Supporting Information

Turano et al. 10.1073/pnas.0908082106

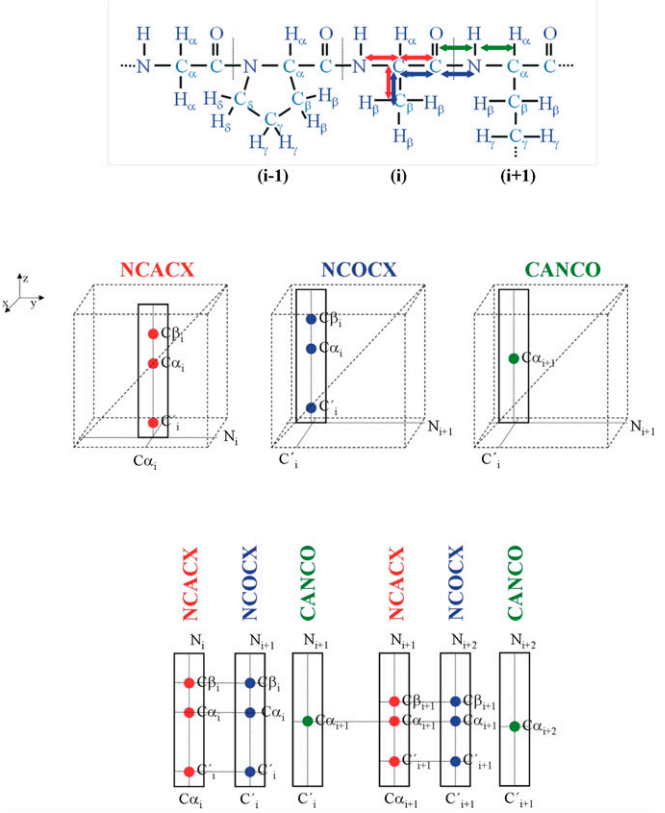

Fig. S1. Schematic representation of the possible connectivities detected in the 3D experiments NCACX (red), NCOCX (blue), and CANCO (green) used for the sequential assignment at the solid state. A peak in the NCACX resonates at the frequencies of N_i-$C\alpha_i$-CX_i, where CX_i can be one of the ^{13}C nuclear spins of residue i (C'_i, $C\alpha_i$, $C\beta_i$, etc.). A peak in the NCOCX resonates at the frequencies of N_{i+1}-C'_i-CX_i. A peak in the CANCO resonates at the frequencies of $C\alpha_{i+1}$-N_{i+1}-C'_i. Independently of the acquisition dimension, the 3D spectra are oriented with the ^{15}N dimension along the x axis. Each strip is therefore a portion of a ^{13}C-^{13}C plane (y, z), centered at the ^{13}C frequency reported in the bottom of each spectrum and extracted from a plane at the indicated ^{15}N frequency. The vertical strips are then visualized next to one another (as in Figs. S2 and S3) and used to build up sequential patterns. A strip at a given ^{15}N frequency in the NCACX permits the identification of intraresidue (residue i) $C\alpha$, $C\beta$, and C'. Comparison with the following NCOCX strip allows the identification of the backbone ^{15}N frequency for the following amino acid ($i + 1$). With this ^{15}N frequency, in the CANCO we can identify the $C\alpha$ of the ($i + 1$) residue.

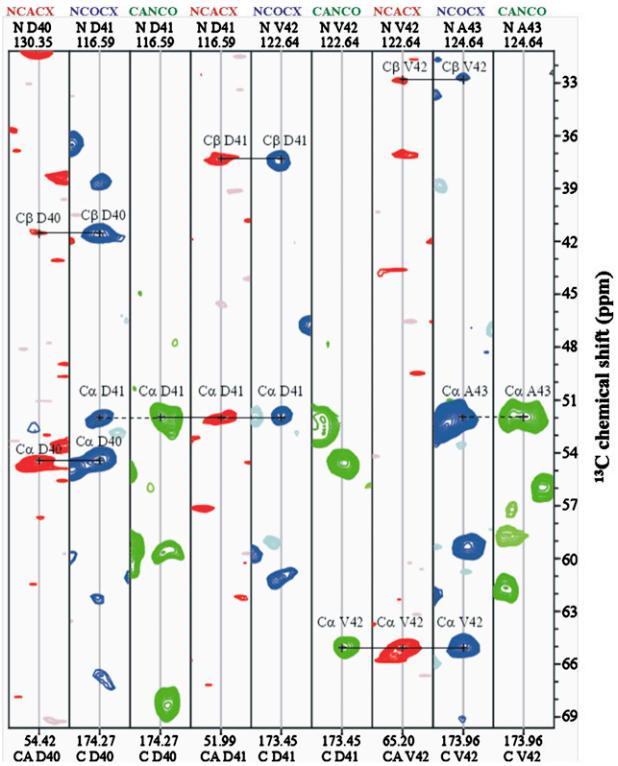

Fig. S2. An example of a sequential walk for residues 40–43 through strips constructed from NCACX (red traces), NCOCX (blue traces), and CANCO (green traces) experiments. The type of spectrum from which strips are extracted is reported on the top of each strip, together with the ^{15}N frequency of the plane from which they are extracted. Each strip is centered at the ^{13}C frequency reported on the bottom. Strips in the CANCO and NCACX with the same ^{15}N chemical shift and containing the same Cα allow the sequential assignment through the C′. Peaks in the NCOCX connected to those of CANCO through dashed lines are sometimes observed, as reported by Oschkinat and coworkers (1).

1 Krabben L, et al. (2009) Loop 3 of short neurotoxin II is an additional interaction site with membrane-bound nicotinic acetylcholine receptor as detected by solid-state NMR spectroscopy. *J Mol Biol* 390:662–671.

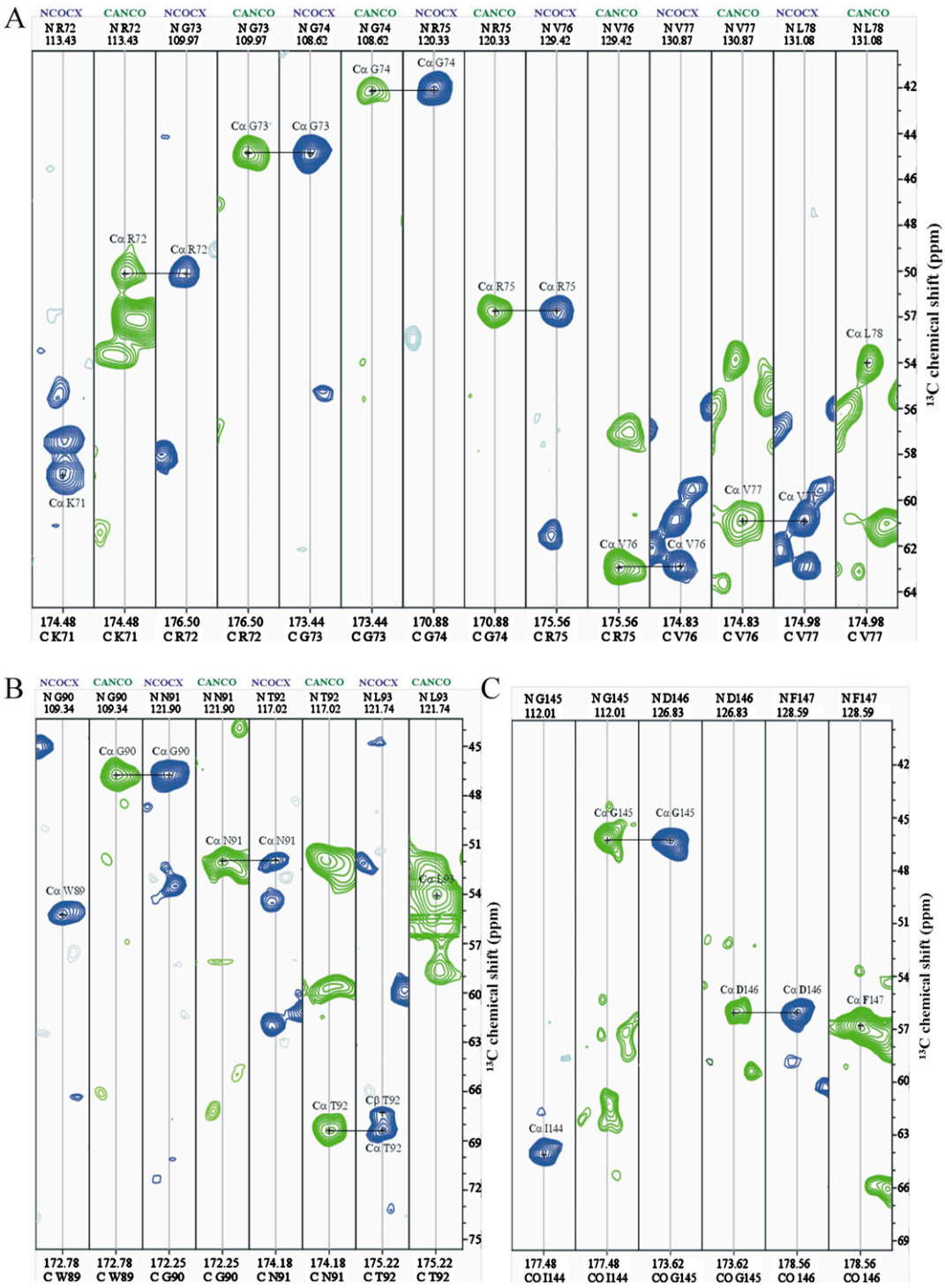

Fig. S3. Examples of the sequential walk connecting residues 71–78 (*A*), 89–93 (*B*), and 144–147 (*C*). The representation is the same as in Fig. S2, but for sake of clarity, only the NCOCX and CANCO strips are shown.

Table S1. Magnetic properties (per subunit) of iron-loaded ferritin at different degrees of metallation, as deduced from Evans measurements at 298 K

Number of iron atoms per ferritin subunit	χ_M ($m^3\ mol^{-1}$)	μ_{eff} (μ_B)	μ_{eff} per iron atom (μ_B)
2	$1.12 \pm 0.11 \times 10^{-7}$	4.49 ± 0.22	2.24 ± 0.11
4	$1.75 \pm 0.18 \times 10^{-7}$	5.61 ± 0.28	1.40 ± 0.07
6	$2.61 \pm 0.26 \times 10^{-7}$	6.85 ± 0.34	1.14 ± 0.06
8	$3.43 \pm 0.35 \times 10^{-7}$	7.85 ± 0.39	0.98 ± 0.05
10	$4.38 \pm 0.44 \times 10^{-7}$	8.87 ± 0.45	0.89 ± 0.04
12	$5.32 \pm 0.54 \times 10^{-7}$	9.78 ± 0.49	0.81 ± 0.04
14	$6.40 \pm 0.65 \times 10^{-7}$	10.70 ± 0.54	0.76 ± 0.04
16	$7.37 \pm 0.74 \times 10^{-7}$	11.50 ± 0.58	0.72 ± 0.03

Table S2. Time-dependence of the μ_{eff} values per iron atom of iron-loaded ferritin at different degrees of metallation, observed by Evans measurements at 298 K

Number of iron atoms per ferritin subunit	t_{stab} (hours)	μ_{eff} per iron atom (μ_B) $t = 0$	μ_{eff} per iron atom (μ_B) $t = t_{stab}$
2	1.5	2.36 ± 0.12	2.24 ± 0.11
4	24	1.56 ± 0.08	1.40 ± 0.07
6	24	1.24 ± 0.06	1.14 ± 0.06
8	24	1.05 ± 0.05	0.98 ± 0.05
10	24	0.93 ± 0.05	0.89 ± 0.04
12	24	0.85 ± 0.04	0.81 ± 0.04
14	24	0.78 ± 0.04	0.76 ± 0.04
16	24	0.74 ± 0.04	0.72 ± 0.03