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antimicrobial peptides showing promising properties for possible application. We investigated two of these peptides, C16-KGGK and C16-KAAK in two different lipid environments, one more resembling mammalian membranes (POPC) and the other closer to bacterial membranes (POPE/POPG 2:1). Investigations were conducted on powder-type samples at a lipid/peptide ratio of 9:1 and a temperature of 303K. First, the host membranes were investigated using ^{31}P solid-state NMR clearly showing no influence of the peptides on the lamellar membrane phase state. Information about the chain dynamics and membrane packing properties was obtained using ^{2}H solid-state NMR. Order parameters of the lipids were slightly reduced upon addition of the peptide. However, the lipid modifications generally exhibit higher order parameters than the surrounding lipids meaning that the length of the peptide lipid modifications is larger than that of the lipid acyl chains. This is in agreement with paramagnetic relaxation enhancement data exhibiting interactions between the amino acids and spin-labeled phospholipids suggesting a peptide backbone location in the headgroup region of the membrane. The dynamics of the lipid modifications were investigated by means of ^{2}H R_{1Z} relaxation rates. While other lipid-modified peptides exhibit square law plots that are bent the ones obtained for the antimicrobial peptides are linear and resemble that of saturated lipids. Therefore the lipid modifications of the antimicrobial peptides are less flexible and longer than that of other lipid-modified peptides allowing the peptide backbone to be located in the lipid headgroup region.

959-Plat
A Comparative Molecular Dynamics Study of the Effect of Alpha-Tocopherol and Cholesterol on Phospholipid Bilayers having Different Levels of Acyl Chain Unsaturation

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Cholesterol and alpha-tocopherol (vitamin E) are important constituents of cell membranes having similar molecular shapes and sizes. These two molecules, however, exhibit very different properties in membranes and are thought to play different roles in the biology of the cell. Cholesterol is found in high concentrations in membranes, especially those composed of saturated phospholipids such as 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), while recent experiments suggest that alpha-tocopherol, which is typically found in much smaller concentrations, preferentially accumulates in membranes containing highly polyunsaturated lipids such as 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (SDPC). Here we examine the structural and dynamic properties of alpha-tocopherol and cholesterol and their effects on phospholipid bilayers using molecular dynamics (MD) computer simulation methods. Six long simulations (hundreds of nanosecond each) have been carried out, one for each neat lipid (DPPC and SDPC), and one for each solute in each lipid. The dramatically different effect on membrane properties that is observed in the simulation is explained in terms of unique solute-lipid interactions arising from polyunsaturation of the lipid acyl chains.

PLATFORM S: Fluorescence Spectroscopy

960-Plat
Tryptophan Fluorescence Modulated by Histidine Quenching During Folding of Small Alpha Helical Peptides: Distance and Solvation Effects

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Tryptophan (Trp) fluorescence has been extensively used to study the folding kinetics of small alpha helical proteins including: the villin headpiece N68H (1yrf), villin headpiece K65(NLE) N68H K70(NLE) (2f4k) and a peptide Ac-Trp-$(Ala)_3$-His^{+}-NH_2(WH5). In these three cases a histidine (His) has been placed in an alpha helical section of the protein four amino acids away from Trp, so that His will quench the fluorescence of Trp as the protein folds. His^{+} quenches the fluorescence of free Trp in solution at a diffusion limited rate by electron transfer from the Trp ring to the His^{+} ring. The rate of electron transfer is known to fall off exponentially due to the exponential decay of the electronic coupling V_{el} with distance. In this work we show that this decay also depends on how the energy gap is affected by distance. Solvation around the imidazole cation at large distances increases the energy gap, since waters around the cation point their dipoles towards the ring, destabilizing the charge transfer state. At short distances, the decreased water accessibility reduces the destabilization of the charge transfer state, enhancing electron transfer from the Trp ring to the His^{+} ring. This is seen in five different proteins: 1yrf, 2f4k, barnase (1a2p), T4-lysozyme (1lyd) and WH5. We also examine the ability of Trp fluorescence to determine folding rates for 1yrf, 2f4k and WH5 by using QM/MM simulations to determine the electron transfer rates in the folded and unfolded states.

961-Plat
Structural Heterogeneity and Quantitative Single Molecule FRET Efficiency Distributions of Polyproline Through a Hybrid Atomistic Simulation and Monte Carlo Approach

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Förster Resonance Energy Transfer (FRET) experiments allow to probe molecular distances via the distance dependent energy transfer efficiency from an excited donor dye to its acceptor counterpart. In single molecule settings, not only average distances, but also distance distributions or even fluctuations can be probed, providing a powerful tool to study structural changes in biomolecules. However, the measured energy transfer efficiency depends not only on the distance between the dyes, but also on their mutual orientation, which is typically inaccessible to experiments. Thus, assumptions on the orientation distributions and averages usually have to be employed, severely limit the accuracy of the distance distributions extracted from FRET experiments.

Here, we demonstrate that by combining FRET experiments with the mutual dye orientation statistics obtained from Molecular Dynamics (MD) simulations, improved estimates of the distance distributions can be obtained. From the time-dependent mutual orientations, the FRET efficiency is calculated and the statistics of individual photon absorption, FRET transfer, and photon emission events is determined from subsequent Monte Carlo (MC) simulations. All recorded emission events are then collected to bursts from which efficiencies are calculated in close resemblance to the actual FRET experiment. For several test systems, we demonstrate the feasibility of our approach by direct comparison to experimental data.

In particular, we have studied a poly-proline chain with attached Alexa488 and Alexa594 dyes. Calculated efficiency distributions from our simulations agreed with the experimental findings and identify the presence of cis-isomers as one source of the experimentally observed heterogeneity. This result demonstrates that dye orientations from MD simulaions, combined with MC photon generation, can indeed be used to improve the accuracy of distance distribution reconstruction from experimental FRET efficiencies.

962-Plat
The Photochemistry of Bacteriophytochrome: Key to its Use as a Deep-Tissue Fluorescence Probe

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Bacteriophytochromes (Bphs) are red-light photoreceptors that regulate a variety of bacterial responses. Their photosensory core consists of PAS, GAF and PHY domains and covalently binds biliverdin (BV). The Bph light activation mechanism involves isomerization around the BV C15=C16 double bond, resulting in a flip of its D-ring. In an important recent development, PAS-GAF variants were engineered for use as near-infrared fluorescent markers in mammalian tissues (Shu et al. Science 2009). Here, we report the photochemistry of two Bphs from Rps. palustris, RpBphP2 (P2) and RpBphP3 (P3) that have distinct photoconversion and fluorescence properties. We applied ultrafast spectroscopy on P3 and P2 PAS-GAF proteins and the P3 D216A and P2 D202A PAS-GAF-PHY proteins. In these mutants a conserved aspartate which connects BV with the PHY domain through extensive hydrogen-bond networks, was replaced by alanine. The excited-state lifetime of P3 and P2 PAS-GAF was significantly larger than their PAS-GAF-PHY counterparts. Mutation of the conserved Asp to Ala in PAS-GAF-PHY had a similar but larger effect. In particular, the fluorescence quantum yield of the P3 D216A mutant was 0.066, higher than that of wild type P3 (0.043) and similar to the engineered Bph of Shu et al. We conclude that elimination of a key hydrogen-bond interaction between Asp and a conserved Arg in the PHY domain is responsible for the excited-state lifetime increase. H/D exchange resulted in a 1.5 - 1.7 fold increase of excited-state lifetime. The results are rationalized with a reaction model where excited-state deactivation of BV proceeds via excited-state proton transfer from the BV pyrrole nitrogens to the backbone of the conserved Asp or a bound water. This work may aid in rational structure- and mechanism-based conversion of P3 and other BPhs into efficient near-IR fluorescent markers.