Supporting Information

Self assembly of organic-inorganic hybrid nanoflower as anefficientbiomimetic catalyst for self-activated tandem reaction

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Experimental Section

*Reagents and materials:*2, 2-azinobis (3-ethylbenzothiozoline)-6-sulfonic acid (ABTS) and Rhodamine B (RhB)were purchased from Sigma-Aldrich. 3,3,5,5-Tetramethylbenzidine (TMB) was purchased from BBI (Ontario, Canada). Glucose was obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). Bovine serum albumin, glucose oxidase and horseradish peroxidase were purchased from Sangon Biotechnology Inc. (Shanghai, China). $CuSO_4$ and hydrogen peroxide were purchased from Beijing Chemicals (Beijing, China). All other reagents were of analytical reagent grade, and used as received. Ultrapure water (18.2 MΩ; Millpore Co., USA) was used throughout the experiment.

*Measurements and characterizations:*The SEM samples were prepared by depositing a dilute aqueous dispersion of the as-prepared samples onto a silicon wafer. TEM images, high-angle annular dark-field scanning TEM (HAADF-STEM) and the energy dispersive X-ray spectroscopy (EDX) were recorded using a FEI TECNAI G2 20 high resolution transmission electron microscope operating at 200 kV. The crystalline structures of the as-prepared samples wereevaluated by X-ray diffraction (XRD) analysis on a on a D8 Focusdiffractometer (Bruker) using Cu Kα radiation (λ = 0.15405 nm). The UV-Vis absorption spectra were recorded using a JASCO V550

UV/Visible spectrophotometer (JASCO International Co., LTD., Tokyo, Japan). The pH measurements were performed with a PHS-3C portable pH meter (Shanghai Precision & Scientific Instrument Co., China).

Preparation of BSA-$Cu_3(PO_4)_2.3H_2O$ nanoflowers and GOx-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanoflowers: BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$nanoflowers: 20 μL of aqueous $CuSO_4$ solution (120 mM) was added to 3 mL of PBS (0.1 M, pH 7.4) containing different concentrations of bovine serum albumin (0.02 mg mL^{-1}, 0.1 mg mL^{-1} and 0.5 mg mL^{-1}). The mixture was then incubated at 25 °C for 3 days.

GOx-$Cu_3(PO_4)_2{\cdot}3H_2O$nanoflowers: 20 μL of aqueous $CuSO_4$ solution (120 mM) was added to 3 mL of PBS (0.1 M, pH 7.4) containing 0.5 mg mL^{-1} glucose oxidase. The mixture was then incubated at 25 °C for 3 days.

Peroxidase-like activity measurements: The peroxidase-like activity of the BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanoflowers (0.5 mg mL^{-1} BSA) were investigated through the catalytic oxidation of the peroxidase substrate ABTS in the presence of H_2O_2. To examine the capability of our nanoflower as a catalyst on the oxidation of ABTS (1 mM): (1) blank control; (2) 20 μg mL^{-1} BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$; (3)25 mM H_2O_2 ; (4) 25 mM H_2O_2 and 20 μg mL^{-1} BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$. All the reactions were incubated in10 mM pH 4.0 phosphate buffer and monitored spectrophotometrically at 417 nm.

*Glucose oxidation reaction and ABTS oxidation reaction:*Gluconic acid, the oxidation product, was verified by reaction with hydroxylamine and Fe^{3+}. The mixture could produce a red color change with an absorbance at 505 nm. In brief, 250 mL of solution 1 (5 mM EDTA and 0.15 mMtriethylamine in water) and 25 mL of solution 2

(3 M NH_2OH in water) were added to the catalytic reaction solution. After 25 min incubation, 125 mL of solution 3 (1 MHCl, 0.1 M $FeCl_3$, and 0.25 M CCl_3COOH in water) was added to the above mixture. The reaction solution was incubated for another 5 min before spectral measurements. In order to prevent the influence of the nanoparticles, the solution was centrifuged to remove the nanoparticles. The other product, H_2O_2 was assayed via the oxidation of 1 mM ABTSin the presence of 0.05 μg mL^{-1}HRP. The oxidation product in 10 mM pH 7.4 phosphate buffer produced a green color with a major absorbance peak at 417 nm.

*Kinetic studies:*Kinetic measurements were carried out in time course mode by monitoring the absorbance change of TMB at 652 nm.20μg mL^{-1}BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanoflowersand 0.8 mMTMBwere added in pH 4.0buffer solution (200 μL, 37 °C) in the presence of a series of concentrations of H_2O_2.The Michaelis-Menten constant was calculated using the Lineweaver-Burk plot: $1/v = (K_m/V_{max}) \cdot (1/C) + 1/V_{max}$, where$v$is the initial velocity, V_{max} is the maximal reaction velocity, and C is the concentration of substrate.[3b, 3d]

*Self-activated cascade reaction:*20 mM glucose and 0.8 mg mL^{-1} GOx-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanoflowers were added initially and the mixed solutions were incubated at 37 °C for 5 h. After that, 4 mM ABTS was added to the above solutions. All the solutions were incubated in 0.1 mM pH 7.4 phosphate buffer.

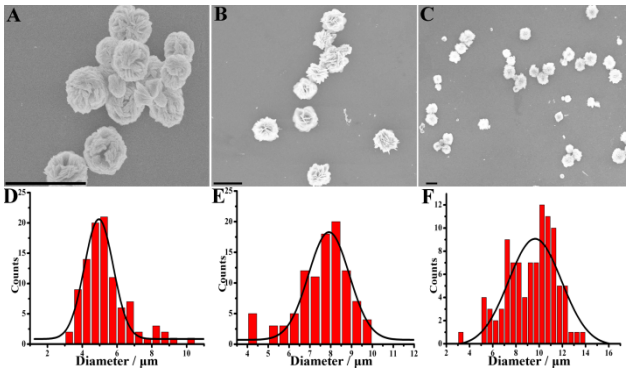

Fig. S1 A-C) SEM images of nanoflowers with different concentrations of bovine serum albumin: A) 0.5 mg mL^{-1}; B) 0.1 mg mL^{-1}; C) 0.02 mg mL^{-1}. Scale bars are 10 μm. D-F) Size distribution histogram of BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ hybrid nanoflowers: D) 0.5 mg mL^{-1}; E) 0.1 mg mL^{-1}; F) 0.02 mg mL^{-1}.

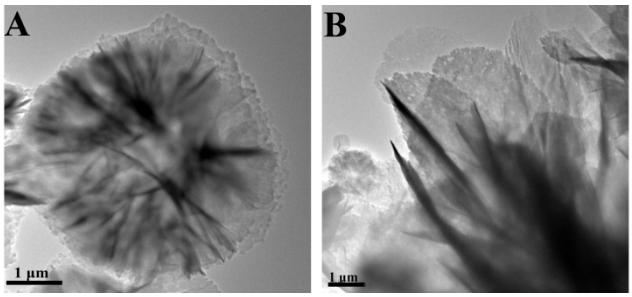

Fig. S2A) TEM images of the nanoflowers; B) High-resolution TEM of the region.

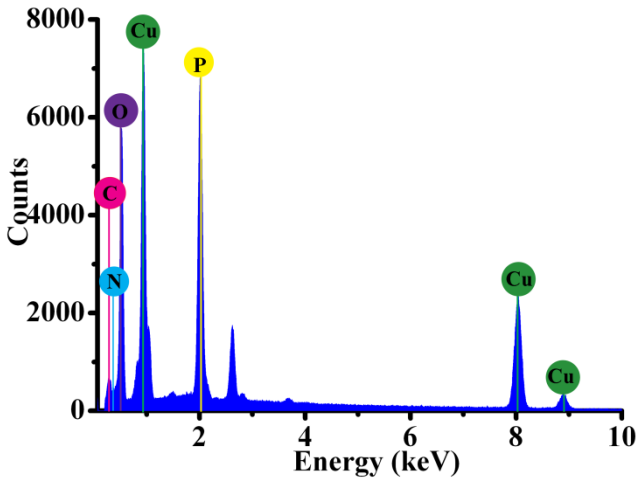

Fig. S3EDX pattern of the hybrid nanoflowers.

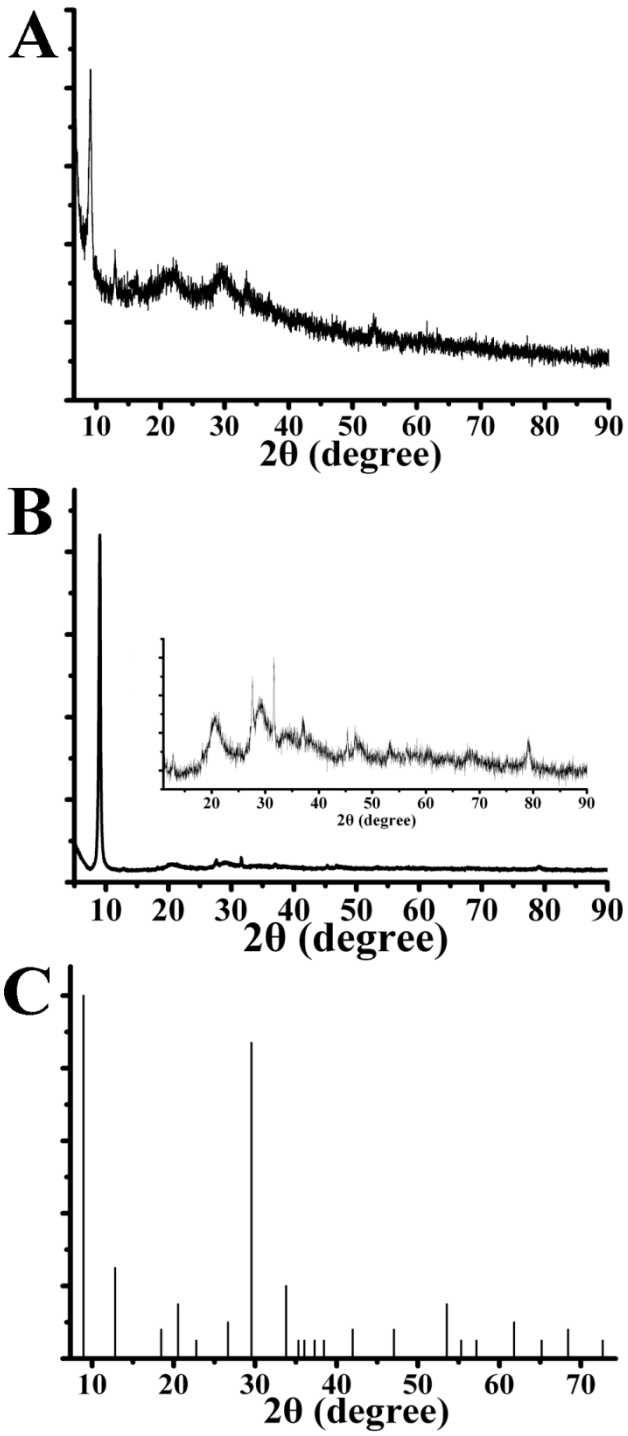

Fig. S4 XRD patterns of samples: A) nanoflowers obtained with BSA; B) particles of crystals obtained without BSA, inset was the partial enlarged image; C) standard $Cu_3(PO_4)_2 \cdot 3H_2O$ (JPSCD 00-022-0548).

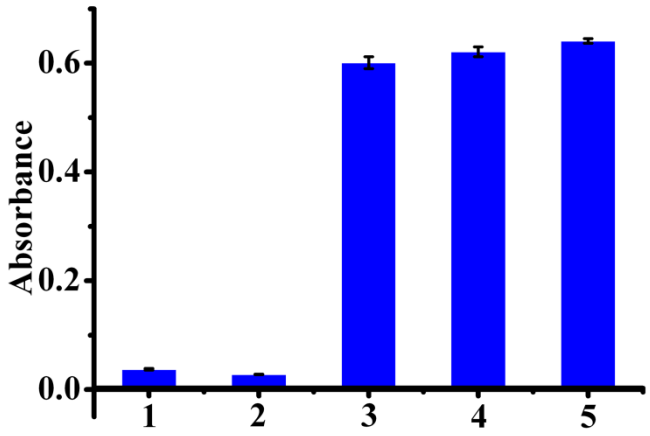

Fig. S5 The exploration of catalytic mechanism of our catalyst. 1) BSA; 2) GOx; 3) $Cu_3(PO_4)_2 \cdot 3H_2O$crystal; 4) BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ hybrid nanoflower; 5) BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ hybrid nanoflower. All solutions were incubated with 1 mM ABTS and 25 mM H2O2 in10 mM 4.0 phosphate buffer at 37 °C.

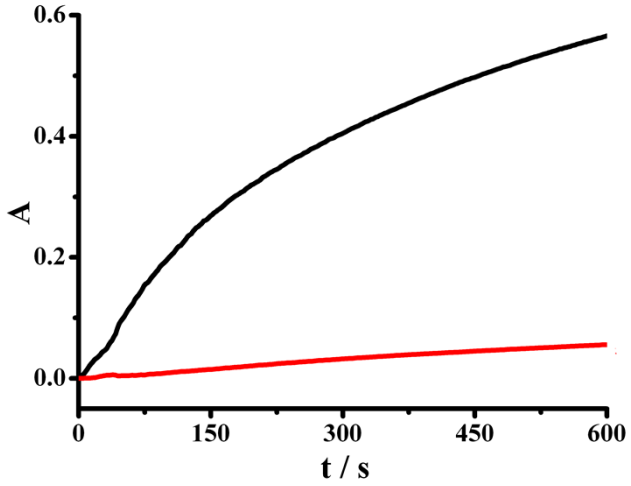

Fig. S6 Demonstration that the catalytic activity of nanoflowers does not result from copper leaching. 20 μg mL^{-1} BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ hybrid nanoflowers were incubated in 10 mM pH 4.0 reaction buffer for 600 s, and then removed. The activity of the leaching solution (red line) was then compared to that of the nanoflowers (black line). Each solution was incubated with 1 mM ABTS and 25 mMH_2O_2.

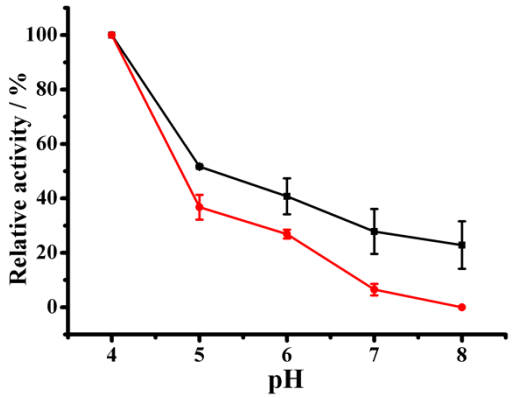

Fig. S7 Effects of pH on the catalytic activities of BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ hybrid nanoflowers (red line) and free HRP (black line). Experiments were carried out using 20 ng mL^{-1} HRP or 20 μg mL^{-1} BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ hybrid nanoflowers. All reactions were implemented in 10 mM phosphate buffer with 1 mM ABTS and 25 mM H_2O_2.

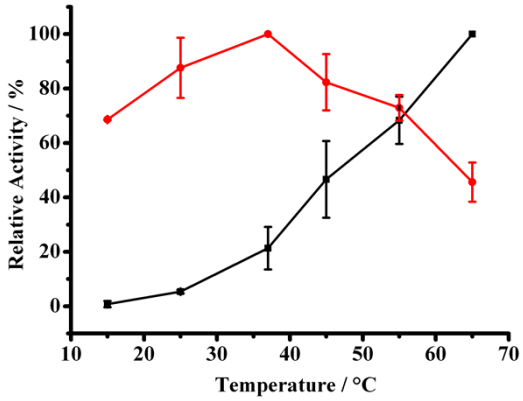

Fig. S8 Effects of operating temperature on the catalytic activities of BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ hybrid nanoflowers (black line) and free HRP (red line). Exprements were carried out using 20 $ngmL^{-1}$ HRP or 20 $\mu g\ mL^{-1}$ BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ hybrid nanoflowers. All reactions were implemented in 10 mM pH 4.0 phosphate buffer with 1 mM ABTS and 25 mM H_2O_2.

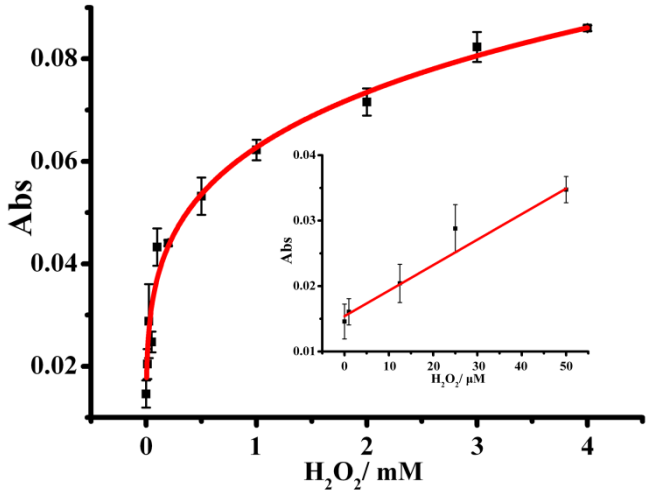

Fig.S9 Liner calibration plot for the detection of H_2O_2.

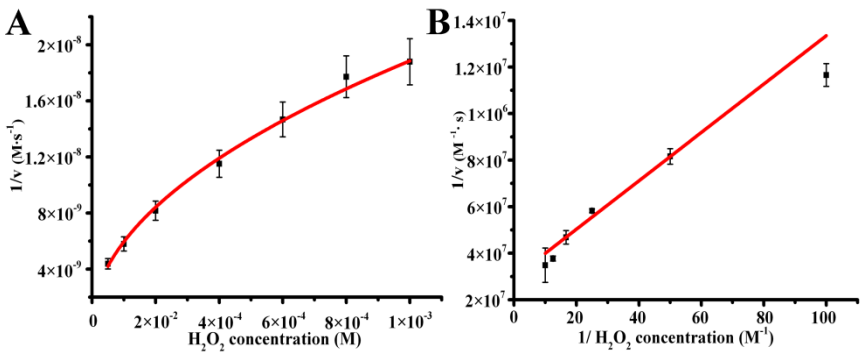

Fig .S10 Lineweaver-Burk plot of the reciprocals of initial rate vs substrate concentration for the determination of kinetic parameters K_m and V_{max} of BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ hybrid nanoflowers with 0.8 mM TMB. The velocity (ν) of the reaction was measured using 20 μgmL^{-1} in 200 μL of 10 mM pH 4.0 phosphate buffer at 37 °C. Error bars were estimated from three independent measurements.

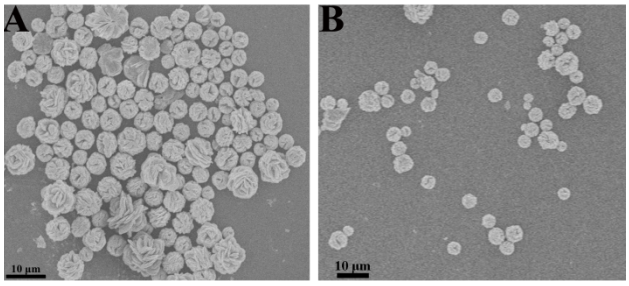

Fig. S11 SEM images of the BSA-$Cu_3(PO_4)_2 \cdot 3H_2O$ nanoflowers (A) after catalytic reactions (37 °C); (B) after high-temperature reactions (65 °C).

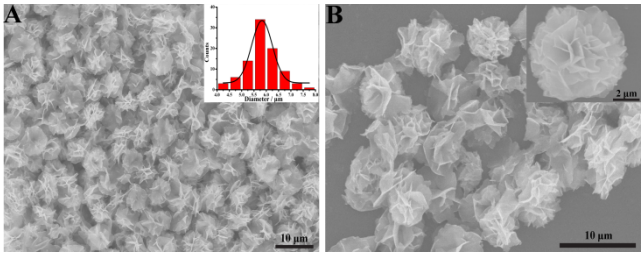

Fig. S12SEM images of the GOx-incorporated nanoflowers. A) before catalytic reactions, inset was the size distribution histogram of nanoflower; B) after catalytic reactions, inset was the partial enlarged image of nanoflower.

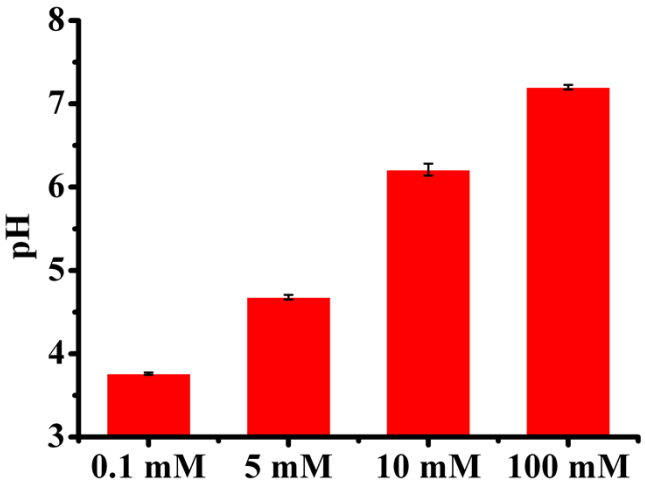

Fig. S13 The influence of buffer concentrations on enzymatic reactions. All reactions were carried out in pH 7.4 phosphate buffer.

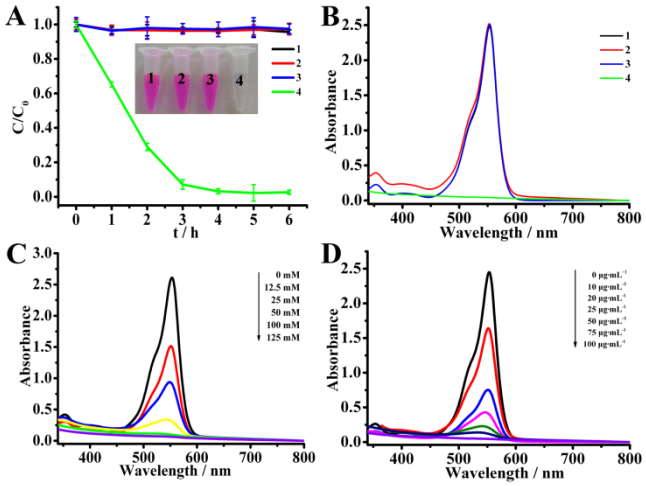

Fig.S14 Removal of toxic organic compounds. (A) RhodamineBdegradation over time with different solutions: 1) blank control; 2) 125 μgmL^{-1}BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanoflower only; 3) 125 mMH_2O_2 alone; 4) 125 μgmL^{-1}BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanoflower and 125 mMH_2O_2. Inset was the typical photograph of corresponding solutions; (B) The absorption spectra of theRhB solution after incubated with different components for 6 h: 1) blank control; 2) 125 μgmL^{-1}BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanoflower only; 3) 125 mM H_2O_2 alone; 4) 125 μgmL^{-1}BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanoflower and 125 mM H_2O_2; (C) Relative absorbance spectraof thesolution containing RhBand 125 μgmL^{-1}nanoflower with different concentrations of H_2O_2; (D) Relative absorbance spectraof thesolutioncontaining RhB and 125 mMH_2O_2 with different concentrations of BSA-$Cu_3(PO_4)_2{\cdot}3H_2O$ nanoflower. All samples were incubatedwith 12.5 μgmL^{-1}RhB in10 mMpH 4.0 phosphate buffer at 37 °C.