SUPPORTING INFORMATION

for the manuscript

MAS Solid State NMR of Perdeuterated Proteins

by

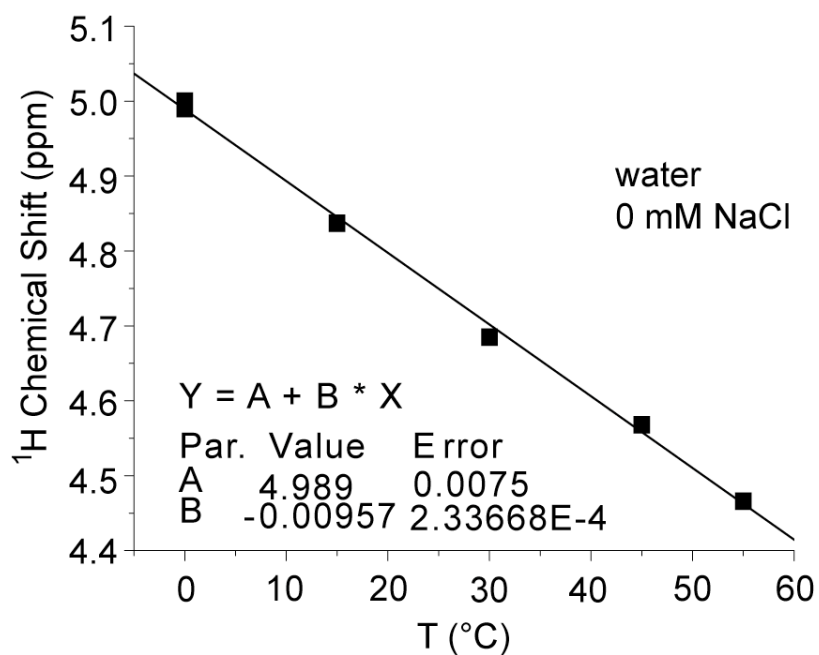Rasmus Linser, Veniamin Chevelkov, Anne Diehl and Bernd Reif

Fig. 1: Calibration for the correlation between chemical shift and temperature (sample without salt). Parameters given refer to a linear regression done in ORIGIN. Acquisition took place after 15 min in order to assure temperature equilibrium. Water chemical shift was set to 5.0 ppm at 0 °C.

Fig. 2: Calibration for the correlation between chemical shift and temperature (sample with 100 mM salt). Parameters given refer to a linear regression done in ORIGIN. Acquisition took place after 15 min in order to assure temperature equilibrium. Water ch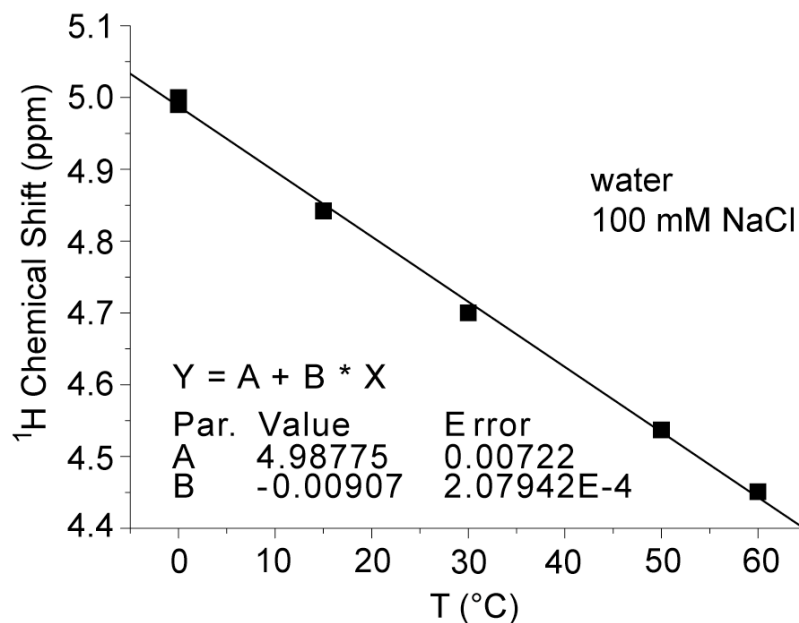emical shift was set to 5.0 ppm at 0 °C. No higher temperatures were taken into account in order to avoid spectrometer damage.
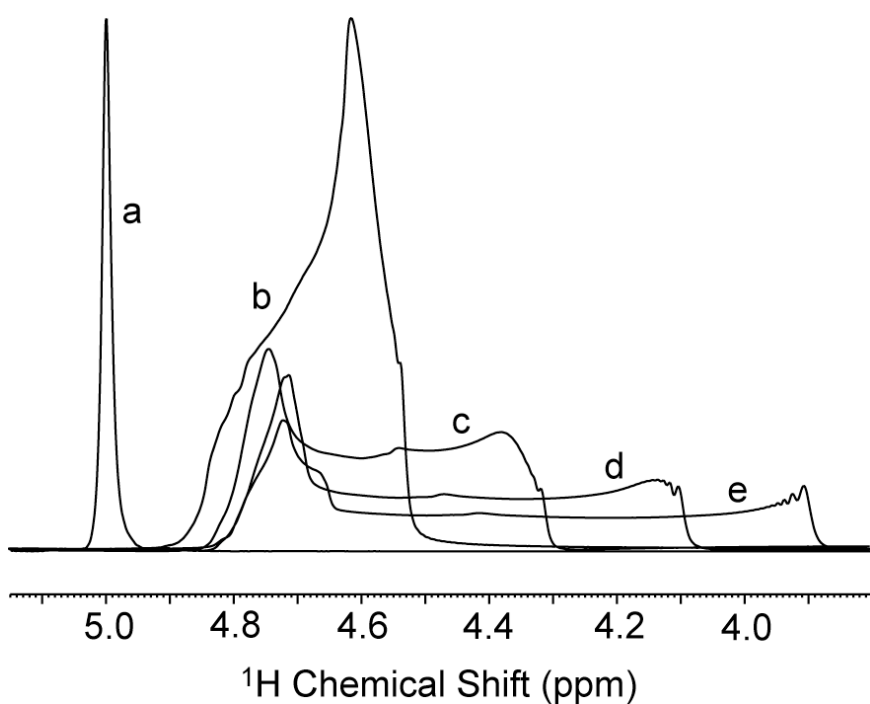
Fig. 3: H_2O spectra to monitor the dynamic temperature increase obtained for a sample containing 100 mM NaCl, employing the pulse scheme represented in Figure 1B of the main manuscript. Spectra b, c, d, and e are obtained upon application of decoupling for $t_1 = 0$, 10, 20, and 30 ms prior to acquisition. The total duration of decoupling during each scan is kept constant and set to 30 ms. Spectrum a is a reference spectrum representing the H_2O chemical shift in the absence of decoupling. Spectra a and b are scaled independently. d_5 is set to 1 ms in the experiment.
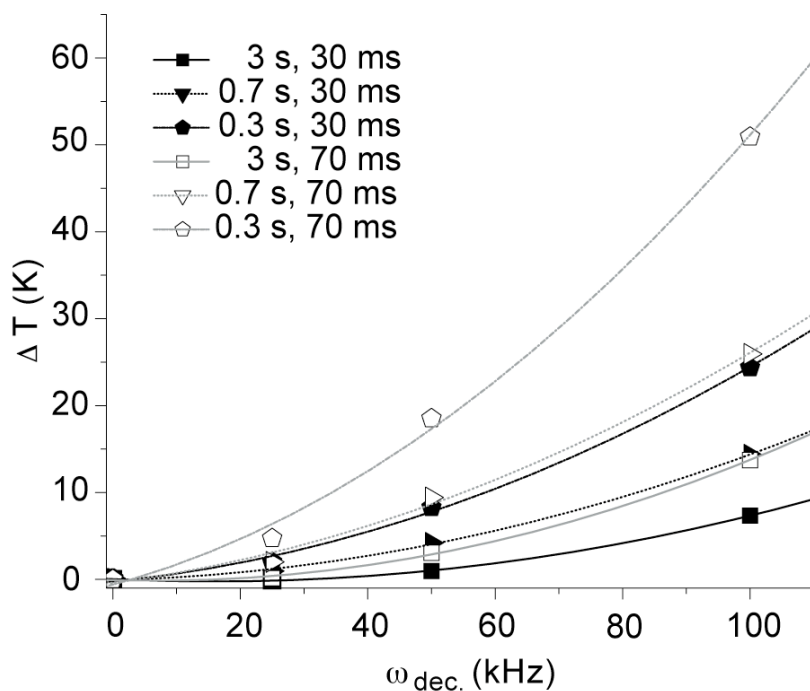
Fig. 4: RF field dependence of the water temperature increase (0 mM NaCl) upon decoupling for 30 ms (black curves, filled symbols), and 70 ms (grey curves, open symbols) for different recycle delays, employing the pulse scheme represented in Figure 1A of the main manuscript. Acquisition was performed 30 ms after decoupling, cooling air was set to 275 K output temperature.

The effects of an increase in decoupling power, as well as the impact of variation of the duration of decoupling, were investigated using the salt-free water sample (Supporting Information Figure 4). The observed temperature increase varies with the employed decoupling power and the use of recycle delays of different length. Due to limited heat dissipation, significantly more heating is observed for shorter recycle delays.

A 10-fold reduction of the recycle delay from 3 s to 0.3 s yields a temperature increase from 1-8 K (decoupling at 50 kHz) and from 7-24 K (100 kHz decoupling) above the temperature measured in the absence of decoupling. The effect is even larger if decoupling is applied for a duration of 70 ms (50 kHz decoupling: temperature increase of 3-17 K, and 100 kHz: temperature increase from 14-51 K above the temperature measured in the absence of decoupling). An acquisition time of 70 ms is required in order to obtain a spectral resolution of 15 Hz. This kind of resolution (or better) is currently achievable in the ^{15}N chemical shift dimension of protein samples subjected to the above described deuteration scheme.
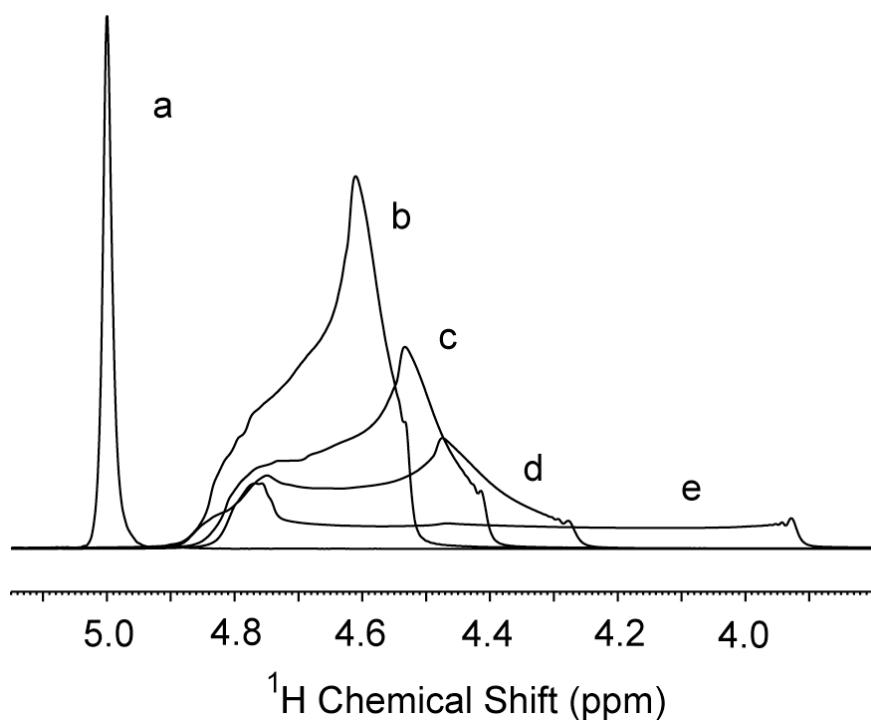
Fig. 5: H_2O spectra to monitor the static temperature decay obtained for a sample containing 100 mM NaCl, employing the pulse scheme represented in Figure 1A of the main manuscript. The duration and strength of the cw-irradation were set to 30 ms and 100 kHz, respectively. (B), (c), (d), and (e) correspond to spectra recorded with a delay d_2 of 2.7 s, 1.7 s, 1 s and 30 ms, respectively. Spectrum (a) shows the water chemical shift without decoupling at 275 K. A recycle delay RD of 3 s was used in all experiments. A flow rate of the cooling gas of 1335 L/h was used for the spectrum (e), whereas the flow rate was set to 935 l/h in (b-d). Resonance (a) is represented not to scale.
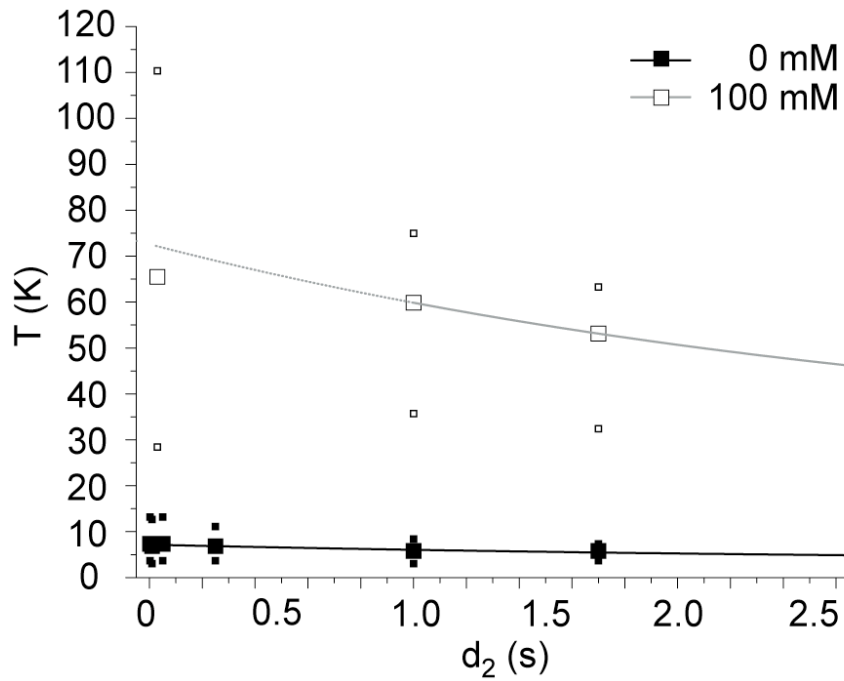
Fig. 6: Temperature decay after decoupling for a water sample containing no salt (black curves and symbols) and 100 mM NaCl (grey curve and open symbols). The recycling was kept constant at 3 s. The flow rate of the cooling air was set to 935 L/h for the water sample containing 100 mM NaCl, with the exception of the first data point (1335 L/h). The dotted line represents the extrapolated curve using only the data points at 1.0 s, 1.75 s and 2.75 s for fitting. The flow rate of the cooling air for the 0 mM NaCl sample was set to 1335 L/h.
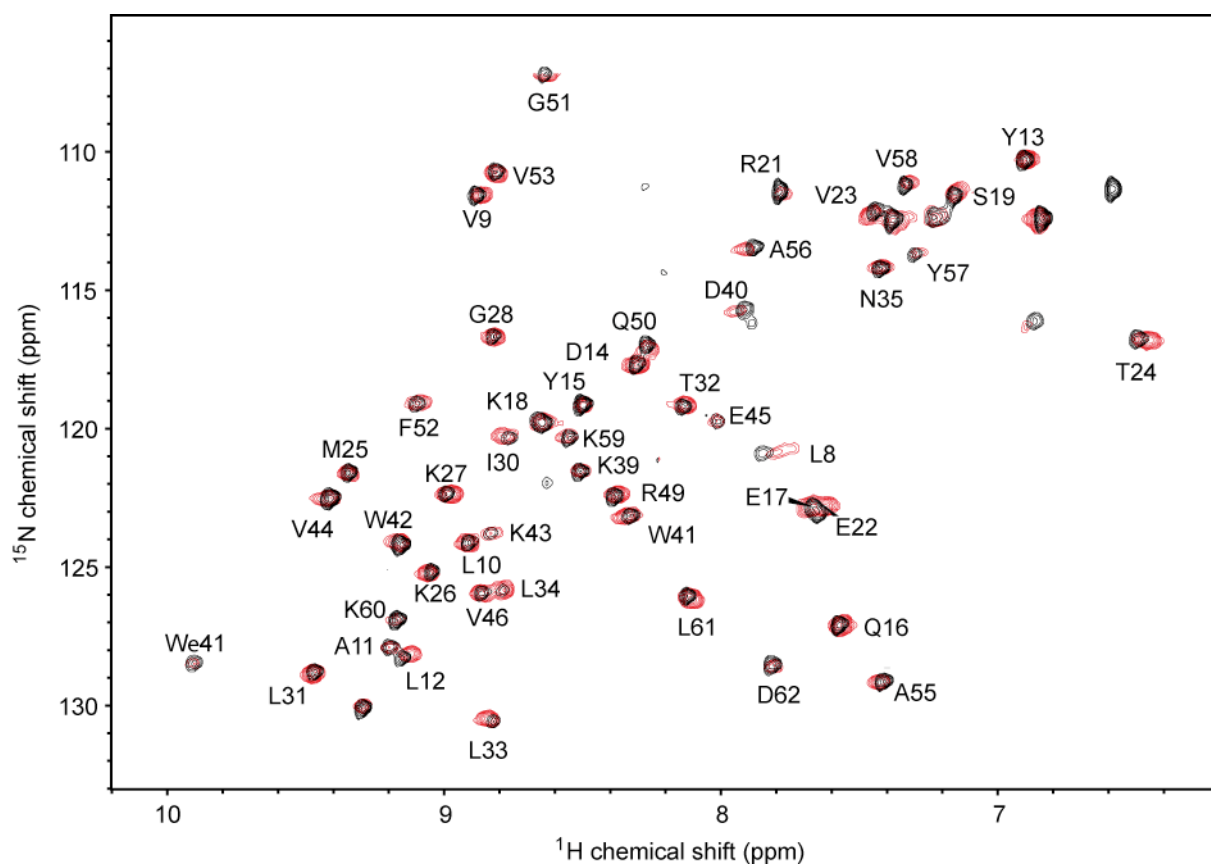
Fig. 7. Superposition of a ^{1}H detected ^{1}H,^{15}N correlation experiment in the absence (black) and presence of 250 mM [Cu(edta)]$^{2-}$ (red). The figure represents the full spectrum.