Fluorescent sensors using DNA-functionalized graphene oxide

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Abstract

In the past few years, graphene oxide (GO) has emerged as a unique platform to develop DNA-based biosensors. This takes advantage of the DNA adsorption and fluorescence quenching property of GO. The adsorbed DNA probes can be desorbed from the GO surface in the presence of target analytes, producing fluorescence signal. In addition to this initial design, many other strategies have been reported including the use of aptamers, molecular beacons, and DNAzymes as probes, label-free detection, using the intrinsic fluorescence of GO and covalently linked DNA probes. The potential applications of DNA-functionalized GO range from environmental monitoring, cell imaging and biomedical diagnosis. In this review, we first summarize the fundamental surface interactions between DNA and GO and the related fluorescence quenching mechanism. Following that, the various sensor design strategies are critically compared. Problems at the current stages are described and a few future directions are also discussed.

Keywords: graphene, DNA, biosensors, adsorption, fluorescence

1. Introduction

Graphene is a single layer of graphite, in which carbon atoms are arranged in a 2D hexagonal lattice [1]. Since its first report in 2004 [2], graphene has attracted tremendous amount of interest due to its unique physical properties [3]. While the majority of the graphene field involves device fabrication, energy storage and catalysis [4,5], graphene has also emerged to be a highly versatile platform for biosensor development [6-15]. In particular, extensive work has been carried out to interface DNA with graphene [16]. Since 2009, more than 300 papers have been published under the keywords of DNA, graphene and (bio)sensors, indicating the versatility of combining DNA and graphene for analytical applications [17].

A biosensor couples a biologically-derived molecule to a signal transduction mechanism to detect and quantify target analytes. The current biosensor field is built on the success story of glucose meters, which mainly use glucose oxidase to detect glucose. Pregnancy test strips represent another commercialized example, where antibodies are employed for recognizing a human hormone. These biosensors have dramatically improved healthcare and quality of life. While proteins are the main players in the current biosensor market, DNA-based detectors have attracted more and more attention in the past two decades. DNA microarrays, molecular beacons and many PCR-based detection methods are already commercialized, all of which detect various nucleic acid targets. With the discovery of aptamers in the early 1990s [18,19], the range of analytes that can be recognized by nucleic acids have expanded significantly. Aptamers are single-stranded DNA or RNA molecules that selectively bind to target molecules. Most aptamers are isolated by a combinatorial biology method called systematic evolution of ligands by exponential enrichment (SELEX) [18,19]. So far, hundreds of aptamers have been selected to bind to metal ions, small organic molecules, peptides, proteins, various surfaces and even whole cells [20,21]. An important application of aptamers is for developing biosensors [22-28][29].

Aptamers are sometimes called chemical antibodies since they are derived chemically and are similar to antibodies in terms of binding affinity and specificity. The range of analytes that can be potentially targeted by aptamers even exceeds that by antibodies since aptamers can be selected against toxic and non-immunogenic compounds (e.g. ATP). At the same time, aptamer production does not involve animals or live cells, avoiding batch-to-batch variation. In addition, DNA aptamers have excellent stability, cost-effectiveness, smaller size, predictable secondary structure and are easy to be modified. With these advantages, aptamers are believed to be a promising analytical platform [22-28].

Many new opportunities arise when DNA and aptamers are interfaced with inorganic nanomaterials [33-35,26,36]. This review summarizes developments in the graphene/DNA sensor field. Due to its hydrophobicity, it is quite difficult to disperse pristine graphene in water. To interface with biomolecules, graphene oxide (GO) is often used. GO is attractive for DNA-based optical sensing for two main reasons: 1) GO adsorbs single-stranded (ss) DNA with the appropriate affinity and 2) GO is a general and efficient fluorescence quencher. This review focuses on fluorescence detection. The papers and reviews related to other detection methods (e.g. electrochemistry) can be found elsewhere [37-39]. We pay particular attention to the intermolecular force between DNA and GO and its effect on biosensing. We hope to critically review the current state-of-the-art that may stimulate future developments. Given the vast number of references in this field, only selective examples are highlighted to illustrate the design principles.

2. Graphene oxide for DNA adsorption and fluorescence quenching

Before reviewing the sensing strategies, fundamental properties of DNA and GO will be first discussed. GO is typically prepared by the Hummer's method to chemically exfoliate and oxidize graphite [40]. This chemical synthesis produces a large quantity of samples. A typical representation of the GO surface is shown in Figure 1A, where many oxygenated species including carboxyl, hydroxyl and epoxy groups are displayed on the surface. The thickness of GO is about 1 nm [41]. This is thicker than

pristine graphene due to the oxygen containing groups. The oxygen content can reach as high as ~40%. These oxygenated groups are not evenly distributed on the GO surface, e.g. carboxyl groups are on the edges. Within the GO plane, highly oxidized regions and crystalline carbon regions co-exist in the nanoscale domains [42,43]. The highly oxidized GO has a yellow color and the level of oxidation can be decreased by adding reducing agents such as hydrazine, glucose or sodium borohydride. Once prepared, GO is readily dispersed in water at high concentration, maintaining colloidal stability even at relatively high ionic strength. The size of GO sheets varies from nanometer scale to tens of microns or even larger.

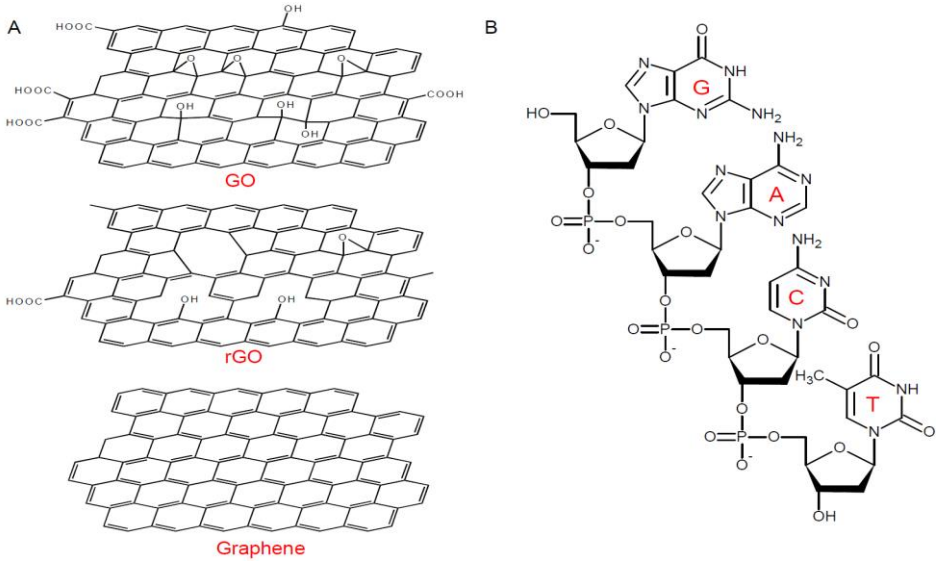

Figure 1. (A) Schematic structure of GO, reduced GO (rGO) and pristine graphene. (B) Structure of a single-stranded DNA.

2.1. DNA adsorption

Before graphene was extensively studied, DNA was already known to adsorb onto other carbon surfaces such as carbon nanotubes (CNTs) and graphite [44]. The surface of GO is featured by 2D planar structure, single atomic layer thickness and heterogeneity, containing both oxidized and carbon-rich domains [42,43]. The sizes of these domains are a few to tens of nanometers, depending on the level of oxidation. It is not surprising that DNA can be adsorbed by graphene since the DNA bases are

aromatic and can stack with the π electrons in graphene. DNA base adsorption by graphene has been studied by various computational methods [45-47]. It is generally accepted that the base rings are parallel to the graphene surface to maximize π-π stacking. Antony and Grimme ranked adsorption energy to be G > A > T > C [45], which is similar to G >A ≅ T ≅ C using density function theory calculations [47]. While calculations are often done on graphene due to its simple structure, experiments for colloidal systems are often carried out using GO. Isothermal titration calorimetry (ITC) indicates a trend of G > A > C > T for GO adsorption [48]. Adsorption energy is also a function of GO concentration and more heat is released with higher GO concentration. For example, when adenosine was titrated to 0.3 mg/mL of GO, 2.26 kJ/mol heat was released. The heat increased to 22.6 kJ/mol with 1.0 mg/mL of GO. This is likely due to surface heterogeneous and certain regions adsorb the bases with higher affinity. At a higher GO concentration, more such high affinity regions are available.

DNA adsorption is different from adsorption of individual bases. First, DNA carries negative charges on its phosphate backbone (see Figure 1B for structure), posing a kinetic barrier to be adsorbed by negatively charged GO. Second, DNA may form various secondary structures due to intramolecular base pairing, competing with adsorption by GO. Simulation shows that adsorption between ss-DNA and GO is mainly from π-π stacking [49]. By using atomic force microscopy to study the force required to peel DNA off graphite, Manohar *et al.* concluded that not all the bases are adsorbed and a diverse range of DNA conformations are likely to present [50]. We followed a 24-mer DNA adsorption by GO using ITC [51]. At the same time, the adsorption capacity was measured to ensure complete DNA adsorption. The heat of adsorption decreased with increasing DNA concentration, also suggesting the effect of surface heterogeneity. The pristine graphene domains are likely to adsorb DNA more tightly, releasing more heat than the highly oxidized domains. In addition to π-π stacking, hydrogen bonding was also suggested to be important [52].

The ζ-potential of GO as a function of pH was reported. The absolute value of ζ-potential decreases almost linearly with decreasing pH from 12 to 2, although the surface remains negatively charged in the whole pH range [53]. Therefore, GO is likely to contain a wide range of acid groups with different acidity and these groups may affect each other depending on their protonation state. To adsorb negatively charged DNA, salt is required. This requirement can be readily achieved with GO since it has good colloidal stability. In comparison, gold nanoparticles suffer more from salt-induced aggregation due to large van der Waals force [54]. Fast DNA adsorption by GO can be achieved with a few mM Mg^{2+} or more than 100 mM NaCl. Since electrostatic repulsion can be screened by adding salt, the same can also be achieved by lowering pH. In a low salt buffer, we observed complete DNA adsorption at pH 4 and 5, while at pH 8 only ~30% DNA was adsorbed [55]. When the length of DNA is concerned, GO adsorbs shorter DNA more rapidly [55].

2.2. Adsorption of ds-DNA

It needs to be noted that even ds-DNA can also be adsorbed by GO [56-58]. Long ds-DNA might partially denature on GO to expose ss-regions. But for short ds-DNA, it might dissociatively adsorb to form two ss-DNA on the GO surface, although no direct evidence is currently available to support this. The assumption is reasonable from the thermodynamic standpoint since DNA adsorption by graphene is more stable than its hybridization to cDNA [59]. Computational studies also suggest the possible mechanisms of ds-DNA adsorption by graphene [60]. The key difference between ss- and ds-DNA adsorption is kinetics, where ss-DNA adsorption takes place much faster.

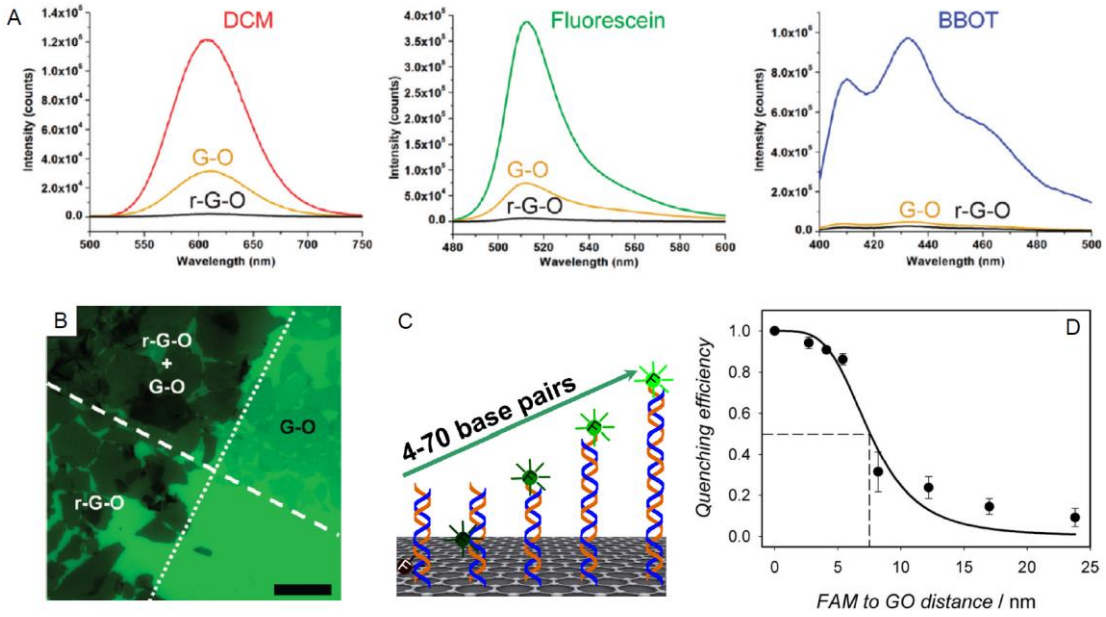

Figure 2. (A) Fluorescence spectra of three dyes and their quenching by GO and rGO. Strong quenching is observed regardless of the emission wavelength or molecular structure of the dye. (B) A fluorescence micrograph showing that rGO has the strongest quenching power. Reprinted with permission from reference [61]. (C) Schematic presentation of using DNA to control the fluorophore-to-GO distance. (D) Distance-dependent quenching of FAM fluorescence by GO. Reprinted with permission from reference [62].

2.3. Fluorescence quenching

Graphene and GO can efficiently quench a diverse range of adsorbed fluorophores, quantum dots and fluorescent metal nanoclusters. Huang and co-workers studied the quenching of three organic dyes by GO and rGO (Figure 2A) [61]. All the dyes are quenched regardless of their emission wavelength, indicating GO is a general quencher. In addition, rGO quenches much more efficiently compared to GO. This was directly observed using a fluorescence microscope (Figure 2B). Fan and co-workers described that the different dyes linked to DNA can also be efficiently quenched by GO [49]. Theoretic calculations show that graphene is a long-ranged dynamic quencher and its quenching efficiency follows d^{-4} dependency, where d is the distance between fluorophore and graphene [63-66,62,67]. In

comparison, molecular quenchers usually follow a d^{-6} dependency, and thus they are more short-ranged quenchers. We used DNA as a rigid molecular rod to control the distance between FAM and GO, and have confirmed this long-ranged energy transfer and quenching by GO (Figure 2C) [62], where the distance for 50% quenching was determined to be 7.5 nm (Figure 2D). This distance is longer for rGO and graphene. Close to complete quenching can be achieved when the fluorophore is adsorbed on the GO surface, which in-turn corresponds to a large signal enhancement upon desorption. The ability to quench all types of fluorophores and a wide range of wavelengths with high efficiency makes GO and graphene a unique platform for optical sensing. As described below, fluorescence quenching provides a convenient tool for studying DNA adsorption and also for biosensor design.

3. Sensor design strategies

GO offers an ideal platform for developing fluorescent sensors. In addition to its superior fluorescence quenching property and DNA adsorption properties, GO has very high specific surface area. Importantly, the adsorption affinity of DNA on GO is optimal, where adsorption is strong enough to achieve a low background, but weak enough to be desorbed by the target DNA or other analytes. For comparison, DNA adsorption by CNTs is much more strongly, while the signaling kinetics is much slower [68,69]. In one case, DNA took more than 10 h to reach desorption equilibrium, while DNA desorption from GO takes usually less than 1 h. An important reason is the 1D rod shape of CNTs, upon which DNA can wrap around to disfavor desorption. While wrapping CNTs, most DNA bases can contribute to adsorption, which also increases the binding affinity. In addition, GO is highly oxidized, posing electrostatic repulsion with DNA and impeding π-π stacking, while CNTs are often oxidized to a lower extent. For these reasons, DNA-functionalized GO has been extensively studied as a fluorescent sensing platform.

3.1. Analyte-induced desorption of fluorescent probes

In this strategy, a probe DNA with a covalently attached fluorophore is first adsorbed by GO, leading to quenched fluorescence. The probe is desorbed by target molecules to produce fluorescence signal. This method is attractive because of its simplicity, high signal and low background noise. It is also possible to detect multiple analytes by adsorbing different probes.

3.1.1. Adsorbed DNA or aptamer probes

The first paper on this topic was reported by Yang and co-workers [17]. As illustrated in Figure 3, a carboxyfluorescein (FAM)-labeled ss-DNA probe is adsorbed by GO. With a high salt concentration (e.g. 10 mM Mg^{2+}), almost-complete probe adsorption and fluorescence quenching took place. Such a low background is important for high sensitivity. In the presence of a target cDNA, the probe is desorbed, forming a duplex with the target and increasing fluorescence. The detection limit is reported to be ~1 nM cDNA. In the same work, the authors also employed fluorescently labeled aptamers for detecting non-nucleic acid targets.

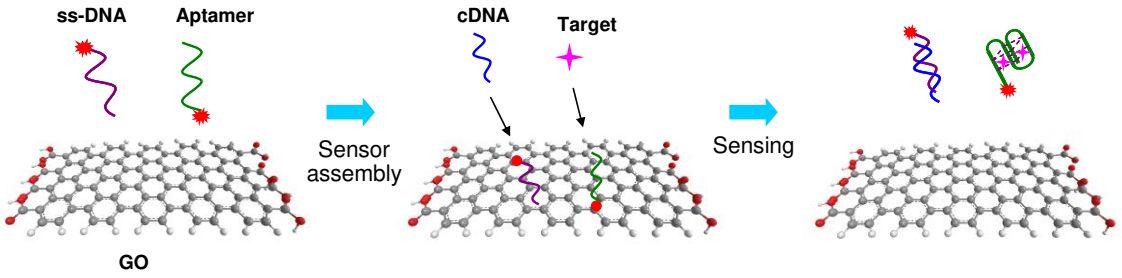

Figure 3. Schematic presentation of assays based on adsorbed DNA probes. Fluorescence is quenched upon probe adsorption by GO. In the presence of target molecules, probes desorb to produce fluorescence.

Similar methods have also been employed by other groups to detect various targets including proteins, small molecules, and metal ions. For example, a sensor for thrombin was reported using a sodium dodecyl benzene sulfonate (SDBS)-modified GO [70]. A FAM-labeled thrombin aptamer was

adsorbed on SDBS–GO, and the quenched fluorescence was recovered after thrombin addition. This sensor has a detection limit of 31.3 pM thrombin, which compares favourably with many other fluorescence based detection methods. Mucin 1 (MUC1) is widely present in epithelial malignancies. Tang and co-workers constructed a fluorescent sensor for MUC1 using an aptamer/GO complex. MUC1 can be detected in a broad dynamic range from 0.04 to 10 μM with a detection limit of 28 nM and good selectivity. Moreover, the results have also been verified for real sample applications using 2% serum containing buffers [71].

Aside from protein targets, Wu and co-workers mixed CdSe/ZnS quantum dot (QD) functionalized aptamers with GO to detect Pb^{2+}. The QD-conjugated aptamer was also adsorbed by GO, resulting in fluorescence quenching. When Pb^{2+} was added, a G-quadruplex structure was formed, shielding its bases and increasing fluorescence. This sensor has a limit of detection of 90 pM Pb^{2+} with excellent selectivity [72]. Since folded DNA quadruplex has a low affinity with GO, Wang and co-workers developed a fluorescence assay for screening quadruplex-binding ligands. Three series of Chinese medicine monomers were investigated, and the flavonoids were found to be potential quadruplex-binding ligands [73].

In this method, since the probe DNA is not covalently linked to GO surface, it is usually difficult to achieve sensor regeneration. Based on the understanding of the pH-dependent DNA adsorption and the high colloidal stability of GO [55], we demonstrated a biosensor regeneration method. At pH 7.5, adsorbed aptamer probes are desorbed by adding target molecules to produce fluorescence signal. At pH 3.5, desorbed aptamers can re-adsorb, releasing target analytes. The probe/GO complexes were harvested after centrifugation for regenerate [74].

3.1.2. Multiplexed detection

The graphene platform is ideal for multiplexed detection by co-adsorbing different DNA/aptamer probes, each carrying a fluorophore with a distinct emission wavelength. Ye and co-workers reported

an example for various targets including DNA, protein (thrombin), metal ions (Ag^+ and Hg^{2+}) and small molecule (cysteine), where the detection limits were 1 nM, 5 nM, 20 nM, 5.7 nM and 60 nM respectively. This system is simple to prepare and it has little background interference compared to traditional molecular beacons [75].

While most of the work employed specific aptamer probes, by using a pattern recognition method, Hu and co-workers adsorbed seven designed DNA probes on GO and tested their responses to a large suite of proteins including subtilisin A, fibrinogen, cytochrome *c*, lysozyme, horseradish peroxidase, bovine serum albumin, lipase, casein, and haemoglobin. Although these DNA sequences are not the specific protein aptamers, their unique response patterns are analytically useful for separating them [76].

3.1.3. Fluorescence anisotropy based assays

In addition to detecting fluorescence intensity change upon probe desorption, Yang and co-workers noticed that fluorescence anisotropy also changes significantly in the same process. Fluorescence anisotropy is a reliable, sensitive, and robust measurement, since it is less affected by fluorophore concentration or quantum yield. When a fluorophore is free in solution, the anisotropy value is low due to its fast tumbling. On the other hand, when associated with a large molecule or material, the anisotropy value increases. For example, when a fluorophore labeled aptamer probe is mixed with GO, its anisotropy value increases to ~0.4, while the free probe has a value of ~0.05. Using this method, ATP was detected down to 1 μM. Importantly, the detection could be achieved in human serum samples [77].

3.1.4. Enhancement of molecular beacons

A molecular beacon (MB) is a DNA hairpin with a fluorophore and a quencher labeled on the two ends (Figure 4A) [78,79]. In the closed state, the fluorescence signal is low. In the presence of the target

cDNA, the hairpin is opened with increased fluorescence signal. MBs not only suppress background signal compared to non-structured linear probes, but also increase specificity for target recognition. MBs are widely used for homogeneous DNA detection. The quencher part in MBs is usually small molecule-based dark quenchers. Since not all the beacons are initially in the closed state, background fluorescence is often observed. Adsorption of molecular beacons by GO may allow further suppression of background.

Fan and co-workers systematically studied the interaction between GO and MBs (Figure 4B) and developed an enhanced MB with improved performance. They found that the background fluorescence of MB was greatly decreased in the presence of GO, which enhanced the signal-to-background ratio as well as the sensitivity. They also found slightly enhanced target selectivity [80]. Since GO is an efficient quencher, it is probable to just the stem-loop structure with a fluorophore labeled but without a quencher (Figure 4C). Yang and co-workers reported adsorption of a stem-loop probe to GO [81]. In the presence of the cDNA, the MB was released from GO, resulting in fluorescence recovery. This system detects targeted DNA at a higher sensitivity and has selectivity for single-base mismatch comparable to that with conventional MBs. Wei and co-workers also reported a quencher-free MB, where only a FAM fluorophore was labeled on the end of a hairpin. This hairpin was adsorbed by GO to achieve fluorescence quenching. The goal was to measure damaged DNA, which solicits a weaker fluorescence enhancement compared to the un-damaged DNA. According to this paper, chlorpyrifos-methyl and three metabolites of styrene were used to study their DNA damage effects. The method was concluded to be reliable, rapid and simple for detecting DNA damage [82].

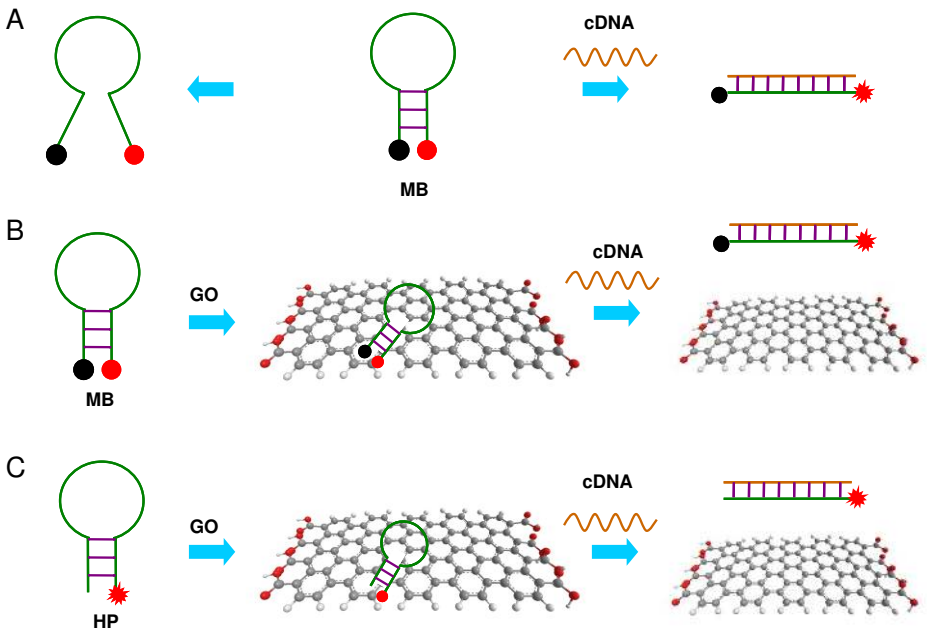

Figure 4. Enhancement of MB performance by GO. (A) A fraction of MB may stay in the open state to produce background fluorescence. In the presence of cDNA, a large fluorescence enhancement is observed. (B) Adsorption of MB probes by GO and detection of cDNA. (C) Adsorption of hairpin probes (HP) by GO and detection of cDNA. This figure is re-drawn with modifications from reference [80].

Kim and co-workers reported an improved quencher-free MB system [83], where the fluorophore is embedded into the loop region. This design has an excellent single mismatch discrimination power but suffers from high background. Using GO as an external quencher, their signal-to-background ratio (S/B) improved from 2.2 to 31.0 [84]. This hybrid system still maintained a high selectivity for fully matched over single-base-mismatched cDNA.

Li and co-workers reported a fluorescence based method for detecting Cu^{2+} using MBs and GO. In the presence of Cu^{2+} and H_2O_2, the MB is cleaved into small fragments, and GO cannot adsorb the fluorophore bearing fragment effectively. Without Cu^{2+}, the hairpin remains intact and can be adsorbed

by GO to produce even lower fluorescence. Although the target is not a DNA, the concept of using GO to suppress MB background still applies [85]. This method offers a detection limit of 53.3 nM Cu^{2+}.

3.1.5. Detection in cells

It is difficult to use nucleic acid-based probes to directly detect intracellular targets, since highly negatively charged DNA or RNA cannot cross the cell membrane on their own. Therefore, either micro-injection or a delivery vehicle is required. Reports have shown that nanoscale GO can be used for drug delivery, and GO can be internalized by many cells [86]. With the unique ability of DNA adsorption, superior quenching capacity and DNA protection from enzyme cleavage, GO is a useful nanomaterial for intracellular detection and imaging of target analytes. In this section, we reviewed a few examples of such applications.

Detecting survivin mRNA as a tumor biomarker is important for early cancer diagnosis. Chen and co-workers discovered that GO can protect DNA oligonucleotides from enzyme-induced degradation, which is important for applications in biological samples. In this study, they used a MB to detect survivin mRNA, and the MB probe was adsorbed by nano-sized GO. With the targeted survivin mRNA, the beacon produced fluorescence that was measured by both fluorescence microscopy and flow cytometry. The signaling mechanism is believed to be similar to that outside cells [87].

Xu and co-workers developed a sensitive on-chip strategy for ultrasensitive detection of survivin mRNA in a single living cell. A micro-channel surface was conjugated with a prostate stem cell antigen (PSCA) monoclonal antibody for prostate cancer cells (PC-3) recognition. Thus, PC-3 cells were specifically captured on the micro-channel surface. After capture, a FITC-labeled antisense DNA (F-S1) adsorbed on GO was used for cell transfection. The F-S1 probe is complementary to a fraction of the survivin mRNA. After being internalized by the captured cell, F-S1 could selectively bind with survivin mRNA to recover fluorescence. The survivin mRNA content in

each PC-3 cell was quantified to be ~4.8×10^6 copies. This system serves for both automated target cell capturing and intracellular detection and has potential in early cancer detection [88].

Huizenga and Szostak selected a DNA aptamer for ATP/Adenosine in 1995 [32], which has been widely used as a model system for studying aptamer-based metabolite detection. For detecting intracellular ATP, Lin and co-workers studied the adsorption of the FAM-labeled ATP aptamer by nano-GO, which resulted in strong fluorescence quenching [89]. They further incubated the sensor with JB6 cells in culture medium, where effective cellular uptake was observed. The internalized aptamer/GO complex reacted with ATP and showed fluorescence signal. This system has a number of advantages including using GO as a carrier, a quencher and a sensing platform, allowing real-time sensing in live cells. The authors also constructed a sensing platform for simultaneous visualization of ATP and GTP in live cells using two aptamers [90].

While intracellular targets can be imaged with such probes, very few systems can reach quantification or semi-quantification in live cells. This is partially due to non-specific probe interactions with the sample matrix. For example, adsorbed DNA probes might be displaced by proteins. To solve this problem, Tan and co-workers employed an internal standard [91]. They first adsorbed an ATP aptamer MB (AAMB) by GO to form a double quenching platform. After co-incubation with cells, this complex can spontaneously enter the cells and detect intracellular ATP. In addition, a control ss-DNA was employed as an internal reference, which can be released nonspecifically from GO by non-target proteins. Based on the control, the ATP level can be semi-quantified using the fluorescence intensity ratio of the AAMB to control (Figure 5).

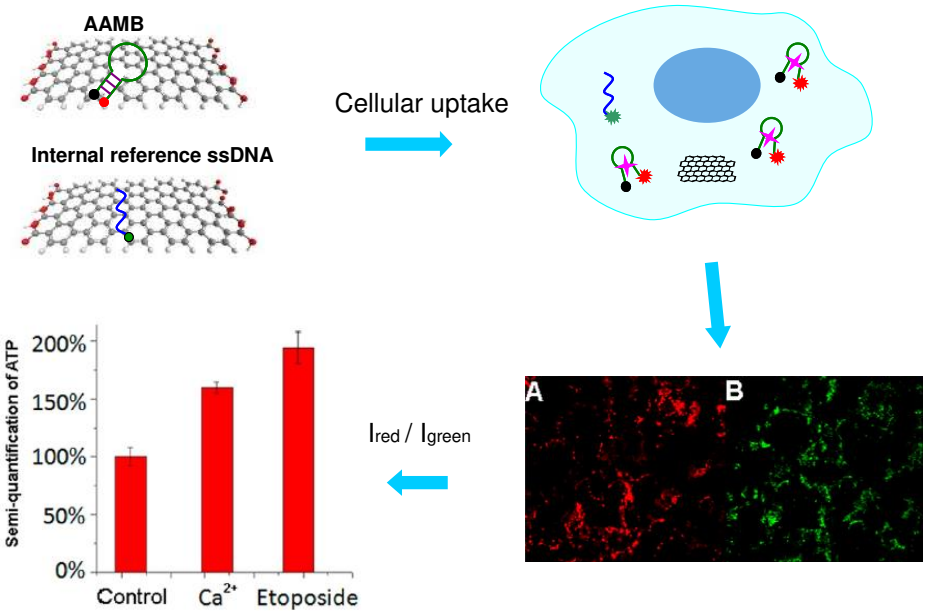

Figure 5. GO-based sensing with an internal reference. Both the MB probe DNA and a reference DNA labeled with a different fluorophore are adsorbed by GO and are delivered to cells. A more accurate result is obtained by measuring the ratio of fluorescence from both DNA, reducing false signals from non-specific probe release from the GO surface. Reprinted with permission from reference [91].

Fu and co-workers fabricated a duplex–triplex switchable DNA/GO nano-machine for measuring intracellular acidification and apoptosis in Ramos cells [92]. The triplex-forming oligonucleotide showed duplex–triplex transition modulated by pH. Detection was made based on the different adsorption affinity between ss-DNA and triplex DNA. This sensor was used to monitor the pH change inside Ramos cells during apoptosis. Fluorescence imaging demonstrated different results between live and apoptotic cells, suggesting the potential of using duplex–triplex switchable system to monitor the complex pH change in live cells.

3.1.6. Modified DNA probes

DNA solid phase synthesis allows the production of a diverse range of modified nucleic acids, where higher target binding affinity and specificity might be achieved. By changing either the

backbone or the sugar ring or the base structure, the interaction of modified DNA with GO is also changed, leading to improved sensors. In addition, modified nucleic acids may also have higher stability against enzymatic degradation.

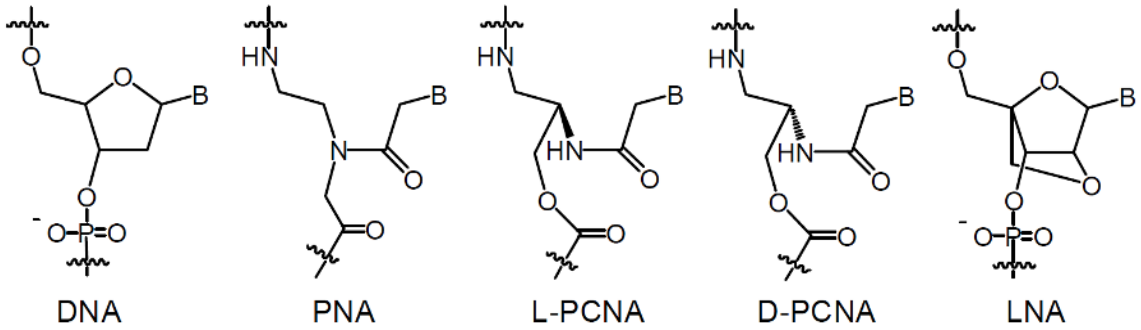

Figure 6. Backbone structures of DNA and a few modified DNA analogs.

Kumar and co-workers used fluorescently labeled octameric poly-thymine sequences of DNA, PNA and PCNA for GO-induced fluorescence quenching studies. The backbone structures of some modified DNAs are shown in Figure 6. Their restored fluorescence by complementary poly-adenine DNA was compared. This was the first systematic study to replace the natural DNA probes with synthetic DNA mimics to interface with GO. These modified nucleic acids demonstrated some excellent properties in forming strong duplexes with cDNA [93].

The backbone of peptide nucleic acid (PNA) is composed by charge neutral polyamides rather than negative phosphates, which can contribute to the stronger hybridization of PNA with its complementary nucleic acid. Zhang and co-workers adsorbed a fluorescently labeled PNA onto GO for fluorescence turn-on detection of DNA. In the presence of target DNA, the adsorbed PNA was released from GO surface, recovering the quenched fluorescence. The detection limit was 0.8 nM DNA. An important advantage is that the PNA sensor has better selectivity against single-base mismatches as well as non-complementary targets [94]. This is mainly due to the higher affinity between PNA and DNA, so that shorter probes are allowed to be used.

Locked nucleic acids (LNAs) refer to the DNA analogues in which the 2′ oxygen in the modified sugar ring is bridged to the 4′ carbon, leading to an excellent binding affinity to target oligonucleotides. Yigit and co-workers studied the interaction between LNA-modified oligonucleotides and its cDNA. The authors demonstrated that LNA modifications did not alter the hybridization yield but the duplex stability dramatically increased. The LNA–GO complex was highly stable even at 90 °C, while desorption was still achieved with cDNA. Detection of single-base mismatches was also demonstrated [95].

3.2. Signaling by inhibition of probe adsorption

Pre-adsorption of probe DNA by GO allows for 'signal-on' sensing of target molecules in a single step. DNA probes are protected by GO from enzymatic degradation. At the same time, GO delivers probe DNA for intracellular detection. However, since the interaction between DNA and GO is quite strong, this method may have compromised sensitivity. In other words, it might be difficult for target molecules to compete with GO for the probe DNA. In addition, physisorbed probes might be non-specifically displaced by proteins or nucleic acids, leading to false signals. An alternative method that might resolve some of these problems is shown in Figure 7A. In this method, a probe DNA is first mixed with the target DNA to form a duplex. Since the adsorption of duplex DNA is much slower compared to the adsorption of ss-DNA, an overall stronger fluorescence is produced. Similarly, if an aptamer probe is associated with its target molecule to form a compact structure, its adsorption kinetics is also reduced.

Fan and co-workers systematically compared the performance of these two strategies (Figure 7A, B) [49]. First, the probe adsorption kinetics is faster than that of target DNA induced probe desorption. This also suggests that DNA and GO have strong interactions. In addition, the signals from these two methods are comparable in the presence or absence of the target DNA. Therefore, the authors decided to use the method in Figure 7A. Using multiple DNA probes labeled with different dyes, the

authors detected multiple DNA targets in the same solution. This method might have better generality, since GO is used to detect the conformation of the probe DNA and most aptamer binding can induce a conformational change. On the other hand, if an aptamer binding affinity is low, its target molecule might fail to remove the probe from the GO surface. A few more examples of this sensing strategy are reviewed below.

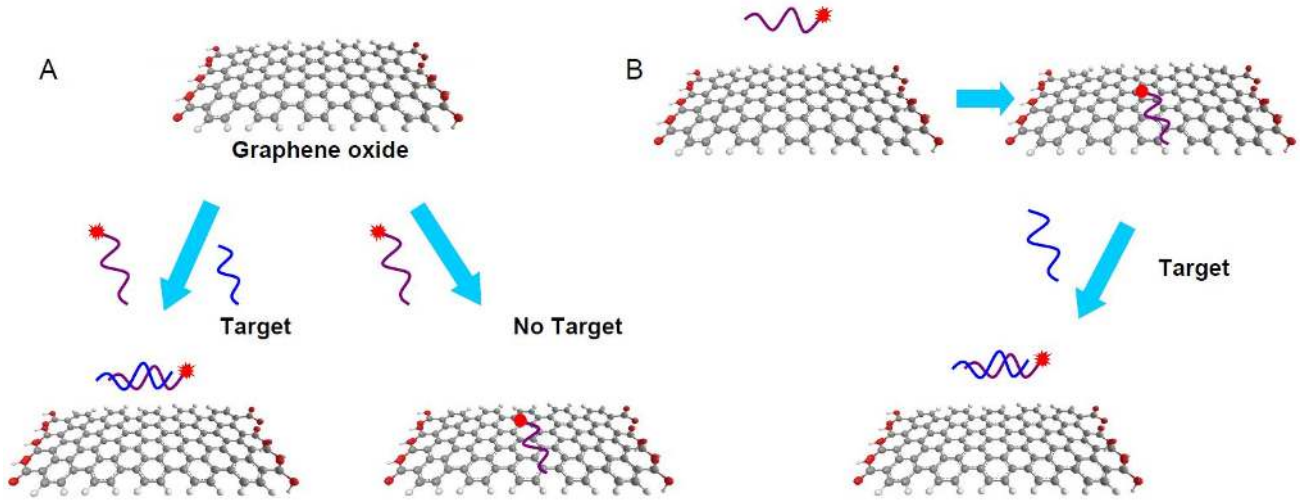

Figure 7. Schematics of signaling by inhibition of probe adsorption (A) and its comparison with target induced probe desorption (B). Re-drawn from reference [49].

3.2.1. Molecular fluorophore labels

Wang and co-workers established an assay to measure fungi toxin (ochratoxin A) generated by *Aspergillus Ochraceus* and *Penicillium verrucosum*. A FAM-labeled toxin-specific aptamer was used, where its adsorption by GO was inhibited by ochratoxin A [96]. The detection limit was 1.9 μM with high specificity. After the surface of GO was blocked by PVP, the detection limit (21.8 nM) was improved by two orders of magnitude.

Fan and co-workers constructed an Ag^+ sensor using a FAM-labeled cytosine (C)-rich DNA. In the absence of Ag^+, the ss-DNA is flexible and quickly adsorbed by GO. While in the presence of Ag^+, a rigid hairpin is formed since Ag^+ stabilizes C-Ag^+-C base pairs, and adsorption by GO is inhibited

[97]. Ling and co-workers developed a fluorescent method for detecting the ds-DNA in *Simian virus 40* (SV40) [98]. SV40 is a simple DNA virus which is often used as a model for the research of tumorigenic and anti-tumor immunity. Interestingly, there is a region of 17 base pairs homopyrimidine homopurine ds-DNA in SV40, which could be targeted via triplex formation. Based on this, a fluorescent biosensor was developed, where triplex formation inhibited adsorption of the probe DNA by GO. Simlar methods were also used to detect IgE [99] and RNA [100].

3.2.2. Nanoparticle fluorophores

In addition to using organic dyes, this method also works for inorganic fluorophores. For example, Ju and co-workers designed an interesting sensor using quantum dots (QDs) [101]. A probe DNA was first conjugated to QDs and the QD fluorescence was quenched upon mixing with GO due to DNA adsorption by GO. In the presence of the target cDNA, the probe formed duplex, inhibiting QD adsorption and resulting in high fluorescence. This sensor is highly sensitive (12 nM detection limit) with excellent selectivity.

In a similar way, Liu and co-workers functionalized upconversion nanophosphors (UCNPs) with an amino-modified ATP aptamer through EDC coupling with the surface carboxyl groups. This complex can be strongly quenched by GO due to the interaction between DNA and GO. For comparison, free UCNPs (without conjugation with the aptamer) was quenched only ~20% because of their weaker interaction with GO. In the presence of ATP, the aptamer is folded and the complex adsorption by GO is inhibited, allowing ATP detection. Excitation of UCNPs allows the use of NIR light, which eliminates background fluorescence from biological sample matrix [102].

3.2.3. Enzyme activity assays

Min and co-workers reported an assay of helicase unwinding activity, where a ds-DNA with a fluorophore label was mixed with GO. In the presence of helicase, the ds-DNA was gradually unwound

and the ss-DNA product was adsorbed on GO, leading to quenched fluorescence. Helicase activity can thus be monitored in real time [103]. Pang and co-workers measured adenosine deaminase (ADA) activity. In the presence of ADA, the substrate adenosine is converted into inosine. Therefore, it cannot inhibit the adsorption of a fluorophore-labeled adenosine aptamer [104]. The as-proposed sensor is highly sensitive with a detection limit of 0.0129 U/mL ADA in a clean buffer, which is more than one order of magnitude better than the previous reports.

While this method is very versatile and can be applied to a broad range of analytes, one disadvantage is that the detection has to be carried out in two steps. In addition, it cannot be directly used for detection in live cells, where the sensor has to be pre-assembled.

3.3. Signaling with DNA staining dyes

Most of the above work involved DNA probes with covalently attached fluorophores. In the past few years, a number of label-free strategies have also been developed using DNA staining dyes for signaling. Label free detection avoids costly DNA labels and can also achieve high sensitivity. Most DNA staining dyes are non-fluorescent when they are free in solution but become highly fluorescent when they bind to ds-DNA.

Li *et al.* reported an interesting assay for detection of single-nucleotide polymorphism. SYBR Green I (SG) dye was intercalated into perfectly matched ds-DNA and resulted in high fluorescence. However, the single mismatched sequence led to a loose duplex, which displayed poor SG binding and low fluorescence. The fluorescence difference is in the order of 10-fold for matched and mismatched targets. Interestingly, after the addition of GO, the fluorescence was well maintained for the perfectly matched target. On the other hand, the mismatched target showed even more quenching, leading to a much better discrimination of single base mismatches (can be as high as 120-fold). In this system, GO plays a quite unexpected role, which might be related to the affinity between SG and GO as well [105].

Similarly, Jiang *et al.* reported a label free probe using ethidium bromide (EB) and GO for detection [106].

MicroRNA (miRNA) refers to a type of small (~22 nucleotides) non-coding RNA for regulating gene expression. Xing and co-workers employed a non-labeled hairpin probe to recognize target miRNA. Once a complex is formed, a strand displacement polymerase acts on the miRNA template with a short primer, leading to a catalytic cycle to continuously generate duplex DNA amplicons, which can be detected by adding SG. The authors used microRNA-21 (mir-21) as the target to study the properties of this assay and the addition of GO allowed a very low background fluorescence [107].

3.4. Sensing mechanism

The sensing scheme in Figure 7A can be readily explained by DNA adsorption kinetics being faster for ss-DNA. However for the sensors based on Figure 7B, there are a few possibilities. First, if the reaction follows the Langmuir-Hinshelwood mechanism, the target DNA is also adsorbed by GO and they hybridize on the GO surface forming a duplex. Desorption of the duplex DNA produces fluorescence. Alternatively, it might be that the adsorbed probe reacts with the cDNA in solution, following the Eley-Rideal mechanism. A third possibility is that there is an equilibrium between the adsorbed probe and the free probe DNA in solution. Hybridization of cDNA with the free probe DNA in solution reduces the concentration of the free probe, inducing a shift of the equilibrium. We designed an experiment to test the hypothesis [59]. It is known that poly-A DNA adsorbs more tightly with GO than poly-T DNA. When FAM-T_{15} was used as a probe, A_{15} generated much stronger signal than T_{15} did (Figure 8A). On the other hand, when FAM-A_{15} was used as a probe, A_{15} still produced more signal, although the overall signal was weaker (Figure 8B).

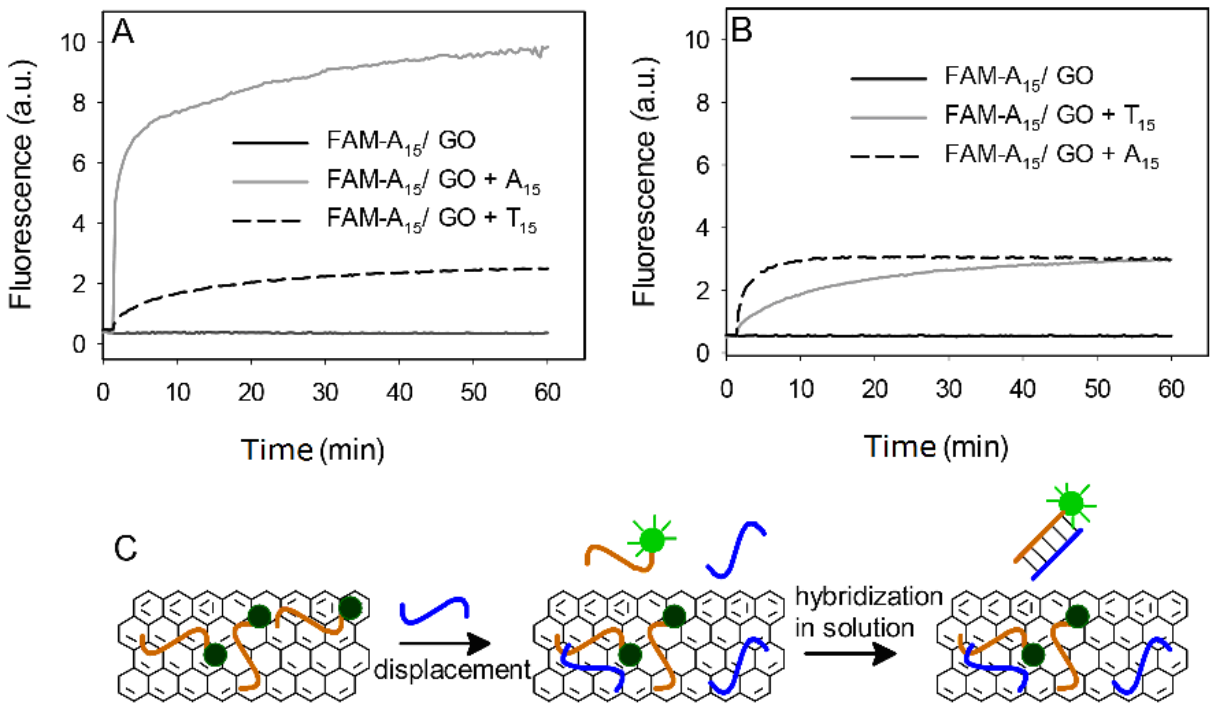

Figure 8. Kinetics of fluorescence enhancement upon adding various DNA to FAM-T_{15}/GO (A) or to FAM-A_{15}/GO (B). The DNA molecules were added at 1 min to induce probe desorption. (C) Schematics of the displacement mechanism for signal generation. Reprinted with permission and modification from reference [59].

Based on this result, all the three mechanisms mentioned above can be ruled out. For example, if the reaction takes place on the surface, then T_{15} should be more effective than A_{15} to desorb FAM-A_{15}. Similarly, if the reaction takes place in solution phase, T_{15} still should be more effective since A_{15} cannot react with FAM-A_{15}. Our result suggests that displacement is an important mechanism in this case (Figure 8C). A fraction of adsorbed probe is first displaced by the cDNA and the displaced probe reacts with target DNA. The reason is that DNA adsorption by GO is much stronger than hybridization energy with cDNA. Thermodynamically, the reaction can only take place via displacement. This predicts a less efficient use of the target DNA. Indeed, based on our calculation, only ~15% cDNA was used to form duplex with the probe DNA, while the rest was used for the non-specific displacement

task. Therefore, one way to improve sensitivity is to selectively block the GO surface to allow more efficient cDNA hybridization.

3.5. Covalently linked probes

All the previous sensors rely on the faster and stronger adsorption of ss-DNA in comparison to ds-DNA (or unfolded versus folded aptamers). While simple adsorption experiments are easy to carry out, they also bare a number of intrinsic problems. For example, adsorbed probes may suffer from non-specific displacement. This is particularly important for detection in biological sample matrix, where a high concentration of proteins and other nucleic acids may compete for the GO surface, leading to a high and unstable background fluorescence. In addition, it is difficult to achieve the sensor regeneration. To solve this problem, various strategies have been tested to form more stable anchors between DNA and GO.

A popular method is to employ a block of adenine in the DNA probe, since adenine interacts with GO strongly. This has been used to perform DNA-directed assembly of GO sheet [108], and to modulate DNA to GO distance [109]. However, this interaction is still not strong enough, and thus displacement might take place. An example is the displacement by the same DNA [59]. To achieve better stability, modified probes are needed.

Furukawa adsorbed the thrombin aptamer to GO via a pyrene label since pyrene forms a strong π-π stacking with the sp^2 GO surface. They observed stronger fluorescence in the presence of thrombin, which was attributed to DNA binding to the target protein and thus directing the fluorophore away from the surface. Note that the DNA molecule was still attached to the GO surface after thrombin binding [110]. Since this aptamer is very short, which contains only 15 nucleotides, the fluorophore is still quite close to the GO surface even after binding to thrombin. GO is a long-range quencher, and therefore only weak signal was obtained. To improve this, the authors further modified their system by adding a DNA spacer between the fluorophore label and the aptamer. With a 20-thymine spacer, the

intensity increased by 6-fold compared to the sensor without the spacer [111]. Recently, a number of other reports also involved pyrene to immobilize DNA probes on GO. Yang and co-workers used molecular dynamics to simulate the interaction of pyrene-polyethylene glycol (Py-PEG) with graphene. They found that Py–PEG can be adsorbed onto the surface of graphene spontaneously with a flat self-assembly morphology [112]. Sun *et al.* studied the adsorption of polycyclic aromatic hydrocarbons (PAHs) such as naphthalene (NAP), anthracene (ANT) and pyrene (PYR) on rGO and GO. The saturated adsorbed capacities was in the order of NAP>PYR>ANT and they may serve as anchors [113]. We recently reported that cisplatin-modified DNA can be adsorbed by GO with a very high capacity and stability, possibly due to the positive charges associated with the Pt/nucleobase complex that can reduce the electrostatic repulsion between DNA and GO [114].

The strongest interaction is covalent attachment. Mohanty and Barry immobilized an amino-modified DNA on GO and via amide bond and observed strong fluorescence on GO upon hybridizing with cDNA containing a fluorophore label [115]. This experiment showed the feasibility of performing covalent probe immobilization on GO. For most analytical applications, it is desirable to label probe DNA instead of the target.

In our previous study [51], we used EDC as a coupling agent to bind an amino and FAM dual-labeled DNA molecule to GO. A scheme of the probe fluorescing on GO surface is shown in Figure 9A. For the covalently linked probe, the sensor became highly fluorescent after adding the cDNA. After sedimentation or centrifugation of the sample, the supernatant was clear and the fluorescence was associated only with the GO sheets (Figure 9B). For comparison, when the physically adsorbed probe was used, the supernatant became even more fluorescent after centrifugation (Figure 9C). A comparison of non-specific displacement was also made. For the covalent probe, displacement by the same DNA sequence (but non-labeled) was completely suppressed (Figure 9D) while for the physisorbed probe, such signal was still quite high (Figure 9E). This is also true for protein-induced probe displacement (Figure 9F). Covalent probes allow high sensitivity by simply increasing sample

volume. For example, our covalent probe has a detection limit of 2.2 nM using a sample volume of 0.05 mL. With a 2 mL sample, the detection limit reached 150 pM.

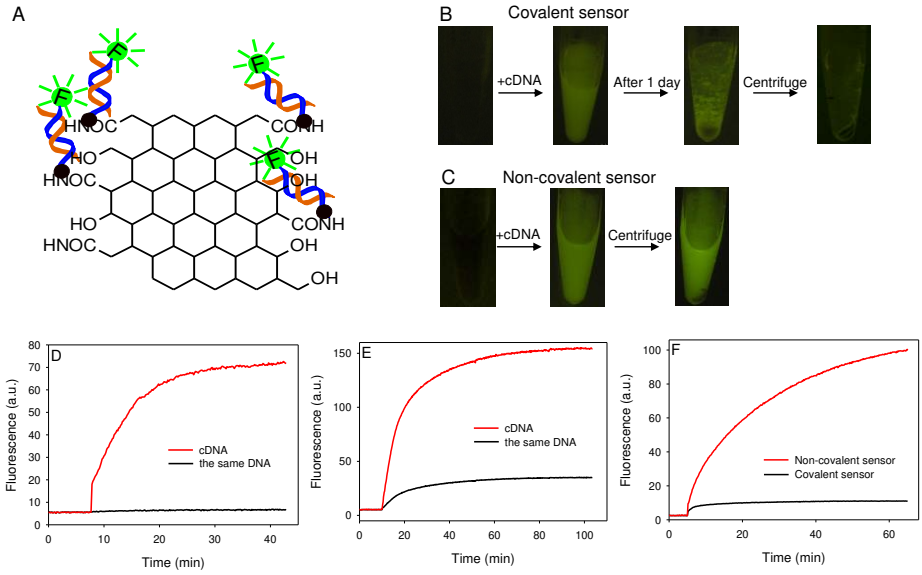

Figure 9. (A) A scheme of covalently attached fluorescently labeled probe hybridized with cDNA. Photographs taken under 470 nm excitation of covalent (B) and non-covalent (C) sensors after reacting with the cDNA and centrifugation. Response of the covalent sensor (D) and non-covalent sensor with physisorbed probe DNA (E) in the presence of cDNA or the same DNA. The intention of adding the same DNA is to test non-specific displacement of probe DNA. (F) Response of the covalent and non-covalent sensors to 0.5% BSA. Reprinted with permission from reference [51].

3.6. Other types of sensor design methods

3.6.1 Using the native fluorescence property of GO

While most sensors involve fluorescence labels and use GO as a quencher, GO has intrinsic fluorescence as well. Unlike graphene, GO contains a number of oxygenated groups including hydroxyl, carboxyl and epoxyl, which create a band gap in GO. This band gap is a function of their chemical nature and the percentage of sp^2 carbon atoms. Therefore, the luminescence property of GO

can be tuned by varying the sp^2/sp^3 carbon ratio. The size distribution and fluorescence property of GO were also studied by flow cytometry [116].

Wu and co-workers developed a label-free fluorescent sensor for Hg^{2+} by covalently attaching an amino-modified thymine-rich DNA to GO. Their GO sheets emitted at ~600 nm and thus appeared orange. When Hg^{2+} was added, a hairpin DNA was formed to position Hg^{2+} nearby the GO surface. As a result, the fluorescence emission of GO was quenched due to electron transfer from excited GO to Hg^{2+}. This sensor has a detection limit down to 0.92 nM Hg^{2+} with excellent selectivity [117].

Seo and co-workers prepared GO sheets with a broad emission range from below 400 nm to over 700 nm (the peak position was at ~546 nm). They explained that photoluminescence of GO is mainly due to the recombination of electron–hole pairs which are localized within the domain of sp^2 carbon embedded in a sp^3 matrix. They covalently attached probe DNA molecules to the GO surface. When hybridized to the target DNA that is attached to gold nanoparticles, the fluorescence was quenched up to 87% [118].

3.6.2. DNAzyme-based detection

DNAzymes are DNA-based biocatalysts. Two Pb^{2+}-dependent RNA-cleaving DNAzymes are shown in Figure 10A and B, respectively. The substrate strands are cleaved into two pieces by the enzyme [119-121]. The cleavage site is a single RNA (rA) and these enzymes are the most active in the presence of low concentration of Pb^{2+}. The DNAzyme in Figure 10C has a DNA substrate, for which Cu^{2+} is required for activity. A unique application of DNAzymes is for metal ion detection [122].

Zhang and co-workers designed Pb^{2+} sensors using two Pb^{2+}-specific DNAzymes. In both cases, the substrate was designed in such a way that the FAM-bearing end contained only 4 nucleotides after the cleavage site, so its affinity to GO is weak after cleavage. The authors reported that in the absence of Pb^{2+}, the DNAzyme complexes were adsorbed by GO, leading to quenched fluorescence. In the presence of Pb^{2+}, the substrate was cleaved and the short FAM-containing DNA piece cannot be

adsorbed by GO, leading to high fluorescence. The difference of fluorescence signal in the presence of Pb^{2+} can reach 16-fold compared to that in the absence of Pb^{2+}, which compares favourably with the typical catalytic DNAzyme beacons using molecular dark quenchers. Since each Pb^{2+} can cleave multiple substrates, this reaction is catalytic with high sensitivity. The authors reported a detection limit of 0.3 nM, which is better than the ones that used molecular dark quenchers. At the same time, the signal enhancement is also higher [123].

Liu *et al.* labeled the 3′-end of the substrate strand of the Cu^{2+}-specific DNAzyme with a FAM and hybridized it with the enzyme [124]. This complex was adsorbed by GO leading to very low background fluorescence. In the presence of Cu^{2+}, the substrate strand is cleaved and the FAM-labeled fragment is released from the GO surface, producing fluorescence signal. With 1 μM Cu^{2+}, the sensor signal increased 32-fold. With such a high signal, the sensor has reached a detection limit of 0.365 nM Cu^{2+}. These papers demonstrated some unique advantages of GO. For example, no additional quencher is needed, and the quenching efficiency is even better than typical molecular dark quenchers. The same DNAzyme was also used for Cu^{2+} detection based on GO amplified fluorescence anisotropy [125].

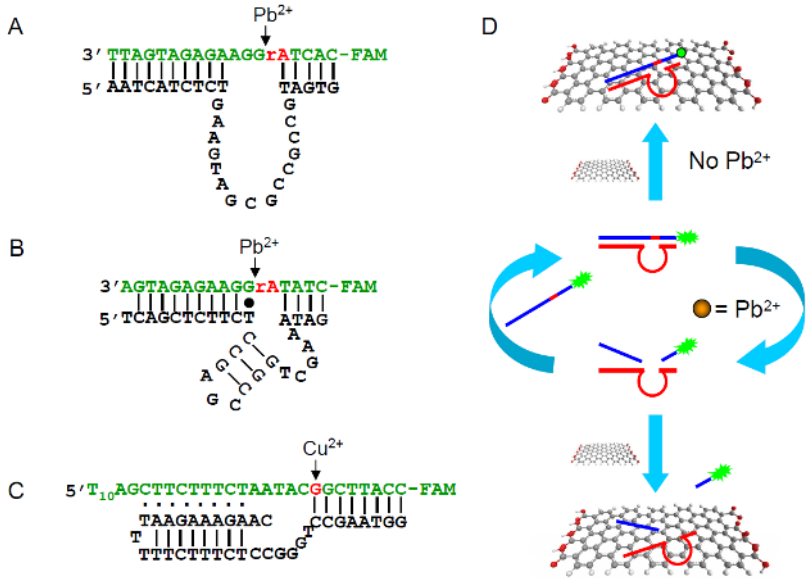

Figure 10. Secondary structures of three metal specific DNAzymes: (A) the GR5 DNAzyme specific for Pb^{2+}; (B) 17E DNAzyme specific for Pb^{2+} and (C) a Cu^{2+}-specific DNAzyme. (D) Schematics of using GO for signaling DNAzyme cleavage reaction for Pb^{2+} detection.

3.7. Signal amplification methods

In most of the above reviewed GO-based assays, each aptamer or DNA probe binds to a single target molecule. While high sensitivity has been realized due to the low background signal enabled by GO adsorption and strong fluorescence quenching, further improvement might be achieved by incorporating signal amplification strategies. In other words, each target molecule may solicit more than one signal molecule.

Yang and co-workers described a catalytic recycling amplified assay. GO protects the adsorbed DNA aptamer for ATP from DNase I degradation. However, the nuclease can cleave the aptamer desorbed from GO, releasing the fluorophore and ultimately liberating the target as shown in Figure 11. The librated target then binds a new aptamer on the GO surface to start a new cycle, which significantly amplified the signal to achieve high sensitivity (40 nM ATP) [126]. For example, the authors reported 330% fluorescence enhancement with the target recycling method, while only 62% was achieved with the normal assay. The authors demonstrated generality by testing aptamer-based cocaine detection, where a detection limit of 100 nM cocaine was reported. Using a similar strategy, Tan and co-workers described a sensitive and simple assay based on aptamer/GO for insulin detection [127]. Taking advantage that DNase I only degrades DNA, Yang and co-workers used this method to detect miRNA, where the target RNA is recycled to allow a detection limit of 25 pM in cell media [128].

DNase I non-specifically degrades both ss- and ds-DNA. New signal strategies have been designed based on other enzymes. For example, Zhao *et al.* developed an exonuclease III based assay. A 5′ FAM-labeled probe was used to hybridize with the target DNA. Exonuclease III does not cleave

ss-DNA but catalyzes stepwise degradation of nucleotides from the blunt 3′ end of the probe in a duplex. Therefore, in the presence of the target DNA, a large number of probe DNA can be degraded, generating free fluorophore that are not adsorbed by GO. This sensor exhibits a high sensitivity towards the target DNA with a detection limit of 20 pM, which was better than the GO-based DNA sensors without enzymatic amplification, and it provides a universal sensing platform for sensitive detection of DNA [129].

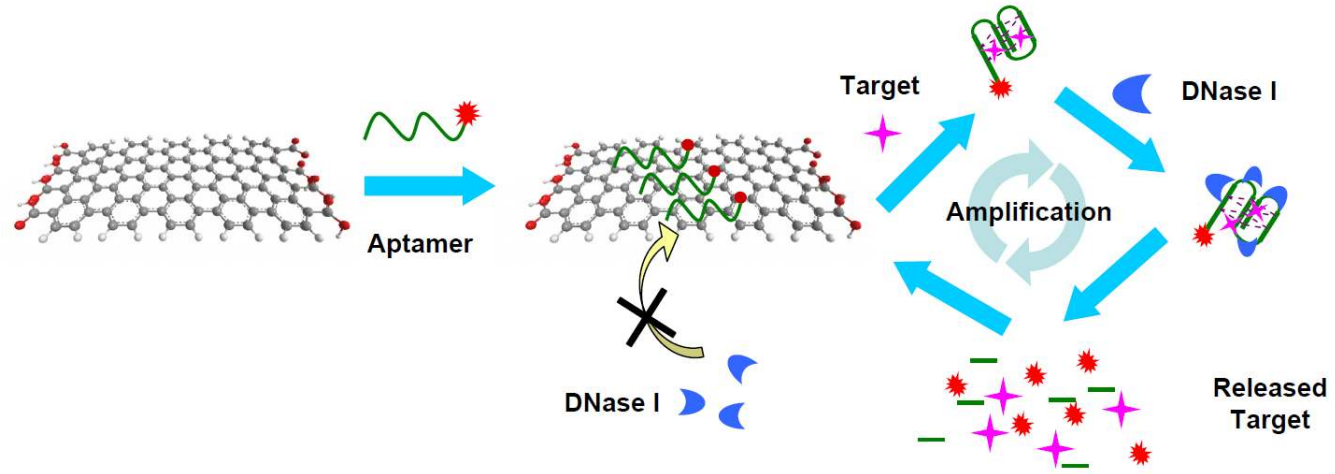

Figure 11. Amplification strategy based on a DNA-protective nanomaterial. The figure was re-drawn from reference [127].

Willner and co-workers also reported exonuclease III based assays for simultaneously detecting multiple analytes. In this case, the probe molecules were labeled with different fluorophores and adsorbed by GO. The target DNA was complementary to a fraction of the probe DNA, leading to their partial dissociation from the GO surface and degradation by the enzyme. This released the target molecule to go to the next catalytic cycle. The authors achieved a detection limit of 5 pM DNA, which was significantly better than the ~1 nM limit without the signal amplification strategy [130].

Yu and co-workers established an exonuclease III aided signal amplification using GO and DNA aptamers for lysozyme detection. A hairpin probe (HP) was designed to shield the lysozyme aptamer in the stem region. The hairpin was opened in the presence of lysozyme target, and the opened

hairpin can hybridize with a FAM-labeled signal probe. Once this duplex is formed, exonuclease III can catalyze the recycling operation as described above, and GO was used to detect the released fluorophore. This assay allows a detection limit of 0.08 μg/mL lysozyme [131].

Li and co-workers developed an enzyme-free signal amplification assay for miRNA detection by employing hybridization chain reaction (HCR) coupled with a GO surface-anchored fluorescence signal readout strategy. A pair of specially designed DNA hairpins was used and each was labeled with a fluorophore. The target miRNA led to their hybridization into a long duplex chain. As a result, their adsorption by GO was inhibited. Since each target miRNA induced a large number of hairpins into the duplex form, signal amplification was achieved. While in the absence of the target, the individual hairpins were adsorbed by GO to quench their fluorescence. This method detects the target miRNA down to 1 pM, which is about three orders of magnitude better than the one without signal amplification [132].

3.8. Graphene oxide like materials

The discovery of graphene has stimulated research on single-layered 2D nanosheets. Interestingly, many of such materials also exhibit excellent fluorescence quenching and DNA adsorption properties, which have been used in biosensor development in a similar way to GO. For example, the Sun's group and many others have tested a diverse range of materials for DNA adsorption and fluorescence quenching [133-138]. We also reported adsorption of DNA by metal oxides [139,140], and nucleotide coordinated nanoparticles [141]. Here we only cover a few examples of monolayers 2D planar materials similar to graphene.

Zhang and co-workers fabricated single-layered MoS_2 nanosheets, in which a positively charged Mo plane was sandwiched between two sulphur planes. Since such materials have been reported to adsorb aromatic compounds and the authors speculated that it has a fluorescence quenching property as well, a FAM-labeled DNA was tested as a probe for DNA detection. The free probe was

quickly adsorbed by MoS_2 sheets to quench fluorescence, while adsorption was inhibited after forming a duplex with the target DNA. This is very similar to the mechanism of GO-based sensing discussed above, and a detection limit of 0.5 nM DNA was reported. The authors further demonstrated the detection of adenosine using the same platform [142].

Yu and co-workers reported a signal amplification method for detecting miRNA by using WS_2 nanosheets. In this work, the miRNA target was hybridized with a FAM-labeled ss-DNA probe to form a duplex. The probe strand of the duplex was cleaved by a duplex-specific nuclease, thus releasing the miRNA target for signal amplification. The degraded FAM-labeled DNA could not be adsorbed by WS_2 while the original full length probe was adsorbed with quenched fluorescence. This method gives a detection limit of 300 fM miRNA[143]. Other graphene-like materials such as BN nanosheets have been tested for nucleobase adsorption and they might also be used in a similar way for DNA detection [144,145]

4. Summary and future directions

In this review, we summarized fundamental interactions between DNA and GO, fluorescence quenching property, and various strategies for sensor design and signal amplification. Impressive progress has already been made for detecting environmental and biomedical targets. It is even possible to achieve detection in live cells. While the research on GO/DNA based sensing started only five years ago, hundreds of papers have already been published in this field. This reflects the advantages of this sensing platform: simple to use, high performance and low cost. GO is a unique material; with a heterogeneous surface and a diverse range of attractive forces for DNA adsorption, a large fraction of DNA probes are adsorbed with an intermediate force. These probes can respond to the presence of target molecules. For example, the similar concept was studied using CNT as quencher even before the use of GO, but the amount of work on GO is much more.

Given these achievements, there are still a few problems to be overcome before this technology can reach its full potential. These problems also point to the future directions. 1) For biosensor development, beyond the proof-of-concept work, it is important to demonstrate real applications to reach the next stages. For example, while detection has been made in live cells, the current sensors employ only physisorbed probes. Even though internal references can be added to deal with the non-specific probe release problem, the sensitivity is likely to suffer from such events. Inside a cell, covalent probes are likely to be more attractive due to the protective effect of GO and also more resistant to non-specific probe displacement. One future direction is to develop covalently linked probes or stronger physisorption mechanisms. 2) On the materials side, a broad range of nanomaterials have already been reported to share similar properties as GO in terms of fluorescence quenching and DNA adsorption, including many carbon-based nanomaterials, metal oxides, organic and polymeric nanoparticles, quantum dots and metal nanoparticles. Though it needs to be noted, the forces of interaction might be quite different in each case. A systematic evaluation is needed to screen optimal materials for this purpose. GO may or may not be the most optimal material. 3) For fundamental studies, a more precise picture of DNA interacting with GO surface is needed in terms of different levels of oxidation, adsorption affinity, types of intermolecular forces involved and target induced desorption. Systematic work is also needed to understand the binding of DNA by different carbon-based materials. Efforts have already been seen in this regard [146].

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