Investigating proton transfer with SERRS and electrophilic addition of isocyanates in the catalytic centre of cytochrome c oxidase.

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Within this work surface enhanced resonance Raman spectroscopy (SERRS) is combined with an electrochemical setup to investigate proton transfer in cytochrome c oxidase (CcO). In this work the protein in its resting state, the fully oxidized "as-isolated" form without any further electron injections, was investigated. While under turnover conditions, each time an electron is donated to the enzyme, a proton is pumped across the cell membrane, presumably triggered by the reduction of heme a, no proton-pumping is observed even though six electron equivalents are donated to bring the protein from its resting state back to the fully reduced state, thus in the presence of oxygen and further electron equivalents back to turn-over-conditions. In the resting state a peroxide bridge is thought to be presence which could suppress the proton pump. By using an excitation-laser with a wavelength of 647nm, which is close to a ligand to metal charge transfer at 655nm visible in UV-VIS, we investigate a Raman vibration at 750cm^{-1}, which could originate in the stretch vibration of a bridging peroxide. Furthermore, advantage is taken of the fact that CcO ends up in a mixed valence state, with heme a being reduced, after the catalytic center is inhibited, when the protein is immersed in aqueous solutions with high concentrations of KCNO. The benefit of this new procedure is that the mixed-valence state is achieved, without any electrochemical treatment, or without the use of a reducing agent, where the electron-donation pathway remains unclear. Furthermore, we used the site-specificity of H/D sensitive propionate vibrational modes by using them as markers for proton accessibility to the two active heme centers.

We could show, by using the above-mentioned procedure, that even though heme a is reduced, the heme's propionates are not protonated when heme a_3 is in a six-coordinated low-spin state, which can be compared to the situation in the resting state, where the peroxide is present. Even though it remains unclear, which role the heme's propionates play in the proton-loading sites of CcO, our results achieved with SERR spectroscopy, can help to better understand, which influence the ligation of the enzyme has on the proton-pumping process, specially in the resting state.

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Immobilization of Cytochrome *c* Oxidase for Spectro-electrochemical investigation

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Cytochrome *c* Oxidase (C*c*O) plays a vital role in most aerobe living organisms. As the terminal enzyme of the respiratory chain it catalyses the last step of electron transport by reducing molecular oxygen to water. The energy gained from this process is harnessed by pumping additional protons across a membrane, building up a gradient, which can in turn be used by the ATP-synthase for ATP recovery. Several methods have been developed to study the well-orchestrated mechanism of electron and proton delivery for the pumping process as well as the catalytic reaction, which is devoid of unwanted side reactions, e.g. hydrogen peroxide formation.

To specifically probe the active site of the enzyme, without an overflow of information from the entire enzyme, Resonance Raman spectroscopy (RRs) is an excellent tool. To further increase signal intensities, the surface enhancement effect of some electrode surfaces can be used. For this, immobilization of C*c*O is crucial but additionally enables direct electron transfer between electrode and enzyme for simultaneous electrochemical studies with the same setup.

Using an amino terminated Self-assembled monolayer (SAM), imitating the lysine rich binding pocket of Cytochrome *c*, good spectroscopic signals were achieved, but voltammetric measurements were not possible with this system. A novel approach to this problem is the use of a hydrophobic SAM, which is formed under applied potential thus yielding a better coverage of the electrode surface, compared to the patchy patterns of the amine terminated SAMs.

Cyclic voltammetry of the dodecanethiol-SAM revealed a good immobilization of C*c*O, showing a distinctive reduction signal and oxidation peaks, which are separable by lowering the scanrate. Spectroscopic measurements of the immobilized enzyme were performed to study the structure of the active site and changes thereof under the influence of different applied potentials.

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A novel setup for time-resolved IR spectroscopy on Cytochrome c Oxidase

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Cytochrome c Oxidase (CcO) is the fourth and terminal complex in the mitochondrial respiratory chain. Physiologically, it uses four electrons provided by cytochrome c to pump four protons into the intermembrane space, while catalytically reducing oxygen to water. The atomic details of the sequential steps that go along with this redox-driven proton translocation are still a matter of debate. We use CcO from *Rhodobacter sphaeroides* to study the correlation between the active center's redox state and the protonation events in the enzyme's catalytic cycle. Time-resolved infrared spectroscopy is a well-established method to investigate transient protonation changes but applying it to CcO poses a plethora of challenges. Our goal is to use a quantum cascade laser (QCL) setup and slow-flowing, CO poised CcO solution in a microfluidics channel transparent to mid-infrared radiation to eventually study oxygen binding on the reduced enzyme after CO flash-off. We report on a novel setup designed for this purpose.

Exploring the reductive phase of Cytochrome *c* Oxidase: assignment of heme's redox states and relative structure changes through potential-resolved FTIR

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Cytochrome c Oxidase (C*c*O) is a protein central to cellular respiration. Together with other enzymes, it contributes to the creation of the proton gradient essential to ATP synthesis. It is well known that C*c*O reduces molecular oxygen to water and pumps protons while undergoing a cycle initiated by electron injection from Cytochrome *c*. At the beginning of this catalytic cycle, the reduced Cytochrome *c* transfers electrons to the Cu_A cofactor of the enzyme. This step is followed, respectively, by the reduction of the metal centers of heme a and heme a_3 and triggers a series of changes in the residues neighboring the active center.
The mechanistic details of this machinery are not yet completely understood, partially because of the deficiency of time-resolved data providing structural information on the physiological cycle.
We combine steady-state attenuated total reflection (ATR) FT-IR spectroscopy with electrochemistry in order to disentangle structural changes coupled to the reduction of single heme cofactors. We can control the redox state of their metal centers by mediated electron injection. After binding carbon monoxide to the active center of C*c*O and raising the applied potential, we monitor the shift of the C≡O stretching vibration, and ultimately its disappearance due to unbinding. CO is utilized both as a marker for the completely reduced state and as a probe for the Vibrational Stark Effect arising from the electric field of the reduced heme a on the oxidized heme a_3. This approach allows us to clearly separate potentials corresponding to the oxidation states of the protein's cofactors and to correlate them with the protein's IR difference spectra.

Conformational and protonation dynamics at the surface of phytochromes

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Phytochromes are red-light photoreceptor proteins that regulate a variety of responses and cellular processes in plants, bacteria, and fungi. They utilize light to control various biological processes and share similar molecular processes, which include light absorption by the chromophore, transient protonation /deprotonation and protein structural changes, eventually leading to activation and signal transduction. Phytochromes act as photochemical switches, which interconvert between a red (P_r) and a far-red (P_{fr}) absorbing state [1]. In this work Cph1 from Synechocystis and Agp1 from Agrobacterium tumefactions are investigated.

We utilize time-resolved fluorescence measurements to study the conformational dynamics of phytochrome. Fluorescence probes are attached to the protein by cysteine labeling and were used to investigate the fluorescence depolarization (fluorescence anisotropy) as a function of pH. Changes in final anisotropy as a function of pH identified conformational changes in the protein that seem to correlate with chromophore deprotonation.

Furthermore, the surface attached pH-sensitive probes were used to study the bulk and surface specific protonation states. Titration experiments of the labeled protein show differences in the protonation behavior between the pH-probe at the protein surface and in solution. To reveal the intermediate states during P_r to P_{fr} transition, the kinetic and protonation signal was measured by time-resolved absorption spectroscopy.

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Title: Single-Frequency IR Spectroscopy with Microsecond Time Resolution for Tracking Electron and Proton Transfer in the D1-V185N Variant of Photosystem II

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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 four semi-stable S-state intermediates. These transiently formed states are involved in ill-understood alternating electron and proton transfer steps [Klauss et al. 2012, *Proc. Natl. Acad. Sci. U.S.A., 109*(40)]. For complete mechanistic understanding of photosynthetic water oxidation, the transiently formed intermediates need to be investigated in detail.

Genetic modification of amino acid residues in the vicinity of the OEC has been repeatedly approached for the cyanobacterium *Synechocystis sp. PCC 6803.* Such manipulations aim to alter the behavior of the PSII photocycle to help identify the involved mechanisms. Recently, retarded O_2 release kinetics, with altered pH and isotope exchange characteristics, have been reported using time-resolved oxygen polarography with an asparagine substitution on the D1-Val185 residue [Bao et al. 2015, *Proc. Natl. Acad. Sci. U.S.A., 112(45)*].

An important tool to study such protonation and oxidation states changes is infrared spectroscopy. Our novel quantum cascade laser based single-frequency infrared absorption setup produces a time-resolution of 1 µs and a spectral resolution of 0.5 cm^{-1}. With a tuning range of 1300 cm^{-1} to 1650 cm^{-1}, encompassing much of the characteristic carboxylate and amide II bands, and an integrated ns pulsed 532 nm excitation laser, the time evolution of transitioning PSII complexes can be observed. This contribution describes the early work of a collaboration geared at a comparison of transients of the wild type *Synechocystis sp. PCC 6803* and the mutant D1-Val185Asp absorption bands to investigate the dynamics of the water cavity and the OEC.

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**Time-resolved IR absorption spectroscopy:
tracking photosynthetic water oxidation in Photosystem II**

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Photosystem II (PSII) is one of the two light-activated photosynthetic proteins and performs water oxidation in order to reduce the mobile membrane bound electron carrier plastoquinone. At the catalytic center, a Mn_4Ca-oxo cluster, four oxidizing equivalents needed for O-O bond formation are accumulated involving a sequence of alternating steps of electron and proton removal from the catalytic site (Klauss et al., J. Phys. Chem. B (2015), 119, 2677-2689).

Understanding of the process at an atomic level may not only answer basic questions of the light reaction of photosynthesis and PSII function but could also provide hints on the development of improved catalysts for artificial water splitting, a clean way of producing storable energy carriers.

We designed an infrared absorption experiment with a continuous-wave quantum cascade laser tunable from 1300 to 1650 cm^{-1} and thus covering the amide I and II regions, the symmetric and asymmetric COO^- stretching region as well as bands of the quinones and the redox-active tyrosine (denoted as Y_Z). The current time resolution is in the tens of ns range, which enables us to observe pivotal structural changes and proton transfer dynamics in PSII.

We will provide an overview of the complex dynamics of PSII trackable at the various wavenumbers. This includes the electron transfer from the tightly bound reduced quinone Q_A^- to the exchangeable quinone Q_B, the multiphasic kinetics of the oxidation of Y_Z by the special chlorophyll unit P680 and the proton coupled electron transfer events around the Mn cluster. The latter data is measured on PSII-enriched membrane particles of spinach as well as core complexes of the cyanobacterium *Synechocystis sp.* PPC 6803. Because we can track all these processes in functional proteins with only one data set, time resolved IR measurements on PSII will provide new insights on their nature in the near future.

The influence of water analogues on the oxygen-evolution step in Photosystem II

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Photosystem II (PSII) harbors an active site Mn4Ca-oxo cluster that facilitates the multi-step water oxidation reaction involving four events of excitation by light, intercalated by electron transfer and deprotonation steps (Kok's S-state cycle) [Dau et. al 2012, Curr Opin Chem Biol, *16*(1-2), 3-10]. Being water both the solvent and the substrate for this reaction, the detailed mechanistic aspects become very demanding to investigate and are currently still insufficiently understood. In particular, the likely crucial role of the water cluster neighboring the metal-oxo core at the catalytic site and the identity of the 'substrate' water molecules, which is closely related to the identification of the actual O-O bond formation mechanism, are still unknown [VINYARD et. al 2017, Annu Rev Phys Chem, 68]. The use of water analogues, molecules sufficiently similar to water that could either replace substrate water molecules or other water molecules in the vicinity of the Mn-complex, like ammonia (NH3) or Methanol (MeOH), are one of the ways to approach this question. NH3 is a reported inhibitor [SCHUTH et. al 2017, Biochemistry, *56*(47)] that has at least two binding sites, being at least one directly to the Mn-complex, most likely W1 [NAVARRO et. al 2017, Proc Natl Acad Sci U S A, *110*(39)]. Methanol is reported to have two binding sites closely to the Mn-complex, but may not replace any of the substrate waters [NAGASHIMA et. al 2017,J Phys Chem Lett, *8*(3)]. Using time-resolved measurements on PSII membrane particles, we aim to understand how water analogues affect the kinetics of the oxygen evolution step.

Time-Resolved Single-Frequency Infrared-Spectroscopy on Photosystem II in H_2O and D_2O: Tracking Protonation Dynamics

Sarah Mäusle, Philipp Simon, Holger Dau

Photosystem II (PSII) is a large protein complex in plants and cyanobacteria which catalyzes the oxidative splitting of two water molecules into four electrons (reducing equivalents), four protons, and one dioxygen molecule. Following the absorption of a photon, charge separation takes place at a special chlorophyll pair, P680, allowing an electron to move to the acceptor side of PSII. The resulting positive hole leads to the oxidation of a redox-active tyrosine residue Y_Z and subsequently of the manganese-calcium (Mn_4Ca) complex. After accumulating four oxidative equivalents at the Mn_4Ca cluster, dioxygen is formed. The four semi-stable redox states of the Mn_4Ca cluster are referred to as the S-states (S_0 to S_3) (Dau, H. & Haumann, M. (2008) *Coord. Chem. Rev. 252*(3-4): 273-295). The process leading up to O_2 formation is still not fully understood on an atomic level.

Structural and electrostatic changes that occur in PSII during the S-state cycle influence its vibrational modes and can thus be observed via difference infrared spectroscopy. Our time-resolved single-frequency IR setup allows for the observation of electron transfer as well as protonation dynamics with a high temporal resolution (up to 50 ns). H_2O/D_2O exchange can help to identify events involving proton movement by investigation of the kinetic isotope effect (KIE = τ_D/τ_H). A KIE exceeding unity suggests involvement of protons, while the specific value can hint at the type of protonation dynamics involved (Krishtalik, L. I. (2000) *BBA-Bioenergetics: 1458*(1): 6-27).

Time traces at various wavenumbers, such as 1395 cm^{-1} (symmetric COO^- vibrations), 1515 cm^{-1} (CO stretching band of $Y_Z^\bullet$), and 1544 cm^{-1} (Amide II band), were recorded for PSII membrane particles from spinach. The respective time traces were fitted with multi-exponential functions and the extracted time constants were then used to calculate the KIE, allowing us to identify and analyze protonation dynamics that eventually lead to O_2 formation.

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Investigations on microbial channelrhodopsins studied by time-resolved FT-IR an UV/Vis Spectroscopy

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The discovery of light-gated ion channels opened up the field of optogenetics, the combination of optical and genetic tools in order to control single cells. Two of the best-studied ChRs are *Cr*ChR2 from *Chlamydomonas reinhardtii* and *Ca*ChR1 from *Chlamydomonas augustae*. In *Cr*ChR2 first results on a molecular level were accomplished. However, there are two photocycle models derived from experiments performed under multiple turnover conditions (electrophysiology) and single turnover conditions (spectroscopy). We could show that the long-lived P4480-intermediate, which accumulates under multiple turnover conditions, is photosensitive and its photoexcitation leads into a secondary photocycle. Our results are capable of combing the two photocycle models.
Whereas advanced results have been achieved for *Cr*ChR2, we know less about *Ca*ChR1. I performed time-resolved FT-IR step scan experiments to provide time-resolved molecular insides into the protein over a broad spectral range.

Optimizing Crystal Size of Photosystem II by Macroseeding: Toward Neutron Protein Crystallography

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Photosystem II (PSII) catalyzes the photo-oxidation of water to molecular oxygen and protons. The water splitting reaction occurs inside the oxygen-evolving complex (OEC) via a Mn4CaO5 cluster. To elucidate the reaction mechanism, detailed structural information for each intermediate state of the OEC is required. Despite the current high-resolution crystal structure of PSII at 1.85 A and other efforts to follow the structural changes of the Mn4CaO5 cluster using X-ray free electron laser (XFEL) crystallography in addition to spectroscopic methods, many details about the reaction mechanism and conformational changes in the catalytic site during water oxidation still remain elusive. In this study, we present a rarely found successful application of the conventional macrosccding method to a large membrane protein like the dimcric PSII core complex (dPSIlcc). Combining microsccding with macrosecding crystallization techniques allowed us to reproducibly grow large dPSIlcc crystals with a size of ~3 mm. These large crystals will help improve the data collected from spectroscopic methods like polarized extended X-ray absorption fine structure (EXAFS) and single crystal electron paramagnetic resonance (EPR) techniques and arc a prerequisite for determining a three-dimensional structure using neutron diffraction.[1]

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Coworkers: Luiz Schubert, Ramona Schlesinger, Joachim Heberle

“Spectroscopic investigations on the light-driven inward H^+ pump xenorhodopsin”

Ion gradients across the cell membrane drive the synthesis of adenosine triphosphate (ATP). In several archaea, bacteria and unicellular eukaryotes these ion gradients are established by light-driven rhodopsins. Recently, a new class of proton-pumping bacterial rhodopsins, named xenorhodopsin (XeR), has been discovered. Although it has been shown, that the crystal structure of XeR from Nanosalina (NsXeR) is similar to the outward proton pump bacteriorhodopsin (bR), the vectoriality of proton translocation is inverted.

We investigated NsXeR by means of time-resolved spectroscopy to get an insight in it´s photocycle. UV/Vis flash photolysis experiments indicate at least 4 intermediates. Similar to the photocycle in bR, a blue shifted M intermediate is observed. Upon rise of the M intermediate, a proton release to the bulk solution is associated. Future time-resolved FT-IR step scan and rapid scan experiments will provide a primary structural insight into XeR´s pumping process.

Title: Spectroscopic investigation of variants of Channelrhodopsin-1 from *Chlamydomonas augustae*

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Abstract

Channelrhodopsins are photoreceptors located in the eye-spot of green algae, which cause phototactic responses depending on light conditions in the surrounding. Channelrhodopsin-2 from *Chlamydomonas reinhardtii* (*Cr*ChR2), a light-gated cation channel, is used in the neurophysiological field to optically control cellular processes. Channelrhodopsin-1 from *Chlamydomonas augustae* (*Ca*ChR1) is yet another promising optogenetic tool with mechanistic differences to CrChR2. To understand which amino acids are involved in the process of channel opening upon light activation, we created variants of *Ca*ChR1 and analyzed them by means of time-resolved UV-vis spectroscopy and FTIR difference spectroscopy. Here, especially we focused on the role of cysteines.

Residues involved in the protonation of the biliverdin chromophore of Agp2

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Key sentence: Conserved tyrosine 165 and arginine 211 are essential for the complete photo-conversion of Agp2, but play different roles in this process.

Key words: phytochromes, photo-conversion, Raman and IR spectroscopy, UV-vis spectroscopy

Agp2 is a bacterial phytochrome from the plant pathogen *Agrobacterium tumefaciens*. It belongs to the group of bathy phytochromes, which, unlike prototypical phytochromes, have a Pfr dark state with the biliverdin (BV) chromophore in the ZZEssa conformation. It changes due to photo-isomerisation to ZZZssa in the first step of the photoconversion to the Pr state. During the transition from Pfr to Pr, several (de)protonation events take place at the BV chromophore. Tyrosine 165 and arginine 211 are strongly conserved among all phytochromes and are located in the chromophore-binding pocket in close proximity to the essential protonation sites of the biliverdin chromophore. FT-Raman and FTIR measurements on Agp2-Y165F and -R211A variants showed that the photocycle was incomplete in both variants, going as far as meta-F intermediate state. The deprotonation of the propionic side chain of the BV-ring C was impaired and no keto-enol tautomerization was observed at the carbonyl group of ring D. The latter is pH-dependent in the wild type Agp2 and was proposed to be essential for the dark Pr-to-Pfr reversion. UV-vis spectroscopic kinetic measurements showed that indeed dark reversion was extremely slow and pH-independent in the Agp2-Y165F variant, while the R211A variant showed a behavior much more similar to the wild type protein. Based on the presented results as well as structural data we propose specific roles for the two amino acid residues. Y165 helps to conduct the immediate deprotonation of the ring C propionic side chain, while R211 locks the deprotonated propionate residue thus preventing it from re-protonation in a reverse process.

Electrostatic pKa calculations of the tetrapyrrole chromophore in phytochromes

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Phytochromes are biological red-light photoreceptor and can be found in bacteria and plants. All phytochromes utilize a covalently attached tetrapyrrole chromophore (TPC) that enables photoconversion between red-absorbing (Pr) and far-red-absorbing (Pfr) states. An accurate characterization of the protonation state of the TPC and all titratable residues in the chromophore binding pocket (CBP) is essential for understanding the signal transduction mechanism at atomic resolution. In this work, we applied the electrostatic approach based on the solution of the linearized Poisson Boltzmann (LPB) equation, implemented in the Karlsberg2+ software, to two phytochromes species. The TPC was divided into three fragments: the propionic side chain on ring C (psC), the propionic side chain on ring B (psB) and the chromophore core. The titratable psB and psC fragments were treated in the same way as the carboxylic group of an aspartic acid. In order to treat the TPC as a titratable site, atomic partial charges of the TPC were generated with the quantum chemical program Jaguar v.7.7 using the B3LYP DFT functional and 6-31G** basis set. The TPC geometry was optimized quantum chemically and then, the electrostatic potential of the TPC was computed based on the electronic wave function and charges of the nuclei using the same method as for geometry optimization. Atomic partial charges of the TPC were generated based on this electrostatic potential, using a two-stage restraint-electrostatic-potential(RESP) procedure. Finally, pKa values were computed by combining electrostatic energy calculations and MD simulations using Karlsberg2+. Conclusively, we present here a new methodology to determine the protonation state of the TPC and all titratable residues in the chromophore binding pocket.

Long dynamic simulations of Deinococcus Radiodurans bacterial phytochrome

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Phytochromes are bimodal photoswitches found in plants, bacteria, cyanobacteria, algae and fungi. They can reversibly photoconvert between two states, the red-light absorbing state (Pr) and the far-red-light absorbing state (Pfr), upon illumination and thermal relaxation. By means of long molecular dynamics (MD) simulations we investigated the dynamical properties and correlated motions of Deinococcus Radiodurans bacterial phytochrome in the Pr state. Conventional classical Molecular Dynamic (cMD) was complemented by accelerated Molecular Dynamic (aMD) in order to enhance the sampling of the conformational space. Furthermore, reproducibility of the resulting trajectories was evaluated by comparing different force fields (Amber and Charmm). The dynamic differences between monomer and dimer in the long time simulations are confronted and discussed.

Dynamic hydrogen-bond networks of channelrhodopsin variants

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Channelrhodopsins are membrane-embedded proteins that couple photo-isomerization of the retinal chromophore with proton transfers and passive flow of cations. A key open question is how protons are transferred across long distances in the polar environment of channelrhodopsin variants with different amino acid sequences. To address this question, we perform extended all-atom simulations of channelrhodopsin variants embedded in hydrated lipid membranes, and implement algorithms for the efficient analysis of large data sets on dynamic hydrogen-bond networks. The analyses indicate that the interior of channelrhodopsin hosts extensive networks of protein/water hydrogen-bond networks that rapidly respond to perturbations such as mutation or changes in the protonation state.

Acknowledgements

Financial support was provided in part by the DFG Collaborative Research Center SFB 1078 Project C4 (to A.-N.B.) and by the Freie Universität Berlin within the Excellence Initiative of the German Research Foundation. An allocation of computing time was provided by the HLRN, the North-German Supercomputing Alliance (bec00063).

The study of proton transfer in photosystem II

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The proton transfer in Photosystem II is the crucial element of the proteins main function, which is the light driven water splitting reaction. Recent crystallographic studies of photosystem II [1-2] reveal interactions between the dimers of the protein suggesting dimer-dimer interactions having impact on the release of protons from the reaction centre. [3] To properly investigate the intrinsic influence of dimers on this process, simulations of dimer-dimer system is required. The system proposed for this study would consist of over 2.5 million atoms. To this aim we perform force-field parametrization of the manganese metal cluster of the oxygen evolution complex via quantum mechanical calculations and classical mechanical calculations of the dimer of dimers of photosystem II protein.

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Reaction Path Prediction in Proton Transfer Systems

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The translocation of protons from one side of a biological membrane to the other is an exceptionally important process in nature. To receive a comprehensive picture of the proton translocation through individual proton transfer channels the well orchestrated interplay of various degrees of freedom, acting on different time and length scales, needs to be elucidated. Transition Networks translate complex reactions into networks of simpler transitions, thereby allowing bias-free investigations using path optimization methods. A challenging aspect in calculating TNs is the exponential increase of stationary points on the energy surface with increasing system size, rendering a direct inclusion of all degrees of freedom, involved in the proton translocation process, infeasible. Hence, several re-calculations of the Transition Networks need to be performed with varying configurations of the unsampled degrees of freedom to gain a comprehensive understanding of this exceptionally important process. Here, we present a method, which determines coarse-grained Transition Networks, using simple graph theoretical algorithms, for different configurations of the unsampled degrees of freedom, starting from an initial Transition Network calculation, thereby reducing the re-calculation costs by up to 50 %, while maintaining important network properties, e.g. the minimax barrier of the proton translocation process.

Title: Analysis of Networks in Androgen and Glococorticoid receptors in complex with DNA

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We compared the interaction of the glucocorticoid (GR) and the androgen (AR) receptor in complex with a direct (DR) or an inverted (IR) repeat response element with the help of MD simulation. For the comparison we mainly used the first and last 100ns of the 500ns simulations to calculate different metrics for communication. We showed that H-bonds and correlations are important metrics to consider. Community analysis revealed that the complexes consist of five bigger core communities and several smaller communities. The main communities are all connected to each other and thus imply that the two monomers, the dimerisation region and the two hexamers of the DNA can act as allosteric ligands for one another. For communication between the hexamers and the diagonal monomer the spacer region is an important mediator.

Besides similarities among the AR systems and the GR systems, we also found some for AR-DR and GR-IR, and AR-IR and GR-DR, suggesting a higher specificity of the former because GR does not bind to DR. Monomer A of GR-DR is tilted slightly around the DNA and shows in the end much higher van der Waals and H-bond interaction. Mainly responsible for that is a strong H-bond between HSP472 and the DNA that cannot be found for the other systems. The establishment of that strong H-bond follows the distance of the zinc ions of the second zinc fingers. This is made possible after the H-bond between HSP472 in B and DNA breaks.

From time lagged generalised correlation analysis we learned that the whole complexes, especially the monomers, are constantly in motion as the residues always influence each other in their movement.