Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Structure advantage and peroxidase activity enhancement of deuterohemin-peptide-inorganic hybrid flowers

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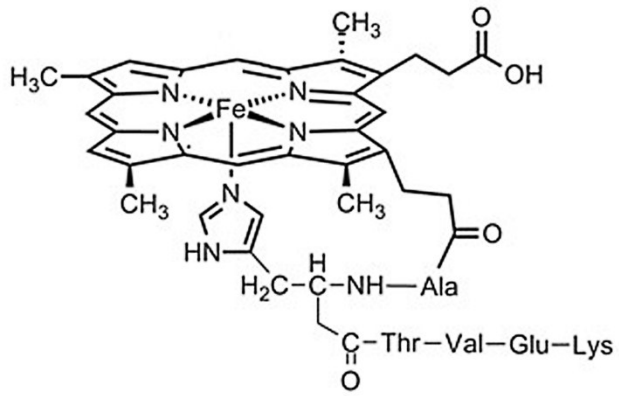

Fig. S1 The structure of DhHP-6 with molecule weight of 1128.

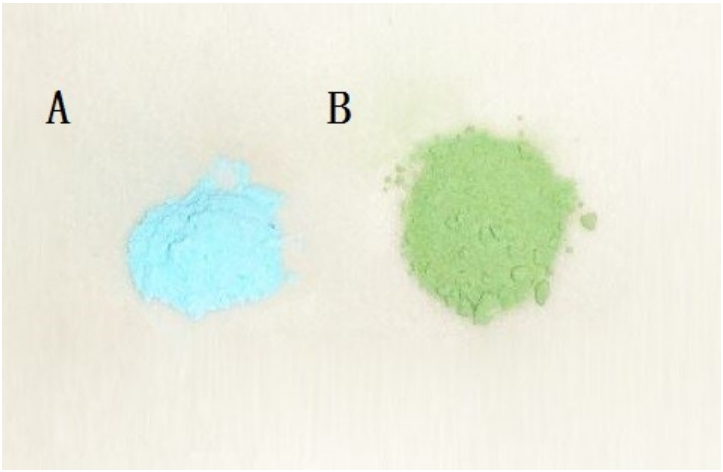

Fig. S2 Powder photographs of (A) $Cu_3(PO_4)_2$ (left, blue precipitate) and (B) DhHP-6-$Cu_3(PO_4)_2$ hybrid flowers (right, green precipitate), prepared with and without DhHP-6 respectively.

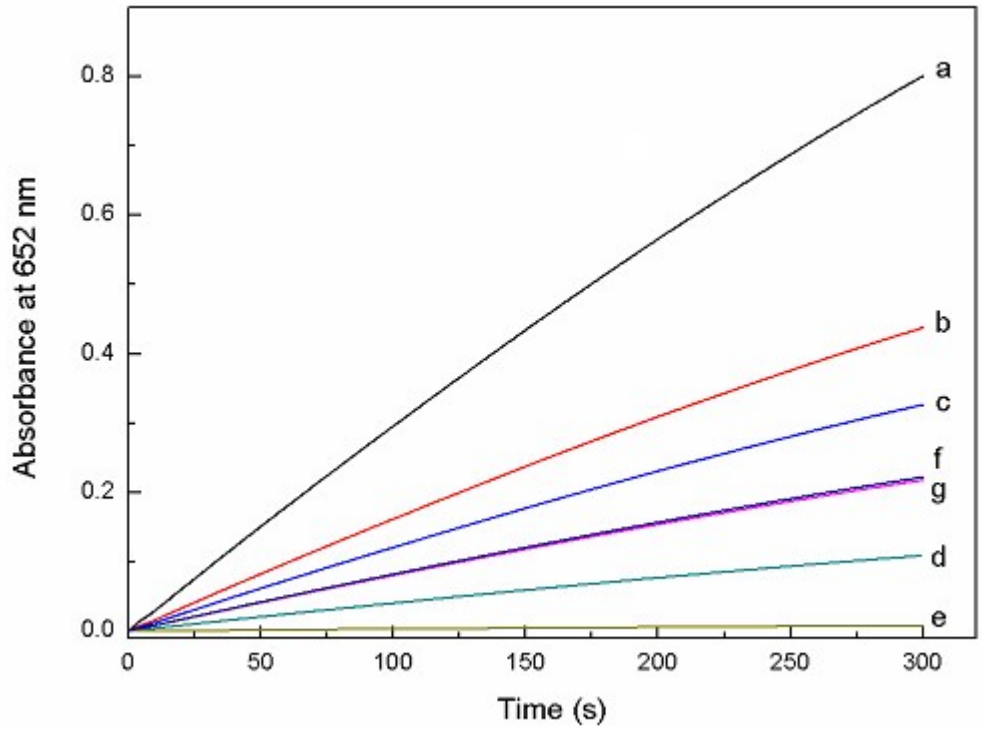

Fig. S3 Absorbance of oxidized TMB (652 nm) as a function of time in catalytic reactions of DhHP-6-$Cu_3(PO_4)_2$ prepared from starting concentrations of DhHP-6 at (a) 0.006 mg mL^{-1}; (b) 0.012 mg mL^{-1}; (c) 0.024mg mL^{-1}; (d) 0.06 mg mL^{-1}; (e) 0 mg mL^{-1}; (f) free DhHP-6; (g) free DhHP-6 and $Cu_3(PO_4)_2$. Experimental conditions: (a) - (d) and (f) - (g) at equivalent amount of DhHP-6 (3.8µg). The 1 mL reaction mixture contained 0.8 mM TMB and 2 mM H_2O_2 at pH 6.0, 35°C.

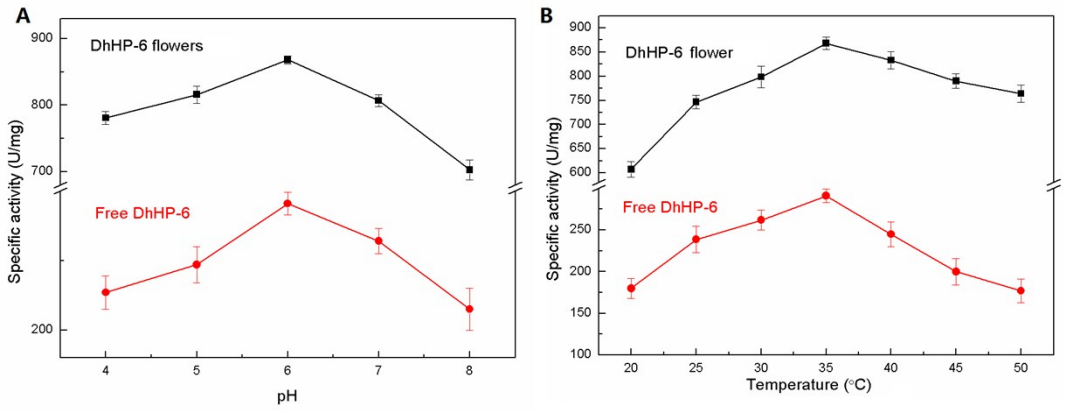

Fig. S4 Peroxidase-like activity of DhHP-6-$Cu_3(PO_4)_2$ flowers (black) and free DhHP-6 (red) as a function of (A) pH at temperature 35 °C and (B) temperature at pH 6.0. One unit specific activity (U/mg) of DhHP-6-$Cu_3(PO_4)_2$ flowers or free DhHP-6 is defined as 1 mg of DhHP-6 that catalyzes 1.0 µmol of H_2O_2 per minute.

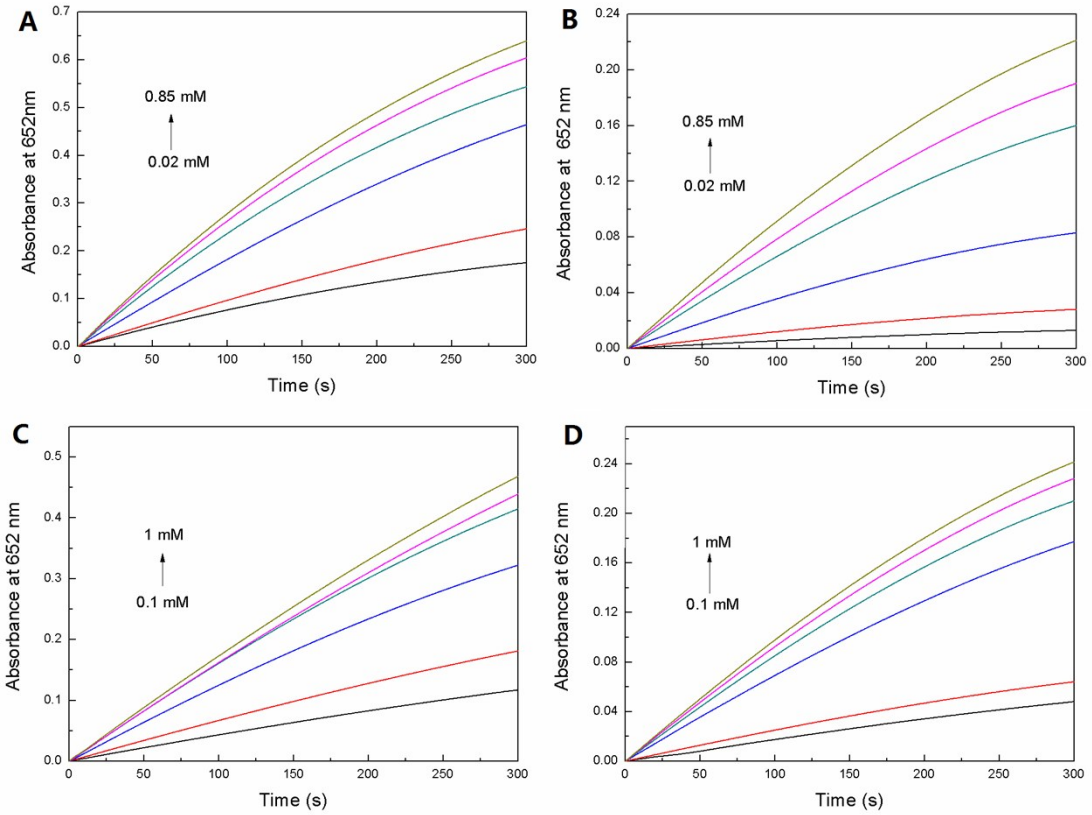

Fig. S5 Time-dependent absorbance changes of the absorbance at 652nm with different concentrations of substrates. Conditions: (A) H_2O_2 (2 mM) and 100 μg DhHP-6-$Cu_3(PO_4)_2$ flowers with different concentration of TMB (0.02 mM-0.85 mM) in 1mL PBS (pH 6.0); (B) H_2O_2 (2 mM) and 3.8 μg DhHP-6 with different concentration of TMB (0.02 mM-0.85 mM) in 1 mL PBS (pH 6.0); (C) TMB (0.8 mM) and 100 μg DhHP-6-$Cu_3(PO_4)_2$ with different concentration of H_2O_2 (0.1 Mm-1.0 mM) in 1 mL PBS (pH 6.0); (D) TMB (0.8 mM) and 3.8 μg DhHP-6 with different concentration of H_2O_2 (0.1 mM-1.0 mM) in 1 mL PBS (pH 6.0).

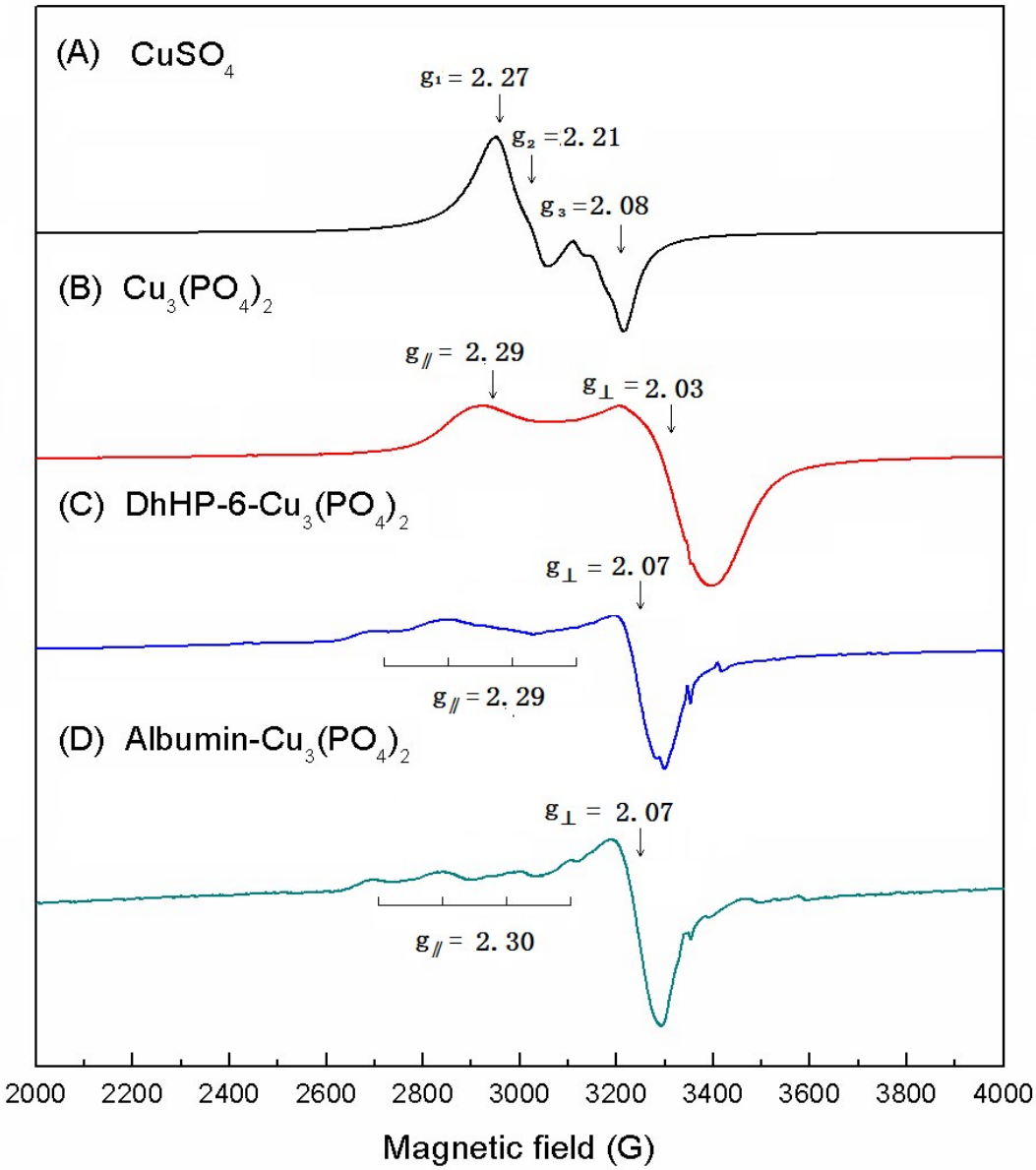

Fig. S6 EPR spectra of (A) $CuSO_4$, (B) $Cu_3(PO_4)_2$, (C) DhHP-6-$Cu_3(PO_4)_2$ flowers and (D) Albumin-$Cu_3(PO_4)_2$ at room temperature.

Table. S1 From left columns 1 - 3: calculated initial DhHP-6 concentrations，measured actual starting concentrations and unreacted concentrations of DhHP-6 determined from absorbance at 389 nm (ε = 54520 M^{-1} cm^{-1}) columns 4 -5: Encapsulation yield (defined as the ratio of the amount of immobilized DhHP-6 to the total amount of DhHP-6 employed in percentage) and weight percentage of DhHP-6 in the flowers. The weight percentage of protein in flowers was calculated according to the ref. 1. The specific activity of free DhHP-6 is 291 U/mg in PBS and 284 U/mg in PBS with physically mixed $Cu_3(PO_4)_2$.

DhHP-6 concentration (mg mL^{-1})			Encapsulation yield (%)	Weight percentage (%)	Specific activity (U/mg)
Targeted	Actual	Unreacted			
0.006	0.00589	0.00118	79.97	3.78	867.2
0.012	0.0118	0.00291	75.29	6.95	584.9
0.024	0.0237	0.00953	59.72	10.85	436.5
0.060	0.0589	0.0296	49.74	18.75	122.2

Reference

1 J. Ge, J. Lei and R. N. Zare, *Nat. Nanotechnol.*, 2012, **7**, 428-432.