Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

SSNMR of biosilica-entrapped enzymes permits an easy assessment of preservation of native conformation at atomic detail

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Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information available

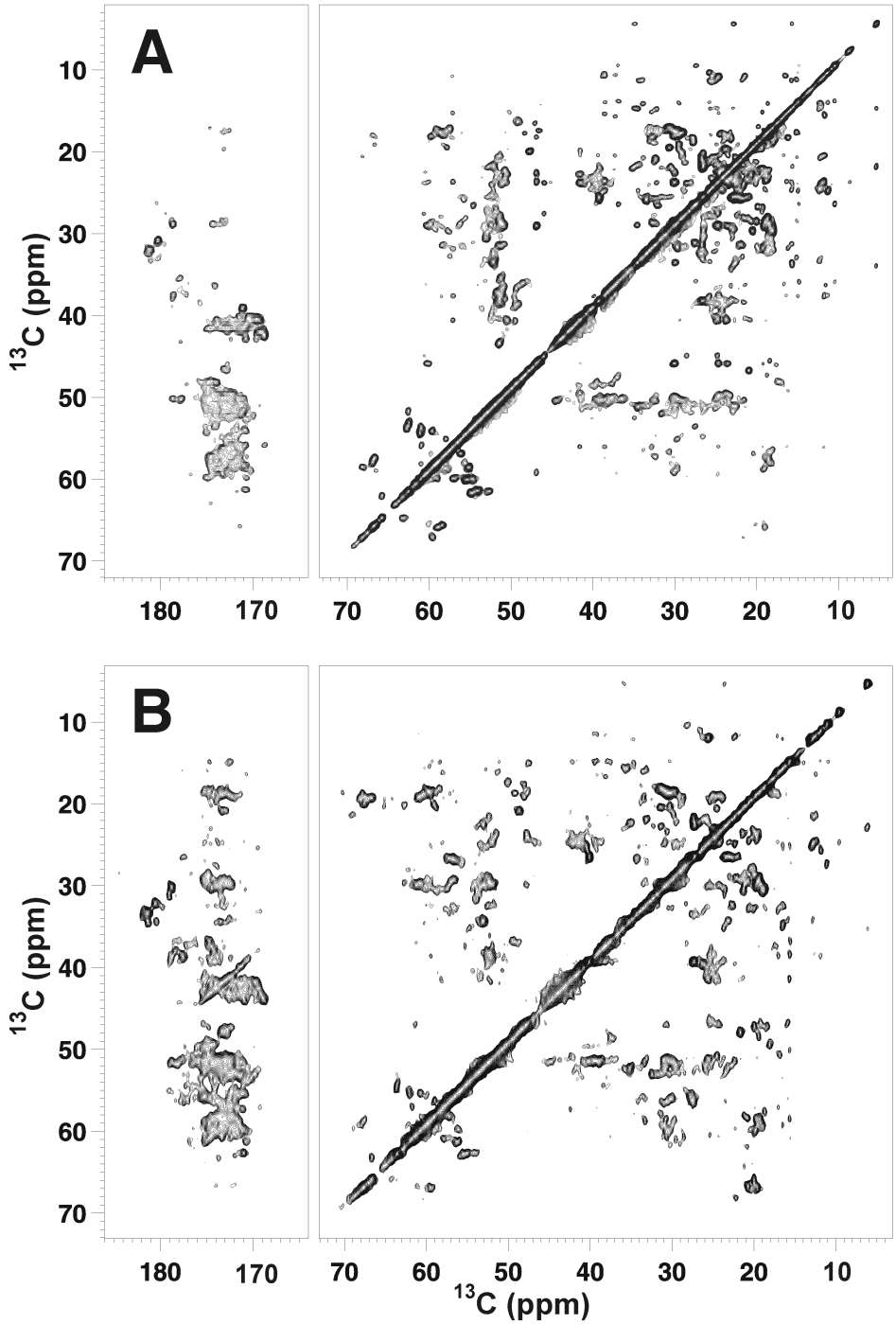

Figure S1. Two-dimensional ^{13}C-^{13}C correlation spectrum of SOD (molecular mass 32 kDa): **A**) PDSD (700 MHz, 15 kHz MAS) at 50 ms mixing of the microcrystalline preparation (48 scans) and **B**) DARR (700 MHz, 11.5 kHz MAS) at 25 ms mixing of the biosilica entrapped protein (256 scans).

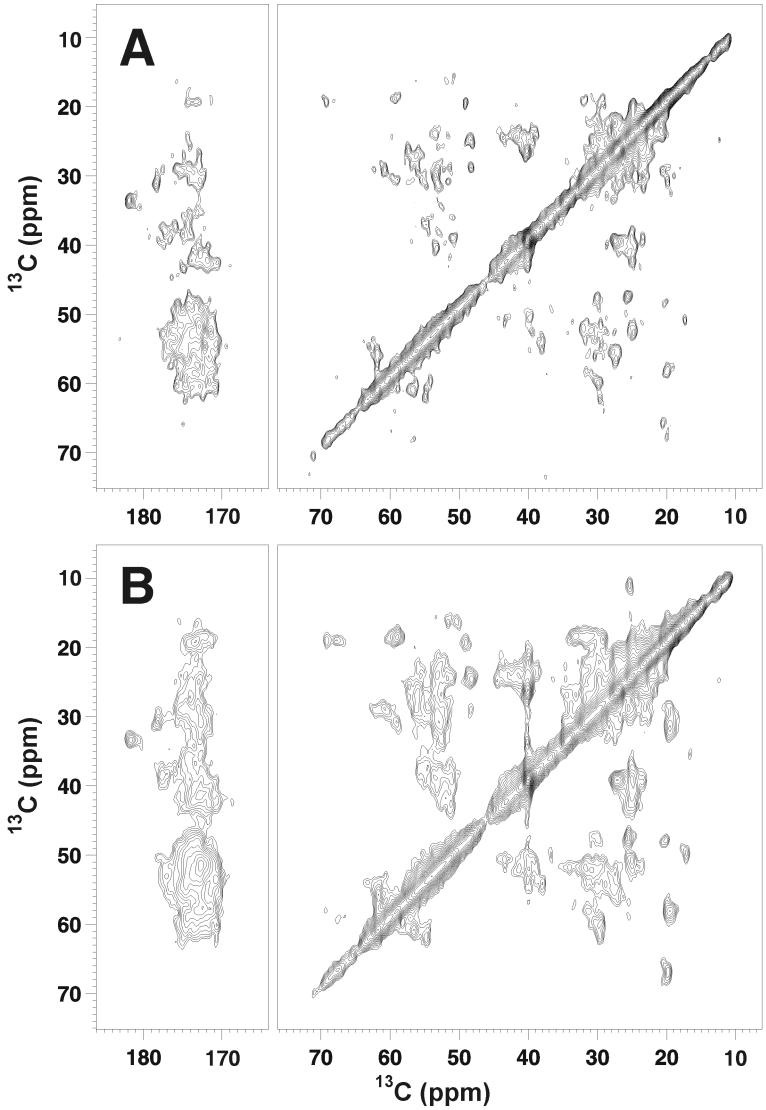

Figure S2. Two-dimensional ^{13}C-^{13}C correlation spectra (700 MHz, 11.5 kHz MAS) of the CAII protein (molecular mass 29 kDa): **A**) DARR at 25 ms mixing of the biosilica entrapped protein (256 scans), after 24 hours and rotor filled with 25 µL of biosilica material; **B**) after 72 hours and rotor filled with additional 25 µL of biosilica material.

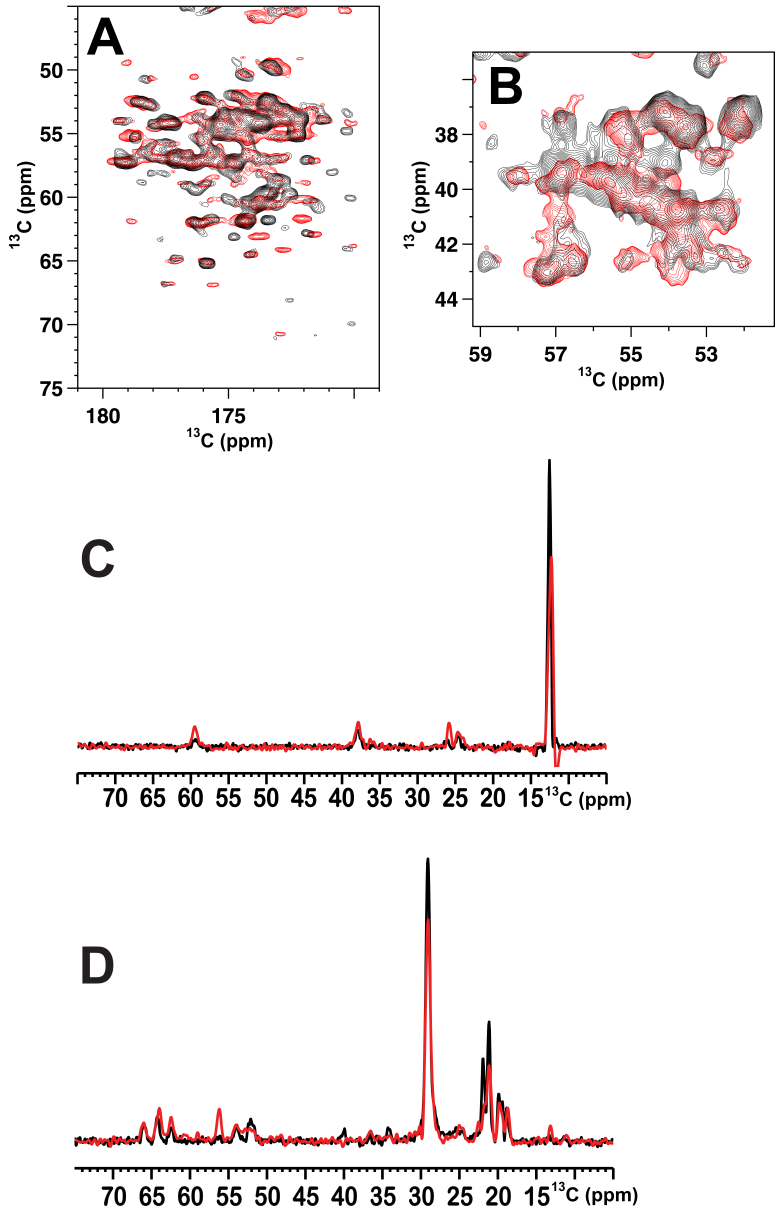

Figure S3. Comparison of two regions of the ^{13}C-^{13}C spectra (Cα-C' correlations in Panel **A**, and Cα-Cβ correlations, in Panel **B**) in both microcrystalline (red) and biosilica-entrapped (black) MMP-12. **C**) and **D**) One-dimensional traces through the same spectra at δ1=12.6 and 29 ppm. Differences in relative intensity are mainly attributable to the different mixing scheme employed.

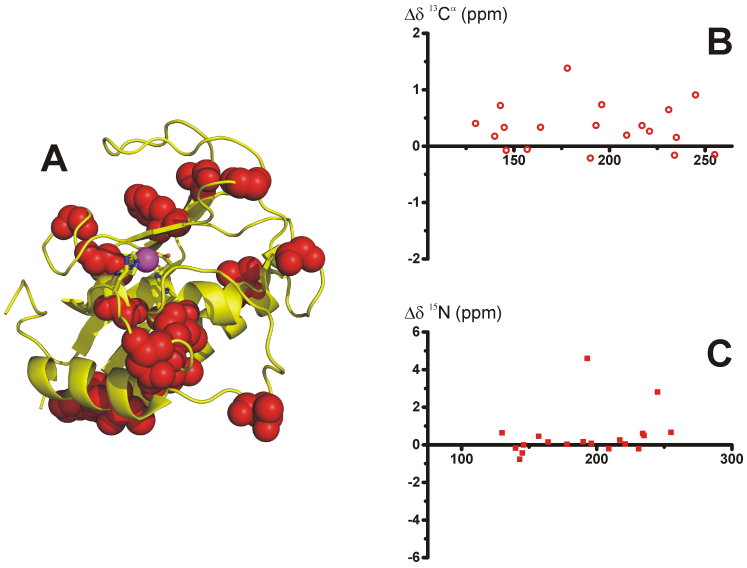

Figure S4. Cartoon representation of the catalytic domain of MMP-12, with the residues experiencing changes in the backbone chemical shift represented as red spheres (A). Plot of the ^{13}C (A) and ^{15}N (B) chemical shift differences between the microcrystalline and silica-entrapped samples from ^{15}N-^{13}C correlation spectra.

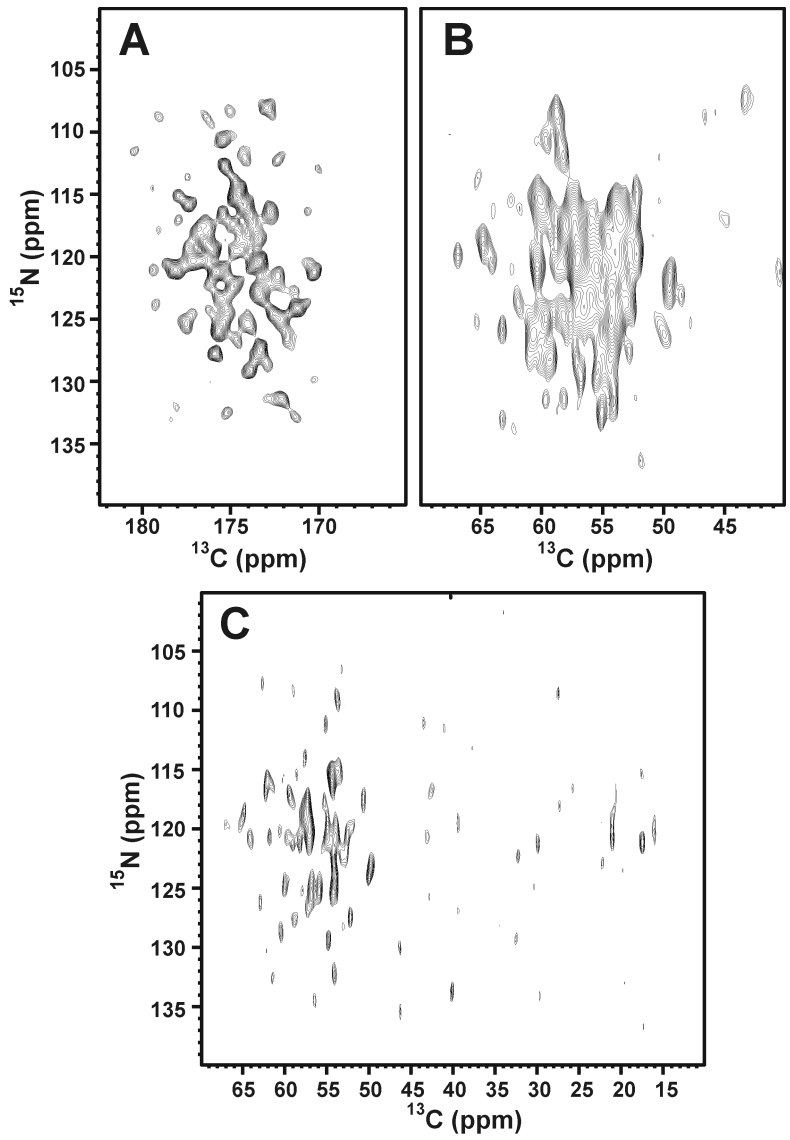

Figure S5. Two-dimensional ^{15}N-^{13}C correlation spectra (700 MHz, 11.5 kHz MAS) of MMP-12: **A**) NCO of the biosilica entrapped protein (192 scans); **B**) NCA of the biosilica-entrapped protein (192 scans); **C**) Aliphatic region of the NCOCX projection on the NCX plane of the biosilica-entrapped protein (192 scans).

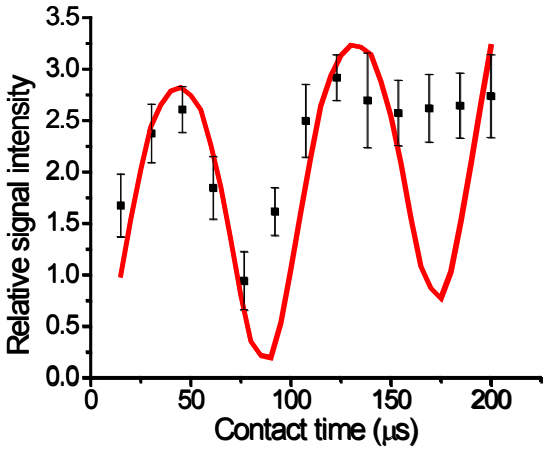

Figure S6. Experimental ^{1}H-^{13}C CP build-up curve compared with the theoretical curve for a $^{1}H_{\beta}$-$^{13}C_{\beta}$ pair of a threonine residue. Deviation at long contact times is due to spin diffusion that is not accounted for in the simulation.

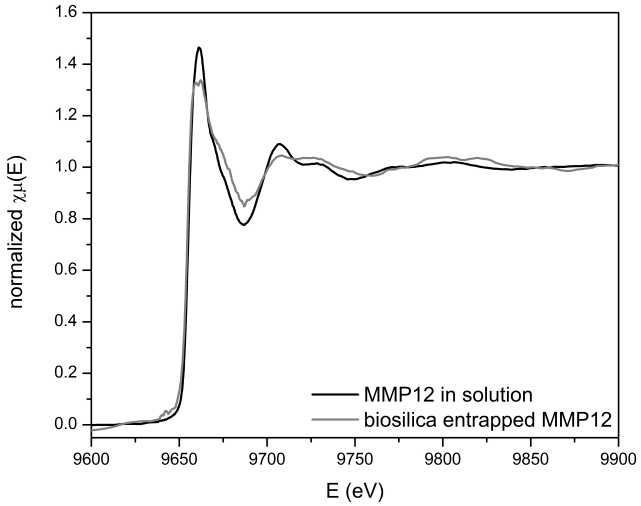

Figure S7. Normalized raw x-ray absorption edge spectra of MMP-12 in solution and entrapped in biosilica matrix.

Table S1. **Nonlinear curve fitting data analysis parameters of MMP12 samples**. Presented are the best-fit parameters of the first coordination shell, where N is the coordination number, R is the zinc-ligand bond distance and σ^2 is the Debye-Waller factor[a].

Sample	χ^2	N	R	σ^2
biosilica entrapped MMP12:NNGH[b]	8.28	4.96±0.64	1.97±0.01 (4xN/O)	3.64E-4±1.28E-3(4xN/O)
			1.78±0.03 (1xN/O)	7.1E-4±3.0E-3(1xN/O)
MMP12:NNGH	83.77	5.14± 0.48	2.02±0.01	6.73E-3±1.50E-3

[a]The raw EXAFS spectra were processed and analyzed following reported procedures (Grossman et al. Nat Struct Mol Biol. 2011 Sep 18;18(10):1102-8))

[b]NNGH - N-Isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid, a broad spectrum MMP inhibitor.

Supplementary text

The catalytic domain of MMP12 has been encapsulated in silica matrix by using poly-L-lysine as catalyst. The protein was inhibited with NNGH (N-Isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid) to reproduce the experimental conditions used to solve both the solution and the microcrystalline structure. 200 µL of MMP-12 (45 mg/mL in buffer A: 20 mM tris pH 7.2, 50 mM NaCl, 10 mM $CaCl_2$, 0.1 mM $ZnCl_2$), 50 µL of poly-L-lysine (10 mg/mL in H_2O, MW: 4000-15000 Da) were added in 1.5 mL vials and shaken for 10 min. To the resulting solution, 250 µl of silicic acid 100 mM in buffer A (freshly prepared from a stock solution of 1 M silicic acid obtained by addition of 850 µL of 1 mM HCl to 150 µL of pure tetramethyl orthosilicate, TMOS) were added and the mixture let react for 10 min at room temperature. After protein entrapment, a 4 mm rotor was filled with 14 vials of the biomineralized MMP12 for the SSNMR analysis. Only a relatively small fraction of the protein in solution was entrapped, the remaining fraction was recovered as such and could be used for the preparation of other samples.

The SOD and CAII enzymes were encapsulated using the same experimental procedure of MMP-12. Biosilification of SOD and CAII was carried out starting from 30 mg/mL of protein in 50 mM phosphate buffer at pH 7. SOD was purchased from Giotto Biotech (www.giottobiotech.com).

SSNMR Spectra were recorded on a Bruker Avance II spectrometer operating at 700 MHz ^1H Larmor frequency, equipped with a standard Bruker 4 mm DVT triple resonance probehead. MAS frequency was set to 11.5 kHz and the temperature was set to 270 K. The DARR spectrum of the biosilica entrapped protein shown in Figure 1, recorded was recorded with at 25 ms mixing time, 256 scans and 1024 indirect points, with an overall experimental time of about 7 days. NCA and NCO experiments were also recorded: the NCA transfer had a 15% transfer efficiency, while the NCO had 42%. Both experiments were acquired with 192 scans and 96 indirect points, with a total experimental time of about 12 h each (Figure S5).

The PDSD spectrum of MMP12 crystals was taken from reference 38. The PDSD spectrum of SOD1 crystals, taken from reference 40, was kindly provided by Prof. Roberta Pierattelli. Overall, the DARR mixing was preferred in the spectra acquired for the current manuscript because it ensures higher transfer efficiency.

X-ray absorption spectroscopy (XAS) - Samples were loaded into aluminum sample holders covered with Mylar tape and were frozen immediately in liquid nitrogen. The samples were then mounted inside a Displex closed-cycle helium cryostat, and the temperature was maintained at 20 K. XAS data collection was performed at the National Synchrotron Light Source at Brookhaven National Laboratory, beam line X3B. The spectra were recorded at the zinc K-edge at low temperature (20 K). The raw EXAFS spectra were processed and analyzed following reported procedures and the results are summarized in Table S1. Theoretical phases and amplitudes were calculated based on a model constructed from a three

dimensional structure of MMP-12 (pdb code: 1Y93). The analysis was performed on 1RMZ and 1Y93 PDB models and showed that the parameters are independent of the model used. The best fit was chosen. The various parameters were varied and fixed to examine the stability of each fit.