

The amphipacity of the natively unstructured amyloid-beta (Ab40) peptide may play an important role in its aggregation into beta-sheet rich fibrils that is linked to the pathogenesis of Alzheimer's disease. Using the air/water interface as an ideal hydrophobic interface, we characterized Ab's surface activity and the structure, assembly, and morphology of Ab adsorbed to the air/water interface. Ab dissolved in water readily adsorbed to the air/water interface to form a contiguous film with a surface pressure of approximately 14 mN/m and showed an apparent critical micelle concentration of about 100 nM. Adsorbed Ab was composed of a single molecular layer extending approximately 20 Å into the aqueous subphase with in-plane ordering that gave rise to X-ray diffraction peaks. Analysis of the diffraction peaks showed that the air/water interface induced the otherwise unstructured Ab peptides to self-assemble into nano-size clusters with Ab peptides folded in a beta-sheet conformation. The presence of these clusters was further confirmed by imaging the morphology of the Ab film using atomic force microscopy. The formation of these ordered clusters was not affected by solution pH, ionic strength, or the presence of cosolutes sucrose and urea at concentrations that are known to stabilize and denature native proteins in solution, suggesting that the hydrophobic interface-driven Ab folding and assembly is robust and strongly favorable. Furthermore, Ab adsorbed at the air/water interface can seed fibril growth in solution when re-introduced into the bulk. Our results implicate that that interface-induced Ab folding and self-assembly may serve as a mechanism by which Ab aggregation occurs *in vivo*.

#### 2927-Plat

##### Thermodynamic and Kinetic Characterization of MST1 and Rassf5 conserved Sav/Rassf/Hpo (SARAH) Domain Interactions

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The molecular switch Ras exhibits its biological function- control of growth, differentiation and apoptosis through the interaction with a multitude of different effectors. It is apparent that growth-inhibitory properties of Ras are mediated via noncatalytic polypeptides of Rassf (Ras Association Domain Family). Tumour suppressor Rassf5 (also termed Nore1) binds directly to active Ras via the Ras Binding Domain (RBD). It is also known to form self-associated complex as well as heterodimers with the proapoptotic serine/threonine Mammalian Sterile 20-like kinase (MST1), the human ortholog for Hippo (Hpo), through their common conserved C-terminal Sav/Rassf/Hpo (SARAH) domains [1, 2]. This unique interaction motif connects the proteins involved in the recent discovered pathway mediated by proteins of the MST family, which promotes apoptosis and restricts cell proliferation [3-6].

For a better understanding of MST1 and Nore1 homo- and hetero- interactions via the SARAH domains, we have investigated the thermodynamics and kinetics of association/dissociation as well as the unfolding mechanism of this domain by use of different biophysical and biochemical methods, such as Differential Scanning Calorimetry (DSC), size-exclusion chromatography, artificial chemical cross-linking, Isothermal Titration Calorimetry (ITC), Circular Dichroism Spectroscopy (CD). MST1 and Nore1 SARAH domains are shown to form not only homodimers, but also higher oligomers. Nevertheless, the heterodimers rather than homodimers are preferentially formed. Finally, we propose a possible mechanism for the thermal unfolding of MST1 and Nore1 SARAH homo- and heterocomplexes.

##### References:

- [1] S. Ortiz-Vega et al., *Oncogene* 21, 1381-1390 (2002).
- [2] E. Hwang et al., *PNAS* 104, 9236-9241 (2007).
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- [4] S. Wu et al., *Cell* 114, 445-456 (2003).
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- [6] H. Scheel et al., *Curr. Biol.* 13, 899-900 (2003).

#### 2928-Plat

##### Chromophore Isomerization Has Large Effects On The Residual Structure Of The Fully Unfolded State Of The Blue Light Receptor Photoactive Yellow Protein

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Protein folding occurs between a well-defined fully folded native state and a structurally much less studied fully unfolded state. We use denaturant *m* values and changes in heat capacity  $\Delta C_p$  to probe folding transitions in photoactive yellow protein (PYP). PYP is a bacterial photoreceptor that exhibits rhodopsin-like photochemistry based on the *trans* to *cis* photoisomerization of its covalently attached *p*-coumaric acid (pCA) chromophore. We report strong

effects of the isomerization state of the pCA on the residual structure of the "fully unfolded" state of PYP by comparing the unfolding of two partially unfolded states of PYP: the acid-denatured state pB<sub>dark</sub>, which contains *trans*-pCA, and the partially unfolded pB photocycle intermediate, which contains *cis*-pCA. Our characterization of pB<sub>dark</sub> by circular dichroism spectroscopy and quenching of aromatic fluorescence indicates a strong loss of tertiary structure in pB<sub>dark</sub>. Despite its low tertiary structure content, pB<sub>dark</sub> retains considerable cooperativity for unfolding. As expected, the unfolding of pB<sub>dark</sub> is associated with values for denaturant *m* value and  $\Delta C_p$  that are smaller than those for the native pG state of PYP. A range of published studies show that the pB state is partially unfolded. We characterize the pB state based on its specific cold denaturation. Despite its partially unfolded nature, we find that the denaturant *m* values and  $\Delta C_p$  values for unfolding of the pB state are essentially the same as those for the native pG state. These results provide experimental evidence that pCA *trans* to *cis* photoisomerization causes significant loss of residual structure in the "fully unfolded" state of PYP. Such large changes in the residual structure of the fully unfolded states have important implications for describing and understanding protein folding.

#### 2929-Plat

##### Pressure Induced Denaturation in Proteins: Stability and Kinetics

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Intricate interplay of temperature and pressure on protein folding leads to interesting phase diagram. In addition, kinetics of pressure induced folding exhibit complex behavior. Here, we propose a simple mesoscopic model, a combination of landscape theory and microscopic details based on polymer physics to investigate this interesting phenomenon. The model is applied to experimental data to understand physical principles of pressure induced denaturation.

## Platform BF: Exocytosis & Endocytosis

#### 2930-Plat

##### Massive Endocytosis (MEND) Activated by Ca and Polyamines in Fibroblasts and Cardiac Myocytes: Dependencies on nucleotides, PIP<sub>2</sub>, cholesterol, clathrin, and other factors

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We describe four protocols that result in internalization of ~50% of the surface membrane of BHK fibroblasts and cardiac myocytes within <1 min. To do so, we use patch clamp with large pipette tips for cell dialysis and Na/Ca exchangers to evoke cytoplasmic Ca transients (5 to 200 μM Ca<sup>2+</sup>) for 1-5 s. Endocytosis is monitored via capacitance and/or optically by standard membrane dyes. In the first protocol, ATP is depleted from the cytoplasm, a Ca transient is evoked, and MEND is then activated by replenishment of ATP and GTP. GTP alone is not sufficient, Ca transients are required, and AMPPNP does not substitute for ATP. Second, when membrane cytoskeleton is stabilized with phalloidin, MEND is made 'available' for 1 to 3 min, and it occurs within 5 s during a Ca transient without ATP depletion. Third, high ATP concentrations (4 to 8 mM) promote MEND to occur within 20 to 60 s after (but not during) a Ca transient. Fourth, polyamines, spermine or spermidine, at physiological concentrations (1 mM) cause MEND to occur within <5 s during Ca transients without ATP depletion. MEND is not blocked by protein domains and other interventions that block clathrin-dependent endocytosis or by tyrosine kinase inhibitors. MEND is blocked by cholesterol depletion, GTPγS, and PIP<sub>2</sub> phosphatases, and MEND is promoted by perfusion of PIP<sub>2</sub> into cells when ATP and GTP are depleted. In neonatal myocytes, transient GTPγS perfusion substitutes for Ca transients in permissive steps leading to MEND activation upon ATP perfusion. We conclude that MEND is a regulated and massive cell stress response that can remove large fractions of the cell surface of multiple cell types by clathrin-independent mechanisms.

#### 2931-Plat

##### Distinct Dynamics Of Endocytic Clathrin Coated Pits And Coated Plaques

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Clathrin is the scaffold of a conserved molecular machinery that has evolved to capture membrane patches, which then pinch off to become traffic carriers. These carriers are the principal vehicles of receptor-mediated endocytosis and are the major route of traffic from plasma membrane to endosomes. We report here the use of *in vivo* imaging data, obtained from spinning disk confocal and total internal reflection fluorescence microscopy, to distinguish between two modes of endocytic clathrin coat formation, which we designate as "coated pits" and "coated plaques". Coated pits are small, rapidly forming structures

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 processes 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 quantitative 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  $\alpha$ -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 proper-

ties 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.

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[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