Supplementary Information

^{43}Ca MAS-DNP NMR of frozen solutions for the investigation of calcium ion complexation

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Table of Content.

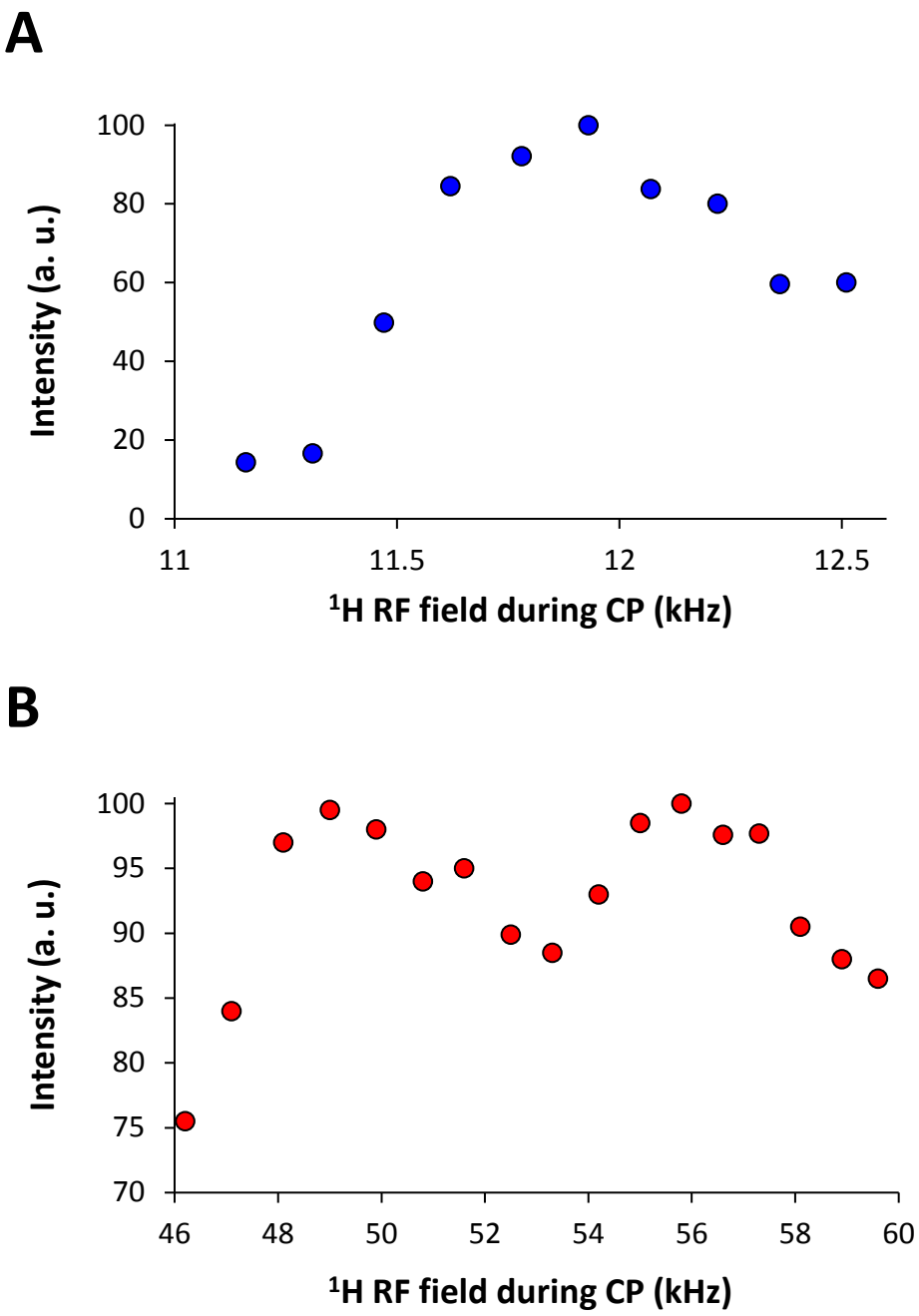


Figure S1. Optimization of LP (A) and HP (B) Hartman and Hahn conditions by varying the ^{1}H RF field during CP. ^{43}Ca RF fields were respectively set to 0.8 and 32 kHz.

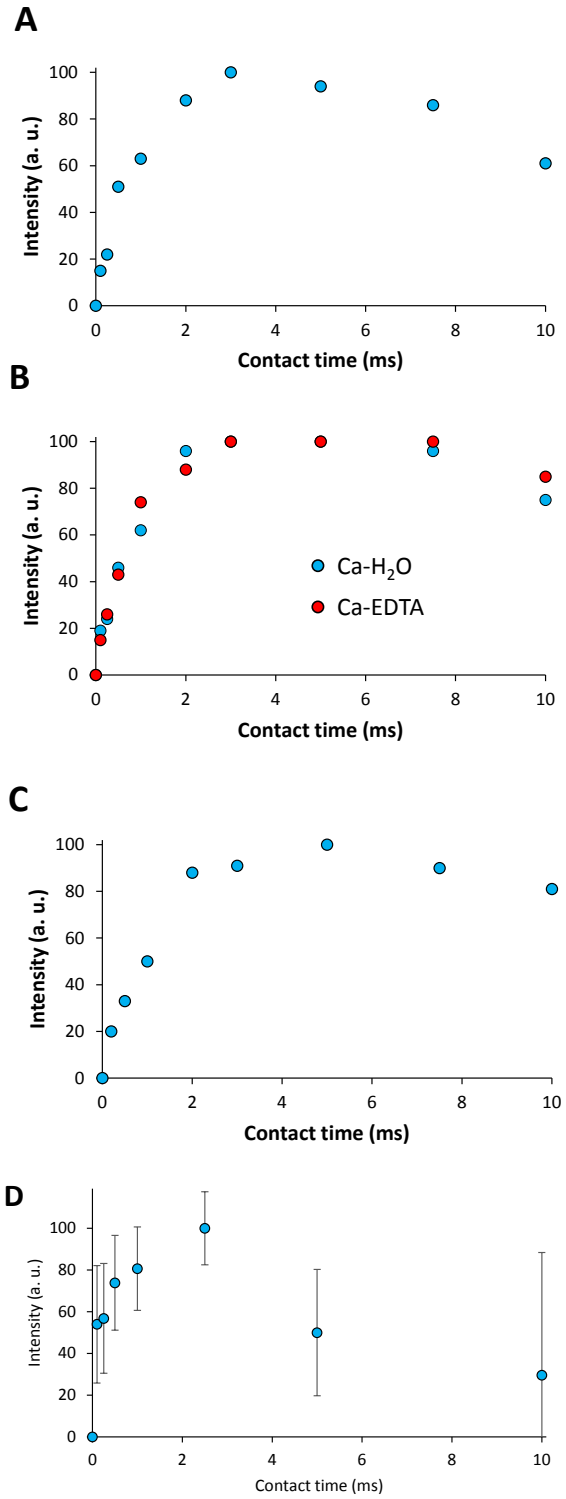


Figure S2. Contact time optimization for (A) Ca-H_2O, (B) Ca-EDTA-50 and (C) Ca-LAsp-100 using HP CP DNP MAS NMR at 100K. (D) Contact time optimization for Ca-H_2O using LP CP DNP MAS NMR at 100K

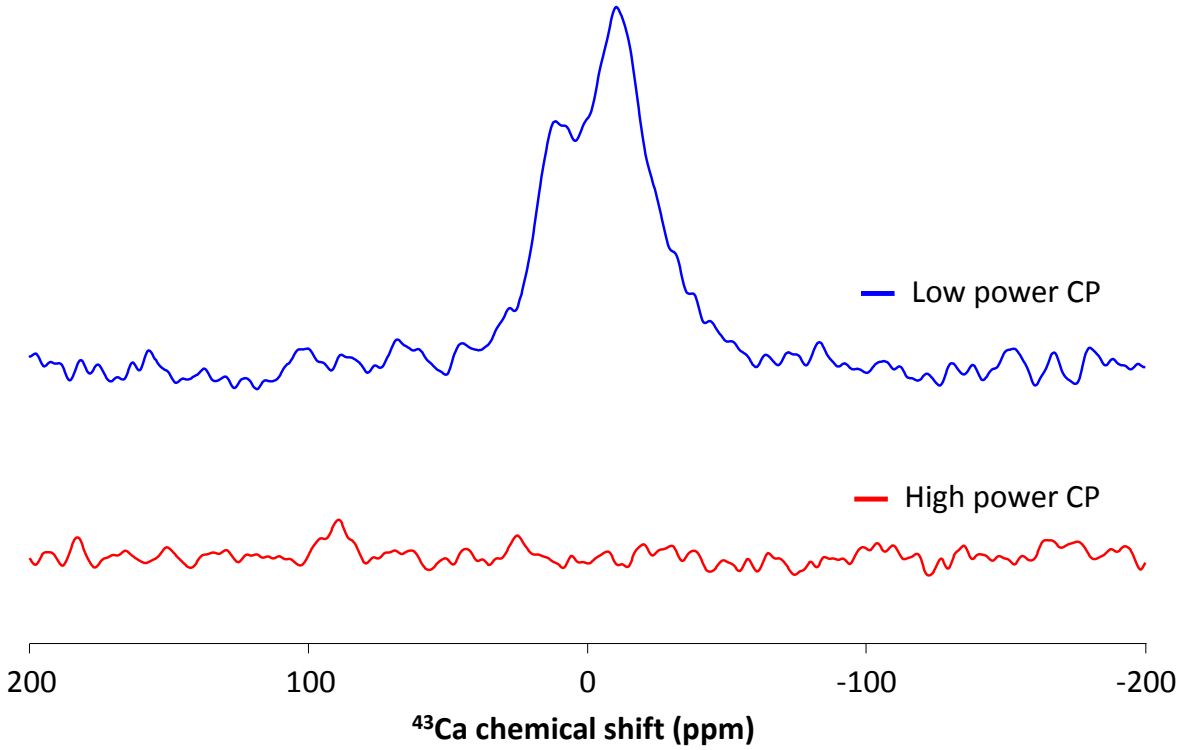


Figure S3. ^{43}Ca MAS-DNP NMR normalized spectra of ^{43}Ca-labelled hydroxyapatite sample acquired with HP (32 scans) and LP (160 scans) CP conditions. ^{43}Ca-labelled hydroxyapatite was prepared according to Wang et al. (*Nat. Mater.* volume 12, pages 1144–1153 (2013); DOI: 10.1038/nmat3787) and the corresponding powder impregnated with Glycerol-d8/D_2O/H_2O (volume ratio 2/3/5) containing 15 mM of Amupol.

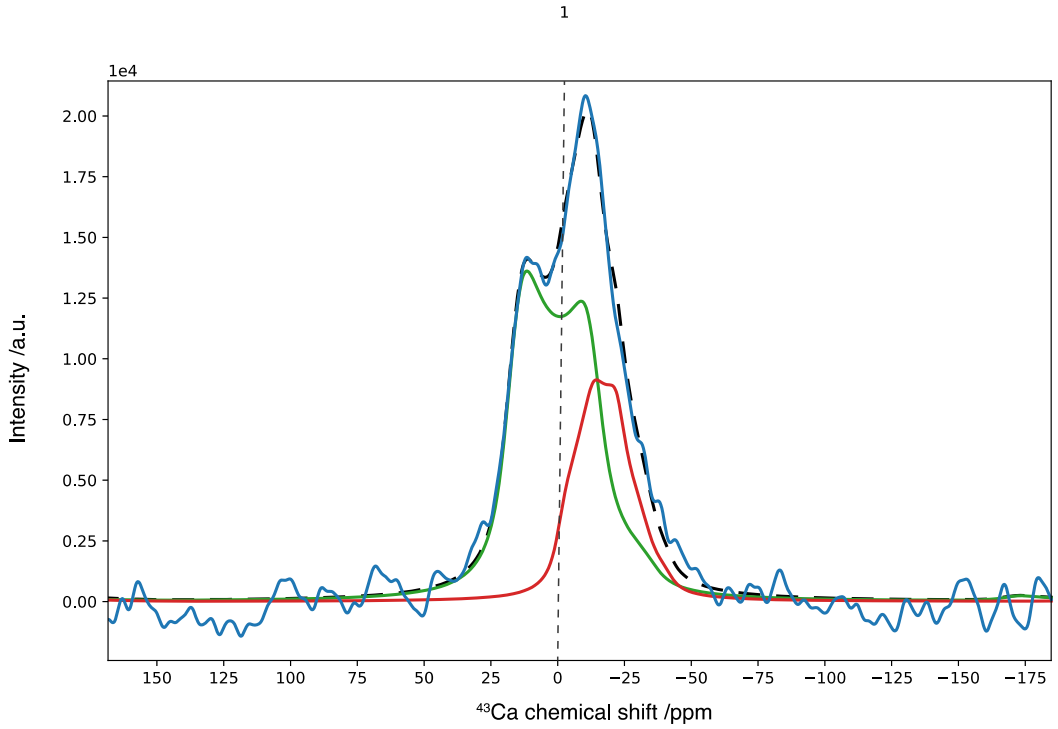


Figure S4. ^{43}Ca MAS-DNP NMR spectrum of the CT line shape of ^{43}Ca-labelled hydroxyapatite sample acquired with LP CP conditions (blue curve) and its best fitting: total spectrum (black dotted curve) and individual sites Ca(I)(red) and Ca(II) (green). Good quality fitting could only be obtained after including 2 Ca sites, in agreement with previous studies (Lee, D. *et al. Nat Commun* 8, 14104 (2017). DOI : 10.1038/ncomms14104 and Laurencin et al. *Magnetic Resonance in Chemistry* 2008, *46*(4), 347-350. DOI: 10.1002/mrc.2117). The best-fitting parameters for the two Ca sites are displayed in Table S1. The ^{43}Ca chemical shift was referenced by setting the center of gravity of the ^{43}Ca signal of HA at 9.4 T to 0 ppm (see main text for discussion).

Table S1. Best fit parameters obtained for the CT line shape of of ^{43}Ca-labelled hydroxyapatite sample. The fit was realized using ssNake (Van Meerten et al. *Journal of Magnetic Resonance* 2019, *301*, 56-66 ; DOI: 10.1016/j.jmr.2019.02.006).

HA CT best fit parameters	**δ_{iso} ^{43}Ca (ppm)**	**C_Q (MHz)**	**η_Q**
Ca(I)	2±3 11±9[a] 4.5±0.8[b]	2.3±0.2 2.6±0.4[a] 2.6±0.4[b]	0.5±0.1 0.4±0.3[a] 0.4±0.2[b]
Ca(II)	26±3 25±5[a] 17.5±0.8[b]	2.7±0.2 2.6±0.4[a]; 2.6±0.4[b]	0.2±0.1 0.4±0.1[a] 0.6±0.2[b]

[a] Lee et al. Nat. Comm. 2017, *8*(1), 14104. DOI : 10.1038/ncomms14104
[b] Laurencin and Smith *Progress Nucl. Magn. Reson.* 2013, 68, 1-40. DOI : 10.1016/j.pnmrs.2012.05.001

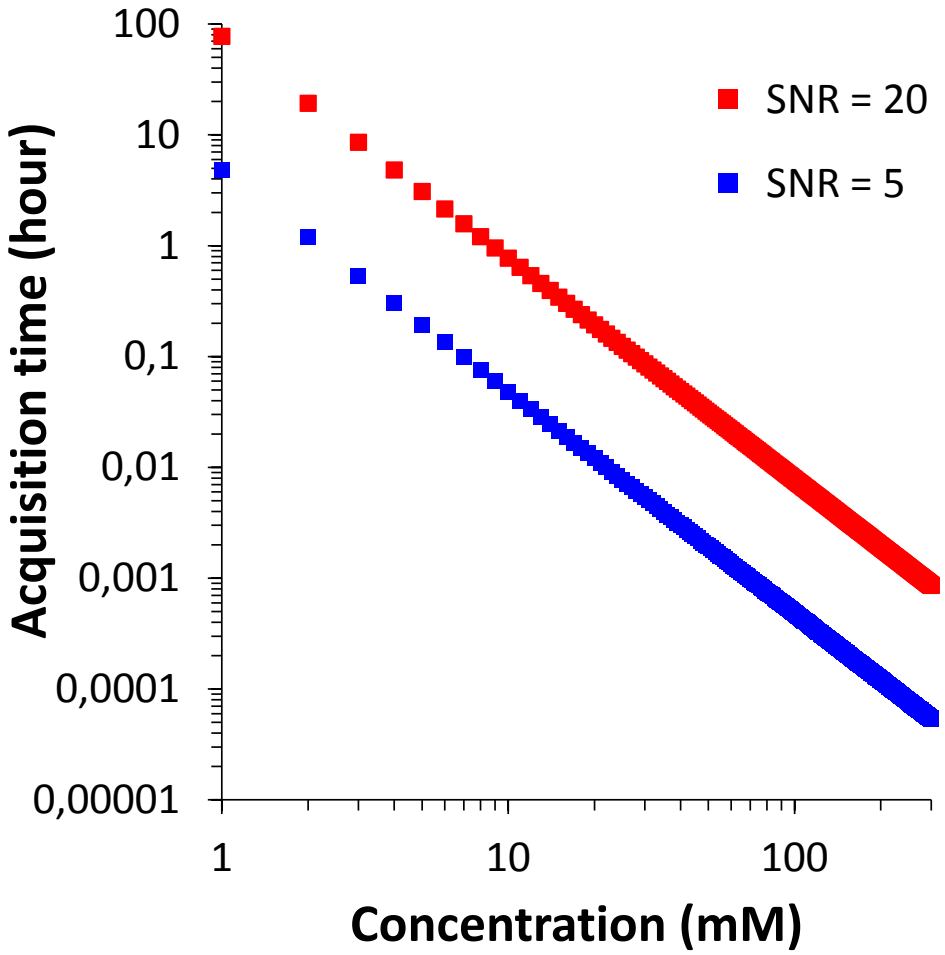


Figure S5. Theoretical acquisition time of a 1D ^{43}Ca MAS-DNP NMR spectrum for SNR = 20 (red squares) and SNR = 5 (blue squares) in HP CP conditions depending on the Ca^{2+} concentration (for 62,2% ^{43}Ca-labelled samples). For physiological concentrations of ^{43}Ca (*i.e.* between 10 and 2.5 mM), the acquisition of 1D spectrum with a SNR = 20 (*i.e.* allowing to further record 2D spectra) would take from 46 min. to 3 days, while a 1D spectrum with a SNR=5 (*i.e.* allowing a straightforward analysis of 1D spectra) would require from 3 min. to 4 hours. This simple calculation shows that analysis of Ca^{2+}-complex solutions at natural abundance would be feasible at high concentrations but extremely difficult in physiological concentration. As an example, a SNR = 5 for 1D ^{43}Ca NMR spectrum of 300 mM Ca^{2+} solutions would be obtained in 12h at natural abundance, in our conditions. Whereas the same SNR for a 10 mM Ca^{2+} solutions would be obtained after more than 1 year, justifying ^{43}Ca-labelling approach.

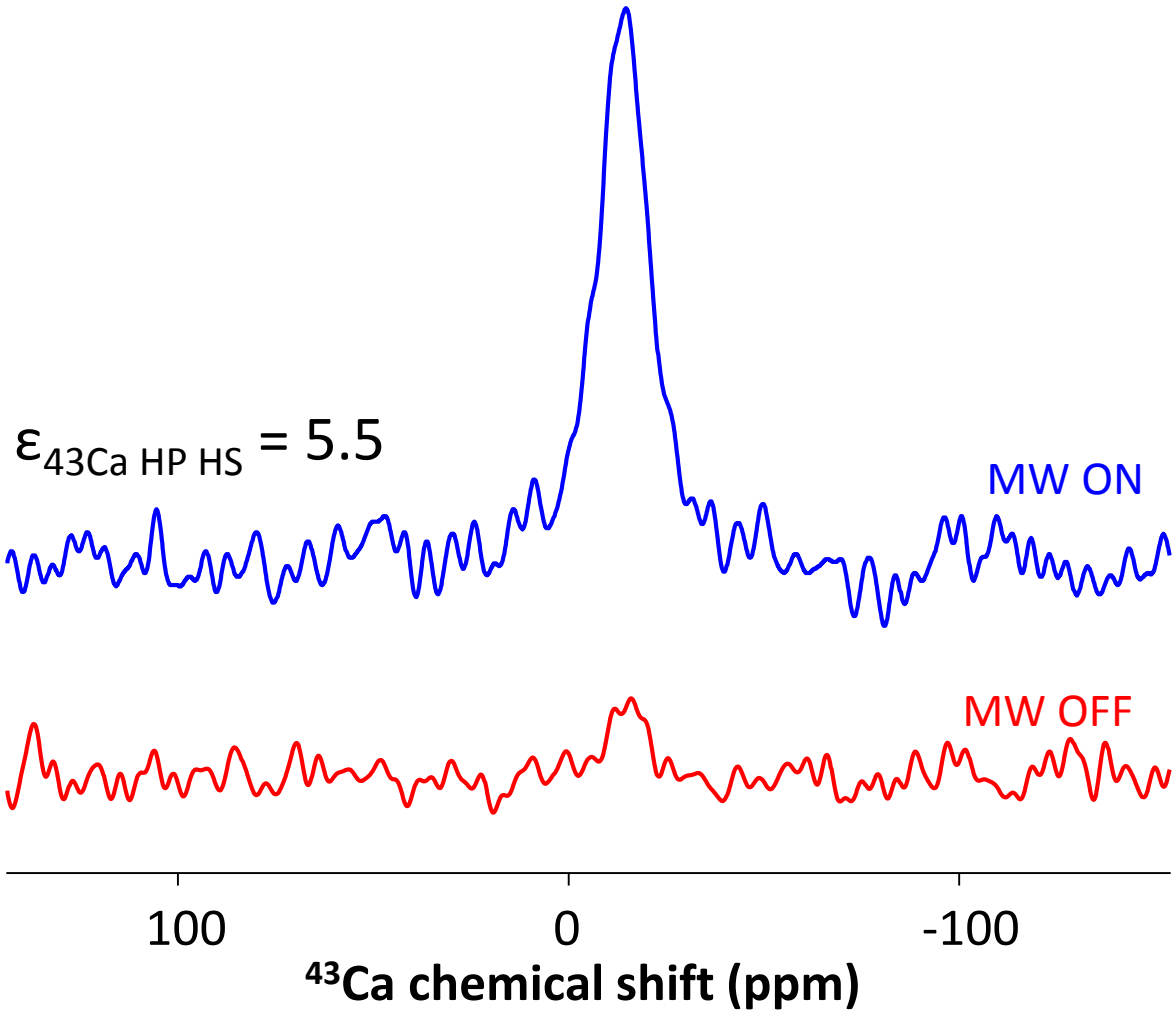


Figure S6. ^{43}Ca hyperbolic secant spectra of Ca-H_2O sample at 100K recorded with (top) and without (bottom) MW. Enhancement factor: $\varepsilon_{43Ca\ HS}$ = 5.5.

Interestingly, the HS experiment provides a significant DNP enhancement factor. Such ^{43}Ca enhancement probably arises from direct ^{43}Ca excitation that might be due to the large EPR linewidth of the AMUPol radical used here, which guarantees efficient polarization transfer over a broad range of nuclear Larmor frequencies.

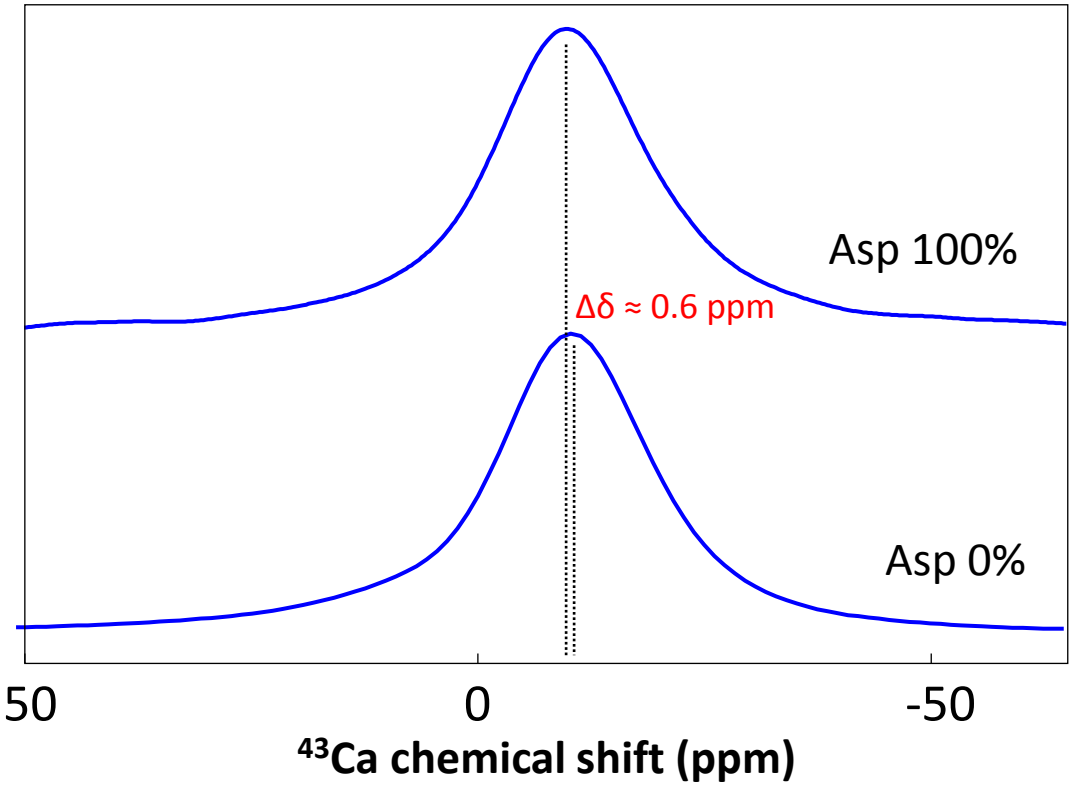
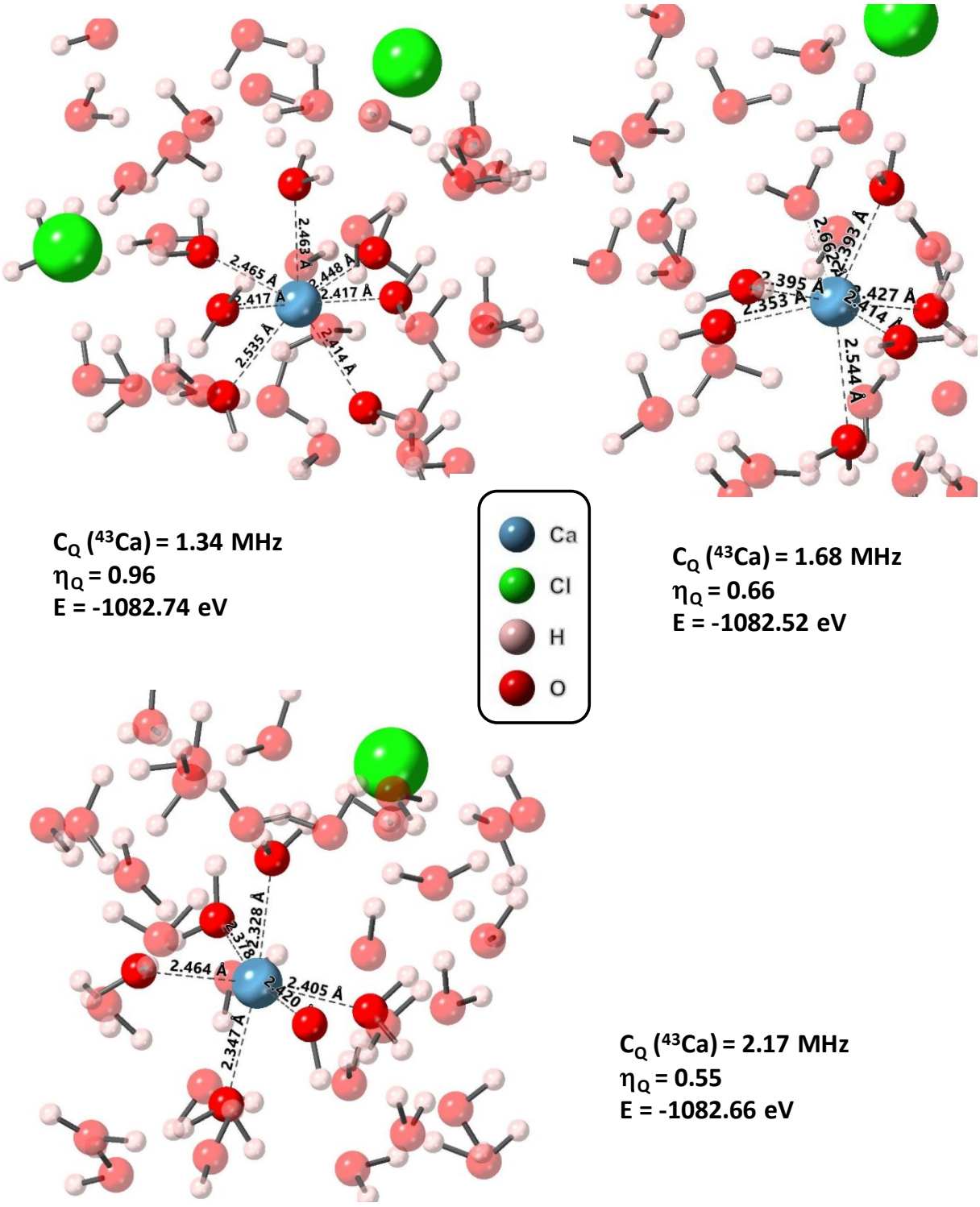


Figure S7. ^{43}Ca CPHP DNP MAS NMR spectra of Ca-Asp100 (top) and Ca-H_2O samples (bottom).

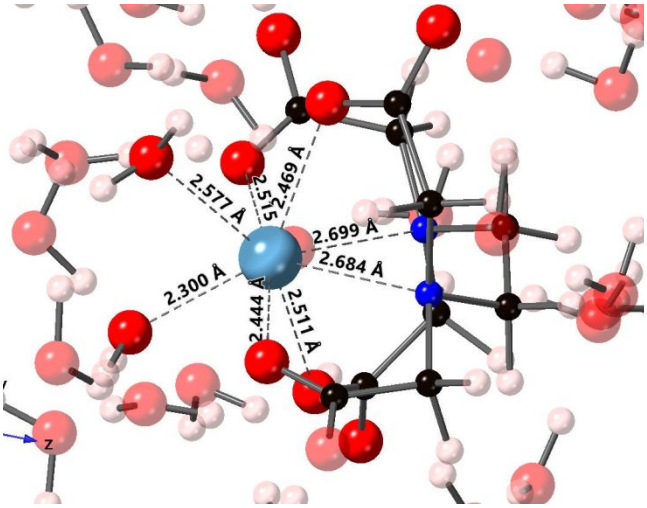


C_Q (^{43}Ca) = 1.34 MHz
η_Q = 0.96
E = -1082.74 eV

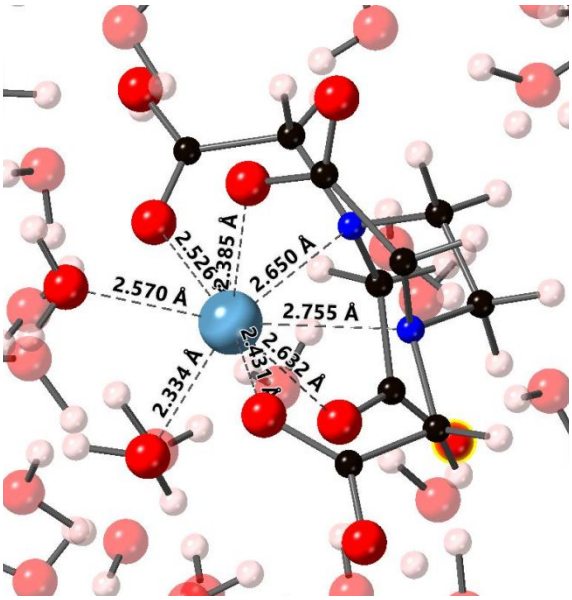
C_Q (^{43}Ca) = 1.68 MHz
η_Q = 0.66
E = -1082.52 eV

C_Q (^{43}Ca) = 2.17 MHz
η_Q = 0.55
E = -1082.66 eV

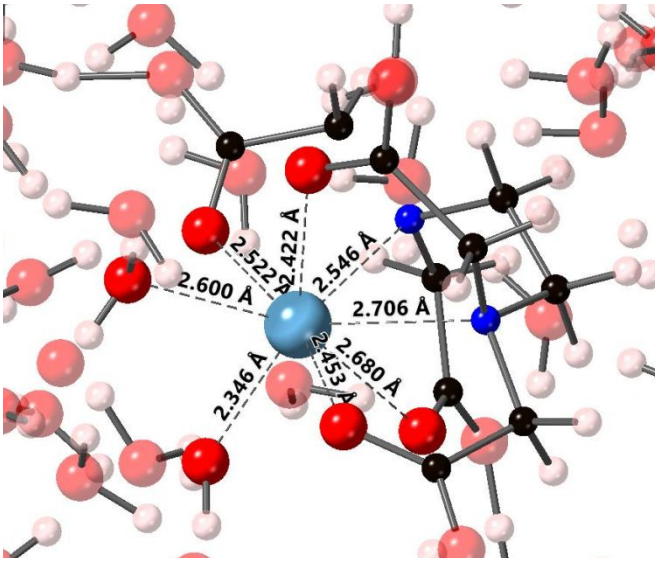
Figure S8. Low energy configurations obtained by DFT and corresponding calculated ^{43}Ca quadrupolar parameters for Ca^{2+} complexed with water molecules. Characteristic distances around Ca^{2+} are shown.



C_Q (^{43}Ca) = 1.30 MHz
η_Q = 0.53
E = -1003.59 eV



C_Q (^{43}Ca) = 1.04 MHz
η_Q = 0.49
E = -1003.70 eV



C_Q (^{43}Ca) = 0.92 MHz
η_Q = 0.93
E = -1003.95 eV

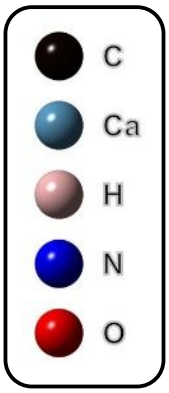
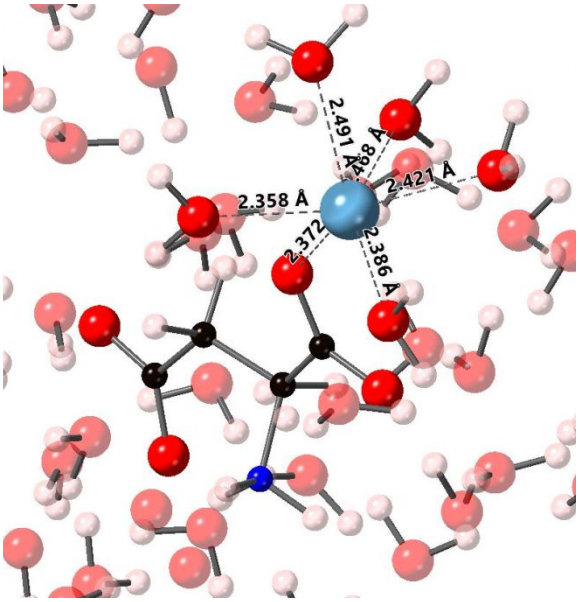
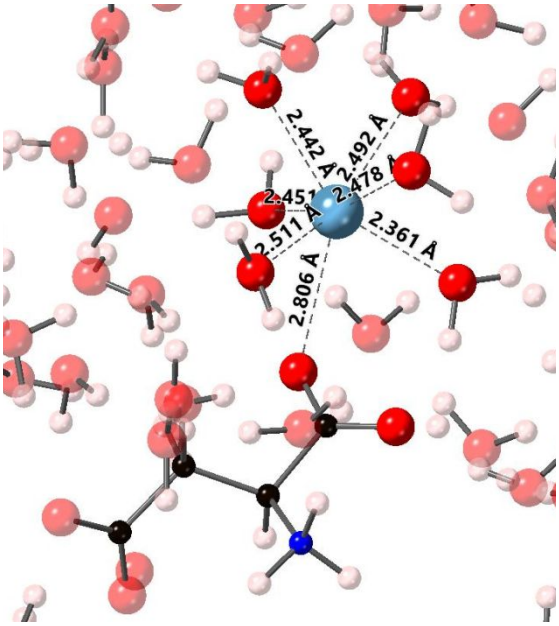


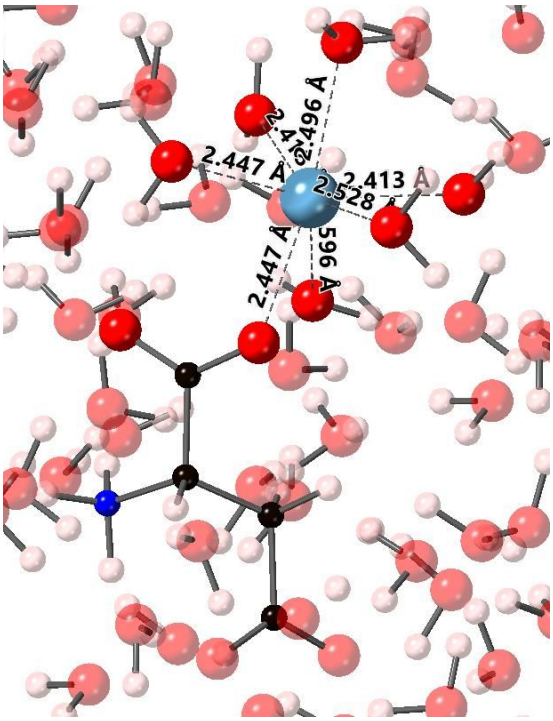
Figure S9. Low energy configurations obtained by DFT and corresponding calculated ^{43}Ca quadrupolar parameters for Ca^{2+} in interaction with EDTA and water molecules. Characteristic distances around Ca^{2+} are shown.

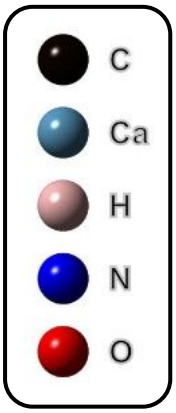


C_Q (^{43}Ca) = 2.55 MHz
η_Q = 0.82
E = -709.38 eV



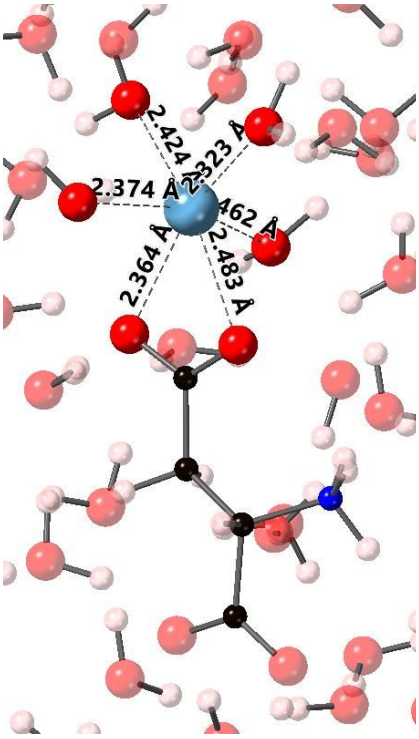
C_Q (^{43}Ca) = 1.37 MHz
η_Q = 0.51
E = -711.62 eV



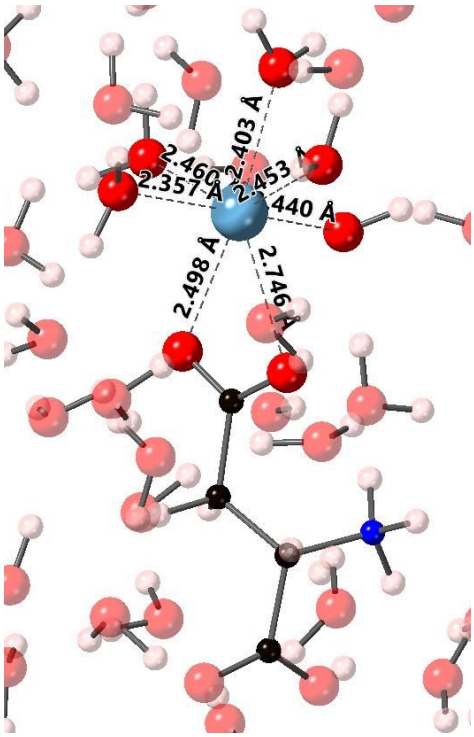


C_Q (^{43}Ca) = 1.29 MHz
η_Q = 0.72
E = -711.62 eV

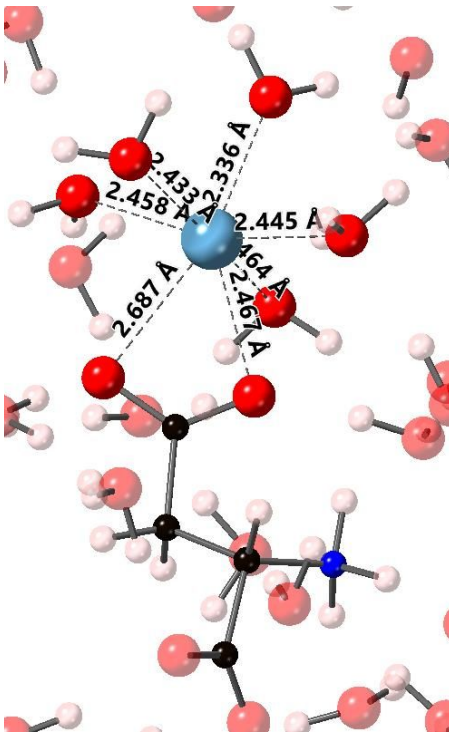
Figure S10. Low energy configurations obtained by DFT and corresponding calculated ^{43}Ca quadrupolar parameters for L-Asp in interaction through C_1OO^- with Ca^{2+}. Characteristic distances around Ca^{2+} are shown.

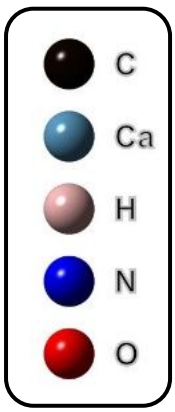


C_Q (^{43}Ca) = 2.42 MHz
η_Q = 0.33
E = -709.56 eV



C_Q (^{43}Ca) = 2.42 MHz
η_Q = 0.33
E = -709.56 eV





C_Q (^{43}Ca) = 1.90 MHz
η_Q = 0.61
E = -711.86 eV

Figure S11. Low energy configurations obtained by DFT and corresponding calculated ^{43}Ca quadrupolar parameters for L-Asp in interaction through C_4OO^- with Ca^{2+}. Characteristic distances around Ca^{2+} are shown.