Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Enzyme-conjugated ZIF-8 particles as efficient and stable Pickering interfacial biocatalysts for biphasic biocatalysis

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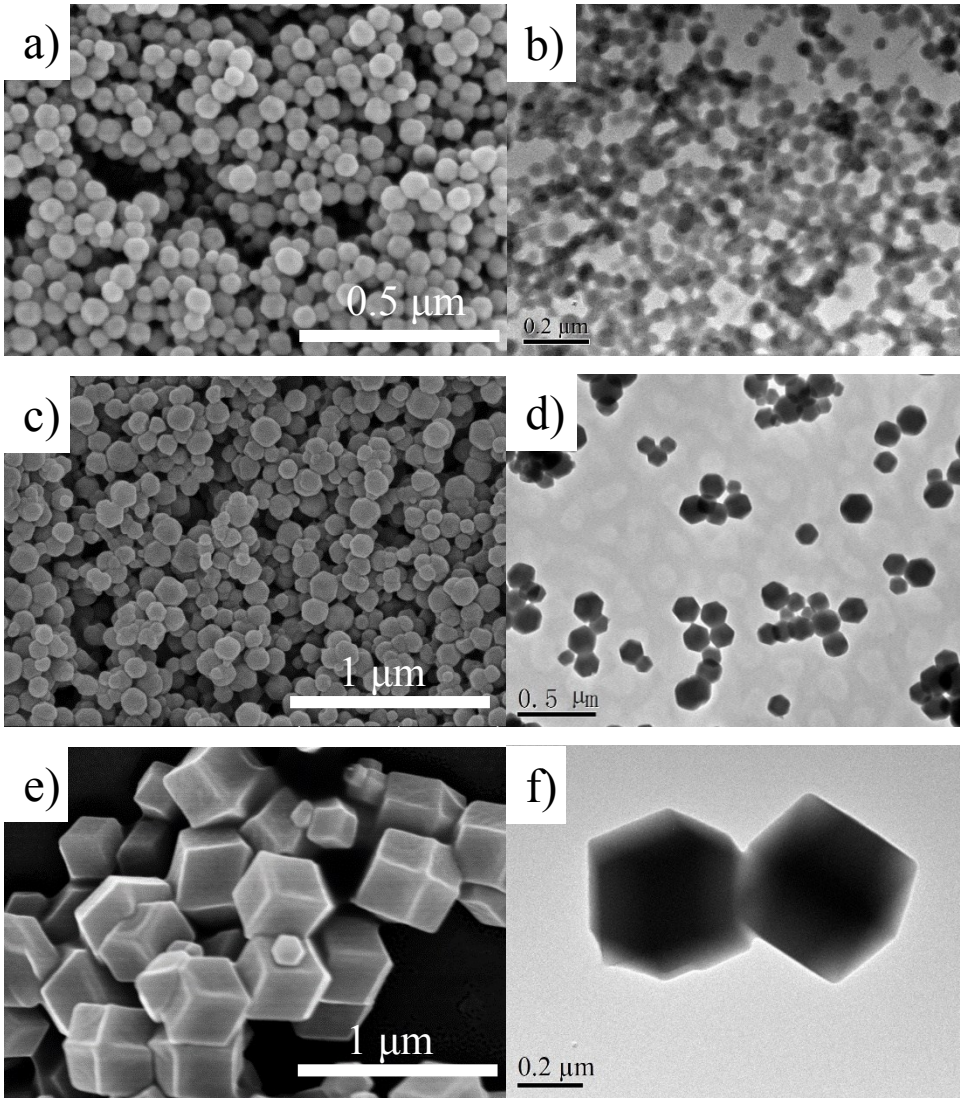

Figure S1. (a, c, e) SEM and (b, d, f) TEM images of ZIF-8 particles with different size (a, b) 50 nm; (c, d) 150 nm; (e, f) 500 nm).

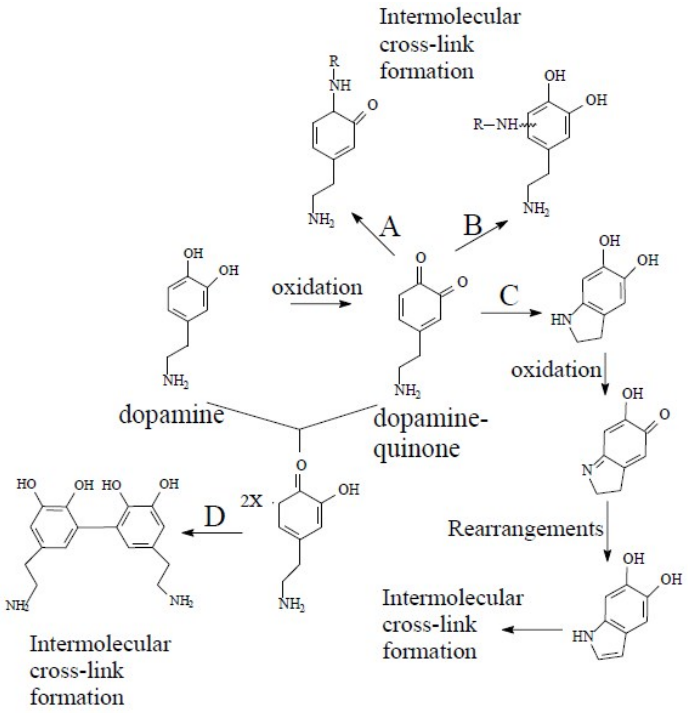

Figure S2. Possible structural evaluation and polymerization mechanism of dopamine. (A) Schiff based substitution; (B) Michael-type addition; (C) Intramolecular cyclization; (D) Aryl-aryl coupling.[1]

Actually, complex reactions, including Schiff based substitution, Michael-type addition, intramolecular cyclization, aryl-aryl coupling, *etc.*, contributed to the formation of polydopamine. Amongst, Michael addition and Schiff-base reaction played two major roles, which could be described as Pathway B and A in **Figure S2**.[1-3]

References

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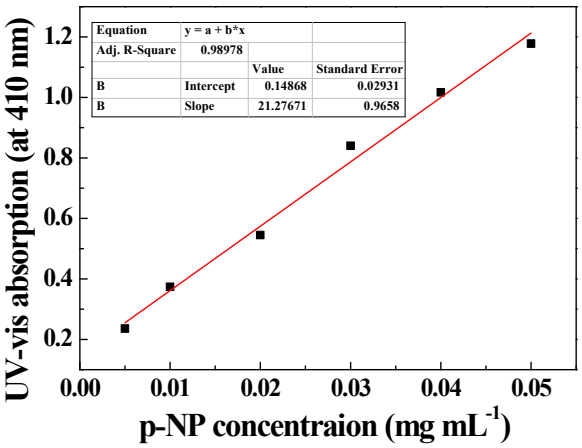

Figure S3. Standard curve (UV-vis *vs.* concentration) of p-NP in Na_2CO_3 aqueous solution.

Table S1. Calculation details about the specific activity of CRL on the modified ZIF-8 particles with different size.

Pickering interfacial catalyst	UV-vis intensity	Calculated concentration of p-NP[a] (mg mL^{-1})	Concentration of p-NP[b] (mg mL^{-1})	Content of p-NP (μmol)	Specific activity (U mg^{-1} (CRL))
CRL-conjugated ZIF-8 (2h)	0.487	0.0159	0.159	2.314	0.257
CRL-conjugated ZIF-8 (4h)	0.459	0.0146	0.146	2.125	0.236
CRL-conjugated ZIF-8 (6h)	0.437	0.0135	0.135	1.952	0.217
CRL-conjugated ZIF-8 (8h)	0.428	0.0131	0.131	1.898	0.211

The standard curve (UV-vis *vs.* concentration) of p-NP in $NaCO_3$ aqueous solution was plotted in **Figure S3**. The specific activity was calculated on the basis of the initial 1-min reaction according to Eq. (S1):

$$\text{Specific activity (U mg}^{-1}\text{ (CRL))} = \frac{M_{liberated\ p\text{-}NP}}{T_{initial} \times W_{CRL}} \qquad \text{(S1)}$$

where $M_{liberated\ p\text{-}NP}$ (μmol) was the mole content of liberated p-NPP into aqueous phase; $T_{initial}$ (min) and W_{CRL} (mg) were the initial reaction time (1 min) used for calculating the specific activity and weight content of CRL used in the reaction. More specifically, the UV-vis spectra of liberated p-NP during the reaction catalyzed by CRL-conjugated ZIF-8 particles with dopamine modification time of 2, 4, 6 and 8 h

were, respectively, 0.487, 0.459, 0.437 and 0.428. According to the standard curve (**Figure S3**), the concentrations of liberated p-NP converted by CRL-conjugated ZIF-8 particles with dopamine modification time were 0.0159, 0.0146, 0.0135 and 0.0131 mg mL^{-1}, respectively. Since the concentration of p-NP was diluted into 10 folds for the UV-vis spectra measurement. The concentration and mole content of liberated p-NP could be then calculated as shown in **Table S1**. Since CRL used in the reaction was fixed at 9 mg and the initial reaction time was 1 min, the specific activity could be calculated based on Eq. (S1).

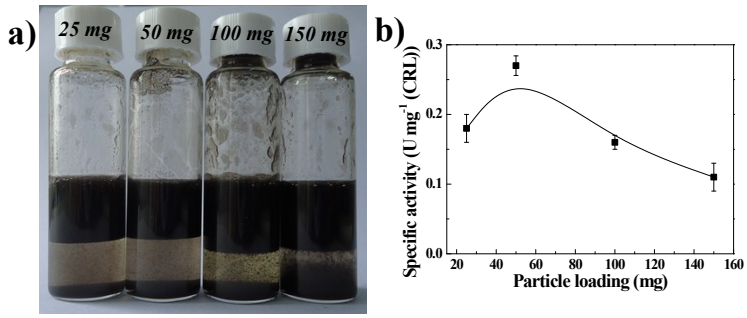

Figure S4. (a) Emulsification property and (b) catalytic performance of CRL-conjugated ZIF-8 particles as a function of particle loading (Pickering interfacial catalysts used in the reaction at the four particles loadings were fixed at the same CRL loading capability.).

The emulsification property and catalytic performance of the Pickering interfacial catalysts as a function of particle loading was investigated. As shown in **Figure S4a**, Pickering emulsions could be formed at all the particle loadings. However, when the particle loading increased up to 100 and 150 mg, some coalescence were formed, which could be as a result of the excess of particles added into the medium. Accordingly, the specific activity (**Figure S4b**) of the Pickering interfacial catalysts upon different particle loadings (25, 50, 100 and 150 mg) were, respectively, 0.18, 0.27, 0.16 and 0.11 U mg^{-1} (CRL). The decreased activity primarily owing to the excess amount of particles added to the reaction medium.

Table S2. Immobilization efficiency, loading capacity and specific activity of CRL on ZIF-8 particles with different size (CRL concentration initially added: 9.0 mg mL^{-1}).

Particle size (nm) (50 mg)	Specific activity (U mg^{-1} (CRL))	Immobilization efficiency (%)	Loading capacity (mg g^{-1})
50	0.26	77.2	278
150	0.25	71.3	257
500	0.23	65.2	235

As previously suggested for enzyme immobilization, reducing the size of the support could provide increased exposed surface for enzyme adsorption, leading to an enhanced enzyme loading.[1, 2] As shown in **Table S2**, under a specific immobilization condition for the three supports (particle amount: 50 mg, CRL concentration: 9.0 mg mL^{-1}), the immobilization efficiency of CRL decreased from 77.2 to 65.2% as the particle size enlarged from 50 to 500 nm. After calculation, the enzyme loading capacities for these three particles were, respectively, 278, 257 and 235 mg g^{-1}. Then, we adopted these immobilized CRL for catalytic hydrolysis of p-NPP. The results showed that immobilized CRL with smaller particle size exhibited higher specific activity. Nonetheless, it could be observed that immobilized CRL with the particle size of 50 and 150 nm had a similar specific activity, which seemed to be contrary to the traditional theory. Basically, immobilized enzyme with smaller support size would result in higher catalytic activity mainly owing to the much more contact opportunities between enzyme and substrate in the reaction medium. Herein, the similar specific activity between the two immobilized CRL (50 and 150 nm) may be as a result of the much higher aggregation of smaller-sized immobilized CRL during the preparation and reaction process. Therefore, in the main text, the immobilized CRL with a particle size of 150 nm was selected for the subsequent experiment.

References

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