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PII: S0039-9140(16)30621-X
DOI: http://dx.doi.org/10.1016/j.talanta.2016.08.043
Reference: TAL16807

To appear in: *Talanta*

Received date: 29 June 2016
Revised date: 6 August 2016
Accepted date: 16 August 2016

Cite this article as: Weidan Na, Qing Liu, Bowen Sui, Tianyu Hu and Xingguang Su, Highly sensitive detection of acid phosphatase by using a graphene quantum dots-based förster resonance energy transfer, *Talanta* http://dx.doi.org/10.1016/j.talanta.2016.08.043

# Highly sensitive detection of acid phosphatase by using a graphene quantum dots-based förster resonance energy transfer

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**ABSTRACT**

A novel and effective fluorescence strategy was developed for sensitive and selective detection of acid phosphatase (ACP). A förster resonance energy transfer (FRET) biosensor was established by attaching nile red (NR) to graphene quantum dots (GQDs) via lecithin/β-Cyclodextrin (lecithin/β-CD) complex as the linker. The introduction of lecithin/β-CD would brought GQDs–NR pair close enough through both electrostatic interaction and hydrophobic interaction, thereby making the FRET occur and thus resulting in the fluorescence quenching of GQDs (donor) and meanwhile the fluorescence enhancement of NR (acceptor). The presence of ACP in the sensing system would catalyze the hydrolysis of lecithin into two parts, resulting in the GQDs–NR pair separation. Meanwhile, considerable fluorescence recovery of GQDs and decreasing of NR was observed due to the inhibition of FRET progress. In this method, the limit of detection (LOD) is 28 μU $mL^{-1}$ which was considerably low for ACP detection. Using the GQDs-based fluorescence biosensor, we successfully performed in vitro imaging of human prostate cancer cells.



## Graphic Abstract

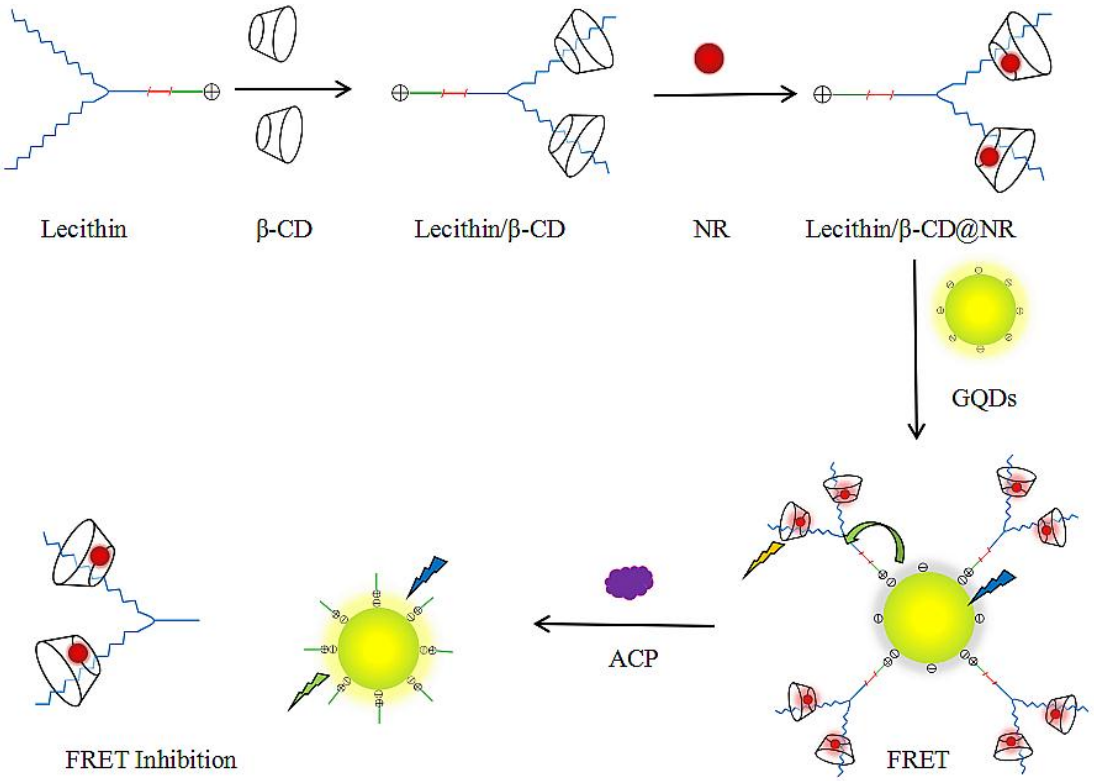


Illustration of the optical biosensor for the detection of ACP based on FRET between donor GQDs and acceptor Nile Red

*Keywords*: Graphene quantum dots; Förster resonance energy transfer; Acid phosphatase; Fluorescence detection

**1. Introduction**

Acid phosphatase, a ubiquitous digestive enzyme, has a broad specificity for catalyzing the hydrolysis of phosphate esters under acidic environment [1]. Although human ACP is normally found to be normally low in mammalian cells, some diseases, such as prostate cancer, Gaucher disease, and disorders related to kidneys, veins, and bones are usually accompanied by changes in the concentrations of ACP [2,3]. Clinically, the measurement of ACP activity has been used for diagnosing cancers and for monitoring cell viability [4]. Due to its biological and clinical importance, the precise concentration measurement of ACP in blood and cells have been regarded

as an essential factor in the pathologic diagnosis of these relative diseases. The currently common methods, such as spectrophotometry [5], immunoassay [6] and and electrochemical methods [7] are quite sensitive. However they usually need the separation of be-surveyed substance. Furthermore, some ACP assays need alkaline pH condition, which could be disrupted by alkaline phosphatase. Among these methods, the fluorescent methods for ACP assay will offer new alternatives since they are rapid, continuous, and in real time.

FRET is a photophysical process involving the nonradiative energy transfer from a donor fluorophore to an acceptor fluorophore (also called FRET pairs) in a close distance through long-range dipole–dipole coupling [8]. Donor in the excited state can transfer excitation energy to acceptor being situated at a distance of less than 10 nm [9]. The main effect of this transfer is the decreasing of the donor light emission and increasing of the acceptor light emission [10]. FRET is a widely used mechanism employed in the bioassays, predominantly due to its high sensitivity to the distance separating the donor from the acceptor. There are numerous reports of FRET effect between II–VI semiconductor quantum dots (SQDs) and organic dyes on their surface. However, the SQDs have suffered from intrinsic limitations such as heavy metals potential toxicity and environmental hazards. Graphene quantum dots (GQDs) are usually considered as a class of zero-dimensional nanomaterials, which exhibits unique optical properties due to the quantum confinement and edge effects. GQDs have many advantages such as narrow emission spectra, broad absorption spectra, negligible photobleaching, and high photochemical stability. They also possess excitation-dependent fluorescence emission spectra, which allow for the accommodation of spectral overlap between donors and acceptors by simply adjusting the excitation wavelength [11]. Relatively nontoxicity of GQDs makes it a good replacement to II–VI semiconductors based

on cadmium ions. Nevertheless, to the best of our knowledge, the use of GQDs as donor FRET also remains largely unexplored. Therefore, making them especially useful in assays that involve multiple FRET pairs.

In this study, we present a facile route for the detection of ACP via a FRET technique. The FRET-based detection biosensor consisted of three components: GQDs as the donor, Lecithin/β-CD complex as the linker and NR as the acceptor, which is illustrated in Scheme 1. The lecithin molecule contained three domains termed as I, II and III according to their different functions. Region I is the positively charged choline which could attach to the surface of GQDs through electrostatic interaction, region II is phosphate ester which could be hydrolyzed by ACP and region III were two alkyl chains. The poor solubility in water of lecithin was due to its two long alkyl chains which was hydrophobic. β-CD could form host-guest complexes (Lecithin/β-CD) with lecithin by including the long alkyl chains of lecithin into β-CD cavities. Lecithin/β-CD would further form a ternary complex (Lecithin/β-CD@NR) after the introduction of NR, which would induce the FRET occur upon non-covalent bonding with negatively-charged GQDs. The presence of ACP in the sensing system could catalyze the hydrolysis of lecithin into two parts, resulting in the GQDs–NR pair separation. Meanwhile, considerable fluorescence recovery of GQDs and quenching of NR were observed due to the inhibition of FRET progress.



## 2. Experimental section

*2.1 Materials*

All reagents were of at least analytical grade. The water used in all experiments had a resistivity higher than 18 MΩ $cm^{-1}$. β-CD, Graphite powder (100 mesh), $H_2SO_4$ (98%), $H_2O_2$ (30%), $KMnO_4$ and nile red were purchased from HuaCheng Biological Co., Ltd (ChangChun).

Lecithin and ACP were purchased from Beijing Dingguo Biotechnology Company. 0.1 mol $L^{-1}$ Tris-HCl (pH 5.8) was used for the detection process.

*2.2 Apparatus*

The fluorescence spectra were obtained by using a Shimadzu RF-5301 PC spectrofluorophotometer equipped with a xenon lamp using right-angle geometry. UV-vis absorption spectra were obtained by a Varian GBC Cintra 10e UV-vis spectrometer. In both experiments, a 1 cm path-length quartz cuvette was used. FT-IR spectra were recorded with a Bruker IFS66V FT-IR spectrometer equipped with a DGTS detector. All pH measurements were made with a PHS-3C pH meter (Tuopu Co., Hangzhou, China). Transmission electron microscopy (TEM) experiments were performed on a Philips Tecnai F20 TEM operating at 200 KV acceleration voltage.

*2.3 The preparation of GO by the modified Hummers method*

Graphene oxide (GO) was prepared from graphite powder by the modified Hummers method [12]. Typically, 1.0 g of graphite powder was added to 25 mL $H_2SO_4$ (98%) and stirred 30 min. Afterwards, 3.5 g $KMnO_4$ was added and the mixture was stirred for 30min in an ice bath, and then stirred at 35 ℃ for 1h. Next, the mixture was diluted by 90 mL distilled water and the temperature was increased to 90 ℃ stirred for another 30 min. Subsequently, another 90 mL diluted water was added and then 10 mL of 30% $H_2O_2$ solution was added. The color of the mixture changed to bright yellow quickly. The resulting mixture was washed with distilled water several times and purified in a dialysis bag with a molecular weight cutoff of 10000 Da against deionized water until the pH of the solution became neutral. After that, the solution was dried under vacuum to remove water. Finally, some black GO powder was obtained.

*2.4 The preparation of GQDs by the chemical oxidation of GO*

1.0 g GO was dissolved in 30 mL $H_2SO_4$ (98%) and stirred 15 min, Afterwards, 5.0 g $KMnO_4$ was added and the mixture was stirred for 30min in an ice bath, and then stirred at 40 ℃ for 1h, Next, the mixture was diluted by slowly dropwise adding 120 mL distilled water, afterward, 10 mL of 30% $H_2O_2$ solution was added. The solution became transparent and color of the solution changed to bright yellow quickly. Finally, the solution was purified in a dialysis bag with a molecular weight cutoff of 1000 Da against deionized water to obtain a pure GQDs solution. Finally, A 2.7 mg $mL^{-1}$ GQDs solution was prepared and stored under ambient condition. The fluorescence spectra were recorded from 500 nm to 700 nm with the excitation wavelength of 470 nm. The slit widths of excitation and emission were both 10 nm.

*2.5 Fabrication of the FRET fluorescent biosensor*

The biosensor, GQDs-lecithin/β-CD@NR, was prepared and the process was performed as follow. Briefly, lecithin/β-CD was produced in an aqueous solution by the inclusion reaction of β-CD with lecithin powder at a molar ratio of 2:1 first. Later NR powder was added to the lecithin/β-CD solution and the molar ratio of lecithin/β-CD to NR was fixed at 1:2 followed by the thoroughly shaking and equilibrated for 4 hours. After that, the as-prepared GQDs were added into lecithin/β-CD@NR solution followed by the thoroughly shaking and equilibrated for 3 minutes. The final concentrations of all the substances were 1.35 mg $mL^{-1}$ GQDs, 31.25 μmol $L^{-1}$ lecithin/β-CD@NR. At last, the fluorescence spectra were recorded from 500 nm to 800 nm with the excitation wavelength of 470 nm. The slit widths of excitation and emission were both 10 nm.

*2.6 Detection of ACP through FRET system*

A typical ACP detection process was performed as follow. 0.1mol $L^{-1}$ Tris-HCl buffer

solution (pH 5.8, 150 μL), 1500 μL GQDs-lecithin/β-CD@NR and various amounts of ACP were successively added into a 2 mL calibrated centrifuge tube. The mixtures were diluted to the 2.0 mL mark with deionized water and mixed thoroughly. 90 minutes later, the fluorescence spectra were recorded from 500 nm to 800 nm with the excitation wavelength of 470 nm. The slit widths of excitation and emission were both 10 nm.

*2.7 In vitro cell imaging*

PC-3M cells were seeded into a six-well plate at a density of 3 × 104 cells per well and incubated at 37 °C. When the cultured cells reached about 70% confluence, the plates were washed with phosphate buffer saline (PBS) three times. A certain amount of ACP was added to the PC-3M cell wells. After incubation for 2 h at 37 °C in the presence of ACP, free ACP was removed from ACP-uptaken PC-3M cells by washing three times with PBS. Before in vitro sensing experiments, IMDM medium was replaced by a fresh serum-free medium. The cells were incubated under the condition of serum starvation with lecithin/β-CD@NR and GQDs-lecithin/β-CD@NR (0.5 μg $mL^{-1}$ GQDs and 15 nmol $L^{-1}$ lecithin/β-CD@NR) for 90 min. The cell imaging of control experiments was also performed: non-ACP-uptaken PC-3M cells and PC-3M cells incubated with GQDs-lecithin/β-CD@NR. The cells were then washed three times with PBS for 5 min each. Finally, cells were fixed with 4% paraformaldehyde for 15 min and washed at least three times with PBS. Fluorescent photos of the cells were obtained by an inverted fluorescence microscope equipped with a Nuance system.

**3. Results and discussion**

*3.1 Feasibility of the ACP assay system*

The proposed novel platform for ACP determination was based on the FRET between GQDs and NR. Fig.1 is the fluorescence spectra of GQDs, NR, GQDs after addition of NR, GQDs after addition of NR in the presence of β-CD/Lecithin, and GQDs after addition of NR, β-CD/Lecithin in the presence of ACP. It can be seen that GQDs had an emission maximum at 544 nm with 470 nm excitation (black line). NR had an emission maximum at 646 nm with the excitation wavelength at 544nm (red line). Therefore, excitation at 470 nm would result in FRET between the GQDs and NR molecules. However, when GQDs was mixed with NR, the FRET phenomenon was not obvious since the distance between most GQDs and NR was further than 10 nm (green line). After the introduction of Lecithin/β-CD, effectively fluorescence quenching of GQDs and a significant fluorescence enhancement with a maximum at 660 nm (typical for NR emission) was observed (blue line). In fact, lecithin/β-CD was acted as a linker for GQDs and NR, which shorten the distance between them and made the FRET process occur. Moreover, the fluorescence emission peaks of GQDs blue-shifted from 544nm to 527nm, which resulted from the decrease of surface vacancies of GQDs [13]. Meanwhile, the fluorescence emission peak of NR was red-shifted from 646 nm to 660nm. The reason could attribute to the formation of dimer due to dipolar interaction between the NR molecules in water [14]. The introduction of ACP resulted in the enhancement in GQDs fluorescence which was accompanied by a decrease in NR fluorescence (purple line). This indicated that the acceptor NR was far away from the donor GQDs and the FRET process was inhibited. Such phenomenon might meaningfully confirm the evidence aforementioned in the representation of Scheme 1. Therefore, it was expected that the ACP triggered release of NR from GQDs could be monitored because of the efficient fluorescence change from both GQDs and NR.

*3.2 Spectral properties of GQDs and NR*

As a well-known mechanism, the efficiency of FRET corresponds to the appreciable overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor. The more overlap, the higher efficiency of energy transfer [15]. The absorption and emission spectra of GQDs and NR were shown in Fig. 2(A) , respectively. It could be seen that the absorption spectrum of GQDs was broad, and the maximal emission peak was at 544 nm. The maximal absorption and emission peaks of NR were at 564 nm and 654 nm, respectively. So there was appreciable overlap between the emission spectrum of the GQDs (donor) and the absorption spectrum of the NR (acceptor). The effect of the excitation wavelength on the fabrication of FRET was also investigated as shown in Fig. 2 (B). The broad absorption spectrum of GQDs contributes to the flexibility in choosing a suitable excitation wavelength to excite the GQDs and allows FRET-based target detection with a low background. In order to make the contribution of the fluorescence of NR coming from direct excitation of NR as little as possible, the FRET detections were performed by exciting the donors at 470 nm.

*3.3 The fabrication of the ternary inclusion complex lecithin/β-CD@NR*

β-CD, as a well-known host molecule, is an oligosaccharide composed of seven glucose units. It can selectively bind various molecules in their cavities to form stable host–guest inclusion complexes [16]. Firstly, the self-assembly behavior of lecithin/β-CD mixed system was observed. It can be seen that the solution of lecithin is white precipitate at a concentration of 0.25 mmol $L^{-1}$, whereas the white precipitate was gradually disappeared upon the addition of β-CD, indicated that β-CD can form inclusions with the alkyl chains part of lecithin. According to previous report, the size of the β-CD cavity matches the size and structure of the NR molecule, facilitating the

formation of inclusion complexes [17]. The concentrations of NR was fixed by changing the ratios of NR: lecithin/β-CD were shown in Fig. 3(A) It can be seen that the fluorescence intensity increased sharply with the increase of the concentration ratio of lecithin/β-CD to NR and was nearly consistent when the ratio reached 1:2. The results indicated the formation of a ternary inclusion complex (lecithin/β-CD@NR) made the NR molecule dispersed in water. Fig. 3(B) showed the effect of incubation time on the fluorescence intensity of NR during the formation of lecithin/β-CD@NR. It can be seen that the fluorescence intensity sharply decreased and then gradually increased. The fluorescence intensity remained constant after 4 hour, evidently due to the completely formation of ternary inclusion complexes. Thus, 4 hour was chosen as the incubation time for the formation of lecithin/β-CD@NR.

*3.4 Optimization for ACP detection*

As GQDs and NR were closely linked via lecithin/β-CD, resulting a donor-linker-acceptor pair as a biosensor, the fluorescence from GQDs was transferred to the acceptor NR due to the close proximity. An increase in emission peak of NR would be readily observed. From Fig. S1, it could be seen that, with the increasing acceptor concentration, the fluorescence of GQDs is effectively quenched while Nile Red fluorescence emission is gradually increased. The spectra clearly indicate the presence of the FRET process between GQDs and Nile Red. The optimal concentration of NR was chosen to be 62.5 μmol $L^{-1}$. The conjugation of GQDs and NR in this work was based on electrostatic interactions, in which the negatively charged GQDs form a complex with the positively charged lecithin/β-CD@NR and therefore brings the NR fluorophores in close proximity to the GQDs. It is also worth noting that the electrostatic interaction is a more labour-saving and cost-saving way to connect FRET pairs than conventional covalent linking.

pH value was also an important parameter relevant to good performance of the ACP detection system. The effect of pH on the FRET system was investigated in the pH range from 5.8 to 9.0. As shown in Fig. 4(A), the efficiency of FRET system was seriously influenced by the change of pH value. From the Inset of Fig. 4(A), it could be seen that the relative fluorescence $F_1/F_2$ dramatically increased with the increasing of pH value from 5.8 to 6.6 and sharply decreased when the pH value further increased from 6.6 to 9.0. Moreover, under acidic pH condition the detection could avoid the interference from alkaline phosphatase (ALP) and where the catalytic capability of ACP is the strongest [18]. Hence, the physiological condition pH 5.8 was chosen as the ideal reaction pH throughout the whole experiment. The effect of hydrolysis time on the ACP assay performance was investigated with three different ACP concentrations. As shown in Fig. 4(B), the hydrolysis rate of lecithin was increased with the enhancement of ACP concentration, and the relative fluorescence intensity ($F_1/F_2$) of the biosensor increased with the increase of the reaction time, and then became stable after the reaction time reached 90 minutes. The optimal incubation time was chosen as 90 minutes in the further experiments.

*3.5 FRET between GQDs and NR*

The energy transfer efficiency E is a key factor to evaluate whether the FRET system is good or not. So in all the chemistry experiments related to FRET, the value of E has to be calculated in order to get a relative accurate value of the efficiency of energy transfer [19,20], E can be calculated according to the equation (1):

$$E = 1 - F/F_0 \quad (1)$$

where *F* and $F_0$ are the fluorescence intensity of the donor in the presence or absence of the acceptor, respectively. The relationship between E, r and $R_0$ can be expressed though equation (2):

$$E = R_0^6/(R_0^6 + r^6) \quad (2)$$

where $r$ is distance between energy donor and energy acceptor. $R_0$ is the Förster Radius where the FRET efficiency is observed at 50%, which can be expressed in equation (3):

$$R_0^6 = 8.8 \times 10^{-25} k^2 n^{-4} \Phi J(\lambda) \quad (3)$$

$K^2$ denotes a dipole-dipole interaction between donor and acceptor, typically $k^2$ =2/3. $n$ is the refractive index of the medium ($n$ = 1.33 in water). $\Phi$ is the quantum yield of the GQDs, which was calculated as 1.04% by the integrated emission of the GQDs samples in solution compared with that of rhodamine 6G (QYs, 95%) in ethanol under identical optical density.[20] $J(\lambda)$ is the associated spectral overlap integral, expressing the degree of spectral overlap between the absorption of acceptor and the emission of donor, which can be calculated from equation (4):

$$J(\lambda) = \int F_0(\lambda)\, \varepsilon_A(\lambda)\, \lambda^4 d\lambda / \int F_0(\lambda)\, d\lambda \quad (4)$$

$F_0(\lambda)$ is the fluorescence intensity of the donor at the wavelength of $\lambda$, and $\varepsilon_A(\lambda)$ is the molar absorption coefficient of the acceptor at the wavelength of $\lambda$. The $J(\lambda)$ is calculated to be 9.97 $\times 10^{-13}$ $cm^3$ L $mol^{-1}$ for this FRET system by integrating the spectra according to Fig. 2(A). In this study, the energy transfer efficiency (E) was calculated to be 70.60 % , then $R_0$ was calculated to be 4.56 nm and the distance between the donors and the acceptor (r) was calculated to be 2.80 nm. According to the value of "r", we can see that the distance between GQDs and NR was small enough, which also indicates that the energy transfer from GQDs to NR could occur with high probability.

*3.6 The detection of ACP*

When ACP was added to the GQDs-lecithin/β-CD@NR system, the lecithin of biosensor would be cleaved and consequently the linkage between GQDs and NR would be destroyed. As the

fluorescence intensity ratio $F_1/F_2$ ($F_1$ and $F_2$ refer to the fluorescence intensity of GQDs and NR, respectively) consequently represented the degree of cleavage and thus highly correlates to the concentration of ACP, an accurate quantification of ACP can be achieved. Fig. 5 was the fluorescence spectra of GQDs-lecithin/β-CD@NR system in the presence of different concentrations of ACP. The fluorescence intensity of GQDs increased dramatically and the fluorescence intensity of NR decreased gradually with the increasing concentration of ACP, indicating the FRET was inhibited and the NR was far away from the GQDs. As plotted in Fig. 5 Inset, a good linear relationship was presented between the fluorescence intensity ratio ($F_1/F_2$) and the concentration of ACP. The linear regression equation is $F_1/F_2$ = 1.49299 + 0.00224[ACP], μU $mL^{-1}$. The corresponding determination coefficient ($R^2$) is 0.9912, and the detection limit for ACP is 28 μU $mL^{-1}$. The detection limit is defined by the equation LOD=3σ/s, where σ is the standard deviation of the blank signals (n=11) and s is the slope of the calibration curve. Some analytical parameters in other methods for the detection of ACP were listed in Table 1 [21-25]. Compared with those reports about ACP assay, our present method offered a comparable or super detection limit and dynamic range.

*3.7 Interference study*

Selectivity is a very important parameter to evaluate the performance of a new biosensor, especially for ones with potential applications in biomedical samples, a highly selective response to the target over other potentially competing species is necessary. Therefore, we further evaluated the selectivity of our biosensor with various coexistence substances added. Table S1 showed the interference effect of some biological molecules and common inorganic ions on the determination of ACP, a relative error of 5.0% was considered to be tolerable. Tolerable concentration was

defined as the concentrations of coexisting substances causing less than 5.0% relative error. As shown in Table S2, the tolerable concentration ratios of coexisting substances to 100 μU $mL^{-1}$ ACP was over 10-fold for ATP, ALP, Try, Lys and GSH. The concentration of NaCl, KCl and $Zn(NO_3)_2$, $Ca(NO_3)_2$ was 2.0 mmol $L^{-1}$ and the concentration of glucose was 5.00 mmol $L^{-1}$. The concentration of $FeCl_3$ and $FeCl_2$ was 0.1 and 0.2 mmol $L^{-1}$. The results showed that there was little interference from commonly existing substances. Thus, the present method was suitable for selective detection of ACP.

*3.8 In vitro study*

To demonstrate the potential of the GQDs-Lecithin/β-CD@NR system for sensing applications, the GQDs-Lecithin/β-CD@NR system was employed to detect ACP in vitro. The fluorescence image in Fig. 6(A) showed a significant red fluorescence from the lecithin/β-CD@NR when incubated with PC-3M cells. From Fig. 6(B), we could see also see the red fluorescence when the GQDs-lecithin/β-CD@NR was incubated with PC-3M cells, such red fluorescence was attributed to the NR emission after excitation of the GQDs and the fluorescence energy transfer to the NR. The GQDs-Lecithin/β-CD@NR appeared to be fully internalized throughout the cell, indicating the GQDs-Lecithin/β-CD@NR could penetrate through the cell membrane and disperse in the cytoplasm. As shown in Fig. 6(C), when GQDs-Lecithin/β-CD@NR was incubated with ACP-uptaken PC-3M cells, the fluorescence microscopic image showed a strong intracellular green fluorescence signals of GQDs. The strong green fluorescence were attributed to the fact that the lecithin was effectively hydrolyzed and the FRET process was inhibited and the fluorescence of GQDs was obviously recovered. The corresponding fluorescence color change in Fig. 6(B) and Fig. 6(C) clearly indicated that the high

sensitivity of the GQDs-Lecithin/β-CD@NR could serve as a potential biocompatible biosensor for ACP activity detection in the intracellular region.

**4. Conclusion**

In conclusion, a novel and sensitive biosensor was successfully fabricated for ACP detection via the FRET between GQDs and NR. By employing lecithin/β-CD complexes as the linker, the phenomenon of FRET between GQDs and NR was observed. The addition of ACP significantly reduced the efficiency of the FRET system, due to its catalytic ability for the hydrolysis of lecithin. This biosensor showed a high selectivity to ACP among other coexistence which was due to the selectivity nature of enzyme. The proposed method was applied to the fluorescence imaging of ACP in PC-3M cells with satisfactory results.

**Acknowledgments**

This work was financially supported by the National Natural Science Foundation of China (Nos. 21075050 and 21275063), the Science and Technology Development project of Jilin province, China (No. 20150204010GX)

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**Scheme 1** Illustration of the optical biosensor for the detection of ACP based on FRET between donor GQDs and acceptor Nile Red.

**Fig. 1** Fluorescence spectra of GQDs (black line), NR (red line), GQDs after addition of NR (green line), GQDs after addition of NR in the presence of β-CD/Lecithin (blue line) , GQDs after addition of NR, β-CD/Lecithin in the presence of ACP (purple line).

**Fig. 2** (A) Normalized absorption (black dash line) and emission spectra (black solid line) of GQDs in water excited at 470 nm, and the normalized absorption (red dash line) and emission spectra (red solid line) of the Nile Red in water excited at 544 nm. (B) The effect of the excitation wavelength in the range from 420 to 490 nm on the emission spectra of GQDs-NR pairs.

**Fig. 3** (A)The fluorescence spectra of the lecithin/β-CD@NR soultion of fixed NR level with the variation of the ratio between NR and lecithin/β-CD. (B) The effect of incubation time on the

fluorescence of lecithin/β-CD@NR soultion. Inset: The normalized fluorescence intensity of lecithin/β-CD@NR soultion in different incubation time (0, 40, 80, 120, 160, 200, 240 min).

**Fig. 4** (A) The fluorescence spectra of GQDs-lecithin/β-CD@NR system in the pH range from 5.8 to 9.0. Inset:The effect of pH on the relative fluorescence intensity $F_1/F_2$ of GQDs-lecithin/β-CD@NR system after incubation of 20 minutes. (B) The relative fluorescence intensity $F_1/F_2$ of GQDs-lecithin/β-CD@NR system in the presence of (a) 50, (b) 375 and (c)750 μU/mL ACP at different incubation time. $F_1$ and $F_2$ were the fluorescence intensity of GQDs and NR with the excitation wavelength at 470 nm.

**Fig. 5** Fluorescence spectra of the GQDs-lecithin/β-CD@NR (62.5 μmol $L^{-1}$) in the presence of ACP at various concentrations (0, 75, 187.5, 375, 562.5, 750, 937.5, 1125, 1312.5, 1500 μU $mL^{-1}$). Inset: The relationship between $F_1/F_2$ ($F_1$ and $F_2$ refer to the fluorescence intensity of GQDs and NR, respectively) and the concentration of ACP.

**Fig. 6** The fluorescent microscopy images of PC-3M cells counterstained with (A) lecithin/β-CD@NR with excitation at 550 nm, (B) GQDs-lecithin/β-CD@NR with excitation at 470 nm and (C) GQDs-lecithin/β-CD@NR in the presence of 1 μU $mL^{-1}$ ACP, followed by further incubation for 90 min.

**Table 1** The comparison of different method for the detection of ACP.

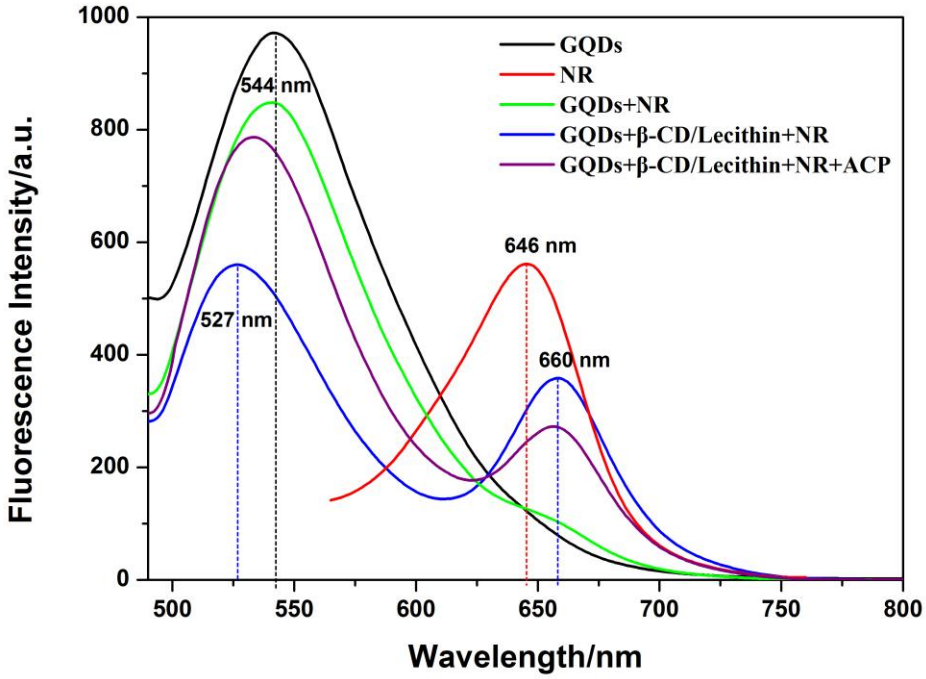
| Detection method | LOD | Linear range | Ref |
|---|---|---|---|
| magnetoelastic | 1.5 mU $mL^{-1}$ | 1.5-15 mU $mL^{-1}$ | [21] |
| Fluorometry | 5.5 mU $mL^{-1}$ | 18.2-1300 mU $mL^{-1}$ | [22] |
| Fluorometry | 8.9 μU $mL^{-1}$ | 0.02-3 mU $mL^{-1}$ | [23] |
| Fluorometry | 0.45 μU $mL^{-1}$ | 1.0-50 μU $mL^{-1}$ | [24] |

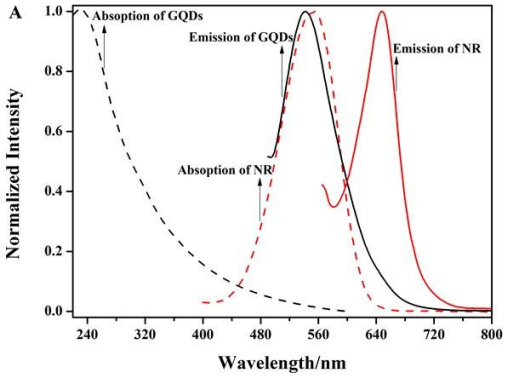
| | | | |
|---|---|---|---|
| Fluorometry | 1.0 nM | 0-30 nM | [25] |
| Fluorometry | 28 μU $mL^{-1}$ | 0-1500 μU $mL^{-1}$ | This work |

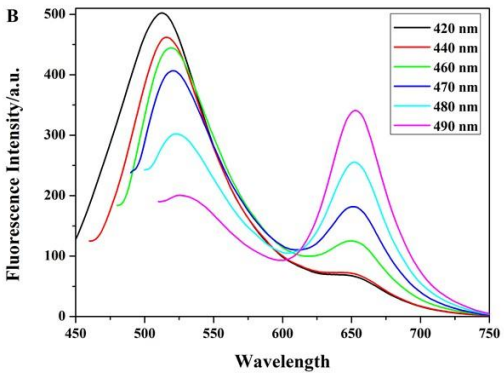
The ACP in [25] was obtained from Sigma (USA). 1 nM, which is corresponding to 0.3-1 mU/mL.

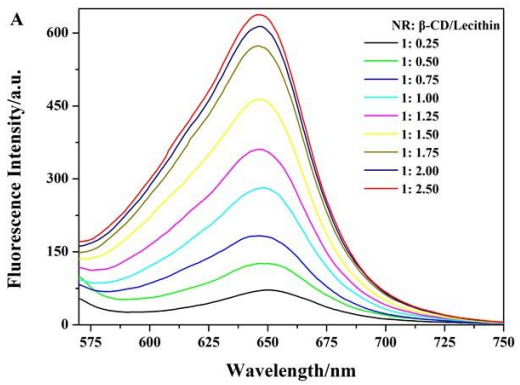
Highlights

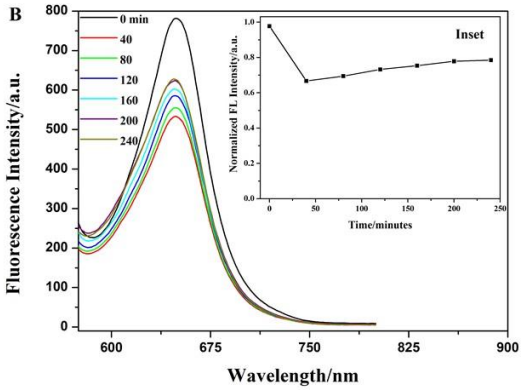
- A novel biosensor was successfully fabricated for ACP detection via the FRET between GQDs and NR.
- By employing lecithin/β-CD complexes as the linker, the phenomenon of FRET between GQDs and NR was observed.
- A good linear response for ACP was found with a much lower detection limit.
- Using the GQDs-based biosensor, we successfully performed in vitro imaging of human prostate cancer cells

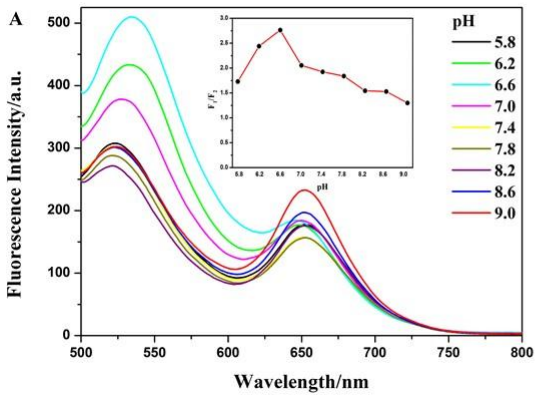

GQDs
NR
GQDs+NR
GQDs+β-CD/Lecithin+NR
GQDs+β-CD/Lecithin+NR+ACP
544 nm
646 nm
527 nm
660 nm
Fluorescence Intensity/a.u.
Wavelength/nm

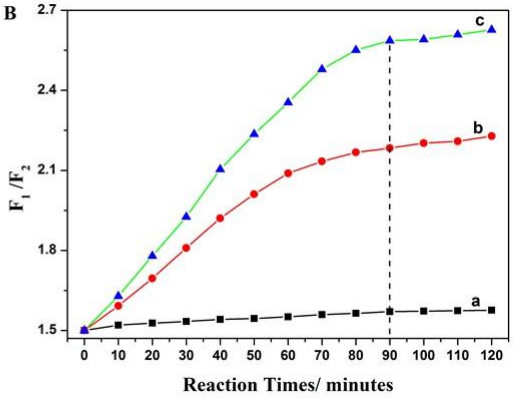

A
Absoption of GQDs
Emission of GQDs
Emission of NR
Absoption of NR
Normalized Intensity
Wavelength/nm

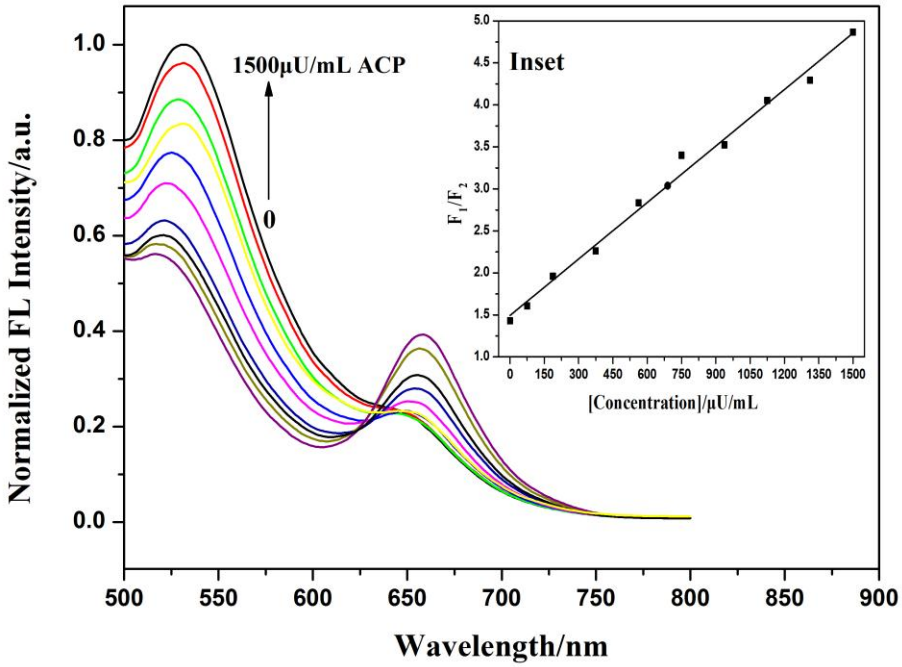

B
420 nm
440 nm
460 nm
470 nm
480 nm
490 nm
Fluorescence Intensity/a.u.
Wavelength

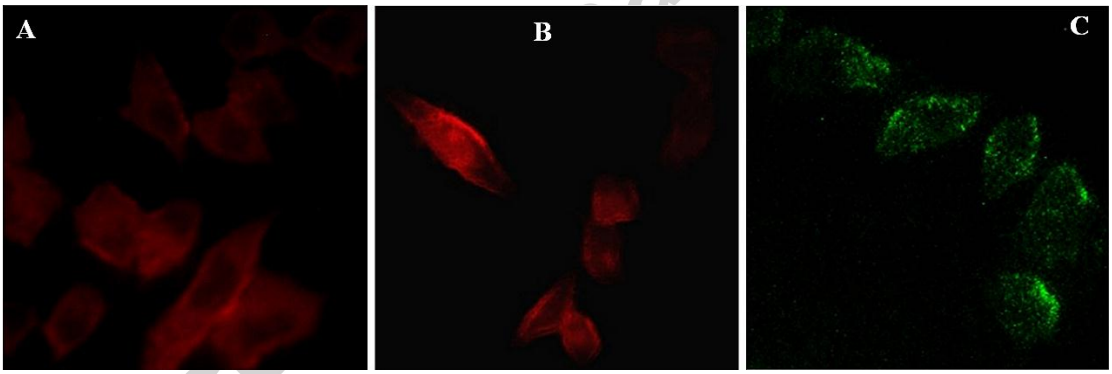

A
NR: β-CD/Lecithin
1: 0.25
1: 0.50
1: 0.75
1: 1.00
1: 1.25
1: 1.50
1: 1.75
1: 2.00
1: 2.50
Fluorescence Intensity/a.u.
Wavelength/nm


B
0 min
40
80
120
160
200
240
Inset
Normalized FL Intensity/a.u.
Time/minutes
Fluorescence Intensity/a.u.
Wavelength/nm


A
pH
5.8
6.2
6.6
7.0
7.4
7.8
8.2
8.6
9.0
Fluorescence Intensity/a.u.
Wavelength/nm


B
c
b
a
F1 /F2
Reaction Times/ minutes



Scheme 1