that deform the underlying membrane by progressive recruitment of clathrin, adaptors and other regulatory proteins. They ultimately close off and bud inward to form coated vesicles. Coated plaques are larger, less sharply curved, longer-lived structures; their clathrin lattices do not close off, but instead move uniformly inward from the cell surface shortly before membrane fission. Local remodeling of actin filaments is essential for the formation, inward movement and dissolution of plaques, but it is not required for normal formation and budding of coated pits. We conclude that there are at least two distinct modes of clathrin coat formation at the plasma membrane – classical coated pits and coated plaques – and that these two assemblies interact quite differently with other intracellular structures.

2932-Plat
The Language of Shape: Biological Reactions are Dramatically Affected by the Shape of Lipid Membranes
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A plethora of biological process are taking place on the surface of lipid membranes. As a rule membranes in vivo are curved in a variety of complex geometries. Here I will present a quantitave study on the influence of membrane curvature on protein-membrane and membrane-membrane interactions. To gain systematic access to a continuum of membrane curvatures we immobilized liposomes on a surface at dilute densities. Using confocal fluorescence microscopy we imaged single liposomes of different size, and therefore different curvature, and monitored their interaction with a binding partner (proteins or other liposomes).
I will discuss unpublished data on two important classes of biomolecular interactions that exhibited dramatic curvature dependence: A) SNARE-mediated docking and fusion B) anchoring of peripheral proteins.
The following references provide partial information on the single-liposome assay:
B. Lohse et al., JACS. in press.
A. H. Kunding et al., Biophysical Journal. 2008. 95 (3).
S. M. Christensen and D. Stamou. Invited review Soft Matter, Cover Page Article. 2007. 3 (7)
D. Stamou et al. Angewandte Chem.-Int. Edition, Cover Page Article. 2003. 42 (45).

2933-Plat
Cortical Tension Affects the Spatial Heterogeneity of Clathrin-Coated Pit Dynamics
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Clathrin-mediated endocytosis (CME) in mammalian cells is critical for many cellular processes including cell surface receptor down-regulation and nutrient uptake. From analyses of protein interaction networks, the actin polymerization machinery is a modular component within the endocytic interactome. However, the precise role of actin in CME is still under debate. Live cell microscopy has revealed a wide variation in the dynamics of clathrin-coated pits (CCPs). To gain insight of the heterogeneity of CCP dynamics and how cortical actin might influence this heterogeneity, we applied total internal reflection fluorescence microscopy to live cells grown on micro-fabricated substrates patterned with adhesive and non-adhesive regions. Cells on patterns showed overall longer CCP lifetimes compared to cells on chemically uniform surfaces, possibly the result of increased cortical tension. CCP lifetime distributions were also significantly different between adhesive and non-adhesive regions. When the structure of cortical actin is weakened by application of an actin monomer sequestering drug latrunculin A (latA), we found that the CCP lifetimes were homogenized to the level of the non-adherent regions. The decrease in CCP lifetime on adherent regions suggests that cortical actin filaments act as barriers at the adherent surface in CME.

2934-Plat
Screening the Sensing of Membrane Curvature by BAR domains on Single Liposome Arrays
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Membrane traffic relies on the preferential binding of protein domains to high curvature areas. The BAR domain is a banana shaped α-helical homodimer found in several proteins families that play a major role in endocytosis, actin regulation and signaling.[1] It is shown to sense and/or induce lipid membrane curvature by peripheral binding. While most attention have been aimed at curvature induction[2], we investigate the molecular mechanism of curvature *sensing* by performing a thorough study on the whole superfamily of BAR domain proteins including NBARs, FBARs, IBARs. We compared the sensing properties of 9 different BAR proteins and also measured on numerous truncation or point mutation variants.
We developed a high-throughput single liposome assay[3] to test the curvature dependent binding properties of these BAR proteins. Fluorescence intensities of immobilized vesicles allowed us to measure accurately their size/curvature and the respective densities of BAR proteins. Combining selectivity curves with the mutagenesis studies enabled us to evaluate the contribution of dimer structure, electrostatics and helix insertion to membrane curvature sensing by BAR domain proteins.
Our results prompt a thorough reevaluation of the membrane curvature *sensing* mechanism of BAR domain proteins.
[1] McMahon, H. T. & Gallop, J. L. Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 438, 590-596 (2005).
[2] Frost A. *et al.* Structural Basis of Membrane Invagination by F-BAR domains, Cell, 132, 807-817 (2008).
[3] Stamou, D., Duschl, C., Delamarche, E. & Vogel, H. Self-assembled microarrays of attoliter molecular vessels. Angewandte Chemie-International Edition, Cover Page Article 42, 5580-5583 (2003).

2935-Plat
Computational Delineation of the Bioenergetics of Protein-Mediated Orchestration of Membrane Vesiculation in Clathrin-Dependent Endocytosis
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Internalization of extracellular cargo by eukaryotic cells via the clathrin-dependent endocytosis (CDE) is an important regulatory process prominent in several cellular functions. Subsequent to receptor activation, a sequence of molecular events in CDE is responsible for the recruitment of various accessory proteins such as AP-2, epsin, AP180, eps15, dynamin, amphiphysin, endophilin, and clathrin to the plasma membrane to orchestrate membrane vesiculation. While the involvement of these proteins have been established and their roles in membrane deformation, cargo recognition, and vesicle scission have been identified, current conceptual understanding falls short of a mechanistic description of the cooperativity and the bioenergetics of the underlying vesicle nucleation event which we address here using theoretical models based on an elastic continuum representation for the membrane and atomistic to coarse-grained representations for the proteins. We employ the surface evolution approach to describe membrane geometries by minimizing the Helfrich Hamiltonian in a curvilinear coordinate system and address how the energetics of vesicle formation in a membrane is impacted by the presence of a growing clathrin coat. We consider two limiting scenarios: (1) the clathrin assembly model in which the clathrin coat induces membrane curvature by forming a curvilinear scaffold; (2) the accessory curvature-inducing protein assembly model, in which the clathrin lattice merely serves as a template to spatially pattern curvature inducing proteins such as epsin which collectively induce membrane curvature. Analyzing the energy required for vesicle formation from a planar bilayer, we demonstrate the role of the CDE protein assembly in driving membrane vesiculation. Furthermore, using a time-dependent Ginzburg-Landau formalism along with the thermodynamic method of free energy perturbation, we calculate the free energy the nucleated vesicle and quantify the finite-temperature corrections to the energy landscape of vesicle nucleation in CDE.

2936-Plat
The Dynamics Of Secretion-associated Plasma Membrane Changes Visualized With Polarized TIRFM
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The morphological dynamics of the plasma membrane were visualized in bovine adrenal chromaffin cells using polarized total internal reflection fluorescence microscopy (TIRFM). This method is based on monitoring the fluorescence of an oriented membrane probe (the carbocyanine dye, DiI) excited by a polarized evanescent field created by TIR illumination. DiI has been shown to embed in the membrane with its transition dipole moments nearly in the plane of the membrane. Thus, by monitoring the pixel-by-pixel ratio of the membrane-embedded DiI fluorescence excited by the two polarizations (***p*** - perpendicular to substrate; ***s*** - parallel to substrate) over time, regions of membrane curvature are vividly highlighted. To relate the orientation of the membrane with exocytosis, granules were labeled with the marker neuropeptide (NPY) - cerulean. In response to high KCl depolarization, fusion of granules coincided with 15-20% increases in DiI-membrane *p/s* values at locations of NPY-Cer release. The *p/s* values then often declined over several seconds to approximately pre-fusion levels. In other instances, the *p/s* values declined more slowly providing evidence of longer-lasting membrane curvature. Some granules were associated with areas of the membrane with increased curvature (larger *p/s* values) prior to stimulation. These granules were significantly more