The Old Man and the Membrane
Membranes are Jewish
From “The Statue Within” by F. Jacob (circa 1954): Jacques Monod to Francois Jacob with regard to Mel Cohn’s and Annamari Torriani’s postulate regarding the existence of a lactose permease.

“Eef vee haf to zink about silly zings like permeabeelité, zees field eez ‘opeless--let’z forget zees mutant.”
THE MOST INTERESTING AND IMPORTANT MEMBRANE PROTEINS ARE **TRANSPORT PROTEINS** BECAUSE THEY TRANDUCE ENERGY INTO WORK IN THE FORM OF A CONCENTRATION GRADIENT. THEY ARE CALLED “PUMPS” ONLY BY CHUMPS WHO DON’T KNOW ANY BETTER.

IN CONTRAST, **CHANNELS** ARE BORING HOLES, WHICH MERELY ALLOW IONS, WATER, UREA, AMMONIA, ETC. TO FLOW DOWN THEIR ACTIVITY GRADIENTS. THE ONLY INTERESTING ASPECT OF CHANNELS IS GATING, SINCE GATING IS SIMILAR TO TRANSPORT.

**RECEPTORS** ARE OBVIOUSLY FACOCKTED TRANSPORTERS THAT BIND LIGANDS, BUT FORGOT HOW TO TRANSPORT THEM ACROSS THE MEMBRANE.

**ELECTRON TRANSFER PROTEINS AND LIGHT HARVESTING PHOTOSYNTHETIC REACTION CENTERS** ARE JUST SCAFFOLDS THAT HOLD COFACTORS AND METALS IN PLACE.
Lactose/H⁺ Symport

LacY is an almost perfect thermodynamic machine

A. Active transport

B. Influx

C. Efflux

Friday, May 1, 2009

- Functionally and structurally a monomer
- Conformationally extremely dynamic
- Complete Cys-Scanning Mutagenesis
- Battery site-directed techniques
  -- Seven critical residues
  -- Model for helix packing
Overall structure- C154G mutant

Abramson J., Smirnova, I. et al. (2003) Science
Overall structure-C154G mutant

Abramson J., Smirnova, I. et al. (2003) Science
BOUND PHOSPHOLIPID CRITICAL FOR CRYSTALLIZATION OF WILD-TYPE LacY
Superposition of apo structures of wild-type and C154G LacY
Superposition of apo structures of wild-type and C154G LacY

CRYSTALS AIN’T EVERYTHING!
Superposition of apo structures of wild-type and C154G LacY

CRYSTALS AIN’T EVERYTHING!

Friday, May 1, 2009
Large cavity open to cytoplasm
Sugar Binding Site

Specificity in galactopyranosyl ring
Anomeric moiety increases affinity

N-terminal helices--blue
C-terminal helices--red
H+ Translocation
Residues involved in $H^+$ translocation do not form a pathway through LacY
$K_D > \text{pH 10}$

Smirnova et al (2008) *PNAS*
ALTERNATING ACCESS MODEL
ALTERNATING ACCESS MODEL
Five independent lines of support for the alternating access model

1. Initial evidence was obtained from site-directed thiol cross-linking, which underestimated distance on the cytoplasmic side of LacY [Abramson, A., Smirnova, I., Kasho, V., Verner, G., Kaback, H. R. and Iwata, S. (2003) Structure and mechanism of the lactose permease of Escherichia coli. Science 301, 610-615.]


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A Remarkable Experiment
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<td>2. MTS-6-MTS</td>
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<td>3. MTS-8-O2-MTS</td>
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<td>4. MTS-11-O3-MTS</td>
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<td>5. MTS-14-O4-MTS</td>
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<td>6. MTS-17-O5-MTS</td>
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<tr>
<td>7. NDM</td>
<td>~12 Å</td>
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Friday, May 1, 2009
A. 

control (MTS)$_2$-3 (~5Å) 
(MTS)$_2$-6 (~9Å) 
(MTS)$_2$-8-O2 (~11Å) 
(MTS)$_2$-11-O3 (~15Å) 
(MTS)$_2$-14-O4 (~17Å) 
(MTS)$_2$-17-O5 (~22Å) 
NDM (~12Å) 

C. 

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Friday, May 1, 2009
Electrophysiology of LacY

(Solid supported membrane electrode)
Garcia-Celma, J.-J., Smirnova, I., Kaback, H.R., Fendler, K.


Friday, May 1, 2009
Through the glass brightly—the future for membrane proteins, as seen by me!

**Expression, folding & targeting**—prokaryotic—generally okay; eukaryotic—still big problem. **BARRIERS** to folding/targeting!

**Structure**—anyway you can get it! *Single conformation* essential but not sufficient for function. Crystallography, *NMR, EM*

**Bottom line**—low resolution structure (>4 Å) is sufficient for threading into a prototype higher-resolution structure for family and molecular dynamics modeling. Then judicious Cys-scanning mutagenesis, followed by functional studies, site-directed spectroscopy and cross-linking to test structure/function.
Darwin was wrong!

Natural selection is not the driving force for evolution. Rather, the true driving force for evolution is the desire of Mother Nature to screw the investigator.