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Graphene Oxide-Based Biosensors

Lingwen Zeng, Shilin Cao, Hang Yin, Jun Xiong and Donghai Lin

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<http://dx.doi.org/10.5772/intechopen.78222>

Abstract

In this chapter, the latest developments in graphene oxide-based biosensors are presented. These biosensors are complexes of graphene oxide and biomacromolecules, including enzymes such as glucose oxidase, horseradish peroxidase, laccase, and nucleic acids such as DNA and RNA. The structure, design and preparation process (immobilization process) of the above graphene oxide-biomacromolecule composites were summarized. Some typical examples of immobilization of biological macromolecules are described. The immobilization efficiency and electrochemical performance of immobilized biomolecules based on graphene oxide were discussed, which may guide designing better graphene oxide-based biosensors.

Keywords: graphene oxide, biosensor, enzyme, nucleic acid

1. Introduction

Graphene is a new kind of two-dimensional single-atom carbon sheet with a single atom thick [1]. Nowadays, this so-called “thinnest in our universe” material [2] has attracted more and more attention, because of its unique properties such as unique electronic properties [3].

Graphene oxide (GO), one of the nanomaterials from graphene family, contains many reactive oxygen functional groups, such as hydroxyl group, a carboxyl group, an epoxy group [4]. It has been extensively used for biosensor research and application. In order to enhance the electrochemical properties of the GO-based biosensor, GO can be modified with other materials, such as macromolecules, small mass organic molecules, metallic oxide, and metallic/nonmetallic simple substances. In this chapter, we describe GO-based biosensors containing various composites of these materials with references such as GO-chitosan nanocomposites, GO-based

glucose oxidase, chitosan-ferrocene/graphene oxide/glucose oxidase, metal oxides, HRP, multi nanomaterials, quantum dots, multiwall carbon nanotubes, DNA, miRNA, etc. We not only describe the relevant preparation process of the above biosensors but also introduce their electrochemical properties to provide more guidance for designing suitable GO-based biosensors.

2. Enzyme/graphene oxide based biosensor

2.1. GOD/graphene oxide (GO) based biosensor

Glucose oxidase (GOD) is an oxidoreductase, which can oxidize glucose to D-glucono- δ -lactone and form hydrogen peroxide. GOD has shown great potential in glucose biosensor, forage, medicine [5] and biocatalysis [6].

Improving the dispersion of GO ensures the efficient use of the GO-based biosensors. Chitosan is a biopolymer with unique physical/chemical properties and can be well soluble in aqueous acidic solution [7]. Kang et al. [7] firstly mixed graphene with chitosan solution to form a hybrid nanocomposite of graphene-chitosan. Then, this hybrid nanocomposite was coated onto the surface of a glassy carbon electrode (GCE). Finally, this electrode was incubated with GOD solution to form a GOD/graphene/chitosan sensor (**Figure 1**). The result showed that chitosan could improve the dispersion of the graphene and GOD enzyme molecules. The as-prepared GOD/graphene/chitosan sensor exhibited excellent sensitivity ($37.93 \text{ AmM}^{-1} \text{ cm}^{-2}$) and a much higher enzyme loading ($1.12 \times 10^{-9} \text{ mol cm}^{-2}$). Also, this biosensor could retain more than 95% of the enzyme activity after store of 7 days at 4°C .

Chitosan can be used not only as a stabilizing agent but also as reducing agent. Sun et al. [8] designed a graphene platelet-glucose oxidase (GP-GOD) biosensor (**Figure 2**). GO was dispersed in H_2O and mixed with 0.5 M chitosan solution. After stirring for 30 min at room temperature, this mixture was heated at 90°C for 2 h to form a graphene platelet composite (GP)

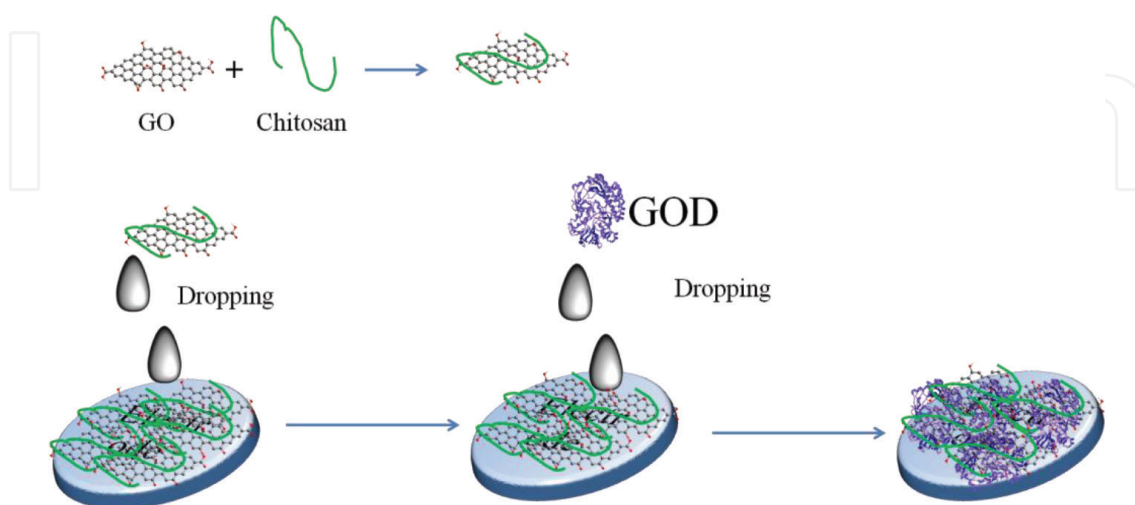


Figure 1. The scheme of GO-chitosan/GOD bioelectrode [7].

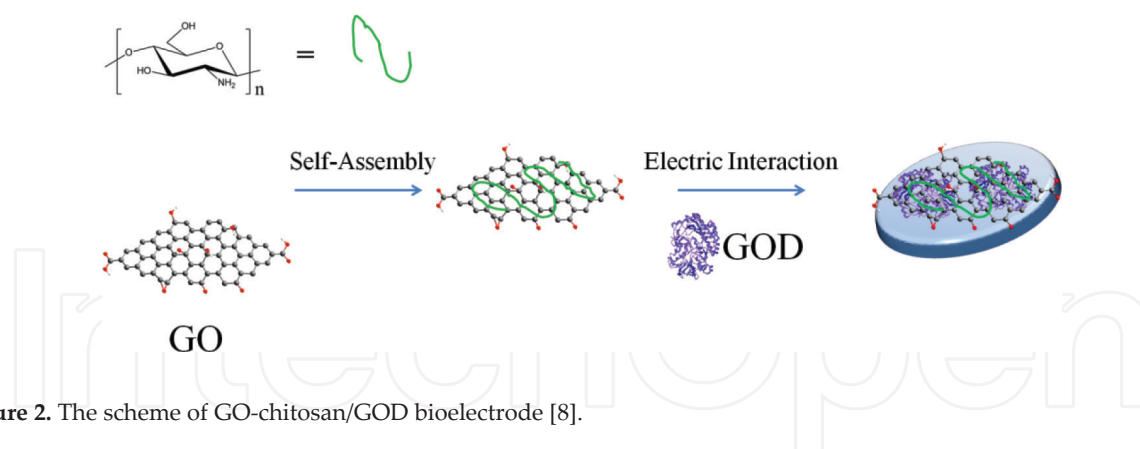


Figure 2. The scheme of GO-chitosan/GOD bioelectrode [8].

dispersion. Then, the GP dispersion was mixed with GOD solution under ultrasonic treatment and kept at 4°C for 12 h to obtain a large amount solid-state GP-GOD product. The glucose biosensor was constructed by deposition of the as-prepared GP-GOD product on a glassy carbon electrode. The linear relation against the concentration of glucose ranged from 2 to 22 mM ($R = 0.9987$) with an estimated detection limit to be 20 M at a signal-to-noise ratio (S/N) of 3.

Luo et al. [9] developed a GO-based glucose biosensor by a direct electro-deposition process. The graphene oxide, chitosan, and GOD are directly electrodeposited onto a glassy carbon electrode (GCE) by using electrochemical reduction under controlled direct electrical potential. This direct electro-deposition process is rapid (several minutes) and can produce uniform, controllable and reproducible films. The GO-chitosan-GOD composite was formed by dispersing GO (5 mg) in chitosan solution (0.2% w/v) and 5 mg mL⁻¹ GOD added stepwise. The GCE was then immersed in the GO-chitosan-GOD solution while a fixed potential of -1.0 V was applied for 400 s. When the electrodeposition time increased from 100 to 400 s, the amount of the GOD entrapped in the film and their current response increased as well. However, while the electrodeposition time further increased from 400 to 900 s, the current response was not a significantly improved. The reason is that excessively thick films have negative effects on the GOD activity and prolong the response time. The as-prepared biosensor film indicated fast response (<3 s), a lower detection limit (0.4 M), and a linear range from 0.4 M to 2 mM towards glucose.

The direct electron transfer between electrode surface and active center of enzyme is commonly hindered. This is mainly because that the active center of the enzyme is buried in the globular structure of the protein molecule. To overcome this drawback, enzymes can be composited with conducting or redox polymers. Qiu et al. [10] designed a homogeneous chitosan-ferrocene/graphene oxide/glucose oxidase (CS-Fc/GO/GOD) nanocomposite film as a novel platform for glucose biosensor. The ferrocene branched chitosan (CS-Fc) was prepared by the following steps (**Figure 3**): (1) chitosan aqueous solution and ferrocenecarboxaldehyde (FcCHO) methanol solution was mixed at room temperature for 2 h to form the Schiff-base; (2) the NaCNBH₃ was added to the above mixture and CS-Fc. (3) the biosensor was constructed by covering the mixture onto the GCE and dried in air at room temperature (**Figure 4**). This CS-Fc/GO/GOD sensor exhibited a wide linear range, excellent sensitivity, good reproducibility, and long-term stability.

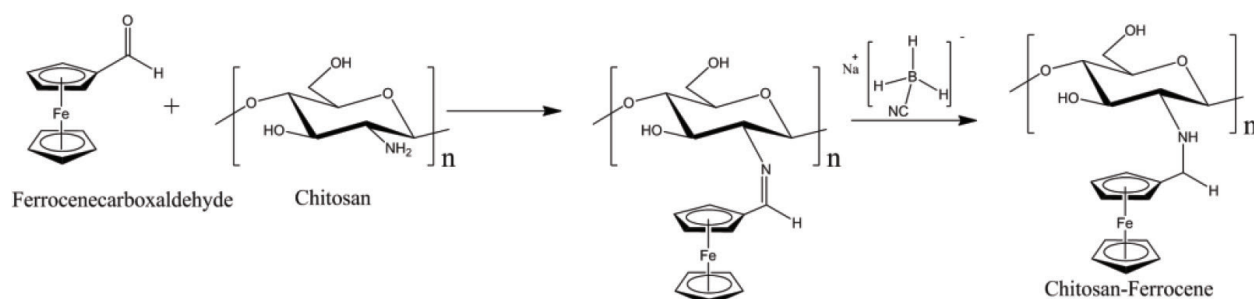


Figure 3. The preparation scheme of the ferrocene branched chitosan [10].

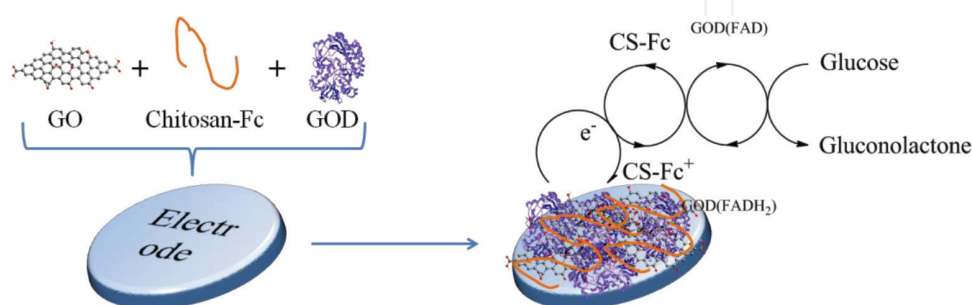


Figure 4. The preparation scheme of CS-Fc/GO/GOD-based glucose biosensor [10].

Apart from chitosan, metal oxides were also used to facilitate the immobilization of GOD onto GO to form biosensors. ZnO is a nontoxic material with good conductivity. It has a high isoelectric point at about 9.5. Thus, the electrostatic interaction between ZnO and GOD (with an isoelectric point at 4.2) can occur. Chen et al. [11] prepared ZnO-microflowers on reduced graphene oxide (RGO) modified GCE by using simple electrodeposition (**Figure 5**). This positively charged ZnO/RGO composite self-assembled with negatively charged GOD and fabricated an RGO/ZnO/GOD biosensor. The linear range of the biosensor was 0.02–6.24 mM with a detection limit of 0.02 mM and sensitivity of 18.97 $\mu\text{A mM}^{-1}$.

Carbon nanotubes are cylindrical nanostructural carbon allotropes with unique electronic, optical properties. Incorporation of carbon nanotubes into GO can enhance direct electron transfer in a biosensor. However, the carbon nanotubes are difficult to disperse homogeneously

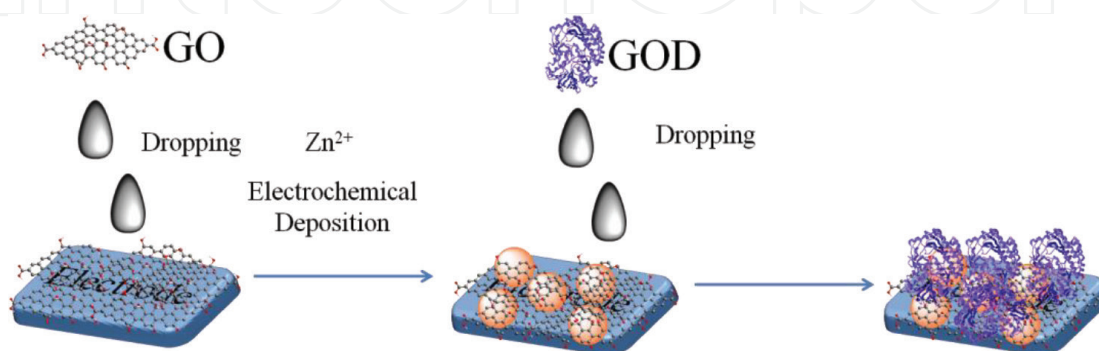


Figure 5. The preparation scheme of ZnO/GO/GOD-based glucose biosensor [11].

in aqueous solution because of its hydrophobic surface and internal van der Waals interactions [12]. Surface modification of carbon nanotubes by GO is a way of solving this problem. Chen et al. [13] dispersed carbon nanotubes with GO aqueous homogeneous suspension to obtain stable carbon nanotubes/GO composite (**Figure 6**). The GOD was positively charged, and carbon nanotubes/GO composite is negatively charged. Thus, the GOD was immobilized by carbon nanotubes/GO composite through the electrostatic interaction as well as physical adsorption. The as-prepared biosensor is reproducible with enhanced direct electron transfer. The linear range of the biosensor was 0.1–19.82 mM with a detection limit of 0.028 mM.

2.2. Horseradish peroxidase/GO based biosensors

HRP is extensively used in clinical diagnosis. It can oxidize chromogenic substrates to colored products by using hydrogen peroxide [13]. The characteristic color change can be easily detected by spectrophotometric methods [14].

Combining GO sheets with chemiluminescence (CL) reagents can facilitate the preparation of a sensitive sensor with attracting CL property. In the work of Liu et al. [15], N-aminobutyl-N-ethylisoluminol (ABEI) functionalized GO hybrids (ABEI-GO) was first synthesized by adding the ABEI alkaline solution into a stable GO suspension for 24 h at room temperature with stirring. HRP buffer solution was then mixed with the ABEI-GO suspension to form an ABEI-GO@HRP hybrid (**Figure 7**). In this strategy, there might be two assembly ways between HRP and ABEI-GO: (1) strong electrostatic interaction between HRP and GO, (2) interactions with hydrogen bonding. The results suggested an excellent CL properties for the detection of H_2O_2 , exceeding those of previous reports. The ABEI-GO@HRP sensor showed a detection limit of 47 fM at physiological pH condition.

GO-based biosensors constructed with multi nanomaterials have also been investigated [16]. For example, many researchers have validated the remarkable electrocatalytic properties and biocompatibility of graphene-gold nanocomposite (G-AuNP). CdTe-CdS, the core-shell quantum dots, could significantly enhance the charge transfer, enabling nanosensor exploiting high intensity. Taken together, Gu and co-workers [16] fabricated a biosensor to detect hydrogen peroxide, integrating the benefits of G-AuNP, CdTe-CdS, and AuNPs (**Figure 8**). Such a biosensor was constructed by successively dropping casting G-AuNP, CdTe-CdS, AuNPs,

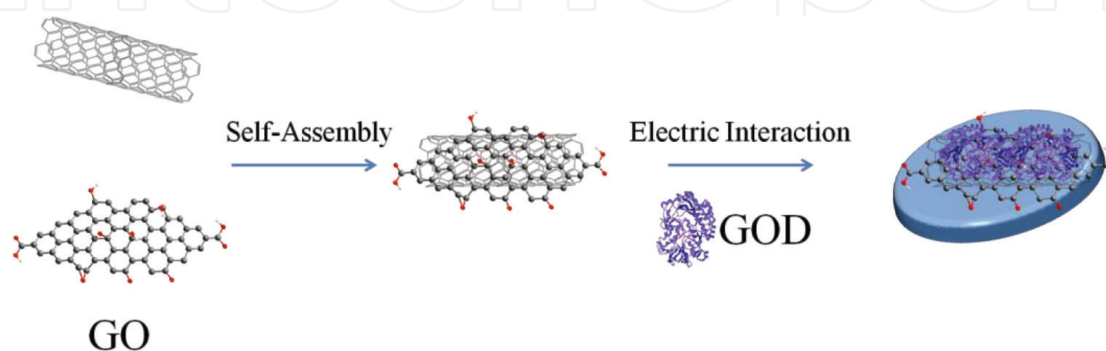


Figure 6. The preparation scheme of carbon nanotube/GO/GOD-based glucose biosensor [12].

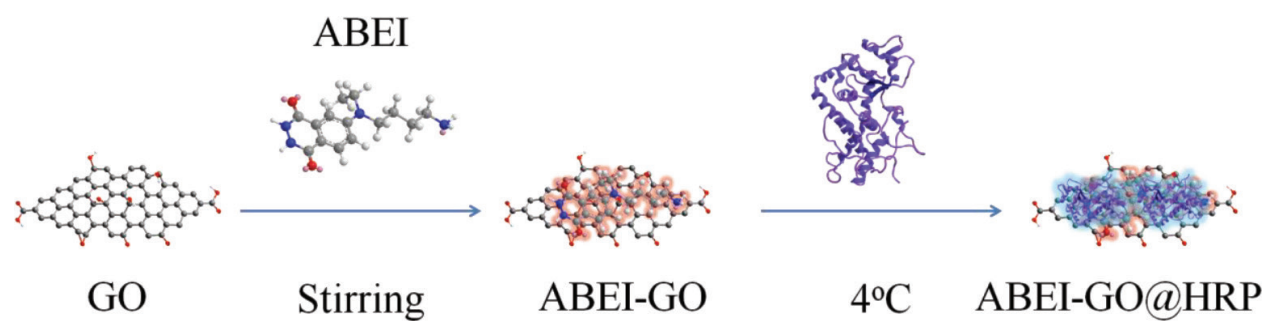


Figure 7. Scheme of the preparation process of the ABEI-GO@HRP composite [15].

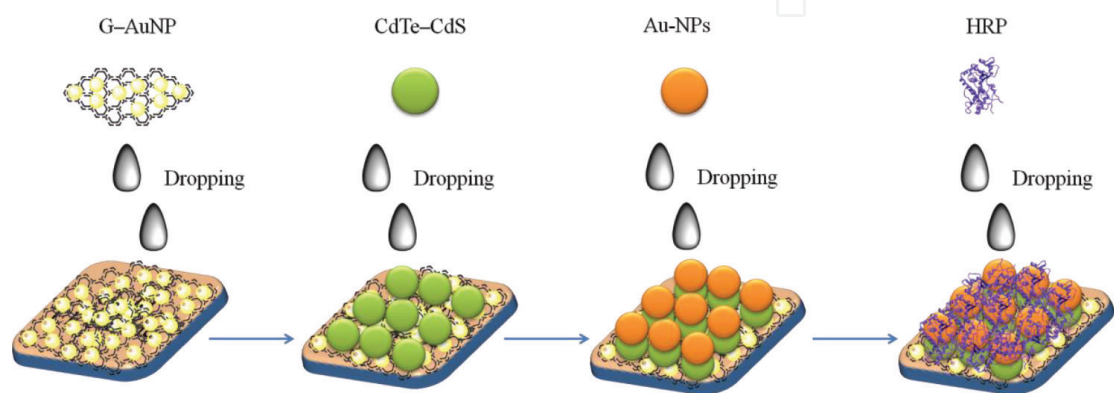


Figure 8. Scheme of the preparation process of the HRP/AuNPs/CdTe-CdS/G-AuNP/GE [16].

and HRP onto the surface of the gold electrode step by step. The Au-NPs were synthesized by citrate reduction in the presence of glutin [17] (**Figure 9**). The G-AuNPs were prepared by in-situ reduction of the HAuCl_4 -loaded GO (**Figure 10**). The result exhibited that the biosensor displays an admirable sensitivity, low detection limit ($S/N = 3$) (3.2×10^{-11} M), wide calibration range (from 1×10^{-10} to 1.2×10^{-8} M) and good long-term stability (20 weeks).

Multiwall carbon nanotubes (MWNTs) have a poor solubility in water, which limits their application in biosensors. To overcome this obstacle, Zhang et al. [18] synthesized a well

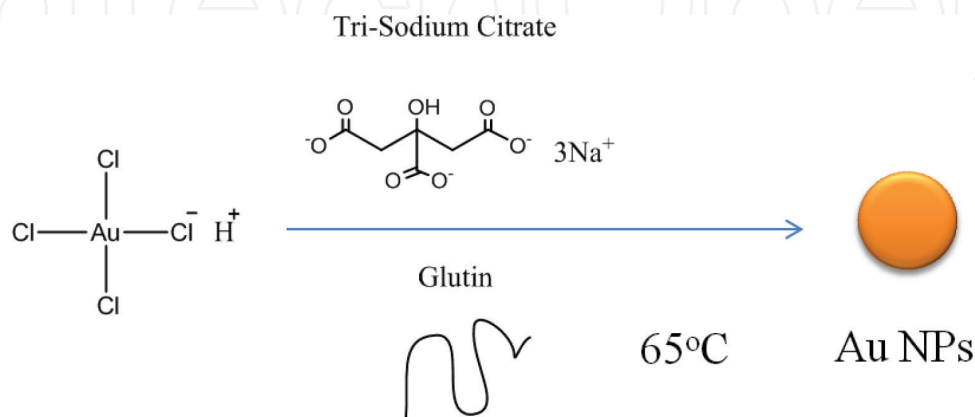


Figure 9. Scheme of the preparation process of the Au NPs by citrate reduction [17].

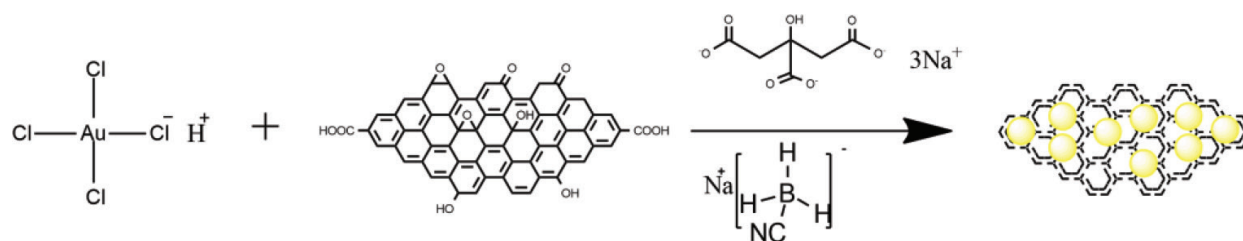


Figure 10. Scheme of the preparation process of the G-AuNPs were synthesized by in-situ reduction of the HAuCl_4 -loaded GO [17].

Carbon Nanotube

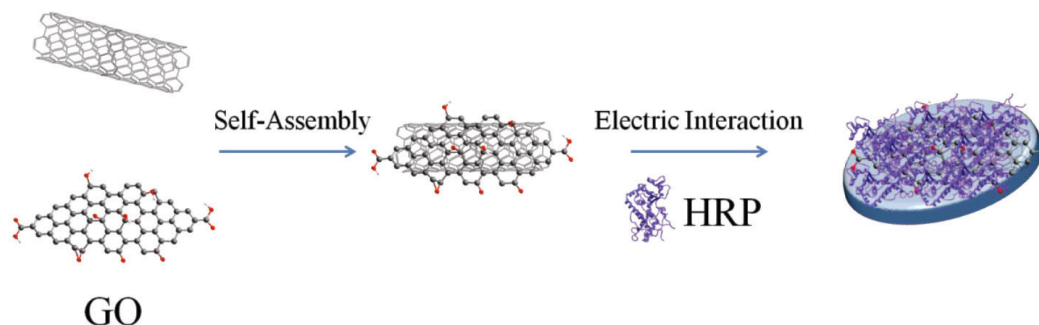


Figure 11. The preparation scheme of carbon nanotube/GO/GOD-based glucose biosensor [18].

depressed GO-MWNT hybrid nanomaterial aqueous solution that carried a negative charge. Subsequently, the as-prepared GO-MWNT aqueous solution was dropped onto the GC electrode, followed by adding HRP onto the GO-MWNT/GC (**Figure 11**). The result indicated that the direct electron transfer between immobilized enzyme and the GC electrode was enhanced by GO-MWNT composite. For detection of H_2O_2 , the detection limit for the sensor was $1.17 \mu\text{M}$ on S/N-3, and the sensitivity of HRP/GO-MWNT/GC electrode was $563.7 \text{ mA cm}^{-2} \text{ M}^{-1}$. For the reduction of NaNO_2 , the sensitivity and the detection limit was $0.6 \text{ mA cm}^{-2} \text{ M}^{-1}$ and 12 mM (S/N = 3), respectively. Furthermore, this novel electrode showed excellent stability for less than 5% activity of 15 days.

Nafion, a commercial tetrafluoroethylene-perfluoro-3, 6-dioxa-4-methyl-7-octenesulfonic acid copolymer, has also been used to modify the GO electrode. In the work of Zhang et al. [19], Nafion solution was mixed with GO by ultrasonication, then HRP was added into the prepared mixed solution, following by casting onto the GCE. The as-prepared electrode, HRP/GO/Nafion/GCE, was proven to have a favorable electrocatalytic response with excellent linear relationships from $1.0 \mu\text{M}$ to 1.0 mM and the detection limits of $4.0 \times 10^{-7} \text{ M}$ (S/N = 3). Furthermore, the HRP/GO/Nafion/GCE biosensor showed satisfactory stability for less than 5% of reduced activity after 4 weeks of storage.

An excellent GO-based biosensor means to possess good electron-transfer property. Co-immobilizing Cytochrome *c* (Cyt *c*) and HRP on GO-chitosan nanocomposite were tried to fabricate a bi-protein electrode by Wan et al. [20]. Firstly, GO-chitosan nanocomposites were

synthesized by stirring GO solution and chitosan solution. Then the HRP-Cyt *c*/GO-chitosan/Cyt *c*/MUA-MCH/Au electrode was produced by a layer-by-layer technique. It was found that the as-prepared biosensor can have an effective response to detecting H_2O_2 within 2 s, along with the linear range from 20 to 330 μM and detection limit of 6.68 μM ($\text{S/N} = 3$). Moreover, the electrode retained most of the activity for 2 weeks.

Owing to the good biocompatibility and large surface area of Co_3O_4 nanosheets (**Figure 12**), Co_3O_4 nanosheets can be used to enhance the electric transfer between enzyme and electrode in a biosensor. Herein, Liu et al. [21] firstly mixed Co_3O_4 suspension with HRP, following with addition of rGO (**Figure 13**). Then the mixture was dropped cast onto the GCE to form the Co_3O_4 -HRP/rGO/GCE electrode successfully. This electrode held a higher HRP loading (with a concentration of $1.48 \times 10^{-10} \text{ mol cm}^{-2}$) than that of monolayer coverage. Furthermore, the as-modified biosensor presented an excellent electronic response with a linear a range from 1 to 5400 μM , a limit of detection of 0.21 μM and a limit of quantification of 0.58 μM for detection of NaNO_2 . Though the study of the stability of electrode was at 4°C for 4 weeks, they validated that electrode can hold 94.1% of its activity.

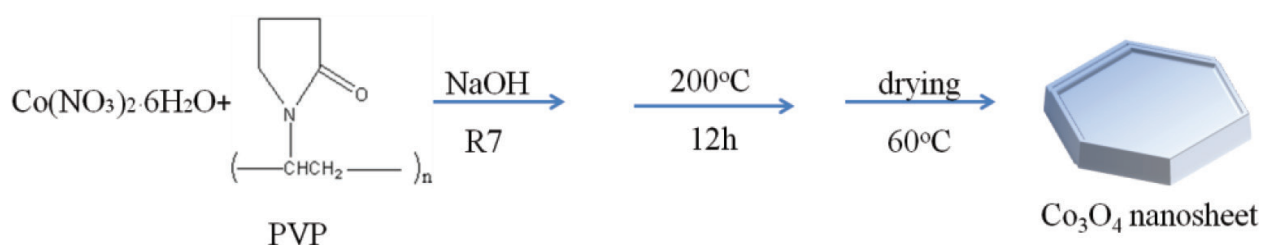


Figure 12. The preparation scheme of Co_3O_4 nanosheet [21].

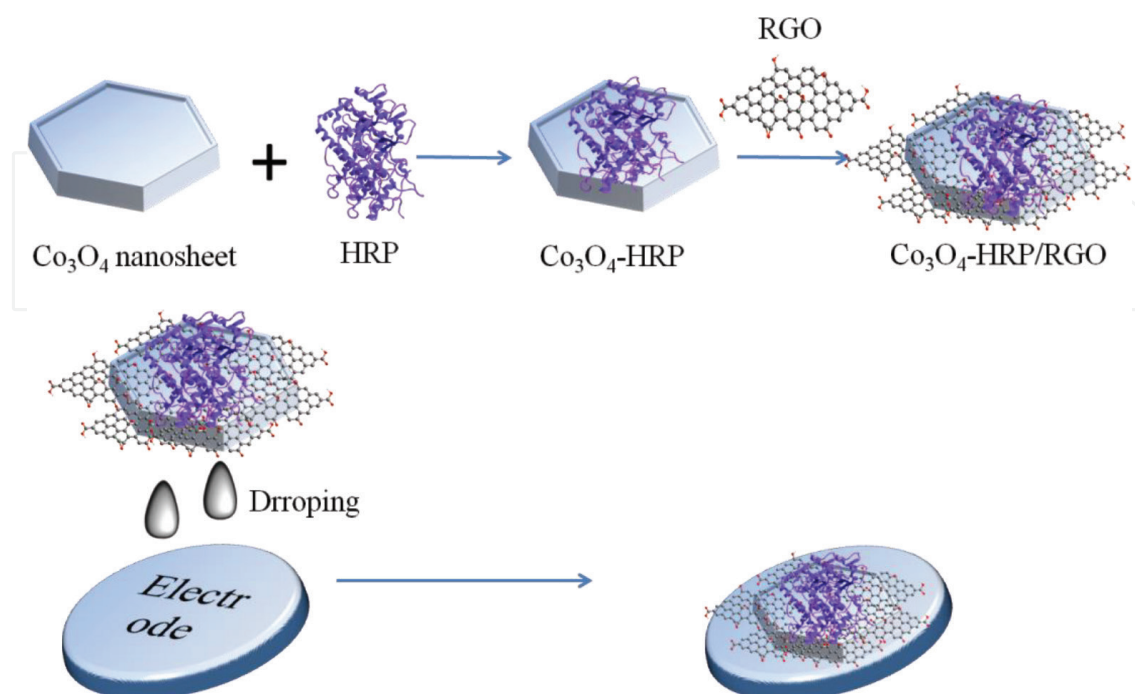


Figure 13. The preparation scheme of Co_3O_4 nanosheet/RGO/HRP biosensor [21].

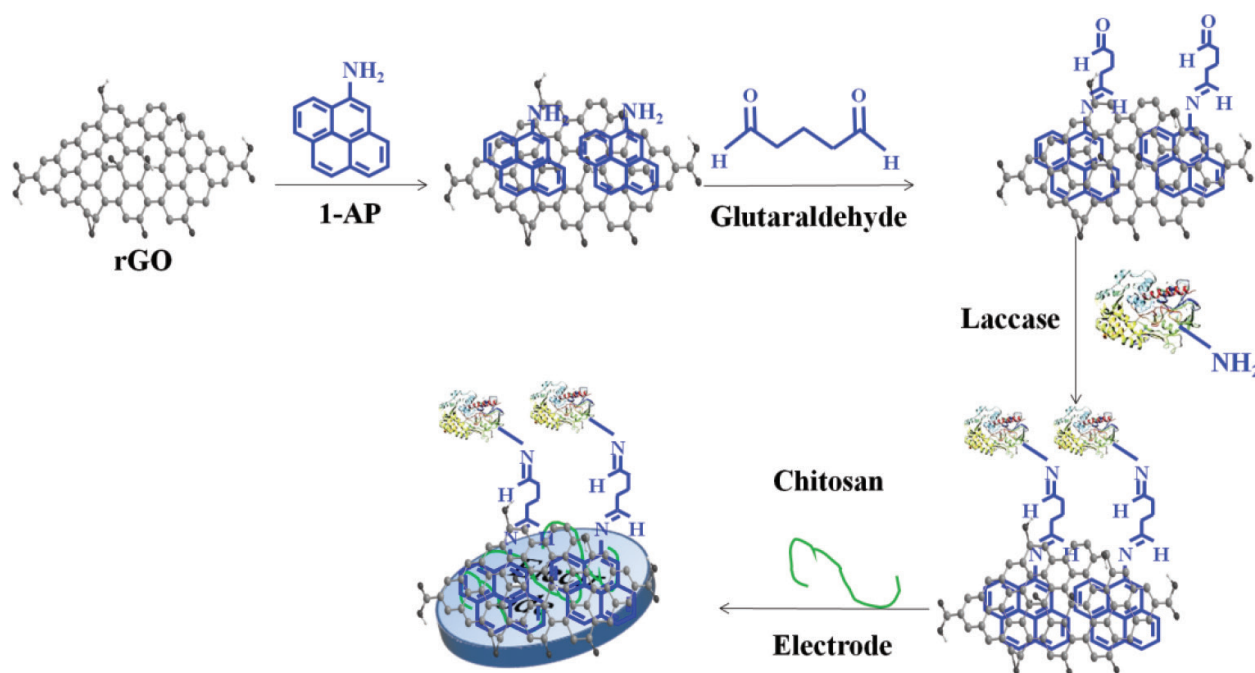


Figure 14. The preparation scheme of AP-rGOs/chitosan/laccase biosensor [23].

Palanisamy et al. [22] reported a novel electrode synthesis based on a screen-printed carbon electrode (SPCE) for the detection of H₂O₂. The electrode was fabricated by dropping GO-HRP composite onto SPCE to form ERGO sensor. The result showed that ERGO had an excellent enzyme loading, and the surface coverage concentration of HRP onto SPCE/ERGO-HRP was calculated to be $7.32 \times 10^{-10} \text{ mol cm}^{-2}$. Moreover, the linear range of detection was 9–195 μM , and sensitivity of the sensor is $0.09 \mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$.

2.3. Laccase/graphene oxide (GO) based biosensor

Laccase is a kind of blue multi-copper oxidase and can catalyze phenols in the presence of oxygen. Thus, this enzyme can be used for the fabrication of phenols detection [23].

Zhou et al. [23] prepared a 1-aminopyrene-reduced graphene oxides (AP-rGOs) composite via the interaction between the pyrenyl group of 1-aminopyrene and graphene (**Figure 14**). Then they covalently immobilized the laccase onto the AP-rGOs form Lac/AP-rGOs by using glutaraldehyde as cross-linker. After mixing chitosan with Lac/AP-rGOs, the Lac/AP-rGOs/chitosan stock solution was dropped onto GCE. The biosensor was used for the detection of phenols in water samples. The result showed that the biosensor exhibited a fast response time (<5 s), high stability (retained >97% activity after 7 days of storage).

3. Nucleic acids/graphene oxide-based biosensor

Previous literature indicated that the GO-chitosan composite could also be used for DNA biosensor fabrication [24]. The GO-chitosan electrode was activated by glutaraldehyde and covalently cross-linked with *Salmonella typhi* specific 5'-amine labeled single-stranded (ss)

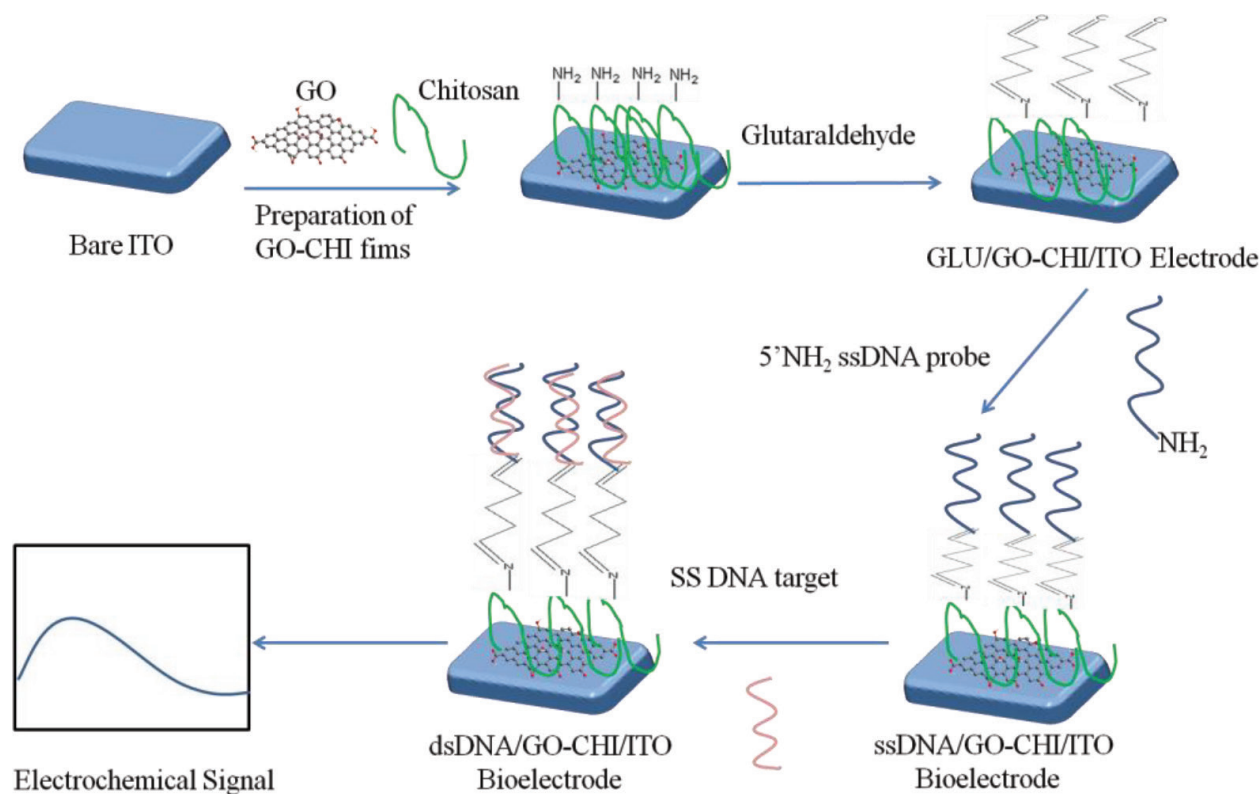


Figure 15. The scheme of ssDNA/GO-chitosan/ITO bioelectrode [24].

DNA probe (5'NH₂-ssDNA probe) (**Figure 15**). This DNA biosensor exhibited good ability to detect both complementary and non-complementary target. The linear range of detection was 10 fM–50 nM and the detection limit was 10 fM.

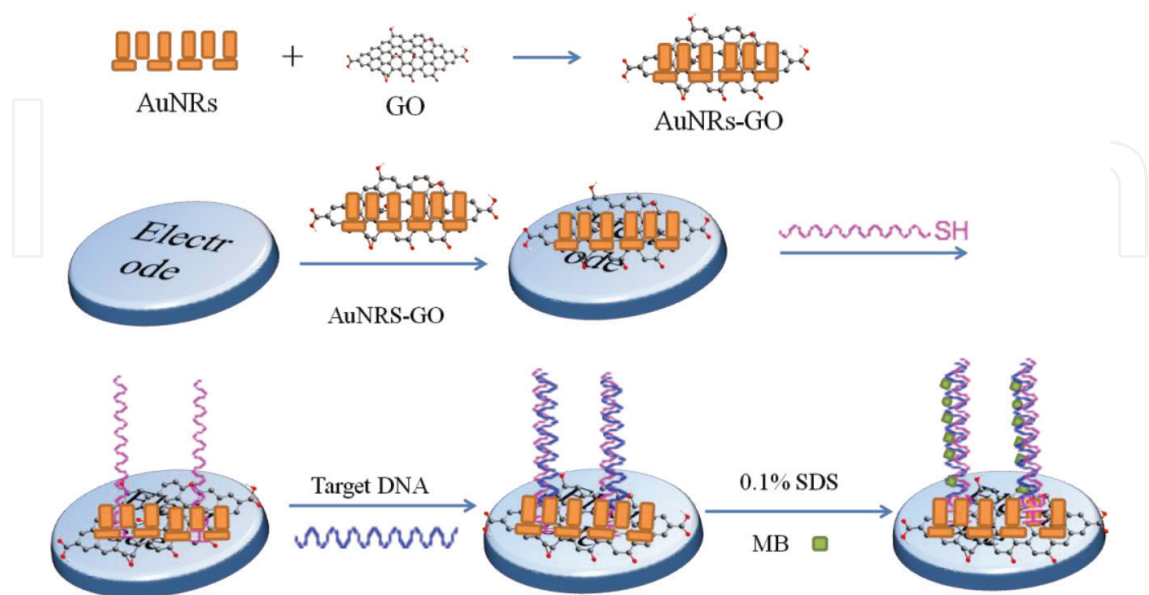


Figure 16. The scheme of DNA/AuNRs/GO biosensor [25].

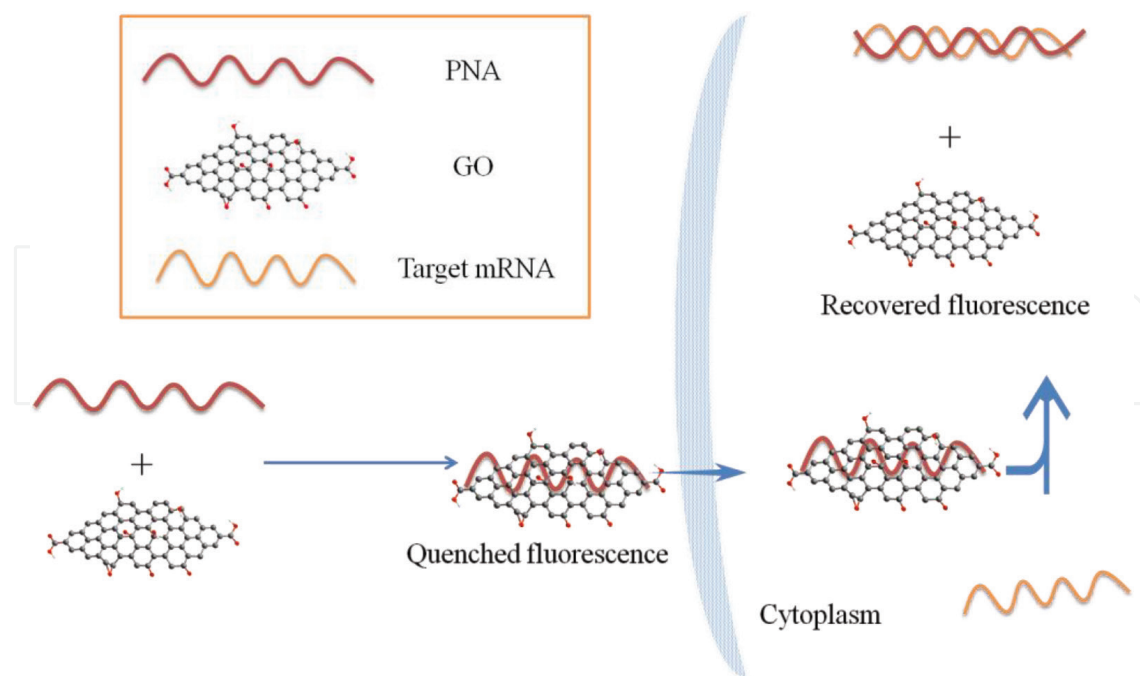


Figure 17. The scheme of PNA/NGO biosensor [26].

Zhang et al. [25] decorated gold nanorods (Au NRs) onto GO sheets and constructed a DNA biosensor (**Figure 16**). The AuNRs were prepared via a seed-mediated method and then composited with GO via electrostatic self-assembly. This biosensor exhibits significant selectivity and can distinguish complementary DNA in the presence of the 100-fold amount of single-base mismatched DNA.

Min et al. [26] designed a nano graphene oxide (NGO) based miRNA biosensor on evaluating target miRNA expression levels in living cells (**Figure 17**). The dye-labeled peptide nucleic acid (PNA) probes were binding onto the surface of NGO. In this biosensor, NGO and PNA acted as fluorescence quencher and probe, respectively. The miRNA expression levels can be evaluated by detecting the fluorescence quenching of the dye-labeled on PNA. The results showed that the biosensor exhibited a low detection limit (1 pM) and can detect the dynamic change in expression levels of the specific miRNA in stem cell differentiation [26].

4. Conclusion and outlook

Graphene oxide is one of many unique carbon materials, which displayed potential applications in the development of next-generation biosensors owing to its various physical and chemical properties. The functionalization of GO leads to the adsorption of various biomacromolecules, including enzymes such as glucose oxidase, horseradish peroxidase, laccase, and nucleic acids such as DNA and RNA for biosensing applications. The major prospect to be addressed in the future is the increasing demand for the engineering of biosensors

based on GO that allow monitoring and detecting analytes with high selectivity and sensitivity at low cost. GO-based biosensors should also be fabricated as point-of-care devices for better in situ clinical diagnosis or as an in-situ sensing platform for environmental analysis.

Acknowledgements

High-Level Talent Start-Up Research Project of Foshan University (gg07016).

Conflict of interest

The authors declare no competing financial interest.

Notes/Thanks/Other declarations

The authors want to thank Bao-Yan Yang, Wei-Ming Gu, and Lin-Hao Lai from FoSU for their kindly help on editing presentation schemes and figures.

Acronyms and abbreviations

AP	1-aminopyrene
5'NH ₂ -ssDNA probe	5'-amine labeled single-stranded (ss) DNA probe
Cyt <i>c</i>	cytochrome <i>c</i>
DNA	deoxyribonucleic acid
ERGO	electrochemically reduced graphene oxide
Fc	ferrocene
FcCHO	ferrocenecarboxaldehyde
GCE	glassy carbon electrode
Au NRs	gold nanorods
GOD	glucose oxidase
GO	graphene oxide
GP	graphene platelet
G-AuNP	graphene-gold nanocomposite

HRP	horseradish peroxidase
Lac	laccase
MWNTs	multiwall carbon nanotubes
ABEI	N-aminobutyl-N-ethylisoluminol
NGO	nano graphene oxide
RGO	reduced graphene oxide
RNA	ribonucleic acid
S/N	signal-to-noise ratio
SPCE	screen printed carbon electrode

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