Supporting information

MOF based Tri Electrode Aptasensor Platform for Effective Detection of Sepsis Markers with Minimal Cross Interference

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Materials/reagents, and synthesis procedure

Copper nitrate hydrate, Cu (NO_3).2H_2O, cobalt acetylacetonate (II), zinc nitrate (II) hexahydrate, erbium chloride (III), ytterbium chloride (III), Trypan blue solution (0.4%), benzene tetra carboxylic acid (H_4BTC), 3-phosphonopropionic acid (3-PPA) technical grade, 94%, N-hydroxy succinimide, 98%, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide ≥97.0% (T), dimethyl formamide (DMF), and phosphate buffer (PBS, pH 7.4) were obtained commercially from Sigma Aldrich Chemical Co. USA (St. Louis, MO, USA). Standard acetate buffer (pH 5) was obtained from VWR, Radnor, PA, USA. The bovine whole blood samples were obtained from Lampire Biological Laboratories, Pipersville, PA, USA. Streptavidin lyophilized powder, MES buffer, 0.5 M (pH 5.5), procalcitonin (PCT), C-reactive protein (CRP), and interleukin-6 (IL-6) were obtained from Thermo Fisher Scientific, Waltham, MA, USA. Aptamers specific to CRP, IL-6, and PCT, such as 5' Amino Modifier C6, 5' Amino Modifier C12, and 3' Biotin Oligos, were obtained from Integrated DNA Technologies, Newark, New Jersey, USA.

Aptamers specific to CRP, IL-6, and PCT were selected from the literature depending upon their affinity for respective biomarkers. Aptamer sequences such as:

PCT - 5'-CCG CGG CAG TTC CGT AAT GTT AAT GCC-biotin/3'

CRP - 5'/ 5AmMC6-CGG TTA CAG ATG ATC AGG CT-3'

IL-6 - 5'/ 5AmMC12-GGT GGC AGG AGG ACT ATT TA-3'

The lab-ready aptamer stock solutions were obtained in the following forms:

5' Amino modified - Anti - IL6 aptamer: HPLC purified with a yield of 4nmol, normalized to 100 mM in IDTE pH 8.0) IDTE Buffer pH 8.0 (10 mM Tris-HCl/0.1 mM EDTA))

3' Biotin modified - PCT aptamer: HPLC purified with a yield of 2nmol, normalized to 100 mM in IDTE pH 8.0) IDTE Buffer pH 8.0 (10 mM Tris-HCl/0.1 mM EDTA

5' Amino modified - CRP aptamer: HPLC purified with a yield of 4nmol, normalized to 100 mM in IDTE pH 8.0) IDTE Buffer pH 8.0 (10 mM Tris-HCl/0.1 mM EDTA)

The stocks were stored at -20°C, and before use, and the solutions were diluted to the desired concentration (1-5 mM) with MES buffer.

Synthesis of Cux (Try)x. Trypan blue (Try) (100 μL) and Cu (NO_3).2H_2O (100 mg) were dissolved in 3 mL of DMF; this step was followed by 15 min sonication and then stirring at 110 °C for 24 h. The product was centrifuged from the mixture and washed repeatedly with ethanol; the product was then dried in a vacuum oven at a temperature of 100 °C for a period of 12 h.

Synthesis of Cux Coy (Try)x. Trypan blue (Try) (100 μL), Cu (NO_3).2H_2O (100 mg), and Co (NO_3)2 (100 mg) were dissolved in 3 mL of DMF; this step was followed by 15 min sonication and then heating at 110 °C for a period of 24 h. The product was centrifuged from the mixture and

washed repeatedly with ethanol; the product was then dried in a vacuum oven at a temperature of 100 °C for a period of 12 h.

Synthesis of Cux Ery (Try)x. Trypan blue (Try) (100 μL), Cu $(NO_3).2H_2O$ (100 mg), and Er $(Cl_3)_3$ (100 mg) were dissolved in 3 mL of DMF; this step was followed by 15 min sonication and then heating at 110 °C for 24 h. The product was centrifuged from the mixture and washed repeatedly with ethanol; it was then dried in a vacuum oven at 100 °C for 12 h.

Synthesis of Cux Yby (Try)x. Trypan blue (Try) (100 μL), Cu $(NO_3).2H_2O$ (100 mg), and Yb $(Cl_3)_3$ (100 mg) were dissolved in 3 mL of DMF; this step was followed by 15 min sonication and then heating at 110 °C for 24 h. The product was centrifuged from the mixture and washed repeatedly with ethanol; it was then dried in a vacuum oven at 100 °C for 12 h.

Development of Triad POC device and aptasensor fabrication

Typically, the device incorporates three T-shaped cavities with a total depth of 6.5 mm each (3 mm central well and 2.5 mm deep outer circle) to estimate the binding of PCT, CRP, and IL-6. We optimized the well ratios and channel patterns to achieve reliable mechanical properties and effective analytical performance. Channel patterns were engraved in the stock PMMA material to connect the central wells and packed with modified MOF paste, which served as the working electrode. Each outer is well accommodated with a 30 μl reaction volume, ensuring that detection reactions occur independently.

Later, the aptasensor was formulated as a paste and packed in the wells of a PMMA-based laser-scribed microdevice as described previously. The 3-PPA modified conductive MOFs were chemically functionalized with biomarker-specific aptamers to yield three distinct aptasensors. These aptasensors were analyzed with voltammetric and impedimetric techniques. We have optimized the electrode behavior across different MOF pastes (consisting of Cu/Co-TB, Cu/Zn-TB, and Cu/Er-TB) and identified the best-performing system. In particular, the Cu/Zn-TB system was used, followed by modification of the carboxylic group with 3-PPA. Subsequent chemical treatments, including EDC / NHS activation, streptavidin binding, and immobilization of specific DNA oligos, were carried out within the wells.

Instrumentation

Scanning electron microscopy (SEM) data were obtained using a Model SU3900 variable pressure scanning electron microscope (VPSEM) (Hitachi, Tokyo, Japan). A DropSens SPELEC system (Metrohm AG, Herisau, Switzerland), in conjunction with a PalmSens portable sensor bit potentiostat that contained a PSTrace Bluetooth user interface, was used in a three-electrode configuration to record voltammograms and impedance measurements in a multiplex format. A laser scriber was used to develop the PMMA platform.

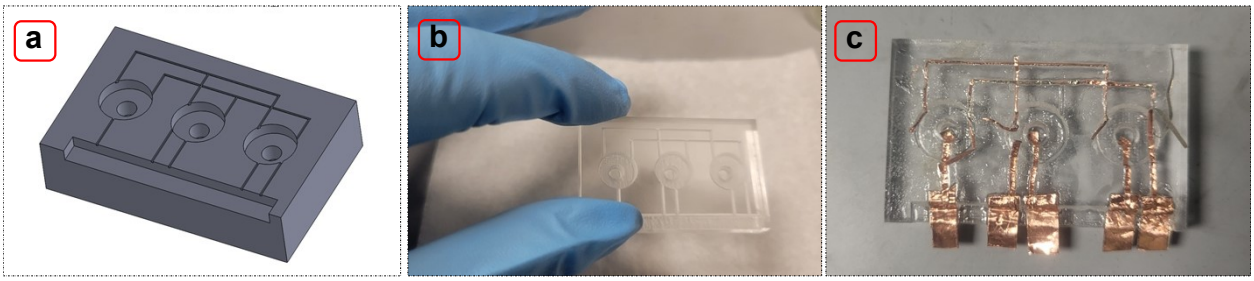

Figure S1. (a) Computer-aided 3D design of triad platform, PMMA prototype before (b), and (c) after establishing connection pads.

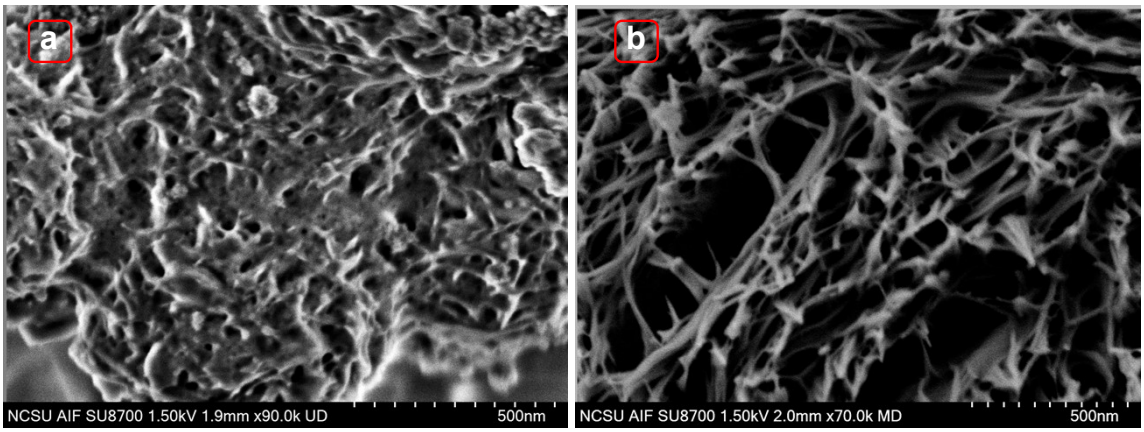

Figure S2. SEM micrographs of Cu / Er - TB MOF before (a) and after (b) 3-PPA modification.

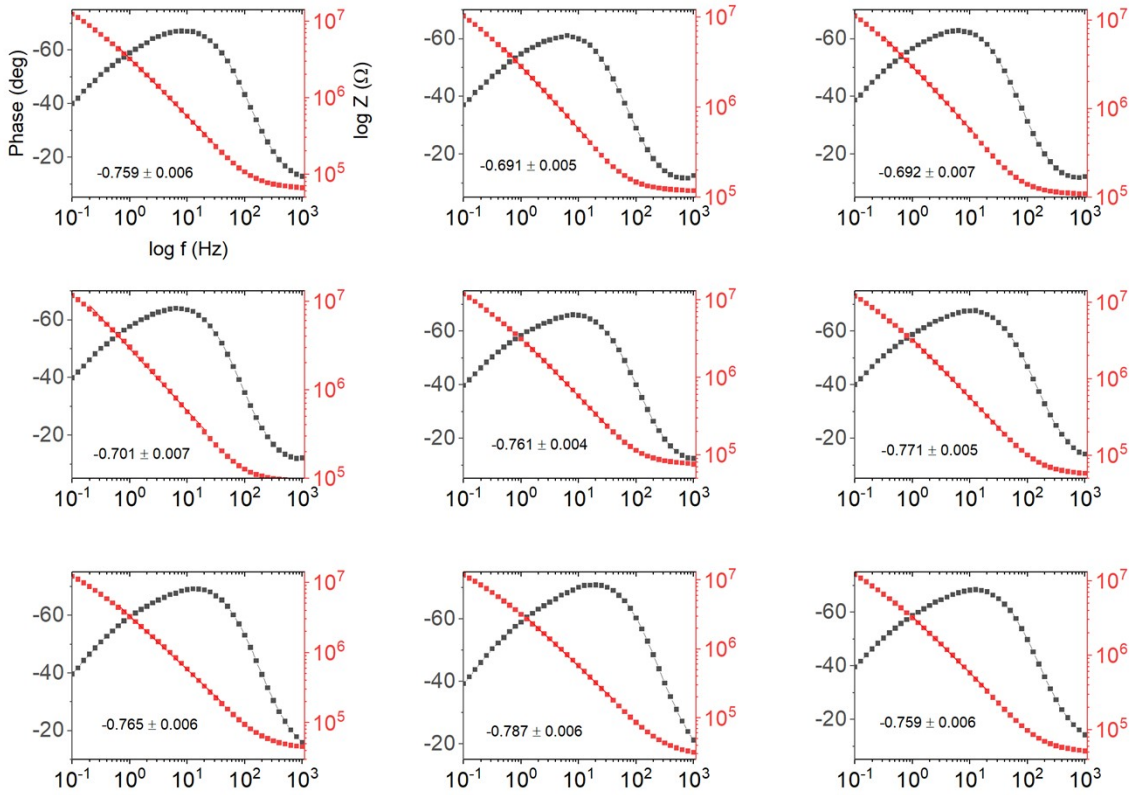

Figure S3. Bode plots showing the optimization of the MOF-PPA mixtures.

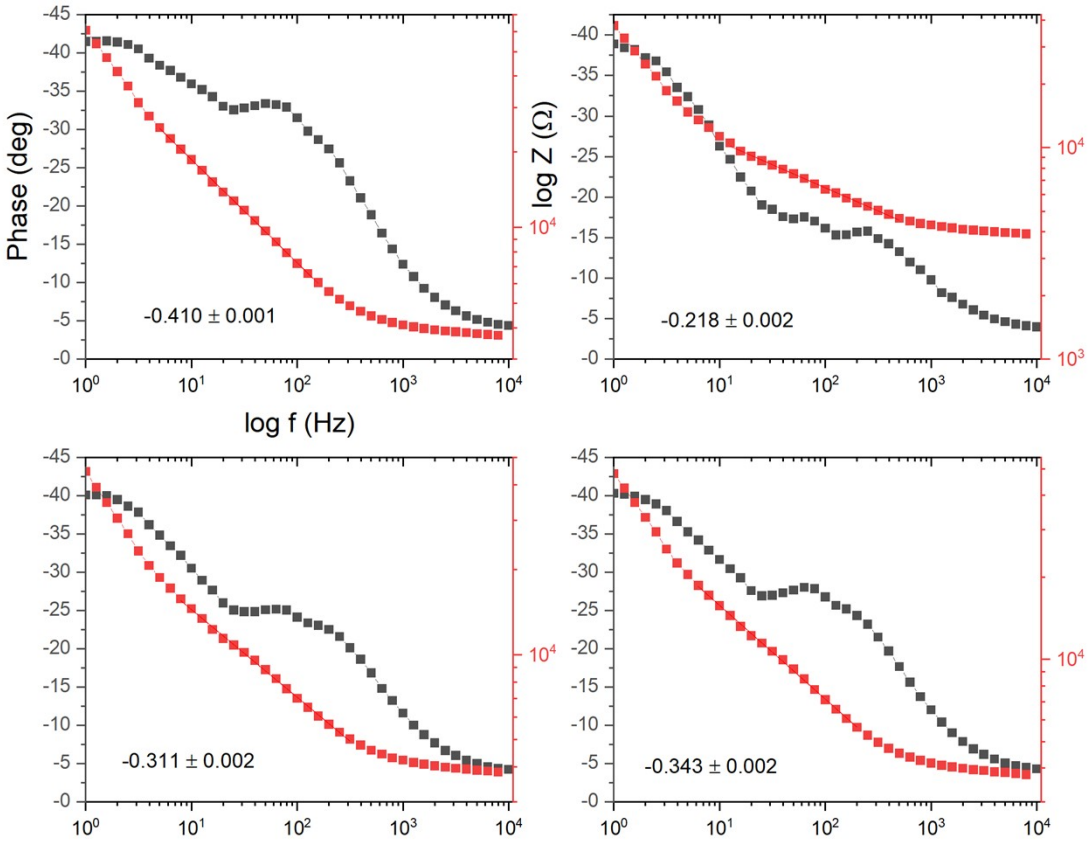

Figure S4. Bode plots corresponding to EDC/NHS linking on the electrode surface.

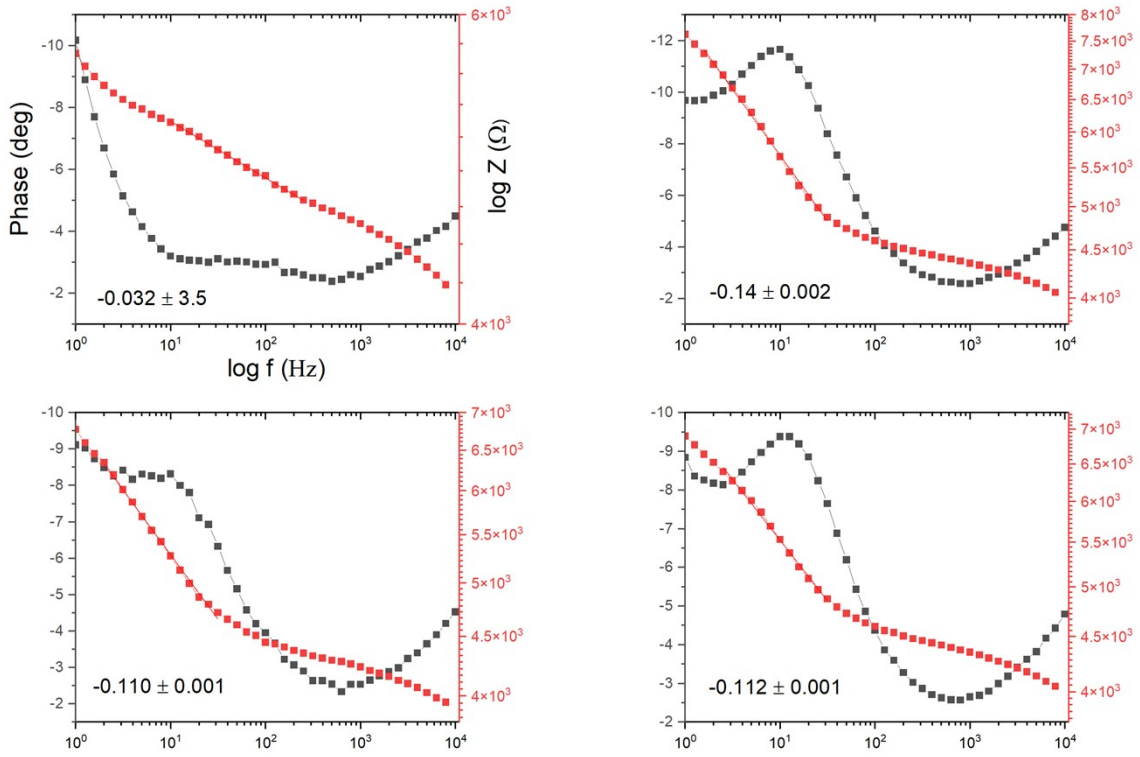

Figure S5. Bode plots depicting the aptamer immobilization on the electrode surface.

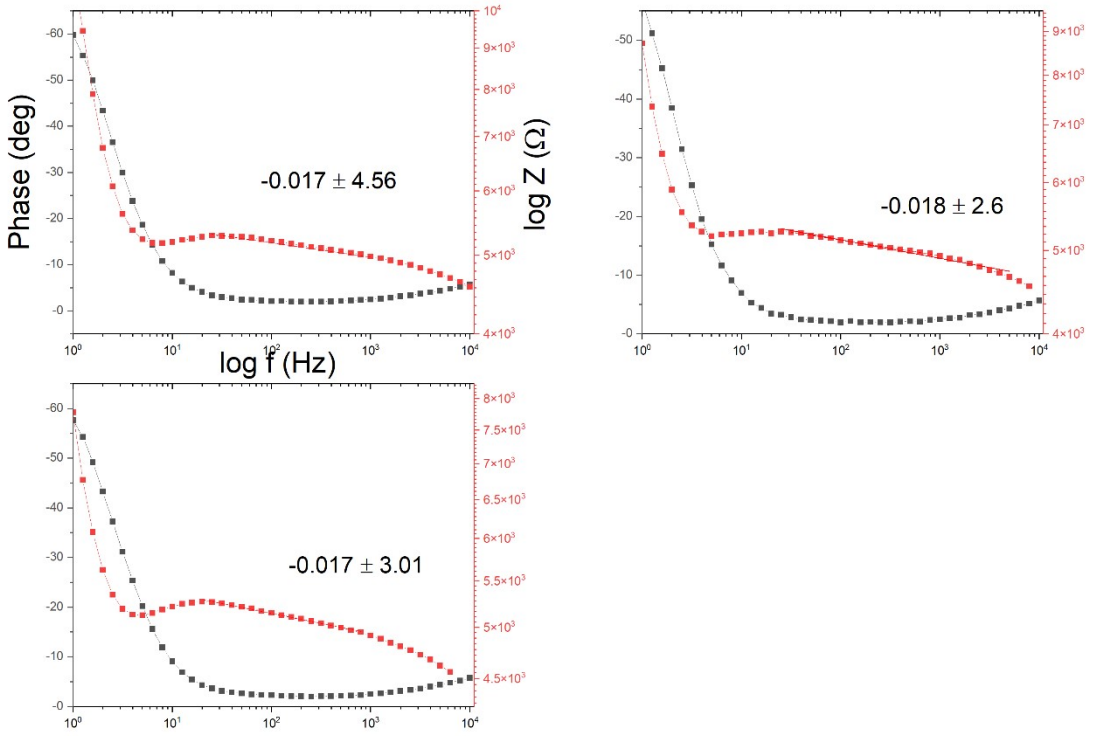

Figure S6. Bode plots depicting the aptamer-target biomarker coupling on the electrode surface.

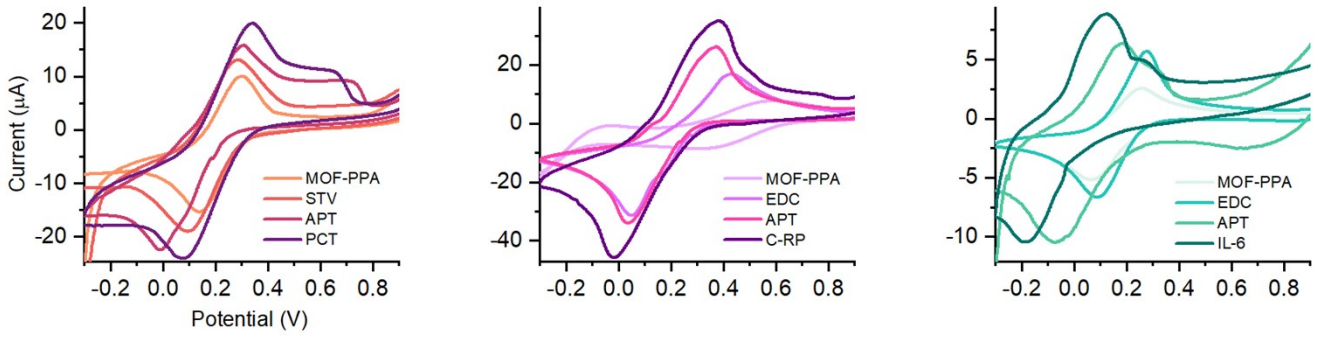

Figure S7. CV curves for PCT, CRP, IL-6, recorded upon carboxylic acid functionalization of MOF with 3-PPA, EDC / NHS & STV surface activation, subsequent aptamer immobilization, and respective target binding.

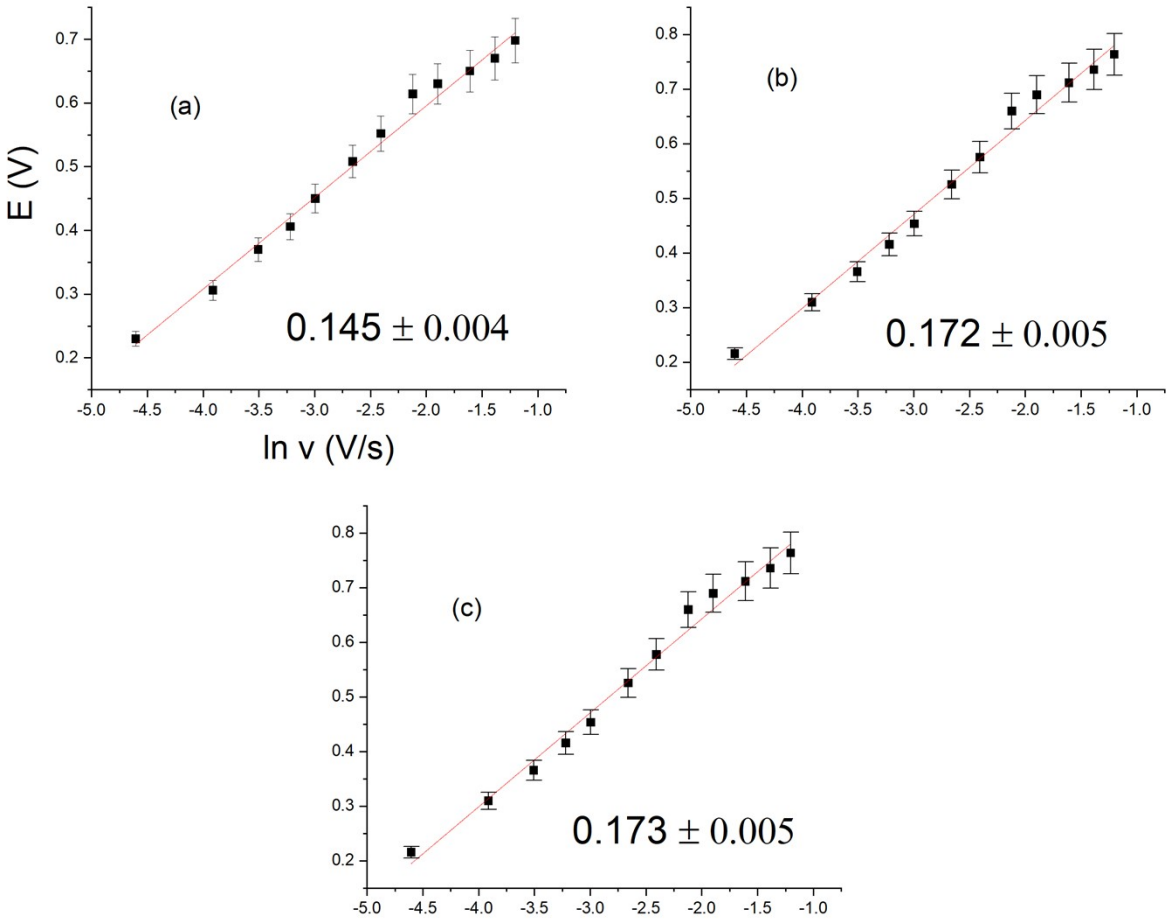

Figure S8. Ep vs. ln v plots for (a) aptamer immobilization, (b) CRP 0.001 mg mL^{-1}, (c) CRP 0.5 mg mL^{-1}.

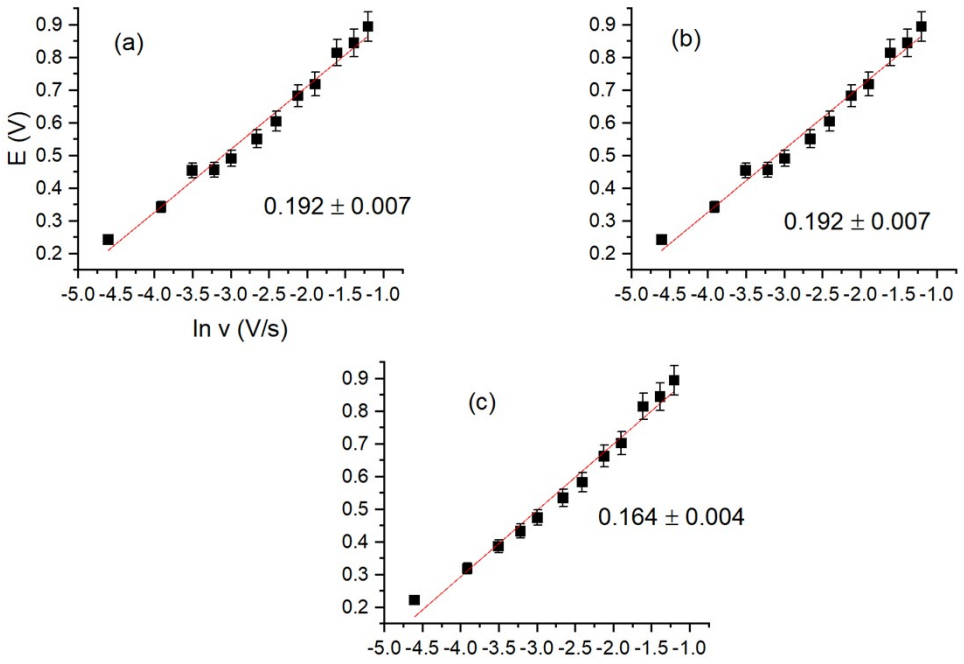

Figure S9. Ep vs. ln v plots for (a) aptamer immobilization, (b) IL-6 0.01 ng mL^{-1}, (c) IL-6 50 pg mL^{-1}.

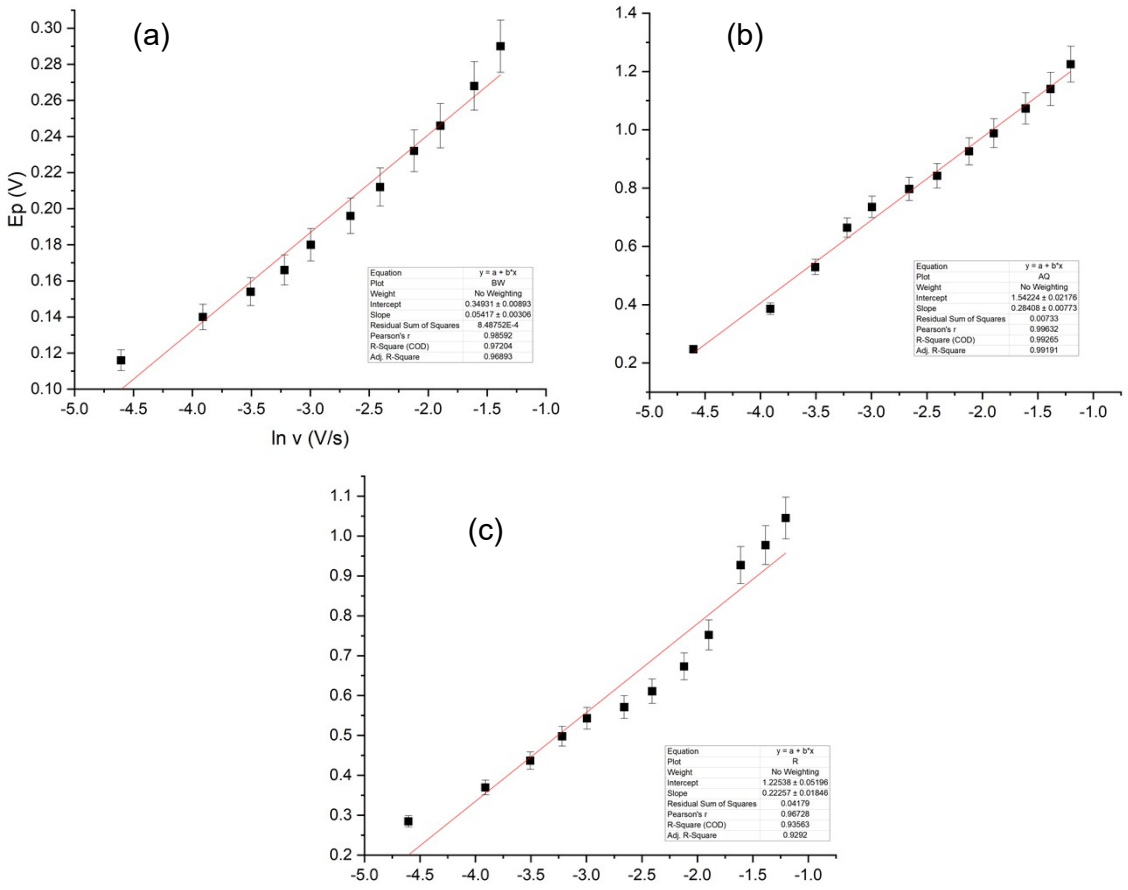

Figure S10. Ep vs. ln v plots for PCT, CRP, and IL-6 detection in the presence of (a) CRP + IL-6 (b) PCT + IL-6, and (c) PCT + CRP.