Surface modification of Nanoporous Alumina towards bio-sensing applications in optical nano-fluidic system

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Abstract

The surface modification of nanoporous anodic alumina (NAA) by grafting functional molecules is a key step in its application as a biosensing probe platform.
Aptamers are single-stranded nucleic acid molecules that bind with high affinity and specificity to their targets. They are a promising class of compounds since their 3D structure leads to bind with a wide variety of biomolecules down to the femtomolar range [1]. In this work we propose and study a path for the atachment of aptamers to NAA based on three steps, as illustrated in Figure 1a. Furthermore, we demonstrate its validity for biosensing based on the reflectance spectroscopy method in a flow cell. The attachment of aptamers is performed in three steps. In the first one, NAA is functionalized by grafting aminopropyl triethoxysilane (APTES) and glutaraldehyde (GTA). This method has already been demonstrated for protein attachment on NAA[2]. Furthermore, it has also been used for DNA attachment to detect ATP [3]. The second step consists of the covalent attachment of streptavidin to the APTES-GTA while the final step is the grafting of the biotinylated aptamer to the streptavidin.
The study of each surface modification step is carried out by FTIR spectroscopy (Figure 1b) where we observe C-H peak of APTES, amide bounds and one broad peak of Streptavidin whose signal intensity depends on the Streptavidin concentration. With experiments by in-situ reflectance spectra monitoring in the flow cell we demonstrate that this surface modification is suitable to use in biosensin probes since when Biotin is injected on the streptavidin-modified NAA signal increase ocur wich mantains when is injected PBS (Figure 1c).

References

[1] D. J. Patel, A. K. Suri, F. Jiang, L. Jiang, P. Fan, R. A. Kumar, and S. Nonin, *J. Mol. Biol.*, vol. 272, no. 5, 645–64

[2] M. Baranowska, A. J. Slota, P. J. Eravuchira, G. Macias, E. Xifré-Pérez, J. Pallares, J. Ferré-Borrull, and L. F. Marsal, *C. Sur.B Bio.*, vol. 122, 375–383

[3] Y. Jiang, N. Liu, W. Guo, F. Xia, and L. Jiang, *A. Chem. Soc.*,vol. 134, 15395−15401.

Figures:

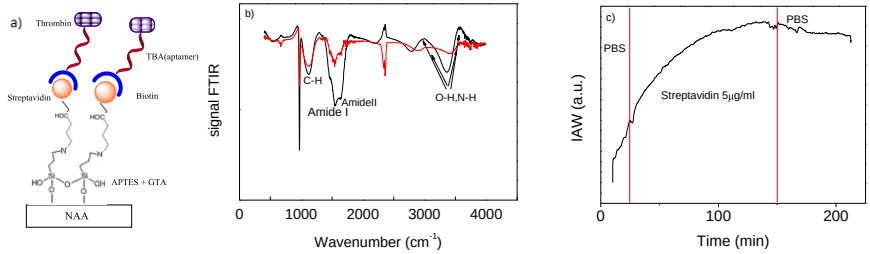

Figure 1: a) Scheme of surface modification b) FTIR spectra with APTES+GTA+ Streptavidin 100μg/ml and 10μg/ml c) Processed reflectance spectra by Average over Wavelength (IAW) of pre-incubated Streptavidin (10 μg/ml) with injection in fluid cell of Biotin (5 μg/ml)