Supporting Information

An efficient and recyclable enzyme catalytic system constructed through the synergy between biomimetic mineralization and polyamine-salt aggregate assembly

Jiafu Shi\textsuperscript{a, c}, Zhongyi Jiang\textsuperscript{b, c, *}

\textsuperscript{a} School of Environmental Science and Engineering, Tianjin University, Tianjin 300072, China

\textsuperscript{b} School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China

\textsuperscript{c} Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300072, China.

*To whom correspondence should be addressed. E-mail: zhyjiang@tju.edu.cn; Fax: 86-22-23500086; Tel: 86-22-27406646 (Zhongyi Jiang)
Supporting information for Experimental section:

**Enzyme immobilization:** The enzyme immobilization yield (%) of silica NPs were calculated according to the equation (S1), respectively:

\[ \text{Enzyme immobilization yield (\%) } = \frac{M_{\text{enzyme, immobilized}}}{M_{\text{enzyme, added}}} \times 100\% \quad (S1) \]

Where \( M_{\text{enzyme, added}} \) was the mass of enzyme added in the solution (μg), and \( M_{\text{enzyme, immobilized}} \) was the mass of immobilized enzyme (μg). \( M_{\text{enzyme, immobilized}} \) was calculated by measuring the concentration of enzyme in the supernatant before and after immobilization (room temperature: 20±2°C). The enzyme concentration was determined through Coomassie Brilliant blue binding method at 595 nm using a UV spectrophotometer.

Under identical condition (that refers to the condition described in the section of "Synthesis of enzyme-encapsulated silica NPs or pristine silica NPs" of the manuscript), the enzyme immobilization yield was ca. 87.0%.

Besides, the mass of immobilized enzyme in the NAMCs was calculated upon the enzyme-encapsulated silica NPs. Notably, since nearly all enzyme-encapsulated silica NPs could be assembled on the surface of NAMCs, the mass of enzyme utilized for conducting the catalytic reaction should be equal to the total mass of enzyme in the enzyme-encapsulated silica NPs that we added.
Supporting information for Results & discussion:

Figure S1. SEM image of as-synthesized silica NPs.
Figure S2. Optical image of a) PAH aqueous solution, b) PAH-citrate aqueous mixtures (stirring time 1 min) and c) PAH-citrate aqueous mixtures (stirring time 15 min); and d) SEM images of the resultant PAH (5.0 mg mL\(^{-1}\))-citrate microaggregates.

As shown in Figure S2a, the PAH aqueous solution was transparent. Once added with sodium citrate, the resultant mixture became turbid (Figure S2b). After continuous stirring for \(\text{ca.}\) 15 min, the solution was changed to be transparent (Figure S2c). From the SEM images, numerous PAH-citrate microaggregates could be observed and exhibited spherical shape in Figure S2d. Notably, the size of the microaggregates (ca. 4 \(\mu\)m) was much smaller than that of the corresponding NAMCs (ca. 5-10 \(\mu\)m, as shown in Figure 2c in the manuscript). This phenomenon could be explained as follows: the size of PAH-citrate microaggregates would increase as the stirring time was prolonged, which has been proven by previous reports.\[^{[S1, S2]}\]

In the present study, it would take some time to adsorb silica NPs for completely covering the surface of PAH-citrate microaggregates after the silica NPs and PAH-citrate microaggregates were mixed together. Therefore, the pristine NAMCs could grow up to a much larger size during this period.

Figure S3. a-c) Optical and d-f) SEM images of NAMCs synthesized from different PAH concentrations: a, d) 0.5 mg mL$^{-1}$, b, e) 1 mg mL$^{-1}$ and c, f) 5.0 mg mL$^{-1}$. Inset in Figure S3f is the surface morphology of NAMCs synthesized from PAH concentration of 5.0 mg mL$^{-1}$.

Capsule structure, especially the size, may directly govern the recyclability of NAMCs-based enzyme catalytic systems. In previous literatures,[S1, S2] the size of PSAs was proven to be affected by the initial polyamine concentration, which would finally determine the particle size of NAMCs. Therefore, three different concentrations of PAH aqueous solution was utilized for generating NAMCs. As shown in Figure S3a-S3c, when the PAH concentration increased from 0.5 to 5.0 mg mL$^{-1}$, the resultant NAMCs grew from hundreds of nanometers to 5~10 μm as evidenced by optical microscopy. After frozen dried, the morphologies of these NAMCs were characterized by SEM. As shown in Figure S3d, lower PAH concentration would lead to the collapsed capsules with a size of ~300 nm. Increasing PAH concentration from 0.5 to 1 mg mL$^{-1}$, the size of NAMCs was changed to ~1.6 μm (Figure S3e). Additionally, once the PAH concentration was up to 5.0 mg mL$^{-1}$, the as-acquired NAMCs showed spherical shape with a particle size of 5~10 μm.
(Figure S3f). After enlarging the surface region, numerous closely packed NPs of about ~25 nm could be observed from the inset of Figure S3f. It should be noted that the larger the capsule size was, the easier to recycle the enzyme catalytic system would be. Therefore, in this study, the largest microcapsules were adopted for the construction of enzyme catalytic systems.

**Figure S4.** Chromatographic spectra for reduction of formaldehyde to methanol. Curve (1) refers to the chromatographic spectrum of the substrate solution; curve (2) refers to the chromatographic spectrum of the reaction solution after reacting for 3 min.

In the present study, the reaction substrate (*formaldehyde*) and product (*methanol*) were analyzed on an Agilent Micro GC 6820 with an HP-INNOWAX capillary column (Hewlett–Packard Company, 30 m×0.32 mm×0.50 μm) equipped with a flame ionization detector (FID).

As shown in **Figure S4**, after reacting for 3 min, the peak intensity of formaldehyde decreased, while the peak of methanol appeared. This strongly indicated the successful conversion of formaldehyde to methanol catalyzed by the NAMCs-based enzyme catalytic system.
Figure S5. DLS analysis of a) the silica nanoparticles and b) the microcapsules.

The dynamic light scattering (DLS) experiments of the silica NPs and the NAMCs were conducted. As shown in Figure S5, the average particle size of the silica NPs and the NAMCs was about 25.6 nm and 5~10 μm, respectively. Meanwhile, the detected polydispersities of the silica NPs and the NAMCs were, respectively, 0.505 and 0.781.
The kinetic parameters (including the $V_{\text{max}}$, $K_{MA}$, $K_{MB}$, $k_{cat}$) of free and immobilized YADH

In the present study, the reaction of YADH (either free or immobilized YADH) catalyzing conversion of formaldehyde to methanol coupled with the oxidation of NADH to NAD$^+$ was used to evaluate the catalytic activity of the free and immobilized YADH.

$$HCHO + NADH + H^+ \xrightleftharpoons{} CH_3OH + NAD^+$$

The assay was typically carried out in a neutral aqueous solution of 10 mM formaldehyde and 0.133 mM NADH at 25 °C for 5 min. The enzyme activity was determined spectrophotometrically by directly measuring the decrease in absorbance of NADH at 340 nm.

The Michaelis constant ($K_m$) and the maximum reaction rate ($V_{\text{max}}$) were determined at 25 °C, and pH 7.0. The concentrations of NADH and formaldehyde ranged from 0.020 to 0.133 mM and 2 to 10 mM, respectively. The $K_m$ and $V_{\text{max}}$ for the free and immobilized YADH were calculated according to Dalziel's equation$^{[S1-S3]}$:

$$[E_0]/V = \Phi_0 + \Phi_A/[A] + \Phi_B/[B] + \Phi_0/([A] \times [B])$$

$$V_{\text{max}} = [E_0]/\Phi_0$$

$$K_{MA} = \Phi_A/\Phi_0$$

$$K_{MA} = \Phi_B/\Phi_0$$

where $\Phi_0$, $\Phi_A$, $\Phi_B$ and $\Phi_0$ were kinetic coefficient, $[E_0]$, $[A]$ and $[B]$ represented the concentrations of YADH, NADH and formaldehyde, respectively. $K_{MA}$ and $K_{MB}$ represented the Michaelis constant calculated based upon NADH and formaldehyde, respectively.

After conducting the kinetic experiment, the kinetic parameters (including the $V_{\text{max}}$, $K_{MA}$ and $K_{MB}$) of free and immobilized YADH were presented in Table S1.

Table S1. Kinetic parameters of free and immobilized YADH.

<table>
<thead>
<tr>
<th></th>
<th>$V_{\text{max}}$ (μM$^{-1}$ s$^{-1}$)</th>
<th>$K_{MA}$ (μM$^{-1}$)</th>
<th>$K_{MB}$ (μM$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme in free form</td>
<td>0.80</td>
<td>5.6</td>
<td>40</td>
</tr>
<tr>
<td>Enzyme-encapsulated</td>
<td>0.66</td>
<td>7.0</td>
<td>51</td>
</tr>
<tr>
<td>silica NPs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzyme catalytic system</td>
<td>0.60</td>
<td>7.5</td>
<td>57</td>
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Moreover, as illustrated in the manuscript, the amount of YADH (Mₜ=141 kDa) utilized in this study was 0.1 mg, and the reaction solution volume was 10 mL. Based on the equation of $k_{cat}=V_{max}/[E_0]$, the $k_{cat}$ for the enzyme in free form, enzyme-encapsulated silica NPs and enzyme catalytic system were, respectively, 11.28, 9.31 and 8.46 s⁻¹.

References: