3208-Plat
Structures of Blood Coagulation Factor VIII in Solution and Membrane-Bound
Alexey Y. Koyfman[1,2], Jaimy L. Miller[3], Daniela Dalm[3], Kirill Grushin[3], Svetla Stoilova-McPhie[2,3].
[1]Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX, USA, [2]Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, Galveston, TX, USA, [3]Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, TX, USA.
In this study we apply Cryo-electron tomography (Cryo-ET) to resolve the structural changes of coagulation Factor VIII (FVIII) upon membrane binding. Despite the pivotal role FVIII plays in blood coagulation, information for its membrane-bound structure is incomplete. Cryo-ET is a powerful approach to investigate the functional structure of protein complexes close to the physiological state.
Coagulation Factor VIII (FVIII) is a multi-domain blood plasma glycoprotein. Defect or deficiency of FVIII is the cause for Hemophilia type A - a severe bleeding disorder. Recombinant FVIII concentrate is the most effective drug against Hemophilia type A. Human and porcine FVIII concentrates are commercially available. Porcine FVIII is highly homologous to human FVIII (86% sequence identity) and is used as replacement therapy in patients who develop inhibitory antibodies against the human form, as it also forms active complexes with human Factor IXa.
During blood clotting, FVIII undergoes proteolytic activation. Activated FVIII then binds to Factor IXa on the negatively charged platelet membrane to enhance thrombin generation and secure timely coagulation. Upon binding to a membrane, FVIII undergoes structural changes critical for its function.
The resolved structural differences between the porcine FVIII in solution and on the lipid membrane define the critical interfaces for its function. Structural understanding of Factor VIII binding to the membrane provides the basis for the design of novel therapeutic interventions against both hemophilia and thrombosis.
Acknowledgments
This work is supported by a National Scientist Development grant from the American Heart Association: 10SDG3500034 and UTMB-NCB start up funds to SSM. The authors acknowledge the Cryo-EM and Scientific Computing facilities at the Sealy Center for Structural Biology at UTMB (www.scsb.utmb.edu).

Platform: Dynamics of Ligand Binding and Coupled Motions

3209-Plat
Mapping Allosteric Communication Pipelines in GPCRs from Micro-second Timescale Molecular Dynamics Simulations
Supriyo Bhattacharya, Nagarajan Vaidehi.
Immunology, City of Hope National Medical Center, Duarte, CA, USA.
G protein coupled receptors (GPCRs) are 7 transmembrane (TM) helical proteins that signal through effector proteins bound at the intracellular (IC) interface. Agonist binding at the extracellular (EC) domain triggers conformational change at the IC domain leading to receptor activation. However, the mechanism of allosteric communication and the amino acids involved in the pipelines of allosteric communication (allosteric pathways) are not known yet. In this study, we have mapped the allosteric pipelines of communication in β_2-adrenergic receptor (β_2AR) using microseconds timescale Molecular Dynamics trajectories. Using mutual information in the internal coordinates, we have compared the allosteric pipelines among three β_2AR conformations; inverse agonist bound inactive state, agonist bound intermediate state, and agonist and G protein bound active state. Strong allosteric communication along the TM domains stabilize the receptor in the inactive and active conformations. However the agonist bound intermediate state is dynamic showing weakened communication between the EC and IC domains. We have identified the residues that mediate multiple allosteric pipelines as allosteric hubs. These allosteric hubs have also been identified with the mutations that alter the receptor efficacy without altering the ligand binding. The role of such mutations were not previously understood.
We have identified the residue networks involved in a continuous communication pipeline from the EC loops to the G protein coupling site. One of the termination points of the activation signal is the engineered Zn^{2+} binding site at the IC interface, which is a known positive allosteric modulator binding site in β_2AR. We have shown how our method in conjunction with MD simulations can identify allosteric pockets which can then be used for screening allosteric drugs in GPCRs.

3210-Plat
Molecular Dynamics Simulations of the Catalytic Subunit of Protein Kinase a Reveal New Insight into the Catalytic Process
Jianhui Tian[1], Loukas Petridis[2], William T. Heller[1].
[1]Structure and dynamics of soft matter, Oak Ridge National Lab, Oak Ridge, TN, USA, [2]Center for Molecular Biophysics, Oak Ridge National Lab, Oak Ridge, TN, USA.
Protein kinases chemically add phosphate groups to target proteins. Phosphorylation usually results in a functional change of the target protein, and serves as a regulatory mechanism to elicit a response to a stimulus at the cellular or organism level. Protein kinase A (PKA) was first characterized in 1968 and is considered to be the prototype for the entire kinome. The inactive form of PKA is a heterotetramer of two catalytic subunits (PKAc) that are bound to each member of a regulatory subunit (PKAr) dimer. PKA activates when two cyclic-AMPs bind to each of the PKAr, which releases the PKAc to perform the phosphorylation reaction on a polypeptide substrate. PKAc has two lobes that flank the active site: a small N-terminal lobe that is primarily associated with binding and positioning ATP, and a large lobe that provides a docking surface for substrates or inhibitor proteins. PKAc has three major conformational states: open, intermediate and closed. Despite its early discovery, its activation and deactivation is still not fully understood. Although NMR spin relaxation experiments have probed local dynamics, the movements of the PKAc domains, that may be related to its biological activity, have not been directly examined. To gain new insight into the role that PKAc dynamics plays in the phosphotransfer reaction, we performed microsecond long molecular dynamics simulations of apo-PKAc, PKAc-ATP (intermediate) and PKAc-ATP-substrate peptide (closed, reactive) and PKAc-ATP-inhibitor peptide (closed, nonreactive) complexes. By applying a network-based analysis method, we found conformational communities for PKAc in the various states that made it possible to identify critical residues and structural transitions involved in the catalytic process.

3211-Plat
Oxygen-Affinity of Hemoglobin is Regulated by Effector-Linked Dynamic Modulations of High-Frequency Thermal Fluctuations
Takashi Yonetani.
Univ. Pennsylvania, Philadelphia, PA, USA.
Heterotropic effectors reduce the O2-affinity of hemoglobin (Hb) as much as >10\3-folds: P50 = 0.4 → 200 mmHg, Klow = 0.3 → 0.004 mmHg-1, and Khigh = 10 → 0.004 mmHg-1. However, there are no detectable changes in either static T/R-quaternary and associated tertiary structures or the stereochemical and electronic structures of the heme coordination that are attributable to such changes in the O2-affinity of Hb, upon binding of the heterotropic effectors to either T(deoxy)- or R(oxy)-Hb1,2, indicating that the O2-affinity of Hb is not regulated by static T/R-quaternary and associated tertiary structures of Hb and that the reactivity (or the O2-affinity) of the heme Fe itself is not altered1, in sharp contrast to the mainstream idea of the heme-centric regulation of the O2-affinity in Hb3. Our 6ns-molecular dynamics simulations4,5 indicated that the amplitudes of very high-frequency (>GHz) thermal fluctuations of the globin matrix are entropically enhanced by the dissociation of O2 from oxy-Hb and/or binding of heterotropic effectors to deoxy- and oxy-Hb, in parallel to the reduction of the O2-affinity. The rate of dissociation of O2 from oxy-Hb is enhanced by the effector-linked enhanced thermal fluctuations, which simultaneously reduce the rate of geminate-recombination of O2. This results in the reduction of the apparent O2-affinity of Hb by heterotropic effectors.
References: 1 Yonetani & Kanaori, BBA 1834 (2013) 1873-1884; 2 Kanaori et al., BBA 1807 (2012) 1253-1261; 3 Perutz, Nature 228 (1970) 726-739; 4 Laberge & Yonetani, Biophys. J, 94 (2008) 2737-2751; 5 Yonetani & Laberge, BBA 1784 (2008) 1146-1158.

3212-Plat
Discovering and Manipulating Protein Conformational Heterogeneity and Function
Daniel Keedy[1], Henry van den Bedem[2], Justin Rettenmaier[1], James Wells[1], James Fraser[1].
[1]UCSF, San Francisco, CA, USA, [2]Joint Center for Structural Genomics, Stanford Synchrotron Radiation Lightsource, Stanford, CA, USA.
Proteins must transition between multiple conformations to accomplish their functions in the cell, yet the dominant three-dimensional perspective involves a static crystal structure representing the most populated single conformation. Recent advances have revealed previously "hidden" alternative conformations with the potential to bridge dynamic descriptions from NMR spectroscopy with the static descriptions from X-ray crystallography. However, it remains challenging to determine which of these alternative conformations are