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Two New Types of Polymer Nanodiscs for Membrane Protein Studies

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Lipid nanodiscs (LNDs) are discoidal nanostructures consisting of a lipid bilayer membrane patch encased by a belt formed by membrane scaffold proteins (MSPs). Styrene-maleic acid (SMA) copolymers have also been used for solubilization and reconstitution of MPs into nanodiscs. These polymer-encased nanodiscs (SMALPs, for SMA lipid particles) are promising platforms for studies of membrane proteins (MPs) in a near-physiologic environment without the use of detergents. One drawback of SMA available copolymers is their limited buffer compatibility and flexibility for various applications. In addition, the development of nanodiscs for biotechnology and biomedical applications is undermined by the fluidic and labile nature of the lipid bilayer. Here, we 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) does 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 present data on the solubilization capability of different zSMA copolymers that sets the bases for the development of new zSMAs. Finally, we introduce polymer nanodiscs (PNDs) as discoidal amphiphilic block copolymer membrane patches encased by MSPs, which are more stable than LNDs and amenable for chemical modification. We were able to produce PNDs with different copolymers, including PNDs with an hydrophobic core based on polystyrene. We expect that the higher mechanical and chemical stability of block copolymer membranes and their chemical versatility will open new opportunities for applications built on the reconstituted MPs, or involved with drug targeting and delivery.This work was supported in part by NSF grants DMR-1623241 and CBET-1623240.

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ADP-Regulated MiD51-Phospholipid Interactions Couple Cellular Bioenergetics to Mitochondrial Membrane Remodeling

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Mitochondria are dynamic organelles that constantly undergo fission and fusion. A key player in mitochondrial fission, dynamin-related protein 1 (Drp1), is recruited from the cytosol to pre-destined fission sites at the mitochondrial outer membrane by membrane-anchored protein adaptors, including mitochondrial dynamics protein of 51 kDa (MiD51). The nucleotidyl transferase domain of MiD51 does not exhibit any enzymatic activity, yet selectively binds ADP. The role of ADP binding to MiD51 is unclear. We have used microscale thermophoresis (MST) as a primary technique to reveal the direct, selective interaction of MiD51 with cone-shaped phospholipids that induce negative curvature stress to achieve local membrane constriction. We found that MiD51 specifically binds cardiolipin (CL) and phosphatidic acid (PA), both of which play critical roles in the regulation of Drp1-mediated membrane fission. Interestingly, ADP competitively inhibits MiD51-CL binding suggesting that CL and ADP, both of which possess dianionic phosphates at physiological pH, bind overlapping binding sites in MiD51. We corroborated this result using a nucleotide-binding disruption mutant of MiD51, which also showed reduced cardiolipin binding affinity. Further, the absence of interactions with shorter acyl chain 6-phosphatidic acid (6-PA) indicated that longer acyl chains (or greater hydrophobic surface area) are required for efficient MiD51-phospholipid interactions. Overall, our results suggest that ADP negatively regulates MiD51-CL interactions, which likely functions to couple the energetic state of the cell (i.e. ATP/ADP ratio) to the balance of mitochondrial fission and fusion.

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Probing the Effect of Cardiolipin on the Redox-Partner Recognition between Cytochrome C_2 and Cytochrome Bc_1 Complex

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Cytochrome c (cyt. c) and cytochrome bc_1 (bc_1) complex are redox proteins that participate in the electron transport chain of the mitochondria, with cyt. c shuttling electrons from bc_1 complex to complex IV. Cyt. c and bc_1 complex reside in the inner membrane of the mitochondria, which is rich in the negatively-charged cardiolipin (CL). CL concentration in mitochondria varies upon different physiological conditions, and in some cases, a depletion of CL leads to a reduced bc_1 complex activity. In this study, the redox-partner recognition between cyt. c and bc_1 complex is examined using molecular dynamic (MD) and Brownian dynamics (BD) simulations with variations in CL concentration and salinity. First, MD simulations were performed to equilibrate cyt c_2 and bc_1 complex with membranes of different CL concentrations to obtain atomistic representations of their *in situ* configurations. Then, for each CL concentration, the electrostatic potential profile as well as hydrophobic interactions under different salinities were determined; to serve as input parameters for BD simulations. Then, for each redox state of cyt. c_2 and that of bc_1 complex, as well as for each of the above-mentioned CL concentration, a set of 10 independent 200 microsecond BD simulations were performed to sample the binding likelihood between the two proteins. Our result shows that a match between the redox state of cyt. c_2 and that of bc_1 complex promotes the binding of the two proteins in all different conditions. However, the ability of cyt. c_2 to distinguish bc_1 complex from the membrane surface is susceptible to the corresponding electrostatic potential profile, which can be significantly modified by variations in CL concentration and salinity.

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Is the Human Domain Swapped Dimer of Cytochrome *C* the Peroxidase in Apoptosis?

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Cardiolipin (CL) is oxidized by Cytochrome *c* (cyt*c*) as an early step in the intrinsic pathway of apoptosis. X-ray structures of domain-swapped dimers (DSDs) of cyt*c*, both from our lab and others, demonstrate that the DSD of cyt*c* could act to oxidize CL. We hypothesize that the DSD has evolved into a switch that provides tight regulation of the intrinsic pathway of apoptosis. Data presented here will show that the human DSD is kinetically more stable than horse or yeast DSDs. Circular dichroism data show that the DSD adopts a similar structure to monomeric cyt*c* when it binds to CL nanodiscs at high lipid-to-protein ratios. Peroxidase activity data show that there is a seven-fold increase in activity of the Human DSD compared to monomeric cyt*c*. Additionally, fluorescence correlation spectroscopy (FCS) data show that dimerization of Human cyt*c*enhances binding to CL nanodiscs: monomeric cyt*c* binds with a dissociation constant in the micromolar range while the dimeric version binds with a dissociation constant in the submicromolar range.

1842-Pos

Impact of Lipid-Protein Interactions on Alpha-Helical Membrane Protein Fold

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Despite decades of membrane protein research, little is known about the role that the lipid solvent plays in stabilizing membrane protein structures. Several hypotheses are currently under debate, including the opposing views that lipids are either (1) not required for membrane protein stability or (2) that they form a boundary layer crucial for maintaining fold and facilitating conformational rearrangement within the bilayer. In addition to the possibility that a lipid boundary impacts membrane protein fold and dynamics, there is also evidence that suggests alpha-helical membrane protein structures are sensitive to hydrophobic matching between the transmembrane region and surrounding environment. To identify a set of unifying principles that govern membrane protein fold and stability within lipid systems, a model alpha-helical transmembrane protein was studied in lipid-detergent bicelles (with a lipid to protein molar ratio of 0.3–0.7). Continuous-wave electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) spectroscopy were used to investigate secondary and tertiary protein structure as well as the presence of a lipid boundary in both mixed and segregated lipid environments. To examine the potential role of pre-formed lipid interactions, protein samples were either purified directly in bicelles or initially reconstituted in lipid vesicles, followed by detergent addition to form bicelles. Specific lipid-protein interactions and physical properties of proteobicelles varying in lipid concentration and segregation were also explored. These results have important implications for cellular processes involving membrane proteins that may utilize surrounding lipids for functions such as signaling and regulation, molecular assembly, and lipid metabolism.