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

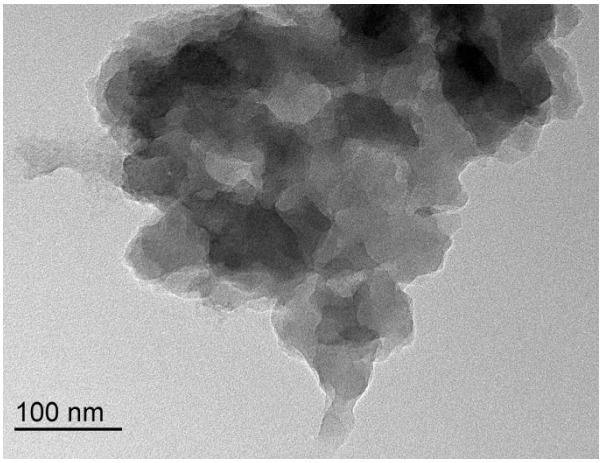

Figure S1. TEM image of GDY.

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

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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

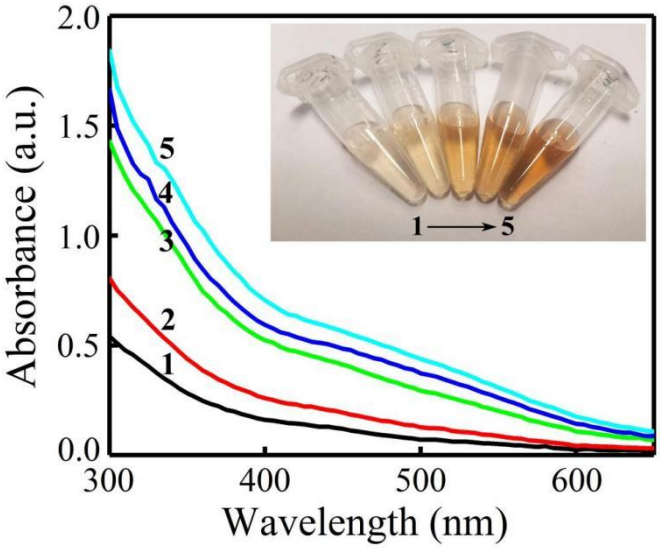

Figure S2. Adsorption spectra of H/GDYO-catalyzed oxidation of dopamine under the presentation of H_2O_2 incubated in HAc-NaAc (0.1 M, pH 4.0): H_2O_2 (10 mM) + H/GDYO (10 μg mL^{-1}) + dopamine with variable concentrations (From 1 to 5 are 0.1, 1, 3, 5 and 10 mM, respectively).

It is reported that nanomaterials with peroxidase-like activity could mediate the oxidation of dopamine (DA) to aminochrome by H_2O_2.[1] We performed dopamine oxidation test in H_2O_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_2O_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_2O_2.

S3) Optimization of reaction conditions of H/GDYO nanozyme

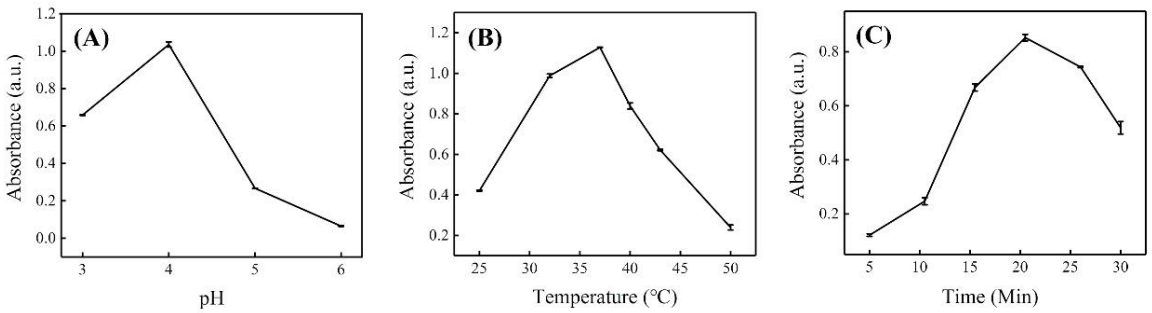

Figure S3. Effects of (A) pH, (B) temperature and (C) time for the catalytic reaction in H_2O_2 (10 mM)-TMB (10 mM) system.

To achieve the high catalytic activity, the main reaction conditions were explored for H/GDYO by using the TMB-H_2O_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_2O_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

1 W. H. Chen, M. Vazquez-Gonzalez, A. Kozell, A. Cecconello and I. Willner, *Small*, 2018, **14**, 1703149.