Supporting Information for

High frequency, calibration-free molecular measurements in situ in the living body

Hui Li[†#], Shaoguang Li[†#], Jun Dai[⊥], Chengcheng Li[†], Man Zhu[†], Hongxing Li[†], Xiaoding Lou[†], Fan Xia*[†] and Kevin W. Plaxco*[‡§]

[†]Engineering Research Center of Nano-Geomaterials of Ministry of Education, Faculty of Materials Science and Chemistry, China University of Geosciences, Wuhan 430074, China

[⊥]Department of Obstetrics and Gynecology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430074, China

[‡]Department of Chemistry and Biochemistry, University of California Santa Barbara, Santa Barbara, California 93106, United States

[§]Center for Bioengineering, University of California Santa Barbara, Santa Barbara, California 93106, United States

Materials and methods

Materials

Kanamycin was purchased from Gold Biotechnology (MO, USA), ATP from Sangon Biotech (Shanghai) Co., Ltd, 6-Mercapto-1-hexanol, tris(2-carboethyl)-phosphine hydrochloride (TCEP), and cocaine hydrochloride from Sigma-Aldrich (MO, USA); all were used as received. Bovine whole blood in heparin were purchased from Shanghai Yuanye Bio-Technology Co., Ltd (China) and used as received. 2 and 7/8" microcloth, 1 μm and 0.05 μm alumina powder were obtained from CH Instruments Ins. Gold wire (0.2 mm diameter) and tungsten wire (0.2 mm diameter) for electrode fabrication were purchased from Wuhan Xinshenshi

Chemical Technology co. LTD. Teflon tubes used as insulating materials were purchased from Zeus Industrial Products. The relevant methylene blue, anthraquinone and thiol-modified DNA aptamer sequences (sequences as reported in references 1 and 2) were synthesized by LGC Biosearch Technologies, purified by C18 HPLC and PAGE, confirmed by HPLC profile and mass spectrometry. These were dissolved in TE buffer (1×) (10 mM tris(hydroxymethyl)aminomethane, 1 mM EDTA, pH 8.0) to a final concentration of 100 μM, aliquoted and stored at -20°C prior to use. The sequences used in this study are:

Cocaine aptamer:

5'-HO-$(CH_2)_6$-S-S-$(CH_2)_6$-dT(anthraquinone, AQ)AGACAAGGAAAATCCTTCAAT GAAGTGGGTCG-$(CH_2)_7$-methylene blue (MB)-3'

ATP aptamer:

5'-HO-$(CH_2)_{11}$-S-S-$(CH_2)_{11}$- dT(anthraquinone, AQ)ACCTGGGGGAGTATTGCGGA GGAAGGT-$(CH_2)_7$-methylene blue (MB)-3';

Kanamycin aptamer:

5'-HO-$(CH_2)_{11}$-S-S-$(CH_2)_{11}$- dT(anthraquinone, AQ)GGGACTTGGTTTAGGTAATG AGTCCC-$(CH_2)_7$-methylene blue (MB)-3'

Gold electrodes (2 mm in diameter), fritted Ag/AgCl electrodes, and platinum wires were purchased from CH Instruments, Inc. (TX, USA).

To mimic the circulatory system the whole blood was circulated using a miniature gear pump (Model: 74013-55, Cole-Parmer; IL, USA) through a closed loop

consisting of a 30 mL sample reservoir and two 4 mm-diameter and 25 cm-long plastic tubes.[1]

Sensor fabrication We followed the same procedure for sensor fabrication as we previously reported[2]. Gold wire electrodes were made using the following procedure: the wire is composed of two parts: gold wire (5-6 mm, 0.2 mm diameter) and tungsten wire (8 cm, 0.2 mm diameter). These two components were first cold soldered using electrically conductive silver epoxy adhesive. The tungsten wire was then insulated using a heat shrinkable teflon tubing leaving the gold wire exposed for DNA modification and 1 cm tungsten wire at the other end for connection to the potentiostat. Following the insulation step, the gold portion of these was electrochemically roughened in order to increase the surface area. Briefly, the sensors were immersed in 0.5 M sulfuric acid and rapidly pulsed between $E_{initial}$ = 0.0 V to E_{high} = 2.0 V vs Ag/AgCl for 400,000 times with each pulse being of 2 ms duration.

Immediately prior to sensor fabrication we prepared a solution of thiol-and-methylene-blue-modified DNA in phosphate buffered saline buffer (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.0) by incubating a solution of 100 μM DNA and 20 mM tris-(2-carboxyethyl) phosphine hydrochloride (1:200) for 1 hr at room temperature followed by dilution with PBS to 200 nM as confirmed by UV-Vis spectroscopy. We then immersed freshly cleaned electrodes in this solution for 1 hr at room temperature. The resulting sensors were washed with deionized water and then incubated in 20 mM phosphatidyl choline solution in PBS

overnight at 4°C before being rinsed with water prior to use.

Electrochemical measurements

Electrochemical measurements were performed at room temperature using a multichannel CHI1040C potentiostat (CH Instruments, Austin, TX) and a standard three-electrode cell containing a platinum counter electrode and a Ag/AgCl (3 M KCl) reference electrode. Square wave voltammetry (SWV) was performed using a potential window of -0.1 to -0.5 V, a potential step of 0.001 V and an amplitude of 0.05 V. We interrogated the cocaine-detecting sensors using 10 Hz for both calibration-free and traditional calibration approach. We interrogated ATP- and kanamycin-detecting sensors using 120 Hz and 30 Hz for the measurements, respectively.

All whole blood experiments were conducted in a closed-loop system with a continuous flow of whole blood (1 mL/s) using a circulator pump.[1]

In vivo Experiments

Surgery. For the anesthetized preparation, rats were anesthetized using isoflurane gas inhalation (2.5%) and monitored throughout the experiment using a pulse oximeter (Nonin Medical, Plymouth, MN) to measure heart rate and %SpO2 to insure depth of anesthesia. After exposing both ventral jugular veins, a simple catheter made from a SILASTIC tube (Dow Corning, Midland, MI, USA) fitted with a steel cannula (Plastics One, Roanoke, VA, USA) was implanted into the left jugular vein. 0.1-0.3 mL of heparin (1000U/mL, SAGENT Pharmaceuticals, Schaumburg, IL, USA) were immediately infused through the catheter to prevent blood clotting. The sensor was

inserted into the right jugular vein and secured in place with surgical suture. Following drug infusions, animals were euthanized by overdose on isoflurane.

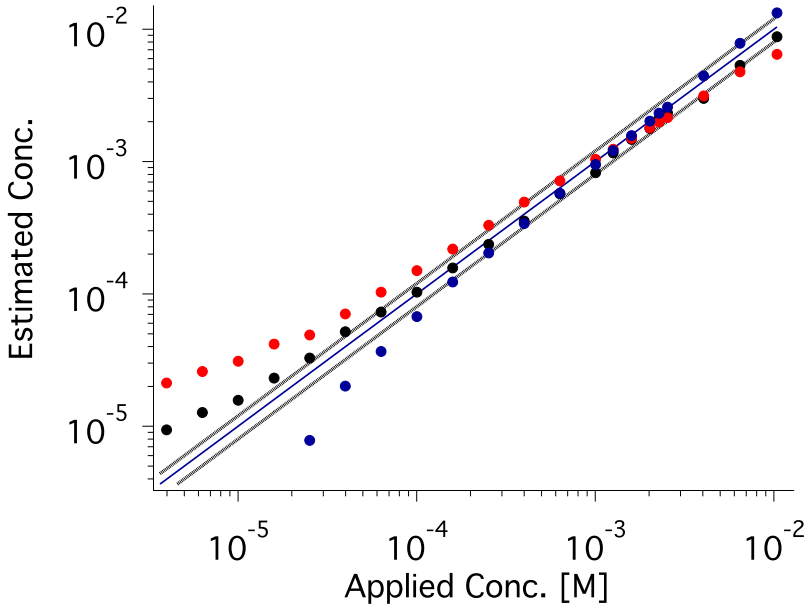

Figure S1. Accurate, precise cocaine measurements via calibration-free approach. Here we present the data for the individual sensors shown as an average of calibration-free curve in Fig. 2C. As shown, this dual-reporter calibration free approach produces cocaine concentration estimates (also in undiluted whole blood) within 20% of the actual concentration of the drug over a two order of magnitude concentration range.

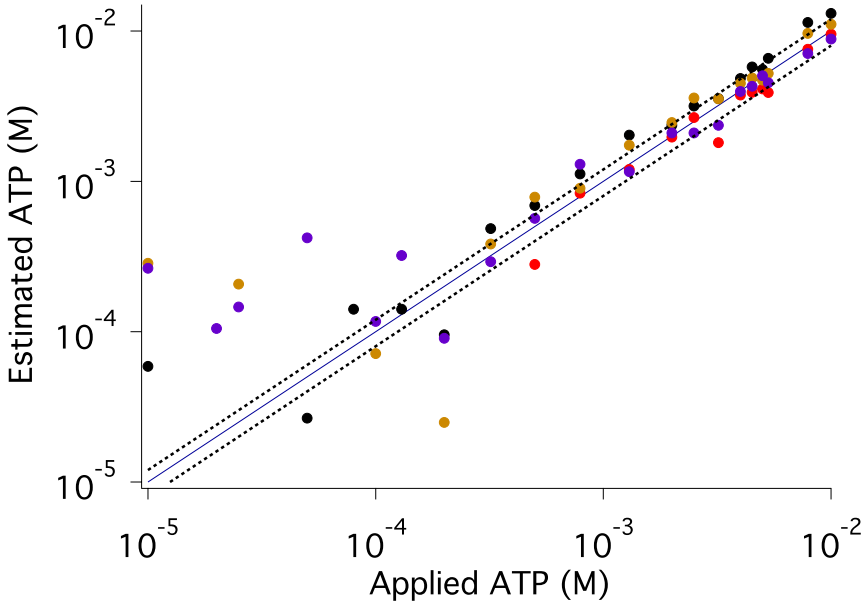

Figure S2. Accurate and precise ATP measurements via calibration-free approach. Shown are data for the individual sensors shown as an average of calibration-free curve in Fig. 3C. We achieved ATP concentration estimates (in whole blood) within 20% of the actual (spiked) concentration across an approximate 30-fold concentration range.

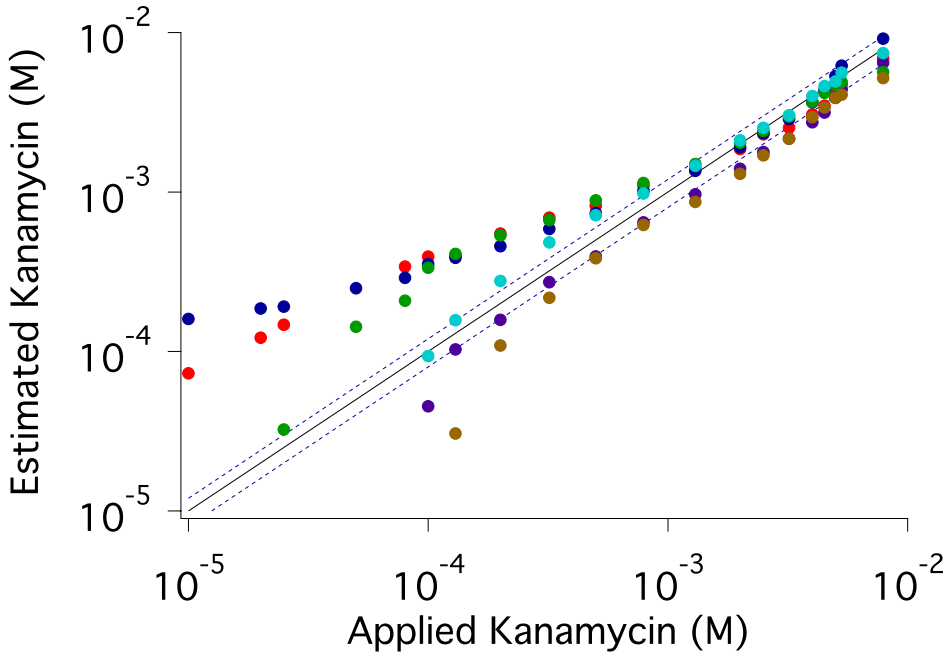

Figure S3. Accurate and precise kanamycin measurements via calibration-free approach. Shown are data for the individual sensors shown as an average of calibration-free data in Fig. 4C. Here we show six independently hand-fabricated sensors interrogated in undiluted whole blood, under which the estimated concentrations are accurate to within 20% of the actual (spiked) concentration of the drug across an approximate 15-fold concentration range.

Table S1. The parameters r_{min}, r_{max}, $K_{1/2}$ and n_H for the E-AB sensors.

Parameters \ Aptamer	Cocaine	ATP	Kanamycin
r_{min}	0.17±1e-6	0.62±0.005	2.20±0.041
r_{max}	0.45±2e-6	2.21±1.321	172130±2.79e3
$K_{1/2}$ (M)	0.00266±2e-5	0.032±0.045	4348±8.62e2
n_H	0.65±0.004	0.86±0.131	0.82±0.164

References

1. Li, H., Arroyo-Currás, N., Kang, D., Ricci, F. & Plaxco, K.W. *J. Am. Chem. Soc.* 2016, **138**, 15809-15812.
2. Li, H.; Dauphin-Ducharme, P.; Arroyo-Curras, N.; Tran, C. H.; Vieira, P. A.; Li, S.; Shin, C.; Somerson, J.; Kippin, T. E.; Plaxco, K. W. *Angew. Chem. Int. Ed.* **2017**, *56*, 7492-7495.