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学 位 論 文 の 内 容 の 要 約
Summary of doctoral dissertation content

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学位の種類 Doctoral degree	博士（工学）
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指 導 を 受 け た 大 学 University	東京農工大学
学位論文題目 Thesis title	ナノポアによる相補鎖 DNA の unzipping を用いた標的分子検出に関する研究

【論文の内容の要約】[Summary of the contents of the doctoral dissertation]

This paper presents an approach to detect microRNA (miRNA) expression patterns by unzipping DNA duplexes through a biological nanopore. MiRNAs have been identified as specific markers for cancer diagnosis, so it is crucial to develop simple strategies for miRNA pattern recognition. Chapter 1 summarizes previous research on nanopore-based miRNA detection technology. The proposed system in Chapter 2 enables pattern recognition of five types of miRNAs that are overexpressed in bile duct cancer (BDC). The BDC miRNA patterns are encoded in diagnostic DNAs (dgDNAs) and electrically decoded by nanopore analysis. The system successfully detects miRNA expression patterns from the plasma of BDC patients without the need for labeling. In addition, the dgDNA-miRNA complexes can be detected at sub-femtomolar concentrations (~10^{-16} M), a significant improvement over previously reported detection limits (~10^{-12} M) for similar analytical platforms. The detection of concentrations below the detection limit of conventional nanopore measurements was assumed to be caused by an excess amount of dgDNAs relative to the target miRNAs. Based on a theoretical estimate, we found that the higher concentration of our diagnostic DNA relative to the target RNA molecules plays a critical role in this phenomenon. Our finding should be an intriguing physicochemical phenomenon that has never been proposed before, and it will be an essential concept for low-concentration detection using nanopore technology. In Chapter 3, I reproduced the ultra-low concentration detection phenomenon found in a more straightforward system and identified the dgDNA sequence conditions and concentration that improved sensitivity. It was found that the complementary part of the probe sequence had the most significant influence on sensitivity and that, for the detection of fM levels, sensitivity was significantly improved by using probes at a concentration of approximately 107 times that of the target nucleic acid molecule. The sensitivity enhancement by the excess

amount of dgDNA shown in this study may lead to the development of more sensitive nanopore measurement techniques, which can be combined with other techniques to improve sensitivity. Chapter 4 provides a summary and outlook. Nanopore decoding of dgDNA-encoded information is a promising tool for simple and early cancer diagnosis. The proposed system would be potentially valuable for medical applications if integrated into a commercially available nanopore sequencer. In addition, several biological nanopores other than αHL have been used for nanopore sensing. Nanopore decoding will have the potential to be applied to the different types of nanopores. It is hoped that the results of this study will lead to the further development of nanopore sensing and form part of the basis for the development of practical technology, including pathological diagnosis technology.