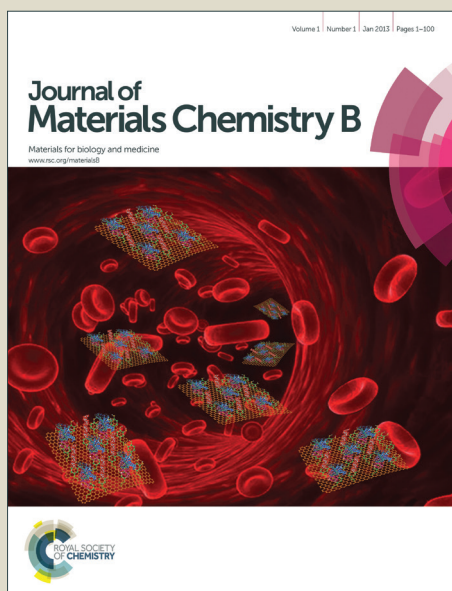


# Journal of Materials Chemistry B

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

*Accepted Manuscripts* are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

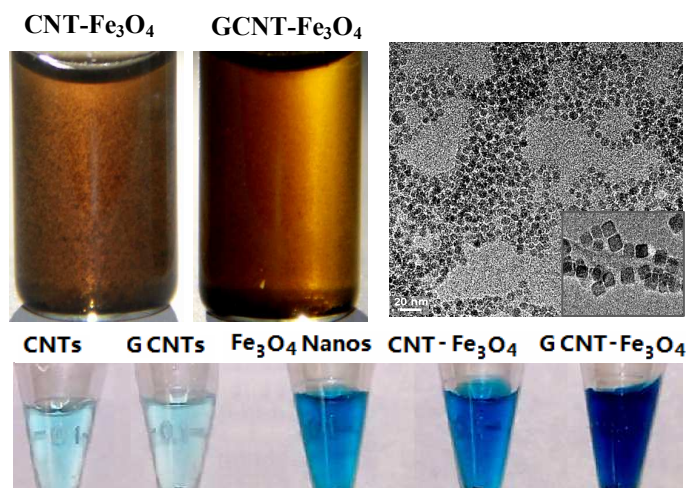
You can find more information about *Accepted Manuscripts* in the [Information for Authors](#).

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard [Terms & Conditions](#) and the [Ethical guidelines](#) still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

## The Table of Contents Entry:

### Recyclable Mimic Enzyme of Cubic $\text{Fe}_3\text{O}_4$ Nanoparticles Loaded on Graphene Oxide-Dispersed Carbon Nanotubes with Enhanced Peroxidase-Like Catalysis and Electrocatalysis

Hua Wang,<sup>\* a</sup> Shuai Li,<sup>a</sup> Yanmei Si,<sup>a</sup> Zongzhao Sun,<sup>a</sup> Shuying Li,<sup>a</sup> Yuehe Lin<sup>\* b</sup>



$\text{Fe}_3\text{O}_4$  nanocatalysts loaded on graphene oxides (GO)-dispersed CNTs for  $\text{GCNT-Fe}_3\text{O}_4$  nanocomposites with enhanced aqueous stability and peroxidase-like catalysis and electrocatalysis.

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

# Recyclable Mimic Enzyme of Cubic Fe<sub>3</sub>O<sub>4</sub> Nanoparticles Loaded on Graphene Oxide-Dispersed Carbon Nanotubes with Enhanced Peroxidase-Like Catalysis and Electrocatalysis

Hua Wang,<sup>\*,a</sup> Shuai Li,<sup>a</sup> Yanmei Si,<sup>a</sup> Zongzhao Sun,<sup>a</sup> Shuying Li,<sup>a</sup> Yuehe Lin<sup>\*,b</sup>

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

Fe<sub>3</sub>O<sub>4</sub> Nanos as nanocatalysts may present peroxidase-like catalysis activities and high electrocatalysis if loaded on conductive carbon nanotubes (CNTs) supports, however, their catalysis performances in aqueous system might still be challenged by the poor aqueous dispersion of hydrophobic carbon supports and/or low stability of loaded iron catalysts. In this work, amphiphilic graphene oxide nanosheets (GO) were employed as “surfactant” to disperse CNTs to create a stable GCNT supports in water for covalently loading cubic Fe<sub>3</sub>O<sub>4</sub> Nanos with improved distribution and binding efficiency. Comparing to original Fe<sub>3</sub>O<sub>4</sub> Nanos and CNTs loaded Fe<sub>3</sub>O<sub>4</sub> nanocomplex, the so prepared GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposite could achieve higher aqueous stability and especially much stronger peroxidase-like catalysis and electrocatalysis to H<sub>2</sub>O<sub>2</sub>, presumably resulting from the synergetic effects of two conductive carbon supports and cubic Fe<sub>3</sub>O<sub>4</sub> nanocatalysts effectively loaded. Colorimetric and direct electrochemical detections of H<sub>2</sub>O<sub>2</sub> and glucose using the GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposite were conducted with high detection sensitivities, demonstrating the feasibility of practical sensing applications. Such a magnetically recyclable “mimic enzyme” may circumvent some disadvantages of natural protein enzymes and common inorganic catalysts, featuring the multi-functions of high peroxidase-like catalysis, strong electrocatalysis, magnetic separation / recyclability, environmental stability, and direct H<sub>2</sub>O<sub>2</sub> electrochemistry.

## 1. Introduction

Enzymes have been widely applied as biological catalysts in environmental, industrial, medical, and biosensing fields.<sup>1-3</sup> Natural enzymes like protein ones, however, can encounter some disadvantages regarding protease digestion, easy denaturation, and catalytic activity inhibition especially in some complex media (i.e., wastewater). Moreover, natural enzyme-based electrochemical sensors can be additionally challenged by the redox center of enzymes deep inside the insulate protein shells, making it necessary of electronic mediators/relays.<sup>4-7</sup> Alternatively, increasing efforts have been contributed to the development of inorganic catalysts that possess the catalysis capacities of natural enzymes but address some of their catalysis limitations.<sup>8-19</sup> In particular, the development of peroxidase-like nanocatalysts has attracted many attentions.<sup>13-18</sup> Fe<sub>3</sub>O<sub>4</sub> nanoparticles (Fe<sub>3</sub>O<sub>4</sub> Nanos) as the most known example have been established to possess intrinsic peroxidase-like catalysis activities through converting Fe<sup>2+</sup>/Fe<sup>3+</sup>.<sup>13, 17, 18</sup> Fe<sub>3</sub>O<sub>4</sub> catalysts, however, may suffer from limited catalysis performances, low suspending stability, and poor conductivity of the metal oxides especially for electrocatalysis applications. Accordingly, many conductive materials typically as carbon nanotubes (CNTs) have been introduced as supports for Fe<sub>3</sub>O<sub>4</sub> loading, due to they

possess high electric conductivity, large surface area, mechanical strength, and chemical inertness.<sup>7, 18, 20-25</sup> Gracefully excellent as many works are, CNTs were mostly employed to increase the electrocatalysis capacities of Fe<sub>3</sub>O<sub>4</sub> nanocatalysts so as to achieve low-potential sensing of H<sub>2</sub>O<sub>2</sub> or glucose<sup>26</sup> or their electromagnetic behaviour,<sup>27</sup> while the fact that CNTs might improve the intrinsic peroxidase-like catalysis of Fe<sub>3</sub>O<sub>4</sub> nanocatalysts is thereby ignored to some degree. Some researchers have introduced CNTs to obtain the enhanced catalysis of Fe<sub>3</sub>O<sub>4</sub> nanocatalysts,<sup>18</sup> nevertheless, their catalysis performances and large-scale applications can still be challenged by the poor dispersion of hydrophobic carbon supports and low stability of iron catalysts mixed in aqueous system. Therefore, improved spatial distribution and well-dispersion stability of CNT supports should be one of the key requirements to obtain further enhanced peroxidase-like catalysis and electrocatalysis of Fe<sub>3</sub>O<sub>4</sub> nanocatalysts.

Up to date, two ways of oxidation treatment<sup>28</sup> and surfactant addition<sup>22</sup> are normally used to improve the aqueous dispersion of hydrophobic CNTs, but with still limited success. In recent years, there has emerged another amazing carbon material of graphene in materials science and biotechnology fields.<sup>16, 29-32</sup> The reduced graphene oxides (GO) nanosheets have been also

used to attach Fe<sub>3</sub>O<sub>4</sub> Nanos achieving excellent electrocatalytic activities.<sup>33</sup> Particularly, GO nanosheets are amphiphilic with an edge-to-center distribution of hydrophilic and hydrophobic domains,<sup>30</sup> implying that they may serve as a wonderful “surfactant” for CNTs to create a stable colloidal dispersion in water.<sup>30-32</sup> In addition, it is well established that the catalysis activities of inorganic nanocatalysts could be shape- and size-dependent,<sup>34-37</sup> i.e., cubic platinum nanoparticles have been demonstrated to show strong peroxidase-like catalysis.<sup>36</sup> Therefore, in the present work, cube-shaped Fe<sub>3</sub>O<sub>4</sub> Nanos have been synthesized and further covalently bound onto the GO nanosheets-dispersed CNTs (defined as GCNTs), aiming to develop a new kind of magnetic nanocatalysts with enhanced peroxidase-like catalysis and electrocatalysis performances towards extensive applications. Comparable investigations were carried out to demonstrate the improved support dispersion, high environmental stability, and especially enhanced peroxidase-like catalysis and electrocatalysis activities of the as-prepared GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites by optical and electrochemical measurements, including the feasibility for recyclable sensing applications for H<sub>2</sub>O<sub>2</sub> and glucose detections.

## 2. Experimental Section

### 2.1. Materials and reagents

Multiple-walled carbon nanotubes (CNTs) functionalized with carboxyl groups were purchased from Carbon Solutions, Inc. (Riverside, CA). Graphene oxides nanosheets (GO) were obtained from Graphene Supermarket (USA). Glucose oxidase (GOD), glucose, human erythrocyte AChE, bovine serum albumin, Nafion (5.0%), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC), N-hydroxysuccinimide (NHS), FeCl<sub>3</sub>, sodium oleate, MES buffer, tetramethylammonium hydroxide (TMAH), and peroxidase colour substrate were the products of Sigma-Aldrich including 3,3',5,5'-tetramethylbenzidine (TMB), o-phenylenediamine (OPD), 3,3'-diaminobenzidine (DAB), p-aminophenol (PAP), and hydroquinone (HQ). Other reagents were of analytical reagent grade.

### 2.2. Synthesis of cubic Fe<sub>3</sub>O<sub>4</sub> Nanos

Cubic Fe<sub>3</sub>O<sub>4</sub> Nanos were synthesized by following the procedures of thermal decomposition of iron-oleate complex.<sup>37, 38</sup> Typically, FeCl<sub>3</sub> (5.0 mmol) and sodium oleate (15 mmol) were added to a mixture containing ethanol (10 ml), water (7.5 ml), and hexane (17.5 ml). The mixture was then refluxed at 60 °C for 4 h. After being separated and evaporated out the organic reagents, the resulting iron oleate was dissolved in an appropriate amount of 1-octadecene containing sodium oleate (2.5 mmol). The mixture was further heated to 300 °C for 40 min under nitrogen. The cooled solution was precipitated by 2-propanol, then washed with hexane and ethanol. The precipitate of Fe<sub>3</sub>O<sub>4</sub> Nanos was subsequently resuspended in 1.0 M tetramethylammonium hydroxide (TMAH) as the stock suspension.

### 2.3. Preparation of and GOD modification of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposite

The GO-dispersed CNTs (GCNT) were prepared with the mass ratio of GO nanosheets to CNTs being 1:10. An aliquot of 100

mg CNTs, which were treated to be derivatized with carboxyl groups, were ultra-sonicated in 50 ml deionized water for 30 min, and then 10 mg GO solution was added into the CNT solution to be sonicated until a visually homogeneous dispersion was formed. The resulting GCNT mixture was sonicated for 16 h (60 s on and 5 s off cycles) and then centrifuged and washed to get GCNTs. Furthermore, the GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposite was synthesized by the EDC/NHS cross-linking chemistry. An aliquot of carboxyl groups-derivatized GCNTs above were re-dispersed in MES buffer (pH 5.0) containing 100 mM EDC and 80 mM NHS to be stirred overnight at room temperature. The activated GCNTs were then separated and washed twice by centrifuge. Moreover, an aliquot of cubic Fe<sub>3</sub>O<sub>4</sub> Nanos of the desired amount in TMAH above was magnetically separated and washed for three times. The yielding precipitate of amine-derivatized Fe<sub>3</sub>O<sub>4</sub> Nanos was then mixed with the activated GCNTs suspension for 3 h (pH 7.0), followed by magnetic separation and washing. The resulting GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites were finally dispersed in 2-propanol at different concentrations. In addition, the CNT-Fe<sub>3</sub>O<sub>4</sub> nanocomplex was prepared accordingly except for GO addition.

In addition, an amount of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocatalysts (1.0 mg) was dispersed in 1.0 ml PBS containing 0.5 % Triton X-100. GOD (10 mg) was added into an aliquot of GCNT-Fe<sub>3</sub>O<sub>4</sub> suspension (1.0 mg ml<sup>-1</sup>) pre-activated by EDC/NHS chemistry to be incubated overnight at 4 °C. Then, the mixture was magnetically separated and washed twice. Subsequently, GOD-loaded GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites were dispersed in PBS containing 0.5 % Triton X-100 to be stored at 4 °C.

### 2.4. Colorimetric catalysis experiments

Colorimetric tests were performed to comparably examine the peroxidase-like catalysis activities among different suspensions (0.01 mg in 1.0 ml PBS, pH 4.5) of CNTs, GCNTs, Fe<sub>3</sub>O<sub>4</sub> Nanos, CNT-Fe<sub>3</sub>O<sub>4</sub>, and GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocatalysts, which were separately added into 0.15 ml TMB-H<sub>2</sub>O<sub>2</sub> substrates. Moreover, the organic peroxidase substrates (2.0 mM) of TMB, DAB, OPD, PAP, and HQ were catalytically oxidized by mixing with 4.0 mM H<sub>2</sub>O<sub>2</sub> using the 0.01 mg ml<sup>-1</sup> GCNT-Fe<sub>3</sub>O<sub>4</sub> suspension to produce different colors of reaction products. In addition, the colorimetric detections for glucose and H<sub>2</sub>O<sub>2</sub> of different concentrations were conducted in acetate buffer (0.2 M, pH 4.5) containing 4.0 mM TMB using GOD-loaded GCNT-Fe<sub>3</sub>O<sub>4</sub> GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites (0.01 mg ml<sup>-1</sup>), respectively. Moreover, the dynamics parameters (i.e., K<sub>m</sub> and V<sub>max</sub>) of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites were measured accordingly to plot Michaelis-Menten curves with different concentrations of H<sub>2</sub>O<sub>2</sub> and TMB.

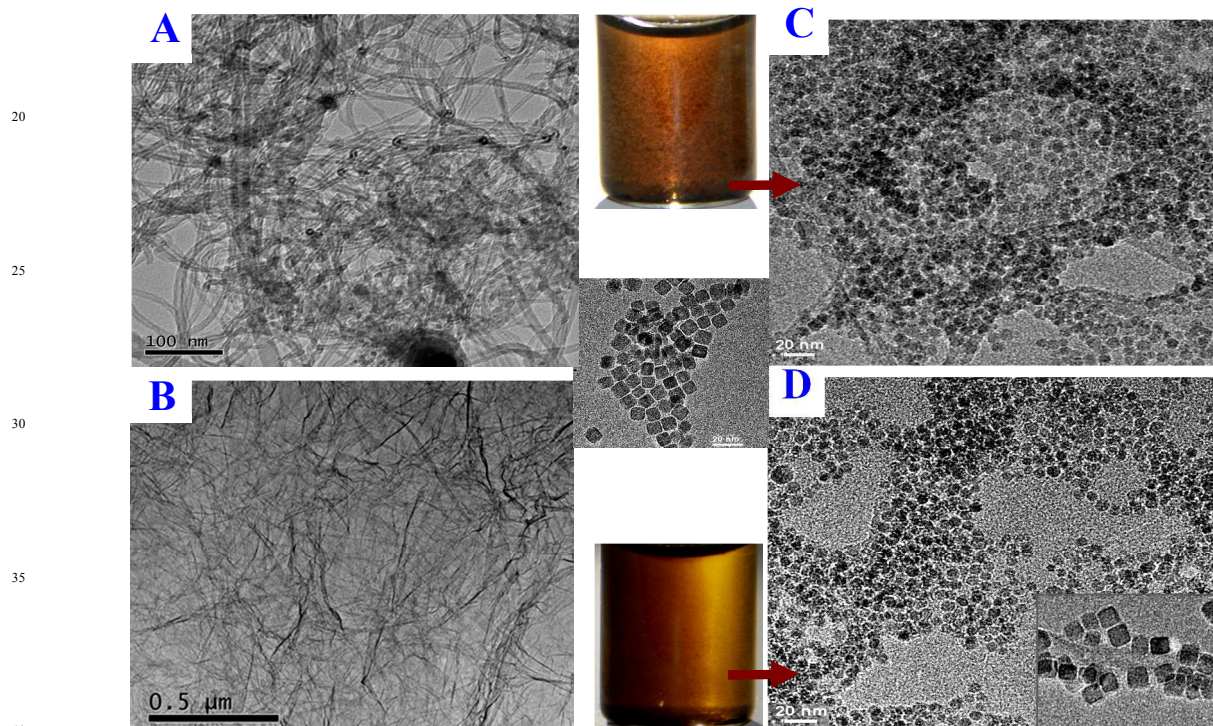
### 2.5. Electrochemical catalysis experiments

The electrocatalytic investigations of the GCNT-Fe<sub>3</sub>O<sub>4</sub> modified electrode for H<sub>2</sub>O<sub>2</sub> were performed using an electrochemical analyzer CHI 760 (CH Instruments, Austin, TX). A three-electrode configuration (CH Instruments) consisting of a glassy carbon working electrode, an Ag/AgCl reference electrode, and a platinum counter electrode was employed. Herein, 5 μl of the suspensions (0.010 mg in 1.0 ml 0.25 % Nafion) of CNTs, GCNTs, Fe<sub>3</sub>O<sub>4</sub> Nanos, and GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposite was separately dropped onto the electrodes to be incubated overnight

at 4°C. Following that, 5  $\mu\text{L}$  of 0.05 % Nafion was added onto the electrodes and left for 1 h at room temperature.

Furthermore, cyclic voltammetry (CV) measurements of the resulting electrodes were conducted in PBS solution containing the same  $\text{H}_2\text{O}_2$  concentration. Also, the direct electrochemical responses of the GCNT- $\text{Fe}_3\text{O}_4$ -modified electrode to successive additions of  $\text{H}_2\text{O}_2$  with a step of 1.0 mM were recorded at the same CV conditions. Moreover, glucose was electroanalyzed

with the GOD-GCNT- $\text{Fe}_3\text{O}_4$  modified electrode. Herein, an appropriate amount of the GOD-labeled GCNT- $\text{Fe}_3\text{O}_4$  nanocatalysts was first dispersed in PBS buffer with 0.25% Nafion. An aliquot of 5.0  $\mu\text{L}$  of the mixture was added onto the electrodes to be incubated overnight at 4°C and washed twice. The amperometric responses to glucose with different concentrations for the resulting enzyme electrodes were recorded at an applied potential of - 0.3 V.



**Fig. 1** Typical TEM images of (A) CNTs, (B) GO, (C) CNT- $\text{Fe}_3\text{O}_4$ , (D) GCNT- $\text{Fe}_3\text{O}_4$  nanocomposite (Insert: partially amplified view of a nanocomposite), and free cubic  $\text{Fe}_3\text{O}_4$  Nanos (middle), including the photographs of CNT- $\text{Fe}_3\text{O}_4$  and GCNT- $\text{Fe}_3\text{O}_4$  suspensions indicated.

### 3. Results and Discussion

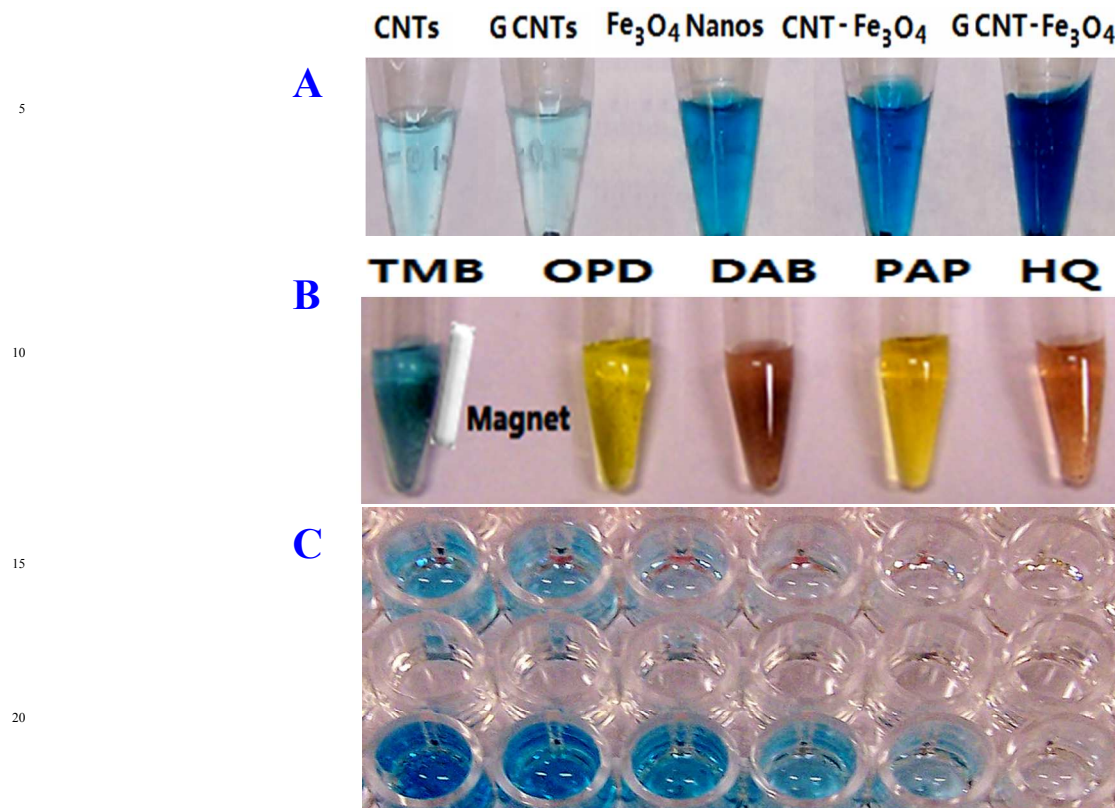
#### 3.1. Synthesis and characterization of GCNT- $\text{Fe}_3\text{O}_4$ nanocomposite

Cube-shaped  $\text{Fe}_3\text{O}_4$  Nanos were synthesized by the thermal decomposition of iron-oleate complex,<sup>37, 38</sup> and stabilized in TMAH solution to be derivatized with amine groups.<sup>38, 39</sup> The yielded  $\text{Fe}_3\text{O}_4$  Nanos were further separately coupled onto the carboxyl groups-functionalized CNTs and GO nanosheets-dispersed CNTs (GCNTs, mass ratio of GO to CNTs being 1:10) by the EDC/NHS cross-linking chemistry, resulting in GCNT- $\text{Fe}_3\text{O}_4$  nanocomposites. **Fig. 1** shows the comparison of topological structures among different nanomaterials by transmission electron microscopy (TEM) imaging. One can note that cubic  $\text{Fe}_3\text{O}_4$  Nanos ( $\sim 13$  nm in diameter) could be largely loaded onto CNTs and GCNTs to form the CNT- $\text{Fe}_3\text{O}_4$  (**Fig. 1C**) and GCNT- $\text{Fe}_3\text{O}_4$  (**Fig. 1D**), respectively. However, the latter could display much higher dispersion and suspension stability than the former, as clearly shown in their solution photographs. Moreover, the TEM images manifest that  $\text{Fe}_3\text{O}_4$  nanocatalysts

were well distributed and aligned with desirable density on the GCNT- $\text{Fe}_3\text{O}_4$  nanocomposites (**Fig. 1D, Insert**), in contrast, they were stacked and coagulated on the CNT- $\text{Fe}_3\text{O}_4$  nanocomplex. Obviously, the GCNT supports could enable better binding distribution and dispersion stability of  $\text{Fe}_3\text{O}_4$  Nanos on the nanocomposite, confirming the vital role of GO “surfactant”. The synergetic effects of carbon supports of high conductivity or electron-transferring and cubic  $\text{Fe}_3\text{O}_4$  Nanos of intrinsic catalysis activity were thereby expected to facilitate both enhanced peroxidase-like catalysis and electrocatalysis of GCNT- $\text{Fe}_3\text{O}_4$  nanocomposite for recyclable sensing applications afterwards.

#### 3.2. Colorimetric investigations of peroxidase-like catalysis activities of GCNT- $\text{Fe}_3\text{O}_4$ nanocomposite

We first employed the peroxidase substrate of TMB- $\text{H}_2\text{O}_2$  to investigate the peroxidase-like catalysis activities of the obtained GCNT- $\text{Fe}_3\text{O}_4$  nanocatalysts (**Fig. 2**). **Fig. 2A** shows the comparison of colorimetric TMB- $\text{H}_2\text{O}_2$  reaction results among CNTs, GCNTs,  $\text{Fe}_3\text{O}_4$  Nanos, CNTs- $\text{Fe}_3\text{O}_4$ , and GCNT- $\text{Fe}_3\text{O}_4$  nanocomposite so prepared. One can find that both of CNTs and



**Fig. 2** Colorimetric comparison investigations of catalysis activities of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocatalysts: (A) comparison of catalytic activities for TMB-H<sub>2</sub>O<sub>2</sub> reaction (5.0 mM TMB and 2.0 mM H<sub>2</sub>O<sub>2</sub>) among CNTs, GCNTs, Fe<sub>3</sub>O<sub>4</sub> Nanos, CNT-Fe<sub>3</sub>O<sub>4</sub>, and GCNT-Fe<sub>3</sub>O<sub>4</sub> materials normalized with the same iron amount; (B) GCNT-Fe<sub>3</sub>O<sub>4</sub>-catalyzed H<sub>2</sub>O<sub>2</sub> oxidization of various organic peroxidase substrates (2.0 mM) of TMB, OPD, DAB, PAP and HQ; and (C) colorimetric detection of glucose (up) and H<sub>2</sub>O<sub>2</sub> (down) ranging from 0.050 to 1.0 mM (from right to left) using GOD-loaded and pure GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocatalysts (0.010 mg/mL), respectively.

GCNTs could show a little bit of catalysis behaviour, which might result from the existence of catalysis-active iron segments of CNTs,<sup>18, 23, 40</sup> and/or the intrinsic peroxidase-like catalytic activity of GO nanosheets.<sup>16</sup> But Fe<sub>3</sub>O<sub>4</sub> Nanos showed stronger catalysis than the commercially-available CNTs or GCNTs in terms of colour densities of TMB-H<sub>2</sub>O<sub>2</sub> reaction products. More importantly, when Fe<sub>3</sub>O<sub>4</sub> Nanos were loaded on GCNTs, the resulting GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites could show much higher catalysis activity than Fe<sub>3</sub>O<sub>4</sub> Nanos and carbon supports. Also, they could exhibit much stronger peroxidase-like catalysis than the CNT-Fe<sub>3</sub>O<sub>4</sub>, demonstrating the apparent difference in catalyst supports. Again, the use of GO “surfactant” could significantly improve the aqueous dispersion and stability of the supports to allow for better distribution and more effective loading of cubic Fe<sub>3</sub>O<sub>4</sub> nanocatalysts, which may in turn facilitate greatly enhanced peroxidase-like catalysis activities of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites by faster conversion of Fe<sup>2+</sup>/Fe<sup>3+</sup>.<sup>13</sup>

Furthermore, the kinetic characterization of peroxidase-like activities of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites was carried out by plotting the typical Michaelis-Menten curves (**Fig. S1, ESI**). According to the Lineweaver-Burk equation, the Michaelis constant ( $K_m$ ) and the maximal reaction velocity ( $V_{max}$ ) were obtained and shown in **Table S1, ESI**, comparing to those of natural enzyme of horseradish peroxidase (HRP) reported elsewhere.<sup>13</sup> It is found that the  $K_m$  value of the GCNT-Fe<sub>3</sub>O<sub>4</sub> with H<sub>2</sub>O<sub>2</sub> substrate (2.52 mM) is a little lower than that of HRP

(3.7 mM), showing that they could have compatible affinity to H<sub>2</sub>O<sub>2</sub>. But the GCNT-Fe<sub>3</sub>O<sub>4</sub> presented much lower  $K_m$  with TMB substrate (0.118 mM) than HRP (0.434 mM), indicating higher affinity to TMB was achieved for the GCNT-Fe<sub>3</sub>O<sub>4</sub>, presumably resulting from its carbon carriers with strong adsorption to TMB.

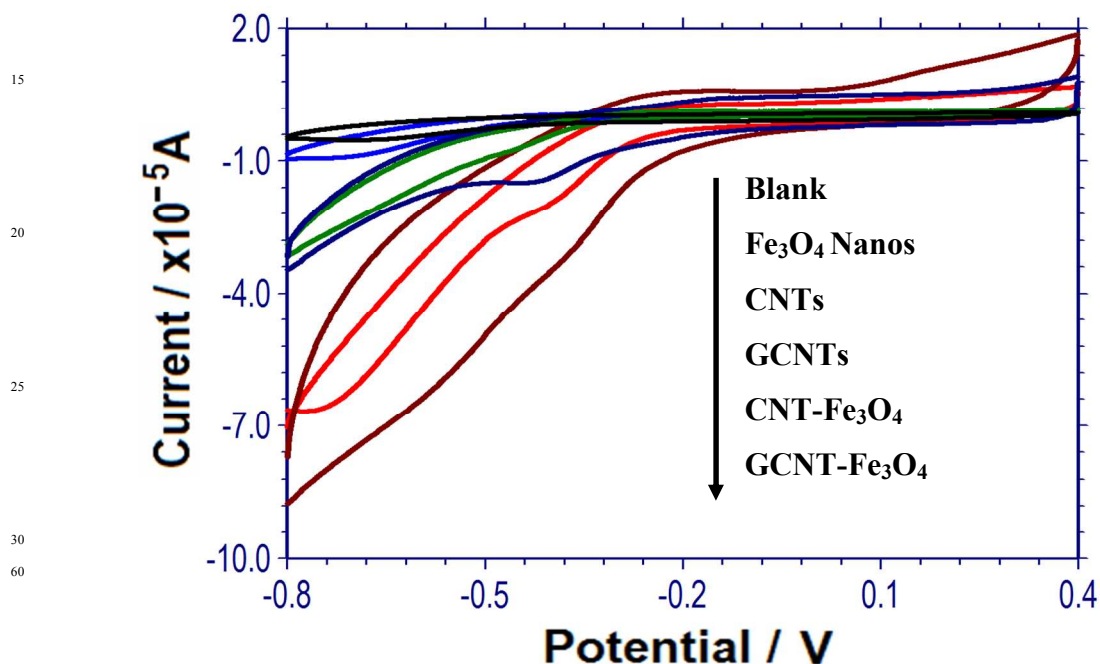
### 3.3. Colorimetric sensing applications of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposite

The peroxidase-like catalysis activities of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocatalysts was first examined by the H<sub>2</sub>O<sub>2</sub>-induced catalytic oxidation of some organic peroxidase substrates commonly used, including TMB, OPD, DAB, PAP, and HQ. **Fig. 2B** shows the photographs of the colorimetric reaction products. It was discovered that these originally-colourless organic substrate solutions could be rapidly turned into various colours, i.e., the DAB solution presented a brown colour. Obviously, the GCNT-Fe<sub>3</sub>O<sub>4</sub> enzyme mimic may display strong catalysis degradation of typical organic peroxidase substrates. Note that when a magnetic stirring bar was applied to the side of the tube after color reactions, magnetic nanocatalysts could be effectively collected from the substrate media, as exemplified in TMB tube in **Fig. 2B**. Therefore, the strong magnetism of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocatalysts could additionally enable the inorganic nanocatalysts to be recovered or recycled after usage, indicating the possibility of extensive applications in the field of environmental chemistry.

For example, they may be tailored for the magnet-aided separation and recyclable catalysis degradation or removal of organic toxicants such as phenols<sup>18</sup> in wastewater treatments.

Furthermore, TMB-H<sub>2</sub>O<sub>2</sub> substrate reactions catalyzed by the developed GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposite were found to be pH-dependent, which was colorimetrically tested by changing the pH values from pH 0.50 to pH 14 (data not shown). The optimal pH value of the reaction was found to be ~ pH 4.5, as determined by the fastest reaction of blue substrates. Additionally, when the GCNT-Fe<sub>3</sub>O<sub>4</sub> catalysts were placed at 90 °C for 1 h or stored at room temperature for six months, no significant change was observed in their catalysis performances for TMB-H<sub>2</sub>O<sub>2</sub> reactions

(data not shown). The results indicate that the inorganic mimic enzyme can display high thermal and long-term-storage stabilities. Subsequently, the feasibility of catalysis applications for the colorimetric H<sub>2</sub>O<sub>2</sub> and glucose detections was probed using GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocatalysts and GOD-GCNT-Fe<sub>3</sub>O<sub>4</sub> ones, respectively (Fig. 2C). A proportional change of colour densities of TMB chromogens were obtained for H<sub>2</sub>O<sub>2</sub> and glucose both with the linear concentrations ranging from 0.050 to 1.0 mM by separately plotting the absorbance values vs H<sub>2</sub>O<sub>2</sub> and glucose concentrations (data not shown), indicating the potential of the sensitive colorimetric detections of H<sub>2</sub>O<sub>2</sub> and glucose.



**Fig. 3** Comparison of direct voltammetric responses to H<sub>2</sub>O<sub>2</sub> (3.0 mM) among the electrodes separately modified with GCNTs, CNTs, Fe<sub>3</sub>O<sub>4</sub> Nanos, CNT-Fe<sub>3</sub>O<sub>4</sub>, and GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites, scanning from -0.80 to 0.40 V at a scan rate of 0.10 V s<sup>-1</sup>.

### 3.4. Electrochemical investigations of electrocatalysis activities of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposite

The GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocatalysts were coated onto the electrodes to conduct the direct electrocatalysis to H<sub>2</sub>O<sub>2</sub> reduction, in comparison with the electrodes modified separately with CNTs, GCNTs, Fe<sub>3</sub>O<sub>4</sub> Nanos, and CNT-Fe<sub>3</sub>O<sub>4</sub> nanocomplex. Fig. 3 illustrates the typical CVs of these electrodes, in which significantly different changes in H<sub>2</sub>O<sub>2</sub> response currents were observed upon adding 3.0 mM H<sub>2</sub>O<sub>2</sub>. Interestingly, the current response of the CNTs-modified electrode was higher than that of the Fe<sub>3</sub>O<sub>4</sub> Nanos-modified electrode, but was lower than that of the electrodes modified with GCNTs or CNT-Fe<sub>3</sub>O<sub>4</sub>. Compared with CNTs, semiconductive Fe<sub>3</sub>O<sub>4</sub> Nanos might be packed more tightly on the electrode leading to lower electron transportation, although they had high peroxidase-like catalysis activity in the colorimetric experiments above. As for the GCNTs-modified electrode, a relatively high electrocatalysis to H<sub>2</sub>O<sub>2</sub> was observed presumably resulting from the intrinsic electrocatalysis activities of CNTs and/or GO, as evidenced elsewhere.<sup>16, 22, 33, 41-43</sup> From

Fig. 3, one can find that the H<sub>2</sub>O<sub>2</sub> reduction response of the GCNT-Fe<sub>3</sub>O<sub>4</sub>-modified electrode was ~2.5 times higher than that of the electrode with Fe<sub>3</sub>O<sub>4</sub> Nanos or GCNTs alone. Also, it could exhibit much larger responses to H<sub>2</sub>O<sub>2</sub> than the CNT-Fe<sub>3</sub>O<sub>4</sub> modified one. Accordingly, stronger electrocatalysis to H<sub>2</sub>O<sub>2</sub> was verified for the GCNT-Fe<sub>3</sub>O<sub>4</sub> electrode including higher electron-transferring capacities, presumably benefiting from the high electrocatalysis of carbon supports,<sup>16, 18, 22, 23, 33</sup> and the intrinsic peroxidase-like catalysis of Fe<sub>3</sub>O<sub>4</sub> Nanos aforementioned.

### 3.5. Electrochemical sensing applications of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites

Fig. 4 manifests the direct voltammetric responses of the GCNT-Fe<sub>3</sub>O<sub>4</sub> modified electrode to successive additions of H<sub>2</sub>O<sub>2</sub>. It is observed that the CV responses could increase proportionally with increasing H<sub>2</sub>O<sub>2</sub> levels linearly ranging from 0.010 to 0.50 mM. Of note, the interferences of other electroactive substances such as ascorbic acid and uric acid can be largely excluded if H<sub>2</sub>O<sub>2</sub> was detected at such a low over-potential.<sup>22, 33, 44</sup>

Moreover, the electrochemical detections of glucose by the

electrode modified with GCNT-Fe<sub>3</sub>O<sub>4</sub>-loaded GOD were conducted (Fig. 5). Herein, GOD was covalently loaded onto the GCNT-Fe<sub>3</sub>O<sub>4</sub> modified electrodes forming the enzyme sensor for glucose analysis through amperometric detection of liberated H<sub>2</sub>O<sub>2</sub>. A typical current-time response curve was obtained for the enzyme sensor to successive additions of glucose at applied potential of -0.3 V (Fig. 5A). One can note that the current responses could increase with increasing glucose concentrations,

in which the steady-state signals were reached in less than ~10 s. Fig. 5B describes the calibration curve of the GOD sensor for glucose samples with different concentrations. The linear concentrations of glucose were achieved ranging from 0.050 to 5.0 mM, with the detection limit of 0.022 mM. Accordingly, the developed electrode could exhibit much better performances of sensing to glucose than the electrodes modified with CNTs-loaded GOD documented previously.<sup>22</sup>

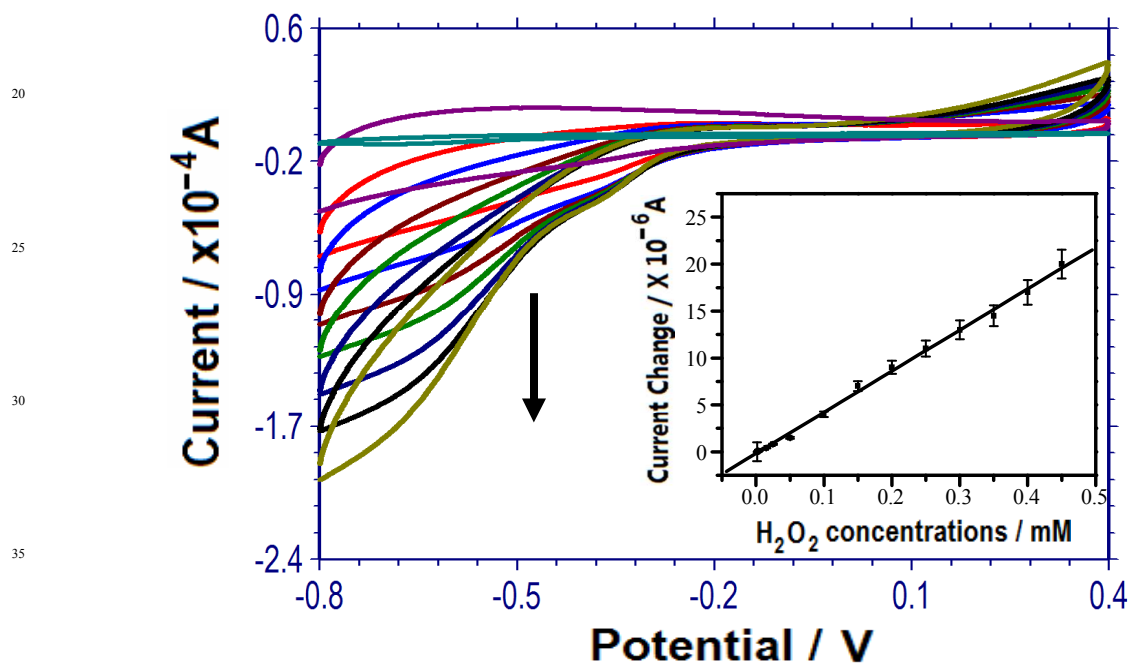


Fig. 4 Direct voltammetric responses of the GCNT-Fe<sub>3</sub>O<sub>4</sub> modified electrode to successive addition of H<sub>2</sub>O<sub>2</sub> with a step of 1.0 mM. Insert: the calibration curve of amperometric responses to H<sub>2</sub>O<sub>2</sub> with different concentrations linearly ranging from 0.01 to 0.50 mM.

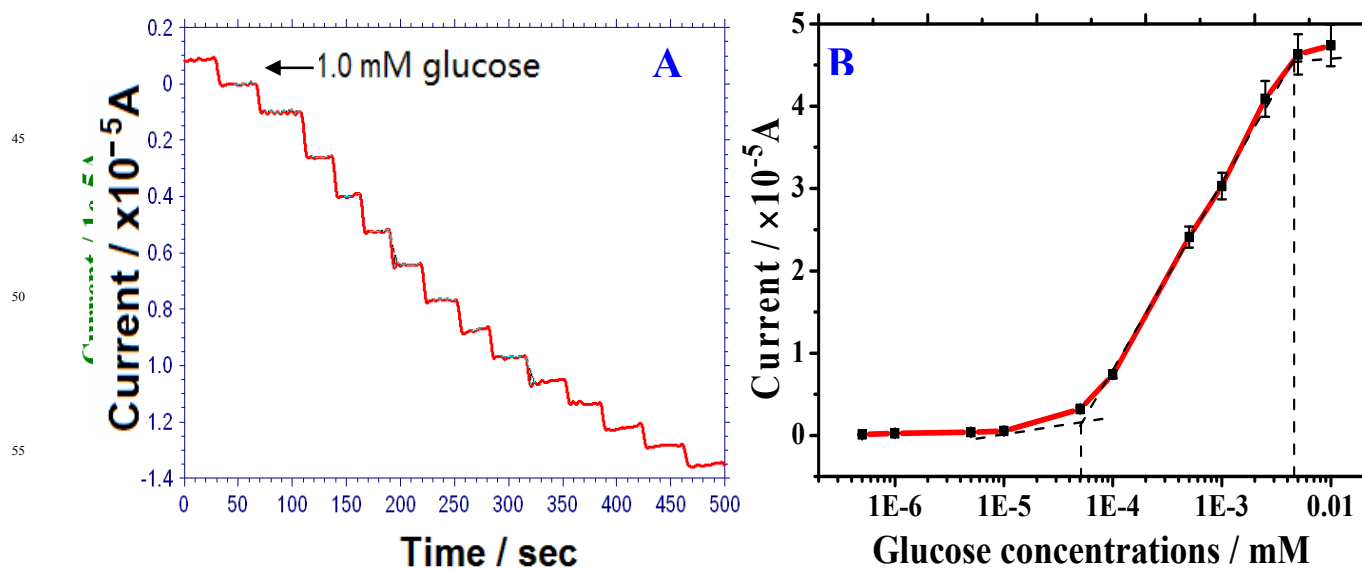


Fig. 5 (A) Typical current-time responses of the GCNT-Fe<sub>3</sub>O<sub>4</sub> modified electrode combined with GOD to successive addition of glucose with a step of 1.0 mM, measured at an applied potential of -0.3 V; (B) the steady-state calibration curve of amperometric responses to glucose with different concentrations ranging from 0.050 to 5.0 mM.



#### 4. Conclusions

In this study, cubic Fe<sub>3</sub>O<sub>4</sub> Nanos were successfully synthesized and covalently bound onto the GO nanosheets-dispersed CNTs supports leading to the GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites with enhanced peroxidase-like catalysis and electrocatalysis activities. On the one hand, the introduction of GO nanosheets could significantly improve the aqueous dispersion of hydrophobic CNT supports to allow for more effective and stable loadings of Fe<sub>3</sub>O<sub>4</sub> nanocatalysts. On the other hand, the synergetic effects of two carbon supports of high conductivity / electron-transferring and cubic Fe<sub>3</sub>O<sub>4</sub> Nanos of intrinsic catalysis activity could facilitate greatly improved peroxidase-like catalysis and electrocatalysis activities. Comparing to natural enzymes or even common iron-based nanocatalysts like CNT-Fe<sub>3</sub>O<sub>4</sub> nanocomplex, the resulting GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites could possess some advantages of: (1) strong peroxidase-like catalysis to catalyze the H<sub>2</sub>O<sub>2</sub>-induced oxidization of typical peroxidase substrates to enable the sensitive H<sub>2</sub>O<sub>2</sub>-based detections; (2) high electron-transferring and electrocatalysis capabilities to allow for direct electrochemistry to H<sub>2</sub>O<sub>2</sub> at a low over-potential; (3) unique magnet-aided separation and organic catalysis degradation abilities to facilitate recyclable catalytic detoxification or removal of organic pollutants (i.e., phenols) in the environment and wastewater treatments; and (4) high environmental stability against high temperature and long-term storage. The outstanding catalysis performances of the GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocatalysts have been subsequently demonstrated by colorimetric and direct electrochemical sensing of H<sub>2</sub>O<sub>2</sub> and glucose with high detection sensitivity. This recyclable mimic enzyme holds great promise of intensive applications in recyclable biological catalysis, magnetic separation, toxicant removal or degradation, and enzyme-based sensing analysis fields. Significantly, such a synthesis protocol of “artificial enzyme” may open a new door towards multifunctional catalyst design by dispersing magnetic nanocatalysts onto two high-surface-area carbon supports.

#### Acknowledgments

This work is supported by the National Institutes of Health Counter ACT Program through the National Institute of Neurological Disorders and Stroke of USA (award # NS058161-01), the National Natural Science Foundation of China (No. 21375075), and the Taishan Scholar Foundation of Shandong Province, P. R. China.

#### Notes and References

<sup>a</sup> Shandong Province Key Laboratory of Life-Organic Analysis, School of Chemistry and Chemical Engineering, Qufu Normal University, Qufu City 273165, P. R. China.

<sup>b</sup> \* E-mail: [huawangqfnu@126.com](mailto:huawangqfnu@126.com);

<sup>b</sup> Pacific Northwest National Laboratory, Richland, WA 99352, USA. Tel: +01 509 371 6241; Fax: +01 509 371 6242.

\* E-mail: [yuehe.lin@wsu.edu](mailto:yuehe.lin@wsu.edu)

† Electronic Supplementary Information (ESI) available: The kinetic characterization of peroxidase-like activities of GCNT-Fe<sub>3</sub>O<sub>4</sub> nanocomposites comparing to HRP. See DOI: 10.1039/b000000x/

1. E. Torres, I. Bustos-Jaimes and S. Le Borgne, *Appl. Catal., B- Environ.* 2003, 46, 1-15.
2. A. Mueller, *Mini-Rev. Med. Chem.* 2005, 5, 231-239.
3. N. Q. Ran, L. S. Zhao, Z. M. Chen and J. H. Tao, *Green Chem.* 2008, 10, 361-372.
4. A. E. G. Cass, G. Davis, G. D. Francis, H. A. O. Hill, W. J. Aston, I. J. Higgins, E. V. Plotkin, L. D. L. Scott and A. P. F. Turner, *Anal. Chem.* 1984, 56, 667-671.
5. G. Arai, K. Shoji and I. Yasumori, *J. Electroanal. Chem.* 2006, 591, 1-6.
6. J. Wang, *Analyst.* 2005, 130, 421-426.
7. J. Wang, *Chem. Rev.* 2008, 108, 814-825.
8. V. Nanda and R. L. Koder, *Nat. Chem.* 2010, 2, 15-24.
9. A. J. Kirby, *Angew. Chem-Int. Edit.* 1996, 35, 707-724.
10. R. Breslow, X. J. Zhang and Y. Huang, *J. Am. Chem. Soc.* 1997, 119, 4535-4536.
11. L. Fruk and C. M. Niemeyer, *Angew. Chem-Int. Edit.* 2005, 44, 2603-2606.
12. A. Asati, S. Santra, C. Kaittanis, S. Nath and J. M. Perez, *Angew. Chem-Int. Edit.* 2009, 48, 2308-2312.
13. L. Z. Gao, J. Zhuang, L. Nie, J. B. Zhang, Y. Zhang, N. Gu, T. H. Wang, J. Feng, D. L. Yang, S. Perrett and X. Yan, *Nature Nanotech.* 2007, 2, 577-583.
14. W. C. Ellis, C. T. Tran, M. A. Denardo, A. Fischer, A. D. Ryabov and T. J. Collins, *J. Am. Chem. Soc.* 2009, 131, 18052-18053.
15. B. Meunier, In *Biomimetic Oxidations Catalyzed by Transition Metal Complexes*, Imperial College Press, Covent Garden, 2000, pp. 171-214.
16. Y. J. Song, K. G. Qu, C. Zhao, J. S. Ren and X. G. Qu, *Adv. Matter.* 2010, 22, 1-5.
17. H. Wei and E. Wang, *Anal. Chem.* 2008, 80, 2250-2254.
18. X. L. Zuo, C. Peng, Q. Huang, S. P. Song, L. H. Wang, D. Li and C. H. Fan, *Nano Res.* 2009, 2, 617-623.
19. A. T. Bell, *Science.* 2003, 299, 1688-1691.
20. J. Wang and Y. H. Lin, *TrAC, Trends Anal. Chem.* 2008, 27, 619-626.
21. R. H. Baughman, A. A. Zakhidov and W. A. de Heer, *Science.* 2002, 297, 787-792.
22. J. Wang, M. Musameh and Y. H. Lin, *J. Am. Chem. Soc.* 2003, 125, 2408-2409.
23. B. Sljukic, C. E. Banks and R. G. Compton, *Nano Lett.* 2006, 6, 1556-1558.
24. H. Yan, H. J. Zhou, P. Yu, L. Su and L. Q. Mao, *Adv. Mater.* 2008, 20, 2899-2906.
25. R. Laocharoensuk, J. Burdick and J. Wang, *ACS Nano.* 2008, 2, 1069-1075.
26. S. W. Qu, J.; Kong, J. L.; Yang, P. Y.; Chen, G., *Talanta.* 2007, 71, 1096-1102.
27. L. Zhang, Q. Q. Ni, T. Natsuki and Y. Q. Fu, *Appl. Sur. Sci.* 2009, 255, 8676-8681.
28. M. S. Saha and A. Kundu, *J. Power Sources.* 2010, 195, 6255 -6261.

29. X. L. Li, X. R. Wang, L. Zhang, S. Lee and H. J. Dai, *Science*. 2008, 319, 1229-1232. 65
30. L. J. Cote, J. Kim, V. C. Tung, J. Luo, F. Kim and J. Huang, *Pure Appl. Chem.* 2011, 83, 95-110. 65
31. J. Ma, L. Zhou, C. Li, J. H. Yang, T. Meng, H. M. Zhou, M. X. Yang, F. Yu and J. H. Chen, *J. Power Sources*. 2014, 247, 999-1004. 70
32. L. L. Tian, M. J. Mezzani, F. S. Lu, C. Kong, Y. L. Cao, T. J. Thorne and Y. P. Sun, *ACS Appl. Mater. Inter.* 2010, 2, 3217-3222. 70
33. H. Teymourian, S. A. and S. Khezrian, *Biosens and Bioelectron.* 2013, 49, 1-8. 75
34. T. S. Ahmadi, Z. L. Wang, T. C. Green, A. Henglein and M. A. El-Sayed, *Science*. 1996, 272, 1924-1926. 75
35. F. F. Peng, Y. Zhang and N. Gu, *Chin. Chem. Lett.* 2008, 19, 730-733.
36. M. Ma, Y. Zhang and N. Gu, *Colloids Surf. A: Physicochem. Eng. Aspects*, 2011, 373, 6-10. 80
37. M. V. Kovalenko, M. I. Bodnarchuk, R. T. Lechner, G. Hesser, F. Schaffler and W. Heiss, *J. Am. Chem. Soc.* 2007, 129, 6352-6353.
38. J. Jiang, H. W. Gu, H. L. Shao, E. Devlin, G. C. Papaefthymiou and J. Y. Ying, *Adv. Mater.* 2008, 20, 4403-4407. 85
39. L. E. Euliss, S. G. Grancharov, S. O'Brien, T. J. Deming, G. D. Stucky, C. B. Murray and G. A. Held, *Nano Lett.* 2003, 3, 1489-1493.
40. J. Kruusma, N. Mould, K. Jurkschat, A. Crossley and C. E. Banks, *Electrochem. Commun.* 2007, 9, 2330-2333. 90
41. C. E. Banks, T. J. Davies, G. G. Wildgoose and R. G. Compton, *Chem. Commun.* 2005, 7, 829-841. 25
42. K. P. Gong, S. Chakrabarti and L. M. Dai, *Angew. Chem. Int. Edit.* 2008, 47, 5446-5450. 95
43. I. Dumitrescu, P. R. Unwin and J. V. Macpherson, *Chem. Commun.* 2009, 6886-6901.
44. K. Yamamoto, G. Shi, T. S. Zhou, F. Xu, J. M. Xu, T. Kato, J. Y. Jin and L. Jin, *Analyst*. 2003, 128, 249-254. 100

35

105

40

110

45

115

50

120

55

125

60

130