Electrochemical SELEX approach and its application to voltametric COF based-aptasensor for the diagnostics of cystic fibrosis

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Chemicals and Materials for SELEX

N-hydroxysuccinimide (NHS) pre-activated resin beads were purchased from Purolite, Life Sciences (PA, USA). Ethylenediaminetetraacetic acid (EDTA), sodium bicarbonate, sodium chloride, sodium azide, Tris-base, urea, magnesium chloride, hydrochloric acid, sodium acetate, Tetramethylethylene diamine (TEMED), acrylamide/bis-acrylamide (30% solution) were purchased from Sigma Aldrich (St. Louis, MO, USA). Spin-X with CA membrane, pore size 0.45 µm was purchased from Corning life sciences (Tewksbury, MA, USA). 3K centrifugal filters (Amicon Ultra, 0.5 mL) utilized for DNA desalting and concentration, were obtained from EMD Millipore (Alberta, Canada). PCR reagents: (10x buffer, dNTPs, Taq polymerase, loading dye, and 100 bp ladder) were purchased from ACE Biotech (Riyadh, Saudi Arabia). The DNA library (5'-TCCCTACGGCGCTAAC-40 N -GCCACCGTGCTACAAC-3'), fluorescent primer carboxyfluorescin (5'-FAM-TCCCTACGGCGCTAAC3'), Poly A primer (5'-poly-dA20-hexaethyleneglycol-GTTGTAGCACGGTGGC-3'), unlabeled primers, M13 primers, and the aptamer sequences were synthesized by Metabion International AG (Planegg, Germany). Aptamer sequences were prepared in binding buffer 50 mM Tris at pH 7.5, 2 mM $MgCl_2$, and 150 mM NaCl. Cloning Kit with One Shot TOP10 and the DH5α T1R chemically competent E. coli cells

were purchased from Invitrogen Inc. (New York, USA). The X-Gal utilized in cloning steps was purchased from Bio Basic Inc. (Toronto, Ontario, Canada). Agarose powder, 50X Tris base, and ampicillin were purchased from Bio-Rad (California, USA). Culture media were acquired from Saudi Prepared Media Laboratory (SPML; Riyadh, Saudi Arabia). Milli-Q water was used for the preparation of all the reagents in this study.

NanoDrop 2000C Spectrophotometer from Thermo Scientific (Ottawa, Canada) was used to measure the DNA concentration and the protein absorption. A powerpack-current power supply from Bio-Rad (California, United States) was used for the electrophoresis. UVP BioDoc-It Imaging system (UK) was used in the DNA band analysis. T100 Thermal Cycler was used to carry out the PCR amplification.

SELEX process:

N-hydroxysuccinimide (NHS) pre-activated resin beads were used as a solid matrix for the CFTR protein. 2 ml of NHS beads were washed multiple times with 2 ml of 1 mM HCl to eliminate preservatives. Subsequently, 200 µg/mL of CFTR prepared in (0.2 M $NaHCO_3$, pH= 8.3) were incubated with the beads at 4 °C overnight by end-over-end rotation. The beads were then washed with 0.1 M tris-HCL (pH= 8.3) to block free active sites and rotated at room temperature for 2 h. Afterward, the beads were washed six times with 0.1 M sodium acetate, NaCl (pH 4), and 0.1 M tris-HCl, NaCl (pH 7.5), alternatively. Finally, the conjugated beads were stored in TE (Tris-EDTA), 0.02% Sodium azide at 4 °C, for further use.

To remove preservatives, the CFTR-coupled beads were washed five times with 300 µL binding buffer using Spin-X cellulose acetate centrifuge filter tubes, then incubated with the ssDNA library for 2 h at room temperature with end-over-end rotation. The ssDNA solution was

heated at 90 °C for 5 minutes, followed by cooling at 4 °C for 10 minutes, and then at room temperature for 5 minutes before incubation with CFTR-coupled beads. Subsequently, beads were washed with binding buffer to remove unbound ssDNA until no fluorescent was detected. To elute bound ssDNA, 300 µL urea elution buffer was added and heated at 90 °C for 10 minutes, followed by centrifugation until no fluorescent ssDNA was detected. The eluted ssDNA was collected and desalted by using an ultrafiltration 3 kDa cut-off membrane tube and washed multiple times to remove urea and salts that can inhibit the PCR. The fluorescence of eluted ssDNA was measured in each cycle to follow the recovery. After the sixth cycle, a counter-selection step was performed using blank beads. Following the same protocol, ssDNA collected from cycle five was incubated with blank beads for two hours, then washed five to six times to collect ssDNA, which was then concentrated, heated, and incubated with CFTR-coupled beads.

At the end of each cycle, PCR was performed to amplify the selected sequences in 20 reaction tubes with a 50 µl reaction mixture. Each mixture was 1x Taq buffer, 2 mM $MgCl_2$, 200 µM dNTPs, 0.2 µM of forward and reverse primers, and 2 units of Taq polymerase. The conditions of the PCR were as follows: 10 min at 95 °C, then 30 sec at 95 °C for 15 cycles, 30 sec at 54 °C, 45 sec at 72 °C, and finally, an extension step for 10 min at 72 °C.

To concentrate the amplified DNA volume, ethanol precipitation was performed where 0.1 volume of 3 M sodium acetate and three volumes of cold 100 % ethanol were added and mixed by vertexing, then kept at -80 ˚C for 1 h. Subsequently, the sample was centrifuged at 13000 x g for 30 min at 4 °C. The supernatant was removed, and the DNA pellet was washed with 75 % ethanol and then dried for 5 minutes. The pellet was resuspended in a solution of water and loading dye in formamide and then heated at 90 °C for 5 mins. The DNA was then subjected to 10 % denaturing polyacrylamide gel electrophoresis (PAGE) for ssDNA separation. The fluorescent ssDNA band

was cut from the gel, added to TE buffer, and subjected to a freeze−thaw cycle where it was chilled at -80 °C for 30 mins and then heated at 90 °C for 10 minutes, followed by end-over-end rotation overnight at 37 °C . Finally, the ssDNA was concentrated and desalted using ultrafiltration, quantified by UV spectrophotometry, and used for the next selection cycle.

To clone the DNA obtained from the last round, in which the recovery nearly plateaued, the DNA was first amplified using a set of unmodified primers, followed by cloning using the TOPO TA Cloning Kit and the 1-TOPO vector. The colonies were grown on LB-agar medium involving IPTG, X-Gal, and ampicillin. Then, the white and light blue colonies were observed. These colonies were picked up one by one, and PCR was run using forward and reverse M13 primers on each colony to amplify the ssDNA inserts. Finally, PCR products underwent Sanger sequencing analysis.

Supporting figures and tables:

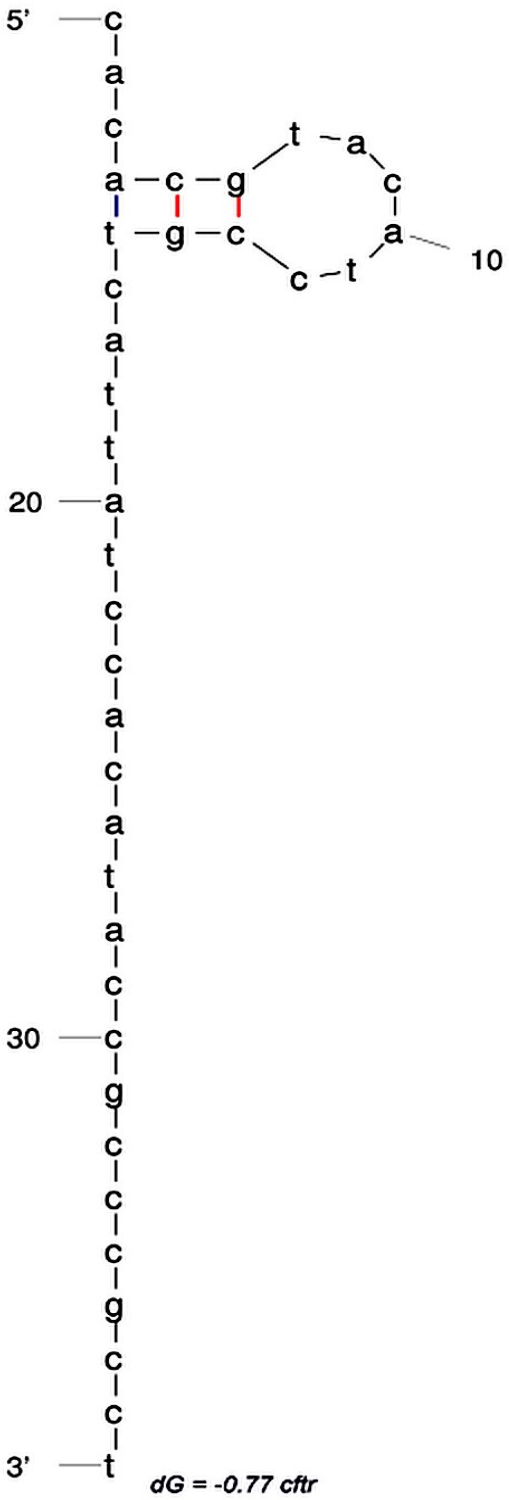

Figure S.1: Prediction of the secondary structure of CF1 aptamer by using mfold.

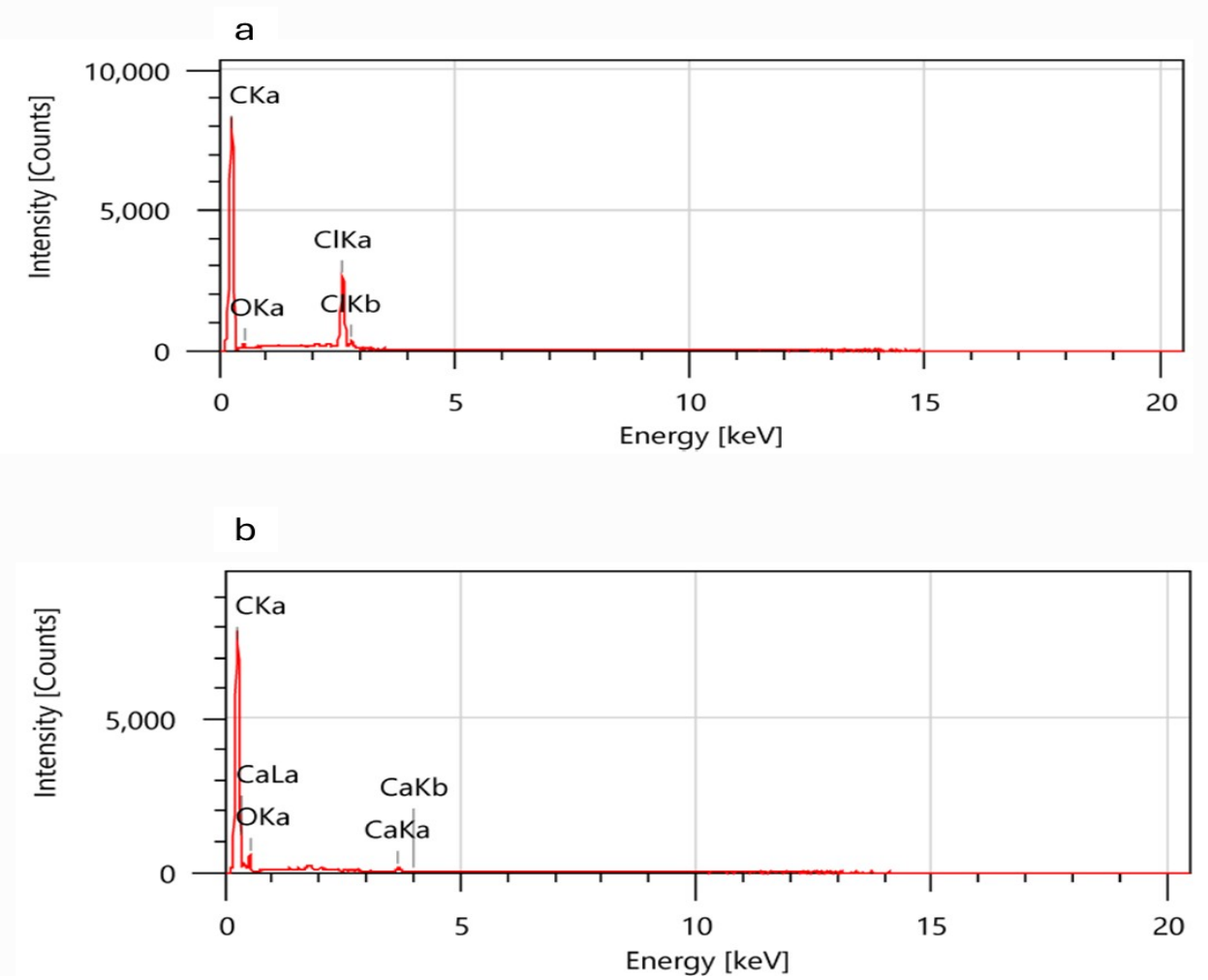

Figure S.2: EDX analysis of the screen-printed carbon electrodes before **(a)** and after deposition of the TPT-PDA-COF **(b)**.

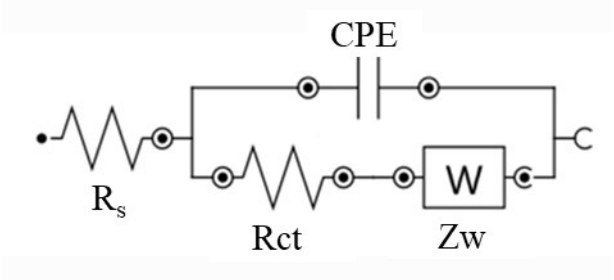

Figure S3: Schematics of the circuit used for fitting EIS results.

Table S.1: The EDS analysis results obtained for the bare SPCE and the COFs/SPCE

Element	Percentage of individual elements for the electrode before and after modification with COFs			
	Electrode before modification		Electrode after modification	
	Mass (%)	Atom (%)	Mass (%)	Atom (%)
C	88.75+0.16	94.89+0.17	85.06+0.16	88.91+0.17
O	2.35+0.07	1.88+0.05	13.59+0.21	10.66+0.17
Cl	8.90+0.06	3.22+0.02	-	-
Ca	-	-	1.35+0.06	0.42+0.02
Total	100	100	100	100