Supplementary Material (ESI) for PCCP

Supplementary Information

Probing the calcium and sodium local environment in bones and teeth using multinuclear solid state NMR and X-ray absorption spectroscopy

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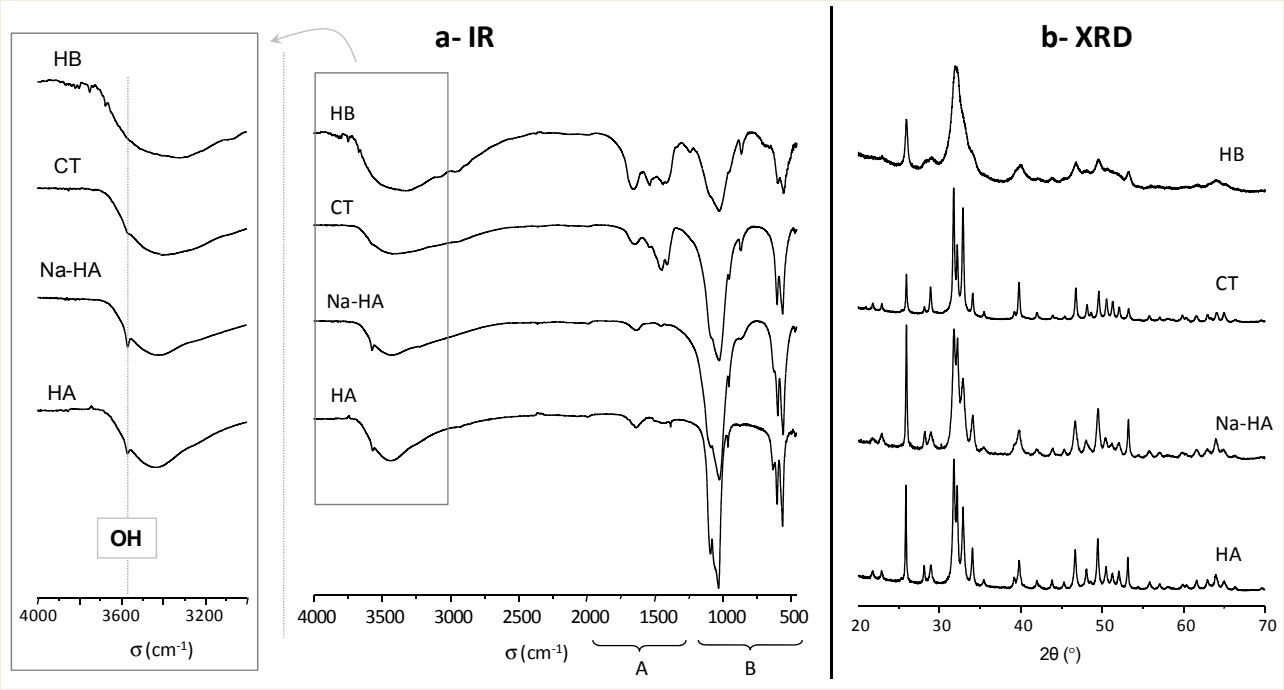
A- *"Background" Characterizations of HB, CT and the synthetic models HA and Na-HA*

B- *Additional analyses of the calcium environment in HB, CT, and other synthetic models*

C- *Additional analyses of the sodium environment in HB, CT and Na-HA*

Figure S1:
(a) IR spectra and (b) XRD powder patterns of HA, Na-HA, HB and CT.



Description of the data:

On the IR spectra, "A" and "B" correspond to sections where vibrations are mainly due to the organic and mineral components, respectively.

According to XRD, the mineral phase of the tooth sample (CT) is very crystalline, presumably because of the presence of enamel. Interestingly, the relative intensities of the diffraction peaks for the tooth differ from those of the synthetic hydroxyapatites. This could result from the presence of substituting ions in the lattice, and/or from preferred orientations of the crystallites.

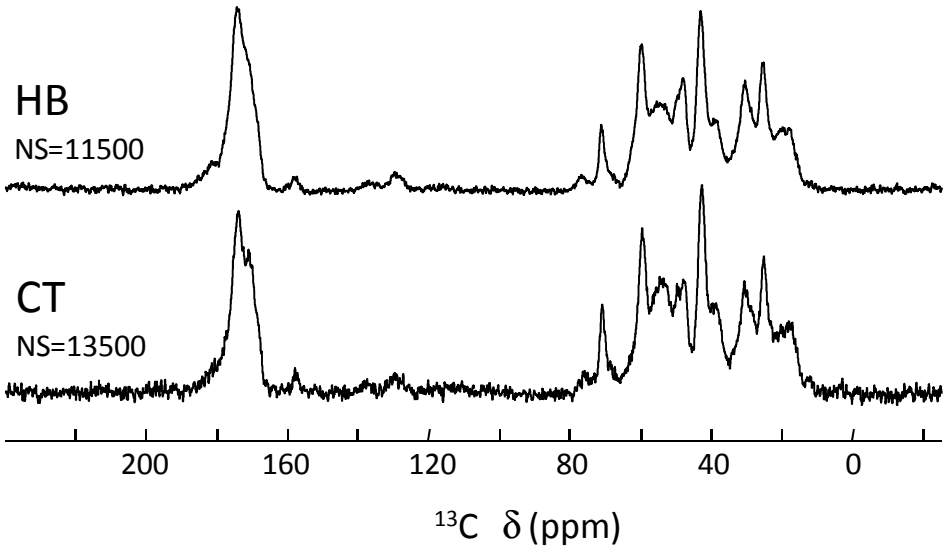
It is noteworthy that in the case of Na-HA, only peaks belonging to a hydroxyapatite phase are detected by XRD, and that the sample is crystalline.

Experimental Details:

IR spectra were recorded from KBr pellets on a Perkin Elmer GX FTIR spectrometer between 400 and 4000 cm^{-1}.

X-Ray diffraction patterns were collected at room temperature with a PAnalytical X'Pert Pro MPD X(G23) diffractometer using Cu-Kα radiation. Measurements were performed in the 2θ range between 20° and 70°, with a step size of 0.013°. In the case of Ca-HA, Na-HA and CT, the counting time per step was 2 s, whereas in the case of HB, a counting time per step of 12 s was used, because of the much lower crystallinity of the sample.

Figure S2: $^{13}C\{^{1}H\}$ CPMAS NMR spectra of HB and CT.



Description of the data:

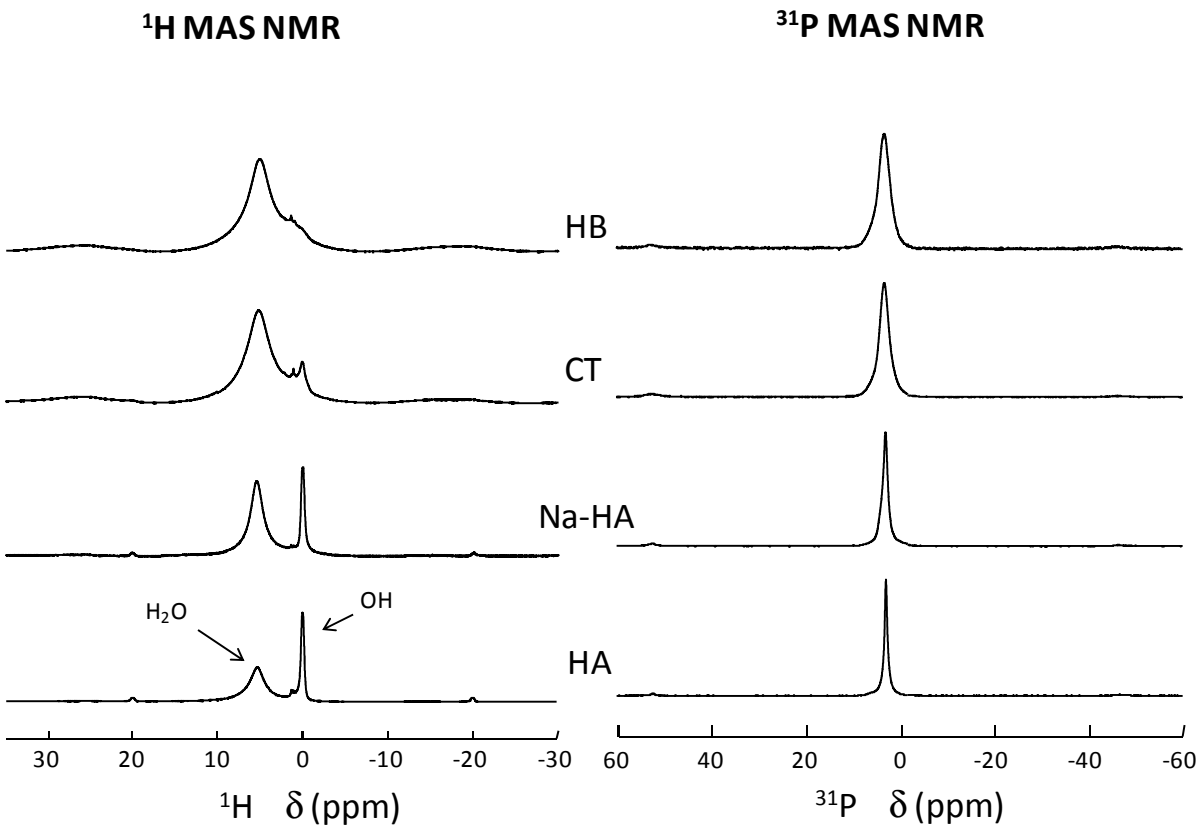
These spectra show that HB contains more collagen than CT (in agreement with IR spectra in Figure S1). This is not surprising given that in the tooth sample analysed, the enamel (which is ~97% apatite mineral) had not been separated from the dentin and cement.

Experimental details:

$^{13}C\{^{1}H\}$ CPMAS (Cross Polarisation MAS) spectra of HB and CT were recorded at 7.1 T, using a Bruker 4 mm MAS probe spinning at 8.5 kHz, with a contact time of 2 ms, and 100 kHz TPPM ^{1}H decoupling (A. E. Bennett, C. M. Rienstra, M. Auger, K. V. Lakshmi and R G. Griffin, *J. Chem. Phys.,* 1995, **103**, 6951). A recycle delay of 5 s was used, and the number of transients (NS) acquired for each sample is indicated next to each spectrum.

The ^{13}C NMR spectra are referenced to L-alanine (carbonyl at 177.8 ppm)

Figure S3: ^{1}H and ^{31}P MAS NMR spectra of HA, Na-HA, HB and CT at 11.75 T.



Description of the data:

In the case of HA and Na-HA, the ^{1}H NMR water signal (at ~5 ppm), which corresponds mainly to the surface water molecules (see J. P. Yesinowski and H. Eckert, *J. Am. Chem. Soc.,* 1987, **109**, 6227), is clearly distinct from the strong OH peak at ~0 ppm. In contrast, because water is one of the main constituents of bone and teeth, its signal is much broader and more intense on the ^{1}H NMR spectra of HB and CT, and partly overlaps the peaks corresponding to the organic and mineral phases (see references 24 and 44 in the main text).

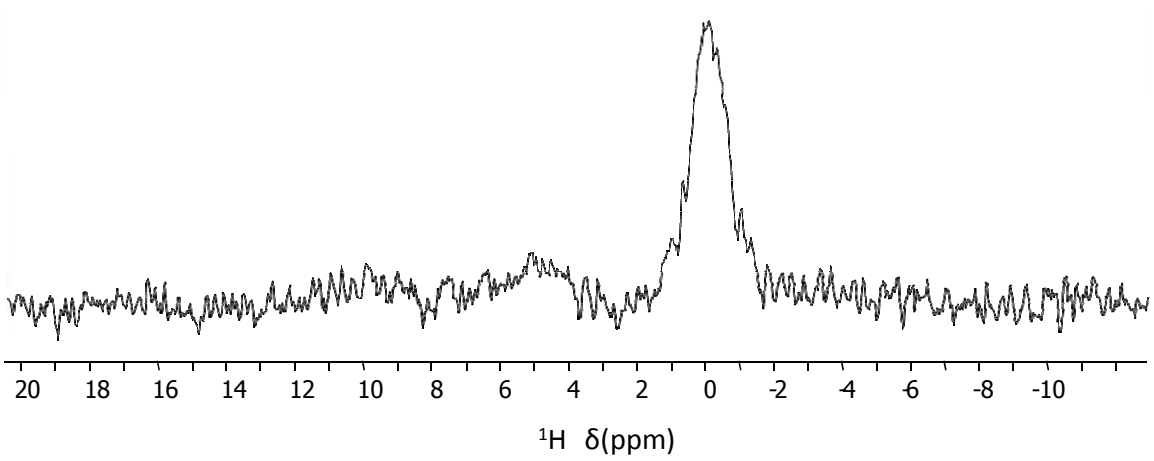
In agreement with previous ^{31}P MAS NMR studies of bone and teeth, the ^{31}P NMR lineshapes of HB and CT are broader than for the synthetic compounds.

Experimental details

^{1}H and ^{31}P magic-angle spinning (MAS) NMR spectra were recorded at 11.75 T using a Bruker 4 mm MAS probe spinning at 10 kHz. For the ^{1}H spectra, 8 transients were acquired with a recycle delay of 15 s. In the case of ^{31}P, 16 transients were acquired with a recycle delay of 60 s.

The ^{1}H and ^{31}P NMR spectra were referenced to TMS (at 0 ppm) and solid $NH_4H_2PO_4$ (at 0.9 ppm), respectively.

Figure S4:
$^{1}H\{^{31}P\}$ CPMAS NMR spectrum of HB at 14.1 T.



Comment:

In contrast with CT, the OH peak for HB is not detectable by IR. However, the hydroxyl peak at ~0 ppm clearly appears on this $^{31}P\{^{1}H\}$ CPMAS NMR spectrum.

Experimental Details:

The $^{31}P\{^{1}H\}$ CPMAS NMR spectrum was recorded using a contact time of 2.5 ms (see T. Isobe, S. Nakamura, R. Nemoto, M. Senna, and H. Sfihi, *J. Phys. Chem. B;* 2002, **106**, 5169). The spectrum was recorded at 14.1 T with a Bruker 4mm probe spinning at 10 kHz. 720 transients were acquired with a recycle delay of 60 s. The ^{1}H NMR spectrum is referenced to TMS (at 0 ppm).

Table S1. Na/Ca molar ratios in Na-HA, HB and CT, as determined by ICP-MS.

	Na-HA	**Cow Tooth**	**Horse Bone**
x(Na)/x(Ca) (± 0.004)	0.122	0.054	0.081

Comment:

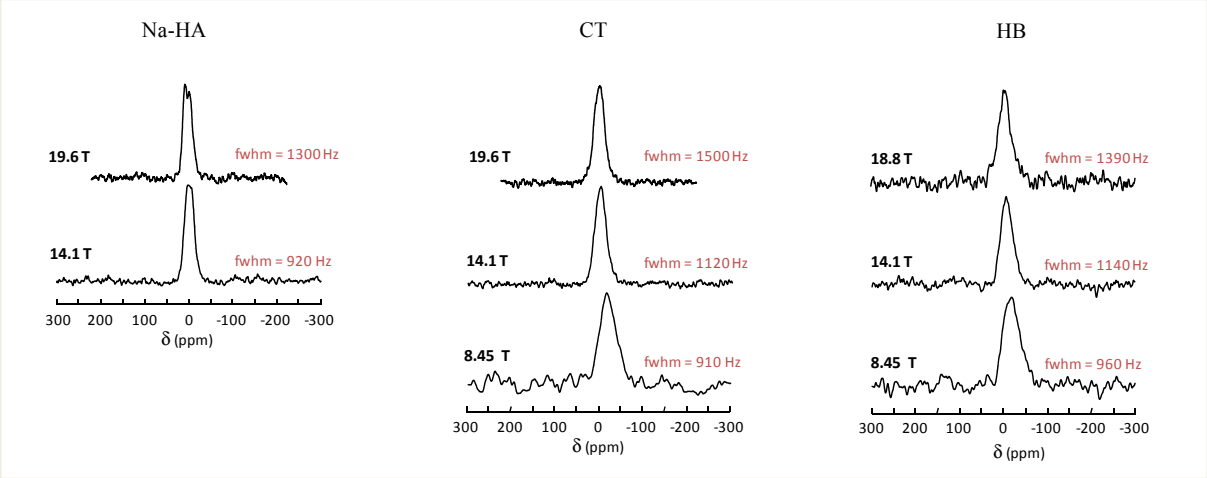
The presence of sodium in the natural apatite was first detected by EDXS, but accurate Na/Ca molar ratios were determined by ICP-MS.

In the case of HB and CT, it is noteworthy that no trace of NaCl (which could have come from residual body fluid in the HB and CT samples) was detected by ^{23}Na MAS NMR.

Experimental Details

Measurements were performed by *ICP MS* on an Agilent 7500CX instrument.

Figure S5: ^{43}Ca MAS NMR spectra of Na-HA, CT and HB at different magnetic fields.



Comment:

The full width at half maximum (fwhm) is indicated next to each spectrum. Errors on these values are 20 Hz.

Details on the recording conditions can be found in the Experimental section of the text.

Figure S6: ^{43}Ca MAS NMR spectra of HA, Na-HA, CT and fluoroapatite at 19.6 T.

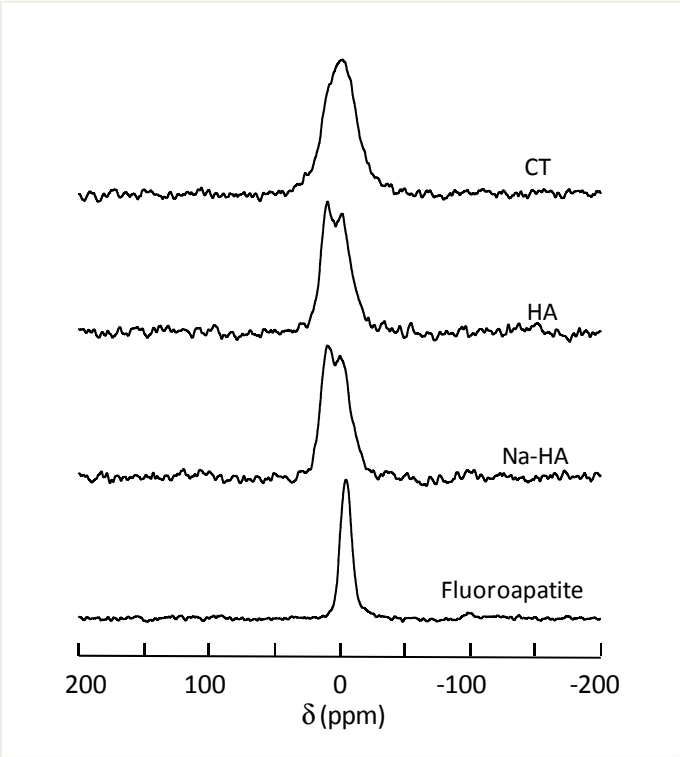


Table S2 Fitting parameters of the Ca K-edge EXAFS spectrum of HA, CT and HB (Figure 3).

For each compound, the fits were performed supposing an apatite-like environment around the calcium. In each shell, the number of atoms was kept constant (and equal to those found in crystalline HA), while the average distance R and the Debye Weller factor $2\sigma^2$ were varied.

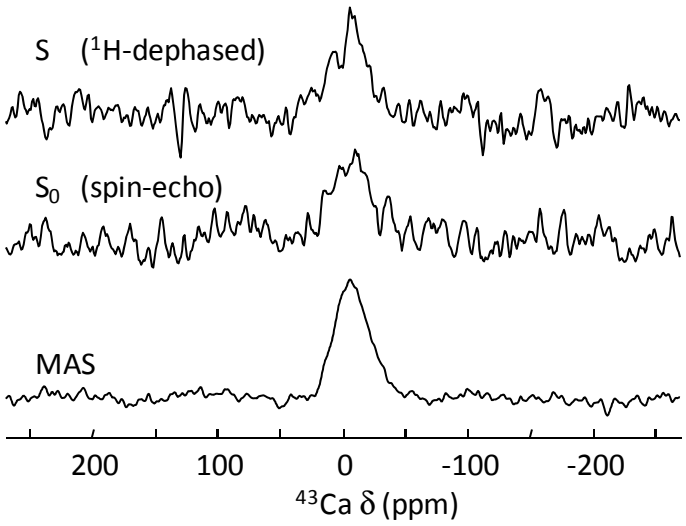
For HA, the shells used for the fit are very similar to those proposed in ref. 79, and the quality of the fit is comparable.

For HB and CT, due to the loss of long-range ordering, only 3 shells (2 Ca and 1 P) were considered (like in the EXAFS study of amorphous calcium phosphate – see J. E. Harries, D. W. L. Hukins and S. S. Hasnain, *J. Cryst. Growth,* 1987, **84**, 563), and the R and $2\sigma^2$ values of the Ca-O shells were optimized. It is noteworthy that when fitting the spectra of HB and CT with only 1 Ca-O sphere, the quality of the fit is strongly reduced.

Typical errors on the distances and Debye Weller factors in these fits are ±0.02 Å in R and 20% in $2\sigma^2$.

	Atom	N (atoms)	R (Å)	$2\sigma^2$ (10^{-2} nm^2)
HA	**O**	5.4	2.37	0.012
	O	3.0	2.55	0.018
	P	2.4	3.15	0.018
	Ca	0.8	3.51	0.011
	O	6.0	3.93	0.050
	Ca	8.4	4.07	0.049
	O	4.8	4.51	0.023
	O	6.6	4.73	0.050
	Average Ca-O distance in the 1st coordination sphere		**2.43**	
CT	**O**	5.4	2.38	0.017
	O	3.0	2.58	0.029
	P	2.4	3.15	0.024
	Average Ca-O distance in the 1st coordination sphere		**2.45**	
HB	**O**	5.4	2.39	0.033
	O	3.0	2.65	0.032
	P	2.4	3.17	0.022
	Average Ca-O distance in the 1st coordination sphere		**2.48**	

Figure S7: Natural-abundance $^{43}Ca\{^{1}H\}$ REDOR NMR spectra of HB.



Description of the data:

Spin echo (S_0) and ^{1}H dephased (S) REDOR spectra were recorded in ~ 40 hours each, and processed using the same phase parameters. The MAS spectrum of bone is represented for comparison.

There seems to be a very slight decrease in intensity of the high frequency component of the signal on the ^{1}H dephased spectrum.

Experimental Details:

The natural abundance $^{43}Ca\{^{1}H\}$ REDOR experiment was carried out at 14.1 T on HB, using a Doty 7 mm MAS probe spinning at 5500 ± 5 Hz. The ^{43}Ca $\pi/2$ and π pulses were 18 and 36 μs, respectively. ^{1}H-dephasing π pulses of 16.2 μs were used with a dephasing period of 3.63 ms. These pulses were phase cycled according to the XY-8 scheme. 2800000 transients were collected with a recycle delay of 0.05 s. ~40 hours were needed to record each spectrum.

Figure S8: ^{23}Na MAS NMR spectra of Na-HA, HB and CT at different magnetic fields.

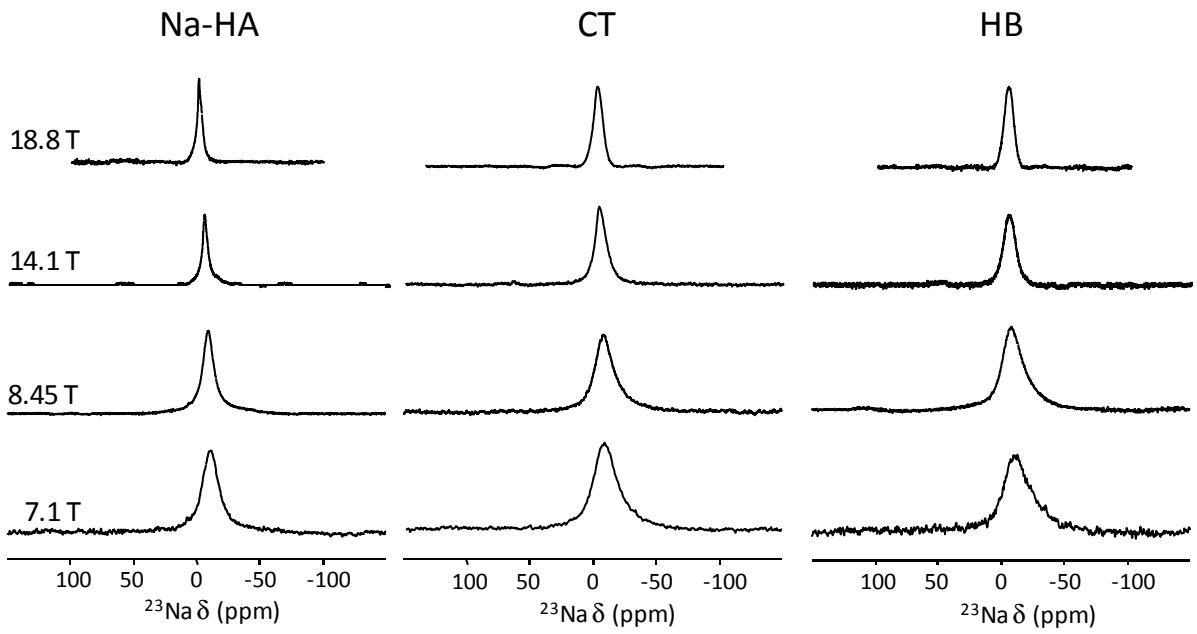
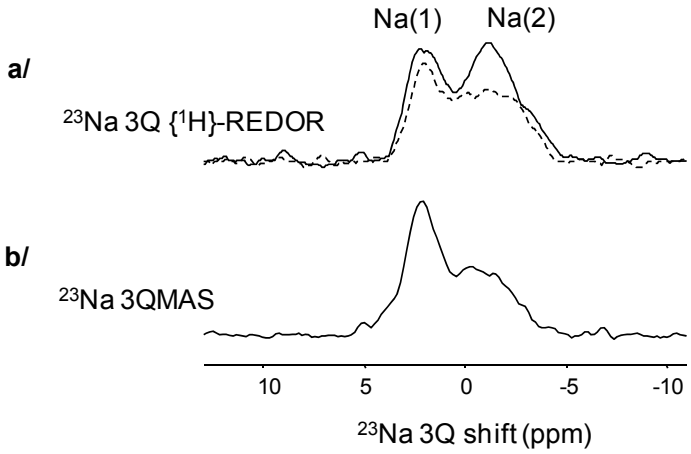


Figure S9: ^{23}Na {^{1}H} 3Q-REDOR study of Na-HA

(a) 3Q projections of 2D ^{23}Na {^{1}H} 3Q-REDOR spectra. The spectrum with a solid line corresponds to a normal spin-echo, and the dashed one was obtained after reintroducing π pulses on the ^{1}H side.
(b) 3Q projection of the ^{23}Na 3QMAS spectrum represented in Figure 5b.



Comment:

The comparison of the two ^{23}Na {^{1}H} 3Q-REDOR spectra shows that only the low-frequency component is affected by ^{1}H recoupling pulses, meaning that it corresponds to sodium cations in close proximity with protons. This is in agreement with the ^{1}H{^{23}Na} R^{3}-HMQC spectrum of Na-HA shown in the article.

The comparison of the 3Q projections of the ^{23}Na 3QMAS (bottom) and of the ^{23}Na {^{1}H} 3Q-REDOR spectrum in the *absence* of ^{1}H pulses, which thus corresponds to a spin-echo (top, solid line), demonstrates that the two sodium sites also have different T_2 relaxations.

Experimental Details:

The ^{23}Na{^{1}H} 3Q-REDOR (triple quantum Rotational Echo Double Resonance – C. Fernandez, D. P. Lang, J. P. Amoureux and M. Pruski, *J. Am. Chem. Soc.,* 1998, **120**, 2672) spectra of Na-HA were collected at 14.1 T with a Bruker 4 mm MAS probe spinning at 10 kHz. The ^{23}Na 3Q excitation and conversion pulses were 4.5 and 2.0 μs, respectively, with a solid-rf of ~100 kHz. The solid-rf of the selective π/2 and π pulses were 14 and 80 kHz. A rotor-synchronized 1.2 ms ^{1}H-dephasing period was used, with π pulses of 7.75 μs, which were phase cycled according to the XY-8 scheme. The spectral widths for both f_1 and f_2 were 20 kHz. 32 t_1 increments were collected with a dwell time of 50 μs. A total of 11520 transients were collected by the States method for each t_1 increment, and a recycle delay of 0.05 s was used.