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**Interactions of graphene and graphene oxide with proteins and peptides**

**Abstract:** This review selectively describes the recent progress in the interactions of proteins (enzymes) and shortchain peptides with graphene and graphene oxide (GO). Particularly, the advances of the immobilization mechanisms of enzymes on graphene and GO, the catalytic properties of the immobilized enzymes, and their applications are summarized in detail. The interfacing of the peptides with graphene and GO, the as assembled conjugates, and their potential applications are discussed briefly. The possible ongoing development for the assembly of conjugates of graphene and GO with proteins and peptides in a controlled manner is speculated upon.

**Keywords:** enzyme; graphene; graphene oxide; interaction; peptide; protein.

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**1 Introduction**

Graphene, a single atomic layer of carbon atoms that are chemically bonded with a hexagonal symmetry, shows a true two-dimensional (2D) crystalline structure and unique properties [1–3]. Ever since the first laboratory production [4], graphene and its derivatives have attracted great research and technological interests. Significantly, the discovery of graphene in the laboratory ended satisfactorily the long time argument about whether the true 2D crystals could exist in nature or not [5, 6]. Meanwhile, the unique properties of the pristine graphene sheet, such as the theoretical specific surface area of ~2630 m² g⁻¹, Young’s modulus of ~1.0 Tpa, intrinsic mobility of ~2×10⁴ cm² V⁻¹ s⁻¹, thermal conductivity of ~5000 Wm⁻¹ K⁻¹, and good optical transmittance of ~97.7%, in a wide wavelength range afford graphene and its other related materials pronounced potentials for many applications [7–15]. For instance, using graphene as active or scaffold materials [16, 17], electronic devices [18–20], energy storage systems (super capacitors, secondary lithium ion batteries) [21–25], energy conversion devices (fuel cells, solar cells) [26–29], and bio/chemical sensors have been produced already [30–37]. Up to now, several approaches, including micromechanical exfoliation of graphite [4], chemical vapor deposition [38–42], and solution-based chemical reduction of graphene oxide (GO) to graphene have been developed successfully for preparing the graphene sheets at laboratory scale [43–48], but the mass-scale production of high-quality graphene sheets remains to be exploited. It is fair to say that, as electronic or electrical active materials, the graphene sheets have several advantages [49–51]. However, similar to the carbon nanotube, C60, and other fullereine materials, the lack of surface functionalities (Figure 1A), which can serve as anchoring sites for the external molecules and other species, is a drawback to the certain practical applications of pristine graphene, such as sorption-based sensors [52–54]. Therefore, laborious work has been conducted to modify/functionalize the surface of graphene sheets [55–58]. Additionally, the poor dispersion capabilities of graphene sheets in aqueous solution and most organic solvents make the processing of the graphene sheets extremely hard, especially for use in biological systems [43, 59].

In contrast, GO sheets (Figure 1B) have abundant surface oxygen-containing groups, such as epoxide, hydroxyl, and carbonyl groups. Additionally, the GO sheets could be prepared through the oxidative intercalation and exfoliation of graphite in mass scale [45, 46, 60]. Owing to the enriched surface functionalities, the GO sheets have good solubility in aqueous solution and some polar organic solvents, and can be processed easily through a wet chemical procedure [61–64]. The surface functional groups can also provide plenty of reaction sites for linking the external species, such as small molecules, polymers, biomacromolecules, and inorganic nanoparticles without using any cross-linking reagents or additional surface modification [55, 65–71]. The unique large and atomically flat surface of GO provides a platform for studying the as loaded external species through high-
resolution surface analytic techniques such as atomic force microscopy (AFM) etc. This is essential for studying the interaction mechanism between GO and loaded species and also for their conformational change [65]. The oxygen-containing groups on GO sheets afford them with moderate biocompatibility, thus, favorable to their biological and biomedical applications [72–75].

Reviewing the rapidly expended literature pool of the researches on graphene and GO, there have been more than 77 review articles documented by SciFinder database alone. Review topics covered the advances of preparation and characterization of graphene and GO [76–78], graphene or GO-based chemical/biosensors [31, 79–81], drug delivery systems using graphene or GO [82, 83], biosafety of graphene and GO [84], graphene or GO reinforced polymer composites [85–87], electronic and optoelectronic devices [88–90], energy conversion and storages, and so on [91–93]. Nevertheless, the study considering the interactions of protein (enzymes) and peptide with graphene and GO, and the properties and applications of the as obtained conjugate systems, have not been reviewed yet, though there are large number of publications that have appeared during the last few years in this area.

In fact, it has been illustrated that the individual GO sheets could serve as an ideal solid substrate for enzyme immobilization. It was demonstrated that enzyme molecules could be directly immobilized on GO without using any coupling reagent due to the intrinsic surface functional groups of GO, π–π stacking, and/or hydrophobic interactions [65, 66, 94–96]. Having the atomically flat surface of GO, the loading density and the conformation of the immobilized enzyme molecules on GO could be studied in situ using AFM, which is not easy to perform on other solid substrates, especially the nanoscaled materials used for enzyme immobilization. The advantages of using GO as a solid substrate for enzyme immobilization also stimulated the explorations of the properties and potential applications of the GO-immobilized enzymes. Therefore, this review will selectively focus on the recent progresses of the interactions of proteins (enzymes) and peptides with graphene and GO. We will first describe the protein immobilization on the GO. This part will cover the enzyme immobilization mechanism, characterizations of conformation and properties/activity variation of the enzymes after the immobilization, and performance of the immobilized enzymes. Then the applications of the proteins/GO conjugates will be discussed. Additionally, the interactions of peptides with GO and graphene and their applications will also be summarized. Finally, we will give a brief outlook about the future research in the area.

2 Immobilization of proteins/ enzymes on GO

Immobilization of enzymes on certain solid substrate is an efficient way to improve their performances. The immobilized enzymes have several advantages over the free ones, such as high-thermal and storage stability, easy separation from the reaction mixture [97]. Therefore, the immobilized enzymes can usually find more practical applications as industrial biocatalysis [97–99], biosensors [100–105], and in biotechnology [106, 107]. So far, a range of materials, including glass, polymers, porous materials, and more recently nanosized materials, have been employed as
the solid matrixes for enzyme immobilization [108–116]. It has been illustrated that the composition, morphology (including the dimensions), and surface functionalities of the solid substrates play important roles in regulating their interaction with enzyme molecules, the conformation of the enzymes, and the catalytic activity of the immobilized enzymes [109, 114]. In addition to that, the solid substrate could also serve as a platform for direct observation of the immobilization procedure and the catalytic reaction process. Having unique surface functionality and structural characteristics, GO sheets have been used as a solid substrate for enzyme immobilization through non-covalent adsorption and covalent binding in recent years.

2.1 Noncovalent adsorption of the protein/enzyme molecules on the GO

GO used as a matrix for enzyme immobilization was first reported by Zhang et al. [65, 66]. Without using any cross-linking reagents, horseradish peroxidase (HRP) and lysozyme molecules were immobilized onto GO simply by incubating the GO sheets in a phosphate buffer containing the HRP or lysozyme. As shown in Figure 2, the loading density of HRP on GO depends on the initiation concentration of the HRP in the buffer solution. More importantly, it was illustrated that the immobilized enzymes could be observed clearly with AFM imaging, which is hard to realize on other nanoscaled solid substrates. With these images, the approximate dimensions of the immobilized enzymes were estimated, 140×140×15 Å, roughly comparable to the size of free HRP, 30×60×75 Å. By varying the immobilization conditions for both HRP and lysozyme, the authors demonstrated that the HRP and lysozyme immobilizations on the GO were dominated by the electrostatic interaction between negatively charged GO sheets (in the pH range of 4–11) and enzyme molecules. The GO-immobilized enzymes showed an improved thermal stability and a wide active pH range. The immobilized enzymes also exhibited a high removal efficiency to several phenolic compounds, such as 2,4-dimethoxyphenol and 2-chlorphenol (major components of industrial wastewater). The results showed undeniably the exceptional potential of GO as a solid substrate for enzyme immobilization.

In fact, the interactions between enzyme molecules and GO could be very complicated because the charge status of the surface functional groups of the enzyme depends strongly on the environmental conditions, including the pH value, and the ionic strength of the buffer. The surface density of the oxygen-containing groups on the GO also varied with the preparation procedure and storage conditions. Therefore, through electrostatic interaction,
different enzymes could exhibit different enzyme loadings and stabilities on the GO. On the other hand, the functional groups could also be unfavorable to the enzyme loading if the electrostatic repulsion occurred. The basal plane of the GO enriched with $\pi$ electrons (each carbon atoms of GO bonded with three adjacent carbon atoms with sp$^2$ hybridized orbitals forming robust $\sigma$ bonds, and the rest of the electrons in the $p$ atomic orbitals are delocalized all over the basal plane of the GO sheet forming a superb $\pi$ bond) making it possible for the GO to interact with the enzyme through $\pi-\pi$ stacking interaction.

To test the possible hydrophobic interactions between the enzyme and GO, Guo and his colleagues prepared chemically reduced GO sheets (abbreviated as CRGO), with different reduction extent, and accordingly with different hydrophobicity [94]. Using CRGO as a substrate, and HRP and oxalate oxidase (OxOx) as model enzymes, it was found that HRP and OxOx both could be adsorbed onto CRGO more efficiently compared to that on GO. The enzyme loading on CRGO is tenfold higher, and the maximum loadings reach 1.3 and 12 mg mg$^{-1}$ for HRP and OxOx, respectively. It was also demonstrated that the more CRGO was reduced, the higher was the enzyme loading (Figure 3). This result implies that the interaction between the enzyme and CRGO should be dominated by the hydrophobic interaction. The results also indicate that the hydrophobic interaction between the enzymes and CRGO, in this case, is stronger than the electrostatic interaction between the enzymes and GO [94]. Similarly, Lee et al. [95] reported that heparin can also be absorbed on the surface of graphene through hydrophobic interaction. They found that the hydrophobic interaction strongly depends on both electron density and geometry of heparin. The lower electron density of the low-molecular weight heparin (LMWH) resulted in the decrement of hydrophobic interaction. With LMWH instead of unfractioned heparin (UFH), the conjugation between graphene and LMWH is not effective. Thus, the hydrophobic interaction between the hydrophobic graphene plates and heparin backbones contribute to the effective graphene/heparin conjugation. These results suggest that for proteins with a more hydrophobic surface, CRGO and graphene are better solid immobilization substrates than GO. Actually, the strong hydrophobic interaction between the graphene sheets and protein molecules can be applied in graphene preparation.

As shown in Figure 4, by using the strong hydrophobic interaction between hydrophobic protein hydrophobins (HFBI) and graphene, the graphite could be exfoliated, and the graphene sheets functionalized with HFBI were generated simultaneously [117]. In water, a monolayer of amphiphilic HFBI is spontaneously adsorbed on the hydrophobic surface of graphite. The adsorption lowered the surface energy of graphite and improved the contact between water and graphite. The detachment of graphene and ultrathin graphite sheets coated with HFBI occurs

![Figure 3](image)

**Figure 3** HRP (A) and OxOx (B) loadings on GO and CRGO as a function of the total amount of enzyme. GO and CRGO weights are all 1 mg [94].

![Figure 4](image)

**Figure 4** (A) Structure of the HFBI protein. The molecule has a well-defined structure with aliphatic hydrophobic side chains exposed on one face of the surface (green patch), over an area that accounts for 19% of the total surface area. The diameter of the molecule is about 2 nm, and the molecular weight is 7.3 kDa. The N terminus, to which sequences were added in the engineered variants, is indicated by an arrow. (B) HFBI-facilitated exfoliation of graphene [117].
when the noncovalent adsorption of protein/enzyme onto GO was also studied theoretically using molecular dynamics simulation method [118]. The theoretical result indicated that the π-π stacking between the GO backbone and the aromatic residues of the enzymes might play a key role in the enzyme immobilization on the GO. Figure 5 showed a theoretical model of HP35’s (protein villin head-piece) adsorption on GO through π-π stacking. Theoretical simulation also showed that the softness of GO could help the protein binding by adapting its own shape to fit better with the aromatic residues of the protein forming stronger π-π stacking. This phenomenon was observed experimentally by Alwarappan et al. [119]. They demonstrated that a strong π-π interaction existed between the individual hexagonal cells of the GO basal planes and the glucose oxidase. This is similar to the interactions between the protein molecules and CNTs where the π-π stacking interaction is a dominated factor [112, 120].

Apparently, the enzyme immobilization onto GO might be a result of the synergic effect of the different interactions. De et al. [96] studied the interaction between GO and chymotrypsin and found that GO could strongly inhibit the activity of chymotrypsin, which might be due to the coexistence of anionic, hydrophobic, and π-π stacking interactions and a large surface area to mass ratio of GO. As pointed by Duinhoven et al. the enzyme immobilization on solid substrate should be a comprehensive result of several attractive and repulsive interactions, and the exact interaction or “driving force” for it should be different for various classes of enzymes/proteins [121]. No matter which kind of interaction is the driving force, after the enzyme immobilization, the properties of solid substrate and enzyme could both be affected [122].

### 2.2 Covalently binding enzyme/protein on GO

As aforementioned, weak interactions, including hydrophobic, electrostatic, and π-π stacking interactions could drive enzyme immobilization on GO or graphene. The enriched reactive oxygen functional groups of GO should also render it a good solid substrate for enzyme/protein immobilization through covalent binding [54]. Protein molecules have amine and carboxyl groups on their surfaces. Therefore, the covalent immobilization of enzyme/protein on GO could be achieved through the chemical reactions with these functional groups. Among them, the reaction between the free amine groups on the surface of the enzyme/protein and carboxylic groups of GO or CRGO is applied frequently. As schematically illustrated in Figure 6, bovine serum albumin (BSA) was immobilized successfully on GO sheets through diimide-activated amidation reaction of the amine groups of BSA with the carboxyl

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**Figure 5** (A) A typical structure of HP35 adsorbed on the graphene surface. Here, HP35 is shown as a cartoon with red helix and green loop, the graphene is shown as the cyan lines. (B) The superposition of the adsorbed HP35 structure on graphene (red) with its native structure (green). The images were created with PyMOL [118]. The HFBI-modified graphite stack is disturbed with ultrasonic waves.

**Figure 6** Schematic diagram to produce GOs-BSA [123].
groups of GO under ambient conditions [123]. The BSA covalently bonded on the GO still retains its bioactivity properly.

Besides the direct binding between the chemical functional groups of GO and enzyme/protein, the linking molecules (cross-linkers) are also frequently used to covalently immobilize enzymes onto GO. For example, using glutaraldehyde as a cross-linker, alkaline protease was covalently attached on the GO sheets [124]. A more complicated protocol was proposed and used to immobilize trypsin chemically on the GO sheets by Xu et al. [125]. They functionalized the GO with polylysine (PL) and PEG-diglycolic acid (PEG) and, then, used the PEG-PL modified GO as a substrate for trypsin immobilization (see Figure 7). The advantages of the method are that both PL and PEG served as receptors for the adsorption of trypsin. Additionally, as spacers, they suppressed/minimized the direct adsorption of the trypsin onto GO sheets, which was proven useful for maintaining the activity of the enzyme [125].

More interestingly, using a bifunctional molecule, 1-pyrenebutanoic acid succinimidyl ester (PYR-NHS) as a cross-linker, Kodali et al. created well-controlled micropatterns of glucose oxidase and laminin on graphene (see Figures 8 and 9). The aromatic pyrenyl group of PYR-NHS interacted intensely with the basal plane of graphene through \( \pi-\pi \) stacking, thus, the micropatterns of PYR-NHS could be generated on graphene. The reactive succinimide ester groups of NHS could bind specifically with the amine groups of enzyme. As a result, the micropatterns of the enzymes were created on the graphene surface. The procedure involves actually both noncovalent and covalent binding interactions. This strategy should be useful for massive preparations of parallel biosensors, gene chips, electronic devices, and other multifunctional surface nanoarchitectures [126].

3 Catalytic performances of the GO-immobilized enzymes

Generally, the conformation of the enzymes might be changed after being immobilized on solid substrate surface, thus, their activities could be affected upon immobilization [96]. The effect of the solid substrate on the immobilized enzymes in many cases is unpredictable because its surface structure and property are unknown. The activity of the enzymes immobilized on GO or graphene might be affected by a combination of the GO/graphene chemistry, protein intrinsic property, and the
Figure 8  Schematic illustration of a simple approach to functionalizing graphene noncovalently for subsequent immobilization and micrometer-resolution spatial patterning of proteins [126].

Figure 9  Micropatterned proteins (A) glucose oxidase and (B) laminin on epitaxial graphene treated with PYR-NHS. Scale bars are 20 and 10 μm, respectively [126].

immobilization procedure. However, in the case of GO or graphene, they have an atomic level plain surface, and the surface functional groups of GO are well understood. It is possible to elucidate the performance of the immobilized enzymes through different surface techniques. To investigate the effects of the surface chemical functionality of GO on enzyme conformation and activity, conjugates of HRP and OxOx with CRGOs that have different reduction extents were assembled by Zhang et al. [94]. It was found that the enzyme conformations on CRGOs are closely related to the reduction extents of CRGOs. As shown in Figure 10A, the secondary structure of HRP was lost gradually with the CRGO reduction extent increasing. The severe conformation change resulted in the decrease of enzyme activity as predicted (Figure 10B). The results indicate that the chemistry of GO can affect the conformation and activity of immobilized HRP. On the other hand, the activity of the immobilized OxOx on CRGO was even higher than that of the free one, and the reusability of the immobilized OxOx was improved by the increment of the reduction extent of the substrate CRGO [94]. The better performance of OxOx is ascribed to its higher hydrophobic surface compared to that of HRP. Obviously, the performance of the immobilized enzymes on GO could be modulated both by the intrinsic structure property of the enzymes and the surface functionality of GO.

Figure 10  CD spectra (A) and relative activity (B) of the native HRP and HRP bound to CRGOs [94].
Shao et al. also found that the conformation and activity variations of glucose oxidase occurred after the immobilization on GO [127]. They found that the UV-visible spectrum of the flavin adenine dinucleotide (FAD) moiety in glucose oxidase was changed upon binding to GO. In addition to that, with the increase of the concentration of GO, the intensity of FAD bands further increases, suggesting that the FAD moiety of the enzyme in the GOx-GO bioconjugate system becomes more exposed to a solvent than that in native glucose oxidase. In the meantime, they found that the tryptophan residues of glucose oxidase were converted to a more hydrophobic environment after immobilization through fluorescence spectroscopic studies [127]. A theoretical study based on the CD data demonstrated that the interaction with GO induced a structural transformation from $\alpha$-helix to $\beta$-sheet, even to the unfolding of glucose oxidase. The conformation variations resulted finally in a significant decrease in the catalytic activity of glucose oxidase in glucose oxidation (see Figure 11) [127]. A similar phenomenon was also observed by Pavlidis et al. [128]. With lipase and esterase as model enzymes, they investigated the effects of the carbon nanomaterials, including GO and multiwalled carbon nanotubes (MCNTs), on the catalytic behaviors of the enzymes. The interactions between the enzymes and carbon nanomaterials affect significantly the catalytic activity of the enzymes. Notably, an increase up to 60% of the catalytic efficiency of lipases and a decrease down to 30% of the esterase were observed. Additionally, the use of CNTs and GO derivatives, especially the amine-functionalized ones, led to the increased thermal stability of most of the hydrolases tested. They also believed that the altered catalytic behavior of the enzymes in the presence of carbon nanomaterials arises from specific enzyme-nanomaterial interactions, which can lead to significant conformational changes of the enzymes [128]. Recently, Jin et al. explored the interactions between serine proteases, including trypsin, chymotrypsin, proteinase K, and GO functionalized with different amine-terminated polyethylene glycol [129]. It was found that the PEGylated GO could selectively improve the activity and thermotolerance of trypsin, but barely affected chymotrypsin or proteinase K.

As previously mentioned, the enzymatic activity of the immobilized enzymes in most cases were affected; however, their thermostability, storage stability, and reusability of enzymes immobilized on GO sheets were improved. This could be attributed to the intrinsic structure and properties of the enzymes and the unique 2D motif of GO.

### 4 Applications of the conjugates of protein and GO

#### 4.1 Construction of the multifunctional composites

Besides biocatalytic applications, the conjugates of protein (enzyme) and GO are emerging as a template for further inorganic nanoparticle assembly that affords the as prepared nanocomposites with more feasible structures and unique properties over the composites of bare GO or inorganic nanoparticles [67–69]. As shown in Figure 12, using GO immobilized with BSA as a template, a series of metal nanoparticle-BSA-GO composites were synthesized [130]. In comparison with bare GO sheets, the GO/BSA template showed an extremely versatile and highly efficient assembly capability to the inorganic nanoparticles. Similarly, Lu et al. prepared Au nanoparticle-$\beta$-lactoglobulin-GO nanocomposite and found that the as prepared complex system could yield a strong surface-enhanced Raman scattering (SERS) for Rhodamine 6G (see Figure 13) [131].

Additionally, on the basis of GO/protein or graphene/protein conjugates, some biomimetic nanocomposites have been prepared. Using the conjugate of graphene and hydrophobin as a matrix, Linder et al. prepared the biomimetic nanocomposites with a new approach (Figure 14) [132]. The hydrophobin of the system was connected with nanofibrillated cellulose (NFC) forming biomimetic nanocomposites with remarkably good mechanical properties (modulus: 20.2 GPa, strength: 278 MPa, strain-to-failure: 3.1%, and work-of-fracture 579 kJ m$^{-2}$) [132].

![Figure 11](image-url) Effects of GO on the enzymatic activity of glucose oxidase in glucose oxidation [127].
Figure 12  TEM images of (A) BSA-RGO, (B, C, D) AuNP-BSA-RGO, (E) PdNP-BSA-RGO, and (F, G) PtNP-BSA-RGO. AuNPs and PtNPs had average diameters of 6 and 4 nm, respectively. PdNPs had a worm-like shape. Approximately 4 nm in one dimension [130].

Figure 13  (A) TEM images of BLG-RGO/Au hybrid and the inset showing a higher-resolution TEM image of the same sample. (B) Raman spectra of solid R6G (black curve) and SERS spectra on BLG-RGO (red curve) and BLG-RGO/Au hybrid (blue curve) [131].

4.2 Improvement of the biocompatibility and biodegradation of GO and graphene

Along with the increasing exploration on the potential applications of graphene and GO in many areas including biomedicine, advanced materials, and electronic products, the issues regarding the biocompatibility, safety, and possible degradation (removal) of graphene, GO, and their derivatives have also attracted great attentions. The adsorption of the protein (enzyme) molecules on graphene and GO, in turn, may improve their biocompatibilities or accelerate their chemical process or biodegradation.
Kotchey et al. studied systematically the enzymatic oxidation of GO and CRGO by HRP in the presence of hydrogen peroxide \[133\]. As shown in Figures 15 and 16, with electron microscopy, it was found that the HRP could catalyze the oxidation of GO, but not the CRGO. The computational docking study showed that the active site, heme, of HRP was in a closer proximity to GO compared to the CRGO owing perhaps to the surface functionalities of GO wherein the HRP adsorbed more closely to it than that of the CRGO. Though the oxidation is not very efficient, the study provided a route to degrade GO when they are not needed.

Following the enzymatic degradation of GO, the biodegradable nanocomposites of amyloid fibrils and GO were reported by Li et al. (see Figure 17) \[134\]. Amyloid fibrils are naturally occurring protein aggregates with a good stability in solution under highly hydrated conditions, have well-organized supramolecular structures, and outstanding strength. The as prepared nanocomposites of amyloid fibrils and GO show high conductivity and can be enzymatically degraded entirely. They can reversibly change shape in response to variations of humidity and can be used in the design of biosensors for quantifying the activity of enzymes.

### 4.3 Other applications

Herceptin, a well-known humanized IgG monoclonal antibody against the extracellular domain of the human epidermal growth factor receptor 2 (Her2), shows a good binding affinity to GO and CRGO. On the basis of this observation, Guo et al. developed a herceptin/CRGO conjugate by simply incubating the CRGO and herceptin under an alkaline condition (Figure 18) \[135\]. Interestingly, it was found that the herceptin/CRGO conjugate exhibited a constant fluorescent intensity. Additionally, as shown in Figure 19, the stability of herceptin/CRGO conjugate under high ionic conditions, near-infrared excitation characteristics, and nonphotobleaching properties render it an excellent bioprobe for live cell imaging.

The crystal structure of a protein is important to determine or understand its detailed structure, function, and also the application. The diverse interaction of graphene/GO with protein has also been applied in protein crystallization. Gully et al. showed that graphene and GO could improve both the crystal nucleation and crystalline output of protein at a lower protein concentration \[136\]. As nucleating agents, graphene and GO showed that they are universally applicable for many proteins under...
Figure 15  TEM micrographs of GO after 0, 5, 8, 10, 12, and 20 days of incubation with HRP and 40 μM H₂O₂ [133].

Figure 16  TEM micrographs of RGO after 0, 10, and 20 days of incubation with HRP and 40 μM H₂O₂ [133].

Figure 17  AFM images of the GO/amyloid fibril composites with GO to amyloid fibril ratios of (A) 1:2, (B) 1:5, and (c) 1:8 [134].

Figure 18  Schematic of herceptin-directed reduction and functionalization of GO and its application for intracellular fluorescent imaging [135].
different conditions (Figure 20). This work actually offers a novel approach for the determination of additive efficiency in protein crystallization owing to the well-studied surface functionality and unique single atomic-layered morphology.

In most cases, the individual sheetlike morphologies of GO and graphene are preserved properly when the enzyme and protein molecules are immobilized on them. However, using hemoglobin (Hb) as a cross-linker, Huang et al. creatively prepared a GO/hemoglobin composite supramolecular hydrogel by simply mixing Hb and GO under ambient conditions (Figure 21) [137]. Using pyrogallol oxidation by hydrogen peroxide in an organic solvent as a model reaction system, they demonstrated that the GO/hemoglobin composite hydrogel has a higher catalytic activity and stability than free hemoglobin or GO [137].

Additionally, a more complicated GO-biotin-streptavidin complex system was assembled by immobilizing biotin and streptavidin stepwise on the GO and was used for protein affinity application. The complex system shows a strong biotin recognition capability and an excellent loading capacity. It can capture biotinylated DNA, fluorophores, and Au nanoparticles demonstrating the usefulness as a docking matrix for the affinity purification of protein and other biomolecules [138].

Figure 19  (A) Confocal fluorescence lifetime image (top) and the corresponding fluorescence intensity image (bottom) of dried herceptin/CRGO conjugate. Scale bar is 10 mm. (B) Fluorescence fluctuation trace of the herceptin/CRGO. (C) TCSPC graph of the fluorescence lifetime decay (black line) and the corresponding fitting curve (red line) [135].

Figure 20  Crystalline material forming conditions in protein crystallization trials with (darker gray) graphene (white), a control, and (lighter gray) GO. The crystalline material includes microcrystals, needles or needle clusters, rods, and single crystals. Error bars represent SE [136].
5 Interactions of graphene and GO with peptides

Similar to proteins (enzymes), many researches showed that the short-chain peptides could also be bonded to graphene and GO. The small peptides assemble preferentially to the edge or planar surface of GO or graphene via electrostatic or π-π interactions [139–146]. For example, Katoch et al. demonstrated that the peptide GAMHLPSWHMGTL (a dodecamer peptide) could be adsorbed onto the graphene surface forming a complex reticular structure (Figure 22). The peptide assumes a helical conformation differing from the α-helix in that it exists in a buffer solution due to its interaction with the graphene surface [145]. Comparing with proteins, the relatively simple structure of peptides makes it possible to theoretically study the detailed interactions to predict the conformational changes during adsorption. It is also possible to measure, experimentally, the interaction between the amino acid residues and the graphene or GO surface. For instance, based on an all-atom molecular dynamic simulation, Ou et al. showed that when α-helical peptides were adsorbing to the graphene surface, they unfolded and assembled into amorphous dimers, and almost no β-sheets could be formed at the graphene surface [147].

The simulation indicated also that perhaps owing to the strong interactions between Arg13-Ile14-Lys15 and the graphene surface, the adsorption and the α-helix unfolding are initiated from the C-terminal region, and the extent of unfolding depends on the interaction strength. More specifically, using the density functional theory and the Møller-Plesset second-order perturbation theory (MP2) within the linear combination of atomic orbital-molecular orbital (LCAO-MO) approach, Kawazoe et al. studied, theoretically, the interaction of phenylalanine (Phe), histidine (His), tyrosine (Tyr), and tryptophan (Trp) molecules with graphene and CNTs. It was shown that the aromatic rings of these amino acids prefer to orient parallel with the plane of graphene and CNTs through π-π interactions with a strength order of Trp > Tyr > Phe > His [148]. Meanwhile, Ye et al. investigated experimentally the interactions of lysine (Lys), His, arginine (Arg), Trp, Tyr, and Phe with GO [142]. They illustrated that the binding strength between the amino acids and the GO surface followed the order of Arg > His > Lys > Trp > Tyr > Phe, which is a little bit different from the theoretical data obtained by Rajesh et al. [148]. The reason might be that besides the π-π interactions, the amino acids Lys, His, and Arg have positively charged side chains, which can be interacted with GO via electrostatic interaction, additionally. These results elucidated that, similar to the adsorption of enzymes and
proteins, the adsorptions of short-chain peptides onto the GO should also be a result of the multifunctional interactions between peptide molecules and the GO. Moreover, the peptide-GO conjugates were also used as templates to assemble gold or other nanoparticles to build multifunctional nanoarchitectures. The GO-peptide complex was developed as an ultrasensitive sensor for detecting small molecules such as TNT [139]. The dye-labeled peptides/GO conjugates have been used as a sensor to probe biomolecules and live-cell imaging [140–142].

6 Summary and outlook

In summary, we have highlighted the recent advances of the researches on the interactions of proteins (enzymes) and short-chain peptides with graphene and GO. As novel 2D materials, the atomic flat surface, abundant surface functional groups, and ultralarge aromatic structure render GO and graphene as an ideal platform for elucidating the immobilization mechanisms of enzyme, protein, and short-chain peptides on them, theoretically and experimentally. It has been illustrated that enzymes, proteins, and short-chain peptides can be immobilized through covalent and non-covalent interactions. After the immobilization, the conformations of some of the enzymes, proteins, and short-chain peptides were changed, which further affect their bioactivities. Through the interactions of GO and graphene with enzymes, proteins, or short-chain peptides, more complicated supramolecular architectures were assembled. Some of the as obtained conjugates showed unique biological and chemical properties that were found applicable as bio-probes and biosensors.

However, as an emerging area, several fundamental and technical issues remain to be resolved in the area. First, the detailed site distribution of the oxygen-containing groups on the GO and CRGO is unclear at the moment, which is critical for understanding deeply the interaction mechanisms of the proteins and peptide molecules with GO. Second, there is still lack of instrumentation or techniques to follow in situ conformational change of the proteins during immobilization. Third, it is well-known that the graphene and CRGO sheets were enriched with the π electrons, and some enzymes catalyzed reactions that electron transfer has occurred. However, the electron transfer between graphene/GO and the immobilized enzyme has not been observed yet. This question may be solved with model complexes that catalyze the redox reaction. In this review, we mainly focused on the variations of the properties of immobilized proteins (enzyme) and peptides. In fact, the enzyme effects on the intrinsic structure and properties of GO and graphene are also worth exploring, which may be helpful for the application of the conjugates. It is also worth pointing out that combining the unique morphology of graphene/GO and the versatile functionalities of protein and peptide molecules, the conjugates of proteins or peptides and graphene/GO should be a promising platform for the fabrication of novel graphene-based nanoarchitectures.

Acknowledgments: We thank the National Science foundation of China (nos. 91123011, 90923041, 31070742), The State Key Laboratory of Bioreactor Engineering (no. 2060204), 111 Project (no. B07023), the Science and Technology Commission of Shanghai Municipality (no. 11DZ2260600), and the National “973 Program” of China (no. 2010CB933900) for the financial support of this work.

Received November 20, 2012; accepted December 29, 2012

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