Supporting Information for

Amplification of Nuclear Overhauser Effect Signals by Hyperpolarization for Screening of Ligand Binding to Immobilized Target Proteins

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1. Determination of the binding capacity of the bead solution

The binding capacity of the concentrated avidin coated bead solution was estimated following a similar procedure described in ref. 1. It was assumed that the bead solution has the same binding capacity for HABA as for the strong ligand biotin. The binding capacity for biotin was determined based on the measurement of the amount of biotin left when removing the biotin-bound bead after adding a certain amount of biotin. 10 μL of 40 μM biotin solution was added to 30 μL of the bead solution, followed by incubation for 5 min and centrifugation for 5 min. The concentration of biotin left in the supernatant was quantified using the HABA/avidin colorimetric assay.[2] The supernatant was added to the HABA-avidin solution, causing the displacement of HABA from the complex. The decrease of absorbance of the HABA-avidin complex at 500 nm (A_{500}) was measured (Figure S2). A control experiment was performed by replacing the supernatant with 10 μM biotin solution. The binding capacity of the bead stock solution was determined as 8.9 μM.

The manufacturer reported binding capacity is 0.05 nmole biotin per milligram of 0.5 % w/v bead suspension in PBS buffer, which corresponds to a bead concentration of 0.25 μM. The OD_{600} values for the original solution and the concentrated bead solution were measured as 4.13 and 162, respectively. Therefore, the binding capacity of the concentrated bead solution, according to the manufacturer, is 9.8 μM, which is within 10 % of the value determined above.

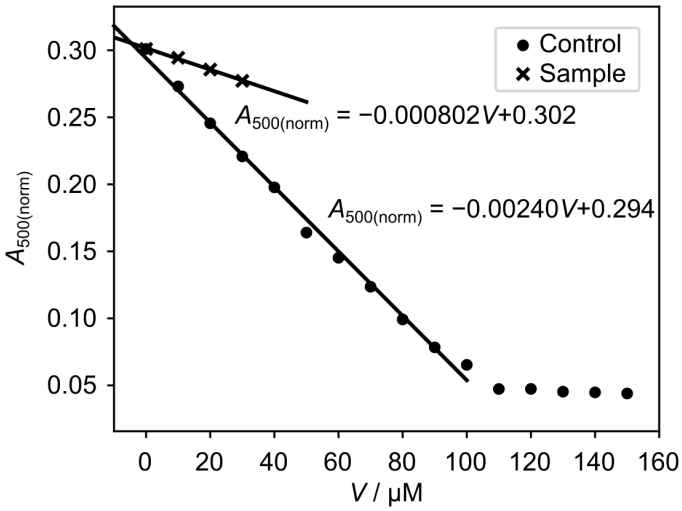

Figure S1. Decrease in absorbance of the HABA-avidin complex at 500 nm (A_{500}) when titrating 150 μL (V_i) HABA-avidin solution with the supernatant from the bead solution and the control biotin solution (10 μM), respectively. V_a represents the volume of the sample/control solution added. The absorbances were normalized by the total sample volumes, as $A_{500(norm)} = A_{500} \times (V_a + V_i)/V_i$. The biotin concentration in the sample supernatant was determined as 3.3 μM.

2. Determination of the cross-relaxation rates from conventional NOESY

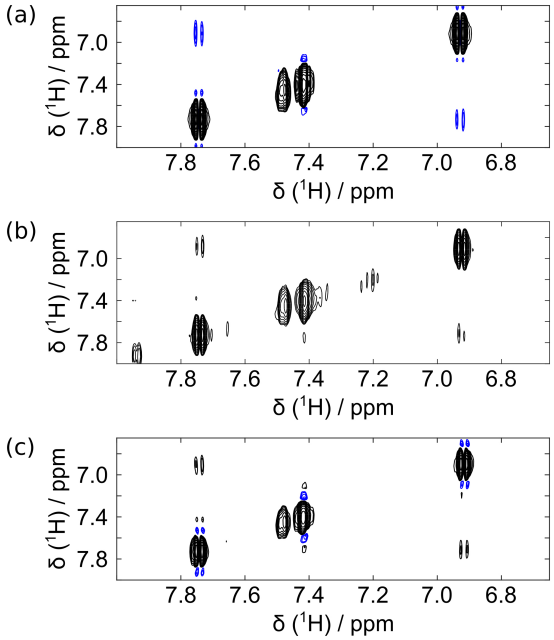

Figure S2. ^{1}H-^{1}H NOESY spectrum of a) 100 μM HABA showing positive NOE b) 100 μM HABA with avidin in solution (20 μM binding site) showing negative NOE c) 100 μM HABA with avidin immobilized on polystyrene beads (2.25 μM binding site) showing negative NOE. The NOE mixing time was 500 ms, and 128×4096 complex points were collected.

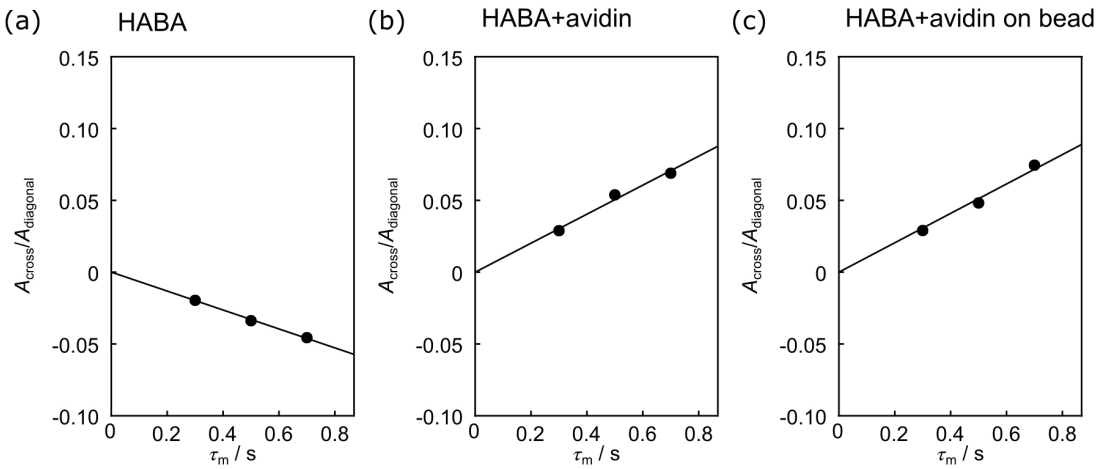

Figure S3. NOE build-up curves in the initial regime measured from ^{1}H-^{1}H NOESY spectra with mixing times of 300 ms, 500 ms, and 700 ms. A_{cross} represents the average integral of the cross peaks between two HABA proton spins, H_a and H_b, while $A_{diagonal}$ is the average integral of the two diagonal peaks for spin H_a and spin H_b. Cross-relaxation rates are obtained by a linear fit of $A_{cross}/A_{diagonal}(\tau_{mix})$.[3] Sample conditions and corresponding fitted cross-relaxation rates are a) 100 μM HABA, σ = 0.066 s^{-1}; b) 100 μM, HABA with avidin (20 μM binding site), $\sigma = -0.101$ s^{-1}; c) 100 μM HABA with avidin immobilized on polystyrene beads (2.25 μM binding site), $\sigma = -0.102$ s^{-1}.

3. Determination of the dissociation constant for ligand HABA binding to protein avidin

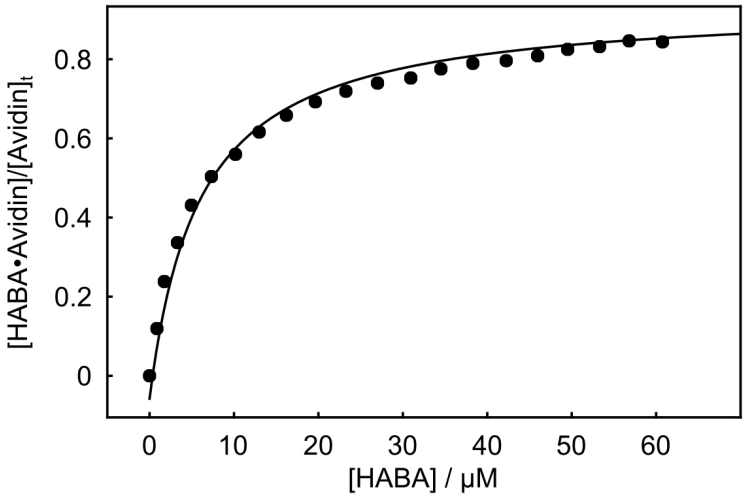

Figure S4. Titration of avidin with HABA. The affinity was determined by measuring the absorbance at 500 nm using UV/Vis spectrophotometry. A molar extinction coefficient of 35500 $cm^{-1}M^{-1}$ for the HABA-avidin complex[4] was used for calculating the complex concentration [HABA•Avidin]. The fraction of bound protein was fitted with the equation $[HABA•Avidin]/[Avidin]_t = [HABA]/([HABA]+K_d)$. The dissociation constant K_d was determined as 5.9 ± 0.6 μM, in agreement with the previously reported value of 6 μM.[5]

4. Simulation of field dependence of the cross-relaxation rates

The degree of deviation in the cross-relaxation rates caused by different field strengths can be estimated using the following equations, which take into account only the dipolar relaxation in a two-spin system[6]

$$\sigma = \left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_{\mathrm{H}}^4 \hbar^2}{10} \frac{1}{r^6} [(-J(0) + 6J(2\omega)] \tag{S1}$$

$$J(\omega) = \frac{\tau_{\mathrm{c}}}{1 + (\omega\tau_{\mathrm{c}})^2} \tag{S2}$$

γ_{H} is the gyromagnetic ratio of the proton, and r is the spin-spin distance. σ values at two different field strengths corresponding to ^{1}H frequencies (ω) of $2\pi \times 400$ MHz and $2\pi \times 500$ MHz were simulated with the molecular correlation time (τ_c) changing from 0.01 ns to 10 ns (Figure S5).

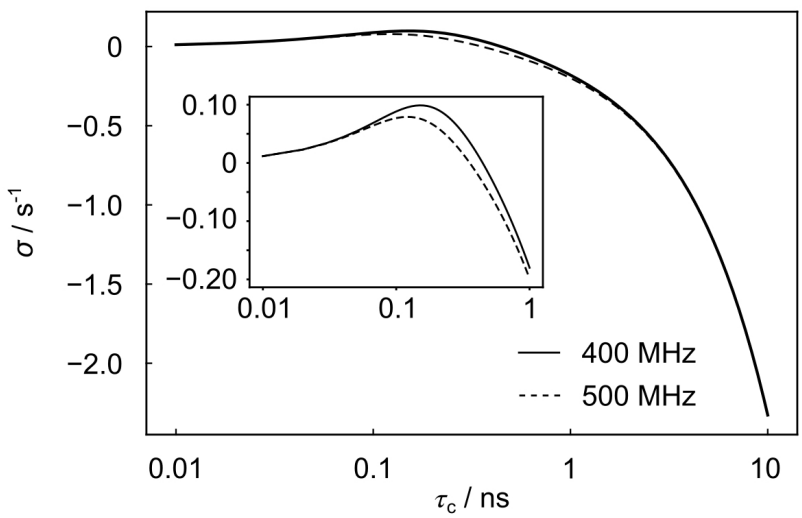

Figure S5. Calculated cross-relaxation rate for a ^{1}H-^{1}H dipolar spin system at 400 MHz and 500 MHz. Eq. (S1) and Eq. (S2) were used to perform the calculations with r = 2.5 Å.

5. Experimental parameters and fitted results of the DNP trNOE experiments

Table S1. Experimental parameters and fitted results of the DNP trNOE experiments with hyperpolarized HABA. Exp no. 1-6: HABA only; Exp no. 7-10: HABA mixed with preloaded avidin coated polystyrene particles; Exp no. 11: HABA with preloaded beads and biotin. The enhancement factor ε for the unsuppressed signal (*a* or *b*) was determined by comparing the peak integral from the first scan of the DNP experiment with the reference spectrum measured under thermal polarization for the unsuppressed peak. r_a, r_b, and σ were obtained from fitting the time evolution of peak integrals simultaneously for signal *a* and *b* using the Solomon equations (Eq. (1) and Eq. (2) in the text). σ_b was determined when immobilized protein is involved, based on the overall cross-relaxation rate σ with determined cross-relaxation rate for free ligand σ_f and the bound fraction X_b (Formula in Experimental Section).

Exp no.	c_{HABA} (μM)	$c_{binding\ site}$ (μM)	X_b	Peak suppressed	ε (a or b)	r_a (s^{-1})	r_b (s^{-1})	σ (s^{-1})	σ_b (s^{-1})
1	66.9	-	-	a	3.26E+03	0.28	0.34	0.066	-
2	65.7	-	-	a	3.67E+03	0.23	0.33	0.057	-
3	66.6	-	-	a	2.84E+03	0.22	0.34	0.056	-
4	69.7	-	-	b	3.12E+03	0.27	0.27	0.058	-
5	69.7	-	-	b	3.06E+03	0.28	0.28	0.056	-
6	67.6	-	-	b	4.01E+03	0.27	0.28	0.059	-
7	63.5	0.83	0.012	a	2.11E+03	1.00	0.75	−0.055	−9.5
8	90.4	0.83	0.009	a	2.59E+03	0.87	0.67	−0.032	−8.5
9	64.7	1.15	0.016	b	1.74E+03	0.76	1.00	−0.074	−10.5
10	68.4	0.90	0.012	b	2.07E+03	0.67	0.84	−0.045	−8.1
11	58.0	0.80	0.012	b	3.13E+03	0.39	0.37	0.017	-

6. References

(1) Janolino, V. G.; Fontecha, J.; Swaisgood, H. E. A spectrophotometric assay for biotinbinding sites of immobilized avidin. *Appl. Biochem. Biotechnol.* **1996**, *56* (1), 1–7.

(2) Green, N. M. [74] Spectrophotometric determination of avidin and biotin. *Methods Enzymol.* **1970**, *18*, 418–424.

(3) Macur, S.; Farmer, B. T.; Brown, L. R. An improved method for the determination of cross-relaxation rates from NOE data. *J. Magn. Reson. 1969* **1986**, *70* (3), 493–499.

(4) Määttä, J. A. E.; Airenne, T. T.; Nordlund, H. R.; Jänis, J.; Paldanius, T. A.; Vainiotalo, P.; Johnson, M. S.; Kulomaa, M. S.; Hytönen, V. P. Rational modification of ligand-binding preference of avidin by circular permutation and mutagenesis. *ChemBioChem* **2008**, *9* (7), 1124–1135.

(5) Green, N. M. Avidin. *Adv. Protein Chem.* **1975**, *29*, 85–133.