Real-Time Interferometric Aptasensor for Thombin in Nanoporous Anodic Alumina

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Aptamers have been reported as a promising class of compounds for biosensing since their 3D structure leads to bind a wide variety of biomolecules and permit detection down to the femtomolar range. Aptamers are single-stranded nucleic acid molecules that bind with high affinity and specificity to their target. The thrombin binding aptamer is one of the mostly studied aptamers in aptabiosensors [1].
Nanoporous anodic alumina (NAA) has been reported as an assuring material to use as a biosensor platform because of its advantageous optical properties [2]. Such properties are exploited in Reflection Interference Spectroscopy (RIfS) for optical biosensing [3]. For such application. the surface chemical functionalization is a crucial step.
In this work, we propose a path for the NAA surface functionalization by the attachment of Thrombin – Binding-Aptamer (TBA) to the pore walls, as illustrated in Figure 1a. By means of RIfS in a flow cell system, we study the binding events during the functionalization progression and the ability of the system to detect the thrombin.
In order to achieve aptamer functionalization NAA is modified in a first step by grafting aminopropyl triethoxysilane (APTES). The second step consists on the covalent attachment of sulfo-NHS-biotin by the formation of amide bond and in the third step the biotin-modified NAA is exposed to streptavidin. Next step is grafting the biotinylated thrombin binding aptamer (TBA) to the streptavidin. Finaly the aptamer modified NAA is exposed to diferent concentrations of thrombin. Figure 1b shows an example of the detection of thrombin at 50 µg/ml concentration. We demonstrate the ability of the system to detect all the binding events during the NAA surface functionalization to achieve an aptasensor against thrombin. We also prove that the system is able to specifically detect thrombin.

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References

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Figures

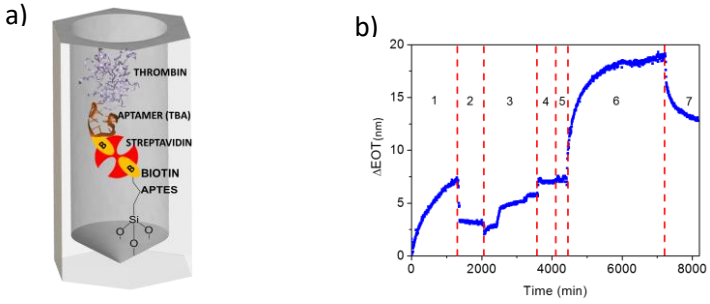

Figure 1: a) Schematic of NAA surface functionalization b) Example of registered change in EOT as a function of time during the NAA functionalization and aptamer-mediated thrombin detection: 1) Sulfo-NHS-Biotin 5 mM, 2) PBS, 3) streptavidin 50 µg/ml, 4) aptamer (TBA) 10mM, 5) PBS, 6) Thrombin 50 µg/ml, 7) PBS