lipid bilayer and control the actin-actin or actin-lipid interactions by the addition of cross-linking proteins. Upon the addition of myosin II motors, we observe the movement of actin and myosin by timelapse confocal microscopy. In the absence of adhesion to the membrane and actin cross-linking proteins, contractility at 10-100 um length scales is only observed for sufficiently long (10 um) actin filaments. To facilitate contraction of short filaments (~ 1 um), the addition of an actin cross-linker protein is required. Increasing adhesion to the lipid bilayer reduces the rate of contraction while facilitating tension build up. Our results demonstrate the roles of actin network connectivity and membrane adhesion in modulating the nature of force transmission in a biomimetic model of the actin cortex.

Platform: Membrane Receptors & Signal Transduction II

1206-Plat
Biochemical Crosslinking and Liquid Chromatography-Mass Spectrometry Demonstrate a Rhodopsin Dimerization Interface Mediated by Helices 1 and 8
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Heptahelical G protein-coupled receptors (GPCRs) can exist as monomers and a ternary complex of ligand, GPCR and heterotrimeric G protein is the basic signaling unit. GPCRs also tend to form dimers and higher-order oligomers in membranes, although the functional consequences of these interactions are in most cases unknown. Furthermore, the precise protein-protein interface(s) in receptor dimers and the mechanisms mediating dimerization remain controversial. Two-dimensional and three-dimensional densities obtained from electron microscopy, as well as X-ray data on packing of rhodopsin crystals, suggest that the primary dimer contacts involve transmembrane helix (H) 1 and cytoplasmic H8. Biochemical crosslinking studies with dopamine D2 receptors hint that this interface may be broadly significant. We crosslinked rhodopsin dimers in native rod outer segment disk membranes to demonstrate the proximity of H8 between adjacent receptors. Four homobifunctional cysteine-reactive crosslinkers were used, two with maleimide groups and two with methanethiosulfonate groups. The formation of dimers and oligomers was verified by SDS-PAGE, size-exclusion chromatography, and immunoblot analysis. We used partial proteolysis and high-resolution liquid chromatography-mass spectrometry (LC-MS) to identify the site of a crosslink between Cys316-Cys316. Cys316 is one of two reactive cysteines in rhodopsin and is located in H8. The spacer length of the crosslinkers that formed the intermolecular Cys316-Cys316 crosslink is consistent with the distance predicted in a H1/H8 dimer model. This result corroborates findings from coarse-grained molecular dynamics (CGMD) potential of mean force (PMF) calculations, which show that the H1/H8 orientation is by far the most stable among the possible dimer orientations tested. Together these results strongly suggest the existence of this interface in native membranes. Given the high degree of homology across class A GPCRs, these results may be relevant for other receptors.

1207-Plat
The Retinal Energy Landscape as a Function of the Rhodopsin Photocycle
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Retinal is the covalently bound inverse-agonist of the prototypical G protein-coupled receptor, rhodopsin. It comprises a β-ionone ring and polyene chain covalently bound to Lys296 of rhodopsin by a protonated Schiff base (PSB). During the course of rhodopsin activation, retinal initially undergoes an 11-*cis* → all-*trans* isomerization, followed by a deprotonation of the Schiff base. Using quantum chemical calculations at the MP2 level of theory and solid state NMR spectroscopy we demonstrate substantial differences in retinal structure and dynamics between the protonated and deprotonated species. The delocalization of positive charge from the PSB results in perturbations of the entire retinal moiety upon deprotonation. For example, methyl rotation barriers are shifted as much as 200% [1]. Surprisingly, deprotonation of retinal drastically affects the energetics of β-ionone ring rotation, producing an extra minimum in the C5=C6-C7=C8 torsional energy surface. This results in a proton affinity (PA), and hence pK_a, that depends on β-ionone ring orientation. Specifically, the PA of retinal is lowered for non-planar conformations of the β-ionone ring, in turn lowering the pK_a and facilitating the deprotonation required for formation of the Meta I pre-activated state of rhodopsin. In order to extend these calculations to account for interactions within the rhodopsin binding pocket we have used the QM data, including MP2 level torsion scans of every dihedral angle, to refine new retinal force fields for both protonation. This data opens the door to future molecular dynamics studies of retinal proteins that include the activated Meta II state. [1] B. Mertz *et al*. (2011) *Biophys. J.* **101**: L17.

1208-Plat
Activation Mechanism of the β2-Adrenergic Receptor
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A third of marketed drugs act by binding to a G protein-coupled receptor (GPCR) and either triggering or preventing receptor activation. While recent crystal structures have provided snapshots of both active and inactive functional states of GPCRs, these structures do not reveal the mechanism by which GPCRs transition between these states. Here we characterize the activation mechanism of the β_2-adrenergic receptor, a prototypical GPCR, using the first simulations in which a GPCR transitions spontaneously from one crystallographically observed state to another (Nature 469:236 (2011); PNAS, in press); the total duration of these all-atom simulations, at over 600 microseconds, is unprecedented for a molecular system of this size. A loosely coupled allosteric network, comprising three regions that can each switch individually between multiple distinct conformations, links small perturbations at the extracellular drug-binding site to large conformational changes at the intracellular G protein-binding site. Our simulations also reveal an intermediate that may represent a receptor conformation to which a G protein binds during activation, and suggest that the first structural changes during receptor activation take place on the intracellular side of the receptor, far from the drug-binding site. By capturing this fundamental signaling process in atomic detail, our results may provide a foundation for the design of drugs that control receptor signaling more precisely by stabilizing specific receptor conformations.

1209-Plat
Dynamic Monomer-Dimer Equilibrium of a Prototypical GPCR, Beta2 Adrenergic Receptor: A Single Molecule Imaging Study
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Recently, by developing special single-molecule techniques and analysis theory, we first time ever succeeded in obtaining the two-dimensional association equilibrium constant in the membrane and fully characterizing the equilibrium by also obtaining both dissociation and association rate constants (Kasai et al., 2011). As an important paradigm for this work, we examined the monomer-dimer equilibrium of a GPCR, formyl-peptide receptor (FPR). Here, we applied this method to a prototypical class-A GPCR, beta2-adrenergic receptor (B-AR), to clarify whether it forms dimers. Although B-AR has been studied extensively, whether it (and other class-A GPCRs in addition to FPR) exists as dimers has been controversial. We found that B-AR does form dimers with rate and equilibrium constants similar to those for FPR (FPR values in parentheses): the monomer-dimer equilibrium constant = 1.6 (3.6) copies/square microns; the dimer dissociation rate constant of 12.6 (11.0) /s [dimer lifetime of 80 (90) ms]; and the monomer association rate constant of 7.9 (3.1)/[copies/square microns]/s. However, under physiological conditions, the behaviors of B-AR and FPR were quite different due to the difference in the expression levels, ~260 and ~2.1 copies/square microns [740,000 (6,000) copies/cell] in dog heart cells and neutrophils, respectively. The majority (95%) of B-AR would exist as dimers, whereas about half (42%) of FPR would do so. Interestingly, their dimer lifetimes are similar, ~80 and ~90 ms, respectively, but their monomer lifetimes would be vastly different, i.e., 0.49 ms and 154 ms, respectively. These results suggest that the dynamic monomer-dimer equilibrium might play a role in signal transduction and/or its regulation.

1210-Plat
Interactions of CCR5, the Main HIV Coreceptor, with Rantes and Other Ligands
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The entry of the human immunodeficiency virus 1 (HIV-1) into host cells requires the sequential interaction of the viral envelope glycoprotein 120 (gp120) with the host-cell factor CD4 and with either CCR5 (CC chemokine receptor 5) or CXCR4, both G-protein coupled receptors (GPCR). This leads to the fusion of viral and host cell membranes. The normal physiological