Supporting Information

Mimicking Horseradish Peroxidase and NADH Peroxidase by Heterogeneous Cu^{2+}-Modified Graphene Oxide Nanoparticles

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Materials

Graphite powder (< 20 μm), copper(II) chloride, hemin, dopamine hydrochloride, luminol sodium salt, glucose oxidase (GOx), glucose, β-Nicotinamide adenine dinucleotide, reduced disodium salt hydrate (NADH), Alcohol Dehydrogenase from Saccharomyces cerevisiae (AlcDH) were purchased from Sigma-Aldrich. Water used was the ultrapure water from a NANOpure Diamond (Barnstead Int., Dubuque, IA) source.

Methods

Preparation of graphene oxide (GO). The GO was prepared using the modified Hummers method.[1] Prior to the GO preparation, an additional preoxidation procedure was conducted. Graphite powder (8 g), concentrated H_2SO_4 (30 mL), $K_2S_2O_8$ (10 g), and P_2O_5 (10 g) were mixed and stirred 6 h at 80 °C. After cooling to room temperature, the mixture was carefully diluted with water, filtered, washed until the filtrate became neutral, and then dried at room temperature over 24 h. The obtained preoxidized graphite, concentrated H_2SO_4 (184 mL), $NaNO_3$ (4 g) were mixed in ice bath and stirred 10 min. Then $KMnO_4$ (24 g) was gradually added with stirring, the temperature of the mixture was kept below 5 °C, and the mixture was stirred another 1 h. The mixture was then stirred 6 h at 35 °C and stayed overnight. Water (368 mL) was added dropwise into the resulting solution over a period of around 30 min. Then water (1120 mL) was added followed by H_2O_2 (20 mL, 30%), and the stirring continued for 10 min to obtain a bright yellow graphite oxide suspension. During this step, H_2O_2 (30%) was used to

reduce the residual permanganate and manganese dioxide to colorless soluble manganese sulfate. The graphite oxide deposit was collected from the suspension by centrifugation at 12000 rpm for 10 min, and dialyzed until the pH was 5~6. Then a sonication (30 W, 30 min) was used to exfoliate the graphite oxide to obtain a GO suspension. A centrifugation at 5000 rpm for 10 min was used to remove all the visible particles. Then the supernatant was further centrifuged at 12000 rpm for 1 h to collect viscous precipitate in which much water existed. Finally, ethanol was added to the obtained viscous precipitate and the formed mixture centrifuged at 12000 rpm for 30 min to remove the water, then dried at 45 °C for 24 h, thus GO was obtained.

Preparation of GO NPs. The GO NPs were prepared as described previously.[2] The obtained GO was dispersed in DMF with the concentrations of 5 mg mL^{-1} under sonication for overnight (30 W). The dispersion was transferred to a poly(tetrafluoroethylene) (Teflon)-lined autoclave (30 mL) and heated at 200℃ for 5 h. After cooling to room temperature, the mixture was under centrifugation at 5000 rpm for 20 min, and brown transparent suspension was collected. The suspension solvent was removed with the aid of a rotary evaporator. The residue was purified by column chromatography on silica gel using water as eluent to provide the desired product, GO NPs.

Preparation of GO with ca. 130 nm. The 130 nm-sized GO was prepared as described previously.[3] The GO sheets were dispersed in water under sonication (30 W, overnight) with concentration for 5 mg mL^{-1} (200 mL), and then was cut into small size under tip sonication for 30 min with a total energy of 175955 J. The GO dispersion was diluted

to 1 mg mL^{-1} and centrifuged at 12000 rpm for 20 min to collect the supernatant. The size of the obtained GO was ca. 130 nm, measured by dynamic light scattering (DLS).

Preparation of Cu^{2+}-GO NPs. The Cu^{2+}-GO NPs were prepared as follows: copper(II) chloride (100 mg) was added to 10 mL of an ethanolic dispersion of GO-NPs (10 mg). After stirring overnight, the mixture volume was reduced to 0.5 mL under vacuum. Then ethyl ether 25 mL was added slowly with stirring, and the concomitant generation of sediment was observed. After stirring for 10 min, the mixture was under centrifugation at 5000 rpm for 10 min to collect the sediment. The sediment was washed with ethanol/ethyl ether mixture solvents for 4 times further, and then dried. Inductively coupled plasma atomic emission spectroscopy (ICP-OES) analysis indicated a coverage of Cu^{2+} ions corresponding to 0.21 mg per milligram of GO NPs. As for the Cu^{2+}-GO NPs with other content of Cu^{2+}, such as 0.13, 0.06, 0.03 mg of Cu^{2+} per 1 mg GO NPs, were prepared using the same procedure but with different amount of copper(II) chloride.

Preparation of Cu^{2+}-GO. The Cu^{2+}-GO were prepared as follows: copper(II) chloride (100 mg) was added to 10 mL of an aqueous solution of micrometer-sized GO (10 mg) or 130 nm-sized GO (10 mg). After shaking for overnight, the mixture was under centrifugation at 10000 rpm for 15 min to collect the sediment. And the sediment was washed with water for 4 times and then dried. ICP-OES analyses indicated a coverage of Cu^{2+} ions corresponding to 0.05 mg per milligram of micrometer-sized GO and 0.06 mg per milligram of 130 nm-sized GO, respectively.

Preparation of hemin-GO NPs. The hemin-GO NPs were prepared as follows: 4 mL hemin (10 mM, DMSO/ethanol = 1:9) was added to 6 mL of an ethanolic dispersion of GO (10 mg). After shaking for overnight, the mixture was under centrifugation at 10000 rpm for 15 min to collect the sediment. And the sediment was washed with ethanol (5 mL) for 4 times and then dried. The loading of hemin evaluated using UV spectrum was 0.67 mg of hemin per 1 mg of GO NPs.

Dopamine Oxidation Studies. For a typical oxidation reaction, 50 μL H_2O, 20 μL MES buffer (50 mM, 2 mM $MgCl_2$ and 10 mM KCl, pH = 5.5), 10 μL Cu^{2+}-GO NPs (100 μg mL^{-1}), 10 μL variable concentrations of dopamine were added, with final concentrations of 0, 0.005, 0.025, 0.1, 0.5, 1, 5, 10 mM, followed by the addition of 10 μL H_2O_2, with a final concentration of 10 mM. The catalytic oxidation of dopamine, was followed spectroscopically by measuring the changes in the absorption of the aminochrome product at λ_{max} = 480 nm for dopamine (ε = 3058 M^{-1} cm^{-1}). In all measurements, a background signal measured at λ = 800 nm was subtracted. UV−vis spectroscopy measurements were performed in a quartz cuvette (path length l = 1 cm) using a Shimadzu UV-2401PC spectrophotometer (the total volume was increased to 150 μL). All the experiments were done for 3 times.

Luminol Oxidation Studies. For a typical luminol oxidation reaction, 105 μL PB buffer (400 mM, pH = 9.0), 15 μL Cu^{2+}-GO NPs (100 μg mL^{-1}), 15 μL luminol (5 mM) and 15 μL variable concentrations of H_2O_2 were added, with final concentrations of 0, 0.05, 0.1, 0.25, 0.5, 1, 1.5 mM. The catalytic oxidation of luminol was followed spectroscopically by measuring the chemiluminescence. The light emission intensity

was measured immediately by a Cary Eclipse Fluorescence Spectrophotometer (Varian Inc.). All the experiments were done for 3 times. For the GOx-catalyzed aerobic oxidation of glucose: 80 μL acetate buffer (10 mM, pH = 5.0), 10 μL GOx (4 U mL^{-1}), and 10 μL variable concentrations of glucose were added, with final concentrations of 0, 1, 2.5, 5.0, 10.0, 25.0 mM, the reaction mixtures were kept in oxygen atmosphere at 25 °C for 0.5 h. After reaction, 15 μL of the reaction mixture was added to the luminol system instead of 15 μL H_2O_2.

NADH Oxidation Studies. For a typical NADH oxidation reaction, 70 μL HEPES buffer (25 mM, 10 mM $MgCl_2$ and 25 mM NaCl, pH = 7.2), 10 μL Cu^{2+}-GO NPs (100 μg mL^{-1}), 10 μL NADH (10 mM) and 10 μL variable concentrations of H_2O_2 were added, with final concentrations of 0, 0.1, 0.2, 0.5, 1, 2, 5, 10 mM. The catalytic oxidation of NADH, was followed spectroscopically by measuring the changes in the absorption of NADH at λ_{max} = 340 nm (ε = 6200 M^{-1} cm^{-1}). In all measurements, a background signal measured at λ = 800 nm was subtracted. All the experiments were done for 3 times. For a typical coupled Cu^{2+}-GO NPs-alcohol dehydrogenase catalytic reaction, 500 μL HEPES buffer (25 mM, 10 mM $MgCl_2$ and 25 mM NaCl, pH = 7.2), 100 μL Cu^{2+}-GO NPs (100 μg mL^{-1}), 100 μL AlcDH (9.0 U mL^{-1}), 100 μL NADH (0.5 mM), 100 μL benzyl alcohol (10 mM), and 100 μL H_2O_2 (100 mM) were added. The reaction mixture was kept at 25 °C for 5.0 h, and the reaction was quenched in the solvent system for HPLC. The composition of reaction mixtures was determined using a Hitachi HPLC system (Sorbent column 100−4.6 mm) developed with

acetonitrile/water (30:70). Benzoic acid, benzyl alcohol, and benzaldehyde were well resolved with elution times of 2.9, 4.8, and 7.6 min, respectively.

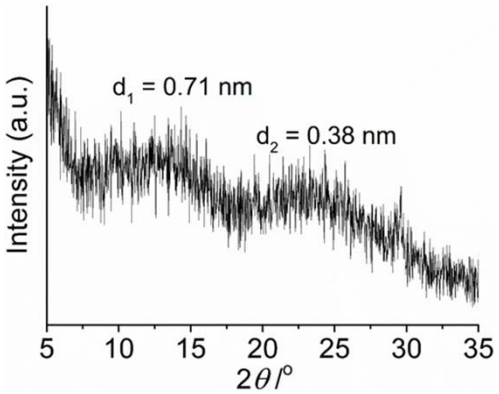

Figure S1. X-ray diffraction (XRD) pattern of GO NPs.

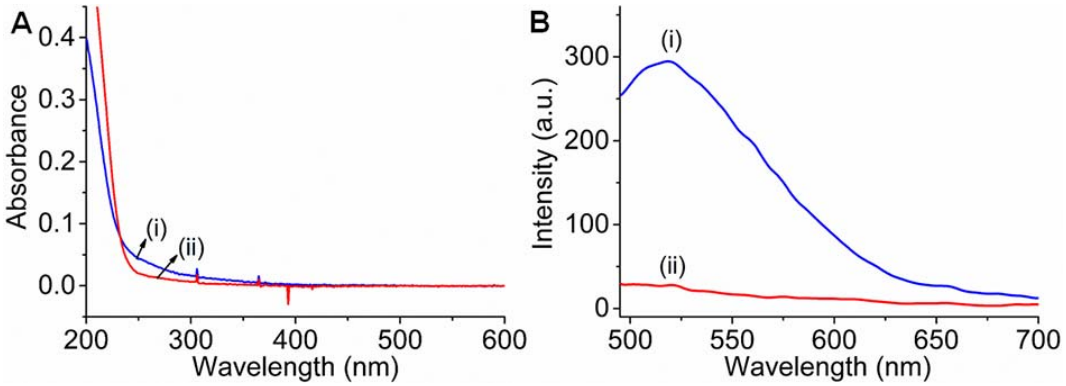

Figure S2. (A) UV absorbance spectra and (B) fluorescence spectra of (i) GO NPs, (ii) Cu^{2+}-GO NPs, the concentration of GO NPs is the same with that of Cu^{2+}-GO NPs (100 μg mL^{-1}). The fluorescence was excited at 235 nm where their absorbances are the same.

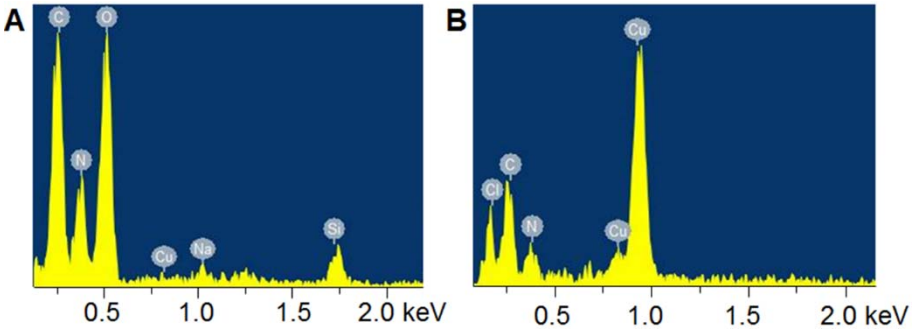

Figure S3. The energy-dispersive X-ray spectroscopy (EDS) of (A) GO NPs and (B) Cu^{2+}-GO NPs on silicon wafer.

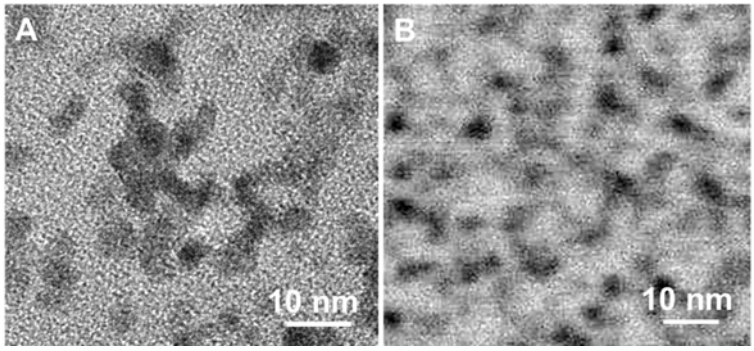

Figure S4. Transmission electron microscopy (TEM) images of (A) GO NPS, (B) Cu^{2+}-GO NPs.

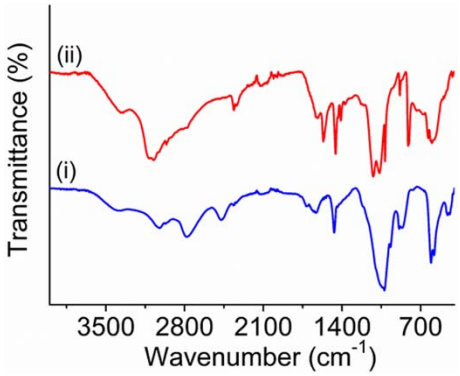

Figure S5. FT-IR spectra of (i) GO NPs, (ii) Cu^{2+}-GO NPs.

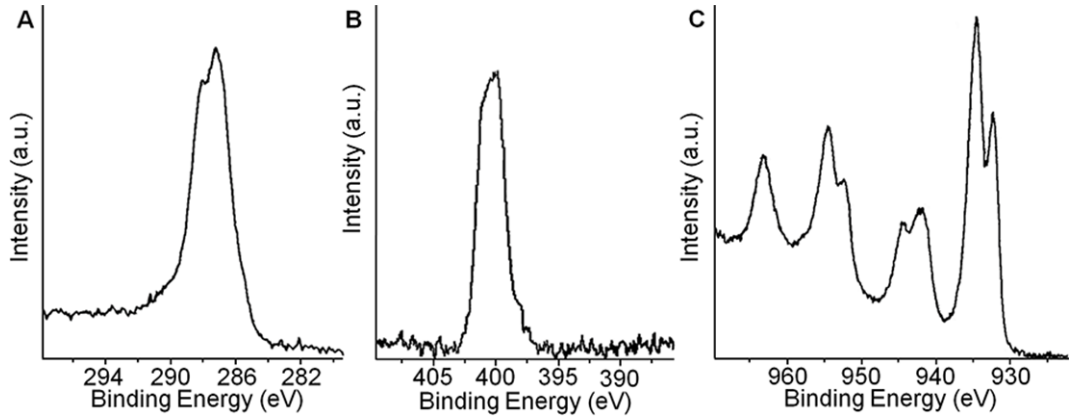

Figure S6. X-ray photoelectron spectra (XPS) of Cu^{2+}-GO NPs: (A) C (1s), (B) N (1s) and (C) Cu (2p).

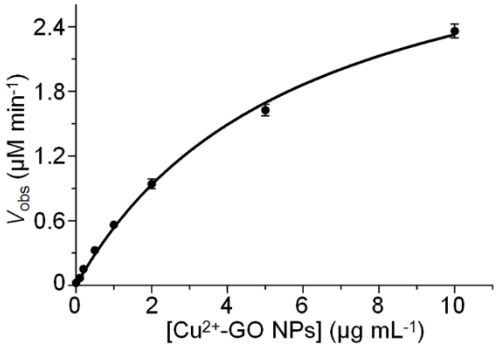

Figure S7. The rates of dopamine (**1**) oxidation by H_2O_2 using variable concentrations of Cu^{2+}-GO NPs in the presence of dopamine 1 mM and H_2O_2 10 mM: 0, 0.1, 0.2, 0.5, 1, 2, 5, 10 μg mL^{-1}, the total volume of the reaction mixture: 100 μL. All the experiments were conducted in MES buffer 10 mM, including 0.4 mM $MgCl_2$ and 2 mM KCl, pH = 5.5.

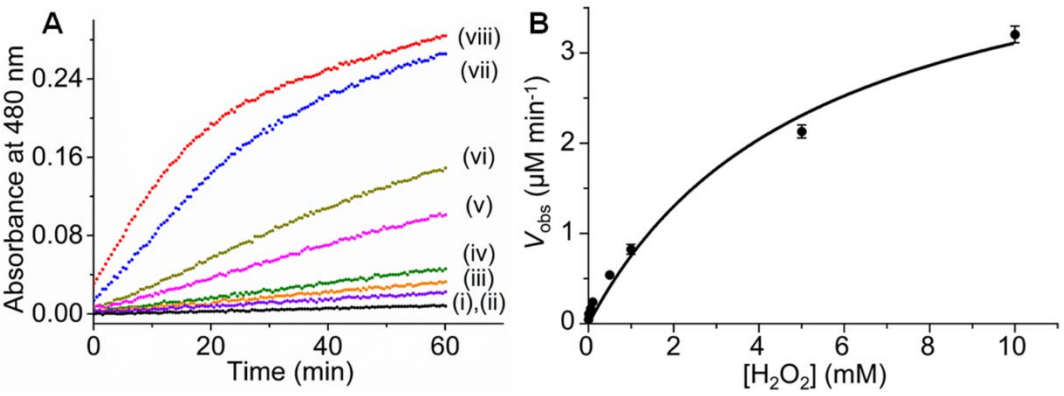

Figure S8. (A) Time-dependent absorbance changes upon the oxidation of dopamine (**1**) by variable concentrations of H_2O_2 in presence of dopamine 10 mM and Cu^{2+}-GO NPs 10 μg mL^{-1}: (i) 0, (ii) 0.01, (iii) 0.05, (iv) 0.1, (v) 0.5, (vi) 1, (vii) 5, (viii) 10 mM, the total volume of the reaction mixture: 100 μL. (B) The rates of dopamine (**1**) oxidation by variable concentrations of H_2O_2. All the experiments were conducted in MES buffer 10 mM, including 0.4 mM $MgCl_2$ and 2 mM KCl, pH = 5.5.

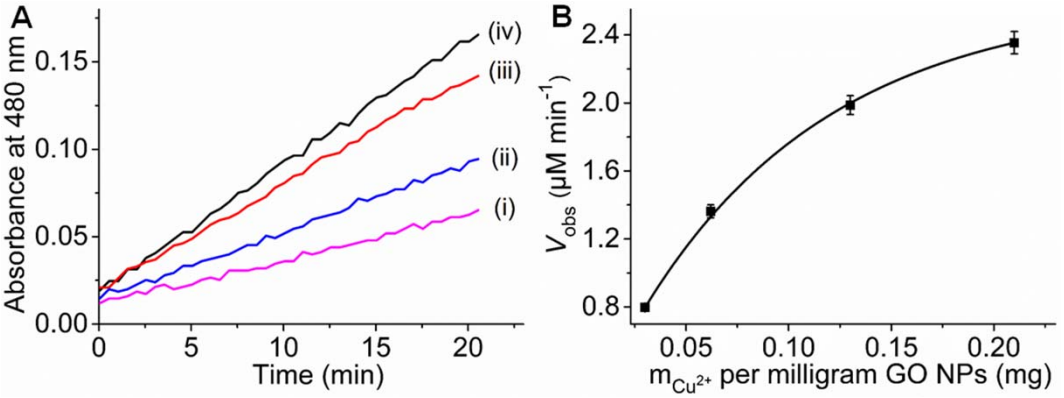

Figure S9. Time-dependent absorbance changes upon the oxidation of dopamine (**1**) by H_2O_2 using Cu^{2+}-GO NPs with different content of Cu^{2+} in presence of dopamine 1 mM and H_2O_2 10 mM: (i) 0.03, (ii) 0.06, (iii) 0.13, (iv) 0.21 mg of Cu^{2+} per 1 mg GO NPs, the GO NPs content is the same as the content associated with Cu^{2+}-GO NPs (0.21 mg of Cu^{2+} per 1 mg GO NPs, 10 μg mL^{-1}), the total volume of the reaction mixture: 100

μL. All the experiments were conducted in MES buffer 10 mM, including 0.4 mM $MgCl_2$ and 2 mM KCl, pH = 5.5.

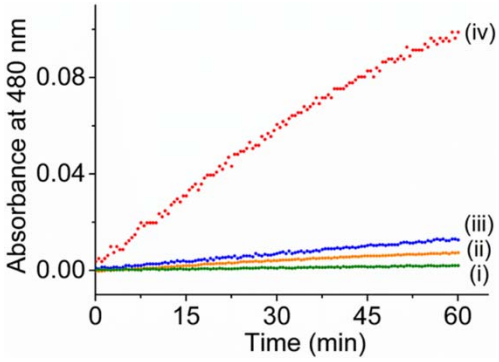

Figure S10. Time-dependent absorbance changes upon the oxidation of dopamine (**1**) by H_2O_2 using different catalysts in presence of dopamine 1 mM and H_2O_2 10 mM: (i) GO NPs, (ii) Cu_2O, (iii) Cu^{2+}, (iv) Cu^{2+}-GO NPs, the GO NPs content is the same as the content associated with Cu^{2+}-GO NPs (1 μg mL^{-1}), and the copper content is the same as the content associated with Cu^{2+}-GO NPs (1 μg mL^{-1}), the total volume of the reaction mixture: 100 μL. All the experiments were conducted in MES buffer 10 mM, including 0.4 mM $MgCl_2$ and 2 mM KCl, pH = 5.5.

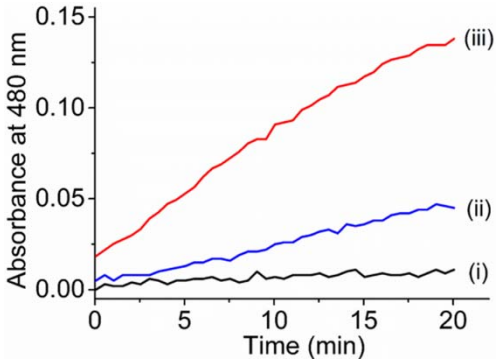

Figure S11. Time-dependent absorbance changes upon the oxidation of dopamine (**1**) by H_2O_2 using different catalysts in presence of dopamine 1 mM and H_2O_2 10 mM: (i)

hemin, (ii) hemin/GO, (iii) Cu^{2+}-GO NPs, the molar content of hemin is the same as the molar content of Cu^{2+} ions associated with Cu^{2+}-GO NPs (10 μg mL^{-1}), the total volume of the reaction mixture: 100 μL. All the experiments were conducted in MES buffer 10 mM, including 0.4 mM $MgCl_2$ and 2 mM KCl, pH = 5.5.

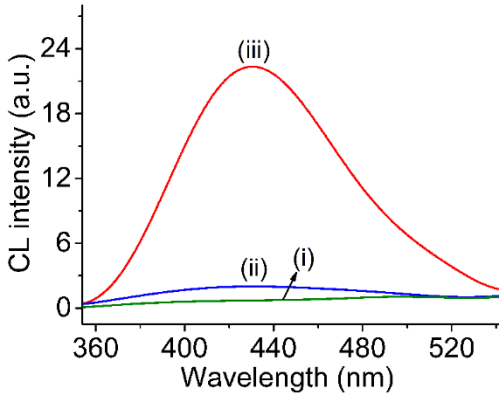

Figure S12. Chemiluminescence spectra generated upon the oxidation of luminol (**3**) using different catalysts in the presence of luminol 0.5 mM and H_2O_2 1 mM: (i) GO NPs, (ii) Cu^{2+}, (iii) Cu^{2+}-GO NPs, the GO NPs content is the same as the content associated with Cu^{2+}-GO NPs (1 μg mL^{-1}), and the Cu^{2+} content is the same as the content associated with Cu^{2+}-GO NPs (1 μg mL^{-1}), the total volume of the reaction mixture: 150 μL. All experiments were conducted in PB buffer 280 mM, pH = 9.0.

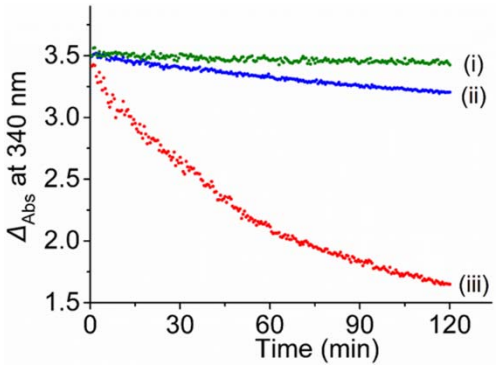

Figure S13. Time-dependent absorbance changes upon the oxidation of NADH (**5**) to NAD^+ (**6**) in the presence of different catalysts, NADH 1 mM and H_2O_2 2 mM: (i) GO NPs, (ii) Cu^{2+}, (iii) Cu^{2+}-GO NPs, the GO NPs content is the same as the content associated with Cu^{2+}-GO NPs (1 μg mL^{-1}), and the Cu^{2+} content is the same as the content associated with Cu^{2+}-GO NPs (1 μg mL^{-1}), the total volume of the reaction mixture: 100 μL. All the experiments were performed in 17.5 mM HEPES buffer solution that included 7 mM $MgCl_2$ and 17.5 mM NaCl, pH = 7.2.

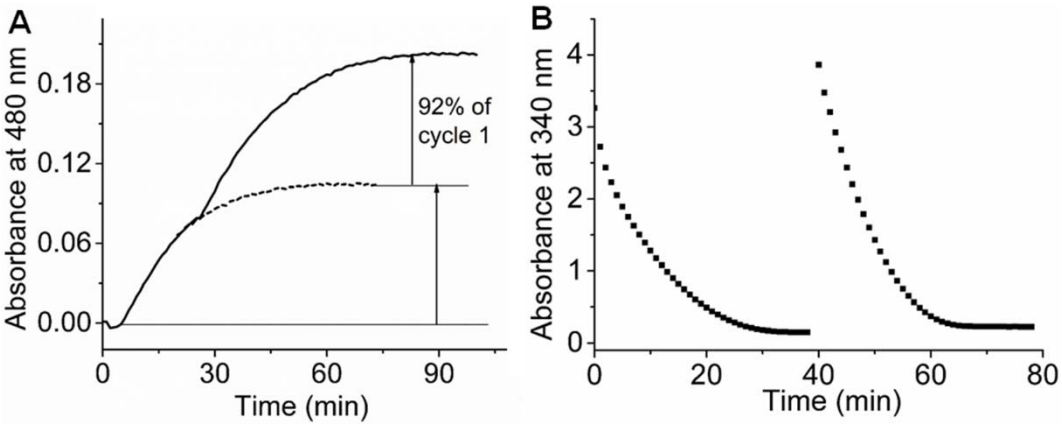

Figure S14. (A) Time-dependent absorbance changes upon the oxidation of dopamine (**1**) by H_2O_2 using Cu^{2+}-GO NPs, 10 μg mL^{-1}, for the first cycle, dopamine 0.1 mM and H_2O_2 10 mM, the total volume of the reaction mixture: 120 μL. For the second cycle, 1.2 μL 1 mM dopamine and 1.2 μL 100 mM H_2O_2 were added. All the experiments

were conducted in MES buffer 10 mM, including 0.4 mM $MgCl_2$ and 2 mM KCl, pH = 5.5. (B) Time-dependent absorbance changes upon the oxidation of NADH (**5**) to NAD^+ (**6**) in the presence of Cu^{2+}-GO NPs, 10 μg mL^{-1}, for the first cycle, NADH 1 mM and H_2O_2 10 mM, the total volume of the reaction mixture: 150 μL. For the second cycle, 1.5 μL 10 mM NADH and 1.5 μL 100 mM H_2O_2 were added. All the experiments were performed in 17.5 mM HEPES buffer solution that included 7 mM $MgCl_2$ and 17.5 mM NaCl, pH = 7.2.

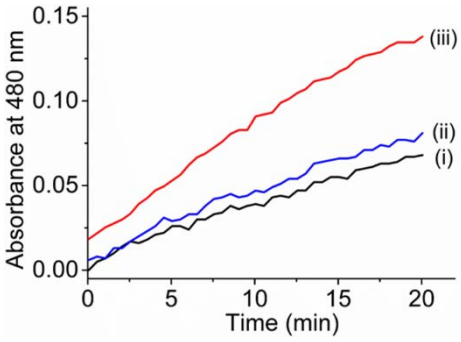

Figure S15. Time-dependent absorbance changes upon the oxidation of dopamine (**1**) by H_2O_2 using different catalysts in presence of dopamine 1 mM and H_2O_2 10 mM: (i) Cu^{2+}-modified micrometer-sized GO, (ii) Cu^{2+}-modified 130 nm-sized GO, (iii) Cu^{2+}-GO NPs, the Cu^{2+} content is the same as the content associated with Cu^{2+}-GO NPs (10 μg mL^{-1}), the total volume of the reaction mixture: 100 μL. All the experiments were conducted in MES buffer 10 mM, including 0.4 mM $MgCl_2$ and 2 mM KCl, pH = 5.5.

Comparison between the activity of Cu^{2+}-GO NPs (6 nm, prepared by the solvothermal method) and other Cu^{2+}-GO matrices towards the H_2O_2-stimulated oxidation of dopamine to aminochrome.

At present, it is impossible to prepare GO NPs of controlled sizes by the solvothermal method. This prohibits the possibility to examine size effects on the catalytic activities of Cu^{2+}-GO NPs. Nonetheless, we compared the catalytic activities of the 6 nm-sized Cu^{2+}-GO NPs to other Cu^{2+}-modified related GO matrices, Figure S15. In these experiments we made use of micrometer-sized GO and 130 nm-sized GO particles (prepared by sonication of the micrometer-sized GO[3]). The two GO samples provide the parent material for the solvothermal preparation of the GO NPs. All three GO samples were modified with Cu^{2+}-ions and the catalytic functions of the different GO matrices were examined while normalizing the samples to contain the same Cu^{2+}-ions content (verified by ICP measurements). While the Cu^{2+}-modified micrometer-sized GO and the 130 nm-sized GO reveal almost similar activities, a ca. 2-fold catalytic enhancement for the 6 nm sized GO NPs is observed. While the observed difference in the catalytic functions is not understood, we believe that the difference is originated from additional surface functionalities, generated by the solvothermal process, that interact with the Cu^{2+}-ions.

REFERENCES

(1) Hummers, W. S.; Offeman, R. E. *J. Am. Chem. Soc.* **1958**, *80*, 1339.

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(3) Wang, S.; Li, H.; Zhang, L.; Li, B.; Cao, X.; Zhang, G.; Zhang, S.; Wu, L. *Chem. Commun.* **2014**, *50*, 9700–9703.