2D IR Spectroscopy of Protein Dynamics Using Two Vibrational Labels - a Site Specific Genetically Incorporated Unnatural Amino Acid and an Active Site Ligand

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Supporting Information: Alternate Gaussian fit to Az region of MbAzCO FTIR spectrum, FT-IR spectrum of AzPhe, full 2D IR spectrum of MbAz, and SDS-PAGE and mass spectroscopic characterization of MbAz

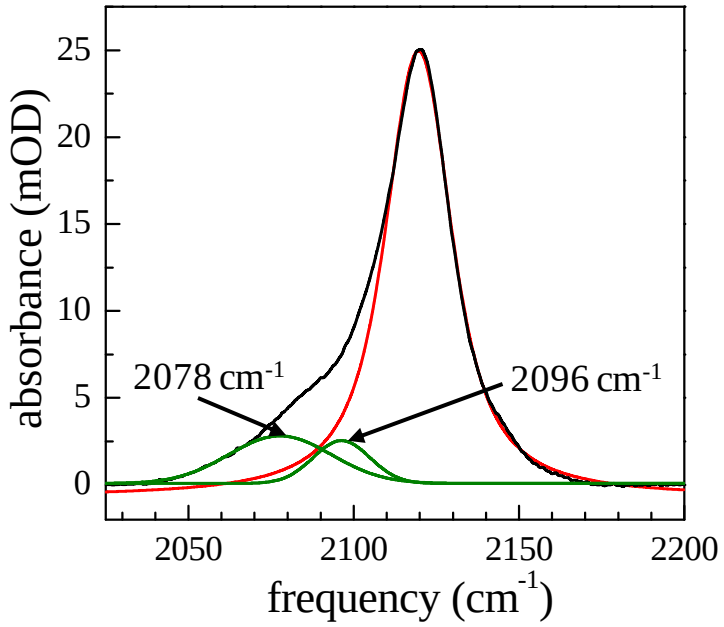

Figure S1: FT-IR spectra of Az in MbAzCO. The experimental data are shown in black, and the Voigt fit to the main band is in red. The two Gaussian fit to the low frequency side of the spectrum are shown in green. The fit with two Gaussians removes the large oscillation in the residuals seen in Figure 2D that occur when the data are fit with a single Gaussian on the lower frequency side of the spectrum.

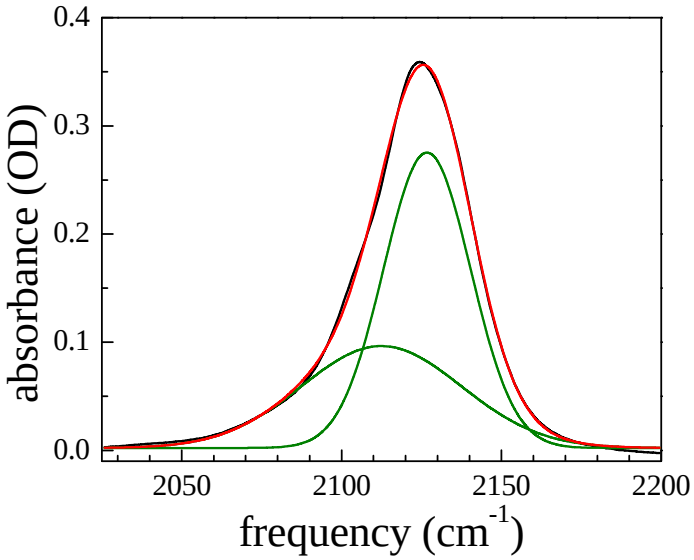

Figure S2: FT-IR spectra of Az of AzPhe in 50% glycerol/PBS. The experimental data are shown in black, the two Gaussian fit to the spectrum is in green, and their sum is in red.

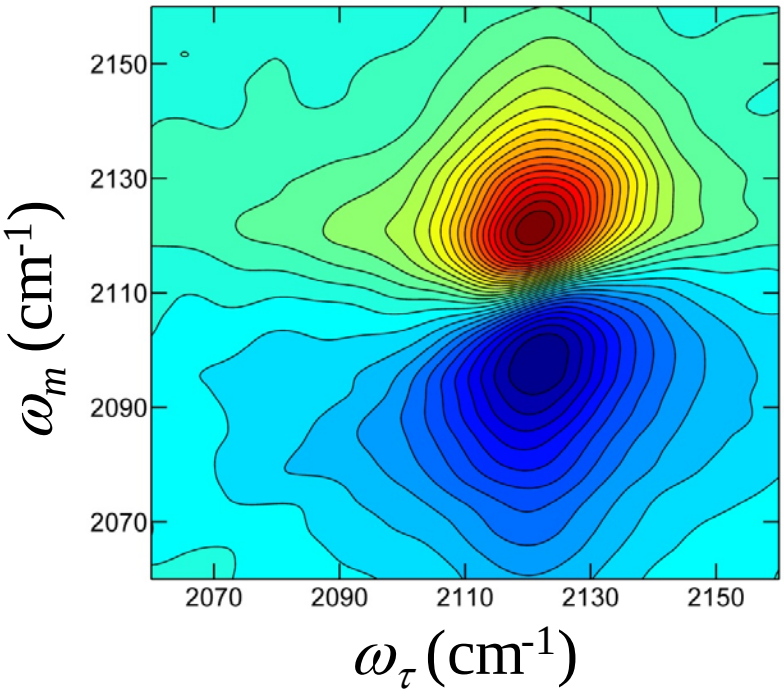

Figure S3: 2D IR spectrum of Az of MbAzCO showing entire frequency range. Neither the low frequency band found at 2075 cm^{-1} in the FT-IR spectrum nor any cross bands are apparent.

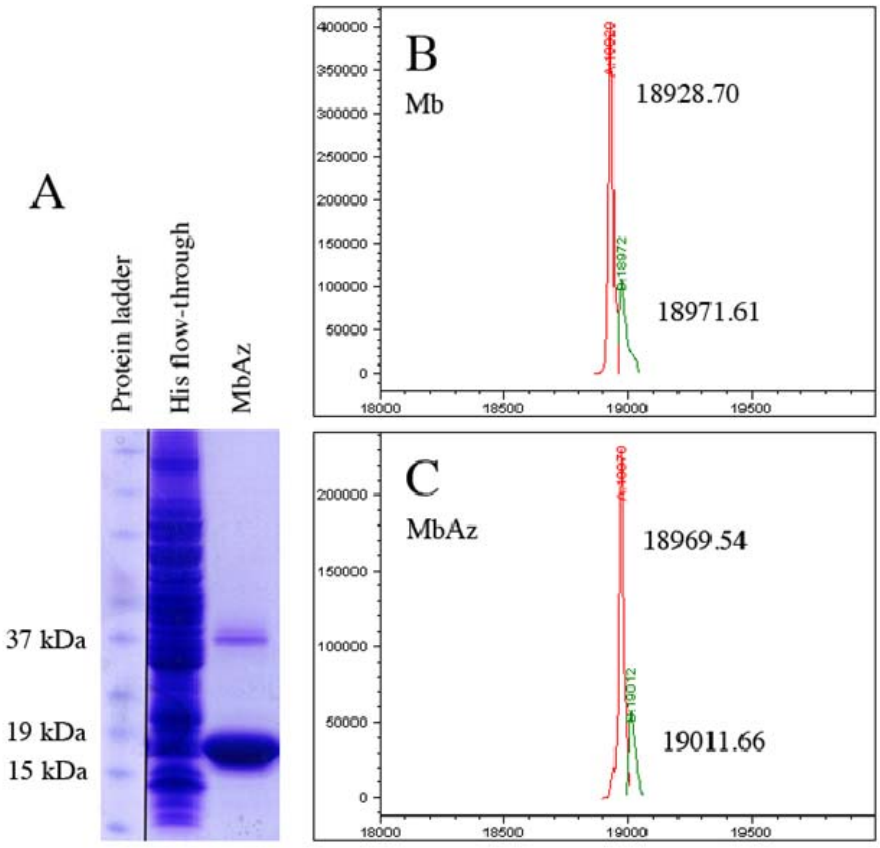

Figure S4: (A) SDS-PAGE gel of His purification flow-through and purified MbAz. A small ~40 kDa band is visible that may be MbAz dimer. Mass spectrometry characterizations of Mb (B) and MbAz (C) show 41 Da mass difference, corresponding to the difference between Phe and AzPhe. In both, the smaller green peak is likely an acetyl adduct.