Electronic suplementary information

Experimental methods

The aptamer synthesis was contracted to Qiagen (Germany) and was HPLC-purified. It contains a 5' nonamethylene thiol which is protected and a 3' heptamethylene amino-spacer giving as final structure: (Thiol-C_9-5'-CCAACGGTTGGTGTGGTTGG -3'-C7-Amine). The aptamer solution was made at 1mg mL^{-1} with autoclaved milliQ water and stored at -20ºC. Freshly prepared solution of 125 µmol of N- hydroxysuccinimide ester and 250 µmol of N, N'-dicyclohexylcarbodiimide in anhydrous dimethylformamide were added to a solution of 100 µmol of ferrocene acetic acid in the same solvent (these reagents were purchased form Aldrich). The final volume was 300 µL. The reaction mixture was stirred at room temperature under argon atmosphere until appearance of a precipitate. The precipitate was removed by centrifugation and the supernatant was added to 90 µL of 0.1M sodium tetraborate buffer at pH 8.5 containing 0.01 µmol of the amino modified oligonucleotide and was left to react for 6 hours at room temperature. The white precipitate was separated by centrifugation and the supernatant was treated with 10 molar excess of dithiothreitol for deprotection. The modified oligonucleotide was purified with G-25 Shephadex column (Amersham Pharmacia Biotech). The product was characterized by UV spectrometry (8453 from Hewlett Packard) and cyclic voltammetry (Autolab PGSTAT10 from Eco Chemie).

Electrochemistry was performed in a home made 20 µL two electrode thin layer cell with a 4mm^2 square gold sheet as a working electrode opposite to a solid state Ag/AgCl reference/counter painted on a plastic substrate. Ag/AgCl ink from Dupont (5874 conductor). The electrochemical surface plasmon resonance (e-SPR) measurements were performed with a double channel Autolab ESPRIT™ equipment from Eco Chemie. The e-SPR electrochemical cell is a three-electrode system, with a 4.8 mm^2 gold layer sensor disk working electrode. The 35 µL cell has a Ag/AgCl reference electrode and a platinum counter electrode.

The gold electrodes were cleaned with "piranha" solution (70% (v/v) H_2SO_4, 30% (v/v) H_2O_2) for 2 hours at 60ºC and then with concentrated KOH for 1 hour at 60ºC. **Extreme caution should be used with this solution since it reacts violently with organic material and all work should be carried out in a fumehood**. Electrodes were stored in a concentrated H_2SO_4 solution. Before use they were cleaned with concentrated HNO_3 solution for 10 minutes and rinsed with distilled water.

In the e-SPR experiments, 35 µl of 10 mM HEPES, 50mM NaCl pH=8 was added into the cell to set the baseline, 35 nM of redox aptamer in 1M NaH_2PO_4 was immobilized for one hour on the gold coated glass surface. The interaction with thrombin (Purchased form Sigma) was performed with incubation of 2.5 µM thrombin in 50 mM citrate buffer pH 5, 100 mM NaCl during 20 minutes. Regeneration of thrombin was carried out with three incubations of 100 mM glycine, 150 mM HCl for 15 minutes each. As a control, 2.5 µM of bovine serum bovine (BSA, Sigma) in 50 mM citrate buffer pH 5, 100 mM NaCl was injected under the same conditions as the thrombin. After each immobilization a cyclic voltammogram was recorded with a scan rate of 10 mV s^{-1} between –0.2 V and 0.2 V.

In the amperometry experiments, gold electrodes were modified with a mixed self-assembled monolayer exposing it to 33 nM of thiol-aptamer-ferrocene in 1M NaH_2PO_4 and 160 µM MP-11 (purchased from Sigma). The interaction with the different concentrations of thrombin or BSA in the case of controls was performed in 50 mM citrate buffer pH 5, 100 mM NaCl was detected by cyclic voltammetry (scan rate of 10 mV s^{-1}, scan between –0.3 V and 0.4 V) and chronoamperometry (-0.1 V was applied in a solution of 1 mM H_2O_2 in 50 mM citrate buffer pH 5, 100 mM NaCl, after 100 seconds different concentrations of thrombin or BSA (control) was added. The interaction was detected in real time.