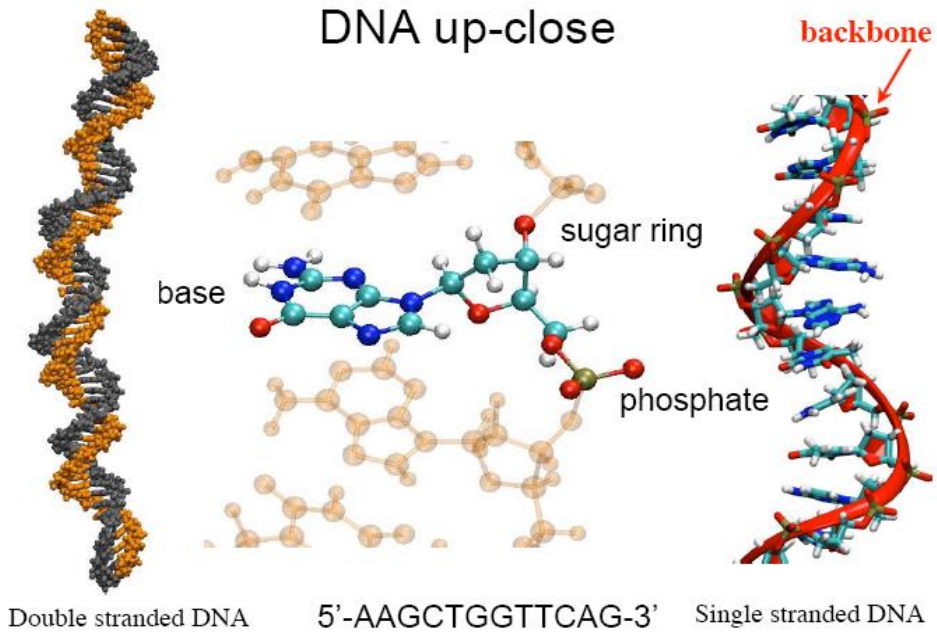
# *DNA DETECTION AND CHARACTERIZATION WITH NANOPORE TECHNOLOGY*

*Craig Wells*
*Department of Physics*
*Clarkson University*

# WHAT IS DNA?




A – adenine, T - thymine
G – guanine, C - cytosine

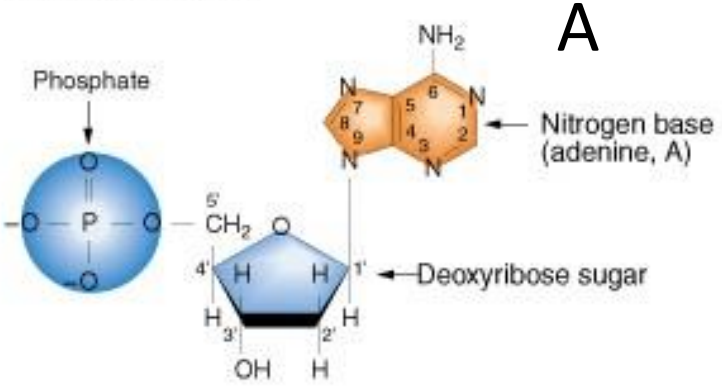
- **Deoxyribonucleic acid** (**DNA**) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms.
- Chemically, DNA is a long polymer of simple units called nucleotides, containing a backbone made of sugars and phosphate groups.
- Attached to each sugar is one of four types of molecules called bases (nucleobase).
- It is the sequence of these four bases along the backbone that encodes information.

# NUCLEOTIDES

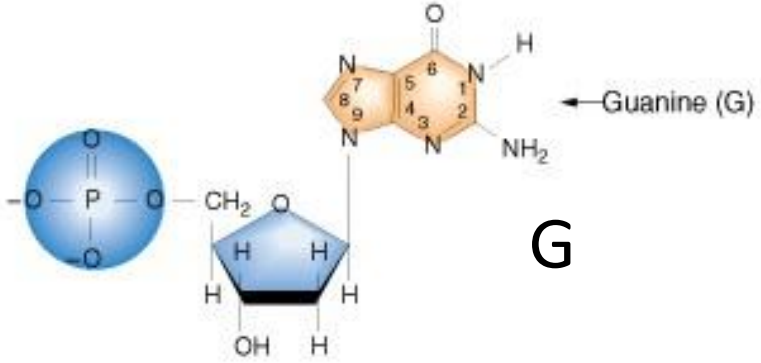
Base + Sugar + Phosphate = Nucleotide

**Purine nucleotides**

A



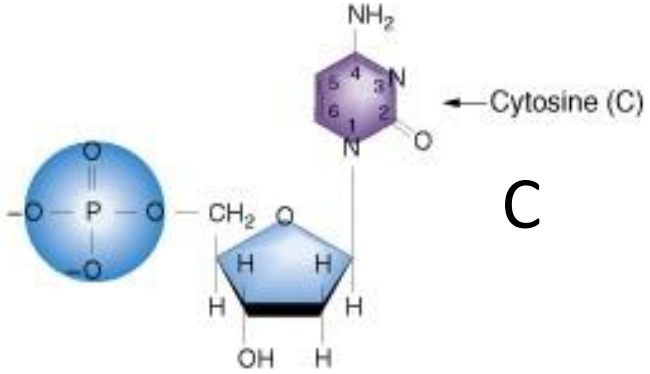

Deoxyadenosine 5'-phosphate (dAMP)




G

Deoxyguanosine 5'-phosphate (dGMP)

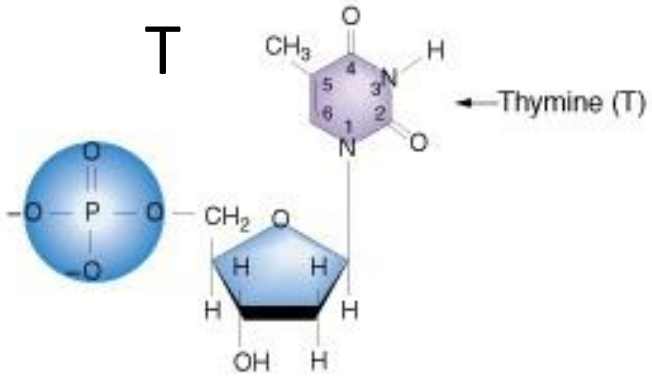
**Pyrimidine nucleotides**




C

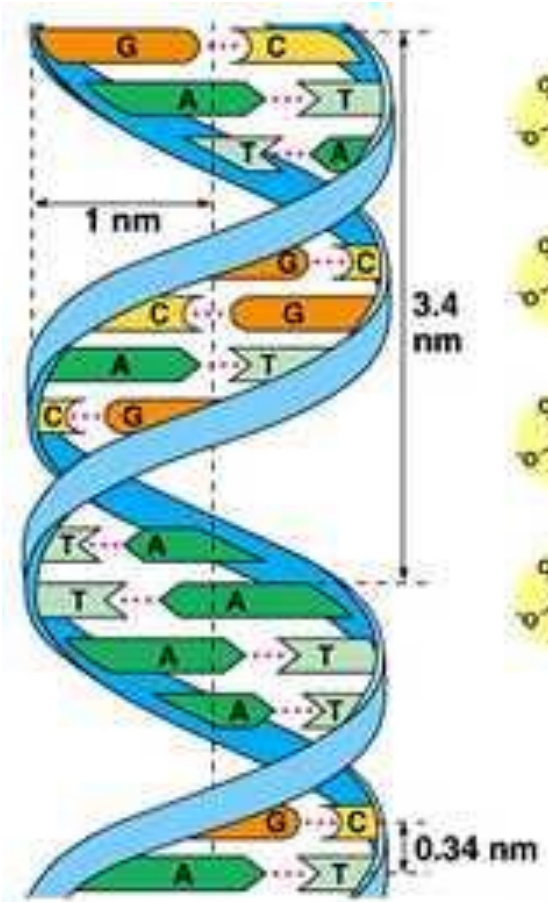
Deoxycytidine 5'-phosphate (dCMP)

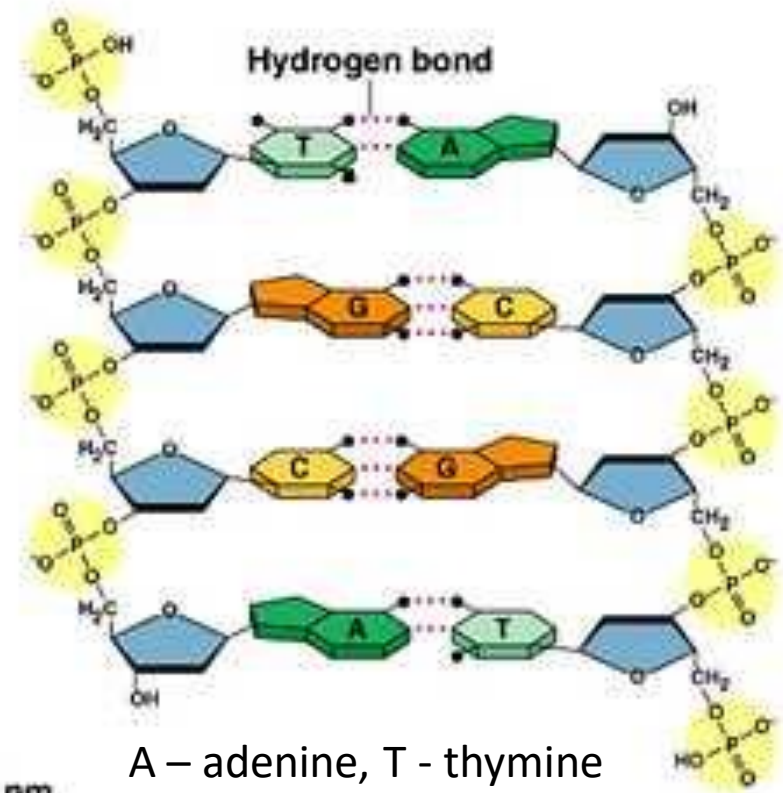
T




Deoxythymidine 5'-phosphate (dTMP)

# DNA STRUCTURE

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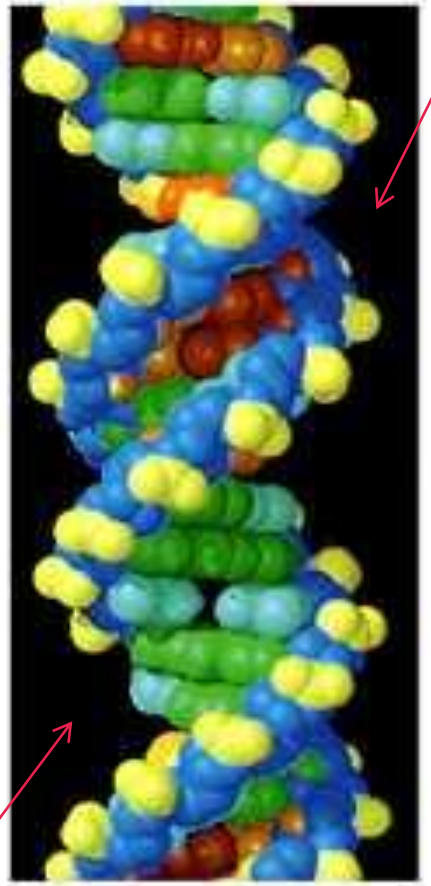
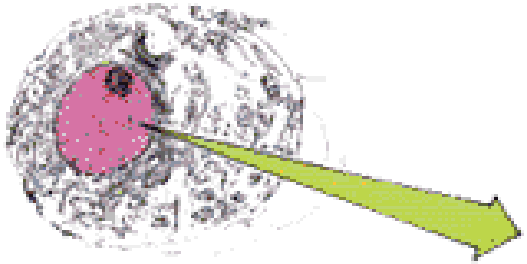



Minor grove

Major grove

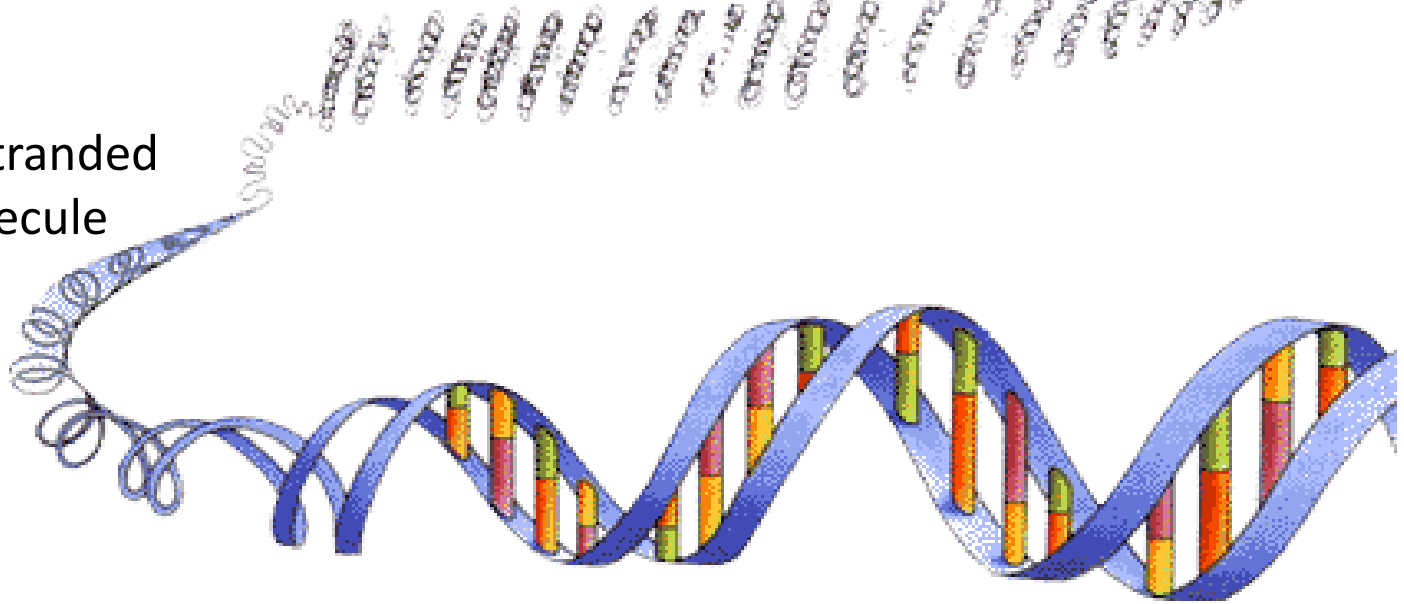
# DNA IN THE CELL



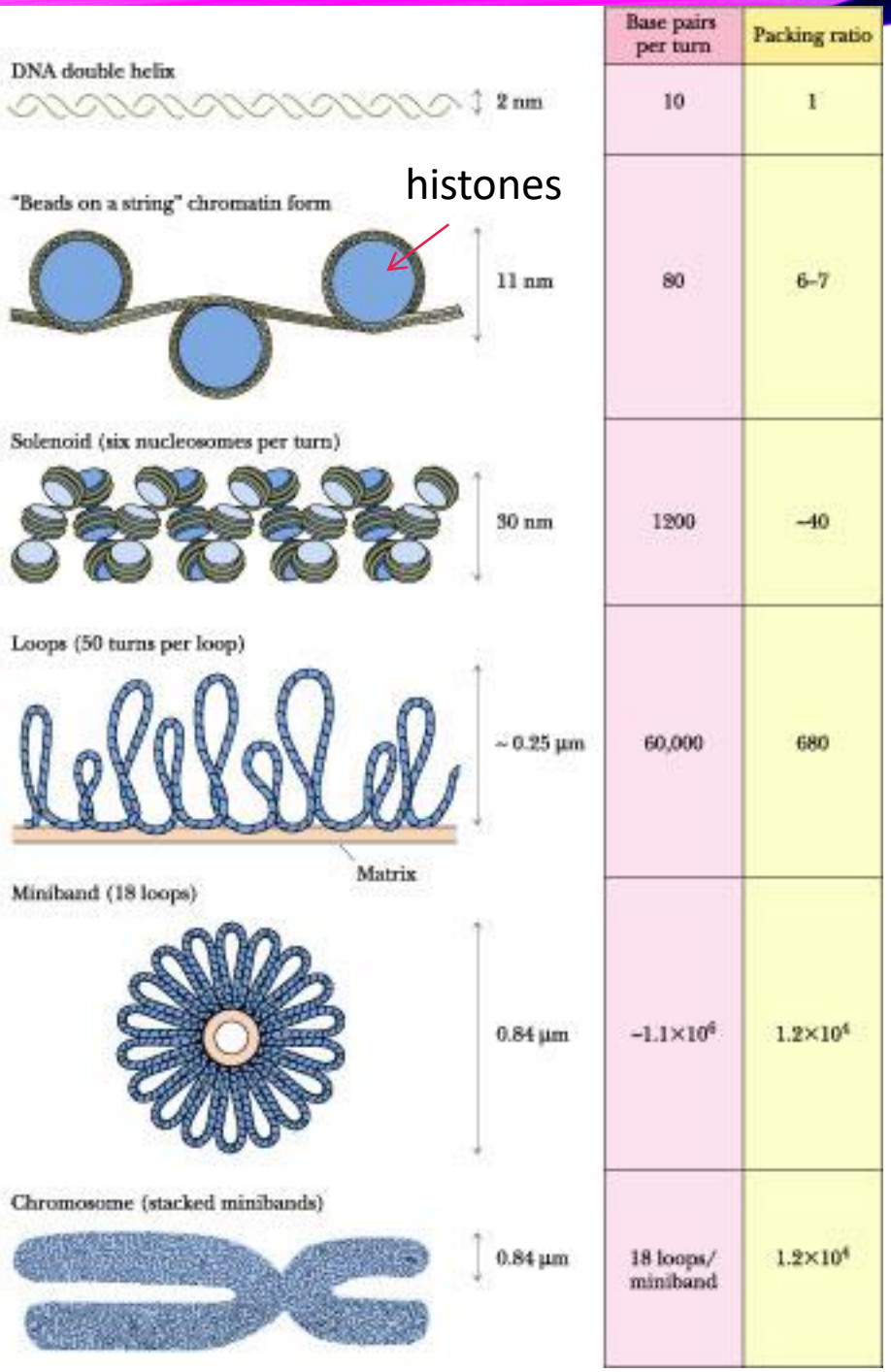
cell nucleus

chromosome

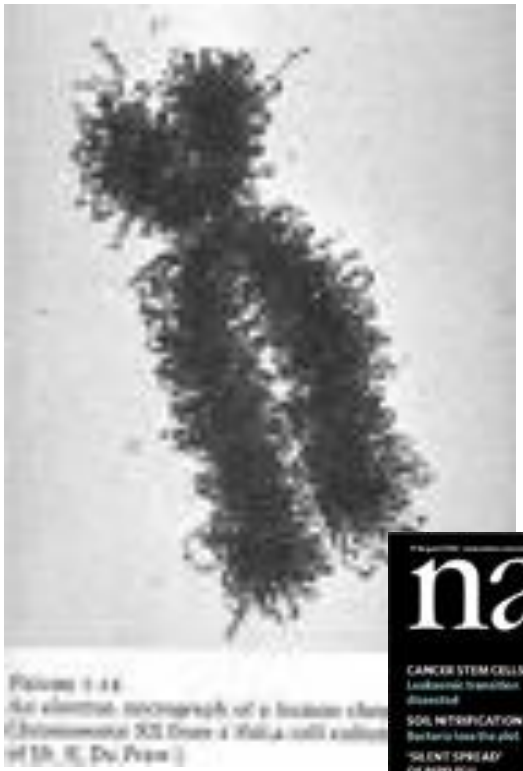
Double stranded
DNA molecule



# DNA STRUCTURE




| Base pairs per turn | Packing ratio |
|---|---|
| 10 | 1 |
| 80 | 6–7 |
| 1200 | ~40 |
| 60,000 | 680 |
| $\sim 1.1\times10^{6}$ | $1.2\times10^{4}$ |
| 18 loops/ miniband | $1.2\times10^{4}$ |



# THE HUMAN GENOME

- is the genome of *Homo sapiens*, which is composed of 23 distinct pairs of chromosomes (22 autosomal + X + Y) with a total of approximately 3 billion DNA base pairs containing an estimated 20,000–25,000 genes.
- TAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACAACCCTAACCCT
  AACCCTAACCCTAACCCAACCCTAACCCTAACCCTAACCCTAACCCTAACCTAACCCCTAACCCTAACCCTAACCCTAACCC
  TAACCTAACCCTAACCCTAACCCTAACCCTAACCCTACCCTAACCCTAACCCTAACCCCTAACCCTAACCCTAAACCCTAAA
  CCCTAACCCTAACCCTAACCCTACCTAACCCCAACCCCAACCCCAACCCCAACCCCAACCCCAACCCTAACCCCTAACCCT
  AACCCTAACCACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCCTAACCCCTAACCCTAACCCTAACCCACCCTA
  ACCCTAACCCTAACCCCTAACCCTAACCCTAACCCTAACCCTCGCGGTACCCTCAGCCGGCCCCCGCCCGGGTCTGACCT
  GAGGAGAACTGTGCTCCGCCTTCAGAGTACCACCGAAATCTGTGCAGAGGACGCAGCTCCGCCCTCGCGGTGCTCTCC
  GGGTCTGTGCTGAGGAGAACGCAACTCCGCCGGCGCAGGCGCAGAGAGGCGCGCCGCGCCGGCGCAGGCGCAGACA
  CATGCTAGCGCGTCGGGGTGGAGGCGTGGCGCAGGCGCAGAGAGGCGCGCCGCGCCGGCGCAGGCGCAGAGACAC
  ATGCTACCGCGTCCAGGGGTGGAGGCGTGGCGCAGGCGCAGAGAGGCGCACCGCGCCGGCGCAGGCGCAGAGACAC
  ATGCTAGCGCGTCCAGGGGTGGAGGCGTGGCGCAGGCGCAGAGACGCAAGCCTACGGGCGGGGGTTGGGGGGGCG
  TGTGTTGCAGGAGCAAAGTCGCACGGCGCCGGGCTGGGGCGGGGGGAGGGTGGCGCCGTGCACGCGCAGAAACTC
  ACGTCACGGTGGCGCGGCGCAGAGACGGGTAGAACCTCAGTAATCCGAAAAGCCGGGATCGACCGCCCCTTGCTTGCA
  GCCGGGCACTACAGGACCCGCTTGCTCACGGTGCTGTGCCAGGGCGCCCCCTGCTGGCGACTAGGGCAACTGCAGGG
  CTCTCTTGCTTAGAGTGGTGGCCAGCGCCCCCTGCTGGCGCCGGGGCACTGCAGGGCCCTCTTGCTTACGTATAGTGGT
  GGCACGCCGCCTGCTGGCAGCTAGGGACATTGCAGGGTCCTCTTGCTCAAGGTGTAGTGGCCTCTGCAGGAGGCTGCC
  ATTTGTCCTGCCCACCTTCTTAGAAGCGAGACGGAGCAGACCCATCTGCTAGCCCTTTCTATAATAACTAAAGTTAGCTGC
  CCTGGACTATTCACCCCCTAGTCTCAATTTAAGAAGATCCCCATGGCCACAGGGCCCCTGCCTGGGGGCTTGTCACCTCC
  CCCACCTTCTTCCTGAGTCATTCCTGCACTTGCTCCCTAACCTGCCCCACAGCCTTGCCTGGATTTCTATCTCCCTGGCTTG
  GTGCCAGTTCCTCCAGTCGATGGCACCTCCCTCCCTCTCAACCACTTGAGCAAACTCCAAGACATCTTCTACCCCAACACC
  AGCAATTGTGCCAAGGGCCATTAGGCTCTCAGCATGACTATTTTTAGAGACCCCGTGTC…
- 455 thousand pages total, A4, 10 pt font.

# DNA SEQUENCING

- It used to cost $10,000,000. Time: 3-4 months.
- Today's cost: ~ $1,000. Time: 1 week.
- $10 million Archon X Prize for genomics "to create technology that successfully maps 100 human genomes in 10 days."
- In-doctor's office diagnostics, personal medicine, research
- Need for ultra fast sequencing techniques

– electronic DNA sequencing utilizing modern *nanotechnology*

*Nanotechnology deals with objects of nanometer scale:*

$1nm=10^{-9}m$

$1nm=0.000000001\ m$

# LENGTH SCALES

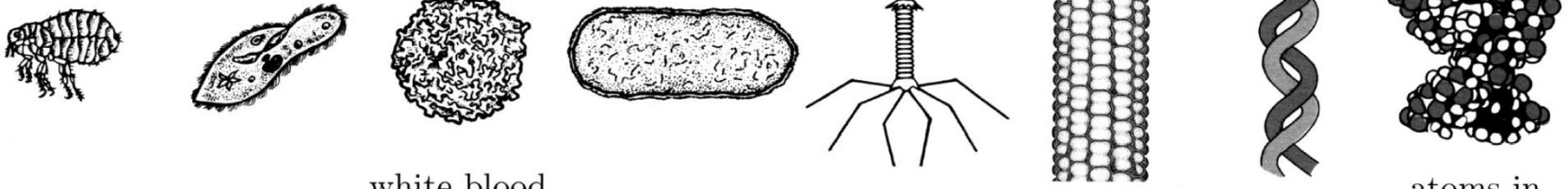
~2m

*DNA is not quite a spaghetti!*
*It is a veeeeery thin and*
*a veeeeery long spaghetti!!*

$10^{-3}$m

$10^{-6}$m

$10^{-9}$m



flea 1 mm

protozoan 0.1 mm

white blood cell 0.01 mm

*E. coli* 1 $\mu$m

T2 phage 0.1 $\mu$m

microtubule 25 nm

DNA 2 nm

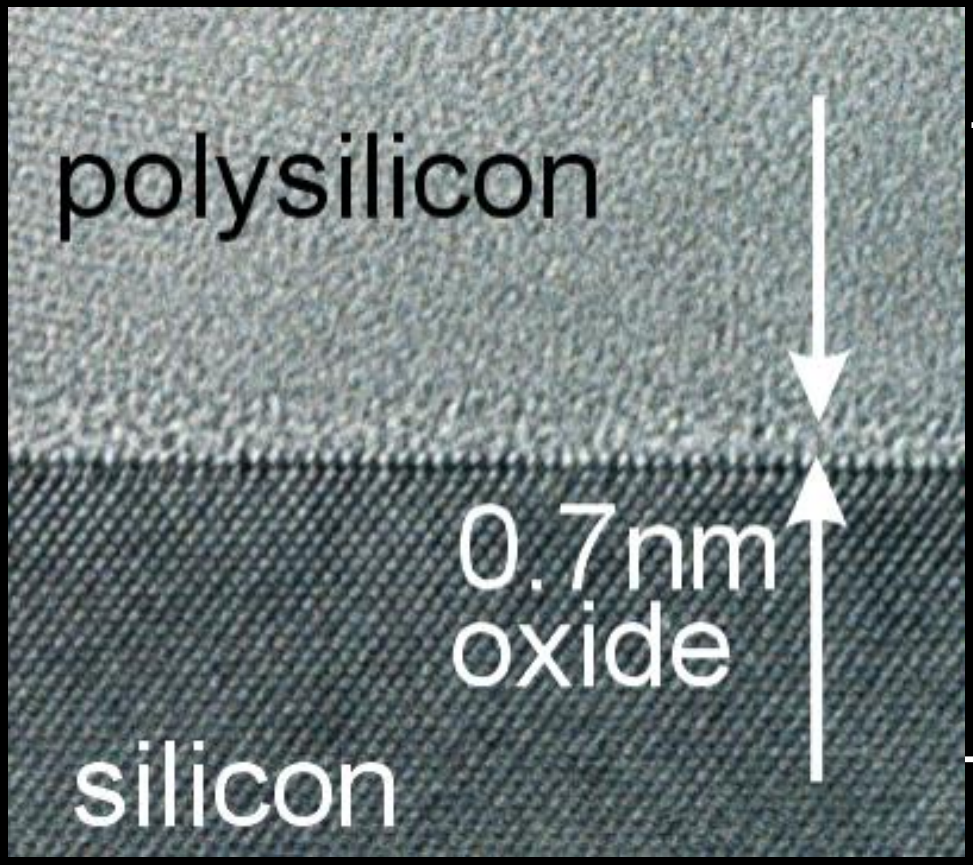
atoms in DNA 0.2 nm

From Biological Physics: Energy, Information, Life (WH Freeman and Company, ©2004 by Philip C. Nelson).

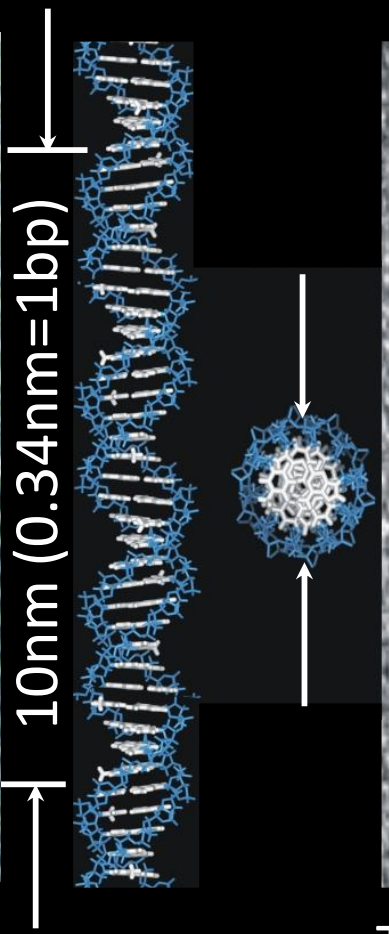
2.1 (Icons.) *Dramatis personæ.* Approximate relative sizes of some of the actors in our story. T2 phage is a virus that infects bacteria, for example, *Escherichia coli.* Much of this book will be occupied with phenomena relevant at length scales from the protozoan down to the DNA helix. [Adapted from Kornberg, 1989.]

# SILICON NANOTECHNOLOGY FOR SEQUENCING DNA

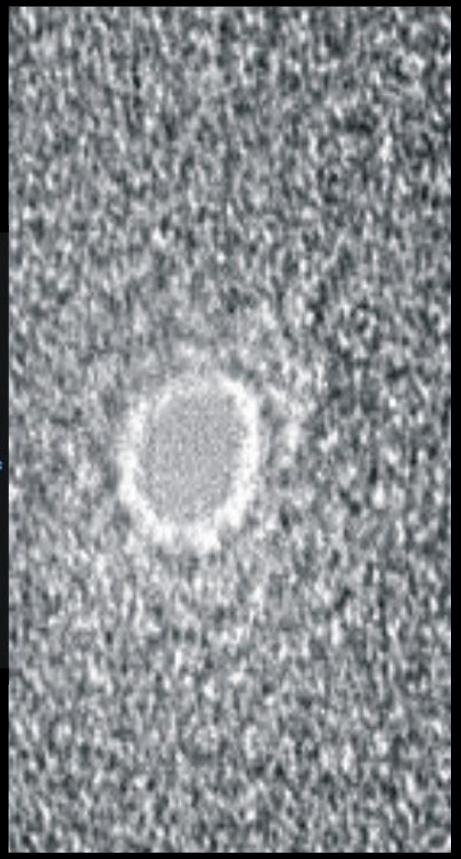
- ultra-thin membranes




TEM X-section through a gate



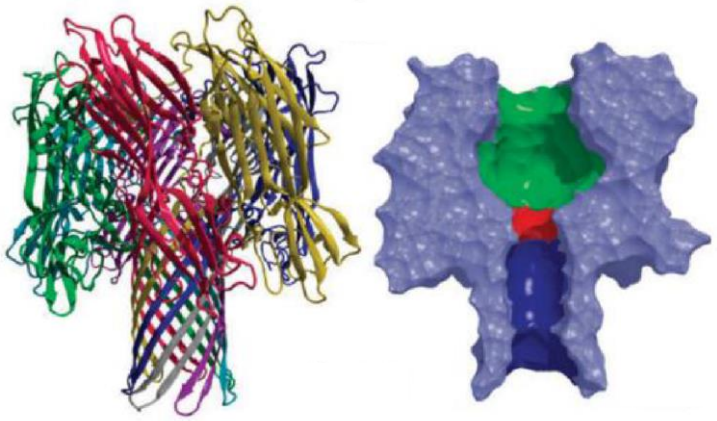

*DNA*



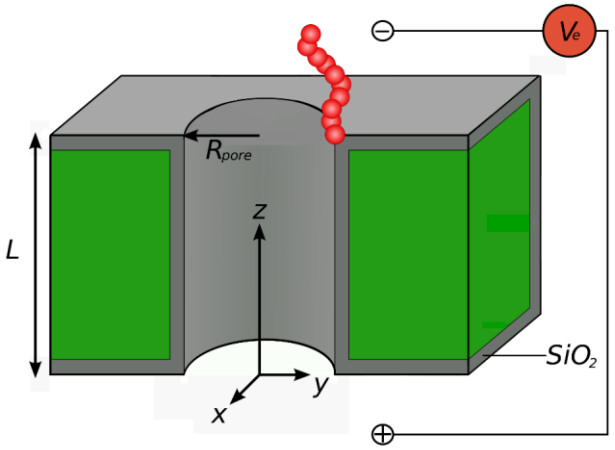
TEM (top-down projection)

Deamer, Branton, Kasianowicz
Golovchenko
Martin

# NANOPORES



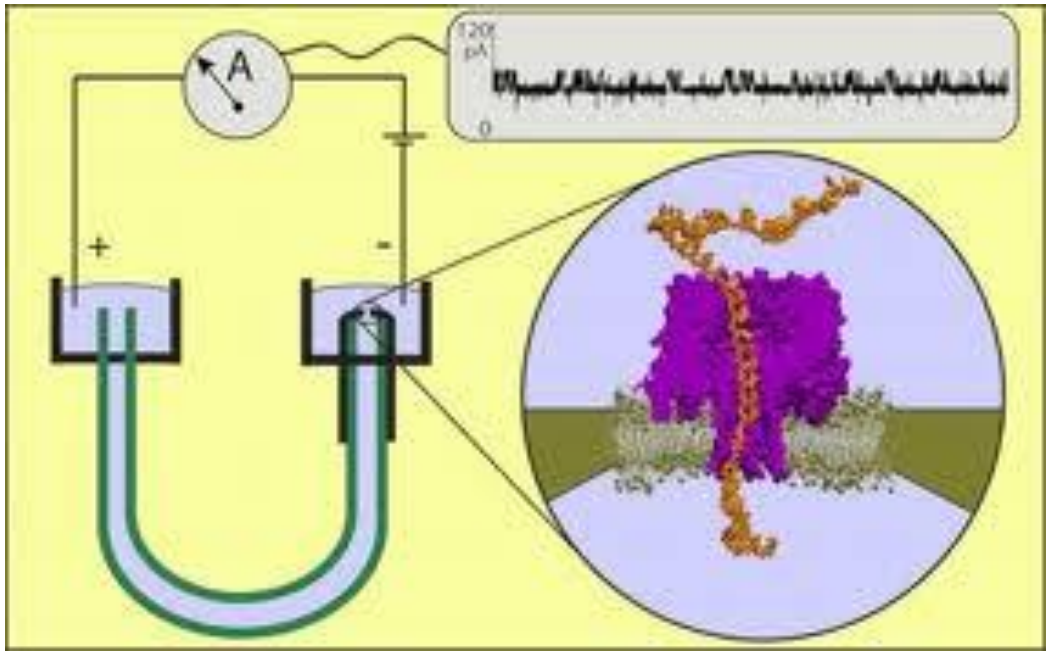
α-hemolysin pore
(biological nanopore)




Silicon dioxide pore (artificial nanopore)

- Nanopores, pores on the nanoscale, can be biological or artificial
- Biological nanopores (pore-forming proteins) are compatible with specific molecules and environments
- Artificial membranes, made from solid-state material, can be constructed for a specific task
- Nanopores can be used for identification/characterization of many molecules, as well as filtering proteins

# BIOLOGICAL PORES FOR DNA SEQUENCING

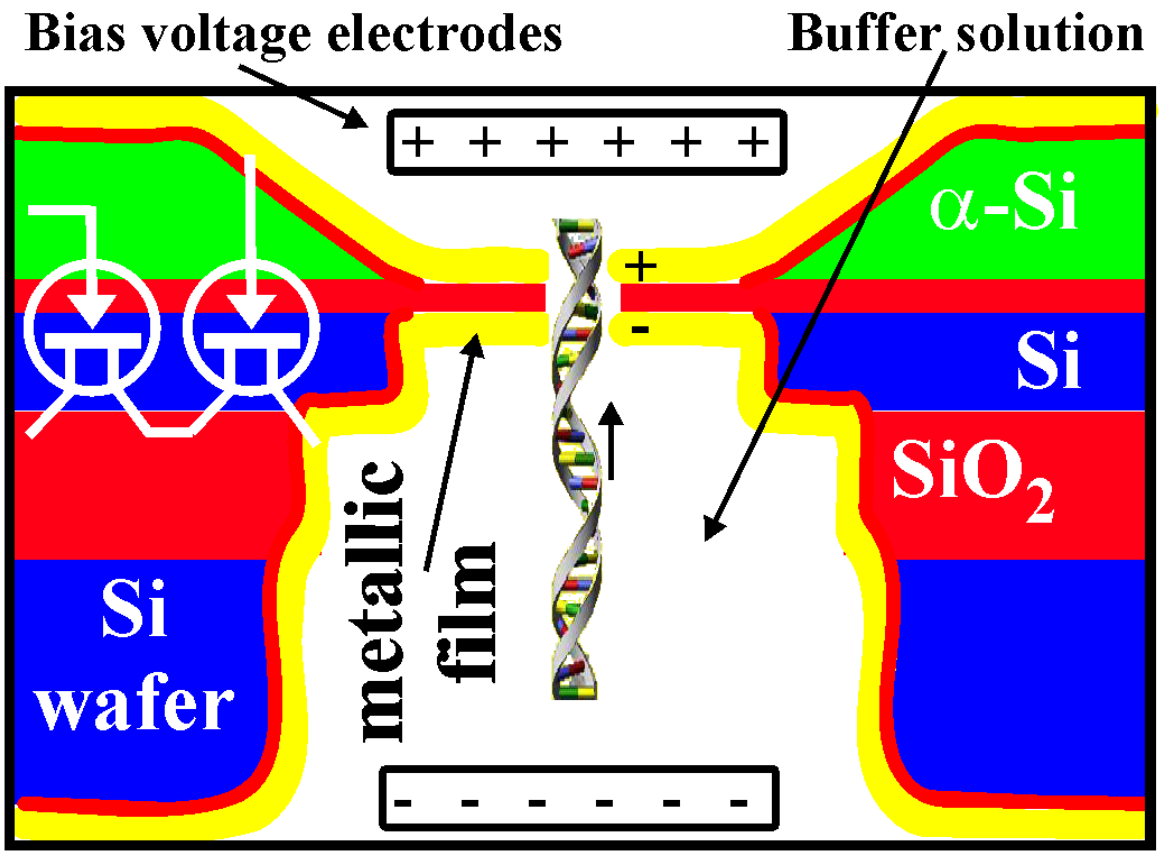



α-hemolysin - bacterial channel

By 1996 a team of researchers from Harvard University and the University of California, Santa Cruz, had found that using one protein in particular, alpha hemolysin (αHL), it was possible to get single strands of DNA to pass through a nanopore. This, they realized, might lead to a new way to sequence DNA. But threading a strand through an αHL pore and detecting the individual bases at the same time is very difficult.

- The electrolyte bias is applied, the electrolyte flows through the pore
- The biomolecule translocates and blocks the ionic current flowing through the nanopore
- The ion current blockades are recorded

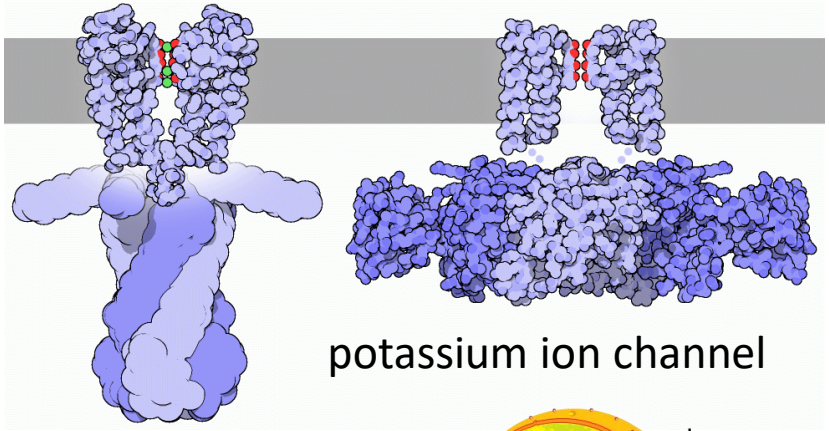
# ELECTRONIC DETECTION OF THE DNA MOLECULAR SEQUENCE



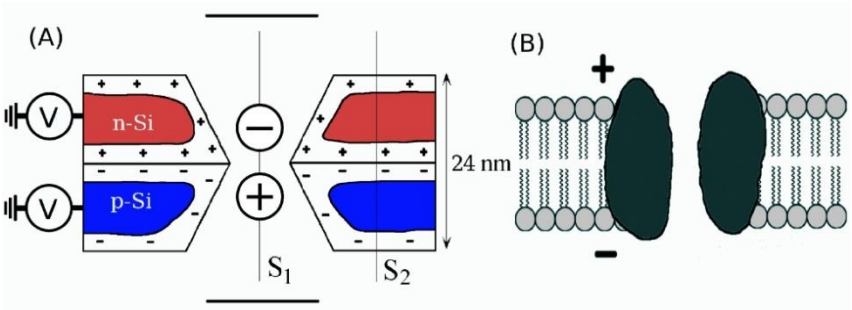

**Record voltage variation in addition to current variation**

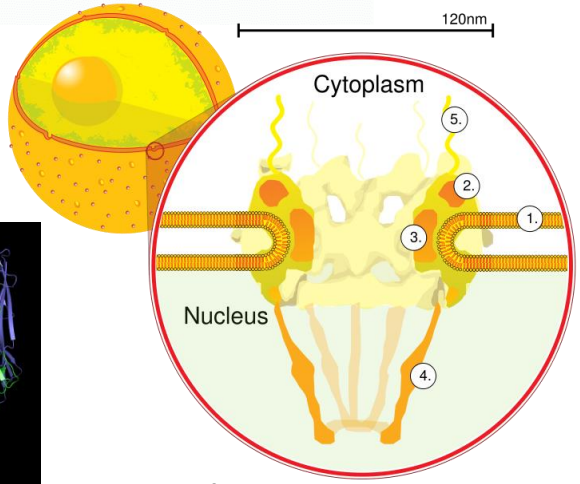
**G. Timp, UIUC**

# REVERSE ENGINEERING OF THE BIOLOGICAL CHANNELS WITH LAYERED SOLID-STATE MEMBRANES

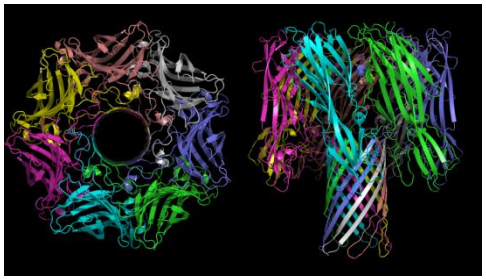


potassium ion channel

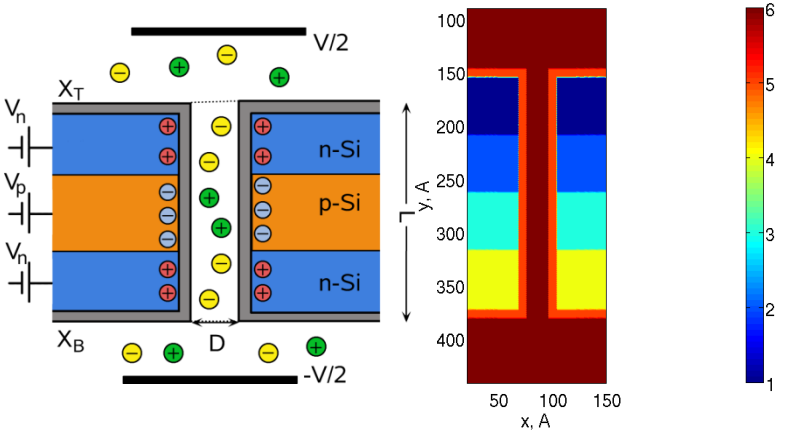



nuclear pore



bacterial toxin:
$\alpha$-hemolysin channel



# NANOPORES IN SOLID-STATE MEMBRANES: WHAT DO WE WANT?

- to duplicate different functions that bio-pores have.
- to have robust devices that perform over the wide range of conditions (pH, T, solution strength)
- to have control over the nanopore (open/closed, size, charge, direction)
- to use nanopores as bio-filters and bio-sensors
- to facilitate ultra-fast electronic DNA sequencing

# MinION

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Oxford NANOPORE Technologies

PRODUCTS SERVICES APPLICATIONS GET STARTED PUBLICATIONS

## Portable, real-time biological analyses

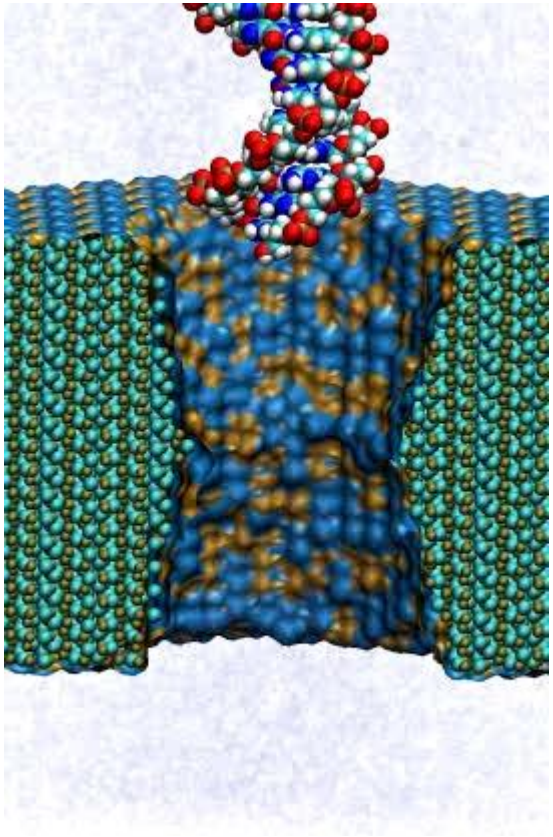
MinION is the only portable real-time device for DNA and RNA sequencing.

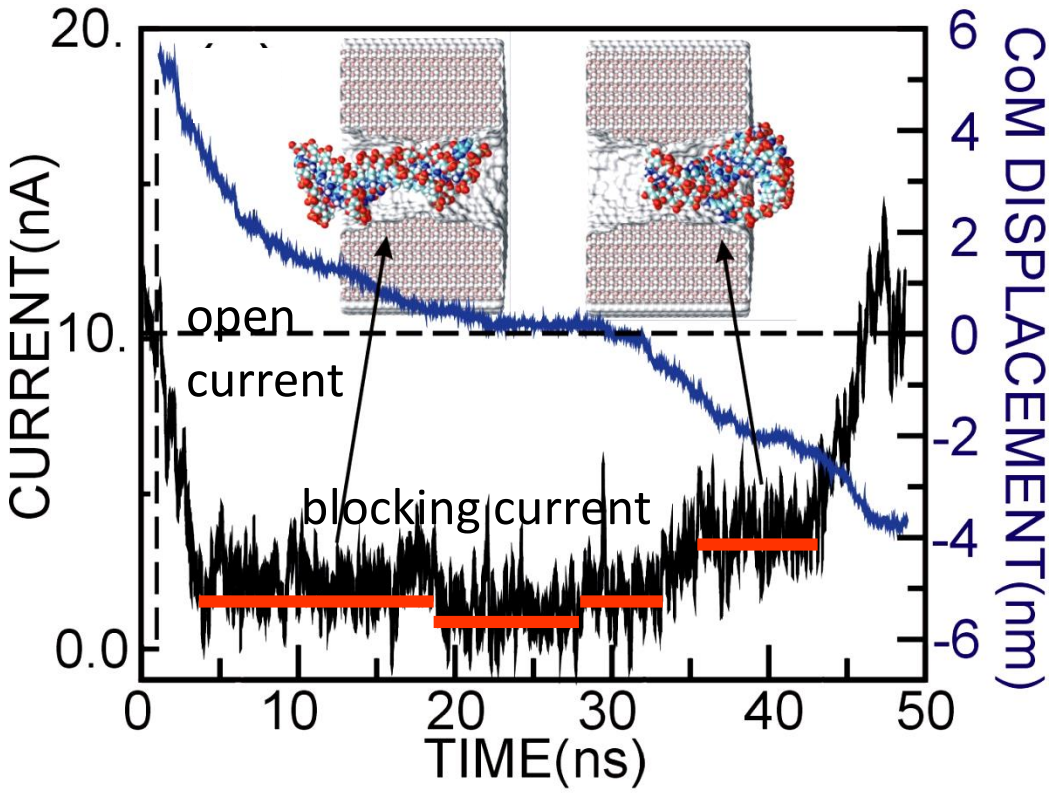
Each consumable flow cell can now generate 10–20 Gb of DNA sequence data. Ultra-long read lengths are possible (hundreds of kb) as you can choose your fragment length. The MinION streams data in real time so that analysis can be performed during the experiment and workflows are fully versatile.

The MinION weighs under 100 g and plugs into a PC or laptop using a high-speed USB 3.0 cable. No additional computing infrastructure is required. Not constrained to a laboratory environment, it has been used up a mountain, in a jungle, in the arctic and on the International Space Station.

Oxford Nanopore Technologies
https://nanoporetech.com/products/minion

# MOLECULAR DYNAMICS OF DNA IN A $Si_3N_4$ NANOPORE




- translocation time: 10nsec-3msec depending on field and pore interactions
- % blocking current correlated with molecular velocity

(• large fields cause the DNA to denature)

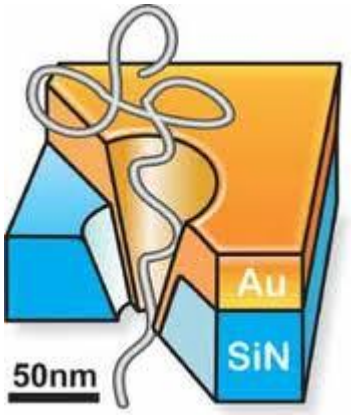
360,000-atom MD simulation A. Aksimentiev

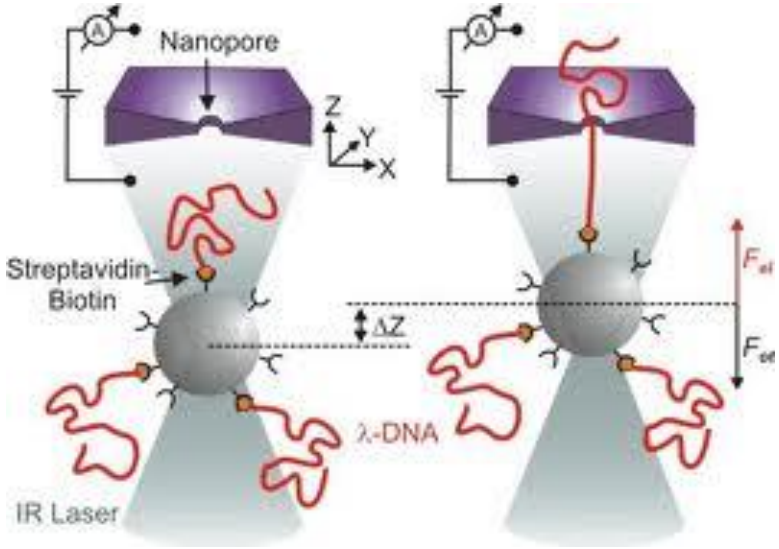
# CHALLENGES FACED BY THE NANOPORE SEQUENCING TECHNIQUES

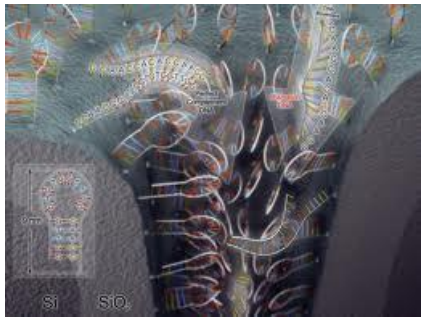
- DNA translocates too fast – 1 base pair/30 ns
- Translocation is not controlled
- Nucleotides are too closely packed
- DNA conformational (positional) noise

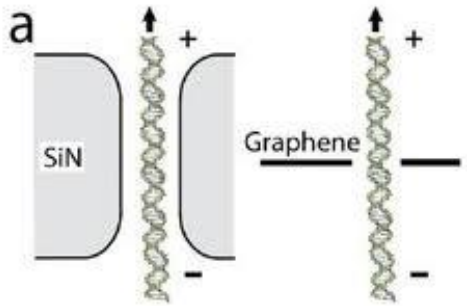
- Slow down DNA
- Must control DNA translocation
- Stretch DNA (with an electric field)
- Collect numerous readings to average out the positional noise

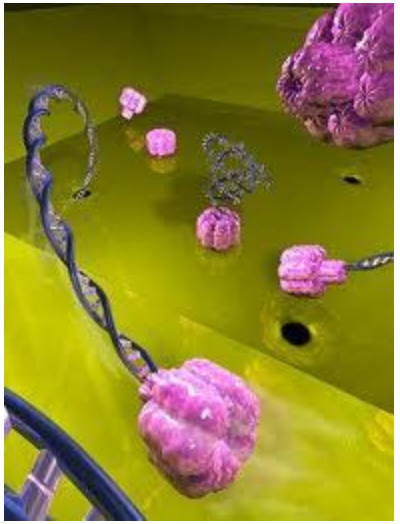
# OTHER SYSTEMS

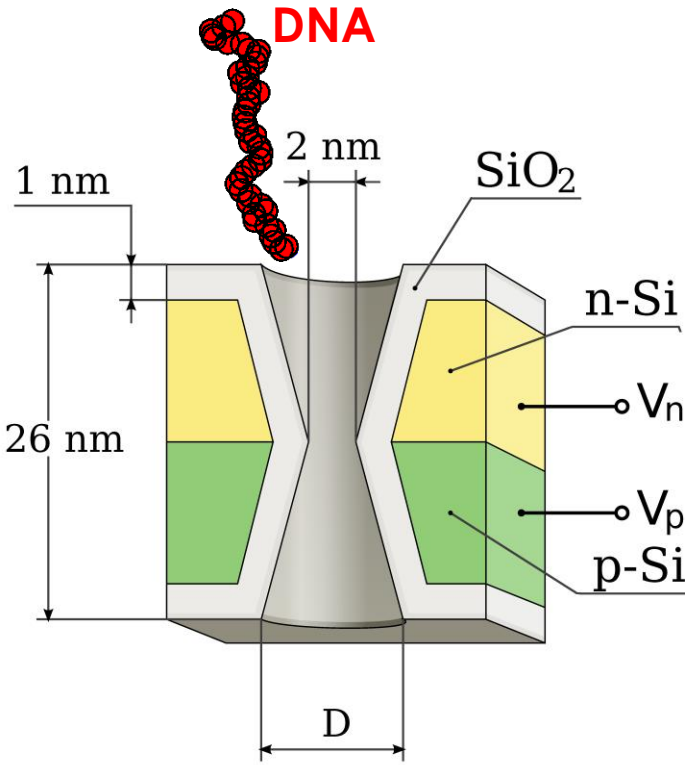





# BROWNIAN DYNAMICS (BD)

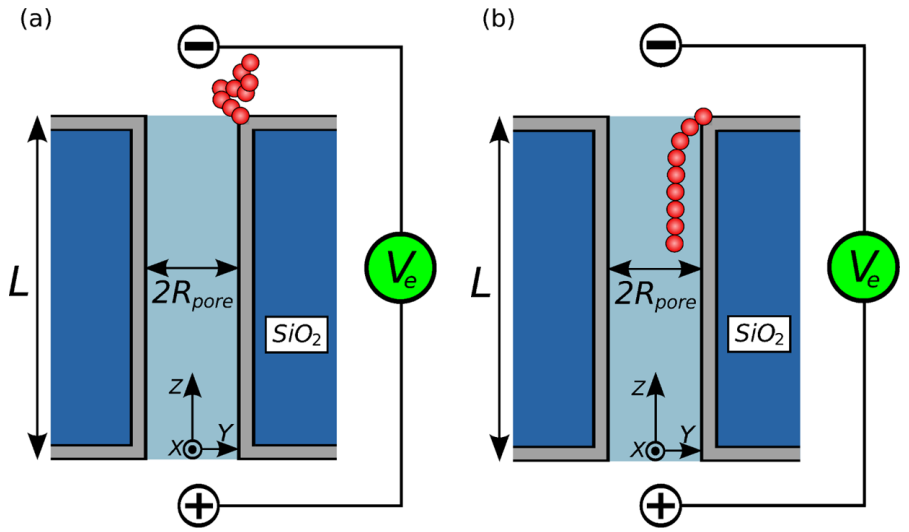



- DNA is a collection of beads.
- Each bead is one nucleotide (charged)
- Their motion is described by several forces and Brownian motion
- Brownian motion is a type of "random walk" which describes the movement of particles in a solution

*Total Force = Volume Exclusion Force + Electric Charge Forces + Bonding Force +* ***Random Force***

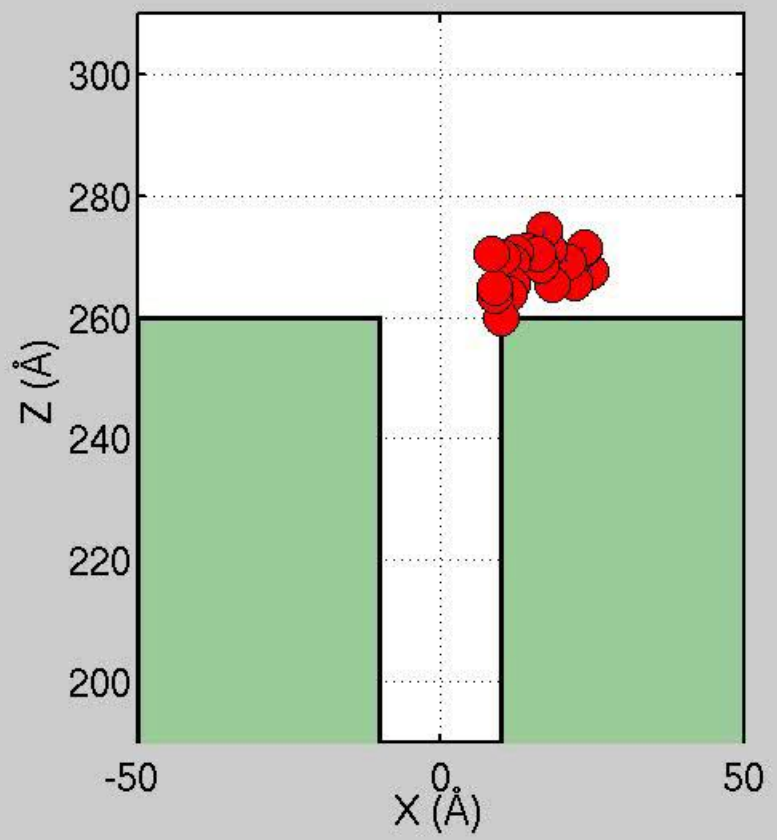
# NANOPORE GATING

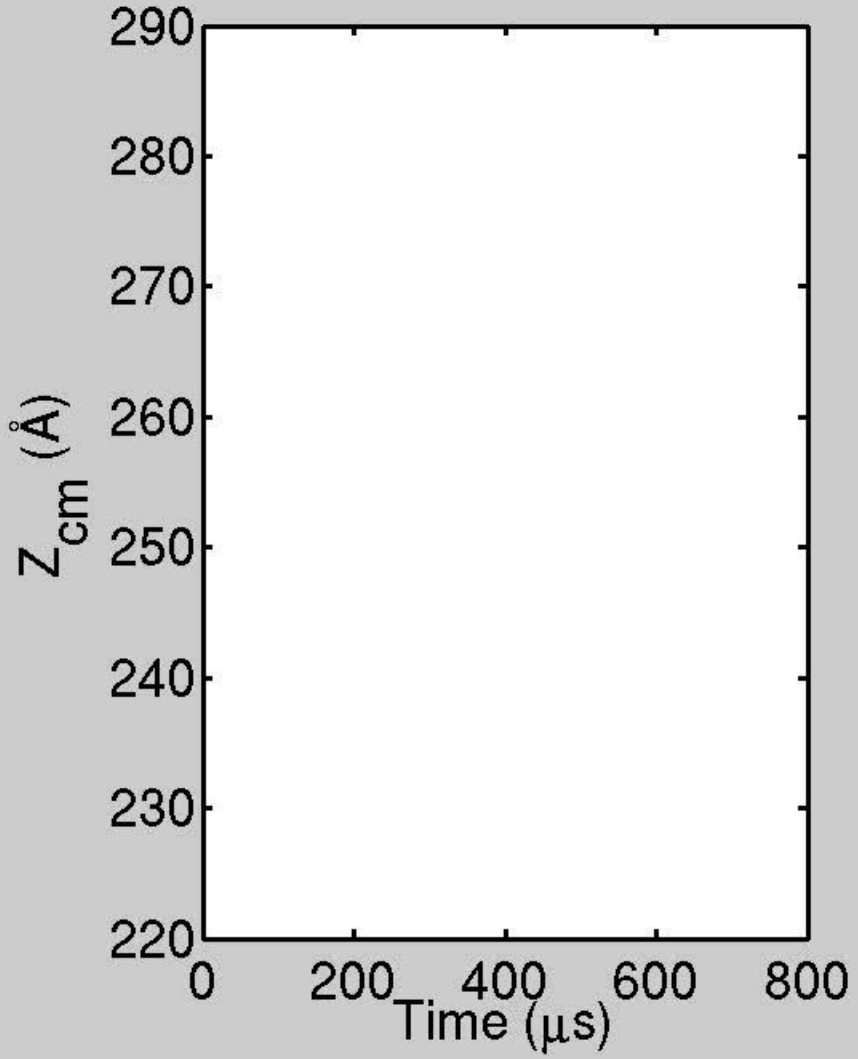
- It is possible to modify the nanopore by attaching single-stranded DNA to the surface of a membrane
- Surface modifications allow for greater selectivity and control in the system by only allowing certain types of molecules to pass




- (a) Zero bias, the chain hovers above the membrane
- (b) Positive bias, the chain extends into the nanopore

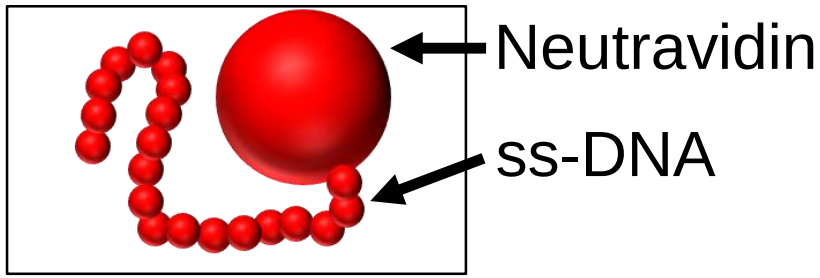
# SINGLE SIMULATION

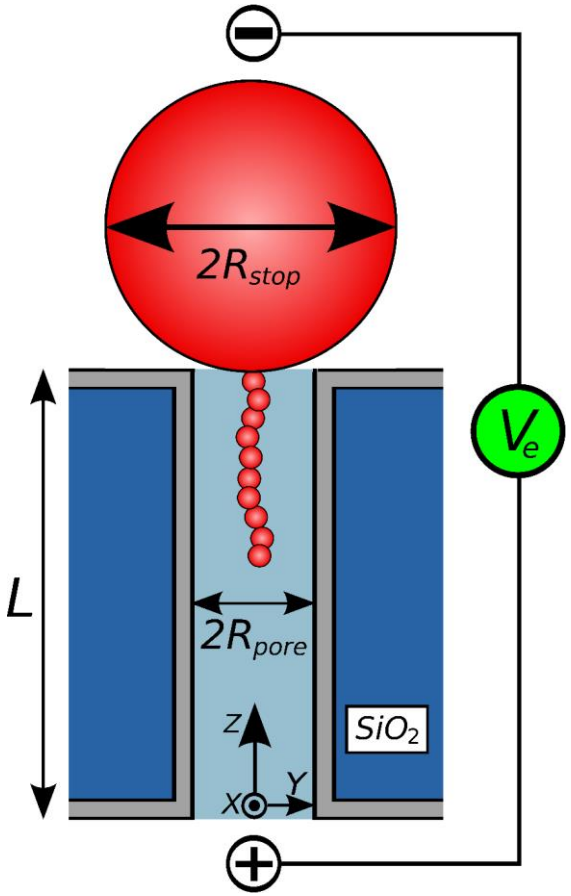


# MOLECULAR STOP

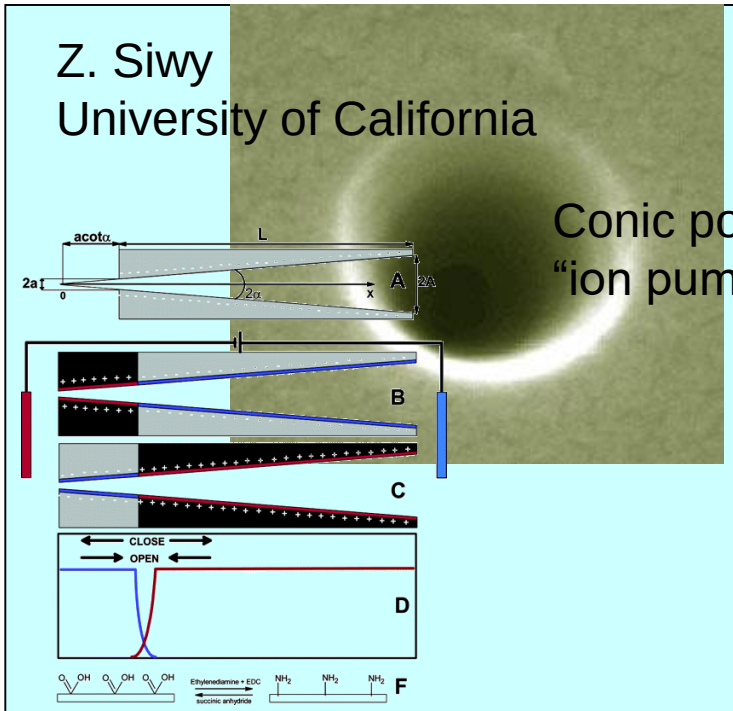
- ss-DNA can be attached to a much larger protein (in this case Neutravidin), preventing translocation (earning the name “molecular stop”)
- These systems greatly increase the resolution of nucleotide position, improving measurement accuracy



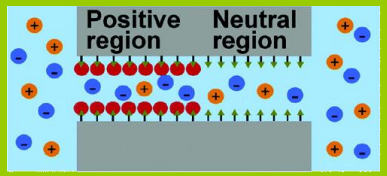



# SOLID-STATE NANOPORES TODAY: ION AND PROTEIN FILTERING

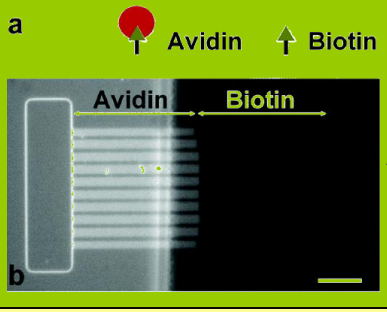
Z. Siwy
University of California




Conic pore
"ion pump"

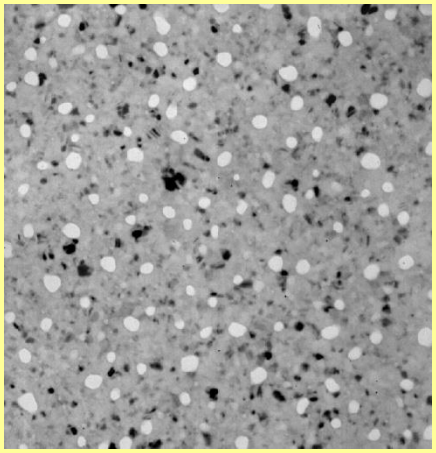


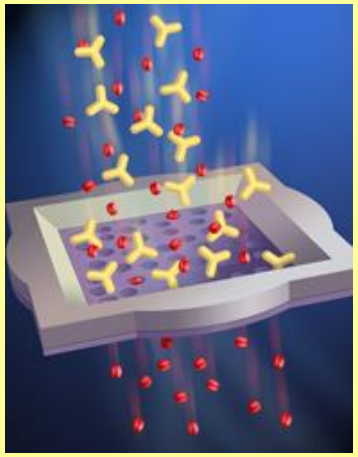

"Nanofluidic diode"



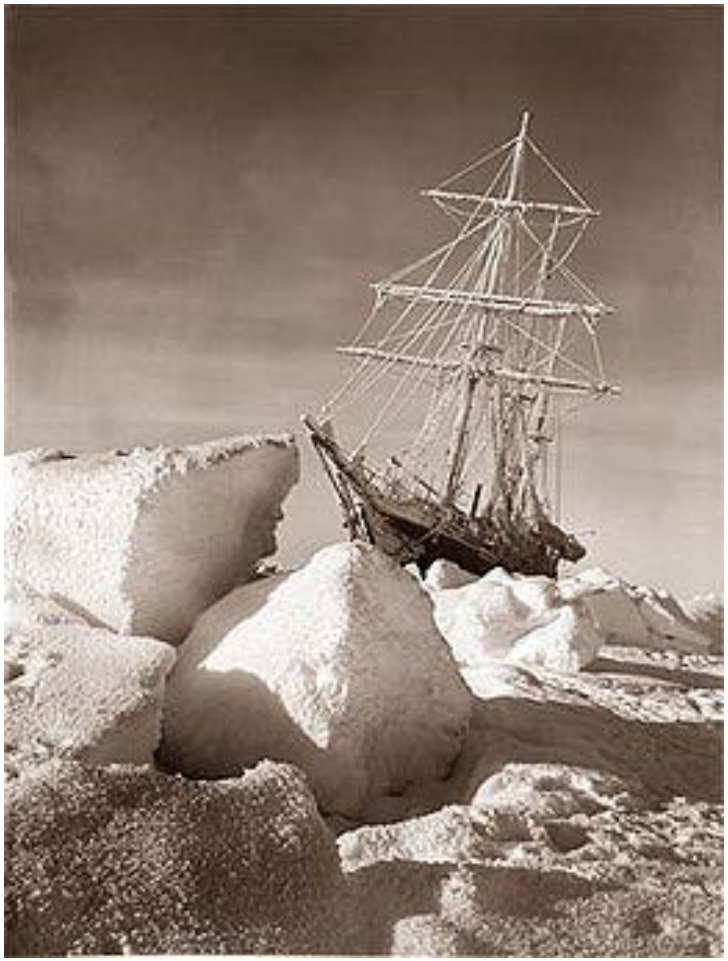

R. Karnik
A. Majumdar
University of California

Ph. Fauchet
University of Rochester





# RESEARCH IN SCIENCE



- *"MEN WANTED: For hazardous journey. Small wages, bitter cold, long months of complete darkness, constant danger, safe return doubtful. Honour and recognition in case of success."*
  *Sir Ernest Shackleton.*

*Ship "Endurance" traveled to the Antarctic but was trapped and lost in the ice on route; the crew was rescued by an open-boat journey to a whaling station at a distant island.*