# Bifunctional Graphene/ $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> Hybrid Aerogels with Double Nanocrystalline Networks for Enzyme Immobilization

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**H**ighly porous hosting materials with conducting (favorable to electron transfer) and magnetic (favorable to product separation) bicontinuous networks should possess great potentials for immobilization of various enzymes in the field of biocatalytic engineering, but the synthesis of such materials is still a great challenge. Herein, bifunctional graphene/ $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels with quite low density (30–65 mg cm<sup>-3</sup>), large specific surface area (270–414 m<sup>2</sup> g<sup>-1</sup>), high electrical conductivity (0.5–5 × 10<sup>-2</sup> S m<sup>-1</sup>), and superior saturation magnetization (23–54 emu g<sup>-1</sup>) are fabricated. Single networks of either graphene aerogels or  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> aerogels are obtained by etching of the hybrid aerogels with acid solution or calcining of the hybrid aerogels in air, indicative of the double networks of the as-synthesized graphene/ $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels for the first time. The resulting bifunctional aerogels are used to immobilize  $\beta$ -glucuronidase for biocatalytic transformation of glycyrrhizin into glycyrrhetinic acid monoglucuronide or glycyrrhetinic acid, with high biocatalytic activity and definite repeatability.

### 1. Introduction

Aerogels are a kind of highly porous three-dimensional architectures with charming properties such as quite low density, large open pore, high specific surface area, etc., and thus have wide applications ranging from lab-scale catalysis

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to industrial-scale thermal-insulation.<sup>[1-3]</sup> They are generally prepared from molecular precursors by sol-gel processing and then by supercritical fluid drving or freeze drving to replace the solvents in the wet gels with air.<sup>[4]</sup> In most cases they are amorphous and metastable from the point of view of their structural and thermodynamic properties.<sup>[5]</sup> So far it is a great challenge for the sol-gel derived aerogels that the parameters of their pores (e.g. size, geometry, order, etc.) and solid walls (e.g. composition, size, geometry, crystallinity, etc.) of the aerogels can be respectively regulated at will by tuning synthetic conditions during sol-gel processing, and thus the structures and functionalities of the resulting aerogels can be adjusted. Interpenetrating organic/inorganic networks of resorcinol-formaldehyde (RF)/metal oxide aerogels,<sup>[6]</sup> obtained in one pot through the catalytic effect of gelling solutions of hydrated metal ions on the gelation of RF have recently been demonstrated to provide such an alternative.

Graphene, the first prepared 2D nanocrystal,<sup>[7,8]</sup> has stimulated ever-increasing attention in recent years owing to its exceptional physical and chemical properties.<sup>[9–13]</sup> It can be envisioned that graphene aerogels have great potentials

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in many fields due to combination of their respective characteristics of the graphene sheets and aerogels. Worsley et al.<sup>[14]</sup> have initially synthesized graphene aerogels with high electrical conductivity by sol-gel polymerization of RF in an aqueous suspension of graphene oxide (GO), and followed by pyrolysis of the organic cross-links and thermal reduction of the GO to graphene occurred simultaneously during pyrolysis after supercritical CO<sub>2</sub> drying of GO-RF gels. Latter on, mechanically strong and electrically conductive graphene aerogels<sup>[15]</sup> have been synthesized in our lab by supercritical drying of hydrogel precursors synthesized from reduction of GO with L-ascorbic acid. In situ incorporating of any other components with a controlled way is a simple but creative strategy to functionalize the as-synthesized graphene aerogels. For example, we have synthesized carbon nanotube/ graphene hybrid aerogels<sup>[16]</sup> in our lab by supercritical CO<sub>2</sub> drying of their hydrogel precursors obtained from heating the aqueous mixtures of graphene oxide and carbon nanotubes with vitamin C without stirring. Yu et al.<sup>[17]</sup> and Yan et al.<sup>[18]</sup> have independently incorporated magnetic Fe<sub>3</sub>O<sub>4</sub> nanoparticles into graphene hydrogel precursors and then freeze-dried the precursors for obtaining the corresponding graphene/ nanoparticle hybrid aerogels. In comparison with supercritical drying, Freeze drying can generally keep the components of the precursors from running off as no extraction with supercritical fluid has happened during drving of the aerogel precursors, however *in situ* growth of the ice crystals can push a lot of mesopores cracked to form macropores along the freeze direction, which leads to low specific surface area (usually less than  $100 \text{ m}^2 \text{ g}^{-1}$ ) of the resulting aerogels.

Fast development of biotechnology requires immobilization of active biospecies, e.g., enzymes or cells on porous matrix including mesoporous activated carbon, glass beads, polymers, mesoporous silica, organic-inorganic hybrids, etc.<sup>[19-22]</sup> The ability to form materials under aqueous, roomtemperature conditions (at which biospecies are active) opens up the possibility to encapsulation of biologicals during sol-gel processing, and the main idea of the encapsulation technique has been easily adopted to aerogels. For example, cytochrome c has been stabilized in an aerogel matrix with over 80% of the protein remaining active in the final aerogel composite.<sup>[23]</sup> Lipase immobilized in silica aerogel has shown very high catalytic activity, in most cases much higher than that of the free lipase as it has a lower tendency to aggregate than that of free lipase when being dispersed in solid aerogel matrix.<sup>[24]</sup> However in most cases the gentle conditions associated with sol-gel chemistry are still too harsh for many biospecies and the entrapped biospecies usually retain only part of their native activity in fresh gels, which drive researchers to develop strategies to immobilize the biospecies within the aerogels after supercritical drying.

In this manuscript we present a rational approach to synthesize crystalline graphene/ $\gamma$ Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel monoliths with conducting and magnetic bifunctionality by using epichlorohydrin as a proton scavenger to initiate hydrolysis and polycondensation of ferric chloride in the presence of GO, and followed by supercritical drying with CO<sub>2</sub> and carbonizing in argon in sequence. The resulting graphene/ Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel monoliths show quite low density

 $(30-65 \text{ mg cm}^{-3})$ , large specific surface area  $(270-414 \text{ m}^2 \text{ g}^{-1})$ , high electrical conductivity  $(0.5-5 \times 10^{-2} \text{ S m}^{-1})$  and superior saturation magnetization (23–54 emu  $g^{-1}$ ). To our surprise, if anyone of two components of the hybrid aerogels have been completely removed by an appropriate treatment, the left one is still in original monolithic shape, indicating double networks existed within the resulting hybrid aerogels. The as-prepared graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels have been used in immobilization of  $\beta$ -glucuronidase for biocatalytic transformation of glycyrrhizin into glycyrrhetinic acid monoglucuronide or glycyrrhetinic acid. Results show that the target enzyme, immobilized to the hybrid aerogels through physical adsorption with the maximum loading amount up to 2.45 mg mg<sup>-1</sup>, has exhibited high biocatalytic activity and definite repeatability. The work presented here, to the best of our knowledge, is the first report on graphene-based aerogels with double networks. More importantly, this strategy may give inspiration to synthesize a series of graphene-based aerogels hybridized with other metal oxides and the resulting bifunctional aerogels may immobilizing various enzymes for biological catalysis, biofuel cell, etc.

#### 2. Results and Discussion

## 2.1. Synthesis and Characterization of the Bifunctional Aerogels

Synthesis of the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels was illustrated in Figure 1. A fix amount of FeCl<sub>3</sub>•6H<sub>2</sub>O was added to GO N,N-dimethylformamide (DMF) suspension to form the uniform mixture, then epichlorohydrin was added to above mixture with stirring for a short while and then standing still over a night to form the gel. Need to point our that, during the gel formation, if no GO existed in the mixture, the resulting gel would be broken at the following solvent-exchange or carbonization step, indicating that the existence of GO can greatly enhance the mechanical property of the resulting gel; and if no ferric chloride existed in the mixture, no gelation would be observed, indicating that epichlorohydrin can not stimulate the gelation of the GO. The resulting graphene/iron oxide aerogel would be obtained as long as the above hybrid gel was processed with solvent-exchange, supercritical  $CO_2$ drying and carbonization under Ar atmosphere in sequence.

X-ray powder diffraction (XRD) has been used to determine the crystalline structure of the resulting graphene/ Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels as shown in **Figure 2**a. There are six peaks at 30.1, 35.5, 43.1, 53.4, 57.0 and 62.7° observed in the hybrid aerogels, consistent with (220), (311), (400), (422), (511) and (440) planes of the standard XRD data for the cubic spinal crystal of the bulk maghemite (namely  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>, see JCPDS NO. 04-0755). Comparing the XRD patterns of the hybrid aerogels with those of the graphene or Fe<sub>2</sub>O<sub>3</sub> powder with the same processing history, we can find that: (1) no conventional peaks of graphene sheets have been detected, suggesting that the graphene sheets are homogeneously dispersed in the Fe<sub>2</sub>O<sub>3</sub> matrix and overlapping of the graphene sheets have not happened<sup>[25]</sup> and (2) the XRD patterns of the hybrid aerogels are the same as that of the Fe<sub>2</sub>O<sub>3</sub>



Figure 1. Illustration of the synthetic route for single-network of the graphene and  $Fe_2O_3$  aerogels and double-network of the hybrid graphene/ $Fe_2O_3$  aerogels.

powder, indicating that the existence of graphene sheets can not influence the crystallization of the iron oxide. Raman spectroscopy has been used to determine the interaction between components of the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels as shown in Figure 2b. Two prominent peaks at 1348 cm<sup>-1</sup> and 1587 cm<sup>-1</sup> can be observed from graphene powder, corresponding to D and G band, respectively. It is well known that D band is ascribed to the edges, other defects and disordered carbon in the graphene sheets, and G band arises from the zone centre  $E_{2g}$  mode, corresponding to ordered sp<sup>2</sup>-bonded carbon atoms in the graphene sheets.<sup>[26]</sup> Raman spectra of graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels show similar D and G bands to that of the graphene powder, indicating that the structure of graphene is maintained after the carbonization procedure.<sup>[27]</sup> The intensity ratios of D to G band  $(I_D/I_G)$  of the hybrid aerogels increase from 0.84 to 0.98 as the mass ratios of graphene to Fe<sub>2</sub>O<sub>3</sub> decrease from 1:1 to 1:5, suggesting a decrease in the size of in-plane sp<sup>2</sup> domains of the graphene sheets as the metal oxide fraction increases, similar to the results reported elsewhere.<sup>[28,29]</sup> Furthermore, the G band of the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels show a visible blue-shift in comparison with that of the graphene, indicating a strong interaction between graphene sheets and iron oxide nanoparticles, consistent with the observations related to metal oxide/ graphene composites in the literature.<sup>[30]</sup> For the iron oxide powder with the same processing history, there are five bands around 348, 500, 700, 1360 and 1580 cm<sup>-1</sup> as shown in Figure 2b and Figure SI1. This is the characteristic of Fe<sub>2</sub>O<sub>3</sub> rather than Fe<sub>3</sub>O<sub>4</sub>. However these characteristic bands of Fe<sub>2</sub>O<sub>3</sub> can't be observed from the hybrid aerogels, which is probably due to either special interaction between graphene sheets and Fe<sub>2</sub>O<sub>3</sub> nanoparticles or strong fluorescence background small

of transition metal elements under laser excitation.<sup>[31]</sup> X-ray photoelectron spectroscopy (XPS) has been further used to determine the phase structure of the iron oxide in the hybrid aerogels. In comparison with GO, an additional Fe peak can be observed from the graphene/iron oxide hybrid aerogel as shown in Figure 2c, suggesting that iron oxide has been embedded within the graphene sheets. Figure 2d presents the Fe2p XPS pattern of the graphene/iron oxide hybrid aerogel, from which it can be seen that  $Fe2p_{3/2}$  and Fe2p<sub>1/2</sub> are 710.7 eV and 724.8 eV, respectively, with a satellite peak at 719.0 eV (indicated with an arrow), characteristic of Fe<sub>2</sub>O<sub>3</sub>.<sup>[32]</sup> Moreover, the changes in chemical composition of the GO in hybrid aerogel before and after carbonization can also be acquired from XPS analysis. For the GO powder, four types of carbon bonds: C-C/C=C (284.5 eV), epoxy and hydroxyl (286.5 eV), carbonyl (287.8 eV), carboxyl (289.0 eV) can be observed<sup>[15]</sup> as shown in Figure 2e. After carbonization, the intensities of the peaks at 286.5, 287.8 and 288.7 eV are much lower, especially

for the peak centered at 286.5 eV as shown in Figure 2f, suggesting that most of the oxygen-containing functional groups in GO have been removed successfully by thermal reduction and majority of the conjugated bonds have been restored accordingly.<sup>[33]</sup>

Morphological structure of the resulting graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels has been verified by scanning electron microscope (SEM) and transmission electron microscope (TEM) as shown in Figure 3. Although the Fe<sub>2</sub>O<sub>3</sub> aerogels synthesized with the same processing parameters as those in our case has been reported elsewhere,<sup>[34]</sup> we have not obtained monolithic Fe<sub>2</sub>O<sub>3</sub> aerogels in our lab probably due to their extremely fragile attribute. However in the presence of graphene sheets (actually its precursor GO before carbonization of the hybrid aerogels) we have easily obtained monolithic graphene/ Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels as shown in Figure 3a, indicating that 2D carbonaceous sheets can greatly enhance the mechanical property of the iron oxide aerogels. A lot of iron oxide nanoparticles with sphere-like morphology in the size of 10-30 nm can be observed from both SEM (Figure 3b) and TEM (Figure 3c) images. High resolution TEM study as shown in Figure 3d has indicated that the resulting iron oxide nanoparticles attached to the graphene sheets are polycrystalline with characteristic diffraction pattern of the \gamma-Fe<sub>2</sub>O<sub>3</sub>, consistent with the results revealed by XRD and XPS analyses. Particularly, even after long time ultrasonication during TEM sample preparation, the Fe<sub>2</sub>O<sub>3</sub> nanoparticles in the hybrid aerogels still can be tightly attached to the graphene sheets, suggesting a strong interaction between graphene sheets and Fe<sub>2</sub>O<sub>2</sub> nanoparticles, which agrees well with above-mentioned Raman spectroscopy observation. We can envision that random deposition of iron oxide nanoparticles onto both sides of the



**Figure 2.** XRD patterns (a) and Raman spectra (b) of the graphene powder (1), graphene/ $Fe_2O_3$  hybrid aerogels with different mass ratios of graphene to  $Fe_2O_3$ : 1:1 (2), 1:2.5 (3), 1:5 (4), and  $Fe_2O_3$  powder (5). All samples have been synthesized with the same processing conditions; and XPS survey profiles of the graphene/ $Fe_2O_3$  hybrid aerogel and GO (c), Fe 2p XPS pattern of the graphene/ $Fe_2O_3$  hybrid aerogel (d), C 1s XPS spectra of the pure GO (e) and graphene/ $Fe_2O_3$  hybrid aerogel (f). The hybrid aerogel used in (c–f) is with the mass ratio of graphene to  $Fe_2O_3$  1:2.5.

graphene sheets have produced a large amount of mesopores (pore diameter in the range 2–50 nm), we will discuss below, in the resulting hybrid aerogels. However we need to point out that there are a great deal of macropores (pore diameter >50 nm) in the order of ca. micrometer can be observed from SEM images, which provides possibility that our hybrid aerogels can accommodate some large biomolecules, e.g., enzyme, in these macropores.

The porous characteristics of the hybrid graphene/Fe<sub>2</sub>O<sub>3</sub> aerogels have been further confirmed by nitrogen sorption tests. As shown in **Figure 4**a, all the adsorption-desorption curves exhibit type-*IV* isotherm with a H<sub>3</sub> hysteresis loop, suggesting a characteristic of open wedge-shaped mesoporous structure.<sup>[35]</sup> The Brunauer-Emmett-Teller (BET) surface areas of the investigated graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels are calculated between 271 and 414 m<sup>2</sup> g<sup>-1</sup>, much higher than that of other graphene/iron oxide composites reported elsewhere (see Figure SI2).<sup>[18,36-40]</sup> When the graphene loading

amount is at ca. 50 wt%, the BET specific surface area of the as-made hybrid aerogel reaches its maximum (see Table SI1). This indicates that more iron oxide attached onto the graphene sheets would decrease the specific surface area of the resulting hybrid aerogels, which is probably due to that the Fe<sub>2</sub>O<sub>3</sub> nanoparticles themselves tend to aggregate owing to their strong magnetic behaviors.<sup>[41]</sup> The pore size distributions obtained from the isotherms as shown in Figure 4b indicate a number of mesopores with the diameters approximately in the range 10~12 nm located in the hybrid aerogels. In addition, the total pore volume increases from 0.636 to 1.192  $\text{cm}^3 \text{g}^{-1}$  with the increase of graphene content. The relative increase in the pore volume in the resulting hybrid aerogel comes from the relatively low amount of Fe<sub>2</sub>O<sub>3</sub> nanoparticles anchoring on graphene sheets.

Magnetic performance of the graphene/ Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels were investigated using a vibrating sample magnetometer (VSM) at room temperature with an applied magnetic field sweeping back and forth between -20 KOe and +20 KOe. It can be seen from Figure 4c that the magnetic hysteresis loops of all samples are S-shape. The saturation magnetization (Ms) of the  $Fe_2O_3$  powder is 67 emu g<sup>-1</sup>, which is smaller than the reported value (74 emu  $g^{-1}$ ) of the bulk Fe<sub>2</sub>O<sub>3</sub>.<sup>[34]</sup> The decrease in Ms value of the Fe<sub>2</sub>O<sub>3</sub> powder can be attributed to size effect of Fe<sub>2</sub>O<sub>3</sub> nanoparticles.<sup>[41]</sup> While the values of saturation magnetization of graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels are smaller than that of the Fe<sub>2</sub>O<sub>3</sub> powder due to incorporation of magnetically inactive graphene

sheets into iron oxide aerogels. The saturation magnetization of the hybrid aerogels are further decreased from 54 to 23 emu $\bullet$ g<sup>-1</sup> with the increase of the mass ratios of graphene to Fe<sub>2</sub>O<sub>3</sub> from 1:5 to 1:1. However the saturation magnetization values of our synthesized hybrid aerogels are much higher than those of graphene/iron oxide composites reported elsewhere<sup>[18,36–39]</sup> (see Figure SI3), which is probably ascribing to double nanocrystalline networks of the hybrid aerogels mentioned below. What's more, all the magnetic remanences (Mr) of the Fe<sub>2</sub>O<sub>3</sub> powder and the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels are nearly zero (the value of the former is 0.83 emu g<sup>-1</sup> and values of the latter are in the range from 0.03 to 0.09 emu  $g^{-1}$ ). This indicates that there is almost no remaining magnetization when the external magnetic field is removed, suggesting that both Fe<sub>2</sub>O<sub>3</sub> powder and graphene/ Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels exhibit superparamagnetic behaviors, consistent with results reported elsewhere.<sup>[18,36-39]</sup> The superparamagnetic performance of the hybrid aerogels provides





**Figure 3.** Digital photo of the hybrid graphene/Fe<sub>2</sub>O<sub>3</sub> aerogel under a magnet (a), SEM (b) and TEM (c,d) images of the typical graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel. Inset in (d) is the selected area electron diffraction (SAED) pattern of the hybrid aerogel.

an easy way to handle them, as illustrated in Figure 3a, by using an external magnetic field.

Conductivity of the resulting graphene/ $Fe_2O_3$  aerogels has been investigated by four-probe method at room temperature. Figure 4d shows typical *I–V* curves of the hybrid aerogels with different mass ratios of graphene to  $Fe_2O_3$ . It can be seen that all the hybrid aerogels present linear *I–V* curves, indicating

that these aerogels are conductive. Restoration of  $\pi$ -conjugated systems from GO sheets upon thermal anneal,<sup>[42]</sup> confirmed by XPS analysis as shown above, is the reason for the electrical conductivity of the hybrid aerogels. However the electrical conductivity values of our synthesized hybrid aerogels are greatly dependent on the mass ratios of the graphene to  $Fe_2O_3$ . When the mass ratio is 1:5, the electrical conductivity of the resulting hybrid aerogel is  $4.97 \times 10^{-3}$  S m<sup>-1</sup>, and when the mass ratio is 1:2.5, the electrical conductivity of the resulting hybrid aerogel is  $4.63 \times 10^{-2}$  S m<sup>-1</sup>, one order of magnitude higher than that of the hybrid aerogel with the mass ratio 1:5. When the mass ratio is further increased to 1:1, the electrical conductivity of the hybrid aerogel is only  $5.23 \times 10^{-2}$  S m<sup>-1</sup>, almost the same as that of the hybrid aerogel with the ratio 1:2.5. Theoretical studies have shown that graphene sheets have a quite low percolation threshold (less than 0.5 wt%) and can form conductive network easily.<sup>[43]</sup> That the hybrid aerogels with the mass ratios of graphene to Fe<sub>2</sub>O<sub>3</sub> 1:2.5 and 1:1 have the close values in conductivity is one of the strong evidence that the graphene sheets have formed conducting network within the resulting hybrid aerogel matrices. However the conductivities of these hybrid aerogels are two orders of magnitude lower than those of our synthesized graphene aerogels reported elsewhere<sup>[15]</sup> probably because of the higher junction resistance of the conducting graphene network, which results from the existence of iron oxide nanoparticles embedded within the graphene network observed from SEM and TEM images.

### **2.2. Revealing of the Double Networks** for the Resulting Hybrid Aerogels

In order to further confirm the existence of the conducting graphene network in the hybrid aerogel matrices, the resulting graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels with the mass ratios of graphene to Fe<sub>2</sub>O<sub>3</sub> from 1:1 to 1:2.5 were etched with 1.0 M HCl

to get rid of the  $Fe_2O_3$  component and followed by secondly supercritical  $CO_2$  drying, the monolithic graphene aerogels were obtained as shown in the inset of **Figure 5**a; while the resulting graphene/Fe<sub>2</sub>O<sub>3</sub> aerogel with the mass ratio of graphene to Fe<sub>2</sub>O<sub>3</sub> 1:5 was processed with the same procedure, no monolithic graphene aerogel was obtained. These results show that the graphene conducting network really



**Figure 4.** Nitrogen sorption isotherms (a), pore size distribution curves (b), magnetization curves (c) and *I*–*V* curves (d) of the resulting graphene/ $Fe_2O_3$  hybrid aerogels with different mass ratios of graphene to iron oxide.



**Figure 5.** SEM images of graphene (a,b) and  $Fe_2O_3$  (c,d) aerogels made by etching and calcining the graphene/ $Fe_2O_3$  hybrid aerogels, respectively. (The insets in (a) and (c) are digital photos of the graphene and  $Fe_2O_3$  aerogels, respectively).

exists in the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels with the mass ratios of graphene to Fe<sub>2</sub>O<sub>3</sub> from 1:1 to 1:2.5, consist with the above results revealed by conductivity investigation. To our surprise, if the resulting graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels with the mass ratios of graphene to Fe<sub>2</sub>O<sub>3</sub> from 1:1 to 1:5 were calcined in air to get rid of the graphene component, the monolithic Fe<sub>2</sub>O<sub>3</sub> aerogels were obtained as shown in the inset of Figure 5c, indicating the Fe<sub>2</sub>O<sub>3</sub> magnetic network really exists in the resulting graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels with the mass ratios of graphene to Fe<sub>2</sub>O<sub>3</sub> from 1:1 to 1:5, which might be the reason that the saturation magnetization values of our synthesized hybrid aerogels are much higher than those of graphene/iron oxide composites reported elsewhere.[18,36-39] The existence of conductive and magnetic networks in the resulting graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel matrix has indicated the resulting hybrid aerogels reported herein are bifunctional ones with double networks.

Figure 5a,b present typical SEM images of the graphene aerogel made by etching of the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel with 1.0 M HCl solution, from which a well-defined and interconnected 3D porous graphene network can be clearly verified. The partial overlapping or coalescence of the graphene sheets has resulted in cross-linking to build the resulting graphene aerogel. Furthermore, there is no obvious Fe<sub>2</sub>O<sub>3</sub> nanoparticles located within the graphene aerogel, suggesting all the  $Fe_2O_3$  nanoparticles attached to the graphene sheets were completely removed and a graphene-only aerogel was generated. Figure 5c and 5d present SEM images of the Fe<sub>2</sub>O<sub>3</sub> aerogel made by calcinating of the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels in air, from which a well-defined and interconnected 3D porous Fe<sub>2</sub>O<sub>3</sub> network can be clearly verified. No obvious graphene sheets existed in the remaining Fe<sub>2</sub>O<sub>3</sub> aerogels, indicating all the graphene sheets embedded within the Fe<sub>2</sub>O<sub>3</sub> have been totally burned out. These observations have

further confirmed the existence of double networks in the resulting graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels. For comparison, Yan et al. have reported 3D graphene/Fe<sub>3</sub>O<sub>4</sub> aerogels elsewhere.<sup>[18]</sup> We have repeated Yan's work and found that a graphene aerogel monolith can be obtained after etching the graphene/Fe<sub>3</sub>O<sub>4</sub> aerogels, suggesting the presence of graphene network within the graphene/Fe<sub>3</sub>O<sub>4</sub> aerogels; while after calcination process, we have obtained Fe<sub>3</sub>O<sub>4</sub> powders instead of Fe<sub>3</sub>O<sub>4</sub> framework, revealing that Fe<sub>3</sub>O<sub>4</sub> network do not exist within the resulting graphene/Fe<sub>3</sub>O<sub>4</sub> aerogels; thereby we can conclude that this graphene/Fe<sub>3</sub>O<sub>4</sub> aerogels do not possess double-network structure. Furthermore, Yu et al. have synthesized graphene/ $\alpha$ -FeOOH aerogel elsewhere,<sup>[17]</sup> however they have claimed that, after etching with HCl to remove  $\alpha$ -FeOOH component, the graphene 3D network structure was broken down due to the severe reaggregation of the graphene sheets, and thus we conclude that this graphene/a-FeOOH

aerogel do not possess the double network, neither. These comparisons have indicated that the double networks of the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels might be unique among the graphene-based composite aerogels.

The graphene and Fe<sub>2</sub>O<sub>3</sub> aerogels obtained from the asmade graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels were further investigated by XPS and XRD analyses as shown in Figure SI4. After calcination of the as-prepared graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel, no C composition can be detected by XPS survey analysis in the remaining product as shown in Figure SI4a, indicating the graphene sheets in the hybrid aerogels were burned out completely, and no obvious difference in XRD patterns has been observed between original graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel as shown in Figure 2a and the remaining Fe<sub>2</sub>O<sub>3</sub> aerogel as shown in Figure SI4b, indicating that the remaining aerogel is  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> instead of  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>. After etching of the asprepared graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel with HCl solution, no Fe composition can be detected by XPS survey analysis in the remaining product as shown in Figure SI4a, suggesting Fe<sub>2</sub>O<sub>3</sub> nanoparticles in the hybrid aerogels have been etched out totally. Furthermore, double diffraction humps have appeared at 24.4° and 44.1° in the XRD pattern as shown in Figure SI4b, which could be the characteristic peaks of the (002) and (100) planes of the graphite,<sup>[44]</sup> indicating that partial overlapping of the graphene sheets has happened during etching.

To further confirm the porous attribute of the resulting graphene and  $Fe_2O_3$  aerogels obtained from the as-made graphene/ $Fe_2O_3$  hybrid aerogels, nitrogen adsorption-desorption isotherm were performed (see Figure SI5). Both graphene and  $Fe_2O_3$  aerogels exhibit a type-*IV* isotherm with a H<sub>3</sub> hysteresis loop, same as the graphene/ $Fe_2O_3$  hybrid aerogels, showing of a mesoporous structure. BET analysis reveals that the resulting  $Fe_2O_3$  aerogel has a rather large



**Figure 6.** (a) Transformation of GL into GAMG and GA catalyzed by  $\beta$ -glucuronidase (PGUS-E), (b) effect of time on the immobilization amount of PGUS-E to the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel, (c) SEM image of the hybrid aerogel after immobilization of the PGUS-E, (d) Nitrogen element mapping image of the hybrid aerogel after immobilization of the enzyme and (e) Raman spectra of the resulting hybrid aerogel (black line), aerogel-GA (red line), aerogel-PGUS-E (blue line) and aerogel-GL (pink line) systems.

surface area of 110 m<sup>2</sup> g<sup>-1</sup>, which is higher than that of the nanocrystalline iron oxide aerogels made at the same temperature reported elsewhere.<sup>[34]</sup> In addition, the total pore volume (0.43 cm<sup>3</sup> g<sup>-1</sup>) of the resulting Fe<sub>2</sub>O<sub>3</sub> aerogel is also higher than that of the nanocrystalline iron oxide aerogels reported elsewhere.<sup>[34]</sup> Similarly, a BET surface area of 441 m<sup>2</sup> g<sup>-1</sup> can be obtained for the resulting graphene aerogel, although the value is a little lower than that of the self-assembled graphene aerogel (13~15 mg cm<sup>-3</sup>) made via etching of the asprepared graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel reported herein is much lighter than that of the graphene aerogel (31 mg cm<sup>-3</sup>) reported in our previous study.<sup>[15]</sup>

### 2.3. Immobilization of Enzyme Biomacromolecules for Biocatalysis

Glycyrrhizin (GL), an important triterpenoid saponin,<sup>[45]</sup> is a drug with multi-pharmacological effects such as antiinflammation, antivirus, anti-tumor, immuno-modulating



etc.<sup>[46,47]</sup> GL is also widely used in food industry as an important natural sweetener. However, if the human body takes in GL too much, it will produce certain side effects, such as reducing the sodium discharge of human body and increasing the potassium discharge.<sup>[48]</sup> GL contains a pentacyclic triterpenoid nucleus and two distal glucuronides. It can be transformed into glycyrrhetinic acid monoglucuronide (GAMG) or glycyrrhetinic acid (GA) by  $\beta$ -glucuronidase (PGUS-E) as shown in Figure 6a. GAMG is a kind of high sweetness with low calorie functional sweetener.<sup>[49]</sup> While GA serves as a very important functional drug, it can inhibit information delivery of the original cancer cells and also possess a superior function of anti-viral infections, especially for the carcinogenic viruses such as hepatitis viruses, Epstein-Barr virus and HIV infection.<sup>[50]</sup> GA also can be used as an additive for cosmetics due to its ability of scavenging oxygen free radical. In the previous study, one of authors (C. Li) of this manuscript and his co-authors have screened a fungal strain. Penicillium purpurogenum Li-3, which can use GL as a carbon source and convert it into GAMG by expressed  $\beta$ -glucuronidase.<sup>[51,52]</sup> C. Li et al. have also cloned the gene (GenBank Accession No. EU095019) and overexpressed PGUS-E in Escherichia coli BL21.<sup>[53]</sup> After transformation of GL, separation of the reaction components from the solution system has been troubled by recovering of PGUS-E. Herein we try to immobilize PGUS-E to the as-made graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid

aerogel and find that PGUS-E immobilized to the hybrid aerogel has shown high catalytic activity and definite repeatability in transformation of GL into GA with easy separation of biocatalyst from the reaction mixture.

Loading capacity of the porous matrix can directly determine the catalytic effect of the immobilized enzyme. To investigate the maximum adsorption capacity of the hybrid aerogel for PGUS-E, we have explored relationship between immobilization amount and adsorption time as shown in Figure 6b. Results show that the PGUS-E can be immobilized to the hybrid aerogel without any pretreatment, and the maximum loading amount is about 2.45 mg mg<sup>-1</sup>, which is higher than those of the horseradish peroxidase immobilized to the GO sheets.<sup>[54,55]</sup> SEM image as shown in Figure 6c has indicated that the hybrid aerogel after immobilization of enzyme biomacromolecules exhibits a smooth and compact morphology, instead of a well-defined porous network as observed in Figure 3b, which is probably due to the fact that the pores within aerogel have been filled with enzyme completely. Nitrogen element mapping as shown in Figure 6d has indicated the uniform distribution of N element within the

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**Figure 7.** (A) Recorded curves of the GL conversion rate vs. reaction time by using the free PGUS-E, the hybrid aerogel and the immobilized PGUS-E to the hybrid aerogel as the catalysts, respectively, (b) Lineweaver–Burk plot for determination of apparent kinetic parameters of the hydrolysis of GL catalyzed by free or immobilized PGUS-E, and (c) Arrhenius plot for calculation of apparent activation energy of the hydrolysis of GL catalyzed by free or immobilized PGUS-E.

hybrid aerogel matrix, indicative of uniform immobilization of PGUS-E into the pores of the hybrid aerogel. To elucidate the driving force for PGUS-E being immobilized to the hybrid aerogel, Raman spectroscopy has been used and the results are shown in Figure 6e. The G band of the graphene/ $\gamma$ -Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel locates at 1594 cm<sup>-1</sup>, after immobilization of enzyme into the aerogel, the G band shows an apparent blue-shift to 1600 cm<sup>-1</sup>, revealing that there is a strong interaction between enzyme and hybrid aerogel, which benefits PGUS-E to move into the pores of the hybrid aerogel. By the way, there are strong interactions as well between either hybrid aerogel and GL or hybrid aerogel and GA as the G bands of the hybrid aerogel after incorporation of either GL or GA show apparent blue-shifts as well. However, blue-shift of the G band for the hybrid aerogel incorporated with GL is bigger than that for the hybrid aerogel incorporated with GA, which means that, during enzyme catalytic reaction in the co-existence of GL and GA, GL still can be adsorbed into the pores of the hybrid aerogel to take place the reaction as the interaction between the hybrid aerogel and GL is higher than that between the hybrid aerogel and GA.

The catalytic performance of the immobilized PGUS-E has been compared with those of the free PGUS-E and the hybrid aerogel as shown in Figure 7a. For the free PGUS-E, the conversion rate of the GL is up to 99.3% after 4-hour reaction, indicating that the PGUS-E is a highly efficient biocatalyst for GL transformation. While for the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel, only 3.74% conversion rate can be obtained after 4 hours, which might be due to the pseudo-conversion of the GL resulting from its physical adsorption to the hybrid aerogel as no target products were observed from the reaction mixture. For the immobilized PGUS-E, the conversion rate of GL is up to 98.0% after the same reaction time, almost the same as that of the free enzyme. These results indicate that, after immobilization to the hybrid aerogel, the target enzyme can keep its full bioactivity and can catalyze the conversion of GL. However all the GL conversion rate catalyzed by the immobilized PGUS-E is lower than that catalyzed by the free enzyme after the same reaction period as shown in Figure 7a, indicating that the reaction catalyzed by the immobilized PGUS-E is slower than that catalyzed by the free enzyme. Diffusion of the raw material (GL) into the porous matrix and diffusion of the products (GAMG and GA) out of the porous matrix might be the main reason for observation of such slowing down transformation of GL into GAMG and GA. Furthermore, the immobilized PGUS-E and free enzyme have shared the same optimum pH and temperature conditions for GL transformation (see Figure SI6), suggesting that the enzyme structure has almost not changed after immobilization of PGUS-E into hybrid aerogel and that each component of the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogel has no effects on the activity of the immobilized enzyme. To further disclose the effect of the immobilization on the activity of the enzyme, a comparative study on the apparent kinetic parameters (K<sub>m</sub>, V<sub>max</sub>) of the reaction has been carried out between the immobilized enzyme system and the free enzyme system. The Lineweaver-Burk plot<sup>[56]</sup> as shown in Figure 7b has suggested that the hydrolysis of GL catalyzed by PGUS-E follows the Michaelis–Menten equation.<sup>[57]</sup> The much lower value of K<sub>m</sub> have been observed from the immobilized PGUS-E system (see Table SI2) in comparison with that observed from the free enzyme system, and the ratio of V<sub>max</sub>/K<sub>m</sub> was about twice higher than that observed from the free PGUS-E system, therefore the affinity capacity between enzyme and GL substrate has been enhanced after enzyme immobilization, that is to say our hybrid aerogel can keep the



**Figure 8.** A digital photo indicating that the graphene/ $Fe_2O_3$  hybrid aerogel containing PGUS-E has been separated from the reaction mixture by a magnet (a), illustration of GL conversion vs. batch cycles when immobilized PGUS-E was used repeatedly (b), HPLC graph of the reaction mixture catalyzed by the immobilized PGUS-E after 2 hours (c) and HPLC graph of the supernatant in c reacted further in the absence of the immobilized PGUS-E after 10 hours (d).

immobilized enzyme in an active conformation. Moreover, the activation energy ( $E_a$ ) of the PGUS-E catalyzed hydrolysis of GL was determined by liner fit of the Arrhenius plot as shown in Figure 7c, from which the  $E_a$  value can be calculated (see Table SI2). The  $E_a$  value for the hydrolysis of GL by the immobilized PGUS-E was much lower than that of the free PGUS-E, revealing that the hybrid aerogel might make the formation of the 'enzyme–substrate complex' easier and more stable due to its porous attribute.<sup>[58]</sup>

Furthermore, PGUS-E immobilized to the hybrid aerogel can be easily separated from the reaction mixture by a magnet as shown in Figure 8a and thus can be re-used for the next batch, which shows great advantages over usual enzyme immobilization strategies as the separation procedure used here avoids long-time and multistep purification process. The catalytic performance of the re-used biocatalyst against re-use times has been plotted in Figure 8b, from which it can be seen that, for the 2nd cycle, the conversion rate of the GL remains 86.6%, while for the 7th cycle, the conversion rate of GL decreases to 34.8%. The reason that GL conversion rate decreases significantly with the increase of the batch cycles probably attributed to the accumulation of the reaction products (GAMG and GA) covered on the surface of PGUS-E immobilized to the porous matrix, which affects the catalytic performance of the immobilized enzyme at the next batch.<sup>[59,60]</sup> Obviously, immobilization of PGUS-E to the hybrid aerogel can be re-used, and the re-use cycles are not very high for the time being, but still superior than those of the other immobilized enzymes reported elsewhere.<sup>[60-62]</sup> To further disclose the essence that the conversion rate of GL decreases with the cycle times when PGUS-E is immobilized to the hybrid

aerogel as a biocatalyst, we have designed and carried out the following experiments: the substrate (2 g L<sup>-1</sup> GL) was catalyzed by immobilized enzyme for 2 h and then biocatalytic reaction was deliberately suspended by separation of the immobilized enzyme from the reaction mixture with a magnet. At this time we could observe the existence of a large quantity of GAMG and a small quantity of GL in the reaction mixture from the HPLC graph as shown in Figure 8c. The supernatant was further stirred for another 10 h in the absence of the immobilized enzyme and then the components of the mixture were analyzed again by HPLC as shown in Figure 8d. It was very interesting to see that almost all substrate GL and intermediate product GAMG have been transformed into the final product GA, which ascribed to that a small amount of the enzyme immobilized to the hybrid aerogel has desorbed into the solution, which has continued to catalyze GL or GAMG transformation into the final product GA. Partial desorption of the immobilized PGUS-E into the solution might be another reason for that GL conversion rate decreases significantly

with the increase of the batch cycles. These observations have also indirectly shown that the loading of the PGUS-E into the highly porous hybrid aerogel has been mainly governed by the physical adsorption instead of the chemical bonding as in the case of the latter the immobilized enzyme would not desorb from the porous matrix to solution.

### 3. Conclusion

We have developed a general and practical approach to synthesize graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels by supercritical fluid drying and carbonizing of their hydrogel precursors, which have been made by hydrolysis and polycondensation of FeCl<sub>3</sub> in the presence of GO and epichlorohydrin in DMF solution. The resulting graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels show quite low density (30-65 mg cm<sup>-3</sup>), large specific surface area (270–414 m<sup>2</sup> g<sup>-1</sup>), high electrical conductivity  $(0.5-5 \times 10^{-2} \text{ S m}^{-1})$  and superior saturation magnetization  $(23-54 \text{ emu g}^{-1})$ . In addition, we have also obtained intrinsic graphene and Fe<sub>2</sub>O<sub>3</sub> aerogels by etching of the resulting graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels with 1.0 M HCl and calcining of the resulting graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels in air atmosphere, respectively, which has directly confirmed the existence of the double skeleton networks in the as-synthesized graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels. PGUS-E, one of the important enzymes, can be immobilized to the graphene/Fe<sub>2</sub>O<sub>3</sub> hybrid aerogels via physical adsorption with high biocatalytic activity and definite repeatability. This synthetic method might be expanded to other graphene-based metal oxide hybrid aerogels with double networks and the as-synthesized

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graphene/metal oxide hybrid aerogels might be used as hosts to immobilize other important enzymes for biocatalysis.

### **Experimental Section**

See the Supporting Information for a full explanation of the experimental materials and methods.

### Supporting information

*Supporting Information is available from the Wiley Online Library or from the author.* 

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