

**Supporting Information:**

**An Adaptive Polynomial Baseline Correction  
Method for Electrochemical Aptamer-based  
Sensor**

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# 1 Sensor Fabrication

## 1.1 Fabrication of E-AB biosensors for cocaine detection on the gold disk platform.

Gold disk electrodes (1.6 mm diameter) were purchased from BASi, US, and cleaned using a modified version of a previously reported method.<sup>7</sup> Initially, the electrodes were electrochemically cleaned in 0.1 M KOH by performing voltammetric scans ranging from  $-1.3V$  to 0 V at a scan rate of  $1V/s$ . This was followed by mechanical polishing on a BASi polishing pad using  $0.3\mu m$  and  $0.05\mu m$  alumina slurries for 1.5 minutes each. Subsequently, the electrodes were sonicated in water for 10 minutes. An additional electrochemical cleaning step was conducted in 0.5 M H<sub>2</sub>SO<sub>4</sub>, using voltammetric scans from  $-0.2 V$  to  $+1.6 V$  at a scan rate of  $0.1 V/s$  until a typical cyclic voltammogram was obtained.

To modify the electrodes with aptamers, disulfide and methylene blue dual-labeled cocaine aptamers were first reduced using 100 mM TCEP for 2.5 h in the dark at room temperature. The reduced aptamers were then diluted with PBS buffer to achieve a final concentration of 150 nM. The electrodes were incubated with this aptamer solution for one hour in the dark at room temperature. Following incubation, the electrodes were thoroughly rinsed with water to remove loosely adsorbed aptamers. Finally, the aptamer-modified electrodes were immersed in a 3 mM MCH solution overnight.

## 1.2 Fabrication of E-AB biosensors for cocaine detection on the disposable platform.

The laser-ablated gold electrodes, manufactured by Mint Diagnostics, UK, were first sonicated in water for 1 minute to remove loosely adsorbed impurities. They were then immersed in 5 % Decon90 solution for 10 min, followed by a thorough rinse with copious amounts of water. Subsequently, the electrodes underwent cleaning in 25 % H<sub>2</sub>O<sub>2</sub> with 8 mM KOH

for 10 min. After another thorough rinse with water, the electrodes were dried under N<sub>2</sub> flow before use. To modify the aptamers onto the electrodes, a mixture of 1.5  $\mu$ L of 10  $\mu$ M aptamer and 1.5  $\mu$ L of 10 mM TCEP was prepared at room temperature for 2.5 h. Then, we added 91  $\mu$ L of PBS buffer and 1  $\mu$ L of 100 mM cocaine solution to the aptamer-TCEP mixture to achieve final aptamer concentrations of 150 nM aptamer and 1 mM target. After a 5-minute incubation, we added 5  $\mu$ L of 300  $\mu$ M MCH to achieve a final MCH concentration of 15  $\mu$ M. Subsequently, 1  $\mu$ L of the aptamer-target-MCH mixture was mounted onto each working electrode and incubated for 1 h. After a thorough rinse with water, the electrodes were backfilled in 3 mM MCH solution in PBS overnight in the dark. The electrodes were thoroughly rinsed with water before use.

### **1.3 Fabrication of E-AB biosensors for THC detection on the disposable platform.**

The disposable platform was fabricated using a similar method with some modifications, except that the laser-ablated gold electrodes were manufactured by FlexMedical Solutions, UK. Briefly, after cleaning, the electrodes were incubated with 15  $\mu$ M MCH for 15 min, followed by thorough rinsing with water. The MCH modified electrodes were then incubated with 150 nM THC aptamer for 1 h at the room temperature in the dark. After a thorough rinse with water, the electrodes were backfilled in 3 mM MCH solution overnight in the dark. The electrodes were thoroughly rinsed with water before use.

#### **1.3.1 Preparation of cocaine hydrochloride aqueous solution.**

In aqueous solution, cocaine hydrolyses to benzoylecgonine and ecgonine methyl ester in water is responsible to cocaine degradation during storage.<sup>39</sup> Storage temperature and pH should be carefully considered.<sup>40,41</sup> Cocaine hydrochloride powder was dissolved in hydrochloric acid (HCl) solution, pH 5.0, to achieve a concentration of 100 mM, and then stored at 4 °C for 6 months.

Table S1: Aptamer sequences used in this study.

Aptamer	Sequences
COC-apt	5'-HS-SH-C6-AGACAAGGAAAATCCTTCAATGAAGTGGG TCG-MB-3'
COC-mut	5'-HS-SH-C6-AGACAAGGAAAATCCTTCAACGAAGTGGG TCG-MB-3'
THC1.2-47	5'-HS-SH-C6-CTTACGACCCAGGGGGGTGGACAGGCG GGG GTTAGGGGGGTTCGTAAG-MB-3'
THC1.2-41	5'-HS-SH-C6-ACGACCCAGGGGGGTGGACAGGCGGGG GTTAGGGGGGTTCG-MB-3'
THC1.2-39	5'-HS-SH-C6-CGACCCAGGGGGGTGGACAGGCGGGGG TTAGGGGGGTTCG-MB-3'
THC1.2-37	5'-HS-SH-C6-GACCCAGGGGGGTGGACAGGCGGGGGT TAGGGGGGTC-MB-3'
THC1.2-35	5'-HS-SH-C6-ACCCAGGGGGGTGGACAGGCGGGGGT TAGGGGGGT-MB-3'

Saliva collection. Human saliva was collected using the passive drool method, which allows the fluid to naturally drool into a 50mL Falcon tube. Human saliva used in this study was collected early in the morning from two volunteers, one female and one male, aged 30 to 35. After combining the saliva samples, the pooled saliva was filtered through a 0.45 $\mu$ m PES filter to obtain approximately 1 to 2mL of filtered saliva. All the saliva samples used in this work were collected fresh every morning and then used immediately without storage. This collection was conducted in accordance with the New Zealand Institute of Public Health and Forensic Science (PHF Science) internal consent procedures for method validation and quality management procedures for method validation accredited by ANSI National Accreditation Board. All the staff members involved were from the Forensic Research and Development Department at PHF Science. They voluntarily donated their saliva and were fully informed about its intended use.

### 1.3.2 Sensor characterization on the gold disk platform.

The electrochemical measurements were carried out using an EmStat3 Blue (PalmSens, Netherlands) by SWV. The optimal responsive square-wave frequency was determined by

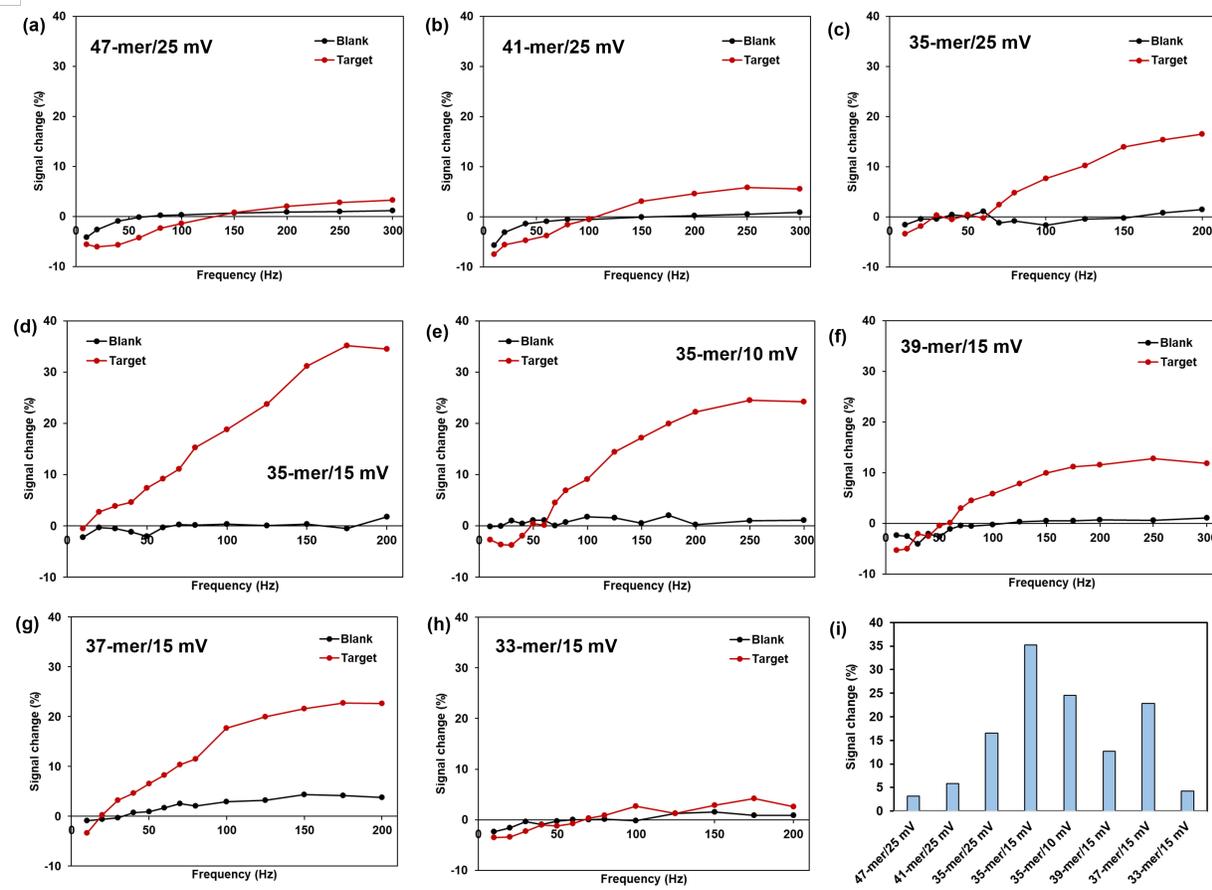


Figure S1: (a - h) Screening the signal change of different THC aptamer truncations across a range of SW frequencies and SW amplitudes. (i) The maximum signal changes of different THC aptamer truncations are compared with the 35-mer truncation of the THC aptamer showing the highest signal change at 15 mV. All the sensors were prepared on the gold disk platform. The sensors were characterized with the presence of  $8 \mu\text{M}$  THC in the binding buffer containing 2.6% ethanol.

screening from 10 to 300  $\text{Hz}$  with 25  $\text{mV}$  amplitude from -0.5  $\text{V}$  to 0 with the presence of 0.5  $\text{mM}$  cocaine in 2  $\text{mL}$  of buffers or pooled saliva respectively. After determining the optimal frequency, target titration was performed to obtain a binding isotherm. This was achieved by titrating a concentrated cocaine stock solution into 2  $\text{mL}$  buffer (20  $\text{mM}$  Tri buffer, 40  $\text{mM}$  NaCl, 5  $\text{mM}$  KCl, pH 7.4) with an incubation time of 30 sec.

## 1.4 Sensor characterization on the disposable platform

Similar principles to that of the gold disk electrode were applied to the disposable gold electrode. The disposable gold electrode was designed to fit a commercialized connector, the All-In-One Drop Cell (MicruX, Spain) (Figure S2). As the reference potential of a pseudo gold reference electrode varies from one condition to another, the screening potential range was adjusted accordingly.



Figure S2: The disposable electrode was connected to the connector with  $5\mu\text{L}$  sample loaded onto it.

## 1.5 Cocaine detection in saliva

In a standard test,  $90\mu\text{L}$  of human saliva was mixed with  $10\mu\text{L}$  of  $200\text{mM}$  Tris buffer (pH 7.4) containing  $3\text{M}$  NaCl, achieving a final NaCl concentration of  $300\text{mM}$  and a saliva percentage of 90 %. After cocaine was added to the mixture,  $5\mu\text{L}$  of it was applied to the chip for measurement.

## 2 Baseline correction plots

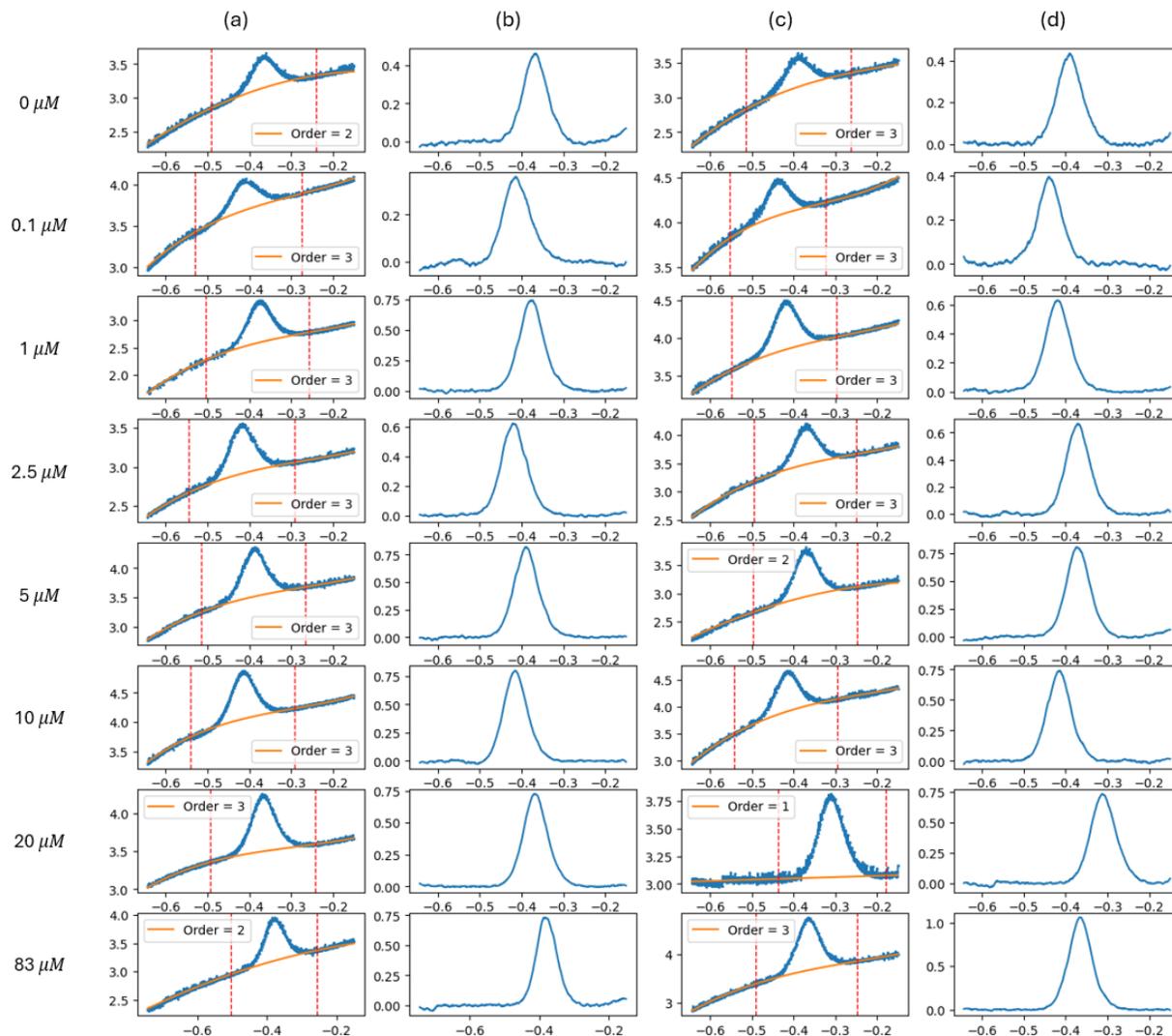


Figure S3: Selected baseline correction plots for the THC aptamer sensor at varying target concentrations with disposable platform. Each row corresponds to a different THC concentration ( $0 \mu M$ ,  $0.1 \mu M$ ,  $1 \mu M$ ,  $2.5 \mu M$ ,  $5 \mu M$ ,  $10 \mu M$ , and  $20 \mu M$  and  $83 \mu M$ ) with two selected replicates in (a) and (c) columns collected by using independent E-AB sensors. (b) and (d) are the normalized signal for (a) and (c), respectively. The blue curves in (a) and (c) represent the original signals and the orange curves are the fitted baseline correction curves.

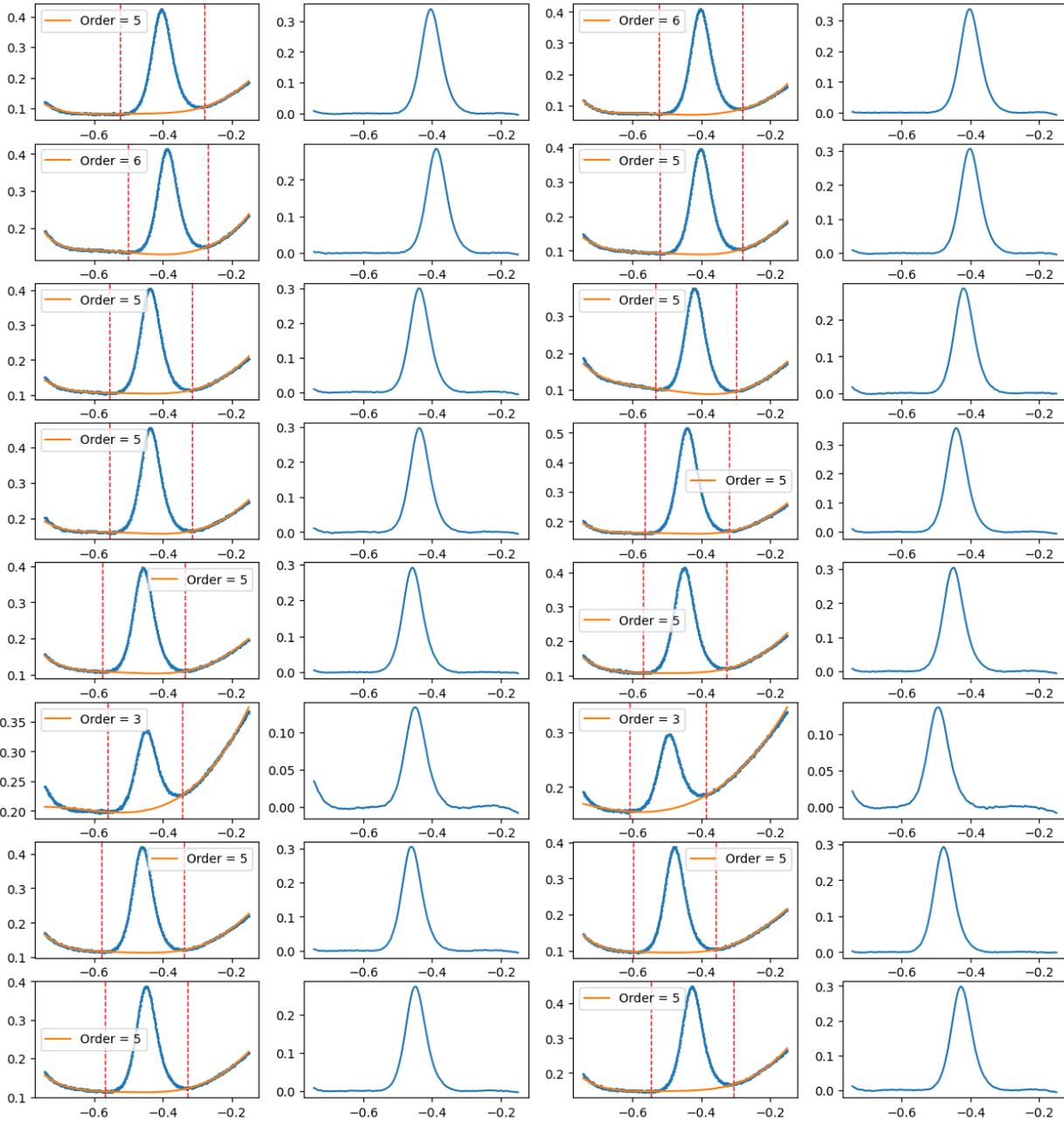


Figure S4: Selected baseline correction plots for the cocaine aptamer sensor at varying target concentrations with disposable platform. Each row corresponds to a different cocaine concentration ( $0 \mu M$ ,  $5 \mu M$ ,  $10 \mu M$ ,  $50 \mu M$ ,  $100 \mu M$ ,  $150 \mu M$ , and  $250 \mu M$ ) with two selected replicates separated by (a) and (c) columns collected by using independent E-AB sensors. (b) and (d) are the normalized signal for (a) and (c), respectively. The blue curves in (a) and (c) represent the original signals and the orange curves are the fitted baseline correction curves.

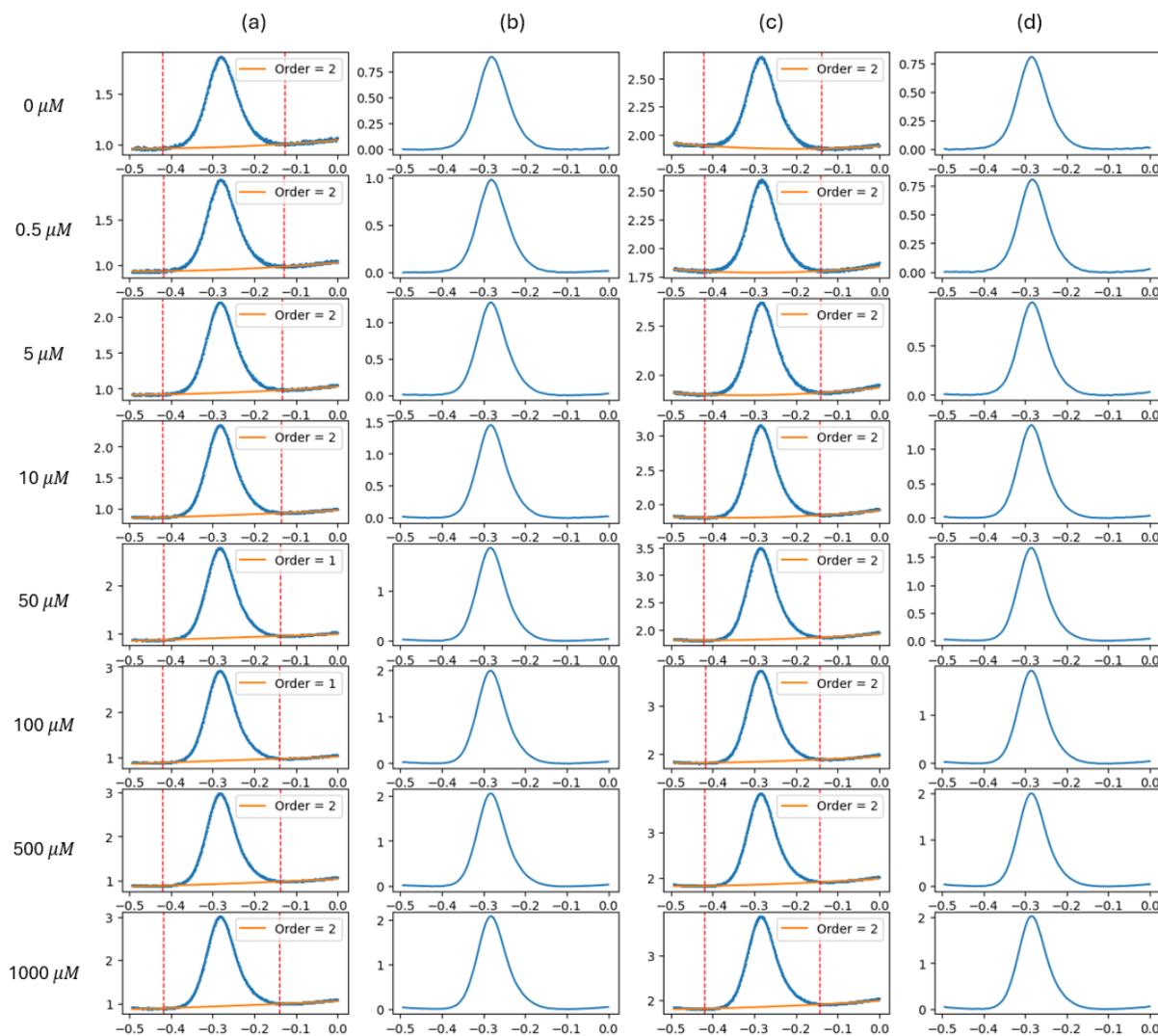


Figure S5: Selected baseline correction plots for the Cocaine aptamer sensor at varying target concentrations with standard platform. Each row corresponds to a different Cocaine concentration ( $0 \mu M$ ,  $0.5 \mu M$ ,  $5 \mu M$ ,  $10 \mu M$ ,  $50 \mu M$ ,  $100 \mu M$ , and  $500 \mu M$  and  $1000 \mu M$ ) with two selected replicates in (a) and (c) columns collected by using independent E-AB sensors. (b) and (d) are the normalized signal for (a) and (c), respectively. The blue curves in (a) and (c) represent the original signals and the orange curves are the fitted baseline correction curves.

### 3 User interface

The methods described above were made accessible via a web application built with the Shiny for Python framework.<sup>S1</sup> The interface code was encapsulated within a Docker container<sup>S2</sup> for deployment via the Azure Kubernetes Service (Microsoft). The interface allows a user to upload signal files collected at responsive and non-responsive frequencies and completes baseline fitting and correction, peak current value determination, and concentration prediction for the provided files. The user can switch views to investigate the plots for different signal files and download a complete summary table of values for all signal files that have been uploaded. Figure S6 shows a screenshot of the interface.

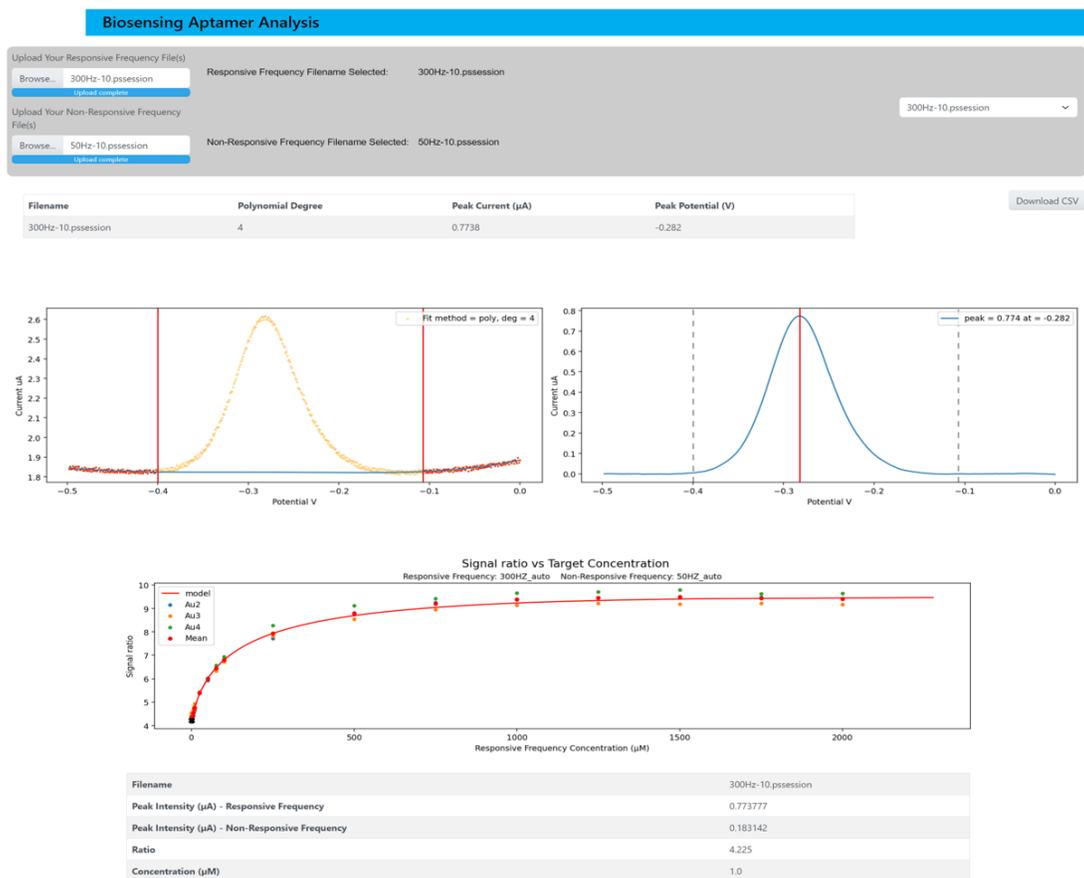


Figure S6: Screenshot of the Analysis Interface showing a test sample of Cocaine

## References

- (S1) Posit Software, PBC Shiny for Python. 2024; <https://shiny.posit.co/py/>.
- (S2) Docker Inc. Docker: Enterprise Container Platform. <https://www.docker.com>, 2024;  
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