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Homogeneous Selecting of a Quadruplex-Binding Ligand-Based Gold Nanoparticle Fluorescence Resonance Energy Transfer Assay

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G-quadruplexes are higher-order DNA and RNA structures formed from G-rich sequences that are built around tetrads of hydrogen-bonded guanine bases. There is considerable interest in the design of ligands that target G-quadruplex DNA because of their potential anticancer activity. We designed a fluorescence resonance energy transfer (FRET) system to identify molecules that stabilize G-quadruplexes in a homogeneous medium using unmodified gold nanoparticles (GNPs) as a fluorescence quencher. The assay exploits the different adsorption abilities of GNPs for single-stranded DNA and double-stranded DNA. Fluorescein-tagged probe DNA adsorbed onto the surface of GNPs can quench the fluorescence of a DNA probe. Intramolecular folding of an oligonucleotide of the human telomeric sequence into a G-quadruplex structure led to fluorescence enhancement in the presence of quadruplex-binding ligands. G-quadruplex formation, induced by specific binding of GDNA ligands, was investigated by CD measurements. Melting of the G-quadruplex was monitored in the presence of putative G-quadruplex-binding molecules by measuring the absorbance at 295 nm. Two series of natural drugs were studied, and flavonoids were shown to increase the melting temperature of the G-quadruplex. This increase in the T_m value was well-correlated with an increase in FRET efficiency. The combined data from fluorescence measurements and melting experiments indicate that the FRET approach offers a simple, sensitive, and effective method to identify ligands with potential anticancer activity.

Teleomeres are essential for the stability and replication of eukaryotic chromosomes.[1,2] Telomeres generally consist of many tandem repeats of a 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] It is known that telomeres gradually shorten with each cell cycle and eventually result in celluar senescence. However, the telomere length in tumor cells is maintained and leads to cellular immortalization. Studies on telomeric DNA discovered that G-quadruplex formation could inhibit the telomere maintenance, which is potentially useful in therapeutic application. Therefore, ligands of G-quadruplex have received great attention because quadruplex-binding ligands have potential applications in genomic studies as well as therapeutic purposes. Several techniques are employed to explore the formation and properties of G-quadruplexes, including X-ray diffraction, NMR spectroscopy, circular dochroism (CD), mass spectrometry, and UV−vis spectroscopy.[5−10] Because of its sensitivity and flexibility, fluorescence spectroscopy, particularly the fluorescence resonance energy transfer (FRET) technique, has been explored as a powerful tool to study G-quadruplexes. On the basis of the conformational transition of G-DNA, it is easy to exploit the FRET technique to study the stability and molecular mechanism of G-DNA with its ligands.[11−17] Lee et al. used single-molecule spectroscopy to probe the dynamics of human telomeric DNA.[13] Folding of the DNA into a compact G-quadruplex structure yielded a smaller average distance between the donor and acceptor and, hence, displayed higher FRET than the unfolded form. Mergny et al. used a fluorescence assay to identify molecules that stabilize G-quadruplexes. Intramolecular folding of an oligonucleotide with

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four repeats of the human telomeric sequence into a G-quadruplex structure led to FRET between a donor and an acceptor covalently attached to the 5′ and 3′ ends of the oligonucleotide, respectively.[14] In general, the DNA strand was labeled with a fluorophore donor and acceptor or fluorophore and quencher at its 5′and 3′ends, respectively. This requires the difficult process of dual labeling on the same DNA strand. The development of a simple method for selecting ligands of G-DNA would be desirable.

Gold nanoparticles (GNPs), as a class of nanomaterials with many unique properties such as colorimetric, conductivity, and nonlinear optical properties, have been explored for potential applications in biomolecular detection.[18,19] GNPs are also quenchers for chromophores through energy-transfer and electron-transfer processes. Furthermore, Rothberg et al. found that single-stranded DNA (ssDNA) and double-stranded DNA have different propensity for adsorbtion of citrate-coated gold nanoparticles.[20] On the basis of the quenching capability of gold nanoparticles to the proximate fluorescent dye, GNPs have been successfully used to construct a FRET system for DNA and proteins detection.[21−24] Rothberg et al. reported a simple method to detect specific oligonucleotide sequences using gold nanoparticles as a fluorescent quencher.[21] In this paper, by taking advantage of GNPs, we have designed a simple FRET system for selecting quadruplex-binding ligands using unmodified GNPs as a fluorescence quencher. Dye-tagged probe DNA sticks to GNPs, so that fluorescence from dye-tagged single-stranded probes is quenched. When quadruplex-binding ligands are added, the formation of G-quadruplexes leads to release of the probe DNA from GNPs, and the fluorescence enhancement is observed. In this way, no ligand labeling is required, and interaction between the probe and ligand is performed in a homogeneous solution under conditions that can be specified independent of the assay. Further, the DNA strand needs only one dye labeled, leading to less laborious and more cost-effective synthesis.

Studies of binding interactions of small molecules with DNA quadruplexes are useful not only for better understanding of molecular recognition but also for the development of anticancer therapeutic agents. A number of promising small molecules have been devised to selectively promote the formation and/or stabilization of such higher-order structures,[25−31] but examples involving traditional Chinese medicine monomers are rare. Here, two series of Chinese medicine monomers were studied, and we try to explore the natural quadruplex-binding ligands. A family of planar flavonoid molecules was shown to specifically interact with an intramolecular quadruplex. 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. $HAuCl_4 \cdot 4H_2O$ was purchased from Sigma (U.S.A.). Daidzein (7,4′-dihydroxyflavone), apigenin (5,7,4′-trihydroxyisoflavone), quercetin (3,5,7,3′,4′-pentahydroxyflavone), luteolin, kaempferol, colchicine, and matrine were purchased from Nanjing TCM Institute of Chinese Material Medica (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 (5′-FAM-GTTCAT GCCGCCCATGCTCG-3′, F-DNA) were synthesized by Shanghai Sangon Biotechnology Co. (Shanghai, China) and used without further purification. The oligonucleotide stock solutions (25 μM) were prepared with a TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.2) and kept frozen. Millipore Milli-Q (18 MΩ.cm) water was used in all experiments.

Instrumentation. UV−visible adsorption spectra were recorded on a UV−vis spectrophotometer (China). Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer. Melting curve measurements were performed on a UV-2450 UV−vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan). CD spectra were measured on a Chirascan Circular Dichroism Spectrometer (Applied Photophysics Ltd., England).

Synthesis of Gold Nanoparticles. Gold nanoparticles were prepared by the citrate reduction of $HAuCl_4$ according to the literature.[32] Briefly, 1.5 mL of 1.0% trisodium citrate was added to 100 mL of boiling 0.01% $HAuCl_4$ solution. The solution was kept boiling and stirring for 15 min, and then the solution was allowed to reach room temperature. Then, it was stored in a refrigerator at 4 °C before being used. The concentration of GNPs was estimated by UV−vis spectroscopy on the basis of an extinction coefficient of 4.2×10^8 M^{-1} cm^{-1} at $\lambda = 523$ nm for 25 nm particles.

Fluorescence Experiments. First, the fluorescence spectra of FAM-labeled GDNA were recorded on a fluorometer (F-4600, Hitachi) with excitation at 480 nm and an emission range from 500 to 600 nm. Then, a certain volume of GNPs solution was added into the FAM-labeled GDNA solution. The fluorescence of this mixture was recorded within 10 min. For the study of drug−GDNA interactions, a drug solution with a certain concentration was added into the above mixture for fluorescence detection.

Melting Temperature (T_m) Measurements. All measurements were performed in 10 mM Tris-HCl buffer solutions (pH 7.2). Concentrations of GDNA and drugs were 3×10^{-6} and 1.0×10^{-4} M, respectively. Absorbance of DNAs was then mea-

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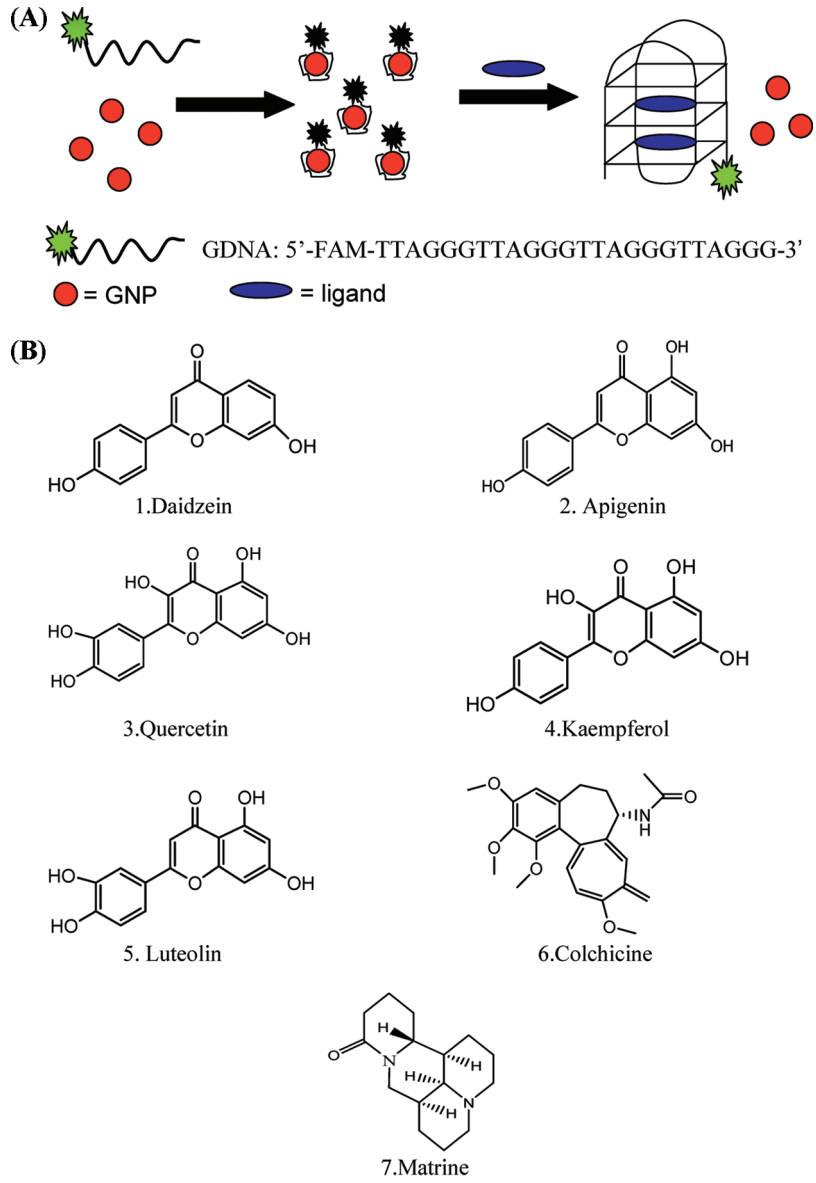

Figure 1. (A) Schematic illustration of the selection of quadruplex-binding ligand-based FRET assay using gold nanoparticles as a fluorescence quencher. (B) Structures of quadruplex ligands used in our assay.

sured at 295 nm as a function of temperature using an UV-2450 UV−vis spectrophotometer equipped with a thermoelectrically temperature controlled micro-multicell holder (eight cells, optical path length = 1 mm). The temperature ranged from 10 to 95 °C with a heating rate of 0.5 °C/min. The resulting absorbance versus temperature curves were differentiated to determine T_m values.

CD Spectroscopy. The CD spectra of DNA oligonucleotides were measured for a 5 μM DNA total strand concentration using a Chirascan circular dichroism spectrometer (Applied Photophysics Ltd., England). 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 μM were dissolved in a 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.

RESULTS AND DISCUSSION

Sensing Mechanism. In this paper, we have designed a FRET system for selecting quadruplex-binding ligands using unmodified GNPs as a fluorescence quencher. An intramolecular G-quadruplex corresponding to approximately four units of the human telomeric repeat sequence was used as probe DNA. Figure 1A describes the mechanism of the present approach for selecting quadruplex-binding ligands in the homogeneous solution. The assay is based on the GNP properties that ssDNA can adsorb onto negatively charged GNPs at a chosen ionic strength, while the G-quadruplex does not at the same ionic strength. When a dye-labeled oligonucleotide single-stranded probe sticks to the gold nanoparticles, the attendant proximity of the dye to the gold leads to fluorescence quenching of the dye. However, in the presence of quadruplex-binding ligands, the formation of G-quadruplexes leads to releasing the probe DNA from GNPs, and the fluorescence enhancement is observed. The feasibility of this FRET protocol was first investigated by adding daidzein (7,4′-dihydroxy-

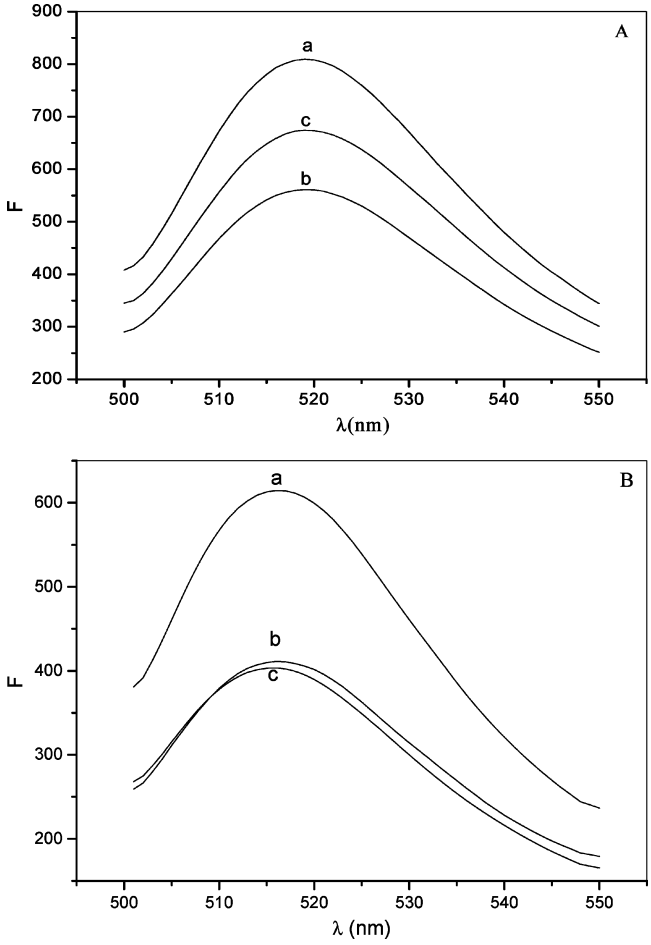

Figure 2. Fluorescence emission spectra of FAM-labeled GDNA at different experiment conditions. (A): (curve a) 20 nM FAM-labeled GDNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA), (curve b) curve a + 0.4 nM GNPs, and (curve c) curve a + 0.4 nM GNPs + 10 nM daidzein. Excitation wavelength was 480 nm. (B): (curve a) 20 nM FAM-labeled GDNA in TE buffer (10 mM Tris-HCl, 1 mM EDTA), (curve b) curve a + 0.4 nM GNPs, and (curve c) curve a + 0.4 nM GNPs + 10 nM matrine. Excitation wavelength was 480 nm.

isoflavone) into the mixed solution of FAM-labeled GDNA and GNPs. Daidzein is a flavonoid and is effective in tumor therapy. Curve a in Figure 2A shows the fluorescence spectra of 20 nM FAM-labled telomere DNA (5′-FAM-TTAGGGTTAGGGTTAGGGTTAGGG-3′, F-GDNA) in a TE buffer (10 mM Tris-HCl, 1 mM EDTA) at pH 7.2. The fluorescence intensity is obviously decreased by ~31% after mixing with unmodified gold nanoparticles (curve b of Figure 2A). To improve the FRET efficiency, the effect of the molar ratio between F-GDNA and GNPs was studied, and the optimal molar ratio is 50:1 with the F-GDNA concentration at 20 nM. The fluorescence signal of F-GDNA was quenched after adding a gold nanoparticle to the F-GDNA solution, which indicated that FRET occurred between the fluorescent dye and gold nanoparticle. That is, FAM-labeled GDNAs efficiently adsorb onto the gold nanoparticles so that their fluorescence is quenched. The adsorption mechanism of ss-DNA on the gold nanoparticle has been reported.[20] Curve c of Figure 2A is the fluorescence spectra of the mixture of F-GDNA with gold nanoparticles after adding 1 μL of daidzein, and the final concentration of daidzein is 50 nM. Fluorescence enhancement is observed after specific binding interaction in the presence of daidzein. The experimental

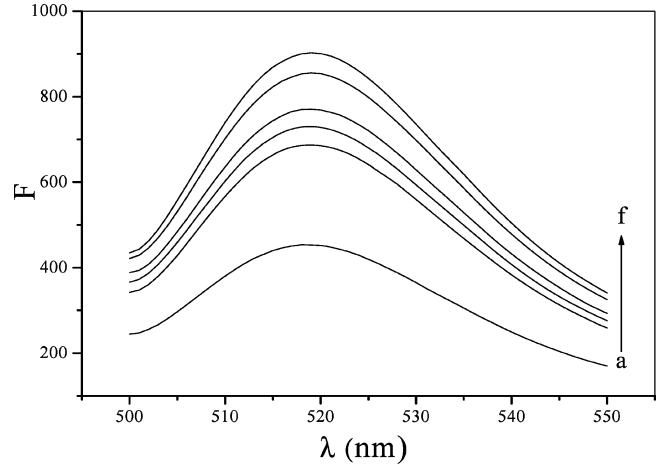

Figure 3. Emission spectra of solutions of F-GDNA/GNP with the addition of daidzein. From curve a to curve f, the concentration of daidzein is 0, 10 nM, 50 nM, 100 nM, 500 nM, and 1 μM, respectively. The concentration of F-GDNA was 20 nM, the concentration of GNPs was 0.4 nM, and the excitation wavelength was 480 nm.

result demonstrated that daidzein is a quadruplex-binding ligand. That is daidzein facilitated the folding of the single-stranded telomeric DNA into a G-quadruplex structure and stabilized the G-quadruplex structure. Prior to application of the FRET system in selecting quadruplex-binding ligands, the effect of ligands on the fluorescent signal of FDNA was first studied in the TE buffer (Figure S1 of the Supporting Information). The fluorescent signal of FDNA has little change with the increasing concentration of daidzein in the absence of gold nanoparticles. Figure S1 of the Supporting Informationindicated that fluorescence enhancement resulted from the releasing of probes from the gold nanoparticle.

The FRET system used gold nanoparticles as fluorescent quenchers by noncovalent adsorption of DNA probes onto the surface of unmodified gold nanoparticles. A control experiment with a negative G-quadruplex binding ligand was performed to prove the specificity of the ligand interactions, which leads to the releasing of the fluorescent probes. Matrine was chosen as a negative G-quadruplex binding ligand. From Figure 2B, we can see that the fluorescent signal was not obviously enhanced after the addition of matrine to the F-GDNA/GNP solution. Control FAM-labeled DNA (F-DNA) was designed to further investigate the specificity of the fluorescence enhancement induced by the selected ligand. The fluorescent signal of F-DNA is obviously decreased after mixing with unmodified gold nanoparticles. However, the fluorescent signal of the FDNA/GNP solution has little change with the increasing concentration of daidzein (Figure S2 of the Supporting Information). These control experiments indicated that the fluorescence enhancement resulted from the specific binding of this G-quadruplex binding ligand to the dye-labeled probe, which leads to the releasing of probes from the gold nanoparticle.

To further validate the feasibility of this FRET system, we studied the relationship between the ligand concentration and fluorescence enhancement. Figure 3 compares the emission spectra observed upon addition of daidzein with different concentration to solutions of F-GDNA/GNP mixtures. From curve a to curve f in Figure 3, the fluorescence of the F-GDNA/GNP solution continuously increased, with the concentration of daidzein ranging

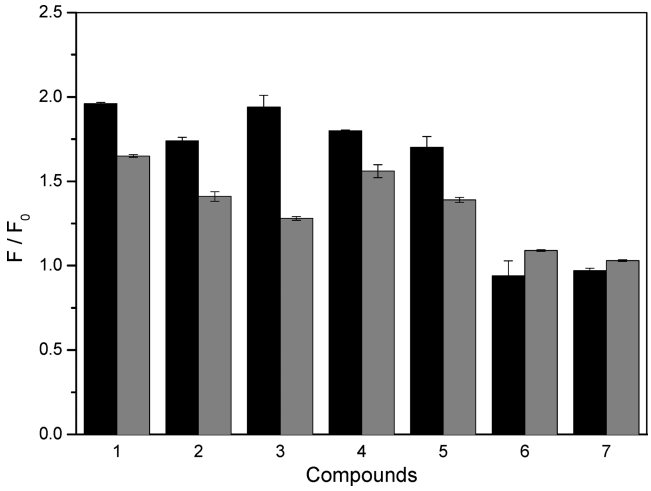

Figure 4. Bar chart of fluorescence response of drugs **1**−**7** to GDNA, where F_0 and F are the FAM fluorescence intensities in the absence and presence of drugs. Black bars: FRET efficiency of different drugs measured in a TE buffer solution (10 mM Tris-HCl, 1 mM EDTA,50 mM KCl, and pH 7.2). Grey bars: FRET efficiency of different drugs measured in a TE buffer solution (10 mM Tris-HCl, 1 mM EDTA, and pH 7.2). The structures of drugs **1**−7 are shown in Figure 1B. Histograms and error bars represent average values and standard deviations, respectively. Relative standard deviations (% RSD) are all less than 8.8%.

from 10 nM to 1 μM, respectively. Upon the addition of daidzein, the fluorescence of the F-GDNA/GNP solution increases significantly, indicating that the ligand of GDNA can modulate the energy transfer between a fluorophore and gold nanoparticle. That is, when the concentration of the ligand increased, more and more single-stranded F-GDNAs formed a G-quadruplex and were released from gold nanoparticles, which induced the enhancement of fluorescence, and the increase of fluorescence intensity can quantitatively reflect the amount of daidzein added. However, the fluorescent signal has little change with an increasing concentration of matrine (Figure S3 of the Supporting Information). The result further verified that the fluorescence enhancement of the FRET system was attributable to specific binding. Therefore, the FRET assay is feasible to use to select the quadruplex-binding ligand.

Identification of Quadruplex Formation. To understand whether G-quadruplex formed in the presence of selected ligands, we measured circular dichroism (CD) spectra. CD can be used to gain information about quadruplex structures of DNAs as well as the effects of sequence, cations, chemical modification, and ligand binding on a quadruplex structure.[33−35] Therefore, CD can be used to judge whether G-quadruplex formed after the addition of ligands to a GDNA solution. We investigated the CD spectral changes of human telomeric DNA (5′-TTAGGGTTAGGGTTAGGGTTAGGG-3′, Human24 DNA) in the presence of daidzein under stabilizing cation deficient conditions. Figure 5 shows the CD spectra for the titration of the Human24 DNA with increasing amounts of daidzein. It is known that CD spectra of linear Human24 DNA show positive peaks near 255 nm and negative peaks near 234 nm, and that antiparallel G-quadruplexes show positive and negative peaks around 295 and 265 nm, respectively, while parallel G-quadruplex structures show positive and negative peaks around 260 and 240 nm, respectively.[36,37] On the basis of this information, the structural type of a G-quadruplex can be determined from a CD measurement. Without any metal cations, the CD spectra of the Human24 DNA at room temperature exhibited a negative band centered at 236 nm, and a major positive band at 255 nm (Figure 5a). Upon the addition of 1 μM daidzein to the Human24 DNA, a dramatic change in the CD spectrum was observed (Figure 5b). The maximum at 255 nm was gradually decreased and shifted to near 245 nm, while the band centered at about 295 nm increased dramatically with an increase of the concentration of daidzein. At the same time, a major negative band at about 260 nm started to appear, indicating formation of a G-quadruplex. As the daidzein concentration increased to 10 μM (Figure 5c), the CD spectrum of this new DNA conformation was virtually identical with the CD spectra of antiparallel G-quadruplexes, where the major positive band was usually observed around 295 nm with a negative band at 265 nm. The CD intensities around 295 and 265 nm increase when 50 μM daidzein was added to Human24 DNA (Figure 5d). These spectral changes were not observed when matrine was added to Human24 DNA (Figure S4 of the Supporting Information). These results suggested that the G-quadruplex structure of human telomeric DNA is induced by a specific ligand, and the FRET system can effectively select quadruplex-binding ligands.

Identification of G4-Specific Ligands. The DNA tetraplexes or G-quadruplexes exhibit four stranded structures containing one or more nucleic acid strands. Four guanines on a plane, interacting via Hoogsteen bonding, form a G-quartet as shown in Figure 1A. Typically, two, three, or four G-quartets are stacked within a quadruplex and held together by $\pi-\pi$ nonbonded attractive interactions. G-quartet formation inhibited the telomerase activity in most cancer cells, and these telomerase inhibitors have been proposed as new potential anticancer agents. So identification of quadruplex-binding ligands is of great theoretical and practical importance. Besides metal cations, a number of promising small organic molecules have been devised to selectively promote the formation and/or stabilization of guanine-quadruplex structures. These ligands have the common feature of an extended planar aromatic electron-deficient chromophore with cationic substituents. Planar aromatic chromophores are key structural features in many G4-based telomerase inhibitors. For this reason, seven traditional chinese medicine monomers, whose formulas are shown in Figure 1B, were investigated as potential ligands using the proposed method. The compounds selected for examination in this study comprised two families of traditional chinese medicine monomers. Daidzein, apigenin, quercetin, kaempferol, and luteolin are flavonoids. Colchicine and matrine are alkaloids. Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show antiallergic, anti-inflammatory, an-

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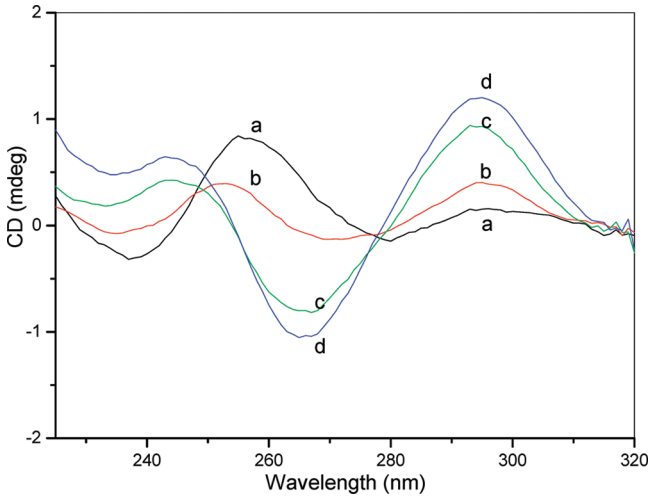

Figure 5. CD spectra of the Human24 DNA (5 μM) in a 10 mM Tris-HCl buffer: (a) without daidzein, (b) with 1 μM daidzein, (c) with 10 μM daidzein, and (d) with 50 μM daidzein.

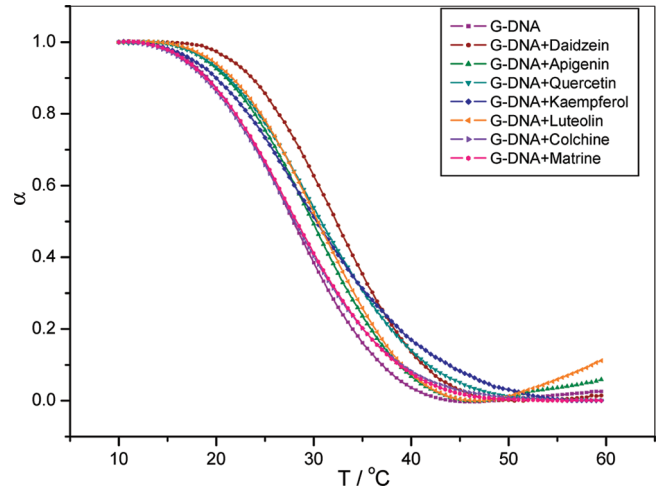

Figure 6. Effect of different drugs on the UV melting curves of TTAGGGTTAGGGTTAGGGTTAGGG. Data have been converted into fraction-folded (α) plots. The samples were prepared in 10 mM Tris-HCl (pH 7.2) and were heated at a rate of 0.5 °C min^{-1}. Oligonucleotide concentration was 3 μmol/L, and the drug concentration was 100 μmol/L.

timicrobial, and anticancer activity.[38,39] The influences of different drugs on fluorescence response were explored by measuring FRET efficiency. FRET efficiency can be obtained by measuring the increase in the fluorescence of the FAM. In Figure 4, the FRET efficiency (F/F_0) for these drugs is compared in the TE buffer solution (10 mM Tris-HCl, 1 mM EDTA, and pH 7.2), where F_0 and F are the fluorescence intensities of FAM in the absence and presence of drugs. The results demonstrated that flavonoids induced fluorescence enhancement, indicating that these flavonoids are G-quadruplex interactive compounds. However, colchicine and matrine are unable to form stable G-quadruplex structures. This is mainly attributed to the structure of colchicine and matrine.

G-quadruplex formation is known to be promoted by the presence of monovalent cations, especially by potassium ions.[40−42] So, the FRET efficiency of these compounds was measured in the TE buffer containing 50 mM KCl. From Figure 4, we found that FRET efficiency of compounds **1**−**7** increased in the presence of K^+. This is attributed to the fact that K^+ facilitates and stabilizes G-quadruplex structures. These are necessary for quadruplex stability because they coordinate the O6 guanine atoms in a G-quartet. Daidzein had the greatest FRET efficiency compared to that of other compounds, suggesting that it forms the most relatively compact structures.

Further Characterization of Quadruplex-Binding Ligands. We tried different approaches to further characterize the interaction of these drugs with quadruplex DNA. The stability of G-quadruplexes is commonly assessed by monitoring melting profiles at different experimental conditions.[43−45] The most common application of the melting curves is to compare closely related oligonucleotides or to assess the relative binding strength of different quadruplex-binding ligands. For a simple comparison, the melting temperature (T_m) of each transition is determined and compared between samples. T_m is the mid-point of a melting curve at which the complex is 50% dissociated. DNA quadruplexes show only small changes in absorbance at their UV maximum (260 nm). However, a greater signal is obtained at 295 nm, at which there is a large decrease in absorbance on melting.[43] This is, therefore, the preferred wavelength for obtaining high-quality melting data that can be used for determining thermodynamic properties of quadruplexes. A typical absorbance melting profile of GDNA is shown in Figure 6 and Figure S5A of the Supporting Information, whereas normalized data were shown in Figure S5B of the Supporting Information. T_m can then be determined accurately by taking the temperature at which the fraction folded ($\alpha = 0.5$). The fraction folded at each temperature is determined by the distance between the unfolded (high-temperature) and folded (low-temperature) states.[44] Comparison of the T_m values of different drugs enables a simple comparison of their relative stabilities. This is illustrated in Figure 7, which compares the UV melting profiles (convert into fraction-folded plots) for a series of quadruplex-binding drugs, where it is shown that the flavonoids are more stable. Drugs **1**−**7** were compared at 3 μM probe concentrations, and the results are summarized in Table 1. T_m values of flavonoids were higher than those obtained with alkaloids. As expected, the flavonoids increase the melting temperature of GDNA. There are several different ways to compare the relative affinities of quadruplex-binding ligands. The simplest is to determine the changes in melting temperature (ΔT_m) produced by different ligands at the same concentration. The data shown in Table 1 suggest that daidzein is a better quadruplex ligand than apigenin and quercetin for quadruplex stabilization under the tested conditions. At the concentration of 100 μM, daidzein

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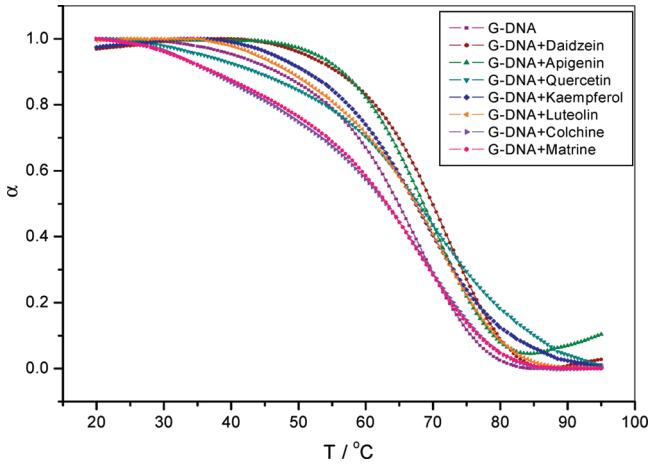

Figure 7. Effect of different drugs on the UV melting curves of TTAGGGTTAGGGTTAGGGTTAGGG in the presence of K^+. Data have been converted into fraction-folded (α) plots. The samples were prepared in 10 mM Tris-HCl (pH 7.2) containing 100 mM KCl and were heated at a rate of 0.5 °C min^{-1}. Oligonucleotide concentration was 3 μmol/L, and the drug concentration was 100 μmol/L.

stabilized the quadruplex by 5.5 ± 1.24 °C. These data indicate that the flavonoids tesed in this work prefer to bind to the F-GDNA. As expected for the dissociation of a G-quartet, T_m was dependent on the nature of the metal ion. A T_m of 65 °C was obtained in a 10 mM Tris-HCl buffer containing 100 mM KCl as compared to 56 ± 0.87 °C in a 10 mM Tris-HCl buffer containing 100 mM NaCl and 28 ± 0.72 °C in a 10 mM Tris-HCl buffer. A substantial increase in the T_m value for the GDNA−drug complex was observed in UV measurements containing K^+, suggesting that they fold into more stable secondary structures. This is consistent with observations with fluorescence measurements.

CONCLUSION

In this paper, we have developed a fluorescence method for homogeneous selecting a quadruplex-binding ligand-based gold nanoparticle FRET assay. FAM-labeled GDNA adsorbs onto gold nanoparticles via electrostatic adsorption, and the fluorescence of FAM was quenched. Intramolecular folding of an oligonucleotide into a G-quadruplex structure breached fluorescence energy resonance transfer between GNPs and fluorescent dye attached to the 5′ end of the oligonucleotide. So, quadruplex-binding ligands made the fluorescence enhanced, which was utilized to identify G4-specific ligands. Two series of Chinese medicine monomers were studied by the proposed method. Fluorescence experiments and T_m measurements indicated that flavonoids were potential quadruplex-binding ligands, and the formation of G-quadruplex was monitored by CD 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.

Table 1. Results (Mean and 95% Confidence Limits) of GDNA and GDNA−Drug T_m Measurements

oligonucleotide	compound	cation	T_m (°C)	ΔT_m (°C)
GDNA	−	−	28 ± 0.72	−
GDNA	daidzein	−	32 ± 1.1	4 ± 1.31
GDNA	apigenin	−	30.5	2.5 ± 0.72
GDNA	quercetin	−	30.5 ± 0.8	2.5 ± 1.07
GDNA	kaempferol	−	30 ± 0.74	2 ± 1.03
GDNA	luteolin	−	30	2 ± 0.72
GDNA	colchicine	−	28 ± 0.62	±0.9
GDNA	matrine	−	28 ± 0.62	±0.9
GDNA	−	K^+	65	−
GDNA	daidzein	K^+	70.5 ± 1.24	5.5 ± 1.24
GDNA	apigenin	K^+	70 ± 1.05	5 ± 1.05
GDNA	quercetin	K^+	68.5 ± 0.78	3.5 ± 0.78
GDNA	kaempferol	K^+	67.5 ± 0.87	2.5 ± 0.87
GDNA	luteolin	K^+	67.5 ± 0.74	2.5 ± 0.74
GDNA	colchicine	K^+	64 ± 1.01	−1 ± 1.01
GDNA	matrine	K^+	63.5 ± 0.87	−1.5 ± 0.87
GDNA	−	Na^+	56 ± 0.87	−
GDNA	daidzein	Na^+	59 ± 0.87	3 ± 1.23
GDNA	apigenin	Na^+	58.5	2.5 ± 0.87
GDNA	quercetin	Na^+	58.5 ± 0.87	2.5 ± 1.23
GDNA	kaempferol	Na^+	58	2 ± 0.87
GDNA	luteolin	Na^+	58.5 ± 0.62	2.5 ± 1.07
GDNA	colchicine	Na^+	55.5 ± 0.75	−0.5 ± 0.97
GDNA	matrine	Na^+	55	−1 ± 0.87

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SUPPORTING INFORMATION AVAILABLE

Additional emission spectra and melting curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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