Molecular Dynamics Simulation of Membrane Channels

Part II. Structure-Function Relationship and Transport in Aquaporins

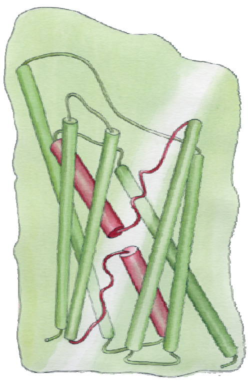
Summer School on Theoretical and Computational Biophysics
June 2003, University of Illinois at Urbana-Champaign
http://www.ks.uiuc.edu/training/SumSchool03/

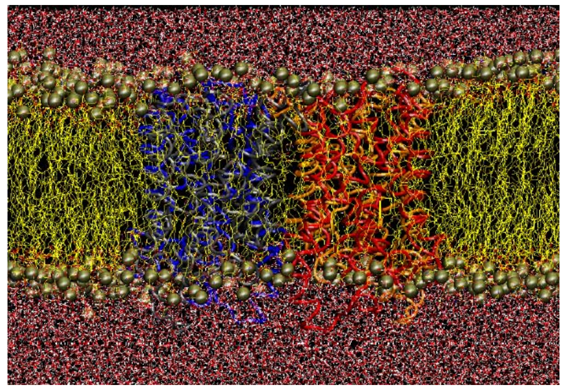
Analysis of Molecular Dynamics Simulations of Biomolecules

- A very complicated arrangement of hundreds of groups interacting with each other
- Where to start to look at?
- What to analyze?
- How much can we learn from simulations?

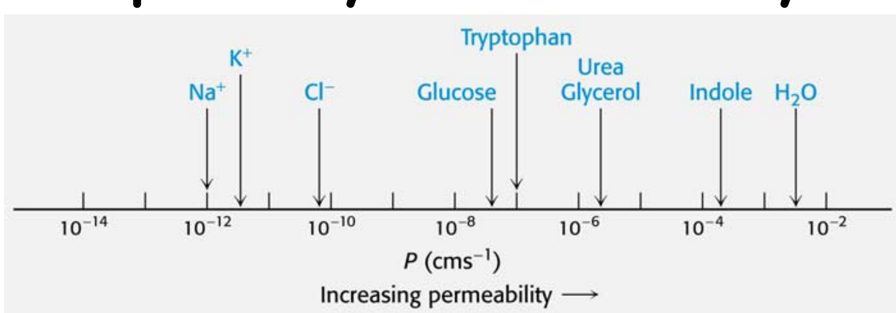
It is very important to get acquainted with your system

Aquaporins
Membrane water channels





Lipid Bilayer Permeability



Water is an exception:

- **Small size**
- **Lack of charge**
- **Its high concentration**

Water Transport Across Cell Membrane

Always passive; bidirectional; osmosis-driven

- Diffusion through lipid bilayers

  slower, but enough for many purposes

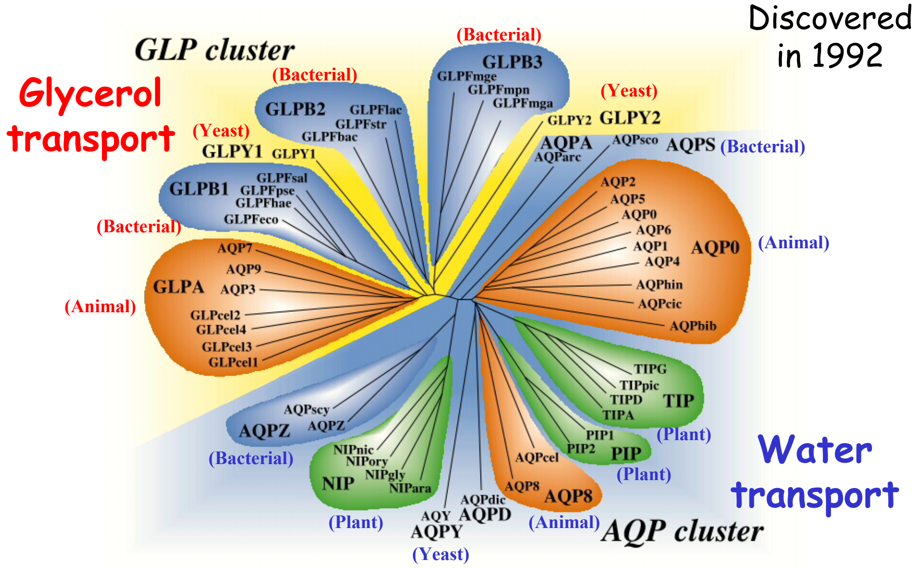
- Channel-mediated

  Large volumes of water needed to be transported (kidneys).

  Fast adjustment of water concentration is necessary (RBC, brain, lung).

The Aquaporin Superfamily

Discovered in 1992

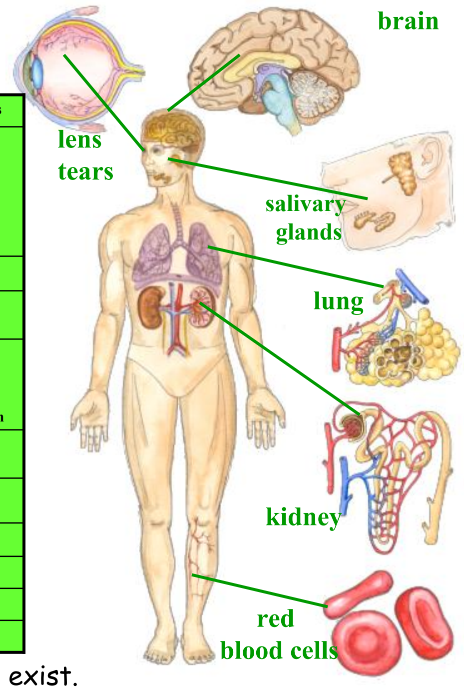


Heymann and Engel *News Physiol. Sci.* **14**, 187 (1999)

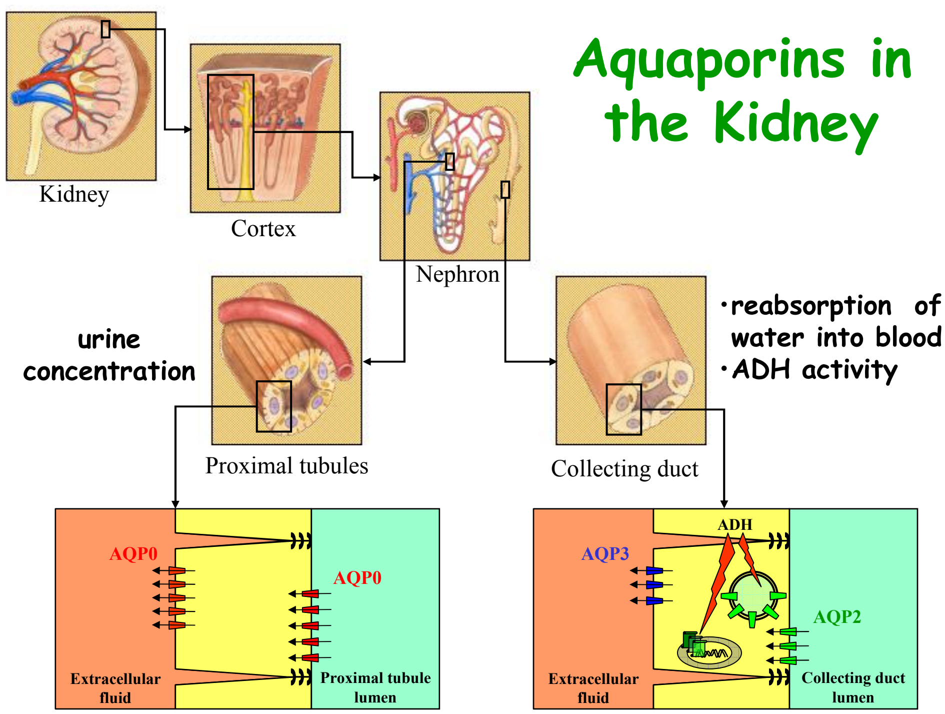
Aquaporins in Human Body

Aquaporin-0	Eye: lens fiber cells	Fluid balance of the lens
Aquaporin-1	Red blood cells Kidney: proximal tubules Eye: ciliary epithelium Brain: choriod plexus Lung: alveolar epithelial cells	Osmotic protection Concentration of urine Aqueous humor Production of CSF Alveolar hydration
Aquaporin-2	Kidney: collecting ducts	ADH hormone activity
Aquaporin-3	Kidney: collecting ducts Trachea: epithelial cells	Reabsorption of water Secretion of water
Aquaporin-4	Kidney: collecting ducts Brain: ependymal cells Brain: hypothalamus Lung: bronchial epithelium	Reabsorption of water CSF fluid balance Osmosensing function? Bronchial fluid secretion
Aquaporin-5	Salivary glands Lacrimal glands	Production of saliva Production of tears
Aquaporin-6	Kidney	Very low water permeability!
Aquaporin-7	Testis and sperm	
Aquaporin-8	Testis, pancreas, liver	
Aquaporin-9	Leukocytes	
Aquaporin-10		

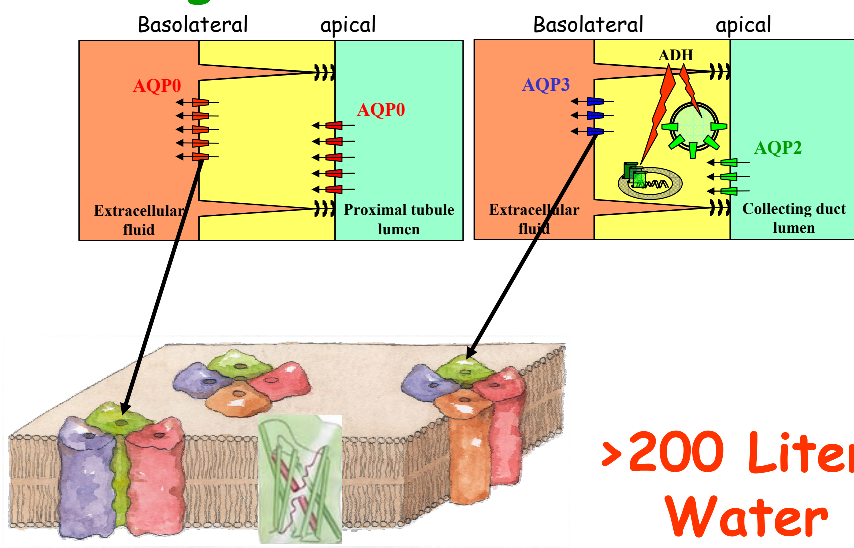
Additional members are suspected to exist.



Aquaporins in the Kidney

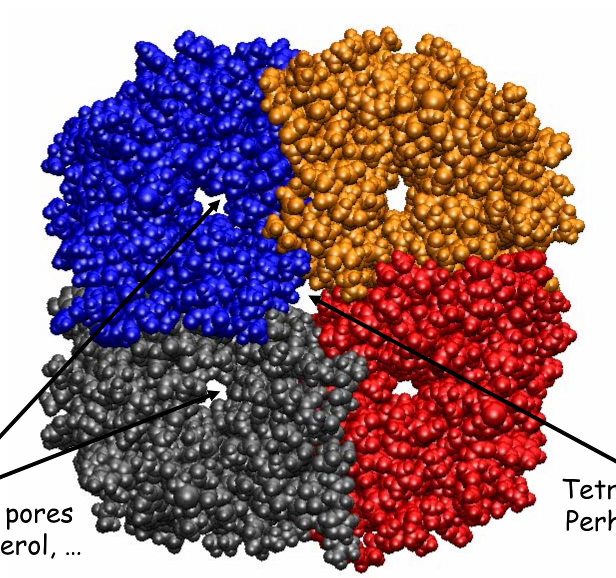


High Permeation to Water



Nephrogenic diabetes insipidus

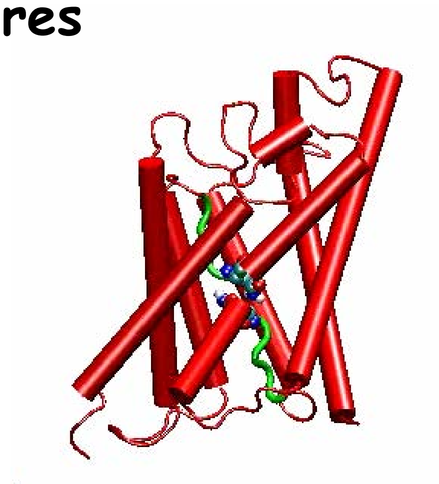
>200 Liters Water Everyday!

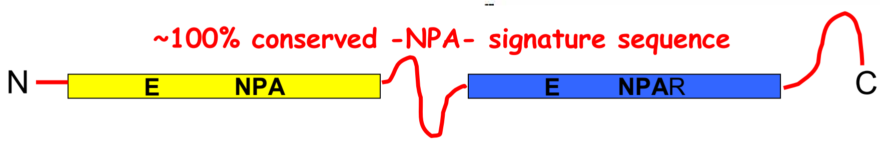


<u>Aquaporins of known structure:</u>
GlpF – E. coli glycerol channel (aquaglycerolporin)
AQP1 – Mammalian aquaporin-1 (pure water channel)

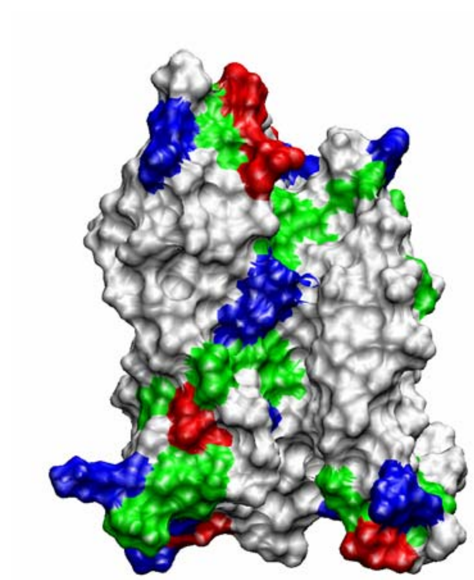
Functionally Important Features

- Tetrameric architecture
- Amphipatic channel interior
- Water and glycerol transport
- Protons, and other ions are excluded
- Conserved asparagine-proline-alanine residues; NPA motif
- Characteristic half-membrane spanning structure



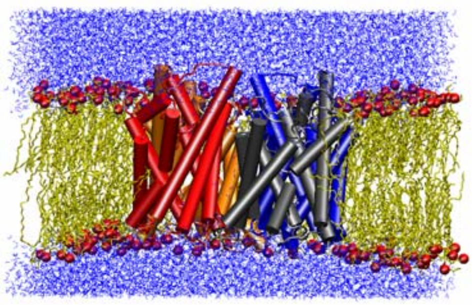


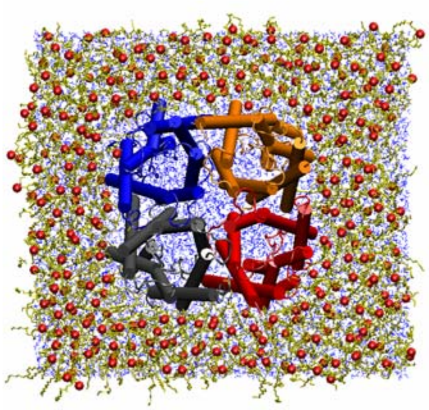
A Semi-hydrophobic channel



Molecular Dynamics Simulations

Protein:	~	15,000 atoms
Lipids (POPE):	~	40,000 atoms
Water:	~	51,000 atoms
Total:	~	106,000 atoms





NAMD, CHARMM27, PME

NpT ensemble at 310 K

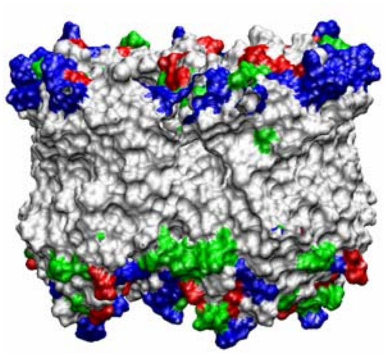
1ns equilibration, 4ns production

10 days /ns – 32-proc Linux cluster

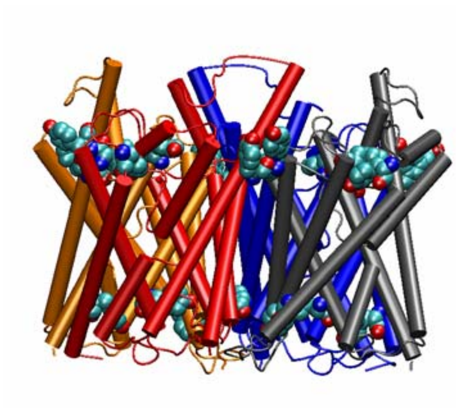
3.5 days/ns - 128 O2000 CPUs

0.35 days/ns – 512 LeMieux CPUs

Protein Embedding in Membrane

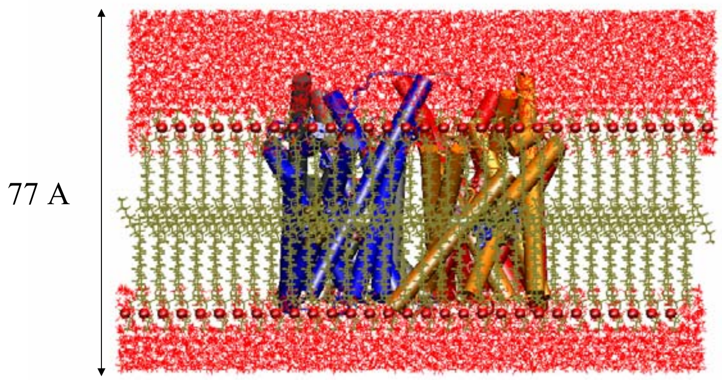


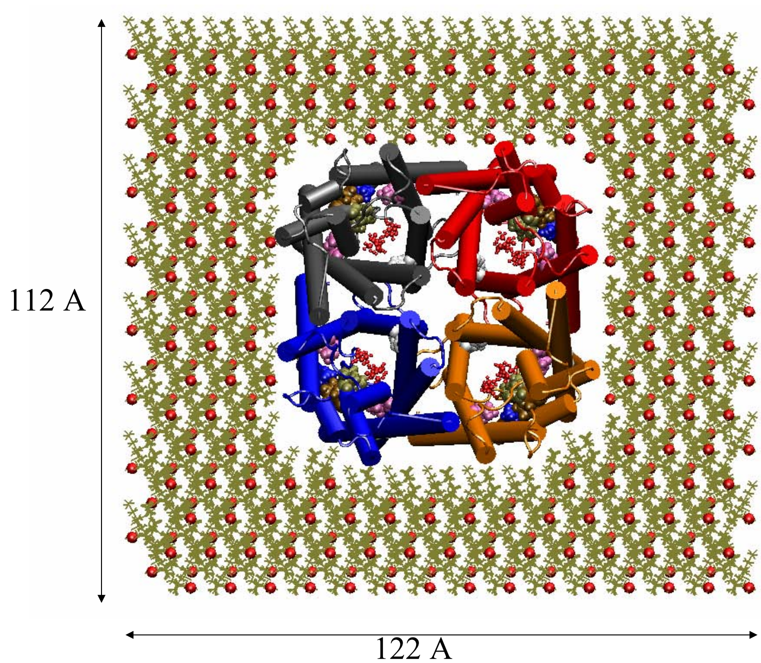
Hydrophobic surface of the protein



Ring of aromatic side chains, specially tyrosines

Embedding GlpF in Membrane

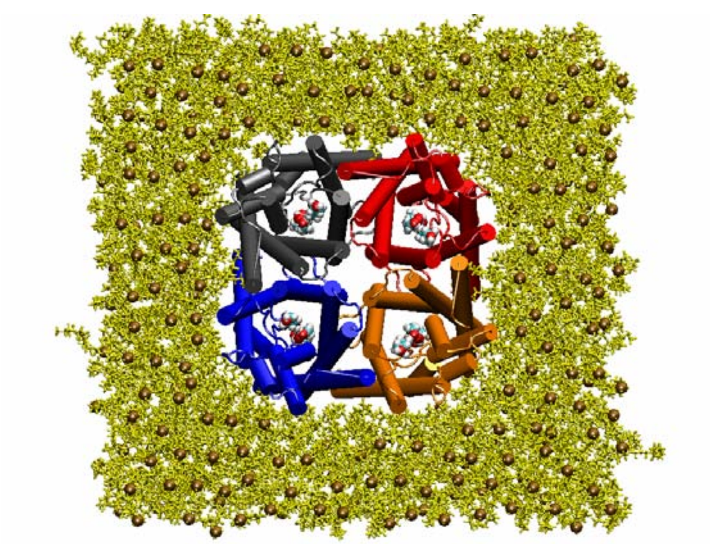




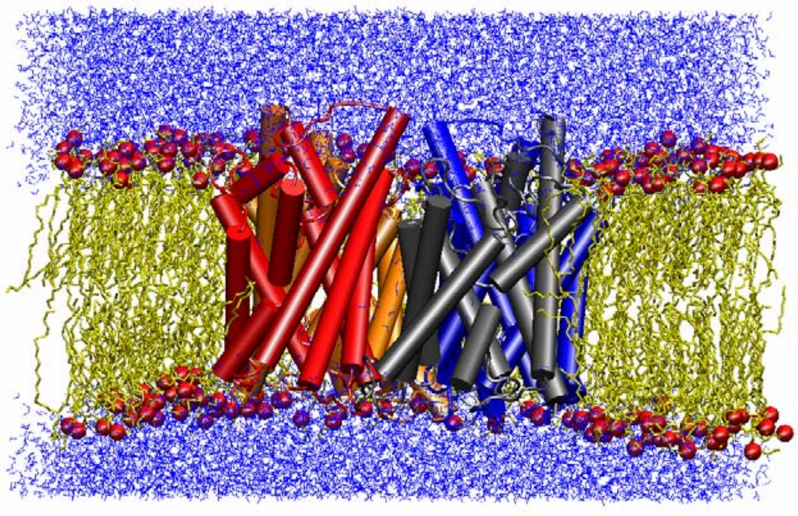
A Recipe for Membrane Protein Simulations

- Insert your protein into a hydrated lipid bilayer.
- Fix the protein; minimize the rest and run a short "constant-pressure" MD to bring lipids closer to the protein and fill the gap between the protein and lipids.
- Watch water molecules; if necessary apply constraints to prevent them from penetrating into the open gaps between lipids and the protein.
- Monitor the volume of your simulation box until it is almost constant. Do not run the system for too long during this phase.
- Now release the protein, minimize the whole system, and start an NpT simulation of the whole system.
- If desired, you may switch to an NVT simulation, when the system reaches a stable volume.

Lipid-Protein Packing During the Initial NpT Simulation

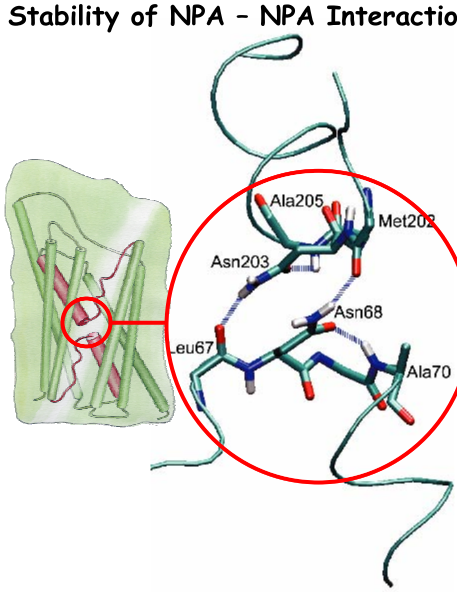


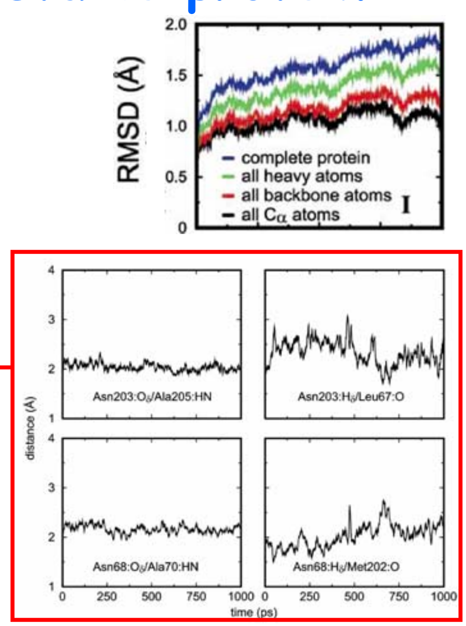
Adjustment of Membrane Thickness to the Protein Hydrophobic Surface



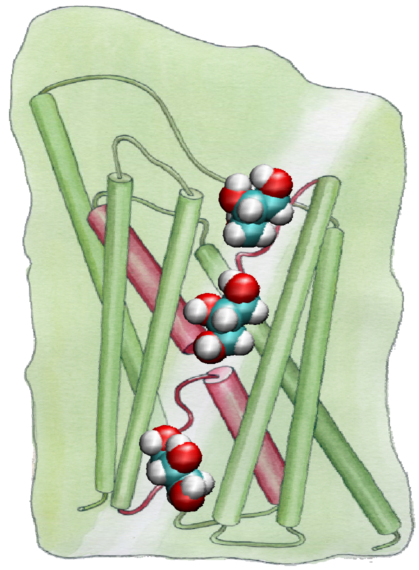
An extremely stable protein

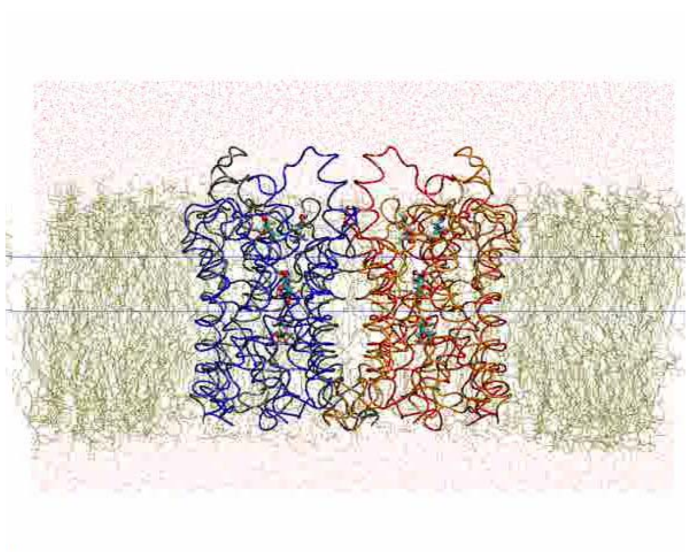
Stability of NPA – NPA Interaction



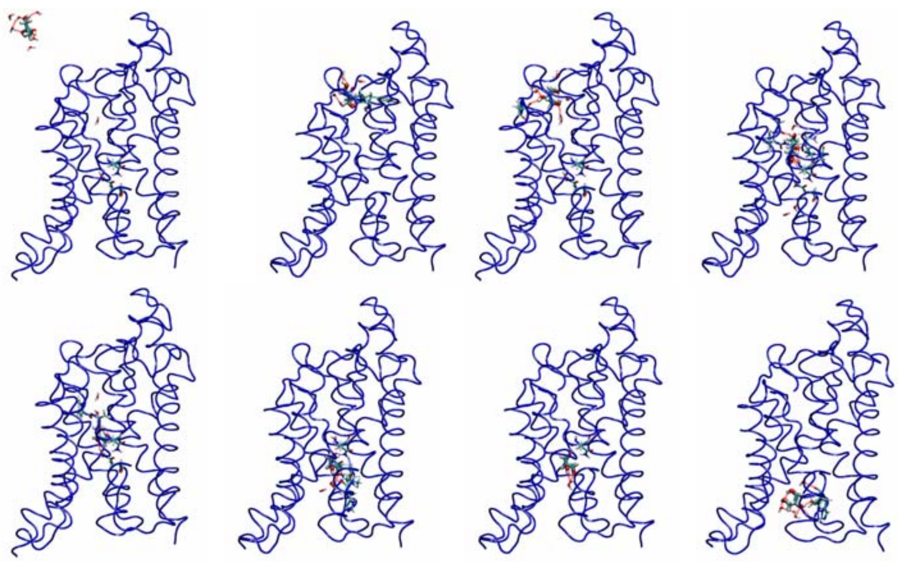


Glycerol-Saturated GlpF





Description of full conduction pathway



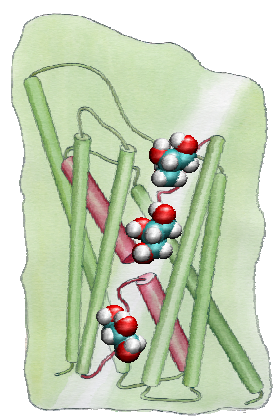
Complete description of the conduction pathway

Details of Protein-Substrate Interaction Are Always Important

- Identify those groups of the protein that are directly involved in the main function of the protein.
- Look at the interaction of these primary residues with other groups in the protein.
- Look at buried charged residues inside the protein; they must have an important role.
- Backbone hydrogen bonds are mainly responsible for stabilization of secondary structure elements in the protein; side chain hydrogen bonds could be functionally important.

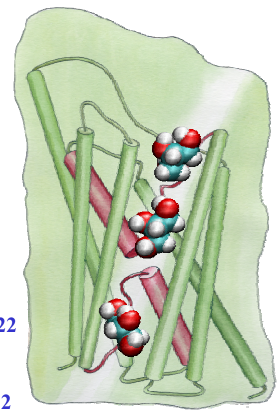
Channel Hydrogen Bonding Sites

```
…
{set frame 0}{frame < 100}{incr frame}{
   animate goto $frame
   set donor [atomselect top
   "name O N and within 2 of
   (resname GCL and name HO)"]
   lappend [$donor get index] list1
   set acceptor [atomselect top
   "resname GCL and name O and
   within 2 of (protein and name HN HO)"]
   lappend [$acceptor get index] list2
}
…
```



Channel Hydrogen Bonding Sites

GLN	41	OE1 NE2	LEU	197	O
TRP	48	O NE1	THR	198	O
GLY	64	O	GLY	199	O
ALA	65	O	PHE	200	O
HIS	66	O ND1	ALA	201	O
LEU	67	O	ASN	203	ND2
ASN	68	ND2			
ASP	130	OD1	LYS	33	HZ1 HZ3
GLY	133	O	GLN	41	HE21
SER	136	O	TRP	48	HE1
TYR	138	O	HIS	66	HD1
PRO	139	O N	ASN	68	HD22
ASN	140	OD1 ND2	TYR	138	HN
HIS	142	ND1	ASN	140	HN HD21 HD22
THR	167	OG1	HIS	142	HD1
GLY	195	O	GLY	199	HN
PRO	196	O	ASN	203	HN HD21HD22
			ARG	206	HE HH21HH22



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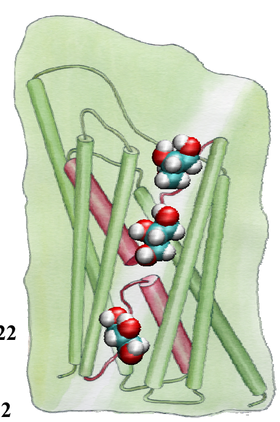
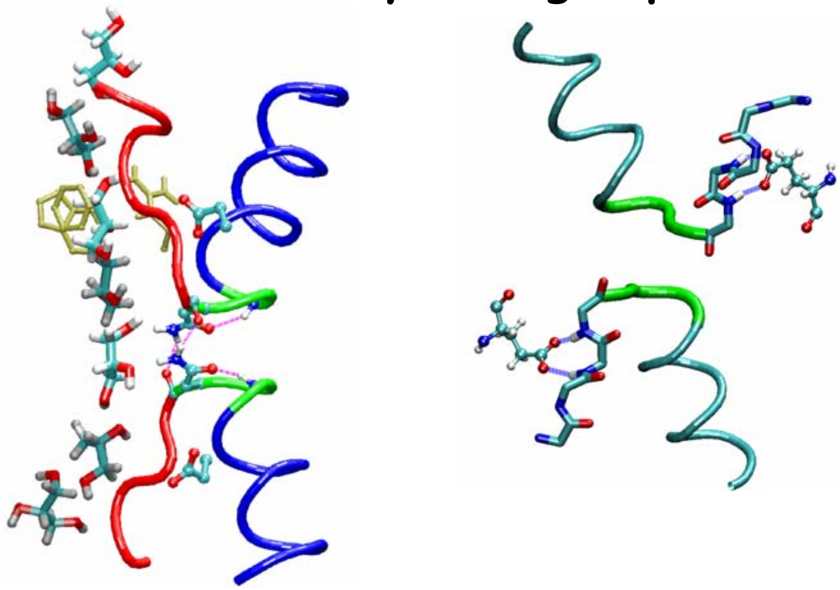
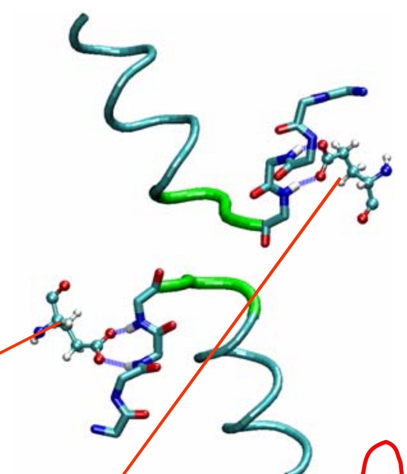


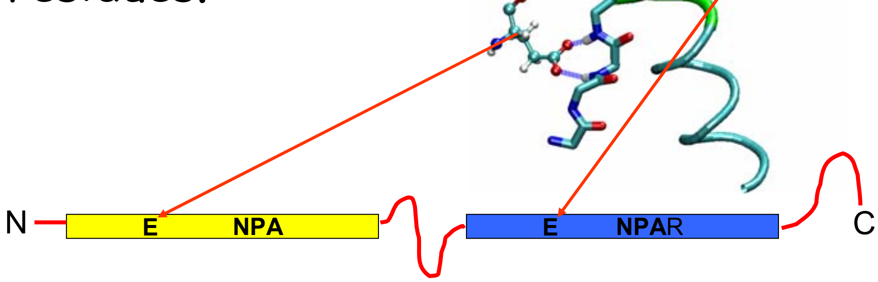
The Substrate Pathway is formed by C=O groups



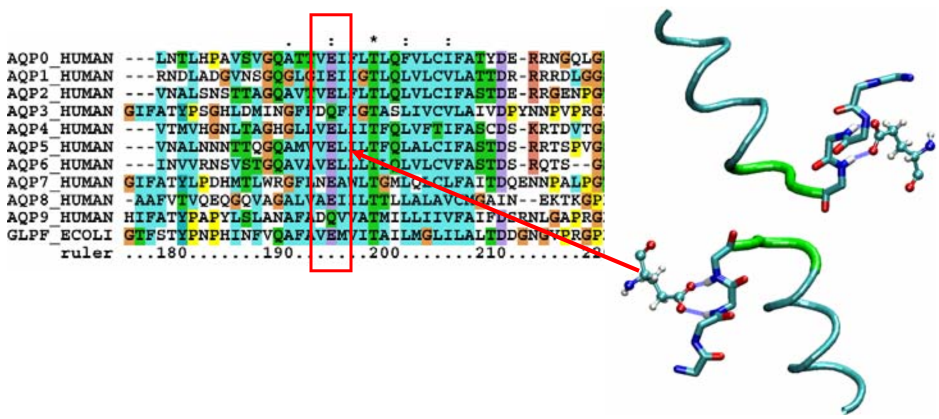
The Substrate Pathway is formed by C=O groups

Non-helical motifs are stabilized by two glutamate residues.

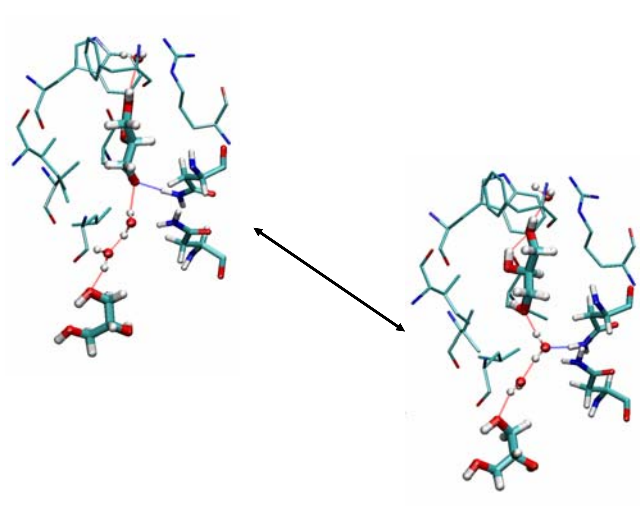




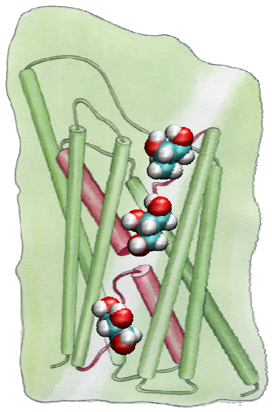
Conservation of Glutamate Residue in Human Aquaporins

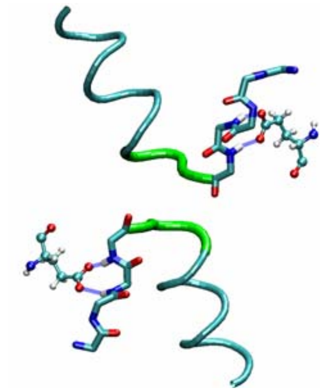


Glycerol – water competition for hydrogen bonding sites

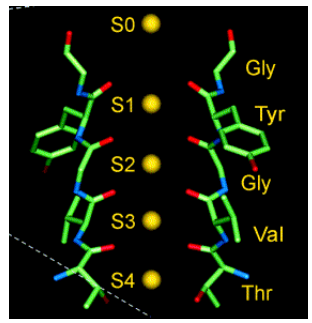


Revealing the Functional Role of Reentrant Loops

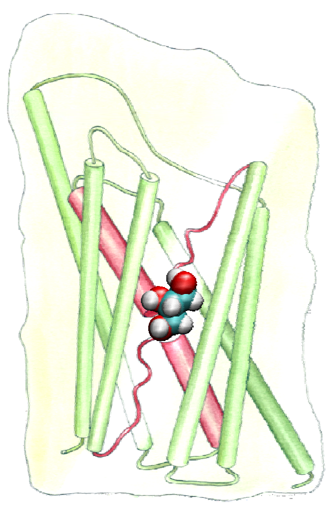


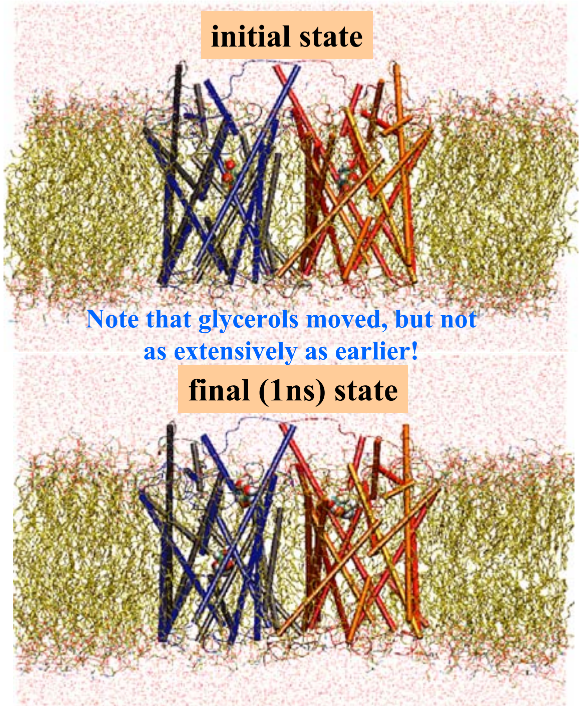


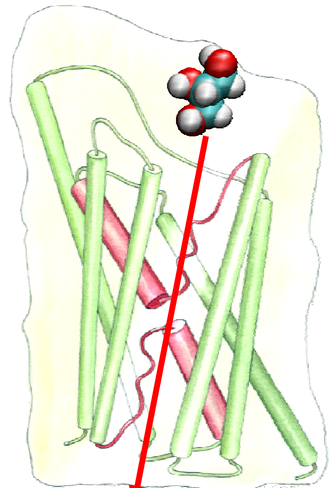
Potassium channel



Single Glycerol per channel

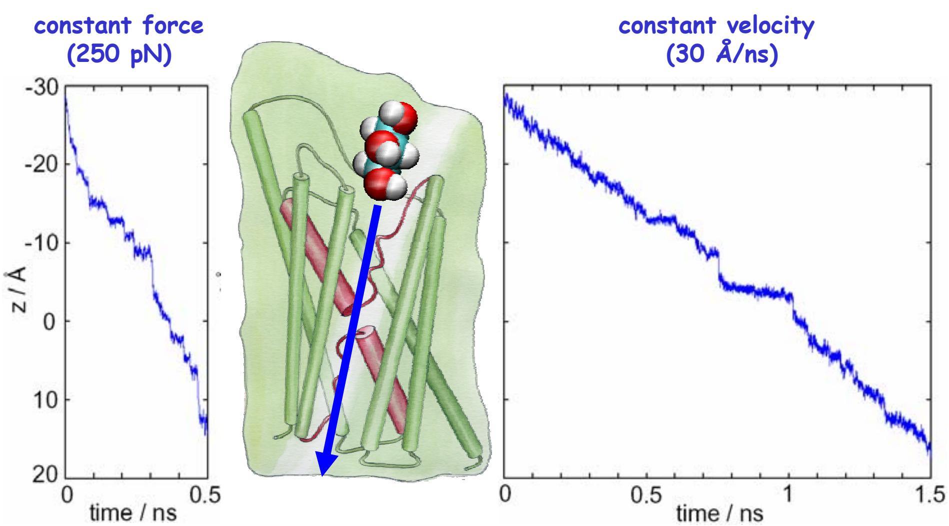




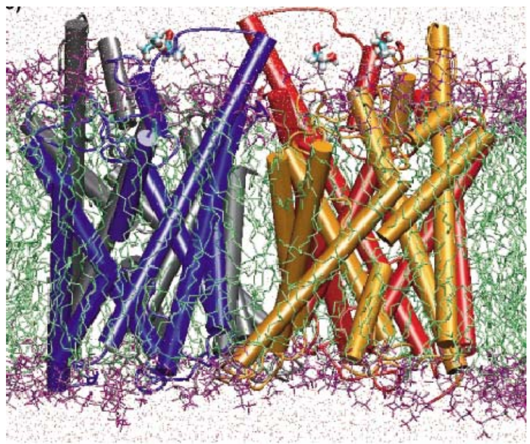


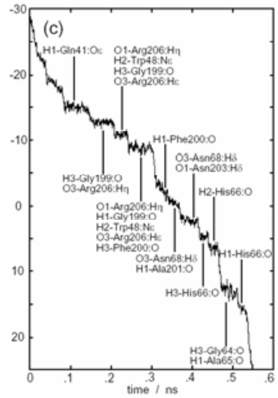
We need to enforce an entire conduction event.

Steered Molecular Dynamics



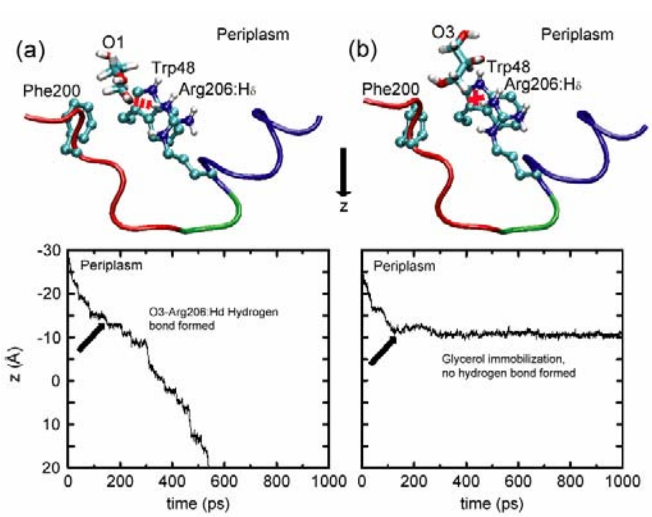
SMD Simulation of Glycerol Passage

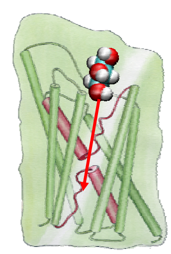


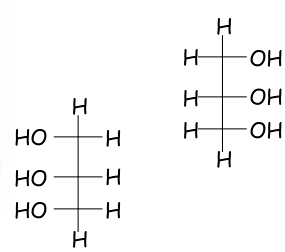


Trajectory of glycerol pulled by **constant force**

Evidence for Stereoselectivity of Glycerol

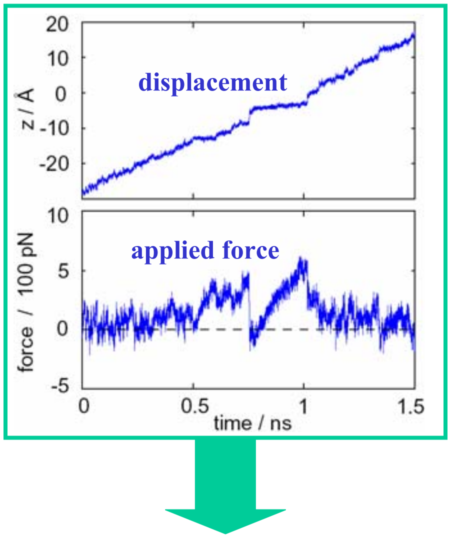






Cannot be verified by experimental measurements

Free Energy Calculation in SMD



SMD simulation
a non-equilibrium process

$$\Delta G \leq \langle W \rangle$$

One needs to discount irreversible work

$$e^{-\Delta G / k_B T} = \left\langle e^{-W / k_B T} \right\rangle$$

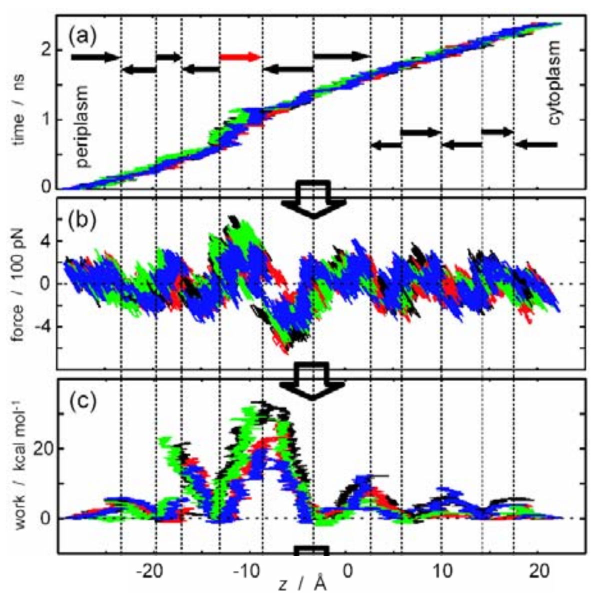
Jarzynski, *PRL* 1997
Hummer, *PNAS, JCP* 2001
Liphardt, et al., *Science* 2002

Constructing the Potential of Mean Force

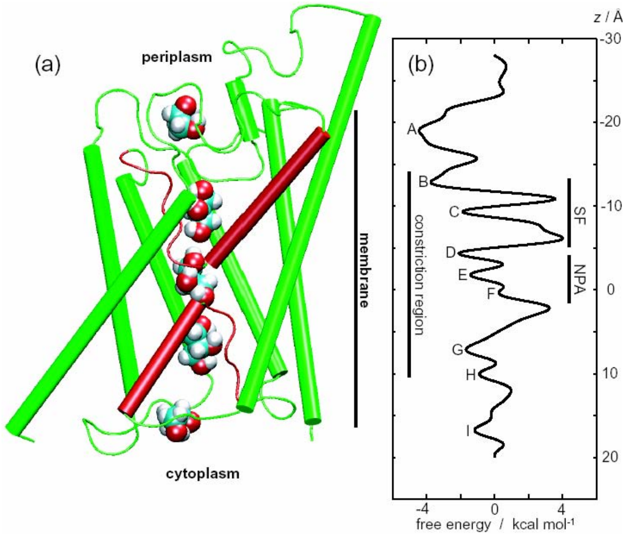
4 trajectories
v = 0.03, 0.015 Å/ps
k = 150 pN/Å

$$f(t) = -k[z(t) - z_0 - vt]$$

$$W(t) = \int_0^t dt' \, v f(t')$$

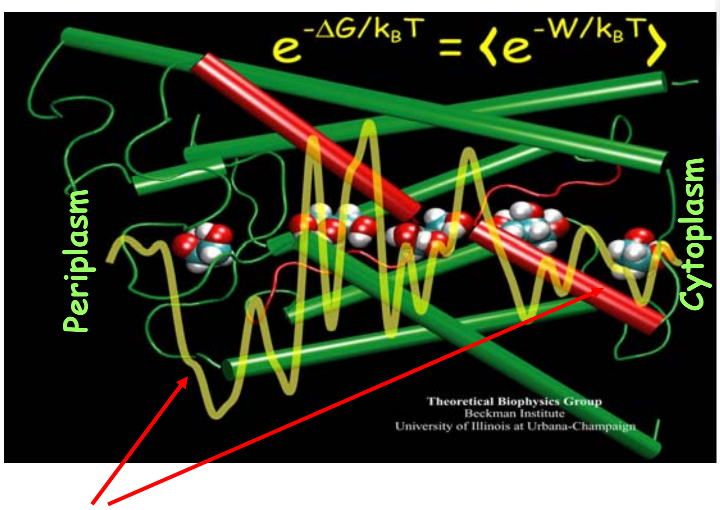


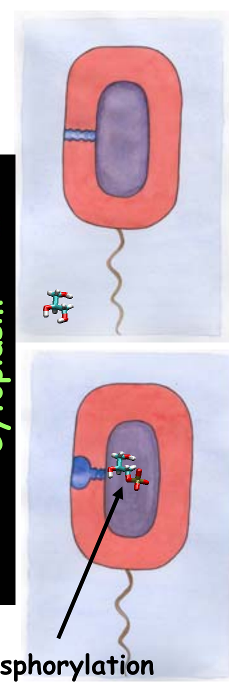
Features of the Potential of Mean Force



• Captures major features of the channel
• The largest barrier ≈ **7.3 kcal/mol; exp.: 9.6±1.5 kcal/mol**

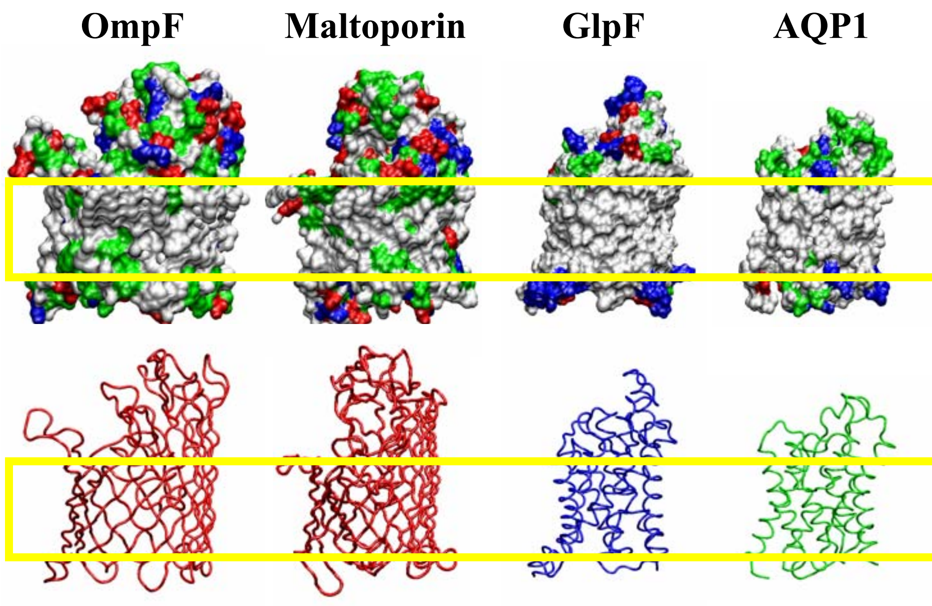
Asymmetry of the Potential of Mean Force



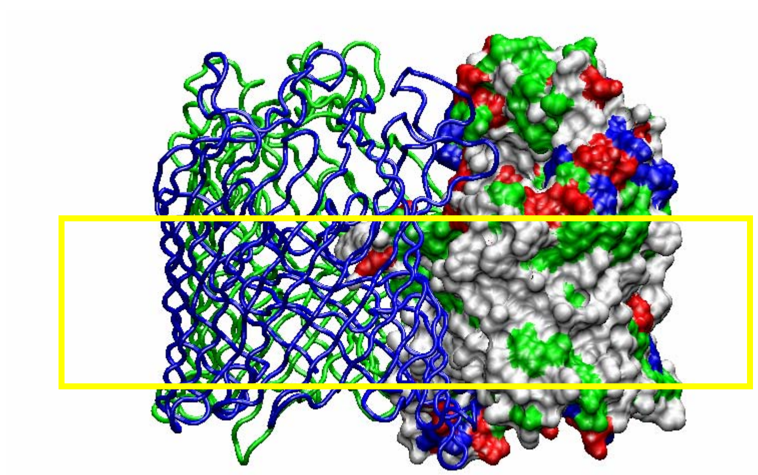


Asymmetric Profile in the Vestibules

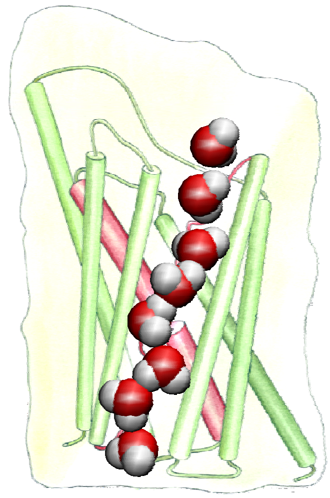
Assymetric structure; biological implication?



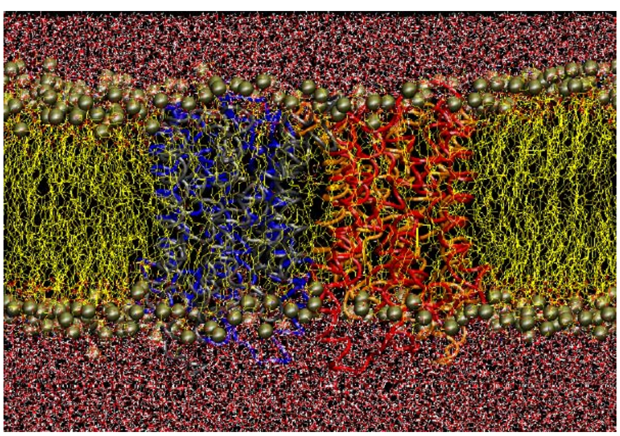
Asymmetric structure of maltoporin



Glycerol-Free GlpF



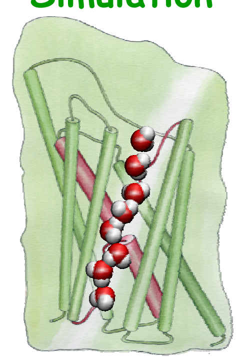
Water permeation



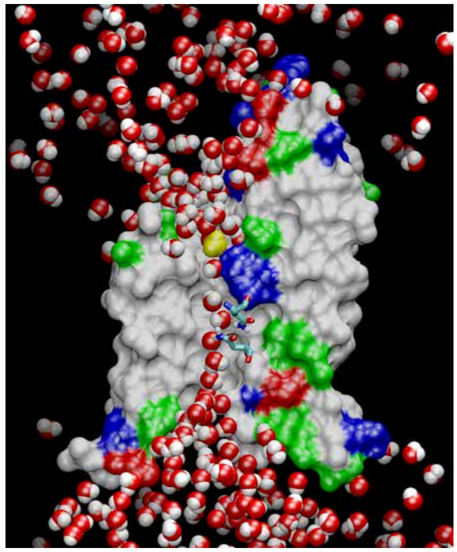
18 water conducted
In 4 monomers in 4 ns
1.125 water/monomer/ns

Exp. = ~ 1-2 /ns

5 nanosecond
Simulation

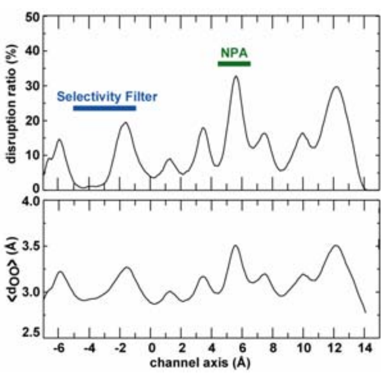


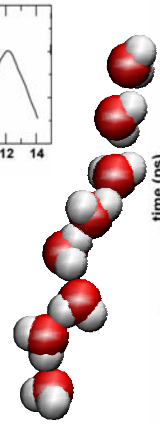
7-8 water
molecules in each
channel

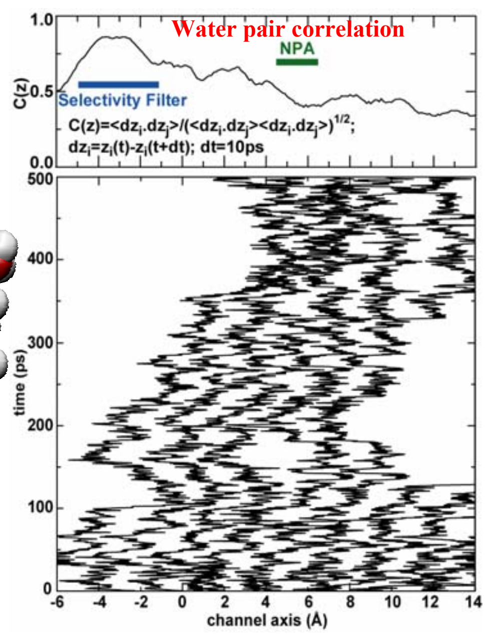


Download the movie from www.ks.uiuc.edu/Research/aquaporins

Correlated Motion of Water in the Channel



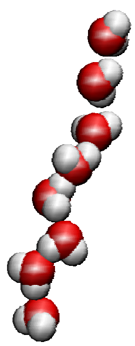


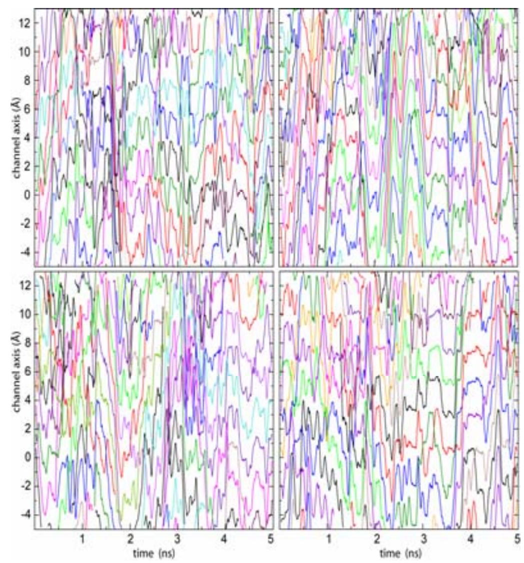


The single file of water molecules is maintained.

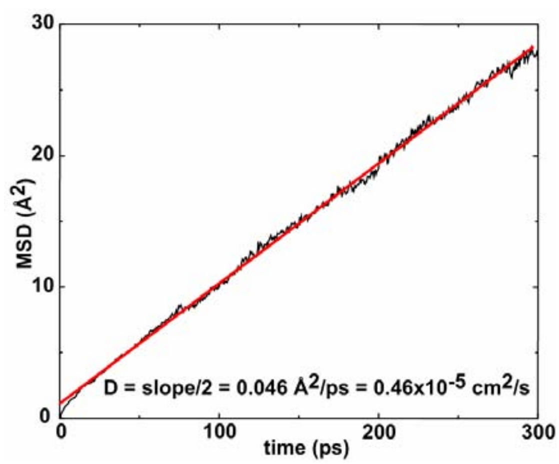
Correlated Motion of Water in the Channel

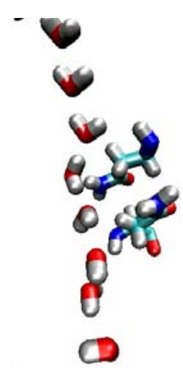
The single file of water molecules is maintained.





Diffusion of Water in the channel

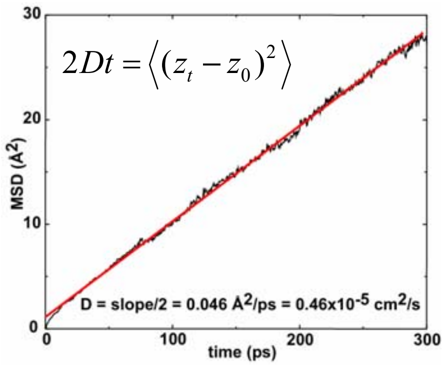


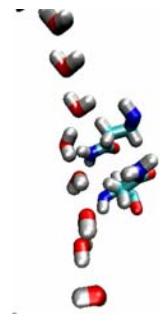


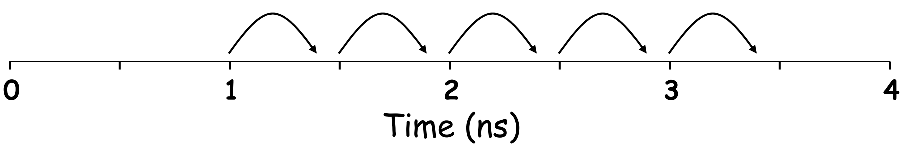
One dimensional diffusion: $2Dt = \left\langle (z_t - z_0)^2 \right\rangle$

Experimental value for AQP1: 0.4-0.8 e-5

Diffusion of Water in the channel

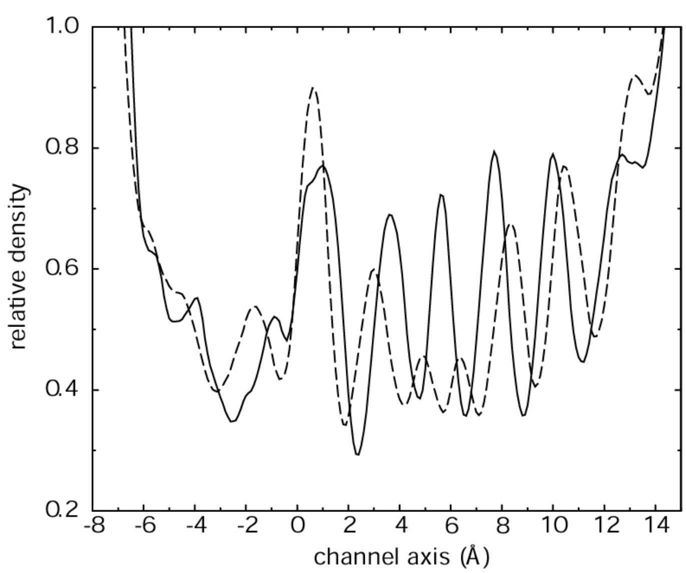




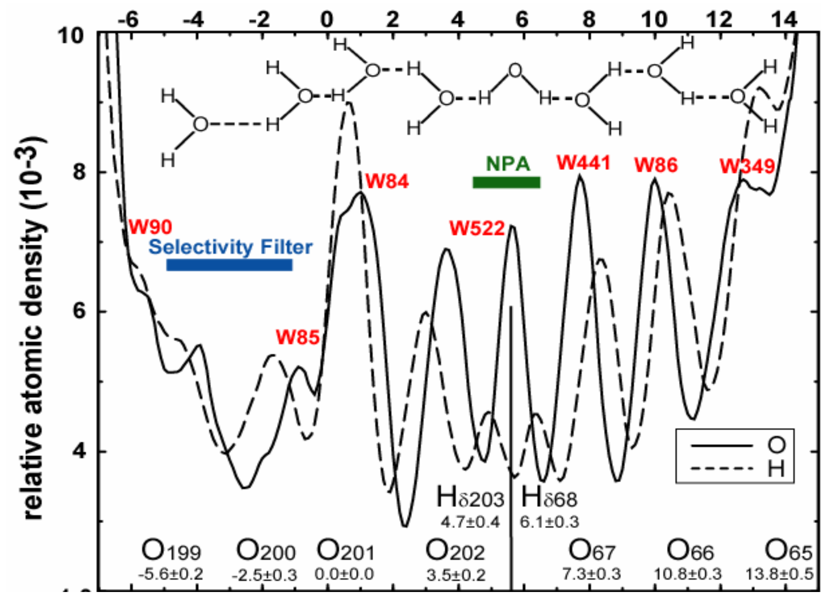


Improvement of statistics

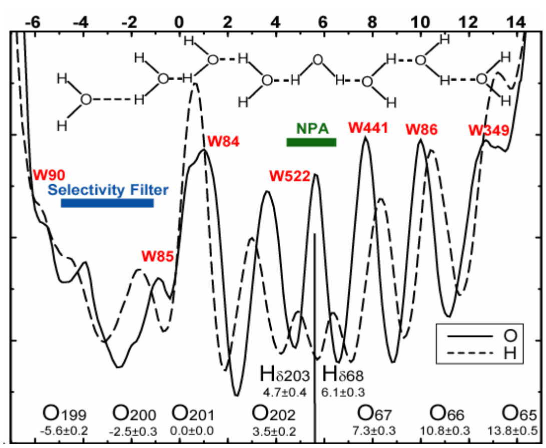
Density of O and H atoms along the GlpF channel

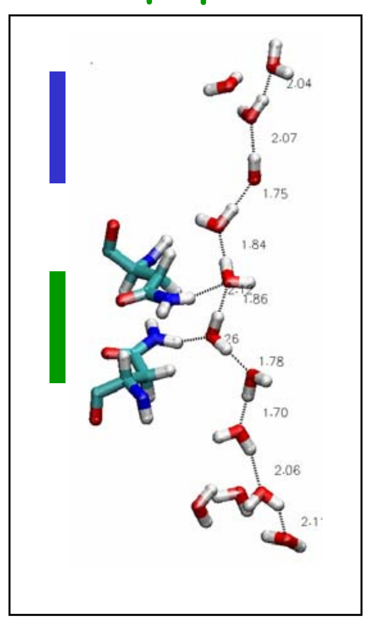


Water Distribution in Aquaporins

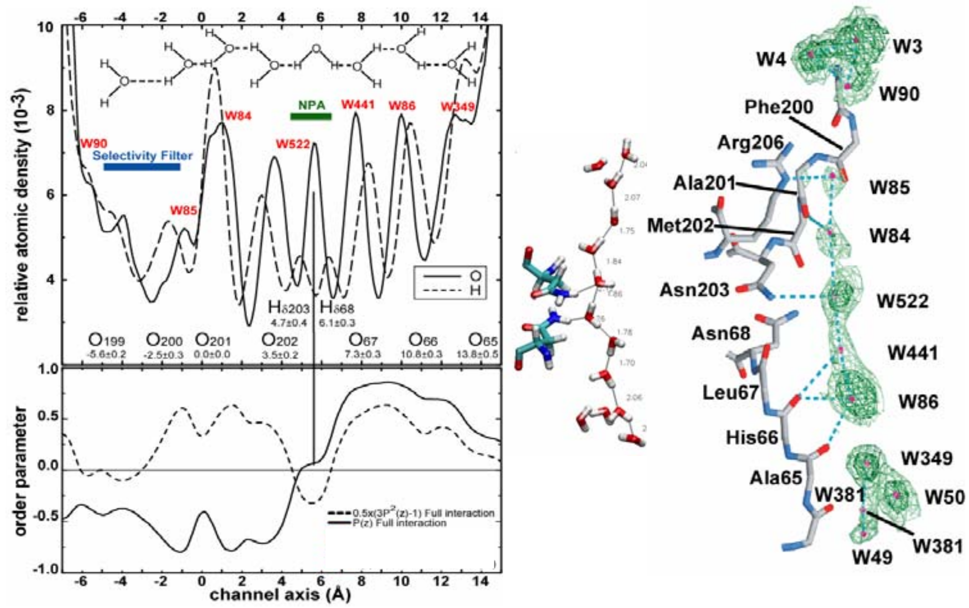


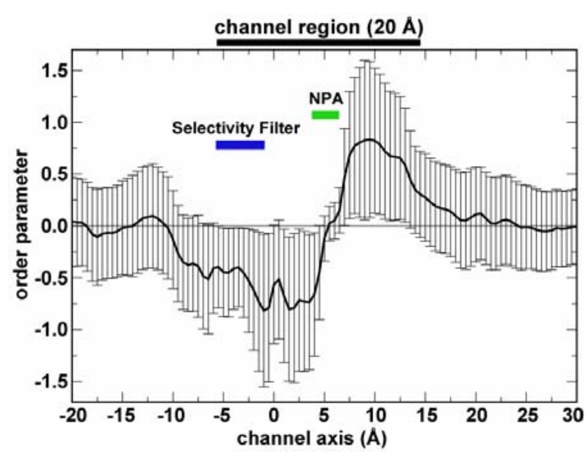
Water Bipolar Configuration in Aquaporins





Water Bipolar Configuration in Aquaporins



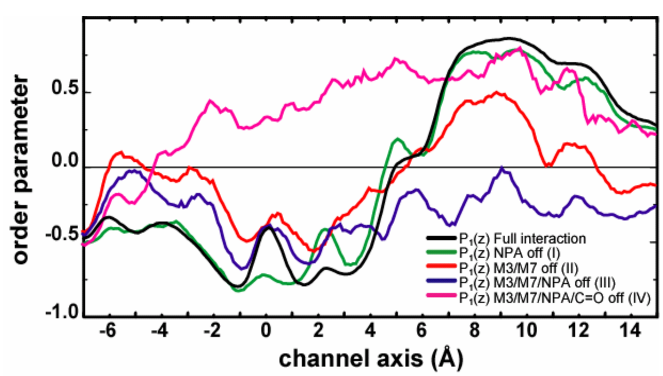


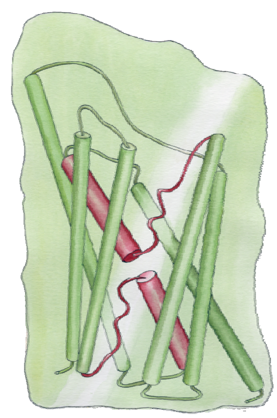
R E M E M B E R:

One of the most useful advantages of simulations over experiments is that you can modify the system as you wish: You can do modifications that are not even possible at all in reality!

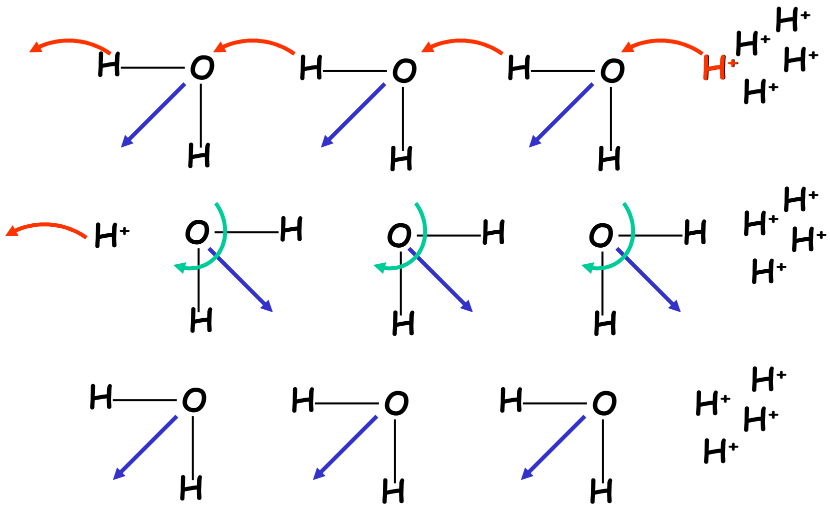
This is a powerful technique to test hypotheses developed during your simulations. Use it!

Electrostatic Stabilization of Water Bipolar Arrangement

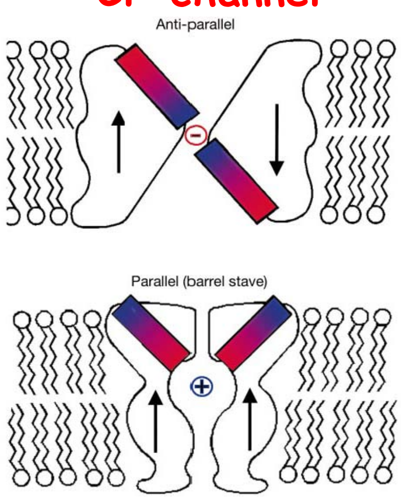




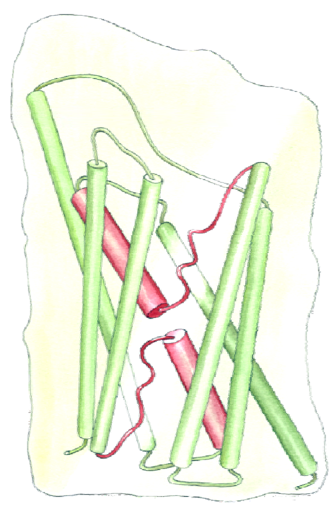
Proton transfer through water



Cl^- channel

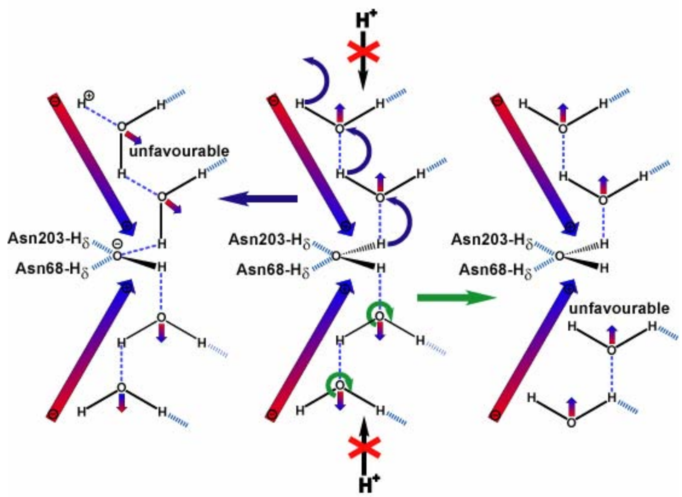


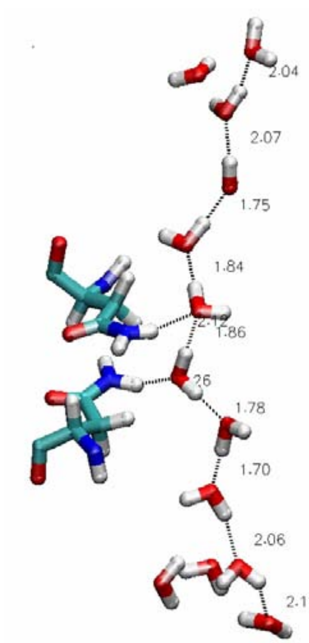
K^+ channel

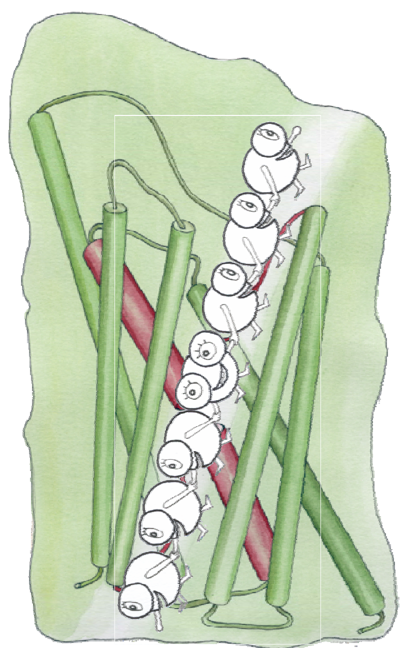


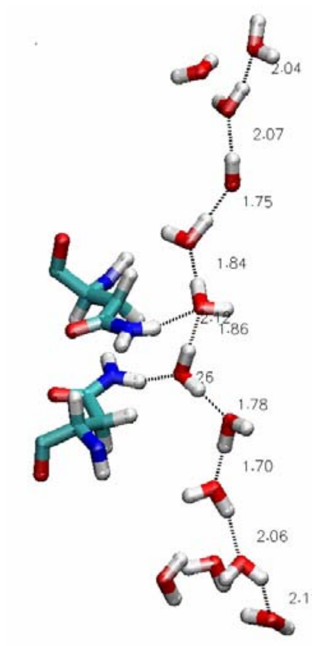
Aquaporins

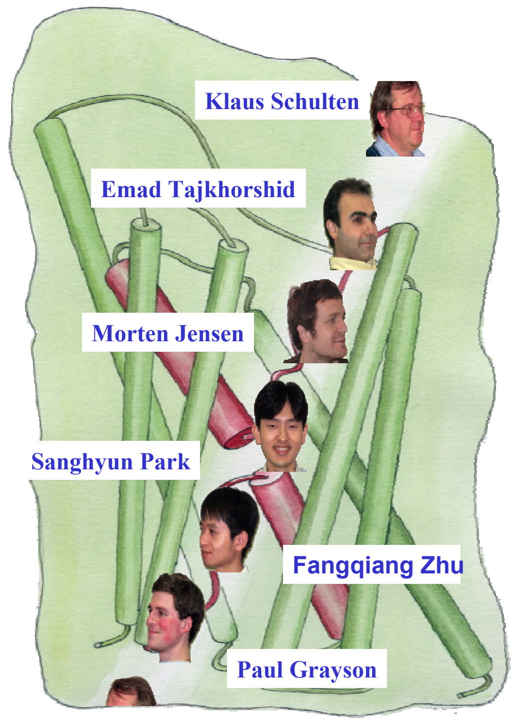
Proton Blocking by a Global Orientation Mechanism








2.04
2.07
1.75
1.84
1.86
1.78
1.70
2.06


Klaus Schulten
Emad Tajkhorshid
Morten Jensen
Sanghyun Park
Fangqiang Zhu
Paul Grayson