Supporting Information for:

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

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Experimental Section

Oligonucleotide Synthesis:

^{Fur}dG and ^{CNPh}dG phosphoramidites and corresponding mTBA oligonucleotides were synthesized as previously reported.[1] Full synthetic details including MS characterization of mTBA oligonucleotides are available in our recent publication and the corresponding ESI.[1]

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:

$$Fraction\ Bound = \frac{(F_{obs} - F_i)}{(F_{max} - F_i)}$$

Where F_{obs} = observed fluorescence intensity, F_i is the initial fluorescence intensity and F_{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.

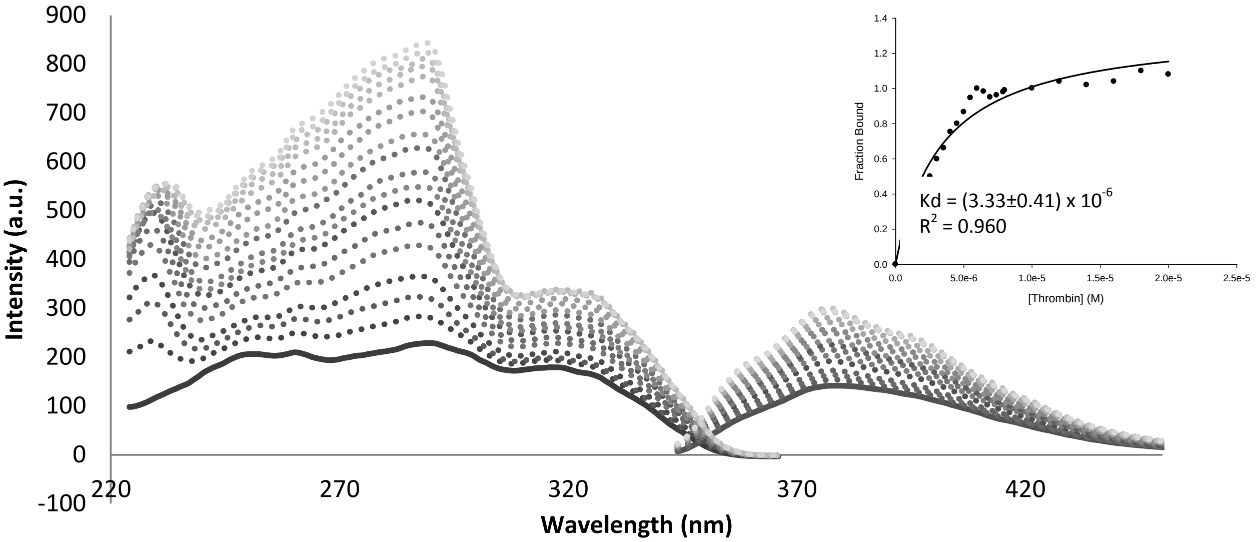

Figure S1: ssDNA to quadruplex fluorescence titration of TBA Fur@5 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

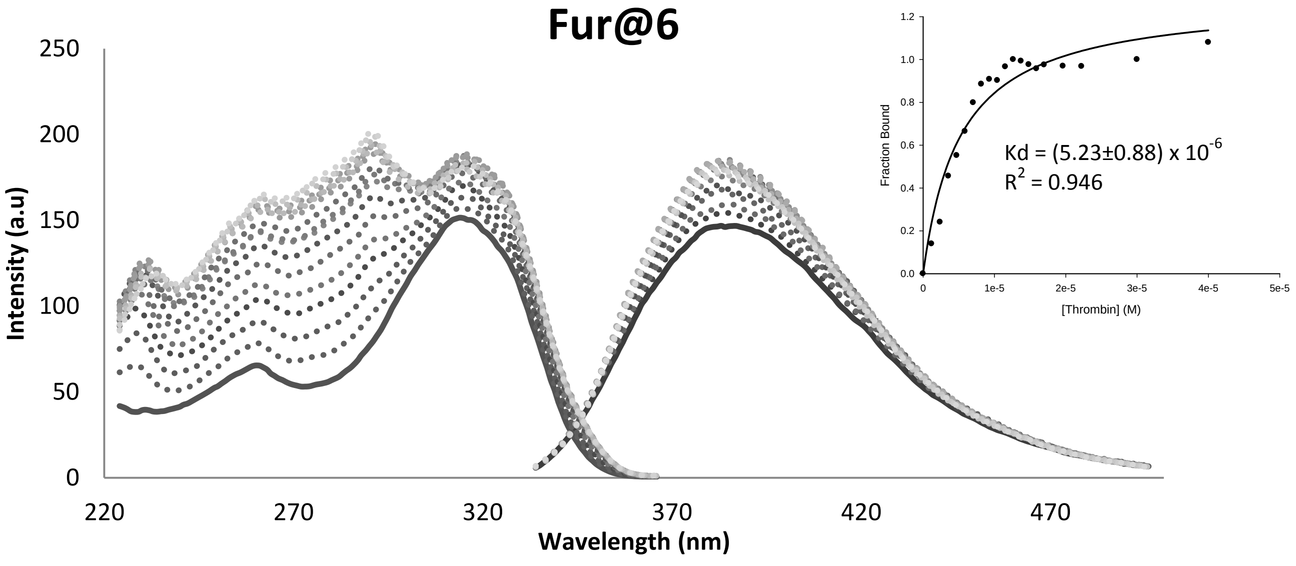

Figure S2: ssDNA to quadruplex fluorescence titration of TBA Fur@6 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

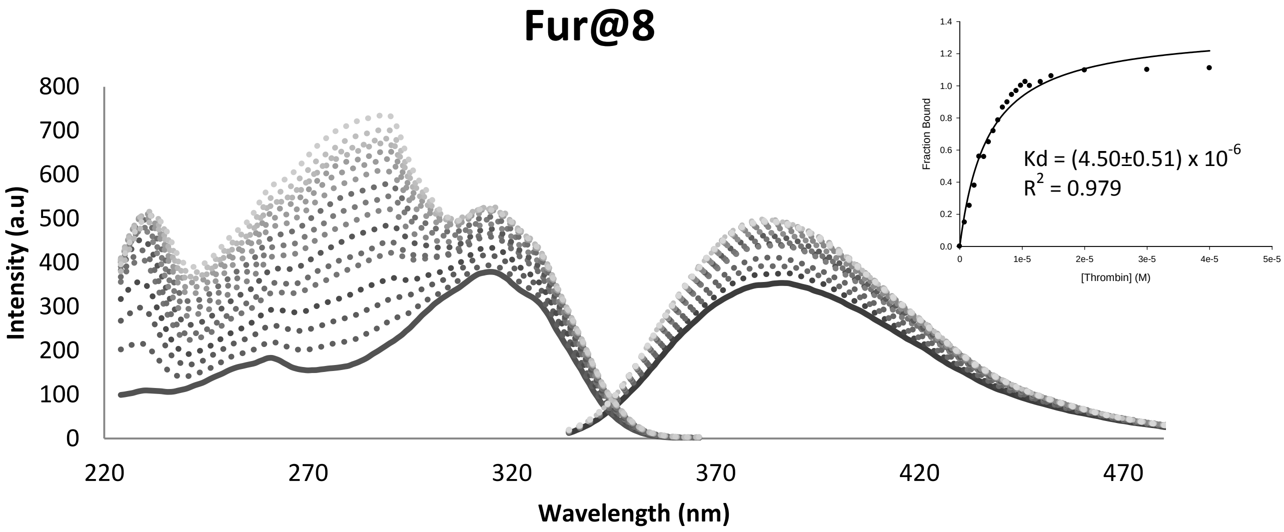

Figure S3: ssDNA to quadruplex fluorescence titration of TBA Fur@8 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

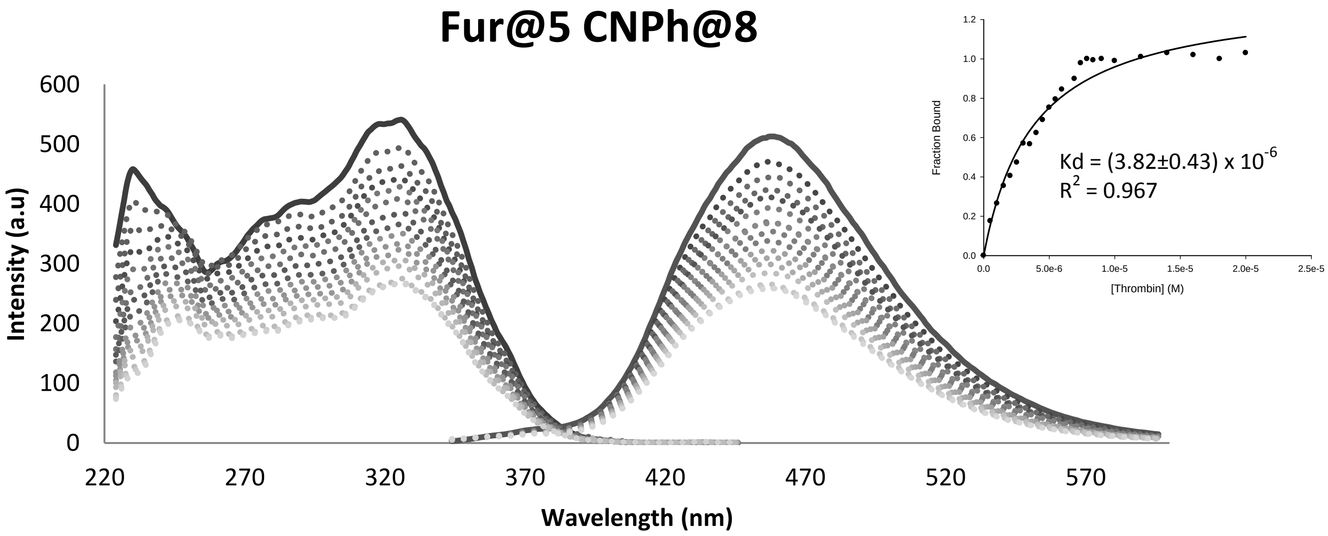

Figure S4: ssDNA to quadruplex fluorescence titration of TBA Fur@5;CNPh@8 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

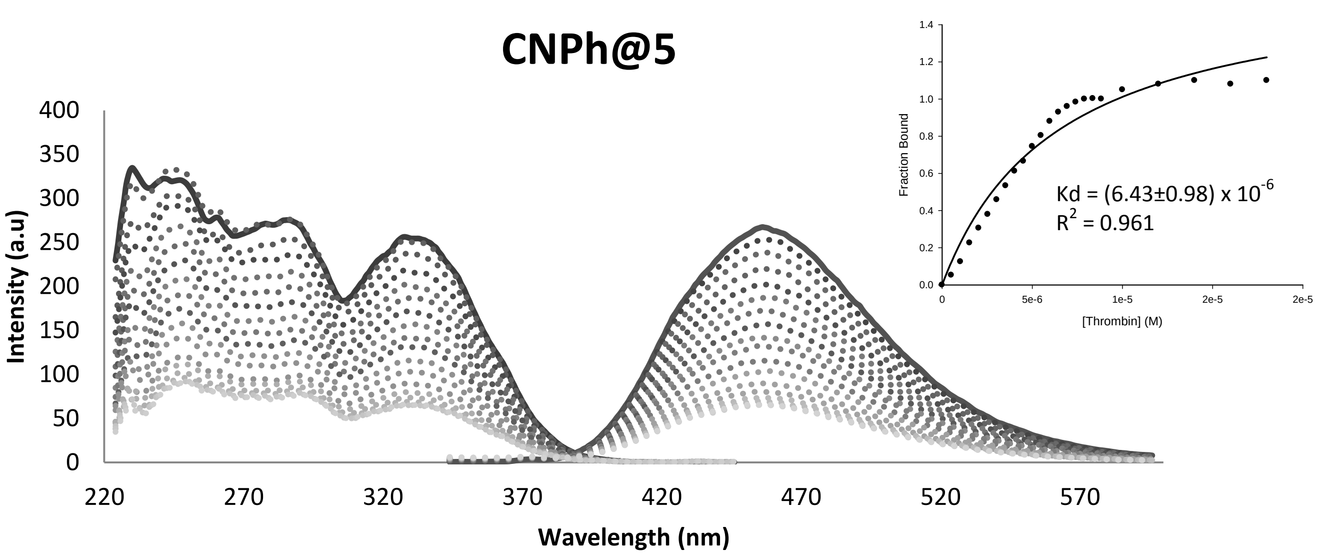

Figure S5: ssDNA to quadruplex fluorescence titration of TBA CNPh@5 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

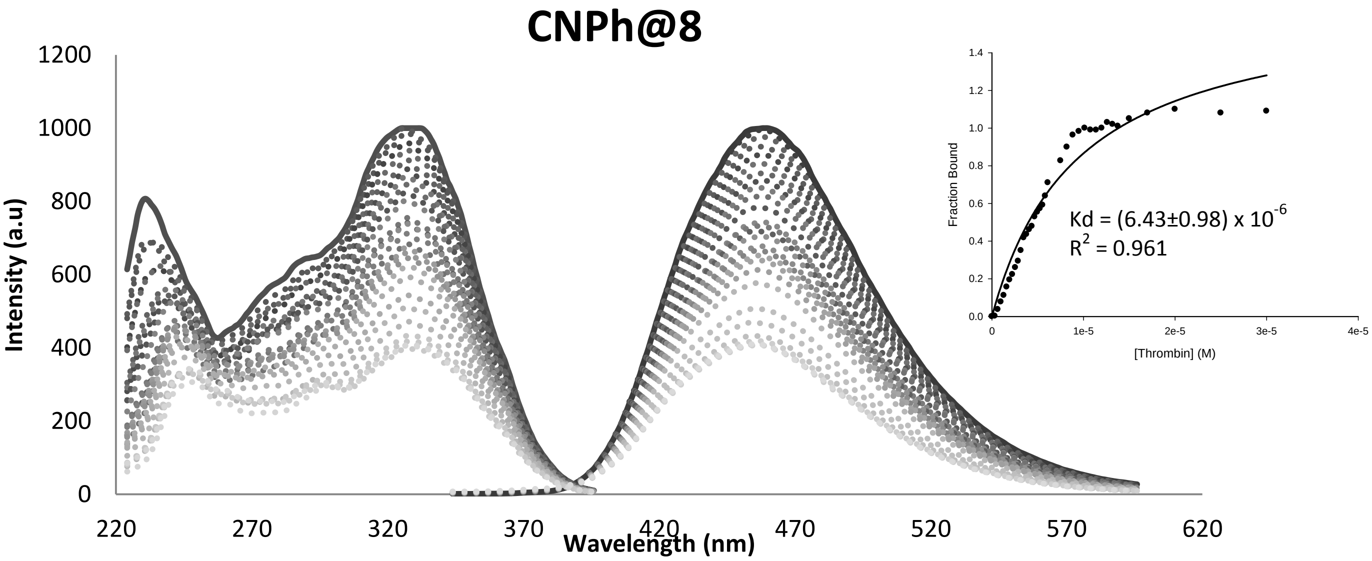

Figure S6: ssDNA to quadruplex fluorescence titration of TBA CNPh@8 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

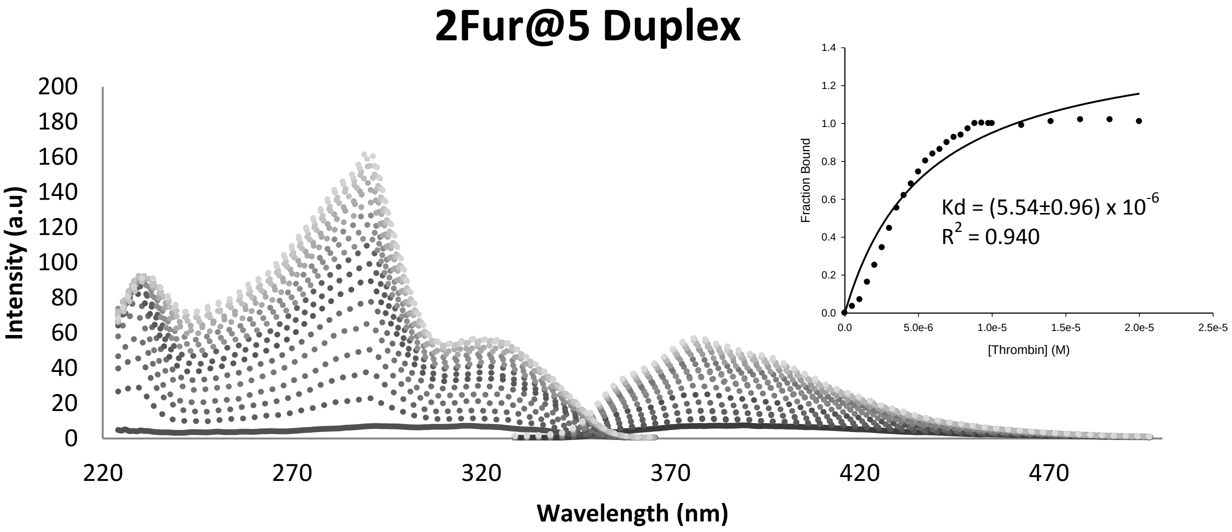

Figure S7: dsDNA to quadruplex fluorescence titration of TBA Fur@5 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

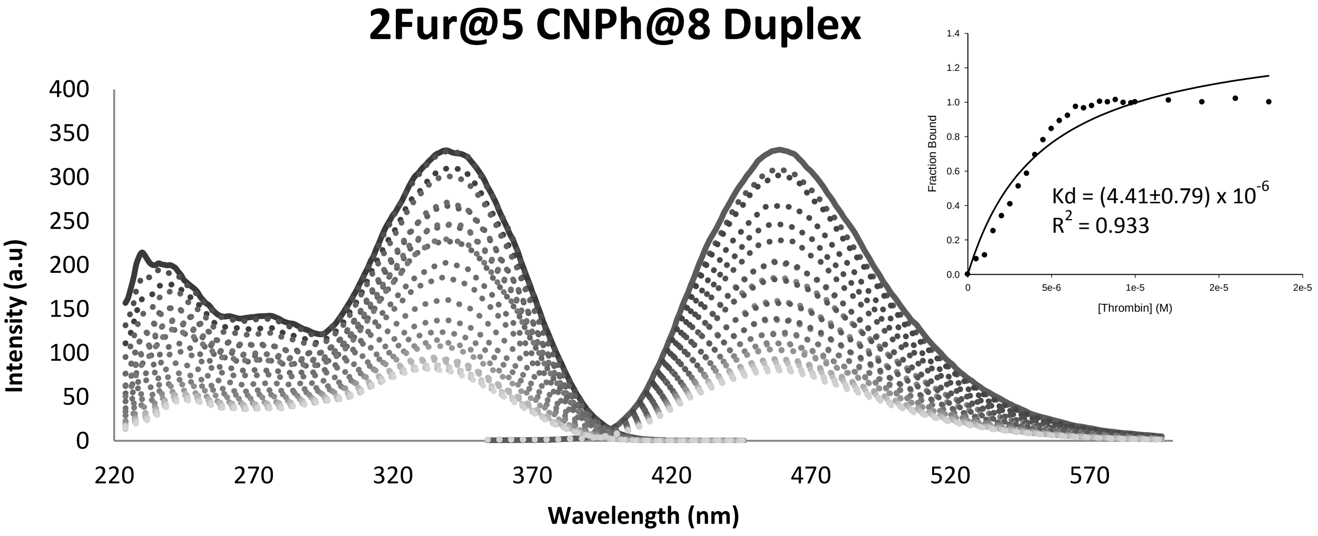

Figure S8: dsDNA to quadruplex fluorescence titration of TBA Fur@5;CNPh@8 with thrombin. Insert: Binding isotherm plotting Fraction of oligonucleotide bound vs. [thrombin] to generate dissociation constants and associated coefficient of determination.

Table S1: Tabulated Dissociation Constants and associated coefficient of determination values for thrombin binding by various ^{Fur}dG and ^{CNPh}dG mTBA oligonucleotides

Starting Structure^a	Modification	K_d (x10^{-6}) ^{b}	Std. Error (x10^{-7})^d	K_a (x10^5) ^{e}	R^2
ssDNA	^{Fur}dG@5	3.33	4.10	3.00	0.960
ssDNA	^{Fur}dG@5;^{CNPh}dG@8	3.82	4.30	2.62	0.967
dsDNA	^{Fur}dG@5;^{CNPh}dG@8	4.41	7.90	2.27	0.933
ssDNA	^{Fur}dG@8	4.50	5.10	2.22	0.979
ssDNA	^{Fur}dG@6	5.23	8.80	1.91	0.946
dsDNA	^{Fur}dG@5	5.54	9.60	1.81	0.940
ssDNA	^{CNPh}dG@5	6.43	9.80	1.56	0.961
ssDNA	^{CNPh}dG@8	9.42	9.98	1.06	0.953

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

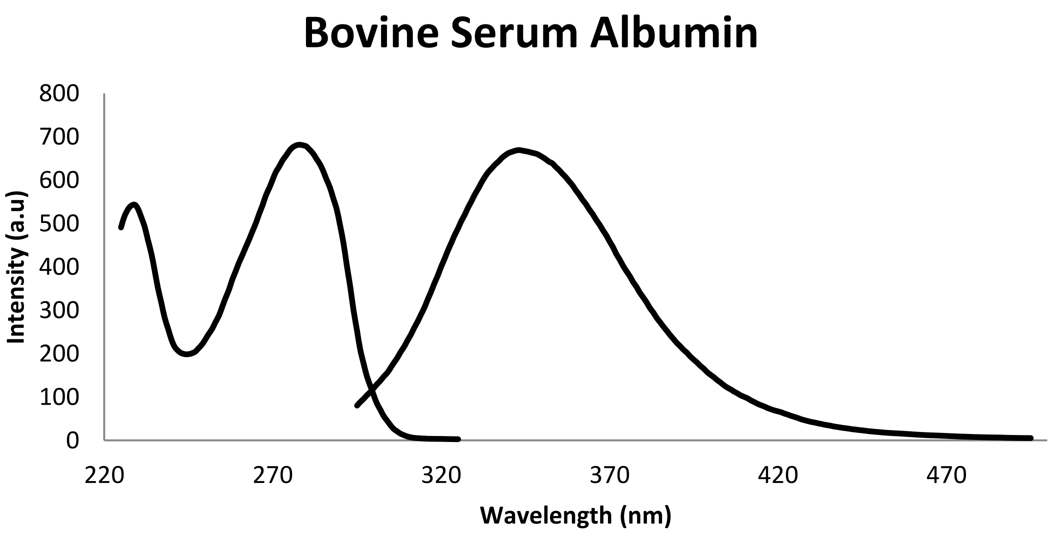

Figure S9: Excitation and emission spectra of 6μM Bovine Serum Albumin solution in H_2O.

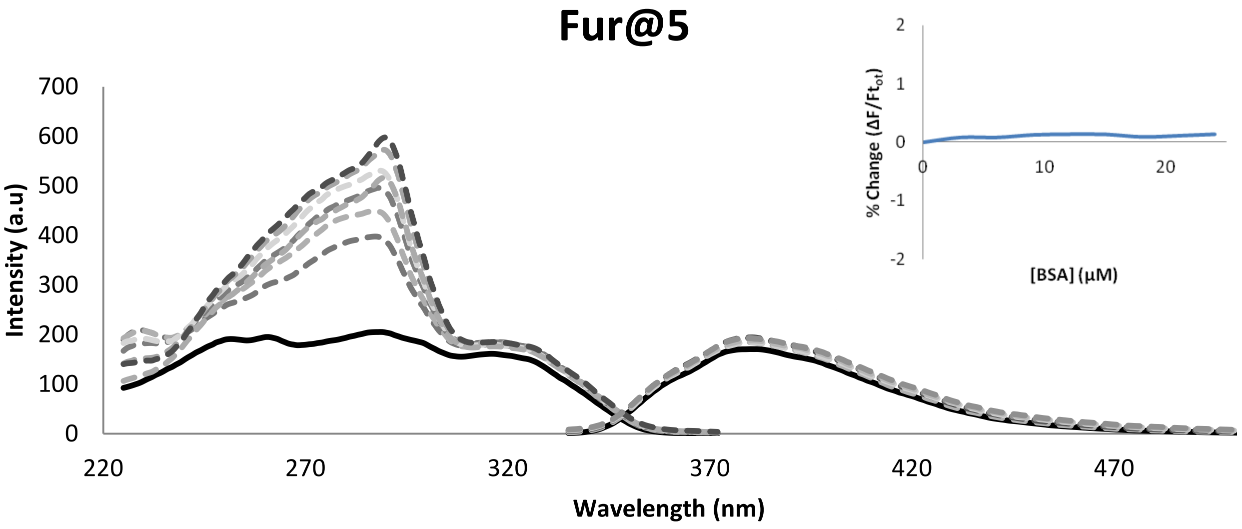

Figure S10: ssDNA to fluorescence titration of TBA Fur@5 with serum albuin. Insert: % Change in fluorescent signal vs. [bovine serum albumin].

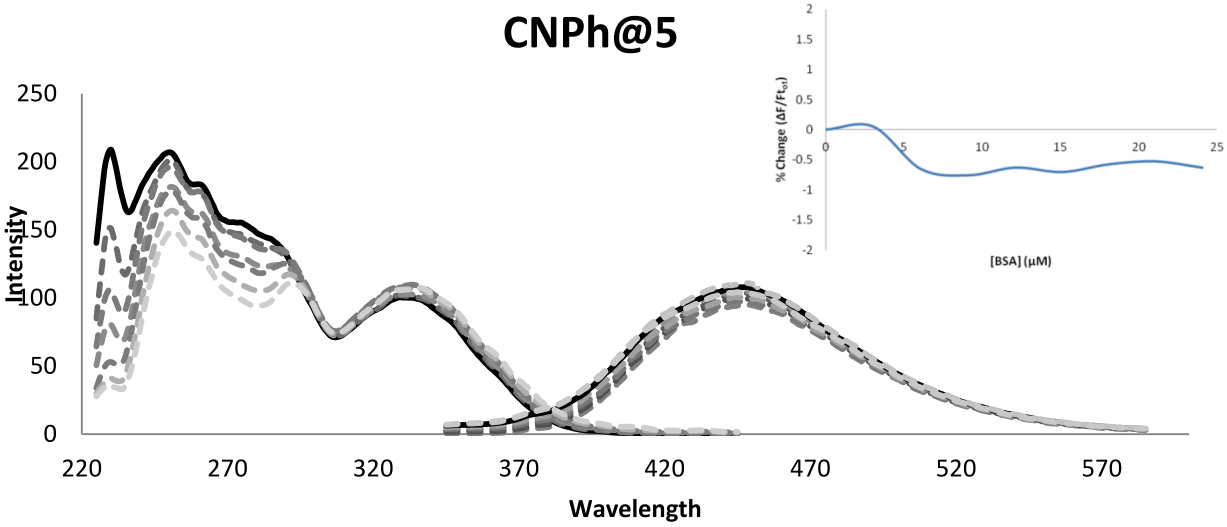

Figure S11: ssDNA to fluorescence titration of TBA CNPh@5 with serum albuin. Insert: % Change in fluorescent signal vs. [bovine serum albumin].

REFERENCES:

[1] M. Sproviero, K. L. Fadock, A. A. Witham, R. A. Manderville, P. Sharma and S. D. Wetmore, *Chem. Sci.* 2014, **5**, 788-796.