been shown that ODPC interacts with cell membranes because of its amphiphilic character[1]. In this work, we investigate how the lipid composition influences on ODPC interaction with phospholipid membranes. To do so, we used giant unilamellar vesicles (GUV) composed of DOPC (dioleoylphosphatidylcholine), DOPC:Chol (8:2) - Chol = cholesterol - and DOPC:SM:Chol (1:1:1) - SM = sphingomyelin. The results showed that ODPC causes a solubilization of the DOPC membrane followed by its disappearance. The presence of Chol intensifies such a phenomenon. On the other hand, GUVs containing Lo-Ld phase separation of DOPC:SM:Chol display a different scenario when in contact with ODPC. The rafts disappear with time evolution (less than 20 minutes) ending up to a complete disappearance of the membranes. Changes in physical membrane properties are being better investigated.
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EPR Spectroscopy

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Investigation of the Potra Domains from Cyanobacterial Omp85 by Peldor Spectroscopy
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Omp85 proteins contain a C-terminal transmembrane beta-barrel and a soluble N terminus with a varying number of polypeptide-transport-associated (POTRA) domains.[1] N-terminal POTRA domains (P1 and P2) of Omp85 from the cyanobacterium *Anabaena sp.* PCC 7120 might have functions in substrate recognition and heterooligomerization.[2] P3 is implied in regulation of protein transport by its L1-loop. Molecular dynamics (MD) simulations predicted that P2 and P3 are fixed in orientation, consistent with a short connection and a large interface between both domains, and that there is a hinge between P1 and P2.[2] In this study we used site-directed spin labeling (SDSL) to investigate the conformational flexibility between POTRA domains by PELDOR (pulsed electron-electron double resonance) spectroscopy.[3-5] The experimental results will be compared with the MD calculations and X-ray structures.[2] Further studies of the interaction of POTRA domains with chaperones and substrates are underway.
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Distance Determination in Multimeric Membrane Protein by Symmetry Constrained Analysis of Double Electron-Electron Resonance Spectroscopy
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Distance determinations are often a core strategy to decipher the molecular mechanism by which membrane proteins execute their biological function. EPR spectroscopy and pulsed Double Electron-Electron Resonance (DEER) methods are unparalleled tools for the measurement of probe-based long-range distances in protein. However, distance determination from echo intensity modulation in pulsed DEER experiments is a moderately ill-posed problem. Here, we show that by using protein symmetry-based geometrical constraints in homo-oligomeric membrane proteins, we are able to greatly facilitate the fitting solution. We modeled the distance distribution with 2 Gaussians, with each mean distance identified by its Gaussian peak. During the fit process, the mean distance ratio was constrained to be within a specified tolerance of the theoretical distance ratio determined by the symmetry of the oligomeric assembly (1:1.4 for tetramers; 1:1.6 for pentamers). We compared this approach against the classical Tikhonov regularization, concluding that our method stabilizes a solution that is much easier to interpret in molecular modeling terms. Importantly, we also show that when the qualities of the dipolar evolution signal increases (more observed periods and higher SNR), there is little or no difference in the distance distribution obtained by our method vs. Tikhonov regularization. This suggests that the geometrically constrained fit does not artificially distort the distance distribution. Our approach was validated on 2 different ion channels of different oligomeric states: CorA, a homopentameric Mg2+ channel and KcsA, a homotetrameric K+ channel. In all cases, the distances obtained by DEER are in excellent agreement with respective crystal structures.

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Room Temperature Electron Spin Resonance Distance Measurements in T4 Lysozyme using Trityl-Based Spin Labels
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Pulsed Electron Spin Resonance (ESR) spectroscopy in combination with site-directed spin labeling is unique in providing nanometer- range distances and distributions in biological systems. . Distance constraints are valuable for determining structure and structural changes, while distance distributions reflect structural heterogeneity. To date, most of the pulsed ESR techniques require cryogenic temperatures to reduce the rapid electron spin relaxation rate and to prevent averaging of electron-electron dipolar interaction due to the rapid molecular tumbling. To enable measurements at physiological temperatures, we are exploring a trityl-based spin label with a relatively long relaxation time where the protein is immobilized by attachment to a solid support. In this preliminary study, trityl radicals were attached *via* disulfide linkages to substituted cysteine residues at positions 65 and 80 or at positions 65 and 76 in T4 lysozyme (T4L) immobilized on Sepharose. Double quantum coherence (DQC) was applied to the two spin-labeled double mutants at room temperature. Distances extracted from DQC show a 2.2 nm and a 1.8 nm spin-spin distance, respectively, close to those expected from models of the trityl label in the T4L structure. Narrow distribution of distances was observed in each case, indicating that the trityl spin label is relatively rigid. The results show that, with the use of trityl spin label and DQC, structural constraints can be obtained to study protein structure/dynamics at physiological temperature. Furthermore, as we show, trityl labels enable micromolar sensitivity DQC distance measurements in the temperature range of 60-100 K, since their distant methyl groups only weakly contribute to the relaxation of electron spins. As an additional advantage less intense microwave pulses are needed as compared to nitroxides.

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Measuring Protein Conformational Exchange Rates with Pressure-Jump Site Directed Spin Labeling EPR Spectroscopy
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The native state ensemble of a protein is comprised of distinct conformational substates in equilibrium, exchanging on the μs - ms time scale. Functionally important substates can be sparsely populated (''excited states'') and invisible to conventional spectroscopic methods, but recent studies have shown that high hydrostatic pressure can populate excited states and slow exchange kinetics to the easily measurable millisecond and longer time window. Thus, hydrostatic pressure can populate excited states for spectroscopic characterization, and pressure-jump relaxation experiments can determine the exchange rates between excited and ground states. Site Directed Spin Labeling (SDSL) - Electron Paramagnetic Resonance (EPR) spectroscopy of proteins containing a paramagnetic nitroxide side chain (a ''spin label'') has been shown to provide information on both structure and dynamics in proteins of any degree of complexity. This makes it an ideally suited spectroscopic technique for these studies. Here we present a pressure-jump EPR system that achieves sub-millisecond pressure jumps or drops of arbitrary magnitude. We use a pressure intensifier capable of generating hydrostatic pressures up to 4 kbar (60,000 psi) to pressurize two reservoirs, and a novel air operated valve to rapidly equilibrate the reservoirs to an intermediate final pressure. Pressure cells and resonators have been developed for both X and Q band operation; the Q band system employs a modified Varian E-110 Q-band bridge. The pressure-jump system and EPR spectrometer are controlled via a user interface constructed with Labview.