Posters: General Protein-Lipid Interactions II

2264-Pos Board B280

SANS Observation of Precrystallization Intermediates of Bacteriorhodopsin in the Lipidic Cubic Phase

Thomas Edgar Cleveland, Paul Butler.
Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD, USA.

Membrane proteins can be incorporated into the lipidic cubic phase (LCP) for crystal growth and structure determination. LCP crystallization has become an important tool in the field of membrane protein crystallography (particularly, but not solely, with GPCRs and other small membrane proteins). However, many details of this process are not well understood. There is little direct experimental evidence for the localization of protein and detergent after incorporation into LCP; the mechanisms of nucleation and crystal growth; and the details of how the cubic phase modifies the interactions between protein molecules. We are using Small Angle Neutron and X-Ray Scattering (SANS/SAXS) to study each step of the cubic phase crystallization process using Bacteriorhodopsin (bR) as a model system.

Using SANS, it is possible to contrast-match the non-protein components of the system, i.e. detergents and lipids. This allows us to measure the protein scattering directly and in isolation, greatly simplifying the data interpretation from these complex multicomponent systems. At high bR concentrations, it is possible to measure structure factors, from which information on protein-protein interactions can be obtained. We have measured the concentration-dependent scattering of bR: (1) in solution; (2) after incorporation into LCP; and (3) as a function of precipitant concentration. Solution structure factor measurements at lower salt concentrations are consistent with a charged sphere interaction model. In contrast-matched LCP at lower concentrations of bR and precipitant, scattering from bR monomers could be observed, similarly to bR in solution. At higher bR and precipitant concentrations, a series of higher-order structures were observed by SANS, as well as protein-dependent Bragg reflections in samples in which macroscopic crystals were later observed.

2265-Pos Board B281

Polymer Nanodiscs as New Platforms for Membrane Proteins

Mariana C. Fiori[1], Yunjiang Jiang[1], Wan Zheng[1], Miguel Anzaldua[2], Mario J. Borgnia[3], Guillermo A. Altenberg[1], Hongjun Liang[1,2].
[1]Department of Cell Physiology and Molecular Biophysics, and Center for Membrane Protein Research, Texas Tech University Health Sciences Center, Lubbock, TX, USA, [2]Department of Chemical Engineering, Texas Tech University, Lubbock, TX, USA, [3]Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.

Lipid nanodiscs (LNDs) are discoidal nanostructures that consist of a lipid bilayer membrane patch encased within membrane scaffold proteins (MSPs) derived from apolipoprotein A1. LNDs are playing increasingly important roles in studies of the structure and function of membrane proteins (MPs). A recent development is the use of styrene-maleic acid (SMA) copolymers for solubilization and reconstitution of MPs into nanodiscs. These polymer-encased nanodiscs (SMALPs, for SMA lipid particles) are promising platforms for studies of MPs in a near-physiologic environment without the use of detergents. However, current SMA copolymers display severe limitations in terms of buffer compatibility and ensued flexibility for various applications. In addition, the development of nanodiscs as a MP-supporting platform, or a drug targeting and delivery vehicle, is undermined by the fluidic and labile nature of the lipid bilayer. Here, we introduce new approaches to address some of the drawbacks of SMALPs and LNDs by using a set of new block copolymers to replace the MSPs and another set of block copolymers to replace the lipid bilayer. Our new family of zwitterionic styrene-maleic acid-derivative copolymers (zSMAs) do not aggregate at low pH or in the presence of polyvalent cations (as commercial SMAs do), and can be used to solubilize MPs and produce nanodiscs of controlled sizes. We also introduce polymer nanodiscs (PNDs), discoidal amphiphilic block copolymer membrane patches encased within MSPs. PNDs are novel two-dimensional nanomembranes that maintain the advantages of LNDs while addressing their stability weakness. We expect that the higher mechanical and chemical stability of block copolymer membranes and their chemical versatility for adaptation will open new opportunities for applications built upon diverse MP functions, or involved with drug targeting and delivery.

This work was supported in part by NSF grants DMR-1623241 and CBET-1623240.

2266-Pos Board B282

The Role of Angiomotin Coiled-Coil Homology Domain Arginine/Lysine Residues in Vesicle Fusion Activity

Seth Sears[1], Ann Kimble-Hill[2].
[1]Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA, [2]Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN, USA.

Angiomotins (Amot) are a family of adaptor proteins that control cellular signaling responsible for cellular differentiation and proliferation. These cellular events have been liked to regulation of invasive ductal carcinoma, the most common form of breast cancer. Their characteristic coiled-coil homology (ACCH) domain is of particular interest because of its capability to selectively bind phosphatidylinositol lipids (PI). These binding events subsequently lead to lipid membrane deformation and juxtanuclear endosomal vesicle fusion to the apical membrane. Our library of arginine and lysine residue mutations in the ACCH domain were screened for a loss of vesicle fusion activity. The mutations at the following residues led to a diminished fusion activity: R40T, K49E, K72E, K76E, R85T, R103G, K111E, K126E, K136E, K187E, R140S, R221Q, R224E, and R234G. Next, we endeavored to characterize how each of these residues participated in vesicle fusion by determining the kinetic rate of vesicle fusion. In this study, fluorescence resonance energy transfer between probes from 2 different lipid populations as a function of incubation time and protein concentration was utilized to determine the rate of fusion. Careful analysis of this data will provide insight into the ACCH domain structural elements that drive membrane fusion events.

2267-Pos Board B283

Membrane Solubilization by Styrene-Maleic Acid Copolymers: Importance of Polymer Length and Comonomer Sequence

Adrian H. Kopf[1], Nelmari Harmzen[2], Juan J. Dominguez[1], Martijn C. Koorengevel[1], Rueben Pfukwa[2], Bert Klumperman[2], Antoinette J. Killian[1].
[1]Membrane Biochemistry & Biophysics, Bijvoet Center and Institute of Biomembranes, Utrecht University, Utrecht, Netherlands, [2]Department of Chemistry and Polymer Science, Stellenbosch University, Matieland, South Africa.

Styrene-maleic acid (SMA) copolymers have emerged as a powerful alternative to detergents for the extraction of membrane proteins from cellular membranes. These polymers can solubilize membranes in the form of nanodiscs that are stabilized by the polymer, allowing characterization of membrane proteins in their native environment. However, our understanding of the parameters that determine the efficiency of solubilization or the properties of the resulting nanodiscs is still limited. This is partly due to the heterogeneity of commercially available SMA copolymers, where there are large variations in both the length distribution of the polymers and the comonomer sequence distribution. Here we successfully synthesized a series of copolymers by Reversible Addition-Fragmentation Chain Transfer (RAFT) mediated polymerization in conjunction with repetitive chain extension. These copolymers were found to have a well-defined length, composition and monomer sequence by size exclusion chromatography (SEC) and DEPT ^{13}C NMR, among others. We investigated the solubilizing efficiency of these polymers as well as the properties of the resulting nanodiscs using both lipid model membrane systems and E.coli membranes. Results of these experiments will be shown and the effects of the polymers will be compared with those of commercially available polymers.

2268-Pos Board B284

Characterizing the Lipid Annulus Surrounding Membrane Proteins with Native Mass Spectrometry of Nanodiscs

James E. Keener, Deseree J. Reid, Dane Evan Zambrano, Ciara Zak, **Michael T. Marty**.
Chemistry and Biochemistry, University of Arizona, Tucson, AZ, USA.

It is challenging to characterize the transient and heterogeneous interactions between membrane proteins and their surrounding lipids. High resolution structural biology techniques can detect tightly bound structural lipids but generally cannot detect weakly bound annular lipids. Spectroscopy techniques can detect relative proximity of lipids but require labels that may disrupt their behavior. By using nondenaturing ionization to preserve noncovalent complexes for mass analysis, native mass spectrometry (MS) provides a label-free strategy for detecting and characterizing