Molecular Aptamer Beacon-based SERS biosensor for the detection of nucleic acids

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Abstract. Nucleic acids are essential biomolecules for the functioning of cells. In past years, nucleic acids have been assessing their role in prognostics and diagnostics. The progress of nanotechnology has allowed the fabrication of various type of nanostructured biosensors able to detect them with high sensitivity and specificity. Among the available sensing mechanisms, the sensor technology based on Surface-enhanced Raman Spectroscopy (SERS) is frequently preferred for identifying nucleic acids. In these sensors, natural or synthetic oligonucleotide sequences, acting as probes to hybridize the target molecules, are immobilized on a plasmonic sensing platform. In particular, aptamers, short DNA/RNA sequences, are emerging as new recognition elements for their chemical stability and specificity. Here, we focus on the combination of a specific type of aptamer, a molecular aptamer beacon, and nanostructured SERS biosensors for a sensitive detection of nucleic acids.

1 Introduction

Nucleic acids, DNA and RNA, are fundamental biomolecules that encode genetic information and direct essential biological processes such as protein synthesis and gene expression, ensuring the proper function of the cells. Due to their role, nucleic acids are gaining importance as disease biomarkers [1]. In the last past years, nanotechnology has provided innovative tools, such as nanostructured biosensors, for detecting nucleic acids offering rapid tests and the possibility to monitor disease progression. Biosensors are analytical tools for detecting various types of molecules and are composed of a biological recognition element and a transduction system [2]. Among the optical biosensors, Surface surface-enhanced Raman Spectroscopy (SERS)-based sensors are frequently preferred for identifying nucleic acids. SERS amplifies nucleic acids' Raman signals, leading to an ultrasensitive detection of the nucleotide bases constituting the nucleic acid sequences [3]. By exploiting the nucleotide base pairing, oligonucleotide probes are immobilized on SERS bionsensor to detect the sequences through hybridization via complementarity. Aptamers are emerging as new recognition elements for their chemical stability and specificity [4]. This work illustrates a possible combination of aptamer-based SERS biosensor, involving the use of a molecular beacon as a capture probe for the detection of a target nucleic acid.

2 Molecular aptamer beacon-based SERS biosensors

Discovered in the early 1990s, aptamers are usually single-stranded short sequence DNA or RNA (10-30 kDa) able to bind several molecules, like DNA, and RNA, but also proteins and other small cellular targets [5]. They are considered "chemical antibodies", obtained via Systematic Evolution of Ligands by EXponential Enrichment (SELEX) process that provides a non-immunogenic and non-toxic fabrication, differently from antibodies preparation. Aptamers have found plenty of applications in biology, imaging and drug delivery. Recently, they have stood out in nanotechnology, showing their potential in biosensors. The aptamer-based biosensors, aptasensors, offer the possibility to carry out analytical assays (electrochemical, electrical, optical and so on) due to their ability to specifically recognize target molecules [6]. The specificity and sensitivity of aptamer-based sensors rely on their structures which are accurately designed for capturing the analyte of interest. Combining a highly sensitive technique such as SERS and selective aptamers, sensitive platforms are obtained [7]. An interesting application is the use of molecular aptamer beacons (MABs) as capture probes in SERS biosensors. In general, MABs are short oligonucleotide sequences that unite the characteristics of molecular beacon and aptamer. Merging the ability of molecular beacons to interact with several types of targets and the binding-specificity of aptamers, the resulting MABs are considered a prestigious tool [8]. MABs can be combined with several available SERS substrates, which usually are nanostructured materials with a high surface-to-volume ratio (e.g. noble metal nanoparticles, metal coated nanostructured, metal colloidal solutions and so on) [4].

2.1 Molecular aptamer beacon design

MABs are characterized by a stem and loop structure. In the case of nucleic acid detection, the loop region of

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MABs is designed to be complementary to target molecule and upon binding, MABs change their structure, leading to the "open" state. The assumed structures of MAB can be simulated using online tools such as, UNAFold, Oligo Analyzer 3.1 and Nupack. We report, as an example, the design of a MAB (5'-CGACTCAACATCAGTCTGATAAGCTACGTCG-3') for the detection of miRNA-21 (5'-UAGCUUAUCAGACUGAUGUUGA-3'), a well-known cancer biomarker [9]. The nucleotide bases of the loop region have been selected to be complementary to the miRNA-21. The stem region should have a high energy in order to maintain the structure in a "closed" state (raging between ΔG= -1.5 and -2 kcal/mol). MAB's assumed structures were obtained using UNAfold online tool. The simulations showed five possible conformational arrangements with different energy levels. We reported MAB best energy level conformation (ΔG=-2.10 kcal/mol) (Fig.1a) and the assumed conformation upon binding with the target miRNA (Fig.1b).

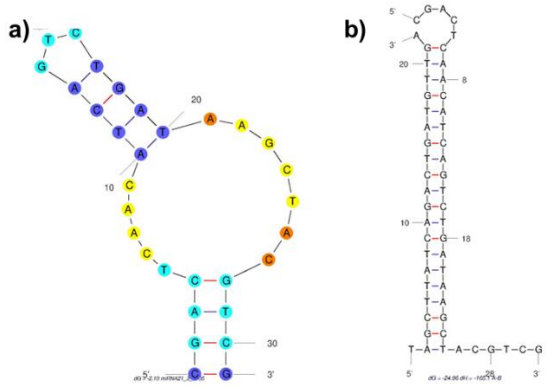

Fig. 1. a) Assumed conformation of MAB designed for miRNA-21. b) Assumed conformation of MAB interacting with target miRNA-21.

2.2 Detection Strategy of Molecular Aptamer Beacon-based SERS biosensor

Generally, two approaches in MAB-based SERS biosensors are used: label-free and labeled. The former consists of following the Raman fingerprint of the MAB conformational change upon its binding with the target nucleic acid. The latter involves the use of a Raman reporter, as a label, which commonly is a fluorophore with a well-known Raman signal easy to track. Due to several advantages such as high distinct signals, reduced background interferences, stability, and specificity, labeling MAB with Raman Reporter is a preferred strategy. When the target analyte is absent, the MAB is in a "closed" state bringing the Raman Reporter closer to the SERS substrate resulting in a high signal. On the other hand, when the target is present, it hybridizes to the loop region, MAB "opens" and the Raman Reporter is brought far from the substrate, leading to a decreased SERS signal (Figure 2). The proposed SERS substrate consists of self-assembled gold nanoparticles on a metal layer by drop-casting. The small gaps among gold nanoparticles are exploited to deliver an enhanced Raman signal of the adsorbed molecules (probes and target) on their surfaces.

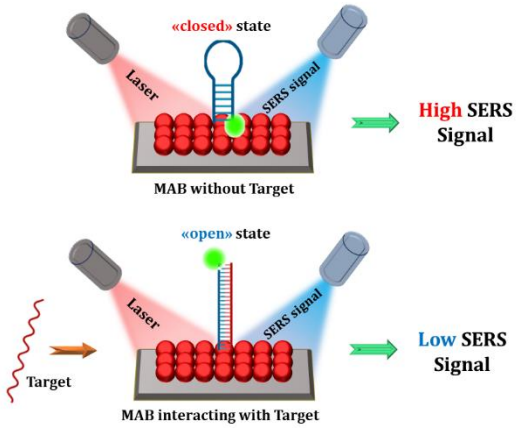

Fig. 2. Schematic representation of detection strategy using MAB labelled with Raman Reporter.

3 Conclusions

In past few years, molecular aptamer beacon (MAB)-based SERS biosensors have attracted the attention in the diagnostic field due to their ability to selectively bind various targets. We proposed a combination of MAB specifically designed for a target of interest (miRNA-21, in this specific work) with a SERS substrate composed by clusters of self-assembled gold nanoparticles on a thin metal film. The presented biosensor configuration can be adapted for various other targets by simply changing the design of the MAB probe.

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