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Supporting Information

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Self-Assembly of a Bifunctional DNA Carrier for Drug Delivery**

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Supporting Information

APTAMER MODIFICATION AND TMPyP4 BINDING WITH G-QUADRUPLEX-APTAMER

In the modified Sgc8 DNA sequence, R-Sgc8, the R consists of the following sequences: a) Biotin-6G-, b) Biotin-T-4G-4T-, c) Biotin-T-4G-4T-4G-4T-, d) Biotin-T-4G-2T-4G-4T-, e) Biotin-T-4G-6T-4G-4T-, f) Biotin-T-3G-4T-3G-4T- and g) Biotin-T-6G-6T-6G-4T-. Sequences a, b and c were used to test G-quadruplex formation in different buffers. Sequences d and e were designed to test the effect of T-linker length on G-quadruplex formation, and sequences f and g were used to test the effect of the length of the G segment on G-quadruplex formation. Biotin-T-4G-4T-4G-4T-TD05 was synthesized as a reference to test the selectivity of the modified aptamer sequences. The biotin was present for binding with streptavidin-PE in the selectivity test.

After the sequences were annealed to form G-quadruplexes, the annealed DNAs were tested by PAGE gel (Figure S1). The PAGE test showed that Biotin-T-4G-4T-4G-4T-Sgc8 in buffer 1 formed larger molecules after annealing (Lane 6 in Figure 2) with a size between 50 bp and 100 bp. Since the size of Biotin-T-4G-4T-4G-4T-Sgc8 is 58 bases, we can project that two molecules of Biotin-T-4G-4T-4G-4T-Sgc8 associated to form a G-quadruplex in buffer 1. Lane 7 also showed that part of Biotin-T-4G-4T-4G-4T-Sgc8 in buffer 2 formed a dimer, most likely in the G-quadruplex conformation. On the other hand, the position of Biotin-T-4G-4T-Sgc8 in buffer 1 and buffer 2 (lane 2, 3) is similar to that of Sgc8-FAM (lane 9), indicating that Biotin-T-4G-4T-Sgc8 did not associate. Therefore, we annealed Biotin-T-4G-4T-4G-4T-Sgc8 in buffer 1 (G-quad-Sgc8) as the TMPyP4 carrier in subsequent experiments. A TEM image of G-quad-Sgc8 (Figure S2) shows that the particle size was 8.3 ±3nm. The annealed Biotin-T-4G-4T-4G-4T-TD05 (G-quad-TD05) was used as a reference.

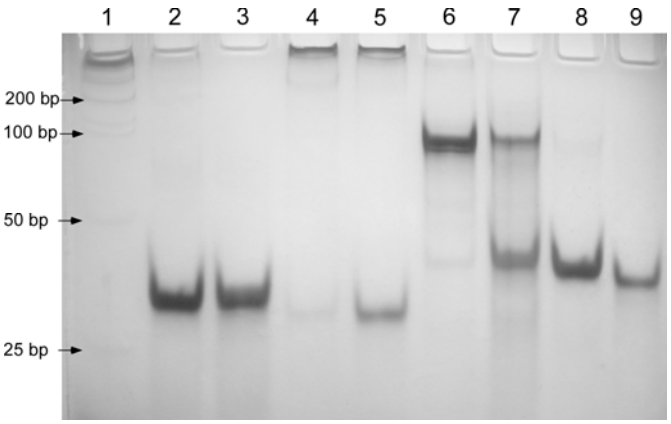

Figure S1. Association of sequences in two different buffers analyzed by PAGE. The modified aptamers were diluted to 40μM in buffers and annealed overnight from 95°C to 21°C and then stored at 4°C for 6 hours. Lane 1, ladder; Lanes 2 and 3, Biotin-T-4G-4T-Sgc8 in buffer 1 and buffer 2; Lanes 4 and 5, Biotin-6G –Sgc8 in buffer 1 and buffer 2; Lanes 6 and 7, Biotin-T-4G-4T-4G-4T-Sgc8 in buffer 1 and buffer 2; Lane 8, Biotin-T-4G-4T-4G-4T-Sgc8 in water; Lane 9, Sgc8-FAM in water. Lanes 6 and 7 showed a band lower than the100 bp position, indicating formation of a dimer after annealing. This dimer is very likely in the G-quadruplex conformation.

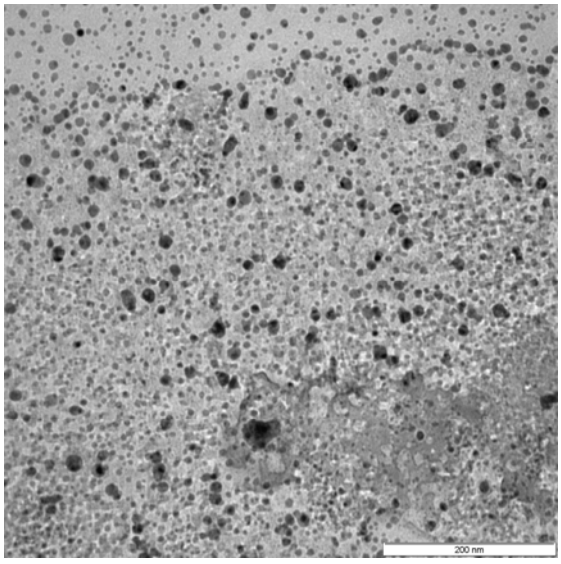

Figure S2.TEM image of G-quad-Sgc8. The scale bar is 200 nm. The particle size is 8.3 ±3nm.

The G-quadruplex conformation of annealed Biotin-T-4G-4T-4G-4T-Sgc8 in buffer 1 was confirmed by circular dichroism (CD) spectroscopy (Figure S3). The CD spectra and the conformations of G-quadruplexes show an empirical relationship. That is, positive CD at 260 nm and negative CD at 240 nm represent parallel strands, while positive CD at 290 nm and negative CD at 260 nm represent anti-parallel strands.[1-3] Formation of the G-quadruplex was further confirmed by analyzing the CD spectra of annealed Biotin-T-4G-4T-4G-4T-Sgc8 before and after binding of TMPyP4. The negative peak at 245 nm and positive peak at 270 in the CD spectra indicated that the annealed aptamer did form a parallel G-quadruplex conformation and furthermore, that this structure was maintained after binding with TMPyP4.

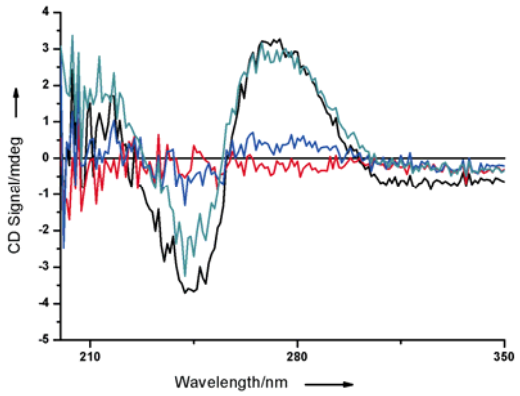

Figure S3. CD spectra of G-quad-aptamer. Black curve: G-quad-Sgc8, light blue: 50μL G-quad-Sgc8+350μL TMPyP4, blue: 15μL G-quad-Sgc8+385μL TMPyP4, red: TMPyP4.

TMPyP4 BINDING WITH ANNEALED APTAMER

To study the binding of TMPyP4 with annealed aptamer, the TMPyP4 was dissolved in buffer 1, and the concentration was determined by measuring UV-Vis absorption at 422 nm with a molar absorptivity of 2.26×10^5 $M^{-1}cm^{-1}$. The peak position of TMPyP4 was at 422 nm (Figure S4), and with the addition of aptamer, the peak position shifted to 438 nm. This 15 nm shift is attributed to the binding of TMPyP4 with the G-quadruplex. However, the peak position did not increase, even with increasing aptamer fraction (the concentration was from 0 to 10 μM), indicating saturation of G-quadruplex binding with TMPyP4.

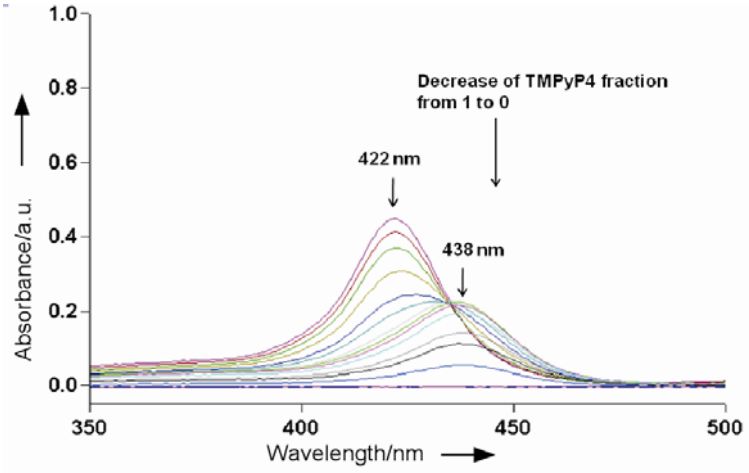

Figure S4. UV-Vis absorption titration spectra of TMPyP4 with G-quad-Sgc8. The peak of TMPyP4 at 10 μM is at 422 nm. The fractions of TMPyP4 are from 1 to 0 with the peak intensity from high to low. The peaks shifted to higher wavelength and reached 438 nm with the addition of G-quad-Sgc8, indicating intercalation of TMPyP4 into the G-quadruplex.

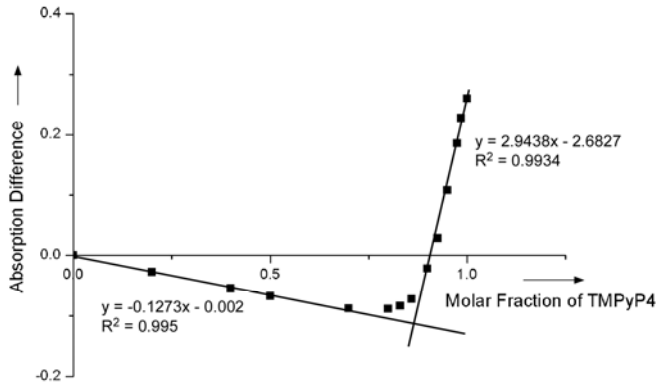

Figure S5. Jobs plot for the binding of TMPyP4 to the aptamer with G-quadruplex. TMPyP4 fraction at intersection is 0.858; the binding ratio of TMPyP4 to Aptamer is 6:1. The binding ratios of TMPyP4 with annealed sequences d and e, 6.9:1, were determined in the same way.

The interaction of TMPyP4 with the annealed aptamer was also studied by steady state fluorescence emission with different mole ratios of TMPyP4 and aptamer. The emission spectra changed in shape and intensity with different concentrations of aptamer (Figure S6). These changes of the emission spectra are indicative of a change in the local environment of TMPyP4 bound to the G-quadruplexes. This is most likely a result of fluorescence quenching by the adjacent guanosine nucleotides, which are known to have electron sharing/donor properties with the adjacent base.[4-5] These results indicate that the TMPyP4 formed a complex with the G-quadruplex after mixing.

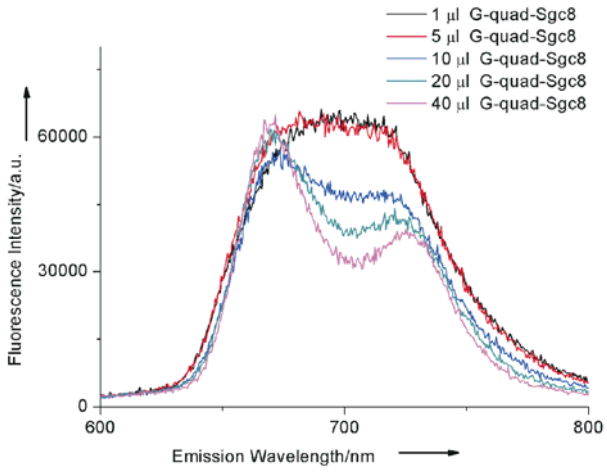

Figure S6. Steady state fluorescence emission of TMPyP4 with the addition of aptamer. 1, 5, 10, 20, and 40 μL of 5 μM aptamer were added to 20 μL of 5 μM TMPyP4. Then the mixtures were diluted to 1000 μL to give a TMPyP4 concentration of 0.1 μM.

THE LEAKAGE OF TYPyP4 FROM THE CARRIER

The leakage of drug from the carrier is a key factor of drug delivery. It may contribute to the toxicity of the drug to the normal cells, which causes consequent side effects. TMPyP4 leakage from the G-quad-aptamer was studied in vitro by monitoring the fluorescence of TMPyP4 in the buffer solution during dialysis with centrifugal filter devices (Figure S7). Over the course of 21.5 hours, the fluorescence intensity of the buffer solution outside of the sample holding tube was about 8,000 for free TMPyP4 and 5,000 for the complex. At 57 hours, both tubes were centrifuged at 14000 rpm for 15 min to make sure the dissociated TMPyP4 entered the dialysis buffer. At that time, the fluorescence intensity was 143,000 for the tube with free TMPyP4 and 24,000 for that of the G-quad-Sgc8-TMPyP4. Assuming that all the free TMPyP4 was centrifuged into the dialysis buffer, only 1/6 of the intercalated TMPyP4 dissociated within 57 hours. This experiment showed a slow leakage of TMPyP4 from the complex.

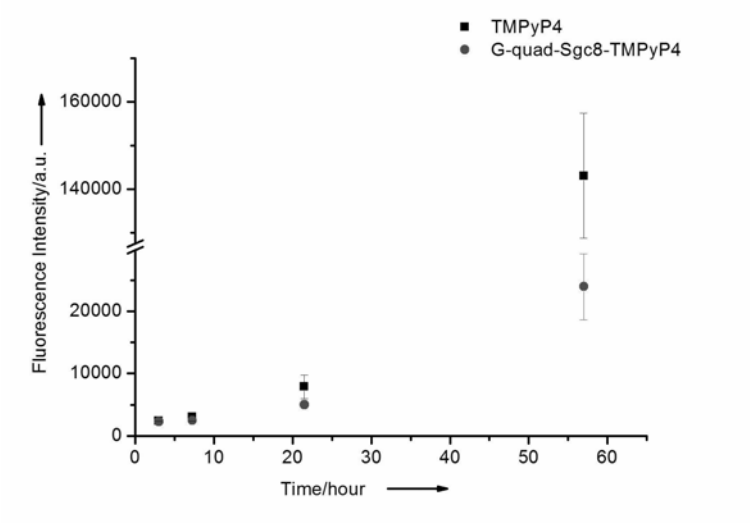

Figure S7. *In Vitro* leakage profile of free TMPyP4 (black square) and complex of G-quad-Sgc8 with TMPyP4 (black circle). The error bar is the standard deviation of 3 replicates.

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