Electronic Supplementary Material (ESI) for Analyst.

Development and characterisation of cysteine-based gold electrodes for the electrochemical biosensing of the SARS-CoV-2 spike antigen

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1. Introduction

Table S1. Electrochemical methods for determination of the SARS-CoV-2 antigen and/or antibody or its fragments.

Biosensor	Method	LOD	Analyte	Biosensor preparation time (h)	Measurement time (min)	References
Cotton-tipped, bovine serum albumin, SARS-CoV-2 nucleocapsid protein, diazonium salt and carbon nanofiber modified screen-printed electrode	Square wave voltammetry	0.8 pg/mL	Nucleocapsid protein	25	30	31
Bovine serum albumin, SARS-CoV-2 spike antigen, glutaraldehyde, cysteamine and gold-clusters modified glassy carbon electrode	Square wave voltammetry	0.01 ag/mL	Spike antibody	3.5	40	32
Graphene and gold nanoparticles conjugated with suitably designed antisense oligonucleotides	Chronopotentiometry	6900 copy/mL	RNA	>30	35	33
Magnetic bead-based immunosensor combined with carbon black-modified screen-printed electrode	Differential pulse voltammetry	19 and 8 ng/mL	Spike and nucleocapsid protein	2	30	34
Graphene, 1-pyrenebutyric acid N-hydroxysuccinimide ester and SARS-CoV-2 spike antibody modified field-effect transistor	Semiconductor analyzer	1 fg/mL, 242 copy/mL	Spike protein and RNA	>7	15	35
Cobalt functionalized TiO_2 nanotube-based screen-printed electrode	Amperometry	0.1 µg/mL	Spike protein	>12	5	36
p-sulfocalix[8]arene, graphene oxide, toluidine blue functionalized gold supersandwich	Differential pulse voltammetry	200 copy/mL	RNA	>30	5	37
SARS-CoV-2 spike antibody, bovine serum albumin, staphylococcal protein A, Cu_2O nanocubes modified screen-printed electrode	Electrochemical impedance spectroscopy	0.04 fg/mL	Spike protein	12	20	38
Bovine serum albumin, SARS-CoV-2spike antibody, 1-pyrenebutyric acid N-hydroxysuccinimide ester modified graphene electrode	Square wave voltammetry	20 µg/mL	Spike protein	5	45	39
Bovine serum albumin, SARS-CoV-2 spike antibody and functionalized graphene oxide modified glassy carbon or screen-printed electrode	Square wave voltammetry	1 ag/mL	Spike protein	2.5	40	40
Aptamer, Chitosan, graphitic carbon nitride, cadmium sulfide, indium tin oxide electrode	Photo-electrochemical method	0.12 nM	Receptor-binding domain protein	17	40	41

Bovine serum albumin, SARS-CoV-2 spike protein, mercaptoethanol, gold-clusters, modified glassy carbon electrode	Square wave voltammetry	0.03 fg/mL	Spike antibody	2.5	30	42
SARS-CoV-2 spike protein, Polydimethylsiloxane, polylactic acid membrane or screen printed elctrode	Potentiometry	1 fg/mL	Spike protein	>29	3	43
Bovine serum albumin, 4-aminothiophenol, 3,3′ -dithiobis [sulfosuccinimidyl propionate, m-phenylenediamine, molecularly imprinted polymer- gold-based thin-film electrode	Differential pulse voltammetry	111 fM	Nucleocapsid protein	2.5	5	44
Bovine serum albumin, SARS-CoV-2 spike antibody, glutaraldehyde/Dicyclohexylcarbodiimide-4-Dimethylamino pyridine, cysteine and gold-flowers modified glassy carbon electrode	Square wave voltammetry	0.93 and 46.3 ag/mL	Spike protein	3	30	This study

2. Preparation of biosensing platforms

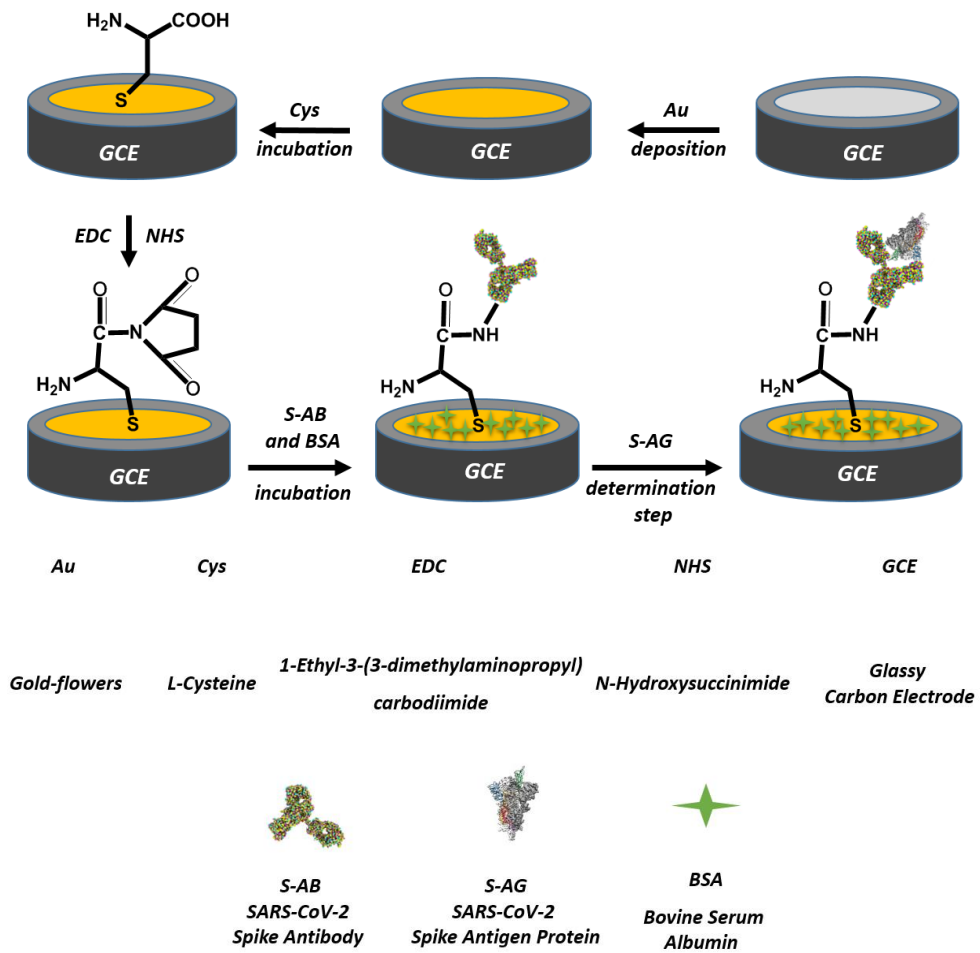


Fig. S1. Schematic presentation of the procedures for preparing BSA/S-AB/GluAl/*f*-1 Cys/Au/GCE.

3. Characterisation of the biosensing platforms

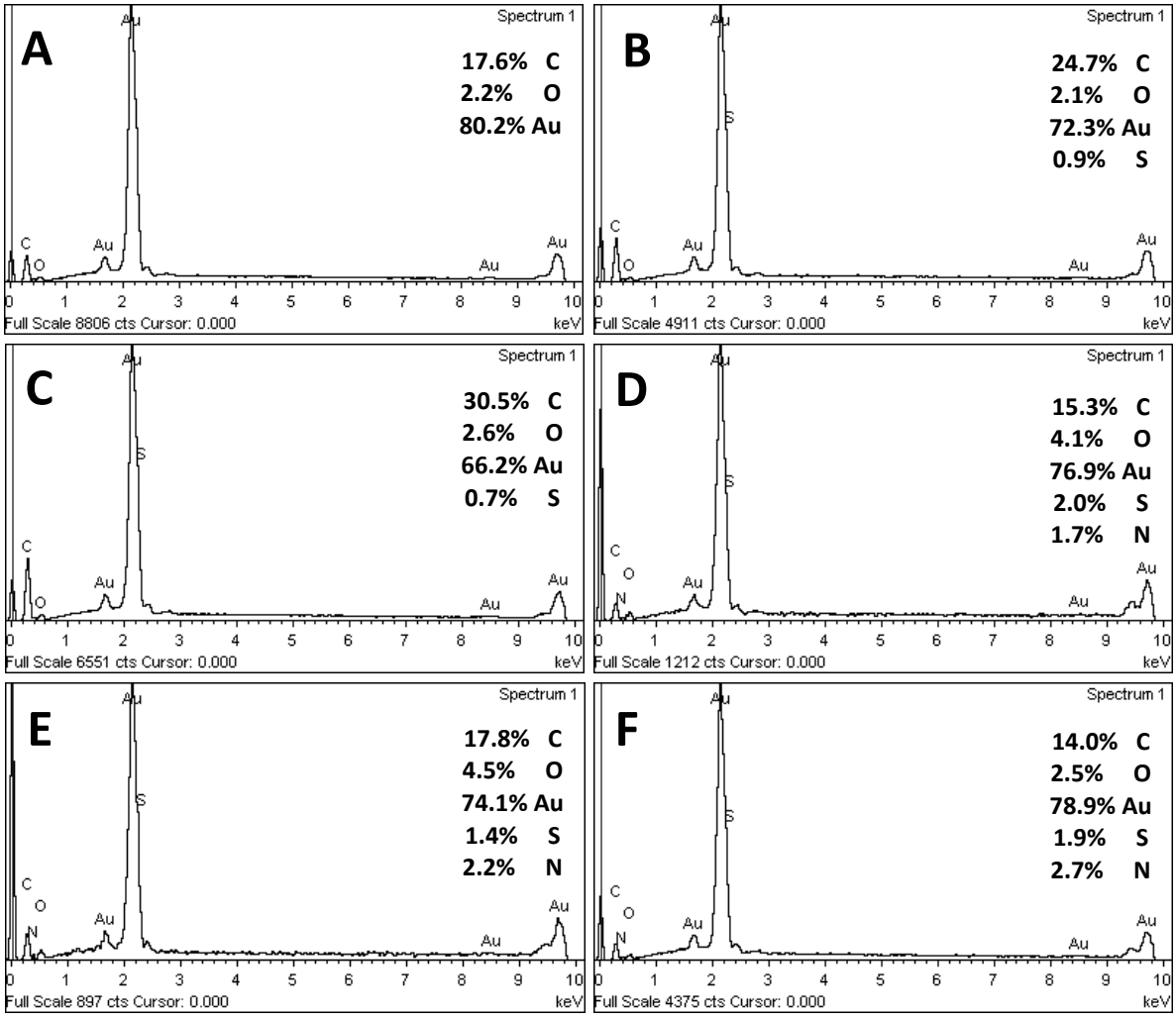
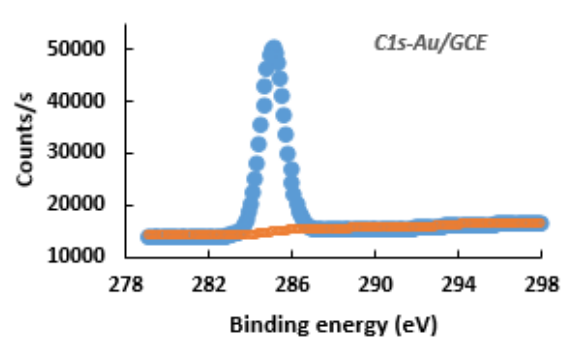


Fig. S2. EDX spectra for (A) Au/GCE, (B) Cys/Au/GCE, (C) GluAl/Cys/Au/GCE, (D) S-AB/GluAl/Cys/Au/GCE, (E) *f*-Cys/Au/GCE and (F) S-AB/*f*-Cys/Au/GCE. EDX analysis: ATW2 window, 30 mm^2, 142 eV at 5.9 keV, INCA software (mass percentages were given in EDX spectra).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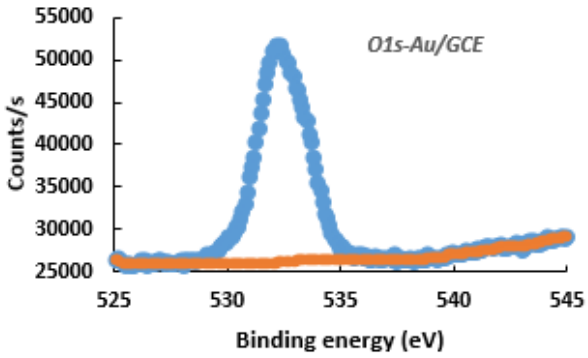
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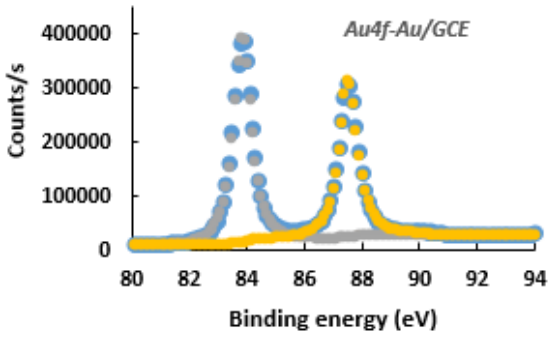
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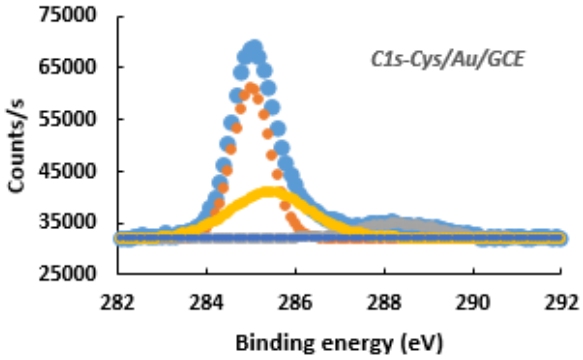
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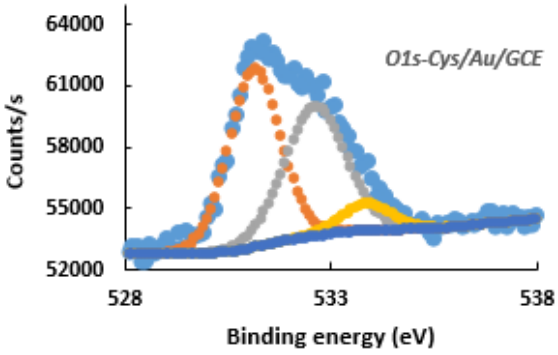
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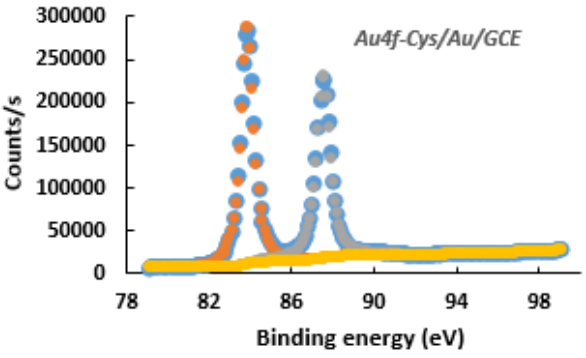
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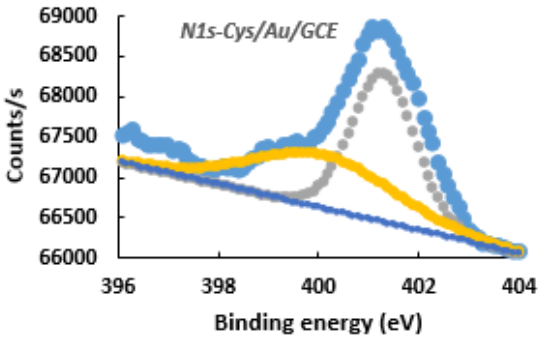
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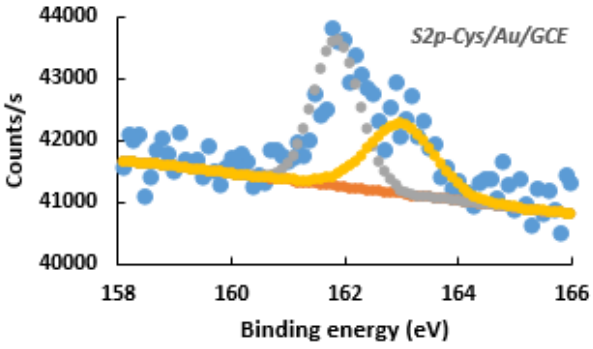
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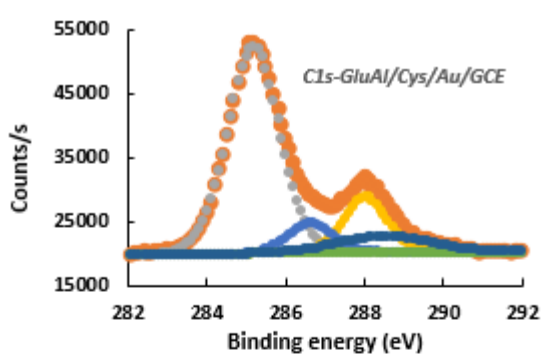
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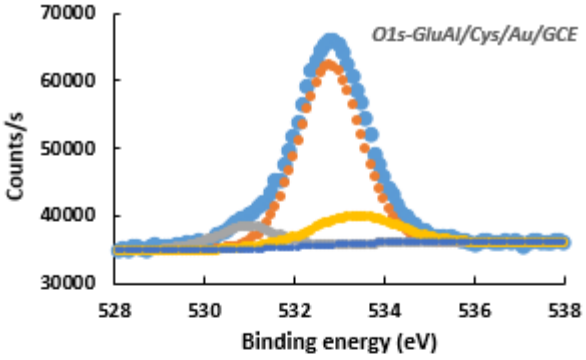
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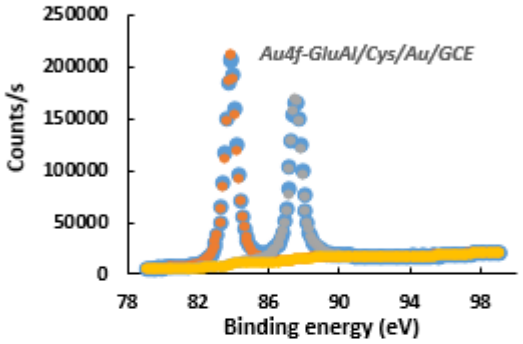
Binding energy (eV)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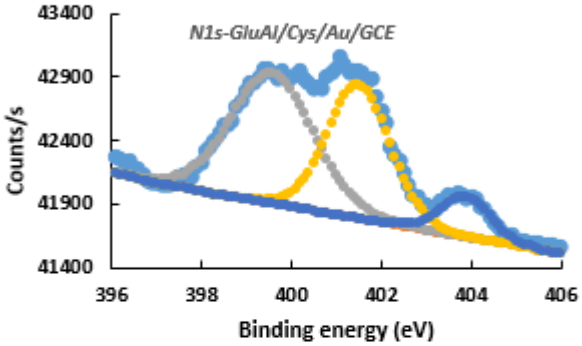
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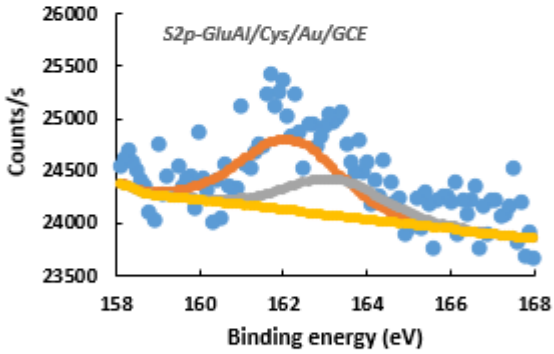
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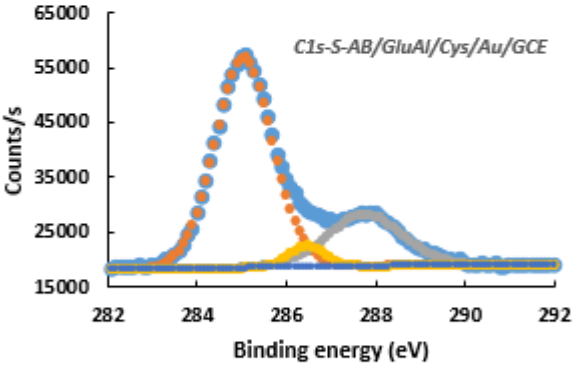
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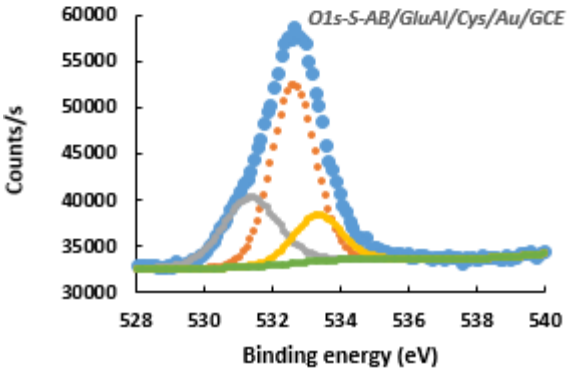
Binding energy (eV)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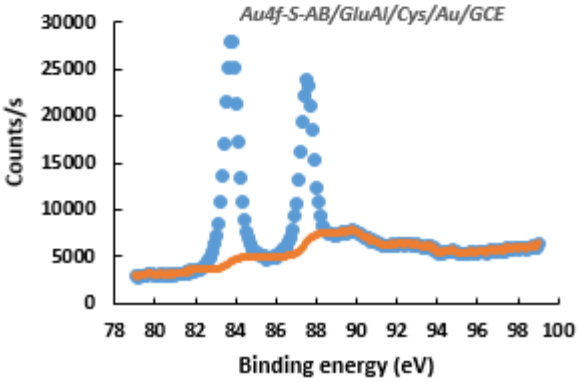
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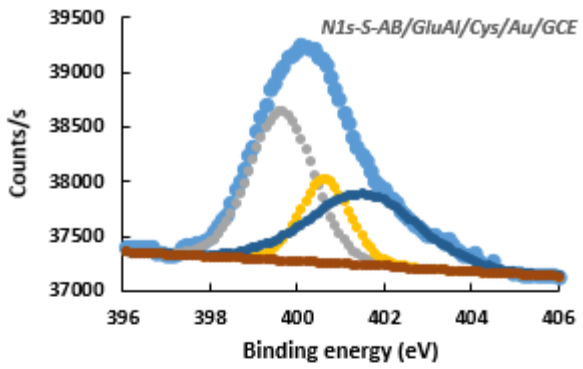
Counts/s
Binding energy (eV)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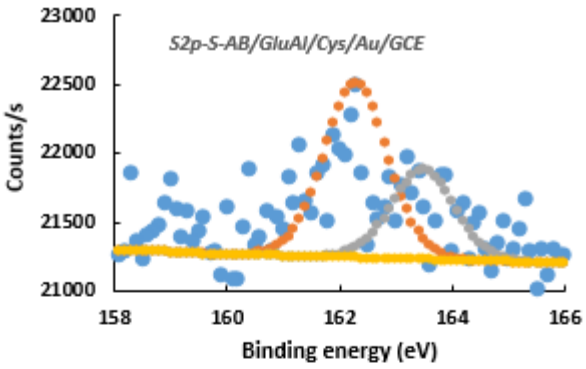
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Counts/s
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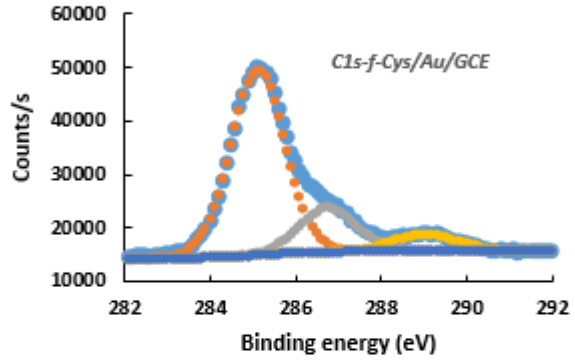
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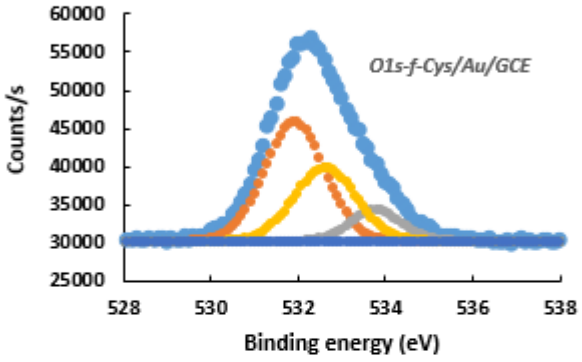
Counts/s
Binding energy (eV)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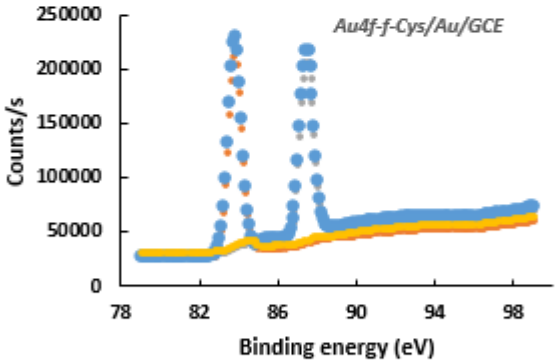
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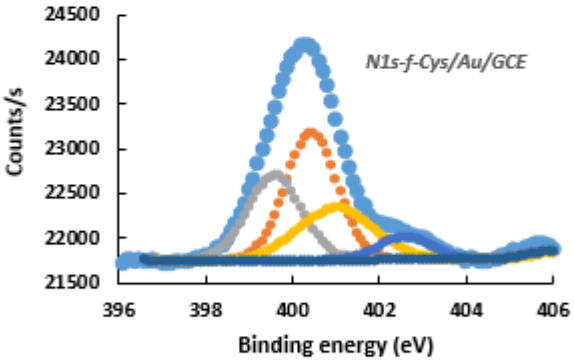
Counts/s
Binding energy (eV)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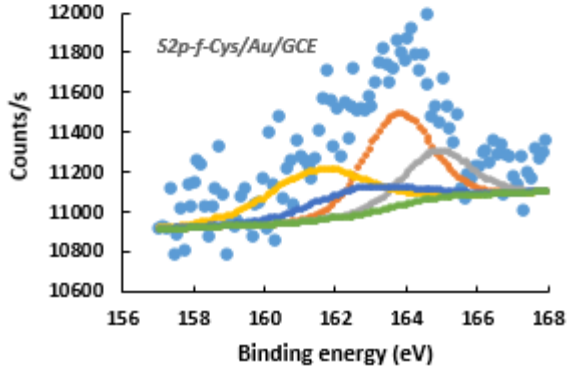
Counts/s
Binding energy (eV)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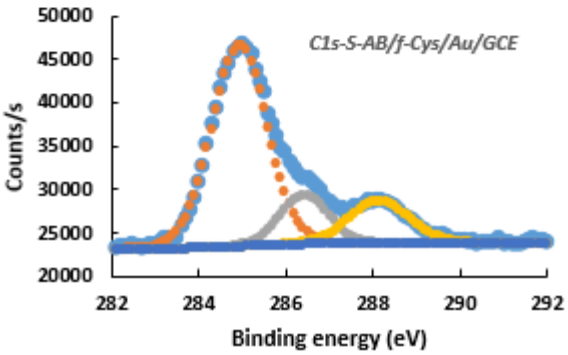
Counts/s
Binding energy (eV)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Counts/s
Binding energy (eV)

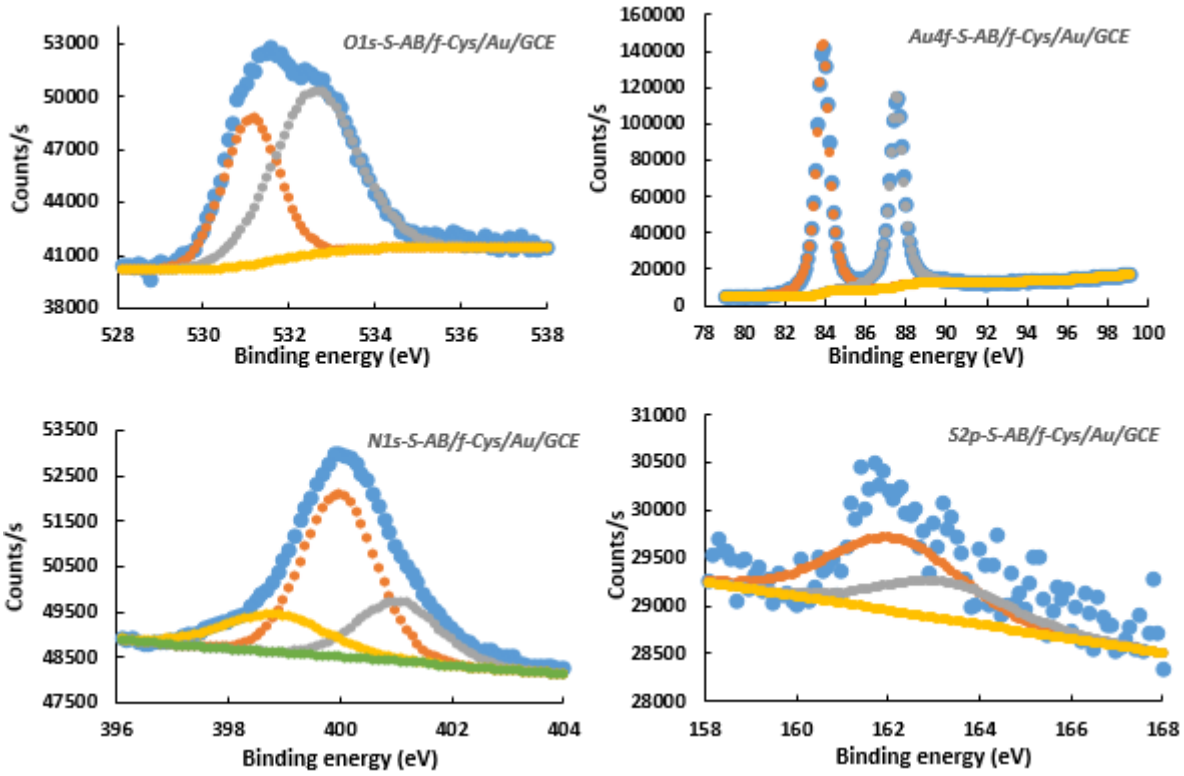


Fig. S3. XPS spectra of the produced platforms. XPS analysis: Al Kα gun, 300 μm spot size, 50 eV pass energy, 0.1 eV energy step size.

4. Cyclic voltammetric characteristics of the developed systems

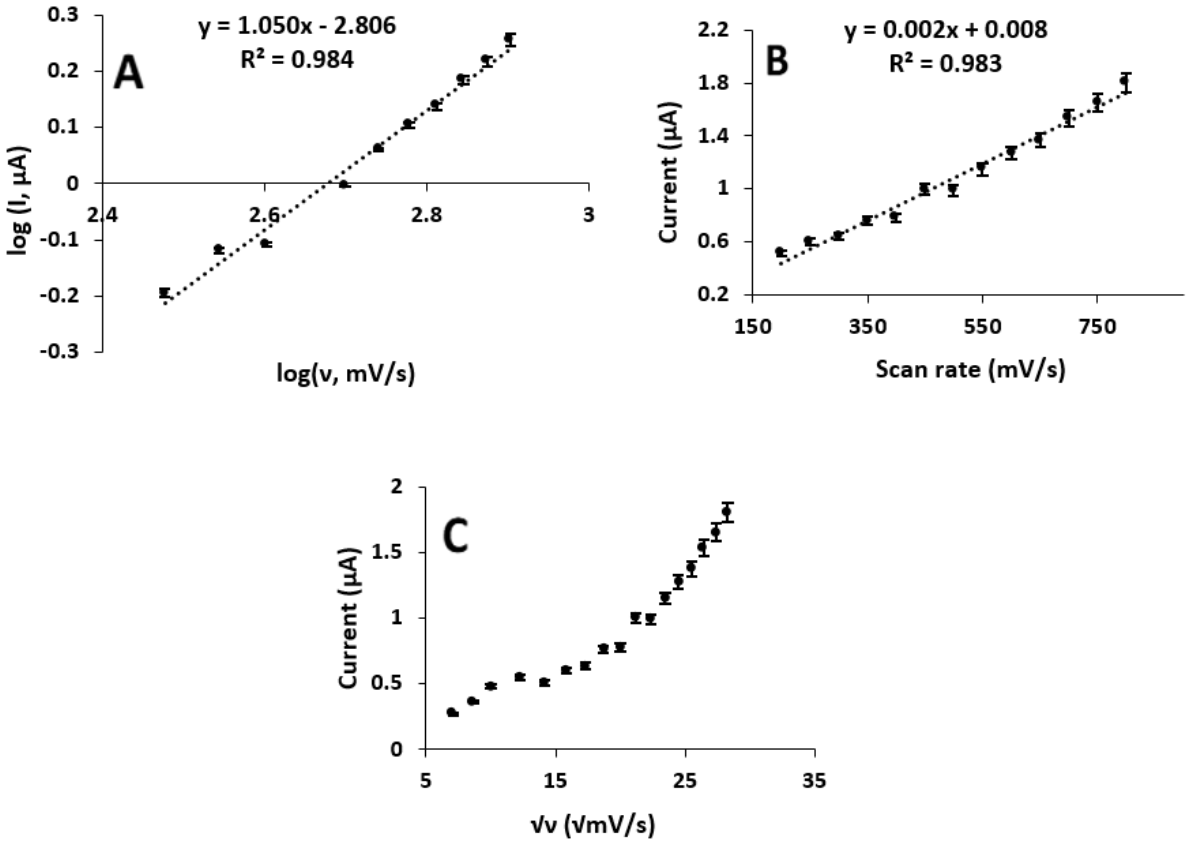


Fig. S4. (A) log(I_p) – log(ϑ) curve, (B) $I_p - \vartheta$ curve, and (C) $I_p - \sqrt{\vartheta}$ curve. Conditions: 100 pg/mL of S-AG in 0.01 M (pH 7.5) of PBS solution by using BSA/S-AB/GluAl/Cys/Au/GCE. E_{start}: 0.85 V, E_{finish}: -0.75 V, step amplitude: 5 mV and pulse amplitude: 25 mV.

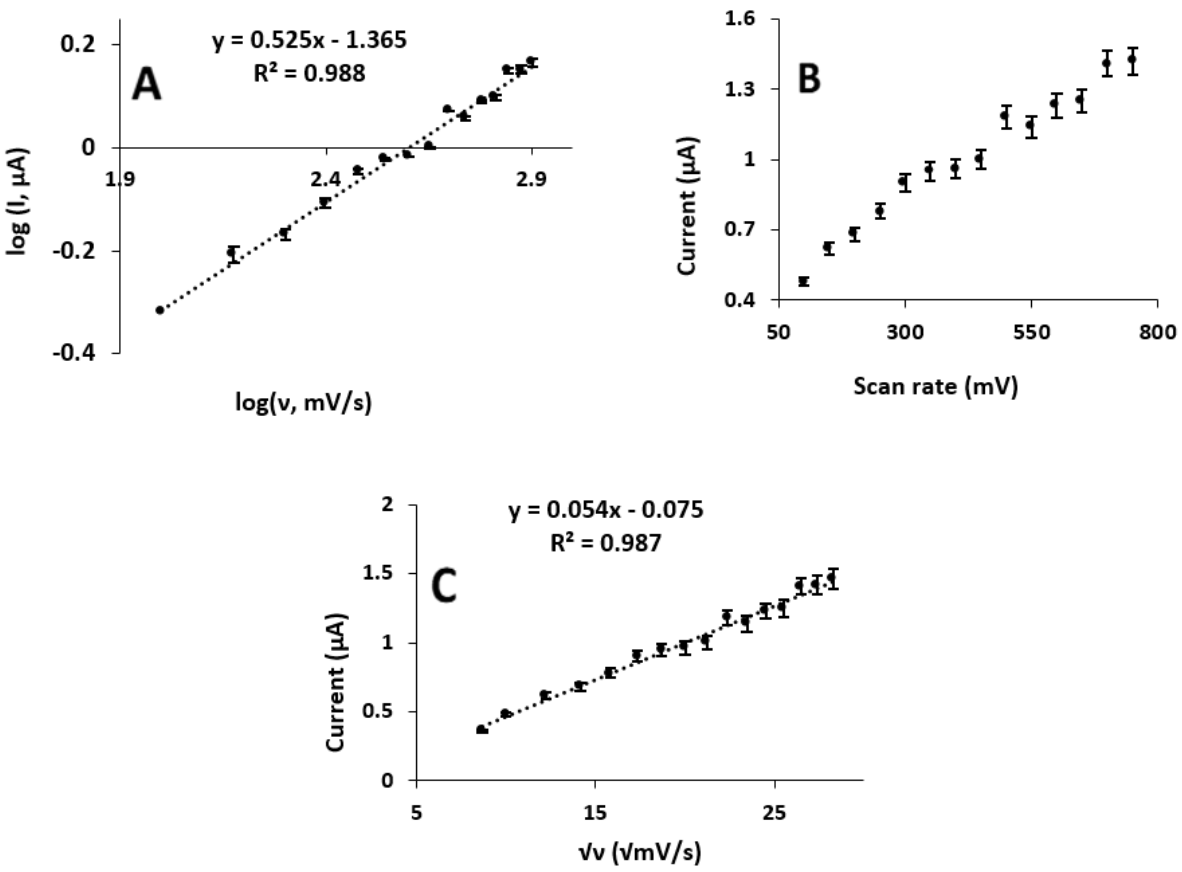
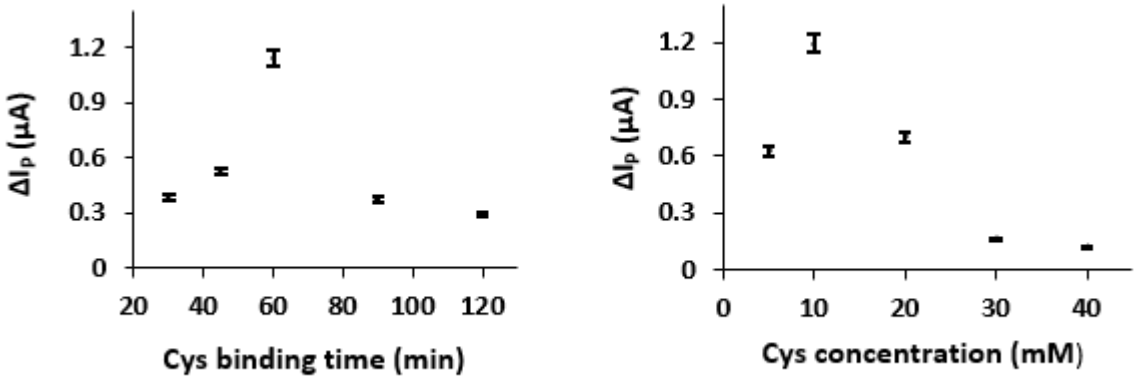


Fig. S5. (A) log(I_p) – log(ϑ) curve, (B) $I_p - \vartheta$ curve, and (C) $I_p - \sqrt{\vartheta}$ curve. Conditions: 100 pg/mL of S-AG in 0.01 M (pH 7.5) of PBS solution, by using BSA/S-AB/*f*-Cys/Au/GCE. E_{start}: 0.85 V, E_{finish}: -0.75 V, step amplitude: 5 mV and pulse amplitude: 25 mV.

5. Optimisation studies



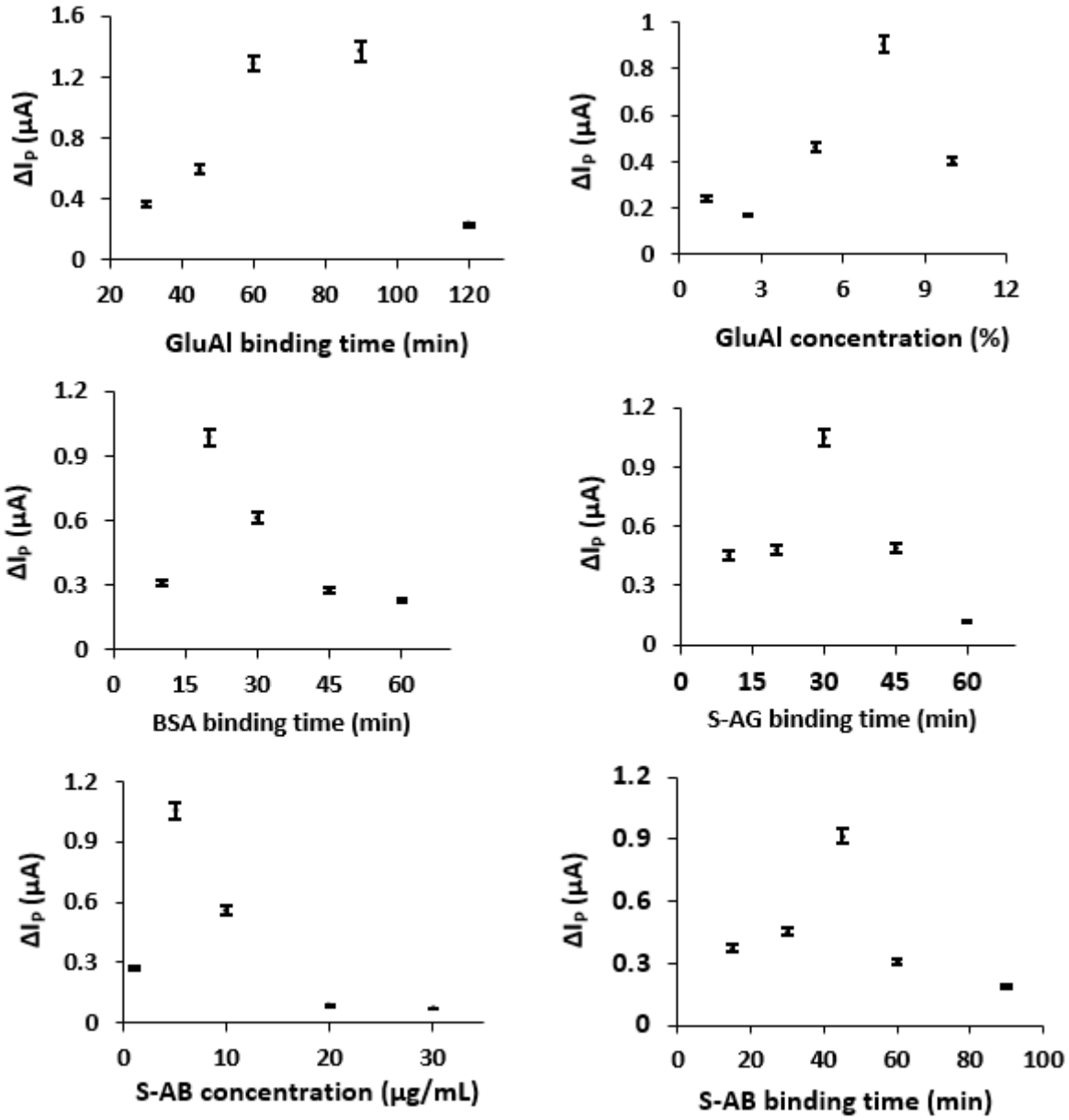


Fig. S6. Effects of the binding times of cysteine, glutaraldehyde, S-AB, BSA and S-AG and the concentrations of cysteine, glutaraldehyde and S-AB on peak height with BSA/S-AB/GluAl/Cys/Au/GCE. Conditions: 0.01 M (pH 7.5) of PBS solution, 1 pg/mL of S-AG. E_{start}: 0.85 V, E_{finish}: -0.75 V, step amplitude: 5 mV, pulse amplitude: 25 mV.

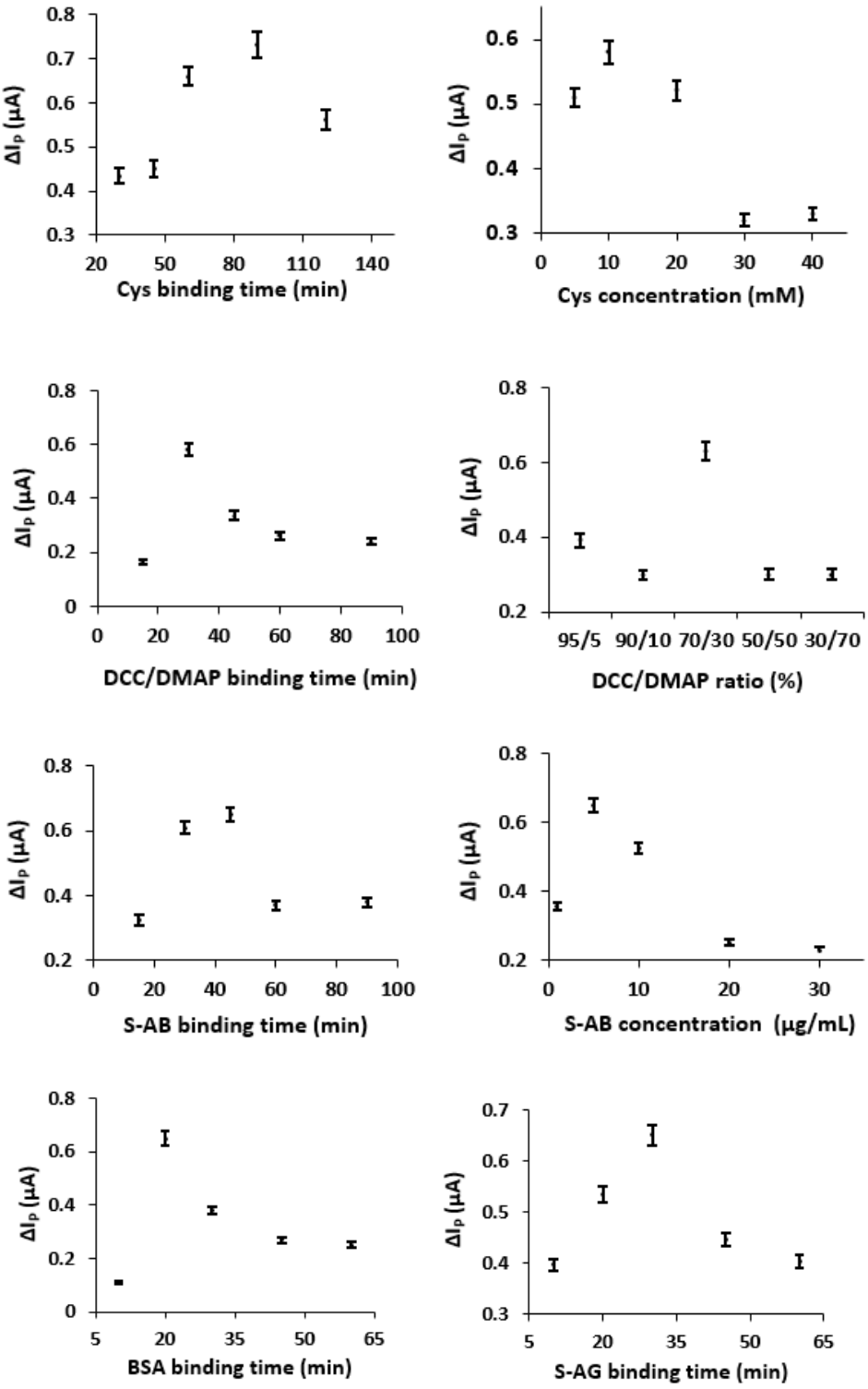


Fig. S7. Effects of the binding times of cysteine, DCC/DMAP, S-AB, BSA and S-AG and the concentrations of cysteine, DCC/DMAP and S-AB on peak height with BSA/S-AB/*f*-Cys/Au/GCE. Conditions: 0.01 M (pH 7.5) of PBS solution, 1 pg/mL of S-AG. E_{start}: 0.85 V, E_{finish}: -0.75 V, step amplitude: 5 mV and pulse amplitude: 25 mV.

6. Validation of the method and sample application

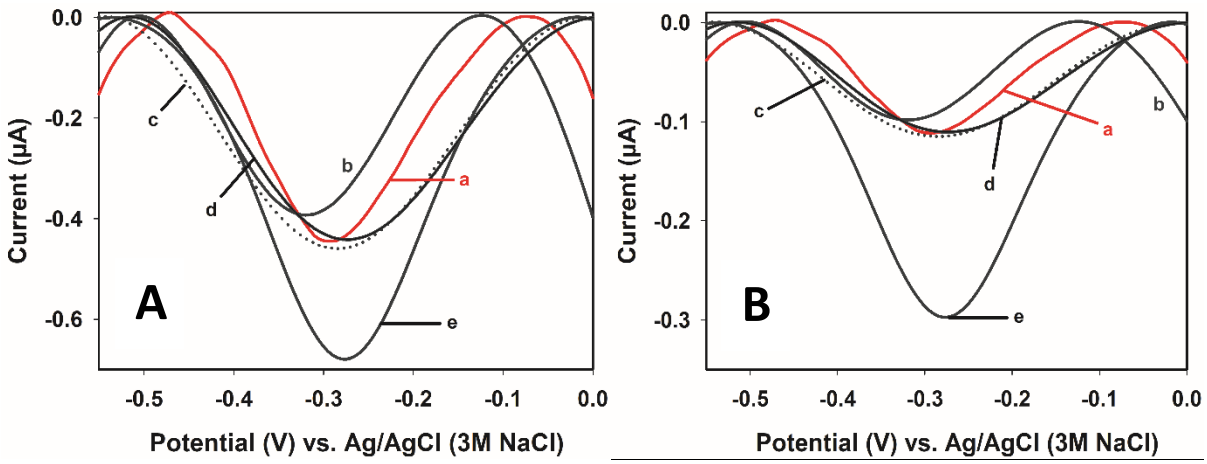


Fig. S8. Selective response of SARS-CoV-2 spike antigen biosensors to (a) 0.01 M (pH 7.5) of PBS solution, (b) influenza A spike antigen, (c) MERS-CoV spike antigen, (d) pneumonia antigen and (e) SARS-CoV-2 spike antigen at (A) BSA/S-AB/GluAl/Cys/Au/GCE and (B) BSA/S-AB/*f*-Cys/Au/GCE. Conditions: (A) 1 ag/mL of interfering or SARS-CoV-2 virus protein, (B) 100 ag/mL of interfering or SARS-CoV-2 virus protein, E_{start}: 0.85 V, E_{finish}: -0.75 V, step amplitude: 5 mV and pulse amplitude: 25 mV.

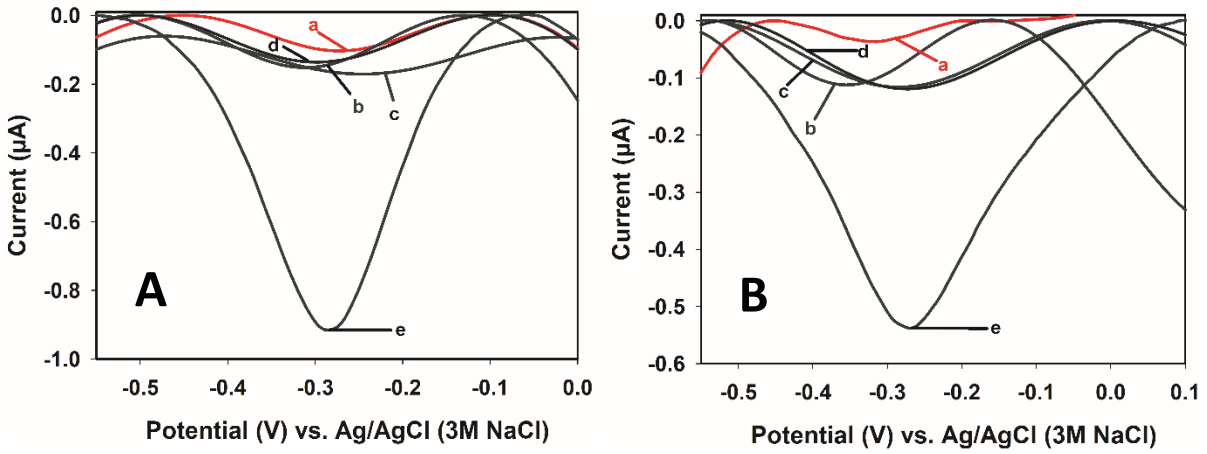


Fig. S9. Selective response of SARS-CoV-2 spike antigen biosensors to (a) 0.01 M (pH 7.5) of PBS solution, (b) influenza A spike antigen, (c) MERS-CoV spike antigen, (d) pneumonia antigen and (e) SARS-CoV-2 spike antigen at (A) BSA/S-AB/GluAl/Cys/Au/GCE and (B) BSA/S-AB/*f*-Cys/Au/GCE. Conditions: 10 fg/mL of interfering or SARS-CoV-2 virus protein, E_{start}: 0.85 V, E_{finish}: -0.75 V, step amplitude: 5 mV and pulse amplitude: 25 mV.

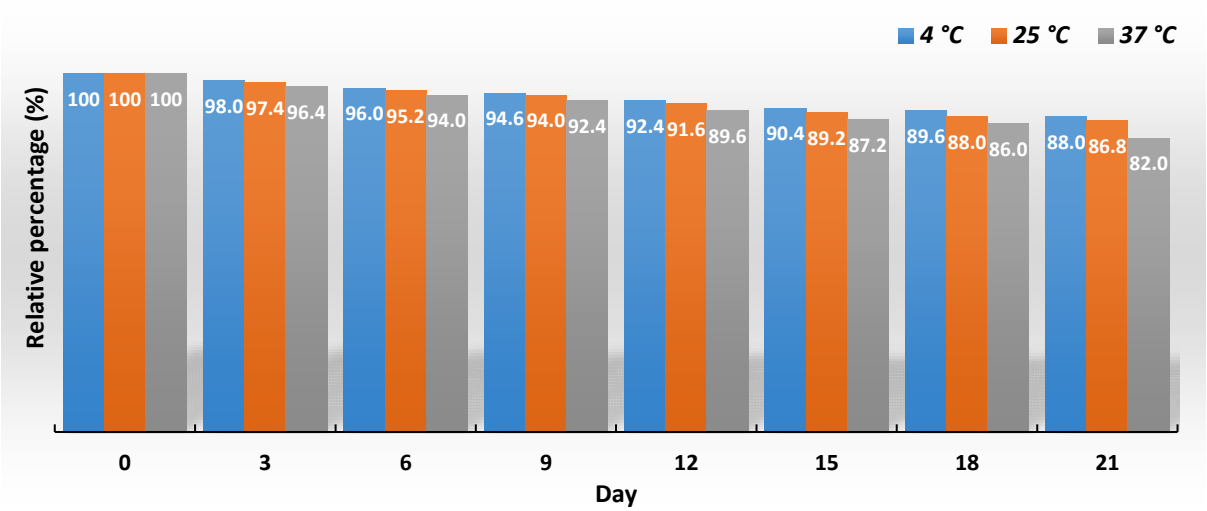


Fig. S10. Sensor stability studies of BSA/S-AB/GluAl/Cys/Au/GCE at 4 °C, 25 °C and 37 °C for 21 days by using square wave voltammetry. Conditions: 0.01 M (pH 7.5) of PBS solution, 100 ag/mL of S-AG. E_{start}: 0.85 V, E_{finish}: -0.75 V, step amplitude: 5 mV and pulse amplitude: 25 mV.

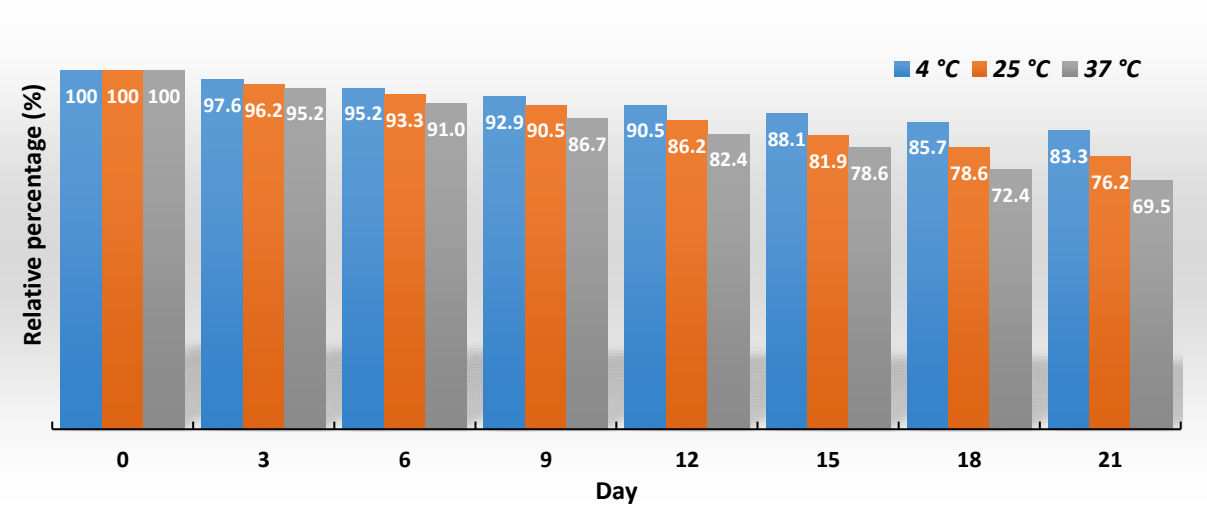


Fig. S11. Sensor stability studies of BSA/S-AB/*f*-Cys/Au/GCE at 4 °C, 25 °C and 37 °C for 21 days by using square wave voltammetry. Conditions: 0.01 M (pH 7.5) of PBS solution, 100 ag/mL of S-AG. E_{start}: 0.85 V, E_{finish}: -0.75 V, step amplitude: 5 mV and pulse amplitude: 25 mV.

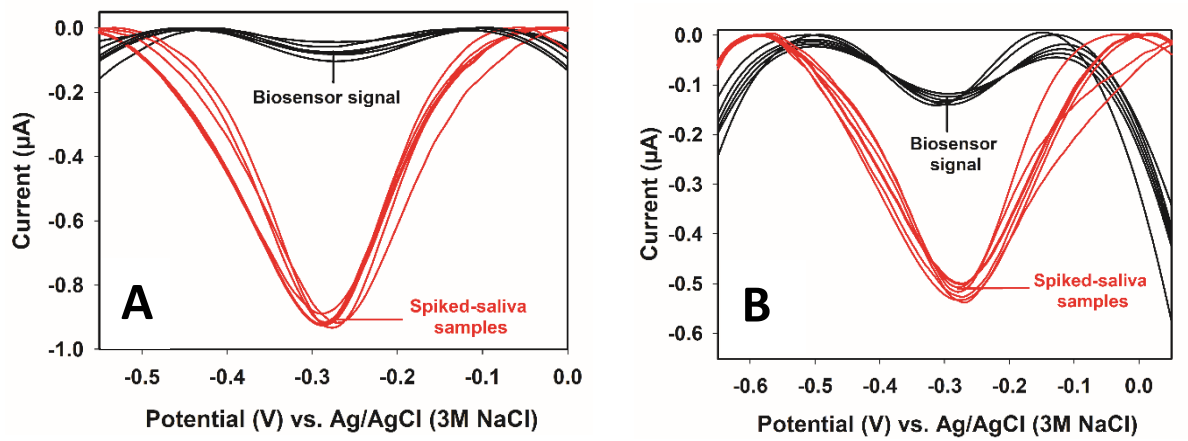


Fig. S12. The obtained square wave voltammograms from six independent measurements for S-AG spiked-saliva samples (A) by using BSA/S-AB/GluAl/Cys/Au/GCE and (B) BSA/S-AB/*f*-Cys/Au/GCE. Conditions: 0.01 M (pH 7.5) of PBS solution, 10 fg/mL of S-AG. E_{start}: 0.85 V, E_{finish}: -0.75 V, step amplitude: 5 mV and pulse amplitude: 25 mV.

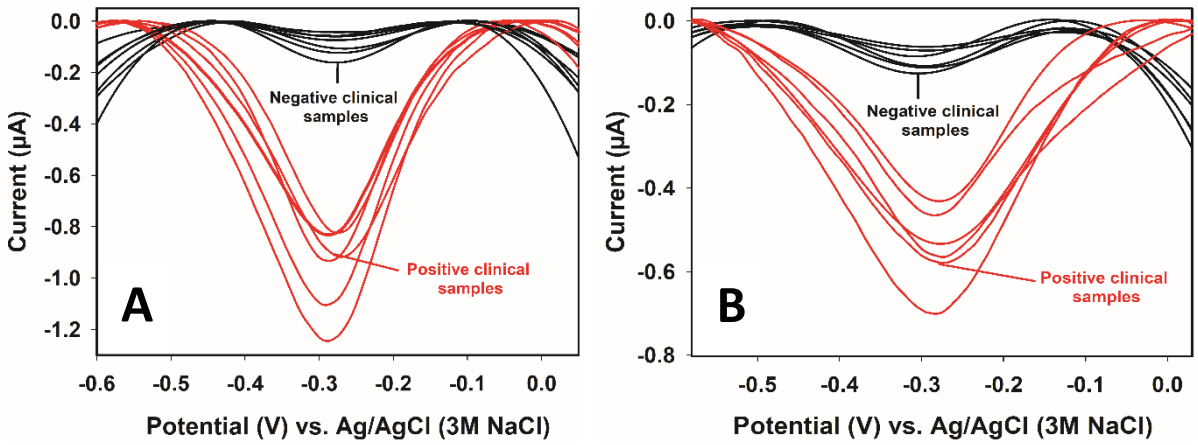


Fig. S13. The obtained square wave voltammograms from six independent measurements for RT-PCR approved positive and negative clinical samples consisting of gargle and mouthwash liquids (A) by using BSA/S-AB/GluAl/Cys/Au/GCE and (B) BSA/S-AB/*f*-Cys/Au/GCE. Conditions: 0.01 M (pH 7.5) of PBS solution, 10 µL of clinical sample. E_{start}: 0.85 V, E_{finish}: -0.75 V, step amplitude: 5 mV and pulse amplitude: 25 mV.