Molecular Dynamics Simulation of Membrane Channels

Part II. Structure-Function Relationship and Transport in Aquaporins

Emad Tajkhorshid

Beckman Institute, UIUC

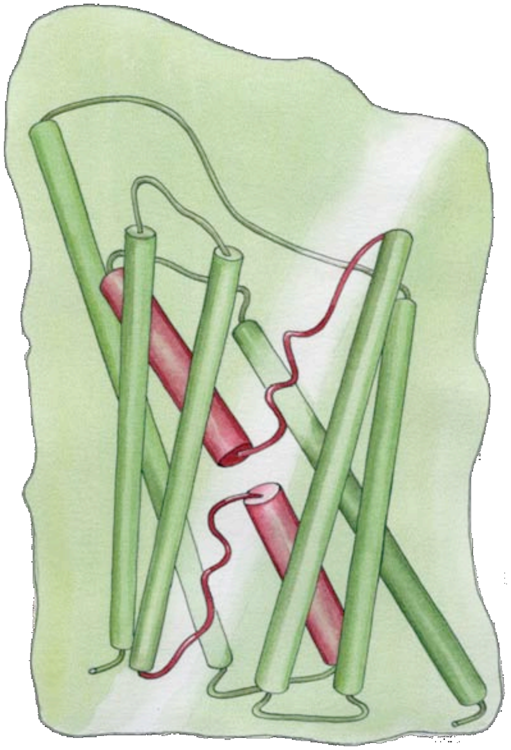
Summer School on Theoretical and Computational Biophysics
June 2004, University of Western Australia

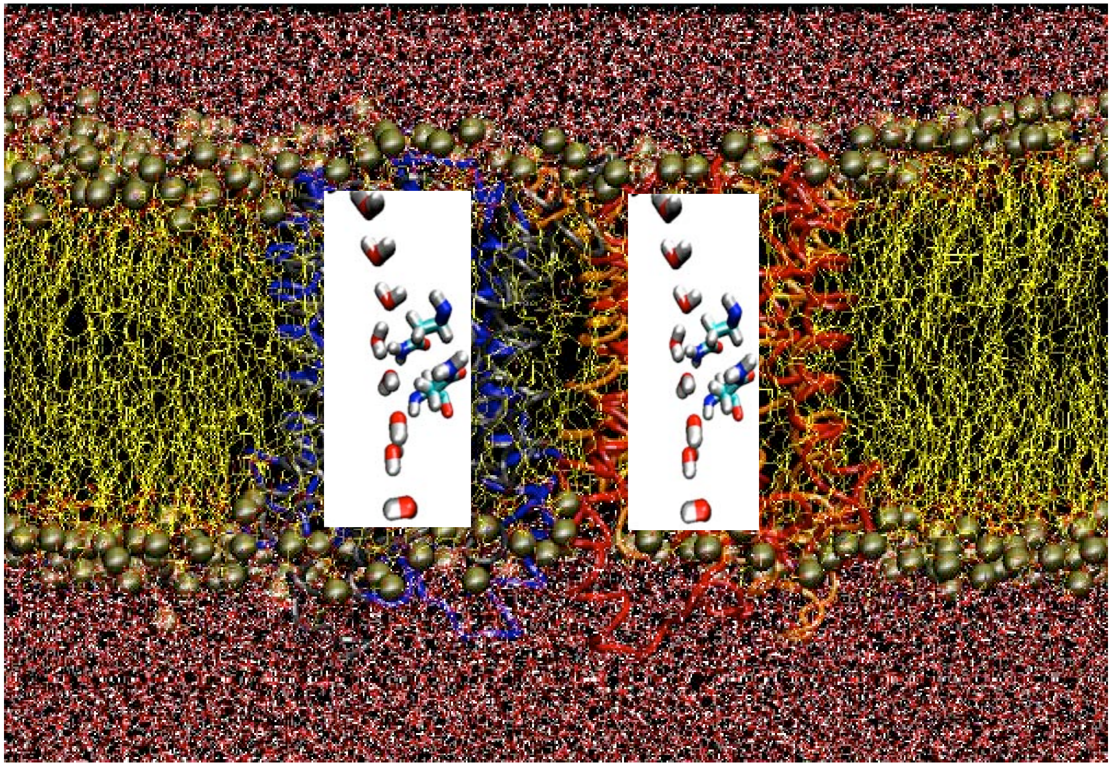
Analysis of Molecular Dynamics Simulations of Biomolecules

- 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?

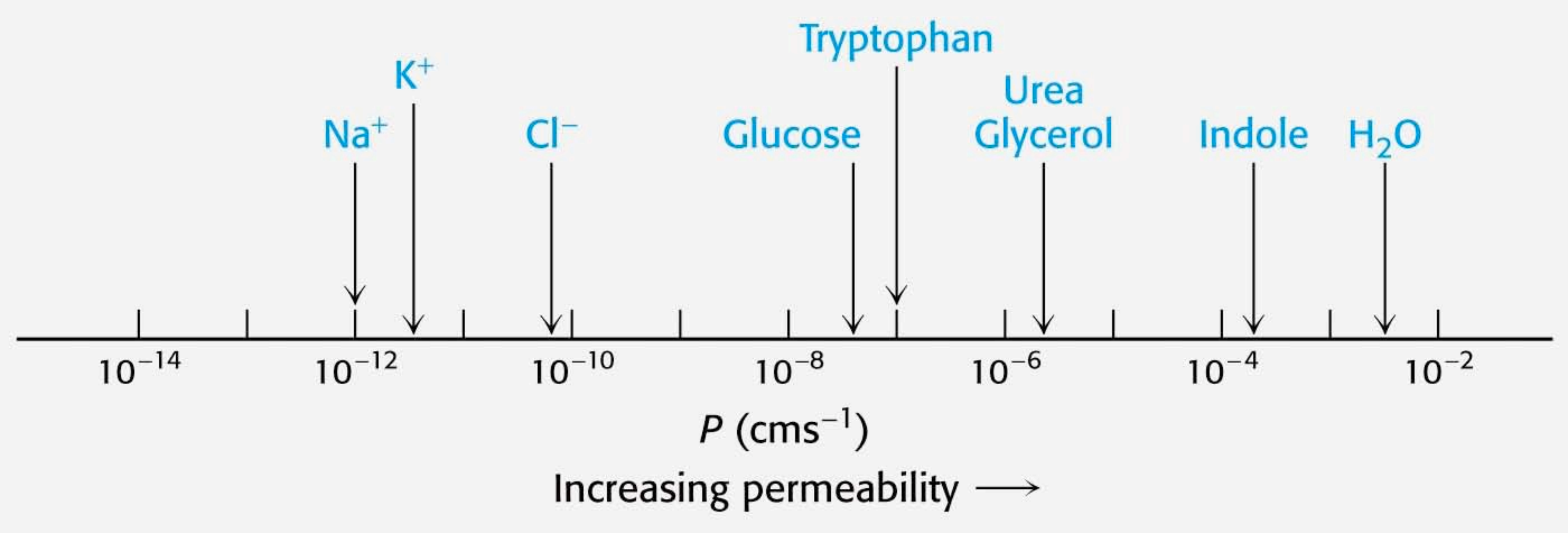
It is very important to get acquainted with your system

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

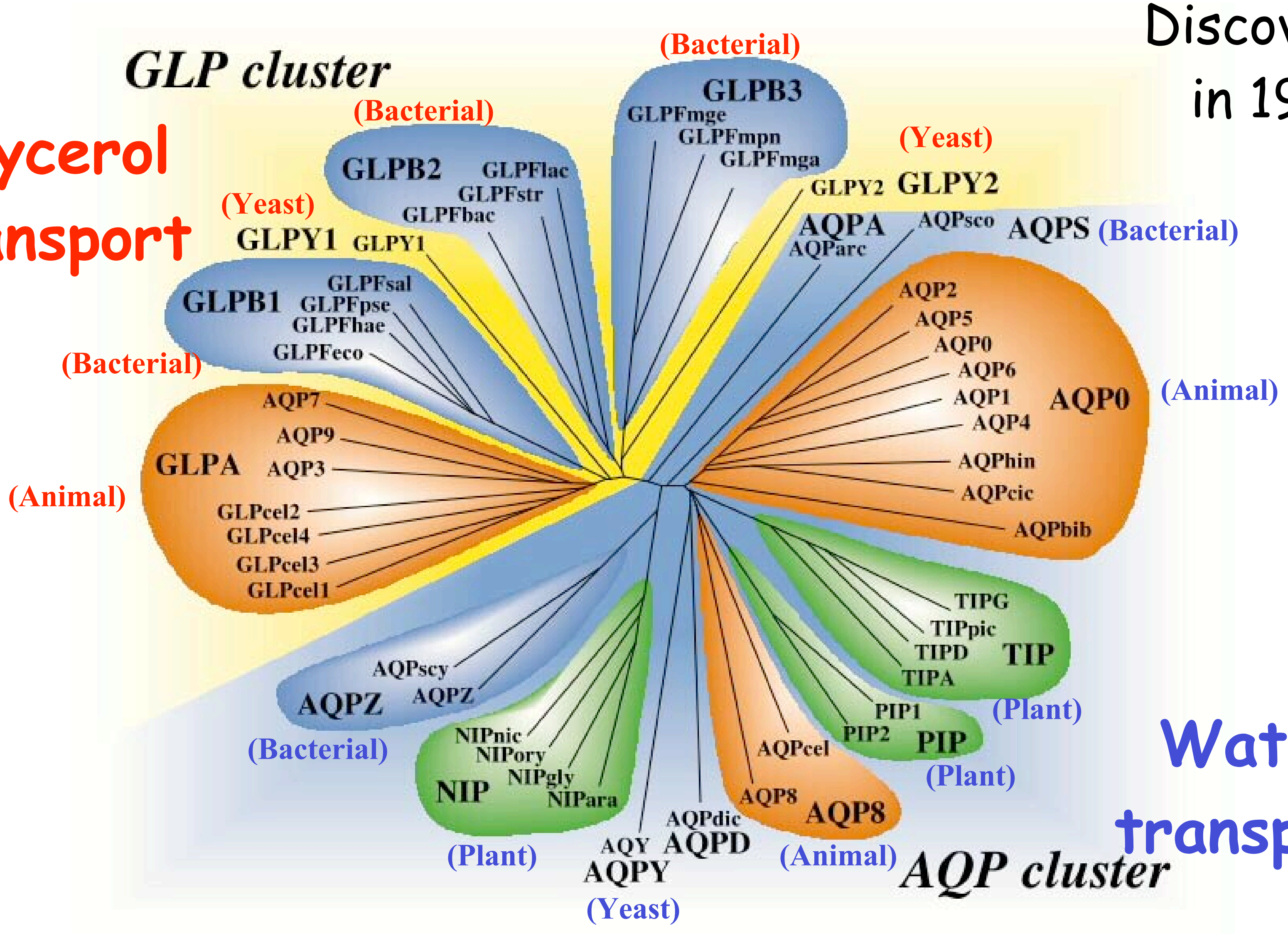
  Large volumes of water needed to be transported (kidneys).

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

The Aquaporin Superfamily

Discovered
in 1992

Glycerol transport



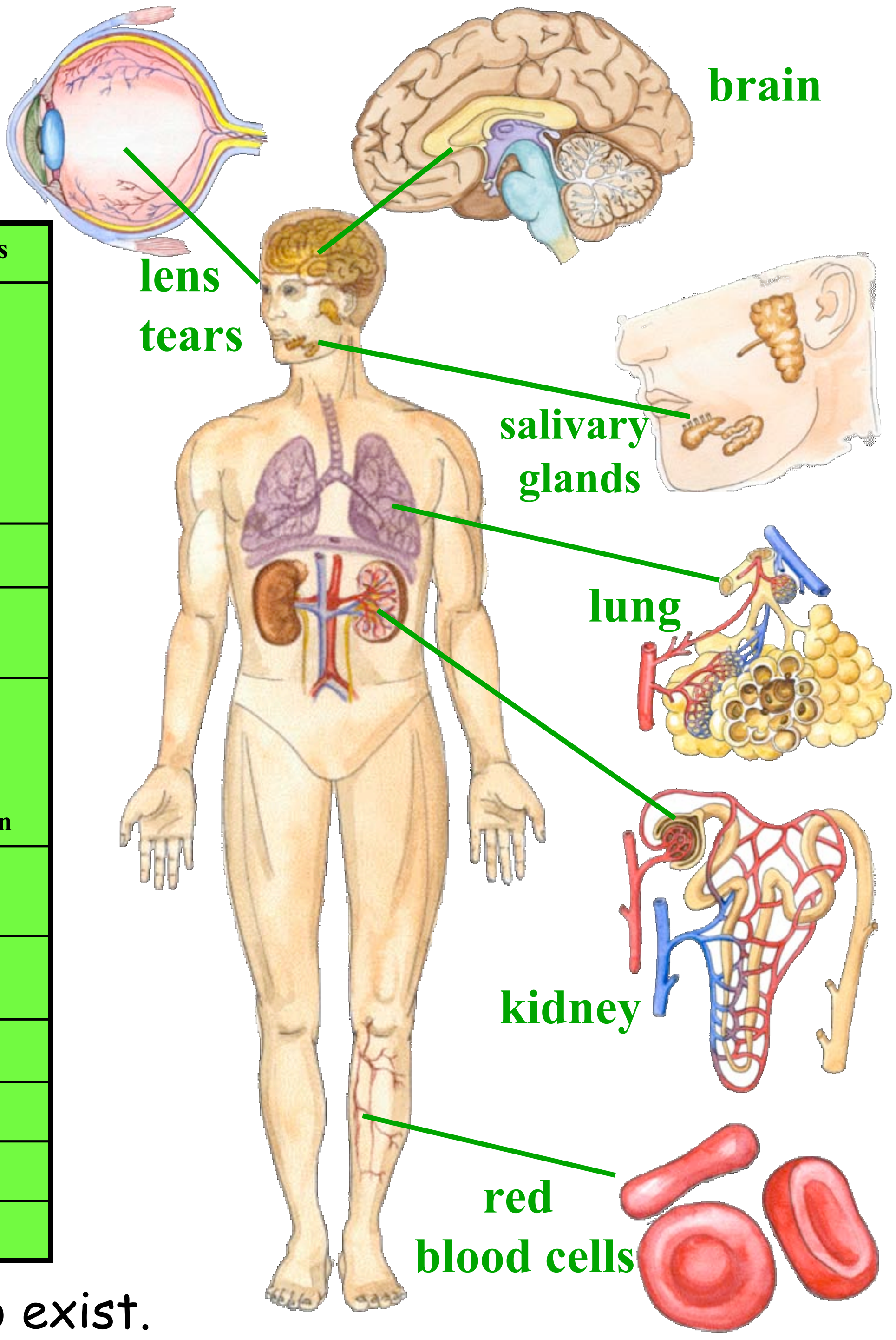
Water transport

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

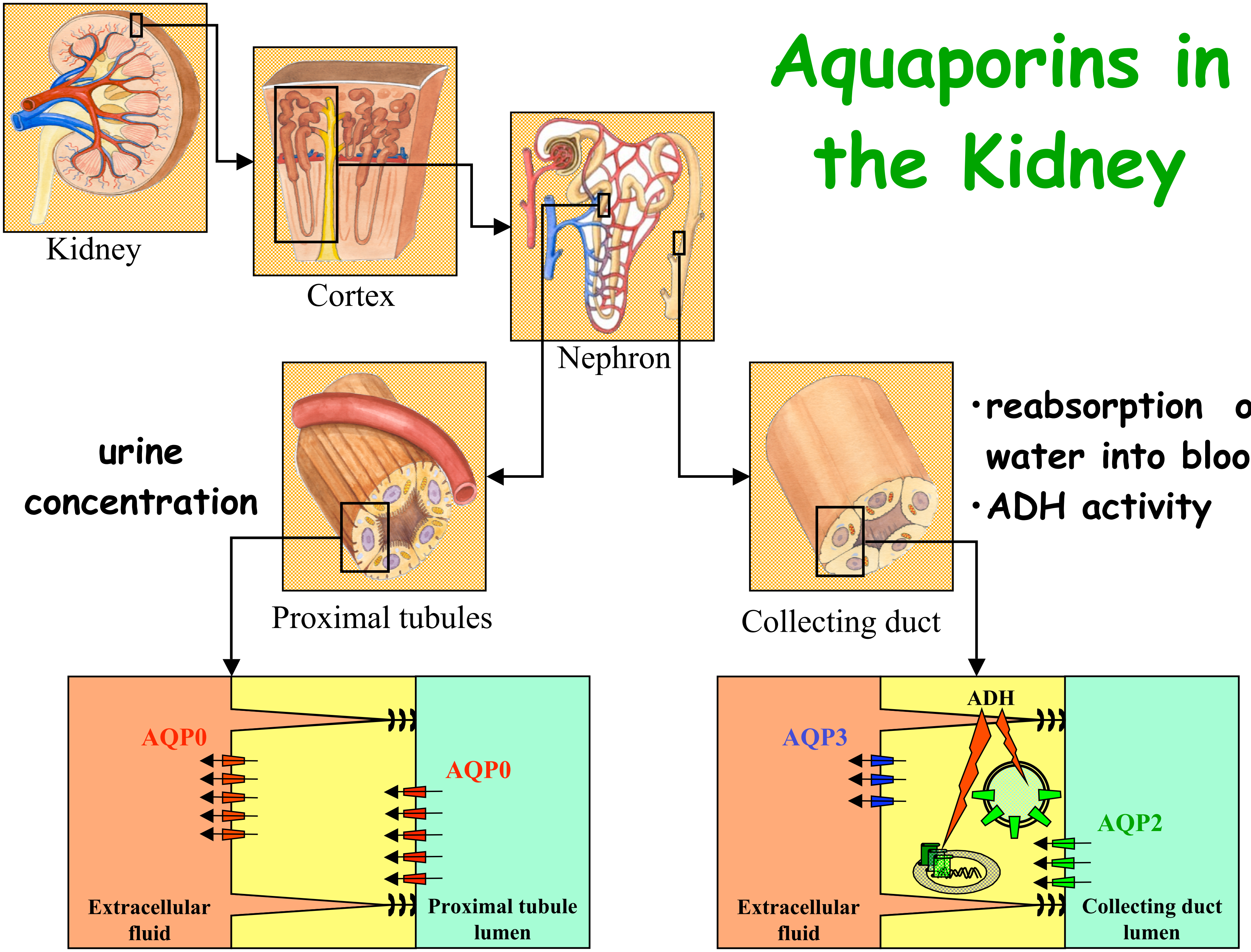
Aquaporins in Human Body

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.



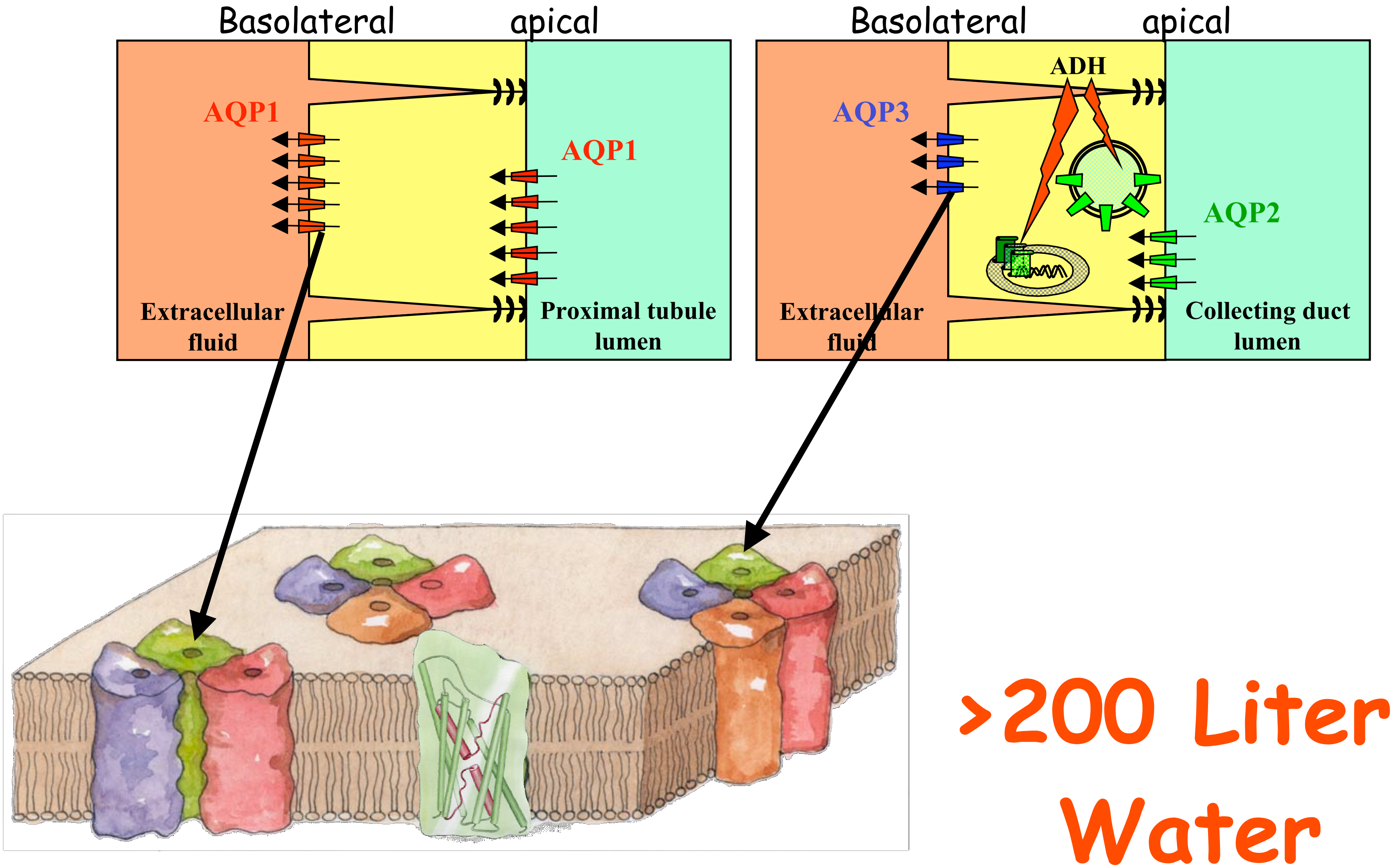
Aquaporins in the Kidney



urine concentration

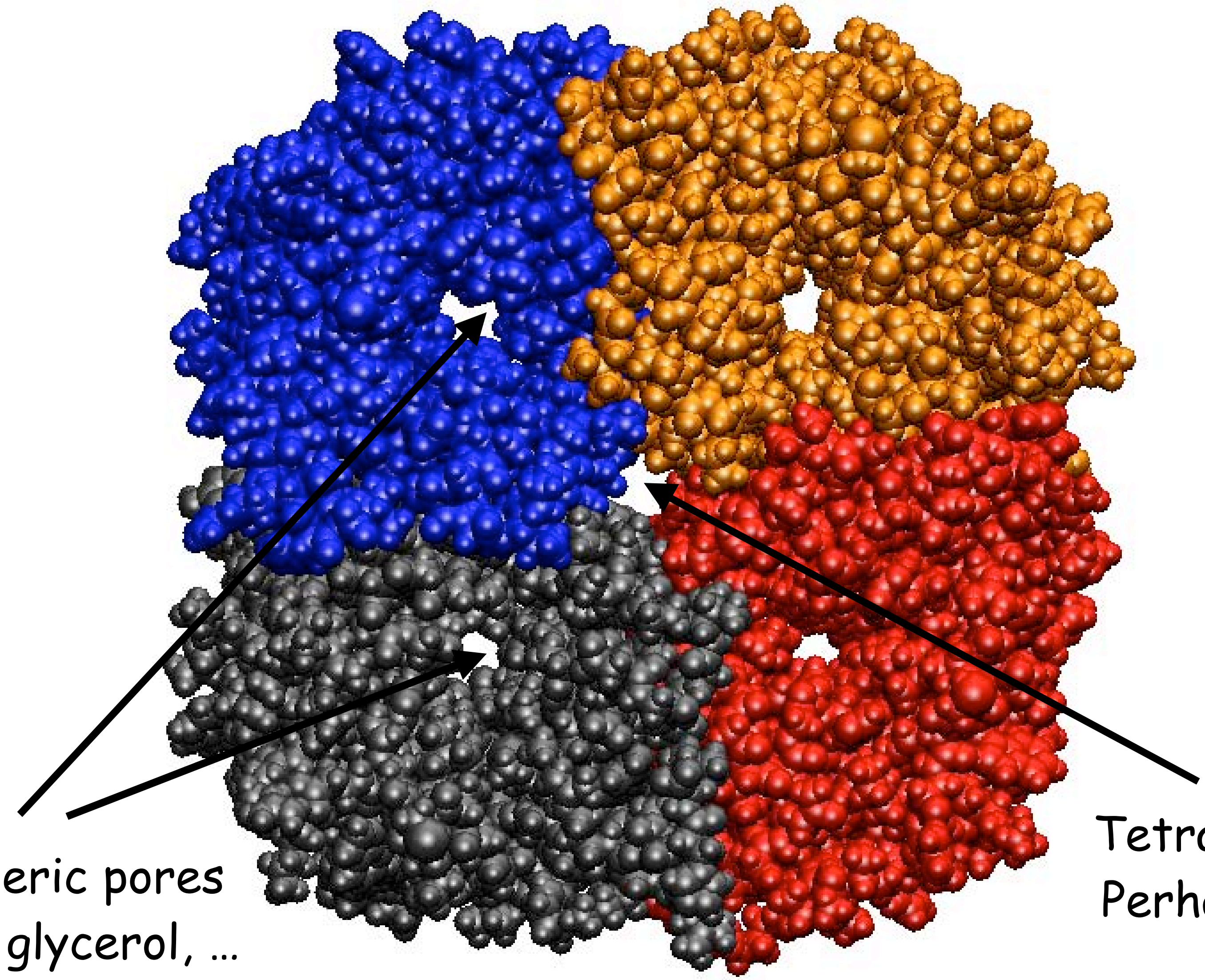
- reabsorption of water into blood
- ADH activity

High Permeation to Water



Nephrogenic diabetes insipidus

>200 Liters
Water
Everyday!



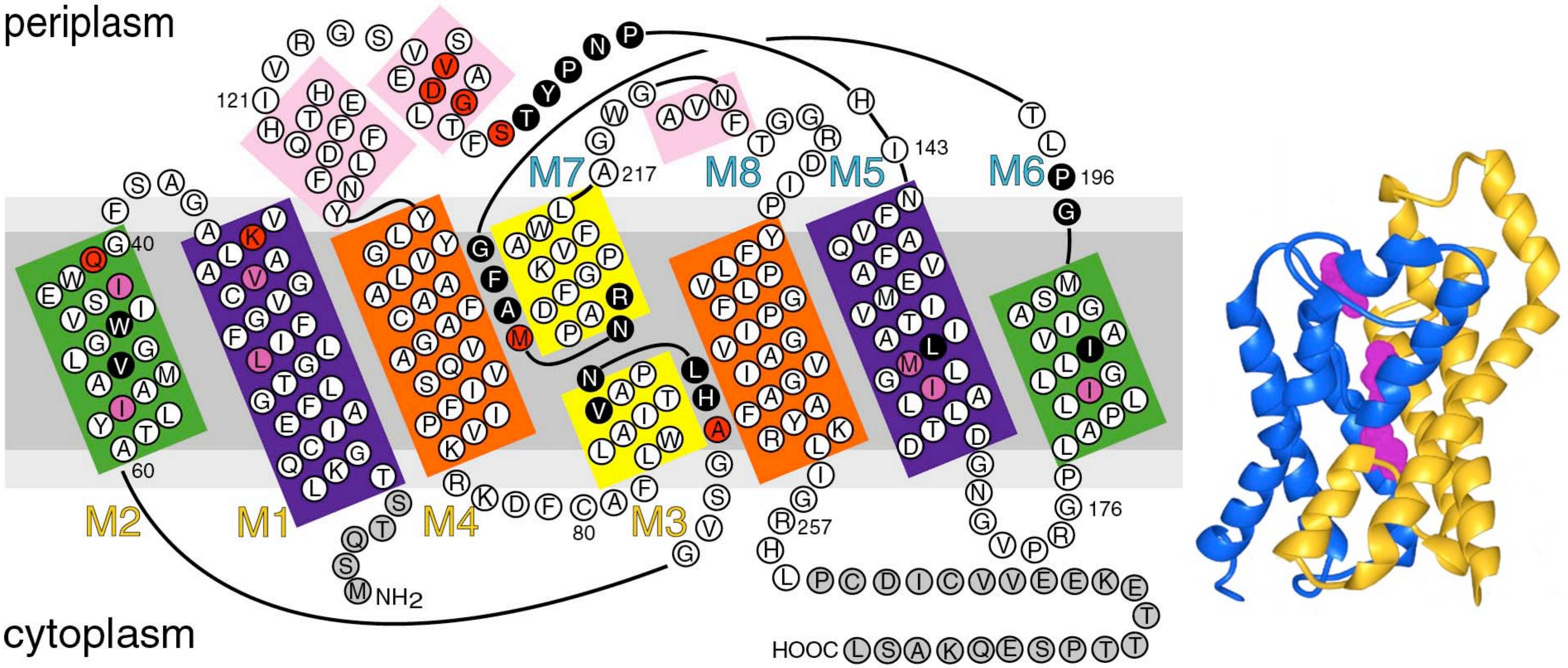
Monomeric pores
Water, glycerol, …

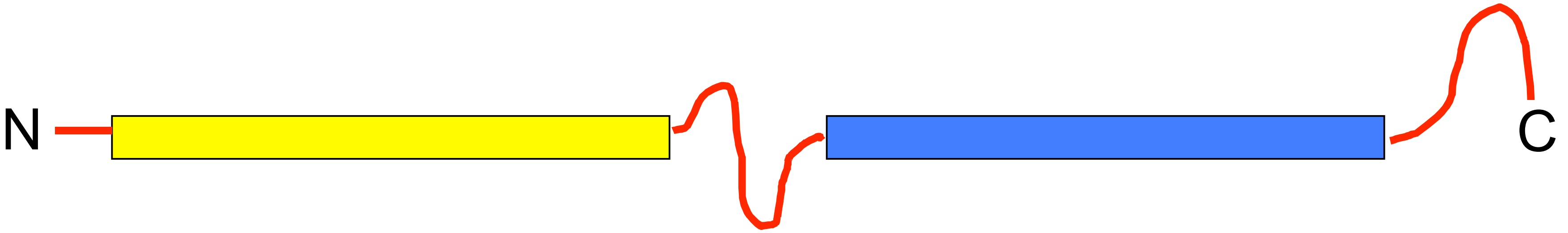
Tetrameric pore
Perhaps ions???

Aquaporins of known structure:

GlpF – E. coli glycerol channel (aquaglycerolporin)
AQP1 – Mammalian aquaporin-1 (pure water channel)

Architecture of the Channel

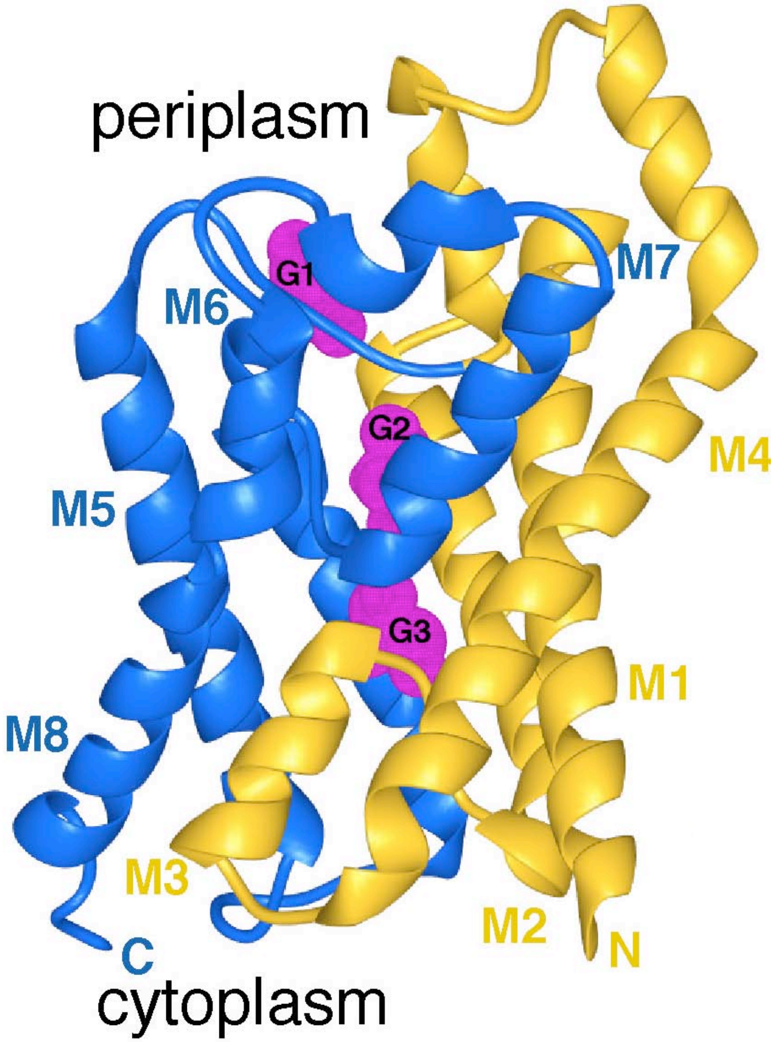


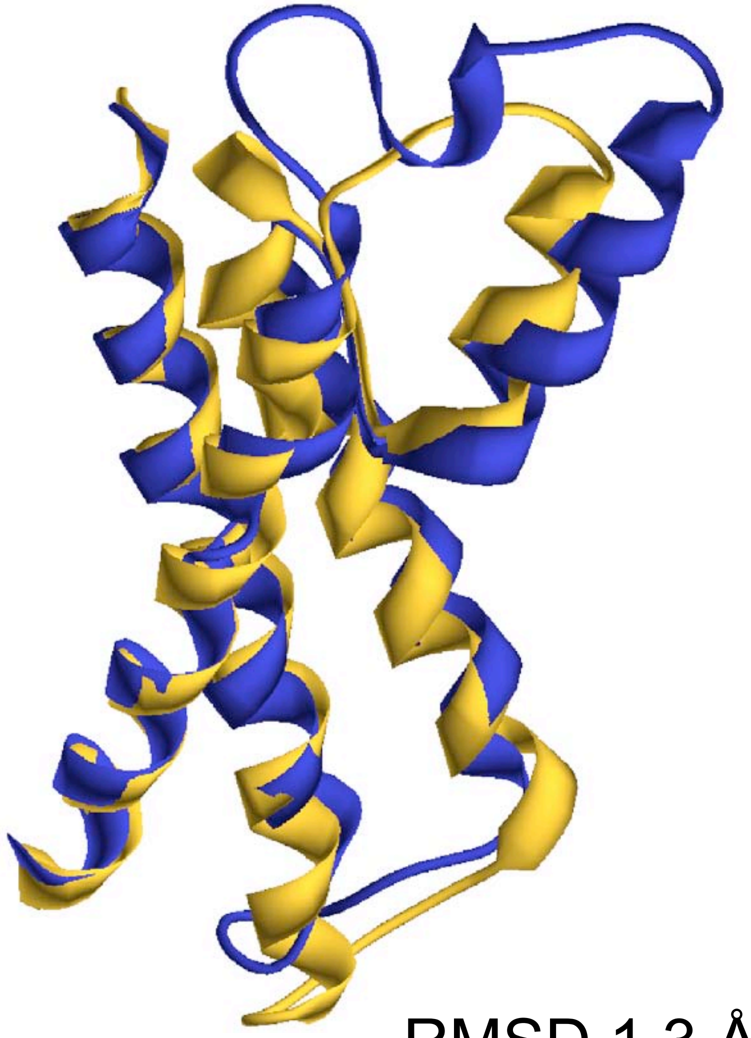


Fu, *et al*, *Science* **290**, 481 (2000)

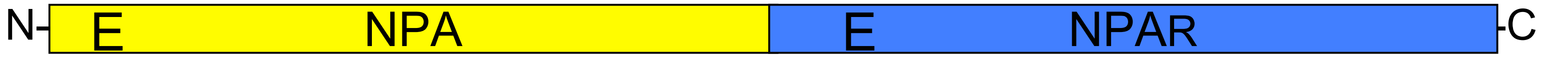
Channel Fold

Fu, *et al*, *Science* **290**, 481 (2000)





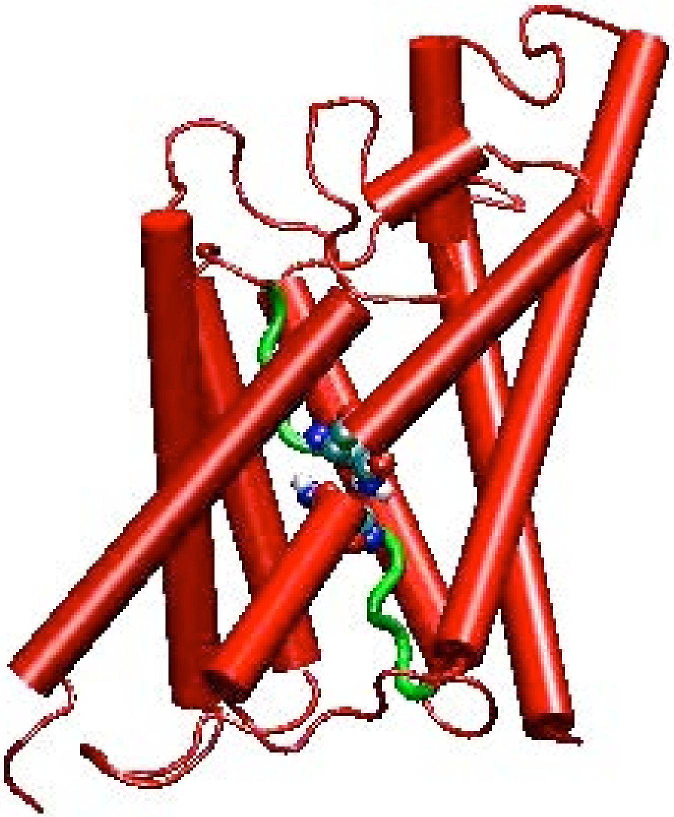
RMSD 1.3 Å

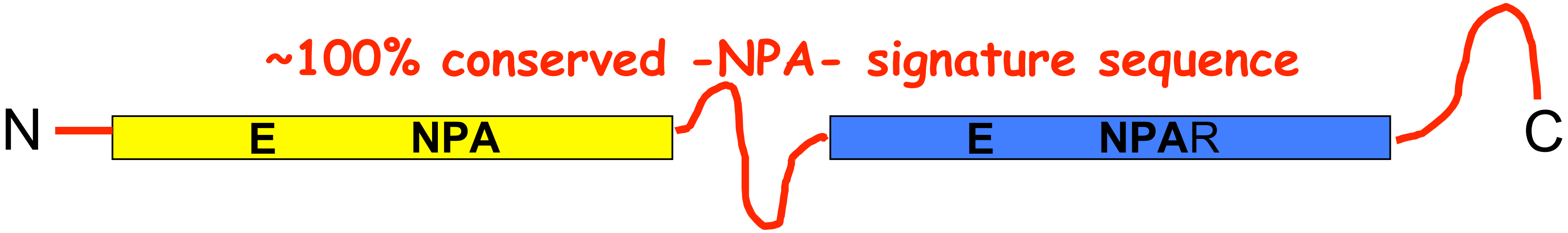


Internal gene duplication

Functionally Important Features

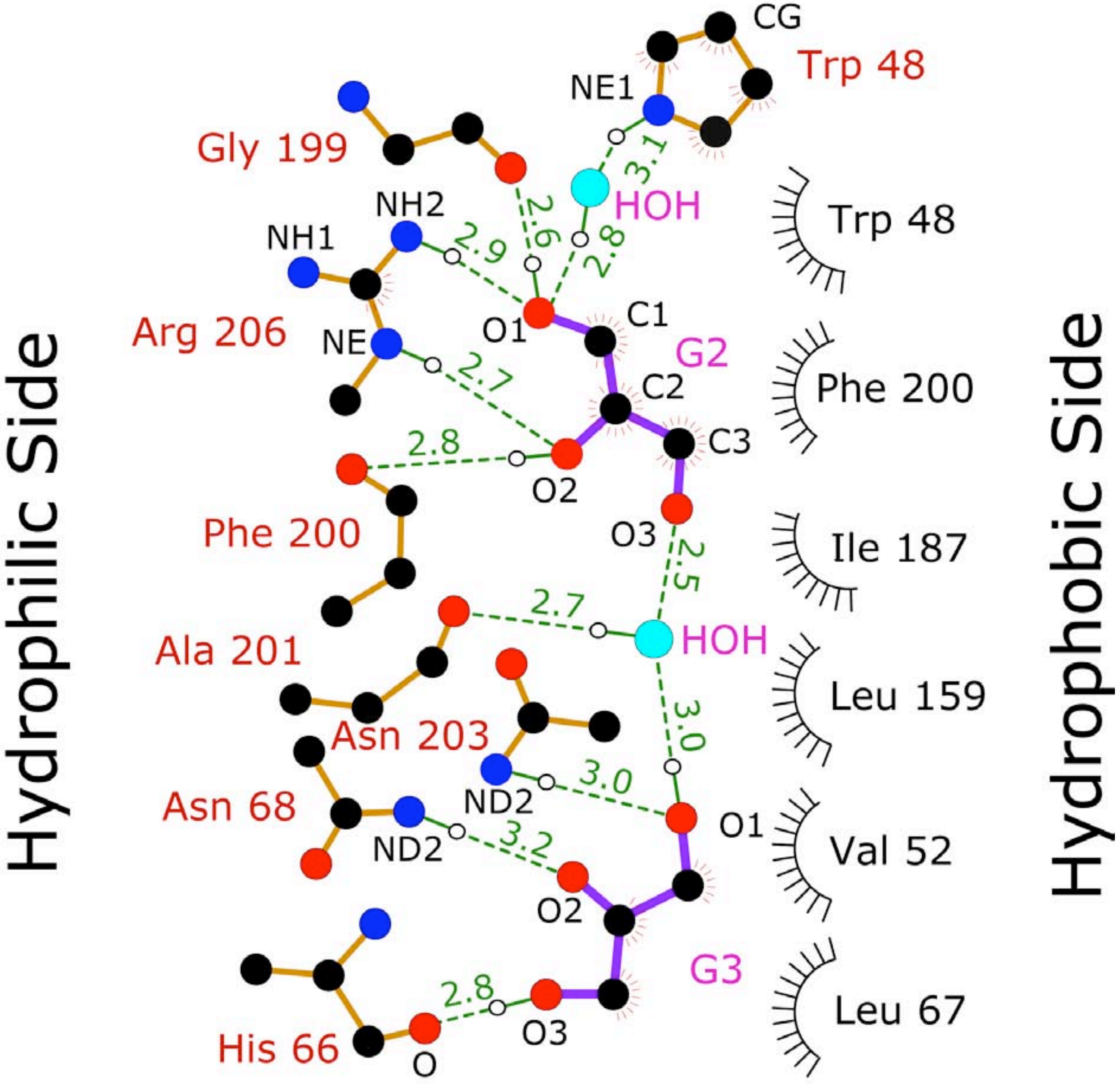
- Tetrameric architecture
- Amphipatic channel interior
- Water and glycerol transport
- Protons, and other ions are excluded
- Conserved asparagine-proline-alanine residues; NPA motif
- Characteristic half-membrane spanning structure





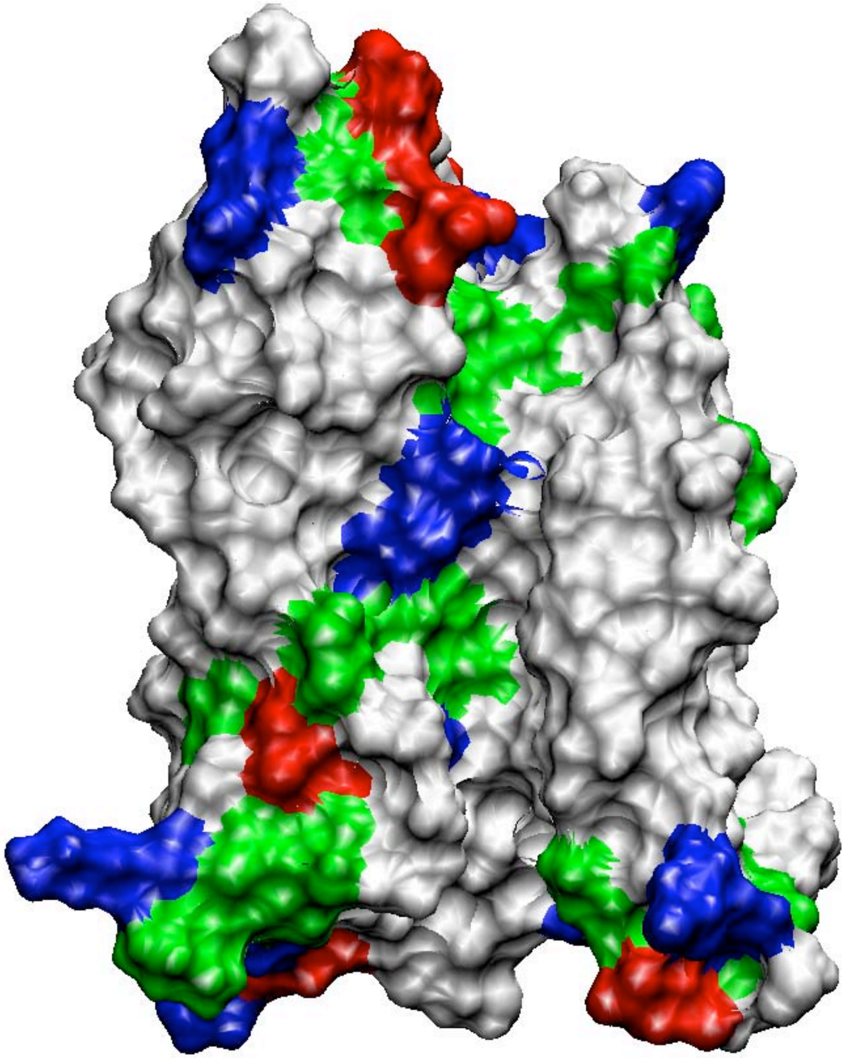
~100% conserved -NPA- signature sequence

A Semi-hydrophobic channel

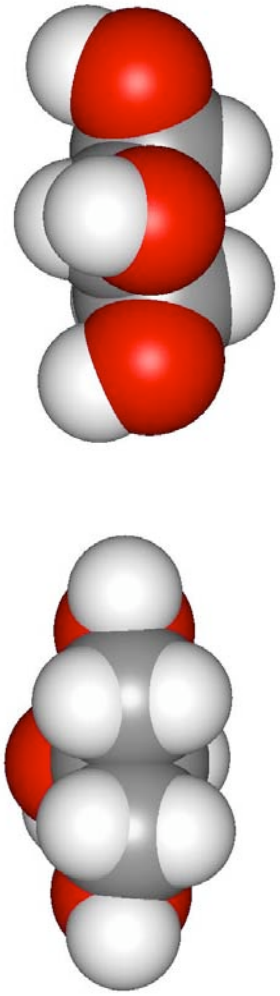


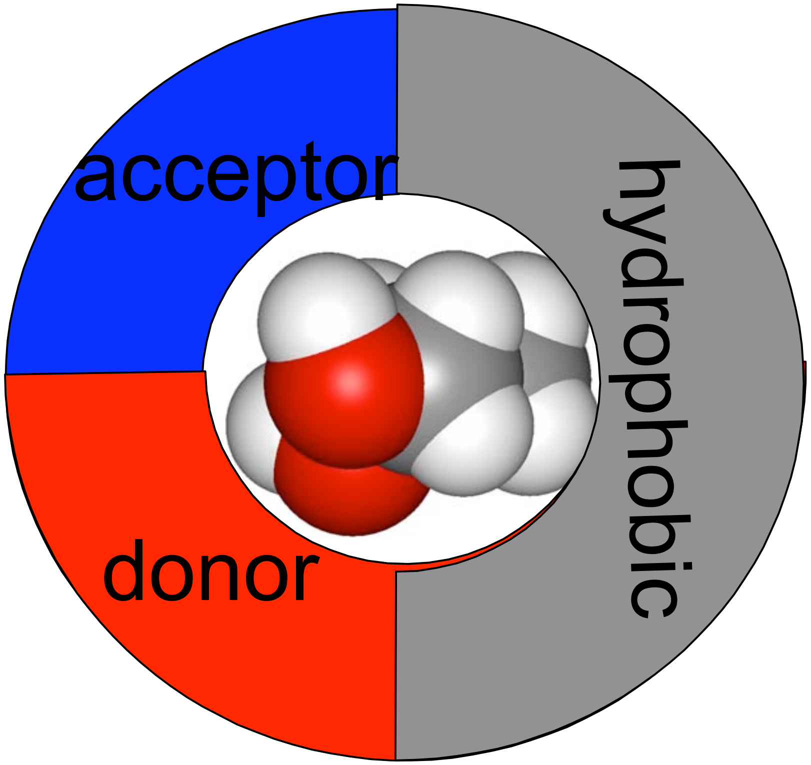
Fu, *et al*, *Science* **290**, 481 (2000)

A Semi-hydrophobic channel

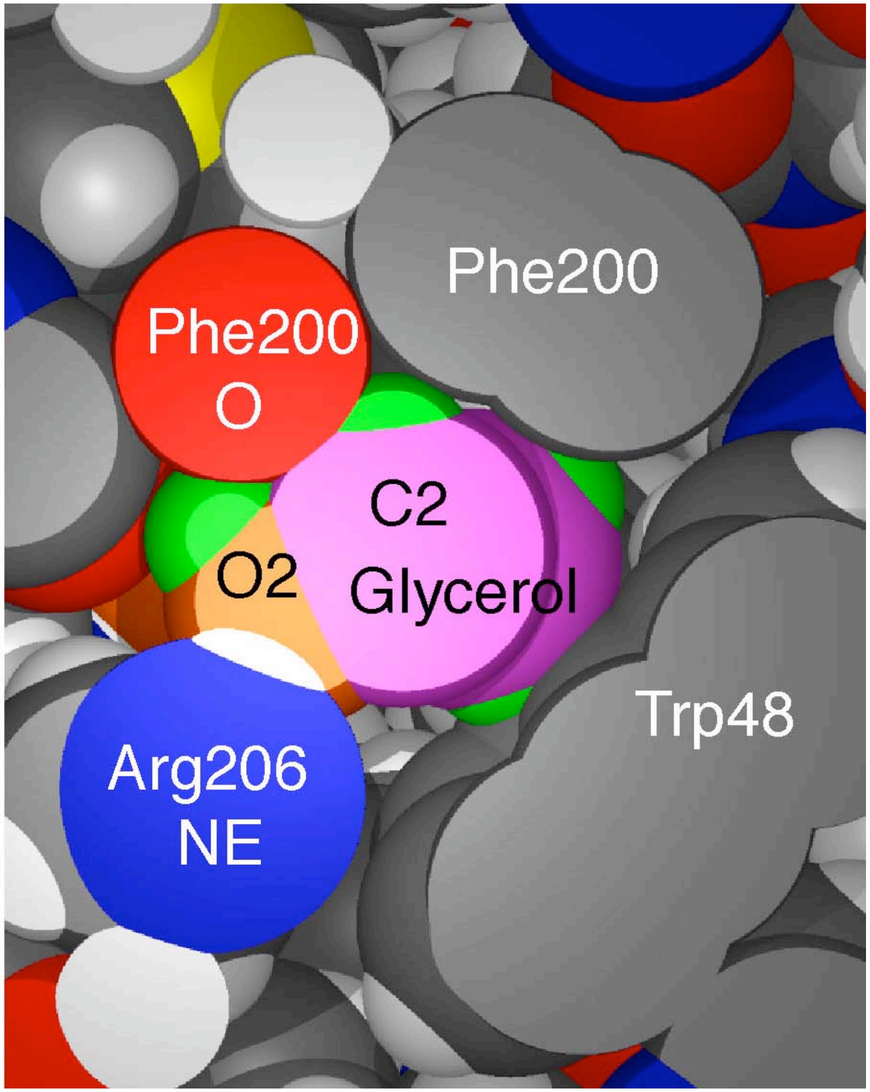


Complementarity
glycerol molecule ↔ channel



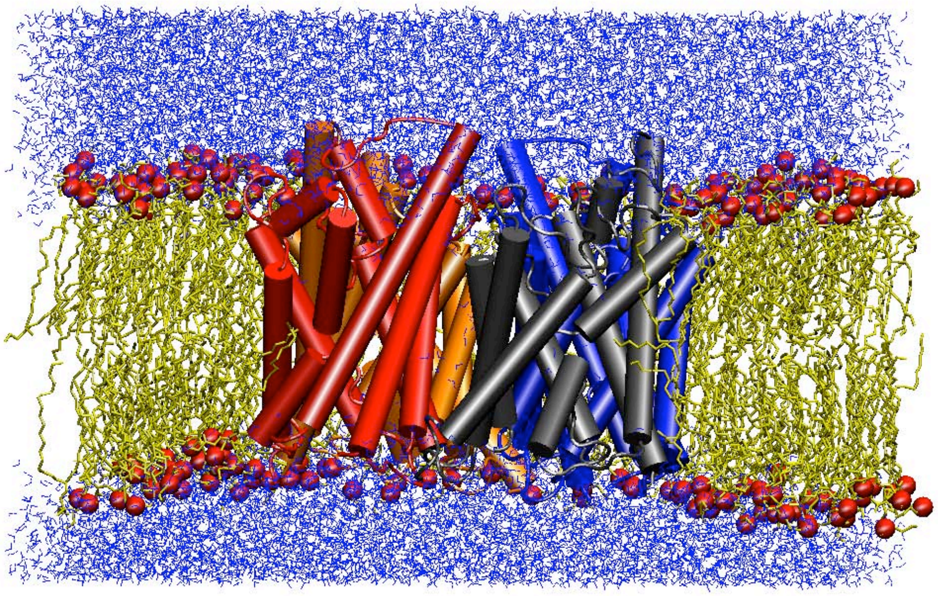


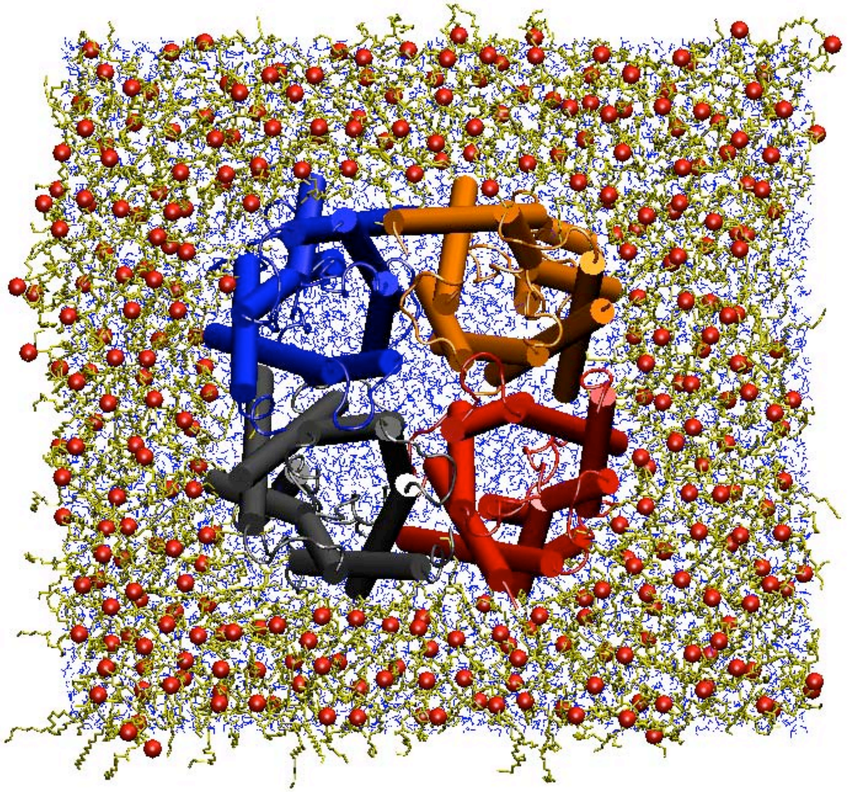
Tight Packing in the Selectivity Filter



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

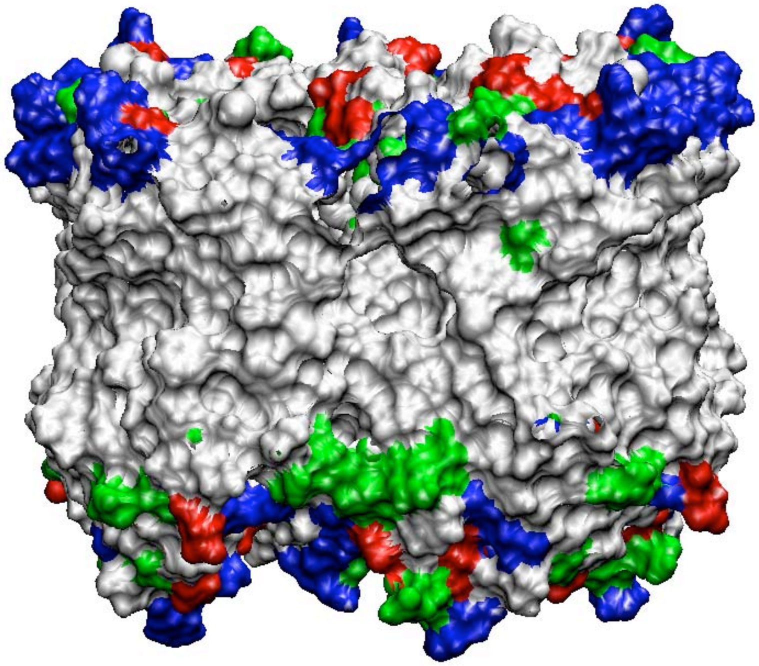
1ns equilibration, 4ns production

10 days /ns – 32-proc Linux cluster

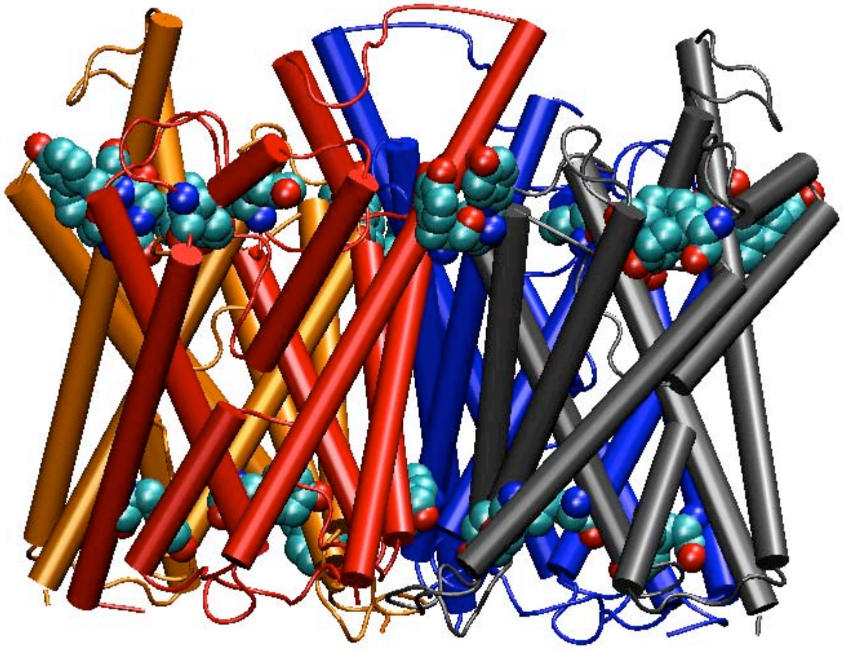
3.5 days/ns - 128 O2000 CPUs

0.35 days/ns – 512 LeMieux CPUs

Protein Embedding in Membrane

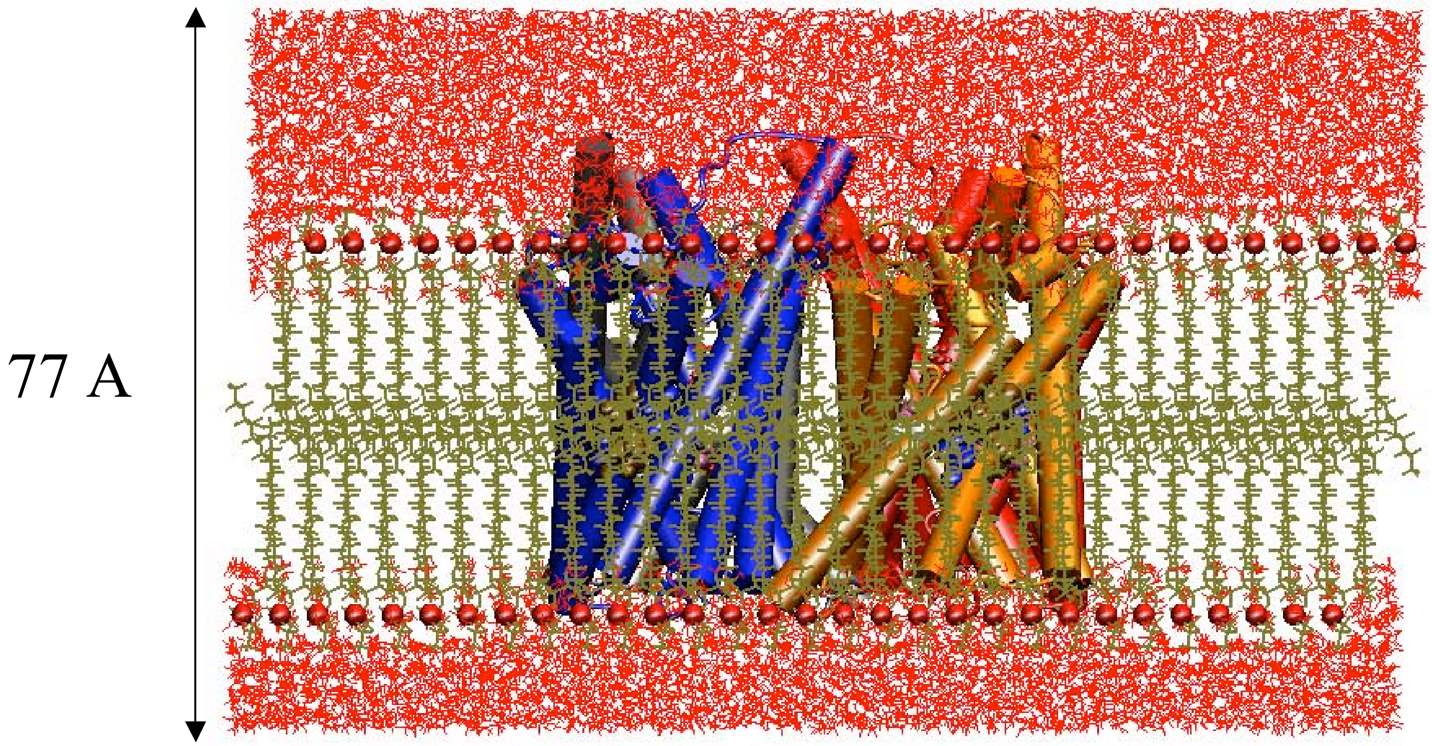


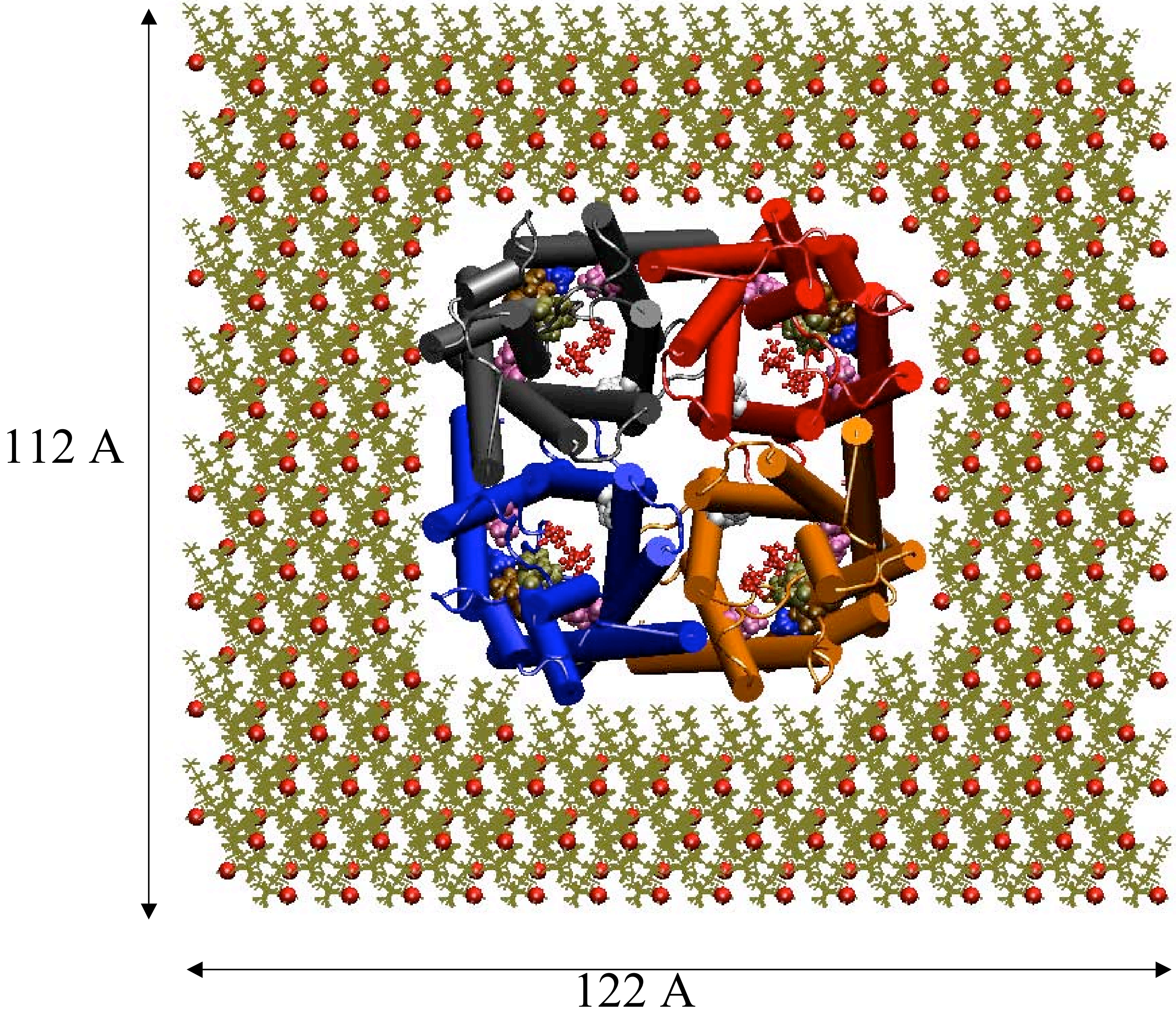
Hydrophobic surface
of the protein



Ring of aromatic
side chains,
specially tyrosines

Embedding GlpF in Membrane

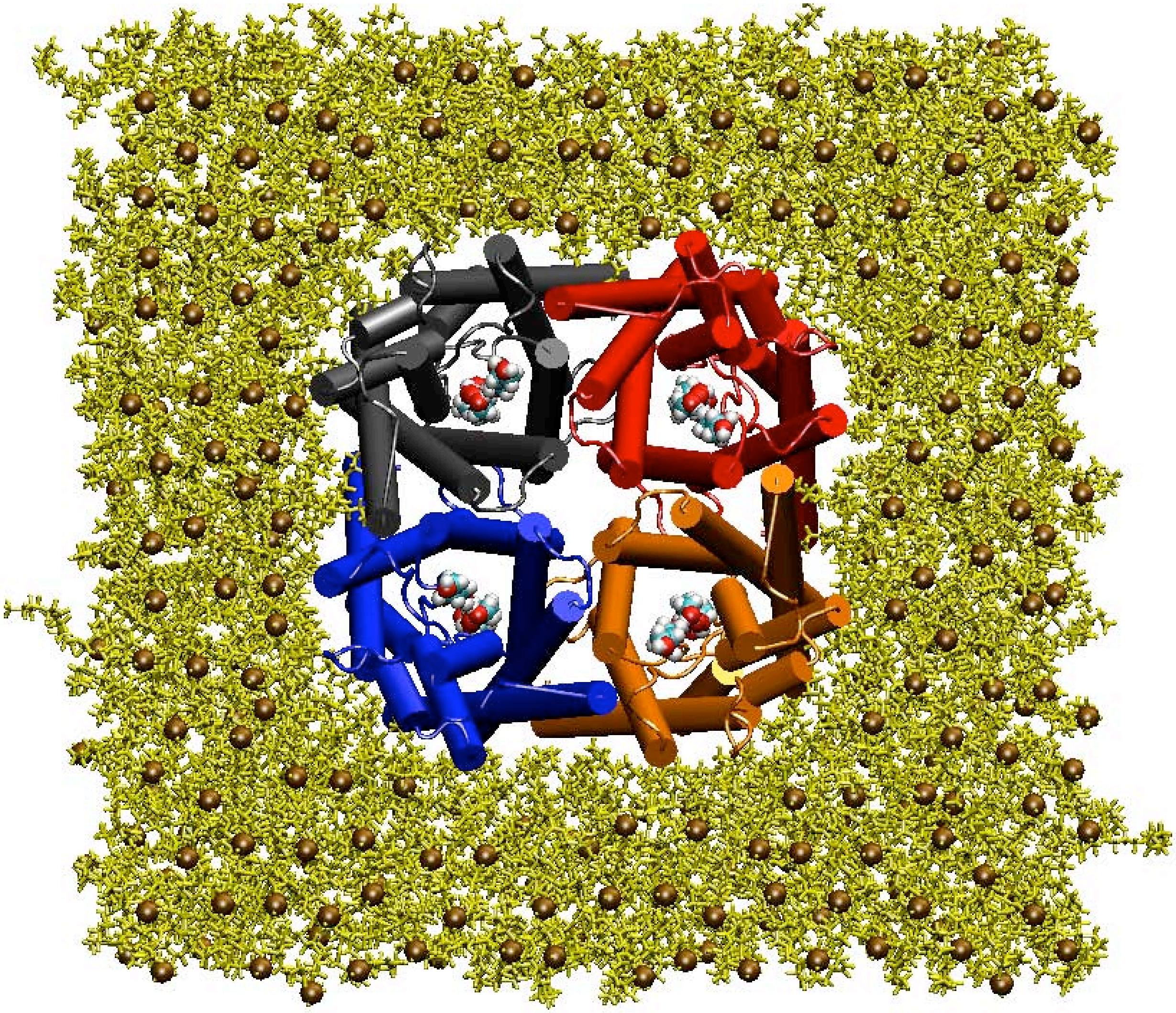



112 A
122 A

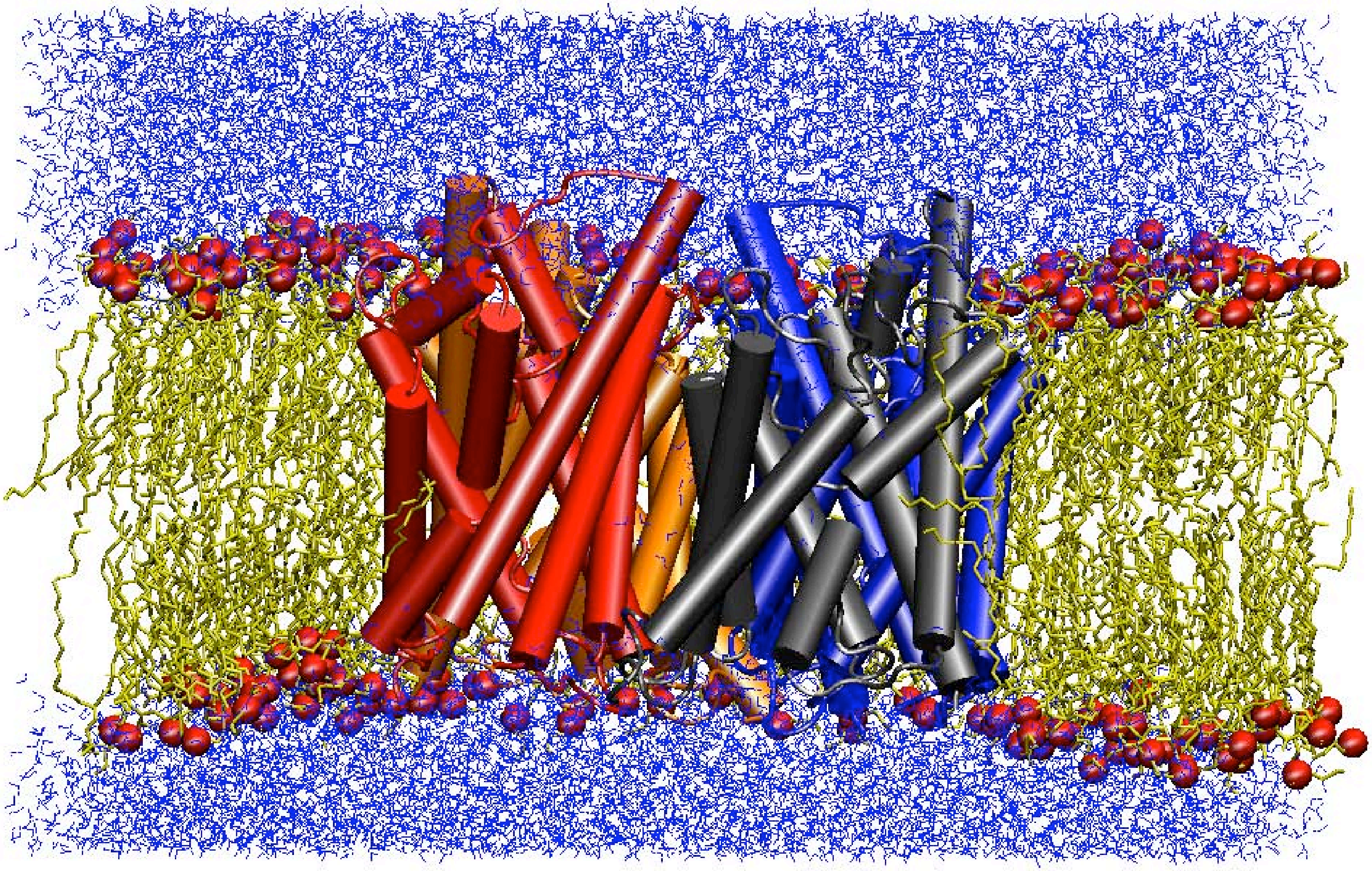
A Recipe for Membrane Protein Simulations

- 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.

Lipid-Protein Packing During the Initial NpT Simulation

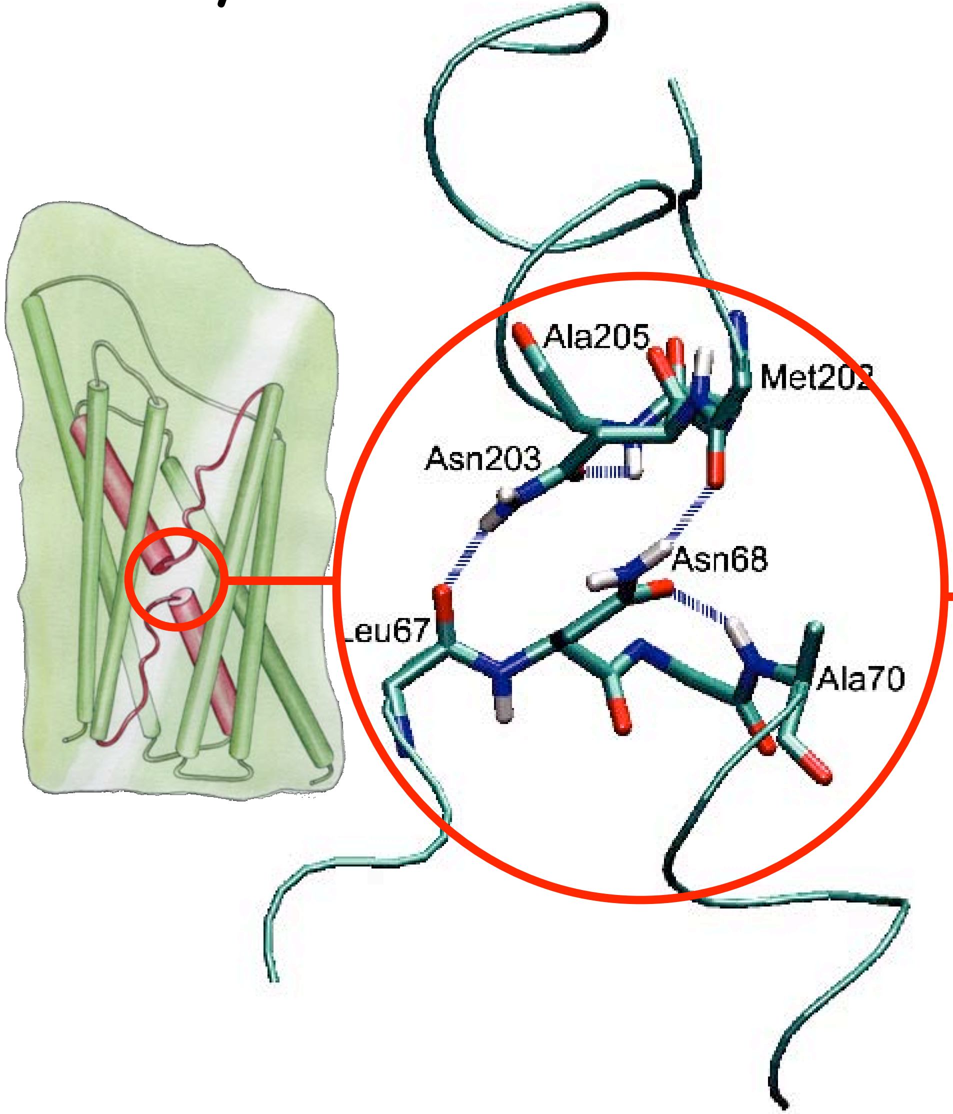


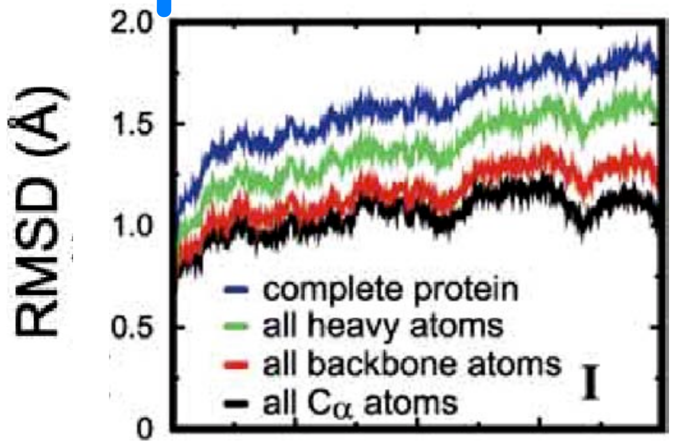
Adjustment of Membrane Thickness to the Protein Hydrophobic Surface

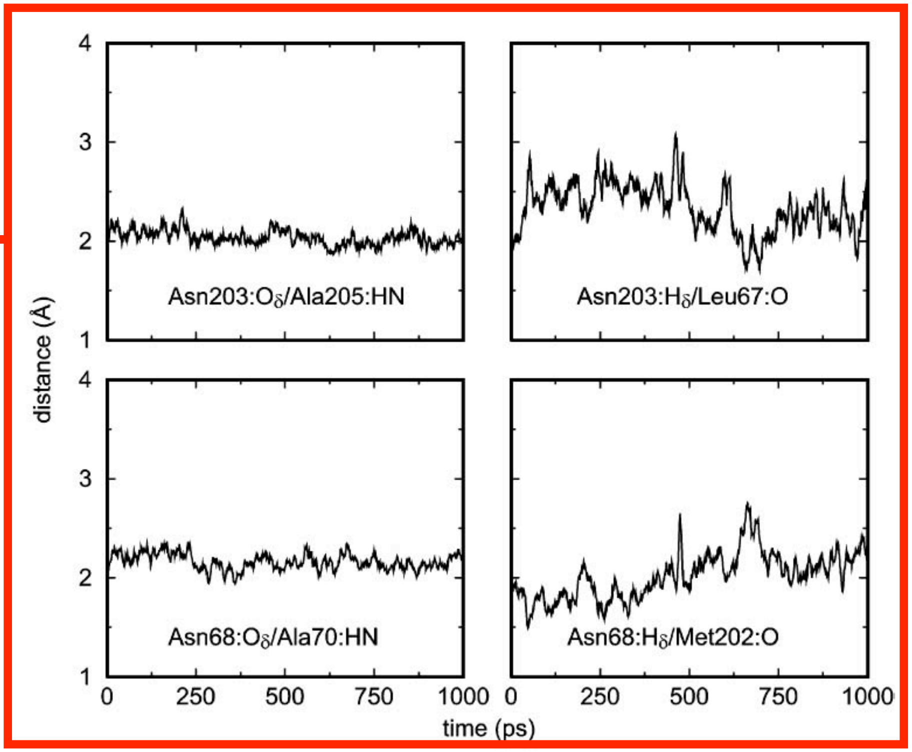


An extremely stable protein

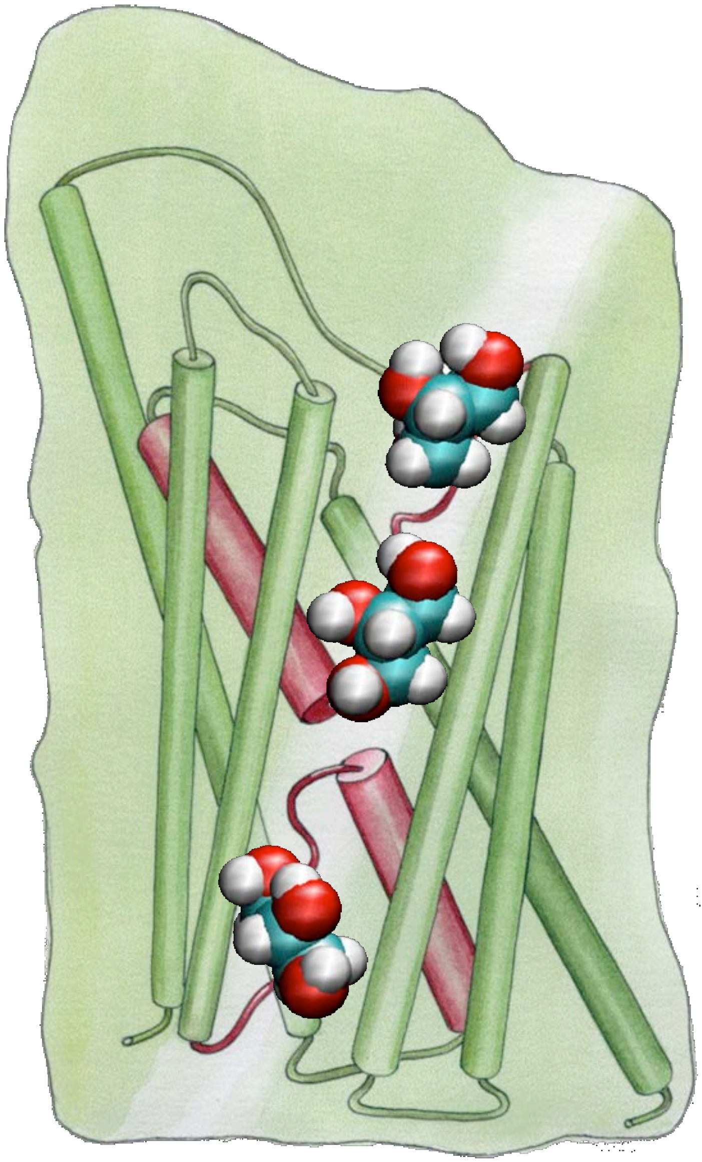
Stability of NPA – NPA Interaction

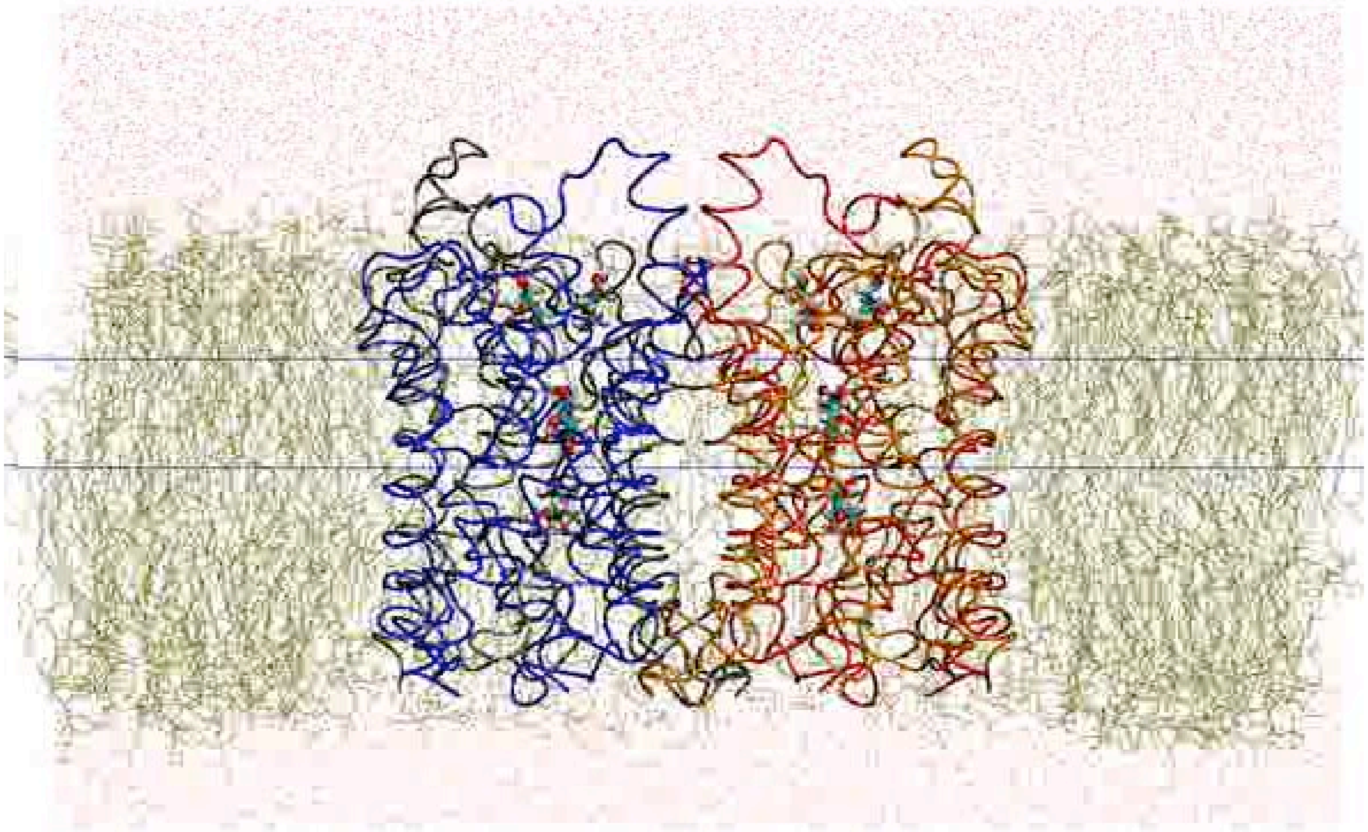






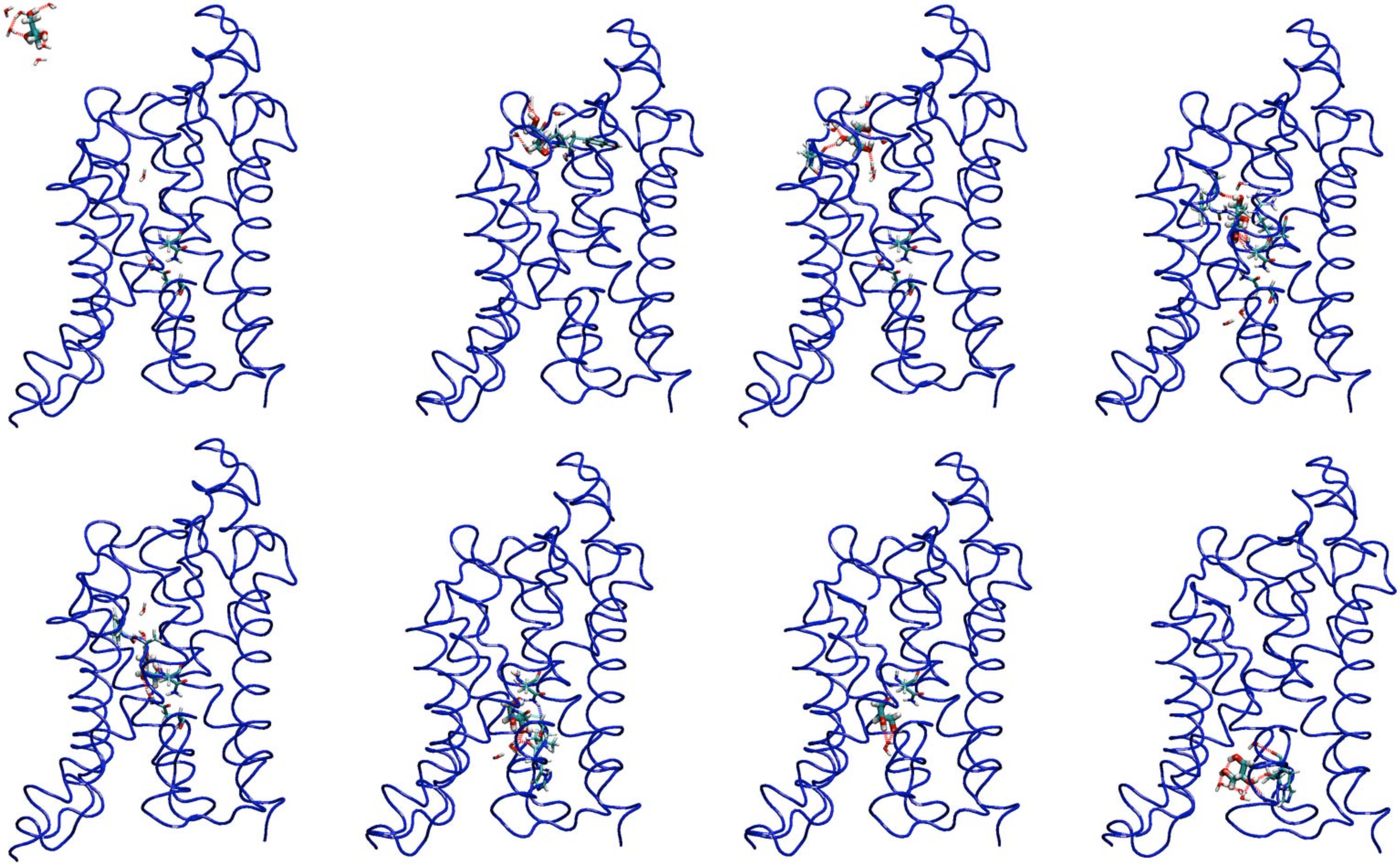
Glycerol-Saturated GlpF





--

Description of full conduction pathway



Complete description of the conduction pathway

Constriction region

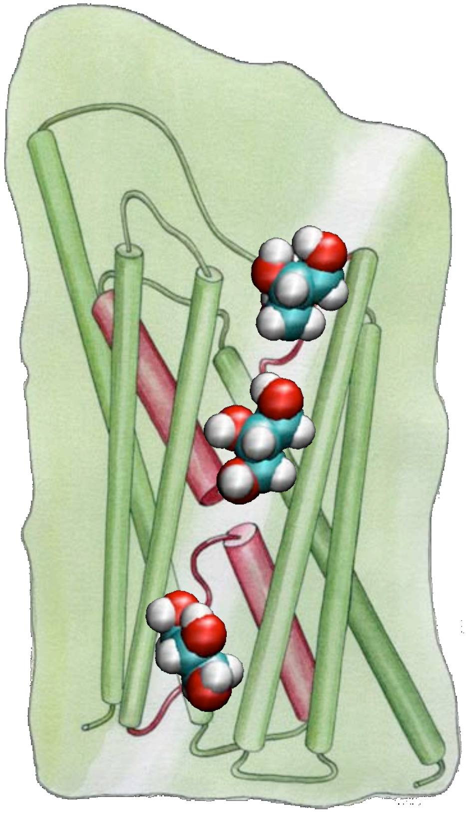
Selectivity filter

Details of Protein-Substrate Interaction are 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

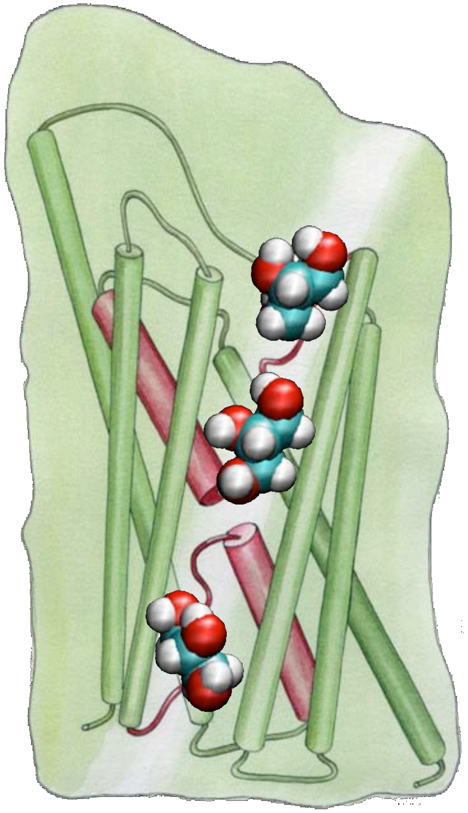
```
…
{set frame 0}{frame < 100}{incr frame}{
   animate goto $frame
   set donor [atomselect top
   “name O N and within 2 of
   (resname GCL and name HO)”]
   lappend [$donor get index] list1
   set acceptor [atomselect top
   “resname GCL and name O and
   within 2 of (protein and name HN HO)”]
 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



Channel Hydrogen Bonding Sites

GLN	41	OE1 NE2
TRP	48	O NE1
GLY	64	O
ALA	65	O
HIS	66	O ND1
LEU	67	O
ASN	68	ND2
ASP	130	OD1
GLY	133	O
SER	136	O
TYR	138	O
PRO	139	O N
ASN	140	OD1 ND2
HIS	142	ND1
THR	167	OG1
GLY	195	O
PRO	196	O
LEU	197	O
THR	198	O
GLY	199	O
PHE	200	O
ALA	201	O
ASN	203	ND2

LYS	33	HZ1 HZ3
GLN	41	HE21
TRP	48	HE1
HIS	66	HD1
ASN	68	HD22
TYR	138	HN
ASN	140	HN HD21 HD22
HIS	142	HD1
GLY	199	HN
ASN	203	HN HD21HD22
ARG	206	HE HH21HH22

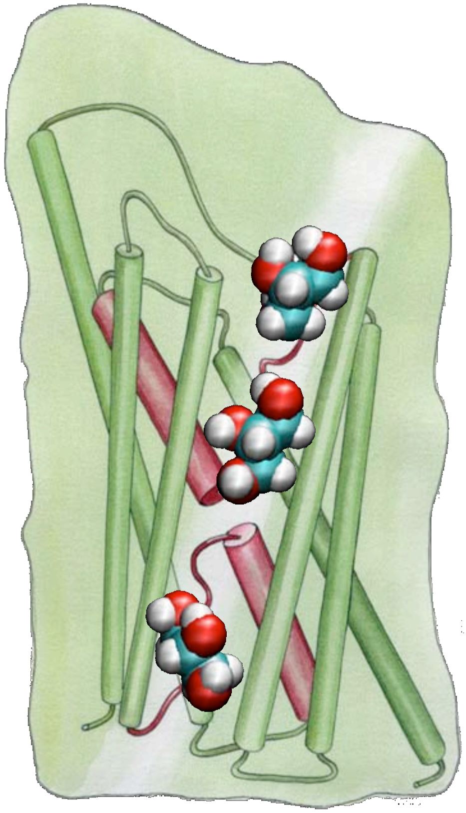


Channel Hydrogen Bonding Sites

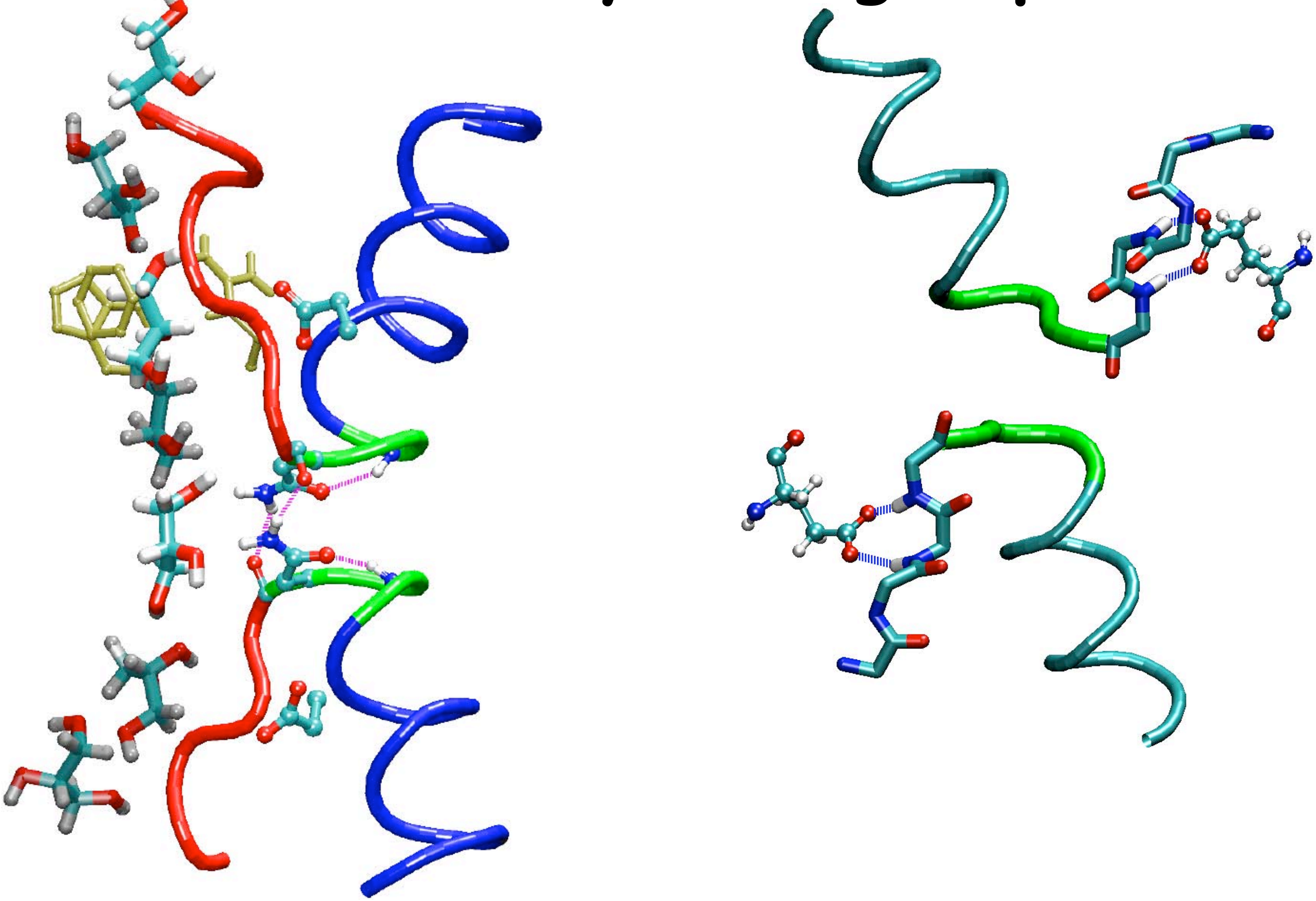
GLN	41	OE1 NE2
TRP	48	O NE1
GLY	64	O
ALA	65	O
HIS	66	O ND1
LEU	67	O
ASN	68	ND2
ASP	130	OD1
GLY	133	O
SER	136	O
TYR	138	O
PRO	139	O N
ASN	140	OD1 ND2
HIS	142	ND1
THR	167	OG1
GLY	195	O
PRO	196	O

LEU	197	O
THR	198	O
GLY	199	O
PHE	200	O
ALA	201	O
ASN	203	ND2

LYS	33	HZ1 HZ3
GLN	41	HE21
TRP	48	HE1
HIS	66	HD1
ASN	68	HD22
TYR	138	HN
ASN	140	HN HD21 HD22
HIS	142	HD1
GLY	199	HN
ASN	203	HN HD21HD22
ARG	206	HE HH21HH22

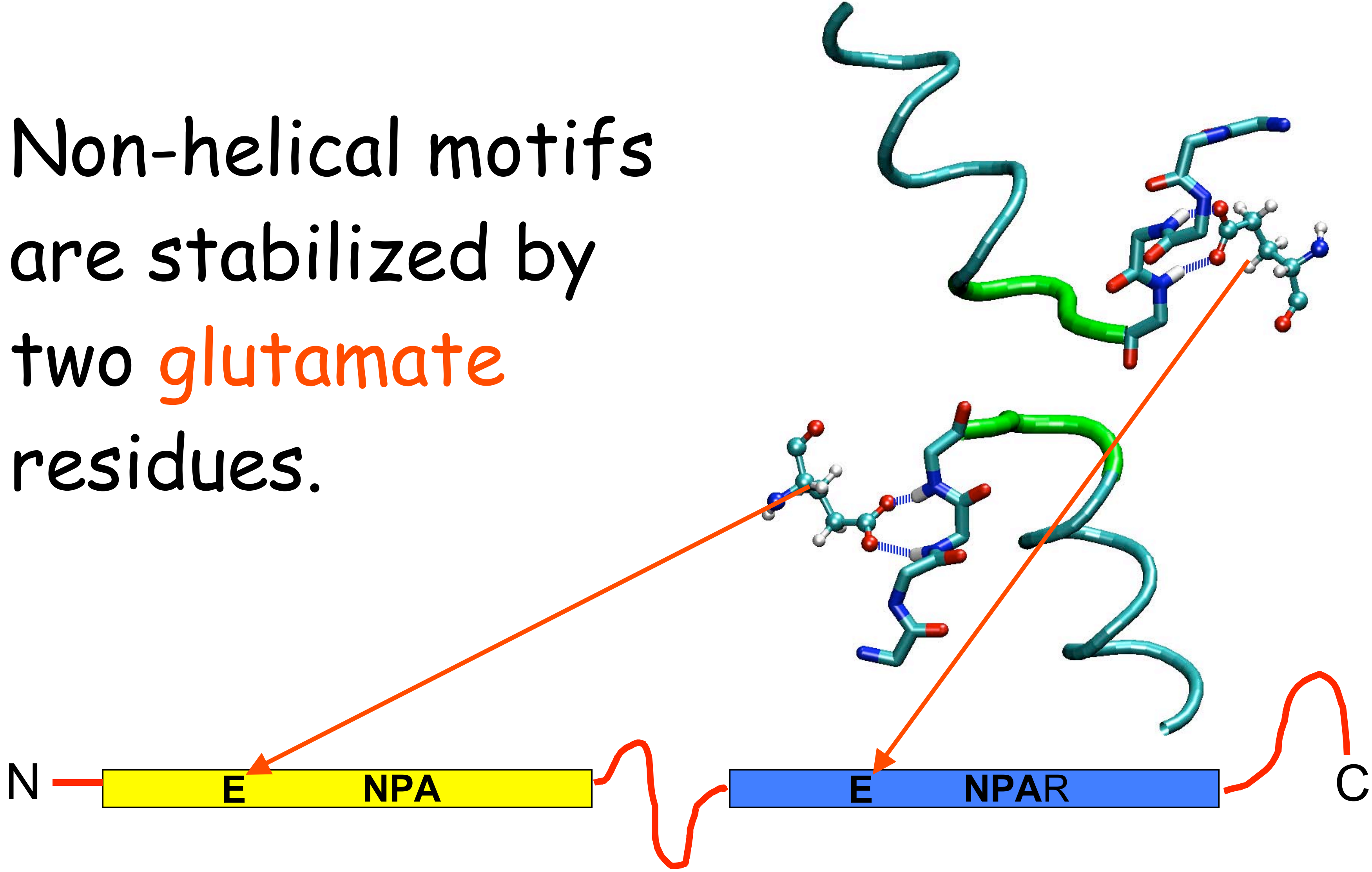


The Substrate Pathway is formed by $C=O$ groups

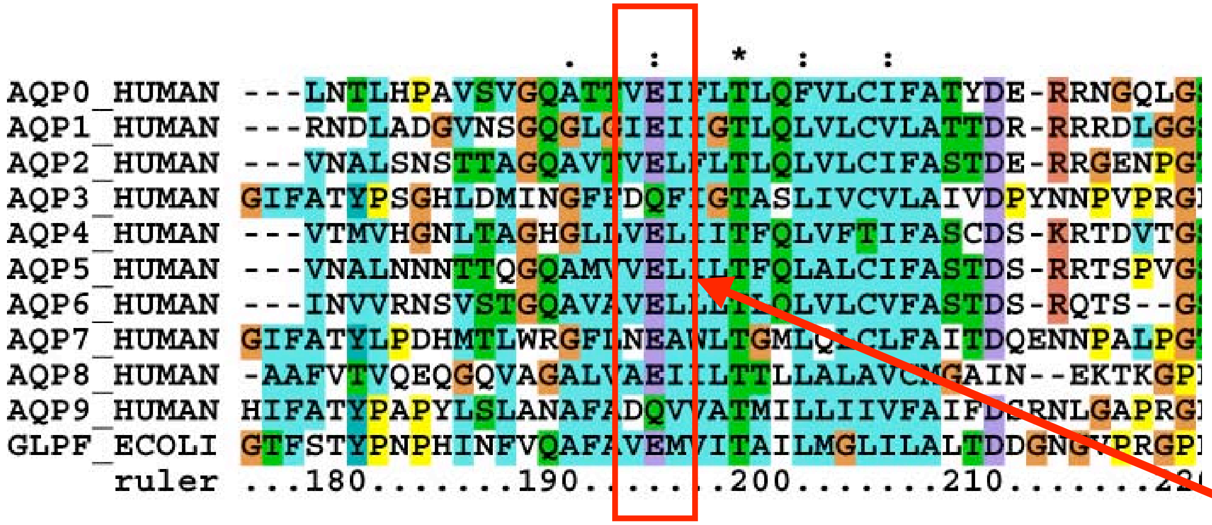


The Substrate Pathway is formed by C=O groups

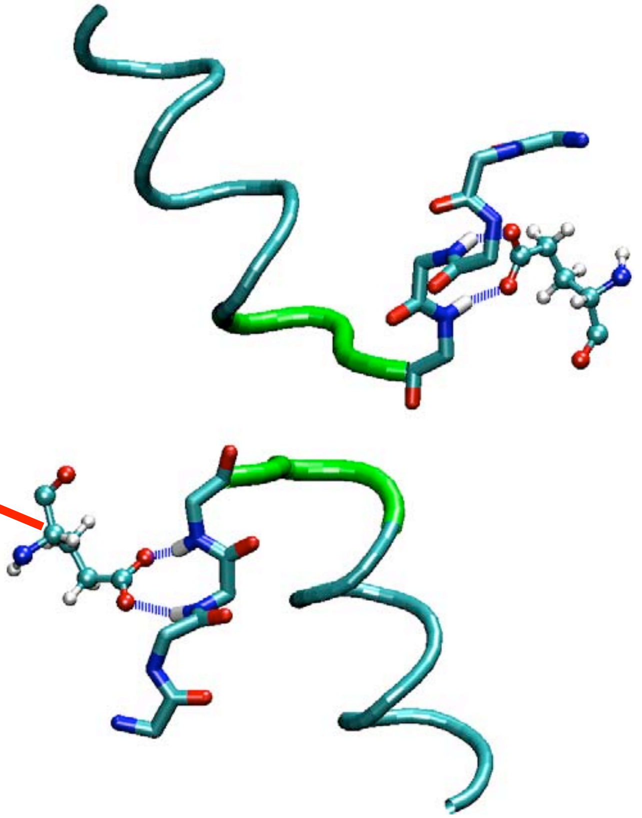
Non-helical motifs are stabilized by two glutamate residues.



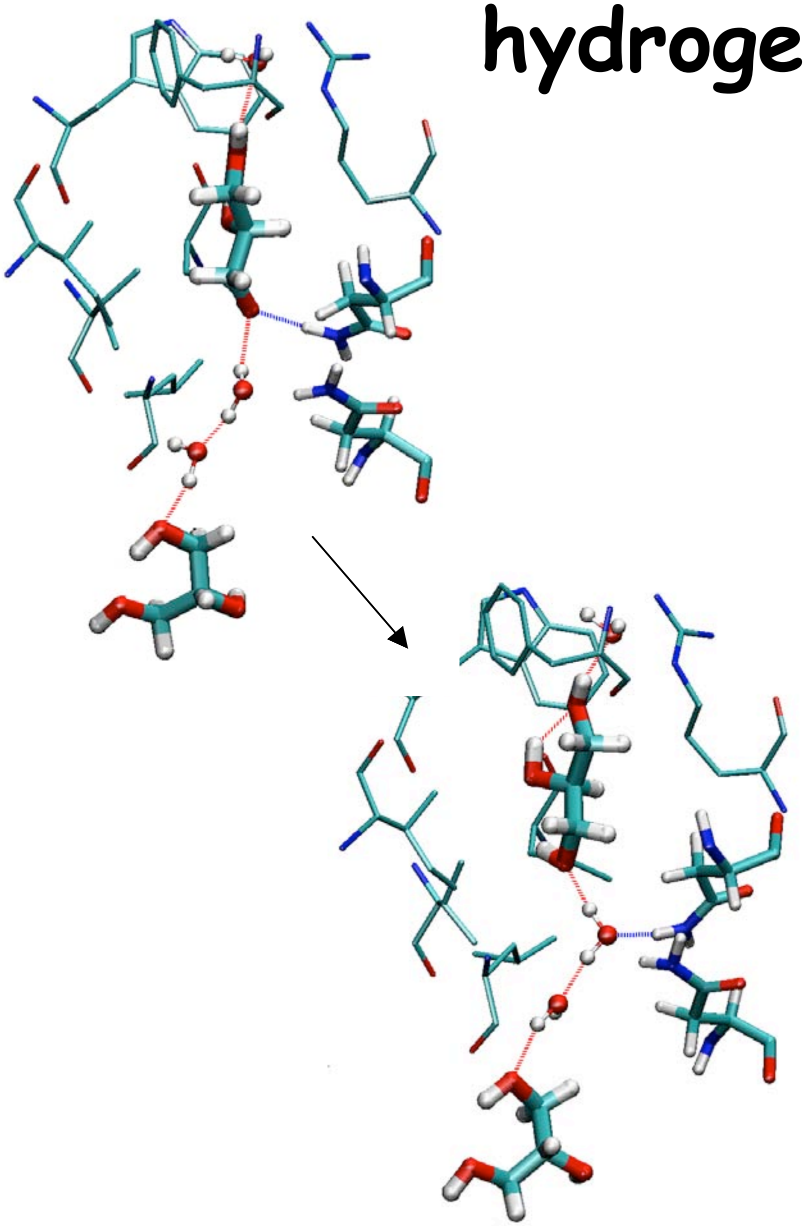
Conservation of Glutamate Residue in Human Aquaporins

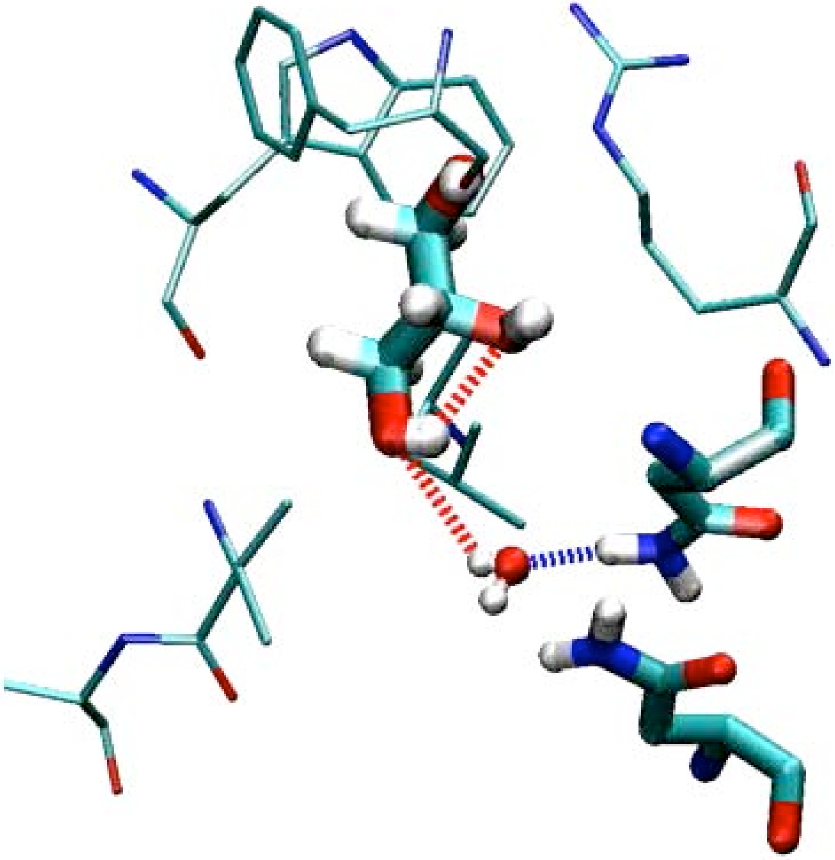


```
                          .   :   *  :   :
AQP0_HUMAN ---LNTLHPAVSVGQATTVEIFLTLQFVLCIFATYDE-RRNGQLG
AQP1_HUMAN ---RNDLADGVNSGQGLGIEIIGTLQLVLCVLATTDR-RRRDLGG
AQP2_HUMAN ---VNALSNSTTAGQAVTVELFLTLQLVLCIFASTDE-RRGENPG
AQP3_HUMAN GIFATYPSGHLDMINGFFDQFIGTASLIVCVLAIVDPYNNPVPRG
AQP4_HUMAN ---VTMVHGNLTAGHGLLVELIITFQLVFTIFASCDS-KRTDVTG
AQP5_HUMAN ---VNALNNNTTQGQAMVVELILTFQLALCIFASTDS-RRTSPVG
AQP6_HUMAN ---INVVRNSVSTGQAVAVELLLTLQLVLCVFASTDS-RQTS--G
AQP7_HUMAN GIFATYLPDHMTLWRGFLNEAWLTGMLQLCLFAITDQENNPALPG
AQP8_HUMAN -AAFVTVQEQGQVAGALVAEIILTTLLALAVCMGAIN--EKTKGP
AQP9_HUMAN HIFATYPAPYLSLANAFADQVVATMILLIIVFAIFDSRNLGAPRG
GLPF_ECOLI GTFSTYPNPHINFVQAFAVEMVITAILMGLILALTDDGNGVPRGP
     ruler ...180.......190.......200.......210.......22
```

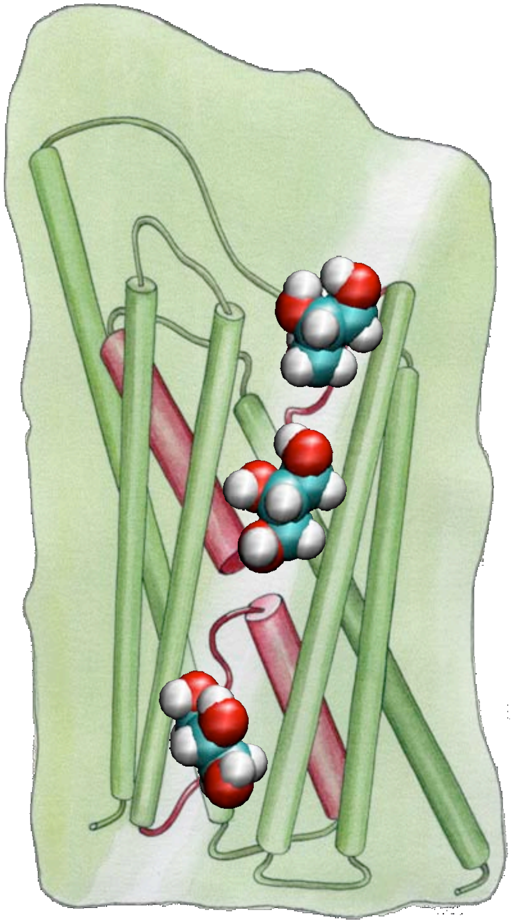


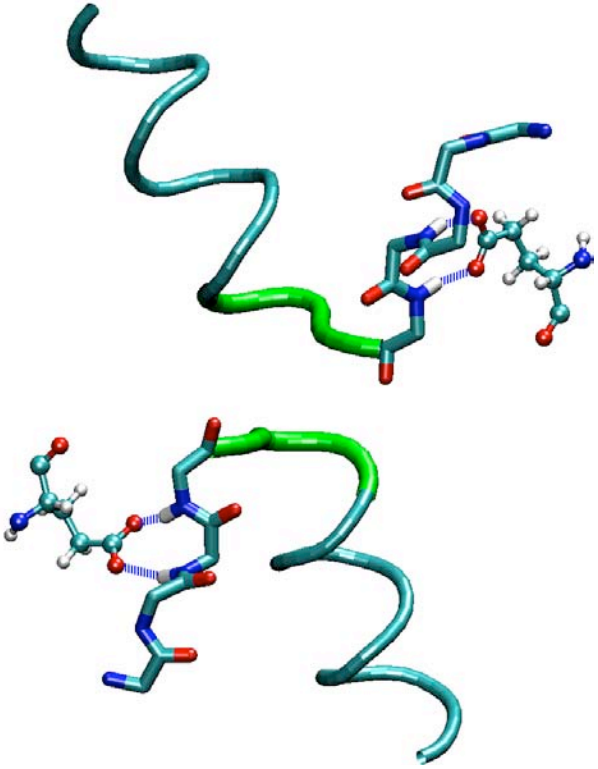
Glycerol – water competition for hydrogen bonding sites



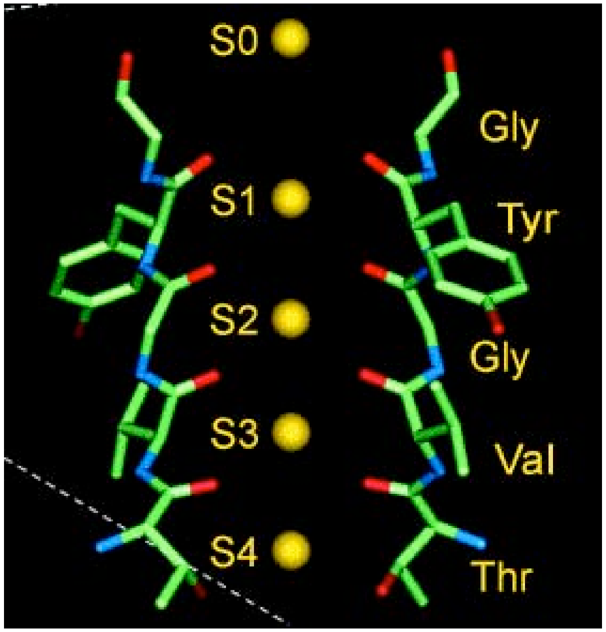


Revealing the Functional Role of Reentrant Loops

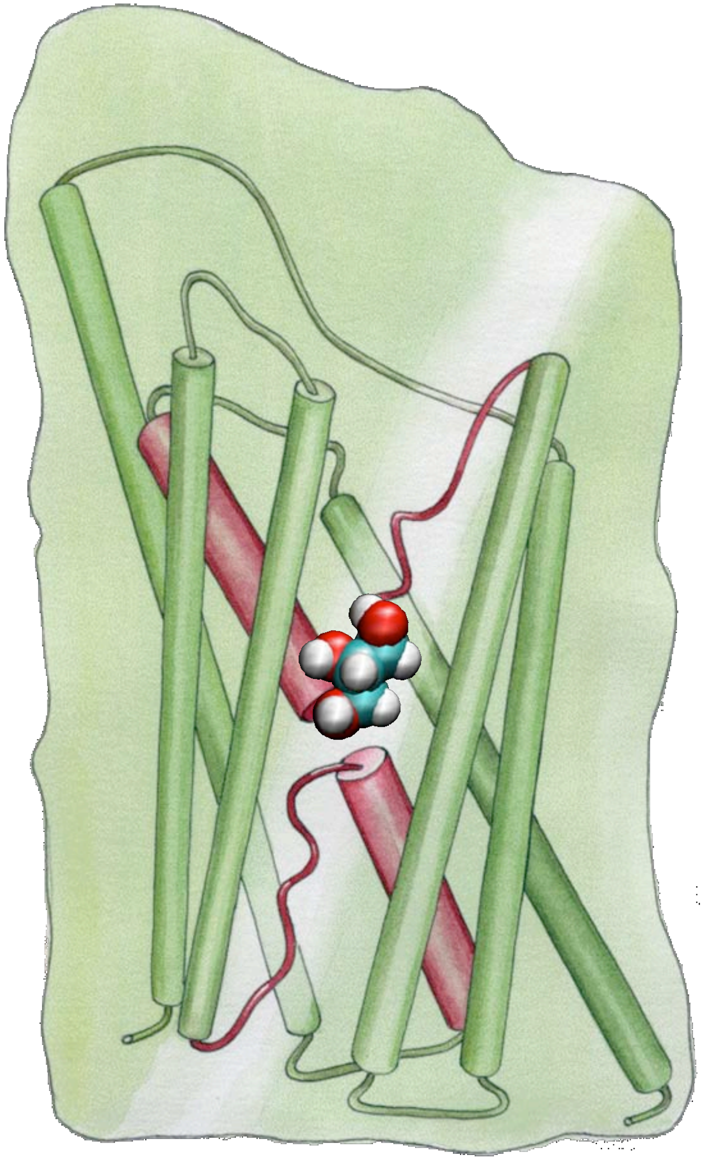




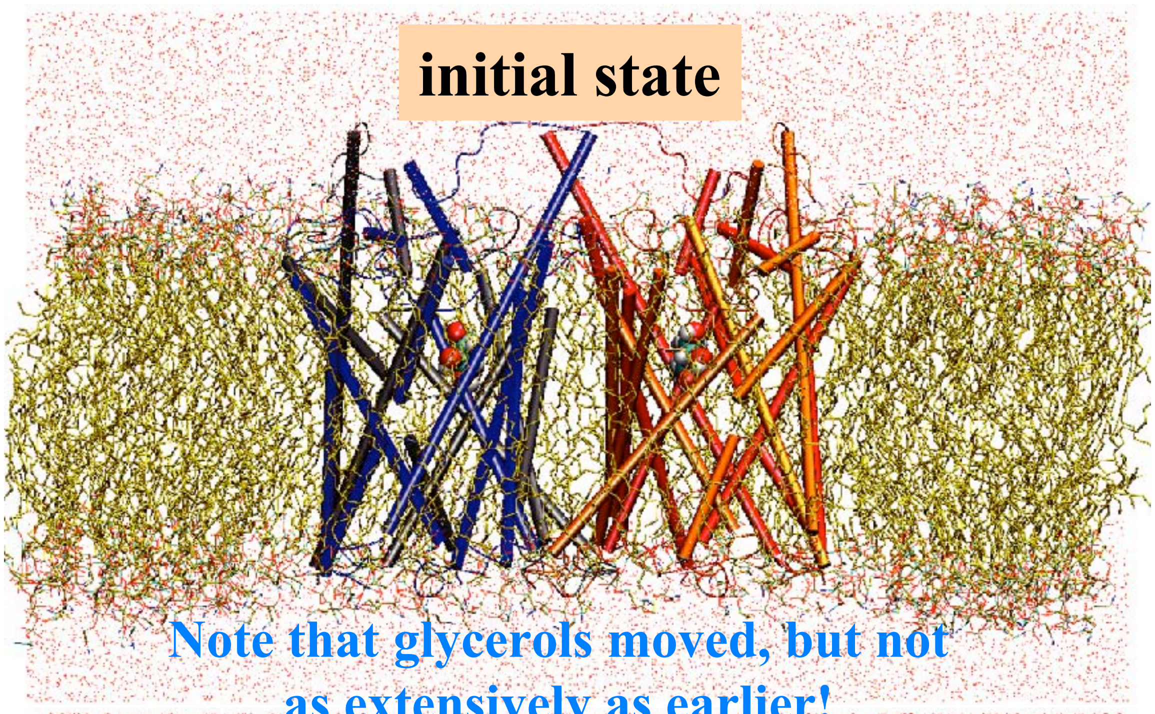
Potassium channel



Single Glycerol per channel

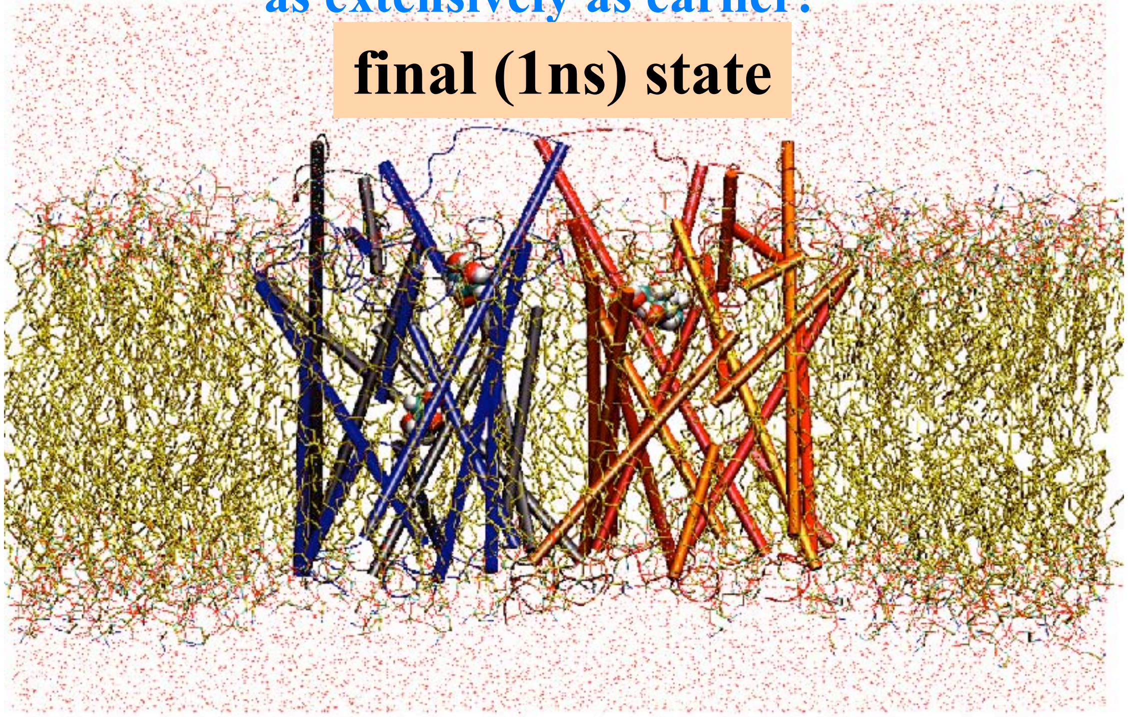


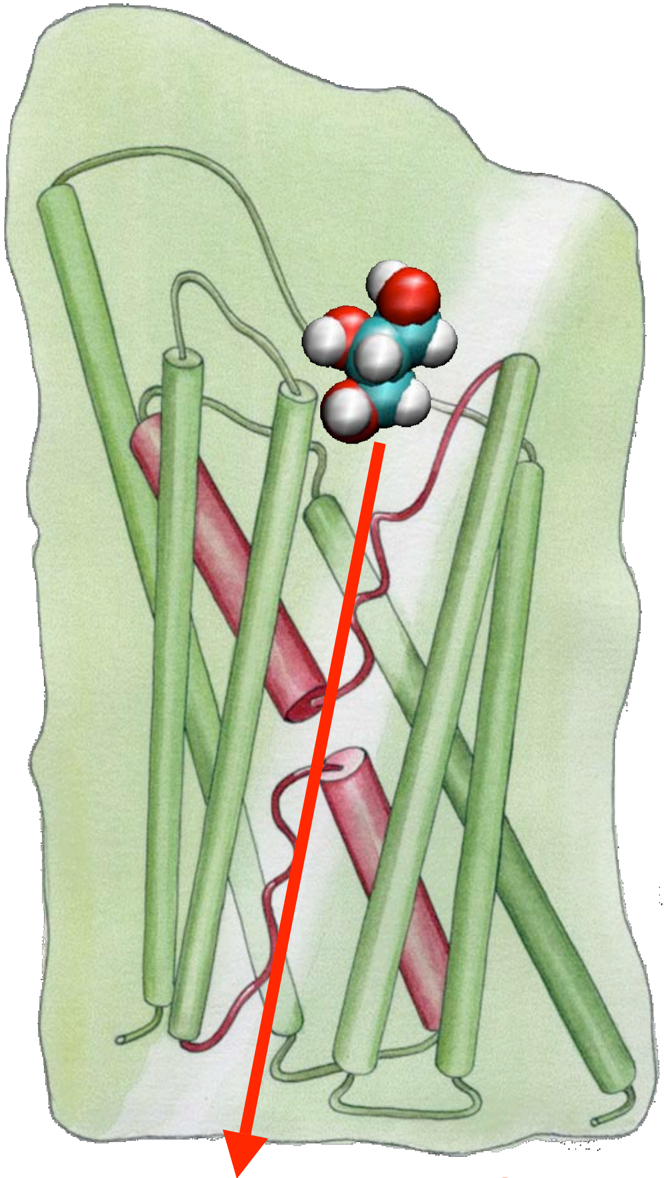
initial state



Note that glycerols moved, but not as extensively as earlier!

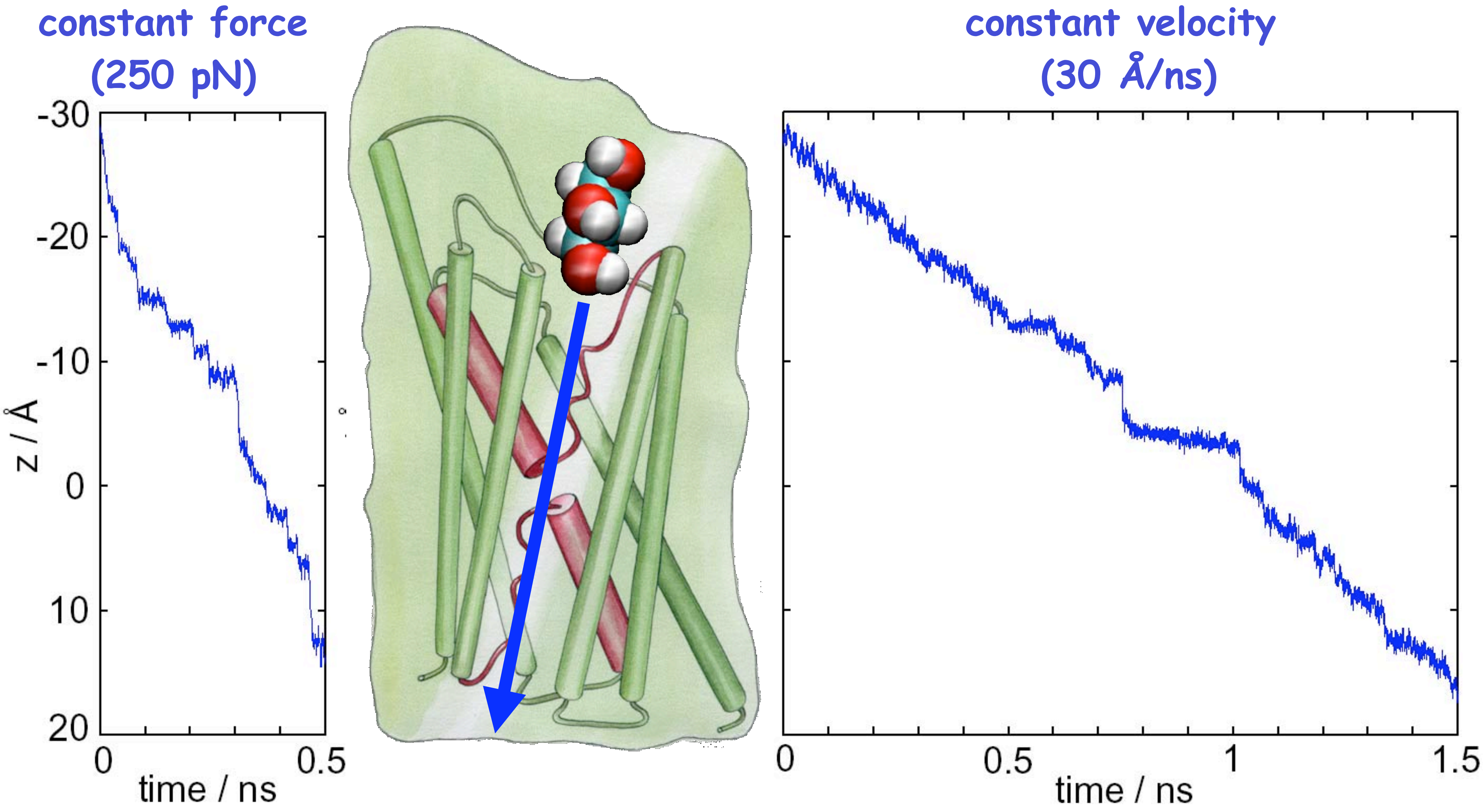
final (1ns) state



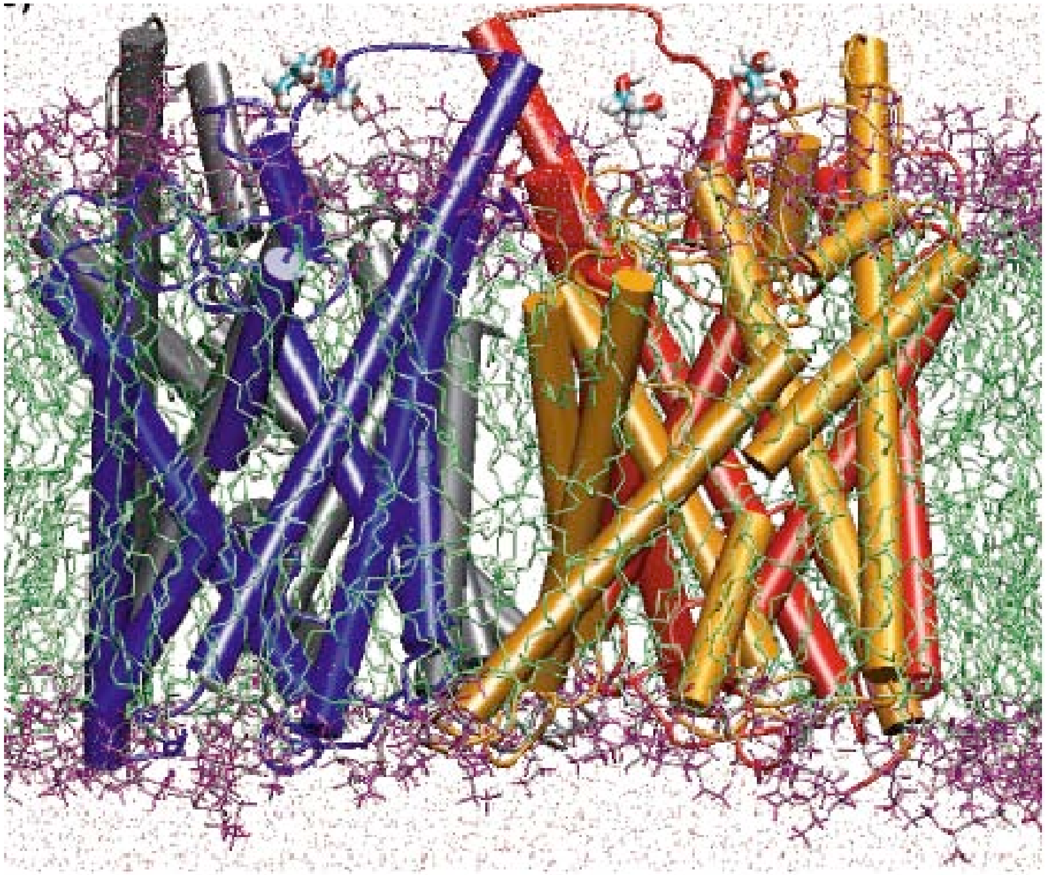


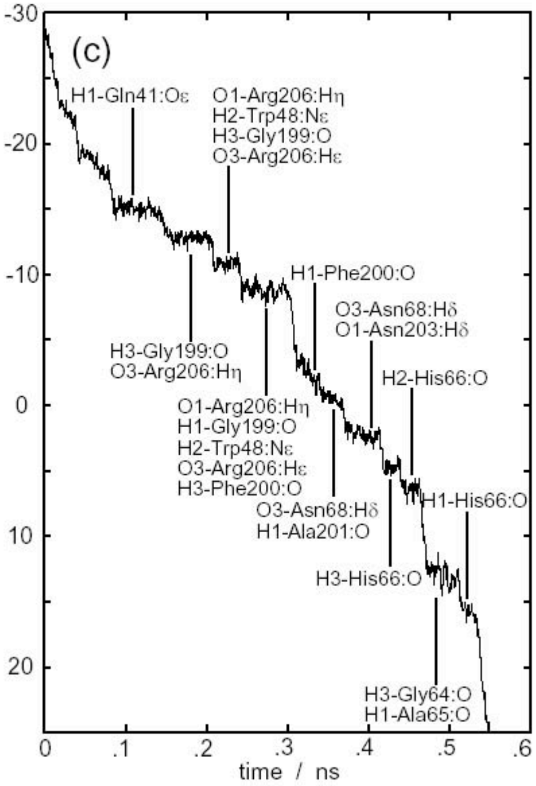
We need to enforce an entire conduction event.

Steered Molecular Dynamics



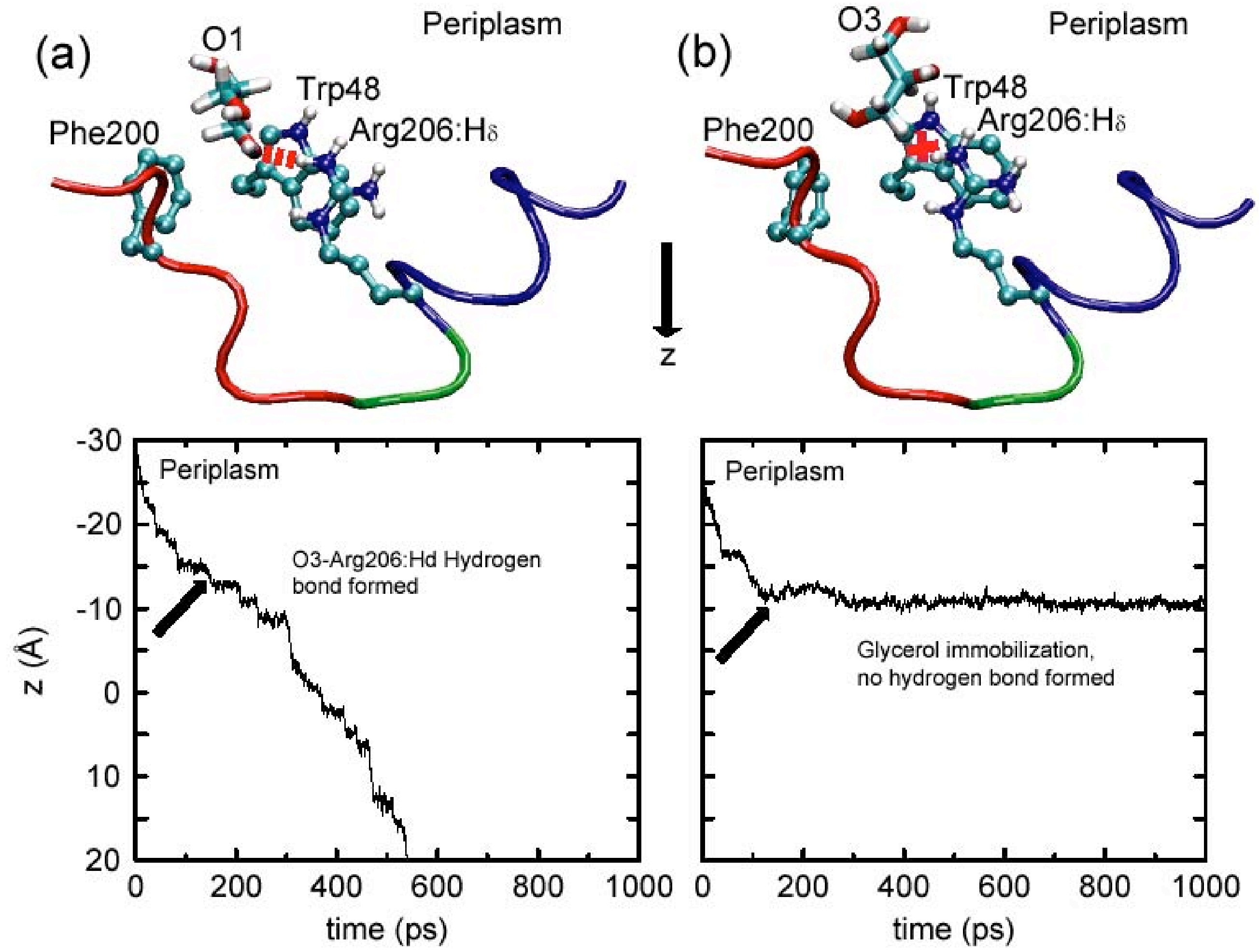
SMD Simulation of Glycerol Passage

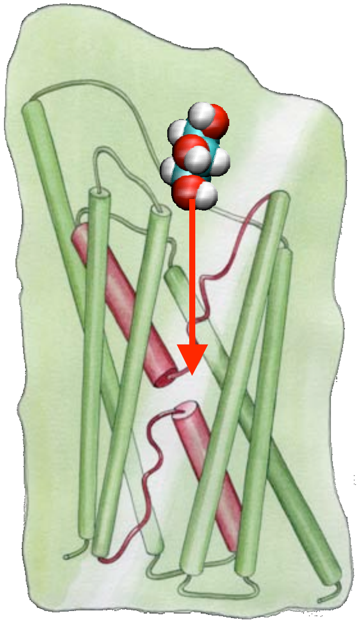


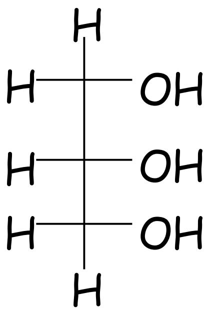


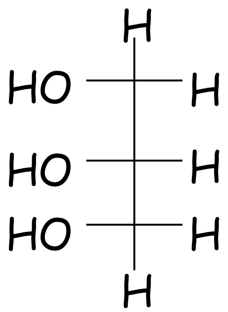
Trajectory of glycerol pulled by **constant force**

Evidence for Stereoselectivity of Glycerol



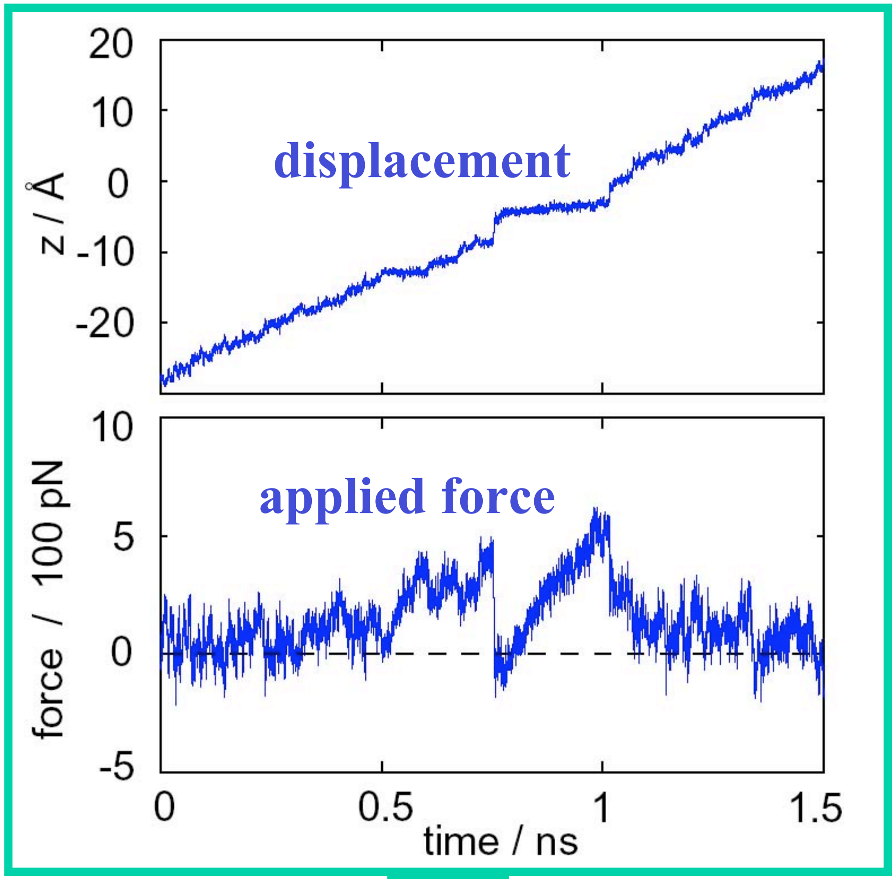






Cannot be verified by experimental measurements

Free Energy Calculation in SMD



Free energy

SMD simulation

a non-equilibrium process

$$\Delta G \le \langle W \rangle$$

One needs to discount irreversible work

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

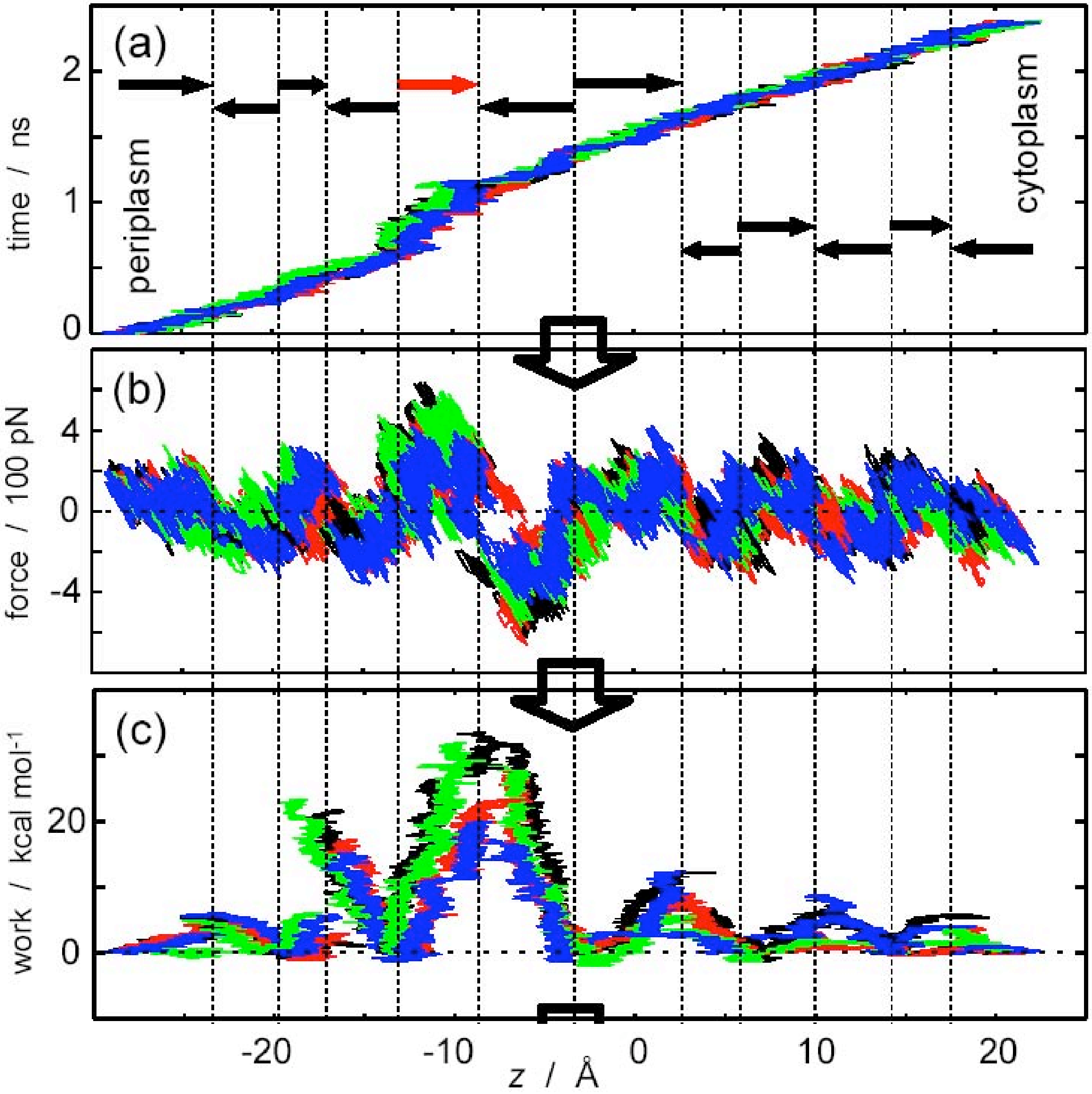
Jarzynski, *PRL* 1997
Hummer, *PNAS, JCP* 2001
Liphardt, et al., *Science* 2002

Constructing the Potential of Mean Force

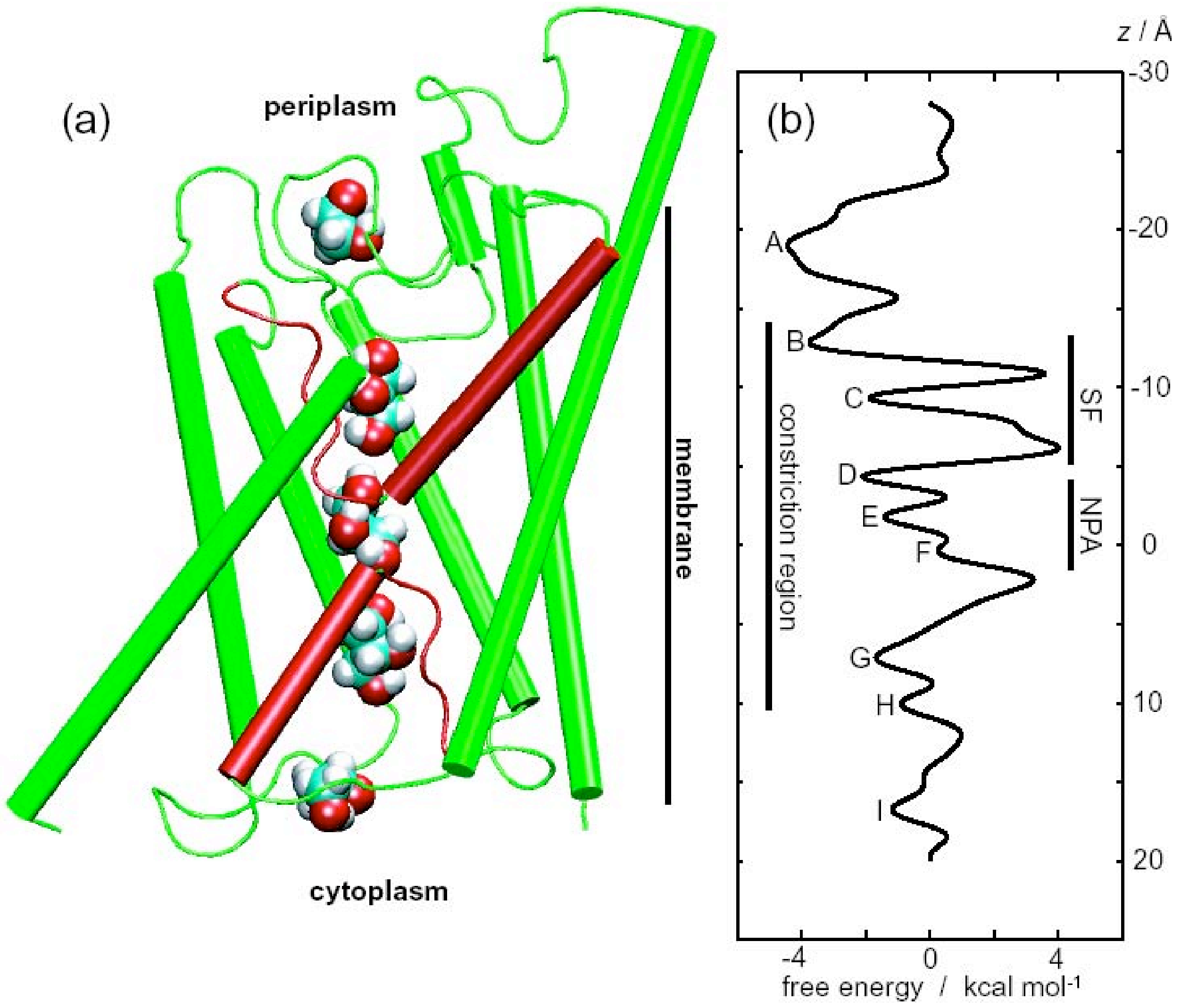
4 trajectories
v **= 0.03, 0.015 Å/ps**
k **= 150 pN/Å**

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

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

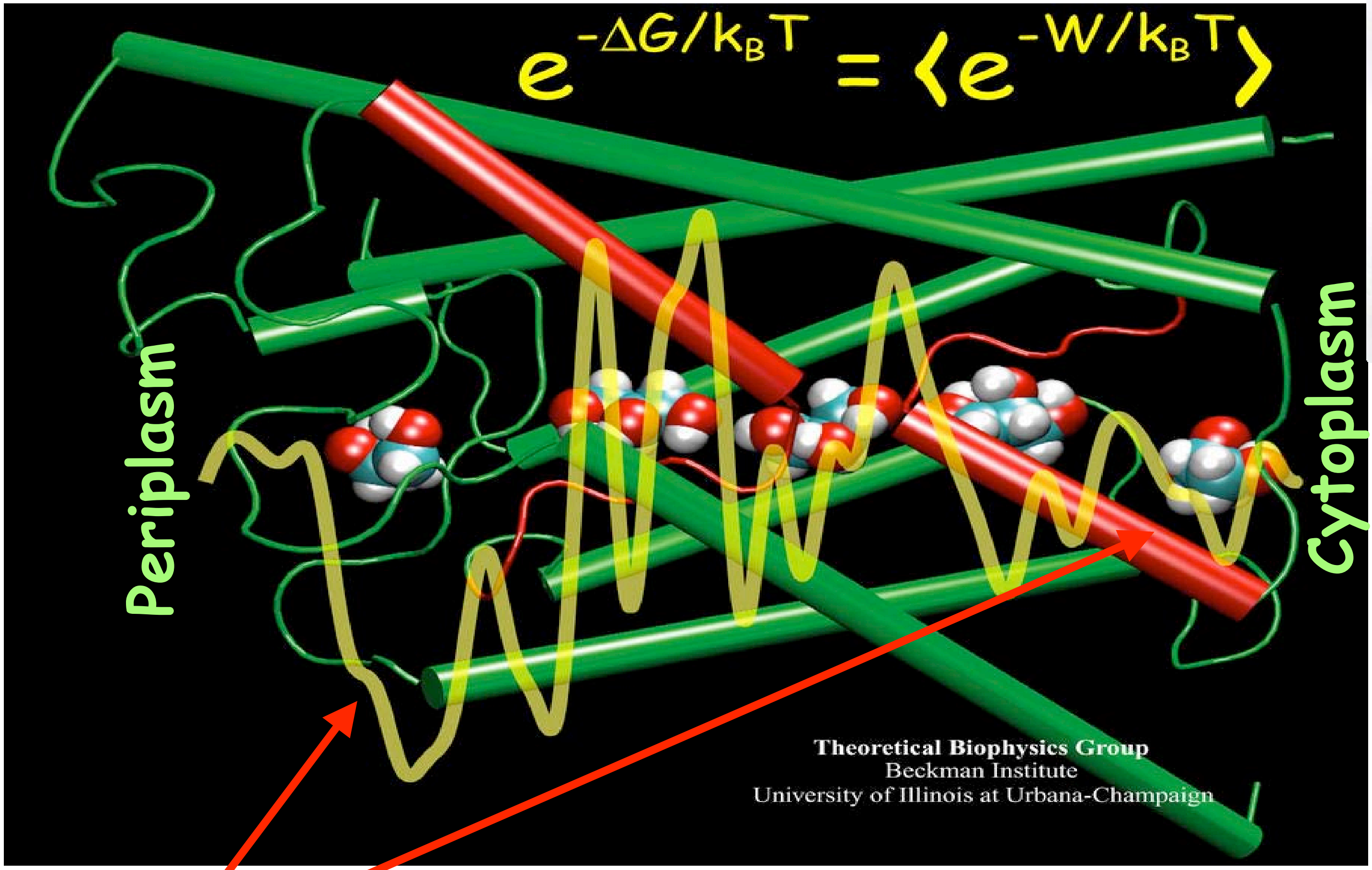


Features of the Potential of Mean Force

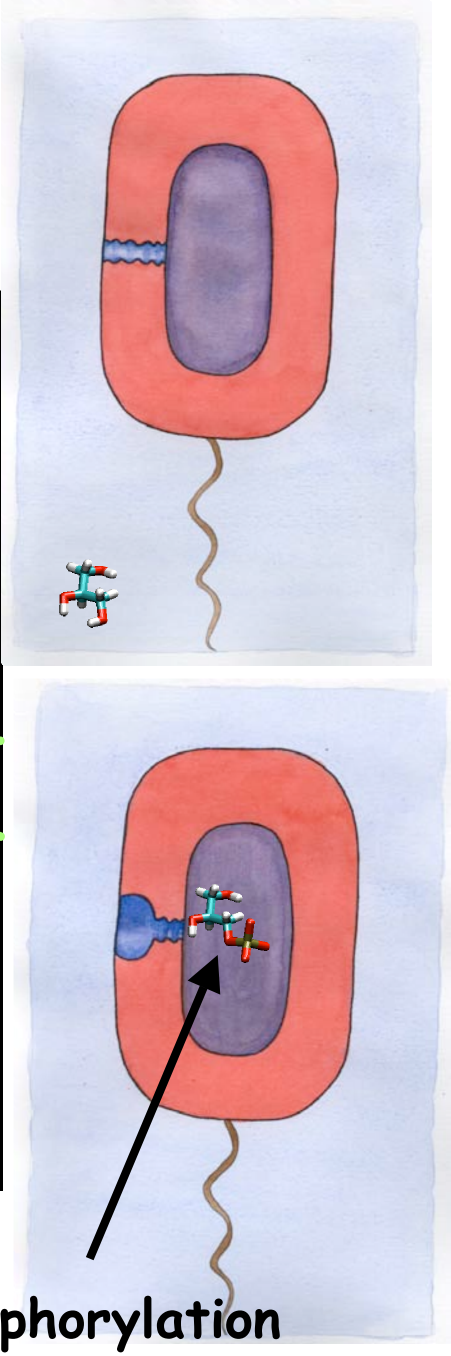


- Captures major features of the channel
- The largest barrier ≈ 7.3 kcal/mol; exp.: 9.6±1.5 kcal/mol

Asymmetry of the Potential of Mean Force



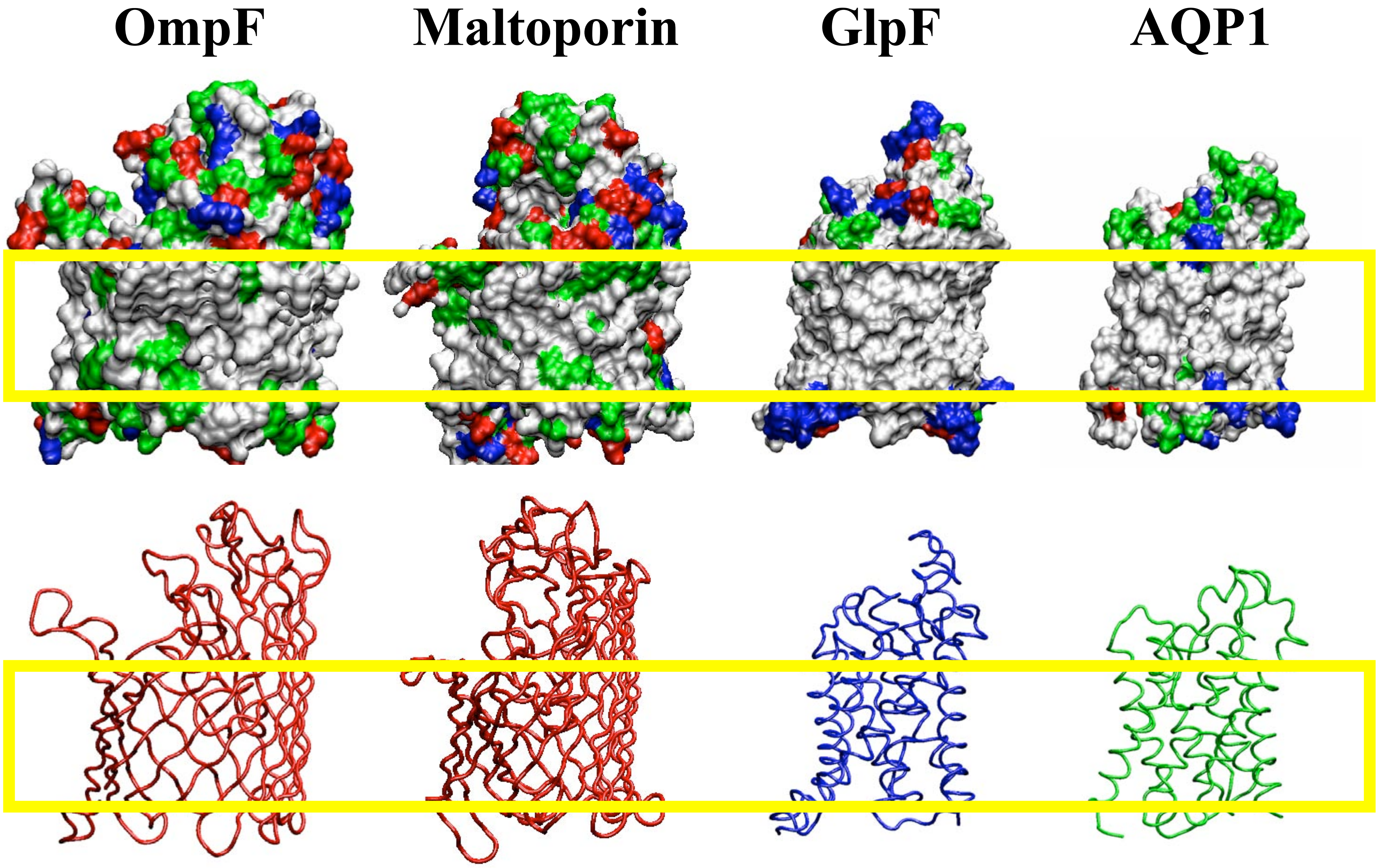
$$e^{-\Delta G/k_BT} = \langle e^{-W/k_BT} \rangle$$



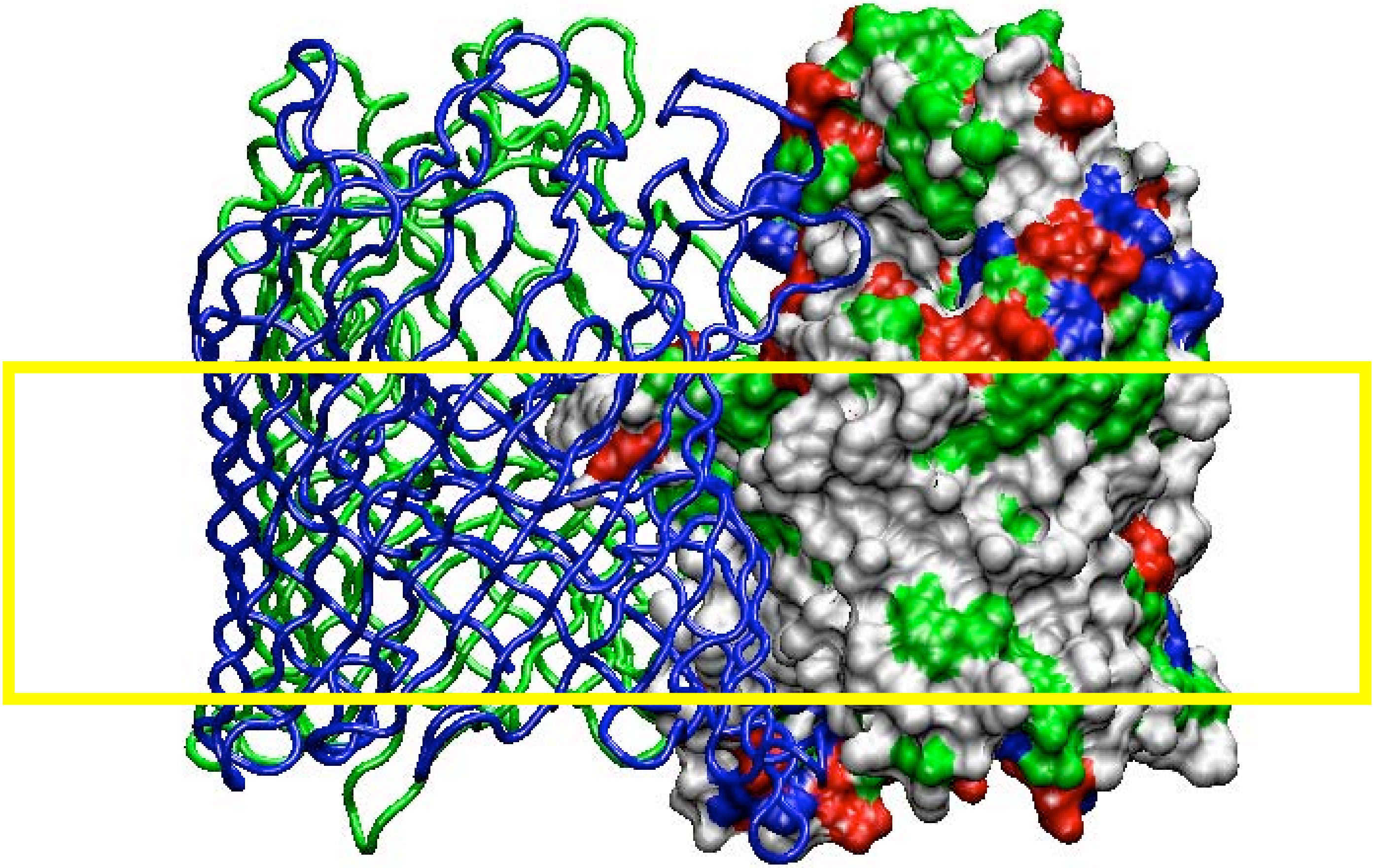
Asymmetric Profile in the Vestibules

phosphorylation

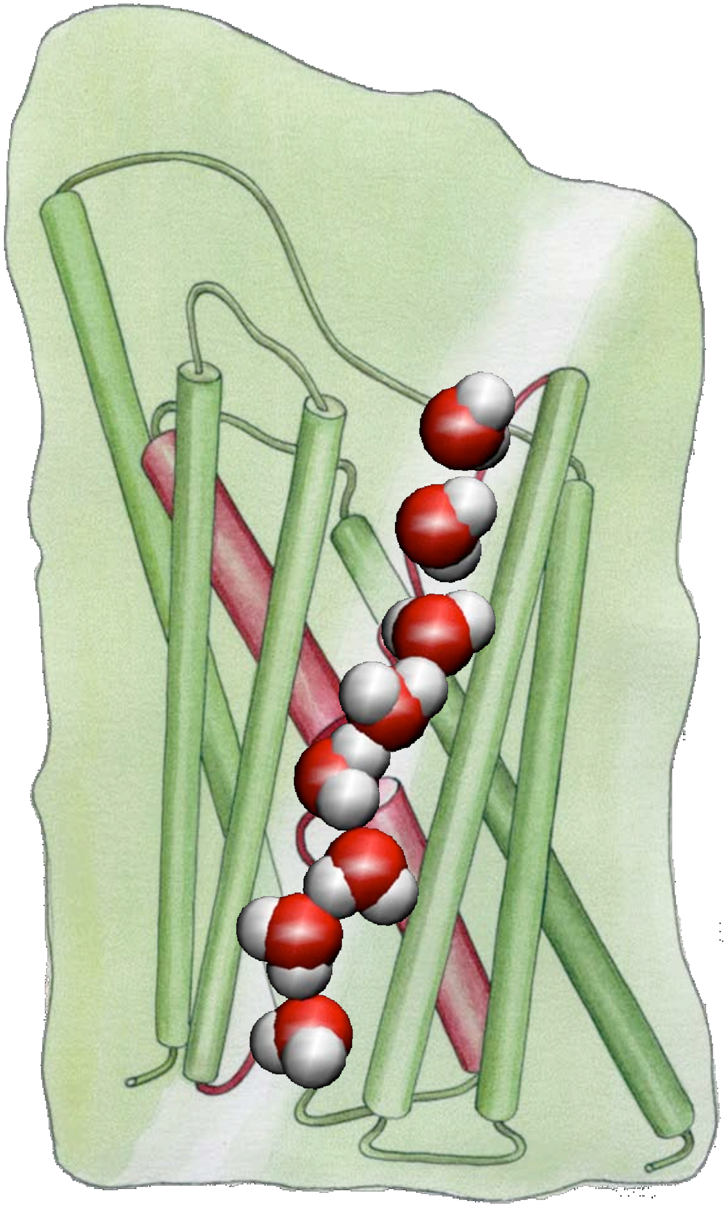
Assymetric structure; biological implication?



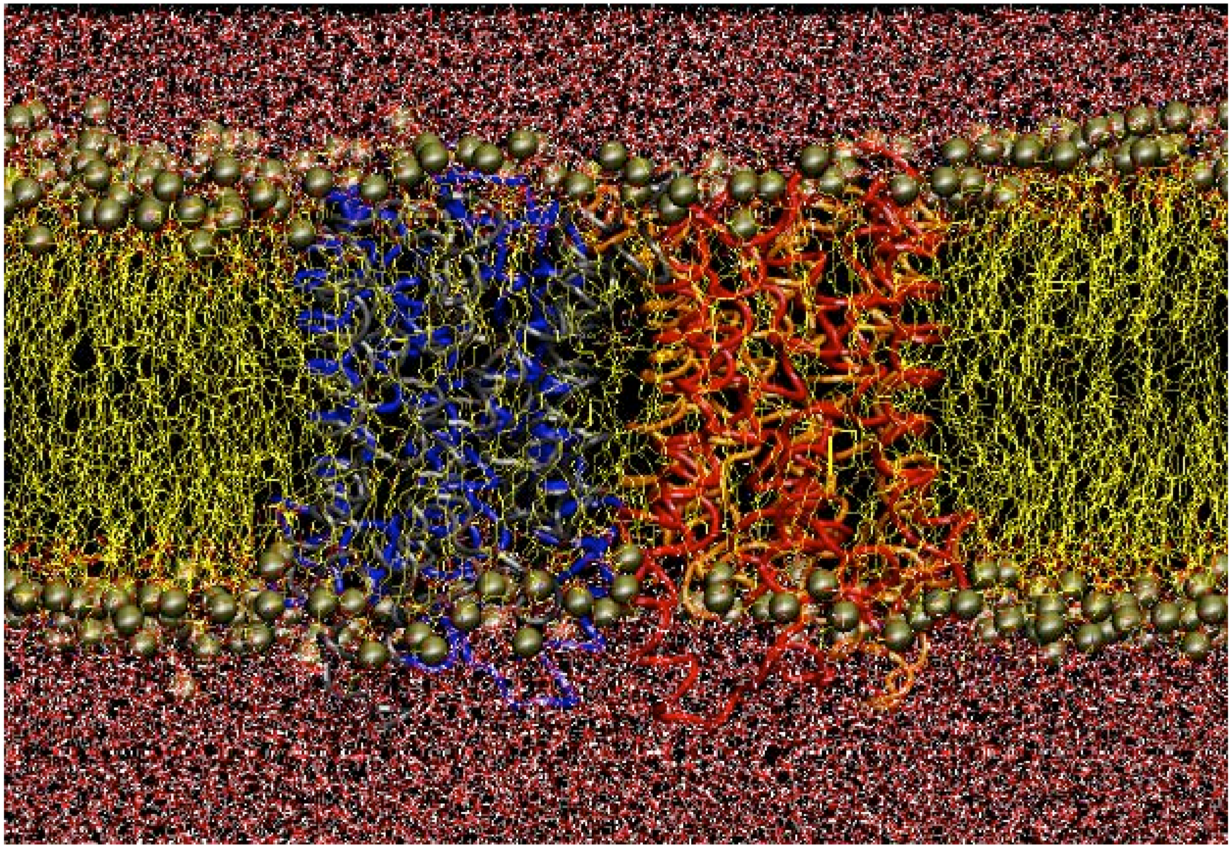
Asymmetric structure of maltoporin



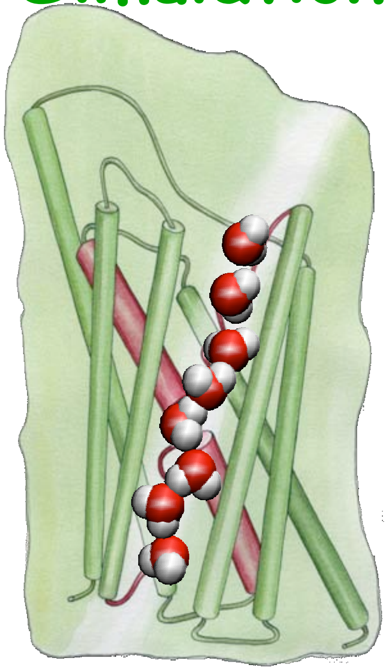
Glycerol-Free GlpF



Water permeation



5 nanosecond Simulation



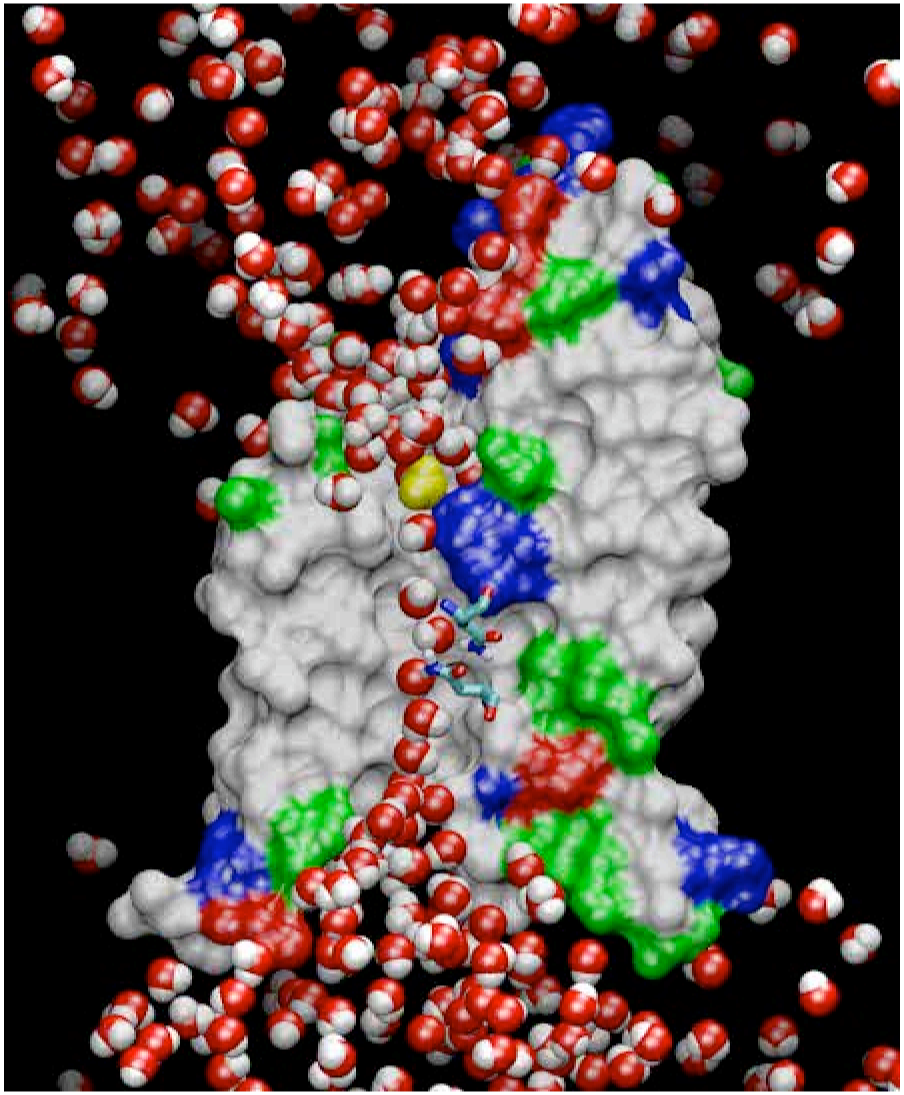
18 water conducted

In 4 monomers in 4 ns

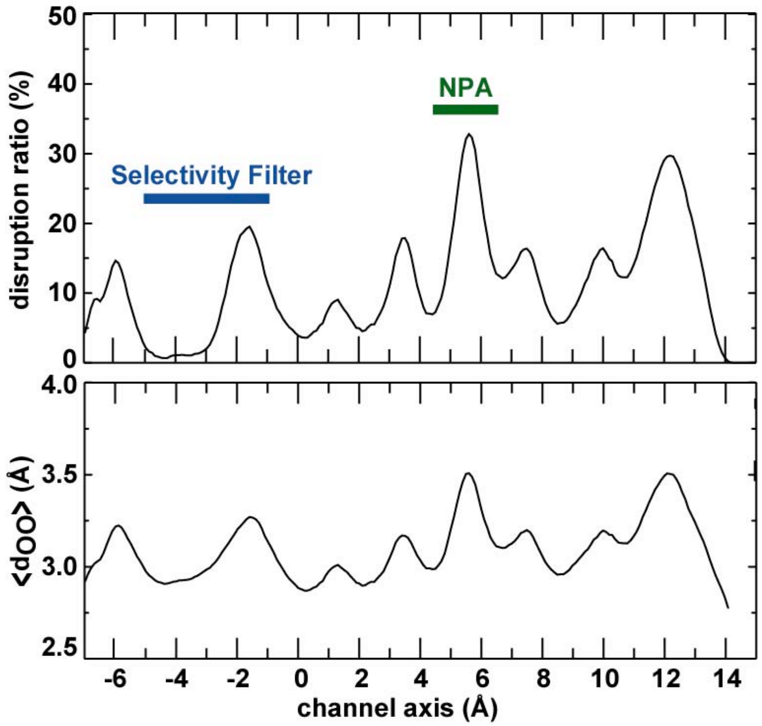
1.125 water/monomer/ns

Exp. = ~ 1-2 /ns

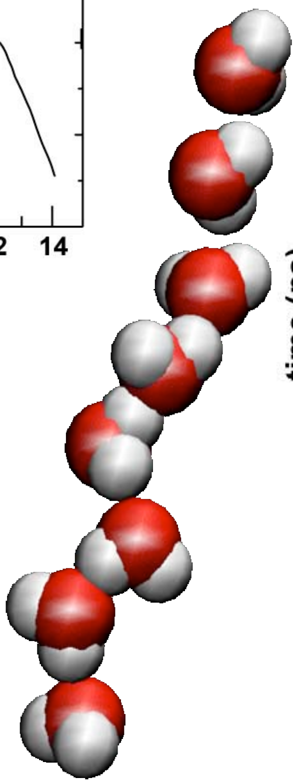
7-8 water molecules in each channel

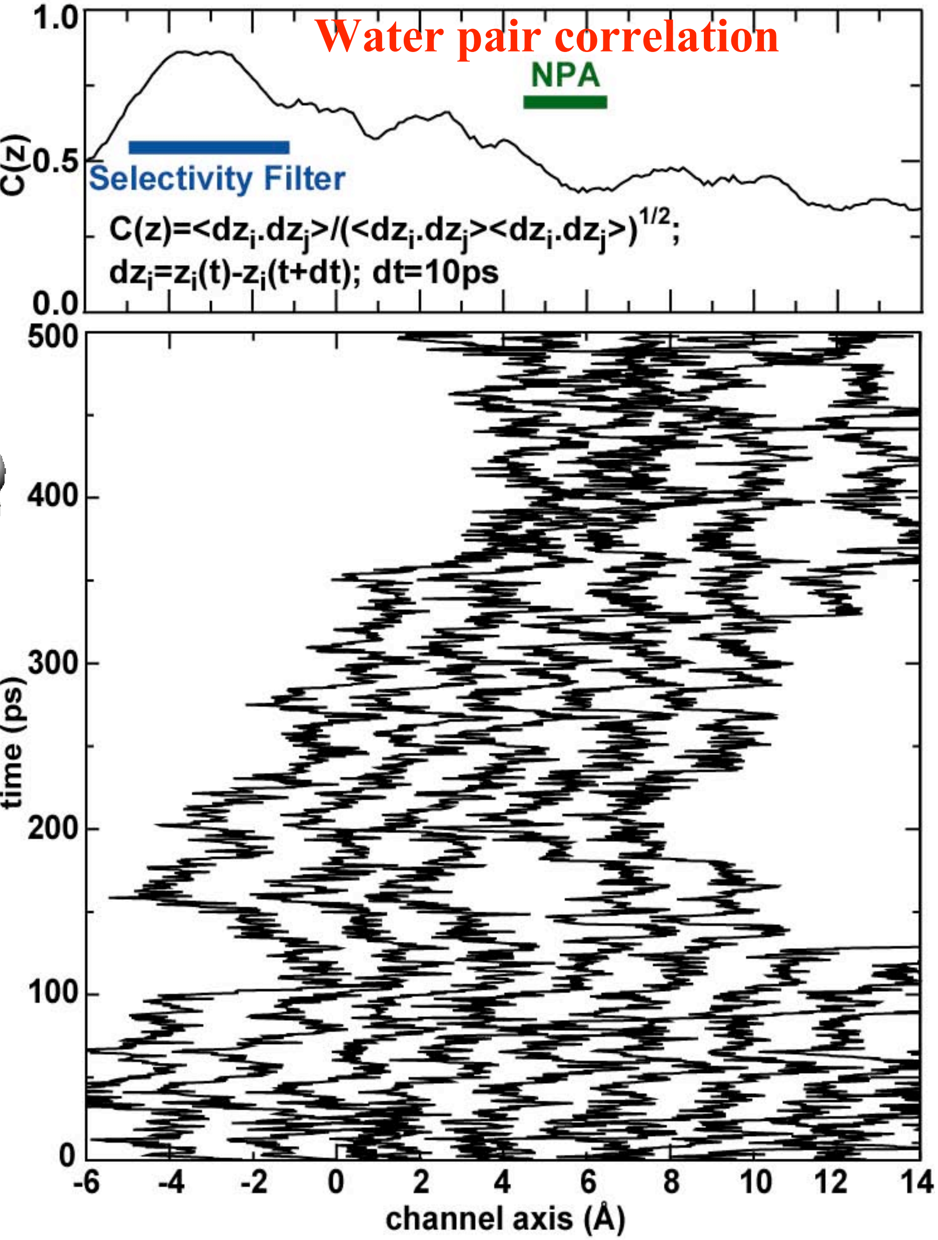


Correlated Motion of Water in the Channel



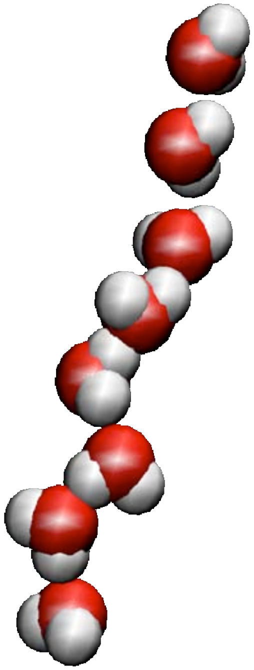
The single file of water molecules is maintained.

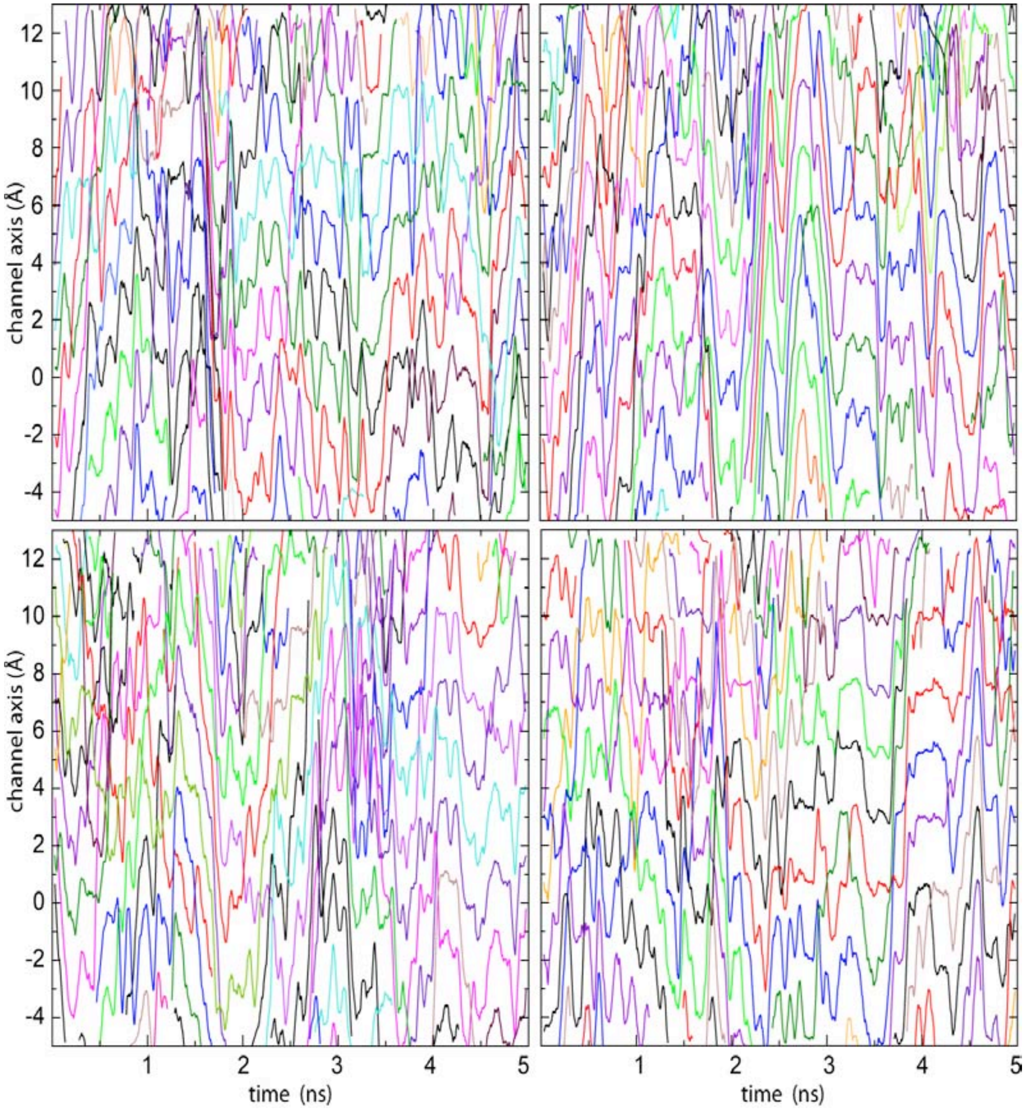




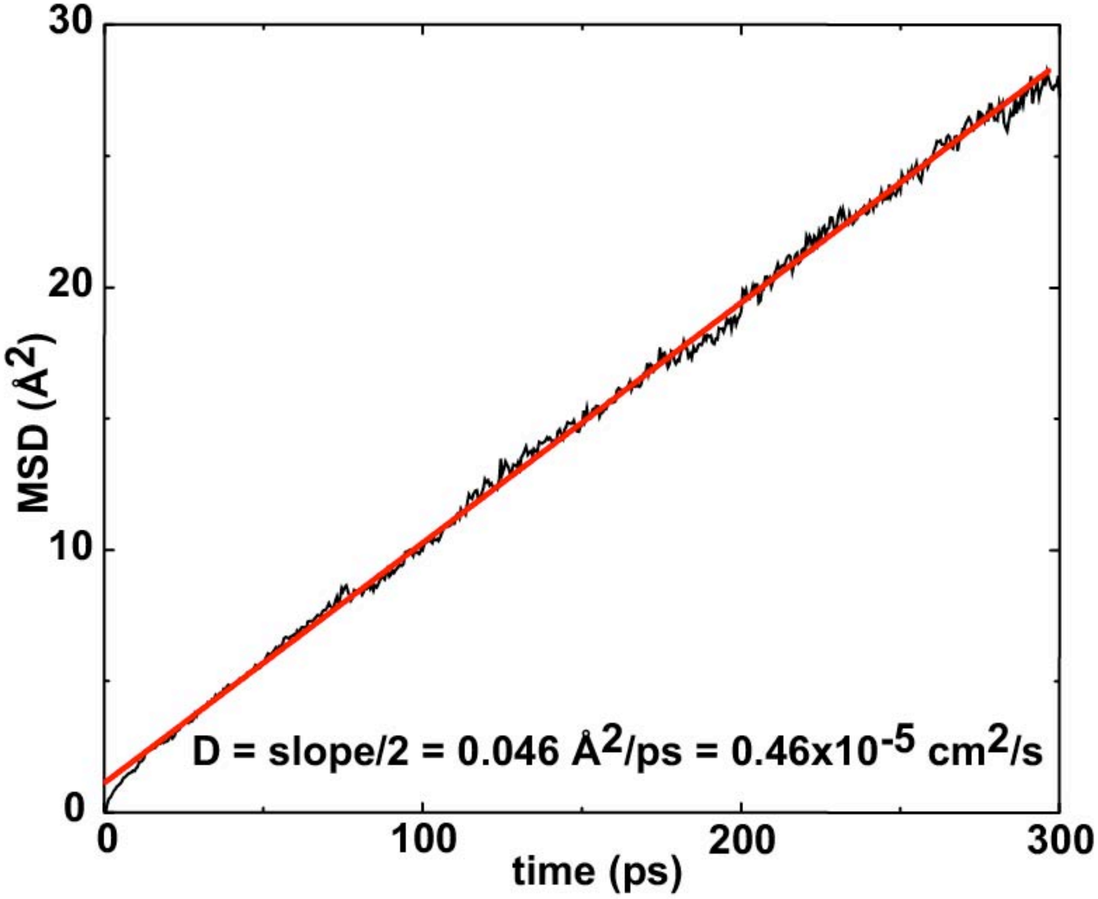
Correlated Motion of Water in the Channel

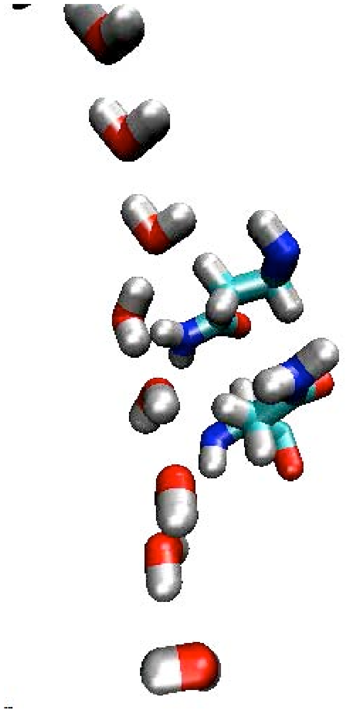
The single file of water molecules is maintained.





Diffusion of Water in the channel

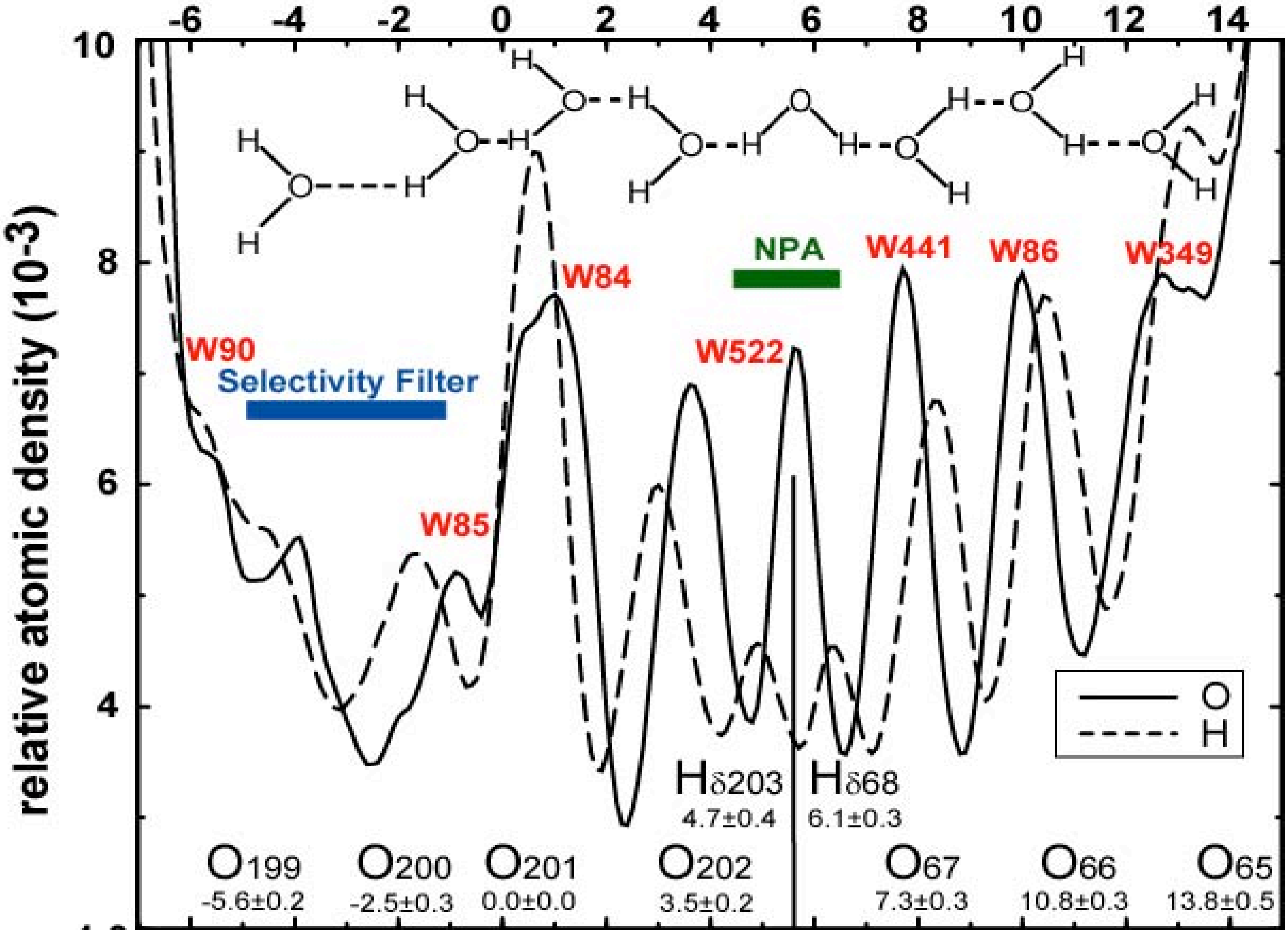




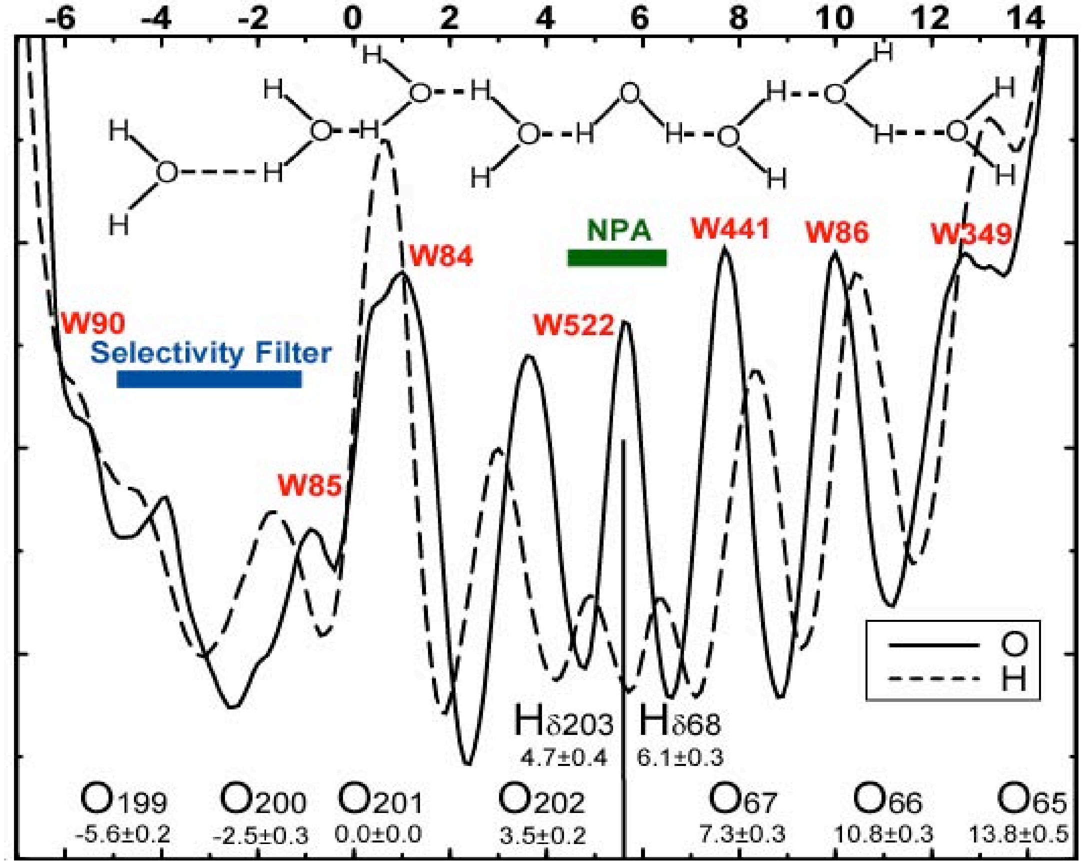
One dimensional diffusion: $\langle \quad \rangle$

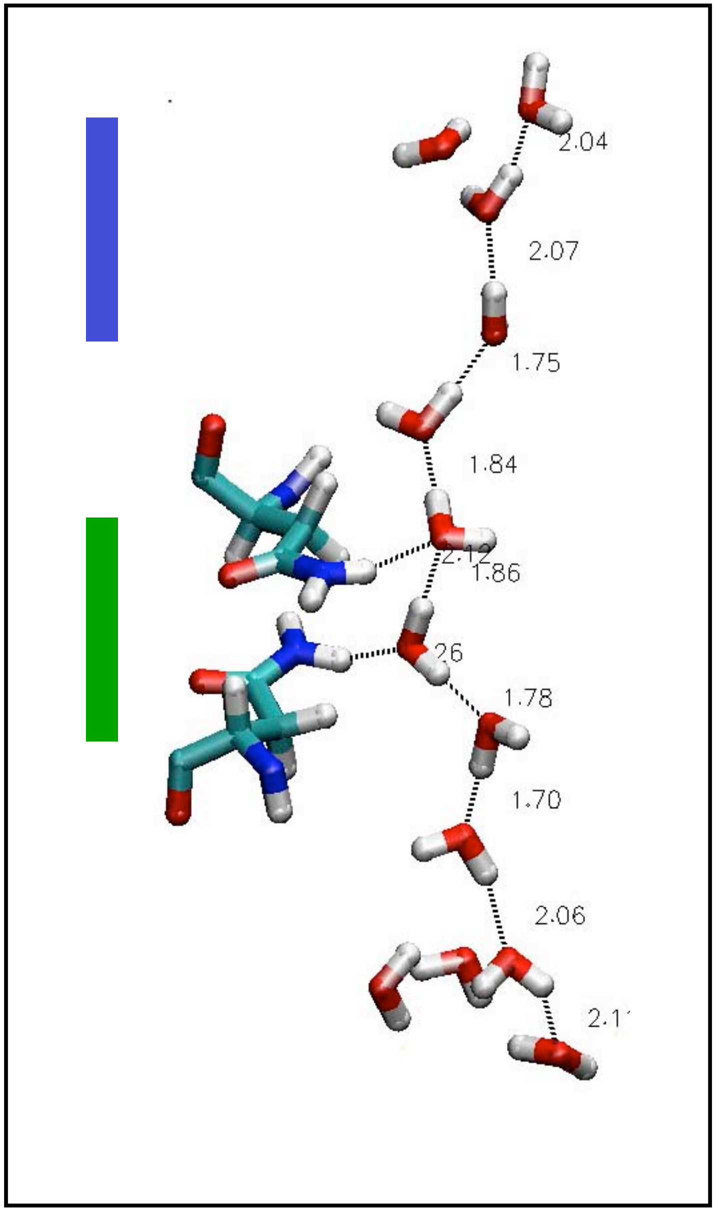
Experimental value for AQP1: 0.4-0.8 e-5

Water Distribution in Aquaporins

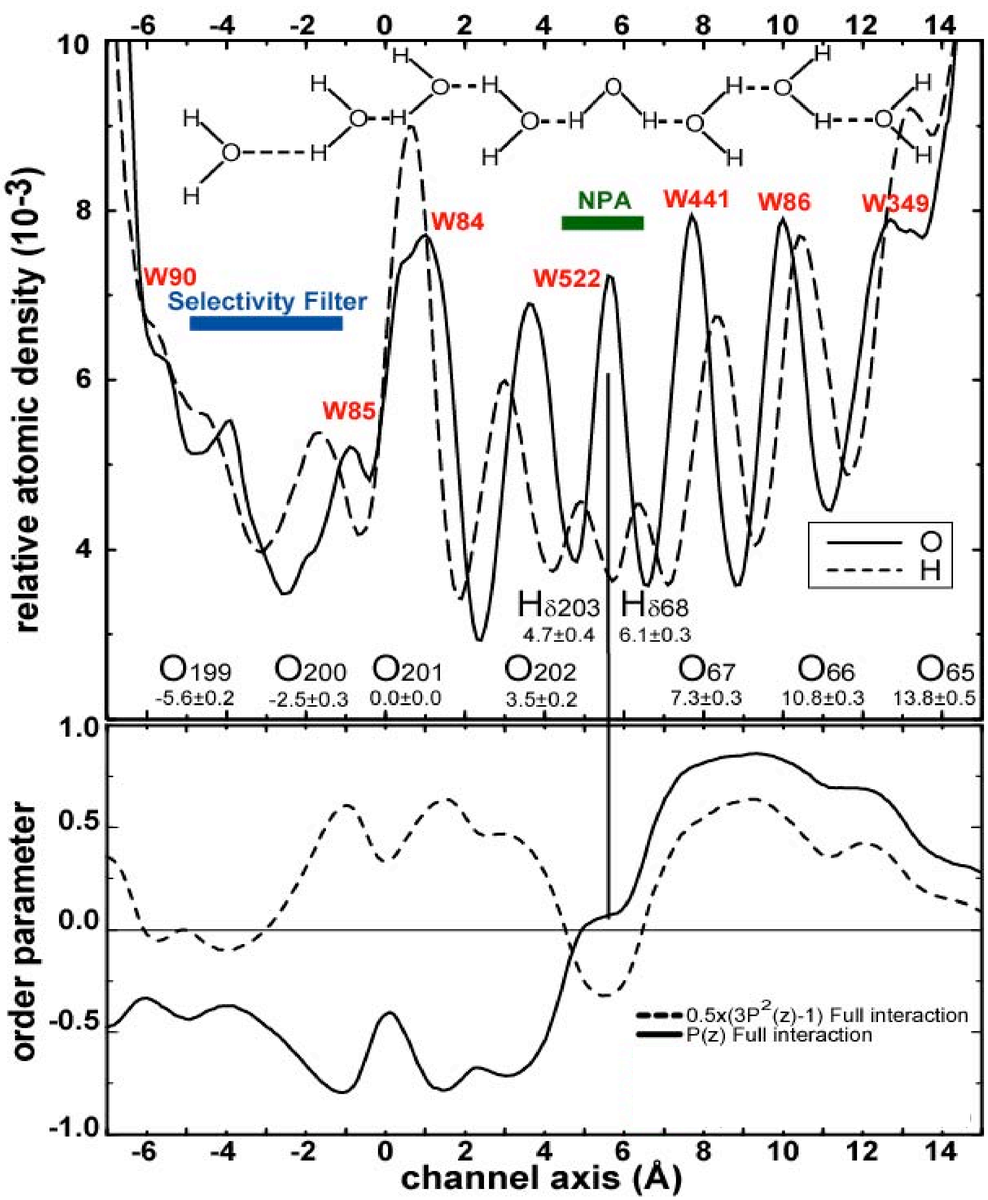


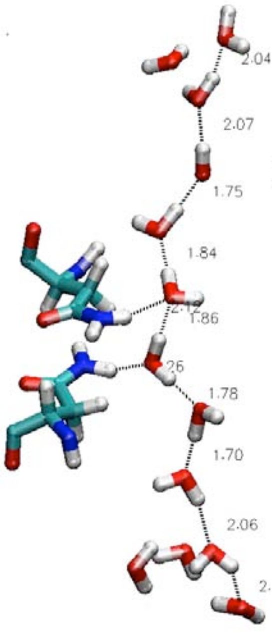
Water Bipolar Configuration in Aquaporins

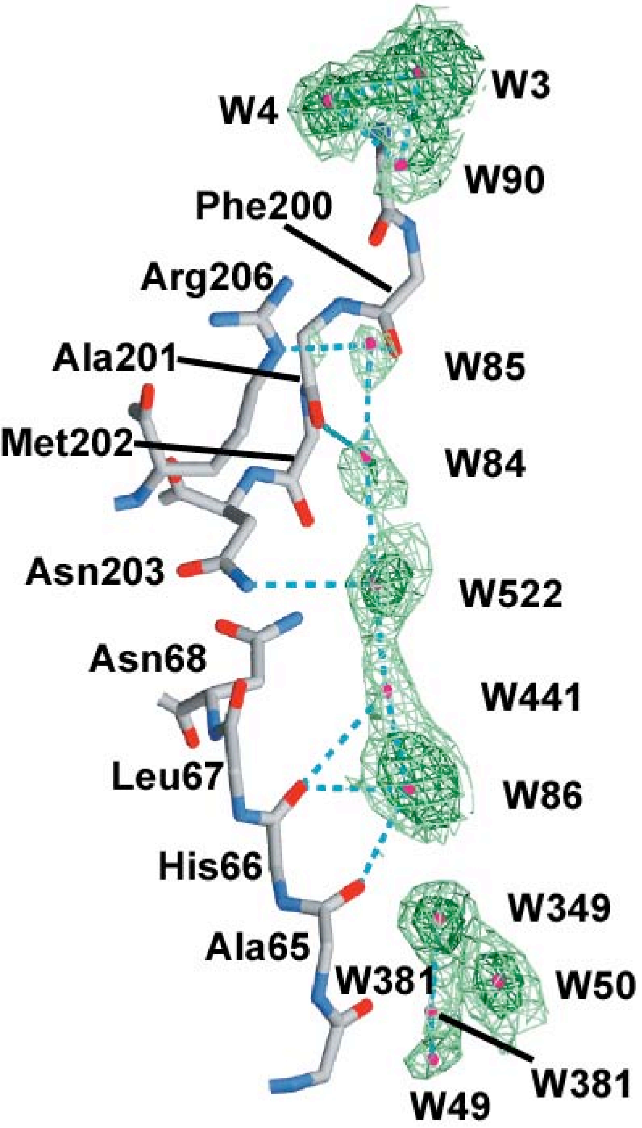


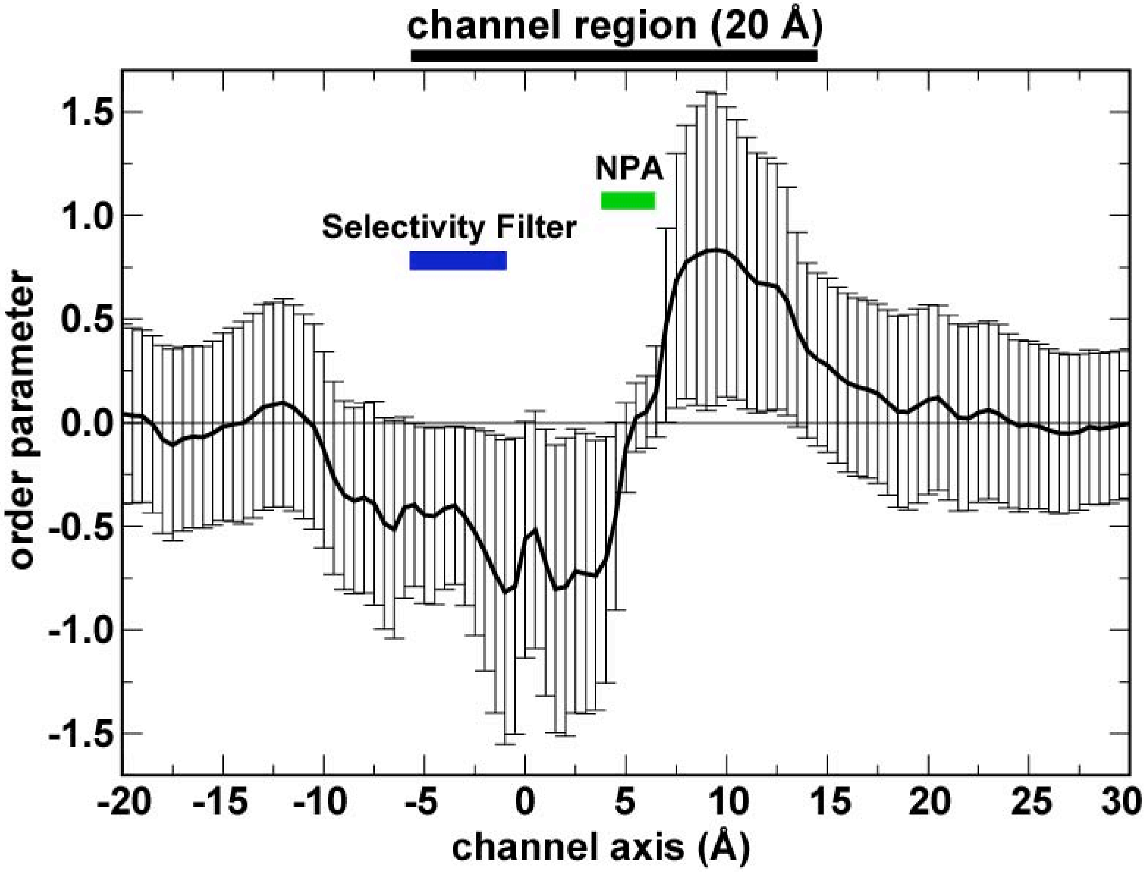


Water Bipolar Configuration in Aquaporins







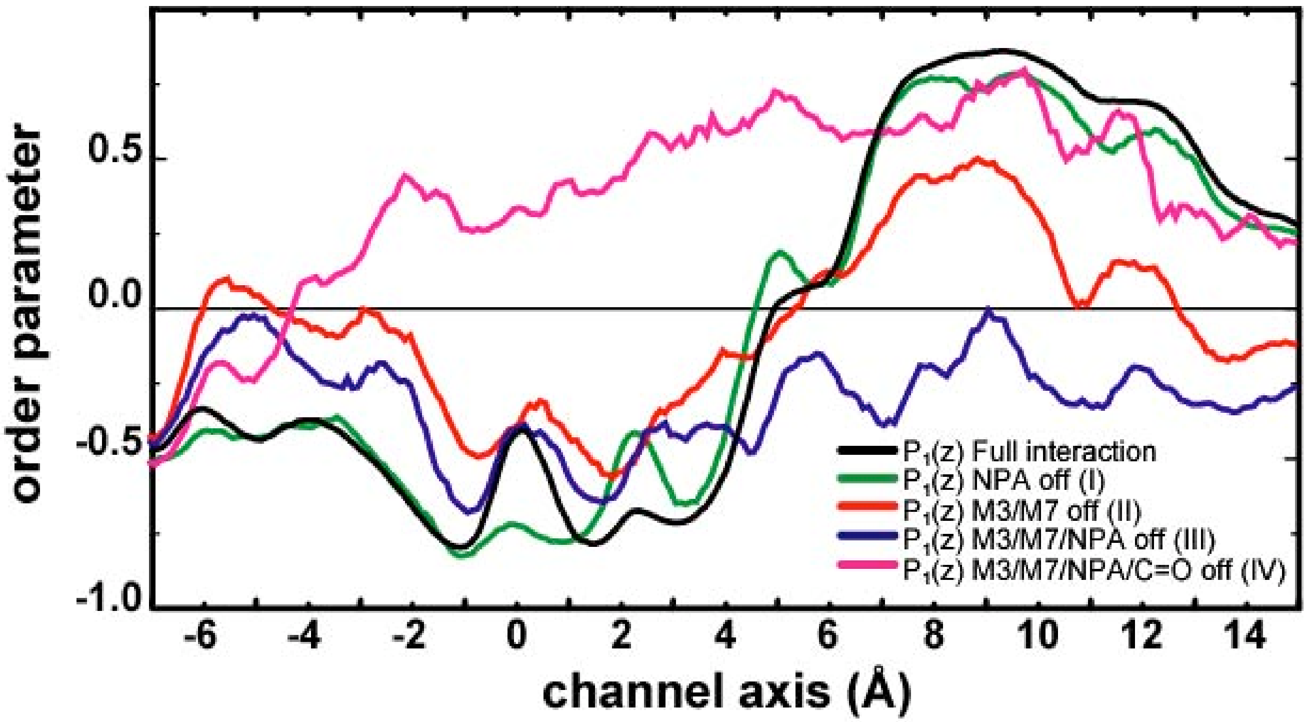

channel region (20 Å)
NPA
Selectivity Filter
order parameter
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-20
-15
-10
-5
0
5
10
15
20
25
30
channel axis (Å)

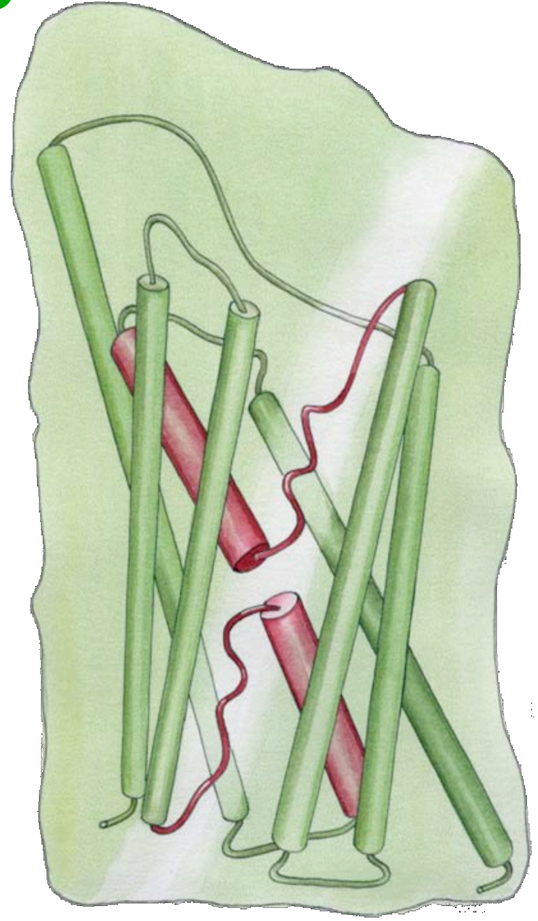
REMEMBER:

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!

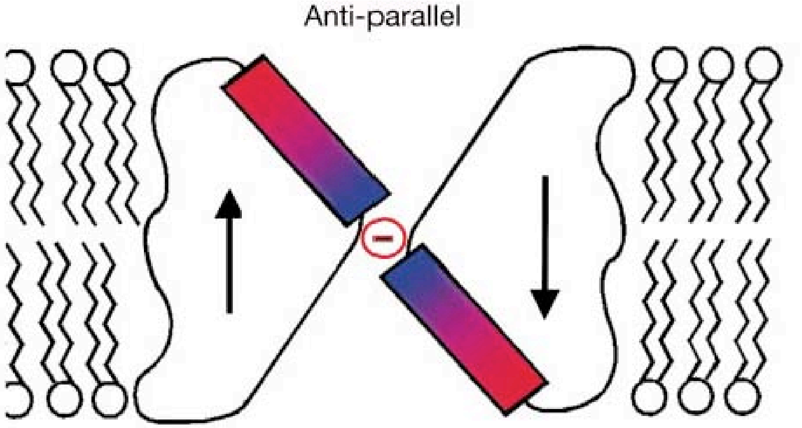
This is a powerful technique to test hypotheses developed during your simulations. Use it!

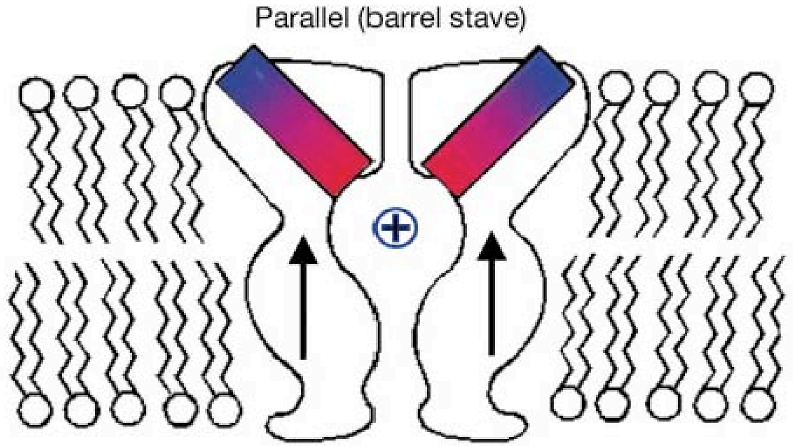
Electrostatic Stabilization of Water Bipolar Arrangement



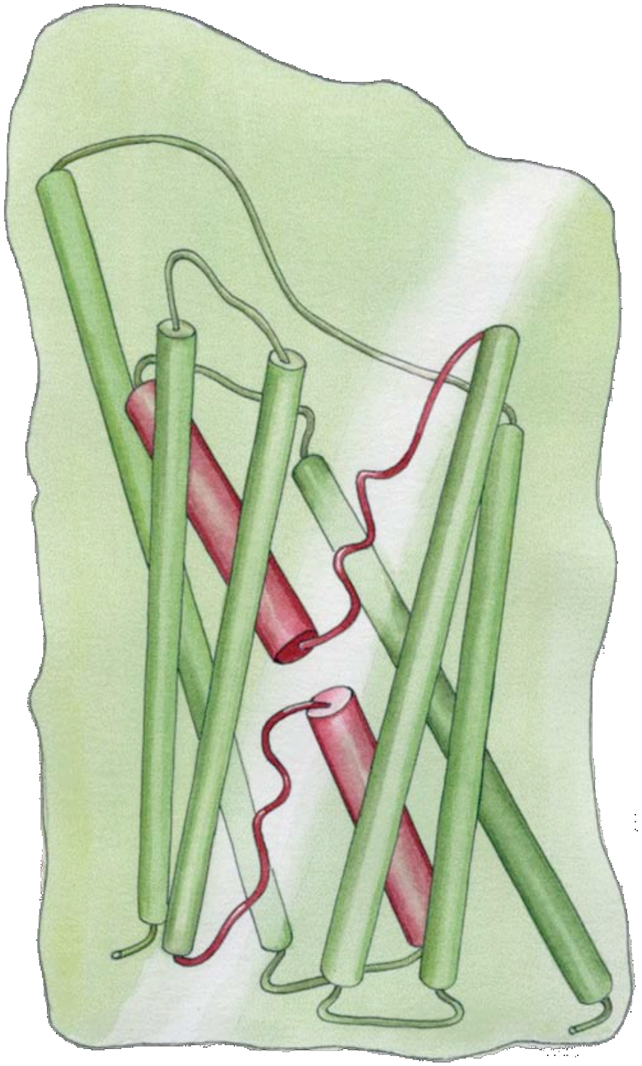


Cl^- channel



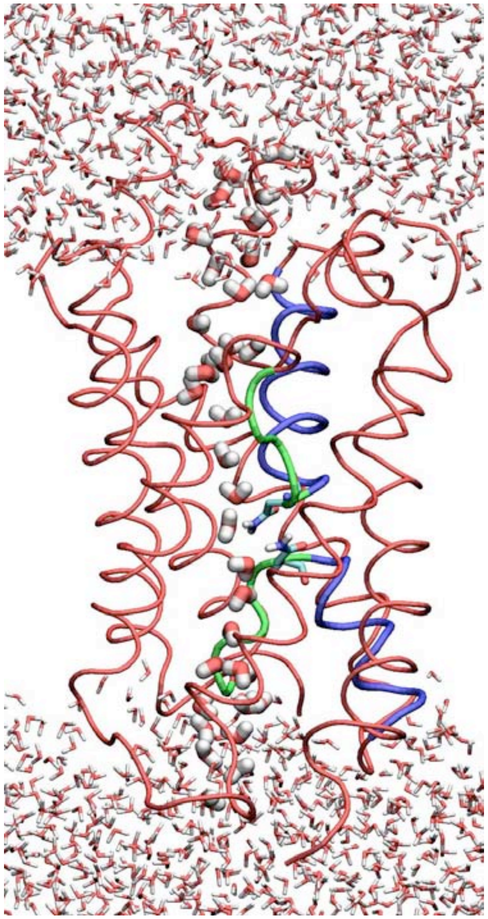


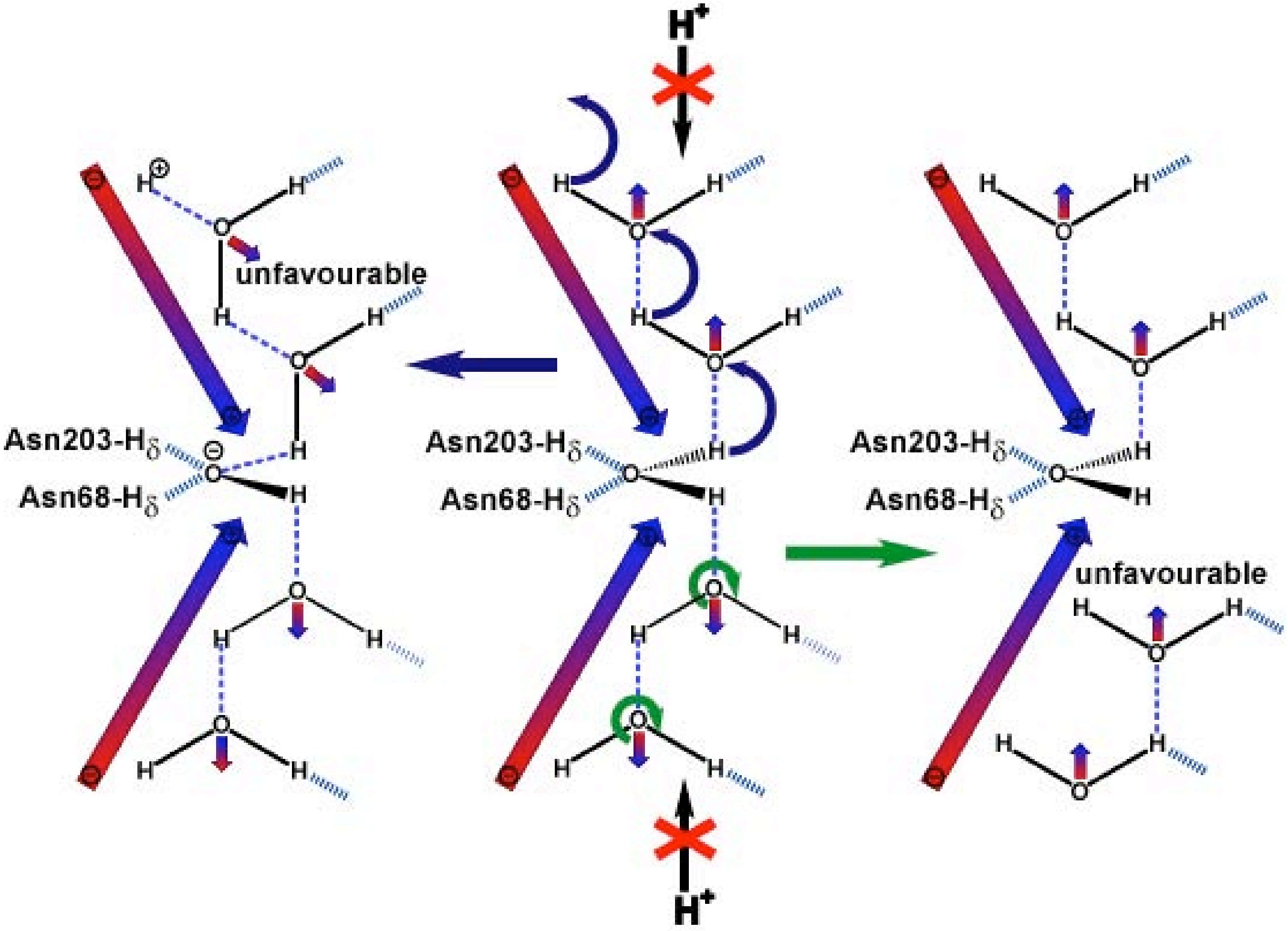
K^+ channel

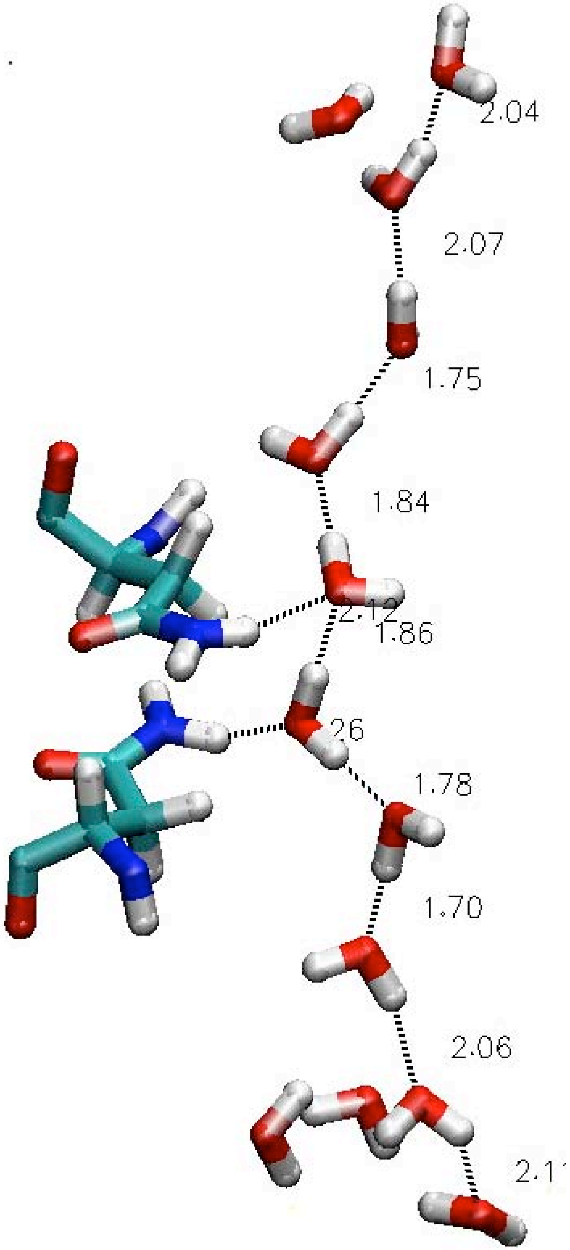


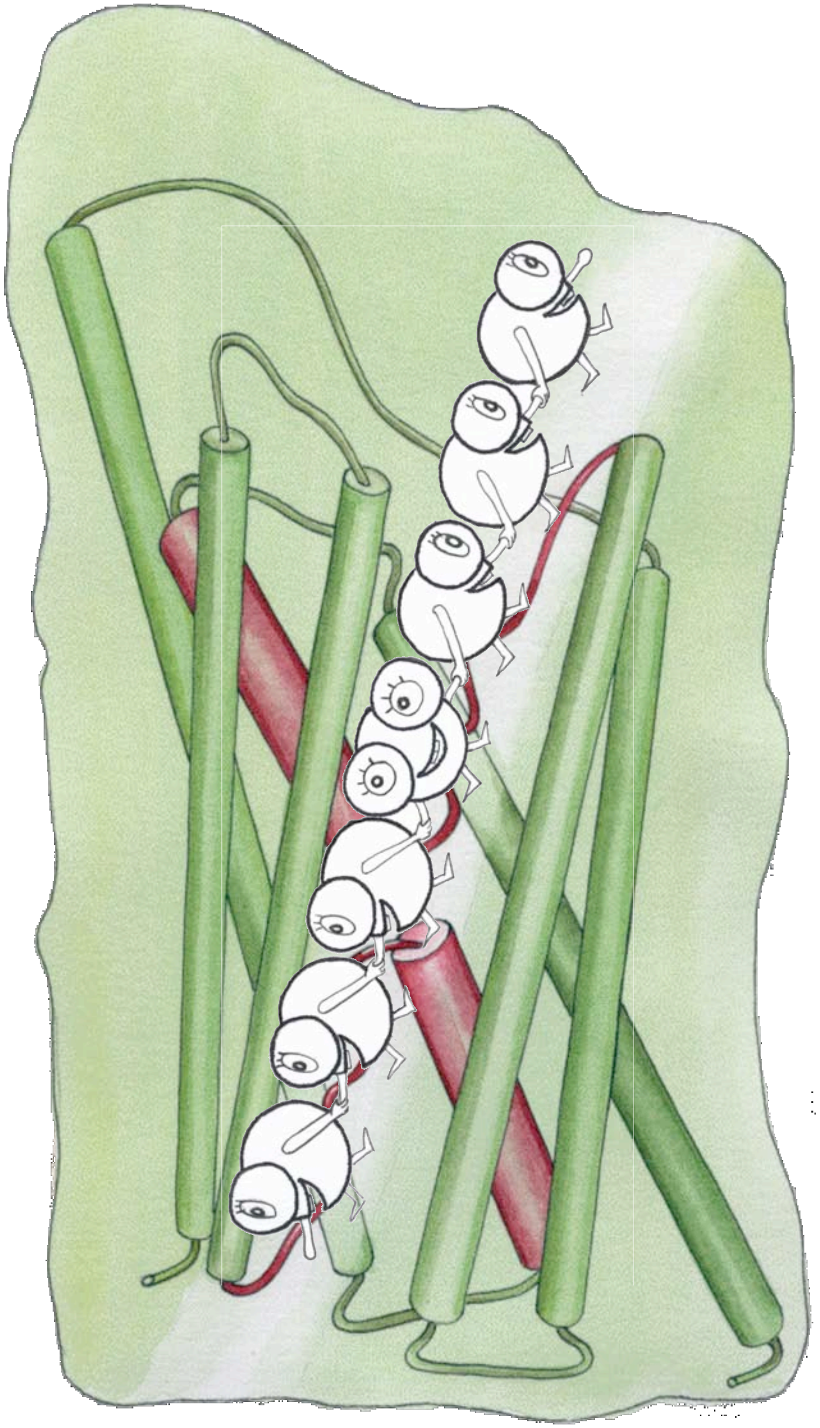
Aquaporins

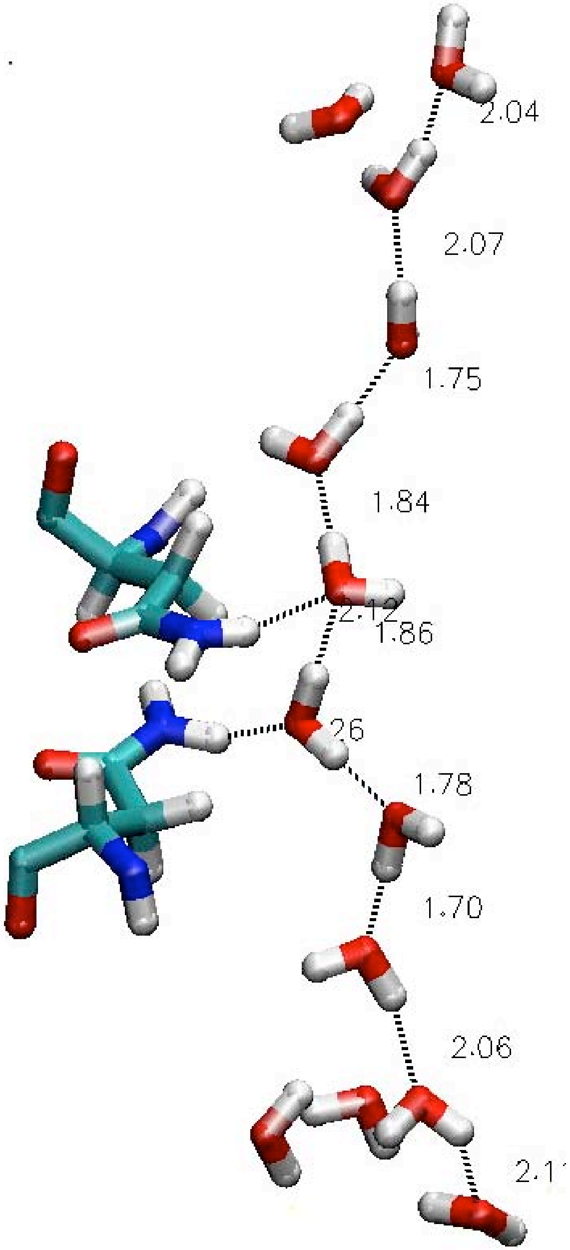
Proton Blocking by a Global Orientation Mechanism






2.04
2.07
1.75
1.84
2.12
1.86
26
1.78
1.70
2.06
2.1




2.04
2.07
1.75
1.84
2.12
1.86
26
1.78
1.70
2.06
2.1


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