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Membrane Induces Contraction but not Collapse of the Denatured State of a Helical Membrane Protein

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fall within previously determined estimates for both the bilayer center and interface and they correlate with the steeply changing profile of water concentration in the membrane. Using these results we have discovered a function that describes the solvation environment of the bilayer with nanometer resolution and how membrane protein energetics are modulated by it. These results allow us to more accurately predict hydrophobic side chain water-to-bilayer transfer free energies, and can be easily implemented in structure prediction algorithms.

**128-Plat**

**Characterization of Protein Folding Dynamics in Membrane-mimetic Environments using Single-molecule Fluorescence Spectroscopy**

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The self-assembly process of linear peptide chains to three-dimensional structures is a crucial prerequisite for the proper functioning of proteins. Key properties of this very intricate folding reaction have been well described by Kramers' theory. In this thermodynamic picture conformational changes are illustrated by diffusion on a free energy surface. In the case of a folding transition, the protein successfully crosses the free energy barrier between the unfolded and folded well. The attempt frequency as well as the transition path time, the part of the folding trajectory when the barrier is crossed, are both crucial parameters to obtain a better understanding of the folding process itself. While many studies shed light on the folding of water-soluble proteins, the folding process of self-inserting membrane-associated proteins still remains elusive.

Here, we study the folding of membrane associated proteins in the complex, anisotropic environment of membrane-mimetic systems. We reveal the influence of head group charge and polarity on the attempt frequency and on the transition path time of membrane-proteins by using single-molecule FRET and nanosecond FCS.

**129-Plat**

**Membrane Induces Contraction but not Collapse of the Denatured State of a Helical Membrane Protein**

Ruiqiong Guo[1], Kristen A. Gaffney[2], Michael D. Bridges[3], Miyeon Kim[1], Wayne L. Hubbell[3], Tobin R. Sosnick[4], **Heedeok Hong**[1,2].
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Defining the conformational features of denatured states of proteins is essential to understanding their folding, chaperone action, degradation and translocation. A central question is whether the denatured state ensemble (DSE) is expanded or collapsed. While a majority of studies have been focused on water-soluble proteins, the DSE of membrane proteins is much less understood. Here we successfully reconstituted the on-pathway DSE of a helical-bundle membrane protein GlpG of *E. coli* in native bilayers using a steric trapping. The method couples spontaneous unfolding of a doubly biotin-tagged protein to competitive binding of bulky monovalent streptavidin. Using our novel paramagnetic biotin derivative and double electron-electron resonance spectroscopy (DEER), we determined the interspin distances between specific biotinylated sites in the native and sterically trapped denatured states. To predict the distances between spin labels, we employed *Upside*, a model for course-grained molecular dynamics simulation of soluble and membrane proteins. *Upside* also provided the limiting distance distributions for the collapsed and fully expanded DSE models in a lipid bilayer. In large bicelles and *E. coli* lipid vesicles, the trapped DSE is expanded by 30-60% relative to the native state but contracted by 10-30% relative to the fully expanded condition. This result indicates that in DSE of membrane proteins, individual helices can partially separate, but not to a fully expanded ''good solvent'' condition.

## Platform: Neuroscience

**130-Plat**

**Antagonists Pharmacologically Chaperone Opioid Receptors**

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More than 100 people die daily in the United States from opioid-related drug overdoses. μ-Opioid receptor (MOR) antagonists, such as naltrexone (Ntx) and naloxone, partially suppress overdose effects, but also induce supersensitivity to MOR activation. Opioid receptors are folded in the endoplasmic reticulum (ER), undergo transport to the Golgi, eventually reach the plasma membrane, and may also later undergo endocytosis. We examined the effects of opioid ligands on MOR trafficking during the earlier processes. Properly folded protein cargo recruits ER exit sites (ERES) and then enters coated vesicles for delivery to the Golgi. We transfected SH-SY5Y cells with fluorescently tagged Sec24 variants to visualize and quantify ERES, and with the strongly ER-retained MOR mutant, MOR[N190K]. We attained sub-μm resolution, for information on ligand effects on ERES levels in live cells. Förster resonance energy transfer (FRET) showed that MOR closely interacts with both Sec24C and Sec24D. Using Sec24D-eGFP to fluorescently mark ERES, we observed that the antagonists increase the fraction of the cytoplasm occupied by ERES. We also found that SH-SY5Y cells overexpressing wild-type δ-opioid receptors have increased ERES levels after exposure to naloxone. These effects most likely occur when an antagonist acts as a pharmacological chaperone of opioid receptors. We rule out the alternative hypothesis that antagonists affect ERES levels via changes in [cAMP] because Ntx did not detectably change [cAMP]. In contrast to these effects of antagonists, several opioid agonists (morphine, fentanyl, buprenorphine, and methadone) lacked detectable effects on ERES levels. Full or partial agonists, but not antagonists, phosphorylate MOR at S375, but SH-SY5Y cells overexpressing MOR[N190K][S375A] showed no change in ERES density in response to agonists. The possibility that antagonists induce supersensitivity by pharmacologically chaperoning opioid receptors could suggest innovative approaches for opioid abuse disorder. Support: DA049140, DA046122.

**131-Plat**

**Changes in Number and Structure of Nerve Receptors (AMPARs) Associated with Memory in Dissociated Hippocampal Neurons**

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Changes in the number and structure of GluA1- and GluA2-type AMPA receptors (AMPAR) are important for long-term potentiation (LTP), in which synaptic strength increases and leads to learning. Electrophysiology, the standard method, is insufficient to quantify those changes because it measures the product of the current per receptor and the number of receptors. Here we used super-resolution fluorescence to quantify the change in the number of AMPARs per synapse. We find that at basal level on a transfected cell, there are $31 \pm 1$ GluA1. With LTP, this value increases by 22% at 5 minutes ($\sim$ 38), and to 29% ($\sim$40) after 20 minutes. Similar (though slightly smaller) changes in GluA2-type is also observed. The increase in the number of synaptic AMPAR (due to LTP), is because of intracellular AMPAR undergoes exocytosis into the synapse while avoiding the dense region called the Post-Synaptic Density. At the same time, the exchange between synaptic and extrasynaptic AMPARs does not significantly change the synaptic portion. This strongly argues against the widely believed lateral movements from extrasynaptic AMPARs. By combining the fluorescence and electrophysiology measurements we find the current increases by 1.6 times per AMPAR from 5 to 20 minutes after LTP, which could be explained by phosphorylation or reduced desensitization of AMPARs. Directly measuring the motion of AMPARs via super-resolution fluorescence clarifies the number of AMPARs that move into the synaptic membrane upon LTP.

**132-Plat**

**Astrocyte Expression of Synapse Promoting Genes is Developmentally Regulated by Neuronal and Astrocyte Activity**

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Astrocytes are crucial regulators of neuronal synapse development and function. In the rodent cortex neurons are arranged in layers, each with stereotyped synaptic connectivity. Cortical astrocytes produce several synapse promoting factors, however, whether they differentially regulate formation of synapses in different layers is unknown. Furthermore, the developmental expression pattern of astrocyte synaptogenic cues, and whether expression is regulated by neuronal or astrocyte activity, is unknown. Here we use genetic mouse models, immunohistochemistry and in situ hybridization to quantify the developmental expression changes of astrocyte-derived synapse promoting genes, such as glypicans and chordin like1, in the mouse visual cortex. The observed expression changes occurred mainly between post-natal days (P) 7 and 14, a