Magnetic Resonance Imaging of Sample in the Flow Cell

One-dimensional images acquired with a pulsed field gradient spin echo sequence (PFGSE) were used to investigate the sample filling into the flow cell [1]. Figure S1 shows images obtained with a z-gradient strength of 0.014 T/m. With a completely filled cell (without gas bubbles present), the smooth feature in the image represented by the dashed line was observed. If gas bubbles are trapped, the image becomes uneven (solid line).

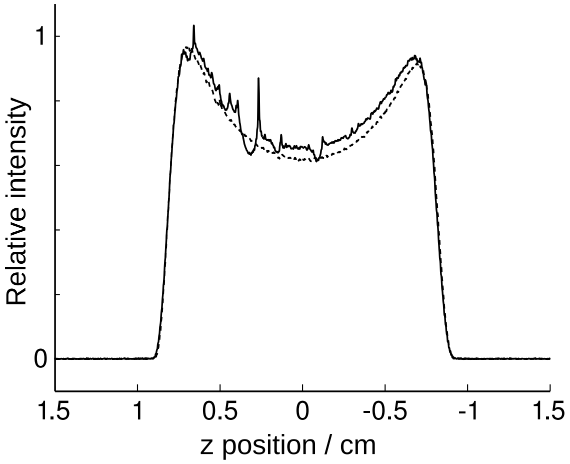

Figure S1: One-dimensional images obtained using a pulsed field gradient spin echo sequence. An image containing irregularities, presumably due to the presence of gas bubbles (solid line), is superimposed with a profile from a homogeneous sample (dashed line).

Pulse Sequence for the Diffusion Measurement

Figure S2a shows the pulse sequence used for diffusion measurements. Echo trains from the first four readouts (m=1 – 4) obtained from this pulse sequence on a stationary water sample are shown in Figure S2b. The acquisition parameters are included in the main text. The different indices n refer to different degrees of winding in the magnetization helix, which is typically quantified by the wavenumber $k = \gamma G_z t_n/(2\pi)$. The variable t_n is the time between the pulses α_n and the $\pi/2$ storage pulse, and is equal to $n\delta + \delta_d$ ($n = N$ for the first pulse and $n = 1$ for the last pulse). The γ and G_z are the gyromagnetic ratio of nucleus of interest and

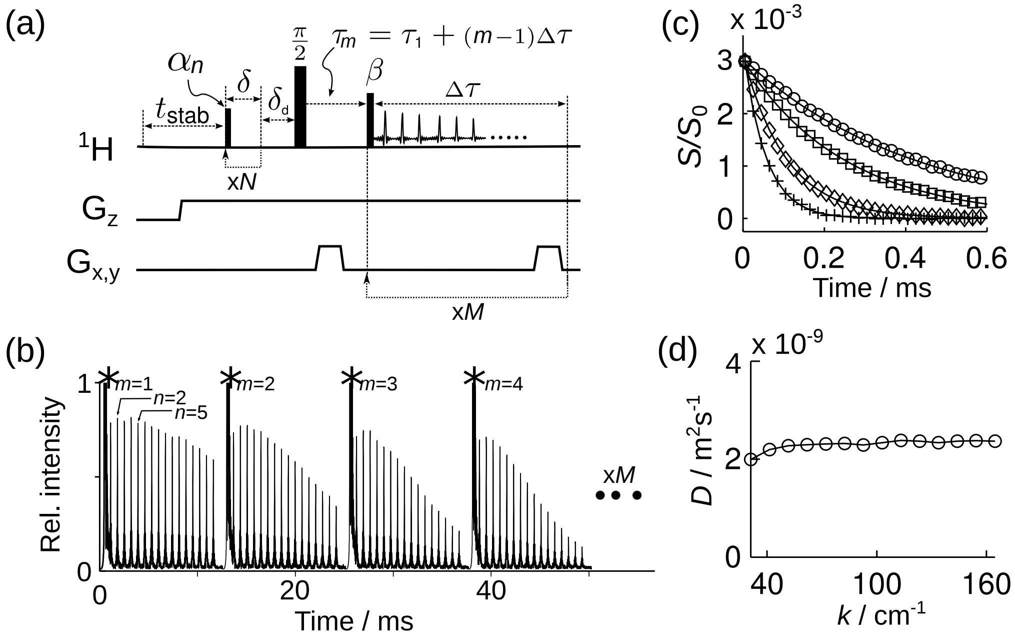

Figure S2: a) Pulse sequence for diffusion measurement in a single scan [2, 3]. b) The first four stimulated echo trains obtained from a stationary sample of H_2O. The symbols $*$ indicate FIDs directly originating from the application of the pulse with flip angle β. c) Amplitude of selected echoes from the same winding of magnetization helices (S/S_0) as a function of time (○: k=31.4 cm^{-1}, □: k=52.3 cm^{-1}, ◇: k=94.0 cm^{-1}, +: k=135.7 cm^{-1}). Solid lines are fit results obtained using the Equation 4 in the main text. d) Diffusion coefficients (D) obtained from the data shown in part in (c).

the gradient strength along the B_0 field applied during the measurement, respectively. Figure S2c shows the signal attenuation of stimulated echoes with different k. Diffusion coefficients derived from fitting data (as selected shown in Figure S2c) to Equation 4 (using n as the independent variable) are summarized in Figure S2d. The values converge to the self-diffusion coefficient of H_2O at 298 K.

Sample Mixing

When using the liquid driven injector, the correct timing to switch the valve V2 (Figure 5a in the main article) is important for achieving the optimal mixing of the hyperpolarized solution with the second sample component. Reagents used to determine the injection parameter (t_{mix}) are bromophenol blue prepared in 10 mM citrate buffer, pH=6 (reagent A) and 200 mM citrate buffer, pH=3 (reagent B). The reagent A is used as a stand-in for the HP sample in a test injection. When 10 μL of reagent A is mixed with the reagent B at correct timing, the instant color change from purple to yellow resulting from the pH jump can be clearly observed as shown in Figure S3. On the other hand, if the reagent B is injected too late, both blue and yellow color will be observed. The mixing ratio of two reagents is, however, best determined from NMR signals.

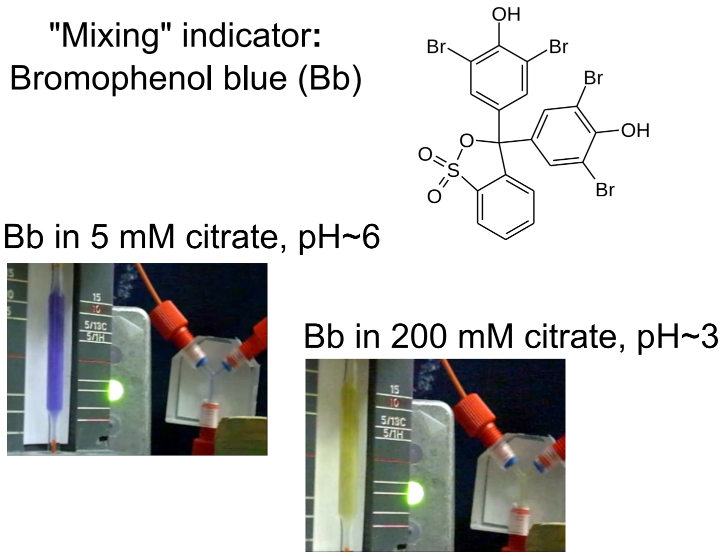

Figure S3: Distinct color change of bromophenol due to a pH change upon mixing, used to determine the timing for injection of the non-hyperpolarized sample in loop L2.

References

[1] P. Callaghan, *Translational Dynamics and Magnetic Resonance: Principles of Pulsed Gradient Spin Echo NMR*, Oxford University Press, **2011**.

[2] S. Peled, C.-H. Tseng, A. A. Sodickson, R. W. Mair, R. L. Walsworth and D. G. Cory, *J. Magn. Reson.* **1999**, *140*, 320–324, doi:10.1006/jmre.1999.1850.

[3] J. Granwehr, R. Panek, J. Leggett and W. Köckenberger, *J. Chem. Phys.* **2010**, *132*, 244507-244513, doi:10.1063/1.3446804.