

## Sensitivity-enhanced detection of non-labile proton and carbon NMR spectra on water resonances

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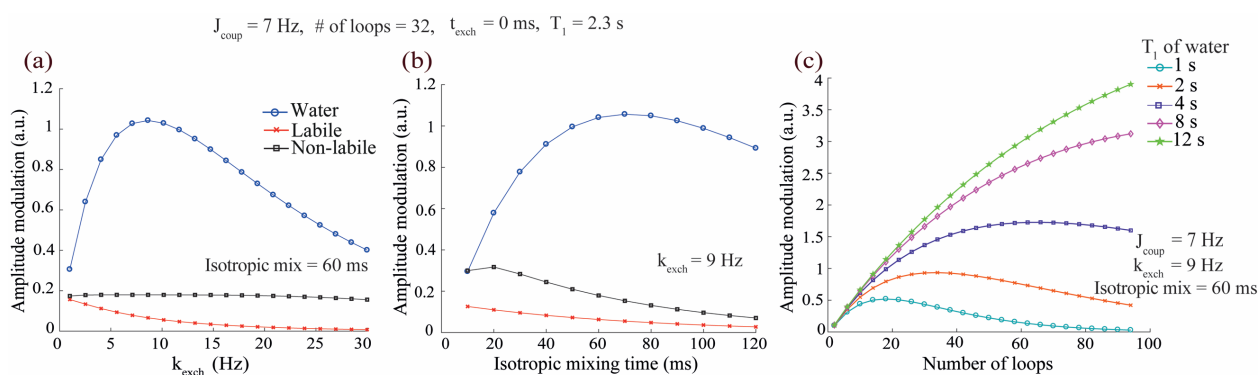
### Supplementary Information

**Parametric dependencies of REFLEX.** To examine the effects of the exchange rate on REFLEX experiments for a given  $J$ -coupling, as well as to optimally tune the parameters of these experiments, a multi-site Bloch-McConnell-based model was put forward focusing on the fate of various longitudinal magnetization components  $M$ . Specifically, it was assumed that

$$\frac{d}{dt} \begin{pmatrix} M_{nl} \\ M_l \\ M_w \end{pmatrix} = \begin{pmatrix} -\frac{1}{T_1^{nl}} - fJ_{coup} & fJ_{coup} & 0 \\ fJ_{coup} & -\frac{1}{T_1^l} - fJ_{coup} - k_{exch} & \frac{k_{exch}}{n_w} \\ 0 & k_{exch} & -\frac{1}{T_1^w} - \frac{k_{exch}}{n_w} \end{pmatrix} \begin{pmatrix} M_{nl} \\ M_l \\ M_w \end{pmatrix} + \begin{pmatrix} \frac{M_{nl}^0}{T_1^{nl}} \\ \frac{M_l^0}{T_1^l} \\ \frac{M_w^0}{T_1^w} \end{pmatrix} \quad (1)$$

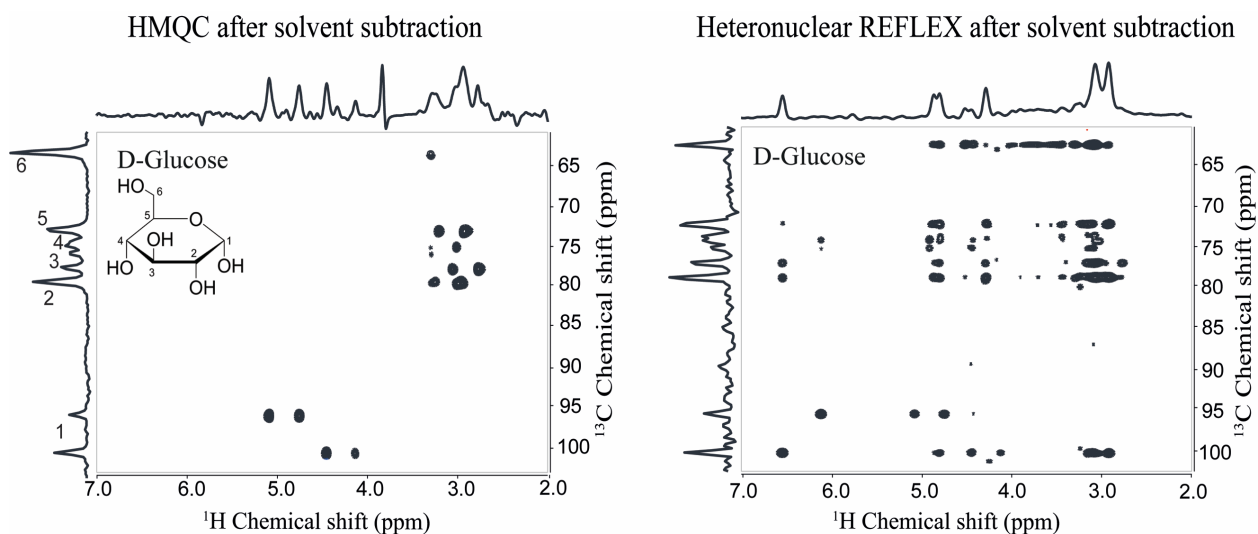
Here  $M_{nl}$ ,  $M_l$ ,  $M_w$  represent magnetizations of non-labile, labile and water species respectively; the same holds for the  $T_1$  relaxation constants as well as for the starting magnetizations represented with a zero superscript.  $J_{coup}$  and  $k_{exch}$  are the coupling constant between the non-labile and labile protons and the exchange rate between the latter and the solvent respectively, and the factor  $f$  accounts for the progressive  $J$ -coupling averaging due to chemical exchange effects. In accordance with Fig. 3b this was assumed as  $f = \exp(-bk_{exch})$ , where  $b = 0.06$  is the value suggested by the data in that experimental Figure. During the isotropic mixing period this model assumes that both  $J_{coup}$  and  $k_{exch}$  are active, while during the exchange time only  $k_{exch}$  is active. Simulations based on all these considerations can successfully reproduce the overall behavior that we observed

experimentally: Supporting Fig. S1 summarizes how different parameters under experimental control, affect the experiment's overall sensitivity.



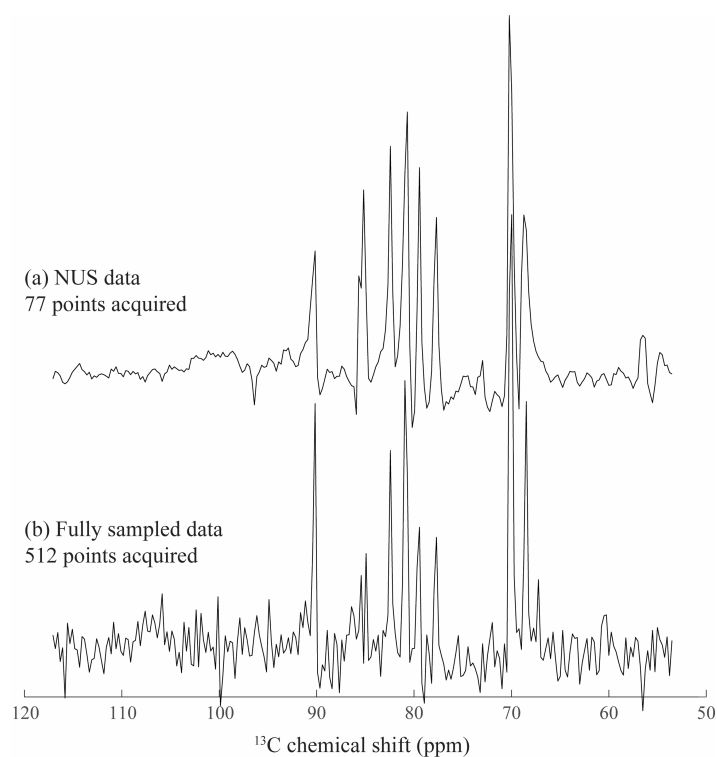
**Figure S1.** Predictions arising from a Bloch-McConnell formalism (Equation 1) describing how different controllable parameters influence the overall sensitivity of Homonuclear REFLEX. In all cases the  $M^0$  magnetizations of the non-labile and labile sites were assumed to be 1 and the water  $M^0$  was assumed to be 270, to reflect the difference in concentrations. (a) Effect of  $k_{\text{exch}}$  on the various magnetizations after 60 ms. (b) Effect of changing the isotropic mixing time of the experiment on all three intervening sites. For both (a) and (b) 32 loops and  $T_1 = 2.3 \text{ s}$  for water were assumed. (c) Effect of looping the number of FLEX processes on the water's modulation for different water  $T_1$  relaxation times. In all figures  $T_1$  of the non-labile species is assumed 1.3 s whereas the one of labile sites is set equal to the corresponding water's  $T_1$ .

**Additional information conveyed by REFLEX.** One of the interesting features of relayed FLEX experiments is their establishment of total correlations between labile sites, non-labile sites, and water. This leads, in addition to the water-detected spectra, to TOCSY-like 2D spectra in the homonuclear correlations and to 2D HMQC-TOCSY in the heteronuclear experiments, including in all cases both labile and non-labile resonances. Figure S2 illustrates this by presenting 2D spectra obtained in HMQC and in heteronuclear REFLEX experiments using the same parameters and conditions as in Figure 2, after subtracting from the latter the solvent signal. Notice how the latter shows, in addition to the directly bound  $^{13}\text{C}$ - $^1\text{H}$  correlations, cross-peaks among all mutually coupled nuclei. This feature of these experiments provides, in addition to the water-detected 1D spectra of the non-labile species, additional information of the kind arising in conventional 2D NMR experiments.



**Figure S2.** 2D HMQC and Heteronuclear REFLEX spectra processed with solvent subtraction, showing on the left-most projection the origin of different  $^{13}\text{C}$  resonances.

### Example of heteronuclear REFLEX acquisitions on natural abundance sucrose



**Figure S3.** Spectrum of 400 mM natural abundance sucrose in  $\text{H}_2\text{O}:\text{d}_6\text{-DMSO}$  (20%:80%) obtained by Heteronuclear REFLEX experiments at 37 °C. (a) Using a conventional 256 points hypercomplex acquisition (512 experiments total). (b) Using only 77 experiments with randomly chosen  $t_1$  values and hypercomplex phases, reconstructed using the Cadzow procedure. The spectra are acquired using 8 scans and spectral width of 8000 Hz.