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Submitted for the degree of Doctor of Philosophy

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Submitted and under review

Under preparation


Conference Presentations


## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>2D</td>
<td>Two-dimensional</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
</tr>
<tr>
<td>BNNP</td>
<td>Boron nitride nanoparticle</td>
</tr>
<tr>
<td>BNNF</td>
<td>Boron nitride nanoflake</td>
</tr>
<tr>
<td>BNNS</td>
<td>Boron nitride nanosheet</td>
</tr>
<tr>
<td>BNQD</td>
<td>Boron nitride quantum dot</td>
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<tr>
<td>C60</td>
<td>Fullerene</td>
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<tr>
<td>CNT</td>
<td>Carbon nanotube</td>
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<tr>
<td>CRGO</td>
<td>Chemically reduced graphene oxide</td>
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<tr>
<td>CV</td>
<td>Cyclic voltammetry</td>
</tr>
<tr>
<td>CVD</td>
<td>Chemical vapor deposition</td>
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<tr>
<td>DOX</td>
<td>Doxorubicin</td>
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<tr>
<td>ECL</td>
<td>Electrochemiluminescence</td>
</tr>
<tr>
<td>ESR</td>
<td>Electronic spin resonance</td>
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<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
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<tr>
<td>GNR</td>
<td>Graphene nanoribbon</td>
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<tr>
<td>GO</td>
<td>Graphene oxide</td>
</tr>
<tr>
<td>GQD</td>
<td>Graphene quantum dot</td>
</tr>
<tr>
<td>h-BN</td>
<td>Hexagonal BN</td>
</tr>
<tr>
<td>IC50</td>
<td>50% growth inhibition concentration</td>
</tr>
<tr>
<td>nGO</td>
<td>Nano-scale graphene oxide</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet visible spectroscopy</td>
</tr>
<tr>
<td>RGO</td>
<td>Reduced graphene oxide</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>TMB</td>
<td>3,5,3',5'-tetramethylbenzidine</td>
</tr>
<tr>
<td>XPS</td>
<td>X-ray photoelectron spectroscopy</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
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Abstract

Two-dimensional (2D) nanomaterials have attracted a lot of attentions due to their unique geometric structure and surface properties. Being ultra-large in lateral size while ultra-thin in layer number, 2D nanomaterials have large surface area on which almost every atom is exposed on their surface, especially single layer nanosheets. Among them, graphene oxide (GO) is a classical form of graphene (perfect 2D nanosheet) derivatives with vast oxygen functional groups on its surface. Since graphene was discovered, GO has been widely explored in numerous research for various applications. It is very interesting to combine the excellent properties of graphene and water processibility. The oxygen functional groups are of great importance to the performance of graphene oxide. However, the determination of oxygen content, hydrophobicity of graphene oxide and the evaluation of reduction efficiency are still difficult tasks because of heterogeneous structure of GO.

First in this project, we describe a detailed understanding of the surface chemistry of GO by studying the interactions at the interface of GO/[Ru(bpy)_3]^{2+} nanocomposites, which further probes the surface tuning of GO. For the first time, we propose a new idea of interaction transitions by surface tuning of GO. Due to the gradual removal of oxygen functional groups on GO, the interaction happens between GO and the ruthenium complex switches from electrostatic attraction to π-π stacking. The spectroscopy and atomic force microscopy give strong evidence of the presence of the non-covalent interactions as well as the interaction transition. We expect this method can provide a universal protocol for the probing of other 2D nanomaterials for better understanding of their surface chemistry.

To disperse GO in low-polar solvents can realize a perfect self-assembly with functional molecules and apply to removal of organic impurities, which only dissolve in low-polar solvents. The surface chemistry of GO plays an important role in its dispersibility in these solvents. Until now, it is still an experimental challenge to directly transfer hydrophilic GO into low-polar solvents. In this study, we designed an interface to transfer GO by simultaneous pushing and pulling the nanosheets into low-polar solvents. Our approach is outstanding due to its ability to obtain monolayers of chemically reduced graphene oxide (CRGO) with designed surface properties in organic phase. Using the transferred graphene dispersions, we have fabricated GO/C_{60} nanocomposites and assessed the abilities of CRGOs
for dye adsorption. We hope our work could provide a universal approach for the phase transfer of other nanomaterials.

Fighting with cancer is a crucial task because cancer kills people. Although the bullets are ready to destroy cancer cells, there are still many problems to be resolved, such as toxic and side effects. Well-controlled, low-toxic and high-efficient delivery of anticancer drugs using nanomaterials as carriers is the key approach to chemotherapy of mortal tumours. Graphene materials, especially GO, have been widely developed for drug delivery due to its biocompatibility and large specific surface area. However, deep understanding of the surface chemistry at the carrier/drug interface is very limited. Here, we design a facile operation on the well-studied surface of GO for highly efficient loading and adjustable release of doxorubicin (DOX). Hydrogen bond and π-π stacking interactions are confirmed between GO and DOX, and then the tuning of surface chemistry is achieved via mild chemical reduction for the improvement of DOX release. This work provides a new idea of drug delivery improved by tuning of the surface chemistry of the nanocarriers.

2D boron nitride nanosheet (BNNS) has drawn a lot of attentions due to its complementary properties and applications to graphene, with advantages in chemical and thermal stability. BNNSs are consider to be chemically inert because of the strong energy of B-N bonds, however, BNNSs with low longitudinal size exhibit catalytic activities. To figure out what are the active sites on BNNSs is of great interest for good understanding of their potential applications. Herein, we investigate the free radicals on BNNSs by stepwise exfoliating bulk boron nitride into smaller dimensions. We discover that the unsaturated boron atoms account for the radicals on BNNSs via a free radical scavenging method. The functional groups contribute to dispersity of BNNSs in solvents but not affect the density of radicals. BNNSs with smaller lateral dimensions provide stronger signals of radicals which are further confirmed by the oxidization of 3,5,3’,5’-tetramethylbenzidine and co-reaction in the electrochemiluminescence of a ruthenium complex.
Chapter 1: Introduction
1.1 What is graphene?

Two-dimensional (2D) nanomaterial is a very novel concept in recent years, with ultrathin thickness, a high degree of anisotropy and chemical functionality.\(^1\) In fact, research on 2D nanomaterials is still in its infancy, with most of the attention on investigating the unique material characteristics and few reports focusing on biomedical applications.\(^2\) 2D nanoparticles such as carbon-based 2D materials (graphene and its derivatives) and hexagonal boron nitride (h-BN) provide enhanced physical, chemical and biological functionalities due to their uniform structure, high surface-to-volume ratios and surface chemistry. In this chapter, I take graphene as a typical example to demonstrate its unique properties, potential applications, and the important role of surface chemistry to 2D nanomaterials.

Although relatively new, graphene has already been extensively utilised in various fields because of its distinctive physical and chemical properties, which include superior electrical conductivity, excellent mechanical flexibility, large surface area plus high thermal and chemical stability.\(^3\) The thinnest material in the world is considered to revolutionise almost every part of everyday life. For example, graphene has been successfully exploited for energy applications, due to its high conductivity, transparency and ultrathin sheets.\(^4\)-\(^7\) Because of their large surface area (2630 m\(^2\) g\(^{-1}\)),\(^8\) excellent mechanical strength and aromatic–rich structure, graphene materials have also been employed as a pollutant adsorbent due to the strong attraction of small molecules to its surface. Furthermore, these properties also contribute to its application as a catalyst or catalytic support for fuels and photo degradation of organics.\(^9\)-\(^12\) To be excellent and holding great potential for practical applications, better understanding of graphene surface chemistry is strongly required. Obtaining more knowledge of graphene surface can teach people what’s on its surface and what would happen by surface functionalisation.

1.1.1 Graphite

Graphite is an accessible 3D material existing widely in nature and can also be synthesised.\(^13\) In natural graphite, vast graphene layers stick together via prominent π-π stacking interactions which contribute importantly to the high thermodynamic stability of graphite. Consequently, the main task that should be overcome for graphite exfoliation is always how to conquer these strong interactions. The stacking
mode of the single sp² layers can either lead to a hexagonal (AB) or rhombohedral (ABC) stacking, or the configuration may be displayed without any regularities within the layer sequence. Figure 1.1A shows the ideal structure of graphite, however, there are many macroscopical fissures and holes on the crude graphite flakes (Figure 1.1C), which influence the chemical reactivity. Graphite is the pristine material that has been widely applied for single sheet exfoliation, namely the top-down method to prepare single layer carbon nanosheets. Compared with 2D nanomaterials, the properties of graphite are limited by its stacking structure, which decreases its surface area and electron mobility.

Figure 1.1. Chemical structure of graphite and graphene. Adapted with permission (Eigler 2014). Copyright © 2014, Wiley Online Library.
1.1.2 Graphene and graphene oxide

It all begun with graphene which is a new member of the nanocarbon family, composed of well separated 2D layers formed by aromatic carbon atoms. The theory of graphene can be dated back to 1947 when Wallace demonstrated it as a starting point for the understanding of electronic properties of graphite. In 1984, the emergent massless Dirac equation was first pointed out theoretically. Ever since 1970, single layer of graphite has been grown epitaxially on top of other materials, but the interactions between the surface of the substrate and graphene were always too strong to exhibit the excellent attributes of graphene. On the other hand, mechanical exfoliation has been attempted to make thin films of graphite since 1990, however, the achieved materials were all over 50 layers thick at that time. Although scientists agreed that one atom thick, 2D crystal graphene existed, no one worked out to extract it from graphite, until 2004 Geim and Novoselov creatively isolated free standing single layer of graphite using adhesive tape at University of Manchester. If you draw with a pencil, you could have already made graphene although you did not know. It is really attractive to physicists and chemists as such a superb nano-sized material with excellent attributes could be obtained by such a simple and inexpensive way. The discovery of graphene terminated the discussion whether the single-layer 2D material can exist stably or not. After isolation of free standing graphene, the stability of this 2D material is thought to be governed by infinitely small fluctuations at low temperatures which are the result of the thermal fluctuations. And another possibility can be attributed to the ripples observed in exfoliated nanosheets that can extend 2D structure into 3D structure. Theoretically, graphene is composed of single layer of carbon atoms, which forms a honeycomb lattice. It looks like plenty of benzene rings linked together (Figure 1.1B). And the stiff sp² covalent bonds should make it totally a planar structure. Actually, real graphene exhibits edges that have both zig-zag and armchair arrangement. Figure 1.1D shows non-functionalised graphene edges can exist in vacuum. And it has been verified that there are continuous wrinkles in the surface structure of graphene materials. These wrinkles resolved the problem of instability of 2D crystalline material, therefore, graphene sheet looks like sea wave rather than a paper. Furthermore, generalized graphene materials are not limited to single-layer carbon sheets, and have been expanded to another three categories namely: bilayer graphene, few layer graphene and multilayer graphene. Each of them has its own advantages in certain area.
For example, the high electrical conductivity and high transparency make graphene a promising transparent material for solar cells and liquid crystal display, especially to use less layered graphene which performs less light absorbance and better electron transfer rate. On the other hand, multilayer graphene is able to build a 3D network which can be fabricated using commercially available expanded flake graphite but is very different with graphite’s stacking structure. In contrast to those systems fabricated from individual exfoliated graphene, these graphene networks exhibit several merits including simple preparation method, low cost, and easy to be scaled up to large area wafers. Furthermore, the porous structure and high specific surface area of the nanoribbon networks make it possible for efficient functionalisation and high sensitivity for gas adsorption.

Graphene oxide (GO) is a single layer of graphite oxide composed of bonded sp² carbon atoms heavily decorated with hydroxyl, carboxyl and epoxy functional groups. In 1860s, Brodie reported the formation of graphite oxide for the first time and the method is call “Brodie method”. The obtained chemical was named as “oxyde de graphite” in the first paper, and “graphitic acid” in the later publications. In 1865, Gottschalk confirmed the results of Brodie in his article in which “graphitic acid” appeared in the title for the first time. Many efforts had been made until in 1958, Hummers and Offeman proposed the most popular method which is a safe and fast oxidizing technology, showing quite high efficiency by applying the mixture of sulfuric acid (H₂SO₄), sodium nitrate (NaNO₃) and potassium permanganate (KMnO₄) for oxidization. Generally speaking, graphite powders are utilised as raw materials which would be oxidized using strong acid and oxidant. The introduced oxygen functional groups greatly improved GO’s dispersive capacity in aqueous solution compared with hydrophobic graphite sheets, making it single-layer dispersed in water after sonication. Although GO are usually less-conductive and defective in sheet structure, it has drawn considerable concerns due to the surprising properties brought by the oxygen functional groups. The carboxyl groups, which only appear at the edges of GO sheets, import negative charges that significantly promote its solubility in polar solvents. Moreover, these hydroxyl and epoxide functional groups in the basal plane spatially prevent the re-stacking between GO sheets, making these sheets separated with each other. Furthermore, GO retains a large area of hydrophobic domain which is non-oxidized and is suitable for non-covalent
modifications by some other groups, such as π-π stacking interaction which has been frequently used for biomolecule immobilisation. In fact, GO is more processable and has better storage stability in aqueous solution than unmodified graphene sheet because it can be single-layer and chronically stable. CRGO, which is generated by the reduction of GO, is the most frequently used graphene alternative due to the low-cost and large-scale production. Its electronical conductivity is much higher than that of GO, but not comparable with perfect graphene due to the residual of oxygen-containing groups and structural defects. However, the improvement of solubility and water processibility, and the combination of properties with other versatile molecules sometimes make CRGO even more eye-catching than pristine graphene. The surface chemistry of GO is of significant importance in this project because 1) GO can be the most concerned graphene derivative, 2) the surface chemistry plays a key role to its performances and 3) more fundamental knowledge is required in this area to have better support before any process on GO.

1.1.3 Nano-scale GO and graphene quantum dot

As discussed above, the most attractive performance of GO may be attributed to its improved solution processability compared to graphene by incorporation of oxygenated groups in its molecular structure. During the whole processing, the resultant GO sheets are not only endowed with oxygen functional groups, but also experienced torn up into smaller pieces. Consequently, the lateral sizes of the as-synthesized GO sheets usually show polydispersity, ranging from a few nanometers to tens of micrometers, which may even exhibit marked differences from synthesis to synthesis. This ununiform size distribution makes it difficult to precisely define its exact structure.

Nano-scale GO (nGO) has similar spectroscopic characteristics and chemical properties with conventional GO, but very different solution properties, such as surface activity and colloidal stability. At the very beginning, nGO sheets (<100 nm) were obtained via density gradient separation which picked out the nGO from the polydisperse GO dispersion. After removing large sheets in micrometer level, small GO has been utilised in cellular imaging due to its photoluminescence from visible to the near-infrared range, and drug delivery studies due to the ultrasmall size making it easily passing cell
membranes.\textsuperscript{53-55} On the other hand, by direct synthesis, the size of nGO can be very uniform, which is as small as 20 nm because the starting material (carbon fibers) for its synthesis has a limited diameter.\textsuperscript{56} Compared with GO, this material has an increased charge to surface ratio which was confirmed by zeta potential measurement.\textsuperscript{57} Therefore, nGO has better water solubility and stability due to the strong electrostatic repulsion between the nanosheets. Moreover, even after reduction reaction, reduced nGO is able to well disperse even in acidic solution (still negatively charged), making it a promising surfactant for carbon nanomaterial solubilisation.\textsuperscript{57} Figure 1.2 shows the apparent differences in size, surface charge and oxygen functional groups between conventional GO and nGO. The ultra-small size and excellent water solubility have given nGO more potential to be used in biomedicines. In fact, nGO has been fabricated into nano carriers of anticancer drugs for controlled or even targeted delivery.\textsuperscript{53,58} The small size makes the drug carriers able to pass through lipid bilayer for drug release and the good water dispersity guarantees nGO can go out of cells and leave tissues through circulation.

\section*{Figure 1.2.} A structural model of conventional GO and nGO. Adapted with permission (Chou 2012).
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Graphene quantum dots (GQDs) are nanometer-scale fragments of graphene sheets, composed of a regular hexagonal lattice of sp² carbon atoms edged with heteroatomic functional groups. In the past few years, because the application of conventional graphene is unfortunately restricted by its nature which is easy to aggregate irreversible, leading to poor dispersion in common solvents, there has been increasing favour to fabricate zero-dimensional (0D) GQDs and study some new performances from GQDs associated with quantum confinement and edge effects. The nanoscale dots are biocompatible, strongly luminescent and well dispersed in various solvents, showing great potential for application in those systems of bioimaging, photovoltaics and photoluminescence. You can even treat it as a large molecule but without a certain molecular weight, because of the greatly reduced lateral size and the initially monolayer thickness. One major path in preparing GQDs is to reduce nGO which is obtained by modified Hummers method. Similar with RGO, GQDs obtained by this way also remain some hydrophilic groups, endowing good water dispersive properties with them. The other idea lies in cutting approach that graphene sheets assemblies are directly cut into 0D GQDs via physical, chemical or electrochemical techniques. For example, Kwon et al. synthesized GQDs by amidative cutting of tattered graphite. Interestingly, the size of the GQDs is controllable and can be tuned between 2 and over 10 nm by adjusting the amine concentration. The energy gaps in such GQDs are narrowed down while increasing the size, therefore, GQD dispersions will show colourful photoluminescence from blue to brown under a UV lamp (Figure 1.3).
Therefore, the shape, the size, the structure, and the surface functional groups significantly influence the properties and real applications of graphene nanomaterials. Having a more fundamental knowledge about its surface chemistry will kindly guide the development direction of 2D nanomaterials.

1.2 Synthesis of graphene based nanomaterials

Due to the value of graphene materials in various applications, the study for mass production of versatile graphene sheets, especially few-layer sheets became an important and challenging task for the users of graphene. Notably, graphene’s attributes can be controlled and purposefully tuned by chemical derivatisation, with important parameters being the synthetic conditions, dimensions, number of layers and atomic doping, which can provide chemical flexibility. Therefore, graphene synthesis approaches should be carefully selected according to specific requirement and mechanism to be used, with a
balanced consideration on performances (e.g., electronic conductivity and surface area), dispersibility, cost and processibility. As a new member of carbon nanomaterials, graphene was firstly obtained by Novoselov and co-workers via the now famed Scotch tape method in 2004, however, the low production capacity failed to satisfy the requirement for mass graphene production for further research and future applications. Nevertheless, it has really provided an idea to prepare this 2D conjugated structure and proved the possibility for it to exist stably in nature, which greatly catch people’s eyes. Up to now but not limited to these, graphene synthesis methods can be classified as exfoliation, thermal decomposition, chemical vapor deposition (CVD), opening carbon nanotubes (CNT), thermal reduction and oxidation-reduction, and some others. Each of these preparation methods has their own advantages and drawbacks, inevitably. In this section, I will have a brief introduction of some present strategies for graphene fabrication and describe the difference in graphene structure introduced by different approaches. To begin with, several examples of mainstream approaches for graphene preparation have been shown in Figure 1.4, and these will be discussed one by one in the following sections.
1.2.1 Oxidation reduction of graphite

Oxidation-reduction might be the most frequently utilised preparation strategy of graphene in large scale (Figure 1.4A). In a typical process, the graphite flasks are firstly oxidized using sulphuric acid and potassium permagnate to afford graphene oxide, which helps in generation of carboxyl groups at the edges and epoxy groups on basal planes which increase the hydrophilicity of the nanocarbon sheets and consequently helps in dispersion of single-layer 2D graphene oxide in water with help of ultrasonication. This method was initially exploited by Hummers and has been modified by users...
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according to their own conditions and purposes\textsuperscript{41,73}. Secondly, graphene oxide dispersed in aqueous solution was converted into graphene (normally called chemically reduced graphene oxide) in the presence of various reductants like hydrazine hydrate (\(\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}\)), sodium borohydride (\(\text{NaBH}_4\)) and hydroiodic acid (\(\text{HI}\)) etc.\textsuperscript{74-77} In order to lower contamination to the environment, some more friendly reducing agents for GO have been reported for the substitution of hydrazine which is carcinogenic, such as L-ascorbic acid,\textsuperscript{77,78} alkyl amines,\textsuperscript{79,80} reducing sugars,\textsuperscript{81} wild carrot root,\textsuperscript{82} metal nanoparticles\textsuperscript{83} and powders,\textsuperscript{84} phytoextract,\textsuperscript{85} green tea,\textsuperscript{86} baker’s yeast,\textsuperscript{87} supercritical alcohols,\textsuperscript{88} and amino acids.\textsuperscript{89} Compared with other methods, the chemical reduction approach is a promising one since it can be scalable in production and versatile in realizing abundant chemical functionalization. In addition, it has been demonstrated that the electrical conductivity of GO can be restored close to the level of graphite by chemical reduction.\textsuperscript{90,91} Interestingly, chemical reduction of GO can be controlled by varying the reaction conditions, such as adjusting reduction time\textsuperscript{92} or changing reductant’s mass,\textsuperscript{93} to tune the surface functional groups of RGO. In addition to chemical reduction of GO, some other reduction methods have been explored for preparing graphene sheets such as thermal reduction,\textsuperscript{94} microwave reduction,\textsuperscript{95} electrochemical reduction\textsuperscript{96} and UV-induced photocatalytic reduction.\textsuperscript{97} Reduced graphene oxide usually suffers from the problem of irreversible aggregation after the oxygen functional groups are removed from its surface, which leads to a gradual decrease of hydrophilicity and surface charge. Moreover, the smaller and heterogeneous size distribution, and also structural defects created during oxidation process affect RGO’s applications. This could be limitation because of poor electrical conductivity, or this could be advantage as people have developed wide application based on structural defects, like vacancies in basal plane and heteroatom doping.\textsuperscript{98} Every coin has two sides, it all depends on how people consider about it.

1.2.2 Other liquid exfoliation techniques

Meanwhile there are many approaches to exfoliate graphene sheets directly from pristine graphite powder in solution.\textsuperscript{99,100} The main barrier of this approach is to overcome the strong interaction (\(\pi\)-\(\pi\) stacking) between each single sheet in graphite 3D network structure. The addition of surfactant is a good idea to help graphene sheets dispersed in water, as its hydrophobic site can tightly grasp graphene
sheets while its hydrophilic groups help increasing solubility. Therefore, graphene sheets can be exfoliated and keep separated with each other in the solution. For example, surfactants such as sodium dodecyl benzene sulphonate has been used to intercalate graphite, followed by sonication yielding single to few layer graphene.\(^{33}\) In one of our previous work, we used a pyrene functionalised block copolymer to exfoliate graphene sheets directly from graphite powder (Figure 1.4B).\(^{101}\) The resultant sheets showed out to be single-layer, defectless and in large size, which can be up to several micrometers. Similarly, when using the exfoliation technique, graphene samples obtained from highly-oriented pyrolytic graphite in organic solvents such as N-methyl-pyrrolidone\(^{102}\) were virtually free of crystal defects, resulting in high carrier mobility.\(^{103}\) Apart from that, a facile and low-cost approach was adopted to obtain graphene sheets by continuous sonication of natural graphite crystals in dimethylformamide (DMF) for large-scale production of high-quality single-layer graphene sheets.\(^{104,105}\) However, this is usually time consuming, what is worse, long time ultrasonication will break graphene sheets into smaller pieces *via* fracture at wrinkles.

1.2.3 CVD and thermal decomposition

Graphene with high structural quality and large surface area can be obtained through low-pressure CVD (Figure 1.4C)\(^{106}\), in which hydrocarbons are supplied in gas form and a metal is used as both catalyst and substrate to grow the graphene layer.\(^{107,108}\) Specifically, single- or multi-layered graphene sheets with high transmittance, electrical properties and large size have also been successfully prepared by the CVD methods on different substrates.\(^{109-112}\) The thermal decomposition of silicon carbide (SiC, Figure 1.4D) is another technique employed for the fabrication and processing of large graphene sheets.\(^{113-116}\) Sutter et al.\(^{117}\) reported that interaction between the first epitaxial graphene layer and metal substrate of ruthenium (Ru) is fairly strong, while the second layer is almost completely detached, showing weak electronic coupling to the metal. This result showed that graphene sheets obtained through this approach retained the inherent electronic structure of graphene. The advantage of these two methods is that the as-prepared graphene sheets are excellent in electrical conductivity, optical transparency and size control, which provide chance for applications in conductive devices. Although sheet thickness could be tuned according to the conditions, it is difficult to peel off the nanosheets from the substrate which
is a great drawback. And the high temperature used in these strategy is another unstable factor which may cause trouble with the purity and crystalline nature of graphene sheets.

1.2.4 Unzipping carbon nanotube

As shown in Figure 1.4E, this approach bases on lengthwise cutting and unraveling of carbon nanotubes (CNTs) with the presence of strong oxidizing agent, producing a nearly 100% yield of graphene nanoribbon (GNR) structures.\textsuperscript{118} Obviously, the edges of resultant sheets will be oxygen groups functionalised, leading to a good water solubility and processibility. An intelligent method for the preparation of graphene ribbons and sheets by unzipping CNT with intercalation of lithium and ammonia followed by exfoliation was reported.\textsuperscript{119} The resulting material consists of multilayered flat graphitic structures (nanoribbons); partially opened multiwalled carbon nanotubes, and graphene flakes. The large number of edge atoms makes them attractive for many applications. By controlled unzipping of CNTs, GNRs can be obtained by plasma etching of CNTs partly embedded in a polymer film.\textsuperscript{120} Specifically, the GNRs synthesized by an argon plasma etching method had very smooth edges and a narrow width distribution (10-20 nm). This method provides a novel idea to synthesize GNRs with huge length-width ratio, however, the difficulty to control the cutting point results in vast defects in sheet structure.

All the above methods have their own advantages and limitations. For example, mechanical exfoliation did produce high quality graphene but suffered from very low output. In the oxidation-reduction method, mass production was achieved while the excellent sp\textsuperscript{2} network was not fully preserved. Therefore, graphene synthesis is still a challenging but urgent task for its users. In consideration of present situation, different strategies should be selected due to the practical applications of the materials. For example, if superb electronic properties are required, mechanical exfoliation or CVD might be the suitable one to produce high quality graphene sheets. If functional groups and water solubility are the favorable attributes of resulting materials, oxidation-reduction exfoliation would be the undisputed choice. In all of my project, I used the oxidation-reduction exfoliation because of the following points. Firstly, I wanted to study the wet chemistry of GO rather than in solid-state, so good water solubility was the vital attribute need to be considered. Furthermore, the introduced oxygen
functional groups on GO provided much more opportunities for functionalisation compared with only carbon-carbon double bond conjugated structure. In a word, oxygen functional groups made simple carbon more active. Lastly, to be mono-dispersed in solution, GO could better utilise its ultra-large surface area, which was especially prominent in sensing and biomedicine applications.

1.3 Surface functionalisation of graphene and GO

The surface chemistry of nanomaterials plays a crucial role with respect to interaction with other molecules. And better understanding the mechanism of graphene surface modification helps further development in sheets functionalization and specific applications. Graphene derivatives have many superior surface properties, which make it satisfactory in the applications of various sensor system. In this section, we will discuss the unique surface properties and modification approaches while taking graphene’s sensing applications as examples to figure out why choose graphene, how to select synthesis and functionalization method, and what is the advantages of graphene over other materials.

To begin with, graphene that is a single-atom-thick sheet of sp² hybridized carbon atoms which are packed in a hexagonal honeycomb crystalline structure, has a conjugated structure with vast delocalised π-electrons, providing it with superior electrical properties (e.g., extremely high carrier mobility which is 200,000 cm² V⁻¹ m⁻¹). This is extremely good in fabrication of electronic device and electrochemical sensor. For example, when utilised as supporting material for molecular detection, graphene’s superb conductivity could extremely accelerate the electron transfer rate, leading to fast response time and strong response signals. Moreover, the large surface area of graphene (2630 m² g⁻¹), which is twice that of single-walled carbon nanotubes and the strong absorptive capacity to various molecules make it a finest loading substrates, which can immobilise small molecules via either direct adsorption or covalent binding with some functional groups on graphene derivatives. With no doubt, higher concentration of probe molecules on each substrate will make the measurement much more sensitive. Thirdly, interesting optical properties endow this 2D material with applications of touch screen and solar cells because of the high transmittance (97.7%) in combination with its high charge mobility. Furthermore, with smart processing, such as oxidation and heteroatom doping, graphene related materials can be rich in functional groups attached to their surfaces and more importantly, at edges,
which helps molecular-level tuning and fabrication of the hybrid nanomaterials. In my opinion, tuning the surface chemistry of graphene materials is the most primary and direct approach to adjust materials’ properties for certain objectives. Functionalised graphene materials are apt to conjugate with different recognition molecules as well as incorporate other functional materials (e.g., metal nanoparticles, proteins or conducting polymers) for electrochemical bioanalysis. Graphene surface is able to be modified via covalent, non-covalent as well as some other methodologies, which meet the specific requirements of different types of applications. Figure 1.5 presents some alternative approaches for surface modifications of graphene sheets.

1.3.1 Covalent modifications

Covalent interaction, which is much stronger than other ways, plays an important role in graphene’s functionalisation. It primarily takes place with the help of covalent bond formation, which happens either on the basal planes or at the edges. Generally speaking, graphene can be covalently functionalised by reaction with unsaturated π bonds of graphene, the oxygen functional groups on GO sheets, organic functional moieties, and heteroatom doping.

In order to carry out couplings with the C=C bonds of graphene sheets, several as-prepared diazonium salts have been used to produce highly active free radicals for addition reactions, which vertically immobilise various aryl-addends on the surface. These reactions convert sp² carbon atoms to sp³ hybridization, generating non-conducting and semiconducting regions in the graphene layers (Figure 1.5B). Furthermore, dienophile compounds can also react with the sp³ carbons. Some types of dienophiles, such as aryne, nitrene and azomethine ylide, have been used for the addition reaction with graphene, creating versatile terminated groups for further functionalisation and modification of graphene materials. This method provided an option for holes creation between each sheet but the conductivity would be pulled down due to the damage of π-conjugated structure. On the other hand, others paid more attention to the covalent linkage between oxygen moities (from GO or RGO) and some functional groups. The carboxyl groups at edges were able to react with the amino groups from small molecules or even proteins catalysed by a well-known carbodiimide procedure (Figure 1.5A). Typically, GO first reacted with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)
or N, N’-dicyclohexylcarbodiimide (DCC) under ambient conditions, producing a stable active ester in the presence of N-hydroxysuccinimide (NHS). Then this ester would react with the amine group of a target molecule (DNA or enzyme, etc.) to form an amide bond. By this way, strong covalent bonds could be generated, granting graphene sheets some expected functions. Till now, graphene derivatives have already been covalently bonded with beta-aminocyclodextrin, poly-L-lysine, protein, and DNA, which can be further employed for the development of electrochemical sensing platforms. The transformation of carboxylates to acyl chlorides was another common method for graphene modification. By this means, graphene (or graphite) oxide surface groups were firstly activated with thionyl chloride, obtaining an acyl chloride–graphene derivative, which subsequently reacted with hydroxyl or amino groups from molecules such as neutral red, cyclodextrin, poly(vinyl alcohol), poly(3-aminobenzene sulfonic acid), amino terminated polypyridyl ruthenium(II) complexes and amino group modified silica spheres. All of these functional molecules extended graphene’s applications in sensing application, and graphene helped improving sensitivity as a solid-state electron mediator. On the other hand, heteroatom doping is a common approach to tailor the electronic properties of graphene materials. The nitrogen and boron atom are most frequently applied in synthesizing heteroatom-doped graphene as they have similar structure with carbon and can act as electron donor or acceptor, respectively. When nitrogen atoms are incorporated into the basal plane of graphene, they denote electrons into graphene leading to n-type doping of graphene while boron-doped graphene would exhibit p-type behaviour. As a result of nitrogen doping, three types of nitrogen bonding configurations were introduced into graphene’s basal plane as ‘pyridinic’, ‘pyrolic’ and ‘graphitic’ nitrogen, which are highly active sites for redox reduction. It has been reported that nitrogen doping can significantly increase the electron conductivity, improve the electron-donor properties and binding ability, and also enhance the biocompatibility and sensitivity of graphene in biosensing applications.
All of the above binding methods for functional molecules to graphene are trustworthy and very strong, making the resultant surface stable in both the structure and activity storage. However, there are drawbacks such as disruption of sp² conjugated structure and being limited to the surface formation of chemical-converted graphene. The task to control reduction reaction is still perplexing RGO users as there is a difficult balance to make between good conductivity and good chemical tunability.

1.3.2 Non-covalent modifications

Although not as strong as the covalent binding, non-covalent reactions have also been utilised to modify graphene materials due to their own merits, or even more popular than covalent binding. Compared with covalent one, this method does not destroy the original sp² conjugated structure of graphene, which furthest retains the high electronic conductivity of graphene sheets. It is truly important for electronic sensing to use unbroken graphene nanosheets. Non-covalent linkages between graphene and other functional molecules can be mainly achieved through π-π stacking, hydrophilic and hydrophobic interactions.
First of all, π–π stacking is an important interaction for graphene functionalization. It is well known that the carbon atoms forming graphene are in sp² C=C hybridization, making graphene sheets a 2D planar structure, theoretically. More interestingly, large quantities of π electrons generate a conjugated delocalization system, giving high conductivity and opportunities for conjugation extension with some aromatic compounds which have similar but smaller π conjugated structure. The extension of this delocalization is called π-π stacking which does not form any covalent bond. A typical example for π-π stacking interactions is that the graphene sheets are held by various pyrene derivatives that are readily dissolved or dispersed in aqueous solution and this property is subsequently utilised to make graphene-based conducting films or sensors. Pyrene is a small planar aromatic compound containing 4 benzene rings which looks like an ultra-small carbon sheet. Usually, sonication helps pyrene groups tightly adhering on graphene sheet through interactions between π electrons, which is even strong enough to exfoliate graphene. Besides that, single stranded DNA can also attach to the graphene surface via π-π stacking, leading to specific detection of DNA molecules or proteins. In fact, nucleic acid bases and graphene can act as a platform for different categories of DNA related detection techniques. Additionally, several amphiphilic molecules, such as sodium dodecyl sulfate and hexadecyltrimethylammonium bromide, are good dispersing agents for graphene in aqueous solution with the hydrophobic tails attached to the surface of graphene and the hydrophilic side improving water solubility. On the other hand, GO, as well as RGO, can be capped by hydrophilic polymers to prevent aggregation in aqueous solution. Several polymers including polyvinyl alcohol, poly (diallyldimethylammonium chloride), polyetherimide and polyvinylpyrrolidone have been widely used as dispersants due to their affinities arising from hydrophilic interactions with and without electrostatic attraction.

Non-covalent interactions are not as strong as covalent interactions, however, they are usually easy to be carried out and the nanosheets can retain its pristine electronic structure, which greatly improves the applications.
1.3.3 Other methods

Inorganic materials were used to modify graphene and induced an additional electrochemical catalytic ability that may provide further facility for functionalization. The combination of graphene and metal nanoparticles in solution (especially in aqueous solution) has caused considerable concern due to their facile synthesis approaches and high potential as enhanced materials for electrochemical and analytical applications. In recent years, various strategies have been employed to prepare graphene–metal nanohybrids, particularly utilising noble metals. For example, the microwave-assisted synthesis of metal nanoparticles (Pd, Cu and PdCu) dispersed on the graphene surface in oleylamine and oleic acid have been realized. This method allows the simultaneous reduction of GO and various metal salts, and produced novel nanocatalysts supported on the large surface area of the thermally stable graphene.

With a wet-chemical approach, Pt nanoparticles and Pt-on-Pd nanodendrites are attached to graphene nanosheets in aqueous phase. A “clean” synthesis, involving reduction of Pd and Au, Pt precursors by GO provides a convenient and direct way to fabricate metal–graphene nanocomposites without capping agents and any additives.

On the other hand, self-assembly approach provides an alternative strategy for preparing high-quality graphene–metal hybrids with the desired nanoparticles. This can be achieved using numerous methods for the synthesis of metal nanoparticles with different sizes, components and shapes. Zhu et al. fabricated a 3D nanocomposite film by alternatively assembling the graphene nanosheets modified by ionic liquid and Pt nanoparticles. In this method, an imidazolium salt-based ionic liquid (IS-IL)-functionalised graphene was synthesized by binding 1-(3-aminopropyl)-3-methylimidazolium bromide onto graphene nanosheets. By introduction of IS-IL on the surface of graphene nanosheets, positive charged graphene nanosheets can be well dispersed in aqueous solution. Self-assembly is a facile and regular approach, making the structure of as-prepared multilayer film highly uniform. Furthermore, the electrocatalytic activity of the films could be further tailored by simply choosing different cycles in self-assembly process, offering a smart route to build electrochemical nanodevices. Deng and coworkers employed a general approach for GO reduction and decoration using bovine serum albumin (BSA). They prepared graphene–metal (Au, Ag, Pt, Pd) hybrid nanosheets through the affinity
between these noble metals and the amine, thiol and imidazole groups on BSA (Figure 1.5C). This is an environmentally friendly, one-step reduction/decoration strategy to assemble nanoparticles with controllable size, shape, composition, and surface property, which are meaningful in graphene surface modifications.

Meanwhile, metal or semimetal oxide nanomaterials have gained considerable attention in the electrochemistry field. Oxides generally exhibit low electrical conductivity therefore converting them to conductive graphene materials will decrease the over potential and increase the current density. Moreover, the utilisation of oxides may bring additional means for further modification of graphene materials. Compounds including Fe₃O₄, Co(OH)₂/Co₃O₄, and MnO₂ can bond with GO via various methods. For examples, Yang et al. fabricated mesoporous SiO₂ coated GO using a wet chemical method. Cationic surfactants, such as cetyltrimethyl ammonium bromide, were selected to electrostatically adsorb and self-assemble onto the surface of GO which is highly negatively charged in alkaline solution. This procedure directed the formation of mesoporous silica around the surface of single-layer GO. Utilising suitable cationic surfactants can not only effectively resolve the incompatibility and aggregation problems between graphene sheets and inorganic materials, but also provide the molecular template for controlled nucleation and growth of mesoporous silica on the surface of GO sheets. A hydrothermal procedure was applied to synthesize Co₃O₄ nanowires on 3D graphene foam. It is reported that this 3D hybrid is capable to deliver high specific capacitance of 1100 F g⁻¹ at a current density of 10 A g⁻¹ with good cycling stability, moreover, it can detect glucose with a high sensitivity of 3.39 mA mM⁻¹ cm⁻² and a remarkable lower detection limit of less than 25 nM (S/N = 8.5). Hydrothermal synthesis is a simple and well-studied method to process functional materials, but the high temperature and high pressure make it dangerous to be performed in the experiment.

1.4 Recent advances in graphene-based applications

Beginning with the first isolation of free-standing graphene sheets, exploration of its practical application has been a hot topic in the field of nanotechnology. Due to the superior properties beyond its precursor-graphite and 1D carbon-CNT, graphene has been a rock star in various domains such as chemical and biosensing, drug delivery, energy conversion and so on. In this section, I take the
application of graphene on sensing and energy area as examples to demonstrate how graphene were used practically and discuss its advantages which make it prominent in these areas.

1.4.1 Graphene in sensing applications

Sensing is to use physical or chemical method to detect a physical presence and convert that data into a signal that can be read by an observer or an instrument. High sensitivity chemical and biomolecules detection using inexpensive sensor devices are important for different industrial, environmental, public safety and military applications.\textsuperscript{123} In the first year of my candidature, we summarized recent research on graphene sensors combined with our own work and published a critical review article discussing the important of surface chemistry of graphene for chemical- and bio-sensing.\textsuperscript{188} Figure 1.6 presents several common substances that have been detected using graphene as matrix. For example, various enzymes have been immobilised on electrode surface with graphene for electrochemical detection of biomolecules due to the redox reaction happens on electrode surface.

\textbf{Figure 1.6.} Graphene-based sensors. Adapted with permission (Liu 2015). Copyright © 2015, Elsevier.
Edges and defects on graphene provide a high electron transfer rate, suggesting that graphene sheets or small flakes of pristine graphene are very suitable for electrochemical detection. GO is able to support the efficient electrical wiring the redox centers of several heme-containing metalloproteins to the electrode because GO can facilitate electron transfer. As the redox centers of proteins are often concealed in folded polypeptide shells with poor charge transfer, metalloproteins suffered from poor electron transfer rate at various surface. GO is an ideal substrate for accommodating proteins and promoting protein electron transfer due to its high electron mobility and excellent protein adsorption ability because of strong hydrophobic interaction. Therefore, GO can promote electron transfer between redox centers and electrode surface. Furthermore, vast oxygen functional groups on the surface of GO facilitate electrochemical activities. We developed an electrochemical detection approach towards single protein molecules (microperoxidase-11, MP-11), which are attached on the surface of graphene nanosheets (Figure 1.7). The non-covalently functionalised graphene nanosheets exhibited enhanced electroactive surface area, where amplified redox current are produced when graphene nanosheets collide with the electrode. We observed stepwise changes in redox current and the charge transferred in electrochemical processes, which was amplified by repeatedly reducing and oxidizing functionalised graphene nanosheets as they randomly diffuse on the electrode surface. We estimated the number of the MP-11 molecules on a single graphene sheet is in the range of 105 ± 18. This facile and highly sensitive detection method may be useful for future biosensing research and investigating single-molecule reactions.
Graphene provides an ultralarge surface for enzyme or other signal molecules immobilisation. Combined with its high electrical conductivity, it can act as an excellent loading platform for sensing applications. But again, graphene varies from graphene, its surface chemistry plays an important role in its performances due to different proposes. More oxygen groups on GO might provide more binding sites to functional molecules, however, the poor electrical conductivity decreased the signal strength and sensitivity. To find the balance between is the key to achieve best performances of graphene, and this needs better understanding of its surface chemistry.

1.4.2 Graphene in energy production and storage

Energy is always a vital global issue in economic development and social stability, especially under the circumstance that the traditional resources (fossil fuels) might run out in the near future. The key solution is not only to exploit renewable and sustainable energy sources but also, possibly even more crucially, to realize efficient energy storage and delivery on demand, often for mobile applications, such as transportation systems and carry-on electronic devices. Higher requirement has already been proposed for new energy systems\textsuperscript{192} which should hold the advantages of portability and energy efficiency whilst being environmentally friendly\textsuperscript{193,194}. Although this research area is beset with

\textbf{Figure 1.7.} Electrochemical detection approach towards single MP-11 molecules. Adapted with permission (Li 2014). Copyright © 2014, RSC Publications.
difficulties, our earth do require advances in the exploiting of novel and even surprising materials. There are three major ways that energy is stored: chemically, electrochemically, and electrically. In recent years, the exceptional physical attributes of graphene have profoundly got the look of researchers as graphene is ideally suitable for these applications due to its low weight, large surface area, unique heterogeneous electron transfer and charge carrier rates, good catalytic activity and low preparation cost. By smart utilisation, graphene materials can show surprising outcomes in various energy systems compared with dominated devices at present. Therefore, graphene will be a promising alternative in future applications to help with technological advances within energy related fields. In this section, we will focus on graphene’s superior properties to figure out why it is suitable for this application.

1.4.2.1 Lightness and surface area

Carbon is the lightest element used for energy storage, which can be tuned into various forms to provide high surface area and energy capacity. A crucial characteristic of a substrate material, especially in energy production and storage, is surface area. By comparison with another outstanding member of carbon nanomaterials—carbon nanotubes (CNTs), we can find advantages of graphene. In the fifteen years, there has been fiery interest in the application of CNTs for energy storage device fabrication. Besides low weight and chemical stability, CNTs exhibit some merits in this area. They have a large surface area, up to 1315 m² g⁻¹ for single-walled carbon nanotubes (much better than 3D graphite, which exhibits a typical surface area of 10–20 m² g⁻¹), can be nanostructured and mass-produced. However, CNTs have their own limitations, such as the presence of toxic residual metallic impurities which are imported while synthesizing and very difficult to remove, and a high manufacturing cost. On the other hand, single layer graphene sheet has a much larger surface area-2630 m² g⁻¹ which is two times of SWCNT’s. Larger surface area leads to more immobilisation of functional molecules, improving the energy conversion rate. Furthermore, among various methods that have been utilised to fabricate graphene, the oxidation-reduction method is mostly used because of the low cost and high production. With appropriate treatment the final product can be metal-free, stable over a large range of temperatures and biocompatible.
1.4.2.2 Electronic properties

Focus has switched to graphene due to its extreme electronic quality. The electrical conductivity of graphene, resulting from its extensive conjugated sp² structure, has been reported to be ~64 mS cm⁻¹ which is more than 60 times of SWCNTs.²⁰⁷,²⁰⁸ At room temperature, graphene displays the half-integer quantum Hall effect, with the effective speed of light as its Fermi velocity \( \nu_F \sim 106 \text{ m s}^{-1} \),²⁰⁹,²¹⁰ and more interestingly graphene is different from its counterparts due to the unusual band structure, leading the quasiparticles in it formally identical to the massless Dirac Fermions. The charge density of graphene can be controlled by means of a gate electrode²¹¹; charge carriers can be adjusted continuously between electrons and holes where electron mobility remains high even at high concentrations in both electrically and chemically doped devices, which translates to ballistic transport on the sub-micrometer scale¹⁹⁷. Furthermore, the ultrafast charge carrier properties are found not only to be continuous, but also performing high crystallinity¹⁸, which means charge carriers are able to transfer a long inter-atomic journey without scattering. Therefore, graphene can be used as an electron transfer channel because of its high-speed operation and low electric energy depletion. And graphene materials are expected to carry super-current due to their exceptional attributes while ambipolar electric field effect has been detected in graphene.

1.4.2.3 Other attributes

Some specific characteristics make contribution to graphene’s capacities in energy field. Compared with 3D graphite, graphene nanosheets are more flexible and processible, providing convenience for fabrication of flexible electronic and energy storage devices.¹⁹⁶ This also means the equipment can be bendable or even stretch, and combined with graphene’s good mechanical strength, the service life and work conditions can be extremely promoted. Single layer graphene sheet is a wonderful optical material due to its ultrahigh light transmittance which is 97.7 % and wavelength-independent.²¹² This is especially meaningful when the Sun acts as the energy resources, by which means many types of solar cell²¹³-²¹⁵ have been designed using graphene sheet as one of the electrode materials. For example, by combining the unique photodiode properties of photosystem I (PS I) with the exceptional optical and electronic properties of graphene, Gunther and co-workers²¹⁶ fabricated a hybrid light-harvesting
electrode. The thickness of this photoactive electrode is less than 10 nm, introducing an interesting approach to convert solar power to electric energy (Figure 1.8).

![Schematic diagram of PS I/graphene coated light-harvesting electrode and the amplified electrochemical signals after adding methylene blue. Adapted with permission (Gunther 2013). Copyright © 2013, ACS Publications.](image)

**Figure 1.8.** Schematic diagram of PS I/graphene coated light-harvesting electrode and the amplified electrochemical signals after adding methylene blue. Adapted with permission (Gunther 2013). Copyright © 2013, ACS Publications.

One invisible characteristic cannot be ignored is that there are vast oxygen functional groups at the edges or basal plane of GO and RGO, thus specific groups can be introduced and play vital roles in electrochemical battery and fuel cells. Graphene derivatives’ electronic and chemical performances could be purposefully tuned by certain modification approaches which are given possibility by carboxyl groups or hydroxyl groups. In a word, graphene exhibits many advantages suitable for energy production and storage, including large surface area, fast electron mobility, high transparency and so on. Furthermore, the strong absorptive capability of graphene suggests a promising future in energy field.

1.4.3 GO in drug delivery

Diseases are always something discomforting human beings, and cancer is even killing people and widely considered to be incurable by the public. In fact, cancer cells can be killed medically by treated
with anti-cancer drugs, such as doxorubicin\textsuperscript{218} which interacts with DNA by intercalation and inhibition of macromolecular biosynthesis.\textsuperscript{219,220} This inhibits the progression of the enzyme topoisomerase II, which relaxes supercoils in DNA for transcription.\textsuperscript{221} However, tumors are always around normal tissues in human body. Without any treatment once we utilise drugs to tumors, they attack normal living cells. That’s the reason why people with cancer get weaker and weaker under chemotherapy. In order to have efficient drug concentrations, a lot of free drugs could be used, which would kill more normal cells. Thus, the development of new drug delivery systems with the ability to improve the therapeutic efficacy while lower the side effects is one of the key projects in modern medicine design.\textsuperscript{222} Using drug carriers, the therapeutic agents can be loaded in large amount and released in the designed way, which meets the real requirement of chemotherapies. The development of novel nanomaterials has led to a number of new drug delivery systems in recent years.\textsuperscript{223}

Graphene has been extensively applied as one of the most popular biomaterials because of its unique characteristics including one-atom thickness, large surface area, excellent electrical and thermal conductivity and good biocompatibility. Apart from these, graphene oxide, especially nano-sized graphene oxide has been designed as a very good material for drug delivery\textsuperscript{44,224} due to the introduction of oxygen functional groups that provide better water solubility and biocompatibility to the nanosheets. Furthermore, the 2D planar structure and ultra-large surface area provide GO awesome loading capacity of drugs which can attach on both sides of the nanosheets. Another important point is that the COOH and OH groups could be the binding sites for further modifications or drug loading. All of these make GO a big and safe vehicle to load and deliver its cargos.

The drug release kinetics from its carrier is usually controlled by the diffusion, it is of great interest to control this process by tuning the nano-bio interface.\textsuperscript{225} This could be achieved by several ways, like pre-modification of GO surface, changing chemical conditions of surrounding buffer or drug binding method. With better and better understanding of GO surface chemistry, more efficient delivery systems can be developed for real use and meet the requirement of controlled release. For example, GO/poly(vinylalcohol) (PVA) composite hydrogel was designed as a pH sensitive delivery system.\textsuperscript{226} The hydrogels exhibited pH-induced gel–sol transition, a property that can be applied for loading and
selectively releasing drugs at physiological pH, while relatively stable under acidic condition (Figure 1.9). In another work, GO nanosheets were covalently functionalised with hydrophilic and biocompatible pluronic F38, Tween80 and maltodextrin for loading and release of a poorly water-soluble antioxidant and anti-cancer drug - ellagic acid. The ellagic acid release rate from the functionalised GO was found to be pH-dependent in an increasing order: water (neutral pH) < pH 4 < pH 10.

Figure 1.9. (a) Drug release using GO/PVA gel (b) XRD patterns of PVA, GO and GO/PVA. Adapted with permission (Bai 2010). Copyright © 2010, RSC publications.

Apart from these, there are some methods which can stimulate the drug release by external field such as electromagnetic fields and ultrasound simulation for the controlled release of drugs. In this thesis, I will focus on the effect of tuning surface properties of GO to the drug delivery behaviour in chapter 5.

1.4.4 Enzyme immobilization using graphene-based nanomaterials

Enzymes are biological molecules that are usually composed of ambient polypeptides and internal catalytic center. They are versatile catalysts in the laboratory and on an industrial scale. Enzymes are sensitive, efficient but fragile in ambient condition, thereby it is challengeable to put them into practical
use in industrial field and they are mostly replaced by non-renewable metals. Using dissociative enzymes does face some problems like easy to be wiped off activity and hard to deliver efficiently. If an enzyme or any other type of catalyst, is dispersed in the reaction solution, it will be very difficult to retain its activity and reuse it. Immobilisation of enzymes on certain substrates can help to overcome these issues. It can help enabling the application of enzymes in different solvents, at harsh pH value and temperature, and high matrix concentrations. Meanwhile substrate-specificity, reactivity and enantioselectivity can be developed. Carbon nanomaterial is a smart choice for enzyme immobilisation because it is non-toxic to biomolecules and has strong interactions with proteins, especially to use graphene derivatives which have ultra-large surface area, tremendously improving enzymes’ loading mass. There have been many approaches using different mechanisms for enzyme immobilisation.

Enzyme immobilisation requires interactions between the two parts, the carrier and the enzyme itself. The surface properties of both can be crucial for assembling. Specifically the polar groups (e.g. amino groups and acid groups), non-polar surface area and sugar moieties play important roles in enzyme surface chemistry. It is usually unpractical to adjust these groups, therefore the decision of what carrier to use is the key to efficient immobilisation of enzymes. And to prepared or modified carriers to match enzymes’ chemistry is of course a good method. There are general requirements for the carriers, including large surface area, good biocompatibility, as well as chemical and mechanical stability. Enzymes should maintain or even improve the catalytic activity after the immobilisation procedures, and this is usually decided by the interactions between the carrier and enzyme itself. However, there are no certain methods applied to the building of any enzymes because of their complicated surface chemistry. The mostly used strategies will be discussed as below, and each with its own advantages and disadvantages.

1.4.4.1 Non-covalent bonds

For enzyme immobilisation, there could be different categories of interaction between carriers and the enzymes, which is strongly influenced by the distribution of their hydrophobic and hydrophilic regions. Enzymes with a large lipophilic surface area tend to interact well with a matrix rich in hydrophobicity, while large hydrophilic surface area of the enzyme will interact with a hydrophilic carrier’s surface.
In the case of Van der Waals’ force, both of carrier and enzyme need to have large lipophilic surface areas. As Van der Waals’ force is relatively weak, it is not the true driving force behind this immobilisation. Instead, it is entropy-driven interaction. As soon as an enzyme molecule is settled, it will replace vast water molecules both from the carrier’s and its own surface. This kind of interaction between two materials via the rise in entropy is considered as hydrophobic interaction. For instance, lipases have been absorbed to many non-polar supports like accurel and macroporous polymer based on methyl and butyl methacrylic esters cross linked with divinylbenzene. GO has been used as the 2D scaffold to study the dependence of the activity of cascade reactions of two enzymes (Figure 1.10). The hydrophobic interactions played important roles in achieving high product conversion rates due to the ability to transfer electrons. Owing to direct molecular channeling, a very short transient time can be achieved when the enzyme molecules self-assembled randomly on GO surface.

**Figure 1.10.** GO-based enzyme nanoarchitectonics for substrate channeling. Adapted with permission (Mathesh 2017). Copyright © 2017, Wiley Online Library.
In fact, hydrophilic amino acid residues always appear on the surface of enzymes. And enzymes may be glycosylated, further increasing the hydrophilicity of the protein. Therefore hydrogen bonds can be easily formed and thus enzymes can be immobilised on hydrophilic sites of various substrates.

The advantage of immobilisation via entropy effects and hydrogen bonds is that enzymes do not need to be pre-treated or chemically modified. It is even possible to use pristine enzyme for these immobilizations. Varying the immobilisation conditions can tremendously affect the results and thus might allow a straightforward manipulation of the enzyme’s properties. However, there is a significant disadvantage of non-covalent adsorption, which is, the enzyme tends to leach readily from the carrier when dispersed in aqueous media because this kind of interaction is weak. This is not a problem if applied in organic media, but enzymes are more suggested to be used practically in water or buffer solution which mimics the biological conditions.

Besides, electrostatic interaction, which depends on pH value affecting the surface charge on the enzyme surfaces, is another valuable form of non-covalent approach for enzyme adsorption. Due to the presence of amino and acid groups, most enzymes have an isoelectric point (pI), making them positively charged in acid solution while negatively charged in basic solution, approximately but not exactly. As discussed above GO and RGO have shown to be negatively charged due to the large quantities of carboxyl groups at edges, which provides possibility for electrostatic attractions with positively charged enzymes. Horseradish peroxidase (HRP) has already been self-assembled on sodium dodecyl benzene sulphonate functionalised graphene sheets by electrostatic attractions, forming a novel hierarchical nanostructure in aqueous solution. The obtained layer-by-layer structure exhibited excellent biocompatibility with entrapped HRP maintaining its native structure in the composites, by which this electrode showed good sensing performance to the reduction of H$_2$O$_2$.

1.4.4.2 Covalent bonds

Covalent binding is very strong interaction by forming a real chemical bond, which means the enzymes can be tightly fixed on matrix. Therefore the problem which happened in aqueous solution called enzyme leaching can be effectively resolved. In general, covalent immobilisation would be considered preferentially when using enzymes in water and denaturing factors exist. This is because the existence
of multiple covalent bonds between proteins and carriers could reduce conformational flexibility and thermal vibrations, which prevent protein unfolding and denaturation. However, there is an obvious drawback of covalent immobilisation, the enzyme is chemically modified, which has been found possible but very difficult. As the size of enzyme molecules are much larger than functional groups for covalent binding, these bindings cannot occur in a uniform manner for all enzyme molecules in one batch. However, multipoint attachment of each enzyme helps to form tight linking to the carrier. This is not only limited to monomeric enzymes but also includes multimeric ones.\textsuperscript{234}

The actual enzymes may have a very low content of protein (\(<5\%\)) due to the various additives such as sugars and polyols, which are widely used as stabilisers. Thereby all components of native enzymes should be taken in consideration while choosing a covalent immobilisation approach, such as the influence of additives and the true terminal groups. Again with the presence of amino groups, covalent bonds such as amide bond can be generated for enzyme immobilisation. The amino group can be used as nucleophile to attack an aldehyde or an epoxide. Interestingly, irreversible immobilisation of aldehyde has been achieved by reducing the imine formed by covalent binding. For the case of epoxide attack, candida rugosa lipase was immobilised on an epoxy activated resin in order to protect its surface amino groups and repeatedly use. This is because amino groups are easy to be poisoned by acetaldehyde which is the side product of the reaction in the lipase catalysed acylation of alcohols in dry organic solvents.\textsuperscript{235}

Crosslinking is a common method for protein immobilisation. Figure 1.11 shows the common procedure of enzyme crosslinking.\textsuperscript{230} In this case, enzyme itself acts as the carrier and actually the force belongs to covalent binding by using a di-functional agent such as glutaraldehyde. The only requirement of the enzymes is that they should be crystallisable. The first step is to form enzyme aggregates or crystals, however, enzyme can also crosslink in solution, resulting in a carrier-free and less-tuned cluster. The addition of precipitants such as acetone is followed by the cross linker.
1.4.4.3 Entrapment

The best way to avoid any negative influence on the structure of an enzyme might be encapsulating it. In this case enzymes tend to just get involved into carriers rather than bind with the carriers. Sol–gel method, which is the mostly used techniques, uses silica materials that are highly porous and readily prepared. Sol-gels are prepared by tetraalkoxysilane which is hydrolysed by acid or base hydrolysis. Properties of these gels can be further tuned by changing the alkene group associated with the sol-gel preparation. This could be explained by the fact that the entrapment of lipase in sol-gel with increased hydrophobicity due to the alkene group exist showed more activity than the one encapsulated in esterification of lauric acid with 1-octanol. Hydrophobic surfaces can contribute to the reactivity of lipases, since they might induce interfacial activation; i.e. the lipase might be in its active conformation. Thereby hydrophobic sol–gels can activate lipases. In addition the rather brittle sol–gel can be mechanically strengthened by including porous glass beads or silica glass fibers during the sol–gel synthesis. In this way high activity and stability can be obtained with lipase preparations. There are several disadvantages of this system including ease of enzyme leakage, loss in activity and denaturation of enzymes due to free radicals.

In summary, both merits and demerits exist in all of these immobilisation methods. While covalent binding suffers from changes of enzyme structure, non-covalent one is relatively weak, resulting in unstable binding. So users should figure out the balance between the above points and select the method...
which is suitable for specific purpose. Furthermore an extremely important task is to explore an efficient and stable matrix used as enzyme carrier. Graphene materials, especially GO and RGO, can be ideal substrates for enzyme immobilisation due to their unique surface chemistry. Firstly graphene has a large specific surface area which allows high concentrated protein loading. Actually, this is always the main factor to consider when choosing a substrate. Secondly graphene materials even GO have 2D planar structure and large area of hydrophobic regions, which is very suitable for non-covalent immobilisation, such as π-π stacking interactions. Furthermore, the oxygen functional groups on GO surface, which make it possible to immobilise enzymes without further surface modification or any coupling reagents, serve for tight binding to enzyme molecules via electrostatic interactions, hydrogen bond and strong covalent binding. The atomically flat surface enabled researchers to observe the immobilised enzyme in the native state directly using atomic force microscopy. For example, the immobilization of HRP and lysozyme, as model enzymes, on monolayer GO sheets has been reported. The rich surface functional groups of GO helped enzymes immobilization processing quickly through electrostatic interaction without using any cross-linking reagents; the flat surface of GO contributed to the observation of native immobilised enzymes in situ using AFM. They found that the catalytic performance of the immobilised enzymes is determined by the full retention of their pristine conformation. In a recent published work from our group, Dr. Mathesh demonstrated that the open and active form of lipase can be achieved and tuned with an optimized activity through CRGO (Figure 1.12). Surface tuning on GO nanosheets resulted in optimised lipase activity while converting hydrophilicity to hydrophobicity of GO. This research is a major step toward designing nanomaterials as a platform for enhancing enzyme immobilization/activity.
1.5 Issues and problems in current studies on surface chemistry of GO

Despite a long history since the 1840s, GO’s precise chemical and geometric structure is still not well defined. Due to the partial amorphous characters of GO, none of the current synthesis methods can provide an acknowledged idea that GO can be achieved with settled characters such as size, C/O ratio and electrical conductivity. Apart from that, the lack of precise analytical techniques to probe GO’s
surface is also a key issue to be resolved.\textsuperscript{241} The progress in this point is impending but having numerous difficulties.

Scientists hold different viewpoints on the structure of GO but many suggest the sheets have discrete repeat units. Figure 1.13 presents several of well-known structural models of GO. While in 1939 Hofmann’s model only consisted of epoxy groups at edges or on basal planes,\textsuperscript{242} Ruess’s structure introduced hydroxyl groups into the sheets inspired by the detected hydrogen content.\textsuperscript{243} Another variation Ruess model was the transition from totally sp\textsuperscript{2} hybridized model to a sp\textsuperscript{3} hybridization–containing system. Scholz and Boehm designed GO model as linked quinoidal units forming a waved structure which was free of epoxide and ether groups.\textsuperscript{244} The Nakajima–Matsuo model built a lattice framework analogous to the structure of poly(dicarbon monofluoride), which formed a stage 2 graphite intercalation compound.\textsuperscript{245} Later this contributed to understanding the surface chemistry of GO by proposing a stepwise oxidation for preparation.\textsuperscript{246} Lerf and Klinowski were the first to use nuclear magnetic resonance (NMR) spectroscopy to characterise GO and built their models based on the reactivity.\textsuperscript{247} They proposed the presence of carboxylic acid on the periphery of the GO platelets in one of their models.\textsuperscript{248} The Dékány model is a mixture of the Ruess and Scholz–Boehm skeleton, including a random distribution of two types of regions: the \textit{trans}–linked cyclohexane chairs and the corrugated hexagon ribbons.\textsuperscript{249} They thought that a slight tilting angle between boundaries of these domains may be the main reason of the wrinkling of the layers seen in transmission electron microscopy (TEM) images. In fact, the general idea on GO is that the oxidized rings contain C–O–C (epoxide) and C–OH groups, while the sheets terminate with C–OH and COOH groups. The exact structure of GO is very difficult to precisely define.
1.5.1 Issues in synthesis approaches

Due to the non–stoichiometric nature and complex changes in the structure of GO, the determination of its complete structure is always perplexing people. At present the specific oxidation of graphite flakes is almost to employ the modified Hummers methods for single sheet preparation.\textsuperscript{34} Although operators made some changes with the initial method, people are following the similar steps.\textsuperscript{41,101} Under different conditions, there are a lot of variations with the properties of resulting sheets, such as the dimension, shape and defects. Actually, even the protocol is totally the same, the attributes of GO may have big distinctions between each experiment. There are several reasons to explain this. Firstly, the starting material – graphite source is usually of indeterminable structure. The initial lateral dimension of
graphite is not the same with each other. It is clear that tightly stacked layers suffer from stronger obstacles with oxidation and more importantly, the following step – exfoliation, with a result of low−efficient functionalization. It is impossible to get the same goal with beginnings of big difference. For example, in order to achieve nGO sheets, graphite nanofibers with a huge thickness/diameter ratio are selected as starting materials and treated with a regular Hummers’ method. The ultrasmall size of this material facilitates its applications in biosensing due to the losing of signal quenching, and in drug delivery because of the ability to travel across cell membranes. Figure 1.14A shows the application of nGO as a good surfactant for carbon nanotubes. The nanoscale confirms the high charge to surface ratio and the π−π stacking interactions between aromatic rings in carbon materials contributed to the surfactant function of nGO. Secondly, the reagents used in each work varied, which resulted in different oxidation results. While the present solution processes usually follow Hummers’ system – a mixture of H$_2$SO$_4$, NaNO$_3$ and KMnO$_4$, some others also nitric acid (HNO$_3$) and potassium chlorate (KClO$_3$) compounds, which shows comparable reactivity. In one of modified Hummers’ methods, NaNO$_3$ is replaced by phosphorus pentoxide (P$_2$O$_5$) to help drying the pre oxidized sheets. Due to the use of concentrated H$_2$SO$_4$, the presence of interlayer water lowers oxidation efficiency. Marcano et al. described a greater amount of hydrophilic oxidized graphene than Hummers’ method by making a little changes with KMnO$_4$ dosages and solvent environment (Figure 1.14B). They declared there were no harmful gases (NOx) generated and the reducing products showed the same performances with normal methods. Gao and co−workers reported a fast operation using a strong oxidant – K$_2$FeO$_4$ to get GO in only 1 h (Figure 1.14C). This approach avoided the introduction of polluting heavy metals and toxic gases into the products, and also enabled the recycling of H$_2$SO$_4$, going green and high efficiency. To think differently, Shen et al. proposed that ordinary graphite was treated directly with benzoyl peroxide (BPO) in solid state, which readily generated stable GO without help of any solvents. The oxidization process was attributed to oxygen decoration and the expansion of CO$_2$ delivered into the interlayers between the GO nanosheets during the reaction is the main force for exfoliation (Figure 1.14D).
There is a crucial nature of GO that we cannot ignore – the defects. Besides the well-known ripples, subsistent graphene has several other types of ‘defect’, including topological defects (e.g., pentagons, heptagons, or their combination), vacancies, adatoms, edges/cracks, adsorbed impurities and so on.\textsuperscript{71} Specifically on GO, we would like to focus on defects as (1) the vacancies (e.g., holes and irregular edges), and (2) those sites where carbon atoms are replaced or functionalised by oxygen or nitrogen groups. Defects are introduced as soon as the generation of hydrophilic sites and would be expanded under overoxidation. Moreover, overmuch mechanical exfoliation may even tear GO sheets \textit{via} the
broken holes or edges, which is the main reason for the decrease of lateral dimension under long–time sonication. High density of defects is treated as negative impact considering the loss of electrical conductivity. Thus, to control and recover defects on GO is a worthy task in certain areas. Higginbotham et al.\textsuperscript{256} developed a method to prepare low–defect GO nanoribbons \textit{via} longitudinal unzipping of multiwalled carbon nanotubes. They investigated the influences of reaction conditions, such as acid content, time and temperature to optimise the experiments, and found the use of a weaker acid, such as trifluoroacetic acid (TFA) or H\textsubscript{3}PO\textsubscript{4} could prevent overoxidation and the subsequent hole formation. Similarly, mild oxidation was utilised to process graphite and the resulting GO exhibited low density of defects which was confirmed by the comparison with highly oriented pyrolytic graphite (HOPG).\textsuperscript{257} On the other hand, researchers found defects could be beneficial when graphene served as a catalyst support or the catalyst itself.\textsuperscript{258} For the case of catalyst immobilization, the oxygen functional groups and topological defects can act as favourable anchoring or nucleation points for the active substances.\textsuperscript{259} The bonding energy of oxygen–doped surface of GO toward metal atoms is much higher than that of non–defective graphene, increasing the stability of supported nanoparticles.\textsuperscript{260} More than being an excellent substrate, GO can be used as a metal–free catalyst which is attributed to the presence of edges/defects or doping atoms. Although the nature of the catalytically active sites in specific reactions remains under debate, scientists believed the defects contribute to the intrinsic catalytic activity of GO. For example, Bao and co–workers demonstrated that nitrobenzene can be reduced at room temperature using rGO as an efficient catalyst.\textsuperscript{261} They suggested that the unsaturated carbon atoms at edges/defects may be the catalytically active centers for the reduction reaction, which was supported by another work.\textsuperscript{262} The multipoint oxidative coupling of amines is affected by the carboxylic acid groups and unpaired electrons at edge/defect sites.\textsuperscript{263} The regeneration of COOH under a neutralization treatment brought a remarkable recovery in the catalytic activity, whereas quenching of the localized spins generated at the edges or defects resulted in a drop in the catalytic activity for several times. Thus both of COOH and defects accounted for GO’s catalytic reactivity. In Su’s study, porous GO served as not only metal catalyst support but also the catalyst itself for tandem catalysis of amines.\textsuperscript{264} Apart from that, there are some other influences of defects to GO’s surface chemistry, such as decrease of surface area and improvement in edge effect.
In brief, the differences between each preparation methods result in the ununiformity of GO surface chemistry. The starting material plays a key role in size control, and the oxidative conditions decide chemical compositions and sheet qualities. Generally speaking, it’s difficult to design a universal approach for the preparation of GO with the same properties, however, a facile method to probe GO surface chemistry and well control of the surface chemistry are meaningful and urgent to be developed.

1.5.2 Limited understanding of the reduction mechanism

The reduction reaction is mostly applied for the surface tuning of GO. The aims of reduction include the removal of oxygen and healing of structural defects. The resultant is given a name of RGO which is considered as an analogue of unmodified graphene, but actually there are some differences between them, such as surface complexity and structural defects. Various strategies have been developed for the reduction of GO, but there are still challenging questions need to be answered. For example, can the oxygen groups be totally removed during reduction? Can the sp³ hybridized carbon be converted to sp² hybridized carbon? Which type of oxygen functional groups is removed first? To answer these questions, clear understanding of the reduction mechanism is required but currently this is very limited. Here I will review the proposed mechanisms of GO reduction and give my opinions on them.

1.5.2.1 Removal of oxygen functional groups by chemical reduction

The binding energies of edge groups to the carbon plane are much stronger than those of basal oxygen, making the former is more difficult to be eliminated. Thus the edge groups are usually reserved after reduction, which has little influence on conductivity but can stabilize GO in the dispersion. The first reduction mechanism using hydrazine was presented by Stankovich and co–workers (Figure 1.15A). They gave an explanation of how to remove epoxide and recover sp² C=C with hydrazine catalysis. Starting with epoxy groups reacts with hydrazine to generate hydrazino alcohols, the intermediate product then experiences the dehydroxylation by heat treatment, forming an aminoaziridine moiety which further undergoes thermal elimination of di–imide, which restores the conjugated graphene network. This theory were then supported by density functional theory (DFT) simulation, whose results showed that hydrazine can only drive the reduction of epoxy groups. They found that the hydroxyl groups attached within the aromatic region can be easily eliminated or migrate to edges,
restoring conjugated structure on the original sites. Figure 1.15B shows two proposed routes in hydrazine de-epoxidations by Gao and co-workers. Actually they are all following very similar pathways with Stankovich’s theory, showing the attack of N₂H₂ to epoxy groups and its release. However, the negative charge, which considerably decreases during chemical reduction, is mostly attributed to COOH groups at GO’s edges. Therefore, a mechanism of COOH elimination should also apply to this reduction and is currently missing.

Figure 1.15. (A) The reaction pathway for epoxide reduction with hydrazine. Adapted with permission (Stankovich 2007). Copyright © 2007, Elsevier. (B) Local atomic structures for stationary points involved in hydrazine de–epoxidations. Adapted with permission (Gao 2010). Copyright © 2010, ACS Publications.
In recent years, more than 50 types of reducing agent have been exploited to deal with GO. In Chua and Pumera’s review paper, these reducing agents were classified into two categories: (I) normal agents with well-known mechanism or at least widely applied in organic chemistry, and (II) unconventional ones with unproved/unknown reducing capacities for carbon–oxygen functionalities in accordance with organic chemistry. Some of the mechanisms have been accepted by scientists, but some are still people’s deduction due to the usage of those reductants on other materials. More efforts are imperious in this area because our target is always the mass production of high-quality graphene materials, which primarily relies on the deep understanding of the synthesis mechanism.

1.5.2.2 Defect repairing

Formation of CO$_2$ and defective regions cannot be avoided during oxidation of graphite. About one CO$_2$ molecule per 35–55 lattice carbon atoms is generated during the oxidation and the resulting GO usually contains one carbonyl groups per 10–12 carbon atoms. The presence of defects introduces a lot of differences between RGO and pristine graphene (e.g. electrical conductivity), thus healing of defects is of great value to obtain high-quality RGO.

Due to the losing of aromatic rings in the sheet structure, the healing of defects needs carbon sources. CVD method used in synthesize carbon nanotubes is a good option. Using ethylene as carbon source, Lopez et al. suggested the reconstruction of graphene’s aromatic rings on a silica substrate. But there is no evidence to support this mechanism. Rozada and co-workers reported a two-step graphitization of RGO to achieve full repair of defects, however, the annealing temperature is too high (1,800 to 2,700 °C) to be applied in normal laboratories. As well as the oxidizing steps, the deoxygenation inevitably leaves behind vacancies and topological defects on the RGO. That is why defect density is always found rising after reduction. Figure 1.16A showed a novel strategy to repair newborn defects with carbon radicals produced by the thermal decomposition of a suitable precursor. Just after oxygen functional groups were removed from GO surface, there remained the adjacent carbon atoms with dangling bonds. At this time, extraneous carbon radicals were added to repair the faulty sites. An enhancement of conductivity was considered to be attributed to the formation of additional sp$^2$ C structures. Apart from that, molecular dynamics (MD) simulation was applied to investigate the
possibility and outcome of defect healing.\textsuperscript{275} Vacancies could be filled up by continuous exposure to CO and NO molecules (Figure 1.16B). A CO molecule could be grasped by a vacancy site and a NO molecule subsequently removes the extra O by forming NO$_2$ (upper part). In another route, N–doping might be achieved by sequential vacancy creation and subsequent exposure to NO molecules at room temperature (bottom part). However, there is no experiment support and it seems the method could only heal single–atom vacancies. There are only a few papers focusing on the mechanism of the defect repair. Both experimental and theoretical study are required to find out if the vacancies can be filled and the edges can be repaired to sp$^2$ hybridized carbon.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure16.png}
\caption{(A) Schematic of GO reduction with the real–time repair of newly generated defects. Adapted with permission (Dai 2011). Copyright © 2011, Springer. (B) Schematic view of the vacancy healing and N–doping process of graphene with vacancies (V) by CO and NO molecules. Adapted with permission (Wang 2011). Copyright © 2011, APS Physics.}
\end{figure}
1.5.3 Issues on characterization techniques for studying surface chemistry of GO

Graphene oxide materials have been investigated by a variety of characterization techniques ranging from composition (C/O ratio and functional groups), morphology (size, thickness, defects, and crystallization) to instinct properties (conductivity, strength and electron transfer speed). Techniques, such as Raman, XPS, FTIR and AFM, are all useful and well-studied tools for GO characterization. However, we would like to present our idea of the current issues that appears in my experiments.

1.5.3.1 Raman spectroscopy

Raman spectroscopy is a standard non-destructive technique to characterise quality of graphene-based nanomaterials, especially examining the ordered/disordered structure, crystal structures and layer numbers.\textsuperscript{276,277} In real Raman spectrum, the predominant features of graphene oxide are the D band around 1350 cm\(^{-1}\) that can be assigned to the degeneration of E\(_{2g}\) photons, and G band around 1580 cm\(^{-1}\) corresponding to K–point phonons of A\(_{1g}\) symmetry,\textsuperscript{277,278} while the intensity of D peak and G peak is constant with the introducing defects and structural defects, respectively. The 2D band, located at 2640–2680 cm\(^{-1}\), is the second order of D peak and decreases in intensity with introduced defects.\textsuperscript{279} Compared with the highly ordered graphite sample, the G band of GO shifts to higher frequency, and decreases back to origin location as thermal reduced to functionalised single graphene sheets (FGS).\textsuperscript{280} The possible explanations for this blue shift is the resonation action of isolated double bonds in GO at higher wavenumbers. More specifically, both D and G bands broaden significantly in GO due to the topological defects and vacancies.\textsuperscript{11} The ratio of D and G bands’ intensity (I\(_D\)/I\(_G\)) obtained from the Raman spectrum can provided a good sense of the extent of disorder and defects that occur on the surface of graphene oxide, which is important for further understanding their properties and applications. It is previously shown that the GO samples exhibited both broad G and D band due to the increasing disorder which intensity is inversely proportional to the crystallite size (L\(_a\)).\textsuperscript{281}

However, there are two issues I found in my experiment. Firstly, the I\(_D\)/I\(_G\) is not very sensitive to the surface tuning of GO. There are only a little changes before and after the chemical reduction of GO. This is not good enough when elaborated and controlled surface tuning is desired. Secondly, the laser used in Raman spectra usually induces the fluorescence of some luminophors. For example, if pyrene
molecules are immobilised on GO, the intensity of excited fluorescence could be so strong that the peaks of GO will be blocked, which makes the sensitivity much worse. Furthermore, the qualitative and quantitative determination of defects in GO and RGO is a significant issue to be addressed. Raman spectroscopy alone is not sufficient to prove the ideality of graphene since defects are known that do not activate the D band.\textsuperscript{282}

1.5.3.2 X−ray photoelectron spectroscopy (XPS)

XPS is a surface−sensitive and semi−quantitative analysis technique that detect the surface chemical properties of graphene−based materials. This approach is very sensitive to chemical composition, impurity presence and bonding condition especially carbon and oxygen atoms, the fascinating fingerprints of GO, which could be the direct evidence of sp\textsuperscript{2}/sp\textsuperscript{3} modulation around 0−10 nm depth usually.\textsuperscript{283,284} In the XPS wide scan survey of GO, the paramount peaks located at about 532 and 285 eV are corresponded to the O1s and C1s peaks, which are the main atoms of graphene oxide materials.\textsuperscript{285} Five distinct components centred at 284.5, 286.2, 286.8, 287.9, 288.9 eV could be assigned to sp\textsuperscript{2}−hybridized carbons, C−O in epoxy and hydroxyl, carbonyls, amides, and carboxylic acids.\textsuperscript{286} Based on the ratio of these two main peaks’ intensity, the ratio of carbon to oxygen elements (C/O ratio) and the extent of oxidation/reduction of GO could be well described.

However, the oxygen content detected in XPS is influenced by H\textsubscript{2}O molecules. Due to the highly hydrophilic surface, there are many H\textsubscript{2}O molecules absorbed on GO nanosheets via hydrogen bonds. This cannot be avoided and could leads to the estimation of too many hydroxyl groups. The same issue was found in the IR spectra which always showed a wide and strong peak around 3400 cm\textsuperscript{-1}.

1.5.3.3 Atomic Force Microscopy (AFM)

AFM can directly determine the layer thickness of GO. This technique is specifically suitable for the characterization of 2D nanomaterials which show large lateral size but ultrathin height. As observed from previous study, the thickness of single unreduced GO sheets is around 1 nm, which could be reduced to 0.6 nm after chemical reduction.\textsuperscript{287} Apart from imaging, thickness and layer number detection, AFM has been applied to measure the mechanical properties of monolayer graphene including the Yong’s modulus of few−layer graphene on a strip of graphene suspended over trenches.\textsuperscript{288}
However, the repulsive force between AFM tips and the functional species on GO surface induces deformation and results in unrealistic height measurement. Electron microscopes are also used to characterise GO but each suffers from limitations. SEM is of low resolution and only applies to the surface morphology of the materials. The edge of GO, performing great significance due to its unique properties, exhibits a dark line in each layer in TEM images, then could be used to count the number of layers and confirm the types of achiral edges of graphene sheets.\(^{289, 290}\) However, under TEM, the functional molecules that can be observed on GO by AFM are not visible due to the high transparency of the nanosheets.

In addition to above mentioned techniques, X–ray absorption spectroscopy, fluorescence quenching microscopy, thermal gravity analysis (TGA), UV–vis spectroscopy, electron energy loss spectroscopy (EELS), conductivity analyser, electrochemical method and other techniques have also been utilised to study GO materials as well as its reduction process.\(^{241, 291-293}\) The key issue is to qualitatively and quantitatively probe the morphology and surface properties. A facile and universal method is required to resolve the current problems.

### 1.6 Surface chemistry of boron nitride nanosheets

Boron nitride (BN) comprises of boron and nitrogen atoms (1:1) is a heat and chemically resistant refractory compound. It exists in various crystalline forms that are isoelectronic to a similarly structured carbon lattice. The hexagonal form corresponding to graphite is the most stable and soft among BN polymorphs, and is therefore used as a lubricant and an additive to cosmetic products. Because of excellent thermal and chemical stability, boron nitride ceramics are traditionally used as parts of high-temperature equipment. Being able to fabricate into nano size, it has potential use in nanotechnology. Nanotubes of BN can be produced that have a structure similar to that of carbon nanotubes, and bulk BN can be exfoliated into 2D planar structure which is similar to graphene nanosheets.

BN nanosheet (BNNS) is a classical member of the graphene-analogous 2D nanomaterials. But it didn’t catch much attentions before the famous graphene was published in 2004. Right in the next year, BNNSs were exfoliated from hexagonal boron nitride (h-BN) by the same group via mechanical exfoliation by simply using a piece of adhesive tape, which is the Scotch tape method.\(^{294}\) h-BN has a
typical layered structure like graphite. BNNS is called “white graphene” because of its nearly identical geometrical structure to graphene. Within a 2D layer, alternating B and N atoms are linked with each other via strong B–N covalent bonds; whereas the 2D layers are held together by weak van der Waals forces. Unlike the case of graphite, the interlayer stacking pattern in the h-BN features its B atoms in every consecutive BN layer siting exactly above or below the N atoms in the adjacent layers. Such structural characteristics imply the polarity of B–N bonds the partially ionic character of the covalent B–N bonds. Electron pairs in sp²-hybridized B–N s bonds are more confined to the N atoms due to their higher electronegativity; and the lone pair of electrons in the N pₓ orbital is only partially delocalized with the B pₓ orbital, in contrast to the equally contributed and evenly distributed electrons along the C–C bonds of graphite layers. BNNSs therefore perform differently with graphene. They have superb mechanical strength and high thermal conductivity, but their bandgap (~4–6 eV) are much wider in comparison to conductive graphene, making BNNSs the insulators. People works in conductive devices might look down upon them, but again, electrical conductivity is not everything to 2D nanomaterials. These attributes make BNNSs attractive for use as thermally conductive but electrically insulating fillers for polymer or ceramic composites, deep ultraviolet light sources, dielectric layers, cosmetic products, microwave-transparent shields, etc.²⁹⁵

1.6.1 Synthetic methods

BNNSs were prepared via both top-down²⁹⁶ or bottom-up²⁹⁷ approaches, which typically refer to the exfoliation of h-BN and the synthesis from B and N precursors, respectively. In fact, these methods all have the same principle with graphene synthesis.

The available top-down methods include mechanical cleavage, surfactant-assisted exfoliation, direct solvent exfoliation, and chemical functionalisation–induced exfoliation. The exfoliated BNNSs can maintain high crystallinity from their h-BN precursors. However, the lateral sizes for the commercially available h-BN particles were typically on the order of only 10 μm or less. This has resulted in limited lateral sizes of exfoliated BNNSs, which were below 1 μm.²⁹⁸,²⁹⁹ On the other hand, top-down methods have been reported on the unzipping of boron nitride nanotubes (BNNTs) into boron nitride nanoribbons (BNNRs).³⁰⁰,³⁰¹ BNNR is a specific form of BNNSs that have definitive widths and thus edge structure
(zigzag or armchair). Prof. Chen’s group prepared BNNSs by solid-state ball milling of commercially available \( h \)-BN and urea powder.\(^{302} \) Excitingly, the products can be colloidal aqueous solution, and fabricated into aerogels and freestanding membranes. The presence of terminal amino groups play a vital role to have it disperse in water. In another work, a facile method was developed to obtain few- or monolayer BNNSs in water by simple ultrasonication.\(^{303} \) BN nanoparticles were exfoliated because its edge B atoms suffered from hydrolysis under water sonication, which introduce OH groups onto the sheet, improving the hydrophilicity (Figure 1.17).

![Figure 1.17](image)

**Figure 1.17.** Exfoliation and cutting of \( h \)-BN in water via sonication-assisted hydrolysis. Adapted with permission (Lin 2011). Copyright © 2011, ACS Publications.

Meanwhile, BNNSs were also synthesised from B and N precursors via bottom-up approaches such as chemical vapor deposition and thermal decomposition. Similar with graphene, the lateral sizes of the synthesized BNNSs could reach several centimeters.\(^{297,304} \) While exfoliated BNNSs are useful for applications that require large quantities of materials such as polymeric nanocomposites, the bottom-up synthesised nanosheets as well as those from the Scotch tape mechanical exfoliation, typically supported on a metal or crystalline substrate, are more suitable for electronic or sensor applications with less sample requirement. With CVD method, a patched or stacked graphene/BN hybrid structure was synthesised.\(^{305} \) Both in-plane junctions and stacked layers were obtained, which was the first report to
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prepare well-ordered graphene/h-BN structure, providing another direction for future 2D materials. There are other approaches developed to synthesise BNNS, most of which are with similar mechanism with graphene methodologies.

BN polymorphs including BNNSs and BNNTs are excellent oxidation-resistant materials. They are stable up to ~850°C in air and inert against oxidative acid treatments. Note that the chemical inertness for BNNSs is relative. As given in the following detailed discussions, BNNSs do have rich noncovalent and ionic chemistry that can render them soluble in various solvents and processable for applications such as nanofillers in polymeric nanocomposites and supports for catalysts or sensors. However, it is relatively difficult to covalently modify the B–N bond network by oxidation reaction. Highly reactive species are required to achieve successful functionalisation.

1.6.2 Surface functionalisation of BNNSs

Although BNNS was reported to be highly chemically inert, many efforts have been made on it and chemical functionalisation was indeed introduced onto BNNSs. Unlike graphene which is composed with carbon, BN functionalisation cannot refer to those classical organic chemistry.

Generally speaking, BN functionalisation on their basal plane has to open its polarized conjugated p bonds, with generated new bonds present at an even number. When a functional group forms a covalent bond with boron or nitrogen atom, a compensating group should also be attached to the unpaired N or B atom to balance the overall charge. On the other hand, some functional groups can form bridging chemical bonds between B and N, similar to C=C bond epoxidation. The simplest functionalised BN structure model usually has two groups added to a neighbouring B–N unit. However, both experimental and theoretical studies on the chemical modification of a conjugated carbon system have revealed that, when the charge and aromaticity are satisfied, the bonded functional groups are not limited to the neighbouring positions (they can be spatially separated). Such phenomena may also be applicable to the BNNSs because of the conjugated structure. Due to the different electronegativity, B and N atoms are partially positively (electron deficient centers) and negatively (electron rich centers) charged, respectively. Thus, the B sites are able to be attacked by nucleophilic groups, while the N sites are
reactive with electrophilic ones. Figure 1.18 summarised the different functional groups that have already been attached to the basal plane or edges of $h$-BN.

For instance, Ian’s group introduced NH$_2$ groups onto BNNS to fabricate the ultralight aerogels.\textsuperscript{302} Using ball milling bulk BN and urea, amino groups appeared at the edges and defects of BNNSs which were exfoliated by shear forces. In another work also using ball milling method for BN exfoliation, OH groups were added to BNNS edges assisted by NaOH.\textsuperscript{309} These hydrophilic groups helped to exfoliate BN into single or few layers as well as gave the resulting BNNSs water solubility which is of great significance. Li et al. developed a novel and effective method to use NaOH and KOH molten salts to
exfoliate h-BN into nanosheets, nanoscrolls were obtained as well. The as-prepared products can be readily dispersed in a wide range of solvents, including water and ethanol, and form stable dispersions. Apart from these, alkyl and some other groups (–OCOR, –NHCOR, –COR, etc.) were also successfully functionalised on BNNSs, which were all summarised in a review article.³⁰⁸

Not only covalent bonds, but also non-covalent interactions can be applied to functionalise BNNSs. There is no doubt that covalent functionalisation effectively modifies BN’s properties, whereas functionalisation via weaker interactions is preferred when it is essential to preserve the intrinsic nanotube properties. The case of BNNT functionalisation is considered as a proper model to be followed in BNNS tasks just like people did on graphene functionalisation following CNTs’ experiences. π-π stacking is one representative mode of non-covalent interactions happened between two conjugated structures, such as 2D nanomaterials and aromatic molecules. This is well known in graphene and CNT functionalisation.¹⁰¹ Considering the structural similarity between graphene and BNNSs, we can reasonably speculate the application of π-π stacking to decorate BNNS surface.³¹⁰ For example, a ‘Janus’ block polymer was fixed onto BNNS surface via π interactions to tune its solubility in organic solvents.³¹¹ This provided more opportunities for BNNS functionalisation with various molecules dissolved in solvents with different polarities. However, the present discussions are theoretical calculations and speculation based on its similar but not the same structure with graphene. There are no direct evidences for the true presence of this π interaction that should be further explored experimentally. By probing this kind of interaction using an ECL molecule, we aim to characterise π-π stacking.

1.6.3 Radicals on BNNSs

The radicals, which provide active sites for various chemical reactions, are the molecules or molecular fragments with single unpaired electrons in their outer shells.³¹² They are generated by homolysis of the chemical bonds, which dissociate a molecule by a process where each of the fragments retains one of the originally bonded electrons. During homolytic fission of a neutral molecule with an even number of electrons, two free radicals will be generated. The number of electrons in the outermost shell of one atom greatly influences its chemical activity. If the outermost shell is full without unpaired electrons,
this atom is stable thus chemically inert. On the other hand, when the outermost shell lose one of the
paired electrons, the atom will be quite active. Thus, it tries to stabilise itself through gaining or losing
an electron to fill or empty the shell. Compared with pristine graphene (pure carbon), boron nitride
bonds are easier to get broken because of the difference in electronegativity of B and N atoms. However,
the energy required to break bonds in this manner is still high, homolysis only occurs under certain
circumstances, like ultraviolet radiation or heat. For instance, radiation can break weak bonds between
electrons to produce unpaired electrons. And the free radicals have the ability to break up the normal
pairing of electrons in neighbouring molecules to obtain a new electron for themselves. This leads to
the creation of more radicals and extensive molecule damage (Figure 1.19).313,314

\[\text{Figure 1.19. The generation of a free radical in oxygen molecule. Adapted with permission (Benjamin}}\]
\[\text{2012). Copyright © 2012, Springer.}\]

It was reported that graphene/graphite is a carrier of radicals as a results of a relatively high edges and
some defects in its structure.315,316 The stability of these radicals benefit from the rigid $\pi$-conjugated

planar structure of graphene which acts as a physical barrier for the radicals and prevents them to react with each other.\textsuperscript{317-319} Because defects affect the magnetic, electrical, chemical, and mechanical properties of the graphene sheets, controlling the formation of defects in graphene nanomaterials offers a means for engineering their properties. This stability is attributed to the conjugation of the residual C-O-C, C-OH, COOH and C=O groups to the large graphene plane.\textsuperscript{320} BNNSs own the similar 2D nanostructure with GO thus some radicals could be also found at edges or defects. Investigation of the radicals on BN nanomaterials, especially unmodified BN is a promising project due to their potential applications in catalyst and co-reaction materials. However, there is only a few work focusing on the radical detection of boron nitride. The oxygen radical functionalisation of BNNSs has been reported theoretically\textsuperscript{321} and experimentally\textsuperscript{322}. Using density functional theory (DFT) calculations, the results indicated that OH groups gained a stronger binding energy with B atoms, and BNNSs were most stable when OH radical coverage reached 60%. Furthermore, the broad band gap of BN decreased with OH functionalisation, which results in the possibility to fabricate it into electrical devices. On the other hand, the influence of oxygen groups to BNNSs’ mechanical properties was investigated experimentally, however, the strength of radicals on the nanosheets and the mechanism was not investigated.

While making surface modification and exfoliation of BN, the chemical bonds (B-N) could be cleaved, with which process there might be some radicals generated at the edges of the resulting BNNS. These could be boron radical, nitrogen radical or oxygen radical if introducing OH groups on it. These radicals will turn inert BNNSs to be active at edges, for example, to initiate polymerisation reactions or to catalyse some conventional reactions.\textsuperscript{323} Apart from that, reactive oxygen species result in food spoilage, oil rancidification, polymer degradation, and destroy biological structures including cell membranes, protein structures, and DNA.\textsuperscript{324} Hence, oxidation prevention which could be initiated by free radicals is significant in nutrition, food and pharmaceutical formulations, biomedical implants, topical protection, cosmetics, metal corrosion, wound healing therapies, and the long-term stabilisation of chemical products. To be a 2D nanomaterials, BNNSs have great potentials for biological applications like drug delivery.\textsuperscript{325} But first of all, its cytotoxicity brought by the possibly introduced radicals should be studied carefully because the active radicals would disrupt the metabolism \textit{in vivo}. 
There are two difficulties needed to be overcome in the radical detection. Firstly, the pristine radicals on BN samples could be too weak to be measured. Surface functionalisation and exfoliation are required to introduce functional groups and defects. Secondly, the radicals are localised onto BN surface and cannot be trapped by detection molecules. Therefore, we planned to use a sensitive radical scavenging approach for the electronic spin resonance (ESR) spectrum measurement, to indirectly detect the radicals on BNNSs.

ESR is based on absorption of microwave radiation stimulated by an electromagnetic field in molecules such as free radicals and transition metal ions with unpaired electrons. ESR detects the absorption of microwave energy, which occurs on transition of unpaired electrons in an applied magnetic field. The amplitude of the ESR signal is proportional to the number of the unpaired electrons present in the sample, allowing quantification of the radicals. One option for ESR measurement is to use the radical scavenging assay. In this method, a stable radical – DPPH was used to measure the intensity of the radicals in BN dispersions. Unlike the radicals fixed at BN edges, DPPH radicals can spread through the whole solution, and react with antioxidant and any radicals in the solution. The reactions are shown below.

\[ \text{DPPH}^- + \text{AH} \rightarrow \text{DPPH-H} + \text{A}^- \]

\[ \text{DPPH}^- + \text{R}^- \rightarrow \text{DPPH-R} \]

By this method, the strength of the radicals from BN can be assessed by the decrease of DPPH peaks. The weaker the DPPH signal is, the stronger the radicals in this solution. The presence of radicals on BN nanomaterials would endow them many applications, such as enzyme-like catalytic reaction and co-reaction in ECL. For example, BN nanoplatelets was reported to promote the aerobic autoxidation of thiols to disulfides. The addition of the radical scavenger resulted in a drop of the catalytic ability, which indicated a radical intermediated process. A probable mechanism is that the radicals on BN nanoplatelets initiate the formation of thiol radicals. As a catalyst for dehydrogenation reaction, BN was developed to selectively convert propane to propene. This was attributed to the adsorption of oxygen molecules to BN edges, where nitrogen radicals were generated to dehydrogenate propane. It is
surprising that BN, which is well known for high chemical stability under oxidative conditions, is catalytically active. To use BN as a catalyst could be a game-changing technology in catalytic chemistry. In addition to the above, the large surface area and monolayer structure of BNNS would further improve the performances.

1.7 Conclusions and project aims

From the literature review, I conclude that 2D nanomaterials, such as graphene and BNNSs hold great potential for profound applications in various areas. All come from the excellent properties they gain from the unique structure – to be one-atom thick and π electron conjugated around the sheet structure. The water-soluble GO is of tremendous interest because of its tunable surface chemistry. Although the advanced properties have enabled GO as an emerging star in both scientific and commercial applications, there are much problems on surface chemistry of GO (or BNNSs) should be answered. In this thesis, there are 3 research questions that I want to answer.

1. How to qualitatively and quantitatively determinate the level of oxygenated species on GO?
2. What are the non-covalent interactions between GO and [Ru(bpy)_3]^{2+} (or DOX)?
3. Can the chemical reduction switch off the surface interactions on GO? And what is the application of surface tuning?
4. Are there active sites on the surface of BNNSs and what are they?

With the following chapters including experimental work and data discussions, I will demonstrate my work on the surface chemistry of 2D nanomaterials (GO and BNNSs). The project aims of each chapter are listed as follows.

- Chapter 3: Probing the tunable surface chemistry of graphene oxide. This work aimed to obtain better understanding of GO surface using an indirect method. A ruthenium complex was used as a probe molecule to functionalise GO surface. By well studying the interactions and morphologies before and after the reduction of GO, we monitored the gradual surface tuning from GO to CRGO. We wish to present a universal method that can be used to probe the surface properties of the other 2D nanomaterials.
• Chapter 4: **Simultaneously “pushing” and “pulling” graphene oxide into low-polar solvents through a designed interface.** We aimed to develop a facile and inexpensive method for GO phase transfer by surface tuning, based on the understanding of surface groups and charges of GO. Two types of designed interface were investigated to transfer GO from water to low-polar organic solvents. Quantified data have been used to optimise the experimental conditions to achieve the dispersion of GO in hexane. Surface chemistry of the designed interface was investigated in detail. On the other hand, a simultaneous process by “pulling and pushing” GOs into hexane was developed to achieve CRGOs with controlled hydrophobicity in hexane. The transferred GO and CRGO were attempted in the applications of fabricating nanocomposites and dye adsorption.

• Chapter 5: **A surface chemistry-driven on/off switch strategy for a controlled doxorubicin delivery using graphene oxide.** In this work, we aimed to find out GO’s application in anti-cancer drug delivery. First of all, the interactions between GO and DOX were well studied to confirm the good loading ability of the drug on the nanocarriers. Then a controlled delivery method was carried out using chemical reduction of GO. Based on the well understanding of GO surface chemistry, we want to present a universal method for the release of drug by switching off the non-covalent interactions at the carriers’ surface.

• Chapter 6. **Radicals on boron nitride nanosheets.** We aimed to investigate the potential radicals on BNNSs, which could come from the break of the chemical bonds during synthesis and modifications. Firstly functional groups were introduced onto BN and the exfoliation of the nanosheets were achieved by sonication. BNQDs were also achieved by solvothermal reaction. The radicals on BN were detected using a free radical scavenging method with ESR. Boron radicals was theoretically and experimentally confirmed to appear on the edges or defects of BNNSs and BNQDs. We also found the decrease of the sheet dimension led to an increase of radical density.
Chapter 2: Methodology
2.1 Introduction

The methods applied in my research project are to use experimental methods to investigate the surface chemistry of 2D nanomaterials in detail. These include the synthesis of various graphene oxide derivatives and boron nitride nanosheets, the surface functionalization of the prepared 2D nanomaterials, characterisations of their properties using different instruments, and the analysis and discussion of the results. In this chapter, I will give out the details about all of the experiments and also brief introduction of the instruments used for characterisations.

2.2 Chemicals

Graphite nanofibers (99%) were purchased from Catalytic Materials. Hydrochloric acid (HCl) was from Chem Supply (32% w/w). Concentrated sulphuric acid (H₂SO₄, 98%) were obtained from Merck Australia. Graphite flakes (99%), L-AA (≥99%), sodium hydroxide (NaOH, ≥98%), H₂O₂ (Sigma-Aldrich, 30%), hexane (98%), oleylamine (≥98%) and other chemicals were ordered from Sigma-Aldrich. Milli-Q water was used in all of the experiments.

2.3 Synthesis of graphene oxide and chemically reduced graphene oxide

Graphene oxide dispersed in aqueous solution was synthesized using a modified Hummers’ Method. In a typical experiment, 2 g of graphite flakes was mixed with 12 mL of concentrated H₂SO₄ and kept stirring at 80°C for 5 h on a heating plate. Then the solution was cooled at room temperature and ultrasonicated using a water bath sonicator (VWR industries, GRANXUBA3) for 5 h to break the larger flakes into smaller flakes. The mixture was diluted with 500 mL of Milli-Q water and left overnight. The settled preoxidized graphite flakes were obtained by filtering the solution with porous filters (200 nm pore size). The residue was dried at 80°C in a drying oven to remove water quickly. To further transform the preoxidized graphite into graphite oxide, the resultant powder was put into 120 mL conc. H₂SO₄. Next, KMnO₄ (15 g) was added slowly (within 1 h) and the mixture was then stirred at room temperature for at least 2 h. The solution was diluted with 250 mL of H₂O very carefully and stirred for a further 2 h, and then 700 mL dH₂O was added. Within a short period of time, 20 mL of H₂O₂ was added to the mixture until the colour turned bright yellow. Ultrasonication was conducted for 4 h in order to exfoliate graphene oxide nanosheets from the oxidized product. The resultant dispersion was
divided into 15 mL batches and centrifuged at 10,000 rpm for 30 min (Eppendorf centrifuge 5810R). Pellets were re-dissolved in 1:10 HCl with vigorous shaking and centrifuged for 10 min to remove unwanted metal ions. This was repeated another two times. Then pellets were collected and dissolved in 10 mL H₂O and centrifuged for 10 min at 10,000 rpm to remove acid. Centrifugation was performed repeatedly with H₂O until the light yellow supernatant was obtained which were GO sheets. Nano-scale graphene oxide (nGO), which is composed of smaller platelets, was synthesized via a similar method using graphite nanofibers as the starting material. A more course-grained liquid crystal graphene oxide (LCGO) dispersion was also prepared following the well-established approach of Wallace and co-workers.

Chemically reduced graphene oxide (CRGO) with different surface hydrophobicity was prepared via a modified literature method. Briefly, 200 mg of L-AA was added to 20 mL of GO dispersion (1 mg/mL) and stirred vigorously for different time intervals namely 1, 6, 12, 24 and 48 h. Once the reduction was carried out for the desired period of time, the reaction was stopped by centrifuging (Eppendorf centrifuge 5810R) at 11,000 rpm for 15 min and washing it thrice with H₂O. The pH of the as prepared CRGO was adjusted to 9 with the aid of NaOH (1 mM). The reduction reaction and surface charge was monitored using UV-Vis and Raman spectroscopy.

2.4 Preparation of graphene/[Ru(bpy)₃]²⁺ nanocomposites in aqueous solution

In a typical experiment, GO or CRGO dispersions were mixed with 0.2 of [Ru(bpy)₃]²⁺ solution (1 mM) with vigorous stirring. The mixture was sonicated for 10 min before any characterisation in order to achieve colloidal dispersion. The pH was adjusted to a little alkaline (around 8) to prevent it from precipitation. This dispersion can be used for study of the spectroscopies which will be discussed in detail later.

Meanwhile the solution of graphene or composites can be used for the preparation of free standing films. 2 mL of GO or CRGOs (1 mg/mL) were vacuum filtered on an isoporous membrane (polycarbonate, hydrophilic, 0.22 μm, 25 mm, white, plain, Millipore Corporation, Australia) using a vacuum filtration unit (RZ6, Vacuubrand Inc., U.S.A.). Additional 4 mL H₂O was added to wash the film surface. Free standing films were able to be peeled off from the membrane after drying. For the
case of composite films, 2 mL of graphene/[Ru(bpy)$_3$]$_2^{2+}$ composite solution was filtrated to obtain a thin composite film which was also washed by water after drying.

2.5 Phase transfer of GO and the influence of surface chemistry

The experiment conditions were adjusted due to the different purposes. Here is an example how GO is transferred. Briefly, GO dispersion was diluted to 1 mg/mL with a pH 7. 1 mL of GO was mixed with 1 mL of hexane which contained 0.5% (v/v) oleylamine. Then 40 µL of ethanol was added into the mixture. With mild shake by hand for 10 s, GO was transferred from water into hexane (from bottom to top phase).

In this experiment, phase transfer of GO or CRGOs were carried out under different conditions to compare their transfer efficiency. To begin with, organic solvents with different polarities were utilised as receiving phase of graphene sheets. Taking hexane as a typical example, 5 mL of hexane (containing 5% (v/v) oleylamine) was added into 5 mL of GO (1 mg/mL, pH 7.0) dispersion to form layered two phases in a 20 mL vial. Then 200 µL of ethanol was added and the vial was shaken vigorously for 20 s. After a while, the solution formed two separated phase again with the organic phase turning brown. Furthermore, to use containers with different bottom diameters, the influence of interface sizes was studied.

For subsequent experiments, the mass ratios between GO and ethanol were maintained as described above. Regular GO, nGO and LCGO were treated with the same conditions to investigate the influence of GO to phase transfer behaviour. The transfer efficiencies of graphene with different sizes were calculated based on the absorbance at 230 nm in UV-vis spectra. The experiment details can be find in Table 2.1.
Table 2.1. Typical experimental details and results showing the GO transfer efficiencies with different lateral dimensions.

<table>
<thead>
<tr>
<th>Oleylamine (v/v)</th>
<th>0.1%</th>
<th>0.3%</th>
<th>0.5%</th>
<th>0.1%</th>
<th>0.3%</th>
<th>0.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>nGO[a]</td>
<td>1.542</td>
<td>1.332</td>
<td>1.262</td>
<td>8%</td>
<td>20%</td>
<td>24%</td>
</tr>
<tr>
<td>GO</td>
<td>2.168</td>
<td>1.326</td>
<td>1.227</td>
<td>10%</td>
<td>44%</td>
<td>48%</td>
</tr>
<tr>
<td>LCGO</td>
<td>1.820</td>
<td>0.746</td>
<td>0.618</td>
<td>18%</td>
<td>68%</td>
<td>74%</td>
</tr>
</tbody>
</table>

[a] Regular GO has the medium lateral dimension, nGO has the smallest size and LCGO has the largest size. The complete transfer of all the samples can be achieved by proper ethanol dosages.

The pH value of initial GO aqueous solution was adjusted to be 3.0, 5.0, 7.0, 9.0 and 10.0, respectively, to study the influence of pH. The experiment details are shown in Table 2.2.

Table 2.2. GO transfer efficiencies at different pH.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Absorbance at 230 nm</th>
<th>Concentrations of GO in hexane (mg/mL)</th>
<th>Transfer efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO in pH 3.0</td>
<td>0.0574</td>
<td>0.473</td>
<td>94.5</td>
</tr>
<tr>
<td>GO in pH 5.0</td>
<td>0.0866</td>
<td>0.458</td>
<td>91.6</td>
</tr>
<tr>
<td>GO in pH 7.0</td>
<td>0.4820</td>
<td>0.260</td>
<td>52.0</td>
</tr>
<tr>
<td>GO in pH 9.0</td>
<td>0.3825</td>
<td>0.320</td>
<td>64.0</td>
</tr>
<tr>
<td>GO in pH 10.0</td>
<td>0.3603</td>
<td>0.300</td>
<td>60.0</td>
</tr>
</tbody>
</table>

Experiment conditions: UV measurement in water, 0.3% oleylamine

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Absorbance at 230 nm</th>
<th>Concentrations of GO in hexane (mg/mL)</th>
<th>Transfer efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO in pH 3.0</td>
<td>0.0210</td>
<td>0.490</td>
<td>98.1</td>
</tr>
<tr>
<td>Sample name</td>
<td>Absorbance at 230 nm</td>
<td>Concentrations of GO in hexane (mg/mL)</td>
<td>Transfer efficiency</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------------</td>
<td>----------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>GO in pH 3.0</td>
<td>0.0192</td>
<td>0.492</td>
<td>98.3</td>
</tr>
<tr>
<td>GO in pH 5.0</td>
<td>0.0322</td>
<td>0.485</td>
<td>97.0</td>
</tr>
<tr>
<td>GO in pH 7.0</td>
<td>0.0644</td>
<td>0.469</td>
<td>93.8</td>
</tr>
<tr>
<td>GO in pH 9.0</td>
<td>0.0516</td>
<td>0.478</td>
<td>95.1</td>
</tr>
<tr>
<td>GO in pH 10.0</td>
<td>0.0410</td>
<td>0.481</td>
<td>96.1</td>
</tr>
</tbody>
</table>

* In all of the experiments, the initial concentration of GO was prepared as 0.5 mg/mL, the volume of GO water solution and hexane were all 0.5 mL, and the dosage of ethanol was 60 µL. All of the experiments were conducted for at least 3 times to get the average data and error bars.

CRGOs under different reduction time were used to study the effect of oxidised degrees. Three concentrations of oleylamine were used in specific experiments to observe the influence of other experiment conditions. Experiment details can be found in Table 2.3.

**Table 2.3.** GO transfer efficiencies with different CRGOs.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Absorbance at 230 nm</th>
<th>Concentrations in water (mg/mL)</th>
<th>Transfer efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>230 nm</td>
<td>water</td>
<td></td>
</tr>
</tbody>
</table>
### Probing the Surface Chemistry of Two-Dimensional Nanomaterials

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Absorbance at 231 nm</th>
<th>Concentrations in water (mg/mL)</th>
<th>Transfer efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO-0.1% Oleylamine</td>
<td>0.3987</td>
<td>0.198</td>
<td>60.4%</td>
</tr>
<tr>
<td>GO-0.3% Oleylamine</td>
<td>0.0121</td>
<td>0.050</td>
<td>90%</td>
</tr>
<tr>
<td>GO-0.5% Oleylamine</td>
<td>0.001</td>
<td>0</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Absorbance at 235 nm</th>
<th>Concentrations in water (mg/mL)</th>
<th>Transfer efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>6h-0.1% Oleylamine</td>
<td>0.4201</td>
<td>0.291</td>
<td>41.8%</td>
</tr>
<tr>
<td>6h-0.3% Oleylamine</td>
<td>0.2870</td>
<td>0.198</td>
<td>60.4%</td>
</tr>
<tr>
<td>6h-0.5% Oleylamine</td>
<td>0.002</td>
<td>0</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Absorbance at 249 nm</th>
<th>Concentrations in water (mg/mL)</th>
<th>Transfer efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h-0.1% Oleylamine</td>
<td>0.9420</td>
<td>NA*</td>
<td>NA</td>
</tr>
<tr>
<td>24h-0.3% Oleylamine</td>
<td>0.3784</td>
<td>0.261</td>
<td>47.8%</td>
</tr>
<tr>
<td>24h-0.5% Oleylamine</td>
<td>0.0227</td>
<td>0</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Absorbance at 257 nm</th>
<th>Concentrations in water (mg/mL)</th>
<th>Transfer efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>32h-0.1% Oleylamine</td>
<td>1.064</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>32h-0.3% Oleylamine</td>
<td>0.4303</td>
<td>0.322</td>
<td>35.6%</td>
</tr>
<tr>
<td>32h-0.5% Oleylamine</td>
<td>0.0570</td>
<td>0.060</td>
<td>87.8%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Absorbance at 257 nm</th>
<th>Concentrations in water (mg/mL)</th>
<th>Transfer efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>48h-0.1% Oleylamine</td>
<td>1.3893</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>48h-0.3% Oleylamine</td>
<td>0.5359</td>
<td>0.336</td>
<td>32.8%</td>
</tr>
<tr>
<td>48h-0.5% Oleylamine</td>
<td>0.1801</td>
<td>0.115</td>
<td>77.0%</td>
</tr>
</tbody>
</table>

* Not available to be measured due to the muddiness made by oleylamine;

The initial concentration of CRGOs was 0.5 mg/mL, the volume of CRGOs and hexane (containing oleylamine) were all 0.5 mL. All of the experiments were conducted for at least 3 times to get average data and error bars.
2.6 Phase transfer of GO via simultaneous surface tuning

In this method, 200 mg of L-AA was dissolved in 40 mL of aqueous GO (0.5 mg/mL) dispersion, under stirring. Immediately thereafter, 40 mL of hexane containing 0.1% (v/v) oleylamine was added to the solution to create a biphase dispersion that was stirred at room temperature. Samples were extracted from the hexane phase at different time intervals. The CRGOs obtained from these samples were named according to these time intervals; for example, CRGO obtained 24 hours after the start of the experiment was labelled as CRGO 24h.

2.7 Fabrication of GO/C60 nanocomposites in chloroform

10 mL of GO aqueous dispersion (1 mg/mL) was mixed with 10 mL of chloroform containing 0.1% (v/v) oleylamine in a vial. By adding 0.5 mL of ethanol, the mixture was shaken for 20 seconds to achieve GO chloroform dispersion. Then the bottom phase was collected by removing the upper water phase. After that, 10 mL of C60 in chloroform (1 mg/mL) was mixed with the previous GO dispersion. This mixture was sonicated for at least 1 h before characterizations.

2.8 Adsorption of dyes in organic solvents using graphene in chloroform

GO, CRGO 24h and CRGO 48h dispersed in chloroform were achieved via the above method with the concentration to 0.5 mg/mL. Taking Sudan red G as an example, 4 mg of it was dissolved in 10 mL of chloroform as the stock solution. This stock was diluted to obtain Sudan red G with concentrations to be 0.0025, 0.01, 0.025, 0.05, 0.08 and 0.10 mg/mL (each 0.5 mL). 0.5 mL of GO or CRGO dispersion was mixed with the diluted dye solutions with violent shake. The resulting dispersions were filtrated to remove graphene while the supernates were collected and measured their absorption intensities at 500 nm. According to the standard curve built by the linear relation between dye concentrations and absorbance at 500 nm, the adsorption ability of graphene can be calculated. On the other hand, the directly dissolved graphene were achieved by dissolving powders of GO, CRGO 24h and CRGO 48h (each 5 mg) in 10 mL of chloroform. The poorly dispersed solutions were also used for dye adsorptions.

Using the directly dissolved graphene to adsorb the dyes, we could only obtain weak dye adsorption at very high concentrated dye/graphene ratio (50 μg/mL). The results are summarized in Table 2.4.
Table 2.4. The adsorption of dyes on graphene directly dissolved in chloroform.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sudan red G concentration</td>
<td>50 μg/mL</td>
<td></td>
</tr>
<tr>
<td>Absorbance at 500 nm (using GO)</td>
<td>0.4029</td>
<td></td>
</tr>
<tr>
<td>Dye adsorbed on GO</td>
<td></td>
<td>0.32 mg/g</td>
</tr>
<tr>
<td>Absorbance at 500 nm (using CRGO 24h)</td>
<td>0.4025</td>
<td></td>
</tr>
<tr>
<td>Dye adsorbed on CRGO 24h</td>
<td></td>
<td>0.35 mg/g</td>
</tr>
<tr>
<td>Absorbance at 500 nm (using CRGO 48h)</td>
<td>0.4017</td>
<td></td>
</tr>
<tr>
<td>Dye adsorbed on CRGO 48h</td>
<td></td>
<td>0.90 mg/g</td>
</tr>
<tr>
<td>Sudan red 7B /graphene ratio</td>
<td>50 μg/mL</td>
<td></td>
</tr>
<tr>
<td>Absorbance at 537 nm (using GO)</td>
<td>0.2710</td>
<td></td>
</tr>
<tr>
<td>Dye adsorbed on GO</td>
<td></td>
<td>0.21 mg/g</td>
</tr>
<tr>
<td>Absorbance at 537 nm (using CRGO 24h)</td>
<td>0.2708</td>
<td></td>
</tr>
<tr>
<td>Dye adsorbed on CRGO 24h</td>
<td></td>
<td>0.23 mg/g</td>
</tr>
<tr>
<td>Absorbance at 537 nm (using CRGO 48h)</td>
<td>0.2688</td>
<td></td>
</tr>
<tr>
<td>Dye adsorbed on CRGO 48h</td>
<td></td>
<td>0.61 mg/g</td>
</tr>
</tbody>
</table>

2.9 Loading of doxorubicin (DOX) on GO surface

2.4 mL of GO dispersion (1 mg mL⁻¹) was added to 14.6 mL of phosphate buffer solution (PBS, 50 mM pH 8.0) and sonicated for 30 min to obtain a well-dispersed brown solution. Then, 3 mL of DOX (2 mg mL⁻¹) was added into GO dispersion and sonicated for 1 h, following with vigorous shake overnight at room temperature in the dark. The product (GO-DOX) was collected after thrice centrifugation (12,000 rpm) and washing with PBS in order to remove unbound or unstable DOX molecules. The resultant residues were protect from light using aluminium-foil paper and kept in refrigerator (4 °C). The mass of unbound DOX was quantified using UV-Vis spectrophotometer, thus the DOX loading efficiency of GO could be calculated. Changes of buffer pH value due to the addition of alkaline GO were restored by adding bits of NaOH and HCl solution. With the similar process, loading of DOX on CRGOs and nGO was carried out as well.
2.10 The measurement and calculation for DOX binding on graphene materials

The amount of DOX in buffer solutions can be quantified using the calibration curve which was generated from the linearity between the absorbance at the wavelength of 480 nm (a characteristic UV-Vis absorbance peak of DOX) and DOX concentrations. The relationship between concentration (C, mg mL\(^{-1}\)) and absorbance (Abs, a.u.) can be described by equation (2).

\[
C = 0.526 \times \text{Abs} + 1.35 \times 10^{-3} \quad (2)
\]

The loading efficiency of DOX can be calculated via equation (3):

\[
\Phi = \frac{M_i - M_s}{M_G} \quad (3)
\]

where \(\Phi\) is the mass ratio of bonded DOX on GO, \(M_i\) is the initial amount of DOX, \(M_s\) is the total DOX amount in the supernate after centrifugation, and \(M_G\) is the amount of GO used for binding.

2.11 In vitro DOX release

The prepared GO-DOX nanocomposites were divided into two equal aliquots and each was dispersed in 6 mL of PBS with pH 7.4 and pH 5.3, respectively. Then, these were transferred into dialysis sacks (MWCO 12,000 Da, Sigma Aldrich) and dialyzed in 20 mL of PBS with relevant pH value at the physiological temperature of 37 °C in a glass vial. The solution was kept stirring on a magnetic stirrer and protected from light with aluminium-foil paper. Samples (0.5 mL each) containing the released drug were withdrawn every 1 h until 6 h, and following collections were made at the 12th, 24th, 36th and 48th hour, respectively. The concentrations of DOX released into buffer solution were calculated using equation (2), and the real-time release efficiency could be obtained from equation (4):

\[
\Psi = \frac{C_s \times V}{M_L} \times 100 \% \quad (4)
\]

where \(\Psi\) is the cumulative percentage of drug release, \(C_s\) is the calculated DOX concentration in buffer solution, \(V\) is the remaining volume of the liquid in the whole system, and \(M_L\) is the initial amount of DOX on GO calculated after the loading process.

In the contrastive experiments, 12 mg or 24 mg of \(\text{LAA}\) was introduced into this system with the other conditions constantly. In brief, \(\text{LAA}\) acid was added into GO-DOX dispersion following with its pH
value adjusted to 7.4 or 5.3 by NaOH (1 mM) titration. Samples were withdrawn and measured 480 nm absorbance with the same time intervals and used for comparison with the above experiments. To exclude the influence of the light absorption from LAA, control samples containing graphene and LAA but without DOX were measured as well.

2.12 Preparation of polyethylene glycol grafted nGO (PEG-nGO) and cellular test

PEG was covalently functionalised onto nGO to further improve its biocompatibility. 20 mL of nGO (0.5 mg/mL) was mixed with 200 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and stirred for 1 h at room temperature. Then 20 mg of N-hydroxysuccinimide (NHS) and 200 mg of PEG diamine were added into the activated nGO solution, and stirred for another 2 h. The resulting product was filtrated and dialysed for 5 days in order to remove unreacted molecules. The powders were re-dispersed in PBS (pH 7.4) by sonication. The loading of DOX on PEG-nGO followed the same method above, and the resulting solution was further filtrated with a 0.22 μm syringe filter.

2.13 Cell culture and viability assay

HT29 cells were purchased from the American Type Culture Collection. Cells were cultured in DMEM (Invitrogen, 12800-017), supplemented with 10% FBS (Hyclone, A50111) and 1× Glutamax (Life Technologies, 35050-061) at 5% CO₂ and 37°C. MTT assay was conducted as previously reported. Briefly, cells were seeded into 96-well plates (2000 cells/well). After 24 h, the culture medium was replaced by medium containing illustrated reagents at indicated concentrations. Free DOX, nGO-PEG, as well as nGO-PEG-DOX and nGO-PEG-DOX-L-AA containing the equivalent DOX as the free drug at indicated concentrations were added into the HT29 colorectal cancer cells. After 48 h of incubation, cells were treated with MTT (Sigma, M5655) for 4 h and the absorbance was measured at the wavelength of 570 nm using a VICTOR™ X5 Multilabel Plate Reader (PerkinElmer Life and Analytical Sciences). The IC50 data were analyzed using GraphPad Prism 3.03 software.

2.14 Preparation of boron nitride nanomaterials

BNNPs and BNNFs were prepared using a controlled ball milling process by Dr. Srikanth Mateti. The milling jar was loaded with 4 g of BN bulk powder and four hardened steel balls (25 mm in diameter).
Argon gas was used in the milling process to produce BNNP-OH. Ammonia gas was used as the milling atmosphere to exfoliate the BNNF-NH$_2$. BNNF-OH was produced by planetary ball milling at the presence of NaOH. The functional groups are given after the name of the materials in the abbreviations.

BNNSs dispersed in water or DMF were prepared by ultrasonication. BNNP-OH, BNNF-NH$_2$ and BNNF-OH were used as starting materials to prepare BNNSs with different functional groups. For example, 10 mg of the BNNP-OH were placed in a vial (20 mL) with 15 mL of Milli-Q water. The mixture was sonicated using an ultrasonicator (30% power, BILON92-IID, Shanghai Bilon Instrument Co., Ltd.) for 6 h keeping the temperature below 20 °C, followed by centrifugation at 3000 rpm for 5 min to remove the precipitates. The resulting dispersion represented a white colour and can keep the state of suspension for a few weeks. The concentration of the resulting BNNS-1 was measured to be 0.3 mg mL$^{-1}$ by weight method. Using the same process, BNNS-2 and BNNS-3 can be obtained by using BNNF-OH and BNNF-NH$_2$ as starting materials.

BNQDs were synthesized using a published method. In brief, 40 mg BNNP-OH and 15 mL of DMF were added in a beaker (20 mL), and kept sonication for 4 h to exfoliate BNNP-OH into BNNS-1 by ultrasonic cell crusher with an output power of 400 W. The obtained BNNS-1 were first degassed with N$_2$ for 30 min to remove the oxygen of the dispersion, then the dispersions were decanted into autoclave with the filling factor of 2/3 and subsequently treated under solvothermal conditions of 180 °C for 10 h in vacuum drying oven, followed by being cooled down naturally to room temperature. Afterward, the resulted suspensions were centrifuged at 10000 rpm for 5 min to remove the precipitation and the supernatant containing the as-prepared BNQD-1 were collected. BNQD-2 and BNQD-3 were synthesized by the same approach using BNNS-2 and BNNS-3 dispersions.

2.15 Detection of the boron radicals on boron nitride nanomaterials

As achieved, the BN powder samples are commercial bulk BN, BNNP-OH, BNNF-OH and BNNF-NH$_2$. The powders were carefully transferred into an Active spectrum 5.0 mm quartz ESR tube (Wilmad-lab glass Co., No. 710-SQ-100M, 5 mm OD) and measured using a benchtop Micro-ESR spectrometer (ACTIVE SPECTRUM).
The radicals of various BN nanomaterials dispersed in water or DMF were evaluated by the radical scavenging assay. Herein, 2, 2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, MW: 394.32) is used as a standard sample with one stable radical (N·) on each molecule. The scavenging of DPPH radicals using BN nanomaterials was carried out according to the methodology designed by Brand-Williams et al. Taking BNNS-1 as an example, the reaction mixture contained 100 uL of BNNS-1 (0.1 mg mL⁻¹ in water) and 100 uL of DPPH (1.0 mM in ethanol). After keeping the mixture in dark for 30 mins, the mixture was transferred into an Active spectrum 1.0 mm quartz tube (Wilmad-lab glass Co., No. 710-SQ-100M, 1 mm OD) and measured using the Micro-ESR spectrometer. The same method was used for the other types of dispersions by maintaining the concentration of BN the same. The densities of the free radicals in each sample can be judged by the intensity decrease of DPPH peaks.

2.16 Oxidization of TMB using BN nanomaterials as catalysts

The redox activity of the radicals was demonstrated by their abilities to oxidize a chromophore – TMB which is a substrate of horseradish peroxidase. For example, 200 uL of TMB (1 mM in ethanol) was mixed 2 mL of BNNS-1 (0.1 mg mL⁻¹) and 5 µL of H₂O₂. The mixture was kept at room temperature in dark for 3 h. The oxidation reaction was monitored by the colour change of the mixtures and measuring UV-Vis absorption at 650 nm.

2.17 ECL of [Ru(bpy)₃]²⁺ using BNNS and BNQD as co-reaction reagents

Relative ECL efficiencies (ΦECL) were evaluated, using a 3 mm diameter glassy carbon electrode (GCE), by comparison of the ECL spectra with that of the standard (0.1 mM [Ru(bpy)₃]²⁺ in 0.1 M PBS pH 7.4). Co-reactant ECL were generated using a 3 mm diameter GCE in a 0.1 mM solution of the complex containing increasing concentration of BNNS-1 or BNQD-1 as the co-reactant. ECL spectra were obtained using an Ocean Optics CCD, model QE6500, UV/Vis fiber optic (length 1.00 m) with a HR 4000 Breakout box trigger in conjunction with a PGstat 12 AUTOLAB potentiostat. The electrochemical cell was encased in a custom-built light-tight faraday cage.

2.18 Computational Details

The spin-polarized calculations for DPPH adsorbed on the BN edge and one N defect (V₅) within a 10 x 10 two-dimensional (2D) h-BN nanosheets were performed based on density functional theory (DFT)
as implemented in the plane wave basis Vienna ab initio simulation package (VASP) code\textsuperscript{336,337} under the framework of projector augment wave (PAW) method\textsuperscript{338}. A dispersion correction of total energy (DFT-D3 method)\textsuperscript{339} was used to incorporate the long-range van der Waals interaction. To study 2D system under the periodic boundary condition, a vacuum layer with a thickness 18 Å was set to minimize artificial interactions between neighboring layers. One dimensional (1D) nanoribbon model is adopted to study the effect of BN edge. The energy cut-off for the plane-wave basis set was set to 500 eV. The structures were fully relaxed until energy and force were converged to $10^{-6}$ eV and 0.001 eV/Å, respectively. Due to the large supercell, single Gamma point ($1 \times 1 \times 1$) was used to sample the 2D and 1D Brillouin zone during geometry optimization. The adsorption energy, $E_{\text{ads}}$ is calculated according to the following equation (s1)

$$E_{\text{ads}} = E_{\text{tot}} - E_{\text{substrate}} - E_{\text{DPPH}}$$

(1)

where $E_{\text{tot}}$, $E_{\text{substrate}}$ and $E_{\text{DPPH}}$ are the total energies of the DPPH/substrate after geometry optimization, the relaxed nanoribbon / 2D-V\textsubscript{N}-nanosheet and the isolated DPPH molecule.

2.19 Instruments and sample preparation

**UV-Visible Spectroscopy.** All the scans were performed in a continuous mode from 800 nm to 190 nm using quartz cuvette of path length 1 mm, with a scan rate of 500 nm/min and data interval of 1 nm using Varian Cary 300. A quartz cuvette with a light path of 1 mm was applied for the measurement. Samples were prepared in aqueous or organic solution. Sonication was used to help the samples better disperse.

**Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy (ATR-FTIR).** ATR-FTIR was performed using Alpha FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) equipped with a deuterated triglycine sulfate (DTGS) detector and a single reflection diamond ATR sampling module (Platinum ATR Quick-Snap\textsuperscript{TM}).\textsuperscript{340} Spectral resolution 4 cm$^{-1}$ with 256 co-added scans were used. Background measurements were obtained before scanning each sample. Secondary derivative and curve fitting was done using OPUS 6.0 software suite. The samples were powders, films or to drop cast the solution and measure when it dried.
**Raman spectroscopy.** Raman measurements were conducted using Renishaw Invia Raman Microspectrometer (Reinshaw plc, Gloucestershire, UK), equipped with 457, 514 and 633 nm laser, 1,800 or 2,400 grating and a thermo-electrical cooled CCD detector. Spectral data was acquired using 20 s exposure time, 5% power together with 4 cm$^{-1}$ spectral resolution. The sample preparation method was to put one drop of diluted solution on the flat aluminum-foil paper. After liquid evaporated, the residues were used for Raman tests using 457 and 514 nm laser.

**Zetasizer.** The particle size and surface charge of CRGO and its composites were measured using Zetasizer nano ZS. Normal two-way cuvette was used for particle size and disposable capillary cell DTS 1061 (Malvern Instruments, Worcestershire, UK) was used specially for zeta potentials. All measurements were carried out at room temperature with certain refractive index and equilibration time of 2 min. All of the measurements were repeated for at least 3 times.

**Photoluminescence spectra.** The photoluminescence spectra were measured using a photoluminescence spectrophotometer (Cary, Eclipse). A quartz microplate with 4 transmission sides was used in all the measurement. The excitation wavelengths were decided by measuring the strongest absorption peak in UV-Vis spectra. Each measurement was repeated for at least 3 times.

**X-Ray Powder Diffraction (XRD).** XRD results were tested using X’Pert Powder Instrument (The Analytical X-ray Company). The operating voltage was set as 40 mV and current was 30 mA. The diffraction angle started with 6° and ended at 70°. All of the samples were made into opaque film and fixed on a glass slide using tape for the measurement.

**AFM.** For AFM imaging purpose, multimode 8 from Bruker biosciences corporation (USA) was used in peak force quantitative nano-mechanical imaging mode. All the high resolution images were obtained at a scan rate of 0.977 Hz, 512 scans/lines and aspect ratio of 1 at room temperature. The image processing was carried out using Nanoscope Analysis (Version 8.1) provided with the instrument and the height profile was obtained by WsXM (Nanotech Electrica, S.L., Spain). The probes used for scanning was also obtained from Bruker (silica nitride, Scanasyst Air). For sample preparation, highly
diluted solution was drop casted on freshly cleaved mica surface and allowed to dry at room temperature with help of spin coater (WS-650MZ-23NPP).

**Cyclic voltammetry (CV).** All voltammetric experiments were carried out using a Biologic SAS, model 1. A three-electrode system was used for CV constituting of a counter electrode (platinum mesh), a reference electrode (Ag/AgCl) and a working electrode (GCE). The scan range and scan rate were decided by the applied redox reaction. For example, to investigate the redox reaction of Ru complex, the scan range was set between 0.6 V and 1.3 V, and the scan rate was 100 mV/s. For sample preparation, the samples were dropped on the surface of GCE and dried at room temperature before the measurement.

**ESR.** X-band continuous-wave ESR spectra were obtained using a Micro-ESR spectrometer (ACTIVE SPECTRUM). The powder samples were directly transferred into the Active spectrum 5.0 mm quartz ESR tube (Wilmad-lab glass Co., No. 710-SQ-100M, 5 mm OD) and start the measurement. The liquid samples were first injected into the ESR capillaries (1.7 mm) and then secured in the 5 mm tube for the measurement. The magnetic field range was set from 3309 to 3650 gauss. The ESR settings were as follows: field center, 3474 G; field sweep, 60 G; microwave frequency, 9.72 GHz; microwave power, 20 mW; magnetic field modulation, 100 kHz; modulation amplitude, 2.0 G; conversion time, 655 ms; and detector time constant, 655 ms.

**ECL.** ECL experiments were performed with an Auto lab PGSTAT128N potentiostat. The light was detected using an Ocean Optics QE65Pro spectrometer with HC-1 (300 l/mm) grating and Hamamatsu S7031-1006 back-thinned CCD (Quark Photonics, Vic., Australia) via optical fiber (1.0 m length, 1.0 mm core diameter) and collimating lens (Ocean Optics 74-UV, 200–2000 nm), positioned under the transparent base of the electrochemical cell described above, and vertically aligned with the face of the working electrode that was 2 mm above the base of the cell. The spectrometer was fitted with a 200 mm entrance slit, which provided a spectral resolution of 6.5 nm (FWHM). Acquisition was triggered using a HR 4000 Break-Out box in conjunction with the potentiostat. The spectra were corrected for the change in instrument sensitivity across the wavelength range (including absorption from the optical fiber and the lens, features in the grating response and the CCD detector response) using correction factors (one for each slit width setting) that were established using an HL-2000 Ocean Optics light
source directed onto a WS-1-SL diffuse white reflectance standard. The spectra were integrated to determine the relative ECL intensities.
Chapter 3: Probing the tunable surface chemistry of graphene oxide
3.1 Introduction

Despite its short history since 2004, graphene has been widely explored through functionalising this extraordinary two-dimensional material. Among various derivatives, graphene oxide is the most popular form of functionalised graphene, which incorporates carboxylic, hydroxyl and carbonyl groups at its edges, and epoxy and hydroxyl groups on its basal plane. These oxygen-containing groups impart a negative surface charge that inhibits irreversible sheet aggregation in solution. The corresponding processibility and monolayer dispersion in aqueous solution are the most attractive points to researchers, as well as the further chemical modification sites they provide, allowing, for example, amidation reactions. Although GO has gained considerable application value in many areas, more work is still being performed to improve its surface properties via chemical reductions, particularly due to conductivity problems. There are many reducing agents that could deoxidise GO according to well-supported mechanisms or proposed mechanisms based on knowledge of organic chemistry. Determination of amount of different oxygen-containing groups, hydrophobicity of GO surface and the evaluation of the efficiency of chemical reactions are still difficult tasks because of heterogeneous structure of GO. L-Ascorbic acid, which possesses a mild reducing capacity and nontoxic nature, is frequently applied as a reductant in biochemistry. The most interesting thing is that this reduction process can be controlled by tuning reaction time and concentration, which give us great opportunity to design the surface of graphene nanosheets.

Herein we present a detailed surface chemistry study of graphene oxide using tris(2,2'-bipyridine)ruthenium(2+) ([Ru(bpy)₃]²⁺) as a detection probe, exploring the interactions between GO and this complex. [Ru(bpy)₃]²⁺ has been reported to be able to functionalise various matrixes via electrostatic adsorption and hydrophobic interactions. Detailed study of surface interaction between GO and the probe molecule has been carried out. A controlled chemical reduction of GO by L-ascorbic acid was used to adjust the oxygen content on its surface. This was monitored by the transition of surface interactions of the chemically reduced graphene oxide (CRGO).
3.2 Results and discussions

Our hypothesis for this work is displayed in Figure 3.1. The GO surface is full of oxygen functional groups, but there is a gradual removal of oxygen by the controlled chemical reduction. Not surprisingly, the surface charge (-) of GO will drop, and therefore the hydrophobicity of the carbon nanosheets will be restored to some extent.\textsuperscript{340} This control of surface properties facilitates convenient binding of graphene materials with various modifiers \textit{via} covalent or non-covalent binding.\textsuperscript{129} To be specific, the probe molecule – \([\text{Ru(bpy)}_3]^{2+}\) is able to interact with GO surface \textit{via} both electrostatic interactions and \(\pi-\pi\) stacking. Due to the different kind of binding sites, there is a difference in phosphorescence under UV excitation. The \(\pi-\pi\) stacking results in the photoluminescence quenching of \([\text{Ru(bpy)}_3]^{2+}\) while the electrostatic interaction has less influence to this. With this work, we aim to measure the performance change of \([\text{Ru(bpy)}_3]^{2+}\) which gives information of GO surface tuning, therefore, it is an indirect probing method.

\textbf{Figure 3.1.} Schematic of the surface chemistry tuning of graphene oxide.
[Ru(bpy)₃]²⁺ is amphipathic molecule with three bipyridine ligands binding to a ruthenium metal center, in a three-dimensional spatial arrangement (Figure 3.2). We used the coordinate figures to estimate its size to be around 1.0 nm across, which is useful to our AFM analysis. [Ru(bpy)₃]²⁺ was selected as the modification molecule because it is able to probe the graphene surface through two categories of non-covalent binding, namely electrostatic interaction and π-π stacking. Interestingly, the mechanisms of these two binding categories are somewhat contradictory, with the former based on surface charge, while the latter is dependent on the hydrophobicity of the graphene sheets. Thus, I explored the interactions that existed between GO and [Ru(bpy)₃]²⁺ for a better understanding of its surface chemistry. Self-assembly was applied here to prepare all of the samples by simple chemical mixing and slight shaking.

![Figure 3.2. Chemical structure of [Ru(bpy)₃]²⁺ molecules (a) drawn using ChemDraw Ultra 8.0 and (b) generated by Chem3D Ultra 8.0.]

3.2.1 Study of the interactions between GO and [Ru(bpy)₃]²⁺

UV-Vis absorbance spectra were frequently applied to investigate the interactions happen between graphene surface and some luminescent molecules. Figure 3.3 showed that the absorbance intensities of [Ru(bpy)₃]²⁺-GO hybrids were positively correlative to the dosage of GO. However, the peak at 456
nm, attributed to the metal-to-ligand transition of [Ru(bpy)$_3$]$^{2+}$, was decreased by GO binding. This is due to the new pathway of electron transfer introduced by GO, in which the Ru metal center directly delivered an electron to the GO sheets via COOH or OH, indicative of their electrostatic interaction. When more and more electrons are directly transferred onto GO sheets, less UV light will be absorbed.

![Figure 3.3. UV-Vis absorbance spectra of GO/[Ru(bpy)$_3$]$^{2+}$ composites.](image)

This was further characterised by the particle size and zeta potential test (Figure 3.4). The addition of [Ru(bpy)$_3$]$^{2+}$ into GO solutions caused the sudden decline of surface charge ((-) from GO). This simultaneously brought a sharp increase in particle size (almost double). The drop of zeta potential is obvious because of the neutralisation of surface charge of GO by adding positively charged molecules. The increase of particle size might due to the restacking of GO sheets or crosslinking by the ruthenium complex.
On the other hand, photoluminescence quenching provided strong evidence of π-π stacking interactions on the graphene surface.\textsuperscript{101} The orange phosphorescence ($\lambda_{\text{max}} \sim 610$ nm) from ruthenium(II) diimine complexes arises from the excitation of an electron from the metal-based $d(\pi_m)$ orbitals to ligand-based π* antibonding orbitals,\textsuperscript{354} (i.e. metal-to-ligand charge-transfer, MLCT), followed by intersystem crossing to the lowest excited triplet state.\textsuperscript{355} The adhesion of the bipyridine ligands on GO sheets via π-π stacking introduces a new pathway of electron-transfer from the Ru metal center to GO π-orbitals, inhibiting the generation of phosphorescence. Although GO (prior to chemical reduction) contained many oxygen functional groups, it was still able to quench the phosphorescence of $[\text{Ru(bpy)}_3]^{2+}$ due to π-π stacking on its hydrophobic regions (Figure 3.5). With an increase of GO dosage, there will be less and less free $[\text{Ru(bpy)}_3]^{2+}$ in the mixture, leading to the stronger quenching of phosphorescence. Therefore, strong π-π stacking was observed between the GO surface and the bipyridine ligands of $[\text{Ru(bpy)}_3]^{2+}$.
Raman spectra were measured with a laser excitation wavelength of 457 nm under ambient conditions. It is a sensitive method to characterise the change in the surface of graphene. The samples were prepared by dropping the solution on the flat aluminum foil, which did not show any signal, and then let it dry. The characteristic peaks of $[\text{Ru(bpy)}_3]^{2+}$ could be clearly seen in the red curve (Figure 3.6). Specifically, there were four sharp peaks at 1318, 1488, 1555 and 1600 cm$^{-1}$. In the case of the GO/$[\text{Ru(bpy)}_3]^{2+}$ composite, two additional peaks at 1360 and 1580 cm$^{-1}$, the typical D band and G band from graphene materials, were observed. The peaks belonging to $[\text{Ru(bpy)}_3]^{2+}$ exhibited a slight red-shift (2-3 cm$^{-1}$) which can be more easily seen in the inset graph. This was evidence of the interaction of $[\text{Ru(bpy)}_3]^{2+}$ and GO. The D band for GO indicates the disorder of carbon sheets while the G band stands for the structure of the in-plane $sp^2$ bond. $I_D/I_G$ of GO was calculated to be 0.98. After mixed with $[\text{Ru(bpy)}_3]^{2+}$, this ratio decreased slightly to 0.93. The reason for this might be the remarkable increase in size and extended $sp^2$ $\pi$ conjugation that was introduced by $[\text{Ru(bpy)}_3]^{2+}$ via $\pi-\pi$ stacking interactions.$^{356}$

**Figure 3.5.** Phosphorescence spectra of GO/$[\text{Ru(bpy)}_3]^{2+}$ composites.
Figure 3.6. Raman spectra of [Ru(bpy)$_3$]$^{2+}$ (red curve) and GO/[Ru(bpy)$_3$]$^{2+}$ composite (blue curve).

Attenuated total reflectance - Fourier transform infrared spectroscopy (ATR-FTIR) is very useful to confirm the specific functional groups whether from small molecules or on large substrate. Here we used FTIR to verify the successful self-assembly of [Ru(bpy)$_3$]$^{2+}$ on GO (Figure 3.7). The pattern of GO (black curve) showed characteristic peaks at 1722 cm$^{-1}$ and 1614 cm$^{-1}$, respectively, both of which experienced a blue-shift after the composite material was formed (blue curve). This indicated the addition of [Ru(bpy)$_3$]$^{2+}$ affected the chemical environment of both COOH and C=C bonds, with Ru metal center close to COO$^-$ groups and bipyridine ligands attached on C=C conjugated structure. Therefore, electrostatic interactions and π-π stacking were both verified again by FTIR.

Figure 3.7. ATR-FTIR results of GO/[Ru(bpy)$_3$]$^{2+}$ composites.
GO and its ruthenium composite were made into films by water filtration for X-ray diffraction (XRD) analysis (Figure 3.8). The spectrum of GO exhibited a sharp (002) peak at 10.6° revealing a d-spacing of 0.83 nm between the adjacent sheets. When introducing the ruthenium complex, this peak shifted to 9.3° and became weaker, and this was attributed to increased interlayer spacing and disorder. The distance between crystal carbon atoms increased after Ru modification, revealing that the ruthenium complex stacked tightly onto GO sheets. This was even stable after vast water washing, indicating the successful binding of ruthenium complex onto GO. Through all of these results, the interactions between GO and [Ru(bpy)₃]²⁺ have been confirmed to include both electrostatic and π-π stacking interactions, as well as the electron transfers between them.

![Figure 3.8. XRD (d) results of GO/[Ru(bpy)₃]²⁺ composites.](image)

3.2.2 Controlled reduction of graphene oxide

The chemical reductions of GO were carried out using L-ascorbic acid as the reducing agent, and were recorded in real-time by UV-Vis and Raman spectra (Figure 3.9). Five different CRGO samples were synthesised by controlling the reduction time (1h, 6h, 12h, 24h and 48h). Stepwise red-shifts of the GO peak (230 nm) were observed in the UV-Vis spectra (Figure 3.9a), corresponding to gradual removal of oxygen containing groups (also evidenced by thickness decrease in atomic force microscope results.
Figure 3.10. However, even the most reduced sample (48h, peak centered at 244 nm) retained oxygen groups when compared with highly reduced GO, which commonly exhibits maximum absorbance at 268 nm. In Raman spectra (Figure 3.9b), there was an obvious increase of D band intensity, due to the introduction of more defects in the graphene sheets during reductions. We would suggest that the as-prepared CRGOs should have similar functions as GO in terms of binding methods; however, we needed to ascertain what happened on their surface and how the properties changed.

![Graph showing UV-Vis and Raman spectra](image)

**Figure 3.9.** UV-Vis spectra (a) and Raman spectra (b) of CRGOs with different reduction time (1h, 6h, 12h, 24h and 48h).

To continue with, atomic force microscope (AFM) is an excellent method for 2D material characterisations because it can scan a beautiful image showing the large lateral size and ultra-small thickness. AFM images and corresponding height profiles were obtained to observe the morphology change of CRGOs after reduction reactions. In this work, the hydrophilic GO sheets interacted well with the mica surface and form stable and smooth surface on it. Figure 3.10a showed the decrease in height of CRGO sheets under longer and longer reduction time. With step-by-step removal of surface oxygen groups, the thickness of monolayer CRGO dropped from 1.0 nm to 0.7 nm. And as mentioned in the main paper, Figure 3.10b was used to examine the heights of [Ru(bpy)_3]^{2+} on CRGO sheets, showing a decrease of average figures with longer reduction times. Compared with the theoretical figure
of unmodified graphene whose thickness is 0.34 nm, GO and CRGO are much thicker due to the presence of oxygen groups at edges and basal plane. These oxygen groups could extend into vertical space, putting up GO sheets, which made them thicker.

Figure 3.10. AFM images of (a) CRGOs and (b) monolayer [Ru(bpy)₃]²⁺ on CRGOs.
To summarize, the monolayer GO has been successfully synthesised by this modified Hummers’ method. The exfoliated GO nanosheets are fully of oxygen functional groups and structural defects. Furthermore, controlled chemical reduction is also achieved with gradual removal of the oxygen groups as well as the surface charge on it.

3.2.3 Probing the surface tuning of GO

In this work, we aimed to probe the surface chemistry (such as surface charge, oxygen content and surface hydrophobicity) of GO using [Ru(bpy)₃]²⁺. The preparation of fine graphene/[Ru(bpy)₃]²⁺ mixture is easy by self-assemble, which just mixes the two solution and stirs at a gentle speed. Firstly, in the UV-Vis absorbance spectra (Figure 3.11a), we observed a blue-shift in the 286 nm peak in [Ru(bpy)₃]²⁺/CRGO composites, indicating an important change in the CRGO surface. This is strong evidence of the restoration of graphene’s hydrophobicity, which means larger surface area, lower oxygen content, and stronger ability to bind by π-π stacking. Interestingly, this was achieved through spectral shift of probe molecule rather than direct detection of graphene surface. The 286 nm peak of [Ru(bpy)₃]²⁺ corresponded to the π to π* transition of the bipyridine ligands. Its shift therefore demonstrated another type of π electron delocalization from π-π stacking with the GO surface. Importantly, we found that CRGOs subjected to longer reduction-time shifted this peak further (reaching 280 nm by the 48 h reduction sample), indicative of stronger π-π stacking. Obviously the restoration of hydrophobicity of the graphene material facilitated π-π stacking interactions on its surface. This was further verified using phosphorescence emission spectra (Figure 3.11b), in which CRGOs showed different degrees of phosphorescence quenching. With the same [Ru(bpy)₃]²⁺ concentration in all mixtures, the more reduced sample proved to be the stronger quencher (from red curve down to the dark-blue curve and from left to right in the inset), again corresponding to stronger π-π stacking. This is theoretically correct but people failed to confirm it. Furthermore, I believe that the electrostatic interaction should get weaker because the oxygen functional groups were partly removed. Less oxygen content led to a decrease of surface charge. Therefore, the final result of the reduction reaction is that π-π stacking was improved while the electrostatic interactions weakened, here we define it as the interaction transition from hydrophilic to hydrophobic. This could a novel concept as we could switch
the interactions between compounds by some smart treatment in a simple system. We could design a surface for molecule loading and do some simple work to help it release the cargo.

In Figure 3.11c, we have shown our viewpoints of the morphology of the [Ru(bpy)$_3$]$^{2+}$ monolayer bound onto GO nanosheets, referring to our AFM results. The measured thickness of [Ru(bpy)$_3$]$^{2+}$ on the GO sheets ranged from 1.0 nm to 1.5 nm (Figure 3.11d, e). I would suggest the height fluctuations was caused by the influence of different modes of binding as I discussed above. [Ru(bpy)$_3$]$^{2+}$ has a 3D structure (already shown in Figure 3.2) and thus a spatial barrier appeared when it self-assembled on GO. When it was attached to hydrophobic regions of graphene via π-π stacking interactions, the complex would be expected to remain at the edges or near defects, where the spatial barrier was
minimized. Obviously, one of its ligands must sit parallel with the graphene sheet for the π electron hybridization. We suppose that this type of binding might make its thickness (around 1.0 nm) lower than those linked via electrostatic interactions (around 1.5 nm). The latter took place on oxygen functional groups that had already imparted an increase in height onto the graphene oxide sheets. To examine this notion, monolayer CRGO sheets were obtained on mica substrates by spin coating. In order to get a single layer of \([\text{Ru(bpy)}_3]^{2+}\) on the CRGO surface, a low concentration \([\text{Ru(bpy)}_3]^{2+}\) solution (10 µM) was dropped on the CRGO coated mica surface, kept for 60 s and the droplet was absorbed by wiping paper. The thicknesses of the \([\text{Ru(bpy)}_3]^{2+}\) monolayer on different CRGO samples were determined using AFM (see Figure 3.10b) and corresponding height profiles. The results for the height range and average heights are presented in Table 3.1. All the samples exhibited a height range from 1.0 nm to 1.5 nm, which verified both electrostatic and π-π interactions, consistent with our deduction. Moreover, a decrease in average height from 1.4 nm to 1.2 nm was observed due to the transition from electrostatic interactions to π-π stacking interactions by the oxygen reducing steps.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Height range (nm)</th>
<th>Average height (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRGO 1h + [Ru(bpy)_3]^{2+}</td>
<td>1.0-1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>CRGO 6h + [Ru(bpy)_3]^{2+}</td>
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<td>1.3</td>
</tr>
<tr>
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<td>1.0-1.5</td>
<td>1.3</td>
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<tr>
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<td>1.0-1.5</td>
<td>1.3</td>
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<tr>
<td>CRGO 48h + [Ru(bpy)_3]^{2+}</td>
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<td>1.2</td>
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</tbody>
</table>

A loading experiment of \([\text{Ru(bpy)}_3]^{2+}\) on GO gave further insight into GO reduction (Figure 3.12). The ability to load \([\text{Ru(bpy)}_3]^{2+}\) was calculated using the intensities of the 286 nm absorbance peak of \([\text{Ru(bpy)}_3]^{2+}\). Firstly, the standard curve for \([\text{Ru(bpy)}_3]^{2+}\) was plotted using a wide range of \([\text{Ru(bpy)}_3]^{2+}\)
concentrations. Then, for each sample, 650 µL of GO and 200 µL of [Ru(bpy)$_3$]$^{2+}$ were mixed, shaken vigorously and centrifuged at 14,000 rpm for 1 h. The supernatant was used to measure the concentration of [Ru(bpy)$_3$]$^{2+}$ that was not bound to GO, enabling the loading of [Ru(bpy)$_3$]$^{2+}$ on the graphene material to be calculated. As both the electrostatic and π-π interactions contributed to the binding of [Ru(bpy)$_3$]$^{2+}$ on graphene oxide, its loading capacity would change during the reduction process. From the data we found untreated GO exhibited the greatest ability to accommodate [Ru(bpy)$_3$]$^{2+}$ (0.26 mg/mg), which should attribute to the large amount of oxygen groups on its surface. However, the sharp decrease exhibited by the 1 h reduction-time sample showed that a large decrease in surface charge could be achieved within a short period of time, leading to the poor loading ability. After that with longer reduction time, the loading back to increase and reached 0.21 mg/mg for the 48 h CRGO sample, corresponding to the most extensive restoration of hydrophobicity. From my point of view, the surface charge could be removed very fast by reduction reaction but the restoration of hydrophobicity took long time.

![Figure 3.12](image)

**Figure 3.12.** Loading experiment of [Ru(bpy)$_3$]$^{2+}$ on GO and CRGOs with different reduction times.

Graphene and GO have a lot of applications in the case of electrochemistry, like the previously mentioned sensing and catalyst. Cyclic voltammetry curves (Figure 3.13a) were used to investigate the
electrochemistry of GO/[Ru(bpy)_3]^{2+} composite. Due to the oxidation of [Ru(bpy)_3]^{2+}, there is an oxidation peak around 1190 mV for pure ruthenium complex. In our experiment we found the introduction of GO or CRGOs brought a negative shift of this peak, and the more reduced CRGO had the stronger ability to shift it, moving to 1102 mV in 48 h CRGO. I suggest that the decrease of oxidation potential may be attributed to the decrease of oxidation energy due to the enhanced electron transfer. The rate of heterogeneous electron transfer (HET), namely the transfer of electrons from/to graphene sheets to/from compounds, is related not only to the properties of binding molecules but also to the amount of defects and functional groups presented on graphene surface. We found that the enhanced electron-transfer kinetics could facilitate the redox reactions of [Ru(bpy)_3]^{2+} that usually required high overpotentials to achieve. This could significantly lower the energy consumption in the electrochemical process. Herein, the reduction of GO helped to save energy costs by improving the HET rate. For better understanding of the influence of oxygen functionalities, peak-to-peak separations (ΔE) were plotted against reduction time of GO (Figure 3.13b). The decreasing trend shown in the figures indicated improvement of the HET by removal of oxygen functional groups. Furthermore, the restoration of sp^2 carbon-carbon improved the conductivity of the modified electrode surface, which was evidenced by the increasing oxidation currents with longer reduction time. Based on the reduction peaks, we observed that the redox reaction of [Ru(bpy)_3]^{2+/3+} is not reversible, especially in those less reduced samples (GO, 1 h CRGO, 6 h CRGO and 12 h CRGO). As discussed above, for less reduced CRGO sheets, electrostatic interactions are dominated through the binding of ruthenium compound, and their electron transfers rely on oxygen functional groups (like COO-). In all of these samples, large amount of negatively charged groups might prevent electron from approaching Ru^{3+} due to electrostatic repulsion. Therefore, oxidised ruthenium metal could be only partially reduced, leading to the lower reduction currents. However, this would not react when π-π stacking plays the major part in this binding because electron goes through graphene’s conjugated region rather than oxidised edges (such as 24 h CRGO and 48 h CRGO).
3.3 Conclusions

The surface chemistry of GO is a very important project because we can gain a lot of fundamental knowledge from it. It tells us what is on GO surface and what would happen if we do functionalisation on it. Better understanding give us chance to design the surface of GO for various applications. In this work, a novel analytical approach to probe the surface chemistry of GO has provided new insight into the properties of this important material – GO. Chemical reduction for GO surface tuning was successfully employed to manipulate the surface charge and hydrophobicity, which enabled its surface interactions transfer from the electrostatic attraction to π-π stacking. Our work presents a new method to explore surface chemistry and to control binding modes on modified carbon materials.

Figure 3.13. Cyclic voltammetry curves (a) and peak-to-peak separations (b) of CRGO/[Ru(bpy)$_3$]$^{2+}$. 
Chapter 4: Simultaneously “pushing” and “pulling” graphene oxide into low-polar solvents through a designed interface
4.1 Introduction

The chemically tunable nature of graphene oxide (GO) that arises from the presence of oxygen functional groups means it is very attractive for a range of applications.\textsuperscript{361-363} Its high and versatile surface area enables it to have tunable hydrophilic and hydrophobic properties in solutions.\textsuperscript{267,364-366} In its as-synthesized form, GO has an excellent dispersibility in aqueous solutions and highly polar organic solvents due to the oxygen functional groups that decorate its surface. However, these same groups mean GO does not disperse well in low-polar organic solvents such as hexane and chloroform. This restricts the application of GO in the organic phase.\textsuperscript{367} For example, GO has demonstrated an excellent capacity to adsorb dye in aqueous solution,\textsuperscript{368-370} but there are far fewer reports of dye adsorption in the oil decontamination context.\textsuperscript{371} The preparation of fullerene (C\textsubscript{60})/GO nanocomposites is also difficult to achieve because fullerenes are typically dispersed in low-polar solvents.\textsuperscript{372} Despite some effort by the community, it is still a challenge to directly disperse or transfer hydrophilic GO into low-polar solvents without compromising its desirable surface chemistry.\textsuperscript{371,373,374}

Phase transfer has been explored as a means of dispersing GO in low-polar solvents.\textsuperscript{373,375} The design of the interface used in the phase transfer approach is inspired by the somewhat analogous self-assembly and functionalization of nanoparticles at the water/oil interface.\textsuperscript{227,375-378} A well-known approach to improve GO solubility in low-polar solvents is to covalently functionalise it.\textsuperscript{373} This is, however, relatively complicated and irreversible. Non-covalent functionalization using surfactants is, therefore, preferred.\textsuperscript{232} Such an approach has been widely employed to transfer GO from water to chloroform,\textsuperscript{379,380} for example, and to improve the interfacial interaction of GO with epoxy matrices.\textsuperscript{381} The commercially available oleylamine is one surfactant that is effective in transferring GO into low-polar solvents.\textsuperscript{382} This effectiveness arises in part from the long-chain aliphatic nature of oleylamine, which means it dissolves well in low-polar solvents but poorly in aqueous solutions.\textsuperscript{383} The positively charged terminal amino groups of the surfactant is the other key characteristic as it interacts favourably with the negatively charged GO sheets. There are two drawbacks with the surfactant-based phase transfer approach, however. The first is the cost of surfactants like oleylamine, which arise from their relative complexity. The second is the need for high dosages to effect significant phase transfer that can lead to
the aggregations of the GO once it has transferred into the low-polar phase. These drawbacks mean it is highly desirable to develop a phase transfer method that requires far less surfactant.

The transfer of GO between two immiscible solvents can be further improved through the use of a third ‘carrier’ solvent that is miscible in both. Ethanol has been used for this purpose when seeking to effect phase transfer between water and low-polar solvents. In this case, ethanol forms an intermediate phase between water and the organic phase in which GO and surfactant are suspended before they select which phase to enter according to their surface properties. The introduction of such an intermediate phase could decrease the dosage of surfactants.

The surface chemistry and more specifically, the solution behavior of GO in mixed solvent systems during surface modifications such as covalent and non-covalent functionalization, and the reduction of GO to its conductive form, is not fully understood. In a recent work, we show that the surface chemistry of GO can be tuned by chemical reduction using the mild reducing agent L-ascorbic acid (L-AA). Using this method, we further demonstrate how GO surface properties change upon their exposure to various liquid environments. This leads to the effective use of interfaces for the tunable conversion of GO surfaces and consequently, dispersion into low-polar solvents. As far as we know, the transfer of both GO and chemically reduced graphene oxide (CRGO) has been reported separately but there is no report for a comparison between them or simultaneous phase transfer of GO into organic solvents and chemical reduction.

Herein, we present the design of a new interface to transfer GO from water into low-polar solvents and simultaneously convert it to CRGO by in situ reduction. In this approach, oleylamine anchors the GO at the water/low-polar solvent interface while L-AA gradually converts the GO from hydrophilic to hydrophobic to achieve phase transfer. A good understanding of the interface is achieved by investigating the influence of surface chemistry using ethanol as a carrier without L-AA. The transfer efficiencies of GO with different lateral dimensions, pH and the level of oxygenated species were studied in detail. This approach allows the concentration of oleylamine to be very low (0.1%), facilitating the formation of monolayer nanosheets in the low-polar phase. On the other hand, CRGO in low-polar solvents is achieved by the cooperation of surfactant and surface tuning. The advantages
of this approach include: (i) the obtained CRGO is dispersed as monolayers in the low polar organic phase, and (ii) the surface properties of the transferred CRGO can be controlled by collecting dispersion in organic phase at certain time interval. To explore the applications of the transferred GO and CRGOs, we have fabricated C_{60}/GO nanocomposites by π-π stacking interactions, and an attempt to adsorb dye for oil decontamination is carried out using CRGOs in chloroform.

4.2 Results and Discussion

In the following, we present and discuss the results obtained from the systematic study of the following system characteristics that influence the transfer efficiency of the process illustrated in Figure 4.1A when using oleylamine as the surfactant and ethanol to form the intermediate phase: (a) the polarity of organic solvents; (b) surfactant concentration; (c) intermediate phase concentration; (d) pH of GO dispersions; (e) GO lateral dimensions; and (f) GO oxygen contents. On the other hand, an approach is designed to simultaneously “push and pull” GO nanosheets from water into low-polar solvents (Figure 4.1B). Using the transferred GO and CRGOs in chloroform, we demonstrate the fabrication of GO/C_{60} nanocomposites and dye adsorption.

![Figure 4.1. The mechanism of GO transfer.](image)
4.2.1 Selection of low-polar solvents

Firstly we show this approach is applicable in common laboratory solvents of low polarity. By using oleylamine as a surfactant and creating the intermediate with ethanol at the interface, GO sheets can be transferred from water into five representative low-polar solvents (Figure 4.2a). Using the same concentration of oleylamine (0.5% v/v), complete transfer of GO is achieved in hexane by adding only 160 µL of ethanol, whereas in chloroform, dichloromethane, ethyl acetate and toluene, the critical values of ethanol dosage for complete GO transfer are 360, 760, 1140 and 560 µL, respectively (Figure 4.2b). In order to make the interface uncomplex and clear, utilising the solvent required less ethanol dosage is preferred. Therefore, hexane is selected as a model for the low-polar solvents, which could be due to the high solubility of oleylamine in hexane.

Figure 4.2. (a) Photographs of GO after phase transfer from water (b) ethanol dosages for complete GO transfer into organic solvents; (c) XRD patterns of GO (d) AFM image of transferred GO on HOPG.
4.2.2 Confirmation of oleylamine binding to GO surface

By filtrating the GO dispersed in water or hexane, the GO films are prepared and characterised by X-ray diffraction patterns. Compared with GO film prepared from water phase, there is a left shift of the typical (002) peak (Figure 4.2c) in GO film from hexane phase, which indicates the increase of d-spacing between GO nanosheets due to the intercalation of oleylamine molecules. This is confirmed by the AFM image of transferred GO shown in Figure 4.2d where a thickness of 1.5 nm (larger than that of pure GO which is around 1.0 nm) suggests oleylamine molecules are attached on GO surface.\textsuperscript{365,389} The AFM imaging is only achieved on highly oriented pyrolytic graphite (HOPG) rather than hydrophilic mica, indicating that the transferred GO is highly hydrophobic because the oxygen functional groups have been blocked by oleylamine.

4.2.3 The optimization of oleylamine and ethanol dosage

In order to obtain GO dispersions with less contaminations, the dosages of oleylamine and ethanol are optimized in hexane. As the density of hexane is less than that of water, it appears in the upper layer of the solvent mixture. In our study, 0.1\% (v/v) oleylamine is found to be the lowest concentration that can induce GO transfer. This concentration here is significantly lower when compared with that of oleylamine works for MoS\textsubscript{2} transfer: 2.5\% (v/v).\textsuperscript{383} This should be attributed to the intermediate formed by ethanol which is miscible with both water and hexane. For the dosage of ethanol, the increase of GO concentration requires higher ethanol dosages for effective phase transfer because wider channels are necessary (Figure 4.3a, b). Using tubes with a diameter of 8 mm, we find less oleylamine dosages result in more ethanol requirement (Figure 4.3a). This is reasonable as oleylamine and ethanol cooperate to achieve phase transfer of GO. Once one part of the force weakens, the other part needs to be stronger to accomplish the work. Interestingly, the transfer of GO is affected by the diameter of the reactors. For example, the transfer of GO (at 2 mg/mL) is not successful using small tubes (5\textsuperscript{th} in Figure 4.3c), but can be carried out using vials of larger size (Figure 4.3d). This is further confirmed in Figure 4.3b (red and blue curves) by using reactors with different diameters. GO (2 mg/mL) is successfully transferred into hexane in vials with diameters of 16 and 27 mm. This could be due to the difference of interfacial
area per unit volume of the reactors. Vials with larger diameters can provide wider interfacial area for GO transfer.

![Figure 4.3.](image.png)

**Figure 4.3.** The relationship between ethanol dosages for complete GO transfer and GO concentration.

4.2.4 The influence of GO lateral dimensions, pH, and the level of oxygenated species on GO

The surface chemistry of GO plays an important role in its phase transfer. It is expected that GO lateral dimensions, pH, and the level of oxygenated species on the GO surfaces influence the transfer behavior of GO because these factors affect the electrostatic interactions between GO and oleylamine. Therefore, if this interaction can be enhanced by modulating the GO surface properties and solution conditions in water phase, we should be able to improve the efficiency of GO transfer. To verify our hypothesis, we compare the transfer efficiencies of various samples using different dosages of oleylamine and ethanol under different experimental conditions. The transfer efficiency of each experiment is calculated by measuring the concentration of GO remained in water after phase transfer.
Table 2.1 shows a representative experiment which explores the influence of the GO lateral dimensions on the transfer efficiencies. The lateral dimensions of nGO, GO and LCGO are measured by Dynamic Light Scattering, (Figure 4.4a) exhibiting an increasing dimension distribution with nGO, regular GO and LCGO. We find the transfer efficiency is correlated to GO lateral dimensions whereby larger dimension affords the higher transfer efficiency (Figure 4.4b), which means larger GO is easier to be transferred. This is quite unexpected as it would be reasonable to believe that the transfer of GO with smaller dimension is easier because it is lighter. Smaller GO has larger edge to surface ratio, exhibiting higher density of oxygen functional groups because oxidation is much easier at edges or defects than that on the basal plane. Therefore, due to the strong hydrogen bonds with water molecules, smaller GO is more difficult to escape from water phase than GO with large lateral dimensions.390,391
Figure 4.4. Study of the influence of surface chemistry of GO to the phase transfer.

The effect of pH is investigated via the similar approach. Table 2.2 gives out the details of a representative experiment. Higher transfer efficiencies are obtained in acidic conditions (Figure 4.4c), likely due to the improved electrostatic interactions at a low pH, which arises from the bigger difference between Zeta potentials of oleylamine and GO in acidic conditions than in basic conditions (Figure...
The lowest transfer efficiencies are found at pH 7.0. Under neutral condition, both GO and oleylamine are poorly charged, leading to the weakest binding.

On the other hand, the level of oxygenated species on GO also affects the strength of electrostatic interactions. The density of oxygen functional groups determine the hydrophobicity of GO and CRGO, which affects their interactions with low-polar solvents. GO is chemically reduced using L-AA to achieve CRGOs with different oxygen content by controlling reduction time. We find the transfer efficiencies of CRGOs decrease upon the reduction time of GO (Figure 4.4e). We postulate that the removal of carboxyl group results in a decrease of GO surface charge, leading to weaker electrostatic interactions between GO and oleylamine. The experimental details are showed in Table 2.3. The completely transferred GO and CRGOs dispersions are measured using UV-Vis spectra (Figure 4.4f). Despite of the strong absorbance of oleylamine in UV region, there is an increase in the absorbance with increase of GO reduction time, which is consist with the previous report.

Phase transfer by the surface tuning of GO

Since the surface hydrophilicity of GO nanosheets can be tuned by controlled chemical reduction, we design an interface that allows simultaneous phase transfer and surface tuning of GO. The designed interface and transfer mechanism are displayed in Figure 4.1B. GO nanosheets are first captured by oleylamine at the interface via electrostatic attractions. The chemical reduction of the captured GO is carried out through the subsequent addition of L-AA into the water phase. In this instance, it is hypothesized that the oxygen functional groups that are not blocked by oleylamine would be removed from GO surface to yield a hydrophobic surface and result in a decreased hydrogen bonding with water. This decreased dispersity of CRGOs pushes them out of aqueous phase and oleylamine that prefers organic solvents pulls them towards hexane to complete the transfer process. Control experiments without oleylamine or L-AA indicated that the transfers cannot be achieved, which suggests that both the electrostatic interactions and the surface tuning are necessary for this “pushing” and “pulling” mechanism of phase transfer. This process is also demonstrated in Figure 4.5a, b and c that show the gradual transfer of GO from water to hexane phase at the presence of L-AA. Furthermore, the gradual evolution of color changed from brown to black (i.e. from GO to CRGOs) in hexane over time. The
transferred CRGOs show excellent stability in hexane after 1 month (Figure 4.5d). We note that in this example, the complete transfer is not achieved because a few highly reduced GO were not initially captured at the interface possibly due to the weak interaction with oleylamine. As L-AA has a poor solubility in hexane, it will be removed atomically from the CRGOs’ surface during phase transfer, thus eliminating the need for further washing.393

![Figure 4.5. Photographs of transferred CRGOs after stirring with L-AA for 0 h (a), 6 h (b), 72 h (c) and 1 month (d).](image)

We monitor this transfer process by *in situ* UV-vis absorption and Raman spectroscopy analyses. Figure 4.6a shows that the product concentration and the reduction degree of CRGOs increased with the transferring duration. The increase concentration of CRGO is illustrated by the increase in the absorbance intensity of the $\pi-\pi^*$ transitions, which is quite weak at 6th hour but became much stronger after 96 hours. The peak center shifts from 242 nm (24 h, red curve) to 260 nm (96 h, dark yellow curve) due to the removal of oxygen functional groups which results in the improved $\pi-\pi^*$ transitions. This has also suggested that the reduction degrees increase with the phase transfer duration.92 These results are corroborated by the Raman spectra in Figure 4.6b, which show the reduction of GO results in an increase of $I_D/I_G$ due to the introduction of structural defects.394 AFM images of the CRGOs (Figure 4.6c-e) collected at different time intervals show the thickness of CRGOs is around 1.0 nm, representing the
transferred CRGOs are monolayers. Our results indicate the phase transfer of GO and the tuning of its surface chemistry can be carried out simultaneously by a facile approach.

**Figure 4.6.** Characterizations of CRGOs after phase transfer.

4.2.6 Fabrication of GO/C60 nanocomposites

The high electron affinity and superior ability to transport charge make fullerene an excellent acceptor component for solar cells. Polymer-based photovoltaic systems are applied to fabricate low-cost, lightweight, and flexible devices because they can be processed in solution. GO can be a superior electron donor due to its conjugated structure and oxygen functional groups. However, electron transfer between GO and fullerene in solution has not been achieved due to the poor dispersity of GO in low-polar solvents that can dissolve C_{60}. Here we demonstrate the unique advantages of our method for the preparation of GO/C_{60} nanocomposites by simply mixing the two contents, which would otherwise
Probing the Surface Chemistry of Two-Dimensional Nanomaterials

be not possible if GO is not tuned and transferred into an appropriate solvent. Solution mixing typically involves dispersing the additive phase (the filler), dissolving the matrix in a single or mixed solvent system, and recovering the composites via solvent evaporation or coagulation using non-solvents. This protocol usually leads to better solute dispersion than other processing. We first transfer GO into chloroform which is a good solvent for dispersing C<sub>60</sub>. The C<sub>60</sub> dispersion is then mixed with GO in chloroform, and homogeneous dispersion is obtained by bath sonication for 1 h. In UV-Vis spectra, the blueshift of the π-π* transition peaks, which indicates the electron transfer between the π electrons, is a strong evidence of π-π stacking (Figure 4.7a). The (002) peak of graphene cannot be observed in XRD spectra (Figure 4.7b), which suggests that the C<sub>60</sub> has intercalated into GO layers and disrupts its crystal structure. In Raman spectra (Figure 4.7c), the redshift of C<sub>60</sub>’s peak at 1461 cm<sup>-1</sup> also shows the electron transfer in between GO and C<sub>60</sub>. According to the height profile of AFM (blue line in Figure 4.7d), C<sub>60</sub> appears on the surface of GO monolayers, showing the thickness of 1 to 3 nm. These results illustrate that by using the same solvent (chloroform), C<sub>60</sub> self-assembles well onto GO surface by π-π stacking interactions, allowing for the fabrication of uniform nanocomposites. This approach can be extended to the preparation of other graphene-based nanocomposites such as nanopaints to provide chemical inertness, electrical conductivity and other desirable properties of graphene. For example, graphene nanopaints made from epoxy resin with graphene in low-polar solvents have found many applications in corrosion and oxidation protection.
4.2.7 Dye adsorption using CRGOs in chloroform

To be chemically inert and volatile, chloroform is widely applied in pharmaceutical industry and dye production. However, those dye impurities are usually difficult to be removed because of their good solubility in chloroform. GO is suggested to be an excellent adsorbent due to its large surface area and vast functional groups. But the applications have been limited to water purifications or polar impurity adsorption due to the poor solubility in organic solvents. Here we investigate the adsorption capacities of the transferred GO to two lipophilic dyes in chloroform. GO and CRGOs are transferred into chloroform using the above approaches and mixed with the dyes (experimental details in section 2.16). Sudan red G and Sudan red 7B are selected as model low-polar organic impurities since they are hardly soluble in water but highly dissolved in some low-polar solvents. Figure 4.8
represents the adsorption abilities of GO with different reduction degree to the dyes. In both Figure 4.8a and 4.8b, CRGOs with improved hydrophobicity performed better than unreduced GO samples. Since the adsorbed Sudan red dyes have conjugated structures, the CRGO 48h sample which shows stronger \( \pi-\pi \) stacking interactions \(^{165}\) can absorb more dye molecules. The maximum adsorption of Sudan red G is achieved to be 27.0 \( \mu g/\text{mg} \) (on transferred CRGO 48h), which is 30 times larger than that of CRGO 48h directly dispersed in chloroform (0.90 \( \mu g/\text{mg} \), Table 2.4). The transfer process prevents irreversible aggregation of CRGOs thus significantly enhances the available surface area for dye adsorption. The same situation happens to Sudan red 7B, the transferred CRGO 48h sample gained an ability to absorb Sudan red 7B of 19.6 \( \mu g/\text{mg} \) which is also 30 times larger than that of directly dispersed CRGO (0.61 \( \mu g/\text{mg} \), Table 2.4).

![Figure 4.8. Dye adsorption capacities of GO and CRGO to (a) Sudan red G and (b) Sudan Red 7B.](image)

The adsorption isotherms of Sudan red 7B are fitted with Langmuir and Freundlich model, respectively (Figure 4.9 and Table 4.1). We find Langmuir model can better fit the adsorption of Sudan red 7B on graphene. For the case of dropping abilities with increasing Sudan red G concentrations in Figure 4.9a, we speculate this is because of the positively charged methoxy groups on Sudan red G causes the aggregation of the CRGO nanosheets, decreasing the available surface for the binding. The transferred CRGOs have showed very good adsorption capacities to Sudan red dyes in chloroform. We hope this
will provide an option for solvent decontaminations but needs further exploration to improve the adsorption efficiencies.

**Figure 4.9.** Langmuir and Freundlich fitting model of Sudan red 7B adsorbed on GO (a), CRGO 24h (b) and CRGO 48h (c).

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**Probing the Surface Chemistry of Two-Dimensional Nanomaterials**
The equilibrium adsorption capacity \( Q_e \) represents the intrinsic adsorption capacities of the graphene.

The Langmuir fitting model is based on the assumption of homogeneous monolayer adsorption on graphene surface, described as below:

\[
Q_e = \frac{Q_m K_L C}{1 + K_L C}
\]

where \( Q_m \) (mg g\(^{-1}\)) is the maximum adsorption capacity based on the Langmuir model, \( K_L \) (L·mg\(^{-1}\)) is the Langmuir constant of adsorption, \( C \) is the concentration of Sudan red 7B before adsorption.

The Freundlich model is based on the assumption of heterogeneous adsorption, the equation is as below:

\[
Q_e = K_F (C)^{1/n}
\]

where \( K_F \) and \( n \) are the Freundlich constants of adsorption. \( 1/n \) is a measure for the sorption intensity.

**Table 4.1.** Summary of the fitting results of the adsorption of Sudan red 7B by GO and CRGOs in chloroform based on Langmuir and Freundlich models.

<table>
<thead>
<tr>
<th>Graphene type</th>
<th>Langmuir model</th>
<th>Freundlich model</th>
</tr>
</thead>
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<tr>
<td></td>
<td>( Q_m )</td>
<td>( K_L )</td>
</tr>
<tr>
<td>Unit</td>
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<td>[L mg(^{-1})]</td>
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</tr>
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<tr>
<td>CRGO 48h</td>
<td>24.2</td>
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</tbody>
</table>

4.3 Conclusions

In conclusions, an interface has been designed for simultaneously “pulling” and “pushing” GO into low-polar solvents. Good understanding of the surface chemistry of GO has been achieved through the detailed study of phase transfer conditions. We discover that the transfer efficiencies can be adjusted by varying the pH, lateral dimensions and reduction degree of GO. Through the chemical reduction of
GO at the interface, we can obtain CRGOs with designed surface hydrophobicity in low-polar solvents. The transferred GO shows π-π stacking interactions with C_{60} and the CRGOs are found to be promising adsorbents to dyes in chloroform. Our work provides an effective way to prepare CRGOs well dispersed in organic media with controlled surface properties, which is the key to the fabrication of multifunctional composites derived from colloidal dispersions. We hope our work can provide a universal method for the phase transfer of other nanomaterials.
Chapter 5: Switching off the interactions between graphene oxide and doxorubicin using vitamin C: combining simplicity and efficiency in drug delivery
5.1 Introduction

Cancer cells suffer from abnormal growth with high potential to invade or spread to other normal tissues.\textsuperscript{405} Nowadays more than 100 types of cancers are affecting human beings.\textsuperscript{406} This includes not only the recession in somatic function but also the torment in mind. The abnormal growth of cancer cells plunders resources and living space from neighbours, destroying human tissues.\textsuperscript{407} The current therapies are mainly the chemotherapy and the radiotherapy.\textsuperscript{408} Despite significant advances in chemotherapy, cancer still remains the major cause of death world-wide.\textsuperscript{409} Over the years, a variety of anti-cancer agents have been developed.\textsuperscript{410,411} For example, doxorubicin (DOX) can inhibit the progression of the enzyme topoisomerase II, which relaxes supercoils in DNA for transcription.\textsuperscript{218} However, it also affects normal cells through side effects or toxicities, with the most dangerous one to be cardiomyopathy, leading to congestive heart failure.\textsuperscript{412,413} There is an unmet need to fabricate well-designed nanocarriers for effective drug delivery to minimize side effects.\textsuperscript{414,415} An ideal nanocarrier should be able to deliver high doses of therapeutic drugs with ultralow toxicity to normal cells and be cancer-specific.\textsuperscript{416} However, it is quite difficult to engineer all of these attributes into one system while keeping it uncomplicated. To date, drug-encapsulated nanoparticles have been well studied as chemotherapy agents to achieve safe and effective dosage of drugs and tumour-specific targeting.\textsuperscript{416-419} However, these nanoparticles suffer from complicated synthetic procedures and high cost. The development of facile and efficient methods for the delivery of anticancer drugs is of great interest.\textsuperscript{420}

In recent years, the advances in nanoscience and technologies have enabled the design of various nanomaterials for different purposes.\textsuperscript{222,421} They are designed with different sizes, shapes and chemical composition.\textsuperscript{422} To be a single-atom thick carbon allotrope with various interesting physical and chemical properties, graphene has drawn a lot of attention over the last decade.\textsuperscript{19,423,424} To disperse graphene in water, the oxidation-exfoliation method produces an excellent derivative – graphene oxide (GO) by functionalization and exfoliation of pristine graphite.\textsuperscript{267,425} GO that incorporates vast hydrophilic groups at its edges or defects has been widely applied in wet chemistry because of its large surface area and amphipathy.\textsuperscript{41,42} Importantly, the water-dispersibility and modifiable oxygen functional groups offer many opportunities for drug delivery.\textsuperscript{188,224,426} In previous reports, GO was found to be a
promising nanocarrier for the delivery of anticancer drugs.\textsuperscript{427,428} Targeted drug delivery using specific aptamers\textsuperscript{429,430} and the synergetic effect with photothermal therapy\textsuperscript{431,432} have been reported using GO as a nanocarrier. Much effort has been devoted to improve therapeutic effect,\textsuperscript{433,434} however, a deep understanding of the surface chemistry between the drugs and GOs has not been achieved yet.\textsuperscript{435}

First of all, the biocompatibility and toxicity of GO must be confirmed through extensive \textit{in vitro} and \textit{in vivo} studies before clinical therapies. This question can be quite complicated to be explained because the structure of GO, the climate and the tested animals vary from lab to lab, and there are many factors needed to be investigated systematically. For example, GOs with less oxidation levels resulted in faster immune cell infiltration, uptake, and clearance following both subcutaneous and peritoneal implantation.\textsuperscript{436} It is proposed that surface modification or coating strategies could be helpful to improve compatibility of GO as a component of nanomedicine. Many reports have indicated the promise of GO which showed low cell toxicity, but results achieved so far are inconsistent.\textsuperscript{437-439} People agree that the surface functionalization, to improve dispersity of GO, has been shown to considerably improve their biocompatibility. PEG is a frequently used biocompatible polymer to functionalise carbon nanomaterials. It can reduce the non-specific binding to biological membranes and improve their \textit{in vivo} pharmacokinetics for better tumour targeting.\textsuperscript{427} Graphene and GO covalently modified with PEG have showed negligible in vitro toxicity to many cell lines.\textsuperscript{224,250} Furthermore, the toxicity would express once the doses of GO get too high. 20 µg mL\textsuperscript{-1} of GO was reported to induce toxicity to human lung epithelial or fibroblast cells after 24 h,\textsuperscript{440} while Wang et al. reported that more than 50 µg mL\textsuperscript{-1} of GO was toxic to human fibroblast cells.\textsuperscript{441} The cytotoxicity can arise from direct interactions between cell membranes and GO nanosheets, which are sharp and cause physical damage to the cell membranes. This can be resolved by incubating GO with fetal bovine serum which improves its protein adsorption ability.\textsuperscript{442} Surprisingly, Yang et al. found that PEGylated nGO mainly accumulated in the reticuloendothelial system including liver and spleen after intravenous administration, and could be gradually cleared without causing any appreciable toxicity to mice over a period of 3 months.\textsuperscript{443} Therefore, GO has good biocompatibility by proper treatment and is promising for \textit{in vivo} applications.
The development of an effective surface chemistry-driven on/off switch strategy is critical, as we can decipher the molecular dynamics on GO surface, enabling us to switch on or switch off the interactions for drug loading and release.\textsuperscript{365,444} Herein, vitamin C, which is also known as L-ascorbic acid (L-AA) and an essential nutrient in the repair of tissue, was used as reductant to tune the oxygenated levels of GO. We present our investigations into the non-covalent interactions between GO and DOX, followed by an evaluation of the performance of GO for both loading and release of DOX. Most importantly, surface tuning of GO was carried out to switch off the binding forces between GO and DOX, to realize the accelerated release of DOX.

5.2 Results and discussions

Figure 5.1 gives a brief view of our idea, including the loading of DOX on GO surface by non-covalent self-assembly and the controlled drug release by mild chemical reduction reaction. DOX is an amphipathic molecule with an anthraquinone-like basal plane to be hydrophobic area and terminal hydroxyl and amino groups making there hydrophilic.\textsuperscript{330} Not surprisingly, GO should be a prominent carrier of DOX molecule \textit{via} hydrogen bonding and $\pi$-$\pi$ stacking interactions. After that, we intend to achieve the release of DOX from its surface by a mild chemical treatment. Using L-AA to gradually remove the oxygen functional groups on GO,\textsuperscript{92} DOX will escape from GO sheets \textit{via} two possible mechanism. On the one hand, removal of oxygen functional groups would cut off the linking by hydrogen bond between GO and DOX. On the other hand, the decrease of surface charge\textsuperscript{41} of GO brought by oxygen removal would lead to graphene sheet restacking, which means less graphene’s surface is available for carrying DOX molecules \textit{via} $\pi$-$\pi$ stacking interactions. Both of these two weaken the binding ability of GO to DOX, leading to the faster drug release. The proposed mechanisms have been shown in Figure 5.1.
5.2.1 Surface chemistry between GO and DOX

To gain more knowledge of this drug loading system, detailed study of surface chemistry was carried out by optical spectroscopy analysis. We used attenuated total reflection – Fourier transform infrared spectroscopy (ATR-FTIR, Figure 5.2) to detect the non-covalent bonding on GO surface.101 The peak at 1615 cm\(^{-1}\) which corresponded to C=C vibrations of DOX experienced a redshift to 1620 cm\(^{-1}\) after DOX loading on GO. This was because π-π stacking interactions stabilized π-electron delocalization of GO-DOX, lowering the energy of these vibrations. To discuss oxygen functionalization, we surmised that the electron cloud density of carboxyl on GO weakened because the lone pairs of oxygen atoms donated electrons to the neighbouring hydrogen atoms. This resulted in a decrease of frequency in C=O stretching vibrations, leading to a redshift of this peak from 1725 cm\(^{-1}\) to 1716 cm\(^{-1}\).
Figure 5.2. ATR-FTIR of GO/DOX mixture. The experiments were carried out in the neutral solution (pH 7.0).

Next, fluorescence quenching gave strong evidence of π-π stacking interactions at the surface of graphene materials. The orange fluorescence (λ_{max} \sim 603 \text{ nm}) from DOX arose from the excitation of an electron from the original π orbitals to the excited π* antibonding orbitals, (i.e. π to π* transition), followed by intersystem crossing to a lower excited state. The adhesion of the aromatic region on GO sheets via π-π stacking introduced another pathway of electron transition from the excited π* orbitals to GO’s π orbitals, inhibiting the emission of fluorescence (Figure 5.3). Although GO (prior to chemical reduction) contained many oxygen functional groups, it was still able to quench this fluorescence of DOX due to π-π stacking on its hydrophobic regions.
Probing the Surface Chemistry of Two-Dimensional Nanomaterials

Figure 5.3. Fluorescence spectra of GO/DOX mixture.

In Raman spectra (Figure 5.4), we observed the typical D band and G band of GO (black curve), and the characteristic peaks of DOX (red curve) corresponding to in-plane bending motion of C-O (1262 cm\(^{-1}\)) and C-O-H, C-H bending (1433 cm\(^{-1}\)) and skeletal ring vibration (1579 cm\(^{-1}\))\(^{445}\). The peaks of both GO and DOX were obtained in the spectrum of GO-DOX (blue curve), indicating the binding of DOX on GO. Specifically, we noted that the peaks of DOX experienced red-shifts (details shown at the top-left corner) after this binding. This further indicated the electron transfer between DOX and GO, with –OH and –NH\(_2\) of DOX binding to GO’s hydrophilic groups \textit{via} hydrogen bonds while its aromatic quinone attached on GO’s conjugated domains by \(\pi-\pi\) stacking.

Figure 5.4. Raman spectra of GO/DOX mixture.
UV-Vis absorbance spectra (Figure 5.5) showed that the absorbance intensities of the GO-DOX increased due to the hyperchromic effect of GO. From the spectrum of DOX, there were two specific peaks (centered at 232 nm and 480 nm) that corresponded to π to π* transition of the aromatic domains. We found GO binding resulted in slight spectral shift of both the two peaks, which indicated another type of π electron delocalization between DOX and GO (red curve). The excitation of π electrons of DOX by UV light irradiation was affected by the nearby π electrons of GO, indicating the presence of π-π stacking. Nevertheless, we believed π-π stacking between GO and DOX was very weak as there was only a slight peak shift in the spectrum of GO-DOX (red curve, Figure 5.5). GO was highly hydrophilic with vast oxygen functional groups that hindered π-π stacking but facilitated hydrogen binding between GO and DOX. Thus hydrogen binding should be the dominating interaction. The peak shifted from 232 to 237 nm when GO was chemically reduced for up to 48 h, indicative of the improvement of π-π stacking between chemically reduced graphene oxide (CRGO) and DOX. However, due to the decrease of surface charge after chemical reduction, CRGO were aggregated more easily, driven by π-π stacking between the nanosheets. This decreased the surface area of CRGOs for DOX loading. Therefore, the binding capacities of CRGOs to DOX need to be further assessed because hydrogen bonding is weakened by chemical reduction and at the same time, the surface area of the nanocarrier decreases. In our experiments, the dispersions of CRGO-DOX were sonicated in water at pH 7.0 to prevent sheet aggregations.

Figure 5.5. UV-Vis absorbance spectra of CRGO-DOX dispersions.
To conclude this section, we have provided strong evidence of the interactions on GO/DOX interface, which are considered to be hydrogen bond and π-π stacking interaction. Combined with the large surface area of GO nanosheets, we believe GO can be an excellent nanocarrier for the anticancer drug – DOX.

5.2.2 AFM characterization

AFM was also used to investigate the non-covalent interactions. The morphologies of DOX immobilised on GO and CRGOs were displayed in Figure 5.6. GO or CRGOs were first spin coated on mica to form monolayer substrates, and then followed by dropping low-concentrated DOX solution. GO and CRGOs, which were used as the substrate for DOX loading, showed a thickness from 0.7 to 1.0 nm due to the different degree of reduction of the GO. The thickness of monolayer DOX was estimated to be around 1.5 nm through the vertical direction while lower through the other directions. The white traces with a height under 1.5 nm were all attributed to monolayers of DOX on graphene substrates. According to the height profiles through the white lines, monolayers of DOX on GO had a thickness ranging from 0.5 nm to 1.5 nm and the average height was calculated to be 1.0 nm by measuring 30 points on the white lines. The thickness of DOX on CRGOs showed a decreasing trend from 0.9 nm to 0.6 nm as a function of reduction time. This was because highly reduced CRGOs preferred to interact by π-π stacking which showed a lower height of DOX on CRGOs, rather than to bind by hydrogen bonds. The chemical reduction of GO induced an interaction transition from hydrogen bond to π-π stacking between GO and DOX, by which we were able to switch off the interactions through a controlled process.
5.2.3 *In vitro* loading and release of DOX using GO

In order to efficiently fix DOX on GO surface, we optimized the binding conditions first. In this work, all of the loading and release experiments were determined by the absorbance at 480 nm, which was used for calculations of DOX concentrations in the supernatant. Here we took GO as an example to investigate the effect of pH values for DOX loading (Figure 5.7). With the same initial mass ratio (DOX:GO = 1:1), GO gained its best loading capacity in basic solution (higher than 90 %), which was inconsistent with another work reporting neutral condition was most suitable for binding. In our
opinion, as binding of DOX led to GO sheets aggregations, the available surface area for drug loading was greatly affected by GO’s dispersity. It is well known that GO is more negatively charged in basic solution (inset of Figure 5.8), which helped it better disperse in buffer solution, thus achieved higher loading efficiency.

![Figure 5.7. The influence of pH values to the binding ability of GO.](image)

On the other hand, we investigated DOX loading with different initial mass ratios between GO and DOX as shown in Figure 5.8. The mass of loaded DOX increased linearly with increase of the initial GO/DOX mass ratios (black curve). The loading capacity can be as high as 3.5 mg mg⁻¹, which is comparable or even better than other drug carriers. However, the ratio of stably bonded drug to initial usage was found decreasing (unfilled bars), which meant it gradually reached the maximum of GO’s loading capacity. In our experiments, we set the mass ratio as 1:1 in order to achieve good loading efficiency and less DOX waste simultaneously.
Figure 5.8. The influence mass ratios of GO/DOX to the binding ability of GO.

And we also study the influence of oxygen functional groups to the loading efficiency of GO. Because the densities of oxygen groups would affect the strength of hydrogen bond, and the restoration of hydrophobicity could facilitate π-π stacking, there should be some difference in DOX loading on GO and its reduction product – CRGOs. Figure 5.9 showed the comparison for the loading capacities of graphene with different oxygen contents, which were carried out using chemical reduction of GO. We used the mild and biocompatible reductant – LAA to deal with GO dispersions at room temperature. Well control of surface oxygen groups were fulfilled via adjustment of reduction time. Under the pH value of 8.5, we found that GO was more talented for DOX loading (~83%) than any other CRGOs, which obtained weaker binding capacities as long as the reduction process. We suggest this is due to the weakening of sheet solubility brought by decrease of surface charge. Inset of Figure 5.10 indicated that the introduction of DOX on graphene surfaces did not affect their surface charge much, excluding the influence of electrostatic interactions. Thus, we suggested that the key point for DOX loading efficiency was the dispersive capacity of graphene in aqueous solution. Inspired by this, we attempted to bind DOX using GO (good drug carrier) then free it from CRGO’s surface which could be easier and faster.
To summarise the above, the basic experiment, higher DOX dosage and higher oxygen content on GO can improve the loading behaviour of DOX on GO nanosheets. However, some factors need to be considered, for example, the physical pH is around neutral (7.4) and too much drug dosage leads to high cost. Therefore in the following experiment, we mix GO and DOX within pH 7.4 and a mass ratio to be 1:1.

5.2.4 The *in vitro* release of DOX

The release behavior of DOX from GO surface *in vitro* was studied at a temperature of 37 °C under two pH values: pH 7.4 mimicking the physiological environment and pH 5.3 simulating the micro-environment in tissues of tumors. As shown in Figure 5.10, DOX was very stable on GO at pH 7.4 even the presence of lAA could slightly promote it to 4.2 %. On the other hand, the acidic condition at pH 5.3 was quite suitable for DOX release, reaching 15 % at the 48th hour. More importantly, we could observe the remarkable promotion of DOX release with addition of lAA into the system, which obtained a more than 25 % release of DOX with 24 mg of lAA (which could also be identified by the colour difference of the resulting solution in Figure 5.12b inset). As discussed with Figure 5.1, we would like to explain the functions of lAA as two parts: firstly, removal of the oxygen-containing groups would directly cut off the link *via* hydrogen bond; secondly, the restoration of hydrophobicity made CRGO
sheets restack irreversibly, significantly decreasing their surface area for binding. Thus, the real-time reduction could push DOX leaving from GO surface.

![Graph showing in vitro release behavior of DOX from GO under pH value 5.3 and 7.4, with and without L-AA.]

**Figure 5.10.** *In vitro* release behavior of DOX from GO under pH value 5.3 and 7.4, with and without L-AA.

As regular GO sheets are large in size for transportation into cancer cells, here we employed nano-scale GO (nGO) and investigated its delivery behavior of DOX. NGO has similar chemical and physical properties with regular GO, so the surface chemistry studies should also apply to the case of nGO. We found the release behaviour of DOX was similar with that of regular GO (Figure 5.11).
Figure 5.11. *In vitro* release behavior of DOX from nGO under pH value 5.3 and 7.4, with and without L-α-Ascorbic acid (L-α-AA).

The reason why drug was released fast in the first 6 h was that L-α-AA was consumed gradually. This could be explained by the proposed mechanism displayed in Figure 5.12a. In our experiment, we took only nGO+ L-α-AA as control to study the consumption of L-α-AA (shown in Figure 5.12b). And the inset photograph was placed here to show the colors of resulting drug delivery solution. Obviously, the sample with pH 5.3 and incubated with L-α-AA could release more DOX than others.

**Figure 5.12.** (a) A proposed mechanism for the reduction of epoxide and dihydroxyl groups with L-α-Ascorbic acid (L-α-AA) and (b) relative densities of L-α-AA in the control sample over time (inset: digital photograph of the drug delivery system after 48 h).
In summary of this, GO-DOX composite could release drug molecules faster in acidic condition than in neutral condition, furthermore, the introduction of LAA facilitated this release behavior, especially in the beginning hours. Therefore, our design was confirmed to work well, and next we would try cellular tests to find out whether it’s working in real cells.

5.2.5 Cellular viability

In vitro cell toxicity of the drugs was evaluated by MTT assay. Before the application of nGO with the cancer cells, PEG was introduced onto nGO nanosheets via carbodiimide catalyzed amide formation to evaluate its potential cellular toxicity. FTIR and UV-Vis spectra confirmed the covalent binding between the carboxylic on nGO and amino groups of PEG to achieve the resulting nGO-PEG (Figure 5.13).

![Figure 5.13](image)

Figure 5.13. (a) ATR-FTIR spectra of nGO and nGO-PEG; and (b) UV-Vis absorbance spectra of nGO, PEG, nGO-PEG and nGO-PEG-DOX.

The relative cell viability of the HT29 colorectal cancer cells using DOX with different concentrations after drug treatment for 48 h was demonstrated in Figure 5.14. As illustrated by the black curve in Figure 5.14a, the nanocarrier itself (nGO-PEG) was non-toxic to the cancer cells at a concentration up to 10 mg/L. Work by others has shown that high dosage of L-AA (over 100 µM) can result in
significant cytotoxicity. However, the concentration of L-AA used in our work is under 20 µM, at which there is only marginal impact on cell viability (blue curve in Figure 5.14a). Therefore, the toxicity of the nGO-PEG drug conjugates (red curve) is most likely attributed to the released DOX, rather than the reductant (L-AA) or nGO-PEG. In Figure 5.14b, all of the three different forms of DOX have demonstrated toxicity to the HT29 colorectal cancer cells. In this experiment, the concentration of DOX loaded on nGO-PEG-DOX and nGO-PEG-DOX-L-AA was kept the same as that of free DOX. To begin with, the free DOX was found to have an increasing toxicity with its dosage increasing from 0 to 30 µM, exhibiting a 50% growth inhibition concentration (IC50) of ∼1.2 µM. The nGO-PEG-DOX (Figure 5.14b, blue curve) showed a lower toxicity compared to the free DOX sample, with an IC50 of 5.4 µM. Given that only the released free DOX can interact with DNA molecules to elicit cytotoxicity, the lower toxicity of nGO-PEG-DOX was because less DOX (around 12% according to dark yellow curve in Figure 5.11) was released from nGO-PEG surface at pH 5.3 in 48 h. In fact, the IC50 of the released DOX from nGO-PEG-DOX was estimated to be about 0.8 µM, indicating higher toxicity than free DOX sample. Free DOX suffered from an export effect of drug efflux pumps in drug-resistant cells, thus expressing low toxicity.449 It was reported that GO could enter cancer cells and stay in the nucleus by both non-specific endocytosis and passive diffusion, realizing efficient intracellular DOX release by avoiding being expelled.447,450 In order to improve the drug potency, L-AA was added into the nGO-PEG-DOX system (red curve in Figure 5.14b) to accelerate DOX release in cells. As expected, it presented an improved toxicity than that of nGO-PEG-DOX to the cancer cells, with an IC50 of ∼3.4 µM. These results are consistent with our initial hypothesis that surface tuning by the addition of L-AA facilitates DOX release from carrier surface. To summarize, nGO-PEG-DOX can be an effective nanocarrier of DOX to overcome drug resistance of cancer cells. DOX binds to nGO-PEG tightly at physiological pH but releases fast in cancer cells after endocytosis. The acidic condition in the endosomal-lysosomal compartment is likely to be the main driving force for the release of DOX inside the cancer cells while the introduction of L-AA results in more release of DOX, thus conferring higher toxicity.
5.3 Conclusions

In conclusion, we present a facile method to improve the release efficiency of DOX from GO surface. Based on our understanding of the surface chemistry, DOX self-assembled on GO surface with a high concentration. Spectroscopies and AFM images have provided strong evidence of the non-covalent interactions between GO and DOX. L-AA, which can tune the surface chemistry of GO, was used to facilitate DOX release from the carrier surface. Our work demonstrates a universal approach to achieve drug delivery by tuning the surface chemistry of the drug carriers. We expect such an approach can expand the applications of GO in some other areas.

Figure 5.14. *In vitro* cell toxicity assay with different drug concentration.
Chapter 6: Radicals on boron nitride nanosheets
6.1 Introduction

Besides graphene materials, boron nitride nanosheet (BNNS) has drawn a lot of attentions due to their interesting two-dimensional (2D) structure. The success to mechanically exfoliate bulk boron nitride (BN) gave rise to BNNSs which possess some difference with graphene.

To begin with BN, which is a compound of equivalent boron and nitrogen atoms with excellent thermal and chemical stability, there are various crystalline forms of BN which are isoelectronic to carbon lattices. The lattice of BN follows elemental carbon structures by sharing the delocalized electrons among the conjoint atoms. Like the case of graphite and diamond which are two typical carbon allotropes, the hexagonal form of BN (h-BN) corresponding to graphite is most stable and soft among BN polymorphs, thus it is applied to the lubricant and additives to cosmetic products. The cubic (sphalerite structure) variety analogous to diamond is called c-BN, which is softer than diamond but with better thermal and chemical stability. Due to this prominent thermal and chemical stability, BN is traditionally applied in high-temperature equipment. h-BN became more popular in recent years ever since the exfoliation of single carbon layer – graphene.

BNNSs are monolayers of BN and nicknamed as “white graphene” because some of their physical properties are comparable with those of graphene. For example, the calculated thermal conductivity value for BNNSs ranges from 300-2000 W m\(^{-1}\) K\(^{-1}\) which is just a little lower than that of graphene (1500-2500 W m\(^{-1}\) K\(^{-1}\)). This difference is due to softer phonon modes of BNNSs and the mass difference between B and N atoms. BNNSs are supposed to gain a higher thermal conductivity than few-layered ones because of the reduced interlayer phonon scattering. However, due to the large bandgap (~4-6 eV), BNNSs are insulating materials while graphene with zero bandgap has excellent electrical conductivity. Graphene has sublattice symmetry (2 C in a unit cell) which protects the Dirac point in its band structure, and thus is gapless. On the other hand, in BNNS the sublattice symmetry is broken (1B and 1N in a unit cell), which opens the band gap and makes it insulating. Apart from that, BNNS is chemically inert and thermally stable. It is insoluble in normal acids, only alkaline molten salts and nitrides can dissolve and etch BN. h-BN is only decomposing at temperatures up to 1000 °C.
in air, 1400 °C in vacuum, and 2800 °C in an inert atmosphere. Generally speaking, BNNSs are more stable than graphene under high temperature and other tough conditions.

Similar with graphene preparation, BNNSs can be obtained through top-down or bottom-up methods, in which h-BN are exfoliated into layered structure or the nanosheets are built from boron and nitrogen precursors, respectively. In the top-down approach, the task is to separate BNNSs from each other by overcoming the strong lip-lip interactions between layers, and it is very challenging to achieve complete or high degrees of exfoliation. The low solubility of 2D materials is attributed to their extremely high surface energy, which translates into a strong Van der Waals interaction, and thus a high restacking tendency. In the case of BNNSs, the restacking tendency is more intense because the electron-deficient boron atoms and electron-rich nitrogen atoms lead to a very strong “lip–lip” interaction. Introducing functional groups onto the surface usually helps with sheet exfoliation, however, due to the high chemical stability and insolubility in acid, the Hummers’ method cannot work with BN to achieve “BN oxide” just like work done on graphite. To date, hydroxyl, amino and some other hydrophilic groups have been successfully introduced onto BN nanomaterials to achieve sheets exfoliation. As one typical example using mechanical cleavage, ball milling has been developed to synthesize BN nanoflake (BNNF) and BN nanoparticle (BNNP) from bulk BN. Lei et al. fabricated BN colloidal solutions through a one-step exfoliation and functionalization. NH₂ groups appeared at the edges of BNNFs to give good water dispersity. In another work, BNNPs were directly exfoliated into BNNSs in water due to the sonication-assisted hydrolysis. Hydroxyl groups were introduced to the edges or defect of the nanosheets to realize the dispersibility in water in which BNNSs can be dispersed as mono or few layers rather than to be precipitations. On the other hand, the bottom-up method mostly refers to chemical vapor deposition (CVD). Generally, the CVD precursors for BNNSs could be either separate B and N compounds (e.g. BF₃-NH₃, BCl₃-NH₃, B₂H₆-NH₃) or a single one (e.g. BH₃-NH₃, B₃N₃H₆). The advantage of CVD methods is to be able to prepare large area (up to few cm²) and transferrable BNNSs with less defects.

Free radicals are widely used in many fields such as polymerization, biomedicine and catalysis. However, many of the radical species used by industry are costly, toxic and unstable. To produce stable
probing the surface chemistry of two-dimensional nanomaterials

radicals is of great interest in designing electro-catalysts and initiating of organic chemistry. The challenge is to prevent individual radicals from self-coupling. Graphene is reported as a carrier of free radicals which locate at edges or defective sites of the nanosheets. Both theoretical and experimental study have confirmed the presence of the radicals on graphene. The reaction between individual radicals can be hindered by the planar structure of graphene where the skeleton is physically rigid. The structural defects and π electrons on basal plane of GO are attributed to the free radicals. Having similar structure with graphene, the rigid 2D structure of BNNSs could make it physically difficult for radicals to react with each other, therefore, BNNSs may also serve as the good carrier of free radicals. BN nanotubes were used as substrates for polymer grafting by a surface initiated atom transfer radical polymerization. But the initiators were immobilised on BNNTs prior to polymerization rather than to directly use the pristine radicals on the nanotubes. In another work, BN nanoplatelets have been developed as a solid radical initiator for the aerobic oxidation of thiophenol to diphenyldisulfide. Radical scavenging by N-tert-butyl-α-phenylnitrone support an autoxidation mechanism involving thyl radicals. Using BN catalysts, selective oxidative dehydrogenation of propane to propene has been reported, which also indicates there are active sites on BN. Recently, porous BN was found to be catalytically active in acetylene hydrochlorination due to the ability of polarizing and activating acetylene by its armchair edges. However, the origin of the active sites has not been achieved yet. Recently we reported the biocompatibility of BN nanomaterials is affected by the unsaturated B atoms located at the edges or defects of BN. However, there was no direct evidence showing what the real radical is.

In this work, we investigated the radicals on different BN nanomaterials. Ball milling was first applied to prepare BNNPs and BNNFs with different functional groups from bulk BN powders. By measuring powder samples, radicals can only be detected on BNNPs which have a lot of structural defects and smaller dimension. The dimension reduction was achieved by ultrasonication and solvothermal reaction to obtain BNNSs and BN quantum dots (BNQDs) in DMF. A radical scavenging method was used for the detection of radicals on the prepared BN solutions, and it was found that the density of free radicals improved obviously with the decrease of the lateral dimensions.
Furthermore, the results of UV-Vis spectra and FTIR have showed that the free radicals are attributed to the unsaturated boron atoms which came from the cleavage of B-N bonds during exfoliation and solvothermal reaction. Furthermore, the free radicals were investigated by catalysing oxidization of 3,5,3’,5’-tetramethylbenzidine (TMB)\textsuperscript{470} and co-reaction in the electrochemiluminescence (ECL)\textsuperscript{471} of a ruthenium complex.

6.2 Results and discussions

It has been reported by the theoretical study that the free radicals exist on the zigzag edges of graphene nanoribbons and these radical electrons are strongly promoted by stronger π conjugated area.\textsuperscript{315} The radicals on GO is supposed to be phenalenyl-like radicals and irradiation-induced hydroxyl radicals due to the break of covalent bonds at edges or defects. Similarly, the cleavage of B-N bonds provides opportunities for the production of boron and nitrogen radicals due to ball milling or sonication. Edge functional groups such as OH and NH\textsubscript{2} groups could affect the active sites producing free radicals. Figure 6.1 described the main contents carried out to confirm the presence of boron radicals on BNNSs and the promising applications of radicals in redox reactions and ECL co-reactions.

![Figure 6.1. Main contents in this study of free radicals on BNNSs.](image_url)
6.2.1 Synthesis of BN nanomaterials with different dimensions

Figure 6.2 shows the scanning electron microscope (SEM) and atomic force microscope (AFM) images of BN nanomaterials with different degree of exfoliation. Bulk BN was first exfoliated by ball milling under different experimental conditions to obtain the first stage products – BNNP-OH, BNNF-OH and BNNF-NH$_2$. The suffixes indicate the functional groups at edges or defect sites of the BN nanomaterials. According to the SEM images, BNNP-OH (Figure 6.2a) has a spherical shape with diameters ranging from 100 to 200 nm. BNNF-OH (Figure 6.2b) and BNNF-NH$_2$ (Figure 6.2c) exhibit flake-like structure with a lateral dimension up to 1 μm. These were further processed with ultrasonication to achieve BNNSs dispersed in water or DMF with much smaller sizes. Figure 6.2d-f are the AFM images and height profiles of BNNS-1, BNNS-2 and BNNS-3 obtained by the materials in Figure 6.2a-c, respectively. After strong and long-time sonication, all of them showed a thickness of around 0.9 nm which indicated monolayer of BN has been exfoliated. Interestingly, BNNS-1 (Figure 6.2d) showed a similar lateral dimension with that of BNNS-2 (Figure 6.2e) which ranged from 50 to 200 nm while BNNS-3 (Figure 6.2f) had a relative larger size up to 400 nm. The presence of amino groups provides BNNS-3 with better dispersing ability than BNNSs with hydroxyl groups, therefore, larger nanosheets can well disperse in water and will not be removed by centrifugation process. Finally, BNQDs that were considered to have very small diameter and suffered from most bond cleavages, were synthesized via solvothermal method in DMF. AFM images (Figure 6.2g-i) represented that the prepared BNQDs were much smaller in size (less than 4 nm) than BNNSs. Therefore, we have obtained various BN nanomaterials with different morphologies, dimensions and functional groups, by which we are able to investigate whether there are radicals on BN nanomaterials and what affects the radical density.
6.2.2 Detection of the radicals on BN nanomaterials using ESR spectra

The radicals on BN materials should come from the broken chemical bonds at edges or defects, producing boron or nitrogen radicals. ESR spectra were used to measure the densities of free radicals both in solid state and in solutions. To begin with powder samples, we tested the ESR of bulk BN and partially exfoliated BNNP-OH, BNNF-NH2 and BNNF-OH (Figure 6.3a). We found that only BNNP-OH (red curve) presented a weak and wide ESR peak between 3400 and 3500 gauss. These results
indicate that the shape and size play an important role in densities of active sites on the materials. This is consistent with our previous report,\textsuperscript{468} in which work BNNP-OH induced stronger reactive oxygen species (ROS) than other types of BN. It was supposed that unsaturated boron atoms appeared more at the surface of BNNP-OH and accounted for the cell death (Figure 6.3b). The smaller size and defective spherical structure should be the main cause of the stronger radical density on the nanoparticles. On the other hand, BNNF functionalised by both OH and NH\textsubscript{2} groups did not show ESR peaks. There was a very low edge to surface ratio on the nanoflakes, thus the cleavage of B-N bonds, which happened mostly at edges, was greatly inhibited. As for powders, it seems the morphology of BN decided the density of radicals rather than the functional groups.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.3.png}
\caption{ESR spectra of solid-state bulk BN, BNNP-OH, BNNF-NH\textsubscript{2} and BNNF-OH, respectively; (b) the supposed structure of BNNS, BNNT and BNNP.}
\end{figure}

As described above, BNNP-OH, BNNF-OH and BNNF-NH\textsubscript{2} were further exfoliated into BNNSs by ultrasonication in both water and DMF. BNNS-1 and BNNS-2 were functionalised with OH groups while BNNS-3 was terminated with NH\textsubscript{2} groups. Followed by that, BNQDs were synthesized by solvothermal method in DMF. All of these dispersions were used for ESR tests using a radical scavenging method which detected the density decrease of DPPH free radicals. The nitrogen radical on
DPPH is able to react with one boron radical covalently at edges or defects of BNNSs. This has been investigated by computational simulation (Figure 6.4). As a result, the peak intensity of DPPH in ESR spectra would suffer from a drop due to the quenching of the free radicals.

![Initial structure](image1)

![Optimized structure](image2)

**Figure 6.4.** Computational study of the DPPH anchored on BNNS.

ESR spectra were measured using the mixtures of DPPH and dispersions of different BN nanomaterials. The results are displayed in Figure 6.5. ESR spectrum of DPPH showed 5 sets of symmetric peaks between 3430 and 3500 gauss. Taking Figure 6.5a as an example, free radicals on BNNP-OH were compared with those on its exfoliated forms – BNNS-1 and BNQD-1. The concentrations of BN were kept the same (0.1 mg mL\(^{-1}\)) in each mixture. Using water as the control, BNNP-OH dispersed in water showed a decrease of DPPH radicals (red curve). BNNS-1, which was mono-dispersed in water, was able to provide stronger radicals than BNNP-OH. Furthermore, BNQD-1 was found to scavenge all of the DPPH radicals in the solution, indicating a very strong density of radicals on it. This verified our speculation that smaller dimension of BN leads to stronger radicals on its surface. Firstly, to exfoliate BNNP-OH into BNNS-1 significantly improved their surface area for binding DPPH. Secondly, the cleavage of B-N bonds generated more structural defects to stabilize the boron radicals. The decreasing tendency of radicals by narrowing BN dimensions appeared similarly in the other BN nanomaterials in both water and DMF (Figure 6.5b-d). However, we observed that BNNF-OH (red in Figure 6.5b) and
BNNF-NH$_2$ (red in Figure 5c) were almost free of detected radicals, which is consistent with the results of powder samples. Due to the similar morphology with BNNS-1, BNNS-2 (green in Figure 6.5b) showed a similar capacity to scavenge DPPH radicals with that of BNNS-1 (green in Figure 5a). The density of radicals on BNNS-3 (green in Figure 6.5c) was a little weaker than the others due to its relative larger lateral size. In the blue curve of Figure 6.5a-c, the radicals on BNQDs were measured by mixing DPPH in ethanol and BNQDs in DMF and found to be very strong. In order to exclude the influence of solvents, both DPPH and BN nanomaterials were dispersed in DMF and the ESR spectra were measured by the same method (Figure 6.5d-f). Similar results were obtained as all of the spectra all showed decreasing peak intensity by narrowing the dimensions of BN materials. Compared with BNNFs in water, BNNF-OH and BNNF-NH$_2$ in DMF (Figure 6.5e and 6.5f) expressed a stronger density of free radicals. This could be due to the better dispersity of BNNF-OH and BNNF-NH$_2$ in DMF than those dispersed in water, which prevented the nanoflakes from precipitations. As a result, we believe the radicals on BNNSs are mostly affected by their lateral dimensions and the functional groups contribute to their dispersity in solvents but rarely affect the active sites.
In order to quantify the density of radicals on these BN nanomaterials, we built up the standard curve of DPPH in water/ethanol (1:1) mixture (Figure 6.6a) and in DMF (Figure 6.6b) based on the density differences of the central peaks in ESR of DPPH. It was found that the ESR intensity of DPPH was a
little weaker in water/ethanol mixture than in DMF due to the poor solubility of DPPH in water. The inset equation shows the linear relation of ESR intensity to DPPH concentrations.

Figure 6.6. Standard curves of the intensity difference of the central peaks in ESR spectra of DPPH in (a) water/ethanol (1/1) mixture and (b) DMF.

Therefore, we were able to estimate how many DPPH radicals have been scavenged by adding certain amount of BN nanomaterials. Taking BNNP-OH in water as an example, the intensity difference between the two central peaks was 8.1 which can be read from red curve in Figure 6.5a. So the remaining and scavenging radical densities of DPPH were calculated to be 0.27 mM and 0.23 mM via the standard curve. Since the applied concentration of BNNP-OH was 0.1 mg mL⁻¹, the radical density on BNNP-OH was calculated to be 2.3 mmol/1g BNNP-OH. The density of radicals on the other BNs was calculated by the same method. Table 6.1 summarized the density of free radicals on different BN nanomaterials in both water/ethanol mixture and DMF. We would like to declare that BNNS-1 and BNQD-1 will be used as model samples for the rest of the experiments since the morphologies and radical strengths of BNNSs and BNQDs are very similar.
Table 6.1. Summary of the results of radical density on BN nanomaterials.

<table>
<thead>
<tr>
<th>BN category</th>
<th>Radical density in water/ethanol mixture (mmol g⁻¹)</th>
<th>Radical density in DMF (mmol g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNNP-OH</td>
<td>2.3</td>
<td>3.31</td>
</tr>
<tr>
<td>BNNS-1</td>
<td>3.39</td>
<td>4.03</td>
</tr>
<tr>
<td>BNQD-1</td>
<td>&gt; 5.0</td>
<td>&gt; 5.0</td>
</tr>
<tr>
<td>BNNF-OH</td>
<td>0.23</td>
<td>1.43</td>
</tr>
<tr>
<td>BNNS-2</td>
<td>3.67</td>
<td>3.01</td>
</tr>
<tr>
<td>BNQD-2</td>
<td>&gt; 5.0</td>
<td>&gt; 5.0</td>
</tr>
<tr>
<td>BNNF-NH₂</td>
<td>1.16</td>
<td>1.52</td>
</tr>
<tr>
<td>BNNS-3</td>
<td>3.40</td>
<td>3.64</td>
</tr>
<tr>
<td>BNQD-3</td>
<td>&gt; 5.0</td>
<td>&gt; 5.0</td>
</tr>
</tbody>
</table>

UV-Vis spectra and ATR-FTIR was used to confirm the covalent binding between DPPH and BN nanomaterials. When the active nitrogen radical of DPPH approaches the radicals on BN, a covalent bond will form as indicated in Figure 6.4. Because of a strong absorption band centred at 520 nm (Figure 6.7a, black curve), DPPH radical has a deep violet colour in DMF. This colour will turn to be pale yellow when the radicals are totally neutralized (inset of Figure 6.7a). This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm. We observed BNNS-1 weakened and shifted the peak while BNQD-1 had much stronger influence on the spectrum of DPPH, shifting the peak to about 430 nm. This agreed with the above results describing the BNQD-1 gained more free radicals than BNNS-1. On the other hand, we investigated the FTIR of BNNS-1 before and after DPPH binding (Figure 6.7b). The N-O bending (880 cm⁻¹) and C-H stretching (2853 and 2924 cm⁻¹) vibration on DPPH did not shift after binding indicated that DPPH was not fixed on BNNS-1 via its conjugated structure or nitro groups. The black curve showed the typical B-N stretching peak at 1377 cm⁻¹ and a broad O-H peak around 3300 cm⁻¹. After binding with DPPH, the B-N stretching peak experienced an obvious red shift to 1405 cm⁻¹.
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1, indicating a chemical binding generated at BN skeleton rather than on the functional groups. Nitrogen radicals on BN are unstable and may be terminated with H. Therefore, boron radicals should be the only explanation for the active sites on BN nanomaterials, and this has been simulated by computational study.

Figure 6.7. UV-Vis spectra (a) and ATR-FTIR (b) of DPPH before and after mixed with BN nanomaterials.

6.2.3 The catalytic activity of BNNS-1

The catalytic activity of BNNS-1 was studied using a well-known chromogenic reaction – the oxidization of TMB. The reaction results in a colour change from colourless to blue when converts amino groups to imino groups, and usually is driven by horse radish peroxidase. Herein, BNNS-1 was applied to realize a non-enzymatic oxidization of TMB which was shown in Figure 6.8. In the photographs, the blue colour became much more obvious when using higher concentrated BNNS-1 for 3 h in dark (Figure 6.8a, from left to right), which means the oxidization degree of TMB was higher. UV-Vis spectra (Figure 6.8b) were also applied to study this reaction. Before and after keeping the sample in dark for 3 h, the peak centred at 650 nm, which is a typical character of TMB oxidation, had an increase of intensity. In fact, this chromogenic reaction needs another reactant which is H₂O₂. We
speculated that a small amount of hydroxyl radicals could be released from BNNS-1 due to the cleavage of B-OH bonds by ultrasonication.

**Figure 6.8.** Photographs and UV-vis spectra of TMB+BNNS-1 mixture with increasing concentrations of BNNS-1 after reaction 3 h in dark.

We also investigated this reaction at the presence of H₂O₂. Either BNNS-1 or H₂O₂ was able to cause colour change and increase of absorbance at 650 nm after 3 h. Without catalysts, TMB reacted with H₂O₂ slowly (red curve in Figure 6.9a). When added both of them, the colour turned to be yellow which was due to the formation of a stable state of TMB with a carbonyl group on each benzene ring. We observed an obvious increase of the absorption at 470 nm in the pink curve, corresponding to the yellow colour (Figure 6.9a). Figure 6.9b is a kinetic study of TMB oxidation within 3 h measuring the absorption increase at 650 nm as a function of time. We found that the presence of BNNS-1 was able to speed up this reaction, almost finishing in 80 mins. The cooperation of BNNS-1 and H₂O₂ could improve this absorption by 33% in which process we supposed BNNS-1 worked as a catalyst for TMB oxidization (inset of Figure 6.9b).
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Figure 6.9. (a) Photographs and UV-vis spectra of TMB, TMB+BNNS-1, TMB+H₂O₂ and TMB+H₂O₂+BNNS-1; (b) kinetic of TMB oxidization (absorbance at 650 nm) with BNNS-1 and H₂O₂ (inset shows the mechanism of TMB oxidization).

6.2.4 ECL of [Ru(bpy)₃]²⁺ improved by BNNSs and BNQDs

ECL is an electrochemical method to generate measurable luminescent signals when given a proper potential, in which process electrical energy is converted into radiative energy at the surface of electrodes. It is well reported that [Ru(bpy)₃]²⁺ is a classical complex emitting a strong ECL when co-reacts with some radical holders, such as TPA. Ru²⁺ is firstly oxidized as the voltage of around 1.2 V to obtain Ru³⁺ which is excited by the free radicals of TPA molecules. Then the excited Ru²⁺* will jump back to the ground state and release an orange luminescence at the same time.

Based on the above results, boron radicals are confirmed to be located on BNNSs and BNQDs. These radicals could provide opportunities for the application of BN as co-reactive reagents in ECL of [Ru(bpy)₃]²⁺. Accordingly, we investigated the effect of BNNS-1 to the ECL of [Ru(bpy)₃]²⁺. This ECL behaviour was studied in the presence of increasing concentrations of BNNS-1 (from 3 to 30 µg/mL as shown in Figure 6.10) When the potential was cycled between -0.1 and 1.6 V, an obvious ECL emission at 1.2 V was observed. The addition of BNNS-1 led to an obvious increase of ECL signals (Figure 6.10a). The ECL intensity of [Ru(bpy)₃]²⁺ tripled when 30 µg/mL of BNNS-1 was used (Figure 6.10b). This reflected that BNNS-1 could be an efficient coreactant for the ECL of [Ru(bpy)₃]²⁺. The supposed
anodic mechanism is described with the following equations. Generally, the boron radicals (·BN) was electrochemically oxidized to ·OOBN through the participation of dissolved oxygen (O₂). On the other hand, Ru²⁺ was first oxidized on anode to Ru³⁺ which further reacted with the radical holders (·BN or ·OOBN) to produce Ru²⁺ in excited state. Finally, this complex went back to its ground state with an emission of measurable luminescence.

\[
\cdot BN + O_2 \rightarrow \cdot OOBN
\]

\[
[Ru(bpy)_3]^{2+} - e^- \rightarrow [Ru(bpy)_3]^{3+}
\]

\[
[Ru(bpy)_3]^{3+} + \cdot BN \rightarrow [Ru(bpy)_3]^{2+*} + BN
\]

\[
[Ru(bpy)_3]^{3+} + \cdot OOBN \rightarrow [Ru(bpy)_3]^{2+*} + BN + O_2
\]

\[
[Ru(bpy)_3]^{2+*} \rightarrow [Ru(bpy)_3]^{2+} + hv
\]

**Figure 6.10.** (a) ECL of [Ru(bpy)_3]²⁺ with increasing BNNS-1 concentrations; (b) ECL intensity at the oxidation peaks.
Using BNQD-1 as a co-reactant, we found the improvement of ECL intensity was much stronger (Figure 6.11). However, this needs to be further investigated and the true mechanism is not clear now. Whether BN was co-reacting with ruthenium complex or acted as a carrier of the ECL luminophors is important in this work.

![Graph showing ECL intensity vs. concentration](image)

**Figure 6.11.** (a) ECL of [Ru(bpy)$_3$]$^{2+}$ with increasing BNQD-1 concentrations and (b) ECL intensity at the oxidation peaks.

### 6.3 Conclusions

BN nanomaterials with different dimensions and functional groups have been synthesized by ball milling, ultrasonication and solvothermal reaction. SEM and AFM images showed that BN has been exfoliated step by step. A scavenging method was applied to detect the radicals on BNNSs and BNQDs. Boron radicals are confirmed to be responsible for the active sites on exfoliated BN. To further confirm the presence of free radicals, BNNS-1 was used to catalyse the oxidization of TMB while BNQD-1 was found to be able to markedly improve the ECL of [Ru(bpy)$_3$]$^{2+}$. 
Chapter 7: Summary and perspectives
The surface chemistry of 2D nanomaterials has been studied in detail in this thesis. The presence of oxygen functional groups endow GO with various interesting properties in addition to the excellent nature of graphene. This functionalization not only provides good water solubility and biocompatibility to GO, but also presents active binding sites in defective area. Accordingly, GO is widely applied in wet chemistry for various applications (e.g., drug delivery). However, deep understanding of the surface chemistry and the quantification of the level of oxygen contents of GO are very limited. This study started with probing the amphipathic surface of GO, in which we gained better understanding of GO surface chemistry and tunability. Based on the method developed for the surface tuning, we explored a universal approach to transfer 2D nanomaterials into low-polar solvents, and developed an efficient method for the controlled delivery of DOX. On the other hand, we investigated the boron radicals on the surface of BNNS which is another interesting 2D nanomaterial and explored the potential applications of the detectable free radicals.

7.1 Probing the tunable surface chemistry of GO

In order to have better understanding of the surface of GO, I first studied its tunable surface chemistry by functionalizing GO with a ruthenium complex. The synthesis of GO and the following reduction to prepare CRGOs have been widely used in laboratories all over the world. Research on modified Hummers’ Methods for graphite oxidization and various reduction approaches for GO reduction has made the properties of GO varied from lab to lab. Determination of amount of different oxygen-containing groups, hydrophobicity of GO surface and the evaluation of the efficiency of chemical reactions are still difficult tasks because of heterogeneous structure of GO. The non-covalent interactions are very useful in solution chemistry of GO because they are easy to achieve and tunable. This work provided a common approach to study the surface interactions on GO using a ruthenium complex as the probe molecule. Two non-covalent interactions which are electrostatic interaction and π-π stacking were confirmed by zeta potentials and spectroscopies. More importantly, we could carry out an interaction transition on GO surface from electrostatic interaction to π-π stacking by simple chemical reduction. This work has already been published in Chemical Communications.
This study provides a general idea of probing the surface groups and interactions on 2D nanomaterials in aqueous solution. We speculate that it can be extended to the surface chemistry study of boron nitride nanosheets or other 2D nanomaterials. The confirmation of interactions is very important when self-assembling functional molecules on dispersed nanosheets in wet chemistry. On the other hand, we present a method to tune the interactions on GO surface by controlled chemical reduction. This can make the surface interactions on 2D materials switchable. Therefore we are able to switch on and off the interactions by simple solution treatment. The understanding of GO surface chemistry is the basic throughout the whole thesis.

7.2 Phase transfer of GO into low-polar solvents via a designed interface

Based on the deep understanding of the surface chemistry, I designed a unique interface for simultaneously “pushing” and “pulling” GO into organic phase. It is well known that GO has excellent water dispersity due to the presence of oxygen functional groups, but no solubility in low-polar solvents, such as hexane and chloroform.

To disperse GO in low-polar solvents can realize a perfect self-assembly with functional molecules and apply for removal of organic impurities, which only dissolve in low-polar solvents. The surface chemistry of GO plays an important role in its dispersibility in these solvents. Until now, it is still an experimental challenge to directly transfer hydrophilic GO into low-polar solvents. In this study, we designed an interface to transfer GO by simultaneous pushing and pulling the GO and CRGOs into low-polar solvents. This approach is outstanding due to its ability to obtain mono-dispersed CRGOs with designed surface properties in organic phase. Using the transferred graphene dispersions, we have successfully fabricated GO/C60 nanocomposites and have assessed the ability of CRGOs for dye adsorptions.

This work aims to provide a common approach for the phase transfer of 2D nanomaterials. The idea of particle transfer using chemical reaction is very interesting and makes it possible to finish phase transfer and surface tuning in one step. Good understanding of the interface chemistry has been achieved, however, the follow-up applications should be explored. For example, the well interacted GO/C60 nanocomposite has been successfully prepared in chloroform but fabrication of the solar cells needs
much more efforts by using the nanocomposite. On the other hand, the adsorption of dyes on CRGOs is found to have poor efficiency which adsorbs less than half of the whole concentration. Tuning or functionalization of CRGO surface may help to improve this. To summary, I hope our results can provide a facile and effective method for people who use graphene in low-polar solvents.

7.3 Switching off the interactions between GO and DOX using vitamin C

Based on the understanding of GO surface chemistry and the ability to tune the surface hydrophobicity, I developed a new drug delivery vehicle using GO, in which DOX can be efficiently loaded on GO surface by non-covalent bonding, and controllably delivered into cancer cells by adding a little amount of vitamin C.

Cancer therapy is always a crucial task because human beings suffer from agony or even death from cancers. Although the bullets are ready to destroy cancer cells, there are still many problems to be resolved, such as drug dosage and side effects. Well-controlled, low-toxic and high-efficient delivery of anticancer drugs is the key approach to chemotherapy of mortal tumors. GO has been recognized to be a great nanocarrier of anticancer drug because of its large surface area, excellent water dispersibility and biocompatibility. In this study, GO is applied for loading DOX by non-covalent interactions and released by switching off the interactions via controlled chemical reduction. The surface chemistry of the drug delivery system was studied in detail using spectroscopy and AFM. Hydrogen bond and π-π stacking interactions were confirmed happened between GO and DOX, and then the tuning of surface chemistry was achieved by chemical reduction in order to improve the release of DOX from GO surface.

In this chapter, I emphasize the significance of understanding the surface chemistry between GO and DOX in aqueous solution, and discovered a method to switch off the interactions for drug delivery. I wish this study could enlighten whoever works on drug delivery using nanomaterials where facile and mild chemical reactions could facilitate the release of drug from carrier surfaces.

7.4 Radicals on BNNSs

The radicals on BNNSs have been carefully investigated in this part of work. To be 2D nanomaterials, BNNSs have gain many attentions due to their similar planar structure with graphene. They are
considered to be chemically inert because of the strong energy of B-N bonds, however, BNNSs with low longitudinal size exhibit catalytic activities. To figure out what are the active sites on BNNSs is of great interest for good understanding of their potential applications. In my work, BN nanomaterials with different dimensions and functional groups have been synthesized by ball milling, ultrasonication and solvothermal reaction. A scavenging method was applied to detect the radicals on BNNSs and BNQDs. Boron radicals are confirmed to be responsible for the active sites on exfoliated BN. Because of the presence of free radicals, the oxidization of TMB can be catalysed by BNNSs while BNQDs are good co-reactants in ECL reaction of [Ru(bpy)3]2+.

This study provides valuable information of BNNS surface chemistry, more importantly active radicals have been confirmed on BNNS surface for the first time. This finding can inspire work due to the active sites on BNNSs. The radicals on BNNS may be used to initiate polymerizations or catalyse some certain chemical reactions.

7.5 Perspectives

To incorporate GO into industry is an important and genuine questions for scientists. The study of surface chemistry is of great interests within 2D nanomaterials. It can be a good guide for the design of a device or a useful knowledge to conduct a complicated process. By studying the surface chemistry of GO, we are able to know well about what’s on its surface and what’s happening there. Although our work and literatures have made big progress in this area, there are still some unanswered questions needed to be resolved in the future. The questions are listed as follows:

1. What is the percentage of each type of oxygen functional groups? Is it possible to develop a facile method for the quantification of these functional groups?

2. How do the functional groups affect the pristine characters of graphene? such as the electrical and thermal conductivity, and catalytic activity.

3. What are the functional groups binding to the nitrogen atoms at defective sites on BNNSs?

4. Can the surface chemistry of BNNSs be adjusted in order to switch off the interactions on BNNSs?
More work could be carried out to control the performances of GO by tuning its surface interactions. For example, we may use GO as sensing substrates for the detection of various analytes, light or heat. GO or CRGOs dispersed in low-polar solvents is of great interests when self-assembling functional molecules on the nanosheets. Based on these methods, we could make perfect nanopaints for the applications in anticorrosive, antioxidant and coatings for aerospace plane. On the other hand, the boron radicals on BNNSs make them very active, which is very impressive as people usually consider BN as an inert material. Both theoretical and experimental efforts have been carried out to confirm this speculation. This could open a new branch of chemistry using active sites on BNNSs.

Although exciting progress has been made in the surface chemistry of 2D nanosheets, there are still grand challenges in this research field. At the end of this thesis, I list some proposed future work in the development of 2D nanomaterials

1. To develop a universal approach to synthesize GO or CRGO with designed properties, such as dimensions and surface charge;

2. To develop a facile and effective method to quantify the level of oxygenated content on the surface of GO;

3. To explore the application of GO by switching off its surface interactions between the nanosheets and the functional molecules;

4. To probe the surface chemistry of BNNSs by surface functionalization via either covalent bonding or non-covalent binding;

5. The application of boron radicals in catalysts and in situ polymerization.
Chapter 8: References


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