Development of an electrochemical platform for selective Ara h1 allergen detection

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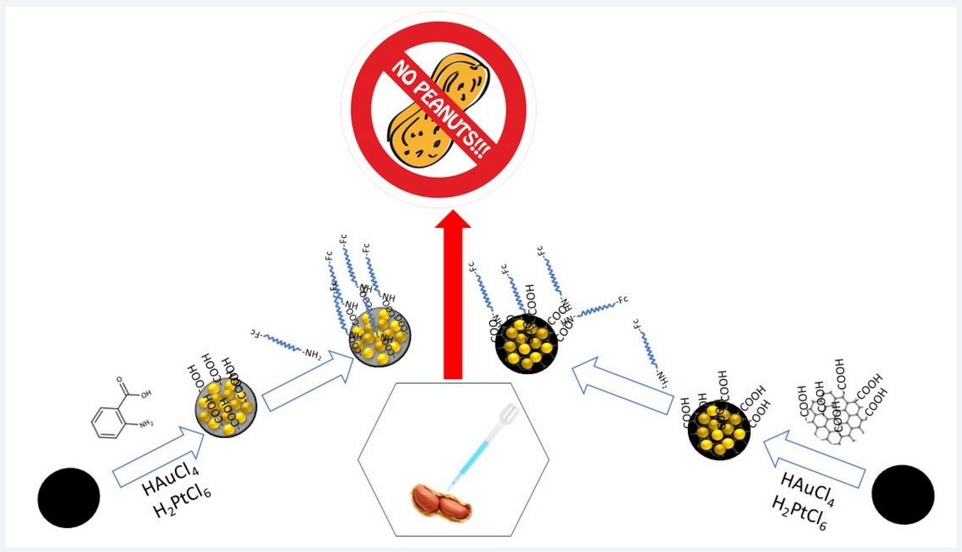
- Ara h1 is one of the major peanut allergens. It is considered one of the most severe, life-threatening food sensitivities since it triggers the highest frequency of severe and fatal reactions, even in trace amounts. Thus, it is extremely important to develop fast, accurate and easy-to-use analytical methods to determine Ara h1 allergen from food products that might contain traces of peanuts [1].
- This poster presents preliminary results in the development of an electrochemical aptasensor for Ara h1 allergen detection.
- Although high porosity is beneficial for sensing, it brings specific challenges, since the properties of nanostructured materials often differ significantly from their bulk counterparts.
- Two approaches to manage the sensitivity and selectivity of the proposed aptasensor were examined. Both platforms used gold and platinum nanoparticles in order to increase the electrocatalytic effect of a screen-printed carbon electrode.
- For the first platform, chemical receptors based on single-walled carbon nanotubes and poly-anthranilic acid were synthesized.
- For the second platform, graphene oxides modified with carboxylic groups were used as carboxyl groups donors with a polymer used to decrease the reactivity of the gold and platinum nanoparticles.
- These findings were used to investigate the immobilization of a 5' amino and 3' Ferrocene modified aptamer

[1] Tran, D.T. et al. *Biosensors and Bioelectronics* **2013**, *43*, 245–251



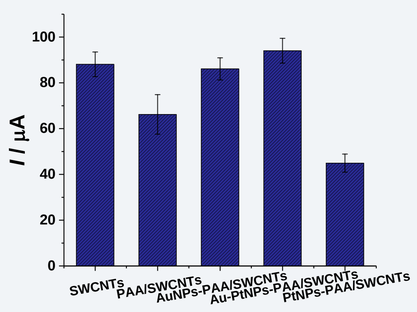
1 Platform developement

Platform	Modification			
	SWCNTs (mg/mL)	Antranylic acid (mM)	$HAuCl_4$ (mM)	H_2PtCl_6 (mM)
SWCNTs	1	0	0	0
PAA/SWCNTs	1	5	0	0
AuNPs/PAA/SWCNTs	1	5	10	0
Au-PtNPs/PAA/SWCNTs	1	5	5	5
PtNPs/PAA/SWCNTs	1	5	0	10

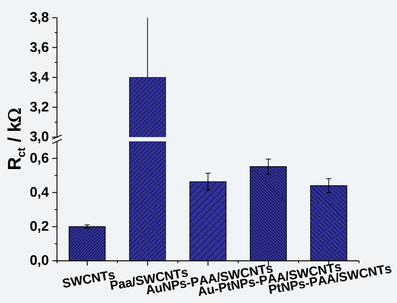
CV: Average current intensities registred in CV (100 mV s^{-1}, 2nd scan) for a 5mM $[Fe(CN)_6]^{3-/4-}$ in 0.1M KCl solution for each developed platform

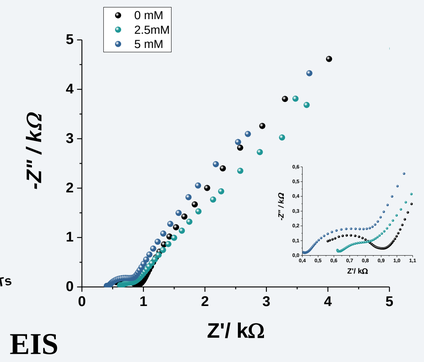
EIS: a)Average Rct (kΩ) for a 5mM $[Fe(CN)_6]^{3-/4-}$ in 0.1M KCl solution for each developed platform

b) EIS spectra in 5mM $[Fe(CN)_6]^{3-/4-}$ in 0.1M KCl for the Au-PtNPs/PAA/SWCNTs using different PAA concentrations



CV





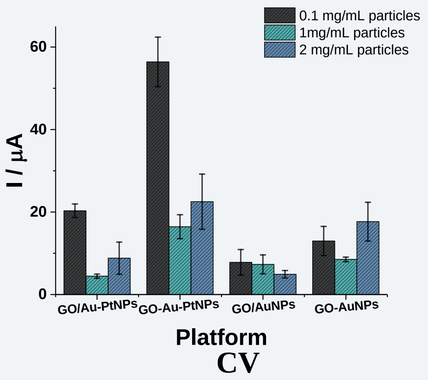
EIS

2

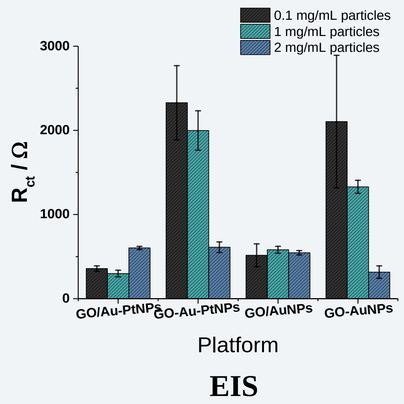
Platform	Modification			
	GO-COOH (mg mL^{-1})	$HAuCl_4$ (mM)	H_2PtCl_6 (mM)	Obs
GO/AuNPs	0.1 - 2	10	0	Electrochemicaly synthetised nanoparticles
GO/Au-PtNPs	0.1 - 2	5	5	
GO-AuNPs	1	10	0	Chemicaly synthetised nanoparticles
GO-Au-PtNPs	1	5	5	

CV: Average current intensities registred in CV (100 mV s^{-1}, 2nd scan) for a 5mM $[Fe(CN)_6]^{3-/4-}$ in 0.1M KCl solution for each developed platform

EIS: a)Average Rct (kΩ) for a 5mM $[Fe(CN)_6]^{3-/4-}$ in 0.1M KCl solution for each developed platform



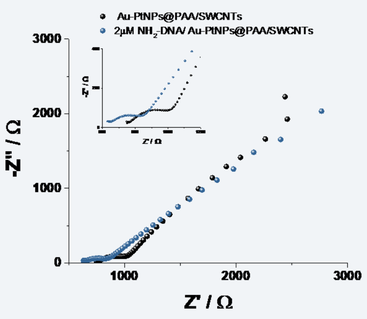
CV



EIS

Aptamer immobilisation

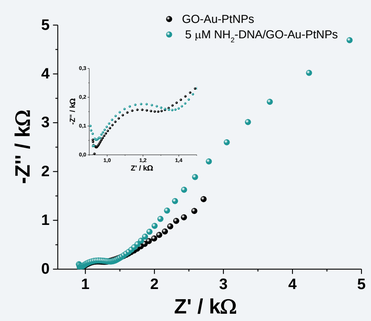
Platform	NH_2-DNA µM	Rct (a) Ω	Rct(p) Ω	ΔRct Ω	Rct (a/p)	RSD %
Au-PtNPs/PAA/SWCNTs	2	398.6	338.3	-60.30	0.85	24.3
	5	398.6	253.5	-145.1	0.64	15.1
	0	398.6	213.1	-185.6	0.53	15.4
Au-PtNPs/SWCNTs	2	278.2	195.3	-82.90	0.7	36.4
SWCNTs	2	221.7	200.7	-21.00	0.9	28.3



Method: 90 min 0.3M EDC/0.1M NHS activation
18 h incubation with NH_2-DNA sequence

Analysis: EIS analysis in a 5mM $[Fe(CN)_6]^{3-/4-}$ in 0.1M KCl was performed on the platform (P) and after DNA immobilisation (A) and the average Rcts were compared.

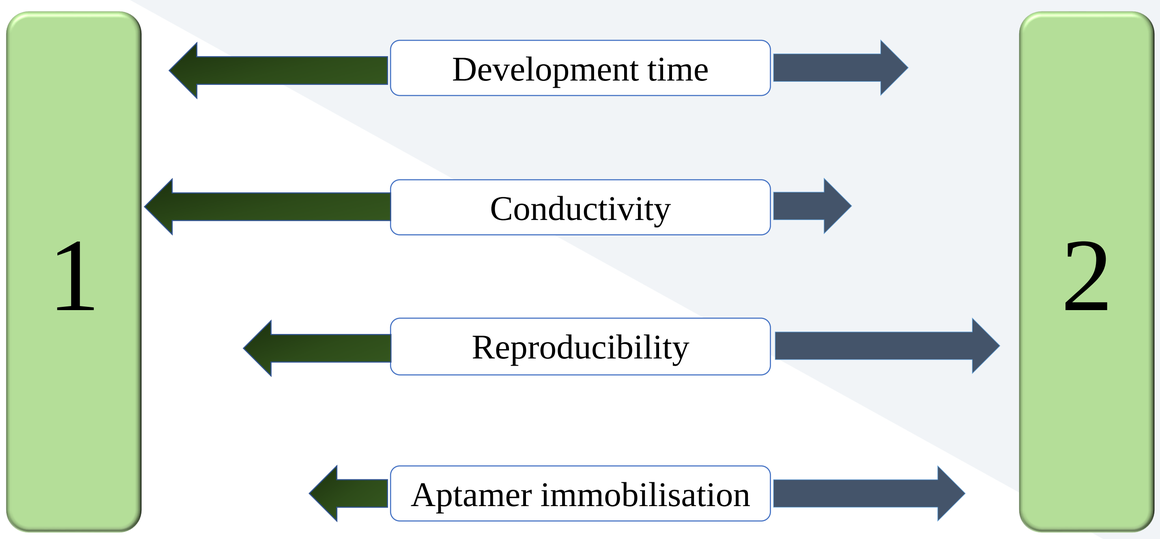
Platform	Rct (p) Ω	Rct (a) Ω	Δ Rct Ω	Rct (a/p)	RSD %
2 mg mL^{-1} GO-Au-PtNPs	544	612.6	68.6	1.13	3.5
2 mg mL^{-1} GO-AuNPs	1920	2188	267	1.15	1.5
1 mg mL^{-1} GO-AuNPs	1305	1003	-302	0.81	43.4
1 mg mL^{-1} GO/AuNPs	461	456	-4.71	0.99	19.1



Method: 90 min 0.3M EDC/0.1M NHS activation
18 h incubation with a 5µM NH_2-DNA in 10mM TRIS buffer pH 7.4

Analysis: EIS analysis in a 5mM $[Fe(CN)_6]^{3-/4-}$ in 0.1M KCl was performed on the platform (P) and after DNA immobilisation (A) and the average Rcts were compared.

Platforms comparison



Conclusions & perspectives

- Platform 1 offers a better conductivity thanks to the combination of SWCNTs and gold and platinum nanoparticles, but offers low reproducibility and a small number of cabroxylic groups offered by poly-antranylic acid and a uneffcient aptamer immobilisation
- Platform 2 does not have a high conductivity due to the electron blocking effect of graphene oxides, that instead offer a high number of carboxylic groups and a more efficient aptamer immobilisation
- Future steps:
  1. Optimisation of aptamer concentration and immobilisation time
  2. Optimisation of a blocking step
  3. Ara H1 detection from buffer solutions
  4. Ara H1 detection from spiked cookie samples
  5. Ara H1 detection from real samples.

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