ELEC3223 Intro to BioNanotechnology
ELEC6205 BioNanotechnology

Single molecule sensing with nanochannels and nanopores
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“The potential and challenges of nanopore sequencing”
Nature Biotechnology 2008

“Continuous base identification for single-molecule nanopore DNA sequencing”
Nature Nanotechnology 2009

Companies: Agilent; IBM; Oxford Nanopore; NABsys
The first human genome sequence was completed in 2003 through the Human Genome Project, a 13-year effort with an estimated cost of $2.7 billion.

In 2008 a human genome was sequenced over a 5-month period for approximately $1.5 million.

In the rapidly evolving field of “next-generation” sequencing (NGS) 5 commercial technologies have emerged during the past 5 years.

The US National Human Genome Research Institute (NHGRI) announced funding in August 2008 for a series of projects as part of its Revolutionary Genome Sequencing Technologies program, which has as its goal the sequencing of a human genome for $1000 (http://www.genome.gov/27527585).

Nanopores are a possible method of doing this.
Why nano-pores?

• Single molecule spectroscopy, dynamics, kinetics
• Single molecule sensing and sequencing
• Single molecule high throughput analysis
• Single molecule sequencing and PCR
• High speed molecular analysis
• Nano-fluidics
Nano bio-sensing

The Coulter Counter; principle
Coulter counter – counting particles

Low frequency (1kHz)
Low voltage (100mV)

Small hole
50μm for cells

NaCl

50μm hole
Aperture is slightly larger than a cell.

Cell transiently blocks current path. Magnitude of change depends on cell size – technique used as a counter and to measure cell size.
Analysis:
Particle is considered an **insulator** which **displaces** a given volume of fluid. This changes the **resistance** of the column of electrolyte, i.e. is there is a change in the current path.

\[ \rho \text{ the resistivity, } = \frac{1}{\sigma}, \text{ where } \sigma \text{ is the conductivity of the fluid} \]

\[ A_c \text{ area of channel} \]

\[ A_p \text{ area of the particle} \]
Consider tube, diameter $D$, length $L$ filled with aqueous liquid, resistivity $\rho$. Resistance of the empty channel is

$$R = \rho \frac{L}{A_c} = \frac{4 \rho L}{\pi D^2} \quad (1)$$

Maxwell showed that the **effective** (new) resistivity of a **suspension** of insulating spheres is given by

$$\rho_{\text{eff}} = \rho(1 + 3\phi / 2) \quad (2)$$

where the volume fraction $\phi$ for a particle in a cylinder is given by

$$\phi = \frac{\text{vol particle}}{\text{vol cylinder}} = \frac{2d^3}{3D^2L} \quad (3)$$
Combine (1), (2) and (3) to give the resistance of a channel (cylinder) diameter $D$, containing a particle, diameter $d$

$$R' = \frac{4\rho L}{\pi D^2} \left( 1 + \frac{d^3}{D^2 L} \right) = \frac{4L}{\sigma \pi D^2} \left( 1 + \frac{d^3}{D^2 L} \right)$$

The change in resistance $\Delta R$ is:

$$\Delta R = R' - R = \frac{4}{\sigma \pi D^4} d^3$$

Proportional to volume of particle and fourth power of the radius of the cylinder.
**Question:** Assume cylinder 200nm long, 50nm diameter. The cylinder is filled with KCl, with $\sigma = 0.5 \text{ S/m}$

- Calculate the DC current flowing through the cylinder at 1V.
- Calculate the change in current when a 5nm particle passes through the channel.
**Example:** Assume cylinder of KCl, with \( \sigma = 0.5 \text{Sm}^{-1} \) \((\rho = 2 \Omega \text{m})\)

Channel is 200nm long, 50nm diameter. Resistance of channel is

\[
R = \frac{1}{\sigma A_c} = \frac{4}{0.5 \pi (50 \times 10^{-9})^2} \frac{200 \times 10^{-9}}{2.04 \times 10^6 \text{Ohm}}
\]

Introduce a 5nm diameter protein molecule (insulator)

\[
\Delta R = R' - R = \frac{4}{0.1 \pi (50 \times 10^{-9})^4} \frac{(5 \times 10^{-9})^3}{2.55 \times 10^5 \Omega}
\]

which is around 1 in 10 change in resistance
For an applied voltage of 1V, current in empty channel is

$\frac{1}{(2.04 \times 10^6)} = 0.49\text{uA}$ or $490\text{nA}$.

CHANGE in current of around 1 in 10 is easy to measure, around $50\text{nA}$
Nanochannels:

- Biological – $\alpha$-haemolysin
- Synthetic - nanopores or nanochannels made in materials such as Au, Si or polymer
- Can make pores selective: e.g. binding charged molecules at the entrance.
- A positively charge molecule would repel molecules of equal charge.
Stochastic sensing using molecular pores

Protein pores - $\alpha$-haemolysin

Current fluctuates as ion passage is blocked by molecules.

The molecule to be detected is a transient blocker. It interacts with protein - binds and dissociates, hence stochastic current events.
Concept for sequencing DNA by using a single protein pore. A single-stranded DNA (or RNA) molecule moves through the pore in the electric field. As it passes a "contact site", each base produces a characteristic modulation of the amplitude in the single channel current.

The amount of current which can pass through the nanopore at any given moment varies depending on whether the nanopore is blocked by an A, a C, a G or a T (in theory).