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# A fluorescence turn-on biosensor based on graphene quantum dots (GQDs) and molybdenum disulfide ($MoS_2$) nanosheets for epithelial cell adhesion molecule (EpCAM) protein detection

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**Abstract**

This paper presents a "turn-on" fluorescence biosensor based on graphene quantum dots (GQDs) and molybdenum disulfide ($MoS_2$) nanosheets for rapid and sensitive detection of epithelial cell adhesion molecule (EpCAM) proteins. PEGylated GQDs were used as donor molecules, which could not only largely increase emission intensity but also prevent non-specific adsorption of PEGylated GQD on $MoS_2$ surface. The sensing platform was realized by adsorption of PEGylated GQD labelled EpCAM aptamer onto $MoS_2$ surface via van der Waals force. The fluorescence signal of GQD was then quenched by $MoS_2$ nanosheets via fluorescence resonance energy transfer (FRET) mechanism. In the presence of EpCAM protein, the stronger specific affinity interaction between aptamer and EpCAM protein could detach GQD labelled EpCAM aptamer from $MoS_2$ nanosheets, leading to the restoration of fluorescence intensity. By monitoring the change of fluorescence signal, the target EpCAM protein could be detected sensitively and selectively with a linear detection range from 3 nM to 53 nM. The limit of detection (LOD) of this FRET biosensor was around 3 nM.

**Keywords:** graphene quantum dots (GQDs), molybdenum disulfide ($MoS_2$), fluorescence resonance energy transfer (FRET), epithelial cell adhesion molecule (EpCAM)

## 1. Introduction

Epithelial cell adhesion molecule (EpCAM) is a glycosylated membrane protein expressed on the surface of circulating tumor cells (CTCs) (Baeuerle and Gires, 2007). EpCAM protein is considered to be the most frequently and intensely studied tumor-associated antigens because of its overexpression in most cancer cells, including colorectal cancer (Dalerba et al., 2007), breast cancer (Gastl et al., 2000), gallbladder cancer (Varga et al., 2004), pancreatic cancer (Fong et al., 2008) and liver cancer (Yamashita et al., 2010). In a perspective view, EpCAM protein has been regarded as a prognostic tumor biomarker for cancer diagnosis, prognosis and therapy (Baeuerle and Gires, 2007). Previously, cytometry technique, polymerase chain reaction (PCR), or a combination or both approaches have been widely used for EpCAM based CTCs detection (Ntouroupi et al., 2008; Lambrechts et al., 1999). However, these methods are all suffered from the disadvantages of long analytical time, labor-intensive operation and expensive instruments, which hamper the development of point of care diagnosis (Ntouroupi et al., 2008; Lambrechts et al., 1999; Gubala et al., 2011). Besides, the majority of EpCAM-based diagnostic and therapeutic approaches are relied on anti-EpCAM antibody, which fails to provide objective clinical response because of the large size and instability in physiological environment (Schwartzberg, 2001; Armstrong and Eck, 2003). Therefore, small sized aptamer with the merits of ease of synthesis, good stability, fast tissue penetration and low toxicity has raised much attention as a perfect alternative of antibody (Tan et al.,

2013). The specific affinity interaction between aptamer and different biomolecular targets also stimulates the extensively application of aptasensors (Song et al., 2013).

Fluorescence resonance energy transfer (FRET), relying on the energy transfer between donor and acceptor, is a powerful tool to monitor biomolecular interactions in nano-scale. The FRET based aptasensor is very promising due to its direct response and feasible quantification (Choi et al., 2006; Bagalkot et al., 2007). Traditional FRET pairs, such as fluorescent dyes and proteins, are mainly limited by poor photobleaching resistance and low chemical stability. Some novel nanoparticles, such as semiconductor quantum dots (QDs) (Zhang et al., 2005) and upconversion nanoparticles (UCNPs) (Ye et al., 2014), are photo-stable fluorescence probes but hampered for biological applications by its high toxicity. The emergence of graphene quantum dots (GQDs) as fluorescence probes perfectly solves these problems (Sun et al., 2013). GQDs are well-confined 0D graphene fragments with high brightness, good photo-stability and excellent biocompatibility, which ensure their application as FRET donors in long-term bio-detection (Zhu et al., 2012; Shi et al., 2015a).

In addition, graphene oxide (GO), the water-soluble graphene derivative equipped with super quenching ability, has been used as efficient fluorescence acceptors in FRET assay (Shi et al., 2015b). Recently, much focus has been paid to other 2D materials such as transition metal dichalcogenides (TMD) due their graphene analogous structure (Xu et al., 2013; Yang et al., 2015). Among them, molybdenum disulfide ($MoS_2$) has attracted tremendous attention due to its easy exploitation, unique electronic and optical properties. Single-layered $MoS_2$ nanosheet is made up of

a hexagonal layer of molybdenum atoms sandwiched between two layers of sulfur atoms (Ataca et al., 2011). Due to its high quenching capability and good biocompatibility, $MoS_2$ nanosheets started to be used as quenchers in FRET assays for biosensing applications. Zhu et al. firstly reported the high fluorescence quenching capability of $MoS_2$ nanosheet for fluorescent dye labelled ssDNA and demonstrated the different affinity to ssDNA versus dsDNA (Zhu et al.,2013). After that, $MoS_2$ nanosheet based FRET platforms have been used for protein detection including DNA methyltransferase (MTase) activity (Deng et al., 2015), human a-thrombin (Ge et al., 2014) and prostate specific antigen (PSA) (Kong et al., 2015). However, the current $MoS_2$ FRET assays mainly used fluorescent dyes as donor molecules. The FRET assay with GQD as donor and $MoS_2$ as acceptor for protein detection has not been explored.

Herein, we reported a novel GQD-PEG-aptamer/$MoS_2$ based FRET assay for EpCAM protein detection with GQD as donor and $MoS_2$ nanosheet as quencher. The FRET assay was established by attachment of GQD labelled aptamer on $MoS_2$ nanosheets, triggering FRET phenomena due to close proximity between GQD and $MoS_2$ nanosheets. The fluorescence signal was maintained at "off" status due to the fluorescence quenching of GQD by $MoS_2$ nanosheets. In the presence of EpCAM protein, the specific affinity between aptamer and EpCAM protein would detach GQD labelled aptamer from $MoS_2$ surface, which turned "on" the fluorescence signal. By monitoring the change of fluorescence signal, EpCAM protein can be detected

sensitively. The proposed FRET assays displayed a linear range from 3 nM to 53 nM with a limit of detection (LOD) around 3 nM.

## 2. Materials and Methods

### *2.1 Materials*

Carboxylated graphene quantum dot (GQD-COOH) solution was purchased from Nanjing XFNANO Materials Tech Co., Ltd. (Nanjing, Jiangsu, China). Molybdenum disulfide ($MoS_2$) nanosheets were purchased from Nanjing Mknano Science and Technology Co., Ltd. (Nanjing, Jiangsu, China). Amine-PEG-Amine (MW=2000) was purchased from Laysan Bio, Inc. (Arab, Al, USA). 1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) and N-hydroxysulfosuccinimide (Sulfo-NHS) were purchased from Sigma Aldrich (St.Louis, Mo, USA). All of these chemicals were used as received without further purification. Aptamer was synthesized and purified by Integrated DNA Technologies (IDT) Inc. (Coralville, IA, US). A 48-base hairpin-structured aptamer modified with carboxyl group was used as the probe (5′-/5carboxy1/-CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG-3’) (Song et al., 2013). EpCAM recombinant human protein (hIgG1-Fc Ta) purchased from Sino biological Inc. (North Wales, PA, USA) was used as EpCAM-positive protein target. All the aptamer and protein were dissolved in ultrapure water to prepare stock solution.

*2.2 Synthesis of PEGylated GQDs*

Briefly, 2.4 mg of 1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC) and 3.6 mg of N-hydroxysulfosuccinimide (Sulfo-NHS) were added into 1 mL carboxylated graphene quantum dot (GQD-COOH) solution (1 mg/mL) and the mixture was stirred for 15 min. Then, 10% Amine-PEG-Amine diluted with PBS was added and incubated with activated GQD-COOH overnight at room temperature. Finally, the PEGylated GQDs solution was purified and concentrated by ultrafiltration (Amicon Ultra-0.5, 3KD, Millipore).

*2.3 Synthesis of aptamer conjugated PEGylated GQDs*

Carboxyl modified aptamer was then covalently conjugated onto the amine functionalized GQDs-PEG through EDC/NHS method. Initially, the carboxyl modified aptamer was pretreated with EDC/NHS for 15 min. After that, the activated aptamer with final concentration of 1.95 μM was added into amine functionalized GQDs-PEG solution. The mixture was then shaken overnight at room temperature. In order to remove excessive EDC, NHS and aptamer, the final product, GQD-PEG-aptamer, was purified and concentrated by ultrafiltration (Amicon Ultra-0.5, 3KD, Millipore).

*2.4 FRET assay establishment and EpCAM protein detection*

Fluorescence spectra of FRET quenching efficiency and EpCAM protein detection were recorded by an Edinburgh FLSP920 spectrophotometer equipped with

a 450 W steady-state xenon lamp at room temperature. In a typical experiment, the final product of GQD-PEG-aptamer (50 µL) was incubated with $MoS_2$ nanosheets (50 µL) against a series of final concentrations ranging from 10 µg/mL to 400 µg/mL. After 20 min incubation, the sample was measured with the excitation and emission wavelengths fixed at 360 nm and 466 nm, respectively. For EpCAM protein detection, GQD-PEG-aptamer/$MoS_2$ nanocomplex was incubated with an increasing concentration of target protein (3 nM, 9 nM, 18 nM, 36 nM, 53 nM). After 2 h incubation at 37 °C in the dark envionment, the fluorescence signal was measured, respectivley. The control experiments were conducted by measuring the fluorescence signal of sample after addition of the same volume of PBS. All the fluorescence spectra were recorded under the same condition.

*2.5 Characterization*

The morphology and size of GQDs, GQD-PEG and $MoS_2$ nanosheets were characterized using a JEOL-2100F transmission electron microscopy (TEM) equipped with an Oxford Instrument EDS system, operating at 200 kV. The absorption spectra of GQDs, GQD-PEG, $MoS_2$ nanosheets were characterized by a UV–vis spectrophotometer (Ultrospec 2100 pro). The Zeta potential and size distribution of $MoS_2$ nanosheets were determined at neutral pH environment with a Zetasizer Nano Z system from Malvern Instruments Ltd.

## 3. Results

### *3.1 Mechanism of GQD-PEG-aptamer/MoS2 based FRET biosensor*

The sensing mechanism of GQD-PEG-aptamer/$MoS_2$ based FRET biosensor for EpCAM protein detection is shown in Fig. 1. GQD is utilized as the FRET donor, which can emit intense blue light at 466 nm under UV light excitation of 360 nm. 2D $MoS_2$ nanosheets with good quenching ability are served as the sensing platform and the FRET acceptor. In order to prevent the non-specific absorption, amine functionalized PEG is firstly conjugated onto GQD through EDC/NHS method. PEGylated GQD can also emit much stronger fluorescence as compared to bare GQD due to the quantum confinement effect. Carboxylated aptamer is then conjugated onto PEGylated GQD by EDC/NHS chemistry. The sensing platform is realized by the adsorption of aptamer on $MoS_2$ nanosheets via van der walls force, which brings GQD and $MoS_2$ into close proximity. Under 360 nm excitation, the fluorescence of GQD is highly quenched by $MoS_2$ due to the ultra-high quenching capability of $MoS_2$. In the presence of EpCAM protein, the stronger binding affinity between aptamer and EpCAM protein could detach GQD labelled EpCAM aptamer from $MoS_2$ nanosheets, leading to the restoration of fluorescence signal. By monitoring the change of fluorescence signal, the target EpCAM protein can then be detected.

**Here Fig. 1**

*3.2 Characterization of GQDs and AuNPs*

TEM experiments were performed to characterize the morphology and size of PEGylated graphene quantum dots and $MoS_2$ nanosheets. As shown in Fig. 2a, bare GQDs are homogeneous with diameter of 2.8 ± 0.5 nm (Fig. 2b). After PEGylation of GQDs, the collected GQDs-PEG are monodispersed with an obviously increased size (Fig. 2c). The average size increased to 4.2 ± 0.8 nm (Fig. 2d). To further confirm the conjugation between GQD and PEG, UV-vis absorption spectra were measured before and after PEGylation (Fig. 2e). Before PEGylation, GQD has an absorption peak at 336 nm, which is assigned to n-π* transition of C=O. After PEGylation, the peak at 336 nm disappears and the threshold wavelength of GQD-PEG notably shifts from 328 to 276 nm. This can be explained by the energy gap increase of GQD via surface passivation. Fig. 2f shows $MoS_2$ nanosheets with graphene-like 2D structure, which makes them to be ideal nano-sensing platforms with high biomolecule loading rate. The average size of $MoS_2$ nanosheets is around 345 nm (Fig. S1). Selective area electron diffraction (SAED) on the $MoS_2$ nanosheets indicates the $MoS_2$ nanosheets are polycrystalline with some discrete diffraction spots arranged on the concentric rings (Inlet, Fig. 2f). The Zeta potential of $MoS_2$ nanosheets is around -38 mv, indicating their negative surface charge (Fig. S2). Fig. 2g shows the obtained graphene-PEG-aptamer-$MoS_2$ composites in TEM image. The enlarged high resolution TEM (HRTEM) image clearly shows that functionalized GQDs are well adsorbed on the surface of $MoS_2$ nanosheets. Energy dispersive X-ray (EDX) spectrum confirms the formation of graphene-PEG-aptamer/$MoS_2$ composites with

the presence of elements of Mo, S, C, O and P. Here, element of P is from aptamer on GQD surface (Fig. S3).

**Here Fig. 2**

*3.3 Emission spectra of GQDs-PEG and adsorption spectra of $MoS_2$ nanosheets*

Under 365 nm laser emission, both GQDs and GQDs-PEG emit blue light with a slight emission peak shift from 468 nm (GQDs) to 478 nm (GQD-PEGs) (Fig. 3a). Both GQDs and GQDs-PEG disperse well in water solution due to the abundant oxygen-containing functional groups or hydrophilic PEG chains on the surface, which is demonstrated by the uniform blue color distribution in GQD solution. The PL intensity of GQDs-PEG is obviously higher than bare GQDs with a brighter blue emission in the photographs (Inlet, Fig.3a). The enhanced PL intensity is attributed to the surface passivation of GQDs with more quantum confinement of emission energy trapped on GQDs (Shen et al., 2011). The absorption spectrum of $MoS_2$ nanosheets mostly centers in UV region and extends the adsorption band to the NIR range. The spectra overlapping between emission spectra of GQDs and absorption spectra of MoS2 nanosheets ensured the feasibility of this FRET biosensor (Fig. 3b).

**Here Fig. 3**

*3.4 Construction of GQD-PEG-aptamer/$MoS_2$ based FRET biosensor*

In order to optimize the sensing performance, this GQD-PEG-aptamer/$MoS_2$ based FRET biosensor was explored by measuring the fluorescence signal of

GQD-PEG-aptamer after addition of $MoS_2$ nanosheets with various concentrations. Herein, a fixed 50 µL of as-prepared GQD-PEG-aptamer (1 mg/mL) was incubated with 50 µL of a series concentration of $MoS_2$ nanosheets ranging from 10 µg/mL to 400 µg/mL. The fluorescence signals of GQD-PEG-aptamer/$MoS_2$ nanocomplex were then measured. The fluorescence intensity of GQD-PEG-aptamer solution mixed with the same volume of PBS was regarded as control group ($F_0$). The photoluminescence spectra of GQD-PEG-aptamer/$MoS_2$ nanocomplex at different ratio is shown in Fig. 4a. It was observed that the fluorescence signal of GQD-PEG-aptamer gradually decreased with the increasing concentrations of $MoS_2$ nanosheets from 10 µg/mL to 400 µg/mL. This fluorescence quenching was induced by the adsorption of GQD labelled aptamer on $MoS_2$ nanosheets, which brought GQDs close enough to $MoS_2$ nanosheets to trigger the energy transfer from GQDs to $MoS_2$ nanosheets. The energy transfer efficiency is calculated by the equation of $QE=(F_0-F_q)/F_0$, where $F_0$ stands for the original fluorescence signal of control group (50 µL of GQD-PEG-aptamer solution with 50 µL of PBS), and $F_q$ is the fluorescence intensity of GQD-PEG-aptamer after quenching by $MoS_2$ nanosheets. The calculated quenching efficiency and normalized fluorescence intensity is shown in Fig 4b. The fluorescence quenching efficiency reached a maximum of 92.3% and the normalized fluorescence intensity ($F_q/F_0$) decreased to a minimum of 7.6% when $MoS_2$ nanosheets concentration increased to 400 µg/mL. This high quenching verified the excellent quenching ability of $MoS_2$ nanosheets.

**Here Fig. 4**

*3.5 Detection of EpCAM proteins*

After investigating the quenching efficiency of $MoS_2$ nanosheets on GQD, GQD-PEG-aptamer/$MoS_2$ based sensing platform was utilized for the detection of EpCAM protein. In order to achieve optimal sensing performance, GQD-PEG-aptamer/$MoS_2$ based nanocomplex was prepared by using $MoS_2$ nanosheets with the concentration of 400 μg/mL, which ensured the maximum quenching efficiency (92.3%) and lowest background signal. Upon addition of a series of concentrations of target EpCAM proteins, the fluorescence emission spectra of GQD-PEG-aptamer/$MoS_2$ was measured to quantify the fluorescence recovery signals. Fig. 5 shows averaged PL spectra of GQD-PEG-aptamer/$MoS_2$ nanocomplex against an increasing concentration of target EpCAM proteins. The fluorescence signal gradually recovered with the increasing concentration of target EpCAM protein. The reason was that the stronger affinity interaction between aptamer and target protein would detach GQD labelled aptamer from $MoS_2$ nanosheet surface. As the distance between GQDs as donors and the $MoS_2$ nanosheets as acceptors increased, the fluorescence signals were recovered due to the disappearance of FRET effect.

Fig. 6a shows the relative peak fluorescence signal recovery rate ($(F_r-F_q)/F_q$) with the change of EpCAM protein concentrations. Where $F_r$ is the recovered fluorescence intensity after addition of target EpCAM protein, and $F_q$ is the fluorescence intensity of prepared GQD-aptamer-PEG/$MoS_2$ assay after quenching. As shown in Fig. 6b, a logarithmic curve was obtained from 3 nM to 53 nM with the equation of y = 5.3

ln(x)-4.3. The limit of detection (LOD) for EpCAM protein detection is around 3 nM based on the background signal plus 3 times of standard derivation.

**Here Fig. 5**

**Here Fig. 6**

## 4. Conclusion

In this paper, a novel FRET biosensor based on GQD-PEG-aptamer and $MoS_2$ nanosheet pairs was developed for EpCAM protein detection. The FRET assay was established by the adsorption of GQD-PEG-aptamer on $MoS_2$ nanosheets via van der walls force, which brought GQDs and $MoS_2$ into close proximity to trigger FRET phenomena. PEGylated GQDs showed much higher fluorescence emission intensity compared with bare GQDs. The established FRET assay was successfully used for EpCAM protein detection with a logarithmic linear detection range from 3 nM to 53 nM and a limit of detection of 3 nM. This GQD-PEG-aptamer/$MoS_2$ FRET biosensor has the potential to be used as a simple and sensitive aptamer-based sensing platform for various biomarker detection.

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**Figure Captions**

**Fig. 1** The sensing mechanism of GQD-PEG-aptamer/$MoS_2$ based FRET biosensor for EpCAM protein detection.

**Fig. 2** (a) TEM image of bare GQDs; (b) Bare GQDs have an average size of 2.8 ± 0.5 nm; (c) TEM image of PEGlyted GQDs; (d) PEGlyted GQDs have an average size of 4.2 ± 0.8 nm; (e) UV-Vis absorption spectra of GQDs before and after PEGylation; (f) TEM image of $MoS_2$ nanosheets. (Inset: SAED pattern taken on the $MoS_2$ flakes); (g) TEM image of the GQD-PEG-aptamer/$MoS_2$ nanosheet composite; (h) An enlarged HRTEM image of the selected area in (g).

**Fig. 3** (a) PL spectra of GQDs and GQDs-PEG dispersed in water. Inset: photographs of GQDs and GQDs-PEG in water; (b) Spectra overlapping between emission spectrum of GQDs and absorption spectra of $MoS_2$.

**Fig. 4** (a) Fluorescence quenching spectra of GQD-PEG-aptamer with addition of increasing concentrations of $MoS_2$ nanosheets ranging from 10 to 400 μg/mL; (b) Quenching efficiency $QE=(F_0-F_q)/F_q$ and normalized PL intensity versus a series of $MoS_2$ nanosheet concentrations.

**Fig. 5** Fluorescence recovery spectra of GQD-PEG-aptamer/$MoS_2$ based sensing platform in addition of target EpCAM protein with various concentrations from 3 nM to 53 nM.

**Fig. 6** (a) The recovered peak fluorescence signal versus a series of target oligo concentrations target EpCAM protein; (b) Relative peak fluorescence signal recovery rate ($(F_r-F_q)/F_q$) versus the change of EpCAM protein concentratins with a fitting

logarithmic curve with the equation of $y = 5.3 \ln(x)-4.3$.