CRISPR-Cas Systems

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Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based diagnostics have emerged as promising tool to revolutionize molecular diagnostics. Despite notable progress, challenges exist in utilizing CRISPR platforms for point-of-care (POC) applications, such as amplification steps and a focus on non-nucleic acids target detection. We propose an innovative approach, exploiting a DNA circuit, to detect antibodies with an amplification-free CRISPR-based assay.

The demand for clinical analyses in hospital is growing, and proteins and nucleic acids are the most relevant biomarker categories for diagnostics. The gold standard techniques for quantitative analysis of these biomarkers require expensive, bulky, and complicated instrumentation to use, making them inaccessible to the average end user at home.

At CSEM we are developing a novel POC platform that utilizes a CRISPR assay to detect monoclonal antibodies. The sensing method is based on a DNA circuit for the recognition of the antibody and the successive activation of the ribonucleoprotein (RNP) made of CRISPR RNA sequence and the CRISPR-associated protein (Cas) system.

The circuit used for recognition consists of four different single-stranded DNAs: 1) the translator, 2) two splits and 3) a reporter.

1) The translator is in a hairpin conformation. This conformation is achieved using the complementary sequence of the Protospacer Adjacent Motif (PAM) to prevent cleavage by RNP.
2) The splits are used to activate of the translator. They are made of the complementary sequences to the translator and are functionalized with peptide nucleic acid (PNA). The PNA is the recognition element that specifically binds the antibody of interest.
3) The reporter is a short single-strand DNA in a molecular beacon conformation, labelled with a fluorophore and a quencher.

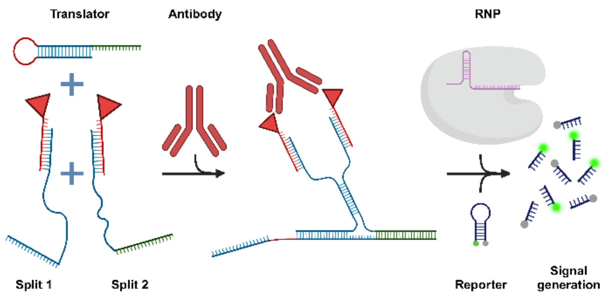

Figure 1: Assay sketch. The activation and the detection are occurring only in presence of the monoclonal antibody contained in the sample.

In our patent [1] we exploited the above-mentioned DNA circuit to activate the Cas in presence of a target antibody: when the antibody binds the recognition elements brought by the splits, it co-localizes them, starting the displacement of the translator and consequently activating it by the formation of a double-strand PAM sequence. The active translator can then recognized by the ribonucleoprotein, initiating the cleavage of both the translator (cis-cleavage) and the reporter (trans-cleavage), and generating the fluorescent signal.

In the present work, we successfully demonstrated the concept of CRISPR-Cas system for the detection of a model monoclonal antibody: anti-hemagglutinin (αHA) from human influenza A virus. In addition, we proved that the usage of our detection system can be simplified and fastened thus indicating compatibility with its integration in future easy to use POC devices.

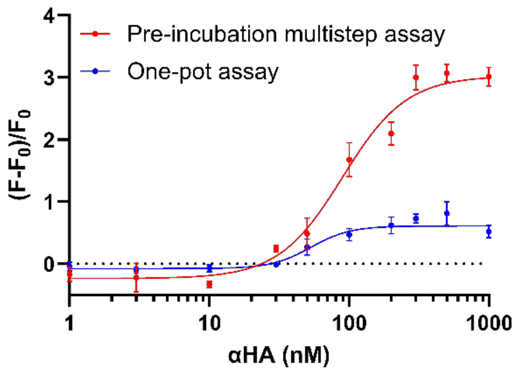

Figure 2. Calibration curves obtained from the multistep CRISPR-based assay for the quantification of the anti-hemagglutinin (αHA) monoclonal antibody. N=3.

Figure 2 shows calibration curves obtained for the detection of αHA. The red curve is the calibration curve obtained for a multistep assay where each component is pre-incubated and added sequentially as depicted in Figure 1. This complex process enables to obtain linear range from 27 ±5 to 342 ±84 nm within an assay time of 90 min. The simplified process results are depicted by the blue calibration curve. These results evidence that despite a lower sensitivity and a reduced detection range from 25 ±6 to 136 ±48nM, similar detection limits could be achieved by adding all the different components: translator, splits, reporter, RNP and sample at once in a one-pot assay.

This major success paves the way for the usage of this CRISPR-Cas detection system in POC solutions. Currently ongoing work on this system relates to various topics: optimizing the assay conditions, extending the detection process to other analytes as well as translating the detection method to electrochemical process to enhance the method sensitivity, speed, and range as well as to develop a sensing method which can be produced on industrial scale and easily interfaced with electronic readout solutions.

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[1] "CRISPR-Cas based method for the detection of non-nucleic acid targets" P-530275-EP.