SUPPORTING INFORMATION

Hyperpolarized ^{89}Y Complexes as pH sensitive NMR Probes

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Synthesis of yttrium complexes. The yttrium complex of DOTP was prepared as previously described.[1] The ligand DO3A-NTs was synthesized as reported.[2] The yttrium complex was prepared by reacting DO3A-NTs and freshly prepared yttrium hydroxide (50% excess) for several days while maintaining the pH of the reaction mixture around 6. The pH was then raised to around 8.5 and the excess yttrium hydroxide was filtered off. Approximately 5% excess ligand was added to the mixture to ensure full complex formation and the pH was adjusted to 7.5. The solution was freeze-dried to give the final complex. The yttrium content of the final product was 10.3% (by ICP-MS). ^{89}Y NMR, H_2O-D_2O, pH 7.5, δ, ppm: 156 (s). MS-ESI (negative), *m/z*: found, 628.20 (100%), calcd. for $[M-H]^-$, 628.11.

Acquisition of NMR spectra. NMR data were collected at a field strength of 9.4T using a Varian VNMRS Direct Drive console. Hyperpolarized ^{89}Y and ^{31}P and thermally polarized ^{1}H NMR data were collected on an Oxford unshielded 89 mm widebore magnet, and thermally polarized ^{31}P NMR data was collected using a Varian premium shielded 54 mm narrow bore magnet. A 10mm MR Resources low gamma probe was used to acquire hyperpolarized ^{89}Y and ^{31}P data, while a Varian 5mm Auto-Switchable 4-Nucleus probe and a Varian 5 mm AutoX Dual Broadband probe were used to collect thermally polarized ^{1}H and ^{31}P data, respectively. The ^{89}Y, ^{31}P and ^{1}H spectra were referenced to YCl_3 at 0 ppm, phosphoric acid at 0 ppm, and *tert*-butanol at 1.2 ppm, respectively. With the exception of T_1 experiments, all free induction decays were acquired using a 90° hard pulse in the case of both hyperpolarized and thermally polarized experiments.

Hyperpolarization experiments. General remarks and sample preparation. For hyperpolarization experiments, samples were polarized in an Oxford DNP Hypersense at 1.4K in a 3.35 T field, subject to 94.125 GHz of continuous microwave irradiation at 100 mW power. 160 μL samples composed of 15 mM OX63 (tris{8-carboxyl-2,2,6,6-tetra[2-(1-hydroxyethyl)]-benzo(1,2-d:4,5-d')bis(1,3)dithiole-4-yl}methyl sodium salt) trityl radical in a H_2O-glycercol (75:25) mixture with either 176 mM YDOTP or 143 mM YDO3A-NTs were used. During the final dissolution step, the frozen sample was dissolved into 4 mL of boiling water.

We have been able to consistently achieve improved polarization levels as compared to previously reported figures by pre-freezing the samples outside of the Hypersense, prior to placing them into the cryostat for irradiation. This was done by pre-cooling the DNP cup in a bath of liquid nitrogen, and then pipetting the 160 μL of solution directly into the cup. Through empirical observation of the transparency and color of the frozen solid, we have been able to judge if a proper glass was formed. Glassing is necessary to achieve optimum polarization levels. Although we do not currently have an analytical method to verify if a proper glass has indeed formed, we have through trial and error been able to consistently produce higher levels of polarization, as compared to figures reported before. We have found that if the solid formed is extremely transparent and changes from a dark green color (due to the OX63 radical) to a light green, then adequate glassing is achieved.

T_1 measurements using hyperpolarized samples. The hyperpolarzied ^{89}Y spectra were acquired using a 20° hard pulse and repetition time of 20 seconds. The T_1 values were obtained by fitting the signal intensity (normalized) as a function of time via the equation [1,3]:

$$I(t) = M_p \cos^{\frac{t}{TR}} \theta \sin\theta e^{-\left(\frac{t}{T_1}\right)}$$

where M_p is the longitudinal magnetization, TR is the recycling time, and θ is the flip angle.

We were unable to quantitatively evaluate the T_1 of YDO3A-NTs at either high or low pH (where 89Y signals are detected) because the hyperpolarized signal decayed much too rapidly. One can however estimate that the T_1 YDO3A-NTs is < ~60 s based on the observation that the magnetization was completely undetectable one minute after dissolution.

Collecting the ^{89}Y chemical shift - pH dataset for YDOTP. The relatively long T_1 of YDOTP provided a significant advantage in hyperpolarized ^{89}Y data acquisition - the entire data set was acquired from a single batch of hyperpolarized solution. Of the 4 mL of hyperpolarized YDOTP solution that was ejected from the Hypersense hyperpolarizer, 3.6 mL was divided evenly into six 10 mm NMR tubes, each of which contained a previously prepared 400 μL 1M buffer solution of pH 4, 5, 6, 7, 8, and 9 (acetate, acetate, MES, MOPS, TRIS, Bis-TRIS, respectively). This 1 mL mixture was then thoroughly shaken to ensure proper mixing. The final concentration of YDOTP was about 4mM. Each tube was then placed in the magnet, one at a time, and a 90° hard pulse was administered. Upon completion of the experiment, the pH in each tube was measured with a pH meter and the results were within 1% of the original buffer value.

Thermally polarized ^{31}P NMR experiments with YDOTP. Thermally polarized ^{31}P experiments were conducted using a 37 mM sample of YDOTP in H_2O-D_2O (85:15) in a 5 mm NMR tube. A 90° hard pulse was administered, and only one transient per pH value was collected, as the signal to noise was sufficient. pH was varied using NaOH or HCl solution. The ^{31}P chemical shift dispersion of YDOTP as a function of pH is shown in **Figure S1**.

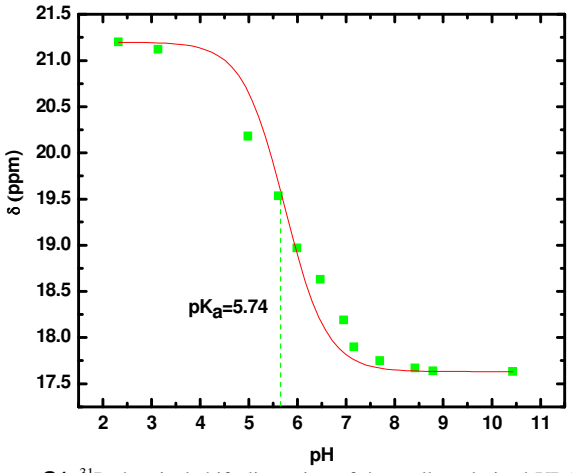

Figure S1. ^{31}P chemical shift dispersion of thermally polarized YDOTP measured at 9.4T and 25°C, as a function of pH. The ^{31}P chemical shifts are referenced to phosphoric acid (0 ppm).

Collecting the ^{89}Y chemical shift - pH dataset for YDO3A-NTs. In direct contrast to YDOTP, hyperpolarized YDO3A-NTs data was collected from multiple experiments as the T_1 values obtained were significantly shorter. Of the 4 mL of hyperpolarized YDO3A-NTs solution that was ejected from the Hypersense, 3 mL was evenly divided into three 10 mm NMR tubes, each of which contained a previously prepared 500 µL buffer solution of different pH – either 4, 5, 6, 7, 8, or 9. This 1.5 mL mixture was then thoroughly shaken to ensure proper mixing. Each tube was then placed in the magnet, one at a time, and a 90° hard pulse was administered. We were generally only able to obtain data for two different pH values, as the hyperpolarized signal had reached a thermal Boltzmann equilibrium by then. This process took approximately a minute, and hence we conclude that the T_1 must be below a minute. Upon completion of the experiment, the pH in each tube was measured and the results were within 1% of the original buffer value.

Variable pH ^{1}H NMR experiments on YDO3A-NTs. As described in the main text of the paper, no hyperpolarized YDO3A-NTs signal was observed for the biologically relevant pH

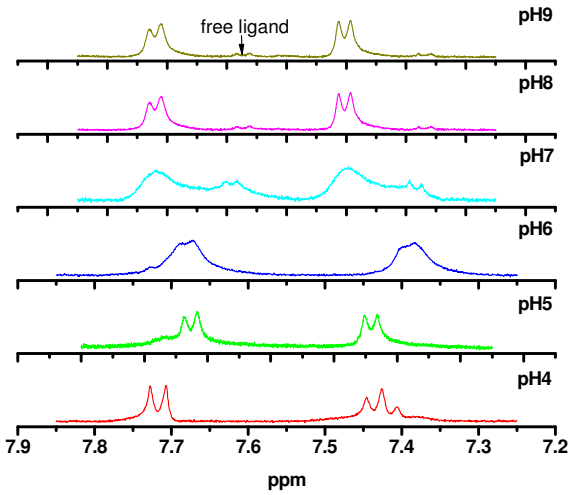

Figure S2. ^{1}H spectra of thermally polarized YDO3A-NTS acquired at 9.4T and 25°C, as a function of pH. The spectra are referenced to *tert*-butanol internal standard (1.2 ppm).

range between 5 and 7; hence, thermally polarized ^{1}H NMR experiments of the compound were conducted at several pH values to study exchange processes that may occur over in this pH range. A 2 mL solution of 51.6 mM YDO3A-NTs in H_2O-D_2O (85:15) was prepared. The pH of the solution was adjusted to 4, 5, 6, 7, 8, and 9 using NaOH and HCl and a pH meter. 200 µL samples were then taken and placed in a 3mm NMR tube at each respective pH value. 20 µL of 5% tert-butanol was added to each NMR tube to provide a pH invariant internal reference (at 1.2 ppm) The 1H NMR spectra at each pH were acquired with a repetition time of 20 seconds to ensure full relaxation (**Figure S2**). The doublets visible at approximately 7.63 and 7.37 ppm are due to excess free ligand, as verified in a separate experiment. Interestingly, the ^{1}H NMR spectrum at pH 4 shows the formation of a protonated species, which may explain the unexpected drop in the ^{89}Y chemical shift of YDO3A-NTs going from pH 5 to pH 4.

Determination of pK_a values from the pH dependence of the ^{89}Y-chemical shift. The pK_a values were obtained by fitting the ^{89}Y chemical shift – pH datasets to the following equation:[5]

$$\delta_{obs} = \frac{\delta_{low} + 10^{pK_a - pH}\,\delta_{high}}{1 + 10^{pK_a - pH}}$$

Here, δ_{obs} represents the observed chemical shift in ppm, pH is the actual pH value, and δ_{low} and δ_{high} are the chemical shifts at the lowest and highest pH (bounding the curve), respectively.

Hyperpolarized ^{31}P experiments with YDOTP. The ^{31}P hyperpolarized spectrum of YDOTP was also acquired to measure ^{31}P enhancement with respect to Boltzmann equilibrium. The dominant mechanism of DNP in the presence of trityl radicals is thermal mixing.[1] Under these DNP conditions all NMR active nuclei in the sample reach a common spin temperature (T) and the polarization (P_i) for each nuclei can be expressed as

$$P_i = tanh\left(\frac{\hbar\gamma_i B_o}{2kT}\right)$$

so the ^{31}P enhancements should theoretically be higher than those obtained for ^{89}Y.[4] With our current instrumentation setup, we could not measure the time-dependent ^{89}Y and ^{31}P polarization build-up curves in the frozen sample, but the measured ^{31}P signal enhancement for YDOTP after dissolution was significantly lower (650-fold compared to thermal equilibrium at 25°C) than that for ^{89}Y (3000-fold). This is likely due to the much more rapid decay in hyperpolarized magnetization (shorter T_1) of ^{31}P compared to ^{89}Y during sample dissolution and transfer.

T_1 measurements of hyperpolarized YDOTP in human blood serum. In order to estimate the T_1 *in vivo*, we measured the T_1 of hyperpolarized YDOTP in human blood serum (**Figure S3**). Of the 4 mL of hyperpolarized YDOTP solution that was ejected from the Hypersense hyperpolarizer, 700 µL was extracted and pipetted into a previously prepared 10mm NMR tube filled with 300 µL of the serum. No buffer was added to this solution so as to preserve the natural pH of the serum. This 1 mL mixture was then thoroughly shaken to ensure proper mixing. The final concentration of YDOTP was about 4mM. T_1 measurements were conducted using the same parameters described previously (20° hard pulse and TR = 20 sec). A T_1 of 75 seconds at pH 7.8 was obtained from the fitting of the data. The T_1 of hyperpolarized YDOTP at pH 8 was found to be 94 seconds, which amounts to only a 20% decrease in serum.

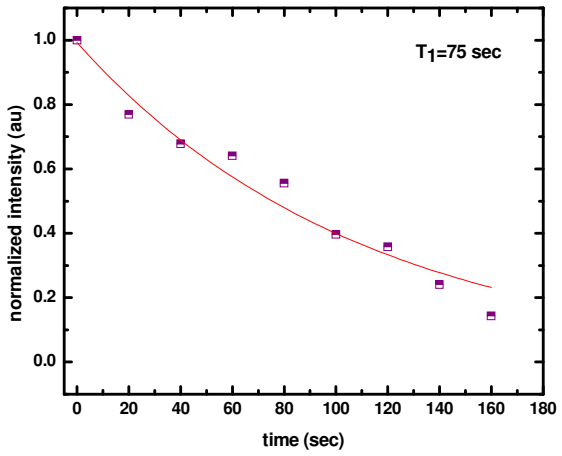

Figure S3: T_1 decay curve of hyperpolarized YDOTP in human blood serum of pH 7.8, measured at 9.4T and 25°C.

The feasibility of hyperpolarized ^{89}Y-complexes as *in vivo* MR spectroscopy an dimaging probes. The receptivity of an NMR nucleus is defined as:

$$R = \frac{\gamma^3}{I(I+1)} \cdot (\textit{natural abundance})$$

where γ is the gyromagnetic ratio and I is the nuclear spin quantum number. Under thermally polarized conditions, the NMR receptivity of ^{89}Y is approximately 1.2 x 10^{-4} that of ^{1}H. However, the 3,000 fold enhancement with respect to thermal Boltzmann equilibrium for hyperpolarized YDOTP essentially makes the receptivity of hyperpolarized ^{89}Y 0.375 that of ^{1}H. A signal enhancement of 10,000 would effectively render the receptivity of ^{89}Y the same as that of ^{1}H. Apart from potentially even higher signal enhancements, one of the practical advantages of hyperpolarized ^{89}Y imaging is essentially the complete elimination of any background ^{89}Y signal, as the final concentrations we have used (after dissolution into 4mL of water and then into 400 μL of buffer) are approximately 4 mM for YDOTP and 3.2 mM for YDO3A-NTs. Under thermally polarized conditions, this concentration is far too low for single shot acquisition and would require a tremendous amount of time averaging to produce any discernable signal. The SNR that we have currently been able to obtain using only a 4 mM concentration of hyperpolarized YDOTP in a 1 mL volume at pH 7, subjected to a train of 20° hard pulses with a repetition time of 20 seconds (as applied for T_1 measurements), has been as high as 37 for the first pulse and is still easily discernable even fifteen pulses later, with an SNR of 6. The linewidth that we have measured for hyperpolarized YDOTP in blood serum was approximately 20 Hz, with a pH dispersion of 0.29 pH unit / ppm and a resolution of 0.06 pH unit in the physiologically relevant pH range between 6 and 8 at 9.4T. The pH dispersion of 3-aminopropyl phosphonate, a ^{31}P MRS probe used to measure extracellular pH, is approximately 0.73 pH unit / ppm between pH 6 and 8.[6] The ^{31}P *in vivo* linewidth for this compound is approximately 0.2 ppm (9.4T) Thus, the resolution (assuming that peaks can be distinguished 20% of the linewidth apart) is approximately 0.03pH unit. At lower field strengths, the resolution would be obviously be less, as the resolution, or ability to distinguish between two chemical shifts, is directly proportional to the magnetic field B_0.

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