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Electronic Supplementary Information

A Label-free Fluorescent Molecular Switch for DNA Hybridization Assay Utilizing a G-quadruplex-selective Auramine O

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Experimental section

Chemicals and Apparatus

Due to its limited solubility in water, AO (Fluka, Switzerland) was dissolved in ethanol when high concentrations were required. All oligonucleotides (sequences shown in Table S1) were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and each stock solution was made with ultrapure water (18.2 MΩcm, Milli-pore Milli-Q purification system). All other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Metal ions in salt nitrates were used as delivered, and stock solutions were prepared by using ultrapure water. All working solutions were prepared with Tris-HCl buffer solution (pH 7.4).

UV-visible absorption spectra were recorded on a Carry 60 UV/Vis spectrophotometer (Varian, Inc. Agilent Technologies). A circular quartz cell with a path length of 1 cm was used. All fluorescence measurements were performed on a Cary Eclipse fluorescence spectrofluorometer (Varian, Inc. Agilent Technologies). Under the excitation wavelength of 430 nm, the fluorescence spectra were recorded from 450-650 nm. Both the excitation and emission slits were set for 5 nm and the scan rate was 300 nm/min. pH was measured by a pHS-3C acidometer (Shanghai Precision & Scientific Instrument Co., Ltd, China).

The CD spectra were collected on a Bio-Logic MOS-450 CD instrument (Bio-Logic, France), of which the lamp was always kept under a stable stream of dry purified nitrogen (99.99%) during experiments. The spectra were measured in the wavelength range 220 −500 nm using a quartz cuvette with 1.0 cm path length. The scanning speed of the instrument was set to 100 nm/min, and the response time used was 2 s. The background of the buffer solution was subtracted from the CD data.

Uv-vis Characterization of the Interaction between AO and Human Telomeric DNA Sequence (htDNA)

For evaluation of the interaction between AO and G-rich ssDNA with Uv-vis spectroscopy, a 20 μL (10 μM) aliquot of the AO solution was added with a micropipet into the quartz cell containing 2 mL Tris-HCl buffer solution and 50 μM htDNA. Uv-vis spectra were recorded immediately after fully mixing AO with the G-rich ssDNA.

AO Induced and Stabilized G-quadruplex and Fluorescence Hybridization Assay

For evaluation of the AO induced and stabilized G-quadruplex and fluorescence enhancement, a 20 μL (10 μM) aliquot of the AO solution was added with a micropipet into the quartz cell containing 2 mL Tris-HCl buffer solution, and then htDNA or G4 MS was added with definite concentrate gradient. Fluorescence emission spectra were recorded immediately after fully mixing nucleic acid target, AO and the htDNA or G4-MS.

Designation	**Sequence (from 5′ to 3′)**
htDNA	AGGGTTAGGGTTAGGGTTAGGG
Capture DNA	GGGTTAGGGTCCTTTGTTTGTGGGTTAGGG
ctDNA	CACAAACAAAGGA
smDNA	CACAAAAAAAGGA
rDNA	CACGGGAGGGGGA

Tabel S1 DNA sequences used in the work.

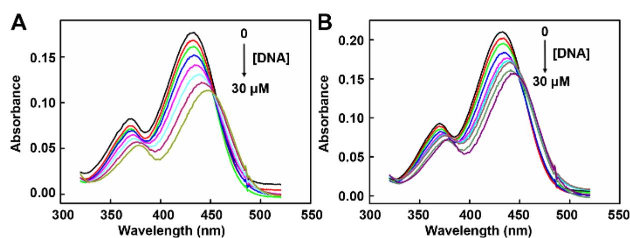

Fig. S1 The absorption spectra of 10 μM AO caused by different concentrations of htDNA in buffered Tris-HCl (10 mM, pH 7.4) solutions in the presence (A) and absence (B) of 50 mM K^+.

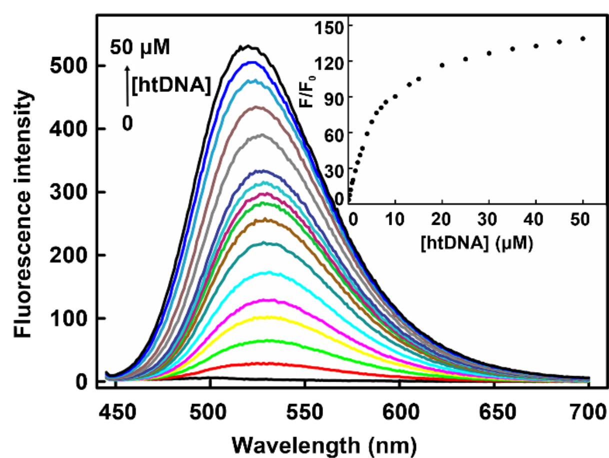

Fig. S2 Fluorescence titration of 10 μM AO in Tris-HCl buffer solutions by different concentrations of htDNA in the absence of K^+. Inset: Fluorescence intensity enhancement (F/F_0) was plotted toward different concentrations of htDNA. F and F_0 are fluorescence intensities of DNA solutions with and without htDNA, respectively.

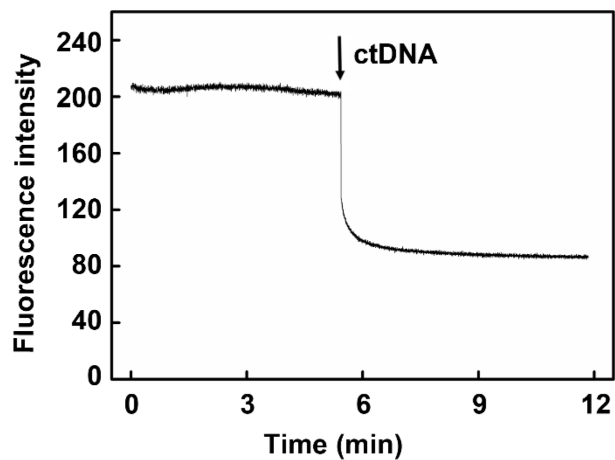

Fig. S3 Advancement of the hybridization of G4-MS with AO as a function of time after addition of 5 μM ctDNA. Fluorescence intensities were recorded at 510 nm with an excitation wavelength of 430 nm.