

Investigation of a Plasmonic Aptamer-Based Biosensor for Heavy Metal Ion Detection

Rebeka Kovács¹, André P. G. Rocha², Tomáš Lednický², Baihui Li², Andrea Csáki², Attila Bonyár¹

¹ *Department of Electronics Technology, Faculty of Electrical Engineering and Informatics, Budapest University of Technology and Economics, Budapest, Hungary,*

² *Leibniz Institute of Photonic Technology, Albert-Einstein-Str. 9, 07745 Jena, Germany
kovacs.rebeka@vik.bme.hu*

Summary:

Optical biosensing devices offer a cost-effective approach to examining molecular interactions and detecting specific molecules; therefore, they are widely used for the detection of proteins, nucleic acids, and heavy metal ions. In this research, a localized surface plasmon resonance (LSPR) biosensing measurement device is studied to investigate the presence and quantity of arsenic ions in water samples, with the help of plasmonic nanostructures and aptamers, which can specifically bind to a target molecule to enable the selective detection of toxic arsenic(III)-ions in water samples.

Keywords: Plasmonic biosensing, localized surface plasmon resonance (LSPR), Microfluidic devices, refractive index sensing, heavy metal ion detection

Background, Motivation and Objective

Agricultural activities and increasing industrial and transport emissions are causing a global issue by polluting surface and ground waters with heavy metal ions, which can accumulate in animal and plant tissues consumed by humans. These ions are non-biodegradable and chronic poisoning can lead to severe health problems: damage to the central nervous system, liver, kidneys, reproductive organs, mucus tissues, and can result in carcinogenic effects in humans and animals. The monitoring of the level of toxic heavy metal ions is indispensable in environmental protection, disease prevention and effective treatment of patients [1, 2]. In most biological and environmental samples, many interfering matrix components affect the reliability of detection; therefore, it is crucial to develop a highly selective approach. [3]

Localized surface plasmon resonance (LSPR) is a phenomenon caused by the interaction of visible light and metal nanoparticles. The wavelength of the absorption peaks is very sensitive to changes in the refractive index of the surrounding medium; hence, the presence of the target molecules can be observed in a real-time, label-free manner. To selectively capture the target molecules/ions, aptamers can be utilized (optical aptamer sensors are called aptasensors). These are artificial oligonucleotide probes created by an *in vitro* process, called SELEX (systematic evolution of ligands by exponential enrichment) [4]. Their main advantages are high

binding affinity, excellent sensitivity and selectivity, and stability. These nucleic acid molecules, with a defined 3D structure, can be modified to bind a wide range of target molecules, cells, ions, and proteins, making them ideal for heavy-metal ion sensing [5, 6, 7]. Single-stranded deoxyribonucleic acid (ssDNA) and ribonucleic acid (RNA) aptamers are often used in biosensing devices. They can be chemically modified with certain ligands (thiols, amino groups); however, DNA aptamers are more stable [8]. Aptamers can bind heavy metal ions via electrostatic interactions, and, with different three-dimensional structures, they can exhibit specific affinity for certain ions, such as arsenic [9, 10]. These aptamers are attached to the surface of the gold nanoparticles, and when a bounding event to the ions occurs, a local refractive index change can be observed in the change of the LSPR extinction peak wavelength.

Description of the New Method or System

The measurement was carried out in an LSPR setup consisting of a *Tungsten* halogen light source, an *Ocean Insight* UV/VIS spectrometer. The plasmonic chip was integrated into a microfluidic channel, and the precise fluid control was achieved using a *Masterflex Ismatec Reglo* peristaltic pump. The plasmonic chip contained 80 nm gold (Au was deposited on chemically-modified cover slips) [11]. The samples are cleaned by O₂ plasma etching prior to each experiment. Arsenic aptamer aliquots were prepared by dilution with ultrapure water. The assay was

performed using 0.95× CB buffer (coupling buffer), with 10 mM MOPS (3-(N-morpholino)propanesulfonic acid, pH 7.4, supplemented with 2 mM MgCl_2) as the running buffer. Aptamer (5 μM) was introduced and the system was equilibrated, followed by CB and buffer exchange to MOPS. Surface blocking was carried out using 1 mM MCH (Mercaptohexanol, 1.37 μL in 10 mL MOPS). The system was incubated for 3 h to allow aptamer folding. Salmon sperm DNA (2 μL in 198 μL MOPS) was then added, followed by rinsing. A 10 μM As(III) standard solution (100 μL) was introduced, and the assay concluded with a final MOPS rinse.

Results

In the first phase of the experiment, the CB buffer was pumped into the microfluidic chip to ensure the correct pH and environmental conditions for the aptamer immobilization step, during which the aptamers attached to the nanoparticle surface, resulting in a distinct shift in the spectra. Then a passivation step is needed to ensure that only the target molecule can interact with the aptamer, while the other parts of the surface were blocked. A long conformational folding step is required for the aptamers to reach their proper 3D structure therefore the experiment was paused for 3 hours. In the last section, the green circles represent the binding of the As(III)-ions to the folded aptamers, which resulted in a 0.82 nm change in the stabilized final step compared to the signal caused by the folding of the aptamers.

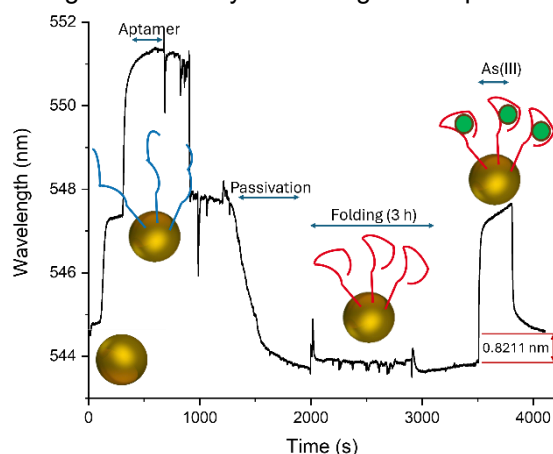


Fig. 1. The arsenic(III) detection process calculated from a centroid-based analysis

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