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Live Action of Escrt III Machineries in Membrane Remodelling: Membrane Deformation & Membrane Scission

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Endosomal sorting complexes required for transport (ESCRT) are involved in many cellular membrane remodelling processes including membrane deformation and fission. Examples of such processes are the biogenesis of endosomal vesicles, virus budding, cytokinesis and nuclear envelope closure. These machineries are also vulnerable to cancerous growth and neurodegenerative diseases. In membrane fission, first a budded neck is formed assisted by ESCRT III filaments that stabilize the highly curved membrane neck. In the final stage, these filaments accumulate to set the stage for membrane fission, in cooperation with vacuolar protein sorting-associated protein 4 (VPS4). The small size and the dynamic nature of these machineries present challenges for scientists to gain a comprehensive understanding of these processes. Therefor, we sought to understand the role of such ESCRT III machineries at the single-molecule level. Specifically, we scrutinize the first and the last steps of membrane budding and fission using High Speed –Atomic Force Microscope (HS-AFM). The unique ability of HS-AFM to study bio-molecules in near-to physiological conditions and with high spatio-temporal resolution makes it a successful tool to study the dynamics of single molecules at millisecond resolution. Our results reveal, for the first time at nm resolution, the dynamics of CHMP2A/CHMP3 constriction by Vps4, as a stepping stone to membrane scission. Furthermore, we scrutinize the role of CHMP2B and CHMP4B in membrane remodelling and discuss possible pathways for the initiation of vesicle budding.

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Driving Forces Stabilizing Cellular Prion Protein (Prp^C) Monomers and Dimers on the Cell Surface

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The conversion of the prion protein PrP^C to its infectious form PrP^{Sc} via autocatalytic misfolding is critical to the development of a group of neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs). To gain insight on the factors that influence the PrP^C to PrP^{Sc} conversion, it is pertinent to identify how PrP^C interacts with its environment. PrP^C is attached to the extracellular side of the cell membrane via a glycosylphosphatidylinositol (GPI) anchor and can be found in monomer or homodimer form. To investigate the driving forces that stabilize PrP^C monomers and dimers on the cell membrane surface, we used molecular modeling techniques. From our analysis, we identified stable dimer conformations and characterized the dimer interface using residue interaction network analysis and residue contact maps. We then implemented a series of molecular dynamics simulations to mimic the effects of changing membrane lipid composition on the protein-lipid interface. Our results indicate that PrP^C dimers are stabilized by hydrophobic interactions along the β-sheets and that dimer stability is affected by the orientation of PrP^C α-helices on the cell membrane surface. We will propose a mechanism by which PrP^C dimers with hydrophobic interfaces inhibit PrP^{Sc} propagation due to the mobility constraints that GPI anchors place onto the dimer structures that do not allow the presentation of a PrP^{Sc} binding domain.

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Influence of Charged Lipids on Glutamic Acid Containing Transmembrane Helices

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Among the crucial components of the cell are transmembrane proteins. These proteins play major roles in many biological activities and are essential for proper cell functioning. An important aspect of these proteins is their respective ionization properties. For glutamic acid, an important consideration is its interaction with lipids in the cell membrane, as little is known about the properties of residues that can titrate from neutral to negative within the hydrophobic environment. In our strictly outlined peptide-lipid system, we substitute a glutamic acid residue in the L14 position of the helical model peptide GWALP23 (acetyl-GGALWLALALALAL^{14}ALALWLAGA-amide). The substitutions of glutamine and aspartic acid will serve as controls. The GWALP23 derivatives are placed in different lipid environments containing negatively or positively charged lipids among the primary zwitterionic lipids of the membrane. The purpose is to create an environment similar to a cell membrane that can also accommodate the glutamic acid side chain. Core alanine resides are labeled with deuterium to determine helix characteristics by solid-state NMR. The peptide-lipid samples will include DMPC doped with 10% charged lipid, as lipid bilayer formation has been confirmed for these lipid mixtures by solid state ^{31}P NMR. The charged lipids will consist of the negatively charged lipid, DMPG, and the positively charged lipid DMTAP. Samples with a 20% charged lipid mixture do not form adequately oriented bilayers with the E14 peptide present. Initially, these experiments will monitor changes in the quadrupolar splittings of the neighboring deuterated alanine residues at positions 15 and 13 as the sample pH is varied. Samples containing E14 and 10% cationic lipid buffered between a pH range of 5.5-8.1 form isotropic peaks in both ^{31}P and ^{2}H-NMR observables. Additional experiments are underway in different lipid mixtures to monitor changes in helix behavior.

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Position Dependent Orientation Difference of Transmembrane Peptides Flanked by Single or Multiple Histidine Residues

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GWALP23, a constructive low-dynamic model peptide framework, widens the range of protein-lipid interactions that can be investigated when potentially charged residues are present. In this context, we have examined the effects of single or pairs of histidine residues. To this end, we substituted residues H8 and H16 into the parent sequence (acetyl-GGALW5LAL8ALALALA-L16ALW19LAGA-amide) of GWALP23. The ^{2}H-NMR spectra of ^{2}H-labeled core alanine residues show, interestingly, no titration dependency from pH 2-8, yet a difference in bilayer thickness-dependent orientation. In DLPC lipid bilayers, a quadrupolar wave analysis indicates a tilted transmembrane helix similar to that of the parent GWALP23 peptide. With respect to DOPC membranes, nevertheless, the peptide Ala residues display a wide range of ^{2}H quadrupolar splittings (about 1.5 −30 kHz). The helix adopts a surface bound orientation on DOPC membranes, however alanine A7 does not fit within the core helix. Additional single histidine peptides, with H2 or H22 substituted into the parent sequence, display tilted transmembrane orientations in bilayers of DLPC or DOPC, with little or no pH dependence of the orientations from pH 2 to pH 8. Results from H^{2,22}WALP23 will be compared with those observed when only H2 or H22 is present. The more central histidines 8 and 16 can lead to a helix transition from membrane-spanning in DLPC (and most likely also in DMPC) to a surface orientation when the lipid bilayer is the thicker DOPC.

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Helix Fraying and Orientation of a Transmembrane Peptide having a Long Hydrophobic Core and Anchored by Interfacial Arginine Residues

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Transmembrane peptides are often flanked by interfacial aromatic residues that potentially play a role in anchoring the peptide to assist in the stabilization of a tilted transmembrane orientation. The peptide GWALP23 (acetyl-GG^{2}-AW(-LA)$_6$WLAG^{22}A-amide) containing two interfacial Trp(W) residues has a stable transmembrane orientation in several lipid bilayers. A related peptide RWALP23 (acetyl-GR^{2}-AW(LA)$_6$WLAR^{22}A-amide) has been employed to investigate the interplay between interfacial arginines and tryptophans. Here we replace the tryptophans of RWALP23 with A5 and A19, to investigate arginines alone with respect to helix fraying and orientation in varying lipid bilayers. Deuterated alanines incorporated into the core sequence of the peptide allow the orientation and stability of the core helix to be assessed by means of solid -state ^{2}H NMR in lipid bilayers DOPC, DMPC and DLPC. The alanines located outside of the core region of the peptide are sensitive to helical integrity. The new alanines, A5 and A19, will provide new information about the length of the core helix and the possible onset of unraveling of the terminals. In DLPC bilayers, ^{2}H resonances from A3 and A7 reveal lower quadrupolar splitting values than those for the R^{2,22}W^{5,19} helix, suggesting that the R^{2,22}A^{5,19} helix might adopt a different orientation. The ^{2}H quadrupolar splittings for A3 and A7 also differ for the two helices in DMPC and DOPC bilayers. Future experiments will be directed toward full orientation calculations based on GALA and Gaussian dynamic methods using all core alanine data points along with known A5 and A19. Further comparisons of quadrupolar splittings of A3, A5, A19 and A21 wave plots for the core helix may give insights into the onset of helix fraying for both N and C terminals of the RWALP23 transmembrane peptide.