

Optimization of ssDNA Immobilization for a CRISPR/Cas12a Electrochemical Biosensor for Friedreich's Ataxia

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Summary:

An electrochemical biosensor based on CRISPR-Cas12a was developed by optimizing ssDNA immobilization on gold electrodes. Electrochemical impedance spectroscopy (EIS) was employed to characterize each modification step and the collateral cleavage activity. The optimized interface allowed Cas12a accessibility and resulted in a decrease in charge transfer resistance after collateral DNA cleavage. These results demonstrate that precise interface control is essential for developing CRISPR-based diagnostic platforms.

Keywords: CRISPR Cas12a, electrochemical biosensor, DNA immobilization, gold electrode

Introduction and Objective

Friedreich's ataxia is a rare inherited neurodegenerative disorder. However, their early diagnosis remains a critical challenge due to the low concentration of biomarkers [1]. Electrochemical biosensors emerge as a powerful analytical tool to overcome this limitation, offering outstanding sensitivity and specificity [2]. Nevertheless, the performance of DNA-based electrochemical sensors relies heavily on the structural organization of the sensing interface. Specifically, controlling the DNA probe density and immobilization dynamics is crucial to optimize signal transduction and ensure accessibility for Cas12a collateral activity, a mandatory requirement for CRISPR-based platforms. In this work, we investigate the influence of ssDNA surface density and immobilization time on the electrochemical behavior of screen-printed gold electrodes.

Methodology

The miniaturized platform consists of a gold working electrode, a counter electrode, and an Ag reference electrode. To modulate the surface coverage, a thiolated ssDNA probe (27-mer) was immobilized at varying concentrations (2.5 and 5.0 μM) and incubation periods (1 and 2 h). Subsequently, the sensing interface was backfilled with 6-mercapto-1-hexanol (MCH) to ensure proper probe orientation and prevent nonspecific binding. The stepwise interfacial modifications were monitored by

electrochemical impedance spectroscopy (EIS) using a $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe. The schematic illustration of the working principle for detection by a CRISPR/Cas12a system are illustrated in Figure 1.

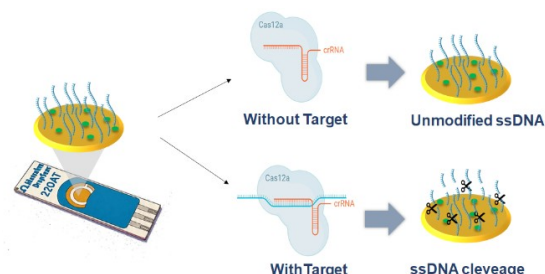


Fig. 1. Schematic illustration of the working principle for detection by a CRISPR/Cas12a system.

Results

Polyacrylamide gel electrophoresis (PAGE) was used to evaluate the molecular components of the CRISPR/Cas12a system before electrochemical characterization. Individual lanes confirmed the integrity of the crRNA, ssDNA reporter and the target oligonucleotide. The control reactions lacking either the target DNA or the crRNA showed electrophoretic profiles distinct from the complete CRISPR reaction, indicating that activation of Cas12a depends on the simultaneous presence of both components. The different migration pattern observed for the complete reaction is consistent with target-dependent activation of Cas12a and supports the subsequent electro-chemical evaluation of the sensing platform.

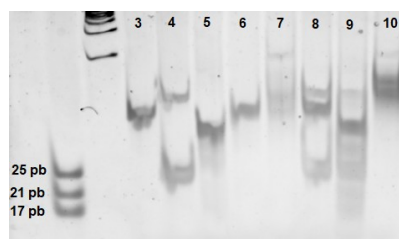


Fig. 1. 20% PAGE analysis of the CRISPR/Cas12a system. Lanes 1–2: DNA ladder; lane 3: crRNA; lane 4: ssDNA reporter; lane 5: target DNA; lane 6: crRNA/Cas12a; lane 7: crRNA/Cas12a + target DNA; lane 8: crRNA/Cas12a + ssDNA reporter; lane 9: CRISPR reaction without crRNA; lane 10: complete CRISPR/Cas12a reaction.

To optimize the sensor fabrication, the effect of ssDNA reporter concentration was first investigated (Figure 2a). The higher charge transfer resistance (R_{ct}) obtained with 2.5 μM ssDNA suggests the formation of a well-oriented and homogeneous DNA/MCH mixed monolayer, which effectively repels the anionic redox probe through electrostatic and steric hindrances. In contrast, a higher probe concentration (5 μM) may induce steric crowding during immobilization, hindering proper molecular rearrangement and efficient MCH backfilling. This can lead to a less organized interfacial layer with disordered DNA alignment, allowing easier access of the redox probe to the electrode surface and resulting in lower R_{ct} values. Using the optimized ssDNA concentration (2.5 μM), the effect of immobilization time was evaluated (Figure 2b). Although a slightly lower R_{ct} was observed after 2 h compared to 1 h, the longer incubation time was selected because it may favor the rearrangement of the immobilized DNA into a more ordered monolayer. Such structural reorganization can improve probe orientation and produce a more homogeneous sensing interface without necessarily increasing the R_{ct} . Therefore, an immobilization time of 2 h was adopted for the subsequent experiments.

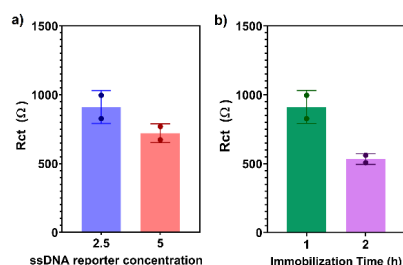


Fig. 2. Optimization of sensor modification conditions (R_{ct}): (a) ssDNA reporter concentration (fixed time: 2 h); (b) immobilization time (fixed concentration: 2.5 μM).

The electrochemical response of the sensor during the stepwise modification process is shown in Figure 3. The initial immobilization of the ssDNA reporter on the bare gold electrode led to a characteristic impedance profile, confirming successful probe attachment. Subsequent surface backfilling with MCH resulted in a slight R_{ct} increase, indicating the successful displacement of nonspecifically adsorbed DNA segments and the passivation of remaining bare gold sites. This modification ensures proper probe orientation while preventing nonspecific interactions. After incubation with the activated CRISPR/Cas12a complex, a decrease in R_{ct} was observed (Figure 3, blue curve), consistent with the collateral cleavage of the immobilized ssDNA reporter. This partial removal of the negative DNA layer facilitates electron transfer of the redox probe, confirming the efficiency of the Cas12a trans-cleavage activity at the sensing interface.

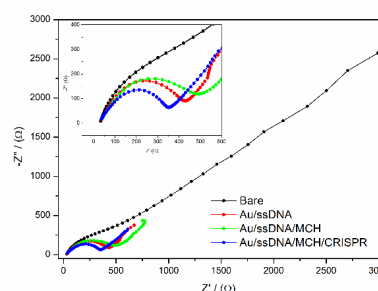


Fig. 3. Nyquist plots for the stepwise modification procedure. The inset shows an enlarged view of the modification steps.

The proposed platform was applied to the detection of a model target, demonstrating its potential for CRISPR-based electrochemical detection, with potential applications in genetic disorders such as Ataxia de Friedreich. These results highlight the critical role of probe density and surface organization in the design of efficient electrochemical CRISPR biosensors.

Acknowledgements

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References

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