Supporting Information

Milliseconds time-scale controlled freeze-quench for solute intermediates analysis by solid-state NMR

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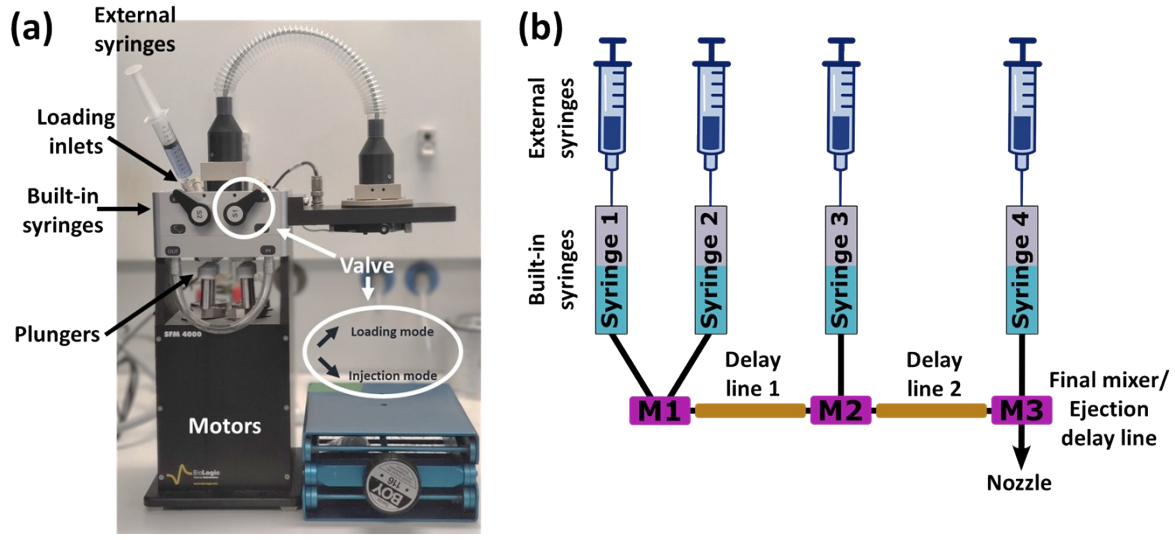

Figure SI1. (a) An image of the SFM-4000 used in this work with a schematic diagram is shown in (b). The solutions are transferred from **external syringes** (10 mL) through the **loading inlets** to the **built-in syringes** (10 mL) with the help of the **plungers** controlled by the stepping **motors**. This process is managed by the BIO-KINE software (BioLogic). After loading the **built-in syringes**, the **valves** are changed to the injection mode (from facing up to down positions). Solutions are transferred to the **mixers (M1, M2 or M3)**. Finally, the mixture is pushed to the **nozzle** and sprayed out in cold isopentane.

In this work, only two solutions were mixed using syringes 3 and 4, where they were mixed in a **mixer M3 (final mixer/ ejection delay line)**.

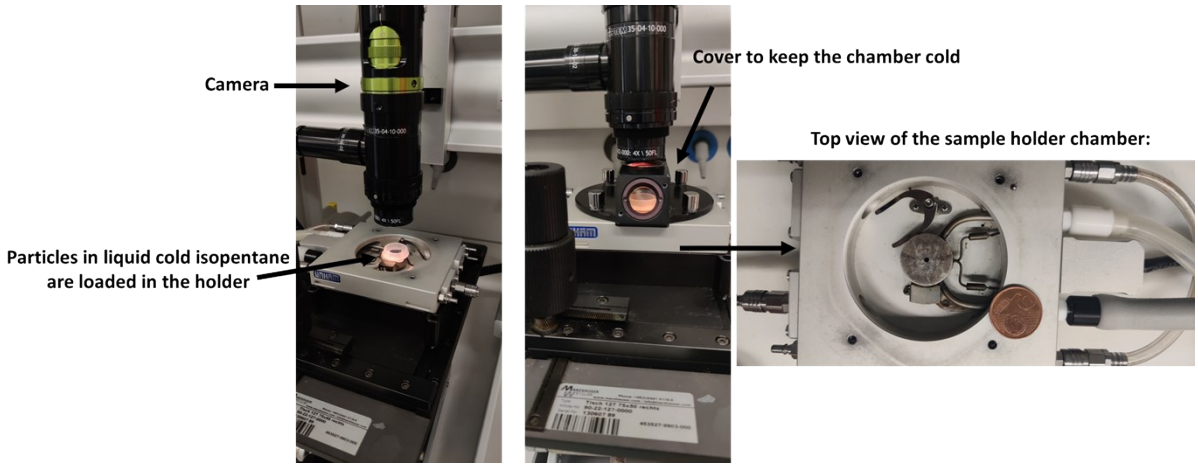

Figure SI2. Home-built setup for imaging the particles. The solution of cold isopentane containing frozen particles is loaded on the sample holder stored in a chamber. This chamber contains a stage that is cooled down to ensure the sample holder reaches -100 ^{0}C. The cover is used to maintain the chamber cold. Cooled nitrogen gas is passed through this chamber to maintain the temperature around -100 ^{0}C.

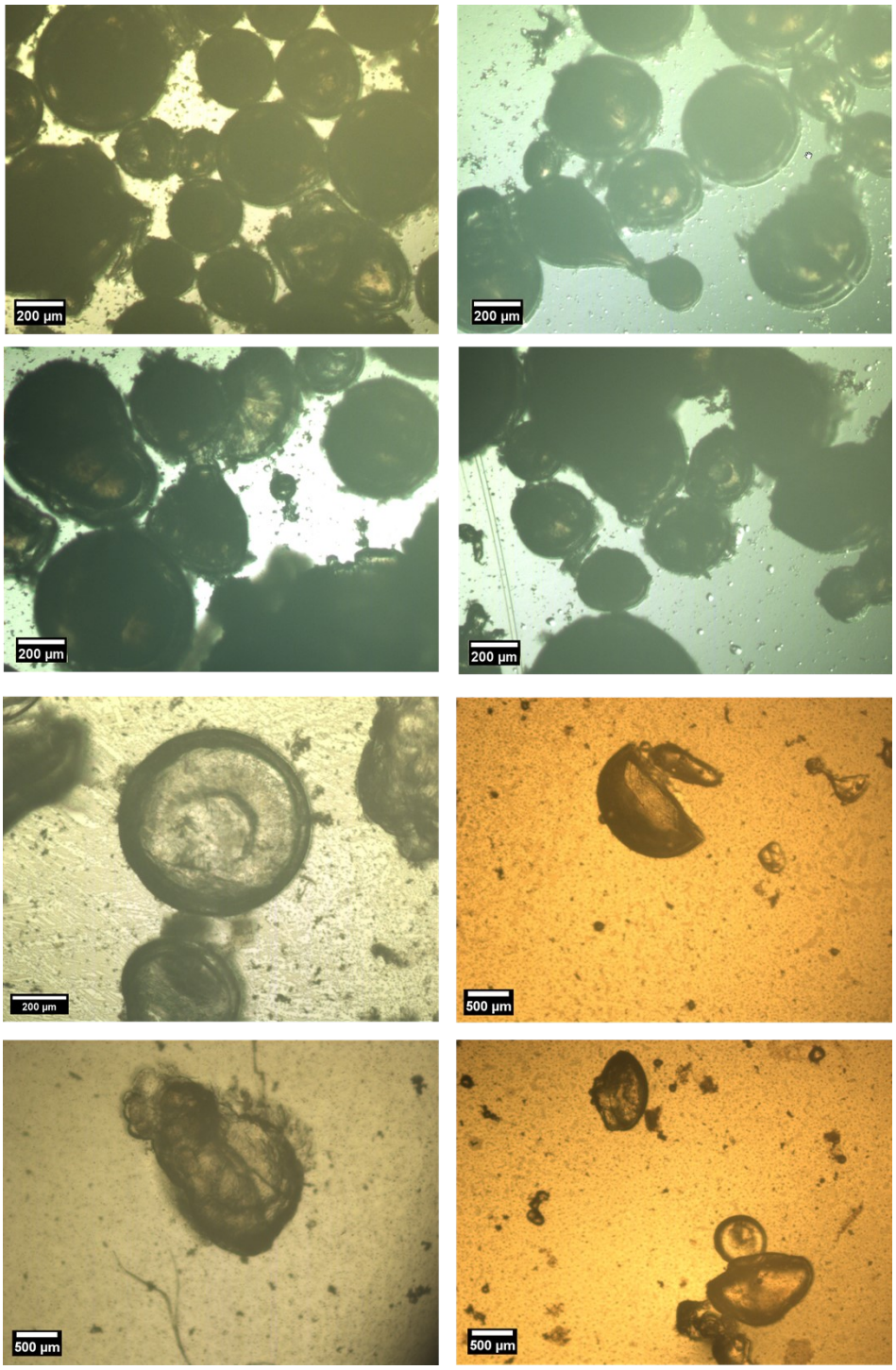

Figure SI3. Optical microscopy images of frozen particles in cold isopentane at ~ -100 ^{0}C (using the home-built light microscope described in Figure SI2)**.**

NMR rotor packing tools and setup

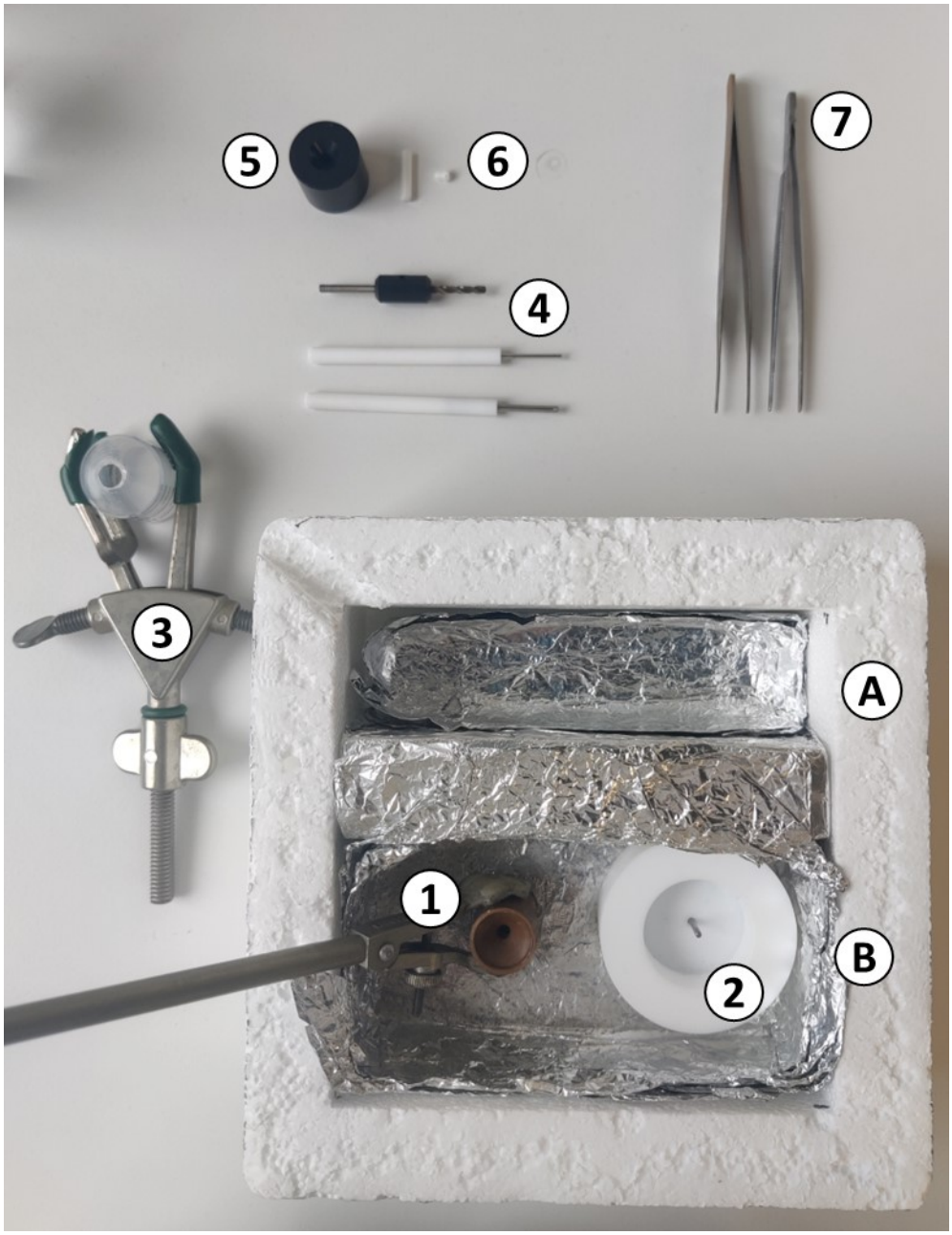

Figure SI4. Tools used for packing NMR rotor. (1) Copper filling funnel, (2) holder with a needle used to lift the rotor out from the filling funnel, (3) 50 mL Falcon tube cut in half and bottom removed used to transfer frozen droplets into copper packing tool, (4) tools used to pack frozen droplets into the NMR rotor, (5) holder used to put the cap on although copper packing tool can also be used to mount the cap, (6) 4 mm zirconia rotor with a cap, (7) tweezers used to help with the packing. A polystyrene box is divided into two sections: (A) and (B). The top compartment (A) is filled with liquid nitrogen, where packing tools (No. 4 and 7) can be stored. The bottom compartment (B) is filled with cold isopentane, where the rest of the packing tools (No. 1 and 2) can be stored.

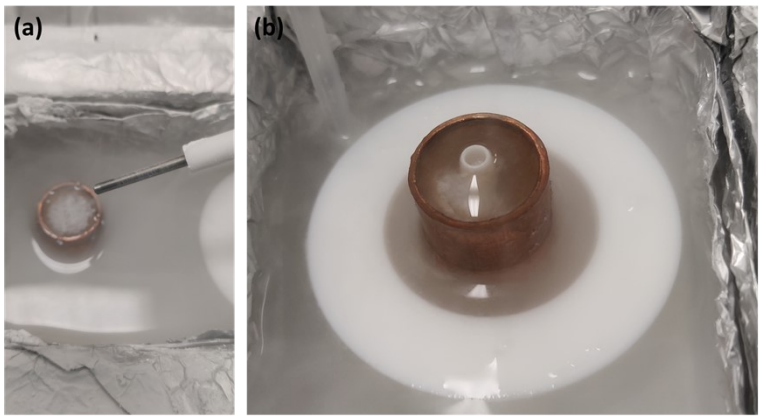

Figure SI5. (a) A filled funnel with frozen droplets is shown. This sample is packed inside the 4 mm rotor using appropriate packing tools that are cooled down before liquid nitrogen. (b) The rotor is lifted partially from the filling funnel using the needle placed in a white Teflon holder (No. 2 in Figure SI4). The residual solid particles are removed using a pipette. Only the top part of the rotor with no solid is exposed to the air, while the rest of the rotor is still inside the copper funnel, maintaining the cold temperature. Then, the level of the filling is checked to ensure enough space for the cap.

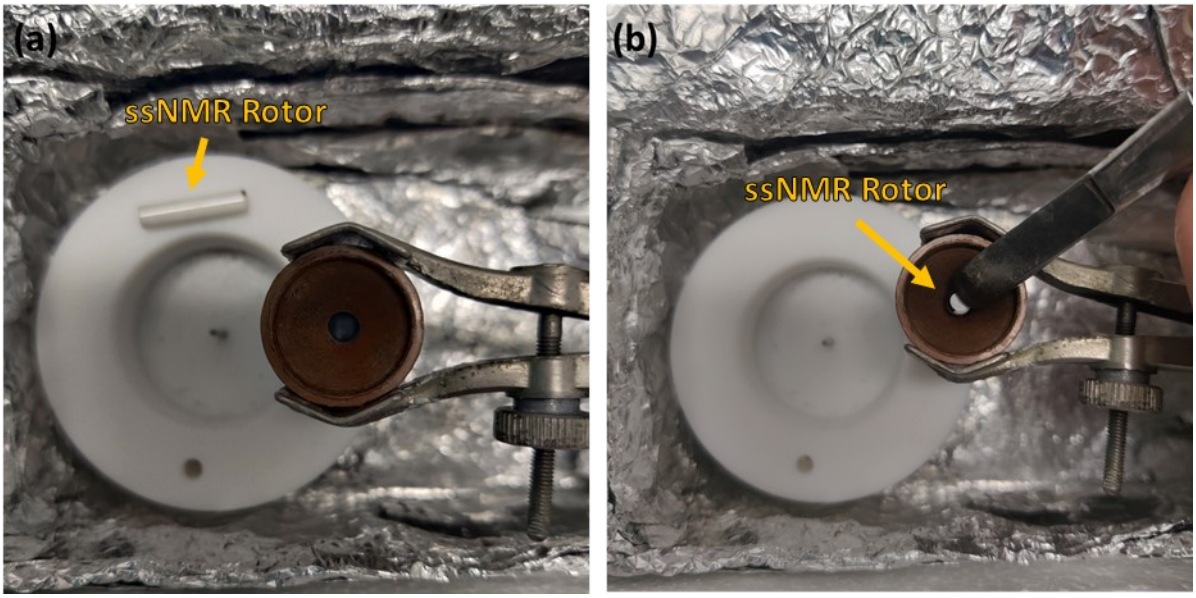

Figure SI 6. (a) The rotor is removed using cold tweezers and placed on the white Teflon holder (yellow arrow), still kept in a cold isopentane bath (not shown in this image for simplicity). The rotor cap is placed inside the packing funnel. (b) The rotor is turned around and slid into the copper funnel. The bottom of the rotor is pushed gently towards the cap using tweezers (yellow arrow) to allow the cap to be mounted on the rotor. The rotor can then be removed and stored in cold isopentane afterward.

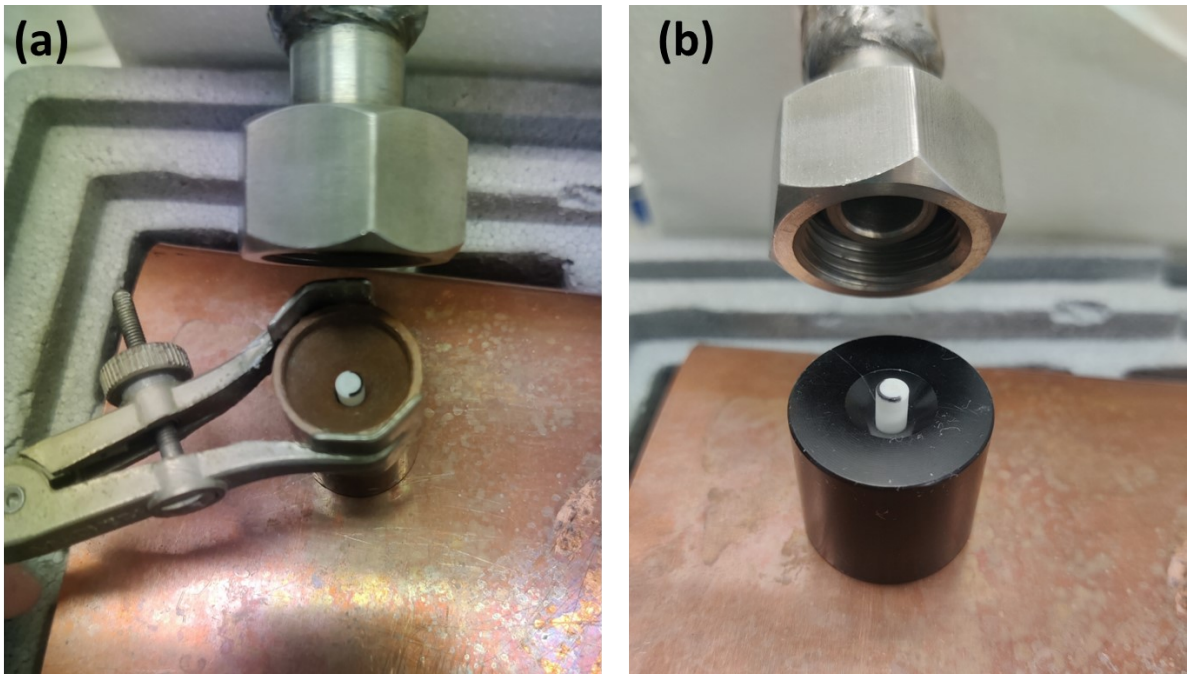

Figure SI7. Rotor cap mounting under cold nitrogen gas (-145 ^{0}C). Cold nitrogen gas is produced by passing it through a metal tube that is immersed in liquid nitrogen. Packing is done on top of a cold copper plate (77K) that is placed in a minimum amount of liquid nitrogen. (a) The cap can be mounted similarly as shown in Figure SI6, using the copper packing tool. The excess isopentane at the opening of the rotor can be removed by holding the rotor with cold tweezers under cold nitrogen flow using tissue and dabbing it gently on the rotor. (b) An alternative way of mounting the rotor cap is shown using a rotor cap closing tool that has been cooled to -145 ^{0}C prior to use and kept under the cold nitrogen flow.

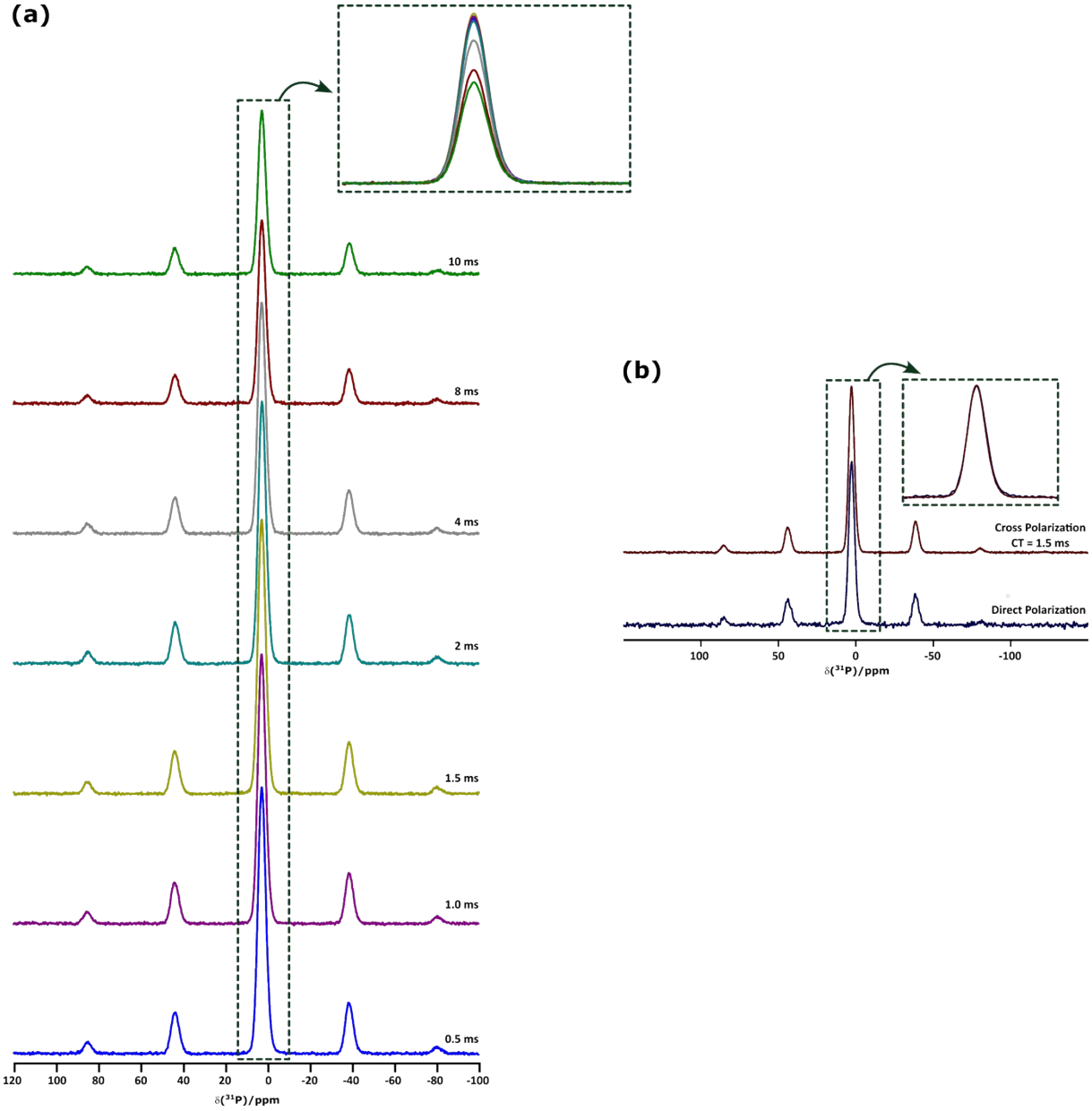

Figure SI8. (a) ^{1}H-^{31}P CP MAS NMR spectra recorded with various contact times. (b) Comparison of ^{31}P direct polarization with ^{1}H-^{31}P CP MAS experiment. We conclude the relative intensities of the HPO_4^{2-} and $H_2PO_4^-$ species present in the spectra at pH 7.4 are identical regardless of the acquisition sequence used (CPMAS or direct acquisition). Thus, ^{31}P CPMAS experiments can be used as a semi-quantitative approach to quantify the proportions of phosphate species in the cryofixed solutions.

The ^{31}P NMR spectra were recorded for a 1.5 M phosphate solution at pH 7.4. High concentration was selected to ensure high sensitivity for safe comparison. We also note that cryo-fixation is efficient for high phosphate concentrations up to 1.5 M.

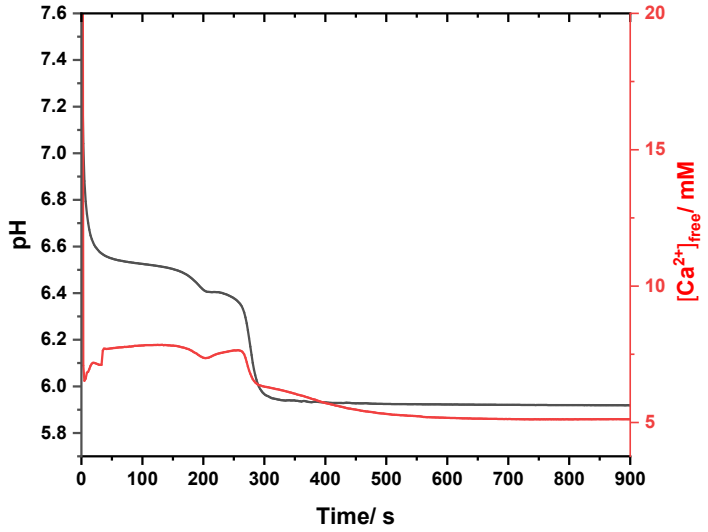

Figure SI9. Calcium potentiometry and pH measurement after mixing 50 mM Ca and Pi containing solutions. After addition, the $[Ca^{2+}]_{free}$ drop down to ~7.5 mM and the pH decreases from 7.4 to ~6.55. Cryo-TEM observations indicate the formation of ACP upon mixing (see Figure SI10).

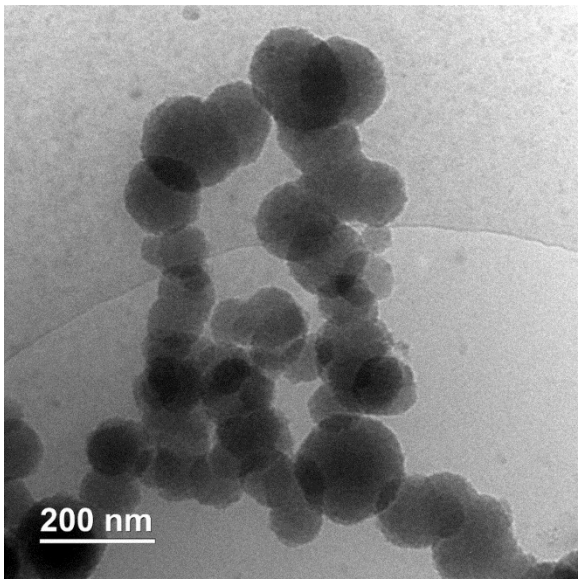

Figure SI10. Cryo-TEM image of the calcium phosphate solution sampled instantly after the mixing. The image shows the presence of amorphous calcium phosphate (ACP) spheres. The grid was prepared as rapidly as possible after mixing the solutions (*i.e.*, 30-40 sec)

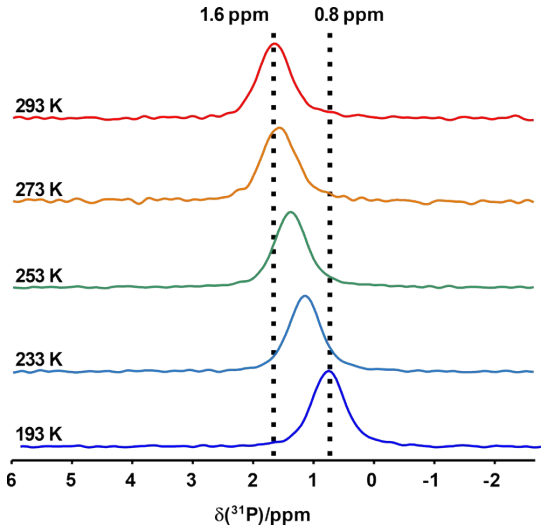

Figure SI11. ^{1}H-^{31}P CP MAS NMR spectra of brushite $CaH_2PO_4 \cdot 2H_2O$ recorded at various temperatures, illustrating the change in ^{31}P chemical shift with temperature. The spectra were acquired at a MAS frequency of 5 kHz, with a contact time of 0.5 ms, 16 scans, and a recycle delay of 1 s.

Table SI1.

Average CSA parameters per each phosphate species deduced from the fitted NMR parameters listed in TableSI2 corresponding to the 31P NMR spectra shown in Figure 5.

Species	δ_{iso}/ ppm	Δ_{CSA}/ ppm	η_{CSA}
PO_4^{3-}	4.4 ± 0.4	|11|± 5*	-*
HPO_4^{2-}	2.6 ± 0.4	57 ± 3	0.4 ± 0.05
$H_2PO_4^-$	1.0 ± 0.2	-64 ± 5	0.75 ± 0.10
H_3PO_4	1.2 ± 0.1	-53 ± 1	0.34 ± 0.05

(*) For PO_4^{3-}, the sign of Δ_{CSA} as well as the η_{CSA} value are not impacting the fitting.

The CSA parameters are defined as follows:

$\delta_{iso} = 1/3\ (\delta_{11}+\delta_{22}+\delta_{33})$

$\Delta_{CSA} = \delta_{33} - \delta_{iso}$

$\eta_{CSA} = (\delta_{22} - \delta_{11}) / (\delta_{33} - \delta_{iso})$

δ_{11}, δ_{22}, δ_{33} are the principal values of the chemical shielding tensor and are ordered as follows:
$|\delta_{33} - \delta_{iso}| \geq |\delta_{11} - \delta_{iso}| \geq |\delta_{22} - \delta_{iso}|$.

Table SI2

Fitted CSA parameters for the ^{31}P NMR spectra shown in Figure 5, with the proportion of phosphate species at different pH.

pH	Species	δ_{iso}/ ppm ± 0.4	Δ_{CSA}/ ppm ± 5	η_{CSA} ± 0.1	Molar fraction ± 0.10
12	PO_4^{3-}	4.8	|11| *	- *	0.82
	HPO_4^{2-}	2.4	53	0.36	0.18
10	PO_4^{3-}	4.1	|11| *	-*	0.03
	HPO_4^{2-}	2.8	59	0.36	0.97
8	HPO_4^{2-}	2.9	58	0.35	0.90
	$H_2PO_4^-$	1.1	-69	0.78	0.10
7.4	HPO_4^{2-}	2.7	58	0.37	0.77
	$H_2PO_4^-$	1.1	-69	0.76	0.23
6	HPO_4^{2-}	2.7	55	0.40	0.28
	$H_2PO_4^-$	1.3	-67	0.70	0.72
5	HPO_4^{2-}	2.6	58	0.40	0.04
	$H_2PO_4^-$	0.9	-65	0.67	0.96
4	$H_2PO_4^-$	1.1	-64	0.79	1.00
3.5	$H_2PO_4^-$	1.0	-59	0.77	0.95
	H_3PO_4	1.2	-53	0.34	0.05
2	$H_2PO_4^-$	1.0	-59	0.77	0.70
	H_3PO_4	1.2	-53	0.34	0.30
0.5	H_3PO_4	1.2	-53	0.34	1.00

(*) For PO_4^{3-}, the sign of Δ_{CSA} as well as the η_{CSA} value are not impacting the fitting. (see also Table SI1).