Strategies to address the non-specific adsorption in electrochemical aptasensors for clinically relevant proteins

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The non-specific adsorption (NSA) is a major issue in biosensors, one that if often overlooked. It leads to lack of accuracy, selectivity, stability and reproducibility as well as to reduced sensitivity. The problem is particularly important in complex media such as serum or in samples containing oligomeric aggregates with exacerbated adsorption at interfaces compared to the monomeric forms. Modifying the surface with a self-assembled monolayer (SAM) of aptamer and filling the gaps in the surface coating with chemisorbed thiolated molecules such as mercaptohexanol or including polyethyeneglycol moieties is a very popular strategy for obtaining simple yet efficient electrochemical aptasensors for proteins. Nonetheless, low dilutions of serum samples or protein aggregates still pose significant practical challenges related to NSA. While the ionic strength, the composition of the interaction buffer help minimize the adsorption of unwanted compounds, they also affect the aptamer conformation and its interaction with the target protein.

We hereby present our investigation with respect to several thiolated compounds that were used for the prevention of NSA from serum samples and from lysozyme aggregation mixtures. We have found that a short methylated thiol, PEG4-SH was the most efficient among the tested compounds for preventing the adsorption of lysozyme amyloid fibrils to a gold surface. The evaluation of the adsorption effects was done based on cyclic voltammetry measurements, by comparing the intensities of anodic and cathodic peaks of the ferrocyanide/ferricyanide couple before and after the incubation with complex samples.

In a second strategy, the adsorption of lysozyme to gold surfaces was evaluated by coupled electrochemical and SPR measurements. Thiol-blocked, aptamer functionalized gold interfaces were compared with "blank" sensors, covered with thiol SAMs only. The specific and non-specific binding of 200 µg/mL lysozyme was evaluated at three values of the applied potential, -0.65 V, 0 V and +0.2 V and it was found that applying a negative potential maximizes the specific over the non -specific binding. The results are discussed.

Combining a thiol coating that includes ethylene glycol groups with the application of an electrical field during the aptamer-protein interaction might prove a successful strategy for other aptasensors and other proteins' detection using a similar sensor design.

Acknowledgements

Financial support by the Romanian Executive Agency for Higher Education, Research, Development and Innovation (UEFISCDI), PN-III-P4-ID-PCE-2020-2297 E-MAP project, contract 84/0.9.02.2021 (for A.V and RMB) is gratefully acknowledged.