Real-time monitoring of calcium phosphate pre-nucleation clusters formation by dissolution dynamic nuclear polarization

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The precipitation of solid calcium phosphate (CaP) phases is an important process that caused widespread interest in research ranging from geology via biomineralization to bone-repair applications. Despite its importance, the underlying molecular mechanisms are still being debated. In particular, the *pre-nucleation* events (prior to nucleation of the first CaP precipitate) remain obscure. The existence of so-called prenucleation clusters (PNCs) was only recently suggested (Gebauer *et al*. *Science* **2008**; Pouget *et al*. *Science* **2009**). PNCs initiate precipitation events and are considered to be the rate-limiting step in biomineralization processes. PNCs can be described as subnanometer-sized solutes constituted of loosely coordinated ions in dynamic equilibrium with the corresponding free solvated species. In the context of CaP biomineralization, only 3 studies (Dey et. al. *Nat*. *Mater*. **2010**; Habraken *et al*. *Nat*. *Commun*. **2013**; Wang *et al*. *CrystEngComm* **2012**) report on PNCs. Structural information about CaP PNCs is very scarce and their dynamical behaviour remains unclear.

We believe that the early onset of CaP precipitation remains elusive, due to a lack of appropriate methodologies that enable access to suitably short time scales at the molecular level. Here we present NMR spectroscopy boosted by dissolution dynamic nuclear polarization (D-DNP) as a method that enables to follow the interaction of phosphate with calcium in real time on a timescale of seconds at atomistic resolution.

Hence, we present unprecedented insights into the formation of pre-nucleation clusters (PNC), which are of high, timely interest and subject to many speculations as they represent a key stage in calcium-based biomineralization processes. With D-DNP-NMR we demonstrate the possibility to determine i) the kinetics of PNC formation, ii) the PNC size and iii) the stability of PNCs via exchange dynamics between free phosphate in solution and phosphates bound in PNCs – a process that we counterintuitively find to depend on the calcium ion concentration in solution.
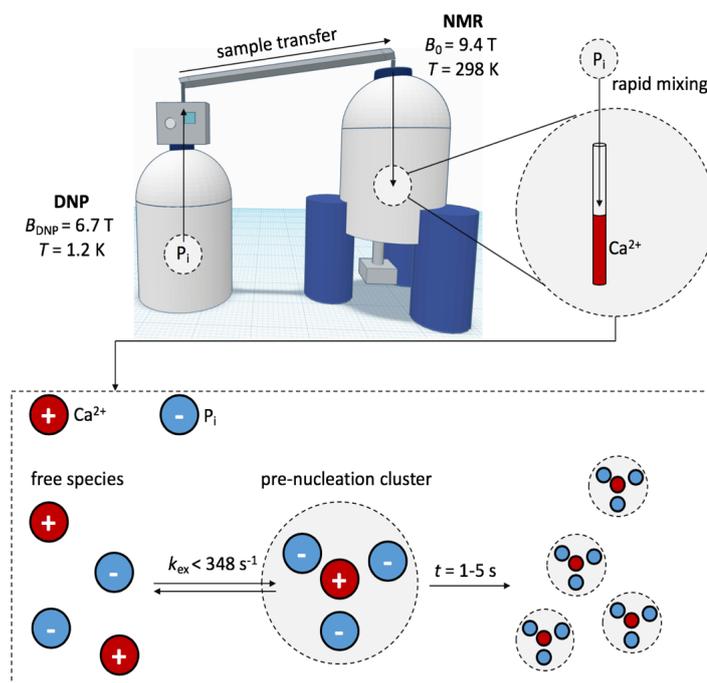

Figure 1. Sketch of the D-DNP experiment for real-time monitoring of CaP PNC formation. Inorganic phosphate P_i is hyperpolarized by DNP at 1.2 K to boost ^{31}P NMR signals. The hyperpolarized sample is then rapidly dissolved and transferred to an NMR spectrometer where it is mixed with a Ca^{2+} solution that is waiting in an NMR tube. After mixing, free and PNC-bound P_i are in dynamic equilibrium, while the PNC formation is monitored in real time. The process takes place on a 1-5 s time scale at 25°C and pH 7.8.