# Electronic Supplementary Information

## Self-repairing metal-organic hybrid complexes for reinforcing immobilized chloroperoxidase reusability

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## Materials

Chloroperoxidase (CPO; EC 1.11.1.10; from Caldariomyces fumago: 4699 units/mL) was purchased from Sigma-Aldrich, USA. CPO was used without further purification, and the stock solution is a 20-fold dilution. Bovine serum albumin (BSA) was purchased from Biotopped, China. Modafinil and 2-(diphenylmethylthio) acetamide was purchased from Energy Chemical, China. Hydroquinone was purchased from Tianjin Fuchen Chemical Reagents Factory. The enzyme substrate 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonicacid Ammonium salt) ($ABTS^{2-}$) was obtained from aladdin, China. Sodium alginate (SA) was purchased from Sinopharm Chemical Reagent, China. Tert-butyl hydroperoxide (TBHP) was purchased from XIYA Reagent, China., Trifluoracetic acid (TFA), acetonytrile, methanol, isopropanol and cyclohexane used for analytical

HPLC were chromatographic grade and obtained from Honeywell Burdick & Jackson. $CaCl_2$, $Na_2HPO_4$, $NaH_2PO_4$, HCl, $H_2O_2$ and other reagents and solvents were obtained from Beijing Chemical Works and were of analytical grade.

## Preparation of metal-organic hybrid complexes

BSA water solution (1 mg/mL, 100 μL) or CPO (12 U) and PBS buffer (10 mM, pH 7.4, 900 μL) were mixed together, followed by adding aqueous $CaCl_2$ solution (180 mM, 100 μL). After incubating for 24 h, the mixture was centrifuged at 7378 g for 5 min to obtain the complexes. The encapsulation efficiency was determined by the Bradford's method.[S2]

## Preparation of SA coated metal-organic hybrid complexes

The precipitation of BSA@$Ca_3(PO_4)_2$ or CPO@$Ca_3(PO_4)_2$ was re-dispersed into a PBS buffer (pH 6.5, 20 mM, 600 μL), then adding 400 μL of SA solution (20 mg/mL). The mixture was incubated at room temperature for the formation of self-repairing metal-organic hybrid composites. After 12 h, the solution was centrifuged at 10625 g for 5 min to obtain SA coated composites.

## Characterization

Transmission electron microscopy (TEM) was performed on a Hitachi H-800 transmission electron microscope. The sample was prepared by pipetting a drop of the aqueous solution of the samples onto a 230 mesh holy carbon copper grid and drying on a filter paper. Scanning

electron microscope (SEM) was performed on a Hitachi S-4700 Scanning electron microscope. A drop of the suspension of the prepared samples was added to a cover glass pieces for SEM and dried at room temperature. Confocal laser scanning microscopy (CLSM) was performed on a LEICA TCS SP8 confocal laser scanning microscopy. Isothiocyanate (FITC)-labeled BSA was prepared according to the method reported by Wu and the co-authors[S1].

Powder X-ray diffraction (XRD) patterns were recorded using a D8 Advance X-Ray diffractometer with a Cu Kα anode (λ= 0.15406 nm) at 40 kV and 40 mA. Fourier transform infrared spectroscopy (FTIR) spectra of BSA, $Ca_3(PO_4)_2$, BSA@$Ca_3(PO_4)_2$ and SA-coated BSA@$Ca_3(PO_4)_2$ composites were performed on a Nicolet 8700/Continuum XL Imaging Microscopy with measuring wavelength range from 4000 to 400 $cm^{-1}$.Each sample was lyophilized before XRD measurement.

## Activity assay of free and immobilized CPO

The activity of chloroperoxidase was established by following the decrease of absorbance at 414 nm due to the conversion of $ABTS^{2-}$ to $ABTS^{\bullet-}$. The activity assay was carried out at room temperature in 10 mM pH 2.75 phosphoric acid buffer solution (10mM, 400 μL, prepared by phosphoric acid and $NaH_2PO_4$), 0.25 mM $ABTS^{2-}$, 4.4 mM $H_2O_2$, and 0.5 U CPO. Immobilized CPO (containing about 0.5 U of enzyme) was introduced in the same assay medium used for determining the activity of

free enzyme. The absorbance at 414 nm was performed on a Shimadzu UV-2450 (Kyoto, Japan) UV-visible Spectrophotometer using quartz cuvettes.

## The kinetic parameter of free and immobilized CPO

To determine the enzymatic kinetic parameter of free CPO, CPO@$Ca_3(PO_4)_2$ and SA-coated CPO@$Ca_3(PO_4)_2$ (containing about 0.5 U of enzyme) was added to 400 μL of pH 2.75 phosphoric acid buffer solution (10 mM) containing 0.25 mM $ABTS^{2-}$ and various concentrations of $H_2O_2$ (9-90 μM). The activity of both free and immobilized CPO was calculated by the initial reaction rate from the slope of changes in absorbance versus time.

The kinetic parameters of $K_m$ and $K_{cat}$ were calculated using the Lineweaver-Bruke plot:

$$\frac{1}{\theta} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

## Enzyme stability test

Thermal stability was carried out by measuring the residual activity of the enzyme exposed to various temperatures (30˚C-80˚C) for 30 min. Storage stability was investigated by measuring their remaining activities after being stored at room temperature for a certain period. The activity assay of free and immobilized CPO was measured by the aforementioned method.

## Reusability of metal-organic hybrid complexes

The recycling use of enzyme catalysts was performed by ABTS method. The composites (including 0.5 U CPO) were mixed with pH 2.75 phosphoric acid buffer solution (10 mM, 400 μL) containing $H_2O_2$ (4.4 mM) and $ABTS^{2-}$ (0.25 mM). After 10 minutes, the mixture was centrifuged at 7378 g for 5 min. The supernatant was separated and the absorbance was determined at 414 nm on a UV/Vis spectrophotometer. The precipitates of enzyme composites were used for the next batch of the enzymatic reaction.

## Synthesis of Modafinil

Asymmetric sulfoxidation of 2-(diphenylmethylthio) acetamide to (R)-modafinil was chosen as the target reaction to investigate the catalysis and the recycling of the hybrid composites. The 2 mL of 10 mM phosphoric acid buffer solution (pH 4.5) containing 0.2 mM 2-(diphenylmethylthio) acetamide and the enzyme containing 6 U/mL CPO were mixed. Finally, added 12 mM TBHP to start reaction at room temperature. The supernatant was separated by centrifugation at 7378 g, 5 min for monitoring the product formation.

Reverse phase HPLC was used to quantitatively analyze the product formation with a SHIMADZU 15C serial HPLC apparatus equipped with reversed-phase C-18 column (250 × 4.6 mm, 5 μm, Diamodsil™) and UV detector at 225 nm. The solvent system consists of 30% acetonytrile and 0.2‰ TFA in water. The column temperature was maintained at 30°C. The

flow rate was 0.5 mL/min, and 20 μL portions were injected into the column. The retention time for modafinil was 7.3 min, for 2-(diphenylmethylthio) acetamide was 8.6 min. The epimeric purity was determined by chiral HPLC with AD-H column (250 × 4.6 mm, 5 μm, Daicel). All the aqueous samples were extracted by ethyl acetate and collected the organic phase. The eluent was methanol with a flow rate of 0.5 mL/min. The retention time was 8.7 min for (R)-modafinil, 10.6 min for (S)-modafinil. The procedure was repeated to determine the reusability of enzyme catalysts.

## The degradation of hydroquinone

A solution of 0.45mM hydroquinone and the hybrid composites containing 0.7 U CPO in 183 μL of pH 3 phosphoric acid buffer solution (10 mM) was mixed at room temperature. Finally, 4 μL $H_2O_2$ (88.3 mM) was added to start reaction. After 20 min, the mixture was separated by centrifugation at 7378 g, 5 min. The sedimentation for continue to use in the next time and the supernatant for monitoring the degradation spectrometrically at 290 nm.

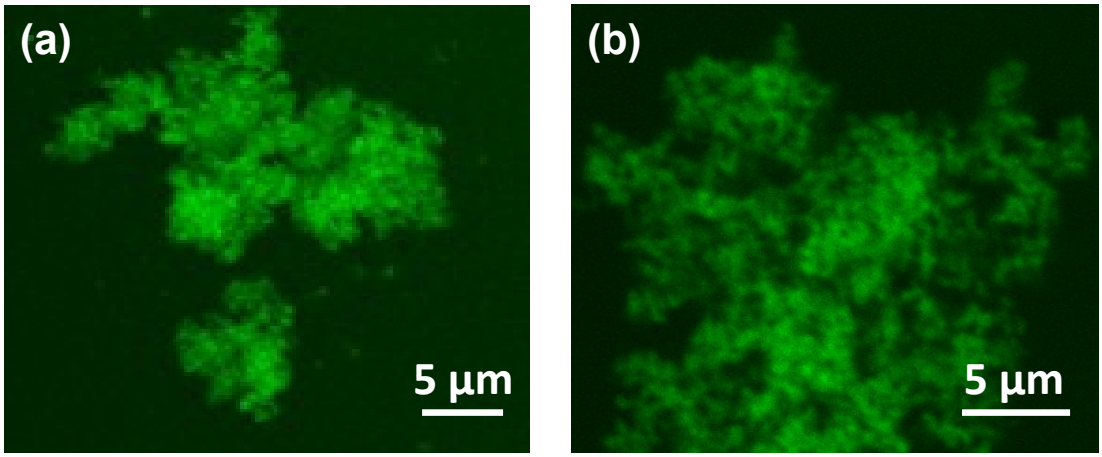


Fig.S1 CLSM micrograph of (a)BSA@$Ca_3(PO_4)_2$ and (b)SA-coated BSA@$Ca_3(PO_4)_2$.

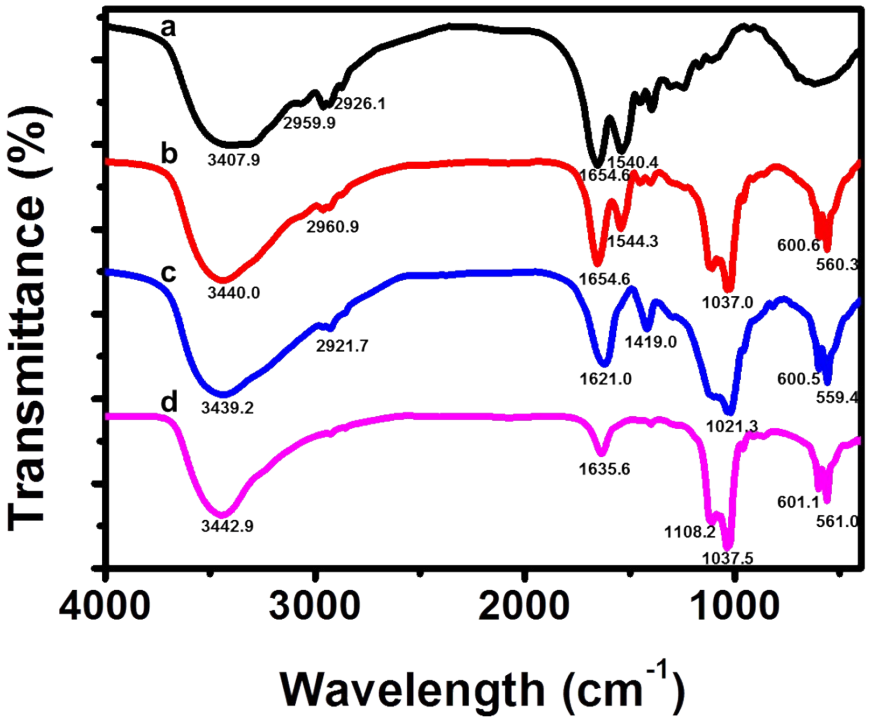


Fig. S2 The FTIR spectra of BSA (Curve (a)), BSA@$Ca_3(PO_4)_2$ (Curve (b)), SA-coated BSA@$Ca_3(PO_4)_2$ (Curve (c)), and $Ca_3(PO_4)_2$ (Curve (d)).

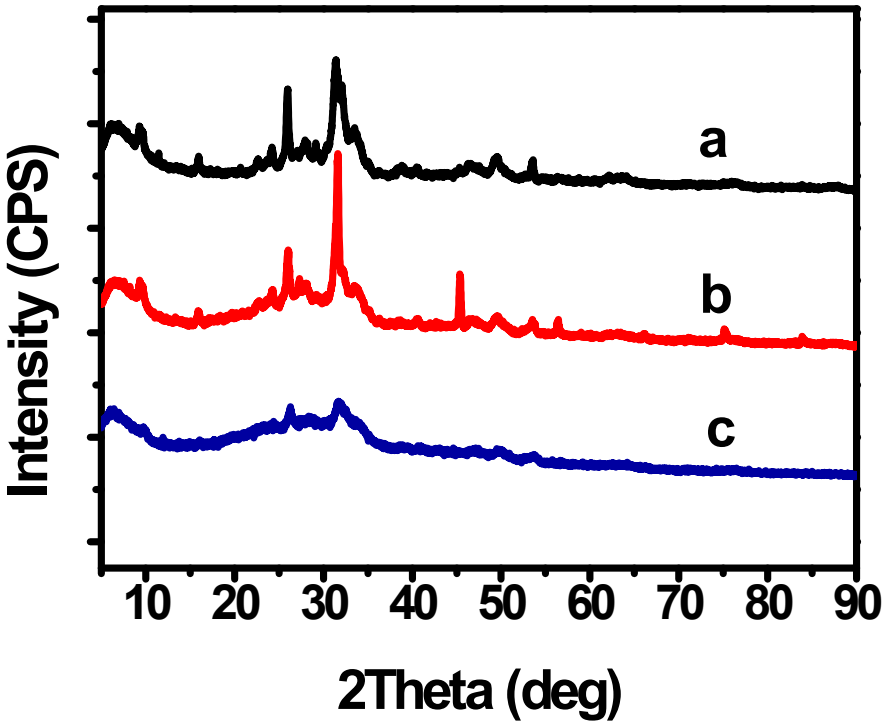


Fig. S3 X-ray diffraction (XRD) spectra of $Ca_3(PO_4)_2$ (Curve (a)), BSA@$Ca_3(PO_4)_2$ (Curve (b)): SA-coated BSA@$Ca_3(PO_4)_2$ (Curve (c)).

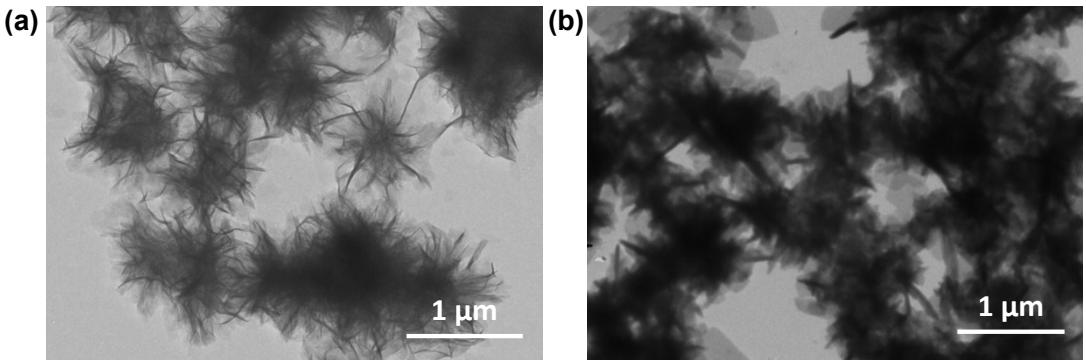


Fig. S4 TEM photos of CPO@$Ca_3(PO_4)_2$ hybrid composites with or without SA coating.

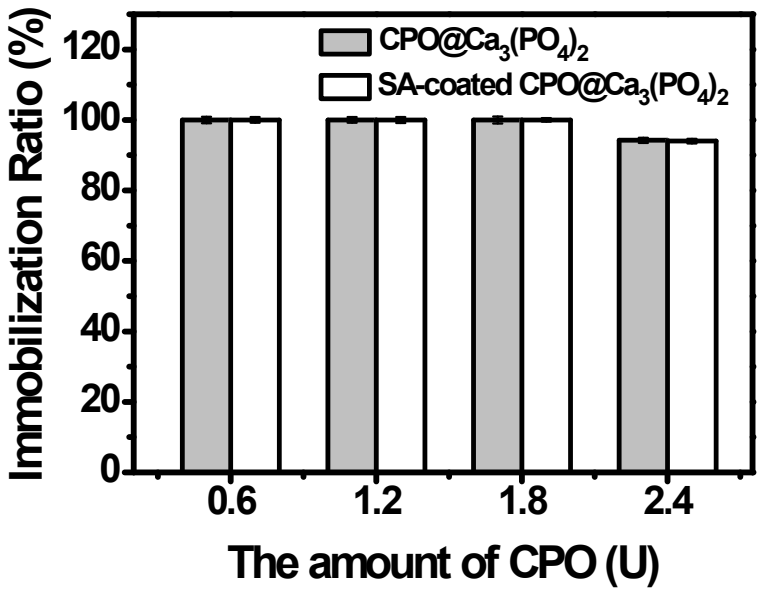


Fig. S5 The immobilization capacity of $CPO@Ca_3(PO_4)_2$ and SA-coated $CPO@Ca_3(PO_4)_2$ hybrid composites. (Experiment conditions: PBS buffer (10mM, pH 7.4, 180 μL), aqueous $CaCl_2$ solution (180 mM, 20 μL), containing different amount of CPO.)

Table S1. Kinetic parameters for free CPO and SA-coated $CPO@Ca_3(PO_4)_2$

| | $K_m$(μM) | $K_{cat}$ ($s^{-1}$) |
|---|---|---|
| **free CPO** | **91.42±0.024** | **5.54±0.012** |
| **SA-coated $CPO@Ca_3(PO_4)_2$** | **90.64±0.036** | **6.18±0.007** |

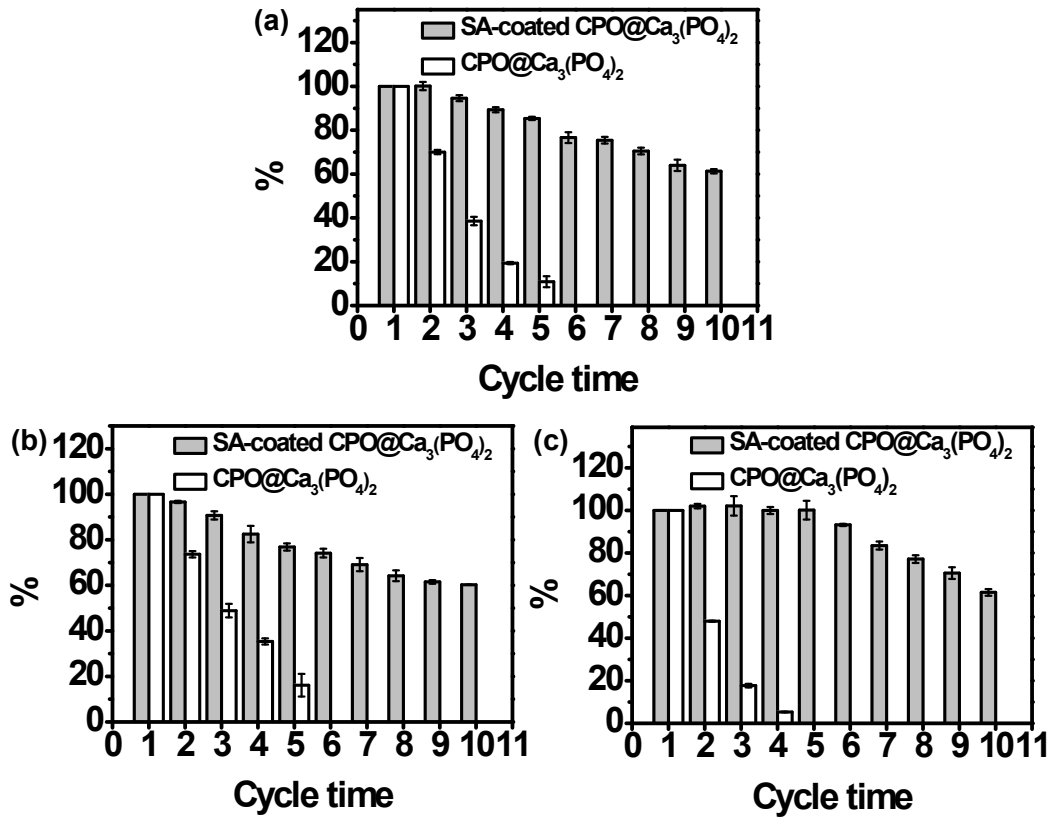


Fig. S6 The reusability of SA-coated CPO@$Ca_3(PO_4)_2$ and CPO@$Ca_3(PO_4)_2$ in different buffer solution. (a) citrate buffer; (b) acetate buffer; (c) citric acid-$Na_2HPO_4$ buffer. CPO activities were measured as follows: Experiments were carried out at room temperature using 0.25 mM $ABTS^{2-}$ in 400 μL of phosphoric acid buffer (pH 2.75, 10 mM) with 0.5 U CPO and 4.4 mM $H_2O_2$.

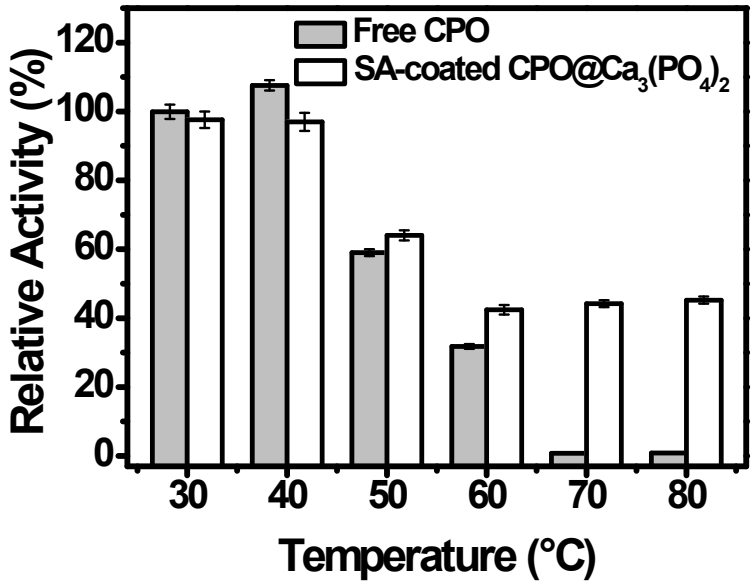


Fig. S7 Thermal stability of SA-coated CPO@$Ca_3(PO_4)_2$ and free CPO. The residual activity of the enzyme was measured as follow: 400 μL of phosphoric acid buffer solution (pH 2.75, 10 mM) containing 0.5 U CPO and 0.25 mM $ABTS^{2-}$, 4.4 mM $H_2O_2$ followed by reaction for 10 min at room temperature.

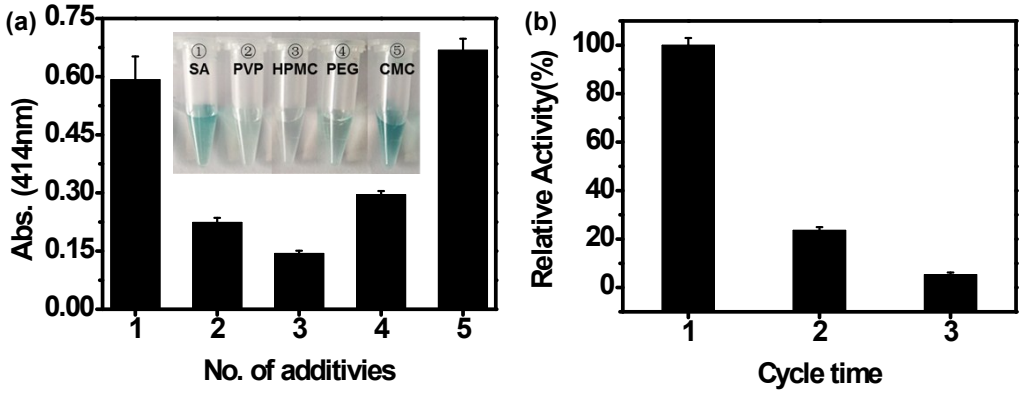


Fig. S8 (a) The enzyme activity with of different additives coating CPO@$Ca_3(PO_4)_2$, inset: photographs showing visible detection; (b) The reusability of CMC-coated CPO@$Ca_3(PO_4)_2$. (For the CPO activity assay, 400 μL of phosphoric acid buffer solution (pH 2.75, 10 mM) containing 0.5 U CPO and 0.25 mM $ABTS^{2-}$, 4.4 mM $H_2O_2$ followed by reaction for 10 min at room temperature).

## Supplementary Reference

[S1] J. G. Xiaoling Wu, Cheng Yang, Miao Hou and Zheng Liu, *Chem. Commun.* **2015**, *51*, 13408-13411.

[S2] M. M. Bradford, *Analytical Biochemistry* **1976**, *72*, 248-254.