difference between iC3b and IgG consumption in a qualitative sense. Phagocytic studies can supply useful information for drug treatments involving passage of the drug through the cell membrane to reach its activation site. My research showed that opsonin density and type affect phagocytosis tremendously.

463-Pos Board B228
A Supported Tubulated Bilayer System Shows Effects of Synaptotagmin-7 on Membrane Curvature
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Fusion and fission of cellular membranes involve dramatic, precisely regulated changes in membrane curvature mediated by a number of proteins. The mechanisms by which proteins influence membrane curvature are not well understood, and current methods for investigating curvature changes using well-controlled systems are limited. We have developed a system based on supported lipid bilayers in which lipid tubules spontaneously form in a manner that can be tuned by varying the ionic character and strength used during bilayer deposition and imaging. Using this supported tubulated bilayer system, which we term ''STuBS,'' we have investigated membrane-targeting C2 domains from synaptotagmin-7, a member of the synaptotagmin protein family that triggers exocytosis in neurons and neuroendocrine cells. We find that addition of purified synaptotagmin-7 C2AB domains, but not synaptotagmin-1 C2A, leads to a Ca^{2+}-dependent disappearance of tubules with concomitant formation of vesicles. These studies demonstrate that synaptotagmin-7 can alter membrane morphology by driving changes in membrane curvature. STuBS is a novel experimental system useful for monitoring solute- and protein-mediated effects on membrane topology in aqueous media and in real time.

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Exosomes Fractionation by Biophysical Properties
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Exosomes are small extracellular vesicles containing nucleic acid and protein, which have shown a great potential for cancer diagnostics and therapeutic applications. Characterization of exosomes is challenging due to their inherent heterogeneity and complexity. A fractionation step is necessary to provide narrow-sized fractions to enable a more accurate subsequent size analysis. Asymmetrical Flow Field-Flow Fractionation (AsFlFFF) is a high resolution elution technique for fractionation of macromolecules and biological nanoparticles based on their hydrodynamic sizes. In this study the AsFlFFF system was interfaced with Multi Angle Static Light Scattering (MALS) detector to characterize the MCF-7 tumor exosome sample. The analysis revealed a size distribution between 30-120 nm in diameter. Several narrow fractions were collected along the size distribution and were analyzed by PCR. The study showed that the population of tumor exosomes in circulation are heterogeneous in their cancer biomarker miR21.

465-Pos Board B230
Membrane Recruitment Enables Weak Binding Endocytic Proteins to Form Stable Complexes
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Membrane targeting and assembly of proteins is required for vesicle trafficking and receptor mediated signaling, but it is not known to what extent the proteins recruited to these events may have evolved to exploit the 2D surface for assembly, versus pre-assembling in solution. We show that the phospholipid targeting proteins of clathrin-mediated endocytosis dramatically enhance their effective binding strength and subsequent complex formation to one another after surface recruitment in yeast and metazoans. For proteins such as clathrin that do not directly bind lipids, the enhancement is still achieved by using three distinct binding sites to stabilize the clathrin to peripheral membrane proteins on the surface. We derive simple formulas that quantify the degree of binding enhancement as a function of the protein and lipid concentrations, binding constants, and critically, the ratio of volume to membrane surface area. Our results thus apply to any cell type or geometries, including *in vitro* systems and the targeting of internal organelles from the cytoplasm. With a sufficient concentration of lipid recruiters, such as PIP2, we show that the effective binding strength is enhanced by orders of magnitude and becomes, surprisingly, independent of the protein-protein binding strength. We quantify how this effect varies for proteins involved in later stages of vesicle trafficking and cell division in yeast. Coupled with detailed spatially and structurally resolved simulations, we have further measured the effect of membrane recruitment on controlling the speed of assembly, and influences of crowding and diffusion on this process.

466-Pos Board B231
Mechanoregulation of Clathrin-Mediated Endocytosis in Isolated Cells and Developing Tissues
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Clathrin-coated assemblies bear the largest fraction of the endocytic load from the plasma membrane of eukaryotic cells. However, dynamics of clathrin-mediated endocytosis (CME) have not been established within tissues of multicellular organisms due to experimental and analytical bottlenecks in determining the lifespan of clathrin-coated structures. We found that clathrin coat growth rates obtained from fluorescence microscopy acquisitions can be utilized as reporters of CME dynamics. Growth rates can be assembled within time windows shorter than the average clathrin coat lifetime and, thereby, allow probing the changes in CME dynamics in real time. Furthermore, this novel approach is applicable to tissues as it is not prone to particle detection and tracking errors, which result in underestimation of the clathrin coat lifetimes. Exploiting these advantages, we detected spatial and temporal changes in CME dynamics within *Drosophila* amnioserosa tissues at different stages of embryo development. We also found that increased membrane tension impedes CME through inhibition of formation and dissolution of clathrin-coated structures. Therefore, the parameters defining clathrin coat dynamics (i.e., lifetime, formation density and growth rates) can be utilized to monitor the spatio-temporal gradients of the plasma membrane tension during cell migration and spreading.

467-Pos Board B232
Regulation of Lysosomal Exocytosis by Oxidative Stress and Calcium Ions
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Lysosomal exocytosis has emerged as an important mechanism of cellular repair and clearance. Cellular reactions supported by lysosomal exocytosis include membrane repair and expulsion of various toxins. Lysosomal exocytosis involves delivery of lysosomes to the plasma membrane followed by SNARE-dependent fusion. The processes of lysosomal delivery and fusion require calcium ions. How these processes are regulated is not completely understood, especially how they are modulated by pathological conditions. Our data show that lysosomal exocytosis is biphasically regulated by reactive oxygen species. Low levels of reactive oxygen species increase lysosomal exocytosis, while high levels of oxidative stress suppresses it. Activation of lysosomal exocytosis by reactive oxygen species requires both the lysosomal ion channels TRPML1 and calcium entry across the plasma membrane. High levels of oxidative stress suppressed lysosomal acidification and significantly the ability of calcium to stimulate lysosomal exocytosis. We propose that stimulation of lysosomal exocytosis by oxidative stress is a cytoprotective mechanism that limits lysosomal permeabilization aos cells death under oxidative stress condition. Suppression of lysosomal exocytosis by oxidative stress is a pathologic mechanism probably driven by inhibition of lysosomal delivery to the plasma membrane.

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Local Turgor Pressure Reduction via Channel Clustering
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The primary drivers of yeast endocytosis are actin polymerization and curvature-generating proteins, such as clathrin and BAR domain proteins. Previous work has indicated that these factors may not be capable of generating the forces necessary to overcome turgor pressure. Thus local reduction of the turgor pressure, via localized accumulation or activation of solute channels, might facilitate endocytosis. The possible reduction in turgor pressure is calculated numerically, by solving the diffusion equation through a Legendre polynomial expansion. We find that for a region of increased permeability having radius 45 nm, as few as 60 channels with a spacing