Assessing the onset of calcium phosphate nucleation by hyperpolarized real-time NMR

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Supporting Information

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D-DNP reference experiments

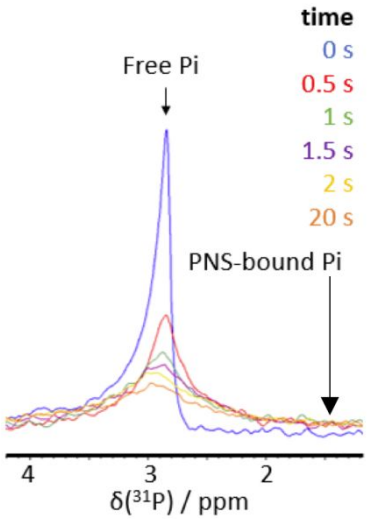

Figure S1. ^{31}P spectra detected after injection of a 13 mM $CaCl_2$ solution into a P_i solution without D-DNP. The mixing process was completed at t = 0 when NMR detection with a 2 Hz sampling rate was initiated. Without hyperpolarization, no significant trace of PNS-bound P_i could be detected (expected frequency indicated by an arrow). The signal of free Pi is decreasing due to CaP precipitation. All spectra were phase-corrected identically. The shape of the blue line is distorted due to turbulences during the mixing process.

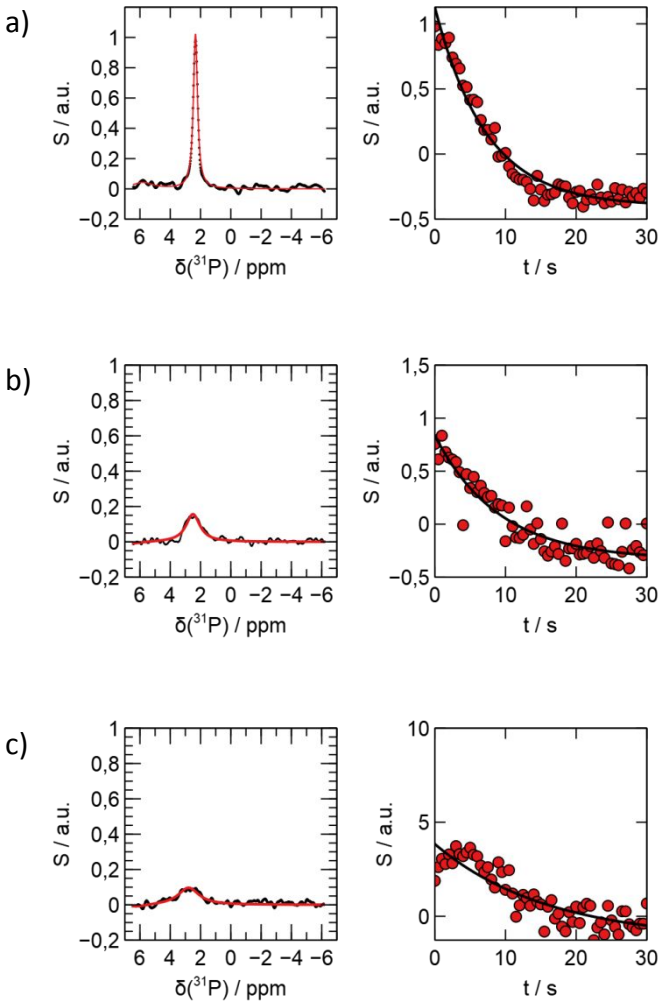

Figure S2. D-DNP ^{31}P NMR spectrum of various Pi and Ca^{2+} solutions (recorded at 2 s after mixing) and time dependence of the ^{31}P signal intensity. At elevated concentrations, the PNS aggregation proceeds too fast for real-time monitoring by solution-state NMR. a) Data for injection of P_i into a 25 mM $CaCl_2$ solution. The CaP precipitation is so fast that PNS signal is not observed after the mixing process. Instead only free P_i remains in solution for which monotone signal decay is observed. b) Same as in (a), but at a concentration of [Ca^{2+}] = 50 mM. The ^{31}P signal is weaker due to facilitated aggregation and precipitation of CaP at higher concentration (cf. turbidimetry results of the main text). The signal decay remains clearly monoexponential. However, line shape analysis become unreliable due to the low SNR. c) Same as in (b) but at [Ca^{2+}] = 100 mM. The signal-to-noise ratio drops further and now even the signal decay becomes difficult to analyze.

Fitting results

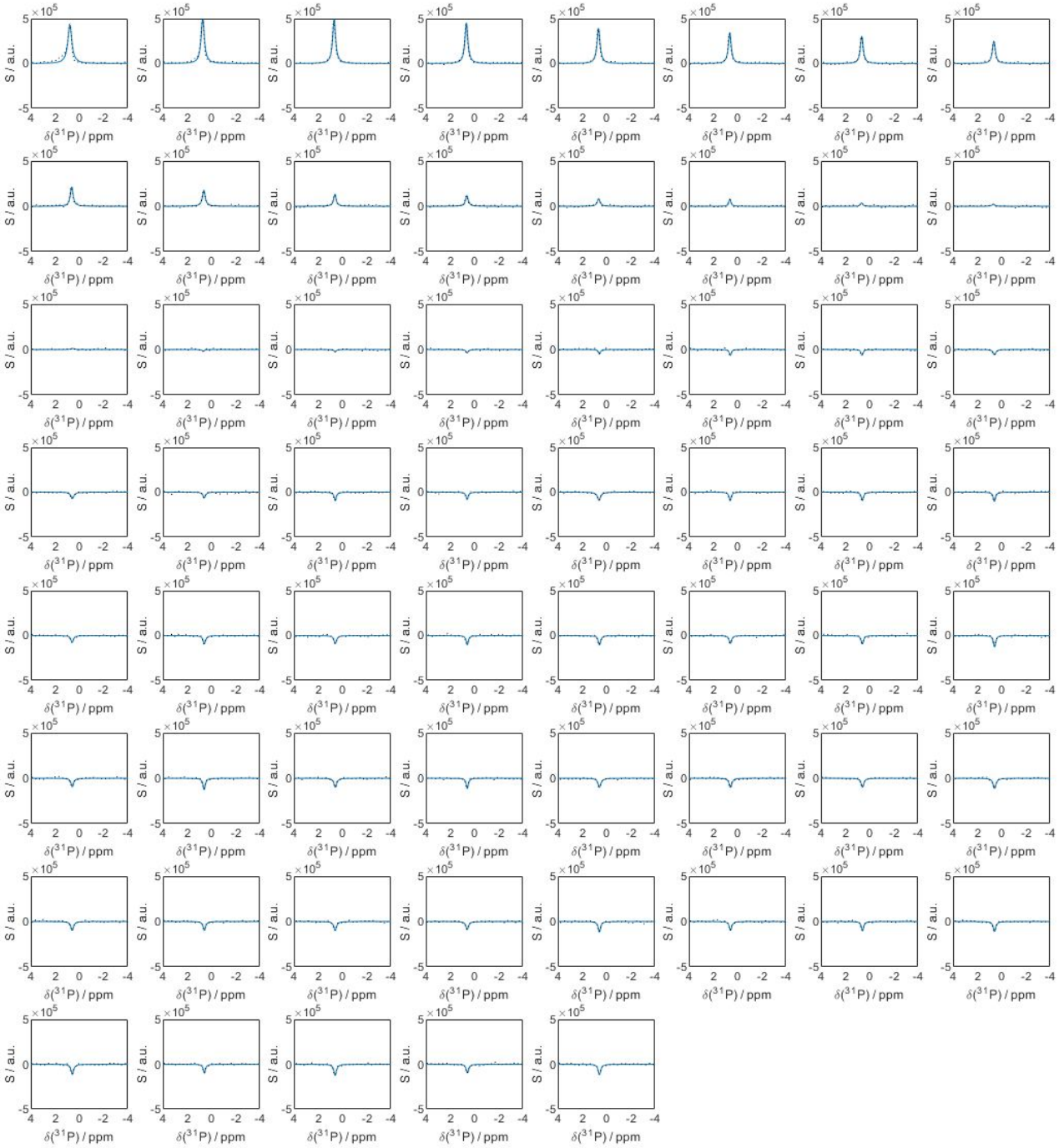

Figure S3. Spectra (black dots) and monomodal fits (blue lines) of P_i after dissolution and mixing with neat buffer (reference experiment with [Ca^{2+} = 0]). The delay between each detection was 0.5 s. Note that he linewidth is constant throughout the experiment showing that biases due to shim instabilities or turbulences are negligible. Negative DNP was used. The signal amplitude therefore switches sign.

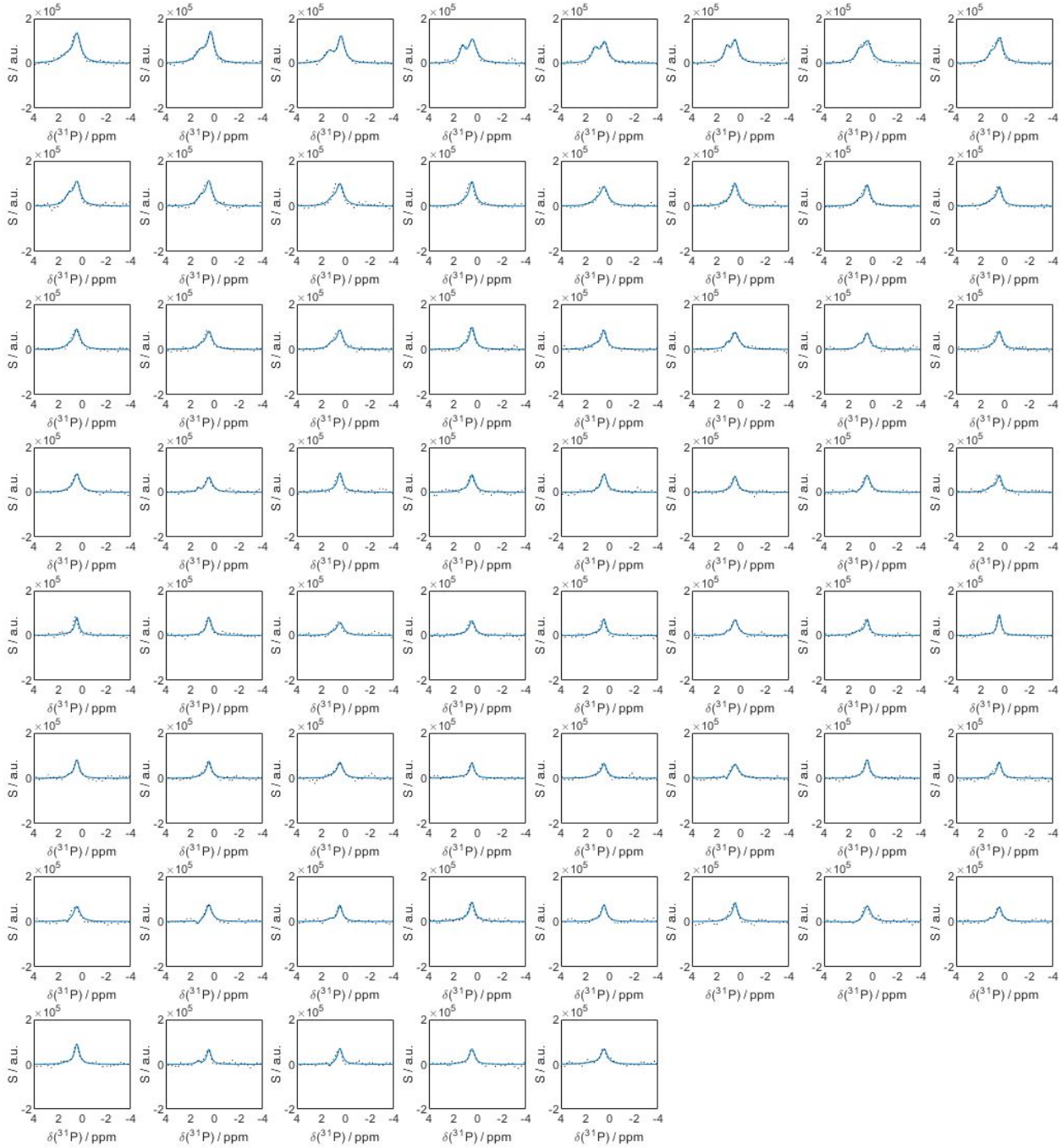

Figure S4. Spectra (black dots) and bimodal fits (blue lines) of P_i after dissolution and mixing with an 8 mM $CaCl_2$ buffered solution. The delay between each detection was 0.5 s. Positive DNP was used. Note that the signal amplitude does not pass through 0 as in Fig. S3 due to the opposite sign of the hyperpolarization in this experiment (here positive, but negative in Fig. S3).

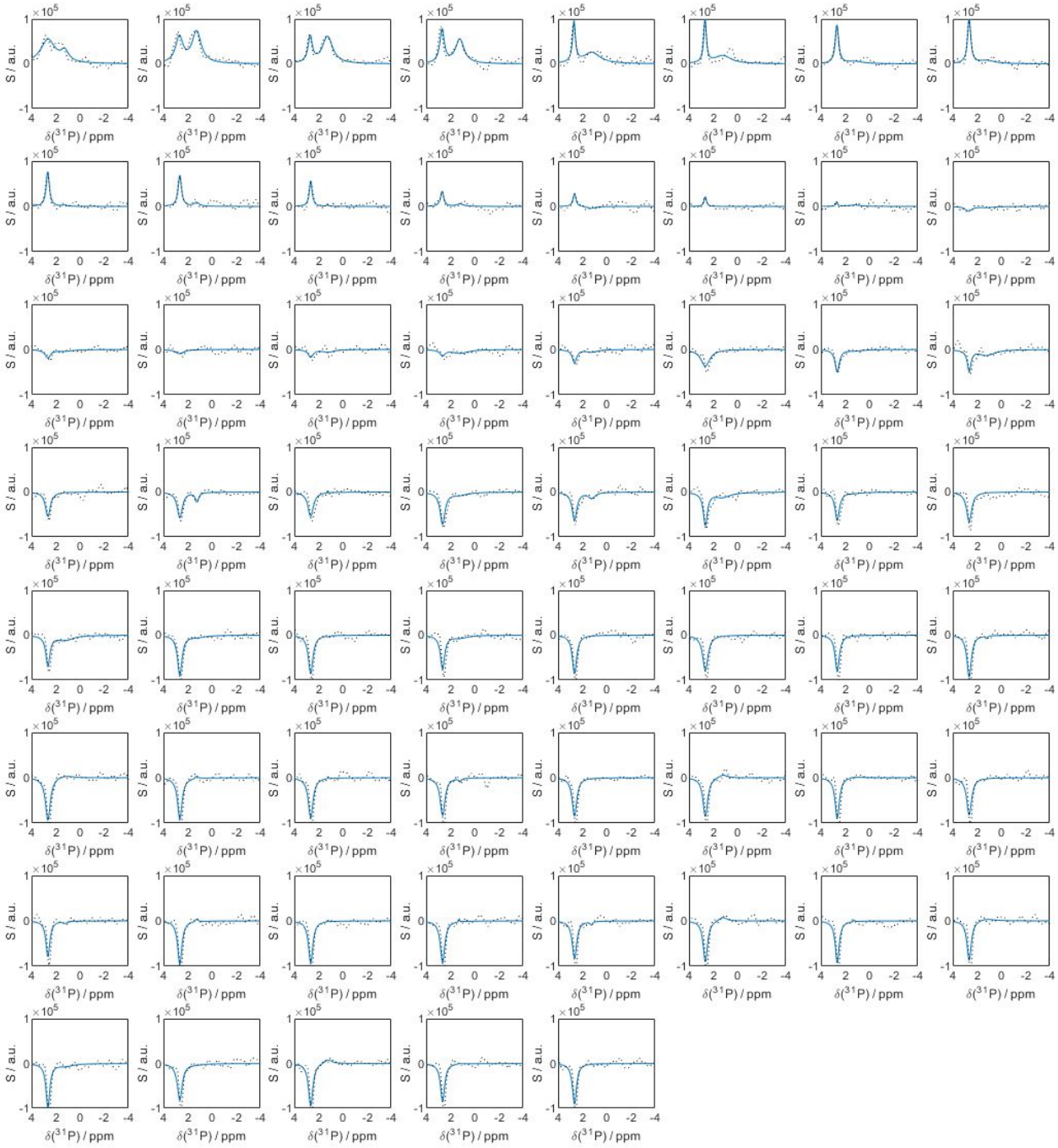

Figure S5. Spectra (black dots) and bimodal fits (blue lines) of P_i after dissolution and mixing with a 13 mM $CaCl_2$ buffered solution. The delay between each detection was 0.5 s. Negative DNP was used. The signal amplitude therefore switches sign.