Electronic Supplementary Information

The effect of amorphous calcium phosphate on protein protection against thermal denaturation

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Experimental details

Mineralization procedures. 0.02 mg/mL CAT (76049 U/mg Protein, Worthington, USA) in Tris-HCl buffer solution (pH 8.0) was incubated with extra-added $CaCl_2$ (5 mM) and $MgCl_2$ (5 mM) for 30 min. This solution was then mixed with Na_2HPO_4 (3 mM) for 4 h to trigger *in situ* ACP precipitation with CAT to generate the ACP-CAT nanocomposites. The resulted nanocomposites could be collected by centrifugation at 16,000 g for 10 min (Beckman Coulter Allegra 64R, USA). The procedures of lysozyme mineralization modification were the same as those of CAT. Beside Mg^{2+}, a macromolecule, polyacrylic acid (PAA) was also applied as ACP stabilizer. PAA stabilized ACP-CAT was prepared by mixing CAT (0.02 mg/mL), $CaCl_2$ (5 mM), PAA (50 μg/mL) and Na_2HPO_4 (3 mM).

Au Labelled CAT. Au labelled CAT was synthesized as described previously.[1] Typically, aqueous $HAuCl_4$ solution (5 mL, 10 mM, 37°C) was added to CAT solution (5 mL, 50 mg/mL, 37 °C). NaOH solution (0.5 mL, 1 M) was then added and the reaction was performed at 37 °C for 24 h.

Characterizations. ACP-CAT nanocomposites were dried in vacuum at 30°C. Their morphology was observed under TEM (Hitachi HT9500, Japan). The mineral phase was examined by FTIR (Shimadzu FTIR-8400, Japan), XRD (X'Pert PRO, $Cu_{K\alpha}$ =1.54 Å, PANALYTICAL, Netherlands). The Au labelled CAT in ACP nanoparticles was characterized by HRTEM (JEOL JEM-2100F, Japan).

Enzyme Activity Assays. CAT assays were performed on a UV/Vis Spectrometer (PERSEE New Century T6, China). The enzyme activity was determined spectrophotometrically by using H_2O_2 as the substrate.[2] Briefly, the free or mineralized enzyme was added to HEPES buffer solution (20 mM, pH 6.5, 25 °C) with a H_2O_2 concentration of 10 mM. The absorbance at 240 nm was recorded with time and the activity was determined according to following equation,

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2 \qquad (1)$$

Lysozyme activity assays were performed with Micrococcus lysodeikticus cells according to previous literature.[3]

Thermostability test. Free or mineralized enzyme was incubated at different temperatures. The samples were collected periodically and their remained activity was examined by enzyme activity assay.

Circular dichroism measurement. CD spectra were performed on an Applied Photophysics Chirascan Spectrometer (MOS 450, Bio-Logic, France). CAT (0.2 mg/mL) was examined in a UV region of 200 to 250 nm in a 0.1 cm path length quartz cell at a step size of 0.5 nm. The concentrations of encapsulated enzyme were calculated based on the activity assay.

H/D exchange assays. H/D exchange was performed by MALDI-MS (Bruker Daltonics UltrafleXtremeFree, Germany). CAT (0.5 mg/mL) and ACP-CAT were equilibrated overnight in a buffer (10 mM Tris-HCl at pH 8.0) prior to 20-fold dilution into D_2O buffer (10 mM Tris-HCl at pD 8.0) to initiate H/D exchange. Exchange times used for labelling at D_2O buffer spanned the range from 1 min to 12 h. After a given exchange time, an aliquot of the deuterated sample was diluted 2-fold into an ice-cold and low pH SA MALDI matrix solution. This step quenched the H/D exchange and prepared the sample for MALDI analysis. The saturated SA matrix solution was prepared by dissolving SA in 45% MeCN/54.9% H_2O/0.1% TFA (v/v, pH 2.0). 2 μL aliquot of the sample in the quenched H/D exchange buffer was spotted onto a stainless steel MALDI target and allowed to dry under a gentle flow of air before the sample was introduced into the vacuum of the MALDI-MS.

Thermal gravimetric analysis. TGA was performed by a thermal gravimetric analyzer (SDT Q600, TA, America) with a heating rate of 10 °C min^{-1} under a constant gas flow rate of air 100 mL min^{-1}. Detailed studies of water dehydration in ACP nanoparticles were performed after annealing samples at specific temperatures for 2 h.

References

1 J. P. Xie, Y. G. Zheng, J. Y. Ying, *J. Am. Chem. Soc.*, 2009, 131, 888-889.

2 S. A. Cetinus, H. N. Oztop, *Enzyme Microb. Tech.*, 2003, 32, 889-894.

3 L. N. Subbaraman and L. Jones, *J. Biomat. Sci. polym. Ed.*, 2010, 21, 343-358.

Supporting Figures

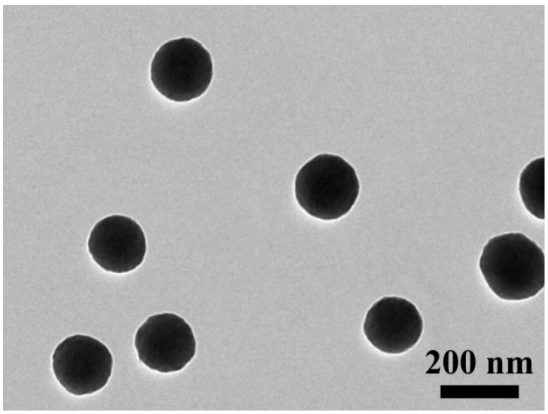

Fig. S1 TEM image of ACP-CAT nanoparticles. The particles were dispersive nanospheres with uniform size distributions of 150-200 nm.

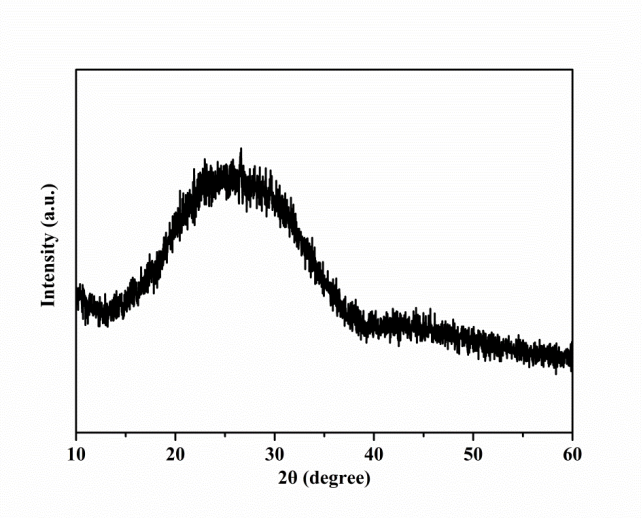

Fig. S2 XRD analysis of ACP-CAT nanocomposites. The resulted mineral phase was amorphous.

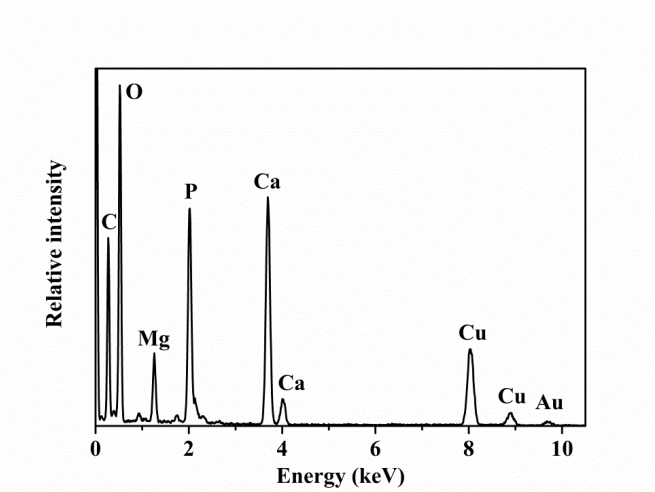

Fig. S3 Energy dispersive spectroscopy (EDS) analysis of ACP-CAT nanocomposites conducted during HRTEM characterization. The molar ratio of Mg/Ca in the nanocomposites was ~0.2. The presence of carbon in the EDS spectrum was consistent with a precipitate consisting of an organic/inorganic composite of proteins in ACP matrix.

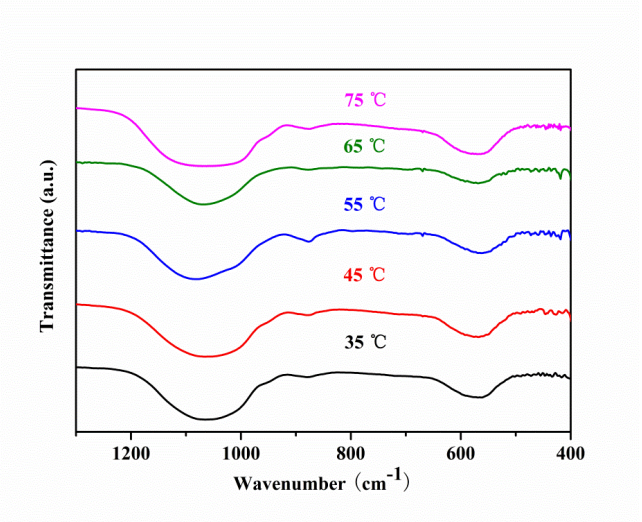

Fig. S4 FTIR of ACP-CAT nanoparticles after different temperatures heat treatment for 30 min. It indicated that the mineral phase was amorphous during thermal treatment.

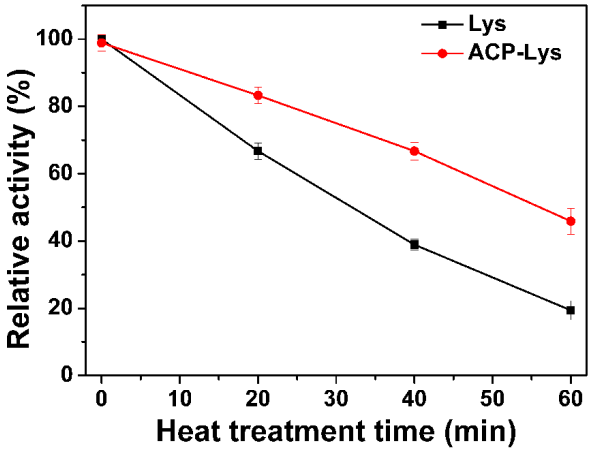

Fig. S5 Thermal inactivation curves of free lysozyme and ACP biomineralized lysozyme at 90°C. The experiment showed that ACP could improve the thermal stability of lysozyme, demonstrating a universal availability of the mineralization-based strategy for protein thermal improvement.

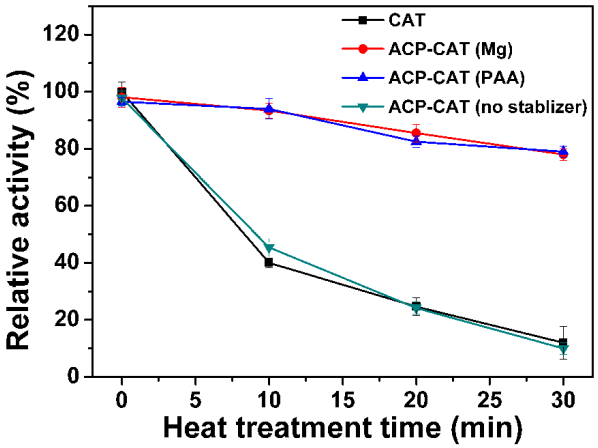

Fig. S6 Activity loss curves of free CAT, Mg^{2+} stabilized ACP-CAT, PAA stabilized ACP-CAT and ACP-CAT without stabilizer at 65 °C. It indicated that either Mg^{2+} or PAA stabilized ACP could enhance protein thermal stability significantly, while unstable ACP had no protective effect.

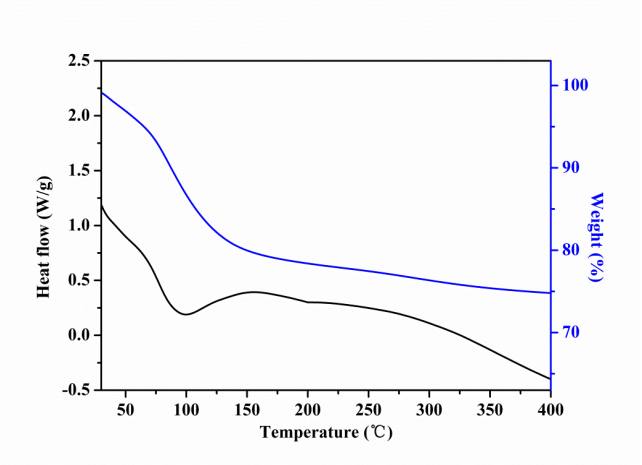

Fig. S7 TGA/DSC of ACP nanoparticles. The endothermic peak in DSC spectra was considered to be the dehydration of structural water present in the interstices of small fragments of CaP ion clusters.

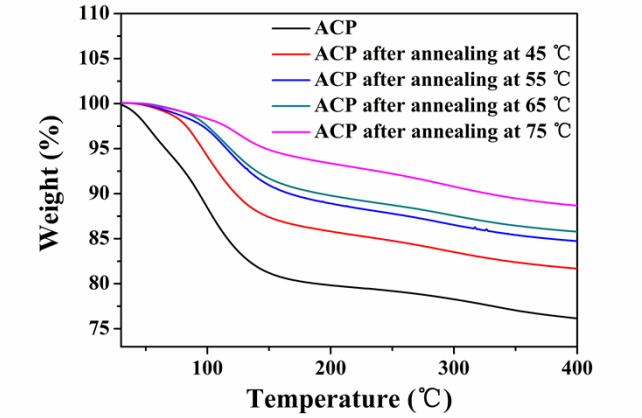

Fig. S8 Detailed studies of structural water dehydration by TGA of ACP nanoparticles after annealing at specific temperatures for 2 h. The dehydration content was calculated by the temperature range of the endothermic peak in DSC spectra.