Prussian blue nanoparticles as novel red-ox specie for sensitive label-free immunosensing using nanochannels: application to parathyroid hormone –related protein (PTHrP) detection

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Abstract

In this work the use of Prussian blue nanoparticles (PBNPs) as novel red-ox indicator agents for the improvement of the sensitivity of a label-free electrochemical immunosensing system based on nanoporous platforms is presented for the first time. Recently our group developed a protein [1,2] and DNA [3] an electrochemical detection technology based on solid-state nanochannel arrays with interest for rapid and cost-effective diagnosis tools [4].. The detection principle is based on the monitoring of $[Fe(CN)_6]^{4-}$ diffusion through anodized aluminum oxide (AAO) membranes attached onto a screen-printed carbon electrotransducer and its differential pulse voltammetry signal change upon biomolecule recognition. However, the label-free methodology suffers of lack of sensitivity, probably due to the small size of the $[Fe(CN)_6]^{4-}$ ions, which requires a high quantity of protein so as to be blocked making necessary sandwich based strategies, with inherent drawbacks in terms of time of analysis and reagents consumption beside its rather low efficiency for small proteins. For this reason, alternative red-ox indicators with bigger size than the ionic ones would be potentially useful for the improvement of the label-free approach. In this context, Prussian blue nanoparticles (PBNPs) appear as ideal candidates for this purpose. Stable and narrow-sized (around 4 nm) PBNPs, protected by Polyvinylpyrrolidone, exhibited a well-defined and reproducible red-ox pair and were successfully applied for the voltammetric evaluation of the nanochannels (20 nm pore sized) blockage due to the immunocomplex formation (Figure 1). The bigger size of the PBNPs compared with ionic indicators such as the $[Fe(CN)_6]^{4-/3-}$ system and the consequent increase in the steric effects which hinder their diffusion to the electrodes allows to improve the detection limits of model proteins (human IgG) from 200 µg/mL to 1 ng/mL levels. The sensitivity of the developed approach for the presence of small proteins captured inside the nanochannels is successfully applied for the detection of parathyroid hormone-related protein (PTHrP), a protein that exerts relevant functions in normal tissues and cancer, achieving its label-free detection at levels of 50 ng/mL.

References

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Figures

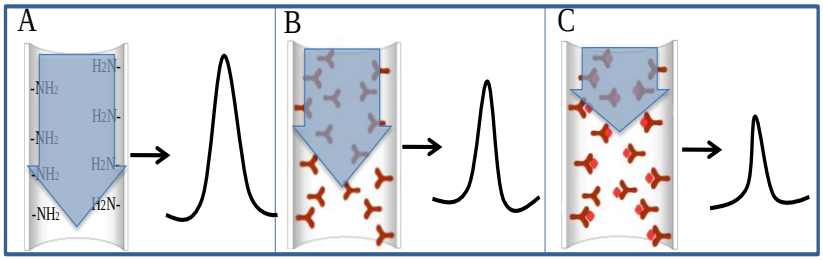

Figure 1. Schematic representation of the sensing principle. The formation of the immunocomplex blocks the diffusion of the Prussian Blue nanoparticles, giving rise to a decrease in their voltammetric signal.

Acknowledge

We acknowledge MINECO (Madrid) for the project MAT2011-25870, the EU's support under FP7 contract number 246513 ''NADINE''. Marisol Espinoza-C., acknowledge to Ministerio de Educación del Programa de Formación de Profesorado Universitario AP2010-5942. The authors also wish to thank Fundació Privada Cellex.