Indirect Detection of Labile Solute Proton Spectra via the Water Signal Using Frequency Labeled Exchange (FLEX) Transfer

*Joshua I. Friedman, Michael T. McMahon, James T. Stivers, and Peter C.M. Van Zijl**

Supporting Information 1: Selective excitation pulses in the label transfer modules.

Selective excitation pulses in the FLEX experiment must be a compromise between the competing requirements of being of short-duration (to minimize exchange losses) and frequency selective (to avoid water excitation leading to radiation damping during tevol and signal loss when detecting water). We chose to employ soft square pulses both for their simplicity and short durations. The off resonance effective field generated by these pulses is given by the equation $\Delta\omega_{eff} = \sqrt{\omega_1^2 + \Delta\omega_{s01}^2}$ which is the simple vector addition of the resonance offset, $\Delta\omega_{so1}$, and the B1 field strength ω_1. By choosing $\Delta\omega_{so1}$ such that the product $\omega_{eff} \bullet \tau_{90}$ is 2π radians on water and nearly $\pi/2$ for the exchanging peaks of interest, water may be selectively returned to its equilibrium population, and the resonances of interests are left transverse by the end of the pulse. This condition is satisfied when[s1]

$$\Delta\omega_{so1} = \omega_1 \cdot \sqrt{15} \qquad [S1]$$

In practice, offset o1 is chosen based on the combined requirements of not wanting to excite any water and keeping the selective pulses short to avoid major loss of exchangeable protons during the pulse. These requirements limit our sampling possibilities. The FID decays with an exponential rate constant of $[k_s+1/T_2^*]$ and, for k_s = 1000Hz, a factor of 2.7 in signal will already be lost for a t_{evol} of 1ms. If we want the pulse length to be at most 100 μs, it can be calculated that ω_1 for a 90 pulse ($\pi/2 = \omega_1 \cdot t_{pulse}$) is 15,700 rad.s. Using eqn. S1, this leads to 9,600 Hz offset for o1 in order to minimize water excitation. In our effort to maximize signal to noise for these rapidly

decaying resonances, we collected as many data points as possible in the early part of the FID (dwell times 10-25 μs), leading to oversampling with respect to the Nyquist-Shannon requirements. The need for placing the carrier frequency far off-resonance, under the above conditions, combined with the short dwell time, conveniently allowed us to forgo quadrature detection, but that intent was not the reason for moving the offset. In the future, nonlinear time sampling methods, such as the maximum entropy method,[s2] could be employed to drastically reduce the experimental collection time while allowing one to preferentially sample the early data points. Placing the carrier downfield of the exchanging resonances also has the advantage of moving carrier noise away from the peaks of interest (without needing to resort to time proportional phase incrementation techniques).

The use of a soft square pulse to selectively excite the peaks of interest results in a frequency dependent phase shift of the transverse magnetization. Assuming all the exchangeable protons in the spectra are near to each other in frequency space they will have a similar phase offset and application of a zero-order phase correction is generally sufficient to make all peaks absorptive. Alternatively one could apply a first-order phase correction to properly phase the frequency spectrum or simply use time domain analysis to extract signal components as was done in this paper. Notice that the commonly used approach of correcting for this phase shift by choosing the zero evolution point to be $1/(2SW) - 4t_{pulse}$ is not possible here due to the small dwell time and large pulse width.

In order to determine the scaling factor λ_s, it is needed to either measure or calculate the fractional amount of transverse magnetization ($M_{T,\Delta\omega_{so1}}$) created by single pulse $\Delta\omega_{so1}$ Hz from the frequency of interest. This fraction must then be squared to obtain λ_s, in order to account for

two selective pulses per LTM). For the case of the square soft pulses applied off-resonance in this paper, $M_{T,\Delta\omega_{so1}}$ is described by the equation below as derived from the Bloch equations[1]

$$M_{T,\Delta\omega_{so1}} = \sqrt{(\sin(\theta)\sin(\alpha))^2 + ((1-\cos(\alpha))\sin(\theta)\cos(\theta))^2} \qquad [S2]$$

where $\theta = \arctan\left(\frac{\omega_1}{\Delta\omega_{so1}}\right)$ and $\alpha = \frac{\pi}{2\sin(\theta)}$

The λ_s attenuation factor was determined experimentally for the quantification experiments by measuring the amount of water signal excited by a single soft excitation pulse as a function of frequency offset from water. The fraction of transverse magnetization generated is obtained by normalizing the water signal as a function of offset to the maximal value obtained when $\Delta\omega_{so1}$ = 0 Hz and then subtracting every point from the maximum unity signal. A comparison of experiment (blue crosses) and theory (red line) are depicted in *Figure 1* below.

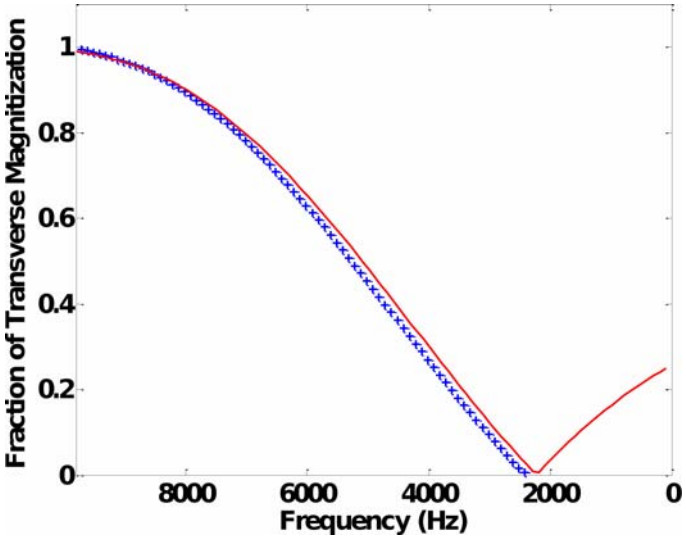

Figure S1. **Excitation profile of the 'selective' soft pulses used in the FLEX experiment**. Experimental data are in blue, the simulated curve derived from equation [S2] is in red. Data is for a 2.5 kHz B1 field applied 9.8 kHz from water on a 11.4T magnet at 10C.

One consequence of using selective excitation of the downfield region is that no NOEs were detected in the FLEX spectrum. The 90-90 pulse sequence prepares for both exchange and NOE transfer and, in principle, FLEX transfer is sensitive to NOE effects. This can be loss of

magnetization to neighboring protons or bound water, or, inversely, exchange-relayed NOE transfer to water. However, the time scale of the NOE buildup is generally much slower than the exchange time scale. A typical LTM t_{exch} value used for detection of rapidly exchanging protons will be about 2-20ms, after which most of the proton magnetization will reside on water. The build-up time for intra- and intermolecular NOEs is generally much slower, unless large macromolecules are studied. In order to detect sensitivity-enhanced NOE effects from aliphatic protons to the exchangeable amide and imino protons in the FLEX spectrum, one would have to excite the portion of the spectrum upfield from water to encode them.

References

(S1) Cavanagh, J.; Fairbrother, W.J.; Palmer III, A.G.; Skelton, N.J. In *Protein NMR spectroscopy: principles and practice;* Academic Press: 2007.

(S2) Hoch, J.C.; Stern, A.S.; Donoho, D.L.; Johnstone, I.M. *J. Mag. Res.* **1990**, *86*, 236

Supporting Information 2**: Processing of the FLEX data**

This processing is illustrated in three figures:

Figure S2: Acquisition of spatial profiles and processing of their integral modulation

Figure S3: Frequency domain analysis of water signal modulation as a function of (t_{evol}).

Figure S4. **Time domain quantification of FLEX data for the DNA sample at 10 ˚C**

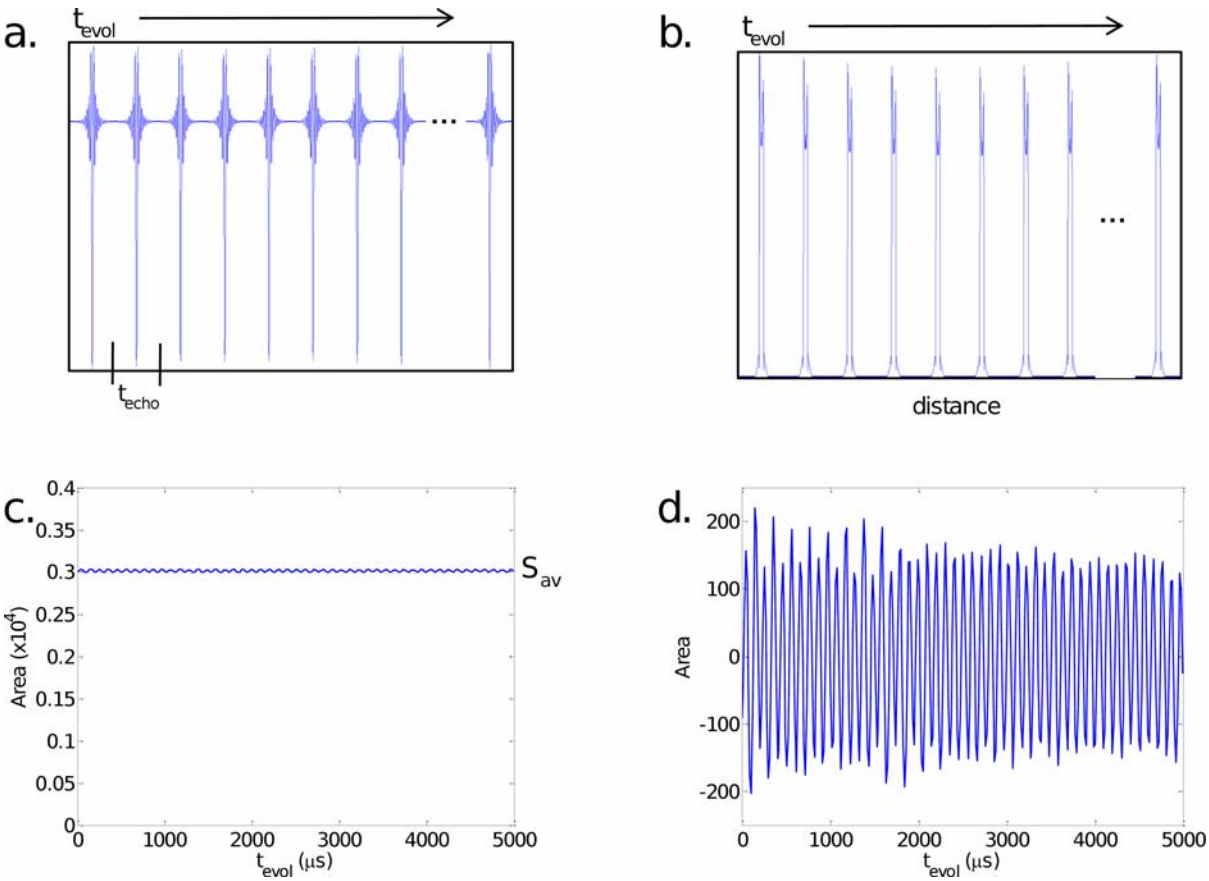

Figure S2: Acquisition of spatial profiles and processing of their integral modulation. (a) FLEX data is recorded as an array of gradient recalled echoes in the directly detected time domain. Each individual echo encodes information about the magnetization of water for a specific evolution time t_{evol}. (b) To quantify this magnetization as a function of t_{evol}, each gradient echo is first Fourier transformed and the magnitude of this data is used to generate a projection of the water volume. (c) Numerical integration of these projections provides a plot of water intensity as a function of the indirect time dimension t_{evol}. (d) S_{av} is then subtracted from each data point to remove the DC offset, producing an interferogram of the chemical shift frequencies of the exchangeable protons plus residual water excited in the label transfer modules.

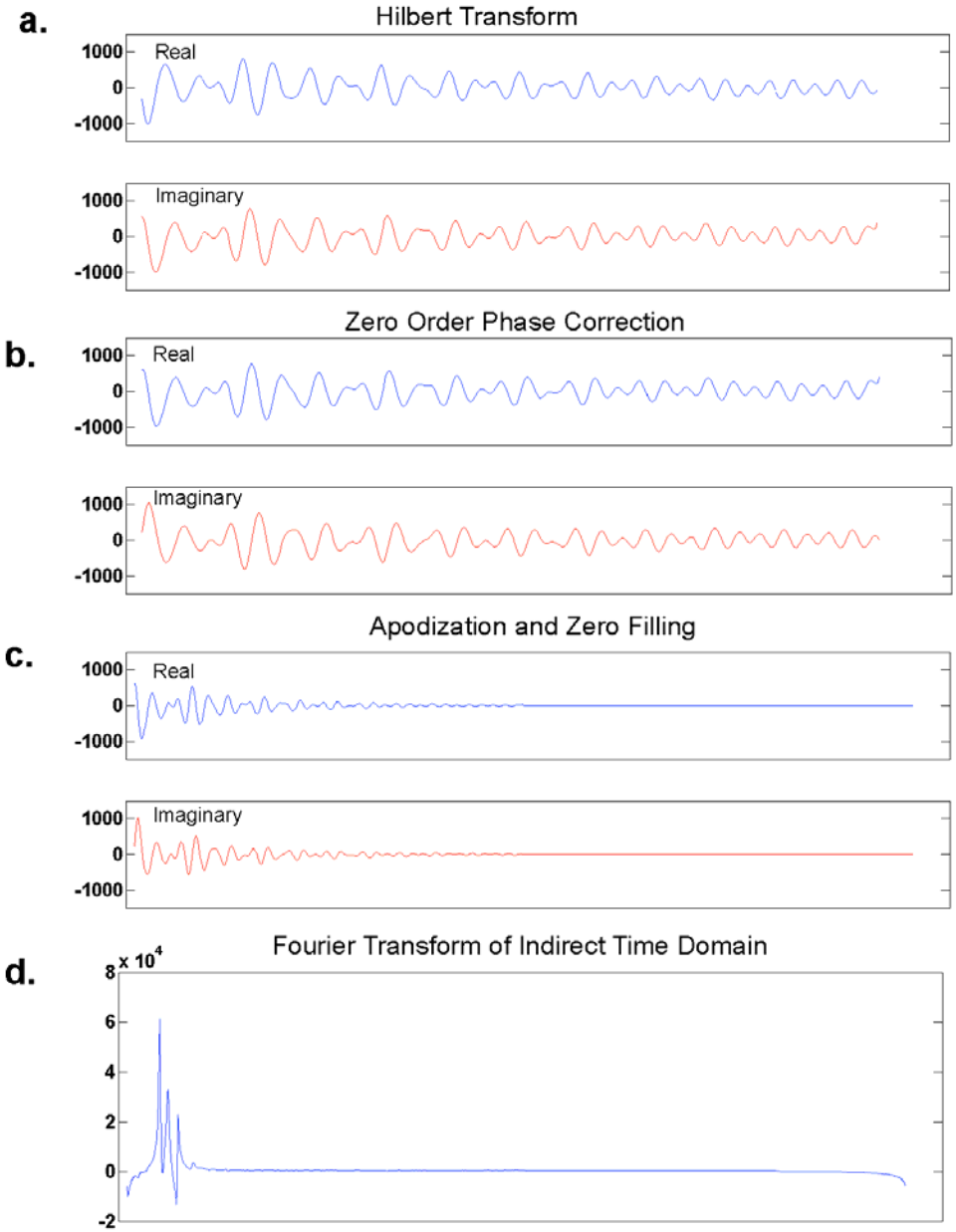

Figure S3: Frequency domain analysis of water signal modulation as a function of (t_{evol}). (a) To generate the frequency domain spectra a Hilbert transform is first applied to the acquired data to synthesize the imaginary component of the signal. (b) A phase correction is then applied to allow later generation of absorptive peaks in the frequency domain. (c) Signal to noise is optimized by exponential broadening with 0.5 times the initial linewidth, followed by zero filling to increase digital resolution in the frequency domain. (d) The exchange edited frequency domain spectrum is then reconstituted by Fourier transformation.

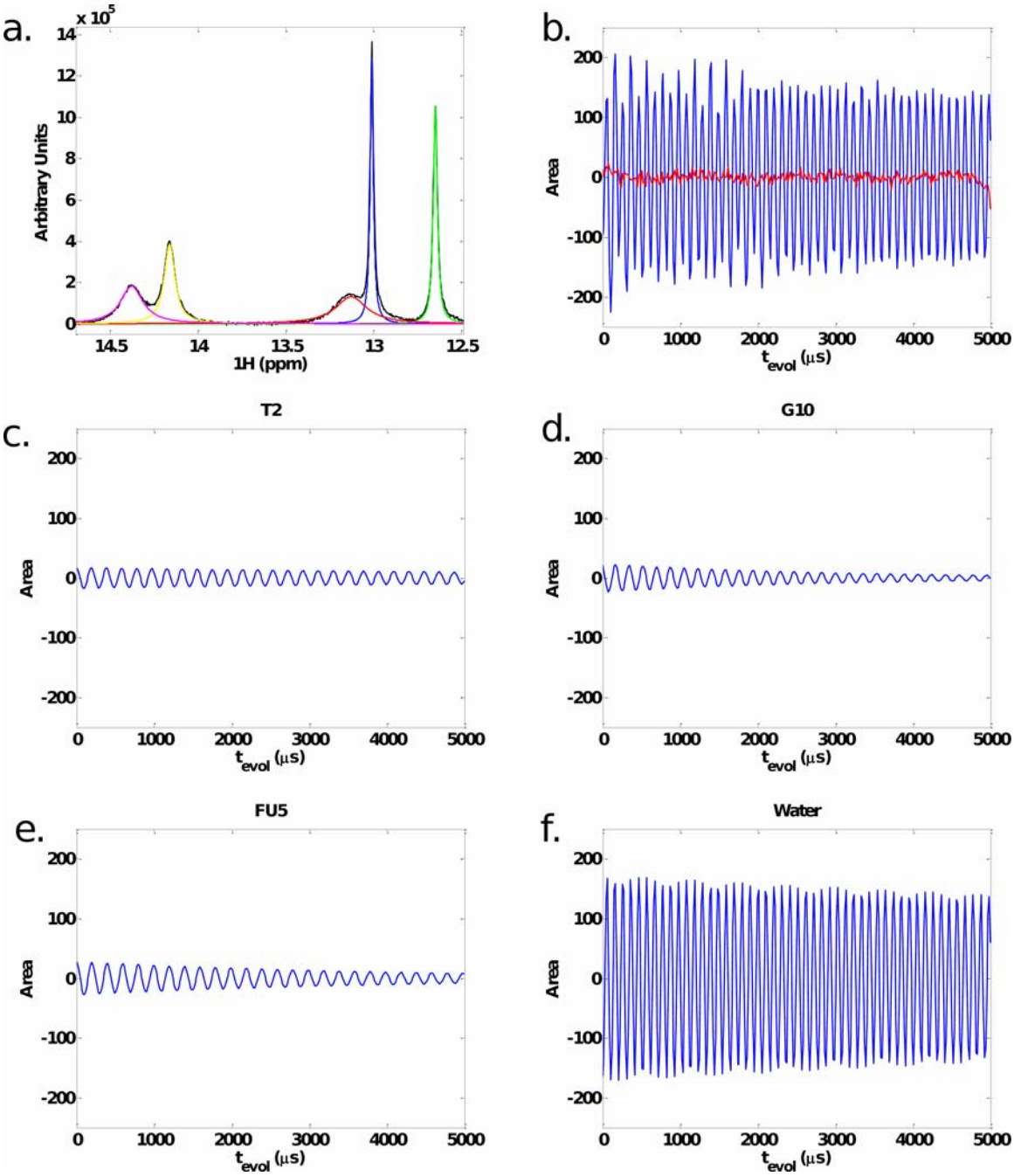

Figure S4. **Time domain quantification of FLEX data for the DNA sample at 10 ˚C.** (a) The exchange rates and resonance frequencies for fitting the FLEX data were obtained by fitting an un-apodized jump-return spectra of the DNA imino region to a 5 term Lorentzian function in the least squares sense. As each imino resonance was present with 1:1 stoichiometries the fitted peak models were constrained to all have the same total area. (b) The FLEX data was fit to equation 4 in the main text with four signal components and an additional phase factor. The phase and amplitude, (PTR_s) were allowed to float in the fitting procedure. The best fit line in blue and residual in red are shown in panel. (c-d) The four signal components corresponding to the resonances T2 (c), G10 (d), FU5 (e) and residual water (f) .