Supplementary information for

Physical Origin Underlying the Prenucleation Cluster Mediated Nonclassical Nucleation Pathways for Calcium Phosphate

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Table S1. Summary of the simulations designed in the present study for investigating the PNC-mediated nonclassical nucleation mechanisms for calcium phosphate. The total cumulative simulation time is about 5.2 μs.

Simulation methods	Simulation systems	Simulation setup	Total time
US[1]	Ca-P_1	6 ns (NPT)+2 ns (NVT)+34×30 ns[3]	2.994 μs
	CaP_1-P_2	6 ns (NPT)+2 ns (NVT)+34×30 ns[3]	
	CaP_1P_2-P_3	6 ns (NPT)+2 ns (NVT)+31×30 ns[3]	
MD[1]	$CaP_1P_2P_3$	6×10 ns (NVT)[4]	0.060 μs
MTD[1]	Ca-P_1	100 ns (NVT)	0.700 μs
	Ca-P_1P_2	300 ns (NVT)	
	Ca-$P_1P_2P_3$	300 ns (NVT)	
MD[2]	Ca/P=1:3	40 ns (NPT)	0.200 μs
	Ca/P=1:1	2×40 ns (NPT)[5]	
	Ca/P=2:1	2×40 ns (NPT)[5]	
US[2]	PNC-PNC	6 ns (NPT)+2 ns (NVT)+35×6 ns	1.290 μs
	PNC-Ca	6 ns (NPT)+2 ns (NVT)+44×6 ns	
	PNC_Ca-PNC	6 ns (NPT)+2 ns (NVT)+33×24 ns	

[1]The simulation methodologies are designed to investigate the PNC formation pathways and the thermodynamic stability of the obtained intermediate states. [2]These simulations are used to unravel the mechanisms along which the highly charged PNCs aggregate to form the new phase, such as the polymetric aggregates and the ACP. [3]A total number of about 34 window simulations for each PMF profile (Table S1) are carried out. [4]Six PNC configurations corresponding to the free energy minimum are exacted from the US trajectories to prepare the initial simulation systems. [5]Two simulation systems are prepared at different ion concentrations in solution to explore the dependence of the obtained aggregation trends on the experimental conditions.

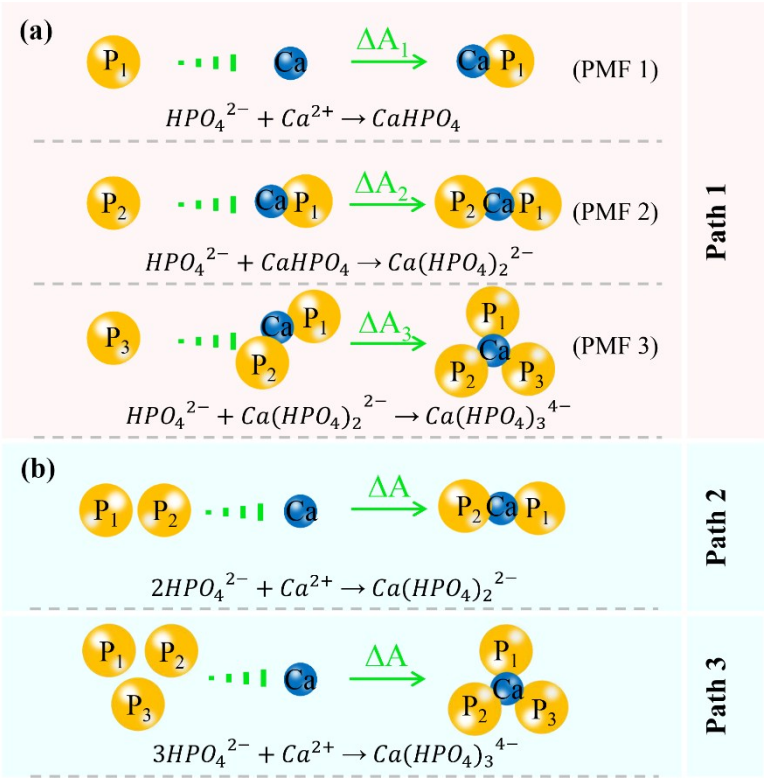

Fig. S1. The pathway-specified strategies for free energy calculations to investigate the thermodynamics for the PNC formations. (a) the phosphate ions are proposed to progressively bind to one calcium ion in a multi-step fashion (Path 1). US together with WHAM is used to obtain PMF profiles; (b) two or three phosphate ions are simultaneously sequestered to one calcium ion (Path 2 and Path 3). The Well-tempered MTD simulations are employed to calculate the multi-dimensional free energy surface.

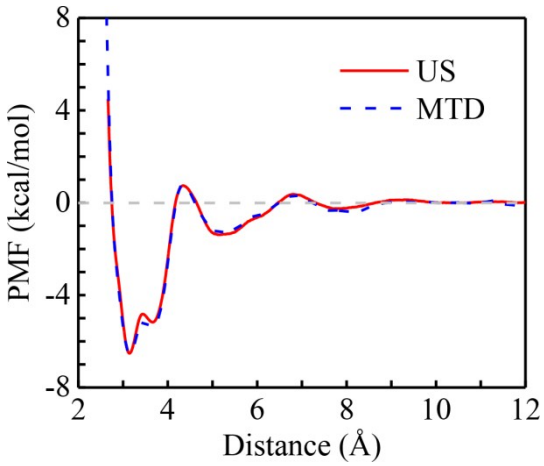

Fig. S2. A comparison of the PMF profiles for the Ca^{2+}-HPO_4^{2-} pairing interaction computed by the US and MTD simulations.

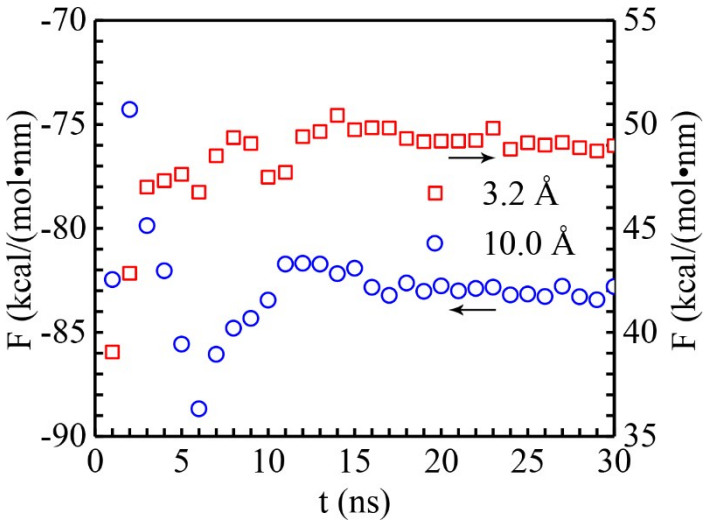

Fig. S3. The mean forces exerting on the ions collected as an average over a series of configurations from the US simulations as a function of simulation time for the interaction between $Ca(HPO_4)_2^{2-}$ and HPO_4^{2-} at two selected separation distances (3.2 and 10.0 Å). A well converged result for the force averaging is obtained after 20 ns.

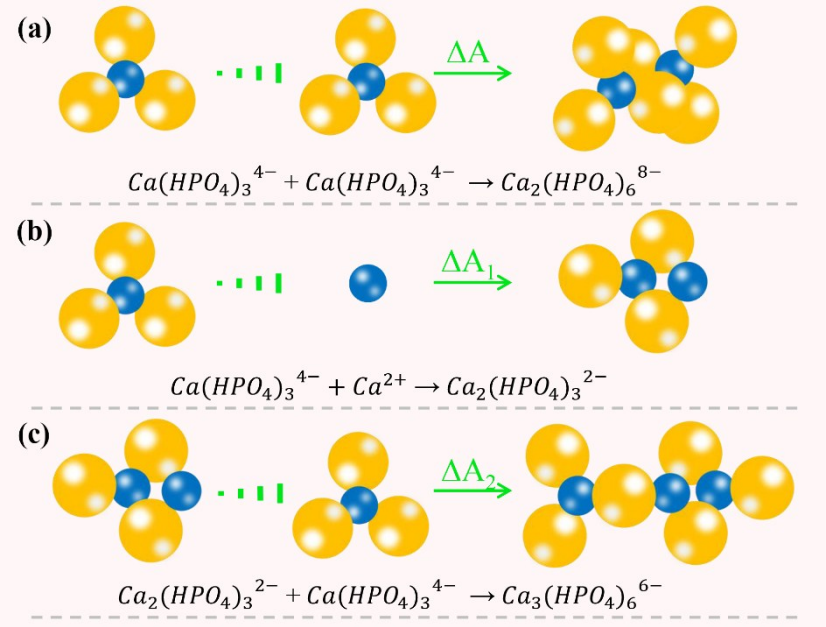

Fig. S4. The pathway-specified strategies for the free energy calculations to investigate the molecular mechanism underlying the aggregations of the highly charged PNCs. (a) the PNC-PNC interaction (b) the coordination of the $Ca(HPO_4)_3^{4-}$ to a free Ca^{2+} in solution, leading to the formation of $Ca_2(HPO_4)_3^{2-}$. (c) The self-assembly of $Ca(HPO_4)_3^{4-}$ and $Ca_2(HPO_4)_3^{2-}$ to form $Ca_3(HPO_4)_6^{6-}$, representing the aggregation process of the PNCs.

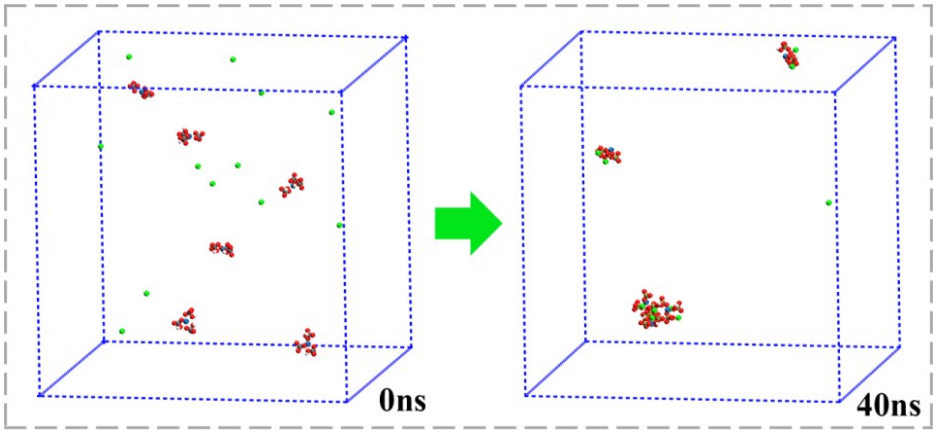

Fig. S5. The representative snapshots from the initial and the final stages of the PNC aggregation (Ca/P = 1.0) at the low ion concentration. The free PNCs successfully aggregate into small clusters.