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Recent advances in chemical functionalisation of graphene and sensing applications

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Abstract: Graphene, a one-atom-thick, sp^2-hybridised allotrope of carbon, has attracted massive interest due to its outstanding electrical, mechanical, thermal and optical properties. The material of graphene is remarkably stable, with a huge potential for developing various types of sensors, including biomedical sensing where enhanced specificity, sensitivity, label-free nature and cost effectiveness for rapid point-of-care detection of diseases are paramount. This is due to the simplicity with which its electronic properties can be modified since each atom in the structure directly interacts with the sensing environment. These unique characteristics can be exploited for several kinds of sensing applications such as electrochemical and electrical sensors as well as optical sensors. While pristine graphene is desirable for applications that require high electrical conductivity, many other applications require functionalised graphene for optimal performance. Therefore, the functionalisation of graphene is a significant step towards tuning its structure for various sensing applications. In this review, we report recent technological progress in the chemical functionalisation of graphene and its sensing applications.

Keywords: grapheme; sensors; biosensors; functionalisation; point-of-care detection.

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1 Introduction

Graphene, a two-dimensional (2D) sp^2-hybridised allotrope of carbon has attracted significant attention in recent times to emerge as the most intensely researched material (Geim and Novoselov, 2007). Following its isolation in 2004 via mechanical cleavage (Novoselov et al., 2004), graphene has been employed for highly diversified applications (Liu et al., 2012b; Kuila et al., 2011), owing to its outstanding properties which include good electrical conductivity, large surface-area-to-volume ratio and good mechanical plasticity (Shao et al., 2010). Graphene has shown great promise for use in energy applications, ultra-thin and transparent sheets (Su et al., 2011; Biswas and Dizal, 2010; Wang et al., 2010a), pollution control (Dreyer et al., 2009), polymer composites (Du and Cheng, 2012; Kuilla et al., 2010), drug delivery systems (Liu et al., 2013b; Yang et al., 2013), liquid crystal devices (Iwan and Chuchmala, 2012), nanoelectronics (Areshkin and White, 2007; Li et al., 2010), sensors and biosensors (Kulkarni et al., 2014; Ma et al., 2013a) and supercapacitors (Pumera, 2010, 2011; Maiti et al., 2014b).

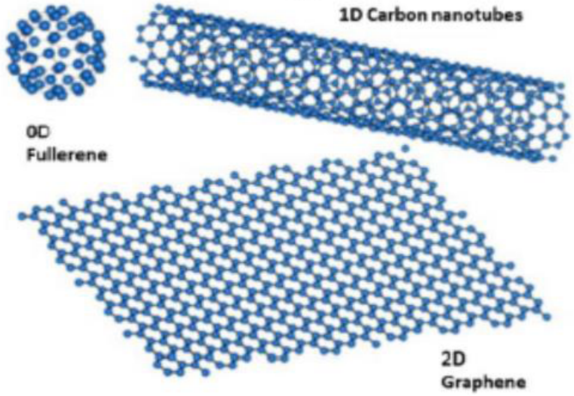
The intrinsic characteristic of graphene to go into and subsequently remain in single sheets in varying matrices is a key attribute with huge application potentials. Therefore, the unvaried dispersion and incorporation of graphene into motley matrices requires the conservation of its intrinsic electronic properties. The presence of van der Waals complex in the graphene sheets inhibit seamless dispersion of graphene in common solvents. In addition, the same complex tends to cause graphene monolayers to re-aggregate following inceptive dispersion and exfoliation. Therefore, the solubilisation of graphene in different solvents is essential in realising its varying applications. In particular, the structural functionalisation via atomic doping, biomolecular immobilisation, and molecular impregnation requires well defined architecture for graphene derivatives. These functionalisation techniques have the potential to further reveal novel applications for graphene, ranging from bioimaging to solar cell harvesting. The literature is replete with reports of various efforts to improve the dispersion of graphene sheets in organic osmotic and aqueous solutions via appropriate functionalisation of graphene surface engineering (Ramanathan et al., 2008; Schniepp et al., 2006; Xu et al., 2008). For this purpose, covalent (Liu et al, 2008b; Xu et al., 2009a) and noncovalent (Bai et al., 2009) functionalisation methods are the most preferred.

In covalent functionalisation, exterior species such as inorganic nanoparticles, molecules, biomolecules, and polymers are covalently bonded in the absence of any cross-linker or agents. The active sites for such functionalisation mechanism are provided by functional groups of reduced graphene oxide (RGO) and graphene oxide (GO). In addition, the atomically oriented flat surface of graphene and GO provides a platform for the reservoir of assorted chemical species. Noncovalent functionalisation, on its own, employs a chemical approach for graphene functionalisation. This can be accomplished via decoration with biomolecules, aromatic compounds, and polymers or alternatively via secondary interactions such as π-π interaction and van der Waals forces. The ability of noncovalent functionalisation technique to enrich graphene with additional functionalities while maintaining and preserving its structural integrity and electronic conductivity makes it advantageous over covalent functionalisation method. The literature is replete with reports of several review work detailing the functionalisation and modification of graphene nanomaterial (Liu et al., 2012a; Maiti et al., 2014a; Kim et al., 2015; Hirsch et al., 2013; Park and Yan, 2013; Quintana et al., 2013; Georgakilas et al., 2016). This latest review attempts to discuss recent and novel developments in the application of functionalised graphene as a platform for various sensing functions.

Before now, several zero-dimension (0D) and one-dimensional (1D) nanomaterials had been the target for novel sensor developments and applications (Huang and Chen, 2010; Huang et al., 2011; Roy and Gao, 2009). These include carbon nanotubes (CNTs) (Maeshashi and Matsumoto, 2009; Sinha et al., 2006), quantum dots (QDs) (Frasco and Chaniotakis, 2009), nanowires (Chen et al., 2011a) and nanoparticles (Luo et al., 2006a). Graphene has shown extraordinary potential in sensing applications (Zor et al., 2015) despite its short research history. In fact, graphene has demonstrated enormous potentials for assorted forms of novel sensors that exploit its unique physical and chemical properties which include large surface-area-to-volume ratio, high carrier mobility, good electron transfer kinetics, single atom thickness and seamless fluorescence quenching ability. These properties are the direct result of 2D, sp^2 hybridised nature of graphene. Interestingly, graphene is shown to possess mechanical rigidity that is two orders of magnitude higher than steel, two orders of magnitude of surface area greater than single-walled carbon nanotubes (SWCNTs) (Noh et al., 2015), good thermal conductivity, excellent quantum Hall effect and good bandgap tunability (Geim and Novoselov, 2007), all of which are considered excellent markers for sensing applications.

In addition, by modulating any of doping concentrations, the quantum of layers, synthetic conditions and dimensionality, an adjustable graphene with super-potentials for motley applications can be developed (Zhu et al., 2010c). These features can significantly boost the opportunities for further functionalisation of graphene surface engineering thereby enriching the already versatile graphene with additional sensing, biosensing, and biomedical functionalities. Therefore, graphene will continue to be a star in sensing applications.

Figure 1 Allotropes of carbon: fullerene (0D), CNTs (1D) and graphene (2D) (see online version for colours)



Source: Soldano et al. (2010)

Figure 2 Schematic illustration of various synthesis methodologies for grapheme

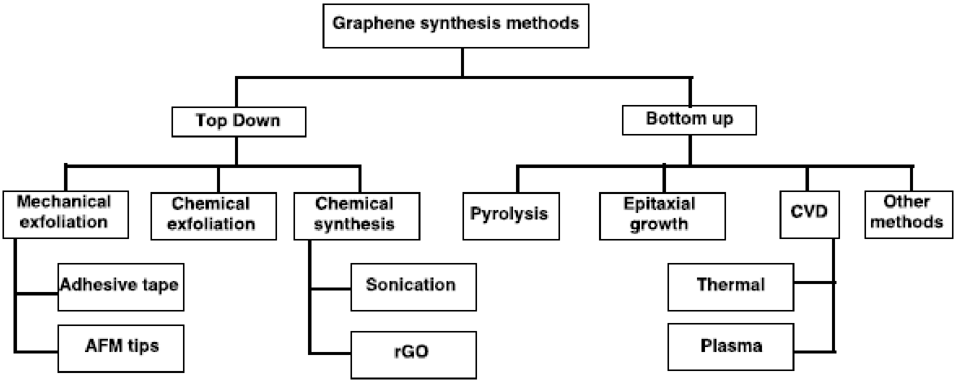
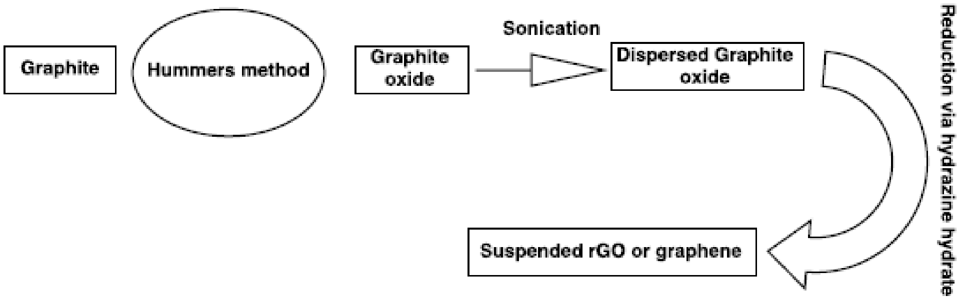


Figure 3 Schematic of the process flow mechanism for graphene synthesis derived from graphite



2 Structure and synthesis of graphene

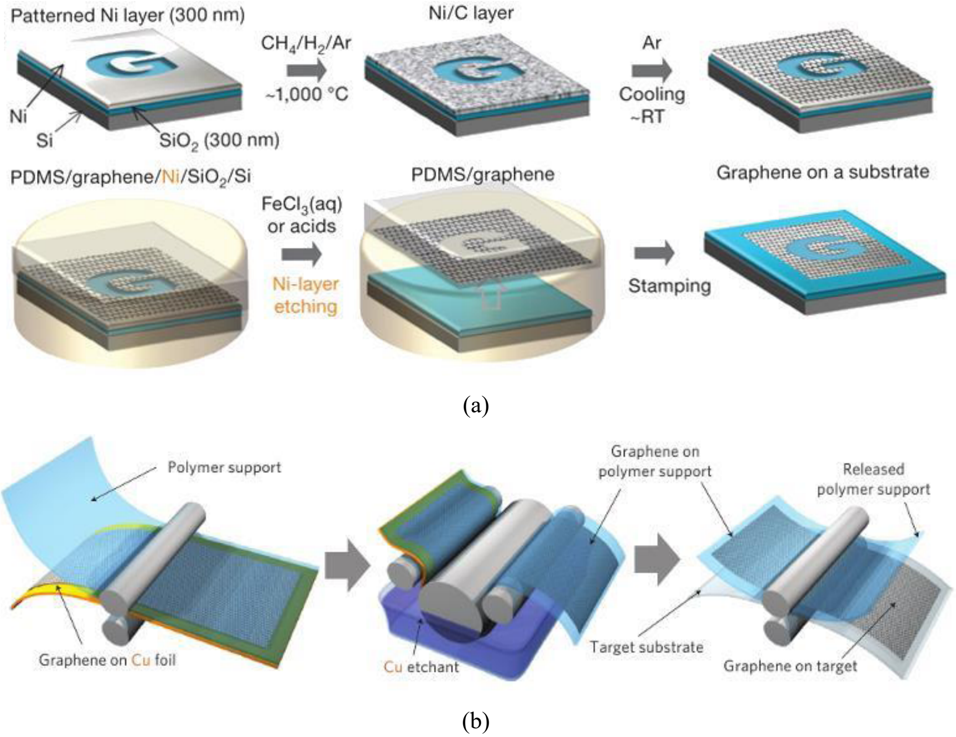
2.1 Structure of graphene

Structurally, graphene is a fundamental material of allotropes of carbon, which include fullerenes, graphite, CNTs and carbon coal (see Figure 1), that is single-atom-thick with planar sheets of sp^2-hybridised carbon atoms arranged in a honeycomb lattice. Graphene can be considered as an infinitely massive aromatic molecule, which positions it as a rare member of the aromatic compounds including flat polycyclic aromatic hydrocarbons. The outstanding properties and highly diversified projected applications of graphene have informed the development of novel synthesis techniques, stimulating the birth of various substrates and methodologies. The bulk production of graphene exploits both bottom-up and top-down approaches, which include but not limited to, mechanical exfoliation, chemical vapour deposition (CVD), thermal decomposition, thermal reduction, exfoliation and reduction-oxidation processes. Figure 2 shows an overview of graphene synthesis techniques while Figure 3 illustrates the process flow mechanism of graphene synthesis as derived from graphite oxide. The various developed methods allow for trade-offs in terms of dimension, cost, and quality of service in the course of their applications (Soldano et al., 2010).

2.2 Chemical vapour deposition

The bulk preparation of graphene is achieved via CVD through the application of appropriate metal exteriors at elevated temperatures (Lotya et al., 2009; Zhang et al., 2013). In this process, the carbon material is interfaced with a metal substrate, followed by precipitation on the substrate via a cooling process. For example, CVD has been achieved by incorporating a nickel (Ni) substrate in a CVD chamber with the assembly held at a temperature below 1,000ºC and under a vacuum, with a diluted hydrocarbon gas (Mattevi et al., 2011). This method, which is similar to carburisation can modulate the thickness dimension and orientation of the resultant graphene layers via the concentration of nickel-carbon complex and cooling rate. Figure 4 shows CVD which is a major preparation method for graphene.

Figure 4 (a) Schematic of CVD preparation of graphene and transfer via polymer support, the carbon interfaces with the Ni during CVD, and forms graphene on the exterior after cooling (b) Roll-to-roll assembly of graphene films grown on Cu foils and transferred on a target substrate (see online version for colours)



(a)

(b)

Source: Krane (2011)

2.3 Thermal decomposition

Other methods to synthesise graphene via material exfoliation include high-boiling point solvent heating and gradient ultrasonication (Krishnamoorthy et al., 2013). These methods have reputation for the production of defect-free graphene. While the use of high-boiling solvent limits the scale of the resulting deposited graphene sheets, the employment of surfactants, sodium dodecyl benzene sulphonate for instance, was found to enhance graphitic exfoliation, with the potential hill generated via coulombic repulsion between the surfactant-decorated sheets causing the monolayer graphene to stabilise against the aggregation effects (Hernandez et al., 2008).

2.4 Thermal exfoliation and reduction-oxidation

Graphene can also be synthesised via thermal exfoliation and reduction of graphite oxide. In this process generally referred to as thermally reduced graphene, the attached oxide functional groups are eliminated via a quick heat treatment method, with the exfoliation phase occurring at the exterior following heating at a temperature of 1,050ºC leading to the evolution of carbon dioxide (McAllister et al., 2007). Photochemical and photo-thermal reduction methods have shown tremendous promise for the development of RGO as the process is independent of both toxic chemicals and elevated temperature. In addition, high-quality graphene has been developed via irradiation of graphite oxide with excimer lasers, sunlight and ultraviolet light (Rao et al., 2010).

The oxidation of graphite via a strong oxidising agent interfaced with exceptionally concentrated inorganic acids is another attractive method for high yield of graphene. The preparation of graphite oxide which exploits the top-down approach can be achieved via any of Hummers (Hummers and Offeman, 1958), or tours (Marcano et al., 2010) methods which utilise concentrated sulphuric acid (H_2SO_4) in combination with appropriate oxidants. The above methods yield graphene with congruent properties and therefore could be employed interchangeably.

2.5 Chemical exfoliation

Another well-established technique for large-scale preparation of graphene is the chemical exfoliation method (Varchon et al., 2007). Here, the graphite interlayer spacing is increased to weaken van der Waals forces at the interlayer edges. To achieve this, various active functional moieties are created at the intersection of graphene layers to generate graphene-intercalated compounds (Park and Ruoff, 2009).

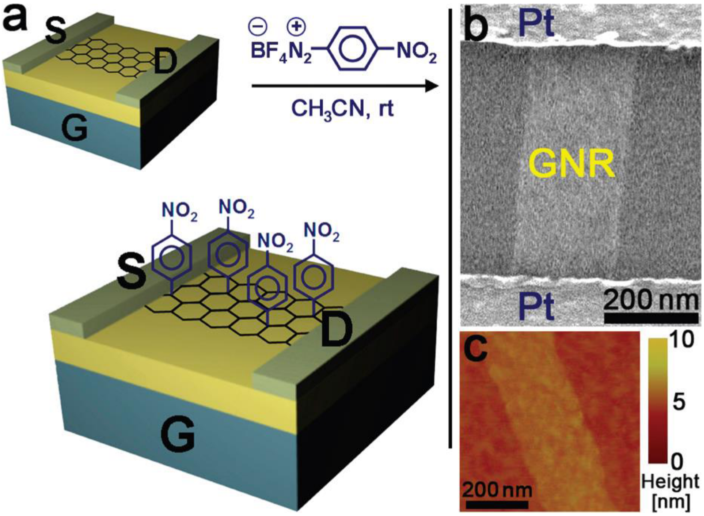
3 Functionalisation and surface chemistry of graphene

Generally, the exterior structure of nanomaterials is important in determining their chemistry with other molecules (Verma and Stellacci, 2010). The outstanding surface properties of graphene are essential in realising their various sensing functionalities (Geim, 2009). The delocalised π-electrons due to the conjugation of the hexagonally ordered honeycomb sp^2 hybridised carbon atoms drive graphene to become conductive (Song, 2013), while significantly improving the transfer rate, resulting in small response time and strong micromolar sensing range (Zhang et al., 2014b). In addition, the massive surface span can be achieved by interfacing graphene derivatives with other functional groups or via direct adsorption (Kybert, 2015). As expected, the sensitivity of the detection system is enhanced via increasing the concentration of the probe molecules. Furthermore, graphene-based derivatives can be greatly enriched with functional groups at the intersection of their edges or exteriors via the application of a smart processing method such as heteroatom doping and oxidation, leading to the development of hybrid sensing devices and molecular level adjustment (Gong et al., 2014). Therefore, tuning graphene exterior chemistry is a fundamental step towards engineering graphene derivatives for various applications. The ability of functionalised graphene to conjugate with varying recognition molecules and incorporate other functional devices is particularly important in bioanalysis (Shao et al., 2010). While various functionalisation methods exist, all of which meet the particular requirements for various types of sensors, the methods of covalent and noncovalent functionalisation are predominant.

3.1 Covalent functionalisation of graphene

As noted earlier, graphene exterior can be chemically modified via either covalent or noncovalent procedure. Graphene can be covalently modified via the interaction of organic functional moieties, unsaturated π-bonds of graphene, oxygen on GO (Georgakilas et al., 2012) and heteroatom doping. Moreover, the presence of carboxylic acid and hydroxyl groups at the intersection of the edges or on graphene basal plane helps to redesign its surface for functionality-specific applications. In the next section, we explore the various routes developed for covalent functionalisation of graphene.

Figure 5 Chemical modification of graphene with 4-nitrophenyl groups, (a) schematic illustration (b) top-view SEM image of graphene nanoplatelet sandwiched by Pt electrodes (c) AFM image of a fragment of graphene monolayer (see online version for colours)



Source: Sinitskii (2010)

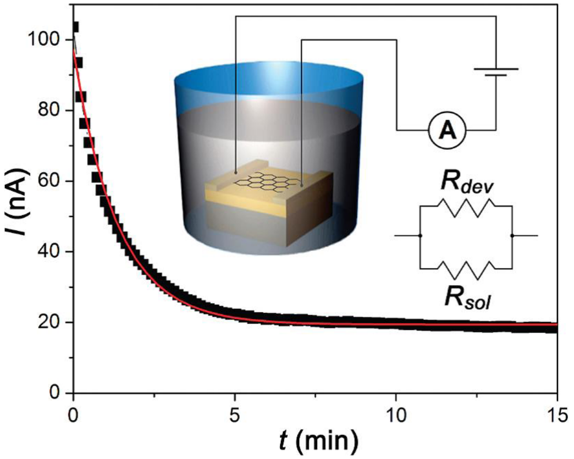
3.1.1 Addition of free radical to sp^2 carbon atoms of graphene

Graphene nanomaterials are commonly treated with free radicals to promote enhanced bandgap and modulate solubility. To this end, radicals generated from diazonium salts and benzoyl peroxide are usually employed. The transport of electron from graphene to photoactivated benzoyl peroxide or aryl diazonium ions yields adaptable varieties of aryl radicals that further add to graphene to form covalent adducts (see Figure 5). At elevated temperatures, diazonium salts yield highly reactive radicals that can go into covalent association with sp^2 hybridised carbon atoms of the graphene. This method has been exploited to redesign graphene via nitrophenyl groups (Sinitskii et al., 2010).

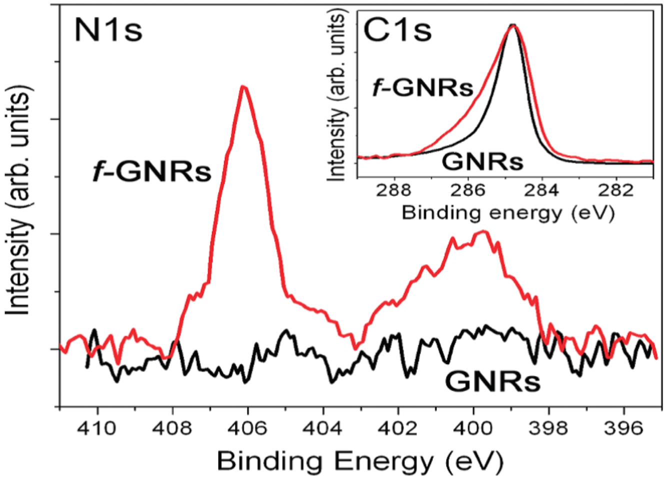
The conductivity of the graphene sheets can be modulated by varying the reaction time, with longer times producing graphene with miniaturised conductivity. Moreover, the degree of functionalisation and reaction time can be employed to modulate the bandgap, potentially creating a nanomaterial-based semiconductor material.

The covalent attachment of 4-nitrophenyl groups to the surface of graphene was verified using X-ray photoelectron spectroscopy (XPS) as shown in Figure 6.

Figure 6 Performance of graphene diazonium salt on a time vs current scale, (a) schematic representation of a two-parallel resistor derived from graphene and solution (b) XPS spectra of GNRs: N1s and C1s before and after chemical functionalisation (f-GNRs) (see online version for colours)



(a)

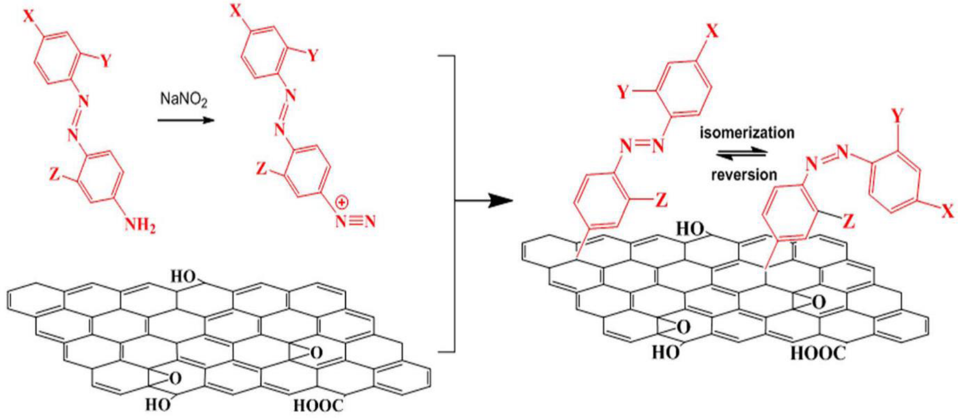


(b)

Source: Sinitskii et al. (2010)

The binding energies of 400 eV and 406 eV functionalised spectrum of N1s XPS graphene, with peaks at 400 eV and 406 eV binding energies are shown to peak at 400 eV and 406 eV, which corresponds to the nitrogen (N) of the nitrogen dioxide (NO_2) and the partially reduced N product. In addition, diazonium salts have been utilised to enable the functionalisation of thermally or chemically converted graphene. Graphene sheets consisting of single layers were synthesised from bulk graphite via mechanical cleavage, and epitaxial graphene (Li et al., 2011).

Figure 7 The preparation route and chemical structures of RGO-para-AZO and RGO-ortho-AZO hybrids synthesised via diazotisation (see online version for colours)



ortho-AZO: X=H, Y=SO_3H, Z=OH
para-AZO: X=SO_3H, Y=H, Z=H

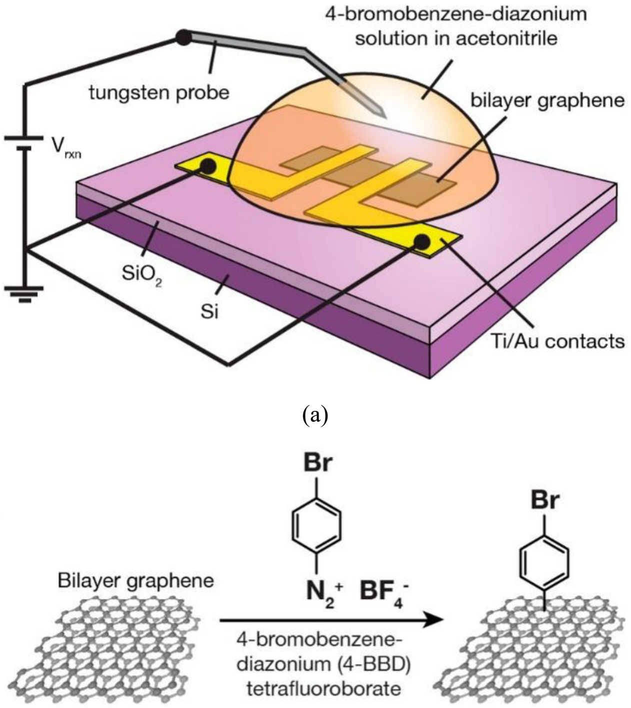
Source: Fang et al. (2009)

Recently, hydroxylated aryl groups have been covalently bonded to graphene via diazonium-mediated addition reaction (Fang et al., 2009). The hydroxylated aryl sites were subsequently attached to polymer chains via atomic transfer radical polymerisation (ATRP). In this event, the covalent linkage of the polymer to the surface of graphene is catalysed by diazonium radicals which act as initiators. Using free radical diazotisation, Feng et al. (2013b) synthesise hybrids of RGO-azobenzene (RGO-AZO) for long-term energy applications via covalent functionalisation (see Figure 7). Furthermore, Liu et al. (2009) report graphene surface modified with phenyl groups via a free radical addition, which involves the deposition of graphene isolated from Kish graphite on a silicon substrate, and immersing same in an initiator solution, usually toluene/benzoyl solution. The reaction of the graphene sheets in the solution was facilitated via an argon (Ar) ion which acts as a catalyst.

The inclusion of the phenyl group was validated through the existence of a robust D band at 1343 cm^{-1}. The D-bands signal the evolution of sp^3-hybridised carbon atoms at the intersection of graphene basal plane, which is created following the addition of phenyl groups. The resultant materials are potential candidates for the fabrication of field effect transistors (FETs) devices. Here, the observed decline in the conductivity of the device can be attributed to the additional sp^3 carbon atoms generated during the attachment of the phenyl groups.

Strano (Wang et al., 2013) study the electronic and physical properties and structures of two-layer graphene subjected to chemical modification using aryl diazonium salts. This scenario is shown in Figure 8. The characteristic changes in the conductive AFM and Raman spectra when the two-layer graphene is employed were noted.

Figure 8 Covalent electrochemical functionalisation of two-layer grapheme, (a) schematic of the experimental setup. Two-layer graphene generated via mechanical exfoliation is on SiO_2/Si wafer and is grounded via Ti/Au metal contacts. a droplet of 4-bromobenzene-diazonium (4-BBD) tetrafluoroborate solution in acetonitrile covers the two-layer flake. A tungsten probe is inserted into the solution droplet to apply a bias voltage during the reaction, Vrxn (b) reaction scheme for the covalent modification of 4-BBD on bilayer graphene (see online version for colours)



(a)

(b)

Source: Wang et al. (2013)

3.1.2 Covalent functionalisation of graphene using dienophiles

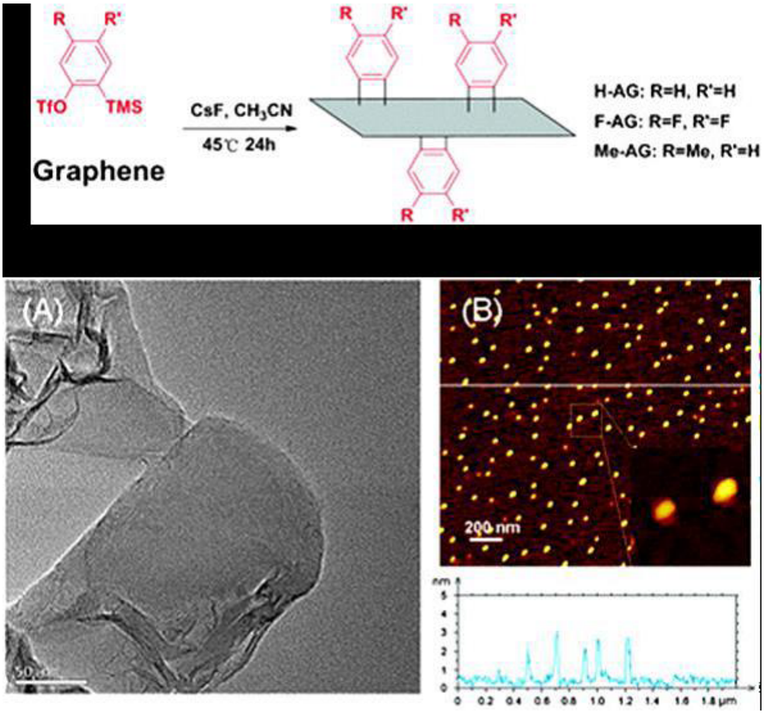
In addition to free radicals-initiated reaction with sp^3-hybridised carbon atoms, the sp^2-hybridised carbon atoms in graphene can react with dienophiles. In this aspect, azomethine ylide, which reacts with 1,3-dipolar cycloaddition is often preferred. The 1,3-dipolar cycloaddition has been employed to modify carbon nanostructures, such as nanotubes, with their derivatives finding applications in several areas, such as nanoelectronics, composites films, solar cells and drug delivery (Georgakilas et al., 2010). Georgakilas et al. (2010) demonstrate graphene sheets synthesised from graphite dispersed in organic osmotic, and coated with pyrrolidine bands via 1,3-dipolar cycloaddition interfaced with azomethine ylides. These graphene sheets are then functionalised with dihydroxyl phenyl groups using the pyrrolidine bands. These groups can be bonded orthogonally to the surface of graphene through the addition of azomethine ylide precursors, which are formed by condensing 3,4-dihydroxybenzaldeyde with sarcosine. This response appends a hydroxyl group to the graphene sheets, enhancing its dispersibility in polar solvents, such as N, N-dimethylformamide (DMF) and ethanol. This enhancement in dispersibility of graphene sheets significantly improves the intensity of G band/Raman intensity of D band (IG /ID) ratio and peak broadening. These changes are attributed to the increase in the degree of compactness of the sp^3-hybridised planar carbon atoms. Comparably, graphene flakes have been coupled to a conjugated polymer using copper-catalysed Huisgen 1,3-dipolar cycloaddition reactions as reported in Castelain et al. (2012).

Quintana et al. (2010) exploit another functionalisation route utilising a 1,3-dipolar cycloaddition by employing a specifically developed NH2-terminated α-amino acid and paraformaldehyde. These amino groups are shown to bind selectively with gold (Au) nanorods. The C-C bonds in graphene react with phenyl and alkyl azides group to form a reactive nitrene intermediate. Reactions between graphene and organic azides have been employed successfully in research to form motley graphene derivatives.

Graphene sheets become enriched with various functionalities via aziridine ring after photochemical and thermal activation of varying para-substituted per fluorophenyl azides (PFPA) (Liu et al., 2010b). The modifications transform the surface energy and solubility-dispersibility of graphene derivatives. Additionally, nitrene has also been employed to functionalise graphene surface. Furthermore, a graphene nanosheet containing lone phenylalanine substituent per thirteen carbon atoms have been synthesised from Boc-protected azido phenylalanine in o-dichlorobenzene (o-DCB). Xu et al. (2013) introduce cyclodextrin on the surface of graphene using alkyl azides (RGO-g-CD) to facilitate the linkage of biopolymers towards the realisation of a thermosensitive nanodevice. The RGO-g-CD nanosheets can further be functionalised with ferrocene-transformed hyperbranched polyglycerol (HPG-Fc) and poly(N-isopropyl acrylamide-co-vinyl ferrocene(II) (PNIPAM-Fc) via a host-guest inclusion between the ferrocene moieties and CD to form RGO-g-CD/Fc-HPG and RGO-g-CD/Fc-PNIPAM nanohybrids. On the dispersibility and stability criteria, both RGO-g-CD/Fc-HPG and RGO-g-CD nanohybrids were found to fare better in a wide range of osmotic than the RGO-g-CD precursor nanosheets, with RGO-g-CD/Fc-HPG nanohybrids exhibiting low cytotoxicity towards 3T3 fibroblasts in a cell viability assay involving a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT).

He and Gao (2010) report an adaptable technique to covalently modify the surface of graphene with polymeric chains and various functional groups via nitrene cycloaddition. This route allows graphene to be covalently attached to various polymers and functional moieties, resulting in 2D macromolecular brushes and functional graphene sheets. The functionalised graphene sheets are shown to exhibit enhanced thermal and chemical stability relative to GO and can be further functionalised via various chemical reactions, including reduction of metal ions, surface-initiated polymerisation, and amidation. The resultant modified graphene sheets were found to be highly processable and dispersible in solvents, and electrically conductive. Park and Yan (2013) demonstrate covalent bond formation between epitaxial graphene and thermally generated nitrenes. Here, the quantum of nitrene in the reaction can be used to tune the band gap of the modified epitaxial graphene. Zhong et al. (2010a) report the cycloaddition of aryne to the surface of graphene by exploiting 2-(trimethylsilyl)aryl triate as a precursor to the reactive benzene intermediate. The scenario is depicted in Figure 7.

Figure 9 Graphene sheets functionalised with an aryne cycloaddition (top), (a) HRTEM image of graphene sheets (with scale bar = 50 nm) (b) tapping-mode AFM image and corresponding height profile of methyl substituted aryne-functionalised graphene sheets (bottom) (see online version for colours)

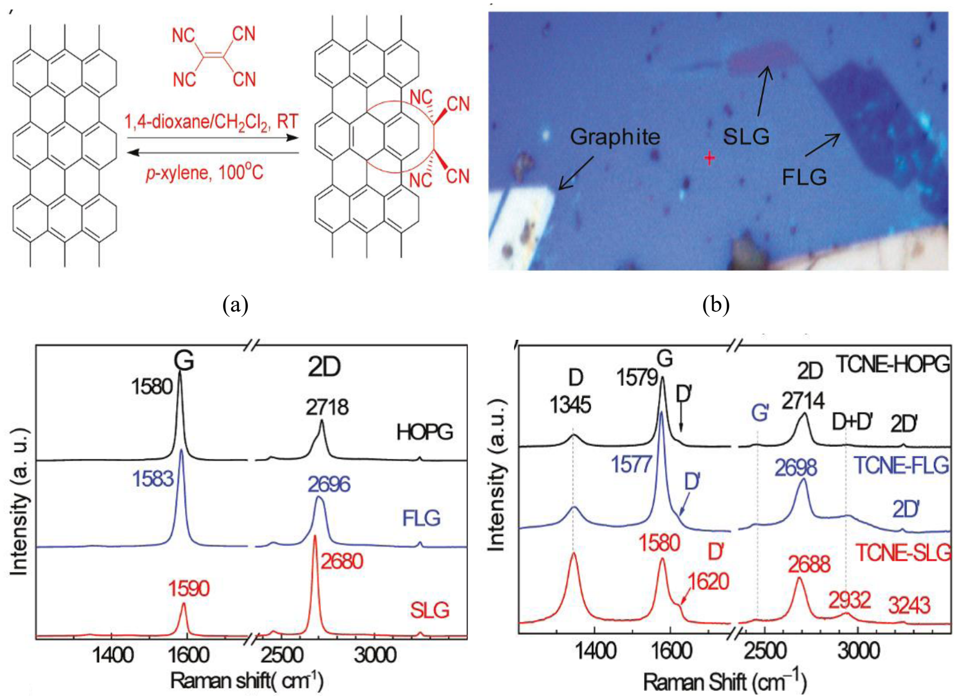


Source: Sarkar et al. (2011)

The structure of the aryne and pristine modified graphene was investigated using atomic force microscopy (AFM) and high-resolution electron microscopy (HRTEM). The HRTEM image in Figure 9(a) shows graphene sheets in the nanometre range dimension of 100–200 nm sise. The AFM investigation of methylated aryne functionalised graphene (Me-AG) indicates average heights dimension of 1.3 to 1.6 nm, revealing few layers of graphene in Me-AG as shown in Figure 9(b) (bottom). The response generated a four-unit ring connecting the graphene surface to the aromatic aryne rings. The aryl-modified graphene groups are dispersible in ethanol, DMF, chloroform, o-DCB and water.

Sarkar et al. (2011) demonstrate the use of graphene as a dienophile and as a diene. The Diels-Alder reacts with a dienophile [tetracyanoethylene (TCNE)] and each unit of graphene at room temperature. This procedure is demonstrated in Figure 10. The progress of this reaction is monitored via Raman spectroscopy, with the spectra of single-layer graphene (SLG), pristine HOPG and few-layer graphene (FLG) showing characteristic G and 2D bands [Figure 10(c)], and the D, D0 and DpD0 peaks appearing in the materials' spectra following the reaction with TCNE [Figure 10(c)]. While the 2D band was found to be significantly weakened, the D band occurring at 1345 cm^{-1} became the most outstanding feature of the Raman spectrum in SLG [Figure 10(b)].

Figure 10 Room temperature Diels-Alder (DA) reaction between graphene (diene) and tetracyanoethylene (TCNE, dienophile), (a) schematic illustration of the reaction (b) micrograph showing a large piece of HOPG, SLG, and FLG on a Si substrate (c)–(d) Raman spectra of HOPG, FLG and SLG; (c) before and (d) after a DA reaction with TCNE (see online version for colours)



(a) (b)

(c) (d)

Source: Sakar et al. (2011)

3.2 *Functionalisation of partially reduced graphene*

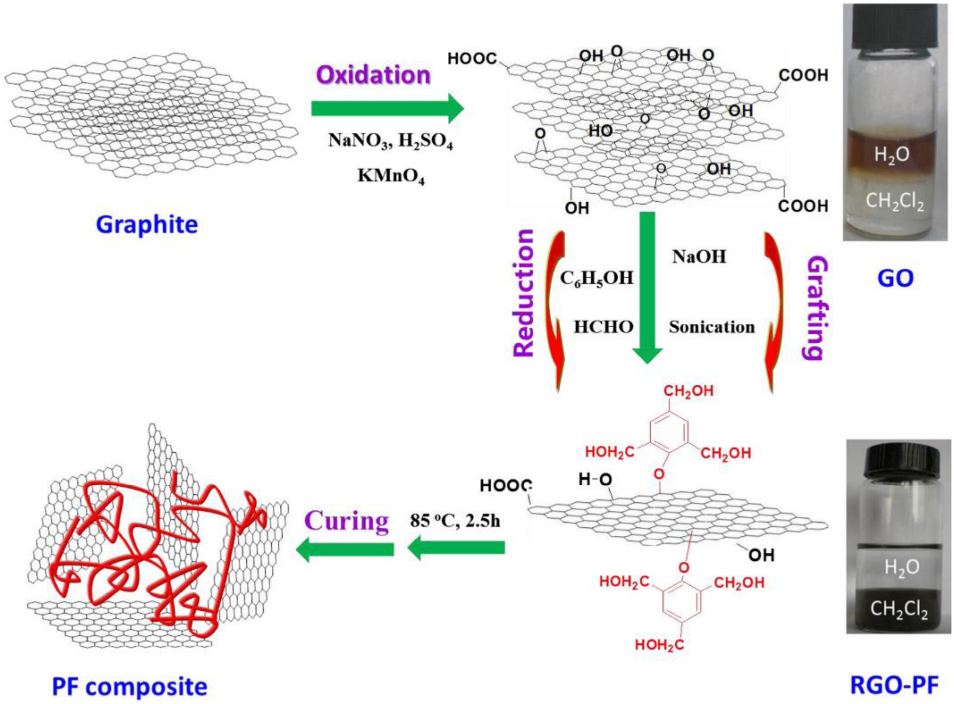
The reduction of GO to graphene through different routes, particularly via chemical means, is convoluted due to the presence of residual hydroxyl and epoxy functional groups on the RGO basal plane (Gilje et al., 2010). The complete removal of oxygen functional groups from the surface of graphene has been confirmed to be impossible during thermal or chemical or combination reductions, consistent with the study of the deoxygenation of graphene structure via density functional theory (DFT) (Gao et al., 2010).

Recently, Iqbal et al. (2014) report an uncatalysed modification of thermally reduced graphene (TRG) using 3-aminopropyltriethoxy silane (APTS). Here, the silane groups are attached to the TRG surface via the residual surface-bound carbonyl and phenolic groups. Similarly, the employment of organic solvent during silylation reaction has the effect of not only increasing the grafting yield from seven to sight atomic percent of Si but also aligns the APTS groups to the intersection of TRG edges.

Cornut (Azevedo et al., 2014) recently demonstrated a novel localised modification technique involving GO deposited on Si oxide surface. The modification is triggered via reduction of GO exploiting electrogenerated naphthalene radical anions. This is followed by the electrografting of the RGO using a diazonium salt carrying a protonated amino species capable of immobilising Au nanoparticle via simple immersion.

Similarly, partially RGO synthesised via solvothermal reduction has been used to generate a graphene organic framework (GOF) (Srinivas et al., 2011). Ajayan (Sudeep et al., 2013) report crosslinked 3D interconnected GO, in what is today known as poly-GO. These 3D engineered nanoporous structures are developed by covalently cascading GO via polymerisation and chemical cross-linking using resorcinol and glutaraldehyde. The poly-GO specimen was found to exhibit robust adsorption capacity for carbon dioxide storage.

Figure 11 Schematic of the simultaneous functionalisation and reduction of GO during in situ polymerisation of a PF composite (see online version for colours)



Source: Yuan et al. (2014)

3.3 Functionalisation of GO

In addition to the classical modification methods herein discussed, graphene can also be functionalised via covalent attachment. Yuan et al. (2014a) demonstrate the functionalisation of GO with phenol formaldehyde via simultaneous reductions that produce ether linkages. This scenario is shown in Figure 11. The functionalisation of GO occurs during a nucleophilic substitution reaction involving the hydroxyl groups of phenol and epoxide groups of the GO under intrinsic conditions. Furthermore, the resultant cured composite was found to exhibit robust thermo-mechanical properties.

Similar to graphene, GO also played role in nitrene chemistry. Moreover, nitrene chemistry was utilised to synthesise G-OH by treating 2-azidoethanol with GO for further modification to form amphiphilic nanoscale ionic materials (NIM) (Wu et al., 2014c). In addition, GO has been covalently functionalised with phenothiazinyl species and its photochemical activity studied (Ballesteros-Garrido et al., 2014). Zhao (Zhang et al., 2014a) demonstrate the functionalisation of GO employing water-soluble pillarenes for in vitro dual-mode fluorescence and Raman bioimaging. Also, an aromatic nitrogen moiety (ANM) in the form of five-unit pyrazole has been successfully synthesised via hydrazine treatment on GO (Park et al., 2012).

4 Non-covalent functionalisation of graphene

In this section, various techniques for noncovalent functionalisation between graphene and other molecular species are reviewed. Graphene has been noncovalently modified via π-π interactions, hydrogen bonding interactions, electrostatic interactions and hydrophobic attraction between graphene and a surfactant, among others. Here, the functional properties of specific materials are also outlined and prospected. The noncovalent modification of graphene and other organic molecules is of interest to the scientific community because these approaches functionalise the surface of graphene without diminishing the outstanding properties of 2D, sp^2-hybridised network (Yang et al., 2013). In addition, the subtle changes in the electronic properties of the π system can engineer structural disruptions to the graphene sheets (Kozlov et al., 2012). Therefore, the noncovalent functionalisation of graphene via macromolecules, surfactants, and ionic liquids (ILs) has provided a platform for particularly stable dispersions of individual graphene sheets in both organic and aqueous media without disrupting the structural integrity of graphene. The noncovalent modification of graphene surface via various chemical groups is discussed in the next section.

4.1 Biomolecules and macromolecules

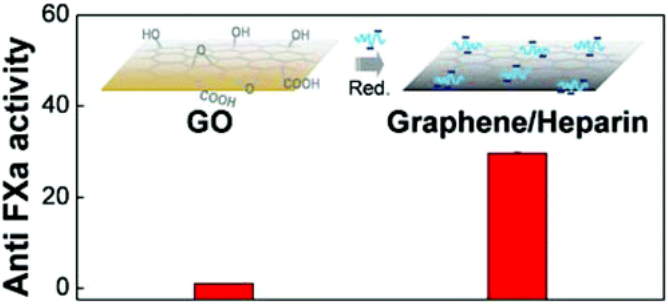
The dispersion of reduced graphene in varying organic osmotic can also be synthesised via noncovalent modification with functional polymers. Kim (Choi et al., 2010) exploited organic graphene dispersed in various solvents, and mediated via noncovalent modification with end-functional polymers. Here, the protonated amine terminal species of the end-functional polymers react with the residual oxygenated functionalities on the surface of graphene that readily transports the graphene from the aqueous phase to the organic via simple sonication. Chen et al. (2016) employ biopolymers, such as epichlorohydrin (ECH) and cellulose, to functionalise graphene creating aqueous-dispersible graphene. The immobilisation of enzyme on the surface of graphene is also receiving a great deal of attention towards improving enzymatic activities. The enzymes can be immobilised on GO in the absence of coupling reagents due to the inherent surface functional groups of π-π stacking, GO and/or hydrophobic interactions (Simsikova and Sikola, 2017).

Jiang et al. (2014) and Hernandez-Cancel et al. (2015) demonstrate the immobilisation of enzymes in the absence of cross-linking reagents. In this aspect, lysozyme molecules and horseradish peroxidase (HRP) have been immobilised onto GO via incubation of GO sheets in a phosphate buffer containing lysozyme or HRP. Guo

(Zhang, 2010) test the possible hydrophobic association between GO and enzymes that was diminished to varying degrees to modulate its hydrophobicity. Interestingly, the enzyme was found to exhibit higher loading as GO was reduced, revealing the predominance of hydrophobic interactions between the enzyme and CRGO. The result is consistent with previous observation of stronger hydrophobic interactions between enzymes and RGO compared with electrostatic interaction. Zhang et al. (2012b) recently demonstrated protein-relevant biosensors anchored on SLG, taking advantage of the kinetic stability of a tripod monolayers. The SLG monolayers of tripod three or pyrene butyrate four and bare SLG are able to capture an anti-E. coli antibody, with added capacity to feature the N-hydroxysuccinimidyl (NHS) esters required for bioconjugation reactions. While the antibody-modified tripod monolayers bind E. coli cells at above background threshold, the antibodies attached to four or adsorbed on SLG show comparable E. coli recognition to control monolayer with a mismatched antibody supported on three.

The findings are consistent with that observed for lectin protein concanavalin A (ConA). The tripod-bound ConA was found to retain its capacity to recognise both oligosaccharides of *Bacillus subtilis* cell walls and dissolved oligosaccharides, with the protein showing no indication of these effects when adsorbed directly onto SLG. These further demonstrate the robustness of noncovalent binding species to preserve the role of proteins on SLG, which are otherwise susceptible to harmful denaturation. In a similar study, Lee et al. (2011) show the absorption of heparin on graphene surface via hydrophobic interactions (see Figure 12). Interestingly, the anti-FXa activity of the graphene/heparin conjugate was found to significantly increase. Other molecules with potentials for immobilisation or interaction with graphene exterior include proteins (Kodali et al., 2011), and chymotrypsin (De et al., 2011).

Figure 12 Blood compatible graphene/graphene conjugate formulated via noncovalent interactions between chemically reduced graphene and heparin (see online version for colours)



Source: Lee et al. (2011)

4.2 Polynuclear aromatic rings

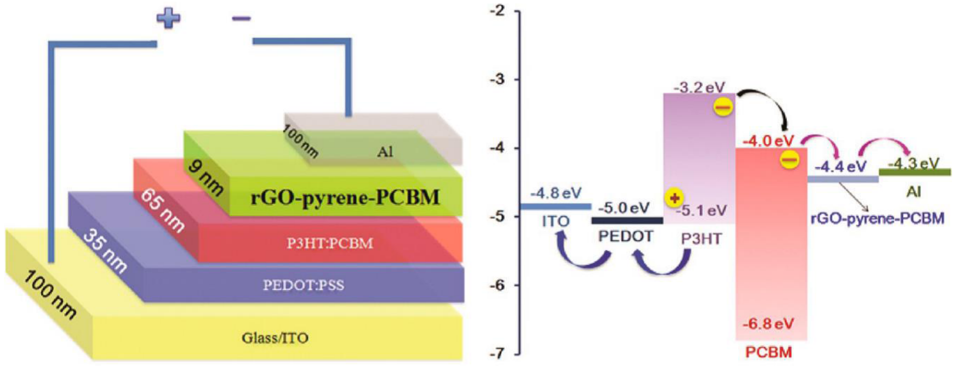
The high affinity for π-π interactions and added functionality make polynuclear aromatic molecules attractive for the dispersion of graphene sheets in organic and aqueous media. In fact, polyaromatic rings, such as pyrene, aromatic dimers of benzene, anthracene, and naphthalene functionalised with polar or flexible side chains have been found to be particularly effective. The pyrene moiety is shown to exhibit a robust affinity for graphite basal plane via π-π stacking. In a study of the functionalisation of various pyrene derivatives, Green (Parviz et al., 2012) demonstrated the ability of these species to stabilise single- and FLG flakes in aqueous dispersions. Moreover, this technique has the potential to significantly increase the yield of graphene/stabiliser when compared with traditional nanomaterial stabilisers, such as polymers or surfactants. The stabilisation mechanism of pyrene derivatives is investigated by reviewing various indicators. Our findings show a marked difference between the polarity of the stabiliser and the solvent for adsorption onto the graphene surface.

The role of the functional species on the pyrene derivatives during the stabilisation mechanism was also investigated. The more electronegative functional species were found to significantly drive the adsorption of stabilisers onto the graphene layers than their less electronegative counterparts. The sodium salts of 3, 4, 9, 10-perylenetetracarboxylic diimide bisbenzenesulphonic acid (PDI) and pyrene-1-sulphonic acid (PyS) have been employed as electron acceptors and electron donors respectively. Their work reveal the presence of large planar aromatic structures in PyS and PDI which anchors them on the hydrophobic exterior of graphene sheets via π-π interactions without perturbing the electronic conjugation of graphene. In addition, the polyaromatic moiety interacts with graphene via π-π stacking, while the acceptor moiety enhances the solubility of the sheets, eliminating the problem of reaggregation. Similarly, water-soluble 1-pyrenebutyrate (PB) has been employed as a stabiliser to prepare unvarying aqueous dispersions of graphene nanoplatelets (Xu et al., 2008). Graphene has been modified with pyrene butyric acid in the presence of a base to form a hydrazine-reduceable product. The AFM images show functionalised graphene nanoplatelets and GO that are 1.7 nm and 1.3 nm thick respectively, with the flexible graphene film exhibiting higher order conductivity comparative to GO precursor. Bose et al. (2011) investigate the noncovalent modification of graphite via 9-anthracene carboxylic acid (9-ACA) to yield water-dispersible graphene with exceptional electrochemical activities, revealing the capacity of 9-ACA to bind to the graphite surface via π-π interactions.

Again, porphyrin-modified graphene sheets have been suspended in an aqueous medium to synthesise highly conductive graphene films (Geng and Jung, 2010). In addition, the appendage of other derivatives of porphyrin, such as haemin (Xue et al., 2014) and 5, 10, 15, 20-tetrakis (1-methyl-4-pyridinio) porphyrin (TMPyP) (Xu et al., 2009a) has been reported.

Recently, Yang (Qu et al., 2013) report the synthesis of a novel graphene-fullerene composite by attaching [6, 6]-phenyl-C61-butyric acid methyl ester (PCBM) to RGO (rGO) via noncovalent modification (see Figure 13). The hybrid morphology of the rGO-pyrene-PCBM was verified via various spectroscopic techniques. Comparatively, the dispersity of the rGO-pyrene-PCBM in DMF was found to be greatly enhanced than in its pristine form due to the intramolecular π-π stacking interactions between pyrene-PCBM and the graphene sheet. In addition, rGO-pyrene-PCBM provided an excellent platform for electron extraction on P3HT:PCBM BHJ-PSC devices, ultimately enhancing efficiency by 15%. The resultant hybrid material exhibited enormous potentials in polymer-based solar cells. In other similar studies, polynuclear aromatic derivatives have been employed as an efficient tool for varying applications (Lin and Buehler, 2013; Schlierf et al., 2013).

Figure 13 Schematic architecture, (a) energy level diagram (b) of ITO/PEDOT:PSS/P3HT:PCBM/RGO-PYRENE-PCBM/AI BHJ-PSC device an incorporated RGO-pyrene-PCBM electron extraction layer (see online version for colours)



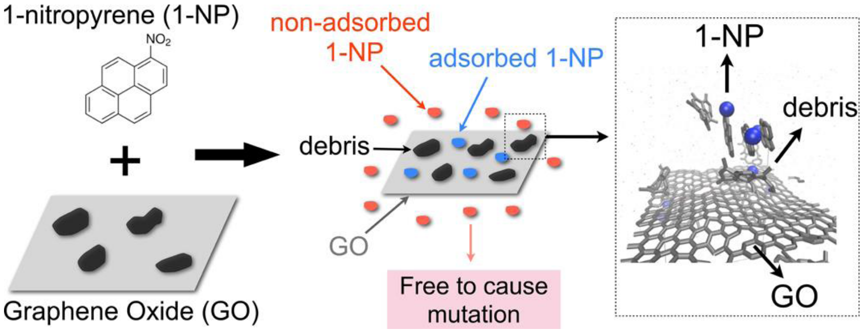
(a) (b)

Note: The left panel shows the dimensionality of each layer.

Source: Qu et al. (2013)

The noncovalent modification of Ni(II) complex containing 5, 7, 12, 14-tetramethyldibenzo-1, 4, 8, 11-tetraazacyclotetradeca-3, 5, 7, 10, 12, 14-hexaene Ni(II)-tetramethyl dibenzo tetra-aza annulene (NiTMTAA) on a graphene surface has been reported (Basiuk et al., 2014). This modification represents a noncomplex model for complex phthalocyanines and porphyrins. The theoretical adsorption of neutral (poly)-aromatic, anti-aromatic and π-conjugated systems on graphene via π-π stacking based on van der Waals density functional theory (vdw-dft) was demonstrated by Persson (Bjork et al., 2010). In addition, the total number of per total atoms was normalised by the π-π interactions, with the carbon atoms registering significant increase proportionate to the amount of hydrogen atoms in the system.

Figure 14 Schematic illustration of the noncovalent interactions between GO and aromatic compounds (see online version for colours)



Note: The role of oxidative debris is also shown.

Source: Coluci et al. (2014)

However, the presence of highly oxidised polyaromatic carboxylated fragments, such as oxidised debris on the surface of the oxidised graphene presents a challenge (Rourke et al., 2011). Coluci et al. (2014) demonstrate the importance of the adsorbed oxidative debris on the surface of graphene during the noncovalent interactions between aromatic compounds and GOs, with those compounds in the 1nm range obstructing the adsorption sites on the surface of GO. This scenario is illustrated in Figure 14.

4.3 Appendage of nanoparticles

In addition to polymers, surfactants, and biomolecules, inorganic nanoparticles have also been attached to graphene surface via noncovalent functionalisation. Most of these techniques employ bridging molecules that are capable of initiating simultaneous hydrophobic, hydrogen bonding and π-π interactions between graphene surface and nanoparticles. Recently, poly (amidoamine) (RAMAM) dendrimer has been employed as a bridge to append silver nanoparticles (AgNP) onto the surface of graphene via noncovalent interactions (Liu et al., 2014). In addition, the employment of RAMAM stabilisation was found to significantly promote unvaried dispersion of AgNP on the graphene surface. Also, Lu et al. (Lu et al., 2009) report a facile noncovalent functionalisation of graphene using Ag nanoparticles. An electrostatic force driven assembly was employed under arc plasma at room temperature. For their part, Liu et al. (2013) Zhu et al. (2010b) report Fe_3O_4 nanoparticles assembled on both sides of RGO with adaptable density initiating 2D nanoparticle composite films. The resultant hybrid material was found to exhibit substantial mechanical robustness, with outstanding capacity to sustain solvothermal and supersonic treatment. Another instance of such nanoparticle appendage to graphene include, arene stabilised palladium nanoparticles (Chen et al., 2013), polyvinylpyrrolidone (PVP) stabilised Fe_2O_3 (Fullerton et al., 2014), polyoxyethylene sorbitol anhydride monolaurate (TWEEN 20) stabilised Au nanoparticles (Lu et al., 2011) and PVP-stabilised AgNP (Dutta et al., 2013).

4.4 Surfactant and ILs

Surfactants are commonly employed to modify various nanoparticles, rendering them more hydrophobic or hydrophilic. Similarly, graphene is modified via noncovalent procedures, such as decorating them with ILs or surfactant-supported functionalisation. Mullen (Liang et al., 2009) report an environmentally-friendly and scalable technique to synthesise chloroform-soluble graphene via a surfactant-facilitated functionalisation. For this procedure, the authors employed the salts of amphiphilic molecules, particularly the quaternary ammonium salts of tetra dodecyl ammonium bromide (TAB), tetradecyltrimethylammonium bromide (TTAB) and di-dodecyl trimethylammonium bromide (DDAB) with one, two and four alkyl chains. Moreover, the extended aromatic conjugation of the reduced graphene was found to be preserved as a result of the ionic interactions, resulting in the high conductivity of the cast composite films. In addition, Loyta et al. (2009) demonstrate a scalable and inexpensive procedure for synthesising unvarying dispersion of graphene at up to 0.3 mg/mL concentration in the water/surfactant system. The dispersion concentration was found to increase sublinearly with the sonication time when facilitated with a sodium cholate surfactant (Pu et al., 2012). Tascon (Fernandez-Merino et al., 2012) report various surfactants employed to stabilise graphene via UV-Vis spectroscopy. The chemical morphology of the surfactants and the essential ILs form attractive candidates for graphene functionalisation without sacrificing their structural integrity.

Before now, various ILs have been explored as functionalising vehicles for graphene towards highly diversified applications (Ma et al., 2014). Moreover, the hydroxyl functionality arising from the ILs has been successfully employed as a ring opening polymerisation plateform for graphene/polymer nanocomposite synthesis. The resulting nanocomposites/graphene exhibited exceptional mechanical characteristics. Furthermore, the localisation of ILs on the surface of graphene yielded a functional hybrid that could be employed as a biosensor.

5 Functionalised graphene as a novel platform for chemical/biosensors

Graphene-based materials are highly appealing for sensing applications in an aqueous environment due to their unique physical and chemical properties. In this section, the complete landscape of recent developments in the role of functionalised graphene as sensing platforms is presented, with the goal of isolating its merits over other non-engineered nanomaterials. In particular, we provide a critical and comprehensive overview of electronic, electrochemical and optical properties of functionalised graphene since these are most relevant to sensor applications. Furthermore, various species of graphene precursors, such as graphene nanoribbon, chemically reduced graphene and large-sised graphene grown via CVD, their consequence on device fabrication and sensing performances are also discussed in this section.

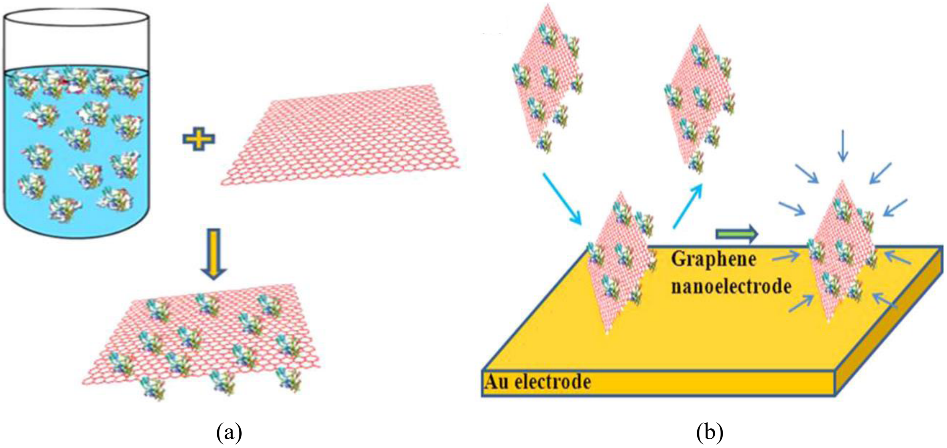
5.1 Graphene-based electrochemical sensors

As noted above, graphene has always shown great potential in electrochemistry (Hernandez and Ozalp, 2012), and exhibits several noteworthy advantages over its closest competitor, CNTs. For one, the presence of metallic impurities in CNTs which interferes with their electrochemical functionality as reported for glucose (Jiang, 2014a), hydrazine, hydrogen peroxide (H_2O_2) (Jiang et al., 2014), amino acids and halothane (Dai et al., 2006) even at thresholds below 100 ppm (Pumera and Miyahara, 2009), are not present in graphene. Moreover, graphite – a precursor for graphene fabrication is both inexpensive and accessible. The use of graphene in electrochemical sensing applications has developed rather quickly. The number of published papers reporting the employment of graphene in sensing and biosensing applications has been exponential since the appearance of the first article (Sundaram et al., 2008). Due to its large electrochemical potential window (Ambrosi et al., 2014), graphene can be used to detect molecules with either high reduction or oxidation (redox) potential.

The presence of edge-plane-like defective sites on graphene promotes good electron transfer kinetics, offering minuscule flakes of pristine graphene or graphene sheets unique chemistries for electrochemical detection. As we already know, the electrochemistry of multi-wall CNTs (MWCNTs) can be tuned via the addition of graphite microparticles or nano graphite impurities, which produces much higher weight/edge-plane ratio than CNTs (Goyal et al., 2010). Consequently, the redox species of $Fe^{3+/2+}$ at RGO/glassy carbon electrodes (RGO/GCE) exhibit ten times higher electron transfer rate than $Fe^{3+/2+}$ at bare glassy carbon electrode. Being an inner-sphere model,

$Fe^{3+/2+}$ possesses an k^o_{app} that is highly sensitive to the exterior carbon-oxygen functionalities, particularly surface carbonyl groups provided by RGO. Moreover, the effective electrical wiring the redox centres of several heme-containing metalloproteins to the electrodes is well supported by GO due to its well-established capacity to facilitate electron transfer kinetics (Boopathi et al., 2014). The screening of the redox centres of proteins in folded polypeptide shells with poor electron transfer kinetics normally result in poor electron transfer rate at the various exterior of metalloproteins. Moreso, the excellent protein adsorption ability and high electron mobility arising from strong hydrophobic interaction project GO as an ideal substrate for accommodating proteins and advancing protein electron transfer. Therefore, GO can advance electron transfer between the surface of electrodes and redox centres of action. Furthermore, extensive oxygen functional species on GO facilitate electrochemical activities (Wu et al., 2012a). Recently, a novel approach for the electrochemical detection of single protein molecules, which are coupled to the surface of graphene sheets was developed (Li et al., 2014), as shown in Figure 15. Here, the noncovalently modified graphene nanosheets manifested increased electroactive surface area, which synthesises to an amplified redox current following a collision between the graphene nanosheets and the electrodes. In addition, stepwise transformations in redox current and the transferred charges in electrochemical activities were observed, which was magnified by frequently oxidising and reducing functionalised graphene nanosheets as they nonspecifically diffuse on the surface of the electrodes. Additionally, the number of the MP-11 molecules playing out on a single graphene nanosheet is at 105±18 range scale. This facile and ultrasensitive sensing technique may be attractive for investigating single-molecule interactions and future biosensing research.

Figure 15 (a) Schematic of self-assembly of MP-11 with graphene sheets (b) Schematics of lone graphene sheet converting to nanoelectrode upon contact with Au electrode (see online version for colours)



(a) (b)

Source: Li et al. (2014)

RGO possesses intrinsic catalytic activity towards several miniature enzymatic products, such as Nicotinamide adenine dinucleotide (NADH) and hydrogen peroxide (H_2O_2), making it highly attractive for enzyme-based sensors. Given its large surface area, electrodes based on graphene nanosheet provide high enzyme loading capacity and a large reaction base (Zhang et al., 2010b). Also, the high surface-to-volume ratio makes it suitable for functional composites, where a minuscule percentage of graphene nanosheet is able to yield percolating conduits for charge conduction (Zhong et al., 2010a). The presence of numerous chemical moieties on graphene surface which afford graphene massive flexibility for functionalisation to boost sensor performance, and the abundance of edge-plane-like defective sites which facilitate charge transfer kinetics, advancing high electrochemical activity, make RGO highly attractive for graphene-based electrochemical sensors. In addition, the electrical and chemical properties of RGO are highly tunable (Jung etal., 2008). Wu et al. (2014a) exploit a one-pot solvent exfoliation to synthesise high-quality graphene sheets and further apply same in electrochemical sensing of various analytes. Graphene synthesised in this form showed strong signal enhancement compared with RGO, making it attractive for advanced sensing functionalities.

Before now, enzymes have been extensively employed as principal bioelement for electrochemical sensing. For instance, the quantitative computation of glucose levels in blood samples plays a significant role in clinical diagnosis and management of diabetic patients (Liu et al., 2012b). In this aspect, glucose oxidase (GOx) has been well employed as the recognition element or mediator (Deng et al., 2009). Biosensors based on glucose have been synthesised via the immobilisation of GOx on graphene sheets using various approaches (Viswanathan et al., 2015). The immobilisation of GOx on graphene nanosheets exploiting various species of noble metal nanoparticles for highly stable and sensitive glucose sensors have been developed (Chang and Tang, 2014; Hossain and Park, 2014). In addition, a metal-free synthesis of mesocellular graphene foam (MGF) for unbroken electrochemistry of GOx and ultrasensitive glucose sensing has been developed (Wang et al., 2014b). MGF has the potential to accelerate the immobilisation mechanism of electrocatalytic active GOx and advance unbroken and fast electron transfer owing to its combined properties of high conductivity, large surface area, and superior pore volume. This work yielded a facile and inexpensive technique to sense glucose with a limit of detection (LOD) of 0.25 mM. Furthermore, Zheng et al. (2013) design multiple layered GOx enzyme electrodes with supervised alternate graphene layers and enzymes, which required initial functionalisation of GOx with pyrene functionalities to achieve self-assembly onto graphene basal plane through noncovalent π-π stacking interaction. Multi- and single- layered enzyme electrodes with supervised biocatalytic activity can be readily fabricated via alternate layer-on-layer of pyrene and self-assembled graphene functionalised GOx. These enzymatic electrodes were found to exhibit enhanced biocatalytic activity as the number of GOx and graphene layers is increased. Such electrodes with supervised nanostructure manifested reliable applications in the analysis of human serum specimen with repeatability, good stability, and high sensitivity.

In addition to GOx based nanodevices, several non-enzymatic glucose sensors based on metal nanomaterial-functionalised graphene electrodes have been synthesised. In particular, copper nanoparticles (Fan et al., 2013), alloy (Yuan et al., 2014b), platinum (Zhou et al., 2013), and nickel oxide (Subramanian et al., 2014) have been employed to synthesise non-enzymatic glucose based biosensors, revealing superior sensitivity and selectivity. Table 1 summarises various graphene-based sensors for glucose detection.

Wang et al. (2011b) demonstrate the mobilisation of acetylcholinesterase (AChE) onto graphene towards the detection of pesticides. AChE is shown to catalyse the hydrolysis of acetylcholine (Ach) resulting in the formation of choline acetate as indicated in the following reaction: ACh + H2O → choline + acetate. Here, the serine phosphorylation at the active centre of the enzyme is a marker for the inhibition of AChE by organophosphate species. Consequently, the blocking of ACh hydrolysis is anticipated as the phosphorylated enzyme is highly unvarying and inactive (Fukuto, 1990).

Table 1 Analytical figures of merit for varying electrode material used for glucose sensing

	Limit of detection	*Linear range*
Electrode materials (based on GOx)		
RGO/multi-layered GOx (GE)	0.154 mM	0.2–40 mM
RGO/polyvinylpyrolidone (GCE)	NA	2–14 mM
RGO/Ag (GCE)	0.16 mM	0.5–12.5 mM
RGO/polypyrrole (GCE)	3.0 µM	2–40 µM
RGO/PdPt (Au)	1.0 µM	2–12 mM
Graphene/polyaniline/Au (GCE)	0.6 µM	4 µM–1.12 mM
GO/ Fe_3O_4	0.2 µM	0.5–600 µM
N-doped graphene	0.01 µM	0.1–1.1mM
AuNPs/GO/CNTs (GCEs)	4.8 µM	NA
Electrode materials (based on metal)		
RGO/$Ni(OH)_2$ (Au)	15 µM	15 µM
RGO/Cu nanowires (GCE)	1.6 µM	0.005–6 mM
N-doped graphene/Cu (GCE)	1.3 µM	0.004–4.5 mM
Pt/Ni-Co nanowires (GCE)	1.0 µM	0-0.2mM and 0.2-8mM
Pt/poly(glutamic acid) (GCE)	11.0 µM	0.05–5.95 mM

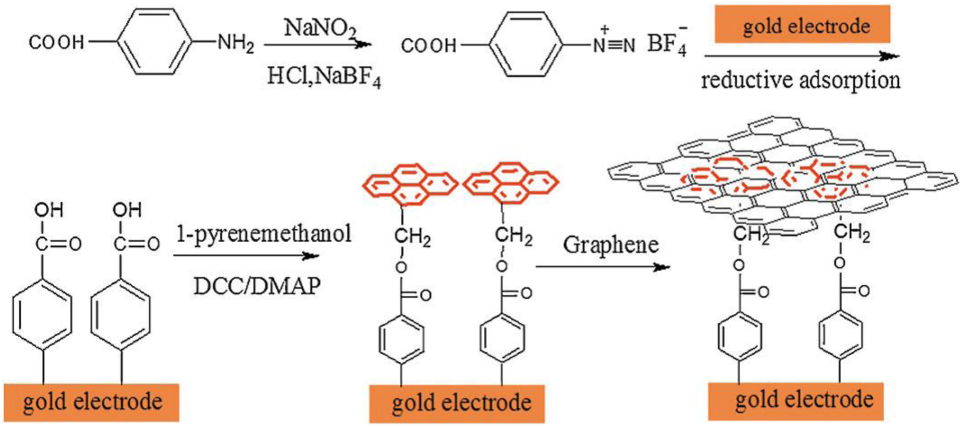
Notes: Classification of electrodes is shown in brackets. Here, GCE stands for glassy carbon electrode and GE stands for graphite electrode.

Source: Liu et al. (2013a), Palanisamy et al. (2014), Xu et al. (2014b), Alwarappan et al. (2010), Hossain and Park (2014), Wang et al. (2010c), Chang and Tang (2014), Yu et al. (2014), Mahshid et al. (2013), Subramanian et al. (2014), Fan et al. (2013), Jiang et al. (2014b), Wang et al. (2011b) and Zhou et al. (2013)

A highly conductive precursor with irreversible absorptive capacity for AChE and large surface area can be synthesised from graphene-based materials, making them attractive substrates for the immobilisation of AChE species enabling precise tracking of deleterious organophosphates pesticides, such as paraoxon (Yang et al., 2014), carbaryl (Li et al., 2013), monocrotophos (Zhang et al., 2012) and dichlorvos (Wu et al., 2013b). Zhai et al. (2014) develop an AChE biosensor based on calcined layered double hydroxide and graphene-Au nanocomposite. The LOD of chlorpyrifos was 1.4×10^{-10} mM revealing its excellent sensitivity relative to previous AChE-based biosensors (Liu et al., 2011b). Additionally, highly dispersed nanoparticles based on zinc oxide (ZnO) have been synthesised on carboxylic graphene to create a hydrophilic exterior for the adhesion of AChE. This assembly was employed to detect carbofuran and chlorpyrifos, registering a LOD of 5.2×10^{-13} mM and 5×10^{-14} mM respectively, showing superior sensitivity for continuous monitoring of pesticide compared with the work of Zhai (Wu et al., 2013b). The exceptional biocompatibility, conductivity, and catalytic activity were the outcomes of the synergistic effects of Nafion, ZnO, and carboxylic graphene. ZnO is biocompatible with a towering isoelectric point (IEP), enabling seamless absorption of low IEP proteins, AChE for example. Also, the carboxylation of graphene particularly improves its conductivity and dispersibility. The conductivity of carboxylic graphene-ZnO can be enhanced when decorated with Nafion, which also imparts protective membrane to the electrode surface in order to circumvent possible leaking of enzyme molecules.

The graphene-based enzymatic electrochemical sensors have been extensively synthesised for the sensing of motley biomolecules, such as nitrate (Liu et al., 2010a), maltose and trichloroacetic acid (Sun et al., 2013). Each of these systems has advantages as well as limitations. For instance, Sun et al. (2013) employ graphene/hemoglobin/TiO_2 composite functionalised carbon IL electrode to sense trichloroacetic acid. While this electrode presents some advantages such as superior ionic conductivity, ease of preparation, wide electrochemical window and high electrochemical stability, its low detection limit (0.22 mmol L^{-1}) is a drawback. However, this can be overcome via additional modifications, such as protein loading and increase in electron transfer kinetics. Furthermore, Park et al. (2014) demonstrate the detection of antigen 15-3, a biomarker of breast cancer by employing a two-enzyme/GO electrode. A detection limit of 0.1 UmL^{-1} was obtained when measured with GO/ITO electrodes and two-enzyme (tyrosinase and β-galactosidase) scheme.

Figure 16 Schematic of chemical functionalisation of the gold electrode with a graphene nanosheet (see online version for colours)



Source: Kong et al. (2013)

The presence of heavy metal ions in food samples and portable water is a major health concern and their detection is, therefore, paramount for food safety (Liu et al., 2012b). Consequently, the highly sensitive, rapid and selective detection of heavy metal ions has been investigated from various analytical perspectives (Yu et al., 2013). Sensors based on graphene nanomaterial have been employed to monitor and detect the presence of heavy metal ions, such as Cu^{2+}, Pb^{2+}, Hg^{2+} and Cd^{2+} (Kong et al., 2013). In this aspect, graphene functionalised Au electrode has been synthesised via noncovalent interaction towards the detection of Cu^2 and Pb^{2+} (Kong et al., 2013).This engineered Au electrode was synthesised initially via functionalisation with benzoic acid moieties, followed by coupling of pyrene and culminating into graphene nanosheet immobilisation through noncovalent π-π interaction. This mechanism is indicated in Figure 16. Gong et al. (2010)

report a graphene-based sensor for the detection of mercury ions (Hg^{2+}), which regularly distributes monodispersed gold particles across the graphene sheet. Surface-functionalised graphene with 1-octadecanethiol has also been proposed for possible detection of Hg^{2+} (Zhang et al., 2010c). The 1-octadecanethiol molecules are silicon oxide supported and self-assembled to yield massive, highly ordered monolayers on the 2D graphene nanosheet. Similarly, alkanethiol functionalised graphene field effect transistor (fGFET) manifested a sensitivity for Hg^{2+} detection at 100 ppm, opening novel opportunities for graphene-based electronics as heavy metal sensors. Also graphene-based sensors for the detection of Ag^{+}, Cd^{2+} and Cu^{2+} have been reported (Gupta et al., 2013). In addition, an RGO/SnO_2 nanocomposite functionalised GCE for the selective and simultaneous detection of ultra-trace concentrations of Hg^{2+}, Cd^{2+}, and Cu^{2+} in drinking water has been reported (Wei et al., 2009).

The utilisation of graphene-based DNA sensors to detect DNA, cysteine, proteins and metal ions can be accomplished by both transfers of fluorescence energy and electrochemical sensors (Gao et al., 2014). The perfect 2D property of graphene coupled with its large surface area can function as a DNA carrier, loading either by direct absorption via π-π interaction or via other binding techniques through functional species from functionalised graphene surface. Furthermore, the superior conductivity of graphene enables fast electron transport between DNA and electrodes. Huang et al. (2014) demonstrate the detection of target DNA molecules. For example, a 2D graphene analog tungsten sulphide-graphene (WS2-Gr) composite for DNA detection has been synthesised (Huang et al., 2014). Graphene can be employed as a 2D conductive support material for an exceptionally electrolytic accessible surface. Compared with graphene materials, WS2 offers much-limited sensing applications due to its low electronic conductivity. However, the detection of DNA can be achieved via non-DNA modified electrodes (Lu et al., 2014). Lu et al. (2014) describe a graphene and hypocrellin A (HA) sensor system with perylene quinonoid drug which can efficiently disable tumor cells. The graphene-HA based electrodes have been successfully applied to track and detect telomere DNA in abdominal fluid and human blood serum. For the first time, the chemistry between telomere DNA and HA was investigated via the electrochemical mechanism. The nonemployment of DNA probe sequence on the electrode provided a cost advantage but sacrificed the solubility of HA in water when combined with graphene sheets, which exerts a limit to the amount of HA that can be fixed on graphene surface.

The precise measurement of hydrogen peroxide (H_2O_2) is of fundamental importance in clinical analysis, biology, chemistry and environmental safety (Zhou et al., 2010). Electrochemical procedures are vital for H_2O_2 detection (Lv et al., 2016). For instance, Cui et al. (2012) synthesise a sensor with excellent sensitivity for organic peroxide via the application of enzyme-free amperometry for the reduction of tertbutyl hydroperoxide (TBHP) through the utilisation of nano-cobalt phthalocyanine on graphene. The sensor has been employed to determine TBHP threshold in body lotion, revealing a linear calibration range scale of 0.026–4.81 mM with a LOD of 5 μM and a sensitivity of 13.64 A M^{-1}. Additionally, many novel and useful non-enzyme H_2O_2 biosensors have been demonstrated (Liu et al., 2011b). To this end, Dey and Raj (2010) synthesise an ultra-sensitive biosensor based on the hybrid complex derived from nanoscale Pt particles and graphene for the detection of cholesterol and H_2O_2. This sensor exhibited high sensitivity and a linear response of 12 mM towards H_2O_2, with 0.5 nM LOD in the absence of any enzyme or redox mediator. The connection of catalytically active Pt nanoparticles and superconductive graphene nanosheets accelerated electron transport for the oxidation of H_2O_2.

The plasma membrane-bound protein Nicotinamide adenine dinucleotide (NADH) oxidase, is responsive to activation by insulin and epidermal growth factor and other molecular effects (Nishikawa et al., 2000). Graphene electrodes modified with ILs are shown to be excellent platforms for both biosensing of ethanol and detection of NADH (Shan et al., 2010). The IL functionalised-graphene-based sensor showed remarkable analytical figures of merit towards NADH along with inexpensive and convenient preparation.

Recently, electrochemiluminescence (ECL) has been receiving a lot of attention as an important detection tool in analytical chemistry owing to its high sensitivity, low background signal and simplicity. ECL sensors based on graphene are shown to be ultra-sensitive due to the outstanding electrical conductivity and large surface area of graphene (Wang et al, 2014a). Qu (Wu et al., 2012b) report a label-free ECL sensor based on graphene-porphyrin nanocomposite functionalised electrode for the detection of telomerase activity. The advantages of free polymerase chain reaction (PCR), ultrasensitivity, low cost and ease of operation make ECL sensors attractive compared with colorimetric sensors. Recently, ECL sensors for the detection of cancer have been synthesised via RGO nanocomposite-based peptide sensor and carbon nanodot complex derived from Ag/graphene hybrid. In fact, electrochemical sensors based on grapheme nanomaterials have been employed to sense a variety of commercially and biologically paramount molecules, including telomerase (Wu et al., 2014b), cancer cells (Feng et al., 2012), dopamine (Wu et al., 2012b), and in medical diagnostics (Feng et al., 2013a).

5.2 Graphene-based electrical sensors

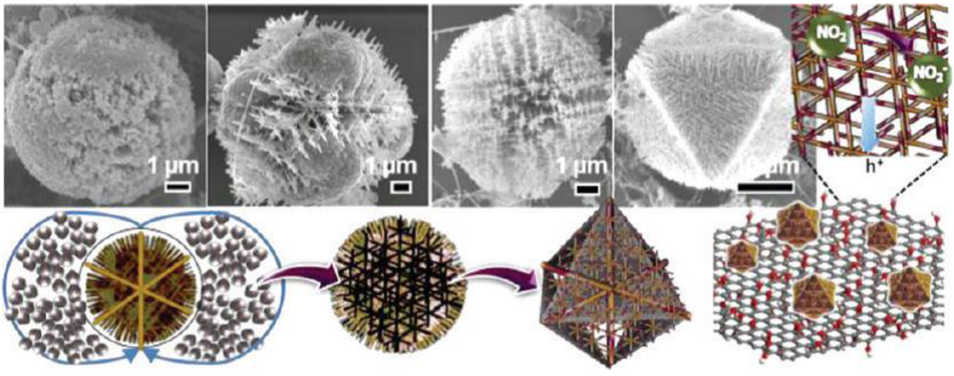
Following the synthesis of the first SLG via mechanical cleavage of graphite (Cao et al., 2014), pristine graphene has enabled the illumination of fundamental properties at condensed matter physics level. Single layer graphene is quasi-metallic with outstanding properties that are particularly functional for electronic sensor fabrication. Due to its zero bandgap, graphene exhibits remarkably high carrier density (Geim and Novoselov, 2007), low intrinsic noise (Pal and Ghosh, 2009), high carrier mobility (Tan et al., 2007), room temperature hall effect (Zhang et al., 2005) and ambipolar field-effect characteristics. 1D nanomaterial-based electronic sensors such as CNTs and silicon nanowires (SiNWs) represent a novel frontier in sensor technology, offering an assortment of opportunities for high temporal resolution, label-free detection, and compatibility with lab-on-a-chip devices (Huang and Chen, 2010). Perhaps, SiNWs are the most researched 1D nanomaterial for nanoelectronic sensing applications (Patolsky et al., 2006a, 2006b). However, the over-reliance of SiNW sensors on the induced field effect limits their nanoelectronic sensing applications (Chen et al., 2011a), making them only suitable for the detection of charged analytes or electrogenic phenomena (Patolsky et al., 2006b). Graphene has been applied for highly diversified applications due to its exceptional electrical features (Liu et al., 2012b).

The electrical reaction of graphene to different chemical groups has been reported to be indistinguishable to CNTs (Zheng et al., 2013). Consequently, just like CNTs, sensors based on graphene have stimulated enormous research attention for the detection of several target species in the vapour phase (Jeong et al., 2010). For the first time, Novoselov et al. (2004) demonstrate a graphene-based sensor for the detection of nitrogen dioxide (NO2), with recent perspective coming from Wang et al. (2016). This sensor, based on the mechanical cleavage of graphene at the intersection of the oxidised Si wafer surface has been utilised to detect NO_2 by gauging the difference in the source-drain resistance. Graphene-based devices have also been reported to detect NO_2 (Jeong et al., 2010). For example, RGO conjugated Cu_2O nanowire mesocrystals synthesised under hydrodermal conditions via unclassical crystallisation in the presence of o-anisidine and GO have been explored as sensors for the detection of NO_2 (Deng et al., 2012). This composite sensor exploits the combined rich interdendritic space within the mesocrystals and the enhanced electrical conductivity of RGO to enable a 3D conducting architecture, as shown in Figure 17.

Graphene-based nanomaterials have also been used as an excellent platform for sensing of NH_3 (Paul et al., 2012). Lu (Ruitao et al., 2015) demonstrate a fast, low temperature and a repeatable RGO-based sensor which is able to detect NH_3. Their work shows that the application of substantial positively polarised gate voltage (Vg) to the RGO FETs caused them to function in the n-type mode, exhibiting faster recovery for NH_3 and instantaneous response relative to their performance in p-type mode. This was attributed to the induced effects of Vg and the ambipolar transport of RGO. The sheer Vg dependent, RGO initiated NH_3 sensing mechanism holds great potential in room temperature gas detection.

Graphene-based gas sensors have also be employed to detect CO_2 (Yoon et al., 2011), H_2O (g) (Chen et al., 2011b), H_2 (Johnson et al., 2010) and NO (Li et al., 2011). For example, Pd-modified multi-layer graphene nanoribbon networks have been utilised in the detection of H_2 gas. The porous structure and the high specific surface area of these nanonetworks are believed to advance efficient functionalisation and superior sensitivity towards H_2. Table 2 shows various select graphene-based sensors for gas detection.

Figure 17 Schematic of SEM images showing the time-dependent architecture of Cu_2O nanowire mesocrystal at 200ºC, a schematic illustration of the Cu_2O crystallisation mechanism facilitated via GO and o-anisidine, and schematic for the mechanism of NO_2 sensing of RGO-Cu_2O (see online version for colours)



Source: Jeong et al. (2010)

Table 2 Selected graphene-based electrodes for gas sensing

Analyte	*Electrode materials*	*Limits of detection*	*Response (%)*
Ethanol	Al_2O_3/RGO	1.5 µg Ml^{-1}	NA
CO_2	GS	NA	0.17/ppm
NH_3	Ethanol-graphene- based (Ti/Au)	160 ppb	0.71/ppm
NO_2	Ozone treated graphene (Au)	1.3 ppb	17
NO_2	S-G or EDA-G (Pt)	3.6 ppm	NA
NO_2	RGO-conjugated Cu_2O	64 ppb	67.8
NO	CVD-grown graphene (Ni)	2 ppb	28

Notes: Here, S-G stands for sulphonated RGO, EDA-G stands for ethylenediamine-modified RGO.

Source: Jiang et al. (2011), Yoon et al. (2011), Paul et al. (2012), Chung et al. (2012), Yuan et al. (2012), Deng et al. (2012) and Li et al. (2011)

As noted, the exceptional electrical properties of graphene promote a high signal to noise (S/N) ratio in detection, with its conductance exhibiting extreme sensitivity to local chemical and electrical agitation as individual atoms of graphene are unsheltered to the outside environment. Moreover, the modulation of Fermi level of zero bandgaps of graphene via the gate voltage leads to the generation of charge carriers (electrons and/or holes). Generally, a large bandgap is desirable for field effect based detection systems (Jin et al., 2016). The opening of bandgap engineering of graphene can be achieved via atomic or molecular dopants (Wei et al., 2009) or by reducing its dimensions to the nanoscale (Barone et al., 2006). Moreso, the provision of wider detection range, and unvaried exterior for efficient functionalisation make graphene advantageous over 1D nanostructured sensing materials (Dreyer et al., 2009). In addition, the 2D structure of graphene makes interfacing with flat cell membranes possible (Ma et al., 2011). Furthermore, graphene has shown promise for cell growth and adhesion due to its biocompatibility (Kalbacova et al., 2010). For instance, Kalbacova et al. (Kalbacova et al., 2010) link the capability of graphene to support cell growth and adhesion to its excellent biocompatibility.

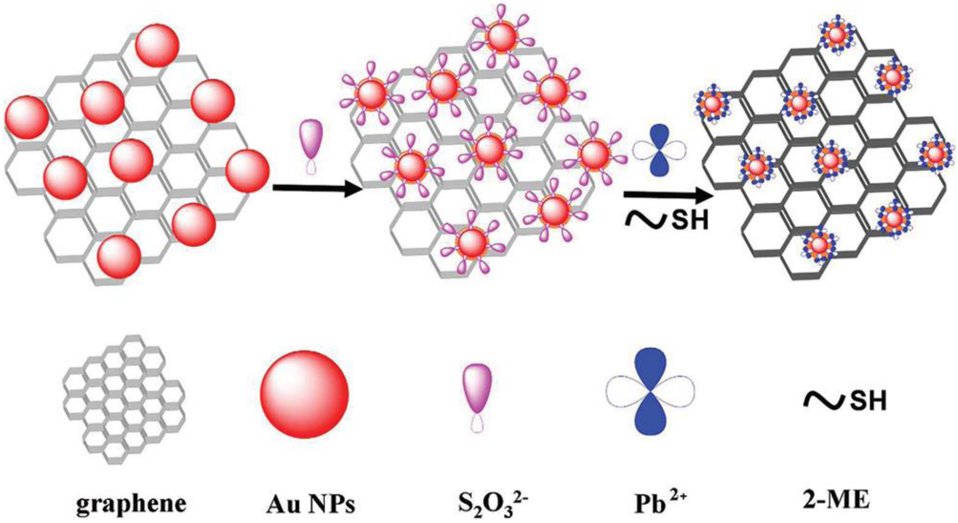
The dynamic activities of living cells, such as adipocytokines (Pui et al., 2009a, 2009b), circulating breast cancers cells (Ning et al., 2008), bioelectricity (Pui et al., 2009a) and triggered secretion of proinflammatory cytokines (Pui et al., 2011) can be detected via appropriate interface with 1D biosensing nanostructures. Consequently, the nanoelectronic-cell interface assumes a new dimensionality owing to the unique properties of graphene, with potential for multiple applications at the device level. In addition, the cell membrane can faithfully interact with the flat graphene as it is also 2D structured (Wang, Y. et al., 2010). This is in contrast to cell membranes interfaced with nanostructures other than graphene where the interaction is inhomogeneous and slack, leading to the disruption of the cell functions as a result of the induced local curvature on the thin cell membrane by nano-topographic structures (Zhang et al., 2009). As a result of the robust interactions between the cell membrane and graphene, any cellular activity-induced chemical and electrical oscillations in the nanogap at the intersection of the two surfaces could substantially transform conductance. Lieber (Cohen-Karni et al., 2010) demonstrate a graphene field effect transistor that is able to extracellularly detect activity potential from lone electrogenic cardiomyocytes. Here, mechanical exfoliation is employed to fabricate nanodevices via e-beam lithography. In addition, the graphene FET was found to register enhanced sensitivity over their traditional metallic microelectrodes counterpart (Heer et al., 2007), but become comparable to those of SiNW FETs (Yeung et al., 2001). The device feedback mechanism was excited by the field effect owing to the transient potential fluctuations across the nano interface of graphene and the membrane arising from the current through the membrane ion channels. Although less prominent than the field effect of SiNWs, the graphene FETs showed comparable signal-to-noise ratio (Meric et al., 2008). This may be attributed to the large interface area at the intersection of graphene-cell complex. Furthermore, the large bandgap in graphene nanorods plays a key role in bioelectricity detection, offering high sensitivity and selectivity via its unique spatial resolution and enhanced field effect arising from their lateral nanoscale dimension.

5.3 Graphene-based optical sensors

In addition to its excellent electrical properties, graphene also exhibits a variety of optical properties (Loh et al., 2010). GOs or other carbonaceous materials are known to fluoresce at a vast range of wavelengths in contrast to zero-gap graphene (Zhengtang et al., 2009). This is the outcome of the disoriented oxygenated functional species on GO confining π-electrons in the sp^2-hybridised carbon nanodomains, leading to the creation of local energy slot that scales inversely with domain dimensionality. In this perspective, graphene could play a role as a universal fluorescence label for optical imaging (Sun et al., 2008). In addition, GO also possesses the intrinsic capacity to quench fluorescence (Liu et al., 2008a). GO exhibits stellar quenching efficiency in comparison with traditional organic quenchers. In this aspect, GO has been able to quench fluorescence even at a distance as small as 30 nm (Kim et al., 2009). In the light of its quenching and fluorescence potentials, GO can be employed in fluorescence resonance energy transfer (FRET), where it could function as either energy acceptor or donor. The optical properties of GO, such as quenching efficiency and fluorescence wavelength can be adapted via regulating the genre and extent of its oxygenation (Wang et al., 2015).

Recently, the highly sensitive and selective fluorescence capability of graphene-based sensors to detect lead ions (Pb^{2+}) has been recognised (Chang et al., 2014). For instance, a graphene-DNAzyme for the magnified switch on fluorescence sensing of Pb^{2+} has been reported (Tang et al., 2015). This sensor operates on the basis of the difference in affinity of ssDNA and the duplex alongside its target towards GO. Here, the hybrid fluorophore carboxyfluorescein labelled DNAzyme substrate played role as both molecular identifier and signal reporter with GO as a super quencher. Fu et al. (2012) demonstrate an Au nanoparticle-functionalised graphene for the detection of Pb^{2+} in aqueous solution using a novel and active switch on fluorescence sensor. In this work, both the ability of AuNPs to quench luminescence and its optical characteristics were exploited. The fluorescence was able to reappear and increase as a result of the addition of Pb^{2+}, this being attributed to the target species accelerating the leeching of AuNPs from the surface of graphene in the presence of a mediator, thiosulphate ($S_2O_3^{2-}$) and 2-mercaptoethanol (2-ME) for instance, as illustrated in Figure 18. This has the potential to revolutionise the complete landscape of sensitive and selective detection of Pb^{2+}.

Figure 18 Schematic illustration of the sensing mechanism of the detection of Pb^{2+} ions based on accelerated leaching of gold nanoparticle on the graphene surface (see online version for colours)

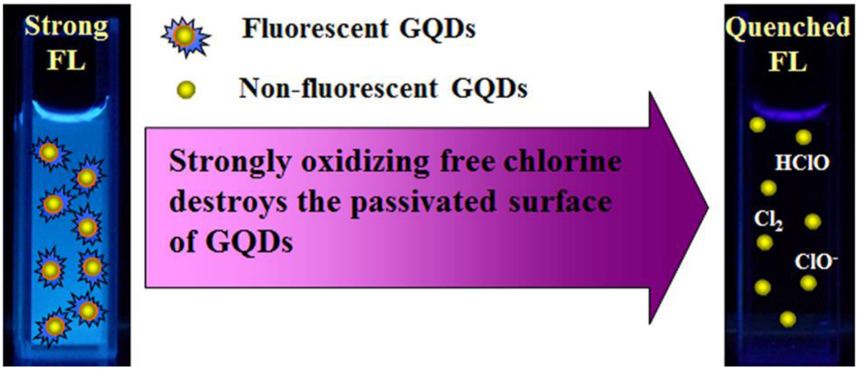


Source: Fu et al. (2012)

Various species of graphene-based sensors have been developed to detect other metal ions. For example, Zhang et al. (2012a) fabricate a highly sensitive and selective biosensor based on graphene with DNA duplexes of poly (dT) for fast fluorescence sensing of Hg^{2+}. This system showed a LOD of 0.5 nM for Hg^{2+} under prevailing optical experimental setup, with no interference from other metal ions. Similarly, a switch on fluorescence Cu^{2+} biosensors utilising a DNA cleavage-reliant graphene-quenched DNAzyme has been developed (Liu et al., 2011a). Here, graphene was employed as both a quencher and scaffold of the Cu^{2+}-dependent DNAzyme facilitating the configuration of the self-assembled graphene-quenched DNAzyme. The role of functionalised QDs in the sensitive and selective optical detection of Cu^{2+} and Ag^{+} has been reported. Moreover, QDs offer a myriad of advantages, such as excellent biocompatibility, good photostability against blinking and photobleaching, and low toxicity in comparison with semiconductor QDs and organic dyes. Furthermore, QDs with graphene morphology, exhibit sedate hot-carrier relaxation and extreme photoluminescence due to the edge effects and quantum confinement which distinguish them from traditional graphene nanosheets.

Graphene-based fluorescence sensors have also been employed to monitor and detect other important molecules. For example, an integrated thiol and GO-activated DNA metallisation for the effective and sensitive switch on fluorescence detection of cysteine has been demonstrated (Lin et al., 2011). Wang et al. (2010b) report a GO-FRET based sensor that is able to detect Cyclin A2, an early stage cancer identifier. Here, a LOD of 0.5 nM was obtained which is 10 times less than that observed for SWCNT-based sensors. Again, fluorescence sensors have been utilised to detect some biotic molecules (Dong et al., 2012). Dong et al. (2012) synthesise a surface-passivated QDs with high fluorescence potential by incorporating partially carbonised citric acid. As the surface passivation is susceptible to destruction from chlorine molecules (Cl_2) leading to substantial quenching of the fluorescence signals, a facile and green QDs sensor for unrestricted Cl_2 in drinking water was designed based on this effect. This mechanism is shown in Figure 19. Veigas et al. (2015) synthesise a general, fast, sensitive and label-free dye-sensor based on RGO/fluorescein complex for the detection of synthetic biotic dyes via competitive adsorption. This assay principle is operated on the basis of the reversible interaction between the fluorescence dye and reduced graphene, and therefore suitable for the detection of various synthetic biotic dyes. Similarly, a fluorescent switch on sensor based on 8-amino-quinoline modified GO via a photo-induced electron transport signalling mechanism was synthesised to detect D-glucosamine, exhibiting exceptional sensitivity and selectivity (Cheng et al., 2012). Additionally, fluorescence-based colorimetric droplet sensor with capability for simultaneous determination of DNA specimen by employing GO nanoprobe tagged with carboxyfluorescein quencher for the ssDNA probe tagged with 6-carboxy-X-rhodamine has been demonstrated. The analytical consideration of the fluorescence intensities of the dyes in droplets yields a quantitative assessment of the target DNAs. This sensing platform exhibits moderate depletion of reagents and is well-suited for high throughput applications. This fabrication technique for DNA-based biosensors has the potential to advance an innovative procedure for the development of robust, novel and extremely sensitive biosensors.

Figure 19 Schematic of detection mechanism of free chlorine based on the fluorescence quenching of QDs (see online version for colours)



Source: Dong et al. (2012)

The various time-depleting purification stages and the requirements of tedious fluorophore labeling processes present a challenge to fluorescence sensors (Ohno et al., 2009). However, some simpler and uncomplicated optical sensors have been developed to overcome this limitation (Tainaka et al., 2010). The advantages of low cost, simplicity, and label-freeness associated with colorimetry are attractive for target detection (Tao et al., 2013b). The last few years have witnessed increased employment of functionalised graphene derivatives in the development of colorimetric biosensors towards the detection of glucose (Ul Hasan et al., 2012), intracellular H_2O_2 and cancer cells. Carboxyl-functionalised GOs possess peroxidase-like characteristic that is able to accelerate peroxidase substrate 3, 3', 5, 5'-tetramethylbenzidine (TMB) reaction in the presence of H_2O_2 to yield a blue coloured complex (Tao et al., 2013b). This opens novel opportunities for the application of functionalised GO nanomaterials in biological sensing and medical diagnosis. This find has enabled the development of synergistic Au-GO nanocluster hybrid which exhibited exceptional peroxidase-like activity via simple electrostatic interaction. Moreover, Au-GO nanocluster is an attractive alternative to the enzyme with advantages of facile preparation, stability, and affordability. In addition, an extreme catalytic activity over a wide range of pH, even in neutral media was observed. Furthermore, the presence of various functional oxygen species on the surface of GO is offers a promising pathway with ease of functionalisation and excellent absorbability for fluorescence molecules. Additionally, GO can be employed as a robust sensing platform for label-free detection of a wide range of analytes, including small molecules, metal ions and DNA via exploiting the DNA-facilitated assembly of GO-hemin (GH) hybrid. Hemin has been employed as a mimetic enzyme for labeling antibody and antigen reactions (Song et al., 2011). A transparent supernatant was observed after the DNA-GH hybrid was separated at the nadir of the tube following a centrifugation procedure. Here, the colorimetric signal of the centrifugal supernatant suffered significant attenuation compared with that in the absence of targets. This may hold great promise for quantitative detection of assorted analytes.

Despite the enormous benefits that graphene offers in a wide range of sensing applications, much work needs to be done before the actualisation of commercial sensors. Firstly, a high-quality graphene source must be readily available on a wafer scale. Secondly, the size, structure of electronic band-gap, and the level of oxidation and shapes and thickness features of graphene have a visible effect on the total performance of biomolecule immobilisation on the surface of graphene. The reliable fabrication of graphene would achieve the required reproducibility for perfect biosensor applications in drug or gene delivery and DNA detection. Thus, alternative techniques of synthesising graphene sheets should be adopted for gaining high-quality graphene-based nanomaterials. The novel graphene-based biosensors must allow for improved control over differentiation of stem cell, the targeting and distribution of the hybrid body, in addition to lower limits of attainable selectivity and sensitivity for biosensing.

Thirdly, extensive work is required to be evaluated regarding the fabrication of portable devices for early detection and diagnosis of various diseases with graphene-based biosensors. Furthermore, most published articles have shown that only a single target can be detected for a single DNA sensor GO solution. If multiple targets could be detected by a single DNA sensor using GO, then the detection throughput will be increased. Consequently, researchers would have to concentrate on the surface chemistry throughout the course of converting graphene to GO, as the conversion of graphene to GO leads to disruption of the electron transport properties to a certain degree (Zheng et al., 2014). Finally, biocompatibility and likely toxicity issues of graphene need to be addressed in order to prevent any possible health hazards in the human body system. Many functionalised chemical remedies and graphene-based nanomaterials are already proved to be biocompatible. Nevertheless, additional studies are encouraged so as to experiment the cytotoxicity over a longer duration of time (Vashist et al., 2012).

6 Conclusions and future perspectives

Graphene has drawn significant research interest in many fields due to its unique morphological properties which can be harnessed for highly diversified applications in sensing. The recent perspectives in graphene-based biological and chemical sensors for the detection of varying analytes, including proteins, gases, biomolecules and heavy metal ions have been discussed. The useful and uncommon properties of graphene still find applications in many areas of science and technology, helping provide solutions in areas such as rapid medical analyses, and food security and safety. However, as a newly discovered material, graphene faces a number of challenges, including an extensive understanding of graphene surface engineering, improved synthesis methods and extending the applications in varying practical paradigms.

The fundamental synthesis methods, such as CVD, mechanical exfoliation, reduction of graphite oxide, thermal deposition, and unzipping CNT are being developed and redesigned. As slight variation in the preparation method can lead to significant variation in output, it is necessary to carefully select an appropriate procedure to synthesise graphene for varying purposes. For each chosen preparation method, there are advantages as well as limitations, as earlier discussed. There is increased momentum by the scientific community to improve on the existing methodologies, including large-sized, high-quality and defect-free graphene. To date, two key challenges for the synthesis of novel graphene materials for the large-scale production of graphene-based products are recognised: on-demand tailoring of graphene properties, and access to a large quantum of high-quality unvarying graphene. Therefore, a better understanding of the physics and chemistry of graphene surface engineering, and the interactions of biomolecules and chemicals at the interface of graphene surface particularly when applied as nano-scaffold in chemical/biosensing and catalysis is paramount. An improved understanding of the workings of graphene at the molecular level will ultimately advance graphene surface engineering and its applications in sensor development and technology.

There is still room for continuous improvement in the design and fabrication of graphene-based sensors. Some graphene-based materials such as QDs have not been fully prospected despite their potentials as sensing materials. New graphene-based materials with entirely novel properties are being developed and created by scientists, researchers, and nanoengineers. The hybridisation of graphene with other inorganic or organic materials such as nanoparticles, polymers, and CNTs has the potential to considerably extend the possibilities in sensor development. Novel and improved functionalisation techniques are being prospected and developed, although much work is needed to fully understand and harness graphene in sensor development and application.

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