Additives Influence the Phase Behavior of Amorphous Calcium Carbonate by a Cooperative Ion-association Process

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Supporting information

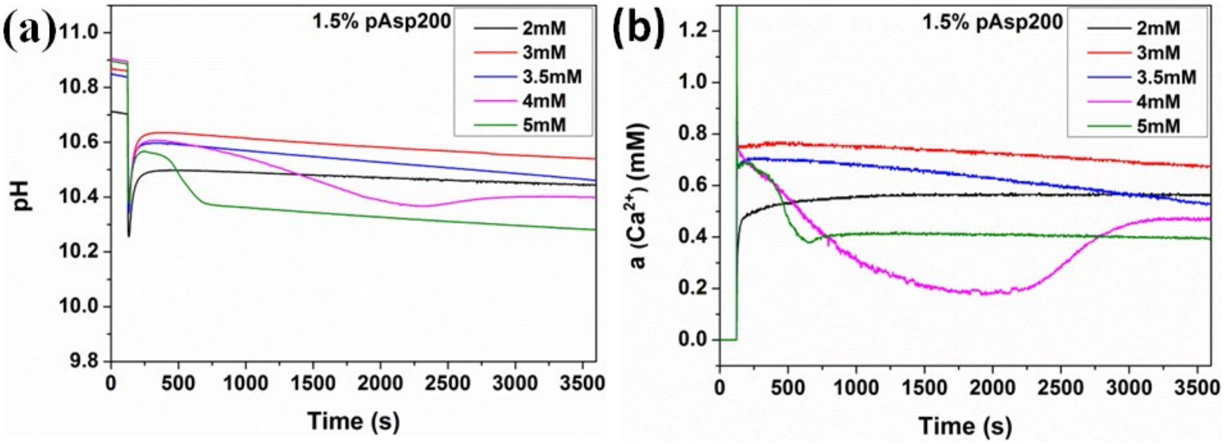

Figure S1. The pH (a) and Ca^{2+} activity (b) of the reaction solution in the presence of 1.5% of pAsp200 at calcium carbonate concentrations from 2 mM to 5 mM. At 5 mM, ACC was precipitated immediately after reaction at 120 s, which was relatively stable for about 300 s and then crystalline calcium carbonate phases formed, as indicated by the drops in both pH and Ca^{2+} activity of the solution. This was followed by the dissolution of ACC, which resulted in a slight increase in Ca^{2+} activity at ~750 s. At 4 mM, a continuous decrease in Ca^{2+} activity was observed, and it was also followed by an increase of Ca^{2+} activity, suggesting the transformation of ACC into crystalline phases. When the concentration was at 3.5 mM or lower, no precipitation could be observed. These results suggest that the spinodal line lies at ~ 3.5 mM.

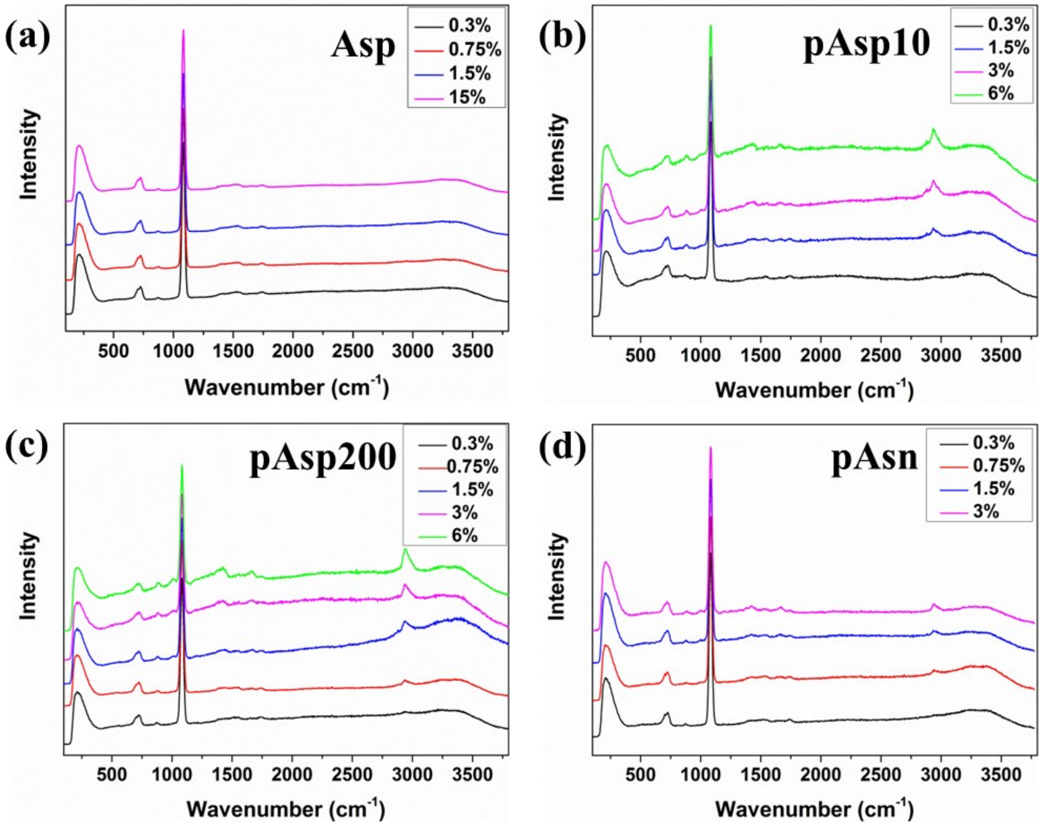

Figure S2. Raman spectra of ACC prepared at 5mM in the presence of different amount of Asp (a), pAsp10 (b), pAsp200 (c) and pAsn (d), showing that Asp monomer does not incorporate into ACC, while pAsp with chain length 10 and 200 and pAsn can incorporate into ACC.

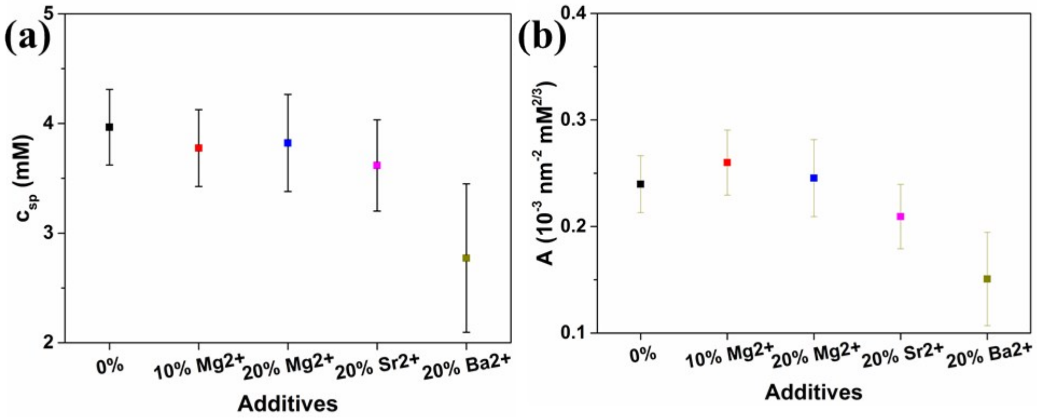

Figure S3. The derived concentrations c_{sp} at the spinodal line (a) and the normalization factor A (b) for the samples prepared in the presence of different additives.

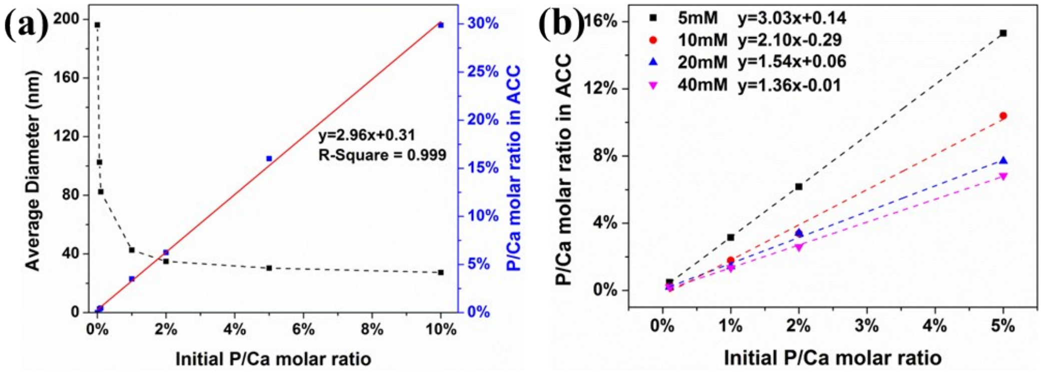

Figure S4. (a) The average diameter and P/Ca molar ratio in the ACC prepared at a calcium concentration of 5 mM in the presence of phosphate. (b) The P/Ca molar ratio in the ACC as a function of initial phosphate content in solution at different concentration of calcium carbonate (5 – 40 mM).

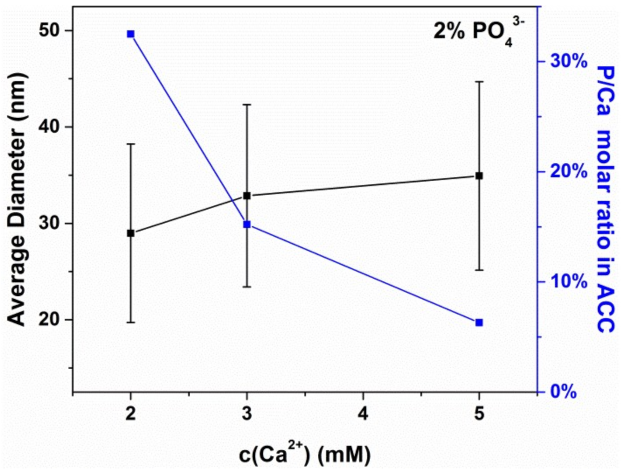

Figure S5. Average diameter and the P/Ca molar ratio in ACC prepared at low concentration of calcium carbonate (2 – 5 mM) in the presence of 2% PO_4^{3-}.

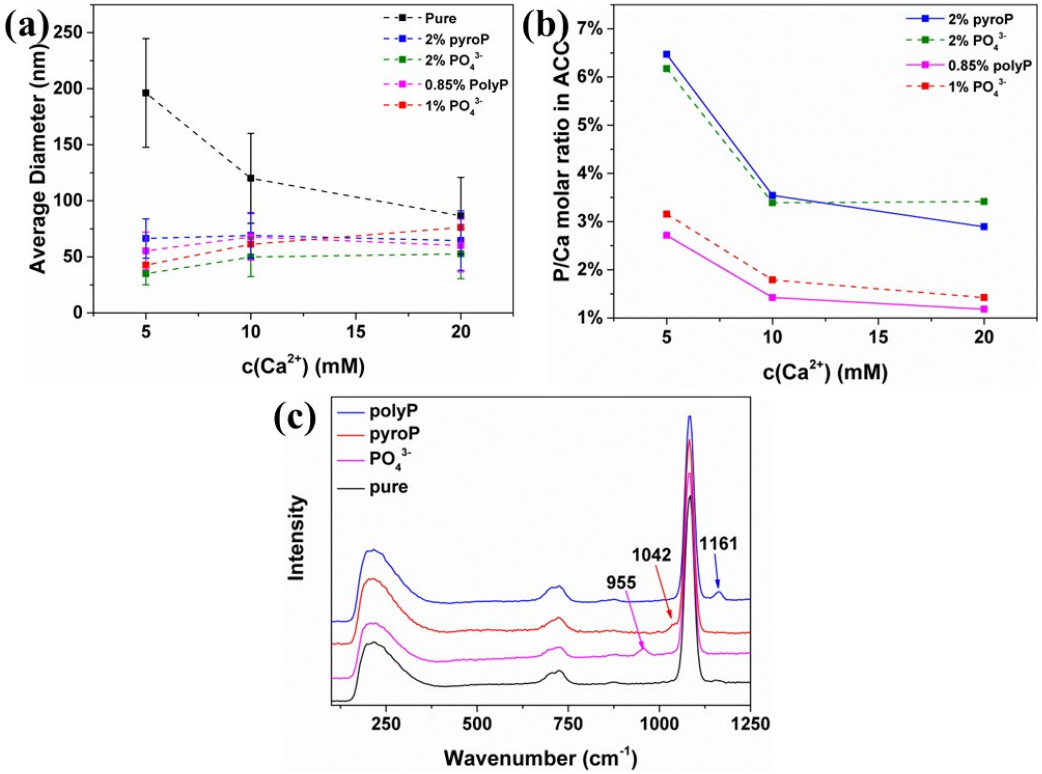

Figure S6. (a) Average diameter of ACC prepared at calcium carbonate concentrations of 5-20 mM in the presence of 2% (P/Ca molar ratio) of pyrophosphate (pyroP) and 0.85% of polyphosphate (P/Ca molar ratio). (b) The corresponding P/Ca molar ratio in ACC measured from amino acid analysis. (c) Typical Raman spectra of ACC prepared in the presence of pyroP and polyp.

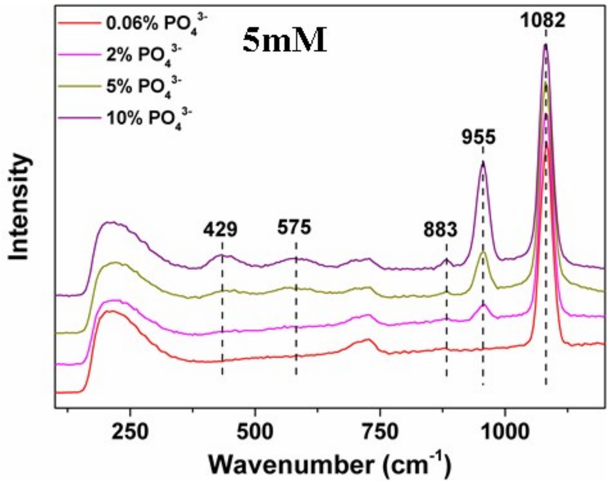

Figure S7. Raman spectra of ACC sample prepared in the presence of PO_4^{3-} ions. The broad peak at 955 cm^{-1} (FWHM width of ~28 cm^{-1}) in the Raman spectra (Figure 3f) suggests that the PO_4^{3-} ions in ACC existed in an amorphous state.[1] No sign of HPO_4^{2-} was observed in these spectra.[2]

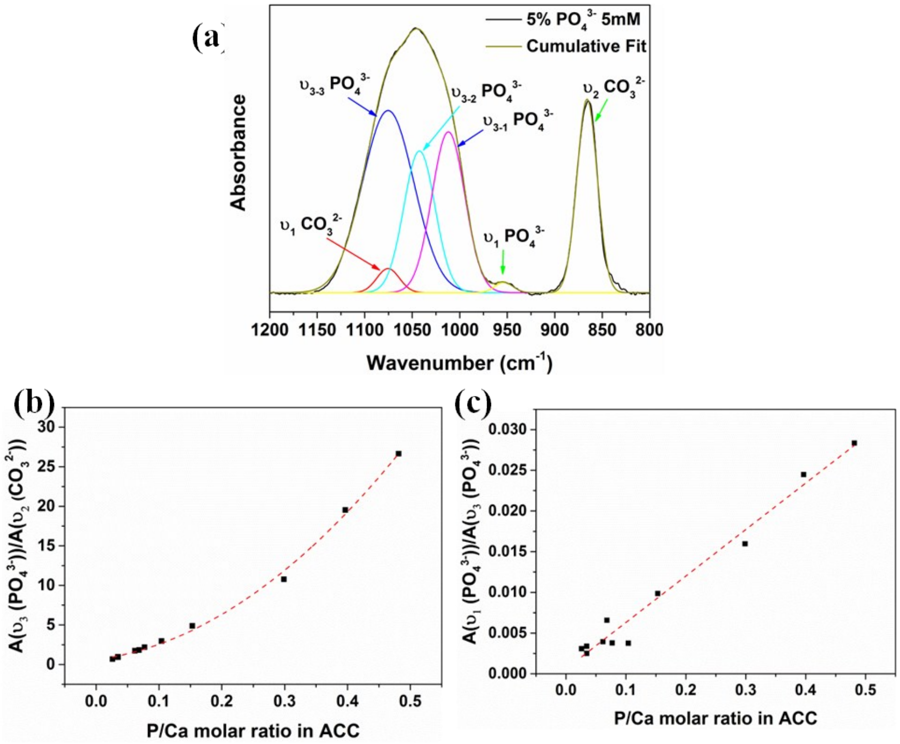

Figure S8. (a) Deconvolution of the ν_3 of PO_4^{3-} into 3 peaks at ~ 1010 cm^{-1}, 1040 cm^{-1} and 1075 cm^{-1} and all peaks were fitted with a Guass function for the FTIR spectrum of ACC spheres prepared at a carbonate concentration from 5 mM in the presence of 5% of PO_4^{3-}, (b) the ratio of peak area between the ν_3 of PO_4^{3-} and the ν_2 of CO_3^{2-}, (c) the ratio of the peak area between the ν_1 of PO_4^{3-} the ν_3 of PO_4^{3-}. Note that during the deconvolution the area of the ν_2 of CO_3^{2-} was fixed according to the area ratio between the ν_1 and ν_2 of CO_3^{2-} in the pure ACC.

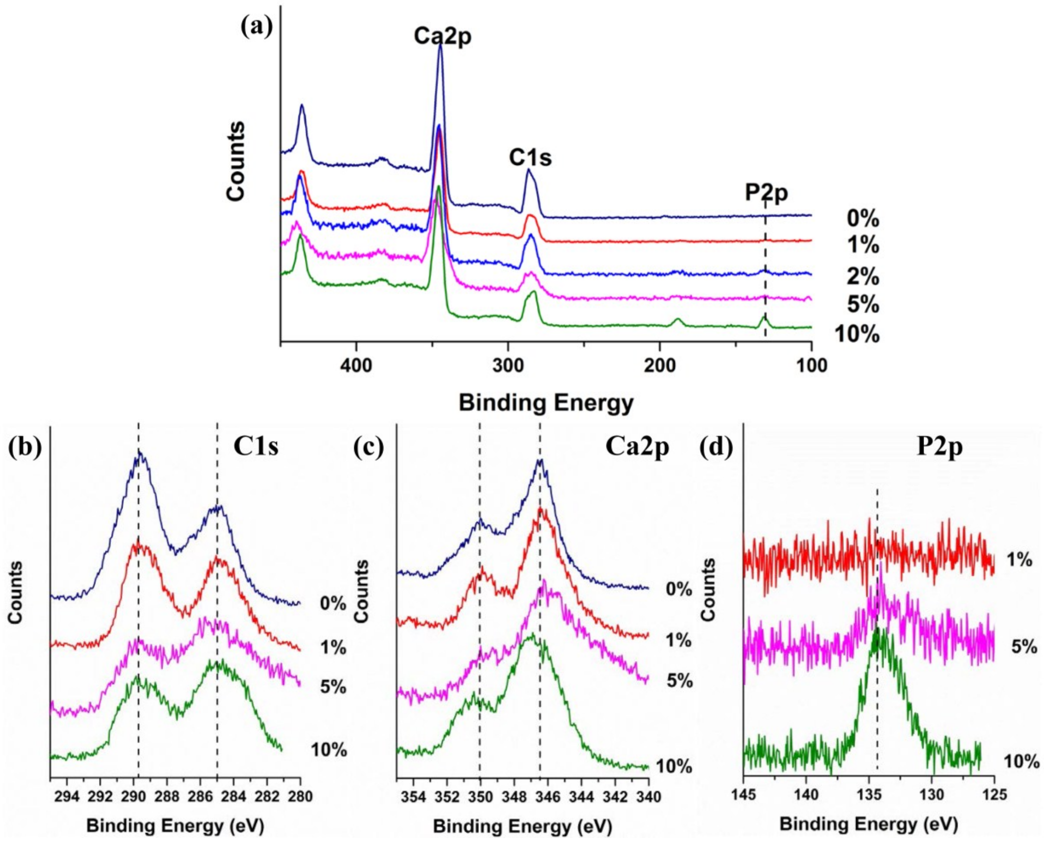

Figure S9. (a) XPS survey spectra of the ACC samples prepared in the presence of 0-10% of P. High resolution XPS spectra for the C1s (b), Ca2p (c) and P2p (d) signal of the ACC samples prepared in the presence of 0%, 1%, 5% and 10% of P. High resolution XPS for C1s line (Figure 4b) clearly indicated a change of the chemical environment of carbon for the samples with different P concentrations, where the relative intensity of the peak 289.8 eV increased with increasing P concentrations. High resolution XPS for Ca2p line (c) also showed a change of the chemical environment of calcium, especially for the samples with P concentrations of 5%, whereas for the 10% P samples, the chemical surrounding of calcium was more comparable with pure ACC, but shifted to higher binding energy. In addition, no difference in the peak position of high resolution XPS for P2p line (d) could be observed, but their intensity increased with increasing P concentrations.

References

1. Amini, S.; Masic, A.; Bertinetti, L.; Teguh, J. S.; Herrin, J. S.; Zhu, X.; Su, H.; Miserez, A., Textured fluorapatite bonded to calcium sulphate strengthen stomatopod raptorial appendages. *Nat. Commun.* **2014,** 5, 3187.

2. Bennet, M.; Akiva, A.; Faivre, D.; Malkinson, G.; Yaniv, K.; Abdelilah-Seyfried, S.; Fratzl, P.; Masic, A., Simultaneous Raman Microspectroscopy and Fluorescence Imaging of Bone Mineralization in Living Zebrafish Larvae. *Biophys. J.* **2014,** 106, (4), L17-L19.