Supplementary Information

Multifunctional Electrochemical Aptasensor for Aptamer Clones Screening, Virus Quantitation in Blood and Viability Assessment

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Aptamer selection. Aptamer selection comprises ten rounds of positive selection against VACV followed by two rounds of negative selection against heat-inactivated virus, blood cells, and human blood plasma. The first ten rounds of positive selection were performed according to the following protocol. The selection was started with a synthetic ssDNA library (Integrated DNA Technologies, U.S.) which consists of a randomized region of 40 nucleotides (A:T:C:G = 25%:25%:25%:25%) flanked by two constant primer-hybridization sites, 5′-CTC CTC TGA CTG TAA CCA CG -N40- GC ATA GGT AGT CCA GAA GCC-3′. Prior to selection experiments, the ssDNA library and aptamer pools were denatured by heating for 5 min at 95 °C in Dulbecco's phosphate buffered saline, containing $CaCl_2$ and $MgCl_2$ (DPBS, Sigma-Aldrich, U.S.) and then renatured on ice for 10 min. Next, 2×10^{10} plaque forming units (PFU) of VACV (Jennerex Inc., Ottawa, Canada) were washed with DPBS, re-suspended in the buffer solution and used for the successive rounds of selection. In the first round, VACV was incubated with 1 µM (0.1 nmol or 6×10^{13} molecules) ssDNA library in 100 µL DPBS for 30 min at 25 °C and then centrifuged at 10 000 × g for 10 min at 15 °C, to remove unbound aptamers, followed by rinsing twice with DPBS. In addition, vials were changed after each centrifugation cycle to remove non-specific aptamers bound to the vial wall. Afterward, VACV was re-suspended in 35 µL of 10 mM Tris-HCl buffer containing 10 mM EDTA, pH 7.4 (TE) buffer (Integrated DNA Technologies, U.S.), and heated for 10 min at 95 °C, to release the aptamers bound to the virus. Subsequently, the viral debris was removed by centrifugation at 14,000 × g for 15 min at 4 °C and the supernatant, which contains the aptamers, was collected and stored at -20 °C. Virus-bound aptamers were amplified using symmetric and asymmetric PCR cycles, respectively. For the symmetric PCR: 5 µL of the aptamer pool in TE buffer was mixed with 45 µL of symmetric PCR Master Mix, containing the following reagents in the final concentrations: 1X PCR buffer (Promega Corporation, U.S.), 2.5 mM $MgCl_2$, 0.028 U μL^{-1} GoTaq Hot Start Polymerase

(Promega Corporation, U.S.), 220 µM dNTPs, 500 nM forward primer (5′- CTC CTC TGA CTG TAA CCA CG-3′), and 500 nM reverse primer (5′- GGC TTC TGG ACT ACC TAT GC-3′). Subsequently, asymmetric PCR was carried out where 5 µL of the symmetric PCR product in TE buffer was mixed with 45 µL of the asymmetric PCR Master Mix containing the same reagents as above, except 1 µM forward Alexa-488 primer (5′-Alexa488- CTC CTC TGA CTG TAA CCA CG-3′), and 50 nM reverse primer. Amplification was performed according to the following PCR program: preheating for 2 min at 95 °C, 15 cycles for symmetric PCR or 10-15 cycles for asymmetric PCR of 30 s each at 95 °C, 15 s at 56.3 °C, 15 s at 72 °C, and hold at 4°C. Afterward, the fluorescently labeled ssDNA was separated from the PCR mixture, primers and dNTPs with 30 kDa cut-off filters (Nanosep, PALL, U.S.) by centrifugation at 3 800 × g for 13 min at 15 °C followed by 3-times washing with 200 µL of the DPBS buffer. The purification quality was controlled using 3% agar gel electrophoresis. Subsequently, the purified aptamer pool was diluted in 100 µL DPBS and its concentration was measured by NanoDrop-2000 UV-VIS spectrophotometer (NanoDrop, U.S.) then stored at -20 °C before continuation to the next round. Finally, 100 nM of the evolved aptamer pool was utilized for the next nine rounds of selection following the same procedure.

Two rounds of negative selection were carried out using the aptamer pool (#8) which showed the highest binding affinity to VACV, according to the flow cytometry data. Firstly, the aptamer pool was incubated with heat-inactivated VACV at 37 °C for 30 min and the supernatant was collected by centrifugation at 14 000 × g for 15 min. Importantly, heat inactivation of VACV was carried out at 95 °C for 30 min. Secondly, aptamers in the supernatant were incubated with human and mouse blood cells for 30 minutes at 37 °C followed by centrifugation to collect the bound aptamers. In the last step, unbound aptamers were incubated with human blood serum for 5 min and then VACV was added directly to this mixture and incubated for another 15 min. Finally, VACV-bound aptamers were separated from the virus and collected as described above.

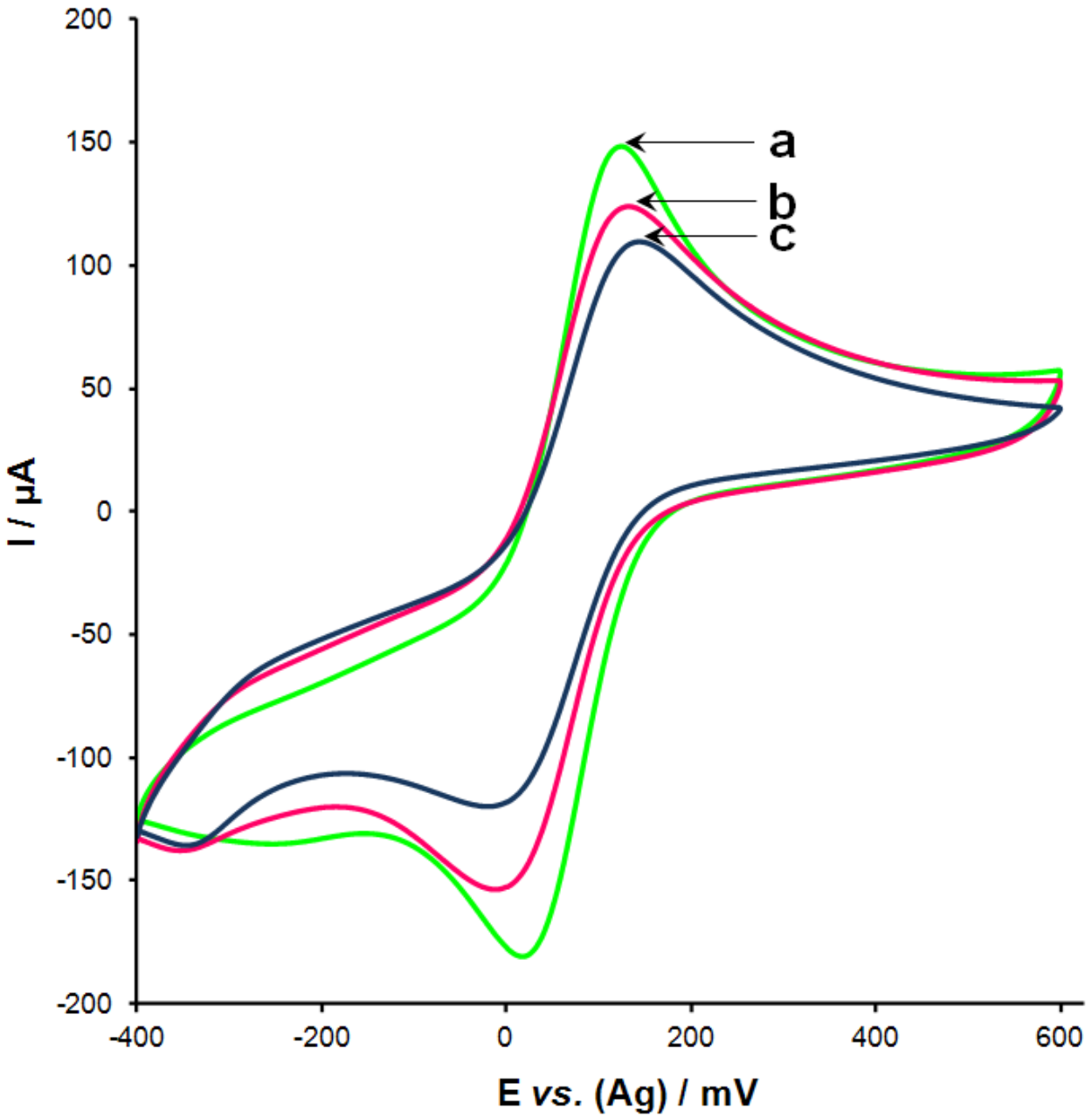

Figure S1. Cyclic voltammograms of the VACV aptasensor after each immobilization or binding step. Cyclic voltammograms were recorded at a scan rate of 100 mV s^{-1} *vs.* pseudo Ag reference, where (*a*) bare GNP-SPCE; (*b*) after coating with the hybridization product of thiolated primer and VACV-specific aptamer; (*c*) after back-filling with 1 mM 2-mercaptoethanol. Electrochemical measurements were performed in 2.5 phosphate buffer (pH 7), containing 4 mM $K_3[Fe(CN)_6]$ and 10 µM $[Ru(NH_3)_6]Cl_3$.

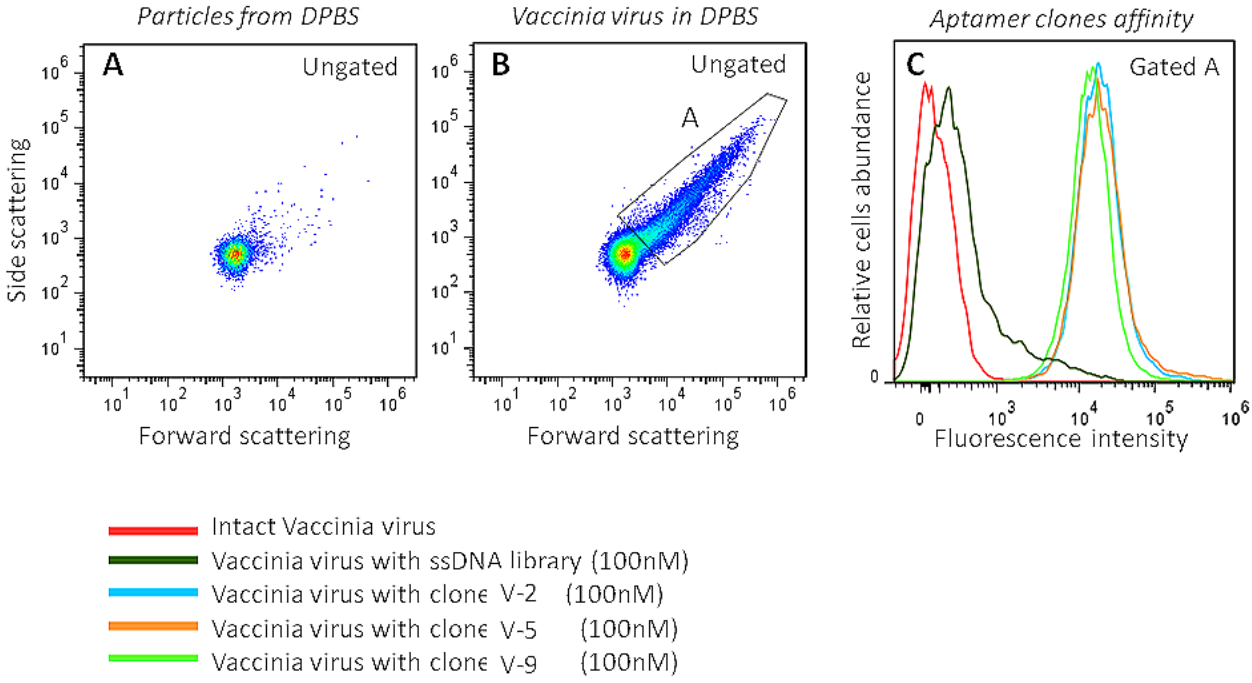

Figure S2. Flow cytometric binding analysis of 100 nM FAM-labeled aptamer clones to 1×10^7 PFU of VACV. **(A)** Two-dimensional plot of the side and forward scattering of buffer particles, **(B)** Gate A for VACV particles, and **(C)** Histograms of the binding between VACV and ssDNA library and aptamer clones (V-2, V-5, and V-9).

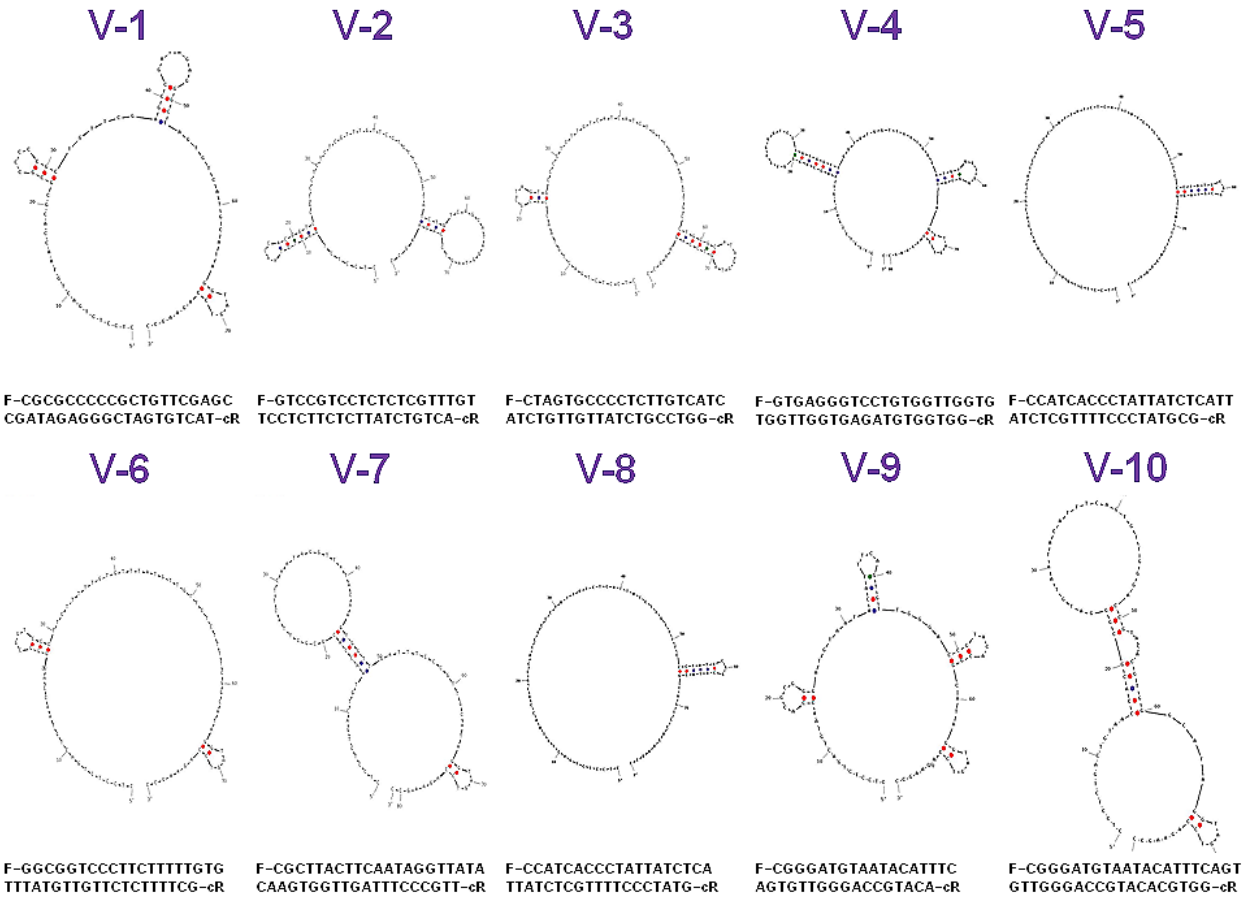

Figure S3. Sequences and predicted secondary structures of the clones isolated from the aptamer pool (#12) and showing the highest affinity to VACV, where F: CTC CTC TGA CTG TAA CCA CG (the forward PCR primer) and cR: GCA TAG GTA GTC CAG AAG CC (the reverse-complement of the reverse PCR primer).

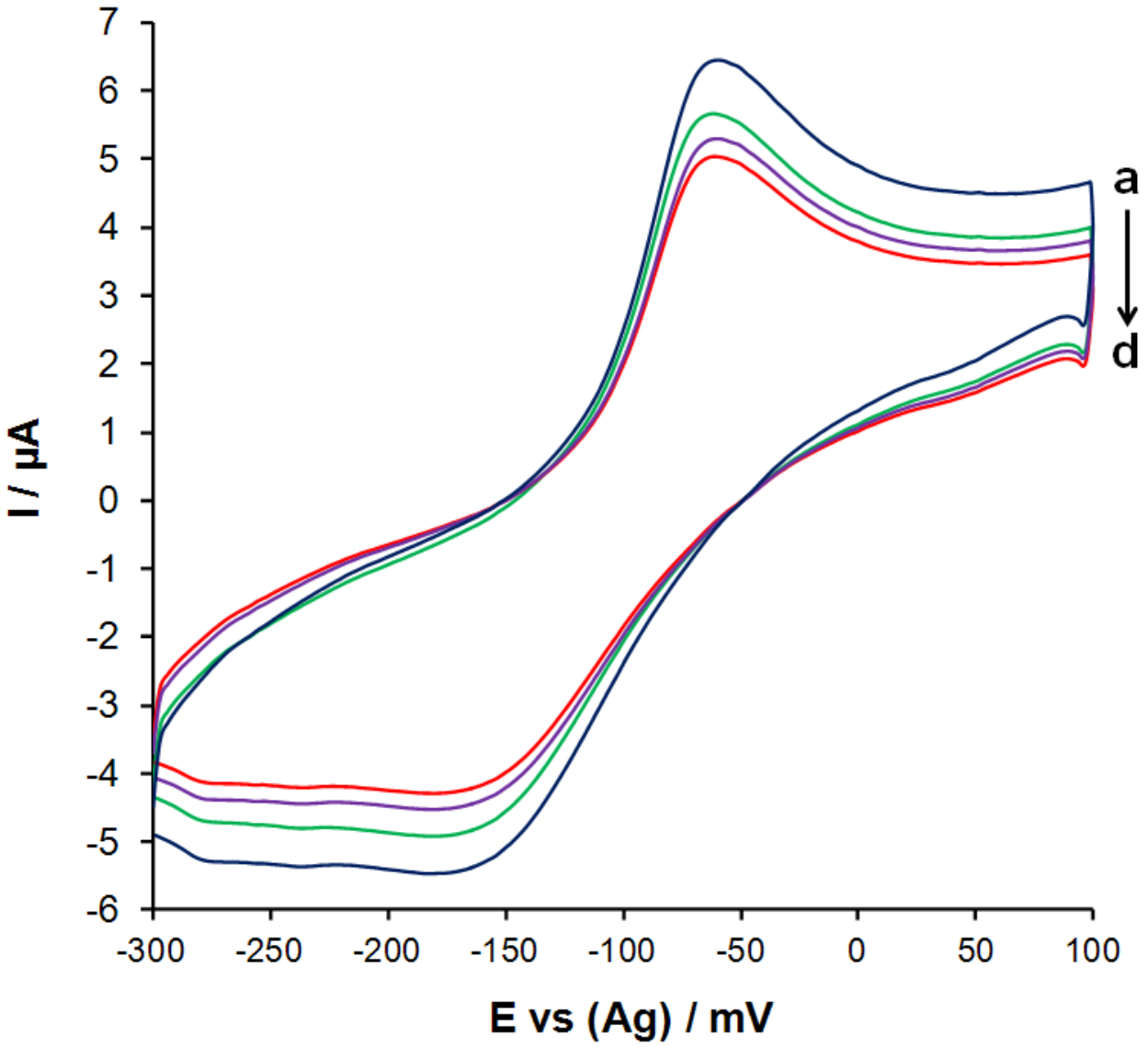

Figure S4. Cyclic voltammograms of four GNP-SPCEs in 1.0 mM $K_4[Fe(CN)_6]$/1.0 M KCl solution, using a scan rate of 100 mV s^{-1} *vs.* pseudo Ag reference.

Table S1. Equivalent circuit element values for the developed VACV aptasensor, in the presence of increasing number of virus particles

Number of virus particles (PFU)	*Rs* *(Ω)*	CPE_{ef} (µF)	n_1	R_{ef} *(Ω)*	*W* ($\mu F^{0.5}$)	CPE_f (µF)	n_2	R_f *(Ω)*
150	88.4	2.72	0.93	2318	734.1	172.7	1	21.5
300	88.3	2.16	0.91	2142	949.8	107.7	1	219.8
450	87.6	2.57	0.91	1747	111.8	104.8	0.99	705.6
600	88.3	2.49	0.9	1368	106.6	128.1	1	147
750	80.9	1.91	0.98	535.5	974	371.6	1	44.3
900	84.3	1.97	0.91	347.5	106.2	0.28	1	119.9