Analytica Chimica Acta 985 (2017) 24–40

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Review

Designed graphene-peptide nanocomposites for biosensor applications: A review

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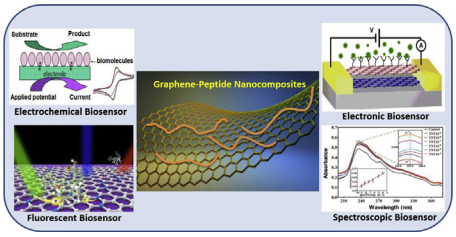
HIGHLIGHTS

- A comprehensive review on the fabrication and application of graphene-peptide nanocomposites was presented.
- The design of peptide sequences for biofunctionalization of various graphene materials was presented.
- Multi-strategies on the fabrication of biosensors with graphene-peptide nanocomposites were discussed.
- Designed graphene-peptide nanocomposites showed wide biosensor applications.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 April 2017
Received in revised form
20 June 2017
Accepted 30 June 2017
Available online 6 July 2017

Keywords:
Graphene
Peptide
Biofunctionalization
Nanocomposites
Biosensors

ABSTRACT

The modification of graphene with biomacromolecules like DNA, protein, peptide, and others extends the potential applications of graphene materials in various fields. The bound biomacromolecules could improve the biocompatibility and bio-recognition ability of graphene-based nanocomposites, therefore could greatly enhance their biosensing performances on both selectivity and sensitivity. In this review, we presented a comprehensive introduction and discussion on recent advance in the synthesis and biosensor applications of graphene-peptide nanocomposites. The biofunctionalization of graphene with specifically designed peptides, and the synthesis strategies of graphene-peptide (monomer, nanofibrils, and nanotubes) nanocomposites were demonstrated. On the other hand, the fabrication of graphene-peptide nanocomposite based biosensor architectures for electrochemical, fluorescent, electronic, and spectroscopic biosensing were further presented. This review includes nearly all the studies on the fabrication and applications of graphene-peptide based biosensors recently, which will promote the future developments of graphene-based biosensors in biomedical detection and environmental analysis.

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http://dx.doi.org/10.1016/j.aca.2017.06.054
0003-2670/

Contents

1. Introduction

Graphene is a one-atom-thick two-dimensional (2D) nanomaterial with innovative structural, electrical, mechanical, thermal, and optical properties [1,2]. Some related graphene materials in the graphene family, like graphite surface from highly oriented pyrolytic graphite (HOPG) [3], graphene layer created by chemical vapor deposition (CVD) [4], chemically prepared graphene oxide (GO) [5], reduced graphene oxide (RGO) [6], and graphene quantum dots (GQDs) [7], have been widely used for the applications in reinforced materials [8], nanodevices [9], energy materials [10], catalyst materials [11], biomedical materials [12], and biosensors [13,14].

Graphene-based materials show great advantages for using as a biosensing platform than other traditional nanomaterials [14]. They have various nanostructures (GQDs, nanotubes, nanobelts, nanosheets, nanoscaffolds, and others) [15,16] with enhanced specific surface area, unique conductivity, excellent capability to bind with biomolecules, and easy chemical modification (especially with GO) [17,18]. In addition, GO and RGO have exhibited the ability to quench the fluorescence of dye labels, and therefore have been widely used for fluorescent sensors [19]. If only graphene materials were used for the fabrication of sensors, some conditions cannot be well-controlled. For example, in one way the biocompatibility of graphene materials is very low [20], which will affect the biorecognition process. Most importantly, the sensitivity and selectivity of graphene-based sensors are limited. Firstly, the electrocatalytic activity of pure GO and RGO is very low, and sometimes metal and metal oxide nanoparticles (NPs) have been conjugated with graphene for improving the electrochemical performances [21]. Secondly, graphene materials are easy to form aggregations, which will reduce the conductivity of graphene-based electrode materials. Thirdly, as graphene shows various interactions with different biomolecules [17], it is hard to evaluate the selectivity of graphene-based sensors if there is no biofunctionalization of graphene.

To extend the sensing applications of graphene materials with enhanced performances, some biomolecules like DNA [22,23], protein [24], enzyme [25], and peptide [26] have been conjugated onto graphene materials for fabricating novel biosensors, among which the modification of graphene with peptide for the creation of graphene-peptide nanocomposites has exhibited extremely important roles [17,27]. Firstly, the peptide chain can provide improved biocompatibility for graphene materials, which makes it possible to detect cells, proteins, and enzymes in live cells. Secondly, the design of peptide sequences could improve the selectivity of biosensors. For example, the specific interaction between analyte and peptide chain as well as the analyte-caused cleavage of peptide chain can promote the unique selectivity of sensors. Thirdly, peptide provides easy modification and strong binding with graphene surface by motif design. Finally, peptide shows self-assembly ability to form peptide superstructures (PS) like nanofibrils (PNFs), nanowires (PNWs) and nanotubes (PNTs) [28–30], which mediated the development of some novel graphene-based nanomaterials for various biosensors [31].

Previously, a few review articles on the biological interactions between graphene and biomolecules, as well as the sensing application of graphene-based materials have been reported. For example, Sanchez et al. demonstrated several interaction modes between graphene-family materials and nucleic acids, lipid bilayers, and small molecular drugs and dyes, and discussed the specific material properties related to the biomolecular and cellular interactions [32]. Ma et al. summarized the studies (before 2012) on the fabrication of graphene- NP nanocomposites for optical and electrochemical biosensors [33]. In another case, Liu and coworkers discussed all covalent and non-covalent strategies for the surface modification of graphene and demonstrated the molecularly engineered graphene surface for electrochemical, electrical, and optical sensing applications [34]. Although many works on the biological modification of graphene with macromolecules have been summarized, there are still much space left on the modification of graphene with designed peptide molecules for various biosensing applications due to the unique functions and diversity of

peptides.

In this review, we focused on the design of peptide sequence for the binding with graphene to fabricate novel and functional graphene-peptide nanocomposites in one way, and in another way we demonstrated and discussed on the biosensor applications of graphene-peptide nanocomposites. For the first attempt, various strategies for constructing electrochemical, fluorescent, electronic, and spectroscopic biosensors with graphene-peptide nanocomposites were discussed in detail especially. This work will be very helpful for understanding the design of graphene-binding peptides, the creation of novel graphene-peptide nanocomposites, and the fabrication of high performance biosensors for biomedical and bioanalytical applications.

2. Fabrication of graphene-peptide nanocomposites

Peptide molecules can be conjugated onto graphene surface by both covalent (amide reaction) and non-covalent (for example hydrophobic, electrostatic, and π-π stacking) interactions [17,18,35]. In this section, firstly we will introduction the conjugation strategies (covalent and non-covalent) of graphene-peptide nanocomposites, and then present and discuss mainly the designing of peptide sequences for non-covalently binding peptide onto graphene surface to create graphene-peptide monomer (graphene-PM), graphene-peptide superstructure (graphene-PS), and graphene-peptide-NPs nanocomposites.

2.1. Conjugation strategies of graphene-peptide nanocomposites

In order to improve the biosensing sensitivity of graphene-peptide nanocomposite-based biosensors, it is necessary to conjugate more peptide probe molecules onto graphene surface. Due to the unique 2D hexagonal honeycomb crystalline structure of graphene and the rich functional groups at their surface or edges, it is possible to modify graphene materials with peptides through both covalent and non-covalent conjugation strategies [17,34,36].

2.1.1. Covalent conjugation

Previously, a few typical strategies (pyrene- and coupling reaction-based methods) have been widely utilized for the modification of graphene with peptides. For the pyrene-based conjugation, firstly a bifunctional pyrene-based N-hydroxysuccinimide ester (NHS) molecule was utilized to modify graphene surface by pyrene-graphene interaction, and then peptide could be bound onto graphene by the formation of an amide bond [37]. For instance, Komori et al. reported the covalent binding of heme peptide onto graphene by covalent modification [38]. Firstly, graphene was modified with 1-pyrenebytyric acid N-hydroxysuccinimide ester (PANHS), and then heme peptide could be conjugated onto graphene by covalent reaction.

Another conjugation strategy of graphene-peptide nanocomposites is based on the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and NHS reaction. In this reaction, the carboxyl groups of graphene surface or edges are reacted with the amino groups of peptide molecules via a well-known ENC/NHS coupling reaction. This method could be used for the covalent binding of amino group-having molecules (NH_2-DNA, protein, peptide, and others) onto graphene [34,39–41]. For example, Kanchanapally and co-workers reported the conjugation of an antimicrobial peptide onto GO membrane by the EDC/NHS strategy [40].

The binding of peptides onto graphene surface by covalent modification could produce graphene-peptide nanocomposites with high stability. However, the covalent bonding could cause inevitable rehybridization of sp2 carbon atoms to sp3 configuration, which will greatly affect the electronic and optical properties of graphene materials. Therefore, more and more alternative non-covalent conjugation strategies have been developed for the creation of graphene-peptide nanocomposites for various applications [17].

2.1.2. Non-covalent conjugation

The non-covalent conjugation of peptides onto graphene surface could be achieved by a few main modes of actions like the π-π interaction, electrostatic force, hydrophobic force, and hydrogen bonding. Compared to the covalent conjugation, the non-covalent one does not need to destroy the original structure and properties of graphene, and therefore the 2D morphology, conductivity, and optical property of graphene can be retained.

The π-π interaction between peptide molecules and graphene surface is the widely used strategy for creating graphene-peptide nanocomposites. For instance, Wu and co-workers designed a pyrene-linked peptide and fabricated GO-peptide nanohybrids by the π-π interaction between pyrene groups with graphene surface [42]. Feng et al. synthesized a isothiocyanate-labeled peptide and conjugated the created peptide onto GO surface through π-π and electrostatic interactions [43]. In another case, Wang et al. reported the binding of phenylalanine-rich peptide with graphene surface, and found that the π-π interaction between phenylalanine and graphene could promote the formation of highly water-soluble graphene-peptide nanocomposites [44].

The electrostatic interaction could also be utilized for creating graphene-peptide nanocomposites. For example, Wang et al. fabricated the RGO-PNF nanocomposites by the electrostatic conjugation between polymer-modified positive-charged RGO and negative-charged PNFs [45]. In another case, Zeng and co-workers reported the binding of Vpr13-33 peptide onto GO surface by molecular simulations, and proved that the binding is ascribed to both electrostatic and π-π interactions [46].

Due to the flexibility to design a peptide sequence, it is possible to select specific amino sequence that with strong binding towards graphene surface and to fabricate graphene-peptide nanocomposites easily. Usually, besides the π-π and electrostatic interactions, van der Walls and hydrophobic interactions contribute to the binding of peptide onto graphene [47], which is much weaker than the interactions (mainly π-π interaction) between ssDNA and graphene surface.

Previously, a few nice review articles on the functionalization of graphene with biomolecules for various applications have been reported [17,34,35]. It is recommended for the readers to study these references if they want to get more details on the biomolecular engineering of graphene with biomolecules.

2.2. Graphene-PM nanocomposites

The Naik group first investigated the chemical functionalization of graphene surface with the phage displayed peptides [48], and found that some designed peptides, such as GAMHLPWHMGTL [47,48], EPLQLKM [48–50], HSSYWYAFNNKT [49,50] showed preferential binding onto the edges and plane of graphene. For example, in a typical study Katoch and co-workers investigated the binding behavior of GAMHLPWHMGTL towards graphene and graphite surface with multi structural characterizations and molecular dynamics (MD) simulations [47]. At lowest potential energy, the conformation of native peptide molecule in vacuum exhibited a highly ordered α-helix structure (Fig. 1a), and the ordered helical structure in power form was transformed to a distorted α-helix structure when putting the peptide into a water box (Fig. 1b). The peptide formed a complex reticular structure when adsorbing onto graphene surface, showing a helical conformation (Fig. 1c) that is different from the α-helix structure in Fig. 1a. The binding of this

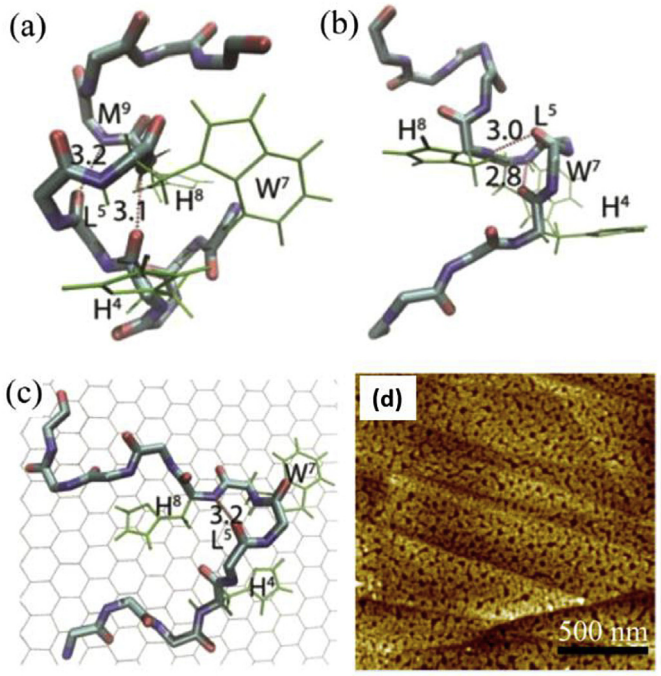

Fig. 1. Binding of graphite-specific peptide onto graphene surface: (a–c) MD simulation on the conformations of peptide in (a) vacuum and (b) water, as well as on (c) graphene sheet. (d) AFM image of peptide monolayer on graphene surface. Reproduced with permission from Ref. [47]. Copyright 2012, American Chemical Society.

designed peptide onto the freshly cleaved graphite surface indicated the formation of a mesh-like monolayer on graphite surface, as identified by atomic force microscopy (AFM) (Fig. 1d). Their studies provided important experimental and theoretical methods to identify graphene-binding peptides, guiding the way to design novel graphene-peptide biosensors with high sensitivity and selectivity.

In another study, Qu and co-workers also investigated the adsorption, conformation transition, and dimerization of α-helix peptide (DELERRIRELEARIK) on the graphene surface at the atomic level by the MD simulations [51]. They found that the graphene surface could induce the α-helix to β-sheet conformation transition of this peptide and the subsequent β-sheet polymerization. Other graphene-binding peptides like GGGRKRIHIGPGPAFYTT, GGGNSWGCAFRQVC [52] and SGVG(VPGVG)$_{50}$VPGHNWYHWWPH [53] also showed strong binding affinity with graphene surface.

2.3. Graphene-PS nanocomposites

For the fabrication of graphene-PS nanocomposites, two potential strategies could be utilized. Firstly, graphene-binding peptide with designed sequence could be adsorbed onto the graphene surface and then the graphene surface could induce the molecular self-assembly to PNWs and PNFs. For example, Chen and co-workers investigated the self-assembly of ionic-complementary peptides with sequence of AEAEAKAKAEAEAKAK [54] and FEFEFKFKFEFEFKFK [55] and the formation of PNWs on graphite surface. Li et al. reported the assembly of Aβ$_{33-42}$ peptide (GSGLMVGGVVIAAAA) and the formation of PNFs on GO [56]. In a recent study, Hayamizu et al. [57] investigated the binding and self-assembly of a few graphene-binding dodecapeptides (for instance wild-type IMVTESSDYSSY and its mutants) on 2D atomic single layer materials like graphite and graphene surface (Fig. 2a). They found the wild-type peptide showed both strong binding and self-assembly ability on graphite, graphene, and other 2D materials. The AFM characterization indicated that a monolayer was formed on graphite surface (Fig. 2b), but the PNW structure with six-fold symmetry was formed on graphene monolayer (Fig. 2c).

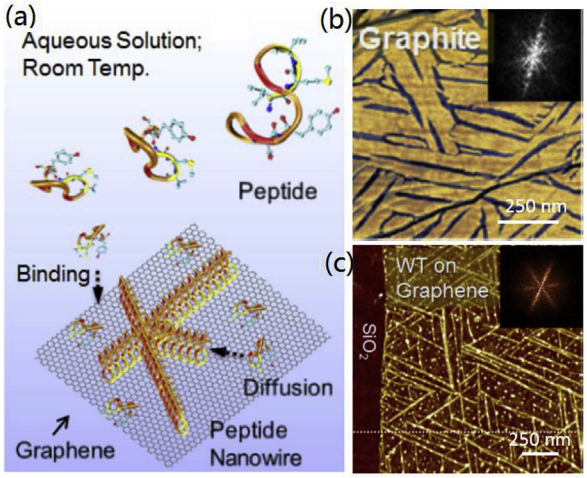

Fig. 2. Preparation of graphene-PS nanocomposites: (a) Self-assembly mechanism of peptide on graphene. (b,c) AFM images of graphene-binding peptide (IMVTESSDYSSY) on (b) graphite and (c) graphene monolayer. Reproduced with permission from Ref. [57]. Copyright 2016, Nature Publisher Group.

Secondly, graphene-PS nanocomposites could be fabricated by binding the pre-assembled PS (PNFs, PNWs, and PNTs) onto graphene materials. For instance, previously, Han et al. reported the synthesis of graphene-PNT hybrid nanocomposites with core/shell nanowire structure by using diphenylalanine (FF) PNTs with RGO [58]. Adhikari and co-workers reported the self-assembly formation of PNFs with short peptides (Fmoc-YD, Fmoc-FD, and pyrene-FFA-OMe) could be incorporated with RGO to form RGO-PNF hydrogel [59] and organogel [60].

2.4. Graphene-peptide-NPs hybrid nanocomposites

Based on the created graphene-PM and graphene-PS nanocomposites, novel hybrid nanomaterials with specific functions could be synthesized by adding various NPs onto the graphene-peptide materials. For example, previously the non-covalent biofunctionalization of GO with PMs (GAMHLPWHMGTL and FEFEFKFKFEFEFKFK) and the subsequent synthesis of GO-PM-PtNPs [61] and GO-PM-AgNPs [44] nanocomposites for biosensor applications have been reported. By using the molecular self-assembly and graphene-binding properties of designed peptides, several PNF nanostructures have been conjugated onto GO or RGO and utilized for further synthesis of graphene-PNF-NPs hybrid nanocomposites [45,62–64]. For example, Wang et al. demonstrated that the motif-designed trifunctional peptide (RGDAEAKAEAKY-WYAFAEAKAEAKRGD) could form PNFs to be decorated with GQDs for simultaneous targeting and imaging tumor cells [63]. In a recent study, they further reported the synthesis of GO-PNF-GQD nanocomposites by peptide design and controllable supramolecular self-assembly [64], as shown in Fig. 3. In their study, a bifunctional peptide sequence, AEAKAEAKYWYAFAEAKAEAK, was designed to create multifunctional PNFs, in which the motif of AEAKAEAK is responsible for the self-assembly formation of PNFs, and the center motif of YWYAF is for the GO- and GQD-specific binding. Therefore, the self-assembled PNFs could first bind with GQDs (Fig. 3a) and then GO for the final formation of GO-PNF-GQD nanocomposites (Fig. 3b).

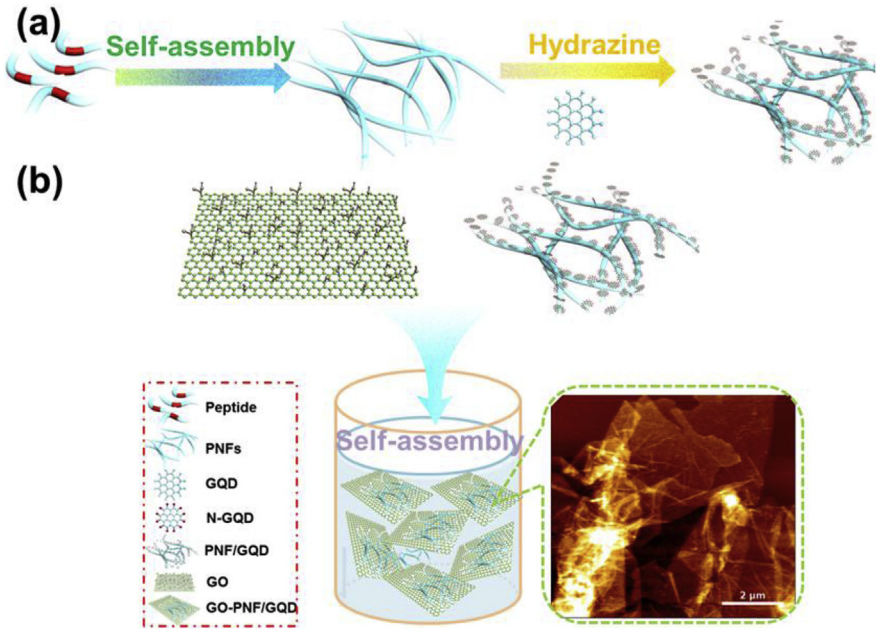

Fig. 3. Synthesis of GO-PNF-GQD nanocomposites by self-assembly: (a) Molecular design and formation of PNF-GQD nanohybrids, and (b) synthesis of GO-PNF-GQD nanocomposites. Reproduced with permission from Ref. [64]. Copyright 2017, Wiley-VCH Verlag GmbH &Co.

Based on the above discussion, it can be known that the design of peptide sequence plays crucial role for the biofunctionalization of graphene-based materials. To make it more clear, we summarized the potential amino acid sequences with strong binding to graphene surface for the formation of graphene-peptide nanocomposites (Table 1).

3. Biosensor applications of graphene-peptide nanocomposite platforms

In this section, the biosensor applications of graphene-peptide nanocomposites in electrochemical, fluorescent, electronic, and spectroscopic detections will be demonstrated in detail.

3.1. Electrochemical biosensors

Electrochemical biosensors show great advantages than other biosensors, such as easy operation, flexible design of sensor architecture, high sensitivity, and low cost. By using graphene-peptide nanocomposites, electrochemical biosensors based on four types of electrochemical techniques, cyclic voltammetry (CV) [38,44,45,61,64–69], differential pulse voltammetry (DPV) [70,71], electrochemical impedance spectroscopy (EIS) [72,73], and electrochemiluminescence (ECL) [74,75] have been fabricated for various sensing applications.

3.1.1. CV-based biosensors

CV is one of the important electrochemical techniques that can

Table 1
Summary on the graphene-peptide (PS and PM) nanocomposites by designing different peptide sequences.

Composites	Graphene Type	Peptide Sequence	Interactions	Ref.
Graphene-PM	graphene/graphite	GAMHLPWHMGTL	π-π stacking	[47,48]
	graphene	EPLQLKM	π-π stacking	[47,49]
	graphite	HSSYWYAFNNKT	π-π stacking	[49,50]
	graphene	DELERRIRELEARIK	hydrophobic	[51]
	GO	GGGRKRIHIGPGPAFYTT	π-π stacking &	[52]
	GO	GGGNSWGCAFRQVC	hydrophobic	[52]
	RGO	SGVG(VPGVG)$_{50}$VPGHNWYHWWPH	hydrophobic	[53]
Graphene-PNF/PNT	graphite	AEAEAKAKAEAEAKAK	hydrophobic	[54]
	graphite	FEFEFKFKFEFEFKFK	π-π stacking	[55]
	GO	GSGLMVGGVVIAAAA	hydrophobic	[56]
	graphene/graphite	IMVTESSDYSSY	hydrophobic	[57]
	RGO	FF	π-π stacking	[58]
	RGO	Fmoc-YD, Fmoc-FD	π-π stacking	[59]
	RGO	pyrene-FFA-OMe	π-π stacking	[60]
Graphene-PNF-NPs	GO	GAMHLPWHMGTL	π-π stacking	[61]
	GO	FEFEFKFKFEFEFKFK	π-π stacking	[44]
	RGO	VIAGASLWWSEKLVIA	electrostatic	[45]
	GO	AEAKAEAKYWYAFAEAKAEAK	π-π stacking	[62]
	GQDs	RGDAEAKAEAKYWYAFAEAKAEAKRGD	π-π stacking	[63]
	GO + GQDs	AEAKAEAKYWYAFAEAKAEAK	π-π stacking	[64]

be used to study the oxidation and redox reactions of electroactive species, and therefore can be utilized for fabricating various electrochemical sensors and biosensors with easy operation and quick determination [21,76]. As one of the important components of graphene-peptide nanocomposites, peptide has two main typical actions to fabricate CV-based biosensors.

Firstly, peptide could be used to fabricate enzymatic biosensors. For example, Komori et al. reported that a heme undecapeptide modified carbon nanotubes (CNTs)/graphene hybrid film could be used for highly sensitive CV-based electrochemical detection of hydrogen peroxide (H_2O_2) [38]. Li and co-workers reported that graphene-PNF nanocomposites showed enhanced amperometric biosensing of dihydronicotinamide adenine dinucleotide (NADH) with a detection limit of 1.2 μM [65]. Wang et al. demonstrated that poly(Lys)-modified graphene could be further functionalized with hemoglobin for enzymatic detection of H_2O_2 [66]. Qu and co-workers demonstrated that the tyrosinase-modified graphene-silk peptide nanocomposites could be a new material to fabricate sensitive amperometric biosensor for phenolic compounds [67].

Secondly, peptide could be used as binding reagent to modify graphene surface and then directly used as non-enzymatic biosensors [68,69]. In a typical study, Guo et al. reported that the RGD-peptide functionalized graphene film could be used for the real-time detection of nitric oxide molecules that released from the live cells [68]. In their study, the selected peptide has the ability to bind with cells with good cell adhesive activity. GO was first non-covalently modified with an aromatic chemical, pyrenebutyric acid (PA), and then a PA-functionalized RGO film was fabricated by a direct assembly approach through filtration. After that, RGD peptide was bound onto the surface of PA-RGO film. Finally, the RGD-PA-RGO film was formed and immersed into the cell medium for cell adhesion (Fig. 4a). Therefore, the concentration of nitric oxide released from the live cells could be real-time detected. When there was no nitric oxide, the obtained CV curve exhibited only typical capacitive behavior (curve 1 of Fig. 4b). In the cell medium with 10 μM nitric oxide, the RGD-PA-RGO film showed good electrocatalytic ability towards the oxidation of nitric oxide, as shown in the curve 2 of Fig. 4b. The logarithmic plot of the current and the concentration of nitric oxide indicated that the constructed biosensor had a linear detection range from nM to μM with a detection limit of 25 nM (Fig. 4c). In addition, the fabricated RGD-PA-RGO film-based biosensor showed a good selectivity on the detection of nitric oxide against those species with a similar oxidation potential to nitric oxide (Fig. 4d), which could be ascribed to the electrostatic repelling towards negative charged nitrite and ascorbic acid caused by the negative charged carboxyl groups on the surface of the hybrid film. This work provides a novel idea to monitor the adhesion and growth of live cells on biomaterials and measure the time-dependent cell density by detecting the released nitric oxide.

In another case, Castillo and co-workers reported the detection of cancer cells by using a PNT-folic acid (PNT-FA)-modified graphene electrode [69]. In the presence of $[Fe(CN)_6]^{3-/4-}$, the binding of cancer cells onto the electrode surface caused an obvious decrease of the faradaic current, as indicated by the CV tests. The developed PNT-FA-graphene based sensor showed a detection limit of 250 HeLa cells/mL with an incubation time of 10 min. In addition, this biosensor was able to detect the folate receptors as low as 8 nM with a linear range to 13 nM towards.

Besides, the created graphene-peptide nanocomposites could be further modified with NPs for non-enzymatic biosensors. For instance, the peptide sequences of GAMHLPWHMGTL [61] and FEFEFKFKFEFEFKFK [44] have been utilized to modify GO non-covalently and chemically synthesize PtNPs and AgNPs for CV-based sensing H_2O_2. By using self-assembled PNFs, Su et al. created RGO-PNF-AgNPs [45] and GQD-PNF-GO [64] nanohybrids and further fabricated biosensors for sensing H_2O_2.

3.1.2. DPV-based biosensors

In the DPV measurement, the current of electrode is measured with the change of applied potential, and therefore the difference of current can be plotted as a function of potential [77]. This technique has been widely used for getting the optimal applied potential for the CV-based biosensors [78,79]. The addition of analytes into a sensor system causes the change of current, which could be linearly fitted to calculate the corresponding detection limit and linear range of the sensor [78].

In a typical study, Chen et al. developed a sensitive assay for the detection of caspase-3 activity with GO-peptide composite-assisted electrochemical DPV sensing [70]. Firstly, an acetylated peptide (Ac-GGHDEVDHGGGC) and 6-hydroxy-1-hexanethiol (MCH) were utilized to modify the gold electrode to create the well-aligned peptide monolayer. In this case, GO had no chance to be attached onto peptide because the formation of amide bonds between GO and peptide was blocked. However, the peptide chain can be cleaved in the presence of caspase-3, causing the exposure of the free N-terminal amino group (Fig. 5a). Therefore, GO could be conjugated onto peptide chain by using the NHS/EDC couple linking. Finally, the electroactive molecule, methylene blue (MB), was added to bind with GO through noncovalent π-π interaction. The DPV signals before and after adding MB could be obtained. After adding caspase-3 with various concentrations, the intensity of the DPV peak increased in response to caspase-3 (Fig. 5b) within a linear range from 0.1 to 100 pg/mL (Fig. 5c). The fabricated DPV biosensor showed a low detection limit of 0.06 pg/mL, which is about much more sensitive (about 4 orders of magnitude) than the fluorescent biosensors [80]. The signal amplification of this biosensor is ascribed to the large surface area to volume ratio of GO and the strong interactions between GO and MB.

In another case, Wang and co-workers reported the fabrication of RGO-silk peptide (RGO-SP) composite-based electrochemical DPV immunosensor for the detection of prostate specific antigen (PSA) [71]. The immunosensor was produced by mixing RGO-SP nanocomposites and monoclonal antibody of PSA (anti-PSA) and then depositing onto a GCE. After binding with PSA, the peak current was found to decrease at an applied potential of 0.24 V. The DPV measurements indicated that the fabricated PSA immunosensor has two separated linear detection ranges of 0.1–5.0 and 5.0–80.0 ng/mL with a detection limit of 53 pg/mL. The high performance of this immunosensor is due to the integration of SP with GO, providing high specific surface area, high conductivity, and abundant active sites for further modification.

3.1.3. EIS-based biosensors

EIS is an electrochemical method that highly suitable for measuring the electronic changes of electrode surface, which is capable for probing the complex bio-recognition events with sensitive, label-free, and rapid analysis [81]. By using the EIS technique, each step of the bio-recognition reaction could be readily analyzed by measuring the electron-transfer caused resistance change.

Graphene-peptide nanocomposites could be utilized for the fabrication of EIS biosensors. Previously, Feng and co-workers realized the label-free EIS detection of cyclin A_2 in cancer cells by using GO sheets functionalized with a hexapeptide (RWIMYF) [72]. To achieve the aim, the GO surface was firstly non-covalently modified with a water-soluble porphyrin molecule, meso-tetra(4-carboxyphenyl)porphyrin (TCPP), and then the specific hexapeptide and Tween 20 were coated onto the TCPP-modified GO surface to create the sensor architecture (Fig. 6a), in which the hexapeptide provides specific binding with cyclin A_2, and Tween 20 could

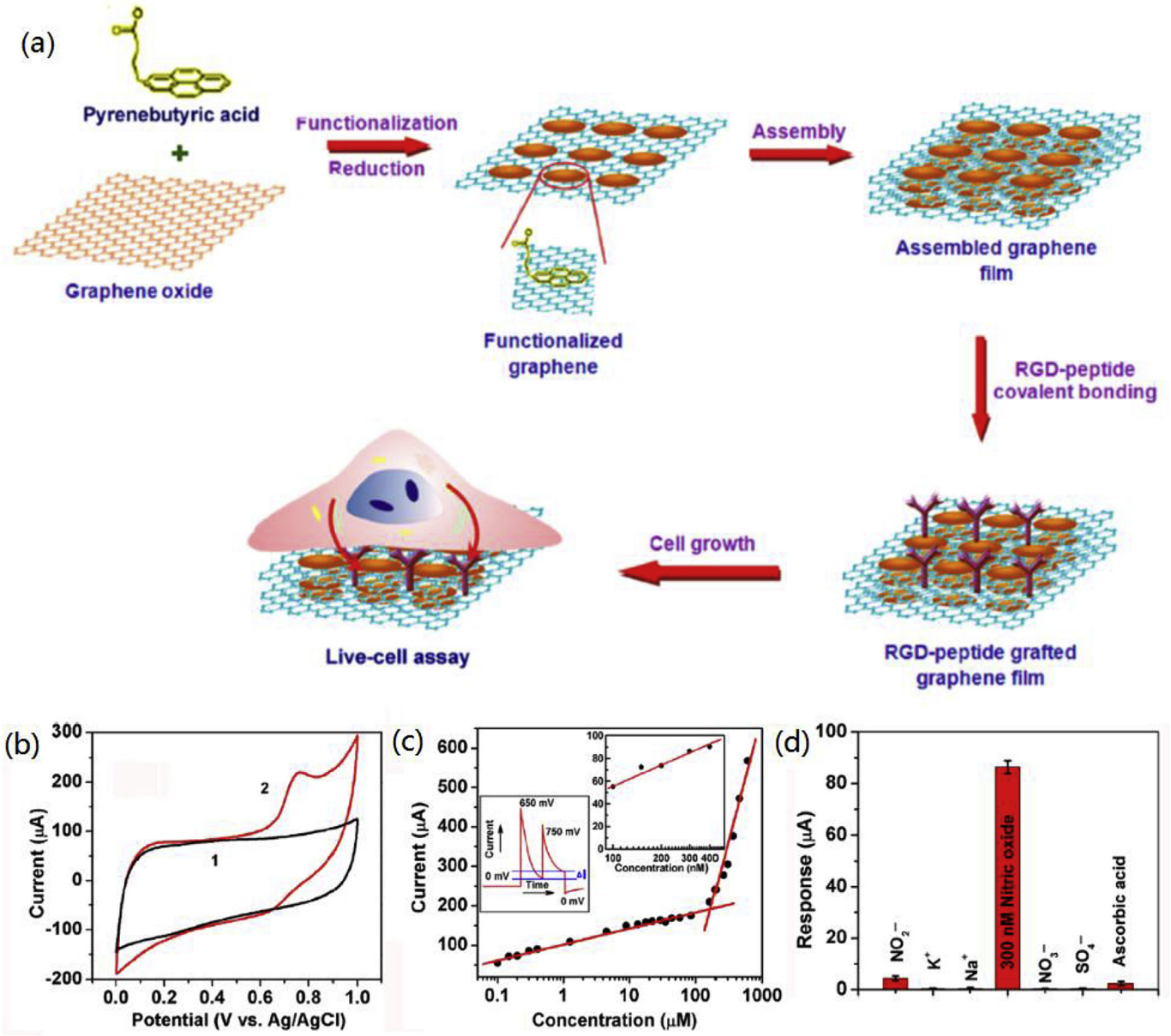

Fig. 4. Graphene-RGD peptide nanocomposites for real-time detection of nitric oxide in live cells. (a) Fabrication and sensing mechanism of biosensor. (b) CVs of graphene-peptide in cell medium in the (1) absence and (2) presence of 10 μM nitric oxide. (c) The linear fitting of the current vs the concentration of nitric oxide. (d) Selectivity of biosensor. Reproduced with permission from Ref. [68]. Copyright 2012, American Chemical Society.

prevent the nonspecific binding of targets (cyclin A_2) with GO surface. After the specific binding of cyclin A_2 onto GO-peptide nanocomposites, the bound cyclin A_2 blocked the accessibility of the redox probe, $[Fe(CN)_6]^{3-/4-}$, onto the electrode surface, causing the changes of EIS signal. The corresponding EIS curving and Nyquist data analysis indicated that the modification of bare GCE with GO, GO-peptide, and subsequently adding cyclin A_2 could change the EIS curves and electron-transfer resistance (R_{re}), as shown in Fig. 6b. When adding cyclin A_2 with increasing concentrations to the biosensor system, the R_{re} value showed linear increasing (Fig. 6c). This EIS biosensor showed a detection limit of 0.32 pM for the sensing of cyclin A_2.

In another work, Gutés and co-workers reported the fabrication of a EIS biosensor based on graphene modified with gold nanoparticles (AuNPs) and designed peptide (Au-graphene-peptide nanocomposite) for sensing decabromodiphenyl ethers (DBDE) [73]. The CVD-prepared graphene was firstly modified with AuNPs and then DBDE-binding peptide (WHWNAWNWSSQQ) to form the Au-graphene-peptide structure. After adding DBDE to the sensor system, the formed Au-graphene-peptide-DBDE structure could prohibit the accessibility of $[Fe(CN)_6]^{3-/4-}$ and cause the EIS signal, as introduced by Feng et al. [72]. The created biosensor showed good linear response of 5% to the saturated limit range from about 5 to 100 ppt.

3.1.4. ECL-based biosensors

It is well known that the ECL generated by an oxidation or reduction reaction on the electrode surface could be a useful tool to investigate the bio-recognition reaction [82], which can be utilized as a novel technique to fabricate biosensors for label-free detection. For instance, previously Wu et al. demonstrated a label-free, ultrasensitive, and highly selective ECL biosensor of cyclin A_2 by

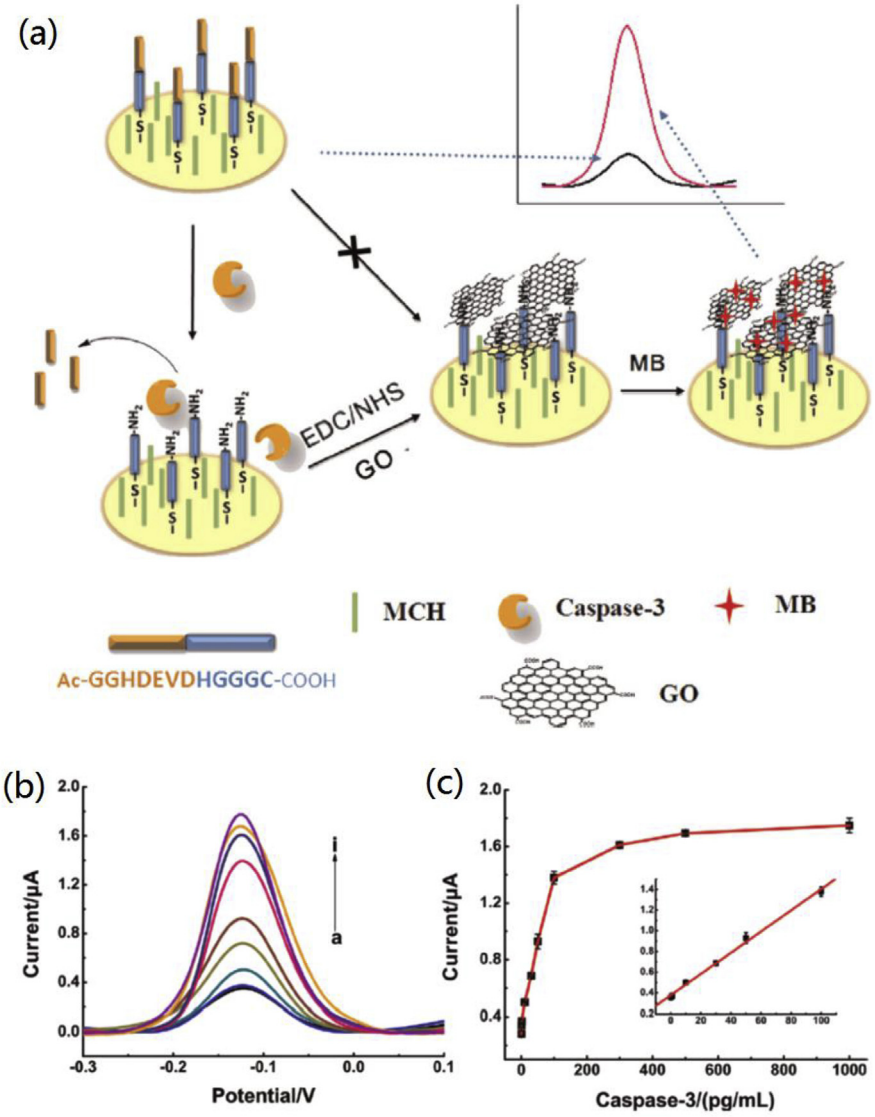

Fig. 5. DPV biosensors based on GO-peptide nanocomposites for sensing caspase-3. (a) Fabrication of GO-peptide biosensors and sensing mechanism. (b) DPV changes with various concentration of protein kinase. (c) Linear fitting. Reproduced with permission from Ref. [70]. Copyright 2015, Elsevier Ltd.

grafting a PEGlyted hexapeptide (PEG-RWIMYF) onto the pre-prepared RGO-NP hybrids [74], as shown in Fig. 7a. Firstly, upconversion nanoparticles (UCNPs) were in-situ synthesized on the RGO surface to form a RGO-UCNPs nanohybrid, which served as the novel ECL emitter. Then the PEG-RWIMYF peptide was linked to the RGO-UCNPs-modified GCE, which acted as the detection probe of cyclin A_2 and the inhibiting agent of non-specific binding events. After adding cyclin A_2 into the biosensing system, the binding of cyclin A_2 with peptide hindered the access of the ECL coreactant ($S_2O_8^{2-}$) to the electrode surface, causing the extremely decrease of ECL signal. With the increasing of the concentration of cyclin A_2 (100 fM-10 nM) in the sensing system, the ECL intensity decreased correspondingly (Fig. 7b), which means that the obtained ECL intensity depended on the surface coverage of cyclin A_2 on the electrode surface. Based on the linear fitting of the obtained ECL data, a detection limit of about 10.5 fM (0.52 pg/mL) was achieved for cyclin A_2 by the fabricated biosensor (Fig. 7c). The fabricated RGO-UCNP-peptide based ECL biosensor displayed great performance for not only directly detecting cyclin A_2 in cancer cell extracts, but also discriminating normal and cancer cells. In addition, this ECL biosensor could be potentially used for the drug screening and early-stage monitoring of cancers.

In another work, Zhao and co-workers reported a novel ECL biosensor based on AuNPs, GQDs, and peptide for the highly sensitive detection of protein kinase activity [75]. The GCE was firstly modified with chitosan and then covalently modified with GQDs, and then peptide with specific binding towards protein kinase A (PKA) was bound onto GQDs by the amide reaction. Finally, PKA and adenosine 5'-triphophate (ATP) were added to cause specific binding with peptide, and AuNPs were then conjugated onto the peptide chain to fabricate biosensor architecture. It was found that the addition of AuNPs could greatly decrease the ECL signal of GQDs due to the ECL resonance energy transfer, but the ECL signal of luminol in solution could be enhanced due to the high electrocatalytic activity of AuNPs, therefore the fabricated ECL biosensor could be used as a dual-potential rationmetric system for monitoring the activity of PKA and developing inhibition assay of PKA simultaneously. The fabricated PKA biosensor showed a linear detection range from 0.01 to 10 U/mL with a detection limit of 0.005 U/mL.

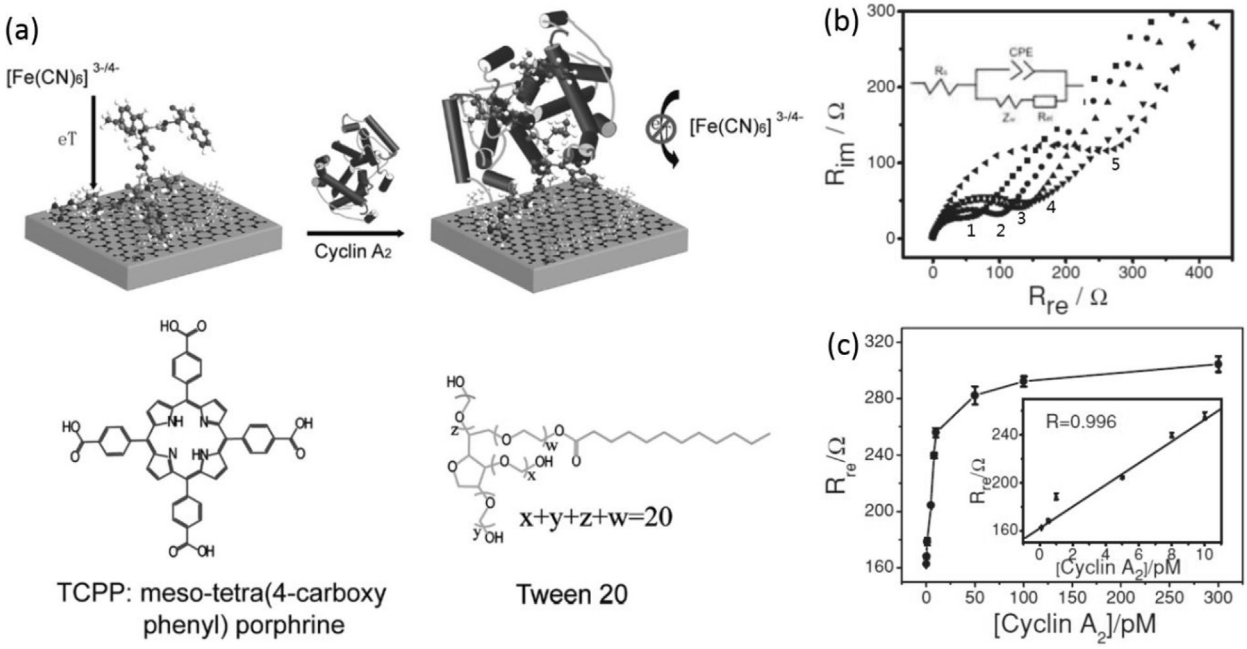

Fig. 6. EIS biosensor for sensing cyclin A_2. (a) Fabrication of GO-peptide EIS biosensor architecture and sensing mechanism. (b) EIS curves with various electrodes: 1) bare GCE, 2) GO modified GCE, 3) GO -peptide modified GCE, 4) GO -peptide modified GCE in Tween 20, and 5) after adding 100 nM cyclin A_2. (c) Nyquist data of the GO -peptide modified GCE after incubating in cyclin A_2 with different concentrations. Inset is the linear fitting. Reproduced with permission from Ref. [72]. Copyright 2012, Wiley-VCH Verlag GmbH &Co.

3.2. Fluorescent biosensors

3.2.1. Biosensors by using graphene as acceptor of fluorescence resonance energy transfer (FRET)

Graphene materials like graphite surface, GO, and RGO are good quencher for various fluorescent labels [83,84], and therefore can be utilized to construct many kinds of fluorescent biosensors for detecting DNA, protein, antigen, antibody, enzyme, cells, and others [85,86]. For the fabrication of graphene-peptide nanocomposite-based fluorescent biosensors by using graphene as quencher (acceptor of FRET), a few alternative strategies could be utilized (Fig. 8).

The first assay is based on the cleavage of peptide by analytes (protein, enzyme, and antigen), which causes the “turn-on” fluorescence of the labels. In this method, the fluorescence-labeled peptide was bound onto the graphene surface, and the fluorescence of labels was turned off, but the cleavage of peptide chain made the “turn-on” fluorescence. For instance, Wang et al. for the first time utilized GO-ZDEVD-fluoromethylketone conjugate as an intracellular biosensor for the detection of caspase-3 [87]. In another typical study, Shi and co-workers demonstrated a GO-peptide nanocomposite based FRET biosensor for the ultrasensitive detection of botulinum neurotoxin A (BoNT/A) [41], and the construction of biosensor architecture is as shown in Fig. 8a. A peptide, SNAP-25 (DEANQRATKMLGSG), was first labeled with green fluorescence protein (GFP), and then was conjugated to GO by covalent reaction to quench the fluorescence. After cleavage by BoNT/A, the GFP labels were released from the GO surface and then “turn on” the fluorescence. This method provides a high selectivity and low detection limit of 1 fg/mL, which was 4–6 orders of magnitude lower than the traditional immunoassays and commercial available FRET kit.

Besides, a lot of other peptide sequences labeled with various dyes, like KCALNNGSGFPRGRAK [26], CALNNGPLGVRGRAK [88], GPLGVRG [89], HSSKLQ [43], DEVD [90], GGWGC [91], and others [92] have been conjugated onto GO or RGO to construct biosensors for sensing protein, enzymes, and their activity. In another study, GO-peptide-QDs (peptide sequences: GKGGLVPPGSGC and GPLGVRGC) nanocomposites has also been utilized for the FRET-based detection of protease activity [93].

Another assay is based on the detachment of fluorescence-labeled peptide from graphene surface due to the stronger interactions between peptide and analytes (protein, cell, antibody, and others) than that of peptide and graphene surface, causing the detected fluorescence from “turn-off” to “turn-on” mode. In a typical case, Feng and co-workers introduced a GO-based fluorescent biosensor for sensing anti-octreotide (AOC) by using fluorescein isothiocyanate (FITC)-labeled octreotide [94]. The FITC-octreotide showed high affinity to bind with GO surface, but the specific binding with AOC caused the release of FITC-octreotide from the GO surface, exhibiting “turn-on” fluorescence, as shown in Fig. 8b. This biosensor reveals a detection limit of 2 ng/mL towards AOC. With the similar strategy, FITC-labeled peptides like HAKRRLIF [95] and PPLRINRHILTR [96] have been utilized to construct GO-based fluorescent biosensors for detecting cyclin A_2 and human chorionic gonadotropin, respectively. Other kind of dye-labeled peptides, such as pyrene-RGD [97], pyrene-GGGRKRIHIGPGPAFYTT [52], pyrene-GGGNSWGCAFRQVC [52], and KKNYSSSISSIHC-tetramethylrhodamine [98] have also been used to create GO-peptide nanocomposite-based fluorescent biosensors for detecting integrin αvβ3 [97], protein [52], and lipopolysaccharide [98], respectively.

In a recent study, Sun et al. reported a novel “turn-off” biosensor to detect the aline phosphatase (ALP) activity based on GO-peptide nanocomposites [99]. They found that the designed FITC-GGGYpR has no affinity with GO surface, and the fluorescence was on, but the remove of phosphate group from the peptide chain with enzymatic hydrolysis caused the formation of FITC-GGGYR, which has strong interaction with GO surface. Therefore, the fluorescence signals of FITC could be turned off by GO. The fabricated fluorescent ALP biosensor showed a linear detection range from 0.2 to 5 nM with a detection limit of 0.08 nM.

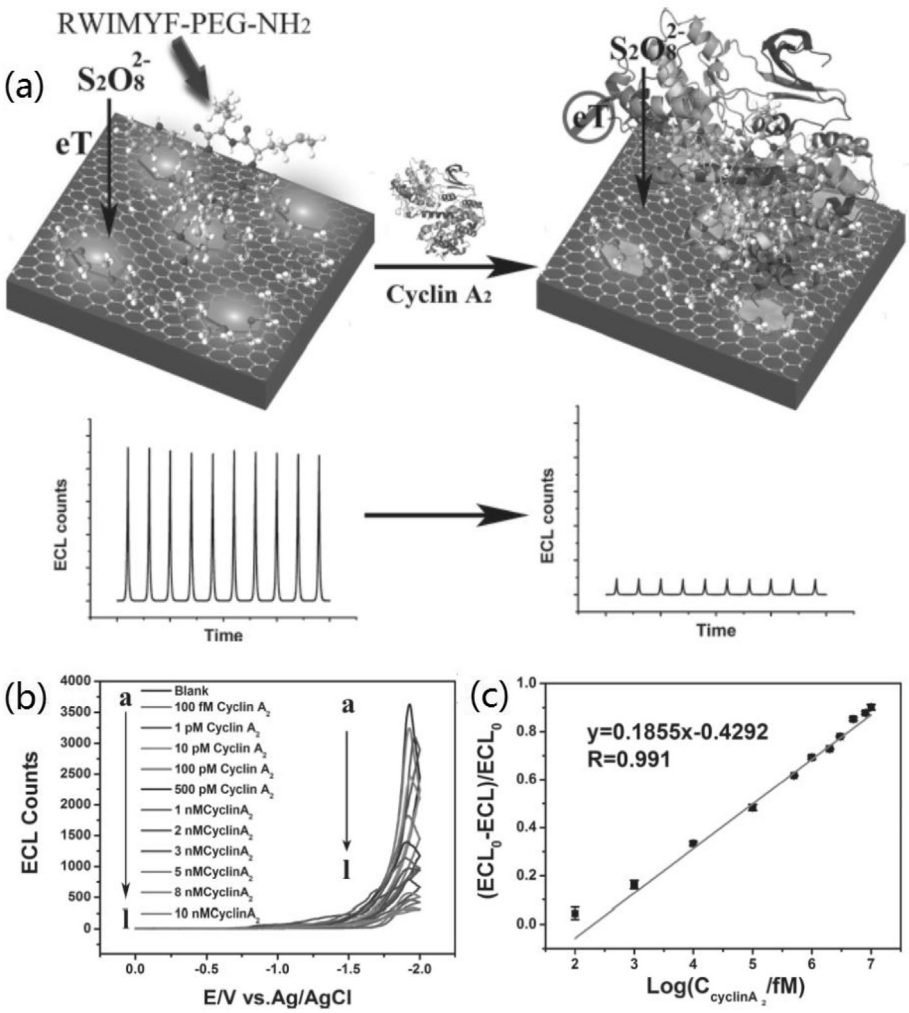

Fig. 7. ECL biosensors based on RGO-UCNPs-peptide nanocomposites. (a) Fabrication of RGO-UCNPs-peptide biosensor architecture and sensing mechanism. (b) ECL intensity vs. cyclin A_2 concentration, and (c) linear fitting. Reproduced with permission from Ref. [74]. Copyright 2014, Wiley-VCH Verlag GmbH &Co.

3.2.2. Biosensors by using graphene as donor of FRET

Besides the fluorescent quencher for FRET biosensors, graphene (GO and GQDs) can also be used as donor (fluorophore) for the fabrication of fluorescent biosensors. Previous studies indicated that GO could be a new type of fluorophore with strong photoluminescence (PL) in the visible and near-infrared regions [100,101]. In addition, the nanoscale GQDs have adjustable PL, high photostability, good solubility, low cytotoxicity, good biocompatibility, and high specific surface area, and therefore are promising candidates for biosensing and bioimaging applications [102–104].

Kwak et al. demonstrated the fabrication of a luminescent GO and peptide quencher-based fluorescent biosensors for simple, rapid, and sensitive detection of proteases, chymotrypsin and matrix metalloproteinase-2 (MMP-2) [105]. In order to apply GO as an emitting donor for the fabrication of FRET-based biosensors, two potential quencher candidates for PL of GO like metalloprotoporphyrin derivatives (MePPs) and QXL570 were utilized. It was found that the PL of alkyne-modified GO could be quenched by binding a QXL-linked peptides (AAPF and GPLGVRG for chymotrypsin and MMP-2, respectively) onto the GO surface, but the GO PL was recovered by adding protease to proteolytic cleave the peptide chain to remove the Q quencher, as shown in Fig. 9. The designed GO-peptide-QXL system showed high sensitivity and selectivity for detecting chymotrypsin (0.16 μM) and MMP-2 (0.76 μM), and exhibited potential application for in-situ determination of MMP-2 (2.0 ng/mL) in living human hepatocytes HepG2 cells.

Wang et al. reported a novel fluorescent biosensor by using the PL quenching of GQDs induced by the coupling linking of GQD-peptide nanocomposites [106]. In their study, the sensor architecture was constructed by covalently binding the designed peptide chain ($RRRADDSD_5$) to the surface of GQDs. After casein kinase II (CK2) and ATP were added into the sensor system, the bound peptide was phosphorylated by CK2. The further introduction of Zr^{4+} ion could link the phosphorylated sites of peptide and induce the extensive aggregation of GQD-peptide nanocomposites, causing the PL quenching of GQDs. Based on the analysis of PL quenching, the activity of CK2 can be monitored and measured with very high sensitivity and selectivity. The fabricated CK2 biosensor showed a linear detection range from 0.1 to 1.0 U/mL with a detection limit of 0.03 U/mL. This novel biosensing strategy exhibited high potential for the high-throughput screening of protein kinase inhibitors and drug analysis.

3.3. Electronic biosensors

Electronic determination of bioanalytes with field-effect transistor (FET) biosensors by using carbon nanomaterials like CNTs and

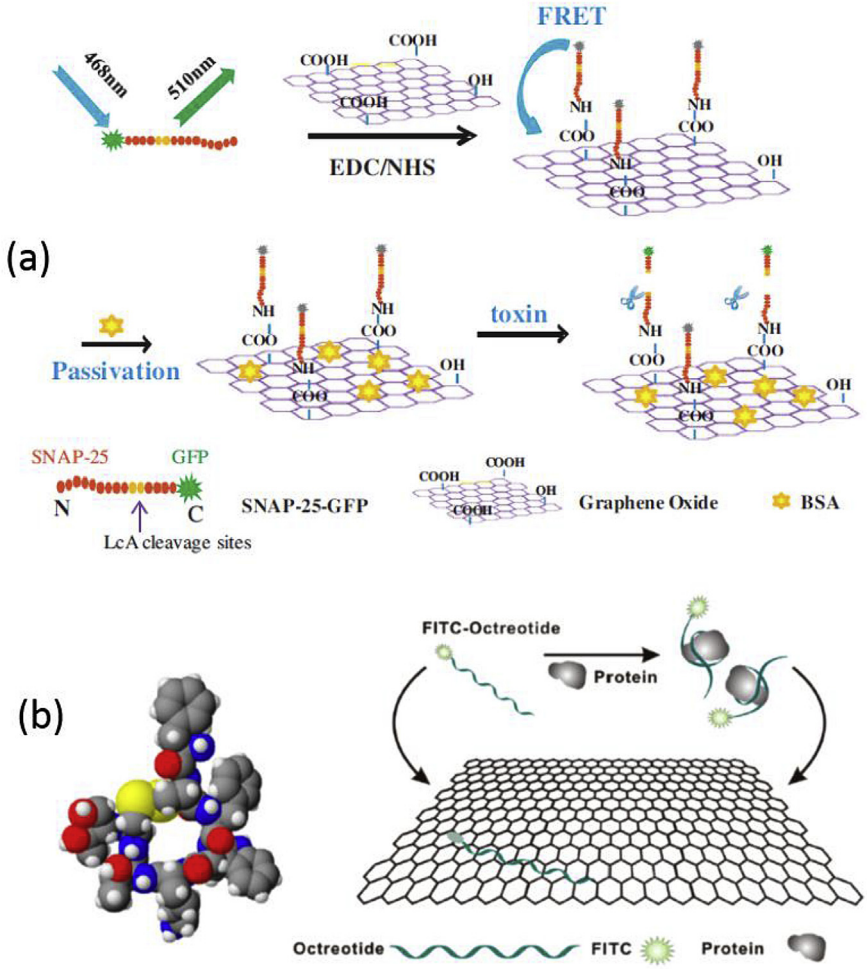

Fig. 8. Two types of fluorescent biosensors based on graphene quenching with (a) cleavage-based and (b) detachment-based biosensing assays. Image (a) was reprinted with permission from Ref. [41]. Copyright 2015, Elsevier Ltd. Image (b) was reprinted with permission from Ref. [94]. Copyright 2013, American Chemical Society.

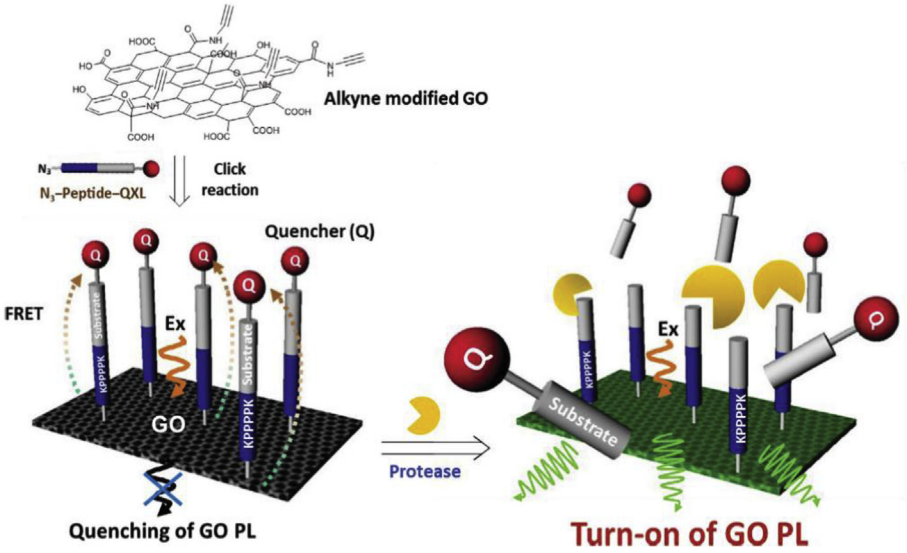

Fig. 9. GO-peptide quencher based fluorescent biosensor for detecting protease. The PL of GO is quenched by the peptide-QXL, but the cleavage of peptide chain with protease removes the Q quencher and causes the turn-on of GO PL. Reprinted with permission from Ref. [105]. Copyright 2014, Wiley-VCH Verlag GmbH &Co.

graphene could achieve in very high sensitivity because the nanomaterials are very sensitive to the electronic effect after binding biomolecules onto the material surface. Previously, FET biosensors based on 2D graphene sheets for the quantitative

detection of protein [107], DNA [108], antigen [109], and the analysis of protein-antibody interaction [110] have been reported.

If a biomolecule like DNA or protein is captured by the graphene FET biosensors, the change of resistance could be detected even at the single-molecular level. Therefore, a target-specific molecular probe, like a designed peptide sequence [111] or peptide nucleic acid (PNA) [112] could be the potential candidates for functionalizing the graphene FET biosensors for selective and sensitive biosensing. In a typical study, Khatayevich et al. demonstrated a graphene-peptide nanocomposite-based FET biosensor for selective detection of streptavidin (SA) against another protein background [111]. To fabricate the FET biosensor architecture, the engineered dodecapeptide bio-GrBP5 with an amino acid sequence of bio-IMVTESSDYSSY was used for the biofunctionalization of graphene biosensor (Fig. 10a), in which the aromatic peptide motif YSSY is responsible for the binding onto graphene surface and the amphiphilic peptide motif IMVTESSD is responsible for the self-assembly formation of monolayer on graphene surface [113]. The bio-modification (biotin) could provide specific interaction with SA. A four-probe graphene FET biosensor architecture (Fig. 10b and c) modified with the above introduced peptide was fabricated and employed for detecting SA, and a detection limit of about 30 μg/mL was achieved (Fig. 10d). In addition, the FET biosensor modified with mixed peptides (bio-GrBP5 and SS- GrBP5) showed high selectivity against 10 mg/mL bovine serum albumin (BSA). This interesting study showed the possibility for controlling the biofunctionalization of FET biosensor surface to construct multifunctional sensors capable of high selectivity and sensitivity.

In another study, Cai and co-workers reported the fabrication of RGO-PNA based FET biosensor for ultrasensitive label-free detection of DNA and PNA-DNA hybridization [112]. PNA is a DNA mimic sequence, which has a negatively charged deoxyribose phosphate backbone replaced by a neutral peptide backbone [114]. The RGO-PNA FET biosensor was fabricated by drop-casting RGO sheets onto the sensing channel as the conducting material, and then a linker molecule, 1-pyrenbutanoic acid succinimidyl ester (PASE), was utilized to modify RGO surface by π-π interaction. Then the PNA probe, N-AACCACACAACCTACTACCTCA-C, could be bound onto the RGO surface by the covalent interaction with PASE linker. After adding the target DNA molecules, the change of electrical signals could be measured by using a silver wire as the gate to realize a liquid-gated FET biosensor. The fabricated RGO-PNA FET biosensors exhibited a great detection limit (100 fM) due to the high sequence-specific affinity and stability of PNA to DNA molecules.

Besides the above introduced FET biosensors, graphene-peptide nanocomposites could be used for fabricating advanced electronic nanosensors. For example, Mannoor and co-workers showed that graphene can be printed onto water-soluble silk membrane to create a fully biointerfaced nanosensor platform for wireless detecting bacteria on tooth enamel [115], as shown in Fig. 11a. In order to enhance the selectivity of the fabricated bacterium biosensor, an antimicrobial peptide (AMP, molecular structure in Fig. 11b) with bifunctions (HSSYWYAFNNKT-GGG-GLLRASSVWGR-KYYVDLAGCAKA) was used to modify the graphene surface to form graphene-AMP composite. The graphene-binding head motif of this AMP, HSSYWYAFNNKT, showed strong noncovalent interactions with graphene surface [49], and the tail motif, GLLRASSVWGR-KYYVDLAGCAKA, showed specific activity towards both the Gram-negative bacteria and Gram-positive bacteria [116]. The fluorescent image in Fig. 11c indicates the high binding affinity of AMP with graphene surface by labeling the adsorbed peptides with a fluorescent probe. After the specific binding of bacterial targets by the immobilized AMP, the electrical conductivity of the graphene film could be modulated by wirelessly monitored with an inductively coupled radio frequency reader device. Fig. 11c shows the resistance (upper) and optical (below) data of the fabricated biosensor for simultaneous detecting and imaging single E.coli bacterium, which indicates that the fabricated nanosensor has high selectivity and sensitivity.

3.4. Spectroscopic biosensors

The binding of graphene oxide with peptide could affect the ultraviolet (UV) absorbance, and therefore the created GO-peptide nanocomposites could be used as a potential platform for spectroscopic biosensing. For example, Zhang et al. fabricated a graphene-based UV-vis spectroscopic biosensor functionalized with peptide for label-free detection of 2,4,6-trinitrotoluene (TNT) [117]. To fabricate the GO-peptide based biosensor platform, a TNT-specific peptide with a sequence WHWQRPLMPVSI was first bound onto GO surface by the NHS/EDC crosslinked coupling, as shown in Fig. 12a. When TNT was added into this biosensor system, the bound peptides would specifically capture TNT molecules by the donor-acceptor and π-π interactions, and caused the changes in optical properties of GO. They further proved that the addition of TNT into GO-peptide nanocomposite solution could affect the absorption intensity in the obtained UV-vis spectra of GO-peptide. It was clear that the increasing of TNT concentration caused the decrease of the intensity of absorption peak of GO-peptide nanocomposites, as indicated in Fig. 12b. This fabricated TNT biosensor

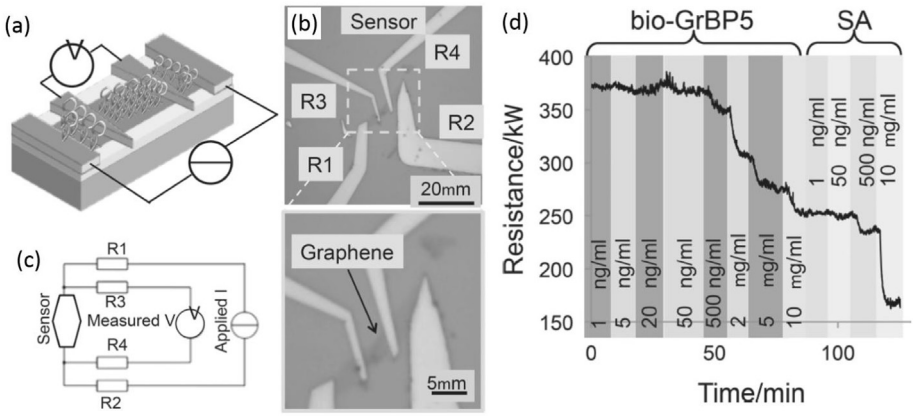

Fig. 10. Graphene-peptide FET biosensors for protein detection: (a) Fabrication of graphene-peptide FET biosensor, (b) Optical image of sensor architecture, (c) Equivalent electrical circuit of sensor, and (d) Detection of SA. Reproduced with permission from Ref. [111]. Copyright 2014, Wiley-VCH Verlag GmbH &Co.

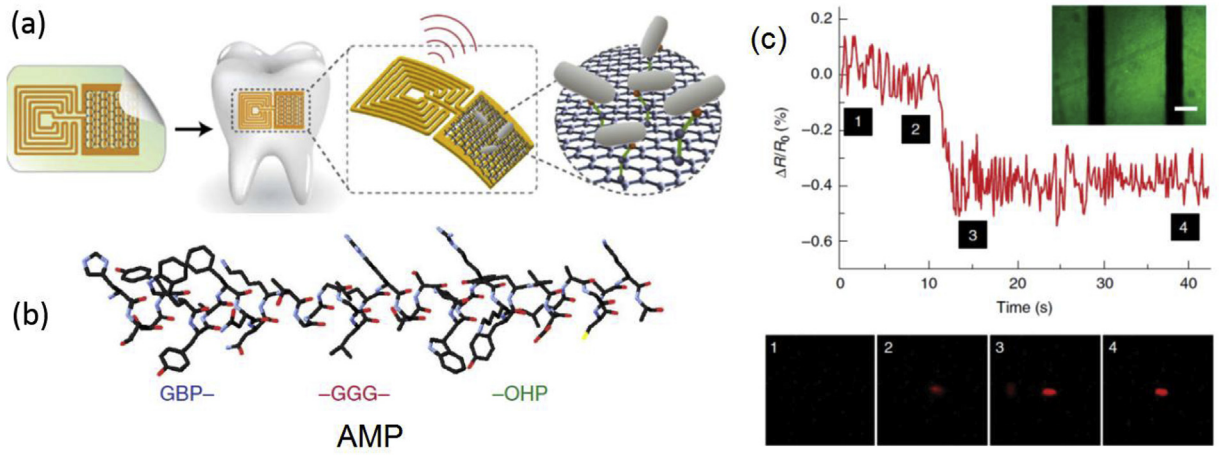

Fig. 11. Electronic biosensors based on GO-peptide nanocomposites. (a–c) Graphene-peptide-based electronic wireless detection of bacteria on tooth enamel: (a) Fabrication of biosensor and sensing mechanism, (b) molecular structure of AMP, and (c) single bacterium detection. Reproduced with permission from Ref. [115]. Copyright 2012, The Nature Publishing Group.

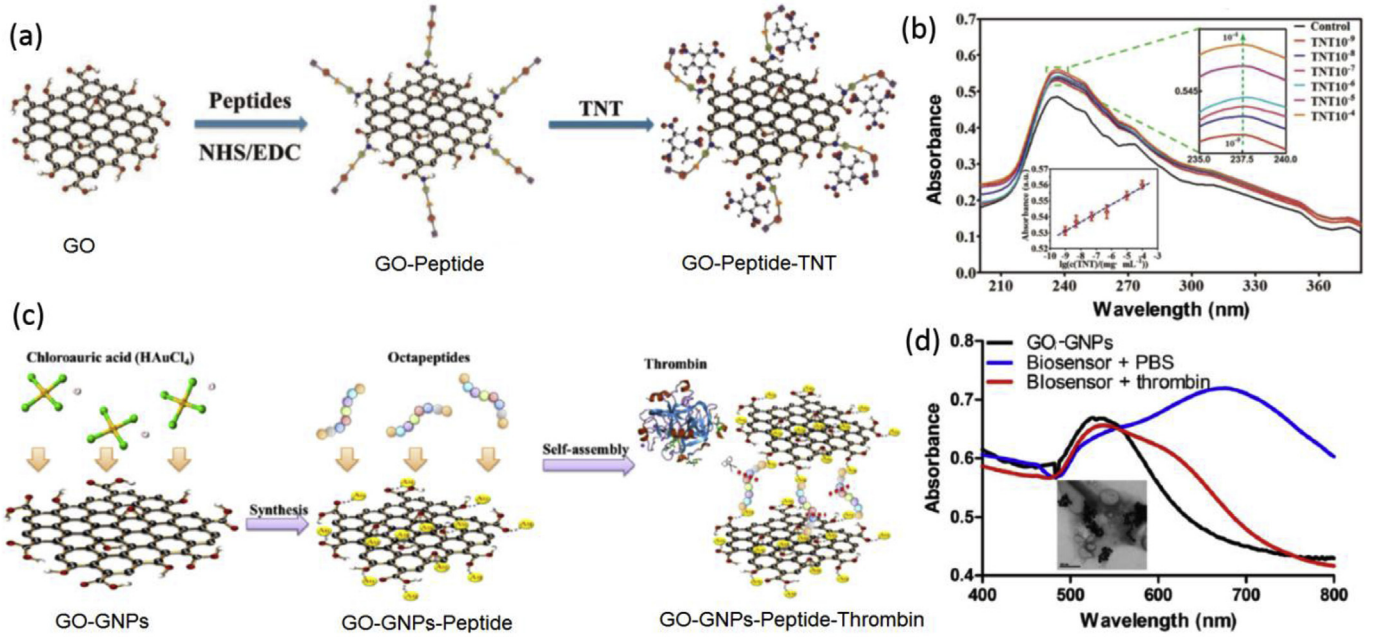

Fig. 12. Advanced spectroscopic biosensors based on GO-peptide nanocomposites. (a,b) TNT biosensor: (a) Fabrication of GO-peptide and the sensing mechanism of TNT, and (b) UV-vis spectroscopic change by adding TNT with various concentrations. (c,d) Thrombin biosensor: (c) Fabrication of biosensor and sensing mechanism, and (d) UV-vis spectroscopic change by adding thrombin. Images were reprinted with permission from Ref. [117]. (a,b). Copyright 2015, and Ref. [118]. (c,d). Copyright 2017, Elsevier Ltd.

showed a detection limit of 4.40×10^{-9} mM with high selectivity, and revealed great potentials for constructing a portable device for easy and stable detection of explosive chemicals.

Based on this study, currently they further designed another spectroscopic biosensor for detecting thrombin by utilizing the GO-gold nanoparticles (GNPs)-peptide nanocomposites [118]. Firstly, they created GO-GNPs hybrids by introducing gold salt to GO surface and the subsequent chemical reduction to GNPs. After that, specially designed peptides with sequences CLVPRGSC or CRGC were added into the GO-GNPs hybrids for the self-assembly of peptides and formation of GO-GNPs-peptide biosensors (Fig. 12c). The designed peptides could bind spontaneously with GO-GNPs hybrids through the formation of Au-S bonds between cysteine and GNPs, in another way this peptide can be cleaved by thrombin. The spectroscopic biosensing was achieved for rapid and high-throughput measurements based on the optical properties of the GO-GNPs-peptide nanocomposite system. After adding thrombin into the designed biosensor system, it was found that the absorption spectrum showed obvious increase at the peak of 528 nm and decrease at the wavelength larger than 600 nm (Fig. 12d). The change of absorption intensity showed a linear dose-dependence and stable response towards the added thrombin. By using this spectroscopic measurement, the fabricated biosensor exhibited a detection limit of as low as 0.5 fM to thrombin with high selectivity against other proteins like human serum albumin (HSA) and insulin.

3.5. A summary of the performances of graphene-peptide based biosensors

In the above sections, we presented and discussed on the construction and electrochemical, fluorescent, electronic, and spectroscopic biosensing with graphene-peptide nanocomposite-based biosensors. To make it more clear, we summarized the material type, peptide sequence, and sensing performances of the above mentioned biosensors, as shown in Table 2.

Based on the above summary, it can be concluded that the designed peptides played great importance for the sensitivity and selectivity of graphene-based biosensors. In order to improve the biosensing performances of the fabricated biosensors, several key factors on the design of peptides should be considered. Firstly, the affinity of peptides with graphene surface should be high enough to make a dense loading of peptides onto graphene, which could greatly increase the detected signal. Secondly, the designed peptides should have high solubility, which could promote the dispersity of graphene materials. Thirdly, the design of peptides with specific recognition towards analytes could enhance the selectivity of biosensors. Finally, the design of peptide motifs for bioinspired synthesis of metal nanomaterials could also improve the sensitivity and selectivity of the fabricated biosensors.

It is clear that electrochemical and fluorescent graphene-peptide based biosensors have revealed wider applications than the electric and spectroscopic biosensors. The fabrication of electrochemical graphene-peptide based biosensors is simple, and all other electronic active materials like metal NPs, CNTs, and GQDs could be added into the sensor system to cause high performances. In addition, the electrochemical biosensors could be utilized for the detection of many analytes from small molecules, organic compounds, to macromolecules. Fluorescent graphene-peptide biosensors could also be utilized for various detections of drug, protein, enzyme, and antigen, but the main problem is that the peptide chain should be firstly labeled with a fluorescent group. The electronic graphene-peptide biosensors showed high sensitivity, but the fabrication technique of biosensors is not easy accessible. The spectroscopic biosensors have not been well developed, which could be only utilized for sensing a few specific analytes like TNT and thrombin.

4. Conclusions and perspectives

In this review, we discussed the synthesis of graphene-peptide nanocomposites and their applications as electrochemical, fluorescent, electronic, and spectroscopic biosensors. Peptide plays very important roles in the construction and enhanced sensing performance of the GO-peptide nanocomposite-based biosensors. The combination of peptide with graphene materials could not only improve the biocompatibility and bio-recognition ability of the constructed biosensors, but also enhance the selectivity due to specific peptide-analyte interactions. In addition, the self-assembly ability of designed peptides made it possible to create novel graphene-PS nanocomposites. The strategies on the design of peptide sequence and the construction of biosensors shown in this review will be helpful for the future developments of graphene-based biosensors in biomedical detection and environmental analysis.

Although great achievements have been obtained, there are still some works could be done in the related fields. Firstly, the binding

Table 2
Comparison of the fabrication and sensor application of various graphene-peptide nanocomposite based biosensors.

Sensor Type	Composites	Analytes	Linear range	Detection limit	Ref.
Electrochemical	peptide-CNTs/graphene	H_2O_2	0.1–30 μM	–	[38]
	graphene-PNF	NADH	50-400 μM	1.2 μM	[65]
	Poly(Lys)-graphene	H_2O_2	10-80 μM	0.1 μM	[66]
	graphene-silk peptide	phenol	0.0015–21.1 μM	0.35 nM	[67]
	RGD-PA-RGO	nitric oxide	nM-μM	25 nM	[68]
	graphene-PNT-FA	receptor	–13 nM	8 nM	[69]
	GO-peptide-PtNPs	H_2O_2	0.2–15 mM	12 μM	[61]
	GO-peptide-AgNPs	H_2O_2	0.02–18 mM	0.13 μM	[44]
	RGO-PNF-AgNPs	H_2O_2	0.05–5 mM	10.4 μM	[45]
	GQD-PNF-GO	H_2O_2	0.01–7.2 mM	0.055 μM	[64]
	GO-peptide	caspase-3	0.1–100 pg/mL	0.06 pg/mL	[70]
	RGO-SP	PSA	0.1–5/5-80 ng/mL	53 pg/mL	[71]
	GO-peptide	cyclin A_2	–	0.32 pM	[72]
	Au-graphene-peptide	DBDE	5-100 ppt	–	[73]
	RGO-UCNPS-peptide	cyclin A_2	100 fM-10 nM	10.5 fM	[74]
	GQDs-peptide-AuNPs	PKA	0.1–10 U/mL	0.005 U/mL	[75]
Fluorescent	GO-ZDEVD	caspase-3	7.25–362 ng/mL	7.25 ng/mL	[87]
	GO-SNAP-25	BoNT/A	1 fg/mL-1 pg/mL	1 fg/mL	[41]
	GO-peptide	thrombin	–	2 nM	[26]
	GO-peptide	MMP-2	10-150 ng/mL	2.5 ng/mL	[88]
	GO-peptide	MMP-2	0.2–2 nM	50 pM	[89]
	GO-peptide	PSA	0.5–3 nM	0.3 nM	[43]
	RGO-polymer-peptide	chymotrypsin	–	–	[91]
	GO-peptide-QD	MMP-2	–	0.5 nM	[93]
	GO-FITC-octreotide	AOC	0-20 ng/mL	2 ng/mL	[94]
	GO-CNTs-peptide	cyclin-A_2	–	0.5 nM	[95]
	GO-peptide	$\alpha v\beta 3$	–	–	[97]
	GO-peptide	LPS	0-20 nM	130 pM	[98]
	GO-FITC-GGGYR	ALP	0.2–5 nM	0.08 nM	[99]
	GO-peptide-QXL	MMP-2	–	2.0 ng/mL	[105]
	GQD-($RRRADDSD_5$)	CK2	0.1–1 U/mL	0.03 U/mL	[106]
Electronic	Graphene-bio-GrBP5	SA	–	30 μg/mL	[111]
	RGO-PNA	DNA	–	100 fM	[112]
	Graphene-AMP	bacteria	–	Single molecule	[115]
Spectroscopic	GO-WHWQRPLMPVSI	TNT	4.4(10^{-9}-10^{-4}) mM	4.4×10^{-9} mM	[117]
	GO-GNPs-CLVPRGSC	thrombin	1 fM-10 pM	0.5 fM	[118]

mechanism of peptide with graphene and the conformation transition of peptide induced by graphene should be understood in deeper way. Computer simulation could be a simple and effective method for this aim. Secondly, as many peptides showed strong binding ability towards graphene surface, it is important to understand the design protocols of peptides for creating graphene-peptide nanocomposites with high stability and biocompatibility. Thirdly, more studies could be done to fabricate biosensors to realize the in-situ and real-time monitoring and detection of analytes in/on living cells. Finally, it is possible to investigate the biomedical and tissue engineering applications of the designed graphene-peptide nanocomposites.

Acknowledgments

LW acknowledges the financial support from the National Natural Science Foundation of China (Grant No. 21505049). YJZ and AGW thank the financial supports by the Project for Science and Technology Service of the Chinese Academy of Sciences (KFJ-SW-STS-172), the Hundred Talent Program of the Chinese Academy of Sciences (2010735), the Natural Science Foundation of China (U1607111and U1507104), and the Zhejiang Provincial Natural Science Foundation of China (Grant R5110230). GW thanks the financial supports from the Deutsche Forschungsgemeinschaft under grants WE 5837/1-1.

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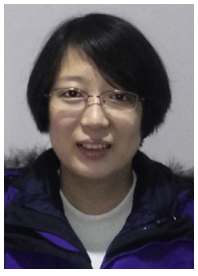

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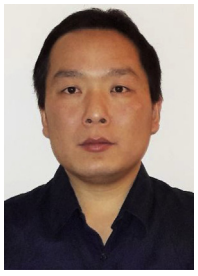

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