

Supporting Information

Hg(II)-mediated “Signal-on” Electrochemical Glutathione Sensor

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MATERIALS AND METHODS

Materials and Reagents

6-mercapto-1-hexanol (C6-OH), tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), sulfuric acid (H₂SO₄), sodium perchlorate (NaClO₄), hydrochloric acid (HCl), sodium hydroxide (NaOH), glutathione (GSH), L-glutathione oxidized (GSSG), and mercury (II) nitrate monohydrate were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. All other chemicals were of analytical grade. Synthetic human stimulated parotid saliva was purchased from US Biocontract (San Diego, CA). All solutions were made with deionized water (DI H₂O) purified through a Millipore Synergy system (18.2 MΩ·cm, Millipore, Billerica, MA). The probe immobilization buffer (Phys2) contained 20 mM Tris, 140 mM NaCl, 5 mM KCl, 1 mM MgCl₂, and 1 mM CaCl₂ (pH 7.4). Target interrogation was performed in a 10 mM phosphate buffer saline (PBS) solution supplemented with 0.5 M NaClO₄ (pH 8.5) or 1:1 PBS: saliva.

The biosensing probe used in this study was a thiol and methylene blue (MB)-modified linear DNA probe purchased from Biosearch Technologies, Inc., Novato, CA. The DNA probe was modified at the 5' end with a C6-disulfide linker (HO-(CH₂)₆-S-S-(CH₂)₆-5'-DNA) and at the 3' end a MB redox label. It contained 15 thymine (T) bases, thus enabling complexation with Hg(II) via the known T-Hg(II)-T interactions. The part of the probe designed for Hg(II) recognition is underlined; the extra bases at the 3' end were added to improve probe flexibility. A DNA-free MB probe purchased from Xaia Custom Peptides (Göteborg, Sweden) was used in the control experiment.

DNA probe: 5' HS-(CH₂)₆-TTT TTT TTT TTT TTT GGC GTA-C7-MB 3'

MB probe: (n) HS-(CH₂)₆- lysine-MB (c)

Instrumentations

Electrochemical measurements were performed at room temperature using a CH Instruments 1040A Electrochemical Workstation (Austin, TX). A platinum wire and a Ag/AgCl (3.0 M KCl) electrode served as the counter and reference electrode, respectively. The working electrodes were polycrystalline gold disk electrodes (CH instruments, Austin, TX) with a geometric area of 0.0314 cm². Sensor interrogation was performed in a conventional electrochemical cell containing PBS or 1:1 PBS: saliva. Both alternating current (AC) voltammetry and cyclic voltammetry (CV) were used in sensor interrogation. AC voltammograms were collected over a wide range of frequencies (1 - 500 Hz) with an amplitude of 25 mV. CV scans were recorded using scan rates between 0.01 - 75 Vs⁻¹.

Electrode Preparation and Sensor Interrogation

Prior to monolayer formation, the gold electrodes were polished with 0.1 μm diamond slurry (Buehler, Lake Bluff, IL), rinsed with DI H₂O and sonicated in a low power sonicator for ~5 min to remove bound particulates. The working electrodes were then electrochemically cleaned by a series of oxidation and reduction cycles in 0.5 M H₂SO₄. The real area of each electrode was determined from the charge associated with the gold oxide stripping peak obtained after the cleaning process in 0.05 M H₂SO₄.¹ The real area of the electrodes used in this study ranged from 0.0345 to 0.0440 cm².

For DNA probe immobilization, 2 μL of 200 μM DNA probe solution was mixed with 2 μL of 10 mM TCEP and left at room temperature in dark for 1 hr to reduce the disulphide bonds. This solution was then diluted with Phys2 to reach a probe concentration of 5 μM. After the cleaning process, the gold disk electrodes were rinsed with DI H₂O and dried with N₂. The diluted probe solution was then dropcasted onto the cleaned gold electrodes for 1 hr. The electrodes were rinsed with DI H₂O and placed in a 2 mM C6-OH solution made in PBS for ~15 hr. Prior to target interrogation, the DNA probe-modified electrodes were allowed to equilibrate in PBS; AC voltammograms were collected at an interval of 5 min until the MB current remained constant. 1.5 μM Hg(II) was then added to the buffer solution; the change in MB current was monitored using AC voltammetry for ~40 min. Next, the electrodes were rinsed briefly using PBS to remove the non-specifically adsorbed Hg(II). These electrodes were placed in a new aliquot of PBS without Hg(II). The sensors were allowed to equilibrate in this buffer until no change in the MB current was observed. 1 μM GSH was then added to the solution.

The calibration curve was obtained by sequential addition of increasing concentrations of GSH (5, 10, 50, 100, 250, 500, 750, and 1250 nM). The sensors were allowed to equilibrate for 60 min after each addition of GSH. Sensor regeneration was accomplished by placing the electrodes in an aliquot of PBS containing 1.5 μ M Hg(II). The regenerated sensors were then rinsed with PBS and placed in an aliquot of PBS without Hg(II), ready for the second interrogation. At the end of each experiment, the monolayer was desorbed by repeated scanning from -0.2 to -1.4 V at a scan rate of 20 mV/s in 0.5 M NaOH. After monolayer desorption, anodic stripping voltammetry was performed in 1 M HCl to remove any Hg(II) that was deposited onto the electrodes.²

The density of electroactive DNA probes (Γ) on the electrode surface was determined from the integrated charge under the MB reduction peak in CV scans collected at slow scan rates (20, 50 and 100 mV/s) using the following equation (equation 1):

$$\Gamma = Q/nFA \quad (1)$$

Where Q is the integrated charge under the reduction peak, n is the number of electrons transferred ($n = 2$ for MB), F is the Faraday's constant, and A is the real electrode area.

The sensor response (% signal enhancement) was determined using the following equation (equation 2):

$$\text{Signal Enhancement (\%)} = [(I - I_0)/I_0] * 100 \quad (2)$$

Where I is for the baseline-subtracted peak current obtained in the presence of GSH, and I_0 is the baseline-subtracted peak current in the absence of GSH (i.e., Hg(II)-bound sensor).

A DNA-free probe (MB probe) was also used to rule out problems such Hg(II) deposition onto the sensor surface. In brief, cleaned gold electrodes were first exposed to a 15 μ M MB probe solution made in DI H₂O for 1 hr, followed by a 4-hr incubation in a 2 mM C6-OH solution made in DI H₂O. The sensors were allowed to equilibrate in PBS prior to the addition of 1 μ M Hg(II). AC voltammograms were collected both before and after the addition of Hg(II).

To determine sensor specificity, the Hg(II)-bound sensors were placed in an aliquot of PBS without GSH, 10 μ M GSSG was then added to the solution. The sensors were allowed to equilibrate until no change in the MB current was observed. 1 μ M GSH was subsequently added to the same solution to confirm the sensors' activity and specificity for GSH. The sensors' response to GSH in a complex matrix such as 50% saliva was also evaluated. The sensors were first equilibrated in this 1:1 PBS:saliva mixture, followed by the addition of 1 μ M GSH. AC voltammograms were collected every 10 min until the signal had reached saturation. Sensor regeneration was performed using the aforementioned protocol.

References

1. H. Angerstein-Kozłowska, B. E. Conway, A. Hamelin, L. Stoicoviciu, *J. Electroanal. Chem.*, 1987, **228**, 429.
2. G. V. Guerreiro, A. J. Zaitouna, R. Y. Lai, *Anal. Chim. Acta*, 2014, **810**, 79.

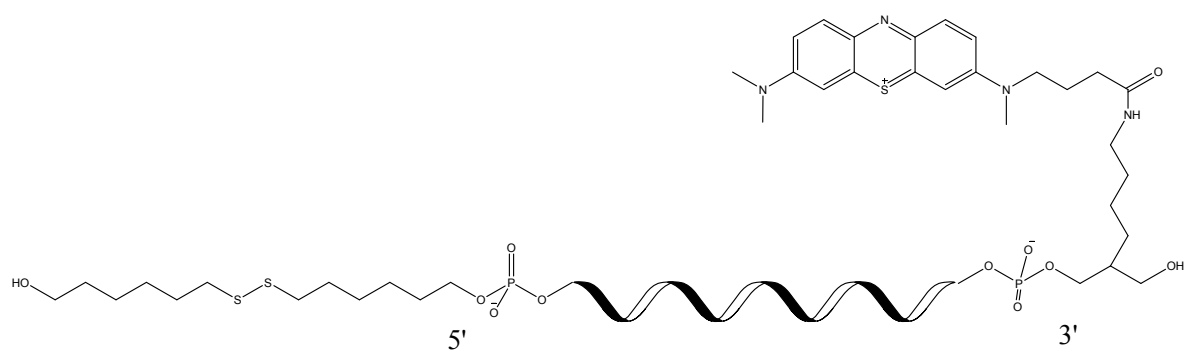


Fig. S1. Structure of the DNA probe used in this study.

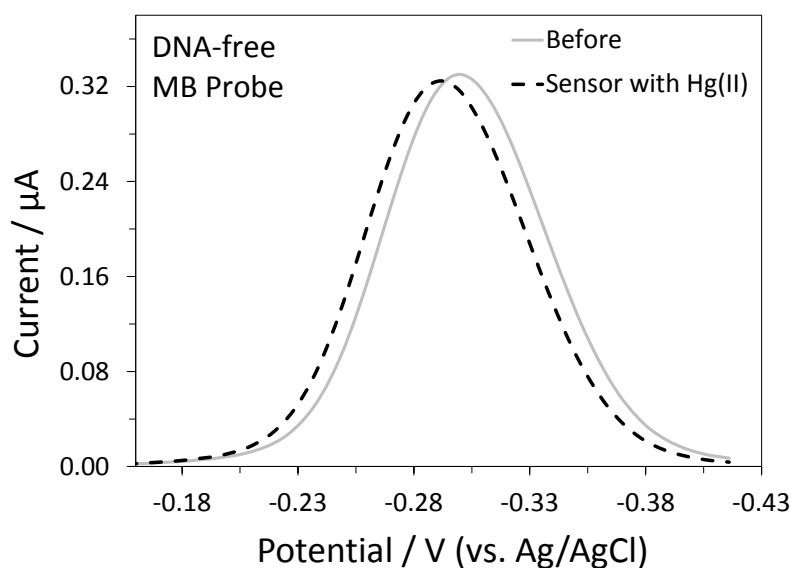


Fig. S2. AC voltammograms of the sensor fabricated with a DNA-free MB probe before and after the addition of 1 μM Hg(II) in PBS. Addition of Hg(II) resulted in a slight shift in the peak potential, but negligible %SS was observed, verifying that the %SS observed with the DNA probe in the presence of Hg(II) is due to the formation of T-Hg(II)-T complexes and not sensor degradation brought on by Hg(II) deposition. Deposition of Hg(II) onto the sensor surface does not appear to be a major issue under the current experimental conditions.

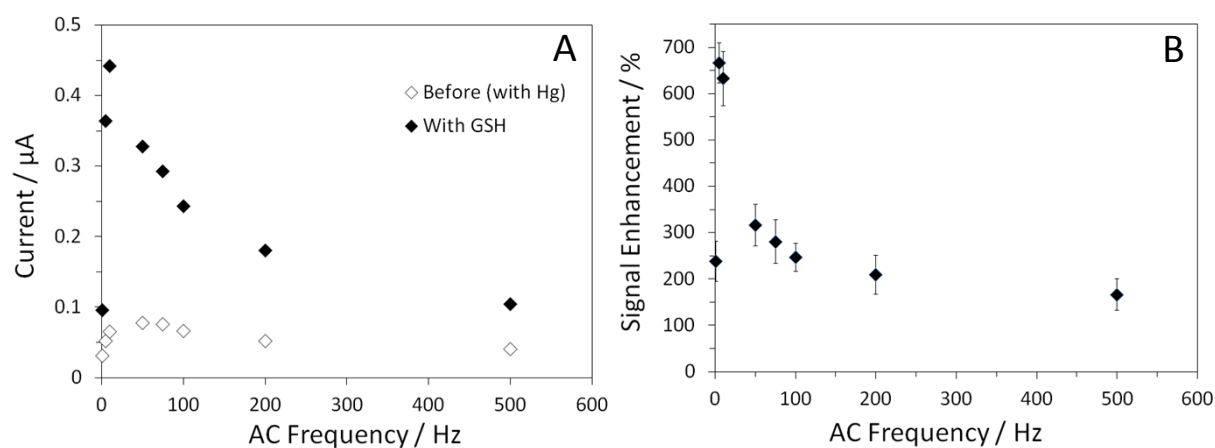


Fig. S3. Representative AC frequency-dependent MB currents in the absence and presence of 1 μM GSH (A). Also shown is the AC frequency-dependent % signal enhancement (%SE) in the presence of 1 μM GSH. These data were averaged from three different sensors (B).

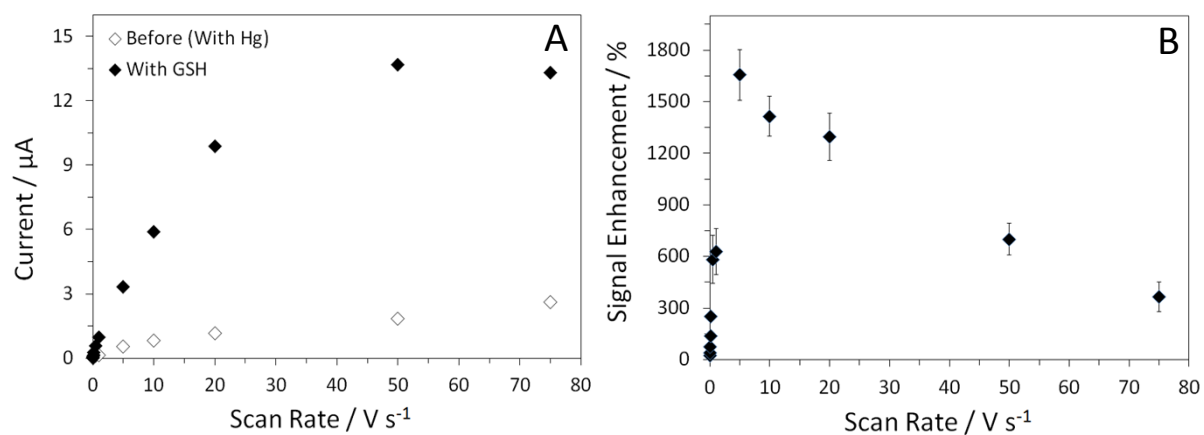


Fig. S4. Representative CV scan rate-dependent MB currents in the absence and presence of $1 \mu\text{M}$ GSH (A). Also shown is the CV scan rate-dependent %SE in the presence of $1 \mu\text{M}$ GSH. These data were averaged from three different sensors (B).

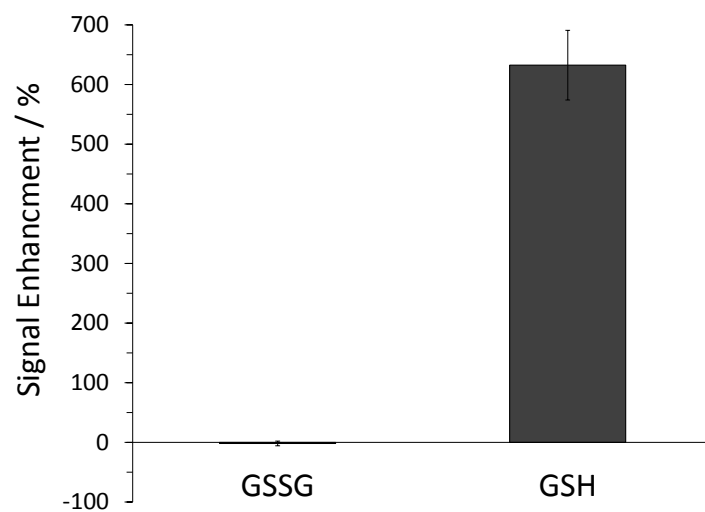


Fig. S5. % signal enhancement recorded in the presence of 10 μM GSSG and 1 μM GSH in PBS. These data were obtained using AC voltammetry at 5 Hz.