Electronic Supplementary Information

A new biomimetic nanozyme of hemin/graphdiyne oxide with superior peroxidase-like activity for colorimetric bioassays

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S1) TEM characterization of GDY

TEM characterization of GDY was performed in order to verify the successful synthesis of GDYO. From the TEM image in Figure S1, it can be clearly seen that graphdiyne was

Figure S1. TEM image of GDY.

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composed of nanoparticles with abundant porous and multilayer structure, and the dispersion is uneven and easy to agglomerate.

S2) Results of dopamine oxidation catalyzed by H/GDYO

![Figure S2](image)

**Figure S2.** Adsorption spectra of H/GDYO-catalyzed oxidation of dopamine under the presentation of H$_2$O$_2$ incubated in HAc-NaAc (0.1 M, pH 4.0): H$_2$O$_2$ (10 mM) + H/GDYO (10 μg mL$^{-1}$) + dopamine with variable concentrations (From 1 to 5 are 0.1, 1, 3, 5 and 10 mM, respectively).

It is reported that nanomaterials with peroxidase-like activity could mediate the oxidation of dopamine (DA) to aminochrome by H$_2$O$_2$.$^1$ We performed dopamine oxidation test in H$_2$O$_2$-DA system to verify the same characteristics of H/GDYO as peroxidase-mimicking nanozyme. The oxidation catalytic determination of dopamine was carried out as follows: 20 μL H/GDYO (1 mg mL$^{-1}$), 100 μL of H$_2$O$_2$ (10 mM) and 100 μL variable concentrations (0.1, 1, 3, 5, 10 mM) of dopamine were added into 500 μL of HAc-NaAc buffer (0.1 M, pH 4.0), the mixture was incubated at 37 °C for 1 h. The oxidation of dopamine was monitored with an UV spectrophotometer at a wavelength of 480 nm. As shown in Figure S2, the absorption spectrum at 480 nm increased with the increase of dopamine concentration. Meanwhile, the color photographs of the catalytic oxidation of dopamine at different concentrations were also
shown in the inset. The results show that H/GDYO peroxidase-mimicking nanozyme can catalyze the oxidation of dopamine to aminochrome in the presence of H$_2$O$_2$.

**S3) Optimization of reaction conditions of H/GDYO nanozyme**

![Figure S3](image)

**Figure S3.** Effects of (A) pH, (B) temperature and (C) time for the catalytic reaction in H$_2$O$_2$ (10 mM)-TMB (10 mM) system.

To achieve the high catalytic activity, the main reaction conditions were explored for H/GDYO by using the TMB-H$_2$O$_2$ reaction system. As shown in Figure S3, we optimized the key assay parameters, including the pH of HAc-NaAc buffer, reaction temperature and reaction time, which could have a significant impact on the analysis. Figure S3A displayed the catalytic performance of H/GDYO at different pH values. Obviously, H/GDYO was more active in the weakly acidic condition and the optimal pH was 4.0. Furthermore, the dependence of the peroxidase-like activity of H/GDYO catalyst on temperature was investigated (Figure S3B). Maximal absorbance was observed at 37 °C, above which the catalytic activity of H/GDYO sharply decreased. In addition, the optimal time of H/GDYO as nanozyme for TMB-H$_2$O$_2$ reaction system was discussed by changing the reaction time (Figure S3C). Apparently, H/GDYO could achieve the best catalytic effect within 20 minutes.

**Reference**