311-Pos Board B80

Developing an Assay to Probe Activtion and Conformational Dynamics of β2-Adrenergic Receptor on Single Molecule Level

Salome Veshaguri[1], Marijonas Tutkus[1], Christian V. Lundgaard[1], Asger Tønnesen[1], Signe Mathiasen[1], Sune K. Jørgensen[1], Lars Iversen[1], Nikos S. Hatzakis[1], Søren G. Rasmussen[2], Dimitrios Stamou[1].
[1]University of Copenhagen, Bio-Nanotechnology and Nanomedicine Lab, Copenhagen, Denmark, [2]University of Copenhagen, Department of Neuroscience and Pharmacology, Copenhagen, Denmark.

G-Protein Coupled Receptors (GPCRs) are structurally flexible membrane proteins(1), that mediate a host of physiological responses to extracellular ligands like hormones and neurotransmitters(2). Details of the dynamic structural behavior are hypothesized to encode functional plasticity seen in GPCR activity(1), where ligands with different efficacies can direct the same receptor towards different signaling phenotypes. Although the number of GPCR crystal structures is increasing(3-5), the receptors are characterized by complex and poorly understood conformational landscapes(6). Therefore, we have developed a fluorescence microscopy assay to study the activation and dynamics of single β2-Adrenergic Receptors (β2ARs) reconstituted in liposomes. Conformational fluctuations are monitored by changes in intensity of a small fluorescent molecule conjugated to an endogenous cysteine located at the cytoplasmic end of the sixth trans-membrane helix of the receptor. By imaging arrays of surface-tethered proteoliposomes, we can read out the dynamic properties of hundreds of single β2AR reconstituted in a lipid membrane. Our data reveal subtle changes in β2AR conformational dynamics with agonist stimulation, which would be undetectable in bulk assays(7-9).

References:
1. Kobilka & Deupi. Trends Pharmacol. Sci. (2007) 28:397-40
2. Maudsley. et al. JPET. (2005) 314:485-94
3. Granier & Kobilka. Nat.Chem.Biol. (2012) 8:670-3
4. Rosenbaum. et al. Nature. (2009) 459:356-63
5. Rasmussen. et al. Nature. (2011) 477:549-555
6. Huber & Sakmar. Trends Pharmacol. Sci. (2011) 32:410-9
7. Hatzakis. et al. Nat.Chem.Biol. (2009) 5:835-41
8. Bendix. et al. PNAS. (2009) 106:12341-6
9. Hatzakis. et al. JACS. (2012) 134:9296-302

312-Pos Board B81

***E. Coli* Heptosyltransferase I: Exploring Function and Dynamics to Create Better Inhibitors for GT-B Enzymes**

Daniel J. Czyzyk, Cassie Liu, Shreya S. Sawant, Manju Hingorani, Erika A. Taylor.
Wesleyan University, Middletown, CT, USA.

Heptosyltransferase (Hep) enzymes are essential for the formation of bacterial biofilms in Gram negative bacteria, making the Hep enzymes an important target for the development of biofilm inhibitors. Our research is directed toward the detailed characterization of both the chemical mechanism of HepI, as well as the protein dynamics. We have already demonstrated the ability of this enzyme to accept a variety of simplified substrate analogues with catalytic efficiencies as good or better than with the native substrate. GTs of the GT-B structural fold, like HepI, are characterized by having two beta/alpha/beta rossman-like domains connected by a linker region, requiring a conformational change from open to closed states in order for catalysis to occur. Currently our lab is exploring these conformational changes through intrinsic tryptophan fluorescence. Using stopped-flow and steady state fluorescence analysis, in conjunction with mutagenisis, we are attempting to elucidate conformational changes that occur during the catalytic cycle. By combining analyses of the chemical mechanism with an understanding of protein dynamics in this system, this work is anticipated to lead to enhancements in drug discovery for this and other GT enzymes.

313-Pos Board B82

Single-Molecule Analysis of Conformational Transitions in XPD Helicase

Mohamed K. Ghoneim[1,2], Maria Spies[2].
[1]Center for Biophysics and Computational Biology, University of Illinois at Urbana - Champaign, Urbana, IL, USA, [2]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA, USA.

Modular organization of DNA helicases is crucial for their cellular functions. ATP binding and hydrolysis governs sequential conformational transitions within the conserved motor core of a helicase, allowing it to transform the chemical energy stored in ATP into mechanical work or directional motion on the DNA lattice. Auxiliary modular domains incorporated into the helicase core determine substrate specificity and often modulate activity of the helicase. XPD (Xeroderma pigmentosum complementation group D) is a DNA helicase with a role in nucleotide excision repair and transcription. It is also a model for understanding the fundamental molecular mechanisms of a family of iron-sulfur (Fe-S) cluster containing helicases, which includes FANCJ, RTEL1, and CHLR1. These enzymes contain two characteristic auxiliary domains, Fe-S and ARCH, incorporated into the motor core. Previous structural and biochemical studies suggested that these auxiliary domains are involved in XPD-DNA interaction and may undergo significant conformational rearrangements during the helicase cycle. Such rearrangements will likely result in spatial separation of the Fe-S and ARCH domains. Here, we addressed whether XPD auxiliary domains are indeed dynamic and whether their mobility is correlated with DNA and ATP binding by the helicase. Single, fluorescently labeled XPD molecules were tethered to the surface and monitored using total internal reflection fluorescence microscopy. Conformational transitions of XPD were detected by following Fe-S-mediated quenching of a fluorescent dye that was site-specifically positioned in the ARCH domain. Dual excitation with green and red lasers was used to correlate conformational dynamics of XPD helicase with binding of the DNA substrate. This approach allowed us to separate and analyze the conformational dynamics of XPD in absence and in presence of DNA.

314-Pos Board B83

SPIM-FCCS: A Novel Technique to Quantitate Protein-Protein Interaction in Live Cells

Anand P. Singh[1], Jan Krieger[2], Agata Pernus[2], Jörg Langowski[2], Thorsten Wohland[1].
[1]Departments of Biological Sciences and Chemistry and Centre for Bioimaging Sciences, National University of Singapore, Singapore, [2]German Cancer Research Center (DKFZ), Biophysics of Macromolecules (B040), Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany.

The combination of Single Plane Illumination Microscopy with Fluorescence Correlation Microscopy (SPIM-FCS) allows the multiplexed measurement of spatio-temporal correlation functions. The light sheet in SPIM-FCS is easily measured and the dimensions of the array detector (EMCCD, sCMOS) are exactly known. The theory for SPIM-FCS with a light sheet of constant thickness and intensity over the pixel observation area has been derived and the observation volume can be exactly calculated. Therefore, calibration of the system is straight forward and SPIM-FCS provides absolute diffusion coefficients as verified by parallel measurements with confocal FCS, single particle tracking (SPT), and the determination of concentration gradients in space and time. Absolute concentration measurements are at the moment only possible with calibration by a standard solution since the cameras used to date do not operate in photon counting mode but are analog detectors. However, we show that over a wide concentration range a linear relationship holds between the number of particles detected and the actual concentration in the solution. We demonstrate that diffusion and concentration maps can be acquired within living cells and organisms, using the example systems c-Fos/c-Jun and CDC42/IQGAP1. Combining two excitation lasers and parallel two-channel fluorescence detection we can measure two-color cross-correlations that allow to determine a spatially resolved map of protein interactions within the sample.

315-Pos Board B84

TNFR1 Signaling is Associated with Backbone Conformational Changes of Receptor Dimers Consistent with Overactivation in the R92Q TRAPS Mutant

Andrew K. Lewis, Christopher C. Valley, Jonathan N. Sachs.
University of Minnesota - Twin Cities, Minneapolis, MN, USA.

The widely accepted model for tumor necrosis factor 1 (TNFR1) signaling is that ligand binding causes receptor trimerization, which triggers a reorganization of cytosolic domains and thus initiates intracellular signaling. This model of stoichiometrically driven receptor activation does not account for the occurrence of ligand independent signaling in overexpressed systems, nor does it explain the constitutive activity of the R92Q mutant associated with TRAPS. More recently, ligand binding has been shown to result in the formation of high molecular weight, oligomeric networks. Although the dimer, shown to be the preligand structure, is thought to remain present within ligand-receptor networks, it is unknown whether network formation or ligand-induced structural change to the dimer itself is the trigger for TNFR1 signaling. In the present study, we investigate the available crystal structures of TNFR1 to explore backbone dynamics and infer conformational transitions associated with ligand binding. Using normal-mode analysis, we characterize the dynamic coupling between the TNFR1 ligand binding and membrane proximal domains and suggest a mechanism for ligand-induced activation. Furthermore, our data are supported experimentally by FRET showing that the constitutively active R92Q mutant adopts an altered conformation compared to wild-type. Collectively, our results suggest that the signaling competent architecture is the