Supporting Information to:

Hyperpolarized Water to Study Protein-Ligand Interactions

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Preparation of hyperpolarized water.

The sample consisted of a 1:1 v:v mixture of deuterated glycerol and H_2O with 25 mM TEMPOL as polarizing agent. This mixture easily formed a transparent and homogeneous glass upon freezing in liquid nitrogen with good DNP properties. 200 µL of this mixture were transferred to a Vespel sample holder, which was placed in a home-built polarizer at 6.7 T, initially at 4 K, later lowered to 1.1 K by reducing the pressure to ca. 70 Pa. Irradiation by frequency-modulated microwaves with the carrier at 187.8 GHz (power at the source output 100 mW, modulation frequency 10 kHz, modulation amplitude 50 MHz) lead to a build-up of the proton polarization to $P(^1H) > 30\%$ after 5 minutes. The sample was subsequently dissolved by injecting 5 mL of D_2O with or without 100 mM ascorbate at 450 K and 1 MPa and transferred to the 500 MHz NMR spectrometer within 4.5 s through a 1.5 mm id PTFE tube, using gaseous helium at a pressure of 0.6 MPa. During most of the transfer, the sample is exposed to a magnetic field above 0.9 T in a magnetic tunnel made of a Halbach-like array of permanent magnets.

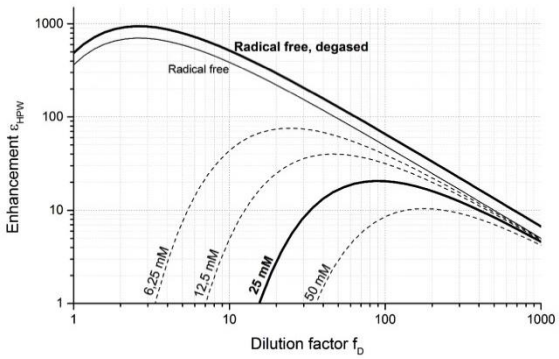

Figure S1. Predicted magnetization enhancement of the hyperpolarized water as function of the dilution factor and concentration of radicals. Predictions are shown for different initial concentrations of TEMPOL in the DNP sample containing 100 µL of frozen water, subsequently diluted in D_2O during the dissolution process with a dilution factor f_D. For information, the same curve but with a degassed and non-degassed radical-free solution is also presented. The relaxation rates R_1 of water were extrapolated from experimental measurements of several solutions with different H_2O/D_2O ratios and different concentrations of TEMPOL. The initial polarization in the solid state was assumed to be $P(^1H) = 0.38$, the time of transfer 10 s, and the thermal polarization of protons at 500 MHz and 300 K $P(^1H) = 4.0\ 10^{-5}$. In practice only dilution factors above 20 are accessible.

During the transfer time from the polarizer to the NMR magnet, water polarization relaxes towards the Boltzmann equilibrium state. The relaxation rate is enhanced in the presence of radicals from the hyperpolarization solution, *i.e.* TEMPOL, and of triplet O_2 dissolved in the water. Ascorbate can be used to reduce TEMPOL[1], if ascorbate does not damage the protein, and O_2 can be removed by degassing of the water. Apart from minimizing the concentration of radicals, the relaxation rate of water can be modulated by the transfer time and the magnetic field profile during the transfer. Additionally, the relaxation rate can be reduced by dilution with D_2O, although this also reduces the proton magnetization injected into the sample. The relaxation properties of water in various mixtures were studied to model relaxation during transfer and to optimize the sample for the DNP-water-LOGSY (Fig. S1). Theoretically, the highest amount of resulting water polarization in the NMR sample can be obtained by removing all radicals after dissolution, by transferring the hyperpolarized water through a magnetic tunnel and by dissolving it in D_2O in a 1:3 ratio. In practice, however, at least a 10-fold excess of superheated D_2O is needed in our setup to dissolve the frozen hyperpolarized H_2O. In the absence of ascorbate, the H_2O should be diluted at least 70-fold with D_2O for optimal results. With these parameters a maximum enhancement of the water magnetization to $P(^1H) \approx 2\ \%$ was achieved.

Adaptation of the Water-LOGSY experiment to D-DNP.

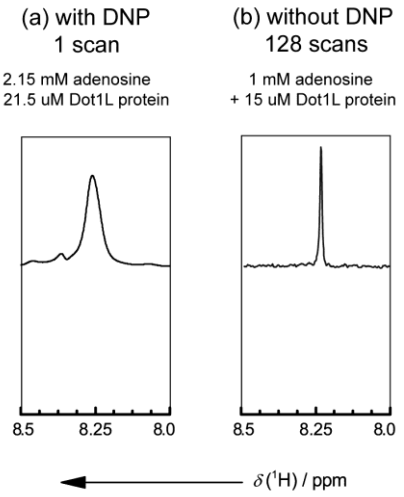

Figure S2. Water-LOGSY signal measured (a) with DNP in a signal scan, and (b) without DNP in 128 scans (20 minutes).

After the transfer, 450 µL of hyperpolarized water was injected into a 5 mm NMR tube waiting in a 500 MHz Bruker spectrometer containing 250 µL of a solution of 6 mM adenosine with or without 60 µM of the protein Dot1L (in 40% H_2O, 60% D_2O, 25 mM deuterated TRIS buffer, 200 mM NaCl, pH ≈ 8), resulting in final adenosine and Dot1L concentrations of 2.15 mM and 21.5 µM respectively. The acquisition was triggered by the injection, and free induction decays were observed at 1 s intervals. Odd scans used selective pulses to excite signals of both ligand and protein while the solvent signal was suppressed. Even scans used hard pulses with 0.01° nutation angles to monitor the water magnetization. Figure S2 shows Water-LOGSY signals measured with DNP, and a reference spectrum without DNP for comparison, obtained using PO-Water-LOGSY[2]. Conventional Water-LOGSY experiments are well documented in the literature[2-7]. The experiment usually begins with an interval where the ligand magnetization is saturated, followed by a mixing time to allow the water magnetization to be transferred by exchange and NOE to the ligands, either directly or indirectly via a protein, and finally by the excitation and acquisition of the signal of the ligands of interest. In the DNP-Water-LOGSY experiment, the initial saturation step was simply replaced by the injection of hyperpolarized water into a solution of ligand(s) and protein waiting in the NMR spectrometer at thermodynamical equilibrium. The ligand signals were then excited by means of selective pulses (PC9 and Rsnob in our case)[8-9]. These pulses must be optimized to prevent any perturbation of the water magnetization, so as to preserve it for subsequent scans and to avoid RD effects. In order to obtain the entire ^1H spectrum, two concatenated spin echo sequences were applied upfield and downfield of the water signal. This allows one to collect several scans where the magnetization is transferred from water to solute, until the water magnetization has fully relaxed and returned to its thermodynamical equilibrium.

Preparation of the protein Dot1L.

Residues 2–332 of human Dot1L were cloned into a modified pET-15b (Novagen) plasmid with kanamycin resistance containing an N-terminal His_6-tag followed by a HRV-3C cleavage site. *E.coli* BL21 star cells (LifeTechnologies) were use as expression host. The protein was expressed using auto-inducing media based on 2-fold terrific broth in 1.5 L fermentation volume. The protein was purified in a buffer containing 50 mM Tris pH 8.0, 200 mM NaCl, 10 mM imidazole and 10% glycerol by Ni-capture (Ni-NTA, GE), elution with 250 mM imidazole, buffer exchange to imidazole-free buffer, tag cleavage overnight with HRV-3C protease and subsequent removal of the His_6-tag on another Ni column. Bound SAM was replaced by an excess of SAH, and the complex was treated with MTAN to hydrolyze SAH under dialysis against 20 mM d-Tris pH 8.0, 200 mM NaCl, 1 mM d-TCEP, 1 mM EDTA. Finally, the protein was purified using a size exclusion column (superdex 75, GE) and concentrated in NMR buffer (20 mM d-Tris pH 8.0, 200 mM NaCl, 1 mM d-TCEP, 1 mM EDTA) to 72 µM. Concentration was determined with HPLC-UV215 (Agilent).

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