

Public Abstract

First Name:Yong

Middle Name:

Last Name:Wang

Adviser's First Name:LiQun (Andrew)

Adviser's Last Name:Gu

Co-Adviser's First Name:

Co-Adviser's Last Name:

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Title:CHARACTERIZATION NUCLEIC ACIDS UNWINDING AND EXPLORING ITS APPLICATION IN MIRNA DETECTION IN THE NANOPORE

MicroRNAs (miRNAs) are a class of short (~14-27 nucleotides) noncoding RNAs that regulate gene expression at the post-transcriptional level. As powerful gene regulators, miRNA binding induces either translational repression or cleavage of target mRNAs. MiRNAs play important roles in development, cell differentiation, and regulation of cell cycle, apoptosis and signaling pathways. Aberrant expression of miRNAs has been found in all types of tumors. Different cancer types have distinct miRNA expression profiles. Reverse transcription real-time polymerase chain reaction (qRT-PCR) and microarrays have been developed for detecting microRNA; however, these methods need labeling and amplification, as they also suffer from cross-hybridization, low selectivity and lack of valid internal controls.

The development of nanopore sensors for microRNA detection is a new effort. One of the superior properties of nanopore is that the ion current in a nanometer-scaled pore structure is very sensitive to the presence, location and conformation of single target molecules occupying the ion pathway. This sensitivity allows elucidating single molecule kinetics from characteristic changes in the pore conductance, and further, quantifying the target from the occurrence of single molecule signature events.

Here we show that a nanopore sensor based on the alpha-hemolysin protein can selectively detect microRNAs at the single molecular level in the plasma samples of lung cancer patients without labeling and amplification of microRNAs. First, we uncovered a signature current pattern that can be used to electrically track the double-stranded DNA (dsDNA) unzipping process. With the signature signals, we can also distinguish the release of dsDNA without unzipping. Second, based on the electrical signatures we identified, we have designed a nanopore-based microRNA sensor that uses a programmable oligonucleotide probe to generate a signature electrical signal for the direct and label-free detection of target microRNA in a fluctuating background, such as plasma RNA extracts from clinical samples.

This sensor can quantify picomolar levels of cancer-associated microRNAs and can distinguish single-nucleotide differences between microRNA family members. This nanopore method can be a useful tool for quantitative studies of microRNAs, which are important for non-invasive screening and the early diagnosis of diseases such as cancer.