Electronic Supporting Information

Electrochemical Detection of pM-Levels of Urokinase Plasminogen Activator by Phosphorothioated RNA Aptamer: Improved Affinity and Suppression of Interference from Nonspecific Adsorption of BSA

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Figures

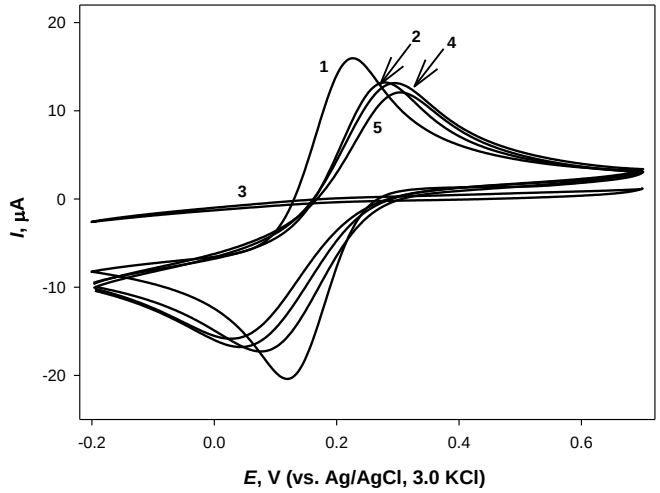

Figure S1. The CVs of (1) bare gold, (2) MC_6OH-modified, (3) BSA-modified, (4) ssDNA/MC_6OH-modified, and (5) ssDNA/MC_6OH/BSA-modified electrodes. The measurements were conducted in 20 mM PBS (containing 0.15 NaCl, pH 7.4) in the presence of 5 mM $K_3Fe(CN)_6$ as a redox indicator. BSA-modified electrodes were prepared by 30 min incubation of the corresponding electrodes in 2 mg/L BSA solution in 20 mM PBS under the lid at rt. Then the electrodes were washed in PBS and used for measurements.

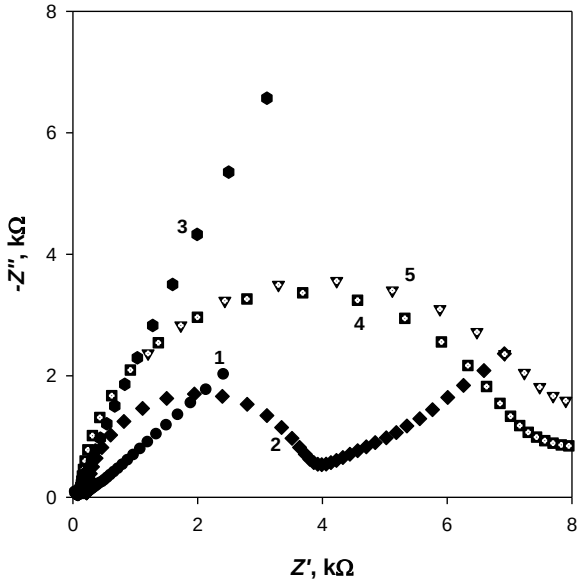

Figure S2. The Nyquist plots of (1) bare gold, (2) MC_6OH-modified, (3) BSA-modified, (4) ssDNA/MC_6OH-modified, and (5) ssDNA/MC_6OH/BSA-modified electrodes recorded in 20 mM PBS (containing 0.15 NaCl/KCl, pH 7.4) in the presence of 5 mM $K_3Fe(CN)_6$ as a redox indicator. Measurements potential was 0.2 V.

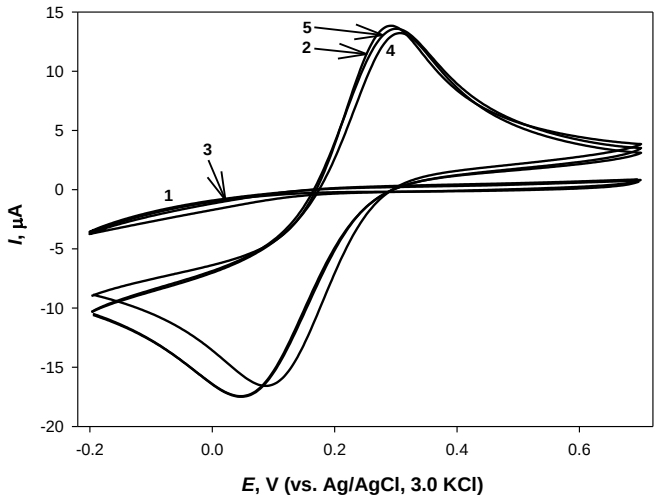

Figure S3. The CVs of (1) bare gold, (2) MC_6OH-modified, (3) BSA-modified, (4) ssDNA/MC_6OH-modified, and (5) ssDNA/MC_6OH/BSA-modified electrodes recorded in The 10% human serum diluted by 20 mM PBS (containing 0.15 NaCl, pH 7.4) in the presence of 5 mM $K_3Fe(CN)_6$ as a redox indicator.

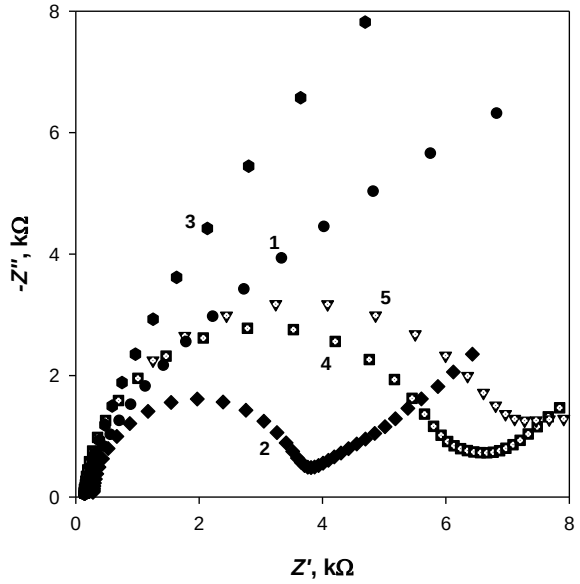

Figure S4. The Nyquist plots of (1) bare gold, (2) MC_6OH-modified, (3) BSA-modified, (4) ssDNA/MC_6OH-modified, and (5) ssDNA/MC_6OH/BSA-modified electrodes recorded 10% human serum solution in 20 mM PBS (containing 0.15 NaCl/KCl, pH 7.4) in the presence of 5 mM $K_3Fe(CN)_6$ as a redox indicator. Potential: 0.2 V.

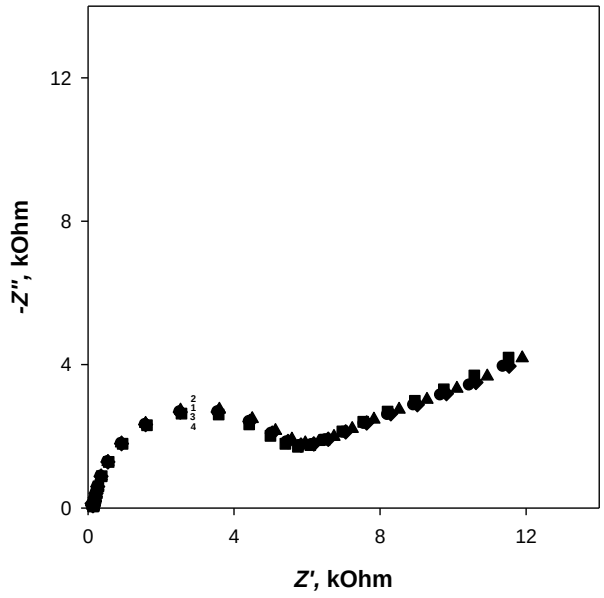

Figure S5. The EIS recorded of the MC_6OH-modified electrode recorded in 20 mM PBS, pH 7.4, containing 2 mM $K_3Fe(CN)_6$ as a redox indicator, (1) before and after 30 min incubation in (2) 1 nM, (3) 10 nM and (4) 100 nM uPA. See Experimental for more details.

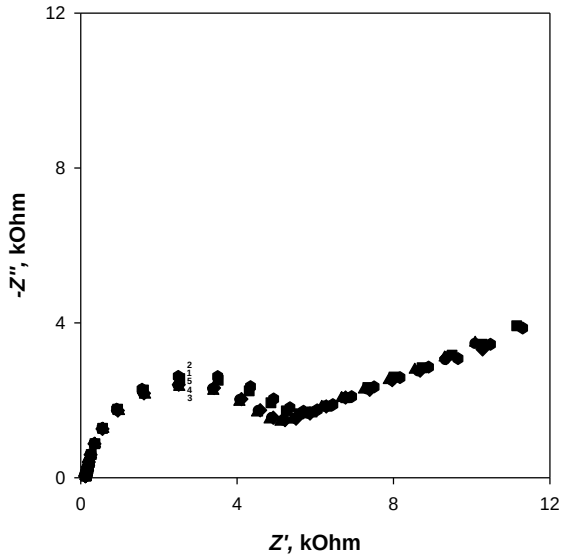

Figure S6. The Nyquist plots of the MC_6OH-modified electrode recorded in 2 mM $K_3Fe(CN)_6$ solution in (1) 20 mM PBS, after 30 min incubation in solution of (2) 100 nM BSA, (3) 1 nM uPA and 100 nM BSA, (4) 10 nM uPA and 100 nM BSA, (5) 100 nM uPA and 100 nM BSA. Potential: 0.2 V.

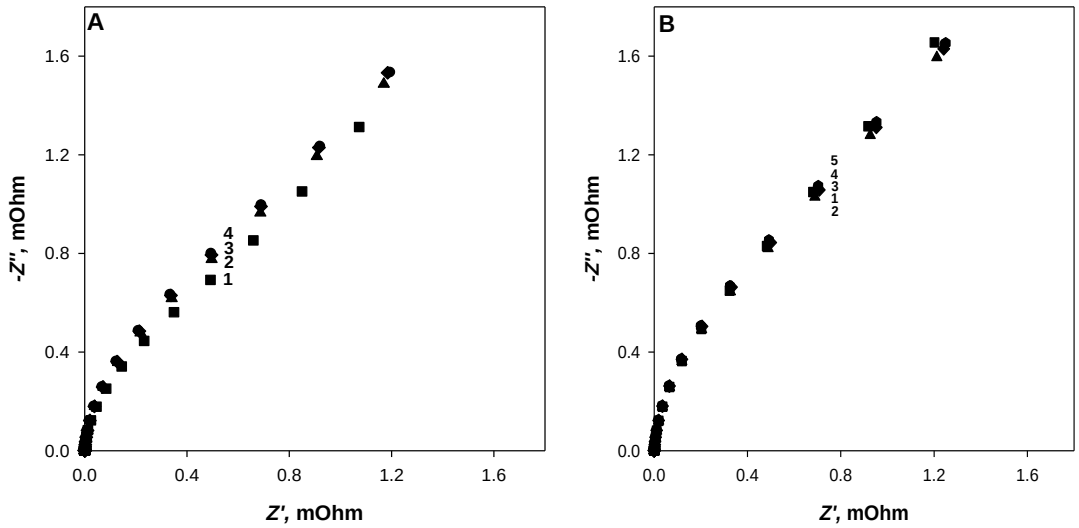

Figure S7. The Nyquist plots of SH-C_{11}-$(EG)_3$-OH modified Au electrodes recorded in 2 mM $K_3Fe(CN)_6$ solution in (A) (1) 20 mM PBS and after 30 min incubation in (2) 1 nM, (3) 10 nM, and (4) 100 nM uPA; (B) (1) 20 mM PBS, and after 30 min incubation in (2) 100 nM BSA, (3) 1 nM uPA and100 nM BSA, (4) 10 nM uPA and 100 nM BSA, and (5) 100 nM uPA and 100 nM BSA. Potential: 0.2 V.

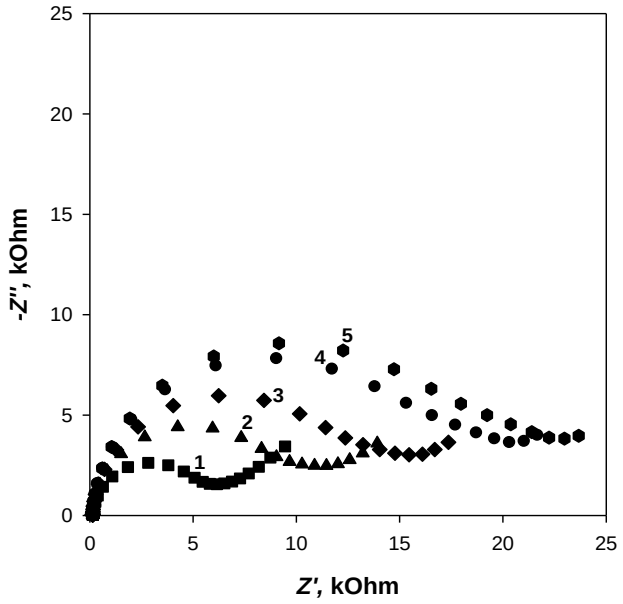

Figure S8. The Nyquist plots of the aptamer/MC_6OH-modified electrode recorded in 2 mM $K_3Fe(CN)_6$ in (1) 20 mM PBS, and after 30 min incubation in solution of (2) 100 nM BSA, (3) 1 nM uPA and 100 nM BSA, (4) 10 nM uPA and 100 nM BSA, (5) 100 nM uPA and 100 nM BSA.

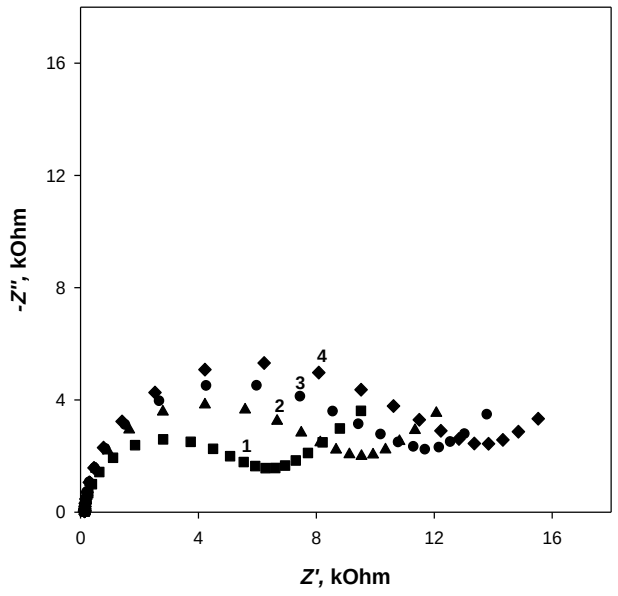

Figure S9. The Nyquist plots of the aptamer/MC_6OH-modified electrode recorded in 2 mM $K_3Fe(CN)_6$ in (1) 20 mM PBS and after 30 min incubation in (2) 1 nM BSA, (3) 10 nM BSA, and (4) 100 nM BSA.

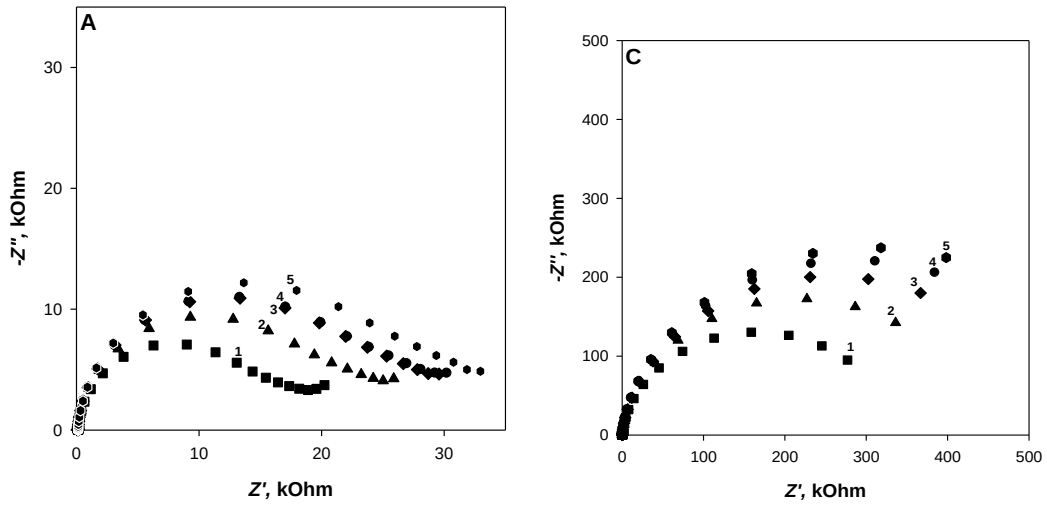

Figure S10. The Nyquist plots of (A) the aptamer/MC_6OH and (C) aptamer/SH-C_{11}-$(EG)_3$-OH-modified electrodes recorded in 2 mM $K_3Fe(CN)_6$ solution in (1) 20 mM PBS, before and after 30 min incubation in (2) 100 nM BSA, (3) 1 nM uPA and 100 nM BSA, (4) 10 nM uPA and 100 nM BSA, (5) 100 nM uPA and 100 nM BSA. Potential: 0.2 V.

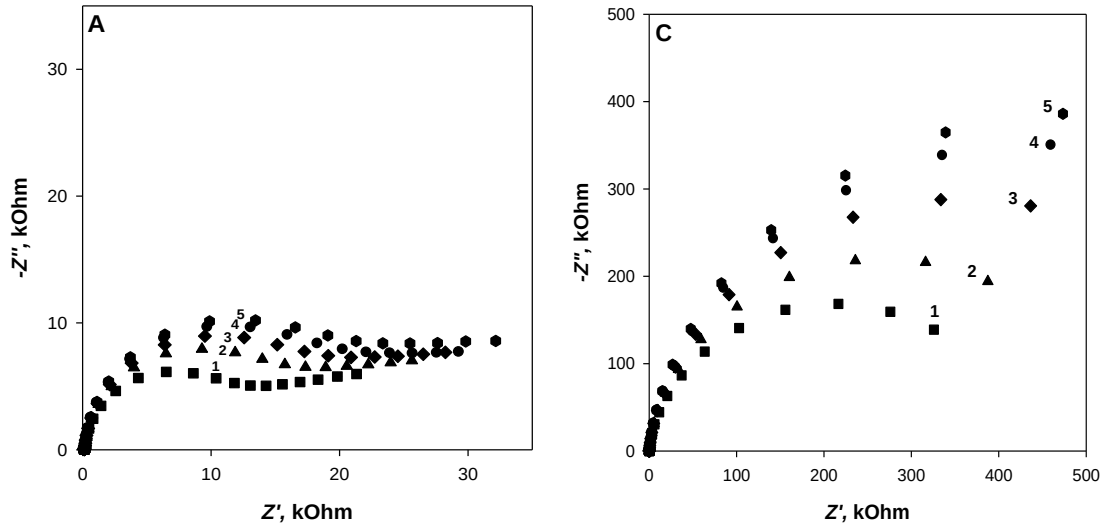

Figure S11. The Nyquist plots of (A) the aptamer/MC_6OH and (C) aptamer/SH-C_{11}-$(EG)_3$-OH-modified electrodes recorded in 2 mM $K_3Fe(CN)_6$ solution in (1) 20 mM PBS, after 30 min incubation in solution of (2) 100 nM BSA and 15 µM SDS, (3) 1 nM uPA, 100 nM BSA and 15 µM SDS, (4) 10 nM uPA, 100 nM BSA and 15 µM SDS, (5) 100 nM uPA, 100 nM BSA and 15 µM SDS. Potential: 0.2 V.

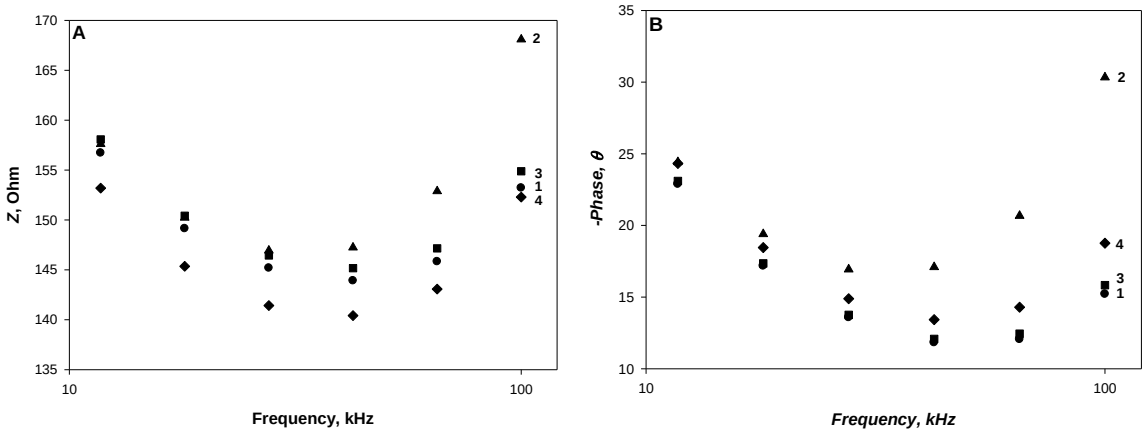

Figure S12. Dependence of (A) the impedance and (B) phase shifts on the frequency (a high frequency range) recorded with the aptamer/MC_6OH-modifed electrode in 2 mM $K_3Fe(CN)_6$ solution in (1) 20 mM PBS, before and after 30 min incubation in (2) 1 nM, (3) 10 nM and (4) 100 nM uPA. Potential: 0.2 V.

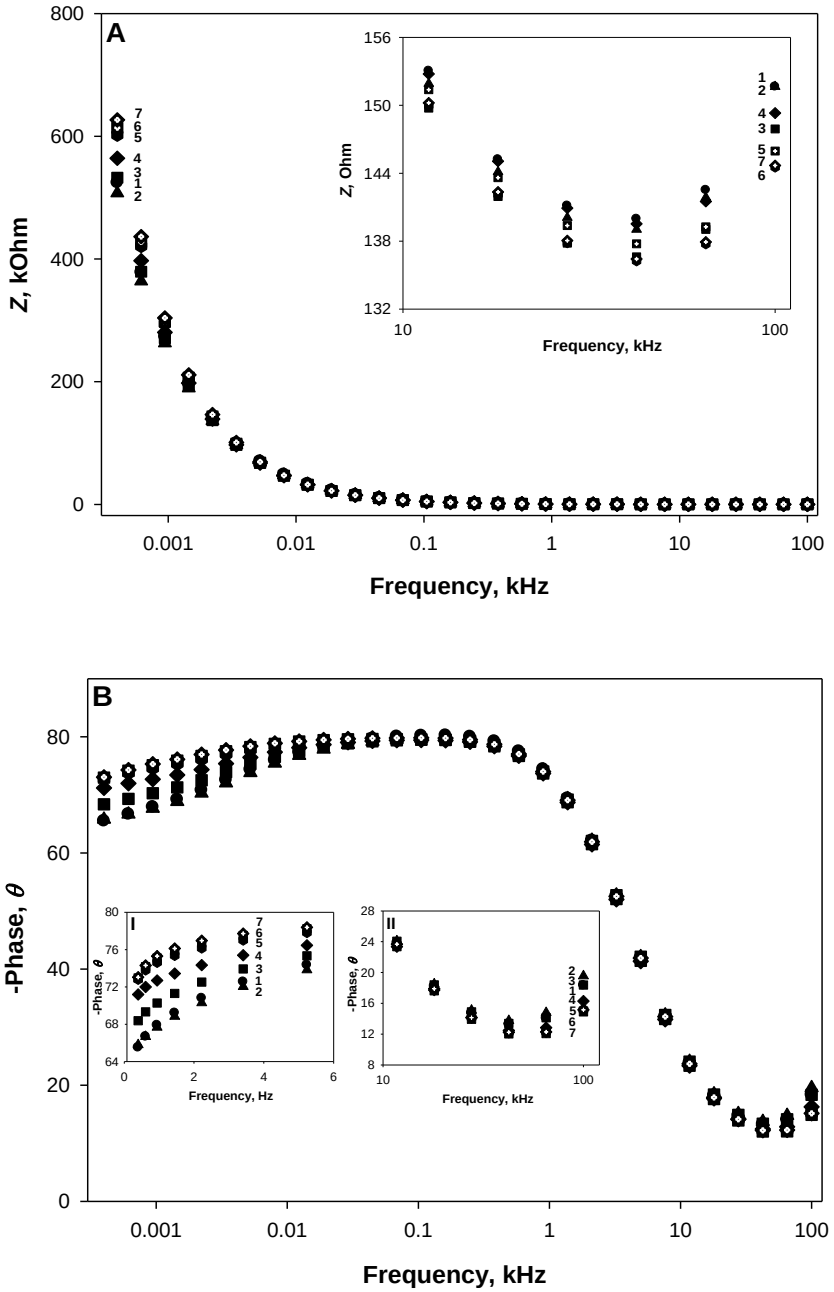

Figure S13. Dependence of (A) the impedance and (B) phase shifts on the frequency recorded with the aptamer/MC_6OH-modifed electrode in 1 μM MB solution in (1) 20 mM PBS, pH 7.4, before and after 30 min incubation in (2) 1 pM, (3) 10 pM, (4) 100 pM, (5) 1 nM, (6) 10 nM and (7) 100 nM uPA. Potential: -0.225 V.

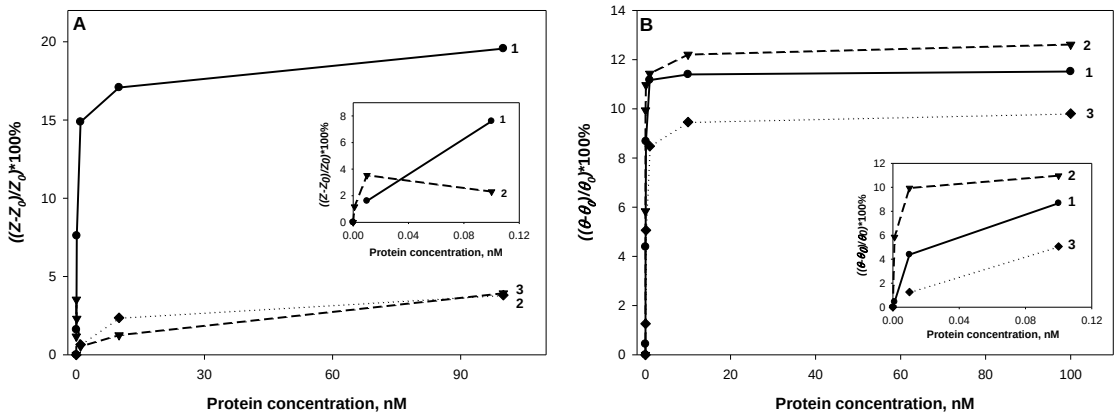

Figure S14. Dependences of (A) the normalised total impedance variation and (B) phase angle change on protein concentration, at the frequency of 0.4 Hz. Potential: -0.225 V. Original spectra were recorded with the aptamer/MC_6OH-modified electrode in 1 μM MB solution in 20 mM PBS, pH 7.4, before and after incubation in solutions of (1) uPA, (2) uPA and 100 nM BSA and (3) 100 nM BSA.

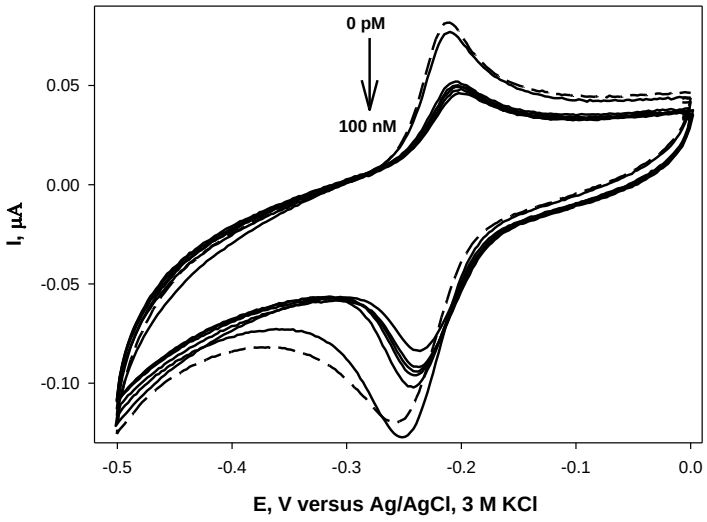

Figure S15. Representative CVs recorded with the aptamer/MC_6OH - modified electrode in 1 μM MB solution in 20 mM PBS, pH 7.4, (dashed line) before and (solid lines) after incubation in uPA solutions of different concentrations in presence of 100 nM BSA, potential scan rate 0.1 V s^{-1}.

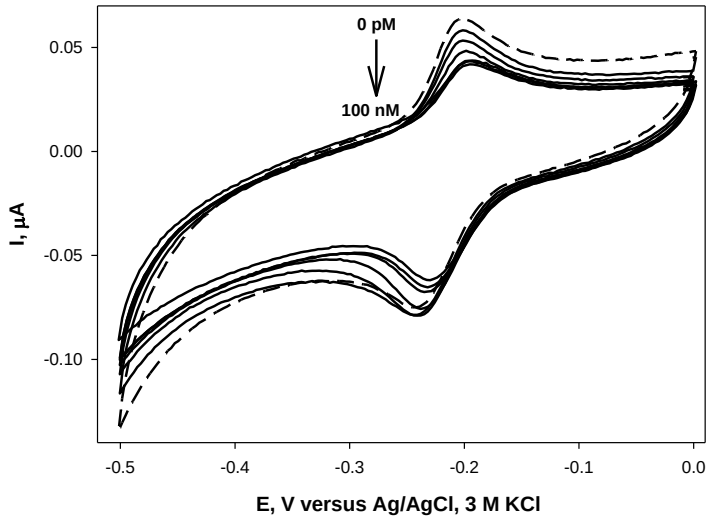

Figure S16. Representative CVs recorded with the aptamer/MC_6OH - modified electrode in 1 µM MB solution in 20 mM PBS, pH 7.4, (dashed line) before and (solid lines) after incubation in BSA solutions of different concentrations, potential scan rate 0.1 V s^{-1}.

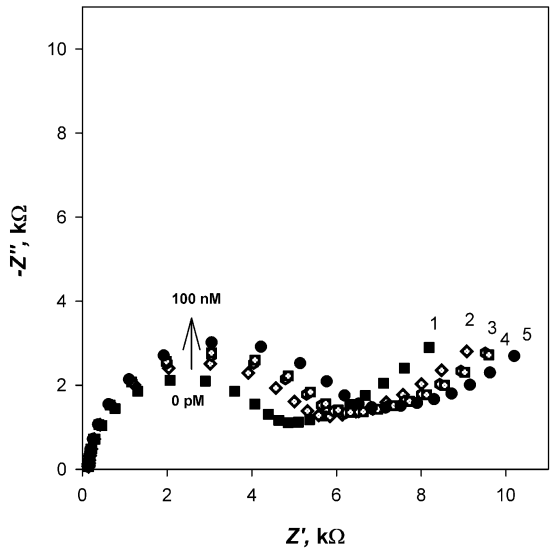

Figure S17. The Nyquist plots of the aptamer/MC_6OH modified electrodes recorded in 2 mM $K_3Fe(CN)_6$ solution in (1) 20 mM PBS, after 30 min incubation in solution of (2) 10% serum, (3) 1 nM uPA, 10% serum, (4) 10 nM uPA, 10% serum, (5) 100 nM uPA, 10% serum. Potential: 0.2 V.

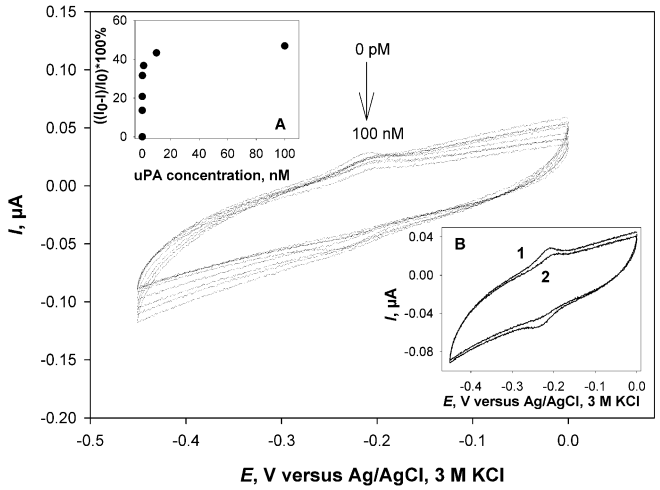

Figure S18. Representative CVs recorded with the aptamer/MC_6OH - modified electrode in 1 µM MB solution in 20 mM PBS, pH 7.4, (1) before and (2) after incubation in 10% serum solutions (inset B) containing different concentrations of uPA (1, 10, and 100 pM, 1, 10, and 100 nM), potential scan rate 0.1 V s^{-1}. Inset A:

Tables

Table S1. Comparison of dissociation constants (K_d) obtained for the aptamer/MC_6OH modified electrode at negatively charged (q-, methylene blue as redox indicator) surface.

(q^-)	$\boldsymbol{K_d}$ / nM from $\Delta\|Z\|$	$\boldsymbol{K_d}$ /nM from $(\theta_0-\theta)/\theta_0)*100\%$
uPA	0.15	0.019
uPA + 100 nM BSA	-	0.001
BSA	6.66	0.09