Cytochrome c oxidase (CcO) from R. sphaeroides was investigated by Modulated Excitation Surface-Enhanced IR-Absorption Spectroscopy (ME-SEIRA) and Phase Sensitive Detection (PSD) 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-Scan 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 bands 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.

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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 nanofluidification 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-HSQC’s 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 0842814 and NIH postdoctoral fellowship GM087099 to V.N.N.