Supporting Information

An Innovative Electrochemical Immuno-Platform for Monitoring Chronic Conditions Using the Biosensing of Hyaluronic Acid in Human Plasma Samples

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Table S1. Some important and widely used methods for the detection of HA.

Technique	Advantages	Disadvantage and limitation	Ref.
Radioimmunoassay	Simple, convenient, noninvasive credible, low-cost method, small sample volume	Low specificity, low automation, absence of separation step, short half-life label, high health hazards due to the radioactivity	[1]
Fluorescent-Based Immunoassays	High specificity due to unique optical properties of molecule, measures analyte concentrations in terms of fluorescence and decay times, good reproducibility	Susceptible to interference due to pH changes and oxygen levels, costs are substantially high, skilled personnel, fluorescent labelling	[2]
ELISA	Sensitive, rapid, low-cost method, time-saving, strong affinity	Need for a large sample size, antibody variability, cross reactivity, time consuming, not sensitive enough to detect sample volumes that are too small, false positive	[1]
Colorimetric	Fast, low-cost method, small sample size, ability to customize array for specific analyte, flexible array size, potential to analyze liquid samples	Low reproducibility of imaging and printing, sample application may vary, low selectivity, low stability	[3]

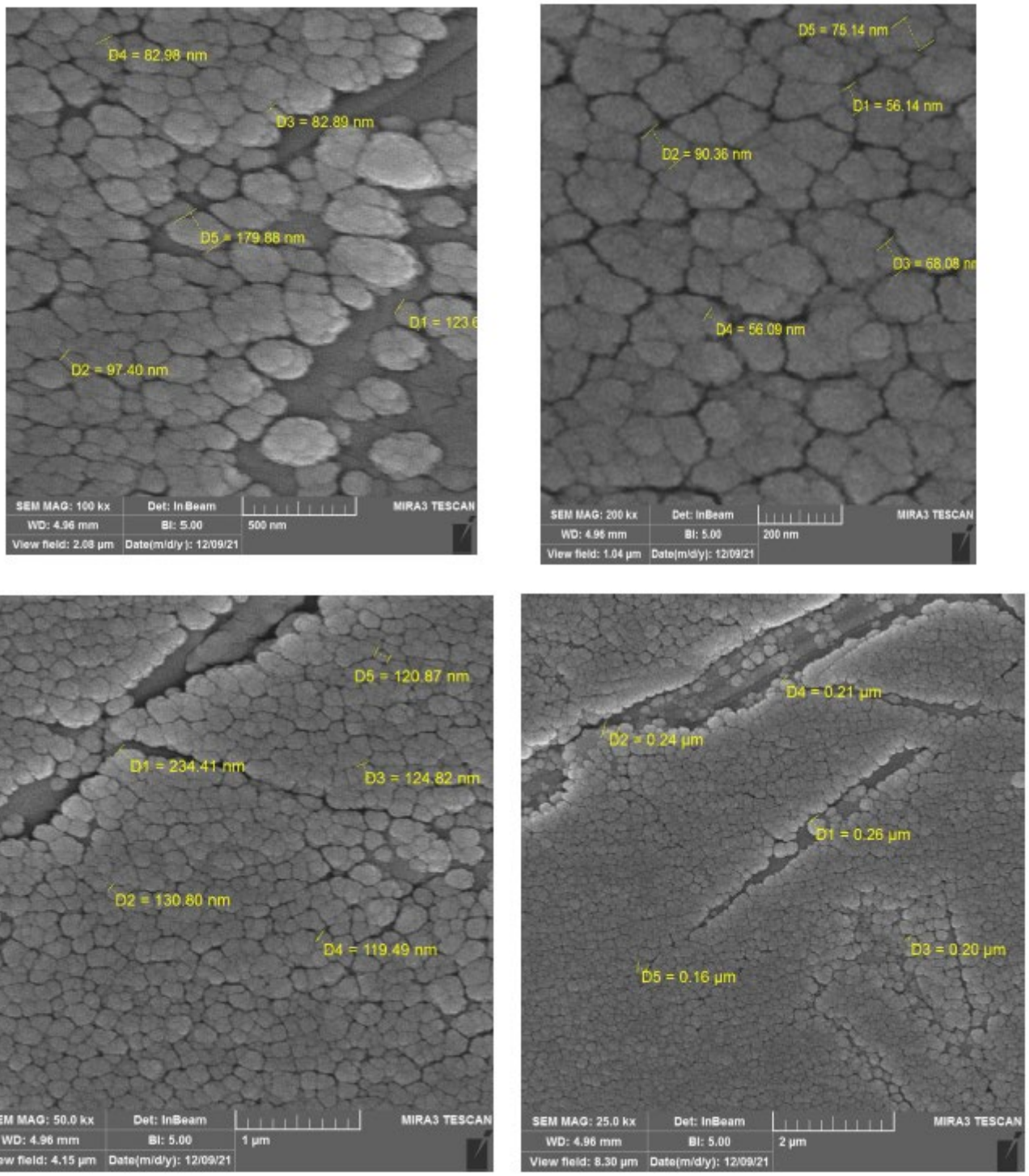

Figure S1. FE-SEM of synthesized nanocomposite (Au@Pt) on the surface of gold electrodes at different magnifications.

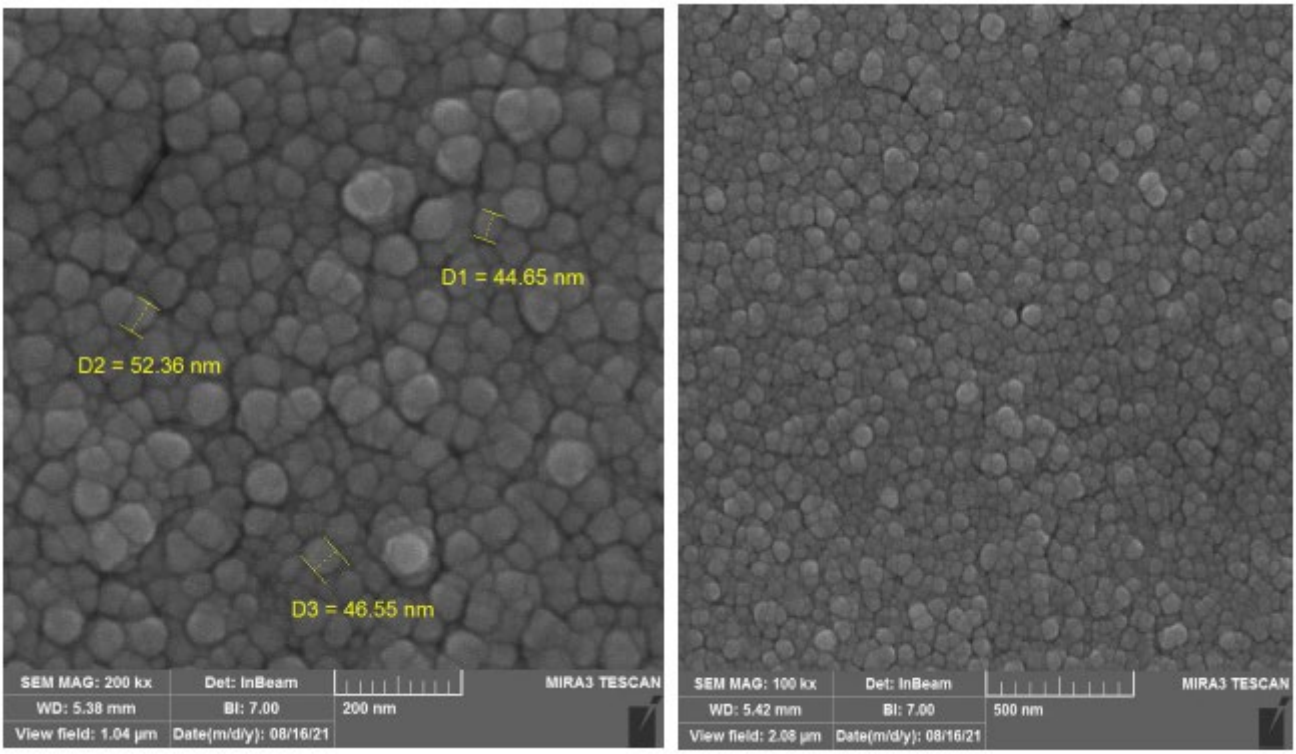

Figure S2. FE-SEM of the HA antibody-BSA at different magnifications.

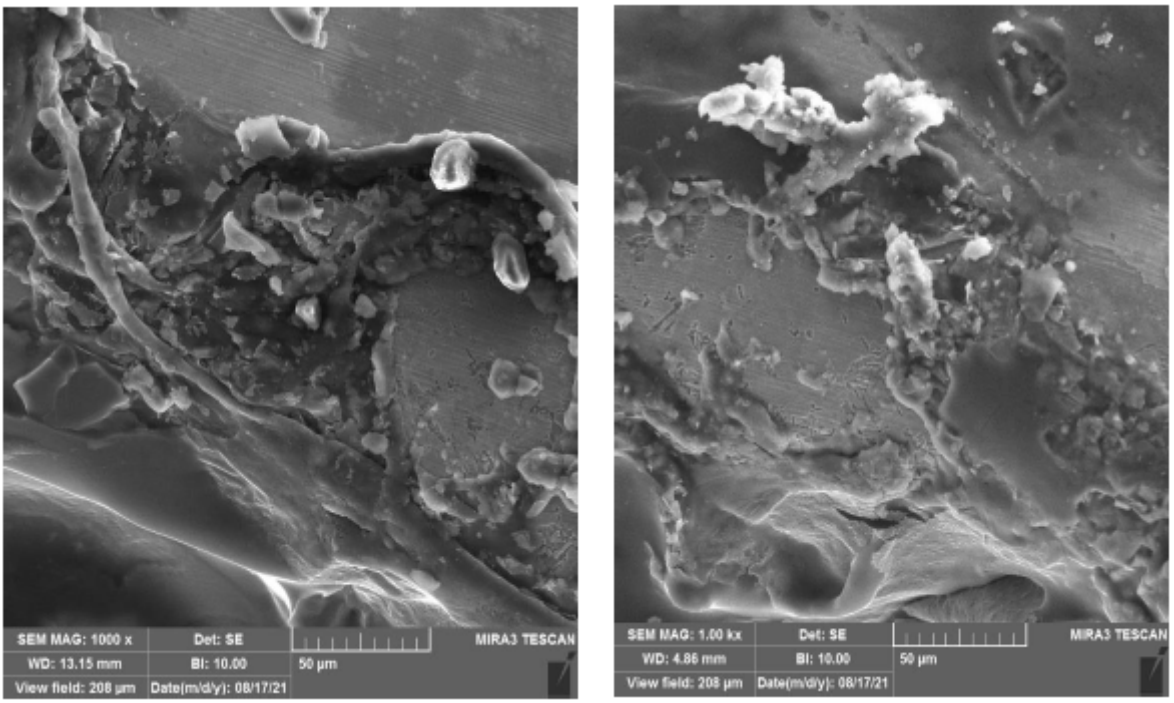

Figure S3. FE-SEM of the HA Antibody-BSA-Ag at different magnifications.

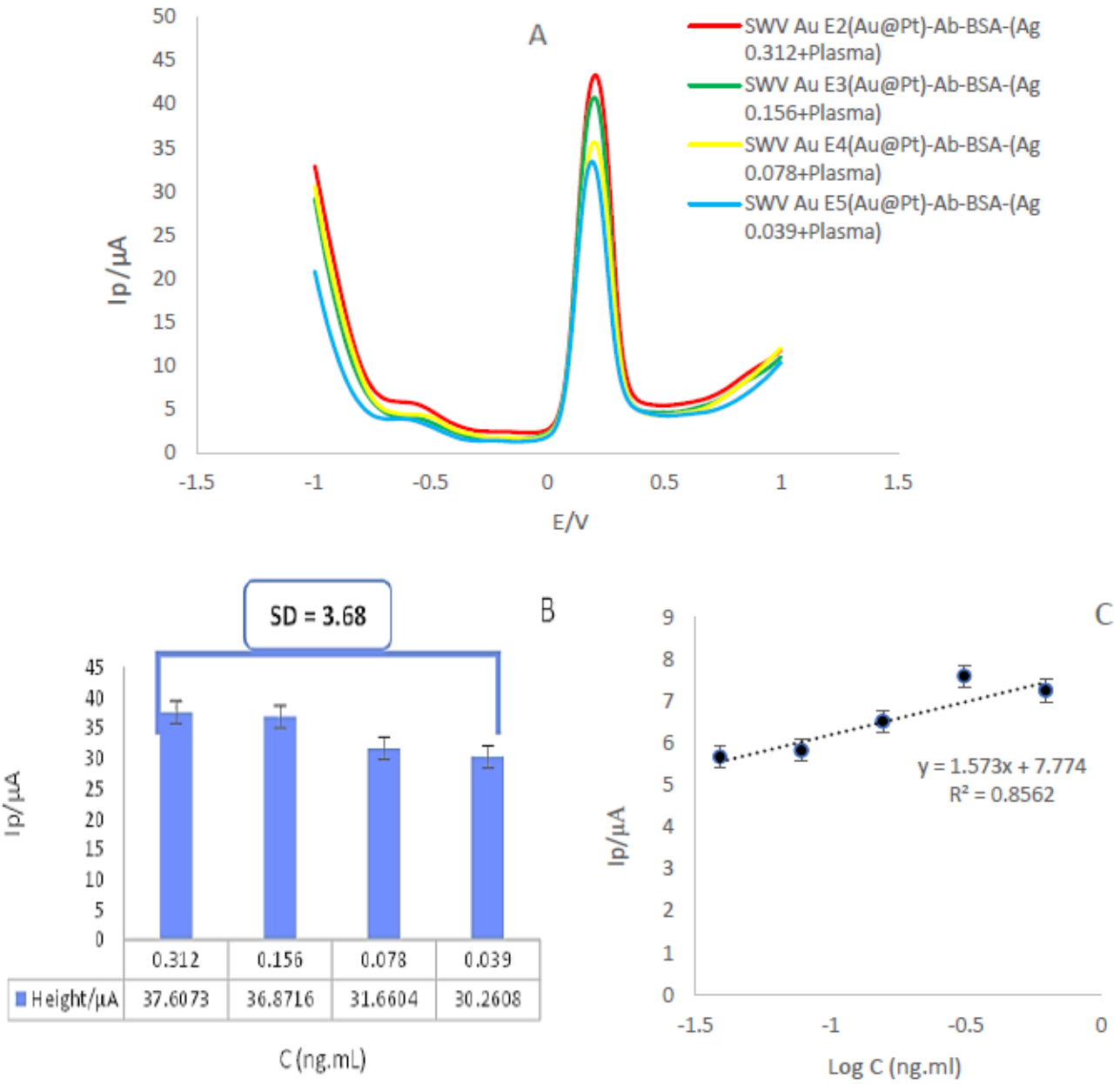

Figure S4. (**A**) SWVs of the engineered immune-platform in the presence of various concentrations (0.312, 0.156, 0.078, and 0.039 ng.mL^{-1}) of HA in human plasma samples. (**B**) Histogram of the peak current of various concentrations of HA. (**C**) Calibration curve (n = 3; SD = 3.68).

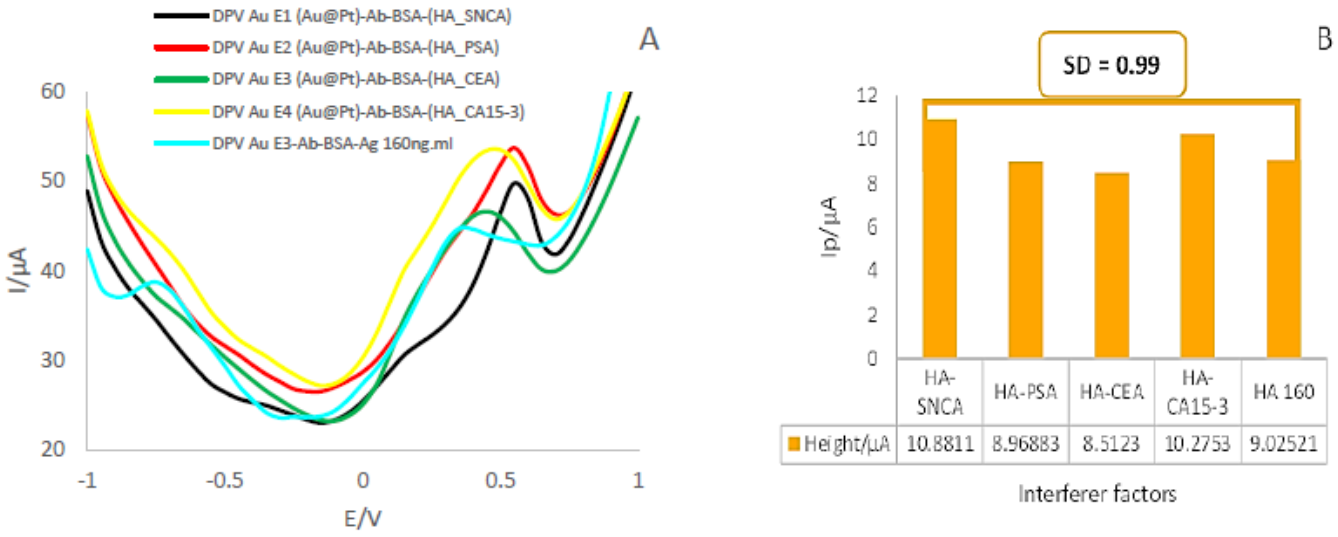

Figure S5. (**A**) DPV of the immunosensor in the presence of various interfering agent (PSA, CEA, CA15-3, and SNCA) techniques for the investigation of selectivity ($T_{equilibration}$: 2 s; E_{begin}: −1.0 V; E_{end}: 1.0 V; Estep: 0.1 V; E_{pulse}: 0.005 V; T_{pulse}: 0.2 s; scan rate: 0.1 V.s). (**B**) Histogram of the peak current of the immunosensor versus interfering species (n = 3; SD = 0.99).

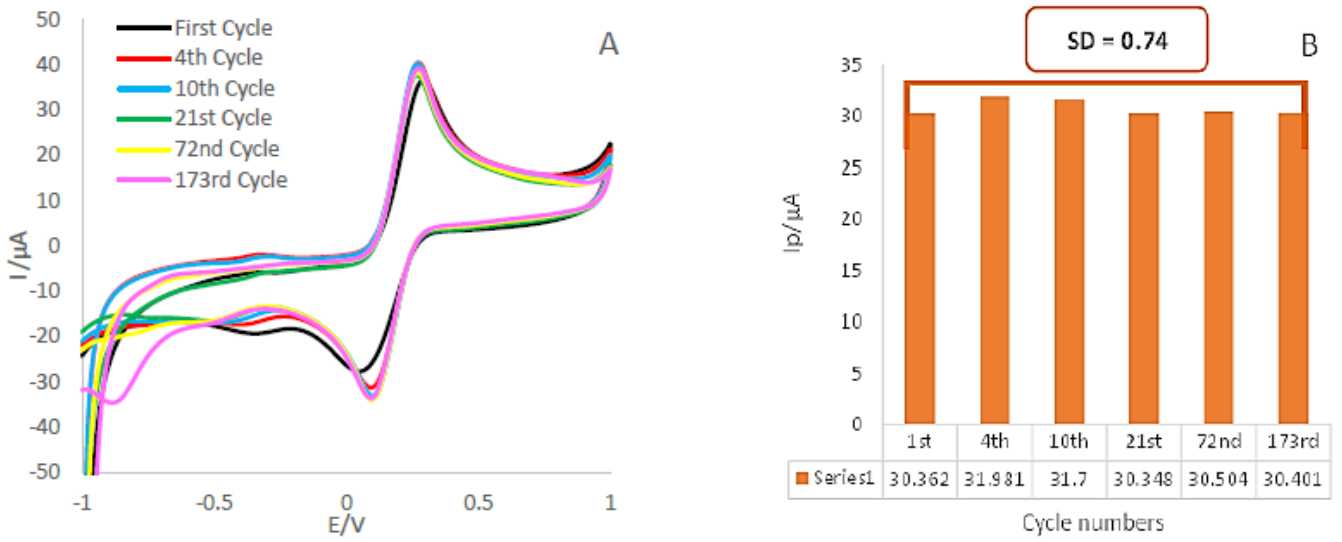

Figure S6. (**A**) CV of the Au-(Pt@Au) nano-alloy technique for the investigation of stability (1–173 cycles). CV technique with data of the following: $E_{quilibration}$: 0 s; E_{begin}: −1.0 V; $E_{vertex1}$: 1.0 V; $E_{vertex2}$: −1.0 V; E_{step}: 0.01 V; scan rate: 0.1 V.s. (**B**) Histogram of the peak current of the Au-(Pt@Au) nano-alloy (n = 3; SD = 0.74).

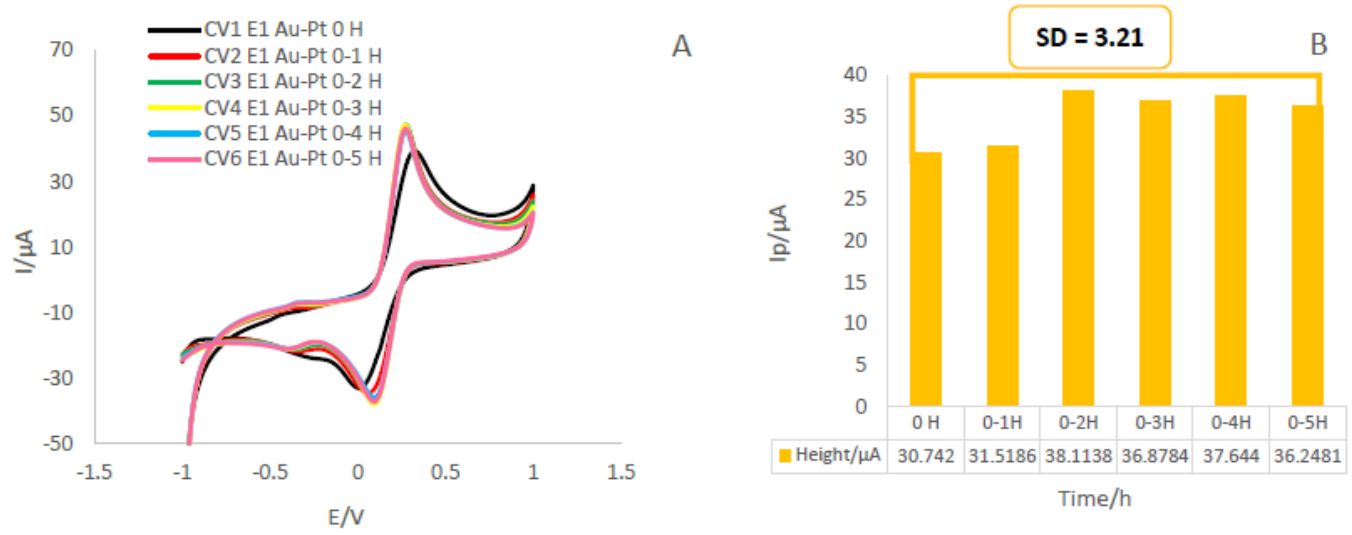

Figure S7. (**A**) CVs of Au-(Pt@Au) nano-alloy when using different storage techniques for the investigation of stability (0–5 hour). CV technique with data of the following: $T_{equilibration}$: 0 s; E_{begin}: −1.0 V; $E_{vertex1}$: 1.0 V; $E_{vertex2}$: −1.0 V; E_{step}: 0.01 V; scan rate: 0.1 V.s. (**B**) Histogram of inter-day stability. Supporting electrolyte is 0.5 mM of $K_4Fe(CN)_6$ /$K_3Fe(CN)6$ (n = 3; SD = 3.21).

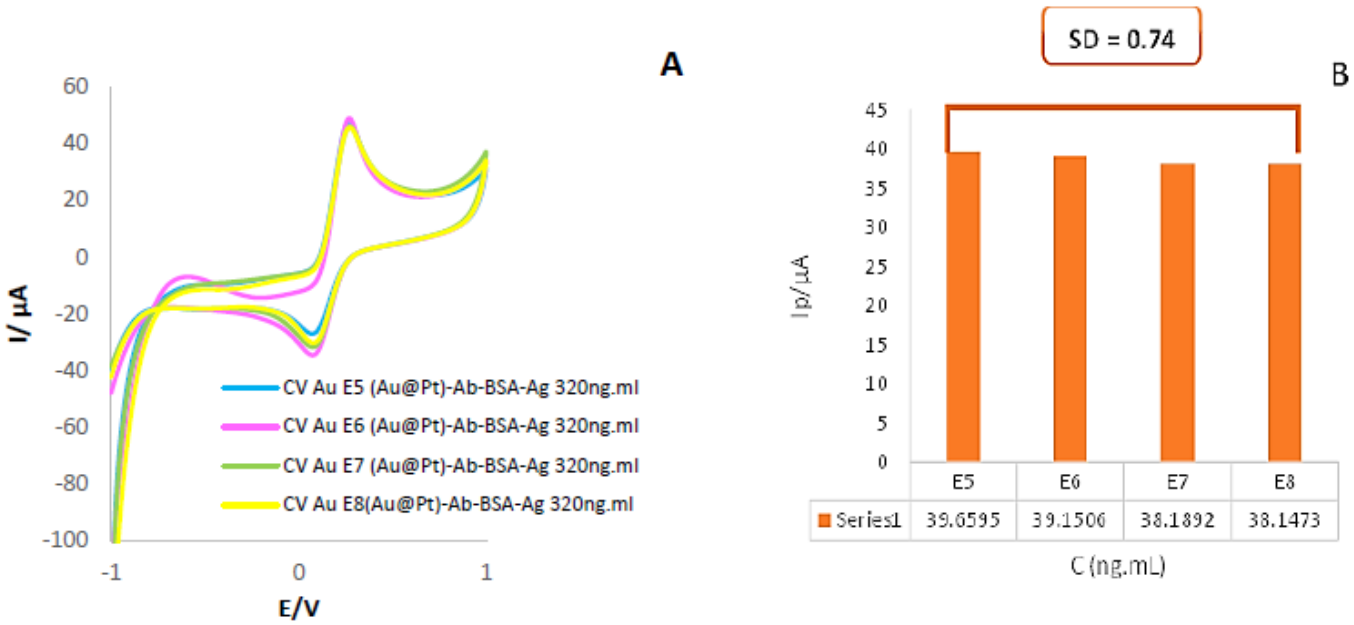

Figure S8. (**A**) CVs of the Au-(Pt@Au) nano-alloy at the same concentration of Ag for the investigation of reproducibility. CV technique with data of the following: $T_{equilibration}$: 0 s; E_{begin}: −1.0 V; $E_{vertex1}$: 1.0 V; $E_{vertex2}$: −1.0 V; E_{step}: 0.01 V; scan rate: 0.1 V.s. (**B**) Histogram of reproducibility. Supporting electrolyte is 0.5 mM of $K_4Fe(CN)_6$ /$K_3Fe(CN)6$ (n = 3; SD = 0.74).

References

1. Hokputsa, S.; Jumel, K.; Alexander, C.; Harding, S.E. A comparison of molecular mass determination of hyaluronic acid using SEC/MALLS and sedimentation equilibrium. *Eur. Biophys. J.* **2003**, *32*, 450–456. https://doi.org/10.1007/s00249-003-0299-6.
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