Supporting Information for

A New Nanobiocatalytic System Based on Allosteric Effect with Dramatically Enhanced Enzymatic Performance

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a***Experimental section***

Chemicals and materials. α-Amylase (the weight percentage is ca. 85%) and 2-chloro-4-nitrophenylmaltotrioside (Cnp-G3) were obtained from Sigma-Aldrich. Calcium chloride ($CaCl_2$, >99%), potassium dihydrogen phosphate (KH_2PO_4, >99%), twelve hydrated disodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$, >99%), ethanol (CH_3CH_2OH, 95%), phosphoric acid (H_3PO_4, >85%), and Coomassie brilliant blue G-250 ($C_{47}H_{50}N_3NaO_7S_2$, >99%) were purchased from Shanghai Chemical Reagent Co. Led. All chemical reagents were used as received without further purification. All aqueous solutions were prepared using deionized (DI) water with a resistivity of 18.2 $M\Omega \cdot cm^{-1}$.

Synthesis of $CaHPO_4$-α-amylase hybrid nanostructures. Hybrid nanoflowers were synthesized through a calcification approach. In a typical synthesis, an aqueous solution of $CaCl_2$ (200 mM, 100 μL) was added into 5 mL of phosphate buffered saline (PBS) solution (3 mM) containing 0.2 mg/mL α-amylase at pH=6.8. The reaction was then allowed to proceed at room temperature for 12 h. The products were centrifuged at 15,000 rpm for 5 min and washed with water three times. The calcification yield of Ca^{2+} was determined to be ~88% by inductively coupled plasma atomic emission spectroscopy (ICP-AES). By comparison, the synthesis of hybrid nanoplates was conducted through only increasing the concentration of PBS to 30 mM. Parallel hexahedrons were produced when the concentration of α-amylase was augmented to 2 mg/mL, while other conditions remained unchanged.

Evaluation of the encapsulation yield of α-amylase. For preparation of Branford assay, 100 mg of Coomassie brilliant blue G-250 was added into 50 mL of ethanol solution, followed by the injection of 100 mL of phosphoric acid solution and 200 mL of DI water. The solution was then diluted five times and stored at 4 ^oC.

To establish the calibration curve for the concentration of α-amylase, a series of PBS solutions (4 mL, 3 mM) containing 0, 0.01, 0.1, 0.2, and 0.5 mg/mL α-amylase were prepared, followed by the addition of 4 mL of Brandford assay. After 15 min, the absorbance at 595 nm was monitored for each solution.

The concentration of α-amylase that was not immobilized was tested in the following manner. 4 mL of supernatant solution was taken from each synthesis, followed by the addition of 4 mL of Brandford assay. The absorbance of this mixed solution was then monitored at 595 nm to determine the concentration of free α-amylase based on the calibration curve. The amount of

encapsulated α-amylase can be calculated by subtracting the amount of free α-amylase from the total amount of α-amylase introduced in a synthesis and the encapsulation yield of α-amylase can be obtained by dividing the amount of total α-amylase by that of the encapsulated one.

Catalytic studies. We used the hydrolysis of Cnp-G3 as a model system to quantitatively evaluate the catalytic activity of the free and immobilized α-amylase. In a typical process, an aqueous solution of Cnp-G3 (50 μL, 9.45 mM) was quickly injected into 1 mL of a PBS solution containing different types of catalysts in a quartz cuvette to continuously measure the curves of extinction *versus* time at 405 nm. The concentrations of α-amylase were kept at 0.04 mg/mL for all catalytic systems except the reaction without any catalyst. To test the recycling of the nanoflowers, the first measurement was conducted as described above, and the reaction rate constant (*k*) for the first measurement was set as 100%. Then, the suspension was immediately centrifuged at 15,000 rpm for 10 min, and the precipitate was washed with PBS and centrifuged again. The precipitate was then subjected to the next catalytic cycle.

Instrumentation. SEM images were obtained with a Supra 40 FE-SEM operated at 5 kV. TEM and high-resolution TEM images were collected on a JEOL-2010 field-emission transmission electron microscope operating at 200 kV accelerating voltage. The energy-dispersive X-ray spectroscopy analysis was also done with a JEOL-2010 TEM. XRD characterization was performed using a Philips X'Pert Pro X-ray diffractometer with a monochromatized Cu Kα radiation source and a wavelength of 0.1542 nm. TGA analyses were performed on a Shimadzu TGA-50 thermogravimetric analyzer at a constant heating rate of 10 $K \cdot min^{-1}$ under ambient air with a gas flow of 25 $mL \cdot min^{-1}$. UV-vis spectra were recorded on a U-4100 at room temperature (Hitachi, Japan). ICP-AES (Atomscan Advantage, Thermo Jarrell Ash, USA) was used to determine the concentration of Ca^{2+} ions.

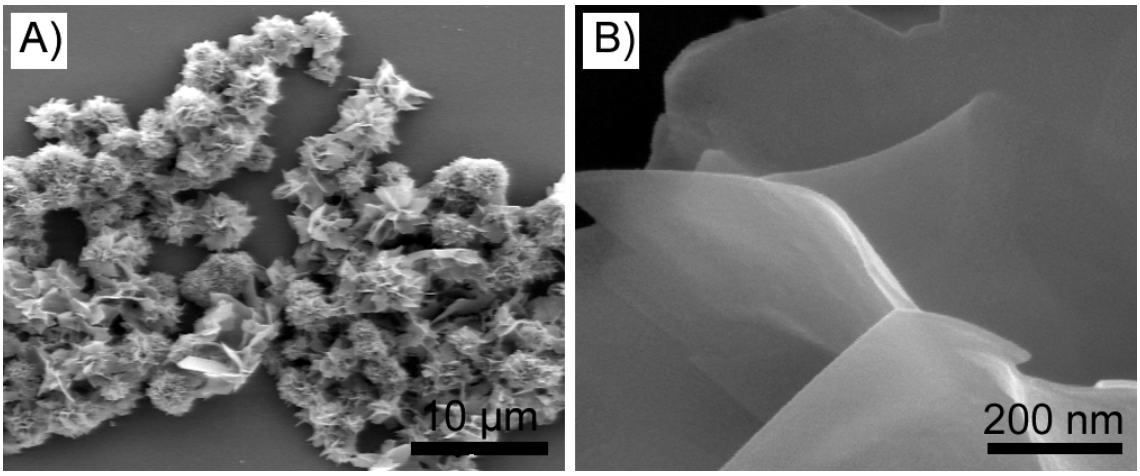

Figure S1. SEM images of the $CaHPO_4$-α-amylase hybrid nanoflowers.

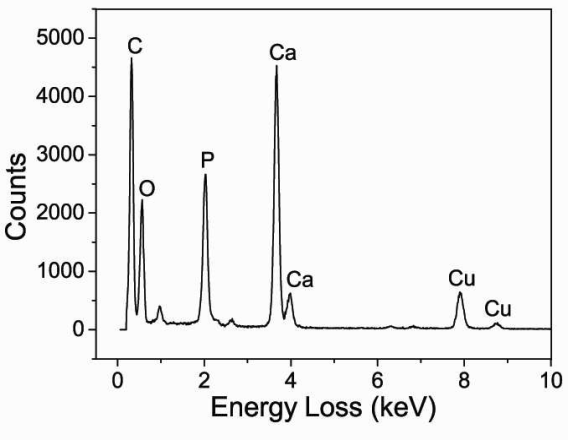

Figure S2. EDX spectrum of the $CaHPO_4$-α-amylase hybrid nanoflowers.

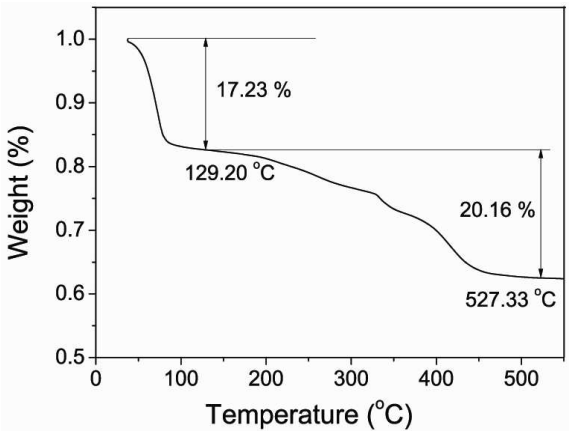

Figure S3. TGA spectrum of the nanoflowers. The weight loss between RT and 129.20 ^{o}C was 17.23%, corresponding to the loss of crystallization water of $CaHPO_4 \cdot 2H_2O$. As temperature increased from 129.20 to 527.33 ^{o}C, the weight loss was 20.16%, resulting from pyrolytic decomposition of α-amylase.

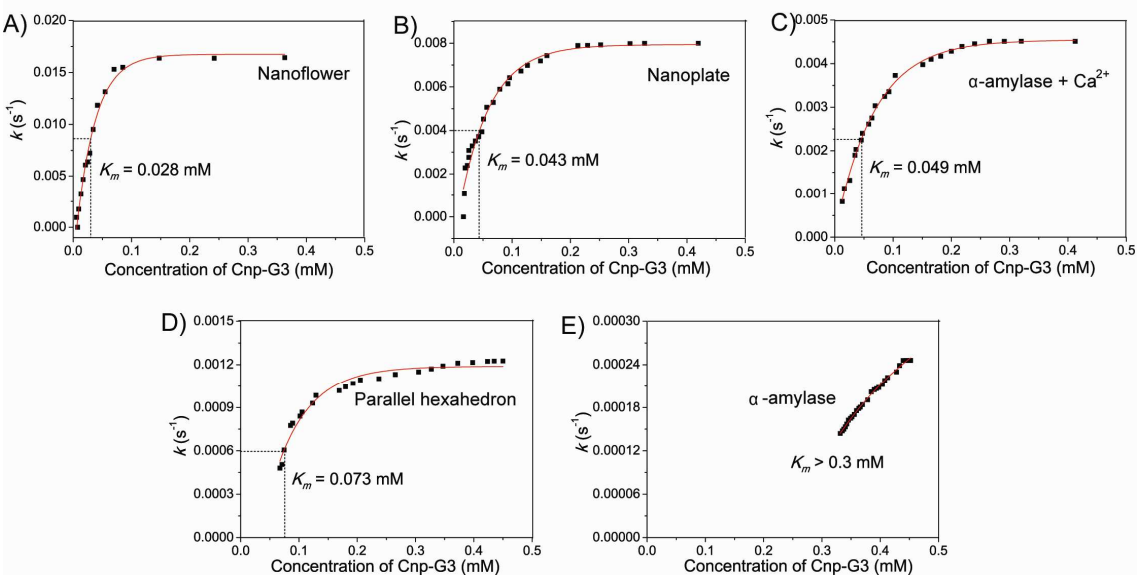

Figure S4. Plots of reaction rate constant (k) against the concentration of Cnp-G3 for five catalytic systems. The K_m values determined from these plots are 0.028，0.043，0.049，and 0.073 mM for nanoflowers, nanoplates, free α-amylase with Ca^{2+}, and parallel hexahedron, respectively. The K_m value for free α-amylase is bigger than 0.3 mM, though the exact value is hard to determine.

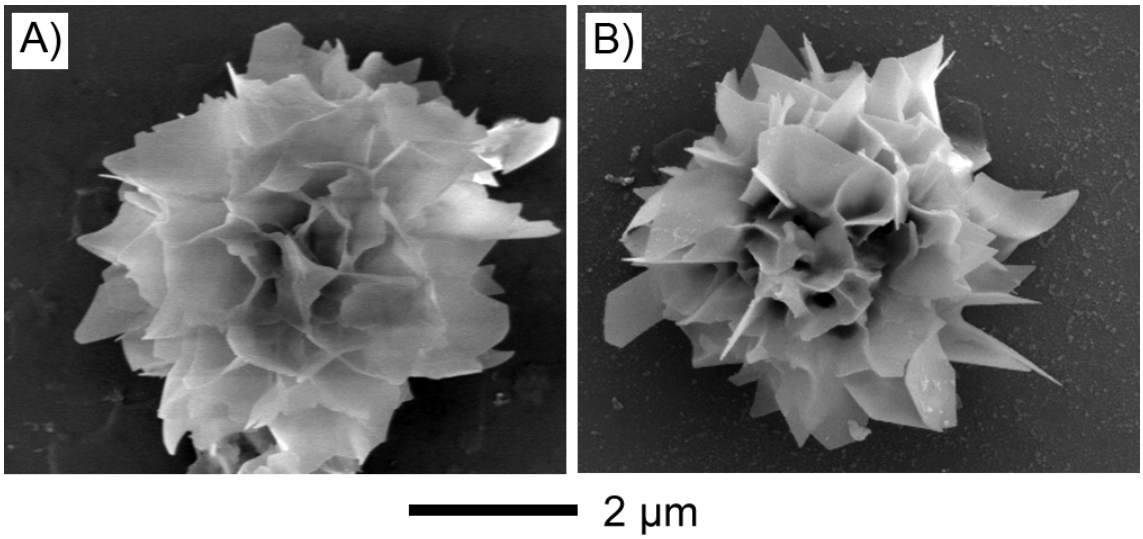

Figure S5. SEM images of the nanoflowers (A) after eight rounds of successive catalytic reaction and (B) stored in PBS for 25 days.