Supporting Information

Water penetration into protein secondary structure revealed by hydrogen-deuterium exchange 2D IR spectroscopy

Lauren P. DeFlores and Andrei Tokmakoff*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

tokmakof@mit.edu

Absorptive 2D IR spectra were acquired using methods and instrumentation that is described in detail elsewhere.[1] The experiments used 1 μJ, 90 fs pulsed mid-IR radiation centered at 1600 cm^{-1}. The pulse bandwidth of 185 cm^{-1} (FWHM) is centered to cover both the fundamental (v=0→1) and anharmonically shifted (v=1→2) overtones of both the amide I/I' and amide II modes of the proteins shown in Figure 1 and 1S. Data is collected in frequency (ω_3) and time (τ_1) and numerically transformed along τ_1 to obtain ω_1. The resolution along ω_3 is dictated by grating dispersion and the size of the 64 pixels on the MCT array detector, and in this experiment is 4 cm^{-1}. ω_1 resolution is determined by the total time delay swept in τ_1 and is 0.5 cm^{-1}. Spectra shown were acquired in the crossed (ZZYY) polarization geometry to enhance to relative intensity of the cross peaks.[2]

Two-dimensional infrared spectra were taken of seven protein systems with various degrees of secondary structural motifs: concanavalin A (Con A, β-protein), β-lactoglobulin (BLG, α/β β-sandwich protein), ribonuclease A (RNaseA, α/β-protein), ubiquitin (α/β-protein), lysozyme (α/β-protein), Myoglobin (α-protein), and serum albumin (α-protein). Experimental conditions were chosen to minimize H/D exchange through the course of data collection (~20 minutes). Samples are held between two CaF_2 windows with a 50 μm spacer in a water cooled brass sample cell. Proteins were monitored at various temperatures over a 2 hour period using FTIR spectroscopy to ensure the amide II band did not change. The ideal temperature was determined to be 5 ˚C for all proteins. The samples were rapidly prepared at 10 ˚C to avoid condensation of atmospheric water onto the sample cell and windows, and then cooled to 5 ˚C once in the dry air purged spectrometer. Con A, BLG, RNaseA, and myoglobin were prepared in a pD = 7.6 phosphate/D_2O buffer, lysozyme in a pD = 5.5 phosphate/D_2O buffer, albumin in a pD = 7.3 phosphate/D_2O buffer, and ubiquitin was prepared at pH = 1.0 using DCl/D_2O. Sample concentrations ranged between 20-30 mg/ml such that the OD of the amide II transition is ~0.1.

The FTIR and absorptive 2D IR spectra for all proteins are shown in Figure 1S to show trends in the cross peak region. The 2D IR spectra presented in this manuscript have not been corrected for pulse spectral intensity in either ω_1 or ω_3 dimensions, creating an enhancement of features around 1600 cm^{-1}.

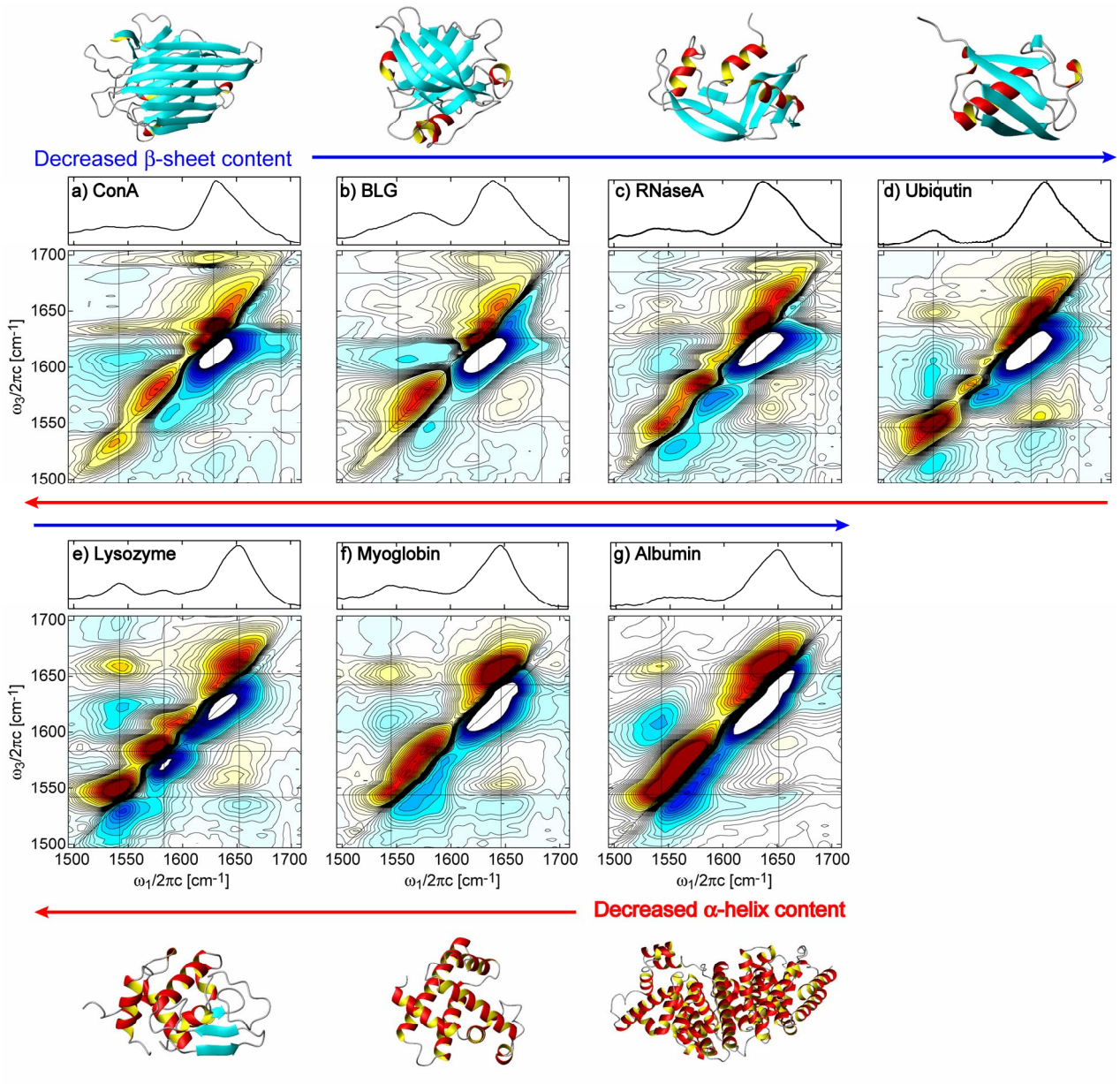

Figure 1S. FTIR and absorptive 2D IR spectra of proteins concanavalin A, β-lactoglobulin, ribonuclease A, ubiquitin, lysozyme, myoglobin, and albumin.

Contour levels are plotted to emphasize the cross peak region of the spectra. Contours for Con A, BLG and RNaseA are plotted in 1% increments for ±8% and 4.3% from ±8% to ±60% peak amplitude of the amide I fundamental. Ubiquitin, lysozyme, myoglobin and albumin are plotted with 1.5% increments for ±12% and 4% from ±12% to ±60% peak amplitude of the amide I fundamental.

To confirm the protonation of the peptide groups in amide I/II couplings and the persistence of Arg side chain absorption seen in the 2D IR spectra, consecutive 2DIR spectra of partially and fully exchanged ubiquitin were taken (Figure 2S). The sample was prepared as described above and the partially exchange protein was taken at 5˚C. The sample was then heated to 65˚C at which the protein fully unfolds and left to exchange for 30 minutes. The temperature was then cooled to 5˚C and the fully exchanged spectra was taken (Figure 2Sb). These spectra show the loss of the amide II doublet at $\omega_1=\omega_3=1550$ cm^{-1}. No diagonal or off-diagonal features involving AmII are observed in the fully exchanged spectrum (Figure 2Sb). Additionally, the side chain absorption of symmetric/asymmetric guanidinium vibrations of the Arg near ~1585 cm^{-1} persist after complete deuteration of the protein.

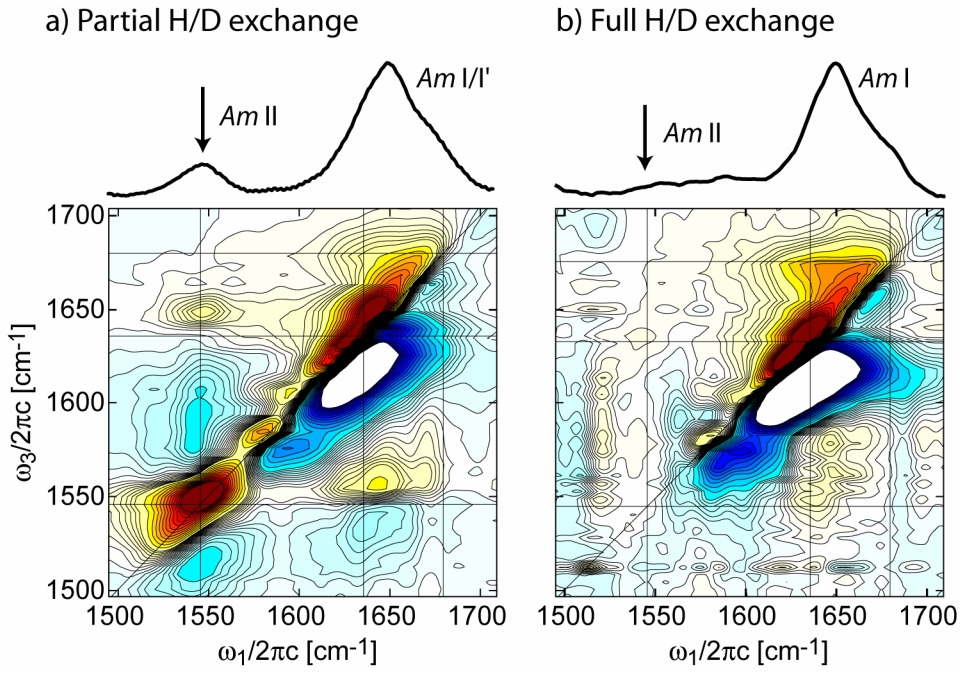

Figure 2S. FTIR and 2DIR spectra of Ubiquitin before exchange and after full exchange. Note disappearance of the amide II peak which is only present when the amide mode is protonated.

(1) Khalil, M.; Demirdoven, N.; Tokmakoff, A. *J. Phys. Chem. A* **2003**, *107*, 5258-5279.
(2) Golonzka, O.; Khalil, M.; Demirdöven, N.; Tokmakoff, A. *J. Chem. Phys.* **2001**, *115*, 10814-10828.