

Supplementary Materials

An Impedimetric Biosensing Strategy Based on Bicyclic Peptides as Bioreceptors for Monitoring h-uPA Cancer Biomarkers

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Table S1. Comparison of biosensing strategies developed recently for h-uPA detection spiked buffer solutions and biological fluids.

Biorecognition layer components	Detection strategy	Matrices tested	Dynamic range (ng mL ⁻¹)	LOD (ng mL ⁻¹)	LOQ (ng mL ⁻¹)	Sample volumes (μL)	Assay time*	Reference
Bicyclic peptide	Impedimetric	PBS**	10–1×10 ²	9	30	20	~ 45 min	Present
Bicyclic peptide	Voltammetric (DPV)	PBS + Diluted human serum	0–5×10 ²	32.5	-	10	~ 1 h	[1]
Antibody	Fluorescence	PBS + Fetal Bovin Serum +	3.3–3.3×10 ³	3.3	-	20	~ 1 h	[2]
Antibody	Photoelectrochemical	PBS + Human serum	1×10 ⁻⁴ –1×10 ³	3.3×10 ⁻⁵	-	30	~ 1 h	[3]
Aptamer	Impedimetric Voltammetric	PBS + Diluted human serum	3.3×10 ⁻² –33	3.3×10 ⁻²	-	-	~ 30 min	[4]

*Assay time is referred to the time of incubation of h-uPA and analysis. ** Phosphate buffer saline

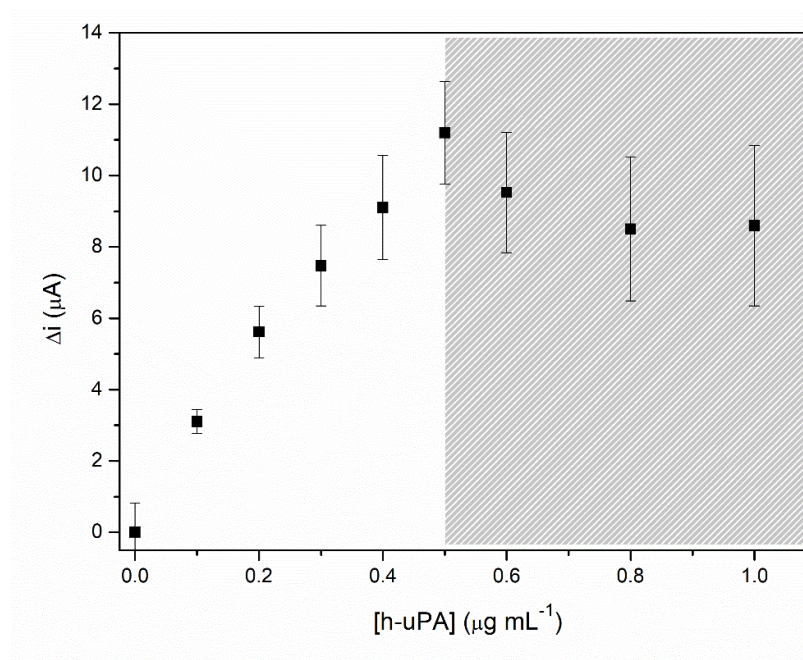


Figure S1. The response of P₃-based assay in presence of h-uPA concentrations ranging 0.1 to 1 μg mL⁻¹.

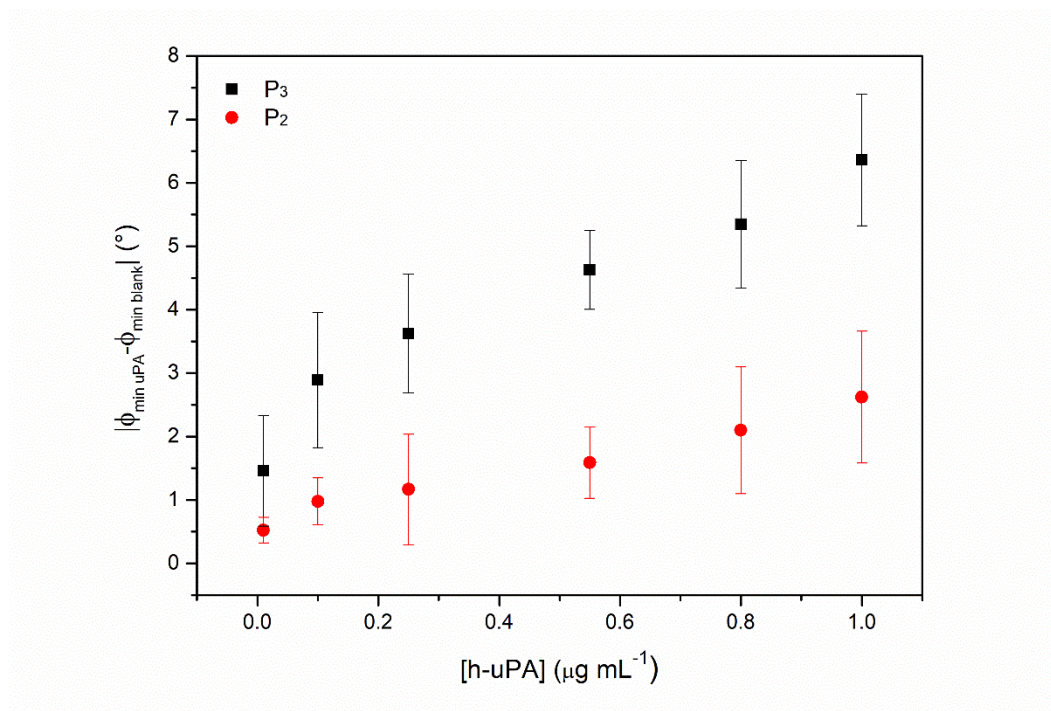
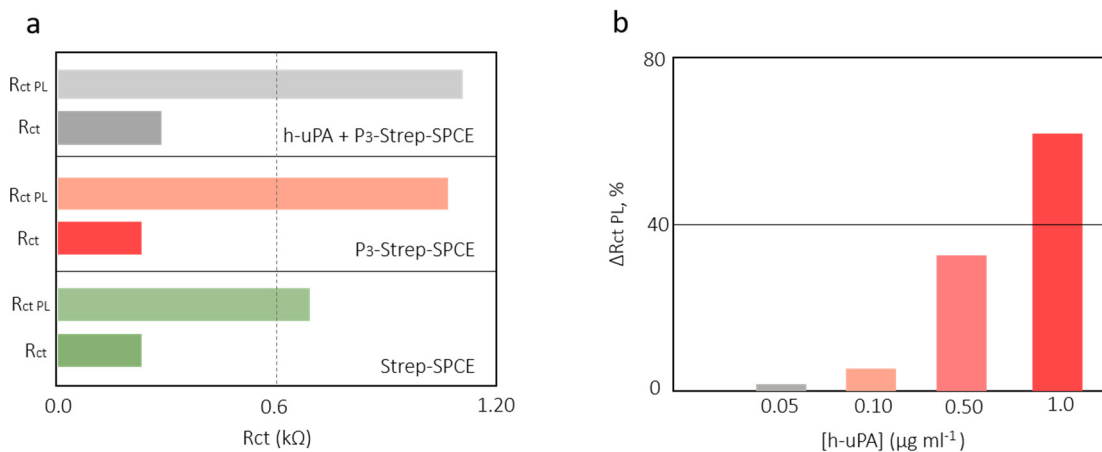


Figure S2. Comparison between the calibration plots obtained with P₂ (red circles) and P₃ (black squares) as bioreceptors in this impedimetric-based assay. The calibration curve of P₃-based assay shows a greater linear slope compared to P₂ one. This trend is consistent with the ones observed for the voltammetric sandwich type assay presented in **Figure 3**. The choice of P₃ as bioreceptor provides a higher sensibility to the platform compared to P₂. The error associated to the response of the two platforms expressed as standard deviation has the same order of magnitude for both P₂ and P₃.



Type of SPCE	Rs, Ω	Rct, kΩ	Rct PL, kΩ
Bare-SPCE	41.2 ± 2.3	2.10 ± 0.07	-
Strep-SPCE	36.1 ± 0.7	0.25 ± 0.01	0.75 ± 0.05
P₃-Strep-SPCE	31.2 ± 1.8	0.27 ± 0.01	1.17 ± 0.07
h-uPA (0.1 µg mL⁻¹)	36.4 ± 2.4	0.34 ± 0.01	1.20 ± 0.08

Figure S3. (a) Comparison of the Rct and RctPL values of Strep-SPCE, P₃-Strep-SPCE, h-uPA-P₃-Strep-SPCE. The values were obtained fitting the Nyquist plots in Figure 4a with the EECs in Figure 4c–e described in the main text. (b) Relative variation of Rct PL upon incubation of samples spiked with increasing concentration of h-uPA. (c) Summary of the values of all resistance components present in the EECs used to fit the EIS data.

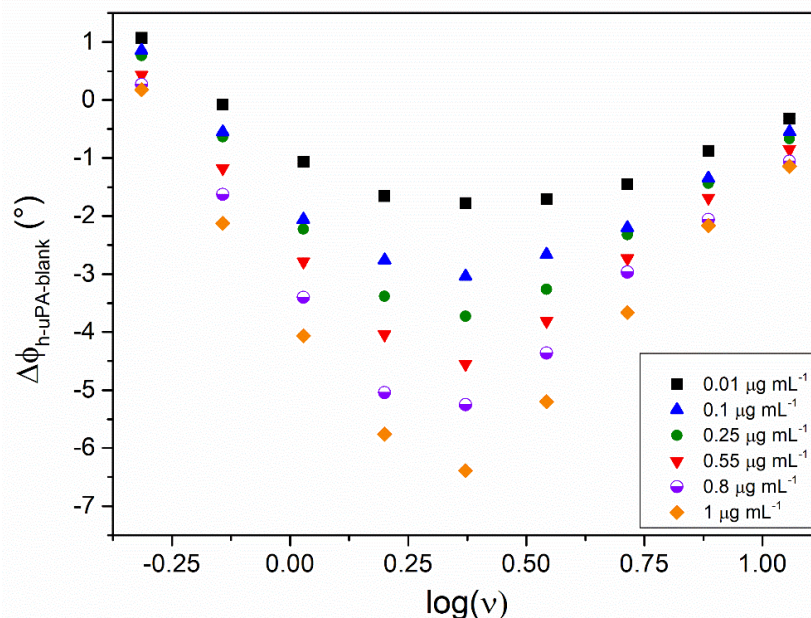


Figure S4. Bode phase peaks, subtracted from the respective blanks, of the 6 h-uPA concentrations tested in the impedimetric P₃-based platform.

References

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