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Nanopore Sensing for Single-Molecule Glycomics

Melissa Morris
University of Rhode Island, xmorrisx@my.uri.edu

THE UNIVERSITY OF RHODE ISLAND

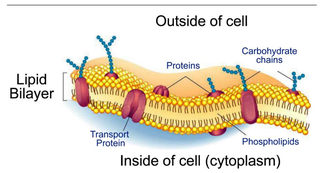
Nanopore sensing for single molecule glycomics

Melissa Morris, Chemistry

Jason R. Dwyer, Department of Chemistry, University of Rhode Island

1. The biological importance and complexity of sugars

Structure of the Cell Membrane



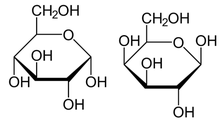
David Gable via thinglink.com/scene/750816659610009601

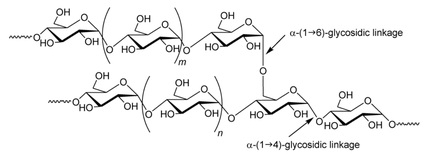
- Carbohydrates include mono-, oligo- and polysaccharides, and their derivatives.
- Carbohydrates act as receptors for bacteria, toxins, viruses, and other cells.
- Their molecular recognition capabilities make them potential stars in drug delivery systems.[1]

- Monosaccharides often only differ in one or two linkages, making their structures difficult to distinguish.
- Carbohydrate chains can have different lengths, different isomers, and complex branching.

Hypothesis

Using a reporter molecule complex, can we better detect sugars with single molecule devices?

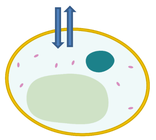




Amylopectin (branched glucose polymer)

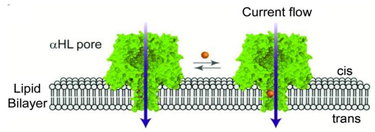
2. Inspired by nature: the birth of solid state nanopores

Cell Membranes



- Special proteins allow molecules to pass in and out of the cell body, as shown by the blue arrows.
- The proteins self-assemble to form narrow channels the width of a single molecule.
- Many of these proteins have been isolated for laboratory use.

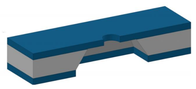
Biological Nanopores



Bayley, Clinical Chemistry **2015,** *61*, 26

- Suspension in a lipid bilayer aligns the proteins, but adds an element of fragility to the system; the lipid bilayer is only stable in a small range of pH values and temperatures.
- Biological nanopores can be toxic compounds, but are extremely stable.
- Detecting single molecules as they pass through the nanopore is a matter of measuring small fluctuations in current.

Solid-State Nanopores



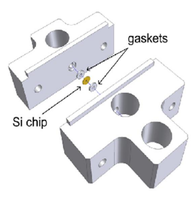
Dwyer, J. R., et. al. App. Spec. **2017,** *71*, 2064.

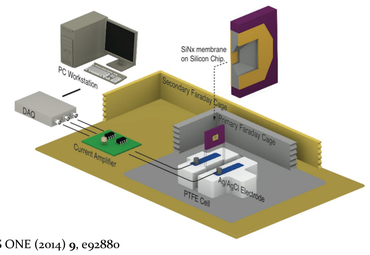
- Solid state nanopores have a robust 10 nm thick layer of silicon nitride(SiN_x) freestanding over the cavity of a silicon support layer!
- A nano-sized hole is manufactured in the thin layer of silicon nitride via a high electric field.
- The chemistry of the pore is quite plain, involving only Si, O, N, H.

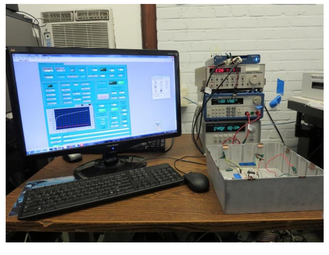
So, how big is a nanopore?

A 10 nm nanopore is **1/10,000** the diameter of a strand of hair.

3. Nanopore fabrication via dielectric breakdown

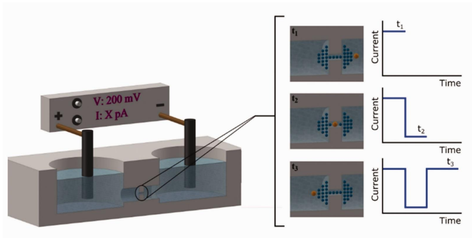




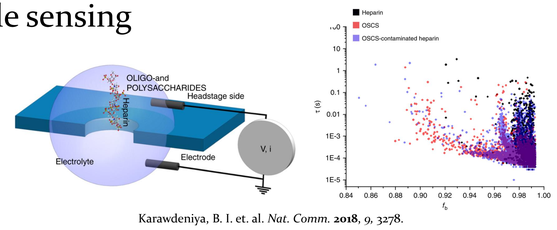


Kwok et. al. PLoS ONE (2014) **9**, e92880

4. Nanopore-based single molecule sensing



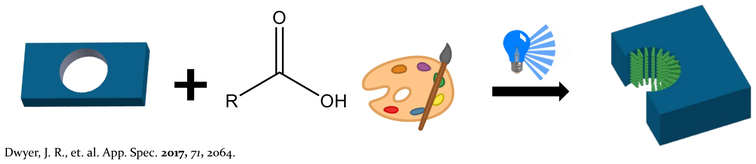
Dwyer, J. R., Harb M. App. Spectroscopy (2017) **71,** 2064.



Karawdeniya, B. I. et. al. *Nat. Comm.* **2018**, *9*, 3278.

- Solid state nanopores were recently used in the first single molecule glycomics study.
- The detected sugar was a common anti-coagulant, heparin, which in 2008 was contaminated with a similar sugar that unfortunately killed many people.
- This work laid the foundation for nanopore-based drug quality assurance.

5. Surface modifications of SiN_x nanopores



Dwyer, J. R., et. al. App. Spec. **2017,** *71*, 2064.

Molecular Painting

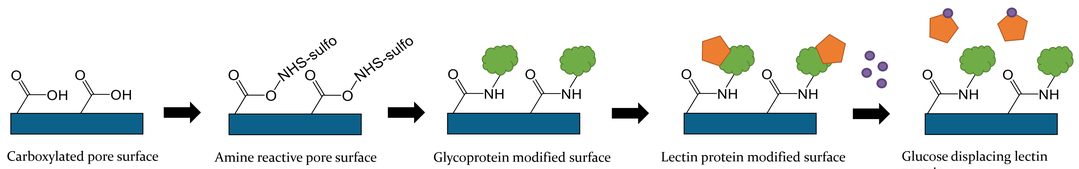
- With the help of UV radiation, certain molecules bind covalently to SiN_x.
- Modifying the chemistry of the pore provides control over the charge and chemical reactivity of the surface.
- By attaching carboxylic acids, a carboxylated pore surface is formed and can serve as a platform for more complex reactions, as seen below.

6. Biomolecular conjugation inside a nanopore

Horse radish peroxidase (Glycoprotein) 3.0 x 3.5 x 6.0 nm[5]

Concanavalin A (Lectin protein) 6.0 x 7.0 x 6.0 nm[6]

Glucose



Carboxylated pore surface | Amine reactive pore surface | Glycoprotein modified surface | Lectin protein modified surface | Glucose displacing lectin protein

Adapted from Ali et. al. Nanoscale (2011) 3, 1897.

Biomolecular recognition

- As shown above, a four step reaction was carried out to attach molecules to a carboxylated nanopore surface.
- The addition of the glycoprotein horse radish peroxidase to immobilize the lectin protein concanavalin A inside a nanopore was first demonstrated by Ali et. al.[3]
- Through a well-known interaction, immobilized concanavalin A will selectively bind to glucose, and detach from the surface.

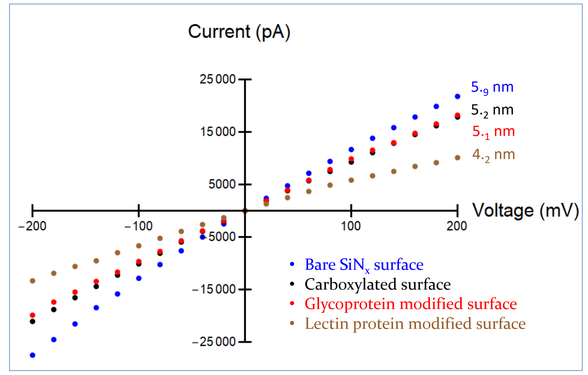
Conductance-based size profiling

- The molecular cartoons above are not to scale, however each attachment decreases the radius of the nanopore:

$$I = KV\left(\int \frac{dz}{\pi(r(z))^2}\right)^{-1}$$

I = current
K = solution conductivity
V = voltage
r = radius of nanopore

- The plot on the right shows IV curves taken after key steps in the reaction, with listed radius values on the left.



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(9) Dwyer, J. R., *App. Spectroscopy* **2017,** 71, 2064.
(10) Karawdeniya, B. I. et. al. *Nat. Comm.* **2018**, *9*, 3278.
(11) David Gable via thinglink.com/scene/750816659610009601

Acknowledgements

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National Science Foundation WHERE DISCOVERIES BEGIN

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Contact Information

Melissa Morris
Richard E. Beaupre Center for Chemical & Forensic Sciences
140 Flagg Rd, Kingston, RI 02881
xmorrisx@my.uri.edu