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Probing disease-related proteins with fluorogenic composite materials

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Probing disease-related proteins with fluorogenic composite materials

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Construction of composite materials based on the self-assembly of fluorescently labeled biomolecules with a variety of micro- or nano-quenching materials (by the Förster Resonance Energy Transfer mechanism) for the fluorogenic recognition of disease-related proteins has become a dynamic research topic in the field of fluorescence recognition. Here we summarize the recent progress on the composition of fluorescence dye-labeled biomolecules including sugars, peptides and nucleotides with organic (graphene and carbon nanotube) and inorganic (gold nanoparticle) materials. Their application in the fluorescence detection of proteins and enzymes on both the molecular and cellular levels is discussed. Perspectives are proposed with respect to the future directions of employing these composite materials in the recognition of pathological proteins.

Key learning points:

1. Principles to construct fluorogenic composite materials (FCMs)
2. Use of graphene, carbon nanotube and gold nanoparticle as quenching materials
3. Scope of using FCMs for fluorescence detection of disease-related proteins
4. Future improvement and extension of FCMs towards disease theranostic

Introduction

Receptor-type proteins and enzymes, which distribute on the cell membrane or in certain cell organelles, play a pivotal role in numerous physiological processes. For example, the G-protein coupled receptors (GPCRs) found in eukaryotes can recognize and bind to small molecules, peptides and proteins, activating myriads of important signaling pathways within cells. However, over-expression or malfunction of these protein receptors may cause fatal human diseases. An estimation indicates that around 40% of all commercial drugs target a GPCR.[1] Protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) modulate reversibly the phosphorylation of the tyrosine residue of biologically important proteins and peptides.[2] Many small-molecules that quench the catalytic activity of disease-related PTKs have been approved as anticancer drugs. Meanwhile, inhibition of malfunctional PTPs is becoming a promising strategy towards the treatment of a number of human diseases.[3]

Given this evidence, ingenious detection and revelation of the recognition between biomolecules and their pairing disease-related proteins may facilitate not only disease diagnoses but also modern drug research & development. Current laboratory and clinical protocols to probe these sensory events largely depend on the immunofluorescence technique (such as the enzyme-linked immunosorbent assay - ELISA). Despite its modularity, reproducibility and high sensitivity, this technique generally requires onerous and elaborate manipulation procedures, long detection time (repeated incubation, blocking and rinsing steps) and high detection cost (use of expensive antibodies).

As a consequence, alternative methods for probing disease-related proteins with simplified detection procedures have been actively developed in recent years. These including surface plasmon resonance, quartz crystal microbalance, electric field effect and electrochemistry techniques have made possible the detection of *label-free* proteins in a sensitive manner. Nevertheless, these techniques still need derivatization before detection and generally employ costly facilities. In contrast, the fluorescence technique, owing to its high sensitivity and simplicity in manipulation, has extensively been used in the interpretation of chemo- as well as bio-recognition events. It is also a mainstream technique employed for the signal readout of fluorescently labeled proteins in conventional biochemical antibody-based sandwich assays.

Recently, composite materials that consist of fluorescence (FL) dye labeled biomolecules assembled with a quenching material (such as graphene, carbon nanotube and gold nanoparticle) have been developed for the simple *fluorogenic* detection of nucleotides as well as proteins and enzymes (Fig. 1). Comparison with the conventional methods, these materials

are of substantial merit in terms of the following factors: 1, The fluorescently labeled biomolecules can spontaneously adhere to the surface of a material to generate a fluorogenic composite material (FCM) using the Förster Resonance Energy Transfer (FRET) mechanism;[4] 2, These composite materials can be used to detect label-free proteins with short detection time and easy-to-manipulate detection procedures; 3, Clustering biomolecules at a material interface can enhance the binding avidity with proteins, which overcomes the inherent low binding affinity between small molecules and a protein; 4, Since most of these detection strategies are solution-based, live cells and pathogens that express a pathological protein can be captured sensitively in a label-free manner, and even *in vivo*.

Here we summarise the recent progress made with respect to the ingenious detection of disease-related proteins using FCMs. This simple approach may provide promising tools for the advancement of disease theranostics.

General principle for the construction of FCMs

The general principle on which to construct the FCMs depends on the *self-assembly* of fluorophore labeled biomolecules to the surface (which does not need derivatization prior to functionalization) of quenching material including carbon nanotube (CNT), gold nanoparticle (AuNP) and, particularly graphene (Fig. 1). We note that graphene oxide (GO) is commonly used due to its satisfactory water solubility and better biocompatibility. The assembly process is based on non-covalent contacts such as π-stacking, electrostatic interactions and van der waal forces, producing the composite materials in a spontaneous manner.

The FCMs when formed exhibit a quenched FL (OFF) because of the FRET effect between the dye-labeled biomolecule (FRET donor) and quenching material (FRET acceptor). The broad adsorption band of the quenching materials can effectively overlap the emission band of the fluorescence dyes used. Meanwhile, the quantum yields of the materials are so low that no FL emission from the materials is available upon excitation of the dyes.[5]

When a target that selectively recognizes the biomolecule is added, the FL can be recovered (ON). This is due to the fact that the target may competitively form a new complex with (such as a protein receptor) or cleave (such as an enzyme) the dye-labeled biomolecule, resulting in decomposition of the FCMs. Nucleotides and oligopeptides are such widely used biomolecules that can strongly bind to the surface of the materials due to their structural complexity. In contrast, small-molecule ligands such as sugars have insufficient 'binding groups'. Therefore, the covalent attachment of a fluorescence dye may provide not only the detection signal but also a 'binder' to the surface of the materials.

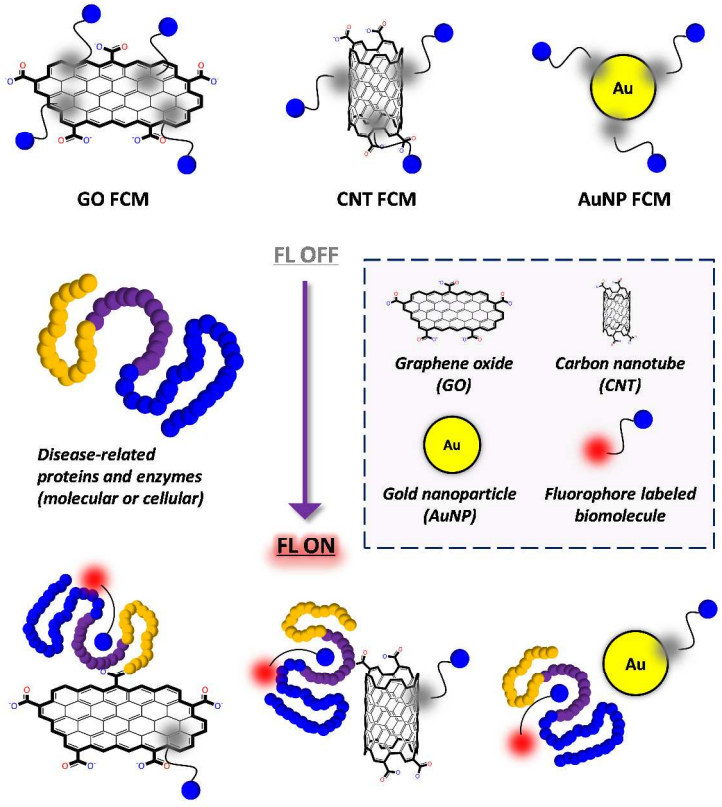

Figure 1 Cartoon depicting the construction of fluorogenic composite materials (FCMs) based on graphene oxide (GO), carbon nanotubes (CNT) and gold nanoparticles (AuNP) for probing disease related proteins via the Förster Resonance Energy Transfer (FRET)-based fluorescence (FL) 'OFF-ON' mechanism. The blue, diffuse red and gray balls represent biomolecule, emissive fluorophore and quenched fluorophore, respectively.

Graphene oxide (GO)-based FCMs

The ideal graphene is a pure atomic thin flake (nano- to micrometers in lateral size) with exceptionally high mechanistic strength and good heat and electric conductivity, as first discovered by the Geim and Novoselov lab.[5] Because of its broad absorbance spectrum, graphene has been proven as a universal quencher of nearly all organic dyes. Its oxidized form, GO, has enhanced water solubility and good biocompatibility compared to the pristine graphene. As a result, GO has received much interest for the development of biomaterials and probes for drug delivery and, especially FL detection of biomolecules. In these systems, GO serves as both the quenching reagent and a platform to cluster the probe molecules, facilitating the sensitivity of the fluorogenic recognition.

GO-FCMs based on protein-ligand binding. Thrombin is a serine protease implicated in the development, metastasis and angiogenesis of tumor cells. Timely monitoring of the protein may give insights into the role of thrombin in cancer biology. The GO-based FCMs (GO-FCMs) for thrombin detection are mainly based on thrombin-aptamer (specific oligo-peptide or -nucleotide based protein, peptide or small molecule binders) interactions (Fig. 2).

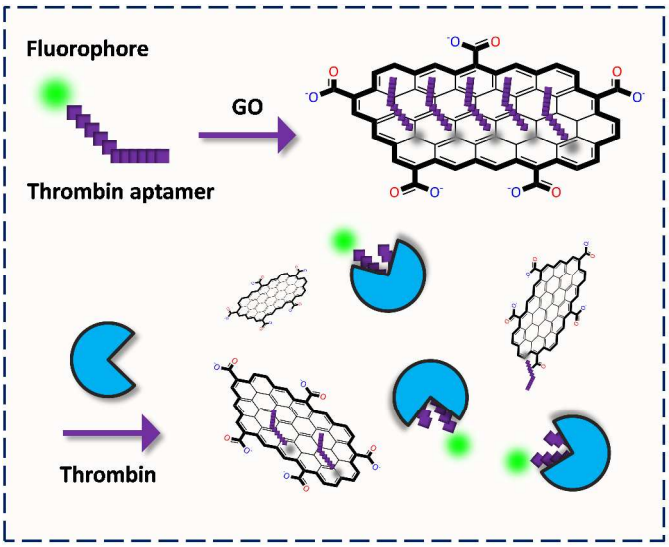

Figure 2 Fluorogenic composite materials based on aptamer covalently labeled with a fluorophore for detection of thrombin.

Yang and co-workers[6] first described an aptasensor fabricated by stacking of a specific human thrombin aptamer labeled with 6-carboxyl fluorescein (FAM) with GO. On the basis of the strong binding affinity between the aptamer and the protein, the GO-FCM could sensitively and selectively detect thrombin with a limit of detection (LOD) of 2 nM. Then Li and co-workers[7] reported a similar aptamer based GO-FCM that achieved picomolar detection of thrombin in phosphate buffered saline (PBS), goat serum and bovine serum. Both studies proved that using GO as a quenching platform is superior in terms of sensitivity to using CNT. This could be possibly reasoned by the broader contact surface of GO (two-dimensional) than CNT (one-dimensional) for binding dye-labeled biomolecules, thereby enhancing the aptamer-protein interaction. The use of aptamers as a precursor to composite with GO for biorecognition opens an effective venue for the fluorescence detection of proteins.

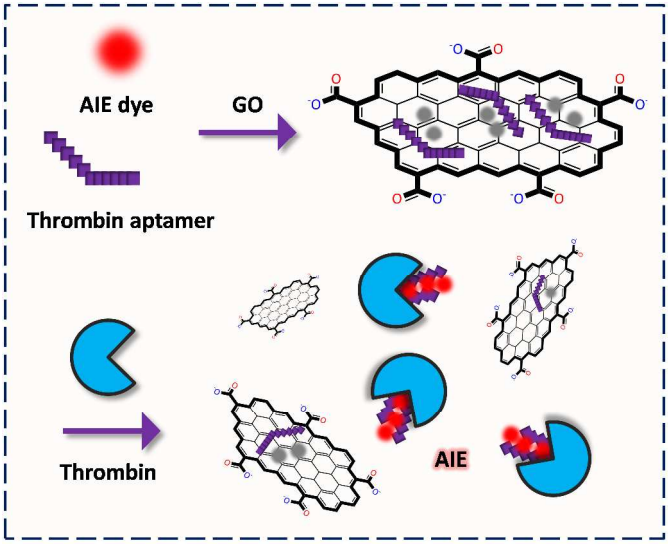

Figure 3 Fluorogenic composite materials based on the composition of label-free aptamer, an aggregation-induced-emission (AIE) fluorophore and GO for detection of thrombin.

Bi and co-workers[8] presented a strategy employing the chemiluminescence resonance energy transfer (CRET) between a luminol-H_2O_2-horseradish peroxidase complex and the FAM dye. Upon thrombin-aptamer binding, the FL could be recovered with a satisfactory LOD of 0.1 pM. This strategy takes advantage of the activation of a luminescent substrate without the requirement of external light excitation. And the reaction-based detection protocol provides the most sensitive GO-FCM so far for thrombin detection. Hah and co-workers[9] demonstrated the applicability of peptide nucleic acids (PNAs) as aptamers in the construction of GO-FCM for thrombin. Ye and co-workers[10] developed a molecular beacon-like FCM consisting of three ss-DNAs tagged with different FL dyes for the simultaneous sensing of thrombin, sequence-specific DNA and heavy metal ions. This study proves that GO can be used as a versatile central platform for the concomitant binding and recognition of multiple targets.

Subsequently, two independent groups revealed new criteria for developing GO-FCM based thrombin probes. On the one hand, an aggregation-induced-emission (AIE)-based[11] GO-FCM aptasensor was developed by Tian and co-workers.[12] In this system the quenched FL of a free AIE dye indicator originally stacked on GO could be released upon intercalation into the quadruplex aptamer-thrombin complex (Fig. 3). Notably, the detection does not require the labeling of either the analyte or the bio-ligand, facilitating a 'double-label-free' detection criterion for thrombin as well as for other proteins. Indeed, the AIE mechanism, being opposite to the conventional aggregation-caused-quenching, has emerged as a promising tool for the design of fluorogenic biosensors. AIE-based sensors produce a

specific FL 'OFF-ON' signal upon aggregation with the analyte, which does not require extensive synthetic efforts of the sensors.

On the other hand, an interesting solid-support detection of thrombin was devised by Furukawa and co-workers.[13] They conjugated the FAM-labeled aptamer to pyrene that is a strong 'binder' to the surface of GO, and the GO-FCM was further deposited onto a silicon surface. Upon recognition, the FL of FAM was recovered without detaching from the surface (due to the presence of the strong pyrene binder), as visualized by atom force microscope (AFM). This system was successfully applied in the microchannel detection of thrombin, paving the way for future solid-phase high-throughput monitoring of the protein.

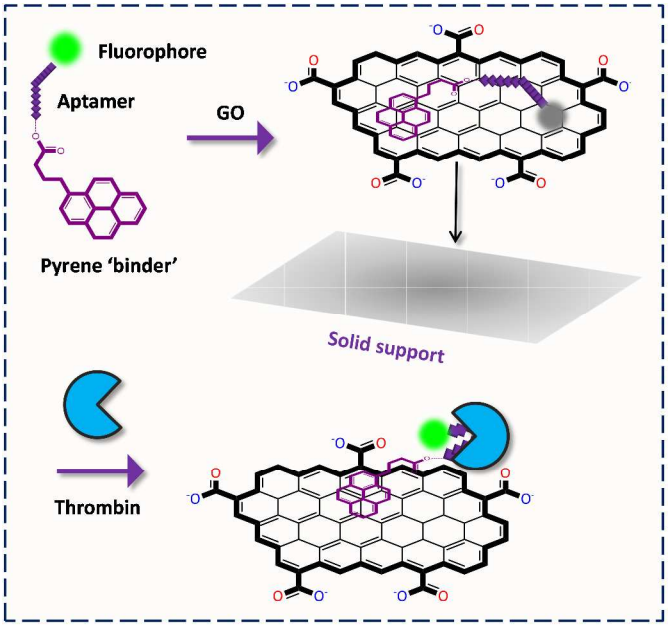

Figure 4 Thrombin detection on a solid support without detachment of the fluorophore-labeled biomolecule from the GO surface..

Detection of other disease-related proteins using the versatile GO-FCMs was also achieved. Yang and co-workers[14] developed a general pyrene-conjugated peptide-GO FCM for the detection of the antibody of a glycoprotein gp120 that is pivotal for HIV-1 invasion. An aptasensor was developed by Li and co-workers[15] for the detection of an oncoprotein, platelet derived growth factor-BB (PDGF-BB), that regulates growth and division of tumor cells. This FCM could selectively discriminate between the highly homogenous PDGF-BB and -AA.

Qu and co-workers[16] constructed a GO-FCM that consists of a fluorescein isothiocyanate (FITC)-labeled peptide ligand for sensing a prognostic indicator of early-stage cancer, cyclin A_2 (Fig. 5). This platform was manifested to be 1200-fold more sensitive than a well-known Tb^{3+} chelating macrocycle-based probe and 10-fold better than a CNT-based probe. Alzheimer's disease (AD) is currently an incurable and irreversible disease affecting millions of people every year. The same researchers reported the construction of a GO-FCM composed of thioflavin-S (ThS) that is a clinical staining dye of the amyloid β (Aβ) fibrils toxic to neuronal cells of AD patients.[17] Complexation of Aβ with the material activated the FL of ThS. Subsequently, near infrared (NIR) irradiation quenched the FL by dissociating the Aβ fibrils because of the strong NIR absorption ability of nano-GO, producing a localised temperature increase. The FCM was demonstrated to be useful in complex cerebrospinal fluid. This study exemplifies the theranostic potential of GO-FCMs for real biological samples.

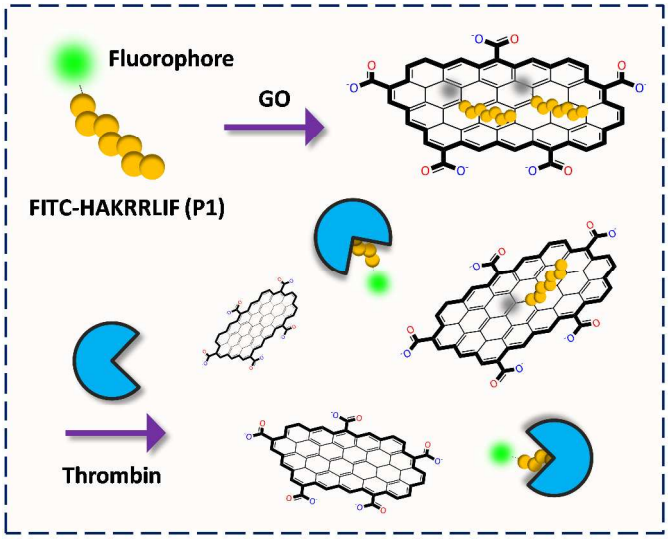

Figure 5 Cyclin A_2 detection using GO composited with a fluorophore-labeled peptide (**P1**).

We reported the fabrication of a resveratrol (a natural product abundantly found in the red wine to bind selectively with Aβ) confined GO-FCM for the sensitive detection of both Aβ monomers and fibrils (Fig. 6).[18] The composite material was used to stain senile plaques in mice brain sections. Comparison with the conventional immunofluorescence antibody staining, the cost of the former was lowered by more than 1000-fold and the detection time lowered by more than 20-fold. We also fabricated a FCM formed between boronate receptors and GO for the selective detection of fructose, offering a new technique for probing disease-related glycoproteins.[19]

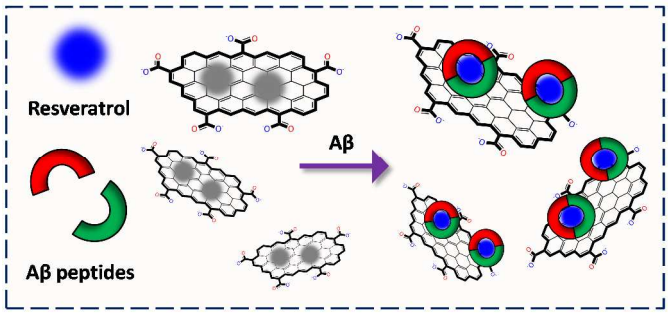

Figure 6 Detection of Aβ peptides using resveratrol-confined GO.

GO-FCMs for live cells. The potential of the FCMs for clinical use has been well-supported by some recent investigations

using the material for probing receptor proteins or endogeneous species expressed by a live cell.

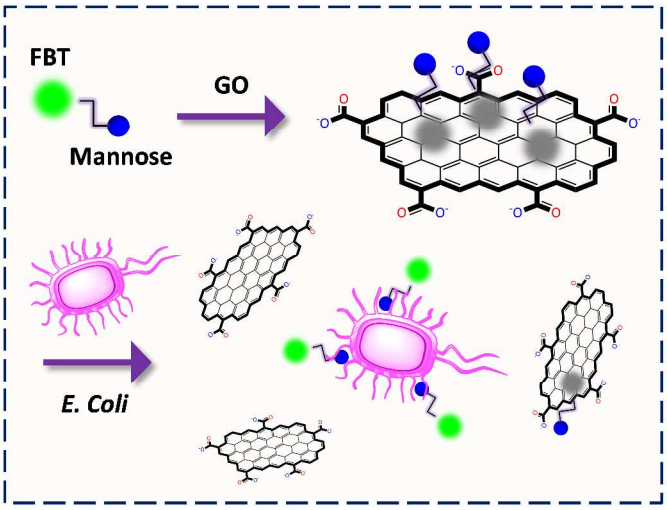

Figure 7 Capturing *E. Coli* expressing mannose receptors by a GO-FCM confined with mannoside labelled with an FBT polymer.

Sugars found on the cell membrane are significant signaling molecules modulating a number of biological as well as patholgical events through interaction with their pairing receptors (lectins). Ingenious detection of sugar-lectin interactions at the cellular level may offer invaluable tools for deciphering glycomics.[20,21]

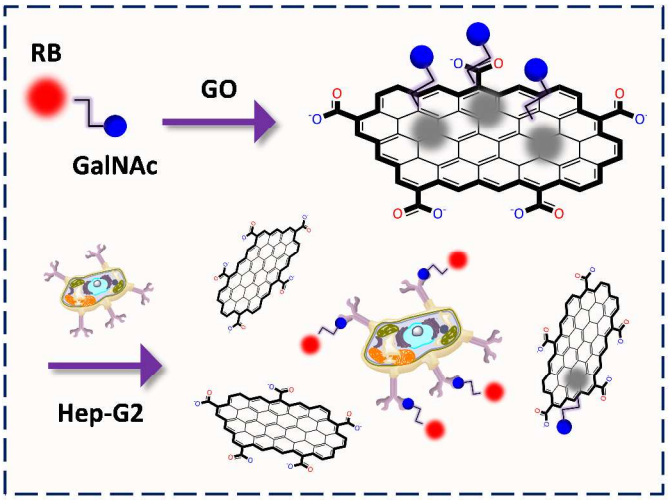

Figure 8 Capturing Hep-G2 expressing the asialoglycoprotein receptors by a GO-FCM confined with GalNAc-rhodamine B (RB).

Liu and co-workers[22] customised a tetrameric mannoside tagged with a neutral benzothiadiazole conjugated polymer, FBT. The glycopolymer itself responded to a lectin with increased FL probably due to a change of the micro-environment from hydrophilic to hydrophobic. In contrast, composition of which with GO led to full suppression of the strong background FL of the compound and, notably, improvement of the sensitivity. More importantly, the FCM could detect an *E. Coli* (a gram-negative bacterium) stain that expresses a mannose receptor (MG1655) in a fluorogenic manner while showing slight response to that without expression of the receptor (Top 10). This paves the way for FCM-based detection of microbes.

We described the preparation of an *N*-acetyl-galactosyl rhodamine B (GalNAc-RB) confined FCM for the fluorogenic probing of the asialoglycoprotein receptors (galactoside-selective) expressed on the membrane of a hepatoma cell, Hep-G2.[23] The RB dye moiety acts not only as a reporter unit but also as a binder facilitating the clustering of the non-aromatic GalNAc to GO surface probably by, π-stacking and electrostatic interactions. We then observed that change of the sugar to *N*-acetyl-glucoside (GlcNAc) as well as knockdown of the receptor caused obvious decrease of the FL signal. This unravels the promise of using these materials for the selective analysis of sugar-lectin interactions at the cellular level. Notably, conventional techniques are unable to sense live cells since the cells need lysis prior to detection. This drawback has been overcome using the GO-FCMs due to their ability to spontaneously produce the detection signal upon recognition.

Peptide-based FCMs were also constructed to probe receptor-type biomarkers on the surface of cancer cells. Chen and co-workers[24] prepared a pyrene-tagged RGD cyclic peptide confined FCM that showed sharp fluorogenic response to MDA-MB-435, a cancer cell line that over-expresses integrin which binds the peptide. Negligible response to MCF-7 cells with low integrin expression level. FCM confined with FITC-tagged octreotide was developed by Huang and co-workers[25] to target the somatostatin receptor subtype 2 over-expressed on AR42J cancer cells. In contrast, the FCM showed no FL response to a control CHO cell line due to the lack of the cancer-specific receptor. These studies demonstrate that the fluorogenic detection is dependent on the selective ligand-receptor interactions, even with live cells.

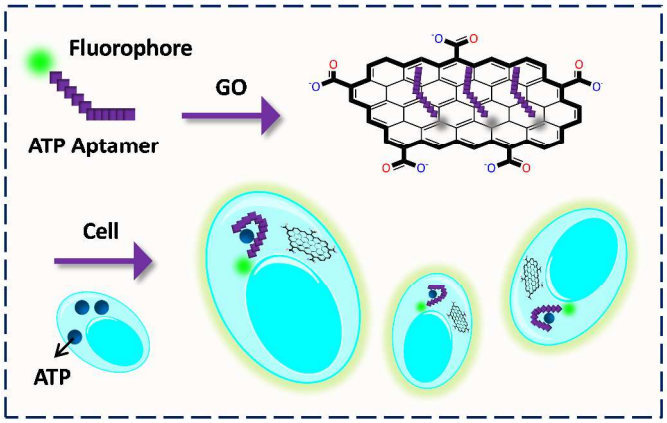

Figure 9 FCM confined with FAM-aptamer for intracellular probing of ATP.

In addition to the transmembrane proteins, Lin and co-workers[26] prepared an FCM confined with ATP aptamers for probing ATP within cells. They demonstrated that GO in this system could serve as an efficient cargo to deliver the aptamer into mice epithelial cells. The FL of FAM then occurred by binding to the intracellular ATP. Also, GO was shown to protect the aptamer from being cleaved during the process of entering the cell, providing a new tool for the tracking of endogeneous species expressed by pathological cells.

GO-FCMs based on enzyme cleavage. Enzymes are another important class of proteins that catalyze and accelerate metabolic processes in the life cycle. However, the over-expression or malfunction of certain enzymes are closely implicated in the pathogenesis of human diseases. A recent review written by Min and co-workers[27] has highlighted comprehensively the development of GO-FCMs for probing the activity of various enzymes including nucleases, methyltransferases, protein kinases and helicases.

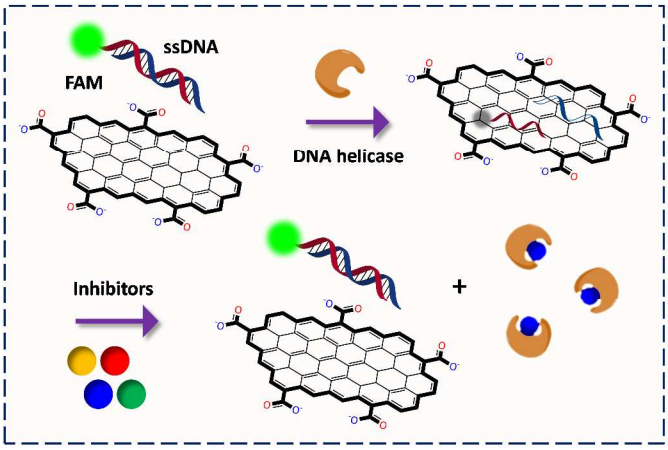

Figure 10 FCM for the fluorogenic screening of helicase inhibitors.

While the general detection principal relies on the cleavage/release of the fluorophore-labeled portion of an enzyme substrate from GO, some pioneering work is noteworthy. The first GO-FCM based enzymatic activity assay was developed by Min and co-workers[28] for the detection of helicase using GO mixed with FAM-labeled dsDNA. Once unwound by the enzyme, the resulting ssDNAs with or without FAM labelling could adsorb onto the GO surface, quenching the FL. The authors then exploited this 'ON-OFF' mechanism to screen inhibitors of helicase in a fluorogenic manner (since competitive binding of the inhibitor with the enzyme weakened the unwinding of the fluorescent dsDNA, Fig. 10). Comparison with the conventional ^{32}P based helicase assay, use of the GO-FCM is advantageous for its conciseness in manipulation, low cost, high shelf-stability and potential to be used in high-throughput drug screening.

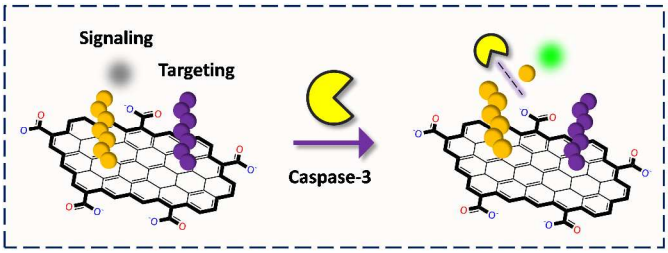

Figure 11 Covalently linked GO-peptides for *in vivo* detection of caspase-3.

Then the same group reported an extended study on the construction of a GO-FCM tethered with two ssDNAs labeled with different fluorophores for the simultaneous detection of two helicases of hepatitis C virus.[29] With this platform concurrent fluorogenic screening of drug candidates of the two pathological enzymes was realised. Ye and co-workers[30] demonstrated the first non-ssDNA based GO-FCM confined with a peptide. Once cleaved by thrombin the FL was released with nanomolar LOD.

Chu and co-workers[31] developed a covalent tactic by linking two different peptides to the surface or edge carboxylic acids of GO through the amide bond (Fig. 11). One FAM-labeled peptide served as substrate of caspase-3, a central mediator for initiation and propagation of apoptosis. The other served as a targeting reagent for cancer cells. The authors observed that the covalent GO-peptide conjugate could fluorescently image cancer cells treated with an apoptosis inducer (to overexpress caspase-3). Interestingly, a non-covalent complex of GO-peptide did not produce any FL, implying that the peptides without coupling to GO was not stable enough to be carried into the cells in this case.

Prostate-specific antigen (PSA) exists in the body fluid as a biomarker for prostate cancer. Ma and co-workers[32] stacked an FITC-labelled peptide that can be degraded by proteolytically active PSA with GO. Thus, a GO-FCM was produced for quantification of a urine sample spiked with PSA. This provides a more rapid and economic tool for the simple clinical determination of a cancer biomarker than the conventional ELISA-based methods. Exonuclease III (Exo III)-aided signal amplification for detection of lysozyme (Lys), an enzyme associated with a range of diseases including monocytic leukemia, was developed by Yu and co-workers.[33] In the strategy, Exo III served as a cleaver for the exposed single-stranded tail sequence of a hairpin probe aptamer-Lys complex. Recyclable aptamer-Lys fluorogenic interactions were thus made possible to improve the sensitivity.

Protein tyrosine phosphatases (PTPs) participate in the pathogenesis of various fatal human diseases. Li and co-workers[34] stacked an FITC-labelled phospho-peptide with GO for evaluation of the catalytic activity of PTP1B (the first identified PTP member related to type II diabetes and breast cancer). In the absence of PTP1B the peptide on the GO surface could not be degraded by chymotrypsin due to existence of the phosphate 'blocker'. However, presence of which led to substrate dephosphorylation, and thus cleavage of the peptide releasing the fluorophore. This research provides a new chemical tool for probing the PTP pathology and facilitating PTP-based drug discovery.[3]

Carbon nanotube (CNT)-based FCMs

Carbon nanotubes can be viewed morphologically as a curled graphene flake. These single-walled (SW) or multi-walled cylindrical nano-carbon allotropes possess unique properties suited for use in nanotechnology.[35] Photophysical investigations reveal that SWNTs are general quenching materials for FL dyes. Despite this, CNT-FCMs have been developed for the analyses of DNA and RNA, whereas relatively less examples were available for detection of disease-related proteins.[36,37]

The pioneering work by Tan and co-workers[38] described an FCM formed between an FAM-thrombin aptamer and SWNT. The fluorescent oligonucleotide-based aptamer could twine against the wall of the NT to quench the FL, whereas binding of thrombin with the aptamer recovered the FL (Fig. 12). Xu and co-workers devised SWNT-based *dahlia*-like single-walled car-

bon nanohorn (SWCNH) as a quenching platform to produce CNT-FCMs. This unique FCM composed of FAM-aptamers has been used to detect thrombin based on either cleavage-[39] or binding-induced[40] FL recovery with pM LOD. The satisfactorily low LOD could be a result of the exceptional morphological effect of the SWCNH. These studies also highlight the importance of structural effect on the activity of carbon material-based FCMs.

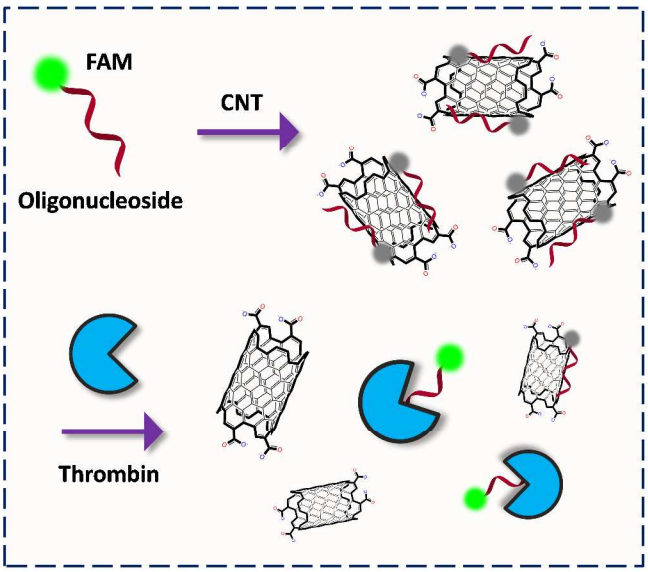

Figure 12 Single-walled carbon-nanotube (SWNT) based probe for the fluorogenic detection of thrombin..

Liang and co-workers[41] developed a multicolor CNT-based nanoprobe for the simultaneous detection of three cancer-related proteases. The peptides labeled separately with three different organic dyes were covalently linked to the CNT by amidation, with quenched FL. The probe showed excellent sensitivity with low interference from competing analytes towards the multicolor detection of the enzymes (Fig. 13). This study reveals that, similar to GO, CNT can also serve as a versatile platform to bind multiple biomolecules for multi-target biosensing.

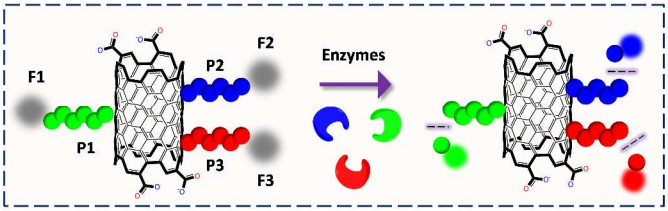

Figure 13 SWNT FCM functionalized covalently with three peptides (P) labelled with different fluorophores (F).

Gold nanoparticle (AuNP)-based FCMs

Gold nanoparticles (AuNPs) are a class of inorganic nanomaterials broadly employed in the construction of chemo- and bio-probes. The simplest detection criterion employing the AuNPs is the 'naked-eye' colorimetric approach based on the analyte-modulated dispersion and aggregation of the modified particles. Localized surface plasmon resonance is another widely exploited property of AuNP for ultrasensitive molecular sensing.[42] Given that they are good quenchers of organic dyes, AuNP-FCMs were developed for FL analysis of proteins and enzymes.

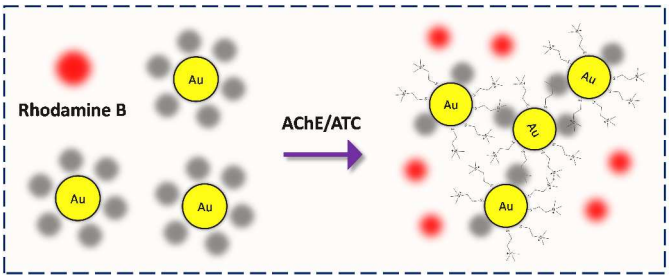

Figure 14 Rhodamine B-attached AuNP FCM for the detection of acetylcholinesterase (AChE) that deacetylatesacetylthiocholine (ATC).

Jiang and co-workers[43] took advantage of the electrostatic interaction between RB and citrate-coated AuNP to produce a RB-coated FCM for the detection of acetylcholinesterase (AChE) in the cerebrospinal fluid of AD transgenic mice. AChE could cleave acetylthiocholine to generate positively charged thiocholine that replaces the RB attached on the particle surface, releasing the FL. This fluorogenic detection was also accompanied by a sharp colour change (Fig. 14). This study provides a dual-signal detection criterion using the unique optical properties of AuNP.

A similar replacement-based immunoassay was developed by Zhao and co-workers.[44] Replacement of an FTIC-labeled antigen by the analyte antigen in a serum sample led to fluorogenic determination of immunoglobulin M (IgM) with picomolar LOD. Liang and co-workers[45] presented a strategy by functionalising a single AuNP with three different antibodies tagged with different organic dyes. The FCM was able to generate multicolor FL signals in both solution and human serum samples by competitive surface binding of the analyte peptides against that of dye-labeled peptides.

AuNP covalently functionalised with a protease substrate labeled *via* a near infrared (NIR) dye, Cy5.5, was prepared by Ahn and co-workers (Fig. 15).[46] This material was used to detect the enzymatic activity of matrix metalloprotease (MMP) which plays a key role in promoting cancer progression. Notably, due to the NIR emission, the FCM was further applied for the sensitive monitoring of MMP activity in a tumor-bearing mice model with or without treatment with an inhibitor. This suggests the applicability and promise of AuNP-FCMs for *in vivo* theranostics.

Human telomerase in cancer cells cannot be regularly shortened, leading to indefinite division of cells. In metastatic cancer tissues, over-expression of the enzyme is detected. Ju and co-workers[47] designed a molecular beacon containing a nick dividing the beacon in to a shorter telomerase primer sequence paring partially with a Cy5-labelled longer sequence that forms a loop on AuNP. In the presence of telomerase the primer was elongated from the 3' end producing a repeated sequence complementary to the 3' end stem of the longer sequence. This caused substantial hybridization which unfolds the loop, and turns on the FL.

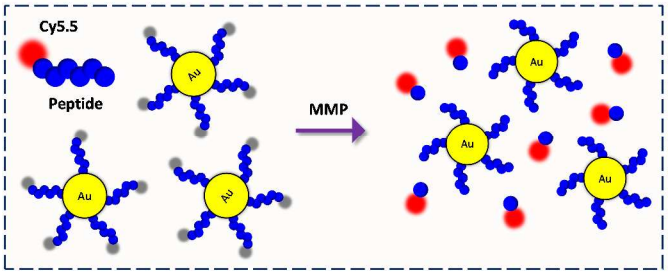

Figure 15 AuNP-FCM for detection of matrix metalloprotease (MMP).

Summary and perspective

The rapid development of modern nanotechnology has made possible the fruitful production of organic and inorganic nano-material-based devices and probes for addressing biological systems. This review summarises recent progression of the use of fluorogenic composite materials (FCMs) for probing disease-related proteins. Three general quenching materials for organic dyes, i.e. gold nano-particle (AuNP), carbon nanotubes (CNT) and, graphene oxide (GO, ranging from nano- to micro-size) have all been shown to be suitable building blocks for the construction of FCM probes. The materials serve both as a quencher and as a platform that clusters dye-labelled biomolecules (or in some cases provides a cell-permeable carrier) for the detection of an analyte protein with high sensitivity and selectivity. Because of the solution-based detection rationale, proteins expressed on the cell surface, within cells as well as in living animals can be captured by these FCMs.

Despite the promise of the FCMs, some important problems still need to be overcome in future systems. Firstly, the particle size and structural effect on the activity of FCMs is always neglected, unfortunately. Huang and co-workers[48] revealed in a recent study that GO of different nano-sizes impacted significantly on the quenching of fluorescent biomolecules as well as the sensing performance of the resulting FCMs for a metal ion. This interesting phenomenon was also observed during our recent research regarding GO-FCMs for detection of sugar-lectin interactions on both the molecular and cellular levels (unpublished results). As a consequence, we propose that the size and structural effects should be investigated and optimized in future studies where FCMs are developed. Likewise, the relationship between the oxygen content of the carbon materials and their sensing performance for an analyte should be elaborated.

Secondly, a number of previous studies have suggested that the two-dimensional GO has better sensitivity than the one-dimensional CNT in bio-sensing, and even superior to the zero-dimensional AuNP.[49] However, the fundamental rationale behind these observations, other than the intuitive morphological distinction, should be delineated. Furthermore, unlike oligo-peptides and -nucleosides, small-molecule ligands such as monosaccharides and oligosaccharides can hardly self-assemble to the surface of the carbon materials due to the absence of sufficient 'binding' groups (such as aromatic groups). In these specific cases, the choice and the coupling manner of a dye with the ligand should be carefully designed, since both factors may impact the self-assembing efficiency. In addition to boronate-based receptors,[19,50] we also envisage the use of sugar aptamers in the future development of FCMs for probing glycomes in the body fluid and on the surface of cells and tissues.

Last but not the least, the majority of the currently developed GO and CNT-based FCMs dissemble upon recognition to produce the fluorogenic signal, especially in the detection of receptor-ligand associations. This fact is a result of the fragile non-covalent attachment of the dye-labelled biomolecules with the materials *via* non-specific absorptions. Although this composition manner is simple, one cannot imagine its stability and hence suitability with complex biological systems (such as in cells and *in vivo*). Covalent functionalization of fluorescent bio-ligands to the carbon materials is certainly a good solution, but will increase the fabrication cost and complexity of the probe. Alternatively, mimicking the solid gold-thiol bonding in fabricating AuNP-FCMs, introduction of an additional 'binder' that strongly immobilizes the ligands to the surface of GO or CNT could prove a promising strategy. Pyrene owing to its planarity and aromaticity might be a good choice as exemplified by Furukawa and co-workers.[13]

Although there remain a number of problems to be resolved for the real application of these next-generation materials, we are confident that the first commercial FCM for clinical disease diagnosis or for industrial high-throughput drug discovery will be achieved in the near future.

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