

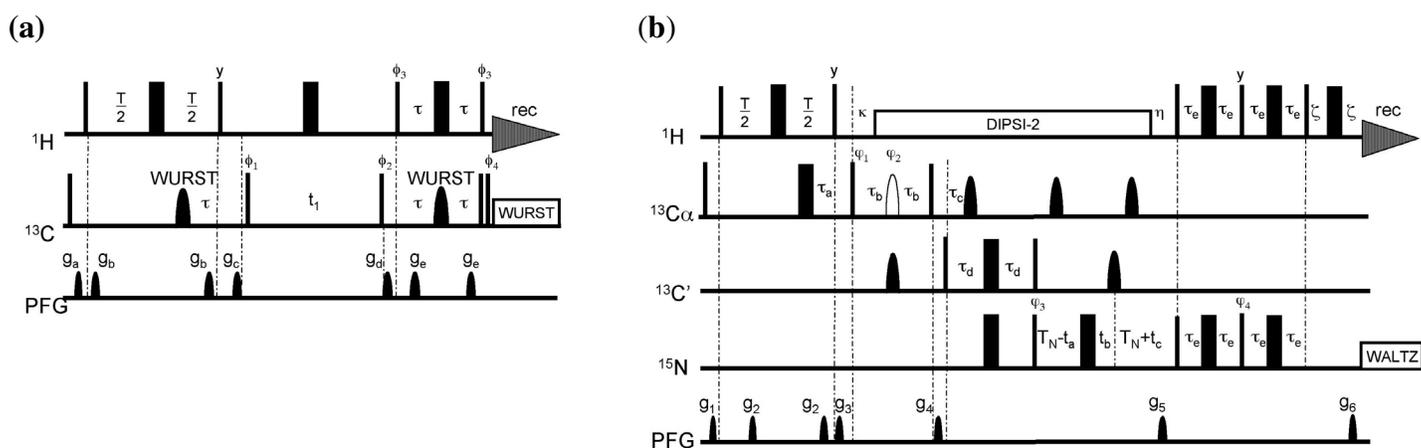
## Supporting Information

### Ensemble Approach for NMR Structure Refinement Against $^1\text{H}$ Paramagnetic Relaxation Enhancement Data Arising from a Flexible Paramagnetic Group Attached to a Macromolecule

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#### 1. NMR experiments to measure $^1\text{H}\text{-}\Gamma_2$ on $^{13}\text{C}$ -attached protons



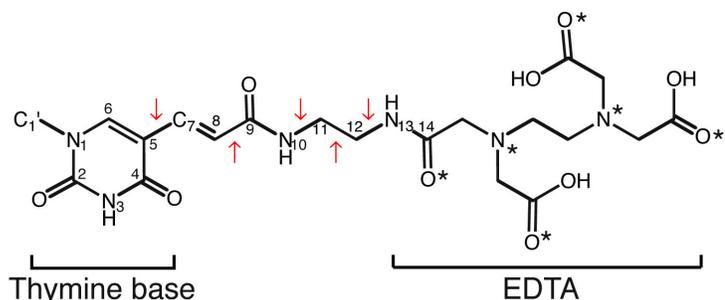
**Figure S1. Pulse sequences to measure  $^1\text{H}\text{-}\Gamma_2$  on  $^{13}\text{C}$ -attached protons**

The pulse sequences shown above are modified versions of (a) 2D  $^1\text{H}$ - $^{13}\text{C}$  HSQC and (b) 2D (HCACO)NH experiments to measure  $^1\text{H}\text{-}\Gamma_2$  on  $^{13}\text{C}$ -attached protons. The delay  $T$  is for  $^1\text{H}$ -transverse relaxation and several different values of  $T$  are used for the measurement of  $^1\text{H}\text{-}\Gamma_2$ . Values of  $^1\text{H}\text{-}\Gamma_2$  were determined with Eq. 20 in the main text using data recorded on  $\text{Mn}^{2+}$ (paramagnetic)- and  $\text{Ca}^{2+}$ (diamagnetic)-chelated states. Note that the effects of  $J_{HH}$ -modulation during the delay  $T$  are cancelled out in Eq. 20. Thin and bold rectangles represent  $90^\circ$  and  $180^\circ$  pulses, respectively, along  $x$  unless indicated. Other details of these experiments are described below. Experiments with  $T=3.4$  ms and  $T = 18.8$  ms were measured in an interleaved manner. Data were recorded on the same  $^1\text{H}_2\text{O}$  samples used for measurement of  $^1\text{H}_\text{N}\text{-}\Gamma_2$ .

(a) This experiment is for simultaneous measurement of aliphatic and aromatic  $^1\text{H}\text{-}\Gamma_2$ .  $^{13}\text{C}$ -WURST pulses were employed at the INEPT transfers to observe both aliphatic and aromatic signals with a good sensitivity using  $^{13}\text{C}$ -carrier frequency at 63ppm. The delay  $\tau$  was set to 1.6 ms. The maximum length of  $t_1$  evolution was set to 15.6 ms, so that peaks appear as singlets in the  $^{13}\text{C}$ -dimension. The experiments were performed at high magnetic fields ( $^1\text{H}$  750 MHz or 800 MHz) to minimize  $J_{CC}$  splitting width in ppm units. A WURST-MLEV composite pulse train was applied for  $^{13}\text{C}$ -decoupling during acquisition. Phase cycles are:  $\phi_1 = x, -x$ ;  $\phi_2 = 4x, 4(-x)$ ;  $\phi_3 = x, y, -x, -y$ ;  $\phi_4 = 8x, 8(-x)$ ; receiver =  $x, y, -x, -y, -x, -y, x, y$ . Quadrature-detection for the  $^{13}\text{C}$ -dimension was obtained using States-TPPI on  $\phi_1$ .

(b) This experiment is for the measurement of  $^1\text{H}\alpha\text{-}\Gamma_2$ . Delays are as follows:  $\tau_a = 1.6$  ms,  $\tau_b = 3.5$  ms;  $\tau_c = 4.6$  ms;  $\tau_d = 11.0$  ms;  $\tau_e = 2.3$  ms,  $\kappa = 2.3$  ms,  $\eta = 5.5$  ms,  $T_N = 16.4$  ms; and  $\zeta = 0.40$  ms. The white bell represents a  $^{13}\text{C}\alpha$ -selective REBURP pulse. Black bells represent  $^{13}\text{C}\alpha$ - or  $^{13}\text{C}'$ -selective sinc-shaped pulses. A semi-constant time scheme was employed for the  $^{15}\text{N}$  dimension. The time increments for this pulse scheme are  $\Delta t_a = T_N / (\text{total time points})$ ,  $\Delta t_b = \Delta t_c - \Delta t_a$ ,  $\Delta t_c = 0.5 / (^{15}\text{N} \text{ spectral width in Hz})$ . Phase cycles are:  $\phi_1 = x, -x$ ;  $\phi_2 = 4x, 4y$ ;  $\phi_3 = 2x, 2(-x)$ ;  $\phi_4 = -y$ ; receiver =  $x, 2(-x), x, -x, 2x, -x$ . Rance-Kay quadrature-detection was used for the  $^{15}\text{N}$ -dimension, alternating  $\phi_4$  between  $-y$  and  $y$  and the polarity of the gradient  $g_5$ . Lengths and strengths of gradients were:  $g_1$ , 1.0 ms, 15 G/cm;  $g_2$ , 0.53ms, 15 G/cm;  $g_3$ , 3.0 ms, 15 G/cm;  $g_4$ , 2.4 ms, 15 G/cm;  $g_5$ , 2.4ms, 12.5 G/cm;  $g_6$ , 0.3 ms, 10 G/cm.

## 2. Geometrical parameters for the conjugated EDTA-Mn<sup>2+</sup> group



**Figure S2.** Chemical structure of the dT-EDTA. Rotatable bonds in the linker region are indicated with red arrows. The asterisks indicate nitrogen and oxygen atoms involved in coordination of Mn<sup>2+</sup>. Note there is a slight difference between the chemical structure of dT-EDTA from Glen Research (Sterling, VA) used in the current study and the original dT-EDTA reported by Dreyer & Dervan (*Proc. Natl. Acad. Sci. U.S.A.* **1986**, 82, 968-972) in so far that the bond between C7-C8 is a double bond in the former but a single bond in the latter.

The chemical structure of the conjugated EDTA group is shown in Figure S2. The multiple structures for the linker-EDTA-Mn<sup>2+</sup> part were generated for the portion beyond the C5-C7 bond. Since we treat the EDTA-Mn<sup>2+</sup> group as a rigid body using the crystal structure coordinates (Richards, S. *et al.*, **1968**, *Inorg. Chem.* **3**, 27-33), we only list the covalent geometry parameters employed for the linker region. The values for the bond lengths and angles employed in the simulated annealing calculation are provided below. The values were taken from those for similar chemical structures.

### Bond Lengths (Å):

C <sub>5</sub> -C <sub>7</sub>	1.507	C <sub>7</sub> =C <sub>8</sub>	1.330	C <sub>9</sub> -O <sub>9</sub>	1.216
C <sub>9</sub> -N <sub>10</sub> , N <sub>13</sub> -C <sub>14</sub>	1.348	N <sub>10</sub> -C <sub>11</sub> , C <sub>12</sub> -N <sub>13</sub>	1.465	C <sub>11</sub> -C <sub>12</sub>	1.530
C <sub>7</sub> -H, C <sub>8</sub> -H	1.080	N <sub>10</sub> -H, N <sub>13</sub> -H	1.020	C <sub>11</sub> -H, C <sub>12</sub> -H	1.090

### Bond Angles (°):

C <sub>5</sub> -C <sub>7</sub> -C <sub>8</sub>	120.00	C <sub>7</sub> -C <sub>8</sub> -C <sub>9</sub>	120.00	C <sub>8</sub> -C <sub>9</sub> -O <sub>9</sub>	120.00
C <sub>8</sub> -C <sub>9</sub> -N <sub>10</sub>	120.00	O <sub>9</sub> -C <sub>9</sub> -N <sub>10</sub>	120.00	C <sub>9</sub> -N <sub>10</sub> -C <sub>11</sub> , C <sub>12</sub> -N <sub>13</sub> -C <sub>14</sub>	120.00
N <sub>10</sub> -C <sub>11</sub> -C <sub>12</sub> , C <sub>11</sub> -C <sub>12</sub> -N <sub>13</sub>	109.47	C <sub>5</sub> -C <sub>7</sub> -H	109.47	C <sub>8</sub> -C <sub>7</sub> -H	109.47
C <sub>9</sub> -N <sub>10</sub> -H, C <sub>14</sub> -N <sub>13</sub> -H	120.00	C <sub>11</sub> -N <sub>10</sub> -H, C <sub>12</sub> -N <sub>13</sub> -H	120.00	N <sub>10</sub> -C <sub>11</sub> -H, N <sub>13</sub> -C <sub>12</sub> -H	109.47
C <sub>12</sub> -C <sub>11</sub> -H, C <sub>11</sub> -C <sub>12</sub> -H	109.47	H-C <sub>11</sub> -H, H-C <sub>12</sub> -H	109.47		

Improper torsion angle terms were applied to maintain planarity for bonds on nitrogen atoms in the peptide groups and *sp*<sup>2</sup> carbon atoms. The peptide N-C and C=C bonds were restrained to the *trans* configuration. All covalent energy terms for the conjugated EDTA-Mn<sup>2+</sup> group were applied with the following force constants: 1000 kcal·Å<sup>-2</sup>·mol<sup>-1</sup> for bond lengths; 500 kcal·rad<sup>-2</sup>·mol<sup>-1</sup> for bond angles; and 500 kcal·rad<sup>-2</sup>·mol<sup>-1</sup> for improper torsion angles. These values are the same as those employed for the rest of the molecule (i.e. protein and DNA).

A quartic van der Waals repulsion term (Nilges, M. *et al.*, **1988**, *Prot. Eng.* **2**, 27-38) was used for the non-bonded contacts. The values for van der Waals radii of the C, N, and H atoms within the EDTA group were the same as those commonly used in Xplor-NIH simulated annealing calculations; the van der Waals radius for Mn<sup>2+</sup> was set to 1.60 Å. For interactions between the EDTA group represented by multiple structures and the rest of the molecule, the force constant for the van der Waals repulsion term was scaled by the inverse of the number of the conformers to avoid overwhelming repulsive forces against the macromolecule as a consequence of increasing the number of conformers. The terms for van der Waals interactions between the members of multiple-conformers were turned off so that they can overlap.