Supplementary Materials: Miniaturized Aptamer-Based Assays for Protein Detection

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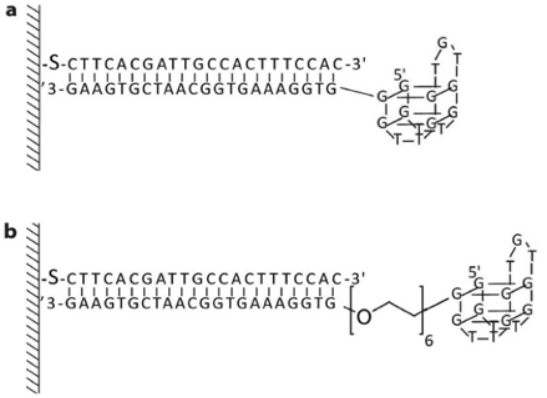

Figure S1. Thrombin aptamers sequences, with (**a**) and without (**b**) hexaethilenglycol-spacer, hybridized to the complementary ssDNA on the surface.

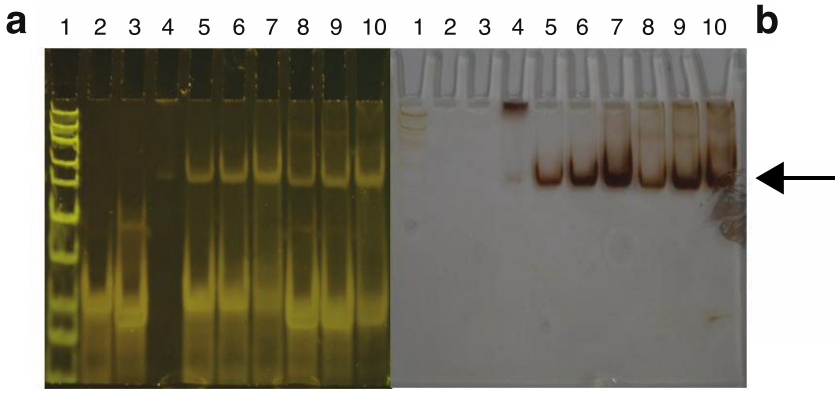

Figure S2. Electrophoretic mobility shift assay with thrombin aptamer designs for DDI, Sybr Green staining (**a**); silver staining (**b**). Lanes corresponding to: 1-DNA ladder; 2-F9-HEGL-aTHR (2 mM); 3-F9aTHR (2 mM); 4-THR(2 mM); 5-7-F9-HEGL-aTHR (2 μM) + THR (0.5, 1, 2 mM); and 8-10-F9aTHR (2 mM) + THR (0.5, 1, 2 mM). The arrow shows bands corresponding to aptamer/protein complex.

EMSA Assay

To run the assay the aptamer constructs were first activated. F9aTHR and F9-HEGL-aTHR were prepared as 10 μM solutions in the binding buffer (THR buf) (20 mM Tris pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$) were first denaturated for 10 min at 95 °C, cooled immediately on crushed ice for 10 min, and left at room temperature for 15 min to restore their functional conformation. Human thrombin prepared in the concentration range 0.5–2 μM was then incubated with aptamers (1 μM final concentration) for 20 min at room temperature and orbital shaking 500 rpm. Next, 5 μL 30% glycerol was added to 20 μL of aptamer-protein samples, mixed and loaded on 12% polyacrylamide gel, 8 × 8 cm, prepared with Tris Borate buffer with 10 mM KCl. EMSA was then performed on a Mini Protean system (Biorad, Laboratories, Inc., Berkeley, CA, USA), at 110 V for 60 min, at room temperature. After electrophoresis, gels were first stained with a DNA intercalating fluorescent dye, Sybr Green, (Invitrogen Corp., Carlsbad, CA, USA), following manufacturer instruction, and visualized at 470 nm excitation wavelength on a transiluminator Safe Imager

(Invitrogen). To visualize the proteins, gels were finally stained with a silver stain kit, ProteoSilver (Sigma-Aldrich, Corp., Saint Louis, MO, USA).

The aptamer/protein complex can be seen on the silver stained gel as an identifiable shift in the protein band. In this regard the F9-HEGL-aTHR was observed to bind more efficiently to THR compared to F9aTHR. The smear visible above the aptamer/protein complex in F9aTHR samples (Figure S2a, lanes 8–10) indicates, in fact, a less intensive binding of the aptamer with the protein. Moreover, an additional band observed in the sample (Figure S2b, lane 3) suggests partial formation of higher molecular weight multimer DNA complexes, as a consequence of partial hybridization of F9aTHR monomers.