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Supporting Information

Experimental methods

Materials

Cesium acetate, cesium chloride, cesium hydroxide, [1-^{13}C]acetic acid and ethylene glycol were all purchased from Sigma-Aldrich. Trityl radical OX063 was obtained from GE Healthcare. Gadolinium contrast agent Prohance was obtained from Bracco Imaging.

Synthesis of cesium [1-^{13}C]acetate

Cesium [1-^{13}C]acetate was prepared by dissolving cesium hydroxide in water and titrating to pH 6.5 with 1-^{13}C acetic acid after which the mixture was lyophilized. An NMR investigation of the product showed equimolar amounts of ^{133}Cs and ^{13}C.

DNP sample

A DNP sample stock solution was prepared by dissolving cesium [1-^{13}C]acetate (or unlabeled cesium acetate) (105 mg, 540 µmol) in ethylene glycol (81 mg) yielding 155 µL solution. To this solution was added trityl radical Ox063 and (to some of the samples) gadolinium contrast agent Prohance to yield concentrations 20 mM and 1.5 mM respectively. For the solid state polarization experiments approximately 390 µmol cesium 1-^{13}C-acetate was used. For the dissolution experiments an amount of the DNP sample stock solution corresponding to 85 µmol cesium acetate was hyperpolarized and dissolved in 4 ml solvent (H_2O or D_2O).
A DNP sample stock solution of cesium chloride was prepared by dissolving CsCl (185 mg, 1.1 mmol) in ethylene glycol (210 µL, 233 mg) yielding a solution with density ~ 1.56 (268 µL). To this solution was added trityl radical Ox063 (8.0 mg, 5.6 µmol) and Gadoteridol (0.4 µmol, from a 100 µmol / g solution in water) to make a solution with the following concentrations: [^{133}Cs] 4.1 M, [Ox063] 21 mM, [Gd] 1.5 mM.

DNP and NMR experiments

Hyperpolarization of the samples were performed on a 3.35 T Hypersense polarizer (Oxford Instruments) working at 1.4 K and applying microwaves at 94 GHz.
A pulse angle calibration of ^{133}Cs and ^{13}C was performed after completed polarization buildup by turning off the microwaves and recording pulses once per second. The pulse angle was calculated by assuming negligible effect on the signal decay from the longitudinal relaxation. The pulse angle was calculated to 5 degrees for both ^{133}Cs and ^{13}C.
The solid state longitudinal relaxation time constant (T_1) was measured for ^{133}Cs and ^{13}C by turning off the microwaves after completed polarization buildup and recording the polarization decay by small

pulses (5 degrees) every 10 min (to avoid influence by the pulse). No significant difference in T_1 was detected for ^{133}Cs and ^{13}C. Gadolinium containing samples had slightly shorter T_1 values (Table S.1)

Table S1. Longitudinal relaxation time constants for the different samples

Sample	T_1 (s)
^{13}C	4650
^{13}C, Gd	4000
^{133}Cs	4470
^{133}Cs, Gd	4050

The DNP samples employed in this study (with 20 mM of the trityl radical Ox063) all reaches equilibrium polarization after approximately 1 h. The ^{13}C polarization buildup follows a mono-exponential path with an approximate time constant of 1000 s. The ^{133}Cs polarization buildup is clearly bi-exponential. To record details of the faster part of the ^{133}Cs buildup of polarization it was sampled with pulses (5 degrees) every 30 s (Figure S.1). The differing hyperpolarization buildup paths indicate differences in the DNP mechanism between ^{133}Cs and ^{13}C which, however interesting, this finding is beyond the scope of this study.

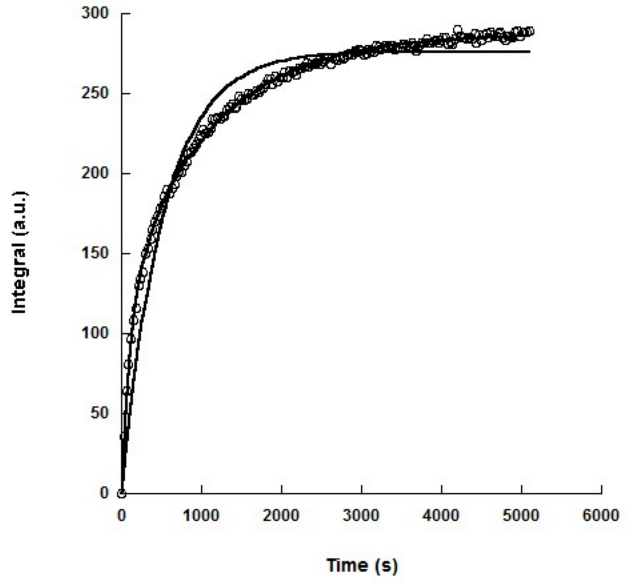

Figure S.1 Buildup of ^{133}Cs signal (20 mM Ox063, no gadolinium added) sampled every 30 s to show bi-exponentiality. The time constants for this buildup were 114 s (fraction of 41 %) and 1070 s (fraction of 59 %). A mono-exponential function is fitted to the data to show the deviation.

All liquid state NMR experiments were performed with a Varian Inova 400 MHz spectrometer fitted with either a 5 mm broad band probe (temperature and ions strength experiments) or a 10 mm broad-band probe (cell experiments).
In the cell uptake experiments a series of 20 degree pulses were acquired every 0.5 s (acq=0.495s, d1=0.005 s) over 30 s. Data was extracted using MNova software (MestreLab Research S.L., Santiago de Compostela, Spain)

Calculation of signal strength

The magnetization of a sample can be calculated from equation S.1:

$$M_0 = \frac{N\gamma^2\hbar^2 I(I+1)}{3k_{\mathrm{B}}\mathrm{T}}B_0 \qquad \text{(eq. S.1)}$$

The signal strength of a sample will however depend also on the induction in the coil, which is proportional to the frequency and hence to the γ of the nucleus. Hence, the signal strengths of a sample with equimolar amounts of ^{133}Cs and ^{13}C will be proportional to $\gamma^3 I(I+1)$, which yields a signal ratio of approximately 2.95,

Calculation of polarization

The absolute polarization (within the high temperature limit $T >> \gamma\hbar B_0/k_{\mathrm{B}}$ of a sample of nuclei with spin I can be calculated according to equation S.2 [1]:

$$P = \frac{\gamma\hbar B_0}{3k\mathrm{T}}(I+1) \qquad \text{(eq. S.2)}$$

The thermal polarization of ^{133}Cs at 9.4 T, 310 K (liquid state experiments) is 12.2 ppm. An enhancement factor of 13000 times thus amounts to approximately 16 % absolute polarization.

In the solid state the thermal signal was recorded for a larger sample comprising 20 mM radical (no gadolinium). After establishing thermal equilibrium (16 h, microwaves turned off, T_1 of sample ~ 4500 s) a train of ten 5 degree pulses were applied after which the microwaves were turned on and the sample was fully hyperpolarized. The solid state enhancement and polarization were then calculated with the thermal signal corrected for the train of pulses. Figure S.2 shows the comparison of a sum of 10 thermal spectra and the fully hyperpolarized spectrum at 3.35 T, 1.4 K. The calculated enhancements were 225 and times amounting to 21 % polarization (without Gd^{3+}) and 54% (with Gd^{3+}), respectively.

According to S.2 the ratio between the ^{133}Cs and ^{13}C polarization should be 1.56 at thermal equilibrium or if an equal spin temperature for the two spins was established. Experimentally, we observed a higher

value of ~ 2.5 (without Gd^{3+}) and of ~ 1.9 (with Gd^{3+}). This discrepancy should be further investigated as it may hold insight to the hyperpolarization mechanism. 133Cs does not have a large quadrupolar moment, and quadrupolar splittings are on the order of a few Hz to some hundred Hz.[2,3] We observe a dipolarly broadened resonance due to solvent proton couplings with a line width of approx.. 4 kHz. We are thus not able to resolve the different quadrupolar transitions in the solid state spectrum. The RF magnetic field strength is only approx. 1 kHz. With the applied short pulse length (20 us, approx. 5 degree) it is expected that all transitions are excited.

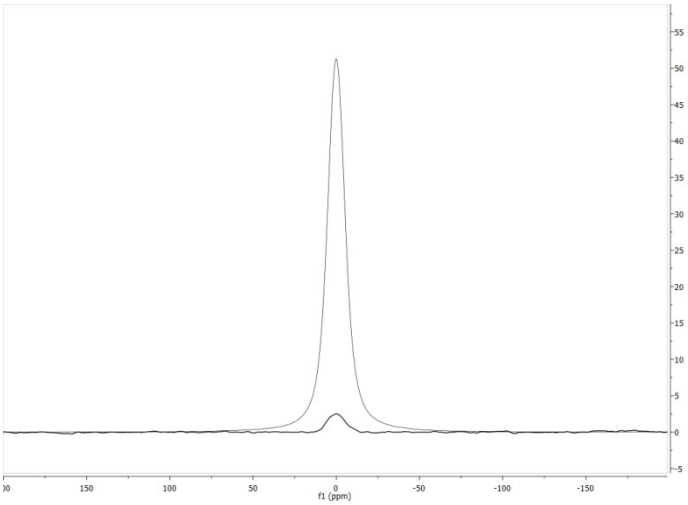

Figure S2. Comparison of the sum of (10) thermal equilibrium spectra with spectrum after full hyperpolarization

Ion strength and temperature experiments

The ion strength of the sample was controlled by addition of sodium chloride to the dissolution solvent. In the temperature experiments the dissolved sample was split in two and the temperature was measured outside of the magnet at the exact time the NMR experiment was started. Cooling towards the set temperature in the NMR spectrometer was calculated using calculation of the exponential rate constant from change in chemical shift and applying Newton's law of cooling.

Membrane impairment experiments

Yeast cell protocol

Yeast strain BY4743 was grown from colonies in an overnight culture in YPD (sigma Y1375). The over night culture was diluted into 50 mL YPD to an OD of 0.17-0.22 in a 500 mL flask and placed at 30 °C and shaking at 200 rpm. 5-7 hours later the yeast cells were harvested at OD 1 (exponential phase). Culture corresponding to a total of OD 50 was used in all experiments.

OD 50 yeast cultures were spun down, pelleted and washed twice in 20 ml water, where after it was pelleted and resuspended in a total of 0.5 mL water.

Electroporation

The 0.5 mL yeast cell suspension was electroporated in batches of 250 uL using standard protocol for yeast culture.

Uptake experiments with hyperpolarized 133CsCl in yeast cells followed by NMR
Yeast cell suspension (either electroporated or not were transferred to a 10 mm NMR tube equipped with a susceptibility plug and inserted into a 9.4T magnet calibrated to 303 K.

6 µmol, 60 µmol and 300 µmol hyperpolarised ^{133}CsCl from preparation described above was dissolved in 4 ml water. 1 mL of these solutions were injected into the 10 mm NMR tube containing 0.5 mL yeast suspension resulting in Cs concentrations of 1 mM, 10 mM and 50 mM, respectively.

A series of 1D 20 degree pulses were acquired every 0.5 s (acq=0.495 s, d1=0.005 s) over 30 s.

References

1. Stupic, K. F., Cleveland, Z. I., Pavlovskaya, G. E. & Meersmann, T. Hyperpolarized 131Xe NMR spectroscopy. *J. Magn. Reson.* **208,** 58–69 (2011).
2. Einarson, L., Kowalewski, J., Nordenskjöld, L. and Rupprecht. 133Cs NMR of oriented CsDNA and Multiple-Quantum spectroscopy of I=7/2. J. Magn. Reson. 85, 288-234 (1989)
3. Kuchel, P. W., Chapman, B. E., Muller, N., Bubb, W. A., Philp, D. J., and Torres, A. M. Apparatus for rapid adjustment of the degree of alignment of NMR samples in aqueous media: Verification with residual quadrupolar splittings in ^{23}Na and ^{133}Cs spectra.