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Study of aptamer immobilization on plasmonic surfaces by SERS analysis

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Surface-enhanced Raman Scattering (SERS) is an excellent technique that can be used to develop diagnostic assays for the sensitive detection of small molecules in complex samples, taking advantage of plasmonic nanostructures [1]. This sensitivity can be further enhanced by combining SERS with the most advanced molecular recognition systems, such as aptamers, DNA or RNA sequences that fold into a 3D structure, creating an artificial receptor able to bind specific target molecules [2-3].

In this work, the immobilization of a model aptamer, previously used for mycotoxin detection [4], was studied by SERS analysis, taking into account the different Raman signal acquired when oligos approach the plasmonic nanostructures with different orientations. In particular, different synthetic oligos carrying a thiol group at the 3' (Fig. 1a) or at the 5' terminal (Fig. 1b) were grafted on porous silicon membranes, decorated with Ag nanoparticles (NPs), by adopting previously optimized methods [5]. The immobilization was assessed by SERS mapping, using both labelled and unlabelled aptamers. The presence of a Raman reporter at the free terminal allowed to understand that the two aptamers bind to the surface with different orientations, preserving their folded structure, predicted by bioinformatics tools. Indeed, in the label-free configuration, it was possible to recognize that the two aptamers reached a similar surface coverage (Fig. 1c, top). However, the results obtained by grafting the labelled aptamer at its 5' end, showed that the Raman signal of the reporter was significantly higher in comparison to the 3' terminal (Fig. 1c, bottom). This can be explained by a lower distance of the reporter from the Ag NPs for the 5' thiolated sequence in comparison to the other one, suggesting that the aptamers bind according to the schemes in Figure 1b and 1a, respectively. This effect is related to the higher electromagnetic enhancement for molecular oscillators close to the plasmonic NPs.

The uniformity of the coverage was then investigated by comparing the SERS signal variability over the mapped surface. A greater fluctuation of the SERS intensity was detected in case of the 5' thiolated aptamer, probably due to a higher conformational variability for the oligo interacting with the silver surface. For this reason, a third aptamer, whose sequence was shortened at the 3' terminal, maintaining the target binding site, was tested. The reduced distance of the Raman reporter from the surface and the vertical orientation of the aptamer boosted the SERS intensity, while keeping a good signal uniformity, showing that such functionalization is suitable for the subsequent use in competitive SERS assays for aflatoxins detection.

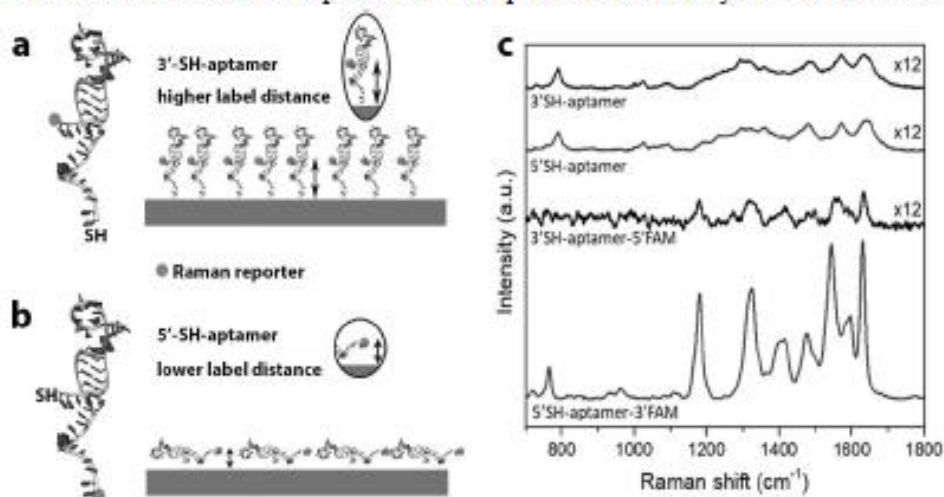


Figure 1: Scheme of the aptamer immobilization at the 3' (a) or 5' end (b). (c) Average SERS spectra acquired on the samples functionalized with the 6-FAM labelled and unlabelled aptamers.

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