Supporting Information

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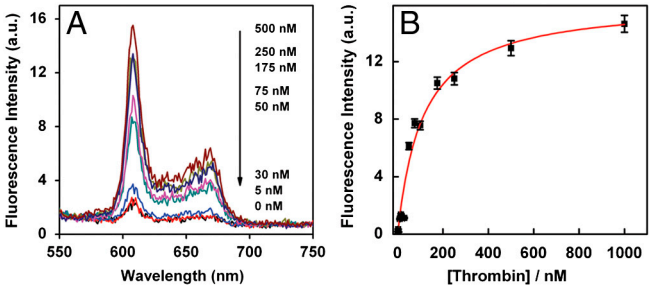

Fig. S1. Self-reporting aptamer (SRA)_2 acts as an effective fluorescent reporter of target-binding events. (*A*) Fluorescence measurements of antisense-hybridized SRA_2 upon the addition of thrombin at different concentrations. The intensity of fluorescence increased monotonically with increasing thrombin concentrations. (*B*) Fluorescence calibration curve indicating thrombin affinity for SRA_2 ($K_d = 83 \pm 17$ nM).

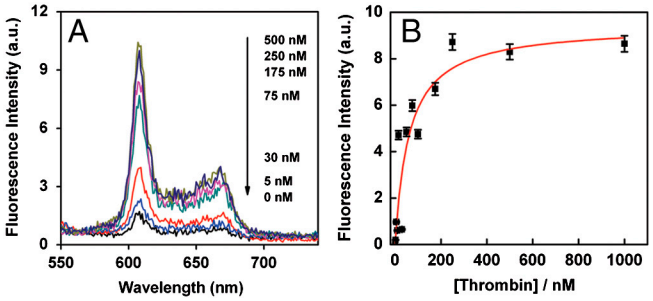

Fig. S2. SRA_3 acts as an effective fluorescent reporter of target-binding events. (*A*) Fluorescence measurements of antisense-hybridized SRA_3 upon the addition of thrombin at different concentrations. The intensity of fluorescence increased monotonically with increasing thrombin concentrations. (*B*) Fluorescence calibration curve indicating thrombin affinity for SRA_3 ($K_d = 76 \pm 22$ nM).

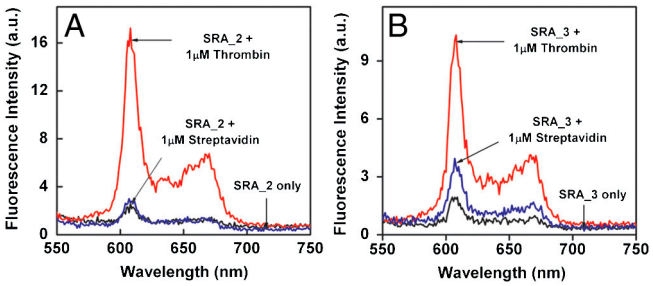

Fig. S3. SRA_2 and SRA_3 demonstrate great target specificity. (*A*) In comparison to duplexes challenged with thrombin, only very weak fluorescence was emitted by antisense-hybridized SRA_2 duplexes alone or challenged with streptavidin. (*B*) Similar characteristics were observed for SRA_3.

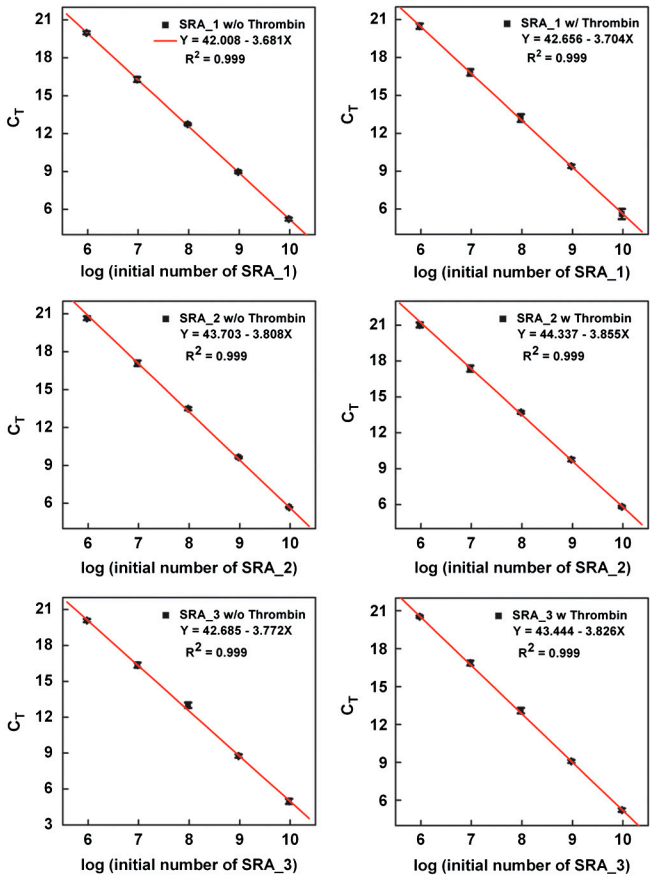

Fig. S4. Threshold cycle number (C_T) is plotted as a function of the logarithm of the initial aptamer copy number. The plots show C_T values for each aptamer in the absence (*Left*) or presence (*Right*) of 1 μM of thrombin. Linear regression equations generated for each dataset indicate negligible difference in slopes between aptamers tested with and without thrombin, demonstrating that the PCR efficiency is not affected by the presence of the target molecule.

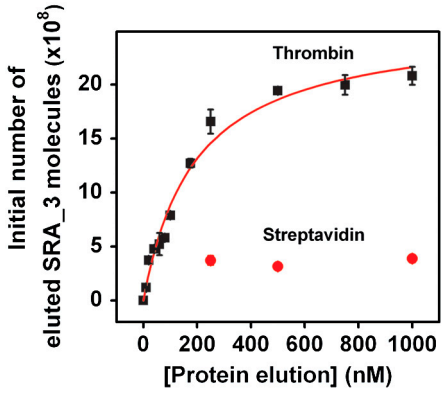

Fig. S5. Determination of the dissociation constant for SRA_3 via RT-PCR yields a K_d value of 156 ± 19.8 nM for thrombin. The aptamer also exhibits clear specificity for thrombin, and the signal from 1 μM streptavidin is ~17% of that observed with 1 μM thrombin.

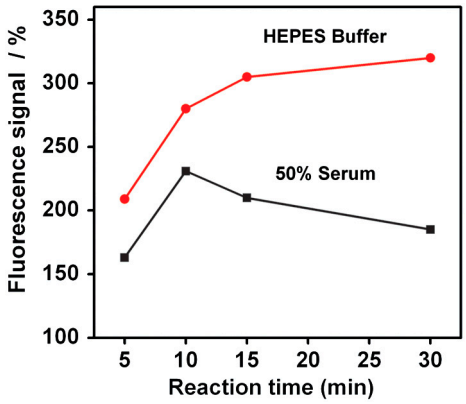

Fig. S6. Time-course experiments reveal the kinetic response of antisense-hybridized SRA_1 to 750 nM thrombin in Hepes buffer or 50% serum. The signal generated by SRA_1 challenged with thrombin in Hepes increases over longer periods of incubation time. In contrast, the signal in 50% serum decreases after 10 min, presumably because the SRA molecules are being digested by serum nucleases.