Ben Snyder
Bioanalytical Chemistry
Drossman

POGIL - Nanopore DNA sequencing

Model 1 – A Look at α-Hemolysin

1. What is a nanopore? Can you think of any naturally existing nanopores? What are their functions?

2. Where are almost all naturally occurring nanopores found?

3. How does this general location define the function of a nanopore? Could the function of a nanopore be defined in the absence of these structures? Hint: think *cis* and *trans*…

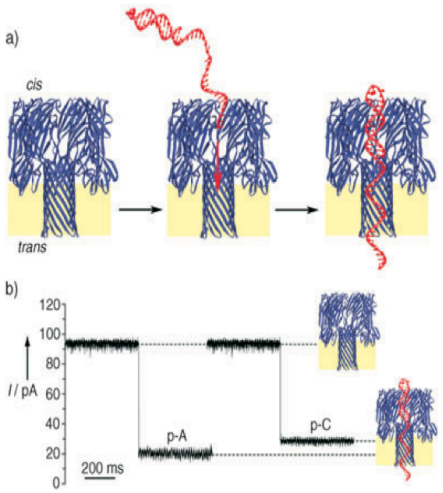
4. Information carrying biological molecules exhibit directionality. With this in mind, what structural element must be present when studying the action of nanopores *in vitro*?

5. The Ashkenasy article outlines the use of α-hemolysin as a nanopore. α-Hemolysin is a bacterial exo-toxin that lyses blood cells by inserting itself into the cell membranes of blood cells. With this in mind, why do you think α-hemolysin was chosen for use in this study of hundreds of other porins?

6. α-Hemolysin has no specific affinity for DNA. The interaction between DNA and the pore of hemolysin is probably not particularly favorable as a result. The researchers solved this problem by running a current across a membrane in which a single molecule of hemolysin was embedded. Why was this an effective solution to the problem outlined above?

7. Practically speaking, why do you think it was necessary to use a single molecule of hemolysin?

8. This study demonstrates a connection between the sequence of a molecule of DNA and the magnitude of the drop in the previously mentioned cross-membrane current that occurs as it 'plugs' a nanopore. Considering that the current flowing across the membrane had a constant potential of ~170 mV, can you think of how pulling a molecule of DNA through a nanopore would result in a current change? Hint: V=iR.



9. With this in mind, can you think of another reason why it was important to use a single molecule of hemolysin?

10. How might this present a significant setback towards the goal of sequencing the human genome for less than $1000 using this method?

Model 2 – Analyzing the Methods and Results Presented in the Ashkenasy Article

1. Take a look at the following DNA strand:

   T^{T}GCAAGCTGACCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3,
   T_{T}CGTTCGACTGGG-5,

   The authors admit that, at this moment, there is no easy way of controlling the passage of ssDNA through a nanopore using this method. As a result, ssDNA tends to move through a pore far too quickly for it to generate a useful signal. dsDNA, on the other hand, is not capable of traveling through the pore of α-hemolysin. The authors were able to generate a useful signal using the DNA shown above. How is it that this molecule is capable of generating a signal, while ssDNA and dsDNA are not?

2. The diagram to the right shows the residual cross-membrane current (as % applied current) observed when each of the four DNAs to the right was inserted into the single molecule of α-hemolysin embedded in that membrane. What region of each DNA molecule was interacting with the most constricted region of the nanopore?

3. Can you think of a reason for the high residual current associated with strands 7 and 8 relative to strands 5 and 6? Remember: V=iR

4. This paper looks at the different signals generated by DNAs containing adenine and cytosine. It even demonstrates that it is possible to generate signals with single-base resolution using these strands. With your answer to the last question in mind, can you think of any problems that might arise when trying to sequence a DNA containing all four bases?

5. Look at the units of the y-axis of the chart to the right. Is the data presented here encouraging with respect to the goal of accurately sequencing many bases?

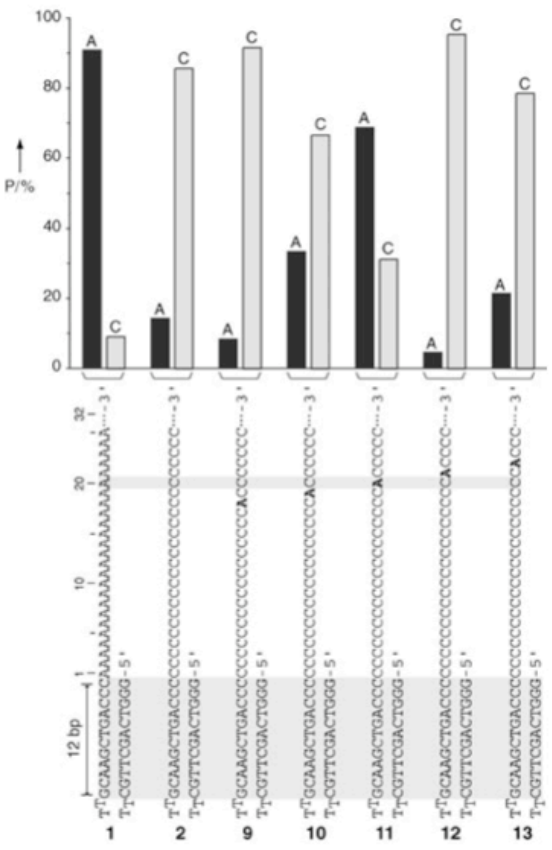


Figure 3. Percentage probability (P) of the A-type ($I_R < 27\%$) and the C-type ($I_R > 27\%$) residual currents for single deoxyadenosine-substituted poly-d(C) DNA strands captured inside the α-HL pore as a pseudorotaxane. Percentage probabilities were calculated by the number of A-type or C-type events measured at 170 mV divided by the total number of events (n) recorded for a given strand ($n = 11$, 21, 22, 32, 22, and 14 for strands **1**, **2**, **9**, **10**, **11**, **12**, and **13**, respectively). The percentage probabilities of A-type and C-type events for each strand are shown as black and gray bars, respectively.

6. Consider that the signal resolution for the sequencing of the human genome was, on average, 1 mistake per 1,000,000 bases (?). What single-base resolution does this work out to? How does this compare to the accuracy of the method outlined in this paper?

Model 3 – Addressing the Problem of Resolution in Nanopore Sequencing

1. Soni and Meller took an interesting first step in improving the resolution of nanopore sequencing: they modified the *information* they were trying to detect instead of just trying to improve the resolution of their instrumentation. Using a strand of DNA as a template, they created a new strand carrying all of the information found on the old strand expressed in binary units. For example, guanine could be translated into the binary sequence 1,0, with 1 represented as CGGGCGGCAA and 0 represented as ATTTATTAGG. What was the purpose of replacing a single base with twenty bases?

2. Why was a single-bit (binary) translation chosen over a two-bit (base four) translation?

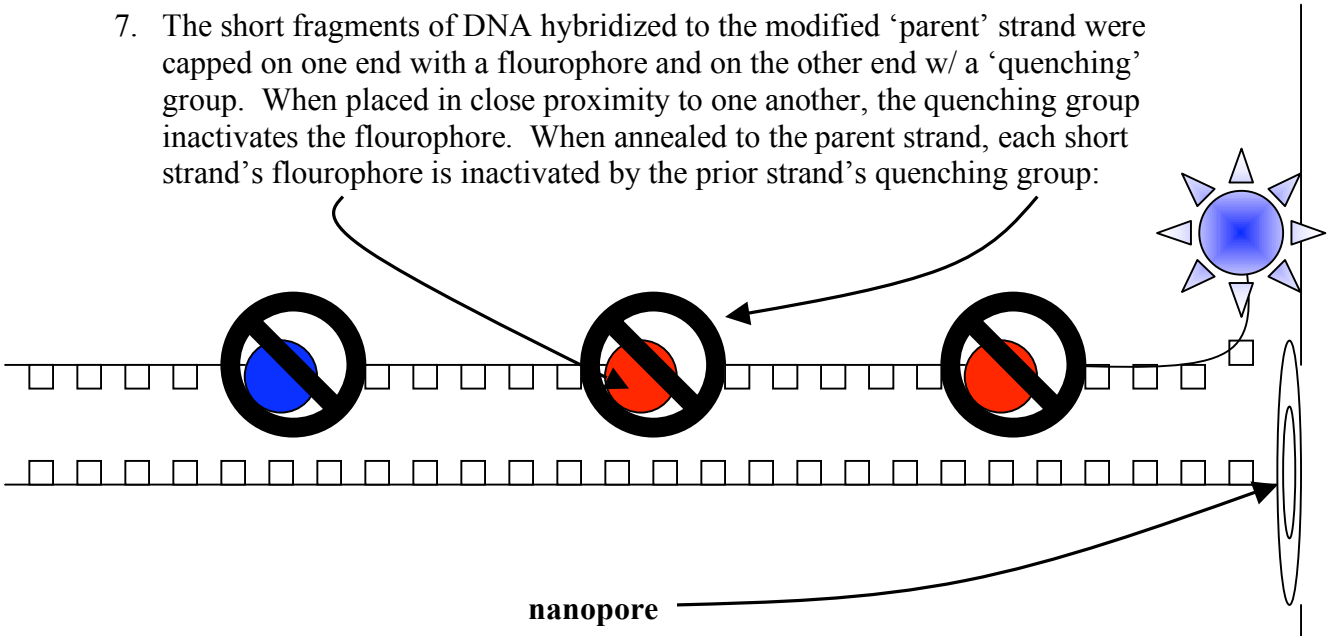
3. Can you think of any problems that might arise from this necessary translation?

4. This paper outlines the use of solid-state nanopores – e.g. small holes burned through an inorganic membrane w/ a laser. Why might a solid-state nanopore be favored over a biological nanopore (e.g. a porin embedded in a lipid bilayer)?

5. How does the use of solid-state nanopores allow for parallel processing?

6. The modified DNA analyzed using this method was hybridized with complementary10bp sequences, yielding a fully double-stranded DNA. The nanopore used was only wide enough to accommodate the passage of ssDNA, however. As the dsDNA was sucked through the nanopore by an electric field, it was forced to 'unzip' and shed these 10bp fragments in a sequential fashion. How do you think this affected the speed at which the DNA passed through the pore? If the DNA was drawn through the pore w/ an electric field, how do you think the speed at which it passes through a nanopore could be modulated?

7. The short fragments of DNA hybridized to the modified 'parent' strand were capped on one end with a flourophore and on the other end w/ a 'quenching' group. When placed in close proximity to one another, the quenching group inactivates the flourophore. When annealed to the parent strand, each short strand's flourophore is inactivated by the prior strand's quenching group:



What happens to the flourophore of each strand as the strand before it is 'peeled' away?

8. There is yet another way in which these flourophores may be inactivated by a quenching group. What might this be? Hint: Consider the sequence of one of these flourophore containing strands. Look at the bolded letters…

(quenching group)-**A**T**T**T**A**|**T**T**A**G**G**-(flourophore)

9. Why is it important that these flourophore-containing DNAs be capable of quenching themselves when not hybridized with the strand of interest?

10. The authors report a 99.9% single base accuracy using this method. How many bp long can a DNA be before this method presents an average error of 1bp? Is this accuracy (or lack thereof) acceptable?