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A highly efficient nanozyme system, termed hollow multipod Cu(OH)$_2$ superstructure (HMPS), has been developed via direct conversion from irregular nanoparticles. The HMPS displayed body size around 150 nm and branch lengths in the range of 150°-250 nm. Based on the excellent catalytic property of HMPS, we developed a simple and highly sensitive colorimetric assay to detect urine glucose, and the results are in good agreement with hospital examination reports.

Nanomaterial-based enzyme mimetics, called nanozymes, have attracted considerable interest by their unique properties, such as high stability, low cost, and excellent catalytic activity. In particular, materials with three-dimensional (3D) hierarchical superstructures exhibit excellent performance in applications, including drug delivery, live cell imaging, and theranostic application. The individual properties of the building blocks are preserved, and the presence of the secondary architecture also contributes to performance, i.e., chemical stability, uniform porosity, and resistance to aggregation of nanomaterials, all of which can be improved in 3D superstructures. Self-assembly is a powerful approach to create these unique superstructures. For example, uniform twinning superstructures connected by pairs of parallel ZnSe nanorods were generated by a self-limited assembly process. Helical Fe$_3$O$_4$ superstructures were obtained through template-free self-assembly of magnetite. Crosslinking dimers with well-controlled interparticle distance and relative orientation were prepared through self-assembly of Au nanodumbbell building blocks. However, these processes may involve relatively weak hydrogen bonds, dipole-dipole or Van der Waals interactions between the subunit components, which, in turn, limit stability, integrity and application of superstructures.

Here, we reported the development of a facile process for the preparation of hollow multipod Cu(OH)$_2$ superstructures (HMPS). These superstructures, which are composed of many tiny branches, are transformed at room temperature from Cu(OH)$_2$ nanoparticle (NP) precursors (Scheme 1). Benefited from the large surface area and unique configuration endowed by the 3D superstructure, these HMPS offer more active sites to trap the reactive molecules inside and increased the collision probability between these active molecules. This results in a high catalytic activity towards 3,3',5,5'-tetramethylbenzidine (TMB) and H$_2$O$_2$. Based on their excellent catalytic activity, we developed a simple colorimetric assay with high sensitivity (limit of detection = 1 nM) to detect urine glucose.
detect urine glucose, and the results stand in good agreement with hospital examination reports.

In a typical procedure, amorphous Cu(OH)$_2$ NPs (Figure S1-S3 in ESI†) and polyvinylpyrrolidone (PVP) were mixed by sonification. Then, certain amount of Cu(NO)$_3$·3H$_2$O was added to the above solution under vigorous stirring. After stirring at room temperature for 15 minutes, the resulting products were collected by centrifugation (see details in the Experimental Section in ESI†).

Based on low-magnification transmission electron microscopy (TEM), three-dimensional (3D) architectures with average edge length around 350 nm were formed (Figure 1a). High-magnification TEM (Figure 1b) images further revealed that these architectures are hollow and composed of many tiny branches with lengths ranging from 150 to 250 nm. The crystal phase of the superstructure was attributed to Cu (OH)$_2$·H$_2$O (JCPDS card no. 35-0505) based on the corresponding powder X-ray diffraction (XRD) pattern shown in Figure 1a. The observed lattice fringe of 0.263 nm in the high-resolution TEM (HRTEM) image (Figure 1c) corresponds to the spacing of the (002) lattice planes in orthorhombic CuOH$_2$. The ring-type selected-area electron diffraction (SAED) pattern (inset in Figure 1c) indicates the polycrystalline nature of these hollow multipod Cu(OH)$_2$ superstructures (HMPS). To investigate specific surface area, full nitrogen sorption isotherms of the HMPS were measured. According to the Brunauer-Emmett-Teller (BET) model and the data in Figure 2b, the specific surface area of HMPS was 136.8 m$^2$/g.

The formation mechanism of HMPS is illustrated in Scheme 1 and Figure S4 (ESI†). When the mixture of Cu(NO)$_3$·3H$_2$O and NH$_3$·H$_2$O is added to Cu(OH)$_2$ NPs, surface hydrated copper ions ([Cu(H$_2$O)$_{6}$]$^{2+}$) coordinate with NH$_3$·H$_2$O to generate [Cu(NH$_3$)$_{2}$](OH)$_2$. n NH$_3$·H$_2$O $\leftrightarrow$ [Cu(NH$_3$)$_{2}$](OH)$_2$. n H$_2$O, NH$_3$·H$_2$O $\leftrightarrow$ NH$_3$ + H$_2$O) (Figure S4a, ESI†). However, generated OH$^-$, which has much stronger affinity than NH$_3$ to coordinate with Cu$^{2+}$ ions, replaces NH$_3$ in [Cu(NH$_3$)$_{2}$](OH)$_2$. n H$_2$O to form a chain structure on the particle surface, i.e., [Cu(NH$_3$)$_{2}$](OH)$_2$. n H$_2$O $\rightarrow$ [Cu(NH$_3$)$_{2}$](OH)$_2$. n H$_2$O $\rightarrow$ [Cu(NH$_3$)$_{2}$](OH)$_2$ (Figure S4b, ESI†). As a consequence of coordination between OH$^-$ and Cu$^{2+}$, the chain structure in Figure S4b evolves into a one-dimensional (1D) structure (tiny branches) (Figure S4c-d, ESI†). Meanwhile, Cu$^{2+}$ migrates from the inner NPs to the surface, leaving a faintly cavity in the original NPs (Figure S4e, ESI†) and forming tiny branches on the surface. Further increase of NH$_3$·H$_2$O solution to 800 µL resulted in slight breakage of tiny branch in the superstructures, in which some branches were dissociated and cavities were visible at the center (Figure S4e-f, ESI†).

Nanozymes are always of great interest in biomimetic chemistry. They possess many unique advantages, such as low cost, high operational stability, facile preparation, and tunable catalytic activity. With the large surface area endowed by the 3D superstructure (Figure 2b), these HMPS nanozymes offer more active sites and increased collision probability between active molecules trapped inside. For the first set of experiments, we investigated whether these HMPS could mimic peroxidase activity for H$_2$O$_2$ and 3,3',5,5'-tetramethylbenzidine (TMB) (Scheme 2). As shown in our experiment, HMPS rapidly catalyzed the reduction of H$_2$O$_2$ in the presence of TMB, generating blue oxidized TMB within 3 minutes at room temperature (Figure S5, ESI†), which in other word, indicating excellent catalytic capability. Next, to decide whether the catalytic activity of HMPS shows similar pH and temperature dependence with that of natural enzymes, we tested catalytic activity of HMPS while varying the pH from 1 to 12 and...
the temperature from 22 °C to 65 °C, respectively. For comparison, the activity of horseradish peroxidase (HRP), one of the most utilized natural enzymes for biocatalysis, was also tested (Figure 3a-b). As shown in Figure 3a-b, high catalytic activity (above 85%) can be achieved with pH in the range of 4 to 5 and temperature in the range of 25 °C to 40 °C. The optimum pH and temperature for HMPS catalysis are approximately 4.5 and 25 °C, respectively, which are very close to the values obtained from HRP (Figure 3a-b).

Table 1: Comparison of the kinetic parameters of HMPS and HRP. $k_m$ is the Michaelis constant, and $V_{max}$ is the maximal reaction velocity.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$k_m$(nM)</th>
<th>$V_{max}$ (10^8 M s^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMPS</td>
<td>1.335</td>
<td>42.1</td>
</tr>
<tr>
<td>HMPS</td>
<td>0.379</td>
<td>39.1</td>
</tr>
<tr>
<td>HRP</td>
<td>0.434</td>
<td>10</td>
</tr>
<tr>
<td>HRP</td>
<td>3.700</td>
<td>8.71</td>
</tr>
</tbody>
</table>

A typical dose-response curve for glucose detection under optimal conditions (i.e., pH 4.5, 25 °C) was shown in Figure 4b and the color change during the reaction was shown in Figure S5 (ESI†). The concentration of glucose that can be detected was as low as 1 nM, and the linear range is from 1 nM to 50 nM (inset in Figure 4b).

We attempted to detect glucose in urine stock solution by using the above method in Figure 4c-d. According to the calibration curve (inset in Figure 4b), the glucose concentrations in two patients were 626.72 μM (11.28 mg/dL) and 4.86 mM (87.48 mg/dL), respectively, which are both close to the values reported in clinical examinations (normal, NEG ≤ 100 mg/dL) (Figure S7-S8, ESI†). Thus, our colorimetric method based on HMPS offered precise detection of urine glucose of these two patients. Therefore, this colorimetric method shows promise for clinical applications to monitor diabetes.

We have developed a highly efficient nanozyme system, termed 3D hollow multipod superstructure (HMPS), via direct conversion from irregular Cu(OH)2 nanoparticles. The HMPS displayed body size around 150 nm and branch lengths in the range of 150~250 nm. Kinetic analysis indicates that the nanozyme system exhibits much higher catalytic activity to H2O2 than that of natural enzyme HRP. The HMPS nanozyme system shows several advantages over HRP, particularly its high specificity and activity.
such as facile preparation, low-cost, and stability. By leveraging the
color changes caused by the nanozyme, HMPS can be utilized in the
analysis of urine glucose concentration at a low limit of detection
(1 nM). Based on these excellent catalytic properties, we developed a
simple and highly sensitive colorimetric assay to detect urine
glucose, and the results are in good agreement with clinical
examination reports. Therefore, the HMPS nanozyme system holds
potential in such clinical applications as diabetes monitoring.
Furthermore, successful demonstration of this work will facilitate
development of more nanozyme systems with high catalytic activity
for medical diagnostics.

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Notes and references