Ternary Composite of Hemin, Gold Nanoparticles and Graphene for Highly Efficient Decomposition of Hydrogen Peroxide

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A ternary composite of hemin, gold nanoparticles and graphene is prepared by a two-step process. Firstly, graphene-hemin composite is synthesized through π-π interaction and then hydrogen tetrachloroauric acid is reduced in situ by ascorbic acid. This ternary composite shows a higher catalytic activity for decomposition of hydrogen peroxide than that of three components alone or the mixture of three components. The Michaelis constant of this composite is 5.82 times lower and the maximal reaction velocity is 1.81 times higher than those of horseradish peroxidase, respectively. This composite also shows lower apparent activation energy than that of other catalysts. The excellently catalytic performance could be attributed to the fast electron transfer on the surface of graphene and the synergistic interaction of three components, which is further confirmed by electrochemical characterization. The ternary composite has been used to determine hydrogen peroxide in three real water samples with satisfactory results.

Natural enzymes have been extensively investigated and widely applied because of their excellent properties, including high substrate specificity and catalytic efficiency. However, high costs of preparation, purification and storage restrict their widespread applications. Therefore, great attention has been paid to the construction and discovery of novel enzyme mimetics. Among them, many peroxidase mimetics, such as Fe3O4 nanoparticles1, graphene oxide (GO)2, porphyrin3, etc., have been successfully developed and used for H2O2 detection. Hemin (iron protoporphyrin) is the active center of heme-proteins, such as cytochromes, peroxidases, myoglobins, and hemoglobin, which has the peroxidase-like activity4. However, its catalytic activity and stability is inferior to natural enzymes5. Therefore, in recent years, large numbers of reports have dedicated to improve the performance of hemin. For example, hydrogel-embedded hemin6 and more elaborate hemin complexes7. They all show activities significantly better than that of hemin, however, still orders of magnitude inferior to natural enzymes, not to mention the difficulties in the synthesis of such kinds of complex hemin conjugates. Therefore, a simple and efficient way to promote ability of hemin still remains a challenge.

Graphene, a new class of two-dimensional carbon nanostructure, possesses unique electronic, optical and mechanical properties8–10. In addition, given its large specific surface area11 and high adsorption capacity, graphene and its derivatives can be used as valuable substrates to interact with various species. These composites can then be used in solar cells12, memory devices13, and even drug delivery14. Most recently, Qu et al.2 reported that GO had an intrinsic enzyme mimetic activity similar to that of natural peroxidases such as horseradish peroxidase (HRP). It also has been found that graphene-hemin composite through π-π interaction has a higher catalytic ability for decomposition of hydrogen peroxide5.

Noble-metal nanoparticles (NPs) have received tremendous interest, especially because of their fascinating catalytic activity, and potential applications in a wide range such as surface plasmonics15, biosensor16, diagnostics17, and catalysis18. Recently, Li and co-workers reported that the positively-charged gold NPs exhibited highly intrinsic peroxidase-like activity19. In addition, Xu and co-workers found that gold NPs bound on microgel particles could enhance the performance of HRP20. There are also some reports that a low concentration of silver NPs (or gold NPs) can improve the activity of HRP21.

Although the synergistic effect of the two materials to promote the decomposition of hydrogen peroxide was reported by several research groups, there are still some problems including low catalytic activity and complicated
synthetic steps. In this report, we prepared the hemin-graphene-gold (H-RGO-Au) ternary composite by a simple method. Firstly, Hemin-graphene nanosheet (H-RGO) was synthesized by simple wet-chemical strategy through the π-π interaction. Then hydrogen tetrachloroauric acid was reduced \textit{in situ} on the surface of H-RGO by ascorbic acid. To our surprise, this new composite has superior performance for decomposition of hydrogen peroxide than that of three components alone or the mixture of three components with the same concentration. Moreover, it also shows higher catalytic activity than that of HRP. This excellent performance is attributed to the high electron transfer rate of graphene and the synergistic interaction of three components. On the basis of these findings, we have, for the first time, employed the composite as a novel peroxidase mimetic to offer a simple, sensitive and selective colorimetric method for H$_2$O$_2$ detection in real water samples.

**Results**

**Synthesis and structure characterization of H-RGO-Au.** GO was prepared according to the modified Hummer’s method$^{13,14,22}$. Two steps were adopted to synthesize H-RGO-Au. Firstly, H-RGO was synthesized by refluxing a mixture of hemin and GO in ammonia in the presence of hydrazine. In this process, GO was reduced by hydrazine and formed a composite of H-RGO by π-π interaction. In the second step, the ternary composite of H-RGO-Au was obtained through reducing hydrogen tetrachloroauric acid \textit{in situ} (Fig. 1). The resulted suspension of H-RGO-Au has a good stability in a certain range of pH from 3 to 9 (Fig. S1a). However, when the pH of suspension was altered to 2, the suspension became agglomerated gradually, which could be attributed to the surface electrostatic effect. As the pH is adjusted to 2, the zeta potential of H-RGO-Au changes into positive and this electrostatic repulsion is weakened. Hence, the suspension is unstable and finally complete precipitation. The zeta potential of as-obtained H-RGO-Au is negative in the pH range from 3 to 9 which leads to a good dispersion due to the intermolecular repulsion force (Fig. S1b).

Fig. 2a shows the transmission electron microscopy (TEM) image of the synthesized GO. It can be seen that there is a layer of GO with a wrinkled and folded morphology. The atomic force microscopy studies have shown that only a single layer of GO was formed$^{22}$. In addition, selected-area electron diffraction (SAED) is performed to determine its crystalline nature (Fig. 2a inset). The well-defined hexagonal diffraction pattern, which is similar to that of single-layer graphene prepared by manual peeling off from graphite, confirms the crystalline structure of graphene-sheet$^{23}$. Fig. 2b shows TEM image of H-RGO. Obviously, we can see some aggregated hemin particles on the surface of RGO and the surface of RGO is not smooth due to the adsorbed hemin. Furthermore, the SAED of H-RGO (Fig. 2b inset) shows well-defined diffraction pattern, which further indicates the crystalline nature of the RGO. Intensity difference in the diffraction spots suggests the formation of few-layered RGO due to its intrinsic nature to form stacks$^{24}$. The elements of the materials in Fig. 2b were further investigated by X-ray energy dispersive spectroscopy (EDS). Compared with GO (Fig. S2a), H-RGO (Fig. S2b) shows a small amount of Fe and Cl, which indicates the existence of hemin on the surface of the RGO. Fig. 2c show TEM image of H-RGO-Au. The gold NPs are dispersed on the surface of H-RGO and the measured size is about 8 ± 3 nm based on size distribution in Fig. S3 a–d. The SAED pattern acquired from the sample is shown as an inset in Fig. 2c. We identified the structure as face centered cubic (fcc) gold space group (JCPDS card NO: 65-2870) by indexing the diffraction pattern. At the same time, we can also see the diffraction spots of few-layered RGO. From high-resolution TEM (Fig. 2d), we find the...

Figure 1 | Schematic for the preparation of H-RGO and H-RGO-Au. Hemin is absorbed on the surface of graphene due to the π-π interaction, then hydrogen tetrachloroauric acid is reduced \textit{in situ} by ascorbic acid.
detailed lattice fringe oblique intersection at an angle of 60° of the gold crystallites. In addition, interplanar spacings were measured to be 0.21 and 0.238 nm (Fig. S3e and f), which corresponds to (200) and (111) planes in gold, respectively. The EDS of H-RGO-Au further confirms that we obtained the composite of H-RGO-Au (Fig. S2c).

Shown in Fig. 3a is the ultraviolet-visible (UV-vis) absorption spectra of aqueous dispersions of GO, hemin, H-RGO and H-RGO-Au. GO exhibits a strong peak centered at 233 nm and a shoulder peak at 300 nm corresponding to $\pi-$-$\pi^*$ transitions of aromatic C=C band and n-$\pi^*$ transitions of C=O band, respectively. On the other hand, the peak at 233 nm red-shifts to 262 nm, the shoulder peak at 300 nm disappears, which indicates that GO was reduced by hydrazine and the restoration of the $\pi$-conjugation network within RGO during the synthesis of H-RGO. In the absence of RGO, the spectrum of hemin solution contains a strong peak at 388 nm attributed to the Soret band of hemin, as well as a group of weak peaks between 500 and 700 nm ascribed to the Q-bands of hemin. In the presence of RGO, maximum of the Soret band of hemin red-shifts from 388 to 413 nm, which could be attributed to the adsorption of hemin molecule on RGO driven by $\pi-$-$\pi$ stacking interaction between the porphyrin moiety and RGO. In addition, there is a new absorption peak at about 541 nm for H-RGO-Au, corresponding to the surface plasmon absorption of gold NPs.

Fig. 3b further explored the effect of gold concentration on the composite structure. With increasing concentration of HAuCl₄, more gold NPs were produced. Therefore, the absorption peak intensity at 541 nm gradually increased. The absorption peak intensity of RGO at 262 nm also increased which could be attributed to the further reduction of graphene by ascorbic acid. The concentration of gold NPs which is considered to be the oxidation products of ascorbic acid containing oxygen functional groups. The absorption of hemin molecule at 413 nm gradually disappeared due to the increasing concentrations of the other two components to cover up this peak. All results indicate that there would be a strong interaction among three components.

Fig. 4a shows the XRD patterns of the as-synthesized GO, H-RGO and H-RGO-Au, all of them show a broad peak around 24.3° which corresponds to the characteristic diffraction peak (002) of the hexagonal structure of graphene. In addition, there are five strong diffraction peaks in H-RGO-Au which could be indexed to the (111), (200), (220), (311) and (222) planes of the Au NPs. Compared with the pattern of bulk Au from the Joint Committee on Powder Diffraction Standards (JCPDS), the diffraction peaks from the synthesized Au NPs are located in the same angles, strongly indicating the formation of Au NPs. Based on the Scherrer formula: $D = \frac{K\lambda}{b\cos{\theta}}$, where $D$ is the mean diameter of gold NPs, $b$ is the full-width at half-maximum of the peak at $2\theta$, $K$ is shape-dependent Scherrer’s constant of 0.94 and $\lambda$ is radiation wavelength (1.5406 Å). The calculated size of gold NPs is 8.2 nm, which is consistent with the measured size by TEM (8 ± 3 nm).

Raman spectroscopy is widely used to detect the ordered and disordered crystalline structure of graphene. Fig. 4b shows the Raman spectra of GO, H-RGO and H-RGO-Au. For all three samples, the two peaks at about 1347 and 1588 cm⁻¹ can be assigned to the D- and G-band, respectively. The D-band is related to the vibrations of $sp^2$ carbon atoms of disordered graphene nanosheets, while the G-band arises from vibrations of $sp^2$ carbon atom domains of graphite. From Fig. 4b, the intensity ratios of D- and G-bands ($I_D/I_G$) from H-RGO and H-RGO-Au are obviously larger than that of GO (0.973 in GO, 0.991 in H-RGO and 1.005 in H-RGO-Au), which
confirms that most of the oxygenated groups would have been removed during the reduction process. In addition, D- and G-bands in H-RGO and H-RGO-Au produce a small red shift compared with GO, which strongly suggests an interaction between graphene and the other two components.

**Peroxidase-like activity of H-RGO-Au.** It is well known that hemin is the active site in peroxidase and exhibits the peroxidase-like activity. Therefore, we further investigate the catalytic properties of H-RGO, H-Au and H-RGO-Au. The peroxidase-like activity was evaluated in the catalytic oxidation of peroxidase substrate 3,3'-5,5'-tetramethylbenzidine (TMB). The TMB cation free radical, a one-electron oxidation product, would be formed with exposure of TMB to peroxidase and H$_2$O$_2$. It is responsible for the blue color (maximum absorbance at 652 nm) that develops during TMB oxidation. The catalytic reaction can be detected by monitoring absorbance change of oxidation products of TMB at 652 nm.

Fig. 5a is the absorbance change with time in the mixture of TMB, H$_2$O$_2$ and H-RGO-Au. The increase in absorbance at 652 nm is considered to be gradually increased oxidation product of TMB. Fig. 5b demonstrates that both of H$_2$O$_2$ and as-obtained H-RGO-Au cannot alone oxidize TMB efficiently. Therefore, TMB oxidation is resulted from the decomposition of H$_2$O$_2$ by as-obtained H-RGO-Au. In order to compare the effect of different catalysts, we measure the time-dependent absorbance curve under the same condition. Fig. 5c illustrates the catalytic activity of different materials with the same concentration. The hemin itself has a higher catalytic activity than that of RGO and gold NPs. There is also a weak synergy between gold and RGO which has been reported by other researchers. Furthermore, the hemin modified with gold NPs or RGO has a higher catalytic activity than that of hemin itself, which could be attributed to the synergistic effect between hemin molecule and nanomaterials. Much to our surprise, gold and RGO can also work together to promote the performance of hemin due to the synergistic effect of three components which will be confirmed in follow. Fig. 5d compares the catalytic activity of ternary composites, simple mixing of three components and three components alone. This experiment was performed according to the following steps. First, the content of hemin and gold NPs in H-RGO-Au is calculated to be about 11 wt% and 32 wt%, respectively, which can be obtained by thermal gravimetric analysis (TGA) (Fig. S4a and b). Then a simple mixing of three components (H + RGO + Au) is obtained by only mixing of 11 wt% hemin, 32 wt% gold NPs and 57 wt% RGO in solution which the final concentration is same as H-RGO-Au. At last, the catalytic properties of 11 wt% hemin, 32 wt% gold NPs and 57 wt% RGO were investigated, respectively. Obviously, we find from the inset that the absorbance at 652 nm for H-RGO-Au has a highest value.
than that of addition of three components alone ($A_{\text{Hemin}} + A_{\text{RGO}} + A_{\text{Au}}$) or simple mixing of three components ($A_{\text{Hemin}} + A_{\text{RGO}} + A_{\text{Au}}$). In addition, the value of $A_{\text{Hemin}} + A_{\text{RGO}} + A_{\text{Au}}$ is a little higher than that of $A_{\text{Hemin}} + A_{\text{RGO}} + A_{\text{Au}}$, due to a weak synergy when three components are simply mixed. The result shows that there might be strongly synergistic effect of three components to improve the catalytic activity of H-RGO-Au.

To further explore the effect of RGO and gold NPs on the catalytic properties of H-RGO-Au, a series of experiments were performed. Fig. S5a is the time-dependent absorbance changes of H-RGO-Au with different concentrations of GO and the constant concentrations of HAuCl$_4$. Fig. S5b demonstrates that the optimally initial concentration of GO in H-RGO-Au is 0.25 mg mL$^{-1}$. Fig. S5c is the time-dependent absorbance changes of H-RGO-Au with different loading of gold nanoparticles in H-RGO-Au and the constant concentrations of GO. Fig. S5d shows that the optimal loading of gold NPs in H-RGO-Au is 32 wt%.

Fig. S6a demonstrates that the reaction rate increases with the H-RGO-Au concentration increasing to 0.5 µg mL$^{-1}$. Similar to peroxidase, H-RGO-Au catalytic activity is dependent on pH, temperature, and H$_2$O$_2$ concentration. From Fig. S6b, c and d, the optimal pH, temperature, and H$_2$O$_2$ concentration are pH 4.0, 40°C, and 200 mM, respectively, which are close to the values for natural enzyme HRP.

For further analyzing the catalytic mechanism and acquiring kinetic parameters, the catalytic activity of H-RGO-Au was studied by enzyme kinetics experiments with H$_2$O$_2$ and TMB as substrates. A series of experiments were performed by changing the concentration of one substrate and fixing the concentration of another (Fig. 6a and d). In a certain range of substrate concentrations, typical Michaelis-Menten curves can be obtained as shown in Fig. 6b and e for TMB and H$_2$O$_2$, respectively. Based on the different oxidation rates with variable substrate concentrations, a Lineweaver-Burk plot can be obtained with a nearly perfect linear relationship (Fig. 6c and f), from which the important kinetic parameters such as $K_m$ and $V_{max}$ can be derived (Table 1). $K_m$ was identified as an indicator of enzyme affinity to substrates. A low $K_m$ represented a strong affinity and vice versa. By comparing the apparent kinetic parameters, the apparent $K_m$ value of H-RGO-Au with H$_2$O$_2$ as the substrate is 1.6 times lower than that of hemin, 49.7 times lower than that of Fe$_3$O$_4$ magnetic nanoparticles and 1.2 times lower than that of HRP. The $K_m$ value of H-RGO-Au with TMB as the substrate is also 10.14 times lower than that of hemin, 1.32 times lower than that of Fe$_3$O$_4$ MNPs and 5.82 times lower than that of HRP. In addition, we calculate the equivalent data for HRP by calculating the kinetic parameters ratio between different materials and HRP (Table 1 brackets). The $K_m$ of H-RGO-Au with TMB as substrate is lower than that of other catalysts, which could be attributed to high surface-to-volume ratios of RGO that could absorb TMB efficiently.

In addition, graphene could serve as a support to anchor hemin and gold NPs and restrict the substrates in a nanoscale region, which could greatly enhance the catalytic activity of H-RGO-Au.
Furthermore, the $V_{\text{max}}$ of H-RGO-Au is higher than that of the other reported catalysts. Taken together, originating from the synergetic effect among RGO, gold NPs and hemin, the materials indeed showed a higher peroxidase-like activity than that of other reported catalysts.

It is necessary to further investigate the thermodynamics of the catalytic reaction for $\text{H}_2\text{O}_2$ by H-RGO-Au. The catalytic reaction is carried out at different temperatures from 25°C to 39°C by monitoring absorbance change of the oxidation product of TMB at 652 nm. Then we can obtain the value of rate constant ($k$) through fitting the

![Figure 6](https://www.nature.com/scientificreports)
The peroxidase catalytic activity of H-RGO-Au is 1.92 times higher than that of H-GO-Au and hemin. The apparent activation energy (E_a) of this system is calculated by changing scan rates from 0.05 to 5 V s^{-1}. The E_a of this system is calculated to be 20.37 kJ mol^{-1}, which is lower than that of few-layer graphene (28 kJ mol^{-1}), iron oxide (60 kJ mol^{-1}), bimetallic Pt-Pd (36.4 kJ mol^{-1}) and ferrihydrites (76.13 kJ mol^{-1}), indicating a higher catalytic activity for the as-prepared H-RGO-Au. The entropy of activation (ΔS) can also be obtained on the basis of the value of pre-exponential factors. The calculated values were listed in Table 2.

To further explore the mechanism of high catalytic activity, we have designed a series of experiments. Firstly, we compared the electrochemical active surface area of H-RGO-Au and H-GO-Au with the same concentration of 0.5 mg mL^{-1}. The peroxidase catalytic activity of H-RGO-Au is 1.92 times higher than that of others, which also means that it had a higher catalytic activity toward H_2O_2 than that of H-GO-Au and hemin.

CVs at increasing scan rates from 0.1 to 3 V s^{-1} were also recorded to investigate electrocatalytic activity of H_2O_2 by different modified electrodes (Fig. S10 a–c). By comparing and calculating ratio of the slope of current vs square root of scan rate (Fig. S10 d–f), the electrode surface area of H-RGO-Au is about 1.7 times higher than that of H-GO-Au and 2.07 times higher than that of hemin, which further confirms that H-RGO-Au would have higher conductivity than that of H-GO-Au and hemin.

Application of H-RGO-Au. On the basis of the intrinsic peroxidase-like property of H-RGO-Au, we developed a simple colorimetric method to detect H_2O_2. The concentration response curve of H_2O_2 to the absorbance data to pseudo-first-order kinetics (Fig. S7a, b, c). The result was listed in Table 2, we can find that the rate constant increases with increasing temperature. Furthermore, the apparent activation energy (E_a) and the pre-exponential factor (A) can be obtained from the slope and intercept of the linear ln(1/k) vs 1000/T dependence (slope = E_a/R, intercept = -lnA) based on Arrhenius equation (Fig. S7d). The E_a of this system is calculated to be 28 kJ mol^{-1}, which is lower than that of few-layer graphene (28 kJ mol^{-1}), iron oxide (60 kJ mol^{-1}), bimetallic Pt-Pd (36.4 kJ mol^{-1}) and ferrihydrites (76.13 kJ mol^{-1}), indicating a high catalytic activity for the as-prepared H-RGO-Au. The entropy of activation (ΔS) can also be obtained on the basis of the value of pre-exponential factors. The calculated values were listed in Table 2.

Table 1 | Summary of the reaction rate constant (k), activation energy (E_a), pre-exponential factors (A), and the entropy of activation (ΔS)

| T [K] | 1000 [K^{-1}] | k [min^{-1}] | E_a [kJ mol^{-1}] | A [min^{-1}] | ΔS [J mol^{-1} K^{-1}] |
|-------|--------------|-------------|-----------------|-----------|---------------------|
| 298.15 | 3.354        | 0.236 ± 0.01 |                 |           |                     |
| 305.15 | 3.277        | 0.281 ± 0.013|                 |           |                     |
| 312.15 | 3.204        | 0.341 ± 0.015|                 |           | 20.37               |

The effect of scan rates on the electrochemical response of K_3Fe(CN)_6 at the different electrodes was investigated for further understanding the catalytic mechanism of H-RGO-Au. In Fig. S9 a–c, CVs of the K_3Fe(CN)_6 were performed at different electrodes by changing scan rates from 0.05 to 5 V s^{-1}. With the square root of scan rate increasing, both of anodic and cathodic peak currents increase linearly (Fig. S9 d–f). Therefore, the electrochemically active surface area can be calculated roughly from the formula listed in methods. The electrochemically active surface area of H-RGO-Au (0.034 cm^2) is nearly 1.42 times higher than that of H-GO-Au (0.024 cm^2) and 2 times higher than that of hemin (0.017 cm^2). Therefore, the higher conductivity of H-RGO-Au than that of H-GO-Au and hemin might result from the high surface area. On the other hand, we also investigated the electrocatalytic activity of H_2O_2 by H-RGO-Au, H-GO-Au and Hemin (Fig. 7b). Among three different electrodes, H-RGO-Au-modified electrode shows a higher current intensity than that of others, which also means that it had a higher catalytic activity toward H_2O_2 than that of H-GO-Au and hemin.
decomposition of hydrogen peroxide are performed in order to explore the mechanism. From these experimental results, we find that the ternary composite has the highest catalytic activity than that of three components alone or the mixture of three components at the same condition. The excellently catalytic performance of the ternary composite could be attributed to the fast electron transfer on the surface of graphene and the synergistic interaction of three components. The kinetics and thermodynamics of H$_2$O$_2$ decomposition by H-RGO-Au indicate that it was superior to the other reported catalysts, which might be also ascribed to the synergistic interaction. The substrates could be absorbed effectively on the surface of H-RGO-Au due to the high surface-to-volume ratio of graphene. Then, the active sites of the gold NPs and hemin absorbed on graphene sheets might react with substrates more directly. This mechanism is similar to that of natural enzymes in which the extraordinarily high catalytic efficiency is largely due to the ability to bring substrates into proximity with their active sites. Furthermore, the electrochemical experiments show that an interleaved electron transfer highways could be built up in H-RGO-Au due to the introduction of graphene. Therefore, we conclude that the excellent peroxidase-like activity of H-RGO-Au could be attributed to the high conductivity and the synergistic interaction of three components.

In summary, we have prepared a new catalyst H-RGO-Au by a simple process. The most important discovery is that H-RGO-Au shows a higher catalytic activity for decomposition of hydrogen peroxide than that of three components alone or the mixture of three components. Kinetic and thermodynamic analysis indicates that the catalytic activity of H-RGO-Au was higher than that of other reported catalysts. The high catalytic activity of H-RGO-Au could be attributed to its high conductivity and the synergistic interaction of three components. At last, the as-prepared H-RGO-Au was used to determine hydrogen peroxide concentration in three real water samples with satisfactory results. Furthermore, H-RGO-Au is a promising candidate as enzyme mimics with advantages of easy preparation, low-cost, and stability. We envision that the engineered catalytic graphene-based material will hold great promises in potential applications, such as biocatalyst, nanosensors and molecular carriers.

### Methods

**Synthesis of H-RGO-Au and H-GO-Au.** The synthesis of GO was described in our previous reports. Briefly, GO (50 mg) was loaded in a 500 mL dried four-necked flask, and 200 mL of H$_2$O was then added. After stirring and ultrasonication for 30 min, 300 mL of ammonia solution and 50 mg of hemin were added to the mixture, followed by the addition of 50 mL of hydrazine solution. The solution was heated in a water bath at 60°C for 4 h. The final product was filtered and washed with water for several times and dried in vacuo to obtain H-RGO-Au. Additionally, the preparation of H-GO-Au was similar to H-RGO except no adding hydrazine. H-RGO-Au/H-GO-Au was synthesized by reduction HAuCl$_4$ with ascorbic acid (AA) in the presence of H-RGO/H-GO at room temperature based on a simple and conveniently wet-chemical method. In the process, HAuCl$_4$ (0.06 mM) and H-RGO/H-GO (6 mg mL$^{-1}$) at room temperature for 5 min. This stable H-RGO-Au/H-GO-Au solution was dialyzed overnight to remove excess AA, HAuCl$_4$, and other byproduct.

**Kinetic and thermodynamic analysis.** Kinetic measurements were carried out in time course mode by monitoring the absorbance change at 652 nm on a TU-1901 UV-Vis spectrophotometer. Catalytic experiments were performed using 0.5 µg mL$^{-1}$ of H-RGO-Au in a reaction volume of 600 µL acetic acid-sodium acetate buffer solution (0.02 M, pH 4, 25°C) with 600 µM TMB as substrate, or 10 mM H$_2$O$_2$, unless otherwise stated. The Michaelis-Menten constant was calculated using the Lineweaver-Burk plot: $1/v = K_m/V_m(1/[S] + 1/K_m)$ where $v$ is the initial velocity, $V_m$ represents the maximal reaction velocity, [S] corresponds to the concentration of substrate and $K_m$ is the Michaelis constant.

Thermodynamics measurements were carried out by fitting the absorbance data of different temperatures to pseudo-first-order kinetics: $y = y_0 + y_1e^{-kt}$, where $k$ is the reaction rate constant. The value of $E_0$ and $A$ was calculated using the Arrhenius equation: $lnk = lnA - E_0/RT$, where $A$ is pre-exponential factor, $E_0$ is the apparent activation energy, $R$ is the ideal gas constant, $T$ is the absolute temperature. The entropy of activation (AS) was obtained according to the equation: $lnA = AS/R$.

**Preparation of catalyst-modified electrode.** Prior to modification, the gold electrode (9 x 2 mm) was polished with 0.3 and 0.05 µm alumina slurry, rinsed thoroughly with doubly distilled water between each polishing step, then washed successively with 1 : 1 nitric acid, ethanol, and doubly distilled water in an ultrasonic bath and dried in air. Then 10 µL of H-RGO-Au (3 µg mL$^{-1}$) dispersion was dropped onto the Au electrodes to prepare catalyst-modified electrodes. Then the electrodes were dried naturally overnight. 2 µL of the 0.5% Nafion in ethanol was spread on the H-RGO-Au/gold surface as a binder to hold the film on the electrode surface stably. The H-GO-Au/gold electrode and hemin/gold electrode were prepared in the same way by using H-GO-Au and hemin instead of H-RGO-Au. The electrochemically active surface area was calculated by the following formula. $I_p/A = (2.69 \times 10^5) \cdot n^{1/2} \cdot D^{1/2}$

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| Table 3 | Determination of H$_2$O$_2$ in water samples (n = 3) |
|---------|----------------------------------|
| **Water** | **Added [µm]** | **Detection [µm]** | **Recovery [%]** | **RSD [%]** |
| Top water | 10 | 9.97 | 99.7 | 1.6 |
| | 20 | 20.04 | 100.2 | 0.9 |
| | 30 | 30.44 | 101.5 | 1.6 |
| | 10 | 10.26 | 102.6 | 2.7 |
| Lake water | 20 | 19.74 | 98.7 | 2.3 |
| | 30 | 30.94 | 103.1 | 1.3 |
| | 10 | 10.26 | 102.6 | 1.1 |
| Commercial water | 20 | 20.21 | 101.1 | 1.5 |
| | 30 | 30.44 | 101.5 | 0.6 |
H₂O₂ detection was realized as follows. A series of different concentrations of H₂O₂ were prepared. 300 μL of TMB (120 μM) and 100 μL of H-RGO-Au (3 μg mL⁻¹) stock solution were added to 200 μL of H₂O₂. The mixed solution was used to perform the time course measurement at the wavelength of 652 nm.

H₂O₂ detection in water samples. H₂O₂ solutions of 30, 60, 90 μM were prepared with three different water samples: tap water from laboratory, lake water from Furuong Lake, and commercial water from supermarket, respectively. No other pretreatment process was performed before the use of three water samples. Experiments were carried out by adding 300 μL of TMB (120 μM) and 100 μL of H-RGO-Au (3 μg mL⁻¹) dispersion to 200 μL of H₂O₂ solution prepared with three different water samples. Then the absorption at 652 nm of mixed solution was measured at different times. Every experiment was carried out for three times parallelly.

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Author contributions
J.W. conceived the work. X.C.L. performed the experiments. All authors analysed the data and prepared the manuscript.
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