

▼ Long Read 1

5 students

 Kathryn Bosley	⋮
 Andre Tischler	⋮

▼ Long Read 2

5 students

 Murat Aci	⋮
 Abraham Steinberger	⋮

▼ Long Read 3

5 students

 Chase Krug	⋮
 Oliver Schlegel	⋮

▼ Long Read 4

5 students

 Manashri Bhor	⋮
 Qiansu Ding	⋮

▼ Long Read 5

4 students

 Isaias Ariza Hernandez	⋮
 Alejandra Quinones	⋮

Warm up question:

Group 1 name one sequencing technology, the next group can either share the read-length from that technology, or name a different sequencing technology



Introduction



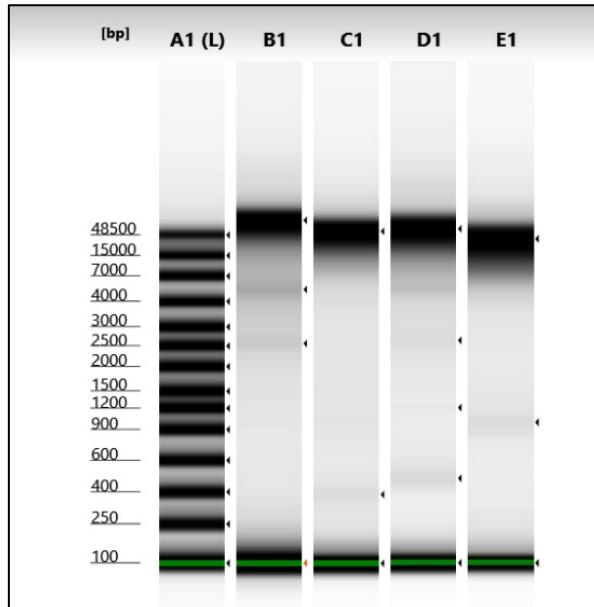
Goals for today

1. Become familiar with DNA requirements, library prep options, and flowcell loading
2. Know enough about how Nanopore sequencing works to troubleshoot
3. Basecall raw Nanopore data
4. Align reads to a reference genome
5. Think about where un-mappable reads are coming from



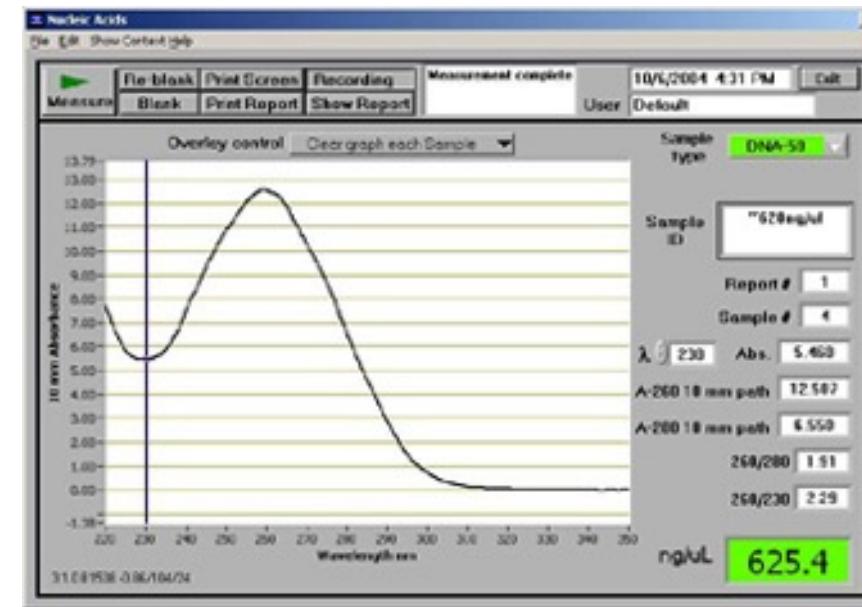
It starts with purified nucleic acid (DNA or RNA)

How can you tell if you've isolated high molecular weight DNA?



Agarose gel
Size selection
Tape Station/Bioanalyzer

Is my DNA pure enough?

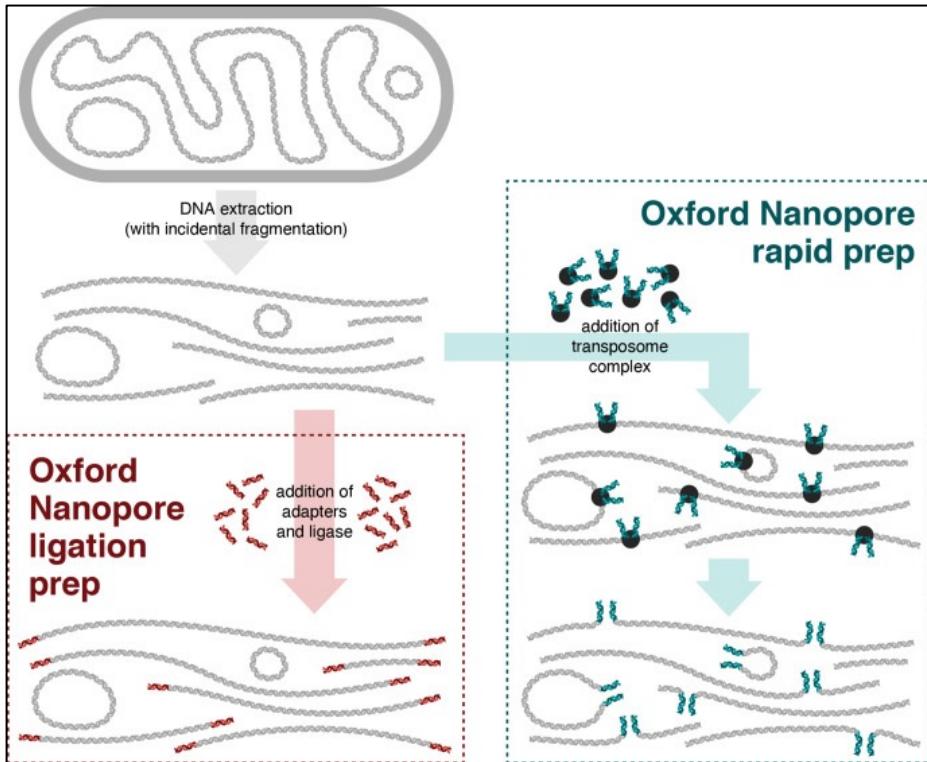


What are optimal 260/280 and 260/230 ratios?
What do low ratios indicate?
Can I trust the Nanodrop quantification?

You have good DNA – time to order some sequencing supplies

<https://nanoporetech.com/products/kits>

Library prep kit



Microb Genom. 2021 Aug;7(8):000631.
doi:10.1099/mgen.0.000631.

Flowcell/Hardware

Choose your flow cell type

Flowcell Type	Channels	TMO (Gb)	Suitable for
Flongle	126 channels	TMO 2.8 Gb	<ul style="list-style-type: none">Library QCPlasmid, viral and bacterial sequencingLow-cost, disposable flow cell from \$90
MinION	512 channels	TMO 50 Gb	<ul style="list-style-type: none">10-20 Gb of Ultra-long readsMultiplex small genomesLow-pass sequencing of larger genomesFrom \$500
PromethION	2,675 channels	TMO 290 Gb	<ul style="list-style-type: none">The highest output flow cells for nanopore sequencingSequence large genomes to high coverageFrom \$600

MinION



MinION Mk1C



MinION Mk1D



GridION



P2 Solo



P2



PromethION 24



PromethION 48

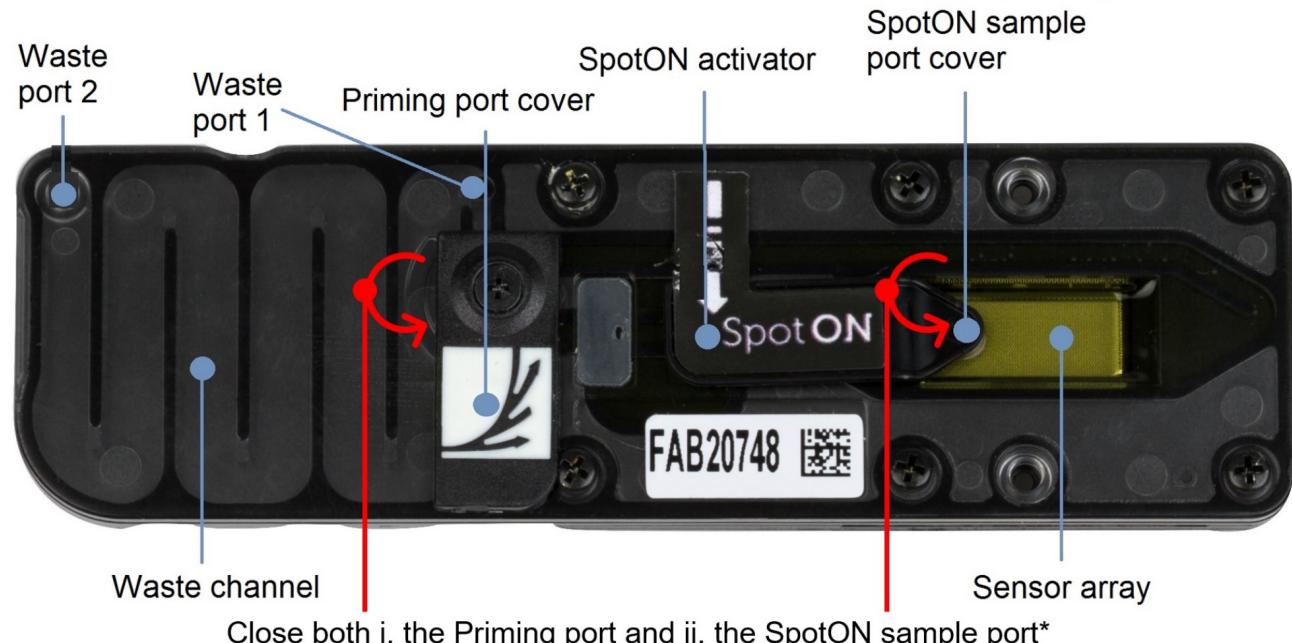


MinION and Flongle Flow Cell compatible

PromethION Flow Cell compatible

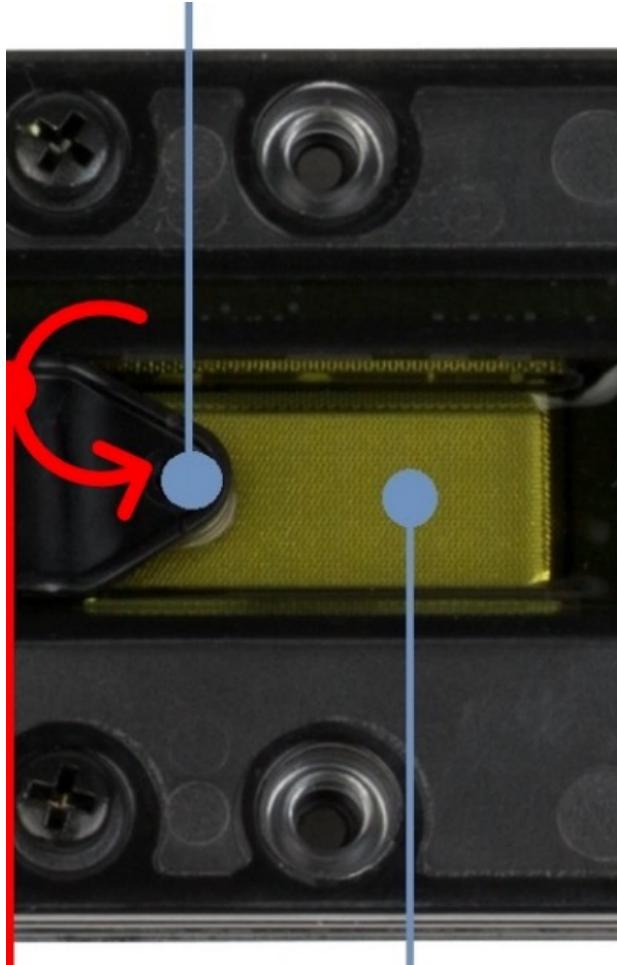
Configuration	Platform				Techniques		Tech specifications		
Number of flow cells per device	1	1	1	5	2	2	24	48	
Maximum number of channels per flow cell	512	512	512	512	2,675	2,675	2,675	2,675	
Run time	72 Hours	72 Hours	72 Hours	72 Hours					
Device TMO ^t	50 Gb	50 Gb	50 Gb	250 Gb	580 Gb	580 Gb	~7 Tb	~14 Tb	
Maximum number of flow cells per year*	104	104	104	520	208	208	2,596	4,992	
Offer sequencing as a service	No	No	No	Yes	Yes	Yes	Yes	Yes	

Let's take a
break and
check out a
flow cell

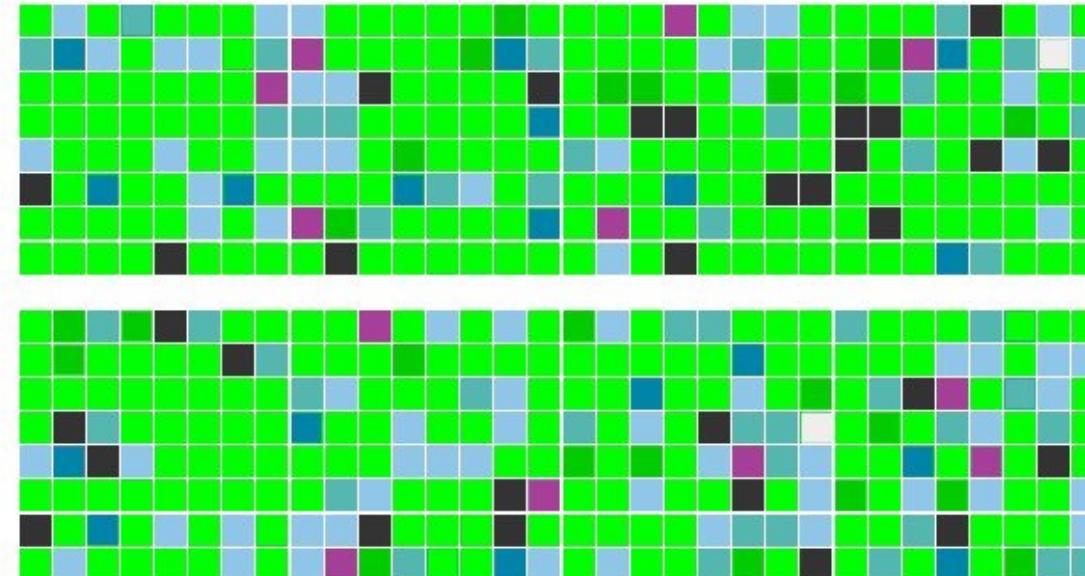


*Both ports are shown in a closed position

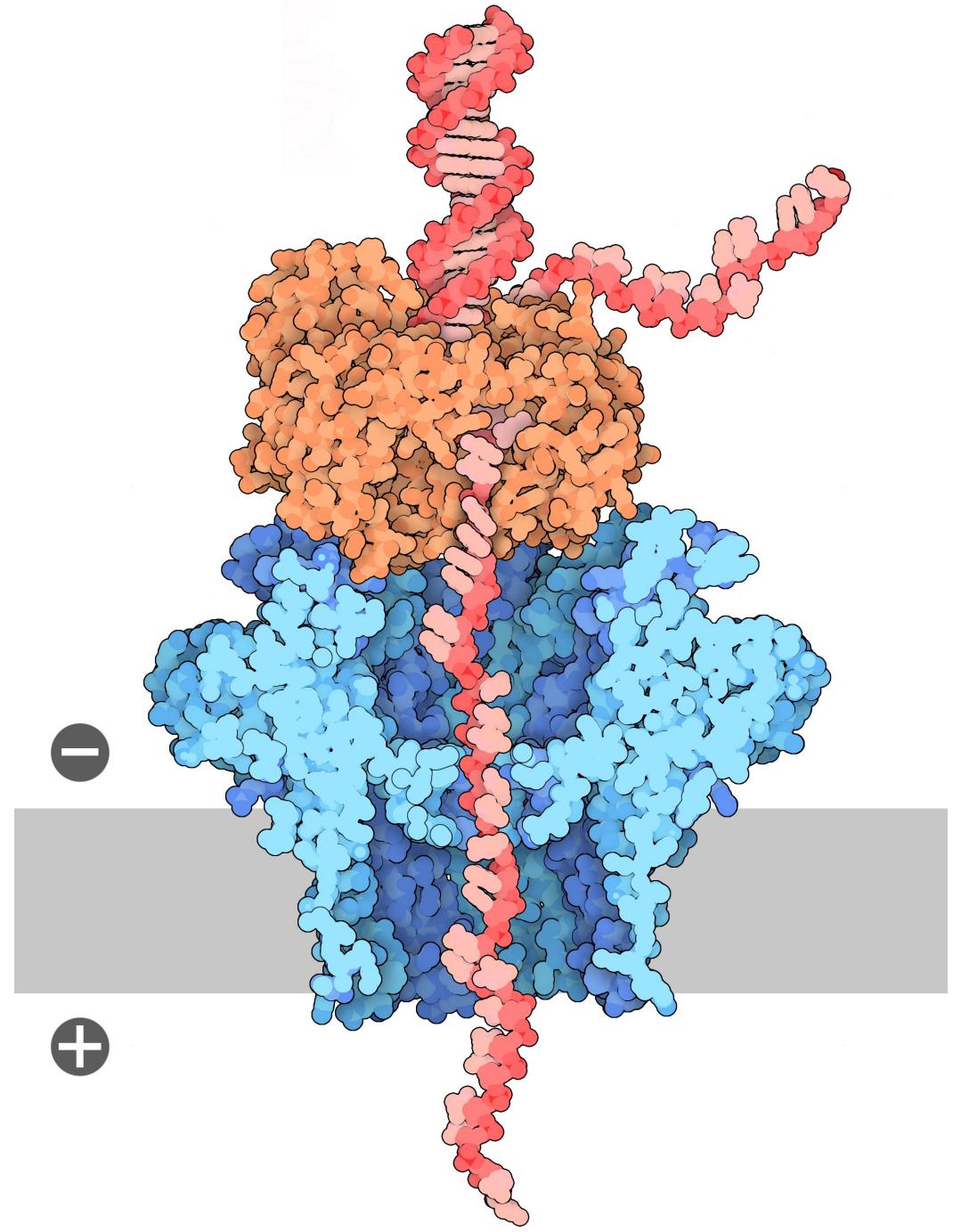
What's really going on in there?



- 512 channels – up to 4 nanopores at each channel
- 2,048 is the theoretical maximum, but only 1 pore/channel can be sequencing at a given time



Behold, the nanopore...

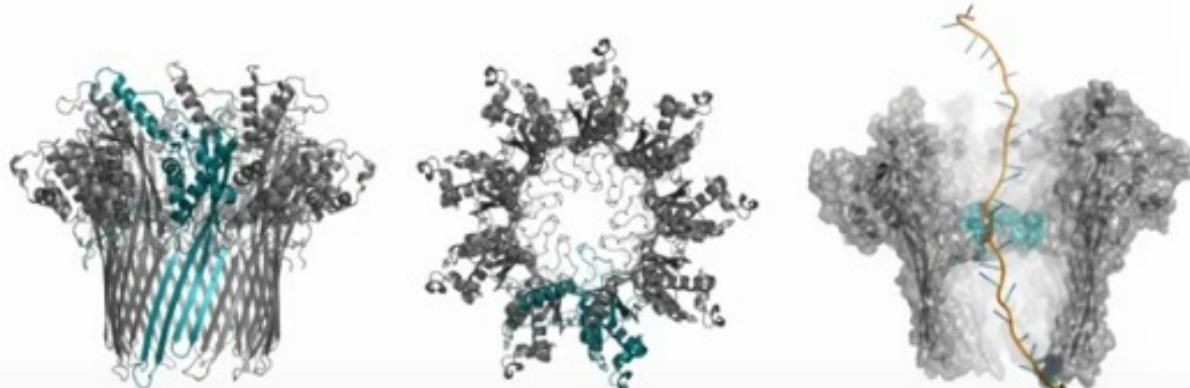


A view of the pore in 2016

'HOLEY GRAIL' PORE – R9

R9 is.....CsgG PORE FROM E.coli

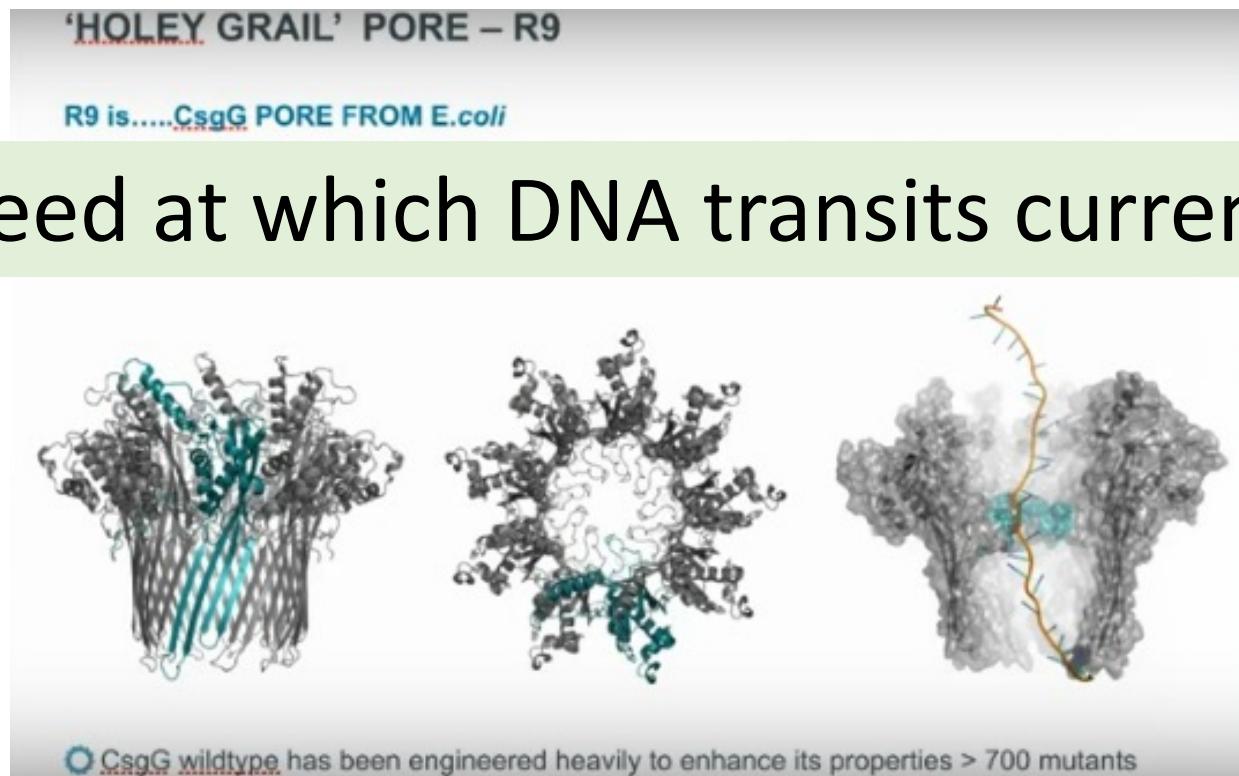
- Nonameric lipoprotein (nine subunits) with a 36 stranded Beta-barrel
- Shape, dimensions, and the position of the constriction of R9 make it a better pore for DNA sequencing



○ CsgG wildtype has been engineered heavily to enhance its properties > 700 mutants

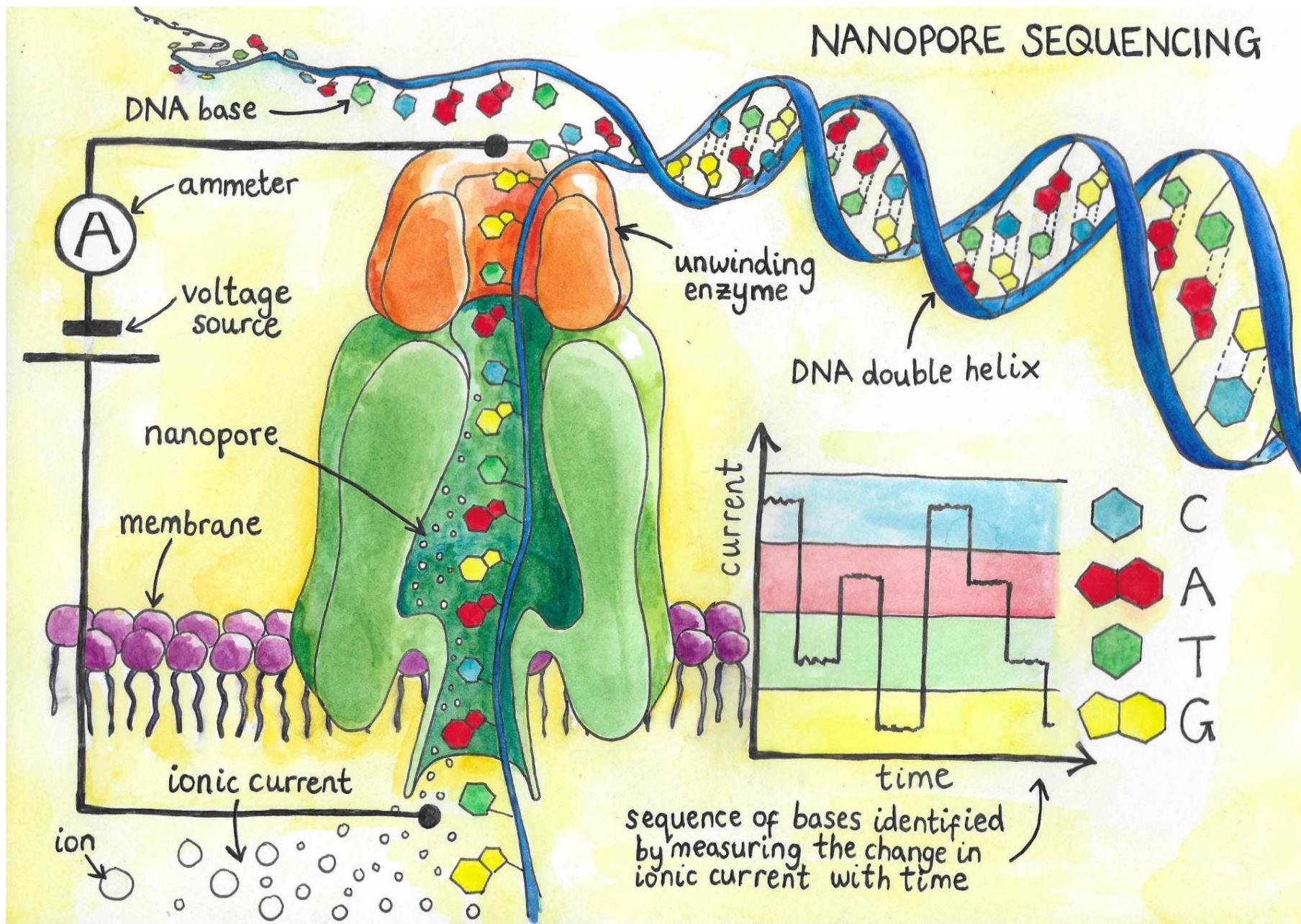
Furthermore, fast mode is now going to a reality: with the new kits (going to developers this month and the whole community next month, with R7 phased out shortly thereafter), speeds of 280 bases per second (or about 3-fold higher than the current 80bps). The system is believed to be capable of even faster; Oxford plans to roll out software updates to up the speed to closer to 500 bps (perhaps in steps) as they gain confidence in the new system.

A view of the pore in 2016

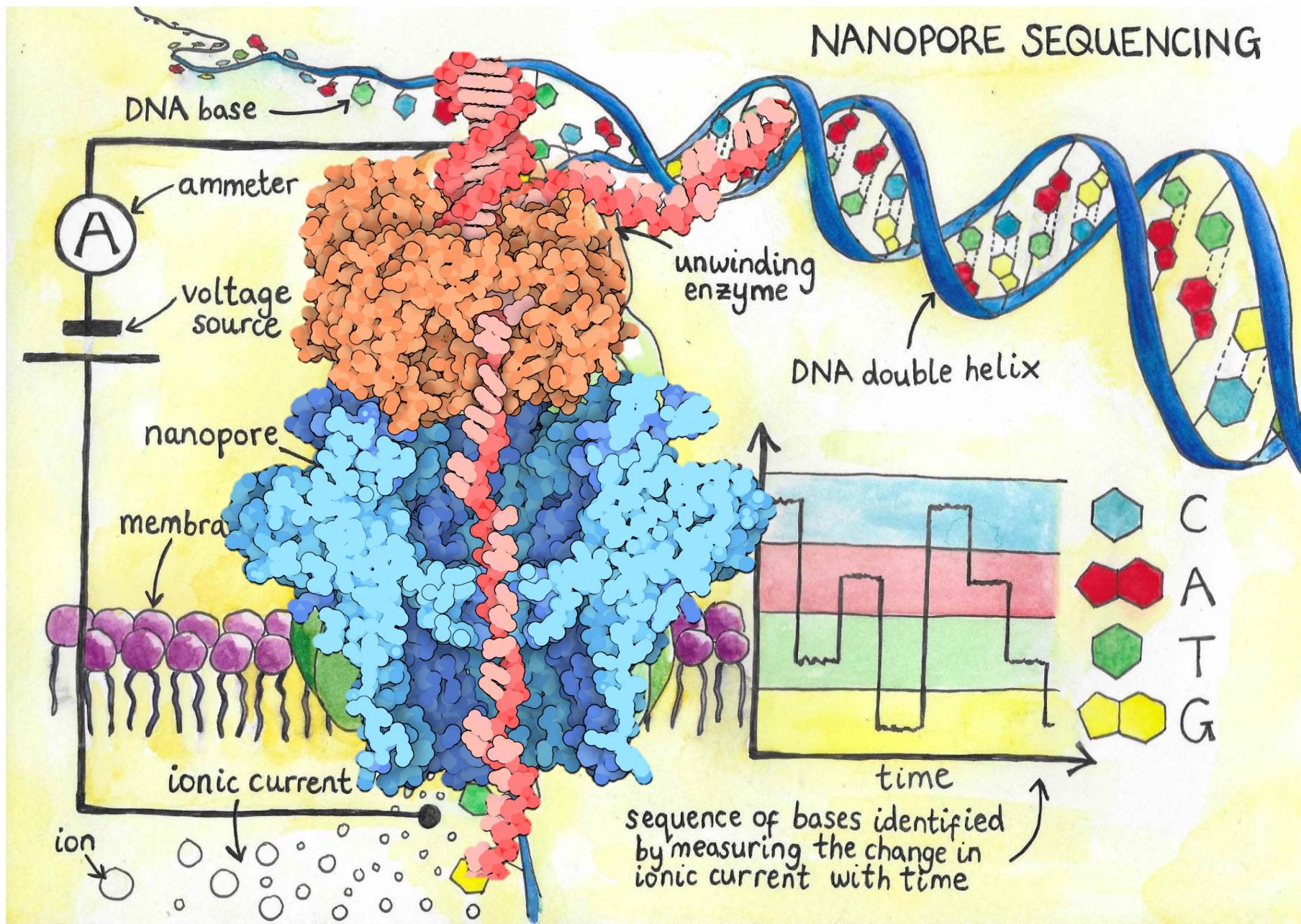


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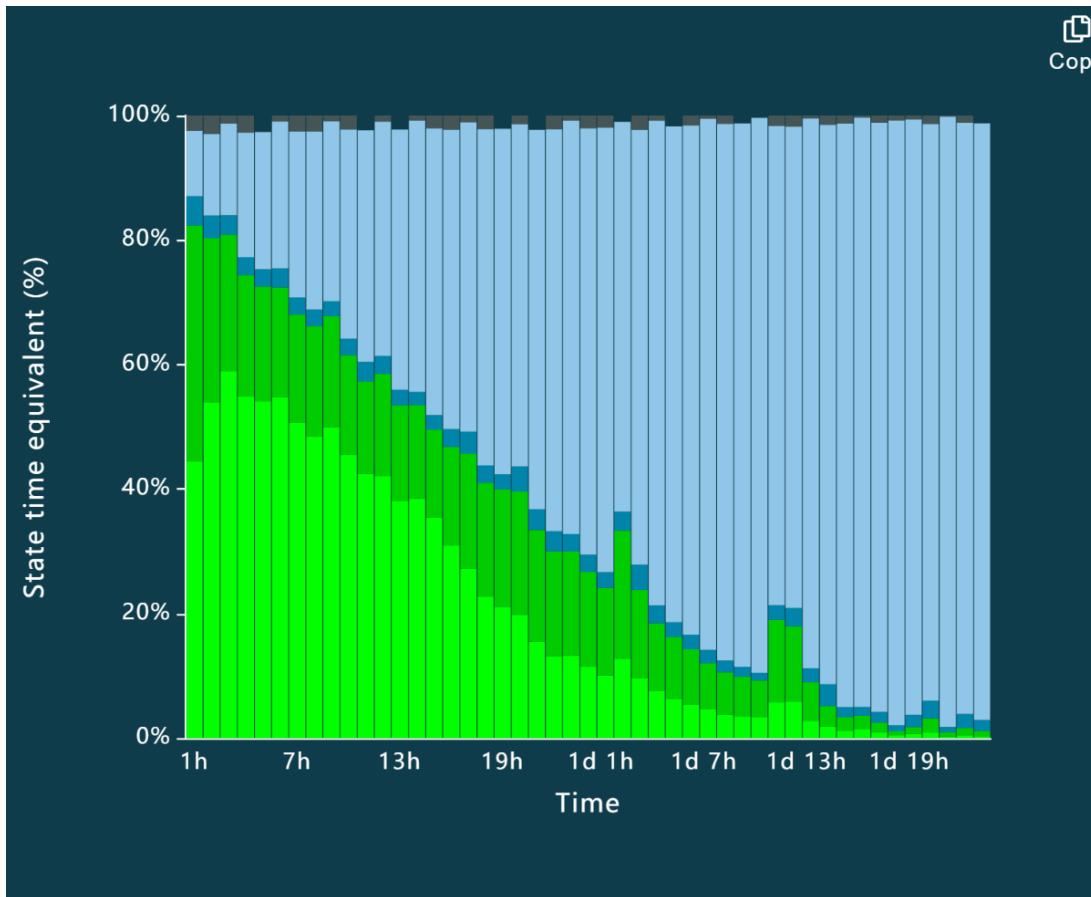
NANOPORE SEQUENCING



NANOPORE SEQUENCING



Monitoring flowcell progress

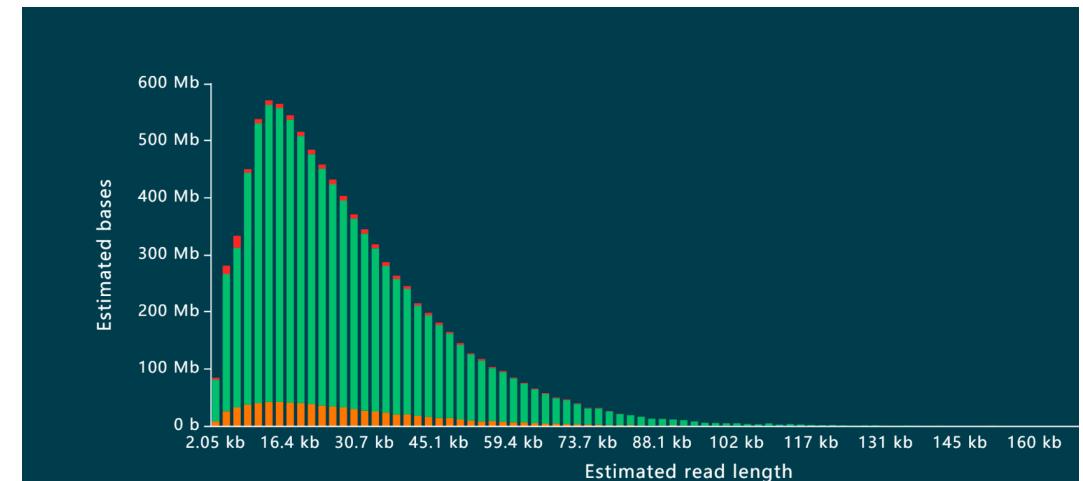


Active pore count reduces over time

What might cause this?

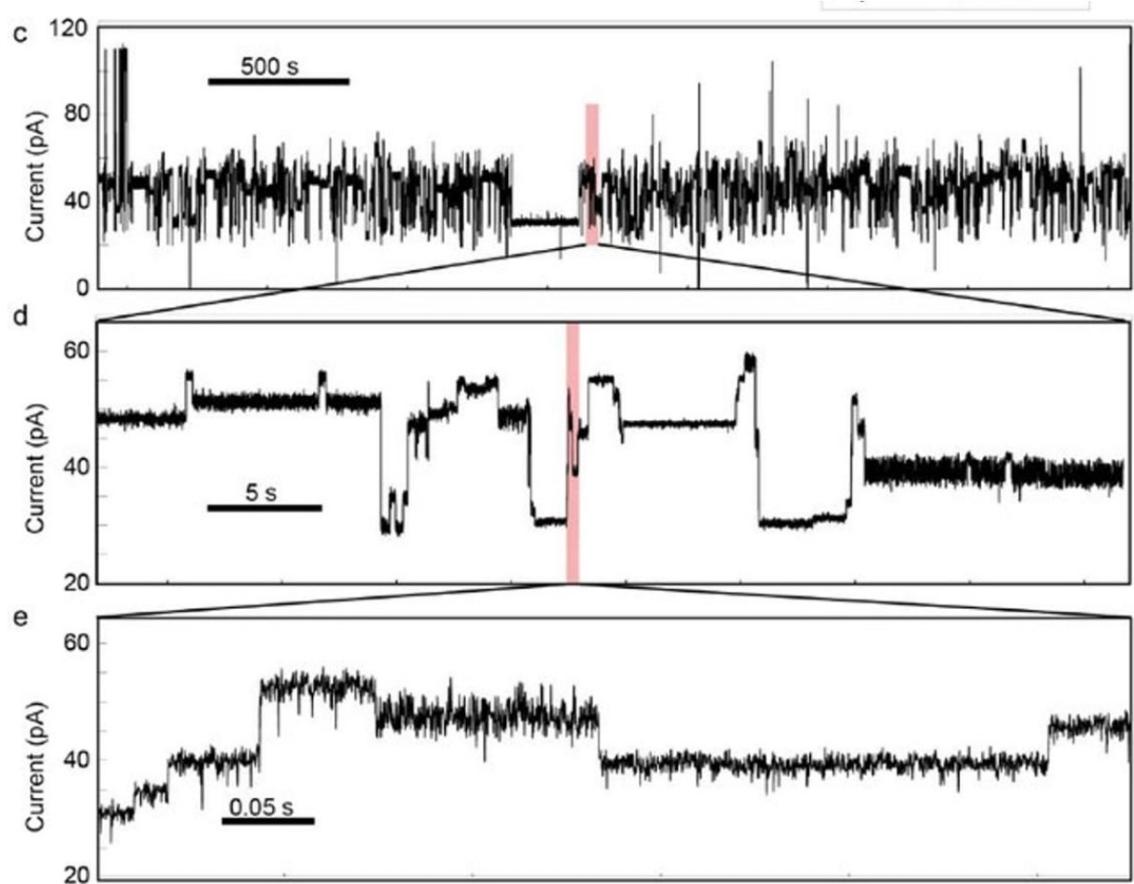
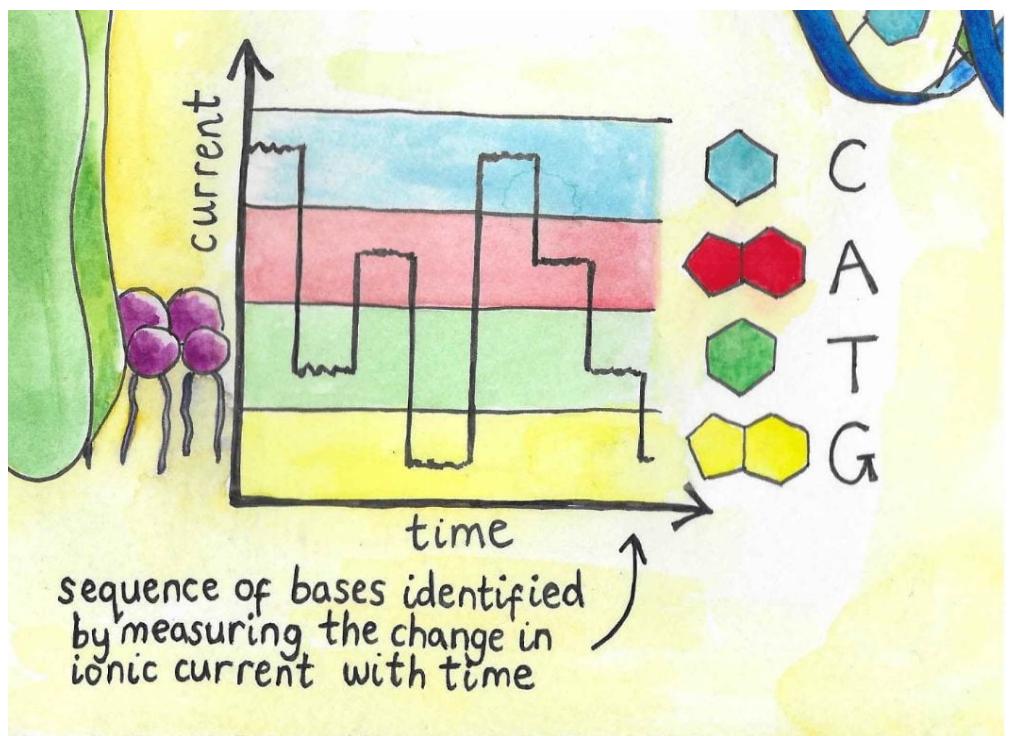
Why are there some increases in active pore count?
(at 1d 13h for example)

Read length distribution



Note: Estimated read length

What's with the "squiggle"?



Converting the squiggle to accurate base-calls is challenging!

This is an area where Nanopore has made huge improvements (more data = better algorithms)

The raw data is stored in .fast5 file format

Let's take a shot at base-calling some raw data!

https://github.umn.edu/agro5431-2023/Lab5_NanoporeSequencing

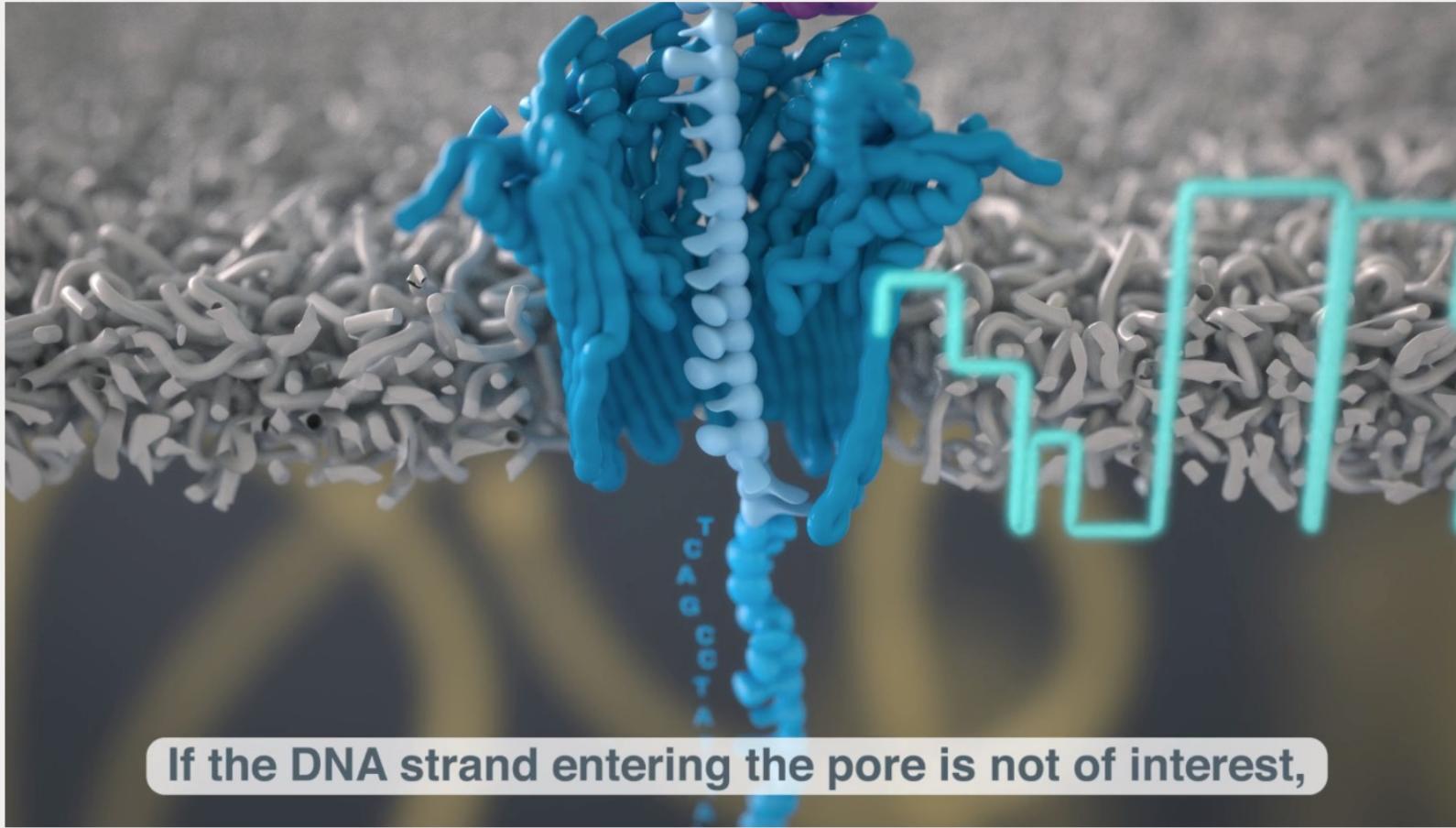
Nanopore is
always
innovating



Adaptive Sequencing – Enrich for sequences you're interested in

Adaptive sampling with Oxford Nanopore

[Get started](#) [Talk to us](#) [Subscribe](#)

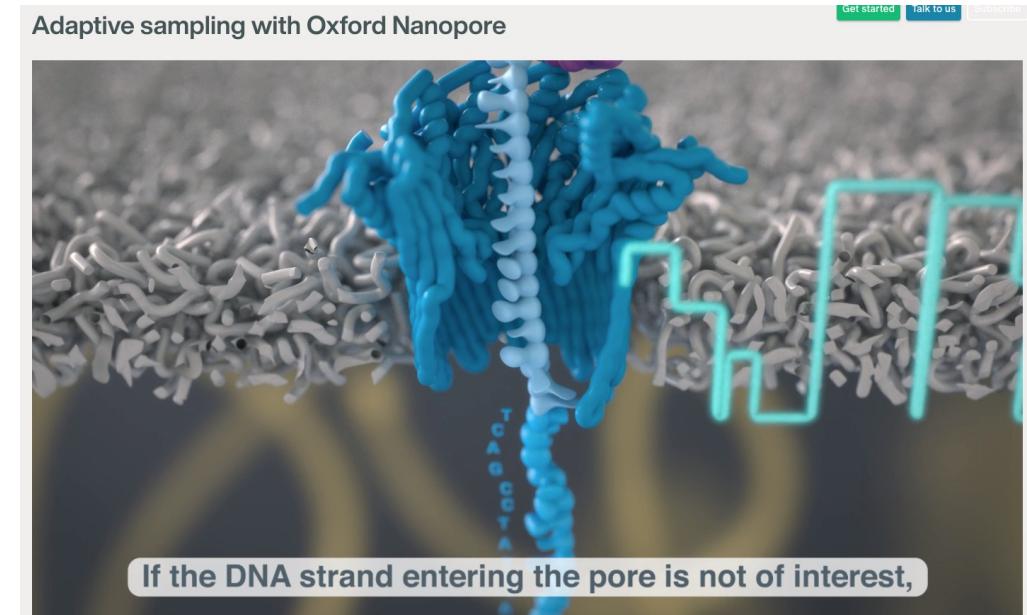


<https://nanoporetech.com/resource-centre/adaptive-sampling-oxford-nanopore>

Adaptive Sequencing – Enrich for sequences you're interested in

What is actually happening?

1. ssDNA passes through the pore
2. basecalling occurs at the same time – this requires a GPU
3. basecalled read is mapped to reference genome
4. if it maps to a region of interest, it continues, if not the current is reversed, kicking out the read



When would this be useful?

Improving accuracy with duplex reads

The image is a composite of two parts. On the left, a woman is speaking at a podium during the 'Nanopore Community Meeting'. The podium and background are blue with the Nanopore logo and text. On the right, there is a scientific diagram titled 'What is duplex sequencing?'. The diagram shows a DNA duplex with a grey template strand and a pink complement strand. A blue arrow points from the start to the middle of the duplex. At the top, a purple nanopore is shown binding to the template strand. In the middle, the nanopore has moved to bind to the complement strand. Another blue arrow points from the middle to the end of the duplex. Labels indicate 'template strand sequencing' for the first stage and 'complement strand sequencing' for the second stage.

What is duplex sequencing?

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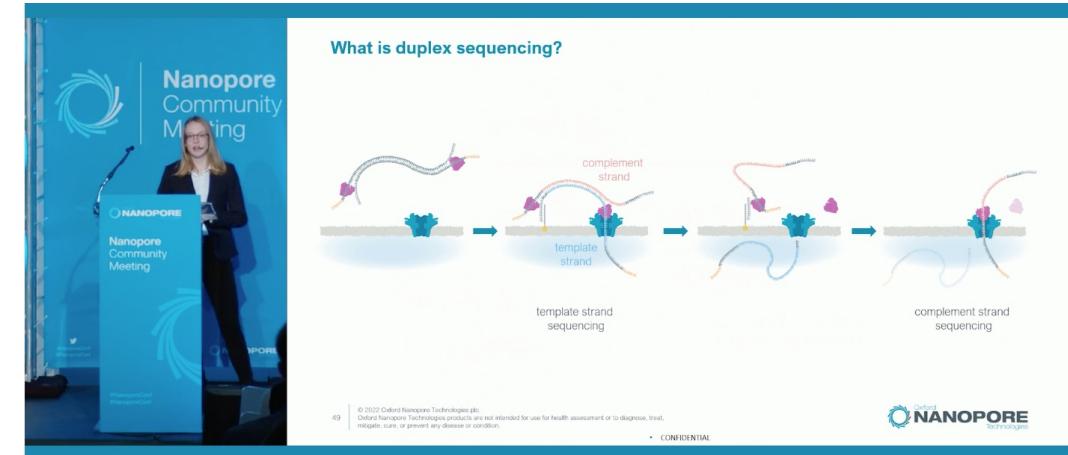
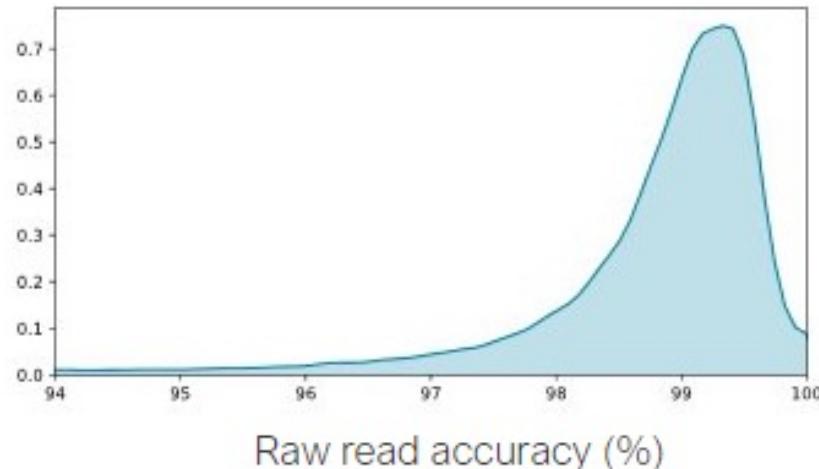
* CONFIDENTIAL

Oxford **NANOPORE** Technologies

<https://nanoporetech.com/resource-centre/video/ncm22/advances-in-duplex-basecalling>

Improving accuracy with duplex reads

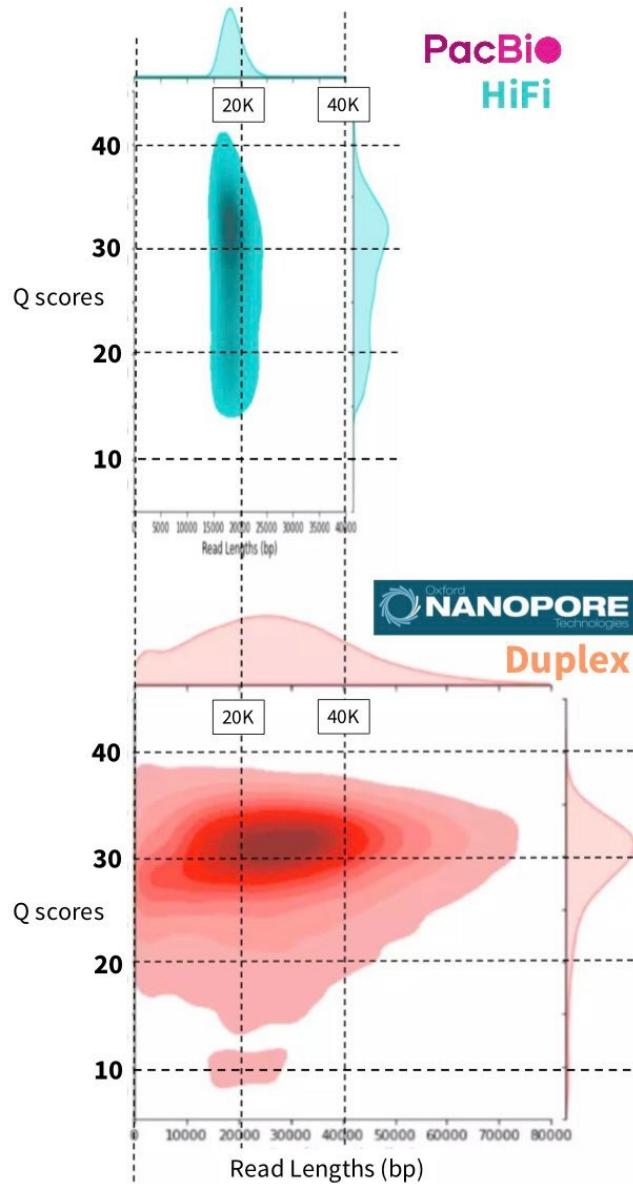
Raw read modal 99.3%, >Q20



Using both strands to basecall improves accuracy,
But not all reads will be duplex in a given run

<https://nanoporetech.com/resource-centre/video/ncm22/advances-in-duplex-basecalling>

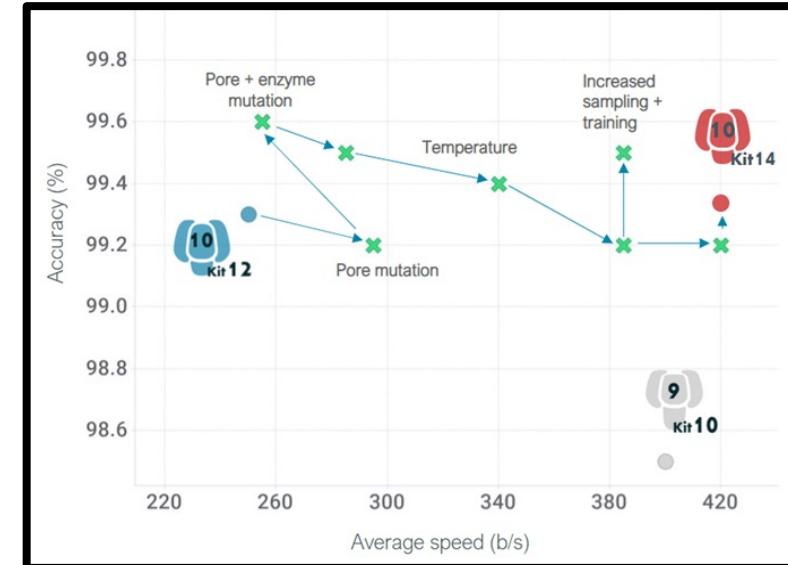
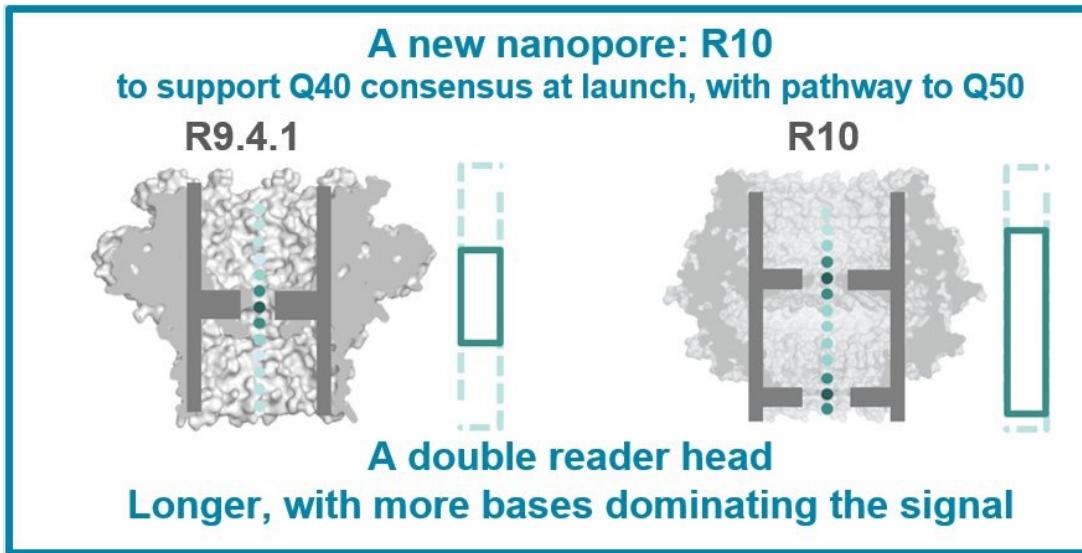
Improving accuracy with duplex reads: The sequencing wars...



NOTE: this is from Twitter, not a peer-reviewed publication

What is this plot communicating?

R10 has entered the chat



The R10.x flowcell is the new standard
Changes to the pore topography allow better base-calling
The user can choose high-accuracy or high throughput modes

How to keep up with the latest Nanopore news?

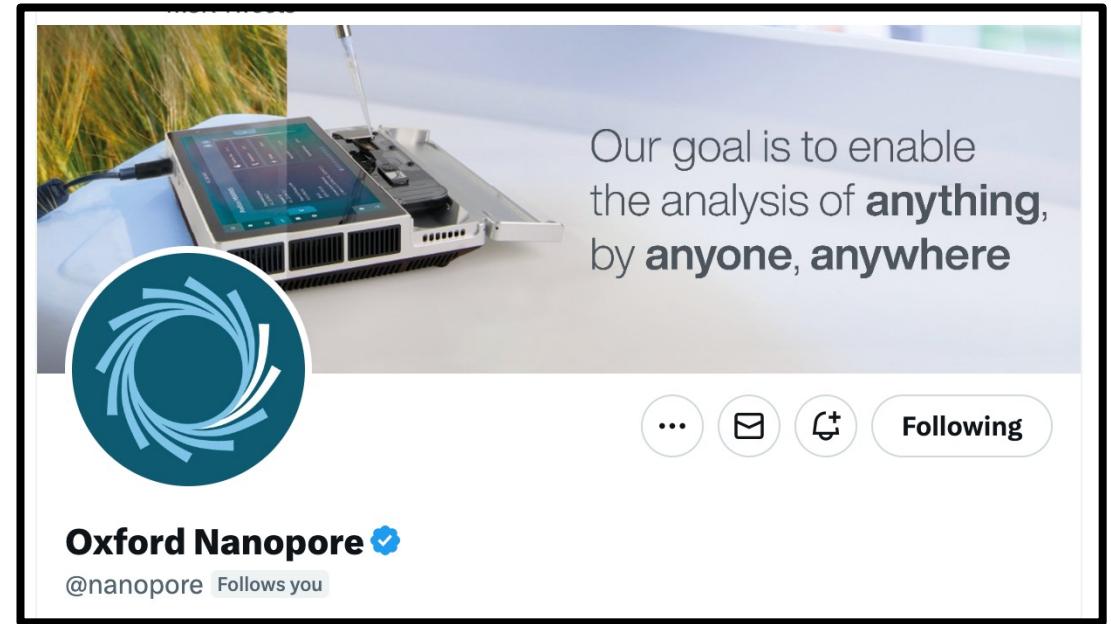
Clive Brown – CTO - @The_Taybor

Alexander Wittenberg – KeyGene - @AW_NGS

Taco Jesse – Rijk Zwaan - @TJesse62

Official account - @nanopore

Sissel Juul – Genomic Applications - @Sisseljuul





Thank you all so much!
Who has questions?

Homework on Canvas

read0094@umn.edu