Electronic Supplementary Information

Enzyme Immobilization in Highly Ordered Macro–Microporous Metal–Organic Frameworks for Rapid Biodegradation of Hazardous Dyes

Shufan Li, Xuejing Zhai, Chong Zhang, Huilin Mo and Shuang-Quan Zang**

College of Chemistry, and Institute of Green Catalysis, Zhengzhou University, Zhengzhou 450001, P. R. China.

Experimental Section

Evaluation of the HRP catalytic activity. The enzymatic activities of free and immobilized HRP were evaluated by their capacity to catalyze oxidation of ABTS in the presence of H_2O_2:

$$H_2O_2 + ABTS \xrightarrow{HRP} H_2O + ABTS^{\bullet +}$$

In the reaction, the concentrations of free HRP, HRP encapsulated in the HRP@SOM-ZIF-8 composite, and HRP bounded on the ZIF-8 surface were set to 0.20 μM. Besides, the concentration of SOM-ZIF-8 alone, SOM-ZIF-8 that entrapped HRP, and microporous ZIF-8 that adsorbed HRP on the surface was set equally. In a typical assay, the composite or free HRP was dispersed in phosphate buffered solution (PBS, 50 mM, pH 7.0), followed by addition of 5.3 mM ABTS solution and a series concentration of H_2O_2 solution. The reaction was performed at room temperature (25 °C) in a total volume of 100 μL. The total reaction time was fixed to 5 min and the reaction was then terminated by 1% SDS. The mixture was centrifuged at 8000 rpm for 5 min to remove the composite, followed by transferring the supernatant into a 96-well plate. The absorbance of oxidative ABTS at 420 nm was measured with the PerkinElmer EnSight HH3400 spectrophotometer. The error bars correspond to the standard deviation of three measurements.

Long-term stability. HRP or HRP@SOM-ZIF-8 (with the same protein concentration of 2.5 μM) was incubated in PBS buffer (50 mM, pH 7.0) and shaken at 100 rpm under room temperature for four weeks. The treated samples were taken at specific time and the residual enzymatic activity was measured as the method mentioned above with 50 μM H_2O_2 solution as substrate. The initial activity of free form and immobilized HRP was considered as corresponding control and to be 100% in this experiment. The relative activity (%) was calculated using Eq. 1:

$$Relative\ activity\ (\%) = \frac{residue\ absorbance}{original\ absorbance} \times 100$$

Leaching study. 2.5 μM HRP@SOM-ZIF-8 was incubated in 1 mL PBS buffer (50 mM, pH 7.0), followed by shaking at 100 rpm at room temperature. A certain amount of mixture was taken at given time, followed by centrifuging at 8000 rpm for 10 minutes. Then the concentration of protein released in the supernatant was measured by BCA protein assay kit. The relative quantity of enzyme was calculated as the ratio of the initial enzyme quantity immobilized in carrier and the enzyme content in supernatant solution at a specific time after immersion.

Enzyme recycling study. For the recycling study, the reaction was performed with 10 μM immobilized HRP or free HRP, 5.3 mM ABTS, 50 μM H_2O_2 in 1 mL of PBS buffer (50 mM, pH 7) at room temperature. After 5 min, the reaction was terminated by 1% SDS, followed by centrifugation (8000 rpm, 10 min) to separate the solid and supernatant. The absorbance at 420 nm of supernatant was determined by PerkinELmer EnSight HH3400 spectrophotometer. Then the recovered enzyme was washed twice with DI water and used for the next reaction by adding similar amounts of substrates as the first cycle. The above procedure was repeated five catalytic cycles. The relative activity was calculated as a ratio of each cycle's activity and first cycle's enzyme activity.

Stability under chelating compound treatment. For chelating compound treatment study, the suspension of immobilized HRP or free HRP was incubated in PBS buffer (50 mM, pH 7.0) containing 1 wt% of EDTA-2Na at room

temperature. Before addition, the pH of EDTA-2Na solution was adjusted to 7.0 by addition of NaOH solution. In the reaction, the concentrations of free HRP, HRP in HRP@SOM-ZIF-8 composite and mixture were set at 10 μM equally, while the content of SOM-ZIF-8 was also the same in composite and mixture. The treated samples were taken at specific time and the relative enzymatic activity was measured as the aforementioned method.

Removal efficiency of dyes. To measure the removal efficiency of dyes by enzyme, the decrease in absorbance at respective absorption band (λ_{max}=465 nm for MO^-, λ_{max}=480 nm for CR^{2-}, λ_{max}=555 nm for RB^+, λ_{max}=530 nm for $R6G^+$) was monitored by PerkinELmer EnSight HH3400 spectrophotometer. In a typical assay, 4 μM HRP that is free or immobilized in SOM-ZIF-8 or bounded on the ZIF-8 surface, 1 mM H_2O_2 and dyes with varied concentrations, were mixed in PBS buffer (50 mM, pH 7.0) at room temperature in a total volume of 200 μL. The control experiment was conducted as mentioned above, where SOM-ZIF-8 or microporous ZIF-8 was added to replace enzymes. The treated samples were taken at specific time. The removal efficiency was obtained as Eq. 2:

$$Removal\ efficiency\ (\%) = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 is the initial absorbance of each dye in control, A_t is the final absorbance of each dye in experiment after reaction.

Reusability of composite for dye removal.

To evaluate the reusability of immobilized enzyme for dye removal**,** the reaction was performed with the mixture containing of 10 μM HRP encapsulated in SOM-ZIF-8, 1 mM H_2O_2 and 100 μM dye in PBS buffer (50 mM, pH 7.0) at room temperature within a total volume of 200 μL. After 2 min, the reaction system was centrifugation (8000 rpm, 10 min), and then 150 μL supernatant was determined by PerkinELmer EnSight HH3400 spectrophotometer. Then the immobilized enzyme was washed twice with DI water and employed in repeated

reuse under the same conditions. The biocomposite was reused at five different times. The relative activity was calculated as Eq. 2.

Supplementary Figures

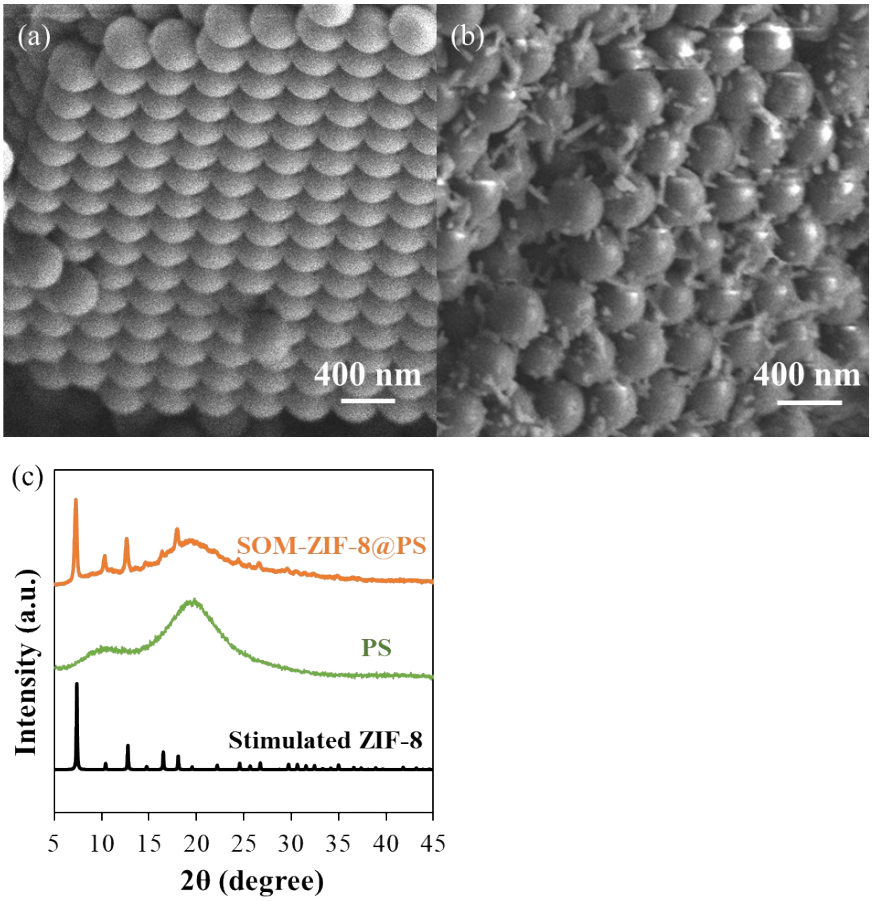

Fig. S1 Representative SEM images of 3D ordered PS templates (a), SOM-ZIF-8@PS (b). PXRD patterns of 3D ordered PS templates and SOM-ZIF-8@PS (c).

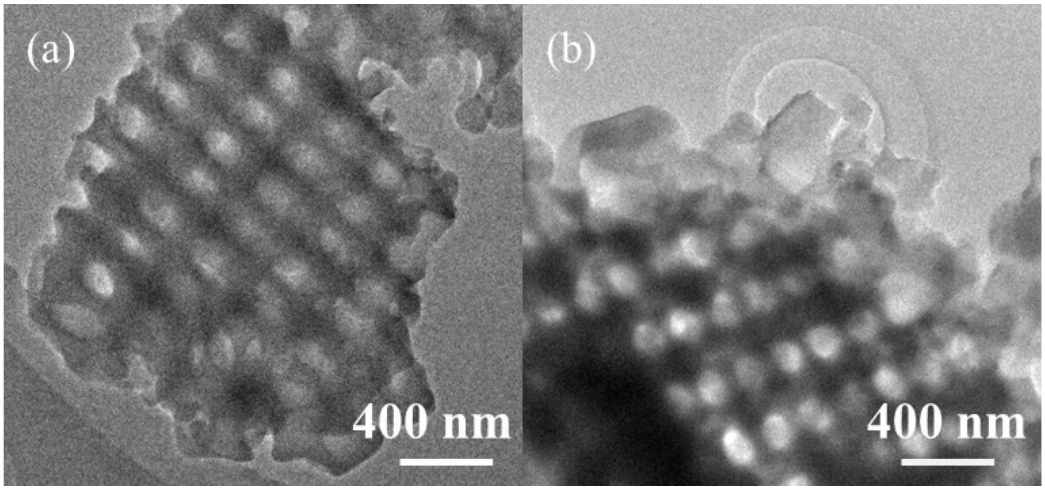

Fig. S2 Representative TEM images of SOM-ZIF-8 (a) and HRP@SOM-ZIF-8 (b).

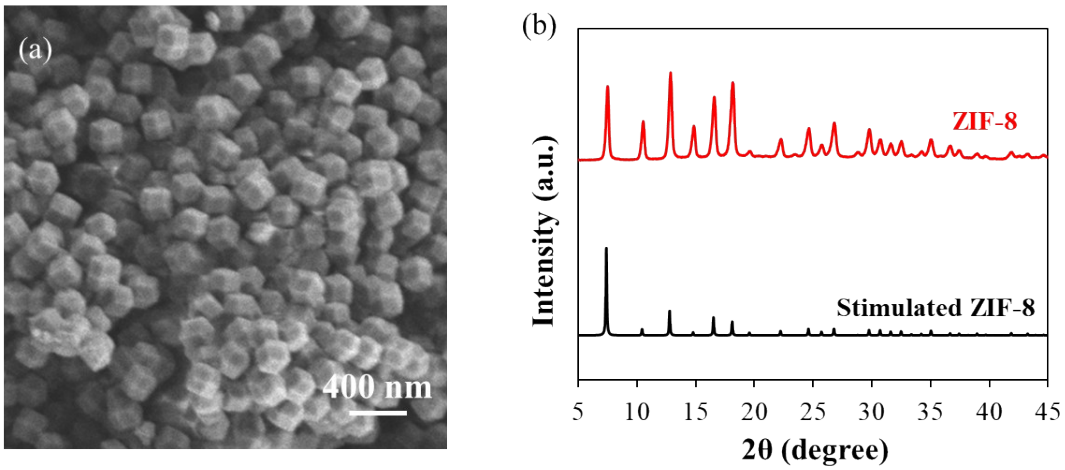

Fig. S3 SEM image (a) and PXRD patterns (b) of ZIF-8.

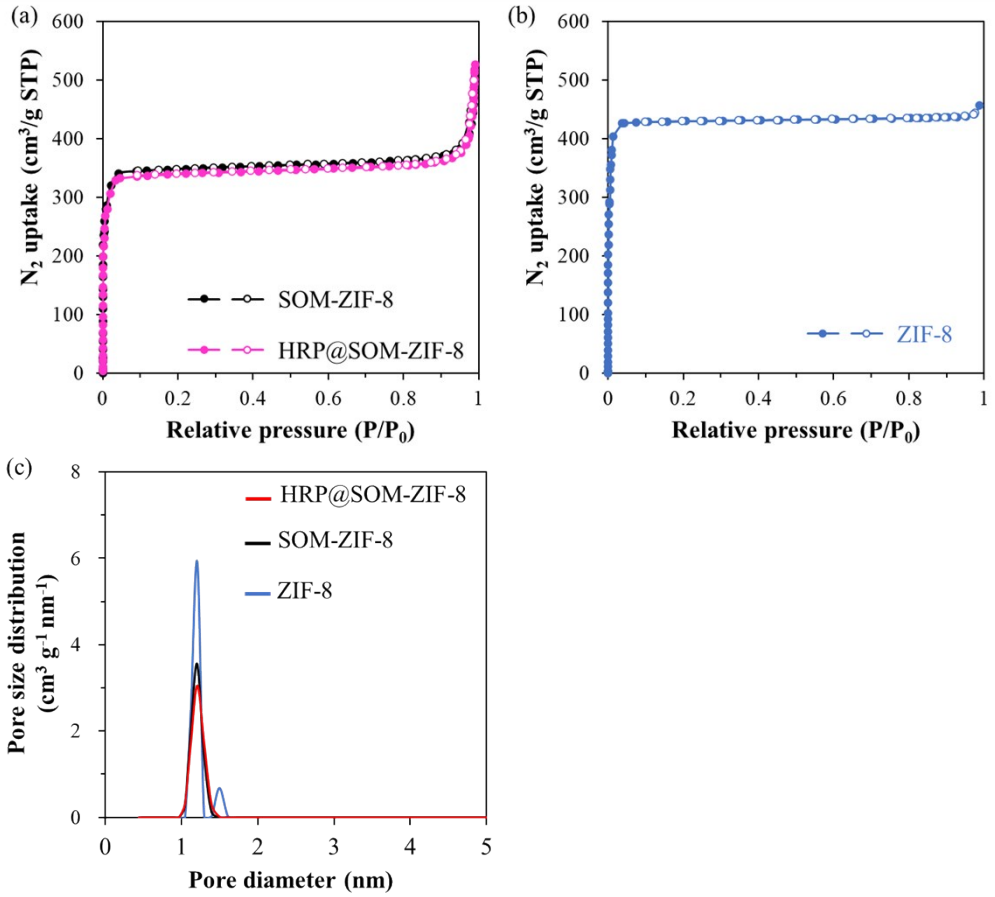

Fig. S4 N_2 adsorption-desorption curves at 77 K for SOM-ZIF-8, HRP@SOM-ZIF-8 (a), and ZIF-8 (b). DFT pore size distributions (micropores) for pure ZIF-8, SOM-ZIF-8 and HRP@SOM-ZIF-8 composite (c).

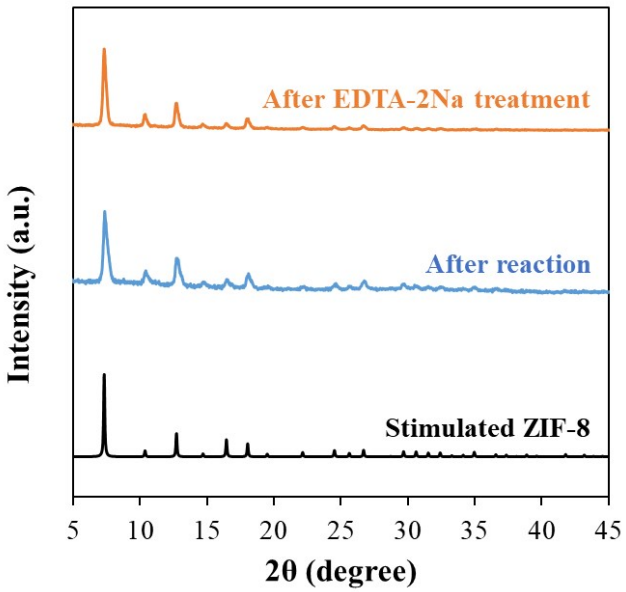

Fig S5. PXRD patterns of HRP@SOM-ZIF-8 after reaction or EDTA-2Na treatment

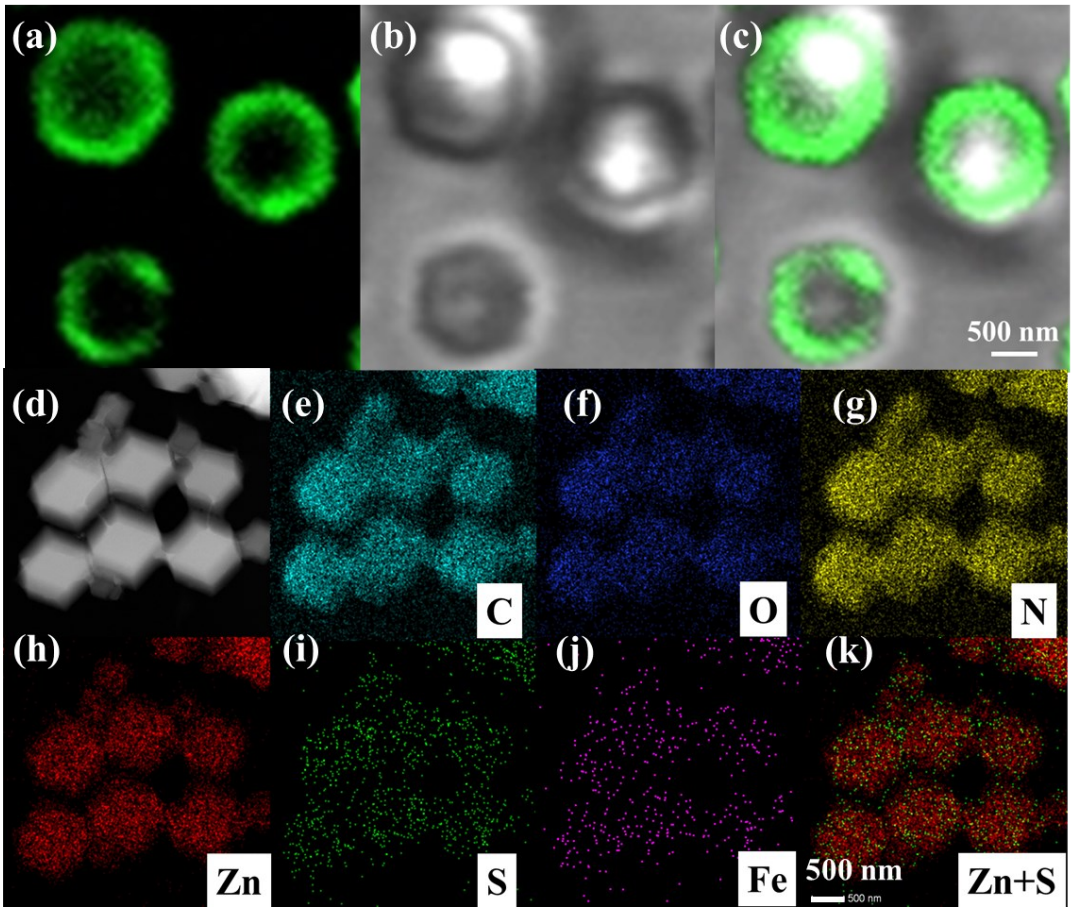

Fig S6. Confocal laser scanning microscopy images for FITC-labeled HRP bounded on ZIF-8 surface: fluorescence properties of the loaded HRP (a); bright-field microscopy image of the ZIF-8 (b); and a merged image of the HRP@ZIF-8 (c). TEM images of pure ZIF-8 (d). EDS mapping of elemental distributions of HRP@ZIF-8 for C(e), O(f), N(g), Zn(h), S(i), Fe(j), and Zn+S(k).

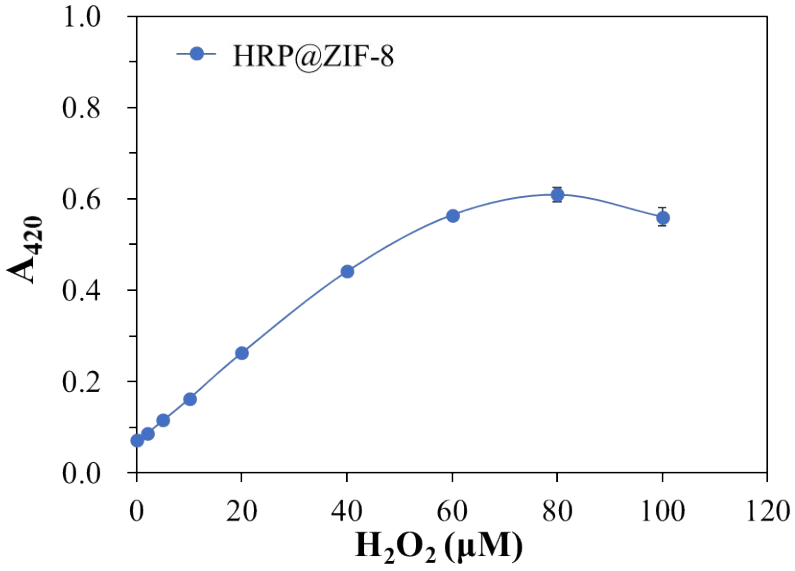

Fig S7. Catalysis of ABTS after 300 s in solution catalyzed by HRP@ZIF-8 at 0-100 μM H_2O_2.

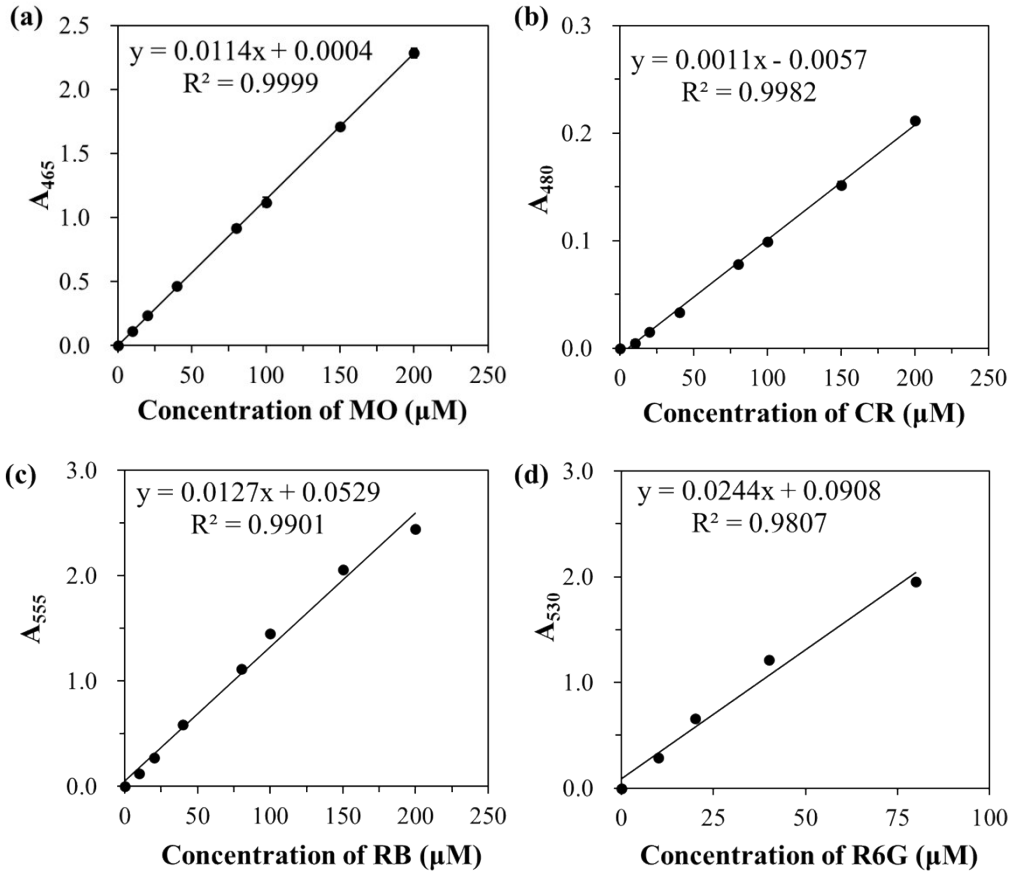

Fig S8. Calibration curve of MO (a), CR (b), RB (c) and R6G (d) at various concentrations.

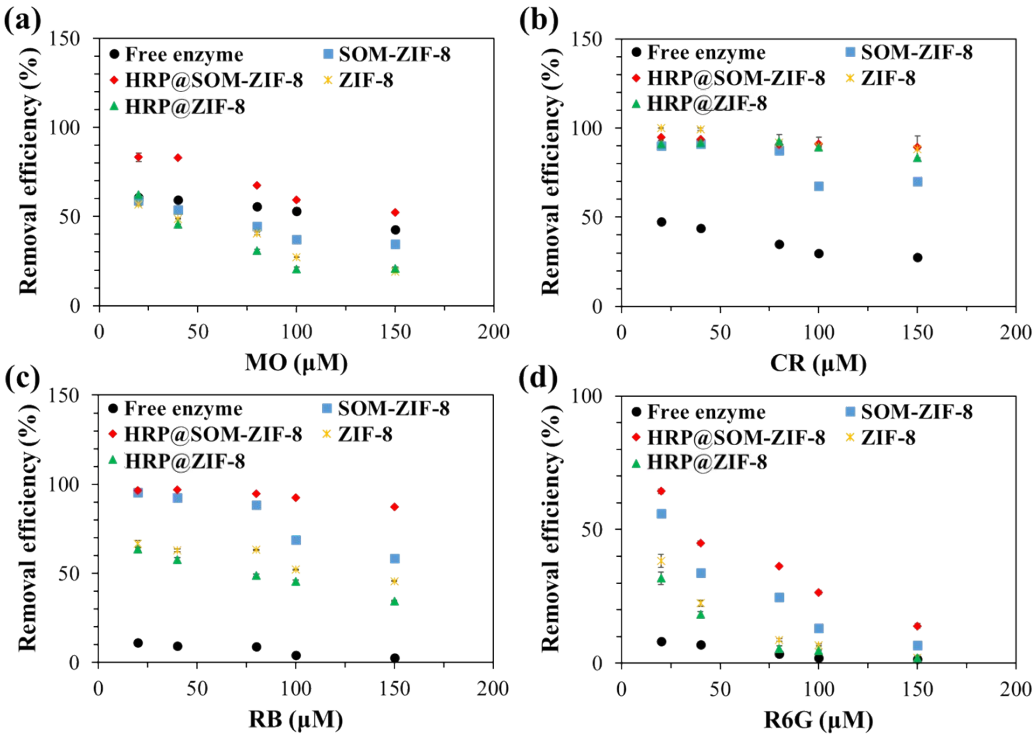

Fig S9. Removal efficiency of MO (a), CR (b), RB (c) and R6G (d) by free HRP, SOM-ZIF-8, HRP@SOM-ZIF-8, ZIF-8 and HRP@ZIF-8 after 2 min reaction.

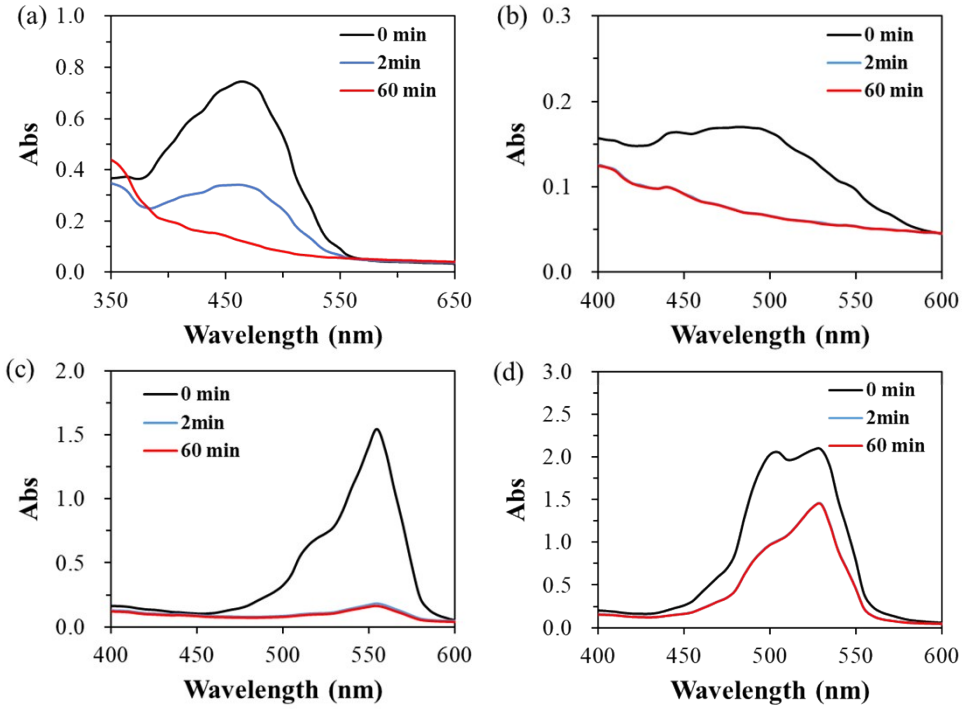

Fig. S10 UV−vis absorption spectra of degradation of 100 μM MO (a), CR (b), RB (c), R6G (d) after treatment with HRP@SOM-ZIF-8.

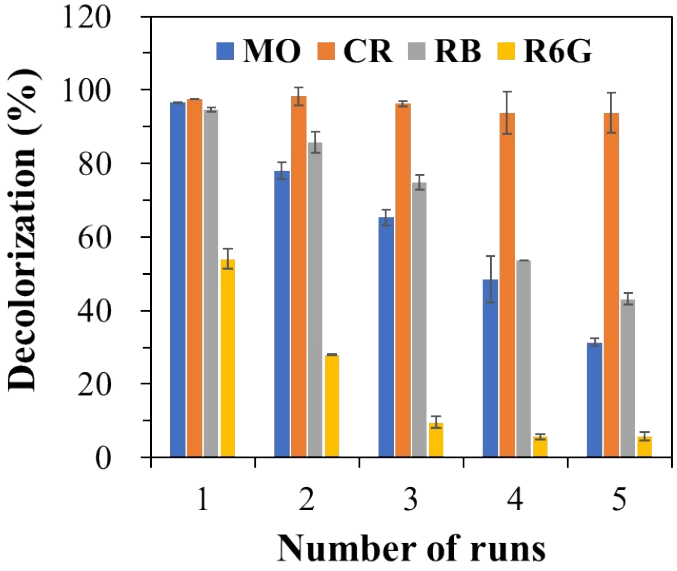

Fig. S11 Reusability study involving the removal of dyes from aqueous solution after 5 runs.