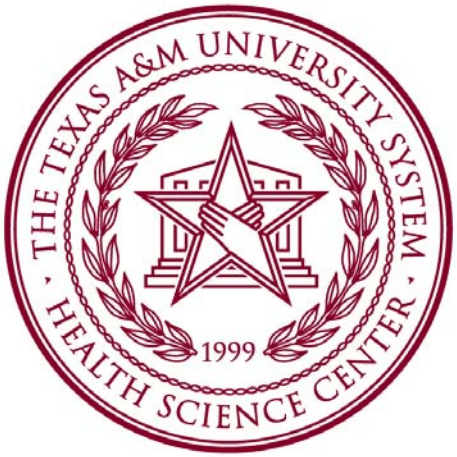
Emerging strategies for engineering channels and pores

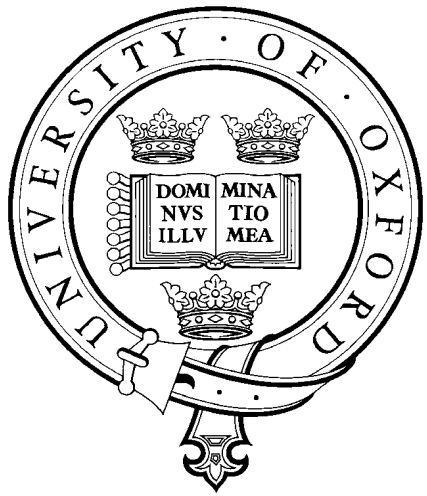
NIH- Nanomedicine Workshop

4th May, 2004

Laboratory of Hagan Bayley

University of Oxford





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OUTLINE

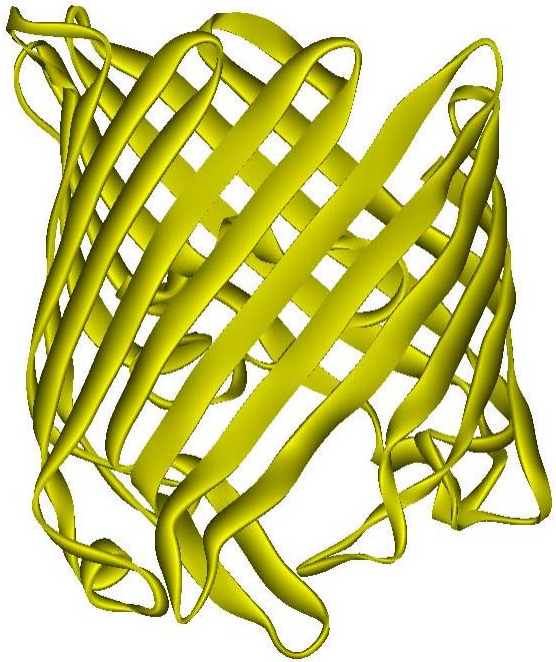
- Emerging applications of nanopores
- Protein engineering as applied to channels and pores
- Review how protein nanopores are made
- Examples of engineered pores
- Additional and improved engineering strategies
- Non-protein nanopores and future applications

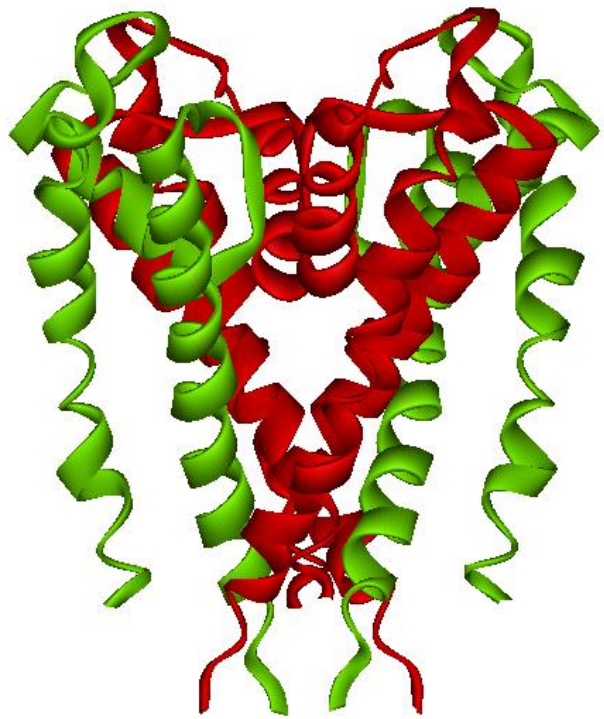
Emerging applications of engineered pores in nanomedicine

- molecular sensing (stochastic sensing)
- drug delivery
- therapeutics
- cryoprotection and desiccation of cells
- components of nanodevices and nanomachines
- tools in basic cell biology

Engineering membrane proteins

- Redesign- change an existing structure (while maintaining the scaffold)
- De novo design
- In vitro evolution/ combinatorial approaches
- Unnatural amino acids





β barrel- favors redesign

- for example, the open structure of the barrel interior has few significant side-chain/ side-chain interactions

- start with something complex and change it

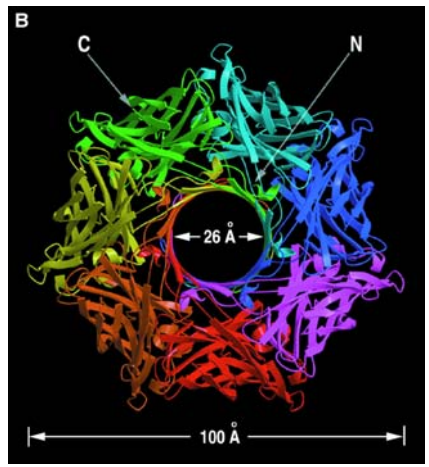
…. or ….

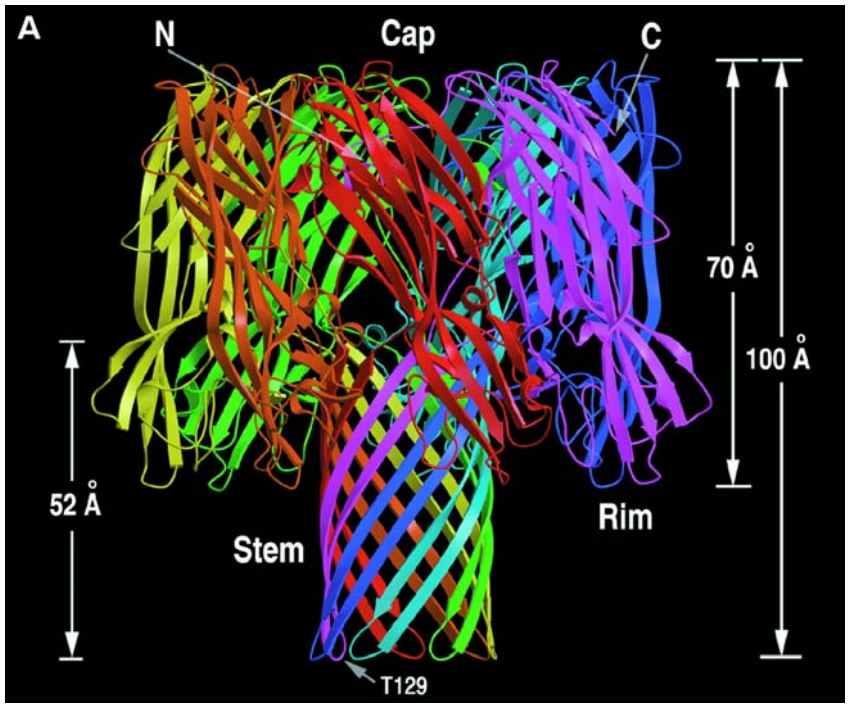
- start with something simple and build function into it

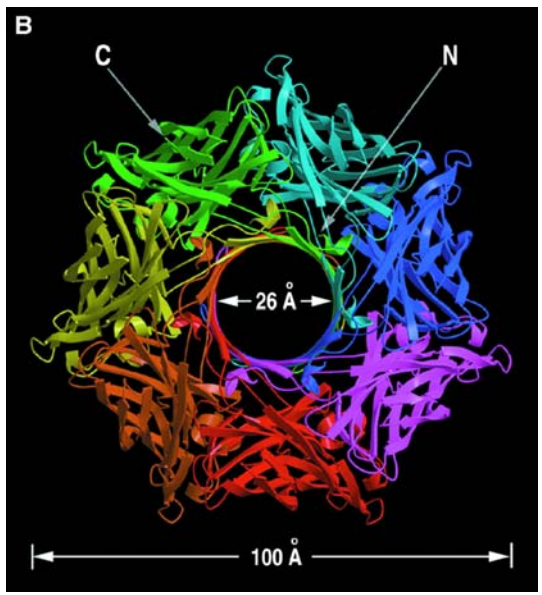
Inside the chimney of a Cornish tin mine

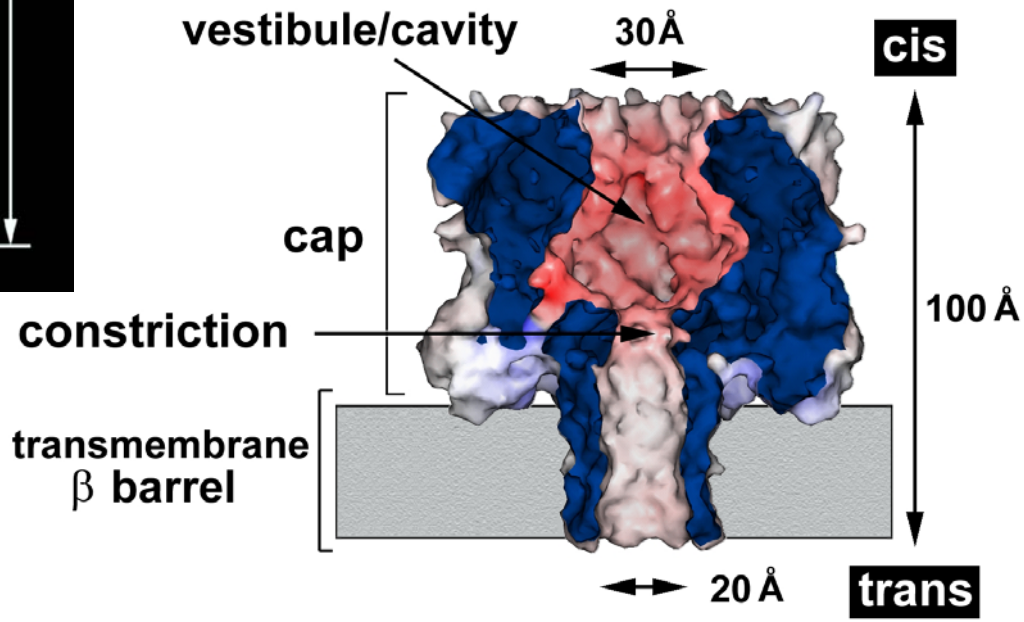
Why is staphylococcal α-hemolysin a good target for engineering?

- easy to express, robust etc.
- high resolution structure
- the wild-type protein doesn't do much- a blank slate
- β barrel is easy to engineer
- the pore stays open- no spontaneous gating









Science 274, 1859-1865 (1996)

Summary of engineered pores

- many made for stochastic sensing

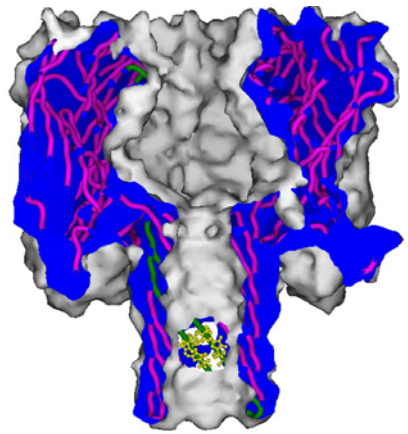
Analyte	Engineering technique	Heteromer or homomer	Site of engineering
divalent metal ions	introduction of His clusters	heteromer	barrel
phosphate esters	introduction of Arg clusters	homomer	barrel
reactive molecules	introduction of Cys residues	heteromer	barrel
organic molecules	use of non-covalent molecular adapters	homomer	barrel
proteins	tethered ligands	heteromer	cavity
nucleic acids	tethered oligonucleotides	heteromer	cavity

Analytes produce partial block or otherwise alter the conductance of the pore

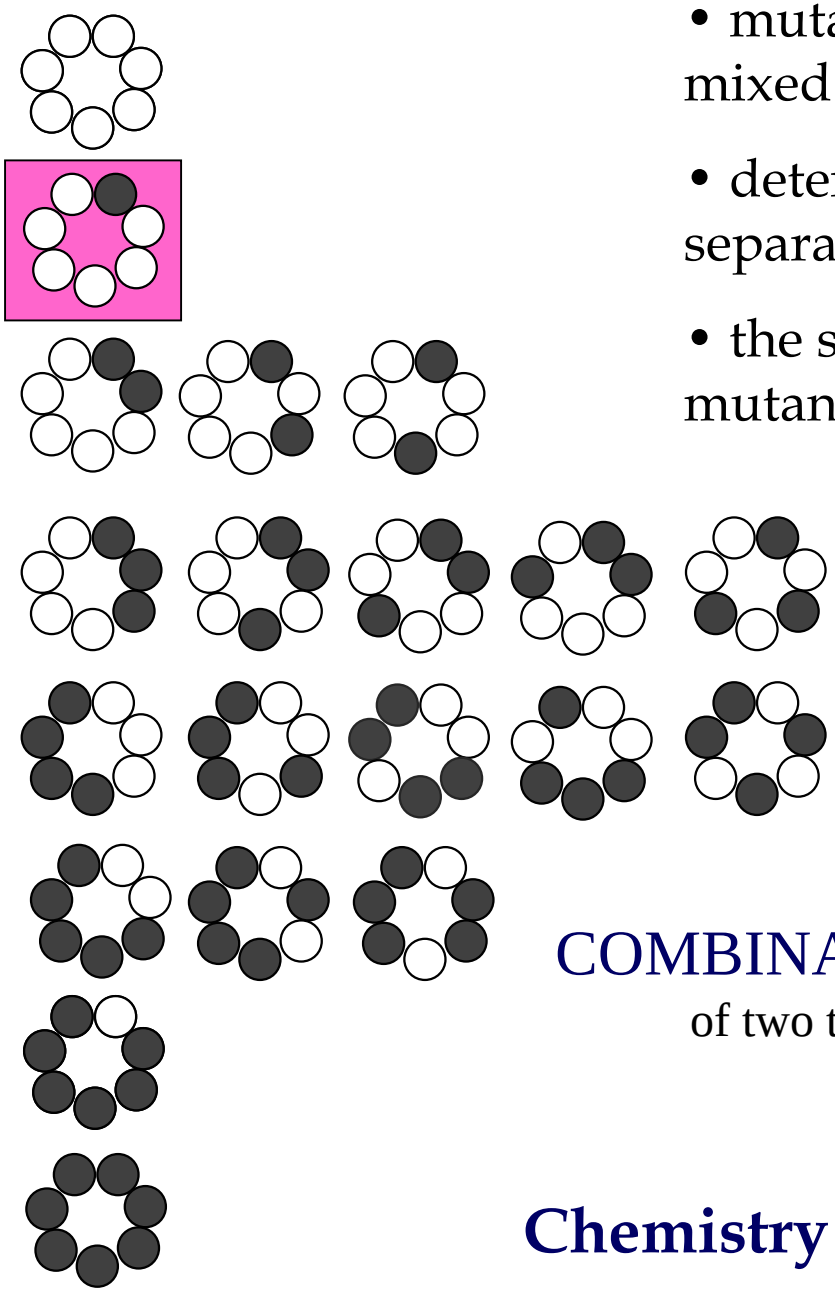
ENGINEERED PROTEIN NANOPORES

VARIOUS ENGINEERING TECHNIQUES

- Genetically engineered binding sites- anions & cations
- Non-covalent modification
  adapters- organic molecules
- Covalent modification
  functionalized polymers- proteins
  oligonucleotides- nucleic acids
- Subunit-subunit interactions
  -pore size



Alterations are inside the barrel or the interior of the cap
In many cases the preparation of heteromers is required



How are heteromers prepared?

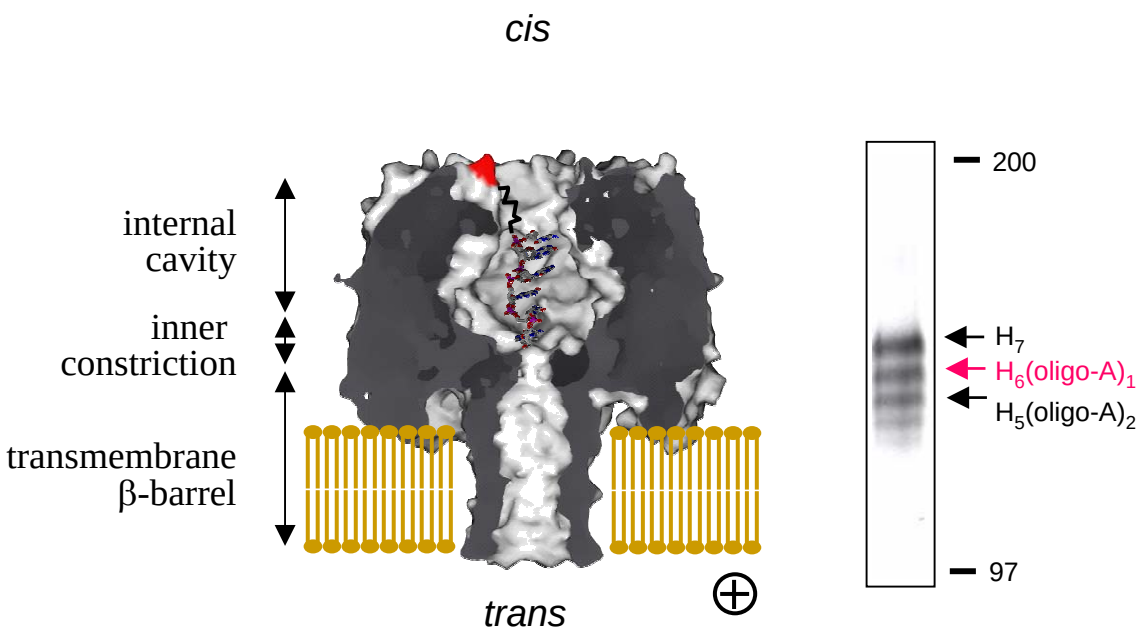
- mutated and unmutated subunits are mixed and assembled
- detergent-resistant heptamers are separated by electrophoresis
- the separation is based, for example, on mutant subunits with oligoaspartate tails

COMBINATIONS and PERMUTATIONS

of two types of subunit in a heptameric ring

Chemistry & Biology 4, 497-505 (1997)

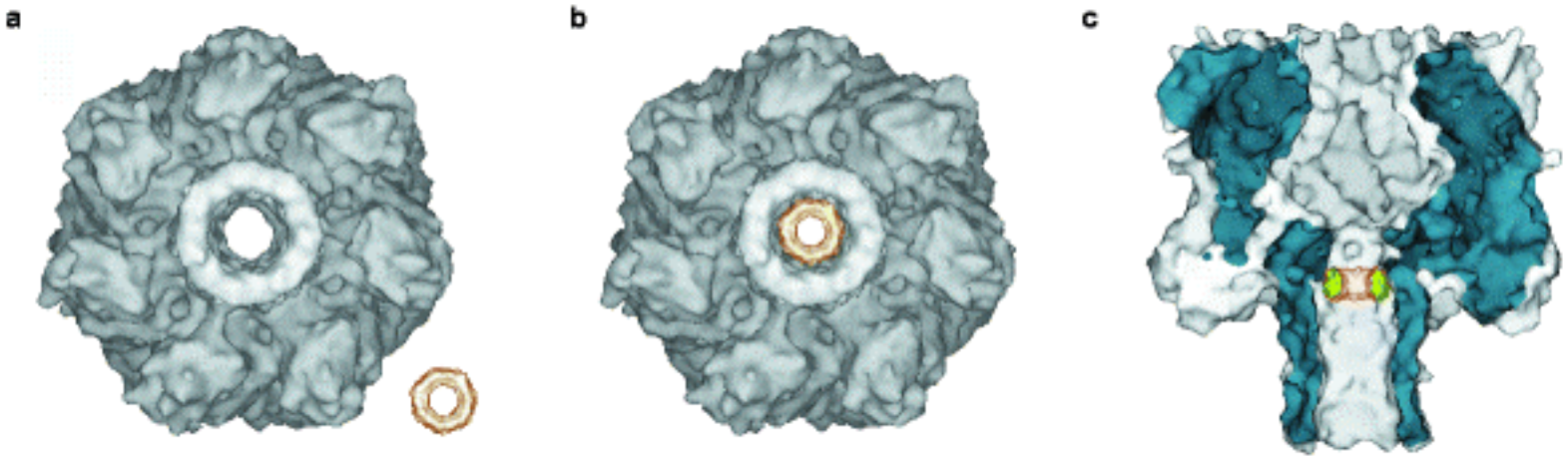
Example:
a heteroheptameric DNA-nanopore



Nature Biotechnology 19, 636-639 (2001)

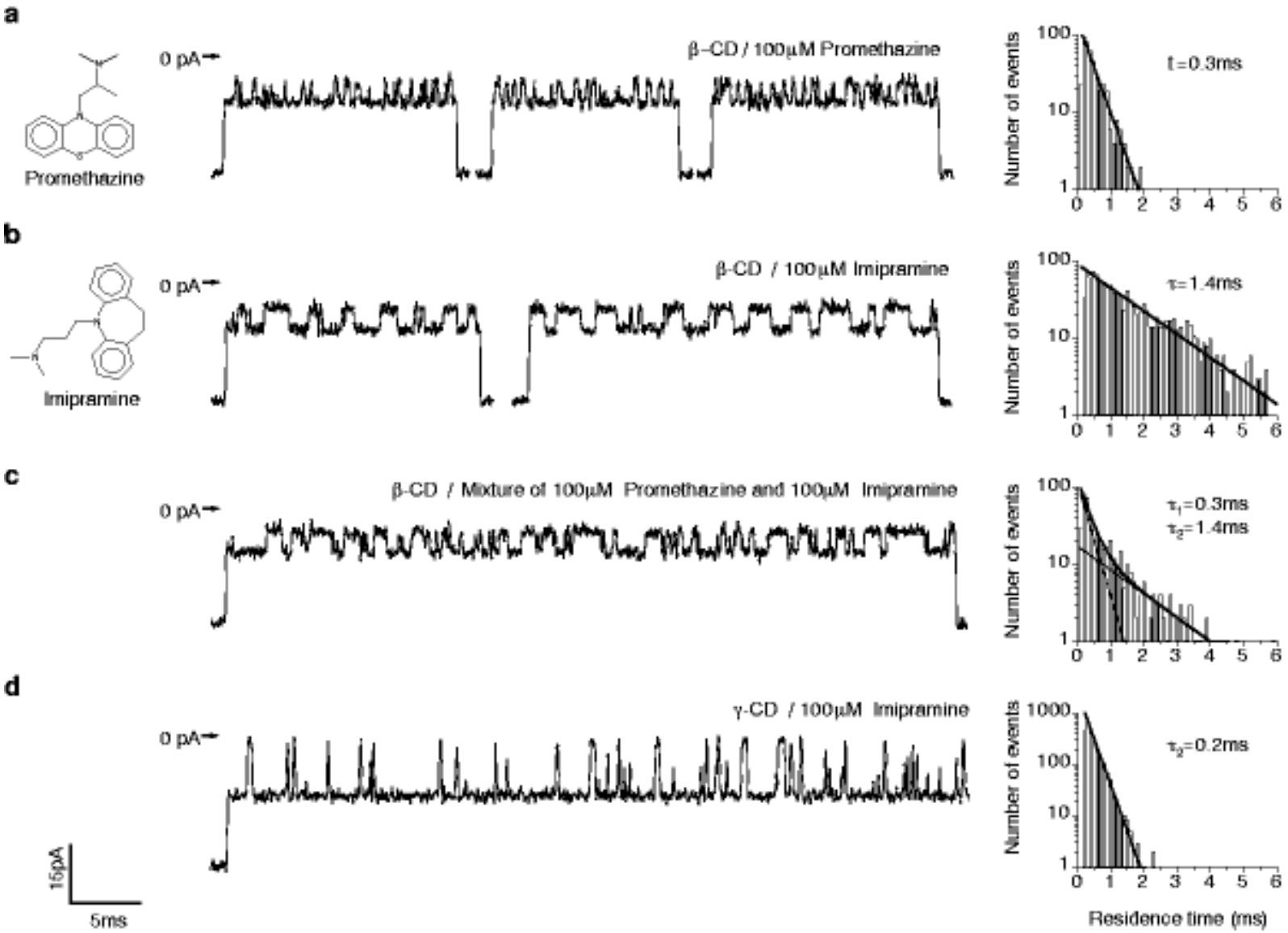
Selected examples of engineered pores

β-Cyclodextrin as a molecular adapter



Nature 398, 686-690 (1999)

Distinguishing tricyclic drug molecules

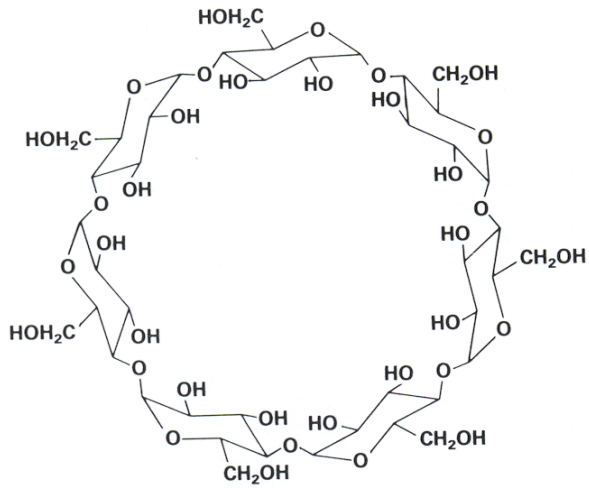


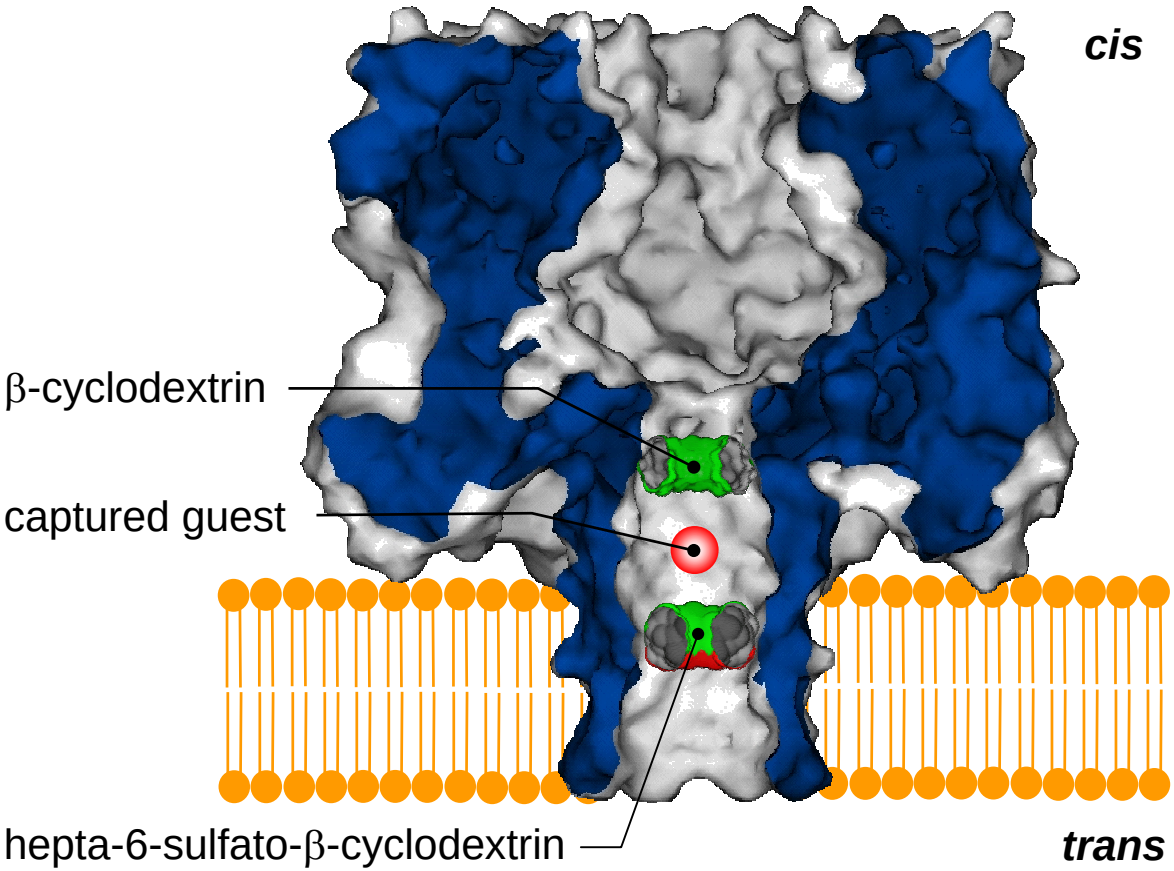
The use of molecular adapters is highly combinatorial

- many different analytes can be detected with one adapter
  binding in stochastic sensing need not be highly specific

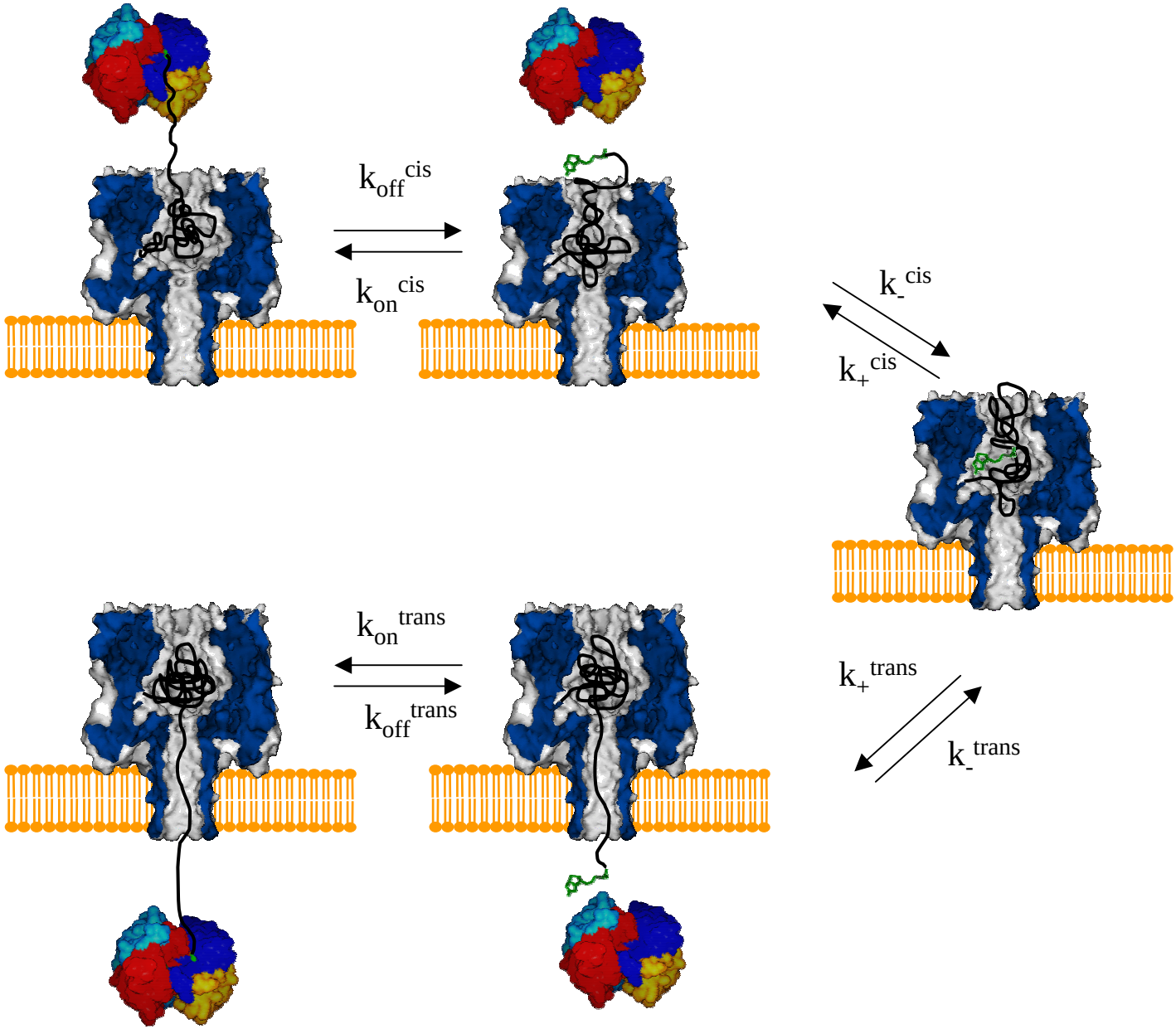
- many different adapters are available

- the pore can be engineered to accommodate the adapters



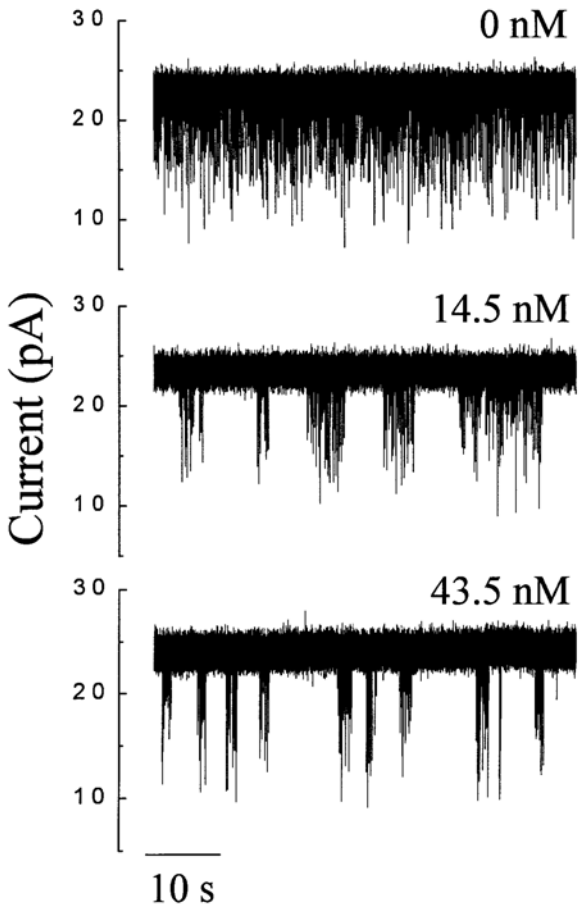


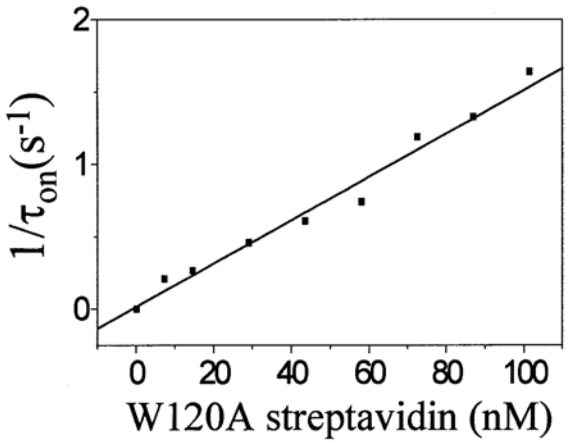
Science 291, 636-640 (2001)



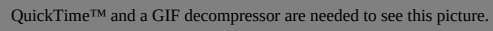
Nature Biotechnology 18, 1091-1095 (2000)

Proteins can be detected at nanomolar concentrations





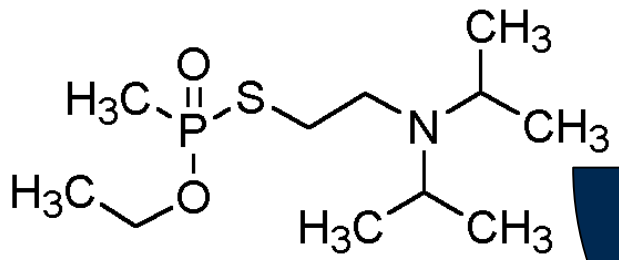
Langevin Dynamics Simulation

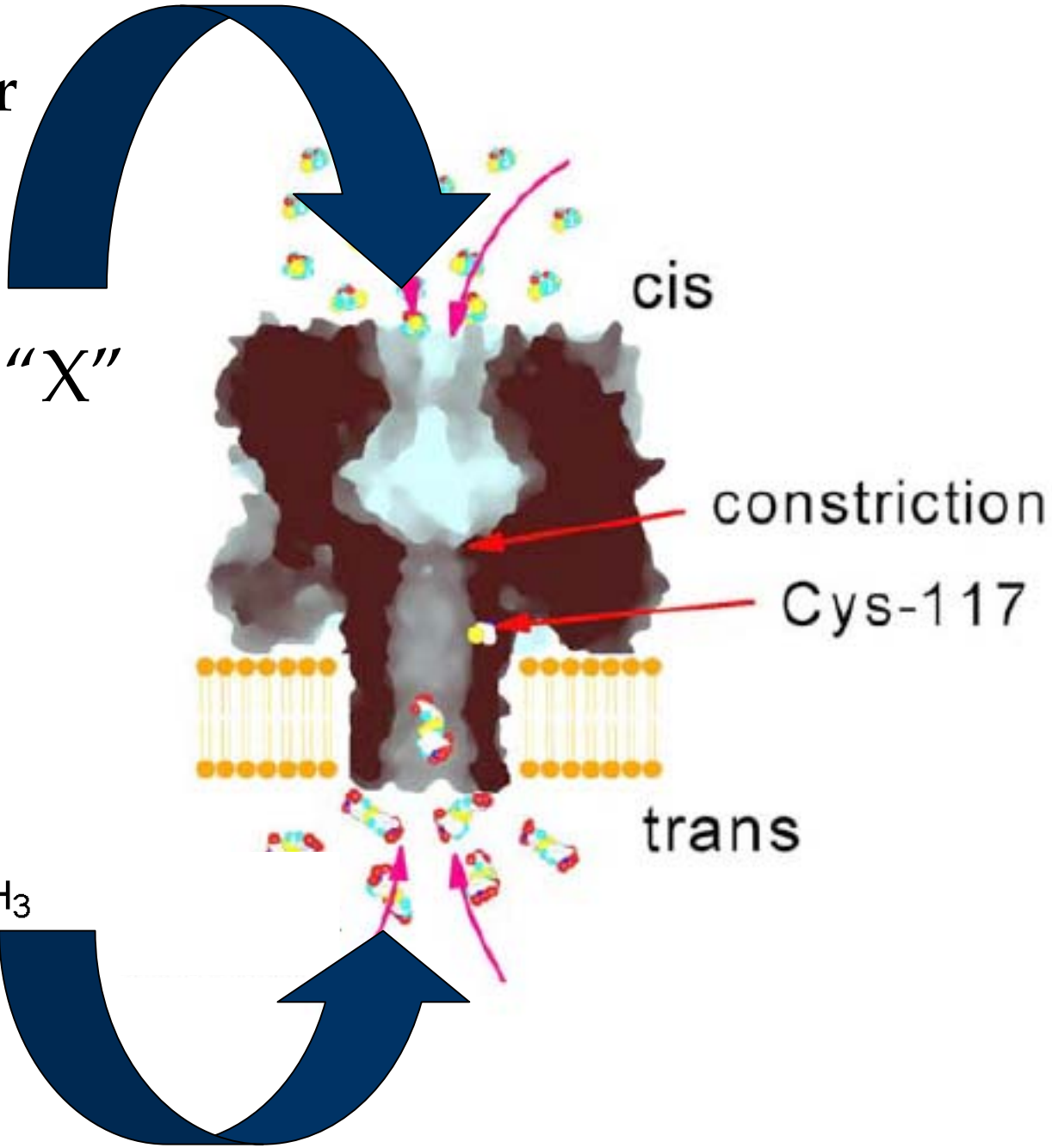


Work of Murugappan Muthukumar- University of Massachusetts at Amherst

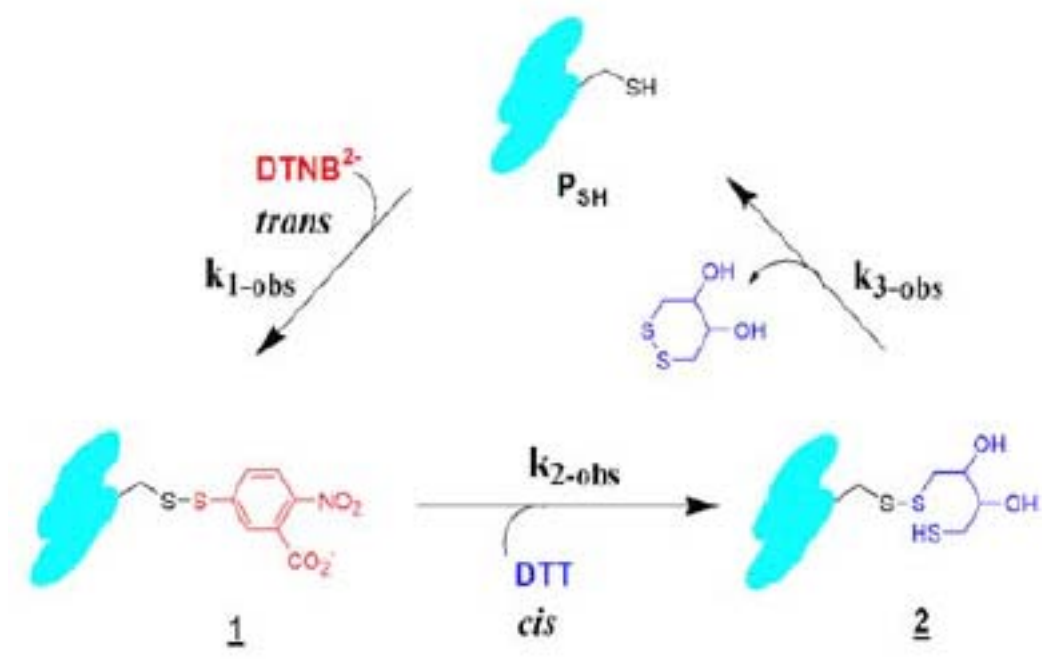
Pore as a **nanoreactor**: for irreversible chemistry with turnover/ detect reactive analytes

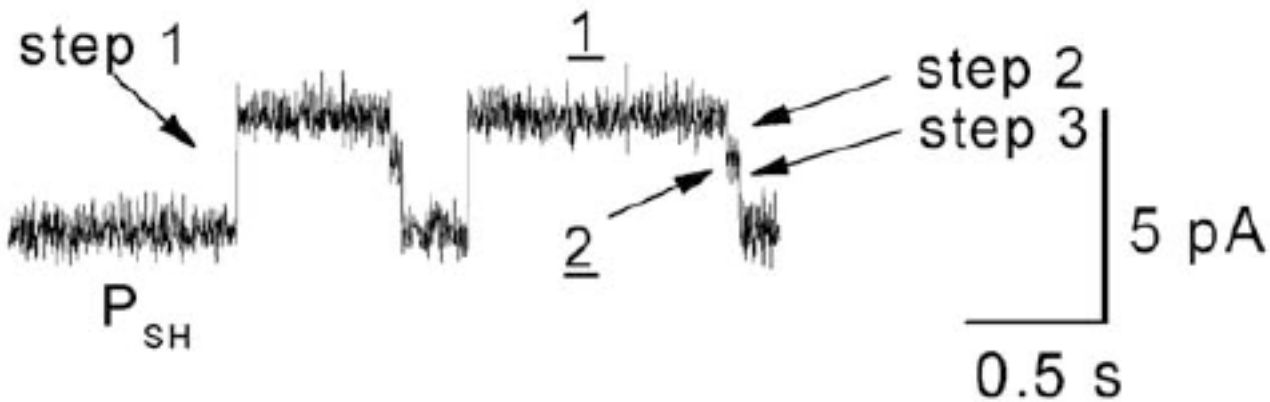
"X"





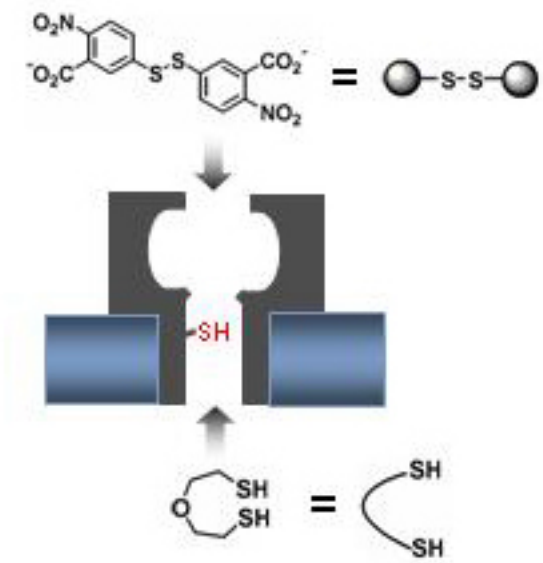
Irreversible chemistry with **turnover**: model reaction

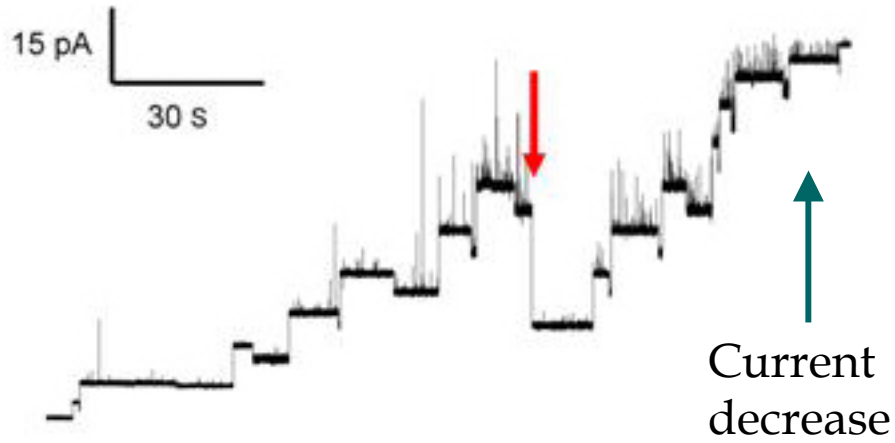


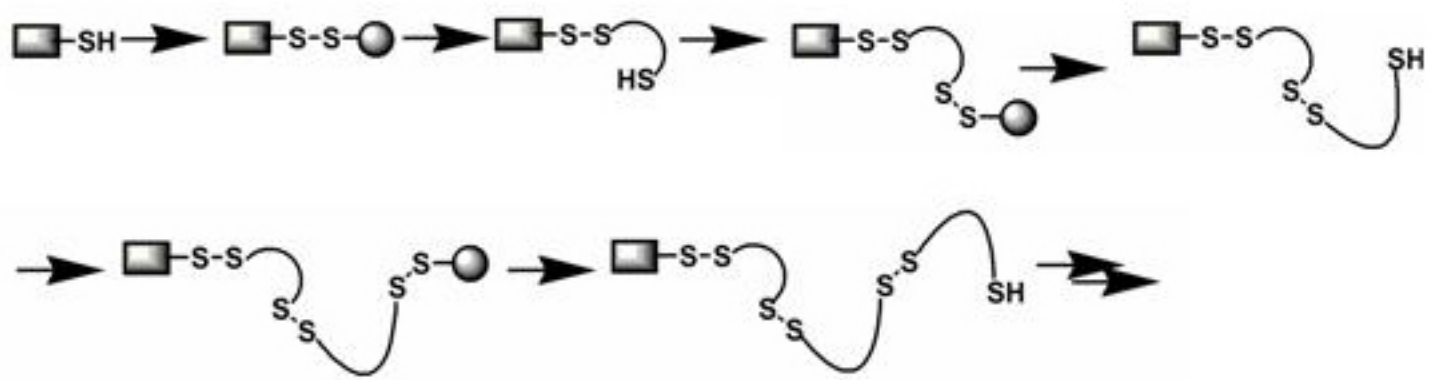


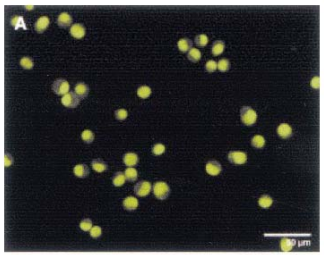
Angew. Chem. Int. Ed. 42, 3766-3771 (2003)

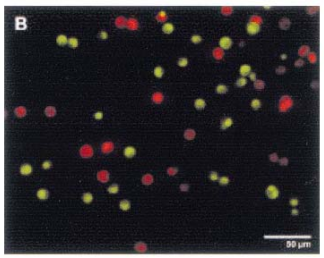
Polymerization

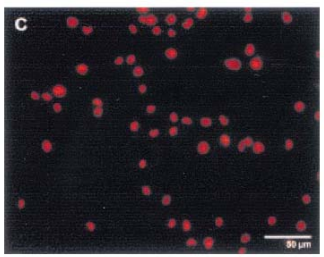






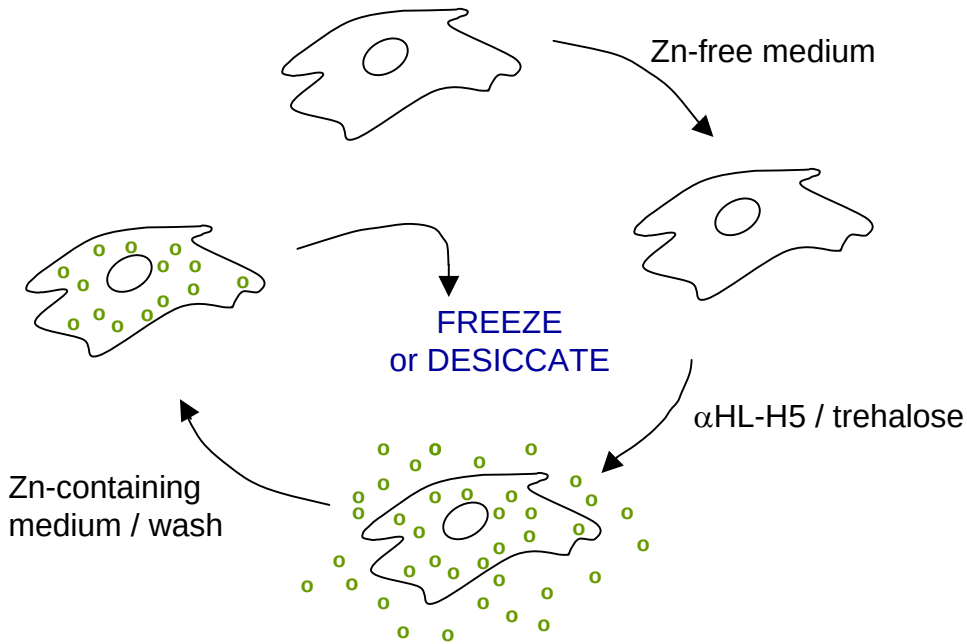






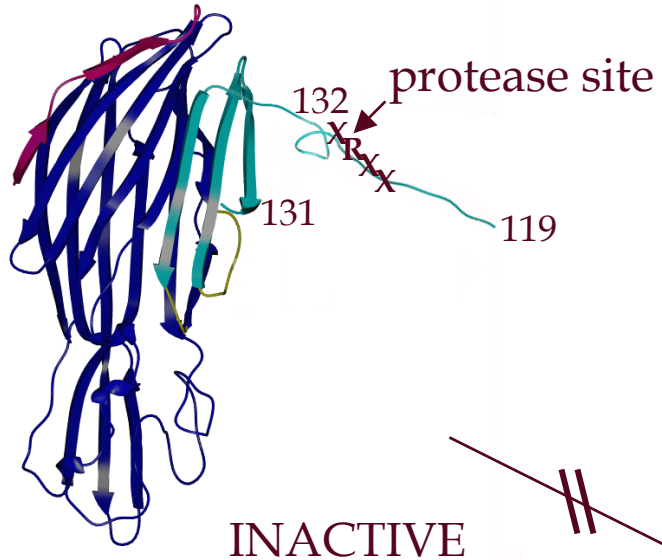
Storage of cells

-Mehmet Toner

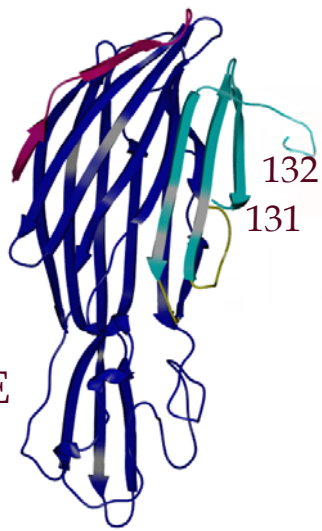


Nature Biotechnology 18, 163-167 (2000)

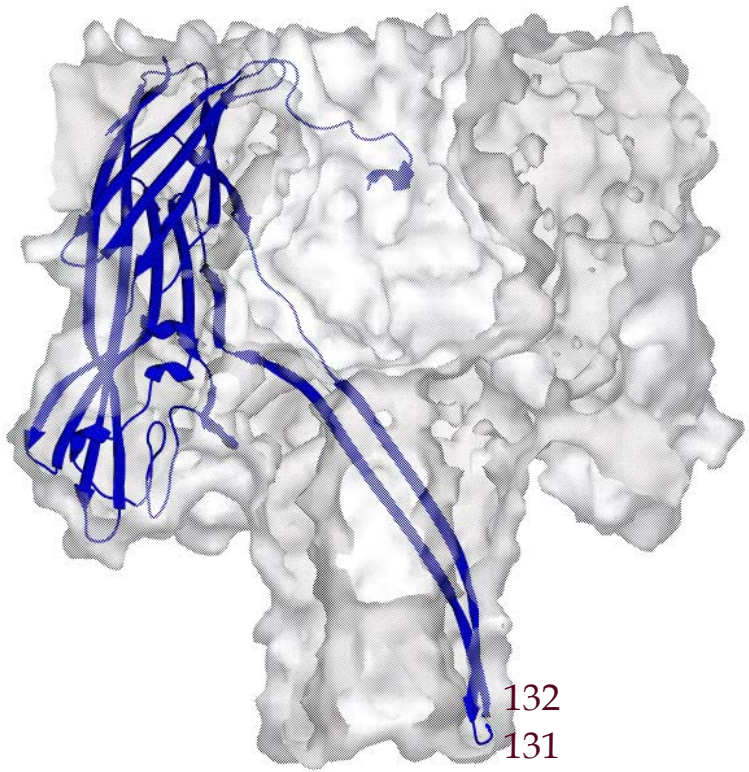
Tumor-protease activation



protease



assembly



FUNCTIONAL PORE (nicked at turns)

Nature Biotechnology 14, 852-856 (1996)

Some engineering techniques developed for protein pores:

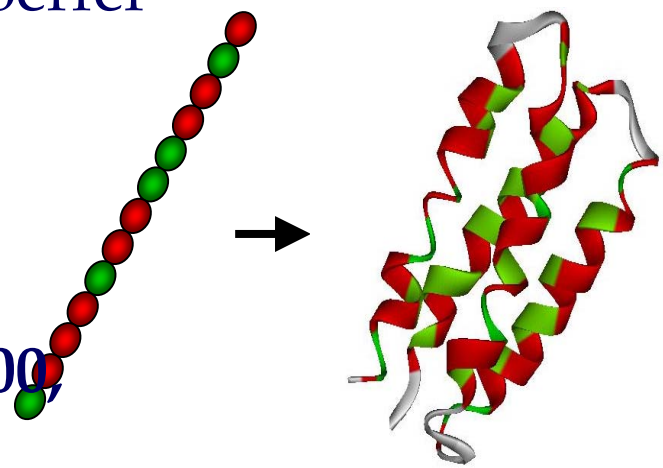
- non-covalent modification (adapters)
- modification with functional polymers
- heteromer assembly and purification
- expression: in vitro synthesis

Additional considerations in membrane protein engineering:

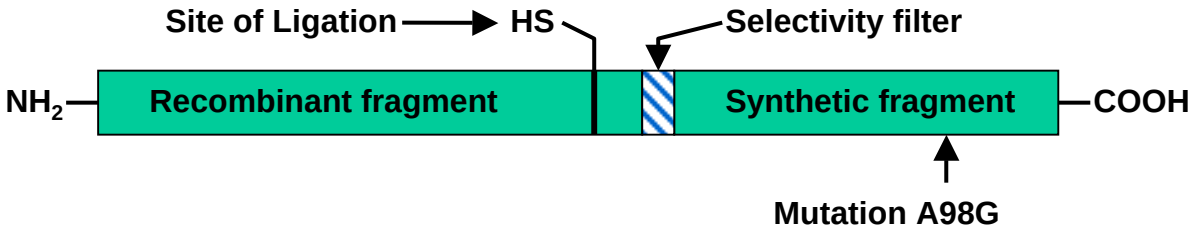
- de novo design, e.g. DeGrado, Engelman

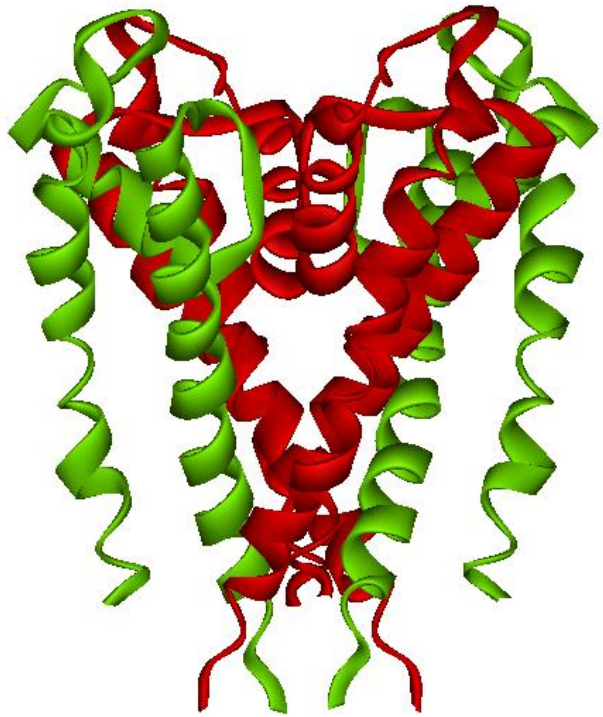
  combinatorial libraries, e.g. Hecht

  computational design of a new fold, e.g. Baker
- unnatural amino acids, e.g. Dougherty, Lester
- total synthesis, e.g. Muir, Kochendoerfer



Wei et al. Proc.Natl.Acad.Sci.USA 100, 13270-13273 (2003)





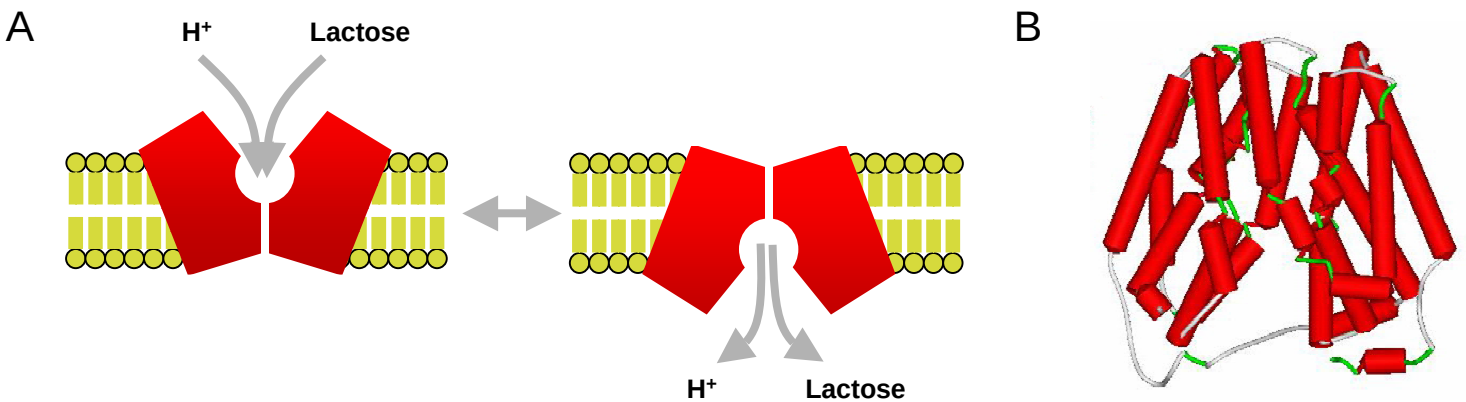
semisynthetic
K channel

Valiyaveetil et al. Angew. Chem. Int. Ed. 43, 2504-2507 (2004)

Structural studies:

blanco.biomol.uci.edu/Membrane_Proteins_xtal.html

- Details- not simply barrels and helix bundles
- Large-scale molecular motions- Rees, MacKinnon, Iwate



Abramson et al. Science 301, 610-615 (2003)

Membrane protein folding

Chen & Gouaux Biochemistry 38, 15380-15387 (1999)

Hong & Tamm Proc.Natl.Acad.Sci.USA 101, 4065-4070 (2004)

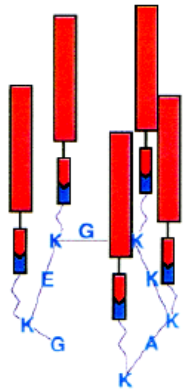
Stable bilayers and other supports

Schiller et al. Angew.Chem.Int.Ed. 42, 208-211 (2003)

Don't forget lipids…

Oliver et al. Science 304, 265-270 (2004)

a)



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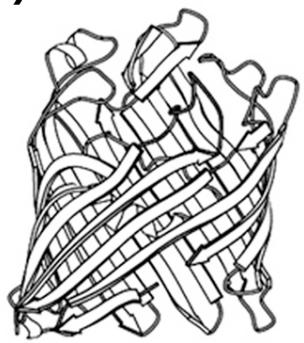

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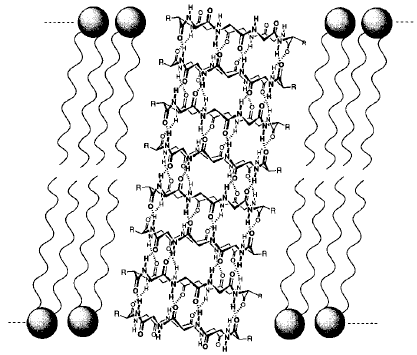

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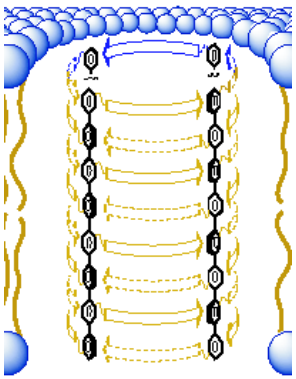

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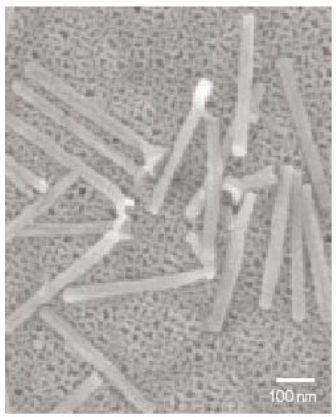
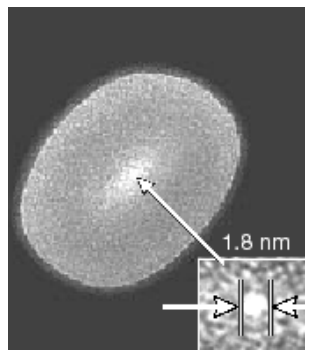
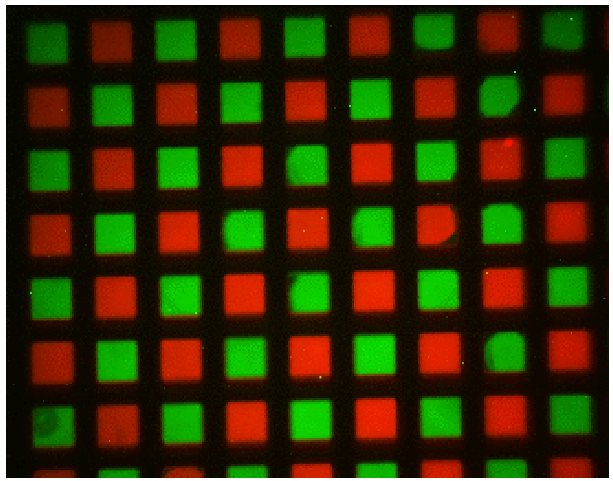


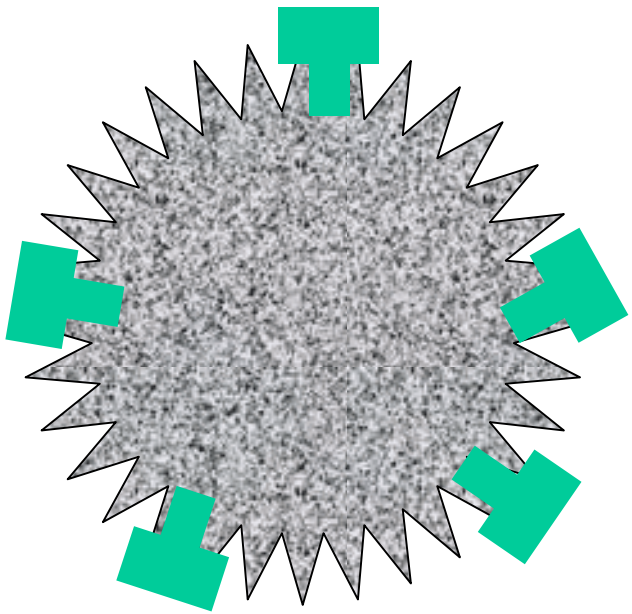
g)



Non-polypeptide pores

Future …





…arrays for single molecule sensing…

…drug delivery, with local activation…

Additional information sources

H. Bayley, H. and P.S Cremer, Stochastic sensors inspired by biology, **Nature** *413*, 226-230 (2001)

H. Bayley, O. Braha, S. Cheley and L.-Q. Gu, "Engineered nanopores", in Nanobiotechnology, C.M. Niemeyer & C.A. Mirkin, eds., Wiley-VCH, pp 93-112 (2004)

H. Bayley and L. Jayasinghe, "Functional engineered channels and pores", **Mol. Membrane Biol.**, in press (2004)

H. Bayley and S.O. Smith, organizers, FASEB Summer Research Conference, " Molecular Biophysics of Cellular Membranes", Tucson, AZ. June 19th- 24th, 2004 src.faseb.org/prog.htm