Supporting Information

Switching the aptamer attachment geometry can dramatically alter the signaling and performance of electrochemical aptamer-based sensors

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Materials and methods:

Reagents

Sodium hydroxide, sulfuric acid, sodium chloride, potassium chloride, potassium dihydrogen phosphate, tris(hydroxymethyl)aminomethane (tris) and 1 M magnesium chloride solution were purchased from Fisher scientific (Pittsburg, PA). Ethylendiaminetetraacetic acid (EDTA), 6-Mercapto-1-hexanol, tris(2-carboxyethyl)phosphine (TCEP), sodium hydrogen phosphate and cocaine hydrochloride were purchased from sigma Aldrich (St. Louis, MO). Doxorubicin hydrochloride was purchased from LC Laboratories. Vancomycin hydrochloride was purchased from GoldBio, Gold Biotechnology (St. Louis, MO).

Stock of phosphate buffered saline was prepared at 10x and diluted 1 in 10 before use in the measurements (working concentration of PBS 1x was 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4 pH 7.4). Assembling buffer was made of 10 mM Na_2HPO_4, 1 mM $MgCl_2$, 1 M NaCl pH 7.0.

The DNA sequences of the aptamers used to fabricate the relevant EAB sensors were as follows:

Name	Sequence
Doxorubicin 5'-anchor	5' C6Thiol S-S - ACC ATC TGT GTA AGG GGT AAG GGG TGG T – MB 3'
Doxorubicin 3'-anchor	5' MB - ACC ATC TGT GTA AGG GGT AAG GGG TGG T – C6Thiol S-S 3'
Cocaine 5'-anchor	5' C6Thiol S-S - GACAA GGAAA ATCCT TCAAC GAAGT GGGTC – MB 3'
Cocaine 3'-anchor	5' MB - GACAA GGAAA ATCCT TCAAC GAAGT GGGTC – C6Thiol S-S 3'
Vancomycin 5'-anchor	5' C6Thiol S-S - CGAGG GTACC GCAAT AGTAC TTATT GTTCG CCTAT TGTGG GTCGG – MB 3'
Vancomycin 3'-anchor	5' MB- CGAGG GTACC GCAAT AGTAC TTATT GTTCG CCTAT TGTGG GTCGG – C6Thiol S-S 3'

All aptamers were purchased as dual-HPLC purification grade with both modifications in place. The doxorubicin- and cocaine-binding aptamers were purchased from IBA lifesciences GmbH (Göttinegen, Germany). The vancomycin-binding aptamer was purchased from Biomers.net GmbH (Ulm, Germany). Upon receipt, each construct was dissolver at 100 µM in a 1 mM EDTA, 10 mM Tris-HCl, pH 8 buffer and stored at -20 °C. Methylene Blue (MB) was conjugated using amide chemistry with a 6-carbon linker on the 5' end and with a 7-carbon linker on the 3' end (Fig. 1B in the main text). Thiol groups in form of dithiol (S-S) on either 5´or 3´ends where introduced via a 6-carbon linker (Fig. 1B).

Sensor fabrication

EAB sensors were fabricated using gold electrodes, following previously described procedures[1]. In brief, polycrystalline gold disk electrodes (2 mm diameter; CH instruments Inc., Austin, TX) were cleaned by polishing with a MicroCloth 2.875 inches (Buehler, Lake Bluff, IL) in a 1-µm monocrystalline diamond suspension oil-based (Buehler, Lake Bluff, IL), sonicated in ethanol, polished using a 0.05-µm aluminum slurry (Buehler, Lake Bluff, IL), and sonicated in ethanol. Subsequently, electrodes were rinsed and electrochemical cleaning performed. First, oxidation and reduction cycling in 0.5 M in NaOH (−0.4 to −1.35 V, 500-1000 scans at 2 V s^{-1}, 0.01 V interval). Second, electrodes are rinsed and transferred to a 0.5 M H_2SO_4 solution and oxidation step (2 V for 5 s) and reduction step (-0.35 V for 10 s), followed by oxidation and reduction scans over -0.35 to 1.5 V for 20 scans at 4 V s^{-1} scan rate and 0.01 V sample interval, then 4 more scans at scan rate 0.1 V s-1 and 0.01 V sample interval. Finally, the gold active area was measured by scanning from -0.35 to -1.35 V in 0.05 M H_2SO_4 at 0.1 V s^{-1} scan rate 0.001 V sample interval and 4 segments[2]. From these scans charge is obtained by integrating the area under the curve, divided by 420 µC cm^{-2} the resulting value is the gold surface area in cm^2. Thiol in the DNA probes must be reduced to cleave the C6-SH protecting groups and permit the DNA immobilization on to the gold surface of the electrode. To do so, the thiolated methylene blue modified DNA aptamers were mixed at a ratio of 5 µL of TCEP10 mM in milliQ water per 1 µL of DNA at 100 µM and incubated for 60 min at room temperature (25 °C) protected from the light. Reduced DNA aptamer probes were diluted in assembling buffer to a deposition concentration of 500 nM. Sensors were immersed individually in the DNA solution for 1 h at room temperature protected from the light. Next, gold exposed in the sensor was passivated with a self-assembled monolayer of 6-mercaptohexanol. To do so, sensors were thoroughly rinsed with milliQ water and immersed, individually, in a 2.8 mM 6-mercaptohexanol in PBS buffer overnight at room temperature protected from the light and tubes sealed with parafilm to prevent evaporation.

Electrochemical measurements

Sensors were removed from the 6-mercapohexanol solution, rinsed with milliQ water and placed in a standard electrochemical cell with external reference and counter electrodes, and containing a known volume of PBS. In the case of the vancomycin aptamers, PBS was supplemented with 2 mM Mg Cl_2. Peak height in square wave voltammetry (SWV) was used as analytical value, relative signal change was calculated in basis of the SWV peak in absence of target. Measurement parameters for each aptamer were performed as previously reported: Doxorubicin[3] 50 mV amplitude and potential step of 3 mV, for cocaine[4,5] 25 mV amplitude and step potential of 4 mV,

for vancomycin[6] 25 mV amplitude and 2 mV potential step, all potential windows used ranged from -0.05 V to -0.5 V.

All electrochemical measurements were performed with a CH multipotentiostat Model 1000C (CH Instruments, Austin, Texas). Polycrystalline gold disk electrodes (2 mm diameter; CH instruments Inc., Austin, Tx) were used as working electrodes. An external reference electrode Ag/AgCl in saturated 3 M KCl and a platinum wire counter electrode (both from CH instruments Inc., Austin, TX) were used in all electrochemical experiments.

Formula to calculate relative signal on/off changes

Relative signal (*on* and *off*) was calculated in basis of the relative changes in the SWV peak, similar as reported in previous work[7].

$$\%\, Signal\ Change = \frac{Current\ peak_{\,i} - Current\ peak_0}{Current\ peak_0} \quad \text{(S1)}$$

Where *Current peak$_i$* is the peak height of methylene blue in SWV at a given concentration of target and *Current peak$_0$* corresponds to the peak height in absence of target. At some square wave frequencies, the former will be smaller than the latter and thus the resulting signal change is negative (Signal-off). Signal gain is defined as the relative signal on/off change seen at the highest target concentration we have employed.

Binding curve fitting

Binding curves of the target titration for the EAB sensors were fitted to Langmuir isotherms:

$$Signal = S_{min} + \frac{[X \cdot (S_{max} - S_{min})]}{(X + K_d)} \quad \text{(S2)}$$

Where *Signal* is the relative signal change measured by SWV, S_{min} the relative signal change seen in the absence of target, S_{max} the relative signal change seen at saturating target, K_d the dissociation constant, and *X* the concentration of target.

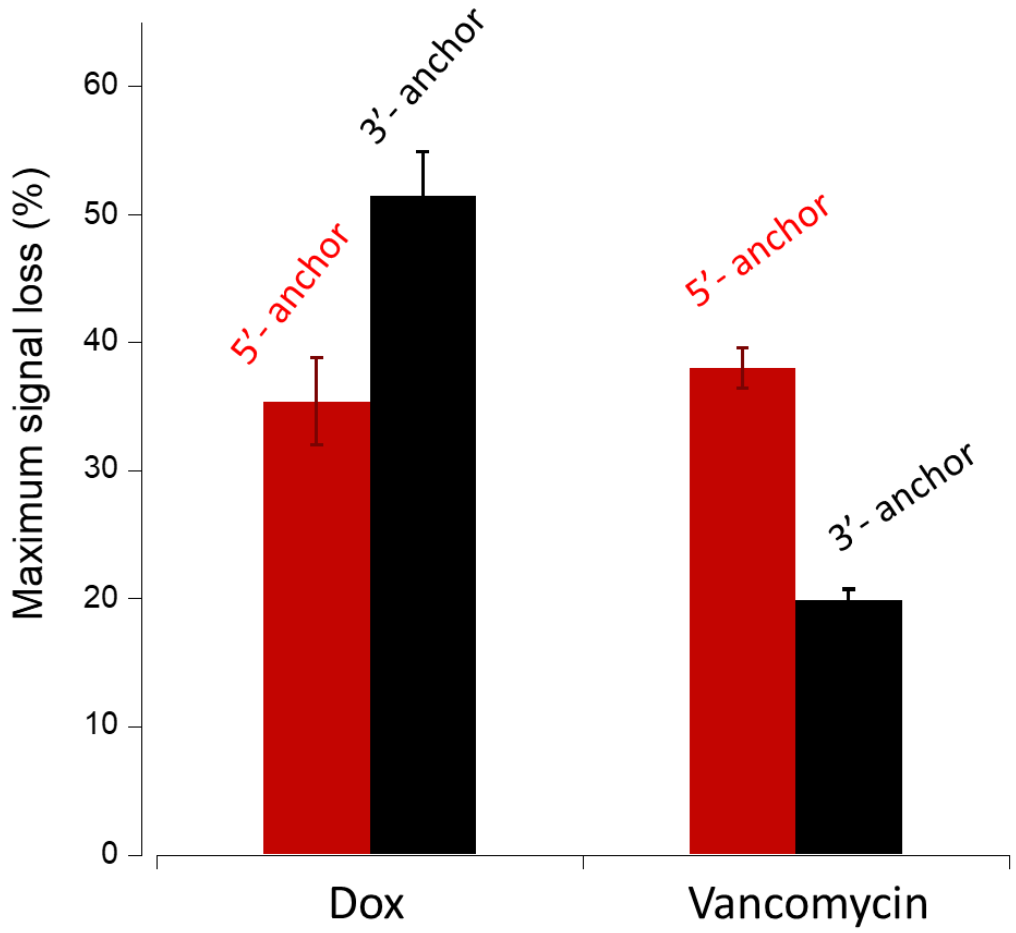

Figure S1: Maximum absolute gain seen at signal-off frequencies also differs between 5' and 3' anchor orientations. Note that neither anchor orientation produced signal-off behavior for the cocaine sensor (Fig. 2B in the main text).

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