



Nanopores for Gene Sequencing

Viktor Stolc, NASA-ARC

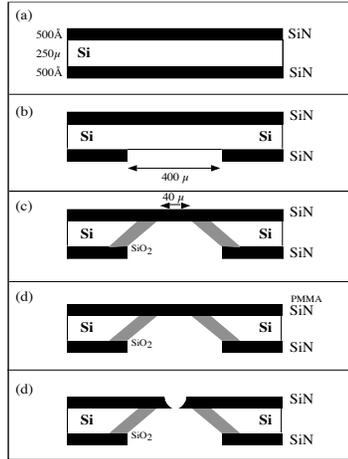
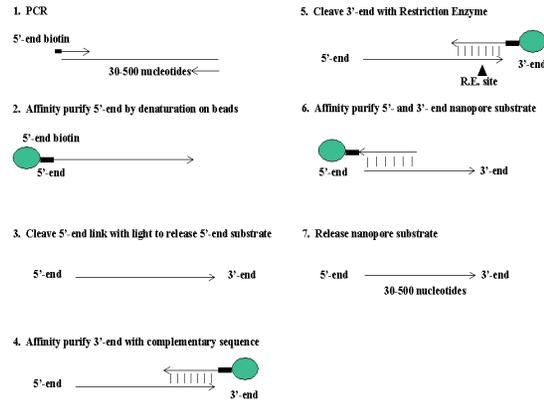


Figure 1. Main processing steps in the fabrication of nanopore.

A schematic diagram of a protocol for selection synthesis of a nanopore DNA substrate.



Description

The objective of this proposal is to develop a device that can sequence single molecules of nucleic acid, DNA or RNA, at a rate of million bases per second by electrophoresis of the charged polymers through a solid-state nanopore channel of molecular dimensions. This will be accomplished with the use of experimentation in a solid-state nanopore made using nanofabrication techniques. The nanopore channel with a diameter and length of less than 5 nm will be made in a silicon-based chip that will have nanoelectrodes placed adjacent to the pore. High-speed electronic equipment with exceptional signal acquisition capabilities will be used to analyze electronic properties of individual subunits of DNA or RNA, to obtain linear composition of each genetic polymer molecule.

Innovative Claims/NASA Significance

Nanopore-based analysis of nucleic acid polymers is revolutionary, because no other technique can determine information content in single molecules of genetic material at the speed of 1 subunit per microsecond. Because individual molecules are counted, the output is intrinsically quantitative. The nanopore approach is more generalized than any other method and in principle may be used to analyze any polymer molecule, including proteins.

The NASA Ames Research Center (ARC) Nanotechnology group wants to develop a solid-state nanopore device with specified geometry and composition of the nanopore. The approach to the development of a solid-state nanopore device is novel in the use of nanofabrication, nanoelectronic components, and high-speed signal acquisition. A novel geometry of the solid-state nanopore (less than 5 nm length and 5 nm diameter) may enable 1-5 nucleotide resolution measurements. This means that maximum resolution will be improved at least 100-fold compared to the (□)-hemolysin ion-channel measurements.

Plans

Year 1:

- Determine the best method for generating small pores, using electron beam etching.
- Examine the use of alternative materials to make small pores, such as generation of mesoporous carbon paper made from nanotubes.
- Demonstrate ability to detect DNA fragments in the form of single stranded and double stranded oligonucleotides in a solid-state pore.

Year 2:

- Test the solid-state nanopore to determine length of oligonucleotides.
- Demonstrate ability to distinguish between single stranded and double stranded oligonucleotides.
- Demonstrate ability to distinguish between oligonucleotide homopolymers of different identity (i.e. polyA, polyC, polyG, polyT, polyU, and combinations of these oligomers).
- Determine feasibility of detecting and analyzing proteins using the solid-state nanopore.
- Demonstrate ability to sequence short strands of DNA (approximately 30 nucleotides).
- Develop methods to detect single nucleotide polymorphism.
- Explore alternative uses of the solid-state nanopore (e.g. for detection of protein-DNA, and/or protein-RNA interaction mapping, DNA, RNA and/or protein modification mapping).
- Develop prototype sequencing device.