



HOKKAIDO UNIVERSITY

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Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Development of Highly Sensitive and Quantitative microRNA Detection System
by a Bipedal DNA Walker-Based Ratiometric Fluorescent Analysis
(二足 DNA ウォーカーを基盤とした蛍光比率解析による
高感度 microRNA 定量検出システムの開発)

DNA walkers enable signal amplification through cyclic enzymatic reactions, offering powerful strategies for the highly sensitive detection of low-abundance microRNAs (miRNAs). However, fluctuations in the cellular microenvironment—such as variations in ion concentration, enzyme activity, temperature, and pH across subcellular compartments—can disrupt amplification efficiency and compromise detection accuracy. To address this challenge, we developed a symmetric bipedal DNA walker design in which one “leg” generates a detection signal while the other “reference leg” synchronously amplifies a dynamic internal reference signal. Unlike traditional static internal reference strategies, this design introduces a real-time, synchronized internal reference during the DNA walking process to correct for system errors. When combined with ratiometric fluorescence-based error compensation, this approach effectively eliminates signal variation caused by nanoparticle loading, strand assembly, and environmental fluctuations, thereby improving detection precision and reproducibility. The system, constructed on gold nanoparticles using an inverted hairpin scaffold, demonstrated robust and consistent performance across varied experimental conditions. Confocal ratiometric fluorescence imaging enabled high-contrast quantification of intracellular miRNA-21 (miR-21) levels across different cell lines. Moreover, spatially resolved fluorescence signals revealed heterogeneous miR-21 distribution within individual cells, providing valuable insights into its subcellular localization and functional relevance. This strategy represents a reliable platform for accurate miRNA quantification in complex biological environments.

The thesis is structured as follows:

- Chapter 1: miRNA as Cancer Biomarkers and Advanced Detection Strategies. This chapter introduces the rationale for using miRNA as a cancer biomarker, discusses the application of DNA walkers in detecting small targets, and explains the importance of ratiometric signals in enhancing detection accuracy.
- Chapter 2: Design of a bipedal DNA walker sensor. This chapter details the design of the bipedal DNA walker sensor, including the rationale behind the symmetric architecture and the selection of detection and reference signal pathways.
- Chapter 3: Construction and responsiveness of the bipedal DNA walker sensor. This chapter describes the fabrication of the bipedal DNA walker sensor and evaluates its responsiveness and stability across various experimental settings.
- Chapter 4: Performance of a BDW Ratiometric Sensor for miR-21 Detection. This chapter presents an evaluation of the BDW ratiometric sensor's performance in miR-21 detection, highlighting key aspects such as sensitivity, selectivity, and anti-interference properties.
- Chapter 5: Visualization and Quantitative Analysis of Intracellular miR-21 Using a BDW Ratiometric Sensor. This chapter demonstrates the application of the BDW ratiometric sensor in visualizing and quantitatively analyzing intracellular miR-21, underscoring its potential utility in cancer diagnostics.
- Chapter 6: Conclusion. This chapter provides a summary of the work conducted and discusses future outlook and potential directions.

We have successfully constructed a bipedal DNA walker-based ratiometric fluorescent sensor for the sensitive and accurate detection of miR-21, but also demonstrated its capability in fluorescence imaging to reveal differential distributions of miR-21 across cancer cell types.