechanics/molecular mechanics (QM/MM) molecular dynamics simulations of the complete reaction cycles of a mono- and dithiol Glutaredoxin (GRX) with a protein and the glutathione/glutathione disulfide (GSH/GSSG) redox couple as substrates.

GRXs are a class of thiol-disulfide oxidoreductases that catalyze thiol-disulfide exchange reactions in many cellular regulatory pathways [1,2]. They can be divided into dithiol GRX, where the active site motif is Cys-xx-Cys, and monothiol GRXs, where the C-terminal cysteine is usually replaced by a serine. Both classes operate through different molecular mechanisms (Fig. 1) which additionally depend on the concentration of the glutathione/glutathione disulfide (GSH/GSSG) redox couple.

In a recent experimental study by Ukuwela et al [3], the different catalytic activities for monothiol and dithiol GRX1 were reported. Using a dithiol/disulfide protein as subtrate, monothiol GRX1 is twice as active as dithiol GRX1 as an oxidase and slightly more active as a reductase, due to different reaction pathways. However, the mechanistic details of the different reaction steps are still not fully understood and remain yet to be demonstrated.

With an ensemble of classical molecular dynamics simulations, we analyzed the regioselectivity of each catalytic step. Subsequently, the free energy profiles of each reaction step were obtained with QM/MM metadynamics [4].

With this information, we were able to identify the catalytic mechanism of mono- and dithiol GRX1 and, moreover, qualitatively reproduced their catalytic activities as oxidoreductases.

P084 DIEDRICH, LUCAS

Lucas Diedrich[1]; Matthias Brosz[1]; Salome Steinke[1]; Tobias Abele[2];
Prof. Dr. Frauke Gräter[1]; Dr. Kerstin Göpfrich[2]; Dr. Camilo Aponte-Santamaría[1]

[1] Heidelberg Institute for Theoretical Studies; [2] Max Planck Institute for Medical Research

Energetics and permeation of photo-resists used for 3D-laser printing across biological lipid bilayers

Photo-induced polymerization of so called photo-resists via 3-dimensional two photon laser printing has recently been used to create complex artificial structures inside of synthetic and living cells (Abele et at. Adv. Matterias. 34: 2106709, 2022). However, little is known about the energetic cost and rates of membrane permeation of this type of molecules across biological membranes. Here, we elucidate the free-energy and major determinants of permeation across biological lipid bilayers of a large set of photo-resist molecules in silico by applying equilibrium and umbrella-sampling molecular dynamics simulations. Our results provide distinct energy landscapes of membrane permeation for the studied molecules in terms of the barriers they must overcome along with preferential localization sites inside the bilayer. Oil/water partition coefficients derived from our calculations correlate well with the corresponding experimental values. Permeation coefficients inferred from the simulations and their comparison with experiments are also underway. Our protocol therefore provides a fast and efficient framework to rationally screen for suitable photo-activatable monomers that can permeate lipid membranes in sufficient amounts and rates to enable 3D printing inside biological compartments defined by lipid bilayers.

P085 DINGELDEIN, LARS

Lars Dingeldein[1]; Dr. Pilar Cossio[2]; Dr. Roberto Covino PhD[1]

[1] Frankfurt Institute for Advanced Studies; [2] Center for Computational Mathematics, Flatiron Institute

Simulation-based inference for single-molecule force-spectroscopy experiments

The self-assembly of biomolecules into their functional form is an essential process in all living organisms since reliably functional biomolecules are necessary for the survival of the organism. One method to study the kinetics of these folding processes on a single molecule level is to use force-spectroscopy experiments. In these experiments, the ends of a biomolecule are attached via linker molecules to a pulling device while the total extension of the device is measured over time. While the pulling device exerts a constant force, the biomolecule undergoes different configurational changes. In the simplest case, the configuration alters between a folded and unfolded state. Since the linker molecules are flexible themselves, the measured extension does not directly represent the molecular extension. Therefore the correct physical properties of the molecular system need to be inferred from the measured extension of the device. We modeled these experiments as a diffusive process on a two-dimensional free energy surface and used Bayesian inference to obtain estimates of the physical properties of the underlying molecular system. We used a simulation-based inference algorithm to learn the posterior distribution, with which we were able to perform Bayesian inference for different ground truth models and reconstruct free energy profiles, diffusion coefficients, and properties of the linker molecule. In future work, we want to perform Bayesian model comparison and model averaging with simulated data to enable the full simulation-based Bayesian workflow.

P086 GASSNER, WENZEL

Wenzel Gaßner[1]; Florian Wilhelm; Prof. Daniel Müller; Dr. Kristyna Pluhackova

[1] Universität Stuttgart

The roles of PIP2 lipids in biased signalling of β2-adrenergic receptor

G protein-coupled receptors (GPCRs) enable cells to react to extracellular stimuli. Given the vast diversity of those stimuli, it is not surprising that GPCRs constitute the largest class of membrane proteins in humans and are a target of more than 30% of all currently distributed drugs. In this respect it is extremely astonishing that all those receptors signal via a handful of different G proteins and are silenced by only two types of arrestin after phosphorylation by one of a few kinases. The fact that all those intracellular interaction partners (i.e., G proteins, kinases and arrestins) bind specifically to the intracellular binding pocket of more than 800 GPCRs hint to a great universality on one hand and to complex modulation mechanisms, e.g. by membrane lipids or allosteric ligands, on the other hand. Yet, the molecular mechanisms of the complex nature of these processes are just becoming to be understood.

Here, we unravel the impact of PIP2 lipids on the structure and dynamics of the β2-adrenergic receptor/ β-arrestin2 complex by all-atom molecular dynamics simulations. Three types of β2-adrenergic receptor/ β-arrestin2 complexes are studied to unveil the impact of receptor activation and phosphorylation on the complex structure and stability: (i) the receptor is not phosphorylated but activated by adrenaline, (ii) the C-terminus of the receptor is phosphorylated but the receptor is inactivated by the antagonist ICI. and (iii) the receptor is both activated and phosphorylated. The complexes are modelled based on diverse X-ray and cryoEM structures of other GPCRs and embedded in model membranes with or without PIP2 lipids. At first, we compare the equilibrated structures of the complexes and then we mechanically separate them by pulling the arrestin away from the membrane embedded receptor in accordance with atomic force microscopy measurements performed on the same systems. Thus, we aim to characterize the role of the β-arrestin2/PIP2 interplay taking into account the relative stability of the complexes, membrane composition, receptor activation and phosphorylation, the dissociation pathways as well as the importance of individual residues.

P087 GLOBISCH, CHRISTOPH

Dr. Christoph Globisch PhD; Prof. Dr. Christine Peter PhD, University of Konstanz

Multiscale simulations of proteins

Coarse grained (CG) models are widely used to study proteins. One of the recurrent challenges in CG modeling of proteins is due to the simplified representation of the system the problem of tertiary structure preservation while still being able to achieve enhanced sampling of the conformational space of the macromolecules. For the MARTINI CG forcefield this is achieved by a uniform supportive elastic network or a supportive GO model. For the MARTINI 2 forcefield derivative ENEDYN we previously developed the IDEN approach, an iteratively adjusted supportive elastic network based on atomistic reference simulations. Due to major modifications in the mapping of the new MARTINI 3 forcefield we adjusted the IDEN and apply it to multi domain proteins like FAT10 and compared it with another supportive approaches.

We performed molecular dynamics (MD) simulations at two levels of resolution: coarse grained (CG) simulations were essential to sample the highly diverse conformational equilibria of the proteins, including the formation of a multitude of protein-protein interfaces between the different subunits. To analyze the vast amount of high-dimensional data, an efficient, highly scalable analysis framework is required. To this end, several dimensionality reduction techniques were developed to obtain low-dimensional representations of the conformational space. We utilized the EncoderMap approach and compared different metrics that allow to assess the (dis)similarity between conformational spaces from simulations at different resolution levels. The density-based clustering algorithm HDBSCAN allows the detection of characteristic conformational states and provides starting points for a possible backmapping of the CG structures which makes it possible to switch back to atomistic resolution and utilize the CG simulations to obtain equilibrated atomistic ensembles. The so-obtained atomistic information is of great importance for the interpretation of experimental data.

P088 HOANG, OANH TU

Oanh Tu HOang

Investigations into Single Transduction Mechanism in Phytochromes

Phytochromes are photosensory receptors in plants, fungis and bacteria. Studying their function is important for the understanding of plant growth and can help to improve economics, such as harvesting. Their function is to regulate the growth and development of the system. There are different classes of phytochromes. Canonical (plants), bathy (bacteria) and prototypical phytochromes (bacteria, fungi). The different phytochromes possesses different parent states, which can either be Pr (red light absorbing) or Pfr (far-red light absorbing). When irradiated with light (700-750 nm), the photo sensor undergoes a cycle. The conversion of Pr to Pfr begins with double bond isomerization at the methine bridge between ring C and D. Followed by several relaxation processes, structural rearrangements in the binding site and protein. A destabilization of the secondary structure occurs through the movement and rearrangement of certain amino acids. Furthermore, to convert the alpha-helix to a beta-sheet a proton transfer from the propionic side chain C to a conserved histidine must take place. In our current study, we are trying to figure out whether the (de)stabilization of the alpha-helix is caused by electrostatic forces. For this purpose, we calculated the dipole-dipole interaction between the chromophore binding pocket and tongue and the continuum electrostatics with the APBS package. Additionally, we calculated the interaction energies between the tongue and the protein environment close to the chromophore and tongue.

P089 JONES, JESSE WILLIAM

Jesse William Jones; Prof. Dr. Maria Andrea Mroginski, TU Berlin

Constant pH Simulations of the Proton Exit Channel in P. Denitrificans Cytochrome c Oxidase

Cytochrome c Oxidase is the terminal enzyme of the cell respiratory chain, catalyzing the reaction of O_2 to $H_2 O$. Proton pumping across the membrane occurs coupled to the reaction. This process converts energy into a membrane potential, which in turn can be used by the ATPase to synthesize ATP from the energy released by the proton backflow through its channel. The reaction at CcO (Cytochrome c Oxidase) is driven by the transfer of electrons from Cytochrome c to CcO, which then move through the enzyme and reduce O_2 to H_2O. To do this, the respective amount of protons needs to be transferred through the membrane too. While the larger part of the mechanism of the CcO reaction has been studied intensively, namely the uptake of protons as well as electron and proton transfer processes, the proton exit into the P-side of the membrane yet has not been studied well. This study aims at providing the necessary knowledge to do some work on the proposed proton exit channels, and maybe find out what happens when the proton gets handed through to the P-side of the membrane, a process taking place even though the protons flow against the overall gradient. To shed light on this process overall, and find out why there is no proton backflow, Constant pH simulations are employed. With Constant pH molecular dynamics, Monte Carlo Evaluations of bonds are utilized in regular intervals inside the molecular dynamic simulation, allowing for bonds breaking and forming during the process observed.

P090 KASPARYAN, GARI

Gari Kasparyan; Prof. Dr. Jochen Hub, Saarland University

Free Energy Simulations of Electroporation

Biological cells are defined as the volume enclosed by a semi-permeable lipid membrane. Forming pores in those membranes plays a role in processes such as membrane fusion and fission, increasing the permeability of the membrane, and others. Electroporation is a method used for decades to help introduce drugs and genetic material in cells or generally as a pore forming modality. Although pores are heavily studied with a variety of methods, the free energy landscape of the initial stages of the pore formation is still not fully understood. We use molecular dynamics simulations to study the mechanisms and energetics of electroporation. We overcome the challenge of exploring the free energy landscape using umbrella sampling along a recently developed reaction coordinate[1, 2]. The potentials of mean force (PMFs) show that electric fields greatly stabilize open pores and lower the barrier for pore formation (as expected). An unexpected discovery is the way in which the pore formation energy barrier is influenced by the applied potential. As a result of that discrepancy between simulations and existing continuum models we propose a novel continuum model of electroporation. To verify our findings we compare two methods for establishing transmembrane potential in an MD simulation – external electric field and charge imbalance.

[1] J. Hub and N. Awasthi, J. Chem. Theory Comput. 2017, 13, 2352-2366

[2] J. Hub, J. Chem. Theory Comput. 2021, 17, 1229—1239

P091 KOLIN, SOFIA

Sofia Kolin[1]; Moritz Marquardt[1]; Patricia Sonnenberg[2]; Dr. Stefan Naumann[2]; Dr. Kristyna Pluhackova[1]

[1] University Stuttgart, Stuttgart Center for Simulation Science, Cluster of Excellence EXC 2075; [2] University Stuttgart, Institute of Polymer Chemistry (IPOC)

Molecular Transport in Mesoporous Carbon Materials

Mesoporous carbon is of interest in many applications due to its high surface area and physicochemical properties, including a high pore volume and good thermal and mechanical stabilities. It provides interconnected channels for the diffusion of organic solvents, which can be used for "flow-through" applications such as purification of drinking water or nanoseparation of proteins. The objectives of this work are to evaluate the impact of pore size, shape and polarity on the permeability of mesoporous carbon materials in comparison to experimental data. Using coarse-grained molecular dynamics we have established molecular models of three 3D structures of carbonaceous materials: (i) hexagonally ordered cylindric pores, (ii) a bicontinuous double primitive structure with two independent cylindrical pore channels and (iii) a cubic system with overlapping spherical pores. Moreover, variation of the pore diameter, surface roughness or percentage of oxidized species of these porous materials enables us to systematically evaluate the impact of the individual material properties on molecular transport of e.g. hydrocarbons or alcohols. Moreover, simulations of molecular transport of mixtures of differently sized solvents, e.g. hexane and dodecane, allow us to unveil details about solvent competition and nanoseparation.

P092 KORN, VIKTORIA

Viktoria Korn; Dr. Kristyna Pluhackova, University of Stuttgart

What the Phos? Parametrizing Protein Phosphorylation for the CHARMM36 and Martini Force Fields

Post-translational modifications (PTMs), among which phosphorylation is the most common one, describe the covalent attachment of molecules to biomolecules, thus rapidly altering their structure and function. Due to their incredibly fast kinetics and reversibility, PTMs play an important role regulating multiple bioorganic pathways such as (de)activating enzymes, translocating proteins, and proteolysis, to name a few. Misplaced, missing, or excessive PTMs are typically linked to disease pathology, including inflammation, cancer, and neurodegenerative diseases, where they are responsible for neurotoxicity and synaptic damage. PTMs modify the bulkiness and the charges of chemical groups, triggering conformational changes of the structure and thereby altering the biomolecules intermolecular interactions and biological function. Molecular dynamics (MD) simulations are an amazing tool that helps us study bio-chemical processes on an atomistic scale. In MD, molecules are described by a priori parametrized force fields. In order to adequately represent chemical structures and properties of biomolecules under natural conditions and reveal the molecular impact of PTMs, parameters for all kinds of atom groups, including PTMs, need to be known. Our goal is to develop accurate force field parameters of phosphorylated amino acids for both the CHARMM36 all-atom and the Martini coarse-grained force field using our own quantum-chemical calculations of solvated complexes and experimental data from the literature.

P093 LAZZERI, GIANMARCO

Gianmarco Lazzeri[1]; Hendrik Jung[2]; Dr. Roberto Covino PhD[1]

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Artificial intelligence sheds light on protein folding dynamics at the atomic scale

Protein folding, misfolding, and conformational changes are ubiquitous in our cells. They play key roles in metabolic pathways and are involved in numerous diseases. In-depth knowledge of the underneath mechanisms is crucial for understanding those processes and can lead to medical applications. However, conformational transitions are rare and fast. In vivo and in vitro observation cannot capture the folding/unfolding dynamics with adequate time and size resolution, while atomistic molecular dynamics simulations are limited by the available computational power.

Transition path sampling (TPS) is a technique for simulating unbiased transition trajectories; the resulting transition path ensemble provides information about the dynamics and mechanism. Yet, TPS leaves other challenges unaddressed. It is especially important to encode the progress of a transition in a reaction coordinate, as it provides quantitative insight into its mechanism. Furthermore, generating transition paths with high efficiency is still a challenge. In our method, AIMMD, we let a neural network control TPS simulations. This significantly speeds up the sampling while learning an optimal reaction coordinate: the committor probability.

We applied AIMMD to the folding of the mini protein chignolin. We collected hundreds of transition paths while learning the protein folding mechanism through the committor. We extracted the most significant features from the committor and turned it into an interpretable and quantitative expression with symbolic regression. We combined TPS, umbrella sampling, and equilibrium simulations to estimate the system's transition rates and the free-energy profile along the committor. Our approach can be directly brought to larger and more complex systems and customized to many conformational changes and structural re-organization processes.

P094 LIU, HAORAN

Haoran Liu, Leibniz-Forschungsinstitut für Molekulare Pharmakologie

Investigation of Ion Permeations through CNGA1 Channels by Molecular Dynamics Simulations

1. Mammalian cyclic nucleotide-gated (CNG) channels are crucial components of numerous signal transduction pathways, most classically in the visual and olfactory sensory systems. High-resolution cryo-EM structures of human CNGA1 channel became recently available. The whole structure is a tetramer consist of 4 subunits including pore area, voltage-sensor domain (VSD), C-linker and cyclic nucleotide binding domain (CNBD) (Figure 1) [1]. Molecular dynamics (MD) simulations were applied to:

(1) determine the conductive state of the CNGA1.

(2) understand non-selective ion permeation mechanism.

(3) identify structural determinants for gating.

Molecular dynamics based computational electro-physiology [2] was employed to simulate ion permeation in the CNGA1 channel. To perform the computational electrophysiology simulations, the system was duplicated along the z axis and transmembrane potential gradients were established by using an ion imbalance of 2 cation ions in different compartments, which is created by two bilayers. Inward and outward ion permeations can be simulated within the same simulation box. In this study, we only employed the pore domain of the whole CNGA1 channel. 5 runs of 500 ns open-state K+ and Na+, respectively, together with 5 runs of 500 ns closed-state K+ simulations were collected so far using the AMBER force field.

1. Conductivity of the CNGA1 channel

(1) Computational electrophysiology simulations demonstrate the open-state of the CNGA1 is conductive, while no ion permeation was observed in the closed-state.

(2) Both outward and inward ion permeations were observed in the simulations (Figure 2A. Ion tracks).

(3) Different from potassium selective channels, only one major ion binding site was found in the selectivity filter (SF) during ion permeation (Figure 2A. 2D and 1D ion occupancy).

2. Gating in the CNGA1 channel

(1) Open- and closed-states of the CNGA1 remains stable during the simulations (Figure 3A).

(2) Except the gating residues, backbone selectivity filter conformation is substantially different for open- and closed- states (Figure 3C/D).

P095 LOKAMANI, MANI

Mani Lokamani; Dr. Jeffrey Kelling; David Pape; Dr. Oliver Knodel; Dr. Guido Juckeland; Prof. Dr. Karim Fahmy, Helmholtz-Zentrum Dresden-Rossendorf (HZDR)

Generalized workflow for automated evaluation of isothermal microcalorimetry traces

The connection between metabolism and growth can be explored by monitoring the heat (enthalpy) release from chemical turnover in metabolizing cells. In contrast to cell number monitoring, Isothermal microcalorimetry (IMC) can measure the heat release in metabolizing cells with unprecedented sensitivity independently from cell number determinations, paving way for dedicated experimental verification of complex growth models. When expressed in the enthalpy- rather than time-domain, as exemplified for cultures of Lactococcus lactis (prokaryote), Trypanosoma congolese (protozoan) and non-growing Brassica napus (plant) cells, general trends and patterns emerge in the IMC traces. The data complies extraordinarily well with a dynamic adsorption-reaction model resembling a Michaelis-Menten equation generalized here to non-constancy of catalytic capacity.

In this contribution, we explore a novel formalism, that reproduces the "life span" of cultured microorganisms from exponential growth to metabolic decline with fundamental consequences for the derivation of toxicity measures and for the transferability of metabolic activity data between laboratories. We also provide a generalized workflow for automated evaluation of IMC traces from different sources.

P096 MAAG, DENIS

Dr. Denis Maag, Karlsruhe Insitute of Technology (KIT)

QM/MM metadynamics of thiol-disulfide exchange using a neural network correction

Molecular dynamics and free energy calculations of chemical reactions in which bonds are broken and formed require a quantum mechanical (QM) description. However, the application of most QM methods is restricted to very short time scales (femtoseconds to picoseconds), due to their high computational cost.

With the semi-empirical Density-Functional Tight Binding (DFTB) method, which is up to 1000x faster than DFT, it is possible to simulate such reactions on longer time scales. However, some classes of reactions suffer from qualitative and quantitative errors due to approximations in DFTB.

In thiol-disulfide reactions [1], a nucleophilic substitution that occurs in a large class of proteins, the transition state is inaccurately described with DFTB [2]:

(i) the sulfur-sulfur distances in the transition state are too long

(ii) the transition state geometries exhibit shallow minima on the free energy landscape, rather than saddle points.

Both artifacts have been corrected by applying a machine learned energy correction with respect to CCSD(T) level of theory [3].

The additional computational effort is comparable to that of DFTB, allowing extensive sampling of thiol-disulfide exchange with ab initio accuracy at low computational cost.

The free energy profiles of thiol-disulfide exchange in a small model system and a blood protein were obtained with QM/MM metadynamics using the machine learned energy correction.

In a next step, the computational efficiency may be further increased by using a neural network instead of DFTB, which learns the energies directly as well as the charges of the QM atoms.

P097 MARTÍNEZ-LEÓN, ALEJANDRO

Alejandro Martínez-León; Prof. Dr. Jochen S. Hub, Saarland University

Overcoming hysteresis in ligand binding free energy calculations

Accurate estimation of ligand binding free energy and elucidation of the corresponded mechanism are key points in drug design. Umbrella Sampling Molecular Dynamic Simulation (US) is one of the most popular enhanced sampling techniques that has tried to deal with this challenge. The performance of US is directly affected by the complexity of the system, selection of reaction coordinate(s), simulation time, selection of restraints, etc. A bottleneck for the accuracy of US is the presences of binding/unbinding hysteresis. Therefore, the range of applicability of US for ligand binding free energy is almost limited to shallow binding pockets and small proteins. Here we test several flavors of US in the pentamer channel Plasmodium-falciparum formate-nitrite-transporter (PfFNT) embedded in a pure POPC membrane. PfFNT had been identified as the malaria parasite's lactate transporter and as a novel drug target. In addition to its biological relevance; this system is very attractive for our proof of concept: presents a buried binding pocket, has more than 126000 atoms, it doesn't undergo dramatic conformational changes and shows hysteresis on the binding/unbinding process. We select the minimalist possible reaction coordinate: the distance between the center of mass of the ligand and the binding pocket. The combination of US with Simulated Annealing, Simulated Tempering, hydrogen mass repartioning and a minimum number of restraints is able to reduce hysteresis from 19±7 kJ/mol to 8±5 kJ/mol. All our work has been highly automated via a Python module, to render the setup transferable to similar systems (https://gitlab.com/md_tools/mdynamic).

P098 PFAENDNER, CHRISTIAN

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Resolution transformation in molecular dynamics: Boosting backmapping via knowledge-driven machine learning

Molecular dynamics simulations provide valuable insights into biological macromolecules with temporal and spatial resolution not currently available to experimental methods. Thereby, MD has helped to uncover biochemical mechanisms important for understanding the development, progression, and treatment of serious diseases such as cancer. Despite advances in high-performance computing, system sizes and simulation times for atomistic simulations are still limited to the nanometer and microsecond scales. To overcome these limitations, sequential multiscale molecular dynamics simulations switch between different levels of resolution of the molecular representation, allowing to study processes over longer timescales and simultaneously recover atomistic details. While coarse-graining is straightforward, the reverse transformation from low to high resolution, also termed backmapping, is a non-trivial task. This is mainly due to the loss of information about the atomistic structure underlying the coarse-grained representation, which must be reintroduced. Available state-of-the-art methods neglect the Boltzmann distribution, get trapped in local minima, might fail to recover the correct stereochemistry, and often require extensive expert knowledge from the user.

Here, we develop a novel chemistry-informed machine learning framework for the reverse transformation of coarse-grained molecular dynamics data to atomistic resolution. Our algorithm, based on Boltzmann distributions of structural features, avoids the drawbacks of current methodologies and shows how hybrid first-principle and certified artificial intelligence-based models can boost MD. Moreover, we provide standardized evaluation criteria to directly compare the performance of different backmapping algorithms, allowing users to choose an appropriate method for a given use case.

P099 SARNGADHARAN, POOJA

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Towards excitonic properties of antenna complexes in Photosystem II

Photosystem II (PSII) is responsible for the oxygen evolution during oxygenic photosynthesis. The whole process is driven by a collective effort of pigment-protein complexes in PSII to create an energy funnel towards the reaction center that transfers the energy from sunlight in the form of excitons. The complex structure of PSII poses a computational challenge to understand the mechanisms that enable efficient excitation energy transfer (EET). Here, we use a computationally efficient scheme to determine the excitonic properties of two antenna complexes showing promising results for the antenna complexes CP43 and CP29 [1,2] similar to those for the LHCII complex [3]. For the ground state dynamics of the system, we employed quantum mechanical/molecular mechanical molecular dynamics (QM/MM MD) based on the density functional based tight-binding (DFTB) [4,5]. The excited-state calculations of the pigments in the light-harvesting complexes were performed using the long-range corrected time-dependent Density Functional tight-binding (LC-TD-DFTB) approach in a QM/MM framework [4]. Using the energy fluctuations, we calculated the spectral densities that correlate well with their experimental counterparts, especially in the high-frequency region. Furthermore, absorption spectra of CP43 complex have been calculated and compared with the experimental ones. We found out that the full second-order cumulant expansion method is a more accurate approximation than Redfield-like formalisms in obtaining the linear absorption due to the existence of the quasi-degenerate states in CP43 [1]. Our computationally efficient method with quite accurate results widen the scope for investigating EET mechanisms in large light-harvesting systems [1,2,3,5].

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P100 SCHÄFER, STEFAN

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Sublytic gasdermin-D pores captured in atomistic molecular dynamics simulations

Gasdermins are the key executing proteins of pyroptosis, a recently discovered type of regulated cell death associated with cell lysis and inflammation. After activation by caspases, they bind the plasma membrane, oligomerize and spontaneously form large membrane pores. These pores are lined with a β-sheet, whose hydrophobic face stabilizes it in the plasma membrane and whose hydrophilic face repels the hydrophobic core of the membrane on the inside of the pore. Fully assembled pores of gasdermin-D (GSDMD) contain about 30 subunits and measure approximately 20 nm in diameter. These pores lead to the efflux of inflammatory cytokines, osmotic shock, and ultimately to cell lysis. The detailed mechanisms of membrane binding, ring assembly, and pore formation remain elusive.

We performed atomistic multi-microsecond molecular dynamics simulations of different-size GSDMD oligomers in pore and pre-pore conformations to study their lipid interactions, dynamics, and structural stability. Using a complex asymmetric plasma membrane mimetic, we were able to identify conformation-specific interactions with acidic lipids that recruit GSDMD to the inner leaflet and may drive oligomerization as well as insertion of the β-sheet. In addition, we found that already small oligomers comprising 2-10 subunits remain stably membrane inserted and form small membrane pores that facilitate water and ion flux across the membrane. Larger oligomers form arc shapes that, beyond a certain size, transition in ring and slit-shaped pores, driven by the tension of the emerging membrane edge. We quantify the membrane edge tension of our plasma membrane mimetic at 86 pN. Lastly, we show that fully assembled pre-pore rings cause membrane buckling that may prime the membrane for concerted insertion of the β-barrel.

Our simulations thus explain the sublytic nonselective ion flux observed in early phases of pyroptotic cell death and the emergence and stability of arc, slit, and ring-shaped pores observed with atomic force microscopy (AFM). They further point towards competing, lipid-dependent assembly pathways by a gradual expansion of sublytic pores at low GSDMD concentrations and a concerted "cookie-cutter" membrane insertion from prepore rings at high concentrations.

P101 SCHERER, KATHARINA

Katharina Scherer[1]; Chetan S. Poojari; Jochen S. Hub

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Effect of Transmembrane Domains on the Free Energy of Stalk Nucleation during Membrane Fusion

The nucleation of the stalk is the first step in membrane fusion. The overall fusion process including the stalk formation is facilitated by fusion proteins anchored in the membrane by transmembrane domains (TMDs). Although TMDs of fusion proteins have been suggested to play an active role during fusion, little quantitative or mechanistic understanding of putative TMD effects has evolved. We used molecular dynamics simulations to analyze the influence of TMDs of the SNARE complex and of viral fusion proteins on the free energy of stalk formation. The stalk free energy was computed highly efficiently via potential of mean force (PMF) calculations along a newly designed reaction coordinate together with the Martini coarse-grained force field [1][2]. The results reveal a decrease in both, the free energy barrier of stalk nucleation as well as the free energy of the final stalk structure, when TMDs are present in the membrane. Further, this free energy decrease scales linearly with the concentration of TMDs in the membrane and strongly depends on the hydrophobic mismatch between the TMD and membrane core as well as on the lipid composition. We could explain the free energy decrease upon insertion of TMDs with an increased disorder in the lipid packing quantified by the order parameter.

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P102 SIKORA, MATEUSZ

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GlycoSHIELD: an online tool to address glycan dynamics and heterogeneity in glycoproteins

The majority of membrane and secreted proteins are post-translationally modified through a covalent addition of complex sugars: glycans. Glycans influence protein-protein interactions and can be hijacked by pathogens like the SARS-CoV-2 virus to sidestep the immune system. The hydrophilic character and lack of secondary structure result in large conformational freedom of glycans, which, together with glycan micro-heterogeneity, hinder the complete structural characterisation of glycoproteins. Molecular dynamics simulations have been instrumental in addressing glycan dynamics and function, but are impractical to study large protein complexes or to elucidate the effects of various glycoforms due to the required computing time.

Taking advantage of the disparity of the time scales of conformational changes of glycans and proteins, we propose a reductionist open-source method to graft ensembles of glycan conformers onto static protein structures: GlycoSHIELD. Through comparisons with extended atomistic simulations of the SARS-CoV-2 spike glycoprotein, we demonstrate that GlycoSHIELD reproduces key features of the shielding effect of glycans at a fraction of the computational cost. We then combine GlycoSHIELD with cryoEM and mass-spectrometry to provide glycosylated structures of spike proteins of 5 coronaviruses, providing novel insights on the role of glycan heterogeneity in controlling virus interactions with other molecules.

GlycoSHIELD, together with a library of over 50 glycan types to date is available for non-expert users at www.glycoshield.eu.

P103 SOUSA, CARLA F.

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Modelling drug permeation using simulated tempering-enhanced umbrella sampling

Drug permeation across biomembranes is a crucial topic for drug development, as many drugs have intracellular targets. Molecular dynamics (MD) simulations can be used to obtain an atomistic-detailed picture of the drug permeation process, providing an accurate physical model for determining the free energy of this process. In this type of permeation studies, umbrella sampling (US) is often used as an enhanced sampling approach, but for bulk drug-like permeants obtaining sufficient sampling is still often a challenge and would require long simulation times, resulting in a high computational cost [1]. Here, we increase the efficiency of the US approach by coupling it with simulated tempering (ST). With ST, the system performs a periodic random walk across a predetermined temperature ladder. Increasing the temperature of the system allows it to escape from local energy minima and to achieve a broader sampling [2].

A set of molecules that covers a wide range of molecular sizes and hydrophobicity was simulated, comprising methanol, ibuprofen, 1-propanolol and atenolol. For the bulkier molecules, the application of ST-US with a temperature ladder from 300 to 348 K (with a ΔT of 6 K) accelerates the convergence of the free energy profile across the bilayer by enhancing the sampling five-fold, when compared to US. For the smallest molecule, methanol, however, the ST-US protocol was hindered by the reduced sampling at the target temperature (300 K). Hence, we developed a modified simulated-tempering protocol that allows increased simulation time at the ground (target) temperature. Specifically, we found that passing 20% of the simulated time at the ground temperature yields rapid convergence of the umbrella histograms.

In this study, we showed that combining simulated tempering with umbrella sampling is a promising approach to improve the efficiency and accuracy of free energy calculations of drug permeation across model membranes. The results of this study pave a way to use ST-US as a conventional method in simulations of drug permeability, increasing the ability for using such studies in a high throughput routine.

P104 SPINETTI, ELENA

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Investigating human Ire1α assembly process via multiscale Molecular Dynamics simulations

In all eukaryotes, the Unfolded Protein Response (UPR) is a molecular program that maintains the protein folding homeostasis in the endoplasmic reticulum (ER). The UPR plays a crucial role in health and disease. Stress sensor proteins on the ER membrane activate the UPR. The evolutionary most conserved sensor is the protein IRE1, which triggers the UPR by forming dimers and larger assemblies. In particular, IRE1's luminal domain interacts with unfolded proteins, and these interactions might promote oligomerization by an unresolved mechanism. A mechanism for direct binding of peptides to IRE1 is hypothesized but still not understood.

My work aims to elucidate the structure and assembly mechanism of large supramolecular assemblies of human IRE1 and probe its direct binding to unfolded proteins. These events are crucial for IRE1's functions and the early stages of the UPR's activation.

We employ a computational multiscale approach, performing atomistic and coarse-grained molecular dynamic simulations.

For investigating the formation of clusters of dimers of IRE1, we use the coarse-grained Martini 3 force field. Preliminary results show that IRE1 dimers can form clusters where contacts are mediated by disordered regions. Peptide binding experiments in atomistic and coarse-grained led us to propose a new model for the direct binding of peptides and unfolded proteins.

In the future, we will expand our model of IRE1 to include the transmembrane domain to investigate how the membrane affects the formation of assemblies.

P105 WALKER-GIBBONS, ROWAN

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The Role of Interfacial Water in Biomolecular Interactions

Molecular dynamics studies have demonstrated that molecular water at an interface displays anisotropic orientational behaviour in contrast to its bulk counterpart. This effect has been recently implicated in the like-charge attraction problem for electrically charged colloidal particles in solution. Here, negatively charged particles in solution display a long-ranged attraction where continuum electrostatic theory predicts monotonically repulsive interactions, particularly in solutions with monovalent salt ions at low ionic strength. Anisotropic orientational behaviour of solvent molecules at an interface gives rise to an excess interfacial electrical potential which we suggest generates an additional solvation contribution to the total free energy that is traditionally overlooked in continuum descriptions of interparticle interactions in solution. We posit that the remit of such behaviour is not limited to macroscopic interfaces. Indeed we expect the same considerations to hold in the interactions of biomolecules and biological interfaces in solution. In the present investigation we perform molecular dynamics simulations to determine the interfacial potential at biomolecular surfaces. We discuss how a free energy contribution from interfacial water may strongly affect the interaction between charged biomolecules in solution under physiologically relevant solution conditions. The study carries broad implications for molecular-scale interactions, with the proposed interfacial mechanism finding relevance in explaining a range of phenomena such as biological phase segregation [1] crystallization and pH-induced gelation, and chromosome packing, or more generally in soft-matter and molecular biological systems that exhibit pH and salt concentration dependent attractive interactions between electrically charged entities.

P106 EICHMANN, VIKTOR

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FTIR spectroscopy on the carbonyl region of variations of photosystem I isolated from cyanobacteria

Some cyanobacteria adapt to low-energy light in a process known as far-red light photoacclimation (FaRLiP). During this process, standard protein subunits of Photosystem I (PSI) are replaced by their far-red variants, and 7-8 of the existing ~100 chlorophylls (chl) a are exchanged by red-shifted chl f. It is currently debated, whether these chlorophylls are part of the electron transfer chain (ETC) of the PSI reaction center, or fulfill a role in light harvesting only as a peripheral chlorophyll. In this study, light-induced FTIR difference spectroscopy was used to investigate the carbonyl region of isolated PSI variants of different cyanobacteria. This region is mainly composed of chlorophyll moieties part of the ETC that are directly or indirectly affected by photo-oxidation.

According to a previous study (Hastings et al., 2019, BBA), the C=O bonds of the P700 chlorophylls after oxidation and possibly intermediate chlorophylls of the ETC were resolved in the difference spectrum. An observed shift in the difference spectra of far-red versus white light PSI of the same cyanobacterial strain could possibly be assigned to a chl f substituting for chl a in this intermediary chl pair, supporting the proposition that chl f is part of the ETC.

To obtain high yields of PSI, a large-scale cultivation system under different conditions and chromatography-based protein purification methods were established.

P107 FISCHER, PAUL

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FTIR and UV-Vis spectroscopic studies of a Rhodopsin Guanylyl Cyclase

Enzymerhodopsins represent a recently discovered class of rhodopsins which includes histidine kinase rhodopsin, rhodopsin phosphodiesterases and rhodopsin guanylyl cyclases (RGCs). The regulatory influence of the rhodopsin domain on the enzyme activity is only partially understood and holds the key for a deeper understanding of intra-molecular signaling pathways. Here, we present a UV-Vis and FTIR study about the light-induced dynamics of a RGC from the fungus Catenaria anguillulae, which provides insights into the catalytic process.

The developed spectroscopic setup supports a wide variety of experimental conditions including temperature control below the boiling point of helium allowing for the investigation of the earliest intermediates. This was complemented by the time-resolved characterization of late photoproducts utilizing slow cycling mutants.

To unravel the mechanism in enzyme regulation, truncated variants were analyzed revealing the involvement of the cytosolic N-terminus in the structural rearrangements upon photo-activation. The incorporated of a high power pulsed laser system enables the use of caged compounds to investigate the catalytic properties of enzymes. Here, we present a real-time monitoring of substrate turnover in RGC and the free GC domain independently on a molecular scale which paves the way for a more general understanding of enzyme activity and protein-protein interactions. Our results show substrate binding to the dark-adapted RGC and GC alike and reveal differences between the constructs attributable to the regulatory influence of the rhodopsin on the conformation of the binding pocket. By monitoring the phosphate rearrangement during cGMP and pyrophosphate formation in light-activated RGC, we were able to confirm the M state as the active state of the protein.

P108 GOETT-ZINK, LUKAS

Dr. Lukas Goett-Zink; Eileen Baum; Prof. Dr. Tilman Kottke, Bielefeld University

Signal Progression in a LOV Photoreceptor Studied by Time-resolved In-Cell Infrared Difference Spectroscopy

FT-IR difference spectroscopy is widely used for studying structural changes of proteins such as enzymes or receptors. However, the proteins are usually investigated under in vitro conditions, which are differing dramatically to those within a host cell. The protein responses can be affected by molecular crowding or binding of metabolites among others [1].

Recently, we established an approach of in-cell infrared difference spectroscopy (ICIRD) to study soluble photoreceptors in living cells with a protein copy number of up to below ~300,000 in the transmission and attenuated total reflection (ATR) configuration [2, 3]. With ICIRD, we aim to understand the impact of the cellular environment on the structural response and signal progression in proteins. Aureochrome1a (Aureo1a) is a blue-light-regulated transcription factor in diatoms consisting of the photosensory light, oxygen, voltage (LOV) domain, the DNA-binding effector domain, a basic region leucine zipper (bZIP), and an N-terminal extension.

Here, we extended ICIRD from a static to a time-resolved method to follow signal progression in Aureo1a. The setup was constructed using the rapid-scan technique, a pulsed laser, and a home-build automatic sample changer yielding a current time resolution of 7.6 ms on a LOV-containing model system in E. coli cells. Investigation of Aureo1a by time-resolved rapid-scan ICIRD resolved the signal progression from the sensory LOV domain to the bZIP effector in vitro and in living cells. LOV dimerization and an alpha-helical unfolding in the bZIP were observed with time constants of tau = 3 s and tau = 6 s, respectively. Such late dimerization let us reconsider the mechanism of light-induced signal progression in aureochromes.

Time-resolved ICIRD expands the range of in-cell methods by a non-invasive and label-free technique, which does not require any purification step and has a limitation in number of copies but no intrinsic limitation in protein size.

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P109 HINZE, WOLFGANG

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Adsorption of shape-directing agents to differently shaped ZnO particles

Zinc Oxide (ZnO) is known to have antimicrobial effects that are, at least in parts, shape-dependent [1]. This makes shape control a relevant topic in the research of ZnO as antimicrobial material. Shape control in ZnO particles can be achieved by using additives in the synthesis [2]. The interaction of particles with such additives is thus of fundamental importance and can be investigated via attenuated total reflection Fourier-transform infra-red (ATR-FTIR) spectroscopy [3]. The goal of this project is, to investigate adsorption behavior of different additives from solution to ZnO particles of different shape. This is done by producing thin films of ZnO particles via an easy and cheap hydrothermal production route and then using these films for time-dependent ATR-FTIR measurements of the adsorption process.

A method to produce ZnO thin films with controlled surface exposure (e.g. prevalently 0 0 1) was established. Adsorption experiments with these films and solutions of additives such as sodium citrate show a stronger adsorption to the ZnO exposing the (0 0 1) face, which is in line with literature [4]. Therefore, this method is a promising approach for tailoring-experiments with the goal of elucidating adsorption mechanisms or finding additives, that suit specific needs for a synthesis route (e.g. high adsorption strength).

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P110 MATTES, LORENZ

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Dual-comb-IR-spectroscopy to study temperature-jump dynamics of polyQ model peptides

Glutamine (Q) rich proteins tend to aggregate and form fibrils. These aggregates can cause neurodegenerative polyQ diseases like Huntington disease. In our research we study the folding dynamics of polyQ rich model peptides with time-resolved IR-spectroscopy at single wavelengths and with dual-combs after a nanosecond laser-induced temperature-jump [1]. The temperature-jump of 5-10 K is induced by a pulsed Ho:YAG laser (IPG Photonics, U.S.A.) at 2090 nm exciting the overtone vibration of the solvent D2O. For single wavelength measurements, a home-built spectrometer with tunable quantum cascade lasers (QCLs) of a MIRcat-QT laser system (Daylight Solutions Inc. U.S.A.) and an MCT-detector (KMPV11-1-J2, Kolmar Technologies, U.S.A.) is already established. The further development of dual-comb QCLs allows measurements with microsecond resolution over a spectral range of 60 cm-1 [2]. Therefore, the beam of an IRis F1 dual-comb-spectrometer (IRsweep, Switzerland) is coupled into the sample cell of the IR-spectrometer. With this dual-comb approach, time-resolved spectra are available in a short time. We get comprehensive insights into structural dynamics by analyzing characteristic amide I′ modes in the same measurement. The impact of individual glutamines on structure and dynamics can be explored.

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P111 MORSHEDI, MAHBOBEH

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The Photo-physics of 2-Cyanoindole probed by Femtosecond Spectroscopy

Indole is the chromophore of the naturally occurring fluorescent amino acid trypto-phane [1]. Therefore, unnatural amino acid derivatives with functionalized indoles are of great interest as fluorescent probes in protein studies. For instance, the fluorescence quantum yield of 2-cyanoindole is strongly sensitive to the solvent. Its fluorescence quantum yield in water is as small as 4.4·10-4 and increases to a value of 0.06 in apolar cyclohexane [2]. 2-Cyanoindole can, thus, represent a promising fluorescence probe in hydration studies of proteins. We have investigated the photo-physics of 2-cyanoindole by time resolved spectroscopy. In water, its fluorescence decays within 8 ps. For short wavelength excitation (266 nm) the initial fluorescence anisotropy is close to zero. For excitation with 300 nm it amounts to 0.2. This is in accordance with the energetic prox-imity of two singlet states (La and Lb) [3]. In water, femtosecond transient absorption reveals that the fluorescence decay is solely due to internal conversion to the ground state (Figure 1a). In less polar solvents, the fluorescence decay takes much longer (acetonitrile: ~640 ps, tetrahydrofuran: ~2.6 ns) and intersystem crossing contributes (Figure 1b).

P112 OLDEMEYER, SABINE

Dr. Sabine Oldemeyer, Freie Universitaet Berlin, Berlin

In-cell Infrared Difference Spectroscopy on the Bacterial Chloride Pump NmHR

The transport of chloride ions across a membrane is necessary to control processes such as cell growth and osmotic pressure and is therefore essential for the survival of organisms. Inward directed halide-pumping rhodopsins (halorhodopsin; HR) are found in halophilic Archaea, such as Halobacterium salinarum and Natromonas pharaonis. Here, they facilitate the generation of the membrane potential together with bacteriorhodopsin, an outward directed proton pump, to produce ATP. In marine bacteria, halorhodopsins such as rhodopsin 3 from Nonlabens marinus (NmHR), were suggested to help sustain the osmotic balance of the cell and are involved in ATP production. NmHR binds a retinal chromophore in the all-trans configuration, which, upon photoisomerization, initiates the transport cycle through a mechanism that was only recently revealed in a time-resolved serial crystallography study. However, all experiments conducted to date were performed on proteins isolated from their natural environment.

In order to gain insight into the structure and mechanism of proteins in their native environment, various in-cell spectroscopic techniques have been recently developed. This is of major importance since in vivo conditions vary substantially from those chosen for in vitro experiments. The protein mechanism can be influenced by the cellular environment through molecular crowding or the binding of cellular metabolites.

A method well-suited for the investigation of the light-induced structural changes of photoreceptors is presented by Fourier transform infrared (FTIR) difference spectroscopy using the attenuated total reflection (ATR) mode, allowing to detect changes in the cofactor, side chains and secondary structure. The success of this approach has been proven for soluble blue-light sensors in living E.-coli cells.

Here, the method is taken one step further and is applied on a membrane protein, the chloride pump NmHR. Light-induced ATR FTIR difference spectra of NmHR overexpressed in E. coli cells show distinct spectral features of the all-trans to 13-cis conversion of the retinal chromophore as well as the response of the apoprotein, confirming that this method is applicable also to membrane proteins. Furthermore, the collected data allows the comparison to spectra gathered from samples prepared in detergent, lipids or reconstituted in nanodiscs, helping to determine the effect of an artificial environment on the photocycle.

P113 SCHLÖTTER, MORITZ

Dr. Moritz Schlötter; Prof. a.D. Georg Maret; Prof. Patrick Müller, Uni Konstanz

CASS – A new tool to investigate dynamics in complex samples

We present a new highly sensitive non-invasive technique which is based on dynamic light scattering. CASS (Cavity amplified speckle spectroscopy) is a tool to observe the real time dynamics of complex samples. Bulk tissue, or samples ranging from proteins to nanoparticles can be studied. Strong enhancement of the light scattering signal is achieved by enclosing the specimen in a turbid, high albedo random cavity.

Minute displacements, or structural changes can be studied with this simple setup, while sample amounts low as 10 micro liters are sufficient. In contrast to conventional light scattering, observation of non-dispersed samples, such as living cells is supported. This extends event to arbitrary cell cultures or bulk tissue. To test the range of its applicability we apply CASS to two living biological samples, as well as solutions of proteins and nanoparticles.

Neuronal signals (Action Potentials), classically do not display optically detectable phenomena in conventional light microscopy. We therefore tested the sensitivity of CASS in isolated ventral nerve cord of earthworms. Induced action potentials by electro-stimulation elicited a robust detectable optical signature. We found an exquisite signal to noise ratio; which has not been described before. Notably, the earthworms axon is wrapped in an opaque myelin sheath. This did not hinder our measurements, even more this is permissive due to the principles of the cavity. We are currently applying CASS to the first hours of embryonal fish egg development. With this well studied system we want to clearly demonstrate the power of a CASS sensor and its benefit in biological research.

In a second experiment series we plan to investigate protein-ligand binding kinetics. Characterization of binding or aggregation of proteins or nanoparticles is a common bottleneck in chemical, pharmaceutical and biological research and industry. A recent publication proofs that CASS can indeed resolve protein binding (doi 10.1117/12.2625017). We aim to perform more systematic pilot experiments to demonstrate the advantages of CASS concerning protein complexation.

CASS is a new tool that can resolve dynamics from sub-milliseconds to many hours with little requirements upon the sample. It is astonishingly versatile, robust and low-cost. We are actively developing this new tool with the aim to make it available for the broad scientific and industrial community.

P114 STEHLE, JULIANE

Juliane Stehle[a], Jörn Weisner[b], Daniel Rauh[b] and Malte Drescher[a], University of Konstanz

The global conformation of the kinase Akt1 monitored by DEER spectroscopy and multilateration

The serine/threonine kinase Akt1 plays a key role in cell signalling and is part of the PI3K/Akt pathway. A dysregulation due to mutations in the protein sequence has been shown to lead to serious diseases including cancer [1]. Akt consists of two domains, the pleckstrin homology (PH) domain and the kinase domain which are connected by a flexible linker. Protein function and activity seem to depend on the relative conformation of these domains to each other [2].

Site-directed spin labelling in combination with DEER distance measurements and subsequent multilateration of the obtained distance distributions proved to be an ideal tool to track conformational changes of kinases [3]. Here, the global conformation of the kinase Akt1 was visualized in its different states of activation. While in the inactive "PH-in" conformation the two domains are close to each other and restricted in their movement, the "PH-out" conformation seems to be a highly flexible state, where the domains are further separated. The apo state shows an intermediate flexibility and distance between the PH and kinase domain. Additionally, it was demonstrated that the cancer-associated E17K mutation has no significant influence on these global conformations of Akt1.

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P115 TELSCHOW, FLORIAN

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Photophysics and photochemistry of a new fluorescent molecular rotor dye: CCVJ boronic acid

The fluorescence yield and lifetime of a class of molecules called "molecular rotors" is environmentally sensitive. In particular, these fluorescent dyes are highly sensitive to viscosity or molecule aggregation. To achieve specific binding to glycoconjugates such as viscous mucins, fluorescent molecular rotor probe 9-[(2-cyano-2-hydroxylcarbonyl) vinyl]julolidine (CCVJ) was derivatized with boronic acids (BA) as they can reversibly bind diols. Here we characterized CCVJ-BA and its properties spectroscopically. This includes comparison of the E-/Z-isomers, determination of fluorescence lifetime and nanoviscosity, emission in protic and aprotic solvents, and binding to the hydrogel-forming mucin that is found in airway cells or in the gastrointestinal tract.

We show that similar to CCVJ [1], CCVJ-BA can be converted by light from the E- to the Z-isomer, of which only the E-isomer is fluorescent. Upon binding of the isomers to mucin, the bound CCVJ-BA appeared to be highly fluorescent, indicating that the E-isomer is preferentially found after mucin binding. We determined the binding constant for CCVJ-BA binding to mucins from the (porcine) gastrointestinal tract (6.5 µM) and from (bovine) submaxillary glands (13.5 µM). The binding constants indicate a slightly higher binding affinity to mucins from the gastrointestinal tract - probably reflecting a different expression of mucin types. Having established the binding to mucin, we aimed at measuring the nanoviscosity of mucin using ps-time-resolved fluorescence spectroscopy. We found high viscosity values above 300 cP using the Förster-Hoffmann equation for viscosity calibration [2]. The presented understanding of the photophysics and photochemistry of CCVJ-BA will foster future spatiotemporal studies of mucus viscosity in living cells and tissue.

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P116 WOLKE-HANENKAMP, STEFFEN

Steffen Wolke-Hanenkamp[1]; Dr. Maximillian Hafer[1]; Florian Eull[1]; Jan-Hannes Schäfer[1]; Dr. Dmitry Shvarev[1]; Dr. Ignacio Moraga Gonzalez PI[2]; Prof. Arne Möller[1]; Prof. Jacob Piehler[1]

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Structural and functional determinants governing constitutive dimerization of an oncogenic gp130 mutant

Class I cytokine receptors (CR) have important functions in the regulation of haematopoiesis, immunity and inflammation, and their dysregulation has been implicated in several types of cancer. A number of related mutations and deletions in the ectodomains (ECD) of the shared CR glycoprotein 130 (GP130) have been identified in patients with hepatocellular carcinoma (HCC), a prevalent form of liver cancer and one of the most common types of malignant tumours worldwide. These GP130 variants constitutively activate downstream signalling via Janus family tyrosine kinases (JAK) and signal transducer and activator of transcription (STAT), but the mechanistic bases of GP130 dysregulation are largely unclear. To tackle this challenge, we have here explored the role of receptor dimerization in signal activation and its dysregulation of GP130 in vitro and in live cells. To this end, we have employed dual-color single-molecule total internal reflection fluorescence (TIRF) microscopy in live cells to quantify receptor dimerization in the plasma membrane by co-tracking analysis. These studies revealed ligand-induced dimerization of wildtype (wt) GP130, but very strong constitutive dimerization of the oncogenic GP130 variant "deltaYY". This truncation lacks Y186-Y190 in the fibronectin III (FNIII)-type D2 domain of GP130, which is critically involved in ligand binding and therefore explain the loss in ligand binding in the mutated receptor chain. Cotransfection assays investigating the influence of associated JAK enforced the hypothesis of a dysregulation solely driven by the extracellular domain. Interestingly, deletion of D1 largely abrogated constitutive dimerization of GP130 deltaYY, suggesting that this domain is involved in the dimerization interface. This could be explained by domain-swapping that is frequently observed for proteins with □-sheet secondary structure such as FNIII, which was confirmed by modelling the dimer by AlphaFold. The purified ectodomains of GP130 wt and deltaYY produced in insect cells also behaved as monomers and dimers, respectively, when analyzed by size exclusion chromatography. Label-free analysis by mass photometric assays clearly identified a monomer-dimer equilibrium for GP130 deltaYY with an equilibrium dissociation constant of ~30 nM. Initial structural analysis by electron microscopy (cryo-EM) confirmed dimer formation, but also indicated loss of a rigid structural organization of the six subdomains that was observed for wt GP130.

P117 BEDNÁR, JUSTUS

Justus Bednár; Dr. Vanessa Maybeck; Prof. Andreas Offenhäusser,
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Surface plasmon resonance microscopy as a platform to assess cell-substrate separation using HEK293 cells as a model

The interface between cells and abiotic materials governs the performance of implants and other biomedical applications. A tight sealing between the cell membrane and an electrode for example is essential for the electronic coupling of bioelectronic implants as well as for in vitro studies of electrogenic cells. To optimize the cell-chip interface with regard to the contact geometry between cell and sensor, our prototype Surface Plasmon Resonance Microscopy (SPRM) setup is able to map the cell-substrate separation with a nanometer axial resolution and an almost diffraction limited lateral resolution, thereby being non-destructive, label free and fast. Unlike other optical methods for this purpose, our SPRM method doesn't rely on the assumption of a constant cytoplasmic refractive index, which can be measured alongside with the cell-substrate separation. In a first study, the adhesion of HEK293 cells is evaluated for gold electrodes with a variety of coatings. High-resolution images of single cell-substrate interfaces are acquired for each electrode coating. The adhesion of cells is assessed as the average cell-substrate separation from low-resolution scans across a multitude of cells. Histograms of cell-substrate separation are compared to data from transmission electron microscopy images of a previous study.

P118 BENNETT, TIMOTHY

Gunnar Kloes; Timothy Bennett; Alma Chapet-Batlle; Ali Behjatian; Andrew Tuberfield; Madhavi Krishnan, Oxford University

Far-field electrostatic signatures of macromolecular 3D conformation

In solution as in vacuum, the electrostatic field distribution in the vicinity of a charged object carries information on its three-dimensional geometry [1]. We report on an experimental study exploring the effect of molecular shape on long range electrostatic interactions in solution, by measuring the effective charge, q_m,of molecules using escape-time electrometry (ETe), see Fig.1. It has been shown that such effective charges, governing interaction energies, are quantitatively very similar to those calculated within charge renormalization theories [2, 3, 4]. Working with DNA nanostructures carrying approximately equal amounts of total charge, but each in a different three-dimensional conformation, we demonstrate that the geometry of the distribution of charge in a molecule has substantial impact on its electrical interactions. For instance, a tetrahedral structure, which is the most compact distribution of charge we tested, can create a far-field effect that is effectively identical to that of a rod-shaped molecule carrying half the amount of total structural charge (Fig. 1). These observations were compared to calculated electrostatic interaction energies of the nanostructures, calculated using an approach which has previously been shown to give remarkably good agreement with effective charges measured for a range of different biomolecules [4, 5]. These comparisons show that with precise measurements of electrostatic energies, such as those obtained using ETe, we are able to not only screen and identify 3D conformations of charged biomolecules in solution, but also shed light on finer-grained attributes such as spacings in ordered molecular charge distributions.

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P119 COLE, FIONA

Fiona Cole[1]; Martina Pfeiffer co-presenter; Dr. Dongfang Wang; Dr. Tim Schröder; Prof. Yonggang Ke; Prof. Philip Tinnefeld

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Controlled mechanochemical coupling in DNA origami arrays

Precisely controlled reaction cascades and allostery are hallmarks of cellular function and important in every biological system. Still, we are only starting to mimic these systems in the laboratory. Here, we introduce an approach to program such biological cascades based on the DNA origami technique. We use a DNA origami domino array structure which – upon addition of trigger DNA strands – undergoes a step-by-step conformational change resulting in a global transformation of the whole structure. We establish a single molecule assay that can characterize the individual steps of the transformation by placing two FRET probes at specific positions in the DNA origami array. With one-second time resolution, we show that most steps in the transformation cascade are strongly coupled and occur simultaneously. By introducing modifications in the structure, the energy landscape of the transformation process can be tailored to selectively weaken the coupling between individual steps and to induce delays. This makes it possible to engineer the energy landscape of the transformation cascade to include coupled and decoupled steps with different time lags between them. Besides altering the coupling between individual steps, we report approaches to globally change the kinetics of the whole transformation cascade and to generate combinations of reversible and irreversible steps. We then apply these approaches to release a cargo DNA strand at a predefined step in the transformation cascade to demonstrate the applicability of this concept in tunable cascades with mechanochemical coupling.

P120 HOLTMANNSPÖTTER, MICHAEL

Dr. Michael Holtmannspötter[1]; Dr. Timo Dellmann[2]; Isabelle Watrinet[1]; Eike Wienbeuker[1]; Prof. Dr. Ana J. García-Sáez[2]; Prof. Dr. Jacob Piehler[1]

[1] Universität Osnabrück; [2] CECAD Research Center

Reversibly labeled HaloTags enable live cell long-term high- and superresolution imaging

Self-labeling enzymes (SLE) such as the SNAP-tag or the HaloTag have emerged as excellent tools to visualize several cellular processes with high spatiotemporal resolution. However, covalent labeling via SLEs suffers from the limitation of a finite pool of labeled proteins and thus irreversible photobleaching. We circumvented this limitation by introducing two different mutations inside the HaloTag, which reintroduce its original dehalogenase functionality. We verified the reversible binding of dye conjugated HaloTag ligands (HTLs) to our two reHaloTag variants in vitro and in cells and obtained different turnover times of ~10 s and ~100 s, respectively. By combining our mutants with fluorogenic substrates, we demonstrate the capability for prolonged time-lapse imaging by confocal and STED microscopy, which was enabled by the dynamic exchange of the fluorophore. In particular for single molecule tracking and localization microscopy (TALM), reversible labeling by the reHaloTags turned out to be ideal to control labeling density at a constant level over extended time periods. We present several applications of these novel labeling tools for time-lapse volumetric imaging with resolution down to single molecule level.

P121 KRISHNASWAMY, VENKAT RAGHAVAN

Venkat Raghavan Krishnaswamy PhD; Susan Wagner PhD; Rico Barsacchi PhD; Moritz Kreysing PhD, Max Planck Institute of Molecular Cell Biology and Genetics.

Screening for GHOSTs (Genetically Enhanced Optically Superior Tissues).

The major limiting factor in live tissue imaging are the inherent light scattering properties of the tissue contributing to the opacity seen in most biological specimens. This significantly impedes our ability to observe and understand the plethora of events that takes place in a living tissue. But remarkably some organisms and living tissues are highly transparent in nature, examples range from several deep-sea creatures to the human retina. Comprehending the mechanisms by which tissue transparency is attained, and replicating it invitro even by few percent would unleash the full capabilities of the optical microscopes and enable us to study the physiological processes in an unprecedented resolution. Recently, we have shown that mammalian cells can be cultured using directed evolution to have less light scattering properties. Here, we propose to unravel the genetic basis of tissue transparency in three dimensional (3D) tissues. We use the immense potential of the high throughput screening and large content image analysis tools to successfully identify compounds that could enhance the optical properties of 3D spheroids. Specifically, we use pharmacologically active compounds to screen for pathways that impact light scattering properties. We further probe into the signaling pathways of these optically superior tissues to pinpoint the underlying molecular mechanisms. The transformative potential of this research would advance our understanding especially in the fields of developmental biology and in functional brain imaging.

P122 KUMPFE, FLORIAN

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[1] Bruker Nano GmbH; [2] Karlsruhe Institute of Technology (KIT)

Studying biomolecular dynamics and structure with high-speed atomic force microscopy

Studying the molecular dynamics and structural conformations is important for understanding the function and biological significance of samples ranging from single membrane proteins to complex macromolecular systems, and further developing appropriate therapeutic applications. Recent atomic force microscopy (AFM) developments have led to unprecedented imaging rates in fluid, enabling temporal resolution on the sub-20-milisecond scale. We will give three examples in which high-speed AFM was applied for studying of structural transitions and biomolecular dynamics in samples, containing bacteriorhodopsin (BR), annexin V (A5), and DNA origami nanostructures (DONs).

BR is a light-driven protein pump in some purple membrane-containing Halobacterium species, where it assembles in a characteristic trimeric bundle. We have studied the structural transition of the described trimers, assembled in a 2D protein crystal. We will demonstrate how photon absorption can lead to a conformational change in the BR trimers, as shown by the reversible transition between a photolyzed and non-photolyzed states.

A5 serves as an important regulator of membrane repair in eukaryotic cells, where it shows a strong Ca2+ binding affinity to phosphatidylserine. We have used high-speed AFM to study the 2D crystal formation in a model system containing supported lipid bilayers and A5 molecules. We demonstrate the lateral dynamics and preferred structural orientations of the mobile A5 trimers.

DONs have emerged as excellent molecular pegboards for the immobilization of ligands on surfaces to study receptor stimulation and early signaling events in adherent cells. These applications take advantage of the effective linkage between receptor ligands and the DONs through high-affinity biotin-streptavidin bridges. Here, we present high-speed AFM data obtained from DONs containing biotin binding sites, imaged in fluid in the presence of streptavidin and analyze the binding dynamics in the system.

P123 LOIDOLT-KRÜGER, MARIA

Evangelos Sisamakis; Marcelle König; Mathias Bayer; Matthias Patting; Marcus Sackrow; Kamil Bobowski; Fabio Barachati; Felix Koberling; Rainer Erdmann; Maria Loidolt-Krüger, PicoQuant GmbH

An easy and reliable way to perform single molecule FRET measurements

Single Molecule studies and – more specifically – single molecule FRET methodologies have become a standard tool for studying dynamic structural changes in proteins and nucleic acids. These types of measurements can reveal dynamic events on time scales covering several orders of magnitude from ~ns to several seconds. This allows studying e.g., chain dynamics, binding, folding, allosteric events, oligomerization and aggregation. The power of these methodologies is highlighted by the study of Intrinsically Disordered Proteins (IDPs) whose biological relevance has been increasingly studied over the recent years.

In this poster we show how easily these measurements can be performed with Luminosa single photon-counting confocal microscope and how all necessary correction parameters are automatically determined requiring no interaction from the user by employing methodologies benchmarked by the scientific community. We will also show how the variable PSF feature can be used in such measurements to fine-tune the observation window of freely diffusing biomolecules.

P124 MELNYK, INGA

Dr. Inga Melnyk, INM – Leibniz-Institute for New Materials

Nanomechanics of molecular motor molecules linked by self-assembled DNA building blocks

Manipulation of forces at molecular scale is of great interest to study living cell functionality in physiological environments. Here we present molecular machines that can apply forces to the cell matrix by using light as energy source. The key actuator is a light-driven rotary molecular motor linked via polymeric chains between the surface and the membrane receptors of the cell. The light-driven actuation induces mechanical twisting of the linker molecules, increases their entanglement, and finally applies a pulling force on the cell membrane. The measured force and time scales are at physiologically relevant magnitudes.[1] Self-assembled DNA building blocks are a powerful platform to design functional devices with nanoscale precision. Well-defined persistence and contour lengths make DNA constructs perfect linkers to study force scales applied by light-driven molecular motors. The pulling forces are monitored by Atomic Force Microscopy technique which provides crucial insides into the operation of these molecular machines but is limited to one measurement at a time. Therefore, parallelized single molecule force measurements with high throughput and piconewton precision are presented via Flow Force Microscopy.[2] Due to highly parallel arrangement of DNA tethered beads in microfluidic-based setup, forces can be measured simultaneously for hundreds of individual motor molecules.

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P125 MUKHOPADHYAY, ANTARA

Antara Mukhopadhyay; Dr. Maximilian Ulbrich, Albert-Ludwigs-University of Freiburg

Dual color subunit counting method using GFP/YFP overlapping spectra

X-ray crystallography, Cryo EM are most used method to determine protein structure. Although efficient, the methods determine protein stoichiometry in vitro. Besides, crystallography requires high density proteins and Cryo-EM uses detergents to solubilize proteins. If we want to study protein structure in its native environment, we need to use optical microscopy. The problem is, the size of proteins are far smaller (~2-5 nm) than the diffraction limit of optical microscope (~20 nm). Hence we cannot determine subunit composition spatially.

Subunit counting method, a robust method, uses photo-bleaching steps of fluorophores to decipher membrane receptor stoichiometry in its native environment [1]. The method counts bleaching steps of fluorophores to count subunits (if all the fluorophores are functional, number of bleaching steps will be directly proportional to the number of subunits present). mEGFP is quite widely used fluorophore in subunit counting as it is photo-stable and its bleaching step counting is easy. The bleaching step count from YFP is also good, it can be excited with the same laser as GFP and it gives emission in different spectral range than GFP but shares overlapping emission spectra. In this research, we established a method to bypass this disadvantage.

We used NMDA receptor, an obligatory dimer of dimer as positive control. We genetically fused YFP with GluN1 and GFP with GluN3A. We expressed them in Xenopus laevis oocytes and after 18-24 hour imaged them using TIRF microscopy in dual view mode. We have used a long pass filter (cutoff wavelength around 520 nm) to divide the emission spectra of GFP and YFP in two different channels.

Hypothesis used to determine a membrane receptor stoichiometry using this method is as follows:

We observed bleaching steps in both channels but with different step heights. This is because a small fraction of GFP emission that is above 520 nm can pass through the filter to be recorded in YFP channel (vice versa for YFP emission). Therefore, the intensity drop of a GFP bleaching step will be higher in GFP channel compared to the YFP channel and vice versa for YFP steps. We can use this difference in step heights to count number of GFP and YFP bleaching steps, which subsequently will be used to determine subunit composition of a receptor.

Here, we successfully reproduced the dimer of dimer stoichiometry of NMDA receptor using this method.

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P126 POLLMANN, CHRISTOPH

Christoph Pollmann; Dr. Maximillian Hafer; Prof. Dr. Jacob Piehler, Osnabrück University

Spatiotemporal dynamics of IL-17 family receptor signaling complexes in the plasma membrane

IL-17 family cytokines have emerged as central mediators of inflammatory and autoimmune conditions. While most family members remain understudied, therapeutic antibodies targeting IL-17A or IL-17 Receptor A have demonstrated high clinical efficacy in psoriasis, psoriatic arthritis and ankylosing spondylitis patients, indicating the high potential of IL-17 cytokines in the treatment of various diseases. A common feature of the IL-17 family cytokines is that all cytokines recruit the shared receptor subunit IL-17RA and a second chain that is assumed to mediate ligand specificity to initiate signaling. Despite being a critical step for the activation of signaling, the assembly of IL-17 cytokine family signaling complexes in the plasma membrane is poorly understood so far. Here, we have explored the spatiotemporal organization and dynamics of IL-17RA, IL-17RB and IL-17RC in live cells by single molecule fluorescence imaging techniques. To this end, dual-color single-molecule TIRF microscopy in combination with nanobody-based labeling was implemented to analyze co-localization and co-diffusion of individual receptor subunits. These studies suggest ligand-induced formation of homo- and heteromeric complexes with higher stoichiometries as compared to homo- and heterodimers that have been previously assumed. In line with recent structural studies, a novel receptor-receptor interface that mediates complex formation spatially distant from the ligand-binding site was confirmed by mutagenesis. Our results support a new paradigm for signaling complexes of the IL-17 family with formation of higher-order complex stoichiometries, which is orchestrated by distinct extracellular receptor interfaces.

P127 SCHEDLER, BENNO RÜDIGER

Benno Rüdiger Schedler; Olessya Yukhnovets; Prof. Dr. Jörg Fitter, RWTH Aachen University

Thermodynamic behaviour of EGFP-Booster-antibody-binding using brightness-gated two color coincidence detection

The thermodynamic behaviour of the antigen-antibody-binding between EGFP (antigen) and Booster (antibody) was analysed using brightness-gated two color coincidence detection (BTCCD). Antigen-antibody-bindings are of great interest for life-science and medicine. One of the most widely known examples is the production of antibodies by the immune system in order to prevent and resolute viral infections by binding to the viral macro molecule [1]. Antigen-antibody-bindings are highly affine and can have a binding constant KD in the pM regime. However, measurements of a binding constant at concentrations in the pM regime are challenging since there are only few suitable techniques like e.g. SPR and microcaliometry. Indeed, in contrast to BTCCD those techniques cannot determine the concentration accurately. BTCCD is an improvement of two color coincidence detection (TCCD), which is a single molecule technique based on fluorescence confocal detection. It measures coincidences of fluorescence molecules that enter the confocal detection volume. However, TCCD underestimates the binding fraction due to a mismatch of both confocal detection volumes. To circumvent this problem BTCCD has been introduced by H. Höfig et al. in 2019 [2]. In BTCCD only the bright bursts of molecules entering the detection volume are selected to calculate the binding fraction. This overcomes the underestimation of the binding fraction compared to normal TCCD measurements. Measurements of the KD at different temperatures reveal the thermodynamic behaviour of a binding, which describes the competition between its entropic and enthalpic part. The KD of the EGFP-Booster-binding ranges from well below 1 pM at 20°C up to above 8 pM at 45°C. An accurate determination of a binding KD prerequisites the measurement of a binding curve. For binding curves one measures the binding fraction at a fixed protein (here EGFP) concentration and different ligand (here Booster) concentrations around the binding constant [3].

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P128 SCHELLNHUBER, KORDULA

Kordula Schellnhuber[1]; Hanna Hübner; Markus Gallei; Roland Bennewitz; Johanna Blass

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Molecular Adhesion and Friction on Porous Membrane

Understanding and controlling the dynamics of polymer-surface interactions are key to design nanoscale objects and to understand biological processes. We study dynamic friction and adhesion at the solid-liquid interface by means of atomic force microscopy (AFM) with focus on entanglement dynamics. As a model system, a single M13mp18 DNA-molecule with a length of 2.5 µm is attached to a colloidal AFM probe using biotin-streptavidin interaction.

Friction measurements are performed by driving the cantilever laterally in parallel to the surface at a height of several hundred nanometers with different tip velocities.

During sliding, the adhesive interaction between the DNA molecule on the AFM tip and nanoporous membrane surface[1] causes a torsional bending of the cantilever. Modulation of the lateral and vertical deflection during sliding will reveal the adhesive interaction between the polymer and the membrane. Additional adhesion measurements are performed at a constant height of several hundred nanometer above the surface with varying waiting time. The retraction curve is analysed for rupture events typical for DNA-membrane interactions to reveal the entanglement kinetics.

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P129 TSUKANOV, ROMAN

Dr. Roman Tsukanov PhD[1]; Dr. Nazar Oleksiievets; Yelena Sargsyan; Jan Christoph Thiele; Nikolaos Mougios; Shama Sograte-Idrissi; Oleksii Nevskyi; Ingo Gregor; Felipe Opazo; Prof. Dr. Sven Thoms; Prof. Dr. Jörg Enderlein

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Fluorescence Lifetime DNA-PAINT for Multiplexed Super-resolution Imaging of Cells

DNA point accumulation for imaging in nanoscale topography (DNA-PAINT) is a powerful super-resolution technique highly suitable for multi-target (multiplexing) bio-imaging. However, multiplexed imaging of cells is still challenging due to the dense and sticky environment inside a cell. Here, we combine fluorescence lifetime imaging microscopy (FLIM) with DNA-PAINT and use the lifetime information as a multiplexing parameter for targets identification. In contrast to Exchange-PAINT, fluorescence lifetime PAINT (FL-PAINT) can image multiple targets simultaneously and does not requires any fluid exchange, thus leaving the sample undisturbed and making the use of flow chambers/microfluidic systems unnecessary. We demonstrate the potential of FL-PAINT by simultaneous imaging of up to three targets in a cell using both wide-field FLIM and 3D time-resolved Confocal Laser Scanning Microscopy (CLSM). FL-PAINT can be readily combined with other existing techniques of multiplexed imaging and is therefore a perfect candidate for high-throughput multi-target bio-imaging.

P130 WÖLL, DOMINIK

Prof. Dr. Dominik Wöll, RWTH Aachen University

Microgels as carrier systems for enzymes studied with a combination of different super-resolution fluorescence microscopy methods

Microgels are soft polymeric nanoparticles[1] that bear the possibility to carry and protect different catalysts and functions,[2] and allow for straight-forward catalyst recycling by separating the microgels from the reaction solution.[3] In contrast to solid particles which allow only access to their surface, the full 3D volume of microgels can basically be loaded with enzymes. This way, interesting catalytic cascade reaction can be realized in small volumes and, additionally, the (thermo)responsive behavior of microgels that are swollen at room temperature and collapse at elevated temperature could be exploited for further reaction control.

Further advances in the application of microgel-based enzymatic systems require a basic understanding of their properties and kinetics. Key questions are: How are enzymes distributed in microgels? What is their (local) activity and how is this related to their (local) environment? These questions can be generally addressed by fluorescence microscopy and spectroscopy. Since the typical microgel sizes are, however, beyond the diffraction limit of light, we used different localization-based 3D super-resolution fluorescence microscopy methods and correlated their results. Fluorescence-labelled enzymes were localized with "direct stochastic optical reconstruction microscopy" (dSTORM),[4] single enzymatic turn-overs of substrates to fluorescent products were investigated with "nanometer accuracy by stochastic catalytic reactions" (NASCA)[5] and the local environment in the microgels[6] was addressed with "points accumulation for imaging in nanoscale topography" (PAINT)[7] using Nile Red as solvatochromic dye.

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P131 YUKHNOVETS, OLESSYA

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Brightness-gated two-color coincidence detection for characterization of picomolar affinity bi-molecular interactions

The topic of specific bi-molecular interaction carries a lot of practical relevance as many biological processes, such as antigen-antibody recognition, enzyme reactions or protein synthesis are based on a specific molecular recognition. In order to describe the nature of the binding, the interaction should be quantified with the equilibrium constant, or KD value. The experimental measurements of KD value are well established for the millimolar to nanomolar concentration regime, but there are only a few methods that can access the low picomolar regime [1]. For example, the major method, surface plasmon resonance requires surface tethering and cannot explicitly monitor the concentration during the measurements, resulting in systematic errors. The brightness-gated two-colour coincidence detection (BTCCD) utilized principles of confocal fluorescence microscopy and can be applied for KD ~ 0.1-10 pM [2]. Although the application range is quite limited, BTCCD does not require surface tethering and can measure the concentration of diffusing molecules in the binding assay.

In the frame of this talk, dissociation-association reaction for two systems, GFP/GFP-Booster antibody and 24 base pairs complementary single strands DNA were studied. The equilibrium binding curve was measured by fixing the concentration of one binding partner and varying the concentration of the other, aiming to cover the range of tenfold above and below the KD. The equilibrium constants for both interactions, depending on the offered conditions (ionic strength of the buffer, temperature) varied from well below 1 picomolar to several picomolar.

Moreover, for high-affinity binding cases, the equilibration of the reaction can take several days to several weeks. However, maintaining the constant concentration at this low picomolar range becomes challenging as well [3]. The strategies and the importance of maintaining proper conditions for a reliable binding study experiment are discussed.

P132 ZOUBOULIS, KONSTANTIN C.

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Single immune-complex detection by Escape-Time electrometry

Antibodies are important biomarkers as they play key roles in disease and are also a successful class of drugs [1]. Existing methods for detection of antibodies such as immunoassays (enzyme-linked immunosorbent assay, ELISA; radioimmunoassay, RIA) and Western Blot are laborious and require several processing steps as well as multiple reagents [1,2]. Here, we present the detection of antigen-antibody protein binding events by Escape-Time electrometry (ETe). ETe enables the rapid measurement of the effective charge of single macromolecules in solution [3]. Fluorescently labeled single molecules are trapped in field-free electrostatic traps and their escape behavior is captured by wide-field fluorescence microscopy. The electrostatic traps are nanoscale features, fabricated in silica and embedded in a multitude of parallel channels in a microfluidic device. Negatively charged molecules experience local electrostatic potential minima in a free energy landscape generated by the negatively charged surfaces. We measure species-specific escape times in order to determine molecular effective charge. So far ETe experiments are mainly performed on nucleic acids apart from pioneering protein ETe measurements [3]. In this study we examine a system consisting of a human IgG antibody and a fluorescently labeled antigen 'bait'. We first determined the effective charge of our antigen molecules that are fluorescently labeled with ATTO 532 dye. Subsequently, we labeled and characterized a human IgG antibody. Finally, we determined the escape times of a mixture of fluorescently labeled antigen and unlabeled antibody in order to distinguish between unbound/free antigens and antigen-antibody protein complexes. Our results suggest that ETe could be deployed as a simple, rapid and highly sensitive approach for in-solution detection of antibodies using fluorescently labeled antigen bait.

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