SFB 1078

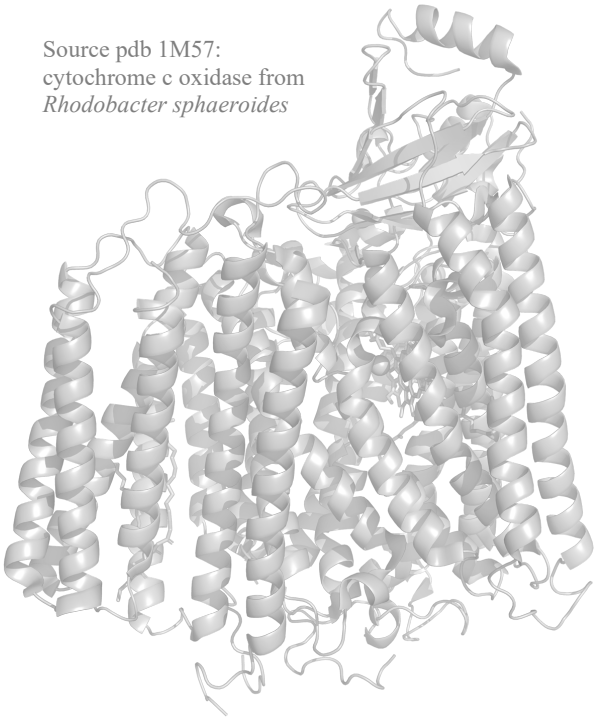
Protonation Dynamics in Protein Function

Final Symposium of the CRC 1078

Collaborative **R**esearch **C**enter / **S**onder**f**orschungs**b**ereich 1078
2013 - 2024

1.-3. September 2024

Berlin



Source pdb 1M57:
cytochrome c oxidase from
Rhodobacter sphaeroides

Updated version from August 29, 2024.

Content:

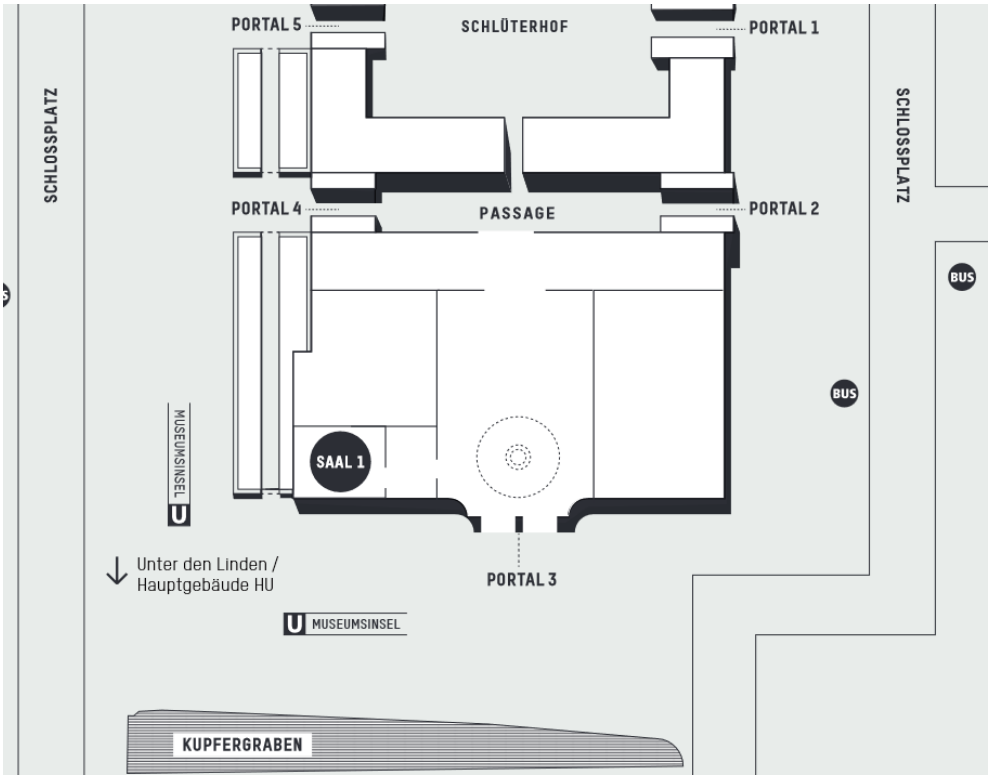
How to get there (1)

1. **Humboldt Forum (*Opening event 1.9.2024*)**
   Address: Saal 1, Schloßplatz 1, 10178 Berlin (entrance via Portal 3)

- **Coming from the Harnack-Haus** use the U3 (direction Warschauer Str.) to station Nollendorfplatz and switch to U2 (direction Pankow) to station Spittelmarkt and short walk (800 m) along the (beautiful) *Kupfergraben* to the venue

- **Optional directly to Museumsinsel next to the venue**: use the U3 (direction Warschauer Str.) to station Hallesches Tor switch to U6 (direction Kurt-Schumacher-Platz) to station Unter den Linden and switch again to U5 (direction Tierpark) to reach Museumsinsel

For alternative routes visit www.bvg.de.



Source: Stiftung Humboldt Forum im Berliner Schloss

Important Information

Please note that entry to the event will only be granted with a personal QR code, which is printed on your registration confirmation.

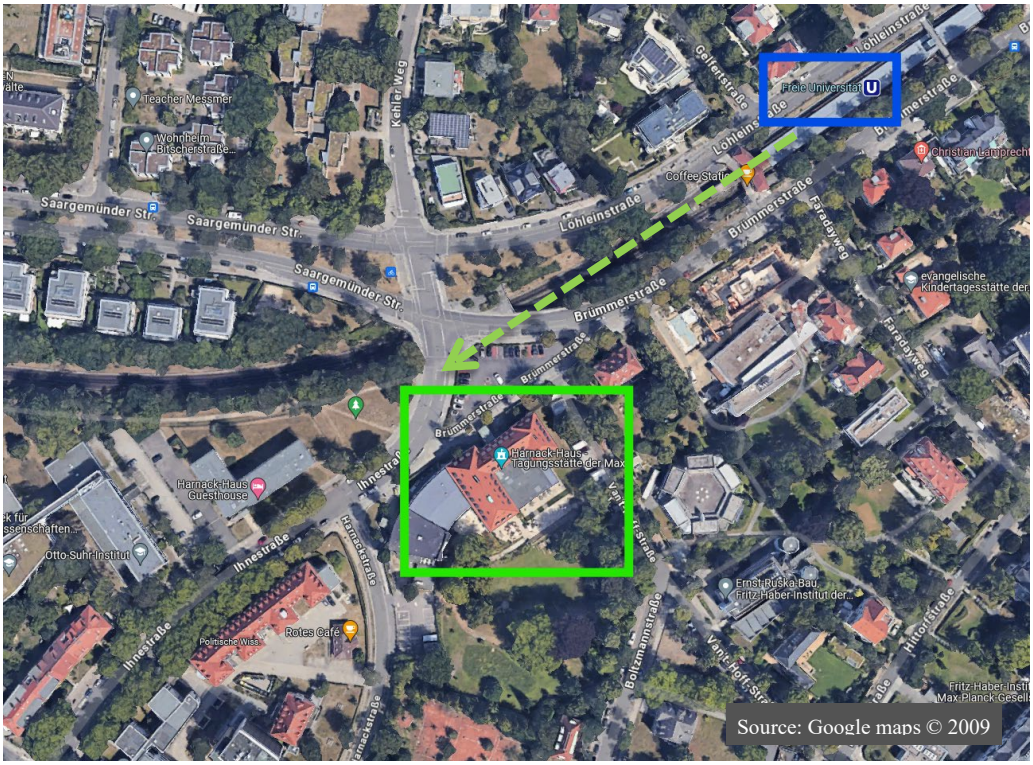
For security reasons, only small bags (smaller than A4 size) and no coats are permitted inside the Berliner Schloss.

A complimentary wardrobe service is available downstairs with sufficient space for luggage.
Lockers are also available for use with a refundable deposit of 1 or 2 €.

How to get there (2)

2. Harnack-Haus (Conference 2.9. and 3.9.2024)

Address: Conference venue of the Max Planck Society, Ihnestr. 16-20, 14195 Berlin



The nearest underground station is **U3 Freie Universität (Thielplatz)** [blue box]: leave the station in train's travel direction and take the left exit (on Brümmerstr.), turn right (south-east) and walk for 170 m; the hotel is on the left side of the street [green box].

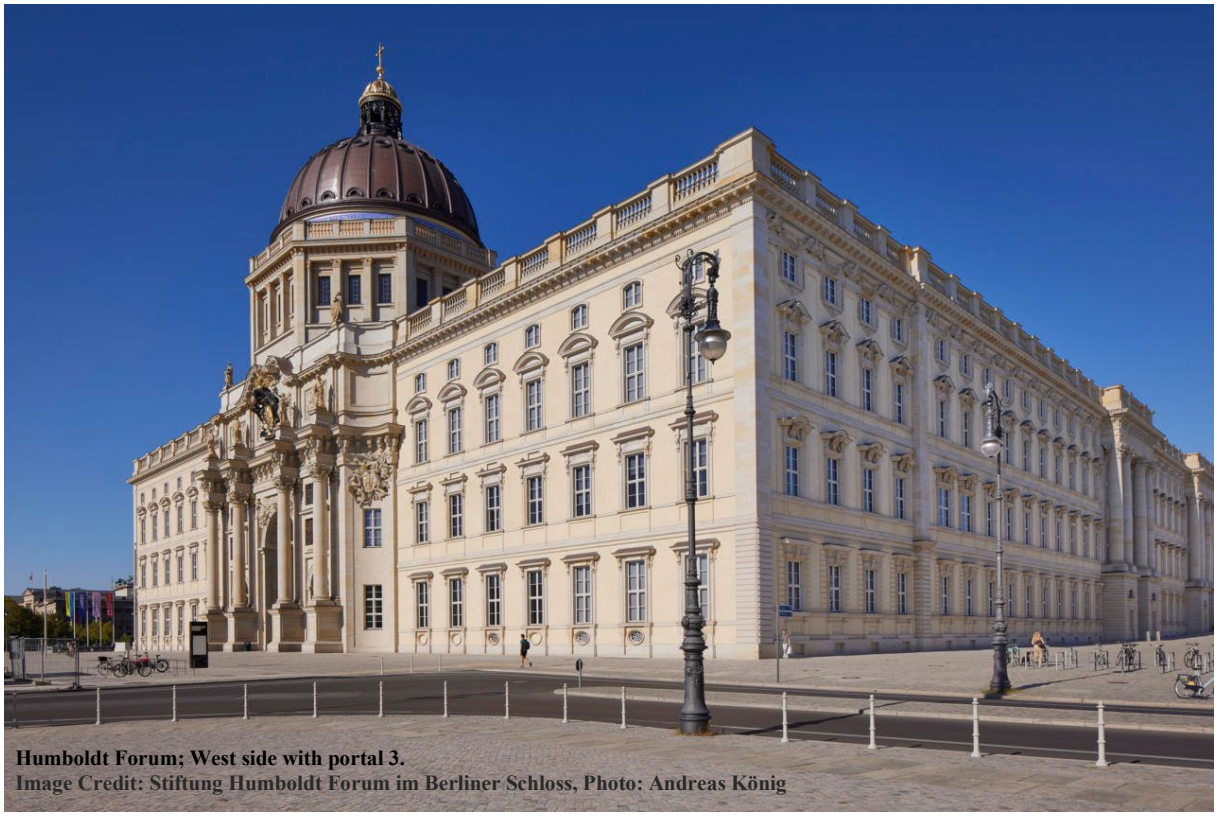
- **Coming from the city**: take U3 (direction Krumme Lanke) to train station Freie Universität (Thielplatz).

- **From BER airport to Freie Universität (Thielplatz):** There are several ways to arrive at and depart from the BER airport. Check out the up to date information at the BER website or the VBB Travel Information.

  One Option: take train (RE7, RB14, RB22, or FEX) towards Hauptbahnhof and get off S Ostkreuz Bhf, change to Ringbahn (S-Bahn) S 41 (⏲) and get off at S+U Heidelberger Platz, change to subway U3, direction of Krumme Lanke, alight at Freie Universität (Thielplatz).

- **From Hauptbahnhof (central station) to Freie Universität (Thielplatz)**: take train S7 (direction of Potsdam) or S75 (Westkreuz) or S5 (Spandau), get off train at Zoologischer Garten, change to subway U9, direction of Rathaus Steglitz, get off subway U9 at Spichernstr., change to subway U3, direction of Krumme Lanke, alight at Freie Universität (Thielplatz).

  For further information on public transportation visit www.bvg.de



Humboldt Forum; West side with portal 3.
Image Credit: Stiftung Humboldt Forum im Berliner Schloss, Photo: Andreas König

Sunday, September 01, 2024

Festive Opening Evening at the Humboldt Forum

From 17:30 — *Admission to the Humboldt Forum in the Berlin Schloss*
--- Please bring your QR code ---

Welcome address

18:00 **Joachim Heberle and Maria Andrea Mroginski**
Welcoming by the Speakers of the CRC 1078

18:20 **Holger Dau**
Résumé Graduate School CRC 1078

Opening talk

18:30 **Menachem Gutman and Esther Nachliel, Tel Aviv University**
Tracing a proton at three-time domains

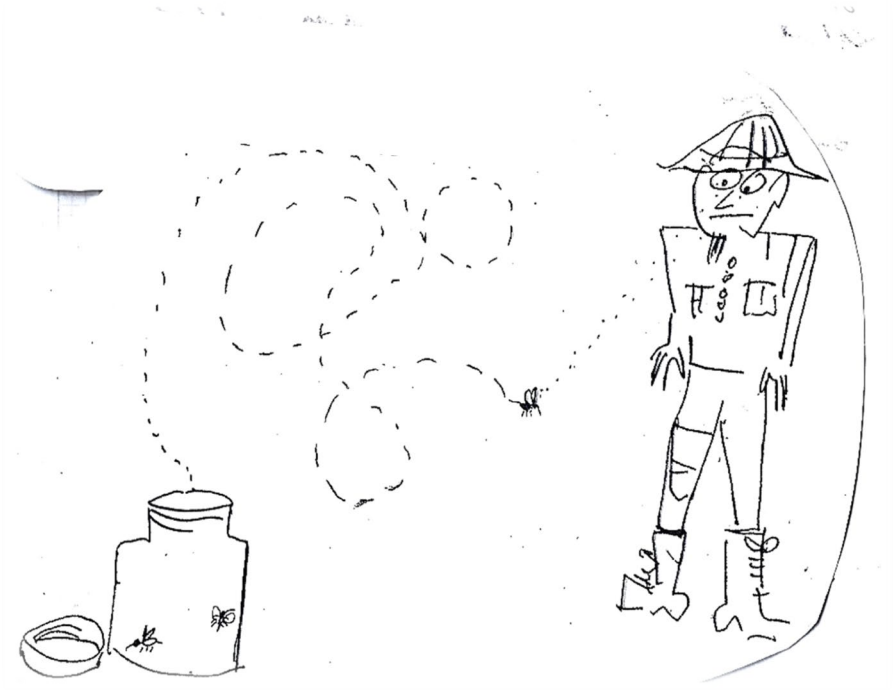
Reception 19:00 – 21:00

Tracing a proton at three-time domains

Menachem Gutman and Esther Nachliel

Biochemistry, Tel Aviv University, Israel.

Once the Proton Motive Force (introduced by Peter Mitchell) was accepted by the bioenergetic community as the mode of energy transfer in cellular metabolism, it became essential to understand the interaction of protons with protein at molecular-time resolved level. At present, the dynamics of proton-solvent-protein-surface interactions are recorded at three-time frames: i. The ps-ns domain; the reaction is initiated by photo-activation of photoacid and the distance covered by a diffusing proton is within the nm scale. ii. The µs time scale observations where the proton is in equilibrium with its environment and the dynamics is compatible with classical chemical kinetic equations. iii. The sub-second sub-mm dimensions experiments are initiated by adding excess acid at one location and the appearance of the protons is monitored at a remote site (~100 µm). The first two methods record the microscopic molecular events with a molecular resolution of the events initiated by the random motion of protons. The third one is a gradient driven macroscopic observation, which reflects macroscopic process besides random walk diffusion.



Detecting a proton far from the source Credit: Menachem Gutman

Monday, September 2, 2024

Conference at the Harnack-Haus

From 08:00	**Planck-Lobby**	**Registration: Please bring sufficient time to receive your booklet, name tag and confirm your attendance.**
09:00 - 09:40	Goethe Saal	**Sharon Hammes-Schiffer**, Princeton University, USA *Proton-Coupled Electron Transfer in Enzymes and Photoreceptor Proteins*
09:45 - 10:10	Goethe Saal	**Inez Weidinger**, TU Dresden, GER *Electron driven protonation in cytochrome c oxidase (CRC 1078)*
10:10 - 10:30	**Planck-Lobby**	**Continued Registration and Coffee Break**
10:30 - 11:10	Goethe Saal	**Junko Yano**, Lawrence Berkeley National Laboratory, USA *Water Oxidation Reaction in Natural Photosynthesis*
11:15 - 11:55	Goethe Saal	**Leonardo Guidoni**, Università degli Studi dell'Aquila, ITA *Protons and electrons movements along intermediate states of oxygenic photosynthesis*
12:00 - 13:30	**Laue Saal**	**Lunch Break**
13:30 - 13:55	Goethe Saal	**Rana Hussein**, Humboldt Universität zu Berlin, GER *Tracking the events in photosystem-II water oxidation by time-resolved spectroscopy and atomic-resolution structural biology (CRC 1078)*
14:00 - 14:40	Goethe Saal	**Ilme Schlichting**, Max Planck Institute for Medical Research, GER *Mechanism and dynamics of fatty acid photodecarboxylase*
14:40 - 15:00	**Planck-Lobby**	**Coffee Break**
15:00 - 15:40	Goethe Saal	**Steven Boxer**, Stanford University, USA *Evolving understanding of electric fields and enzyme catalysis*
15:45 - 16:25	Goethe Saal	**Shy Arkin**, The Hebrew University of Jerusalem, ISR *Viral ion channels: from biophysical models to drug targets*
16:30 - 16:55	Goethe Saal	**Jacek Kozuch**, Freie Universität Berlin, GER *Proton/Ion Channels in Viruses – Understanding the Protonation-Dependent Structure and Function of Viroporins (CRC 1078)*
17:00 - 18:30		**Break for activities** Put up your poster, join us at a guided tour or activity games.
18:30 - 20:00	**Laue Saal**	**Conference Dinner**
19:55	Terrace	Group picture
20:00 - 20:45	Meitner Saal	Poster session ODD numbers
20:45 - 21:30	Meitner Saal	Poster session EVEN numbers
21:30 - open end	**Einstein-Lounge**	**Social get together**

Social activities | Monday

Join us in the gardens of the Harnack House from 5pm to 6:30pm for relaxation and games like table tennis, or a guided tour of the Dahlem Campus.

Sign-Up for a tour on the list at the registration desk (up to 40 spaces).

A Guided Tour: 100 Years of Science in the German Oxford

- **Start:** 5:00 pm
- **Duration:** Up to 90 minutes
- **Language:** English
- **Meeting Point:** Harnack House in front of the main entrance
- **Guide:** Mr. Werner

B Guided Tour: History of the founding of the Dahlem Campus and Freie Universität

- **Start:** 5:00 pm
- **Duration:** Up to 60 minutes
- **Language:** English
- **Meeting Point:** Harnack House in front of the main entrance
- **Guide:** tba

Weather: Tours take place "rain or shine". Umbrellas are available for loan.

Tuesday, September 3, 2024

Conference at the Harnack-Haus

From 08:00	**Planck-Lobby**	**Help desk**
09:00 - 09:40	Goethe Saal	**Hideki Kandori**, Nagoya Institute of Technology, JPN *FTIR spectroscopy of rhodopsins*
09:45 - 10:25	Goethe Saal	**Massimo Olivucci**, Università degli Studi di Siena, ITA *From color-tuning to optogenetics: relationship between red-light absorption and fluorescence intensity in an archaerhodopsin model*
10:30 - 11:00	**Planck-Lobby**	**Coffee Break**
11:00 - 11:25	Goethe Saal	**Joachim Heberle**, Freie Universität Berlin, GER *Proton-coupled ion transfer in channelrhodopsins and related rhodopsins (CRC 1078)*
11:30 - 12:10	Goethe Saal	**John Kennis**, Vrije Universiteit Amsterdam, NL *Reaction dynamics and mechanisms of newly discovered bistable microbial rhodopsins*
12:10 - 13:30	**Laue Saal**	**Lunch Break**
13:30 - 13:55	Goethe Saal	**Karsten Heyne**, Freie Universität Berlin, GER *Phytochromes - Structure, hydrogen bonds, and function (CRC 1078)*
14:00 - 14:40	Goethe Saal	**Janne Ihalainen**, University of Jyväskylä, FIN *Ultrafast Protein Response on Biliverdin Excitation in a Bacteriophytochrome*
14:40 - 15:00	**Planck-Lobby**	**Coffee Break**
15:00 - 15:25	Goethe Saal	**Maria Andrea Mroginski**, Technische Universität Berlin, GER *Unravelling Protonation Dynamics in Proteins: Theoretical Insights and Contributions (CRC 1078)*
15:30 - 16:00	**Goethe Saal**	**Closing remarks**
	Meitner Saal	**Farewell ceremony**

Posters

#	PRESENTER	TITEL
1	Federico Baserga	Cytochrome c Oxidase's conformational dynamics monitored through the bilayer's packing state
2	Stephan Block	Probing the gradient-dependent activity of cytochrome c oxidases at the single enzyme level
3	Janosch Brandhorst	High Time-Resolution P680+/TyrZ Electron Transfer Kinetics Observed by SF-IR
4	Florian Brünig	Ultrafast transient spectral signatures and excess-proton waiting and transfer-path dynamics
5	Jheng-Liang Chen	The Photoreaction of the Proton-Pumping Rhodopsin 1 from the Maize Pathogen Basidiomycete *Ustilago maydis*
6	M. Yahia Dekmak	High time-resolution IR study of the Photosystem-II reaction cycle
7	Manal Ebrahim	Structural Characterization of Phytochromes
8	Linda Feuerstein	Nitrile Groups as Build-in Sensors for Vibrational Stark Effect in Electrocatalytically Active Nitrogen-rich Carbon Materials
9	Ofer Filiba	Photoisomerization of deprotonated Retinal Schiff Base
10	Markus Göbel	Spectro-electrochemical investigation of CcO in nanodiscs and a synthetic mimicry
11	Paul Greife	Tracking water oxidation through time-resolved FTIR Spectroscopy
12	Olli Hart	Methanol Binding Insights in Photosystem II via XFEL Crystallography
13	Luisa Herder	Structural snapshot of a bacterial phytochrome in functional intermediate states
14	Jon Hughes	Pr and Pfr structures of plant phytochrome A
15	Petra Imhof	Interplay of Hydration, Water Mobility, and Proton Transfer in Cytochrome c Oxidase
16	Jesse Jones	Estimation of pKa values in membrane bound proteins
17	Ana P. L. Gamiz Hernandez	Mechanistic principles of proton and electron transfer in mycobacterial supercomplex III2IV2
18	Kirill Kovalev	4D structural studies of the light-driven sodium pump *Er*NaR
19	Mariafrancesca La Greca	Proton dynamics in the light-driven bacterial chloride pump NmHR
20	Pit Langner	Potential-Induced Time-Resolved Surface-Enhanced Difference Absorbance Spectroscopy on Surface-Bound Cytochrome c using Quantum Cascade Lasers
21	Louis Lehmann	Spectroscopic signatures of excess protons in bulk and at interfaces
22	Haoran Liu	Investigation of Nonselective Cation Permeation through CNGA1 Channels by MD Simulations
23	Victor M. Loyo Cruz	Effect of an essential arginine mutation (R473C) on the catalytic reaction of cytochrome c oxidase from *Paracoccus denitrificans*
24	Laura H. Mages	Analysis of the binding behaviour between the electron carrier cytochrome c6 and Photosystem I
25	Sarah M. Mäusle	Substrate water insertion in the S3 → S0 transition traced by time-resolved IR spectroscopy in D1-N298A PSII variant
26	Swantje Mohr	Structural Studies of the Activation Mechanism of M2 using solid state NMR and surface-enhanced infrared absorption spectroscopy
27	Boris Musset	Somatic HVCN1 mutations in cancer: Potential deleterious passenger mutations in the S4
28	Ronja Paschke	Combining vibrational and computational spectroscopy to elucidate the mechanism of the channel opening of Influenza A virus M2
29	David Rosenberger	Joint Forces: Hypothesis generation for transmembrane protein oligomerization by combining AlphaFold2 and MARTINI3
30	Patricia Saura	Electric field effects on the proton transfer reactions in cytochrome c oxidase
31	Miriam Schramm	Oxygen evolution kinetics in far-red adapted Photosystem II variants studied by time-resolved oxygen polarography
32	Luiz Schubert	Proton Release Reactions in the Inward H+ Pump Xenorhodopsin

Poster 1

Cytochrome c Oxidase's conformational dynamics monitored through the bilayer's packing state

Federico Baserga[1], Luiz Schubert[1], Pit Langner[1], Julian Storm[2], Antreas Vorkas[2], Ramona Schlesinger[2] and Joachim Heberle[1]

[1]Experimental Molecular Biophysics, Institute of Experimental Physics, Freie Universität Berlin, Germany

[2]Genetic Biophysics, Institute of Experimental Physics, Freie Universität Berlin, Germany

In nature, integral membrane proteins need a lipidic support in order to perform their function. It is well known that the membrane composition, its curvature, and its lateral pressure can influence the density and function of membrane proteins: Be it in mitochondria, thylakoids, bacteria, or eukaryotic plasma membranes. We show that, for Cytochrome c Oxidase, the opposite effect is also true, as the function of this integral membrane protein also influences lipid packing parameters [1]. Using infrared absorption spectroscopy, we analyze the steady-state and time-resolved influence of protein activation on the lipids immediately surrounding Cytochrome c Oxidase from *Rhodobacter sphaeroides*. The causality rapport is investigated by comparing the lipid behavior with the protonation reactions of specific amino acids and the response of the cofactors during the electronic forward- and back- reactions [2]. Controls are provided monitoring either isotropic thermodynamic parameters, or the local lateral pressure of the membrane thanks to a system employing photoswitchable lipids [3].

1. F. Baserga, A. Vorkas, F. Crea, L. Schubert, J.-L. Chen, A. Redlich, M. La Greca, J. Storm, S. Oldemeyer, K. Hoffmann, R. Schlesinger, J. Heberle, Membrane Protein Activity Induces Specific Molecular Changes in Nanodiscs Monitored by FTIR Difference Spectroscopy, Front Mol Biosci, 9 (2022).
2. P. Ädelroth, P. Brzezinski, B.G. Malmström, Internal Electron Transfer in Cytochrome c Oxidase from Rhodobacter Sphaeroides, Biochemistry, 34 (1995) 2844-2849.
3. J. Morstein, A.C. Impastato, D. Trauner, Photoswitchable Lipids, ChemBioChem, 22 (2021) 73-83.

Poster 2

Probing the gradient-dependent activity of cytochrome c oxidases at the single enzyme level

Stephan Block

FU Berlin, Physical Chemistry, Arnimallee 22, 14195 Berlin, Germany

Heme-copper oxidases (HCOs), such as the cytochrome c oxidase, are terminal oxidases in the respiratory chain and contribute to the generation of an electrochemical gradient across the membrane, which is consumed by ATP synthases to generate ATP from ADP. Due to their high relevance for the bioenergetics of cells, these proteins have been extensively studied, yielding important insights in the intricate interplay of electron uptake, oxygen reduction, and proton translocation, all of which take place in a redox reaction that finally leads to reduction of oxygen to water. Previous research, however, was mainly focused on the turnover of oxygens and electrons by HCOs, while studies reporting the proton turnover rate, that is the rate of proton uptake by the enzyme, are scarce. This talk I will discuss results obtained by a proteoliposome-based assay, in which single cytochrome c oxidases are reconstituted into liposomes containing a pH sensitive dye. This setup allows for quantifying changes of the luminal pH value of single proteoliposomes, which are caused by the proton turnover of single cytochrome c oxidases. Calibration of the physicochemical properties of the proteoliposomes (size, buffering capacity, and proton permeability of the lipid membrane) allows to quantify the proton uptake rate of single enzymes in dependence of the acting proton gradient. The impact of the lipid composition on the activity of single cytochrome c oxidases will be discussed and it will be shown that the dynamics of these enzymes can exhibit a surprisingly complex behavior.

Poster 3

High Time-Resolution $P680^+$/TyrZ Electron Transfer Kinetics Observed by SF-IR

Janosch Brandhorst, Yahia Dekmak and Holger Dau

FU Berlin, Physik, Arnimallee 14, 14195 Berlin, Germany

Photosystem II (PSII) is a cofactor-protein complex that enables plants, algae and cyanobacteria to use water as a source of electrons and protons, using light as an energy source. Dioxygen is produced as a by-product of this water oxidation process. The reaction center of PSII contains a special chlorophyll called P680 which, when light is absorbed by the pigments of PSII, enables charge separation, producing $P680^+$ and providing an electron that can eventually be used for photobiochemistry. Another component of the reaction center of PSII called TyrZ later reduces $P680^+$ again, making it available for further excitation processes. This occurs on a time scale of nanoseconds to microseconds. A suitable method to analyze electron and proton transfer reactions is infrared spectroscopy. Here we present time-resolved single-frequency infrared spectroscopy (SF-IR) measurements that elucidate this process. We use a home-built quantum cascade laser (QCL)-based SF-IR setup with an automatic sample changer to track changes in the absorbance of PSII-enriched membrane particles after laser flash excitation. With this setup, we can perform SF-IR measurements with high time resolution at different wavenumbers, which are characterized by a very good signal-to-noise ratio. We illustrate how we improve the temporal resolution of our setup by applying an approach that takes into account several properties of the measurement hardware. This is done by characterizing the temporal response of our setup to a given signal through an instrument response function (IRF). A deconvolution of our measured signal and the IRF is then performed to extract temporally resolved changes in absorbance from our data that can resolve processes below 100 ns. In this way, we can observe phenomena that are within the 10 – 90 % rise time of our measurement hardware [1]. With this approach, we are able to observe a multiphase, S-state dependent process of TyrZ oxidation by electron transfer to $P680^+$ with rate constants from ~10 ns to 10 µs. These phases are discussed in the context of recent crystallographic snapshot data collected at various times after laser-flash excitation [2].

1. Dekmak MY, Mäusle SM, Brandhorst J, Simon PS, Dau H. Tracking the first electron transfer step at the donor side of oxygen-evolving photosystem II by time-resolved infrared spectroscopy. Photosynth Res. 2023 Nov 23.
2. Li, H., Nakajima, Y., Nango, E. et al. Oxygen-evolving photosystem II structures during S1–S2–S3 transitions. Nature 626, 670–677 (2024).

Poster 4

Ultrafast transient spectral signatures and excess-proton waiting and transfer-path dynamics

Florian Brünig[1], Manuel Rammler[2], Ellen M. Adams[3], Martina Havenith[3], Karsten Heyne[2] and Roland R. Netz[2]

[1]*University of Luxembourg, Department of Physics and Materials Science, 162a avenue de la Faïencerie, 1511 Luxembourg, Luxembourg*

[2]*Freie Universität Berlin, Physik, Arnimallee 14, 14195 Berlin, Germany*

[3]*Ruhr-Universität Bochum, Department of Physical Chemistry II, 44780, Bochum, Germany*

Signatures of solvated excess protons in infrared difference absorption spectra, such as the continuum 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. 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. The continuum band in the 2000 cm^{-1} to 3000 cm^{-1} range is shown to be due to normal-mode oscillations of temporary H_3O^+ complexes. The proton transfer between two water molecules 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 agrees with a band at 1200 cm^{-1} of experimental mid-infrared spectra. The analysis guides the interpretation of our experimental ultrafast infrared spectra of proteins upon photoexcitation. We provide results of QM/MM simulations accounting for excited-state dynamics of the chromophore to elucidate the long-reaching impulsive field effects on the transient spectral signatures.

1. Yang, Y. et al. (2022). Ultrafast proton-coupled isomerization in the phototransformation of phytochrome. Nature Chemistry 14, 823–830.
2. Brünig, F. N., Rammler, M., Adams, E. M., Havenith, M. & Netz, R. R. (2022). Spectral signatures of excess-proton waiting and transfer-path dynamics in aqueous hydrochloric acid solutions. Nature Communications 13, 4210–4210.
3. Brünig, F. N., Hillmann, P., Kim, W. K., Daldrop, J. O. & Netz, R. R. (2022). Proton-transfer spectroscopy beyond the normal-mode scenario. The Journal of Chemical Physics 157, 174116.

Poster 5

The Photoreaction of the Proton-Pumping Rhodopsin 1 from the Maize Pathogen Basidiomycete *Ustilago maydis*

Jheng-Liang Chen, Mariafrancesca La Greca, Luiz Schubert, Jacek Kozuch, Tim Berniser, Joachim Heberle and Ramona Schlesinger

FU Berlin, Physik, Arnimallee 14, 14195 Berlin, Germany

Microbial rhodopsins have recently been discovered in pathogenic fungi and have been postulated to be involved in signalling during the course of an infection. Here, we report on the spectroscopic characterization of a light-driven outward proton pump rhodopsin (*Um*Rh1) from the smut pathogen *Ustilago maydis*, the causative agent of tumors in maize plants. Time-resolved UV/Vis and vibrational spectroscopy indicate a pH-dependent photocycle. The photocycle upon pH change in *Um*Rh1 is significantly different from the well-known bacteriorhodpsin from archaea (*Hs*BR). We applied site-directed mutagenesis on *Um*Rh1 base on the structure and sequence alignment, in order to understand this different behavior of proton pumping. We also characterized the impact of the auxin hormone indole-3-acetic acid that was shown to influence the pump activity of *Um*Rh1 on individual photocycle intermediates. A facile pumping activity test was established of *Um*Rh1 expressed in *Pichia pastoris* cells, for probing proton pumping out of the living yeast cells during illumination. We show similarities and distinct differences to *Hs*BR and discuss the putative role of *Um*Rh1 in pathogenesis.

Poster 6

High time-resolution IR study of the Photosystem-II reaction cycle

Mohamad Yahia Dekmak, Janosch Brandhorst, Sarah M. Mäusle and Holger Dau

FU Berlin, Physik, Arnimallee 14, 14195 Berlin, Germany

In photosystem II (PSII), light drives the oxidation of P680, a protein-bound chlorophyll unit. $P680^+$ oxidizes the oxygen-evolving complex (OEC), consisting of a Mn_4Ca-oxo cluster and surrounding amino acids. Driven by 4 photons, the OEC accumulates the oxidizing equivalents needed to split 2 water molecules, releasing O_2 as a by-product. Infrared (IR) spectroscopy is powerful in investigation of these processes. Monitoring kinetics at the amide-II band provides insight into structural or electrostatic changes coupled to electron and proton transfers (ET, PT). Time-resolved single-frequency IR spectroscopy (TR-SFIR) is a suitable method to monitor difference in absorbance after laser-flash excitation. The IR source is a quantum cascade laser tunable to single frequency between 1300-1900 cm^{-1}. The excitation laser is a frequency doubled Nd: YAG 532 nm with high time-resolution. A home-built automated sample changer allows us to acquire a large amount of data with exceptionally high SNR. A sequence of saturating laser flashes advances the sample through the S-state cycle. Time-resolved spectral information was obtained by monitoring IR difference in absorbance of PSII membrane particles after each excitation flash in the range from 1310 to 1890 cm^{-1} with a resolution of $\sim$ 2 cm^{-1}. This allows to monitor and study IR spectra of fast processes, such as the first electron transfer step at the doner side in PSII membrane particles. A broad upshift above 1750 cm^{-1} was observed and related to $P680^+$ kinetics. The transients in the region between 1750 to 1884 cm^{-1} were averaged and fitted with a sum of exponentials [1]. In addition, we monitored the flash-induced difference in absorbance of spinach PSII membrane particles at the amide-II band at 1544 cm^{-1} [2], allowing us to extract rates for several processes, experiments at 5-30 °C enable estimation of activation energies. The activation energy for a crucial PT step in the $S_2 \rightarrow S_3$ transition agrees with values from the carboxylate-stretching region [3], obtained by constraining the subsequent ET rate to literature values. Steps to solve the significance problem in multi-exponential simulations of single-frequency transients are discussed. In the $S_3 \rightarrow S_0$ an activation energy of ~260 meV was determined, agreeing with earlier results [4]. We conclude that IR absorption changes of the amide-II band reflect various ET and PT kinetics in the PSII photocycle.

1. Dekmak, M.Y., Mäusle, S.M., Brandhorst, J. et al. (2023) Photosynth Res.
2. Noguchi et al. (2003) Biochemistry 42, 6035-6042.
3. Mäusle et al. (2020) J. Chem. Phys 153, 215101.
4. Klauss et al. (2012) PNAS 109, 16035-16040.

Structural Characterization of Phytochromes

Manal Ebrahim[1], Luisa Herder[1], Andrea Schmidt[1], Michal Szczepek[1], Norbert Michael[2], Peter Hildebrandt[2] and Patrick Scheerer[1*]

[1]*Charité – Universitätsmedizin Berlin, Institute for Medical Physics and Biophysics, Group Structural Biology of Cellular Signaling, Chariteplatz 1, 10117 Berlin, Germany*

[2]*Technische Universität Berlin, Institute of Chemistry, Sekr. PC14, Straße des 17. Juni 135, 10623 Berlin, Germany*

**Correspondence address: patrick.scheerer@charite.de*

Phytochromes are modular photoreceptors that use red light as source of information to mediate various physiological reactions in different organisms such as plants, cyanobacteria, fungi and heterotrophic bacteria [1]. The photoswitchable protein usually consists of the highly conserved N-terminal **P**hotosensory **C**ore **M**odule (**PCM**=PAS+GAF+PHY) and a more variable C-terminal output module, which is mostly a histidine kinase [1, 2]. A light-sensitive billin chromophore is incorporated into PAS and GAF domain. It converts reversibly between two stable conformations, the far-red light absorbing Pfr state and the red light absorbing Pr state. The exact mode of action of histidine kinase regulation by light is not known for any bacterial phytochrome system. Our aim is therefore to identify the native structural assembly of bacterial phytochromes in their full-length form and to investigate how the light signal is transmitted through the chromophore via the PHY tongue to the histidine kinase at the output module. Here the bacterial phytochromes Agp1 and Agp2 from *Agrobacterium fabrum* are in the focus of our structural biology studies.

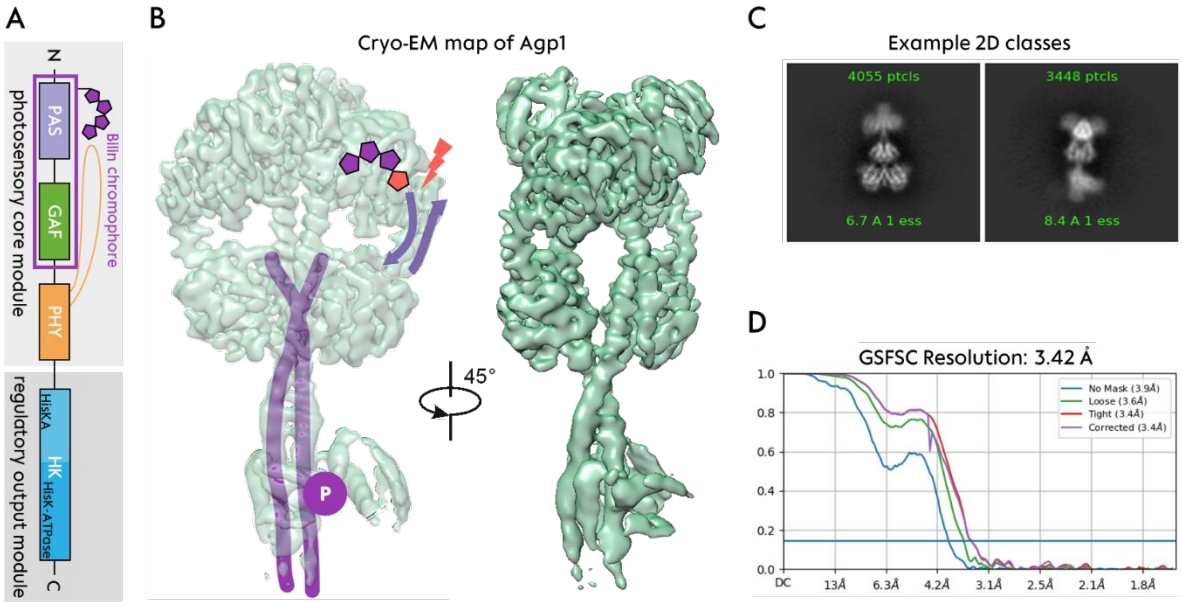


Figure 1: Single-particle cryo-electron microscopy of full-length Agp1: A: Domain architecture of bacterial phytochromes. **B:** Cryo-EM map of full-length Agp1. **C:** Example 2D-classes**. D:** GSFSC resolution plot.

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Poster 8

Nitrile Groups as Build-in Sensors for Vibrational Stark Effect in Electrocatalytically Active Nitrogen-rich Carbon Materials

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Short-ranged electric fields at electrode surfaces play an important role within an electrocatalytic cycle. These field effects can be characterized using the Vibrational Stark Effect, which arises from the interaction between a local electric field and a vibrational mode of surface-bound molecular group and can be measured by spectroelectrochemical methods. Expanding the range of catalytically active systems suitable for electrochemical Stark spectroscopy, we herein present a metal-free carbon-based system that shows electrocatalytic activity for hydrogen evolution reaction (HER) with a straightforward approach in fabrication. The investigated catalyst is an amorphous and porous nitrogen-rich carbon material of C_2N stoichiometry, synthesized via thermal condensation of the hexaazatriphenylene hexacarbonitrile (HAT-CN) precursor material without further treatment and contains numerous -CN groups due to incomplete condensation. [1,2] Using in-situ electrochemical Raman spectroscopy, it is shown that these build-in nitrile functions act as vibrational sensor groups for the Vibrational Stark Effect. Specifically, the ν(C≡N) vibrational mode at ~2235 cm^{-1} exhibits a linear shift in response to the applied electric voltage under non-catalytic conditions in KCl electrolyte. Under HER conditions in HCl electrolyte, deviations from the linear Stark shift indicate rearrangement processes within the electrochemical double layer, affecting the local electric fields at the interface. The observed band shifts are time-dependent and persist over the mid-term (minutes to hours) after the applied voltage is switched back to non-catalytic regions. The results suggest a semi-permanent conditioning of the electrode's surface due to the hydrogen evolution process, which may be of relevance in other catalytic systems.

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Poster 9

Photoisomerization of deprotonated Retinal Schiff Base

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Rhodopsins are transmembrane proteins which function as photoreceptors and have a wide variety of functionalities ranging from sensors to ion pumps. Universally, they share a common chromophore, the retinal as well as a similar secondary structure of seven transmembrane helices. Recently, a new rhodopsin was discovered, which comprise of eight transmembrane helices: Histidine kinase rhodopsin (HKR). The discovery of HKR established a novel sub class of the microbial rhodopsins that is coined enzymerhodopsins. Spectroscopic studies of HKR revealed two switchable and thermally stable isoforms: ultraviolet light-absorbing (Rh-UV) and blue light-absorbing (Rh-Bl). It was determined spectroscopically that the Rh-UV form of HKR carries a 13-*cis*-retinal Schiff base which is deprotonated and undergoes a photoisomerization. Due to the deprotonation of the Schiff base, a lone pair is localized around its nitrogen atom which give rise to n-π^* excited state. This excited state could facilitate an intersystem crossing (ISC) between singlet and triplet states. A small energy gap between singlet and triplet state is essential for successful ISC. In this contribution I will present the results of a hybrid quantum mechanics/molecular mechanics (QM/MM) simulation in the excited state. The energy gap between the low-lying singlet and triplet state is calculated along the isomerization pathway.

Poster 10

Spectro-electrochemical investigation of CcO in nanodiscs and a synthetic mimicry

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Short investigation of Cytochrome c Oxidase reconstituted in nanodiscs on two different self-assembled monolayers. Continued research of the hangman/pacman complex Py2XPFe/Py2CuXPFe modelled after the active centre of CcO.

Poster 11

Tracking water oxidation through time-resolved FTIR Spectroscopy

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In the light-driven reaction cycle of Photosystem II (PSII), two 'substrate' water molecules are oxidized resulting in the release of O_2 at the oxygen-evolving complex (OEC), which consists of a Mn_4Ca-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 (IR) spectroscopy offers unique insight into these processes, as protonation states and even slight structural or functionally crucial H-bond networks can be resolved. However, experiments have restricted themselves to either spectrally extensive Fourier Transform IR (FTIR) investigations of semi-stable intermediates without time-resolution [1] or more recent time-resolved studies including measurements on select IR bands [2,3]. To investigate events of the PSII photocycle, we heavily modified the sample compartments of a commercial FTIR spectrometer to push the experiments beyond previous limits. *Inter alia*, the addition of a custom sample changer allowed for the efficient acquisition and processing of thousands of excitation sequences on dark adapted samples. In a step-scan experiment, more than 230,000 excitation cycles of dark-adapted PSII were recorded to resolve processes from 50 µs to 130 ms over a range of 1300-1800 cm^{-1}, for all S-state transitions. Alongside quantum chemical simulations, analysis of spectral features associated with O_2 evolution identifies a transient carboxylate deprotonation preceding the formation of an oxygen radical at the OEC [4]. These results imply that the rate limiting step in the oxygen evolution transition $S_3 \rightarrow S_0$ is not the O-O bond formation, but a long distance (~10 Å) shuffling of protons towards a relatively close-by glu312/glu65 dyad. This result meshes nicely with impressive crystallographic results identifying the dyad as being involved in the process [5].

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Poster 12

Methanol Binding Insights in Photosystem II via XFEL Crystallography

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Spectroscopic and structural studies have significantly advanced our understanding of the reaction mechanism of water oxidation by Photosystem II (PSII). Nevertheless, the origin of two substrate water molecules inserted during the pivotal S_2-S_3 and S_3-S_0 transitions of the Kok cycle remains elusive [1, 2] While X-ray Free Electron Laser (XFEL) crystallography has provided invaluable insights into the catalytic site's dynamics, specifically identifying the substrate waters within or around the Oxygen-Evolving Complex (OEC) is still challenging. Our study uses a substrate analogue, specifically methanol, to visualize the origin of potential water substrates within the PSII structure. Extensive spectroscopic analysis, especially using Electron Paramagnetic Resonance (EPR), and simulation work suggests that the binding site (or sites) of methanol lies on or close to the OEC [2, 3]. However, direct structural evidence still needs to be provided. Our research leverages cutting-edge room-temperature time-resolved microcrystal diffraction-before-destruction XFEL crystallography to offer preliminary structural insights to collect PSII microcrystals rich in S_3 state, treated with methanol. We aim to investigate the changes in the analogue-structure relative to the methanol-free native state dataset to discern structural deviations attributable to the presence of methanol. Our findings represent a step forward in elucidating the binding site of water substrate analogues proximal to the Mn cluster, potentially unveiling the water insertion path in photosynthetic water oxidation.

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Structural snapshot of a bacterial phytochrome in functional intermediate states

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Phytochromes are modular photoreceptors which were found in plants, bacteria and fungi. It converts light information into biological information and regulates, for example, photosynthesis or motility by a reversible photocycle from the red light absorbing Pr to the far-red light absorbing Pfr state or *vice versa*. [1, 2]
Here we study the activation mechanism of the mutant PAiRFP2 [1, 3] based on *Agrobacterium fabrum* Agp2 and optimized for fluorescence and optogenetic applications at molecular level. We used time-resolved serial femtosecond X-ray crystallography (SFX) conducted at free-electron laser (XFEL) sources to investigate the photoconversion of this photoreceptor. We illuminated our PAiRFPF2 crystals with various exposure times and collected data for seven structural intermediate states. These pump-probe experiments led to time-resolved crystallographic snapshots that reveal detailed insights into changes occurring during the Pfr to Pr transition. These data show structurally the early changes as chromophore isomerization up to later rearrangements such as the beginning of the functionally relevant secondary structure changes.

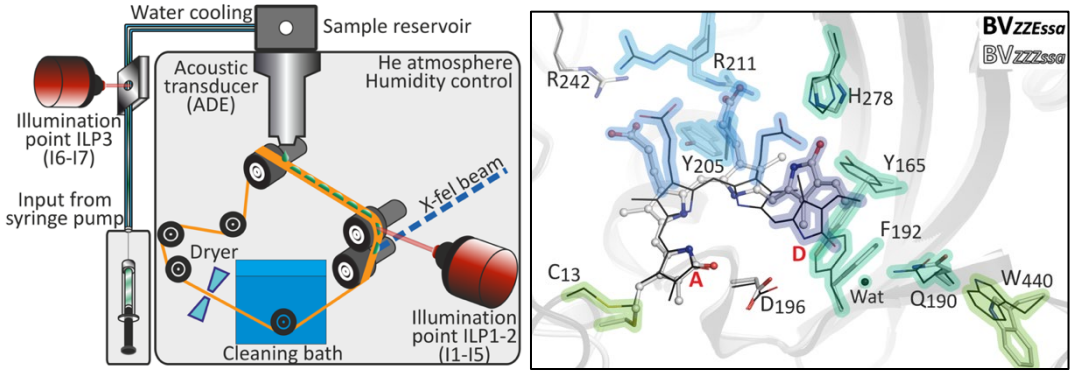

Figure 1: Sample delivery system specific for XFEL measurements (**left**) and close-up view on the chromophore binding pocket to illustrate of time-resolved structural changes (**right**).

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Poster 14

Pr and Pfr structures of plant phytochrome A

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Phytochromes are red/far-red absorbing photochromic biliprotein photoreceptors widespread amongst micro-organisms and ubiquitous in plants where they control developmental processes as diverse as germination, stem elongation and floral induction through the light-induced conversion of inactive Pr to the Pfr signalling state. Although structural models of numerous prokaryotic and several plant phytochromes in the Pr state have been published, detailed Pfr structures are known only for bacteriophytochromes. Here we report 3D crystal structures of the N-terminal chromophore-binding module of soybean phytochrome A, including 2.2 Å structures of both Pr and Pfr states at ambient temperature from X-ray diffraction experiments at European XFEL as well as high resolution cryogenic structures of Pr. In particular, the chromophore D-ring remains α-facial relative to the B-C ring plane following isomerization of the C15=C16 double bond and likely clockwise *Z*→*E* photoflip. Also, the chromophore as a whole rotates slightly within its pocket as the propionate side chains and their partners as well as the side chains of two conserved tyrosines below the D-ring shift radically. Two helices near the chromophore show significant movements that might represent components of the light signal. That many of these changes parallel those in bacteriophytochromes is surprising in view of the extensive sequence divergence, and especially considering that plant phytochromes signal via state-dependent partner interactions primarily with the N-terminal region rather than by regulating C-terminal enzymatic activity as in the case of bacteriophytochromes. Our findings thereby imply that evolution has 'repurposed' fundamental aspects of phytochrome photoactivation to serve the complex demands of photoregulation in the eukaryotic plant.

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Poster 15

Interplay of Hydration, Water Mobility, and Proton Transfer in Cytochrome c Oxidase

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Cytochrome c Oxidase (CcO) also known as complex IV in the respiratory chain is a protein that uses the energy from the reduction of oxygen to water to pump (further) protons through the membrane. For CcO to act as an oxidase and a proton pump, these processes have to be highly regulated. Proton uptake from the inner side of the membrane to the chemical redox centre takes place through two so-called channels, named D or K, after an important Asp or Lys residue, respectively. Our simulations show that the protonation state of the two channels has an impact on the hydration level within the two channels [1] and of the communication within and between the two channels [2]. For the D-channel, the hydration level is lower when the proton has already reached E286 at the end of the channel. This can be explained by a hydrogen-bonded network pointing from E286 to the so-called asparagine gate (formed by N139 and N121), favouring a "closed" conformation [1]. The thus prevented water passage also blocks the most favourable pathway [3] for proton transfer from the channel entrance to its terminus. The D-channel can thus be regarded as auto-regulated, allowing proton passage only when required, that is the proton has not arrived at the upper end of the channel, yet. In the K-channel, the hydration level depends even more critically on the position of the excess proton, suggesting that the proton drags its own hydration sphere with it. Likewise, the conformation of residue E101 at the entrance and K362 in the middle of the channel, are predominantly in an "up" conformation, when protonated [2]. The directionality of the hydrogen-bonded networks and the probabilities for proton transfer are coupled to the conformation of K362 [4,5]. Proton transfer through the entire channel in both directions is feasible only in the "down" conformation and unlikely in the "up" conformation [4]. Similar to the D-channel, this interplay can be regarded as an auto-regulation, preventing back leakage and the transfer of an extra charge, once the proton has reached the upper part of the channel and is therefore close to the redox centre.

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Poster 16

Estimation of pKa values in membrane bound proteins

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Several bioenergetic processes take place in membrane bound complexes that catalyze intricate proton and electron reactions which generate a proton motive force needed for function. Ionizable groups generate proton gradients but are often buried in hydrophobic pockets inside the membrane. Estimation of pKa values of these key residues is of great importance to describe the dynamics of the system and additionally determine proton pathways. Despite major structural, biochemical and computational advances, pKa values of these complexes remain poorly understood and are a major challenge for modern life sciences [1]. While there are many tools to predict pKa values in soluble proteins based either on physical or chemical properties [2], as well as database learning tools with good accuracy [3], membrane bound pKa values remain a challenge. Currently there is no larger database available for pKa values of buried titratable groups due to its complexity, which hinders the development of more reliable, yet typically quick prediction methods. In addition, rare events like wetting-dewetting transitions, which cannot be inferred from e.g. crystal or cryo-EM structure or standard MD simulations alone, are major factors that are missing from the current picture. In this work we review the current state of PBE/MC methodology and available benchmark data to be able to predict pKa values in soluble proteins with similar accuracy, and based on the lessons learned we develop a size-consistent approach to predict pKa values in membrane bound proteins.

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Mechanistic principles of proton and electron transfer in mycobacterial supercomplex III2IV2

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At a molecular level, cellular respiration is performed by membrane bound complexes that catalyze redox-driven proton reactions establishing the proton motive force (pmf) across the membrane that generates ATP, the energy currency of all living systems. In mitochondria, communication between such complexes is achieved via soluble electron carrier proteins, such as cytochrome c that transfers electrons between complex III and complex IV. Interestingly, in mycobacteria, complex III and complex IV express as an obligate supercomplex (SC), where the electron carrier, cytochrome cc is attached to the membrane and stabilizing structurally the interface of complex III and IV. This adaptation suggests that the electron transfer chain between the two complexes is not interrupted between the initial electron donor (quinone) and the final acceptor, O2. In this work, [1] we describe the structure and function of Mycobacteria smegmatis III2IV2 respiratory SC, a model organism to understand the pathogenic Mycobacteria tuberculosis. We describe quinone binding sites that are potential drug targets against tuberculosis by combining Cryo-EM analysis, molecular dynamics and activity assays together with site-directed mutagenesis. Moreover, using QM/MM free energy calculations we provide the principles of the electron transfer bifurcation process that takes place in the Qo site and supports the roll of key residues involved in the first and second proton coupled electron transfer process in complex III. Our combined results provide evidence of functional principles in mycobacteria SC.

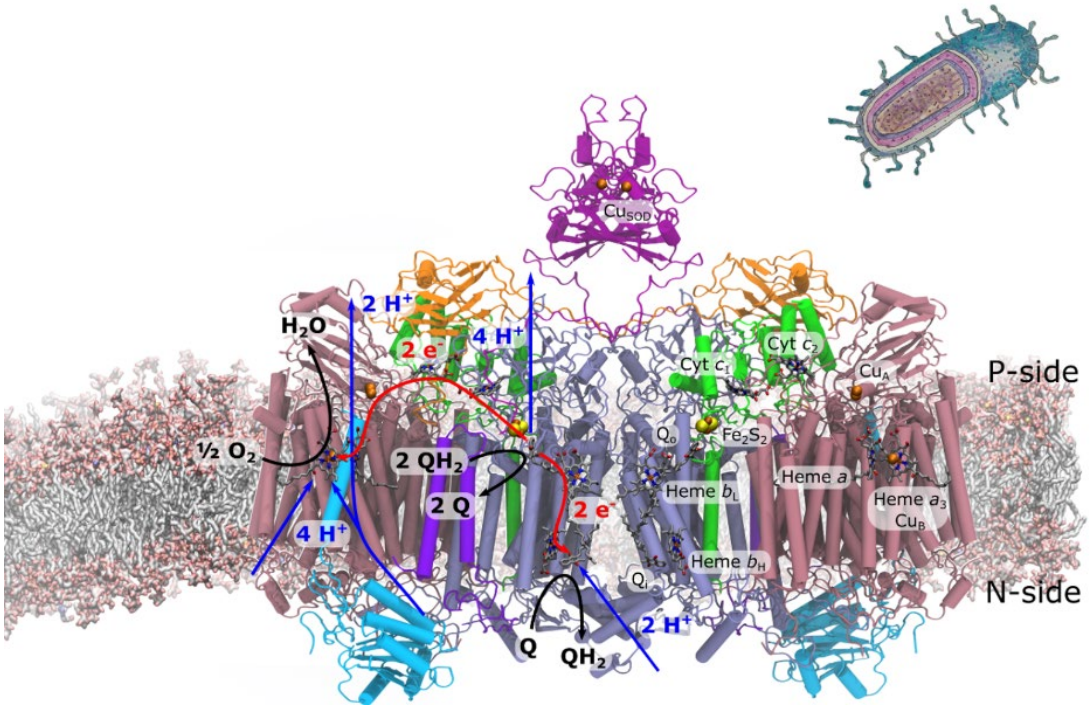

Figure 1: The figure shows the structure and function of the mycobacterial supercomplex III2IV2 from *M. Smegmatis*, a model system to understand long range electron transfer in the pathogenic *M. tuberculosis*. Electron transfer takes place between the electron donor, quinol at the Qo site in complex III2, and the final acceptor, O_2 in complex IV.

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Poster 18

4D structural studies of the light-driven sodium pump *Er*NaR

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Light-driven sodium-pumping rhodopsins are unique natural active ion transporters also being a great model for the understanding of the molecular mechanism of transmembrane sodium translocation against strong electrochemical gradients. The main type of sodium-pumping rhodopsins are proteins with the NDQ motif, such as KR2 from bacteria *Krokinobacter eikastus* [1]. However, even in the case of KR2, the mechanism of sodium translocation remains under debate [2,3]. Recently, we reported a new subgroup of NDQ rhodopsins comprising an additional glutamic acid residue in close proximity of the retinal Schiff base (RSB), and presented the in-depth characterization of one of its members, *Er*NaR from *Erythrobacter sp. HL-111* [4]. The additional glutamic acid (E64 in *Er*NaR) allowed to make the spectroscopic and functional properties of *Er*NaR almost pH-insensitive in the wide range of proton concentration. The structural basis for this affect is suggested to be the low-barrier hydrogen bond (LBHB) between E64 and the main counterion of the RSB, D105 in *Er*NaR, which likely results in a very low pK_a value of D105. However, the mechanism of light-driven sodium pumping in the new subgroup of NDQ rhodopsins involving transient protonation the RSB counterion remains unknown. In our poster, we present the current progress of the structural investigations of *Er*NaR using cryo-electron microscopy and X-ray crystallography, including time-resolved serial femtosecond and millisecond crystallography methods. The ensemble of the high-resolution *Er*NaR structures under various conditions and at fs-ms time delays to the optical excitation of the protein allowed us to propose the molecular mechanism of the *Er*NaR functioning and to show the conformational changes associated with the increase of the pK_a value of D105 allowing for proton translocation from the RSB to the counterion.

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Proton dynamics in the light-driven bacterial chloride pump *Nm*HR

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*Nm*HR, a halorhodopsin from the marine bacterium *Nonlabens marinus*, is an inward chloride pump, discovered less than 10 years ago. Although *Nm*HR and the other two well-known halorhodopsins, *Hs*HR and *Np*HR, transport the same anion, *Nm*HR shows a higher sequence similarity to the outward sodium pump rhodopsin KR2. The latter two share a similar conserved motif of residues involved in the ion transport. Here we report the insertion of the unnatural amino acid p-cyano phenylalanine (pCNF), via amber stop codon suppression. The pCNF probe is an optical probe sensitive to local environmental changes, as the C=N stretching vibration of the cyano group can be used as an infrared marker of local environment, due to its sensitivity to hydrogen bonding and local electric field. We inserted the pCNF by exchanging two conserved tryptophans W99 and W201, respectively, which are close to the chromophore and are involved in the retinal isomerization and chloride pathway. By that we are trying to elucidate the environmental changes around the retinal. Before inserting the unnatural aminoacid pCNF, a first exchange of the two tryptophans has been performed with the canonical phenylalanine, in order to test if the mutation would impair the functionality of the protein. This was accomplished by applying spectroscopic techniques as time-resolved UV-Vis spectroscopy and steady state FTIR. Additionally, by using UV-Vis spectroscopy in the ultrafast range, we are trying to elucidate the putative role of the two tryptophans of NmHR in the very early phase of the photocycle.

Poster 20

Potential-Induced Time-Resolved Surface-Enhanced Difference Absorbance Spectroscopy on Surface-Bound Cytochrome c using Quantum Cascade Lasers

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Understanding protein dynamics and their underlying molecular mechanisms is a crucial part of developing a detailed description and explanation of protein function. Infrared spectroscopy as a label-free technique is perfectly suited to address related questions by directly and non-invasively probing molecular vibrations. While established Fourier-transform infrared spectroscopy methods either struggle with low time resolution or high demands with respect to sample stability, quantum cascade laser (QCL) based approaches were already demonstrated to be capable of overcoming those limitations [1]. Using surface enhanced infrared absorption spectroscopy (SEIRAS) - a technique in which the sample is being probed in attenuated total reflection configuration - we selectively enhance signals from molecules in close vicinity to the surface while also providing a metal surface which can be utilized as working electrode in an electrochemical cell. This allows for application of a potential jump for triggering redox and/or conformational changes within molecules at the surface. In this study we demonstrate the feasibility of this method by investigating kinetics of changes in the protein backbone of surface bound cytochrome c after application of a fast potential jump.

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Spectroscopic signatures of excess protons in bulk and at interfaces

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Understanding acids and bases at interfaces is relevant for a wide range of biophysical phenomena, including proton diffusion in acidic bulk solutions and along bilayer membranes. Proton transfer dynamics in bulk solution is experimentally investigated by FTIR spectroscopy. Based on density-functional theory molecular dynamics simulations, we decompose the FTIR spectrum of excess protons into different characteristic features including transfer-waiting, transfer-path, and normal-mode contributions. We show results for the proton hopping-dynamics between hydronium ions and weak bases, such as the fluoride ion. Hydronium ions adsorb strongly at interfaces due to their hydrogen bonding behavior, where they donate three hydrogen bonds to bulk water molecules. Hence, the presence of hydronium ions induces an anisotropic arrangement of water molecules. This anisotropy can be measured experimentally by sum frequency generation (SFG) spectroscopy. We present depth-resolved SFG profiles and compare pure water with HCl solutions and find characteristic spectroscopic features due to the interfacial hydration structure of hydronium ions and counterions. We verify our simulations by comparison with SFG experiments, where we observe quantitative agreement.

Poster 22

Investigation of Nonselective Cation Permeation through CNGA1 Channels by MD Simulations

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Mammalian cyclic nucleotide-gated (CNG) channels are crucial components of numerous signal transduction pathways, most classically in the visual and olfactory sensory systems. The transmembrane domain of CNG acts as nonselective cation channels, which are opened by the binding of cGMP in the ligand binding domain. Recently, high-resolution cryo-EM structures of human CNGA1 and heteromeric CNGA1/CNGB1 channels became available [1] [2], but the detailed mechanism governing non-selective cation permeation in CNG remains elusive. In this study we used molecular dynamics (MD) to simulate ion permeation process in CNGA1, where a constant transmembrane potential was generated by external electric field. We performed Na^+ and K^+ permeation simulations using different conventional force field parameters. Simulations at nanosecond to microsecond time scales revealed the open-state of the CNGA1 to be conductive, while no ion permeation was observed in the closed-state. Na^+ and K^+ permeated at rational rates, suggesting the cryo-EM structure of CNGA1 represents a true open-state at physiological condition. Different from K^+ channels, monovalent ions occupy two major sites in the selectivity filter. In terms of the gating, simulations showed that the gate residue F389 regulates the ion permeation/conductance by hydrophobic dehydrating effect of the pore. In conclusion, MD simulations revealed valuable insights into non-selective cation permeation and gating in the CNG channels.

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Effect of an essential arginine mutation (R473C) on the catalytic reaction of cytochrome c oxidase from *Paracoccus denitrificans*

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Cytochrome c oxidase (CcO) is a terminal membrane enzyme in the aerobic respiratory chain. CcO pumps protons against the membrane proton gradient and reduces oxygen to water using the energy of the electrons donated by cytochrome c [1]. It has been proposed that reduction of heme a in the catalytic cycle of CcO from *Bos taurus* leads to dissociation of the ion pair formed by an essential arginine and the heme a3 D-propionate, creating a strong field leading protons to a transient proton loading site (PLS) [2]. We mutated the highly conserved amino acid R473 (analogous to R438 in *Bos Taurus*), which forms salt bridges to the D-propionates of heme a and heme a3 in Paracoccus denitrificans [3], to a cysteine (R473C). We investigated the effects of the R473C mutation on the electrostatic environment at the binuclear center. We monitored the changes induced by this mutation through electronic spectroscopy and vibrational spectroelectrochemistry [4]. The R473C mutant shows a decreased catalytic activity, blue-shifted Soret bands in both the oxidized and reduced states and minimal response to electrochemically-induced redox changes.

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Analysis of the binding behaviour between the electron carrier cytochrome c6 and Photosystem I

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Electrocatalytic- and solar-driven fuel synthesis from the greenhouse gas CO_2 is a desirable approach to simultaneously produce sustainable energy carriers, and combat increasing atmospheric CO_2 levels. Formate is a stable intermediate in the reduction of CO_2 and is utilised in a wide range of downstream applications. Recent efforts have focused on using an all-protein, light-triggered, catalytic circuit based on photosystem I (PSI), cytochrome c6 (cyt c6) and formate dehydrogenase (FDH), which would convert CO_2 into formate. However, various challenges remained. Our research addresses the optimization of the structural basis for efficient electron transfer from cyt c6 to a genetically engineered PSI-FDH fusion complex. Due to the transient binding of cyt c6 to PSI it has been challenging to investigate the structural basis for this binding process and to optimize binding affinities [1, 2]. However, structural observations and models propose a specific binding site for cyt c6 near the P700 chlorophyll pair of PSI on the lumenal side [1]. Based on this model, residues likely involved in the binding mechanism were predicted. Here, heterologously expressed cyt c6 variants from the cyanobacterium *Thermosynechococcus vestitus* BP-1 with a higher binding affinity to this suggested binding site were used to increase the electron transfer efficiency to PSI and study the binding mechanism between cyt c6 and PSI. These high affinity variants show increased oxygen and P700 reduction rates compared to the wildtype. Based on these results the binding mechanism will be further specified via structural determination of the Cyt c6-PSI complex by cryo-electron microscopy (cryo-EM).

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Poster 25

Substrate water insertion in the $S_3 \rightarrow S_0$ transition traced by time-resolved IR spectroscopy in D1-N298A PSII variant

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Photosystem II (PSII) is the site of light-driven water splitting in plants, algae and cyanobacteria, a process that produces molecular oxygen as a side product. The inorganic Mn_4CaO_x cluster and its surrounding amino acid residues form the so-called oxygen-evolving complex (OEC). While the OEC catalyses the reaction at high rate, its rate-determining factors are still incompletely understood.

In this study we traced the oxygen-evolving $S_3 \rightarrow S_0$ transition of PSII by time-resolved O_2 polarography [1] and time-resolved infrared spectroscopy using tunable quantum cascade lasers [2] for cyanobacterial photosystems genetically modified at two strategic sites, D1-N298A and D1-D61A. The polarography experiments revealed a severely slowed-down O_2 evolution in the D61A variant as compared to wild-type PSII, explainable by the role of the D61A in proton-coupled electron transfer [1]. In the N298A variant, O_2-evolution is not slowed down. The IR data, however, revealed a slower reaction step also in the N298A variant, which we tentatively assign to water insertion after O_2-formation: Whereas in general the substrate-water insertion step may be unresolvable in time-resolved experiments, here it likely became traceable because of deceleration by genetic modification. Moreover, comparing the rate-determining step in O_2-formation ($S_3 \rightarrow S_4$) of wild-type and D1-N298A variant, activation energy analysis revealed an astonishing entropy-enthalpy compensation. Molecular dynamics (MD) simulations of the N298A variant identified changes in the "water wheel" [3] (a cluster of waters at the end of the O1 channel), which could potentially cause a decelerated water insertion, and supports rationalization of the here reported entropy-enthalpy compensation.

Our results support three rate-determining functions of the protein environment of the metal cluster: acceleration of proton-coupled electron transfer, acceleration of the deprotonation-coupled substrate-water insertion after O_2 formation, and balancing of rate-determining enthalpic and entropic contributions.

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Poster 26

Structural Studies of the Activation Mechanism of M2 using solid state NMR and surface-enhanced infrared absorption spectroscopy

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Viruses and their protein components have been studied extensively in recent years. Nevertheless, some systems appearing in many different virus species yet need to be analyzed in more detail, regarding their essential role in virus function which makes them promising drug targets [1]. One of those systems are viroporins. Present in many different viruses, they are membrane proteins responsible for ion transport, but take part in other steps in the virus life cycle as well [2]. We investigate the structure of viroporins using the M2 proton channel from Influenza A as a model system. A construct of the wildtype M2 containing the transmembrane domain and an adjacent amphipathic helix was reconstituted into proteoliposomes, allowing for experiments in a near native environment. 13C- and 15N-detected magic-angle spinning solid state NMR spectroscopy on the uniformly 13C- and 15N-labeled sample resulted in highly resolved spectra, enabling an assignment of most of the residues. Apart from comparisons to previous studies [3, 4], with this data structural changes induced by different membrane compositions, but also pH values of the environment relevant in the activation of the channel could be monitored on an atomic level. Additionally, deuterated and then back-exchanged samples were used for fast-spinning proton-detected experiments, giving insight into the protonation dynamics and more detailed structural aspects of the protein. Together with data from surface-enhanced infrared absorption (SEIRA) spectroscopy, the activation and proton conduction mechanisms could be studied [5], adding valuable findings to the ongoing discussion on proton-shuttling mechanics in the M2 proton channel.

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Poster 27

Somatic HVCN1 mutations in cancer: Potential deleterious passenger mutations in the S4

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Somatic mutations are prevalent across all types of cancer and can impact any gene. In this study, we focused on HVCN1, which encodes the voltage-gated proton channel Hv1. We identified 197 mutations from three different databases, including 134 missense mutations, 51 synonymous mutations, and 12 mutations introducing a stop codon. While many of these mutations are benign and do not affect channel function, mutations that impact key functional residues, such as those involved in proton selectivity or voltage sensing, can significantly alter channel function and the phenotype of cancer cells. Notably, in addition to mutations in the coding sequence of the central N-terminal part of Hv1, another mutation hotspot was identified in the S4 transmembrane domain, which contains the channel's voltage sensor, and the adjacent C-terminal region. Five somatic cancer mutations within the S4 segment (R205W, R208W, R208Q, G215E, and G215R) were selected for further investigation through electrophysiological analysis and molecular dynamics simulations. Electrophysiological experiments demonstrated that all expressed mutants exhibited proton-selective currents that were time- and voltage-dependent. Compared to the wild type (WT), all mutants showed reduced conductance density, making them inherently less proton-conductive. Analysis of gating kinetics, gating charge, and activation threshold revealed that each mutation affected these parameters differently. Molecular dynamics simulations indicated potential open and closed conformations for each mutant, resulting in structural changes that correlate with the observed functional alterations in HV1 mutants. These mutations likely impact the pH homeostasis of tumor cells, impairing their ability to regulate intracellular pH and reducing the acidification of the extracellular environment.

Combining vibrational and computational spectroscopy to elucidate the mechanism of the channel opening of Influenza A virus M2

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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, and thus present promising targets for antiviral therapy. In fact, it was shown for the M2 proton channel from Influenza A virus (IAV) that its pH-activated proton conductance can be inhibited, which led to its application as an antiviral drug. We employ surface-enhanced infrared absorption (SEIRA) spectroscopy to IAV M2 reconstituted within a solid-supported bilayer lipid membrane, which enables us to track the pH-activated large-scale reorientation of M2's transmembrane α-helices under *in situ* conditions via changes in its distinctive vibrational fingerprint [1]. By calculating computational SEIRA spectra based on a structural model from nuclear magnetic resonance and simulating a channel opening by tilting the transmembrane helices, we obtained an excellent match with the experimental picture. Combining the computational and spectroscopic results, we were furthermore able to quantify the opening angle of the channel transitioning from closed to the activated state. Intriguingly, M2 inhibitors block this mechanical motion. This combined spectroscopic and computational approach presents a tool to quantify structural changes of viroporins from pathogenic viruses of current relevance, possibly providing information on their function and inhibition.

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Poster 29

Joint Forces: Hypothesis generation for transmembrane protein oligomerization by combining AlphaFold2 and MARTINI3

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We present our study on the self-assembly of multiple chains of the transmembrane (M-) protein of the Dengue virus with coarse-grained molecular dynamics simulations using a modified elastic network model for proteins in the MARTINI3 force field [1] and test the stability of the assembled structures by re-introducing atomistic details. We do this to propose a stable structure of a potential viroporin, i.e. a transmembrane ion-channel in viruses, which can serve as a drug-binding target to fight infections with the Dengue virus, which is currently not possible. We compare the results of the self-assembly simulations with direct oligomer predictions using AlphaFold2 and critically discuss the potential of combining protein structure prediction and multiscale modeling to generate hypothesis for the formation of transmembrane protein complexes, which is the first step in the discovery of viroporins as potential drug-binding targets.

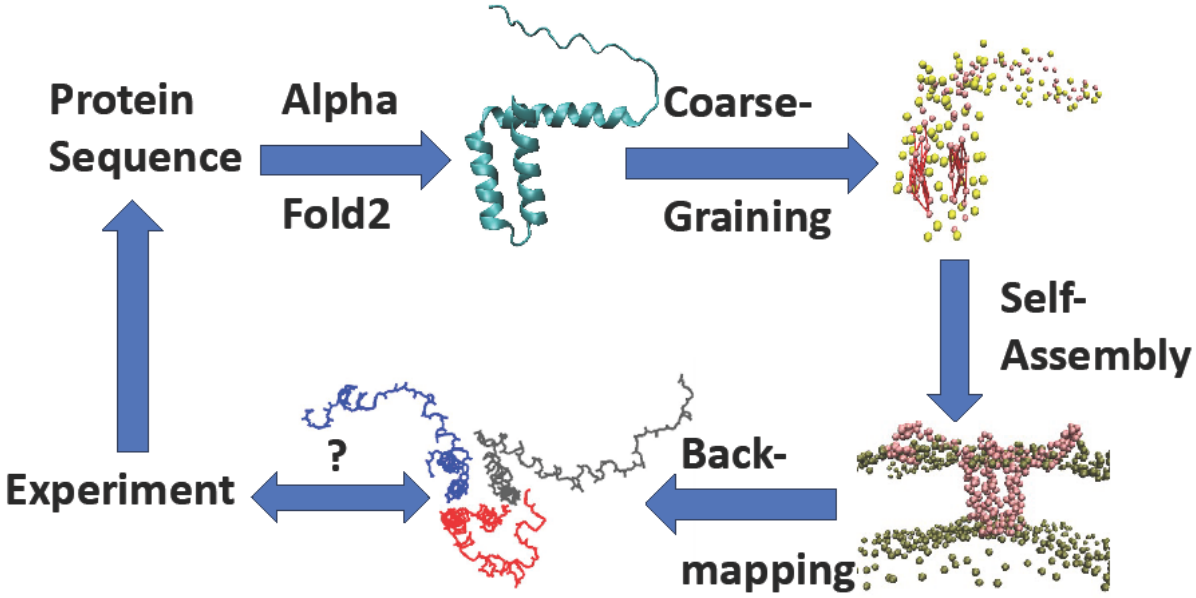

Figure 1: Illustration of a multiscale-modeling approach to predict the structure of a homo-oligomeric protein complex: Given a single protein sequence, we utilize AlphaFold2 to obtain a corresponding structure. This structure is mapped to a coarse-grained representation, which is used in molecular dynamics simulations to study the self-assembly into homo-oligomers. The obtained oligomer structures are mapped back to an all atom representation, which finally can/should be compared to experimentally resolved structures.

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Poster 30

Electric field effects on the proton transfer reactions in cytochrome c oxidase

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Cytochrome c oxidase (CcO) serves as the terminal oxidase in aerobic respiratory chains, catalyzing the conversion of molecular oxygen (O_2) into water, while driving proton pumping across the membrane. However, the molecular principles by which CcO splits the protons along these distinct chemical and pumping pathways are not well understood, despite significant progress in recent decades. In this work, we combine large-scale quantum chemical DFT calculations, hybrid quantum/classical (QM/MM) simulations, and molecular dynamics (MD) studies, to investigate the molecular basis of the protonation reactions in CcO. Our findings show that CcO employs orientated electric fields around its active site to sort the protons along the two distinct pathways, based on redox reactions and coupled conformational changes. We find that reduction of heme a induces a conformational change in a conserved ion-pair that generates a strong electric field along the pumping pathway. This, in turn, couples to the electron transfer to the active site, resulting in an electric field along the chemical pathway for the reduction of molecular oxygen. We propose that similar electric field effects play a role in other energy-converting enzymes, suggesting that these mechanistic principles generally operate in enzyme catalysis [1].

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Poster 31

Oxygen evolution kinetics in far-red adapted Photosystem II variants studied by time-resolved oxygen polarography

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In oxygenic photosynthesis, Photosystem II (PSII) plays a crucial role in converting photon energy from visible light into chemical energy while producing molecular oxygen upon water splitting. Recently, it was shown that in cyanobacteria the previously assumed red limit of 700 nm for this process, emerging from the use of chlorophyll a, can not only be exceeded by chlorophyll d PSII as found in *Acaryochloris marina*, but also by chlorophyll f containing PSII from far- red light acclimated *Chroococcidiopsis thermalis* [1]. Further research showed that the adaptation of PSII to longer, lower-energy wavelengths does not come without costs such as loss of efficiency or increased sensitivity to photodamage [2]. This work focuses on possible changes in the kinetics of photosynthetic oxygen evolution in these two far-red PSII variants. Considering that, time-resolved oxygen polarography was used to examine different types of cyanobacterial PSII, realized by observing thin layers of respective thylakoid membranes previously centrifuged on the surface of a Joliot-type electrode. Measuring over a temperature range from -5 °C to 40 °C enabled the determination of thermal activation energies of two distinct reaction steps. The corresponding oxygen evolution transients showed that the shift from the usual chlorophyll a to chlorophyll d or f photochemistry in PSII coincides with a slowdown of its oxygen evolution kinetics. Biochemical characterisation of the PSII complexes indicated that they are fully intact. The results will be discussed in terms of their implications on the bioenergetics of the systems.

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Poster 32

Proton Release Reactions in the Inward H^+ Pump Xenorhodopsin

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The molecular determinants of directionality are a key subject in the investigation of active ion transport by membrane proteins. The discovery of inward proton pumps [1-3], whose overall structure and membrane topology resemble those from outward proton pumps, has reinvigorated the controversy of vectorial proton transport [4, 5]. We investigated the proton pathway of the inward proton pump from *Nanosalina sp*. (*Ns*XeR) [2] by time-resolved spectroscopy [6]. (FT)IR experiments along with site-directed mutagenesis reveal key steps in the proton pathway on the cytoplasmic side. The identification of D220 acting as a proton release group, raises questions about the identity of the primary proton acceptor of the Schiff base proton. Molecular Dynamics simulations support a protein release pathway via a hydrogen-bonded water wire between the retinal Schiff base and D220.

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