Photosynthesis & Photoreceptors

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Picosecond Fluorescence Of Intact And Dissolved PSI-LHCI Crystals

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Over the last years many crystal structures of photosynthetic pigment-protein complexes have been determined, and used extensively to model spectroscopic results obtained on the same proteins in solution. However, the crystal structure is not necessarily identical to the structure of the protein in solution. Here we studied picosecond fluorescence of Photosystem I-Light Harvesting Complex I (PSI-LHCI), a multisubunit pigment-protein complex that catalyzes the first steps of photosynthesis. The ultrafast fluorescence of PSI-LHCI crystals is identical to that of dissolved crystals, but differs considerably from most kinetics presented in literature. In contrast to most studies, the present data can be modeled quantitatively with only 2 compartments: PSI core and LHCI. This yields the rate of charge separation from an equilibrated core (22.5+/-2.5 ps) and rates of excitation energy transfer from LHCI to core (k_{LC}) and *vice versa* (k_{CL}). The ratio $R=k_{CL}/k_{LC}$ between these rates appears to be wavelength-dependent and scales with the ratio of the absorption spectra of LHCI and core, indicating the validity of a detailed balance relation between both compartments. k_{LC} depends slightly but non-systematically on detection wavelength, the avrage being $(9.4+/-4.9\ ps)^{-1}$. R ranges from 0.5 (below 690 nm) to around 1.3 above 720 nm.

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11-cis Retinol as a Substrate for Cone Dark Adaptation

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We have determined the effectiveness of 11-*cis* retinol as a substrate for visual pigment formation in intact vertebrate cone and rod photoreceptors and measured opsin-mediated transducin activation by 11-*cis* retinol. Methods were of two types. Firstly, visual pigment absorbance spectra were measured microspectrophotometrically in single cone and rod photoreceptor outer segments before and after bleaching of the native visual pigment and following subsequent treatment with 11-*cis* retinal and 11-*cis* retinol. Secondly, we expressed human and salamander cone and rod opsins in COS cells and then tested in a cell free assay the effects of these retinoids on the activation of transducin by opsin. We show that 11-*cis* retinol promotes pigment formation in bleached red and blue salamander cones but not in bleached salamander red or green rods. Transducin activation experiments show that 11-*cis* retinol acts as an inverse agonist of red and green cone opsins, but has no effect on the activity of blue cone opsins. In contrast, 11-*cis* retinol acts as an agonist of rod opsin. We conclude that cones have a mechanism for handling retinoids and regenerating visual pigment that is different from rods. 11-*cis* Retinal and 11-*cis* retinol are usable substrates for cone pigment regeneration and dark adaptation as both retinoids promote pigment regeneration and neither elicits activation of the transduction cascade by opsin. On the other hand, 11-*cis* retinol is not useful for rod function since it does not promote pigment regeneration and its opsin-mediated activation of rod transducin may slow the rate of rod dark adaptation.

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Sampling the unfolding pathways towards the signaling state of Photoactive Yellow Protein

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When receiving a signal trigger, sensor proteins undergo conformational changes resulting in the formation of a signaling state. Photoactive Yellow Protein (PYP) is a bacterial blue light sensor, 125 amino acids in size, including para-coumaric acid as a chromophore. Upon absorbing a blue-light photon, PYP undergoes a series of rearrangements to form a signaling state. The last step in this process is partial unfolding of the protein, occurring on a submillisecond timescale.
Molecular simulation can provide detailed insight into the mechanisms underlying protein conformational changes and is complementary to experiments. Studying a protein folding reaction at atomistic resolution with conventional atomistic Molecular Dynamics (MD) is unpractical due to the long time scales involved. These long time scales originate from the presence of local free energy minima from which it is not trivial to escape.
Advanced simulations enabled us to investigate the equilibrium characteristics, as well as the dynamical pathways of conformational changes linked to the formation of the signaling state of PYP.
Replica exchange MD resulted in the identification of several intermediates during the light induced unfolding. Using these state as input for transition path sampling and subsequent reaction coordinate analysis led to new mechanistic insights in this conformational change. The conformational change starts with the unfolding of a helix in the chromophore binding pocket, followed by the solvent exposure of either the chromophore or glutamate at position 46. Furthermore, our simulations indicate that it is more likely that Glu46 becomes solvent exposed first.
To our knowledge this is the first simulation study of unbiased dynamical pathways of a sub-millisecond timescale process of a biologically relevant protein. This work opens up the way for investigating conformational changes in other interesting systems in high detail.

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Retinal-Salinixanthin Interactions In Xanthorhodopsin: A Study Using Artificial Pigments

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Xanthorhodopsin (xR) is a recently discovered retinal protein which contains in addition to the retinal chromophore a carotenoid chromophore (salinixanthin), which transfers part of the light energy it absorbs to the retinal chromophore. We studied the interactions between the two chromophores by monitoring the UV-Vis and CD spectroscopies of the binding process with synthetic retinal analogues with shifted absorption maxima. We have revealed that the CD spectrum of xR, originated from the carotenoid chromophore as well as the ''pre-pigment'' of retinal, without significant contribution of the retinal chromophore. In addition, because the binding rate process of these analogues is slower compared to the *all-trans* retinal, it was possible to detect and analyze the major alterations in the CD spectrum. It was found that the major alterations occur as a result of binding site occupation by the retinal chromophore, and not due to the formation of the retinal-protein covalent bond.

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Dimer formation in the blue light sensing protein Vivid

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The LOV domain is a signal transducing component found in a large variety of proteins that is responsible for sensing different stimuli such as *l*ight, *o*xygen and *v*oltage. The LOV protein Vivid regulates blue light responses in the filamentous fungi *Neurospora crassa*. We have probed light-induced structural transitions in Vivid using laser excitation coupled with time-resolved small angle x-ray scattering. We observe conformationally distinct monomer states of Vivid that differentially participate in light-induced dimerization. These measurements allow us to propose a mechanism for how light regulates the oligomeric states of LOV domains.

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Design and Signaling Mechanism of Light-Regulated Histidine Kinases

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Signal transduction proteins are organized into sensor (input) domains that perceive a signal and, in response, regulate the biological activity of effector (output) domains. We reprogrammed the input signal specificity of a normally oxygen-sensitive, light-inert histidine kinase by replacing its chemosensor domain by a light-oxygen-voltage (LOV) photosensor domain. Illumination of the resultant fusion kinase YF1 reduced net kinase activity by ~1000-fold in vitro. YF1 also controls gene expression in a light-dependent manner in vivo. Signals are transmitted from the LOV sensor domain to the histidine kinase domain via a 40-60° rotational movement within an α-helical coiled coil linker; light is acting as a rotary switch. These signaling principles are broadly applicable to domains linked by α-helices, and to both chemo- and photosensors. Conserved sequence motifs guide the rational design of light-regulated variants of histidine kinases and other proteins.

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Interactions of Arrestin with Phosphorylated Opsin and the Role of All-trans Retinal

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Visual signal transduction in the dim-light sensitive rod cell begins with light-induced isomerization of the covalently-attached chromophore ligand of

rhodopsin, 11-cis retinal, to all-trans retinal. This event results in formation of the active species Metarhodopsin II (Meta II), which binds and activates several copies of the G-protein transducin, leading to signaling to nearby nerve cells. Metarhodopsin II is also the substrate for rhodopsin kinase, which phosphorylates the receptor to allow binding of the signal-quenching protein arrestin. Metarhodopsin II is not stable and decays within minutes into late photoproducts and ultimately the apoprotein opsin. In a rod cell exposed to the bright light associated with daytime, nearly all rhodopsin photoreceptors are ''bleached'' and exist as a heterogeneous population of these various late-photoproducts. Intriguingly, arrestin has been observed to translocate to the photoreceptor-rich disc membranes of the rod outer segment upon exposure to light, and arrestin remains there for the duration of light exposure.

To better understand the molecular mechanisms involved in this phenomenon, we have studied the interactions of arrestin with phosphorylated opsin (opsin-P) and the effects of all-trans retinal on this interaction. We find that, although arrestin has poor affinity for opsin-P (Kd ~ 40 μM) compared to Meta II-P (Kd ~ 20 nM), the addition of all-trans retinal to opsin-P increases its affinity for arrestin by approximately two orders of magnitude. Given that all rhodopsin photoproducts eventually decay to opsin and free all-trans retinal, this result could explain the persistence of arrestin in the outer segment during constant light-exposure. Finally, we find that arrestin binds opsin-P differently than Meta II-P but undergoes a similar conformational change upon the addition of all-trans retinal. The structural and physiological implications of our results will be discussed.

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Revamped Outer Segment Structure and Photoresponse in Retinal Rods Over-expressing Rhodopsin

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Visual phototransduction begins with photon capture by rhodopsin. Dense packing of rhodopsins in the outer segment membranes improves photon capture but can hinder the photoresponse by impeding the lateral diffusion of transduction proteins on the membrane. We attempted to investigate this effect by over-expressing rhodopsin in rods of transgenic mice. Increased rhodopsin expression was confirmed by Western analyses and by single cell microspectrophotometry. However, electron microscopy revealed that the excessive rhodopsins did not increase the packing density. Rods simply expanded the size of their membranous disks to accommodate the extra rhodopsins without increasing membrane congestion. Rod sensitivity increased due to improved photon capture. The dispersal of transducin and phosphodiesterase within the more spacious outer segment and the greater interdiskal volume delayed the photoresponse onset and reduced the cascade amplification. Flash responses from mutant and wild-type rods were fit with a mathematical model that adjusted for the alteration in outer segment structure.

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Structural Changes of Cephalopod Rhodopsin and β-Arrestin Measured by FTIR Difference Spectroscopy and Isotope Editing

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Invertebrate rhodopsin is the primary photoreceptor found in the eyes of cephalopods. Importantly, unlike vertebrate rhodopsin, invertebrate rhodopsins such as sepia rhodopsin (s-Rh) can be activated by light and then rapidly cycled back to the original state with a second red-shifted photon, thereby facilitating a variety of novel biophysical studies. Additionally, invertebrate rhodopsins can bind to the ubiquitous β-Arrestin2 which is used in regulating signal transduction in many GCPRs. In this study, we used static and time-resolved FTIR difference spectroscopy to investigate the photocycle of s-Rh complexed to β-Arrestin2. In the spectrum of s-Rh alone, difference spectra obtained using two colors to cycle between the ground (rho) and acid meta state show an 11-cis to all-trans photocycle as previously described. Several bands between 1750-1700 cm-1 are assigned using a D2O induced shift to an as yet unknown carboxyl groups. Other large bands are seen especially in the amide I and II regions which indicate significant backbone structural changes. Upon addition of β-Arrestin2, the difference spectrum is altered, especially in the amide I and II regions, reflecting additional structural changes occurring in β-Arrestin2 upon photoactivation. A negative band at 1742 cm-1 was shifted higher in the complex indicating the β-Arrestin2 is perturbing at least one carboxyl group in s-Rh. In order to assign these changes, total 15N isotope labeling of β-Arrestin2 was utilized. Comparison of difference spectra from s-Rh complexes containing unlabelled and 15N labeled β-Arrestin2 reveals ~ 3 cm-1 downshift of a negative/positive feature at 1668/1655 cm-1 indicating that these bands reflect at least partially conformational changes of the β -arrestin involving α-helical structure. These results offer a promising new tool to investigate the molecular mechanism of β-Arrestin interactions with GCPRs to regulate downstream signaling.

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Estimating The Rate Constant Of Cyclic GMP Hydrolysis By Activated Phosphodiesterase In Photoreceptors

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The early steps of light response occur in the outer segment of rod and cone photoreceptor. They involve the hydrolysis of cGMP, a soluble cyclic nucleotide, that gates ionic channels located in the outer segment membrane. This process has been characterized experimentally by two different rate constants beta_d and beta_sub: beta_d accounts for the effect of all spontaneously active PDE in the outer segment, and beta_sub characterizes cGMP hydrolysis induced by a single light-activated PDE. We estimate the experimental values of beta_d and beta_sub from a theoretical model. Considering diffusion in the confined rod geometry, we derive analytical expressions for beta_d and beta_sub by calculating the flux of cGMP molecules to an activated PDE site. We obtain the dependency of these rate constants as a function of the outer segment geometry, the PDE activation and deactivation rates and the aqueous cGMP diffusion constant. Our estimations show good agreement with experimental measurements.

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Revealing The Linear Aggregates Of Light Harvesting Antenna Proteins In Photosynthetic Membranes

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How light energy is harvested in a natural photosynthetic membrane through energy transfer is closely related to the stoichiometry and arrangement of light harvesting antenna proteins in the membrane. Their specific architecture helps them to absorb photons in the primary process of photosynthesis that is subsequently followed by a rapid and efficient energy transfer among the light harvesting proteins (LH2 and LH1) and to the reaction center. In this work, using atomic force microscopy (AFM) imaging, single membrane fragment FRET spectroscopy, spectral fluctuation analysis, and time-resolved spectroscopic analysis, we show the identification of linear aggregates of light harvesting proteins, LH2, in the photosynthetic membranes under ambient conditions. Our results suggest that the light harvesting proteins, LH2, mostly exist in two states, the aggregated and non-aggregated states in the photosynthetic membranes. Our results shed a light on understanding the complex intramolecular energy transfer dynamics and mechanism of the light harvesting in the photosynthetic membranes.

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Prolonged Illumination Up-regulates Arrestin And Two GCAPs: A Novel Mechanism For Light Adaptation

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In vertebrate photoreceptors, light adaptation is mediated by multiple mechanisms but the genomic contribution to these mechanisms has never been studied before. Therefore, we have investigated changes of gene expression using microarrays and real-time PCR in isolated photoreceptors, in cultured isolated retinas and in acutely isolated retinas. In all these three preparations after 2 hours of exposure to a bright light, we observed an up-regulation of almost two-fold of three genes *Sag, Guca1a and Guca1b,* coding for proteins known to play a major role in phototransduction: arrestin and guanylate cyclase activators 1 and 2. Gene up-regulation depends on light intensity and half up-regulation occurs for a light intensity corresponding to 5×10^3 Rh*/sec/rod. Gene up-regulation leads to an increase in the related protein content. Indeed, after three hours of