Instrument-free detection of nucleic acids via gene-targeted four-way-junction sensor coupled with magnetic nanoparticles

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INTRODUCTION

Early diagnosis of diseases is crucial for effective treatment and saving the patient's life [1]. One such a disease, tuberculosis, is now curable with early detection and precise typing [2]. There are many different ways to detect these kinds of pathogens quickly, for example, the gold standard is the PCR. However, PCR requires expensive equipment and highly skilled personnel, which implies a high monetary cost [3]. DNA nanosensors is a rapidly developed technology, which could be suggested as a cost effective alternative to already existing methods, increasing the effectiveness of the diagnostics process [4].

In the presented work, a binary hybridization DNA probe was used to detect the HigA1 gene (**analyte**). The probe has increased specificity provided by the "long" analyte binding arm (f) and increased sensitivity provided by the "short" arm (m) (Fig. 1).

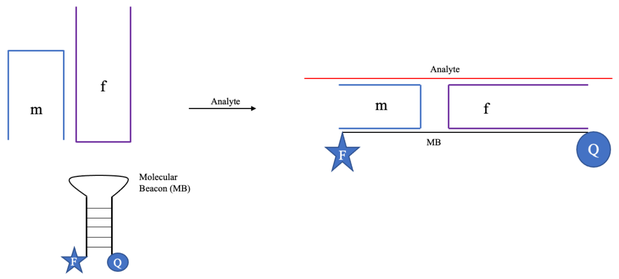


Figure 1. Schematic representation of 4WJ probes and the principle of their hybridization with nucleic acid' analytes.

The DNA probe used in this work is based on a 4-way-junction (4WJ) [5] construction consisting a stem loop hybridization probe (molecular beacon, which unwinds its structure in presence of analyte) with a magnetite nanoparticle functionalized at the 5' end (UMB_NP), and two DNA strands, each having parts complementary to analyte and to UMB.

M. tuberculosis was chosen as the pathogen responsible for various human diseases, such as tuberculosis, pneumonia, neonatal meningitis [6]. For the identification of this organism, we chose specific genome part. The HigA1 gene [7] very often contains a single nucleotide polymorphism in antibiotic resistant strains. The secondary conformation of the selected region is shown in Fig. 2. The blue and green lines correspond to the attachment sites of the m- and f- strands.

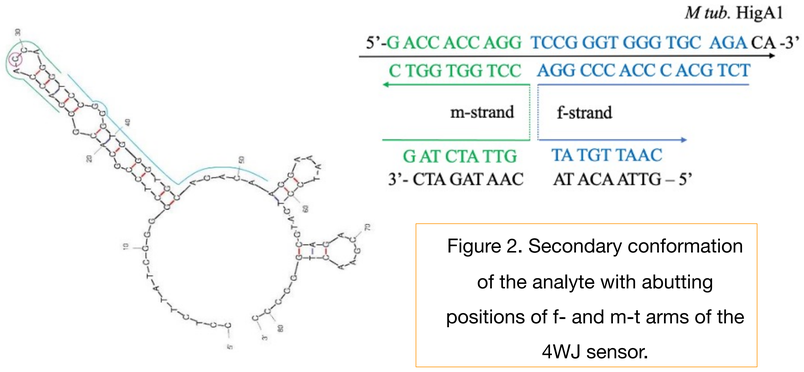
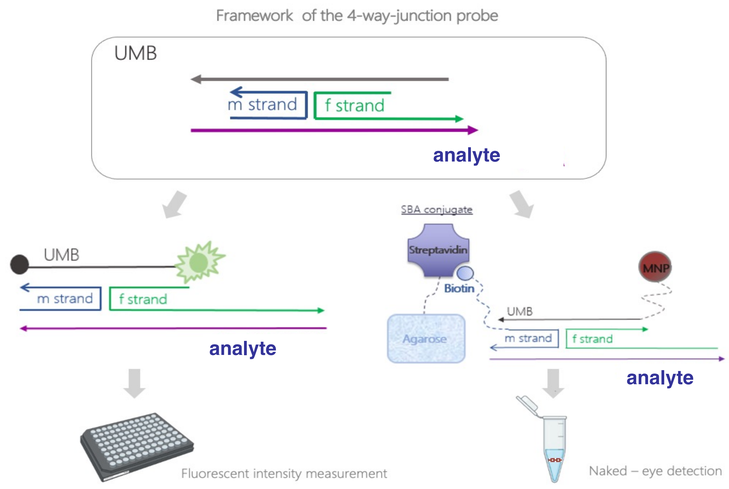


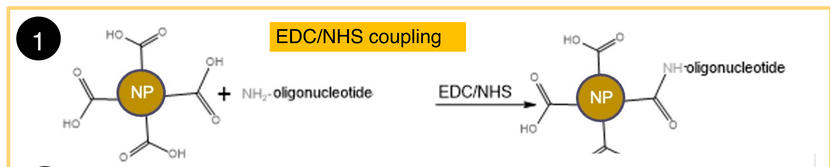
Figure 2. Secondary conformation of the analyte with abutting positions of f- and m-t arms of the 4WJ sensor.

METHODS

Despite all the advantages of the DNA nanosensors, the fluorometric vizualisation method requires expensive equipment which would not fulfil the requirements for the **point-of-care diagnostics'** approach. In this study we propose a new **naked-eye detection** method through higA1 gene-targeted 4-way junction sensor capped magnetic nanoparticles. Two methods of testing 4WJ presented below.



Prior to detection, magnetic NPs were coupled to the UMB using EDC/NHS reaction[8]. The formed complexes were checked via DLS and IR.



COOH-coated magnetic nanoparticles	860 ± 43
COOH-coated magnetic nanoparticles modified via carbodiimide bond	16624 ± 416

Figure 3. IR-spectra of unmodified and modified with UMB COOH-functionalized magnetic nanoparticles. In spectra of modified nanoparticles, a number of peaks in the region of 2850-3000 cm-1 can indicate the presence of organophosphorus groups. A peak at 1690 cm-1 is observed, which may be characterized by the appearance of an amide bond.

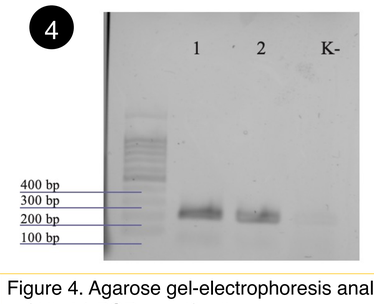
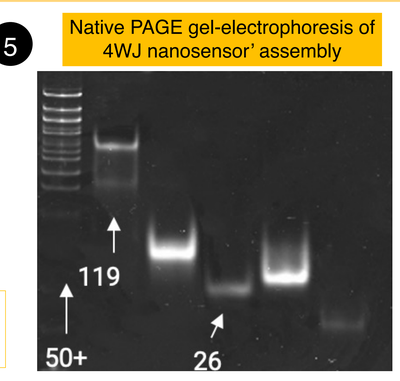


Figure 4. Agarose gel-electrophoresis analysis of HigA1 PCR amplification and assembly of 4WJ



RESULTS

The limit of detection (LOD) of the 4WJ nanosensor on chemically synthesized oligonucleotides of higA1 gene was determined. Using the earlier evaluated concentrations of the sensor's fragment, the minimum amount of the oligonucleotides mimicking higA1 M. tub gene was 0,97 nM.

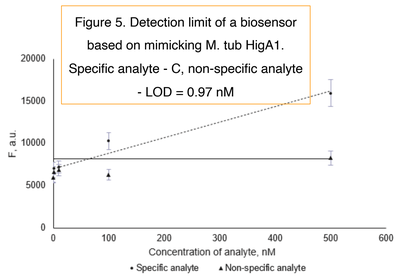


Figure 5. Detection limit of a biosensor based on mimicking M. tub HigA1. Specific analyte - C, non-specific analyte - LOD = 0.97 nM

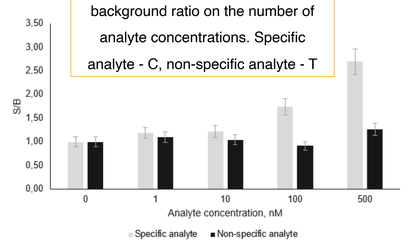


Figure 6. Dependence of the signal-to-background ratio on the number of analyte concentrations. Specific analyte - C, non-specific analyte - T

Fig. 7 shows naked-eye detection of pre-denatured higA1 PCR amplicons. The left tube contains analysed amplicons (due to the sensor assembly, Fig.4), and therefore aggregation happens sooner than in the right tube where nanoparticles are sedimented chaotically solely due to the gravity.

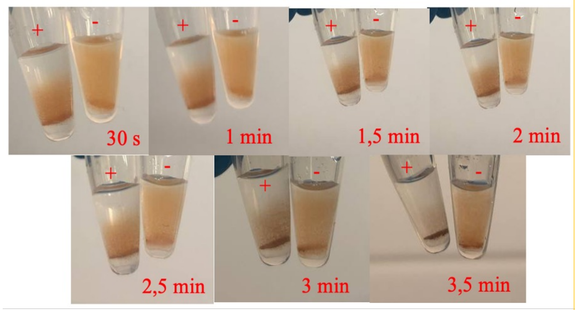
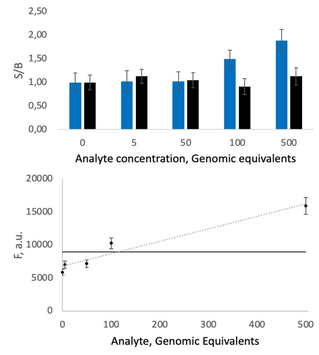


Figure 7. Detection of synthetic higA1fragment; pictures taken with a 30s interval, starting from 1 (30 seconds) to 7 (3.5 minutes); + tube: 4WJ biosensor and the analyte; - tube: only 4WJ sensor



Signal-to-background ratio of the biosensor for *M. tuberculosis* HigA1 gene amplification product depending on the concentration of analyte in genomic equivalents

Limit of Detection of the MB-based 4WJ-nanosensor PCR product of *M. tuberculosis* HigA1 gene after denaturation LOD = 115 GE

Aggregation of magnetic nanoparticles on the agarose beads with *M. tub.* HigA1 PCR product after denaturation (minimal concentration)

CONCLUSIONS

In this work we have introduced a new naked-eye assay for *M.tuberculosis* higA1 gene detection and genotyping based on 4WJ probes attached to magnetic nanoparticles. General principles for constructing 4WJ probes and integrating them into a naked-eye assay have been established. The presence of the pathogen's synthetic DNA and PCR amplicons was reported with S/B > 2.5 without the need of isolation of the amplicons from amplification mixture. The method is versatile since it can be potentially applicable for the analysis of any target DNA sequence, as the probe can be designed for any pathogen. Therefore, this assay can be integrated into point-of-care diagnostics' device.

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