Supplementary Materials

Smartphone-Integrated User-Friendly Electrochemical Biosensor Based on Optimized Aptamer Specific to SARS-CoV-2 S1 Protein

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Chemicals and apparatus

Electrochemical measurements were conducted using an AUTOLAB PGSTAT204 potentiostat controlled by NOVA 2.1.7 software (Eco Chemie, The Netherlands). A conventional three-electrode configuration was employed, consisting of a disposable pencil graphite electrode (PGE) as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl (3 M KCl) reference electrode (BAS, Model RE-5B, W. Lafayette, USA). All measurements were carried out inside a Faraday cage.

The S1 protein-specific optimized aptamer was obtained from Aptamer Group (UK), which developed, optimized, and holds the patent for this sequence targeting the SARS-CoV-2 S1 protein. According to the manufacturer's recommendations, the aptamer should be prepared using a suitable binding buffer. SARS-CoV-2 S1 protein was purchased from Sino Biological. A stock solution of the protein was prepared in ultra-pure water at a concentration of 250 µg/mL and stored at -80 °C until use. Working solutions of the S1 protein were prepared by dilution in phosphate-buffered saline (PBS, 50 mM, pH 7.40). MERS-CoV-S1 (MERS) protein was obtained from Sino Biological. A stock solution was prepared in ultra-pure water at a concentration of 150 µg/mL and stored at -80°C until use. Working solutions were prepared by dilution in phosphate-buffered saline (PBS, 50mM, pH 7.40). Influenza Hemagglutinin (HA) peptide was purchased from Sigma-Aldrich. A stock solution of HA was prepared in ultra-pure water at a concentration of 5 mg/mL and stored at -20°C. Diluted HA solutions were also prepared in PBS (50mM, pH 7.40). N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich. All chemicals used in this study were of analytical reagent grade and purchased from commercial suppliers. Milli-Q grade ultra-pure water was used to freshly prepare all aqueous solutions.

Table S1. Average current values, % RSD values (n=3) and % change values obtained before/after S1P interaction with OPT at the electrode surface and in the solution phase.

	Interaction at electrode surface		**Interaction in solution phase**	
	Average current value (µA), % RSD	**% Change**	**Average current value (µA), % RSD**	**% Change**
PGE	195.38 ± 3.65 1.87%	--	195.38 ± 3.65 1.87%	--
OPT control	45.73 ± 19.26 42.10%	76.59% decrease	69,84 ± 7,42 10.63%	64.25% decrease
OPT-S1P interaction	41.02 ± 11.78 28.72%	10.31% decrease	60,68 ± 3,99 6.58%	13.12% decrease

* Interaction procedure at the electrode surface, average current value for S1 protein (S1P) control group was 208.67 ± 7.75 µA, % RSD= 3.71%, n=3. Interaction procedure in the solution phase, average current value for S1 protein (S1P) control group was 162.26 ± 6.79 µA, % RSD= 4.18%, n=3.

Table S2. Average current values, % RSD values (n=3) and % change values obtained before/after S1P interaction with OPT using different concentrations of redox probe solution.

	2.5 mM Redox Probe Solution		**5 mM Redox Probe Solution**	
	Average current value (µA), % RSD	**% Change**	**Average current value (µA), % RSD**	**% Change**
PGE	195.38 ± 3.65, 1.87%	--	311.99 ± 10.66, 3.42%	--
OPT control	69.84 ± 7.42, 10.63%	64.25% decrease	135.38 ± 3.18, 2.35%	56.61% decrease
OPT-S1P interaction	60.68 ± 3.99, 6.58%	13.12% decrease	116.32 ± 5.72, 4.91%	14.08% decrease

* In the measurements performed with the S1 protein (S1P) control group using 2.5 mM redox probe solution, the average current value was 162.26 ± 6.79 µA, % RSD= 4.18% (n=3). In the measurements performed with the S1 protein (S1P) control group using 5 mM redox probe solution, the average current value was 208.72 ± 18.31 µA, % RSD= 8.77% (n = 3).

Table S3. Average current values, % RSD values (n=3) and % change values obtained before/after S1P interaction with OPT in the presence and absence of EDC/NHS solution prepared in different buffers.

	In the absence of EDC/NHS		In the presence of EDC/NHS prepared in PBS solution		In the presence of EDC/NHS prepared in MES solution	
	Average current value (μA), % RSD	**% Change**	**Average current value (μA), % RSD**	**% Change**	**Average current value (μA), % RSD**	**% Change**
PGE	311.99 ± 10.66, 3.42%	--	311.99 ± 10.66, 3.42%	--	311.99 ± 10.66, 3.42%	--
PGE-EDC/NHS	--	--	328.52 ± 39.21, 11.93%	5.30% increase	444.19 ± 14.36, 3.23%	42.37% increase
OPT control	135.38 ± 3.18, 2.35%	56.61% decrease	191.26 ± 8.46, 4.41%	41.57% decrease	191.88 ± 5.67, 2.95%	56.80% decrease
OPT-S1P interaction	116.32 ± 5.72, 4.91%	14.08% decrease	161.28 ± 18.77, 11.64%	15.98% decrease	160.72 ± 4.76, 2.96%	16.24% decrease

* In the measurements performed with S1 protein (S1P) control group in the absence of EDC/NHS, average current value was 208.72 ± 18.31 μA, % RSD= 8.77% (n=3). In the measurements performed with the S1 protein (S1P) control group in the presence of EDC/NHS prepared in PBS solution, average current value was 289.36 ± 16.99 μA, % RSD= 5.87% (n=3). In the measurements performed with the S1 protein (S1P) control group in the presence of EDC/NHS prepared in MES solution, average current value was 320.32 ± 6.42 μA, % RSD= 2.00% (n=3).

Table S4. Average current values and % RSD values (n=3) and % change values obtained before/after the interaction of OPT prepared at different concentrations with S1 protein.

	Average current value (μA), % RSD (OPT control)	**Average current value (μA), % RSD (after interaction)**	**% Change compared to OPT control**
PGE-EDC/NHS	444.19 ± 14.36, 3.23%	--	--
0.003 nM OPT	169.49 ± 13.48, 7.96%	152.48 ± 20.32, 13.32%	10.03% decrease
0.03 nM OPT	150,41 ± 2,35, 1.57%	143.37 ± 11.28, 7.87%	4.68% decrease
0.3 nM OPT	191.88 ± 5.67, 2.95%	160.72 ± 4.76, 2.96%	16.24% decrease
3 nM OPT	162.28 ± 12.87, 7.93%	151.69 ± 3.69, 2.43%	6.52% decrease
30 nM OPT	150.11 ± 12.99, 8.65%	141.28 ± 23.81, 16.85%	5.88% decrease

* In the measurements performed with S1 protein (S1P) control group, average current value was 320.32 ± 6.42 μA (n=3).

Table S5. Average current values, % RSD values (n=3) and % change values obtained before/after S1P interaction with OPT at different interaction times.

	Average current value (µA), % RSD (OPT control)	Average current value (µA), % RSD (after interaction)	% Change compared to OPT control
PGE-EDC/NHS	444.19 ± 14.36, 3.23%	--	--
5 min interaction	191.88 ± 5.67, 2.95%	160.72 ± 4.76, 2.96%	16.24% decrease
15 min interaction	176.01 ± 4.96, 2.82%	144.16 ± 4.13, 2.86%	18.10% decrease
30 min interaction	171.78 ± 15.49, 9.02%	152.25 ± 4.08, 2.68%	11.37% decrease

*The average current value for S1 protein (S1P) control group at 5 min interaction time was 320.32 ± 6.42 µA (n=3). The average current value for S1 protein (S1P) control group at 15 min interaction time was 322.46 ± 15.22 µA (n=3). The average current value for S1 protein (S1P) control group at 5 min interaction time was 367.95 ± 14.74 µA (n=3).

Table S6. Average current values, % RSD values (n=3) and % change values obtained before/after S1P interaction with OPT at different immobilization times.

	Average current value (µA), % RSD (OPT control)	Average current value (µA), % RSD (after interaction)	% Change compared to OPT control
PGE-EDC/NHS	444.19 ± 14.36, 3.23%	--	--
15 min immobilization	176.01 ± 4.96, 2.82%	144.16 ± 4.13, 2.86%	18.10% decrease
30 min immobilization	181.67 ± 1.56, 0.86%	119.23 ± 7.64, 6.41%	34.37% decrease
60 min immobilization	161.46 ± 8.38, 5.19%	132.86 ± 7.43, 5.59%	17.71% decrease

*After 15 min immobilization, average current value for S1 protein (S1P) control group was 322.46 ± 15.22 µA (n=3). After 30 min immobilization, average current value for S1 protein (S1P) control group was 300.20 ± 20.62 µA (n=3). After 30 min immobilization, average current value for S1 protein (S1P) control group was 362.59 ± 22.06 µA (n=3).

Table S7. Experimental parameters were studied using SARS-CoV-2 S1 specific optimized aptamer-based electrochemical biosensor and the optimized conditions were presented.

Experimental parameters	Conditions	Selected value
Interaction procedure	Interaction procedures in the solution phase and at the electrode surface	Interaction procedures in the solution phase
Redox probe concentration	2.5 mM ve 5 mM	5 mM
EDC/NHS activation	Procedure followed in the presence and absence of EDC/NHS	Procedure followed in the presence of EDC/NHS
Solution for the preparation of EDC/NHS	PBS ve MES	MES
OPT concentration	0.003 / 0.03 / 0.3 / 3 / 30 nM OPT	0.3 nM OPT
Interaction time	5 / 15 / 30 min	15 min
Immobilization time	15 / 30 / 60 min	30 min

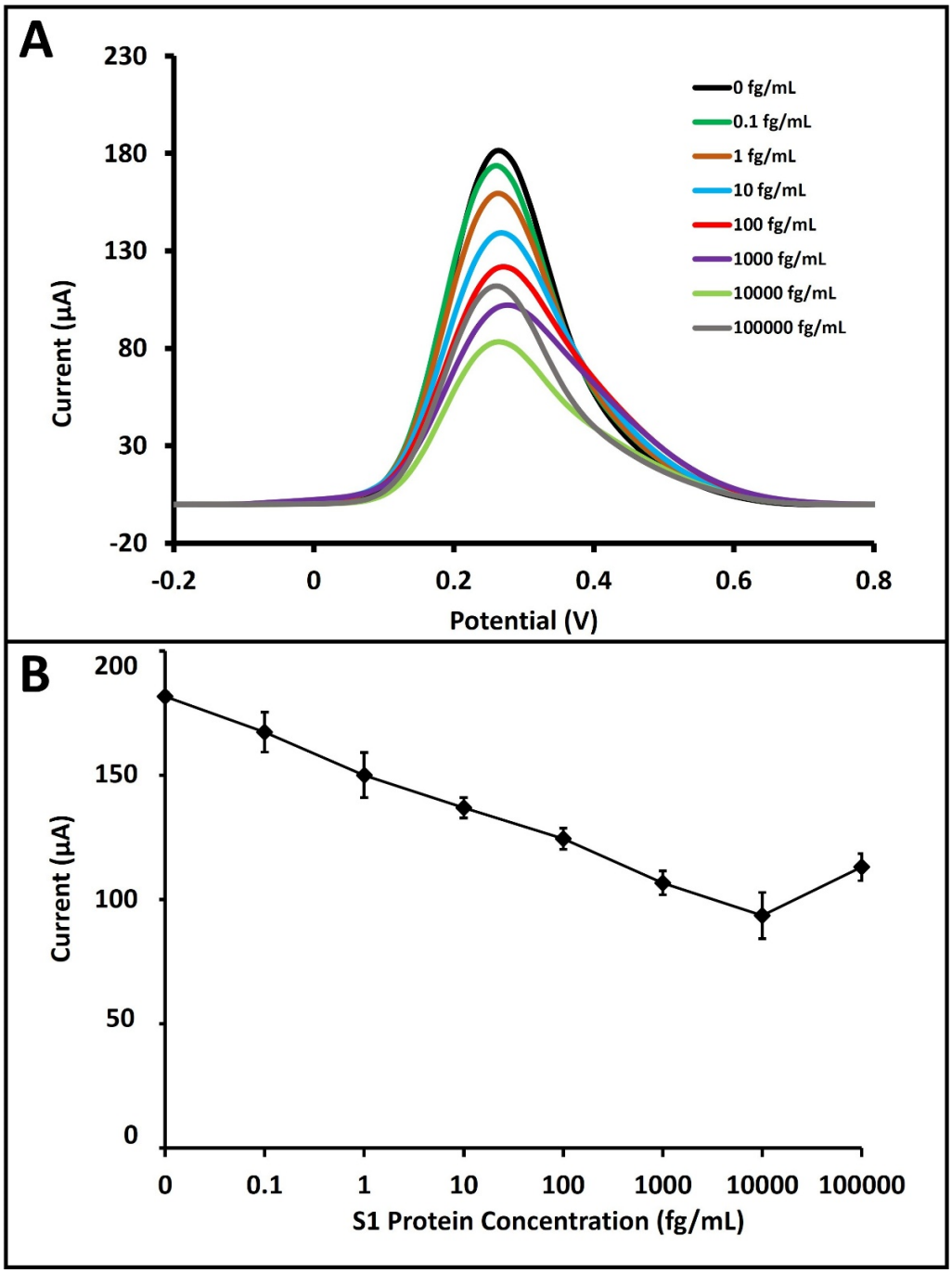


Fig S1. (A) DPV voltammograms and (B) line graph showing the average current values (n = 3) obtained after measurements in 5 mM redox solution across the S1 protein concentration range of 0–100,000 fg/mL in buffer medium.

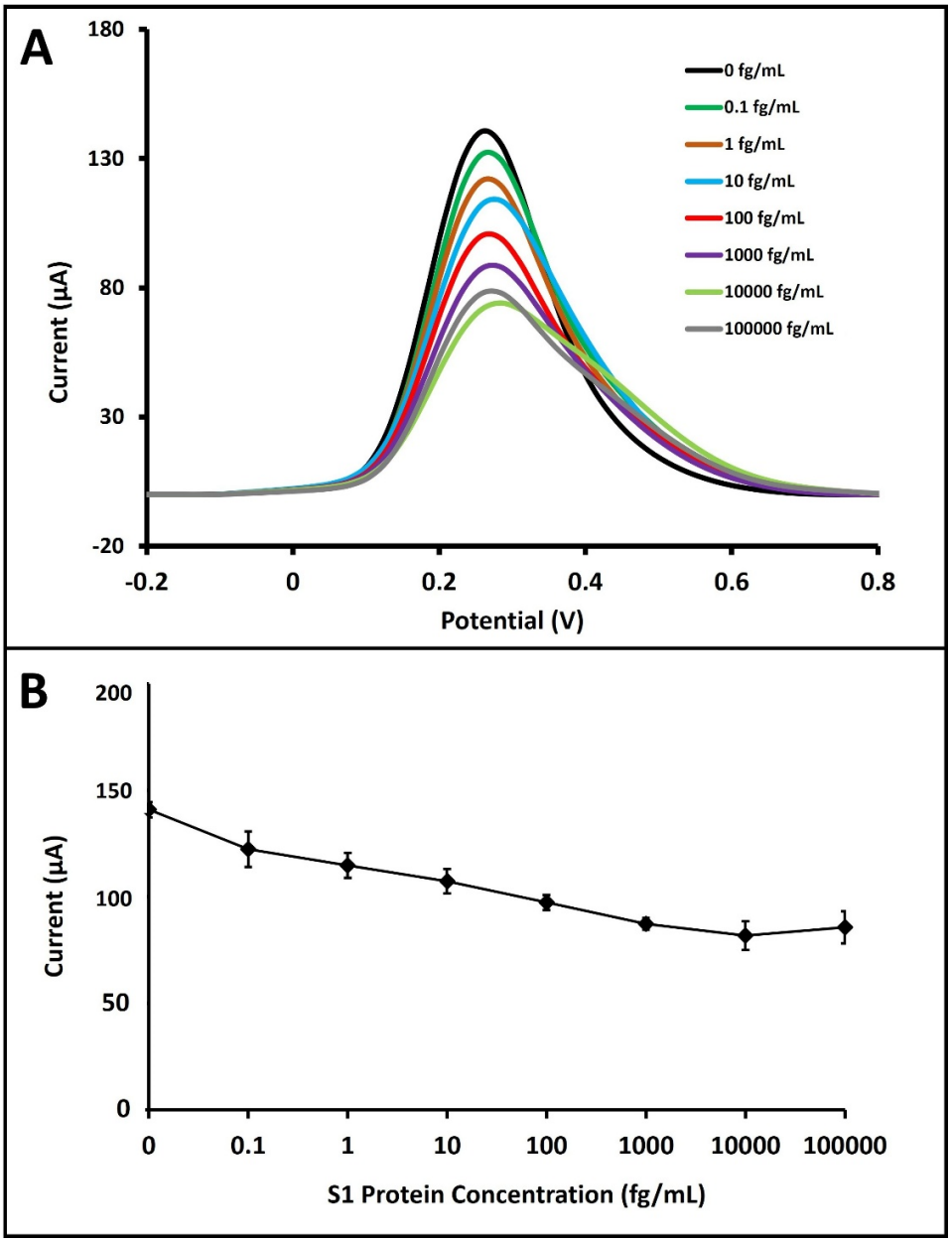


Fig S2. (A) DPV voltammograms and (B) line graph showing the average current values (n = 3) obtained after measurements in 5 mM redox solution over the S1 protein concentration range of 0–100,000 fg/mL in artificial saliva medium.

Table S8. Current responses and percentage changes relative to the control group obtained before and after the interaction of aptamer with 10 fg/mL S1 protein in artificial saliva at different dilution ratios.

Dilution ratio	Before interaction with 10 fg/mL S1 protein (I, n=3) and RSD% (n=3)	After interaction with 10 fg/mL S1 protein (I, n=3) and RSD% (n=3)	Change
1:10	119.26 ± 37.76 31.66%	94.46 ± 11.09 11.74%	20.79% decrease
1:20	140.87 ± 3.63 2.58%	107.27 ± 5.65 5.27%	23.85% decrease
1:50	108.72 ± 19.52 17.96%	103.18 ± 5.23 5.07%	5.10% decrease

Table S9. Relative error values of the developed optimer-based electrochemical biosensor for S1 protein detection in artificial saliva.

Sample (fg/mL)	Found (fg/mL)	Relative error (%)	RSD (%) (n=3)
0.1	0.116	15.58	6.78
1	0.911	8.87	5.10
100	107.732	7.73	3.63

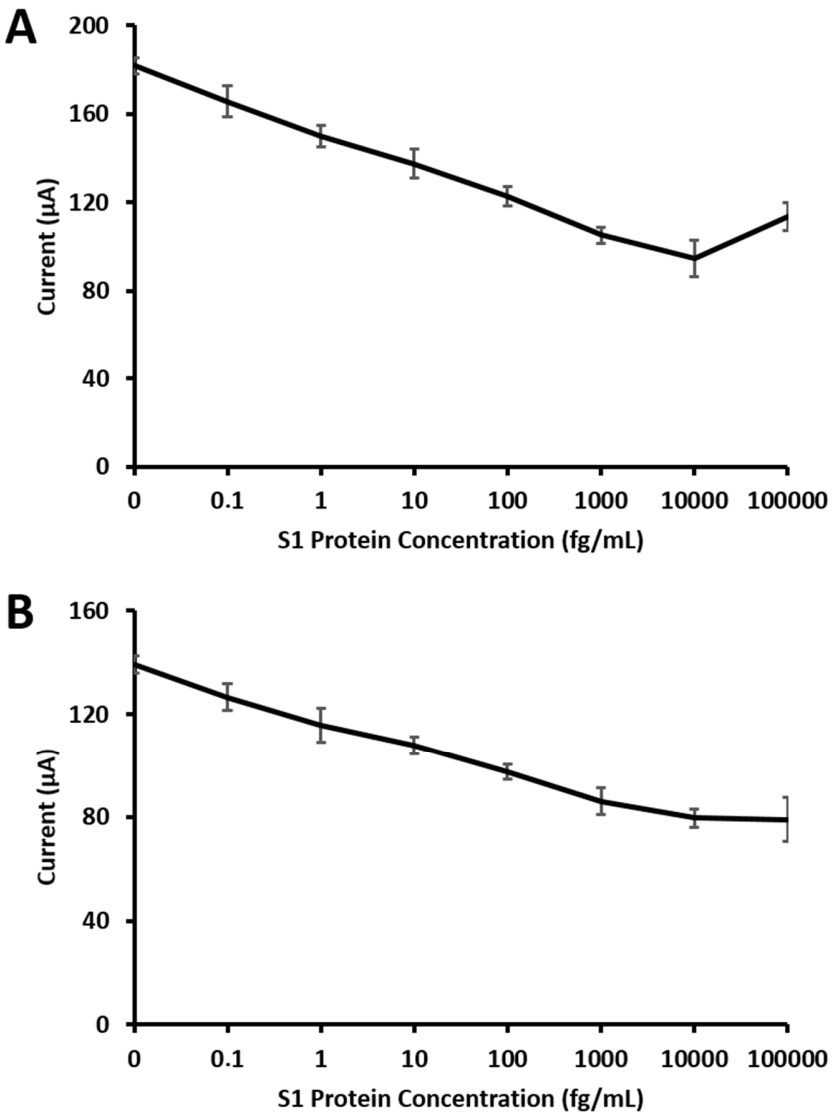


Fig S3. Studies on the determination of S1 protein using a smartphone-integrated potentiostat. (A) and (B) present the line graphs (n=3) obtained after DPV measurements in buffer and 1:20 diluted artificial saliva media, respectively, within the concentration range of 0.1 fg/mL to 100000 fg/mL.

Table S10. Results showing the relative error values of the S1 protein-specific optimer-based electrochemical biosensor developed for the detection of S1 protein in artificial saliva with a smartphone-integrated portable potentiostat.

Sample (fg/mL)	Found (fg/mL)	Relative error (%)	RSD (%) (n=3)
0.1	0.089	10.98	4.08
10	8.144	18.56	3.00
100	100.000	0.00	2.95