

(様式 5)

2024年 3月 28 日
Year Month Day

學位 (博士) 論文要旨

(Doctoral thesis abstract)

論文提出者 (Ph.D. candidate)	工学府博士後期課程 2021 年度入学 (Admission year) 学籍番号 21831007 (student ID No.)	生命工学専攻 (major) 氏名 竹内七海 (Name)
主指導教員氏名 (Name of supervisor)	川野竜司	
論文題目 (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	
論文要旨 (2000 字程度) (Abstract(400 words)) ※欧文・和文どちらでもよい。但し、和文の場合は英訳を付すこと。 (in English or in Japanese) In this paper, an approach to detecting microRNA (miRNA) expression patterns using nanopore-based DNA computing technology is presented. MiRNAs have been identified as specific markers for cancer diagnosis, making it 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 miRNA information from BDC is encoded in diagnostic DNAs (dgDNAs) and decoded electrically through nanopore analysis. The system successfully detects miRNA expression patterns from the plasma of BDC patients without the need for labeling. Additionally, the dgDNA-miRNA complexes can be detected at subfemtomolar concentrations ($\sim 10^{-16}$ M), which is a significant improvement compared to previously reported limits of detection ($\sim 10^{-12}$ M) for similar analytical platforms. The detection of concentrations below the detection limit of conventional nanopore measurements were assumed to be caused using an excess amount of dgDNAs relative to the target miRNAs. Based on a theoretical estimation, we found that the higher concentration of our diagnostic DNA compared 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, and it will be an important concept for low-concentration detection using the nanopore technology. In Chapter 3, I reproduced the phenomenon of ultra-low concentration detection discovered in a simpler system and clarified the dgDNA sequence conditions and concentration that improve the sensitivity. The complementary sequence portion of the probe sequence has the greatest influence on sensitivity, and that in the detection of fM levels, sensitivity was markedly improved by using probes at a concentration of about 10^7 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 by combining it with other techniques to improve sensitivity. Chapter 4 presents a summary and outlook. Nanopore decoding of dgDNA-encoded information represents a promising tool for simple and early cancer diagnosis. The proposed system would be potentially useful for medical applications if this system is integrated into a commercially available nanopore sequencer. Moreover, several biological nanopores other than α HL have also been used for nanopore sensing. The nanopore decoding will have the potential to apply to the different types of nanopores. I hope that the results of this study will lead to the further development of nanopore sensing and will be part of the foundation for the development of practical technology, including pathological diagnosis technology.