

## Electronic Supplementary Information (ESI)

### Quinaldine red as a fluorescent light-up probe for i-motif structures

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#### Reagents

Quinaldine red (2-(4-Dimethylaminostyryl)-1-ethylquinolinium iodide) was bought from J&K Scientific Ltd. (Beijing, China). Quinaldine red stock solutions were prepared with ultrapure water and stored in the dark at -20 °C. DNA oligomers were obtained from Genewiz (Suzhou, China). DNA stock solutions were first prepared with ultrapure water, then concentrations were accurately quantified by 260 nm UV absorbance, and finally stored at -20 °C. Other chemical reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). PB (Na<sub>2</sub>HPO<sub>4</sub>/KH<sub>2</sub>PO<sub>4</sub>, 10 mM) buffer was used in the experiment, if not particularly indicated.

#### Instruments and methods

F-4600 fluorescence spectrometer (Hitachi, Tokyo, Japan) was utilized to monitor the fluorescence spectra of quinaldine red. The excitation wavelength was fixed at 550 nm and emission spectra were recorded from 570 to 800 nm. The absorption spectra of quinaldine red were recorded on a Lambda 25 spectrometer (PerkinElmer, Singapore) from 350 to 700 nm. The circular dichroism (CD) spectra were collected by a Chirascan-plus Circular Dichroism Spectrometer (Applied Photophysics Ltd., Surrey, UK).

#### Samples preparation

Samples were first prepared by diluting DNA stock solutions with PB buffer, then heated to 50 °C for 10 min, and finally cooled slowly to room temperature. Other components, such as QR, were added into the above solutions as required. These samples were incubated for 10 min at room temperature before measurement (absorption, fluorescence and CD data).

#### Melting temperature determination

Melting temperature experiments were performed in 10 mM PB buffer (pH 6.0) using CD. CD signals at 290 nm were recorded from 20 °C to 95 °C at a scan rate of 1 °C /min by using a quartz cuvette of 0.05 cm path length. Melting temperatures were calculated according to the fitted logistic equation generated from the Origin 8.0 software.

## Binding constant ( $K_a$ ) calculation

The binding constant  $K_a$  was obtained based on the fluorescence titration assay. The data from titration assays were analyzed by nonlinear curve fitting to the equation below<sup>1,2</sup>:

$$\frac{F}{F_0} = 1 + \frac{F_{max} - F_0}{2F_0} \left[ \frac{1}{K_a C_{dye}} + 1 + \frac{nC_{DNA}}{C_{dye}} - \sqrt{\left( \frac{1}{K_a C_{dye}} + 1 + \frac{nC_{DNA}}{C_{dye}} \right)^2 - 4 \frac{nC_{DNA}}{C_{dye}}} \right]$$

Where,  $F_0$ ,  $F$  are the fluorescence intensity of quinaldine red in the absence and presence of DNA, respectively;  $F_{max}$  denotes the fluorescence intensity of totally bound quinaldine red and  $n$  represents the putative number of binding sites on a given DNA matrix.

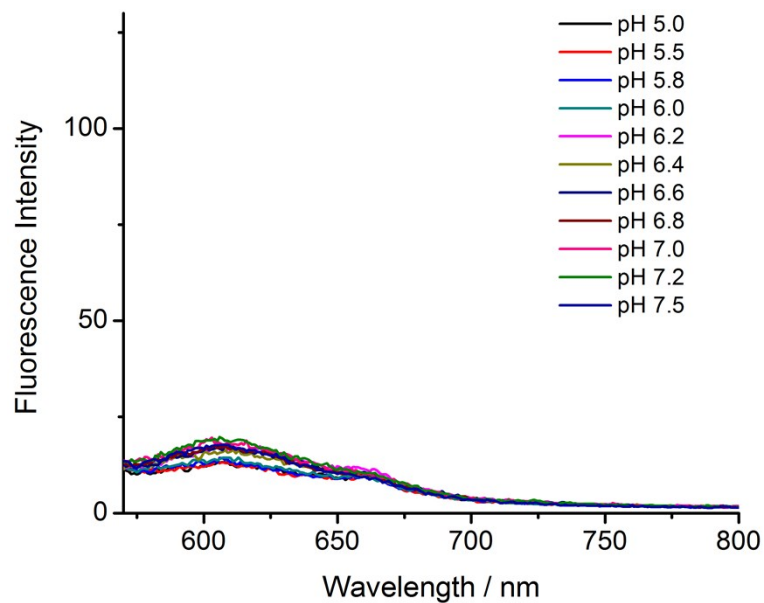
The binding constant ( $K_a$ ) and  $F_{max}$  were obtained using the Origin 8.0 software, where the value of  $C_{dye}$  (quinaldine red) was  $2 \times 10^{-6}$  and the value of  $n$  was 1 based on 1:1 binding.

## Molecular docking

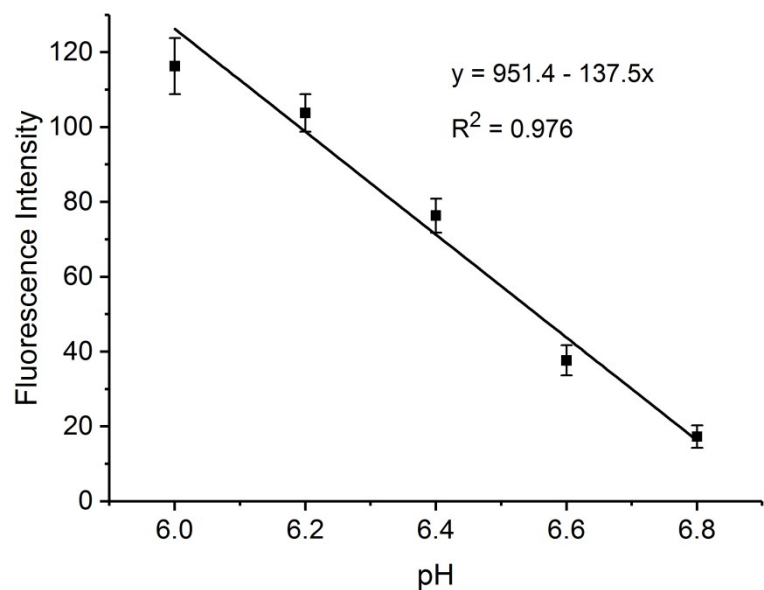
The structure of the modified human telomere fragment d[CCCTA<sub>2</sub>mCCCTA<sub>2</sub>CCCUA<sub>2</sub>CCCT] (PDB: 1EL2) was retrieved from the Protein Data Bank. The structure of quinaldine red was first optimized with the Gaussian 09 package using the B3LYP method with the 6-31G\* basis set.<sup>3</sup> The flexible ligand docking on the entire surface of the rigid receptor was then done by using the Autodock 4.2 program.<sup>4</sup> Docking calculations were carried out using the Lamarckian genetic algorithm (LGA) and the number of GA runs was set as 100. Other parameters were used as default. From the 100 conformations obtained by the docking studies, the most probable conformation with the lowest energy was identified.

**Table S1** DNA sequences used in this experiment

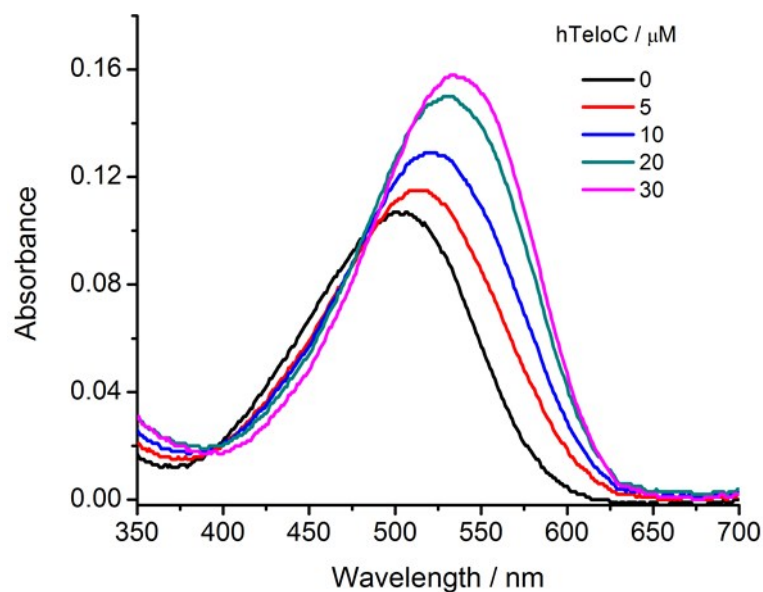
name	DNA sequences (5'-3')
hTeloC	TAACCCTAACCCCTAACCCCTAACCC
hTeloG	AGGGTTAGGGTTAGGGTTAGGG
A12	AAAAAAAAAAAAA
T12	TTTTTTTTTTTTT
ss23a	TATAATACACGTAGCATCTGACA
ss23b	TGTCAGATGCTACGTGTATTATA



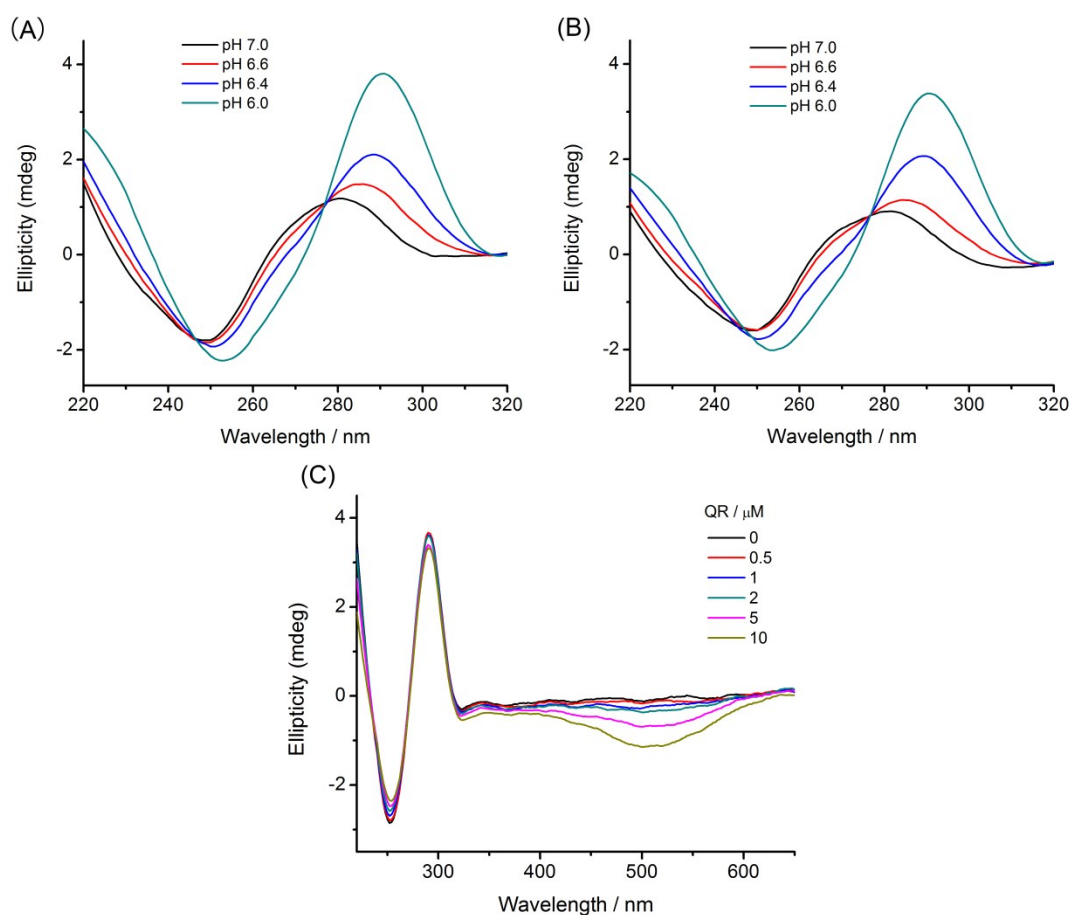
**Figure S1** The effect of pH on the fluorescence spectra of quinaldine red (ca. 2  $\mu$ M) in the absence of DNA.



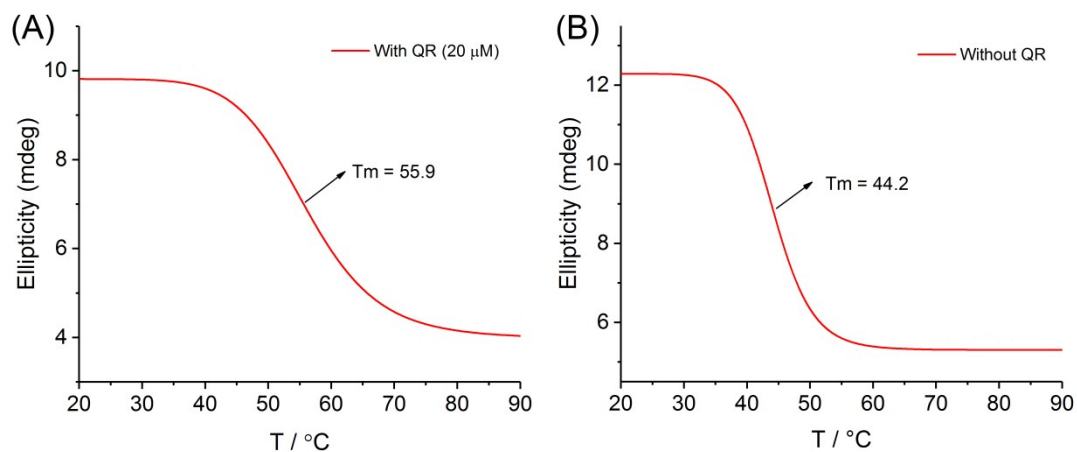
**Figure S2** Linear relationship between pH and fluorescence intensity at 630 nm of quinaldine red (ca. 2  $\mu$ M) in the presence of hTeloC (2  $\mu$ M).



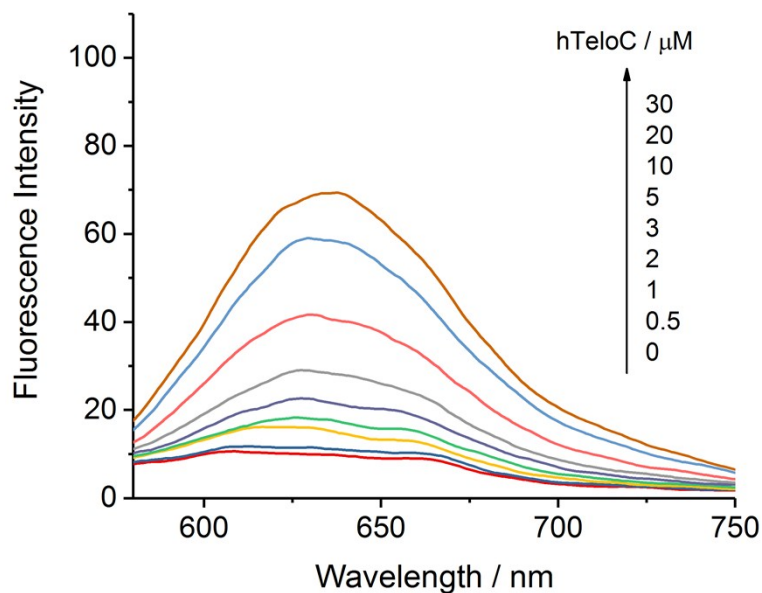
**Figure S3** The absorption spectra of QR (ca. 5  $\mu\text{M}$ ) as the function of the concentration of hTeloC i-motif at pH 6.0.



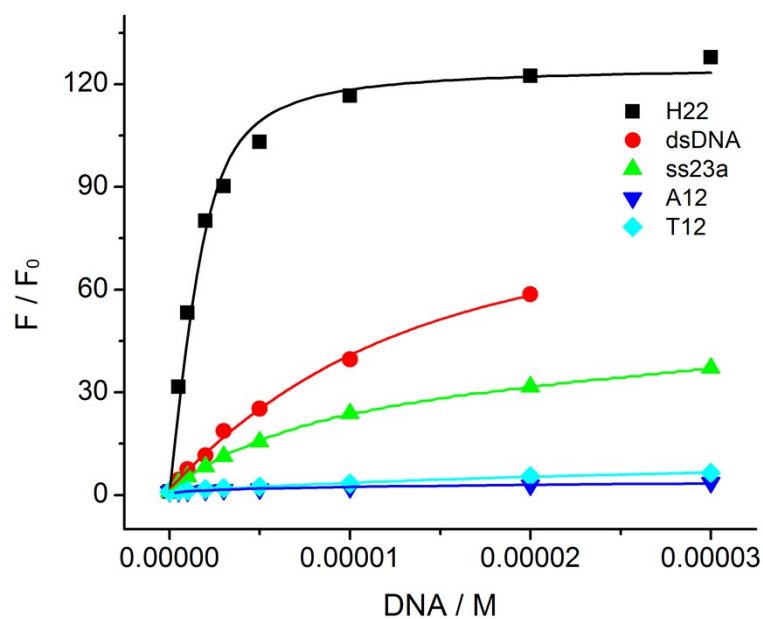
**Figure S4** Circular dichroism (CD) spectra of hTeloC (2  $\mu\text{M}$ ) at different pH values in the absence (A) and presence of QR (ca. 6  $\mu\text{M}$ ). (C) CD spectra of hTeloC (2  $\mu\text{M}$ ) in the presence of different concentration of QR at pH 6.0. CD spectra were recorded in a quartz cuvette of 1 cm path length.



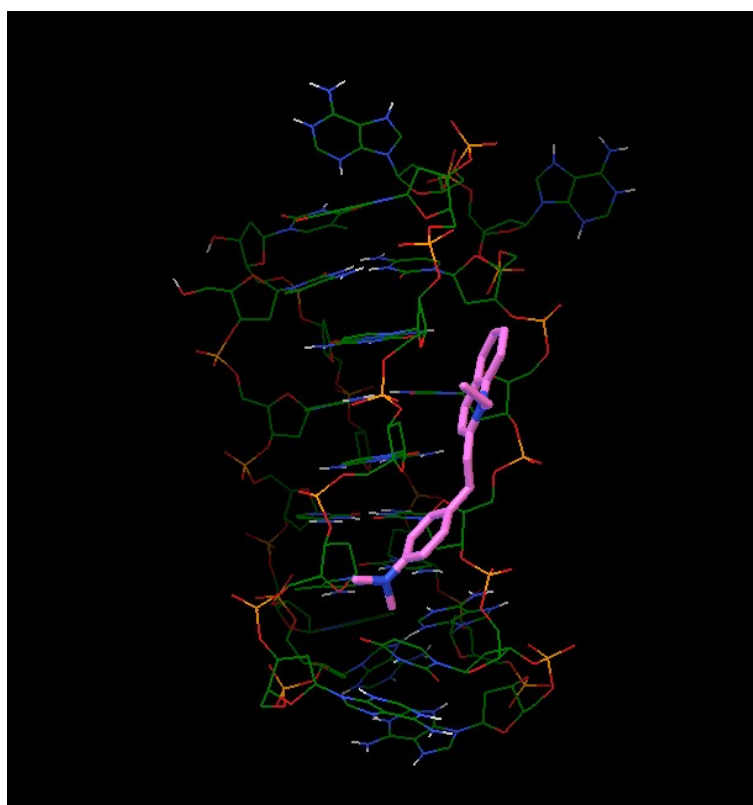
**Figure S5** Melting curves of the hTeloC i-motif (20  $\mu\text{M}$ ) with (A) and without (B) QR (20  $\mu\text{M}$ ) at pH 6.0. The melting temperatures ( $T_m$ ) are 44.3 and 55.9  $^{\circ}\text{C}$  in the absence and presence of QR, respectively. CD signals at 290 nm were recorded in a quartz cuvette of 0.05 cm path length.



**Figure S6** Fluorescence spectra of QR (2  $\mu\text{M}$ ) at pH 7.5 as a function of hTeloC concentrations.



**Figure S7** Fluorescence intensity of QR (ca. 2  $\mu$ M) in PB buffer (pH 6.0) upon titrating with hTeloG G-quadruplex at 635 nm (■), duplex DNA (dsDNA) at 635 nm (●), single-stranded ss23a at 630 nm (▲), A12 at 625 nm (▼), and T12 at 625 nm (◆). The concentration of DNA was presented as the molar strand concentration. Duplex DNA with arbitrary sequences was formed from ss23a and ss23b in PB buffer containing 2 mM MgCl<sub>2</sub>. G-quadruplex hTeloG was formed in 10 mM PB (Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub>) containing 10 mM KCl.



**Figure S8** Stereostructure showing the groove binding mode of QR for the motif.

## References

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