Dyn1 I533A) to generate curvature from model membrane templates in vitro. Here using site-directed fluorescence labeling coupled to multiple, independent spectroscopic, biochemical, 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 binds 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 Dyn1 I533A fails to distinguish membrane curvature in vitro and is defective in directing organized 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.

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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 amphipathic 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 them to giant unilamellar vesicles. using a Förster resonance energy transfer based assay of protein density, developed in our previous studies, we find that the unstructured epsin 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.