Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

Dopamine oxidation promoted by human telomeric DNA models in the presence of a Cu(II) terpyridine chelate

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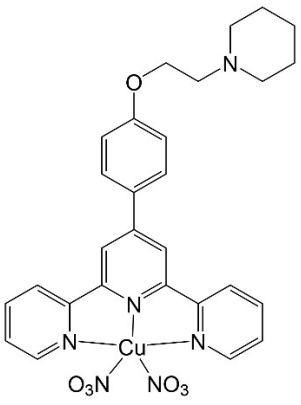
Material and Methods

DNA Samples and Chemicals.

DNA oligonucleotides of human telomere: G-rich single strand (HT21G 5’-GGG TTA GGG TTA GGG TTA GGG-3’) and C-rich single strand (HT21C 5’-CCC TAA CCC TAA CCC TAA CCC-3′), were purchased from Sangon Biotech, Co., Ltd. in a HPLC-purified form. Human telomeric double strand (HT21D) is obtained by hybrid of equimolar HT21G and HT21C. The DNA strand concentration was determined by measuring the UV absorbance at 260nm with the corresponding extinction coefficient provided by the manufacturer. All DNA were heated in 50 mM HEPES buffer (pH 7.5) for 3 min at 95 °C and cooled at 0.5 °C/min to room temperature and then incubated at 4 °C before use. The synthesis details of Terpyridine-Cu(II) complex(CuL2-T) can be obtained from our previous report (Chem. Sci. 2015, 6, 5578). Dopamine hydrochloride(CAS: 62-31-7, 99%) was purchased from Alfa Aesar. 4-(2-hydroxyethyl)piperazine-1-erthanesulfonic acid (HEPES) (CAS: 7365-45-9, 99.5%, Aladdin) was dissolved in H_2O to 50 mM. H_2O_2(CAS: 7722-84-1, 30%) was purchased from Kermel. All chemicals were used without further purification. Milli-Q water (18.2 MΩ·cm) was used for all experiments.



Structure of CuL2-T（L2: 4'-(4-(2-(piperidin-1-yl)ethoxy)phenyl)-2,2':6',2''-terpyridine）

Circular Dichroism (CD) Spectroscopy

Circular dichroism (CD) experiments were carried out on a dual beam DSM 1000 CD spectrophotometer (Olis, Bogart, GA) equipped with a Peltier temperature controller, using a quartz cell of 1.0-cm path length and 2.7 mL volume. The scanning rate was automatically selected by the Olis software as a function of the signal intensity to optimize data collection. The spectra were recorded from 220 to 420nm at 20℃ and subtracted the background CD spectrum of the HEPES buffer (50mM, pH 7.5).

Non-denaturing polyacrylamide gel electrophoresis (PAGE)

DNA samples were incubated in TBE buffer (90 mM Tris, 90mM borate, 2 mM EDTA, pH 7.5). 10μL samples were loaded on the gel. Samples were run on a 20% native polyacrylamide gel (7 cm × 10 cm), made up in 1×TBE buffer containing 20 mM KCl(90 mM Tris, 90mM borate, 2mM EDTA, pH 7.5) for 3 hours at 100 V. Gels were stained with Stains-all (Sigma, 95%) and then destained under the sunlight. Gel pictures were taken by the camera of smart-phone and without any further processing.

Monitoring the Oxidation of Dopamine.

The annealed DNA samples (50 μM) were prepared in HEPES buffer (pH=7.5), and incubated with CuL2-T for 30 minutes at 25 ℃. After the formation of HT21/CuL2-T complexes, dopamine was added, and then H_2O_2 was added to start the reaction. The oxidation of dopamine were tracked by the product formations *via* monitoring the absorbances of 480 nm using a Shimadzu 2600 spectrophotometer. The samples were measured in a quartz cell of 1-cm path length and the total volume of reaction system is 500 μL.

The k_{obs} was obtained from the slope of the initial linear portion (the first 10 seconds) of the plot of absorbance versus reaction time.

Dissociation constant (K_d) measurement.

According to the binding of other copper(II) complexes to the telomeric DNA,[1-3] we use UV-visible spectrophotometric titration experiments to calculate the

dissociation constant(K_d) of three human telomeric DNA and CuL2-T. A spectrophotometric titration of 40 μM CuL2-T with increasing concentrations of DNA in 0 to 30 μM range was performed. The titration was stop until no change of absorbance at λ=332 nm. The saturation curves for the binding of CuL2-T with DNA were determined by plotting fraction of bound CuL2-T (α, determined by equation 1) as a function of DNA concentration and fitting with a one-site binding model to get the K_d value for three DNA:

$$\alpha = \frac{A_x - A_0}{A_\infty - A_0} \quad (1)$$

where A_x is the absorbance at λ=332 nm of CuL2-T incubated with varied concentration of DNA, A_∞ and A_0 are the absorbances at λ=332 nm in the presence of saturating DNA and in the absence of DNA, respectively. Data was fitted by Hill equation by Origin software, and K_d of CuL2-T with DNA could be got from the fitting result.

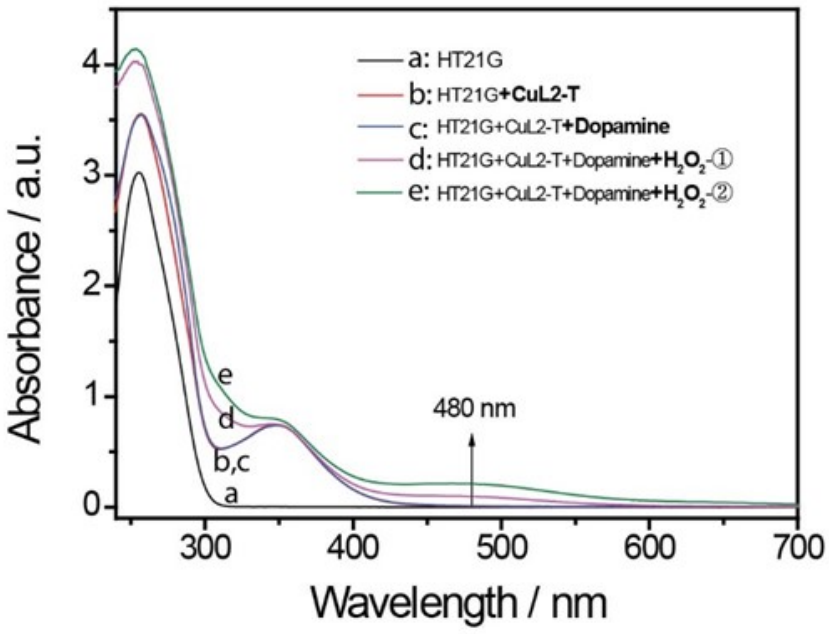


Figure S1 UV-visible spectra of (a) HT21G(50μM), (b) its complex with CuL2-T(300μM), (c) the subsequent addition of dopamine(0.5mM), and (d, e), the final addition of H_2O_2 (20mM) to initiate the reaction. With the addition of H_2O_2, dopamine undergoes an oxidation reaction, evidenced by the appearance of absorbance at 480nm ascribed to oxidation product aminochrome.

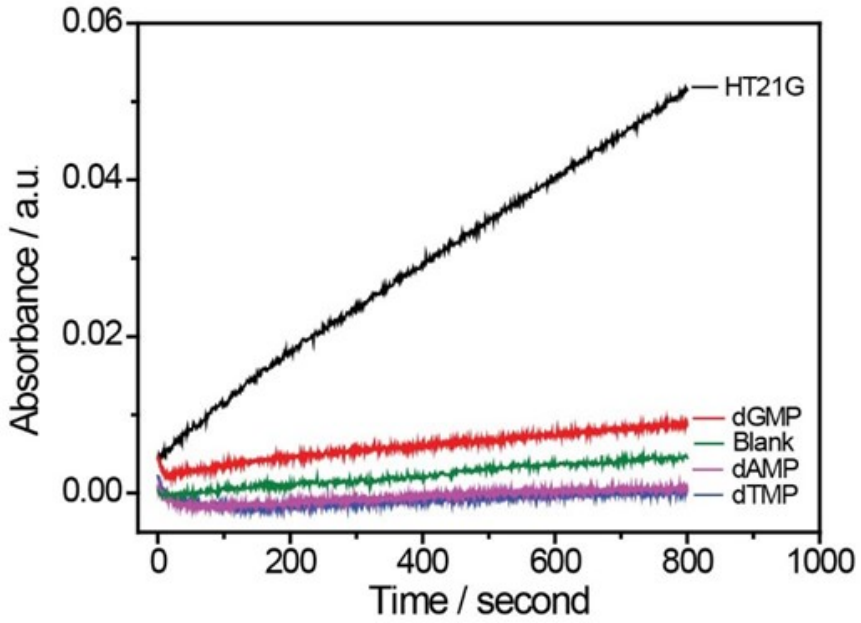


Figure S2 DA oxidation promoted by HT21G(50μM) and its component nucleotides of dGMP, dAMP and dTMP with the concentration of 1.05mM (21*50μM). Blank refers to the DA autooxidation in the presence of H_2O_2. HEPES (50mM, pH=7.5), DA (0.5 mM) and H_2O_2 (20 mM) was used here.

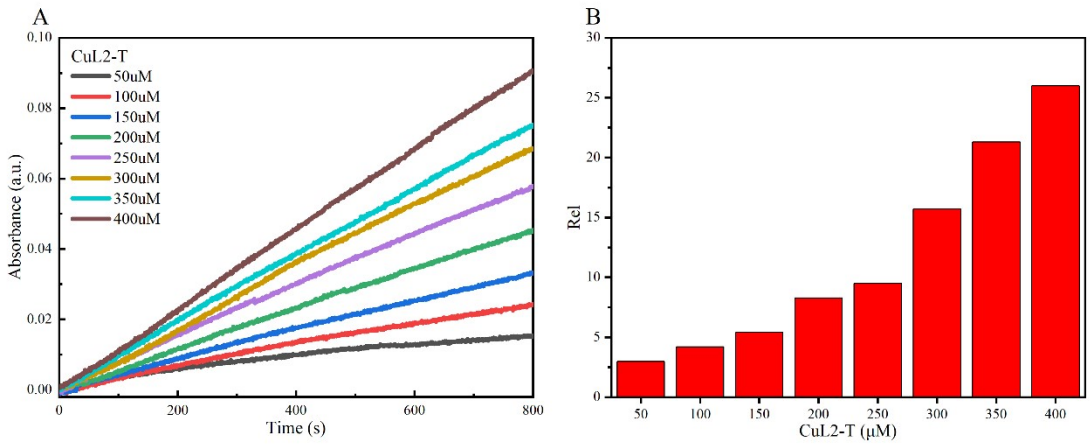


Figure S3 DA oxidation promoted by CuL2-T. A) UV-vis kinetic curve of dopamine oxidation by H_2O_2, in the presence of different concentration of CuL2-T; B) The plot of relative k_{obs} against the different concentration of CuL2-T for promoting DA oxidation. Rel=k_{obs}(CuL2-T)/k_{obs}(blank). All reaction were performed in HEPES buffer(50mM, pH=7.5), DA(0.5mM), H_2O_2(20mM), CuL2-T(50-400μM) were used here.

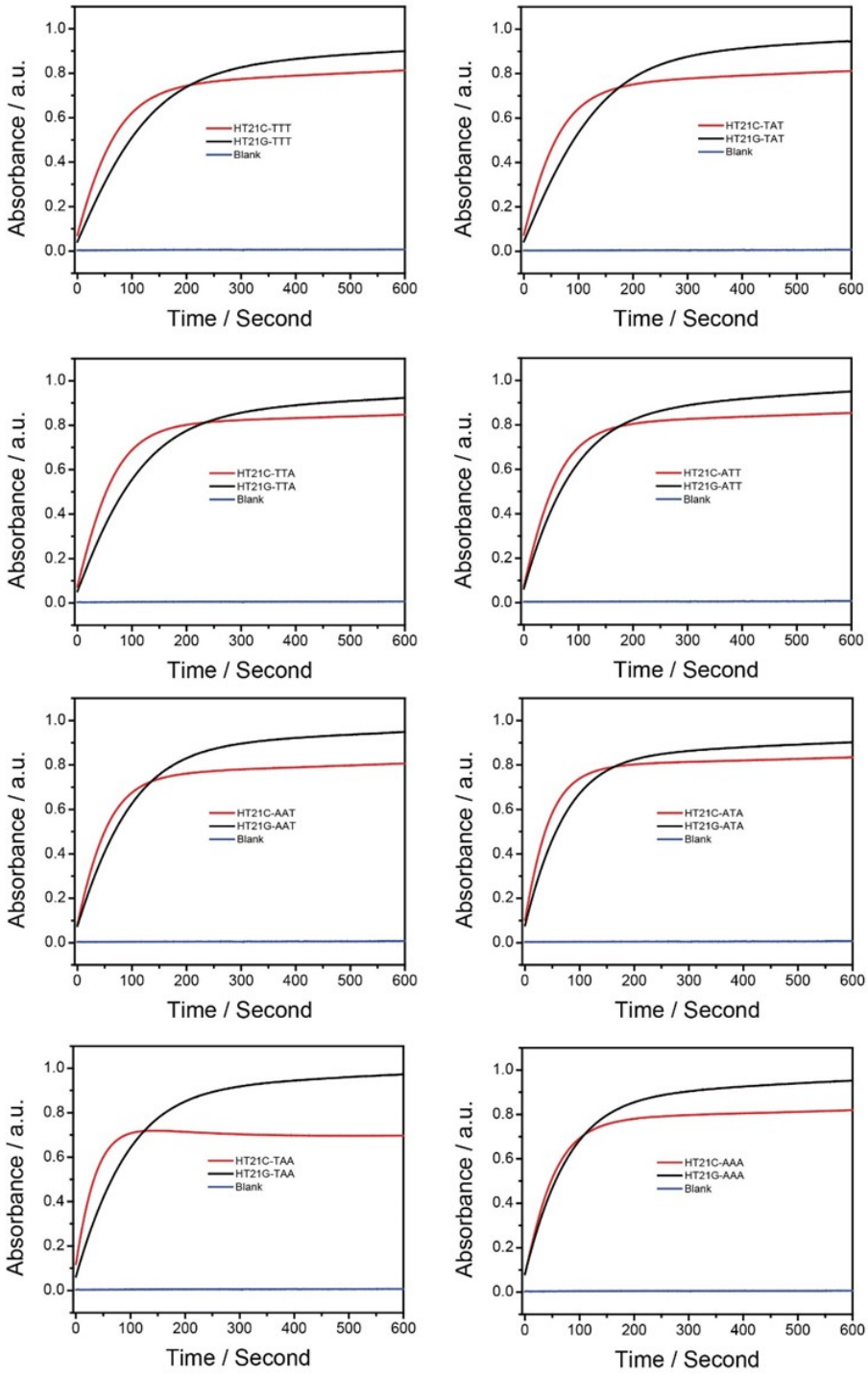


Figure S4 DA oxidation promoted by different loop base sequence of HT21G/C(50μM). Assembly of HT21G and HT21C with CuL2-T in the molar ratio of 1:6 was used here. Blank refers to the DA autooxidation in the presence of H_2O_2. The k_{obs} was calculated by the slope of the line fitted to increase of absorbance change within first 10 second (Table S2). HEPES (50mM, pH=7.5), DA (0.5 mM) and H_2O_2 (20 mM) was used here.

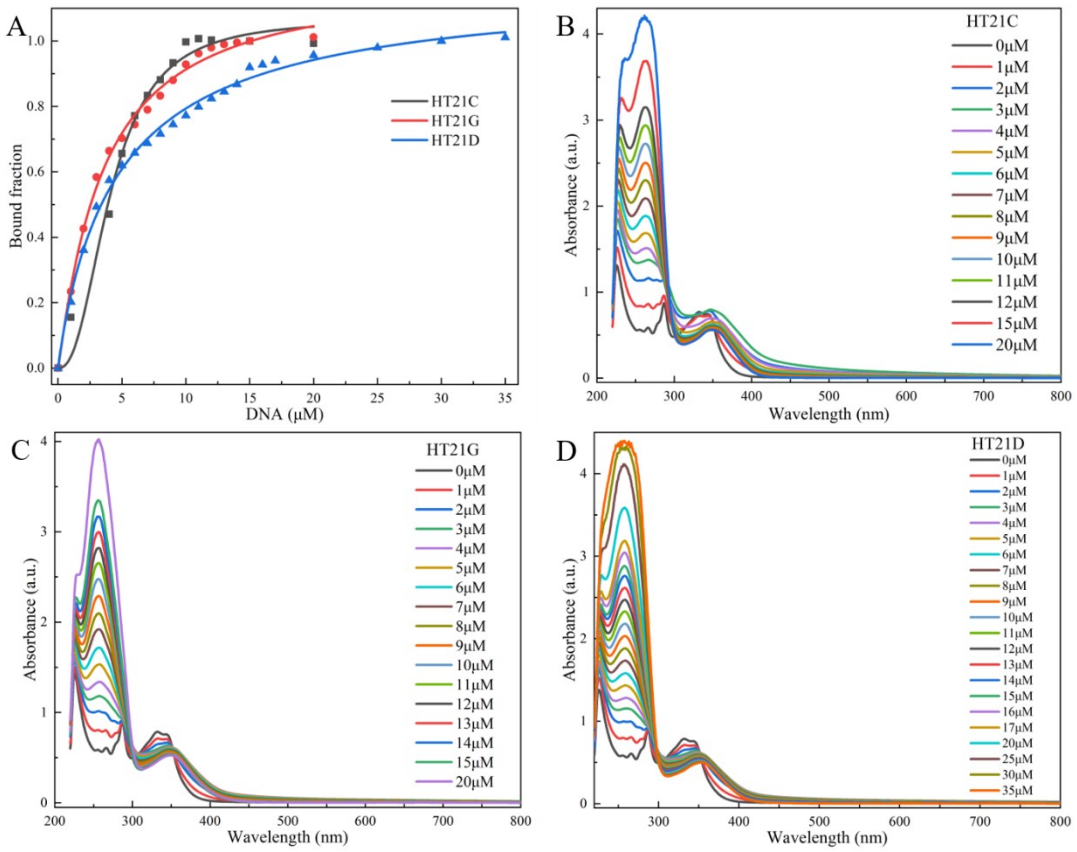


Figure S5 The dissociation constants (K_d) measurement. (A) Plots of bound fraction of CuL2-T to DNA versus DNA concentration. (B-D) UV-vis spectrum of HT21C, HT21G and HT21D titration to CuL2-T. Measurement were performed with CuL2-T (40μM) in 50mM HEPES buffer (pH=7.5) at 25℃.

Note: According to the UV-vis titration experiment, the dissociation constants (K_d) of CuL2-T to HT21G, HT21C and HT21D were calculated as 3.47±0.32μM, 4.11±0.24μM and 4.68±0.37μM, respectively, indicating a binding affinity sequence of HT21G＞HT21C＞HT21D. This is in contrast to the case of HTDNA-CuL2-T promoted DA oxidation (HT21C＞HT21G＞HT21D). Therefore, the different oxidation rate is not due to the different binding ability of HT21G, HT21C and HT21D with CuL2-T.

Table S1 The k_{obs} of different additives for promoting DA oxidation

	HT21G （$\times 10^{-6}$）	HT21C （$\times 10^{-6}$）	HT21D （$\times 10^{-6}$）
Blank	10.01±3.76	10.01±3.76	10.01±3.76
CuL2-T	264.6±3.96	264.6±3.96	264.6±3.96
DNA alone	174.9±5.86	361.3±4.18	273.9±6.35
DNA:CuL2-T=4:1	633.9±4.16	451.2±5.20	37.85±4.66
DNA:CuL2-T=2:1	1030±5.04	1170±5.54	39.22±6.82
DNA:CuL2-T=1:1	1820±6.56	1900±7.11	241.6±5.12
DNA:CuL2-T=1:2	2950±9.23	4490±21.32	565.7±6.27
DNA:CuL2-T=1:4	4120±9.29	7720±37.39	1650±14.33
DNA:CuL2-T=1:5	5300±6.76	9270±38.46	1860±5.02
DNA:CuL2-T=1:6	4980±5.37	9610±30.55	1910±1.55
DNA:CuL2-T=1:7	5430±5.12	8470±7.62	3650±10.99
DNA:CuL2-T=1:8	6270±4.50	8480±6.47	4690±12.08

The change of absorbance at 480nm within first 10 second was used to calculate the k_{obs}. Blank refers to the DA autooxidation in the presence of H_2O_2.

Table S2 The k_{obs} of different loop base sequence HT21G/C for promoting DA oxidation

	HT21G($\times10^{-6}$)	HT21C($\times10^{-6}$)
Blank	10.01±3.76	10.01±3.76
TTT	5560±5.13	8460±29.50
TAT	575±7.06	900±30.26
TTA	6210±7.29	9410±23.48
ATT	8040±21.94	9900±27.14
AAT	7370±14.97	10170±43.02
ATA	9060±34.18	12390±64.33
TAA	7980±21.74	13280±97.78
AAA	8990±35.21	10430±32.08

The change of absorbance at 480nm within first 10 second was used to calculate the k_{obs}. Blank refers to the DA autooxidation in the presence of H_2O_2.