1634-Pos Board B404
Coordination of Bending and Wedging in Membrane Fission
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Protein complexes controlling intracellular membrane scission generally utilize multiple membrane insertions to enforce structural rearrangements of lipid monolayers. In general, the deformation fields created by individual insertions might interact destructively or constructively so that the resulting stress distribution depends strongly on the spatial organization and orientation of the individual insertions. We show here that in cylindrical topology the interaction between two ring-like membrane insertions can be both attractive and repulsive, dependently on the ratio between the curvatures of the membrane and the ring as well as on the insertion parameters. The attractive interaction drives clusterization of the rings leading to substantial stored elastic stress. This stored energy can be further used for localized membrane remodeling if the rings can interact changing the system geometry and boundary constraints. The model was applied to calculate energy barriers for dynamin-mediated membrane fission, substantial reduction of the barrier was found for a particular parameters range, indicating that the molecular design of the fission machinery involves optimization of the membrane insertion geometry.

1635-Pos Board B405
Essential Elastic and Shape Parameters Govern the Dynamics and Energetics of Dynamin-Mediated Membrane Fission
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Membrane fission is the final step enabling the release of vesicles during endocytosis. Dynamin is a protein required for this crucial process in clathrin-mediated endocytosis. It polymerizes into a helix at the neck between the plasma membrane and the endocytic bud. A constriction of the helix occurs upon guanosine triphosphate (GTP) hydrolysis leading to fission. Here we show how the fission mechanism is regulated temporally, spatially and energetically. We perform in vitro experiments where all mechanical and chemical parameters are controlled. Membrane nanotubes are pulled from giant unilamellar vesicles (GUV) using optical tweezers. Membrane tension is set by aspirating the GUVs within a micropipette. Dynamin and GTP are injected near the tube. Tubes always break few seconds after dynamin starts polymerizing. We show that the probability of fission depends on GTP concentration. Membrane tension and bending rigidity are key parameters controlling fission times in a non trivial way. Saddle-like shape favors membrane fission supporting the idea that negative Gaussian curvature plays an important role in the process.

1636-Pos Board B406
Spatio-Temporal Organization of a Minimal Dynamin Machinery Producing Membrane Fission
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Dynamin I, a prototype GTPase controlling membrane remodeling in cells, converts the energy of nucleotide hydrolysis into elastic stress driving membrane fission. The energy transduction requires the concerted action of many dynamin subunits, however, it remains unclear how many subunits, and how they are coordinated in time and space. Using lipid nanotubes pulled from different membrane reservoirs we found that 30-50nm long metastable scaffolds formed by dynamin in the presence of GTP are the most fission-effective. Such scaffolds correspond to just a few rungs of dynamin spiral locally squeezing the tube. The shorter structures fail to create the required elastic stress while the longer ones become self-inhibitory. The optimal scaffold produces elastic stress sufficient to induce fission; the stress is further converted into a hemifission-type intermediate structure by the concerted action of the plecstrin homology (PH) domains of dynamin. The PH domains rearrange during the transition state of the GTP hydrolysis allowing for the reversible closure of the nanotube, resembling the fusion pore flickering. This flickering stage is followed by the complete fission which facilitates disassembly of the dynamin scaffold. In this pathway, changes of dynamin scaffold geometry and rigidity that are associated with the GTP hydrolysis become stochastically coupled to the localized remodeling of the lipid bilayer.

1637-Pos Board B407
Protein-Protein Crowding as a Driving Force for Membrane Bending during Endocytosis
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Highly curved membranes are essential to many cellular functions, motivating an intense effort to discover the mechanisms that shape them. A conserved feature among many proteins that participate in membrane bending is an amphipathic alpha helix that inserts into one membrane leaflet, attaching the protein to the membrane. These insertions are thought to bend membranes by pushing lipid heads apart like a wedge. First reported for the Epsin 1 N-terminal Homology (ENTH) domain, a protein believed to drive curvature during clathrin-mediated endocytosis, this mechanism is thought to shape a wide range of membrane structures, from trafficking vesicles to viral envelopes. However, recent computational studies have questioned the efficiency of the insertion mechanism, predicting that proteins with amphipathic helices must cover nearly 100% of the membrane surface to generate high curvature, an improbable situation given that cellular membranes are densely populated with a diversity of proteins. How then do proteins with amphipathic helices drive efficient bending of cellular membranes? We show that Epsin1 bends membranes via protein-protein crowding rather than via helix insertion. By correlating membrane tubule formation with FRET (Förster Resonance Energy Transfer) lifetime-based measurements of ENTH density on membrane surfaces, we demonstrate that protein coverage above ~20% is sufficient to bend membranes. Whether proteins attach by inserting a helix or by binding lipid heads with an engineered tag, lateral steric pressure generated by bound proteins drives bending. Our results suggest that Epsin1's helix insertion functions primarily to achieve high protein-membrane affinity, enabling binding in a crowded environment. These findings call for a reexamination of the insertion hypothesis and demonstrate a new and highly efficient alternative mechanism by which the crowded protein environment on the surface of cellular membranes can directly contribute to membrane shape change.

1638-Pos Board B408
A Novel Sorting Prevention Mechanism Mediated by HID-1 during Early Biogenesis of Dense Core Vesicles
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Peptide hormones and neuropeptides are packaged and stored in a specialized intracellular organelle called secretory granules (SGs, also known as dene core vesicles, DCV). The molecular mechanisms involved in DCV biogenesis from the trans-Golgi network (TGN) are largely unknown. It is unclear how cargo proteins are correctly sorted or maintained in SGs. In search for mutants with high-temperature-induced dauer formation (Hid) phenotype in *C. elegans*, a gene designated *hid-1* was identified. The *hid-1* gene encodes a highly conserved protein (HID-1) with homologues in *Drosophila melanogaster*, mouse and *Homo sapiens*. Interestingly, the Hid phenotype of *hid-1* mutants is strongly suppressed in *C. elegans* by mutations in *daf-16* gene, which encodes a transcription factor downstream of insulin signalling, suggesting a possible role for HID-1 in the insulin branch of the dauer pathway. Our studies have implicated that HID-1 is involved in the early stages of SG exocytosis. We demonstrated that HID-1 serve as the 'stop sign' to prevent excessive missorting of soluble SG cargoes to lysosomes. We provided evidence that this preventive mechanism is essential for the maturation of SGs and peptide hormone signaling.