expose their anionic lipid content during chilling, we propose that PLA2-IIA is triggered during this process, and that the activity of the enzyme on the fluid domains induces an overall increase in rigidity in the platelet plasma membrane due to an increase in the proportion of ordered lipids. We present a combined FTIR and Laurdan fluorescence spectroscopy study on the thermotropic phase behavior of human platelets, and show that during cold-induced activation membrane rigidity increases. We present results on how the activity of sPLA2-IIA is regulated by the thermotropic phase behavior of the platelet plasma membrane after platelets have been artificially activated through sonication. We show that PLA2-IIA activity increases the rigidity of these activated platelet plasma membranes.

**2556-Pos**

**Neutron Scattering and MD Simulation Study of DOPC and DOPC/cholesterol Bilayers**

**Eduardo Jardon-Valedez**[1], J. Alfredo Freites[1], Ella Mihailescu[2], David Worcester[2], Stephen H. White[1], Douglas J. Tobias[1].
[1]UCI-Irvine, Irvine, CA, USA, [2]NIST Center for Neutron Research, Gaithersburg, MD, USA.

Cholesterol represents about 35% of the lipid content in the plasma membrane. Relevant cellular processes that have been associated with cholesterol-enriched microdomains include, among others, signaling, trafficking, cytoskeleton organization, apoptosis, and cell adhesion. In this work we studied the effects of cholesterol on model bilayers of DOPC using a combination of molecular dynamics simulations and neutron scattering experiments. Taking advantage of the higher scattering length of deuterium relative to hydrogen, the location of labeled components can be determined by difference-structure analysis of the scattering length density profiles. By labeling the terminal methyl groups of DOPC and a cholesterol ring, we determined their respective mass distributions across the membrane in DOPC and DOPC/cholesterol bilayers. The neutron data reveal the terminal methyl groups in a pure DOPC bilayer visit the head group-water interface as observed by the prominent wings in the mass distribution. A narrower terminal methyl group distribution of DOPC in the presence of cholesterol suggests an enhancement of the order of the lipid acyl chains. Cholesterol heads, on the other hand, point toward the aqueous interface, as expected. Order parameters $S_{CD}$ calculated from the MD simulations confirm that DOPC is more ordered in presence of cholesterol. The structural analysis based on MD simulation in concert with neutron diffraction experiments provides valuable data for advancing understanding of role of cholesterol in altering transbilayer communication in cholesterol-enriched microdomains found in biological membranes.

## Membrane Receptors & Signal Transduction II

**2557-Pos**

**Distribution and Dynamics of RBL IgE Receptors (FcεRI) Quantitatively Observed on Planar Ligand-Presenting Surfaces**

**Kathrin Spendier**, Amanda Carroll-Portillo, Keith Lidke, Bridget S. Wilson, Jerilyn A. Timlin, V.M. Kenkre, James L. Thomas.
University of New Mexico, Albuquerque, NM, USA.

There is considerable interest in the signaling mechanisms of immunoreceptors, especially when triggered with membrane-bound ligands. When T cells, B cells, or mast cells bind to monovalent ligands on fluid supported lipid bilayers, receptor clustering, signaling, and receptor redistribution into immunological synapses follow. We have quantitatively studied the kinetics of redistribution of IgE receptors (FcεRI) on RBL-2H3 mast cell surfaces. To separate the kinetics of receptor redistribution from cell spreading, the initial cell-substrate contact time was precisely defined ($\pm 50$ ms) by micropipette cell manipulation. Using total internal reflection fluorescence, the distribution and dynamics of receptor clusters were imaged. We find strong quantitative evidence that initial receptor aggregation occurs at cellular protrusions, which are visible bright spots/regions on cells contacting ligand-free surfaces. The initial size of these regions is independent of the substrate and the presence or absence of ligand, and they were found to be randomly distributed over the interfacial contact area. Using a finite-element diffusion model, we found that the initial rate of accumulation of receptors at the protrusions is consistent with diffusion-limited trapping with $D \sim 10^{-1}$ $\mu m^2/s$. At longer timescales, individual clusters on ligand-bearing membranes were observed to move with both a diffusive and a directed component of motion; clusters eventually coalesced near the center of the contact region (~ 1 minute). The dynamics of the early cluster motion is similar to the dynamics of membrane fluctuations of cells on ligand-free fluid membranes. Thus, the same cellular machinery may be responsible for both processes.

Sandia is a multi-program laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy under Contract DE-ACO4-94AL85000.

**2558-Pos**

**Multi-Color Quantum Dot Tracking to Correlate FcεR1 Aggregate Size with Mobility**

**Patrick J. Cutler**, Bridget S. Wilson, Janet M. Oliver, Keith A. Lidke, Diane S. Lidke.
University of New Mexico, Albuquerque, NM, USA.

The high affinity IgE receptor, FcεRI, is the primary immune receptor expressed on mast cells and basophils. These receptors bind circulating IgE with high affinity and are activated when multivalent allergen crosslinks IgE-bound receptors, initiating a complex signaling cascade that leads to the release of inflammatory mediators by degranulation. It is also well documented that FcεRI crosslinking leads to receptor immobilization. However, the exact mechanism of crosslink-induced activation is not completely understood.
We have used single quantum dot (QD) tracking to characterize the mobility of QD-IgE-FcεRI in the resting and activated state. We have previously shown that crosslink-induced immobilization is depended on the actin cytoskeleton as well as the antigen dose. Furthermore, we have determined that small, mobile clusters are signaling competent and that immobilization is the trigger for internalization rather than signal initiation. In these experiments, a combination of QD-IgE and dark IgE were crosslinked with multivalent antigen; therefore, the cluster size could not be directly correlated with mobility. We have now developed an experimental strategy and algorithmic approach to differentiate between monomers, dimers, trimers, and tetramers. Using a four-color beam splitter, we can simultaneously track FcεRI complexes labeled with four spectrally distinct QDs (525, 585, 655, and 705) at rates up to 30 frames/s. From these data, we use QD localization and channel overlay accuracies to identify monomers, dimers, trimers, and tetramers and determine the diffusion coefficient for each type of aggregate. We demonstrate this approach with both simulated and experimental data.

**2559-Pos**

**Fluorogen Activating Peptides for Single Molecule Localization-Based Superresolution and Single Particle Tracking of FcεRI Subunits**

**Samantha L. Schwartz**.
University of New Mexico, Albuquerque NM, NM, USA.

Fluorogen activating peptides (FAPs) are genetically expressible tags that increase the fluorescence excitation cross-section of dye binding partners by up to four orders of magnitude. The binding of FAPs with corresponding fluorogens is characterized by nanomolar affinity, corresponding to bound lifetimes of up to ten seconds. FAPs exhibit resistance to photobleaching, with single peptides repeatedly binding and activating new dye molecules. The resulting long observation lifetimes for single peptides make the FAP system a convenient, expressible probe for single particle tracking on live cells. The intermittency due to the equilibrium of dye binding and unbinding can be used for localization-based superresolution.
We apply this technology to study the localization and dynamics of the high affinity IgE receptor, FcεRI, the primary multi-subunit receptor on mast cells and basophils. Circulating, allergen-specific IgE molecules bind to FcεRI receptors via the $\alpha$-subunit. Receptor crosslinking by cognate multivalent allergen initiates the immune response by activation of the immunoreceptor tyrosine-based activation motifs on the $\beta$- and $\gamma$-subunits. We have previously characterized the dynamics of the FcεRI by tracking of quantum dot (QD)-labeled IgE bound to FcεRI$\alpha$. To compare the behavior of the $\alpha$- and $\gamma$-subunits, we have generated an FAP-tagged FcεRI $\gamma$-subunit that is expressed on the cell surface of rat basophilic leukemia cells and binds an exogenous fluorogen based on the malachite green dye. We use this probe to determine dynamics of the $\gamma$-subunit during resting and activated states and to generate superresolution images of FcεRI$\gamma$ distribution. We use QD-IgE, in conjunction with the FAP-FcεRI$\gamma$, for two-color single particle tracking to observe the relative spatial and temporal dynamics of the $\alpha$- and $\gamma$-subunits.

**2560-Pos**

**Receptor Cluster Size Affects Signaling in Breast Epithelial Cancer Cells**

**Qian Xu**, Jay T. Groves.
UC Berkeley, Berkeley, CA, USA.

Our purpose is to study a potentially novel mechanical regulatory mechanism in the EphA2 signaling pathway. EphA2 is a receptor tyrosine kinase that is known to be up-regulated in 40% of human breast cancers and plays an active role in metastasis. Activation of EphA2 occurs after binding to its ligand, presented on an opposing cell membrane. Monomer binding is followed by

dimerization of the receptor-ligand complex and then oligomerization. Past research, through predominantly biochemical methods, have concluded that EphA2 signaling depends on the degrees of multimerization of the proteins and the topology of the ligand presentation. However, clustering mechanisms of EphA2 proteins are not well understood because these signaling molecules function in the cell membrane, which is an environment that is difficult to characterize and manipulate. Our hypothesis is that the multi-scale organization of EphA2 in the cell membrane regulates its biochemical function. To mimic the cell-cell junction, we use a supported lipid bilayer - cell membrane hybrid system. Breast cancer cells presenting EphA2 are cultured on a fluid lipid bilayer consisting of ligand fusion proteins, which can stably interact with a subset of capturing lipids within the bilayer. This interaction allows us to control the protein density, precisely image it, and maintain molecular mobility so ligand-induced receptor clustering can occur. Receptor cluster size is varied by changing the cluster size and degrees of oligomerization of its ligand. On the nanometer length scale, antibodies are used to cross link monomeric forms of ligand fusion proteins and thereby vary the degrees of oligomerization. On the micrometer scale, patterned chromium substrates are used to segregate ligands into corrals of variable cluster sizes. Our results suggest that the spatial organization of receptor plays a role in orchestrating the cascade of signaling switches.

**2561-Pos**

**Probing Mechanical Regulation of Receptor Signaling Using a Hybrid Live Cell-Supported Membrane Synapse**

**Pradeep M. Nair**[1], Khalid Salaita[1,2], Debopriya Das[2], Joe W. Gray[2,3], Jay T. Groves[1,2].
[1]University of California, Berkeley, Berkeley, CA, USA, [2]Lawrence Berkeley National Laboratory, Berkeley, CA, USA, [3]University of California, San Francisco, San Francisco, CA, USA.

Recent studies have shown that the spatial organization of cell surface receptors can exhibit regulatory control over their associated signal transduction pathways. The corollary that follows from this observation is that mechanical forces acting on ligands can influence receptor spatial organization and subsequent downstream signaling. Juxtacrine signaling configurations, in which receptor and ligand reside in apposed cell membranes, represent an important class of intercellular communication where physical restriction of ligand organization and movement is evident. Here, we reconstitute the juxtacrine signaling geometry using a hybrid synapse formed between a supported membrane displaying laterally mobile ligands that are natively membrane-anchored and live cells expressing cognate receptors for these ligands. Fluid membrane-tethered ligand presentation induces a global receptor reorganization phenotype. This phenotype is linked to the expression of a subset of proteomic and genomic biomarkers, which suggests an association with disease characteristics. Using nanopatterned substrates to impose mechanical barriers to lateral mobility, it is possible to restrict and guide this reorganization event. Mechanical perturbation of receptor transport within the cell membrane alters the cellular response to ligand, as observed by changes in cytoskeleton morphology and protease recruitment. Our results indicate that receptor reorganization may be a mechanism by which cells respond to the mechanical properties of their environment.

**2562-Pos**

**FGFR1 Interaction with Co-Receptor Klotho-Beta at the Plasma Membrane**

**Eunjong Yoo**[1], Jocelyn Lo[1], Christopher Yip[1], Jonathan V. Rocheleau[1,2].
[1]University of Toronto, Toronto, ON, Canada, [2]University Health Network, Toronto, ON, Canada.

FGF21/FGFR1 signalling modulates the survival and glucose sensitivity of fat and liver cells, properties that make this signalling pathway a potential target in the treatment of diabetes. The majority of FGFs interact with heparin proteoglycans in the matrix for presentation to high-affinity receptors such as FGFR1. In contrast, FGF21 exhibits negligible affinity for heparin. To activate FGFR1, FGF21 requires expression of an alternative co-receptor, Klotho-beta (KLB). To study the molecular interaction between FGFR1 and KLB at the cell membrane, we created fluorescent protein-tagged constructs of KLB and FGFR1. By using fluorescence recovery after photobleaching (FRAP), we show that KLB has a lower diffusion coefficient and mobile fraction than FGFR1. Subsequent addition of lactose, an inhibitor of non-specific galactoside binding in the matrix, increased mobility of KLB with no effect on FGFR1. To determine whether the addition of FGF21 induces FGFR1/KLB association, we are presently examining whether FGFR1 mobility slows to KLB levels in the presence of FGF21. We are also measuring homo-Förster Resonance Energy Transfer (homoFRET) on a Total Internal Refection Fluorescence (TIRF) microscope to reconcile these results by examining the oligomeric state of KLB and FGFR1 at the plasma membrane. Overall, these studies will determine whether FGFR1 associates with KLB in the presence of FGF21 revealing important mechanistic information of a novel endocrine factor.

**2563-Pos**

**Caveolin-1 Boosts Clustering of Mu (μ) Opioid Receptors in the Plasma Membrane**

**Parijat Sengupta**[1], Susana Sanchez[2], Suzanne Scarlata[3].
[1]Washington State University, Pullman, WA, USA, [2]Laboratory of Fluorescence Dynamics, Irvine, CA, USA, [3]Department of Physiology and Biophysics, Stony Brook, NY, USA.

Caveolin-1 (Cav1), is a structural protein component of many mammalian cell plasma membrane and is known to be involved in lipid and protein sorting, receptor desensitization, receptor trafficking, cell migration and many other cellular events. Here we determine if stable expression of Cav1 in cells alters the receptor organization prototype on the membrane. We use two different cell lines for this study: Fisher Rat Thyroid (FRTwt) cells that do not express detectable level of Cav1 and a sister line that is stably transfected with canine Cav1 protein (FRTcav). We express μ opioid receptors (MOR) tagged with either YFP (MOR-YFP) or CFP (MOR-CFP) in cells for different experiments. Förster resonance energy transfer (FRET) measurement between MOR-CFP and Gαi-YFP in FRTwt and FRTcav cells shows receptor sequestration in the presence of Cav1. We find that diffusion of MOR-YFP in plasma membrane of FRTcav cells is slower compared to FRTwt cells by scanning fluorescence correlation spectroscopy (scanning-FCS) experiments. Photon counting histogram (PCH) analyses provide higher average brightness for MOR-YFP in FRTcav cells. Taken together these data provide evidence for caveolin-assisted enhanced clustering of G-protein coupled receptors on the plasma membrane.

**2564-Pos**

**Combinatorial Live Cell Homo- and Hetero-FRET Microscopy of Membrane Proteins**

**Jocelyn R. Lo**, John Oreopoulus, Prerna Patel, Eunjong Yoo, Jonathan V. Rocheleau, Scott D. Gray-Owen, Christopher M. Yip.
University of Toronto, Toronto, ON, Canada.

The structure-function-activity relationships of transmembrane receptors are often mediated not only by ligand-induced signaling but also homo- and hetero-philic binding interactions. Understanding the molecular basis for these interactions is therefore critical for elucidating receptor function. A powerful means of addressing these phenomena is to apply combinatorial microscopies that allow one to probe not only location but also orientation, association, and dynamics. By applying a coupled confocal-total internal reflection fluorescence (TIRF) microscopy imaging scheme, we are examining the distribution, association, and ligand accessibility of two families of transmembrane receptors: carcinoembryonic-antigen-related cell- adhesion molecule 1 (CEACAM1), and fibroblast growth factor receptor 1 (FGFR1), thought to associate with FGF21 co-receptor Klotho-beta (KLB). By using this coupled imaging platform, we can address differences in receptor behaviour, dynamics and structure on the free cell apical surface as well as in the cell itself by confocal microscopy and at the cell-substrate interface by TIRF microscopy. The use of homo- and hetero- Förster Resonance Energy Transfer (FRET) analysis provides us with a powerful means of examining real-time association kinetics of these systems and the effect of soluble ligands on receptor association.

**2565-Pos**

**Studying the NF-κB Signalling Pathway with High-Resolution Fluorescence Microscopy**

**Meike Heidbreder**, Darius Widera, Barbara Kaltschmidt, Christian Kaltschmidt, Mike Heilemann.
University of Bielefeld, Bielefeld, Germany.

Tumour Necrosis factor alpha (TNFα) has long been known to be an important mediator of inflammation, its secretion in cases of lesion or infection a main cellular event. Following activation of TNF receptors 1 (TNFR1) and 2 (TNFR2), the subsequent signal cascade can promote survival (by NF-κB activation) but also cell death (by activation of caspase-8). TNFR1 has been shown to form a trimeric structure in crystallographic studies, which corresponds to that of the native, homotrimeric TNFα. However, the dynamics of TNFR1 upon ligand binding are not yet fully understood.

Here, we use novel techniques from the toolbox of fluorescence spectroscopy and microscopy that enable high temporal and spatial resolution to study the dynamics of TNFα responses in eukaryotic cells. In particular, we use methods