determined that prestin diffuses in and hops between confinement zones on the order of 1 micron in average size. We observe that depletion of membrane cholesterol increases average confinement size and decreases confinement strength. Statistical analysis of squared displacements reveals that depletion of cholesterol removes domains of intermediate size between 142 and 500 nm. From measurements of the initial increase of the mean squared deviation with time, the microscopic diffusion constant was determined to be 0.05 X $10^{-12}$ $m^2$/s, and was unchanged by cholesterol depletion. Our results suggest that membrane cholesterol affects prestin function by changing prestin crowding in confinement zones, consistent with the hypothesis that the microscale organization of prestin in the membrane influences prestin function.

**1390-Pos Board B300**
**Nano-Scale, Microsecond Diffusion Imaging of Membrane Protein - lipid Raft Interaction in the Plasma Membrane**
**Yunhsiang Hsu**, Arnd Pralle.
Spatial membrane domains, such as created by lipid rafts and the membrane cytoskeleton, influence membrane protein mobility and hence membrane bound processes, i.e. cell signaling. However, visualization of these lipid domains in intact cells is challenging because of their small dimension and dynamics.
We have visualized lipid raft and cytoskeleton domains by tracking the diffusion of membrane proteins with thermal noise imaging (TNI). TNI tracks the diffusion of a colloid labeled membrane protein with microsecond and nanometer precision. In addition, an optical trap confines the diffusion to a small area of the membrane providing sufficient statistics for high resolutions maps of the diffusion. We observe millisecond transient confinement zones consistent with 10nm lipid rafts which redistribute after depolymerization of the actin cytoskeleton. Also, we observed larger (~80nm) cytoskeleton caused confinement zones.

**1391-Pos Board B301**
**Modeling the Coalescence Kinetics of Cell Surface Receptor Clusters**
**Kathrin Spendier**, James L. Thomas, Vasudev M. Kenkre.
Cells in a multicellular organism must be ready to respond to a variety of extracellular signals such as ligands on the surface of cells or parasites. To understand these signaling mechanisms, many current experiments investigate transmembrane signaling of immunoreceptors on B cells, T cells, or mast cells, initiated by binding to specific monovalent ligands in fluid membranes. In mast cells, receptor clusters initially form at cellular protrusions through diffusion mediated trapping, undergo primarily diffusive motion with a partial directed element as well, and eventually coalesce at a finite rate to form a large central receptor patch termed synapse (Spendier et al. 2010. Biophys. J. 99:388–397). We are currently developing a coalescence theory to investigate the kinetics of receptor cluster coalescence in detail, which is important in understanding the mechanism and dynamics of cellular signaling. Our coalescence theory is split into trapping considerations, which generalize Smoluchowski's well known theory for arbitrary melding probability, and a feedback idea. Trapping considerations were developed with a unified approach constructed earlier for excitons (Kenkre and Reineker. 1982. Exciton Dynamics in Molecular Crystals and Aggregates. Springer, Berlin) to compute the particle survival probability for any dimension and motion due to a closed or open trapping surface. To build a coalescence theory, we use our trapping prescription to compute the rate at which particles disappear and solve for the time dependent trap radius through a self-consistent approach. We find that our coalescence theory for a finite melding probability is in good agreement with simulations.

**1392-Pos Board B302**
**Time Resolved Fluorescence Anisotropy on Supported Lipid Bilayers**
**Christopher R. Rhodes**, Jay T. Groves.
The diffusion and spatial organization of cell membrane receptor-ligand interactions, such as those involved in the formation of the T-cell immunological synapse, are important physical mechanisms of cell signaling. Although the binding kinetics within these structures have been established at the level of individual microclusters, the time-resolved interactions of single receptor-ligand pairs are generally not accessible by current optical methods. A fluorescence anisotropy method is proposed that has the potential to measure receptor-ligand kinetics without the need for intrinsic labeling of membrane receptors. By measuring the depolarization of fluorescently labeled proteins attached to supported lipid bilayers, nanosecond rotation rates can be used to characterize the proteins' microenvironment. Rotational diffusion of free and hindered model proteins were used to study the flexibility of covalent protein attachment and supported lipid bilayer properties. Progress toward single-molecule measurements of receptor-ligand kinetics in live cells, facilitated by nanoengineered substrates, will also be presented.

## Membrane Receptors & Signal Transduction I

**1393-Pos Board B303**
**Pharmacologically Distinct Ligands Induce Different States of 5-HT2AR and Trigger Different Membrane Remodeling: Implications For GPCR Oligomerization**
**Jufang Shan**, George Khelashvili, Sayan Mondal, Harel Weinstein.
We report on microsecond-scale molecular dynamics simulations of ligand-bound serotonin 2A receptor (5-HT2AR), which demonstrate for the first time at atomic detail how ligands with different measurable effects trigger differential structural perturbations both in the GPCR and the surrounding membrane. In these simulations the full agonist (5-HT), partial agonist (LSD) and inverse agonist (Ketanserin) affect differently the GPCR structural motifs/functional microdomains (e.g., the toggle switch and the ionic lock), stabilizing different conformational states of 5-HT2AR. We find that the dynamics of 5-HT in the binding pocket, moving away from and reentering the binding pocket, are correlated with the dynamics of the ionic lock which prefers the activated state configuration when the agonist is bound. Notably, significant conformational changes occur when 5-HT is replaced by Ketanserin in the activated state, resulting in the stabilization of an inactive-like state of the receptor, consonant with the inverse agonist properties of the ligand. The different states of GPCRs induced by pharmacologically distinct ligands also induce different reorganizations of the lipid matrix surrounding the receptor, and the local membrane perturbations produce different extents of hydrophobic mismatch around the transmembrane (TM) helices of 5-HT2AR. We quantified the energetics of these perturbations with a novel computational procedure (see Sayan Mondal et al., this meeting) for quantitative modeling of anisotropic bilayer deformations around multi-helical TM insertions. To our knowledge, this is the first calculation of differences in membrane remodeling by a GPCR in complex with different ligands, establishing a link between the receptor response to different ligands and the specific membrane deformations. The mechanistic implications of these results point to modes of ligand-induced GPCR oligomerization driven by the hydrophobic mismatch between the receptor and the membrane.

**1394-Pos Board B304**
**Molecular Dynamics Simulations of the Transmembrane Helix of the FGFR3 Receptor in POPC and DPPC**
**Tyler Reddy**, Benjamin Hall, Alan Chetwynd, Mark S.P. Sansom.
Fibroblast growth factor receptor 3 (FGFR3) is a receptor tyrosine kinase that negatively regulates bone growth. Elevated FGFR3 activity results in achondroplasia, the most common form of human dwarfism. In the majority (~98%) of cases the underlying mutation is G380R in the FGFR3 transmembrane domain. We have used coarse-grained molecular dynamics simulations to study the dimerization behaviour of wild-type, heterodimer, and mutant homodimer 33-residue transmembrane FGFR3 constructs in both POPC and DPPC bilayers. FGFR3 dimers are stable once formed in POPC, but dissociations are observed in DPPC. All three FGFR3 constructs exhibit bimodal helix crossing angle distributions, in contrast to the strong preference for right-handed crossing in glycophorin A (GpA) control simulations. We present evidence for a primary FGFR3 dimer interface and a less stable secondary interface. The latter is more pronounced for mutant than wild-type constructs in POPC, but not in DPPC. The helix crossing angle is right-handed at the secondary dimer interface for both heterodimer and mutant homodimer FGFR3 constructs in POPC. G370, A374, and R397 are prevalent FGFR3 dimer contacts, while the same analysis procedure on GpA control simulations selects the most important interfacial residues established by experiment. We suggest subtle differences, relative to wild-type, in the dimerization properties of G380R FGFR3 transmembrane domains.

**1395-Pos Board B305**
**Membrane Insertion and Membrane-Induced Conformational Changes of Talin F2F3 Triggering Integrin Activation**
**Mark J. Arcario**, Emad Tajkhorshid.
Activation of the integrin αβ heterodimer plays an important role in important biological processes such as thrombus formation and tumor metastasis. A well-known activator of intergin is the cyoskeletal-associated protein talin, whose membrane association is suggested to initiate the activation process. However, atomic details of the membrane-binding event and subsequent activation of integrin are largely unknown. We have utilized a novel membrane mimetics system developed in our laboratory, in conjunction with equilibrium molecular dynamics (MD) simulations to describe the membrane-binding event of talin F2F3 and characterize its subsequent interaction with integrin β3. In addition to characterizing the membrane orientation patch of talin F2F3, we were able to elucidate a conserved, Phe-rich hydrophobic anchor which was suggested by mutational studies, but was not evident in previous structural studies.