References
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1145-Pos Board B37
Nanoscale Confinement Effects on the Fast Motions of the Backbone and Side Chains of Ubiquitin
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The prevailing model for solvent dependence of protein motions, the solvent slaving model, predicts that protein motions should be dramatically dependent on the dynamics of the solvent. We have previously measured the dynamics of water near the surface of ubiquitin when encapsulated in reverse micelles. In order to directly investigate the relationship between protein and solvent motions, we have measured the backbone and side chain ps-ns motions of ubiquitin under various reverse micelle encapsulation conditions using nuclear spin relaxation methods. Confinement of the protein in the aqueous core of the reverse micelle produces a strong general damping of the fast backbone motions of the protein. In distinct contrast, the side chain methyl motions are only slightly affected by encapsulation in reverse micelles. No strong correlations between the protein motional character and the dynamics of the protein’s hydration layer were observed, suggesting that the dynamic character of the water near the protein surface have minimal consequences for fast dynamic motions of proteins. Supported by NSF grant MCB 0842814, NIH GM102477, and NIH postdoctoral fellowship GM087099 to N.V.N.

1146-Pos Board B38
Aromatic Amino Acids as Probes of Protein Affinity for Lipid Membranes
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We investigate protein-lipid interactions using the fact that the H1 NMR and UV absorption spectra of aromatic amino acids depend on environment. For calibration, we first generate reference spectra using isopropanol-water solutions to mimic changes in hydrobobicity, and correlate UV with 1H NMR chemical shifts. We then apply this method to measure the interaction of tyrosine (Tyr), tryptophan (Trp) and phenylalanine (Phe) with 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-Dioleoyl-sn-glycero-3-phosphoserine (DOPS) lipid membranes. By measuring characteristic shifts of aromatic residues we can characterize protein affinity to lipid membranes.

1147-Pos Board B39
Downregulation of Nrf2 in Hela Cells by Self-Conditioned Media
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Nuclear factor erythroid 2-related factor-2 (Nrf2) is critical in maintaining intracellular redox homeostasis by promoting transcription of several antioxidant and cytoprotective genes. The Nrf2 signaling pathway has been implicated in malignancies, inflammatory and neurodegenerative diseases. Recent reports have shown that culture-conditioned media can selectively regulate Nrf2 expression in brain cells such as astrocytes and microglia; however, not much is known about how cells self-regulate their endogenous Nrf2 through secretory mechanisms. In our study, HeLa cells were cultured in media containing 0.5% FBS for 24 hours to produce conditioned media. Serum starved cells treated with conditioned media for 3 hours showed lower levels of Nrf2 expression in the nucleus compared to cells treated with unconditioned media. Increasing the proportion of conditioned media resulted in a dose-dependent decrease in Nrf2 expression suggesting the presence of autocrine Nrf2-regulatory elements in the HeLa cell secretome. Exposure to conditioned media did not cause any change in Kelch-like ECH-associated protein-1 (Keap1), the inhibitory binding partner of Nrf2. In summary, our data suggests the existence of a complicated autocrine regulatory mechanism of this pathway at endogenous levels.

1148-Pos Board B40
Protein Dynamics of Cytochrome C Oxidase: Analysis of Vibrational Components by Phase Sensitive Detection Applied to Modulated Excitation-Seirae
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Cytochrome c oxidase (CcO) from R. sphaeroides was investigated by Modulated Excitation Surface-Enhanced IR-Absorption Spectroscopy (ME-SEIRA). Sequential electron transfer (ET) within CcO was initiated by electrochemical excitation. During the modulated excitation by periodic potential pulses with frequencies between 20 Hz and 500 Hz, time-resolved IR spectra were measured by the Step-Sean technique with time-resolution in the millisecond time range. Conformational changes of the protein structure as a result of ET lead to rather complex SEIRA spectra with many overlapping bands embedded in a broad background signal. Phase sensitive detection (PSD) was used to separate single components within the broad band of overlapping structural bands in the amide region. PSD is able to extract the periodic response of single components with the same frequency as the excitation from noise or from static background and therefore enhances the signal-to-noise ratio. Moreover, PSD enables the validation of the fit model utilized for the deconvolution of overlapping bands by analyzing the phase lags of single components acquired at different stimulation frequencies. The phase lags between the evaluated vibrational components and the modulated excitation increase with increasing excitation frequencies, which is an inherent prerequisite of the evaluation method.

1149-Pos Board B41
Site-Resolved Hydration Dynamics of Staphylococcal Nuclease in Reverse Micelles
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Measurements of water dynamics and protein-water interactions are essential to understanding protein folding, structure, function, and dynamics. However, protein-water interactions have historically been difficult to study and have mostly been limited to indirect methods that are unable to measure transient and short-lived interactions. We recently developed a novel method for studying protein-water interactions using NMR spectroscopy by encapsulating proteins of interest in reverse micelles. Appropriate amphiphilic surfactant molecules spontaneously form nanoscale bubbles in the presence of a small volume of water and bulk organic solvent, resulting in reverse micelles with aqueous protein in the interior and organic solvent on the exterior. The removal of bulk water and the effects of nanoco confinement slow protein hydration dynamics allowing for site-resolved measurement of protein-water interactions and dynamics via the nuclear Overhauser effect. Staphylococcal nuclease (SNase) is an extensively studied 16 kD protein with a large number of mutants that have been well classified using standard biophysical techniques. Here we use a pseudo wild-type hyperstable variant (Δ-PHS) and V66E mutant to study surface protein-water dynamics and overall protein hydration. High resolution NOESY-HSQC and ROESY-HSQCs were collected for SNase encapsulated in reverse micelles. Site-specific ratios of NOE and ROE signal intensity at the water chemical shift describe longevity of interacting waters, and can therefore be mapped to the protein structure to determine areas of slow and fast hydration dynamics. Supported by NSF grant MCB 0842914 and NIH postdoctoral fellowship GM087099 to N.V.N.

1150-Pos Board B42
How do Three Proteins Generate Circadian Rhythms? The Detailed Timing Mechanism of the Cyanobacterial Circadian Oscillator
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Virtually all organisms contain a circadian clock that regulates gene expression, metabolic pathway and cell division. These regulations enhance the fitness of organisms by anticipating daily changes. To generate a clock, a stable oscillation must be maintained. Thus, we focus on understanding how molecular components form oscillatory behavior that drive a biological clock.

To study clock mechanism in detail, we use the cyanobacterial oscillator because it is the only system that can be functionally reconstituted in a test tube. Mixing three clock proteins, KaiA, KaiB, and KaiC, and ATP produces a robust 24-hour rhythm of KaiC phosphorylation/dephosphorylation. All other oscillators are difficult to study at the mechanistic level because they cannot be isolated from the complex milieu of living cells. This oscillator has two distinct oscillatory phases: 1) in Phosphorylation Phase, KaiA binds to the C-terminal residues of KaiC known as the A-loop and stimulates KaiC phosphorylation; 2) in Dephosphorylation Phase, KaiA is inhibited from binding to A-loop by KaiB, then KaiC dephosphorylates.

Our central hypothesis is that the KaiA binding site on KaiC (i.e. A-loop) experiences a range of dynamic changes that drive preferential KaiA-KaiC interaction which is critical to the rhythm. Here we will present our latest findings of this unique oscillator to gain a deep mechanistic insight into how these three