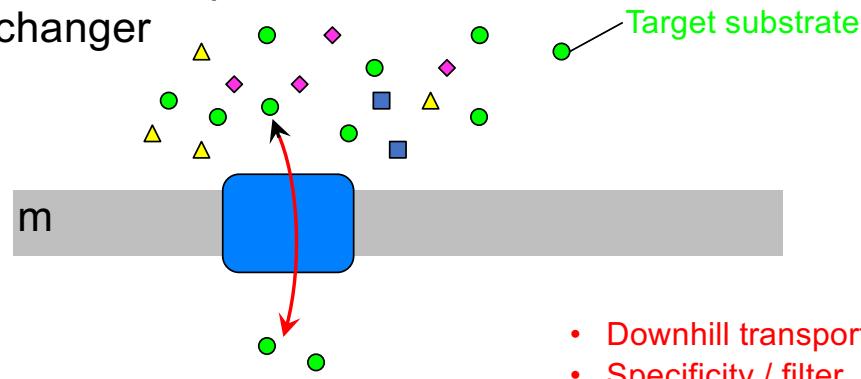
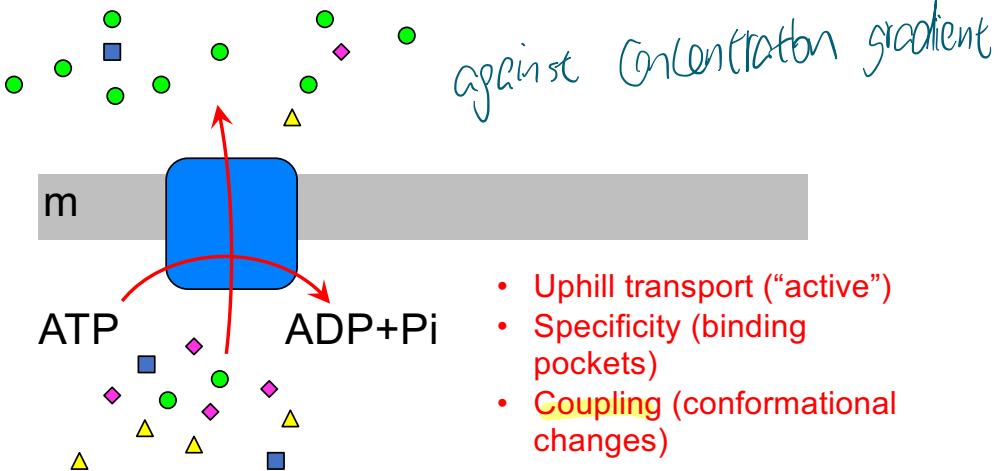


Passive transport: channel / carrier / facilitator / exchanger



Examples: Aquaporin, urea transporter, K⁺ / Na⁺ channels, GLUTs, mitoch. carriers / exchangers, ligand-gated ion channels

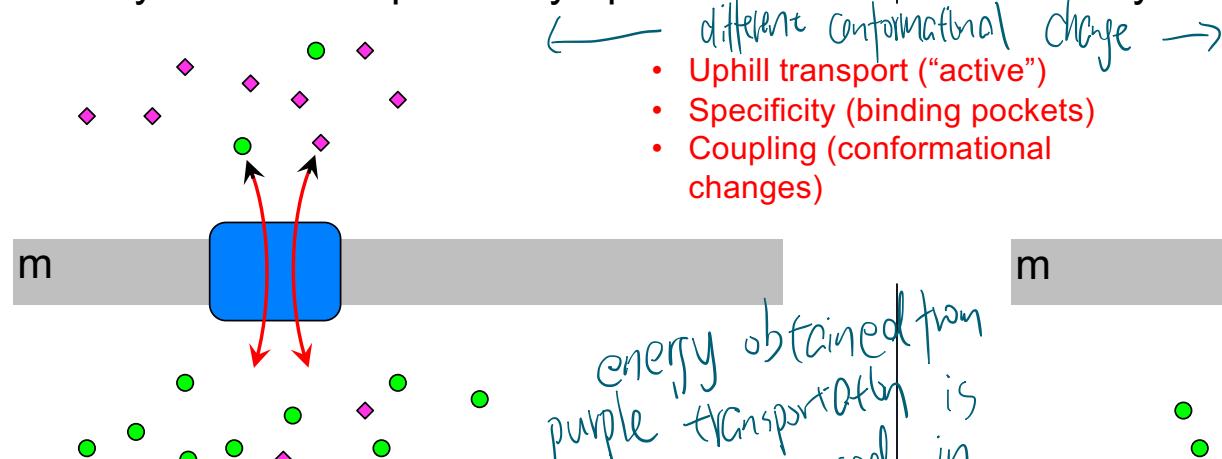
Primary active transport: pump / flippase



against Concentration gradient

Examples: ABC-transporters, P-type ion pumps / flippases

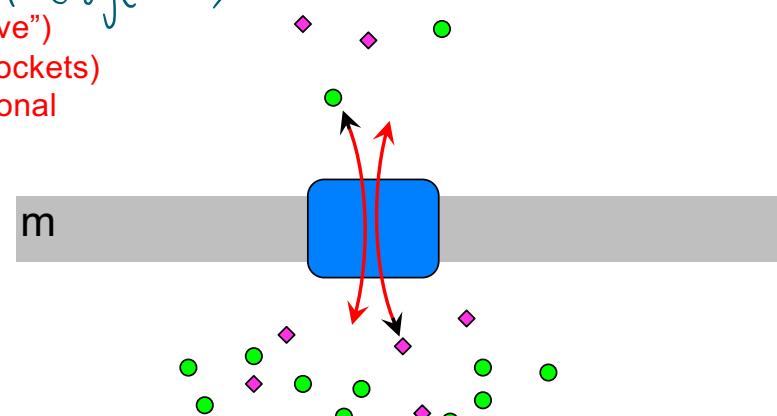
Secondary active transport 1: symporter



Examples: Na⁺-coupled neurotransmitter transporters, LacY

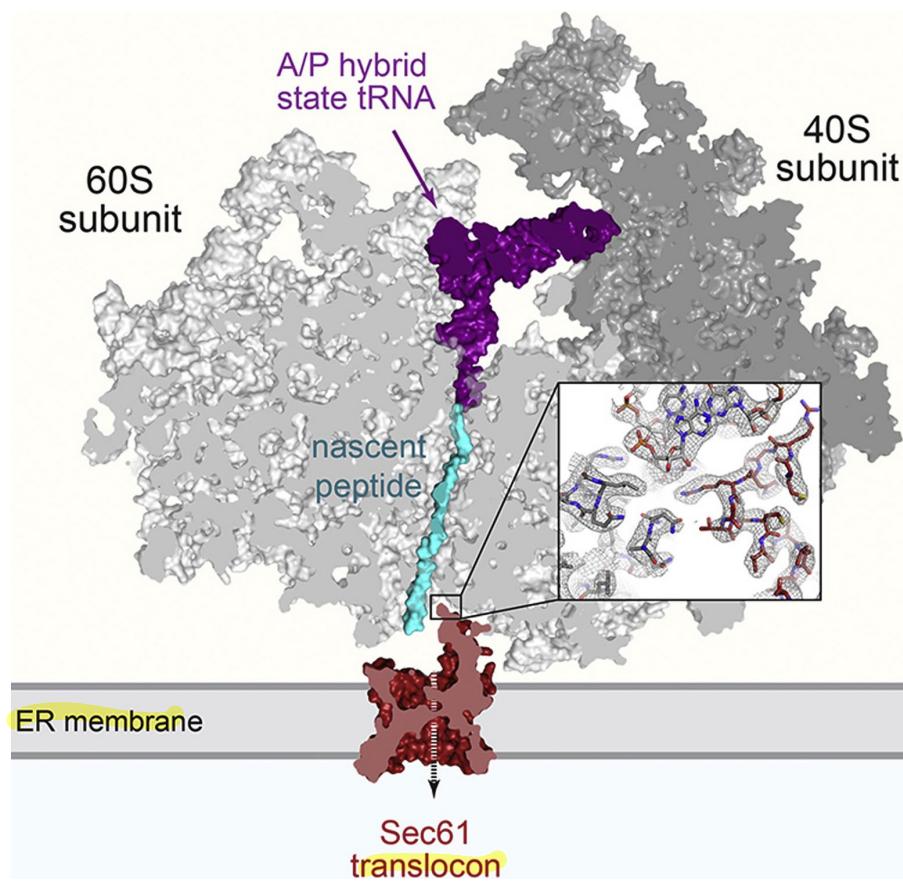
purple transportation
energy obtained from
is used
in green transportation

Secondary active transport 2: antiporter

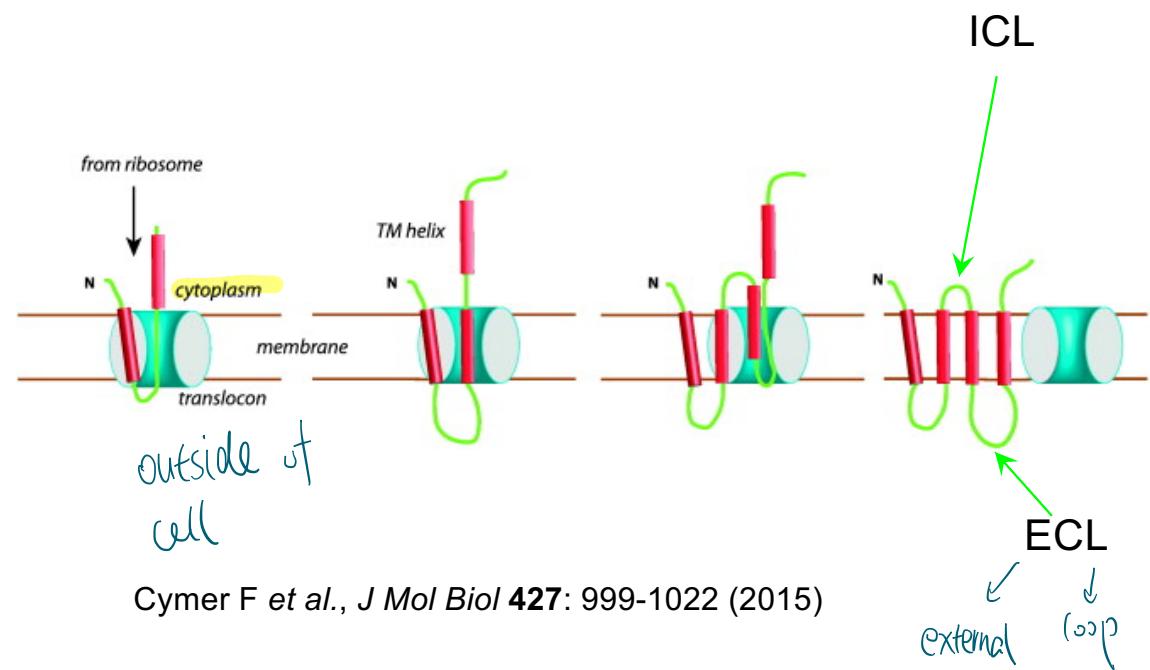


Examples: Glycerol-3-phosphate transporter, Some CIC proteins

Insertion and folding of membrane proteins in lipid bilayer (ER or cytoplasmic membrane of bacteria)



Vorhees RM et al., *Cell* **157**: 1632-1643 (2014)



Cymer F et al., *J Mol Biol* **427**: 999-1022 (2015)

Table 1. Mean residue hydrophobicities for buried and exposed residues.

Protein types	Mean residue hydrophobicities	
	Core ←	Buried
		Exposed
<i>Transmembrane proteins</i>		
11 RC helices	0.19*	0.48
11 RC helices	0.22†	0.36
35 Helices (Table 2)	0.15‡	0.34
<i>Water-soluble proteins</i>		
37 Monomers	0.24†	-0.25
23 Oligomers	0.19†	-0.28
7 Hemoglobin helices	0.17‡	-0.26

Definition of buried residues: * $>80\%$ area buried in helices (13); † $<5\%$ residue area exposed (4).
 ‡Hydrophobic moment (25).

Core of transmembrane and exposed protein is similar

but different in exposed

part

more hydrophilic than core

Hydropathy scale and information used in the assignments

Side-chain	Hydropathy index	$\Delta G_{\text{transfer}}^{\circ}$ (water-vapor) ^a	Fraction of side-chains 100% buried ^b	Fraction of side-chains 95% buried ^c
Isoleucine	4.5	4.4	4.5	5.2
Valine	4.2	4.2	4.3	4.2
Leucine	3.8	4.5	3.2	2.8
Phenylalanine	2.8	2.5	2.5	3.5
Cysteine/cystine	2.5	1.9	6.0	3.2
Methionine	1.9	1.9	1.0	1.9
Alanine	1.8	3.9	5.3	1.6
Glycine	-0.4	—	4.2	1.3
Threonine	-0.7	-0.6	-0.5	-1.0
Tryptophan	-0.9	-0.9	-2.4	-0.3
Serine	-0.8	-0.8	-0.7	-1.0
Tyrosine	-1.3	-1.1	-3.3	-2.2
Proline	-1.6	—	-2.4	-1.8
Histidine	-3.2	-4.2	-3.6	-1.9
Glutamic acid	-3.5	-3.9	-2.8	-1.7
Glutamine	-3.5	-3.5	-4.0	-3.6
Aspartic acid	-3.5	-4.5	-2.5	-2.3
Asparagine	-3.5	-3.8	-3.1	-2.7
Lysine	-3.9	-3.2	—	-4.2
Arginine	-4.5	—	—	—

All values in the last 3 columns result from arbitrary normalization to spread them between -4.5 and +4.5. The normalization functions were:

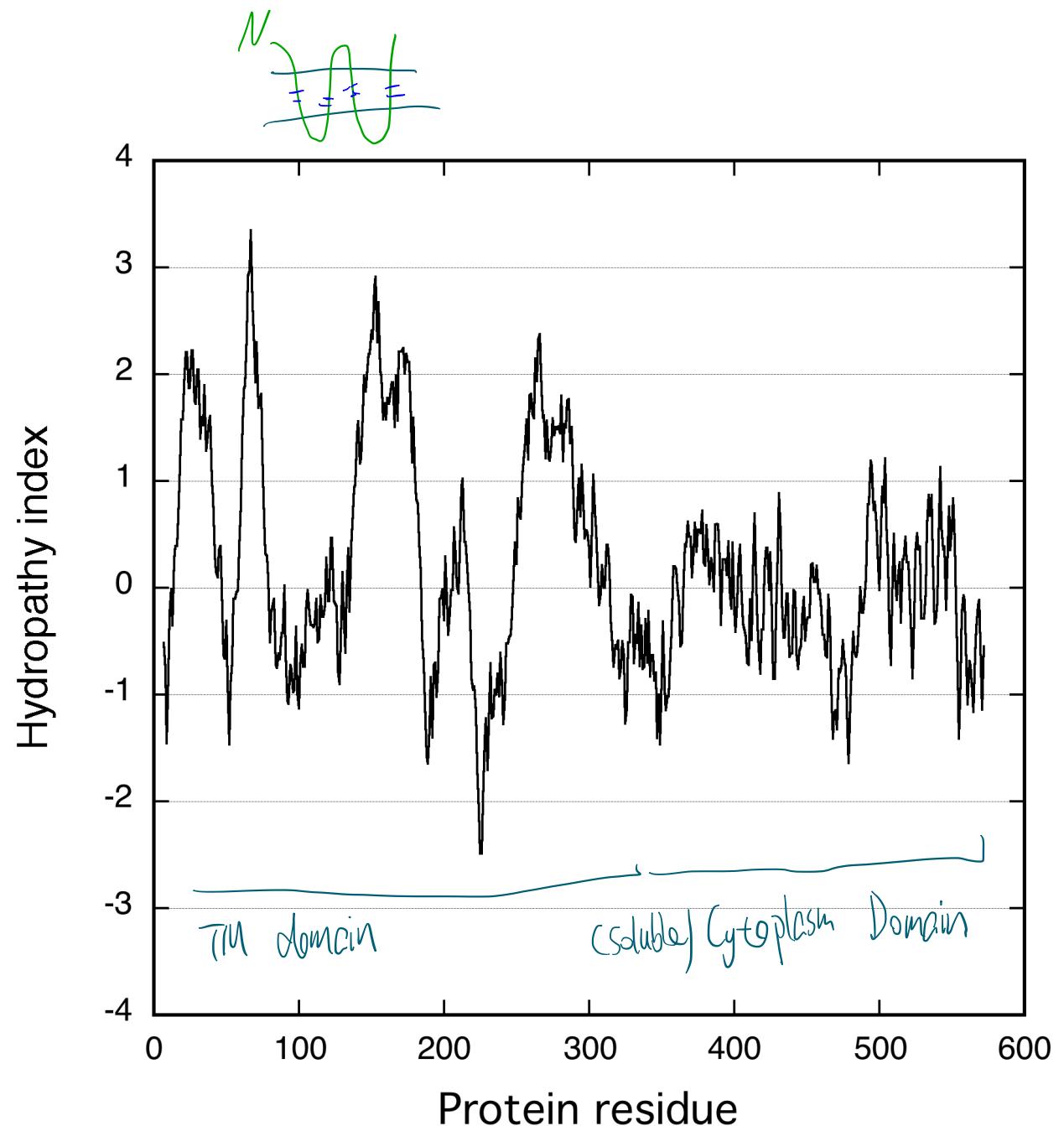
^a $-0.679(\Delta G_{\text{transfer}}^{\circ}) + 2.32$.

^b $48.1(\text{fraction 100\% buried}; \text{Chothia, 1976}) - 4.50$.

^c $16.45(\text{fraction 95\% buried}; \text{Chothia, 1976}) - 4.71$.

Kyte-Doolittle-Plot of multidrug transporter Sav1866

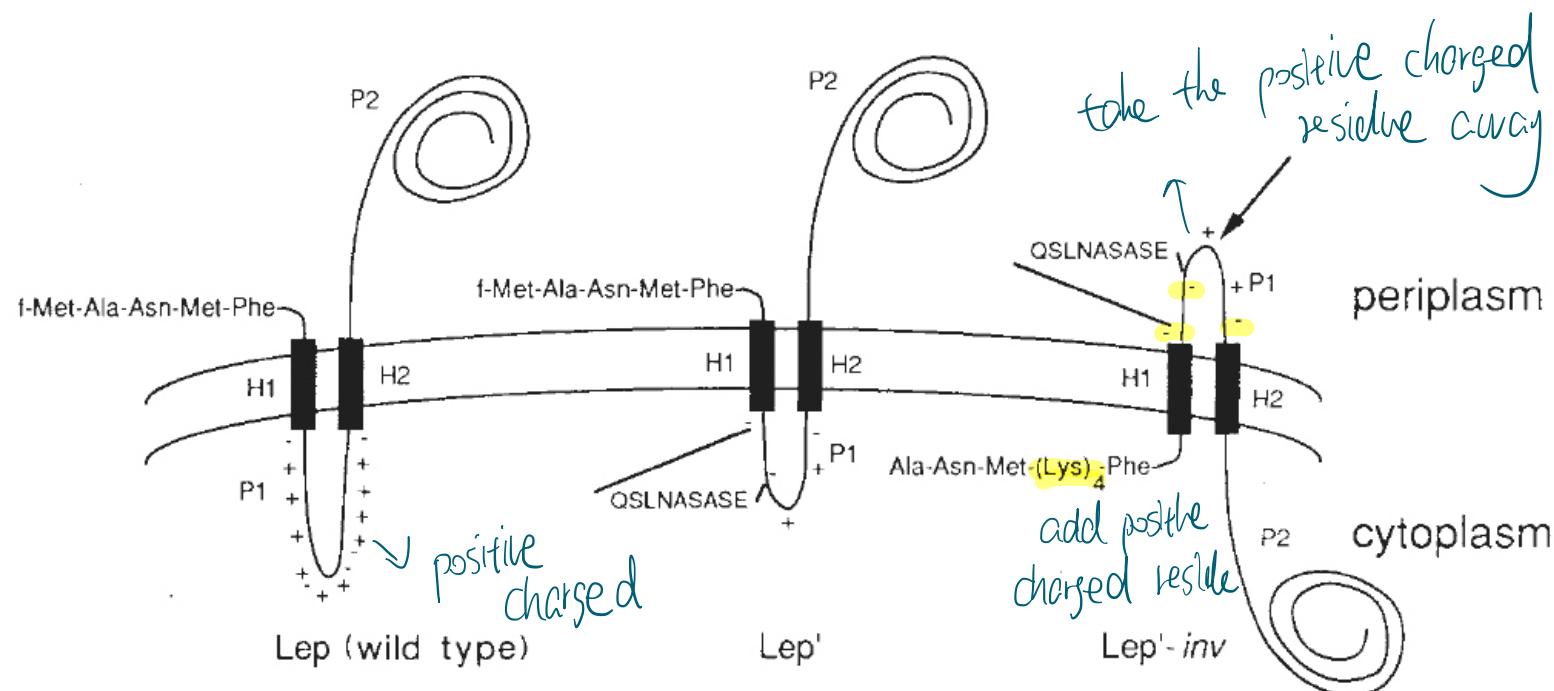
take a slide window
and move one by one
residue - Take the
average.



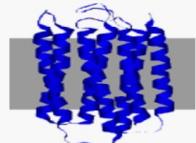


Positive-Inside Rule

FIG. 1 Topology of wild-type *E. coli* leader peptidase, and of the Lep' and Lep'-inv derivatives. The trypsin cleavage site in Lep'-inv is shown by the arrow. N terminal amino-acid sequences, and the nine-residue spacer in Lep' and Lep'-inv, are indicated (single-letter amino-acid code). Mutants of Lep were constructed by oligo-directed insertions and deletions using the Kunkel method¹⁴.



Von Heijne, *Nature* 341: 456 (1989)

TMHMM Server v. 2.0**Prediction of transmembrane helices in proteins**[Instructions](#)**SUBMISSION**Submission of a local file in **FASTA** format (HTML 3.0 or higher) Choose File no file selectedOR by pasting sequence(s) in **FASTA** format:**Output format:**

- Extensive, with graphics
- Extensive, no graphics
- One line per protein

Other options:

- Use old model (version 1)

 Submit Clear**Restrictions:***At most 10,000 sequences and 4,000,000 amino acids per submission; each sequence not more than 8,000 amino acids.***Confidentiality:***The sequences are kept confidential and will be deleted after processing.*

PORTABLE VERSION

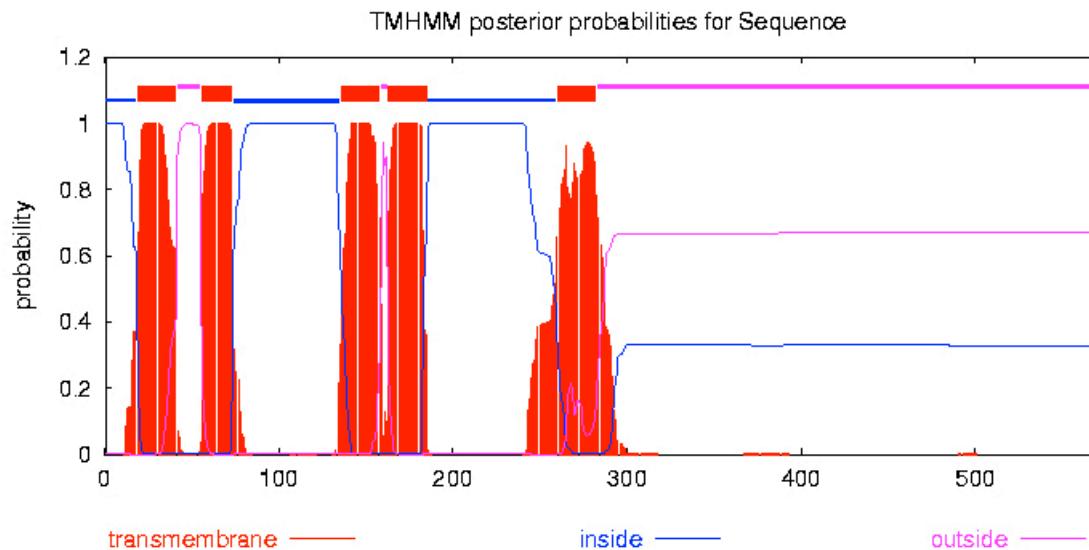
Would you prefer to run TMHMM at your own site? TMHMM 2.0 is available as a stand-alone software package, with the same functionality as the service above. Ready-to-ship packages exist for the most common UNIX platforms. There is a [download page](#) for academic users; other users are requested to contact CBS Software Package Manager at software@cbs.dtu.dk.

GETTING HELPScientific problems: [Anders Krogh](#) Technical problems: [Support](#)

TMHMM result

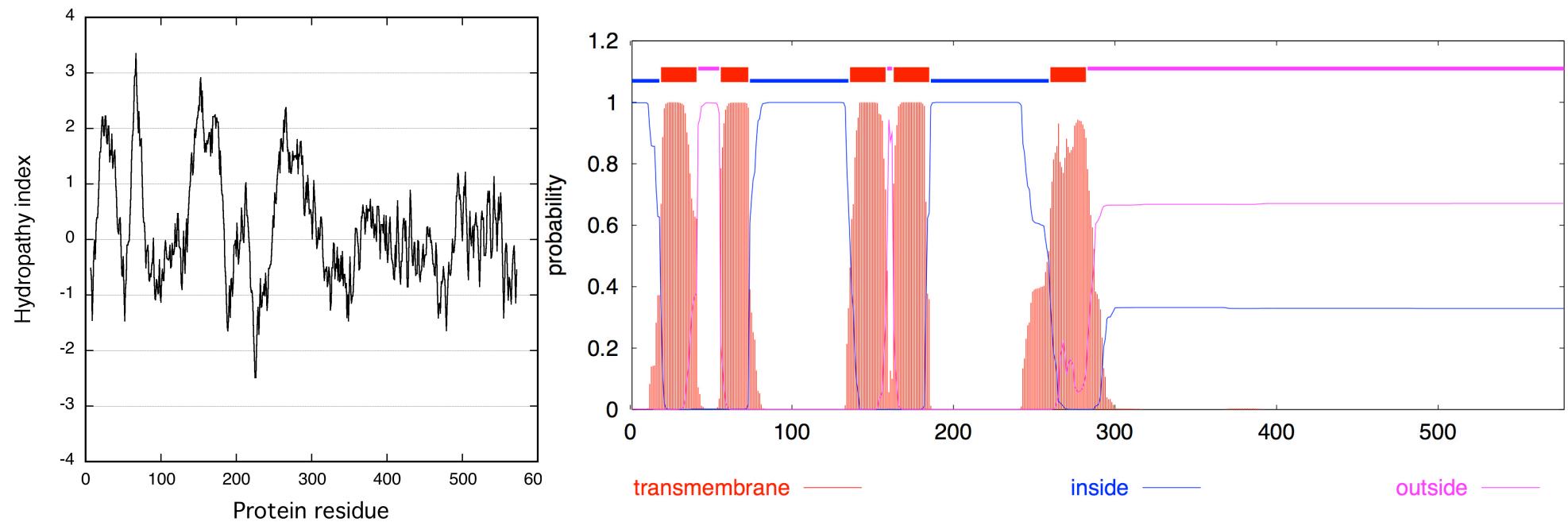
[HELP](#) with output formats

```
# Sequence Length: 578
# Sequence Number of predicted TMHs: 5
# Sequence Exp number of AAs in TMHs: 115.485
# Sequence Exp number, first 60 AAs: 26.86183
# Sequence Total prob of N-in: 0.99835
# Sequence POSSIBLE N-term signal sequence
Sequence      TMHMM2.0      inside     1     18
Sequence      TMHMM2.0      TMhelix   19    41
Sequence      TMHMM2.0      outside    42    55
Sequence      TMHMM2.0      TMhelix   56    73
Sequence      TMHMM2.0      inside    74   135
Sequence      TMHMM2.0      TMhelix  136   158
Sequence      TMHMM2.0      outside   159   162
Sequence      TMHMM2.0      TMhelix  163   185
Sequence      TMHMM2.0      inside   186   259
Sequence      TMHMM2.0      TMhelix  260   282
Sequence      TMHMM2.0      outside  283   578
```

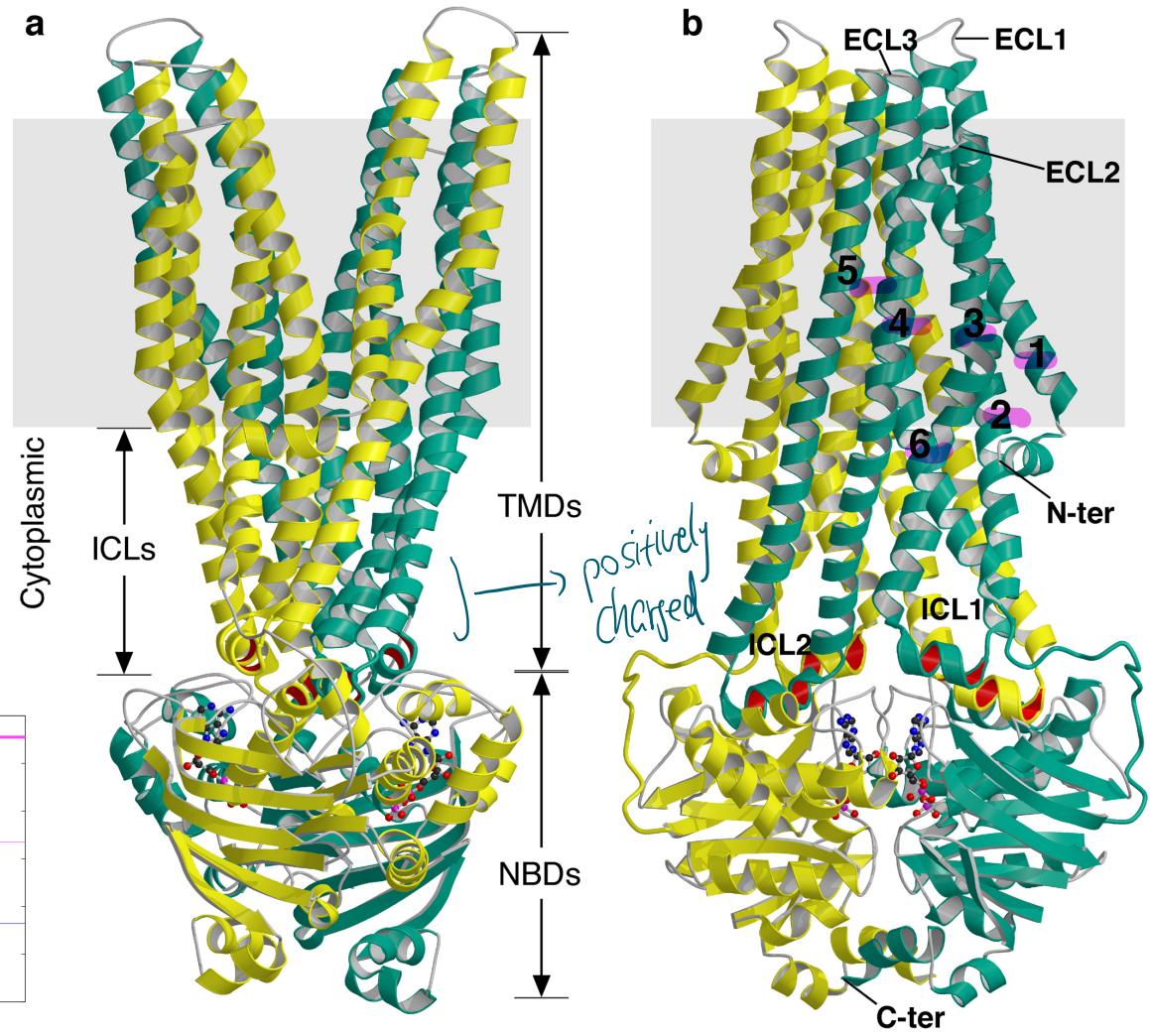
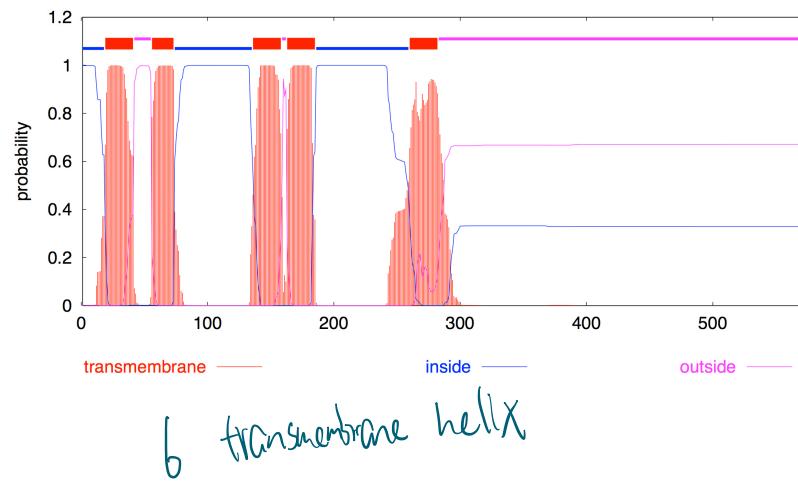
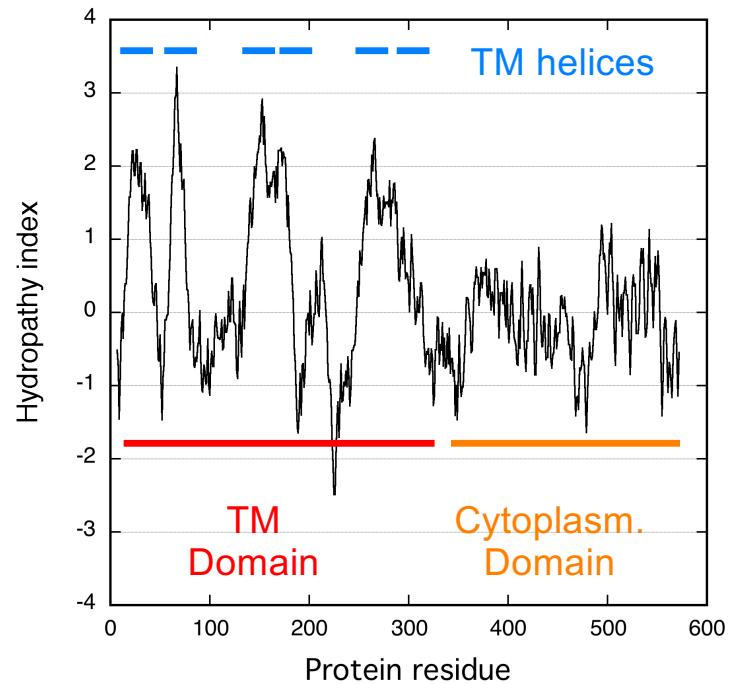


[plot](#) in postscript, [script](#) for making the plot in gnuplot, [data](#) for plot

From Kyte-Doolittle to TMHMM2.0: Prediction of Sav1866 TM topology



Comparison to actual Sav1866 structure



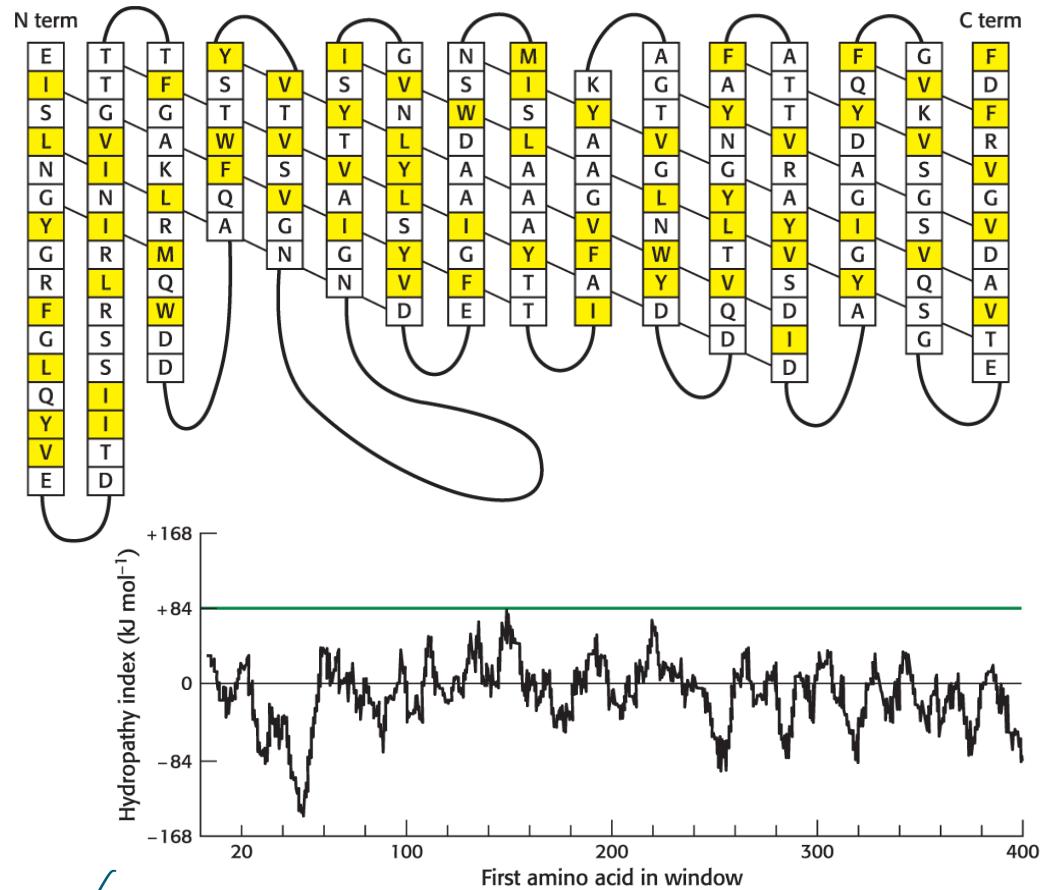
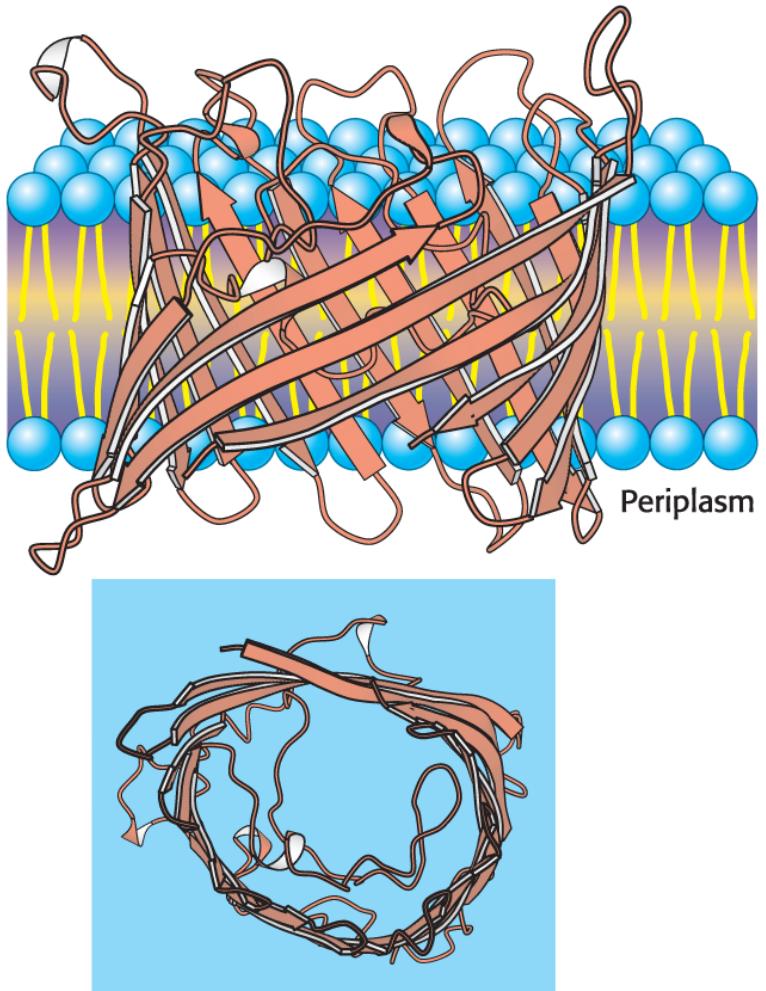
pymol Sav1866

PDB ID: 2HYD.pdb

- Pymol scene 1: Ribbon
- Pymol scene 2: Electrostatic surface potential

Beta barrel membrane proteins: Porins et al.

Another class of membrane protein

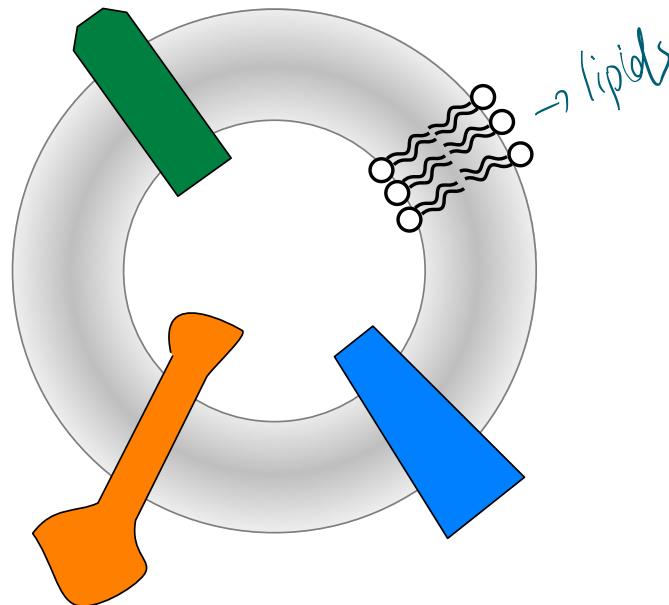


hard to tell where the TM domain is

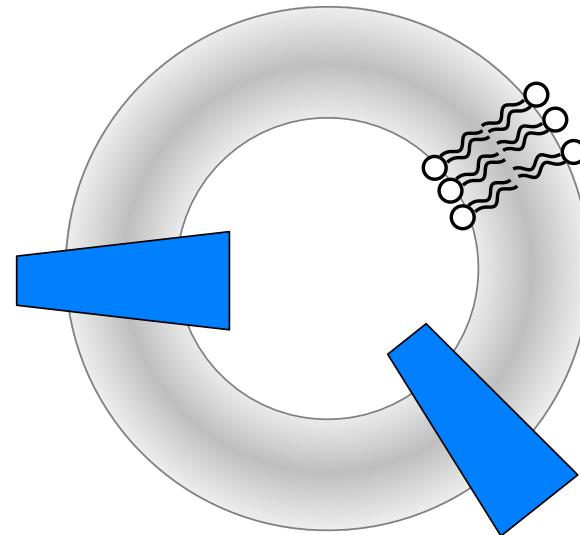
Fig. 12.20mod, 12.21, 12.28 Berg et al., Biochemistry, 9th ed.

Environments for membrane transport / channel protein studies:

1. Full cell: plasma membrane protein
2. Native membrane vesicles (liposomes)



3. Artificial membrane vesicles (LUV), "proteoliposomes"

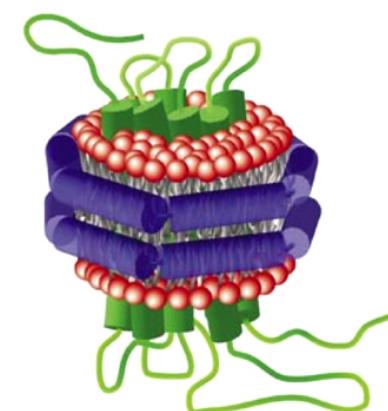


more control

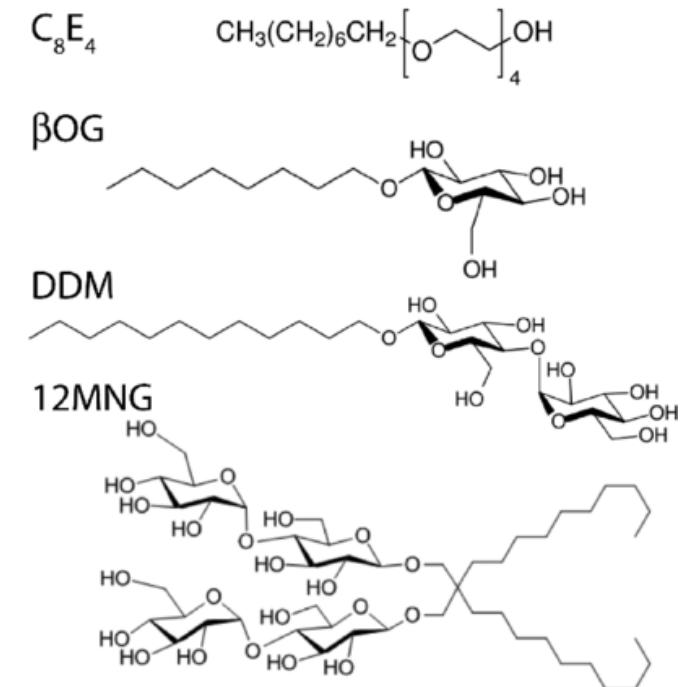
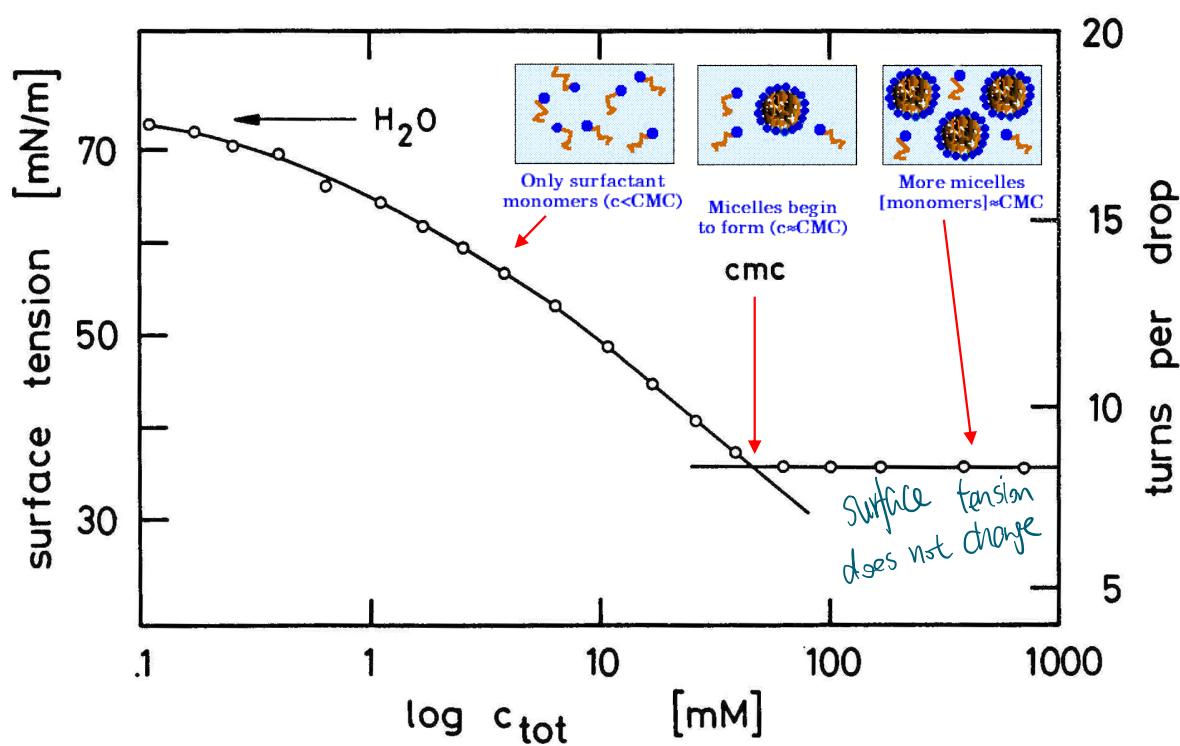
4. Mixed micelles



5. Nanodiscs

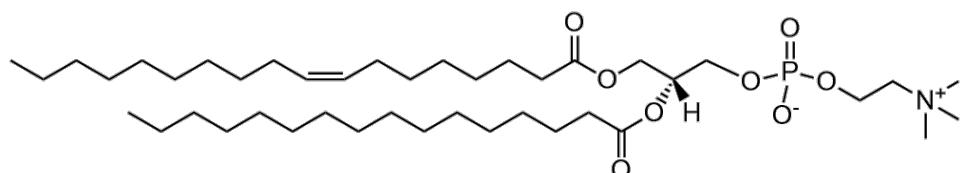
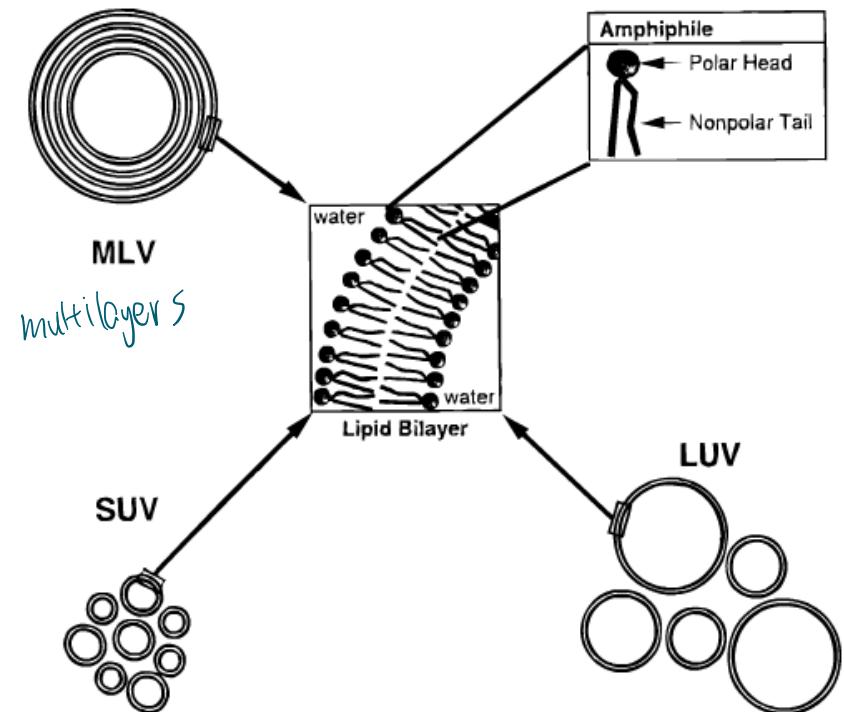
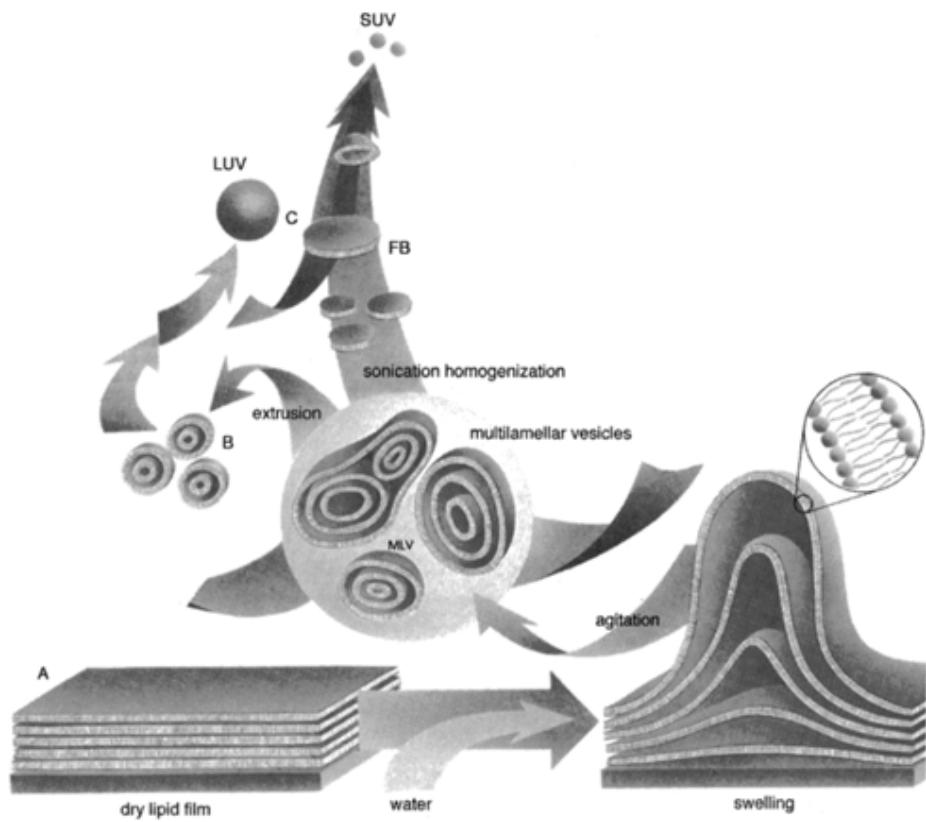


The critical micelle concentration “cmc” of detergents



M. Zulauf in: Crystallization of membrane proteins, CRC Press, 1991

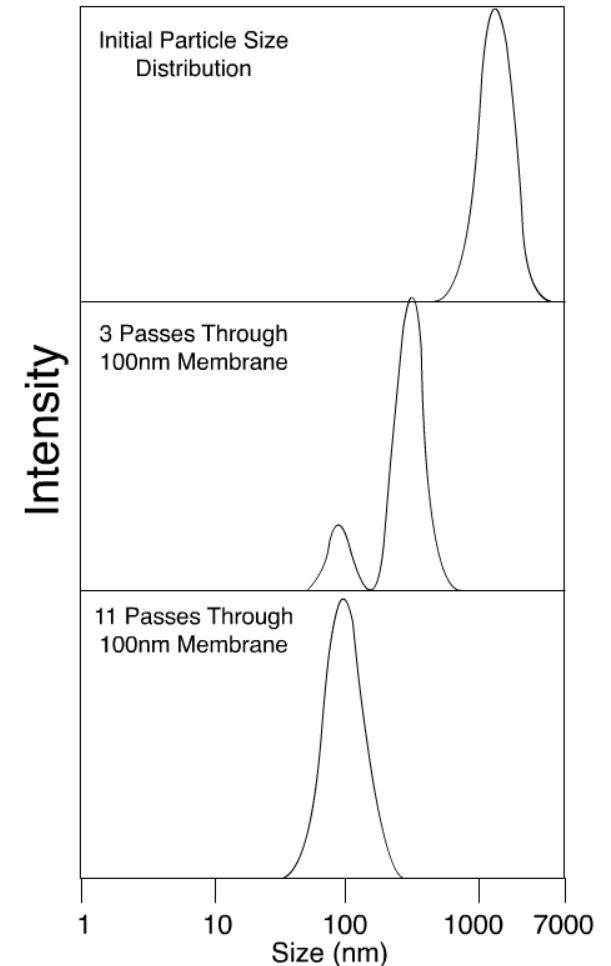
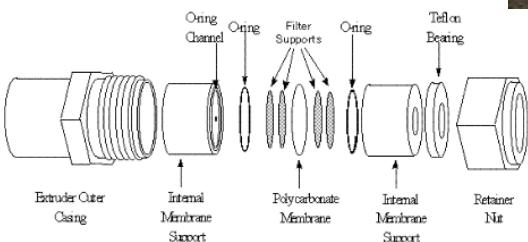
Liposome formation: Lipids



Example: 1-(8Z-octadecenoyl)-2-palmitoyl-sn-glycero-3-phosphocholine

Avanti Polar Lipids, Inc.

Liposome formation by extrusion: hardware



Avanti Polar Lipids, Inc.

Proteoliposomes: Detergent use and removal

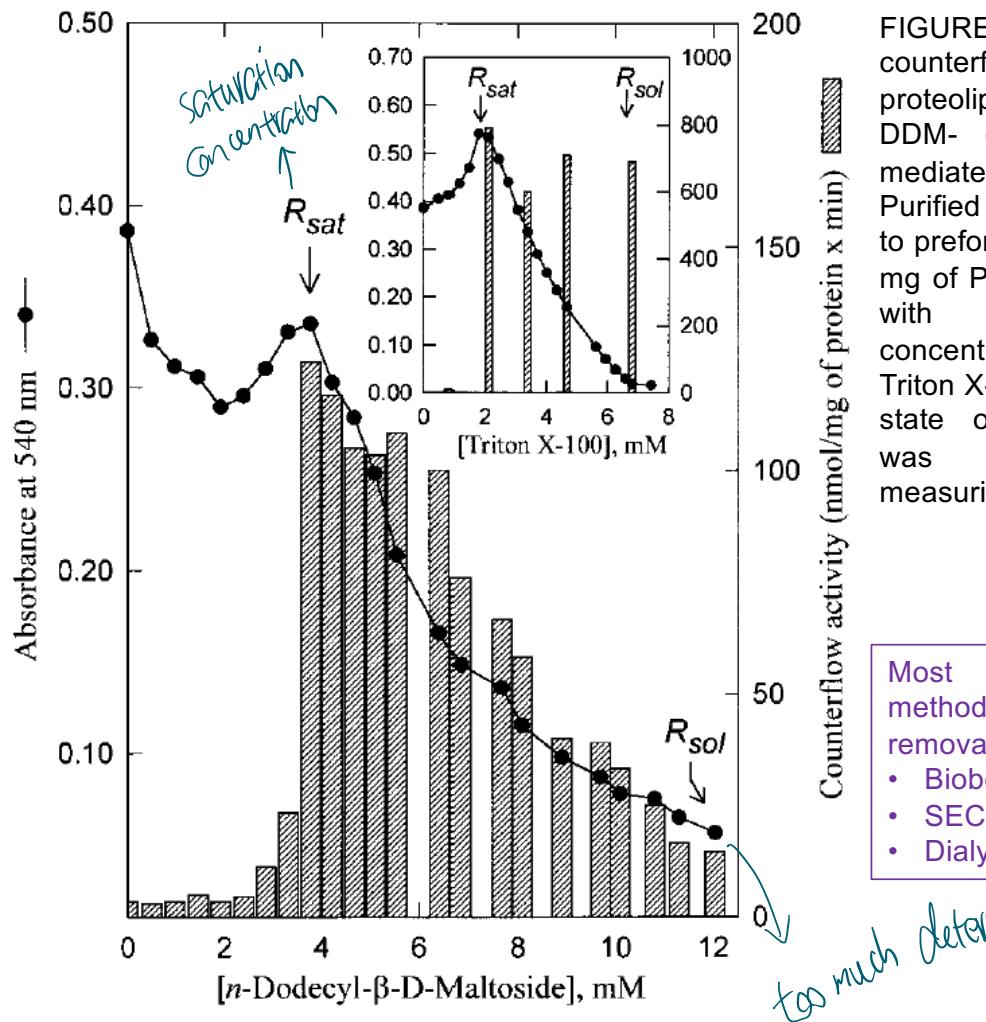


FIGURE 1: Lactose counterflow activity in proteoliposomes made via DDM- or Triton X-100-mediated reconstitution. Purified LacS was added to preformed liposomes (4 mg of PL/mL) equilibrated with different concentrations of DDM or Triton X-100. The physical state of the liposomes was followed by measuring the OD₅₄₀.

Most commonly used methods for detergent removal:

- Biobeads
- SEC
- Dialysis

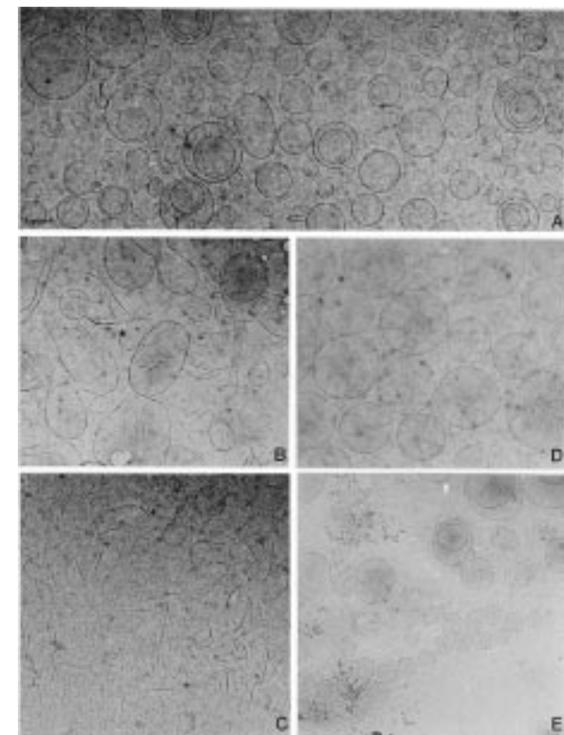
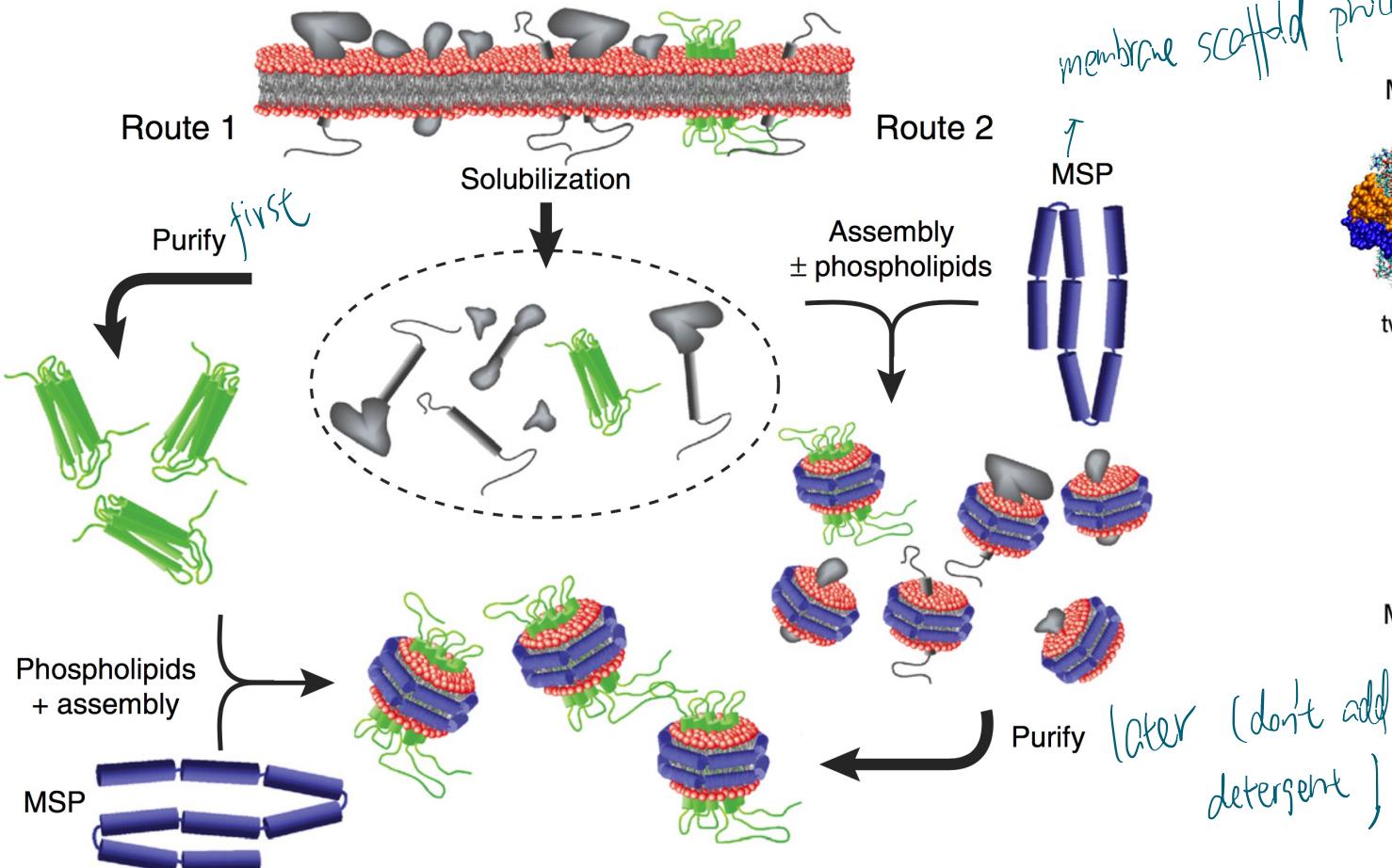


FIGURE 2: Cryotransmission electron micrographs of liposomes titrated with DDM or Triton X-100. Liposomes were diluted to 4 mg/mL and titrated with DDM or Triton X-100. Shown are preformed liposomes without detergent (A) and liposomes treated with 3.8 mM DDM (B), 5.7 mM DDM (C), 1.8 mM Triton X-100 (D), or 2.7 mM Triton X-100 (E). Bar represents 100 nm.

Nanodiscs

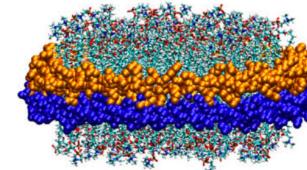
Membrane scaffold proteins, derivative of ApoA-1



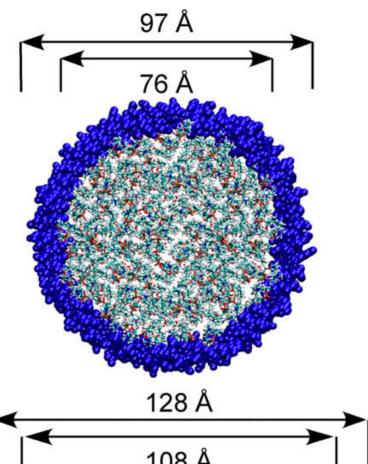
Denisov IG & Sligar SG (2016) *Nat Struct Mol Biol* **23**: 481-486

membrane scaffold proteins

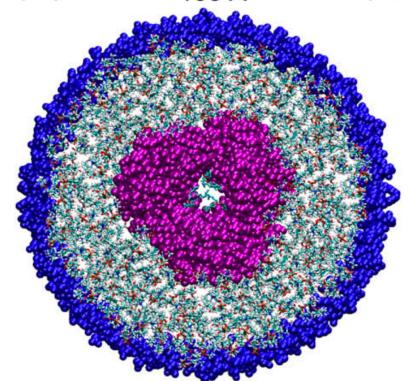
MSP1 Nanodisc



two MSP/Nanodisc



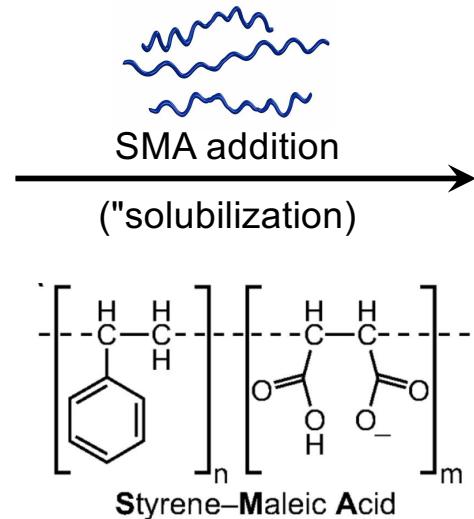
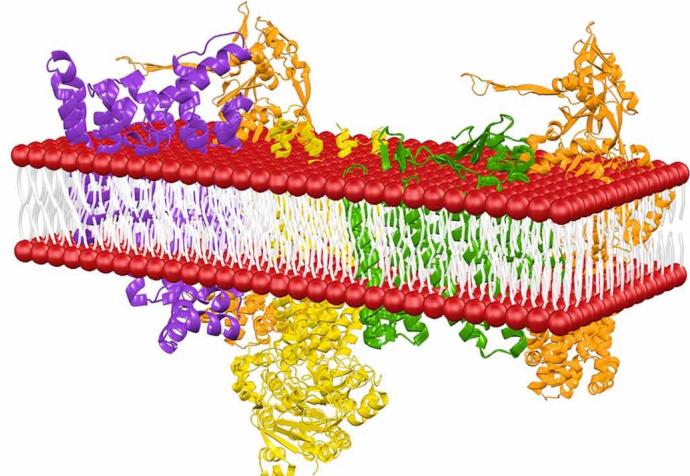
MSP1E3 Nanodisc with bR trimer



Bayburt TH & Sligar SG (2010) *FEBS Lett* **584**: 1721-1727

SMALP-based membrane protein extraction / purification

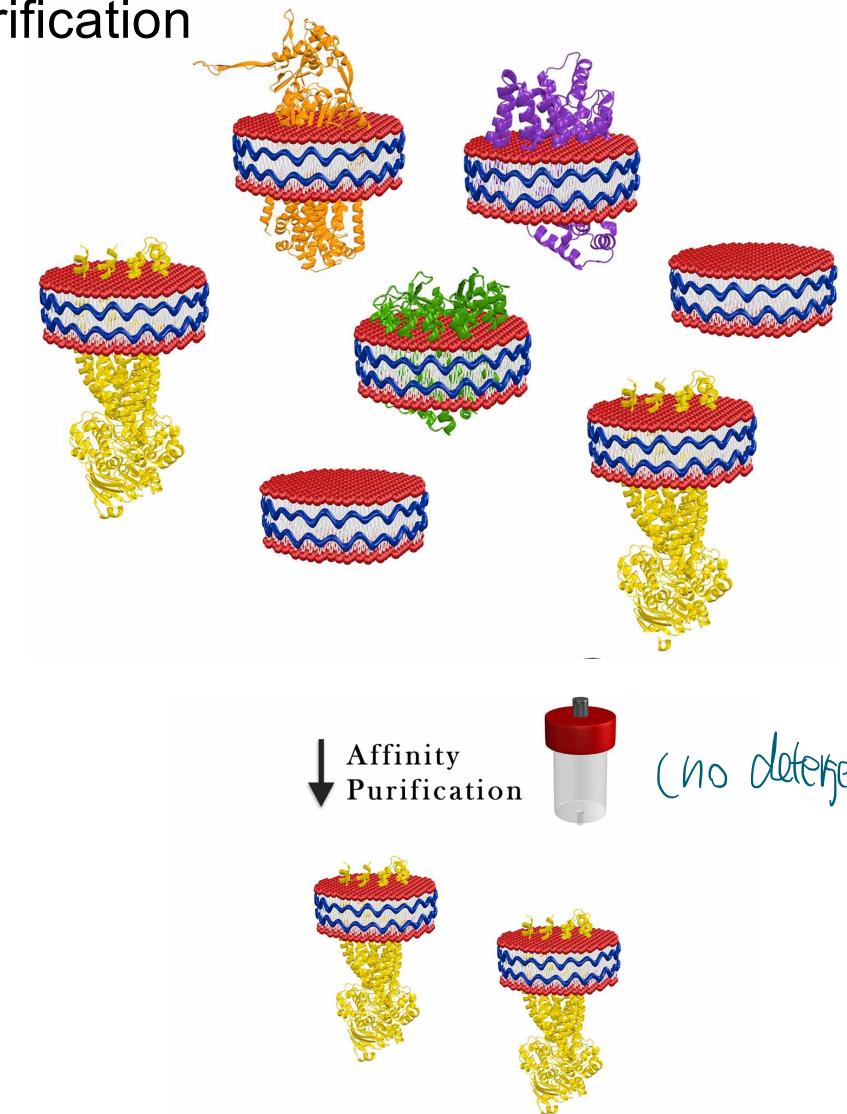
(SMALPs = styrene–maleic acid lipid particles)



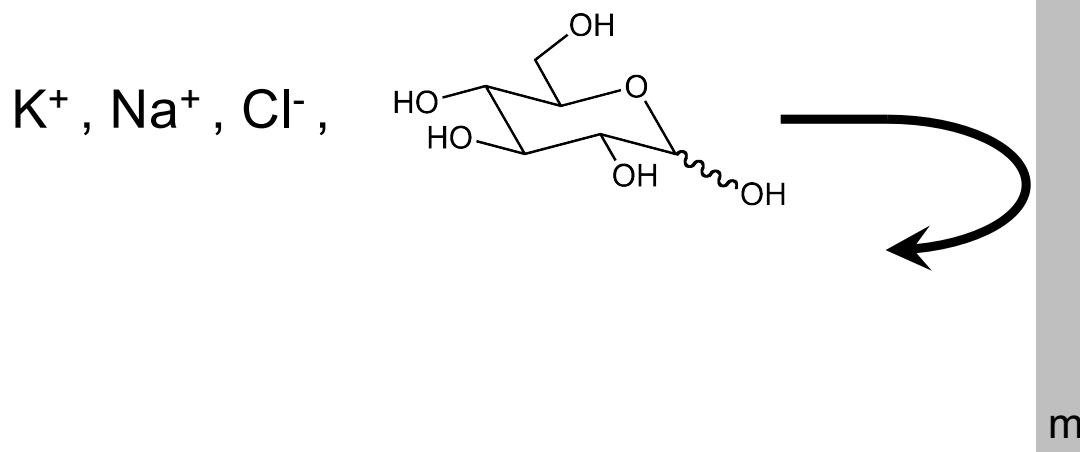
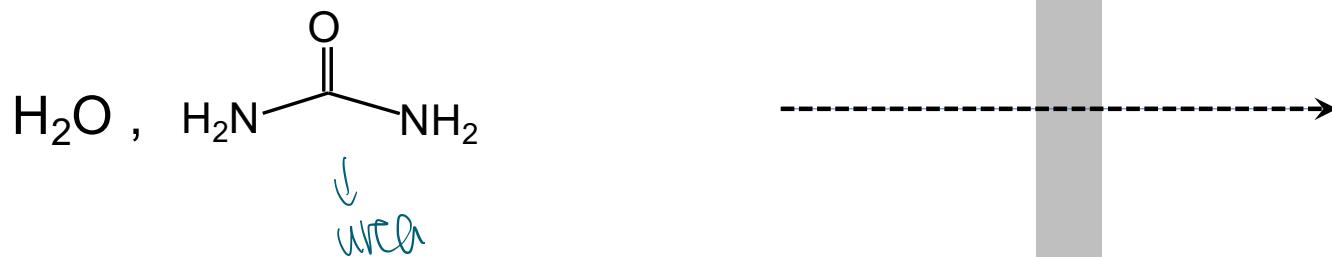
Xiran	n	m
SL25010 P20	3	1
SL30010 P20	2.3	1
SL40005 P20	1.2	1



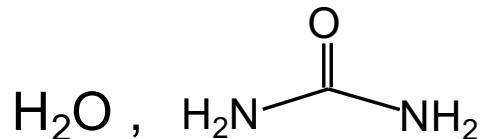
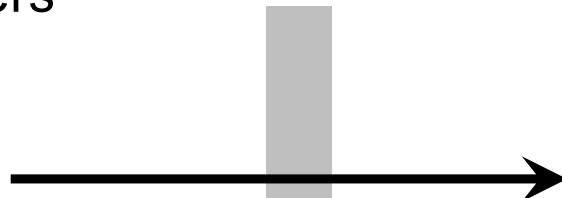
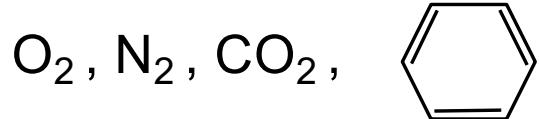
Simon KS et al. (2018) *Biochem Soc Trans* **46**: 1495-1504
Bada Juarez JF et al. (2019) *Chem Phys Lipids* **221**: 167-175



Solute permeation through bilayers

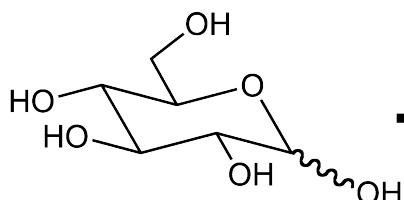


Solute permeation through bilayers

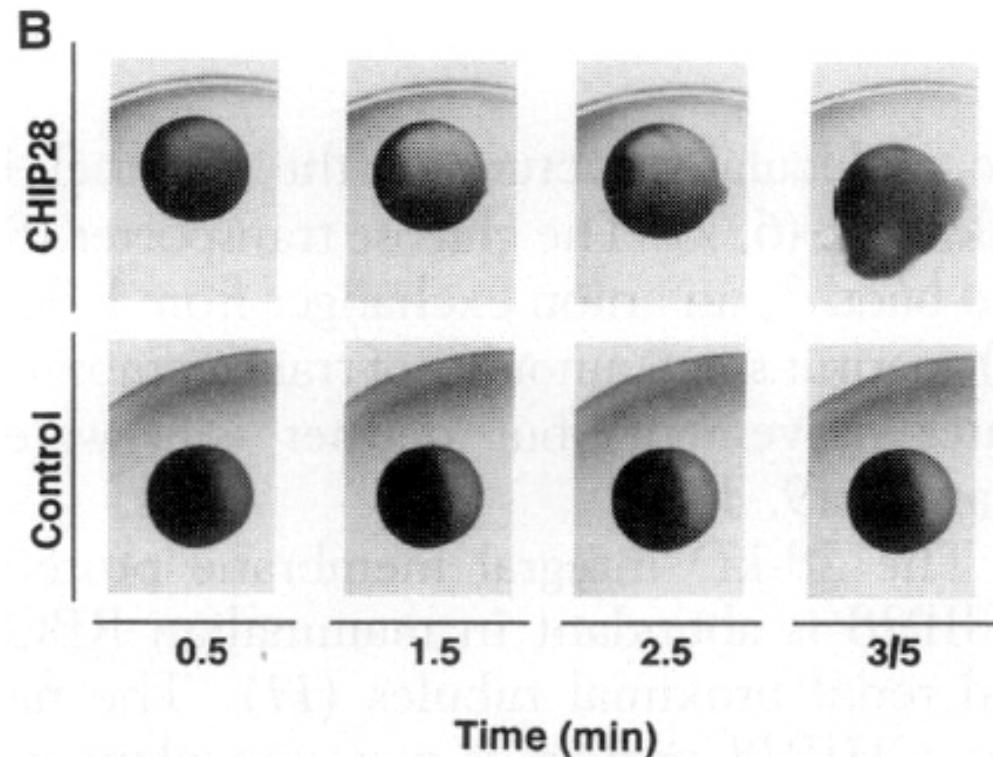


1. Aquaporins

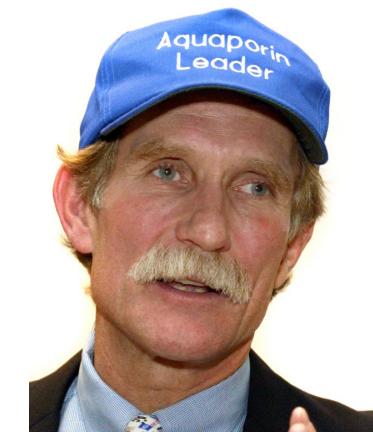
Channel-mediated, high-capacity
 H_2O transport in select cells (e.g.
kidney cells)
 H_2O , but not H^+ transport!



m



Preston et al., Science 256: 385-387 (1992)

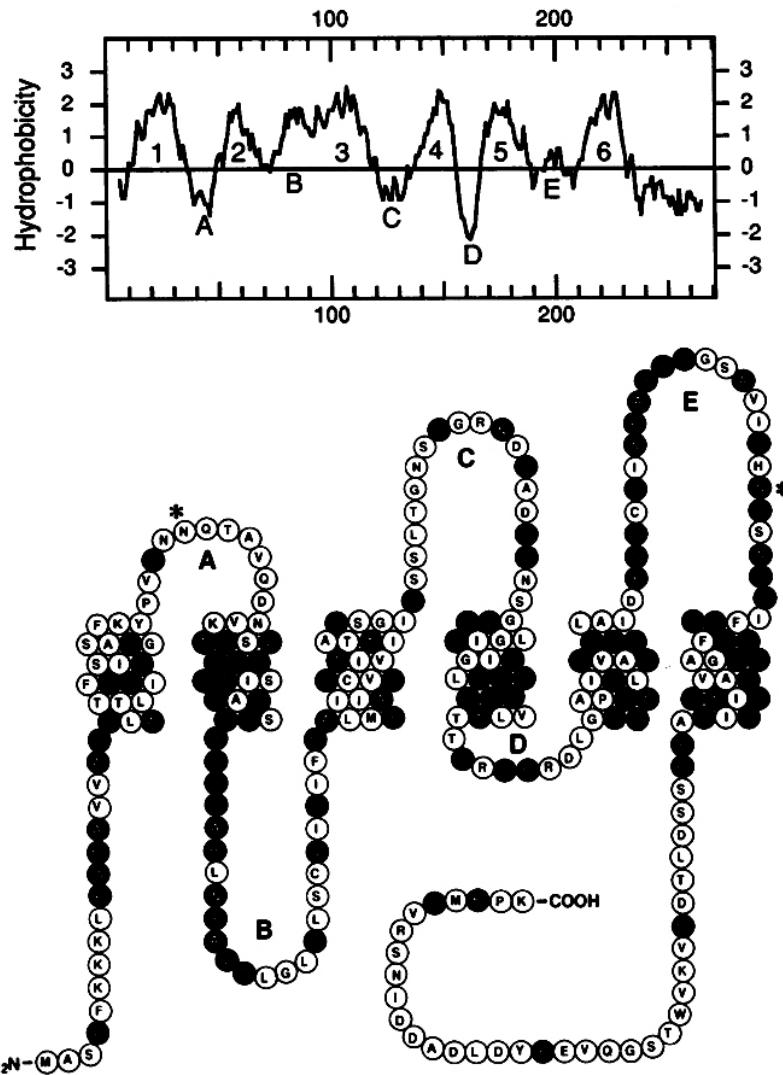


Peter Agre

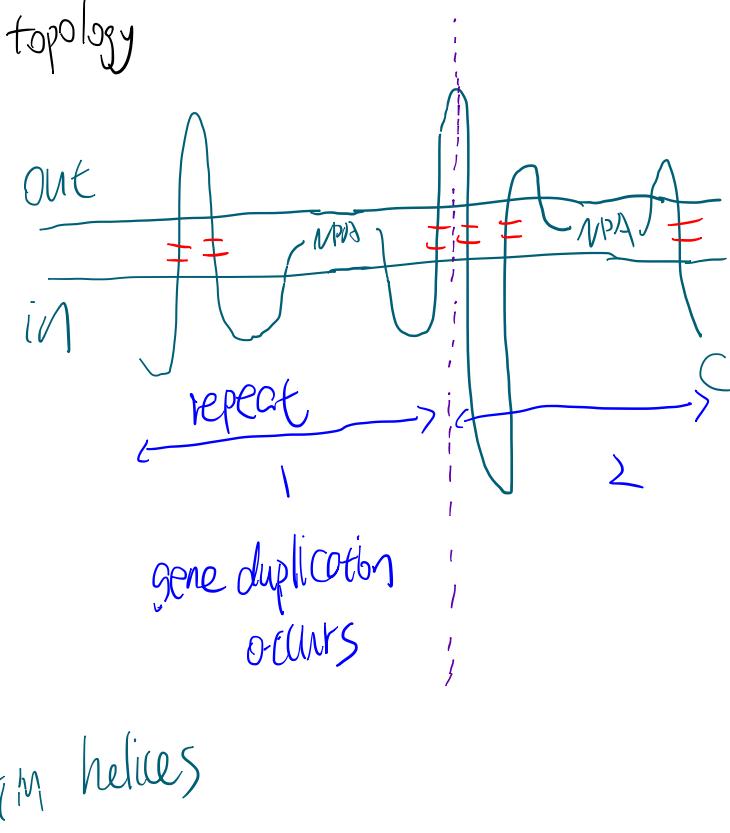
Peter Agre (Nobel lecture): "We used oocytes from the frog *Xenopus laevis*. This convenient expression system was particularly useful, since frog oocytes have very low water permeability and survive even in fresh water ponds. Control oocytes were injected with water alone; test oocytes were injected with 2 ng of cRNA encoding our protein. After three days of protein synthesis, the oocytes appeared essentially identical. Then we stressed the oocytes by transferring them to distilled water, and an amazing difference was immediately apparent. Having exceedingly low water permeability, the control oocytes failed to swell. In contrast, the test oocytes were highly permeable to water and exploded like popcorn. This result produced much celebration in the laboratory; the celebrations continue still."

CHIP28

(Aquaporin)



Actual topology



6 7M helices

Preston & Agre (1991) PNAS 88, 11110-11114

FIG. 3. Hydropathy and proposed membrane topology of CHIP28. (Upper) Deduced amino acid sequence of CHIP28 computer-analyzed for hydrophobicity by using the algorithm of Kyte and Doolittle (19) with a seven-residue window. (Lower) Proposed topology of CHIP28 within the erythrocyte membrane (see text). Loops A, C, and E are exofacial; loops B and D and the N and C termini are endofacial. Two potential glycosylation sites are marked by an asterisk. A black background with white letters denotes residues identical in CHIP28 and MIP26 proteins (7).

Aquaporin topology and implications

pymol aquaporin part 1

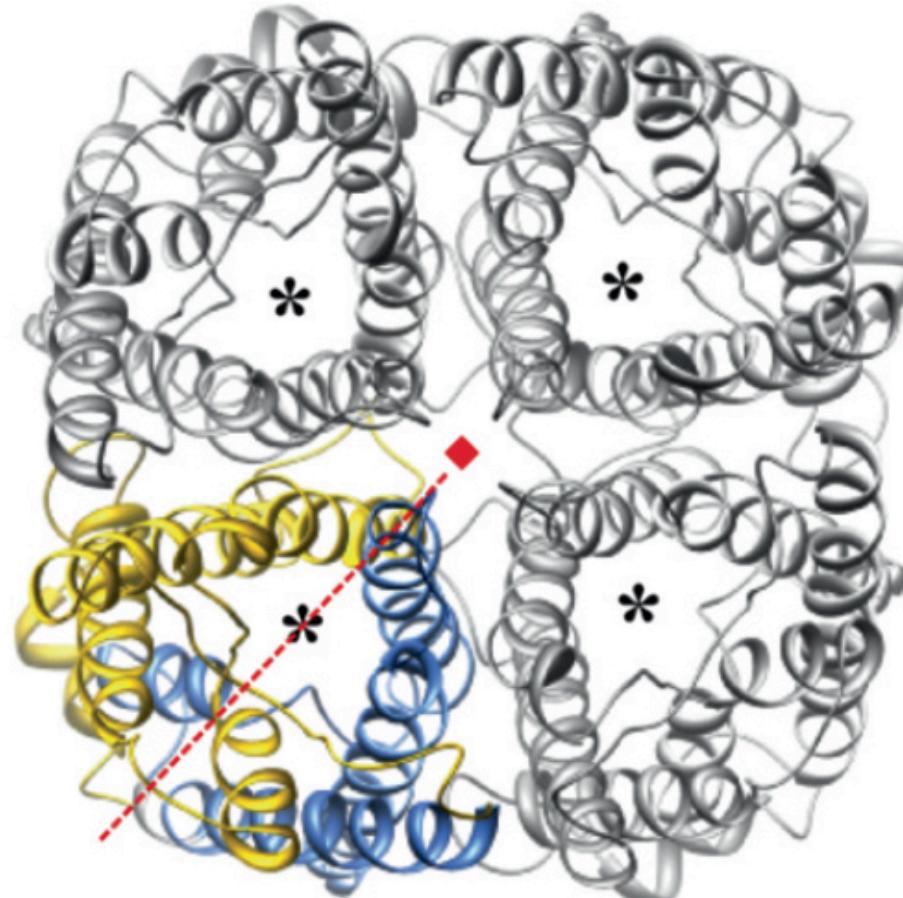
PDB ID: 1J4N.pdb (bovine AQP1)

- Pymol scene 1: ribbon monomer
 - Pymol scene 2: Closeup NPA motif at center → shown as stick
- pseudo two fold symmetry

Aquaporin structure

monomer functions
independently

So that we don't
explore tetramers.



Preston et al., *Handb. Exp. Pharmacol.* 190: 31-56 (2009)



Bing Jap

pdb: 1J4N

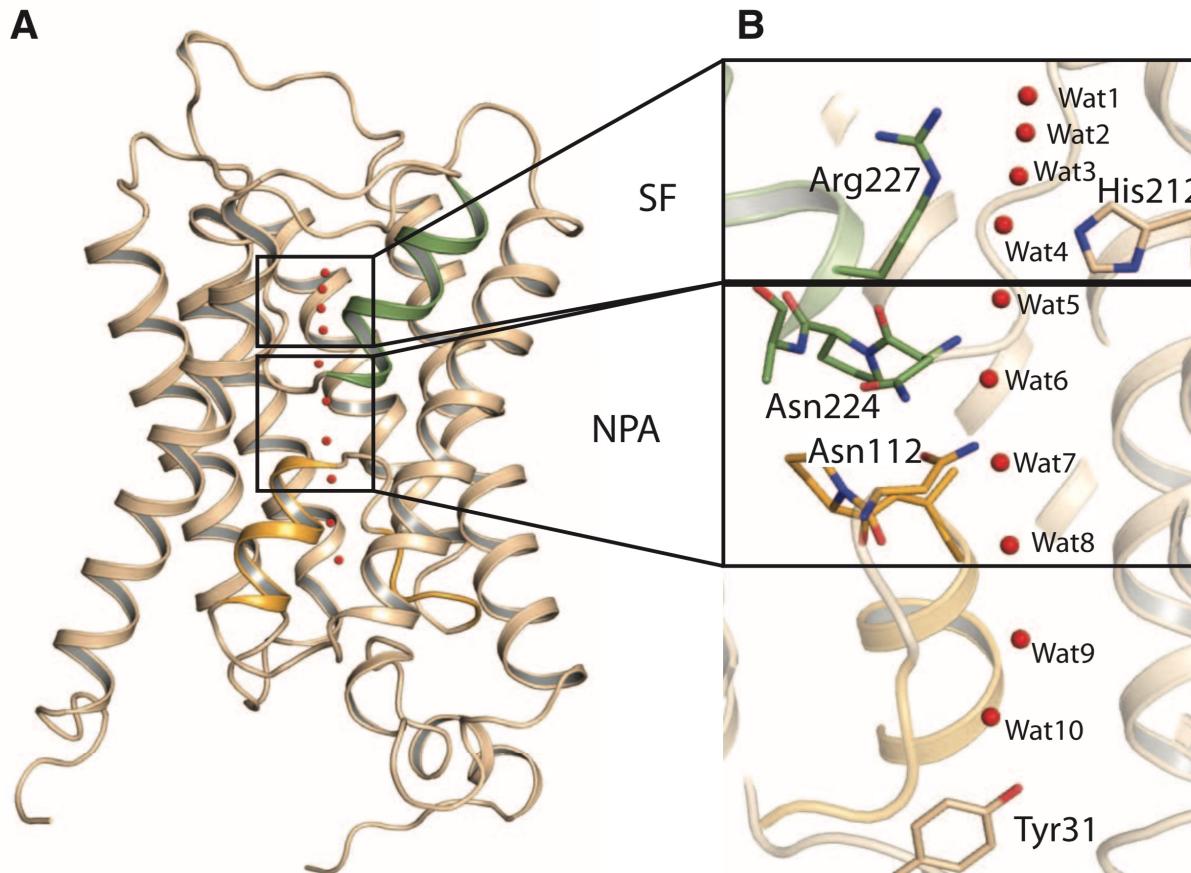
Reference: Sui H et al., *Nature* 414: 872-878 (2001).

pymol aquaporin part 2

PDB ID: 1J4N.pdb, 3ZOJ.pdb

- Pymol scene 1: Tetramer with 1 monomer rainbow colored repeats
- Pymol scene 2: Ribbon monomer view along pseudo two-fold
- Pymol scene 3: Water molecules side view monomer
- Pymol scene 4: Water file through monomer center channel
- Pymol scene 5: Distances water molecules good for hydrogen bonding
- Pymol scene 6: Surface and water molecules
channel is very narrow instead of blocking

Pichia pastoris aquaporin structure at 0.88 Å resolution



Emad Tajkhorshid



Richard Neutze

pdb: 3JOZ

Reference: Eriksson UK et al., *Science* **340**: 1346 (2013).

Pichia pastoris aquaporin structure at 0.88 Å resolution

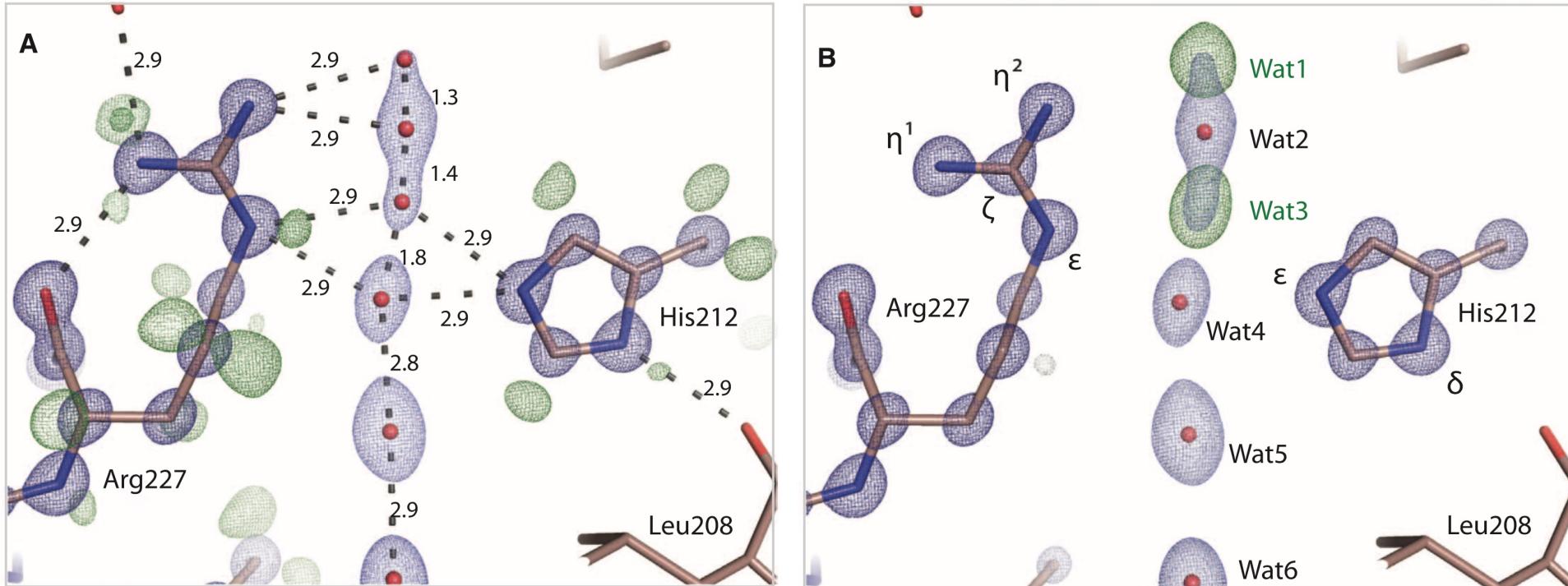


Fig. 3. Electron density within the Aqy1 SF. (A) The $2mF_{\text{obs}} - DF_{\text{calc}}$ (dark blue contoured at $4.3 \text{ e}/\text{\AA}^3$ and light blue at $1.9 \text{ e}/\text{\AA}^3$) and residual $mF_{\text{obs}} - DF_{\text{calc}}$ (dark green contoured at $0.42 \text{ e}/\text{\AA}^3$ and light green contoured at $0.33 \text{ e}/\text{\AA}^3$) electron density associated with His²¹², Arg²²⁷, and water molecules within the SF. Atomic separations (\AA) are indicated. Residual $mF_{\text{obs}} - DF_{\text{calc}}$ electron density reveals that N δ of His²¹² is protonated, whereas N ϵ is not. Connected $2mF_{\text{obs}} - DF_{\text{calc}}$ electron density suggests that the Arg²²⁷ covalent bond from C ζ to N η 2 is preferentially conjugated. Four closely spaced water molecules are modeled within the SF with complementary occupancy (66% occupancy, positions 2 and 4; 34% occupancy, positions 1 and 3). (B) The $mF_{\text{obs}} - DF_{\text{calc}}$ omit electron density map calculated when waters 1 and 3 are removed from the structural model (dark green contoured at $0.65 \text{ e}/\text{\AA}^3$). Positive electron-density features associated with these waters are the strongest within the channel.

Eriksson UK et al., *Science* **340**: 1346 (2013).

→ Cannot coexist (13 / 24)
it is related to forming of
hydrogen bonds

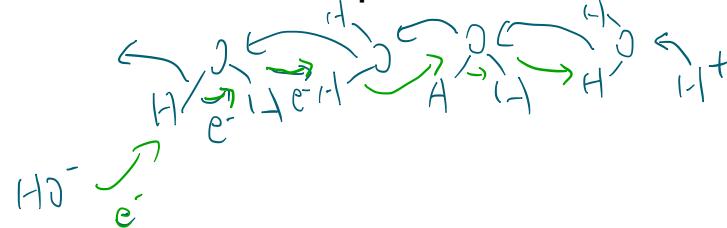


Exclusion of protons by aquaporins:

Three mechanistic proposals

By simulation, it is not realistic

1. Interruption of Grotthus wire



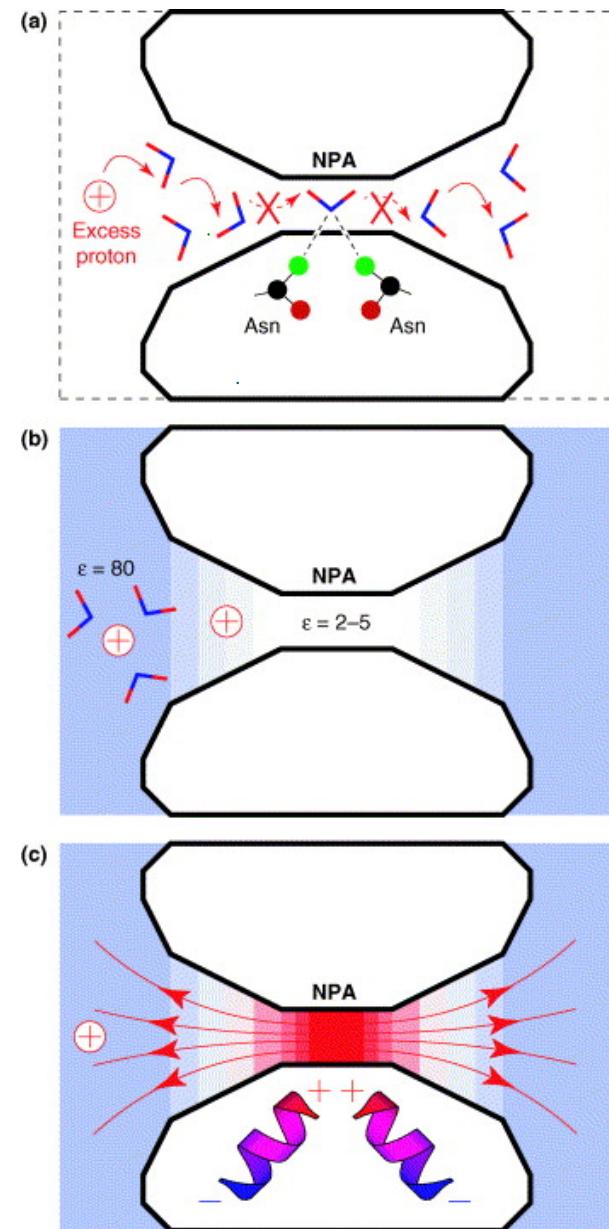
2. Low dielectric region forming barrier for charged species

charged molecules are excluded in hydrophobic area?

3. Electrostatic barrier from short helices forming macrodipoles

most realistic

De Groot and Grubmuller *Curr. Op. Struct. Biol.* 15: 176-183 (2005)



Update.....

"...Proton exclusion can *not* be explained from a discontinuous hydrogen bond network inside the channel, as inferred from the initial X-ray structures. Instead, if a proton is forced into the channel [computationally], remarkably high proton mobility through efficient Grotthus transfers was observed throughout the channel, without any severe interruption. These results contrast with the original picture of an interrupted proton wire. The water molecules inside the pore should in fact *not* be regarded as a static bipolar water column, with the water oxygen atoms pointed toward the channel center at any time. Instead, water molecules rotate inside the channel, and only the average water dipole is pointed toward the channel exits.

The consensus conclusion is that a large electrostatic barrier, rather than proton wire interruption effects, is the dominant mechanism of proton exclusion in aquaporins."

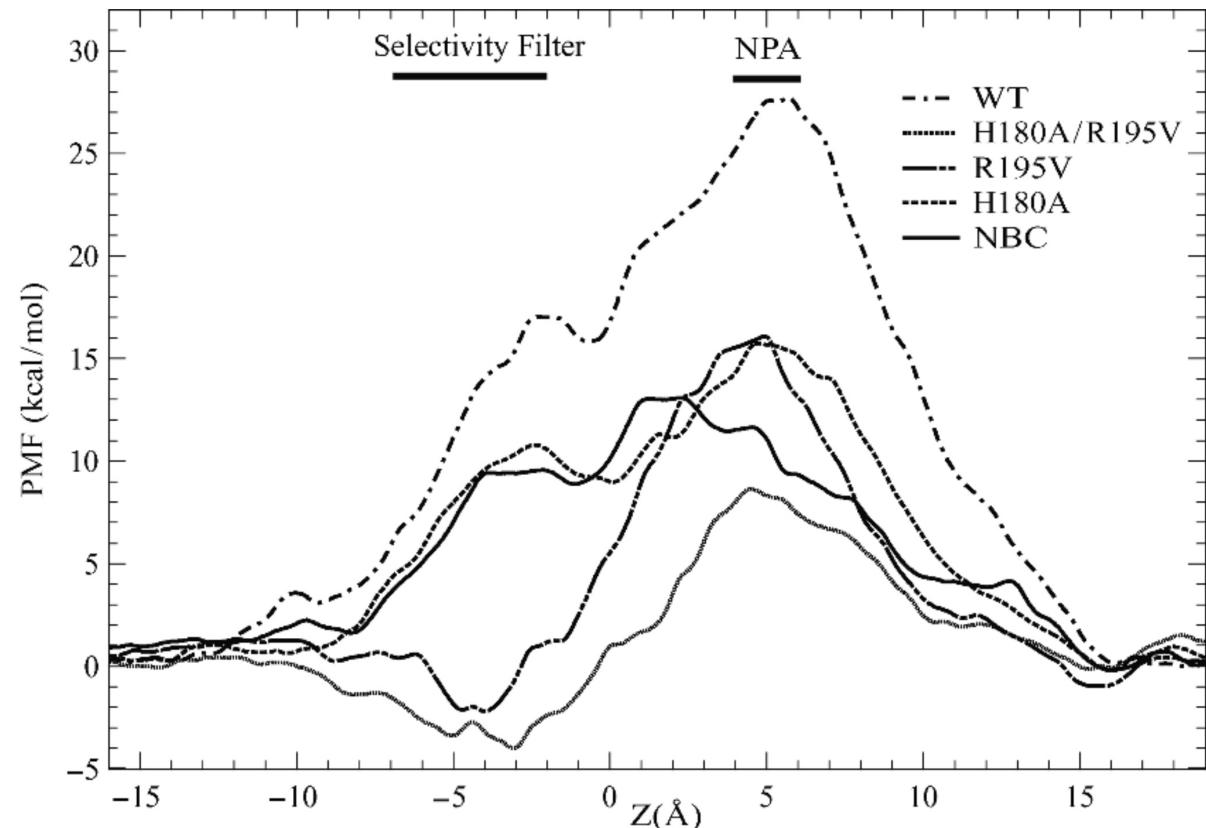


Fig. 4 Potentials of mean force (PMFs) for proton transfer through AQP1 (Chen et al. 2006). In the AQP1 wild type (WT, dotted-dashed curve), a large barrier of 28 kcal mol^{-1} prohibits any proton leakage. Switching off the dipoles of the half helices HB and HE (NBC, *no-backbone-charge*, solid curve) reduces the barrier substantially. Likewise, mutations in the aromatic/arginine region (here termed *selectivity filter*), such as R195V, H180A, R195V/H180A, reduce the barrier and may therefore cause proton leakage, as observed experimentally (Beitz et al. 2006).