

# Gold nanorod-based FRET assay for selection of G-quadruplex-binding ligands

Yan Jin · Guozhen Chen · Yuexia Wang

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**Abstract** Single-stranded telomeric DNA tends to fold into G-quadruplexes made of a stack of G-quartets. Ligands that target G-quadruplex DNA have received considerable attention because of their potential anticancer activity. In this paper, we designed a fluorescence resonance energy transfer system to identify drugs that form G-quadruplexes with human telomeric DNA in a homogeneous medium using gold nanorods (GNRs) as fluorescence quencher. Fluorescein-tagged human telomeric DNA (F-GDNA) adsorbed on the surface of GNRs, which can quench the fluorescence of F-GDNA. In the presence of G-quadruplex-binding drugs, the formation of G-quadruplex further quenched the fluorescence of F-GDNA, which can be utilized to select G-quadruplex-binding ligands. Thirteen natural drugs were studied by this proposed method. G-quadruplex formation induced by drugs was investigated by circular dichroism measurements. Results of research indicated that it offers a simple, sensitive and effective method to identify ligands with potential anticancer activity.

**Keywords** G-quadruplex · Telomeric DNA · Gold nanorod · Natural drugs · FRET

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Y. Jin (✉) · G. Chen · Y. Wang  
Key Laboratory of Applied Surface and Colloid Chemistry,  
Ministry of Education, Key Laboratory of Analytical Chemistry  
for Life Science of Shaanxi Province, School of Chemistry  
and Materials Science, Shaanxi Normal University,  
Xi'an 710062, China  
e-mail: jinyan@snnu.edu.cn

## Introduction

Telomeres are essential for the stability and replication of eukaryotic chromosomes [1, 2]. Telomeres generally consist of many tandem repeats of guanine-rich sequence, and a short 3'-overhang single-stranded sequence tends to form a four base-paired planar structure termed G-quadruplex [3, 4]. In vitro, G-quadruplex blocks the binding of telomerase and inhibits telomere extension. Therefore, ligands of G-quadruplex have received great attention because quadruplex-binding ligands have potential applications in genomic studies as well as therapeutic purposes. Methods for practical, rapid, simple, and sensitive selection of G-quadruplex-binding ligands are in great demand since they can be applied to several fields, including genetics, pathology, and especially in designing and screening of antitumor drugs. In recent years, a large effort has been expended in developing techniques for telomere and G-quadruplex research, including X-ray diffraction, nuclear magnetic resonance spectroscopy, fluorescence, circular dichroism (CD), mass spectrometry, electrochemistry and ultraviolet-visible (UV-vis) spectroscopy [5–10]. Due to its sensitivity and flexibility, fluorescence spectroscopy, particularly resonance energy transfer technique (FRET), has been explored as powerful tool to study G-quadruplexes. On the basis of the conformational transition of G-DNA, it is easy to exploit FRET technique to study stability and molecular mechanism of G-DNA with its ligands [11–17]. Simonsson et al. reported a simple method to study G-quadruplex using fluorescence resonance energy transfer methods [18]. In general, the DNA strand was labeled with fluorophore donor and acceptor or fluorophore and quencher at its 5' and 3' ends, respectively. They require dual labeling on the same DNA strand. A simple method would be desired to be developed for selecting ligands of G-DNA.

With the development of nanotechnology, a number of nanomaterials have received widespread interest in biotechnological systems for diagnostic application and biological imaging. Owing to their unique optical properties and ease of bioconjugation, gold nanomaterial, such as gold nanoparticles and gold nanorods, is a type of promising nanomaterial playing important roles in the design of chemical and biochemical sensing for DNA hybridization [19–21], metal ions [22, 23], antibodies [24, 25], and for cancer cell imaging etc [26, 27]. GNRs are normally passivated by positively charged surfactants, which give not only high stability but also positively charged surface. Therefore, GNRs could easily adsorb many biomacromolecules that possess negative charges through electrostatic attractions, such as DNA, peptide, and even cells. On the basis of the quenching capability of gold nanorods to the fluorescent dye, Gou et al. took advantage of GNRs-based FRET for monitoring human telomere DNA hybridization and G-quadruplex formation [28].

In this paper, we have designed a simple FRET system for selecting G-quadruplex-binding ligands using GNRs as a fluorescence quencher. The fluorescence intensity of fluorescein-tagged human telomeric DNA (F-GDNA) quenched by GNRs after addition of GNRs into F-GDNA solution. When quadruplex-binding ligands are added, the formation of G-quadruplexes increases the space charge density around the DNA macromolecule, which is expected to strengthen the interaction between the GNRs and the DNA. Therefore, the fluorescence of DNA probe was further quenched. In this way, G-quadruplex-binding ligands can be selected without labeling drugs. Furthermore, the DNA strand needs only one dye labeled, leading to less laborious and more cost-effective synthesis. Here, 13 natural drugs were studied by this proposed method and experimental results indicated that the FRET approach offers a simple, sensitive, and effective method to identify ligands with potential anticancer activity.

## Experimental section

**Chemicals** Unless otherwise indicated, all reagents and solvents were purchased in their highest available purity and used without further purification.  $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$  was purchased from Sigma (USA). Daidzein, apigenin, quercetin, luteolin, kaempferol, chrysin, emodin, aloe-emodin, chrysophanol, rhein, berberine, colchicine, and matrine were purchased from Nanjing TCM Institute of Chinese Material Medical (Nanjing, China). Drugs and other reagents were commercially available analytical reagent grade. Fluorescein (FAM)-labeled probe DNA is an oligonucleotide mimicking the human telomeric repeat (5'-FAM-TTAGGGTTAGGGTTAGGGTTAGGG-3', F-

GDNA). F-GDNA and FAM-labeled control DNA (F-DNA, 5'-FAM-GTT CATGCCGCCCATGCTCG-3') were synthesized by Shanghai Sangon Biotechnology Co. (Shanghai, China) and used without further purification. The oligonucleotide stock solutions (25  $\mu\text{M}$ ) were prepared with a TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.2) and kept frozen. Millipore Milli-Q (18 M $\Omega$  cm) water was used in all experiments.

**Instrumentation** Fluorescence spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer. CD spectra were measured on a Chirascan Circular Dichroism Spectrometer (Applied Photophysics Ltd, England, UK). UV-visible adsorption spectra were recorded on UV-2450 UV-vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Transmission electron microscopy (TEM) images were obtained using a JEM-2100 transmission electron microscope.

**Synthesis of gold nanorods** Gold nanorods were synthesized by using a seed-mediated, surfactant-assisted growth method in a two-step procedure [29, 30]. Briefly, colloidal gold seeds were first prepared by mixing aqueous solutions of hexadecyltrimethylammonium bromide (CTAB, 0.1 M, 7.5 mL) and hydrogen tetrachloroaurate(III) hydrate (1%, 0.098 mL). Freshly prepared aqueous solution of sodium borohydride (0.01 M, 0.6 mL) was then added. The colloidal gold seeds solution (0.215 mL) were then injected into an aqueous growth solution of CTAB (0.1 M, 47.6 mL), hydrogen tetrachloroaurate(III) hydrate (1%, 0.788 mL), silver nitrate (0.01 M, 0.3 mL) and freshly prepared ascorbic acid (0.1 M, 0.32 mL). The nanorods were purified by several cycles of suspension in ultrapure water, followed by centrifugation. They were isolated in the precipitate and excess CTAB was removed in the supernatant. Then, it was stored in a refrigerator at 4°C before being used. The nanorods were characterized by absorption spectroscopy.

**Fluorescence experiments** First, the fluorescence spectra of FAM-labeled GDNA were recorded on a fluorometer (F-7000, Hitachi) with excitation at 480 nm and emission range from 500 to 600 nm. Then, a certain volume of GNRs solution was added into the FAM-labeled GDNA solution. The fluorescence of this mixture is recorded within 10 min. For the study of drug-GDNA interactions, drug solution with a certain concentration was added into above mixture for fluorescence detection.

**CD Spectroscopy** The CD spectra of DNA oligonucleotides were measured for 5  $\mu\text{M}$  DNA total strand concentration using a Chirascan Circular Dichroism Spectrometer (Applied Photophysics Ltd, England, UK). CD spectra were

recorded using a quartz cell of 1-mm optical path length and an instrument scanning speed of 100 nm/min with a response time of 2 s at room temperature. CD spectra were obtained by taking the average of three scans made from 200 to 320 nm. All DNA samples at a final concentration of 5  $\mu\text{M}$  were dissolved in Tris-buffer (10 mM, pH 7.2) and heated to 90°C for 5 min, gradually cooled to room temperature, and incubated at 4°C overnight.

**UV measurements** Typically, the UV absorb spectra of GNRs were recorded on UV-vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Then, a certain quantity of GDNA was added into GNRs suspension solution. After thorough mixing, a suitable ligand was added to the mixture. The solution is vortexed thoroughly and used for the UV-vis absorption spectra measurements experiment approximately 10 min after mixing.

## Results and discussion

### FRET between F-GDNA and GNRs

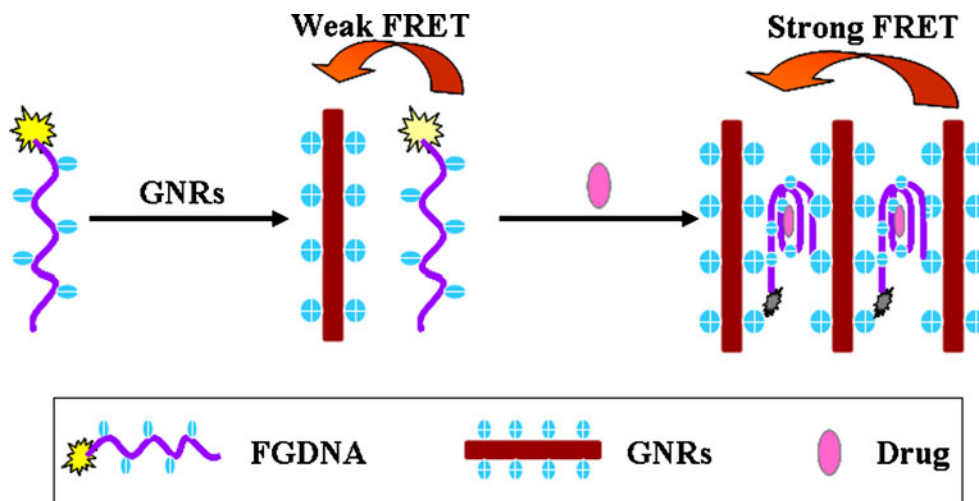
In this paper, we have designed a GNR-based FRET assay for selecting quadruplex-binding ligands using human telomeric repeat sequence as recognition probe. Figure 1 shows the sensing scheme of this approach. Briefly, when a dye-labeled oligonucleotide single-stranded probes sticks to the GNRs, the attendant proximity of the dye to the gold leads to fluorescence quenching of the dye. In the presence of quadruplex-binding ligands, the formation of G-quadruplexes strengthened the interaction between probe DNA and GNRs, resulting in the decrease in fluorescence intensity. The feasibility of this FRET protocol was first investigated by adding emodin into the mixed solution of

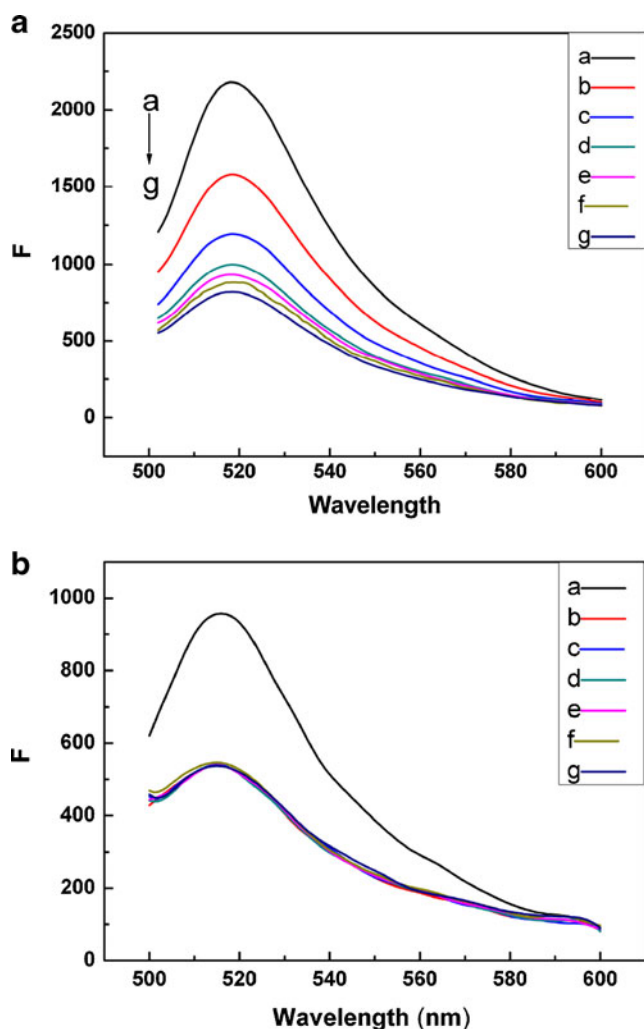
FAM-labeled GDNA and GNRs. Curve a in Fig. 2a shows the fluorescence spectra of 10 nM FAM-labeled telomere DNA (5'-FAM-TTAGGGTTAGGGTTAGGGTTAGGG-3', F-GDNA) in TE buffer at pH 7.2. It was found that the fluorescence intensity is obviously decreased by ~27.5% after mixed with gold nanorods (curve b), which indicated that FRET occurred between fluorescent dye and GNRs. That is, as GNRs was added to the F-GDNA solution, F-GDNA was adsorbed onto the surface of the positively charged GNRs because of the electrostatic interaction between the anionic backbone phosphates of the oligonucleotides and the cationic surfactant bilayer around the GNRs [28]. Upon addition of the emodin, the fluorescence intensity of F-GDNA/GNRs mixture was in an obvious decline. The observed change induced by drug addition can be ascribed to the strengthened interaction between FGDNA and GNRs due to the formation of G-quadruplex in the presence of emodin because G-quadruplex DNA has higher charge density than the ssDNA [24].

To exclude other possibilities that lead to the fluorescence reduction, the effect of ligand on fluorescence intensity of F-GDNA was first studied in the TE buffer (Figure S1, Supporting Information (SI)). Fluorescence intensity of F-GDNA has little change with the increasing concentration of emodin in the absence of GNRs. Figure S1 indicated that fluorescence decrease resulted from the electrostatic interaction between FGDNA and GNRs.

The FRET system used GNRs as fluorescent quenchers by noncovalent adsorption of DNA probes onto the surface of GNRs. A control experiment with a negative G-quadruplex binding ligand was performed to prove the specific recognition interaction between F-GDNA and emodin. According to our previous work, matrine was chosen as a negative G-quadruplex binding ligand. It is apparent from Fig. 2b that the fluorescent signal remained steady after the addition of matrine to the F-GDNA/GNRs

**Fig. 1** Schematic illustration of Gold nanorod-based FRET assay for selection of G-quadruplex-binding ligands

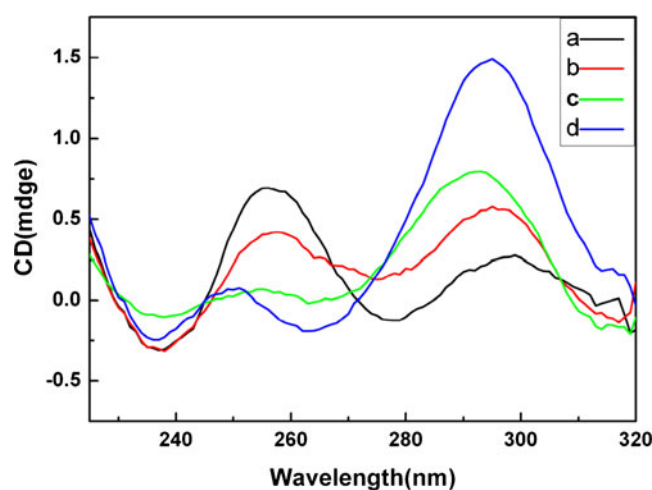




**Fig. 2** Fluorescence emission spectra of FAM-labeled GDNA at different experiment conditions. **a** *a* FGDNA in TE, *b* FGDNA/GNRs, *c–g* FGDNA/GNRs+emodin, the concentration of emodin is 5, 10, 50, 100, and 500 nM, respectively. **b** *a* FGDNA, *b* FGDNA/GNRs, *c–g* FGDNA/GNRs+matrine, the concentration of matrine is 5, 10, 50, 100, and 500 nM, respectively. Fluorescence intensity was recorded at 520 nm with an excitation wavelength of 480 nm

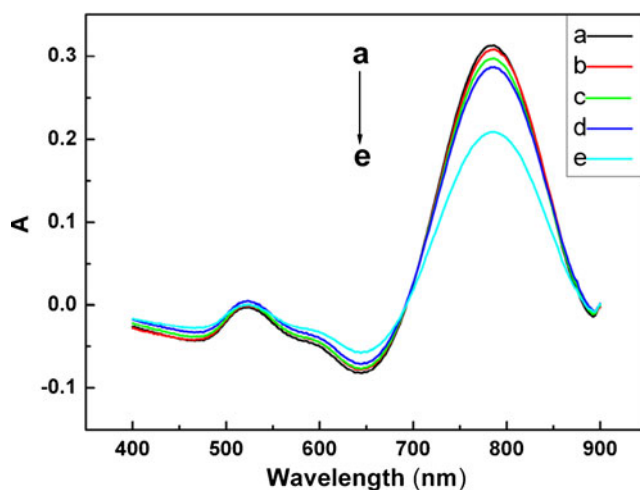
solution. Control F-DNA was designed to further verify the specific molecular recognition between F-GDNA and emodin. The fluorescence intensity of F-DNA obviously decreased after mixed with GNRs. However, there is little difference in fluorescence intensity of FDNA/GNRs solution before and after adding emodin into the FDNA/GNRs (Figure S2, SI). All these control experiments indicated that the fluorescence reduction resulted from the G-quadruplex formation in the presence of emodin, which enhanced the electrostatic interaction between DNA and GNRs.

To further validate the feasibility of this FRET system, the relationship between ligand concentration and fluorescence reduction was studied. From curve *c* to *g* as shown in Fig. 2a, the fluorescence of F-GDNA/GNRs solution continuously decreased with the concentration of emodin



**Fig. 3** CD spectra of the Human24 DNA (5  $\mu$ M) in a 10 mM Tris-HCl buffer: *a* without emodin, *b* with 10  $\mu$ M emodin, *c* with 30  $\mu$ M emodin, and *d* with 60  $\mu$ M emodin

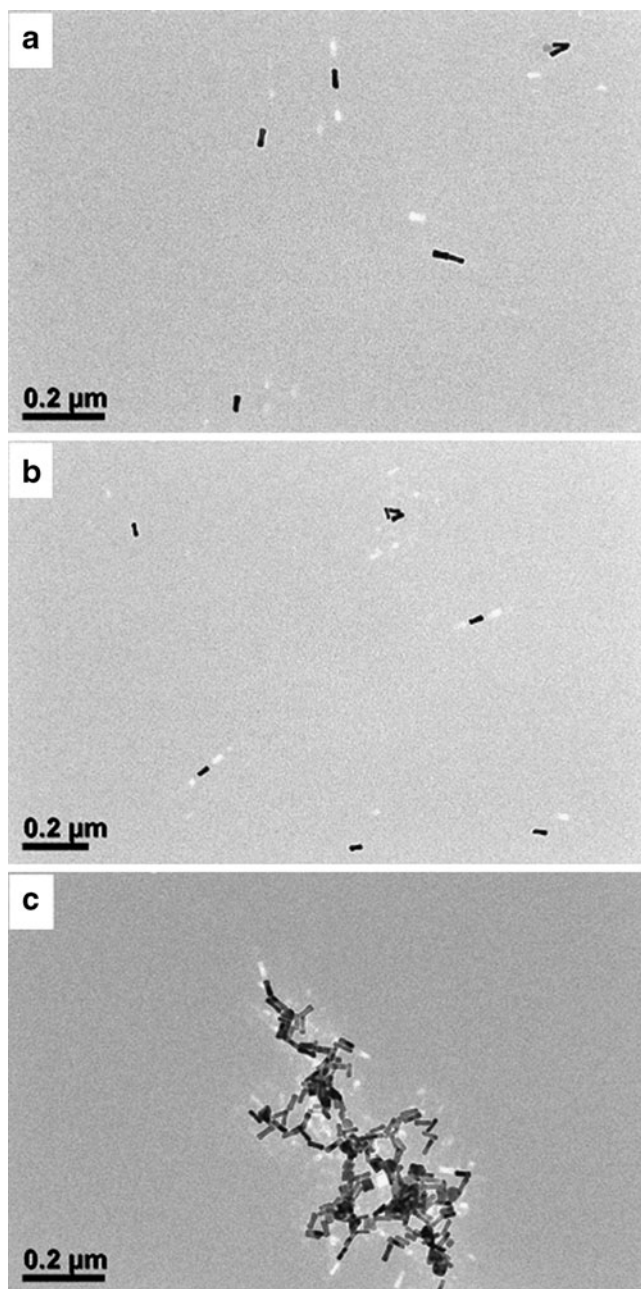
ranged from 5 to 500 nM, respectively. Upon the addition of emodin, the fluorescence of the F-GDNA/GNRs solution decreases significantly, indicating that ligand of GDNA can modulate the energy transfer between fluorophore and GNRs. That is, G-quadruplex formation has been on the increase with the rise of emodin concentration, leading to stronger electrostatic interaction between DNA and GNRs. However, the fluorescent signal has little change with the increasing concentration of matrine (Fig. 2b). The result further verified that the fluorescence reduction of F-GDNA/GNRs solution in the presence of emodin was attributable to specific binding between F-GDNA and emodin. Therefore, this FRET assay is feasible to select the quadruplex-binding ligands.



**Fig. 4** UV absorption of GNRs and GNRs-ssDNA complex in the presence of emodin. *a* GNRs, *b* GNRs/GDNA, *c–e* GNRs/GDNA+emodin, from curve *c* to curve *e*, the concentration of emodin is 250 nM, 500 nM, 1  $\mu$ M, respectively

### Identification of quadruplex formation

CD is a sensitive technology able to study the configuration inversion of DNA and can be used to conveniently monitor quadruplex formation. To understand whether G-quadruplex formed in the presence of selected ligands, we measured CD spectra. As can be seen from Fig. 3, the CD spectra of human telomeric DNA in the absence of emodin are characterized by a positive peaks at 255 nm and a negative peak at 234 nm (curve a). With the addition of

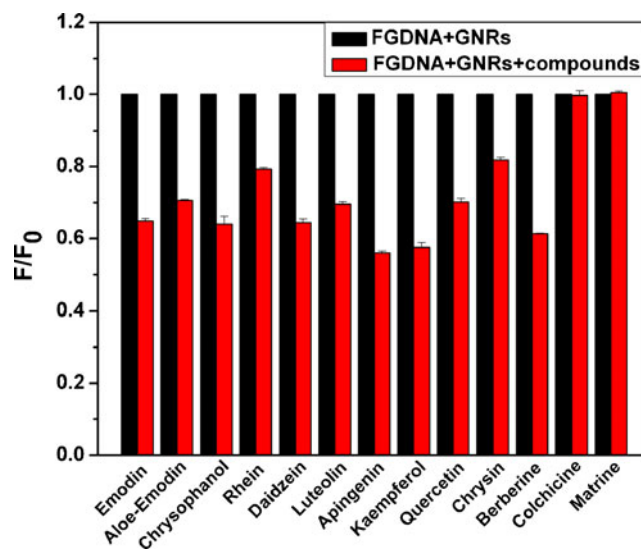


**Fig. 5** TEM images of GNR at different experiment conditions. **a** GNR, **b** GDNA+GNR, **c** GNR+GDNA+emodin. The concentration of emodin is 30 nM

emodin, we observed a concentration-dependent enhancement of the positive peak around 295 nm. At the same time, the positive peaks at 255 nm decreased and a negative peak at about 265 nm start to appear, which indicated G-quadruplex formation. That is, the human telomeric DNA underwent a conformational change and formed the G-quadruplex structure. These spectral changes were not observed when matrine was added to Human24 DNA (Figure S3 of the Supporting Information) [11, 31]. The result is in accordance with FRET measurements. These control experiments indicated that the proposed method is a reliable method to select G-quadruplex-binding ligands.

### Mechanism investigation

It is known that the UV adsorption spectra of GNRs displays two bands assigned to the transversal and longitudinal modes of electronic oscillations, and the locations depends on the aspect ratio. As shown in Fig. 4, the two UV absorb bands for our prepared GNRs are located around 518 and 783 nm. When DNA was added to the GNRs suspension, they were adsorbed onto the surface of the positively charged GNRs because of the electrostatic interactions between the anionic backbone phosphates of oligonucleotides and the cationic surfactant bilayer around the nanorods. However, the electrostatic interaction is not strong enough to change the suspension state of GNRs. So, there is little change in the UV absorb spectra. It is worth noticed that the longitudinal adsorption obviously de-



**Fig. 6** Bar chart of fluorescence response of drugs to F-GDNA/GNRs, where  $F_0$  and  $F$  are the F-GDNA/GNRs intensities in the absence and presence of drugs. *Black bars* the fluorescence response of F-GDNA/GNRs in the absence of drug. *Red bars* FRET efficiency of different drugs measured in buffer solution. *Histograms* and *error bars* represent average values and standard deviations, respectively. Relative standard deviations are all less than 5%

creased after adding emodin to the mixture of GDNA and GNRs suspension. The decrease of longitudinal adsorption indicated the aggregation of GNRs in solution [19]. These variations of adsorption features resulted from G-quadruplex formation, which strengthened the electrostatic interaction between DNA and GNRs. That is, the strong electrostatic interaction induces the aggregation of GNRs. The sensing mechanism has been further verified by TEM images. From images a and b in Fig. 5 we can see, GNRs are dispersed in the aqueous medium in the absence and presence of GDNA. However, GNRs obviously aggregated after that emodin was added into the suspension (image c), which is accordance with the UV-visible adsorption measurements. This is consistent with observations with fluorescence measurements.

**FRET selection of G-quadruplex-binding ligands** A large number of ligands reportedly interact with GDNA to form G-quadruplexes. Folding of telomeric DNA into G-quadruplexes inhibits telomerase by locking the single-stranded telomeric substrate into an inactive conformation that is no longer recognized, nor elongated, by the enzyme [32]. So identification of quadruplex-binding ligands is of great theoretical and practical importance. Here, 13 traditional Chinese medicine monomers were investigated as potential ligands using the proposed method. Daidzein, apigenin, quercetin, luteolin, kaempferol and chrysin belong to flavonoids. Emodin, aloe-emodin, chrysophanol, and rhein belong to anthraquinone. Berberine, colchicine, and matrine belong to alkaloid. They possess anti-allergic, anti-inflammatory, anti-microbial, and anticancer activities. In Fig. 6, the influences of different drugs on fluorescence intensity were demonstrated by comparing the ratios of fluorescence intensity ( $F/F_0$ ) of FGDNA/GNRs mixture, where  $F$  and  $F_0$  are the fluorescence intensity of FGDNA/GNRs in the presence and absence of these drugs, respectively. As shown in Fig. 6, the fluorescence intensity of FGDNA/GNRs further decreased after incubating with daidzein, apigenin, quercetin, luteolin, kaempferol, chrysin, emodin, aloe-emodin, chrysophanol, rhein, and berberine. However, the fluorescence intensity hardly changed after interacting with colchicine and matrine. That is, these natural drugs that enhanced the FRET efficiency between GNRs and FGDNA are G-quadruplex-binding ligands.

## Conclusion

In this paper, we have developed a fluorescence method for selection of G-quadruplex-binding ligands by GNRs-based FRET assay without expensive reagents and complicated

modification. Thirteen natural drugs were studied by the proposed method, indicating that daidzein, apigenin, quercetin, luteolin, kaempferol, chrysin, emodin, aloe-emodin, chrysophanol, rhein, and berberine can induce the human telomeric DNA to form G-quadruplex structure. The sensing mechanism has been verified by the CD, UV, and TEM measurements. The major advantages of this assay are its speed, simplicity, excellent specificity, and convenience. This method provides a useful method to rapid screening antitumor drugs and is of great theoretical and practical importance.

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