

Supporting Information

Signal enhancement of electrochemical biosensors via direct electrochemical oxydation of silver nanoparticle labels coated with zwitterionic polymer.

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Experimental section.

Materials. *N,N'*-dimethyl(methacrylamidopropyl)ammonium propanesulfonate (SPP) was donated by RASCHIG GmbH, Germany. 2,2'-azobis(isobutyramidine) dihydrochloride (AIBA, 97%, Sigma-Aldrich), *N*-morpholino ethane sulfonic acid monohydrate (MES, 97%, Sigma-Aldrich), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 99%, Sigma-Aldrich), *N*-hydroxysuccinimide (NHS, 99%, Sigma-Aldrich), sodium hydrogen phosphate monohydrate (99%, Sigma-Aldrich), silver nitrate (99.8 %, Prolabo), sodium acetate (SA, 99 %, Prolabo), acetic acid (AA, 98 %, Prolabo) and sodium borohydride (98 %, Prolabo) were used without further purification. The RAFT agent, 4-cyanopentanoic acid dithiobenzoate (CTP, 97%) was purchased from Strem Chemicals. Amino-PEG₂-Biotin (biotin), Sulfo-NHS-SS-Biotin, and avidin were obtained from Pierce. Deionized water at 18.2 mΩ (MilliQ, Millipore, France) was used in all experiments.

Synthesis of polyzwitterion, PSPP by RAFT polymerization.

Polyzwitterion, PSPP, was synthesized by RAFT polymerization with the following procedure: Solution containing SPP, CTP, AIBA in acetic buffer (pH 5.2, 0.27 mol L⁻¹ acetic acid and 0.73 mol L⁻¹ sodium acetate) was prepared into a schlenk flask in the following initial concentrations: [SPP]₀ = 0.79 mol L⁻¹, [CTP]₀ = 3.62 mmol L⁻¹, [AIBA]₀ = 0.46 mmol L⁻¹. CTP RAFT agent was neutralized by equimolar aqueous solution of NaOH. The polymerization mixture was degassed by gently bubbling argon for 30 min and subsequently allowed to polymerize at 70 °C in a thermostated oil bath under stirring. After 40 min, the polymerization was quenched by rapid cooling upon immersion of the flask in liquid N₂. The monomer molar conversion was determined by ¹H NMR (D₂O) of the raw solution. The polymerization solution was dialyzed against deionized water for 7 days using a SpectraPor membrane (MWCO: 3 500 Da) to eliminate residual monomers and freeze-dried to yield a pink solid. The polymer was analyzed by size exclusion chromatography (SEC). The chemical characteristics of the synthesized polyzwitterion are reported in Table S1. The presence of the carbonyl on the polymer was checked by ¹³C NMR (D₂O) (Figure S1).

Synthesis of biotinylated zwitterionic polymers, biotin-PSPP.

Biotin-conjugated PSPP polymer was carried out as follows: 60 μL each of fresh NHS solution (28.96 mM in 10^{-2} M MES buffer, pH = 5) and EDC solution (199.70 mM in 10^{-2} M MES buffer, pH = 5) were added into 3 mL of synthesized PSPP polymer ($M_n = 17270$ g mol $^{-1}$, 0.58 mM in 10^{-2} M MES buffer, pH = 5) in a 10 mL flask with a magnetic bar. After 30 min of stirring, 174 μL of biotin solution (10^{-2} M in 10^{-1} M PBS buffer, pH = 7) was added. The reaction mixture was stirred at 25°C for 16 h (Scheme 1). Then, the solution was dialyzed against deionized water for 72 h to remove unreacted biotin and other byproducts using a SpectraPor membrane (MWCO: 8000 Da) and then freeze-dried to yield a pink solid. The PSPP bioconjugation was confirmed by ^1H NMR (D_2O) (Figure S2).

Synthesis of silver nanoparticles, AgNP.

Silver nanoparticles were prepared by reduction of AgNO_3 with sodium borohydride in aqueous media. Briefly, 4 mL solution of 1.0 mM AgNO_3 was added to 60 mL of 2 mM NaBH_4 solution under vigorous stirring. The solution turned bright yellow after the addition of silver nitrate. The reaction mixture was stirred at 25°C for 16 h. The AgNP solution was stored in a dark glass bottle at 4°C for further use. Then silver nanoparticles, AgNP, were analyzed by TEM and UV-vis spectroscopy (see Figure S3 and S4). The prepared AgNP have an average diameter of 12.8 nm and exhibit a surface resonance plasmon, SPR, centered at 393 nm characteristic of well dispersed nanoparticles without any aggregation.

Biotinylated zwitterionic polymer-protected silver nanoparticles Biotin-PSPP-AgNP.

Biotin-PSPP-AgNP were prepared by ligand exchange procedure. 2.0 mL aqueous solution containing 17.9 mg PSPP and 2.0 mg biotin-PSPP was prepared. Next, 347.4 μL of freshly prepared NaBH_4 aqueous solution (10^{-2} M) was slowly injected into the reaction mixture and was left under stirring for 30 min at room temperature. Then ligands exchange was carried out by mixing 24.1 μL of the as prepared solution into 20 mL of AgNP solution under magnetic stirring. After 1 h, the modified AgNP were purified three times by filtration for 10 min to remove the polymer excess using Vivaspin disposable system (cut-off 50 kDa). The purified Biotin-PSPP-AgNP were finally concentrated, redispersed in pure water and then stored at

4°C. Biotin-PSPP-AgNP were analyzed by TEM and UV-vis spectroscopy. The bare AgNP and biotin-PSPP-AgNP exhibit a similar UV-vis spectrum which indicates that any aggregation occurs during the exchange reaction (Figure S4). In addition, Figure S4 also presents a UV-vis spectrum of functionalized AgNP, biotin-PSPP-AgNP, in 1M KCl solution that shows the efficiency of the zwitterionic polymer corona in high ionic strength solution compare to AgNP without zwitterionic polymers. Digital photos of the different bare and functionalized silver nanoparticles in different solution (pure water and 1M KCl) are shown in Figure S4 confirming the efficiency of the zwitterionic polymer corona against aggregation in saline solution.

Electrode modification.

Au/Biotin. The gold electrode disk (0.5 mm diameter) was polished with 0.03 μm alumina paper, and then ultrasonically cleaned in distilled water for 5 min. After drying in air, the electrodes were electrochemically polished by cycling the potential scan between 0 and 1.5 V in 0.5 M H_2SO_4 at a scan rate of 0.1 V s^{-1} (25 cycles). The electrode was then washed with water and dried in air. The clean electrode was immersed in 0.5 mL ethanol/water (9/1) solution of 0.5 mM NHS-SS-biotin for 16 h at room temperature, then washed three times with water under ultrasound to remove free ligands and dried in air.

Au/Biotin/Avidin. Au/Biotin electrode was soaked in 0.5 mL of avidin solution at various concentration (0.5 to 15 nM in 10^{-1} M PBS buffer, pH= 7) for 2 h at room temperature and cleaned three times first with PBS (10^{-1} M, buffer pH= 7) and MES (10^{-2} M, buffer pH= 6.15) solution under ultrasound and dried in air.

Au/Biotin/Avidin/modified AgNP. Au/Biotin/Avidin electrodes were immersed into 0.5 mL of biotin-PSPP-AgNP solution (1.18 nM in 10^{-2} M MES buffer pH= 6.15) for 2 h. Then the electrodes were cleaned three times with MES (10^{-2} M pH= 6.15) solution under ultrasound and dried in air.

Selective adsorption of nanoparticles

Au + modified AgNP and *Au/Biotin + modified AgNP.* The bare and the biotin modified gold electrodes were immersed into 0.5 mL of biotin-PSPP-AgNP solution (1.18 nM in 10^{-2} M

MES buffer pH=6.15) for 2 h. Then the electrodes were cleaned three times with MES (10^{-2} M pH= 6.15) solution under ultrasound and dried in air.

Characterization.

NMR. ^1H and ^{13}C NMR spectroscopy were performed in D_2O in a Bruker 400 (400 MHz) spectrometer.

SEC. The number-average molar mass (M_n), the weight-average molar mass (M_w), and the dispersity ($D=M_w/M_n$) were determined by size exclusion chromatography (SEC) in 0.5 M NaNO_3 aqueous solution at 25 °C and at a flow rate of 1 mL min $^{-1}$ using a Viscotek SEC system equipped with three SHODEX OHpack columns SB-806M HQ (13 μm , 300 x 8 mm). All polymers were injected at a concentration of 2 mg mL $^{-1}$ after filtration through a 0.2 μm pore-size membrane. The setup was equipped with three detectors (refractive index, viscometer and light scattering).

UV-vis spectroscopy. Absorbance measurements were carried out with a UV-vis Hewlett-Packard 8453 spectrophotometer using a quartz cell, in a wavelength range from 200 to 1100 nm and equipped with a temperature controller ($\pm 0.1^\circ\text{C}$).

Microscopy. TEM images were performed with a JEOL 2010 field electron gun microscope operating at an acceleration voltage of 200 kV. Samples were prepared by spreading a drop of sample on an ultrathin 300 mesh Formvar/carbon-coated copper grid and dried in air. The particle size distribution was determined using ImageJ software. Imaging and microanalysis was performed on a SU-70 Hitachi SEM-FEG equipped with a X-Max Oxford EDX detector.

Electrochemical measurements. The electrochemical experiments were performed in a conventional three-electrode cell system using an Ag/AgCl reference electrode and a platinum wire as counter-electrode. AUTOLAB potentiostat/galvanostat interface with NOVA software for cyclic voltammetry (CV) and electrochemical spectroscopy (EIS). All the potentials are given against the Ag/AgCl (3 M KCl). All measurements were performed at $25 \pm 0.5^\circ\text{C}$. The impedance measurements were performed by applying an AC voltage with 10 mV amplitude in a frequency range from 0.1 Hz to 100 kHz under 0.24 V.

Table S1. Experimental conditions for the synthesis of zwitterionic polymers, PSPP.

τ^a	Time	Conversion ^b	$M_{n \text{ theo}}^c$	$M_{n \text{ SEC}}^d$	\bar{D}
(%)	(min)	(%)	(g mol ⁻¹)	(g mol ⁻¹)	
23.3	40	17.6	15350	17270	1.11

^aSolid content in wt %. ^bMonomer conversion was determined by ¹H NMR in D₂O.

^c $M_{n \text{ theo}} = ([M]_0/[RAFT]_0 \times \text{molar mass of monomer} \times \text{conversion})/100 + M_n \text{ of RAFT agent.}$

^ddetermined by SEC in 0.5 M NaNO₃ aqueous solution at 25°C.

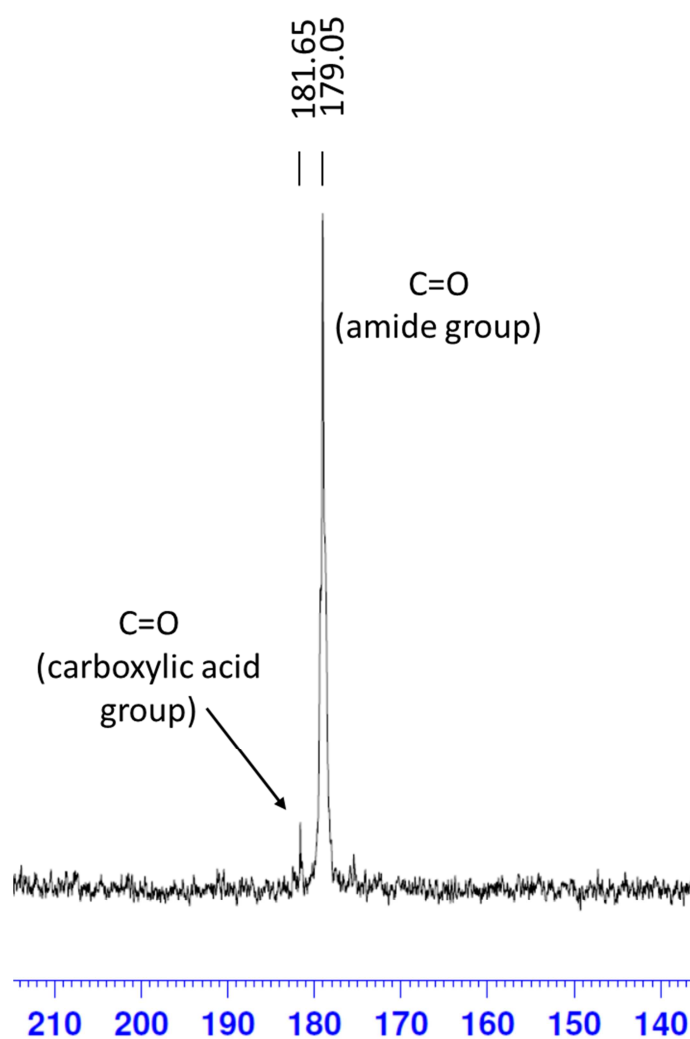


Figure S1. ¹³C NMR spectrum of zwitterionic polymers (PSPP).

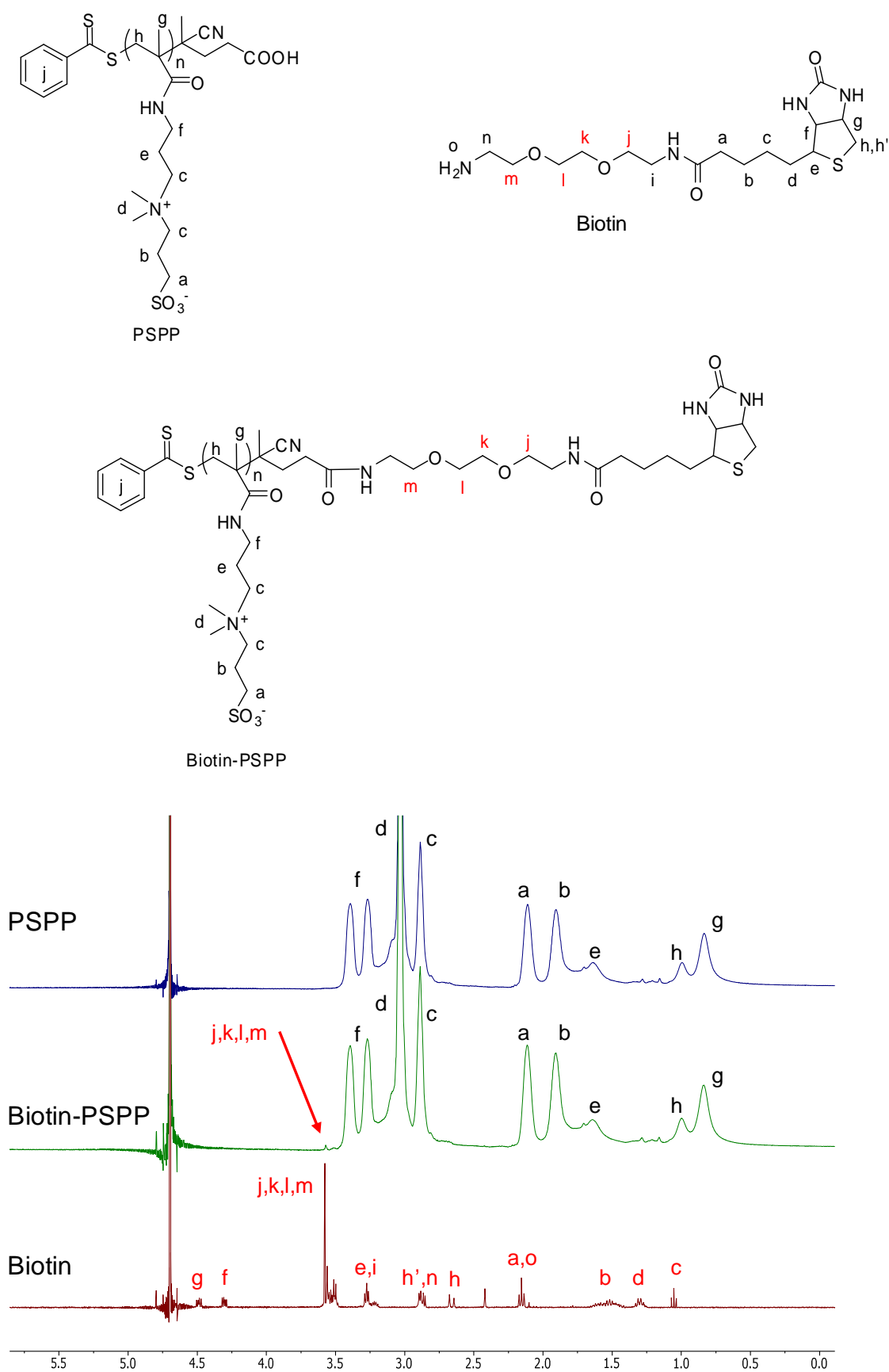


Figure S2. Chemical structure and corresponding ^1H NMR spectra of zwitterionic polymers (PSPP), amino-PEG₂-Biotin (Biotin) and biotinylated zwitterionic polymer (biotin-PSPP).

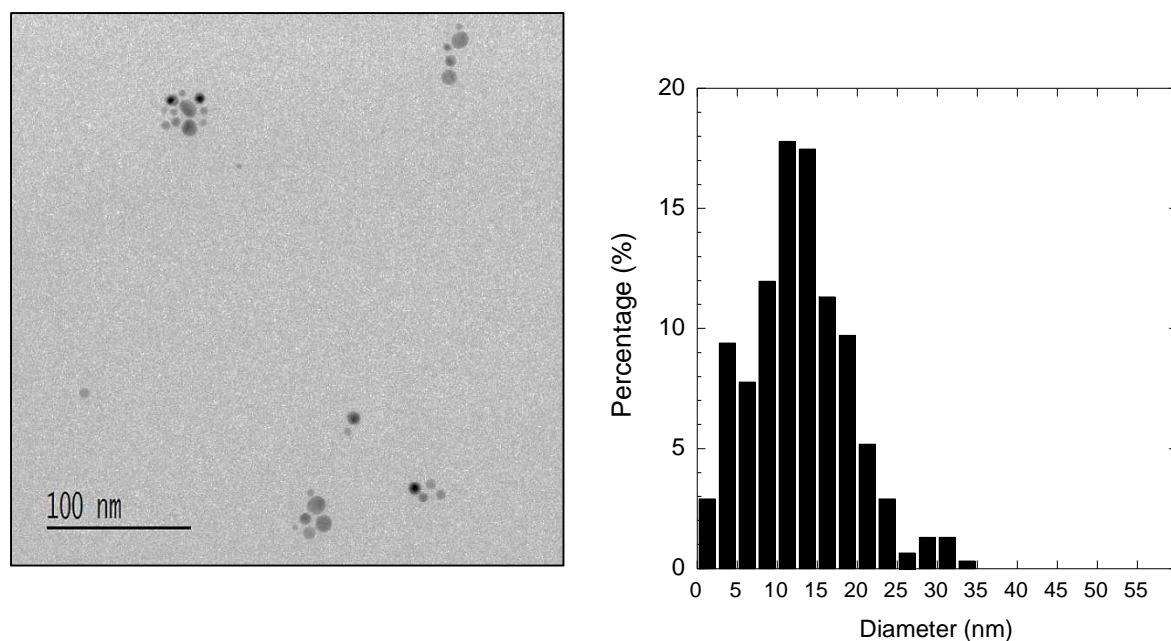


Figure S3. a) TEM image of silver nanoparticles, AgNP. Scale bar is 100 nm. b) Size-distribution histogram calculated from 300 nanoparticles.

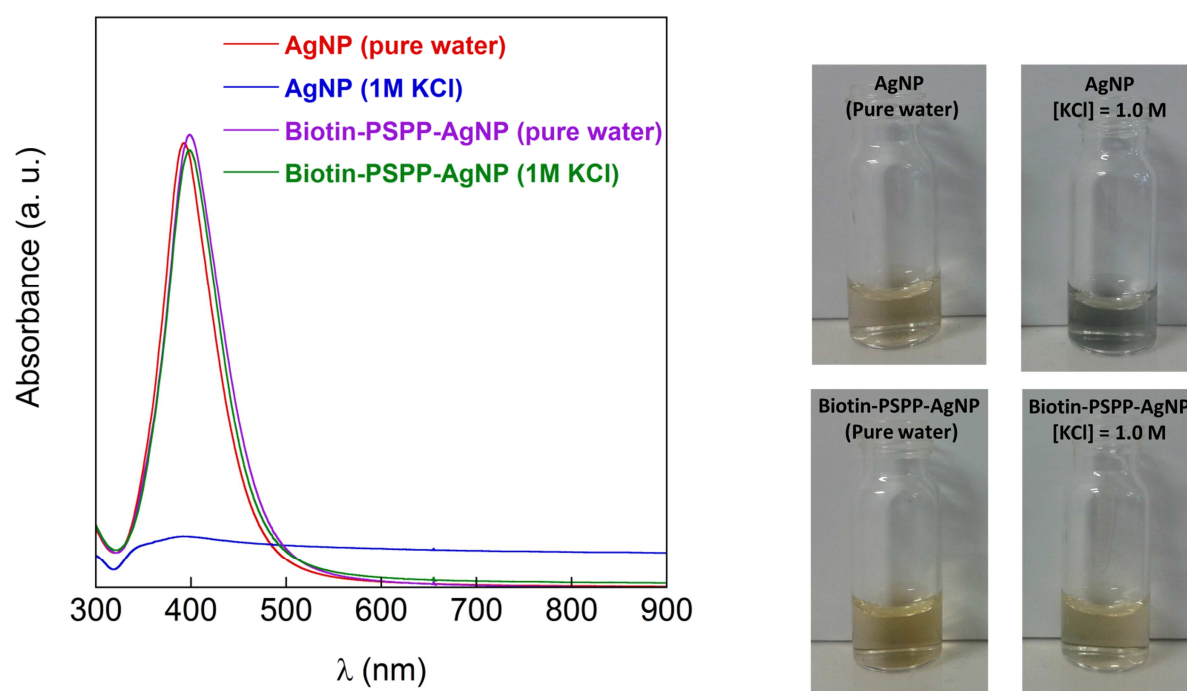


Figure S4. Left: UV-vis absorption spectra of AgNP in pure water, AgNP in 1M KCl solution Biotin-PSPP-AgNP in water and Biotin-PSPP-AgNP in 1M KCl solution. Right: Digital photos of corresponding solutions.

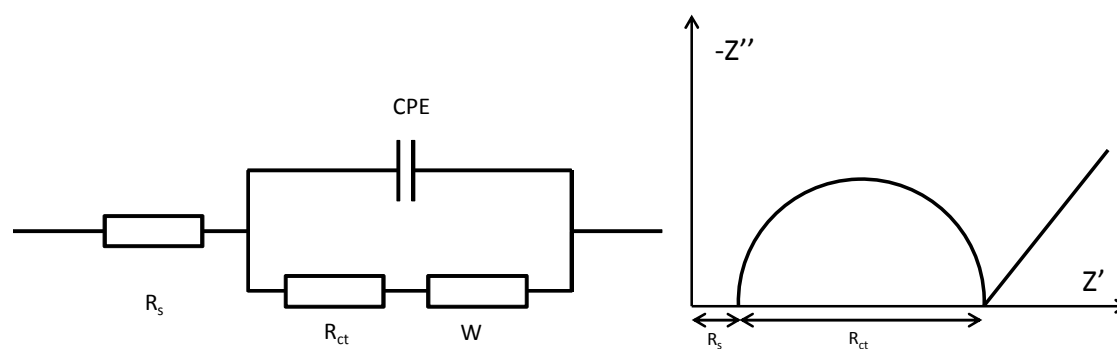


Figure S5. Left: Equivalent electrical circuit diagram of the interface used to fit the impedance spectra. Right: Typical Nyquist plot fitted by the equivalent electrical circuit diagram.

Table S2. Fitted R_{ct} values of gold electrodes after stepwise modification.

Electrode	R_{ct} (k Ω)	+/- (%)
Au	22.8	1.9
Au/Biotin	110	1.3
Au/Biotin/Avidin	500	1.6

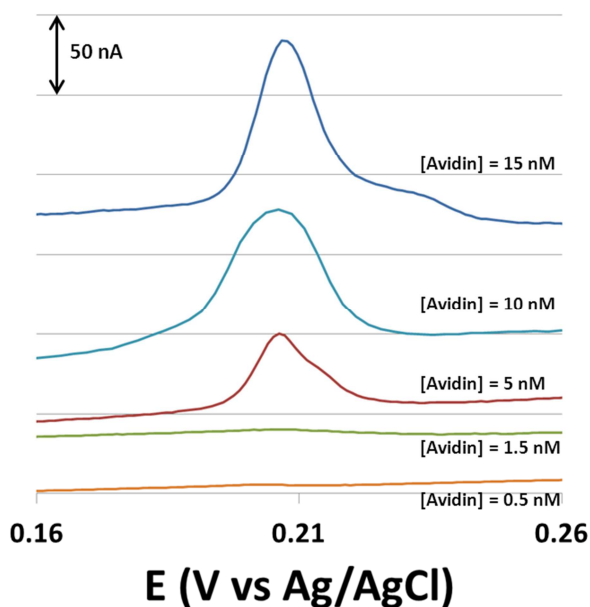


Figure S6. Zoom of the biotin-PSPP-AgNP oxidation peak from cyclic voltammograms for various concentration of avidin. Current are shifted for better reading.

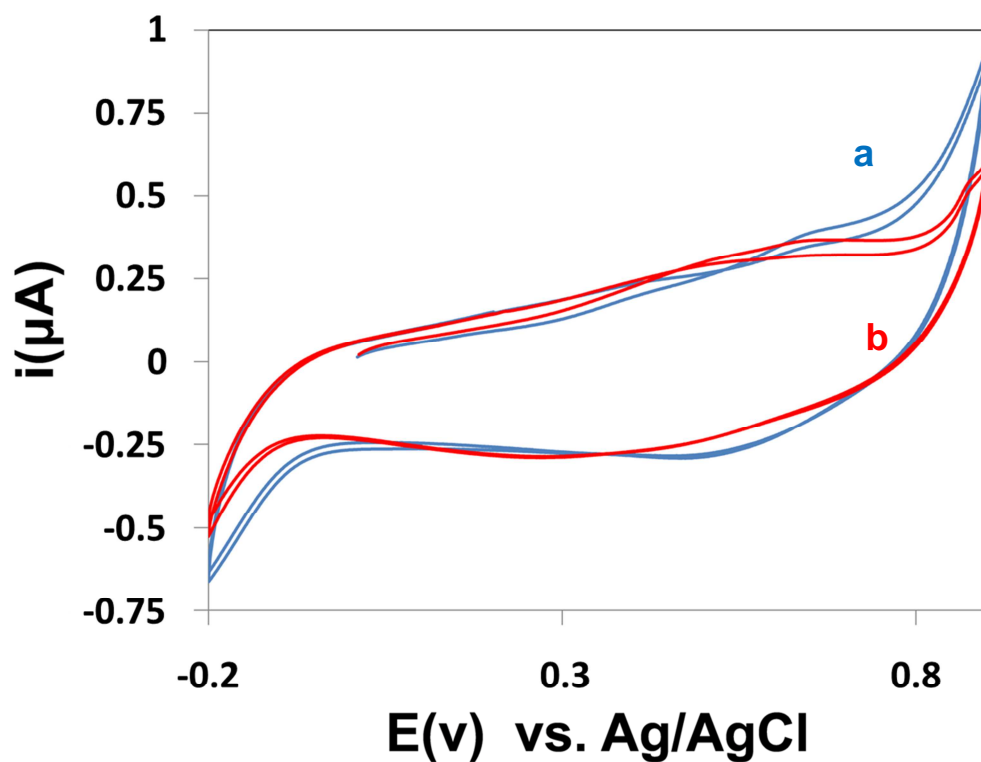


Figure S7. Cyclic voltammograms on (a) Au and (b) Au+Biotin-PSPP-AgNP in 0.1 M PBS pH=7 solution at a scan rate of 50 mV.s⁻¹.

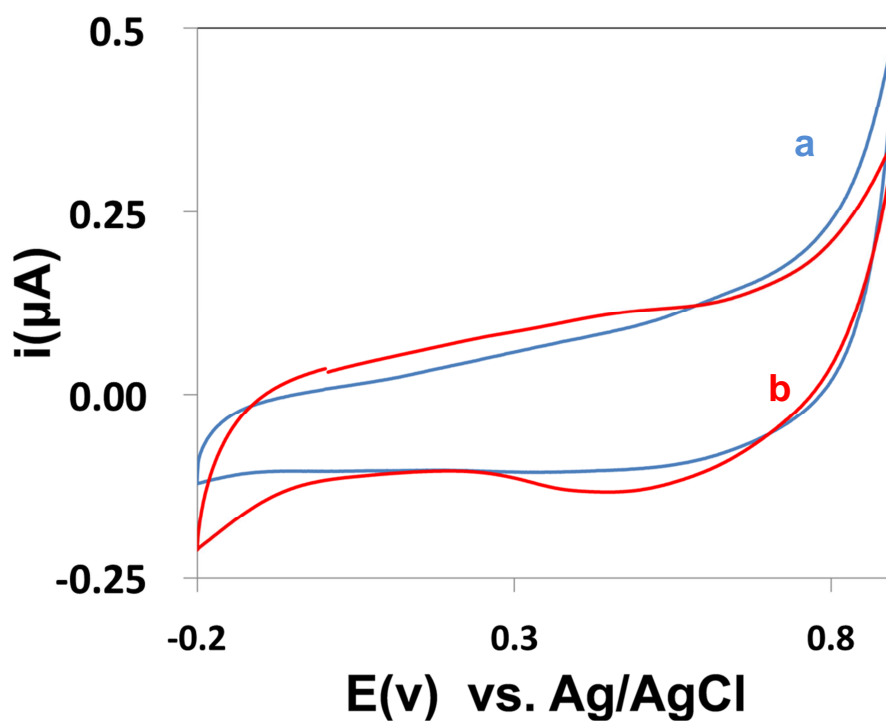


Figure S8. Cyclic voltammograms on Au/Biotin and on Au/Biotin+Biotin-PSPP-AgNP in 0.1 M PBS pH=7 solution at a scan rate of 50 mV.s⁻¹.