CH-413 Nanobiotechnology

Electrical Sensing

Angela Steinauer

March 27, 2025

Learning objectives

1. **FET fundamentals:** Understand how field-effect transistors operate and adapt to nanomaterials (e.g., carbon nanotubes, graphene, nanowires) for sensing.

2. **Biosensor applications:** Apply FET concepts to design and interpret biosensors, focusing on factors such as sensitivity and specificity.

3. **Nanopore sequencing:** Explore how nanopore-based electrical signals enable molecular detection and sequencing.

4. **Real-world context:** Examine practical examples of FET-based sensing and nanopore applications, including a personal research case study.

Definition electrical sensing

*Electrical sensing involves the **detection of changes in the flow or distribution of electric charge**—whether carried by electrons or ions—resulting from biological or chemical interactions.*

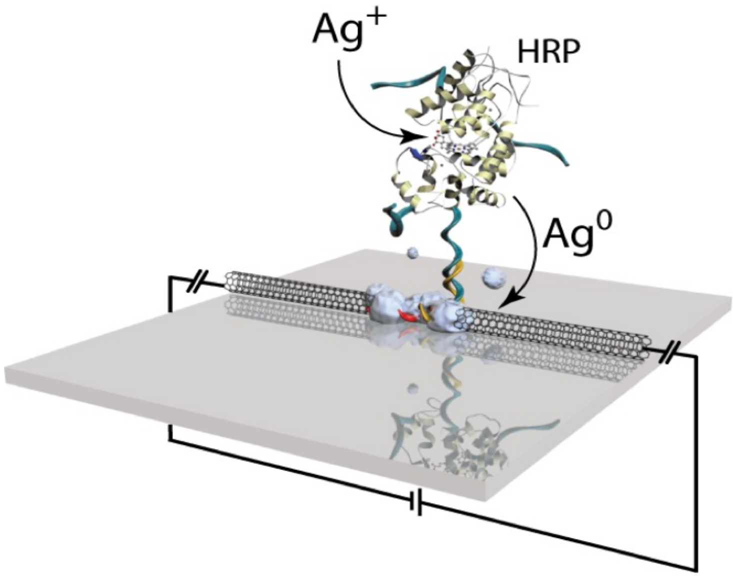


Image: https://swagergroup.mit.edu/research/molecular-and-nanowire-based-sensors

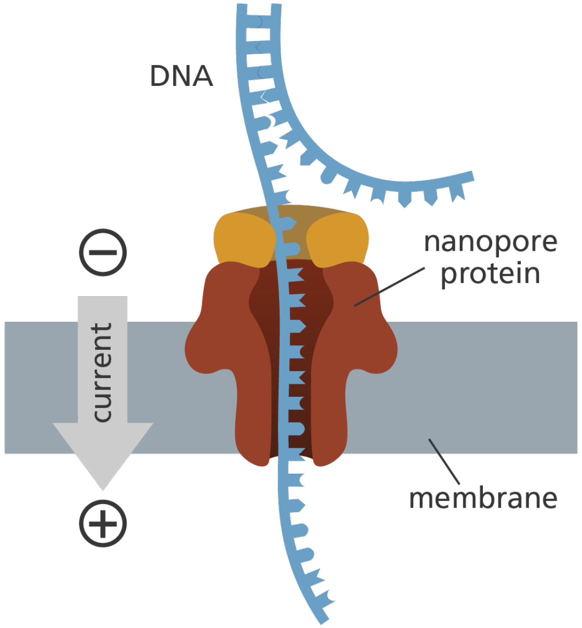


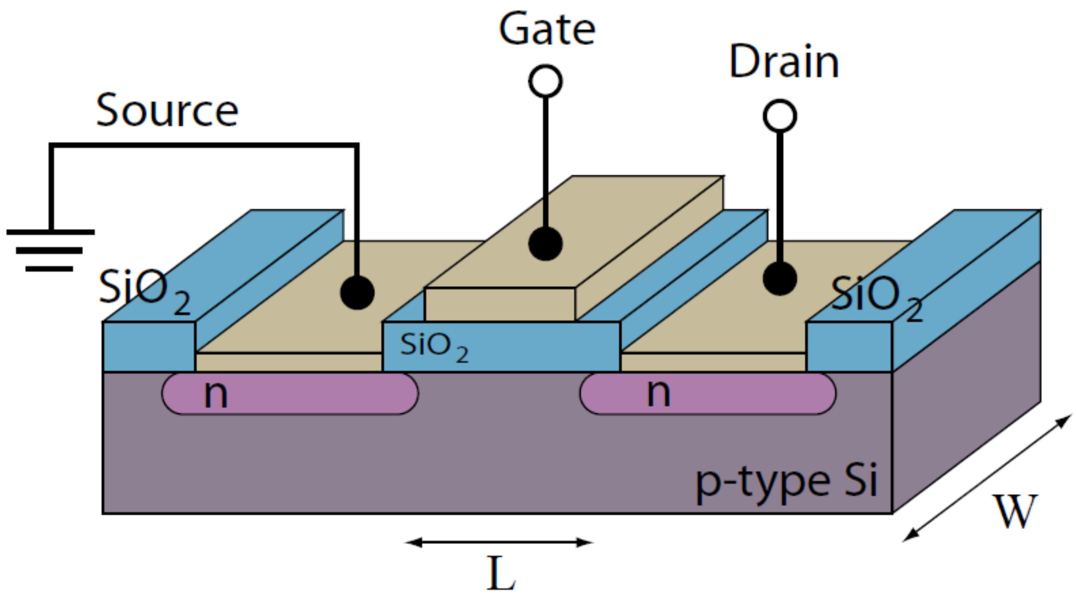
Image: https://www.yourgenome.org/theme/what-is-oxford-nanopore-technology-ont-sequencing/

FET everyday application: dimmer switch

Image: https://www.jumbo.ch/de/wohnen-licht/licht/lampen/trend-lampen/philips-hue-dimmer-switch-weiss/p/6764463

Elements of a field-effect transistor (FET)

OFF state:



MOSFET: metal-oxide-semiconductor field-effect transistor (most common)

Electric current enters through <u>source electrode</u> and exits through <u>drain electrode</u>.

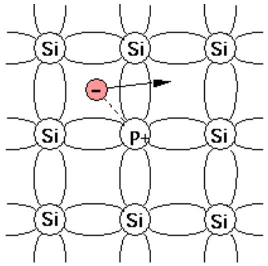
<u>Gate electrode</u>: controls channel between source and drain electrode.

No voltage applied to gate electrode
→ No current detected

Semiconductor layer at bottom:
npn (here) or pnp
→ Controls current flow

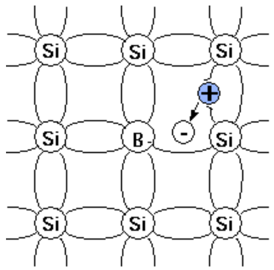
Semiconductors in FETs

- Electrical **conductivity between conductors and insulators**.
- Conducting properties can be **fine-tuned using "doping"**, a process that introduces impurities.
- They are used in FETs to create the **channel between source and drain terminals**.
- The conductivity of the semiconductor channel can be **controlled by applying a voltage to the gate terminal**.
- Semiconductors are essential for the operation of FETs, enabling the modulation of current flow and the amplification of electrical signals.



n-type semiconductor: extra **electrons**
e.g. pentavalent impurities, e^-

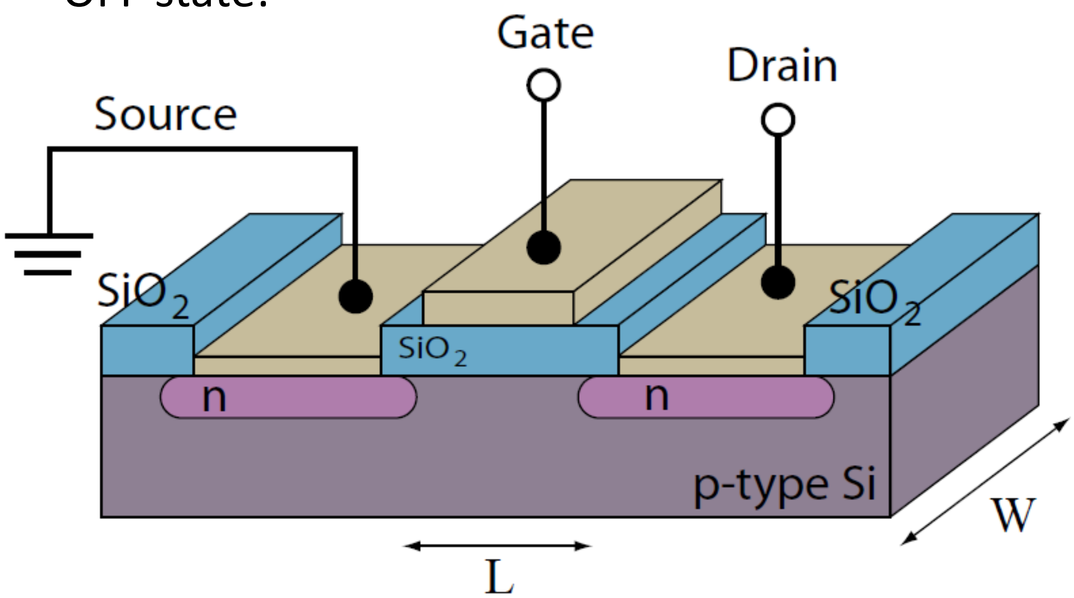
p-type semiconductor: extra **holes** (absence of electrons)
e.g. trivalent impurities, h^+



https://en.wikipedia.org/wiki/Donor_(semiconductors)
https://en.wikipedia.org/wiki/Acceptor_(semiconductors)

Field-effect transistors

OFF state:

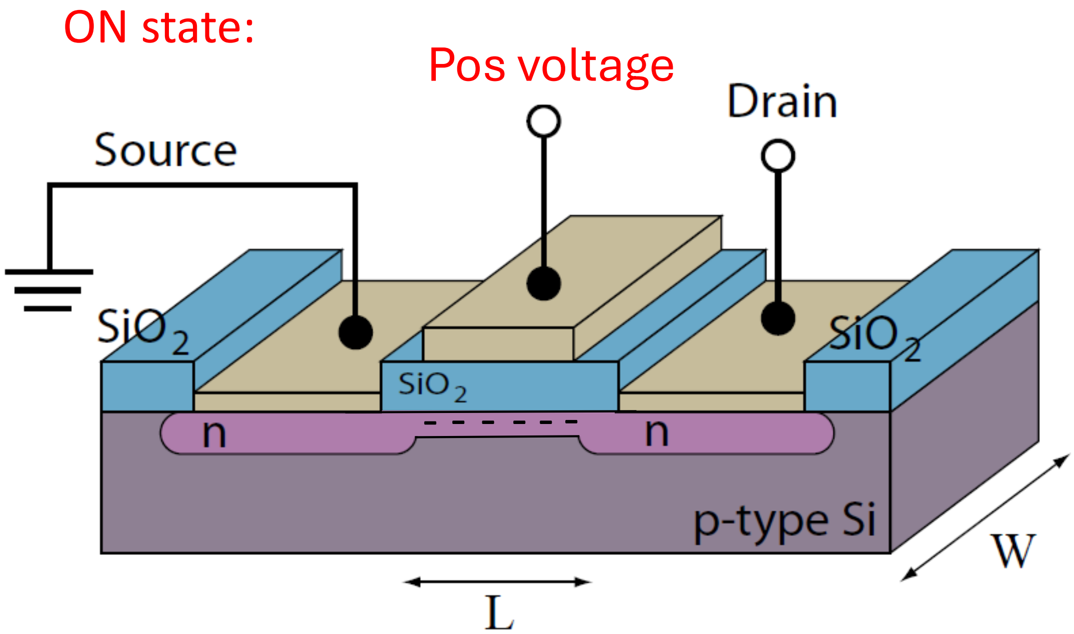


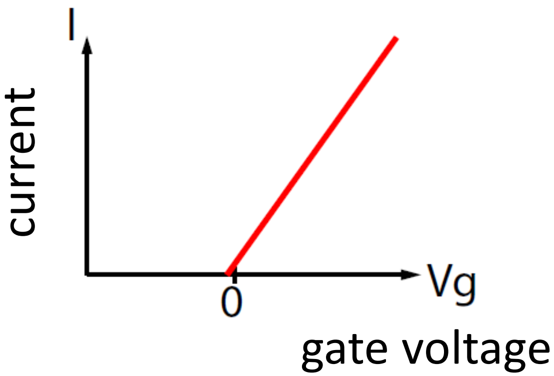
A FET is an electronic switch

- **In <u>n-type FETs</u> (shown here):**
- Current flows between source and drain as electrons.

- Without gate voltage: the p-doted body (containing holes) does not allow e^- flow (depletion) → no current detected

Field-effect transistors





A FET is an electronic switch

- **In n-type FET (shown here):**
- Current flows between source and drain as electrons.
- Without gate voltage: the p-doted body (containing holes) does not allow e^- flow (depletion) → no current detected
- **Positive voltage** at the <u>gate electrode</u> draws electrons to the surface in the body
- → Creates an electric field (E) and a conducting channel: **current flows through transistor**

FET everyday application: dimmer switch

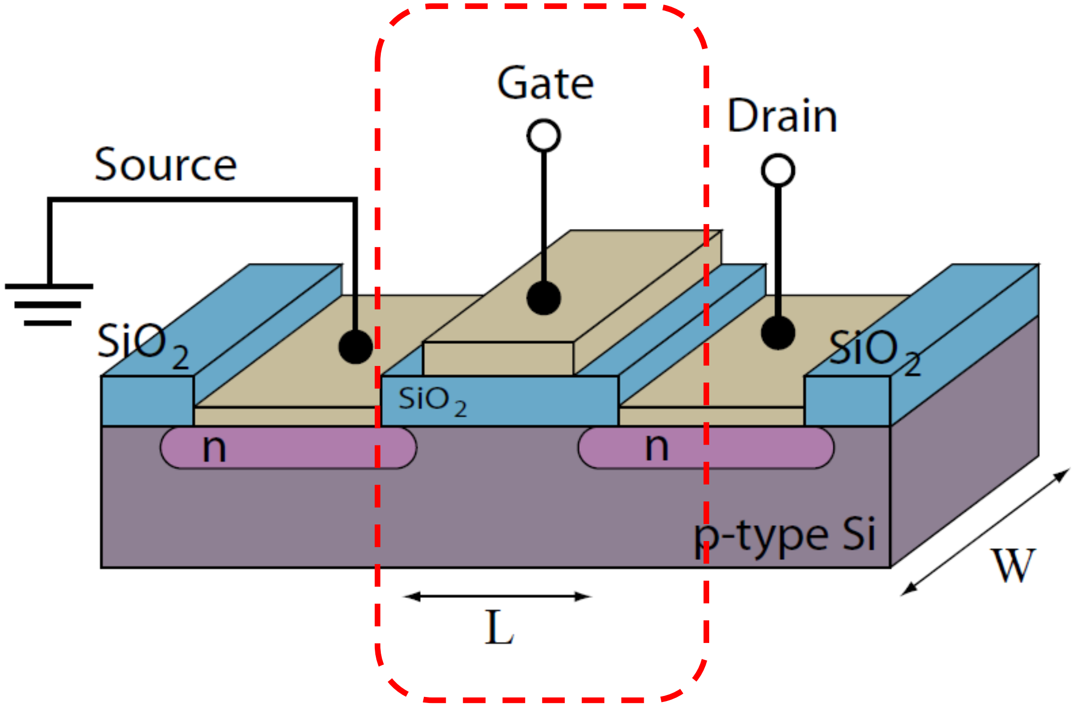
- Dimmer switch at home
- Dimmer controls the brightness of light bulb
- Inside the dimmer switch there is likely an FET
  - Dimmer up: more voltage is applied to the gate of the FET → more electricity flows to the light bulb → light becomes brighter
  - Dimmer down: less voltage applied to the gate of the FET → less electricity → light dims
- The FET controls the amount of electricity flowing to the light bulb

https://www.jumbo.ch/de/wohnen-licht/licht/lampen/trend-lampen/philips-hue-dimmer-switch-weiss/p/6764463

If you want to hear more about FETs

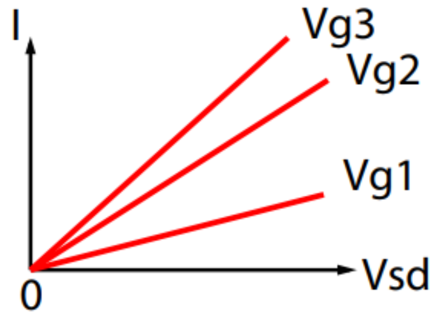
- https://nanohub.org/courses/NT/s2016/outline/unit1transistorfundamentals/l12themosfetasablackbox

Using FETs as sensors



How is gate voltage changed?
Binding of biomolecule to gate area:
Biomolecules are charged particles
→ They can alter the electric field at the gate

The FET acts as sensor by detecting **changes in gate voltage** →



Molecular binding event → **Measurable changes in FET current**

P-channel FETs

https://nanohub.org/courses/NT/s2016/outline/unit1transistorfundamentals/l12themosfetasablackbox

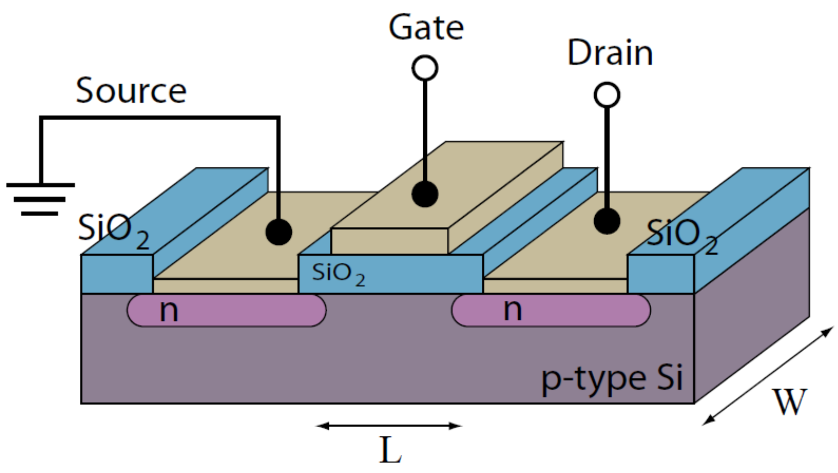
- Watch from 10:53

Carbon nanotube (CNT)FETs are simpler

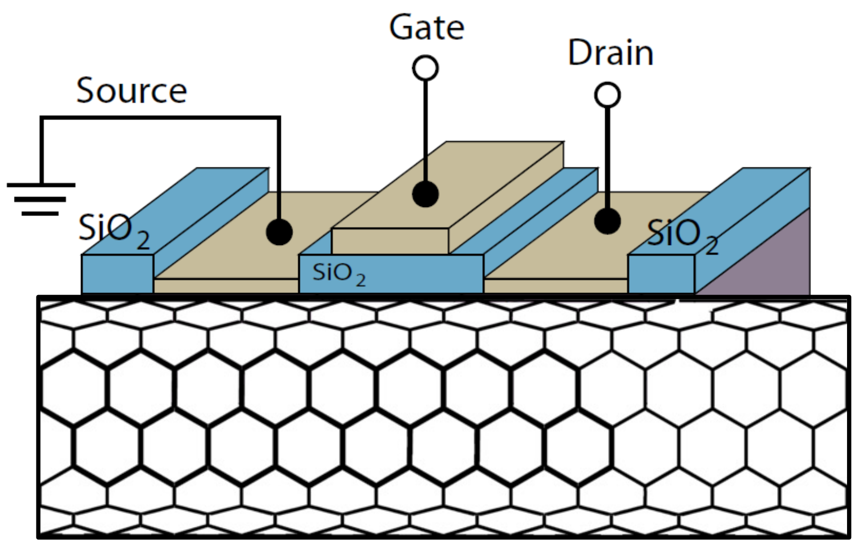
Instead of a npn or pnp semiconductor layer,

they consist of <u>just one p- or a n-layer</u> (often p)

(similar to a Junction FET or JFET)



MOSFET: n-type
(npn layers)



CNTFET: p-type
(p layer)

Nanowires (NWs)

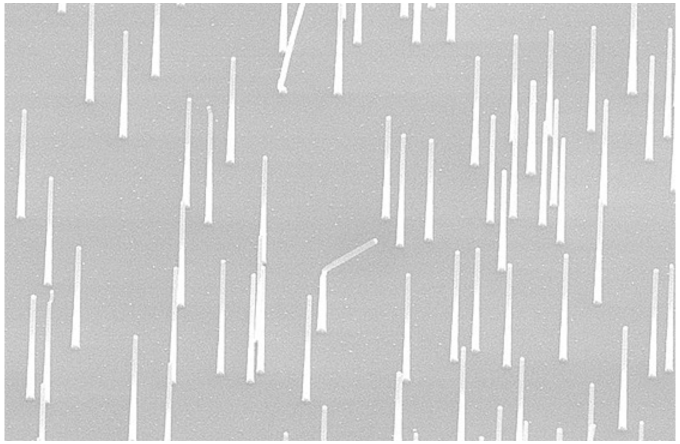
*A **nanowire** is a one-dimensional structure, typically with a diameter on the scale of a few nanometers up to tens of nanometers, and a length significantly larger than its width.*

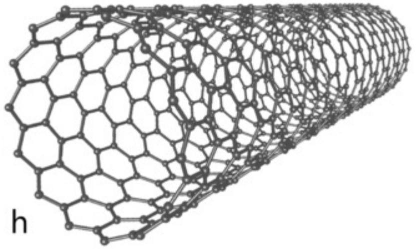
Because of this high aspect ratio, nanowires often display **unique electronic, optical, and mechanical properties** that can be leveraged in nanoscale devices and sensors.

Materials:
- Silicon
- III-V semiconductors (e.g. GaAs, InAs, InP)
- Metal oxides (e.g. ZnO, SnO_2)
- Carbon-based materials (carbon nanotubes, graphene nanoribbons)

Chemical modification of the **nanotubes** or **nanowires** will change local electric field around gate electrode. Will change threshold gate voltage and thus current flowing through device → sensor

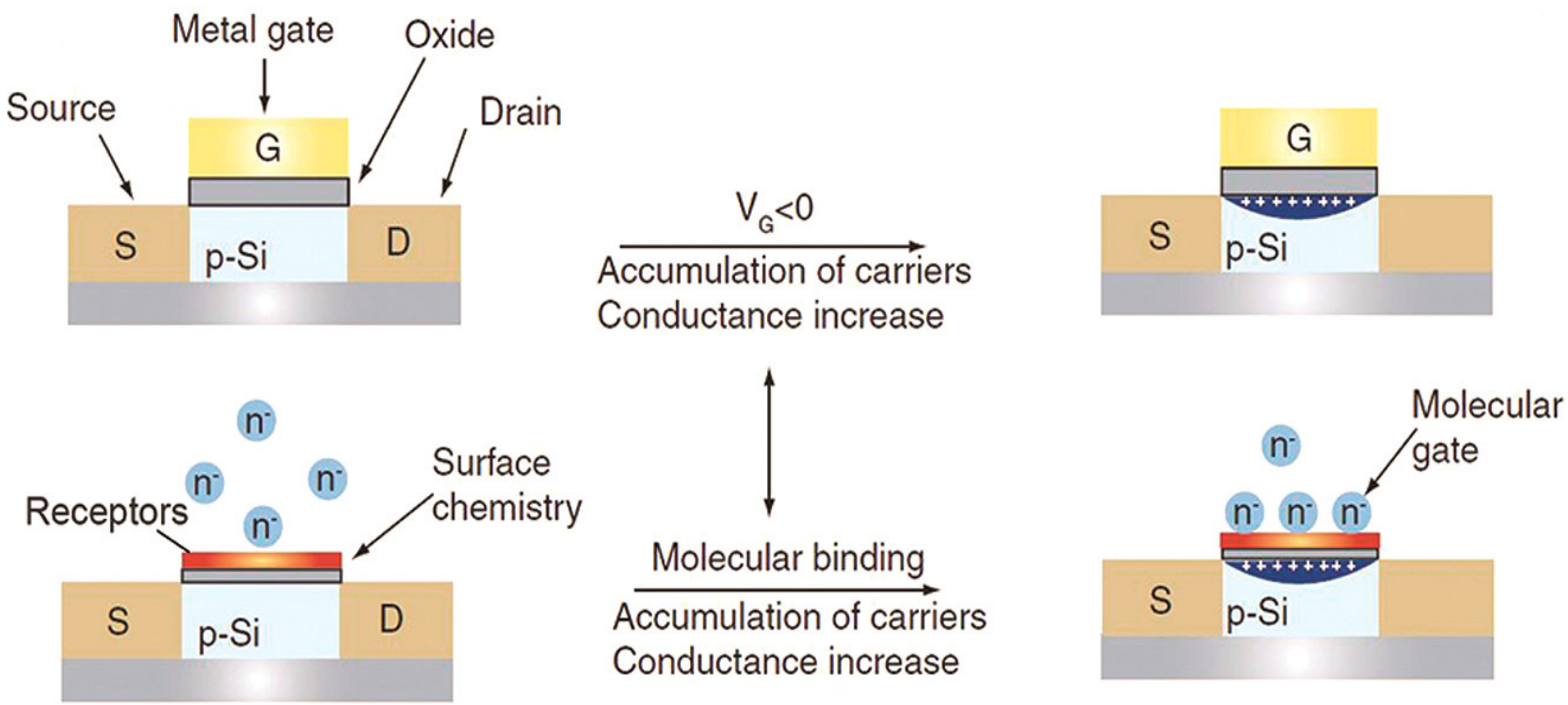
SEM image of nanowires. Source: https://news.mit.edu/2013/explained-nanowires-and-nanotubes-0411





Cui et al. Nano Letters 2003

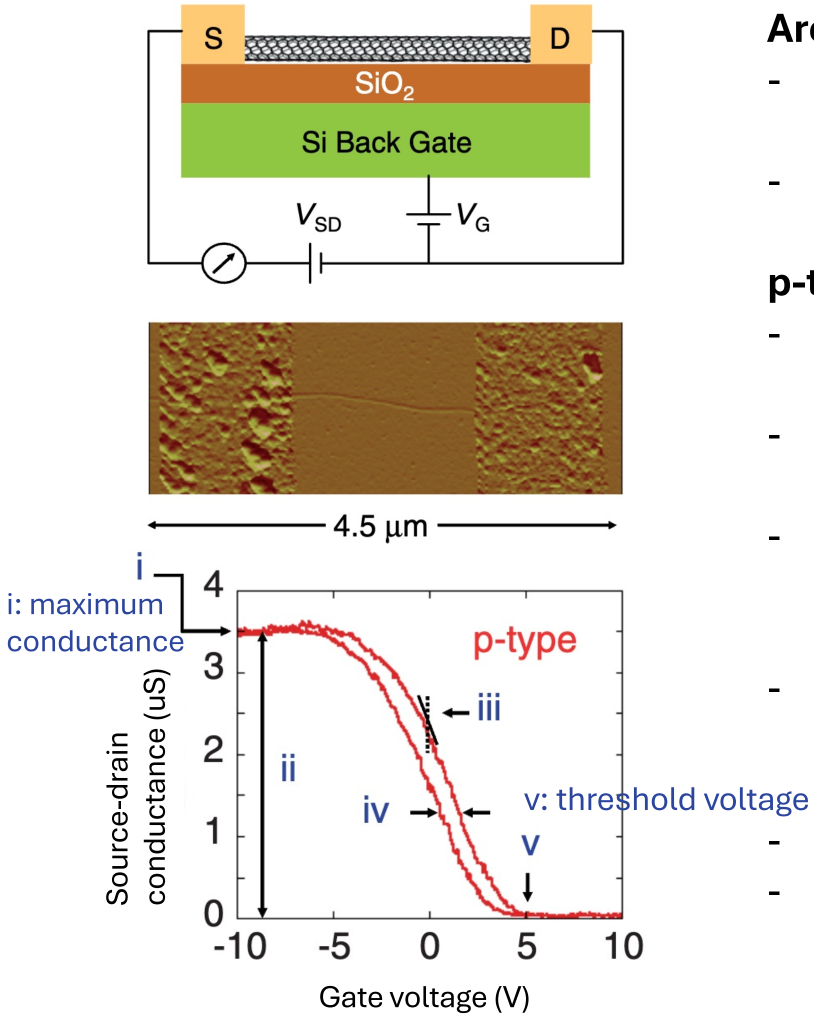
Standard FET vs. FET sensor



Schematic comparison of (top) a standard FET device and (bottom) a silicon nanowire (SiNW) FET sensor. The NW surface is functionalized with a receptor layer to recognize target biomolecules in a solution, which are charged and provide a molecular gating effect on SiNWs.

DOI: (10.1021/acs.chemrev.5b00608)

Gating a nanowire sensor



Architecture:

- Semiconducting p-type, single-walled nanotube (SWNT, black) between source (S) and drain (D) electrodes
- Gate electrode (green)

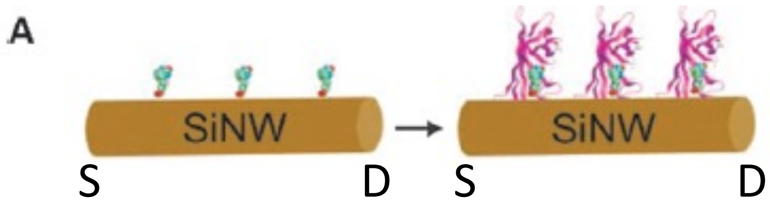
p-type FET:

- **Hole flow**: positive charge carriers (=holes) flow from source to drain
- A sufficiently **negative** gate voltage, below a negative threshold, **creates a channel of holes**
- **Attraction of charge carriers**: negative voltage on drain attracts the positively charged holes from the source, flowing across the channel and out the drain
- **Current direction**: flow of positive carriers (holes) out the drain means the e^- current flows in the opposite direction

- Positive gate voltage (< 5 V): Barrier persists, **OFF-state**
- Lower gate voltage (> 5 V): Decreasing width of barrier and **increasing current**, **ON-state**

Good review about «Carbon Nanotube FET-Based Biosensors»:
Adv. Mater. 2007, 19, 1439–1451. DOI: 10.1002/adma.200602043

Sensing using Si nanowires



S: Source
D: Drain

Concept:

Charges on bound biomolecule generate electric field

P-type

- Similar to applying **gate voltage**
- Results in change in resistance across nanowire / **change in conductance**

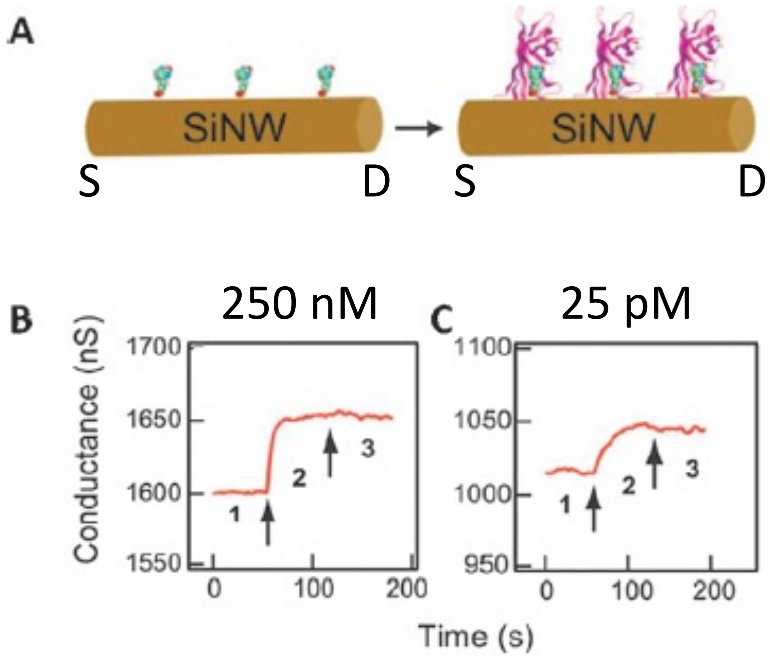
Conductance, G: measured in Siemens (S)

$S = \Omega^{-1}$, $[kg^{-1} \cdot m^{-2} \cdot s^{3} \cdot A^{2}]$

G = 1/R, R: resistance

Cui et al., Science 2001
Swierczewska et al., Chem Soc Rev 2011

Sensing using Si nanowires



Lieber et al.: Biotin decorated NW, showing change in conductance upon avidin binding

Cui et al., Science 2001
Zheng et al., Nat. Biotechnol. 2005
Swierczewska et al., Chem Soc Rev 2011

- Change in conductance: sensing in solution
- **Detection limit**: pM, fM in advanced systems
- **Multiplexing**: by combining different sensors/wires-ab conjugates multiple biomarkers can be read out in serum
- **Limitation:** Ion strength of buffer

Advantages and limitations

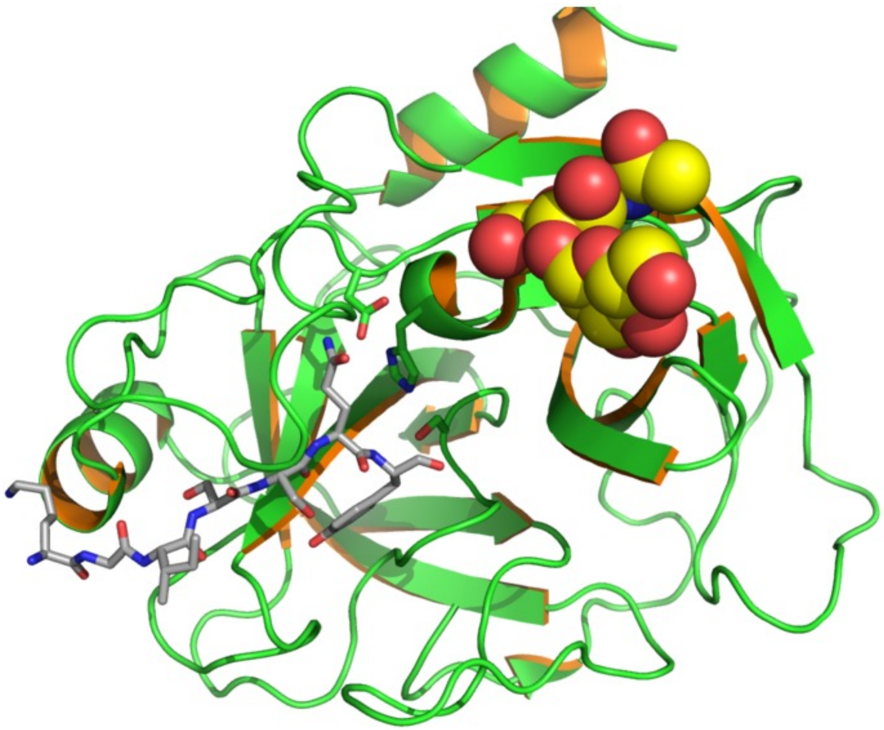
FET sensors:

- High sensitivity
- Surface modifications: high selectivity
- Multiplexing, cost effective
- Fast readout

Disadvantage

- Performance lower in buffer / physiological salt concentration (requires < 1 mM salt)

PSA and prostate cancer



- Kallikrein-3 (KLK3)
- Also known as **prostate-specific antigen** (**PSA**)
- Glycoprotein enzyme

- Secreted by the epithelial cells of the prostate gland
- Elevated levels in patients with **prostate cancer**

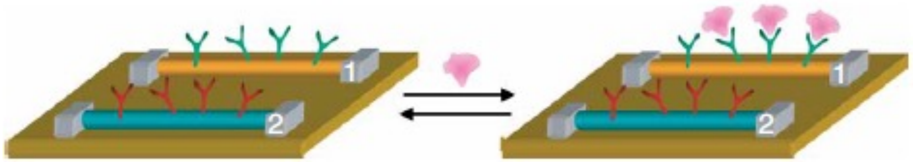
- → **Prostate-specific antigen** (**PSA**)

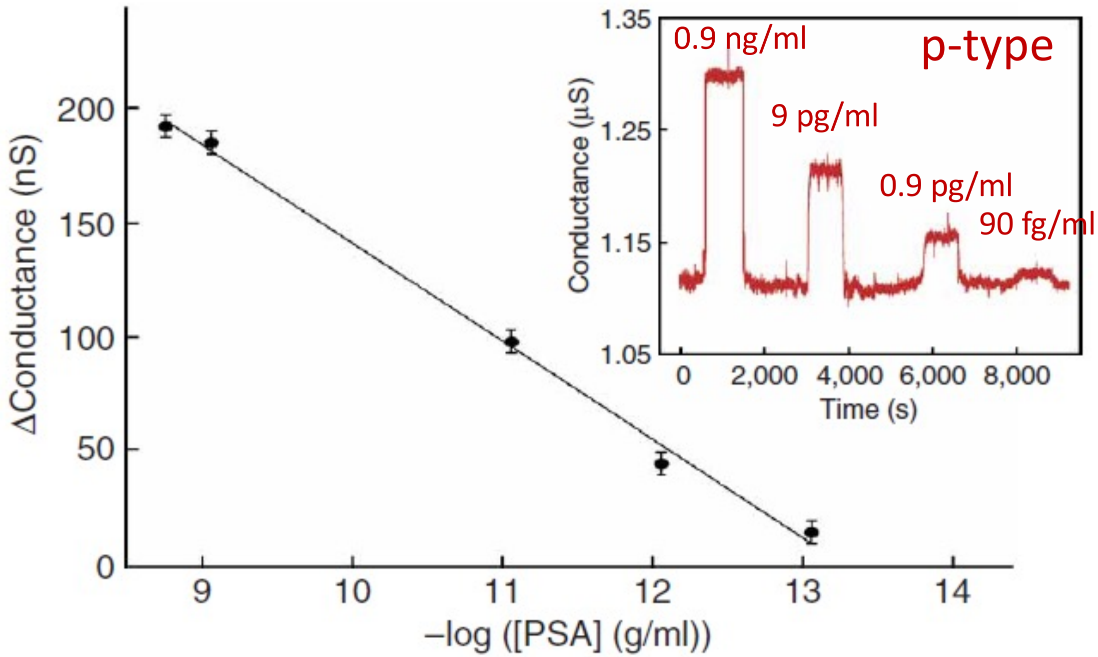
- Normal range: << 4 ng/ml in healthy patients

PSA: pI = 6.8 -> negatively charged under neutral conditions (pH 7.4)

Zheng et al., Nat. Biotechnol. 2005

Nanowire measurement





Protein: PSA

Si nanowires reacted with antibodies

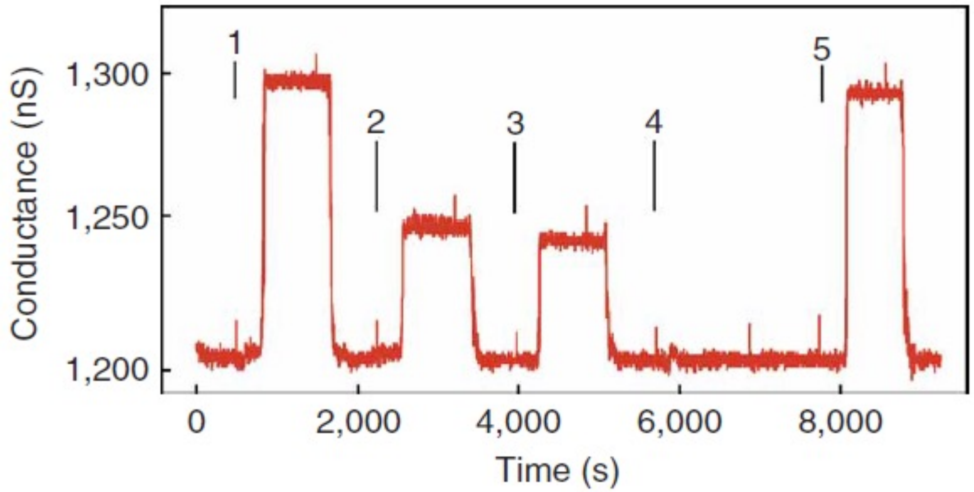
Protein binding alters electric field, which modulates device conductance

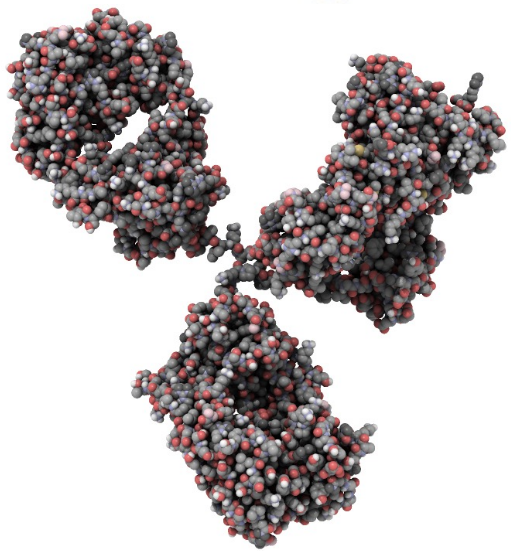
Concentration-dependent voltage readout

PSA: pI = 6.8 -> negatively charged under neutral conditions (pH 7.4)

Zheng et al., Nat. Biotechnol. 2005

Antibody-mediated specificity





Specificity depends on antibody

1. 9 pg/mL PSA
2. 0.9 pg/mL PSA
3. 0.9 pg/mL PSA
4. BSA (no signal)
5. 9 pg/mL PSA

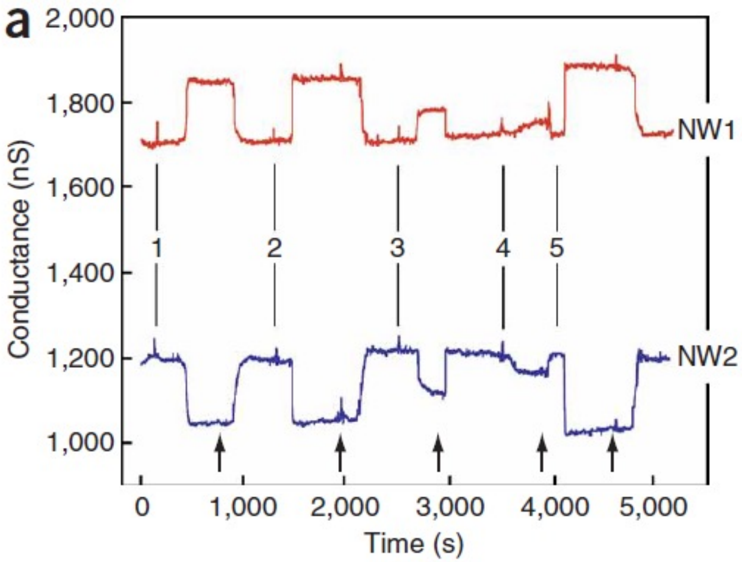
The measurements can be easily repeated

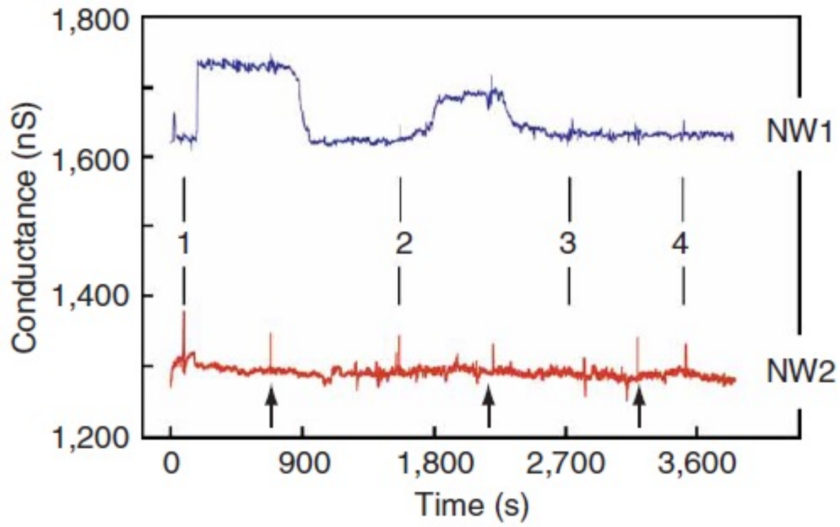
The readout is very selective

→ The antibody must have a fast dissociation rate (k_{off})

Zheng et al., Nat. Biotechnol. 2005

Multiplexed detection





p-type silicon nanowire sensors (NW1, electron holes)
n-type silicon nanowire sensors (NW2, extra electrons)

PSA: pI = 6.8 -> negatively charged under neutral conditions (pH 7.4)

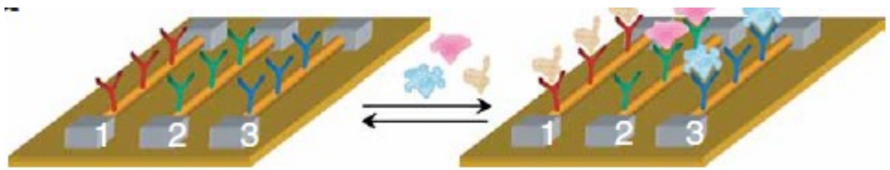
NW1: modified with AB
NW2: ethanolamine blocked

→ internal standard

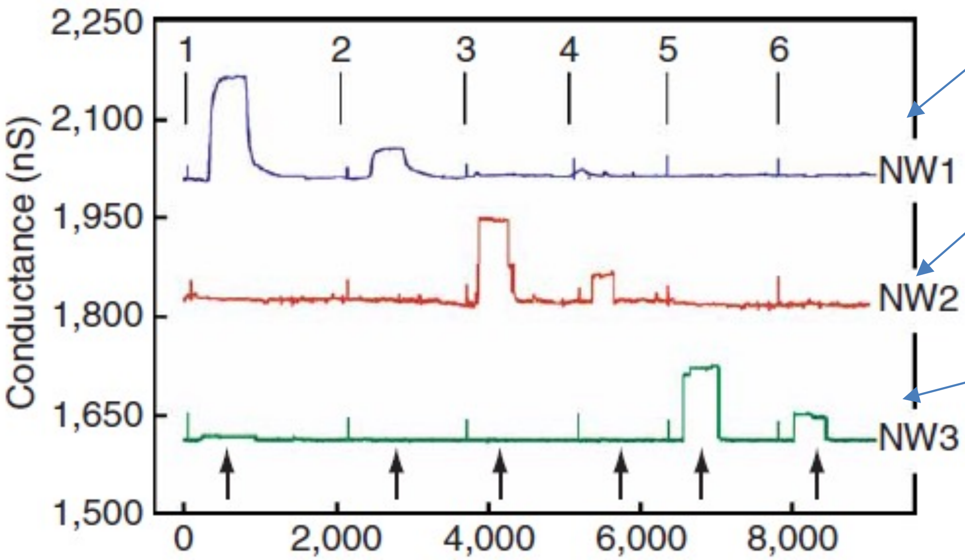
Highest sensitivity: 0.085 pg/mL for PSA

Zheng et al., Nat. Biotechnol. 2005

Multiplexed cancer marker detection



Using differently modified NWs, several cancer markers can be simultaneously detected



(1) 0.9 ng/ml PSA, (2) 1.4 pg/ml PSA, (3) 0.2 ng/ml CEA, (4) 2 pg/ml CEA, (5) 0.5 ng/ml mucin-1, (6) 5 pg/ml mucin-1

PSA (high pg/mL sensitivity)

CEA (Carcinoembryonic Antigen)
colon/rectal cancer (pg/mL sensitivity)

Mucin-1
Overexpression of MUC1 is often associated with colon, breast, ovarian, lung and pancreatic cancers
pg/sensitivity

Also works in serum!

Zheng et al., Nat. Biotechnol. 2005

Conclusions

- Label-free multiplexed detection of cancer markers
- Use of silicon-NW FET devices, integrated into a microfluidic platform

- Detection of cancer markers:
  - PSA
  - CEA
  - mucin-1

  to at least 0.9 pg/mL

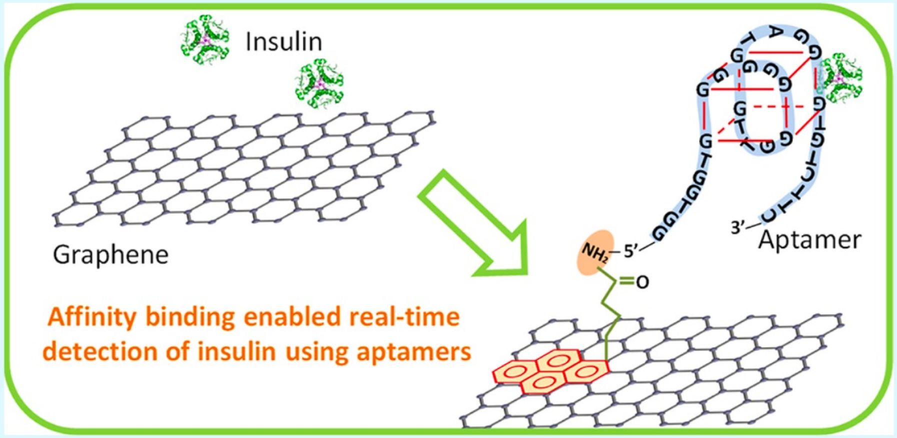
- Functional in undiluted serum samples

Activity

1. Read the abstract on the next slide.
2. Describe the principle of the assay using the schematic on the following slide.
3. Make the connection: How can this assay be incorporated with a field-effect transistor (FET) to measure insulin concentration?
    1. Draw a schematic of the FET that includes the graphene layer used in the assay.
    2. Explain how the sensor works. How are concentrations of insulin measured?

1. Read the abstract.

ABSTRACT: This paper presents an approach to the real-time, label-free, specific, and sensitive monitoring of insulin using a graphene aptameric nanosensor. The nanosensor is configured as a field-effect transistor, whose graphene-based conducting channel is functionalized with a guanine-rich IGA3 aptamer. The negatively charged aptamer folds into a compact and stable antiparallel or parallel G-quadruplex conformation upon binding with insulin, resulting in a change in the carrier density, and hence the electrical conductance, of the graphene. The change in the electrical conductance is then measured to enable the real-time monitoring of insulin levels. Testing has shown that the nanosensor offers an estimated limit of detection down to 35 pM and is functional in Krebs−Ringer bicarbonate buffer, a standard pancreatic islet perfusion medium. These results demonstrate the potential utility of this approach in label-free monitoring of insulin and in timely prediction of accurate insulin dosage in clinical diagnostics.



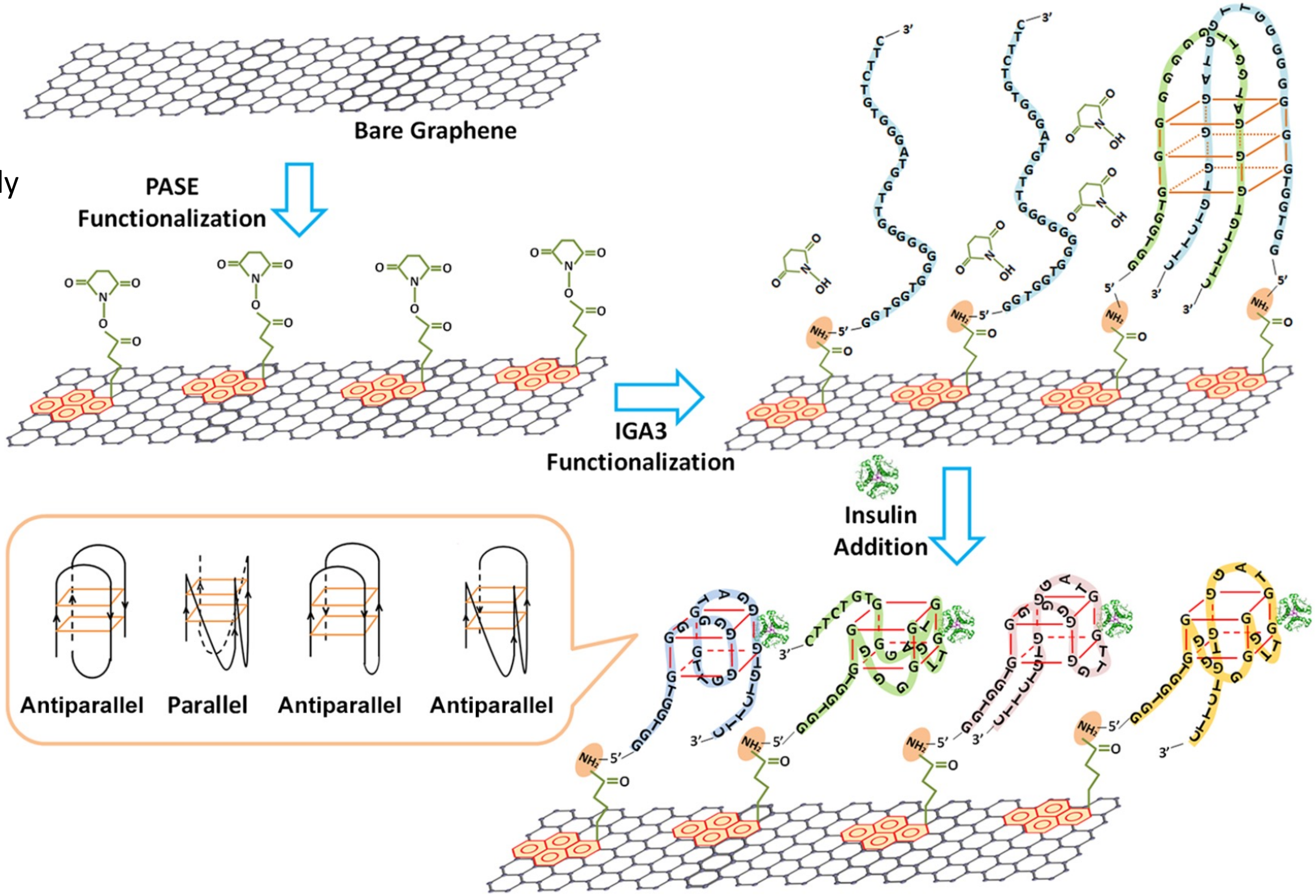
KEYWORDS: *affinity sensing, aptamer, G-quadruplex, graphene field-effect transistor (GFET), insulin*

ACS Appl. Mater. Interfaces 2017, 9, 33, 27504-27511
https://pubs.acs.org/doi/10.1021/acsami.7b07684

2. Describe the principle of the assay.

2) IGA3 aptamer immobilized on the surface via the reaction
of an amine group on the aptamer with the N-hydroxysuccinimide ester on PASE

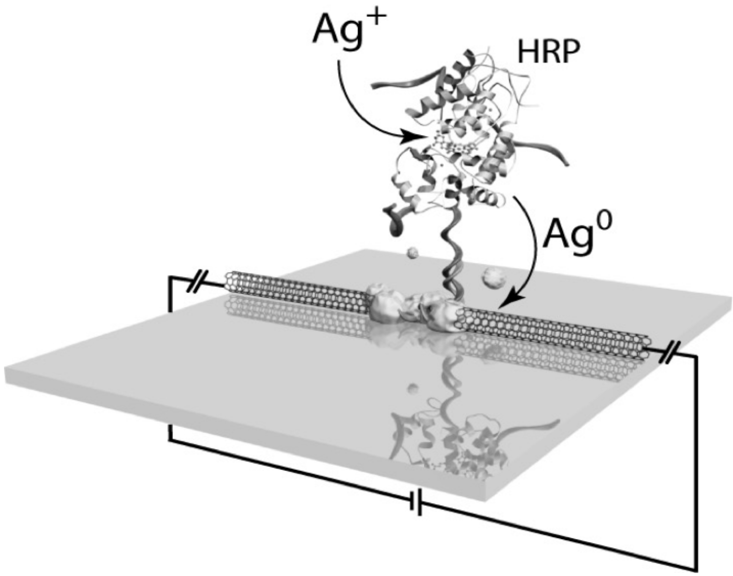
1) PASE noncovalently coupled to graphene via π–π interaction



3) Aptamer binds insulin → formation of both parallel and antiparallel G-quadruplexes → negatively charged insulin and DNA strands closer to graphene surface → negative charge attracts p-type carriers in semiconductor.

Definition electrical sensing

*Electrical sensing involves the **detection of changes in the flow or distribution of electric charge**—whether carried by electrons or ions—resulting from biological or chemical interactions.*



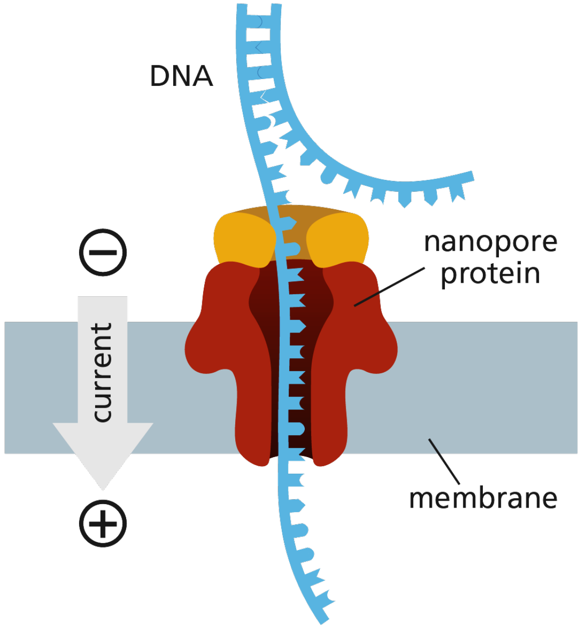
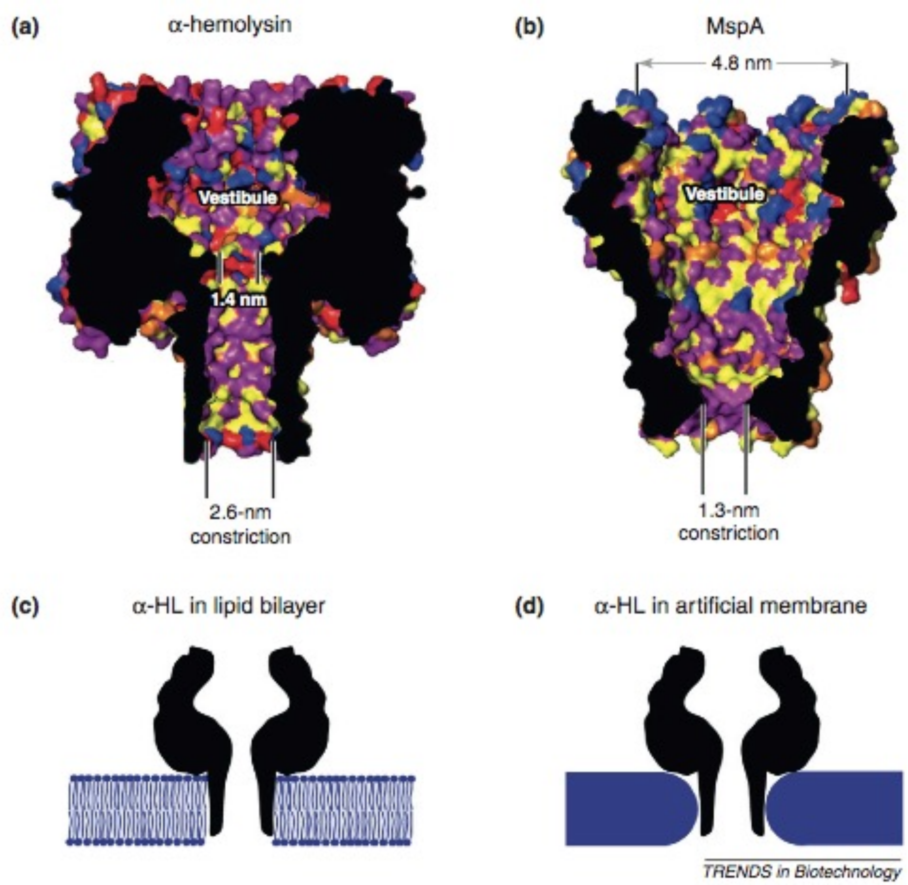


Image: https://swagergroup.mit.edu/research/molecular-and-nanowire-based-sensors

Image: https://www.yourgenome.org/theme/what-is-oxford-nanopore-technology-ont-sequencing/

Nanopores and DNA

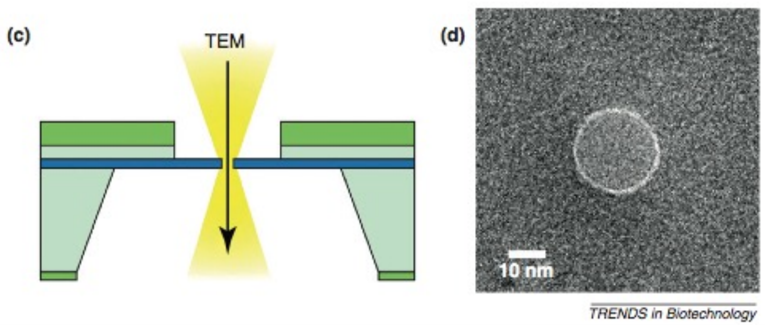
Biological nanopores:



Origin of the pore

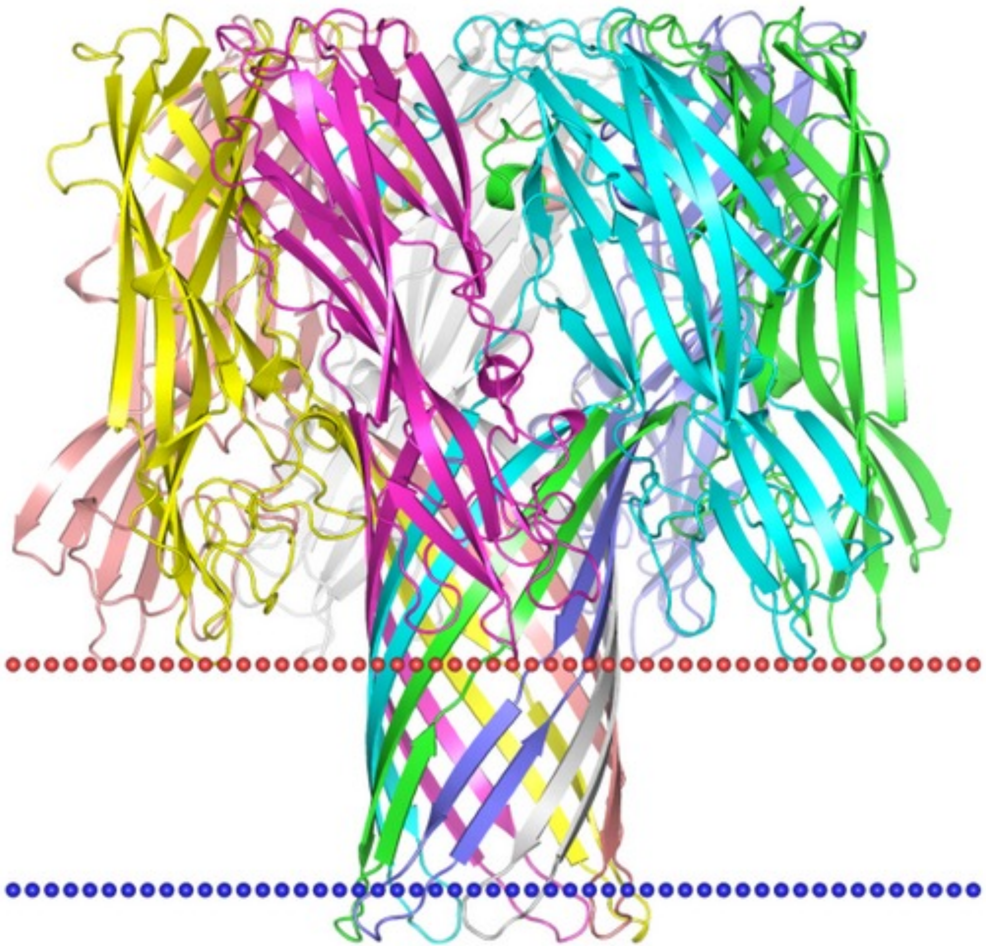
- Synthetic
- Bio-mimetic
- Hybrids

Synthetic nanopore: A single nanopore is drilled using a highly focused electron beam (yellow)



Kowalczyk, S. W., Blosser, T. R., & Dekker, C. (2011). *Trends in Biotechnology, 29(12), 607–614.*

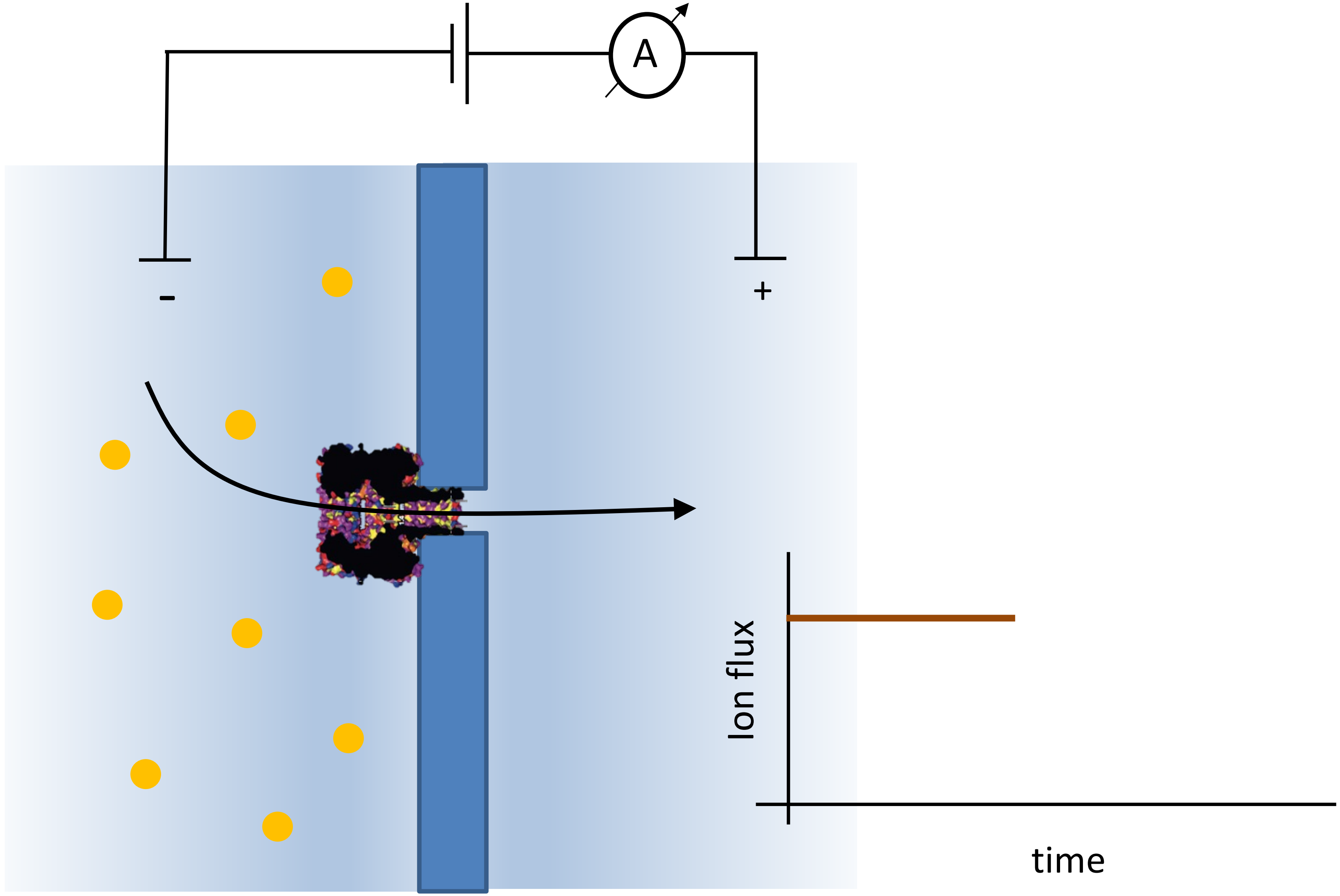
α-hemolysin



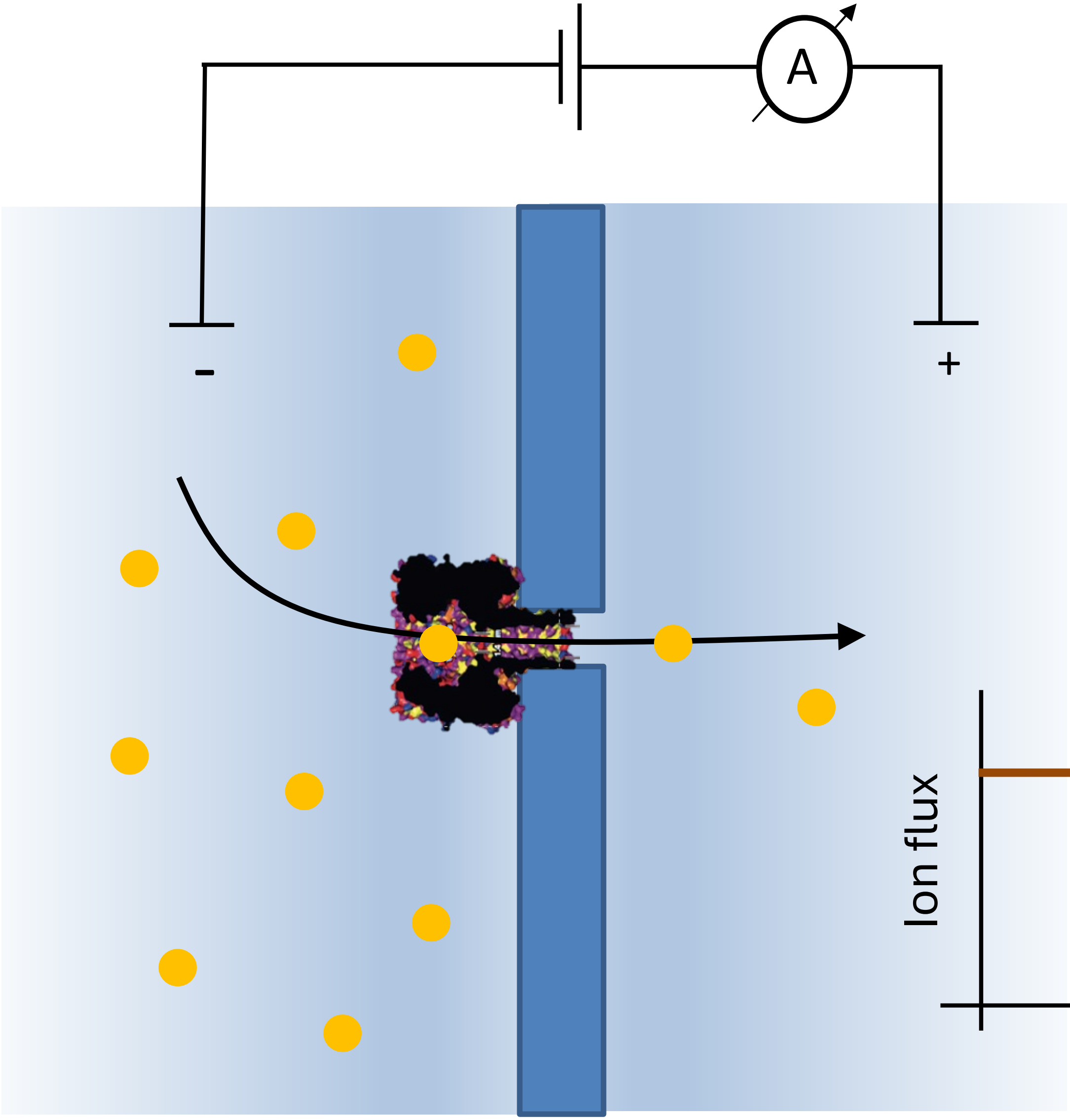
Protein nanopore

- Self-assembling bacterial toxin for example produced in *Staphylococcus aureus*
- 7 subunits membrane protein
- Produces pores in host membrane → cell death and facilitates bacterial infection

Nanopores: measurements

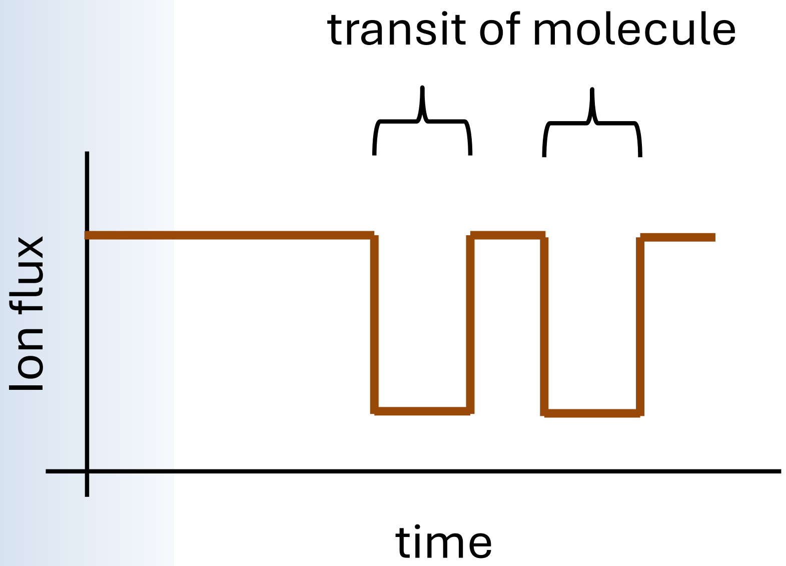


Nanopores: Measurements

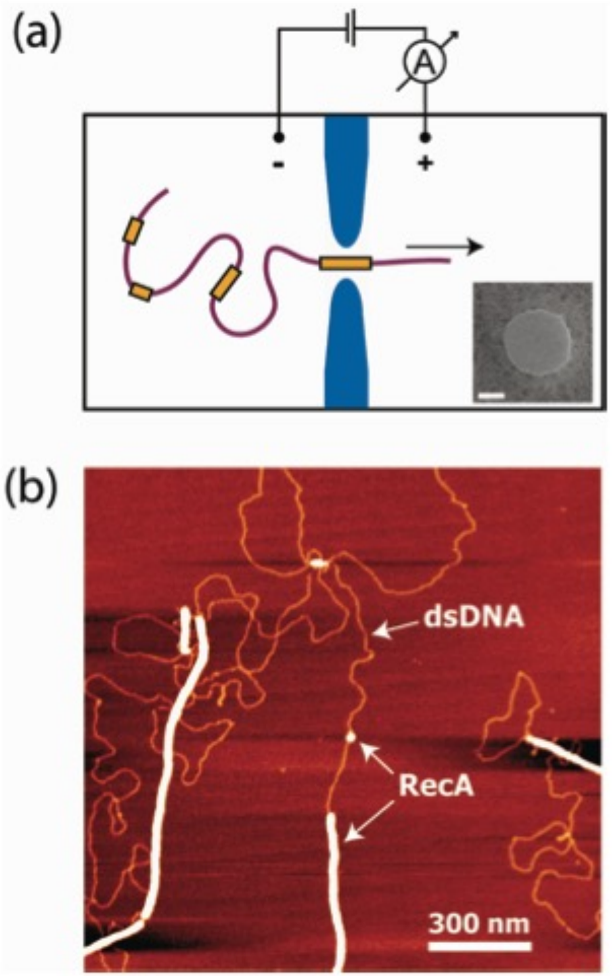


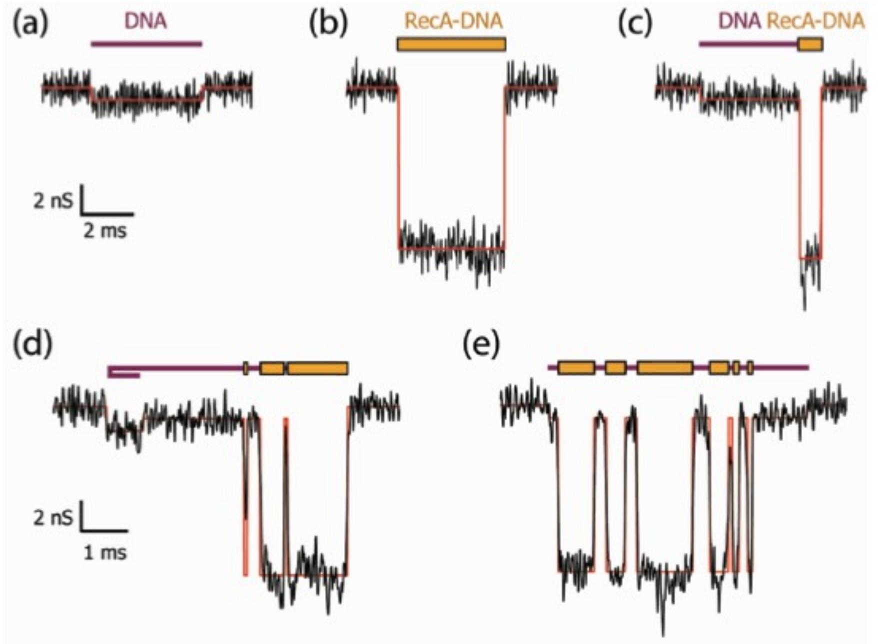
Transitioning molecules produce a change in the current flowing through the pore

They can be directly counted



Nanopore – DNA/protein interactions





Kowalczyk, S. W., Hall, A. R., & Dekker, C. (2010) *Nano Lett., 10(1), 324–328.*

Nanopore – sequencing?

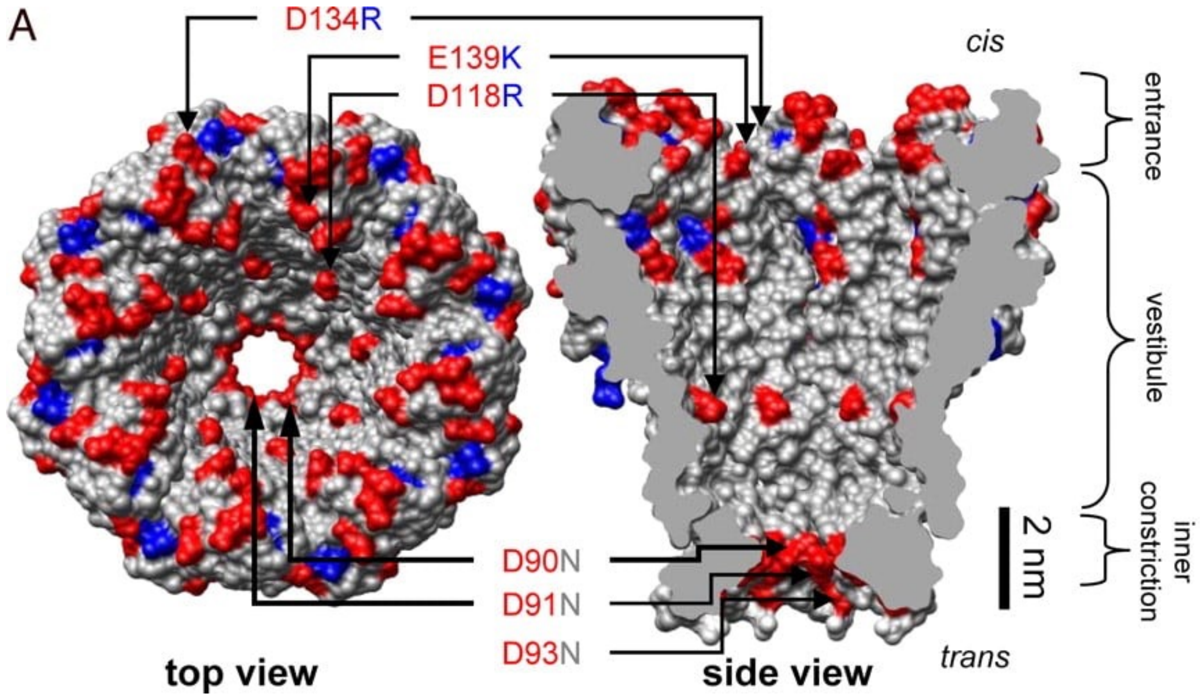
Advantages

- Very fast and possibly very accurate measurement
- Single pore recordings can be done at high frequency with very high signal to noise

Problems

- The DNA translocates too fast through the nanopore
- The difference between bases is too small – resolution problems
- Pores can clog or disintegrate

Nanopore – sequencing!



Mycobacterium smegmatis porin A (MspA) pore

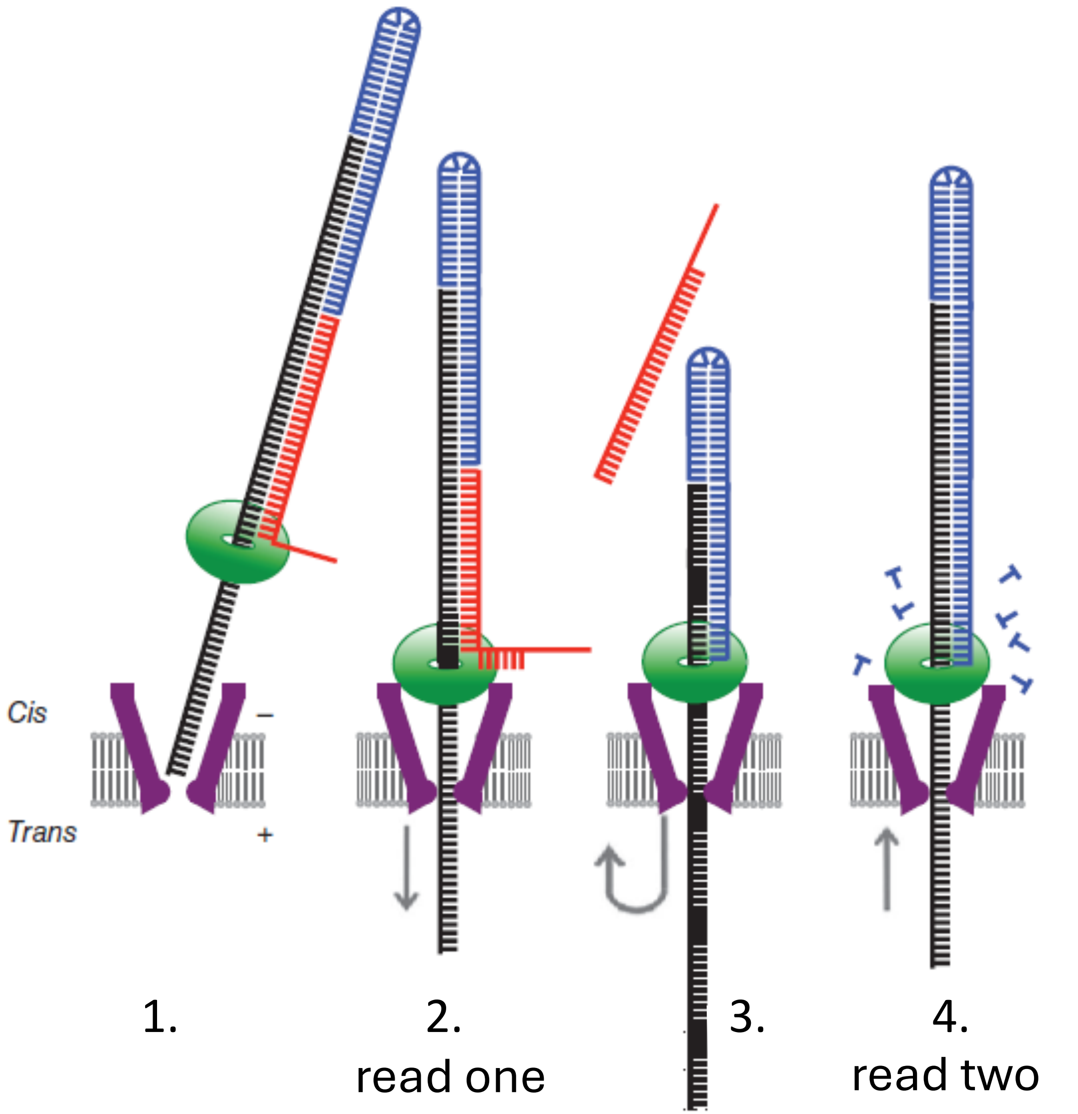
Addition of **positively charged residues** in the vestibule and entrance

Removal of **negative charges** at the constriction

Channel diameter : 1.2 nm at the constriction

Butler, T. Z. et al. (2008) *PNAS, 105 (52), 2064– 20652.*
Manrao, E. A., Derrington, I. M., et al. (2012). *Nature Biotechnology, 30 (4), 349–353.*

Experimental scheme: DNA capture and synthesis

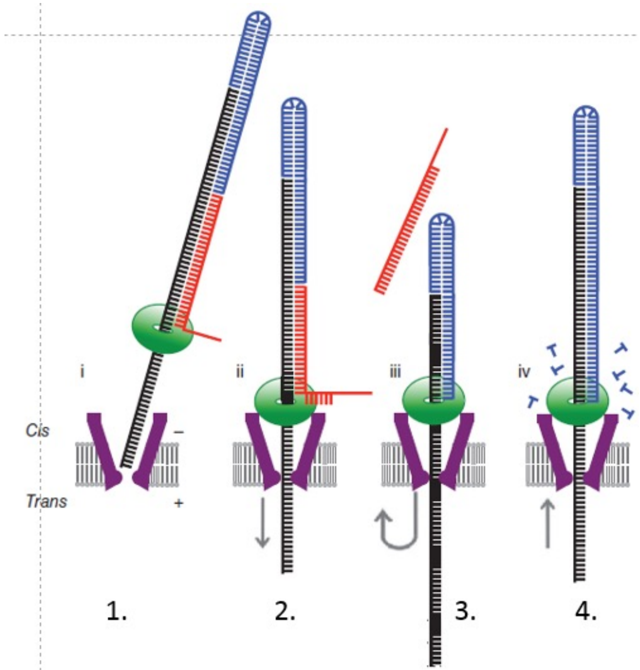


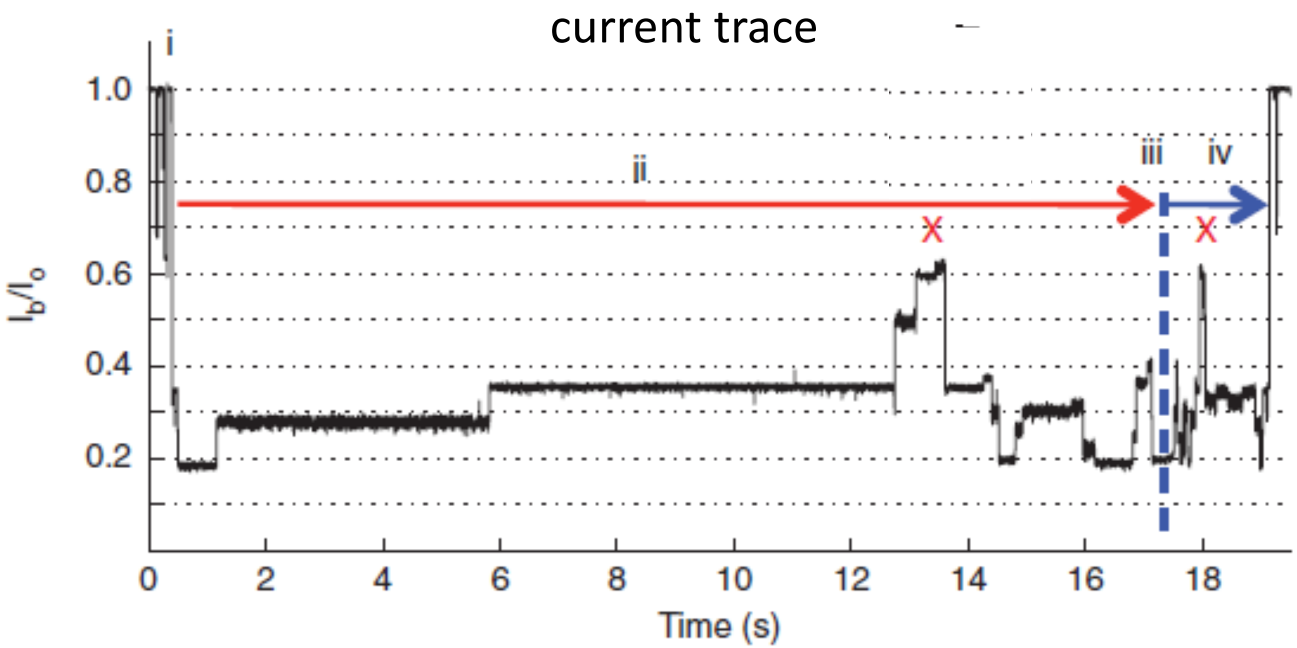
To control transition of DNA through pore: **Use of phi29 DNA polymerase as a molecular motor**

Procedure

1. DNA hairpin (**black-blue**) is hybridized to a **blocking oligomer** and complexed with **DNA pol**. This complex is then threaded into pore
2. Positive potential (on *trans* side) translocates DNA into pore (**read one**), blocking oligomer is released
3. Addition of dNTPs and cofactors allows DNA synthesis with defined rate (**read two**)

Experimental data

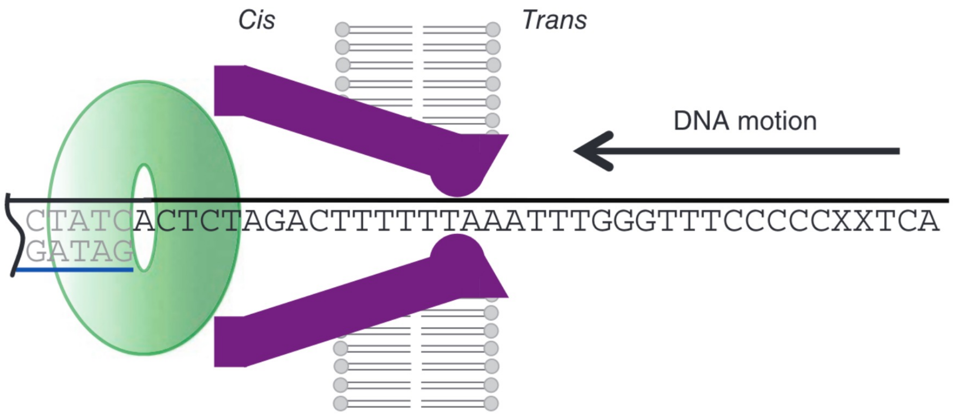




Current trace:

- Start with open pore (I_0)
- Capture with positive voltage on trans side (i)
- Translation, blocking oligomer (ii)
- Direction reversal (iii)
- DNA synthesis by Phi29 RNAP (iv)
- Release (current reverts to I_0)

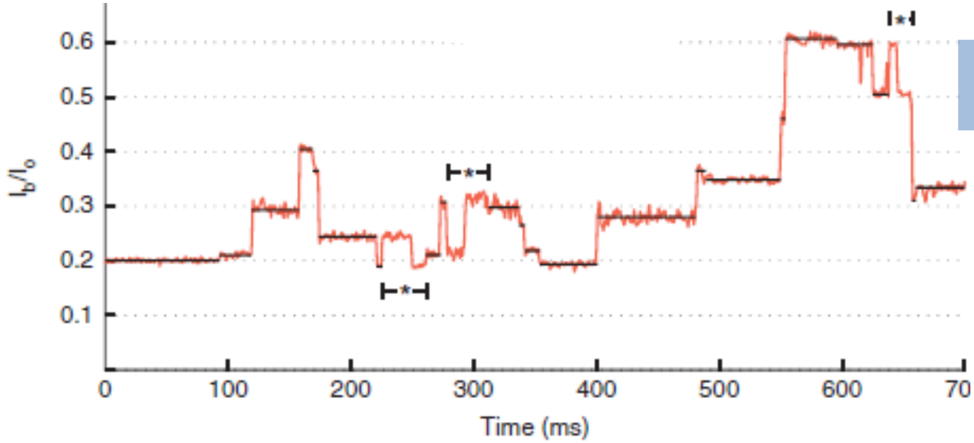
Current trace for polymerase

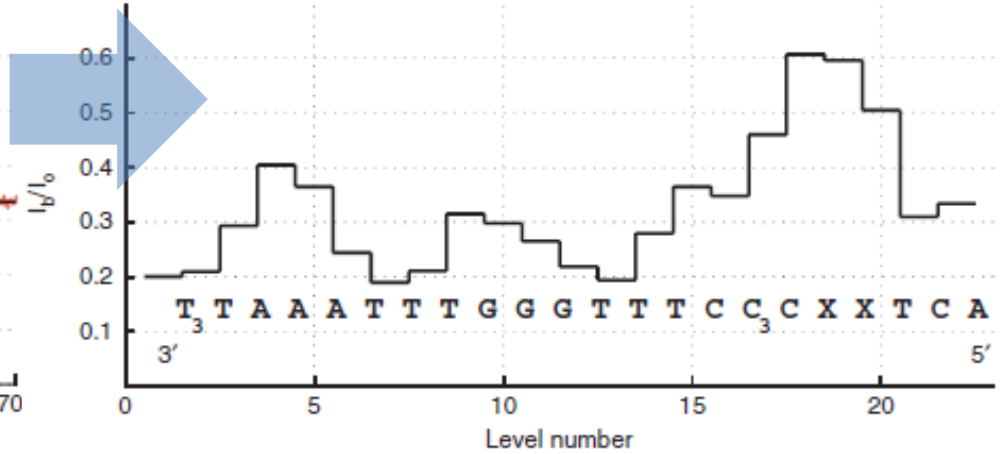


Single-molecule readout of DNA sequence

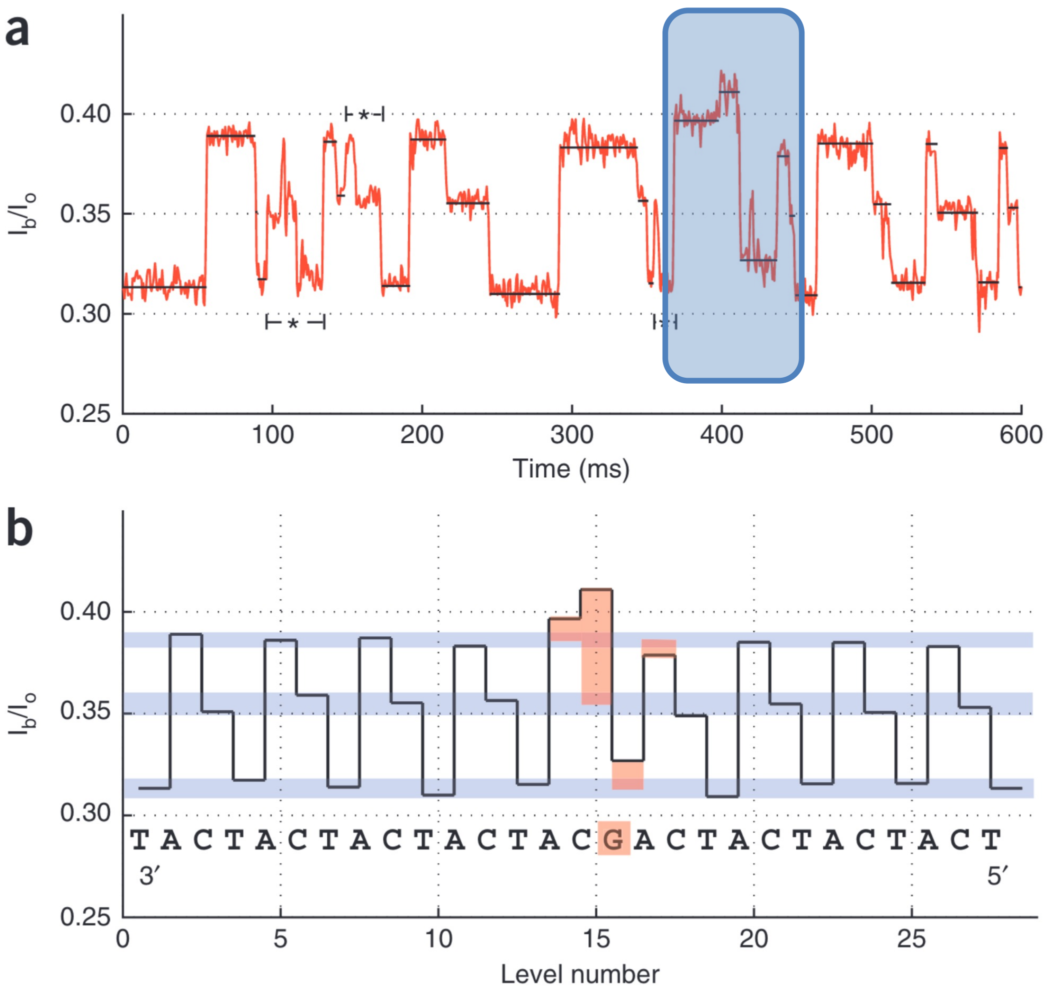
Short sequences of backtracking observed

mapping





Actual sequencing trace, accuracy



82 residue oligonucleotide

- Mostly repetitive sequence (TAC-TAC..) with one G
- Level analysis allows read out of sequence
- "Mutation" is detected in the single molecule trace

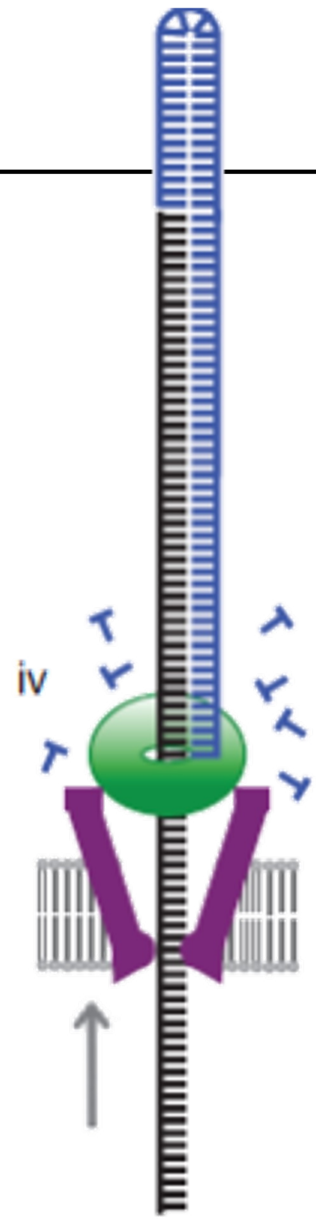
➔ Single molecule sequencing works (in this case)

Important update: basecalling is now a solved issue with AI

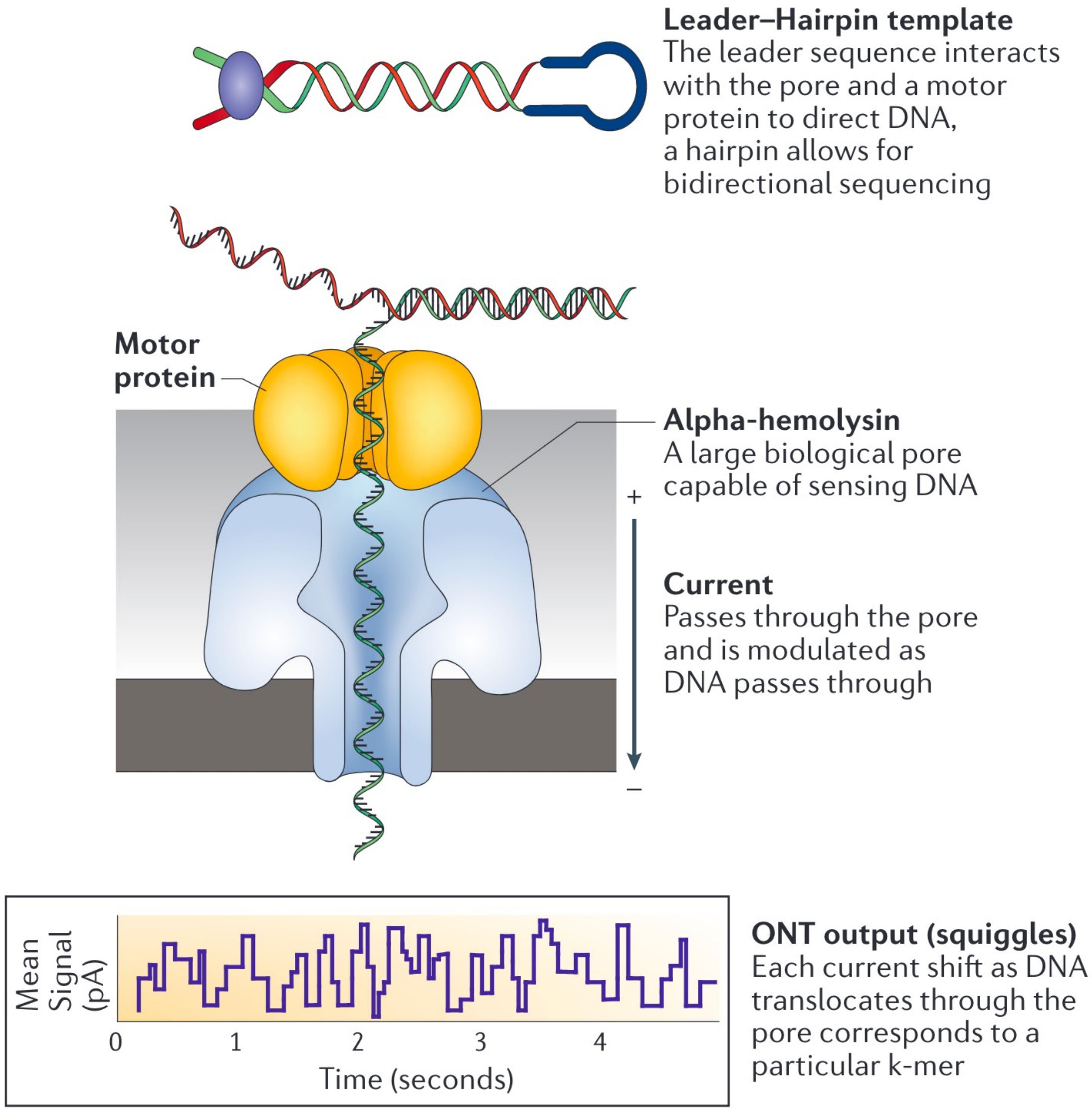
https://doi.org/10.1186/s13059-019-1727-y

Conclusions

- Individual DNA (or RNA) molecules traversing the pore can be sequenced
- Unzipping of a blocking oligomer allows sequencing in one direction (this could also be the antisense strand in dsDNA)
- Phi29 polymerase serves as a **pacemaker** allowing slow transition and sequencing in the other direction
- Both the forward and the reverse strands of DNA can be read: allows two sequencing runs on the same template and increase accuracy



Oxford Nanopore Technologies (ONT)



- DNA is initially fragmented to 8–10 kb.
- Two different adapters, a leader and a hairpin, are ligated to either end of the fragmented dsDNA.
- As the DNA translocates through the pore, a characteristic shift in voltage through the pore is observed.

Applications: Nanopore sequencing

- Very high detection bandwidth
- Instrument requirements: well established
- **Long sequencing** runs possible
- Relying on well-behaved abundant protein
- **Possibility of sequencing DNA modifications (mC and hmC)**

commercial instrument

Challenges:

- Integration into instrument → Oxford nanopore

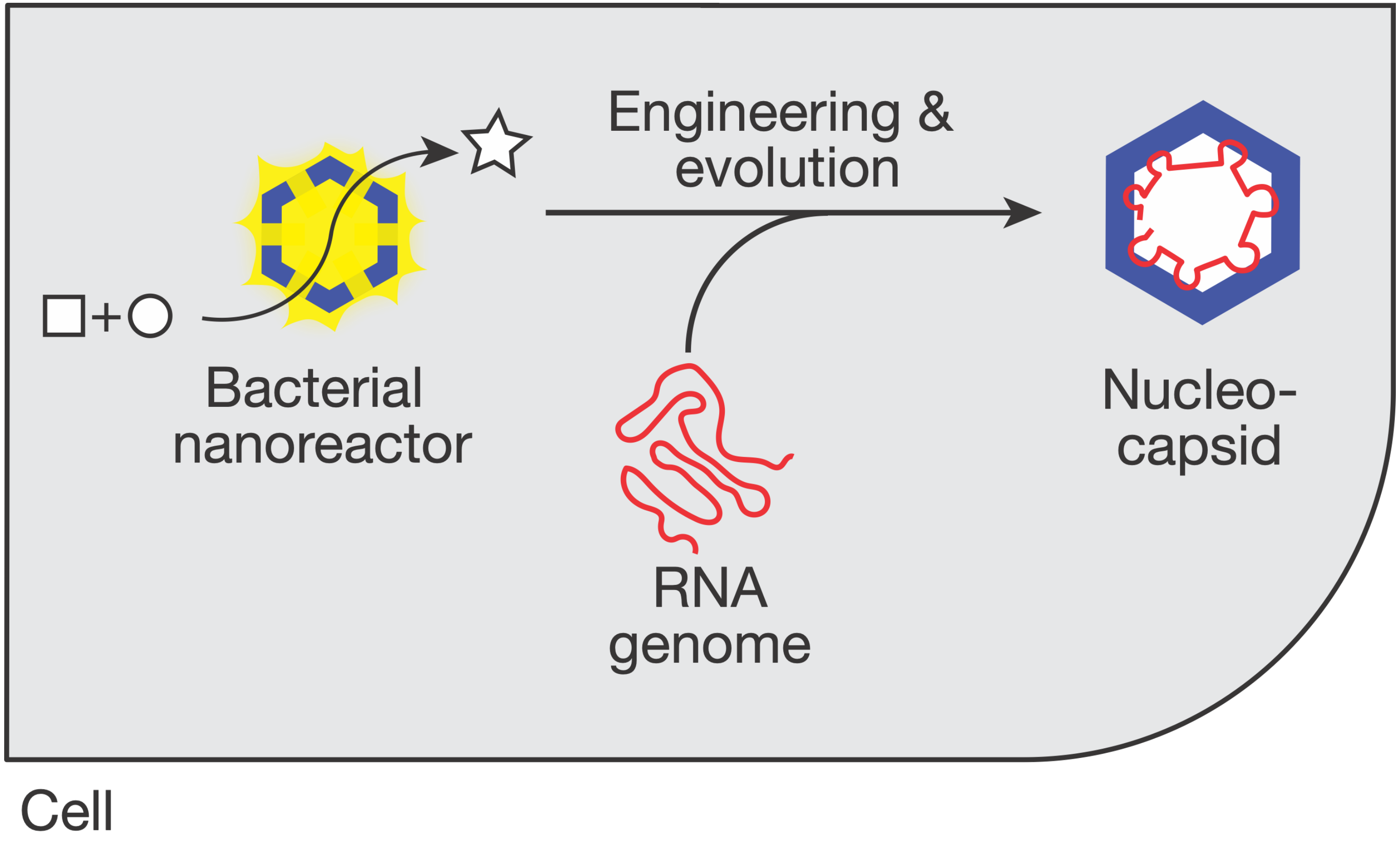
Nanopore sequencing

- https://www.youtube.com/watch?v=qzusVw4Dp8w&ab_channel=OxfordNanoporeTechnologies

- Shorter:
- https://www.youtube.com/watch?v=RcP85JHLmnI&ab_channel=OxfordNanoporeTechnologies

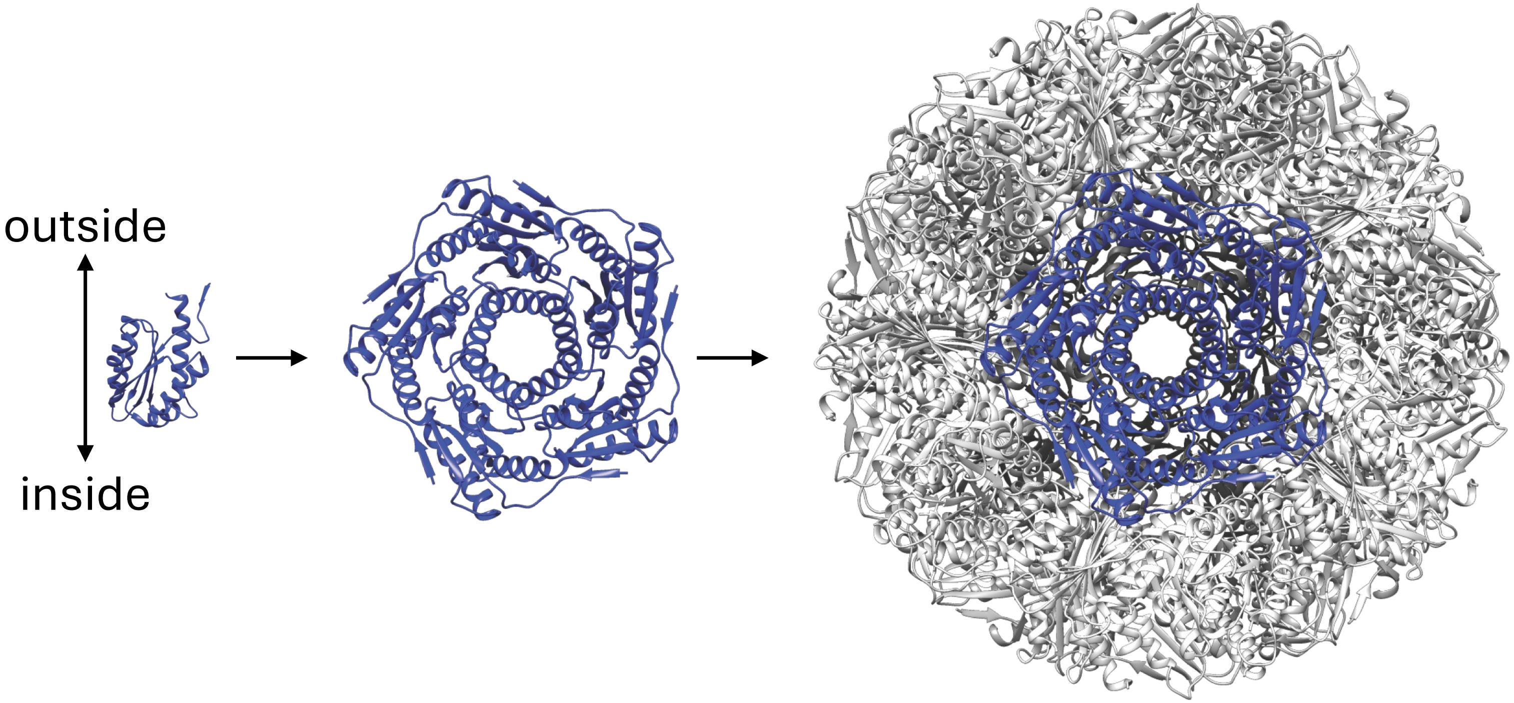
An application of Nanopore sequencing from my own research

Engineering a bacterial protein to bind its own mRNA

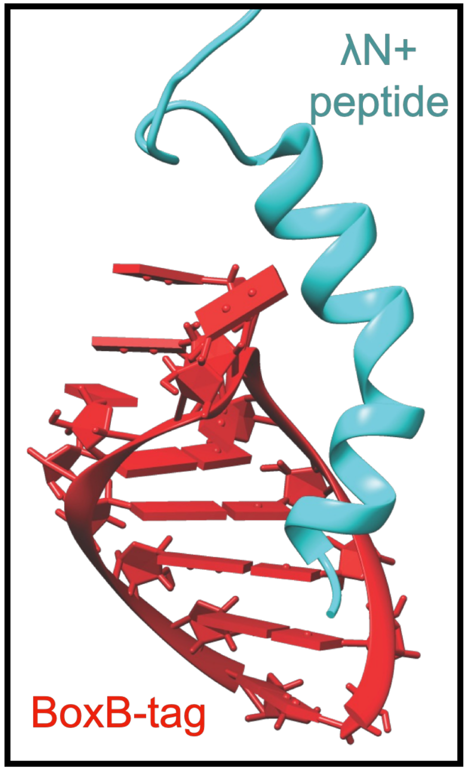


https://www.epfl.ch/labs/libn/

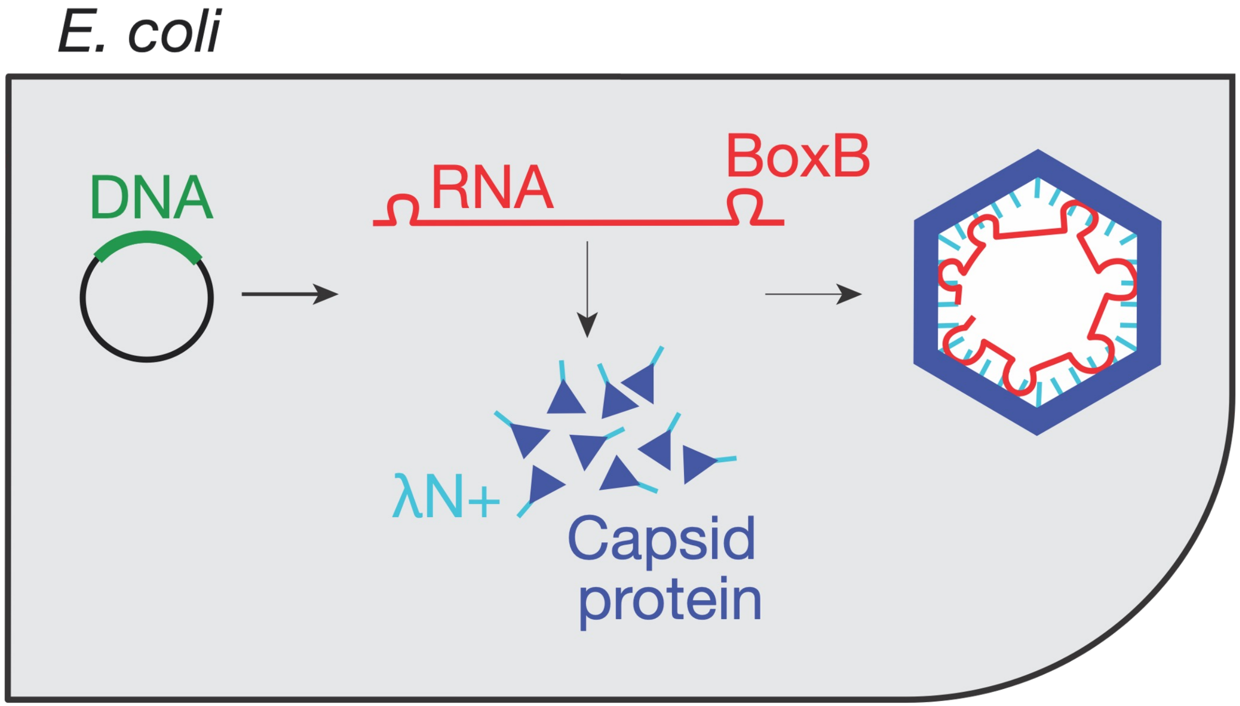
Lumazine synthase from *Aquifex aeolicus*



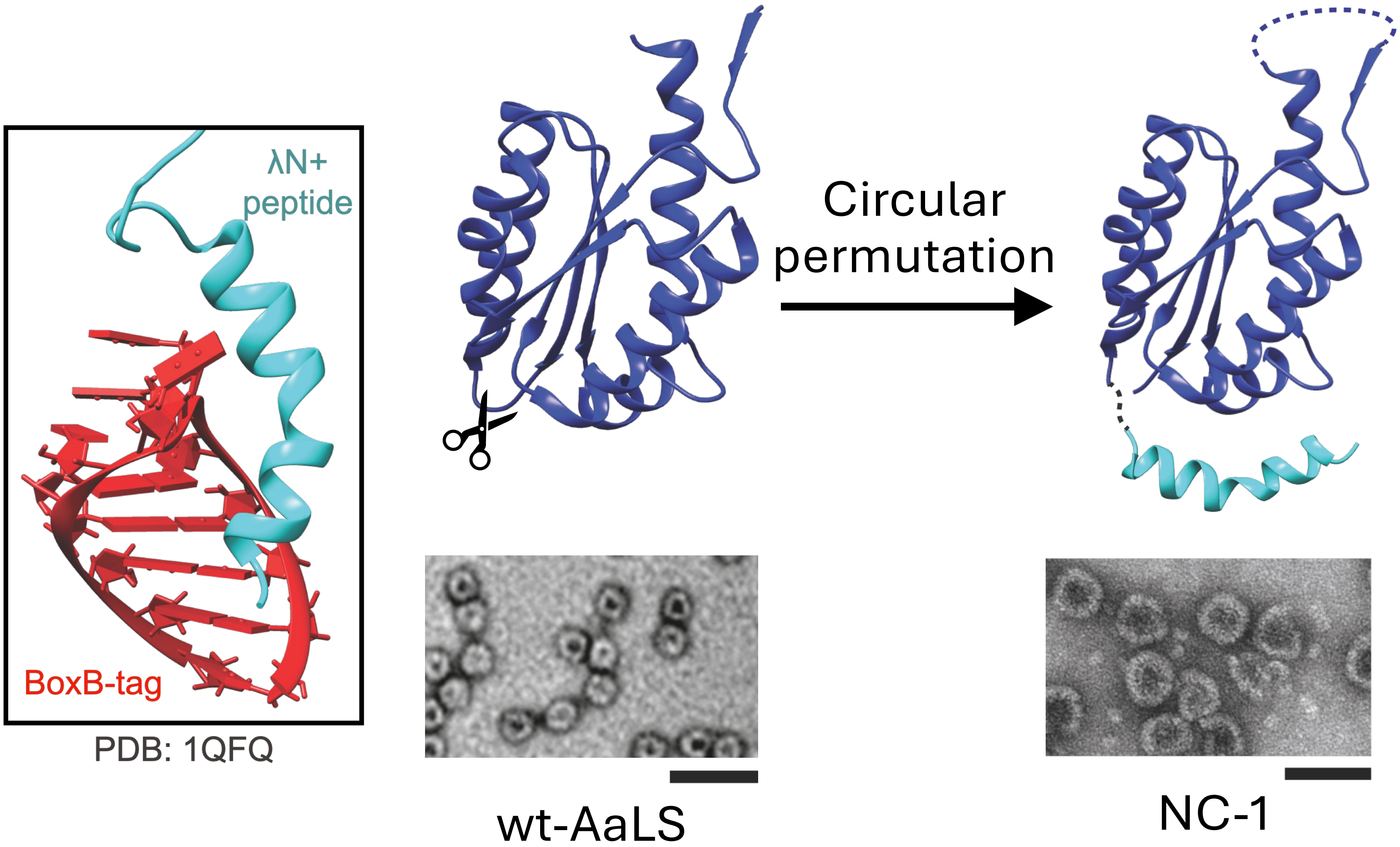
Capsids assemble in bacteria



PDB: 1QFQ

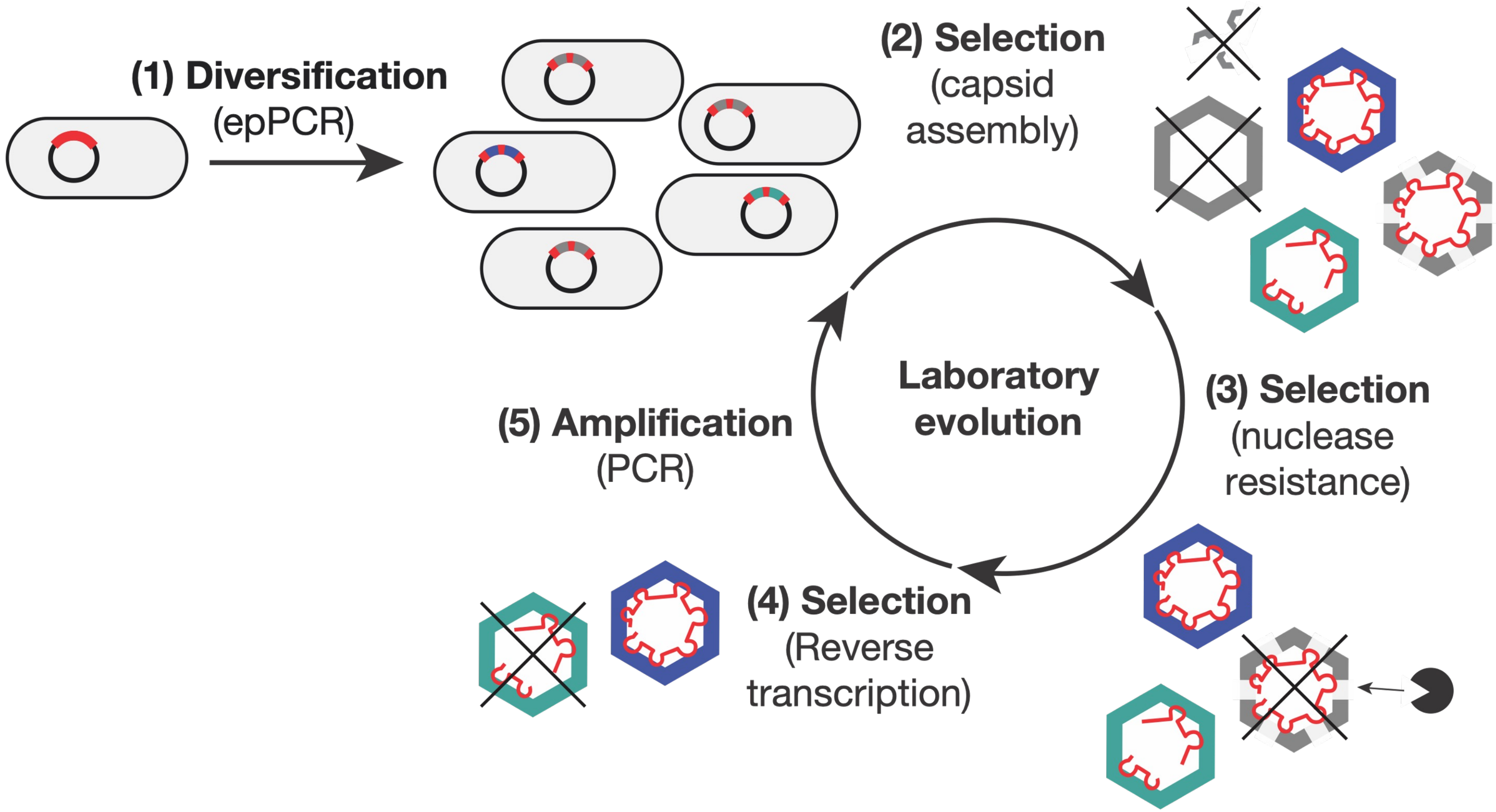


Rational nucleocapsid design



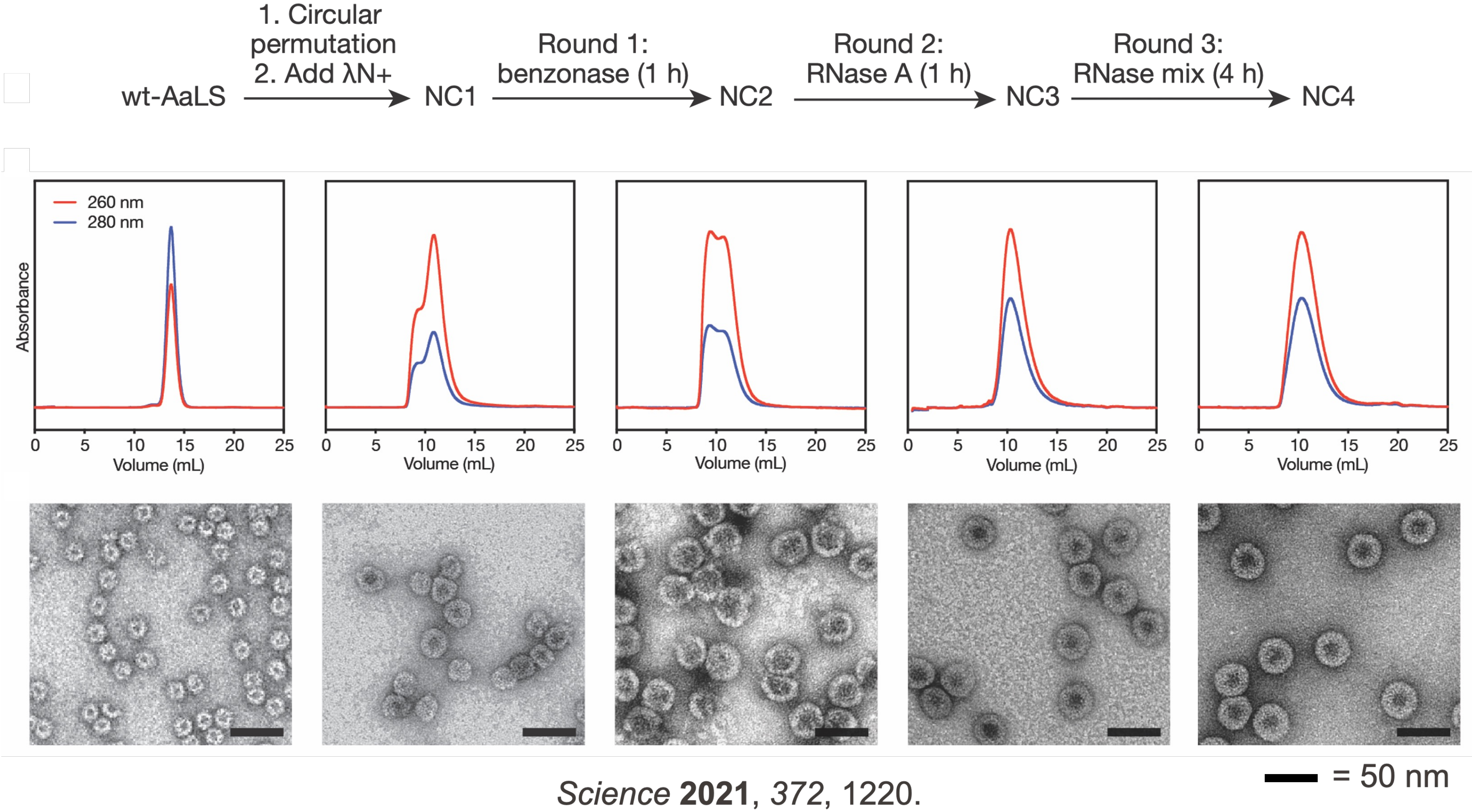
— = 50 nm

Selecting for capsids that package their own encoding RNA



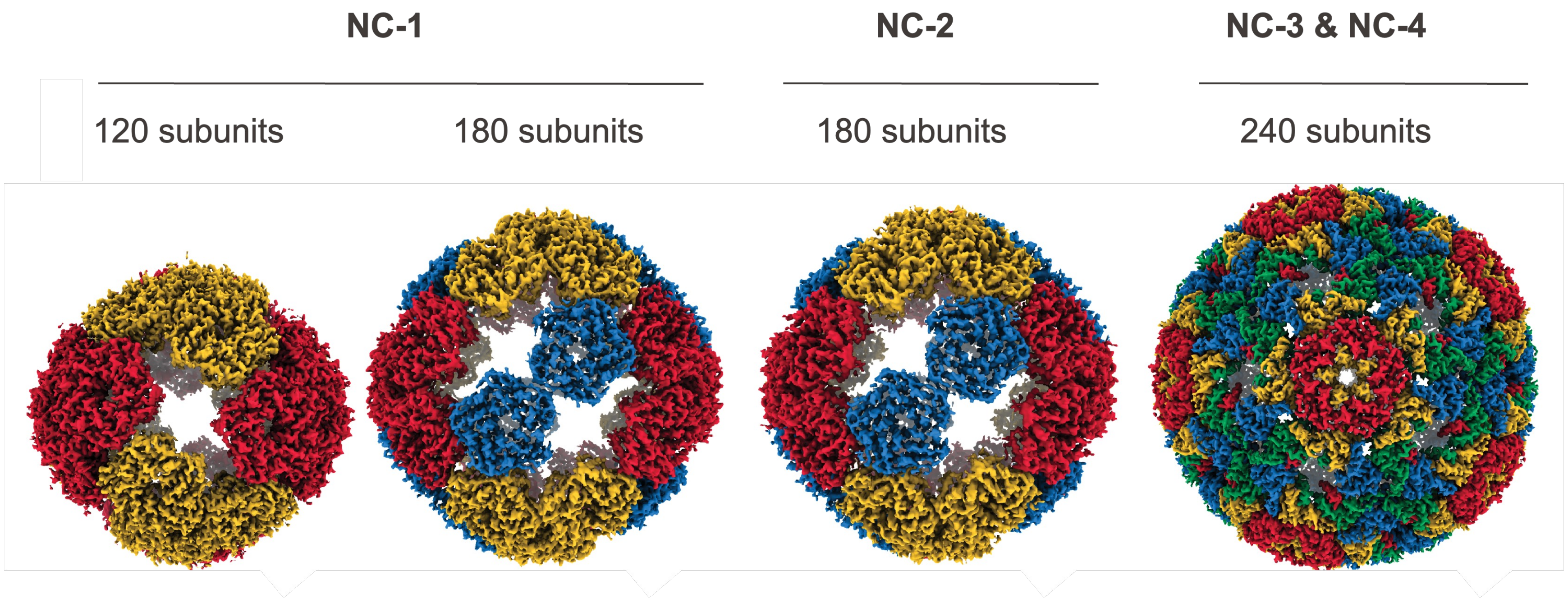
PNAS **2018**, *115*, 5432. *Science* **2021**, *372*, 1220.

Four nucleocapsid generations



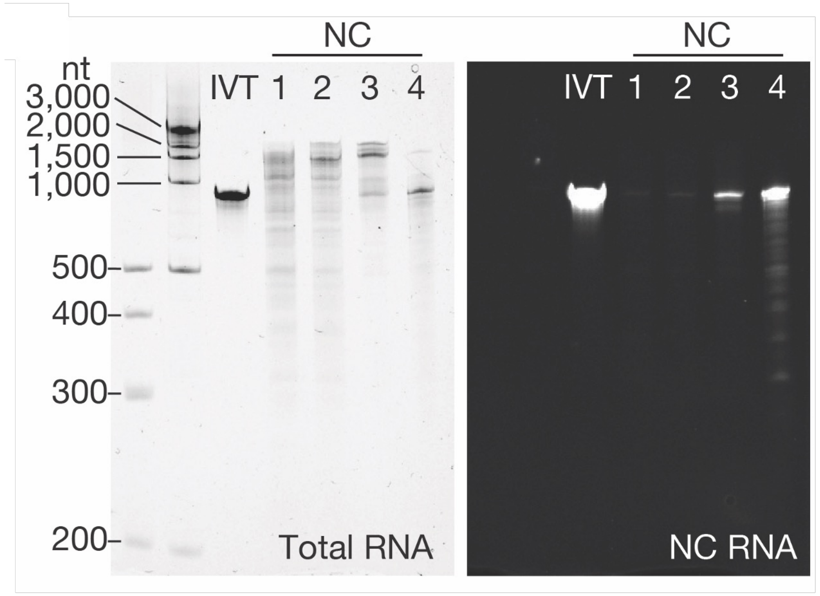
Science **2021**, *372*, 1220.

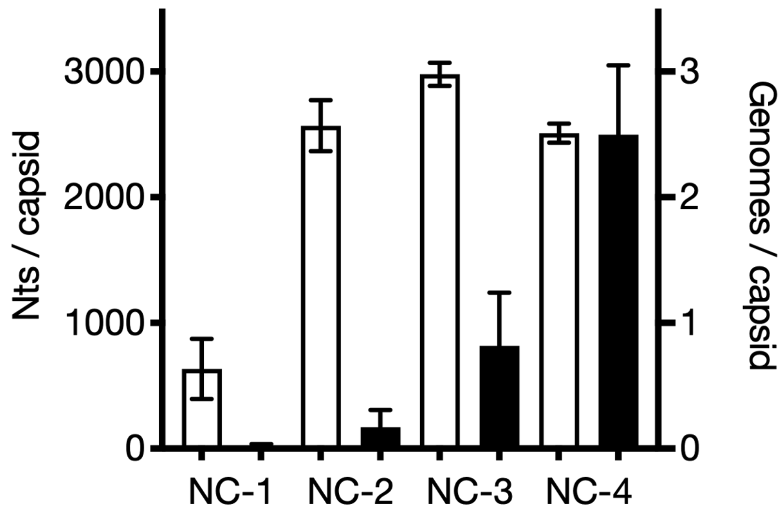
Evolution brings structural change



Science **2021**, *372*, 1220.

Evolution increases self-RNA recognition

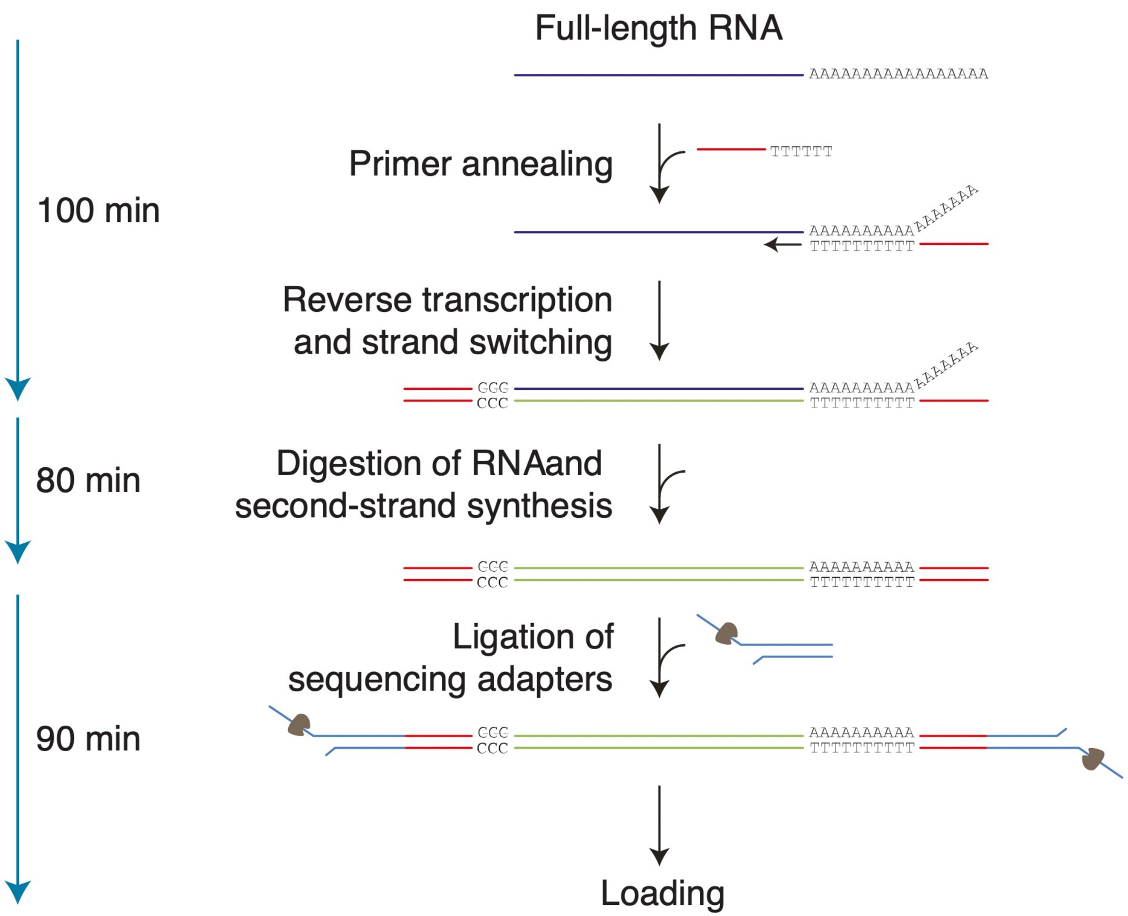




Science **2021**, *372*, 1220.

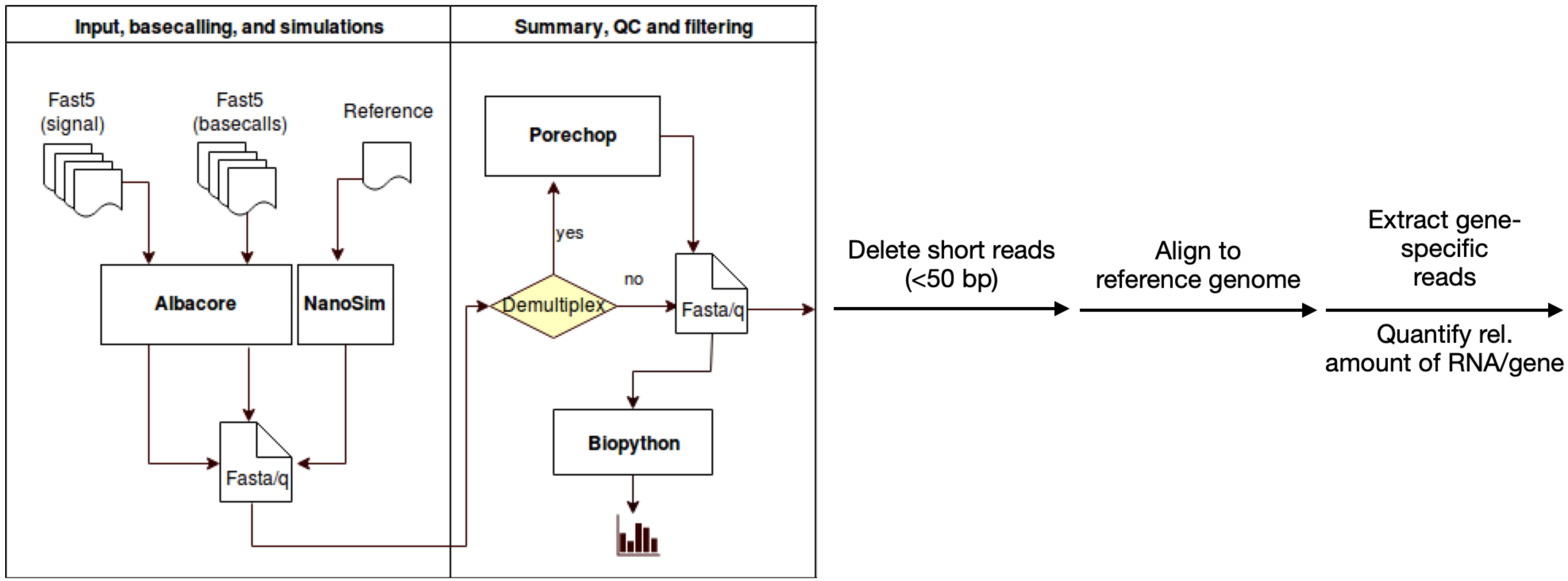
What other RNAs are encapsulated?

Library preparation for Nanopore sequencing

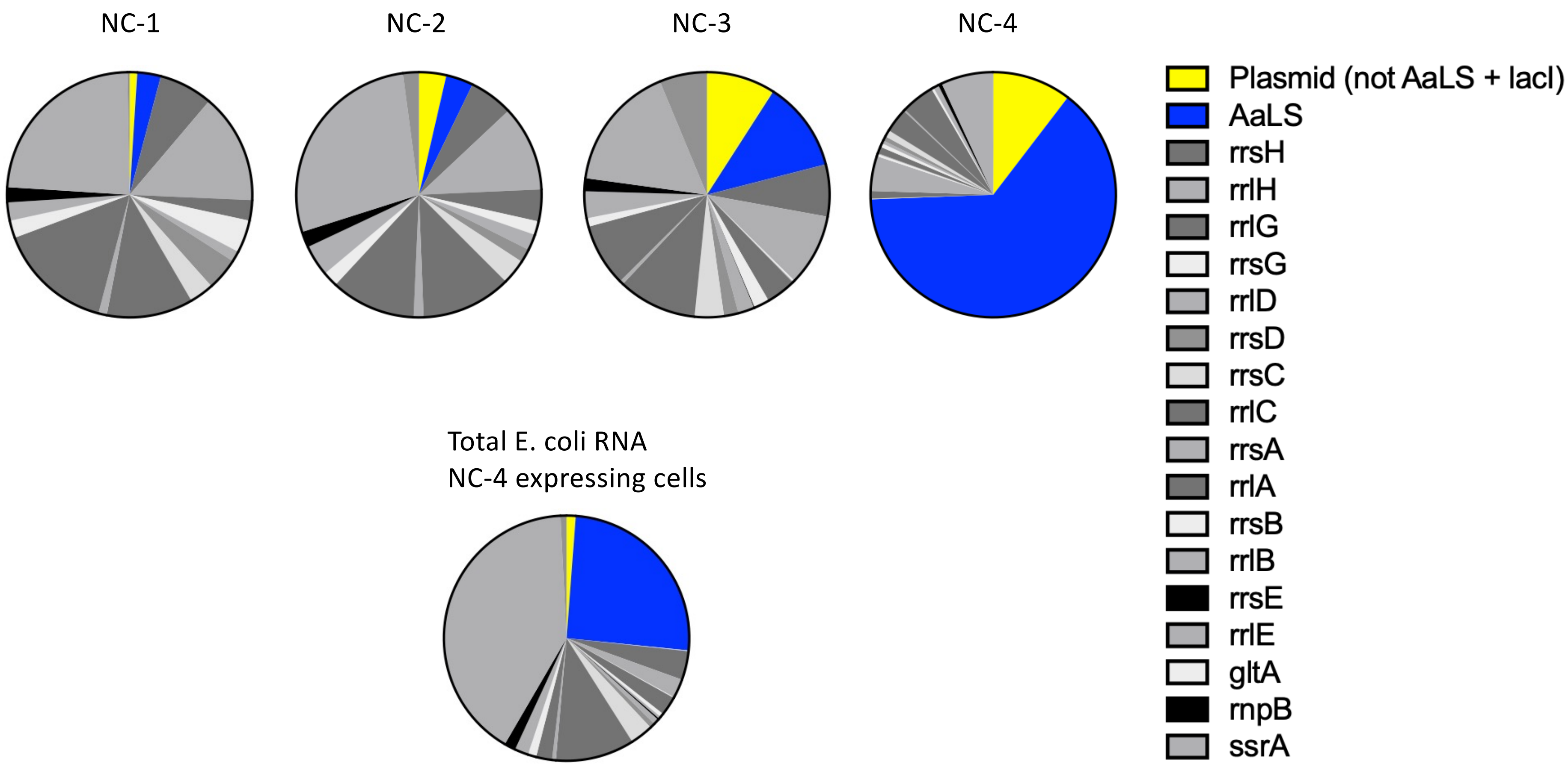


Direct RNA sequencing

Data analysis workflow for Nanopore sequencing



Majority of ”other RNA” is ribosomal RNA



Summary

Electrical sensing involves the detection of changes in the flow or distribution of electric charge—whether carried by electrons or ions—resulting from biological or chemical interactions.

Sensor Type	Charge Carrier	Detection Principle	Electrical Signal
FET	Electrons/holes	Charge-induced modulation of conductance	Change in drain current
Nanopore	Ions	Disruption of ionic current during translocation	Transient change in ionic current

Connections

- https://connections.swellgarfo.com/game/-Nu0ndVlxi8GBgpOOtJ9

Good reviews about today’s topics

- https://www.nature.com/articles/s44222-023-00032-w?fromPaywallRec=false
- https://pubs.acs.org/doi/10.1021/acs.chemrev.8b00172
- https://pubs.acs.org/doi/10.1021/acs.chemrev.7b00088
- https://pubs.acs.org/doi/10.1021/acs.chemrev.5b00608
- https://www.nature.com/articles/s41557-023-01322-x
- https://www.nature.com/articles/s41592-022-01730-w