CH-413 Nanobiotechnology

Electrical Sensing

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Warm-up: Fundamentals of electric principles

- https://www.menti.com/aluc6zjoqrgv

Solutions to warm-up

1. **The electric field is measured in amperes (A), the same unit as current.**

   Answer: False. The electric field is measured in volts per meter (V/m).

2. **If you increase the voltage across a circuit component while its resistance remains constant, the current through it will also increase according to Ohm's Law.**

   Answer: True. According to Ohm's Law, current increases if voltage increases while resistance remains constant.

3. **An electric field can exist even when there is no current flowing.**

   Answer: True. An electric field results from a voltage difference or presence of charged particles, regardless of current flow.

4. **The direction of the electric field is from the point of higher potential to the point of lower potential.**

   Answer: True.

5. **Conductance is inversely proportional to the voltage applied across a material.**

   Answer: False. Conductance is inversely proportional to resistance, not voltage.

6. **The strength of the electric field within a conductor remains constant regardless of the voltage applied across it.**

   Answer: False. The electric field strength in a conductor changes with the applied voltage.

7. **In a circuit, electrons always flow from the positive terminal to the negative terminal.**

   Answer: False. Electrons flow from the negative terminal to the positive terminal.

8. **The electric field has both magnitude and direction, making it a vector field.**

   Answer: True. An electric field is a vector field with both magnitude and direction.

Background to warm-up

1. Electric Field Measurement:

Equation: $E = F/q$

Description: Electric field (E) is defined as the force (F) per unit charge (q). The unit of electric field is volts per meter (V/m), not amperes.

2. Ohm's Law:

Equation: $I = V/R$

Description: Ohm's Law states that the current (I) through a conductor between two points is directly proportional to the voltage (V) across the two points and inversely proportional to the resistance (R).

3. Existence of Electric Field without Current:

Concept: An electric field can exist due to a static charge distribution or a potential difference, even in the absence of a current.

Description: The electric field is present whenever there is a voltage, which can occur without current if the circuit is open or the component has infinite resistance.

4. Direction of Electric Field:

Concept: Electric field lines start on positive charges and end on negative charges.

Description: The direction of the electric field at a point is the direction of the force that would be exerted on a positive test charge placed at that point.

5. Conductance and Voltage:

Equation: $G = 1/R$

Description: Conductance (G) is the reciprocal of resistance (R). It measures how easily electric current can flow through a material and is independent of the voltage applied across the material.

6. Electric Field Strength in a Conductor:

Equation: $E = V/d$

Description: The electric field (E) within a conductor is the voltage (V) divided by the distance (d) between the points. As voltage changes, so does the electric field strength.

7. Electron Flow in a Circuit:

Concept: Electrons flow from lower potential (negative terminal) to higher potential (positive terminal).

Description: In a conventional current perspective, we say current flows from positive to negative. However, the actual charge carriers, electrons, move in the opposite direction due to their negative charge.

8. Electric Field as a Vector Field:

Concept: An electric field is represented as a vector that has both magnitude (how strong) and direction (the way it points).

Description: The electric field vector points in the direction that a positive test charge would move if placed in the field.

Refresher on electric circuits and fields

Voltage vs. Electric Field:

- **Voltage** (V): Measured in volts (V), voltage is the potential difference that causes current to flow in a circuit. In an FET, it's the voltage applied at the gate, source, and drain terminals.
- **Electric Field** (E): Measured in volts per meter (V/m), the electric field is the force per unit charge exerted on electric charges. It's a vector field, meaning it has both magnitude and direction.
- The electric field in the FET is a result of the voltage applied to the gate. It's not equal to the voltage but is related to it; the field strength depends on the voltage and the physical dimensions of the gate structure.

Relationship Between Voltage, Electric Field, and Current:

- The voltage applied to the gate creates an electric field, which in turn modulates the conductivity of the channel by attracting electrons.
- The conductance of the channel, and therefore the current flowing between the source and drain, is controlled by the strength of this electric field.
- Current (I), measured in amperes (A), is the flow of electric charge. In an FET, the amount of current flowing between the source and drain depends on the voltage applied to the gate (which affects the electric field) and the voltage applied between the source and drain.
- Modulating Conductance:

- By varying the gate voltage, you change the strength of the electric field, which alters the density of charge carriers in the channel, thus modulating the current flow between the source and drain.

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/
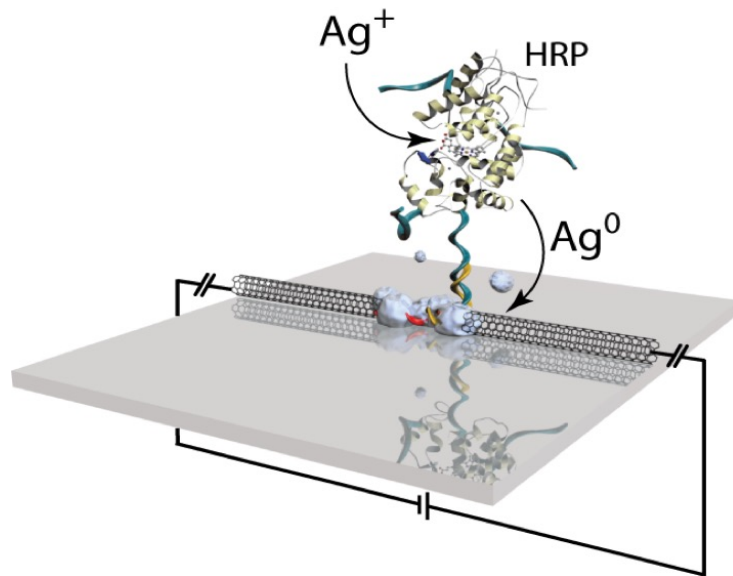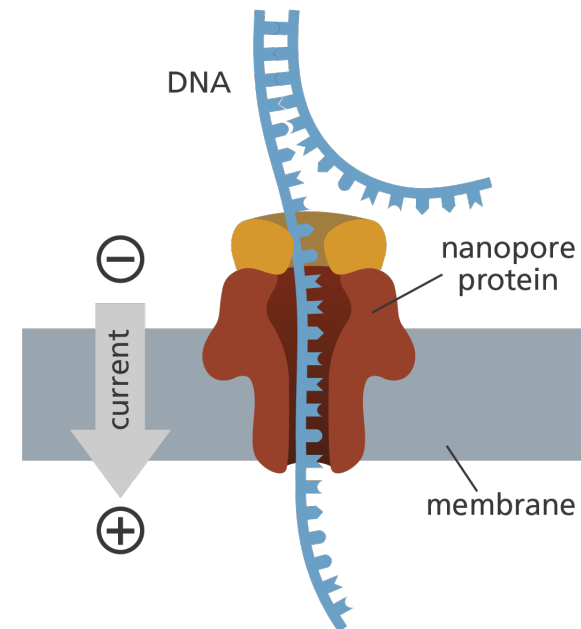
Elements of a field-effect transistor (FET)

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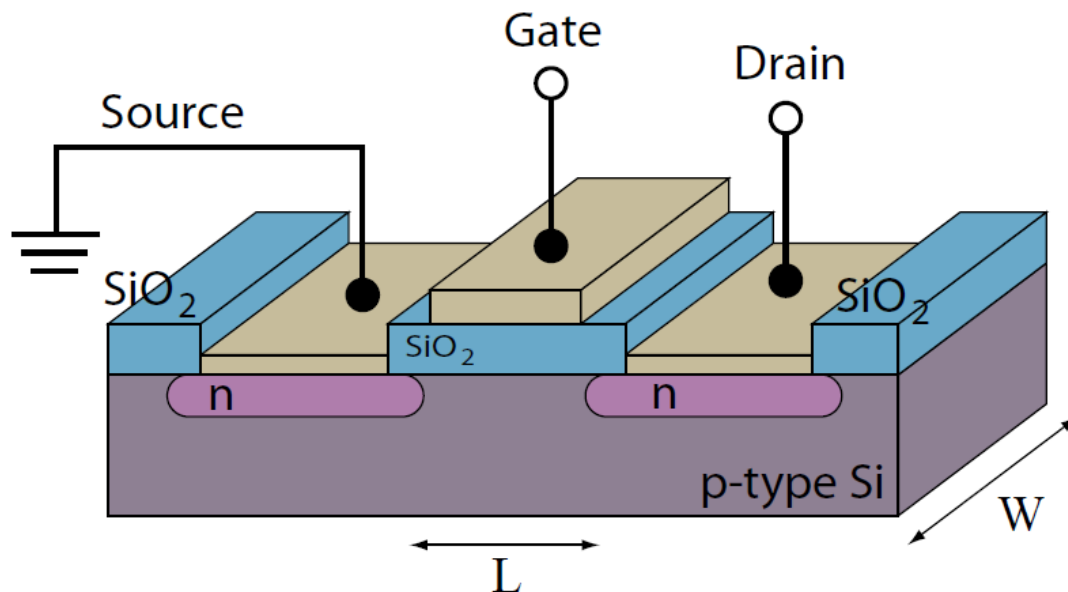
MOSFET: metal-oxide-semiconductor field-effect transistor (most common)

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 (n for negative)**
e.g. pentavalent impurities, e⁻
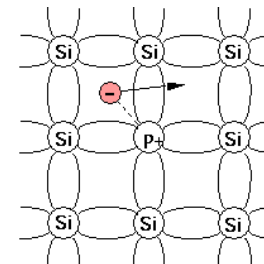
p-type semiconductor: extra **holes** (p for positive, absence of electrons)
e.g. trivalent impurities, h⁺
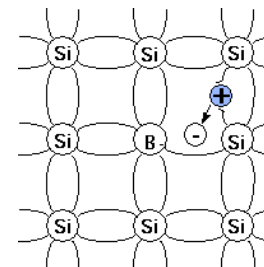
https://en.wikipedia.org/wiki/Donor_(semiconductors)
https://en.wikipedia.org/wiki/Acceptor_(semiconductors)

Field-effect transistors

OFF state:
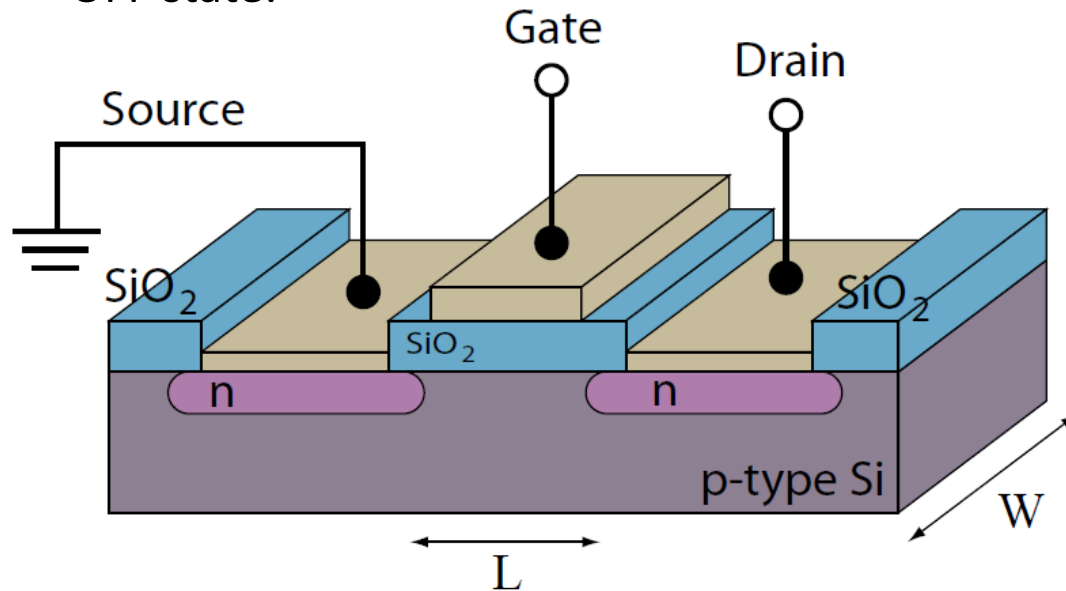
A FET is an electronic switch

- **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

ON state:
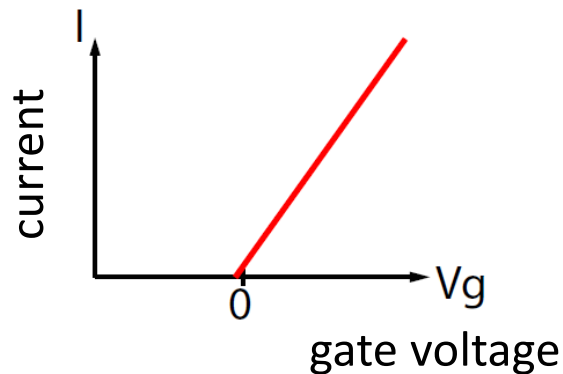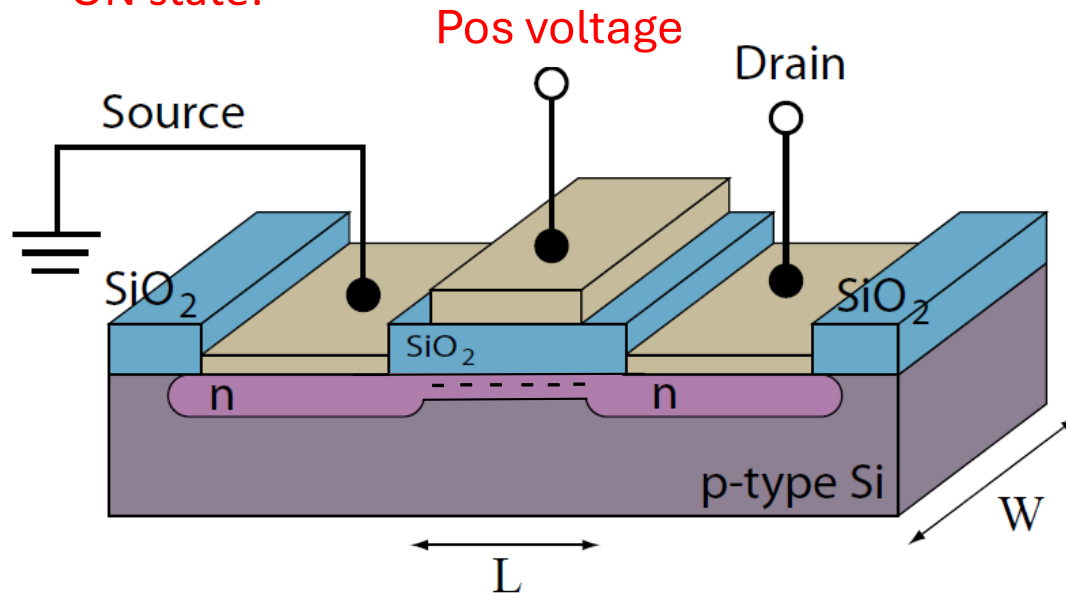
A FET is an electronic switch

- **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
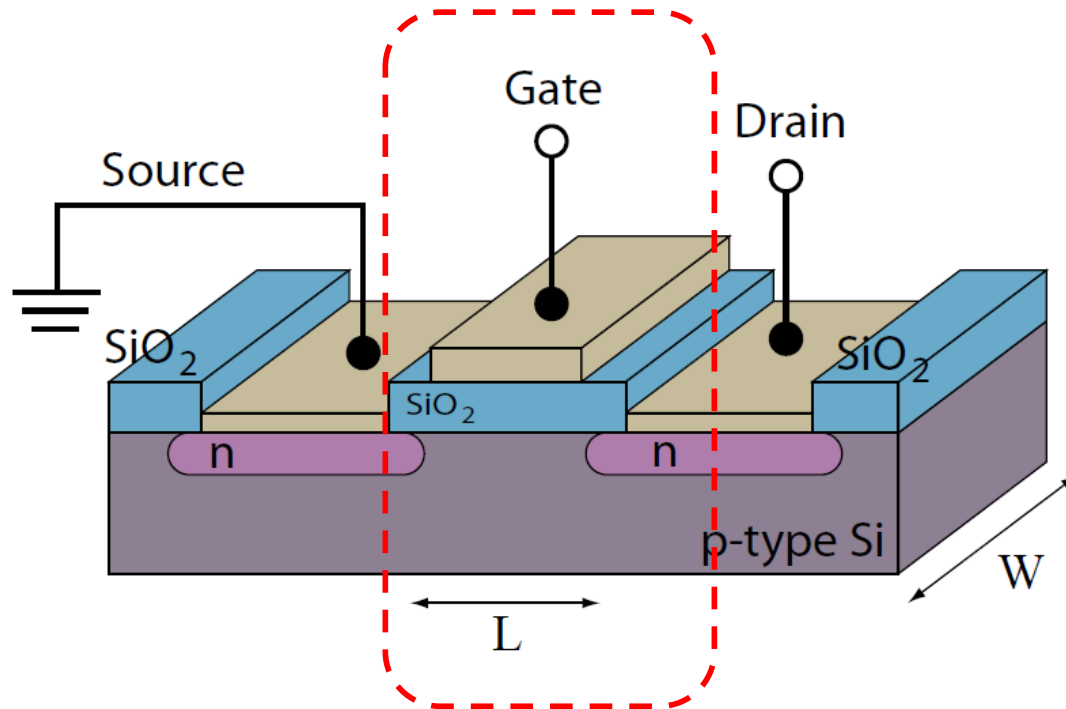
The FET acts as sensor by detecting **changes in gate voltage**
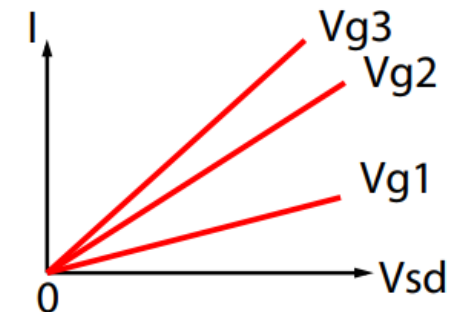
Molecular binding event ⟶ **Measurable changes in FET current**

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)
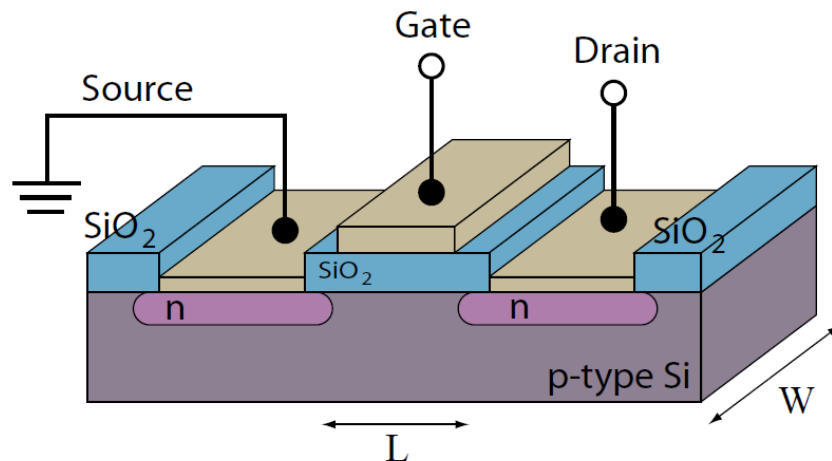
MOSFET: n-type
(npn layers)
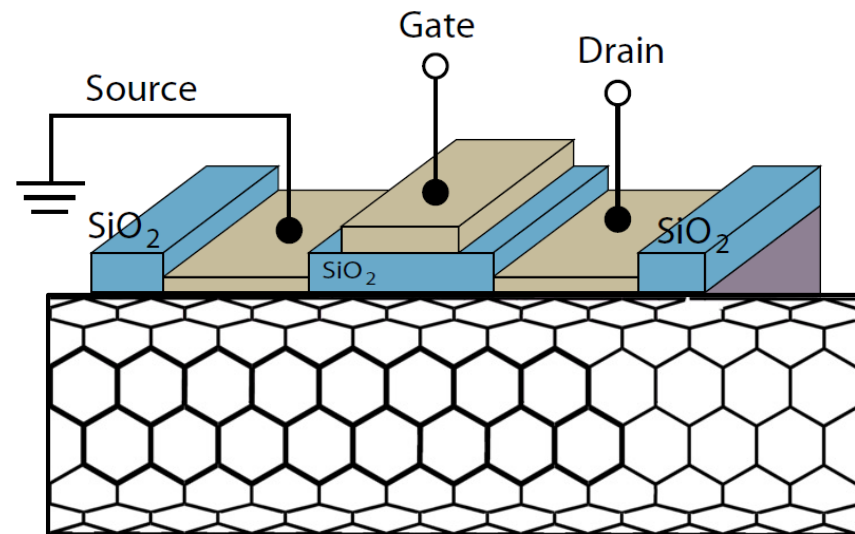
CNTFET: p-type
(one p layer)

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
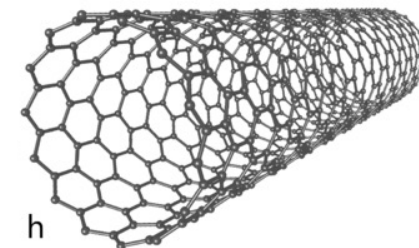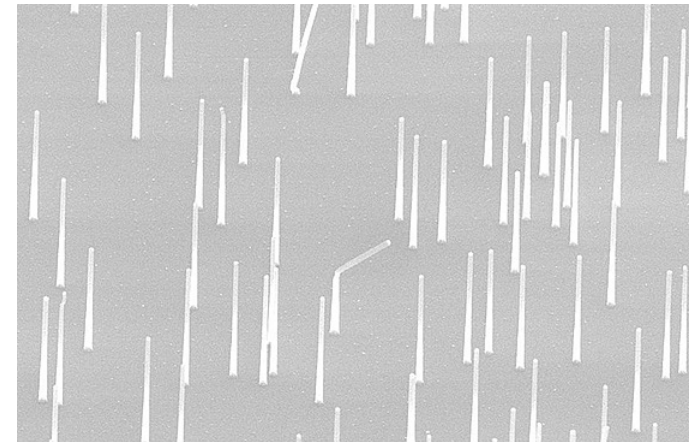
Cui et al. Nano Letters 2003

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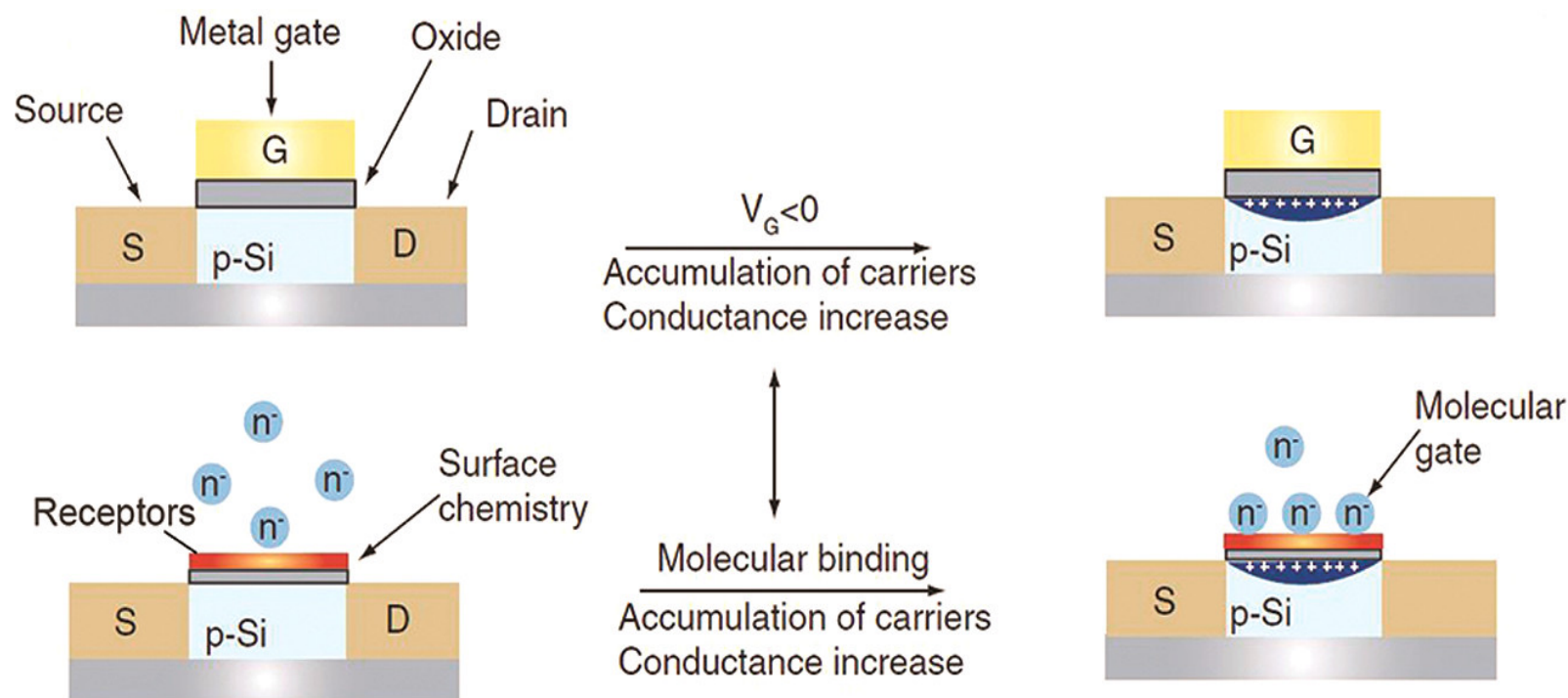
Schematic comparison of (top) a standard FET device and (bottom) a silicon nanowire (SiNW) FET sensor. The NW surface is functionalized with a receptor layer to recognize target biomolecules in a solution, which are charged and provide a molecular gating effect on SiNWs.

Gating a nanowire sensor

i: maximum conductance
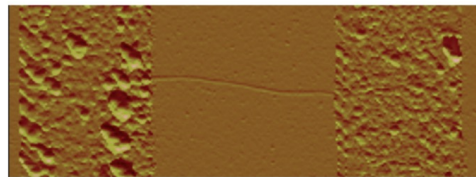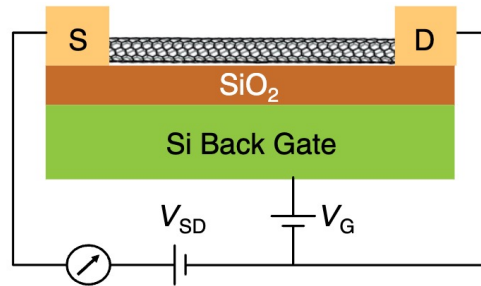
4.5 μm
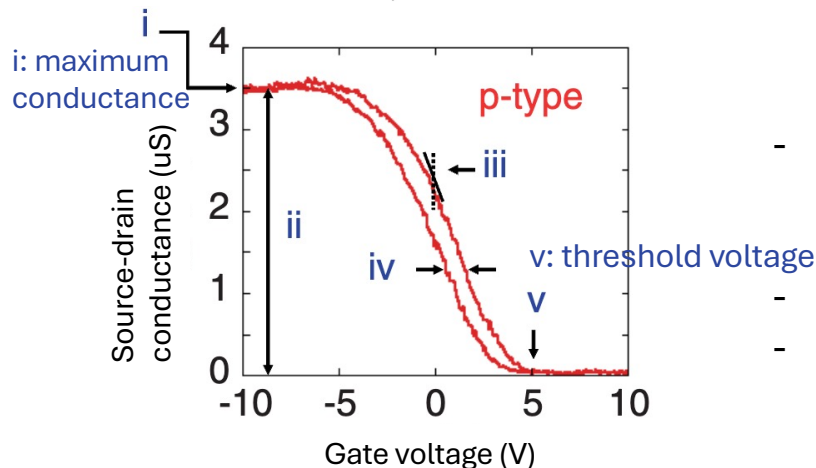
Architecture:
- Semiconducting p-type, single-walled nanotube (SWNT, black) between source (S) and drain (D) electrodes
- Gate electrode (green)

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**

Sensing using Si nanowires
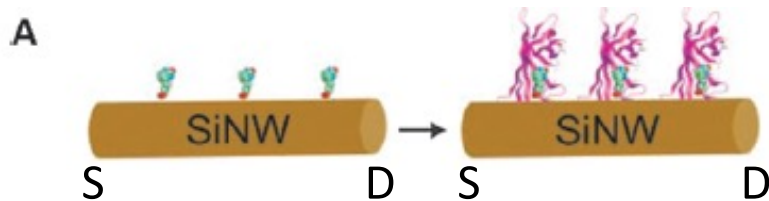
S: Source
D: Drain

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

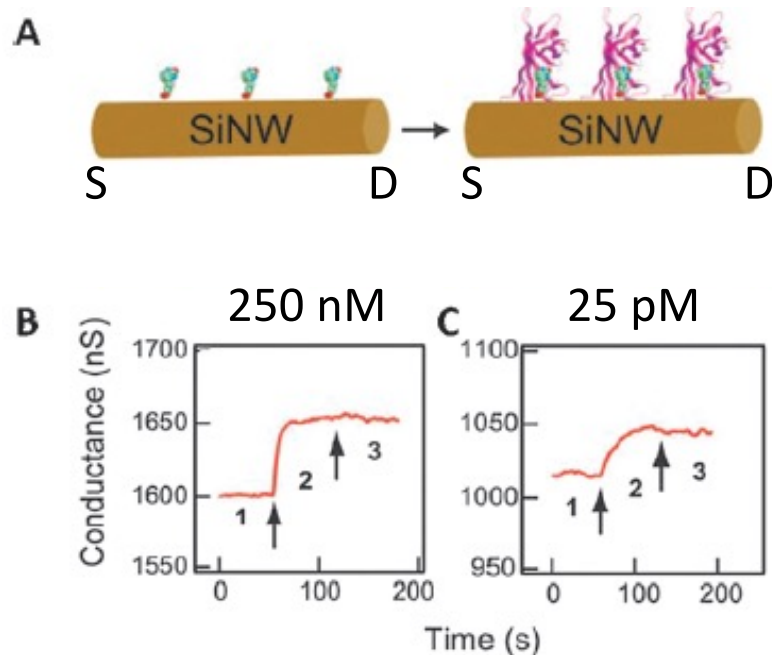measured in Siemens (S)

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

$G = 1/R$, R: resistance

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

Sensing using Si nanowires

A

S   D   S   D

B   250 nM   **C**   25 pM

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

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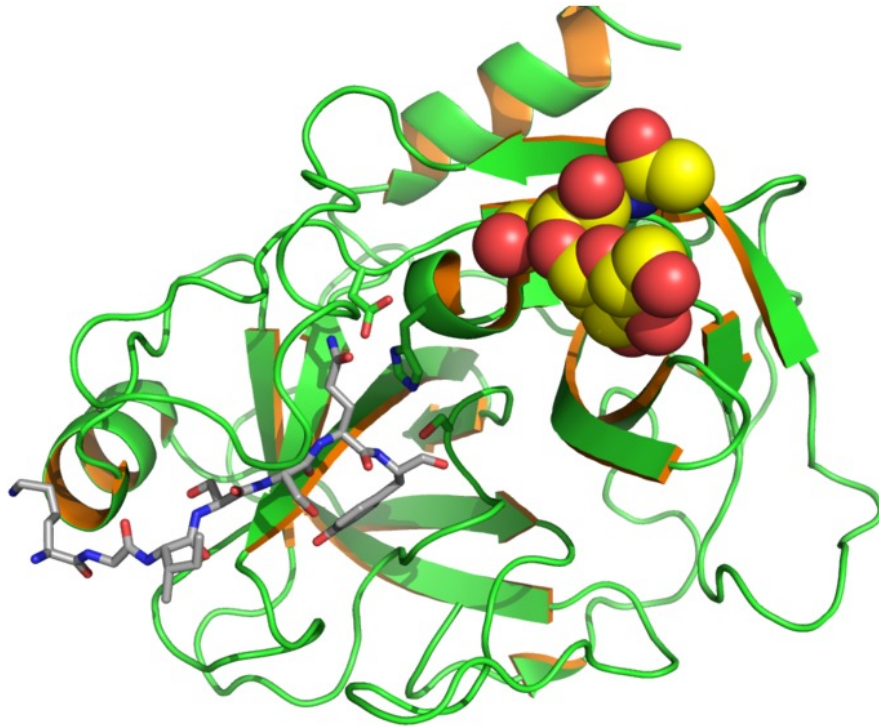
- Kallikrein-3 (KLK3)
- Also known as **prostate-specific antigen** (**PSA**)
- Glycoprotein enzyme

- 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
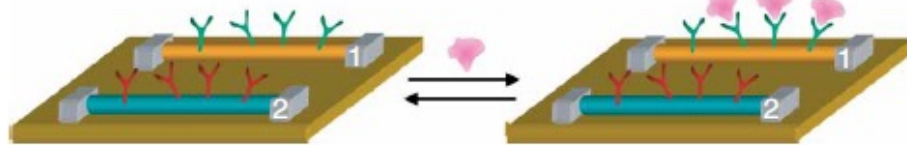
Protein: PSA

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)
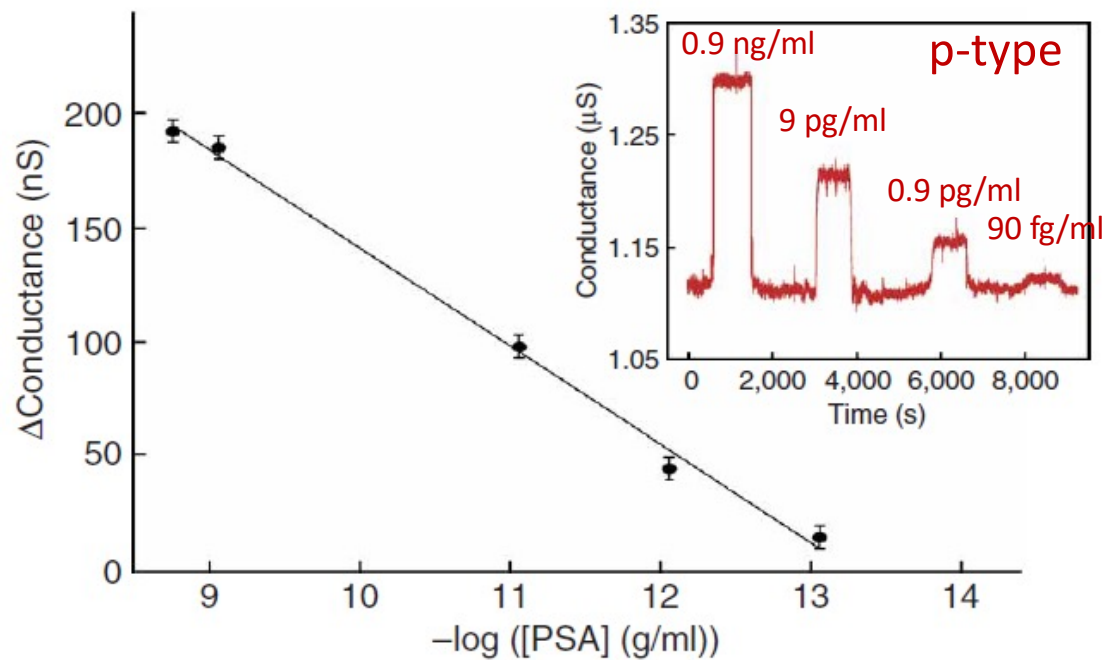
Zheng et al., Nat. Biotechnol. 2005

Antibody-mediated specificity
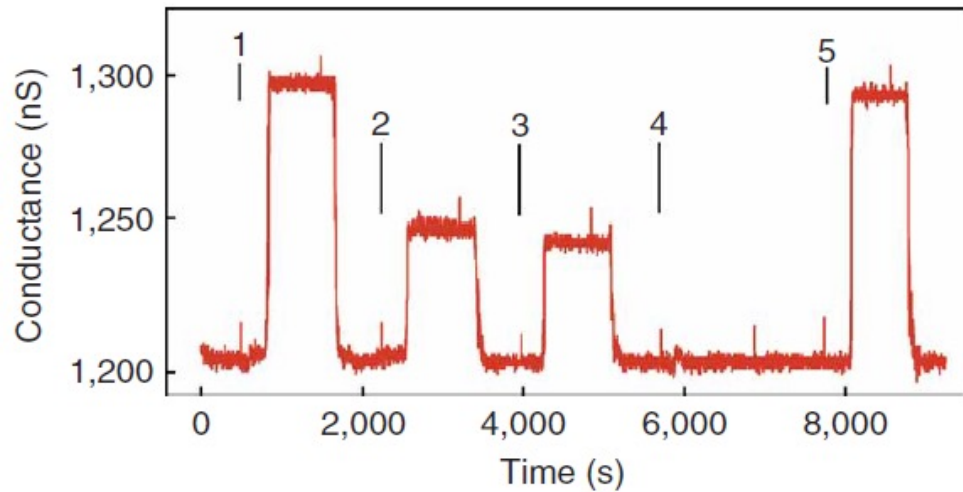
Specificity depends on antibody

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})
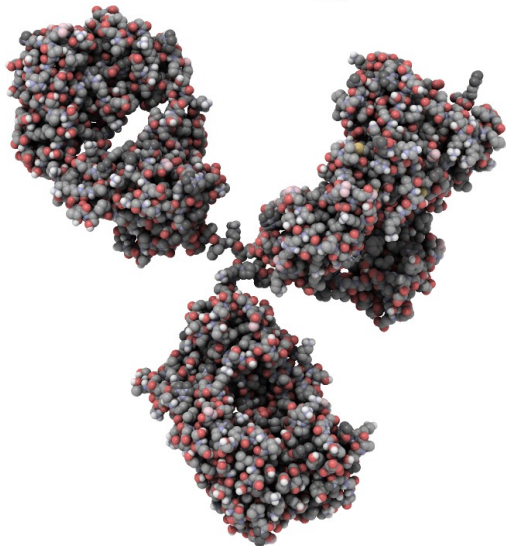
Zheng et al., Nat. Biotechnol. 2005

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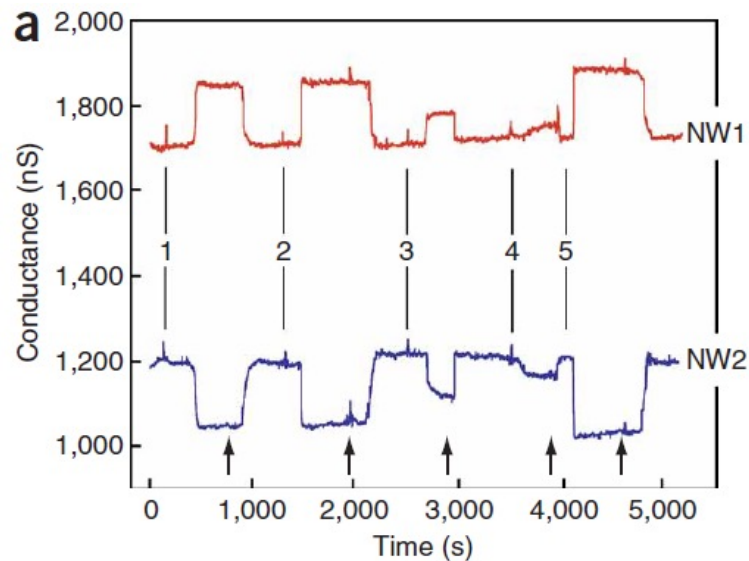
p-type silicon nanowire sensors (NW1, electron holes)
n-type silicon nanowire sensors (NW2, extra electrons)

PSA: pI = 6.8 -> negatively charged under neutral conditions (pH 7.4)
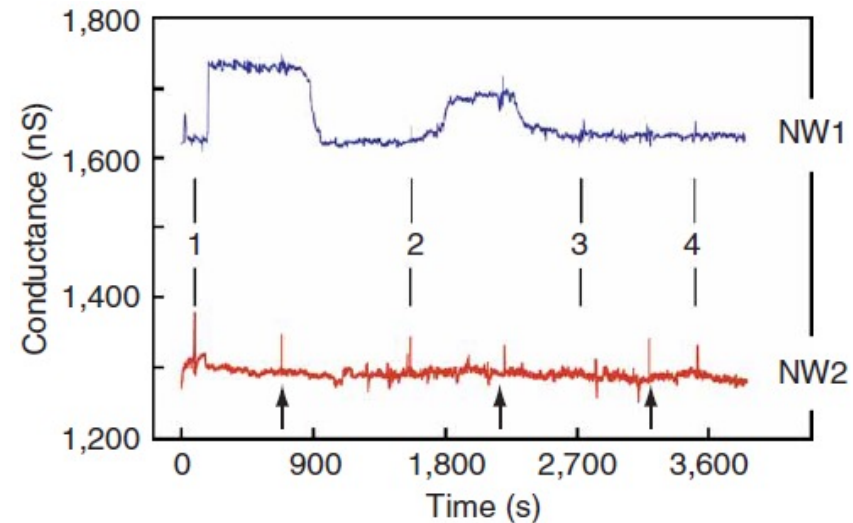
NW1: modified with AB
NW2: ethanolamine blocked

→ internal standard

Highest sensitivity: 0.085 pg/mL for PSA

Zheng et al., Nat. Biotechnol. 2005

Multiplexed cancer marker detection
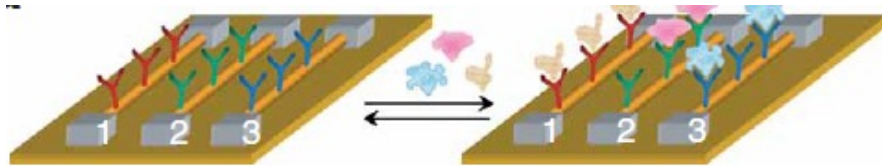
Using differently modified NWs, several cancer markers can be simultaneously detected
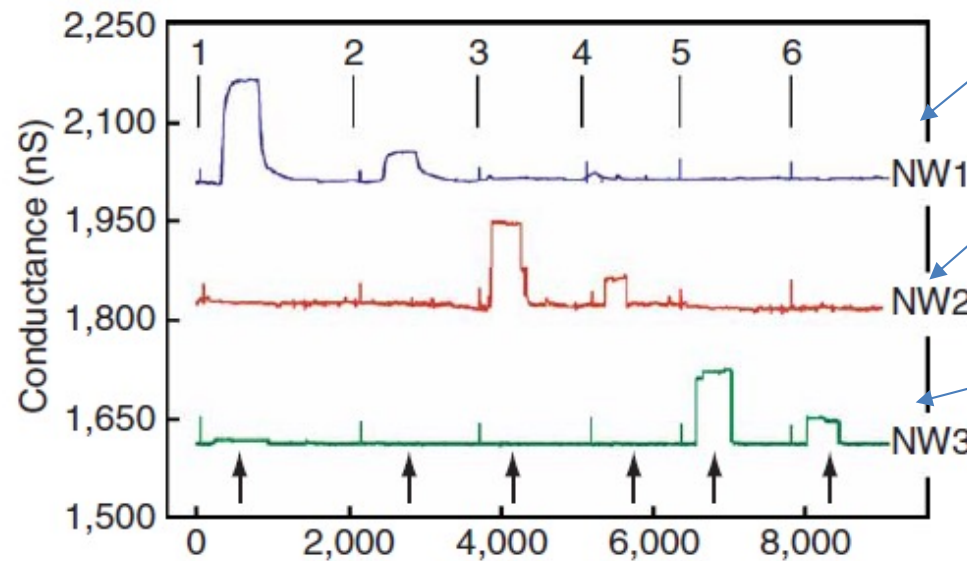
PSA (high pg/mL sensitivity)

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!

(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

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*
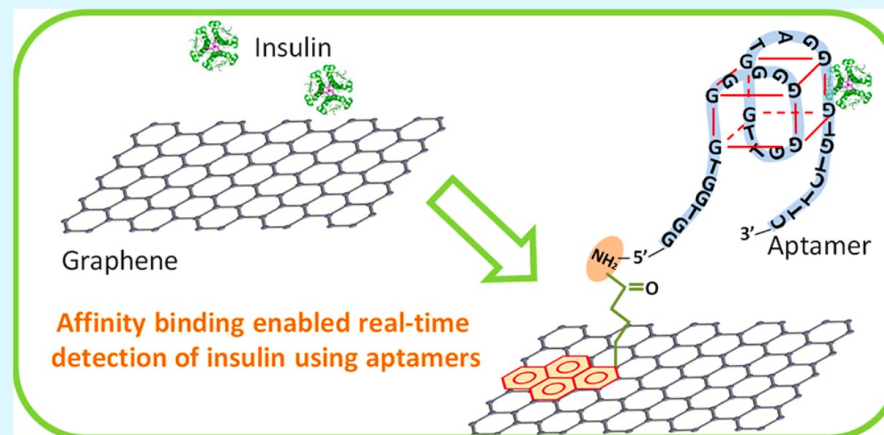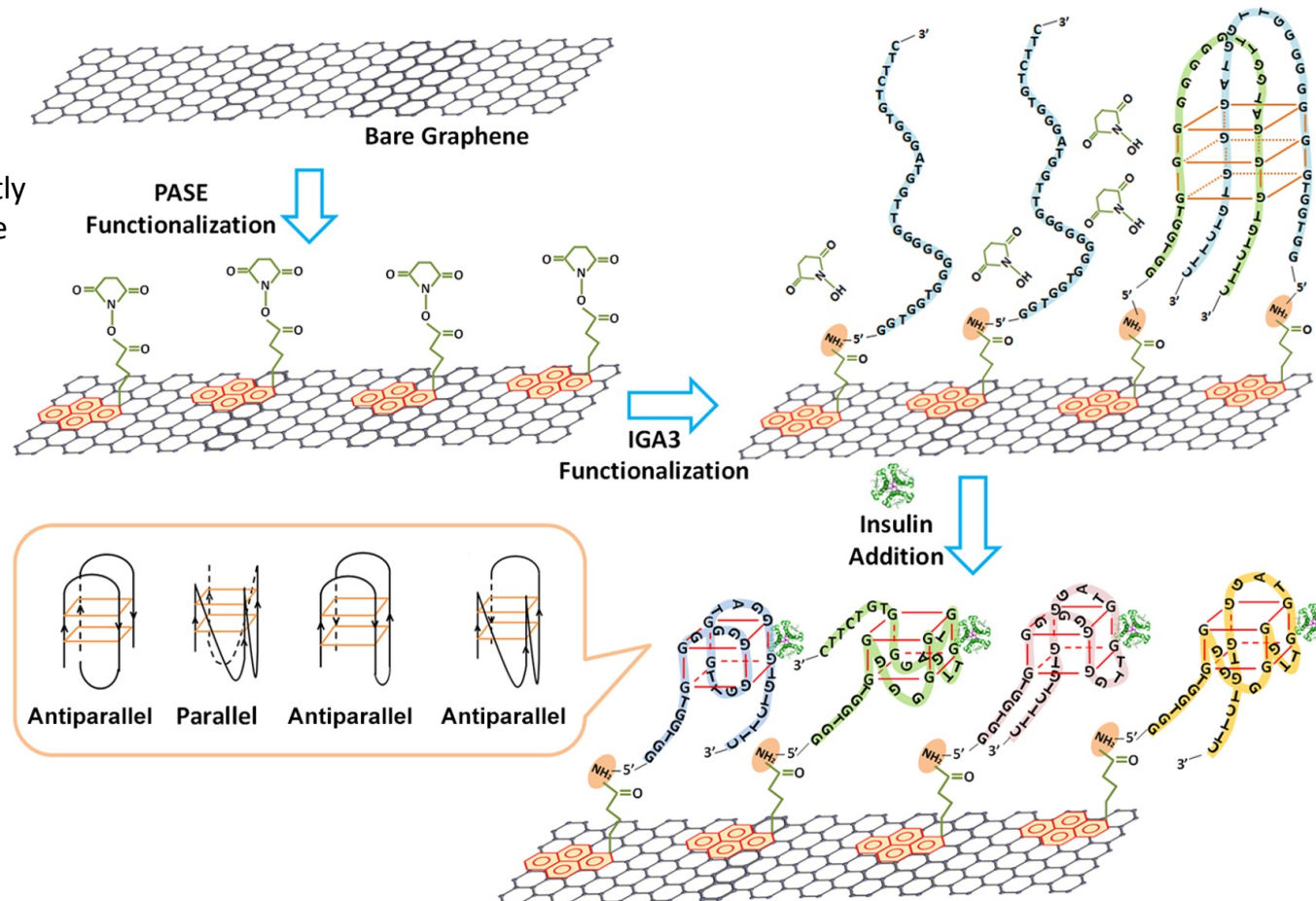
2. Describe the principle of the assay.

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
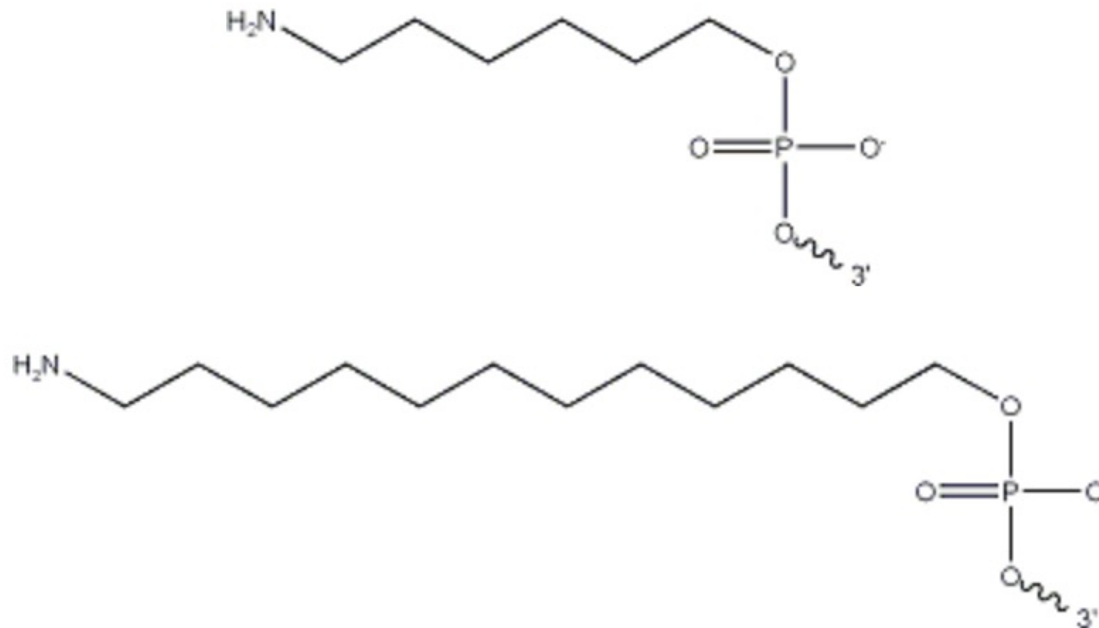closer to graphene surface → negative charge attracts p-type carriers in semiconductor.

Amino modification on aptamer

How did they introduce the amino group on the aptamer?

They ordered 5'-NH$_2$-GGT GGT GGG GGG GGT TGG TAG GGT GTC TTC-3' from IDT.
IDT sells these two types of 5' modifications:

https://eu.idtdna.com/site/Catalog/Modifications/Category/2

IGA3 aptamer origin and structure

Where does the aptamer come from?

It's an insulin-binding aptamer that was previously identified by Yoshida et al. Reference: Biosens. Bioelectron. 2009, 24, 1116–1120.

https://doi.org/10.1016/j.bios.2008.06.016

How are the structural transitions pre- and post-insulin binding affecting the conductance?

"Note that in the absence of insulin, the guanine-rich IGA3 was mostly unfolded in the solution while **G-quartet structure may have formed** as a result of Hoogsteen base pairing.[14] Upon capture of insulin, the existing G-quartet structures can switch to stable, compact parallel or antiparallel G-quadruplex structures.[4,39] These conformational changes brought the electron-rich aromatic nucleotide strands and insulin to the close proximity of the graphene surface,[29] resulting in the direct binding of nucleotide or aromatic amino acids in insulin with the graphene surface and making electrons from either insulin or the deformed IGA3 aptamer more likely to be directly transferred into graphene through the π–π stacking interactions between them[36,40–42] and generating n-type doping to graphene."

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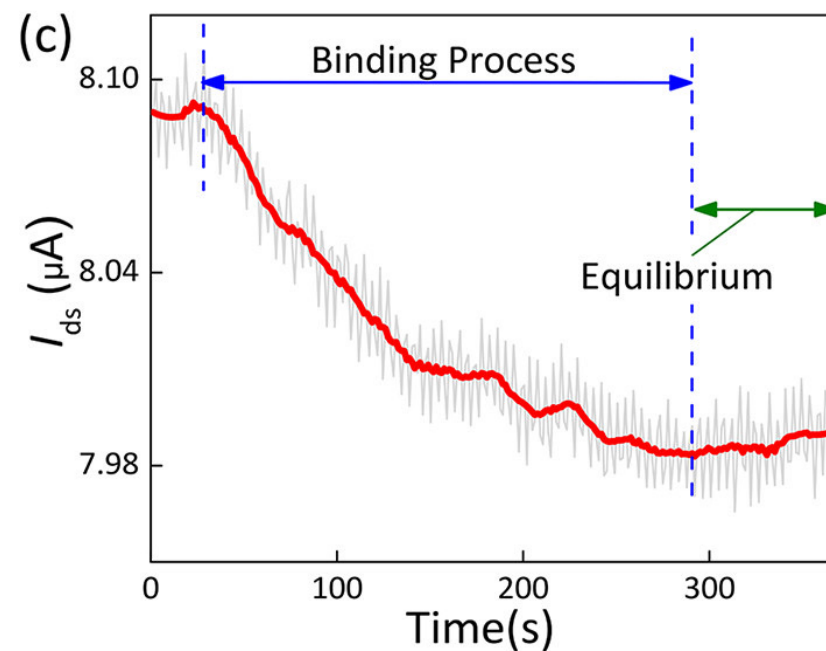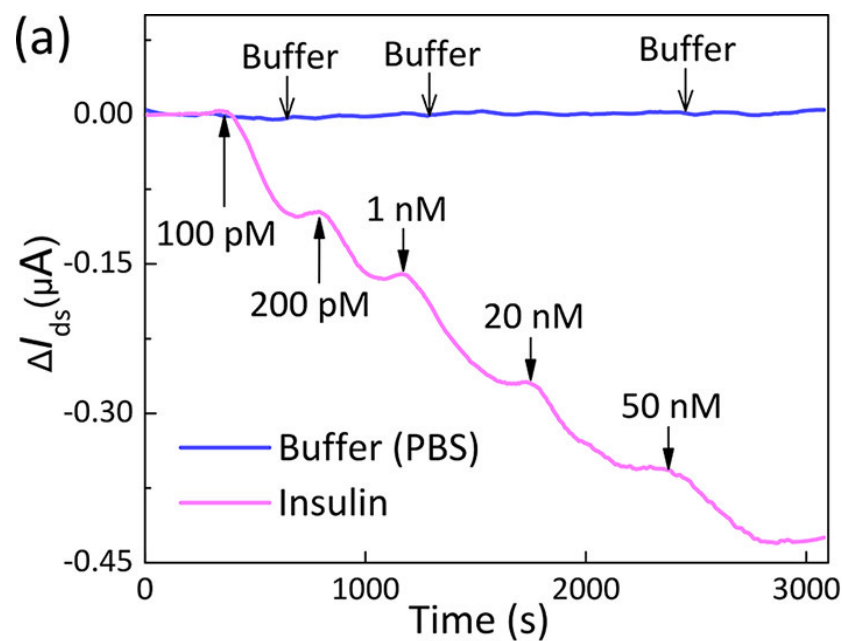
ACS Appl. Mater. Interfaces 2017, 9, 33, 27504-27511
https://pubs.acs.org/doi/10.1021/acsami.7b07684

Graphene FET architecture

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.*
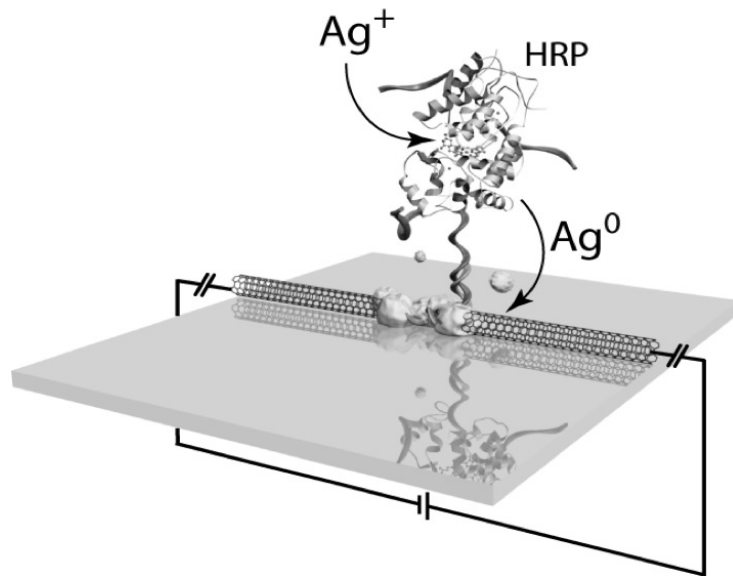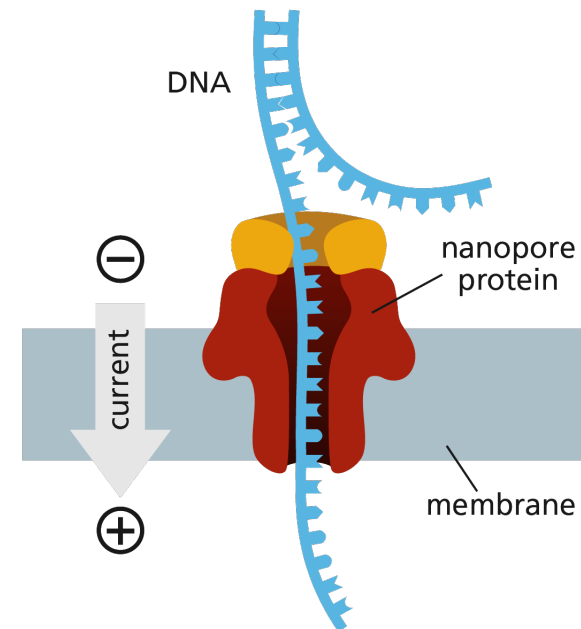

Image: https://swagergroup.mit.edu/research/molecular-and-nanowire-based-sensors

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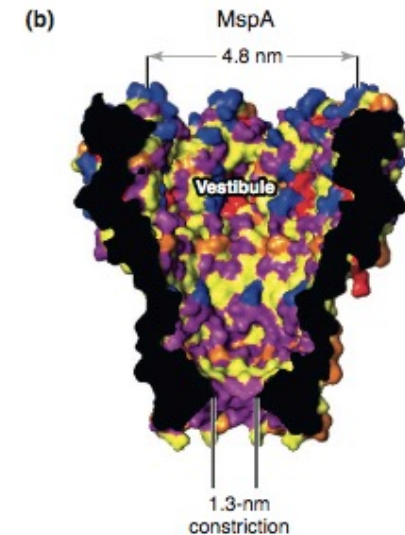
Nanopores and DNA

Origin of the pore

- Synthetic
- Bio-mimetic
- Hybrids

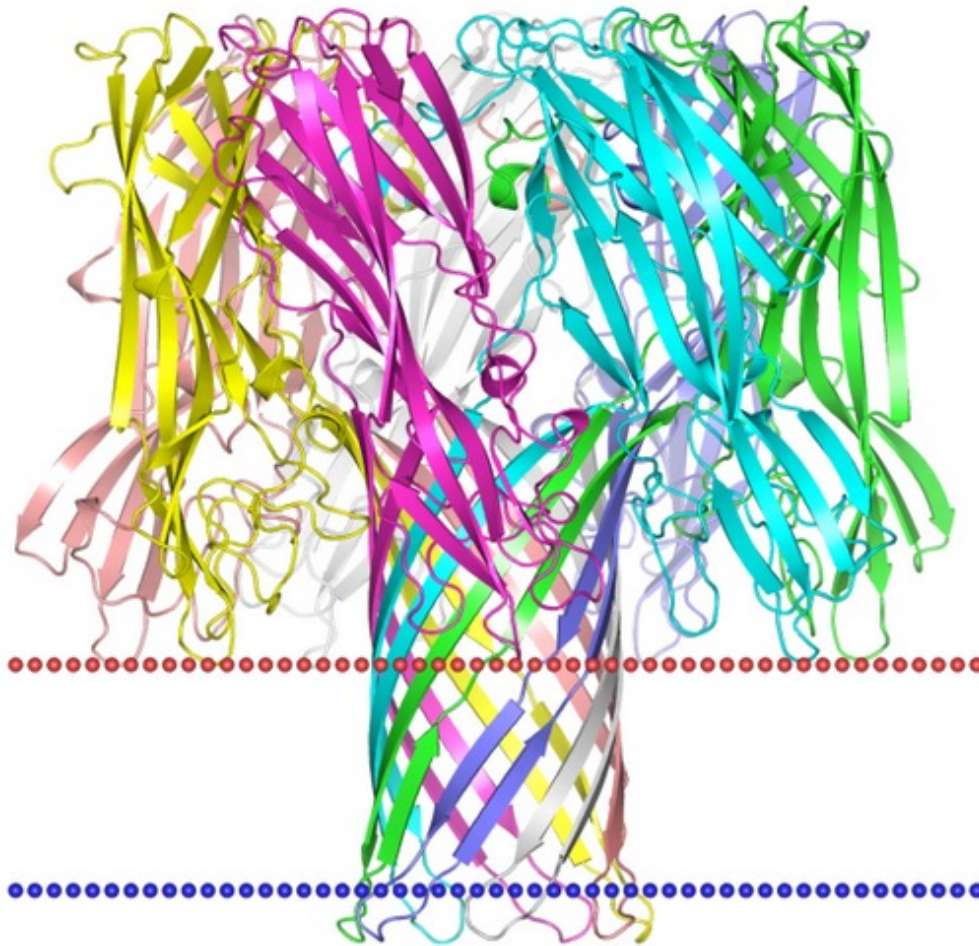Synthetic nanopore: A single nanopore is drilled using a highly focused electron beam (yellow)
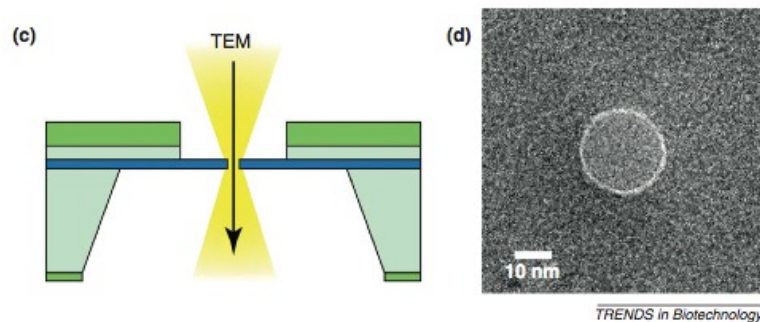
Biological nanopores:
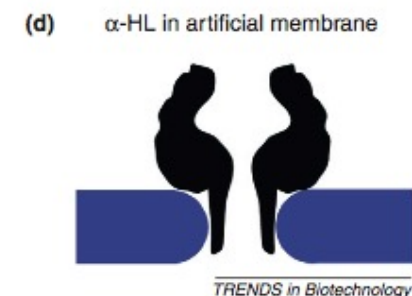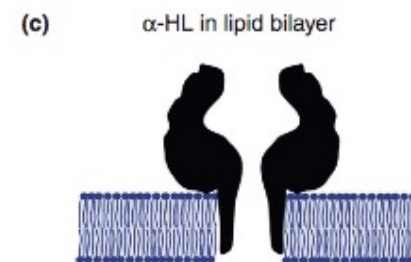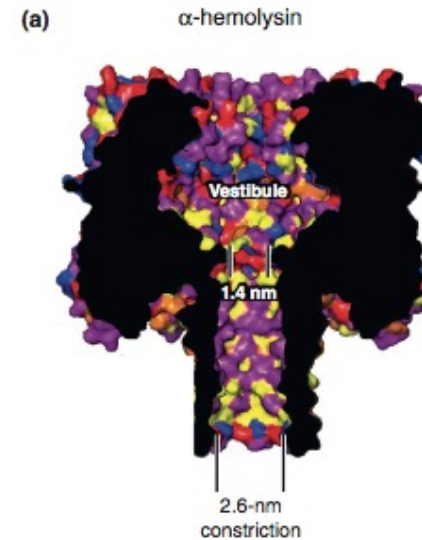
Kowalczyk, S. W., Blosser, T. R., & Dekker, C. (2011). *Trends in Biotechnology, 29(12), 607–614.*
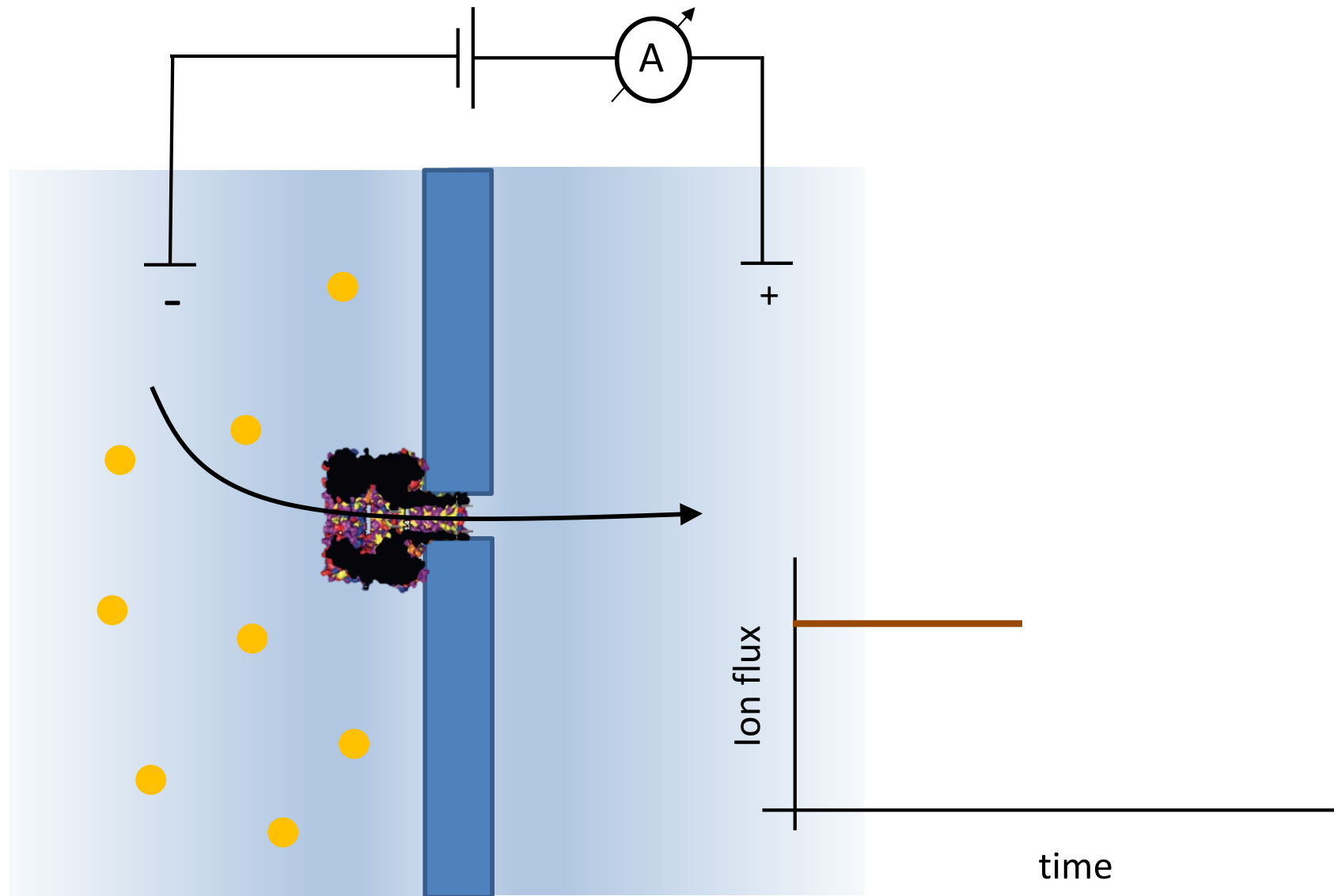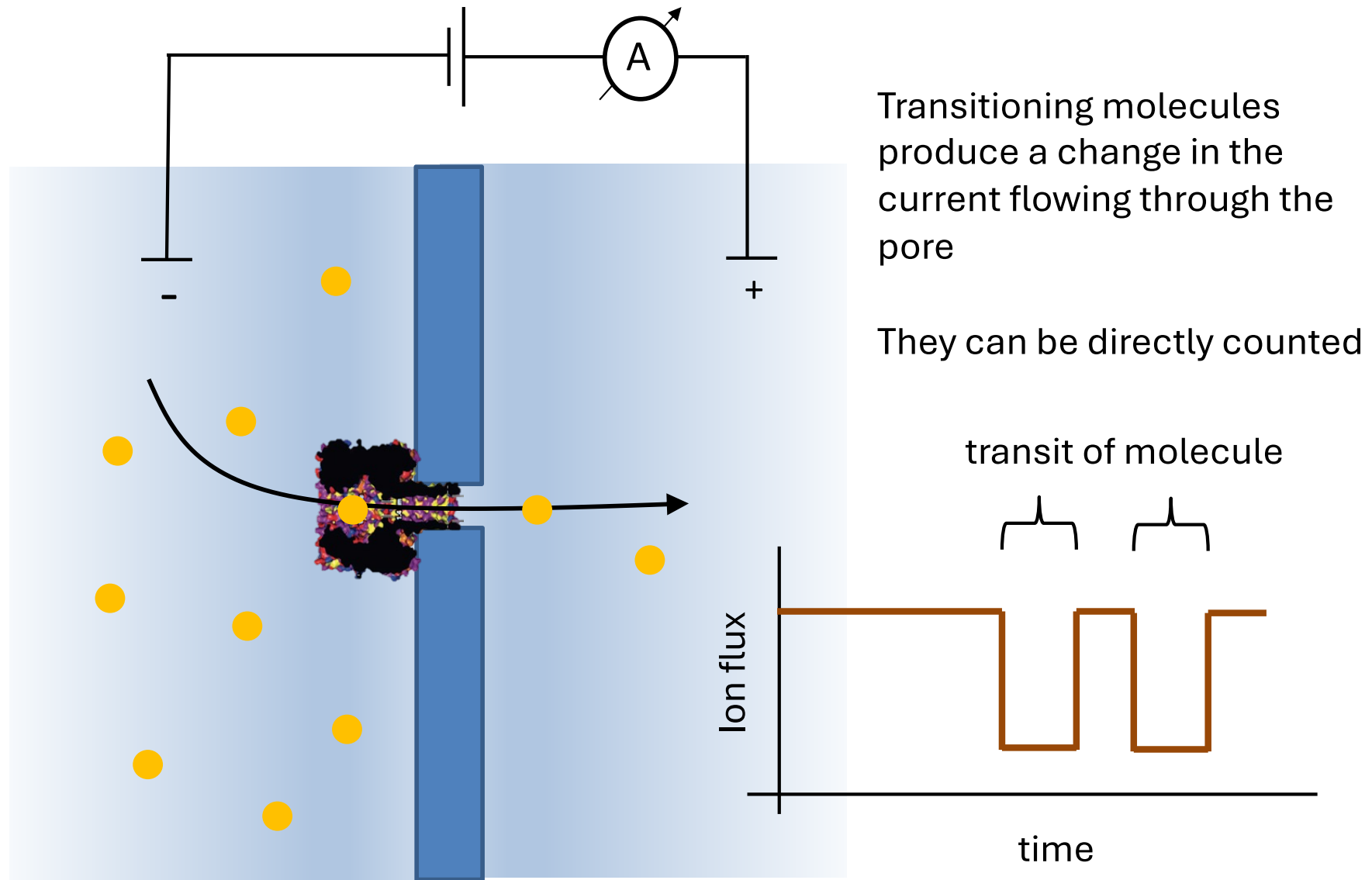
α-hemolysin

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
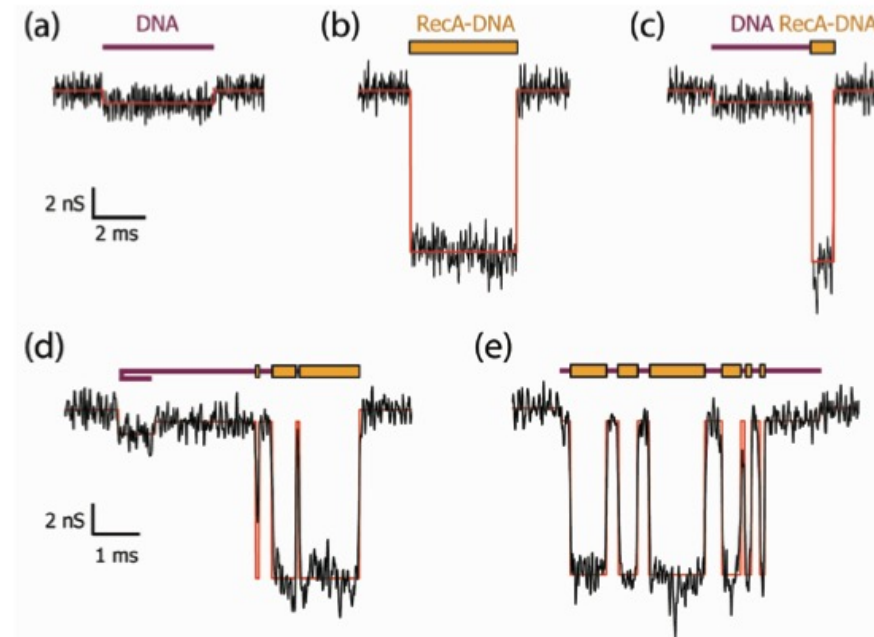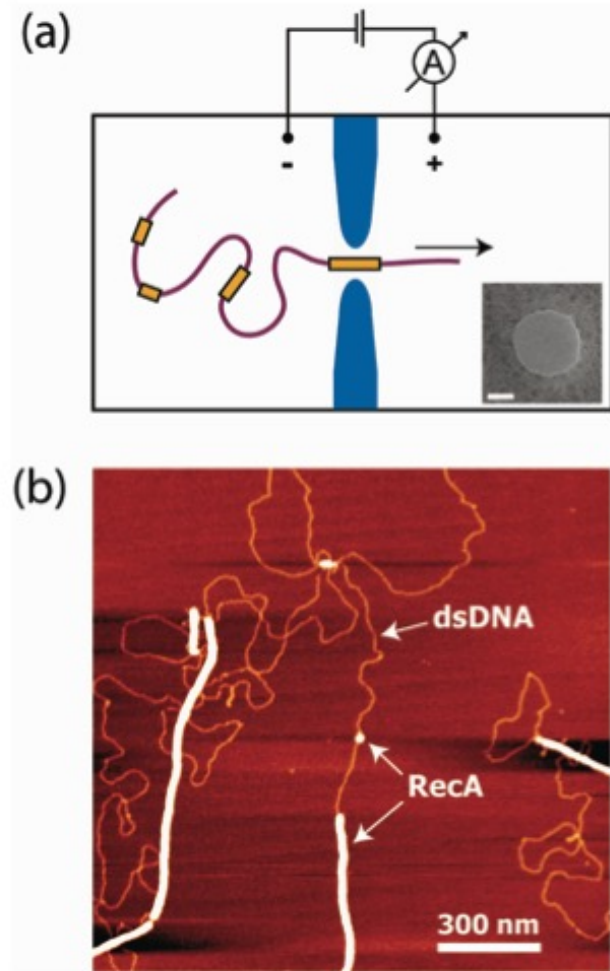
Nanopores: measurements

Nanopores: Measurements

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

They can be directly counted

transit of molecule

Ion flux

time

Nanopore – DNA/protein interactions

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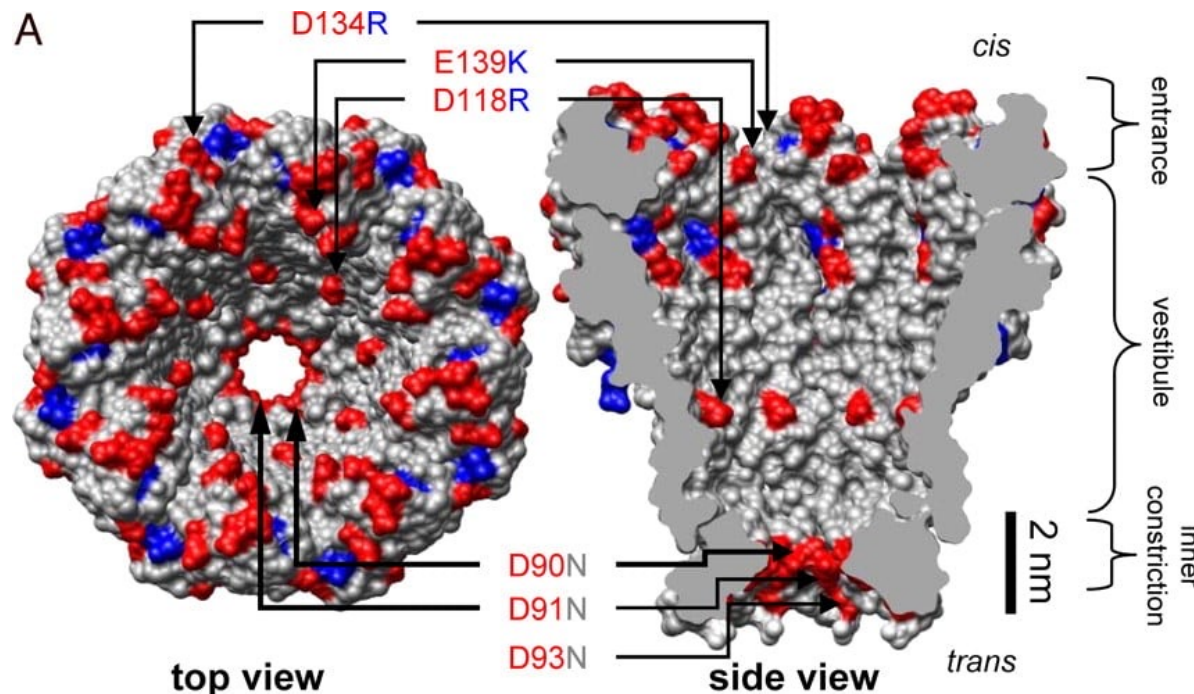
Nanopore – sequencing?

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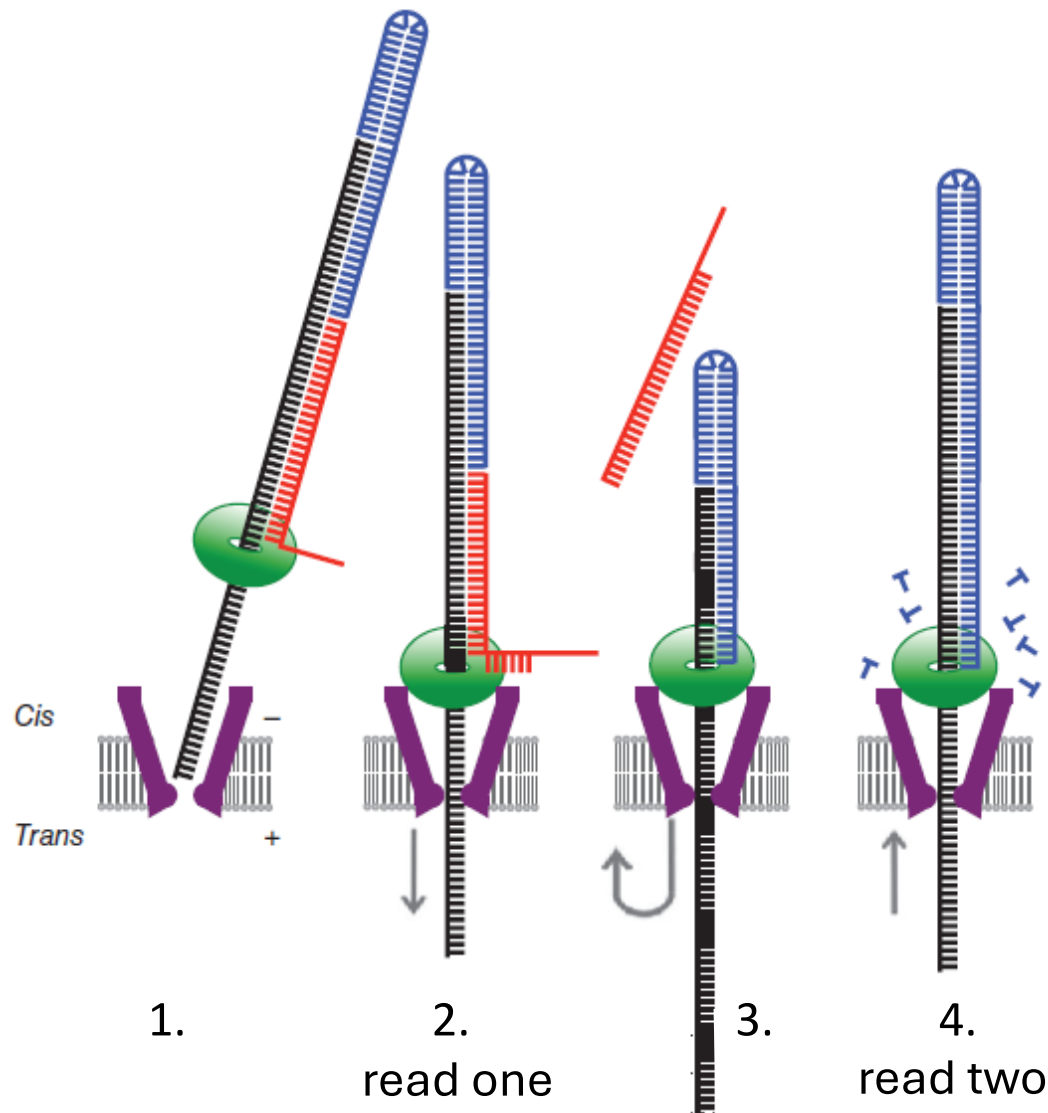
To control transition of DNA through pore: **Use of phi29 DNA polymerase as a molecular motor**

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**)
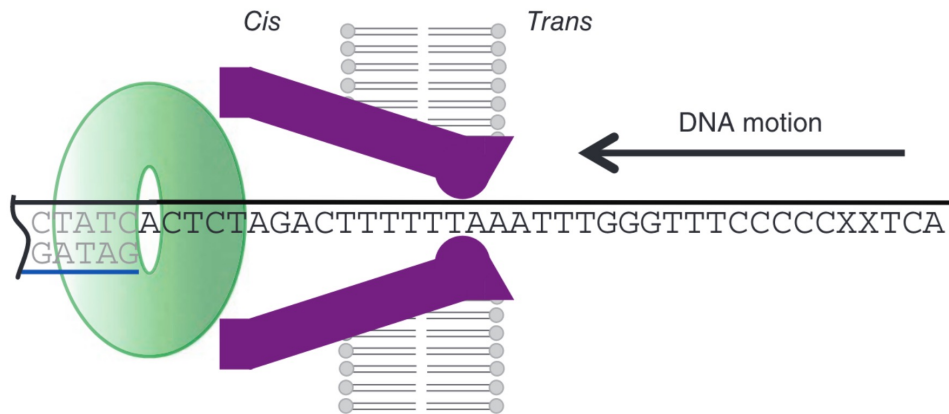
Experimental data
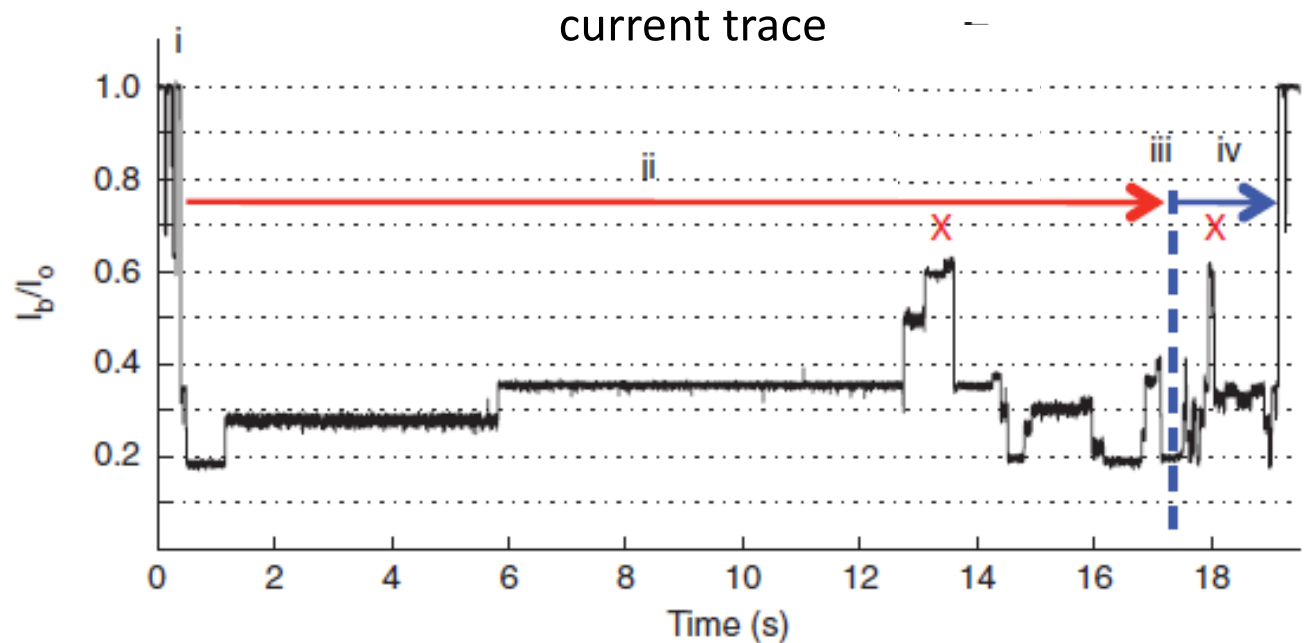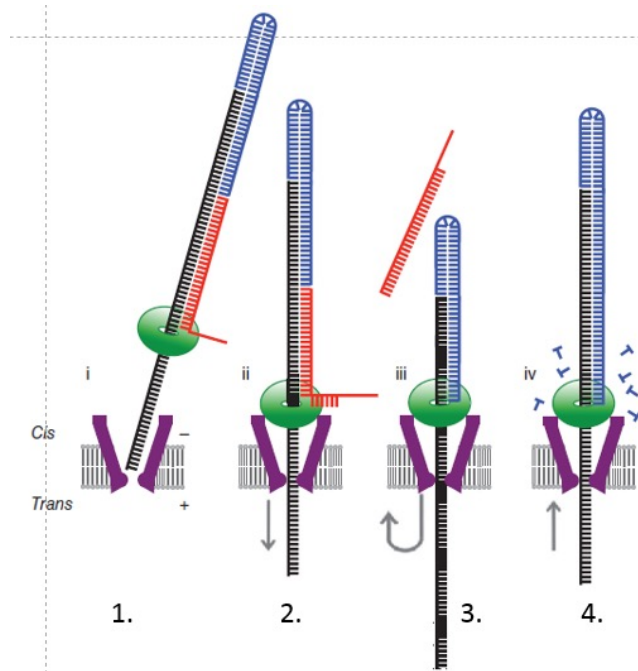
current trace

Current trace:

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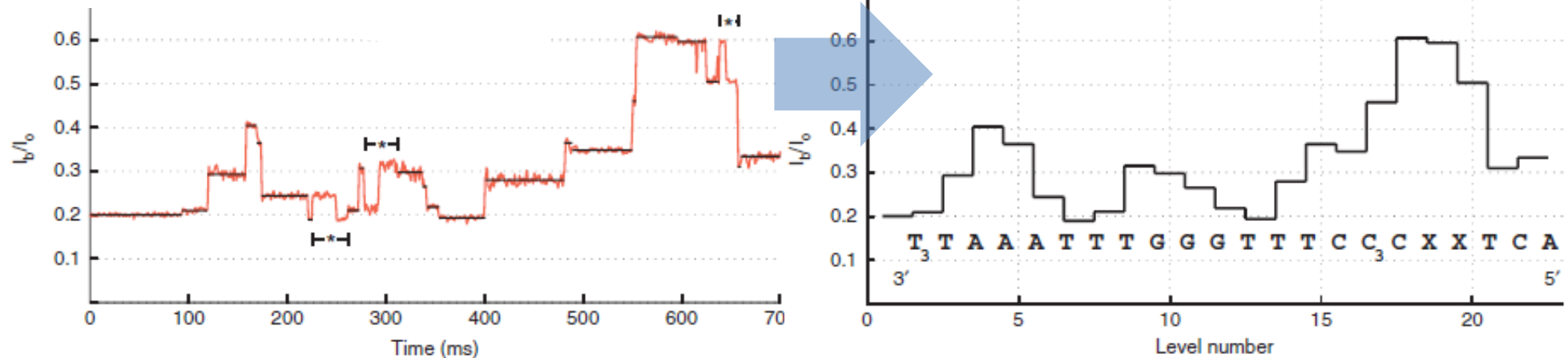- DNA synthesis by Phi29 RNAP (iv)
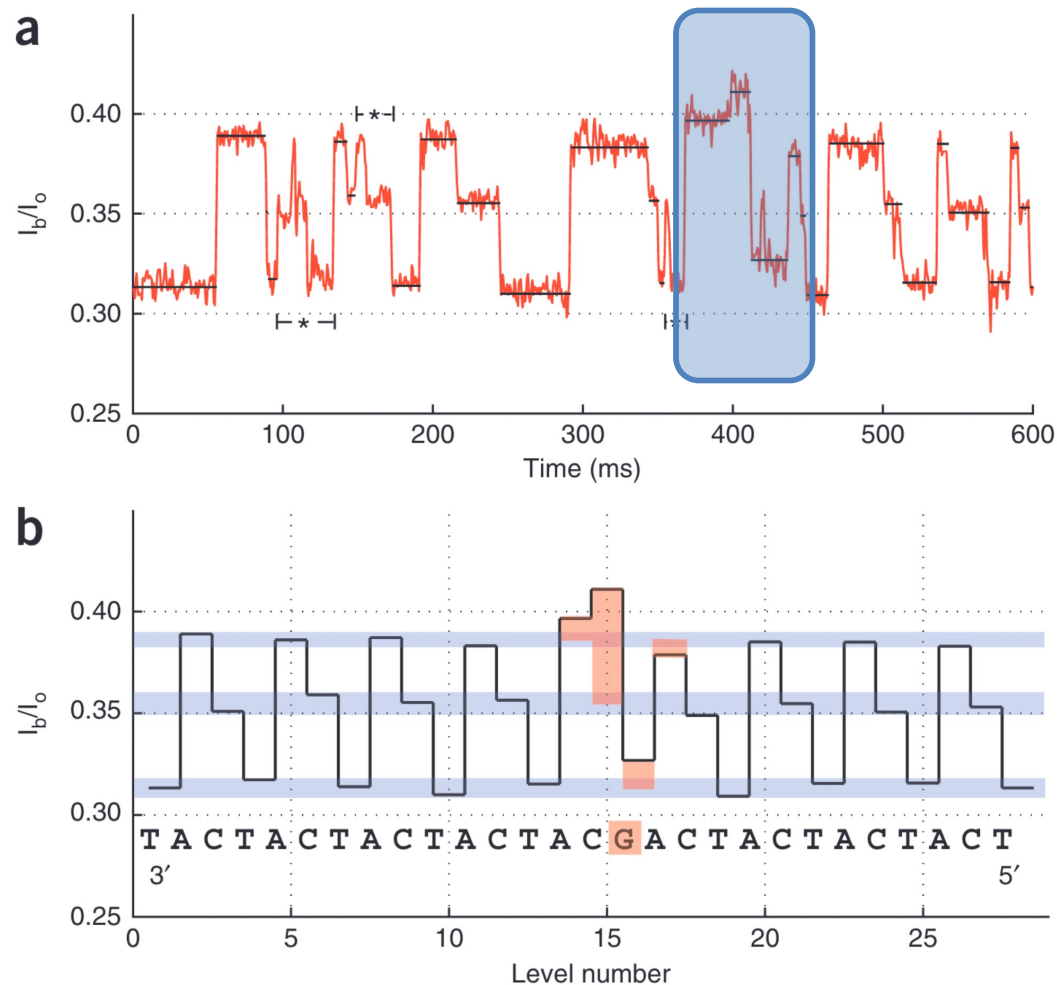- Release (current reverts to I_0)

Current trace for polymerase

Single-molecule readout of DNA sequence

Short sequences of backtracking observed

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
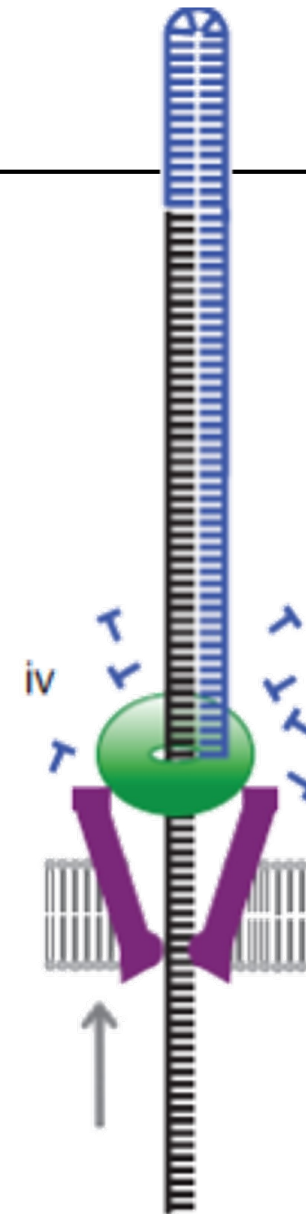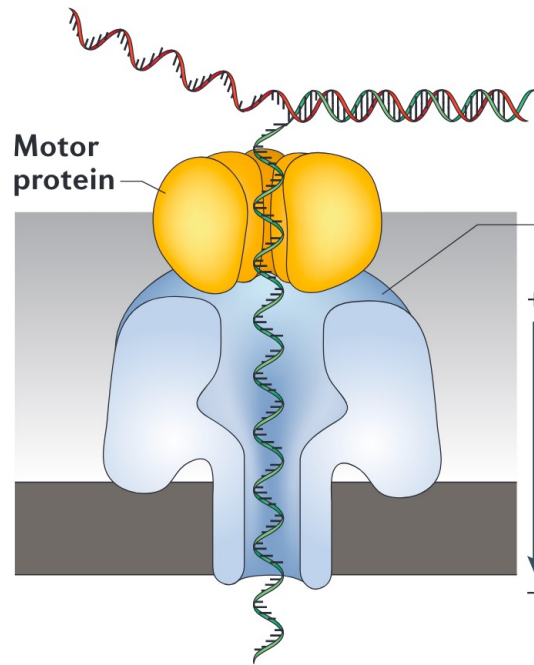
Oxford Nanopore Technologies (ONT)

Leader–Hairpin template
The leader sequence interacts with the pore and a motor protein to direct DNA, a hairpin allows for bidirectional sequencing
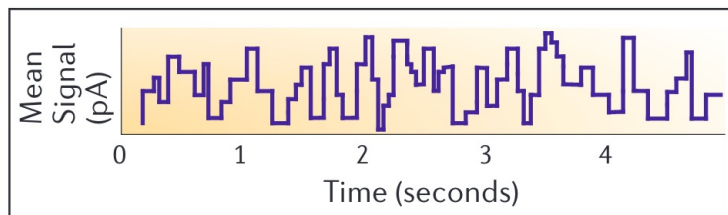
Motor protein

Alpha-hemolysin
A large biological pore capable of sensing DNA

Current
Passes through the pore and is modulated as DNA passes through

ONT output (squiggles)
Each current shift as DNA translocates through the pore corresponds to a particular k-mer

- 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)**

Challenges:

- Integration into instrument → Oxford nanopore

commercial instrument

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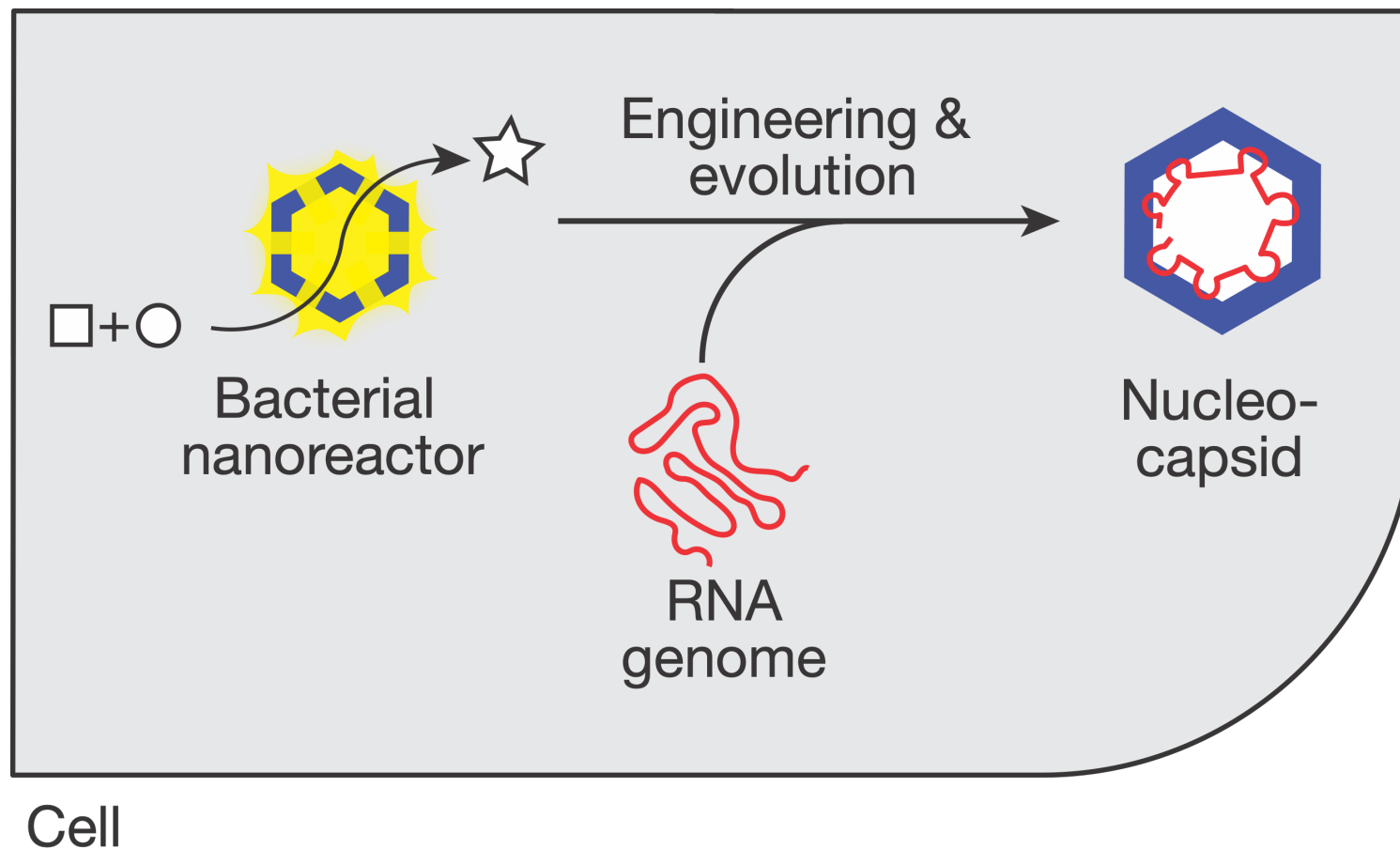
- https://www.youtube.com/watch?v=qzusVw4Dp8w&ab_channel=OxfordNanoporeTechnologies

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

An application of Nanopore sequencing from my own research
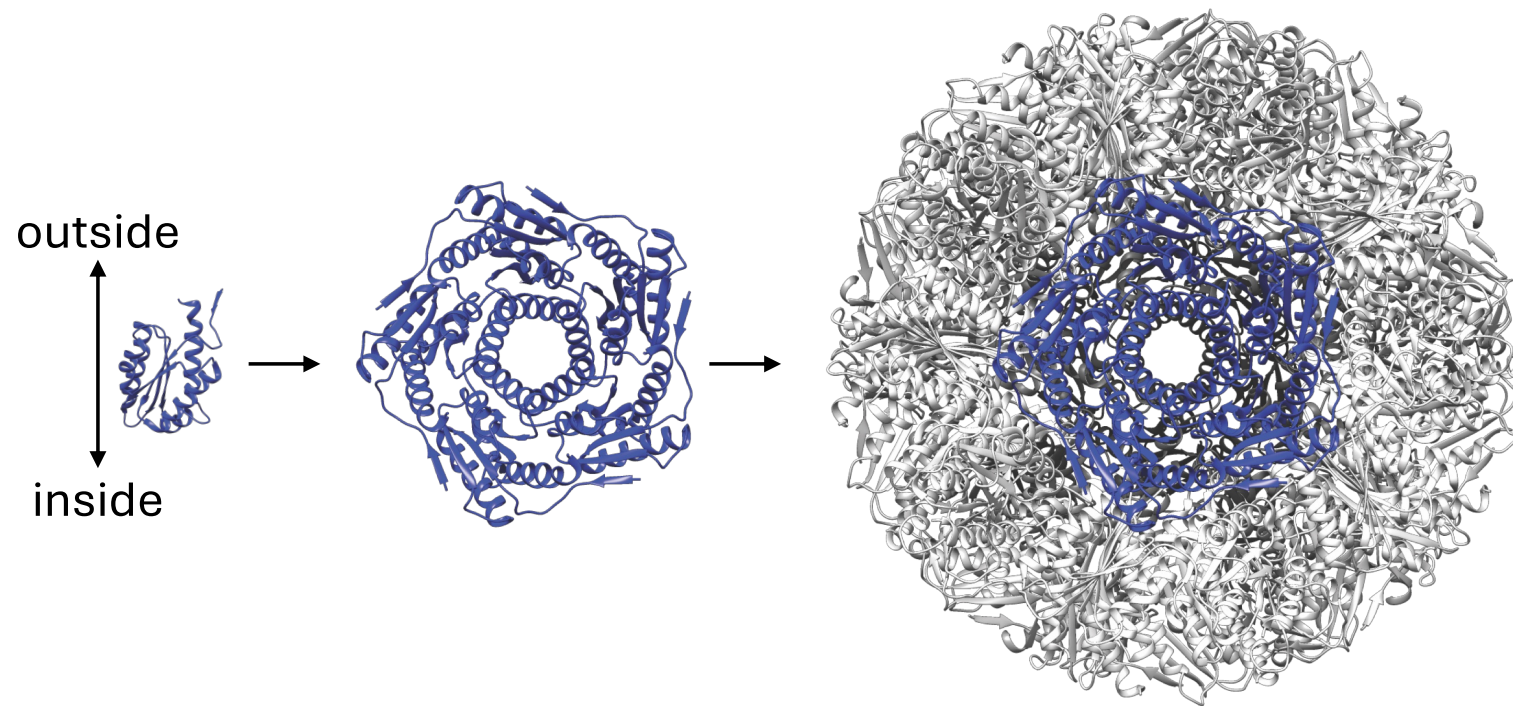
Engineering a bacterial protein to bind its own mRNA

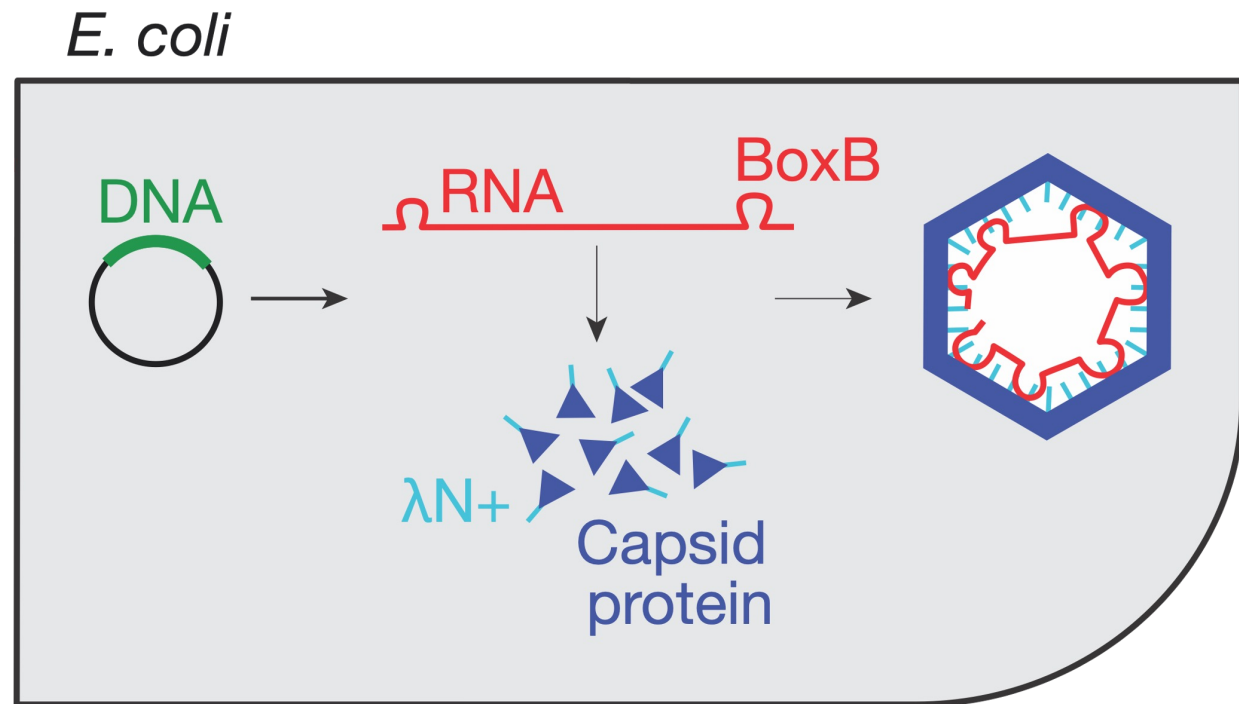# Lumazine synthase from *Aquifex aeolicus*

outside

inside

Capsids assemble in bacteria
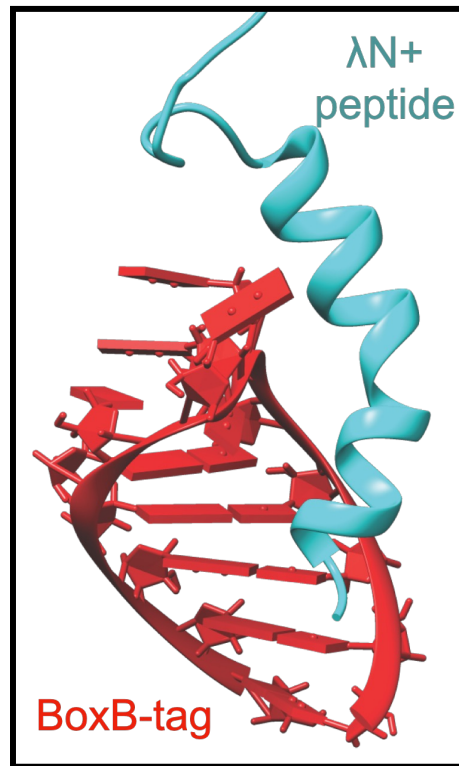
PDB: 1QFQ

Rational nucleocapsid design

λN+ peptide

BoxB-tag
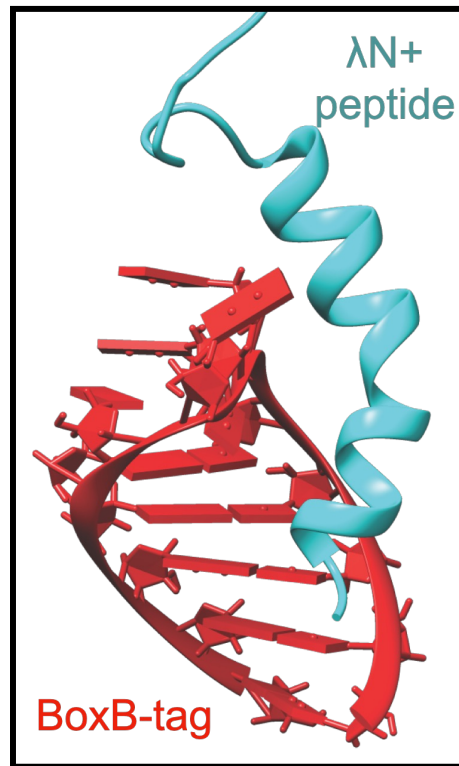
PDB: 1QFQ
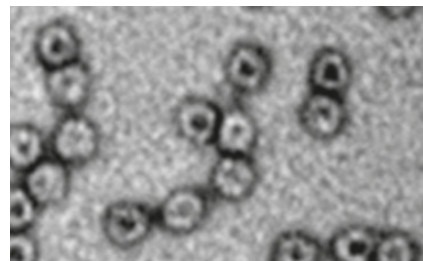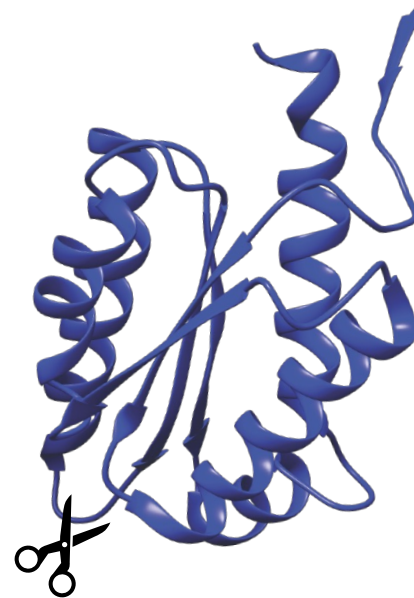
Circular permutation

wt-AaLS

NC-1

━ = 50 nm

Selecting for capsids that package their own encoding RNA
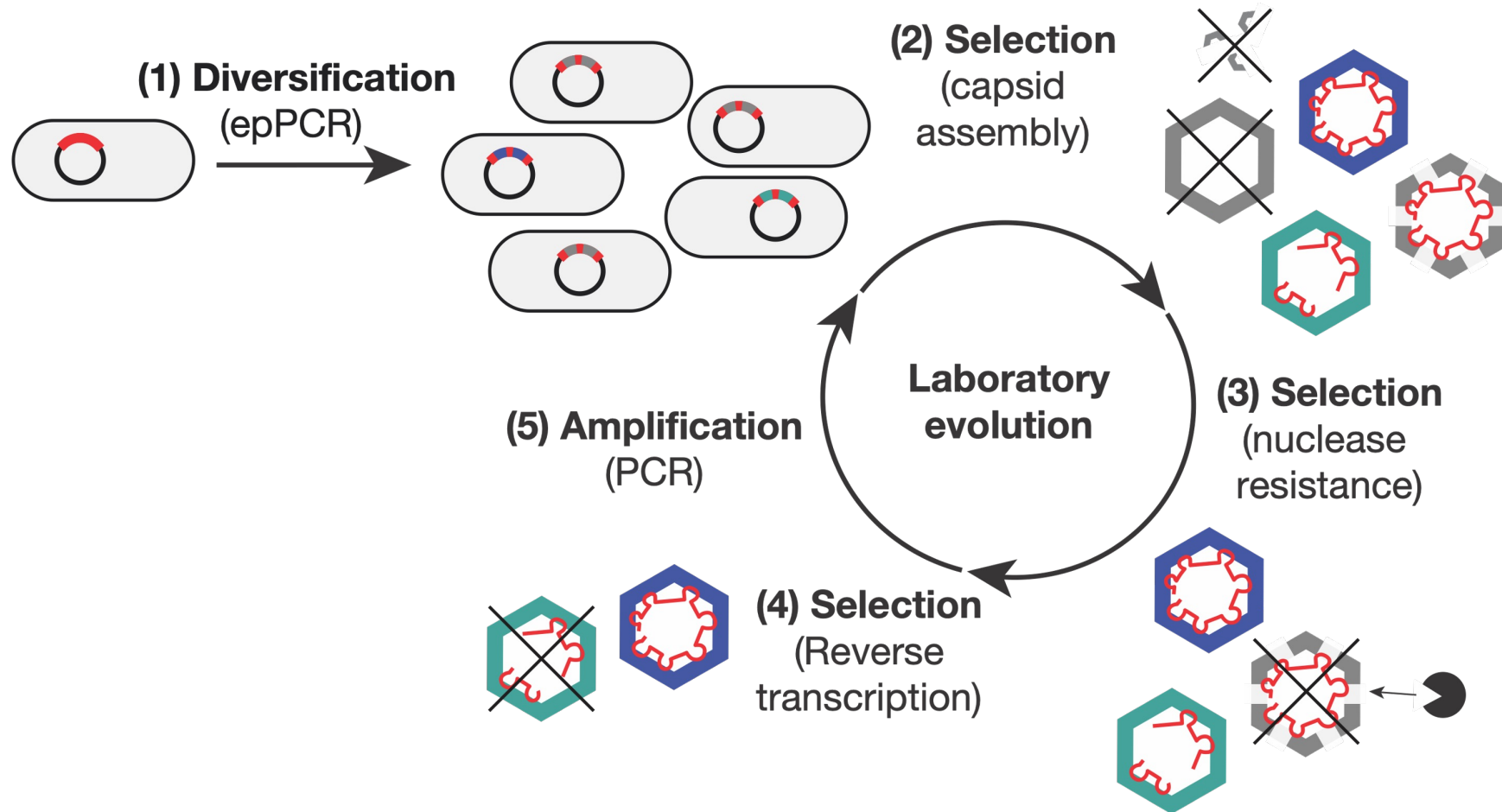
PNAS **2018**, *115*, 5432. *Science* **2021**, *372*, 1220.

Four nucleocapsid generations
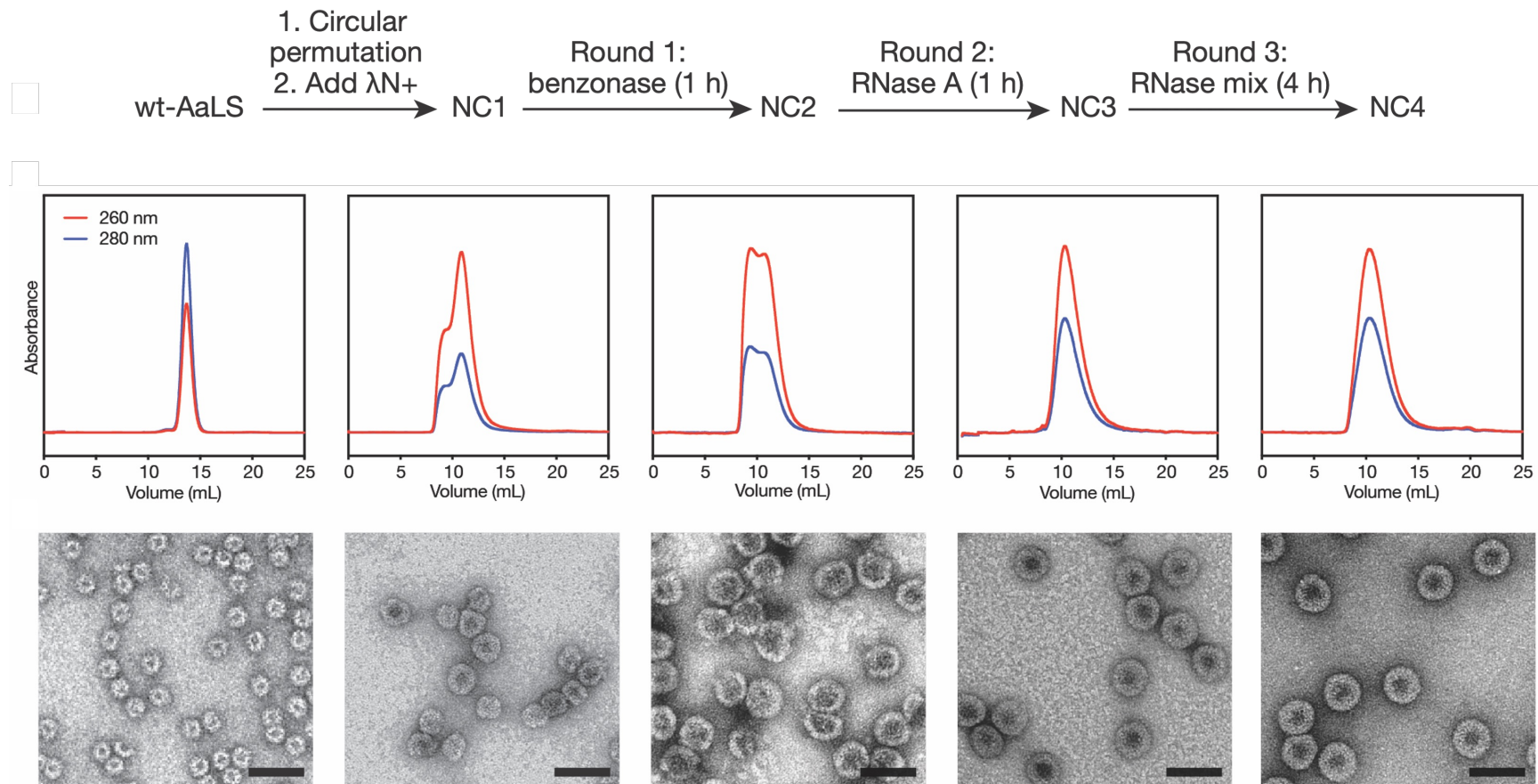
Science **2021**, *372*, 1220.

Evolution brings structural change

NC-1		NC-2	NC-3 & NC-4
120 subunits	180 subunits	180 subunits	240 subunits
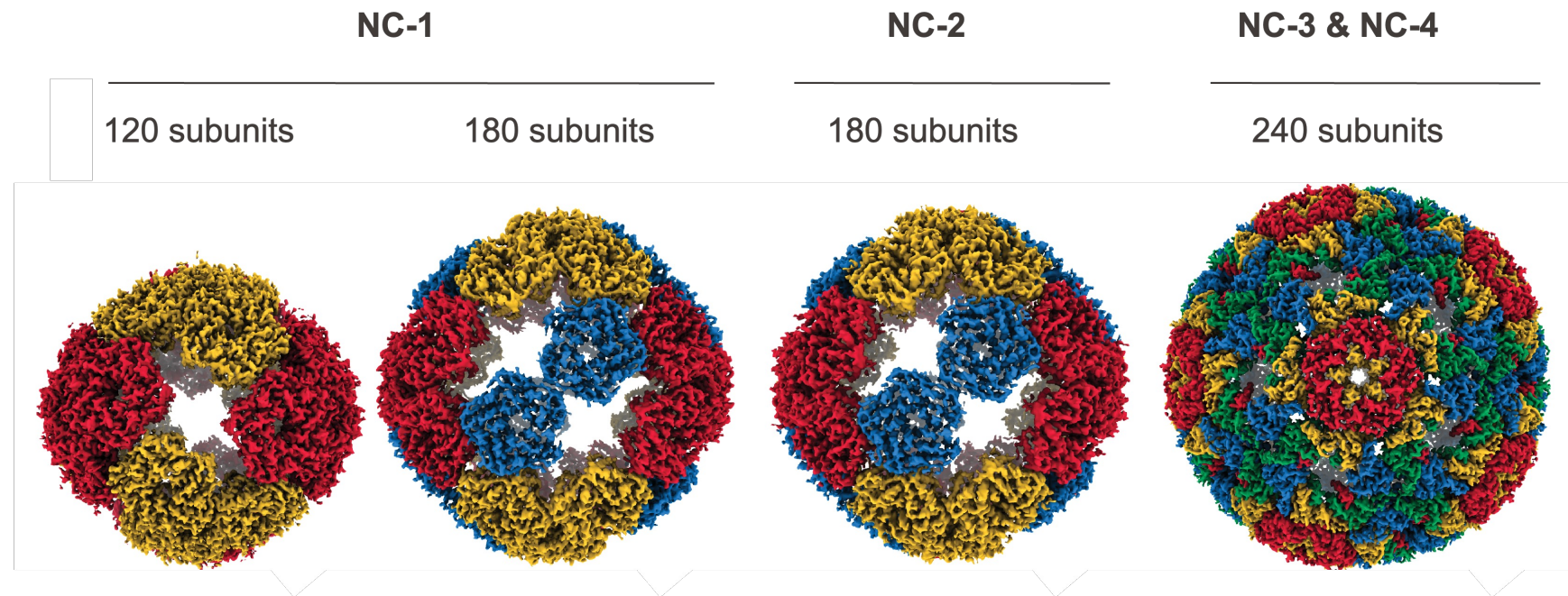
Science **2021**, *372*, 1220.

Evolution increases self-RNA recognition
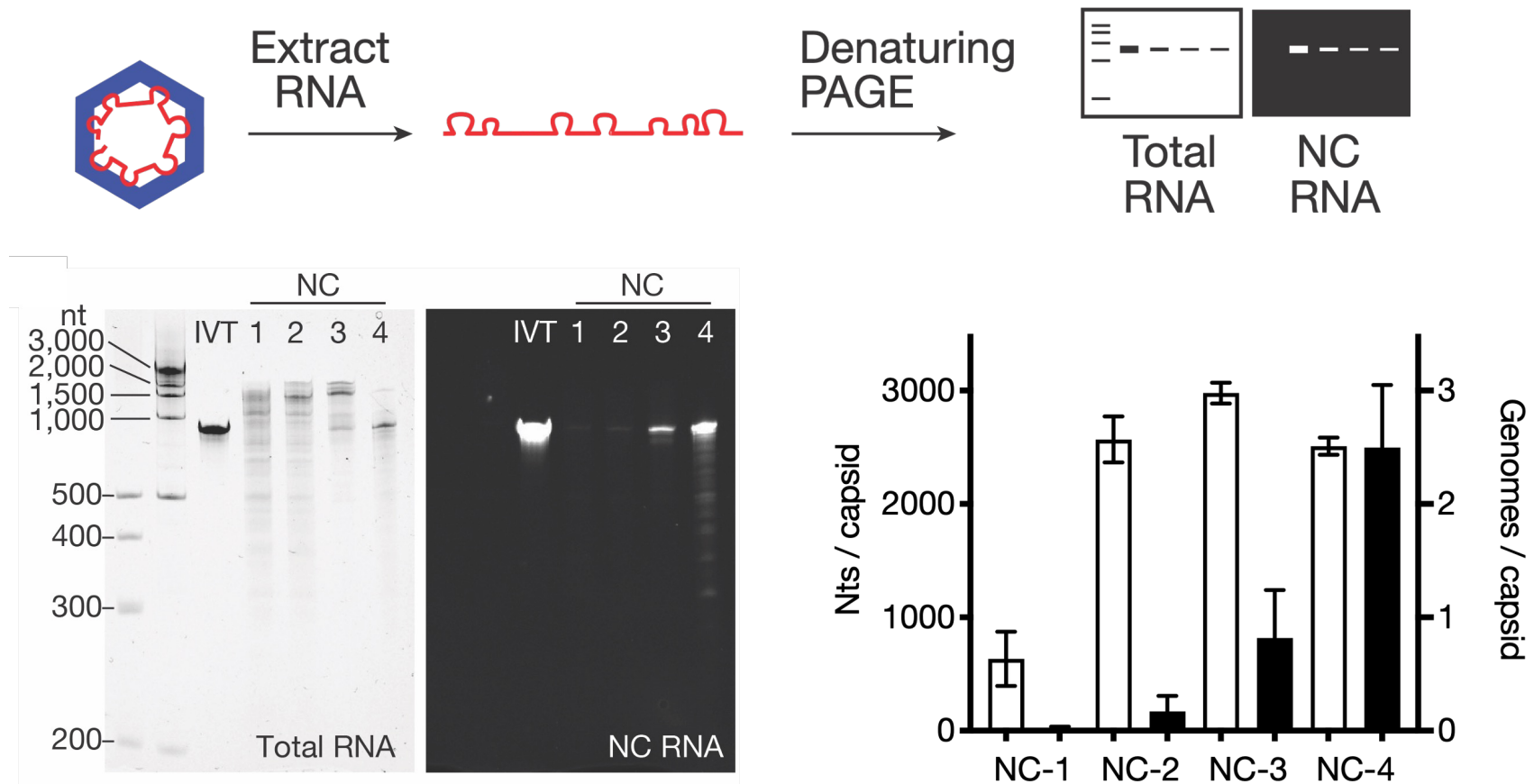
Science **2021**, *372*, 1220.

What other RNAs are encapsulated?

Library preparation for Nanopore sequencing

Full-length RNA

Direct RNA sequencing

100 min — Primer annealing

Reverse transcription and strand switching

80 min — Digestion of RNA and second-strand synthesis

90 min — Ligation of sequencing adapters

Loading
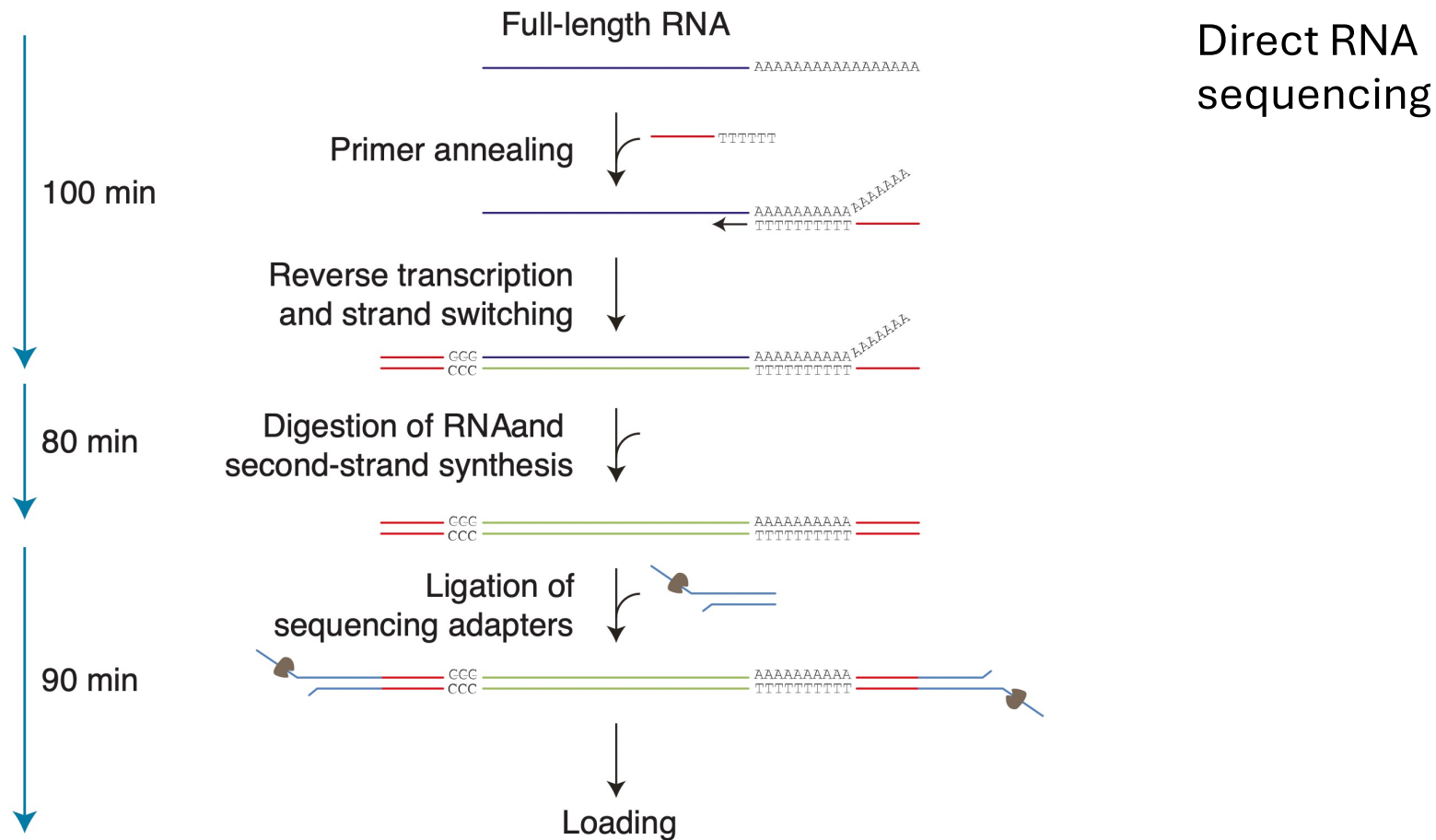
Data analysis workflow for Nanopore sequencing
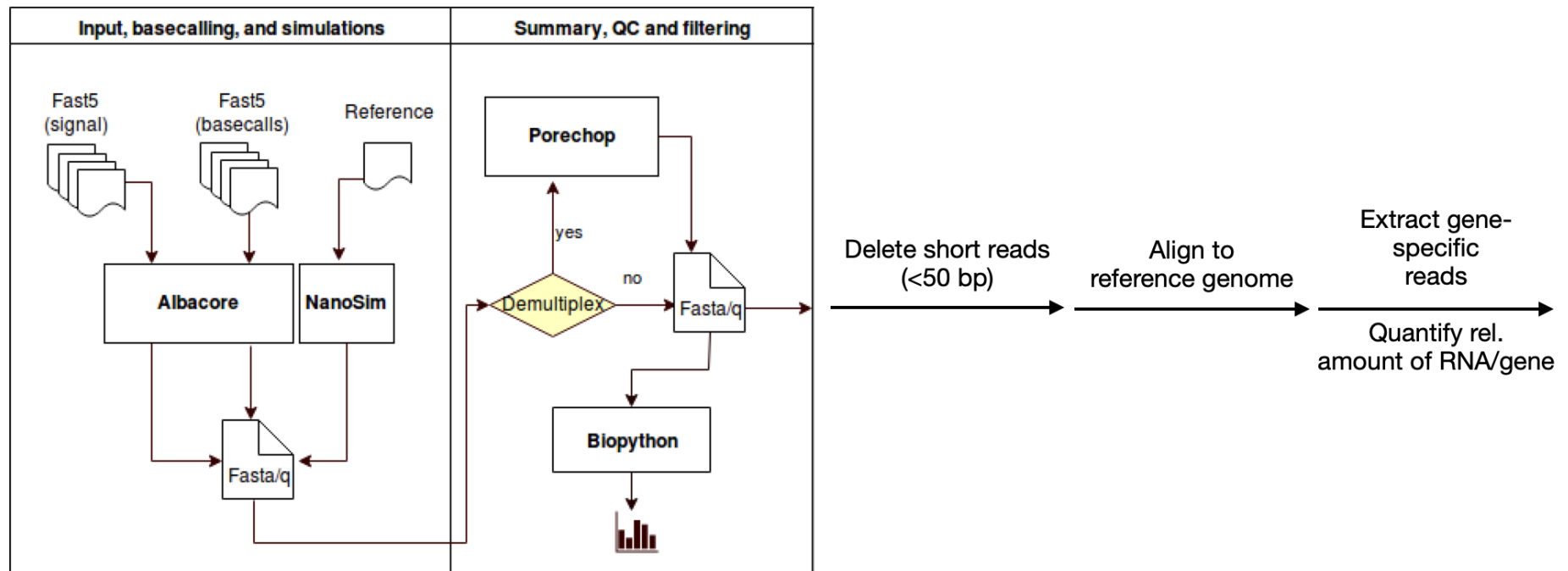
Majority of "other RNA" is ribosomal RNA

NC-1 NC-2 NC-3 NC-4

Total E. coli RNA
NC-4 expressing cells

Legend:
- Plasmid (not AaLS + lacI)
- AaLS
- rrsH
- rrlH
- rrlG
- rrsG
- rrlD
- rrsD
- rrsC
- rrlC
- rrsA
- rrlA
- rrsB
- rrlB
- rrsE
- rrlE
- gltA
- rnpB
- ssrA
- other
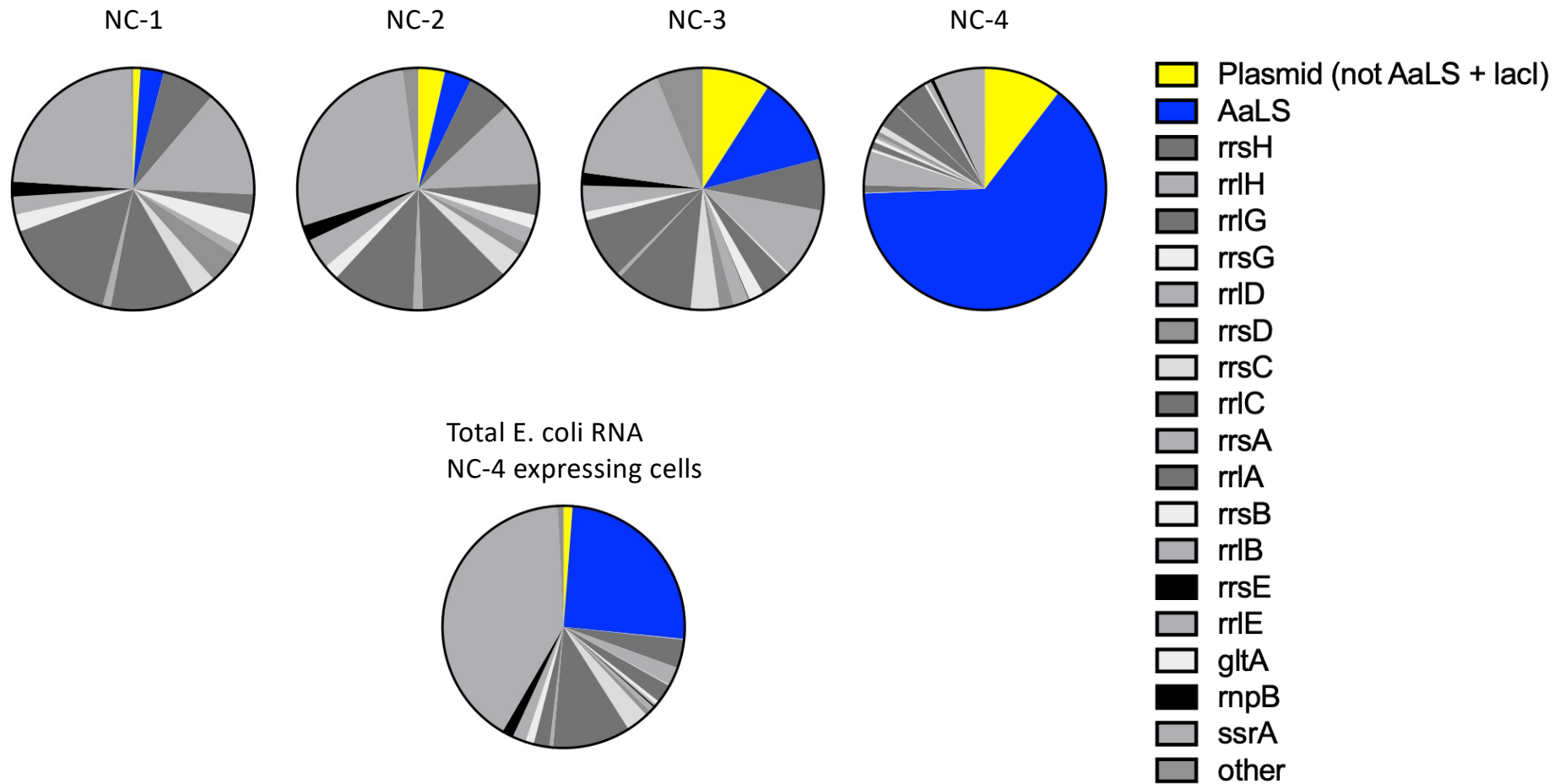
Summary

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

Note about this week's Connections: Category "*Generate(s) an electric field*"

- **Charged particles**: Electric fields are generated by charged particles, such as electrons and protons, due to their electric charge. The electric field radiates outward from positive charges and inward toward negative charges.

- **Dipoles**: Electric fields can also be generated by electric dipoles or separated charges, which consist of two equal and opposite charges separated by a distance. The resulting electric field extends from the positive to the negative charge.

- **Voltage difference**: When there's a voltage difference across two points in a material or in space, it creates an electric field. This is the principle behind the electric field in a circuit or between the plates of a capacitor.

- **Light**: Light and other forms of electromagnetic radiation consist of fluctuating electric and magnetic fields. The oscillating magnetic field component can generate an accompanying electric field, and vice versa. Light can also induce electric fields, particularly in the context of phenomena like Surface Plasmon Resonance (SPR), where incident light interacts with metal surfaces to generate evanescent electromagnetic fields.