Supplementary Figure 1. Pulse sequences used for the PACC approach.

(a) Low-power TPPM

(b) Low-power TPPM

(c) Low-power TPPM

(d) Low-power TPPM

Supplementary Figure 1. Pulse sequences used for the PACC approach. Low-power pulse sequences for (a) 1D $^{13}$C CPMAS, (b, c) 2D $^{13}$C/$^{13}$C chemical-shift correlation and (d) 2D $^{13}$C/$^{15}$N chemical-shift correlation experiments used for the paramagnetic-assisted condensed data collection (PACC) approach under very fast MAS condition. In all the sequences, initial $^{13}$C (a-c) or $^{15}$N (d) transverse magnetization was prepared by cross-polarization (CP) and low-power TPPM (lpTPPM) $^1$H decoupling was employed in order to suppress sample heating and probe arcing problems in fast recycling of the experiments. In (a), after the initial CP sequence, a $\pi$-pulse was applied at the middle of two rotation period ($2\tau_R$), and the signals were acquired at the end of the period. In (b), after the $t_1$ evolution due to $^{13}$C isotropic chemical shifts under lpTPPM $^1$H decoupling, the real or imaginary component was stored along the z-axis. After the unnecessary components in the transverse plane was dephased during a period of $\tau$, $^{13}$C-$^{13}$C dipolar couplings were restored by the fpRFDR sequence without $^1$H rf irradiation. XY-16 pulse sequence was rotor-synchronously applied so that a $\pi$-pulse was applied at the center of every rotor cycle. In the $t_2$ period, $^{13}$C signal was detected under lpTPPM TPPM $^1$H decoupling. In (c), a selective Gaussian $\pi$-pulse was applied at the center of the $t_1$ evolution period for selective J decoupling following Straus et al. To remove the chemical shift evolution of the non-selected spins during the selective pulse period, an extra Gaussian $\pi$-pulse followed by a non-selective $\pi$-pulse was applied before the beginning of the $t_1$ period. In (d), after the $t_1$ evolution period, the real or imaginary component of $^{15}$N magnetization was transferred to $^{13}$C by double quantum $^{13}$C-$^{15}$N ramped cross-polarization, in which the sum of $\omega_C$ and $\omega_N$ was matched to the spinning frequency. The $^{13}$C signals were detected during $t_2$ period under lpTPPM TPPM decoupling. The phase increments were $\phi = -y, -y, -x, -x, \phi_1 = x, x, y, y, -x, -x, -y, -y, \phi_2 = x, x, y, y, -x, -x, -y, -y, \phi_3 = x, x, -x, -x, \phi_4 = x, -x$ or $y, -y.$
**Supplementary Figure 2.** Effects of Cu-EDTA doping on 1D and 2D SSNMR spectra in the PACC approach. Comparison of (a) 1D $^{13}$C CPMAS spectra and (b, c) superimposed 2D $^{13}$C/$^{13}$C chemical-shift correlation solid-state NMR spectra of uniformly $^{13}$C-labeled ubiquitin in microcrystals in the (black) absence and (red) presence of 10 mM Cu(II)-EDTA at a $^1$H frequency of 400.2 MHz. In (red) PACC approach and (black) traditional signal collection for (a-c), the recycle delays of 150 ms and 700 ms were set to 3 times the $T_1$ values, respectively. The green spectrum in (a) is the difference spectrum. The spectrum in (b), which is a magnified image of Fig. 1a with signal assignments, was obtained without J decoupling. The spectrum in (c) was acquired with $^{13}$C-$^{13}$C J decoupling applied on the aliphatic region during the $t_1$ period for enhancing the resolution for $^{13}$CO. All the experiments were performed at a spinning speed of 40 kHz with signal acquisitions under low-power TPPM decoupling at rf fields of 7 kHz for 1.8 mg of $^{13}$C-labeled ubiquitin that was mixed with 2.7 mg of unlabeled one. In (a), signals of 256 scans were accumulated with the total experimental times of (black) 3.1 min and (red) 0.7 min. The total experimental times were (red) 5.4 h and (black) 21.9 h in (b) with 256 scans collected for each $t_1$ real/imaginary point while they were (red) 1.4 h and (black) 5.5 h in (c) with 64 scans. A total of 210 $t_1$ complex points were collected using $t_1$ increments of 48 $\mu$s with $t_1^{\text{max}} = 10$ ms and $t_2^{\text{max}} = 20$ ms in (b, c).
Supplementary Figure 3. Spinning-speed dependence of $^1$H-$T_1$ values for Cu-EDTA doped (a) and undoped (b) uniformly $^{13}$C- and $^{15}$N-labeled ubiquitin samples. $^1$H $T_1$ was measured by detection in $^{13}$C CPMAS signals as described in the text. The samples were prepared as described for the samples used in Fig. 1a. The sample temperature was kept at $\sim 15$ °C by using different temperature for cooling air, depending on the spinning speeds.
**Supplementary Figure 4.** Cu-EDTA concentration dependence of $^1$H-$T_{1p}$ values for unlabeled lysozyme in H$_2$O and D$_2$O.

Supplementary Figure 4. Cu-EDTA concentration ($C_p$) dependence of $^1$H $T_{1p}$ values for unlabeled lysozyme in H$_2$O (red circle) and D$_2$O (green squares). The experiments were performed at 10 kHz MAS detected by $^{13}$C SSNMR. The range of errors for $^1$H $T_{1p}$ is than ±3 ms. The sample in D$_2$O also shows considerable $C_p$ dependence of $T_{1p}$. 
Supplementary Figure 5. Comparison of $^{13}$C CPMAS spectra of unlabeled lysozyme without dopants, with 3 mM Ni(II)-EDTA, and with 10 mM Cu-EDTA.

(a)  

(b)  

(c)  

Supplementary Figure 5. Comparison of $^{13}$C CPMAS spectra of unlabeled lysozyme (a) without dopants, (b) with 3 mM Ni(II)-EDTA, and (c) with 10 mM Cu-EDTA. A total of 41k scans were collected for each spectrum at a spinning speed of 40 kHz, as described for Fig. 1(a) unless otherwise noted. Approximately 6 mg of the protein sample was used for each spectrum. The recycle delays of (a) 1.55 s, (b) 260 ms, (c) 220 ms were matched to $\sim 3T_1$. The total experimental time is (a) 18.9 h, (b) 3.5 h, (c) 2.9 h. The insets denote aromatic regions of the spectra, which show little broadening in (b, c).
**Supplementary Figure 6.** A transmission electron microscopy image of the Aβ(1-40) amyloid fibrils used for this study. The diameter of the fibril is several nm. Samples were negatively stained with uranyl acetate.
**Supplementary Figure 7.** 2D $^{15}$N/$^{13}$CO chemical-shift correlation SSNMR spectra of uniformly $^{13}$C- and $^{15}$N-labeled ubiquitin in microcrystals without and with 10 mM Cu-EDTA dopants.

The sample amounts were (a) 2.5 mg and (b) 1.8 mg. The experimental times were (a) 39 min and (b) 16 min for (a) 12 scans and (b) 24 scans collected in each $t_1$ real/imaginary point with recycle delay of (a) 740 ms and (b) 135 ms, which were matched to three times the $^1$H $T_1$ values. After the first cross-polarization, $^{15}$N signals were observed during the $t_1$ period ($t_1^{\text{max}} = 12$ ms) under low-power TPPM (lpTPPM) decoupling (10 kHz) sequence. The real or imaginary component of the signal was transferred to $^{13}$C by $^{13}$C-$^{15}$N ramped double quantum cross-polarization, for which the conditions used for Fig. 2b were employed. The $^{13}$CO signals were detected during the $t_2$ period ($t_2^{\text{max}} = 16$ ms) under lpTPPM decoupling. It is clear that the sufficient sensitivity was obtained by the PACC approach in 16 mins for less than 2 mg of the sample in (b).
**Supplementary Figure 8.** Comparison of $^{13}$C CPMAS spectra of a 147-residue recombinant protein of the cytoskeletal protein $\alpha$-spectrin II (Sp$\alpha$II(1-147)) without and with 50 mM Cu-EDTA doping.

The samples are uniformly $^{13}$C- and $^{15}$N-labeled. The $^{13}$C shifts are referenced to DSS. A total of 128 scans were collected for each spectrum of (a, b) at a spinning speed of 40 kHz, as described for Fig. 1(a) unless otherwise noted. Approximately 2.5 mg of the protein sample was used for each spectrum of (a, b). The recycle delays were (a) 1.6 s and (b) 360 ms. The molecular mass of the labeled Sp$\alpha$II protein is ~19 kDa. The difference spectrum shows that there are no major differences in the two spectra, despite the considerable reduction of $^1$H $T_1$ by ~4.5 fold with the paramagnetic doping.
**Supplementary Table 1.** Experimental and predicted spinning-speed ($v_R$) dependence of paramagnetic $^1$H $T_1$ ($T_{1p}$)

| $v_R$ (kHz) | 10  | 20  | 30  | 40  |
|-------------|-----|-----|-----|-----|
| Lysozyme $^1$H $T_{1p}$ | 59 (57) | 67 (66) | 74 (72) | 85 (78) |
| **Experimental** (Predicted) (ms) | | | | |
| Ubiquitin $^1$H $T_{1p}$ b) (ms) | 33 (45) | 39 (51) | 44 (55) | 53 (59) |
| Aβ(1-40) $^1$H $T_{1p}$ c) (ms) | 38 ±3 (61) | 54 (85) | 69 (104) | 89 (119) |

a-c) Experimental $T_{1p}$ values were obtained from $1/T_{1p} = 1/(T_1$ for doped sample) – $1/(T_1$ for undoped sample). The estimated errors for $T_{1p}$ are less than (a) ±5 ms, (b, c) ±1 ms, unless otherwise noted. The Cu-EDTA concentrations ($C_p$) were 10 mM and 200 mM for lysozyme/ubiquitin and Aβ samples, respectively. Other samples were the same as those for (b) Fig. 1a and (c) Fig. 1d.

d) The predicted $T_{1p}$ values were obtained from eq. [S3] (Supplementary Data; $T_{1p} = m(V/S)C_p + k(V/S)^2/D$), where $m$, $k$ are constants, $D$ is a spin diffusion constant, $D \propto 1/(v_R)^{0.5}$ is assumed, $V$ and $S$ are volume and surface area of the protein, respectively. Equation [S3] contains $(V/S)$ as an only protein-dependent geometrical parameter, besides $D$ which is likely to be common or similar among different systems. For globular proteins such as lysozyme and ubiquitin, $(V/S) = r/3$, where $r$ denotes a radius of the protein ($r \sim 1.5$ nm for ubiquitin) and $r \propto (MW)^{1/3}$. For a rod like system such as Aβ(1-40) fibrils, $(V/S) = d/4$, where a diameter $d$ of 4 nm was used based on Tycko’s model. For a rod like system such as Aβ(1-40) fibrils, $(V/S) = d/4$, where a diameter $d$ of 4 nm was used based on Tycko’s model. Since $(V/S)$ of 1 nm for Aβ is twice of that for ubiquitin (0.5 nm), the predicted relaxation property of Aβ reproduces that for 70 kDa globular protein (i.e. 8 times the MW of ubiquitin, ~9 kDa).
Supplementary Methods

Reagents

Fmoc-protected amino acids and Wang resins were purchased from Peptide International (Louisville, KY). Uniformly $^{13}$C- and $^{15}$N-labeled amino acids were purchased from Sigma-Aldrich/Isotec (St. Louis, MO). Other reagents for peptide synthesis were purchased from Applied Biosystems (ABI, Foster City, CA). NaCl, NaN$_3$, NaOH and HCl were purchased from Fisher Scientific (Hampton, NH). Purified water (double deionized and distilled) was prepared using a High-Q 103 S water still system (High-Q Corp., Wilmette, IL). The purified water was used for all the sample preparations in the experiments. Uniformly $^{13}$C-labeled and uniformly $^{13}$C- and $^{15}$N-labeled ubiquitin were purchased from Spectra Stable Isotopes (Columbia, MD). Uniformly $^{13}$C- and $^{15}$N-labeled 42-residue Alzheimer’s β-amyloid (Aβ) peptide (Aβ(1-42)) was purchased from rpeptide (Bogart, GA). Unlabeled ubiquitin, 2-methyl-2, 4,-pentanediol (MPD), Cu(II)Na$_2$EDTA (Cu-EDTA), diethylene triamine pentaacetic acid gadolium (III) dihydrogen salt (Gd(III)-DEPTA), and other reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted.

Preparation of ubiquitin protein microcrystals

Ubiquitin samples were precipitated using MPD following the protocol described by Igumenova et al. with minor modifications. For the sample used for Fig. 1a, b, ubiquitin protein stock solution was prepared by dissolving 2.0 mg of $^{13}$C-labeled ubiquitin and 3.0 mg of unlabeled ubiquitin in 0.4 mL of a 50-mM citrate buffer solution at pH 4.4. The unlabeled sample was used as a “filler” to increase the total sample volume for sample handling. The crystallization solution (0.6 mL of MPD) was added to the stock solution with 100 μL increments. The mixed solution was kept at 4°C for about 11 hours in order to produce the desired protein crystals. To prepare protein crystal samples containing Cu-EDTA, about 0.9 mL of the mother liquor was separated as a supernatant from the protein crystals after centrifugation. Then, 4.0 mg of Cu-EDTA was dissolved in the mother liquor. This solution was kept at 4 °C for 2-4 hours, and centrifuged to remove any precipitated proteins due to the salts. The mother liquor containing Cu-EDTA
was reintroduced to the protein crystals, and the crystals were left at 4 °C for 1-2 days to dope Cu-EDTA into protein crystals. The control samples that do not contain Cu-EDTA were prepared in the same manner, but without the addition of Cu-EDTA. The solution containing protein crystals was then centrifuged in a micro-centrifuge tube for 5 min using Eppendorf 5414D micro-centrifuge (Hamburg, Germany) at 1.5×10³ g. The collected protein crystals were packed into a 1.8 mm MAS rotor of 10 μL volume by fitting a 200 μL-pipette tip retaining the protein crystals to the rotor and centrifuging the rotor-pipette tip mounted in a micro centrifuge tube with minimum amount of the mother liquor for 2 - 4 min at 2.5 × 10³ g. The concentration of Cu-EDTA (10 mM) was estimated from the amount of Cu-EDTA used and the total volume of the mother liquor and the protein microcrystals.

The ubiquitin sample used for Fig. 2b was prepared similarly for a 1:9 mixture of 13C- and 15N-labeled and unlabeled ubiquitin samples in a total amount of 4 mg of the protein. Then, 2 mg of the mixture containing 200 μg of the labeled sample was center packed in a rotor and used for the experiment in Fig. 2b.

Synthesis and purification of Aβ(1-40) peptide

The 40-residue Aβ peptide (Aβ(1-40); NH2-DAEFRHDSGY-EVHHQKLVFF-AEDVGSNKGA-IIGLMVGGVV-COOH) was synthesized and purified as described previously using solid-phase synthesis with standard FMOC synthesis and cleavage protocols and HPLC purification. 13C- and 15N-labeling was introduced as described previously by incorporating Fmoc-protected uniformly 13C- and 15N-labeled amino acids at selected residues. Purity of the Aβ samples was determined based on the MALDI-TOF mass spectra collected at UIC Research Resource Center (RRC) to be approximately 95 % after the HPLC purification. The purified peptide sample was stored at -20 °C until it was used. The labeling schemes for 3 samples used for Fig. 3 are as follows (i) Ala-2, Phe-4, Gly-9, Val-18, (ii) Val-18, Phe-20, Ala-21, Ile-31, Gly-33, (iii) Ala-30, Ile-32, Gly-38, Val-39. 15N-labeling was introduced for future experiments. The sample (iii) was also used for Fig. 1d. Fmoc protection of the uniformly 13C- and 15N-labeled amino acids
was performed at the UIC RRC using the protocol of Fields et al. The labeled sample was used without any dilution with an unlabeled Aβ sample for SSNMR measurements.

**Preparation of Aβ(1-40) and Aβ(1-42) fibrils**

A solution of 5 mM Aβ(1-40) was prepared by first dissolving ~ 2.2 mg of Aβ(1-40) peptides in 100 μL of 50 mM NaOH. The peptide mixture was then briefly vortexed, and diluted to a final peptide concentration of 500 μM with purified water containing 0.02 % NaN₃. The pH of the solution was adjusted to 7.4 by using 100 mM HCl. Final solution contains ~ 2 mM NaCl based on the amount of NaOH added and HCl consumed for pH adjustment. The Aβ(1-40) solution was sonicated for 5 minutes in an ice bath and this solution was then centrifuged at 16.1 × 10³ g for 5 minutes to remove any preformed aggregates. The peptide solution of typically 0.8-1.0 mL was then incubated in 1.5-mL microvials at room temperature. Aβ(1-40) fibril formation was confirmed by thioflavin T (ThT) fluorescence assay. The incubated sample was not constantly agitated, but mixed by vortexing for sampling aliquots for the ThT fluorescence measurements (once every 3 days) throughout incubation times. The fibril sample prepared after the incubation of 7 - 10 days was then centrifuged at 16.1 × 10³ g for about 1.5 h (20 min at a time). After removing the supernatant, the gel-like pellet at the bottom of the vial was transferred into the 1.8 mm MAS rotor as described above for the protein microcrystals by centrifugation for about 10 min at 16.1 × 10³ g with minimum amount of Aβ supernatant. Petkova et al. recently reported that Aβ(1-40) peptide can form two distinctive fibril morphologies depending on the agitation conditions during the incubation and that the two samples (named “agitated” and “quiescent” forms) exhibit different ¹³C chemical shifts. The ¹³C shifts for our sample were similar to those for the agitated form, although not identical. The multiple ¹³C shifts observed in the quiescent samples were not observed in our sample except for Ala-21. The ¹³C shifts for Ala-21 are closer to those of the agitated form. Thus, we concluded that the overall conformations of our sample are similar to those of the “agitated form”, although it is possible that our sample morphology (Supplementary Fig. 6) is not identical to the agitated form. The structural model in the inset of Fig. 3 was obtained for the agitated form.
Fibrils of Aβ(1-42) was prepared and packed in a rotor using the protocol described above with minor modifications. A solution of 0.8 mM Aβ(1-42) prepared by first dissolving ~0.4 mg of uniformly \(^{13}\)C- and \(^{15}\)N-labeled Aβ(1-42) peptides in 100 μL of 50 mM NaOH and then diluting the solution to a final peptide concentration of 160 μM in a volume of 500 μL with purified water containing 0.02 % NaN₃. After the adjustment of the pH, the sample was incubated for 15 days at pH of 7.5. Fibril formation was confirmed by ThT fluorescence and electron microscopy.

Cu-EDTA was introduced to the fibril sample in a 10-μL MAS rotor containing the hydrated Aβ(1-40) or Aβ(1-42) sample without Cu-EDTA after collecting the SSNMR data for the control sample. About half of the total volume of H₂O in the rotor was removed by lyophilization of the sample in an open rotor about 10-15 min. Then, in the rotor, we introduced a 400 mM Cu-EDTA solution to retrieve the original sample volume so that the final concentration of Cu-EDTA became ~200 mM. The 400 mM Cu-EDTA solution was prepared by dissolving Cu-EDTA into the Aβ solution separated as a supernatant after centrifuging the Aβ solution containing the fibrils. Then the tightly capped rotor was kept at 4 °C for 1-2 days to dope Cu-EDTA into fibrils. After 1 day of the doping, \(^1\)H \(T_1\) values of the amyloid fibrils typically became constant.

As indicated above, the working Cu-EDTA concentration to achieve suitable \(^1\)H \(T_1\) reduction needs to be optimized for each sample. Although we have not experienced any degradation of the protein microcrystal or amyloid fibril samples due to Cu-EDTA doping even at relatively high concentration (~100 mM), it is advisable that \(^1\)H \(T_1\) values, \(^{13}\)C line widths, and sample stability are tested in \(^{13}\)C CPMAS of unlabeled proteins in the presence of varied Cu-EDTA concentration before experiments are performed for valuable labeled samples. Experimentally, no significant line broadening was observed as described in the article. For the Aβ(1-42) sample, we found a 1D \(^{13}\)C CPMAS spectrum for the undoped sample is nearly identical with that for the doped sample (data not shown).
Preparation of 147-residue fragment of α-spectrin II (SpαII) microcrystals

A 147-residue recombinant protein of the cytoskeletal protein α-spectrin II (SpαII(1-147)) was prepared by following the protocols in the previous works on a similar system SpαII(1-149) by Fung’s group.\textsuperscript{10} SpαII cDNA in the pGEX 2T vector was used to transform E. coli BL21 CodonPlus (DE3) cells (Stratagene, Cedar Creek, Texas). A single colony on an agar plate was used to inoculate 4 mL of LB media containing 100 μg/mL ampicillin and this culture was grown for 6~8 hrs at 37 °C. The cells were pelleted by centrifugation at 4,000 g for 10 min, then resuspended in 150 mL of M9 media containing 3 g/L 99% 13C\textsubscript{6}-labeled D-glucose (Sigma-Aldrich/Isotec), 1 g/L 98% 15N-labeled ammonium chloride (Sigma-Aldrich/Isotec), 2 mM magnesium sulfate, 0.1 mM calcium chloride, 10 μg/ml thiamine, and 100 μg/mL ampicillin. The starter culture was grown at 37 °C overnight. Then, this culture was used to inoculate 1 L of M9 media with the same components. 1 mM isopropyl-β-D-thiogalactopyranoside was added to induce the protein expression when OD600 = 0.7. The cells were grown for 4 more hours before the harvest. The protein was purified by GST affinity and ion-exchange column chromatography as previously described.\textsuperscript{11,12} The purity was about 95%, as determined by SDS gel chromatography and mass spectroscopy.

Microcrystal samples used for SSNMR were obtained by mixing 1.25 mL protein stock solution with the same volume of crystallization solution. The protein stock solution contained 7.5 mg/mL SpαII(1-147) in 25 mM sodium phosphate buffer at pH 7.3, and the crystallization solution contained 10% (w/w) PEG 1000 in water. Before preparation of the sample used for SSNMR, the condition to prepare the microcrystal was screened with handing drop method using 2 μL drop. The mixture was vacuumed by using a centrifugal evaporator (Savant SpeedVac, SC 110) for about 4-6 hours till the total volume was reduced to 1.4 mL. The mixture, in its own container, was sealed in a 50-mL conical tube with 5 mL crystallization solution at room temperature. Normally, microcrystals of ~25 μm in length and <1 μm in width formed in one week. Seeding was done when necessary. Further details of the sample preparation will be discussed in our forthcoming studies.
**SSNMR experiments**

SSNMR experiments were performed at 9.4 T ($^1$H NMR frequency of 400.2 MHz) with a Varian InfinityPlus 400 NMR spectrometer using a 1.8-mm double/triple-resonance MAS NMR probe developed at Dr. Samoson’s lab. All the data were acquired at the spinning speed of 40,000 ± 10 Hz with cooling air at -20 °C supplied through a Varian VT stack at a flow rate of ~66 L/min (~140 cubic-feet/h). At this condition, the actual sample temperature was approximately 15 °C due to frictional heating by fast MAS, which was calibrated using standard samples Cu(II)(DL-alanine)$_2$ and Pb(II)(NO$_3$)$_2$.\textsuperscript{13} Sufficient air flow is required to achieve homogeneous temperature over the sample. All the spectra were referenced externally to TMS using the secondary reference of $^{13}$C adamantane methylene peak at 38.56 ppm, and then, the reference was readjusted to DSS with a 1.7 ppm chemical shift difference between TMS and DSS,\textsuperscript{14} for comparison with the results in the previous publications.\textsuperscript{4,15,16} A recent study reported a refined value of 38.48 ppm for adamantane methylene from TMS and the difference of -1.90 ppm between DSS and TMS references.\textsuperscript{17} As long as rotors are tightly packed, we found no signs of sample degradation in $^{13}$C SSNMR spectra for ubiquitin and Aβ fibrils, at least, for 2-4 months probably because of the low-power pulse sequences, which require much less power than standard sequences using high-power decoupling even in the extremely fast repetition. In case rotor caps were not tight enough, the caps were sealed with instant adhesive such as Krazy Glue (Krazy Glue, Columbus OH), which can be later safely removed by immersing the cap in liquid nitrogen. While we found considerable variation of $^1$H $T_1$ values for undoped samples depending on the hydration level, minor dehydration did not alter $^1$H $T_1$ values of Cu-EDTA doped samples.

**Supplementary Fig. 1a** shows the pulse sequence used for 1D $^{13}$C CPMAS experiments in **Supplementary Figs. 2a, 5, 8.** The $\pi/2$-pulse for proton excitation was 2.5 μs. During the 1 ms CP period, the $^{13}$C rf field was swept from 48 kHz to 76 kHz, while the $^1$H rf field was kept at 102 kHz. The signal was collected during an acquisition period of 20 ms after a rotor synchronous echo period in which a 5 μs $^{13}$C $\pi$-pulse was applied at the middle of the two rotor cycles. Low-power TPPM (lpTPPM) $^1$H decoupling with the rf field intensity ($\omega_1/2\pi$) at 7 kHz was applied with phase alternation ($\Delta\phi$) between ±23° with
intervals of 72 μs (or flip angles of ~180°) during the echo and acquisition periods. We observed this sequence to provide 10-20% better sensitivity than the low-power XiX sequence. The details of the lpTPPM sequence is discussed elsewhere. It was found that lower spinning at 30 kHz considerably degrades the resolution under lpTPPM, in particular for CH₂ groups; thus, spinning speed of 40 kHz or higher is recommended for multi-dimensional SSNMR applications for relatively rigid biomolecules.

To collect the data in Figs. 1a, d and 2a, the pulse sequence for low-power 2D ¹³C/¹³C chemical-shift correlation experiments with fpRFDR mixing (Supplementary Fig 1b) was employed with the same CP condition used for the 1D experiments described above. ¹H lpTPPM decoupling with the rf intensity at 7 kHz was applied during the $t_1$ period, which was incremented with a dwell interval of 48 μs. A real or imaginary component of the signal was stored along the z-axis by a $\pi/2$-pulse, and the unselected component was dephased during the period $\tau$ of 2 ms before the mixing period. During the mixing period of 1.6 ms, we applied the fpRFDR ¹³C-¹³C dipolar recoupling sequence with a train of ¹³C $\pi$-pulses having 13 μs pulse widths without ¹H rf decoupling. Since ¹H-¹³C dipolar couplings are removed in the lowest order average Hamiltonian under the fpRFDR sequence, it was found that elimination of the rf ¹H decoupling does not introduce substantial loss of signals under the very fast MAS condition at 40 kHz. For a model system, uniformly ¹³C-labeled L-alanine, we observed 95, 93 and 98% of signal intensities for the CO₂⁺, CH and CH₃ signals with no ¹H decoupling after 1.6 ms fpRFDR mixing at 40 kHz MAS, respectively, compared with the corresponding signal intensities under 200 kHz cw-decoupling. The signals were detected during the $t_2$ with a sampling interval of 20 μs under the ¹H lpTPPM decoupling. The data were processed using NMRPipe software, and Sparky software (version 3.114) was used to analyze the processed 2D spectra. For the ubiquitin samples in Fig. 1a, signals were collected with $t_1^{\text{max}} = 10$ ms and $t_2^{\text{max}} = 20$ ms, where $t_2^{\text{max}}$ denotes the acquisition length in the $t_2$ domain. The $t_1$ data points were increased to 1.2 times the maximum $t_1$ period ($t_1^{\text{max}}$) by linear prediction. Then, the data were processed with sine-bell window functions with 32° phase shifts in both $t_1$ and $t_2$ domains.

The signal assignments for ubiquitin in Fig. 2b and Supplementary Fig. 2b,c are based on the previous works by Igumenova et al. For the spectra of Aβ(1-40) fibrils in Fig. 1d, $t_1^{\text{max}} = 4$ ms and $t_2^{\text{max}}$
The data were processed with linear prediction on the \( t_1 \) domain to 1.2 times \( t_1^{\text{max}} \) and with a Gaussian window function of 100 Hz in both \( t_1 \) and \( t_2 \) domains. For the spectrum of A\( \beta \)(1-42) fibrils in Fig. 2a, \( t_1^{\text{max}} = 5.7 \) ms and \( t_2^{\text{max}} = 14 \) ms with linear prediction on the \( t_1 \) domain to 1.25 times \( t_1^{\text{max}} \). The data were processed with a Lorentz-Gauss window function of 30 Hz (Lorentz) and 65 Hz (Gauss) in both \( t_1 \) and \( t_2 \) domains.

The 2D \(^{13}\text{C}/^{13}\text{C} \) correlation data with \(^{13}\text{C}-^{13}\text{C} \) J decoupling\(^2\) in Supplementary Fig. 2c were collected using the low-power 2D fpRFDR pulse sequence in Supplementary Fig. 1c in which we implemented selective J decoupling.\(^2\) In Supplementary Fig. 1c, a frequency selective Gaussian shaped \(^{13}\text{C} \) \( \pi \)-pulses were applied to remove the \(^{13}\text{CO}-^{13}\text{C} \) J couplings. The maximum rf intensity in the Gaussian pulse (\( \omega_{\text{max}} \)) was 3 kHz. The pulse widths of the Gaussian \( \pi \)-pulse (\( 4.7\sigma \)) was set to 600 \( \mu \)s width with truncation amplitude of 6\%, where the Gaussian envelope is given by \( \omega_{\text{max}} \exp(-t^2/2\sigma^2) \), where \( -300 \) \( \mu \)s \( \leq t \leq 300 \) \( \mu \)s. The \( t_1 \) data points were increased to 1.2 times the maximum \( t_1 \) period (\( t_1^{\text{max}} \)) by linear prediction.

The data were processed with sine-bell window functions with 32\(^\circ\) phase shifts in both \( t_1 \) and \( t_2 \) domains, and additional Gaussian broadening of 10 Hz was applied in the \( t_1 \) domain. Other experimental and processing conditions are the same as those of Fig. 1a.

The 2D \(^{13}\text{C}/^{15}\text{N} \) correlation spectra were collected using the newly designed low-power \(^{13}\text{C}/^{15}\text{N} \) correlation pulse sequence in Supplementary Fig. 1d. In this pulse sequence, after the first cross-polarization (with a contact time of 0.8 ms), \(^{15}\text{N} \) signals were observed during the \( t_1 \) period under lpTPPM decoupling and then the real or imaginary component of the signal was transferred to \(^{13}\text{CO} \) by double-quantum \(^{13}\text{C}-^{15}\text{N} \) ramped cross polarization, in which the rf intensity for \(^{13}\text{C} \) (\( \omega_{C}/2\pi \)) was swept while that for \(^{15}\text{N} \) (\( \omega_{N}/2\pi \)) was fixed during the contact time of 5 ms. The sum of \( \omega_{N}/2\pi \) and the average of \( \omega_{C}/2\pi \), \( \{\omega_{N} + <\omega_{C}>\}/2\pi \) was matched to the spinning speed, where \( <\omega_{C}> \) denotes the average of \( \omega_{C} \). We also eliminated \(^1\text{H} \) decoupling in the mixing period. The \(^{13}\text{CO} \) signals were recorded for 16 ms under the lpTPPM \(^1\text{H} \) decoupling. The data were processed in the same way as that for Fig. 1a.
$^1$H $T_1$ values were calculated from the data collected by $^1$H inversion recovery experiments detected by $^{13}$C CPMAS, as described in our previous study.\textsuperscript{23} The signal intensities were measured for the highest signals in the $^{13}$CO (160-190 ppm), $^{13}$C$_a$ (40-65 ppm), $^{13}$CH (10-30 ppm) regions to estimate $^1$H $T_1$ values; the average of the $^1$H $T_1$ values estimated for the three regions was used as $^1$H $T_1$ of the sample. For the sample crystallized with MPD in the absence of Cu-EDTA, we observed $^1$H $T_1$ values ranging from 0.23 to 0.30 s with increasing amount (2-5.5 mg) of solvent trapped in the rotor with the sample. We used the sample that exhibits the shortest $^1$H $T_1$ value (230 ms) as a control for the most unfavorable case for our approach. The variation in $^1$H $T_1$ was not observed for the sample incubated with Cu-EDTA.

$^{13}$C $T_1$ values for amyloid was measured by a modified CPMAS experiment in which the $^{13}$C magnetization after the CP period was stored along $\pm z$ axis by a $\pi/2$-pulse, subject to the $T_1$ relaxation during a variable relaxation period ($t_{\text{relax}}$), and then excited by a $^{13}$C $\pi/2$-pulse.\textsuperscript{24} The $^{13}$C signal intensities $\{S(t_{\text{relax}})\}$ were fitted into the exponential function as $S(t_{\text{relax}}) = S(0) \times \exp(-t_{\text{relax}}/T_1)$ to estimate the $^{13}$C $T_1$ values. The standard errors of the $^{13}$C $T_1$ values were calculated based on the uncertainties due to the noise.

**Supplementary Data**

*Transmission electron microscopy characterization of Aβ(1-40) fibrils*

A JEOL JEM-1220 transmission electron microscope (TEM) at an accelerating voltage of 80 kV was used for analysis of morphologies of aggregated Aβ(1-40) peptide. The incubated Aβ(1-40) solution was gently mixed before sampling. The sampled Aβ(1-40) solution of 10 μL was placed onto a carbon-coated Formvar 200-mesh copper grid (Electron Microscopy Sciences, Hatfield, PA) for 1 min. Excess solution was wicked dry with a Whatman filter paper, and then negatively stained with ~10 μL of 2 % uranyl acetate solution for 1 min. Afterwards, excess solution was again wicked dry, the grid was allowed to air dry, and the peptide samples were analyzed.\textsuperscript{25,26} The diameters of the fibrils were estimated from the average widths of the fibrils from the expanded TEM images.
Supplementary Figure 6 shows an TEM image obtained for the Aβ(1-40) fibril sample used for this study. Although we could not obtain high-resolution images for this sample for unknown reasons, somewhat bended morphology for this sample appears to be different from either of the agitated and quiescent forms reported by Tycko et al. The diameter of the sample (~10 nm) is similar to that for the maximum width of the quiescent form (~12 nm) as well as that for a laterally dimerized agitated form (~11 nm). As the agitated form, the fibrils in our sample seem to have tendency to associate literally into multimers. It is noteworthy that the diameter estimated by negatively stained TEM images is usually greater than actual diameter.

Comparison of 1D spectra for ubiquitin

We present a comparative study of 1D $^{13}$C CPMAS for native and relaxation enhanced samples for a given S/N ratio (number of scans) at a spinning speed of 40 kHz. We show 1D $^{13}$C CPMAS spectra (Supplementary Fig. 2a) obtained with lpTPPM decoupling$^{19}$ for hydrated microcrystals of uniformly $^{13}$C-labeled ubiquitin (red, top) with and (black, middle) without 10 mM Cu-EDTA dopants. Spinning at 40 kHz was needed for optimum decoupling performance by lpTPPM. The top spectrum, which is nearly identical with the middle spectrum, was obtained 4.4 fold faster by “condensed” data acquisition in a total time of 0.7 min. As in Fig. 1a, recycle delays (red: 150 ms, black: 700 ms) were set to three times $^{1}$H $T_1$ values, which are notably reduced by doping (red: 50 ms, black: 232 ms). The difference spectrum (green, bottom) indicated minimal intensities. The obtained resolution is comparable to or slightly better than that of the $^{13}$C CPMAS spectrum previously obtained under $^{1}$H decoupling of 80 kHz at the same $^{1}$H NMR frequency (400 MHz).$^4$ This clearly suggests excellent decoupling performance of the lpTPPM sequence.

Comparison of 2D $^{13}$C/$^{13}$C correlation spectra for Cu-EDTA doped and undoped ubiquitin

Supplementary Fig. 2b, c shows a comparison of 2D $^{13}$C/$^{13}$C chemical-shift correlation spectra for uniformly $^{13}$C-labeled ubiquitin (black) without and (red) with 10 mM Cu-EDTA. The data in Supplementary Fig. 2b is a magnified image of Fig. 1a with signal assignments. Supplementary Fig. 2c
shows correlation spectra between $^{13}$CO and aliphatic regions that were collected with $^{13}$C-$^{13}$C J decoupling during the $t_1$ period for resolution enhancement in the $^{13}$CO region.\textsuperscript{22} During the mixing period of 1.6 ms, we applied the fpRFDR sequence for $^{13}$C-$^{13}$C polarization transfer without $^1$H decoupling\textsuperscript{1,27} for the first time for applications to uniformly $^{13}$C-labeled proteins. The total experimental time in Supplementary Fig. 2c by PACC (red) was only 1.4 h, while that by standard acquisitions (black) was 5.5 h. It is clear that the peak positions and the line widths are not affected by Cu-EDTA doping in both (b, c). Unlike SSNMR spectra for previous reported paramagnetic proteins,\textsuperscript{28,29} no signals appear to be missing in the Cu-EDTA doped sample.

**Comparison of 2D $^{13}$C/$^{15}$N correlation spectra for Cu-EDTA doped and undoped ubiquitin**

Supplementary Fig. 7 shows a comparison of 2D $^{13}$CO/$^{15}$N correlation spectra for uniformly $^{13}$C- and $^{15}$N-labeled ubiquitin samples incubated (a) without and (b) with 10 mM Cu-EDTA. A majority of the cross peaks have nearly identical shifts between (a) and (b). The result shows that the spectral information obtained by our approach can be safely utilized to elucidate structural information on secondary structures and conformational distributions, which are reflected in shift positions and line widths, respectively.

**Spinning speed dependence of $^1$H $T_1$ on undoped and Cu-EDTA doped ubiquitin**

In the main text, we proposed the relaxation mechanism that $^1$H polarization relaxed on protein surface by paramagnetic dopants is transferred to $^1$H spins inside the proteins via $^1$H spin diffusion (Fig. 1c). Our data on $^{13}$C $T_1$ values in Fig. 3 suggests the surface specific relaxation is most likely to take place. Although homogeneous $^1$H $T_1$ indicates the effect of spin diffusion, we further tested whether $^1$H spin diffusion is another key element in $^1$H $T_1$ relaxation in the PACC approach by measuring the spinning-speed dependence of $^1$H $T_1$ for (a) Cu-EDTA doped and (b) undoped ubiquitin samples (Supplementary Fig. 3). Since it is known that fast spinning considerably suppresses $^1$H spin diffusion, it is expected that faster spinning slows down the relaxation process. As shown in the figure, for the Cu-EDTA doped sample in (a), $^1$H $T_1$ values increased considerably at faster spinning, while the $T_1$ values were not affected by spinning for
the undoped sample in (b). The data clearly demonstrate that the time required for the $^1$H spin-diffusion process is likely to contribute substantially to $^1$H $T_1$ values in the PACC approach.

**Solvent dependence of $^1$H $T_1$ on Cu-EDTA lysozyme**

Another possible relaxation mechanism in the PACC approach is through exchange of amide protons in proteins with paramagnetically relaxed $^1$H in H$_2$O, as reported for solution NMR.\textsuperscript{30} To examine the effects of this relaxation mechanism, we compared Cu-EDTA concentration dependence of $^1$H paramagnetic $T_1$ ($T_{1p}$) values for Cu-EDTA doped lysozyme in H$_2$O and D$_2$O (Supplementary Fig. 4), where $1/T_{1p} = (1/T_1$ for doped sample) – (1/$T_1$ for undoped one). The effects of concentration dependent relaxation are obvious for the sample incubated in D$_2$O; thus, the hydrogen exchange does not play a major role in the relaxation mechanism in the PACC approach. The data show that the doped sample with D$_2$O (green markers) has moderately higher $^1$H $T_{1p}$ than that for the sample with H$_2$O over all the concentration range. As will be analyzed below, the difference of $^1$H $T_{1p}$ values for the two samples may be attributed to the difference in the spin diffusion rates.

**Modeling the relaxation mechanisms and the limitations in PACC approach**

Although we demonstrated that that this PACC approach is likely to be applicable to various interesting systems including amyloid fibrils, evaluating the limitations of the present approach requires semi-quantitative estimations of $^1$H paramagnetic relaxation rates. Here, we propose a simple relaxation model in order to evaluate $^1$H $T_{1p}$ values at various conditions. For simplicity, we assume that a spin system consists of a bulk spin sink ($^1$H spins inside the protein) and a relaxation sink on the surface ($^1$H spins on the protein surface), at the latter of which $^1$H $T_1$ relaxation takes place rapidly due to paramagnetic dopants, and then $^1$H polarization is transferred via $^1$H spin diffusion to the bulk spin sink. Classical relaxation models through the surface induced relaxation have been studied previously; the analytical solution of the relaxation behavior for systems of various geometries are known to be given as sum of multiple exponential decays.\textsuperscript{31,32} On the other hand, this model does not provide physical insights in the relaxation phenomena in
the PACC approach. Here, we propose a simple alternative approach to represent $T_{1p}$ as a sum of two time constants that represent $T_{1p}$ in two extreme cases.

When the time required for spin diffusion is negligible (the fast-spin diffusion limit), $T_{1p}$ only depends on the time for $^1$H spins in the protein to be relaxed through the relaxation on surface. The relaxation rate ($1/T_{1p}$) is proportional to the concentration of dopant ($C_p$) and the surface area ($S$), and the rate is inversely proportional to the volume of a bulk spin system inside the protein ($V$). Thus, $T_{1p}$ is given by

$$T_{1p} = m(V/SC_p), \quad [S1]$$

where $m$ is a constant that depends on the relaxation rate for a unit concentration of dopants. Here, we assumed that the relaxation takes place only on the relaxation sink having a surface area of $S$. On the other hand, when the spin diffusion is a rate limiting event (slow spin-diffusion limit), the characteristic time constant is given by $^{33}$

$$T_{1p} = k(V/S)^{1/2}/D, \quad [S2]$$

where $k$ is a constant and $D$ is a spin diffusion constant. Although it is known that a $^1$H relaxation curve through the spin diffusion mechanism does not follow a mono exponential behavior, $^{33}$ we assumed an exponential decay for simplicity. In a crude approximation, we represent $T_{1p}$ in a general case as

$$T_{1p} = m(V/SC_p) + k(V/S)^{1/2}/D, \quad [S3A]$$

$$= M/C_p + K, \quad [S3B]$$

where $M$ is a constant that depends on $V/S$, and $K$ is a constant that depends on $V/S$ and $D$. For a spherical protein, $V/S = r/3$ in eq. [S3A], where $r$ denotes a hydrophobic radius of a protein. Because $K \propto r^2$ and $M \propto r$, eq. [S3A] shows that spin diffusion term $K$ contributes much greater for a larger system. Fitting the function in eq. [S3] to the data in Supplementary Fig. 4 shows that $M = 360.4$ [ms mM], $K = 20.9$ ms for the sample in H$_2$O, while $M = 365.9$ [ms mM], $K = 29.6$ ms for the sample in D$_2$O. Thus, in this condition at $C_p \sim 10$ mM, both mechanisms are important for lysozyme. At a higher $C_p$ ($\sim 80$ mM), the spin diffusion should be a dominant rate limiting factor.
Since eq. [S3A] contains a geometrical parameter \(V/S\), this also allows us to assess \(^{1}H\ T_{1p}\) for different systems. For ubiquitin of MW 8.6 kDa and \(r \sim 1.5\) nm (cf. for lysozyme MW \(\sim 14.7\) kDa and \(r \sim 1.8\) nm), we predict that \(T_{1p} = 45\) ms at \(C_p = 10\) mM, which reasonably agrees with the experimental \(T_{1p}\) of 33 ms at \(\nu_R = 10\) kHz, where we assumed ubiquitin and lysozyme as ideal spheres of a common \(^{1}H\) spin density. For a globular protein of MW of 70 kDa, the expected \(T_{1p}\) is 120 ms and 71 ms at \(C_p = 10\) and 50 mM, respectively; both \(T_{1p}\) are reasonably short. The expected \(T_{1p}\) for Sp\(\alpha\)II(1-147) based on the molecular weight (~19 kDa) is ~60 ms, which is considerably shorter than the experimental value (155 ms). Although a molecular structure of this system is not currently available, solution NMR data indicated that the system may form a dimer at a high protein concentration (Fung et al. unpublished).

For a general geometry, \(V/S\) is represented as \(d/2\varepsilon\), where \(d\) is a domain size, and \(\varepsilon\) denotes a “dimensionality” parameter. For a sphere, \(d = 2r\), \(r\) is a hydrophobic radius, and \(\varepsilon = 3\) while \(d\) is a diameter and \(\varepsilon = 2\) for a rod with a square cross section of \((d \times d)\) as amyloid fibrils. Based on a recent Tycko’s fibril model showing a cross section of \(\sim 16\) nm\(^2\) \((d \sim 4\) nm), the predicted \(T_{1p}\) at 200 mM Cu-EDTA is 61 ms, which agrees reasonably with the experimental value of 38 ms at \(\nu_R = 10\) kHz. Since \((V/S) \sim 1\) nm = 3 nm/3 for A\(\beta\), the amyloid system should show relaxation behavior equivalent to that for a globular protein of MW \(\sim 70\) kDa, which has a 3-nm radius. This well explains considerably longer \(^{1}H\ T_{1}\) of the A\(\beta\) fibrils despite higher level of doping.

Spinning speed dependence of \(^{1}H\ T_{1p}\) values is also semi-quantitatively estimated in the framework of eq. [S3]. It is known that the diffusion constant \(D\) is proportional to \(1/(\nu_R)^{n}\), where \(n = 0.5-1\). Our analysis on the lysozyme and ubiquitin samples indicated that \(n \sim 0.5\) yields excellent fittings (Supplementary Table 1). For A\(\beta\), the predicted values also show reasonable overall agreements with the experimental ones. These results suggest that the expression in eq. [S3] appears to be useful for the estimation of \(^{1}H\ T_{1p}\) for unknown samples. Although the predicted values for A\(\beta\) somewhat overestimate \(T_{1}\), this may be attributed to our simplification that the relaxation takes place only on protein surface; at a
higher dopant concentration such as 200 mM used for Aβ, the relaxation effect may reach the inside of the protein assembly.

From the present theoretical model of the $^1$H $T_1$ relaxation, it is possible to discuss the limitations of the PACC approach using paramagnetic doping semi-quantitatively. It appears that this approach provides limited advantage to a hydrophobic globular protein of $r > 6$ nm (MW > 500 kDa). For a 500 kDa protein of diamagnetic $^1$H $T_1$ ($T_{1d}$) of 500 ms, we estimate that $T_{1p} \sim 450$ ms at 100 mM CuEDTA and $\nu_R = 40$ kHz; thus, $T_1$ estimated from $1/(1/T_{1p} + 1/T_{1d})$ yields 230 ms, which still provides acceleration of 2.2 fold. In contrast, for a 100-kDa globular protein of $T_{1d}$ of 500 ms, the estimated $^1$H $T_1$ is $\sim 120$ ms with 100 mM Cu-EDTA and $\nu_R = 40$ kHz. Thus, the present approach should provide sufficient sensitivity advantage up to 100 kDa proteins, which covers most of the current SSNMR applications for globular proteins. For amyloid systems having a long-chain-like geometry, hydrophobic diameter $d$ rather than the molecular weight is a limiting factor. We also predict that reducing $^1$H $T_1$ beyond 2 fold by doping is probably difficult for amyloid systems of $d > 9$ nm. Nevertheless, the PACC approach will be applicable to a variety of amyloid aggregates since many amyloid fibrils have diameters of several nm, as in the case of Aβ(1-40). For larger globular and amyloid systems, it is possible to incorporate paramagnetic labeling inside the systems using EDTA-tags so that the relaxation time is not limited by spin diffusion from the surface of proteins. It is also likely to be feasible to reduce $^1$H $T_1$ values further by enhancing $^1$H-$^1$H spin diffusion by dipolar recoupling or other methods such as switching angle spinning. Further studies will be needed to address such possibilities.

It should be noted that the PACC approach may be applicable to paramagnetic metallo-proteins, for which recent SSNMR studies have offered unique solutions for structural characterization. Although faster $^1$H $T_1$ relaxation has been observed for such paramagnetic proteins, the intrinsic relaxation property may not be optimum for the extremely fast recycling demonstrated in this work. In such a case, paramagnetic doping may provide optimum $^1$H $T_1$ to maximize sensitivity enhancement. On the other hand, modulation of the relaxation properties by paramagnetic dopants may restrict use of the relaxation parameters for structural measurements. Further studies will be needed to examine such possibilities.
Although we demonstrated that Cu-EDTA provides excellent reduction of $^1H\ T_1$ without affecting the resolution, it is probably important to have other choices of paramagnetic dopants in case that Cu(II) is not suitable for a particular application. As one of alternatives, we tested relaxation enhancements by Ni(II)-EDTA on lysozyme microcrystal samples (Supplementary Fig. 5). It was confirmed that $^1H\ T_1$ for lysozyme microcrystals doped with 3 mM Ni-EDTA was reduced to 88 ms from 530 ms observed for an undoped sample. The $T_1$ value is comparable to 73 ms for 10 mM Cu-EDTA doped lysozyme obtained for the same microcrystal sample. A $^{13}C$ CPMAS spectrum of lysozyme doped with 3 mM Ni-EDTA (Supplementary Fig. 5b) shows no major spectral degradation, compared with that of undoped lysozyme (a) and that of lysozyme doped with 10 mM Cu-EDTA (c). We also tested Ni-DO2A, for which we obtained a comparable $T_1$ reduction ($T_1 \sim 96$ ms), but at a higher concentration (15 mM) than that of Ni-EDTA. Although Gd(III) and Mn(II) compounds have been popular as relaxation and contrast agents for NMR and magnetic resonance imaging, such paramagnetic metal ions having relatively long $\tau_s$ ($\tau_s > 10^{-9}$ s) are probably not suitable for our purpose since these ions have more pronounced effects on $R_2$ rather than $R_1$, causing line broadening. As an example, we tested Gd(III)-DEPTA to examine the relaxation property, where DEPTA is an analog of EDTA. Although we found that doping of 1 mM Gd(III)-DEPTA reduces $^1H\ T_1$ of lysozyme to 100 ms, lines of a $^{13}C$ CPMAS spectrum of the Gd-DETPA doped sample were subject to considerable broadening (~2.5 times; data not shown). We also think that paramagnetic ions having large g-anisotropy such as many lanthanide ions may introduce detectable changes in the shift positions through pseudo contact interactions. In these senses, some Cu(II) and 5-6 coordinate Ni(II) compounds are suitable because $\tau_s$ for these compounds are in a range of $10^{-10}$-$10^{-9}$ s and the g-anisotropy for these metal ions is typically small. Further investigation is likely to offer various paramagnetic compounds suitable for the PACC approach.
Additional $^{13}$C SSNMR data for Sp$\alpha$II microcrystals

In Supplementary Fig. 8, we present a comparison of $^{13}$C CPMAS spectra for uniformly $^{13}$C-and $^{15}$N-labeled 147-residue recombinant protein of the cytoskeletal protein $\alpha$-spectrin II (Sp$\alpha$II(1-147))\textsuperscript{10} in (a) the absence and (b) presence of 50 mM Cu-EDTA dopants. The molecular mass of this system is \textasciitilde19 kDa, which is larger than that of ubiquitin. The system is expected to be highly helical protein. Indeed, $^{13}$CO shifts in the figure suggest that the system is highly helical. $^1$H $T_1$ values for the undoped and doped samples were 530 ms and 120 ms, respectively. The spectral features of (a) and (b) are nearly identical. The difference spectrum in (c) shows that no additional chemical shifts or line broadening is detectable from the 1D spectra, despite the considerable reduction in $^1$H $T_1$ due to paramagnetic doping. Our preliminary 2D $^{13}$C/$^{13}$C correlation SSNMR for the samples, which will be discussed elsewhere, also showed nearly identical spectra for the doped and undoped Sp$\alpha$II samples. The results experimentally show that the PACC approach can be effectively implemented for globular proteins of moderate molecular masses.

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