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Nanopore sequencing technology: nanopore preparations

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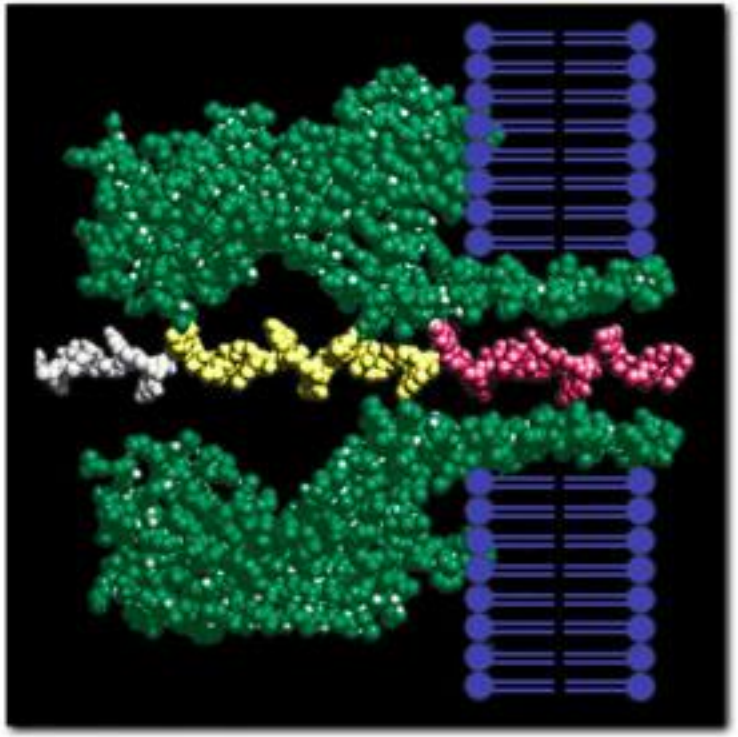
Additional sources:

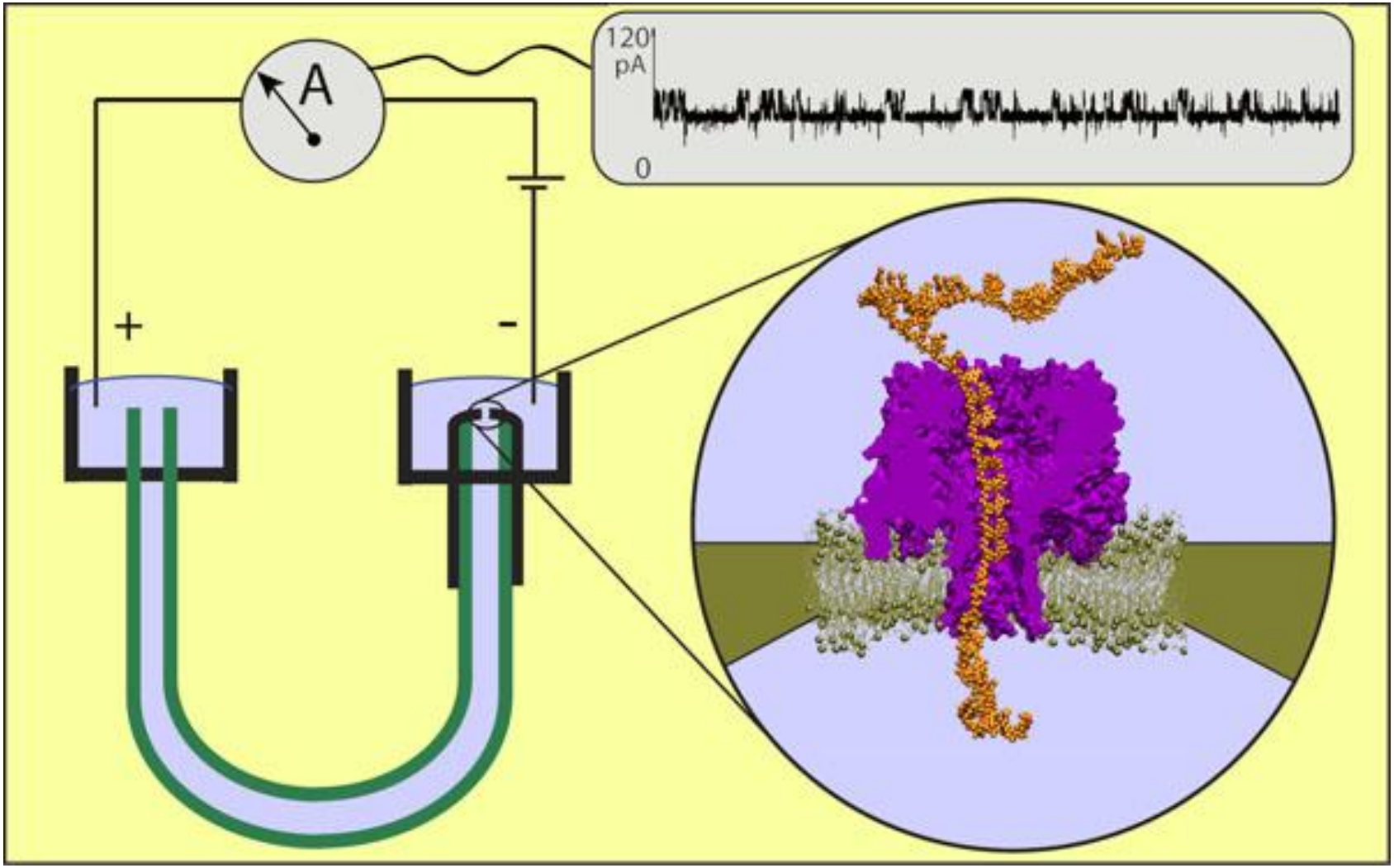
http://www.ks.uiuc.edu/Research/hemolysin/

Using nanopores

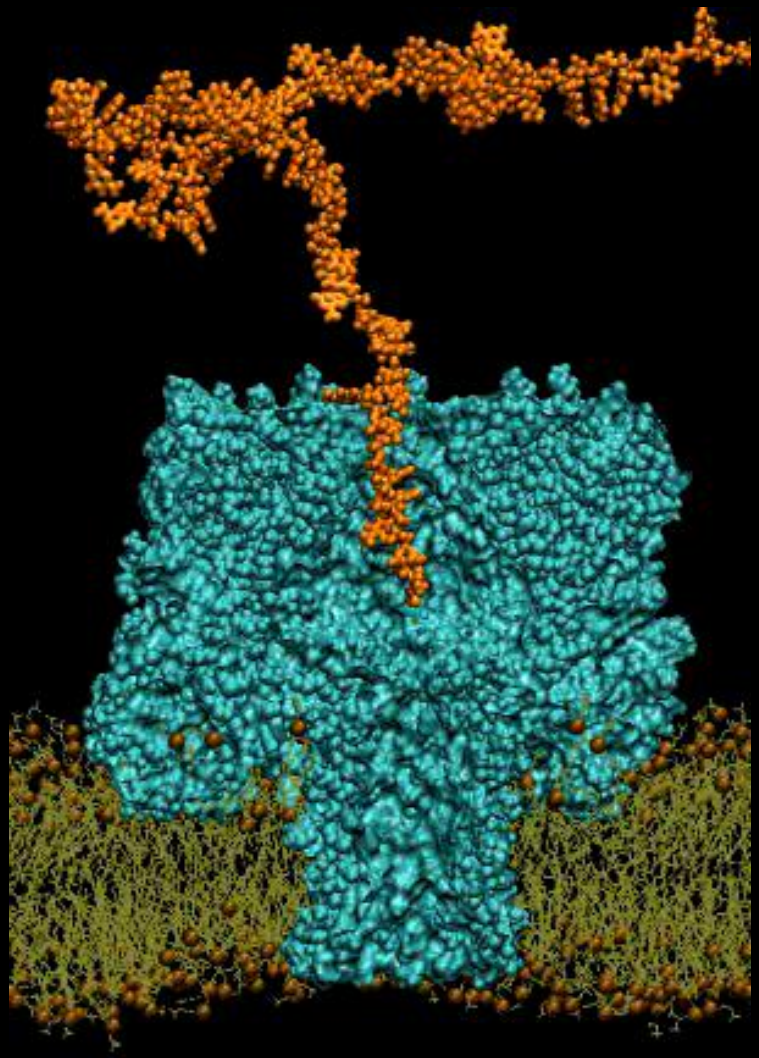
- Proven technique for sensing macromolecules
- A promising DNA sequencing method
  - single molecule sequencing
  - long reads
  - fast
  - simple preparation
- Currently limited success
  - can only discriminate
    - ssDNA / dsDNA
    - DNA / RNA homopolymers
    - single bases in hairpins



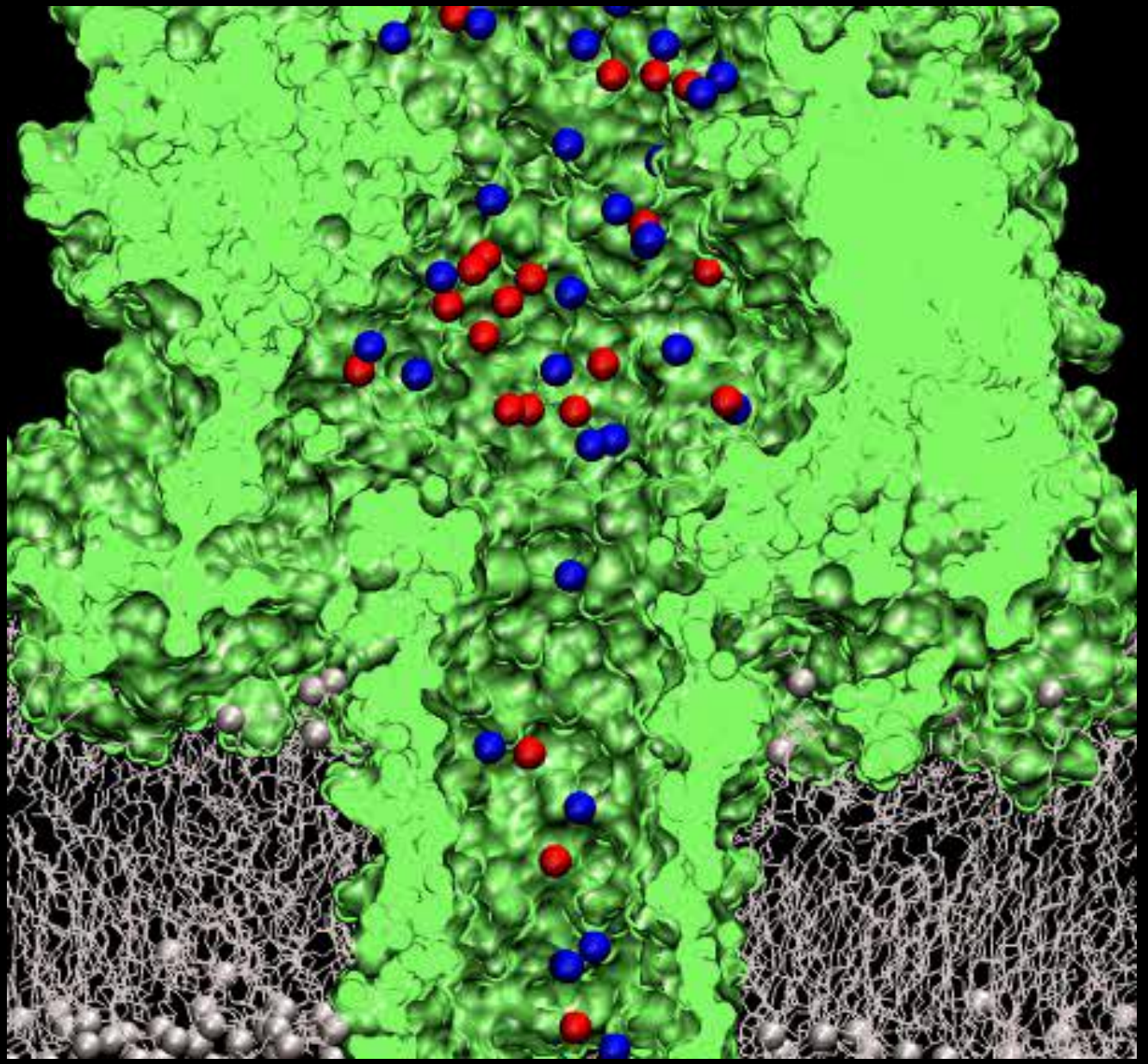
General nanopore detection principle



apply voltage, DNA moves through pore, changes in conductance, measure difference



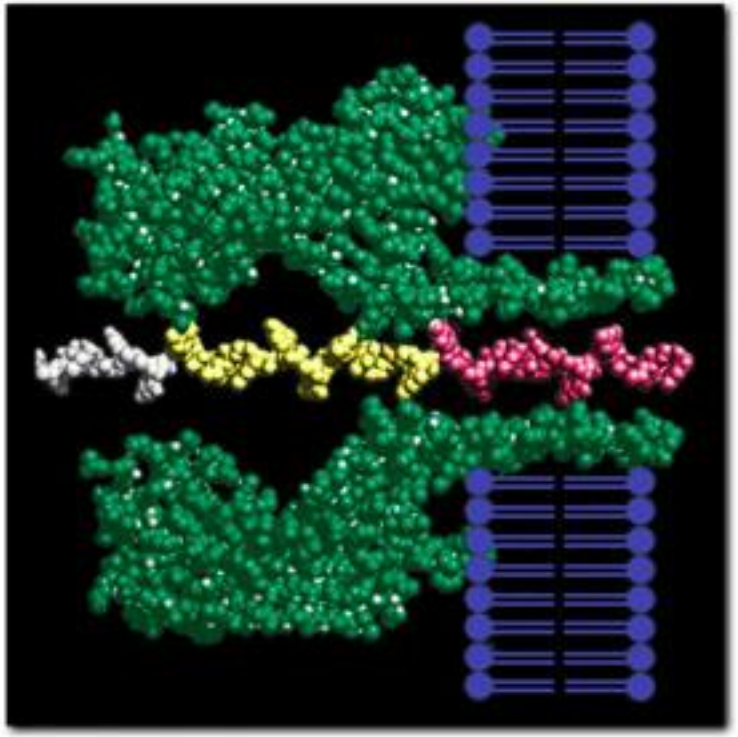
TRANSLOCATION OF DNA THROUGH α-hemolysin PORE



K+ and Cl- ION FLOW THROUGH THE PORE (U = 240mV)

A good pore

- Pore size must match analyte size
- Must be stable under various conditions
  - structural stability
  - electrical stability
- Easy to manufacture
- Possibility to integrate production
- Must be reproducible

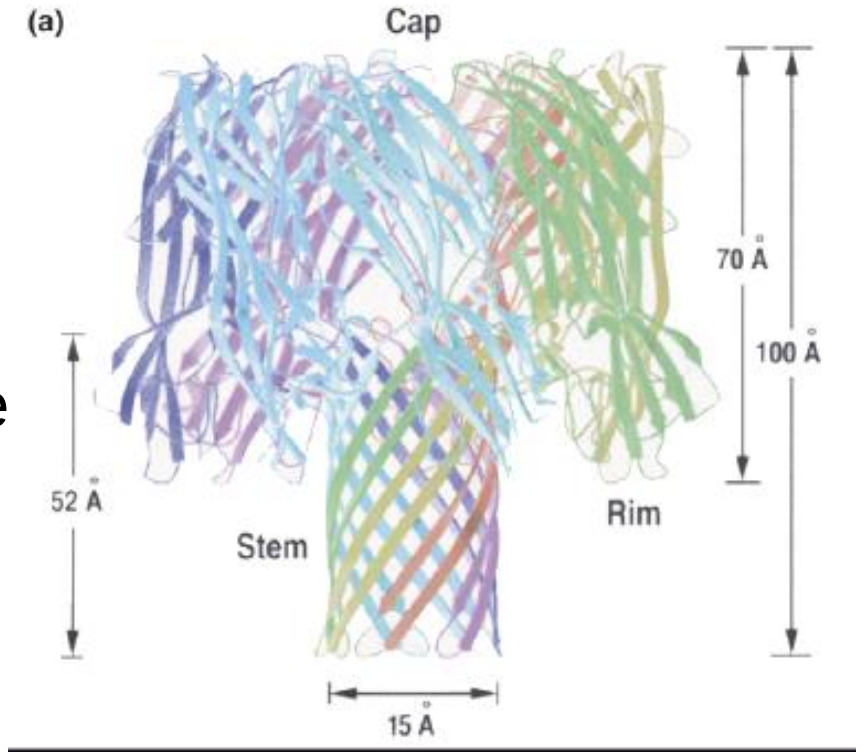


Nanopores for DNA analysis

- Organic nanopores
  - α-hemolysin
    - common usage (well researched)
    - ideal pore size
- Synthetic solid-state nanopores
  - various techniques
    - conventional
    - non-conventional
  - construction more flexible

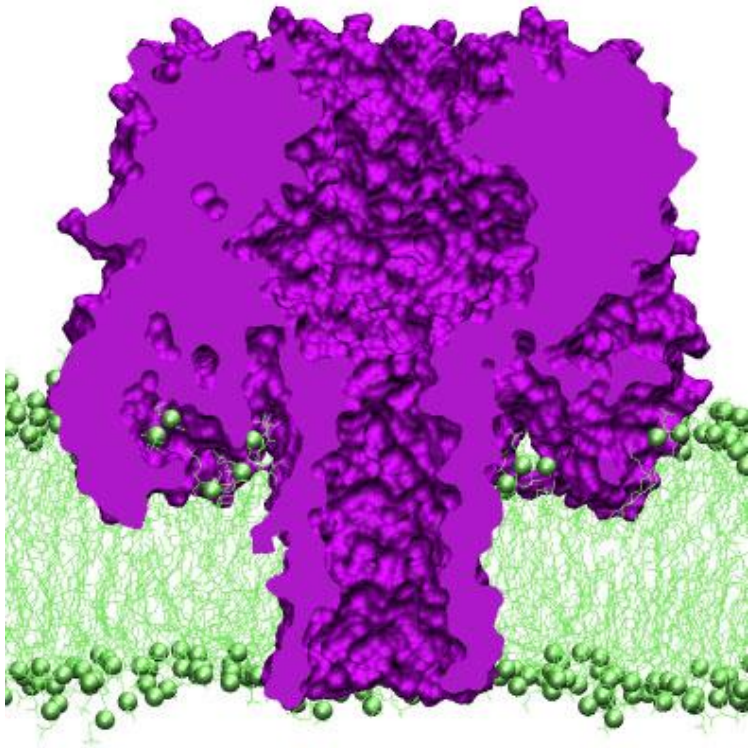
the α-hemolysin pore

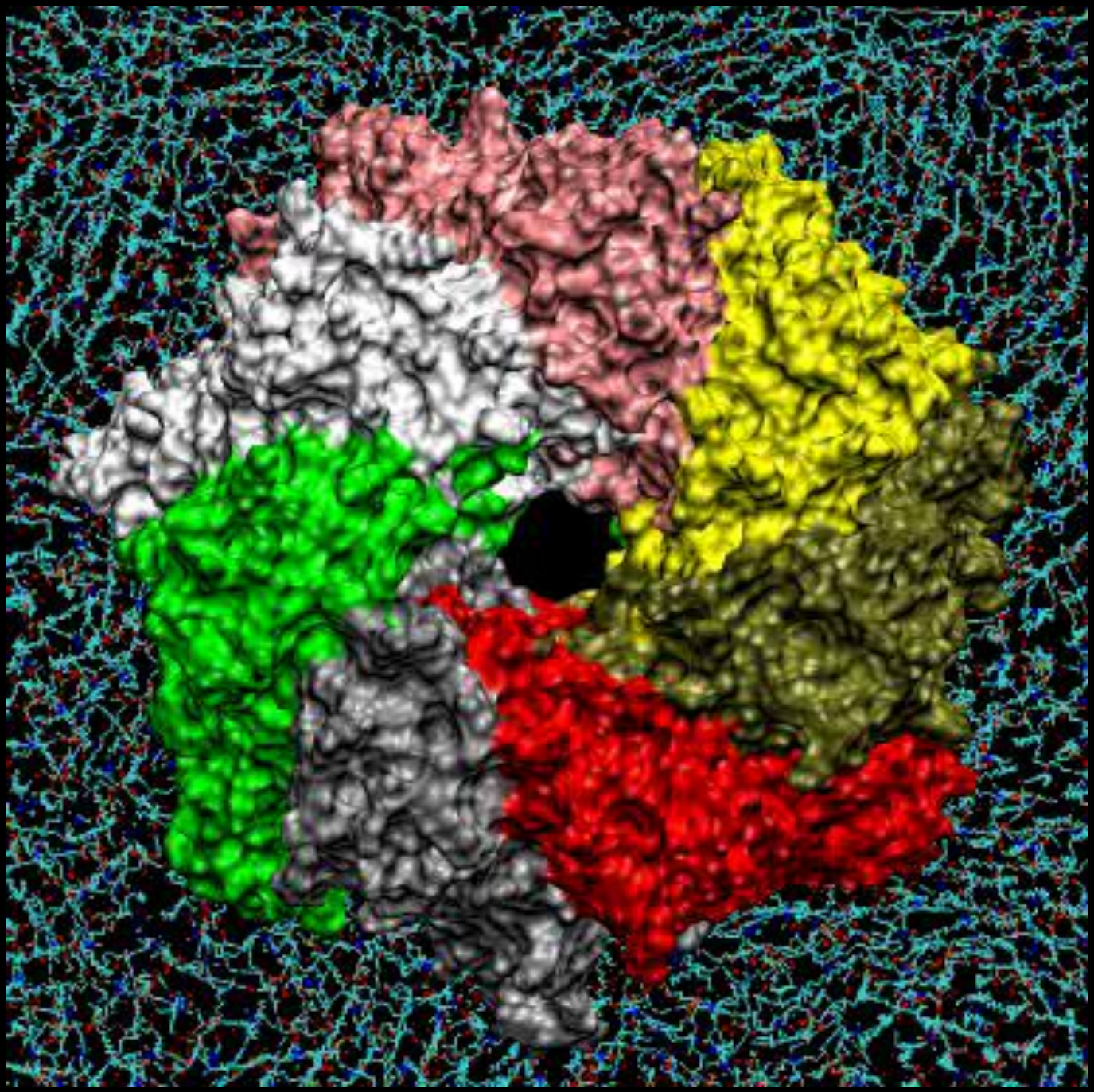
- First candidate for DNA detection
- *Staphylococcus aureus* 33kD monomeric transmembrane protein
- Self-assemble as heptamers on membranes and create a hydrophilic channel
- Pore size 15Å - ideal for 13Å ssDNA analysis
- Steady current over large range – low background noise
- Highly reproducible



α-hemolysin assembly

- Separate buffer with a membrane
- Insert α-hemolysin in buffer
- α-hemolysin will form a channel
- max I = 120pA (120mV)





Other organic pores

- Seek proteins with:
  - different pore sizes
  - less laborious preparation
  - less modification
  - more robust
  - even better reproducibility

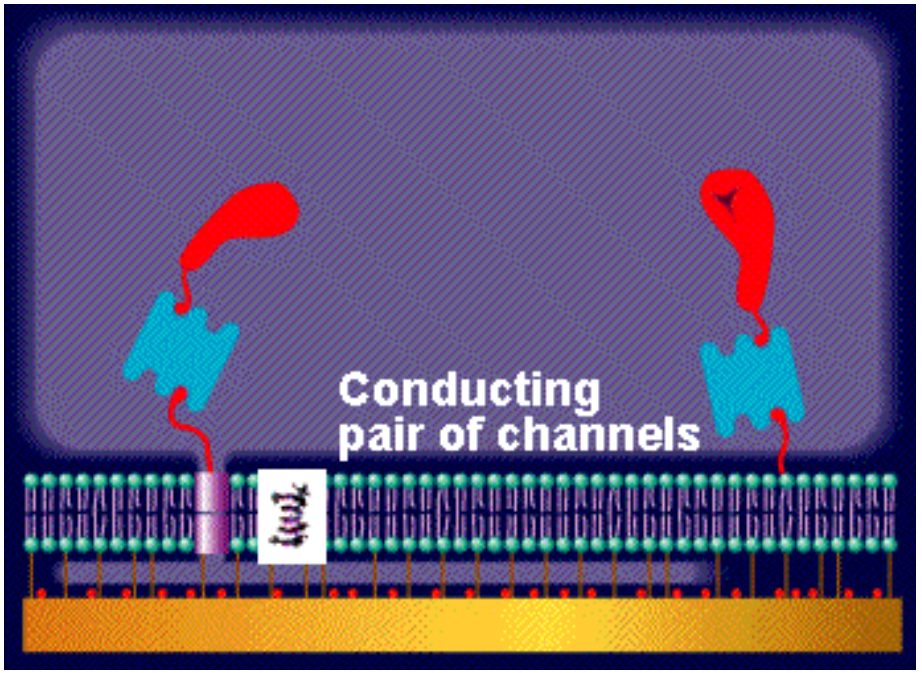
Other organic pores

- Mitochondrial porine (VDAC)
  - monomeric protein in outer m. membrane
  - 30Å aqueous pore (dsDNA)
  - formation similar to α-hemolysin
- Bacillus subtilis vesicle membrane ion channels
  - detect small plasmids
- E. Coli OmpF pores
  - pore size: 10–12Å (~ssDNA)
  - no DNA translocation so far detected

Other organic pores

- Nucleic acid binding protein (NAC) from a rat
  - opened by DNA presence → increase in current

- Gramicidin pores
  - too small to translocate ssDNA
  - channel subunits mobile
  - fasten probe DNA to membrane
  - target DNA will bind to channel
  - on match subunits will be misalgined → current decrease
  - measure hybridisation reaction rates



Synthetic nanopores

- Potential advantages
  - larger choice of sizes
    - protein pore diameter is fixed
  - better stability
    - lipids are fragile
    - proteins withstand only limited electrical oprations
    - simple charge distribution
      - protein conductance = sum of 10-15 nucleotides in pore
  - invariable in performance

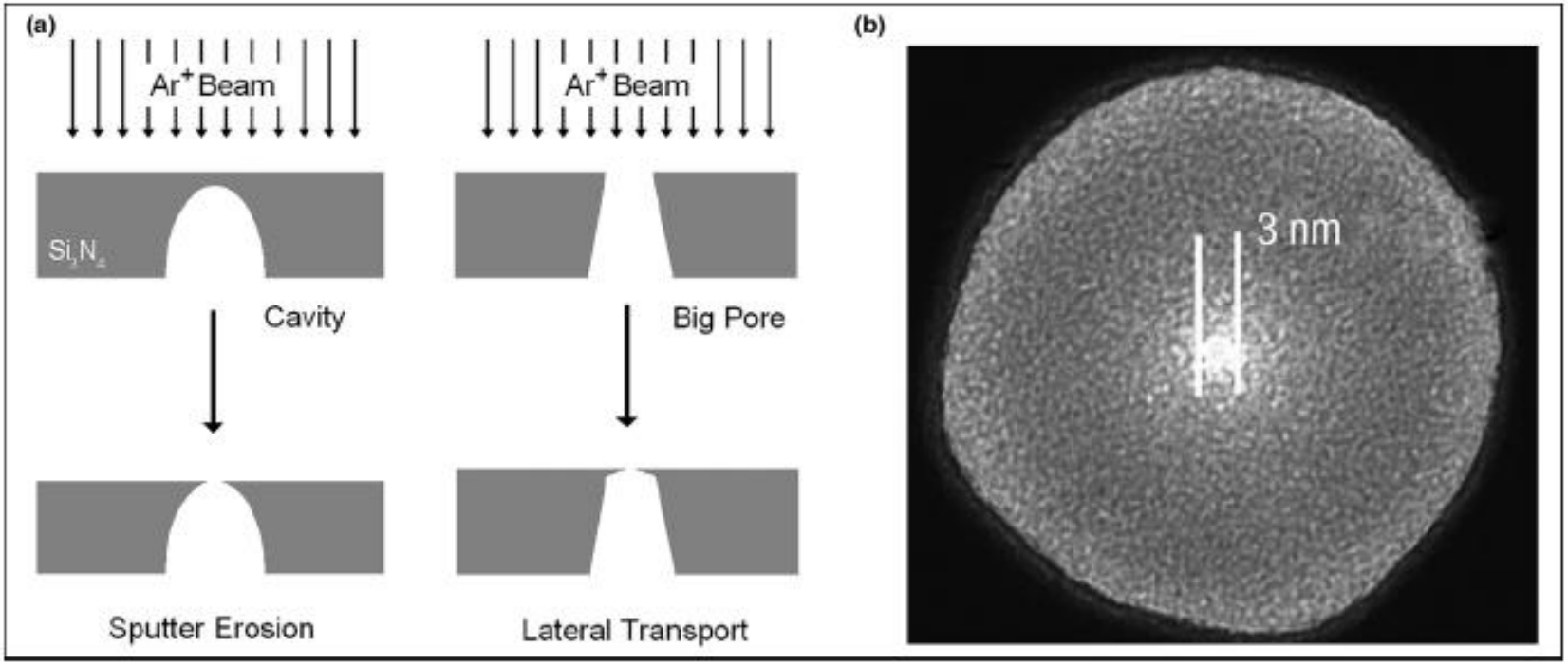
Synthetic nanopores

- State-of-the art photolithography semiconductor technology
  - disadvantage: features > 100Å

- Ion beam sculpting
- Micromolding
- Latent track etching
- Electron beam-induced fine tuning
- Inorganic nanotubes

Ion beam sculpting

- Bombard material with high energy ions (>1000 eV)
  - < 5C → sputtering – surface atoms removed
  - > 5C → lateral transport
- Fine-tune with ALD (atomic layer deposition)
  - fine tune pore size: ~1Å per cycle + add insulating Al_2O_3 coating
  - final result: 18Å min. size



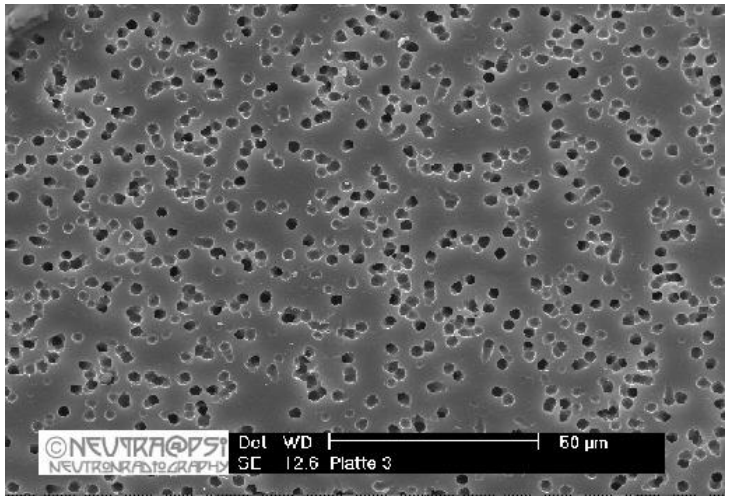
Micromolding

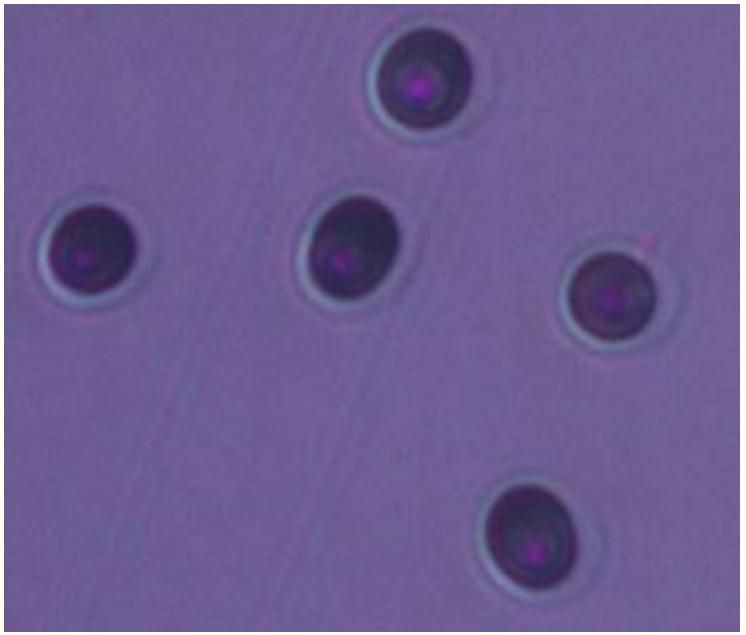
- Conventional lithographic techniques
  - create a reusable master mold
    - pore: electron beam lithography
    - reservoirs: photolithography
    - substrate: silicon
  - cast into poly(dimethylsiloxane slab)

- Results:
  - pore size: 300Å (suitable for larger molecules)
  - simple, fast, reproducible
  - can create arrays of pores for parallel analysis

Latent track etching

- Method
  - Irradiate polymer film with fragments of energetic heavy ions
  - Ion fragments create a track into polymer on impact
  - Track can be etched chemically forming one-pore membrane
  - Coat pore walls with gold solutions → gold nanotubules
- Results
  - 20Å (with gold coating)





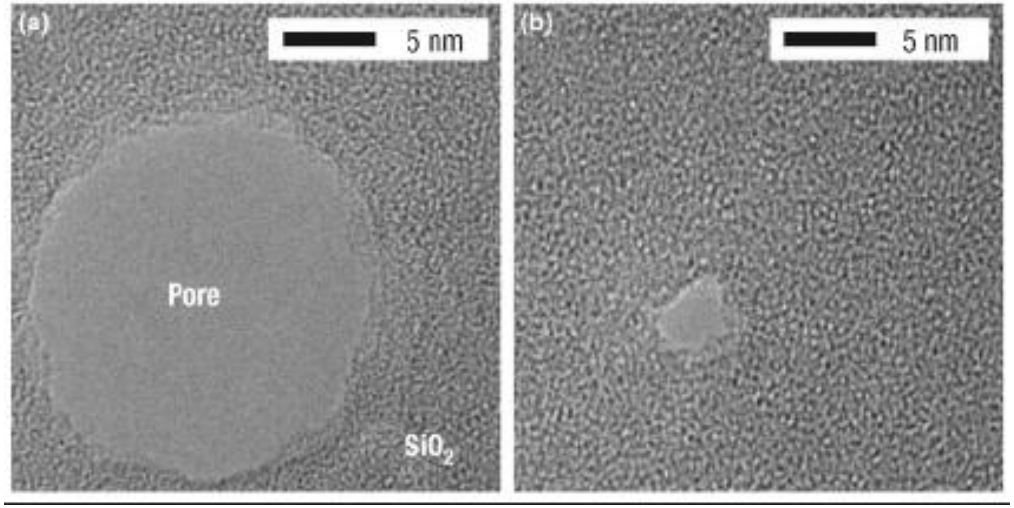
Electron-beam induced fine tuning

- Method
  - Fabricate 340nm thick silicon membranes
  - Create 40nm SiO2 layer on both sides of membrane
  - Remove some SiO2 masks by laser-beams and etching
  - Drill holes with KOH etching + cover with 40nm SiO2
  - Fine tune with 300kV transmission electron microscopy
    - electron beam shrinks pores ~3Å / min
    - shrinkage depends on beam intensity and initial pore size

- Modifications: Ion beam sculpting + E-beam tuning
- Results: 10Å pore size (100Å before tuning)

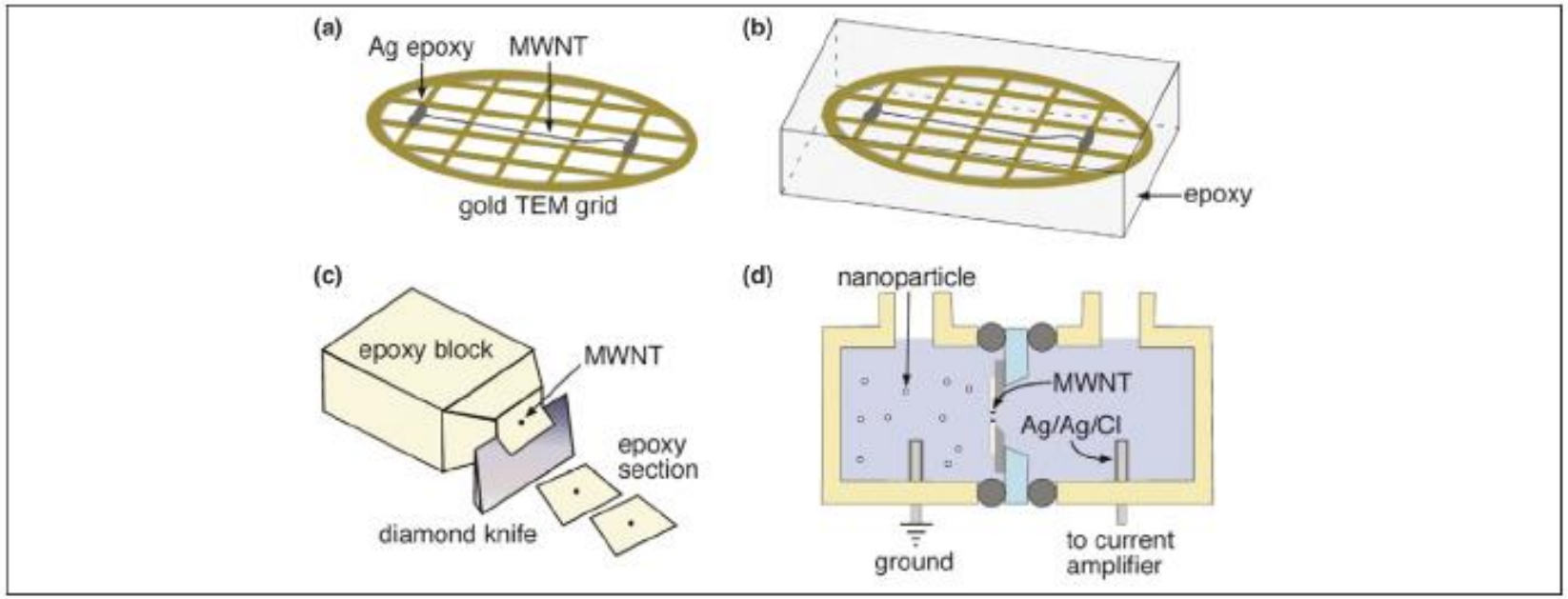
- Advantages:
  - tight focus
  - direct visual feedback
  - variety of usable materials

- Disadvantages:
  - underlying physics not understood
  - might not be well reproducible



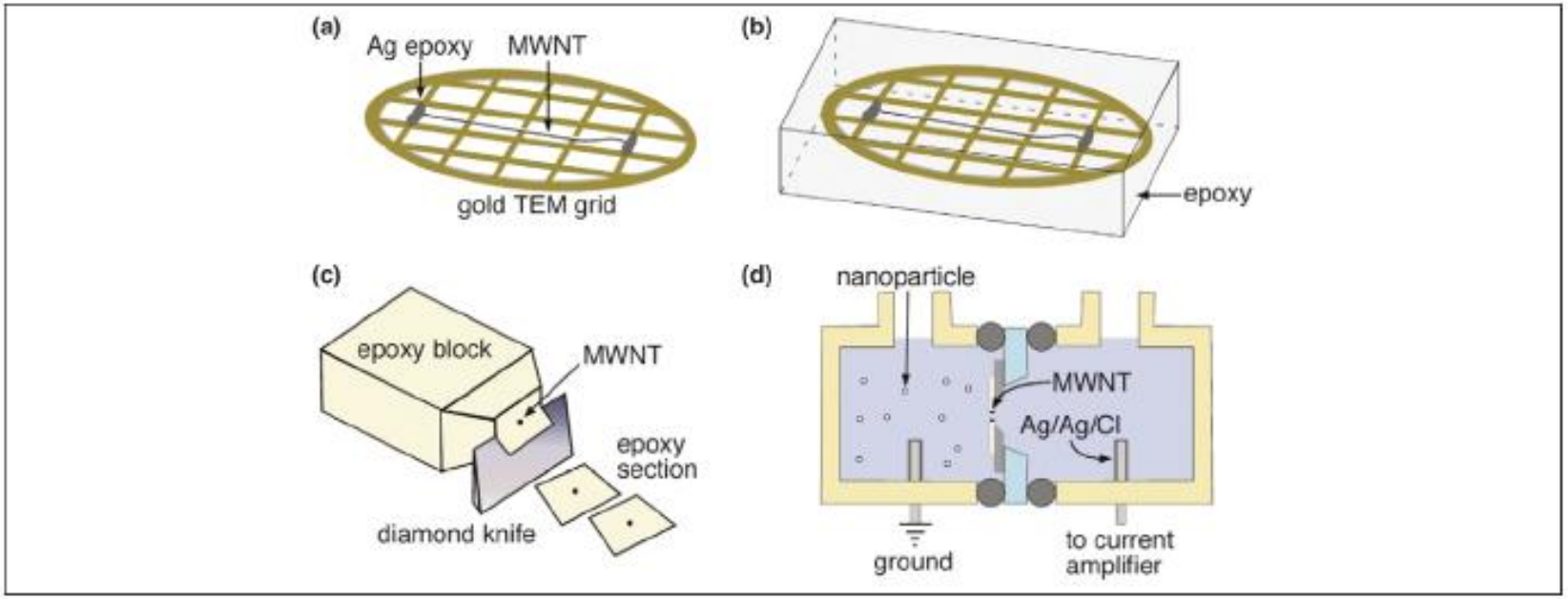
Inorganic nanotubes

- Method
  - Create a carbon nanotube
  - Place on a grid
  - Seal in liquid epoxy
  - Cut thin slices from solid epoxy blocks
- Results
  - 500Å channels



Inorganic nanotubes

- Advantages
  - carbon properties well defined
    - carbon is electrically neutral
    - chemistry, structure known
    - easy to make in various sizes
  - no high energy beams → electric properties unchanged



Summary

- Most analyses performed with α-hemolysin
- Synthetic won't replace α-hemolysine in near future
  - underlying physics complicated
  - can give unreproducible results
- But are worth investigating
  - cheap integrated sequencing systems

Table 1. Nanopores from various preparation procedures

	Protein α-Hemolysin assembly	Synthetic Ion beam sculpting	Micro-molding	Latent track etching	E-beam fine tuning	Nanotubes
Minimum pore diameter reported (nm)	1.5 (fixed)	1.8	80	2.0	1.0	50
Membrane material	Lipid bilayer	Si_3N_4	PDMS	Poly-carbonate	Si, SiO_2, Si_3N_4	Epoxy, Si_3N_4
Pore material	α-hemolysin	Si_3N_4, Al_2O_3		Gold layer		MWNT
Remarks	Mass production	Size tuning	Easy fabrication	Conical shape	Visual fine tuning	Stable, uncharged