Supplementary Information

Gd(III) and Mn(II) complexes for dynamic nuclear polarization: small molecular chelate polarizing agents and applications with site-directed spin labeling of proteins

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Detailed Experimental Section

EPR at 263 GHz

24 µW µw power were used and the field was modulated with a frequency of 5 kHz at 0.1 mT amplitude. For 4MMDPA tag the field was swept by 40 mT over 2024 points at a conversion time of 196 ms; for DOTAM-M respective parameters were 10 mT, 801 points, and 250 ms. The integration time constant was fixed to the conversion time.

DNP at 5 T (140 GHz)

A superconducting sweep coil with a range of ±75 mT allowed for adjusting the field and recording field-dependent DNP profiles using Bloch decay (single-pulse) FID detection. After optimization of the maximum enhancement field position for the respective nucleus the magnet was set to persistent mode and enhancement factors were determined by comparison of NMR intensity with and without µw irradiation using cross-polarization (A. Pines, M. G. Gibby and J. S. Waugh, *J. Chem. Phys.*, 1972, **56**, 1776-1777) to ^{13}C (in case of ^{1}H) or direct read-out of ^{13}C polarization via Bloch decay (F. Bloch, W. W. Hansen and M. Packard, *Phys. Rev.*, 1946, **69**, 680-680). For all experiments radio frequency (rf) pulse amplitudes were set to a nutation frequency of 100 kHz for both ^{1}H and ^{13}C. TPPM was used for broadband decoupling of ^{1}H (A. E. Bennett, C. M. Rienstra, M. Auger, K. V. Lakshmi and R. G. Griffin, *J. Chem. Phys.*, 1995, **103**, 6951-6958). Samples were contained in a 4 mm o.d. sapphire MAS rotor from Insaco (Quakertown, PA). Pulse sequences were preceded by a pre-saturation pulse train consisting of 16 flip (90°) pulses separated by 5 ms and a subsequent polarization delay during which (enhanced) polarization can build up. For the acquisition of build-up curves the polarization delay was varied; all other experiments were

performed using a fixed polarization delay close to 1.26 × T_B (with T_B being the DNP build-up time constant), thus allowing for optimal sensitivity in a given experimental time.

DNP at 9.4 T (263 GHz) and above

Comparison of ^{1}H DNP at 9.4 T and 14.1 T performed at Bruker BioSpin (Billerica, MA): The DNP field profiles were obtained by measuring the enhancements at each magnetic field position. The RF field strength on ^{1}H was 50 kHz during CP and 100 kHz otherwise including TPPM decoupling during acquisition. The DNP enhancements were determined by comparing the NMR signals with and without microwave irradiation. The magnetic field of the NMR magnets were varied by superconducting sweep coils as well as room temperature shim stacks. The sweep ranges of the superconducting coils are 75 mT and 128 mT at 9.4 T and 14.1 T, corresponding to ~8,000 ppm and ~9,000 ppm, respectively. The shim stacks provide fine adjustments to the magnetic field within the range of 240 ppm and 150 ppm at 9.4 T and 14.1 T, respectively.

All other DNP experiments at 9.4 T: The sweepable Bruker Ascent DNP magnet (89 mm) was centered at 9.40 T and contained a superconducting sweep coil with a nominal range of ±75 mT. The sweep coil was energized step-wise to sweep the magnetic field and record DNP field profiles for ^{1}H, ^{13}C, and ^{15}N nuclei. After careful optimization of the respective optimum field position the sweep coil was put into persistent operation and enhancement factors were determined by comparison of NMR intensity with and without μw irradiation. Temperature was read out via a thermocouple inside the MAS stator during experiments. For all experiments radio frequency (rf) pulse amplitudes were set to 100 kHz, 50 kHz, and 40 kHz for ^{1}H, ^{13}C, and ^{15}N, respectively; ^{1}H amplitude was set for to ^{13}C or ^{15}N Hartmann-Hahn matching during CP (S. R. Hartmann and E. L. Hahn, *Phys. Rev.*, 1962, **128**, 2042-2053). SPINAL64 at 100 kHz was used for broadband

decoupling of ^{1}H (B. M. Fung, A. K. Khitrin and K. Ermolaev, *J. Magn. Reson.*, 2000, **142**, 97-101). Bruker 3.2 mm sapphire rotors sealed with silicone soft plug and ZrO_2 drive cap were used.

All pulse sequences were preceded by a presaturation pulse train consisting of 16 flip (90°) pulses separated by 5 ms and a subsequent polarization delay during which (enhanced) polarization can build up. This allowed for reproducible measurement of polarization in a quantitative manner, especially when reaching a quasi-equilibrium using dummy scans was not possible due to extremely long build-up times. For the acquisition of build-up curves the polarization delay was varied; all other experiments were performed with fixed polarization delays between 10 and 60 s unless given otherwise.

Protein expression and labeling

pet-21a plasmids carrying the F4C, A28C, and G75C mutation of human ubiquitin were created using site-directed mutagenesis and were verified by sequencing. The proteins were expressed recombinantly from *E. coli* strain BL21(DE3) in M9 minimal medium containing ^{13}C-glucose and $^{15}NH_4Cl$ as exclusive carbon and nitrogen sources. Purification was performed uniformly for all mutants using the following protocol: cells were resuspended in 25 ml of 50 mM NH_4OAc buffer (pH = 7.0) containing 10 mM 2-mercaptoethanol, lysed, and the lysate acidified to pH = 5 with acetic acid. The lysate was centrifuged and subsequently heated to 85 °C for 15 minutes while inverting several times. After cooling to r.t., the cloudy solution was centrifuged at 10,000 g for 30 minutes. A HiTrap SP HP column was equilibrated with 5 column volumes of 50 mM NH_4OAc buffer (pH = 5.0). The supernatant was loaded, washed with 5 column volumes of the same buffer, and eluted by applying a concentration gradient to 500 mM NH_4OAc buffer (pH = 5.0). For further purification, the sample was loaded to a HiLoad 26/600 gel filtration column and eluted with 50 mM NH_4OAc buffer (pH = 5.0). At the end of the procedure, the buffer was exchanged to

50 mM NH_4OAc buffer (pH = 7.0) for increased stability. Typical yields were about 30 mg per liter of medium used.

For site-directed labeling, 5 equivalents (relative to protein) of 4-mercaptomethyl dipicolinic acid (4MMDPA) or 15 equivalents of 1,4,7,10-tetraazacyclododecane-1,4,7-tris-acetic acid-10-maleimidoethylacetamide (DOTA-M) were added to 0.5 ml of 1 mM protein solution and rocked for 24 h at room temperature. The solution was placed in a PD-10 desalting column and eluted with 50 mM NH_4OAc (pH = 7.0) to remove excess spin label.

Supplementary Figures

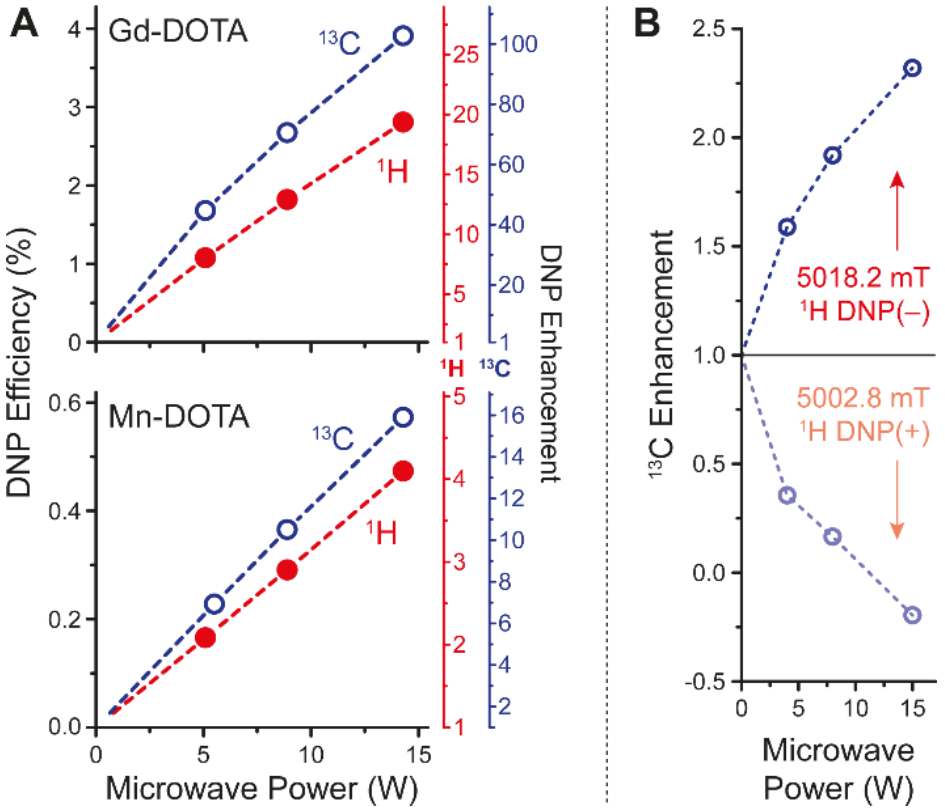

Figure S1. (A) Power dependence of ^{1}H (full circles, red) and ^{13}C (open circles, blue) DNP with Gd-DOTA. Left abscissa represents DNP efficiency relative to the theoretical maximum for each nucleus; right abscissas are ^{1}H (red) and ^{13}C (blue) enhancement factors. (B) Power dependence of "indirect" ^{13}C enhancement at optimum (positive or negative) ^{1}H enhancement.

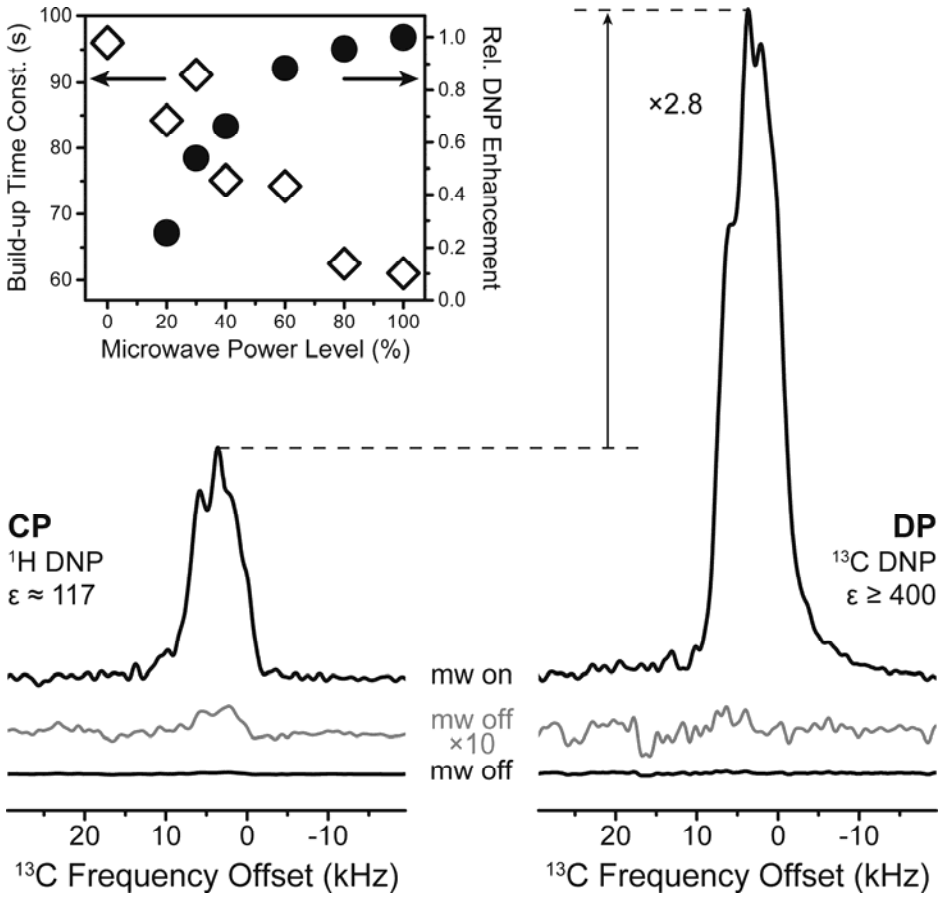

Figure S2. ^{1}H–^{13}C cross polarization (CP, left) and ^{13}C direct polarization (DP, right) spectra of 60% $^{13}C_3$-glycerol in a mixture with 40% H_2O with DNP enhancement by irradiation of 10 mM Gd-DOTA at the appropriate SE field with 120 mW of 140 GHz microwave irradiation at 80 K and under static conditions. To increase µw field strength a TE_{011} resonator was used. 4.2 s and 120 s polarization time was used for CP and DP, respectively. For better visibility the 'µw off' spectra have been multiplied by a factor 10 (gray line). Spectra were averaged over 128 (CP, µw on), 31,104 (CP, µw off), 64 (DP, µw on), and 3,808 (DP, µw off) scans, respectively, and normalized for direct comparability of signal intensity. The inset shows ^{13}C build-up time constant (open diamonds) and relative ^{13}C DNP enhancement (filled circles) as function of µw power level relative to 120 mW.

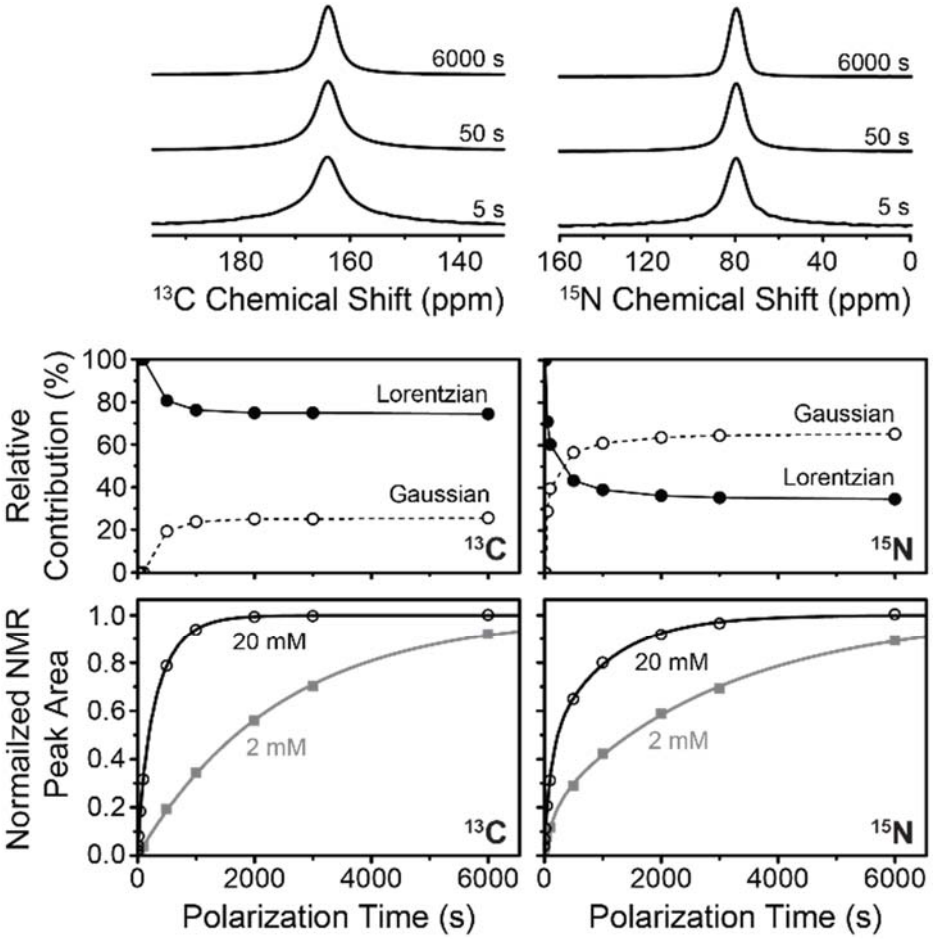

Figure S3. DNP-enhanced ^{13}C (left) and ^{15}N (right) spectra of ^{13}C,^{15}N-urea showing larger degree of broadening at shorter polarization times for a frozen solution containing 20 mM Gd-DOTA. The middle panel shows the relative contribution of homogeneous (Lorentzian) broadening and inhomogeneous (Gaussian) broadening, obtained by fitting with a linear combination of a Lorentzian and a Gaussian line. The lower graphs show the build-up of enhanced polarization obtained by integration over the whole NMR peak in comparison with that of a 2 mM Gd-DOTA solution.

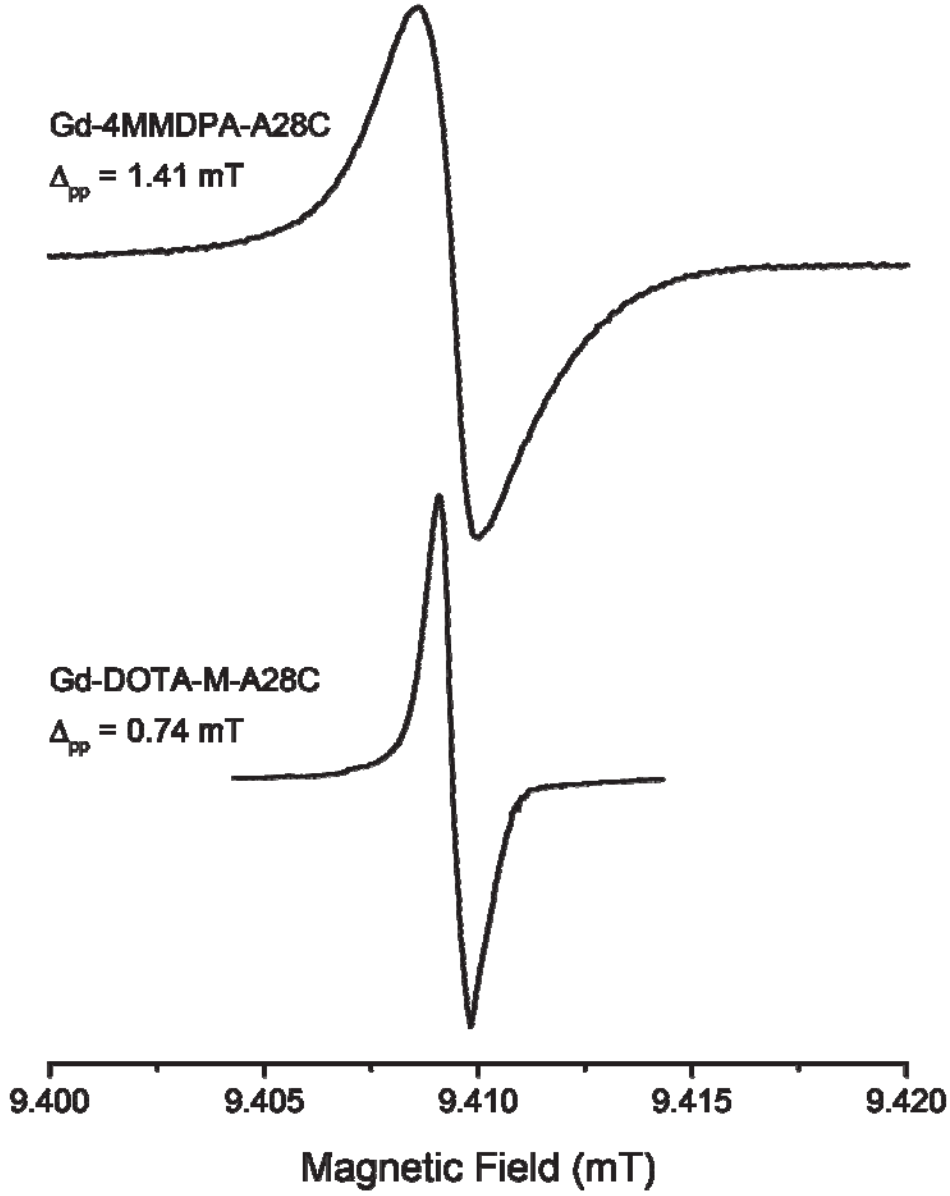

Figure S4. Cw EPR spectra of frozen DNP-solutions of Gd-4MMDPA-labeled and Gd-DOTA-M-labeled A28C mutant of ubiquitin measured at a μw frequency of 263 GHz and a temperature of 100 K. Δ_{pp} is the peak-to-peak linewidth of the cw spectra (*i.e.*, first derivative of the absorption spectra).