Electronic Supplementary Material (ESI) for Analyst.
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Supporting Information for

Specific G-quadruplex structure recognition of human telomeric RNA over DNA by fluorescently activated hyperporphyrin

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Experimental Section

Materials and reagents. RNA and DNA species (Table S1) were synthesized by TaKaRa Biotechnology Co., Ltd (Dalian, China) and purified by HPLC. The nucleic acid concentrations were measured by first dissolving RNA and DNA in pure water and detecting the UV absorbance at 260 nm using extinction coefficients calculated by nearest neighbor analysis. PPIX and TBr_dPP were purchased from Aladdin Industrial Corp. (Shanghai, China) and BioChemPartner Ltd. (Shanghai, China), respectively, while MPIX and ZnPPIX were purchased from Sigma Chemical Co. (St. Louis, USA). TOH_dPP, NMPPIX, TM_dPP, TCPP, and TOHPP were obtained from J&K Scientific Ltd. (Shanghai, China) and used as received. All the structures of the investigated porphyrins were presented in Scheme S1. Milli-Q water (18.2 mΩ; Millipore Co., Billerica, USA) was used in all experiments with a filtration treatment through a BioPak filter (Millipore Corp., Bedford, MA) in order to remove RNase. All other chemicals were analytical-reagent grade (Sigma Chemical Co., St. Louis, USA) and used without further purification.

Fluorescence measurements. Fluorescence spectra were acquired with a FLSP920 spectrofluorometer (Edinburgh Instruments Ltd., Livingston, UK) at 20 ± 1 °C, which was equipped with a temperature-controlled circulator (Julabo Labortechnik GmbH, Seelbach, Germany). Fluorescence was measured in a quartz cell with path length of 1 cm. To prepare the G4 solution, the strand was annealed in a thermocycler (first at 92 °C, then slowly cooled to room temperature) and stored in 4 ^oC overnight. Porphyrins at the specified concentrations were added into the G4 solutions, and the resulting solutions allowed to incubate for 20 min before fluorescence measurements. Tris-HCl (50 mM, pH 7.8) containing 1 mM EDTA, 3 mM $Mg(Ac)_2$, 10% methanol, and 100 mM KCl (or NaCl) was optimized as the appropriate buffer to give the hyperporphyrin spectra.

For the measurement of binding constant, TOH_dPP was titrated with TERRA G4 and the resultant fluorescence intensity at 732 nm was plotted as a function of the G4 concentration. The data were fitted by KaleidaGraph (Synergy Software, PA) according to a 1:2 binding model. The G4

concentration gradually increased and thus finally gave saturation in fluorescence. For the DNA G4, due to the small change in fluorescence upon titration, the fitting for the binding constant was not attempted.

The stoichiometry of TOH_dPP binding to the TERRA G4 was determined by Job's plot analysis. The total concentration of TOH_dPP and TERRA G4 was maintained at 2 μM and the TOH_dPP-to-G4 concentration ratio was sequentially varied.

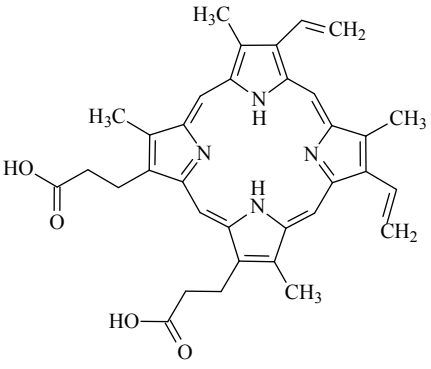
The involvement of the backbone phosphate oxygen atoms of TERRA in the interaction with TOH_dPP was roughly estimated by gradually increasing Mg^{2+} concentration and keeping the K^+ concentration at 0.1 M.

UV/Vis absorption spectra. UV/Vis absorption spectra were determined with a UV2550 spectrophotometer (Shimadzu Corp., Kyoto, Japan) at room temperature using a quartz cell with a path length of 1 cm.

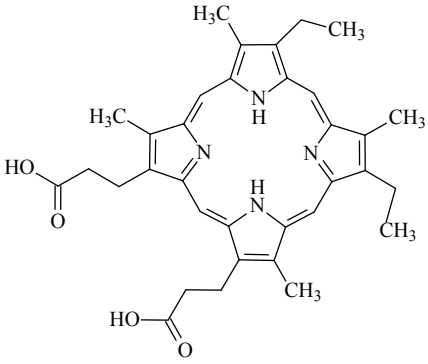
DNA Melting temperature (T_m) measurements. The melting temperatures (T_m) of G4 in the absence and presence of TOH_dPP were determined using a UV2550 spectrophotometer (Shimadzu Corp., Kyoto, Japan), equipped with a TMSPC-8 T_m analysis system which can simultaneously control the chamber temperature and detect up to eight samples with a micro multi-cell. The absorbance of G4 at 295 nm as a function of solution temperature between 5 °C and 100 °C was collected in 0.5 °C increments, with a 30-second equilibration time applied after each temperature increment. In order to give an overall sigmoidal melting curve in the investigated temperature region, the Tris buffer (50 mM, pH 7.8) containing only 20 mM KCl and 1 mM EDTA was used.

Table S1 Oligonucleotides used in this work

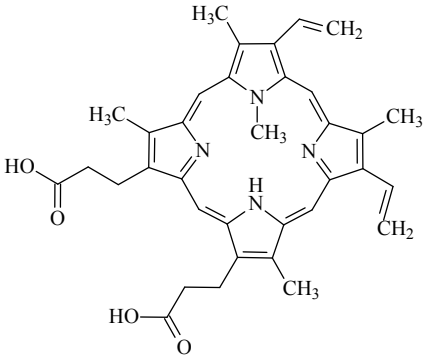
Entry	Name	Sequence	Remark
1	rUAG4	r(5′-UAG$_3$(UUAG$_3$)$_3$-3′)	TERRA G4
2	rAG4	r(5′-AG$_3$(UUAG$_3$)$_3$-3′)	
3	rUUAG4	r(5′-UUAG$_3$(UUAG$_3$)$_3$-3′)	
4	rUUAG4UUA	r(5′-UUAG$_3$(UUAG$_3$)$_3$UUA-3′)	
5	1XAV	5′-TGAG$_3$TG$_3$TAG$_3$TG$_3$TAA-3′	DNA G4
6	TAG4	5′-TAG$_3$(TTAG$_3$)$_3$-3′	
7	AG4	5′- AG$_3$(TTAG$_3$)$_3$-3′	
8	TTAG4	5′-TTAG$_3$(TTAG$_3$)$_3$-3′	
9	TTAG4TTA	5′- TTAG$_3$(TTAG$_3$)$_3$TTA-3′	
10	T$_3$TT	5′-G$_3$TTTG$_3$TG$_3$TG$_3$-3′	
11	T$_3$TT$_3$	5′-G$_3$TTTG$_3$TG$_3$TTTG$_3$-3′	
12	2O3M	5′-(AG$_3$)$_2$CGCTG$_3$AGGAG$_3$-3′	
13	AG4T	5′- AG$_3$(TTAG$_3$)$_3$T-3′	
14	TTAG4T	5′- TTAG$_3$(TTAG$_3$)$_3$T-3′	
15	PS2.M	5′-GTG$_3$TAG$_3$CG$_3$TTGG-3′	



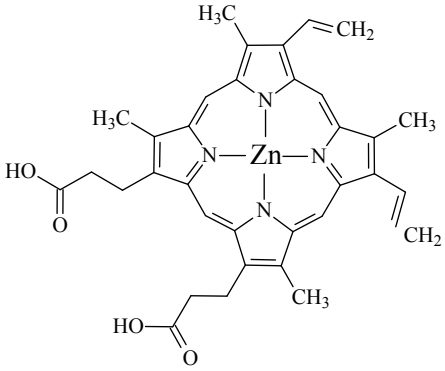
protoporphyrin IX (PPIX)



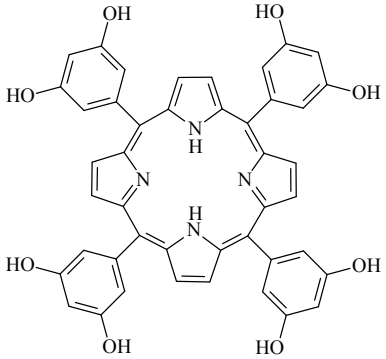
mesoporphyrin IX (MPIX)



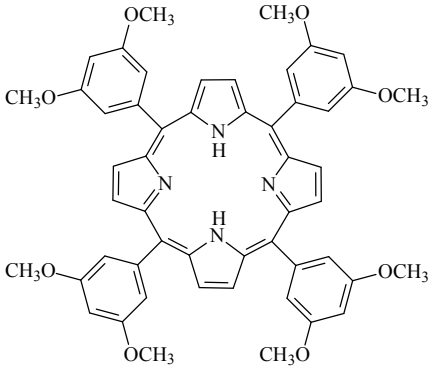
N-Methyl protoporphyrin IX (NMPPIX)



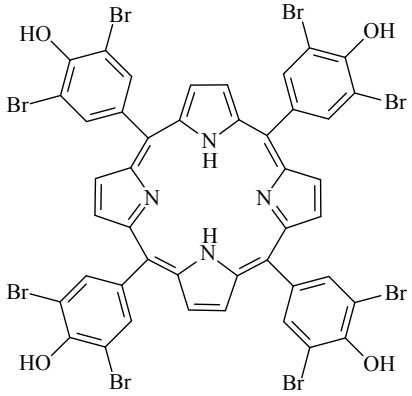
protoporphyrin IX Zinc(II) (ZnPPIX)



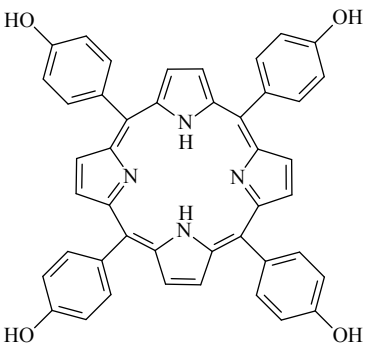
5,10,15,20-tetrakis(3,5-dihydroxy phenyl)porphyrin (TOH_dPP)



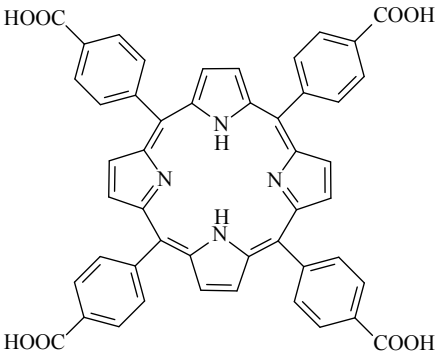
5,10,15,20-tetrakis(3,5-dimethoxy phenyl)porphyrin (TM_dPP)



5,10,15,20-tetrakis(3,5-dibromo-4-hydroxyphenyl)porphyrin (TBr_dPP)



5,10,15,20-tetrakis(4-hydroxy phenyl)porphyrin (TOHPP)



5,10,15,20-tetrakis(4-carboxyphenyl) porphyrin (TCPP)

Scheme S1 Structure of investigated porphyrins.

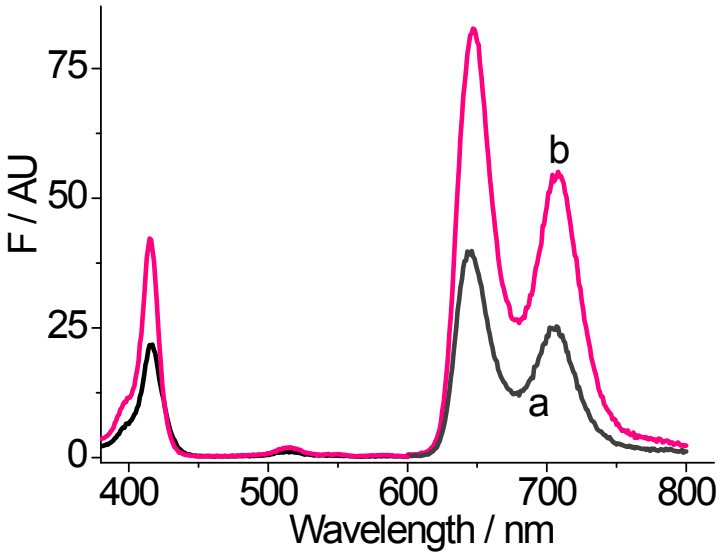


Fig. S1 Excitation and emission spectra of TOH_dPP (4 μM) in the absence (a) and presence of 4 μM TAG4 (b). λ_{ex} = 416 nm; λ_{em} = 646 nm.

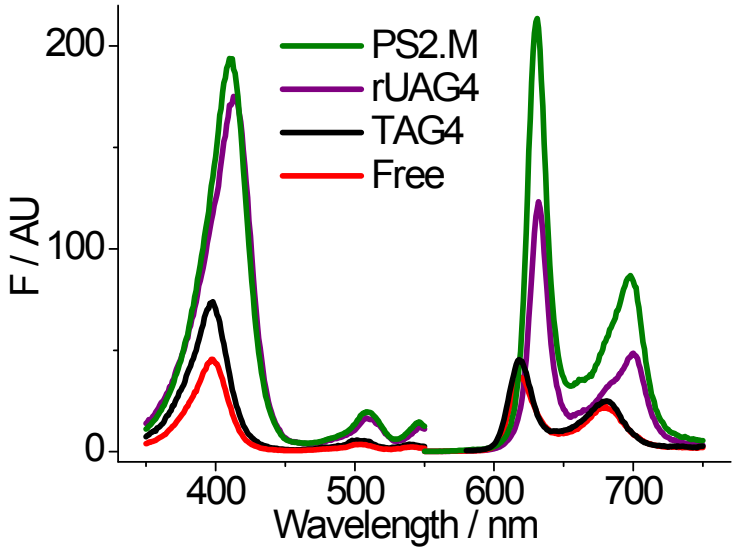


Fig. S2 Excitation and emission spectra of PPIX (1 μM) in 0.1 M K^+ in the absence (red line) and presence of 1 μM PS2.M, rUAG4, and TAG4.

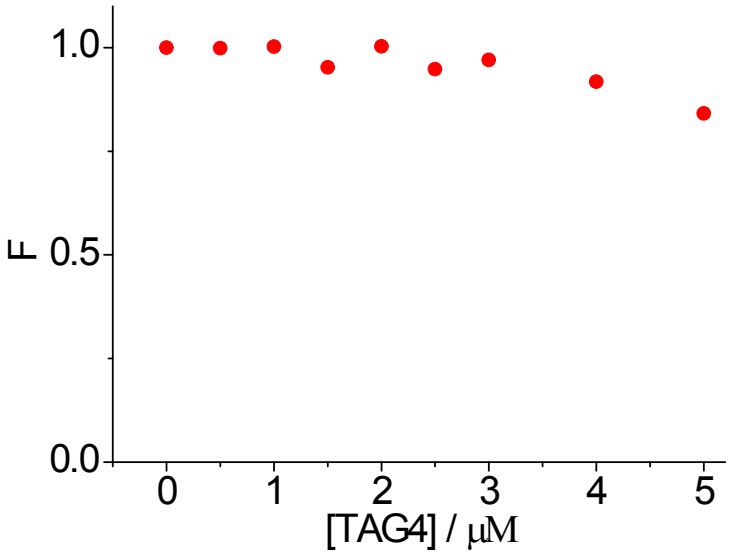


Fig. S3 Effect of the coexistence of TAG4 on the TOH_dPP's fluorescence response towards rUAG4. [rUAG4]= 1 μM; [TOH_dPP]= 3 μM.

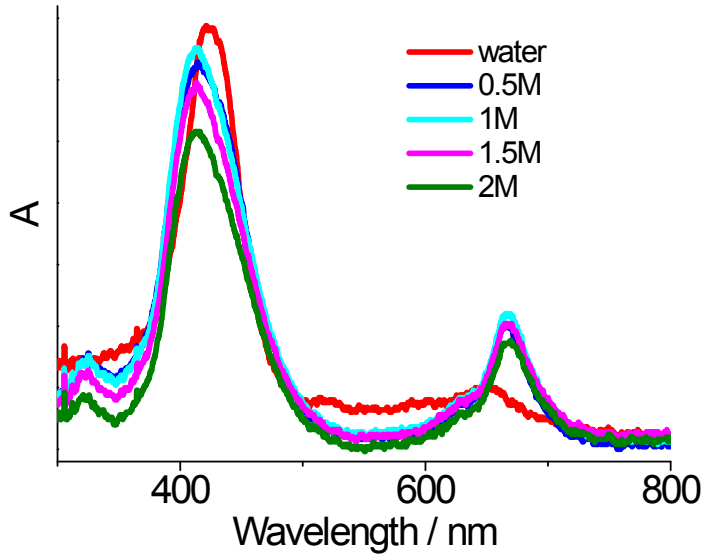


Fig. S4 Absorption spectra of TOH_dPP (4 μM) in water and in the presence of 0.5, 1, 1.5, 2 M HCl.

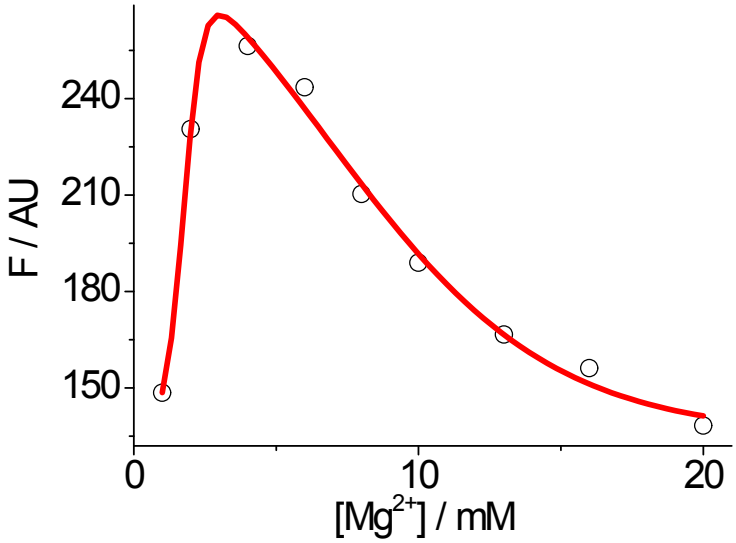


Fig. S5 Effect of increasing Mg^{2+} concentration on the fluorescence intensity of 4 μM TOH_dPP in the presence of 4 μM TERRA. The solutions always contain 0.1 M KCl. A prompt increase in fluorescence was followed by a relatively sluggish fluorescence decrease, suggesting the involvement of the backbone phosphate oxygen atoms of TERRA in the interaction with TOH_dPP.

Table S2 TERRA melting temperature (T_m). The melting temperatures were obtained in a Tris-HCl (50 mM, pH 7.8) containing 20 mM K^+ and 1mM EDTA at 4 μM TERRA.

TERRA	rAG4	rUAG4	rUUAG4	rUUAG4UUA
$\mathbf{T_m}$	67.8	68.4	68.1	65.2