Supporting Information

A tight squeeze: geometric effects on the performance of three-electrode electrochemical-aptamer based sensors in constrained, in-vivo placements

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Experimental

Materials

We fabricated the first "bundled" EAB sensors using 0.2 mm diameter gold wire (99.9% purity, Alfa Aesar), 0.203 mm diameter silver wire (99.99% purity, A-M systems), and 0.254 mm diameter platinum rod (99.95% purity, A-M systems) and insulated with polytetrafluoroethylene heat-shrink (PTFE, 0.02", 0.005", 0.003", black, Zeus Inc., HS Sub-Lite-Wall). The new bundled intravenous sensors consist of 0.2 mm diameter gold wire, 0.125 mm diameter silver wire (99.99% purity, A-M systems), 0.125 mm diameter platinum wire (99.99% purity, A-M systems). In both architectures, the sensors were fed through the lumen of 20G catheters (Becton, Dickinson & Company).

Sodium hydroxide, 6-mercapto-1-hexanol, tris(2-carboxyethyl) phosphine, and sulfuric acid were obtained from Sigma Aldrich. Phosphate buffered saline (PBS) was diluted from a 20x stock purchased from Santa Cruz Biotechnologies. Vancomycin HCl was purchased from VWR. Methylene blue-and-HO-C_6S-S-C_6-modified DNA sequences were purchased from Integrated DNA Technologies. For our in-vitro studies we employed "sensors" in which the "aptamer" was actually an unstructured 37-base sequence ("MB 37" in table S1). For our in-vivo studies we employed a vancomycin-binding aptamer.

Table S1: The DNA sequences employed in this study.

Name	**Sequence and methylene blue location (5' to 3')**
MB 37	HS-C_6-5' ATTAT TTTTT ATTTA TTTTT ATTTT ATTTT ATTTT TT 3' MB
Vancomycin sensor	HS-C_6-5' CGAGGGTACCGCAATAGTACTTATTGTTCGCCTATTGTGGGTCGG 3' MB

Sensor fabrication

We fabricated the intravenous sensors using the dimensions listed in the main text (Figs 1, 5 and 6). All wires were inserted through the heat-shrink tubing, leaving the appropriate length of bare wire exposed prior to heating the insulation. We then immersed the entire sensor with all the exposed ends of three electrodes in household bleach (Clorox, Sodium Hypochlorite 7.5%) overnight to chlorinate the silver electrode. Following this we rinsed the electrodes with Millipore

water and then performed electrochemical cleaning. This cleaning consists of cycling the potential 1000 times between the potentials -1.0 V and -2 V at 2 V/s in a solution of 0.5 M NaOH. We then roughened the electrodes in 0.5 M H_2SO_4 by applying 32,000 alternating, 20 ms pulses at 0 V and 2.2 V. We then cleaned the electrodes by cycling the potential between 1.5 and -0.35 V at 1 V/s twice in the same H_2SO_4 solution.

To modify the electrode surfaces, we first reduced the disulfide bond in the stock alkanethiol-and-methylene-blue-modified DNA by combining 14 μL of 10 mM tris (2-carboxyethyl) phosphine with 2 μL of 100 μM DNA for 1 h in the dark. We then rinsed the freshly cleaned electrodes with distilled, de-ionized water, then fed them through a 20G catheter, and then immersed them for 1 h in 500 nM reduced DNA in PBS. The resulting sensors were then transferred to a 10 mM solution of 6-mercapto-1-hexanol in PBS overnight until use to passivate any uncovered surface on the gold electrode.

Electrochemical measurements

We performed all electrochemical characterization using square wave voltammetry on a CH1040C multipotentiostat. In-vitro tests with the sensors were performed at room temperature in PBS with 25 mV amplitude and square wave frequencies of 5, 7, 10, 15, 30, 50, 70, 100, 200, 250, 300, 600 and 1000 Hz.

We performed in-vivo measurements using square wave voltammetric interrogation at square wave frequencies of 30 Hz and 100 Hz. The voltammograms with these two frequencies were measured in an alternating fashion throughout the duration of the measurement. Signal drift correction was performed using kinetic differential measurement (KDM):

$$KDM = \frac{\left(\frac{i_{100Hz}}{i_{100Hz,\,t=0}} - \frac{i_{30Hz}}{i_{30Hz,\,t=0}}\right)}{0.5 \times \left(\frac{i_{100Hz}}{i_{100Hz,\,t=0}} - \frac{i_{30Hz}}{i_{30Hz,\,t=0}}\right)}$$

where i_{100Hz} and i_{30Hz} are the peak heights at from the 100 Hz and 30 Hz frequencies respectively and $i_{100Hz,\,t=0}$ and $i_{30Hz,\,t=0}$ are peak heights seen prior to target injection.[1] The KDM was correlated

to concentrations using a titration where the electrochemical signals were measured with known target additions into whole bovine blood at 37°C (Fig. S2).

In-vivo measurements

All in-vivo experiments were performed in male Sprague-Dawley rats (4-5 months old, Charles River Laboratories of Santa Cruz, CA). The rats weighed between 350-500 g and were pair-housed in a standard light cycle room (12:12 regular light cycle with lights on at 8AM). They were allowed ad libitum access to food and water and the Institutional Animal Care and Use Committee (IACUC) of the University of California at Santa Barbara approved our experimental protocol which adhered to the guidelines given by the NIH Guide for Care and Use of Laboratory Animals (8th edition, National Academy Press, 2011).

Prior to the measurement, we anesthetized the rats using 4% isofluorane in a Plexiglas anesthesia chamber and then maintained anesthesia via a nose cone during the entire length of the experiment using a 2-2.5% isofluorane/oxygen mixture. We then shaved the neck of the rats and dissected the neck in order to surgically isolate the left and right jugular veins. A small incision was made in each vein using spring-loaded microscissors that allowed us to insert the "bundled" intravenous EAB sensor in the right jugular vein and a catheter with infusion line in the left jugular vein. We anchored both the EAB sensor and the infusion line using two sterile 6-0 silk sutures (Fine Science Tools, Foster City, CA). Prior to any recording, we infused 30 units of heparin through the infusion line. To intravenously dose the rats 30 mg/kg of Vancomycin, we injected a precalculated volume of 0.05 M vancomycin using a syringe pump (KD scientific) as previously described.[2]

Supplementary Data

Square wave voltammograms at intermediate counter electrode lengths

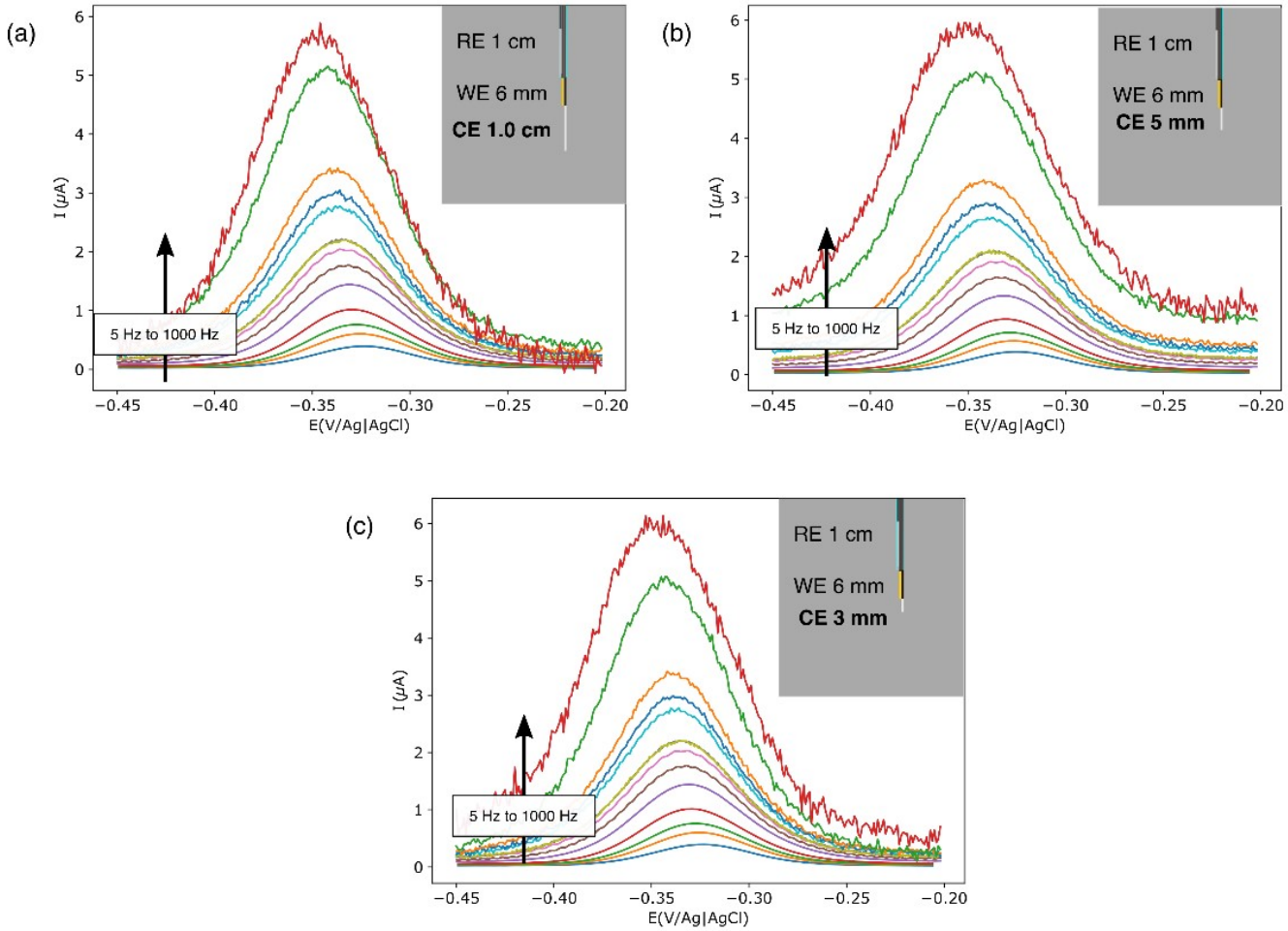

Figure S1. Square wave voltammograms generated at frequencies 5-1000 Hz when using counter electrodes with lengths (a) 1 cm (b) 5 mm and (c) 3 mm. We do not observe significant differences nor a monotonic trend with the electrochemical behavior.

Vancomycin Calibration Curve

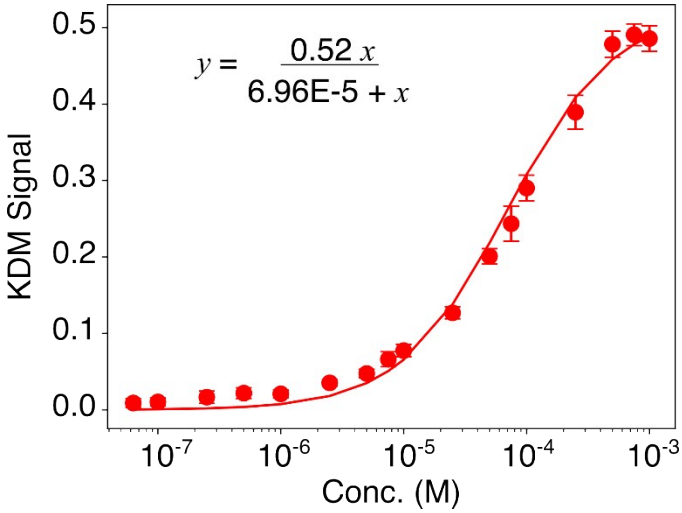

Figure S2. Shown is a calibration curve correlating the concentration of added vancomycin to the resulting KDM signal. These data were collected in whole bovine blood at 37°C. The KDM signal was calculated using data from square wave frequencies 100 Hz and 30 Hz. The Langmuir equation used for fitting is also shown. Error bars indicate the standard deviation of 4 electrodes.

References

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(2) Dauphin-Ducharme, P.; Yang, K.; Arroyo-Currás, N.; Ploense, K. L.; Zhang, Y.; Gerson, J.; Kurnik, M.; Kippin, T. E.; Stojanovic, M. N.; Plaxco, K. W. Electrochemical Aptamer-Based Sensors for Improved Therapeutic Drug Monitoring and High-Precision, Feedback-Controlled Drug Delivery. *ACS Sensors* **2019**, *4* (10), 2832–2837. https://doi.org/10.1021/acssensors.9b01616.