Supplementary Information

A Highly Sensitive Resonance Rayleigh Scattering Method to Discriminate a Parallel-Stranded G-Quadruplex from DNAs with Other Topologies and Structures

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1. Table S1

Sequence of oligomers used in this study

Oligomers	Structure or topology	Topology Ref.	Sequence (from 5' to 3')
ssDNA	single strand		CACACACACACACACACACAC
duplex	linear duplex strands		dT_{20}-dA_{20}
triplex	triplex strands		dA_{20}-dT_{20}-dA_{20}
i-motif	i-motif	S1	CCCTAACCCTAACCCTAACCC
PS2.M	antiparallel-stranded G-quadruplex	S2	GTGGGTAGGGCGGGTTGG
TBA	antiparallel-stranded G-quadruplex	S3	GGTTGGTGTGGTTGG
c-myc (pu18)	parallel-stranded G-quadruplex	S4	AGGGTGGGGAGGGTGGGG
GT DNA	antiparallel-stranded G-quadruplex	S5	GGGGTTTTGGGG
myc-1245	parallel-stranded G-quadruplex	S4	TGGGGAGGGTTTTTAGGGTGGGGA
[B7]3-0	parallel-stranded G-quadruplex	S6	ATTGGGAGGGATTGGGTGGG

2. The optimum experimental conditions for the reaction

To explore the impact of various factors on the usage of RRS method for discriminating a parallel-stranded G-quadruplex from DNAs with other topologies and structures, the effect of Mg^{2+} concentration and the incubation time on RRS intensity were investigated.

Fig. S1 shows the effect of Mg^{2+} concentration on RRS intensity of c-myc solution. When the Mg^{2+} concentration is lower than 0.015 M, the RRS intensity keeps almost unchanged. However, when the Mg^{2+} concentration is higher than 0.015 M, the RRS intensity get an abrupt increase till the Mg^{2+} concentration of 0.03 M and then increase gradually with increasing Mg^{2+} concentration. The RRS intensity reaches the maximum and keeps stable when the Mg^{2+} concentration is higher than 0.1 M. So, 0.1 M Mg^{2+} is chosen in this experiment.

In addition, the effect of incubation time on RRS intensity was also studied. As shown in Fig. S2, the reaction rate is very fast under the experimental condition and it needs only 5 min to accomplish the reaction. So, after mixed thoroughly for 5 min, the solution can be used for the determination of RRS intensity.

Based on above experimental results, the incubation of c-myc with 0.1 M Mg^{2+} for more than 5 min is the best condition for discriminating a parallel-stranded G-quadruplex from DNAs with other topologies and structures.

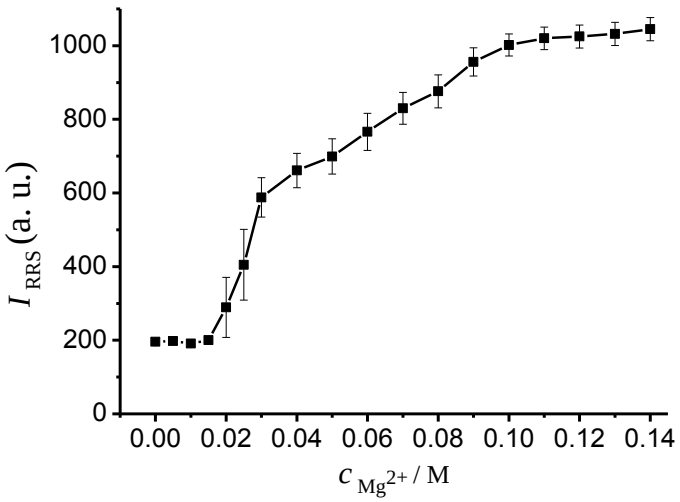

Fig. S1 The effect of Mg^{2+} concentration on RRS intensity. The concentration of c-myc was 0.1 μM. Buffer: Tris-HCl buffer (10 mM, containing 150 mM KCl, pH7.4). Incubation time, 5 min. The error bars represent the standard deviation of three measurements.

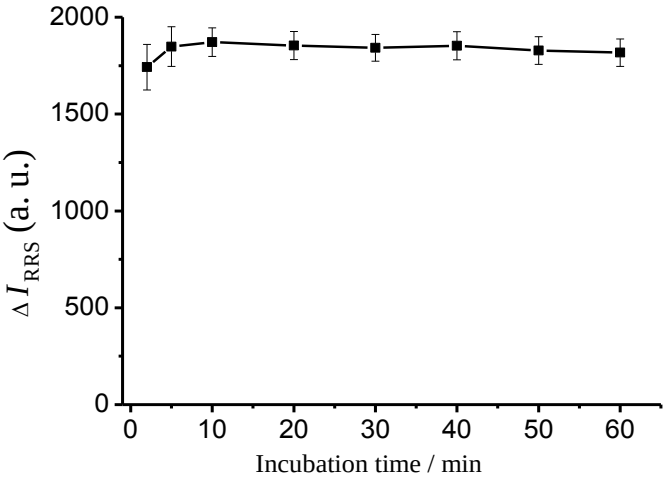

Fig. S2 The effect of incubation time on RRS intensity. The concentration of Mg^{2+} and c-myc was 0.1 M and 0.2 μM, respectively. Buffer: Tris-HCl buffer (10 mM, containing 150 mM KCl, pH7.4). The error bars represent the standard deviation of three measurements.

3. Fig. S3

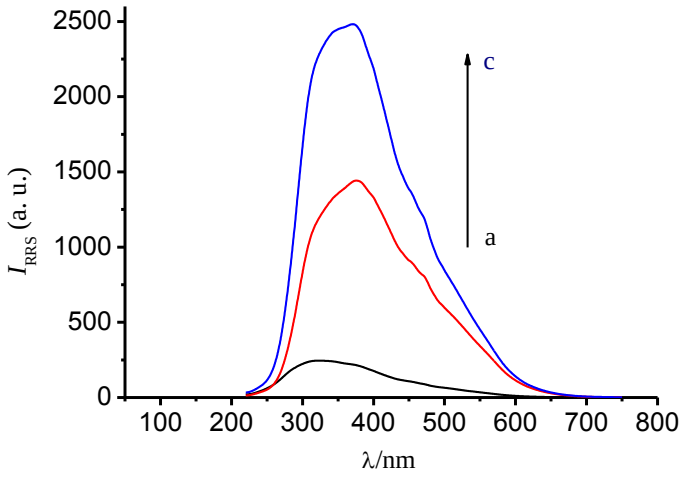

Fig. S3 RRS spectra of myc-1245 and [B7]3-0. (a) Mg^{2+}, (b) Mg^{2+}+myc-1245, (c) Mg^{2+}+[B7]3-0. The concentrations of Mg^{2+} and oligomers are 0.1 M and 0.2 μM, respectively. Buffer: Tris-HCl buffer (10 mM, containing 150 mM KCl, pH 7.4). Incubation time, 5 min.

4. The recognition of nanoscale parallel-stranded G-quadruplex in mixed DNA oligomer sample

Fig. S4 shows the comparison of RRS intensities between mixed DNA oligomer sample (with or without c-myc) and c-myc sample in Mg^{2+} solutions. The mixed DNA oligomer samples contain all the oligomers (with or without c-myc) shown in Table S1. The concentration of each oligomer is the same and indicated by the abscissa of Fig. S4. From the figure, it can be seen that the RRS intensities of the mixed DNA oligomer sample without c-myc exhibits much lower RRS intensity compared to c-myc sample and mixed DNA oligomer sample with c-myc. While, the mixed DNA oligomer sample with c-myc exhibits little difference with c-myc sample. Therefore, the changes of RRS intensities are the result of the increased concentration of c-myc, bearing no relations with the concentration of the other oligomers. Hence, this RRS method can not only be used to discriminate a parallel-stranded G-quadruplex from DNAs with other topologies and structures, but also to recognize nanoscale parallel-stranded G-quadruplex in mixed DNA oligomer sample.

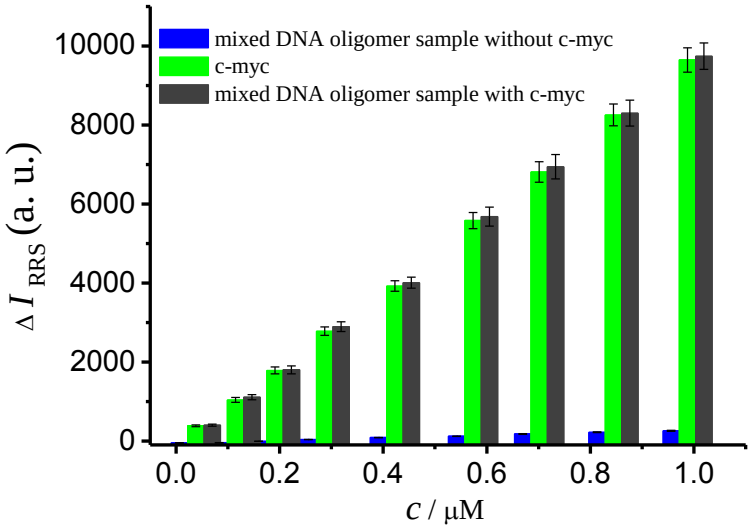

Fig. S4 The comparison of RRS intensities between mixed DNA oligomer sample (with or without c-myc) and c-myc sample in Mg^{2+} solutions. The mixed DNA oligomer samples contain all the oligomers (with or without c-myc) shown in Table S1. The concentration of each oligomer is the same and indicated by the abscissa. The concentration of Mg^{2+} was 0.1 M. Buffer: Tris-HCl buffer (10 mM, containing 150 mM KCl, pH7.4). Incubation time: 5 min. The error bars represent the standard deviation of three measurements.

5. Fig. S5

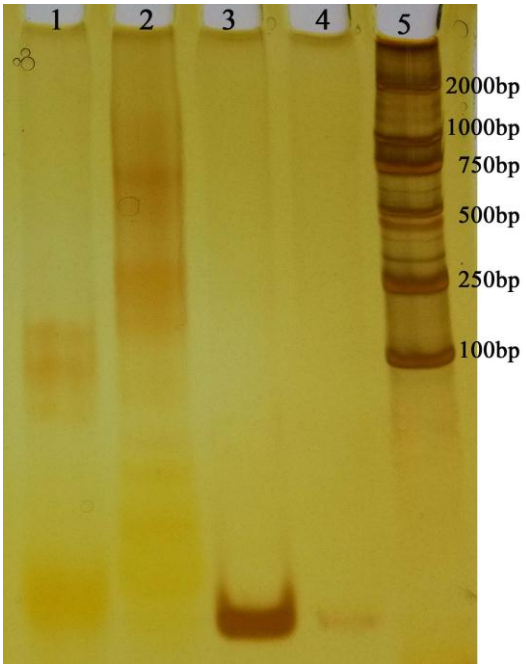

Fig. S5 C-myc and GT DNA resolved on 15 % polyacrylamide gel visualized with silver staining. Lane 1: GT DNA in the presence of Ca^{2+}, lane 2: c-myc in the presence of Mg^{2+}, lane 3: c-myc in the absence of Mg^{2+}, lane 4: GT DNA in the presence of Mg^{2+}, lane 5: a DNA size marker.

6. Fig. S6

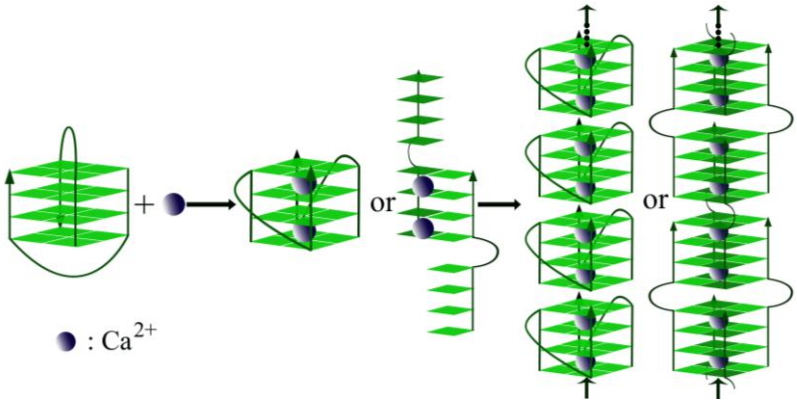

Fig. S6 Schematics for possible mechanism of GT DNA from an antiparallel to a parallel-stranded G-quadruplex by Ca^{2+}. K^{+} is not shown.

7. The effect of incubation time on the structural transition of GT DNA

The effect of incubation time on the structural transition of GT DNA was investigated. It can be seen from Fig. S7 that the RRS intensity of GT DNA is enhanced drastically at first, but reaches the maximum and keeps almost stable 40 min later. So, it needs at least 40 min to accomplish the reaction under the experimental condition and after mixed thoroughly for 40 min, the solution can be used for the determination of RRS intensity. In this article, 1 h was chosen as the incubation time for the structural transition. As the incubation time of GT DNA is longer than that of c-myc, we guess that the course of structural transition and final G-wire formation of GT DNA is time-consuming; and this is also supported by the previous studies[S7].

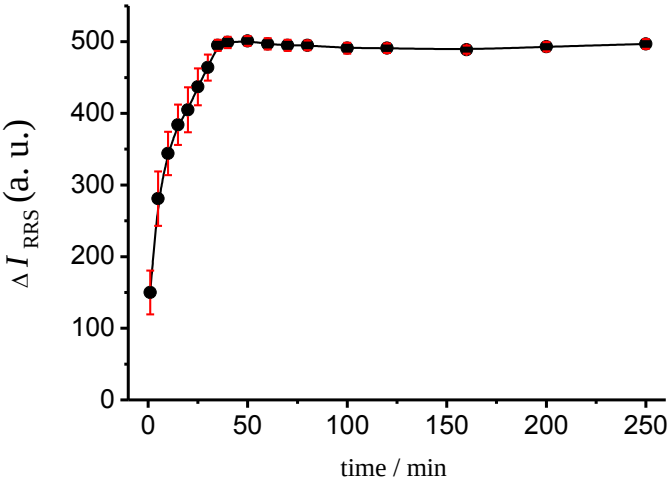

Fig. S7 The effect of incubation time on the structural transition of GT DNA. The concentrations of Ca^{2+} and GT DNA were 0.1 M and 0.9 μM, respectively. Buffer: Tris-HCl buffer (10 mM, containing 150 mM KCl, pH 7.4).

8. Materials and methods

Materials

A Hitachi F-4500 spectrofluorophotometer (Hitachi Ltd., Tokyo, Japan) equipped with a 150-W xenon lamp was used for recording the RRS spectrum and measuring the RRS intensity at a given wavelength with the measurement parameters including the slits (ex/em) of 10/10 nm and the PMT voltage of 400 V.

All the oligomers, potassium chloride, magnesium chloride, and calcium chloride were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and dissolved in Tris-HCl buffer (10 mM, containing 150 mM KCl, pH 7.4). All the oligomers (Table S1) were dissolved in suitable amount of Tris-HCl buffer (10 mM, containing 150 mM KCl, pH 7.4) to a 100 μM solution and heated at 95 °C for 10 min to dissociate any intermolecular interaction followed by a slow cooling to room temperature, respectively. Subsequently, the solution was put in refrigerator at 4 °C for 24 h. The sample at the required concentration was diluted from the stock by using Tris-HCl buffer (10 mM, containing 150 mM KCl, pH 7.4). After this treatment, an aliquot (1 μL) of sample was taken for redetermination of its concentration using the following method. The concentration of oligomer was accurately quantified using UV-vis absorption spectroscopy with the following extinction coefficients (ε_{260} nm, expressed in units of M^{-1} cm^{-1}) for each nucleotide: A = 15400, G = 11500, C = 7400, T = 8700. For the determination of RRS intensity of all the oligomer samples, the final volume was 600 μL. All other chemicals not mentioned here were of analytical reagent

grade and were used as received. All experiments were carried out using ultrapure water.

Methods

Discrimination of a parallel-stranded G-quadruplex: To 500 μL of Tris-HCl buffer solution, 37.5 μL of 1.6 M $MgCl_2$ was added, then suitable amounts of oligomer solution was added, and this solution was then diluted to 600 uL with Tris-HCl buffer solution and mixed thoroughly. After 5 min, the RRS spectrum and intensity of the mixed solution was measured.

Structural transition of GT DNA: To 500 μL of Tris-HCl buffer solution, 37.5 μL of 1.6 M $CaCl_2$ (or $MgCl_2$) was added, then suitable amounts of GT DNA solution was added, and this solution was then diluted to 600 uL with Tris-HCl buffer solution and mixed thoroughly. After 1 hour, the RRS spectrum and intensity of the mixed solution was measured.

RRS Determination: In a common spectrofluorophotometer, the RRS spectra of the solution were recorded by synchronously scanning with λex = λem. The RRS intensity of the oligomer solution and the reagent blank were regarded as I_{RRS} and I_0, respectively, at the maximum scattering wavelength. $\Delta I_{RRS} = I_{RRS} - I_0$.

Gel electrophoresis: The extent of G-wire formation was determined by electrophoresis on 15% native polyacrylamide gels and it was visualized with silver stainning. All the samples were prapared at the concentration of 10 μM and incubated for 2 h at room temperature. The samples were mixed 1:1 with loading buffer (6×Loading Dye) and applied to the gels without boiling. The electrophoresis was run at 10 °C, 120 V in 1× TBE buffer for about 90 min.

9. References

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