SUPPORTING INFORMATION

Single-Stranded DNA Binding Protein-Assisted Fluorescence Polarization Aptamer Assay for Detection of Small Molecules

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Table IS. List of oligonucleotides used.[a]

Oligonucleotides	Sequences and labeling sites
5'-F-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
3'-F-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
T14-F-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
T11-F-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
T3-F-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
T13-F-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
5'-TR-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
3'-TR-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
T14-TR-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
T11-TR-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
T3-TR-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
T13-TR-Apt-A	5'-CCTGGGGGAGTATTGCGGAGGAAGG-3'
Scramble 5'-F-Apt-A	5'-AACGCGGAAGGGGTAGTTGTGGCGG-3'
Split 5'-F-Apt-A (St1*)	5'-ACCTGGGGGAGTA-3'
Split Apt-A (St2)	5'-TGCGGAGGAAGGT-3'
5'-F-Apt-R	5'-GATCGAAACGTAGCGCCTTCGATC-3'
Scramble 5'-F-Apt-R	5'-CGATGGCCAGGTATCACACATGCT-3'

[a]Each labeling site is underlined (green for fluorescein and red for Texas Red)

Table IIS. Comparison of the Ade detection limits for the existing aptasensors.[a]

Assay strategy	Limit of detection	Ref
Fluorescence resonance energy transfer direct assay	10 μM[b]	36
Label-free fluorescent direct assay	10 μM	44
Label-free fluorescent direct assay	20 μM	45
Label-free fluorescent direct assay	3 μM	46
Enzymatic activity inhibition assay	25 μM	47
Label-free fluorescent sandwich-like assay	12 μM	48
G-quadruplex enzyme colorimetric direct assay	6 μM	49
Label-free fluorescent direct assay	2 μM	50
Direct FPAA	10 μM	15
Enzymatic cleavage protection FPAA	5 μM	16
SSB-assisted FPAA	1 μM	This work

[a]Excluding sensing platforms which involved heterogeneous format or sophisticated amplification approach
[b]Estimated from the Ade titration curve

Theoretical model. A competitive displacement model was developed to describe the target-induced FP signal change of the SSB-assisted FPAA. This is derived from the two following binding equilibria that involve the aptamer probe (Apt*), the protein (SSB) and the target (T):

$$SSB + Apt^* \rightleftarrows SSB.Apt^* \quad (1S)$$

$$T + Apt^* \rightleftarrows T.Apt^* \quad (2S)$$

For the binding equilibrium between SSB and aptamer, i. e. Eq (1S), the fraction of the SSB-bound aptamer (f_b) is derived from the measured fluorescence anisotropy r as follows:[S1]

$$f_b = \frac{(r - r_f)}{g(r_b - r) + (r - r_f)} \quad (3S)$$

where r_f and r_b represent the anisotropy for the free and SSB-bound aptamer probe, respectively. The g factor is derived from the ratio of the total emission intensity (I) of the SSB-bound aptamer probe (I_b) to that of the free tracer (I_f), i. e. I_b/I_f.[S1b] It is introduced into Eq (3S) in order to consider possible change in the fluorescence quantum yield of the probe between the two states.[S1a] Both r_b and I_b values can be estimated from the asymptote of the r and I *versus* SSB concentration curves. As frequently observed for DNA-protein associations,[S2] f_b can be linked to the average dissociation constant of the aptamer-SSB complex (K_{dssb}) and the free concentration of SSB ([SSB]) through the Hill binding model:[S3]

$$f_b = \frac{[SSB]^{n_H}}{K_{dssb} + [SSB]^{n_H}} \quad (4S)$$

where n_H corresponds to the Hill coefficient. Under conditions for which the total aptamer probe concentration (c_{apt}) is limiting ($c_{apt} \leq K_{dssb}/5$), the total concentration of SSB can closely approximate its free concentration. The presence of target in the reaction system can be accounted by introducing an additional competitive term into Eq (4S) as follows:[S4]

$$f_b = \frac{[SSB]^{n_H}}{K_{dssb}(1 + \frac{[T]}{K_{dtarget}}) + [SSB]^{n_H}} \quad (5S)$$

where $K_{dtarget}$ and [T] are the apparent dissociation constant of the target-aptamer complex and the free target concentration, respectively. Under limiting aptamer probe concentrations, the total concentration of target can be used in place of its free concentration into Eq (5S). In this relation, the interaction between the folded target-bound aptamer and the SSB protein is considered to be sufficiently weak to be neglected. Thus, combining Eqs (3S) and (5S), and assuming that the fluorescence anisotropy and total emission intensity values are similar for the free and target-bound aptamer states, the change of the probe fluorescence anisotropy when the target concentration varies is described by the following expression:

$$r = \frac{[SSB]^{n_H}\left(gr_b - r_f\right) + r_f\left\{K_{dssb}\left(1 + \frac{[T]}{K_{dtarget}}\right) + [SSB]^{n_H}\right\}}{g[SSB]^{n_H} + K_{dssb}\left(1 + \frac{[T]}{K_{dtarget}}\right)} \qquad (6S)$$

Figure 2S (see below) presents theoretical data, at a given SSB concentration, created from Eq (6S) for various K_{dssb}, $K_{dtarget}$, (r_b-r_f) and g factor values (all other parameters being held constant). The increase in K_{dssb} and (r_b-r_f) values and/or the decrease in $K_{dtarget}$ and g factor values govern an enhancement of the displacement curve slope, i. e. an increase in the assay sensitivity. This is achieved at the expense of the FP signal variation range for the K_{dssb} and g factor effects.

References

(S1) (a) Jameson, D. M.; Ross, J. A. *Chem. Rev.* **2010**, *110*, 2685-2708. (b) Potty, A. S.; Kourentzi, K.; Fang, H.; Schuck P.; Willson, R. C. *Int. J. Biol. Macromol.* **2011**, *48*, 392-397.

(S2) Weinberg, R. L.; Veprintsev, D. B.; Fersht, A. R. *J. Mol. Biol.* **2004**, *341*, 1145-1159

(S3) Wilson, G. M. *Reviews in Fluorescence*; Springer Science: New York, 2005; Chapter 10.

(S4) Hieb, A. R.; D'Arcy, S.; Kramer, M. A.; White, A. E.; Luger, K. *Nucleic Acids Res.* **2012**, *40*, e33.

Figure 1S. Predicted secondary structures for the Apt-A probe and Apt-A-Ade complex (adapted from ref 32).

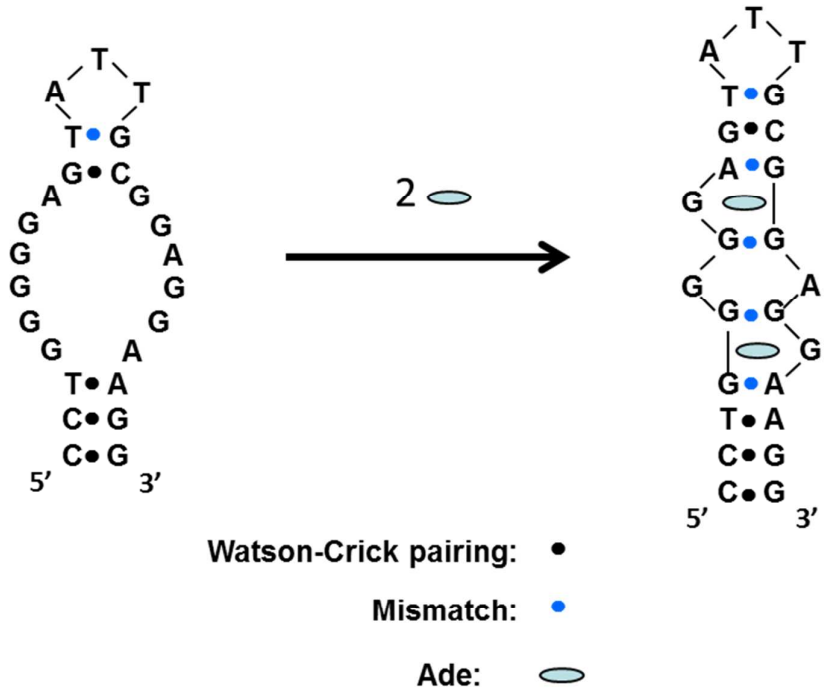
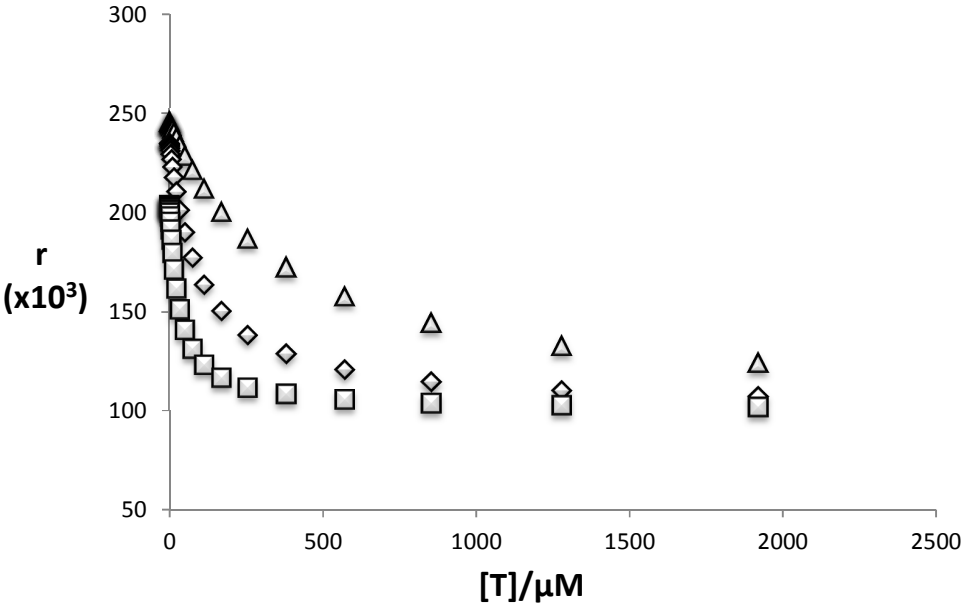
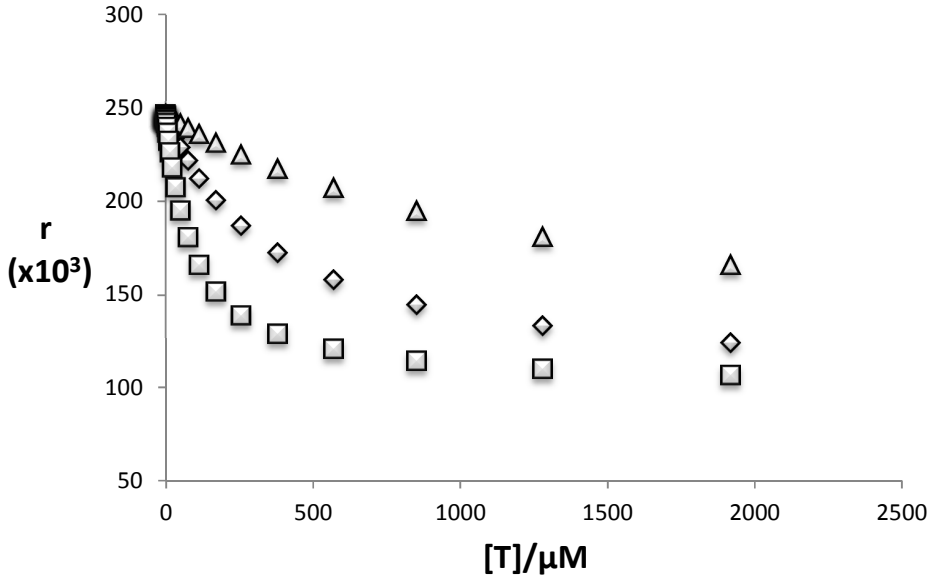


Figure 2S. Theoretical data created from Eq (6S) for different values of **(a)** K_{dssb}: 50 nM (triangles), 100 nM (diamonds) and 150 nM (squares); fixed parameters: r_b = 0.250, r_f = 0.100, $K_{dtarget}$ = 10 µM, *g* factor = 1. **(b)** $K_{dtarget}$: 40 µM (triangles), 10 µM (diamonds) and 2.5 µM (squares); fixed parameters: r_b = 0.250, r_f = 0.100, K_{dssb} = 50 nM, *g* factor = 1. **(c)** (r_b-r_f): 0.200 (triangles), 0.150 (diamonds) and 0.100 (squares); fixed parameters: r_f = 0.100, K_{dssb} = 50 nM, $K_{dtarget}$ = 10 µM, *g* factor = 1. **(d)** *g* factor: 2.5 (triangles), 1 (diamonds) and 0.4 (squares); fixed parameters: r_b = 0.250, r_f = 0.100, K_{dssb} = 50 nM, $K_{dtarget}$ = 10 µM. Total SSB concentration and n_H are fixed to 300 nM and 2, respectively.

(a)

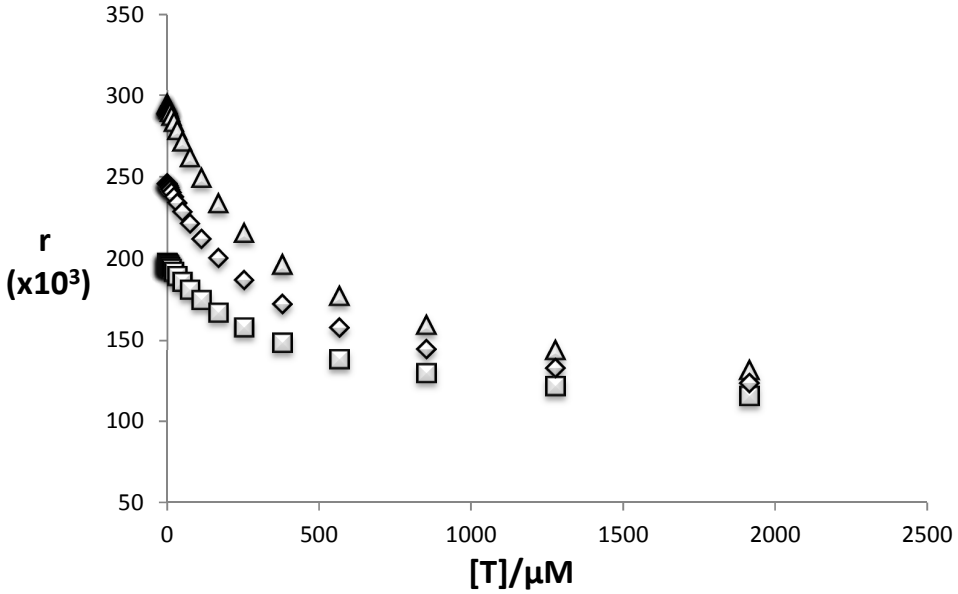


(b)



(Continued)

(c)



(d)

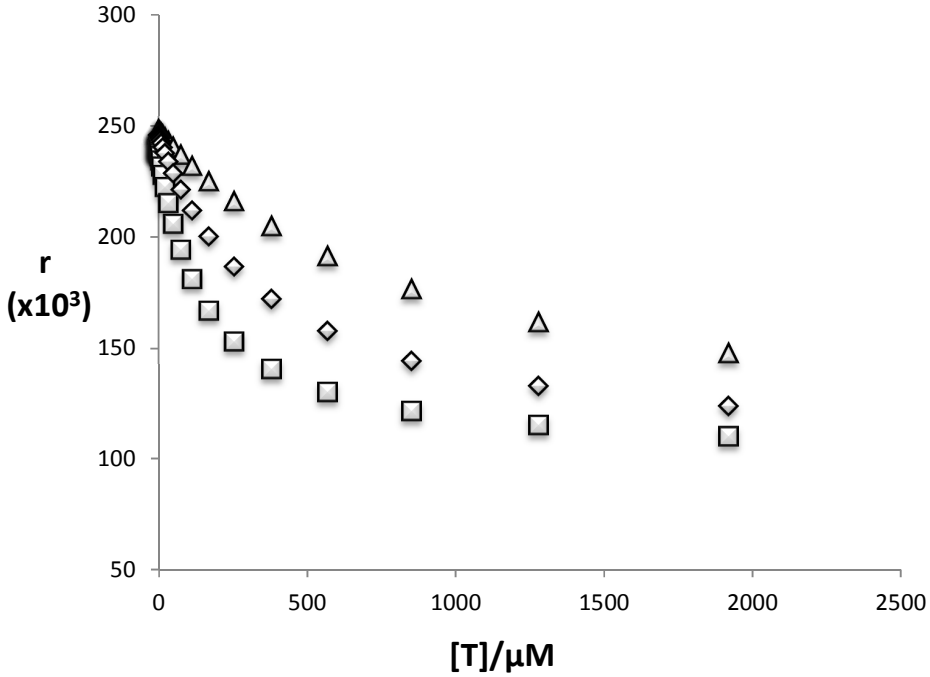


Figure 3S. SSB titration curve using the 5'-F-Apt-A probe. The theoretical curve (——) is recreated using equation parameters (see Figure 2 in the manuscript) obtained by fitting Eq (4S) to f_b. Probe concentration = 10 nM. Binding buffer conditions: 50 mM Tris-HCl, pH = 7.5, 5 mM NaCl, 20 mM $MgCl_2$.

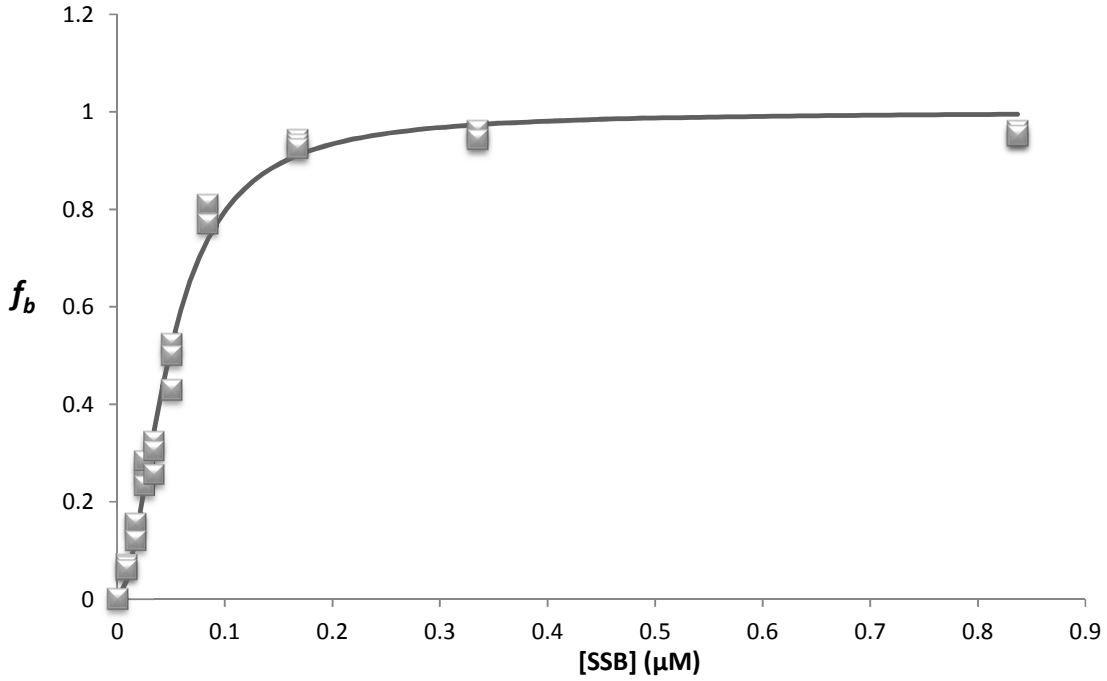


Figure 4S. Representative competitive displacement curves using T14-F-Apt-A (black circles), T13-F-Apt-A (white circles), 3'-TR-Apt-A (black squares) and T11-TR-Apt-A (white squares). The theoretical curves (——) are recreated using equation parameters (see Figure 2 in the manuscript) obtained by fitting Eq (6S) to *r*. Probe concentration = 10 nM. SSB concentration = 0.33 μM. Binding buffer conditions: 50 mM Tris-HCl, pH = 7.5, 5 mM NaCl, 20 mM $MgCl_2$.

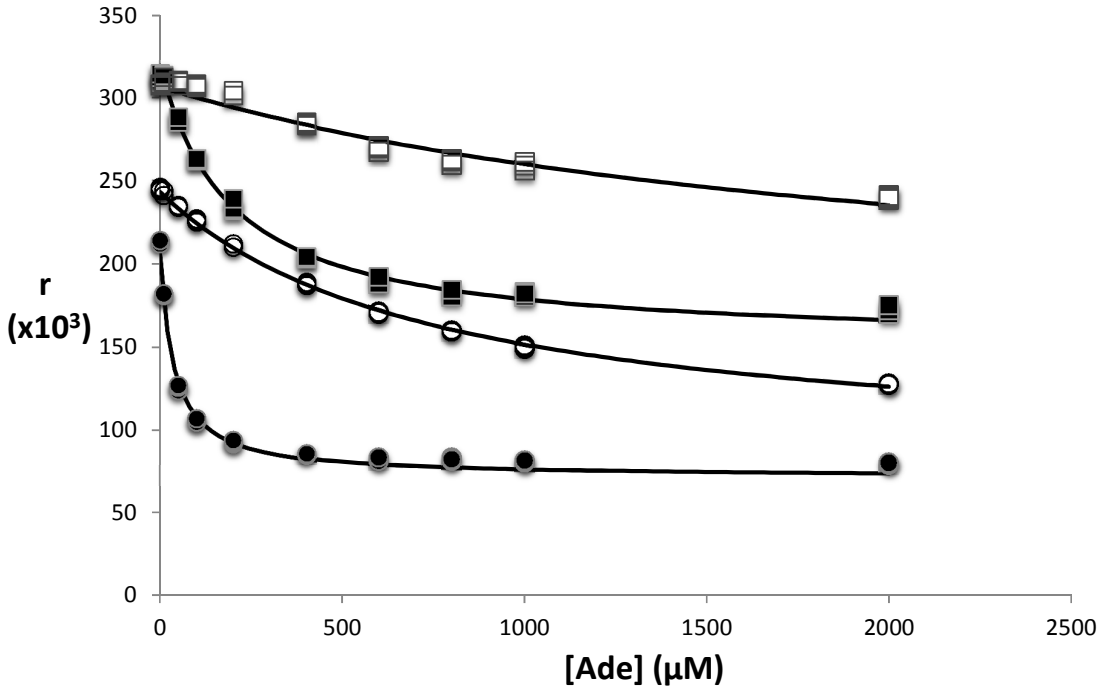


Figure 5S. Calibration curves for Ade using the T14-F-Apt-A probe under 50-fold (squares), 10-fold (triangles) and 5-fold (diamonds) diluted human serum conditions. Probe concentration = 10 nM. SSB concentration = 0.25 µM. $\Delta r = r - r_0$ where r_0 is the fluorescence anisotropy in absence of analyte.

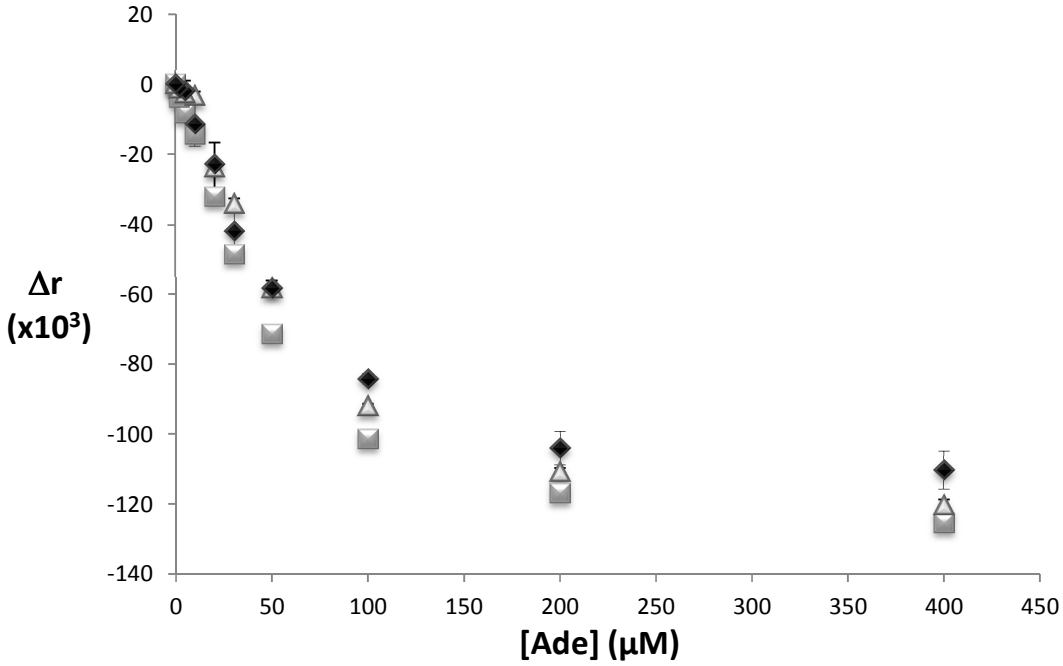
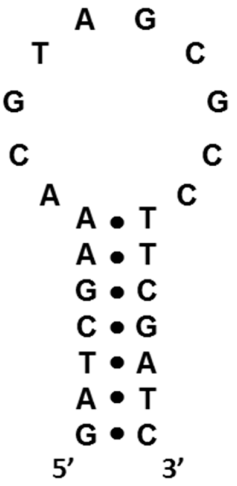


Figure 6S. (a) Predicted secondary structure for the Apt-R probe (adapted from ref 51). **(b)** Titration curves of 5'-F-Apt-R with increasing Rm concentration under SSB-assisted (black symbols) and direct (white symbols) FPAA modes. Grey symbols for SSB-assisted FPAA using the scramble 5'-F-Apt-R. Probe concentration = 10 nM. SSB concentration = 0.33 µM. Binding buffer conditions: 10 mM Tris-HCl, pH = 7.5, 100 mM NaCl. $\Delta r = r - r_0$ where r_0 is the fluorescence anisotropy in absence of analyte.

(a)



Watson-Crick pairing: •

(b)

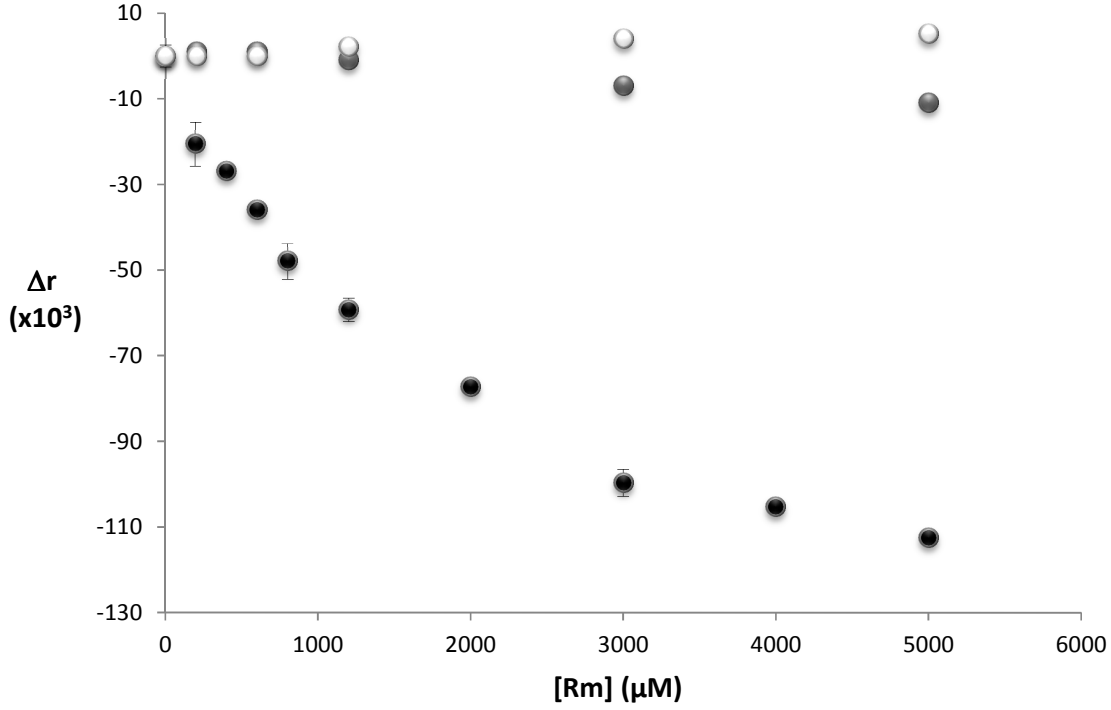
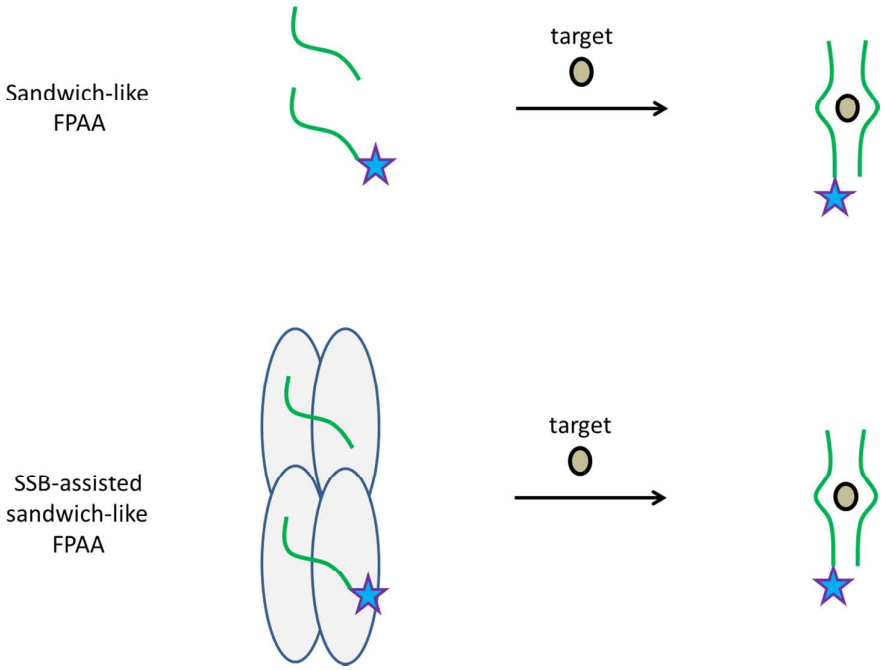


Figure 7S. (a) Schematic representation of the sandwich-like FPAA design with the SSB-assisted format. **(b)** Titration curves of split 5'-F-Apt-A (St1*:St2, 1:8 stoichiometry) with increasing Ade concentration in presence (circles) and absence (diamonds) of SSB protein. St1* concentration = 10 nM. SSB concentration = 1.67 µM. Binding buffer conditions: 50 mM Tris-HCl, pH = 7.5, 5 mM NaCl, 20 mM $MgCl_2$. $\Delta r = r - r_0$ where r_0 is the fluorescence anisotropy in absence of analyte.

(a)



(b)