Supporting Information

Determination of protein-ligand binding modes using fast multi-dimensional NMR with hyperpolarization

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Hadamard reconstruction

The effect of the Hadamard reconstruction can be illustrated by assuming that the ligand protons *a*, *b* and *c* and all other ligand protons (represented by *d*) contribute to polarization transfer to *n* protein protons. The NOE signal for one protein proton *i* can be expressed as $(s_{ai} + s_{bi} + s_{ci} + s_{di})$, where s_{ki} (*k* = *a*, *b*, *c* or *d*) represents the signal intensity corresponding to the relative polarization transfer from each ligand proton. Upon inversion of *a*, *b* or *c*, a reduction of signal intensities would occur accordingly, with the change from s_{ki} to s'_{ki} (*k* = *a*, *b*, or *c*). When there is no NOE correlation between the ligand proton *k* and the protein proton *i*, $s_{ki} = s'_{ki} = 0$. The ligand frequencies are Hadamard encoded with the following matrix

$$[\mathbf{H}]_4 = \begin{bmatrix} 1 & 1 & 1 & 1 \\ 1 & -1 & -1 & 1 \\ -1 & 1 & -1 & 1 \\ -1 & -1 & 1 & 1 \end{bmatrix}$$

The originally obtained protein signals corresponding to different ligand resonances can be arranged into the columns of a matrix as

$$[\mathbf{S}] = \sum_{i=1}^{n} \begin{bmatrix} s_{ai} & s_{bi} & s_{ci} & s_{di} \\ s_{ai} & s'_{bi} & s'_{ci} & s_{di} \\ s'_{ai} & s_{bi} & s'_{ci} & s_{di} \\ s'_{ai} & s'_{bi} & s_{ci} & s_{di} \end{bmatrix}$$

Protein signals in a set of reconstructed spectra can be obtained by decoding the original spectra with the Hadamard matrix:

$$[\mathbf{R}] = [\mathbf{H}]_4^{\mathrm{T}}[\mathbf{S}] = \begin{bmatrix} 1 & 1 & -1 & -1 \\ 1 & -1 & 1 & -1 \\ 1 & -1 & -1 & 1 \\ 1 & 1 & 1 & 1 \end{bmatrix} [\mathbf{S}] = \sum_{i=1}^{n} \begin{bmatrix} 2(s_{ai} - s'_{ai}) & 0 & 0 & 0 \\ 0 & 2(s_{bi} - s'_{bi}) & 0 & 0 \\ 0 & 0 & 2(s_{ci} - s'_{ci}) & 0 \\ 2(s_{ai} + s'_{ai}) & 2(s_{bi} + s'_{bi}) & 2(s_{ci} + s'_{ci}) & 4s_{di} \end{bmatrix}$$

Thereby, the signals correlated through NOE to the 3 ligand resonances are separated into the 3 reconstructed spectra represented by the first three rows in [**R**].

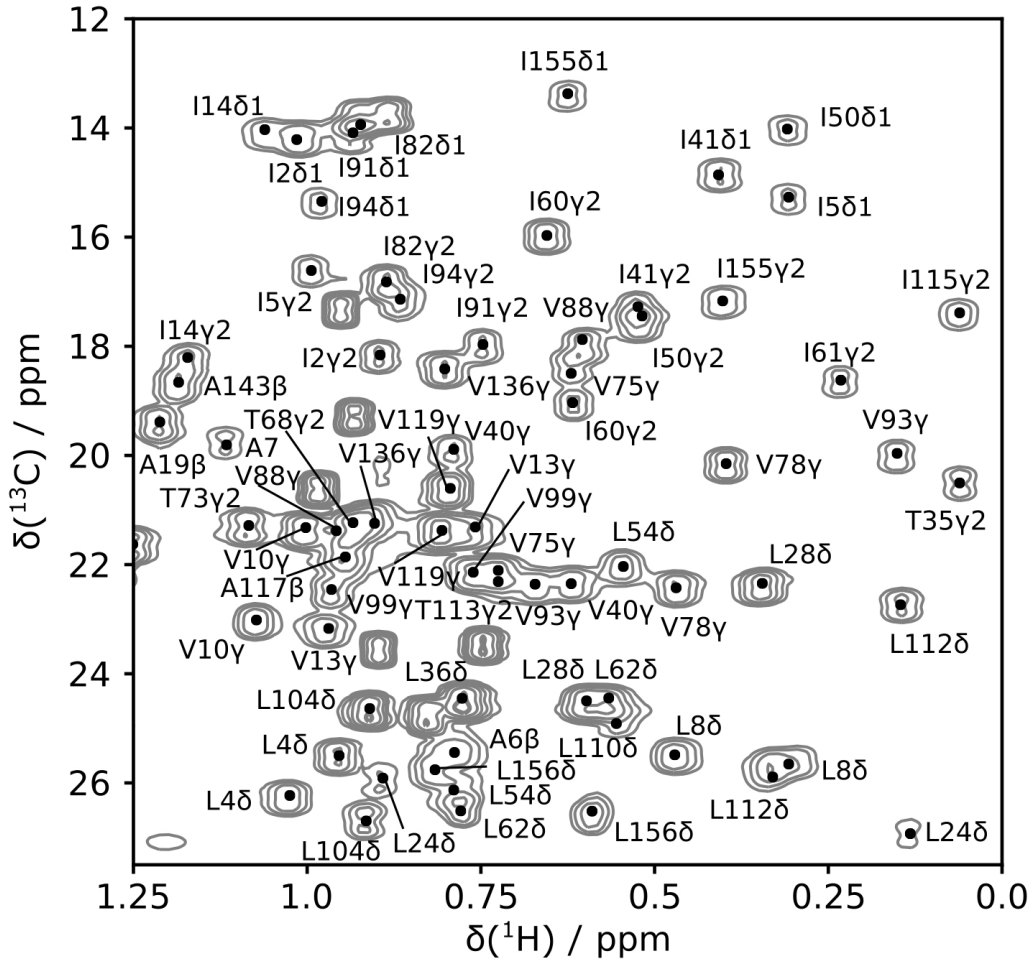

Figure S1. 2D HSQC spectrum of 1.5 mM DHFR in the presence of 15 mM folic acid acquired on a 500 MHz NMR spectrometer equipped with a TCI cryoprobe. The chemical shift assignments were previously determined,[1] adapted from published values[2] to current experimental conditions.

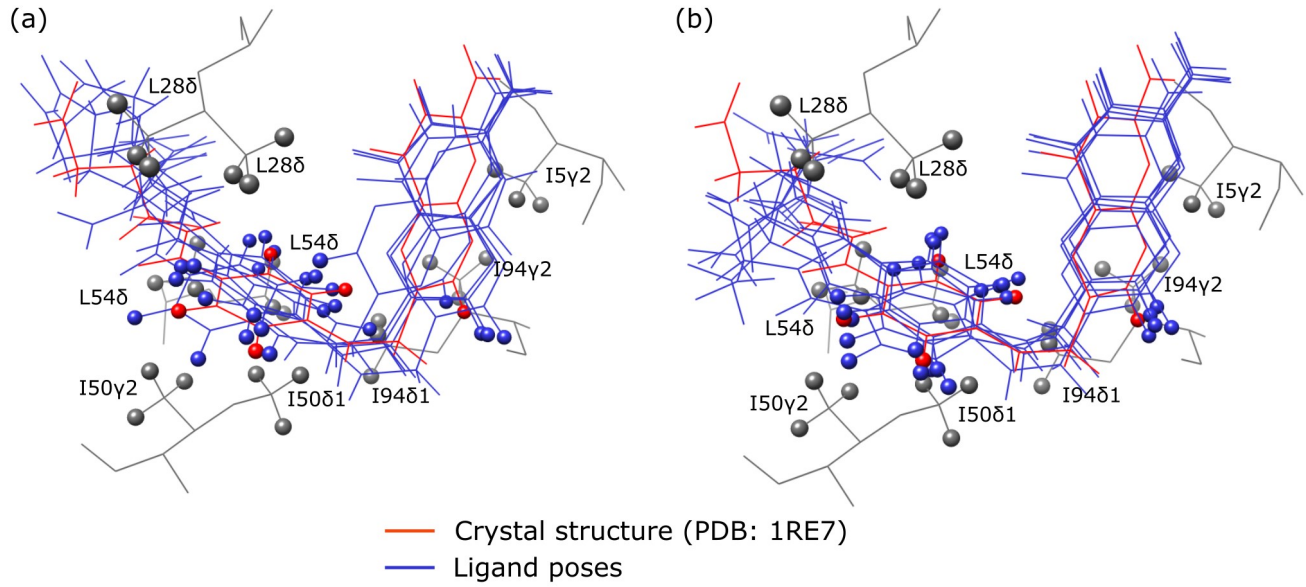

Figure S2. Comparison of the top five poses selected by the Autodock Score and the NOE Score functions. (a) Overlay of the five docked poses with the lowest binding energy calculated by the AutoDock program (blue) and the ligand in the crystal structure (red). The average RMSD value of the 5 selected poses against the reference structure is 1.41 Å when calculating for the 5 encoded protons, and 1.27 Å for heavy atoms in the pteroyl moiety. (b) Overlay of the five poses selected by the NOE Score function (blue) and the reference ligand structure (red). The average RMSD value of the 5 selected poses against the reference structure is 0.76 Å when calculating for the 5 encoded protons, and 0.85 Å for heavy atoms in the pteroyl moiety.

Table S1. Ligand signal enhancement factors and sample concentrations for four DNP-NMR experiments. The signal enhancement was determined by comparing the hyperpolarized ^{1}H signals measured with a 1° excitation with the ligand ^{1}H signals acquired at thermal polarization after the DNP-NMR experiment. * indicates that the peak was selectively inverted and the number in parentheses is the inversion factor, which was determined by comparing the relative enhancement factor for this peak in Exp.1 where no inversion was applied.

Exp.	Enhancement factor								Concentration (mM)	
number	H7	H8	H2'/H6'	H3'/H5'	Hα	Hɣ	Hβ	Hβ'	Folic acid	DHFR
1	650	157	384	491	335	267	138	112	5.06	0.333
2	623	114	-255(0.69)*	-293(0.62)*	321	214	178	145	4.96	0.324
3	-403(0.65)*	131	369	-322(0.68)*	322	212	122	104	4.40	0.365
4	-375(0.60)*	139	-244(0.67)*	469	320	194	170	99	5.30	0.332

References

1. Y. Wang, M. Ragavan and C. Hilty, *J. Biomol. NMR*, 2016, **65**, 41–48.
2. C. J. Falzone, J. Cavanagh, M. Cowart, A. G. Palmer, C. R. Matthews, S. J. Benkovic and P. E. Wright, *J. Biomol. NMR*, 1994, **4**, 349–366.