Supporting Material

Transmembrane Exchange of Hyperpolarized ^{13}C-Urea in Human Erythrocytes: Sub-Minute Timescale Kinetic Analysis

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SECTION S1

Pictorial details of the heat exchange system

The counter-current heat exchanger was designed to ensure that the temperature of the solution of hyperpolarized solute(s) (^{13}C-urea in the present case) would be the same as that of the cell suspension into which it is injected.

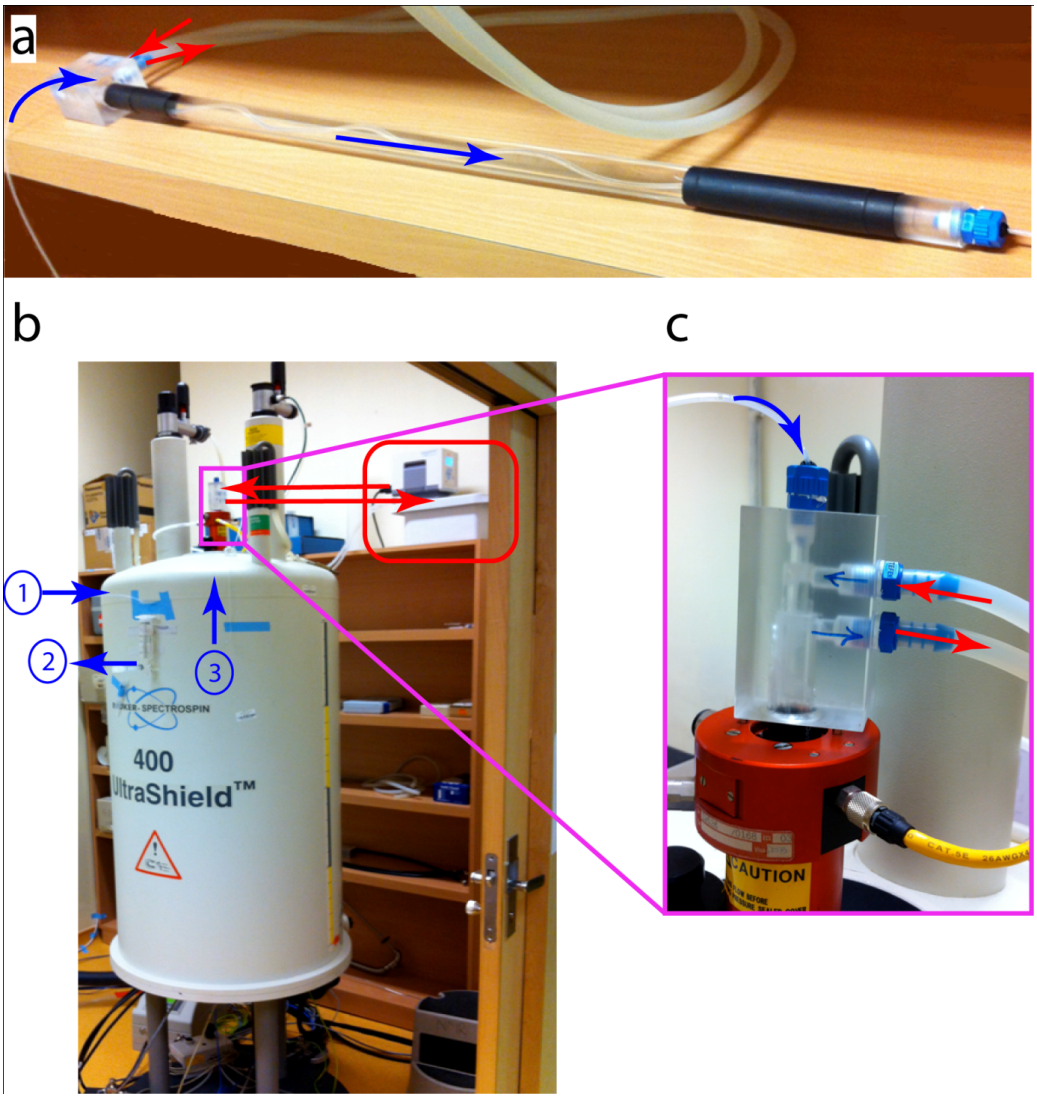

Figure S1: A double-envelope Perspex tube assembly was used to thermostat the solution containing hyperpolarized ^{13}C-urea in the process of injection to the inside of the NMR tube containing a suspension of RBCs that was already thermally equilibrated. The flow directions of the injected solution, and of the water used to thermostat the system, are indicated in blue and red, respectively. (**a**) The double-envelope Perspex tube with a narrow-bore tube inside the inner envelope; and the tubing carrying the water from the thermostating bath. (**b**) The assembly was introduced inside the bore of the magnet, with the flow of hyperpolarized ^{13}C-

urea solution following the sequential numbers: (1) reception of the hyperpolarized ^{13}C-urea solution in the intermediate container after its rapid dissolution; (2) withdrawing the solution, retaining a predetermined amount (typically 2 mL) and then withdrawal the syringe plunger to take 5 mL of air into the barrel to serve as a chaser/mixer following injection of the solution; and (3) injection via the narrow-bore tube to the 10-mm glass sample tube containing the RBCs inside the NMR spectrometer. The water bath used to thermostat the system was located on a shelve next to the magnet. (**c**) A close-up image of the top of the magnet bore.

SECTION S2

1D ^{1}H imaging and evidence of convection in the sample

To investigate if the 3rd peak found by deconvolution in the spectral time courses was due to inadequante mixing of the sample, we conducted imaging studies on the $^{1}H_2O$ in various samples. The imaging pulse sequence entailed a spin echo with a read gradient applied along the *z*-direction ($\mathbf{B}_0$ direction). Note that the coil gradient profile was parabolic and not rectangular.

A problem with mixing a hyperpolarized solution with a dense (high *Ht*) RBC suspension should become evident from variations and irregularities of the ^{1}H imaging profile, compared to an image of a static reference sample. The evolution of the imagining profile after injecting a 'hyperpolarized-like' solution into packed RBCs is shown on Fig. S2. The ^{1}H-image profile changed over the first 60 to 90 s and then settled into a relatively stable parabolic shape. Convection alone will diminish the spin-echo signal intensity as the mixing causes a loss of magnetization-phase coherence in a spin-echo experiment. This phenomenon is independent of whether the solution or suspension of RBCs is chemically mixed or not. In other words, the fact that the integral of the ^{13}C NMR spectrum of ^{13}C-urea in this circumstance remained constant, implied that the amount of the labelled urea in the senstive volume of the NMR probe was constant, even from the earliest of the recorded spectra.

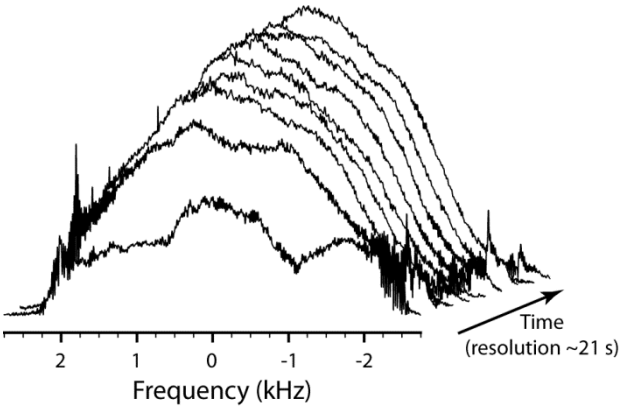

Figure S2: ^{1}H spin-echo based 1D-image time course of $^{1}H_2O$, performed at 37ºC on a suspension of 2 mL of RBCs *(Ht* ~ 0.80) to which 2 mL of 'hyperpolarized-like' solution, heated to 37ºC, was injected. This mimicked the DNP experimental conditions.

To evaluate further whether incomplete sample mixing or simple convection was at play in the early stages of the DNP experiments, we injected isotonic saline containing 0.5% BSA into the same solution. The results are shown in Figs. S3a and b for 25ºC and 37ºC, resepctively. Clearly, at 25ºC that the sample reached flow equilibrium (no convection) in less than 10 s, but a significant amount of convection was evident at the physiological temperature (37ºC). At long times after the injection at 37ºC (not shown; and not at 25^{o}C), the ^{1}H imaging profile was still distorted implying that there were persistent thermal gradients that arose independently of the sample-injection process, leading to convection along the NMR tube.

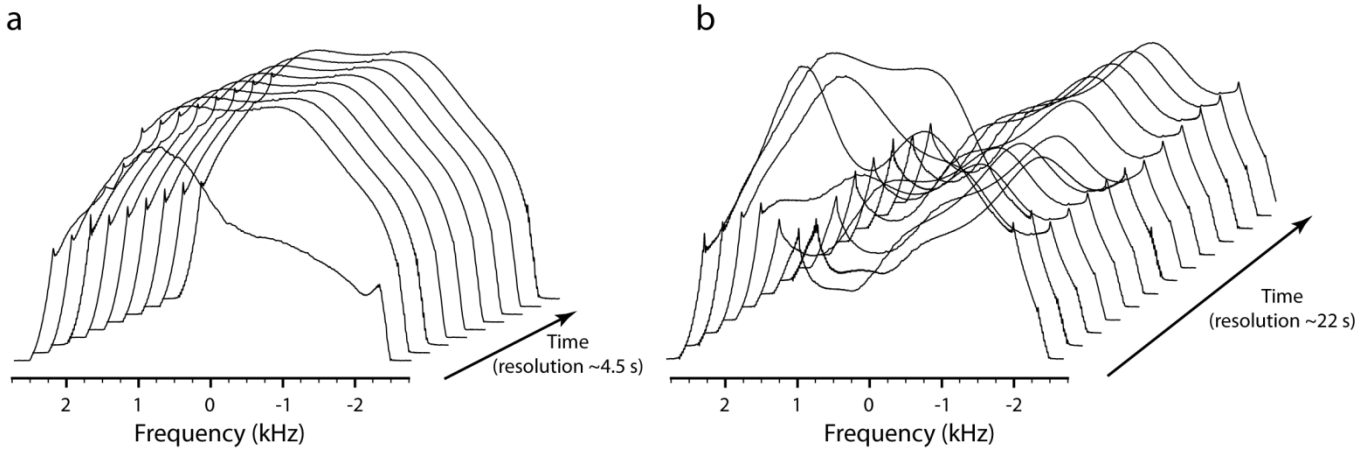

Figure S3: ^{1}H spin-echo based 1D-imaging time course after addition of 2 mL of isotonic saline containing 0.5% BSA into 2 mL of the same solution present inside the NMR magnet at: (**a**) 25ºC; and (**b**) 37ºC. The variations in the 1D profiles at 37ºC were concluded to be due

to thermally-driven convection in addition to that caused by the momentum of the injected solution (that produced streaming) in the process of mixing.

In summary, the ^{1}H imaging profile of solutions free of RBCs evolved over ~60 s after addition of the 'hyperpolarized-like' ^{13}C urea solution. This could have come about from both: (i) heterogeneity in the sample (incomplete mixing); and (ii) macroscopic flow (convection). The latter could arise from streaming (turbulence) due to the momentum of the inflowing liquid, and flow brought about by thermal gradients. This flow, no matter what its origin causes dephasing of magnetization and hence attentuation of the spin-echo signal. We observed a longer time course of evolution at 37^{o}C in the presence of RBCs, which have a greater tendency for coherent streaming (because of the larger 'packet size' of the suspension) than for free solution. Thus we concluded that sample heterogeneity (incomplete mixing of the solvent and solute) had a negligible effect on the ^{1}H imaging profile and that it was due to turbulence brought about by the injection process.

Experimental details

The experimental setup was similar to the one described in the main text. A block-heater was used to pre-warm the added solutions. The pulse sequence was a spin-echo with a *z*-direction magnetic field gradient switched on during the first *t* period (between the 90 and 180º pulse, of duration 46 ms) corresponding to half of the acquisition time, followed by a recovery period of 200 ms. After the 180º RF pulse, the magnetic field gradient was turned on again and the signal acquisition started (acquisition time 92 ms). The gradient intensity was 2% of its maximum available value (~55 G cm^{-1}) leading to an image width of ~5 kHz.

SECTION S3

Hyperpolarized ^{13}C-urea time course after injection into suspensions of RBCs and fittings

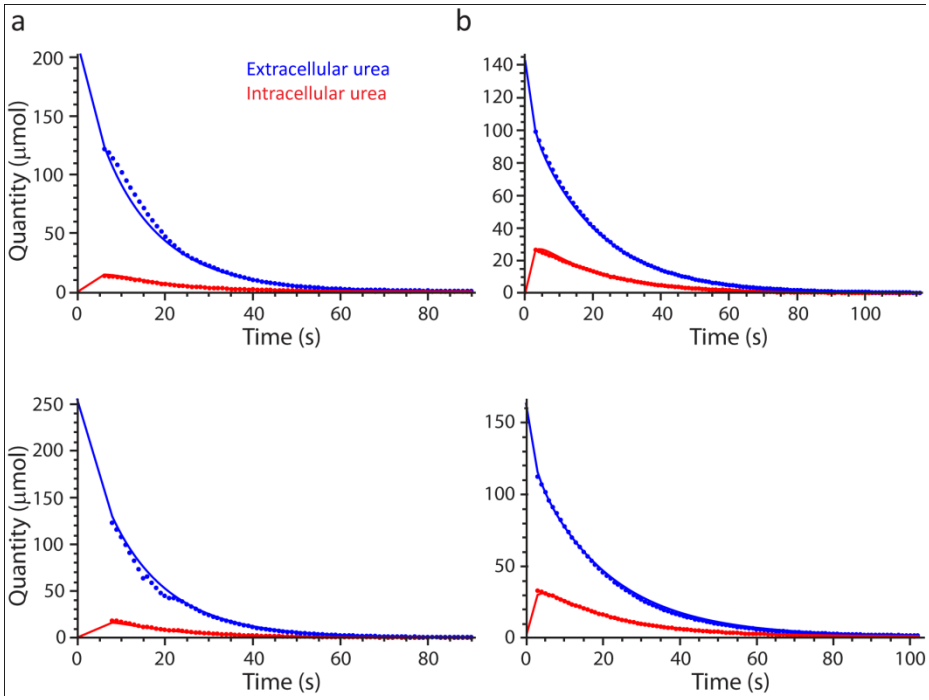

Figure S4: Extra- and intracellular hyperpolarized ^{13}C-urea quantity as a function of time after addition of 2 mL of hyperpolarized ^{13}C-urea to 2 mL of RBCs (data points). The solid lines denote the fit obtained from the averaged values returned by the MCMC data-analysis. Duplicate experiments (top and bottom) were fitted simultaneously. (**a**) 20ºC; and (**b**) 37ºC.

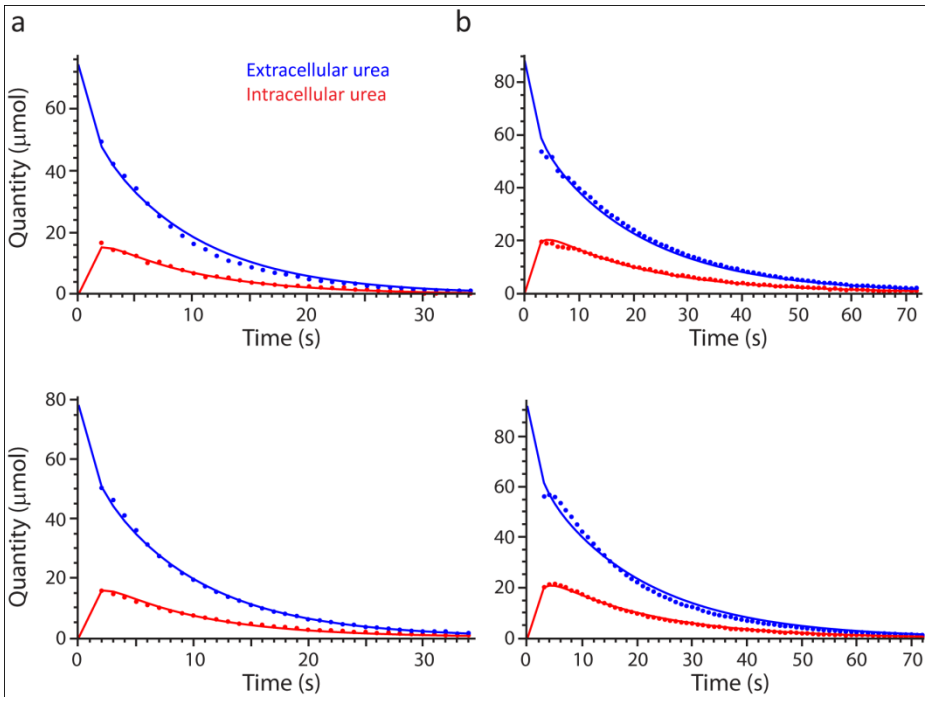

Figure S5: Extra- and intracellular hyperpolarized ^{13}C-urea quantity as a function of time after addition of 1 mL of hyperpolarized ^{13}C-urea to 2 mL of RBCs and 1 mL of buffer (data points). The solid lines dente the fit obtained from the averaged values returned by the MCMC data-analysis procedure. Duplicate experiments (top and bottom) were fitted simultaneously. (**a**) 20ºC; and (**b**) 37ºC.

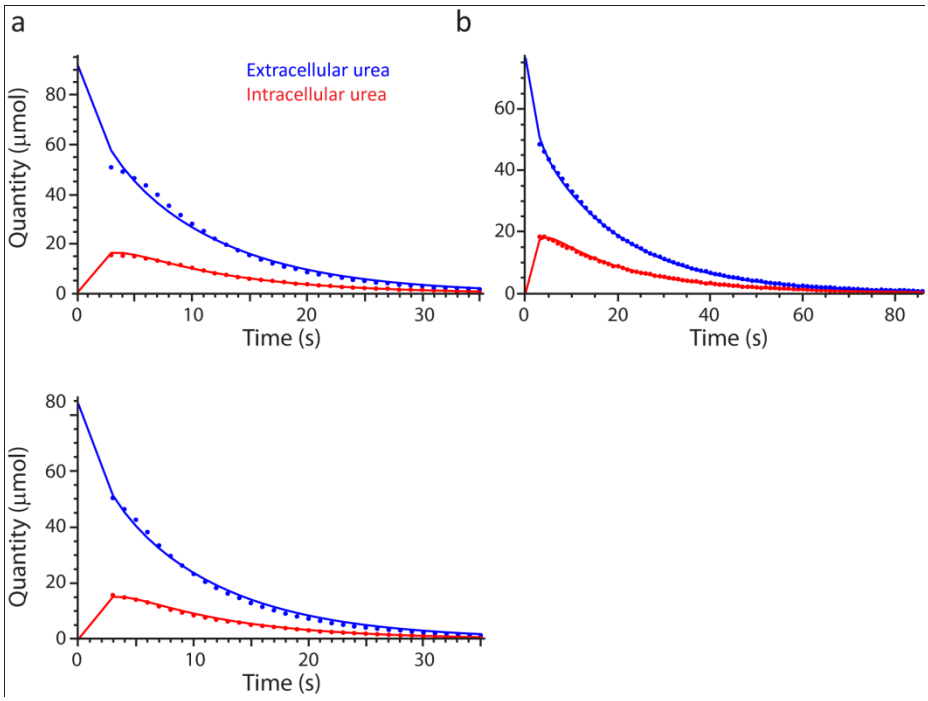

Figure S6: Extra- and intracellular hyperpolarized ^{13}C-urea quantity as a function of time after addition of 1 mL of hyperpolarized ^{13}C-urea to 2 mL of RBCs and 1 mL of buffer containing 40 mM of unlabelled urea (data points). The solid lines denote the fits obtained from the averaged values returned by the MCMC data-analysis procedure. Duplicate experiments (top and bottom) were fitted simultaneously. (**a**) 20ºC; and (**b**) 37ºC.

SECTION S4

Kinetic parameter simulation

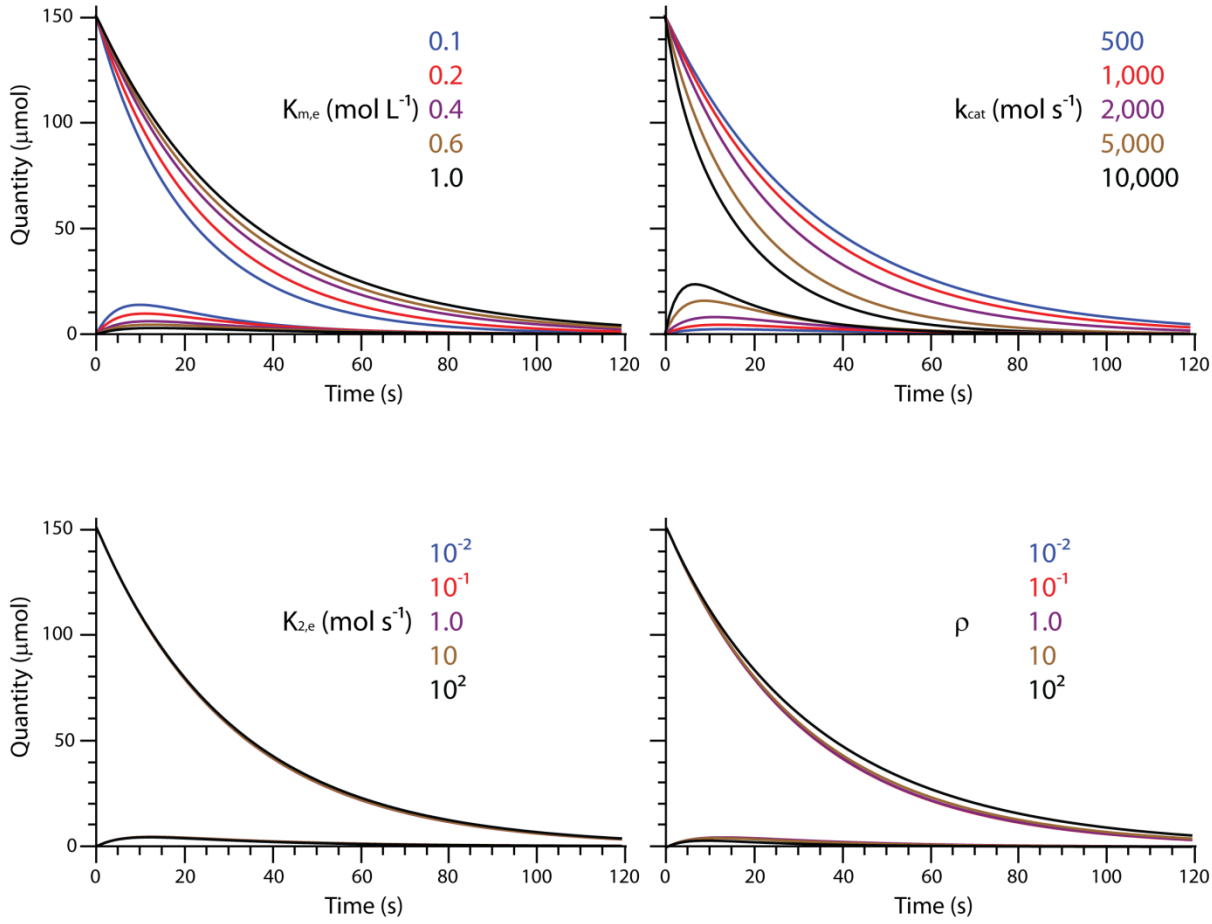

Figure S7: Sensitivities of the responses of a mathematical model of the transport system to changes in each parameter value. The sensitivities were evaluated by simulating signal time courses as the values of individual parameters were varied; one parameter was varied for each graph as indicated in the key in each sub-figure. All other parameters were held constant at the values indicated in Table S1. Changes in the transmembrane enzyme-kinetic parameters, K_{2e} and r had minimal effect on the simulated time courses; and importantly, this was the basis of the decision to reduce the kinetic model to that of a simple product-inhibited Michaelis-Menten system.

Table S1: Parameters and their values used to generate the simulated signal evolution shown in Fig. S5.

$T_{1,e}$ (s)	$T_{1,i}$ (s)	$K_{m,e}$ (mol L^{-1})	$K_{2,e}$ (mol s^{-1})	r	k_{cat} (mol s^{-1})	$Urea_{t=0}$ (mmol)	RF pulse (deg)
43	7	0.6	0.5	0.2	1000	150	4