Supplementary Materials: Unique Properties of Core Shell Ag@Au Nanoparticles for the Aptasensing of Bacterial Cells

Ezat Hamidi-Asl, Freddy Dardenne, Sanaz Pilehvar, Ronny Blust and Karolien De Wael

1. Experimental Section

1.1. Materials

Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), chlorauric acid ($HAuCl_4$), silver nitrate ($AgNO_3$), sodium citrate, hydroxylamine hydrochloride ($NH_2OH.HCl$), sodium borohydride ($NaBH_4$), sodium hydroxide (NaOH), cobalt (II) chloride, poly(vinylpyrrolidinone) (PVP) (M_w ~55000), cetyl trimethylammonium bromide (CTAB), ascorbic acid, glutathione, and cysteamine were obtained from Sigma (Diegem, Flemish Brabant, Belgium). All other reagents were of analytical grade and used without further purification. Ultrapure water obtained from a Millipore water purification system (>8MΩ, MilliQ Millipore) was used in all assays and solutions.

1.2. ssDNA Aptamers

The aptamer cocktail (Apt) was a mixture of three different ssDNA aptamers specific to *E. coli*, reported by Kim et al. [1,2]. A mixture of aptamers was selected in this procedure for several reasons: (1) bacterial cells have many binding moieties on their surfaces for binding to the receptors; (2) aptamers can bind to different components on the cell surface such as proteins, polysaccharides, or flagella; (3) the binding capacity of an aptamer mixture was compared with one of the single aptamers by the research group of Kim et al. and the "mixture" results showed an enhanced sensitivity.

The sequence of each aptamer is as follows: E1 (88-mer), 5′-GCA ATG GTA CGG TAC TTC CAC TTA GGT CGA GGT TAG TTT GTC TTG CTG GCG CAT CCA CTG AGC GCA AAA GTG CAC GCT ACT TTG CTA A-3′; E2 (88-mer), 5′-GCA ATG GTA CG G TAC TTC CCC ATG AGT GTT GTG AAA TGT TGG GAC ACT AGG TGG CAT AGA GCC GCA AAA GTG CAC GCT ACT TTG CTA A-3′ and E10 (90-mer), 5′-GCA ATG GTA CGG TAC TTC CGT TGC A CT GTG CGG CCG AGC TGC CCC CTG GTT TGT GAA TAC CCT GGG CAA AAG TGC ACG CTA CTT TGC TAA-3′. For the selectivity study, a random primer (RP, 90-mer) 5′-NNNNNNNNNNNNNNNNNNNN···N-3′ was used. All aptamers had 5′-SH-$(CH_2)_6$- linkers and were purchased from Eurogentec (Seraing, Liège, Belgium). They were dissolved at a concentration of 100 μM in MilliQ water. For further use, they were diluted in a phosphate buffer solution (PBS) (0.1 M, pH 7.00). Before immobilization of the aptamers at the surface of the modified CPE, a mixture of 5 μM of each aptamer was mixed with 5 mM of TCEP (1:1 *v*/*v*) and kept in the dark at room temperature for 1 h. During this incubation time, disulfide bonds of aptamers were cleaved and then could bind with gold via a covalent bond [3–5]. The TCEP solution should be prepared immediately before use. The final concentration of the aptamer cocktail on the surface of the working electrode is 2.5 μM.

1.3. Bacterial Strains and Culture Conditions

Escherichia coli (KCTC 2571) was used as the target strain and *Staphylococcus aureus* (ATCC 29213) and *Pseudomonas aeruginosa* (ATCC 27853), were used for specificity testing. All of them were grown in a nutrient broth medium. *Escherichia coli* were cultured at 28 °C, while *Staphylococcus aureus* and *Pseudomonas aeruginosa* were cultured at 37 °C. All bacterial strains were obtained from the LM-UGent Laboratory of Microbiology (Ghent, Belgium). The stock solution of bacteria (10^9 CFU/mL) was prepared in PBS 0.1 M pH 7.00 and the other dilution was done in the same buffer.

1.4. Apparatus

Electrochemical measurements were recorded by an Autolab potentiostat controlled by the NOVA 1.10 software package (Metrohm, Utrecht, the Netherlands). The three-electrode system was made up of a CPE as the working electrode, an Ag|AgCl|KCl (3 M) as the reference electrode, and a platinum wire as the auxiliary electrode. A computing double beam UV-Vis spectrophotometer (Cary 100, Varian Inc. (Palo Alto, CA, USA)) was used to measure the absorbance. Morphological investigation of the electrode surface and energy dispersive X-ray analysis was done on a JEOL 6300 Scanning Electron Microscope (Tokyo, Japan).

1.5. Synthesis of Nanoparticles

1.5.1. Gold Nanoparticles (Au)

Gold nanoparticles were prepared according to the chemical reduction procedure described in the literature [6–8]. In this method, 100 mL of 0.01% $HAuCl_4$ were stirred and heated to boiling. Then, 2.5 mL of 1% citrate sodium were added to the solution and it was kept at a boil for 10 min until a deep red color change was observed. The solution was left at room temperature in order to cool down gradually. The nanoparticles solution was kept in a refrigerator and was stable for more than five months.

1.5.2. Silver Nanoparticles (Ag)

The preparation of Ag NP is as follows [9]. All glassware had to be cleaned by soaking in alcoholic KOH before starting the experiments. A 10 mL volume of 1.0 mM silver nitrate was added dropwise (1 drop/s) to 30 mL of 2.0 mM sodium borohydride solution that had been chilled in an ice bath. The reaction mixture was stirred vigorously. The solution turned yellow after the addition of all of the silver nitrate. Then the stirring could be stopped immediately. If stirring continued, aggregation would begin and the yellow solution turned to black. If the drop addition was more than one drop per second, the size of particles would not be in nanometers. The solution of sodium borohydride should be fresh and used immediately after preparation. The very freshly sodium borohydride solution was the key step in this synthesis. The solution of NP could be stable for one month in the refrigerator (4 °C) in transparent glass.

1.5.3. Hollow Gold Nanosphere (HGN)

HGN was synthesized as follows [10,11]: 500 μL of 0.1 M sodium citrate and 100 μL of 0.4 M $CoCl_2$ were added to 100 mL of Milli-Q water and degassed with nitrogen for 1 h in a well-sealed, three-neck flask. Then, 300 μL of a freshly prepared 1 M sodium borohydride solution were added quickly. The solution turned from colorless to brown. The solution was stirred under nitrogen for an additional 45 min to allow the sodium borohydride to completely react. While maintaining nitrogen flow, 50 μL of 0.1 M $HAuCl_4$ solution were added 10 times to reach a final volume of 500 μL. The solution changed from brown to red-purple and, finally, to a deep blue color. The product could be stable for one week in the refrigerator.

1.5.4. Hollow Silver Nanospheres (HSN)

The hollow silver nanospheres were synthesized using the following procedure [12]. An aqueous solution of silver nitrate (1.5 mL, 10 mM) and glutathione (180 μL, 10 mM) was added to 26 mL of ice cold Milli-Q water. The pH of the solution was raised to ~12 by adding an aqueous solution of sodium hydroxide (5.00 mL, 0.1M), while vigorously stirring. After the solution turned pale yellowish, a freshly prepared $NaBH_4$ solution was introduced at once (1.80 mL, 10 mM). The final color should be dark orange and was stable for one week in the refrigerator. If sodium borohydride, the reducing agent, was added at once, the product would be a hollow sphere. If the reductant was introduced gradually to the solution, a silver nanoparticle would result.

1.5.5. Silver–Gold Alloy Binary Nanoparticles (Ag/Au)

The alloy nanoparticles were synthesized via reduction of both of $HAuCl_4$ and $AgNO_3$ at the same time [13,14]. Five hundred microliters of 0.01 M sodium citrate were added to 100 mL of Milli-Q water. Then, 250 μL each of 0.01 M $HAuCl_4$ and $AgNO_3$ were introduced to the solution simultaneously with vigorous stirring. Next, 500 μL of freshly prepared 0.01 M $NaBH_4$ were added all at once. The solution was allowed to stir for an additional 1 min. The final solution color was yellowish red or pale pink and was stable for one month in the refrigerator.

1.5.6. Silver–Gold Core Shell Binary Nanoparticles (Ag@Au)

Because of a spontaneous galvanic replacement reaction between silver nanoparticles and elemental gold as precursor, the synthesis of silver–gold core shell nanoparticles was relatively easy in comparison with other binary nanostructures [15]. In fact, Au shells would be generated on Ag cores. Therefore, the silver nanoparticles had to be prepared first. Next, the binary nanoparticles, and the silver–gold core shell, would be synthesized.

To 10 mL of Ag NP diluted with 30 mL of Milli-Q water, 25 mL of 6.52×10^{-3} M $NH_2OH.HCl$ and 25 mL 4.65×10^{-4} M $HAuCl_4$ were added dropwise (1 drop/s) with two separate pipettes upon vigorous stirring [16,17]. The addition of these two solutions to the Ag NP had to be started simultaneously and finished exactly at the same time. After the addition of two solutions to Ag NP, the stirring should be continued for 45 min. These points were very critical steps in the synthesis of the core shell. Otherwise, agglomeration would start immediately. During the synthesis, the color of the solution changed subsequently from yellow to brown, blue, violet, red, purple, and finally dark reddish pink. The solution was stable for 2–3 weeks in room temperature, kept in a dark place. When the dark reddish pink color of the solution turned to pale purple, the aggregation had started.

1.5.7. Gold–Silver Core Shell Binary Nanoparticles (Au@Ag)

For the synthesis of Au@Ag, the developed protocol is followed [18] with small changes. Forty milliliters of PVP solution (1 wt %) were mixed with 4.0 mL of CTAB solution (0.2 M). The mixture was heated to 60 °C, followed by the sequential addition of 1.0 mL of synthesized Au NP, 1 mL of $AgNO_3$ (4 mM), 2.0 mL of ascorbic acid (0.1 M), and 4.0 mL of NaOH (0.1 M). The solution turned yellow within 1 min and was stirred for 20 min. The product should be kept at room temperature in a dark place and is stable for two weeks.

1.6. Procedure for Preparation of the Aptasensor

The schematic diagram for the whole procedure is shown in Figure 1 and explained step by step as below. The conditions were optimized for the whole procedure and the best one was reported.

1.6.1. Preparation of CPE

The carbon paste electrode used as the working electrode was fabricated in the usual way with graphite powder and paraffin oil in a ratio of 70:30 (*w*/*w*) by hand mixing in a mortar. A portion of the resulting paste was placed firmly into a tube (ϕ: 2 mm) with a geometric surface area of 0.03 cm^2. A copper wire was used for the creation of an electrical connection [19].

1.6.2. Modification of CPE

The surface of the CPE was polished on a weighing paper and washed with distilled water. Then it was pretreated by cyclic voltammetry in PBS 0.1 M pH 7.0 from 0.0 V to 1.0 V (10 cycles) at a scan rate of 0.5 Vs^{-1}. After obtaining a stable cyclic voltammogram, it was rinsed with Milli-Q water; the cyclic scanning was repeated (n = 10) in PBS 0.1 M pH 7.0 containing cysteamine 10^{-3}M from −1.5 V to +2.4 V at 0.1 Vs^{-1}. In this step, the surface of CPE was completely covered by the cysteamine film (Cys-CPE) [20,21]. Then it was rinsed and immersed in a nanoparticle solution overnight to capture

nanoparticles [22]. The outcome of this step was a modified surface: NP-Cys-CPE. For demonstration of surface coverage of CPE by cysteamine, chronoamperometry was designed (Figure S5) [23–25].

1.6.3. Immobilization of Aptamer at Modified CPE

After washing the modified CPE with water, it was immersed overnight in the aptamer cocktail solution. Because of the thiol linker of aptamers, they could form a self-assembly monolayer at the surface of the working electrode. Therefore, Apt-NP-Cys-CPE was made and then it was rinsed and immersed in bacteria solution.

1.7. Electrochemical Measurements

After immersing the aptasensor in bacteria solution for 30 min, it was washed gently in water and electrochemical measurement was done. The cyclic voltammetric studies were performed by scanning the working electrode potential between +0.3 and 1.1 V vs. Ag|AgCl|KCl (3 M) in PBS 0.1 M pH 7.00 at a scan rate of 100 $mV \cdot s^{-1}$. Differential pulse voltammetry measurements were carried out in the same buffer solution by scanning the working electrode potential between 0.5 and 1.2 V vs. Ag|AgCl|KCl (3 M) at pulse amplitude of 50 mV. The electrochemical impedance spectroscopy was done in 0.1 M PBS pH 7.00 containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M NaCl.

References

1. Kim, Y.S.; Chung, J.; Song, M.Y.; Jurng, J.; Kim, B.C. Aptamer cocktails: Enhancement of sensing signals compared to single use of aptamers for detection of bacteria. *Biosens. Bioelectron.* **2014**, *54*, 195–198.
2. Kim, Y.S.; Song, M.Y.; Jurng, J.; Kim, B.C. Isolation and characterization of DNA aptamers against *Escherichia coli* using a bacterial cell-systematic evolution of ligands by exponential enrichment approach. *Anal. Biochem.* **2013**, *436*, 22–28.
3. Xiao, Y.; Lai, R.Y.; Plaxco, K.W. Preparation of electrode-immobilized, redox-modified oligonucleotides for electrochemical DNA and aptamer-based sensing. *Nat. Protoc.* **2007**, *2*, 2875–2880.
4. Zhou, L.; Wang, J.; Li, D.; Li, Y. An electrochemical aptasensor based on gold nanoparticles dotted graphene modified glassy carbon electrode for label-free detection of bisphenol a in milk samples. *Food Chem.* **2014**, *162*, 34–40.
5. Kashefi-Kheyrabadi, L.; Mehrgardi, M.A. Aptamer-conjugated silver nanoparticles for electrochemical detection of adenosine triphosphate. *Biosens. Bioelectron.* **2012**, *37*, 94–98.
6. Raoof, J.B.; Ojani, R.; Ebrahimi, M.; Hamidi-Asl, E. Developing a nano-biosensor for DNA hybridization using a new electroactive label. *Chin. J. Chem.* **2011**, *29*, 2541–2551.
7. Mousavi-Sani, S.Z.; Raoof, J.B.; Ojani, R.; Hamidi-Asl, E. Nano-gold modified genosensor for direct detection of DNA hybridization. *J. Chin. Chem. Soc.* **2013**, *60*, 650–656.
8. Asghary, M.; Raoof, J.-B.; Hamidi-Asl, E.; Ojani, R. A novel electrochemical genosensor based on banana and nano-gold modified electrode using tyrosinase enzyme as indicator. *J. Nanosci. Nanotechnol.* **2015**, *15*, 3394–3404.
9. Mulfinger, L.; Solomon, S.D.; Bahadory, M.; Jeyarajasingam, A.V.; Rutkowsky, S.A.; Boritz, C. Synthesis and study of silver nanoparticles. *J. Chem. Educ.* **2007**, *84*, 322.
10. Schwartzberg, A.M.; Oshiro, T.Y.; Zhang, J.Z.; Huser, T.; Talley, C.E. Improving nanoprobes using surface-enhanced raman scattering from 30-nm hollow gold particles. *Anal. Chem.* **2006**, *78*, 4732–4736.
11. Schwartzberg, A.M.; Olson, T.Y.; Talley, C.E.; Zhang, J.Z. Synthesis, characterization, and tunable optical properties of hollow gold nanospheres. *J. Phys. Chem. B* **2006**, *110*, 19935–19944.
12. Ben Moshe, A.; Markovich, G. Synthesis of single crystal hollow silver nanoparticles in a fast reaction-diffusion process. *Chem. Mater.* **2011**, *23*, 1239–1245.
13. Mallin, M.P.; Murphy, C.J. Solution-phase synthesis of sub-10 nm Au–Ag alloy nanoparticles. *Nano Lett.* **2002**, *2*, 1235–1237.
14. Link, S.; Wang, Z.L.; El-Sayed, M.A. Alloy formation of gold–silver nanoparticles and the dependence of the plasmon absorption on their composition. *J. Phys. Chem. B* **1999**, *103*, 3529–3533.
15. Ma, Y.; Li, W.; Cho, E.C.; Li, Z.; Yu, T.; Zeng, J.; Xie, Z.; Xia, Y. Au@ag core–shell nanocubes with finely tuned and well-controlled sizes, shell thicknesses, and optical properties. *ACS Nano* **2010**, *4*, 6725–6734.

16. Srnová-Šloufová, I.; Lednický, F.; Gemperle, A.; Gemperlová, J. Core–shell (Ag)Au bimetallic nanoparticles: Analysis of transmission electron microscopy images. *Langmuir* **2000**, *16*, 9928–9935.
17. Xin, J.; Zhang, F.; Gao, Y.; Feng, Y.; Chen, S.; Wu, A. A rapid colorimetric detection method of trace cr (vi) based on the redox etching of agcore–aushell nanoparticles at room temperature. *Talanta* **2012**, *101*, 122–127.
18. Cho, E.C.; Camargo, P.H.C.; Xia, Y. Synthesis and characterization of noble-metal nanostructures containing gold nanorods in the center. *Adv. Mater.* **2010**, *22*, 744–748.
19. Hamidi-Asl, E.; Raoof, J.; Meibodi, A.; Bazgir, Z. Celestine blue as a new indicator in electrochemical DNA biosensors. *Sci. China Chem.* **2015**, *59*, 1–7.
20. Hu, G.-Z.; Zhang, D.-P.; Wu, W.-L.; Yang, Z.-S. Selective determination of dopamine in the presence of high concentration of ascorbic acid using nano-Au self-assembly glassy carbon electrode. *Coll. Surf. B Biointerfaces* **2008**, *62*, 199–205.
21. Petkova, G.; Zaruba, K.; Zvatora, P.; Kral, V. Gold and silver nanoparticles for biomolecule immobilization and enzymatic catalysis. *Nanoscale Res. Lett.* **2012**, *7*, 287.
22. Raoof, J.B.; Kiani, A.; Ojani, R.; Valiollahi, R.; Rashid-Nadimi, S. Simultaneous voltammetric determination of ascorbic acid and dopamine at the surface of electrodes modified with self-assembled gold nanoparticle films. *J. Solid State Electrochem.* **2010**, *14*, 1171–1176.
23. Zhang, D.; Zhao, H.; Fan, Z.J.; Li, M.; Du, P.H.; Liu, C.M.; Li, Y.P.; Li, H.T.; Cao, H.B. A Highly Sensitive and Selective Hydrogen Peroxide Biosensor Based on Gold Nanoparticles and Three-Dimensional Porous Carbonized Chicken Eggshell Membrane. *PLoS ONE* **2015**, *10*, 1–14.
24. Majdi, S., Jabbari, A.; Heli, H. A study of the electrocatalytic oxidation of aspirin on a nickel hydroxide-modified nickel electrode. *J. Solid State Electrochem*. **2007**, *11*, 601–607.
25. Eckermann, A.L.; Feld, D.J.; Shaw, J.A.; Meade, T.J. Electrochemistry of redox-active self-assembled monolayers. *Coord. Chem. Rev*. **2010**, *254*, 1769–1802.

Figure S1. Photograph of different synthesized nanoparticles.

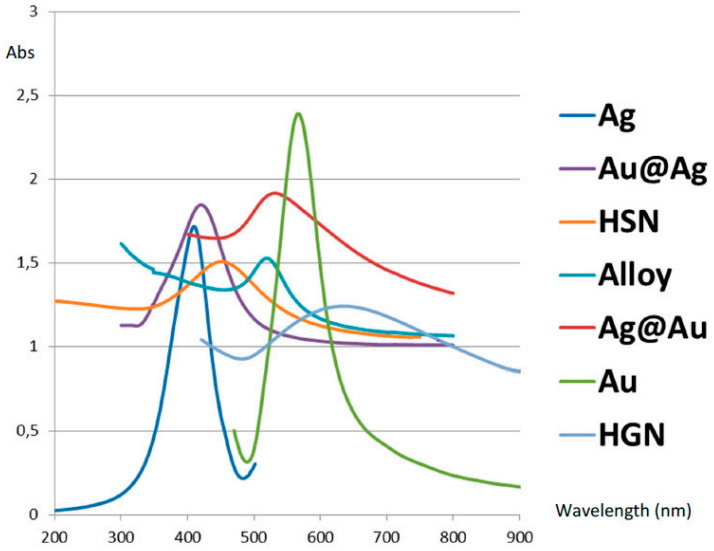
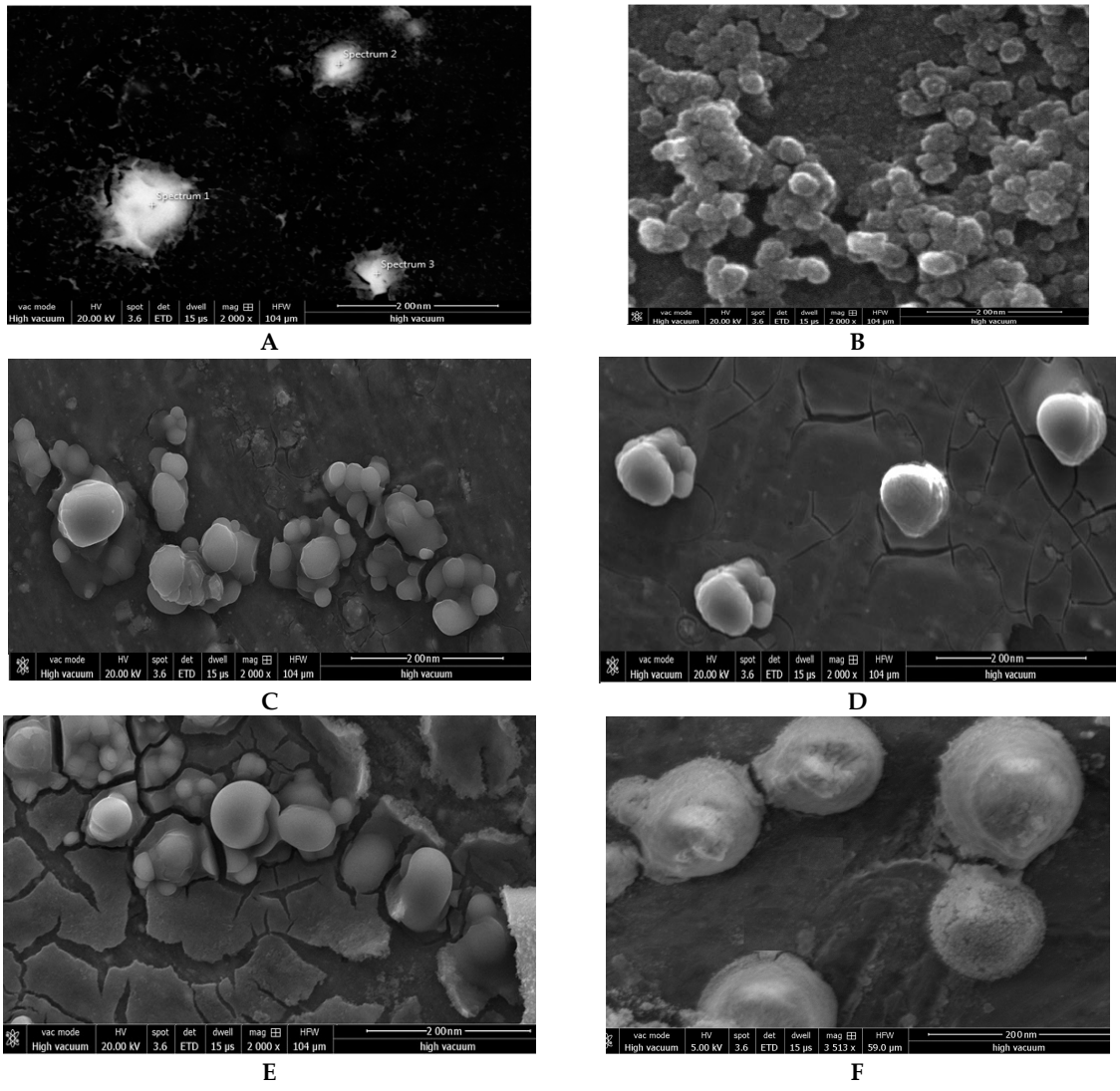
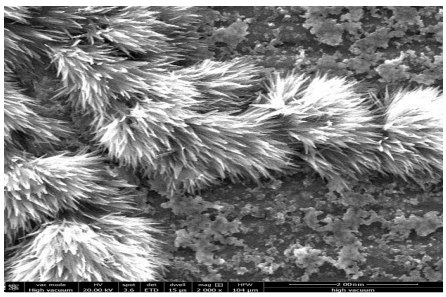


Figure S2. UV-Vis absorption spectroscopy for different synthesized nanoparticles.





G

Figure S3. SEM image for different synthetized nanoparticles. **A**. Gold nanoparticles (Au); **B**. Silver nanoparticles (Ag); **C**. Silver-gold alloy (Ag/Au); **D**. Silver-gold core shell (Au@Ag); **E**. Gold-silver core shell (Au@Ag); **F**. Hollow gold nanospheres (HGN); **G**. Hollow silver nanospheres (HSN).

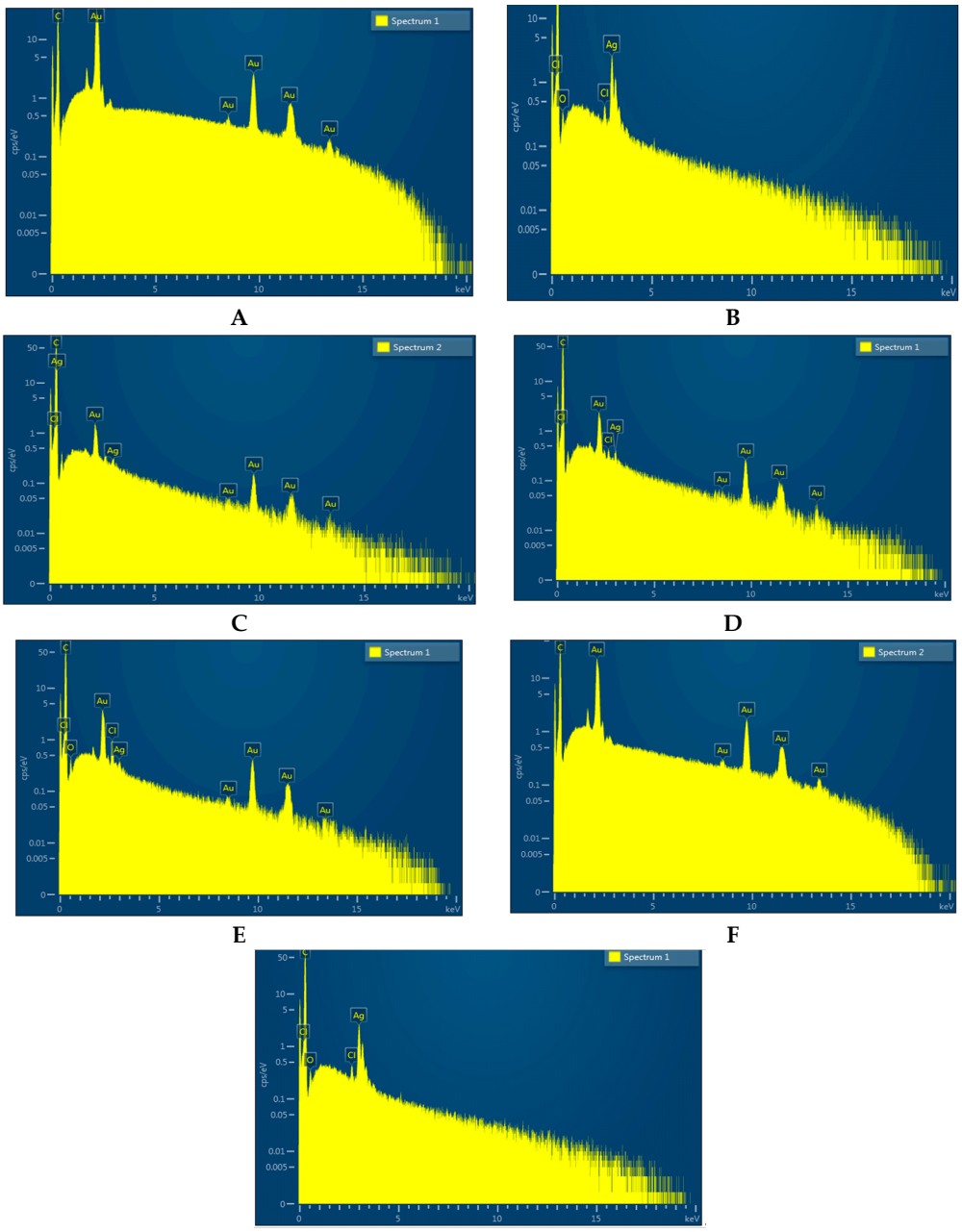


Figure S4. EDX analysis for different synthesized nanoparticles. **A**. Gold nanoparticles (Au); **B**. Silver nanoparticles (Ag); **C**. Silver-gold alloy (Ag/Au); **D**. Silver-gold core shell (Ag@Au); **E**. Gold-silver core shell (Au@Ag); **F**. Hollow gold nanospheres (HGN); **G**. Hollow silver nanospheres (HSN).

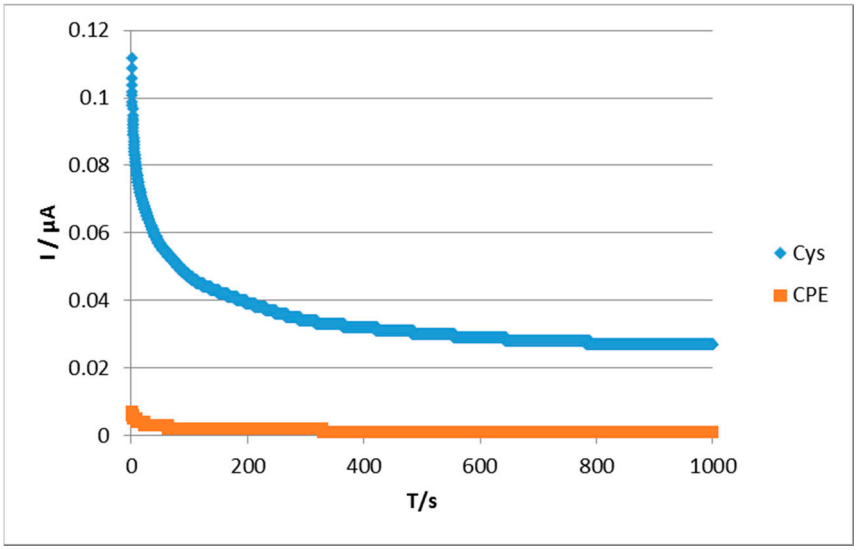


Figure. S5. Amperometric responses of bare CPE and cysteamine modified CPE. The applied potential was +1.0 V in 0.1 M PBS solution (pH = 7.0).