Microchimica Acta (2020) 187:179
https://doi.org/10.1007/s00604-020-4128-8

REVIEW ARTICLE

# Graphene/aptamer probes for small molecule detection: from *in vitro* test to *in situ* imaging

Yi Dong[1] · Ting Zhang[1] · Xiaoya Lin[1] · Jiangtao Feng[1] · Fang Luo[2] · Hong Gao[1] · Yangping Wu[3] · Ruijie Deng[1] · Qiang He[1]

Received: 14 January 2020 / Accepted: 19 January 2020


## Abstract

Small molecules are key targets in molecular biology, environmental issues, medicine and food industry. However, small molecules are challenging to be detected due to the difficulty of their recognition, especially in complex samples, such as *in situ* in cells or animals. The emergence of graphene/aptamer probes offers an excellent opportunity for small molecule quantification owing to their appealing attributes such as high selectivity, sensitivity, and low cost, as well as the potential for probing small molecules in living cells or animals. This paper (with 130 refs.) will review the application of graphene/aptamer probes for small molecule detection. We present the recent progress in the design and development of graphene/aptamer probes enabling highly specific, sensitive and rapid detection of small molecules. Emphasis is placed on the success in their development and application for monitoring small molecules in living cells and *in vivo* systems. By discussing the key advances in this field, we wish to inspire more research work of the development of graphene/aptamer probes for both on-site or *in situ* detection of small molecules and its applications for investigating the functions of small molecules in cells in a dynamic way.

**Keywords** Biosensor · Aptasensor · On-site detection · *In vitro* detection · *In vivo* imaging · Fluorescent assay · Electrochemical assay · Colorimetric assay · Chemiluminescence assay · Electrochemiluminescence assay

## Introduction

Small molecules have been considered as important targets in molecular biology, environmental issues, medicine and food industry. They can act as harmful toxins and carcinogens, or serve as nutrients, drugs or cell signaling molecules. Small molecules are traditionally detected by the methods including high-performance liquid chromatography (HPLC) [1], high-performance liquid chromatography (HPLC-MS), capillary electrophoresis (CE), enzyme-linked immunosorbent assay (ELISA). HPLC and HPLC-MS are the gold methods for detecting small molecules, nevertheless, still suffering from some defects like the requirement of expensive equipment and time-consuming. CE can both allow the detection with few samples and high separation efficiency while lead to poor reproducibility. As a rapid detection technology based on antibody, ELISA has the advantage of high sensitivity, operation simplicity and rapidness [2]. However, small molecules are low-antigenicity, thus it is difficult to obtain their antibody [3–5]. Besides, the complex pre-process, high cost and difficult preservation of antibody may limit the widely application of ELISA. Although many analysis tools are available above, there are also analytical challenges in small molecules detection. Hence, to circumvent the limitations of above methods for small molecules detection, it is imperative to exploit appropriate methods for quantitative detection in a more direct and simpler manner.

Alternative to antibodies, aptamers, species of chemically synthesized recognition molecules, have inherently

Yi Dong and Ting Zhang contributed equally to this work.

✉ Fang Luo
doctorluofang@126.com

✉ Ruijie Deng
drj17@scu.edu.cn

[1] College of Biomass Science and Engineering, Healthy Food Evaluation Research Center and Key Laboratory of Food Science and Technology of Ministry of Education of Sichuan Province, Sichuan University, Chengdu 610065, China

[2] The Center of Gerontology and Geriatrics, West China Hospital, Sichuan University, Chengdu 610065, China

[3] Department of Respiratory and Critical Care Medicine, West China Medical, Sichuan University, Chengdu, China

Published online: 19 February 2020

programmable nucleic acid nature and can be designed into various structure-switching oligonucleotide probes [6–9]. Remarkably, the antigenicity-independent selection process for aptamers makes them well fit for serving as recognition components for low-immunogenic small molecules. Besides, compared with protein-based antibody counterparts, aptamers exhibit the advantage of better chemical stability, synthesis simplicity, convenient regeneration, easy storage and modification [10–14]. Aptamers have exhibited potent applications in fabricating plenty of facile, rapid, robust, and inexpensive detection platforms such as fluorescent assay, electrochemical assay and colorimetric assay for small molecules [3, 13–20]. Furthermore, aptamers are demonstrated to not cause an immune response. Thus, it would be a competing candidate for probing small molecules in living systems. Meanwhile, these are a considerable amount of aptamers being selected for small molecules including mycotoxin, antibiotic, pesticides, hormones, vitamins, pigments and amino acids [6, 21, 22]. Therefore, aptamers for small molecules detection can be widely used in agriculture, medicine, food industry and environmental analysis [23–31].

Graphene is a two-dimensional (2D) nanomaterial comprised of a single layer of carbon atoms that form an ordered hexagonal flat [32, 33]. The flat two-dimensional nanostructure and high surface area of graphene provide abundant of space for binding biomolecules [34, 35]. And graphene oxide (GO) is derived form of graphene [36, 37]. GO is with the addition of oxygen atoms bound with the carbon scaffold. Graphene is hydrophobic in nature while GO is hydrophilic. In addition, GO contains both aromatic ($sp^2$) and aliphatic ($sp^3$) domains, further expanding the types of interactions that can occur with the surface. For simplicity, only the methods using GO were specifically stated, while graphene/aptamer probes imply both graphene/aptamer probes and GO/aptamer probes. Graphene plays key roles in material science and biotechnology, which has attracted much attention for constructing bioassays [38, 39]. They are suitable for the utilization in biosensing system due to their good biocompatibility and low toxicity [39, 40]. Besides, the excellent electronic and optical properties lead them feasible to make a variety of electronic- and optical-based assays [41–43]. Moreover, graphene and GO can conjugate with biomolecules by covalent or noncovalent bindings, thus it is easy to be modified [44].

DNA sequences can adsorb onto the graphene surface by the electrostatic interaction between DNA bases and the graphene surface, or the noncovalent π-π interaction [39, 45, 46]. Graphene is demonstrated to be able to bind well with aptamers like single-stranded DNA (ssDNA) and a hybridized double-stranded DNA (dsDNA), and has been adopted to detect small molecules with high selectivity and sensitivity [43, 47]. Specifically, graphene can serve as the carrier for aptamers to enter cells [48]. Thus, the incorporation of graphene would potentially allow aptamer-based probes to detect small molecules in living cells, even living animals.

In general, graphene is well suitable to combine with aptamers to construct graphene/aptamer probes and to fabricate the sensor platform or assays for small molecules. In addition to *in vitro* detection, graphene/aptamer probes are of great promise for small molecule detection in living cells. There is still no review summarizing the development of graphene/aptamer probes focusing on small molecules. In this review, we will present a brief discussion about how graphene/aptamer probes achieve highly specific, sensitive and rapid detection of small molecules *in vitro* based on different detection platforms, and emphatically highlight the key success in their development for monitoring small molecules in living cells and *in vivo* systems.

## *In vitro* detection

### Fluorescent assay

Fluorescent assays have attracted considerable attention because of the advantages including rapidness, non-destructive and homogeneous reaction conditions. The novel feature of aptamers as a recognition module lies in their programmable and responsive nucleic acid nature. And the structure-switching process of aptamer triggered by the binding of target leads to the change of its affinity towards graphene. Besides, as an excellent energy acceptor in fluorescence resonance energy transfer (FRET) process, graphene can act as superb fluorescent quenchers. The graphene/aptamer probe-based fluorescent assays can confer ultrafast response to target small molecules, and detection proceeds in homogeneous solutions in a test tube. This eliminates the need for complex separation. Based on this principle, graphene/aptamer probe-based fluorescent assays have been constructed for detecting plenty of small molecule species including ATP, ochratoxin A, adenosine, zeatin and so on [53–58].

Xia group [49] designed a label-free fluorescent assay for the detection of aflatoxin $B_1$ in food samples using aggregation-induced emission luminogen and GO (Fig. 1a). Fluorescence increased linearly in the 0–3 ng/mL aflatoxin $B_1$ concentration range with a detection limit of 0.25 ng/mL. The binding of one target molecule, however, only triggers the release of one fluorophore, which may result in relatively low sensitivity. The incorporation of nanomaterials and enzyme catalysis would significantly improve it. Quantum dots (Q-dots), nanocrystals with great photostability, broad excitations and narrow emissions, are frequently used to develop sensitive methods based on GO fluorescence assay. Li et al. [50] developed an aptamer based fluorescence recovery assay for aflatoxin $B_1$ using a quencher system composed of Q-dots and GO (Fig. 1b). The assay possessed a wide dynamic range

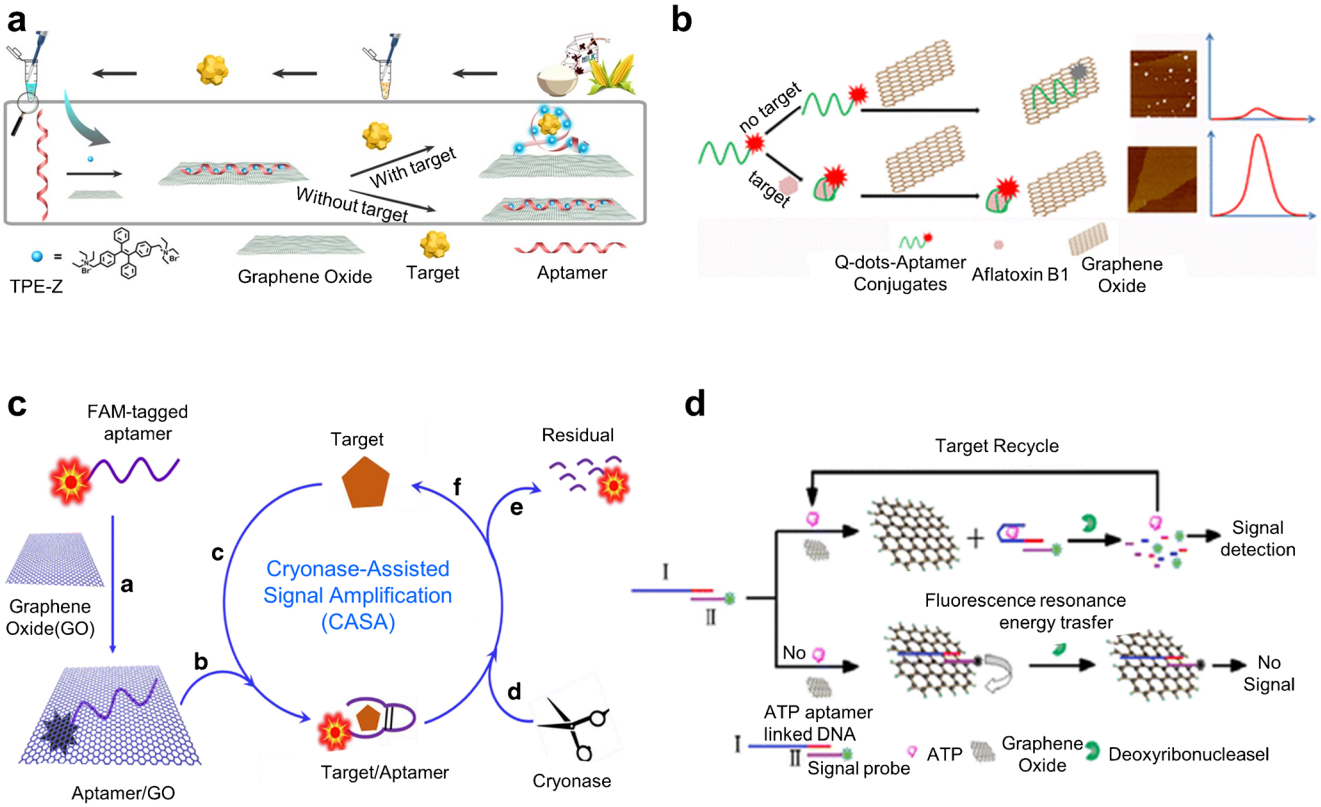



**Fig. 1** Graphene/aptamer probe-based fluorescent assays for *in vitro* detecting small molecules. (a) Fluorescence detection of aflatoxin $B_1$ based on aggregation-induced emission luminogen and GO. Reprinted with permission from Ref. [49]. Copyright 2019 Elsevier; (b) Aflatoxin $B_1$ assay using a quencher system composed of quantum dots and GO. Reprinted with permission from Ref. [50]. Copyright 2019 Springer. (c) A cryonase-assisted signal fluorescent assay for detecting of theophylline and ATP. Reprinted with permission from Ref. [51]. Copyright 2019 Springer; (d) A deoxyribonuclease I-aided target recycling and signal amplification fluorescent assay for determining of ATP. Reprinted with permission from Ref. [52]. Copyright 2017 Springer

(from 3.2 nM to 320 μM) and a low limit of detection (1.0 nM). When performed in peanut oil solution, the dynamic range was 1.6 nM-160 μM, and the limit of detection was 1.4 nM. This was a simple, sensitive and selective method for the determination of aflatoxin $B_1$. To further improve the sensitivity of fluorescent assay, Yu et al. [59] designed a dual strategy for constructing a graphene/aptamer probe. The induce of highly fluorescent nitrogen-doped graphene quantum dots (Q-dots) (with a fluorescence quantum yield up to 30%) and the obtaining of the polarization signal of Q-dots dropped the detection limit down to be 0.029 pM for omethoate detection. Nanomaterials need complex synthesis procedures and are potentially unstable. As an alternative strategy, the cycling of targets by adding enzymes would also attribute to higher sensitivity for small molecules detection with low cost. Cui et al. [51] designed a cyclic fluorometric method for sensitive detection of analytes using aptamers (Fig. 1c). It was based on the usage of GO and cryonase-assisted signal amplification. This cyclic reaction will light-up multiple fluorophore-labeled aptamer probes upon one-target molecule, resulting in significant fluorescent signal amplification. The detection limits were 47 nM for theophylline and 22.5 nM for ATP. Furthermore, Qin group [52] described a fluorometric aptamer based determination of ATP (Fig. 1d). It was based on deoxyribonuclease I-aided target recycling and signal amplification using GO as a quencher. The detection limit was as low as 0.2 nM, and the calibration plot was linear in the 10 nM to 400 nM ATP concentration range. However, the involvement of nanomaterials and enzyme catalysis steps would complex the detection process, which may be detrimental to the accuracy of these assays. Besides, the modification of aptamer probes would further improve the cost and reliability of the detection process. On the contrary, the adoption of DNA intercalation dye would allow label-free fluorescent detection of small molecules [60, 61], obviating the utilization of modified aptamers. There is a summary of fluorescent graphene/aptamer-based probes for *in vitro* detecting small molecules showing in Table 1.

## Electrochemical assay

Attributed to the high electrical conductivity, a large surface area, biocompatibility, and electrocatalytic activity, graphene usually acts as sensitive electrode, signal or nanocarrier

materials. Coupling with the highly specific aptamer, graphene/aptamer probes allow to gain sensitive and selective electrochemical signal responding to target small molecules [79] (Table 2). Electrochemical techniques including differential pulse voltammetry (DPV), square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS) have been used to fabricate aptasensors. These utilization hold great promises for the development of cost-effective, portable, sensitive, selective and disposable electrochemical assays targeting small molecules for diagnostic, biomedical, environmental, and food safety monitoring applications.

DPV, an electrochemical voltammetric technique, allows analysis with high sensitivity. Benefit from the large surface area, graphene can be loaded with plenty of nanomaterials such as Ag nanoparticle, Au nanoparticle, $Fe_3O_4$-AuNPs and PtCu alloy. It can lead to the amplification of the electrochemical signal, in turn improving the sensitivity for small molecules detection [63, 64, 77, 80, 91]. Imani et al. [76] designed an electrochemical assay for staphylococcal enterotoxin B detection based on rGO/AuNUs composition. The assay achieved a wide linear range from 5 fM to 500 fM with the detection limit of 0.21 fM. The assay can be used in spiked food samples with no significant difference compared to the synthetic samples (Fig. 2a). Besides Au nanoparticle, alloy could serve as biosensing substrates, potentially improving the sensitivity of graphene/aptamer probe-based assay. Pei etc. [77] proposed a signal amplification strategy of an ultrasensitive electrochemical assay for the quantitative detection of kanamycin antibiotic based on thionine functionalized graphene/PtCu material. The assay exhibited a high sensitivity and a wider linearity to kanamycin in the range $5 \times 10^{-7}$ μg/mL-$5 \times 10^{-2}$ μg/mL. The detection limit was down to 0.42 pg/mL (Fig. 2b). Similar to the DPV sensors, graphene-based SWV assays were utilized to modify the surface of the graphene electrode. The assays have also been used for detecting drugs and toxins [92]. Besides, EIS sensors can also be fabricated by all kinds of graphene nanomaterials (including GO and modified GO) [64]. To date, EIS sensors have been utilized for detecting small molecules including acetamiprid, ochratoxin A and tetracycline. There still exists one concern that many electrochemical assays performed in "turn-off" mode [65, 77, 80], thus new strategies should be constructed "turn-on" assay to increase their robustness. Qu et al. [67] constructed a "turn on" electrochemical assay using the proximity of two split aptamers. The electrochemical assay allowed for highly sensitive detection of adenosine triphosphate (ATP) and adenosine deaminase (ADA) activity using functionalized graphene as efficient electrochemical label (Fig. 2c). The detection limits for ATP and ADA activity were 13.6 nM and 0.01 U/mL (1.2 nM), respectively. Alternatively, Marty group [78] directly use the conformation response of aptamer to design a "turn-on" assay. Besides, the assay utilized functional graphene oxide (FGO) as the signal-enlarging platform to amplify probe signal (Fig. 2d). In the assay, aflatoxin $B_1$ was permitted to detect in the linear range of 0.05 ng/mL-6.0 ng/mL with a very low limit of detection of 0.05 ng/

**Table 1** Summary of the detection performance of graphene/aptamer probe-based fluorescent assay for *in vitro* detecting small molecules

| Analyte | Probes | LOD | Dynamic range | Samples | Ref |
|---|---|---|---|---|---|
| Aflatoxin $B_1$ | GO/QDs | 1.0 nM | 3.2 nM-320 μM | Peanut oil | [50] |
| Theophylline/ATP | GO/aptamer | 47 nM/22.5 nM | 50 nM-5 μM/0–1.0 μM | Serum | [51] |
| ATP | GO/aptamer | 0.2 nM | 10 nM–400 nM | MRSA cells | [52] |
| Ochratoxin A | PVP-coated GO/aptamer | 1.9 μM | 2 μM–35 μM | Red wine | [53] |
| ATP | GO/carbon dots | 0.08 nM | 0.10 nM–5.0 nM | Yogurt | [61] |
| ATP | GO/aptamer with DNase I | 40 nM | – | – | [62] |
| Digoxin | GO/AgNPs /aptamer | 0.3 pM | 1 pM-100 nM | Plasma | [63] |
| ATP | GO/AuNPs/aptamer | 10 pM | 10 pM-100 nM | Cell lysate | [64] |
| Hemin | graphene/hemin-bound aptamer | 0.64 nM | 1 nM −150 nM | Serum | [65] |
| Microcystin-LR | graphene/hemin-binding-aptamer | 1.9 pM | 0.1 pM −1 nM | Fish extract, Water | [66] |
| Acetamiprid | MWCNT-rGONR/aptamer | 17 fM | 50 fM-10 μM | Water | [67] |
| Tetracycline | GO/aptamer | 29 fM | 0.1 pM-10 μM | Tablet | [68] |
| Ochratoxin A | rGO/aptamer | 0.03 ng/mL | 0.1 ng/mL-200 ng/mL | Red wine | [69] |
| Ochratoxin A | AuNPs–rGO/aptamer | 0.3 pg/mL | 1 pg/mL-50 ng/mL | Wine | [70] |
| Carcinoembryonic Antigen | GO/2-aminopurine | 0.03 ng/mL | 0.05 ng/mL-2 ng/mL | Serum | [71] |
| Salmonella | GO/aptamer | 25 cfu/mL | $10^2$ cfu/mL-$10^7$ cfu/mL | Spiked milk | [72] |
| Lysozyme | GO/$CuInS_2$/ aptamer | 3 pg/mL | 0.01 ng/mL-1.8 ng/mL | Serum | [73] |
| Patulin | GO/aptamer | 0.28 μg /L | 0.5 μg/L-30 μg/L | Apple juice /grape juice | [74] |
| Zearalenone | GO/aptamer | 0.5 ng/mL | 0.5 ng/mL-64 ng/mL | Beer/wine | [75] |

**Table 2** Summary of electrochemical graphene/aptamer-based assays for *in vitro* detecting small molecules

| Analyte | Probes | LOD | Dynamic range | Samples | Ref |
|---|---|---|---|---|---|
| Streptomycin | GR–$Fe^3O^4$–AuNPs/PCNR | 0.028 ng/mL | 0.05 ng/mL–200 ng/mL | Milk | [80] |
| Kanamycin | GR-TH/aptamer | 0.42 pg /mL | 0.5 pg/mL-50 ng/mL | Pork/chicken | [77] |
| Angiogenin | Graphene/AuNPs | 0.064 pM | 0.1 pM −5 nM | Cancer | [81] |
| Interferon-γ | Grapheme/aptamer | 0.065 pM | 0.1 pM- 0.7 pM | Cell media | [82] |
| Aflatoxin $B_1$ | GO/aptamer | 0.05 ng /mL | 0.05 ng/mL–6.0 ng/mL | Beer/wine | [78] |
| TET | rGO-$Fe_3O_4$/aptamer | 0.6 nM | 6 μm-5 mM | | [83] |
| Thrombin | GO/ hemin | 0.062 pM | 0.0001 nM–40 nM | Blood/serum | [84] |
| Alpha-fetoprotein | GR-TH/aptamer | 0.050 μg/mL | 0.1 μg/mL - 100.0 μg/mL | Human serum | [85] |
| Staphylococcal enterotoxin B | GO/AuNu | 0.21 fM | 5 fM–500 fM | Milk/meat | [76] |
| Dopamine | Grapheme-polyaniline | 0.00198 nM | 0.007 nM–90 nM | Serum | [86] |
| Bisphenol A | Graphene /GNPs | 5 nM | 0.01 μM – 10 μM | Milk | [87] |
| Carcinoembryonic antigen | Graphene/hairpin | 80 ag/mL | 80 ag/mL − 950 fg/mL | Serum | [88] |
| Thrombin | Graphene/GPD/aptamer | 200 fM | 0.001 nM–40 nM | Serum | [89] |
| PDGF/ thrombin | Graphene/PtNPs | 8 pM/11 pM | 0.01 nM–35 nM/ 0.02 nM −45 nM | | [90] |

mL. Electrochemical graphene/aptamer-based assays for *in vitro* detecting small molecules were listed at Table 2. These existed graphene/aptamer-based electrochemical assays would allow sensitive detection of small molecules, and may potentially be applied for on-site detection.

## Colorimetric assay

Due to the appealing attributes such as simplicity, rapidness, low-cost and easy to read-out, colorimetric assays are preferred in the application of on-site detection. In the beginning, due to the unique size-dependent surface plasmon resonance properties, gold nanoparticles have been widely used for fabricating colorimetric assays [95]. Later on, some researchers found that hemin and metallic nanoparticle modified graphene can exhibit an intrinsic peroxidase-like catalytic activity, and were utilized as artificial enzymes for detecting small molecules [96, 97]. And the coupling of the graphene nanocomplexes with aptamers allowed the fabrication of colorimetric assays [94, 98]. Wang et al. [98] proposed a colorimetric assay based on the hemin-loaded reduced GO. The hemin-loaded reduced GO would exert high activity for catalyzing 3,3,5,5-tetramethylbenzidine to be with blue color, and be stabilized by acetamiprid aptamers. The binding of acetamiprid would hinder the adsorption of aptamers to GO, leading to its coagulation and lower catalysis activity. The constructed colorimetric assay enabled label-free detection of acetamiprid with a detection limit of 40 nM. Alternatively, platinum/gold nanoparticles were decorated on graphene to endow it with a superior catalytic activity. Wang etc. [93] developed a detection method for patulin based on an enzyme-chromogenic reaction between glucose oxidase and squaric acid. The colorimetric assay exhibited a linear range from 50 pg/mL to 2500 pg/mL, and the limit of detection was found to be 48 pg /mL (Fig. 3a). Sun group [94] developed an ATP assay based on the sandwich structure of GO-platinum/AuNPs (Fig. 3b). The detection limit was low to 0.2 nM, which is superior over many reported visual sensors for ATP detection. The sensitivity of colorimetric assay is highly dependent on the catalytic activity of graphene nanocomplex. And its utility is still hindered by the stability of nanomaterials and its reproducibility. Typical colorimetric graphene/aptamer probes-based assays for *in vitro* detecting small molecules are listed in Table 3.

## Chemiluminescence and electrochemiluminescence assay

Compared to the fluorescence, chemiluminescence (CL) is much cheaper due to its no requirement for an external light source in equipment. Graphene is demonstrated to be able to act as a superb luminescence quencher. Similar to graphene-based FRET assays, the chemiluminescent resonance energy transfer (CRET) assays can be constructed by the adoption of the structure-switching aptamers [108, 109]. Alternatively, Song etc. [104] utilized GO-based instantaneous derivatization to construct a CL assay (Fig. 4a). A CL reagent phenylgyoxal can serve as the signal reporter specifically reacted with guanine bases of aptamers. At the initial state, aptamers adsorbed onto the surface of GO, leading to a strong CL signal. In the presence of ATP, the aptamers binding with ATP molecules would confer a weak binding affinity to GO, resulting in a significant decrease of CL signal. The graphene-based CL assay was applied for ATP detection, achieving a detection limit of 1.4 nmol and a dynamic range of 2 nmol to 80 nmol. Benefited from the utilization of guanine bases

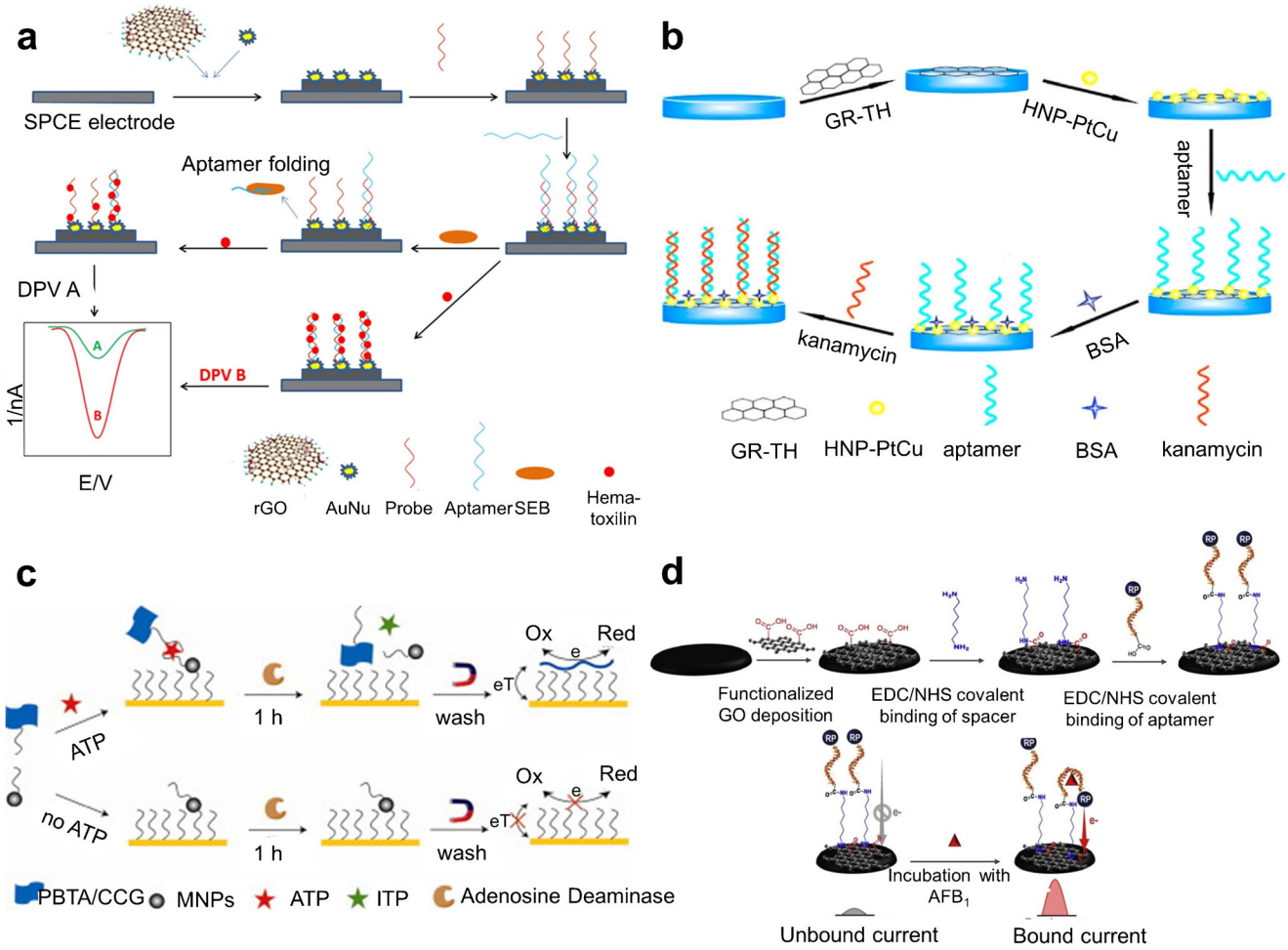



**Fig. 2** Graphene/aptamer probe-based electrochemical assays for *in vitro* detecting small molecules. (a) An electrochemical assay for staphylococcal enterotoxin B detection based on reduced GO and gold nano-urchins. Reprinted with permission from Ref. [76]. Copyright 2019 Elsevier; (b) An amplification strategy of an electrochemical assay for kanamycin based on thionine functionalized graphene/PtCu composition. Reprinted with permission from Ref. [77]. Copyright 2016 Elsevier; (c) Turn-on electrochemical assay using split aptamer for ATP and ADA activity. Reprinted with permission from Ref. [67]. Copyright 2014 Elsevier; (d) An electrochemical aptasensor based on GO assisted electrocatalytic signal amplification of methylene blue for aflatoxin $B_1$ detection. Reprinted with permission from Ref. [78]. Copyright 2017 Elsevier

specific CL reagent, the aptamer-based assay proceeded in a label-free manner. Aptamer composites containing magnetic nanoparticles can exhibit excellent merits for higher selectivity, better stability, and easier separation. To further improve the sensitivity, Luo et al. [105] designed a CL assay for dopamine (DA) detection based on aptamers modified magnetic mesoporous silica @ GO polymers ($Fe_3O_4$•m$SiO_2$•n$SiO_2$@GO). DA could be measured with the linear concentration range of $2.5 \times 10^{-13}$ mol/L to $2.0 \times 10^{-9}$ mol/L with a detection limit of $3.9 \times 10^{-14}$ mol/L (Fig. 4b).

Electrochemiluminescence (ECL) has also been widely used to construct biosensors due to its remarkably high sensitivity and wide dynamic ranges. Graphene can act as both luminescent materials and nanocarriers in graphene-based ECL aptasensors or assays (Table 4). Cui group [106] constructed a bilayer structure of luminescence functionalized graphene hybrids, further increasing the loading of ECL signal molecules, enabling label-free detecting of 2,4,6-trinitrotoluene (TNT) with a very low detection limit of $6.3\times 10^{-13}$ g/mL (Fig. 4c). The graphene/aptamer-based ECL assay has been successfully applied to the detection of TNT in environmental water. Gao etc. [107] developed a tun-on assay for ATP detection in which GO was employed as an indicator for ECL signal generation. The assay was fabricated by self-assembling the ECL probe of a thiolated adenosine triphosphate binding aptamer tagged with a $Ru(bpy)^{32+}$ derivatives onto the surface of AuNP modified glassy carbon electrode. The ECL intensity versus the logarithm of ATP concentration was linear in the wide range from 10 pM to 10 nM with an ultra-low detection limit of 6.7 pM to 4.8 pM, respectively (Fig. 4d). Graphene can pose large amounts of sites for binding with CL or ECL report molecules, thus allow to construct sensitive assays using either nanomaterials or small molecules

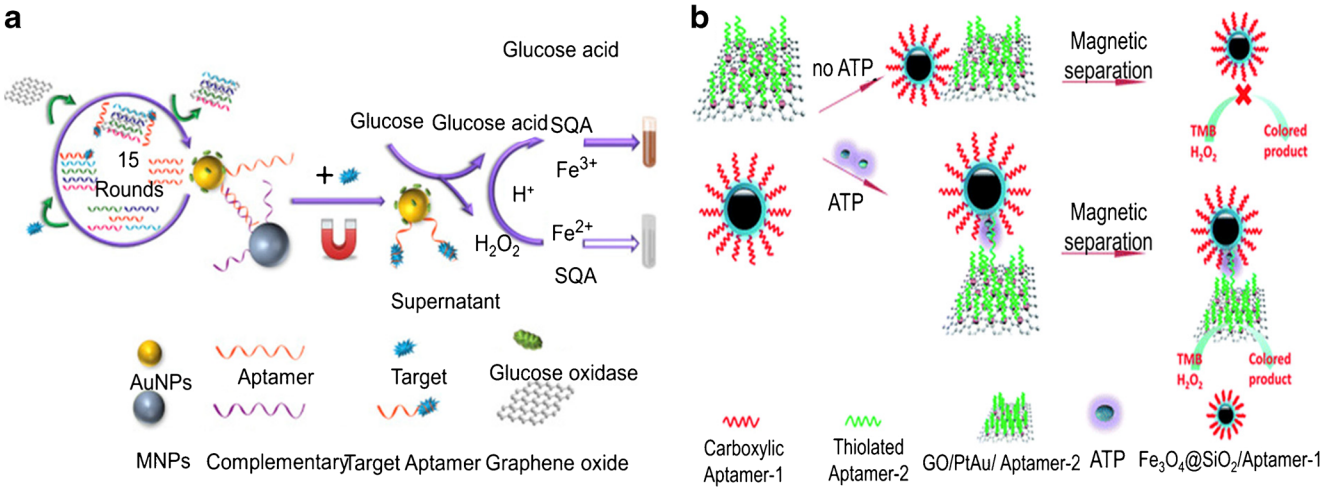



**Fig. 3** Graphene/aptamer probe-based colorimetric assays for *in vitro* detecting small molecules. (a) Colorimetric detection of patulin using AuNPs. Reprinted with permission from Ref. [93]. Copyright 2016 Elsevier; (b) Highly efficient colorimetric detection of ATP utilizing GO Pt/Au nanoparticles. Reprinted with permission from Ref. [94]. Copyright 2015 Royal Society of Chemistry

serving as CL or ECL reporter. CL and ECL assays based on graphene/aptamer are summarized at Table 4.

## *In situ* or *in vivo* detection

### Probing small molecules in living cells

Graphene presents appealing attributes such as the excellent photoluminescence, biocompatibility, leading to plenty applications in biological and medical areas [39, 40, 118]. Specifically, graphene can serve as the carrier of aptamer to enter cells, making it possible to be utilized to detect small molecules in living cells [119–121]. Therefore, the potential for small molecule probing in living cells was explored. In recent decade, the utilization of graphene for the intracellular monitoring and *in situ* molecular probing has made a certain progress [48]. And graphene/aptamer sensoring platforms have been fabricated for small molecules recognition and *in situ* fluorescence monitoring in living cells [120–123].

Lin group pioneered to construct graphene/aptamer probes for small molecule imaging [124]. A graphene oxide nanosheet (GO-nS) based aptamer-carboxyfluorescein (FAM) nanocomplex was fabricated to explore its ability for ATP detection in living cells. *In vitro*, the aptamer probe could detect ATP with high specificity and recovered nearly 85.7% of fluorescence quenched by GO-nS. The significant fluorescence of the FAM-labeled aptamer has be found in mice epithelial cells (JB6 Cl 41-5a) after being incubated with aptamer-FAM/GO-nS for 8 h, manifesting the ability of aptamer-FAM/GO-nS for molecular probing in living cells. Later on, PEGylated graphene nanosheets based aptamer sensor with folate labels has been built for the detection of Cytochrome c (Cyt c) releasing from mitochondria in Hela cells (Fig. 5). This aptamer assay was used as fluorescence imaging tools, allowing the direct visualization of Cyt c translocation in living cells [125]. Although graphene/aptamer probes have been demonstrated to be able to image small molecules in living cells, concerns about the non-specific signals or background and the quantification performance still

**Table 3** Summary of colorimetric graphene/aptamer probe-based assays for *in vitro* detecting small molecules

| Analyte | Probes | LOD | Dynamic range | Samples | Ref |
|---|---|---|---|---|---|
| Patulin | GO /aptamer | 48 pg/mL | 50 pg/ mL-2500 pg/ mL | Food | [93] |
| Acetamiprid | hemin-functionalized GO/aptamer | 40 nM | 100 nM-10 μM | Water | [99] |
| ATP | GO/PtAuNPs/aptamer | 0.2 nM | 0–100 nM | Serum | [94] |
| Folic acid | GO/PtNPs | | 0.17 μg/mL − 6.7 μg/mL | Cancer cell | [100] |
| TMB | rGO/hemin | 40 nM | 100 nM-10 μM | Acetamiprid | [98] |
| Leucine-arginine | GO/Hairpin/Methylene Blue | 219 pM | 0-1000 nM | Water/serum | [101] |
| Thrombin | Graphene/Hemin/aptamer | 2.5 nM | 0–20 nM | | [102] |
| Chloramphenicol | GO/AuNPs/aptamer | 451 pM | 1 nM–120 nM | Milk/serum | [103] |

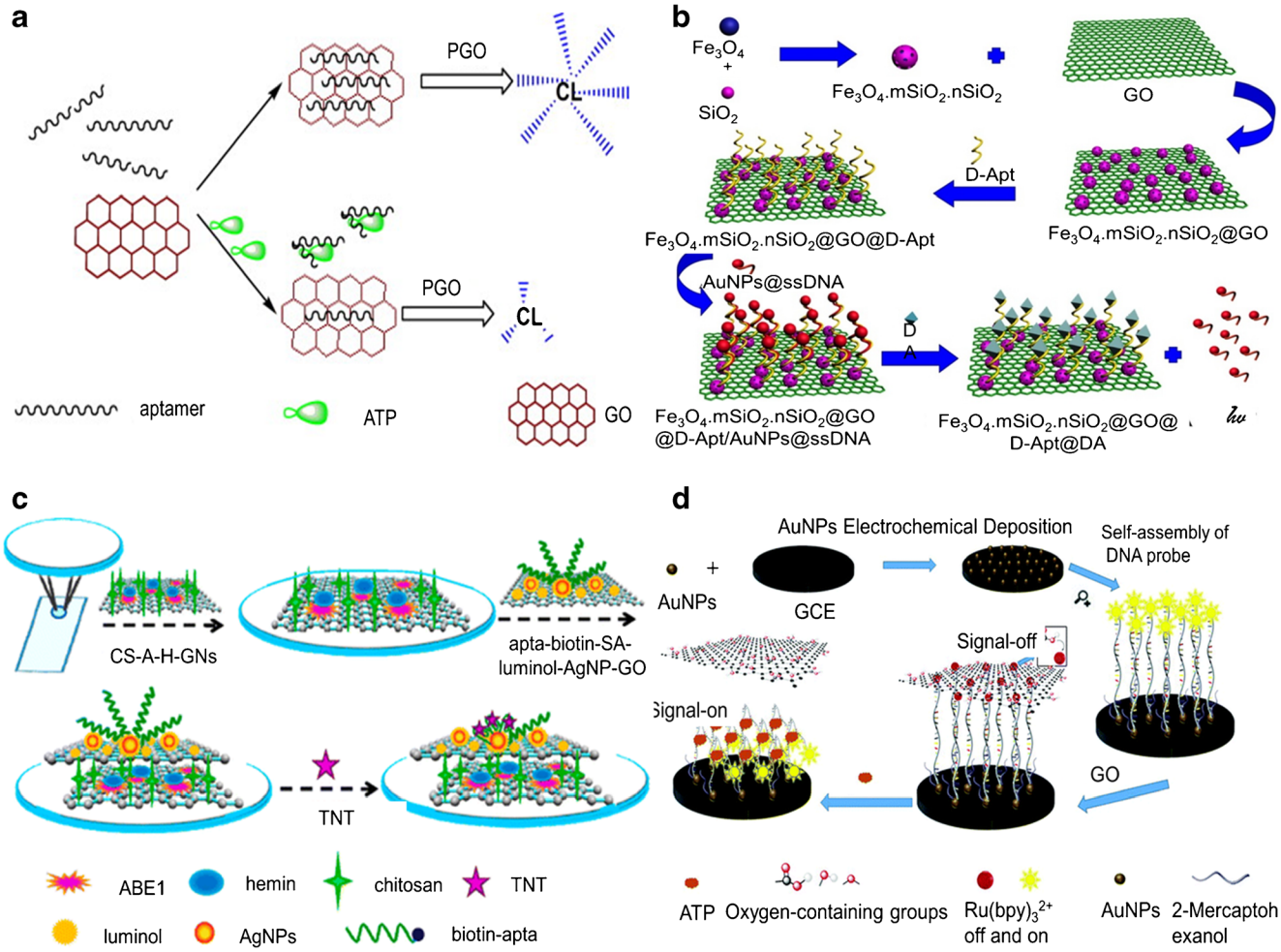



**Fig. 4** Graphene/aptamer probe-based chemiluminescence and electrochemiluminescence assays for *in vitro* detecting small molecules. (a) A graphene/aptamer-based ATP chemiluminescence assay based on graphene oxide and an instantaneous derivatization of guanine bases. Reprinted with permission from Ref. [104]. Copyright 2014 Elsevier; (b) A chemiluminescence assay for dopamine detection based on aptamers modified magnetic mesoporous silica/GO. Reprinted with permission from Ref. [105]. Copyright 2018 Elsevier; (c) Electrochemiluminescent detection of 2,4,6-trinitrotoluene using bilayer structure of luminescence functionalized graphene. Reprinted with permission from Ref. [106]. Copyright 2015 American Chemical Society. (d) An efficient signal-on aptamer-based assay for ATP detection using GO as an electrochemiluminescence signal indicator. Reprinted with permission from Ref. [107]. Copyright 2015 Royal Society of Chemistry

**Table 4** Summary of CL and ECL assays based on graphene/aptamer

| Assay | Analyte | Probes | LOD | Dynamic range | Samples | Ref |
|---|---|---|---|---|---|---|
| CL | Thrombin | GO/aptamer | 0.083 mol/L | 0.25 mol/L- 1 mol /L | Serum | [108] |
| | Dopamine | $Fe_3O_4$·$mSiO_2$·$nSiO_2$@GO | $3.9 \times 10^{-14}$ mol/L | $2.5 \times 10^{-13}$ mol/L-$2.0 \times 10^{-9}$ mol/L | Urine | [105] |
| | ATP | rGO/$SnO_2$-QD | 0.025 pM | 0.1 pM-100 nM, | Serum | [110] |
| | *Staphylococcus aureus* | GO nanosheet | 15 cfu/mL | 50–$1.5 \times 10^5$ cfu/mL | Pork | [111] |
| | Adenosine | HKUST-1/QDs/aptamer | $2.1 \times 10^{-13}$ mol/L | $1.0 \times 10^{-12}$–$2.2 \times 10^{-10}$ mol/L | Urine | [112] |
| | *Escherichia coli* O157:H7 | GO/aptamer | $4.5 \times 10^3$ cfu/mL | $10^4$ cfu/mL–$10^7$ cfu/mL | Skim Milk | [113] |
| | $Hg^{2+}$ | GO/aptamer | 2 nM | – | Stream | [114] |
| ECL | TNT | Graphene /aptamer | 0.63 pg/mL | 1 pg/mL-1 ng/mL | Water | [106] |
| | ATP | GO/aptamer | 4.8 pM | 10 pM-10 nM | Serum | [107] |
| | Thrombin | Magnetic GO/aptamer | $1.3 \times 10^{-9}$ mol/L | $2.0 \times 10^{-9}$ mol/L – $5.0 \times 10^{-8}$ mol/L | Serum | [115] |
| | Cholesterol | MWCNTs-GO-Thi-Au | 50 nM | 0.15 μM–828 μM | Serum | [116] |
| | *V. parahaemolyticus* | GO/ hairpin aptamer | 2230 cells/mL | 4375 cells/ mL-70,000 cells/ mL | – | [117] |

remain. Besides, the success of entrance into the cytoplasm is the key step for imaging of small molecules, as large parts of target small molecules locate in the cytoplasm. The modification of cell-penetrating peptides or the construction of the graphene nanocomponent with positively charged coating may facilitate the entering of probes into the cytoplasm [119, 126], potentially improving the detection efficiency.

## Probing small molecules with reduced background

Non-specific desorption of aptamers from graphene oxide (GO) by competitively binding of non-target biomolecules would occur in a complex system. And the non-specific desorption may result in a false-positive signal [127]. Therefore, the non-specific effect should be paid more attention when the aptamer/GO probe is applied in a complex system containing high concentrations of non-target proteins, such as living cells. Liu group [123] proposed a strategy that a dual-modified aptamer with a fluorophore and an amino group was covalently linked to the carboxyl group on GO (Fig. 6). The coupling efficiency for aptamer reached 50%. Attributed to the covalently binding of aptamers, this dual-modified aptamer/GO probe significantly reduces the non-specific desorption from GO by non-target cellular proteins, in turn enhance the intracellular signal (Fig. 6b). This covalent aptamer probe was used for intracellular ATP imaging in

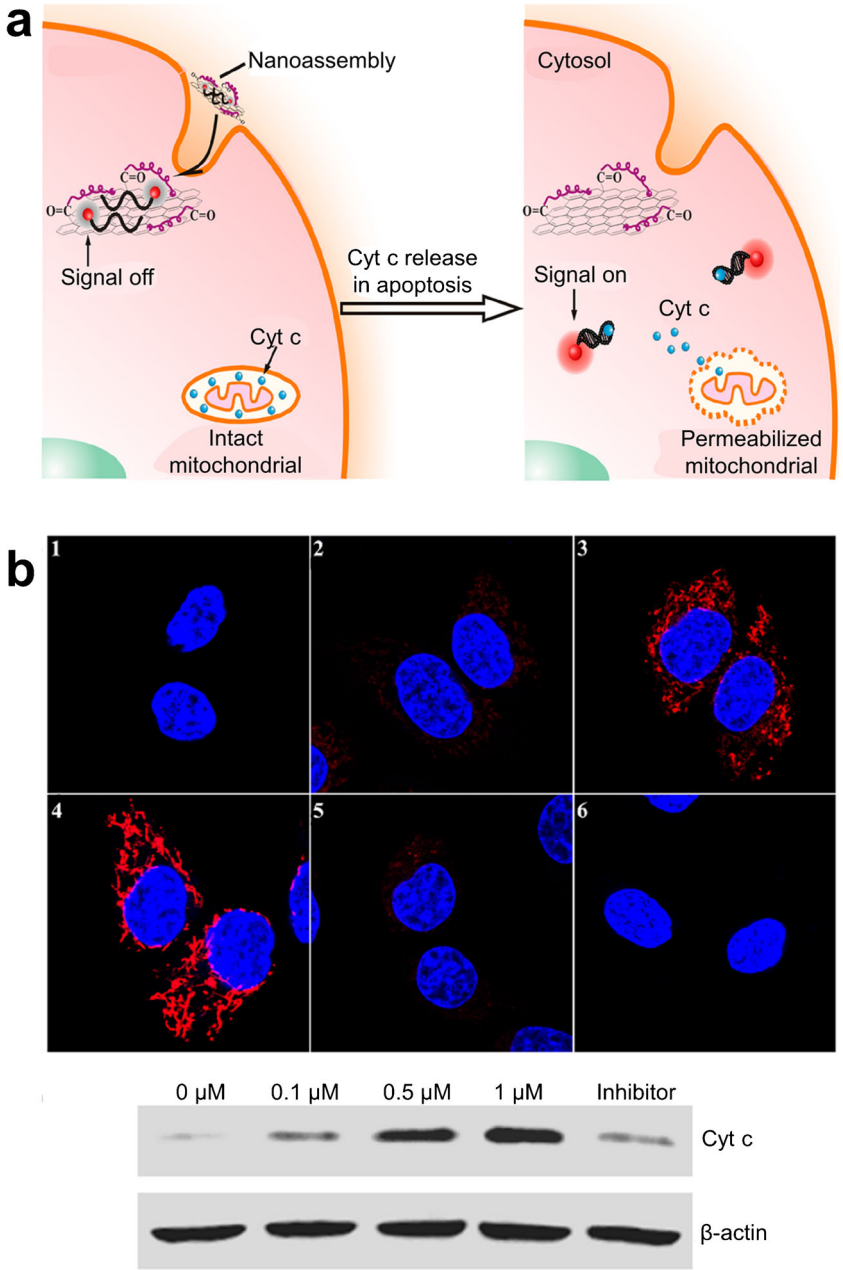
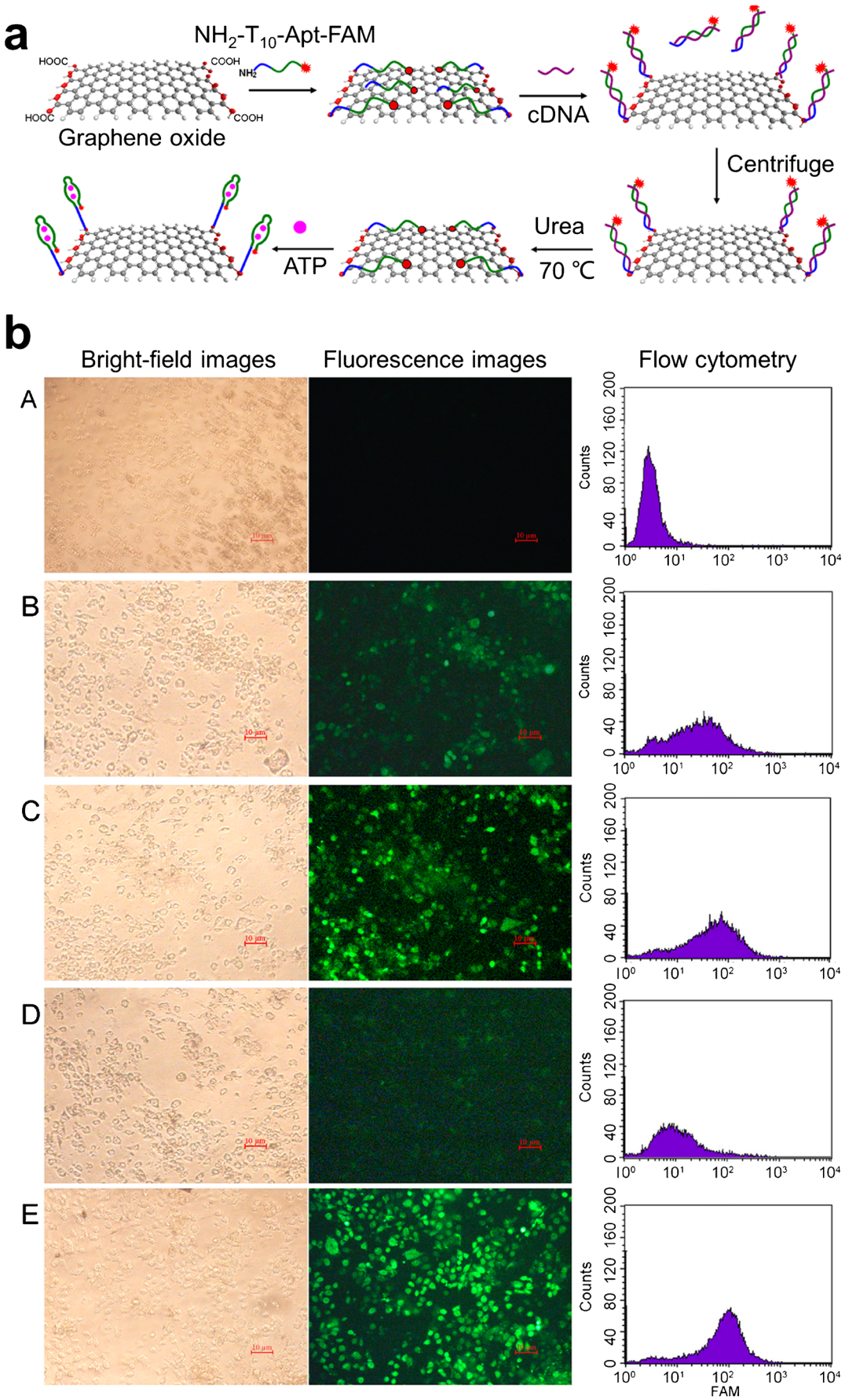



**Fig. 5** Imaging Cyt c release in living cells. (a) Working principle for graphene/aptamer probes for probing Cyt c; (b) Fluorescence imaging of HeLa cells (FR+) incubated with nanoassembly: 1) no treatment, 2) 0.1 μM STS for 1.5 h, 3) 0.5 μM STS for 1.5 h, 4) 1 μM STS for 1.5 h, 5) pretreated with 100 μM pepstatin A for 24 h and incubated with nanoassembly for 1 h followed by 1 μM STS for 1.5 h, 6) incubated with non-aptamer nanoassembly for 1 h followed by 1 μM STS for 1.5 h. The below is the western blotting assay for plasma extracts from corresponding cells. Reprinted with permission from Ref. [125]. Copyright. 2015 American Chemical Society


a
NH2-T10-Apt-FAM
HOOC
COOH
NH2
HOOC
COOH
Graphene oxide
cDNA
Centrifuge
Urea
70 °C
ATP
b
Bright-field images
Fluorescence images
Flow cytometry
A
B
C
D
E
Counts
0 40 80 120 160 200
10^0 10^1 10^2 10^3 10^4
FAM

◀ **Fig. 6** Graphene/aptamer probes against nonspecific-binding. (a) The synthesis and sensing principles of graphene/aptamer probes; (b) Microscopy and flow cytometry results of SMMC-7720 cells: A) medium only, B) physisorbed sensor (T10-Apt1-FAM), C) covalent sensor with $NH_2$-$T_{10}$-Apt1-FAM, D) covalent sensor but with the control aptamer ($NH_2$-$T_{10}$-Apt2-FAM) that cannot bind ATP, E) Cells were treated with $Ca^{2+}$ before the covalent sensor was added. Reprinted with permission from Ref. [123]. Copyright 2014 American Chemical Society

SMMC-7720 cells. Nevertheless, the covalent modification of graphene demands the dual-modified aptamer probe, significantly increased the cost, and the modification efficiency is limited by the amount of functional groups on the planes of graphene. Alternative to the covalent attachment, the discovery of high binding affinity of poly-T and poly-C to graphene would shed light on the strategy for the non-covalent strong modification of aptamer to graphene [128]. The modification of aptamer on graphene may be achieved by simple mixing process, and may also confer the ability to resist the nonspecific binding of proteins and other non-target molecules [127]. Besides, Tan et al. [122] found that the serum-free medium can also help overcoming the problem caused by nonspecific desorption.

## Quantification of small molecules in living cells

The quantification of small molecules in living cells using the graphene/aptamer probes would be challenging due to the uncertainty of the probe concentrations and the varied background in cells. To conquer these problems, Tan group proposed an aptamer molecular beacon probe coupled internal reference to semiquantify ATP in living cells (Fig. 7) [129]. Coupling with the binding of graphene, the aptamer molecular beacon (AAMB) would lead to double quenching platform, thus leading to efficient suppression of the background. A control ssDNA, which may release nonspecifically from GO by nontarget binding, would serve as an internal reference. The intensity ratio of the fluorescence of AAMB and the internal ssDNA was utilized as the signal response to the target, allowing the quantitative analysis of ATP in living cells. Therefore, benefited from the high loading capacity of graphene for probes, the ratiometric graphene/aptamer probes may be constructed by attaching different DNA sequences. However, DNA sequences (including aptamers and internal ssDNA) with different structures may present different binding affinity to graphene. The effects of different binding affinity on the ratiometric quantification performance should further be investigated. On the other hand, motivated by the loading ability of graphene, the ability of multiplexed probing small molecules of graphene/aptamer probes may potentially be achieved by attaching different aptamers onto graphene.

## Multiplexed imaging of small molecules in living cells

Attributed to the high loading ability and full-spectrum quenching property of graphene, the graphene/aptamer probe may potentially be utilized to multiplexed image of small molecules by different fluorescence labelling. Lin group set to explore the multiplexing ability of the graphene/aptamer probes [121]. FAM-labeled ATP aptamer and Cy5-modified GTP aptamer were designed to bind to GO-nS by π-π stacking for fabricating the multiple aptamer/GO-nS sensing platform (Fig. 8a) [120]. The binding of aptamers to GO-nS guaranteed the close proximity of dyes to the graphene surface. Therefore, GO-nS would confer high quenching efficiency for both FAM and Cy5 fluorophores. The aptamer achieved high specificity for ATP and GTP identification (Fig. 8b). This multiple sensing platform can be transported inside the cells via endocytosis, allowing to interact with ATP and GTP in cells. The conformation change of aptamer caused by the binding with target molecules would lead the recovery of the fluorescence of FAM and Cy5, allowing *in situ* multiple fluorescence monitoring of ATP and GTP in MCF-7 breast cancer

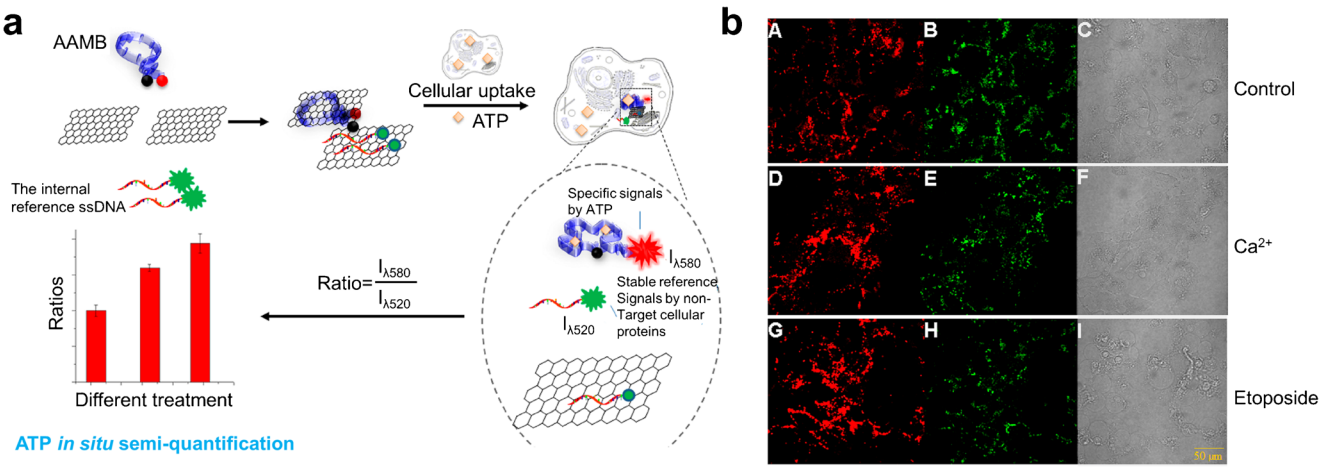



**Fig. 7** Semi-quantification of ATP in living cells. (a)Working principles for aptamer molecular beacon/graphene probes for imaging ATP; (b)ATP imaging with drug stimulation. Reprinted with permission from Ref. [129]. Copyright 2012 American Chemical Society

cells (Fig. 8c). Besides, GO-nS can exert excellent protection for DNA/RNA from enzymolysis in living cells. Thus, the graphene-based aptasenors platform has promising capacities for monitoring of multiple small molecules in living cells, which may promote it to be utilized for cellular simultaneous imaging studies of multiple active molecules.

### Real-time investigation of the cell apoptosis process

The ability of graphene/aptamer probes for monitoring small molecules in living cells would provide us with the dynamic information of cell biological process. Apoptosis is a highly regulated, programs cell death process. And the abnormal apoptosis would trigger serious human diseases such as degenerative disorders and cancers [130]. Chu group developed a graphene/aptamer probe for fluorescence imaging of Cyt c, and utilized the probe for investigating cell apoptosis. Firstly, the colocalization of Cyt c with mitochondria was interrogated to verify that the fluorescence activation was responsive to Cyt c released from mitochondria during cell apoptosis (Fig. 9a). The graphene/aptamer probe was demonstrated to be with rapid response towards target Cyt c (within 5 min), indicating the feasibility of monitoring the release of Cyt c in apoptotic cells. The real-time imaging process indicated that the Cyt c release process was very rapid and completed within ~10 min. The cell apoptosis was also recorded using the commercial intracellular caspase-3 probe. The real-time observation of these two molecules indicated that caspase-3 activation was followed by the cytosolic translocation of Cyt c during the cell apoptosis (Fig. 9b). Besides, the graphene/aptamer probe provided a feasible platform for directly screening critical regulators of apoptotic signaling via fluorescence imaging (Fig. 9c). The demonstration of the application of graphene/aptamer probes for studying the cell apoptosis manifested that they can be applied to monitor the metabolism of small molecules in living cells, thus would be feasible tools for dynamically investigating the cellular process.

### Probing small molecules *in vivo*

Graphene can protect the carried RNA/DNA from enzymatic breakdown, which makes graphene be considered as the excellent carrier material for study *in vivo* [48]. Thus, the graphene/aptamer probes may potentially be constructed to detect small molecules *in vivo*. To improve the imaging depth, and low down the tissue autofluorescence and self-absorption, Tan group [122] utilized the two-photon imaging method for tracking the graphene/aptamer probe (Fig. 10). Aptamer probes were modified with a two-photon dye (TPdye). The two-day-old zebrafish incubated with GO/ATP aptamer-TPdye conjugates presented strong two-photon excitation fluorescence. It indicated that the ATP concentration level was high in the growing zebrafish, while the zebrafish incubated with GO/R-TPdye conjugates showed no fluorescence signal. This research served a way of monitoring target biomolecules in living tissues. Compared to the detection of small molecules in cells, the imaging of small molecules in tissues or living animals would be more challenging. Firstly, the graphene/aptamer probe must be designed to be stable enough to be delivered to the tissues/cells of interest. Besides, the pass through the barriers of vessels and extracellular matrix should be considered if the probes are designed for *in vivo* living animal detection. Nevertheless, the demonstration of imaging ATP in zebrafish implied the likelihood of the application of graphene/aptamer probes for real-time *in vivo* detection of small molecules, which may be utilized to investigate the energy metabolism in muscle or neural activity in brain. A summary the applications of graphene/aptamer probes for *in situ* or *in vivo* detection was listed in Table 5.

## Conclusions and outlook

Small molecules are widely utilized as antibiotics and other important drugs in molecular biology, or as pesticides and preservative in food industry, thus deemed to be key targets. Among existed methods, graphene/aptamer-based assay is considered as a potent tool for small molecule detection due to its high selectivity and sensitivity, speediness and low-cost, as well as the ability of probing small molecules *in vivo*.

The antigenicity-independent selection process for aptamers makes them well fit for serving as recognition components for low-immunogenic small molecules. And the high specificity of aptamers would accommodate aptasensors to identify target small molecules in complex sample matrix, thus may eliminate the complex purification process. Besides, the biocompatibility and specific interaction with DNA/RNA, as well as the superb electrical and optical property, allow graphene and graphene nanocomposites to fabricate different analysis methods including fluorescent assays, electrochemical assays, colorimetric assays, CL and ECL assays. These platforms have been successfully applied for quantifying amount species of small molecules such as cocaine, acetamiprid, theophylline, xanthine, neomycin, folic acid, zeatin, adenosine, ATP and some amino acids.

Remarkably, attributing to the excellent photoluminescence, biocompatibility, probe loading and delivery capacities, graphene/aptamer probes hold great potential for monitoring small molecules in living cells and *in vivo* systems. They have been constructed to image ATP, GTP and Cyt c in living cells. Continuous effects are paid to suppress the non-specific background in living cells, in turn to achieve the quantification of small molecules *in situ* inside cells. Besides, multiplexed aptamer recognition would accommodate to simultaneously monitor different target small molecules, which may allow us to obtain the

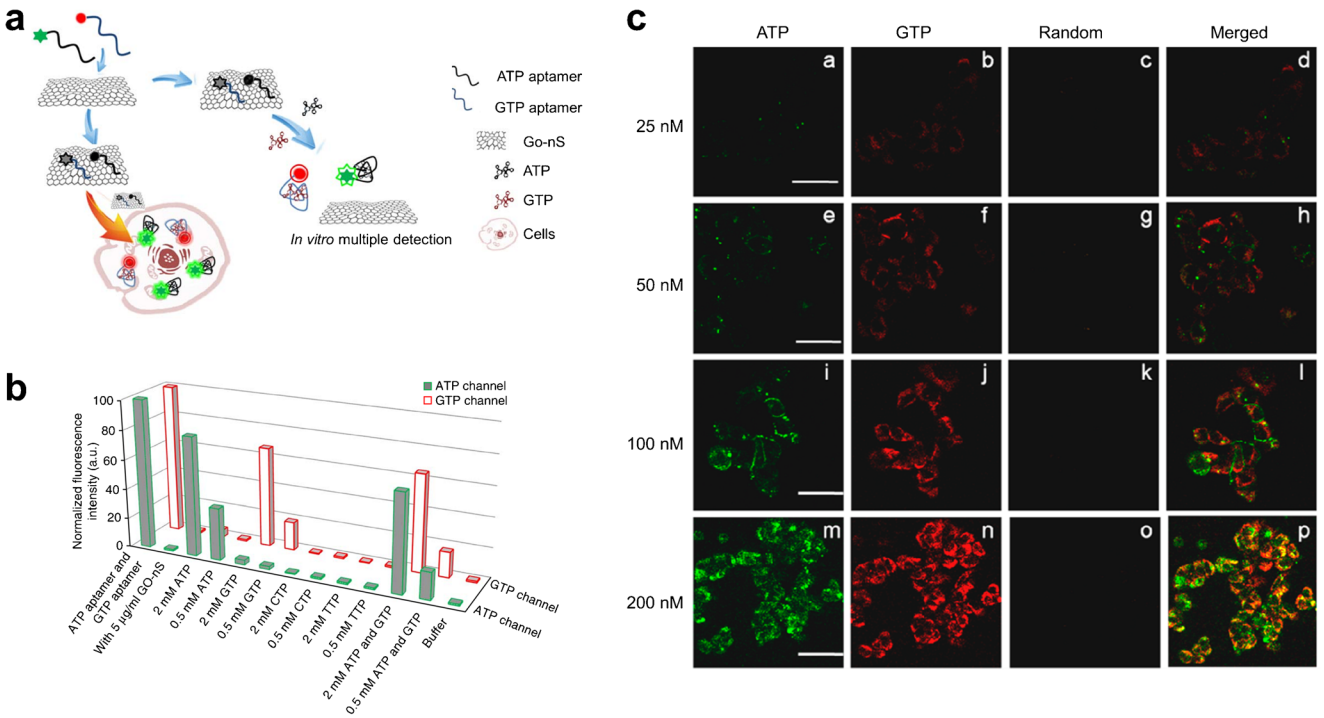



**Fig. 8** Multiplexed imaging small molecules in living cells. (a) Schematic illustration of multiplexed molecular probing in living cells by using the aptamer/GO-nS nanocomplex; (b) Selective response to ATP, GTP, CTP and TTP based on the aptamer/GO-nS sensing platform, Reprinted with permission from Ref. [120]. Copyright 2014 Springer. (c) Simultaneously imaging ATP and GTP in MCF-7 cells. Reprinted with permission from Ref. [121]. Copyright 2013 American Chemical Society

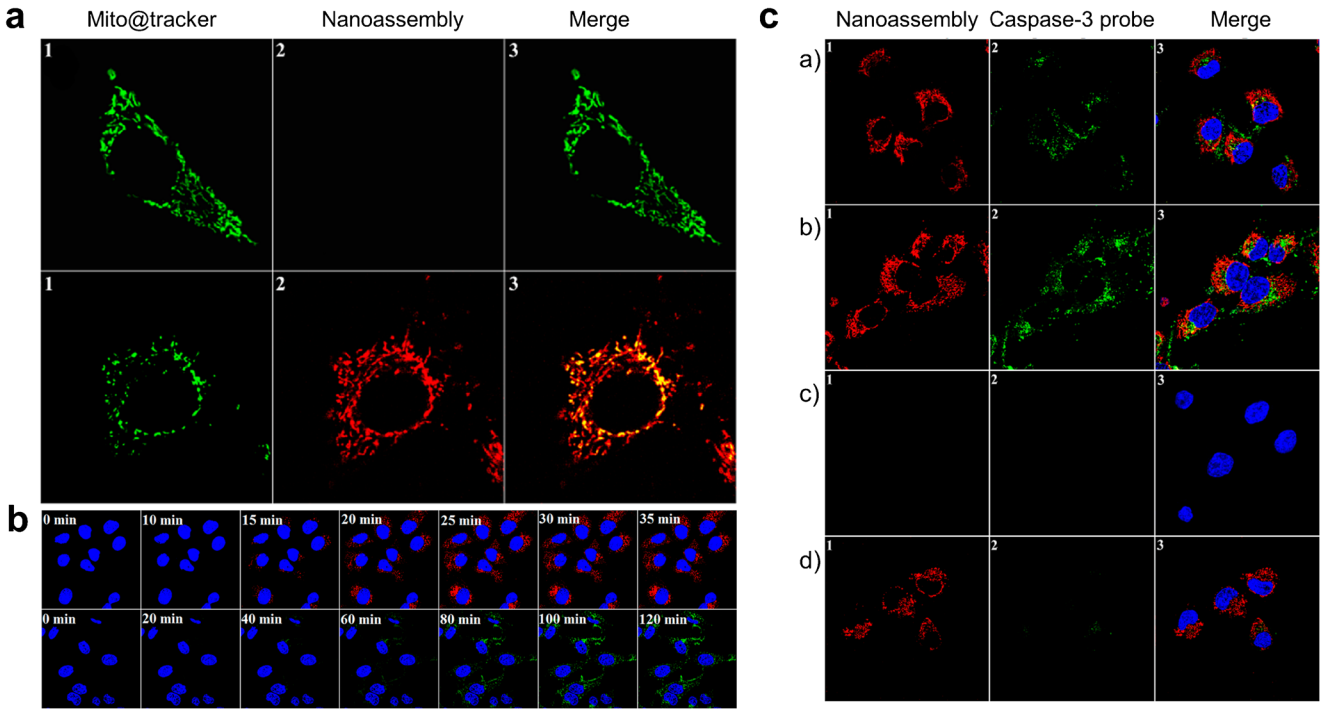



**Fig. 9** Investigating cell apoptosis. (a) Fluorescence imaging of HeLa cells using nanoassembly (graphene/aptamer probes) and Mito@tracker; (b) Real-time fluorescence imaging of cell apoptosis in HeLa cells using nanoassembly (up) and caspase-3 probe (below); (c) Fluorescence imaging of apoptotic signaling: a) HeLa cells (FR+) incubating with nanoassembly and caspase-3 probes followed by 1 μM STS for 100 min; b) Bax overexpressing HeLa cells (FR+) incubating with nanoassembly and caspase-3 probe; c) Bcl-2 overexpressing HeLa cells (FR+) incubating with nanoassembly and caspase-3 probe followed by 1 μM STS for 100 min; d) HeLa cells (FR+) incubating with nanoassembly and caspase-3 probe followed by 100 μM caspase-9 inhibitor I for 1 h and then 1 μM STS for 100 min. Reprinted with permission from Ref. [125]. Copyright 2015 American Chemical Society

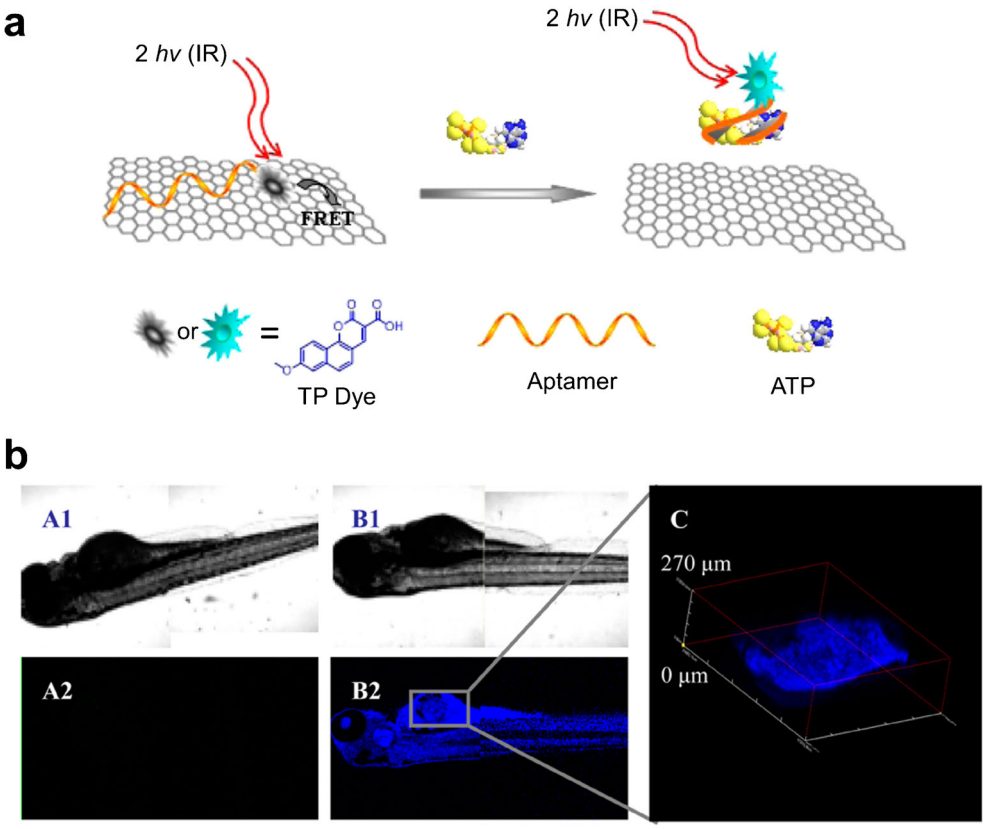



**Fig. 10** *In vivo* imaging of ATP in zebrafishes via the two-photon graphene oxide/aptamer nanosensing conjugates. (a)Working principles of the two-photon graphene oxide/aptamer nanosensing conjugate; (b)Bright-field images and fluorescence images of zebrafishes with the incubation of GO/R-TPdye conjugates and GO/Aptamer-TPdye conjugates. Reprinted with permission from Ref. [122]. Copyright 2014 American Chemical Society

relationship of the metabolism between these molecules (such as ATP and GTP). And the graphene/aptamer probes have been applied to investigate the cell apoptosis process. The ability of rapid response to target enabled real-time imaging of the Cyt c and direct identification of the regulators for apoptosis. Furthermore, the demonstration of imaging ATP in zebrafish implied the likelihood of the application of graphene/aptamer probes for real-time *in vivo* detection of small molecules, which may be utilized to investigate the energy metabolism in muscle, or neural activity in brain.

Despite the noteworthy progress made in developing graphene/aptamer probes for *in vitro* and *in vivo* detection of small molecules, several problems and challenges still exist, especially for *in vivo* detection. Firstly, more attention should be paid to the uniformity of graphene or graphene derives, as the variation of graphene (including the size and the modification state) may exert side effects on the accuracy of quantification. Besides, the non-specific binding of other molecules to graphene would compromise the specificity of aptamers, which may lead to a higher background. The reliable strategy for blocking the surface of graphene is highly demanded in the case of detection in complex samples, such as serum and *in situ* cells.

For the application of *in situ* detection in living cells, the delivery efficiency of probes into cytoplasm should be further improved. The graphene/aptamer probes may be trapped in endosomes, hindering the recognition of small molecules in the cytoplasm (such as ATP). The modification of cell-penetrating peptides or positively charged component may facilitate the entrance of graphene/aptamer probes into the cytoplasm. Besides, up to now, only several species of small molecules such ATP (most probes targeted), GTP and Cyt c can be probed by graphene/aptamer probes. Their application will broaden with the extension of targets. For example, the

**Table 5** Summary of graphene/aptamer probes for *in situ* or *in vivo* detection

| Analyte | Probes | Cell/animal type | application | Ref |
|---|---|---|---|---|
| ATP | GO-nS/aptamer-FAM | mice epithelial (J6B) cells | Real-time imaging ATP in JB6 cells | [124] |
| Cyt c | Aptamer-GO nanoassembly | Hela cells | Investigate the cell apoptosis process | [125] |
| ATP | GO/AAMB | HeLa cells | Semiquantification of ATP in Live Cells | [129] |
| ATP/GTP | aptamer/GO-nS | MCF-7 cells | Multiplexing imaging of small molecules | [120] |
| ATP | GO/ATP aptamer- TPdye | Zebrafishes | Real-time imaging ATP in living zebrafish | [122] |

utilization of graphene/aptamer probes for real-time imaging of mycotoxin in living cells would help us to understand the toxicology of these toxins at the cellular level.

Therefore, the exploration of the full potential of graphene/aptamer probes for small molecules detection, especially *in vivo* detection, requires more attention and continued research support. With these progress, graphene/aptamer probes would facilitate us to understand the roles of small molecules in human health, also benefit the proposal of solutions for environmental issues, medical and food industry.

**Acknowledgements** This work was supported by National Natural Science Foundation of China (No. 21804095), National Science and Technology Major Projects (2018ZX09002008–021), China Postdoctoral Science Foundation (No. 2018 M631079) and the Fundamental Research Funds for the Central Universities (No. 2018SCU12048, No. 1083304121001).

## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

## References

1. McKeague M, Derosa M (2012) Challenges and opportunities for small molecule aptamer development. J Nucleic Acids 14:1–9
2. Yu X, Zhang X, Wang Z, Jiang H, Lv Z, Shen J, Xia G, Wen K (2018) Universal simultaneous multiplex ELISA of small molecules in milk based on dual luciferases. Anal Chim Acta 1001: 125–133
3. Zhang Z, Oni O, Liu J (2017) New insights into a classic aptamer: binding sites, cooperativity and more sensitive adenosine detection. Nucleic Acids Res 45(13):7593–7601
4. Yang K, Barbu M, Halim M, Pallavi P, Kim B, Kolpashchikov D, Pecic S, Taylor S, Worgall T, Stojanovic M (2014) Recognition and sensing of low-epitope targets via ternary complexes with oligonucleotides and synthetic receptors. Nat Chem 6(11):1003–1008
5. Deng R, Dong Y, Xia X, Dai Y, Zhang K, He Q, Zeng W, Ren X, Li J (2018) Recognition-enhanced Metastably shielded Aptamer for digital quantification of small molecules. Anal Chem 90(24): 14347–14354
6. Dunn M, Jimenez R, Chaput J (2017) Analysis of aptamer discovery and technology. Nat Rev Chem 1(10):0076
7. Ma H, Liu J, Ali M, Mahmood M, Labanieh L, Lu M, Iqbal S, Zhang Q, Zhao W, Wan Y (2015) Nucleic acid aptamers in cancer research, diagnosis and therapy. Chem Soc Rev 44(5):1240–1256
8. Deng R, Zhang K, Wang L, Ren X, Sun Y, Li J (2018) DNA-sequence-encoded rolling circle amplicon for single-cell RNA imaging. Chem 4(6):1373–1386
9. Liu M, Song J, Shuang S, Dong C, Brennan J, Li Y (2014) A Graphene-based biosensing platform based on the release of DNA probes and rolling circle amplification. ACS Nano 8(6):5564–5573
10. Zhang L, Wan S, Jiang Y, Wang Y, Fu T, Liu Q, Cao Z, Qiu L, Tan W (2017) Molecular elucidation of disease biomarkers at the interface of chemistry and biology. J Am Chem Soc 139(7):2532–2540
11. Nakatsuka N, Yang K, Abendroth J, Cheung K, Xu X, Yang H, Zhao C, Zhu B, Rim Y, Yang Y, Weiss P, Stojanović M, Andrews A (2018) Aptamer-field-effect transistors overcome Debye length limitations for small-molecule sensing. Science 362(6412):319–324
12. Duan N, Wu S, Dai S, Gu H, Hao L, Ye H, Wang Z (2016) Advances in aptasensors for the detection of food contaminants. Analyst 141(13):3942–3961
13. Li K, Qin W, Li F, Zhao X, Jiang B, Wang K, Deng S, Fan C, Li D (2013) Nanoplasmonic imaging of latent fingerprints and identification of cocaine. Angew Chem Int Edit 52(44):11542–11545
14. Wu S, Liu L, Duan N, Li Q, Zhou Y, Wang Z (2018) Aptamer-based lateral flow test strip for rapid detection of Zearalenone in corn samples. J Agric Food Chem 66(8):1949–1954
15. Li Q, Wang Y, Shen G, Tang H, Yu R, Jiang J (2015) Split aptamer mediated endonuclease amplification for small-molecule detection. Chem Commun 51(20):4196–4199
16. Das J, Cederquist K, Zaragoza A, Lee P, Sargent E, Kelley S (2012) An ultrasensitive universal detector based on neutralizer displacement. Nat Chem 4(8):642–648
17. Xiang Y, Lu Y (2011) Using personal glucose meters and functional DNA sensors to quantify a variety of analytical targets. Nat Chem 3(9):697
18. Zeng H, Zhu Y, Ma L, Xia X, Li Y, Ren Y, Zhao W, Yang H, Deng R (2019) G-quadruplex specific dye-based ratiometric FRET aptasensor for robust and ultrafast detection of toxin. Dyes Pigments 164:35–42
19. Xia X, Wang Y, Yang H, Dong Y, Zhang K, Lu Y, Deng R, He Q (2019) Enzyme-free amplified and ultrafast detection of aflatoxin $B_1$ using dual-terminal proximity aptamer probes. Food Chem 283:32–38
20. Wu K, Ma C, Zhao H, Chen M, Deng Z (2019) Sensitive aptamer-based fluorescene assay for ochratoxin a based on RNase H signal amplification. Food Chem 277:273–278
21. Sheng L, Lu Y, Deng S, Liao X, Zhang K, Ding T, Gao H, Liu D, Deng R, Li J (2019) A transcription aptasensor: amplified, label-free and culture-independent detection of foodborne pathogens via light-up RNA aptamers. Chem Commun 55(68):10096–10099
22. Wu S, Zhang H, Shi Z, Duan N, Fang C, Dai S, Wang Z (2015) Aptamer-based fluorescence biosensor for chloramphenicol determination using upconversion nanoparticles. Toxins 50:597–604
23. Li H, Dauphin-Ducharme P, Ortega G, Plaxco K (2017) Calibration-free electrochemical biosensors supporting accurate molecular measurements directly in undiluted whole blood. J Am Chem Soc 139(32):11207–11213
24. Du Y, Zhen S, Li B, Byrom M, Jiang Y, Ellington A (2016) Engineering signaling aptamers that rely on kinetic rather than equilibrium competition. Anal Chem 88(4):2250–2257
25. Liu M, Zhang W, Zhang Q, Brennan J, Li Y (2015) Biosensing by tandem reactions of structure switching, nucleolytic digestion, and DNA amplification of a DNA assembly. Angew Chem Int Edit 54(33):9637–9641
26. Porchetta A, Vallée-Bélisle A, Plaxco K, Ricci F (2012) Using distal-site mutations and allosteric inhibition to tune, extend, and narrow the useful dynamic range of aptamer-based sensors. J Am Chem Soc 134(51):20601–20604
27. Xia X, Wang H, Yang H, Deng S, Deng R, Dong Y, He Q (2018) Dual-terminal stemmed Aptamer Beacon for label-free detection of Aflatoxin $B_1$ in broad bean paste and Peanut oil via aggregation-induced emission. J Agric Food Chem 66(46): 12431–12438
28. Nguyen V, Kwon Y, Gu M (2017) Aptamer-based environmental biosensors for small molecule contaminants. Curr Opin Biotechnol 45:15–23
29. Xia X, He Q, Dong Y, Deng R, Li J (2020) Aptamer-based homogeneous analysis for food control. Curr Anal Chem 16(1):4–13

30. Carrasquilla C, Kapteyn E, Li Y, Brennan J (2017) Sol-gel-derived biohybrid materials incorporating long-chain DNA aptamers. Angew Chem Int Edit 56(36):10686–10690
31. Yao L, Li Y, Cheng K, Pan D, Xu J, Chen W (2019) Determination of 17β-estradiol by surface-enhanced Raman spectroscopy merged with hybridization chain reaction amplification on au@Ag core-shell nanoparticles. Microchim Acta 186(2):52
32. Ivanovskii A (2012) Graphene-based and graphene-like materials. Russ Chem Rev 81(7):571–605
33. Novoselov K, Geim A, Morozov S, Jiang D, Zhang Y, Dubonos S, Grigorieva I, Firsov A (2004) Electric field effect in atomically thin carbon films. Science 306(5696):666–669
34. Wang L, Zhang Y, Wu A, Wei G (2017) Designed graphene-peptide nanocomposites for biosensor applications: a review. Anal Chim Acta 985:24–40
35. Allen M, Tung V, Kaner R (2010) Honeycomb carbon: a review of Graphene. Chem Rev 110(1):132–145
36. Voiry D, Yang J, Kupferberg J, Fullon R, Lee C, Jeong H, Shin H, Chhowalla M (2016) High-quality graphene via microwave reduction of solution-exfoliated graphene oxide. Science 353(6306):1413–1416
37. Dimiev A, Polson T (2015) Contesting the two-component structural model of graphene oxide and reexamining the chemistry of graphene oxide in basic media. Carbon 93:544–554
38. Zheng P, Wu N (2017) Fluorescence and sensing applications of Graphene oxide and Graphene quantum dots: a review. ChemAsian J 12(18):2343–2353
39. Tang L, Wang Y, Li J (2015) The graphene/nucleic acid nanobiointerface. Chem Soc Rev 44(19):6954–6980
40. Wang Y, Li Z, Wang J, Li J, Lin Y (2011) Graphene and graphene oxide: biofunctionalization and applications in biotechnology. Trends Biotechnol 29(5):205–212
41. Shao Y, Wang J, Wu H, Liu J, Aksay I, Lin Y (2010) Graphene based electrochemical sensors and biosensors: a review. Electroanalysis 22(10):1027–1036
42. Maureen M, Maria C (2012) Challenges and opportunities for small molecule Aptamer development. J Nucleic Acids 2012:748913
43. Zhao Z, Yang H, Zhao W, Deng S, Zhang K, Deng R, He Q, Gao H, Li J (2020) Graphene-nucleic acid biointerface engineered biosensors with tunable dynamic range. J Mater Chem B. https://doi.org/10.1039/C9TB02388G
44. Li D, Zhang W, Yu X, Wang Z, Su Z, Wei G (2016) When biomolecules meet graphene: from molecular level interactions to material design and applications. Nanoscale 8(47):19491–19509
45. Tang L, Chang H, Liu Y, Li J (2012) Duplex DNA/Graphene oxide biointerface: from fundamental understanding to specific enzymatic effects. Adv Funct Mater 22(14):3083–3088
46. Tang L, Wang Y, Liu Y, Li J (2011) DNA-directed self-assembly of Graphene oxide with applications to ultrasensitive oligonucleotide assay. ACS Nano 5(5):3817–3822
47. Sharon E, Liu X, Freeman R, Yehezkeli O, Willner I (2013) Label-free analysis of thrombin or $Hg^{2+}$ ions by nucleic acid-functionalized Graphene oxide matrices assembled on field-effect transistors. Electroanalysis 25(4):851–856
48. Yang Y, Asiri AM, Tang Z, Du D, Lin Y (2013) Graphene based materials for biomedical applications. Mater Today 16(10):365–373
49. Jia Y, Wu F, Liu P, Zhou G, Yu B, Lou X, Xia F (2019) A label-free fluorescent aptasensor for the detection of Aflatoxin B1 in food samples using AIEgens and graphene oxide. Talanta. 198:71–77
50. Lu Z, Chen X, Wang Y, Zheng X, Li C (2015) Aptamer based fluorescence recovery assay for aflatoxin $B_1$ using a quencher system composed of quantum dots and graphene oxide. Microchim Acta 182(3):571–578
51. Lou Y, Peng Y, Luo X, Yang Z, Wang R, Sun D, Li L, Tan Y, Huang J, Cui L (2019) A universal aptasensing platform based on cryonase-assisted signal amplification and graphene oxide induced quenching of the fluorescence of labeled nucleic acid probes: application to the detection of theophylline and ATP. Microchim Acta 186(8):494
52. Yi N, Ke W, Cheng L, Hu J, Qin X (2017) Fluorometric aptamer based determination of adenosine triphosphate based on deoxyribonuclease I-aided target recycling and signal amplification using graphene oxide as a quencher. Microchim Acta 184(6):1847–1854
53. Sheng L, Ren J, Miao Y, Wang J, Wang E (2011) PVP-coated graphene oxide for selective determination of ochratoxin a via quenching fluorescence of free aptamer. Biosens Bioelectron 26(8):3494–3499
54. Pu W, Zhang L, Huang C (2012) Graphene oxide as a nanoplatform for ATP detection based on aptamer chemistry. Anal Methods 4(6):1662–1666
55. Xing X, Liu X, Yue H, Luo Q, Tang H, Pang D (2012) Graphene oxide based fluorescent aptasensor for adenosine deaminase detection using adenosine as the substrate. Biosens Bioelectron 37(1):61–67
56. Qi C, Bing T, Mei H, Yang X, Liu X, Shangguan D (2013) G-quadruplex DNA aptamers for zeatin recognizing. Biosens Bioelectron 41:157–162
57. Bai Y, Feng F, Zhao L, Chen Z, Wang H, Duan Y (2014) A turn-on fluorescent aptasensor for adenosine detection based on split aptamers and graphene oxide. Analyst 139(8):1843–1846
58. Wu K, Ma C, Zhao H, He H, Chen H (2018) Label-free G-quadruplex aptamer fluorescence assay for Ochratoxin a using a thioflavin T probe. Toxins 10(5):198
59. Zhang C, Lin B, Cao Y, Guo M, Yu Y (2017) Fluorescence determination of Omethoate based on a dual strategy for improving sensitivity. J Agr Food Chem 65(14):3065–3073
60. Qiu L, Zhou H, Zhu W, Qiu L, Jiang J, Shen G, Yu R (2013) A novel label-free fluorescence aptamer-based sensor method for cocaine detection based on isothermal circular strand-displacement amplification and graphene oxide absorption. New J Chem 37(12):3998–4003
61. Cheng X, Cen Y, Xu G, Wei F, Shi M, Xu X, Sohail M, Hu Q (2018) Aptamer based fluorometric determination of ATP by exploiting the FRET between carbon dots and graphene oxide. Microchim Acta 185(2):144
62. Lu C, Li J, Lin M, Wang Y, Yang H, Chen X, Chen G (2010) Amplified aptamer-based assay through catalytic recycling of the analyte. Angew Chem Int Ed 49(45):8454–8457
63. Mashhadizadeh M, Naseri N, Mehrgardi M (2016) A digoxin electrochemical aptasensor using Ag nanoparticle decorated graphene oxide. Anal Methods 8(39):7247–7253
64. Zhu L, Liu Y, Yang P, Liu B (2015) Label-free Aptasensor based on Electrodeposition of gold nanoparticles on Graphene and its application in the quantification of adenosine triphosphate. Electrochim Acta 172:88–93
65. Gao L, Xiao Y, Wang Y, Chen X, Zhou B, Yang X (2015) A carboxylated graphene and aptamer nanocomposite-based aptasensor for sensitive and specific detection of hemin. Talanta 132:215–221
66. Eissa S, Ng A, Siaj M, Zourob M (2014) Label-free voltammetric aptasensor for the sensitive detection of microcystin-LR using graphene-modified electrodes. Anal Chem 86(15):7551–7557
67. Feng L, Zhang Z, Ren J, Qu X (2014) Functionalized graphene as sensitive electrochemical label in target-dependent linkage of split aptasensor for dual detection. Biosens Bioelectron 62:52–58
68. Benvidi A, Tezerjani M, Moshtaghiun S, Mazloum-Ardakani M (2016) An aptasensor for tetracycline using a glassy carbon modified with nanosheets of graphene oxide. Microchim Acta 183(5):1797–1804

69. Jiang L, Qian J, Yang X, Yan Y, Liu Q, Wang K, Wang K (2014) Amplified impedimetric aptasensor based on gold nanoparticles covalently bound graphene sheet for the picomolar detection of ochratoxin a. Anal Chim Acta 806:128–135
70. Qian J, Jiang L, Yang X, Yan Y, Mao H, Wang K (2014) Highly sensitive impedimetric aptasensor based on covalent binding of gold nanoparticles on reduced graphene oxide with good dispersity and high density. Analyst 139(21):5587–5593
71. Chen M, Ma C, Zhao H, Yan Y (2019) Exonuclease III-assisted fluorometric aptasensor for the carcinoembryonic antigen using graphene oxide and 2-aminopurine. Microchim Acta 186(8):500
72. Chinnappan R, Alamer S, Eissa S, Rahamn AA, Salah K, Zourob M (2018) Fluorometric graphene oxide-based detection of Salmonella enteritis using a truncated DNA aptamer. Microchim Acta 185(1):61
73. Liu Z, Hua L, Liu L, Su X (2016) Aptamer based lysozyme assay using fluorescent CuInS 2 quantum dots and graphene oxide, and its application to inhibitor screening. Microchim Acta 183(11):1–10
74. Ma L, Guo T, Pan S, Zhang Y (2018) A fluorometric aptasensor for patulin based on the use of magnetized graphene oxide and DNase I-assisted target recycling amplification. Microchim Acta 185(10):487
75. Goud K, Hayat A, Satyanarayana M, Kumar V, Catanante G, Gobi K, Marty J (2017) Aptamer-based zearalenone assay based on the use of a fluorescein label and a functional graphene oxide as a quencher. Microchim Acta 184(11):4401–4408
76. Mousavi S, Nasirizadeh N, Amani J, Halabian R, Imani A (2019) An electrochemical aptasensor for staphylococcal enterotoxin B detection based on reduced graphene oxide and gold nano-urchins. Biosens Bioelectron 127:221–228
77. Qin X, Yin Y, Yu H, Guo W, Pei M (2016) A novel signal amplification strategy of an electrochemical aptasensor for kanamycin, based on thionine functionalized graphene and hierarchical nanoporous PtCu. Biosens Bioelectron 77:752–758
78. Goud K, Hayat A, Catanante G, M S, Gobi K, Marty J (2017) An electrochemical aptasensor based on functionalized graphene oxide assisted electrocatalytic signal amplification of methylene blue for aflatoxin $B_1$ detection. Electrochim Acta 244:96–103
79. Zhang X, Huang C, Jiang Y, Jiang Y, Shen J, Han E (2018) Structure-switching electrochemical aptasensor for single-step and specific detection of trace mercury in dairy products. J Agric Food Chem 66(38):10106–10112
80. Yin J, Guo W, Qin X, Zhao J, Pei M, Ding F (2017) A sensitive electrochemical aptasensor for highly specific detection of streptomycin based on the porous carbon nanorods and multifunctional graphene nanocomposites for signal amplification. Sensor Actuat B-Chem 241:151–159
81. Chen Z, Zhang C, Li X, Ma H, Wan C, Li K, Lin Y (2015) Aptasensor for electrochemical sensing of angiogenin based on electrode modified by cationic polyelectrolyte-functionalized graphene/gold nanoparticles composites. Biosens Bioelectron 65:232–237
82. Yan G, Wang Y, He X, Wang K, Liu J, Du Y (2013) A highly sensitive label-free electrochemical aptasensor for interferon-gamma detection based on graphene controlled assembly and nuclease cleavage-assisted target recycling amplification. Biosens Bioelectron 44:57–63
83. Zhan X, Hu G, Wagberg T, Zhan S, Xu H, Zhou P (2016) Electrochemical aptasensor for tetracycline using a screen-printed carbon electrode modified with an alginate film containing reduced graphene oxide and magnetite ($Fe_3O_4$) nanoparticles. Microchim Acta 183(2):723–729
84. Jing P, Yi H, Xue S, Chai Y, Yuan R, Xu W (2015) A sensitive electrochemical aptasensor based on palladium nanoparticles decorated graphene-molybdenum disulfide flower-like nanocomposites and enzymatic signal amplification. Anal Chem Acta 853:234–241
85. Li G, Li S, Wang Z, Xue Y, Dong C, Zeng J, Huang Y, Liang J, Zhou Z (2018) Label-free electrochemical aptasensor for detection of alpha-fetoprotein based on AFP-aptamer and thionin/reduced graphene oxide/gold nanoparticles. Anal Biochem 547:37–44
86. Liu S, Xing X, Yu J, Lian W, Li J, Cui M, Huang J (2012) A novel label-free electrochemical aptasensor based on graphene-polyaniline composite film for dopamine determination. Biosens Bioelectron 36(1):186–191
87. Zhou L, Wang J, Li D, Li Y (2014) An electrochemical aptasensor based on gold nanoparticles dotted graphene modified glassy carbon electrode for label-free detection of bisphenol a in milk samples. Food Chem 162:34–40
88. Ge L, Wang W, Sun X, Hou T, Li F (2016) Affinity-mediated homogeneous electrochemical Aptasensor on a Graphene platform for ultrasensitive biomolecule detection via exonuclease-assisted target-analog recycling amplification. Anal Chem 88(4):2212–2219
89. Yuan Y, Gou X, Yuan R, Chai Y, Zhuo Y, Ye X, Gan X (2011) Graphene-promoted 3,4,9,10-perylenetetracarboxylic acid nanocomposite as redox probe in label-free electrochemical aptasensor. Biosens Bioelectron 30(1):123–127
90. Bai L, Yuan R, Chai Y, Zhuo Y, Yuan Y, Wang Y (2012) Simultaneous electrochemical detection of multiple analytes based on dual signal amplification of single-walled carbon nanotubes and multi-labeled graphene sheets. Biomaterials 33(4):1090–1096
91. Chen W, Yan C, Cheng L, Yao L, Xue F, Xu J, Bioelectronics (2018) An ultrasensitive signal-on electrochemical aptasensor for ochratoxin a determination based on DNA controlled layer-by-layer assembly of dual gold nanoparticle conjugates. Biosens Bioelectron 117:845–851
92. Mashhadizadeh M, Azhdeh A, Naseri N (2017) 3-Mercapto propionic acid self-assembled on gold nano-particles applied for modification of screen-printed electrode as a new digoxin electrochemical aptasensor using graphene oxide-based signal-on strategy. J Electroanal Chem 787:132–138
93. Wu S, Duan N, Zhang W, Zhao S, Wang Z (2016) Screening and development of DNA aptamers as capture probes for colorimetric detection of patulin. Anal Biochem 508:58–64
94. Zhang S, Wang K, Li J, Li Z, Sun T (2015) Highly efficient colorimetric detection of ATP utilizing a split aptamer target binding strategy and superior catalytic activity of graphene oxide–platinum/gold nanoparticles. RSC Adv 5(92):75746–75752
95. Saha K, Agasti S, Kim C, Li X, Rotello V (2012) Gold nanoparticles in chemical and biological sensing. Chem Rev 112(5):2739–2779
96. Dong Y, Zhang H, Rahman Z, Su L, Chen XHJ, Chen X (2012) Graphene oxide–Fe3O4 magnetic nanocomposites with peroxidase-like activity for colorimetric detection of glucose. Nanoscale 4(13):3969–3976
97. Tao Y, Lin Y, Ren J, Qu X (2013) Self-assembled, functionalized graphene and DNA as a universal platform for colorimetric assays. Biomaterials 34(20):4810–4817
98. Yang Z, Qian J, Yang X, Jiang D, Du X, Wang K, Mao H, Wang K (2015) A facile label-free colorimetric aptasensor for acetamiprid based on the peroxidase-like activity of hemin-functionalized reduced graphene oxide. Biosens Bioelectron 65:39–46
99. Liu M, Zhao H, Chen S, Yu H, Quan X (2012) Interface engineering catalytic Graphene for smart colorimetric biosensing. ACS Nano 6(4):3142–3151
100. Zhang L, Deng H, Lin F, Xu X, Weng S, Liu A, Lin X, Xia X, Chen W (2014) *In Situ* growth of porous platinum nanoparticles

on Graphene oxide for colorimetric detection of Cancer cells. Anal Chem 86(5):2711–2718
101. Abnous K, Danesh N, Ramezani M, Taghdisi S (2017) Colorimetric determination of the microcystin leucine-arginine based on the use of a hairpin aptamer, graphene oxide, and methylene blue acting as an optical probe. Microchim Acta 15:1–7
102. Lin B, Sun Q, Liu K, Lu D, Fu Y, Xu Z, Zhang W (2014) Label-free colorimetric protein assay and logic gates design based on the self-assembly of Hemin-Graphene hybrid Nanosheet. Langmuir 30(8):2144–2151
103. Abnous K, Danesh N, Ramezani M, Emrani A, Taghdisi S (2016) A novel colorimetric sandwich aptasensor based on an indirect competitive enzyme-free method for ultrasensitive detection of chloramphenicol. Biosens Bioelectron 78:80–86
104. Song Y, Yang X, Li Z, Zhao Y, Fan A (2014) Label-free chemiluminescent ATP aptasensor based on graphene oxide and an instantaneous derivatization of guanine bases. Biosens Bioelectron 51: 232–237
105. Sun Y, Lin Y, Ding C, Sun W, Dai Y, Zhu X, Liu H, Luo C (2018) An ultrasensitive and ultraselective chemiluminescence aptasensor for dopamine detection based on aptamers modified magnetic mesoporous silica @ graphite oxide polymers. Sensor Actuat B-Chem 257:312–323
106. Li G, Yu X, Liu D, Liu X, Li F, Cui H (2015) Label-free Electrochemiluminescence Aptasensor for 2,4,6-trinitrotoluene based on bilayer structure of luminescence functionalized Graphene hybrids. Anal Chem 87(21):10976–10981
107. Huang X, Li Y, Zhang X, Zhang X, Chen Y, Gao W (2015) An efficient signal-on aptamer-based biosensor for adenosine triphosphate detection using graphene oxide both as an electrochemical and electrochemiluminescence signal indicator. Analyst 140(17): 6015–6024
108. Lin Y, Li J, Wang Y, Sun Y, Ding C, Sun W, Luo C (2018) A chemiluminescence biosensor for the detection of thrombin based on the aptamer composites. Spectrochim Acta A Mol Biomol Spectrosc 192:153–158
109. Choi H, Lee J (2013) Role of magnetic $Fe_3O_4$ graphene oxide in chemiluminescent aptasensors capable of sensing tumor markers in human serum. Anal Methods 5(24):6964–6968
110. Wang Y, Xu J, Ma C, Li S, Yu J, Ge S, Yan M (2014) A chemiluminescence excited photoelectrochemistry aptamer-device equipped with a tin dioxide quantum dot/reduced graphene oxide nanocomposite modified porous au-paper electrode. J Mater Chem B 2(22):3462–3468
111. Hao L, Gu H, Duan N, Wu S, Ma X, Xia Y, Tao Z, Wang Z (2017) An enhanced chemiluminescence resonance energy transfer aptasensor based on rolling circle amplification and $WS_2$ nanosheet for Staphylococcus aureus detection. Anal Chim Acta 959: 83–90
112. Lin Y, Dai Y, Sun Y, Ding C, Sun W, Zhu X, Liu H, Luo C (2018) A turn-on chemiluminescence biosensor for selective and sensitive detection of adenosine based on HKUST-1 and QDs-luminol-aptamer conjugates. Talanta 182:116–124
113. Khang J, Kim D, Chung K, Lee J (2016) Chemiluminescent aptasensor capable of rapidly quantifying Escherichia Coli O157:H7. Talanta 147:177–183
114. Tian Y, Wang Y, Xu Y, Liu Y, Li D, Fan C (2015) A highly sensitive chemiluminescence sensor for detecting mercury (II) ions: a combination of exonuclease III-aided signal amplification and graphene oxide-assisted background reduction. Sci China Chem 58(3):514–518
115. Jin G, Lu L, Gao X, Li M-J, Qiu B, Lin Z, Yang H, Chen G (2013) Magnetic graphene oxide-based electrochemiluminescent aptasensor for thrombin. Electrochim Acta 89:13–17
116. Wu X, Chai Y, Yuan R, Zhong X, Zhang J (2014) Synthesis of multiwall carbon nanotubes-graphene oxide-thionine-au nanocomposites for electrochemiluminescence detection of cholesterol. Electrochim Acta 129:441–449
117. Kwun J, Yun S, Park L, Lee J (2014) Development of 1,1′-oxalyldiimidazole chemiluminescent biosensor using the combination of graphene oxide and hairpin aptamer and its application. Talanta 119:262–267
118. Georgakilas V, Tiwari J, Kemp K, Perman J, Bourlinos A, Kim K, Zboril R (2016) Noncovalent functionalization of Graphene and Graphene oxide for energy materials, biosensing, catalytic, and biomedical applications. Chem Rev 116(9):5464–5519
119. Qiang W, Hu H, Sun L, Li H, Xu D (2015) Aptamer/Polydopamine Nanospheres Nanocomplex for *in situ* molecular sensing in living cells. Anal Chem 87(24):12190–12196
120. Wang Y, Tang L, Li Z, Lin Y, Li J (2014) *In situ* simultaneous monitoring of ATP and GTP using a graphene oxide nanosheet-based sensing platform in living cells. Nat Protoc 9:1944
121. Wang Y, Li Z, Weber T, Hu D, Lin C, Li J, Lin Y (2013) *In Situ* live cell sensing of multiple nucleotides exploiting DNA/RNA Aptamers and Graphene oxide Nanosheets. Anal Chem 85(14): 6775–6782
122. Yi M, Yang S, Peng Z, Liu C, Li J, Zhong W, Yang R, Tan W (2014) Two-photon Graphene oxide/Aptamer Nanosensing conjugate for *In Vitro* or *In Vivo* molecular probing. Anal Chem 86(7): 3548–3554
123. Liu Z, Chen S, Liu B, Wu J, Zhou Y, He L, Ding J, Liu J (2014) Intracellular detection of ATP using an Aptamer Beacon covalently linked to Graphene oxide resisting nonspecific probe displacement. Anal Chem 86(24):12229–12235
124. Wang Y, Li Z, Hu D, Lin C, Li J, Lin Y (2010) Aptamer/Graphene oxide Nanocomplex for *in situ* molecular probing in living cells. J Am Chem Soc 132(27):9274–9276
125. Chen T, Tian X, Liu C, Ge J, Chu X, Li Y (2015) Fluorescence activation imaging of cytochrome c released from mitochondria using Aptameric Nanosensor. J Am Chem Soc 137(2):982–989
126. Wu Z, Liu G, Yang X, Jiang J (2015) Electrostatic nucleic acid Nanoassembly enables hybridization chain reaction in living cells for ultrasensitive mRNA imaging. J Am Chem Soc 137(21):6829–6836
127. Liu B, Huang Z, Liu J (2018) Polyvalent spherical nucleic acids for universal display of functional DNA with ultrahigh stability. Angew Chem Int Edit 57(30):9439–9442
128. Huang Z, Liu J (2018) Length-dependent Diblock DNA with poly-cytosine (poly-C) as high-affinity anchors on Graphene oxide. Langmuir 34(3):1171–1177
129. Tan X, Chen T, Xiong X, Mao Y, Zhu G, Yasun E, Li C, Zhu Z, Tan W (2012) Semiquantification of ATP in live cells using non-specific desorption of DNA from Graphene oxide as the internal reference. Anal Chem 84(20):8622–8627
130. Ow Y, Green D, Hao Z, Mak T (2008) Cytochrome c: functions beyond respiration. Nat Rev Mol Cell 9:532