bound/dimerized form and the unbound/monomer forms. Using small angle x-ray scattering (SAXS), crystallography, and NMR spectroscopy, we developed an equilibrium model for modulator dependent dimerization. This model demonstrates that a second modulator-binding site produces both an increase in positive cooperativity and a higher apparent affinity. A combination of the crystal structures of the bound modulators and the binding model developed using SAXS data provide new clues for the development of more effective allosteric modulators that may have cognitive enhancing effects.

1690-Symp
Intracellular Domains of NMDA Receptors Control Channel Permeation and Gating Properties
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Among glutamate-gated excitatory channels, NMDA receptors are pivotal to the physiology of central synapses. Their activation initiates cellular processes responsible for synaptic plasticity, the substrate of memory; but it can also awake apoptotic cascades that result in neuronal degeneration. Which cellular pathways are activated hinges critically on the amplitude and time course of the intracellular calcium injected by NMDA receptors. In turn, these features of the calcium transient depend fundamentally on the receptor's ionic conductance, calcium permeability, and gating kinetics. Here, I present new evidence from my laboratory that the NMDA receptor-mediated calcium transient is differentially controlled by the receptor's intracellular domains, with ionic conductance and calcium permeability set by GluN1 and gating kinetics controlled largely by GluN2 subunits. Importantly, the phosphorylation state of GluN1 residues modulates in a reversible and dynamic manner calcium permeability and pore size. This appears to be a heretofore unique case of physiologic regulation of channel permeability by reversible, in-situ post-translational modification. Given the critical role of NMDA receptor calcium transients in synaptic physiology, the mechanisms we discuss here may open the way for new ways to manipulate these for therapeutic gain.

1691-Symp
NMDA Receptors as Dynamic Allosteric Machines
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N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ion channels that are essential mediators of excitatory neurotransmission and synaptic plasticity. NMDARs have also been implicated in a plethora of neuropathological conditions thus receiving strong interest as potential therapeutic targets. Recent years have witnessed major progress in our understanding of the structure, mechanisms and pharmacology of NMDARs, with highlights including the decoding of the first full-length receptor crystal structures and the discovery of complex allosteric interactions between the constitutive domains and subunits. NMDARs form massive (>600 kDa) heterotetrameric complexes that usually incorporate two obligatory GluN1 subunits and two GluN2 subunits, of which there are four subtypes (GluN2A-D). Here, I will present the unique role and structural mechanisms of the large extracellular N-terminal domains (NTDs). These domains are distinct from the agonist-binding domains and lay most distal from the transmembrane pore but have been shown to control receptor activity. I will highlight recent experimental and modeling data showing that the allosteric capacity of NMDAR NTDs is intimately linked to their high conformational mobility, the clamshell-like NTDs undergoing large scale motions that can be sensed by the downstream gating machinery. The NTD allosteric signaling in NMDARs is unique among the ionotropic glutamate receptor family with important implications both for receptor physiology and drug action.

Platform: Single-Molecule Spectroscopy

1692-Plat
NMDA Receptor Ion Channel Dynamics in Living Cells by a Novel Single-Molecule Patch-Clamp FRET Microscopy: Revealing the Multiple Conformational States Associated with a Channel at its Electrical Off State
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Stochastic and inhomogeneous conformational changes regulate the function and dynamics of ion channels. The conformational dynamics is often inhomogeneous and extremely difficult to be directly characterized by ensemble-averaged spectroscopic imaging or only by single channel patch-clamp electric recording methods. We have developed a new combined approaches of using single ion channel patch-clamp electrical recording and single-molecule fluorescence imaging for probing ion channel conformational changes simultaneously with the electrical single channel recording. We were able to probe single ion-channel-protein conformational changes simultaneously with the electric on-off signals, and thus providing an understanding the dynamics and mechanism of ion-channel proteins at the molecular level.(1,2) We have probed NMDA (N-Methyl-D-Aspartate) receptor ion channel in live HEK-293 cell, especially, the single ion channel open-close activity and its associated protein conformational changes simultaneously. Furthermore, we have revealed that the seemingly identical electrically off states are associated with multiple conformational states. Based on our experimental results, we have proposed a multistate clamshell model to interpret the NMDA receptor open-close dynamics. Our results shed light on new perspectives of the intrinsic interplay of lipid membrane dynamics, solvation dynamics, and the ion channel functions.
Reference:
1. Dibyendu Kumar Sasmal, H. Peter Lu, " Single-Molecule Patch-Clamp FRET Microscopy Studies of NMDA Receptor Ion Channel Dynamics in Living Cells: Revealing the Multiple Conformational States Associated with a Channel at Its Electrical Off State," *J. Am. Chem. Soc.*, **136**, 12998-13005 (2014).
2. Suneth P. Rajapaksha, Xuefei Wang, H. Peter Lu, "Suspended Lipid Bilayer for Optical and Electrical measurements of Single Ion Channel Proteins," *Anal. Chem.*, **85**, 8951-8955 (2013).

1693-Plat
Exploring Tau Conformations at the Single-Molecule Level in a Microfluidic Trap
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The conformational dynamics of intrinsically disordered proteins (IDP's) are inextricably linked to their roles in signaling, regulation, folding, and diseases. Single-molecule methods can contribute valuable information on the conformational dynamics of biomolecules because they allow the observation of unsynchronized dynamics and characterization of diverse populations. Typically, target biomolecules are immobilized to allow study over a longer time window. However, biomolecules with more fluid structures, like IDP's, are highly susceptible to having their structure dominated by the immobilization environment. A method of studying single solution-phase biomolecules for prolonged periods of time would be highly useful for elucidating protein dynamics over many timescales.
In this study, we present the use of a microfluidic trap that is capable of canceling Brownian motion to allow the observation of solution-phase dynamics of IDP's over multiple seconds. We will focus on Tau, a protein contributor to the etiology of Alzheimer's disease. Solution-phase conformations of the monomer and small aggregates will be described. The details of the technique, dynamics of the biomolecule targets, and future applications and directions will be discussed.

1694-Plat
3D Tracking of Single Quantum Dots through Off-Focus Imaging
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Recently, tremendous improvements have been achieved in the precision of localization of single fluorescent molecules, allowing localization and tracking of biomolecules at the nm level. Since the behaviour of proteins and biological molecules is tightly related to the cell's environment, a growing number of microscopy techniques are moving from *in vitro* to *live cell* experiments. Looking at both diffusion and active transportation processes inside a cell requires three-dimensional localization over a few microns range, high SNR images and high temporal resolution (ms order of magnitude).
It has been shown that axial localization within few nanometers can be achieved through out-of-focus imaging, by studying the behaviour of the point spread function of probes out of the focal plane. Here we describe a new method, based on this approach, through which the x, y coordinates of the PSF's centre are localized and the radius of the off-focus PSF is automatically measured and related to the axial position of the probe, thus providing a calibration curve for indirect axial position measurement. Our method revealed a non-linear behaviour of this curve for both fluorescent beads and quantum dots. Through our algorithm, simultaneous localization of all three dimensions within 5 nm accuracy can be achieved, over a 2 um range, for 200 nm fluorescent beads. Moreover, by the combination of off-focus imaging with HILO (Highly Inclined and Laminated Optical sheet) illumination we demonstrate 3D tracking of single QDs inside living cells within 10 nm accuracy over 1um range.