

Enhanced IgE Aptasensing via Scaffold-mediated Oriented Immobilization of DNA Aptamers

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Summary:

We developed a scaffold based on a single-chain Cro (scCro)-displaying bio-nanocapsule (scCro-BNC) that enables the oriented immobilization of DNA aptamers, thereby achieving highly sensitive detection in an aptasensor system. Herein, scCro-BNC was applied to a quartz crystal microbalance-based aptasensor for the detection of immunoglobulin E, an important biomarker for allergy diagnosis. This approach improved the sensitivity by approximately 30-fold compared with direct immobilization, demonstrating that scCro-BNC is an effective scaffold for enhancing aptasensor performance.

Keywords: aptasensor, oriented immobilization, scaffold, bio-nanocapsule, scCro

Background, Motivation and Objective

Aptasensors employing DNA aptamers, which have emerged as promising alternatives to antibodies in recent years, are widely applied to the detection of a broad range of target analytes—including food allergens, pathogenic microorganisms, and metal ions—using techniques such as quartz crystal microbalance (QCM), fluorescence resonance energy transfer (FRET), and immunochromatography [1]. For the immobilization of DNA aptamers onto sensor chips, various strategies have been developed, including nonspecific adsorption via electrostatic interactions as well as oriented immobilization using scaffold molecules such as crosslinkers, self-assembled monolayers, and thiol groups [1]. However, complete control over orientation remains challenging, often leading to reduced detection sensitivity.

To address this issue, we focused on single-chain Cro (scCro) [2], a DNA-binding domain that recognizes a specific 17-base pair sequence, and developed a nanoparticle-based bio-nanocapsule (scCro-BNC; Fig. 1) that displays scCro in an oriented manner on its surface [3]. The scCro-BNC is a hollow nanoparticle composed of a lipid bilayer and scCro-L proteins, in which part of the N-terminal region of the hepatitis B virus surface antigen L protein is replaced with scCro, and is produced in *Saccharomyces cerevisiae*. We previously demonstrated that appending a scCro-binding sequence to a thrombin-binding DNA aptamer, followed by its

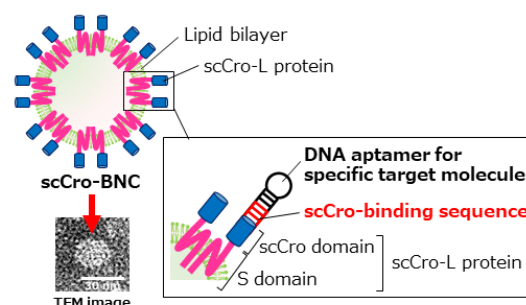


Fig. 1. Capsule structure of scCro-BNC.

oriented immobilization on an scCro-BNC-treated QCM aptasensor, enhanced thrombin detection sensitivity by ~6000-fold compared with direct immobilization method [3].

In this study, we targeted immunoglobulin E (IgE), a key biomarker in allergy diagnostics, and aimed to enhance the sensitivity of an IgE-detecting aptasensor by using scCro-BNC as a scaffold, with the goal of enabling practical applications.

Description of the New Method or System

The IgE-binding DNA aptamer (37-mer) [4] was modified by appending a 17-base scCro-binding sequence to its terminus. The secondary structure of the modified aptamer was predicted by RNAfold WebServer (<http://rna.tbi.univie.ac.at/>).

The binding between the IgE-binding DNA aptamer and IgE was evaluated using a QCM instrument (Single-Q, As One, Osaka, Japan) in 500 μL of phosphate-buffered saline containing 2 mM MgCl_2 at 25°C with stirring at 540 rpm. The QCM sensor chip (As One) was first treated with scCro-BNC (6 $\mu\text{g}/\text{mL}$), followed by immobilization of the DNA aptamer (2 $\mu\text{g}/\text{mL}$). The sensor surface was then blocked with 5% (w/v) bovine serum albumin. IgE (0–6.2 $\mu\text{g}/\text{mL}$) was subsequently added, and a frequency change of less than 3 Hz per minute was considered indicative of signal stabilization. The maximum frequency change was defined as the binding response. As a control, DNA aptamers were directly immobilized onto the sensor chip. A frequency change of 1 Hz (ΔF) corresponds to a mass change of 0.6 ng/cm^2 according to the Sauerbrey equation. All measurements were performed in triplicate.

Results and Discussion

The maximum binding capacities of scCro-BNC and the IgE-binding DNA aptamer were determined using QCM. The amount of scCro-BNC immobilized on the sensor chip was estimated as $223.2 \pm 9.5 \text{ ng}/\text{cm}^2$ based on a frequency change (ΔF) of $-372.0 \pm 15.8 \text{ Hz}$. After BSA blocking, the binding amount of DNA aptamer was $120.0 \pm 21.1 \text{ ng}/\text{cm}^2$ ($\Delta F = -200.0 \pm 35.1 \text{ Hz}$). Based on the molecular masses of scCro-L protein (~48 kDa) and the IgE-binding DNA aptamer (~21.8 kDa), approximately one aptamer molecule was estimated to bind per scCro-L protein, indicating an approximately 1:1 stoichiometric ratio.

IgE binding was evaluated using two immobilization methods: direct immobilization of the DNA aptamer and immobilization via scCro-BNC (Fig. 2). Calibration curves were constructed by plotting IgE concentration against binding amount on a log–log scale, followed by fitting to an exponential function. The direct immobilization method showed linearity in the range of 2.2–6.6 $\mu\text{g}/\text{mL}$, whereas the scCro-BNC method exhibited linearity across a broader range of 0.02–6.6 $\mu\text{g}/\text{mL}$. The limit of detection (LOD) of the QCM system was 2 ng/cm^2 ($\Delta F = -3.3 \text{ Hz}$), corresponding to $\sim 0.61 \mu\text{g}/\text{mL}$ for the direct method and 0.02 $\mu\text{g}/\text{mL}$ for the scCro-BNC method. These results demonstrate that the use of scCro-BNC expands the dynamic range and enhanced detection sensitivity by ~ 30 -fold. Furthermore, the amount of IgE ($\sim 190 \text{ kDa}$) bound per mole of DNA aptamer also increased by ~ 2.5 -fold, suggesting that scCro-BNC promotes oriented immobilization of DNA aptamers on the surface, thereby reducing steric hindrance and enhancing binding efficiency.

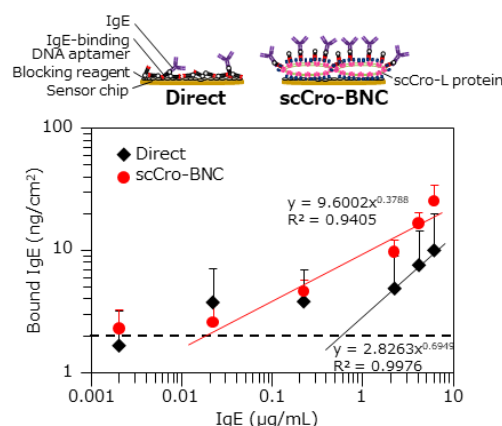


Fig. 2. Enhanced IgE detection sensitivity by scCro-BNC-mediated immobilization in QCM aptasensors.

Conclusions

These findings indicate that scCro-BNC serves as an effective scaffold for enabling highly sensitive detection of a wide range of target analytes in aptamer-based sensors. Future work will focus on extending this approach to various aptasensor platforms, with applications in food quality control, clinical diagnostics, and broader areas of the life sciences.

References

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