Electronic Supporting Information

Sm(III)[12-MC$_{Ga(III)shi}$-4] as a luminescent probe for G-quadruplex structure

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Materials

Preparation of $[Sm(Ga_4)(shi)_4(C_6H_5CO_2)_4(C_5H_5N)(CH_3OH)]\cdot C_5H_6N\cdot C_5H_5N\cdot CH_3OH$
The synthesis of the metallacrown was carried out similarly as reported[22] but with few modifications. Briefly, salicylhydroxamic acid (H_3shi) (153.1 mg, 1.0 mmol) and sodium benzoate (432.3 mg, 3 mmol) were dissolved in a mixture of 40 mL of methanol and 2 mL of pyridine. $Sm(NO_3)_3x6H_2O$ (222.2 mg, 0.5 mmol) and $Ga(NO_3)_3x7H_2O$ (255.7 mg, 1.0 mmol) were added at a time and shortly stirred. The resulting solution was filtered. The slow evaporation yielded the collection of small crystals after 5 days.
Analysis: $[Sm(Ga_4)(shi)_4(C_6H_5CO_2)_4(C_5H_5N)(CH_3OH)]\cdot C_5H_6N\cdot C_5H_5N\cdot CH_3OH$
Yield: 114 mg (25%). ESI-MS, calc. for [M]-, $C_{56}H_{36}N_4O_{20}SmGa_4$, 1515.8; found, 1515.8.

G-quadruplex sequence
Single-stranded HPLC-purified 22-mer deoxyribonucleotide with human telomeric sequence of $AG_3(TTAGGG)_3$ (22Htel) was purchased from Genomed®, Poland, and was used without further purification. The strand concentration was determined at 260 nm in 85°C using reported molar absorptivities of nucleobases[28]. All experiments were performed in a buffer solution consisting of 10 mM sodium cacodylate and 100 mM NaCl (pH 7.2) unless otherwise stated. The sample was incubated at 90°C for 5 min and then cooled down slowly to the room temperature and stored at 4°C overnight to obtain G-quadruplex folding. In the Tb^{3+}-GQ luminescence experiment, 22Htel was incubated as above in the presence of 10mM TRIS buffer, pH 7.0, without alkali metal cations, to be sure of its single stranded form. Quadruplex forming was gained after addition of Tb^{3+} ions.

Regents
Reagents for syntheses of complex were purchased from Sigma-Aldrich (St .Louis, US).
The stock solution of MC was prepared in DMSO and its stability was controlled by recording UV absorption spectra.
Other reagents (Thiazole orange, buffer components, salts) were purchased from Sigma-Aldrich (**St .Louis,** US) and were used without further purification.

Instrumentation

The absorption spectra were recorded by using a Cary 100 UV-Vis Spectrophotometer equipped with a Peltier temperature control accessory (Agilent Technologies, Australia). The CD spectra and CD melting profiles were registered on a J-1500 CD Spectropolarimeter (Jasco, Japan) equipped with a Peltier temperature control accessory. Steady-state fluorescence spectra were taken on a spectrofluorimeter FP-6500 Spectrofluorometer (Jasco, Japan) equipped with a temperature controlled cell holder. Fluorescence measurements for FID assay were performed on a Carry Eclipse Spectrofluorometer (Agilent Technologies

Australia) equipped with a temperature controlled cell holder. Spectra were measured in a 1x0.4 cm quartz cuvette with excitation and emission slits both of 10 nm unless otherwise stated. All experiments were thermostated as indicated in the procedure.

Methods

Circular dichroism (CD) studies

The CD spectra were measured in the spectral range from 215 to 380 nm with 200 nm/min scanning speed and bandwidth of 1 nm. Spectra were registered in quartz cuvette of 1 cm path length and averaged from 2 scans. The CD titration of G-quadruplex was carried out for pre-folded quadruplex (2 μM in strand concentration) with consecutive additions of metallacrown. Each addition of MC was followed by two min of the equilibration time.

Circular dichroism (CD) melting temperature experiments

The experiment was carried out in a 10 mM sodium cacodylate buffer containing 100 mM of sodium chloride. Solution of 2 μM pre-folded G-quadruplex was mixed with metallacrown in the molar ratio of 1:1, 1:2.3, 1:6.9 and 1:12.4 and registered in heating and cooling approach (1.5ºC/min) Melting temperatures (T_m) were determined at 295nm by plotting the tangents.

Fluorescence titration of Sm(III)[12-$MC_{Ga(III)shi}$-4] with GQ/Na^+

Titration of metallacrown with quadruplex was performed in 10 mM sodium cacodylate buffer pH 7.2 containing 100mM NaCl. A $1x10^{-6}$ M solution of metallacrown was titrated with G-quadruplex (up to $1.5x10^{-6}$ M) folded on Na^+ cations. Registration of spectra were followed by 4 minutes equilibration period. Excitation wavelength was set at 309 nm with excitation/emission slits both at 5 nm. This wavelength was chosen due to absorption band of the metallacrown. The probe was thermostated at 25 °C.

Quenching experiment with the GQ- Tb^{3+} luminescence

4 μM terbium (III) ion was added to 1 μM single-stranded 22Htel telomeric DNA in 10 mM Tris-HCl buffer pH 7.0 to obtain GQ-Tb quadruplex. The mixture was excited at 290nm with excitation/emission slits set to 10 nm. Terbium luminescence was monitored at 548 nm. The mixture was titrated with increasing amounts of metallacrown (up to $14x10^{-6}$ M) with 3 min incubation period after each addition. The solution was thermostated at 25ºC.

To calculate association constant of GQ-MC complex (K_{as}), the Stern-Volmer equation for static quenching was used (6):

$$\frac{I^0}{I} = 1 + K_{as}[Q] \qquad (1)$$

- I^0 stands as the luminescence intensity at 548 nm in the beginning of the experiment – (emission of Tb^{3+}/GQ complex)
- I represents luminescence intensity after consecutive addition of MC [Q].

The slope of I^0/I vs. MC concentration gives a Stern-Volmer quenching constant that was considered as association constant (K_{as}).

G-quadruplex fluorescence indicator displacement (GQ FID) assay

The FID experiment was carried out according to procedure described elsewhere[41,42].

The solution of TO (0.5 μM) and pre-folded oligonucleotide (0.25 μM) in 10 mM sodium cacodylate buffer pH 7.2 containing 100 mM NaCl was equilibrated for 10 min before start the experiment. The mixture was titrated with increasing amounts of metallacrown (up to $14x10^{-6}$ M) with 4 min incubation period. Emission spectra of the starting mixture and after every consecutive addition of MC were registered in the spectral range of 490-750 nm with

the excitation wavelength set at 480 nm and excitation/emission slits both at 10 nm. The cuvette solution was thermostated at 20°C.
The percentage of TO displacement was calculated from the following equation:

$$TO_{displ}(\%) = 100 - \left(\frac{FA}{FA_0} * 100 \right) \quad (2)$$

- FA_0 stands for the integrated fluorescence of TO bound to the GQ at the start of the experiment (absence of MC);
- FA is the fluorescence area after consecutive addition of MC;

FA and FA_0 values were calculated by integrating TO spectra in the range of 503 – 750 nm.

The plot of $TO_{displacement}(\%)$ versus MC concentration allow to obtain the value called "$MC_{50\%}$" which stands as a MC concentration causing 50% reduction of TO fluorescence.

The mathematical procedure to calculate the binding constants of metallacrowns (K_{MC}) to 22Htel DNA value was conducted as previously reported[21].
The final equation looks as followed:

$$K_{MC} = K_{TO} \frac{[TO]_{total} - 0.5[GQ]_{total}}{MC_{50\%} - 0.5[GQ]_{total}} \quad (3)$$

- $[TO]_{total}$ stands for the concentration of thiazole orange;
- $[GQ]_{total}$ is the concentration of quadruplex
- $MC_{50\%}$ is MC concentration causing 50% reduction of TO fluorescence, which is defined in previous step;

The binding constant of thiazole orange/22Htel quadruplex system (K_{TO}) of 1.4×10^6 M^{-1} value was calculated using Scatchard analysis of titration data and reported previously[21].

Results

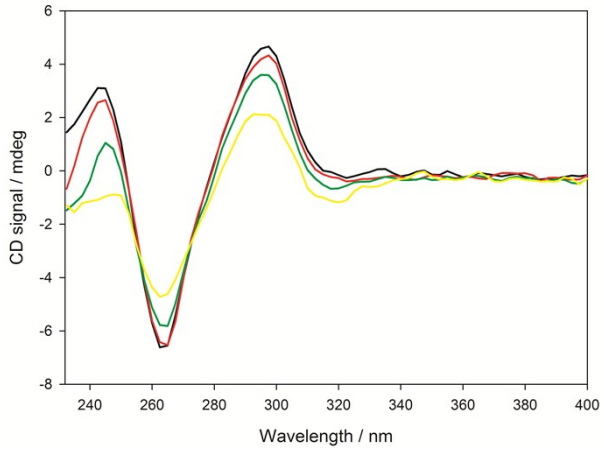

Figure S1. CD spectra of 2 μM 22Htel in the presence of increasing concentration of SmMC in 10mM sodium cacodylate buffer, pH 7.2 and 100 mM NaCl. Ratio GQ/MC equals 1:0 (black line), 1:2.3 (red dash), 1:6.9 (green line), 1:12.4 (yellow line).

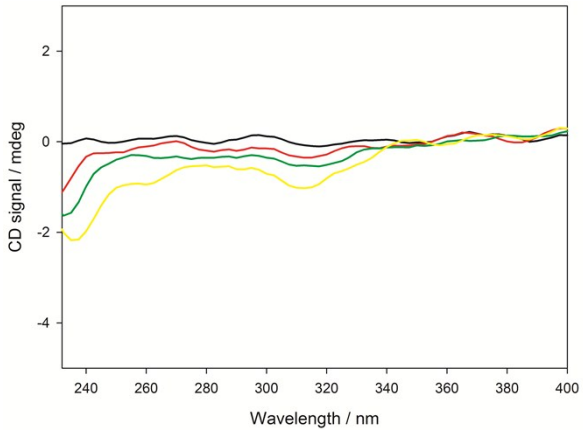

Figure S2 CD spectra of SmMC in 10mM sodium cacodylate buffer, pH 7.2 and 100mM NaCl. MC concentration: 0 μM (black line), 4.5 μM (red dash), 13.4 μM (green line), 23.7 μM (yellow line).

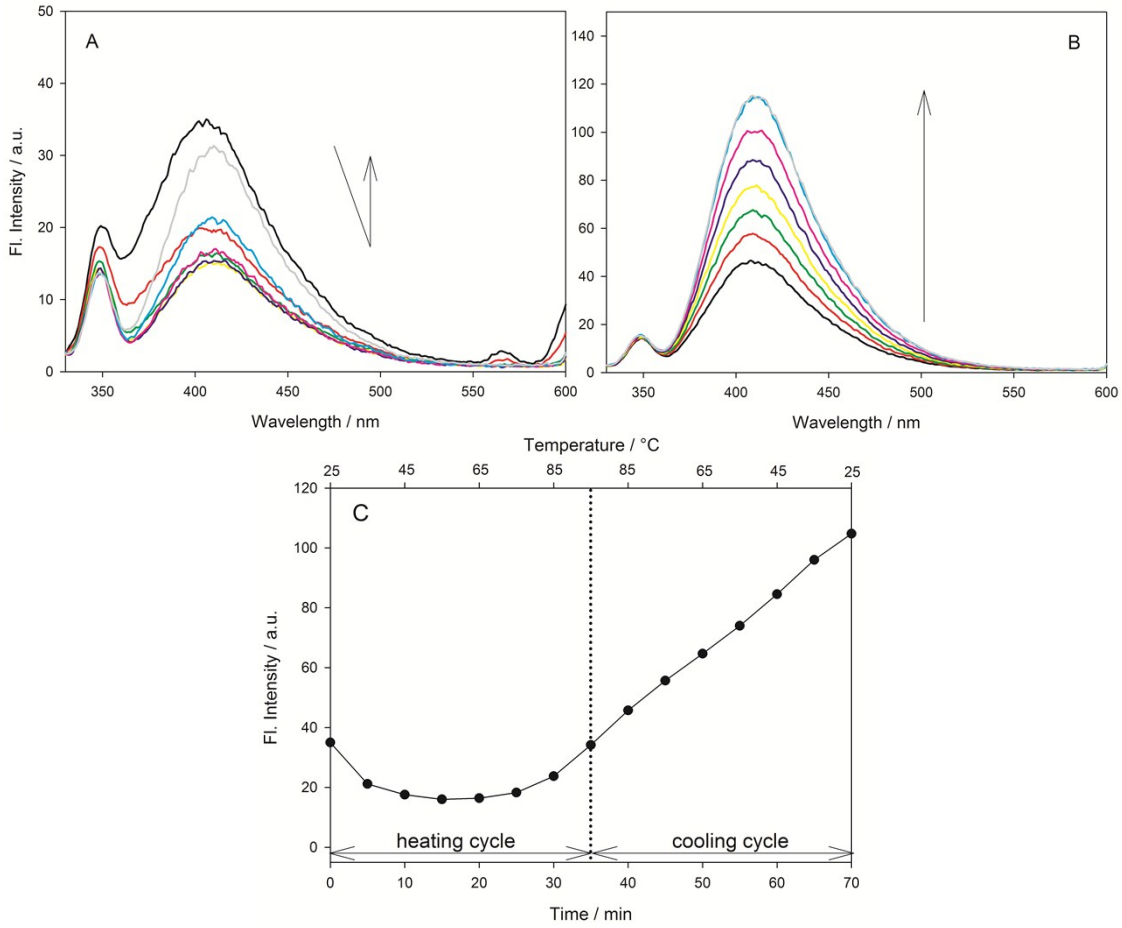

Figure S3. Emission spectra of 4μM SmMC with increasing temperature. Each spectra were recorded after 5 min incubation in the temperature. (A) Heating approach: 25°C (black line), 35°C (red line), 45°C (green line), 55°C (yellow line), 65°C (dark blue line), 75°C (pink line), 85°C (light blue line) and 95°C (grey line). (B) Cooling approach: 85°C (black line), 75°C (red line), 65°C (green line), 55°C (yellow line), 45°C (dark blue line), 35°C (pink line), 25°C (light blue line) and stability in 5 minutes more (grey line). (C) Fluorescence changes at 405 nm for SmMC solution heated to 95 °C and next cooled to room temperature. Parameters: λ_{ex} = 309 nm, ex and em slits both at 10 nm, sensitivity: medium.

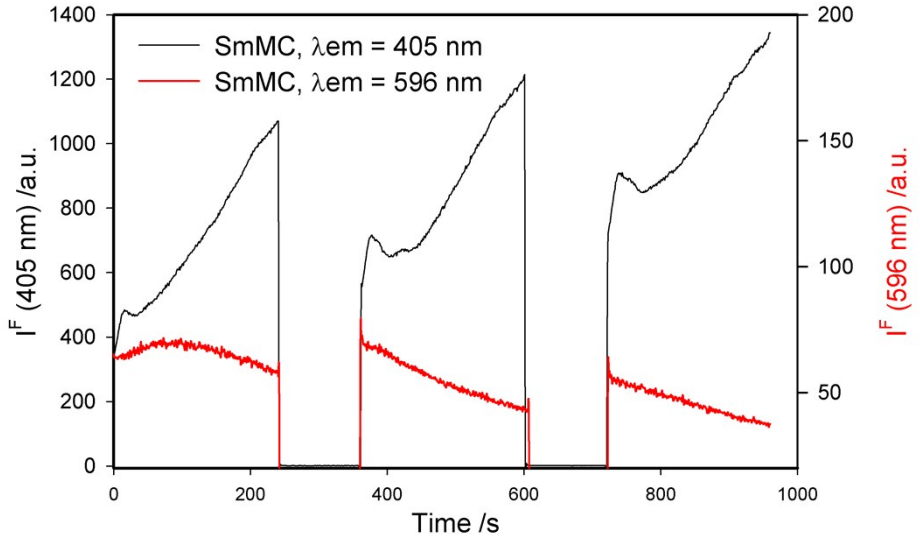

Figure S4 Emission traces of SmMC recorded at 405 nm (black line) and 596 nm (red line) upon repeated irradiation at 309 nm for 4 min with 2 min in dark. Conditions: 10mM sodium cacodylate buffer, pH 7.2 and 100 mM NaCl, excitation/emission slits 5 nm, sensitivity: high.

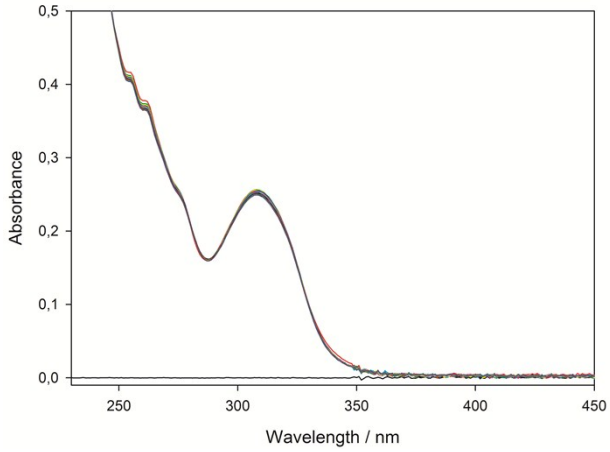

Figure S5. UV-Vis spectra of 15µM SmMC registered in 3h. Each line represents 4 min stability period.