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Electronic Supplementary Information

Amorphous calcium phosphate phase mediated crystal nucleation kinetics and pathway

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Tables

Table S1. The composition of SBF solutions with different calcium and phosphate concentrations and temperatures at pH =7.4. The Ca/P is kept at 1.67.

T=25°C

Ion. C/(mM)	NO.						
	1.	2.	3.	4.	5.	6.	7
H^+	12.7	12.8	12.9	13.0	13.2	13.4	14.0
Na^+	137.4	137.2	137.1	137.0	136.7	136.4	135.6
K^+	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Mg^{2+}	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cl^-	148.0	148.0	148.0	148.0	148.0	148.0	148.0
SO_4^{2-}	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ca^{2+}	4.509	4.676	4.843	5.01	5.344	5.678	6.68
PO_4^{3-}	2.7	2.8	2.9	3.0	3.2	3.4	4.0
$HEPES^-$	10.0	10.0	10.0	10.0	10.0	10.0	10.0
ln(*S*)	27.94	28.21	28.47	28.72	29.19	29.64	30.83
$\ln(S)_{eff}$	27.68	27.69	27.69	27.69	27.69	27.69	27.70

T=37°C

Ion. C/(mM)	NO.							
	1.	2.	3.	4.	5.	6.	7	8
H^+	12.0	12.1	12.2	12.3	12.5	13.2	14.2	17.49
Na^+	138.3	138.2	138.0	137.9	137.6	136.7	135.3	131.0
K^+	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Mg^{2+}	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cl^-	148.0	148.0	148.0	148.0	148.0	148.0	148.0	148.0
SO_4^{2-}	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Ca^{2+}	3.34	3.507	3.674	3.841	4.175	5.344	7.041	12.508
PO_4^{3-}	2.0	2.1	2.2	2.3	2.5	3.2	4.2	7.49
$HEPES^-$	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
$\ln(S)$	25.93	26.30	26.65	26.98	27.60	29.43	31.39	35.45
$\ln(S)_{eff}$	25.30	25.31	25.31	25.31	25.31	25.32	25.33	25.36

T=45°C

Ion. C/(mM)	NO.					
	1.	2.	3.	4.	5.	6.
H^+	11.7	11.8	12.0	12.5	13.0	13.5
Na^+	138.7	138.6	138.3	137.6	137.0	136.3
K^+	5.0	5.0	5.0	5.0	5.0	5.0
Mg^{2+}	1.5	1.5	1.5	1.5	1.5	1.5
Cl^-	148.0	148.0	148.0	148.0	148.0	148.0
SO_4^{2-}	0.5	0.5	0.5	0.5	0.5	0.5
Ca^{2+}	2.839	3.006	3.34	4.175	5.01	5.845
PO_4^{3-}	1.7	1.8	2.0	2.5	3.0	3.5
$HEPES^-$	10.0	10.0	10.0	10.0	10.0	10.0
$\ln(S)$	24.83	25.26	26.05	27.72	29.07	30.19
$\ln(S)_{eff}$	23.82	23.82	23.82	23.83	23.84	23.84

T=55°C

Ion. C/(mM)	NO.					
	1.	2.	3.	4.	5.	6.
H^+	11.6	11.7	11.8	12.0	12.5	13.0
Na^+	138.9	138.7	138.6	138.3	137.7	137.0
K^+	5.0	5.0	5.0	5.0	5.0	5.0
Mg^{2+}	1.5	1.5	1.5	1.5	1.5	1.5
Cl^-	148.0	148.0	148.0	148.0	148.0	148.0
SO_4^{2-}	0.5	0.5	0.5	0.5	0.5	0.5
Ca^{2+}	2.672	2.839	3.006	3.34	4.175	5.01
PO_4^{3-}	1.6	1.7	1.8	2.0	2.5	3.0
$HEPES^-$	10.0	10.0	10.0	10.0	10.0	10.0
ln(*S*)	24.48	24.94	25.37	26.16	27.83	29.17
$\ln(S)_{eff}$	22.07	22.07	22.07	22.07	22.08	22.09

Table S2. The composition of calcium phosphate solution for polymer systems.

Ion.	Na^+	K^+	Mg^{2+}	Cl^-	SO_4^{2-}	Ca^{2+}	PO_4^{3-}	$HEPES^-$	[pH]
C/mM	134.6	5.0	1.5	148.0	0.5	8.0	4.8	10.0	7.4

Table S3. Solubility of ACP at different time at lower supersaturated solutions (as marked in Fig. 1, region I) at 25°C. [Ca] and [P] are the calcium and phosphate concentrations in solutions after the precipitation of ACP, which is determined by ICP-AES; pCa and pPO_4 are calculated activity by VMINTEQ 3.0.

Time (h)	[Ca] (mM)	[P] (mM)	pH	Ca/P	pCa	pPO_4	pKs
1.0	4.563	2.744	7.399	1.50	2.85	8.89	25.84
3.5	4.580	2.738	7.391	1.54	2.85	8.89	25.86
5.0	4.504	2.646	7.388	1.48	2.86	8.91	25.91
Mean(sd)				1.54±0.05			25.87±0.04

Note: The Ca/P ratio of the amorphous phases is in the range 1.54±0.05. The composition of the amorphous phases can therefore be represented by the formula $Ca_3(PO_4)_{1.962}(OH)_{0.114}$, with $pKs=3pCa+1.962pPO_4+0.114pOH$.

Table S4. Solubility of ACP at different time at higher supersaturated solutions (as marked in Fig.

1, region II) at 25°C. [Ca] and [P] are the calcium and phosphate concentrations in solutions after the precipitation of ACP, which is determined by ICP-AES; pCa and pPO_4 are calculated activity by VMINTEQ 3.0.

Time (h)	[Ca] (mM)	[P] (mM)	pH	Ca/P	pCa	pPO_4	pKs
0.5	4.467	2.738	7.397	1.57	2.86	8.89	25.87
2.0	4.284	2.585	7.398	1.49	2.88	8.91	25.97
3.0	4.142	2.532	7.394	1.55	2.90	8.93	26.03
Mean(sd)				1.54±0.04			25.96±0.08

Note: The Ca/P ratio of the amorphous phases is in the range 1.54±0.04. The composition of the amorphous phases can therefore be represented by the formula $Ca_3(PO_4)_{1.962}(OH)_{0.114}$, with $pKs=3pCa+1.962pPO_4+0.114pOH$.

Table S5. The amount of ACP (C_{ACP}) and effective activity of calcium (Ca_{eff}) changing with the increase of apparent supersaturation at pH=7.4.

T=25°C

lnS	27.94	28.21	28.47	28.72	29.10	29.64	30.83
C_{ACP}/mM	0.048	0.101	0.155	0.208	0.315	0.422	0.743
Ca_{eff}/mM	1.206	1.208	1.210	1.212	1.216	1.220	1.233

T=37°C

lnS	25.93	26.30	26.65	26.98	27.60	29.43
C_{ACP}/mM	0.085	0.138	0.192	0.245	0.352	0.726
Ca_{eff}/mM	0.840	0.842	0844	0.846	0.850	0.865

T=45°C

lnS	24.83	25.26	26.05	27.72	29.07	30.19
C_{ACP}/mM	0.113	0.166	0.273	0.540	0.806	1.072
Ca_{eff}/mM	0.672	0.674	0.678	0.688	0.699	0.710

T=55°C

lnS	25.93	26.30	26.65	26.98	27.60	29.43
C_{ACP}/mM	0.232	0.282	0.338	0.445	0.711	0.977
Ca_{eff}/mM	0.522	0.524	0.526	0.530	0.540	0.550

Table S6. The composition of solutions with fixed C_{ACP}=1.648 mM (relative error is less then

±0.8%) and varied pH at 37 °C. The Ca/P is kept at 1.67.

Ion. C/(mM)	pH				
	7.6	7.4	7.2	7.0	6.8
H^+	14.58	14.92	15.40	16.0	16.89
Na^+	134.9	134.4	133.8	133.0	130.7
K^+	5.0	5.0	5.0	5.0	5.0
Mg^{2+}	1.5	1.5	1.5	1.5	1.5
Cl^-	148.0	148.0	148.0	148.0	148.0
SO_4^{2-}	0.5	0.5	0.5	0.5	0.5
Ca^{2+}	7.649	8.216	9.018	10.0	11.52
PO_4^{3-}	4.58	4.92	5.4	6.0	6.89
$HEPES^-$	10.0	10.0	10.0	10.0	10.0
$\{Ca\}_{eff}$	0.747	0.902	1.105	1.373	1.749
C_{ACP}	1.646	1.642	1.653	1.638	1.650
ln(*S*)	33.94	32.53	31.18	29.82	28.59

Figures

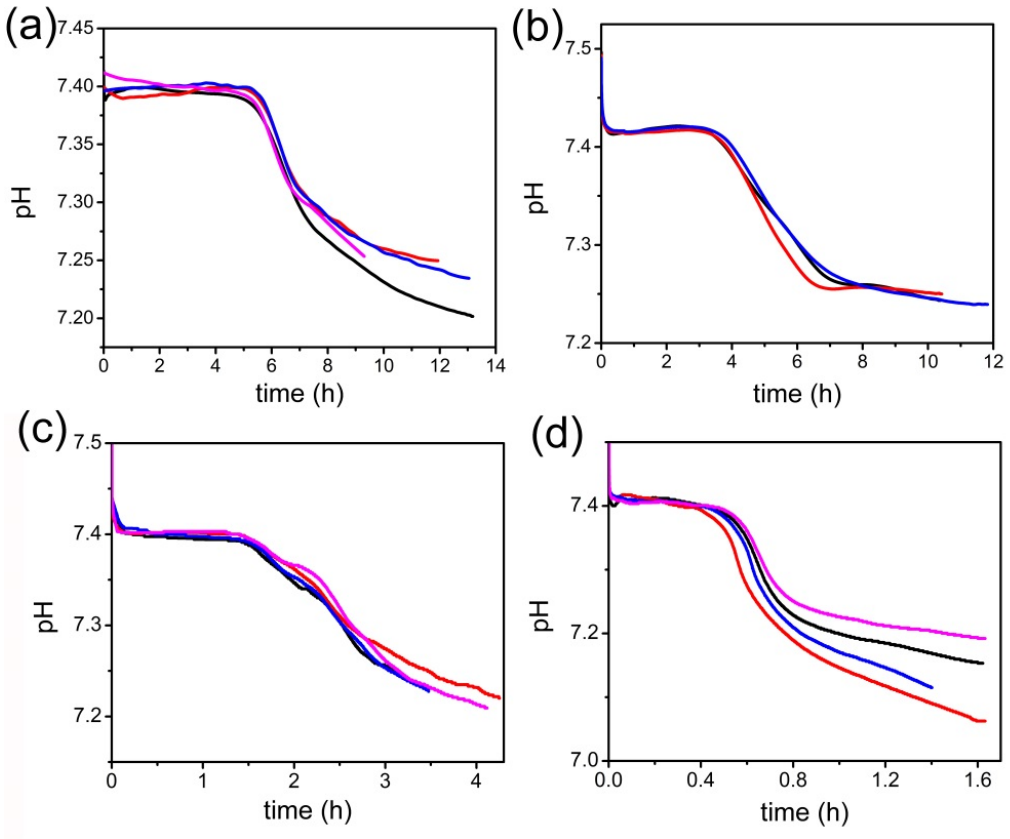

Fig. S1. Examples of pH curves reproducibility at pH=7.4 at different temperatures. At 25°C: (a) lnS=28.47 (b) lnS=29.19; At 37 °C: (c) lnS=29.43 (d) lnS=38.74. Detailed solution compositions see Table S1.

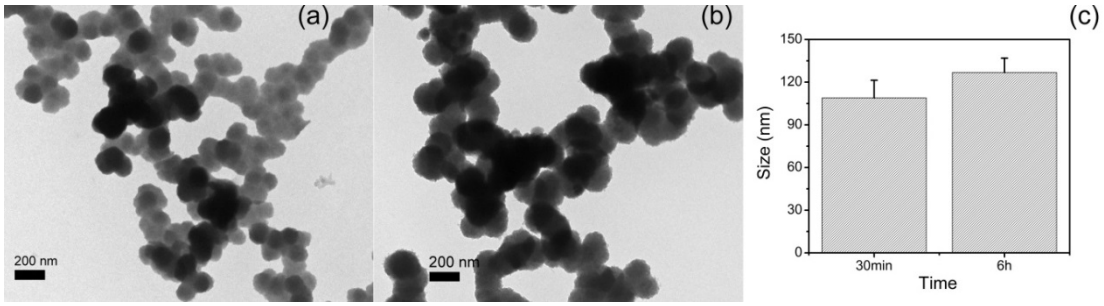

Fig. S2. TEM images of formed ACP particles at 30 min (a) and 6 h (b) at lnS=26.65, T=37°C, pH=7.4. (c) The size of ACP particles obtained from (a) and (b) (N=60)

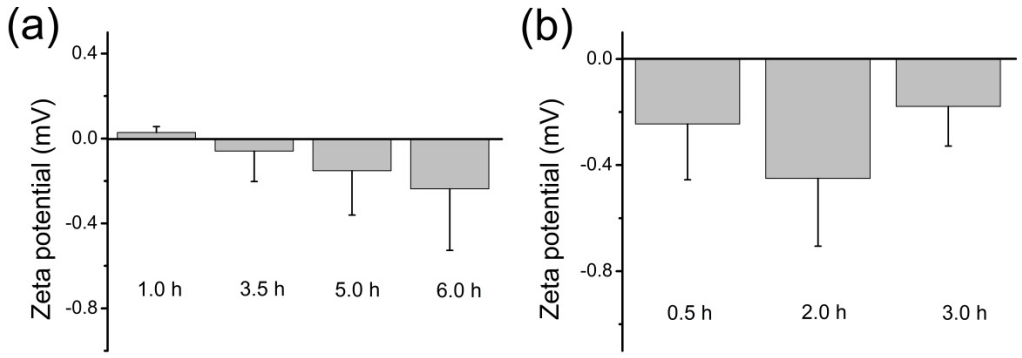

Fig. S3. Zeta potentials of minerals at different time at pH=7.4, T=25°C. (a) at region I, 4.843 mM Ca and 2.9 mM PO_4; (b) at region II, 5.344 mM Ca and 3.2 mM PO_4. I and II were marked by arrows in Fig. 1a. The composition of this two solutions see Table S1 of No.3 and No.5.

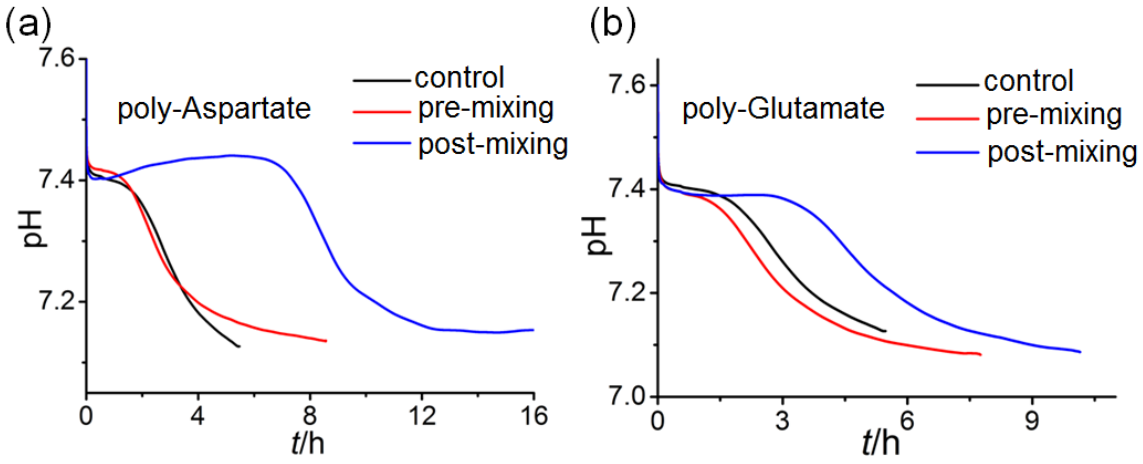

Fig. S4. (a) Representative pH curves of HAP crystallization in the presence (pre-mixing and post-mixing) and absence (control) of (a) poly-Aspartate (20 ppm, M.W. 27k, Alamanda Polymers, US) and (b) Poly-Glutamate (50 ppm, M.W. 2k-15k, GL biochem(Shanghai), China).

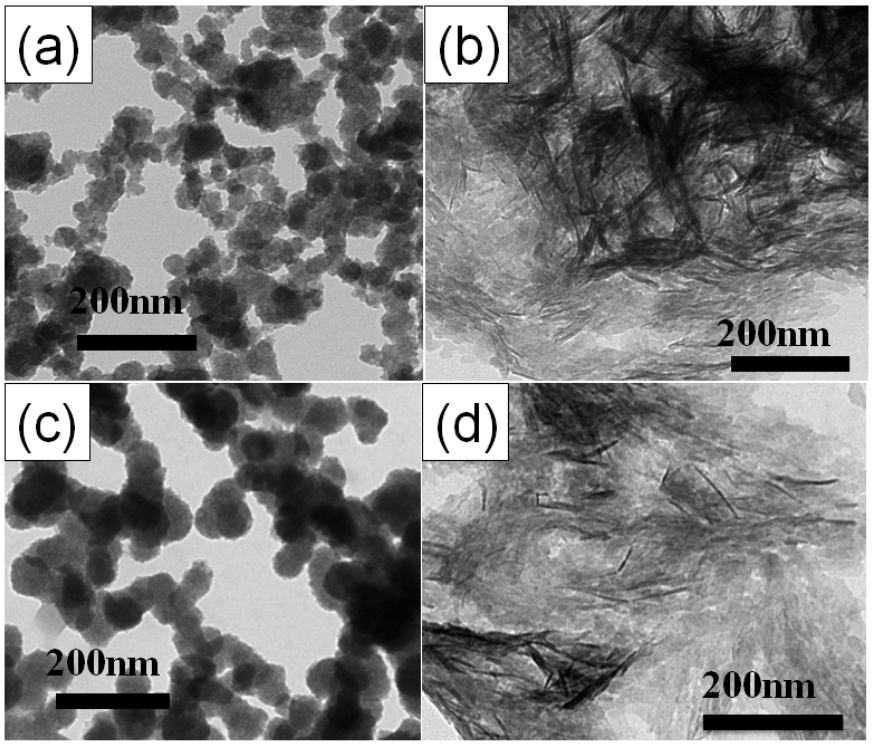

Fig. S5. TEM images of ACP (a,c) and HAP (b,d) precipitations by pre-mixing (a,b) and post-mixing protocols (c,d) .