It is a general opinion that the Meyer-Overton correlation determines biomembrane transport of hydrophobic molecules. This so-called “Overton rule” says that the easier it is for a chemical to dissolve in a lipid the easier and faster it will be transported into a cell. In medical science for example, this passive transport is crucial for the effective delivery of many pharmaceutical agents to intracellular targets. The prediction also concerns CO2 as a hydrophobic molecule. Certainly, the membrane diffusion of CO2 is of critical importance to bacteria, and for plant cells in contrast to many other organisms, plants require CO2 and its availability at the site of CO2 fixation limits the rate of net photosynthesis. In this regard, plants provide an excellent system to study CO2 transport mechanisms. Findings will be presented, which question the applicability of Overton’s rule to specific plant CO2 transport processes. It could be demonstrated that the function of specific membrane proteins, i.e., distinct aquaporins, increased CO2 transport rates. The experiments were performed on synthetic membranes and cell based systems as well as plant tissues and complete plants. Techniques from biophysics, cell biology, molecular biology and plant physiology were employed and it was found that in the analyzed systems CO2 transport rates were limited by the function of these aquaporins. The results could be interpreted in a way that supports alternative cellular CO2 transport mechanisms and a modified model of cellular surfaces. If the findings were of general validity and not specific for plants, our view on diverse transport processes in many living organisms from all kingdoms could be modified.

**3182-Pos Board B43**

**Determinants of Lipid Mixing Membrane Fusion by HIV gp41**  
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A critical step in HIV infection involves membrane fusion between the enveloped virion and the target cell plasma membrane, catalyzed by the viral membrane protein gp41 at or near physiologic pH. Specific global gp41 conformations (i.e. six-helix-bundle and coiled-coil) and specific regions (i.e. fusion peptide) are implicated in steps of fusion catalysis. Our goal is to elucidate the molecular mechanism of gp41 catalyzed membrane fusion. One challenge to biophysical analysis of structure and function in hydrophobic gp41 is solubility. Low pH (~3.0) is utilized to enhance protein solubility by significantly increasing overall positive charge. Interestingly, the cationic six-helix-bundle region enhances/inhibits lipid mixing of anionic vesicles at low/neutral pH, which correlates with close association (~5 Å) between membrane headgroup phosphates and a large/small proportion or population of helical bundles based on solid-state NMR analysis. The apolar trimeric fusion-peptide region contributes to lipid mixing and is predominantly β-sheet (~75%) with a large fraction of β-strands located close to (~5–7 Å) far from (>~10 Å) membrane headgroup phosphates at residues Ala-1 and Ala-14/Leu-7 and Met-19 at low pH. Swapping to neutral pH does not affect fusion-peptide conformation, but does lower the fraction of β-strands close to the membrane surface. Partial shedding of fusion peptides from membranes, poor protein solubility, and protein lipid mixing inactivity at neutral pH are reversed upon pH swap back to low, indicating irreversible aggregation is not occurring at neutral pH. Our solid-state NMR findings indicate that abrogation of lipid-mixing fusion function by gp41 in six-helix-bundle conformation at the physiologic pH of viral fusion is determined primarily by stearic and electrostatic barriers to close membrane apposition imposed by the six-helix-bundle region, and minimally by membrane location or conformation of the fusion-peptide region.

**3183-Pos Board B44**

**Time-Resolved UV-Visible Studies of Rhodopsin Provide Experimental Test of Flexible Surface Model for Lipid-Protein Interactions**  
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Time-resolved UV-Visible absorbance of the retinal chromophore of rhodopsin [1] delivers vital information about key GPCR activation steps in a membrane lipid environment [2]. Following rhodopsin photoexcitation, a minimum of four intermediates equilibrate on the millisecond-time scale [2,3]. The first equilibrium is between the protonated Schiff base (PSB) species Meta I180 and the deprotonated SB species Meta II, and is pH independent. A second equilibrium entails spectrally silent conversion of Meta II to the Meta III substate. The final step is protonation of Glu134 of the ERY sequence in Meta III to yield Meta I180H+ whose pKc characterizes the acid-base equilibrium [3]. Absorbance measurements on the microsecond-to-hundred millisecond-time scale allowed us to study effects of POPC, DOPC, or DOPC/DOPE mixtures on the first equilibrium constant Ki and on the pKc of the final equilibrium. Results were analyzed by singular-value decomposition and were fit by a linear combination of temperature-dependent basis spectra. Notably an increase in Ki was discovered due to either PE head groups or increased acyl chain saturation, whereas little change in pKc was evident. According to the flexible-surface model (FSM) rhodopsin becomes a sensor of negative spontaneous (intrinsically) curvature upon light activation [4]. Competition between the curvature elastic energy and the hydrophobic mismatch at the proteolipid boundary explains the influences of lipid-protein interactions [4]. Both the lipid acyl chains and polar head groups affect the Meta I-Meta II transition, revealing how protein energetics are affected by material properties of the lipid bilayer.


**3184-Pos Board B45**

**Dynamics and Oligomerization Tune Seven-Transmembrane Protein Function: Studies Based on Proteorhodopsin**  
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Seven-transmembrane (7TM) proteins have diverse and important functions, ranging from signaling receptors to ion pumps. They share a reversible switching property, epitomized by the solar-powered microbial proton pump Proteorhodopsin (PR), which uses light energy to facilitate transport by a conformational “switch”. Here, we use PR as a model to capture the elusive details of activation and oligomerization necessary for the function of physiologically important membrane proteins.

We have preliminary spectroscopic evidence that suggests altered photocycle kinetics and shifted pKa values for hexameric and monomeric forms of detergent-solubilized PR. These findings prove that PR function is tuned by self-association, and we apply magnetic resonance together with functional assays of proton transport to further understand dynamics changes that occur upon oligomerization. Our unique magnetic resonance techniques of electron paramagnetic resonance (EPR) and dynamic nuclear polarization (DNP) provide insight into the protein segment mobility and local hydration water dynamics of an amino acid residue spin-labeled with nitroxide-based radicals. Using these methods, we have found that PR’s third cytoplasmic (E-F) loop is a short α-helical segment that experiences conformational change upon photoactivation. This structure is a common motif to the non-homologous G-protein coupled receptors (GPCRs) where it is a docking point for a signal G-protein. Towards understanding how function hinges on dynamics, we developed a PR-Rh chimera by replacing the E-F loop of PR with the corresponding loop of Rh. The chimera successfully expresses and maintains optical properties. We evaluate its capability to activate the G-protein transducin, and apply EPR and DNP to obtain unique information about the biophysics of receptor-G-protein interactions. By controlling the oligomeric form of the PR-Rh chimera, we measure any changes in G-protein activation caused by varying the amount of receptor-receptor interactions.

**3185-Pos Board B46**

**Molecular Mechanism of Activation of IRE1α Cytosolic Domain by Palmitoyl**  
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Inositol-requiring enzyme 1α (IRE1α) is an ER (endoplasmic reticulum) transmembrane containing two enzymatic activities, a Ser/Thr protein kinase and an