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Photosynthesis & Photoreceptors

2693-Pos Board B663

Picosecond Fluorescence Of Intact And Dissolved PSI-LHCI Crystals

Herbert van Amerongen¹, Bart van Oort¹, Arie van Hoek¹, Jan Willem Borst¹, Alexey Amunts², Nathan Nelson², Roberta Croce³.

¹Wageningen University, Wageningen, Netherlands, ²Tel Aviv University, Tel Aviv, Israel, ³University of Groningen, Groningen, Netherlands.

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 average being $(9.4 \pm 4.9 \text{ ps})^{-1}$. R ranges from 0.5 (below 690 nm) to around 1.3 above 720 nm.

2694-Pos Board B664

11-cis Retinol as a Substrate for Cone Dark Adaptation

Masahiro Kono¹, Rosalie K. Crouch¹, P. Ala-Laurila^{2,3}, M.C. Cornwall².

¹Medical University of South Carolina, Charleston, SC, USA, ²Boston University School of Medicine, Boston, MA, USA, ³University of Helsinki, Helsinki, Finland.

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.

2695-Pos Board B665

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

Jocelyne Vreede, Jarek Juraszek, Klaas J. Hellingwerf, Peter G. Bolhuis. University of Amsterdam, Amsterdam, Netherlands.

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 sub-millisecond 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.

2696-Pos Board B666

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

Elena Smolensky, Noga Friedman, Mordechai Sheves.

Weizmann Institute of Science, Rehovot, Israel.

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.

2697-Pos Board B667

Dimer formation in the blue light sensing protein Vivid

Jessica S. Lamb, Brian D. Zoltowski, Suzette A. Palin, Li Li, Brian R. Crane, Lois Pollack.

Cornell University, Ithaca, NY, USA.

The LOV domain is a signal transducing component found in a large variety of proteins that is responsible for sensing different stimuli such as light, oxygen and voltage. 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.

2698-Pos Board B668

Design and Signaling Mechanism of Light-Regulated Histidine Kinases

Andreas Möglich, Rebecca A. Ayers, Keith Moffat.

University of Chicago, Chicago, IL, USA.

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.

2699-Pos Board B669

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

Martha E. Sommer¹, David L. Farrens², Klaus Peter Hofmann¹, Martin Heck¹.

¹Institut für Medizinische Physik und Biophysik, Berlin, Germany, ²Oregon Health & Science University, Portland, OR, USA.

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