

Supporting Information

Rapid assembly of mixed thiols for toll-like receptor-based electrochemical pathogen sensing

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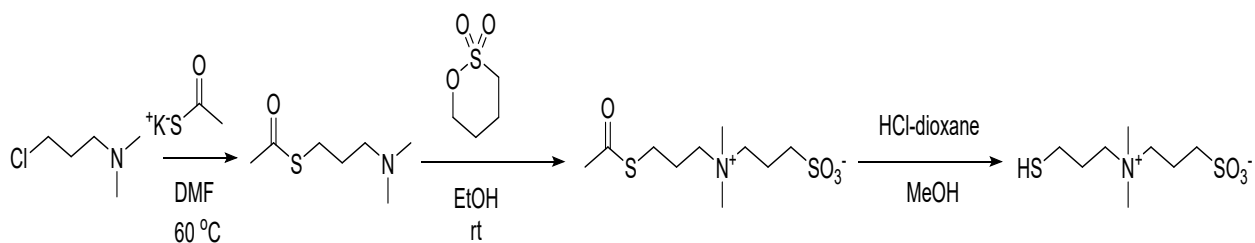
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Synthesis of 3-(dimethylamino)propyl ethanethioacetate

All reagents were procured from Sigma-Aldrich. Potassium thioacetate, 98% purity, (4.5 g, 39.5 mmol) was added to a solution of 3-(dimethylamino)propyl chloride hydrochloride, 96% purity, (2.5 g, 15.8 mmol) in dry DMF, HPLC grade ≥ 99.9 purity, (60 mL), and the mixture was stirred at room temperature for 1h, then heated at 60 °C overnight. The reaction mixture was filtered using Celite® 545 and the solvent was evaporated. Then, 100 mL of H₂O was added and extracted with ACS reagent grade ethyl acetate, ≥ 99.5 % purity, (3 x 50 mL). The ethyl acetate extracts were combined and washed with saturated solution of ACS reagent grade NaCl, ≥ 99 % purity, dried over ACS reagent grade anhydrous Na₂SO₄, ≥ 99 % purity, filtered and then evaporated to afford a red oil as crude. The pure product was obtained after silica-column chromatography using (HPLC grade hexane/ethyl acetate) eluent 1:1 to afford a pure compound as yellow oil (1.8 g, 70 % yield).

¹H NMR (CDCl₃, 600 MHz) δ 2.90 (t, 2H, $J = 7.2$ Hz), 2.56 (t, 2H, $J = 7.2$ Hz), 2.40 (s, 6H), 2.31 (s, 3H), 1.86 (quin, 2H, $J = 7.2$ Hz).



Scheme S1: Synthesis scheme of 3-((3-mercaptopropyl)dimethylammonio)propane-1-sulfonate (DPS)

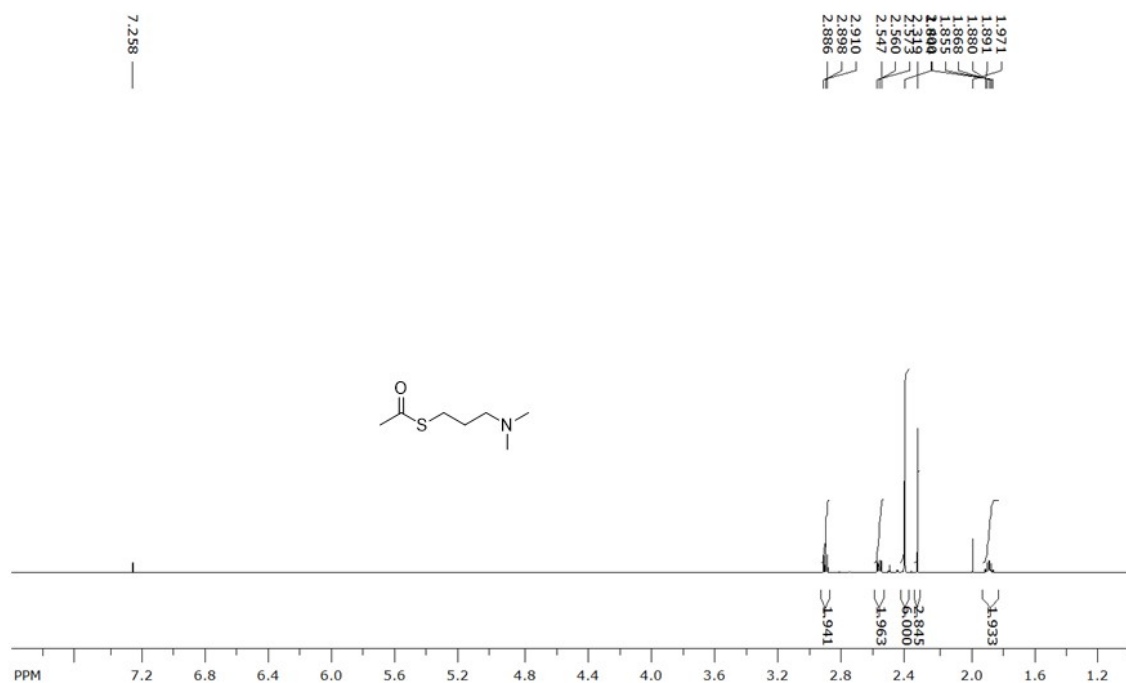


Figure S1a. ^1H NMR spectrum of 3-(dimethylamino)propyl ethanethioacetate

Synthesis of 3-[(dimethylamino)propyl ethanethioacetate]-proylsulfonate

A solution of 1,3-propane sultone (2.3 g, 18.6 mmol), prepared in 5 mL of dry ethanol, was added to a solution of 3-(dimethylamino)propyl ethanethioacetate (1.5 g, 9.3 mmol) in 20 mL of ethanol, and the mixture was stirred under nitrogen at room temperature for 48 h. A brown precipitate was formed, filtered and washed with acetone to afford the pure product as a brown solid (2.1 g, 88 % yield)

^1H NMR (DMSO, 600 MHz) δ 3.40-3.43 (m, 2H), 3.31-3.38 (m, 2H), 3.03 (s, 6H), 2.91 (t, 2H, $J = 7.2$ Hz), 2.49 (t, 2H, $J = 7.2$ Hz), 2.39 (s, 3H), 1.94-2.01 (m, 4H).

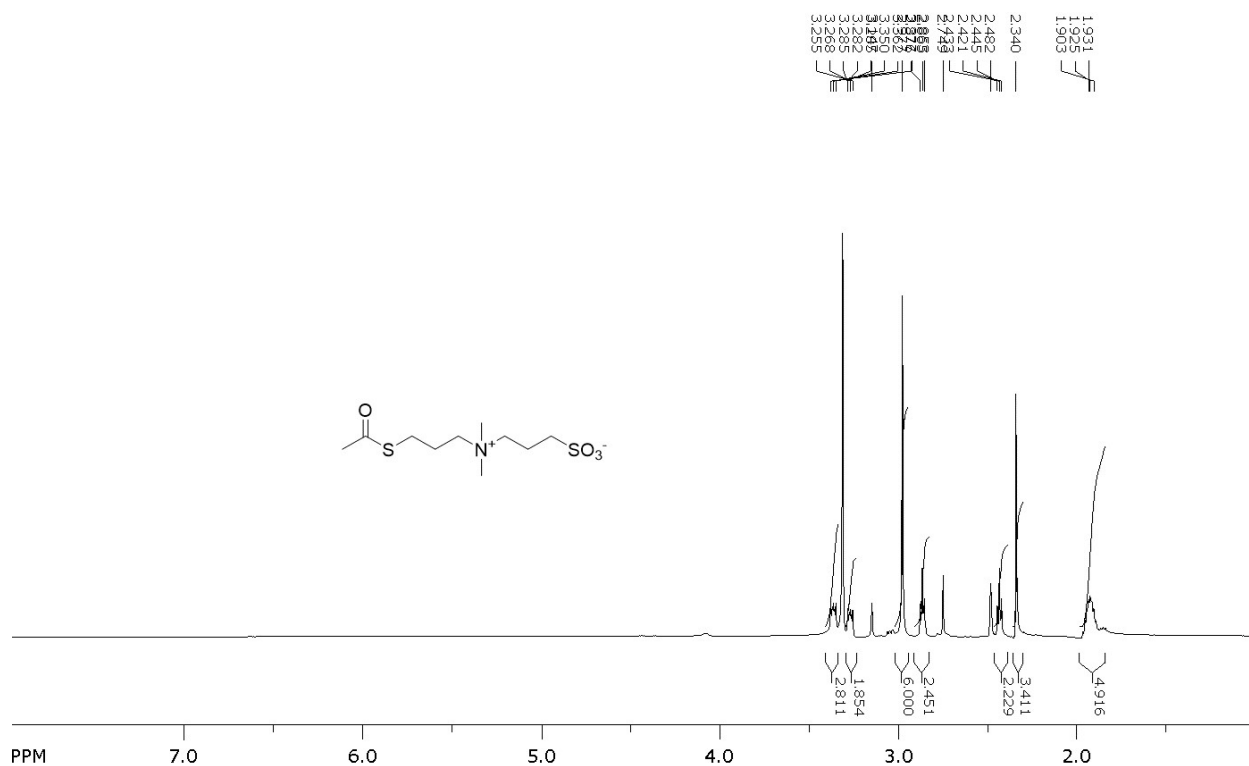


Figure S1b. ^1H NMR spectrum of 3-[(dimethylamino)propyl ethanethioacetate]-propylsulfonate

Synthesis of 3-((3-mercaptopropyl)dimethylammonio)propane-1-sulfonate (DPS)

A degassed solution of HCl (4.0 M in dioxane, 30 mL) was added to a solution of 3-[(dimethylamino)propyl ethanethioacetate]-propanesulfonate (1.0 g, 4 mmol) in methanol and the resulting mixture was stirred under nitrogen at 60 °C for 24 h. The dioxane was then evaporated and a brown oil obtained. The brown product was first dissolved in water (50 mL) and washed with ethyl acetate (3 times 20 mL). The resulting aqueous phase was then evaporated and dried under high vacuum for six days to afford a brown solid (0.8 g, 95 %)

^1H NMR (CD_3OD , 600 MHz) δ 3.50-3.54 (m, 2H), 3.43-3.46 (m, 2H), 3.10-3.14 (m, 6H), 2.86-2.91 (m, 3H), 2.58 (d, 1H, $J = 7.2$ Hz), 2.39 (s, 3H), 2.18-2.24 (m, 2H), 2.05-2.11-2.01 (m, 2H).

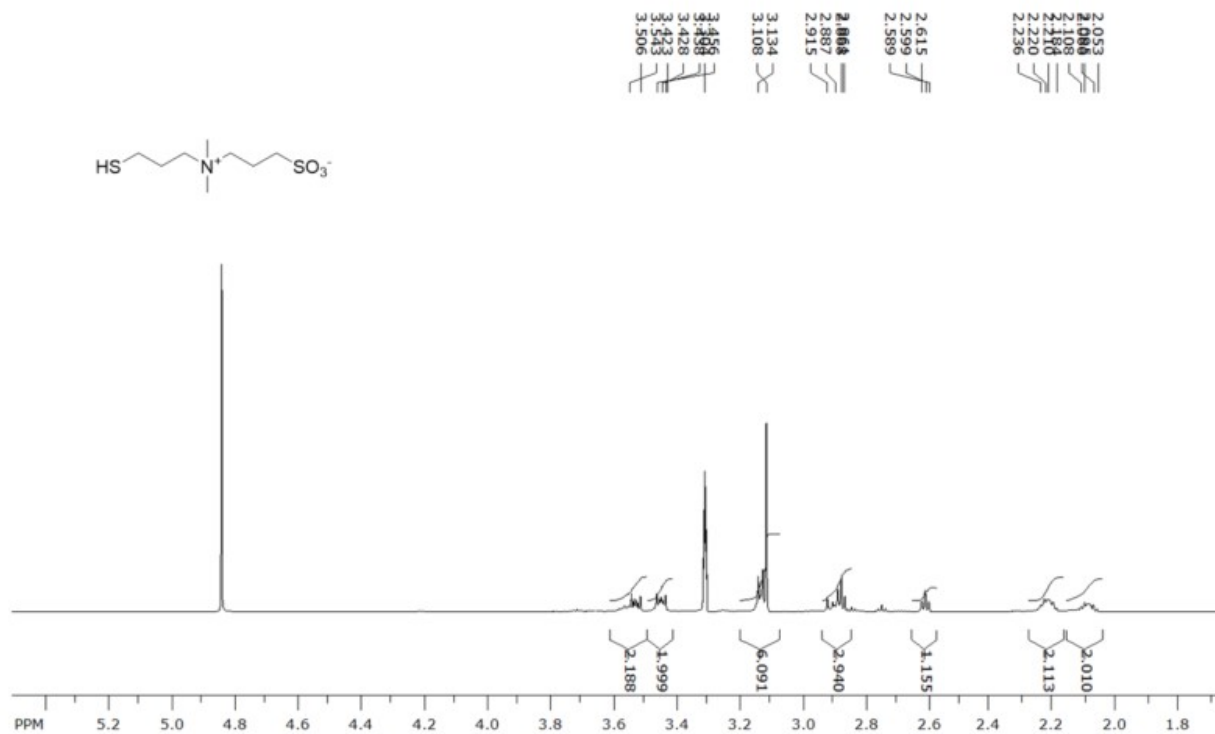


Figure S1c. ^1H NMR spectrum of 3-((3-mercaptopropyl)dimethylammonio)propane-1-sulfonate (DPS).

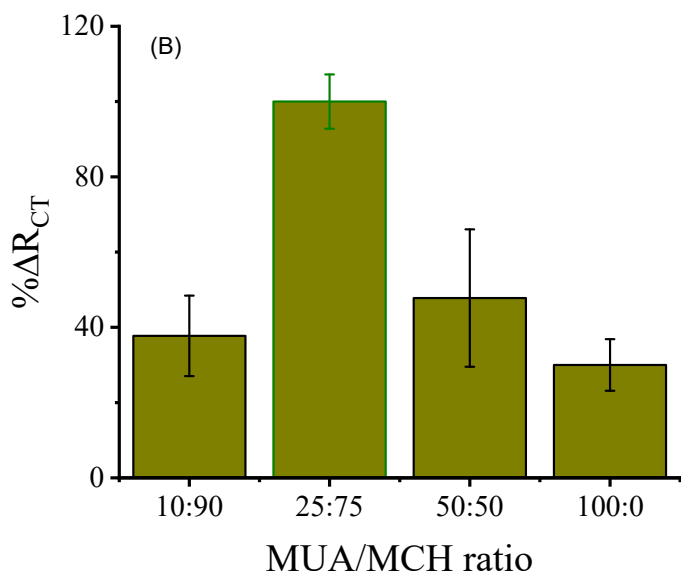
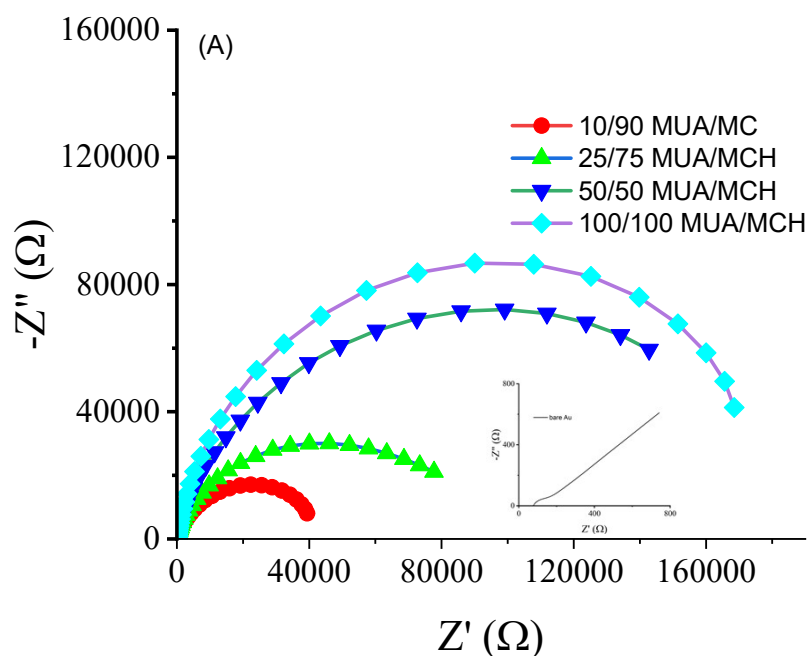


Figure S2. a) Nyquist plot of passive immersion MUA/MCH mixed thiol SAM modified electrodes and b) corresponding histogram of the charge transfer resistance ($\% \Delta R_{CT}$) of the mixed thiol surfaces after covalent attachment of TRL-4, (see Scheme 1 steps b,c,d). Y-axis, $\Delta R_{ct} = [R_{CT}(\text{TRL4-mixed thiol}) - R_{CT}(\text{mixed thiol})]/R_{CT}(\text{mixed thiol})$. The mole composition of thiol varied from 10/90 to 100/0 MUA/MCH, total thiol was 10 mM. TRL4 was used at 5 $\mu\text{g/mL}$.

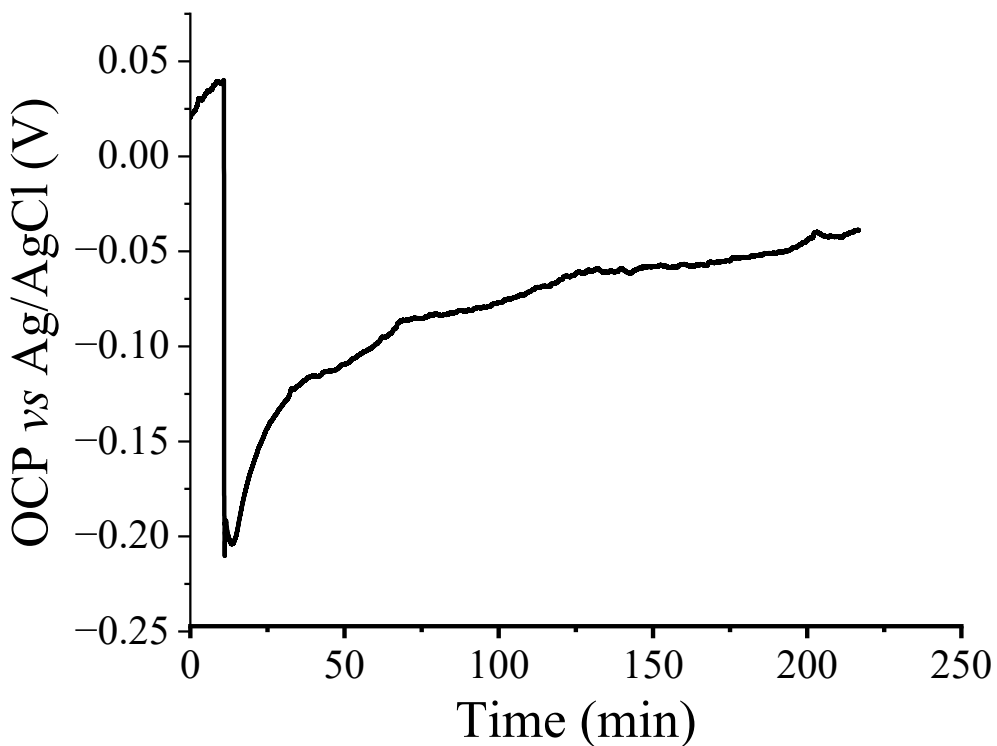


Figure S3. (A) Open circuit potential vs. time plot of a clean gold electrode (0.28 cm^2), initially equilibrated in $0.1 \text{ M LiClO}_4/\text{ethanol}$ solution, after the addition of a solution of 11-mercaptoundecanoic acid (MUA) and 6-mercapto-1-hexanol (MCH) (2.5 mM MUA and 7.5 mM MCH).

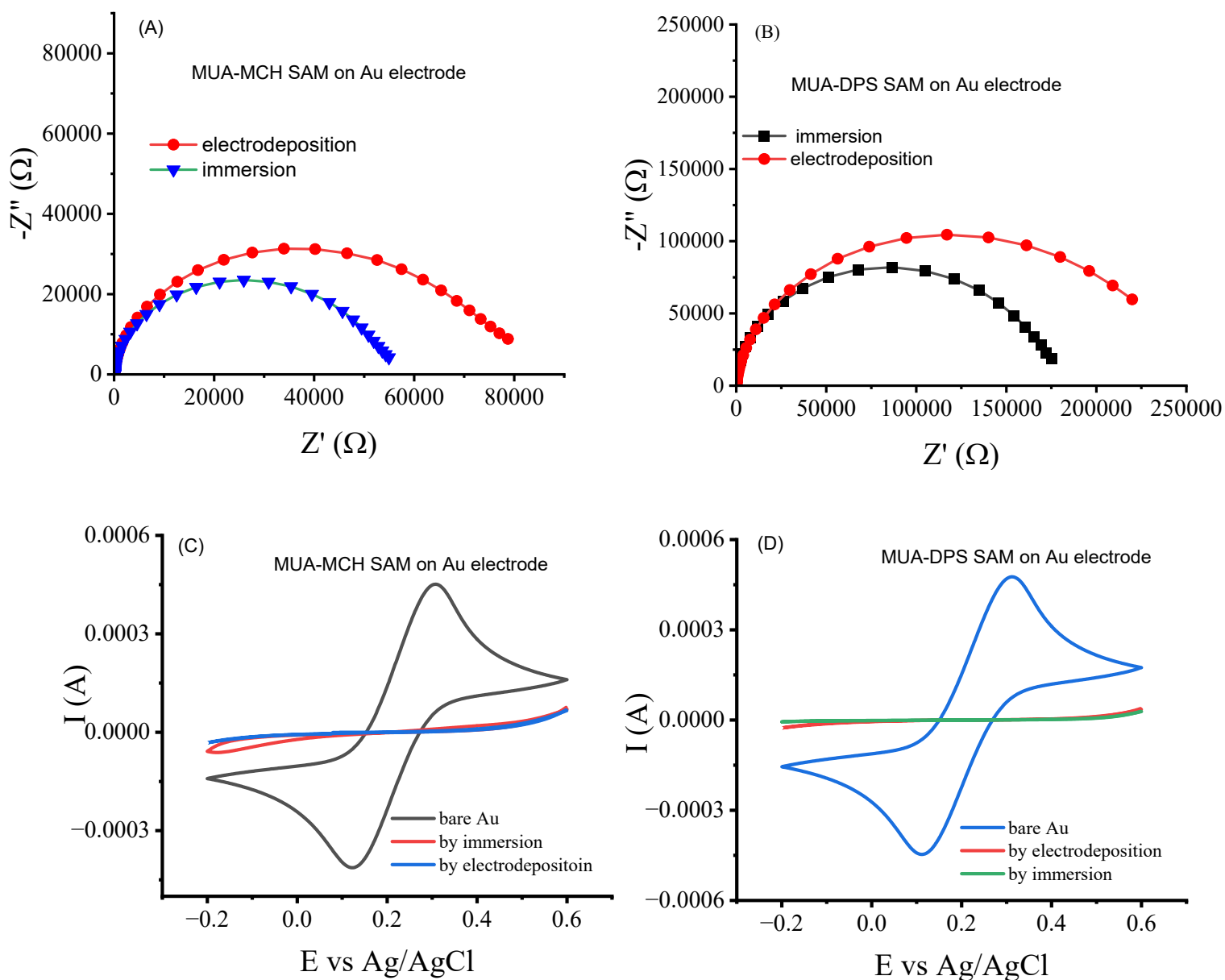


Figure S4. Nyquist plots and CVs of bare gold electrodes and gold electrodes modified with mixed SAMs of MUA and MCH (a, c) and MUA and DPS (b, d) formed by passive immersion and electrodeposition methods. EIS and CV data were collected using 1 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ with prepared in PBS buffer (pH=7.4) or 0.1 M KNO_3 as described in the experimental section.

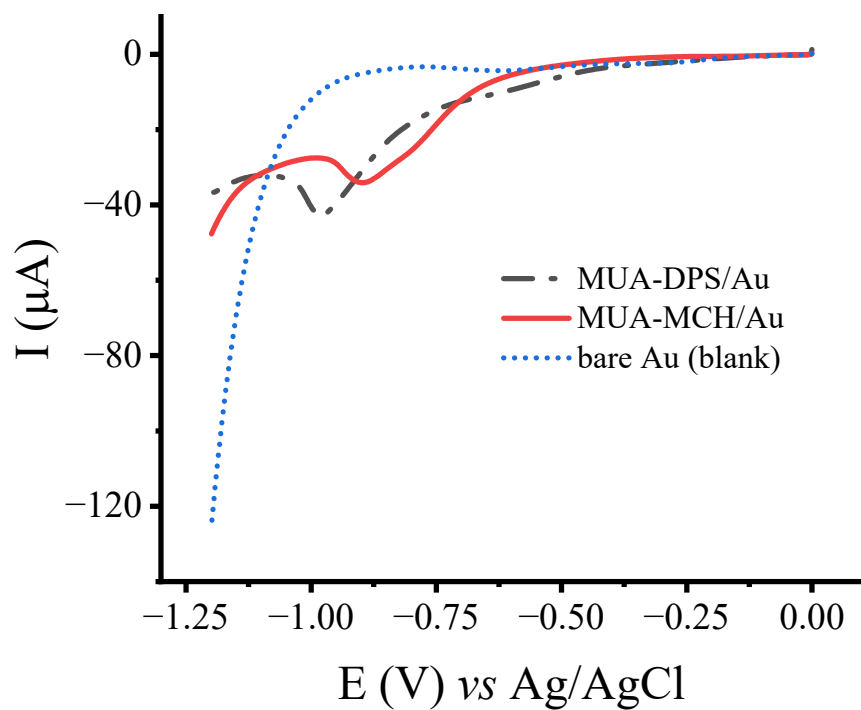


Figure S5. Linear sweep voltammograms of bare Au, voltage-assisted SAMs of MUA-DPS/Au and MUA-MCH/Au at a scan rate of 100 mV/s in the presence of 0.1 M KNO_3 electrolyte.

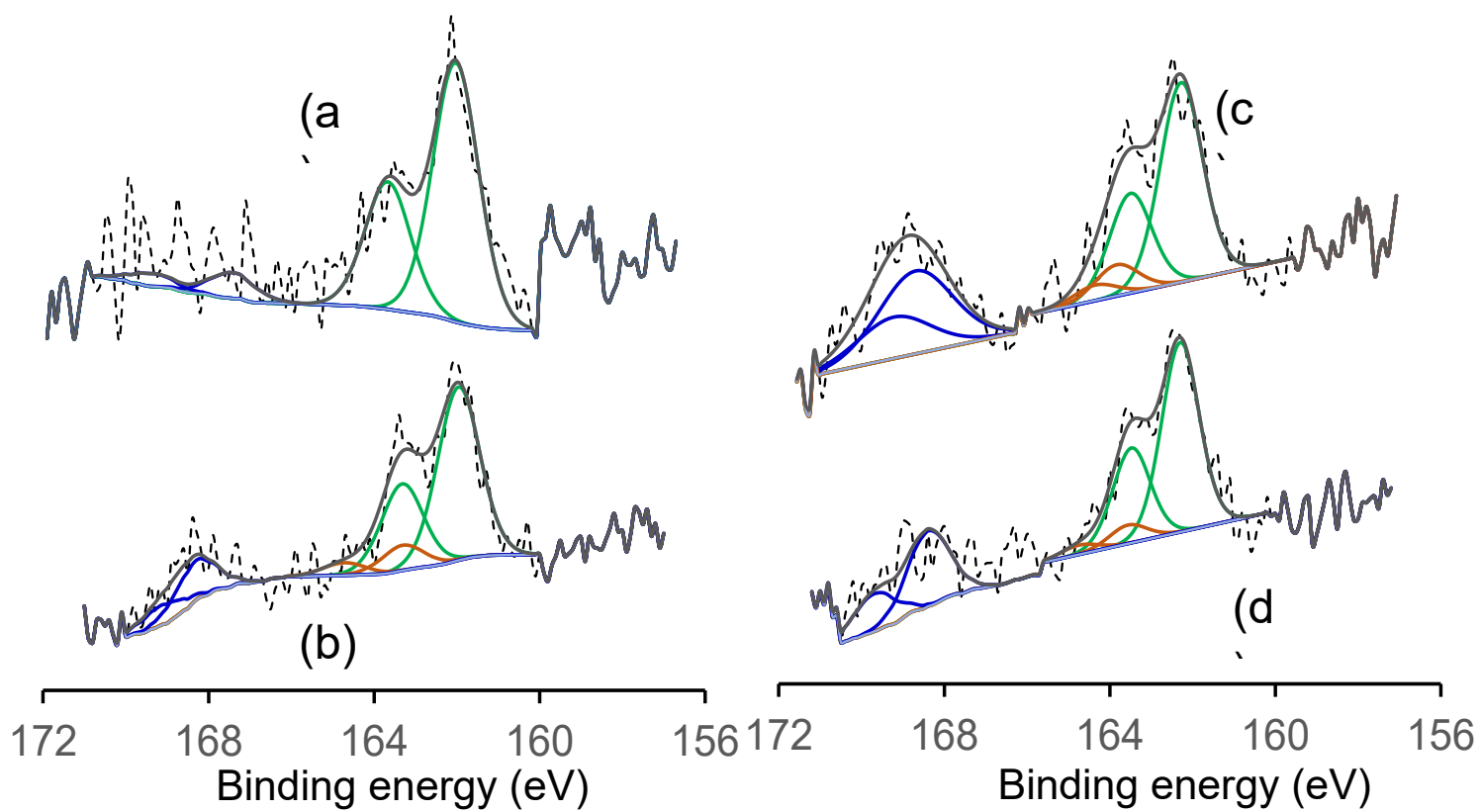


Figure S6. XPS S2p high-resolution spectra of gold electrodes modified with MUA/MCH SAMs (a-b) and MUA/DPS SAMs (c-d) formed by immersion (a, c) and electrodeposition (b, d) methods.

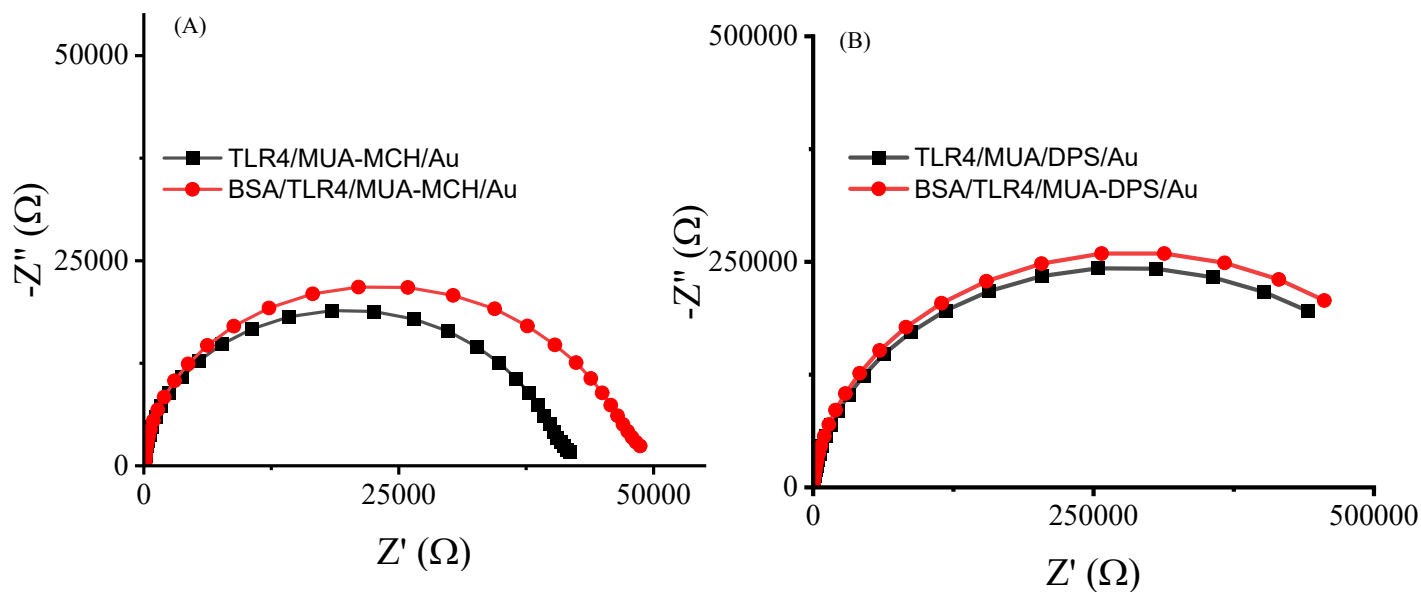
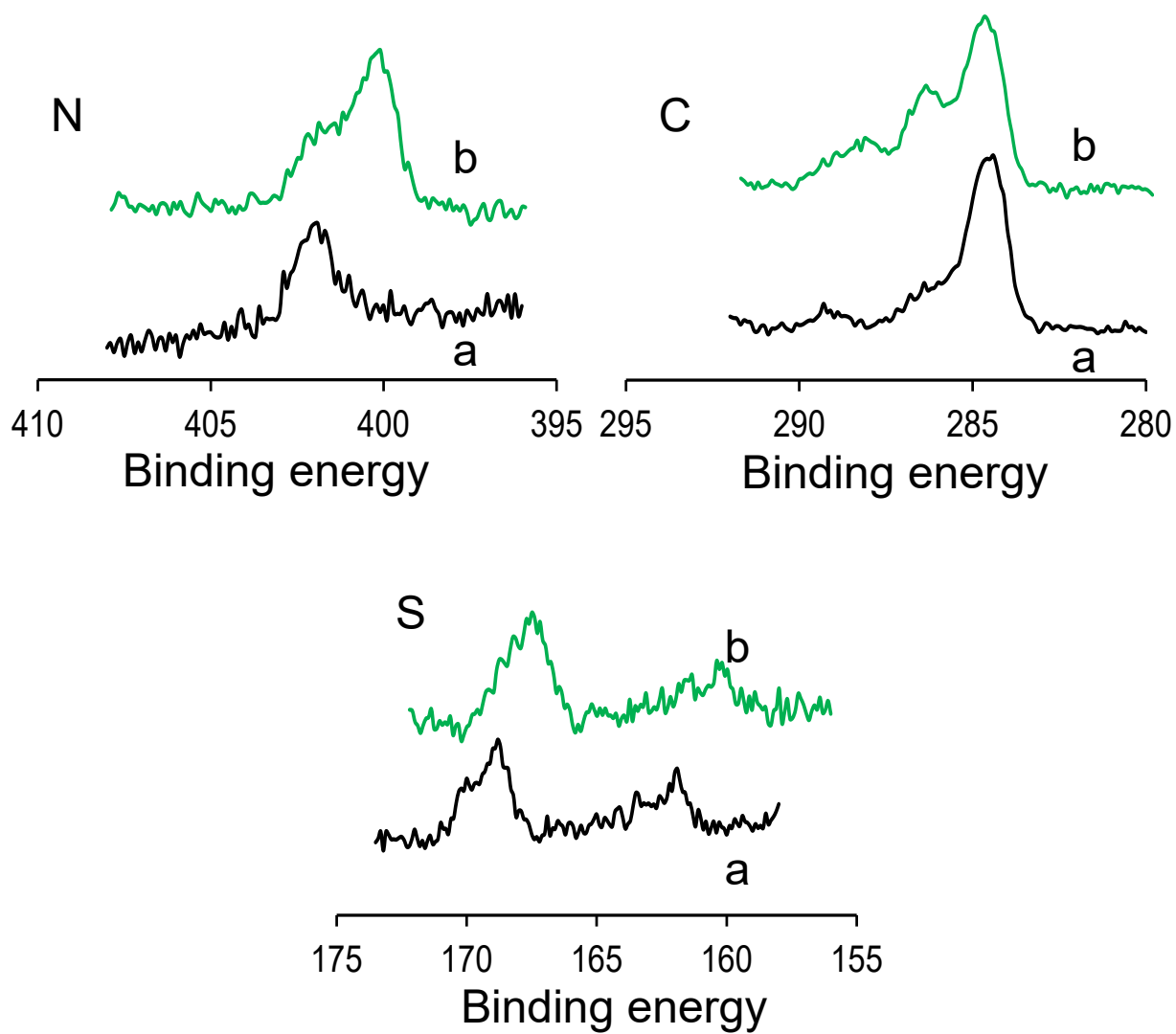


Figure S7. Nyquist plots of MUA/MCH and MUA/DPS modified Au electrodes before and after incubation with 1 mg/mL BSA. The SAM layers were prepared by electrodeposition using 25:75 ratio of MUA/MCH (A) and MUA/DPS (B). The TLR4 and BSA incubation conditions are as discussed in the experimental section.



FigureS8. High-resolution XPS spectra for voltage-assisted deposited MUA-DPS SAM on Au electrode (a) and TLR4 conjugated MUA-DPS/Au surface (b).

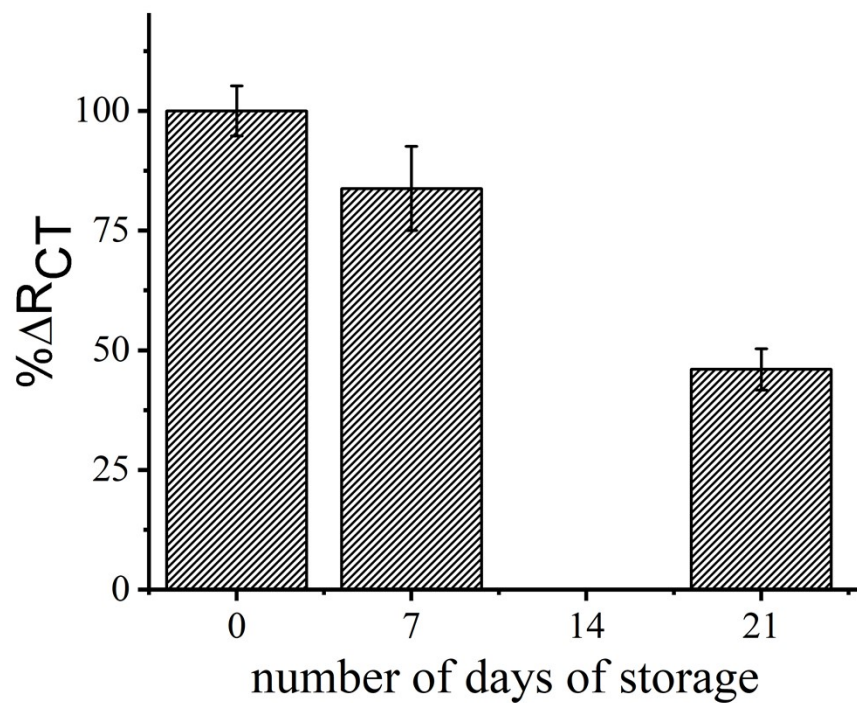


Figure S9. EIS response of TLR4 sensors stored in PBS (pH 7.4) buffer at 4 °C for varying times. The response of a freshly prepared sensor is marked as t = zero day. Each data point is an average of three independently prepared TLR4 sensor exposed to 1,000 ng/mL LPS, with error bars showing standard deviation.

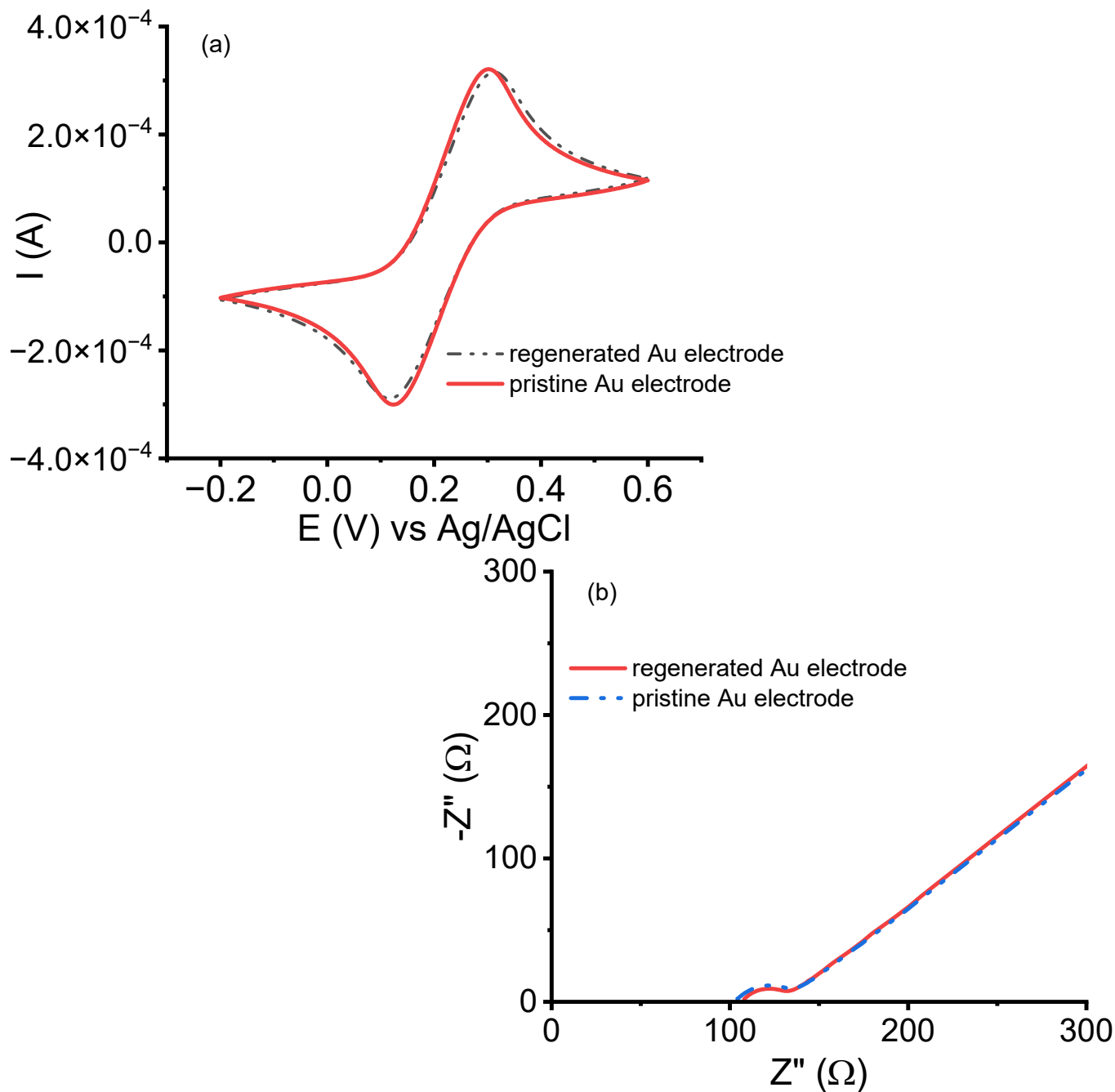


Figure S10. Cyclic voltammogram (a) and Nyquist plot (b) of pristine and regenerated Au electrodes in 1 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ prepared in 0.1 M KNO_3 . Data for the regenerated electrode was obtained after 5 cycles of self-assembly and electrochemical reductive desorption of the TLR9/MUA-DPS/Au sensor. The scan rate for the CV was 100 mVs^{-1} ,

and the frequency for the Nyquist plot varied from 0.1 Hz to 100 kHz. All other experimental conditions are as in Figure 6.