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Graphene and graphene-like two-denominational materials based fluorescence resonance energy transfer (FRET) assays for biological applications

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In the past decades, Förster resonance energy transfer (FRET) has been applied in many biological applications to reveal the biological information at the nanoscale. Recently, graphene and graphene-like two-dimensional (2D) nanomaterials started to be used in FRET assays as donors or acceptors including graphene oxide (GO), graphene quantum dot (GQD), graphitic-carbon nitride nanosheets (g-C_3N_4) and transition metal dichalcogenides (e.g. MoS_2, MnO_2, and WS_2). Due to the remarkable properties such as large surface to volume ratio, tunable energy band, photoluminescence and excellent biocompatibility, these 2D nanomaterials based FRET assays have shown great potential in various biological applications. This review summarizes the recent development of graphene and graphene-like 2D nanomaterials based FRET assays in applications of biosensing, bioimaging, and drug delivery monitoring.

Keywords: Graphene; Graphene-like two dimensional materials; Förster resonance energy transfer (FRET); Biological application

1. Introduction

In recent years, Förster or fluorescence resonance energy transfer (FRET) has been widely used in biosensing area. Generally, FRET is a near-field energy transfer from a fluorescent donor to a fluorescent acceptor within close proximity (Clegg, 1995; Selvin, 2000). Under certain excitation, emission of a fluorescent donor can be absorbed by a fluorescent acceptor nearby, leading to a fluorescence quenching phenomenon. The distance between energy pairs is typically less than 10 nanometers (Forster 1946). Fortunately, many bioreactions in human bodies such as DNA hybridization, antibody-based immunological recognition, and enzyme-catalyzed hydrolysis or transformation occur or cause responses in a similar distance. Due to this high consistency, FRET based biosensor can be a favourable tool for study and detection of such biological reactions (Ha et al., 1996; Heim and Tsien, 1996). Fluorophore pairs also play a crucial role for the performance of FRET assays except for the distance. Excellent fluorescent donor molecules or acceptor molecules with favourable optical properties can substantially enhance the efficiency of energy transfer, resulting in a superior sensitivity of FRET-based biosensors.

Fluorescent donor molecules are mainly divided into two groups: traditional fluorophores such as organic fluorescent dyes (Resch-Genger et al., 2008) or fluorescent proteins (Piston and Kremers, 2007), and emerging nanoparticle-based materials such as semiconductor quantum dots (QDs) (Sapsford et al., 2006), upconversion nanoparticles (UCNPs) (Ye et al., 2014), graphene-based derivatives (Geim and Novoselov, 2007) and graphene-like two-dimensional (2D) based

nanomaterials (Yang et al., 2015). Traditional organic fluorescent dyes are a huge group of molecules with universalities such as low cost, small size, and easiness for modifications (Resch-Genger et al., 2008). However, the poor photobleaching resistance, low chemical stability and relatively short fluorescent lifetime greatly restrict them in broad FRET-based applications. Another representative of traditional fluorophores is fluorescent proteins (FPs) which are commonly used as biolabels for *in-vivo* tracking (Piston and Kremers, 2007). However, the wide range of emission spectra of FPs generates spectral cross-talk and prevents multi-target detection with different colours in FRET assays. Hence, new fluorescent donor materials with high quantum yield, excellent photo-stability, and long fluorescence lifetime are highly in demand to overcome these obstacles.

Recently, many nanoparticles with special optical properties have been used in the design of FRET assays either as donors or acceptors. For instance, nanoparticles including semiconductor quantum dots (QDs), upconversion nanoparticles (UCNPs) are found to have photoluminescence (PL) due to quantum confinement effect (Sapsford et al., 2006; Ye et al., 2014). These photoluminescent nanoparticles are highly photostable with enhanced brightness and long fluorescent lifetime, which make them perfect succedaneum of traditional fluorophores as fluorescence donors. Moreover, some nanoparticles have excellent optical quenching capabilities such as gold nanoparticles (AuNPs) which can be used as efficient fluorescence quenchers in FRET assays (Eustis and El-Sayed, 2006). Most recently, graphene and its derivatives have been widely used in optical based applications due to their tunable band gap,

strong photoluminescence emission and good biocompatibility (Morales-Narváez and Merkoçi, 2012). Moreover, the 2D nanosheet structure with high surface to volume ratio and easy functionalization with biomolecules make graphene-based materials excellent platforms for optical biosensing applications. After the great success of graphene based materials, with deep explorations of graphene properties and widespread applications, graphene-like 2D nanomaterials emerged (Xu et al., 2013). Due to the similar 2D structures and excellent optical properties, graphene-like 2D nanomaterials such as graphite carbon nitride (g-C_3N_4) nanosheets and metal dichalcogenides (TMDs, for instance, MoS_2, WS_2, and MnO_2) are starting to be used in FRET assays (Zhang et al., 2014; Matte et al., 2010; Jariwala et al., 2014)

The purpose of this review is to present the current status of graphene and graphene-like 2D materials based energy transfer systems that use FRET mechanism for various biological applications. We firstly describe FRET mechanism and the development of nanoparticle based FRET assays. We then comprehensively discuss the recent development of graphene and graphene-like 2D materials including graphene oxide (GO), graphene quantum dot (GQD), g-C_3N_4 nanosheets, and TMDs (MoS_2, WS_2, and MnO_2), as donors or acceptors in FRET assays. Specific areas of applications including biosensing, imaging, and drug delivery monitoring are discussed. Finally, the future trends of graphene and graphene-like 2D materials based FRET assays are discussed.

2. FRET assays

2.1 History and mechanism of FRET

The performance of FRET assay is determined mainly by three factors: fluorescence donor, fluorescence acceptor and the distance between donor and acceptor. To construct a FRET assay, a fluorescence donor with a certain range of excitation and emission bands is needed with another fluorescence acceptor whose excitation band should overlap with the emission band of the donor (Forster 1946). Within a typical range on the nanoscale, near-field energy communication occurs which results in the energy transfer from a donor to a nearby acceptor within close proximity (Fig. 1a). The energy transfer efficiency is determined by the equation below (Forster 1946):

$$E = \frac{1}{1+\left(\frac{R}{R_0}\right)^6} \quad (1)$$

Where R is the distance between donors and acceptors, and R_0 is the distance between donors and acceptors when the transfer efficiency is 50% (Fig. 1b). An approximatively inverse ratio exists between FRET efficiency and sixth power of the distance, and the typical value of R_0 is calculated to be at the nanoscale below 10 nm using overlap integral.

The Forster radius (R_0) which is defined as half of the energy transfer efficiency is generally determined by the following equation:

$$R_0{}^6 = \frac{9(\ln 10)}{128\pi^5 N_A}\frac{k^2 Q_D}{n^4} J \quad (2)$$

Where, n is the refractive index of media, Q_D is the quantum yield of the donor without acceptor adsorption, k^2 is the dipole angular orientation factor of donor and acceptor molecules, N_A is the vogadro's number, and J is the spectral overlap integral

of the donor-acceptor pair. So generally, the FRET energy transfer efficiency is dependent on quantum yield of donor molecules, refractive index of media, the spectra overlap degree between donor molecules and acceptor molecules, the donor-acceptor dipole orientation and the distance between donor and acceptor molecules.

Many bioreactions, including DNA hybridization, antibody-antigen recognition and enzyme hydrolysis, commonly happen at the same nanoscale. Therefore, FRET-based assays can be a promising tool qualitatively to detect and monitor such bioreactions. For the biosensing applications, the fluorescence (FL) signal detection depends not only on the overlapping ratio but also on the quantum yield of the donor and the quenching capability of the quencher. High quantum yield means more photons are excited under a certain intensity of excitation, and in macroscopic view the manifestation is a brighter emitted light. High quenching capability can largely change the optical signal due to biological recognition to improve the detection sensitivity.

Here, Figure 1

2.2 Traditional organic fluorescent dyes and fluorescent proteins

Traditional fluorophores including organic fluorescent dyes and fluorescent proteins are widely used in FRET-based biological applications. Organic fluorescent dye is a class of fluorophore with the representatives such as FAM, FITC, Cy3/Cy5 and Texas red (Resch-Genger et al., 2008). They exhibit many advantages such as small

size with plentiful chemical modification sites and high quantum yield. However, organic dyes have disadvantages such as pH sensitivity, easy photobleaching, as well as blinking, which limit the universal use of them in biological applications. Multicolor fluorescent proteins (FP) make living cell imaging possible, which could be used for intracellular protein-protein interaction monitoring (Shimomura et al., 1962). Advantages of fluorescent proteins include high quantum yield and outstanding photostability, which can be used for long-term detection in living cells (Chalfie, 2009). However, the broad spectra of excitation and emission of FP may induce cross-talk effect. Moreover, the detection efficiency is also limited by the large size of FP (Patterson et al., 2000).

2.3 Nanoparticle-based FRET assays

The development of nanomaterials provides promising opportunities to develop multiple types of nanoparticles as donors or acceptors in FRET assays for various biological applications. Many photoluminescent nanoparticles have been used as donors in FRET assays including semiconductor quantum dot (QD), upconversion nanoparticles (UCNP), graphene quantum dots (GQD) and graphene-like 2D nanomaterials based fluorescence nanoparticles. Compared with traditional fluorophores, photoluminescent nanoparticles have strong luminescence, high quantum yield, long fluorescence lifetime and high photostability, which make them perfect candidates as donors in FRET assays (Shi et al., 2014). Moreover, nanoparticles with strong optical absorption capability have been used as efficient quenchers in FRET

assays including gold nanoparticle (AuNP), graphene oxide (GO) and graphene-like 2D nanomaterials. The usage of nanoparticles in FRET assays can bring advantages of high energy transfer efficiency, excellent photo-stability and ultrasensitive detection for various biological applications. In this review, we will mainly focus on the recent development of graphene and graphene-like 2D nanomaterials based FRET assays for biosensing, imaging, and drug delivery monitoring applications.

3. Graphene-based FRET assays

Since graphene was discovered for the first time in 2004 (Novoselov et al., 2004), tremendous attentions have been paid to applications in various areas by researchers because of its excellent thermal, mechanical and electronic properties. Pristine graphene is an ideal one-atom-thick planar sheet structure with sp^2 carbon atoms in a honeycomb lattice, which has excellent quenching capability in FRET assays (Novoselov et al., 2004). However, pristine graphene is highly hydrophobic, which limits its wide applications in biomedical detection. Moreover, the π state electrons on pristine graphene surface induce zero band-gap, resulting in no PL emission under any wavelength excitation. Fortunately, graphene derivatives including graphene oxide (GO)/reduced graphene oxide (rGO), graphene quantum dots (GQDs) can perfectly fill these gaps through particular physical and chemical processes. In this section, the optical properties of graphene derivatives in FRET assays including photoluminescence and quenching capability will be discussed. Table 1 shows a summary of graphene-based FRET assays.

Here, Table 1

3.1 Interaction of graphene and graphene based materials with biomolecules

Graphene and graphene-related materials have been used as substrates for attachment of various biomolecules and cells. Although these biomolecules and cells vary a lot in functions and structures, the functionalization of them onto graphene surface is generally through two approaches: physical adsorption and chemical conjugation.

Physical adsorption is generally quite weak but crucial in nature and enough for biofunctionalization. Among varies types of physisorption, π-π stacking interaction is quite important for biomolecule attachment on graphene surface. Graphene is honeycomb-like carbon structure in a single layer and the abundant sp^2 carbon atoms ensure the high capacity for π-π stacking interaction (Chen et al., 2011). On the other hand, many biomolecules like oligonucleotides, peptides, and some small molecules intrinsically have π bonds. Hence, π-π stacking interaction can be formed between graphene-related materials and such biomolecules without any chemical reactions (Zheng et al., 2003a, b). The strong interaction between single-stranded DNA (ss-DNA)/peptide and graphene related material surface could happen within a few minutes (Zhang et al., 2011a, b). However, graphene materials seldom interact with double stranded DNA (dsDNA) due to the shielding of nucleobases within phosphate backbones of dsDNA (He et al., 2010; Lu et al., 2009; Chang et al., 2010). Another

physisorption force is the electrostatic interaction. Graphene oxide, reduced graphene oxide, and graphene quantum dots are commonly negatively charged (Liu et al., 2015). Therefore, positively charged biomolecules or probes can be easily adsorbed onto graphene surfaces.

Chemical conjugation is another approach for attachment of biomolecules on graphene-related materials. The covalent functionalization for pristine graphene generally uses organic free radicals and dienophiles to react with sp^2 carbon atoms (Kosynkin et al., 2009; Georgakilas et al., 2008). On the other hand, the covalent functionalization for GO is based on the abundant oxygen containing groups on GO surface, including carboxyl groups on the edges and epoxy/hydroxyl groups on the basal plane (Georgakilas et al; Morales-Narváez et al., 2012). Due to the rich chemistry of hydroxyl, carboxyl, and epoxy groups, GO is often chosen as the starting materials to develop other types graphene derivatives through surface functionalization. The most commonly used surface functionalization approach for GO is through carbodiimide chemical reactions. The carboxyl groups (-COOH) on graphene oxide are facile to react with amino groups ($-NH_2$) on biomolecules to form carbodiimide bonds in the presence of EDC and NHS. Peptides or proteins naturally contain amino groups and oligonucleotides can be modified with amino groups at one end. Hence, the carbodiimide chemistry can be broadly used for chemical conjugation between biomolecules and graphene-related materials.

3.2 Fluorescent GO as donor

Fluorescent graphene oxide has been synthesized by many methods including surface oxygen plasma treatment, modified Hummers methods, one-pot electrochemistry method, etc (Gokus et al., 2009; Chen et al., 2010; Lu et al., 2009). Unlike pristine graphene which exhibits no photoluminescence properties due to zero bandgap in the pure sp^2 carbon lattice, GO is occupied by sp^2/sp^3 carbons and oxygen functional groups on the surface or at edges, leading to various photoluminescence properties in visible and near-infrared (NIR) region (Fig. 2a) (Eda et al., 2010). Generally, when pristine graphene is doped with impurities such as oxygen-containing groups, the band gap is opened to facilitate photoluminescence generation (Eda et al., 2010). The recombination of electron-hole pairs in the disrupted π networks containing both sp^2 and sp^3 carbon atoms is the essential reason for the visible and NIR emissions (Gokus et al., 2009; Luo et al., 2009). Hence, the amount and state of sp^3 carbon linking groups is the key of altering the wavelength and intensity of emission, which can be simply manipulated by controlling surface chemistry, solid or liquid state of GO sheets and pH value of the solvent. The photoluminescence of fluorescent GO is quite stable against chemical and photonic bleaching compared to conventional organic fluorophores.

Several studies have been reported since Luo and his coworkers achieved a broad PL from GO for the first time (Luo et al., 2009). Strong photoluminescence was induced in both liquid and solid GO samples with emission from visible to NIR region through oxygen plasma treatment. Compared to liquid samples of GO, spectra of solid GO

shifted more towards to longer wavelength. Under reduction by hydrazine, a red-shift spectrum could be obtained with a relative wavelength increase in IR region. However, the integral quantum yield was decreased due to the nearly completed reduction of GO, resulting in the zero bandgap region. Moreover, PL quantum yield of the above photo-luminescent GO was not high enough for sensing measurement. This was due to the low amount of the recombination electrons in the oxidation groups. Mei et al. achieved a high efficient photoluminescence GO by reacting with various alkylamines (Mei et al., 2010). Intense blue fluorescence was detected attributed to the surface modification of amide and amination of epoxide formation. The quantum yield was enhanced from 0.0002 to 0.13, with the maximum excitation at 350 nm (UV light) and emission at 430 nm. Through such surface modification, GO exhibited tunable hydrophilic and hydrophobic properties which were suitable for further applications in many fields. PH value is another major factor exerting influence on the PL emission. The protons of carboxyl groups vary under different pH values. When pH value was above 8, G-COO^- ions occupied whereas they converted to G-COOH under pH 8. This change affected the emission feature of GO, for example, the spectrum would shift to red when decreasing the pH value reported by Galande and his coworkers (Galande et al., 2011).

Photoluminescent GO-based FRET assays have been used for nucleic acid detection. Liu *et al.* developed a FRET assay for DNA hybridization detection with probe modified photoluminescent GO as a donor and target modified AuNP as an acceptor (Liu et al., 2010). Firstly the amino groups on probe ssDNA(NH_2-ssDNA) reacted with the carboxyl groups on GO through EDC/NHS activation to form the

ssDNA-GO complex. Meanwhile, the target ssDNA with a thiol group at one end was bonded onto AuNPs surface though chemo-adsorption, forming ssDNA-AuNPs composites. After the hybridization between ssDNA-GO and ssDNA-AuNP, the distance between GO and AuNPs was getting close enough to perform the fluorescence quenching. By measuring the fluorescence signal intensity, the limit of detection of target ssDNA-AuNP complex was achieved as low as 200 nM.

As a fluorescent donor, photo-luminescent GO is also used for protein detection. For protein analysis, antibody or aptamer is mainly utilized as specific probes to combine with target proteins. June et al. developed a fluorescent GO-based immuno-FRET array system for rotavirus detection (June et al., 2010). Fluorescent GO was deposited on an amino-modified glass surface. The rotavirus antibodies were then immobilized on GO array surface via covalently binding. Once target rotavirus was captured by the antibodies on GO array surface, AuNP-DNA-antibody was added to form a sandwich structure with target rotavirus and GO array, leading to the fluorescence quenching of GO array. A limit of detection of 10^5 pfu mL^{-1} was achieved for rotavirus detection. Mei *et al.* fabricated a versatile and portable microporous membrane-based sensor immobilized with fluorescent GO for DNA, peptide and protein detection (Mei et al., 2012). GO was treated with *n*-butylamine (NHBu) in advance, resulting in GO-NHBu composites with a blue fluorescence emission at 440 nm. Unlikely general fluorescent GO, GO-NHBu composite is highly photostable and abundant in hydrophilic groups, affording adequate interaction sites for chemical conjugation. Fluorescent GO-NHBu was then jet printed onto a piece of microporous

membrane as the donor with functionalized AgNP as the quencher for detection of different biomolecules. This sensor offered a detection limit of 0.1 pM for IgG, 20 pM for glutathione and cysteine and 1 nM for DNA.

Heavy metal ions like Pb^{2+}, Hg^{2+}, Cu^{2+}, etc. can cause water or food contamination which is seriously harmful to human being. Kundu *et al.* developed a FRET-based approach for Au^{3+} detection in an aqueous medium with highly fluorescent GO/poly(vinyl alcohol) (PVA) hybrid (GO-PVA) as donor and target Au^{3+} ion as acceptor (Kundu et al. 2012). GO/PVA complex had a higher quantum yield compared to GO. The GO/PVA complex has a selective sensing of Au^{3+} ions due to its higher reduction potential compared to other metal ions. The exciton of GO-PVA transferred to Au^{3+} ions, resulting in a reduction of Au^{3+} to Au and the fluorescence quenching. A limit of detection was achieved for Au^{3+} detection as low as 275 ppb. Water safety is not only threatened by heavy ions but also some bacteria which produce highly dangerous toxins. Shi *et al.* designed a FRET assay with fluorescent GO as donor and AuNPs as acceptor on glass slides for microcystins (MCs) detection (Shi et al., 2012). Firstly, GO was spotted onto the APTES modified glass slide through electrostatic adsorption. Then microcystin antibodies were conjugated onto the GO surface through covalent binding. Meanwhile, Au-ssDNA complex was specifically conjugated with microcystins, leading to the sandwich structure (GO-Ab-MC-AuNPs) formation and fluorescence quenching. By monitoring the relative decrease of fluorescence, microcystin-LR and microcystin-RR could be detected with a limit of 0.5 μg/L and 0.3 μg/L, respectively.

3.3 Graphene quantum dot

Graphene quantum dots (GQDs) are small fragments of graphene oxide (GO) with a diameter less than 20 nm in lateral dimension and single or few layers of thickness (Fig. 2b) (Zhu et al., 2012). Due to the small size, GQDs can be regarded as a 0D material. Similar to GO, GQDs contain sp^2 carbon domains and are surrounded with functional groups such as epoxy and carboxyl groups. Because of a larger perimeter-area ratio, more defects of oxygen and other functional groups are possessed on GQD surface which renders GQD excellent photoluminescence due to quantum confinement effect and edge effect (Zhu et al., 2012; Shen et al., 2011). Hence, a combination of limited size and abundant functional groups is the theme of GQDs.

Many approaches have been used for GQD synthesis mainly via two approaches: top-down methods (Pan et al., 2010; Li et al., 2011) and bottom-up methods (Yan et al., 2010; Zhou et al., 2012; Kim et al., 2012). The synthesized GQDs have tunable optical properties (typical PL or upconversion PL), high brightness, excellent photostability, long fluorescence lifetime and exceptional biocompatibility (Shi et al., 2015; Shen et al., 2012; Liu et al., 2013). Basically, the quantum confinement effect and surface effect are the two dominant parameters for photoluminescence (Zhu et al., 2012). Quantum confinement effect is the basic factor of photoluminescence which occurs when GQD is small enough comparable to the exciton Bohr radius. The bandgap of GQDs increases with the decrease in size, resulting in a longer wavelength of emission. In other words, as the size of GQD increase, the emissions under certain excitation will red-shift to

higher energy. Basic study of the bandgap of GQDs was shown by Eda and his coworkers (Eda et al., 2012). The bandgap of benzene, a bring-like structure composed of 6 carbon atoms was calculated to be 7eV. Correspondingly, the bandgap of GQDs with 20 aromatic rings decreased to 2eV, proving GQDs with smaller size have higher energy emission. The edge effect is treated as another significant factor of GQD photoluminescence. Liu et al. analyzed the PL difference between graphene oxide quantum dots (GOQDs) and reduced graphene oxide quantum dots (rGOQDs) (Liu et al., 2013). rGOQDs could be obtained by partially reduction from GOQDs. The emission peak of rGOQDs was shifted to around 420 nm (blue) while that of GOQDs was about 480 nm (green). Amine moieties modified GQDs were reported by Tetsuka et al. (Tetsuka et al., 2012). GQDs were treated with the ammonia-assisted hydrothermal method and a longer wavelength of emission at the same size was achieved. Such phenomenon showed that the PL properties could be altered through various surface modifications. Other parameters such as the excitation wavelength, pH value, and solvent types can exert influence on the emission spectra and quantum yield. For example, the protonation of the free zigzag sites of the GQDs took place in acidic solutions, leading to the destruction of emissive triple carbine state. On the contrary, these free zigzag sites were maintained in alkaline condition and emitted PL (Pal et al., 2015)

GQDs have been used as donors in FRET assays for various biological applications. GQD is firstly employed in FRET assay for DNA detection. Shi et al. developed a FRET assay with GQDs as the donor and AuNPs as the acceptor for specific *Staphylococcus*

aureus gene detection (Shi et al., 2015). To construct the FRET assay, two ssDNA capture probes were conjugated with GQDs and AuNPs, respectively. Interestingly, after the modification with ssDNA, GQDs emitted a much stronger luminescence with an enhancement of quantum yield to 19% compared to unmodified GQDs. Then target bacteria DNA hybridized with two capture probes to bring GQDs and AuNPs into close proximity which triggered FRET effect (Fig. 2c). The limit of detection for *Staphylococcus aureus* gene detection is around 1nM. In another GQD based FRET assay for DNA detection produced by Qian et al. (Qian et al., 2014a), GQDs and carbon nanotubes (CNTs) were used as donors and acceptors, respectively. Bare GQDs were firstly reduced by $NaBH_4$, exhibiting a bright blue fluorescence around 443 nm under UV excitation. The PL quantum yield rose to 20.4% while that of pristine GQDs was only 1.7%, which was promising for sensitive FRET-based DNA detection. A detection limit of 0.4 nM was achieved for DNA detection. Qian et al further realized simultaneous detection of multiple DNA targets by a FRET assay integrating dual-color GQD nanoprobes and carbon nanotubes (Qian et al., 2014b). Blue GQDs and green GQDs were used to construct dual-color probes to recognize two DNA targets respectively. The dual-color GQD probes were assembled on carbon nanotube surface. In the presence of multiple DNA targets, the DNA hybridization between GQD nanoprobes and DNA targets led to the detachment of specific GQD nanoprobes from carbon nanotubes surface with certain color recovery. A detection limit for the two target DNA probes were 3.6 and 4.2 nM, respectively. Qian et al. later used GO to replace AuNP as the acceptor to construct a GQD/GO-based FRET sensor for DNA

detection with an improved detection limit of 75 pM (Qian et al., 2014c).

Here is Figure 2

Zhou et al. designed a GQD based FRET assay for toxic paranitrophenol (4-NP) detection (Zhou et al., 2014). Molecularly imprinted polymer (MIP) was coated on GQD surface for the specific binding to the target 4-NP molecules. Due to the energy transfer from GQD to 4-NP, the fluorescence was quenched. By monitoring the FL change, this method exhibited a good linearity in the range of 0.02-3.00 μg/mL with a detection limit of 9.00 ng/mL. Li et al. developed a GQD based FRET assay for nitrophenol-based explosive substance trinitrophenol (TNP) detection (Li et al., 2015). TNP could quench the fluorescence signal of GQD due to a FRET effect. A linear detection range from 0.1–15 μmol L^{-1} was achieved with a detection limit of 0.091 μmol L^{-1}. Zhang *et al.* developed a FRET assay with ssDNA conjugated GQDs as the donor and silver nanoparticles (AgNPs) as the acceptor for hydrogen peroxide (H_2O_2) and glucose detection (Zhang et al., 2015). Firstly, AgNPs-DNA@GQD composite was prepared and PL of GQD was quenched by AgNPs. In the presence of H_2O_2, H_2O_2 reacted with Ag from the outer sphere and produced hydroxyl radical (•OH) which would decompose DNA into pieces with FL recovery. This FRET assay exhibited a linear detection range from 0.4 to 200 μM with a detection limit of 0.1μM for H_2O_2 detection, and a linear detection range from 2.0 to 100μM with a detection limit of 0.42μM for glucose detection.

3.4 GO as acceptors

Pristine graphene-based structures have sp^2 electronic hybrid orbitals which show high quenching capability of fluorescence. The Hummer's method is commonly used for graphene oxide (GO) synthesis (Marcano et al., 2010), turning 3D graphite into 2D graphene oxide with generated oxygen groups including epoxy, hydroxyls, and carboxyl groups. The synthesized GO is a complex of sp^2/sp^3 carbon atoms and has oxygen groups on the surface or at edges. It has been reported that GO has a lower quenching capability compared with reduced GO. However, the quenching efficacy of 100% of GO has been reported in FRET assays, which demonstrates that GO is still a good quencher. Among graphene-related materials, GO is the mostly used material as acceptor for FRET-based biological applications. This superiority is attributed to the unique heterogeneous structure with abundant functional groups, large surface-volume ratio and good solubility in water.

The unique heterogeneous structure is due to the coexistence of π state sp^2 carbon clusters and σ state sp^3 C-O matrix (Dreyer et al., 2010). Such complicated structure not only provides a broad absorbance from 200 nm to 800 nm, but also supplies a mass of chemical binding sites (Kim et al., 2010). The abundant oxygen-containing groups and rich π-state electrons make it possible for covalent binding and π-π interaction between biomolecules and GO surface, respectively. Typically, antibodies commonly bond onto GO surface via EDC/NHS coupling chemistry, while DNA or peptides can simply interact with GO through π-π stacking. Another advantage is that the large

surface area of GO can offer numbers of binding sites, serving as a platform for various biochemical reactions.

The long range of quenching effect makes GO outstanding from other fluorescent quenchers. In traditional FRET assays, the quenching efficiency is inversely proportional to the sixth power of the distance between donors and acceptors. However, according to Swathi and his coworkers, a fourth-order inverse relation was obtained, resulting in a much longer distance for effective quenching (Swathi amd Sebastian, 2009). Such a long quenching distance provides much more opportunities for large biomolecules detection.

FRET assays with GO as acceptors have been used for nucleic acid detection. For RNA and DNA detection, the typical approach was based on the π--π interaction between nucleotides/deoxynucleotides and sp^2 carbon atoms on GO. The interaction force between GO and ssDNA varied with the length of ssDNA. As the length of SSDNA increased, the force became stronger (Wu et al., 2011). Also, the force between dsDNA and GO was measured to be much weaker compared to that between ssDNA and GO. Utilizing the adhesion strength difference between short ssDNA, long ssDNA and dsDNA, many applications have been developed for RNA/DNA detection based on this mechanism. Generally, fluorescent molecules/materials (FAM (Xing et al., 2013), FITC (Pang et al., 2013), QDs (Liao et al., 2014), GQDs (Qian et al., 2014c), UCNPs (Alonso-Cristobal et al., 2015) etc.) modified ssDNA probe was firstly bonded on GO surface though π-π interaction or covalent binding, triggering the FRET effect between fluorescent molecules/materials and GO. Then, target DNA/RNA was added to

hybridize with ssDNA probe on GO surface. Due to the weaker affinity between dsDNA and GO, the fluorescent labelled dsDNA detached from GO surface causing the FL recovery. Such methods were simple, rapid, well developed with the detection limit from 0.1 pM (Liao et al., 2011) to 0.2 nM (Zhang et al., 2014). For the sake of a more sensitive detection platform, recycle reaction technique was commonly introduced for FL signal amplification (Zhang et al., 2014a; Zhang et al., 2014b; Hwang et al., 2015; Cui et al., 2012). Among them, strand displacement and enzyme catalyzed reaction were commonly used for the amplification.

Zhang et al. employed strand displacement reaction for target DNA detection with amplification (Zhang et al., 2014). In strand displacement reaction, target ssDNA played a role similar as 'enzyme'. In the absence of target DNA, two types of ssDNA, H1 and H2, had the hybridization potential but hardly interacted with each other because of their respective formation of molecular beacon (MB). In the presence of target DNA, H1 opened its hairpin structure due to the hybridization with target DNA, and the stem-opened state of H1 could trigger the displacement of target DNA by H2. The process was similar to DNA branch migration. Then the released target will 'catalyze' another cycle of strand displacement reaction to achieve the signal amplification. H1 and H2 were modified with FAM and quenched in the MB state and fluoresced again after mixed with target DNA. Such method exhibited a detection limit of 0.2 nM and a linear detection range from 0.4 nM to 5 nM. Enzyme catalyzed reaction was introduced by Cui et al for miRNA detection with FAM-labeled ssDNA probe as donor and GO as quencher (Cui et al., 2012). Similar to the mentioned strand

displacement method, target miRNA here performed like a ‘catalyst’ to catalyze the cleavage of FAM-ssDNA by DNase I. DNase I could nonselectively cleave DNA to release oligonucleotide fragments. However, ssDNA adsorption on GO prevented the cleavage, and the PL of FAM was quenched. In the presence of target miRNA, ssDNA was free from GO with FL recovery and decomposed by DNase I, hence the release of ssDNA trigger another cycle of cleavage, leading to the signal amplification. This method showed a linear detection range from 20 pM to 1 nM, and the detection limit was calculated to be 9 pM.

FRET assays with GO as acceptors are also used for small biomolecule detection. Liu et al. reported a homogeneous FRET sensing platform based on the energy transfer from Rhodamine B modified Polyethyleneimine (RB-PEI) to GO for heparin detection (Liu et al., 2015). Heparin has the highest negative charge density compared to any existing biological molecules. Hence an electrostatic attraction could be used as the principle for specific heparin detection. PEI is a polymer which contains high positively charge amine groups while RB is a fluorescence dye. RB-PEI was firstly synthesized by EDC/NHS coupling chemistry and then non-covalently bond to GO surface by electrostatic and π-π interactions. The close distance between RB dye and GO led to the FRET effect. After incubated with heparin, due to the particularly strong electrostatic interaction between PEI and heparin, RB-PEI-GO composite was destroyed and the FL was recovered. The linear detection range was 0.09-09U/mL and the detection limit was calculated to be 0.00132U/mL. In the same manner, Cai et al. built another similar FRET assay for heparin detection based on the electrostatic interaction. Instead of using

the complex of PEI as negative charge donor and RB as FL dye, pyrene-based butterfly shaped conjugated oligoelectrolyte (TFP) was synthesized as both charge carrier and FL donor.

Enzymes are also detected by utilizing FRET-based biosensor with GO as acceptors. Gu et al. developed a FRET immunoassay with FAM-modified peptide as the donor and GO as the acceptor for trypsin detection (Gu et al., 2011). Trypsin, widely known as digestive enzyme, can hydrolyze peptide chains mainly at the carboxyl side of the amino acids lysine or arginine. In this reported method, a piece of peptide composed six arginine residues was labeled with FAM (Arg_6-FAM) at first and then incubated with GO for FL quenching. In the presence of trypsin, Arg6 was hydrolyzed into small fragments and released FAM from GO surface. The FL change was measured and a limit of detection was calculated to around 0.1μM.

Cao et al. fabricated a FRET-based microfluidic chip for circulating tumor cells detection. CCRF-CEM cells were chosen as a representative of circulating tumor cells. In this FRET assay, FAM-modified Sgc8 aptamer was a donor and GO was used as a quencher (Cao et al., 2012). GO was firstly modified on the base of microfluidic channels. Then FAM-Sgc8 was adsorbed onto GO surface and the FL was quenched. When the solution containing CCRF-CEM cells flowed past GO surface, the FAM-Sgc8 conjugated with target cells due to the specific recognition, resulting in the FL recovery. This microfluidic-based FRET assay exhibited a linear detection range from $2.5x10^1$ to $2.5x10^4$ cells/mL with a detection limit of 25 cells/mL. As an efficient platform for CCRF-CEM detection, this FRET-based microfluidic device could provide

a higher sensitivity and selectivity compared to that of other reports.

4. Graphene-like 2D nanomaterials

Over the past few years, numerous efforts have been made on developing graphene-based nanomaterials based devices on various biological applications. Most recently, more attention has been paid to alternative graphene-like 2D nanomaterials which have similar 2D structures and excellent mechanical, electrical and optical properties. Many graphene-like 2D nanomaterials including g-C_3N_4 nanosheets and TMDs (MoS_2, WS_2 and MnO_2) have attracted increased attention in FRET assay based biological applications due to their good biocompatibility, large surface areas and unique optical properties. In this section, we will mainly discuss the applications of g-C_3N_4 nanosheets and TMDs including MoS_2, WS_2, and MnO_2 as donors or acceptors in FRET bioassays. Table 2 shows a summary of grapheme-like 2D materials based FRET assays.

Here, Table 2

4.1 g-C_3N_4 nanosheets based FRET assays

Ultrathin graphitic-phase C_3N_4 (g-C_3N_4) nanosheets with a graphitic plane can be synthesized by sonication-exfoliation of bulk g-C_3N_4 under an acid condition (Thomas et al., 2008). The existence of condensed tri-s-triazine units renders a strong photoluminescence to g-C_3N_4 nanosheets, which also show good stability, high quantum yields, good biocompatibility and low toxicity (Ma et al., 2014)

Due the inherent fluorescence nature, g-C_3N_4 nanosheet could be used as FRET donors. Zhang et al. developed a g-C_3N_4 nanosheet−MnO_2 sandwich nanocomposite

based FRET assay for intracellular imaging of glutathione (Zhang et al., 2014). Here, g-C_3N_4 nanosheet was used as a donor and MnO_2 was used as a quencher in the FRET assay, respectively (Fig. 3a). The FRET assay was established by depositing MnO_2 on g-C_3N_4 nanosheet. Due to the close distance between g-C_3N_4 nanosheet and MnO_2, the fluorescence of g-C_3N_4 nanosheet was quenched due to energy transfer from g-C_3N_4 nanosheet to MnO_2. With the addition of glutathione, MnO_2 was reduced to Mn, leading to the fluorescence signal recovery. The g-C_3N_4 nanosheet–MnO_2 sandwich nanocomposite was also demonstrated to have low cytotoxicity in living cells. Recently, Han et al. developed a g-C_3N_4 nanosheet-based FRET assay for riboflavin (vitamin B2) detection (Fig. 3b) (Han et al. 2016). The bulk g-C_3N_4 nanosheet showed strong blue emission under UV excitation. When riboflavin attached to g-C_3N_4 nanosheet by interaction with functional groups on g-C_3N_4 nanosheet, the blue fluorescence signal from g-C_3N_4 nanosheet was quenched, and a yellow-green signal of riboflavin was strengthened by energy transfer from g-C_3N_4 nanosheet to riboflavin. The linear detection range of from 0.4μM to10 μM was achieved for riboflavin detection with a limit of detection of 170 nM.

Here is Figure 3

Since g-C_3N_4 nanosheet has no obvious absorption in the visible light region, it can not be used as acceptors in FRET assays. Instead, g-C_3N_4 nanosheet was used to quench fluorophores via photoexcited electrons transfer. Wang et al. developed a fluorescence

assay with g-C_3N_4 nanosheet to quench fluorescence dye labelled ssDNA adsorbed on g-C_3N_4 nanosheet (Wang et al., 2013). Upon addition of complementary DNA, the hybridized dsDNA detached from g-C_3N_4 nanosheet, which led the fluorescence signal recovery. A limit of detection of 2.1 nM was achieved. Hu et al. further developed a g-C_3N_4 nanosheet-based multicolour fluorescent nanoprobe for multiplexed analysis of DNA with the similar mechanism (Hu et al., 2015). Moreover, g-C_3N_4 nanosheet was also used for intracellular imaging and sensing. Liao et al. developed a multifunctional g-C_3N_4 nanosheet-based platforms for in-situ fluorescence imaging and intracellular microRNA detection (Liao et al., 2014; Liao et al., 2015). Folate was immobilized on g-C_3N_4 nanosheet via pi-pi stacking interaction for cancer cell targeting. When multifunctional g-C_3N_4 nanosheet platform was transfected to the intracellular environment, hybridization between dye-labelled ssDNA and target microRNA led to the fluorescence signal recovery. By monitoring the fluorescence signal change of cells, multiple microRNAs detections in living cells could be realized. Table 2 shows a summary of g-C_3N_4 nanosheet-based FRET/electron transfer assays.

4.2 TMD nanosheets based FRET assays

Layered transition metal dioxides or disulphides (MoS_2, WS_2, and MnO_2) are another kind of graphene-like 2D materials with properties of the larger surface area, tunable energy band, fast electron transfer rate and fluorescence. Due to the strong optical absorption and fast electron transfer rate, TMD nanosheets are generally used as fluorescence quenchers in constructing FRET-based assays for various biological applications. Recently, TMD based quantum dots are synthesized which can be used as

fluorescence donors in FRET assays. Table 3 shows a summary of TMD nanosheets (MoS_2, WS_2, and MnO_2) based FRET assays.

Here is Table 3

4.2.1 MnO_2 based FRET assays

Manganese dioxide (MnO_2) nanosheets are generally used as fluorescence quenchers in FRET assays due to its strong optical absorption and fast electron transfer. MnO_2 has been widely used for glutathione detection (GSH) detection due to its selective decomposition of MnO_2 to Mn^{2+} ions. GSH is a thiol-containing tripeptide which is generally treated as an essential endogenous antioxidant in cellular defense against toxins and free radicals. Deng et al. firstly reported a FRET assay for rapid detection of glutathione in living cells with upconversion nanoparticles (UCNPs) as donors and single layered MnO_2 nanosheets as acceptors (Deng et al., 2011). With the introduction of glutathione, MnO_2 was selectively reduced to Mn^{2+} ions which recovered the fluorescence signal of UCNPs. By monitoring the fluorescence signal change of UCNPs, intracellular glutathione could be detected. A limit of detection of 0.9 μM was achieved for GSH detection. Later on, various fluorescence nanoparticles-MnO_2 FRET assays were developed for GSH detection. Zhang et al. developed a turn-on FRET sensor for detection of intracellular GSH with g-C_3N_4 as donors and MnO_2 as acceptors (Zhang et al., 2014). A limit of detection of 0.2 μM was achieved in aqueous

solutions. Wang et al. developed a FRET-based sensing platform employing fluorescent carbon dots and MnO_2 nanosheets as energy donor–acceptor pairs for glutathione sensing in human whole blood samples (Wang et al., 2015). A limit of detection as low as 22 nM was reached for GSH detection. He et al. used a similar carbon dots-MnO_2 nanosheet system for intracellular imaging of GSH (Fig. 4). The limit of detection was reported to be 10 nM for GSH detection (He et al., 2015). Wang et al. also reported a fluorescent glutathione probe based on MnO_2 and fluorescent phenol formaldehyde resin (PFR) nanocomposite (Wang et al., 2016). The limit of detection was estimated to be 76 nM. The sensing mechanism of the above FRET assays were all based on the selective reduction of MnO_2 to Mn^{2+} ions, which could recover the fluorescence signals of nanoparticle donors.

MnO_2 based FRET assays are also used for other biomolecules detection. Yuan et al. developed a MnO_2 nanosheet-based homogeneous FRET assay for ochratoxin A (OTA) and cathepsin D (Cat D) detection (Yuan et al., 2013). For OTA detection, fluorophore labelled OTA aptamers were firstly assembled on MnO_2 nanosheet, leading to fluorescence quenching. The combination of OTA and OTA aptamers led to the detachment of fluorophore labelled OTA aptamers from MnO_2 nanosheet surface, resulting in the recovery of fluorescence signals. Cat D protease detection was realized by the cleavage of specific peptide immobilized on MnO_2 nanosheet with $NaYF_4$:Yb, Tm UCNP nanoparticles as donors. The detection limit of this biosensor was as low as 0.02 ng mL^{-1} and 1 ng mL^{-1} for OTA and Cat D, respectively. Wang et al. reported a MnO_2 nanosheets-based fluorescent sensing platform with organic dye labelled ssDNA

as a probe for microRNA and thrombin detection (Wang et al., 2015). The limit of detection (LOD) for miR124a and thrombin were reported as 0.8 nM and 11 nM, respectively.

The application of MnO_2 based FRET assays for multifunctional imaging and drug delivery monitoring was also reported. Zhao et al. developed a novel drug carrier based on multifunctional MnO_2 nanosheet-modified Fe_3O_4@SiO_2/$NaYF_4$:Yb, Er nanocomposites with both MRI imaging and fluorescence imaging functions (Zhao et al., 2014a). The MnO_2 nanosheets served as both drug carriers for Congo red (CR) and quencher for upconversion luminescence to monitor glutathione in the living cells. The MRI imaging function was realized by Fe_3O_4 core of this nanocomposite. In another research group, Zhao et al. also developed a novel dual-activatable fluorescence/MRI bimodal platform for tumor cell imaging using MnO_2 nanosheet−aptamer nanoprobe (Zhao et al., 2014b). In this platform, the MRI imaging was realized by the large amount of paramagnetic Mn^{2+} ions reduced by glutathione from MnO_2.

Here is Figure 4

4.2.2 MoS_2 based FRET assays

Two-dimensional MoS_2 (2D MoS_2) are fabricated by exfoliation of exagonal molybdenum disulfide (2H MoS_2) into one or a few layers (Jariwala et al., 2014; Wang et al., 2012). 2D MoS_2 has good mechanical, electronic and optical properties which make it suitable for many biosensing applications (Kalantar-zadeh et al., 2015).

Especially, unlike graphene which has no bandgap, 2D MoS_2 has a suitable bandgap which renders it photoluminescence (PL) in the visible range directly (Mak et al., 2010). Moreover, PL of 2D MoS_2 has highly tunable optical characteristics which make it suitable for biosensing applications. It is also interesting to know that 2D MoS_2 has been reported to have even lower cytotoxicity compared with graphene and its analogues (Teo et al., 2014).

Fluorescence quenching capability of MoS_2 nanosheets has been investigated. MoS_2 based FRET assays have been used for DNA detection. Zhu et al. developed a FRET assay with MoS_2 nanosheets as acceptors for DNA hybridization detection (Zhu et al., 2013). Fluorophore labelled ssDNA was firstly adsorbed and quenched on basal planes of MoS_2 nanosheet surface (Fig. 5a). Hybridization with target detached the dsDNA from MoS_2 surface and led to the fluorescence recovery. This FRET assay achieved a linear detection range between 0-15 nM with a limit of detection of 500 pM. Later on, other DNA FRET assays with MoS_2 as quenchers have been developed. Huang et al. developed a single-layered MoS_2 nanosheet based microfluidic biosensor for ultrasensitive detection of DNA. A linear detection range was obtained from 0 and 50 nM, with a detection limit of 500 pM (Huang et al., 2015a). Huang et al. reported a novel MoS_2-based fluorescent biosensor for DNA detection via hybridization chain reactions (HCRs) with a linear detection range from 30 pM to 5 nM and a limit of detection of 15 pM (Huang et al., 2015b). Deng et al. developed a simple signal-on fluorescence DNA methyltransferase (MTase) activity assay using MoS_2 nanosheets as fluorescence quenchers (Deng et al., 2015). The linear detection ranges from 0.2 to 20

U mL^{-1} with a limit of detection of 0.14 U mL^{-1}. MoS_2 based FRET assays were also explored for protein detection. Xiang et al. developed a MoS_2-based FRET assay for protein detection via terminal protection of small molecule-linked DNA and exonuclease III-aided DNA recycling amplification (Xiang et al., 2015). A linear detection range from 0 to 600 ng mL^{-1} was achieved with a limit of detection of 0.67 ng mL^{-1} for detection of streptavidin. Ge et al. developed a fluorescence-labelled DNA–MoS_2 FRET biosensor for detecting biomolecular targets such as proteins and small molecules (Ge et al., 2014). A limit of detection of 300 pM was achieved for thrombin detection. Kong et al. developed an aptamer-functionalized MoS_2 nanosheet fluorescent biosensor for sensitive detection of prostate specific antigens (PSAs) (Kong et al., 2015). A linear detection range from 0.5 to 60 ng mL^{-1} was achieved with a limit of detection of 0.2 ng mL^{-1} for PSA detection. Jia et al. used aptamer loaded MoS_2 nanoplates as nanoprobes for intracellular ATP detection and photodynamic therapy (PDT) via ATP-mediated controllable release of singlet oxygen (Jia et al., 2015).

MoS_2 quantum dots are emerging 2D nanomaterial photoluminescent probes which have been synthesized with strong photoluminescence, high quantum yield and good biocompatibility (Dai et al., 2015; Wang et al., 2014; Wu et al., 2015). Wang et al. used MoS_2 QDs as photoluminescent probes to construct a photoluminescence (PL) quenching sensor for detection of 2,4,6-trinitrophenol (TNP) (Wang et al., 2014). The addition of TNP led to the quenching of blue fluorescence of MoS_2 QDs via both resonance energy transfer and electron energy transfer. A wide linear detection range from 0.099 to 36.5 μM was achieved with a detection limit of 95 nM. The role of MoS_2

quantum dots in FRET assays was started to be investigated. Ha et al. synthesized blue luminescent MoS_2 quantum dots using a Li intercalation method (Ha et al., 2014). The role of blue luminescent MoS_2 quantum dots in FRET assays was systematically studied by constructing an Alexa Fluor 430-dsDNA-MoS_2 FRET system. It was found that MoS_2 quantum dots played dual roles in this FRET system. MoS_2 quantum dots could serve as donors with the maximum FRET efficiency of 11.73% at 13 base pair dsDNA (4.42 nm). At other distances larger or smaller than 4.42 nm, the FRET efficiency with MoS_2 quantum dots as donors decreased. MoS_2 quantum dots also served as quenchers in this FRET system with quenching efficiency minimal when the distance was more than 13 base pair dsDNA.

4.2.3 WS_2 based FRET assays

2D layered tungsten disulfide (WS_2) could be synthesized from bulk WS_2 by various methods including liquid-phase exfoliation and Li^+ intercalation (Matte et al., 2010; Halim et al., 2013). Due to the poor water solubility, 2D WS_2 is not as popular as other graphene-like 2D materials such as MoS_2 and MnO_2 in biological applications (Halim et al., 2013). Xi et al. reported a 2D WS_2 nanosheet-based FRET assay for microRNA detection in ethanol solution (Fig. 6a) (Xi et al., 2014). A linear detection range from 0.001 nM to 10 nM was achieved with a detection limit of 300 fM. Yuan et al. synthesized water-soluble WS_2 nanosheet by generating poly(acrylic acid) (PAA) functionalized 2D WS_2 nanosheet through sonication-assisted exfoliation of bulk WS_2 (Yuan et al., 2014). The synthesized water-soluble layered WS_2 nanosheets were then

used to develop a turn-on FRET assay with adsorbed dye-labeled ssDNAs as capture probes for DNA detection. A linear detection range from 0.1 to 50 nM was obtained with a limit of detection of 60 pM. Wang et al. reported a WS_2 nanosheet-based FRET platform for DNA detection via PNA-DNA interaction (Wang et al., 2015). A linear detection range from 1 and 20 nM was obtained with a limit of detection of 500 pM.

2D WS_2 nanosheet-based FRET assays were also used for other biomolecules detection. Qin et al. developed a 2D WS_2 nanosheet-based fluorescence turn-on assay for anticancer drug bleomycin (BLM) detection (Qin et al., 2015). The fluorescence-labeled long ssDNA was firstly adsorbed on WS_2 firmly leading to the quenching of fluorescence signals (Fig. 6b). In the presence of BLM-Fe(II) or S1 nuclease, fluorescence labeled long ssDNA was cleaved into short fragments which led to the detachment of fluorescence labeled short ssDNA fragment from WS_2 nanosheet surface due to the weakening of adsorption force. By observing the fluorescence signal recovery, BLM could be detected. A linear detection range from 0 to 10mM was achieved with a limit of detection of 0.3nM for BLM. Ge et al. developed a WS_2 nanosheet-based FRET sensing platform for highly sensitive detection of T4 polynucleotide kinase (PNK) and its inhibitors (Ge et al., 2014). The fluorescent dye-labeled double-stranded DNA (dsDNA) was firstly mixed with WS_2 nanosheets. Due to the weak adsorption of dsDNA on WS_2 nanosheet, strong fluorescence remained for dsDNA. In the presence of T4 PNK, dsDNA is phosphorylated and degraded into ssDNA, leading to the strong adsorption on WS_2 nanosheet with fluorescence quenching. A linear detection range from 0.01 to 10 U mL^{-1} was achieved with a limit

of detection of 0.01 U mL^{-1}.

5. Comparison between graphene and graphene-like 2D materials based FRET assays

5.1 Comparison of graphene and graphene-like 2D materails as quenchers

Graphene and graphene-like 2D materials are commonly used as quenchers in FRET bio-applications due to their zero or small bandgap. Generally, when the bandgap of an acceptor molecule is small enough, the acceptor molecule will act as a quencher without fluorescence emission and the transferred energy via a FRET mechanism dissipates as heat (Grecco and Verveer, 2011). To achieve an efficient detection platform, the quenching capability of these 2D materials is a critical factor. Among various graphene and graphene-like 2D materials, pristine graphene is regarded as an ideal fluorescence quencher due to its conjugated network consisting of sp^2-hybridized carbon atoms and zero bandgap (Swathi and Sebastian, 2008 and 2009). From both theory calculation and experimental demonstration, the energy transfer efficiency of pristine graphene is demonstrated to have a $(distance)^{-4}$ dependence, while traditional FRET system has a $(distance)^{-6}$ dependence (Swathi and Sebastian, 2008 and 2009). The quenching distance of pristine graphene is as far as 30 nm compared with that of traditional FRET system around 10 nm. However, the low solubility and the difficulty for conjugation limit the application of pristine graphene. Instead, graphene oxide or reduce graphene oxide are adopted, which have weaker quenching capability due to the change the carbon hybridization from sp^2 to sp^3, but still can achieve 100% quenching

efficiency with rapid quenching response within few minutes and a quenching distance as far as 23 nm (Huang and Liu, 2012; Kim et al., 2010). Reduced graphene oxide has better quenching capability compared with graphene oxide due to the higher sp^2 carbon atom ratio (Wang et al., 2010). The main binding force between graphene based nanomaterials and probe molecules is based on pi-pi stacking force between aromatic rings of biomolecules and basal planes.

Many graphene-like 2D materials feature small bandgap which can be used as universal highly efficient quenchers in FRET systems. Graphene-like 2D materials such as TMDs (MnO_2, MoS_2 and WS_2) have been used as good quenchers for nucleic acid based detection (Wang et al., 2013; Zhao et al., 2014b; Zhu et al., 2013; Xi et al., 2014). Generally, fluorophore labelled nucleic acid was adsorbed and quenched on the basal planes of graphene-like 2D materials. The binding force between graphene-like 2D materials and nucleic acid probes is generally based on van der walls force. High quenching efficiencies from 85% to 98% have been achieved with rapid quenching response from 5 minutes to 20 minutes for MnO_2, MoS_2 and WS_2 (He et al., 2014; Huang et al., 2015; Yuan et al., 2014).

To the best of our knowledge, there is very few research to directly compare quenching capability among various graphene and graphene-like 2D materials. Balcioglu et al. compared the quenching capability of GO and MoS_2 nanosheets on fluorescent dye labelled nanoparticle using DNA-length-dependent quenching method (Balcioglu et al., 2014). For the same 20 nM cy5.5 labeled bare iron oxide nanoparticles, 1.8 μg/mL GO achieved a quenching efficiency of 62% and 20 μg/mL MoS_2 achieved

a quenching efficiency of 54%. It is obvious that although the concentration of GO was one-fold lower than that of MoS_2, GO still could achieve a higher quenching efficiency compared with MoS_2.

As a summary, pristine graphene is an ideal fluorescence quencher with super quenching capability. GO and rGO have weaker quenching capability but better solubility in water compared with pristine graphene due to the oxidization of sp2 carbon atoms. The binding force between graphene materials and probe molecules are mainly pi-pi stacking force which is relatively stronger than van der walls force. Graphene-like 2D materials exhibit good quenching capability and the binding force between graphene-like 2D materials and probe molecules are mainly based on van der Waals force. Graphene materials (pristine graphene, GO and rGO) may have better quenching capability compared with graphene-like 2D materials for fluorescence labelled biomolecules. The possible reasons may be due to 1) the intrinsically higher quenching property of graphene materials with special electronic properties, although oxidation of graphene may reduce this high quenching capability; 2) the relatively higher pi-pi stacking binding force between graphene materials with biomolecules compared with the van der walls force between graphene-like 2D materials and biomolecules. More study in this field is needed to systematically compare the quenching capabilities among various graphene and graphene-like 2D materials.

5.2 Comparison of graphene and graphene-like 2D materails as donors

Graphene and graphene-like 2D materials can also be used as donors in FRET

assays due to their photoluminescence properties. Inorganic quantum dots prepared from graphene amd graphene-like 2D materials such as MoS_2 and WS_2 have been demonstrated to be good fluorescence probes due to good photostability, excellent biocompatibility and unique optical properties. As donor molecules, quantum yield (QY) and tunability of emission spectrum are the two important parameters for FRET assays. High quantum yield of donors can lead to high energy transfer efficiency and tunability of emission spectrum of donors can increase versatility to match with various acceptor molecules.

Many methods have been reported to modulate fluorescence emission spectra and increase QY for graphene quantum dots (GQDs). The fluorescence emission spectra of GQDs can be modulated from blue, green, yellow to red by excitation wavelength change, surface functionalization and doping methods (Shi et al., 2015; Zhu et al., 2011; Zhu et al., 2012; Ke et al., 2016; Ge et al., 2014). Up to now, the QY of GQDs can be improved from 19% to 73% by various methods including surface functionalization (Wu et al., 2013; Shi et al., 2015), nitrogen doping (Li et al., 2012), and co-doping with nitrogen and sulfur (Dong et al., 2013). The highest QY of GQDs is reported to be 73% by co-doping with nitrogen and sulfur (Dong et al., 2013). Compared with GQDs, quantum dots prepared from MoS_2 and WS_2 have relatively low QY. The QY of MoS_2 quantum dots is reported from 2.6% to 4.84% (Wu et al., 2015; Wang and Ni, 2014) and the QY of WS_2 quantum dots is reported from 1.8% to 4% (Lin et al., 2013; Yan et al., 2016). The MoS_2 and WS_2 quantum dots also shows excitation-dependent emission. The fluorescence emission spectra of MoS_2 QDs can be modulated from blue, green to

red (Wu et al., 2015) and the fluorescence of WS_2 quantum dots was mainly at the blue to green light region under various excitation wavelength (Lin et al., 2013).

5. Conclusion, Future Perspectives and Challenges

In the past years, graphene and graphene-like 2D nanomaterials including gC_3N_4 nanosheets and TMDs (e.g., MnO_2, MoS_2, and WS_2) have been widely used as donors or acceptors in FRET assays for a wide range of biological applications. This can be attributed to their unique 2D structure, large area to volume ratio, tunable energy band, and good optical properties. In this review, we describe the optical properties of graphene and graphene-like 2D nanomaterials, as well as the recent development of their state-of-the-art FRET assays in biosensing, imaging, and drug delivery monitoring. Especially, the development of FRET assays based on graphene-like 2D nanomaterials is still in its early stage. More efforts are needed to explore their optical properties, develop new biofunctionalization strategies, improve dispersity in water and decrease toxicity in living organisms. It is expected to oversee a rapid development of FRET assays based on graphene-like 2D nanomaterials in the coming years.

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References

Alonso-Cristobal, P., Vilela, P., El-Sagheer, A., Lopez-Cabarcos, E., Brown, T., Muskens, O. L., Rubio-Retama, J., Kanaras, A. G., 2015. ACS Appl Mater Interfaces, 7, 12422-9.

Anne, C., Cornille, F., Lenoir, C., Roques, BP., 2001. Analytical Biochemistry, 291, 253-261.

Balcioglu, M., Rana, M., Robertson, N., Yigit, M.V. 2014. ACS Appl Mater Interfaces. 6, 12100-10.

Cao, L., Cheng, L., Zhang, Z., Wang, Y., Zhang, X., Chen, H., Liu, B., Zhang, S., Kong, J. 2012. Lab Chip, 12, 4864-9.

Cui, L., Lin, X.Y., Lin, N.H., Song, Y.L., Zhu, Z., Chen, X., Yang, C.Y.J., 2012. Chem Commun. 2012. 48, 194-6.

Chalfie, M., 2009. Proceedings of the National Academy of Sciences of the United States of America, 106, 10073-10080.

Chang, H., Tang, L., Wang, Y., Jiang, J., Li, J., 2010. Anal. Chem. 82, 2341-6.

Chien, C.T., Li, S.S., Lai, W.J., Yeh, Y.C., Chen, H.A., Chen, I.S., Chen, L.C., Chen, K.H., Nemoto, T., Isoda, S., Chen, M., Fujita, T., Eda, G., Yamaguchi, H., Chhowalla, M., Chen, C.W., 2012 Angew Chem Int Ed Engl., 51, 6662-6.

Clegg, R.M., 1995. Curr. Opin. Biotechnol., 6, 103-110.

Chen, J., Yan, X., 2010. J. Mater. Chem. 20, 4328-32.

Chen, J.L., Yan, X.P., Meng, K., Wang, S.F., 2011. Anal. Chem. 83, 8787-93.

Dai, W., Dong, H., Fugetsu, B., Cao, Y., Lu, H., Ma, X., Zhang, X. 2015 Small. 11, 4158-64.

Deng, H.M., Yang, X.J. Gao, Z.Q. 2015. Analyst, 140, 3210-3215.

Deng, R., Xie, X., Vendrell, M., Chang, Y.-T., Liu, X., 2011. J. Am. Chem. Soc., 133, 20168-171.

Dong, Y., Pang, H., Yang, H.B., Guo, C., Shao, J., Chi, Y., Li, C.M., Yu, T.Y.Q., 2013. Angew. Chem. Int. Ed., 52,7800-4.

Dreyer, D.R., Park, S.J., Bielawski, C.W., Ruoff, R.S., 2010. Chemical Society Reviews, 39, 228-240.

Eda, G., Lin, Y.Y., Mattevi, C., Yamaguchi, H., Chen, H.A., Chen, I.S., Chen, C.W., Chhowalla, M., 2010. Adv Mater, 22, 505-9.

Eustis, S., El-Sayed, M. A., 2006. Chem. Soc. Rev., 35, 209-217.

Forster, T., 1946. Naturwissenschaften, 33, 166-175.

Galande, C., Mohite, A.D., Naumov, A.V., Gao, W., Ci, L., Ajayan, A., Gao, H., Srivastava, A., Weisman, R.B. Ajayan, P.M., 2011. Sci Rep, 1, 85.

Ge, J., Ou, E.C., Yu, R.Q., Chu, X., 2014. J. Mater. Chem. B, 2, 625-628.

Ge, J., Tang, L.J., Xi, Q., Li, X.P., Yu, R.Q., Jiang, J.H., Chu, X. 2014. Nanoscale. 6, 6866-72.

Ge, J.C., Lan, M.H., Zhou, B.J. Liu, W.M., Guo, L., Wang, H., Jia, Q.Y., Niu, G.L., Huang, X., Zhou, H.Y., Meng, X.M., Wang, P.F. Lee, C.S. Zhang, W.J. Han, X.D. 2014. Nature Communication. 5, 4596.

Geim, A.K., Novoselov, K. S., 2007, Nature Mater., 6, 183-191

Georgakilas, V., Bourlinos, A.B., Gournis, D., Tsoufis, T., Trapalis, C., Alonso, A.M., Prato, M., 2008. J. Am. Chem. Soc.130, 8733-40.

Georgakilas, V., Otyepka, M., Bourlinos, A.B., Chandra, V., Kim, N., Kemp, K.C.,

Hobza, P., Zboril, R., Kim, K.S. 2012. Chem. Rev., 112, 6156–6214.

Gokus, T., Nair, R.R., Bonetti, A., Böhmler, M., Lombardo, A., Novoselov, K.S., Geim, A.K., Ferrari, A.C., Hartschuh, A. 2009. ACS Nano, 3, 3963-8.

Gokus，T., Nair, R.R.，Bonetti, A.，Böhmler, M.，Lombardo, A.，Novoselov, K.S.，Geim, A.K.， Ferrari, A.C.， Hartschuh , A., 2009. ACS Nano, 3, 3963-68.

Grecco, H.E., Verveer P.J. 2011 Chemphyschem., 12, 484-90.

Gu, X., Yang, G., Zhang, G., Zhang, D., Zhu, D., 2011. ACS Appl Mater Interfaces, 3, 1175-9.

Ha, T. Enderle, T., Ogletree, D., Chemla, D., Selvin, P., Weiss, S., 1996. Proc Natl Acad Sci., 93, 6264-6268.

Ha, H.D., Han, D.J., Choi, J.S., Park, M., Seo, T.S., 2014. Small. 10, 3858-62.

Han, J., Zou, H.Y., Gao, M.X., Huang, C.Z., 2016. Talanta, 148, 279–284.

Halim, U.; Zheng, C. R.; Chen, Y.; Lin, Z.; Jiang, S.; Cheng, R.; Huang, Y.; Duan, X. Nat. Commun. 2013, 4, 2213.

He, D.G. Yang, X.X., He, X.X., Wang, K.M., Yang, X., He, X., Zou, Z., 2015. Chem. Commun., 51, 14764-7.

He, D.G., He, X.X., Wang, K.M., Yang, X., Yang, X.X., Li, X.C., Zhou, Z., 2014. Chem Commun. 50, 11049-52.

He, S., Song, B., Li, D., Zhu, C., Qi, W., Wen, Y., Wang, L., Song, S., Fang, H., Fan, C. 2010. Adv. Funct. Mater. 20, 453-9.

Heim, R., Tsien, R.Y., 1996. Curr. Biol., 6, 178-182.

Hu, K., Zhong, T.M., Huang, Y., Chen, Z.F., Zhao, S.L., 2015. Microchim Acta 182,

949–955

Huang, P.J., Liu, J. 2012. Anal Chem, 84, 4192-8.

Huang, Y., Shi, Y., Yang, H.Y, Ai, Y. 2015. Nanoscale. 7, 2245-9.

Huang, J.H., Ye, L., Gao, X., Li, H., Xu, J.B., Li, Z.G. 2015b. J. Mater. Chem. B, 3, 2395-2401.

Huang, P.J.J., Liu, J., 2012 Small, 8, 977-83.

Hwang, S.H., Kim, D.E., Sung, H., Park, B.M., Cho, M.J. Yoon, O.J., Lee, D.H.. 2015. PLoS One, 10, e0136954.

Jariwala, D., Sangwan, V.K., Lauhon, L.J., Marks, T.J., Hersam, M.C., ACS Nano 2014, 8, 1102−1120.

Jia, L., Ding, L., Tian, J., Bao, L., Hu, Y., Ju, H., Yu, J.S., 2015. Nanoscale. 7, 15953-61.

Jung, J.H., Cheon, D.S., Liu, F., Lee, K.B., Seo, T.S. 2010. Angew Chem Int Ed Engl. 49, 5708-11.

Kalantar-zadeh, K., Ou, J. Z., Daeneke, T.; Strano, M. S.; Pumera, M.; Gras, S. L. 2015. Adv. Funct. Mater. 25, 5086−5099.

Ke, C.C., Yang, Y.C., Tseng W.L., 2016, Particle & Particle Systems Characterization, 33, 132–9.

Kim, S.S., Choi, J.Y., Kim, K., Sohn, B.H., 2012, Nanotechnology, 23, 125301.

Kim, J., Cote, L.J., Kim, F., Yuan, W., Shull, K.R., Huang, J.X., 2010. J. Am. Chem. Soc., 132, 8180-86.

Kim, J., Cote, L.J., Kim, F., Huang, J., 2010 J. Am. Chem. Soc., 132, 260-7.

Kong, R.M., Ding, L., Wang, Z., You, J., Qu, F. 2015. Anal Bioanal Chem. 407, 369-77.

Kosynkin, D.V., Higginbotham, A.L., Sinitskii, A., Lomeda, J.R., Dimiev, A., Price, B.K., Tour, J.M. 2009. Nature, 458, 872-6.

Kundu A., Layek RK, Kuila A, Nandi AK. 2012. ACS Appl Mater Interfaces, 4, 5576-82.

Li, Z., Wang, Y., Ni, Y., Kokot, S. 2015. Spectrochim Acta A Mol Biomol Spectrosc. 137, 1213-21.

Li, Y., Hu, Y., Zhao, Y., Shi, G., Deng, L., Hou, Y., Qu, L., 2011. Adv. Mater., 23, 776-780.

Li, Y., Zhao, Y., Cheng, H., Hu, Y., Shi, G., Dai, L., Qu, L. 2012. J Am Chem Soc. 134, 15-8.

Liao, X.J., Wang, Q.B., Ju, H.X., 2015. Analyst, 2015,140, 4245-4252

Liao, X.J., Wang, Q.B., Ju, H.X., 2014. Chem. Commun., 50, 13604-13607.

Liao, Y., Zhou, X., Xing, D., 2014. ACS Appl Mater Interfaces, 6, 9988-96.

Lin, L.X., Xu, Y.X., Zhang, S.W., Ross, I.M., Ong, A.C.M., Allwood, D.A., 2013. ACS Nano, 7, 8214–3

Liu, F., Choi, J.Y., Seo, T.S., 2010. Biosens Bioelectron, 25, 2361-5.

Liu, F., Jang, M.H., Ha, H.D., Kim, J.H., Cho, Y.H., Seo, T.S., 2013.Advanced Materials, 25, 3657-3662.

Liu, J., Liu, G., Liu, W., Wang, Y., 2015. Biosens Bioelectron, 64, 300-5.

Liu, J.S., Liu, G.N., Liu, W.X., Wang, Y.R. 2015. Biosens Bioelectron. 64, 300-5.

Liu, Q., Guo, B., Rao, Z., Zhang, B., Gong, J.R. 2013. Nano Lett. 13, 2436-41.

Lu, J., Yang, J., Wang, J.， Lim, A., Wang, S., Loh, K.P. 2009 ACS Nano, 3, 2367-2375.

Lu, C.H., Yang, H.H., Zhu, C.L., Chen, X., Chen, G.N., 2009 Angew. Chem. Int. Ed. 48, 4785-7.

Luo, Z.T., Vora, P.M., Mele, E.J., Johnson, A.T. C., Kikkawa, J.M. 2009. Applied Physics Letters, 94, 111909.

Ma, T.Y., Tang, Y.H., Dai, S., Qiao, S.Z., 2014. Small, 10, 2382–2389.

Mak, K., Lee, C., Hone, J., Shan, J., Heinz, T. 2010. Phys. Rev. Lett. 105, 136805.

Marcano, D.C., Kosynkin, D.V., Berlin, J.M., Sinitskii, A., Sun, Z., Slesarev, A., Alemany, L.B., Lu, W., Tour, J.M., 2010. ACS Nano, 4, 4806-4814.

Matte, H. S.; Gomathi, A.; Manna, A. K.; Late, D. J.; Datta, R.; Pati, S. K.; Rao, C. N. Angew. Chem., Int. Ed. 2010, 49, 4059−4062.

Mei, Q.S., Zhang, K., Guan, G.J. liu, B.H., Wang, S.H., Zhang, Z.P. 2010. Chem Commun, 46, 7319-21.

Mei, Q., Zhang, Z. 2012. Angew Chem Int Ed Engl, 51, 5602-6.

Morales-Narváez, E., Merkoçi A., 2012. Adv Mater., 24, 3298-308.

Novoselov KS, Geim AK, Morozov SV, Jiang D, Zhang Y, Dubonos SV, Grigorieva IV, Firsov AA., 2004. Science, 306, 666-669.

Pal, S.K., 2015. Carbon, 88, 86-112.

Pan, D., Zhang, J., Li, Z., Wu, M., 2010. Adv. Mater., 22, 734-738.

Pang, S., Gao, Y., Li, Y., Liu, S., Su, X., 2013. Analyst, 138, 2749-54.

Patterson, G.H., Piston, D.W., Barisas, B.G., 2000. Analytical Biochemistry, 284, 438-

440.

Piston, D. W., Kremers, G.J., 2007. Trends in Biochemical Sciences, 32, 407-414.

Resch-Genger, U., Grabolle, M., Cavaliere-Jaricot, S., Nitschke, R., Nann, T., 2008. Nature Methods, 5, 763-775.

Qian, Z.S., Shan, X.Y., Chai, L.J., Ma, J.J., Chen, J.R., Feng, H., 2014a. Biosens Bioelectron. 60, 64-70.

Qian, Z.S., Shan, X.Y., Chai, L.J., Chen, J., Feng, H., 2014b. Chem. Eur. J. 20, 16065 – 16069

Qian, Z.S., Shan, X.Y., Chai, L.J., Ma, J.J., Chen, J.R., Feng, H., 2014c. Nanoscale, 6, 5671-4.

Qin, Y., Ma, Y., Jin, X., Zhang, L., Ye, G., Zhao, S. 2015. Anal Chim Acta. 866, 84-9.

Sapsford, K.E., Berti, L., Medintz, I. L., 2006. Angew. Chem. Int. Ed., 45, 4562-4589.

Selvin, P. R., 2000. Nat. Struct. Biol., 7, 730-734.

Shimomura, O., Johnson, F.H., Saiga, Y., 1962. J Cell Comp Physiol, 59, 223-39.

Shen, J.H., Zhu, Y.H., Chen, C., Yang, X.L., Li, C.Z. 2011. Chem. Commun. 47, 2580-2582.

Shen, J.H., Zhu, Y.H., Yang, X.L., Zong, J., Zhang, J.M., Li, C.Z. 2012. New J. Chem., 36, 97-101.

Shi, J.Y., Tian, F., Lyu, J. Yang, M. 2015. Journal of Materials Chemistry B, 3, 6989-7005.

Shi, J.Y., Chan, C., Pang, Y., Ye, W.W., Tian, F., Lyu, J., Zhang, Y., Yang, M.., 2015. Biosens Bioelectron, 67, 595-600.

Shi, Y., Wu, J., Sun, Y., Zhang, Y., Wen, Z., Dai, H., Wang, H., Li, Z. 2012. Biosens Bioelectron, 38, 31-6.

Swathi, R.S., Sebastian, K.L., 2009 J. Chem. Phys. 130, 086101.

Swathi, R.S., Sebastian, K.L., 2008 J. Chem. Phys. 129, 054703.

Teo, W.Z., Chng, E.L.K., Sofer, Z., Pumera, M.2014. Chem.-Eur. J. 20, 9627−9632.

Tetsuka, H., Asahi, R., Nagoya, A., Okamoto, K., Tajima, I., Ohta, R., Okamoto, A. 2012. Advanced Materials, 24, 5333-5338.

Thomas, A., Fischer, A., Goettmann, F., Antonietti, M., Muller, J.O., Schlogl, R., Carlsson, J.M. 2008. J Mater Chem 18, 4893–4908.

Wang, C.X., Zhai, W.Y., Wang, Y.X., Yu, P., Mao, L.Q., 2015. Analyst, 140, 4021-4029.

Wang, Q., Wang, W., Lei, J., Xu, N., Gao, F., Ju, H.. 2013. Anal Chem. 85, 12182-8.

Wang, Q. H.; Kalantar-zadeh, K.; Kis, A.; Coleman, J. N.; Strano, M. S. 2012. Nature Nanotechnology 7, 699–712.

Wang, S., Zhang, Y., Ning, Y., Zhang, G.J. 2015. Analyst. 140, 434-9

Wang, X., Wang, D., Guo, Y., Yang, C., Liu, X., Iqbal, A., Liu, W., Qin, W., Yan, D., Guo, H.. 2016. Biosens Bioelectron. 77, 299-305.

Wang, Y., Jiang, K., Zhu, J., Zhang, L., Lin, H., 2015. Chem Commun 51, 12748-51.

Wang, Y., Ni, Y., 2014. Anal. Chem., 86, 7463-70.

Wang, Y., Li, Z., Hu, D., Lin, C.-T., Li, J., Lin, Y., 2010 J. Am. Chem. Soc., 132, 9274-6.

Wu, J.Y., Zhang, X.Y. Ma, X.D. Qiu, Y.P., Zhang, T. 2015. RSC Adv., 5, 95178-82

Wu, M., Kempaiah, R., Huang, P.J.J., Maheshwari, V., Liu, J.W., 2011. Langmuir, 27,

2731-8.

Wu, X., Tian, F., Wang, W.X., Chen, J., Wu, M., Zhao, X.J., 2013. J Mater Chem C, 1, 4676-84.

Yan, X., Cui, X., Li, B., Li, L.S., 2010. Nano Lett., 10, 1869-1873.

Yang, G.H., Zhu, C.Z., Du, D., Zhu, J.J., Lin, Y.H., 2015. Nanoscale, 7, 14217-14231.

Ye, W.W., Tsang, M. K., Liu, X., Yang, M., Hao, J.H., 2014. Small, 10, 2390-2397.

Yuan, Y., Li, R., Liu, Z. 2014. Anal Chem. 86, 3610-5.

Xi, Q.; Zhou, D.-M.; Kan, Y.-Y.; Ge, J.; Wu, Z.-K.; Yu, R.-Q.; Jiang, J.-H. Anal. Chem. 2014, 86, 1361−65.

Xiang, X., Shi, J.B., Huang, F.H., Zheng, M.M. Deng, Q.C., Xua, J.Q., 2015. Biosensors and Bioelectronics, 74, 227–232.

Xing, X.J., Liu, X.G., He, Y., Lin, Y., Zhang, C.L., Tang, H.W., Pang, D.W. 2013. Biomacromolecules. 14, 117-23.

Xu, M.S., Liang, T., Shi, M.M., Chen, H.Z. 2013. Chem. Rev. 113, 3766–98.

Yuan, Y.X., Wu, S.F., Shu, F., Liu, Z.H., 2014. Chem. Commun., 50, 1095-97.

Yan, Y.H., Zhang, C.L., Gu, W., Ding, C.P., Li, X.C., Xian, Y.Z., 2016. J. Phys. Chem. C, 120, 12170–7。

Zhang, J., Tao, M., Jin, Y., 2014. Analyst, 139, 3455-9.

Zhang, L., Peng, D., Liang, R.P., Qiu, J.D.. 2015. Chemistry. 21, 9343-8.

Zhang, M., Yin, B.C., Tan, W., Ye, B.C. 2011. Biosens. Bioelectron., 26, 3260-5.

Zhang, M., Yin, B.C., Wang, X.F., Ye, B.C. Chem. Commun. 2011, 47, 2399-2401.

Zhao, P., Zhu, Y., Yang, X., Shen, J., Jiang, X., Zong, J., Li, C. 2014a. Dalton Trans. 43,

451-7.

Zhang, X.L., Zheng, C., Guo, S.S., Li, J., Yang, H.H., Chen, G., 2014. Anal Chem. 86, 3426-34.

Zhang, Z., Liu, Y.F., Ji, X.H., Xiang, X., He, Z.K., 2014. Analyst, 2014. 139, 4806-9.

Zhao, Z., Fan, H., Zhou, G., Bai, H., Liang, H., Wang, R., Zhang, X., Tan, W. 2014b. J Am Chem Soc. 136, 11220-3.

Zheng, M., Jagota, A., Semke, E.D., Diner, B.A., McLean, R.S., Lustig, S.R., Richardson, R.E., Tassi, N.G. 2003. Nat. Mater. 2, 338-42.

Zheng, M., Jagota, A., Strano, M.S., Santos, A.P., Barone, P., Chou, S.G., Diner, B.A., Dresselhaus, M.S., Mclean, R.S., Onoa, G.B., Samsonidze, G.G., Semke, E.D., Usrey, M., Walls, D.J. 2003. Science, 302, 1545-8.

Zhou, X., Zhang, Y., Wang, C., Wu, X., Yang, Y., Zheng, B., Wu, H., Guo, S., Zhang, J, 2012. Acs Nano, 6, 6592-6599.

Zhou, Y., Qu, Z.B., Zeng, Y., Zhou, T., Shi, G.. 2014. Biosens Bioelectron. 52, 317-23.

Zhu, C. F., Zeng, Z.Y., Li, H., Li, F., Fan, C. H., Zhang, H. 2013. J. Am. Chem. Soc., 135, 5998-6001.

Zhu, S., Zhang, J., Tang, S., Qiao, C., Wang, L., Wang, H., Liu, X., Li, B., Li, Y., Yu, W., 2012. Adv. Funct. Mater., 22, 4732-40.

Zhu, S.J., Zhang, J.H., Qiao, C.Y., Tang, S.J., Li, Y.F., Yuan, W.J., Li, B., Tian, L., Liu, F., Hu, R., Gao, H.N., Wei, H.T., Zhang, H., Sun, H.C., Yang, B., 2011 Chem. Commun., 47, 6858-60.

Figure captions

Fig. 1 (a) Scheme of FRET mechanism. (b) Diagram of energy transfer efficiency E as a function of r/R_0.

Fig. 2 (a) Scheme of GO lattice and blue photoluminescence spectra. Reprinted with permission adapted from Eda et al., 2010. (b) Scheme of GQD emission band shift by nitrogen doping and reduction. Reprinted with permission adapted from Zhu et al., 2012. c) Schematic illustration of a FRET assay or the detection of *Staphylococcus aureus* gene between graphene quantum dots and gold nanoparticles. Reprinted with permission adapted from Shi et al., 2015.

Fig. 3 (a) Schematic representation of a FRET assay based on g-C_3N_4–MnO_2 nanocomposite for sensing of glutathione. Reprinted with permission from Zhang et al., 2014. (b) A g-C_3N_4 nanosheet-based FRET assay for riboflavin (vitamin B2) detection. Reprinted with permission from Han et al. 2016.

Fig. 4 Carbon dots-MnO_2 nanosheet based FRET assay for intracellular imaging of GSH. Reprinted with permission from He et al., 2015.

Fig. 5 (a) A single-layer MoS_2 nanosheet based FRET assay for the detection of DNA. Reprinted with permission from Zhu et al., 2013. (b) MoS_2 quantum dots as a photoluminescence sensing platform for 2,4,6-trinitrophenol detection. Reprinted with permission from Wang et al., 2014.

Fig. 6 (a) A WS_2 nanosheet based FRET assay for microRNA detection. Reprinted with permission from Xi et al., 2014. (b) A sensitive fluorescence turn-on assay of bleomycin

and nuclease with WS_2 nanosheet as the platform. Reprinted with permission from Qin et al., 2015.

Table captions

Table 1 Summary of graphene based FRET assays

Table 2 Summary of g-C_3N_4 nanosheet based FRET/electron transfer assays

Table 3 Summary of TMD nanosheet based FRET assays.