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A new light-scattering sensor for screening G-quadruplex stabilizers based on DNA-folding-mediated assembly of gold nanoparticles†

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In this contribution, we constructed a biosensor for screening G-quadruplex stabilizers based on the assembly of gold nanoparticles functionalized with guanine-rich (G-rich) ssDNA using a light-scattering technique. We synthesized a series of metal–terpyridine complexes and investigated their affinity for quadruplex-DNA using the biosensor. Using the ratio of intensity of the light-scattering peak $(I - I_0)/I_0$, it can intuitively present the sequence of ability of stabilizing G-quadruplex DNA as follows: complex **1** > complex **3** > MTX > complex **2** > complex **5** > complex **6** > complex **7** > complex **4** > methyl green > TO > complex **8** > cisplatin. As our method allows the quantitative analysis of stabilizer affinity, EC_{50} values obtained are 4.8, 5.3, 6.1, 6.6, 6.7, 18.2, 20.2, 21.5, 32.2, 41.5 and 48.1 μM for complex **1**, complex **3**, MTX, complex **2**, complex **5**, complex **6**, complex **7**, complex **4**, methyl green, TO and complex **8**, respectively. The results have been verified by G-quadruplex fluorescent intercalator displacement (G4-FID) analysis. The proposed approach is a simple, convenient, intuitive and highly sensitive assay for screening G-quadruplex stabilizers. Our developed biosensor provides a promising tool for screening anticancer drugs.

Introduction

Telomerase has been identified as an attractive target for cancer therapy.[1] Telomeric DNA consists of single-stranded tandem repeated G-rich motifs in the 3′-terminal region and can fold into a G-quadruplex structure in the presence of a stabilizer.[2] A novel anticancer strategy is to stabilize the G-quadruplex conformation by using a G-quadruplex stabilizer thereby inhibiting activity of telomerase. A number of organic molecules and some metal complexes have been reported to efficiently stabilize G-quadruplex structures, but there are few methods for screening G-quadruplex stabilizers. So far, the interaction between the G-quadruplex and the stabilizer has been studied by circular dichroism (CD) spectroscopy,[3] UV-vis spectroscopy,[4] or fluorescence spectroscopy.[5] However, these methods have some unavoidable drawbacks: the CD method requires relatively high DNA and quadruplex stabilizer concentrations; the UV-vis method suffers from absorbance interference or requires many reagents such as ABTS, H_2O_2, hemin or the unstable reactive product; the fluorescence method is limited by cumbersome pretreatments such as thermal denaturation, complicated design, demanding expensive machines or requiring many chemicals including hemin, HPA, H_2O_2. Thus, it is highly desirable to develop a simple, sensitive, and economic method for screening G-quadruplex stabilizing compounds.

In this study, a biosensor for screening G-quadruplex stabilizers based on the assembly of Au-NP-decorated G-rich single-stranded DNA (G-rich ssDNA) using light-scattering spectroscopy was fabricated. Nanoparticles can be excellent probes to monitor biological events and their mechanisms due to their superior optical properties, especially the enhanced scattering properties.[6] In a typical assay, the G-rich ssDNA sequence, which is bound on the surface of the Au NPs, folds into G-quadruplex conformation in the presence of a stabilizer, inducing the assembly of Au NPs with a concomitant red-to-blue color change (Scheme 1).[7] Moreover, the assembly of Au NPs generates an enhanced light-scattering signal for the biosensor. The binding ability of the given compounds to G-quadruplex can be easily monitored according to the increment of light-scattering intensity of the biosensor. Therefore, on the basis of this strategy, a light-scattering method for screening G-quadruplex stabilizers was developed.

To date, metal complexes including Ni(II)-salphen, square-planar platinum(II) phenanthroline complexes, Mn(II)cationic porphyrins, and metal–terpyridine complexes have recently been shown to interact effectively with G-quadruplex structure.[8] As complexes which can inhibit telomerase activity by inducing/stabilizing G-quadruplex formation, G-quadruplex DNA is

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† Electronic supplementary information (ESI) available: Experimental details and supporting figures. See DOI: 10.1039/c3tb20291g

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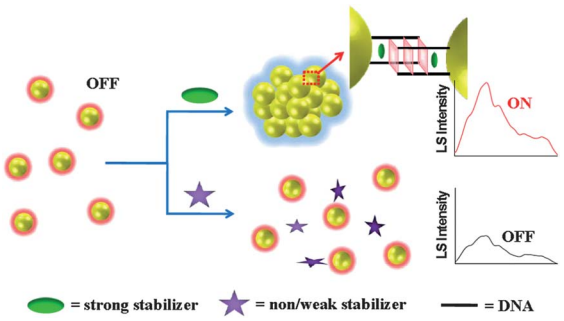
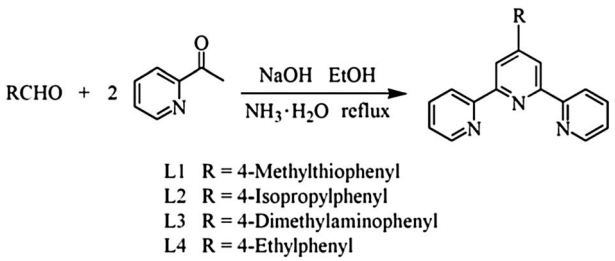


Scheme 1 A schematic illustration of the light-scattering (LS) sensor with the maximum peak located at 388 nm for screening G-quadruplex stabilizers based on the irreversible assembly of Au NPs decorated with G-rich ssDNA at room temperature.



Scheme 2 Reaction scheme for the syntheses of 4′-R-2,2′:6′,2″-terpyridine ligands **L1–L4**.

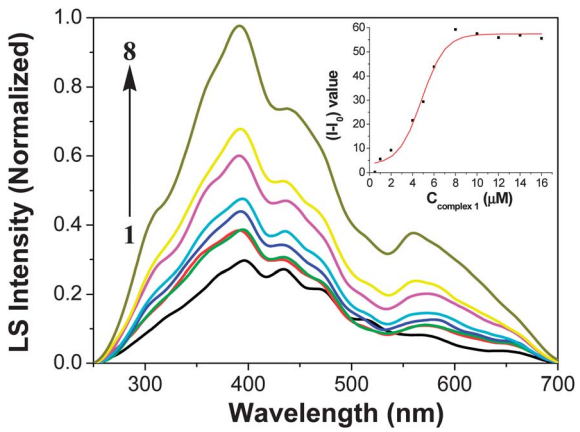


Fig. 1 The turning-on light-scattering (LS) spectra of DNA folding-mediated irreversible assembly of gold nanoparticles in the presence of metal–terpyridine complex **1** in Tris–HCl buffer (10 mM Tris, pH 7.4). The inserted figure displays the curve between the increments of light-scattering ($\Delta I_{LS} = I - I_0$) with the maximum peak located at 388 nm and various concentrations of complex **1** at room temperature. Conditions: (1) the buffer solution (blank); (2) Au NPs functionalized G-rich unit ssDNA; (3)–(8): as for (2) + complex **1**: 0.5, 1.0, 2.0, 4.0, 5.0 and 8.0 μM.

becoming an important target for the development of anticancer drugs.[8b] Numerous metal complexes have been reported as potent telomerase inhibitors which are potential antitumor drugs. Here, we synthesized a series of metal–terpyridine complexes (Scheme 2 and Fig. S1, ESI†) and studied their ability to stabilize G-quadruplex using our proposed method.

Experimental section

Chemicals

The oligonucleotides (HPLC purification) used in this paper were purchased from TaKaRa Biotechnology (Dalian, P. R. China) Co., Ltd and used without further purification. Tetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$), trisodium citrate, tris-(2-carboxyethyl)-phosphine (TCEP) was obtained from Alfa Aesar (UK). All starting materials and solvents were commercially available and used as received without further purification. The solutions were prepared with Milli-Q water (18.2 MΩ cm) from a Millipore system.

The Tris–HCl buffer solution was used to control the acidity of the solution, which was made up of 10 mM Tris solution, and proper volume of hydrochloric acid. The pH values of solutions were adjusted by a pH meter.

Preparation of gold nanoparticles

Gold nanoparticles (16 nm diameter) were synthesized according to our reported procedure.[9a] An aqueous solution of $HAuCl_4$ (100 mL, 0.01%) was brought to a vigorous boil with stirring in a conical flask, and then 3.5 mL of trisodium citrate (1.0%) solution was added rapidly. The color of the solution changed to deep red in seconds and the reduction of $HAuCl_4$ was practically completed after boiling for 7 min. The solution was cooled naturally to room temperature and then diluted to 100 mL. The average diameter of the prepared gold nanoparticles was about 16 nm as characterized by transmission electron microscope (TEM). Besides, the absorbance values at 520 nm in the UV-vis spectrum suggested it was the single size gold colloid.

Preparation of Au NPs functionalized with G-rich ssDNA

Gold nanoparticles functionalized with G-rich unit ssDNA were prepared according to the literature method with a little modification.[9] The Au NPs (4 mL) were first modified with 5′-thiol-capped G-rich ssDNA (100 μL, 10 μM, HPLC grade) which was activated by a TCEP (40 mM, freshly prepared) solution before use. The molar ratio of nanoparticles, G-rich unit ssDNA, and space ssDNA is 1 : 60 : 60. The T_{10} tether in ssDNA is used in the design of the sensor to push a recognition sequence away from the particle surface to enhance recognition and hybridization.[10] The mixture solution was magnetically stirred at room temperature for 24 hours to facilitate the hybridization. Then, the solution was stored in a drawer at room temperature for at least 24 hours. After that, the nanoparticle solutions were centrifuged and redispersed in the buffer solution. The particles were washed three more times and then redispersed in the detection buffer solution. Finally, the solution was kept in dark at room temperature for another 24 hours before used. The final concentrations of the probe solutions were estimated from their measured absorption at 520 nm and published values for extinction coefficients of the unmodified particles.[11] The UV-vis spectra were obtained by monitoring the extinction at 520 nm for the dispersed gold nanoparticles (16 nm).

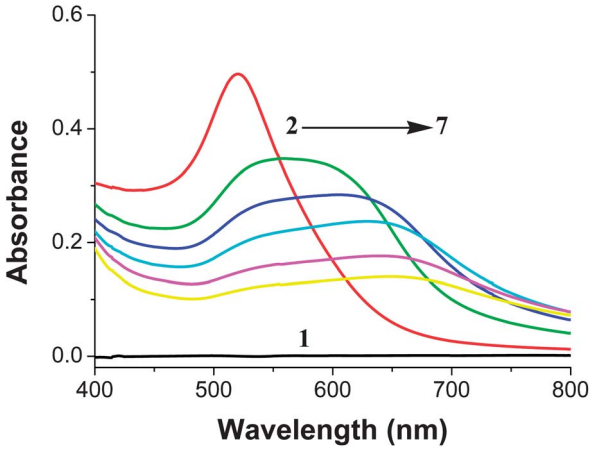


Fig. 2 UV-vis spectra of gold nanoparticles functionalized with human telomeric G-rich ssDNA (TAGGG(TTAGGG)$_3$) in the absence and presence of increasing concentrations of metal complex **1** in Tris–HCl buffer (10 mM Tris, pH 7.4). Conditions: (1) the buffer solution (blank); (2) the solution of Au NPs functionalized with G-rich ssDNA; (3)–(7) as for (2) + complex **1**: 5, 10, 15, 20 and 25 μM.

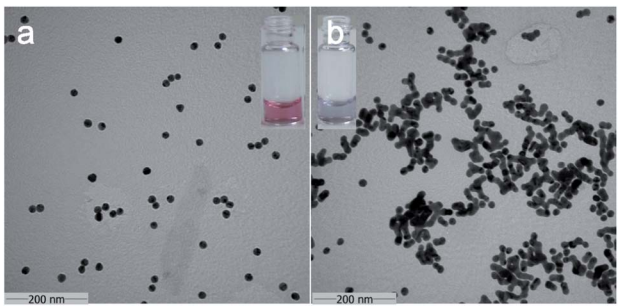


Fig. 3 Typical TEM images of samples of gold nanoparticles in the absence (a) and presence (b) of complex **1**. The inserted photos display the visual color change. Conditions: (a) the biosensor: Au NPs functionalized G-rich unit ssDNA; (b) as for (a) + 1.0 μM metal complex **1**.

Synthesis of ligands and metal complexes

The ligands **L1–L4** and metal–terpyridine complexes **1–8** were synthesized according to previously reported work.[12] The synthesis details are listed in the ESI (see Scheme 2 and Fig. S1†). Stock solutions (4 mM) of metal complexes for titration studies were prepared in DMSO and diluted with Milli-Q water. All the stock solutions were kept at −20 °C in the dark between experiments.

Light-scattering screening assay

A certain volume of G-rich ssDNA-S–Au NPs solution was added into a test tube. Then, a series of dilutions of metal complexes or drugs were pipetted into the test tubes by using microsyringes before light-scattering measurement. The light-scattering spectrum was then obtained by simultaneously scanning the excitation and emission monochromators ($\Delta\lambda$ = 0.0 nm) from 250 to 700 nm with the excitation and emission slits 5 nm. Based on the spectra, LS intensities were measured with the maximum peak located at 388 nm. Spectrum curves were made based on the data collected on the first minute after adding the metal complexes or drugs.

Instrument

^{1}H NMR spectra were recorded on a Bruker AVANCE400 spectrometer and referenced to TMS. Infrared spectra were collected from KBr disk on a Nicolet Avatar 360 FTIR spectrometer in the range of 4000–400 cm^{-1}. Elemental analyses of C, H, and N were determined with a Perkin-Elmer 2400C elemental analyzer. ESI-MS analyses were carried out using an ABI4000 Q TRAP Liquid Chromatography-Mass Spectrometer. The light-scattering spectra were measured with a model LS-55 spectrofluorometer (Perkin-Elmer, USA). Fluorescence spectra were measured with an FLS-920 picosecond fluorescence lifetime spectrometer (Edinburgh Instruments, UK). The TEM images of the colloidal gold nanoparticles were acquired on a JEM-1400 transmission electron microscope (JEOL, Japan). CD spectra were measured using a MOS-450 Circular Dichroism Spectrometer (Bio-Logic, France) equipped with a Peltier temperature controller. UV-vis spectra were recorded on an Agilent 8453 UV-vis spectrophotometer (Agilent Technologies Co. Ltd., USA). Each spectrum is an average of three scans.

Results and discussion

Strategy for screening G-quadruplex stabilizers *via* a light-scattering sensor

In a typical assay, Au NPs functionalized with G-rich unit ssDNA or space ssDNA were used (see Table S1 in the ESI†). Compared to space ssDNA, G-rich unit ssDNA had an additional three-guanine at the termini. By the formation of Au–S covalent bond, Au NPs are decorated with G-rich unit ssDNA with a random distribution in this case. In the absence of a stabilizer, the light-scattering signal is low as an "OFF" state. In this state, gold nanoparticles are well-dispersed. On the contrary, after introducing a stabilizer, it appeared the assembly of Au NPs in a cross-linking manner, which is attributed to the formation of G-quadruplex structure between Au NPs, thereby activating light-scattering signal of the biosensor and representing an "ON" state (Scheme 1).

Characteristics of light-scattering spectra

As shown in Fig. 1, the light-scattering intensity of the biosensor solution (G-rich unit ssDNA-S–Au NPs) is low in the absence of complex **1** in the whole range of scanning wavelengths. Light-scattering, which occurs when a light beam with the wavelength close to the absorption region of species, is usually applied in the detection of bioassembly processes and so on.[13] It is reasonable because the diameter of Au NPs (approximately 16 nm) is smaller than 1/20th of the incident wavelengths in the range of 250–700 nm, therefore the signal would only come from Rayleigh scattering.[14] However, in the presence of complex **1**, the aggregations of Au-NP-based biosensor were rapidly formed, which produces the enhanced light-scattering signals.[15] The light-scattering intensity of the biosensor gradually increases with the addition of increasing

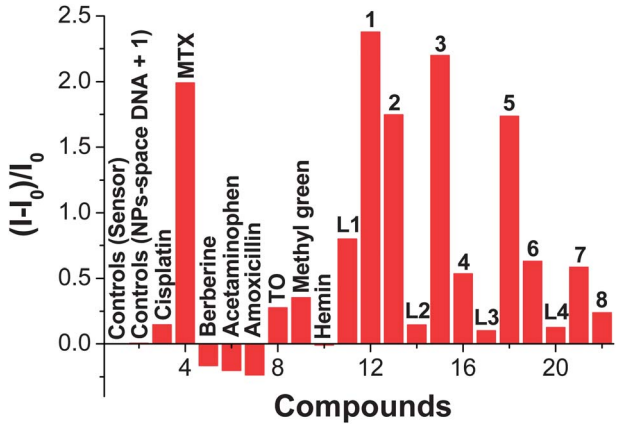


Fig. 4 Bar chart of the light-scattering response of the G-quadruplex stabilizers, where I_0 and I are the intensities in the absence and presence of the G-quadruplex stabilizers. (1) The control group: the biosensor, Au NPs functionalized with G-rich unit ssDNA; (2) the control group: Au NPs functionalized with space DNA in the presence of complex **1** (5 μM); (3) as for (1) + Cisplatin; (4) as for (1) + MTX; (5) as for (1) + Berberine; (6) as for (1) + Acetaminophen; (7) as for (1) + Amoxicillin; (8) as for (1) + TO; (9) as for (1) + Methyl green; (10) as for (1) + Hemin; (11) as for (1) + **L1** ligand; (12) as for (1) + complex **1**; (13) as for (1) + complex **2**; (14) as for (1) + **L2** ligand; (15) as for (1) + complex **3**; (16) as for (1) + complex **4**; (17) as for (1) + **L3** ligand; (18) as for (1) + complex **5**; (19) as for (1) + complex **6**; (20) as for (1) + **L4** ligand; (21) as for (1) + complex **7**; (22) as for (1) + complex **8**. The final concentration of the stabilizers is 5 μM.

concentration of complex **1**, which discloses a concentration-dependent relationship. In other words, complex **1** activates the light-scattering signal of the biosensor. In order to further verify the reasoning, K^+ or Na^+ ions, G-quadruplex stabilizer, was added in the solution of the biosensor. The results showed that an enhanced light-scattering signal also appeared, which suggest that complex **1** has a similar effect to K^+ or Na^+ ions (1000 equal) for promoting the stabilization of G-quadruplex (see Fig. S2 in the ESI†). Besides, we did the experimental assays for the studied gold nanoparticles in the presence of $Cu(NO_3)_2$. The results suggest that Cu^{2+} ions (even 100 equivalents) do not interfere with the detection of metal complex $[Cu(L)(NO_3)_2]$ by the Au-NP-based biosensor. Notably, light-scattering technique is a quite sensitive method for the determination of assembly.[9a,15a,16] Thus, the current method would be a sensitive approach for the detection of G-quadruplex stabilizer.

Characteristics of CD spectra

In order to obtain proof of the assembly of Au NPs decorated with G-rich unit ssDNA, CD spectra were recorded. In the absence of metal complexes or metal cations, CD spectrum of the human telomeric DNA $(TAGGG(TTAGGG)_3)$ exhibited a positive peak at 256 nm and a negative one at 235 nm, which suggests that human telomeric DNA exists as a single-stranded structure under these conditions. However, upon the addition of complex **1**, a dramatic change in the CD spectrum was observed. Specifically, the intensity of the positive CD band at 256 nm decreased gradually from positive to negative with continually blue shift. Meanwhile, a remarkable increase of the CD band at 295 nm and an emergence of a shoulder peak at 265 nm were observed, which reveal that complex **1** can promote G-rich single-stranded oligonucleotide to form and stabilize an antiparallel–parallel hybrid G-quadruplex structure (see Fig. S3 in the ESI†).[17] Considering the existence of steric hindrance in the G-quadruplex formation, it is inclined to form the intermolecular G-quadruplex structure instead of the intramolecular one.[18] In addition, the characteristic peak

Table 1 The comparisons of the proposed method with G-quadruplex fluorescent intercalator displacement (G4-FID) assay

Candidate drugs	Screening results	LS assay (EC_{50}, μM)	G4-FID assay (DC_{50},[a] μM)
Cisplatin	Positive	>70	—
MTX	Positive	6.1	6.3
Methyl green	Positive	32.2	30.5
TO	Positive	41.5	—
Berberine	Negative	—	—
Acetaminophen	Negative	—	—
Amoxicillin	Negative	—	—
Hemin	Negative	—	—
L1	Positive	14.35	15.1
Complex **1**	Positive	4.8	5
Complex **2**	Positive	6.6	6.5
L2	Negative	>70	—
Complex **3**	Positive	5.3	5.6
Complex **4**	Positive	21.5	22.5
L3	Negative	>100	—
Complex **5**	Positive	6.7	6.8
Complex **6**	Positive	18.2	19.5
L4	Negative	>90	—
Complex **7**	Positive	20.2	22.1
Complex **8**	Positive	48.1	50.2

[a] DC_{50} value is expressed as the concentration required to displace 50% of the TO from G-rich ssDNA.

of G-quadruplex does not appear when replacing complex **1** with ligand **L1**. Besides, mimicking the peroxidase-like DNAzyme assay revealed that it appeared G-quadruplex structure after the addition of complex **1** (see Fig. S4 in the ESI†), which is consistent with the results of CD spectra.[19] Therefore, it is concluded that the assembly of the Au-NP-based biosensor is attributed to the formation of an intermolecular G-quadruplex structure between the NPs in the presence of the stabilizer complex **1**.

Characteristics of UV-vis spectra

In order to investigate the interaction between the G-rich unit ssDNA-S–Au-NPs and complex **1**, UV-vis spectra were subsequently carried out in 10 mM Tris–HCl buffer (pH 7.4). As shown in Fig. 2, a persistent bathochromic shift appears for the maximum absorbance peak of the sensor after introducing the increasing concentrations of complex **1**. It is due to the fact that complex **1** stabilizes the formation of G-quadruplex conformation, leading to the aggregation of the DNA-linked Au NPs, and then inducing the red shift of the absorbance peak and the reduction of the absorbance intensity. As a comparison, the effect of complex **2**, K^+, and Na^+ ions on the formation of G-quadruplex was also investigated (see Fig. S5, ESI†). The results are consistent with those of the light-scattering spectra.

Moreover, the ability of complex **1** to bind G-quadruplex was evaluated by the melting temperature (T_m) assay which is based on the monitoring of the stability imparted by a ligand to a G-quadruplex structure. In the presence of complex **1**, the Au NPs decorated with G-rich ssDNA form aggregates and exhibit the characteristic melting transitions with a high melting temperature, which suggests the transition of DNA conformation (see Fig. S6, ESI†).

Characteristics of TEM

The size and morphology of the Au-NP-based biosensor were characterized with transmission electron microscopy (TEM). TEM images (Fig. 3a) showed that gold nanoparticles are generally spherical and well proportioned with an average diameter around 16 nm. The dispersed Au NPs formed large DNA-linked aggregation particles when the addition of the metal complex **1** with a concomitant of red-to-blue colour change (Fig. 3b), which is consistent with the results of light-scattering spectra and UV-vis spectra.

Application-screening as G-quadruplex stabilizers

To test the utility of the proposed method, metal complexes and drugs were used as G-quadruplex stabilizers. The light-scattering spectra of screening G-quadruplex stabilizers are displayed in Fig. S7 and S8.† The first curve is the blank group which is the buffer solution while the second curve is the control group which is the solution of Au NPs functionalized with G-rich unit ssDNA without adding any drugs (or complexes). As shown in Fig. S7,† according to the light-scattering intensity at the maximum peak, the sequence of compounds is as follows: complex **1** > complex **3** > complex **2** > complex **5** > complex **6** > complex **7** > complex **4** > complex **8** > **L1** > cisplatin > **L2** > **L4** > **L3**. The higher the affinity ability for G-quadruplex, the higher the light-scattering intensity was. Thus, it was easy to obtain the sequence of the ability of stabilizing G-quadruplex structure as follows: complex **1** > complex **3** > complex **2** > complex **5** > complex **6** > complex **7** > complex **4** > complex **8** > **L1** > cisplatin > **L2** > **L4** > **L3**. The experimental results displayed that complexes are much more potent G-quadruplex binders than the corresponding free terpyridine ligands. It is due to the electron-deficient planar aromatic system and high polarization through the coordination interaction between metal ions and ligand, which favors π-stacking on the external G-quartet and an electrostatic interaction with negatively charged DNA. Moreover, Cu-complexes (complexes **1**, **3**, **5** and **7**) were found to have higher ability of stabilizing G-quadruplex than Zn-complexes (complexes **2**, **4**, **6** and **8**) (Fig. S7, ESI†). This is due to the different geometries of the complexes: Cu-complexes have geometries of pseudo-square pyramidal structure which features one flat face, promoting π-stacking on the external G-quartet while Zn-complexes adopt trigonal bipyramidal geometries, leading to the steric hindrance of both faces.[20] Fig. S8† suggests that it induced significantly enhanced light-scattering intensity of the sensor in presence of drugs (MTX, methyl green, TO, or cisplatin), which reveals that four drugs are strong G-quadruplex binders. Because of having an extended planar aromatic electron-deficient chromophore, MTX promotes the formation and stabilization of G-quadruplex conformation, leading to the enhancement of light-scattering signal of the biosensor. However, as amoxicillin and acetaminophen only contain one aromatic ring, they could not strongly bind to G-quadruplex.

The influences of these compounds on the light-scattering signal of the biosensor were demonstrated by comparing the ratios of LS intensity $(I - I_0)/I_0$, where I_0 and I are the intensities in absence and presence of G-quadruplex stabilizers. Using the ratio of light-scattering peak intensity $(I - I_0)/I_0$, the sequence of affinity ability of G-quadruplex stabilizers can easily be obtained as follows: complex **1** > complex **3** > MTX > complex **2** > complex **5** > complex **6** > complex **7** > complex **4** > methyl green > TO > complex **8** > cisplatin (Fig. 4). The bar figure suggests that four drugs (amoxicillin, acetaminophen, berberine, and hemin) gave negative results, which were consistent with the previous report.[5b] Moreover, our developed method allows the quantitative analysis of stabilizer affinity. According to the curves of optical density *versus* drug concentration, EC_{50} value, which represent the drug concentration required for 50% enhancement of the light-scattering intensity, can be calculated. The obtained EC_{50} values are 4.8, 5.3, 6.1, 6.6, 6.7, 18.2, 20.2, 21.5, 32.2, 41.5 and 48.1 μM for complex **1**, complex **3**, MTX, complex **2**, complex **5**, complex **6**, complex **7**, complex **4**, methyl green, TO and complex **8**, respectively (Table 1). The results were verified by G-quadruplex fluorescent intercalator displacement (G4-FID) *in vitro* analysis that can evaluate the interaction between the metal complex and the G-quadruplex,[21] which demonstrates the availability of light-scattering screening method (Table 1).

Mechanism

The principle of screening G-quadruplex stabilizers by using a light-scattering biosensor is shown in Scheme 1. In the absence of a stabilizer, Au-NP-based biosensors are well-dispersed, giving the low light-scattering signal. Upon the addition of a stabilizer, the "TTAGGG" at the terminal of ssDNA on the surface of gold nanoparticles can fold into a G-quadruplex between nanoparticles, which promote the DNA-mediated cross-linking assembly of gold nanoparticles (Scheme 1).[7a] The results from the CD spectra, peroxidase-like DNAzyme assay, and the melting transitions assay display the formation of G-quadruplex structure. The quadruplex structures contain guanine quartets (G-quartets). As a G-quartet consists of four guanines, it is only likely to produce the intermolecular G-quadruplex for the "TTAGGG" fragment decorated on gold nanoparticles. The aggregation turns on the light-scattering signal of the biosensor. In our assays, strong G-quadruplex stabilizers can promote G-quadruplex mediated aggregation of Au NPs, which produces a significantly enhanced light-scattering signal. In contrast, non- or weak G-quadruplex ligands cannot form the G-quadruplex structure, which generates a low light-scattering signal. As different stabilizers have different abilities to stabilize G-quadruplex conformation, they create the distinct enhancement of light-scattering. The ability of the stabilizing G-quadruplex can be intuitively monitored according to the increment of the light-scattering intensity of the Au NPs-based biosensor. Therefore, the proposed approach may be used as a primary screening technique for G-quadruplex stabilizers.

Conclusions

In summary, a new method for screening G-quadruplex stabilizers based on Au-NP-based biosensors using a light-scattering technique was proposed. We synthesized a series of metal–terpyridine complexes and investigated their affinity for G-quadruplex DNA by our developed method. As comparisons, clinical drugs were used as G-quadruplex binder candidates in screening assays. The differentiation of G-quadruplex stabilizers including drugs and metal–terpyridine complexes by using the light-scattering sensor was intuitively observed, and was verified by G4-FID analysis. Compared with the reported fluorescence method and UV-vis method, our developed method has the following advantages: first, it is a simple and fast process without requiring many reagents or cumbersome pretreatment. Second, it is a convenient and rapid screening method due to having intuitive screening results. Third, it only requires low DNA and complex concentration. Last but not least, the reactive product is stable. Moreover, as for the anticancer activity of G-quadruplex stabilizers, the nanosensor provides a useful tool for the primary screening of anticancer drugs.

Acknowledgements

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