Materials and Methods

Sample preparation A first series of collagen solutions was prepared by diluting a standard commercial solution (3 mg/mL concentration, Nalgene) with D_2O (CIL, 99.96%). The range of concentrations included 25, 35, 50, 100, 150 μg/ml. Since the solutions were not buffered, the pD values varied continuously from 3.40 to 3.44 for the lowest and highest collagen concentration respectively. A second series of collagen solutions was prepared by diluting the standard commercial solution (3 mg/mL concentration) with deutorated PBS buffer. The deutorated PBS buffer was prepared by mixing 0.066 M Na_2DPO_4 and KD_2PO_4 solutions. These solutions were prepared by dissolving Na_2DPO_4 (CDM Isotopes, 98% of ^2D) and KD_2PO_4 (CIL, 98% of ^2D) with D_2O (CIL, 99.9%). The concentration range was the same as for the first series; the resulting pD was 7.4 for all concentrations. In the discussion below, we refer to the solutions prepared with D_2O as "non-buffered solutions" and the solutions prepared with PBS as "buffered solutions". Deutorated solvents and salts were used for solution preparation to exclude the solvent signals from NMR spectra. All solutions were thoroughly mixed upon preparation and equilibrated for ~ 1 hour after which the first NMR measurement was performed. The solutions were then incubated for ~ 24 hours at 25°C and the second NMR measurement was performed. The 3rd, 4th, 5th, and 6th measurements were performed after 2, 3, 5 and 7 days of incubation respectively. We define freshly prepared collagen solutions (1 hour) as "initial" and those incubated as "aged" in following discussions. The NMR signal intensity of the standard (3mg/mL) solution was also measured at the same conditions as study solutions for reference.

Proton NMR spectroscopy. All NMR experiments were performed at 500.1 MHz on a Varian NMR Systems Inc. spectrometer using a Varian probe (model PFG MR0803P007). The transmitter frequency was adjusted to the Larmor frequency. The probe temperature was stabilized for at least 30 minutes prior to each experiment. The magnetic field stability was adjusted using a deuterium lock. The magnet was shimmed to optimize field homogeneity. Proton NMR spectra were obtained by using a standard PRESAT

sequence to suppress the residual H_2O signal. In this sequence, a long, selective rf-pulse is initially applied at the H_2O frequency to saturate the water signal followed by a non-selective $\pi/2$ rf-pulse and the NMR signal is acquired. The length of the $\pi/2$ pulse was 10 μs for all experiments. To increase the signal-to-noise ratio (SNR), the signal was averaged over 976 scans for each protein spectrum. To confirm that the SNR was sufficient, the spectrum, normalized for the number of averages, was compared to one measured with 4096 scans. These signals were found to be the same within experimental error. The repetition delay between scans was set to 5* T_1, to ensure a complete recovery of spin magnetization after every scan. It should be noted that it is very important to achieve the same saturation level of the residual H_2O signal for all experiments. If saturation level is different, it is hard to establish a consistent and systematic correlation for protein signals. We use the integral intensity of the residual H_2O signal as the criteria of saturation; for all data reported here it varied a maximum of 5% for different experiments.

The spectrum was obtained by Fourier Transform (FT) of the NMR signal. To improve spectrum quality, Lorentzian broadening was applied to the original data before the FT. The 1st order baseline correction was applied to the spectrum after which all spectra were normalized to zero baseline before the analysis. For the data with the same level of water saturation, baseline correction turns out to be invariant to both time and concentration. The residual offset, however, was somewhat concentration dependent. Normalization to zero baseline performed prior to spectrum analysis removed this dependence. Frequency for the corrected spectra was adjusted according to TMS standard.

Figure 1S ^{1}H spectrum of collagen solution