IN FLOW SENSITIVE APTASENSING LYSOZYME USING A POLYMERIC AND METALLIC NANOSTRUCTURED PLATFORM

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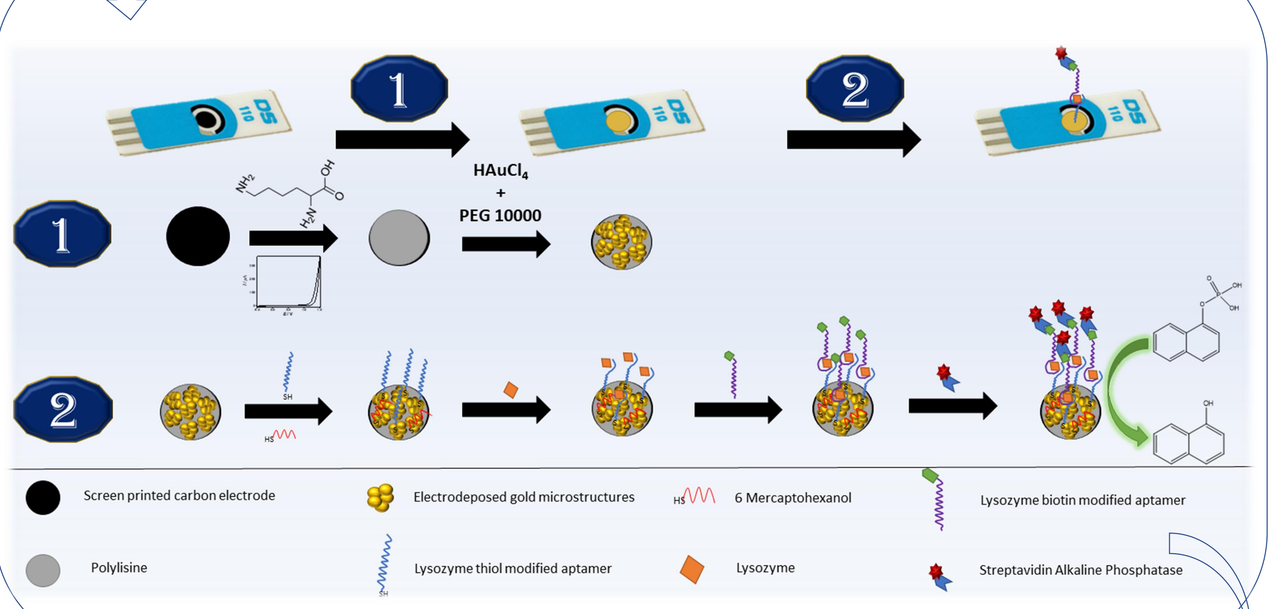
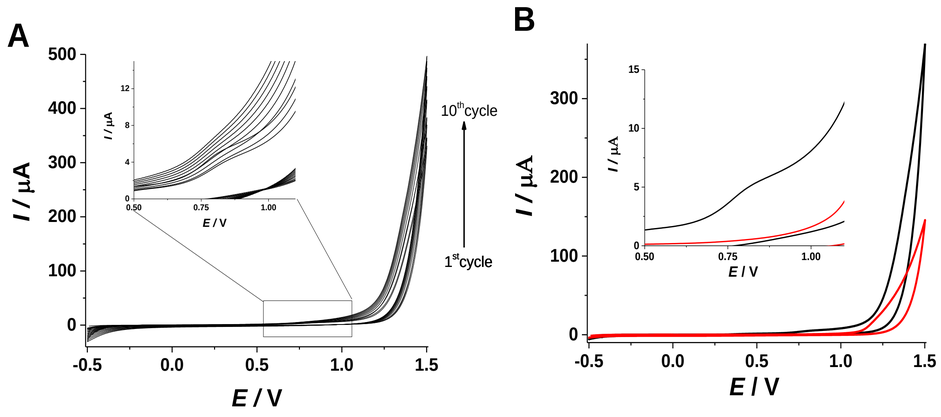
Introduction

- Lysozyme is an enzyme present in multiple organisms where it plays various vital roles. One of the most important relies on its antibacterial activity, being also called the body's own antibiotic. Despite its proven utility, lysozyme can potentially trigger allergic reactions in sensitive individuals, even in trace amounts, thus the need of continue monitoring of lysozyme in products rich in lysozyme like wine or egg white is of high importance [1].
- An electrochemical aptasensor for specific Lysozyme recognition and quantification using a gold nanostructured platform was designed.
- In a first step, Poly-L-Lysine was electropolymerized on a Screen printed carbon electrode (SPCE) cell from a L-Lysine solution in order to obtain a more uniform surface with a better conductivity.
- Secondly, gold was electrodeposited using a multipulse assisted procedure, from a $HAuCl_4$ and PEG 10 000 [2] solution, and compared with a platform containing gold electrodeposited from a solution free of PEG.
- During the platform development, several parameters were optimised:
  - L-Lysine concentration and polymerisation procedure;
  - $HAuCl_4$ concentration;
  - PEG 10 000 concentration.
- Preliminary studies from the electrochemical aptasensor development are presented in this work.

Graphical abstract



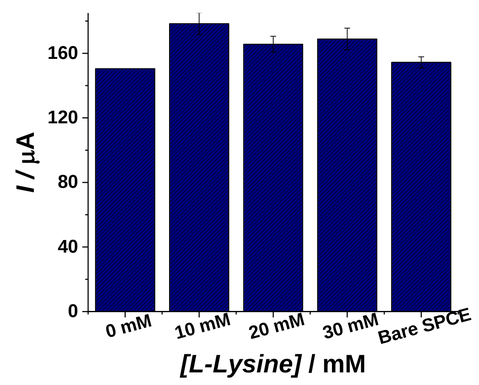
L-Lysine Polymerization



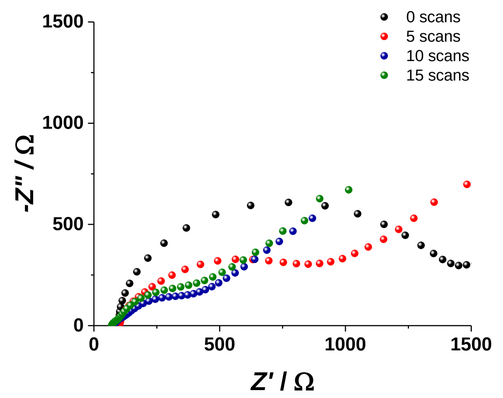
A. Poly-L-Lysine electrodeposition using a CV procedure (10 cycles, -0.5 – 1.5 V, 100 mV s^{-1}) from a 20 mM Lysine in 0.05 M PBS pH 7.4 solution.
B. 1st CV cycle of electropolymerization from a 20 mM L-Lysine in 0.05 M PBS pH 7.4 solution (**black line**) and from a solution free of monomer (**red line**).

Optimized parameters

- **Lysine concentration**
  - 10 mM
  - 20 mM
  - 30 mM
- **CV polymerization scans**
  - 5 scans
  - 10 scans
  - 15 scans

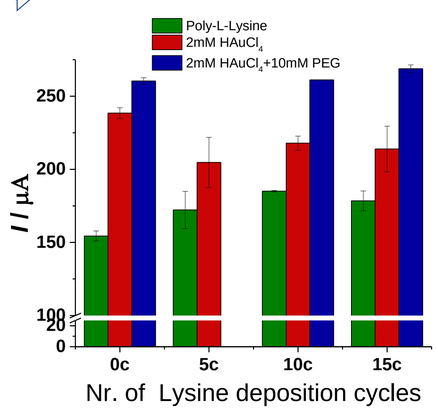


L-Lysine concentration optimisation
Medium current intensity measured in CV (-0.5 - 0.7 V, 100 mV s^{-1}) of a 5 mM $[Fe(CN)_6]^{3-/4-}$ redox probe in 0.1 M KCl.

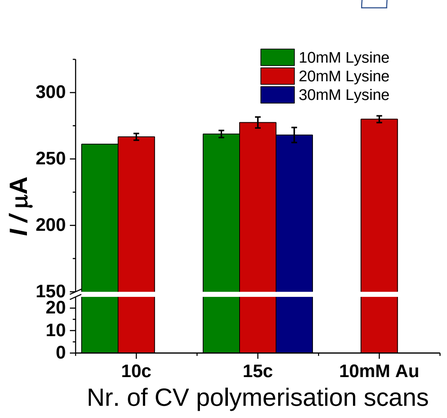


Polymerization cycles optimisation
Nyquist plots obtained in a $[Fe(CN)_6]^{3-/4-}$ redox probe in 0.1 M KCl after a 10 mM poly-L-Lysine electrodeposition (-0.5 – 1.5 V, 10 mM L-Lysine)

Gold electrodeposition

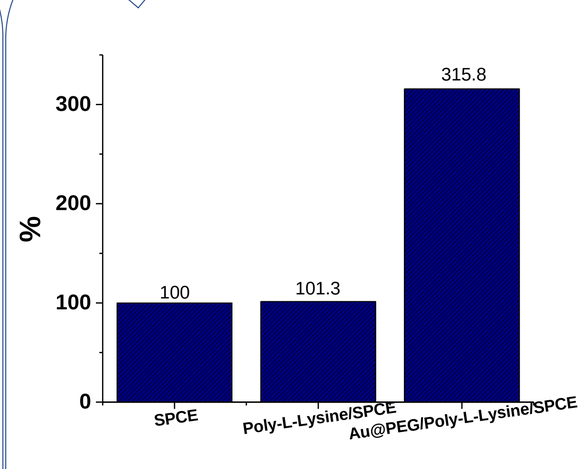


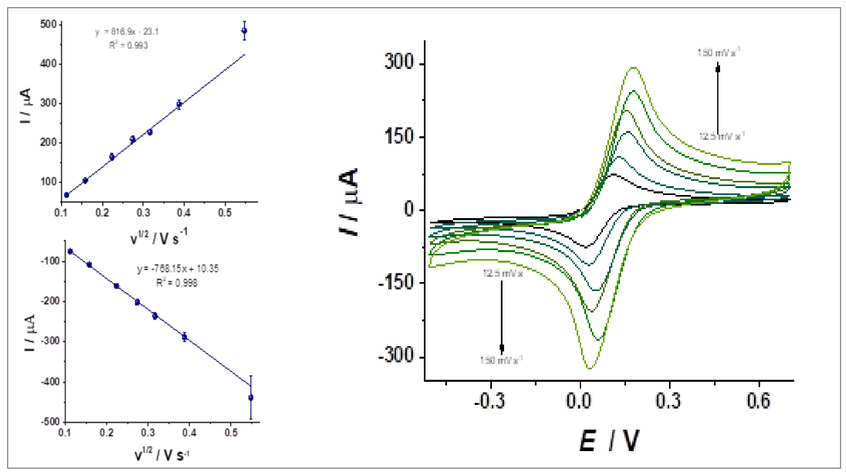
Impact of Lysine polymerization cycles using 10 mM Lysine solution and the effect of PEG addition in a 2 mM $HAuCl_4$ solution, over the electrocatalytic effect of the platform.
The average current intensities of anodic and cathodic response in CV of a 5 mM $[Fe(CN)_6]^{3-/4-}$ redox probe are presented.



Impact of L-Lysine concentration on gold structures deposition using a $HAuCl_4$@PEG mixture solution. The average current intensities of anodic and cathodic response in CV of a 5 mM $[Fe(CN)_6]^{3-/4-}$ redox probe are presented.

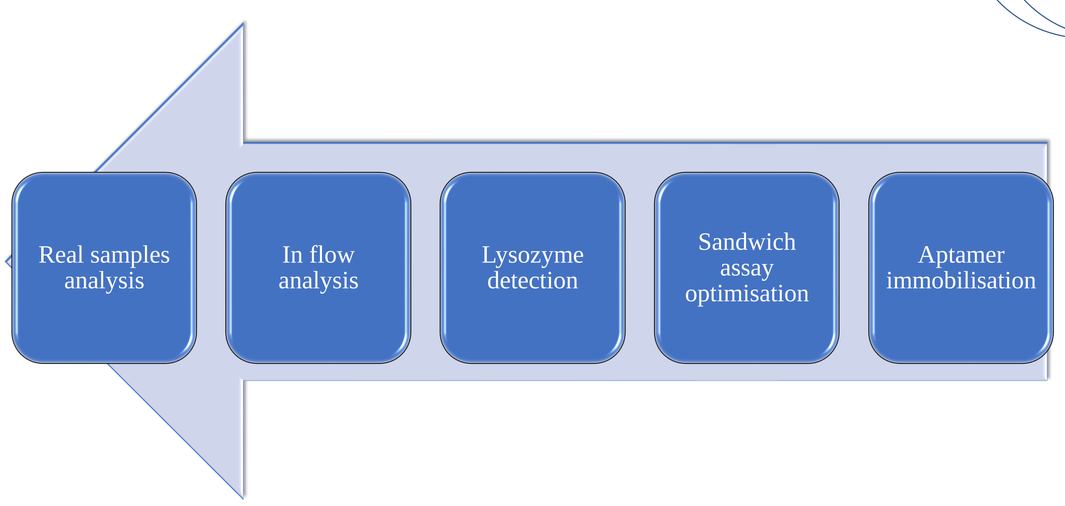
Electrochemical characterisation





Electroactive areas of Au@PEG/poly-L-Lysine/SPCE and poly-L-Lysine/SPCE platforms were calculated and compared with bare SPCE. Cyclic voltammograms were obtained in 5 mM $[Fe(CN)_6]^{3-/4-}$ (-0.5 – 0.7 V , 12.5 – 300 mV s^{-1} scan rates). Plots of anodic and cathodic peak currents versus square root of scan rate at Au@PEG based platform are presented and Randles-Sevcik equation was used to calculate the electroactive area.

Next steps



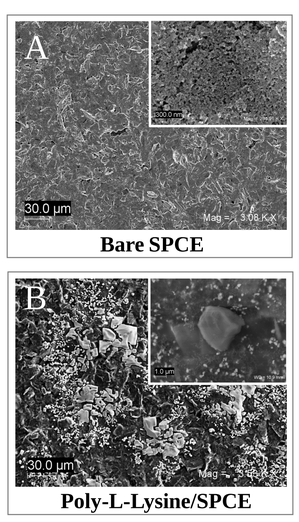
Surface characterisation

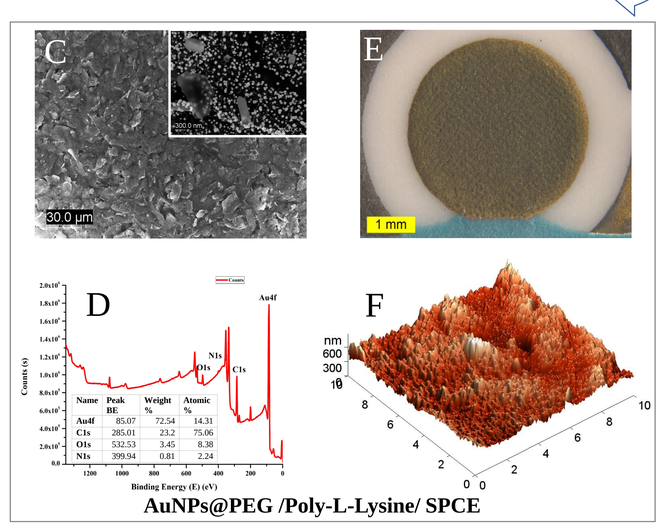
A – C: Scanning Electron Microscopy images at different magnitudes of:
A. Bare SPCE;
B. Poly-L-Lysine/SPCE
C. Au@PEG/ Poly-L-Lysine/SPCE

D: X-ray Photoelectron Spectroscopy results on the Au@PEG/Poly-L-Lysine/SPCE platform.

E: Optical image of the Au@PEG/ Poly-L-Lysine/SPCE platform.

F. Atomic Force Microscopy image of Au@PEG/ Poly-L-Lysine/SPCE platform





References
[1] A. Vasilescu, Q, Wang, M. Li, R. Boukherroub, S. Szunerits, *Chemosensors*, **2016**, *4*(2), 10
[2] M. Negahdary, H. Heli, *Talanta*, **2019**, *198*, 510-517.

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