

Electrochemical Sensing of Food Allergen Lysozyme based on Aptamer-Molecularly Imprinted Polymer Hybrid Platform

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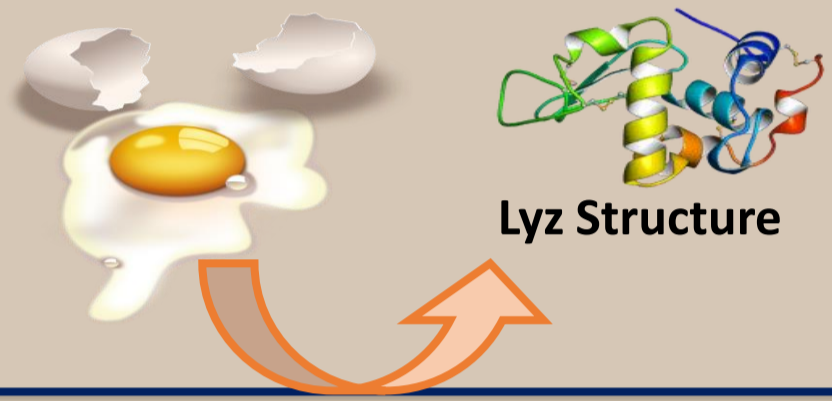
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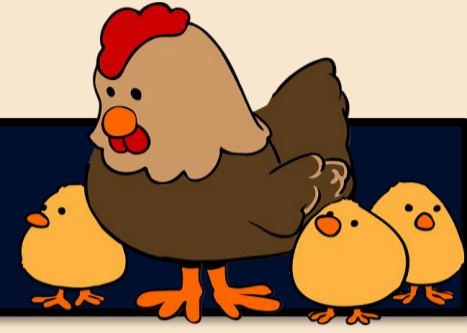


Introduction

- Food allergy is a public health problem that constantly increases in humans today. It has been determined that the main foods that cause this problem are shellfish, tree nuts, eggs, wheat, soy, milk, dairy products, peanuts, and soy. These eight food types are gathered in a «Big Eight» group.
- Food allergy symptoms usually include skin rash, allergic rhinitis, asthma, and anaphylaxis.
- Lysozyme (Lyz) is a glycoside hydrolase enzyme with a compact spherical structure (14.6 kDa). Lyz is commonly found in sweat, cauliflower juice, chicken egg, saliva, and tears.



Apt Sequence:
5'-(SH)-(CH₂)₆-ATC TAC GAA TTC ATC AGG GCT AAA
GAG TGC AGA GTT ACT TAG-3'



Experimental

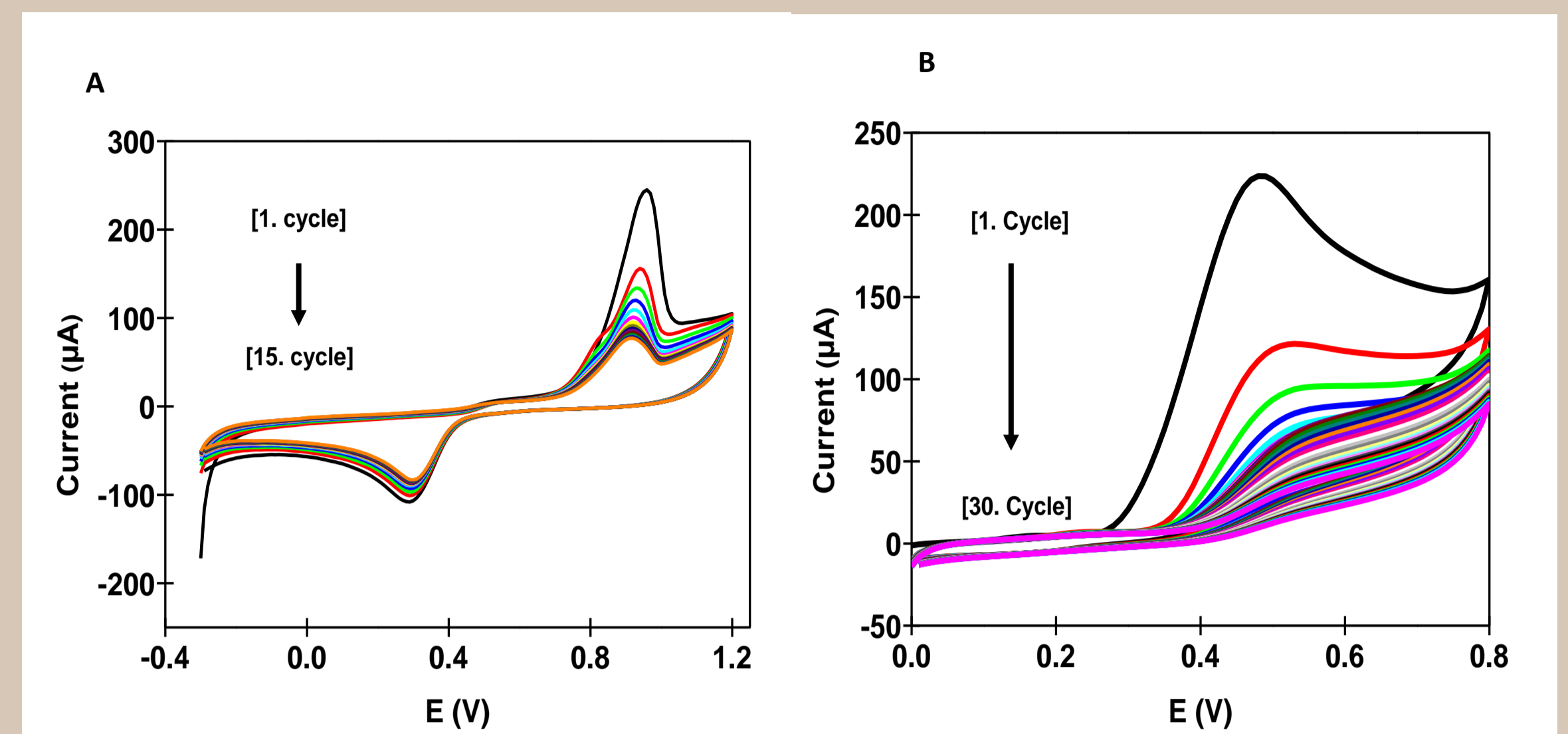
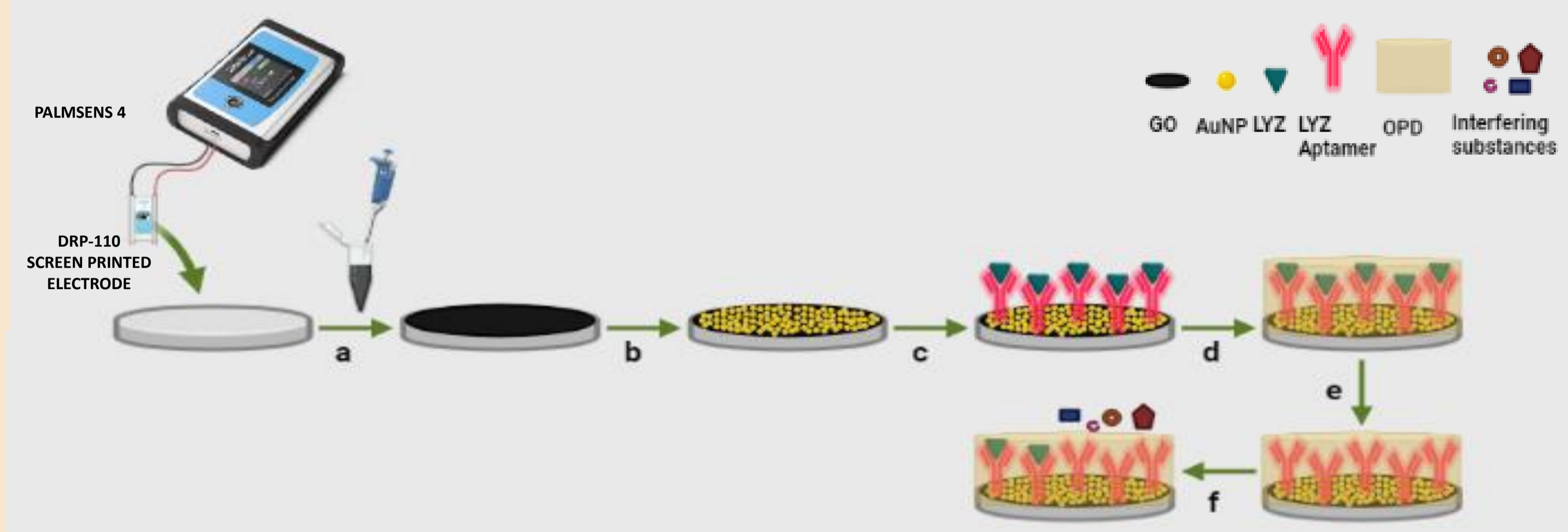


Figure 1. (A) CV curves of 1 mM HAuCl₄ electrodeposition at 15 cycles number, (B) DPV curves of OPD electropolymerization at 30 cycles number

Apta-MIP Sensor Design



- Scheme 1. Preparation of the Apta-MIP sensor for Lyz detection.
- GO modification onto SPE surface with drop-casting method;
 - Electrodeposition of 1 mM HAuCl₄ onto the GO/SPE surface via CV method with 15 successive scans;
 - Immobilization of the [Apt-Lyz] complex (Lyz aptamer is functionalized with -SH group);
 - Electropolymerization of OPD via CV method with 30 successive scans onto the [Apt-Lyz]/AuNP/GO/SPE platform;
 - Washing the modified electrode with SDS:AA solution for removal of the template, LYZ;
 - Rebinding of LYZ as target and other substrates as interfering agents.

Results and Discussion

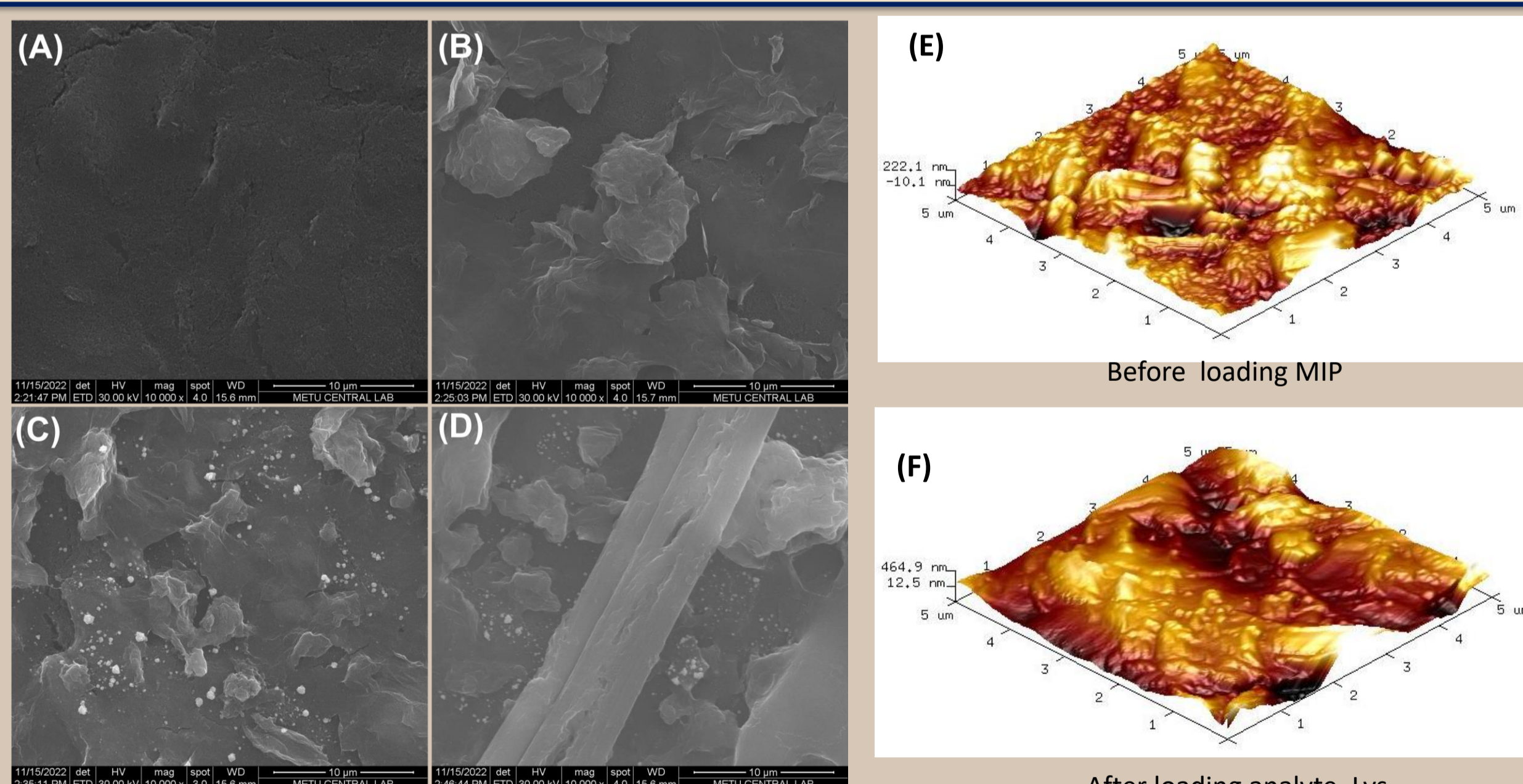


Figure 2. SEM images (A) SPE, (B) GO/SPE, (C) AuNP/GO/SPE and (D) Apt/AuNP/GO/SPE and AFM images of (E) Apta-MIP/AuNP/GO/SPE before loading, (F) Apta-MIP/AuNP/GO/SPE after rebinding with 100 pM Lyz

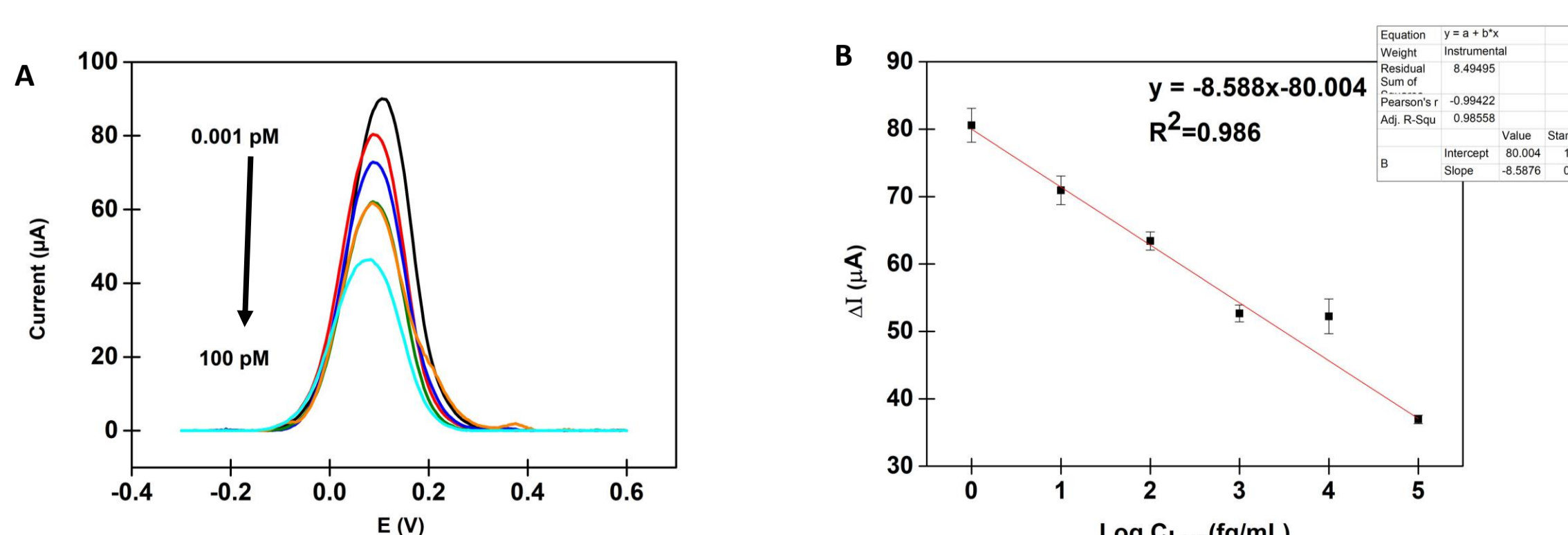


Figure 3. (A) DPV curves and (B) Calibration curves for Lyz in the concentration range of 0.001 pM-100 pM at the Apta-MIP sensor

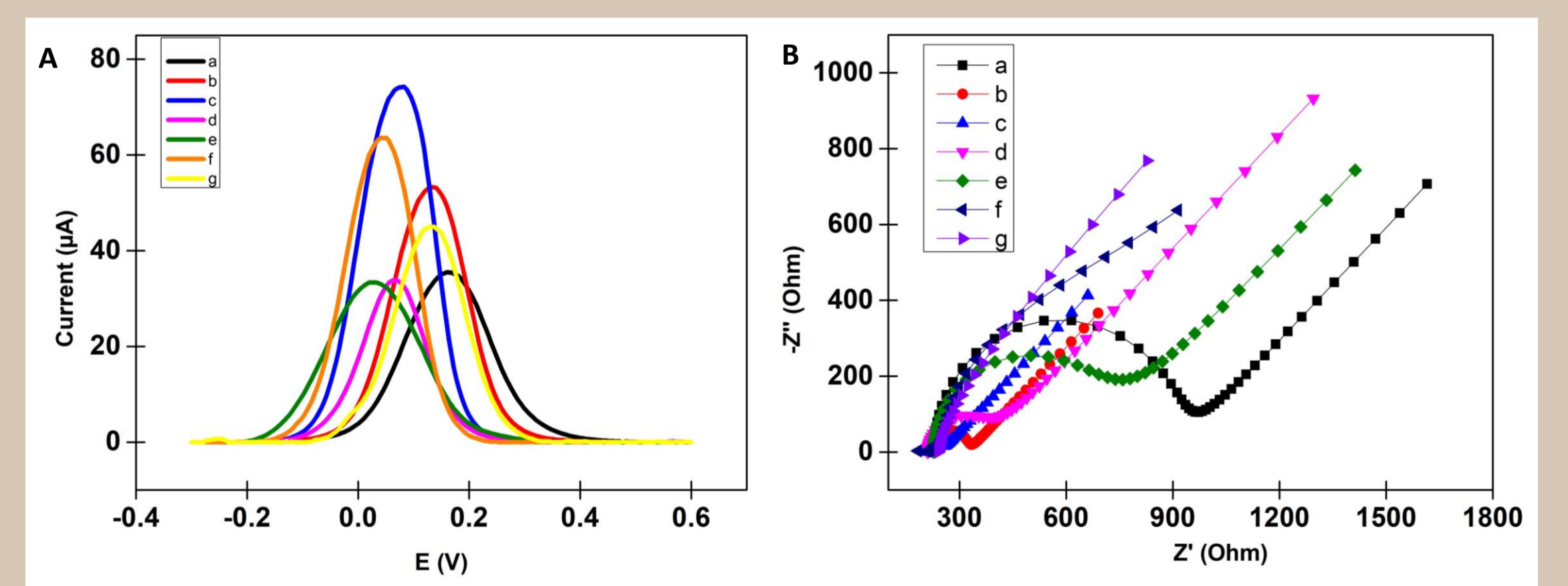
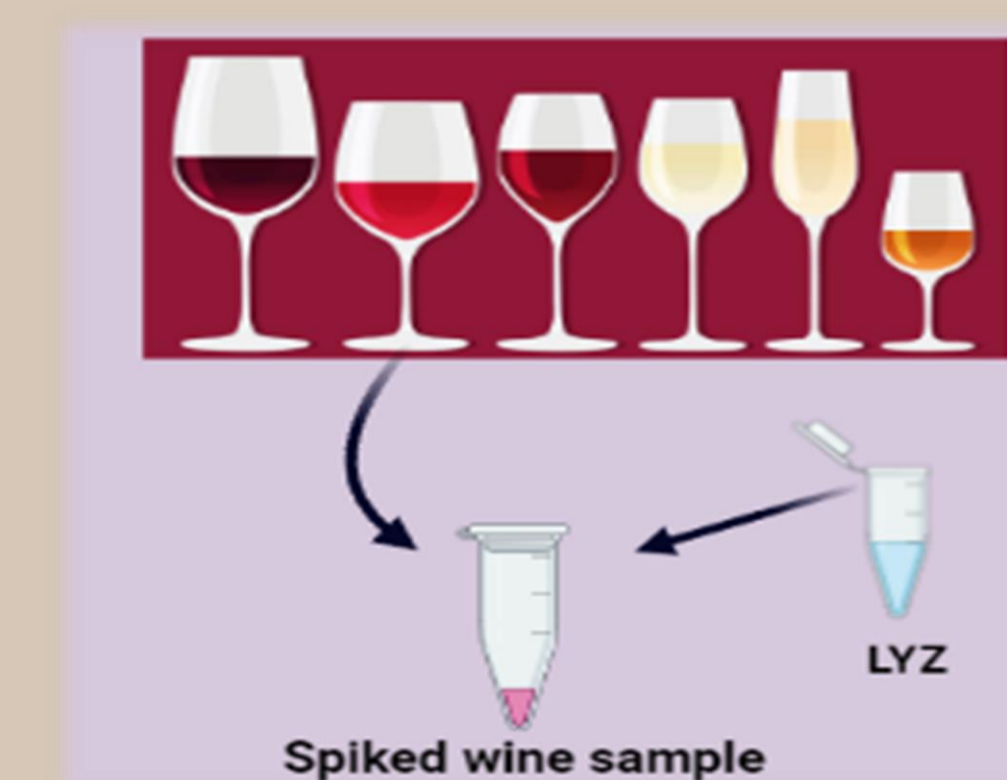


Figure 4. DPV (A) and EIS (B) curves of 5 mM [Fe(CN)₆]^{3-/4-} in 0.1 M KCl at modification of SPE electrode. (a) SPE, (b) GO/SPE, (c) AuNP/GO/SPE, (d) Apt-Lyz/AuNP/GO/SPE, (e) Apta-MIP/AuNP/GO/SPE before washing, (f) Apta-MIP/AuNP/GO/SPE after washing, (g) Apta-MIP/AuNP/GO/SPE after rebinding with 100 pM Lyz.

Future Aspects



To examine the viability and assay accuracy of the Apta-MIP sensor, spiked wine samples with various Lyz concentrations will be analyzed. The suitability of the biomolecule will be tested by the recovery test.

Figure 4. Schematic representation of Lyz detection in wine sample