MICROCONTACT IMPRINTING BASED CAPACITIVE BIOSENSORS FOR REAL-TIME PROTEASE DETECTION/QUANTIFICATION

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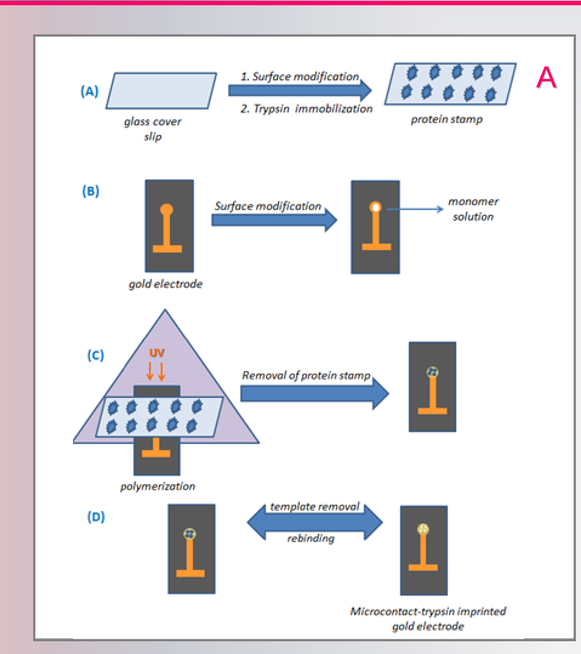
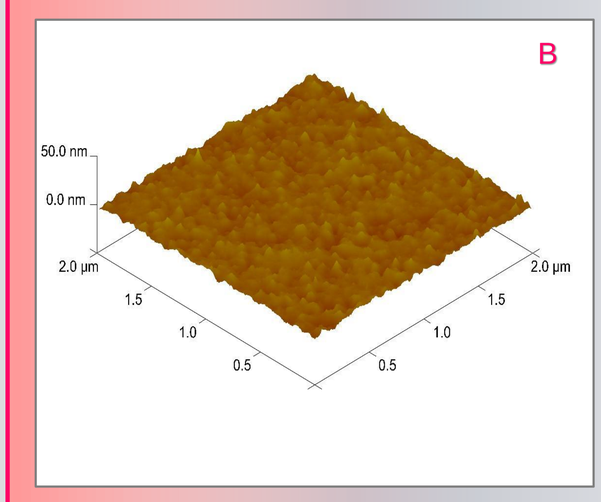
Abstract: Capacitive biosensors are the type of label-free biosensors which are usually referred to the subcategory of impedance biosensors in which the change in capacitance value (ΔC) is measured directly [1]. The main advantages of capacitive systems are the ease of detection, low power consumption, higher sensitivity when compared to other label-free biosensors and the flexibility in sensor size.

Microcontact imprinting technique is based on the microcontact printing which is used to manufacture patterned surfaces [2]. It allows for rapid, parallel synthesis of MIPs in different compositions. Only a few microliters of monomer solution are enough and dozens of samples can be polymerized in the same polymerization batch. A small mass of template is presented as a monolayer to the recognition surface the imprint will be formed in. Consequently, microcontact imprinting technique allows the preparation of highly sensitive, selective, low-cost and stable recognition surfaces useful for e.g. biosensors.

In the study presented, a capacitive biosensor was developed for protease detection. Trypsin was chosen as a model protease. Microcontact imprinting method was used for imprinting of trypsin onto the capacitive sensor electrode.





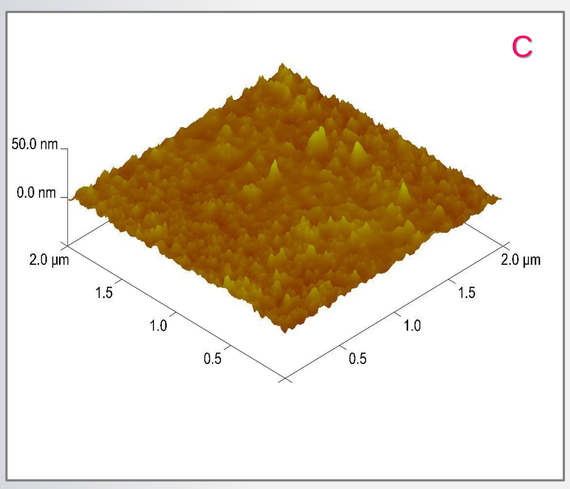


Figure 1. (A) Schematic representation of the microcontact-trypsin imprinted capacitive biosensor. [(A) Preparation of the glass cover slips (protein stamps), (B) Preparation of the capacitive gold electrodes, (C) Microcontact imprinting of trypsin onto the gold electrode surface via UV polymerization, (D) Removal of template protein (trypsin) from the electrode surface]; (B) AFM image of bare gold electrode; (C) AFM image of trypsin-MIP electrode.

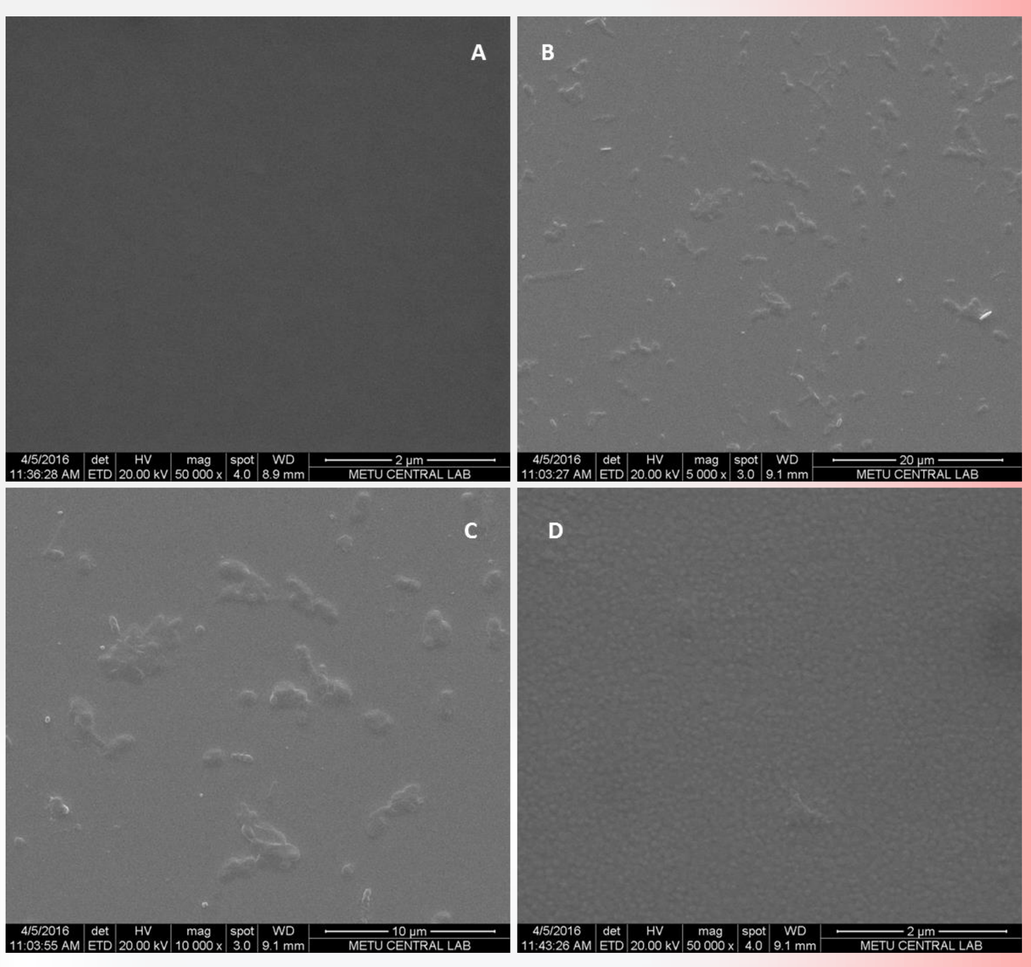
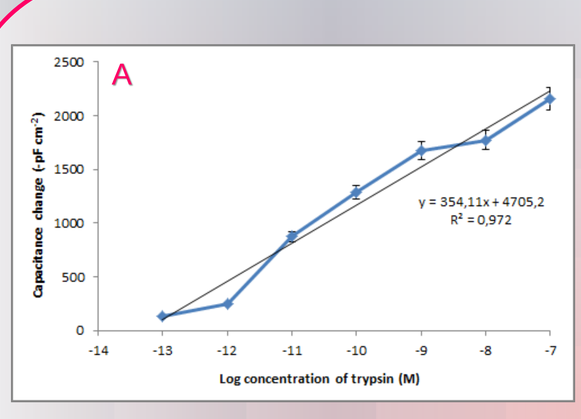


Figure 2. SEM images of bare gold electrode (A-50000 X) and trypsin-MIP electrode in different magnifications (B-5000X), (C-10000X), (D-50000X).



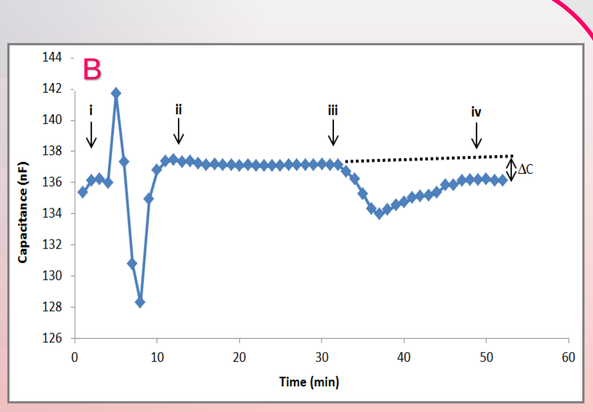


Figure 3. (A) Capacitance change vs. logarithm of trypsin concentrations for trypsin-MIP electrode under optimum conditions, (B) Actual sensorgram showing the capacitance change of trypsin-MIP electrode after injection of trypsin solution (1.0 x 10^{-11} M) under optimum conditions (i: before regeneration, ii: after regeneration, iii: before injection of trypsin solution, iv: after injection and re-equilibration with the running buffer) (flow rate: 100 µL.min^{-1}; sample volume: 250 µL; running buffer: 10 mM phosphate, pH: 7.4; regeneration buffer: 50 mM glycine-HCl, pH: 2.5 ; T: 25 °C).The dotted line is an extension of the stable baseline before injection, and ΔC is the change in signal registered upon injection of a trypsin containing sample.

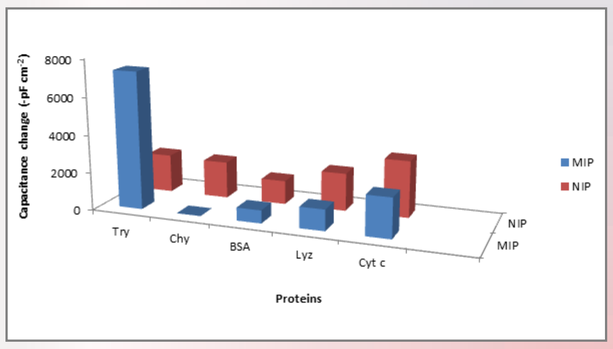


Figure 4. Selectivity of trypsin-MIP capacitive biosensor against competing proteins [chymotrypsin (chy), bovine serum albumin (BSA), lysozyme (lyz) and cytochrome c (cyt c)] in singular manner (protein concentration 1.0 mg mL^{-1}).

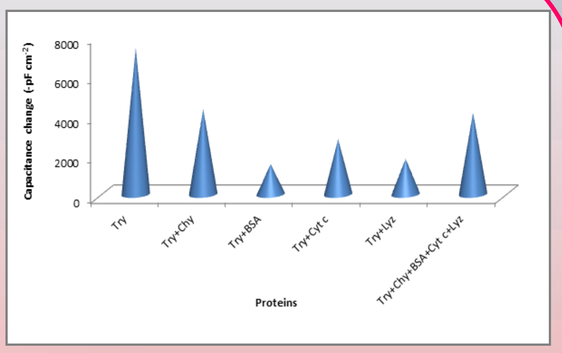


Figure 5. Cross-reactivity of trypsin-MIP capacitive biosensor against competing proteins in pre-mixed solutions (protein concentration 1.0 mg mL^{-1}).

Protein	ΔC (pF) MIP	ΔC (pF) NIP	Selectivity coefficient (k) (MIP)	Selectivity coefficient (k) (NIP)	Relative selectivity coefficient (k')
Trypsin	7331	2044	-	-	-
Chymotrypsin	10	1966	733.1	1.04	705.1
BSA	694	1266	10.56	1.61	6.54
Lysozyme	1128	2011	6.50	1.02	6.39
Cytochrome c	2118	3014	3.46	0.68	5.10

Table. Selectivity coefficients of trypsin-MIP and NIP electrodes.

Discussion: The developed capacitive system might be used successfully to monitor label-free, real-time enzymatic activity of different proteases in a sensitive, rapid, inexpensive manner in biotechnological, environmental, clinical applications.

References

[1] Erlandsson D, Teeparuksapun K, Mattiasson B, Hedström M (2014) *Sensors and Actuators B: Chemical,* 190: 295-304.

[2] Ertürk G, Berillo D, Hedström M, Mattiasson, B (2014) *Biotechnology Reports,* 3: 65-72.

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