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described to promote maximum activity (with Mn as counterion instead of Mg). Using Dynamic Light Scattering (DLS), we will characterize the size of assembled complexes and probe the aggregation state of RTK fragments under the conditions described above. Data will be assessed and correlated with activity data.

1310-Pos Board B261

Patterned Ligand Surfaces Reveal F-Actin and Integrin Organization at EGF and IgE Receptor Signaling Complexes

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We use patterned surfaces to investigate the spatial relationship of ligand-engaged cell surface receptors to their downstream signaling assemblies and consequent cellular responses. We are examining two different receptor systems: the epidermal growth factor receptor (EGFR) on NIH-3T3 cells and the IgE receptor (FcεRI) on RBL mast cells. Arrays of micron-sized features with EGF or IgE hapten, 2,4-dinitrophenyl (DNP), are prepared with lithographic processes and chemical modification. Cells settle on these surfaces, and fluorescence imaging (confocal and TIRF) is used to visualize cellular proteins concentrating at regions of patterned ligand. Radial fluorescence analysis quantifies the intensity of labeled cellular components on and off patterned features and evaluates intensity distributions across different cell areas (e.g., middle vs. peripheral). In NIH-3T3 cells stably expressing EGFR, we find EGFR recruitment to patterned EGF is accompanied by stimulated tyrosine phosphorylation while F-actin and endogenous β1-containing integrins also concentrate, but with differential spatial localizations. F-actin accumulation is phosphatidylinositol 4,5-bisphosphate (PIP_2)-dependent: it is inhibited by pharmacological inhibitors of PIP_2 synthesis and by acute recruitment of a phosphoinositide 5′-phosphatase to the plasma membrane using a rapamycin strategy. Our previous investigations of IgE-FcεRI signaling used patterned lipid bilayers to present DNP. We now find that these surfaces yield more uniform accumulation of IgE-FcεRI and recruited signaling components than patterned DNP-conjugated proteins. This distinction suggests mast cells engaging antigen bound to other immune cells can undergo differences in membrane reorganization: larger, more dense clusters of IgE-FcεRI form when bound to mobile ligand patterns, whereas IgE-FcεRI concentrates at the edges of immobilized ligand patterns. Ongoing work aims to evaluate regulatory roles for F-actin and integrins in RBL mast cells with regard to functional outcomes for spatially localized interactions between activated FcεRI and downstream signaling partners.

1311-Pos Board B262

The Energetics of the Chromophore Regeneration Pathway in Rhodopsin

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The regeneration of rhodopsin after photobleaching (i.e., the binding of 11-cis-retinal) is a key molecular event responsible for dark adaptaion. Here we present a detailed study on the kinetics and thermodynamics of this reaction. We utilized a phospholipid/detergent bicelle system that provided superior opsin thermostability compared with other reconstitution systems. We employed recombinant rhodopsin with a genetically-encoded azido-Phe (azF) [1] that was subsequently modified with Alexa488 fluorophore in a stoichiometric bioorthogonal labeling reaction[2]. The Alexa488-Rho allowed us to develop FRET-based assays to monitor retinal entry into the binding pocket and the subsequent formation of Schiff base bond. We also showed that the diffusion of 11-cis-retinal among the bicelles was not rate-limiting and that the recombination reaction followed a simple second-order rate law. We measured the kinetics of the recombination reaction at different temperatures to assess the enthalpic and entropic contribution of the regeneration reaction. We further used isothermal titration calorimetry to obtain the overall change in enthalpy. To study the reverse reaction (i.e., the dissociation of retinal from opsin) we performed a chromophore exchange experiment in which we observed very slow (~10-7 s-1) exchange of 11-cis-retinal for 9-cis-retinal in rhodopsin. To our knowledge, this is the first in vitro experimental demonstration of the chromophore exchange reaction in rhodopsin. Based on the overall results of the study, we derived an energy diagram for the rhodopsin regeneration reaction.

[1] Huber, T. & Sakmar, T. P. (2014) Chemical Biology Methods for Investigating G Protein-Coupled Receptor Signaling. Chem. Biol.

[2]Tian, H., Naganathan, S., Kazmi, M. A., Schwartz, T. W., Sakmar, T. P. and Huber, T. (2014), Bioorthogonal Fluorescent Labeling of Functional G-Protein-Coupled Receptors. ChemBioChem.

1312-Pos Board B263

Light-Induced Switching of HAMP Domain Conformation and Dynamics Revealed by Time-Resolved EPR Spectroscopy

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In microbial photo- and chemotaxis a two-component signaling cascade mediates a regulated response of the flagellar motor to environmental conditions. Upon activation, photo- and chemoreceptors transfer a signal across the plasma membrane to activate the histidine kinase CheA. Successive regulation of the CheY-phosphorylation level controls the flagellar motor.

In Natronomonas pharaonis a sensory rhodopsin II - transducer complex (SRII/HtrII) mediates negative phototaxis. As the initial signal, a light-induced outward movement of receptor helix F leads to a conformational change of transducer helix TM2, which in turn propagates the signal to the adjacent HAMP domain.

For the HAMP domain, a widely abundant signaling module, several mechanisms were suggested, all comprising two distinct conformational states which we previously observed by two-component cw-EPR spectra at ambient temperatures.

Here, we trace the conformational signal and it's propagation throughout the elongated transducer.(1) We applied cw- and pulse-EPR spectroscopy in conjunction with nitroxide spin labeling. We follow transient changes by time-resolved cw-EPR spectroscopy and compare the resulting spectral changes to simulated EPR difference spectra revealing a shift in the thermodynamic equilibrium between the two states. Structure-based calculations of the expected spectral differences shows agreement with a shift towards a more compact state of the HAMP domain.

To extend the current signaling models to the whole complex, a trimer of *Np*SRII/*Np*HtrII dimers, we carried out molecular dynamics simulations and observed differences between the deactivated and activated complex, ultimately leading to a signaling model that can now be tested experimentally.

[1] Klose, D. et al., FEBS Lett. (2014) http://dx.doi.org/10.1016/j.febslet.2014.09.012 *(in press)*

1313-Pos Board B264

Dynamic Ligand-Protein Interactions Alter Rhodopsin's Conformational Ensemble: Simulations of Rhodopsin and Opsin

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Given their function as transducers of molecular signals across the cell membrane, G protein-coupled receptors (GPCRs) constitute a major target for drugs in a wide variety of physiological scenarios. Understanding the course of structural transitions that allosterically modulate their activation is, therefore, fundamental towards improving rational drug design. Here, we characterize distinct ensembles of all- atom molecular dynamics simulations of class A GPCR, rhodopsin. These ensembles correspond to the active- and inactive-like form of the receptor, with and without ligand, and amount to an aggregate sampling time of ~116 μs. By monitoring ligand orientation within the binding pocket, we observe that retinal adopts diverse, heterogeneous conformations that are consistent with ensemble-dependent dynamics. We also investigate internal hydration within the four ensembles and note how variations in solvation within the core and ligand dynamics may modulate the activity of the receptor.

1314-Pos Board B265

ATRAP (AT1R Associated Protein) Role on Angiotensin II-Mediated NHE3 Activity Modulation

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Angiotensin II (AngII) is one of the most important modulators of fluid transport at proximal tubule, playing a considerable role in the regulation of Na^+/H^+ exchanger isoform 3 (NHE3), which is responsible for the major part of transcelular Na^+ transport at this nephron segment. Such regulatory mechanism is mediated by AngII receptor type 1 (AT1R), a G protein-coupled receptor (GPCR). Considering that the recently identified AT1R interacting protein called ATRAP seems to act as a negative regulator of this receptor, this work aimed to address, by overexpression experiments, how ATRAP affects NHE3 regulation mediated by AngII/AT1R in a proximal tubule cell line (OKP). The NHE3 activity was evaluated by measuring intracellular pH (pHi) recovery after previous acidification with ammonium chloride pulse.