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The amyloid beta peptide and alpha synuclein are the main proteins associated with Alzheimer's and Parkinson's disease respectively. In both diseases, interactions between the relevant protein and lipid membranes are hypothesized to be important in disease onset and propagation. We have in earlier studies developed methodology to study kinetics of amyloid fibril formation with high reproducibility [1] and showed that membrane composition affects the kinetics of amyloid beta peptide fibril formation [2]. We now focus on the protein-membrane interaction and study adsorption of alpha synuclein and the amyloid beta peptide to supported lipid bilayers using techniques such as quartz crystal micro balance (QCM), surface plasmon resonance (SPR) and neutron reflectometry. We see a large dependence on electrostatics and to be able to characterize the contributing effects we vary pH and salt concentration in the solution to alter the protein net charge and the electrostatic screening. The lipid bilayer is composed of zwitterionic POPC with or without incorporation of anionic DOPS or Cardiolipin, which in a biologically relevant way present molecular net negative charge of one and two respectively.

1. E. Hellstrand, B. Boland, D. M. Walsh and S. Linse, Ab aggregation produces highly reproducible kinetic data and occurs by a two-phase process, ACS Chem. Neurosci., 2010, 1, 13-18.
2. E. Hellstrand, E. Sparr and S. Linse, Retardation of Abeta Fibril formation by phospholipid vesicles depends on membrane phase behavior, Biophys. J., 2010, 98, 2206-2214.

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PR3 Interacts Directly to Lipid Bilayers: Evidence from MD Simulations and SPR Experiments

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Proteinase 3 (PR3) is a serine protease of the neutrophils involved in inflammation processes. Its membrane expression is a risk factor for chronic inflammatory diseases. Experimental data led to divergent hypotheses on the binding mode of PR3 to the plasma membrane of neutrophils. While several studies performed on cell lines have identified a number of partner proteins, no direct interaction between hPR3 and any of these potential partners has been demonstrated (reviewed in Hajjar et al, FEBS J., 2010). Differential scanning calorimetry and spectrophotometric measurements, on the other hand, show a direct interaction of PR3 with DMPC vesicles (Goldman et al., Eur J Biochem, 1999).

Using molecular dynamics simulations, we have characterized the membrane-binding site of PR3. Electrostatic surface potential calculations and simulations with an implicit membrane model (IMM1) have showed that PR3 possesses basic amino acids that provide the driving force to orient the protein at the membrane surface, so that a hydrophobic patch can anchor into the hydrophobic region of the membrane. In vitro mutagenesis experiments have confirmed the role of both types of amino acids. All-atom MD simulations and MM/PBSA energy decomposition identify three types of interactions contributing to the anchoring of PR3: hydrogen bonding and charge-pairing with lipid phosphate groups (R177, R186A, K187 and R222), hydrophobic anchoring into the lipid bilayer core (F165, F166 and L223) and cation-π interactions with the choline groups of DMPC (W218).

Surface Plasmon Resonance experiments show a strong binding of Pr3 to DMPC and POPC bilayers.

Altogether our results demonstrate that Pr3 has the ability to directly bind to lipid liposomes and most probably also to the plasma membrane of neutrophils.

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The Role of Protein Context in Disease-Related Huntingtin Protein/Lipid Interface Interactions

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Huntington's disease (HD) is a neurodegenerative disorder caused by an expansion of a poly-glutamine (poly-Q) region near the N-terminus of the huntingtin (htt) protein. Expansion of the poly-Q region above 35-40 repeats results in the disease that is characterized by inclusion body aggregates of mutated protein. The poly-Q region of htt is flanked by a 17 amino acid N-terminal sequence (N17) and a region of proline repeats (P11). As the cell membrane has been proposed to play a role in mediating htt aggregation, Langmuir trough techniques were used to investigate the effects of flanking regions on the surface activity and insertion of htt peptide constructs into a lipid monolayer. Four peptide constructs were tested: N17-Q35-KK, N17-Q35-P11-KK, KK-Q35-KK and KK-Q35-P11-KK, where the additional lysine residues were attached to improve solubility. Surface activity was measured to determine the affinity of each peptide for the air-water interface. The constructs containing the N-terminal sequence had higher surface activities compared to those without. Inclusion of the polyproline region with the N-terminal sequence contributed to the highest surface activity. To determine peptide association with a cell membrane, insertion of each peptide into a monolayer of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) held at a constant surface pressure was also investigated. Peptides with the flanking N-terminus showed appreciable monolayer insertion. The polyproline region only mediated and increased the degree of insertion when the N-terminus was also present. Rates of insertion of the constructs containing the N-terminus were comparable, while those without this region were markedly slower. Experiments were repeated using monolayers of total brain extract to better model a physiological environment with similar results. The amino acid environment surrounding the poly-Q region dramatically affects peptide association with the cell membrane and this may potentially mediate the aggregation process.

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The Uni- to Multilamellar Transition of Mixed Anionic and Zwitterionic Vesicles Induced by Cytochrome-C: A Small Angle X-Ray Scattering Study

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Lipid-protein interactions are regarded as one of the key factors in several biophysical relevant processes. In the present work, we studied the influence of Cytochrome-c (Cyto-c) on unilamellar vesicles composed by mixing an anionic lipid (the 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol, POPG, or the cardiolipin, CL) with the zwitterionic lipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), by means of small-angle X-ray scattering. Cyto-c is a component of the electron transport chain, where it has a pivotal role in transferring electrons in the inner mitochondrial membrane (IMM), which is composed of a significant amount of anionic lipids. CL is a structurally unique anionic phospholipid, containing two phosphatidylglycerol groups and four acyl chains and it is found predominantly in the IMM. The interaction of Cyto-c and CL-containing mixed vesicles has been extensively studied, and it is appointed as the major responsible for the role of Cyto-c in the cell respiration and also in the cell programmed death (apoptosis), as recently evidenced. Present results show that, in the absence of anionic lipids, Cyto-c is not able to change the structural features of POPC unilamellar vesicles. In the mixed systems, however, Cyto-c induces the formation of a multilamellar vesicles, with a bilayer-bilayer repetition distance of 11 nm, circa 4 nm larger than the staking of POPC multilamellar vesicles. This information indicates that Cyto-c, a globular protein with 3.0 nm of diameter, is probably located among the bilayers. Furthermore, such effect takes place as soon as Cyto-c is mixed in the system, as confirmed by absorption spectroscopy. The time-evolution of such process was also investigated and, interestingly, it is faster in POPG-containing vesicles than in the CL ones. Moreover, the number of staked bilayers is larger in the POPG systems.

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Molecular Dynamics Prediction and Refinement of Transmembrane Helix Dimers

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Membrane proteins represent around one fourth of all proteins encoded in genomes. Amongst these transmembrane (TM) proteins span the entire lipid bilayer, and -by allowing information to be transmitted across the membrane-play important roles in cell biology and physiology. Several mechanisms for such signal transduction have been revealed, ranging from conformational changes in protein channels and pumps to alterations in helix structures that regulate the activity of intra- and extracellular domains in TM receptors. We have begun to investigate the role of TM helix association in single pass receptors, such as the Eph and plexin receptor. Often the structures of the TM helix associated states are not known and a number of laboratories have been trying to fill this void by use of molecular modeling and dynamics for structure prediction.

This work explores a plausible route to predicting structures of transmembrane helix dimers that combines two layers of modeling. The method is applied to known helix dimer structures as a validation study. Starting from a pair of unfolded peptides, thorough sampling is performed using an implicit bilayer potential to generate starting configurations that are then relaxed in an explicit bilayer. Helix crossing angles for EphA1 and ErbB1/2 dimers obtained from