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REVIEW ARTICLE

# A review on peptide functionalized graphene derivatives as nanotools for biosensing

Shubhi Joshi[1] · Pratibha Sharma[2] · Ruby Siddiqui[2] · Kanica Kaushal[2] · Shweta Sharma[3] · Gaurav Verma[4,5] · Avneet Saini[2]

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## Abstract

Peptides exhibit unique binding behavior with graphene and its derivatives by forming bonds on its edges and planes. This makes them useful for sensing and imaging applications. This review with (155 refs.) summarizes the advances made in the last decade in the field of peptide-GO bioconjugation, and the use of these conjugates in analytical sciences and imaging. The introduction emphasizes the need for understanding the biotic-abiotic interactions in order to construct controllable peptide-functionalized graphitic material-based nanotools. The next section covers covalent and non-covalent interactions between peptide and oxidized graphene derivatives along with a discussion of the adsorption events during interfacing. We then describe applications of peptide-graphene conjugates in bioassays, with subsections on (a) detection of cancer cells, (b) monitoring protease activity, (c) determination of environmental pollutants and (d) determination of pathogenic microorganisms. The concluding section describes the current status of peptide functionalized graphitic bioconjugates and addresses future perspectives.

**Keywords** Peptides · Graphene · Graphene oxide · Reduced Graphene oxide · Nano-materials · Functionalization · Bioconjugation

## Introduction

Nanotools are capable of being utilised in analytical procedures by replacing the frequently used materials in order to increase the standard of each method and therefore the outcomes. They offer a cost-effective approach in the field of diagnostics. When a nanomaterial is involved in the detection step it is classified as a nanoprobe which is a category of nanotools [1–3].

Nanoparticles based on carbon are the most versatile, amongst the various types of carbon-based nanomaterials, the plausible chemical properties of graphene and its oxidised precursors such as enormous surface to volume ratio [4–7], fluorescence quenching ability [8], high electron mobility [9], cost-efficient and large-scale production [10, 11] makes them desirable candidates for construction of probing devices. Their surface morphology can be further enhanced by biomolecule functionalization [12–16]. Recent reports established that nanoscale graphene derivatives conjugated with low molecular weight peptides have emerged as promising materials for construction of analytical formulations having diagnostic abilities. Graphene oxide (GO) and reduced graphene oxide (rGO) have emerged on the forefront for construction of hybrid scaffolds having immense potential to offer in the field of diagnostics [17–19].

Peptide molecules selected from combinatorial libraries have significantly displayed covalent as well as non-covalent interaction with different abiotic surfaces such as graphene [20–24]. The mechanism of interaction is usually governed by the applicability of the formed nano complex [25–27].

✉ Avneet Saini
avneet@pu.ac.in

[1] Energy Research Centre, Panjab University, Sector 14, Chandigarh 160014, India

[2] Department of Biophysics, Panjab University, Sector 25, Chandigarh 160014, India

[3] Institute of Forensic Science & Criminology (UIEAST), Panjab University, Sector 14, Chandigarh 160014, India

[4] Dr. S.S. Bhatnagar University Institute of Chemical Engineering & Technology (Dr.SSBUICET), Panjab University, Sector 14, Chandigarh 160014, India

[5] Centre for Nanoscience and Nanotechnology (UIEAST), Panjab University, Sector 14, Chandigarh 160014, India

The short structure and specificity of peptides differentiates them from proteins [28]. Peptides offer a useful resource for materials science by taking advantage of its characteristic property of biological specificity and multifunctionality into inorganic or carbon-based nanomaterials while simultaneously controlling structures and properties of nanoparticles [29, 30]. Unlike proteins, peptides do not aggregate on the nanomaterial surface upon interaction [13]. The desirable properties of peptides as listed in Table 1, enable them to retain a controllable and repeatable structure on the inorganic nanomaterial surface thereby providing versatility in forming various tertiary structures [31]. Properties like economical synthesis and capability of recognizing graphitic materials followed by interaction of peptides to form ordered structures on GO and rGO surfaces advocates their use in material interfacing [32–34]. Due to these advantageous properties, peptides are attractive molecules to functionalize graphitic materials and enhancing their inherent chemical and structural properties [35].

Fabrication of material for diagnostic devices requires a multidisciplinary approach and a scrupulous understanding of both an inorganic material and a biological element [41, 42]. The structure of these tools primarily depends on the incorporation of bioactive material participating in the recognition process [43]. Through the medium of this review article we intend to discuss the use of peptide functionalized graphitic nanomaterials as analytical nanotools. This article also aims at providing a clear perspective to readers regarding interaction behaviour of peptides with the abiotic graphitic precursors, thus encouraging application of the conjugate in future real time diagnostic applications.

## Interactions enabling immobilization of peptide to graphitic derivatives

Both covalent and non-covalent interactions facilitate conjugation of peptide biomolecules with graphitic materials. Peptides preferentially bind at the sites containing functional groups either at the edges or at planes thereby modifying the unique topology of graphene-based nanomaterials Figure 1. Table 2 presents the different interactions prevalent amongst peptide and protein molecules. The interaction between peptide molecules and graphitic derivatives involves complicated reactions because the charge status of the surface functional groups of peptide depends robustly on the environmental conditions, pH value, and ionic strength of the buffer. Peptide molecules may possess a negative or a positive charge. Likewise, there is a variation in functional groups possessed by graphene and its oxidised derivatives like GO/rGO mainly because of preparation procedure followed and storage conditions [44–46].

### Immobilization of peptides on graphene

Graphene comprises of $sp^2$ hybridized C-C clouds closely arranged in hexagonal symmetry reflecting a two-dimensional (2D) structure [8, 9]. It does not contain oxygen and is perfectly planar [52, 53]. Due to its intrinsic semi-hydrophilic properties, phage display technique has been employed by researchers to find peptide sequence that specifically bind to graphene [54, 55]. Such studies involve amalgamation of computational designing prior to physical experiments to confirm interaction [56]. Interaction of different peptide sequences with graphitic materials and their applications have been summarised in Table 3.

Covalent interaction is not possible owing to the fact that, there are no reactive groups present and also because of low dispersion in aqueous mediums [56, 63]. Peptides may be immobilized on graphene with the help of non-covalent interaction mechanisms like self-assembly by maintaining mild acidic conditions along with sonication treatment. Peptide molecules exhibit a self organizing tendency after interaction procedure is accomplished [57, 64]. It was observed that, in biosensing applications the self-assembled peptide molecules acted as a bridge between the target molecule and the graphitic surface [47, 59]. Presence of aromatic functional groups enable self-assemble of peptides to graphene by use of π-interactions [58].

Van der Waals forces also encourage the interaction due to water repelling properties possessed by graphene [65–67]. These days researchers are concentrating on cross-linking biomolecules to graphene majorly by application of non-covalent conjugation strategies. Previous studies have convincingly demonstrated that non-covalent method of interaction does not alter the primary architecture of graphene thereby retaining the

**Table 1** Desired properties of peptides for biosensing applications [36–40]

| S. No | Properties |
|---|---|
| 1. | Chemical diversity |
| 2. | Self-Assembly |
| 3. | Robustness |
| 4. | Molecular recognition |
| 5. | Target specificity and high affinity for organic and inorganic compounds. |
| 6. | Ease of synthesis and Conjugation. |

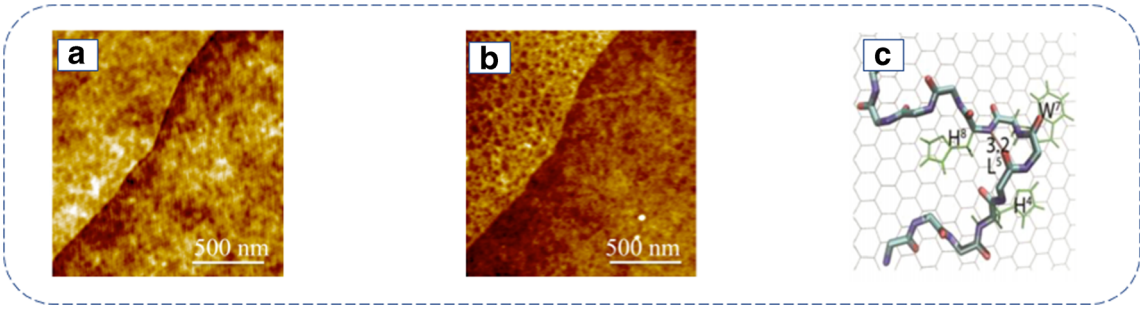



**Fig. 1** AFM image of (**a**) Graphene surface before incubation (**b**) Graphene surface functionalized with self-assembled peptide (**c**) Molecular dynamics-based structure of peptide graphene sheet. [Figures have been reprinted with permission Ref. 25]

physical and chemical structure of the graphitic surfaces and also, it's chemical properties [68]. If the limiting factors of low dispersibility causing difficulty in loading biomolecules is overcome, graphene fabricated with peptide molecules can open novel diagnostic avenues by making use of its commendable flexibility and electron mobility.

## Immobilization of peptides on graphene oxide (GO)

In comparison to graphene, GO is an oxygen-rich carbon material containing many $sp^3$ carbons. They are not perfectly planar. The presence of high density of oxygen functionalities such as epoxide, hydroxyl, and carboxylic enhance its biomolecule biocompatibility [13, 69]. The presence of both aromatic and aliphatic moieties along with numerous defects on its topography encourage biomolecules to form complex on the reaction sites via both covalent as well as non- covalent interaction [70–72]. Table 4 depicts GO binding peptide sequence along with applications of the formed conjugates.

### Non-covalent interactions

The non-covalent absorption of peptides on GO can be accomplished by a fusion of π interaction, electrostatic and hydrophobic force along with interaction of hydrogen molecules [87–90]. These interactions are achieved by following modest protocol of placing aqueous peptide with graphitic material followed by stirring and incubation [91, 92]. Surface phenomenon's such as binding of peptide and diffusion with the abiotic material takes place. Incubation time and peptide concentration are major determinants controlling peptide coverage on GO substrates [37, 93]. It has been observed that peptide upon

**Table 2** Different types of prominent interactions in peptides and proteins [47–51]

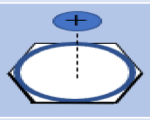
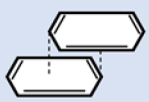
| Interaction Type | Chemical representation | Typical Distance | Free Energy |
|---|---|---|---|
| Covalent Bond | $-C_\alpha-C-$ | 1.5 Å | 356 kJ $mol^{-1}$ |
| Electrostatic interactions | $H_3\overset{+}{N}\cdots\overset{-}{O}-\overset{O}{\overset{\|\|}{C}}$ | 3.0 Å | 5.9 kJ $mol^{-1}$ |
| Hydrogen Bond | $N-H\cdots O=C$ | 2.5-3.2 Å | 2-6 kJ $mol^{-1}$ |
| Van der Waals | $-\overset{H}{\overset{\|}{C}}-H\cdots H-\overset{H}{\overset{\|}{C}}-$ | 3.5 Å | 2-4 KJ $mol^{-1}$ |
| Cation–π interaction |  | 6.0 Å | 2-4 KJ $mol^{-1}$ |
| π stacking interaction |  | 4.9 Å | 8-12 kJ/$mol^{-1}$ |

**Table 3** Studies depicting graphene binding peptide sequence along with their applications

| Peptide sequence | Interactions | Applications | Ref. |
|---|---|---|---|
| DELERRIRELEARIK | Hydrophobic interactions | Diagnostic nano devices. | [25] |
| HSSYWYAFNNKT–GGGGLLRASSVWGRKYYVDLAGCAKA | Non-covalent interactions | Enhances selectivity of the fabricated bacterium biosensor. | [57] |
| AEAEAKAKAEAEAKAK | Hydrophobic interactions | Key role in potential bio-surface engineering. | [58] |
| FEFEFKFKFEFEFKFK | π stacking | Biomolecular sensing and diagnostic applications. | [59] |
| GBP-GGG-OHP (HSSYWYAFNNKTGGGGLLRASSVWGRKYYVDLAGCA-KA) | π stacking | Graphene binding motif linked to the antimicrobial peptide. | [59] |
| EPLQLKM | π stacking | Hybrid material with electronic, optical or catalytic properties. | [60] |
| IMVTESSDYSSY | π stacking | Electronic recognition of bio-analytes using field-effect transistor (FET) biosensor. | [61] |
| IMVTESSDYSSY | π stacking | Biosensing, drug-delivery and tissue engineering | [62] |

incubation with GO undergoes stacking through electrostatic interactions. The aromatic rings present in amino acids have a tendency to interact with the hydrophobic basal planes of GO and organize in a parallel plane through π interactions [94–96]. Similarly, formulation of a nanohybrid comprising of self assembled peptide with GO for biomimetic mineralization of hydroxyapatite (HA) was reported [97]. In this study peptide was interacted with GO in a controlled manner which further enhanced availability of nucleation sites for development of HA crystals.

In this kind of adsorption, the carbon forms networks with the hydrophobic domains of the biomolecule [91]. The hydrophobic effect is a result of dominant directional interactions among water molecules and the complementarity of those reciprocal reactions [98, 99]. Non-covalent interaction between GO and biomolecule greatly relies on factors like electron density and geometry of biomolecule. It has been observed that the hydrogen bonds between GO side groups and biomolecules additionally support surface adhesion [100]. Hence, biomolecules interact with GO through electrostatic

**Table 4** GO interaction with peptide molecules along with applications

| Peptide Sequence | Interactions | Applications | Ref. |
|---|---|---|---|
| KCALNNGSGFPRGRAK | π stacking | Fluorescence-based biosensor. | [73] |
| GGGRKRIHIGPGPAFYTT | π stacking | Molecular recognition, screening drugs, and designing biosensors. | [74] |
| SNAP-25 (DEANQRATKMLGSG) | Covalent interactions | Fluorescent biosensors, high selectivity and low detection limit than traditional immunoassays. | [75] |
| WHWQRPLMPVSI | π stacking | Spectroscopic biosensing. | [76] |
| CALNNDEVDK-FAM | Electrostatic Interactions | Targeted anticancer drug delivery and help in therapeutic self-monitoring. | [77] |
| KKNYSSSISSIHC | Electronic interactions/π stacking | Fluorescence-based sensor for Endotoxin detection. | [78] |
| CLVPRGSC | π stacking | Detection of thrombin protease activity and other proteases related to cancer diseases such as matrix metalloproteinases. | [79] |
| VEVKVEVK (V8); FEFKFEFK (F8) | π stacking | Biomedical application to design novel hybrid peptide hydrogels | [80] |
| MPG, GALFLGFLGAAGS TMGAWSQPK-SKRKV | π stacking | Nano gene carrier, nano drug-loading complex. | [81] |
| NLWAAQRYGRELRRMS DKFVD | π stacking | Sensitive detection of target in cell and fluorescence imaging. | [82] |
| RRRRNLWAAQRYGREL RRMSDKFVD | π stacking | Disease diagnosis, progression tracking and therapeutic evaluation. | [82] |
| RFRFRFRF | π stacking | Surface-tethered peptide, aquaporins mimicking. | [83] |
| FLGVVFKLASKVFPAVF GKV | π stacking | Inhibitory effect against pathogens *Candida albicans* and *Escherichia coli (E. coli)* | [84] |
| ε-poly-L-lysine | Electrostatic Interaction | Isolation and removal of drug resistant pathogens from water. | [85] |
| CGGHSSKLQFWYFWY | Electrostatic Interaction | Biosensing | [86] |

interactions with different degrees of stability. The advantage of physical immobilization of peptides is that the bulk characteristics of GO are least affected and controlled-assembly of peptides is achieved. Self-assembly of peptide on GO surface involves few drawbacks such as low stability due to lack of permanent functionalization and prediction of interaction, these are application specific limitations and can be addressed by following covalent interaction mechanisms.

### Covalent interactions

The presence of enhanced oxygen containing functional groups available on GO facilitate biomolecule immobilization through covalent conjugation mechanisms. Hydrophobic forces along with π interactions are the dominant covalent forces taking place between aromatic and hydrophobic residues of peptides and hydrophobic basal plane of GO surface [62]. During covalent interaction the peptide molecules are bound to GO by the application of cross-linkers like 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N-Hydroxysuccinimide (NHS) and Polyethylene glycol (PEG) [101–103]. The function of these linkers is to induce amidation reaction with the abundantly available surface carboxyl groups to enable conjugation. For instance, ethylene glycol unit of PEG enhances hydrophilic property of GO surface which further enables attachment of peptides in an aqueous medium [104]. Whereas, NHS stabilises the nanocomplex by creating ester functional groups with carboxylates [105]. EDC/NHS were employed as cross-linkers for creating a bond between nisin and GO. Initially GO was conjugated with nisin through amide linkages followed by application of PEG which resulted in the formation of a 3D GO membrane system [106]. Xu G and his colleagues reported interaction of GO with PEG followed by trypsin immobilization, with a purpose of enhancing the immobilization capacity of GO surface along with restricting adsorption of addition biomolecules [107].

Interaction between GO and chymotrypsin was studied and it was found that GO strongly inhibit the activity of

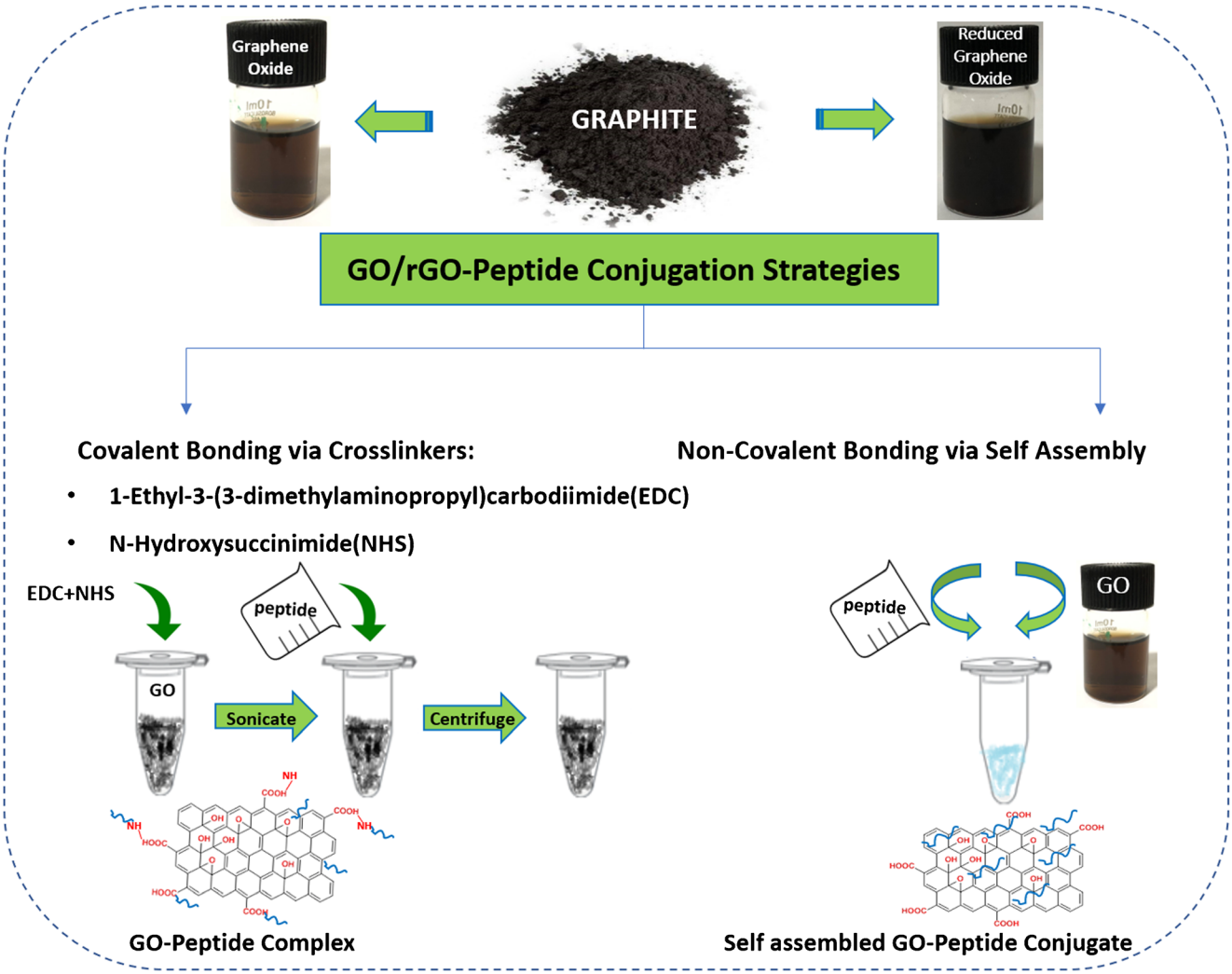



**Fig. 2** Schematic representation of peptide functionalized GO/rGO conjugate

**Table 5** An overview on the properties of commonly used carbon nanomaterials: Gr, GO, rGO

| Carbon material | Electrochemical features | Optical properties | | Advantages | Limitations | Ref. |
|---|---|---|---|---|---|---|
| | | Absorption peaks [$cm^{-1}$] | Functional groups | | | |
| Gr | Zero bandgap Thermal conductivity 3500–5300 W/mK<br>Low resistance $10^{-6}$ Ω cm<br>High electron mobility ~*15,000 $cm^2$/Vs*<br>High transparency<br>Flexible | 1500 $cm^{-1}$ | C=C | ▪ Enables surface assisted assembly of peptide molecules.<br>▪Quick physical adsorption of biomolecules. | ▪Low dispersibility and low stability in aqueous medium. | [126–130] |
| GO | Band gap 3.5 eV<br>Dielectric<br>Resistance $2.10^3$ 6 Ω m | 3200–3700 $cm^{-1}$<br>2900–2800 $cm^{-1}$<br>1670–1820 $cm^{-1}$<br>500–1400 $cm^{-1}$ | O-H<br>C-H<br>C=O<br>O-C-O | ▪Hydrophilic<br>▪High colloidal stability<br>▪Low cost of production | ▪Loss of electronic property. | [131–134] |
| rGO | Resistance $10^{-1}$ to $10^{-5}$ Ω m<br>Conductivity $2 \times 10^4$ S/cm.<br>Band gap ~1.00 to 1.69 eV | 1700–1720 $cm^{-1}$<br>1600–1640 $cm^{-1}$<br>1000–1040 $cm^{-1}$ | C-O-C<br>C=C<br>C-O | ▪Low oxygen containing functional groups.<br>▪Dominant Non-oxidised surface area.<br>▪Restored graphitic domains | ▪Aggregation in aqueous medium.<br>▪Hazardous fumes produced during production.<br>▪Reduced area for biomolecule interaction.<br>▪Low yield | [135–139] |

chymotrypsin, which might be due to the coexistence of anionic, hydrophobic, and π stacking interactions [106, 108]. Zhang and co-workers found that horseradish peroxidase (HRP) and lysozyme were immobilized on GO sheets through electrostatic interactions if the pH level was below the isoelectric point; if the pH level was above the isoelectric point, they suggested that hydrogen bond interactions prevailed [109, 110]. However, the electrostatic interactions are more pronounced on GO during covalent interaction, whereas both van der Waals and electrostatic interactions play a major role in the adsorption of proteins on reduced GO [100]. The increase in the van der Waals interaction on rGO is attributed to the increase in unfunctionalized regions on the surface [111, 112].

Detection protocols require selectivity, these linkers minimise the absorption of unnecessary adsorption of biomolecules. Reports suggest that covalent functionalization tends to improve the inherent characteristics of GO by opening band gap making it stable and soluble in aqueous biological medium [113]. Although covalent immobilization mechanisms deliver exorbitant binding strength and enhanced stability to conjugate during harsh chemical - temperature treatments, there are numerous limitations which restricts its applicability. Most of the covalent conjugation methods are not definite in the occurrence of changes [87]. There is an irreversible rehybridization in $sp^2$ configuration of carbon atoms to $sp^3$ during covalent amide reactions [91]. This type of binding sometimes affects the optical and electronic properties of nanomaterials by disrupting the extensive π bonding on the GO surface [114]. To over come these limitations researchers are using click chemistry, a type of covalent conjugation strategy. It has been proven to be efficient for bioconjugation of nanomaterials with biomolecules as it prevents alteration of peptide activity [26, 115]. Chemical reactions involved in click chemistry are quick in nature, feasible to perform, enhance stability of the reaction and are applicable over molecules with diverse functionalities [116]. Major limitations of click chemistry conjugation reaction involve non availability of click chemistry products, use of copper catalyst in abundance causing copper saturation and production of large quantity by-products [117]. In this context, azide-alkyne click chemistry approach was employed to accomplish functionalization of GO nanoparticles with different biomolecules [118].

## Immobilization of peptides on reduced graphene oxide (rGO)

There are few studies depicting peptide interaction with rGO, a form of GO having reduced oxygen content [119]. It is usually formed by application of reducing agents which in turn increase interlayer spacing [120]. The structure of rGO is compared with graphene but even after reduction it comprises of oxygen functionalities which enable complex formation with peptide molecules [121–123]. Owing to structural

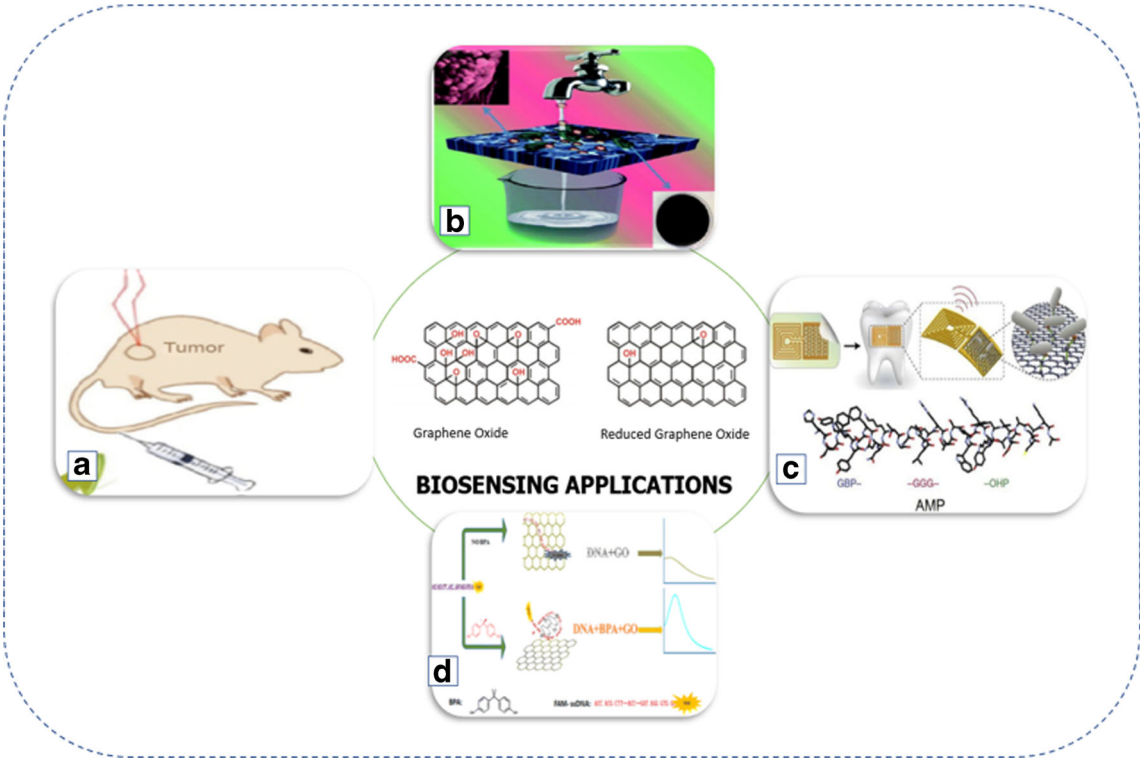



**Fig. 3** Applications of graphene functionalized peptide Complex as a (**a**) Early stage prognostic cancer detector (**b**) Pathogen detector and disinfectant for water (**c**) Detector of Helicobacter pylori (*H. pylori)* on teeth enamel (**d**) FRET sensor for detection of environmental pollutants [Figures have been reprinted with permission Ref. [106, 141, 148]

similarity with graphene, rGO also comprises of π interaction along with increased van der Waals interaction because of prevalent unfunctionalized regions on the surface [124]. In comparison to GO, electrostatic interaction seldom occurs because of decreased surface functionalities [71]. It has been inferred that hydrophobic interactions also enable adsorption of proteins and peptides on rGO. Reduction in biological activity of horse peroxidase (HRP)-GO conjugate was observed by Zhang and co-workers [125]. To overcome this limitation HRP was interacted with rGO via hydrophobic interaction. Following conjugation an enhanced biomolecule loading and prolonged stability was reported. Similarly, formation of rGO-peptide conjugate having large surface area and enhanced chemical stability at high temperatures was reported [12]. Figure 2 illustrates different mechanism for peptide immobilization to GO/rGO. There are few applications which require high oxygen containing functional groups for peptide conjugation whereas some require reduced oxygen functionalities. Investigations revealed that absence of functional surface groups impart a lower amount of perturbation to peptides upon immobilization with rGO [63].

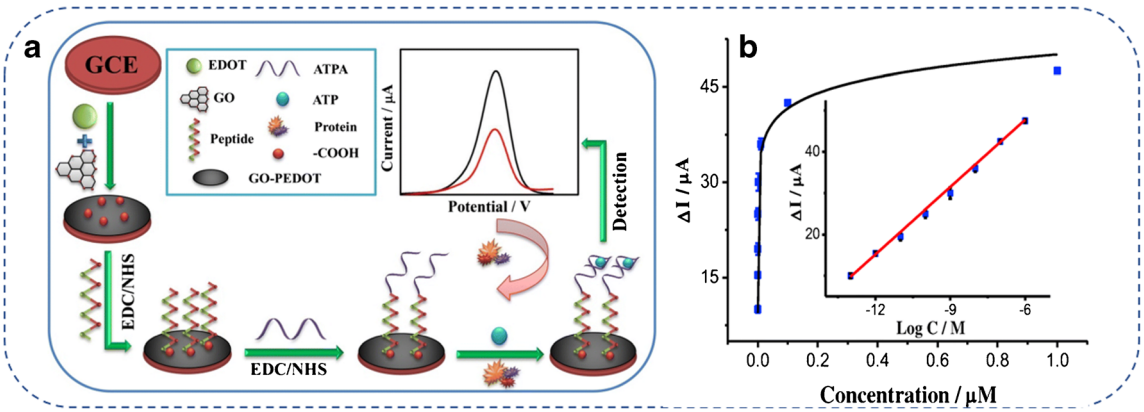



**Fig. 4** **a** Schematic illustration depicting fabrication of an aptasensor for ATP detection. **b** Response plot of aptasensor working at 0.18 V voltage [Reprinted with permission from Ref. 23]

From the above discussion it can be inferred that the selection of graphitic derivative for peptide functionalization depends on the application of the formed nanoconjugate. Table 5 describes the functional groups of different graphitic derivatives along with absorption peaks to provide a better understanding of their physico-chemical property. It should be noted that laboratory scale production of graphitic derivatives results in batch-to-batch variations.

## Applications of peptide functionalized graphitic materials

Graphene and its oxidised derivatives are being studied extensively in distinct disciplines for applications having novel relevance (Fig. 3). Modelling and controlling the fabrication of peptide nanostructures onto single layer may advance the genesis of 2D bio nanosensing devices:

### Detection of cancer cells

For early detection and measurement of an antigen which is found to be proteolytically active (PSA) from urine sample Feng T and co-workers reported a GO-FITC-labelled peptide-based Fluorescence Resonance Energy Transfer (FRET) sensor [140]. Prostate tumor is the prime reason of mortality in males associated with cancer. There is no treatment available to cure prostatic carcinoma but early diagnosis of PSA can reduce the mortality rates [141].The peptide fabricated GO fluorescence sensing technique was constructed by selecting a peptide (HSSKLQ) having PSA-sensitive core substrate a reported by previous research, the mentioned polypeptide is distinctly cleaved by the PSA [140]. Conjugation of peptide with GO single sheet was confirmed by AFM. The fluorescence of the peptide decreased on conjugation with GO which can be attributed to the fluorescence quenching ability of GO. The fluorescence of the dye-labelled peptide was quenched by efficient electron transfer. The kinetic studies were conducted to affirm that absorption of peptide on GO takes place very rapidly. The conjugate was reported to be PSA sensitive. The labelled peptide self-assembled on GO via electrostatic and $\pi$ interactions. The reported method is effective in comparison to other PSA screening techniques.

Formation of a rGO-silk peptide based electrical immunosensor for detection of PSA was described by Wang [122]. In the reported immunosensor, functionalization of peptide with rGO enhanced the surface area enabling successful binding of anti-PSA on the surface of the electrode. The reported immunosensor exhibited elevated selectivity towards PSA in the existence of known interfering species. Immunosensor's performance was quantified at different concentrations of PSA and it was observed that peak current decreased with growing concentration of PSA. The rGO-peptide fabricated sensor is robust and be utilised for rapid retention of tumor markers.

Diagnosing Cyclin $A_2$ in various types of early-stage cancers utilizing graphene conjugated with peptides was evaluated. The study reported utilization of porphyrin to enhance selectivity of graphene by preventing nonspecific binding interactions [142]. On the graphene surface, hexapeptide P0 (RWIMYF) and poly(ethylene glycol) were fixed as Cyclin $A_2$ detection probe and non-specific binding protection agent, respectively. Electrochemical impedance spectroscopy results revealed that the sensing technique was remarkably responsive and selective with the estimated detection limit of cyclin $A_2$ as 0.32 pM. The developed GO-peptide sensor not only detected Cyclin $A_2$ in cell extracts but was also capable of differentiating healthy cells from cancer causing cells in which cyclin $A_2$ was overexpressed.

Research by Castillo and co-workers reported the application of peptide nanotube conjugated with GO electrode modified by folic acid [143]. They described a method for the construction of GO electrode modified with a novel complex composed of peptide nanotubes along with folic acid for the selective identification of human cervical cancer cells [144]. Microscopic techniques like Scanning Electron Microscopy (SEM) and Atomic force microscopy (AFM) confirmed successful functionalization of peptide nanotubes with folic acid. The conjugation of GO electrode with peptide nanotube-folic acid complex produced rush in the current signal. Cyclic voltammograms in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox species indicated that the adherence of the folate receptor from human cervical cancer cells to the peptide nanotube-folic acid modified electrode reduced the electron transfer causing a decrease in the measured current [144]. Control experiments confirmed that the peptide nanotube-folic acid electrode specifically recognized folate receptors and a detection limit of 250 human cervical cancer cells per mL was obtained. Therefore, the formed conjugate may be used for early-stage diagnoses of deadly ailments like cancer or leishmaniasis disease.

### Determination of adenosine triphosphate (ATP)

Determination of ATP level released is vital to regulate metabolic processes of the body. Since release of ATP is associated with several neurological and nervous system related conditions. Zhenjiang and his fellow researchers described a voltammetric method for monitoring ATP content. The reported method used GO-poly(3,4-ethylenedioxythiophene) (PEDOT) conjugate functionalized with peptide molecules [145] (Fig. 4).

Clinical performance of the aptasensor was investigated by sensing presence of ATP in serum extracted from human blood samples by measurements based on electrochemical impedance spectroscopy (EIS). The formed aptasensor

exhibited enhanced selectivity towards ATP detection. The selected substrate, GO comprises of numerous oxygenated hydrophilic groups which enable fabrication of small molecules like peptides. The attached peptides prevent non- specific binding of biomolecules on the GO-PDOT surface by developing a protective hydration coating [146, 147]. The selectivity of the aptasensor can be contributed to electrostatic interactions along with aromatic bond formation. The designed aptasensor overcomes limitations of reduced sensitivity and biofouling usually exhibited by conventional sensing mechanisms.

## Monitoring protease activity

Early detection of protease related disease is a rising concern of the scientific community. Construction of a GO based sensor fabricated with polypeptide molecule was reported [58]. In this study, thrombin was sensed using the GO-peptide conjugate by determining the current response obtained during cyclic voltammetry experiments [59]. GO acted as a favourable nanomaterial for the sensor formation owing to its adsorption properties and characteristic fluorescence quenching ability. Whereas, dye labelled peptide molecule acted as a probe. The interaction mechanism was interpreted by observing the before and after incubation changes between amino acids and GO. Peptide formed a complex with the GO nanosheets by electrostatic interactions along with π interactions. According to the study biomolecules exhibit different driving forces during conjugation with GO. Concentration of peptide along with buffer was optimised and fluorescence intensity was observed. The sensing platform exhibited thrombin at 2 nM detection limit. Random sequences of peptides were analysed to confirm selectivity. According to Zhang M and co-workers the reported concept can also be utilised for construction of different type of protease sensors by changing the peptide sequences.

## Determination of environmental pollutants

In 2015, Zhu Y and co-workers illustrated the construction of a novel aptamer-GO FRET sensor for One-Step detection of Bisphenol A by application of fluorometric assay [148]. Nucleic acid aptamers have drawn enormous interest since discovery [149, 150]. Peptide aptamers are short and comprise of 5–20 amino acid residues. The property of binding on particular sites of the target molecule makes them a desirable candidate for biosensing applications [151]. Bisphenol A (BPA) is used as a monomer during polycarbonate synthesis, poly(vinyl chloride) (PVC) production and as a plasticizer worldwide. It enters the human body by oral exposure, inhalation and transdermal. BPA is a critical causal agent of several endocrine system disorders [152]. The reported biosensor was created using GO and anti-BPA aptamer labelled with Fluorescein amidite (FAM). GO acted as a fluorescence quencher. FRET was produced in the absence of BPA when FAM-ssDNA adsorbed onto GO. The anti-BPA aptamer exhibited observable signal changes on incorporation of BPA. It modifies its configuration thereby obstructing the absorption of nucleic acid aptamer on the GO surface. The application of reported GO−DNA detection approach was analysed by testing spiked water samples with known BPA quantity. Specificity of the sensor was observed by addition of analogs. According to the results the biosensor distinguished the presence of analogs. The developed conjugate showed good detection performance and is rapid, efficient as compared to conventional BPA sensing systems.

Formation of 2,4,6-trinitrotoluene (TNT) optical detector was also reported in 2015, by Zhang [61]. TNT is an explosive extremely toxic to the environment [140]. The reported label-free biosensor comprised of GO covalently bonded with peptides specific to TNT. Cross-linkers EDC and NHS were used to covalently bind to form a complex between the GO and peptide. Covalent binding resulted in modification of GO structure. Since the peptides in the study were TNT specific, they combined with the TNT via hydrogen bonding. 2,4-Dinitrotoluene (DNT) along with isoamyl acetate were used to confirm the specificity of the biosensor towards TNT. The results of the study indicated enhanced absorption for peptide-functionalised GO in comparison to GO alone. The reported biosensor can be employed for detection of explosives even at low concentrations.

## Detection of pathogenic microorganisms

Consumption of unregulated antibiotics has resulted in emergence of multiple drug resistance in disease causing bacteria [153]. Methicillin-resistant strain of *Staphylococcus aureus* (MRSA) is a gram-positive bacterium, capable of causing skin infections usually acquired from hospitals. It is challenging to cure as a result of acquired resistance towards several antibiotics [138].

Kanchanapally and co-workers (2015) investigated and reported the successful eradication of MRSA [106]. Water containing MRSA was passed through GO membrane fabricated with antimicrobial peptide (AMP) nisin. The membrane resulted in complete disinfection of MRSA. It was observed that the 3D GO film only allowed passage of water thereby retaining the MRSA on its surface. Difference in pore size of GO-AMP film i.e. 300 nm and that of MRSA being 1000 nm resulted in efficient capture of the pathogenic microorganism. Nisin is effective in killing the MRSA since it prevents the synthesis of bacterial cell wall on its surface. As reported by the author synergistic effect of the membrane is also among the several reasons behind the effective destruction of the microorganism. The results were justified using microscopic

techniques like SEM and TEM. Reverse transcriptase-polymerase chain reaction (RT-PCR) data indicated 100% elimination of MRSA. Since MRSA is the foremost cause of sepsis associated mortality. Reported nisin conjugated GO membrane can prove to be an inexpensive and effective diagnostic tool.

*E. coli* O157:H7 is an enterohemorrhagic strain of *E. coli* infamous for causing waterborne infections to humans [154]. Zhou C and co-workers (2018) reported the formation of a sensor for effective validation of *E. coli* O157:H7 from different specimens like water and juice. The sensor works on the principle of surface plasmon resonance (SPR). Magainin I altered by cysteine at C terminals was used as a recognition element. The AMP was conjugated with the Silver nanoparticles and rGO non-covalently via self-assembly. The function of rGO in the constructed sensor was to enhance signal amplification. Magainin I-C detected the pathogenic microorganism at $5 \times 10^2$ CFU/mL. The sensor sensitivity was also tested for other non-pathogenic microorganisms. According to the results obtained the detector can be used for disinfection of other *E. coli* species as well. The constructed sensor is highly sensitive, rapid and economical as compared to traditional methods [155]. Its applicability can be extended to detect various food samples for the presence of foodborne pathogens which tend to deteriorate the quality of the product. Further, the rGO conjugated AMP biosensor might be used for early clinical diagnosis owing to its characteristic feature of reproducibility and stability towards different samples.

## Conclusions and perspectives

Interfaces between graphitic nanomaterials and peptides have immense potential to create novel complexes having applications ranging from diagnostics to therapeutics. On the basis of available literature, it can be inferred that, GO has an edge over graphene and rGO due to presence of intrinsic oxygenated functional groups on its planar surface. To form stable complexes with peptide molecules, it is essential to have epoxides, hydroxyl and carboxyl functional groups. Graphene and rGO lack these groups due to which there are not enough reaction sites available for peptide bonding. It was observed that irrespective of the type of immobilization mechanism followed, several conformational changes occur which impacts the inherent structure of peptide and functional chemistry of graphitic material. Conducting in silico studies using various simulation tools followed by physical research provides a precise insight into changes caused by application of covalent and non-covalent mechanisms. This area has not been explored much by researchers as the complex interactions between peptide and graphene derivatives are difficult to interpret and require highly sophisticated instruments. However, an understanding of different interactions between nanoparticle and biomacromolecules is of utmost importance to make a cost-effective and controllable bio-functionalized materials having sensing capability.

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## Compliance with ethical standards

**Conflict of interest** There are no conflicts to declare.

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