Dynamin 2 (Dyn2) to generate curvature from model membrane templates in vitro. Here using site-directed fluorescence labeling coupled to multiple, independent spectroscopic techniques and confocal imaging on Giant Unilamellar Vesicles (GUVs), we have resolved the role of VL1 in dynamin function. Contrary to current understanding, our characterization of the isolated Dyn1 PH domain in comparison to full-length dynamin reveals that the PH domain VL1 is primarily a sensor of membrane curvature that serves in partitioning dynamin to regions of high membrane curvature (i.e. the narrow membrane neck of an invaginated coated pit) in order to direct localized dynamin self-assembly. Similar to full-length dynamin, the isolated PH domain preferentially recruited into highly curved membrane bilayers. However, unlike the full-length molecule, the PH domain was unable to generate curvature from planar membrane templates on its own. Our studies further reveal that in vitro fission-incompetent Dyn 1533A fails to distinguish membrane curvature in vitro and is defective in directing organized self-assembly on curved membrane templates. Our studies provide critical insights into the role of the PH domain VL1 in dynamin function and expand the repertoire of PH domain functionality in protein-protein and protein-membrane interactions.

3179-Pos Board B334
Characterizing MHC-I Delivery to Cell Plasma Membrane: A Spatiotemporal Study
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In our work, we aim at characterizing the delivery of Class I Major Histocompatibility Complex (MHC-I) molecules to the plasma membrane. Three aspects of MHC-I dynamics were investigated: delivery rate, position of delivery events and synthesis and delivery of new molecules.

Using Total Internal Reflection Fluorescence Microscopy (TIRFM) in combination with image analysis, we quantify the delivery rate of MHC-I to the plasma membrane. Furthermore, we demonstrate that inhibition of Dynamin reduces MHC-I delivery rate, a result that leads to the important conclusion that MHC-I molecules are primarily trafficked by Clathrin-coated vesicles. We find that MHC-I molecules are transported to specific, non-random locations on the plasma membrane, with possible implication on the interaction of MHC-I with their receptors on T lymphocyte cell membranes. Finally, comparing experimental Fluorescence Recovery After Photobleaching (FRAP) data with simulated recovery, we show that fluorescence recovery cannot be ascribed to lateral diffusion alone, and has an additional component originating in the delivery of newly synthesized MHC-I to the plasma membrane. These results may shed new light on the rates of synthesis and delivery of MHC-I molecules.

3180-Pos Board B335
Protein Structure Effects on Membrane Bending by Protein-Protein Crowding
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Two major mechanisms of cellular membrane bending during processes such as clathrin-mediated endocytosis have been previously proposed: bending by curved protein scaffolds such as a clathrin coat, and bending by insertion of wedge-like amphiphilic helices into the membrane by adaptor proteins such as epsin1. Recently we have reported a third general membrane bending mechanism: bending by protein-protein crowding, where pressure generated by densely bound proteins drives membrane bending(1). Several endocytic adaptor proteins consist of a folded N-terminal membrane binding domain, and an unfolded C-terminal domain that binds clathrin and other proteins. Due to their lack of structure, the unfolded protein domains have much larger hydrodynamic radii than folded protein domains, potentially increasing the effects of their crowding compared to proteins of equal molecular weight. We have investigated the capability of these unfolded portions of adaptor proteins to bend membranes by binding to them giant unilamellar vesicles. Using a Förster resonance energy transfer based assay of protein density, developed in our previous studies, we find that the unstructured epsin1 C-terminus can bend model membranes at substantially lower densities than the structured epsin N-terminal homology domain, which has traditionally been thought to drive bending. These findings suggest that concentrating unfolded domains of adaptor proteins at endocytic sites may have a previously unappreciated role in promoting membrane bending. We also find that the addition of clathrin can locally increase the concentration of epsin1 on the membrane surface. Our ongoing experiments are investigating how clathrin and adaptor proteins work together to curve membrane surfaces.


3181-Pos Board B336
Polarized-Tirf-Based Monitoring of Sub-Resolution Membrane Curvature Dynamics during Clathrin-Mediated Endocytosis
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Our understanding of clathrin-mediated endocytosis is derived from analysis of live-cell protein recruitment kinetics in combination with static ultrastructure images of coated pit progression. These analyses however, cannot directly correlate membrane curvature dynamics with the arrival and activities of the endocytic machinery. Polarized Total Internal Reflection (pol-TIR) fluorescence microscopy can visualize membrane topology in cells labeled with lipophilic fluorophores whose dipoles align relative to the plasma membrane. We describe a new approach for creating s-polarized and p-polarized TIR fields in a commercial microscope utilizing a 2-dimensional scan head to position polarized...
lasers at orthogonal azimuthal positions in the back focal plane of a high numerical aperture TIR objective lens. This configuration reduces interference fringing of the collection light. Rather, selective excitation polarizations permits visualization of subresolution membrane curvature with subsecond temporal resolution. By monitoring membrane topology relative to the arrival of chromosomally-tagged GFP-dynamin in skin melanoma cells, we were able to define the kinetics of membrane curvature relative to vesicle scission. Blocking the catalytic GTPase cycle of dynamin with the drug, Dynasore permitted visualization of striking curved invaginations, consistent with expected "frozen" clathrin coated pits. Tracking of single dynamin recruitment events provided a region in which to quantify membrane topology. Surprisingly, regions of high curvature were often observed adjacent to clathrin pits. This observation is consistent with a model for actin-mediated membrane protrusions that could drive pit closure. The role for actin was assessed using CFP-LifeAct, and perturbed with the drug, Latrunculin. These studies provide insight into the sequential topological changes during clathrin-mediated endocytosis and move toward a comprehensive understanding of this mechanism.

**3182-Pos** Board B337  
Kv2.1 Cell Surface Clusters Promote Maturation of Clathrin-Coated Pits

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The voltage-gated potassium channel Kv2.1 localizes to stable, micro-domains on the cell surface where it plays a non-conducting role. These surface structures are specialized platforms involved in trafficking of Kv2.1 channels and from the cell surface in hippocampal neurons and transfected HEK cells [Deutscher et al., Nature, 2009]. We examined internalization of Kv2.1 occurring through clathrin-mediated endocytosis and clathrin-coated pits (CCP) localizes adjacent to these micro-domains. This study examines the relationship between Kv2.1 clusters and CCP maturation.

TIRF-microscopy was used to study GFP-tagged-clathrin light chain CCPs in live HEK293 cells. HEK cells do not express endogenous Kv2.1, making them a suitable model system in which to investigate CCPs of Kv2.1 in clathrin-mediated endocytosis. We tracked individual CCPs and measured their lifetimes. This analysis is obtained from the appearance and disappearance of GFP fluorescence within the evanescent field illumination. We compare the dynamics of CCPs in control cells transfected with GFP-CLC and cells co-transfected with Kv2.1 or a Kv2.1 mutant lacking the last 318 amino acids of the C-terminus (ΔC-Kv2.1) necessary for cluster formation.

In control cells, CCPs had a mean lifetime of 12.6 ± 0.3 s (mean±SEM). The lifetime of CCPs in cells co-expressing Kv2.1 and GFP-CLC was reduced by 50%. When GFP-CLC was co-transfected with the non-clustering ΔC-Kv2.1, the lifetime of CCPs increased by 17%. ΔC-Kv2.1 also decreased the rate of channel endocytosis by 12%.

These data reveal that Kv2.1, specifically the C-terminal tail, has a direct effect on CCP lifetimes and thereby maturation. Cells expressing clustering Kv2.1 exhibited more rapidly maturing CCPs as seen by the order of 10-100 fold decrease in CCP lifetimes. Non-clustering Kv2.1 increases CCP lifetimes, and complete loss of Kv2.1 results in even longer lifetimes. Therefore, these results indicate that Kv2.1 cluster formation remodels clathrin-mediated endocytosis.

**3183-Pos** Board B338  
Measuring the Binding Energy between Cargo and Forming Clathrin Coated Pits

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Clathrin mediated endocytosis is the major route of cargo internalization in mammalian cells. The assembly of clathrin coated pits (CCPs) is a multistep process that includes nucleation of a clathrin coat and growth by recruitment of clathrin molecules, adaptors and cargo. This process is terminated either nonproductively (the pit breaks up) or productively (a vesicle forms and is internalized). Even though the association of cargo to CCPs is crucial in the regulation of endocytosis, the study of this interaction in vivo remains challenging.

Here we study the recruitment of cargo by characterizing the interactions of clathrin coated pits with Kv2.1, a potassium channel that is effectively internalized via clathrin mediated endocytosis.

TIRF-based single-particle tracking reveals that Kv2.1 displays a confined subdiffusion type of motion on the cell surface and frequent stalls occur during individual trajectories. Multicolor imaging indicates that these stalls are caused by static CCPs that capture Kv2.1 channels. By monitoring the residence time of Kv2.1 in CCPs, we were able to study the binding strength as a function of the age of a pit, e.g., the time since coat initiation. Due to the dynamic growth of CCPs, the interaction between the pit and cargo is not Poissonian. A kinetic model that takes into account the coat assembly via the recruitment of adaptor proteins leads to nonstationary and nonergodic Kv2.1 dynamics. This model accurately predicts the statistics of binding energies between pit and cargo.

**3184-Pos** Board B339  
Probing the Interior of a Secretory Granule

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Although a great deal is known about events leading to the release of secretory granule contents into the extracellular space, little is known about the physical state of the granule lumenal contents prior to exocytosis. We have used TIRFM as our core technique and combined it with either Fluorescent Correlation Spectroscopy (FCS) or Fluorescent Recovery After Photobleaching (FRAP) to assess the mobility of either Neuropeptide Y (NPY) or tissue plasminogen activator (tPA) inside chromaffin granules (300 nm diameter). Overall granule motion was taken into account by viewing simultaneously in the same granule one subset of protein molecules labeled with cereulean and the other with mCherry. Theories for separating out the effects of granule motion for both FCS and FRAP measurements were derived. The rapidly decaying evanescent fied labeled GFP-Kv1.4-loopBAD channels indicated that this ion channel also trafficked both to and from the cell surface at these microdomains following temperature-sensitive VSV-G protein mutant (YFP-VSV-G-ts045) was delivered to the cell surface at these microdomains following temperature-dependent release from the ER, demonstrating that ER/P MJunctions are trafficking hubs for nascent membrane proteins. Automated tracking of Qdot-labeled G-Fp-Kv1.4-loopBAD channels indicated that this ion channel also trafficked both to and from the cell surface at ER/P MJunctions. To highlight sites of endocytosis we expressed RFP clathrin microscopy and the transferrin receptor fused to a pH-sensitive GFP variant, superelastic pHluorin (TIR-SEP). ER/P MJunctions were defined by the fluorescent ER markers DsRed2-ER or ER-Tracker Green within the TIR illumination field which typically accounted for 20-20% of the cell footprint. Exocytic delivery of TIR-SEP was detected as the transient appearance and subsequent diffusion of bright puncta on the PM. Greater than 80% of these TIR-SEP delivery events were associated with CCPs indicating these microdomains are preferred sites for TIR exocytosis. The temperature-sensitive VSV-G protein mutant (YFP-VSV-G-ts045) was delivered to the cell surface at these microdomains following temperature-dependent release from the ER, demonstrating that ER/P MJunctions are trafficking hubs for nascent membrane proteins. Automated tracking of Qdot-labeled G-Fp-Kv1.4-loopBAD channels indicated that this ion channel also trafficked both to and from the cell surface at ER/P MJunctions. To highlight sites of endocytosis we expressed RFP clathrin light chain (RFP-CLC) which formed dynamic puncta. TIR-SEP formed similar puncta which co-localized with RFP-CLC when expressed together. Endocytosis of both types of puncta was observed frequently suggesting these puncta are endocytic sites. Almost 90% of both types of puncta were present within 0.3 µm of the ER perimeter. Together, these data indicate that both exo- and endocytosis preferentially occur at CCPS and are playing a role more central to basic cell biology than previously appreciated.