Supporting Information for:

Harnessing the G-Tetrad Scaffold for Fluorescent Detection Strategies Within G-Quadruplex Forming Aptamers

Michael Sproviero Richard A. Manderville *

Table of Contents:

1. Experimental Section......................................................................................................S2
2. Figures S1-S8, Fluorescence titrations of mTBA with thrombin...............................S3-S6
3. Table S1, Dissociation/Association Constants for thrombin binding to mTBA........S7
4. Figure S9, Emission and Excitation spectrum of BSA..............................................S7
5. Figures S10, S11, representative fluorescence titrations of mTBA with BSA........S7-S8
Experimental Section

Oligonucleotide Synthesis:

Fur-dG and CNPh-dG phosphoramidites and corresponding mTBA oligonucleotides were synthesized as previously reported. Full synthetic details including MS characterization of mTBA oligonucleotides are available in our recent publication and the corresponding ESI.

Titration Procedure

Titrations were performed in the following way:

All oligonucleotide samples were prepared to a final concentration of 6 μM prepared in 100 μM Sodium Phosphate Buffer pH 7.0 with 0.1M NaCl; duplex samples were prepared using equivalent amounts (6 μM) of the 8-aryl-G modified TBA oligonucleotide and its complementary strand. All measurements were made using quartz cells (Hellma Analytics 119.004F-QS) with a light path of 10 × 2 mm; excitation and emission slit-widths were kept constant at 5 nm. All fluorescence excitation spectra were recorded at the emission wavelength (maximum) of the 8-aryl–dG probe, from 200 to 10 nm below the emission wavelength, while fluorescence emission spectra were recorded at the excitation wavelength (maximum) of the probe, from 10 nm above the excitation wavelength to 600 nm. Spectra were recorded at 25 °C.

Thrombin solutions were prepared to 200 μM stock concentrations. Quantification was performed using absorbance at 280nm and an extinction coefficient ε = 72150 which was obtained from the Sigma Aldrich website. 2 μL additions of thrombin were then added to the fluorescence cuvette containing the DNA solutions. Scans were taken at 5 minute intervals and a total of 20 minutes were allowed to pass before sequential additions.

Fluorescence titration data was transformed into binding isotherms by calculating the fraction bound using:

\[
\text{Fraction Bound} = \frac{(F_{\text{obs}} - F_i)}{(F_{\text{max}} - F_i)}
\]

Where \(F_{\text{obs}}\) = observed fluorescence intensity, \(F_i\) is the initial fluorescence intensity and \(F_{\text{max}}\) is the fluorescence intensity of the oligonucleotide when fully bound by thrombin.

\(K_d\) values were obtained by plotting Fraction bound vs. [Thrombin] to generate a binding isotherm that was subjected to a one site saturation ligand binding analysis within SigmaPlot Version 11.0. \(K_a\) values were calculated to be \(= 1 / K_d\).
Figure S1: ssDNA to quadruplex fluorescence titration of TBA Fur@5 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

\[
K_d = (3.33 \pm 0.41) \times 10^{-6}
\]
\[R^2 = 0.960\]

Figure S2: ssDNA to quadruplex fluorescence titration of TBA Fur@6 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

\[
K_d = (5.23 \pm 0.88) \times 10^{-6}
\]
\[R^2 = 0.946\]
Figure S3: ssDNA to quadruplex fluorescence titration of TBA Fur@8 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

$$K_d = (4.50 \pm 0.51) \times 10^{-6} \quad R^2 = 0.979$$

Figure S4: ssDNA to quadruplex fluorescence titration of TBA Fur@5;CNPh@8 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

$$K_d = (3.82 \pm 0.43) \times 10^{-6} \quad R^2 = 0.967$$
Figure S5: ssDNA to quadruplex fluorescence titration of TBA CNPh@5 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

\[ K_d = (6.43 \pm 0.98) \times 10^{-6} \]
\[ R^2 = 0.961 \]

Figure S6: ssDNA to quadruplex fluorescence titration of TBA CNPh@8 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

\[ K_d = (6.43 \pm 0.98) \times 10^{-6} \]
\[ R^2 = 0.961 \]
Figure S7: dsDNA to quadruplex fluorescence titration of TBA Fur@5 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

\[
K_d = (5.54\pm0.96) \times 10^{-6}
\]
\[
R^2 = 0.940
\]

Figure S8: dsDNA to quadruplex fluorescence titration of TBA Fur@5;CNPh@8 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

\[
K_d = (4.41\pm0.79) \times 10^{-6}
\]
\[
R^2 = 0.933
\]
Table S1: Tabulated Dissociation Constants and associated coefficient of determination values for thrombin binding by various Fur*dG and CNPh*dG mTBA oligonucleotides

<table>
<thead>
<tr>
<th>Starting Structure&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Modification</th>
<th>$K_d$ (x10&lt;sup&gt;-6&lt;/sup&gt;)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Std. Error (x10&lt;sup&gt;-7&lt;/sup&gt;)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>$K_a$ (x10&lt;sup&gt;5&lt;/sup&gt;)&lt;sup&gt;e&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ssDNA</td>
<td>Fur*dG@5</td>
<td>3.33</td>
<td>4.10</td>
<td>3.00</td>
<td>0.960</td>
</tr>
<tr>
<td>ssDNA</td>
<td>Fur<em>dG@5;CNPh</em>dG@8</td>
<td>3.82</td>
<td>4.30</td>
<td>2.62</td>
<td>0.967</td>
</tr>
<tr>
<td>dsDNA</td>
<td>Fur<em>dG@5;CNPh</em>dG@8</td>
<td>4.41</td>
<td>7.90</td>
<td>2.27</td>
<td>0.933</td>
</tr>
<tr>
<td>ssDNA</td>
<td>Fur*dG@8</td>
<td>4.50</td>
<td>5.10</td>
<td>2.22</td>
<td>0.979</td>
</tr>
<tr>
<td>ssDNA</td>
<td>Fur*dG@6</td>
<td>5.23</td>
<td>8.80</td>
<td>1.91</td>
<td>0.946</td>
</tr>
<tr>
<td>dsDNA</td>
<td>Fur*dG@5</td>
<td>5.54</td>
<td>9.60</td>
<td>1.81</td>
<td>0.940</td>
</tr>
<tr>
<td>ssDNA</td>
<td>CNPh*dG@5</td>
<td>6.43</td>
<td>9.80</td>
<td>1.56</td>
<td>0.961</td>
</tr>
<tr>
<td>ssDNA</td>
<td>CNPh*dG@8</td>
<td>9.42</td>
<td>9.98</td>
<td>1.06</td>
<td>0.953</td>
</tr>
</tbody>
</table>

<sup>a</sup> Starting structure refers to the oligonucleotide being present as the single strand or double strand prior to the introduction of thrombin.  
<sup>b</sup> $K_d$ values calculated using SigmaPlot version 11.0 one site saturation simple ligand binding analysis.  
<sup>c</sup> Standard error values associated with the generation of $K_d$ values.  
<sup>d</sup> $K_a$ values calculated as $1 / K_d$.

Figure S9: Excitation and emission spectra of 6μM Bovine Serum Albumin solution in H<sub>2</sub>O.
**Figure S10:** ssDNA to fluorescence titration of TBA Fur@5 with serum albumin. Insert: % Change in fluorescent signal vs. [bovine serum albumin].

**Figure S11:** ssDNA to fluorescence titration of TBA CNPh@5 with serum albumin. Insert: % Change in fluorescent signal vs. [bovine serum albumin].

**REFERENCES:**