**Supplementary information for:**

# Phosphorylated amelogenin N-terminal peptides regulate calcite crystal cluster formation in water-acetonitrile system

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## Characterizations.

**Thermogravimetric Analysis (TG)**. TG (STA 449 F5, NETZSCH, Germany) was used to detect the occlusions amount of amelogenin N-peptide inside calcite crystals. The TGA data were collected under $N_2$ atmosphere from 20 °C to 550 °C at a heating rate of 10 °C/min.

**Raman Spectroscopy (Raman spectra)**. The amelogenin N-peptide adsorbed on calcite surface were detected using a confocal Raman Microscopy (DXR3xi, Thermo Scientific). Raman spectra were collected under a 100× objective using a 532 nm laser at 2.5mV in a laser mode power at 100% with the Raman shift ranging from 200 to 3400 $cm^{-1}$, operating under OMNIC 1.0 with a pinhole aperture of 50 μm and an exposure time of 0.1 s.

**Ultraviolet and visible spectrophotometry (UV-vis)**. The peptide occluded inside calcite crystals was with UV−vis spectroscopy (*Nanodrop One*, Thermo Scientific). Before analysis, the samples were washed with 0.1 M NaOH for 30 s, then the crystals were dissolved in 0.1 M HCl release any occluded amelogenin N-peptide into solution. Before measurements, the instrument with 0.1 M HCl, and the adsorption were recorded at 280 nm. [1]

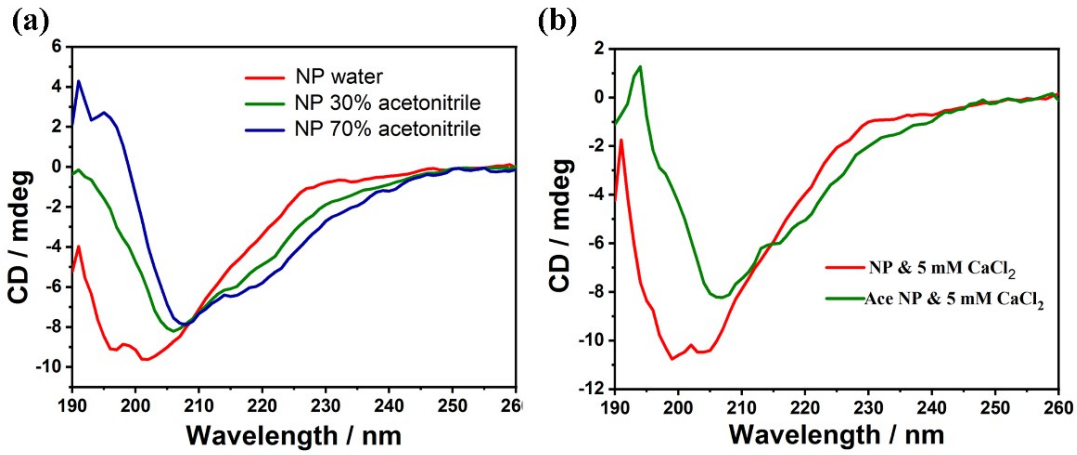


Figure s1 The CD spectra spectrum of NP peptide in different solutions. (a) in $H_2O$, 30% Ace-$H_2O$ and 70% Ace-$H_2O$; (b) in 5 mM $CaCl_2$ solution and 5 mM $CaCl_2$ 30% Ace-$H_2O$ solution.

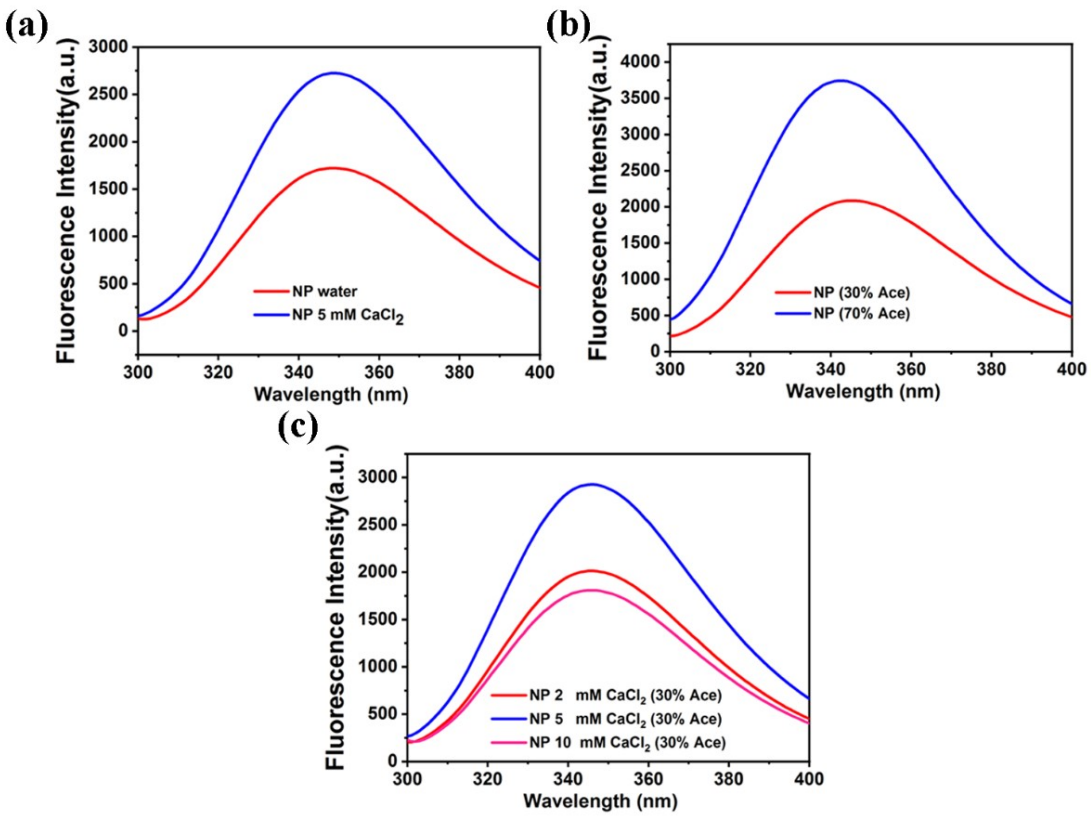


Figure s2 The Fluorescence spectra spectrum of NP peptide in different solutions. (a) in $H_2O$ and 5 mM $CaCl_2$ solution; (b) in 30% Ace-$H_2O$ and 70% Ace-$H_2O$ solutions; (c) in 2 mM, 5 mM and 10 mM $CaCl_2$ 30% Ace-$H_2O$ solutions.

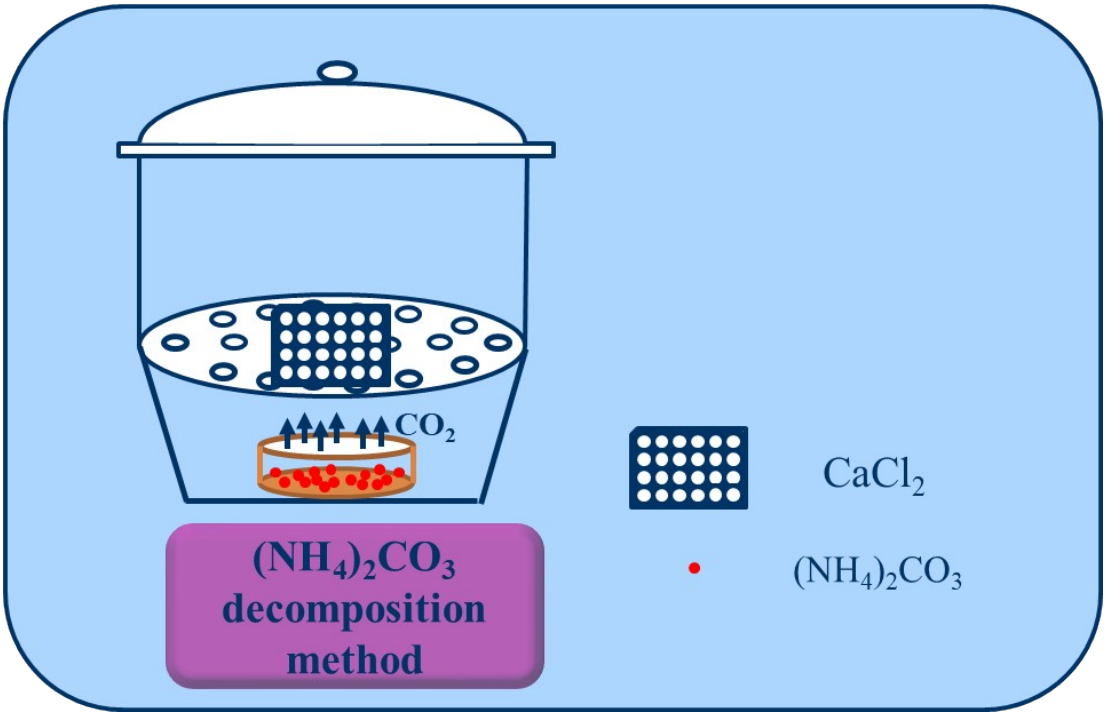


Figure s3 Schematic of calcite formation by $(NH_4)_2CO_3$ decomposition method.

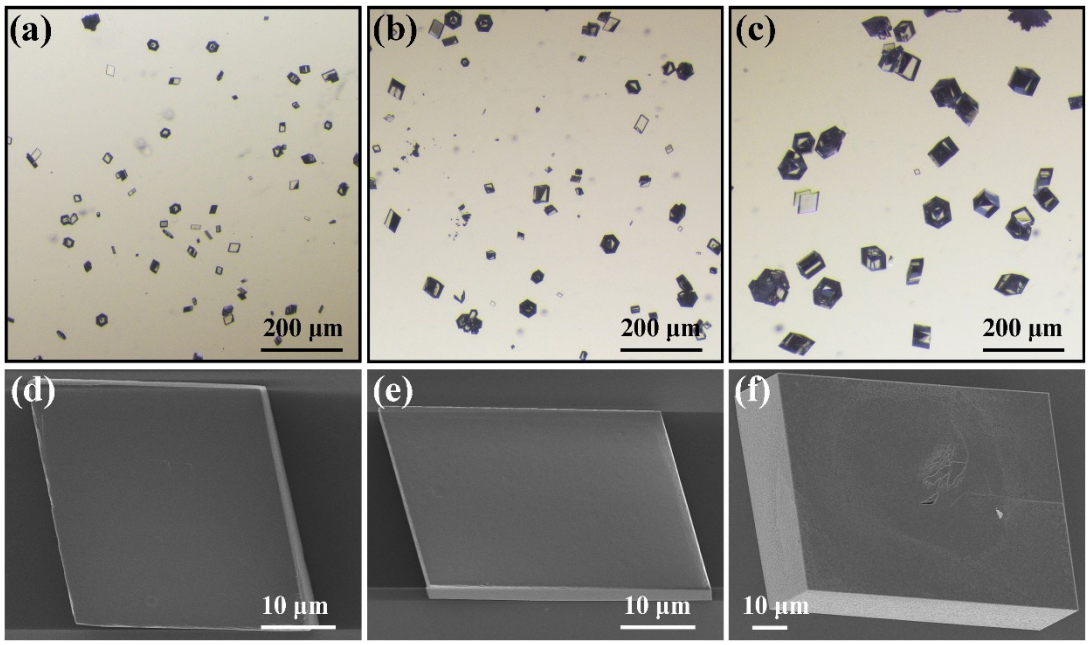


Figure s4 OM and SEM images of calcite formed in $H_2O$. (a), (d) $CaCl_2$ = 2 mM; (b), (e) $CaCl_2$ = 5 mM; (c), (f) $CaCl_2$ = 10 mM.

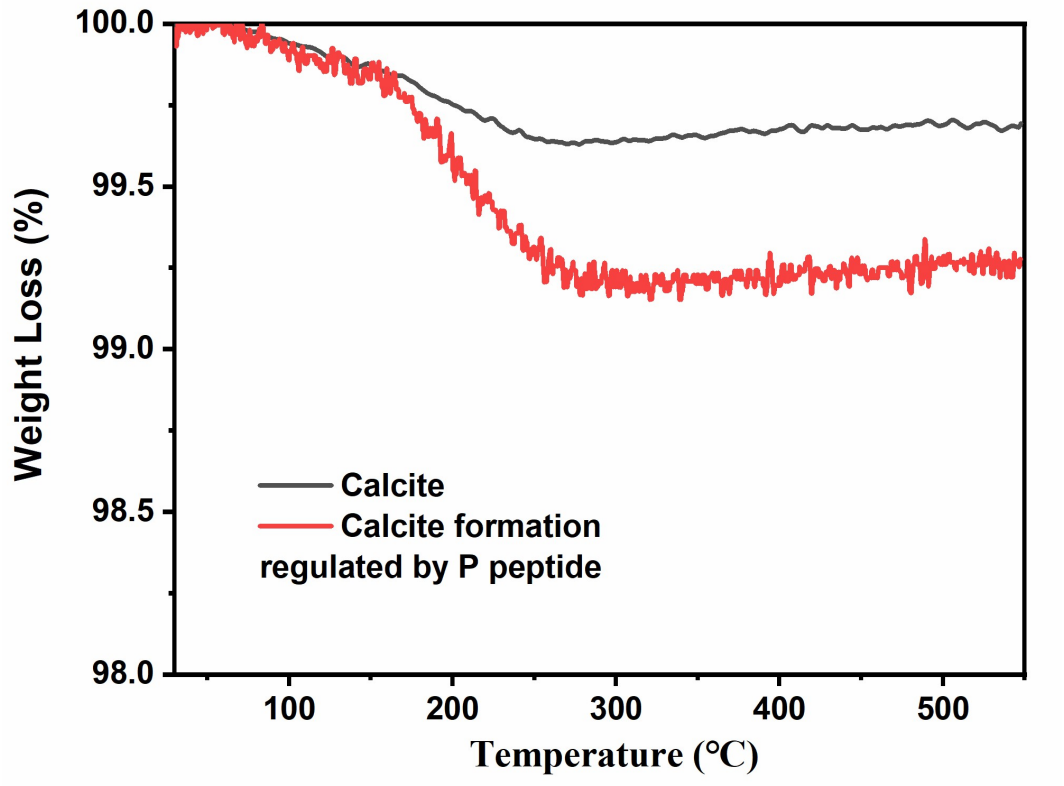


Figure s5 TG images of calcite and calcite formation regulated by P peptide in $H_2O$ (P peptide concentration = 0.2 mg/mL).

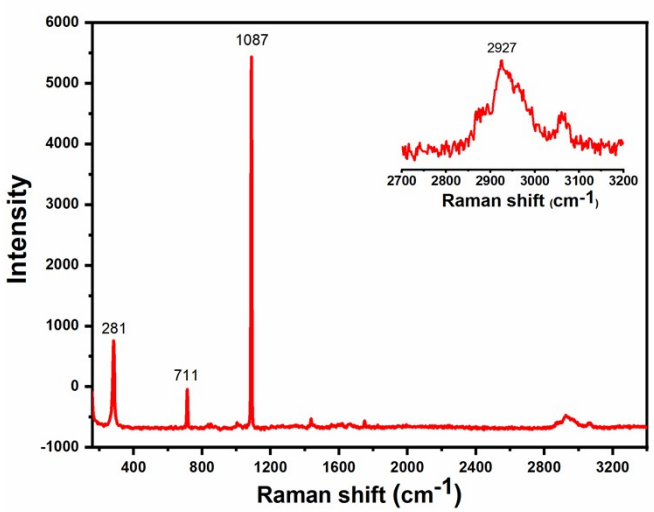


Figure s6 Raman spectra of calcite formation regulated by P peptide in $H_2O$ (P peptide concentration = 0.2 mg/mL).

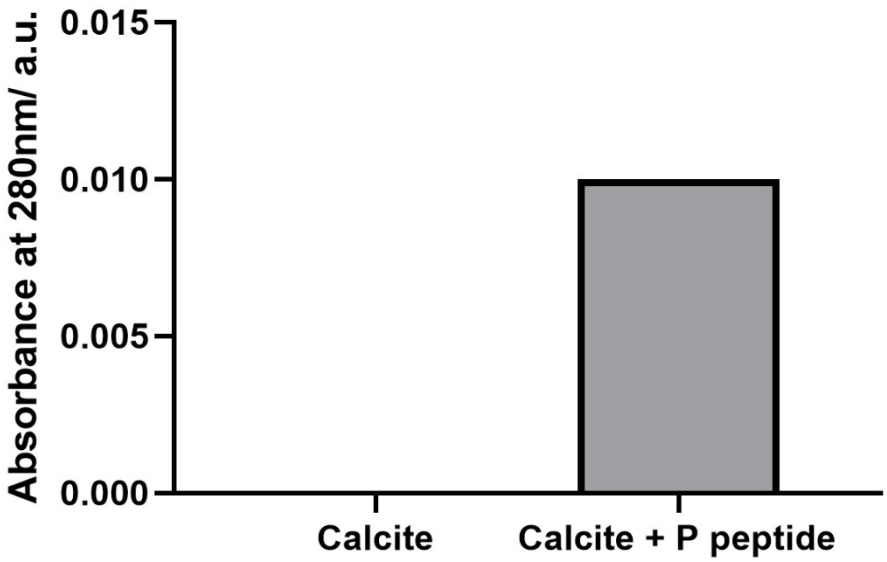


Figure s7 UV-vis absorbance (A280) values of calcite and calcite formation regulated by P peptide in $H_2O$ (P peptide concentration = 0.2 mg/mL).

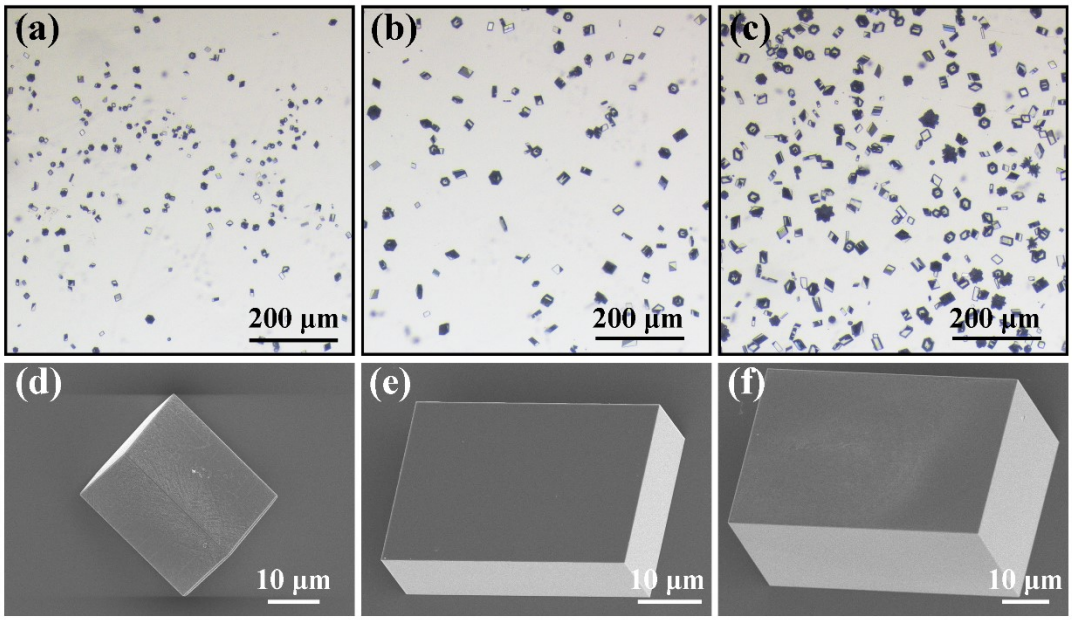


Figure s8 OM and SEM images of calcite formed in 30% Ace-$H_2O$. (a), (d) $CaCl_2$ = 2 mM; (b), (e) $CaCl_2$ = 5 mM; (c), (f) $CaCl_2$ = 10 mM.

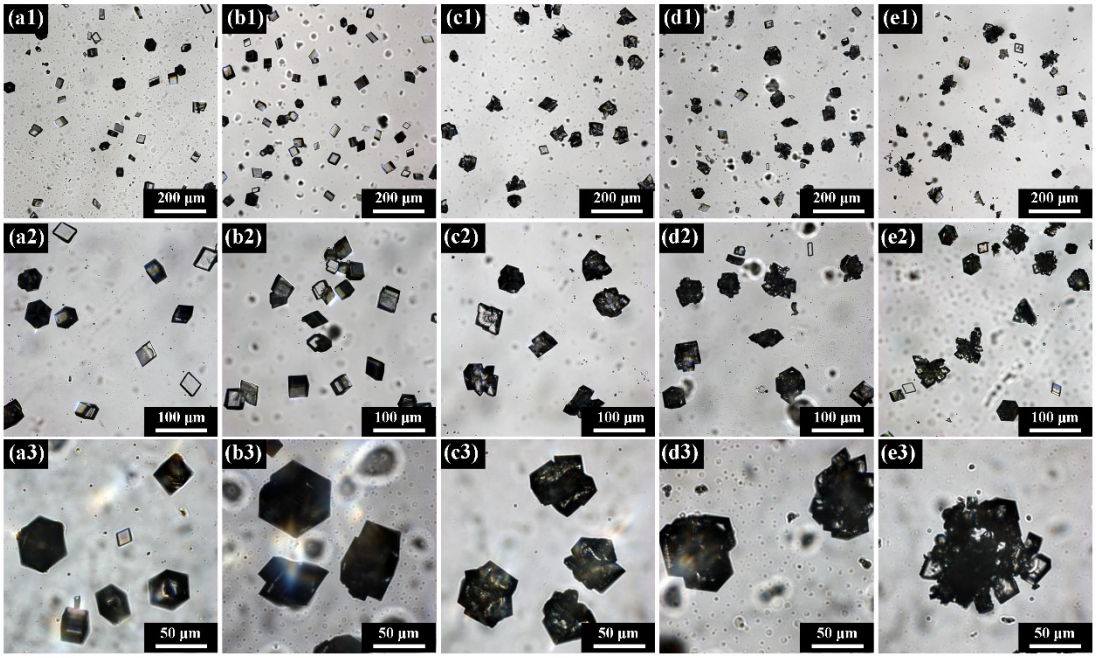


Figure s9 OM images of calcite formed in 30% Ace-$H_2O$ with different P peptide concentrations, $CaCl_2$ = 5 mM. (a1, a2 and a3) P peptide concentration = 0; (b1, b2 and b3) P peptide concentration = 0.01 mg/mL; (c1, c2 and c3) P peptide concentration = 0.05 mg/mL; (d1, d2 and d3) P peptide concentration = 0.1 mg/mL; (e1, e2 and e3) P peptide concentration = 0.2 mg/mL. (a2, b2, c2, d2 and e2) are partial Enlarged View of (a1, b1, c1, d1 and e1). (a3, b3, c3, d3 and e3) are partial Enlarged View of (a2, b2, c2, d2 and e2).

Table s1 Statistics of calcite crystal number in $H_2O$ with different P peptide concentration, $CaCl_2$ concentration = 5 mM.

| P peptide Concentration | Crystals Number |
|---|---|
| 0 | 103±35 |
| 0.01 mg/mL | 156±39 |
| 0.05 mg/mL | 76±5 |
| 0.1 mg/mL | 45±11 |
| 0.2 mg/mL | 61±12 |

Table s2 Statistics of calcite crystal number in 30% Ace-$H_2O$ with different P peptide concentration, $CaCl_2$ concentration = 5 mM.

| P peptide Concentration | Calcite Crystal Number | | | | | total |
|---|---|---|---|---|---|---|
| | Single | dimer | trimer | hexamer | cluster | |
| 0 | 330±6 | 30±6 | 0 | 0 | 0 | 360±92 |
| 0.01 mg/mL | 361±13 | 71±11 | 14±3 | 0 | 0 | 446±57 |
| 0.05 mg/mL | 14±1 | 13±2 | 84±2 | 0 | 0 | 112±7 |
| 0.1 mg/mL | 6±1 | 9±2 | 18±3 | 85±6 | 5±2 | 123±14 |
| 0.2 mg/mL | 10±3 | 43±6 | 32±6 | 6±2 | 27±12 | 119±29 |

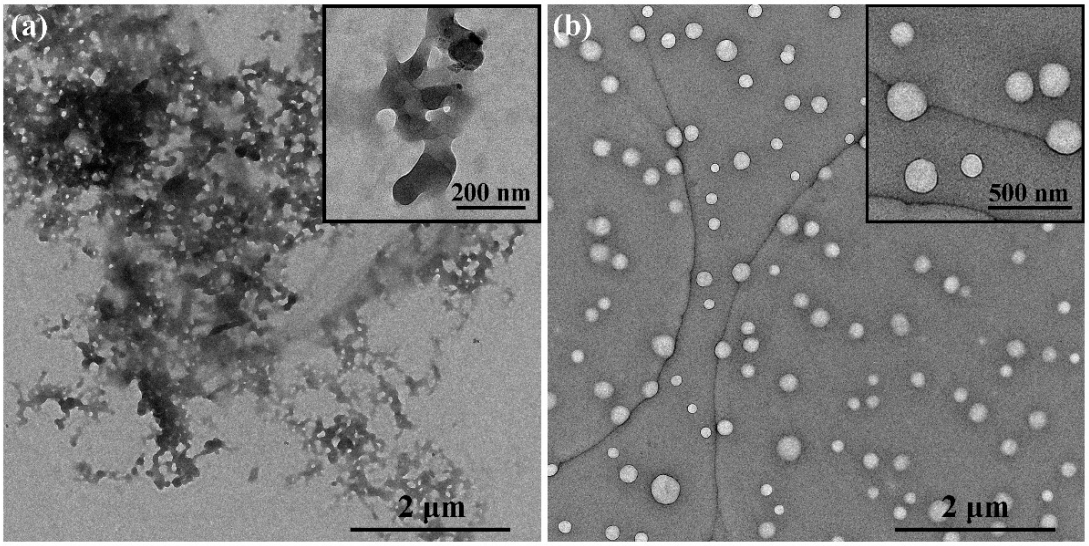


Figure s10 TEM images of NP peptide in $H_2O$ (a) and 30% Ace-$H_2O$ (b).

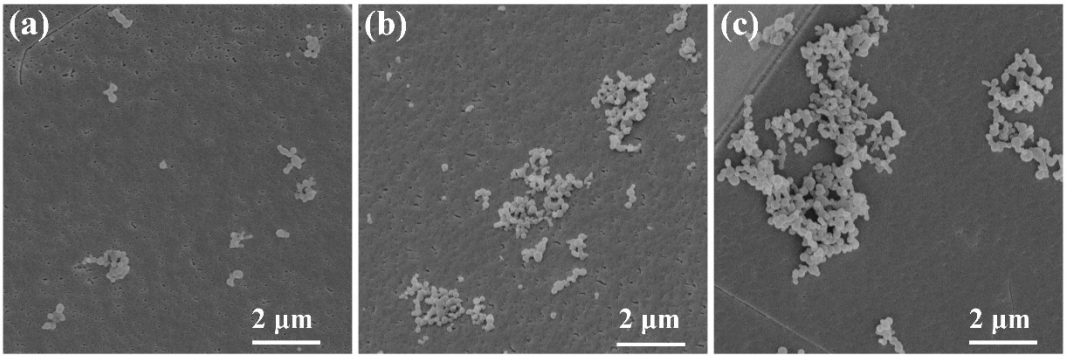


Figure s11 High magnification images of NP peptide particles adsorbed on calcite surface at the concentration of NP peptide (a) 0.01, (b) 0.05 and (c) 0.1 mg/mL.

Table s3 Diffraction intensity ratio of (012), (006), (110), (11-3), (202), (018), (11-6), (208), (0012) to (104) crystal planes of calcite in $H_2O$ with different P peptide concentrations.

| | 0 mg/mL | 0.01 mg/mL | 0.05 mg/mL | 0.1 mg/mL | 0.2 mg/mL |
|---|---|---|---|---|---|
| (012)/(104) | 0 | 0 | 0 | 0 | 0 |
| (006)/(104) | 0.029 | 0.008 | 0.033 | 0 | 0.041 |
| (110)/(104) | 0 | 0 | 0 | 0 | 0 |
| (11-3)/(104) | 0 | 0 | 0.016 | 0.009 | 0 |
| (202)/(104) | 0 | 0 | 0 | 0.011 | 0 |
| (018)/(104) | 0.012 | 0 | 0.012 | 0.008 | 0 |
| (11-6)/(104) | 0 | 0 | 0.025 | 0.015 | 0 |
| (208)/(104) | 0.027 | 0.027 | 0.027 | 0.037 | 0.041 |
| (0012)/(104) | 0.042 | 0.004 | 0.058 | 0 | 0.052 |

Table s4 Diffraction intensity ratio of (012), (006), (110), (11-3), (202), (018), (11-6), (208), (0012) to (104) crystal planes of calcite in 30% Ace-$H_2O$ different P peptide concentrations.

| | 0 mg/mL | 0.01 mg/mL | 0.05 mg/mL | 0.1 mg/mL | 0.2 mg/mL |
|---|---|---|---|---|---|
| (012)/(104) | 0.002 | 0 | 0.032 | 0.085 | 0 |
| (006)/(104) | 0.011 | 0.012 | 0 | 0 | 0 |
| (110)/(104) | 0.003 | 0 | 0.025 | 0.064 | 0.019 |
| (11-3)/(104) | 0 | 0.006 | 0.051 | 0.094 | 0 |
| (202)/(104) | 0 | 0 | 0.031 | 0.048 | 0.009 |
| (018)/(104) | 0.003 | 0.011 | 0.031 | 0.053 | 0 |
| (11-6)/(104) | 0.002 | 0.010 | 0.025 | 0.053 | 0 |
| (208)/(104) | 0.020 | 0.028 | 0 | 0 | 0.016 |
| (0012)/(104) | 0.011 | 0.009 | 0 | 0 | 0 |

1. K. M. Bromley, R. Lakshminarayanan, M. Thompson, S. B. Lokappa, V. A. Gallon, K. R. Cho, S. R. Qiu and J. Moradian-Oldak, *Cryst. Growth Des.*, 2012, **12**, 4897-4905.