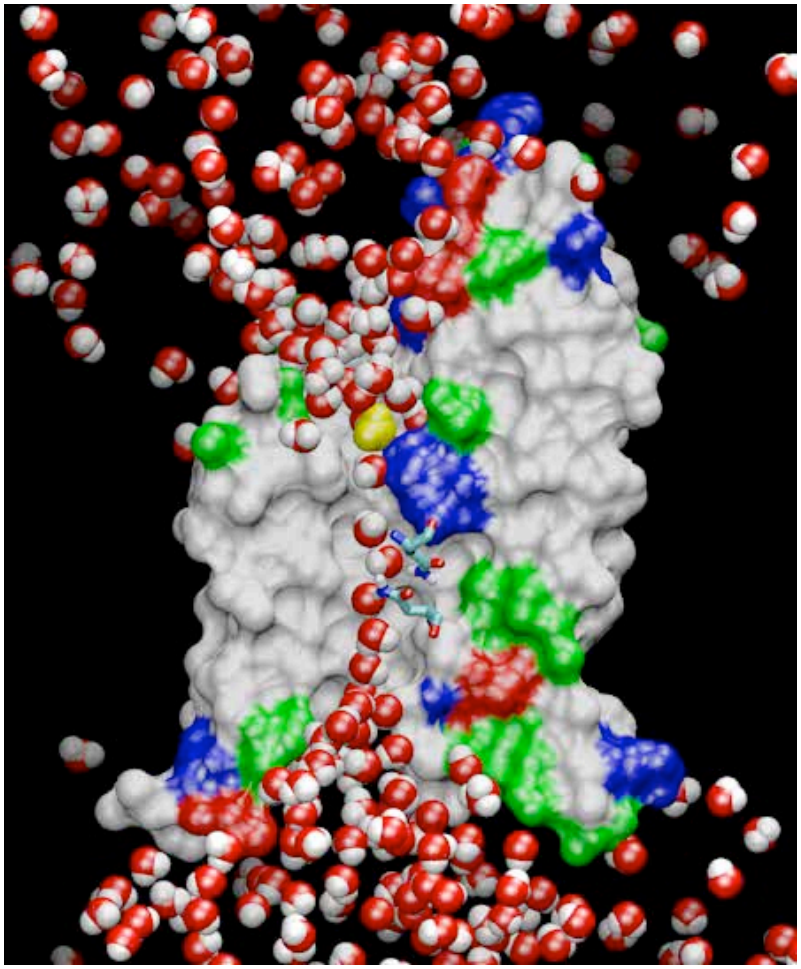
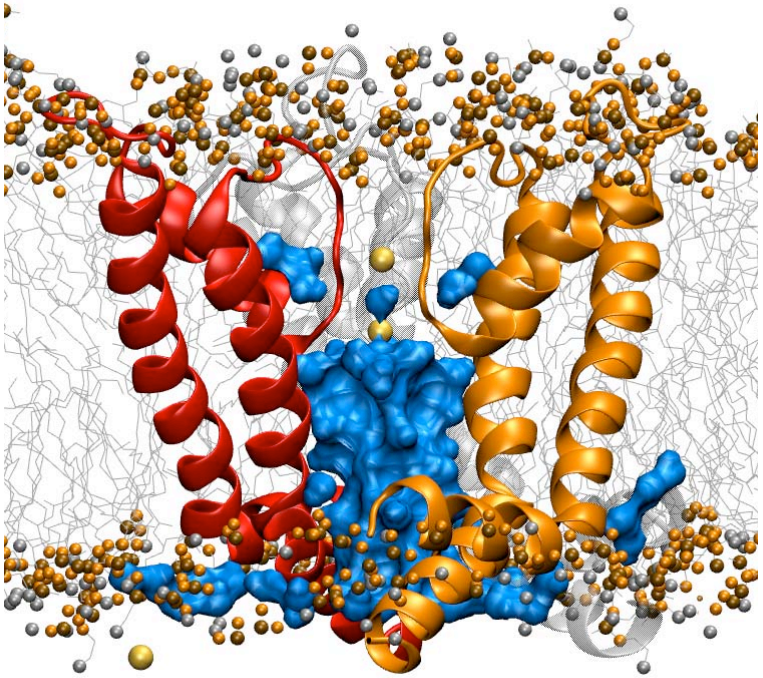
Modeling and Molecular Dynamics of Membrane Proteins



Emad Tajkhorshid

Department of Biochemistry, Center for Biophysics and Computational Biology, and Beckman Institute

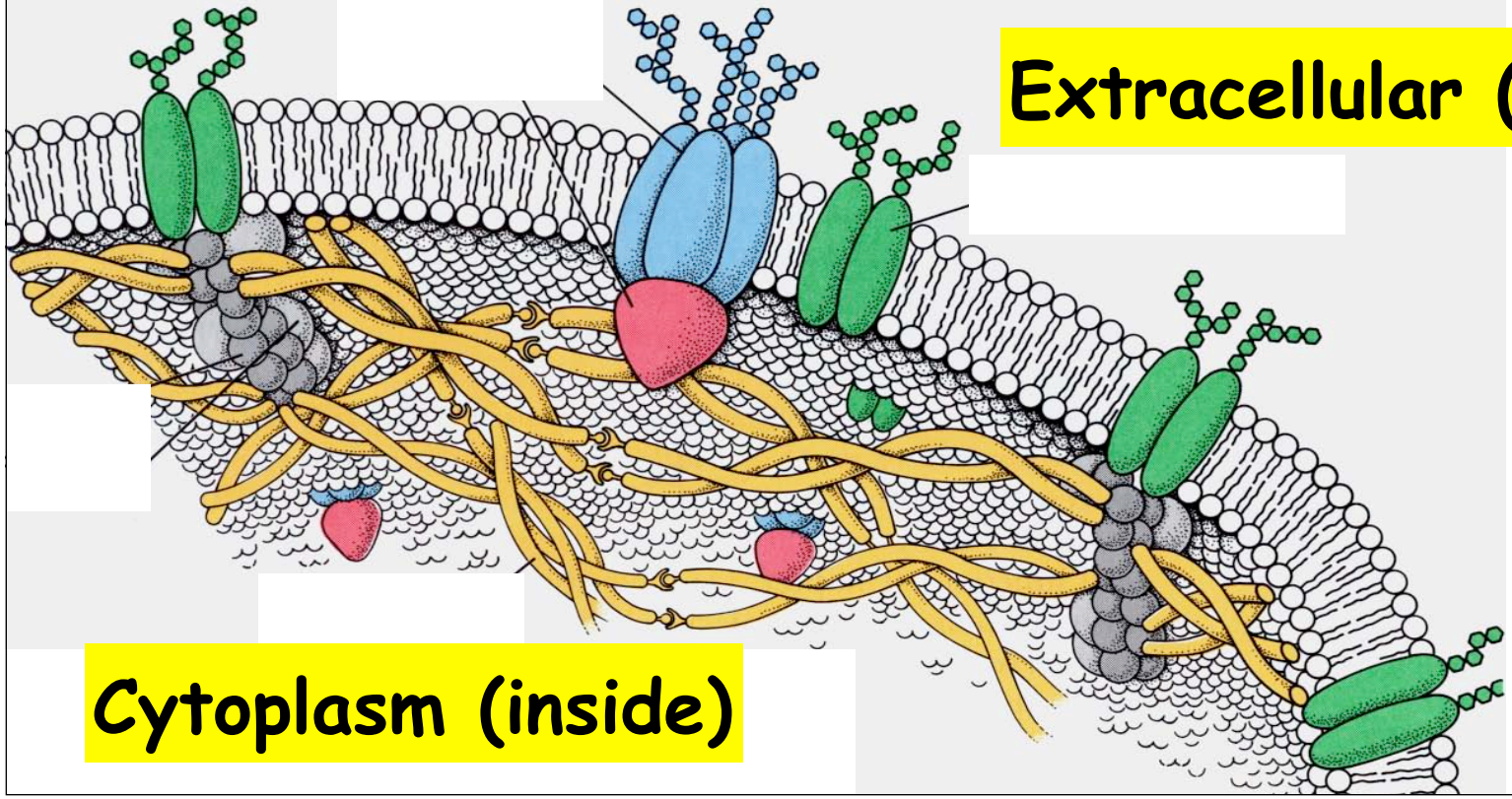
University of Illinois at Urbana-Champaign



Why Do Living Cells Need Membrane

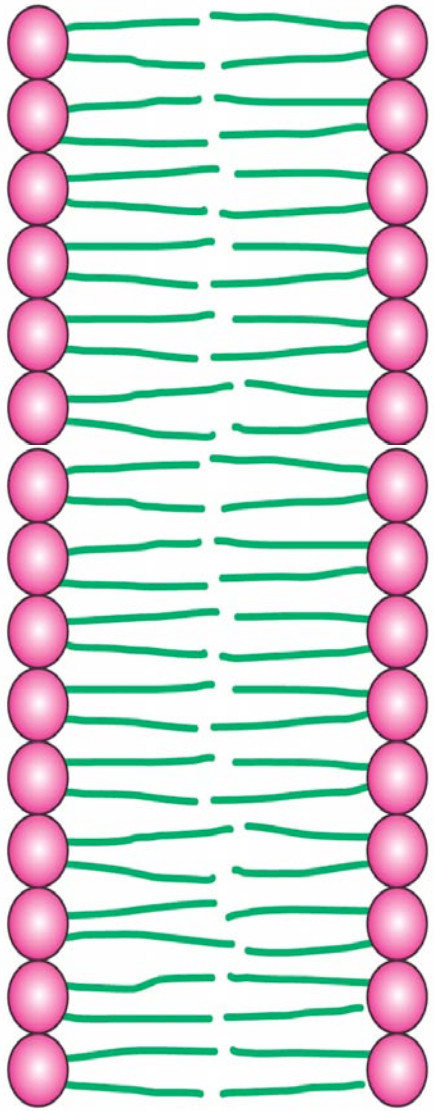
- Living cells also need to exchange materials and information with the outside world

… however, in a highly selective manner.

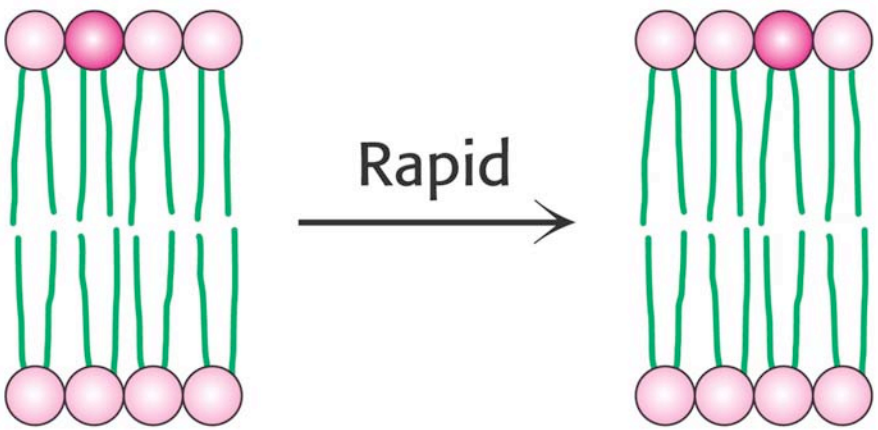


Phospholipid Bilayers Are Excellent Materials For Cell Membranes

- Hydrophobic interaction is the driving force
- Self-assembly in water
- Tendency to close on themselves
- Self-sealing (a hole is unfavorable)
- Extensive: up to millimeters



Lipid Diffusion in a Membrane



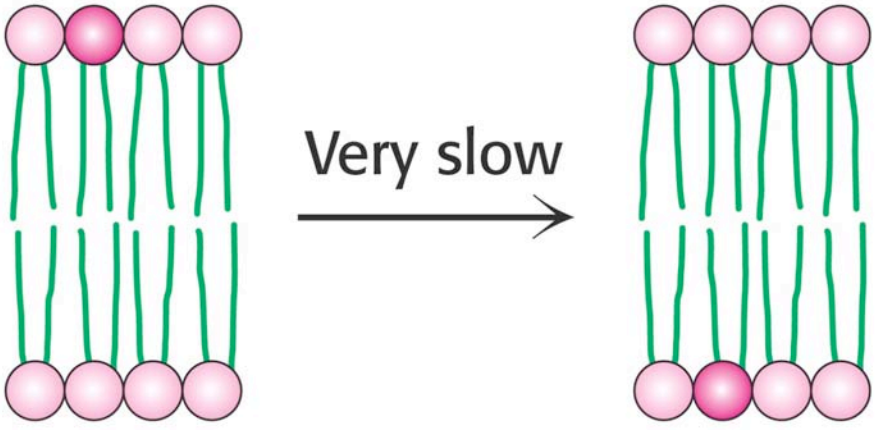
Lateral diffusion

$D_{lip} = 10^{-8}\ cm^2.s^{-1}$

(50 Å in ~ 5 x 10^{-6} s)

$D_{wat} = 2.5 \times 10^{-5}\ cm^2.s^{-1}$

Modeling mixed lipid bilayers!



Tranverse diffusion
(flip-flop)

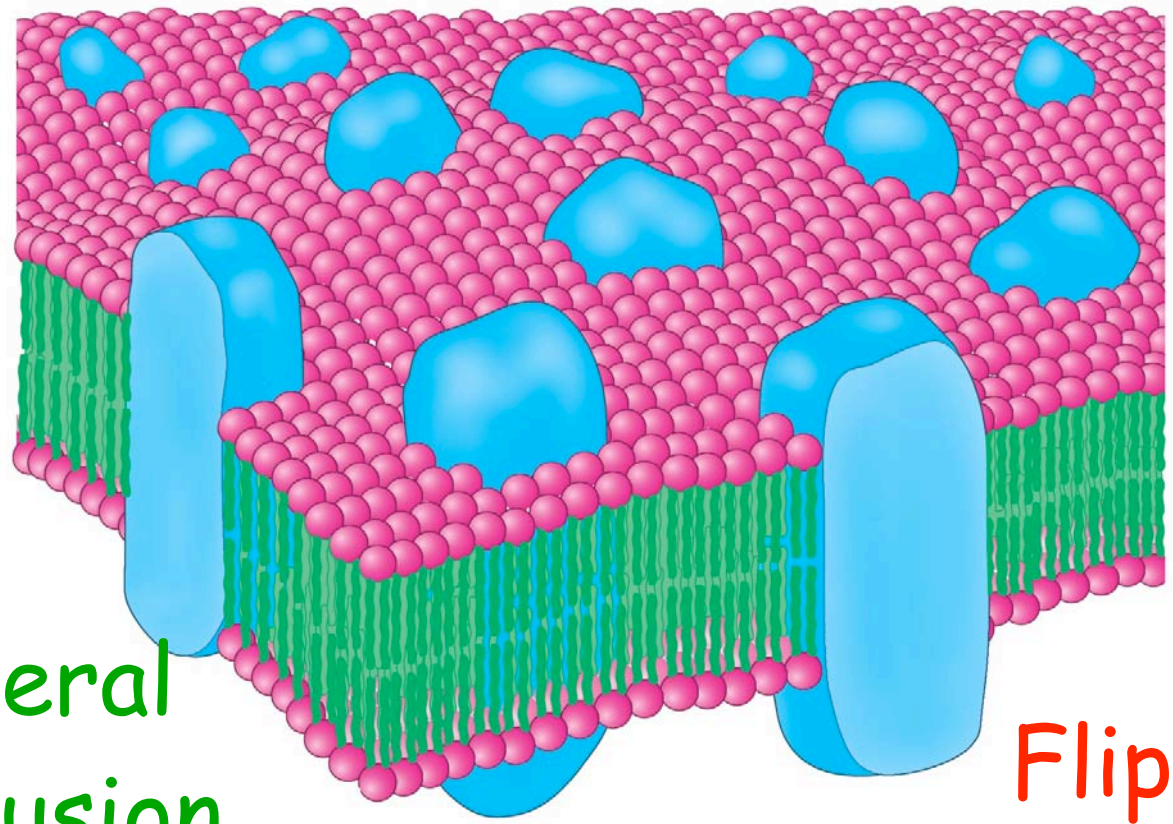
Once in several hours!

(~ 50 Å in ~ 10^4 s)

~9 orders of magnitude slower

ensuring bilayer asymmetry

Fluid Mosaic Model of Membrane



Lateral Diffusion Allowed

Flip-flop Forbidden

Ensuring the conservation of membrane asymmetric structure

Technical difficulties in Simulations of Biological Membranes

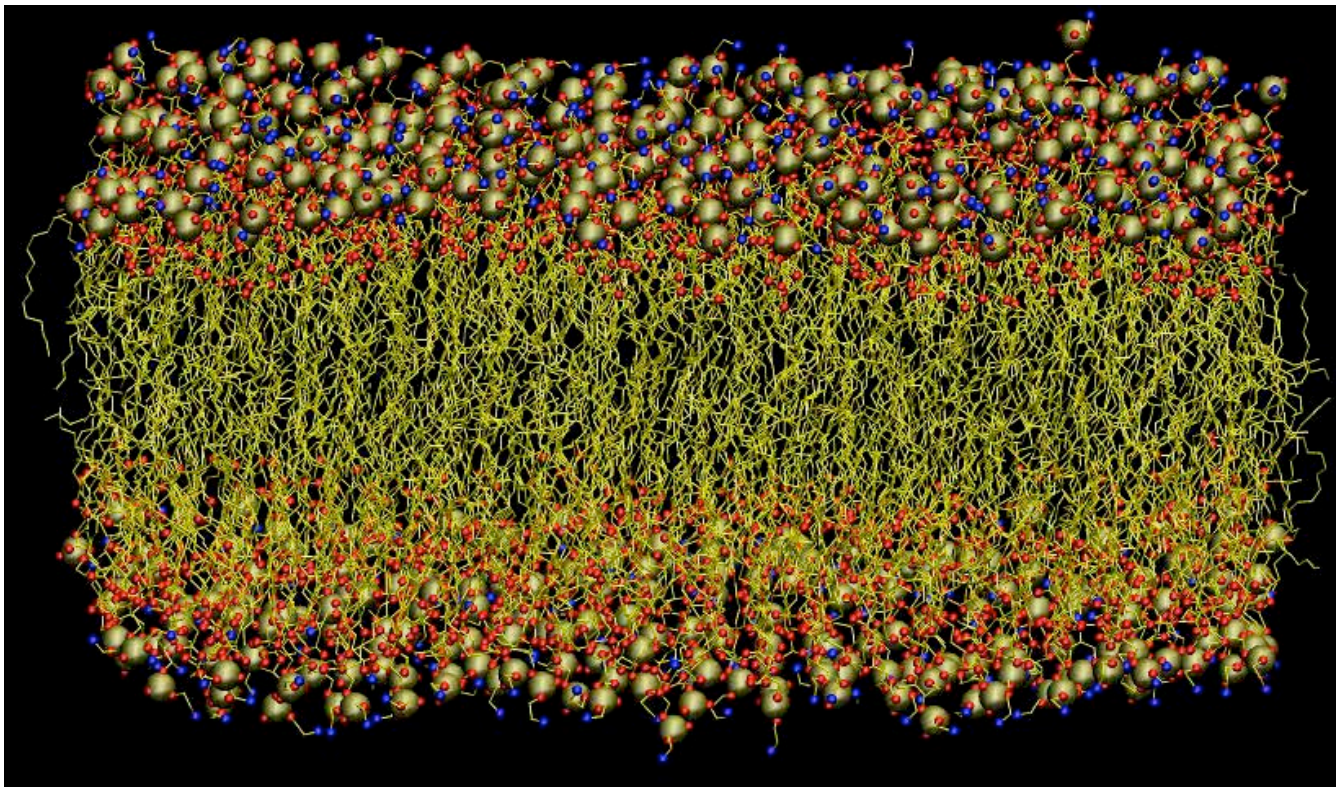
- Time scale
- Heterogeneity of biological membranes ☹

60 x 60 Å

Pure POPE

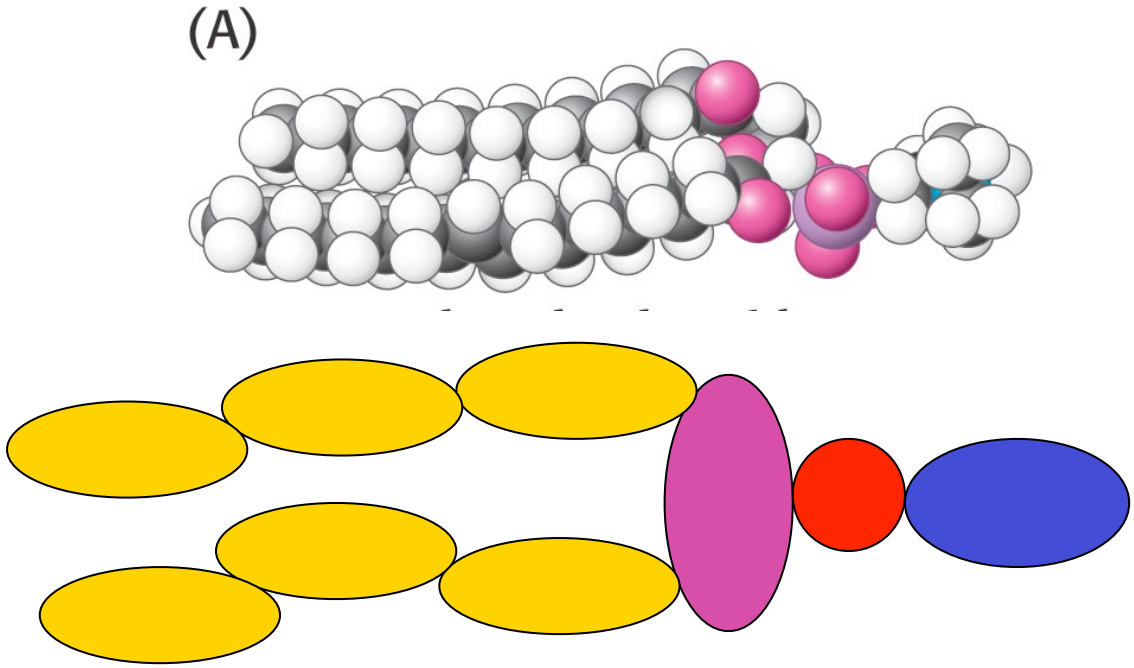
5 ns

~100,000 atoms



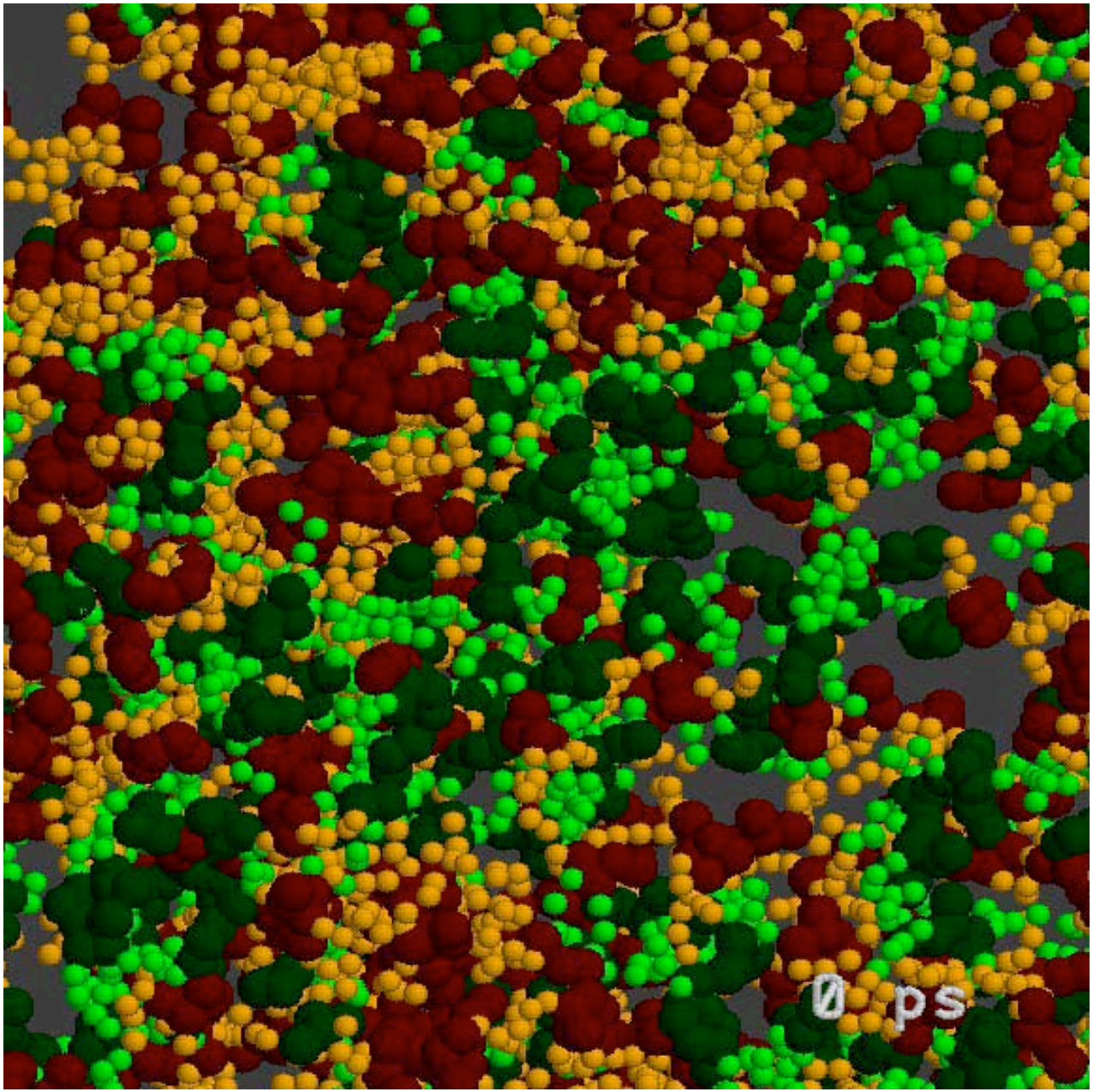
Coarse-grained modeling of lipids

150 particles



9 particles!

Also, increasing the time step by orders of magnitude.



by: J. Siewert-Jan Marrink and Alan E. Mark, University of Groningen, The Netherlands

Protein/Lipid ratio

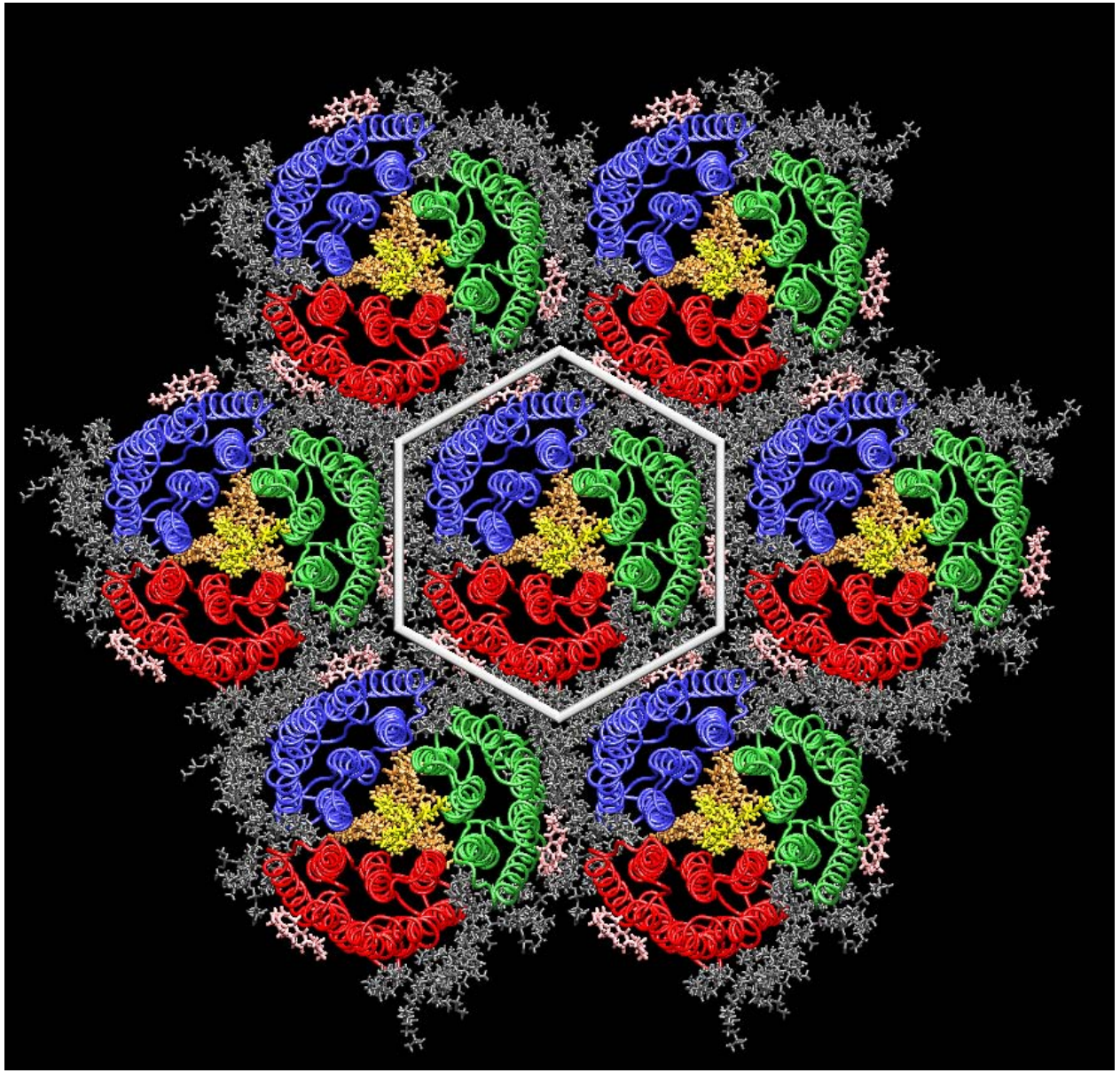
- Pure lipid: insulation (neuronal cells)
- Other membranes: on average 50%
- Energy transduction membranes (75%)

  Membranes of mitocondria and chloroplast

  Purple membrane of halobacteria

- Different functions = different protein composition

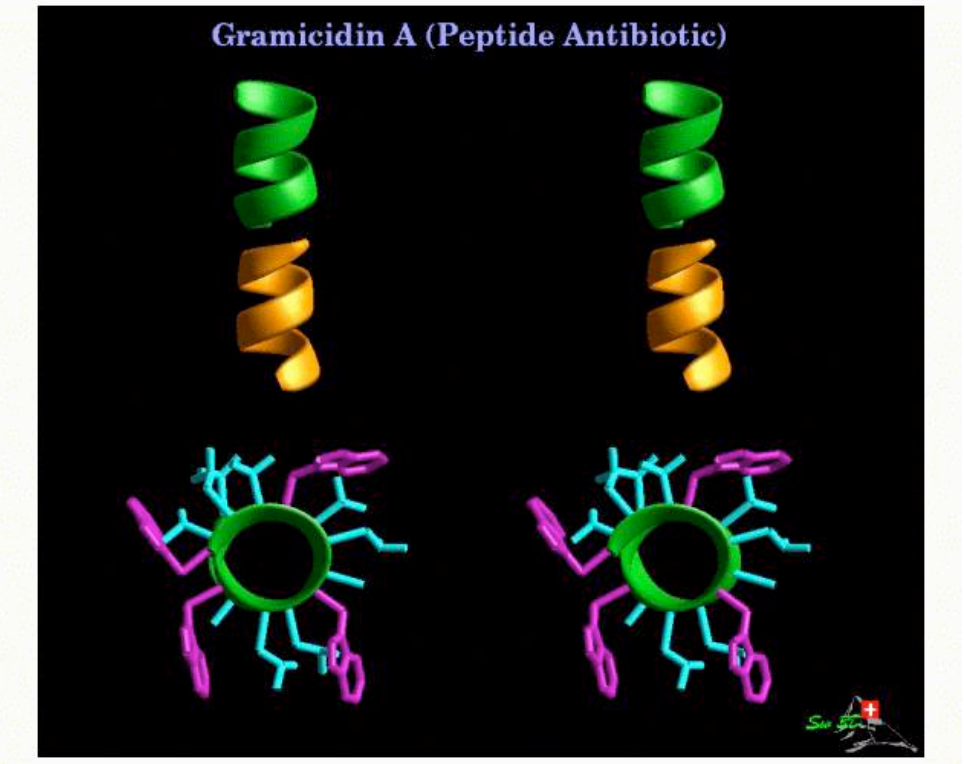
Protein / Lipid Composition

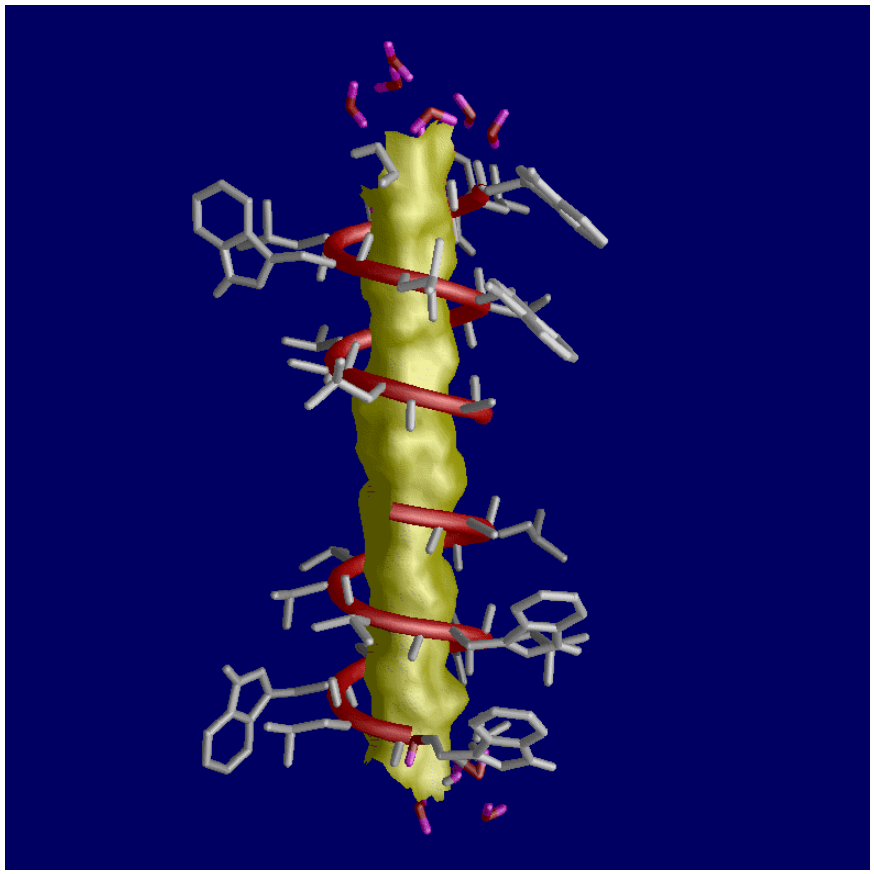


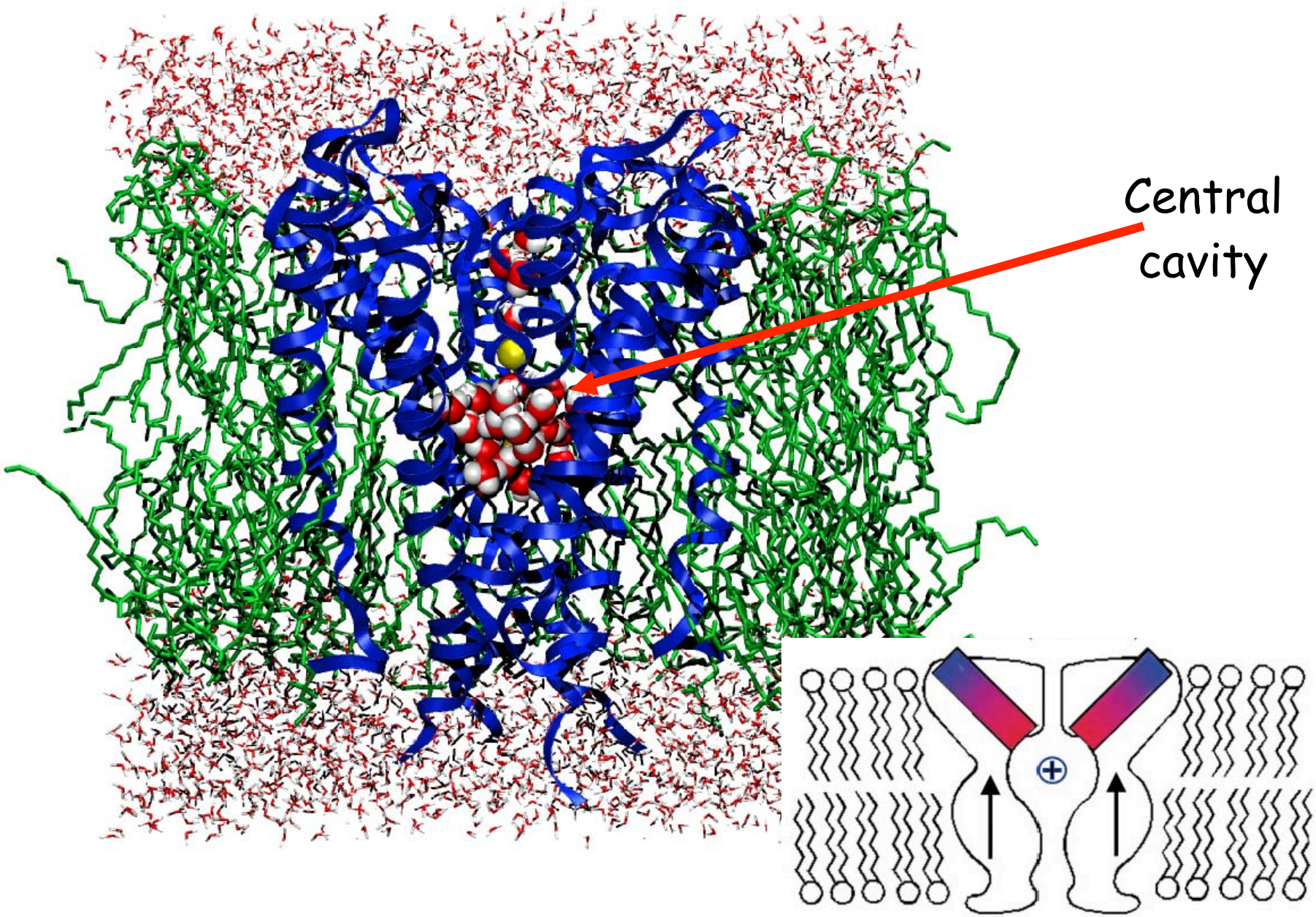
The purple membrane of halobacteria

Gramicidin A

Might be very sensitive to the lipid head group electrostatic and membrane potential





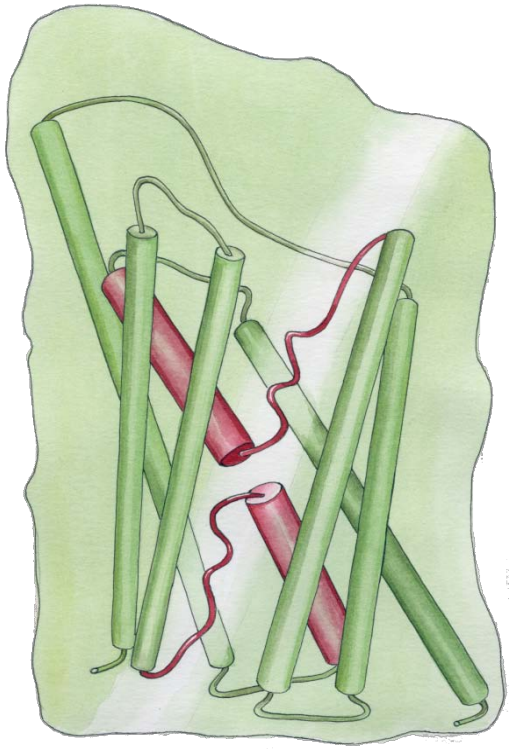

Central
cavity

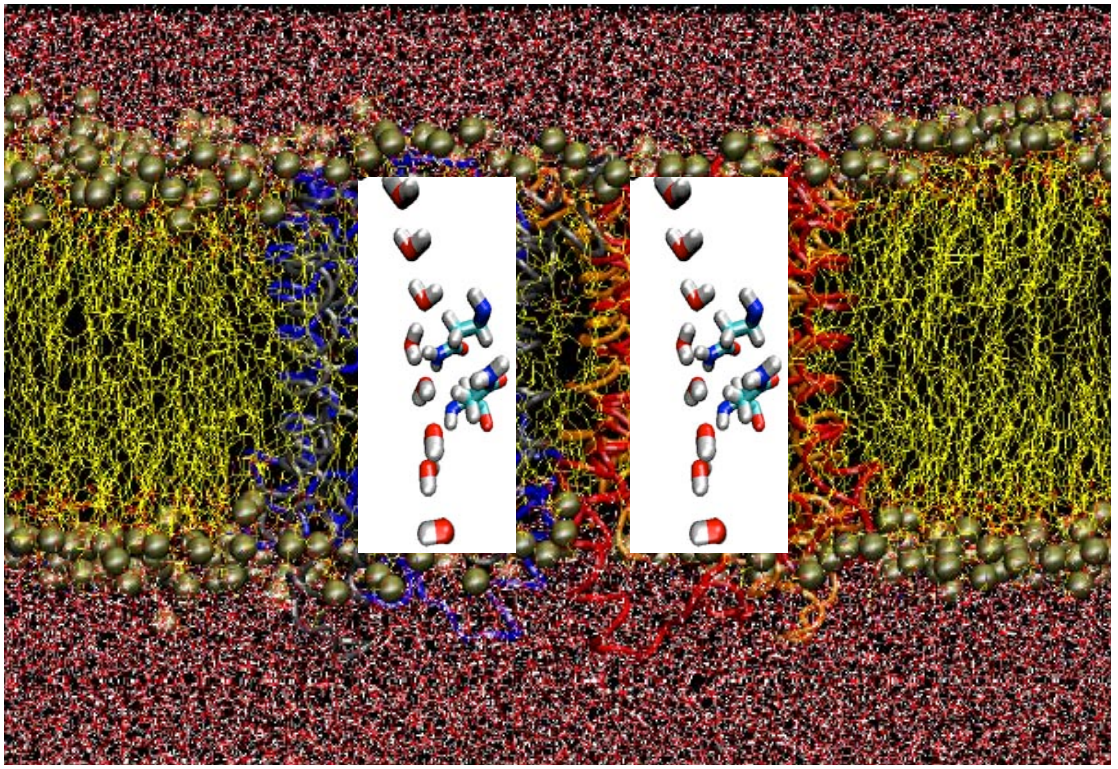
Analysis of Molecular Dynamics Simulations of Biomolecules

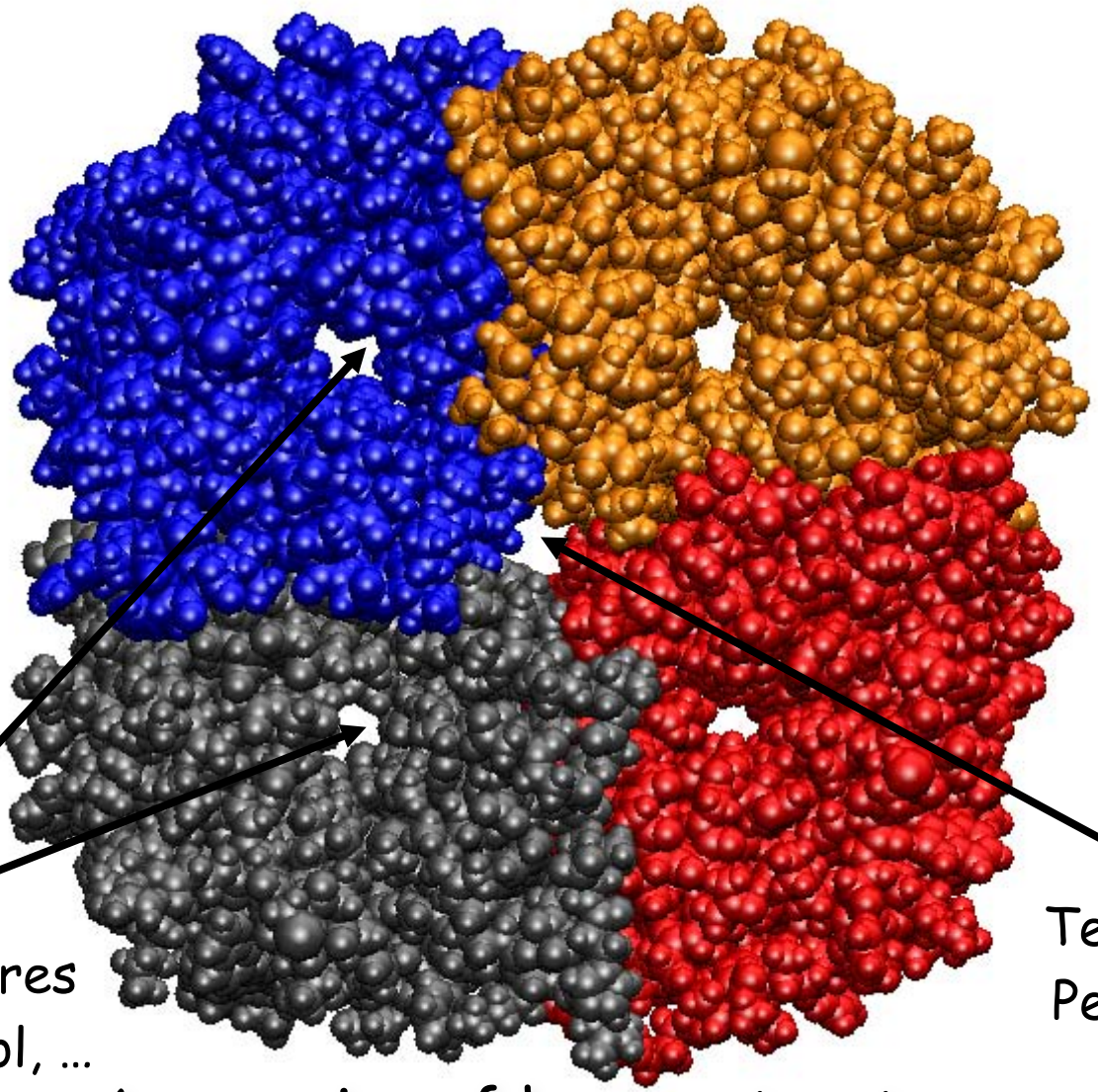
- A very complicated arrangement of hundreds of groups interacting with each other
- Where to start to look at?
- What to analyze?
- How much can we learn from simulations?

It is very important to get acquainted with your system

Aquaporins







Monomeric pores
Water, glycerol, …

Tetrameric pore
Perhaps ions???

Aquaporins of known structure:

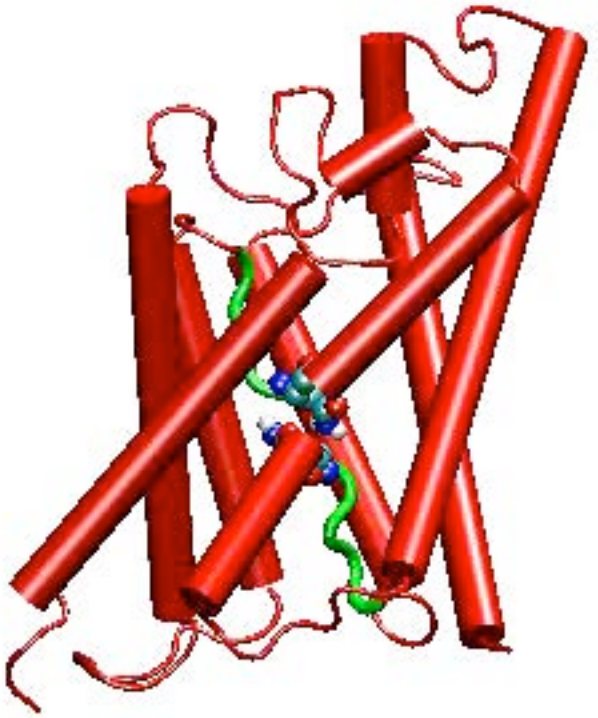
GlpF – E. coli glycerol channel (aquaglycerolporin)

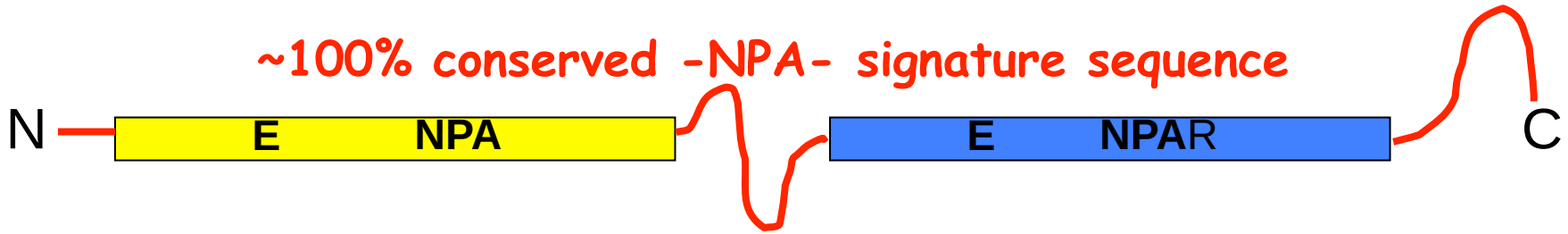
AQP1 – Mammalian aquaporin-1 (pure water channel)

AqpZ and AQP0 (2004)

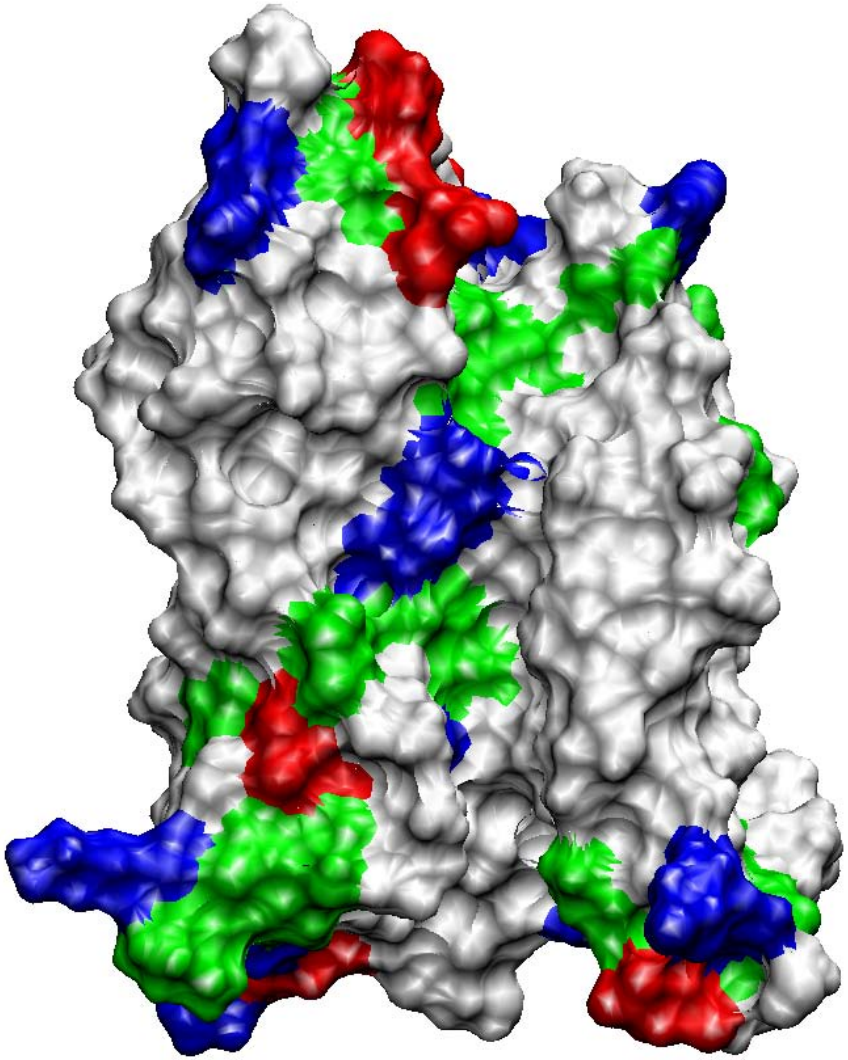
Functionally Important Features

- 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



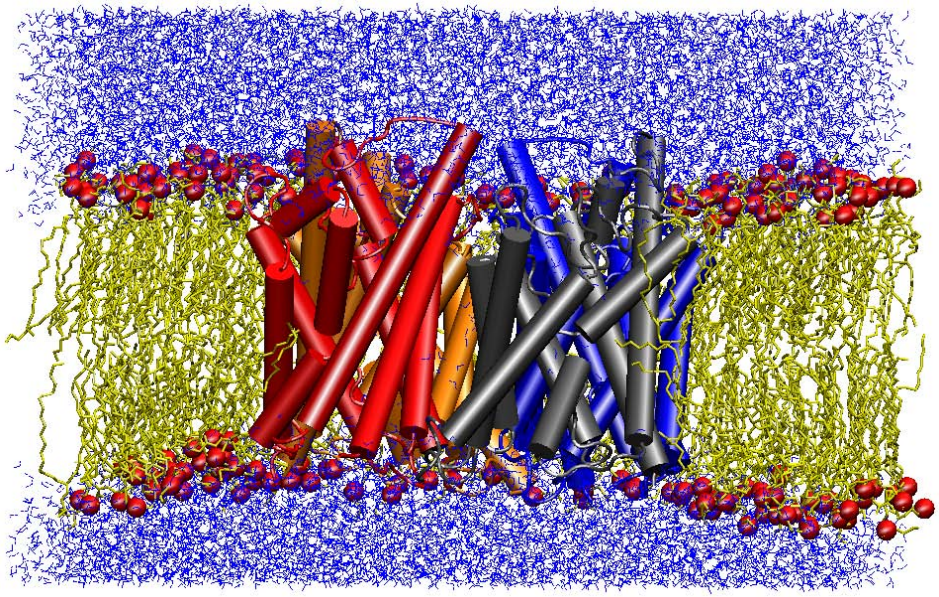


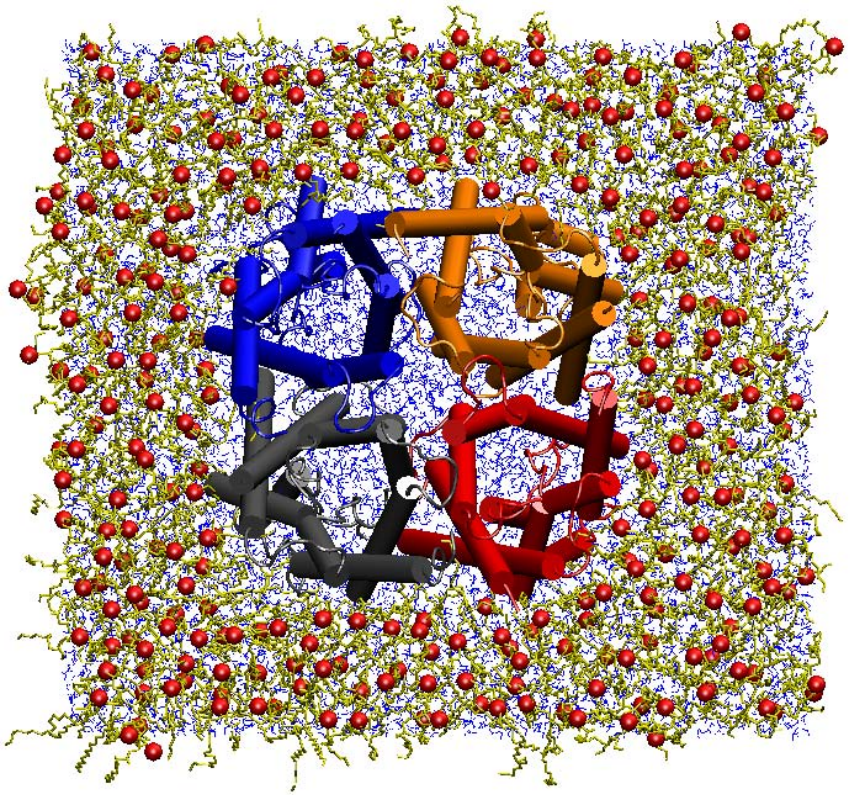
A Semi-hydrophobic channel



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

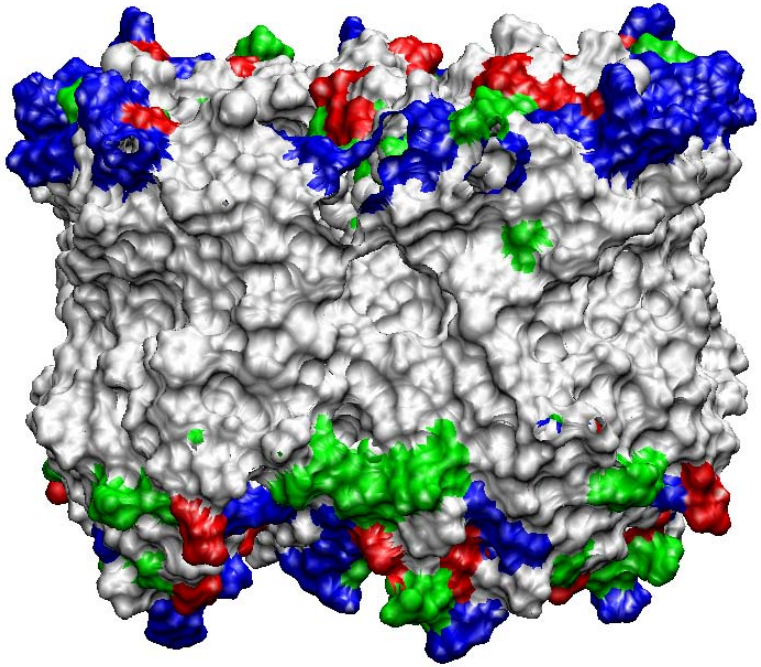
1ns equilibration, 4ns production

10 days /ns – 32-proc Linux cluster

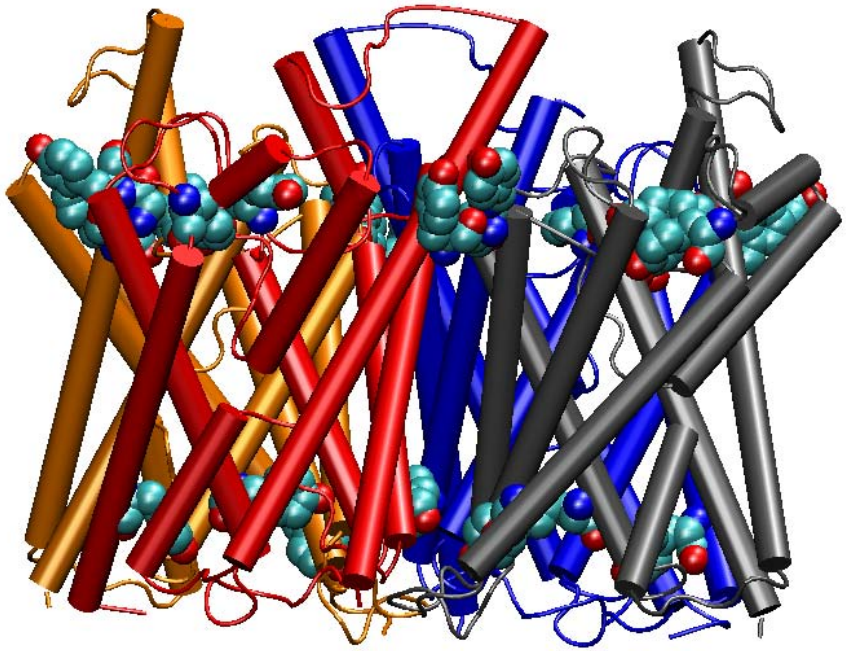
3.5 days/ns - 128 O2000 CPUs

0.35 days/ns – 512 LeMieux CPUs

Protein Embedding in Membrane



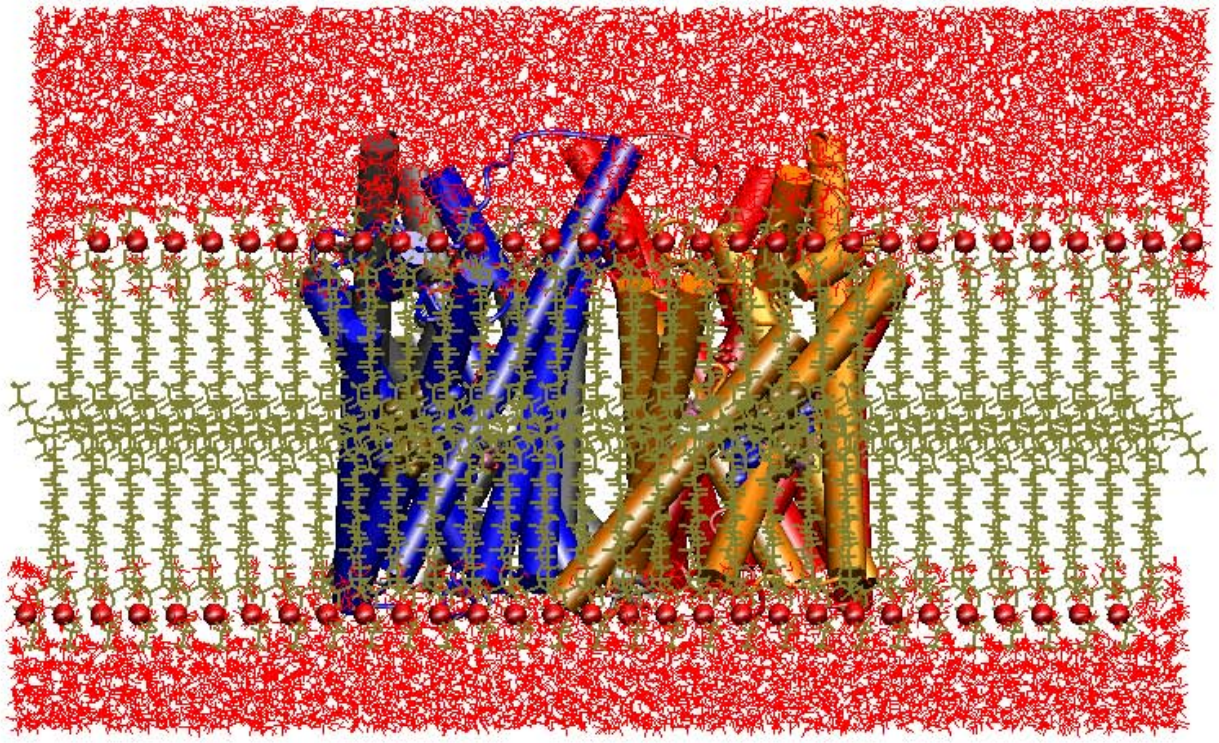
Hydrophobic surface
of the protein

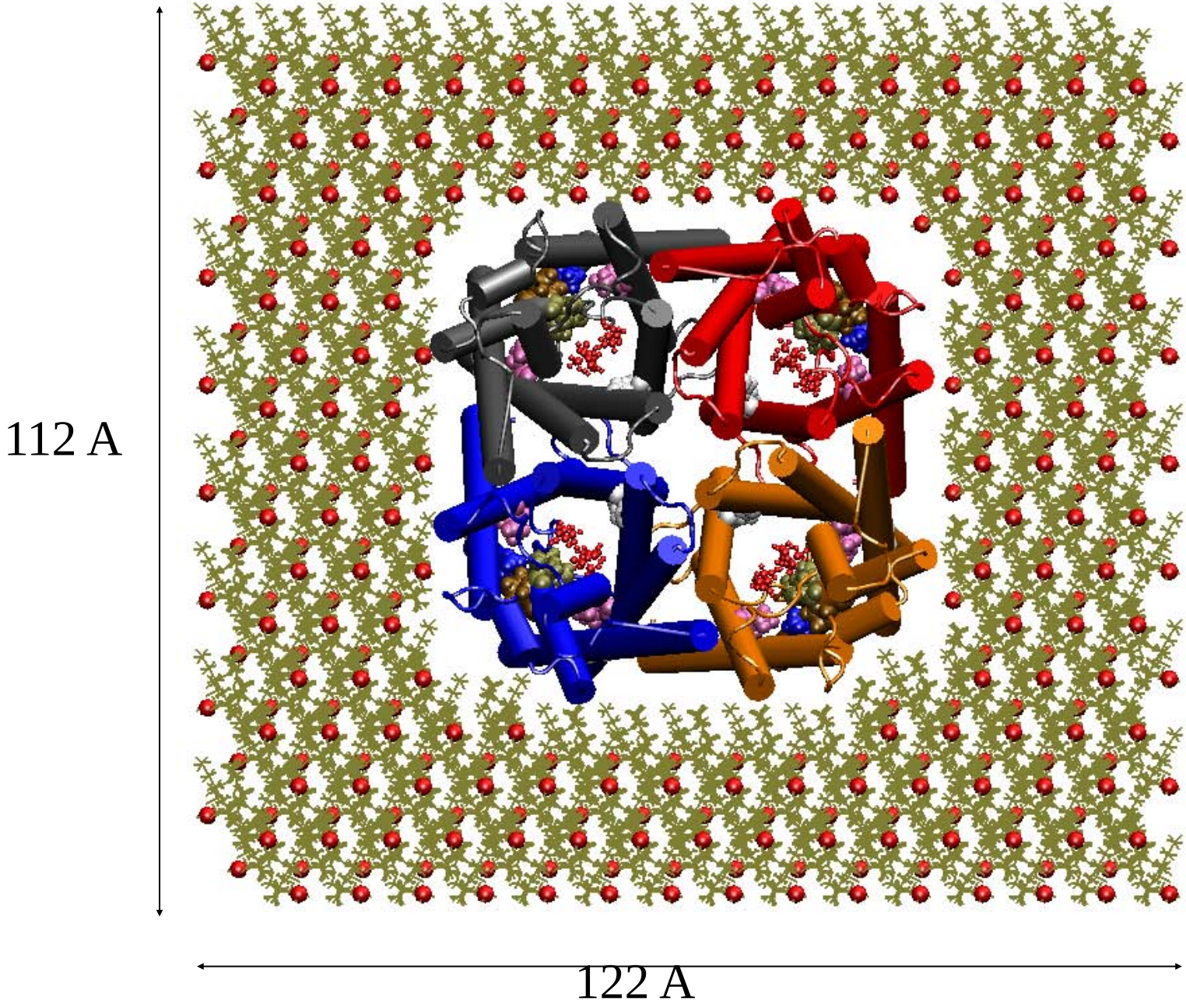


Ring of
Tyr and Trp

Embedding GlpF in Membrane

77 A




112 A
122 A

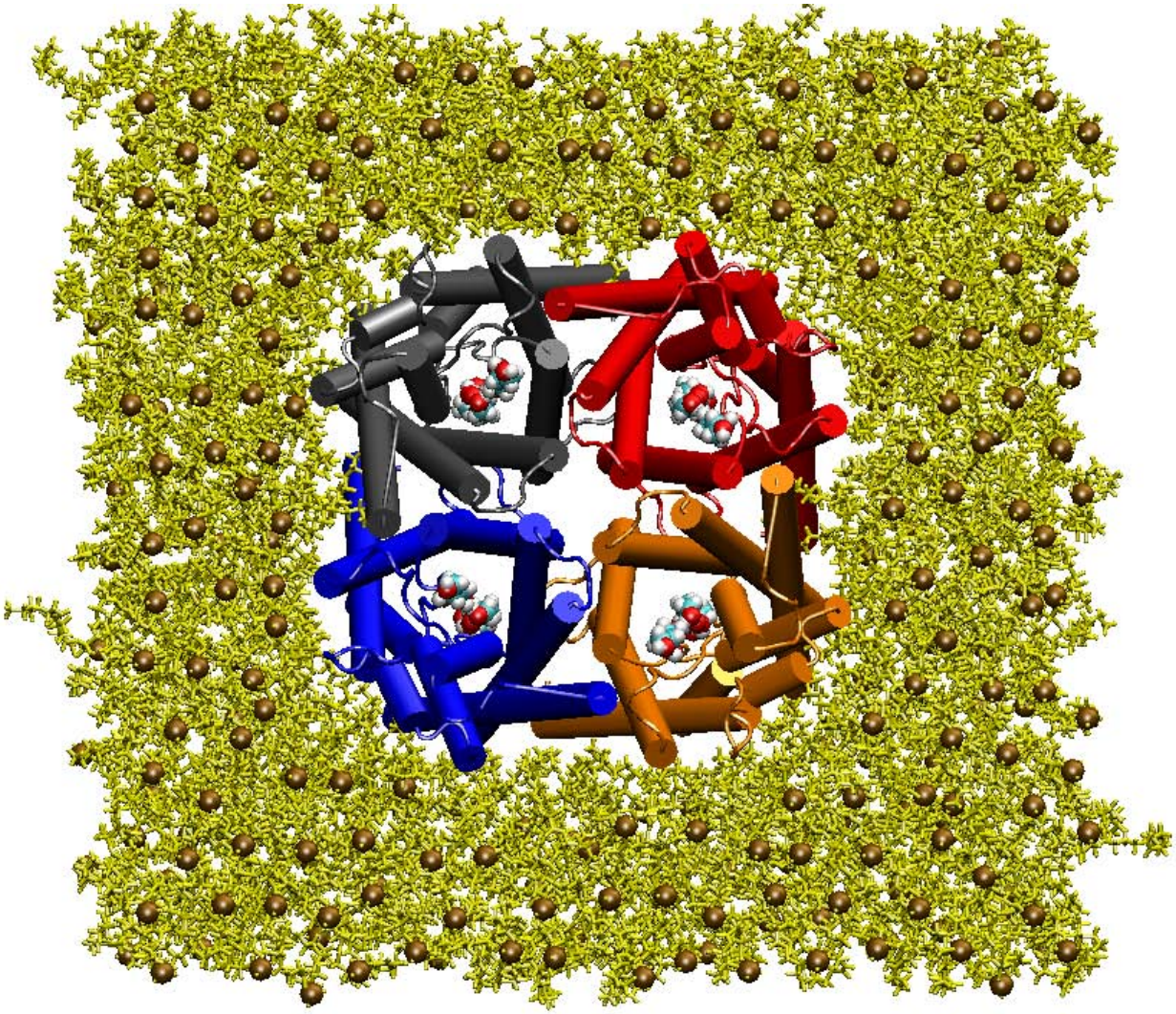
A Recipe for Membrane Protein Simulations

- Align the protein along the z-axis (membrane normal): OPM, Orient.
- Decide on the lipid type and generate a large enough patch (MEMBRANE plugin in VMD, other sources). Size, area/lipid, shrinking.
- Overlay the protein with a hydrated lipid bilayer. Adjust the depth/ height to maximize hydrophobic overlap and matching of aromatic side chains (**Trp/Tyr**) with the interfacial region
- Remove lipids/water that overlap with the protein. Better to keep as many lipids as you can, so try to remove clashes if they are not too many by playing with the lipids. Add more water and ions to the two sides of the membrane (SOLVATE / AUTOIONIZE in VMD)
- **Constrain** (not **FIX**) the protein (we are still modeling, let's preserve the crystal structure; fix the lipid head groups and water/ion and **minimize/simulate** the lipid tails using a short simulation.

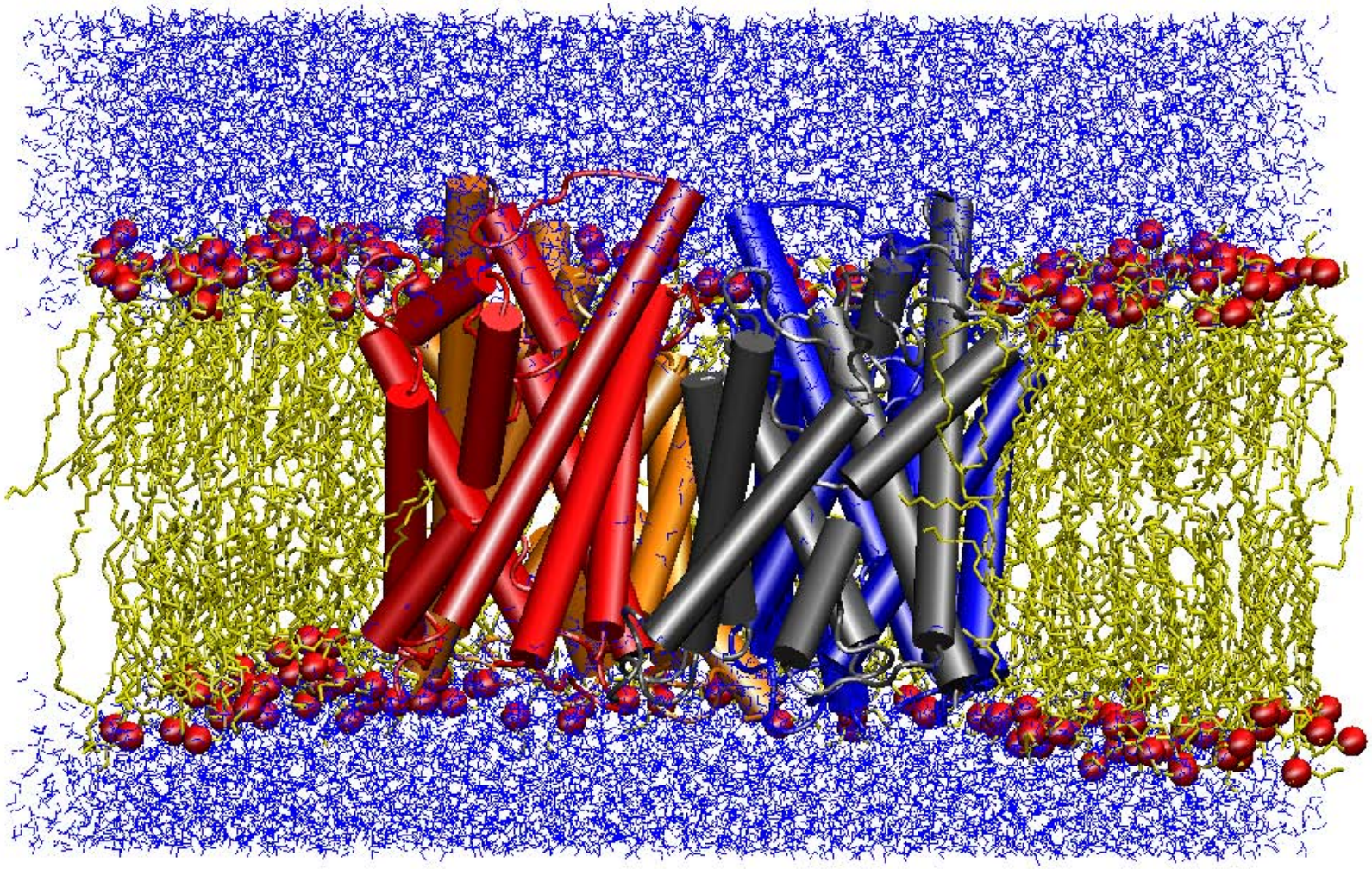
A Recipe for Membrane Protein Simulations

- Continue to constrain the protein (heavy atoms), but release everything else; minimize/simulate using a short "constant-pressure" MD (NPT) to "pack" lipids and water against the protein and fill the gaps introduced after removal of protein-overlapping lipids.
- Watch water molecules; They normally stay out of the hydrophobic cleft. If necessary apply constraints to prevent them from penetrating into the open cleft between the lipids and the protein.
- Monitor the volume of your simulation box until the steep phase of the volume change is complete (.xst and .xsc files). Do not run the system for too long during this phase (over-shrinking; sometimes difficult to judge).
- Now release the protein, minimize the whole system, and start another short NPT simulation of the whole system.
- Switch to an NP_nAT or an NVT simulation, when the system reaches a **stable** volume. Using the new CHARMM force field, you can stay with NPT.

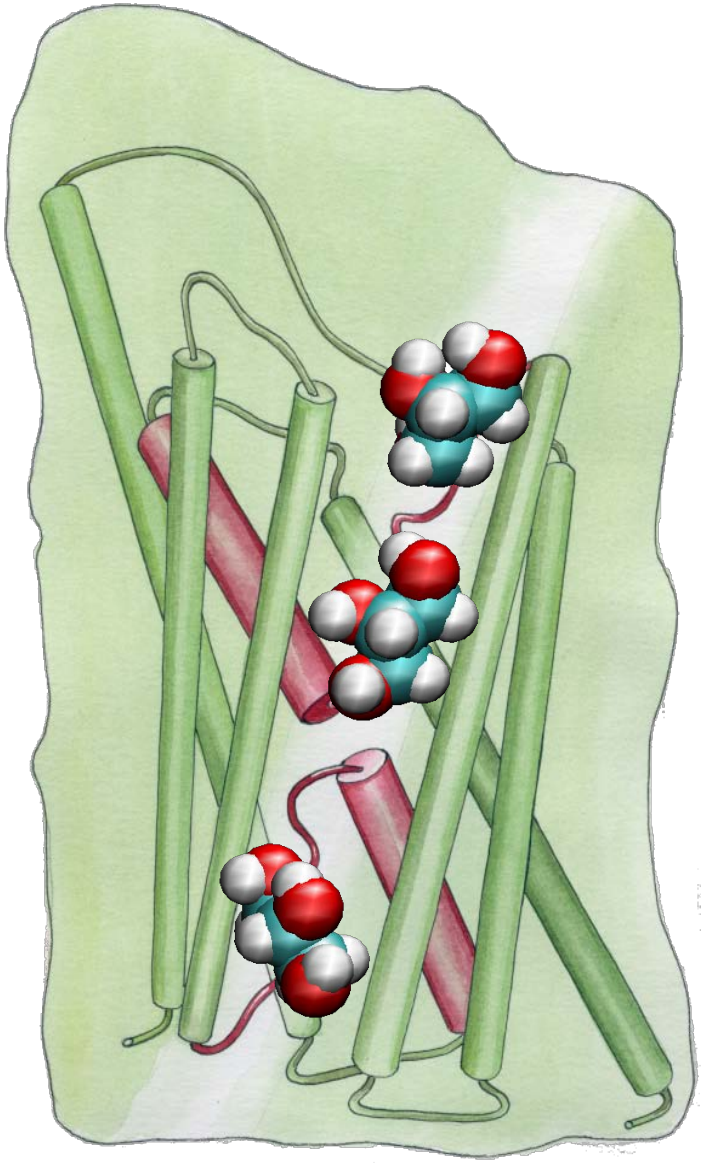
Lipid-Protein Packing During the Initial NpT Simulation

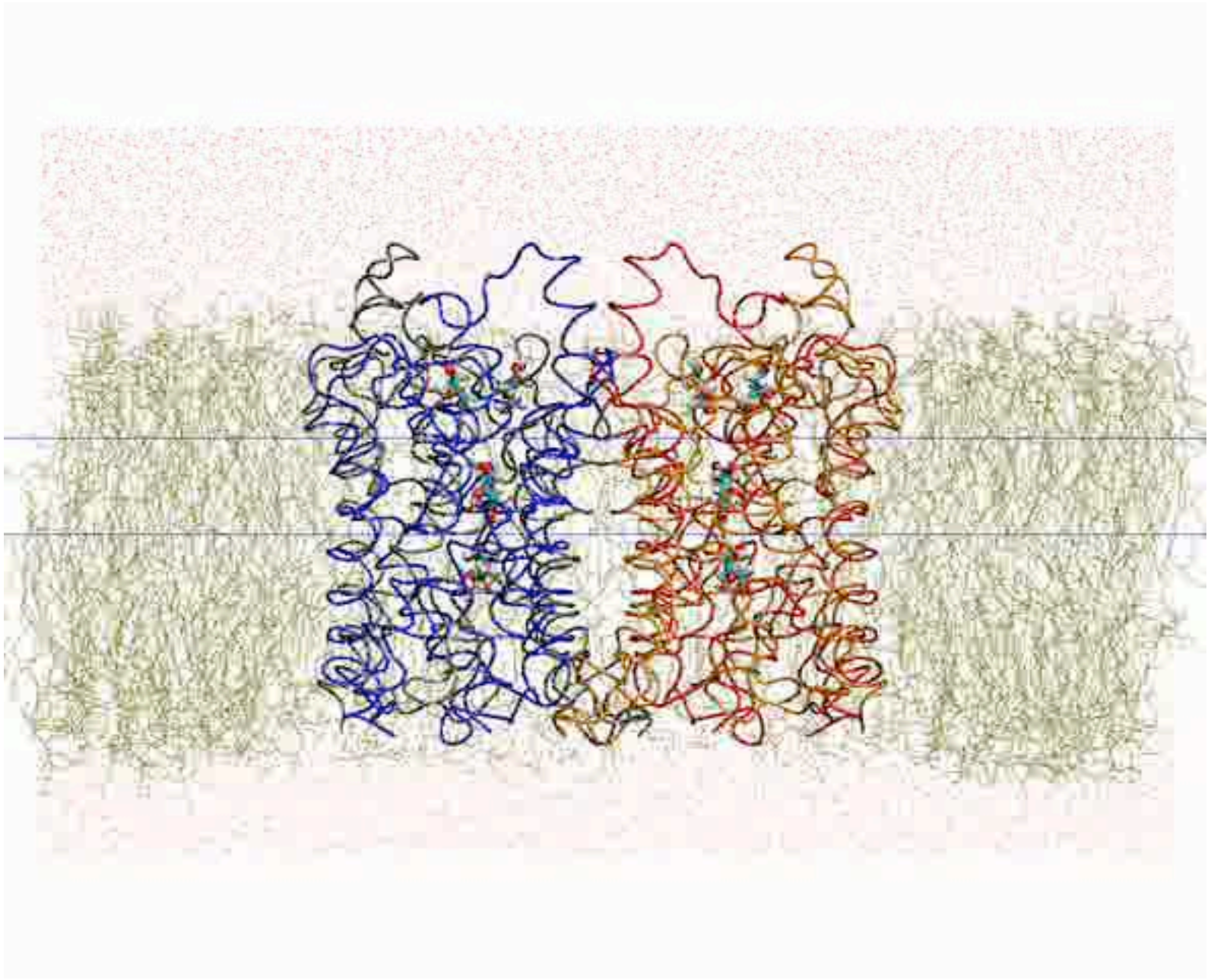


Adjustment of Membrane Thickness to the Protein Hydrophobic Surface

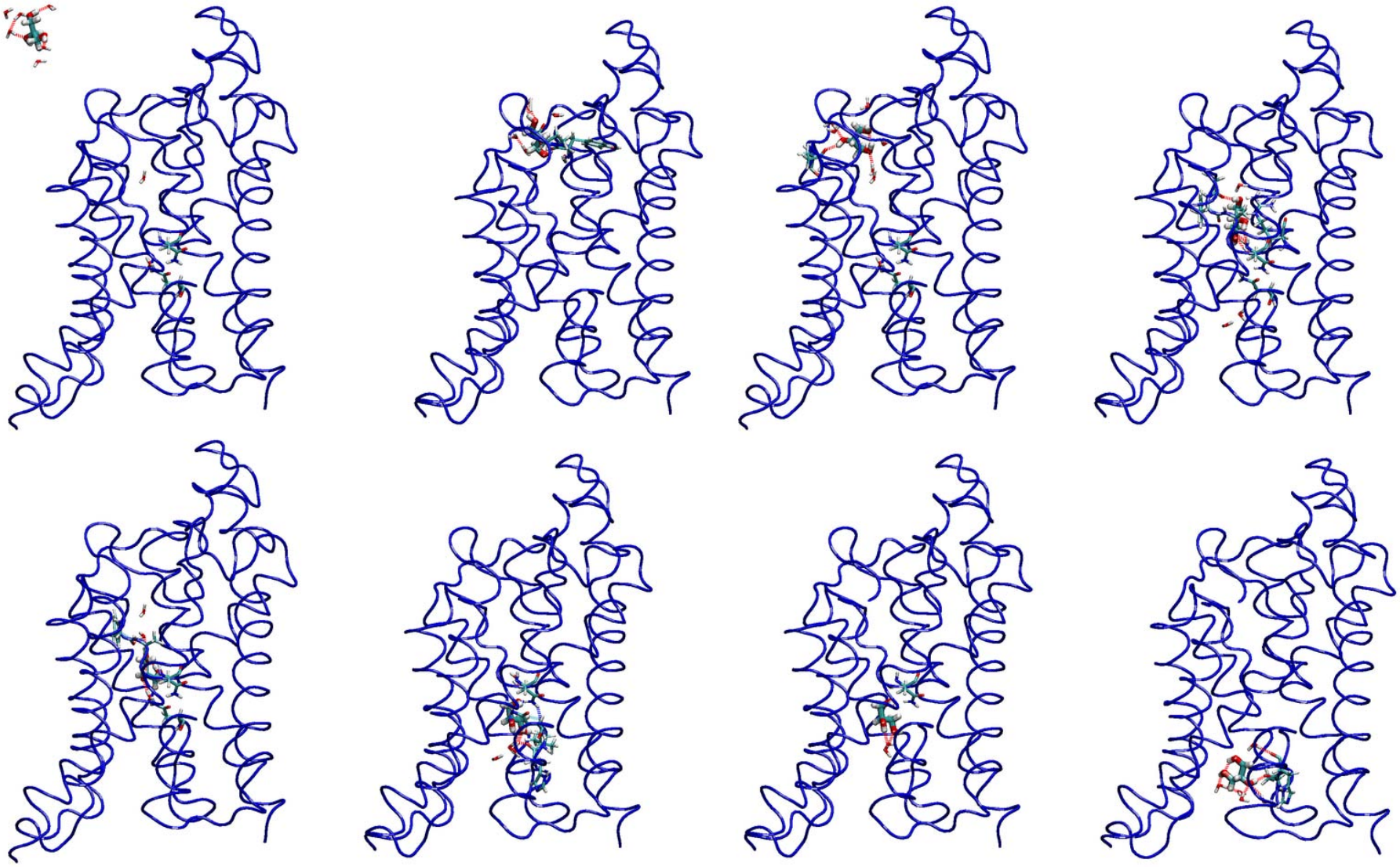


Glycerol-Saturated GlpF

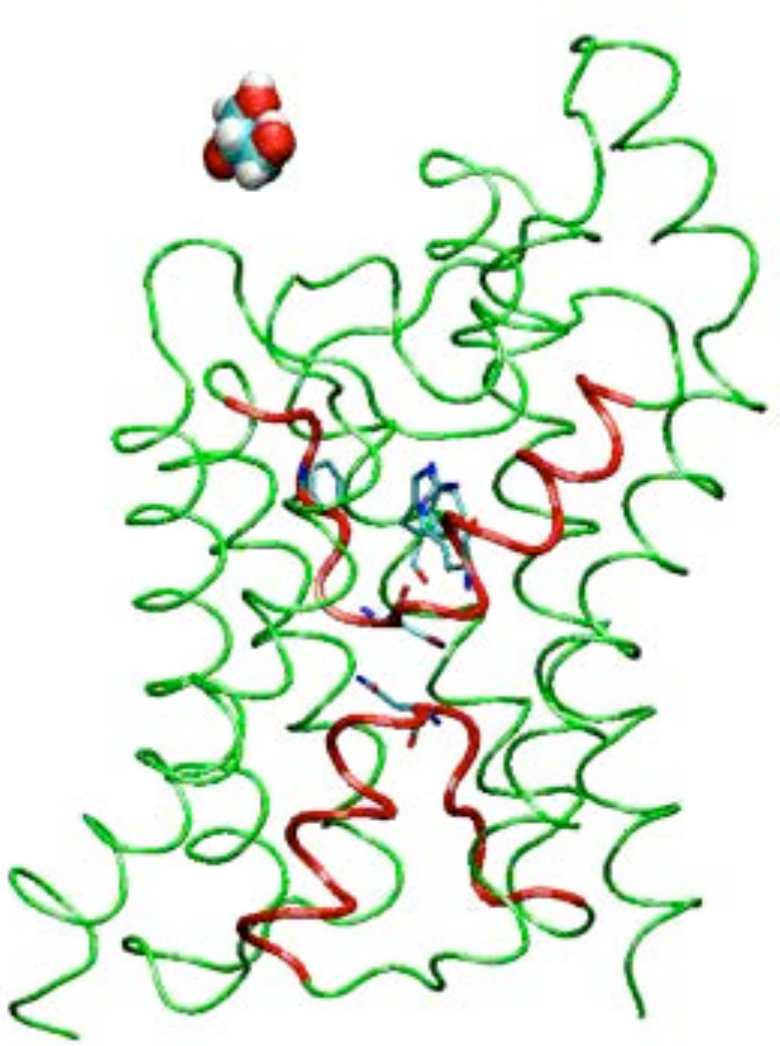




Description of full conduction pathway



Complete description of the conduction pathway

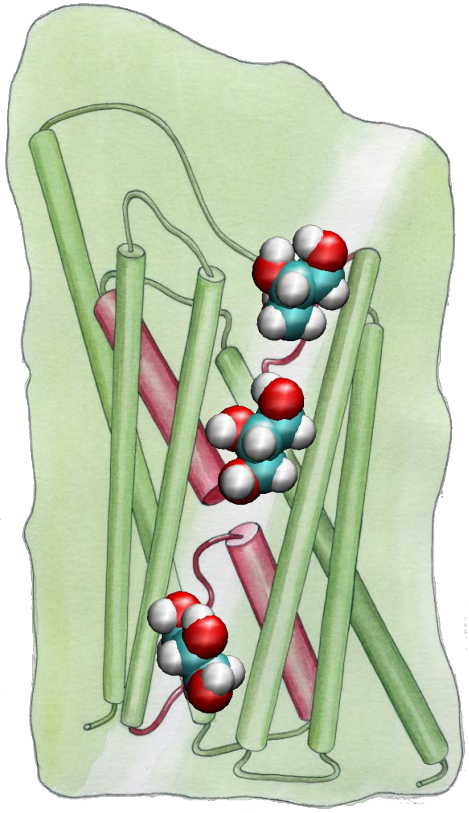


Constriction region

Selectivity filter

Channel Hydrogen Bonding Sites

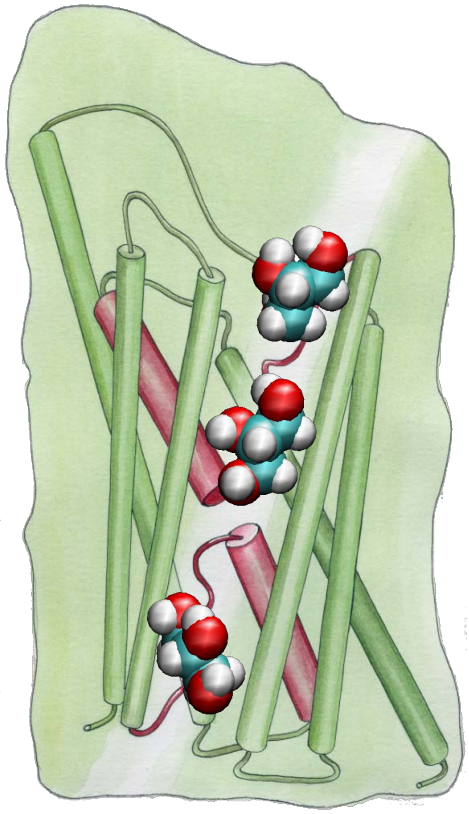
```
…
{set frame 0}{frame < 100}{incr frame}{
    animate goto $frame
    set donor [atomselect top
    “name O N and within 2 of
    (resname GCL and name HO)”]
    lappend [$donor get index] list1
    set acceptor [atomselect top
    “resname GCL and name O and
    within 2 of (protein and name HN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



Channel Hydrogen Bonding Sites

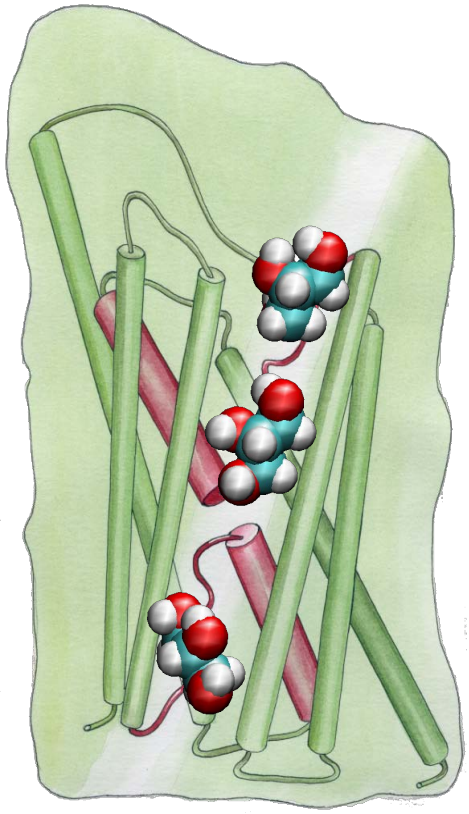
GLN	41	OE1 NE2
TRP	48	O NE1
GLY	64	O
ALA	65	O
HIS	66	O ND1
LEU	67	O
ASN	68	ND2
ASP	130	OD1
GLY	133	O
SER	136	O
TYR	138	O
PRO	139	O N
ASN	140	OD1 ND2
HIS	142	ND1
THR	167	OG1
GLY	195	O
PRO	196	O

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



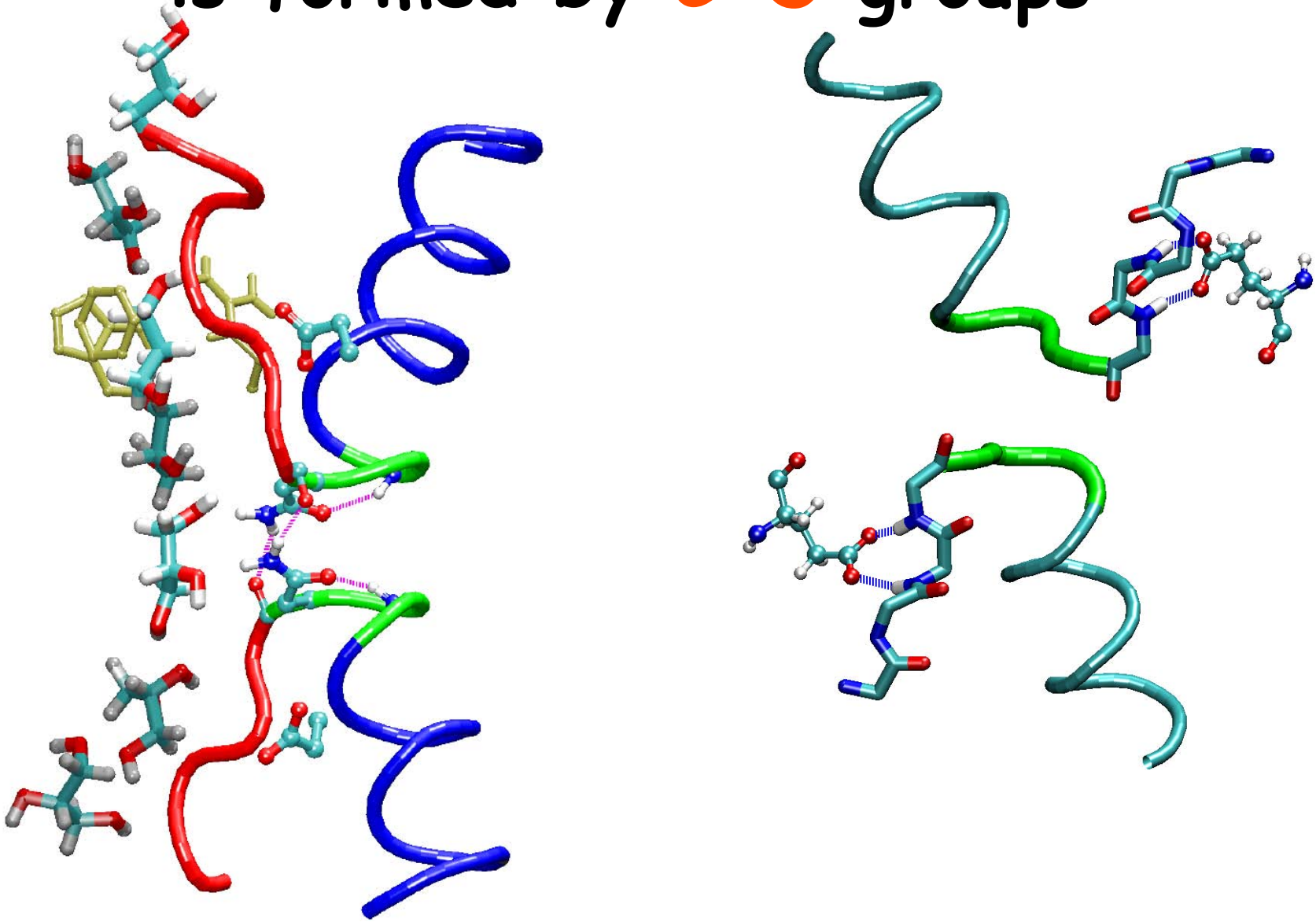
Channel Hydrogen Bonding Sites

GLN	41	OE1 NE2	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



The Substrate Pathway

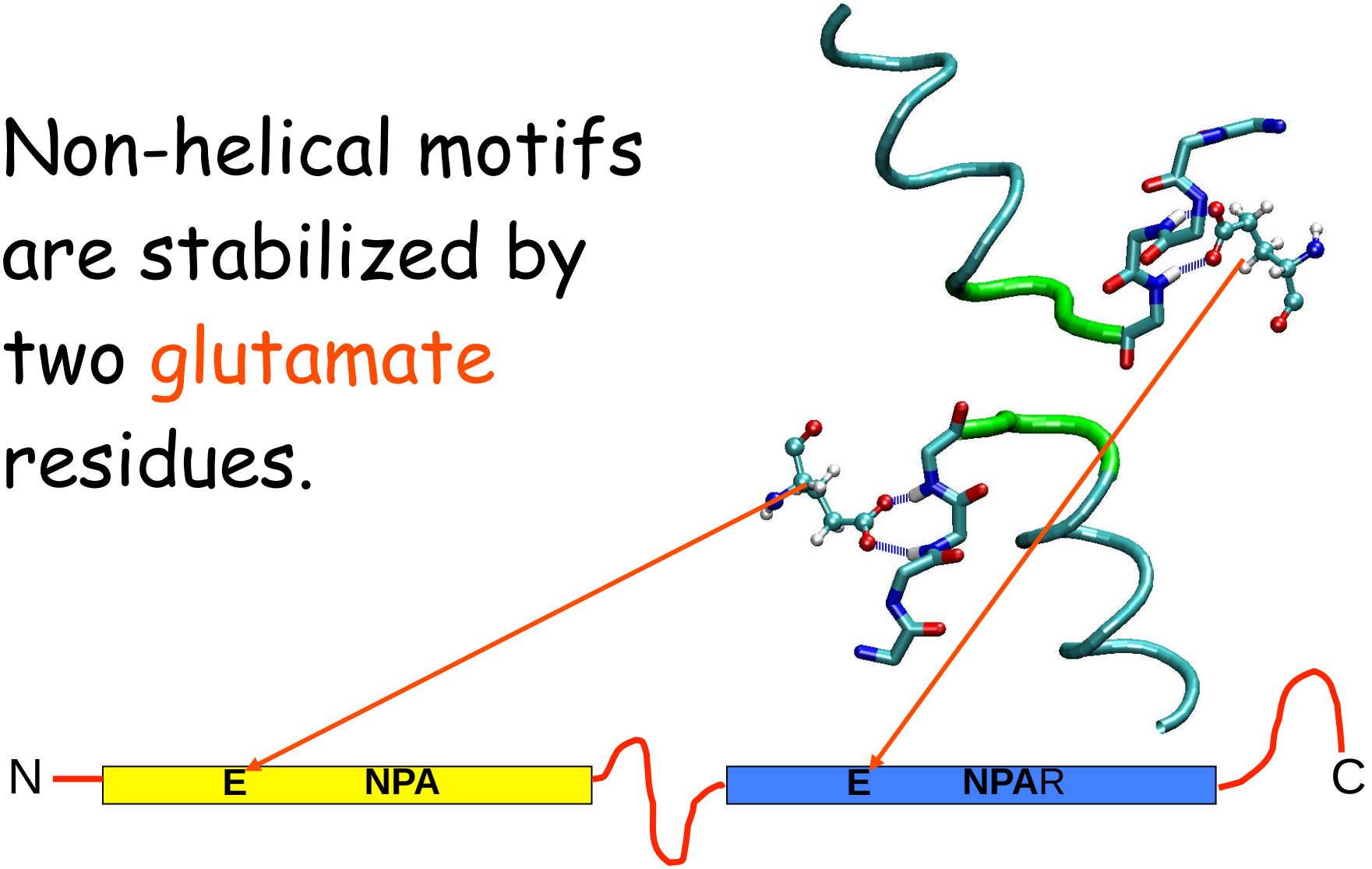
is formed by C=O groups



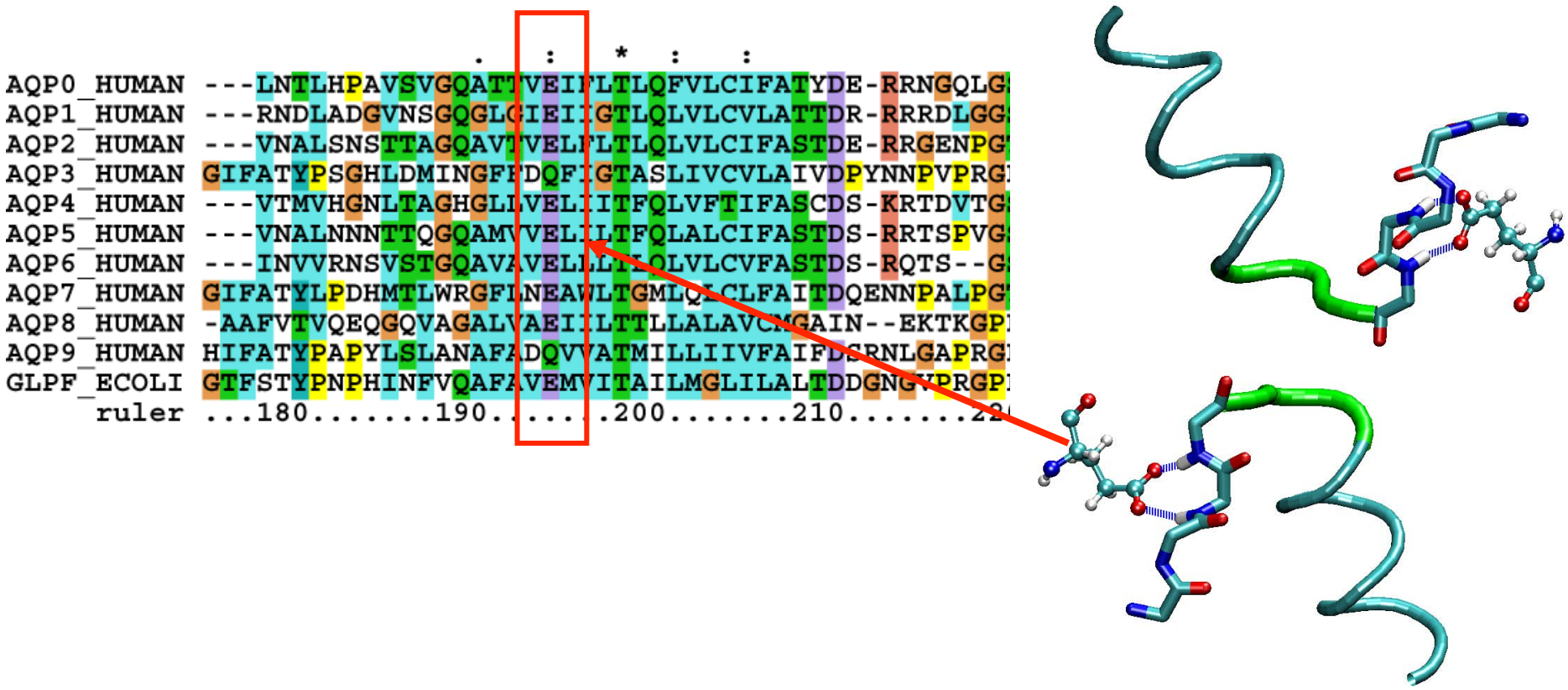
The Substrate Pathway

is formed by C=O groups

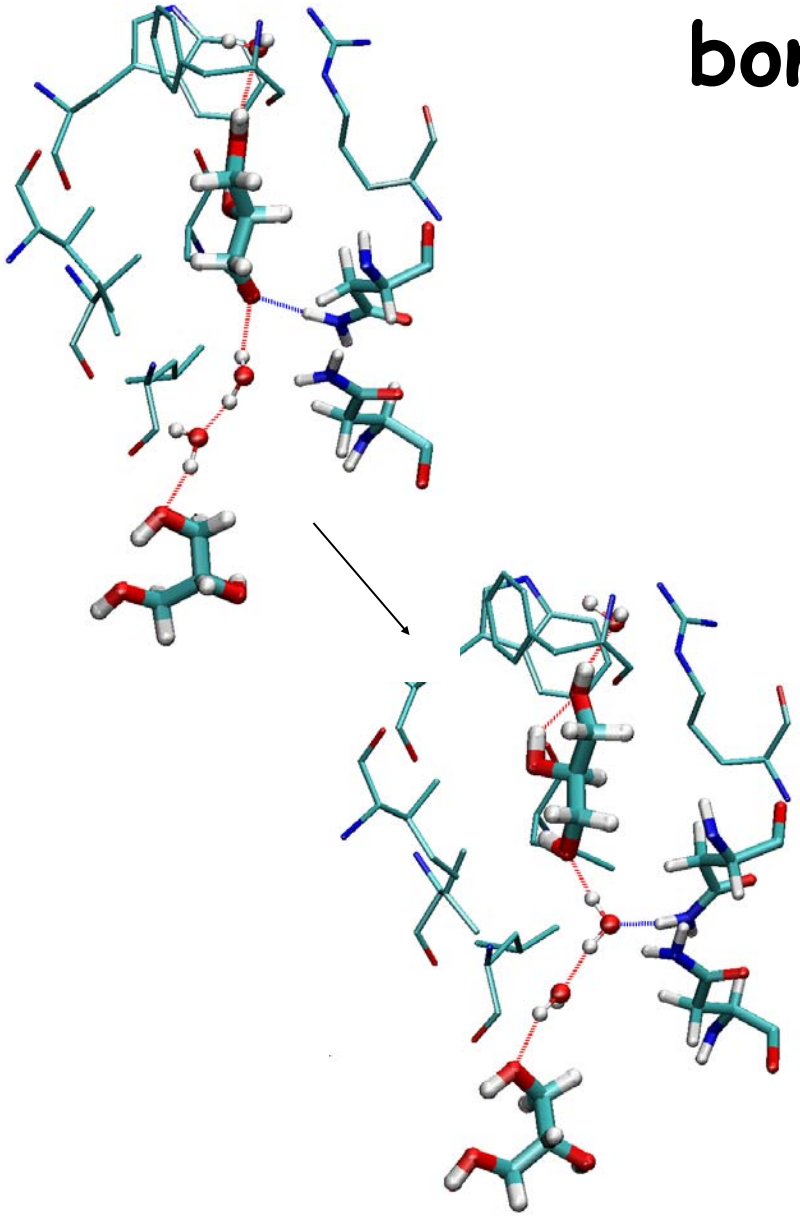
Non-helical motifs are stabilized by two glutamate residues.

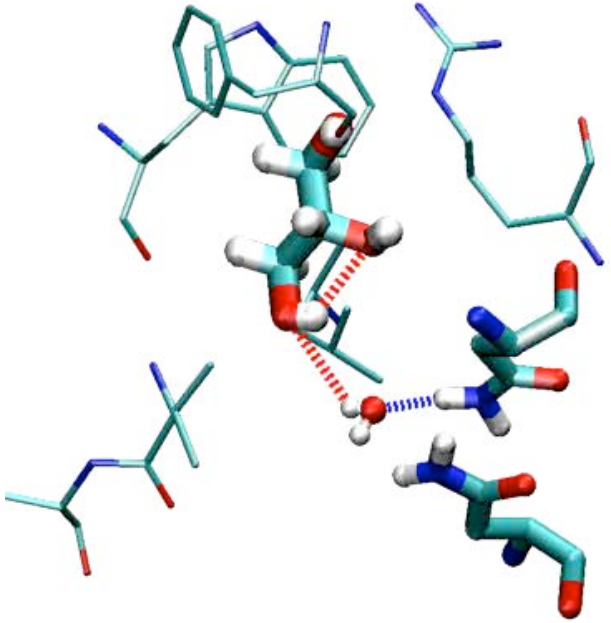


Conservation of Glutamate Residue in Human Aquaporins

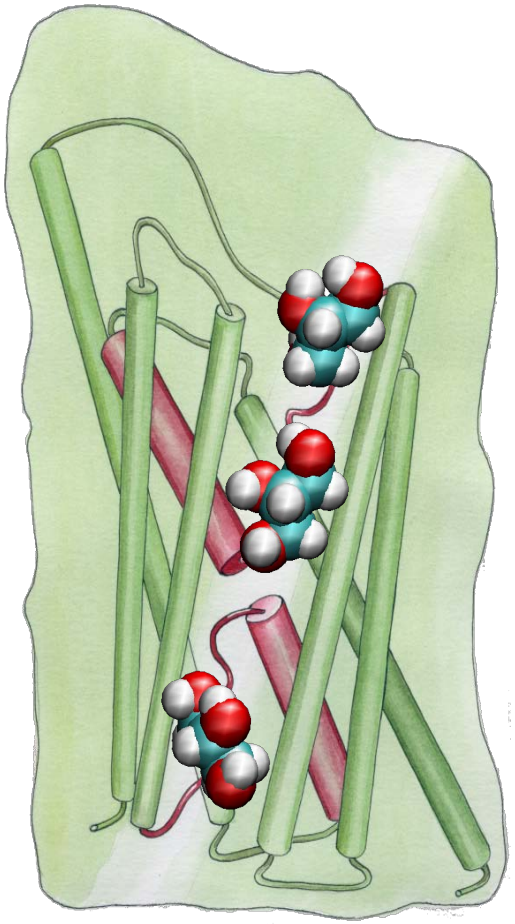


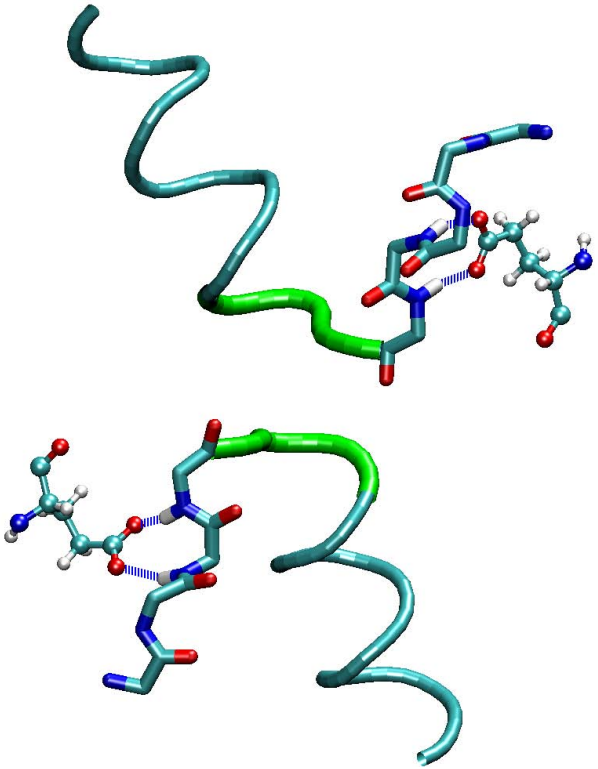
Glycerol – water competition for hydrogen bonding sites



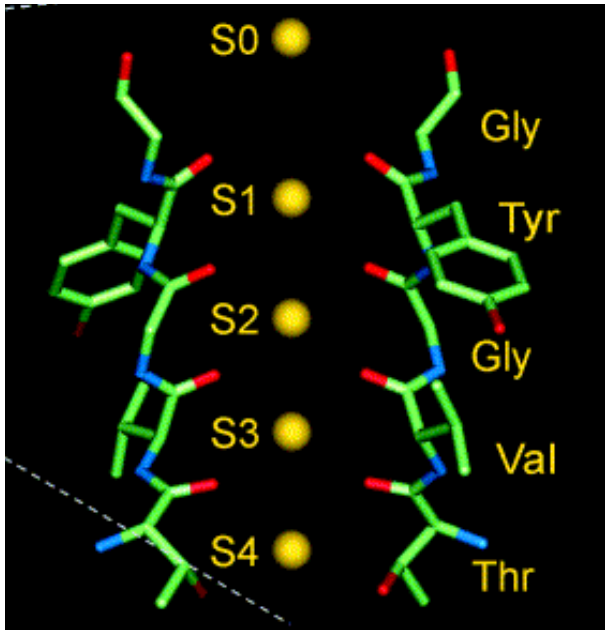


Revealing the Functional Role of Reentrant Loops



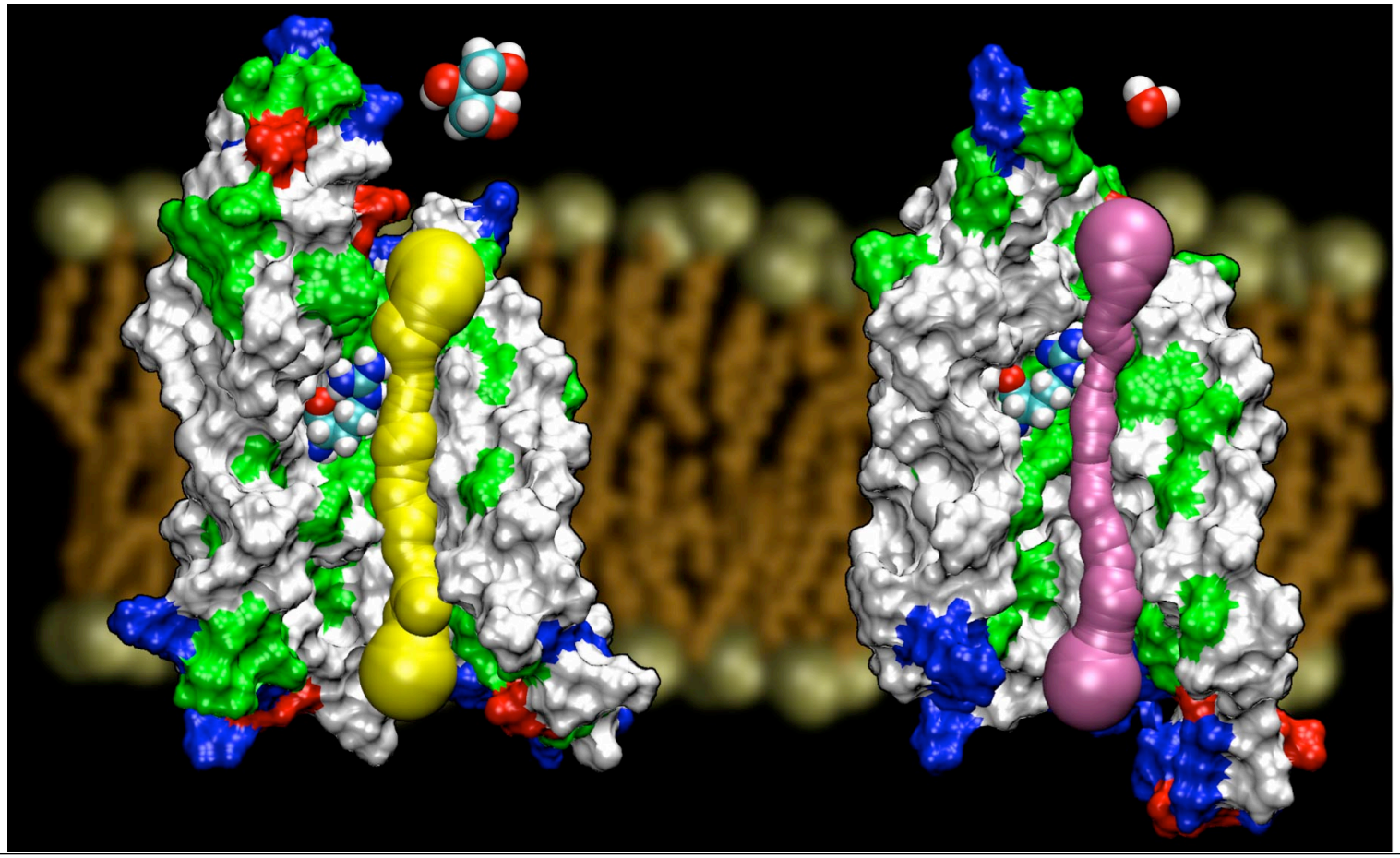


Potassium channel



AqpZ *vs*. GlpF

- Both from *E. coli*
- AqpZ is a pure water channel
- GlpF is a glycerol channel
- We have high resolution structures for both channels



Steered Molecular Dynamics is a non-equilibrium method by nature

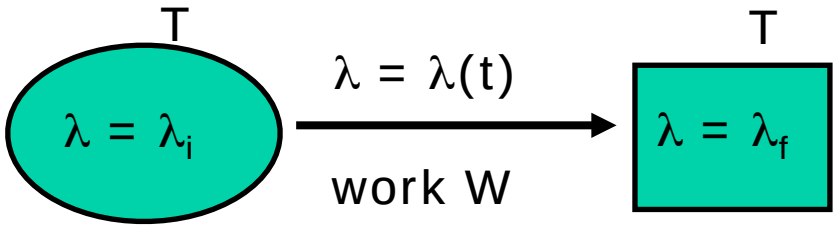
- A wide variety of events that are inaccessible to conventional molecular dynamics simulations can be probed.

- The system will be driven, however, away from equilibrium, resulting in problems in describing the energy landscape associated with the event of interest.

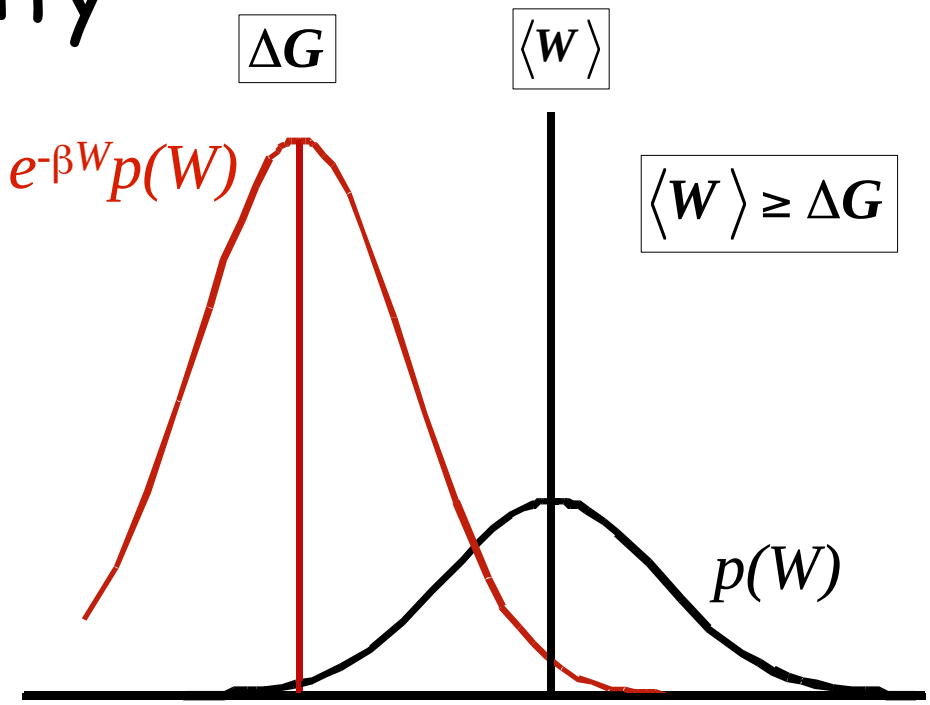
Second law of thermodynamics $\longrightarrow W \geq \Delta G$

Jarzynski's Equality

Transition between two equilibrium states



$$\Delta G = G_f - G_i$$



C. Jarzynski, *Phys. Rev. Lett.*, **78**, 2690 (1997)
C. Jarzynski, *Phys. Rev. E*, **56**, 5018 (1997)

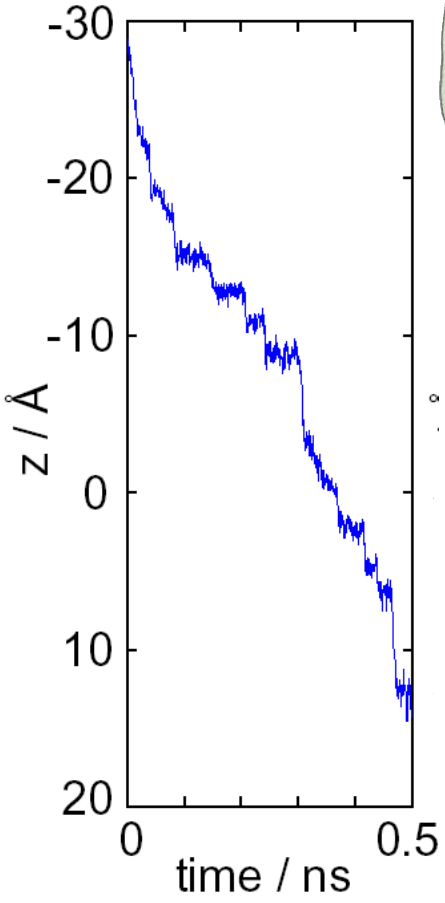
$$\left\langle e^{-\beta W} \right\rangle = e^{-\beta \Delta G}$$

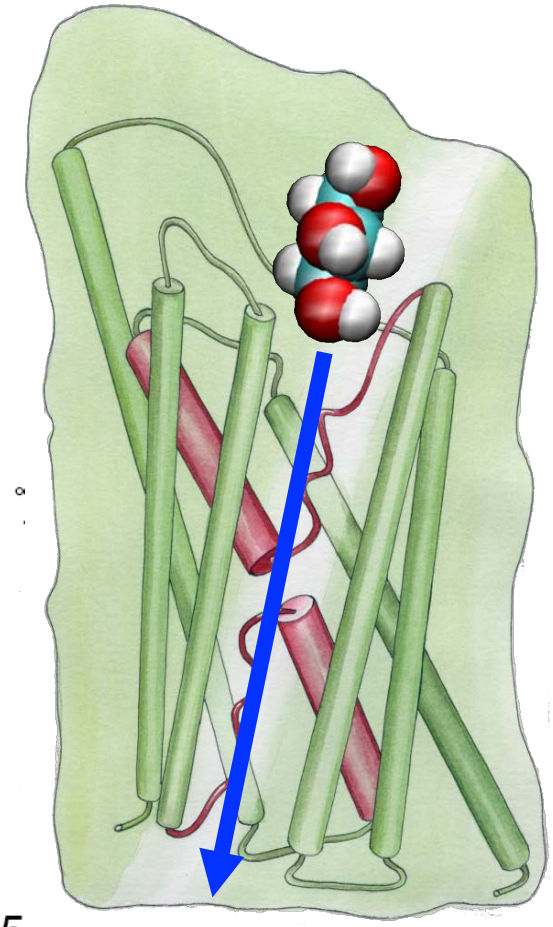
$$\beta = 1/k_B T$$

In principle, it is possible to obtain free energy surfaces from repeated non-equilibrium experiments.

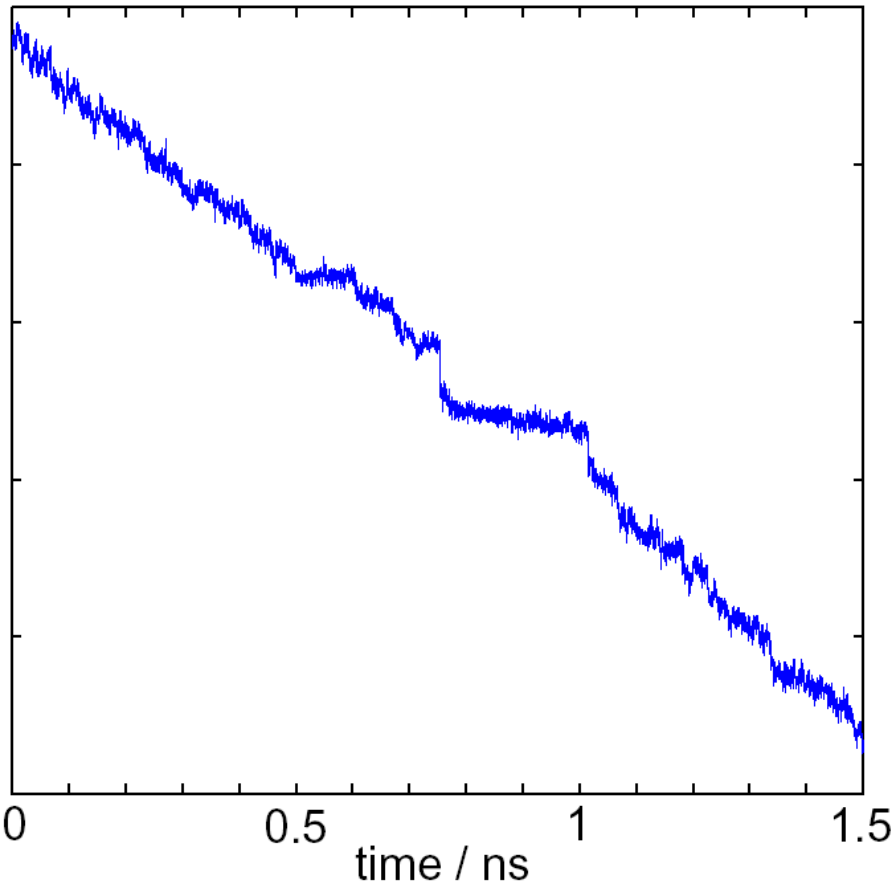
Steered Molecular Dynamics

constant force
(250 pN)

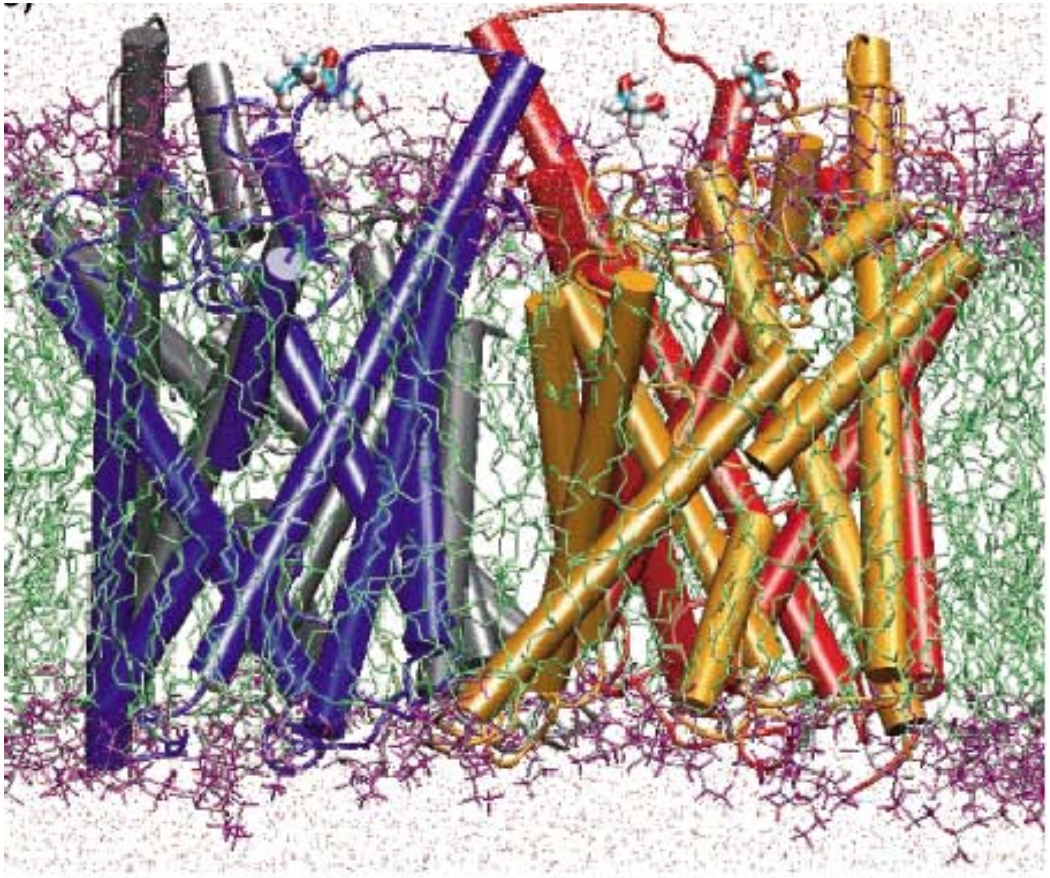


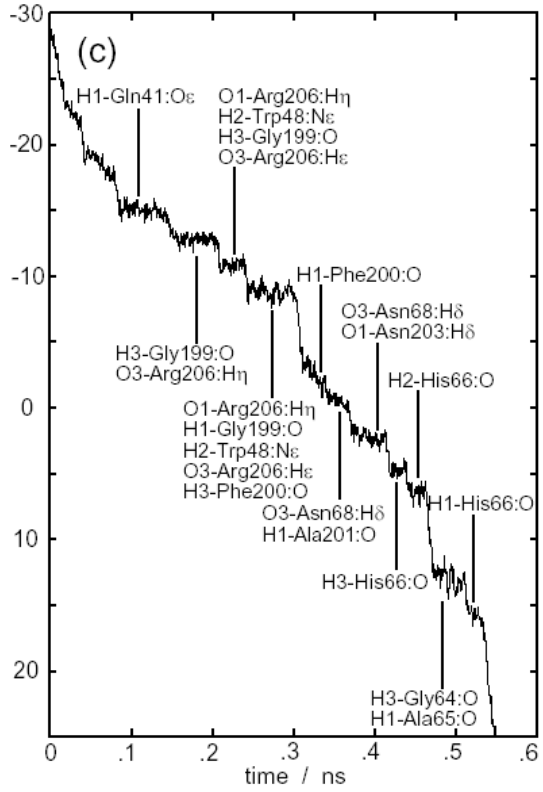


constant velocity
(30 Å/ns)



SMD Simulation of Glycerol Passage





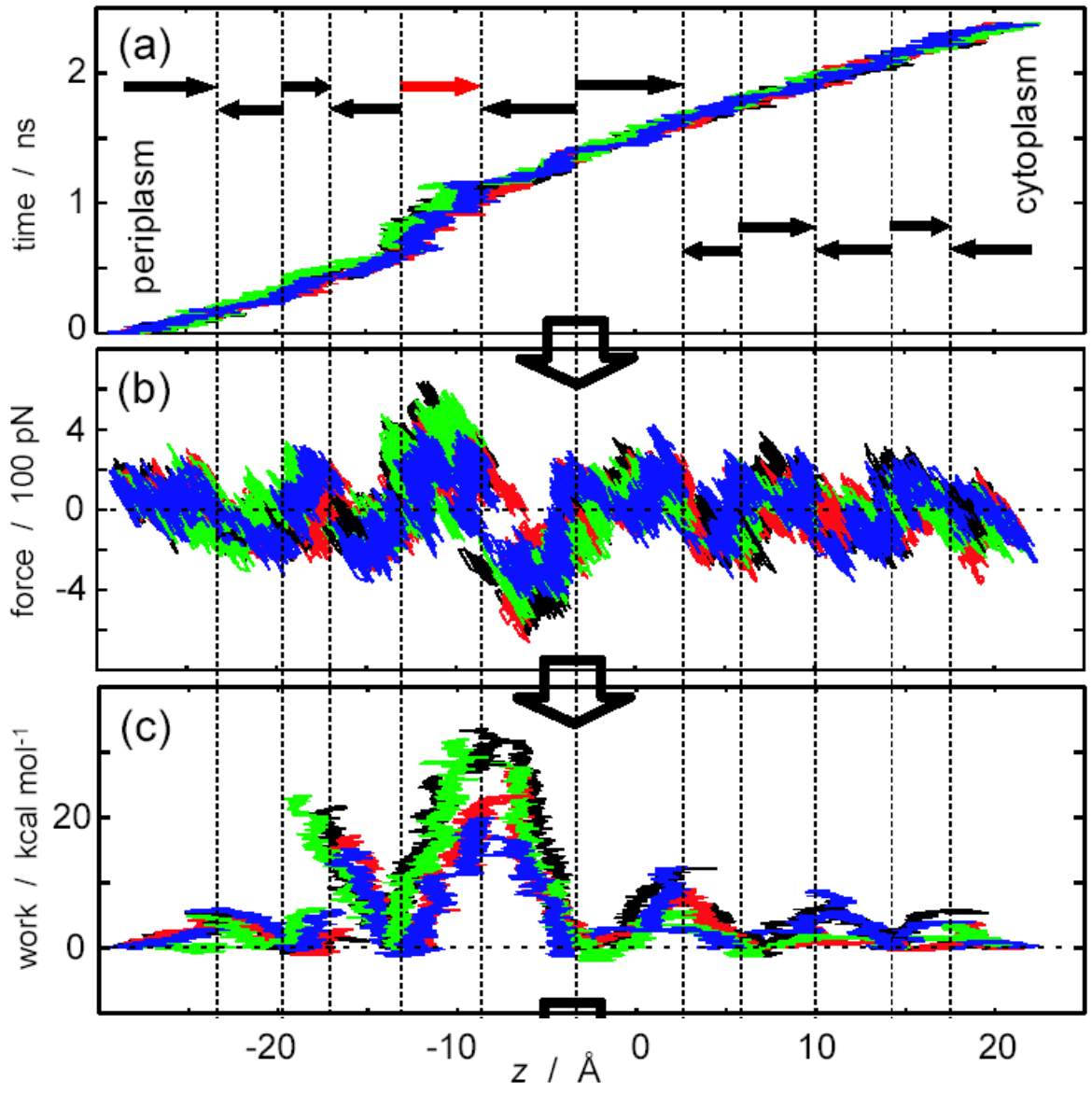
Trajectory of glycerol pulled by **constant force**

Constructing the Potential of Mean Force

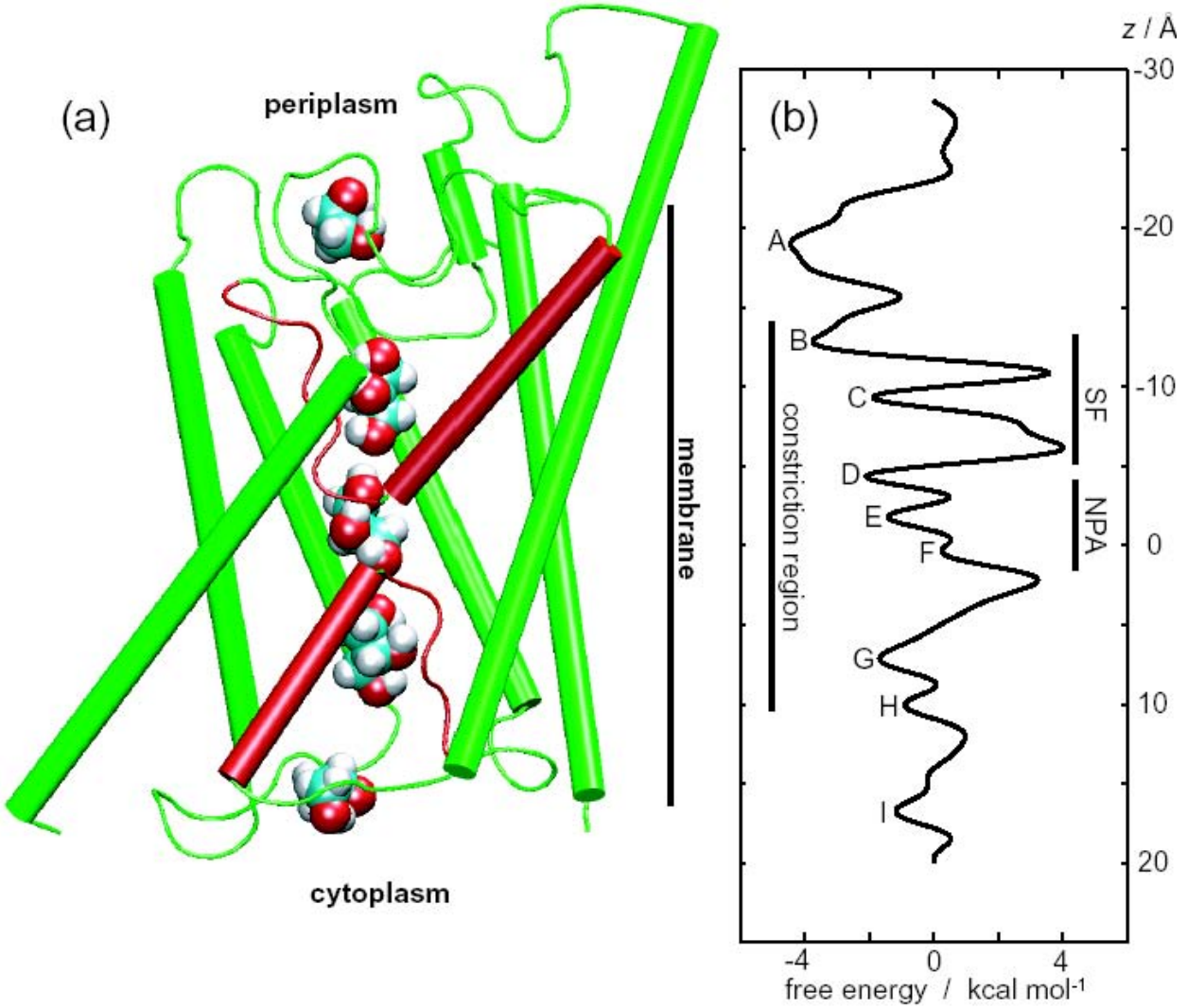
4 trajectories
v **= 0.03, 0.015 Å/ps**
k **= 150 pN/Å**

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

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



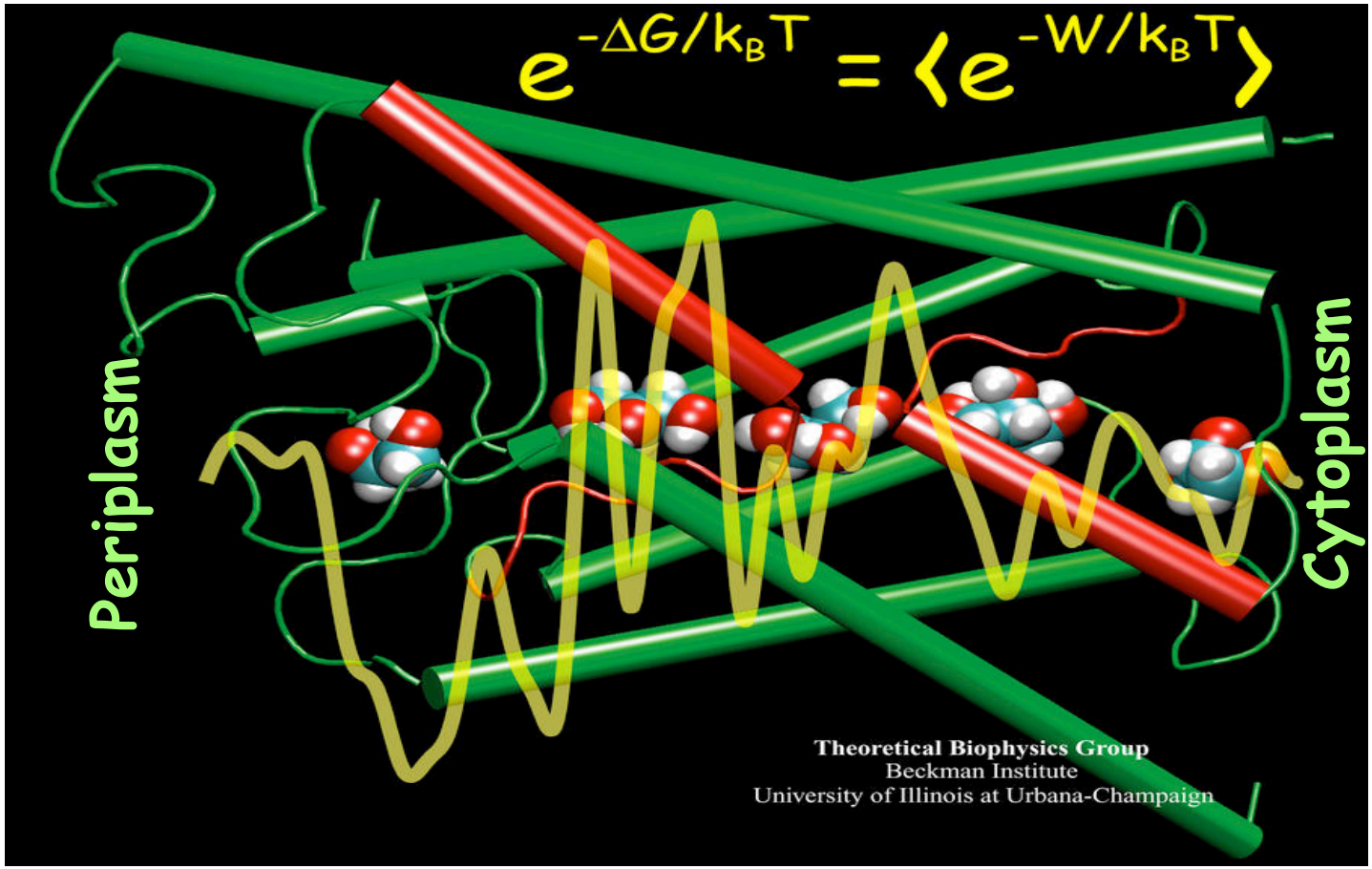
Features of the Potential of Mean Force



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

Jensen et al., *PNAS*, 99:6731-6736, 2002.

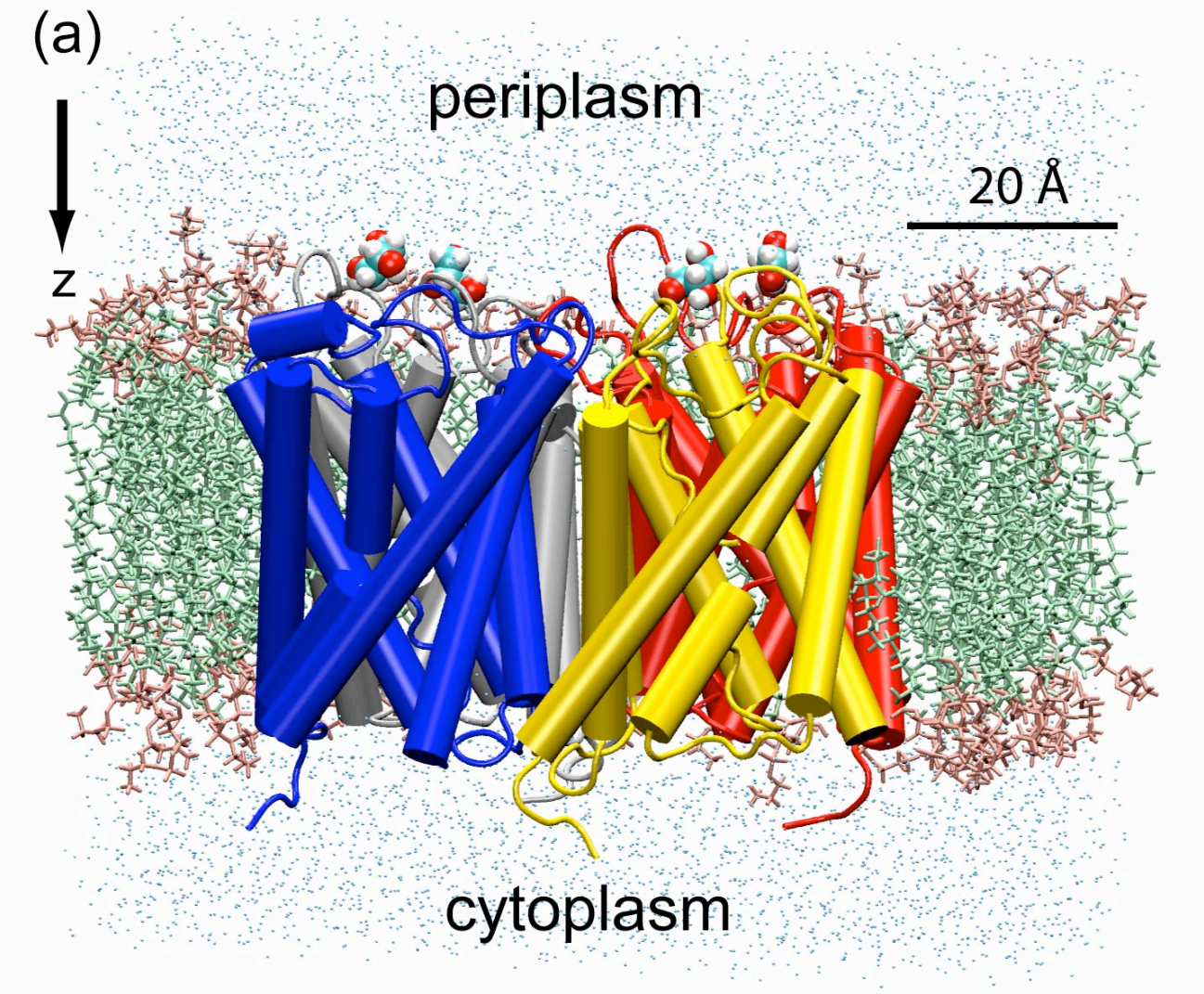
Features of the Potential of Mean Force



Asymmetric Profile in the Vestibules

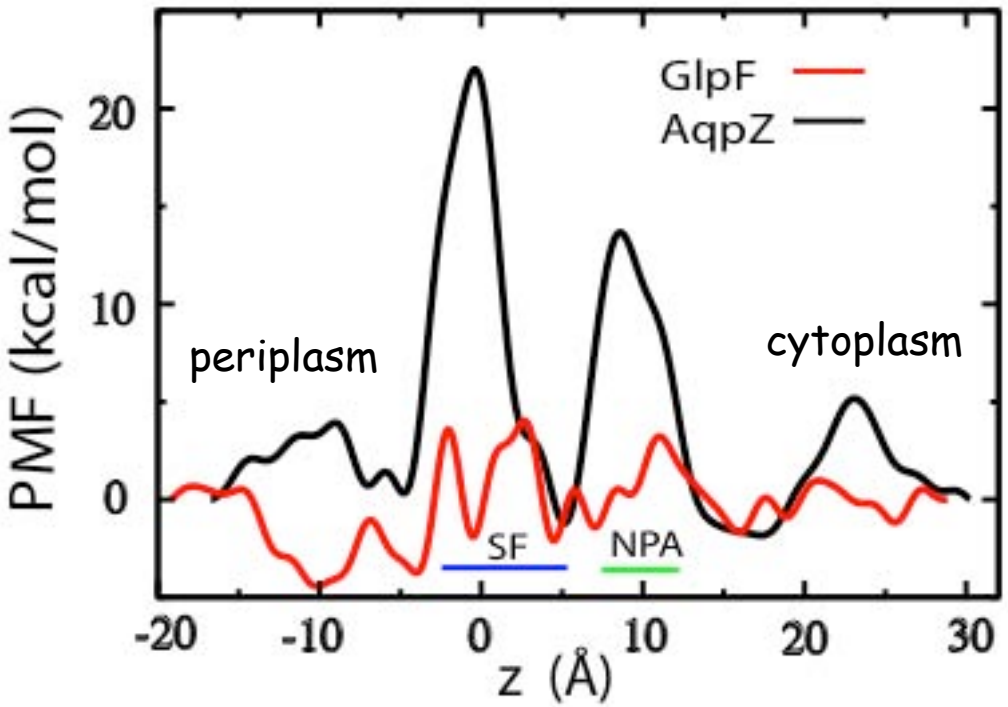
Jensen et al., *PNAS*, 99:6731-6736, 2002.

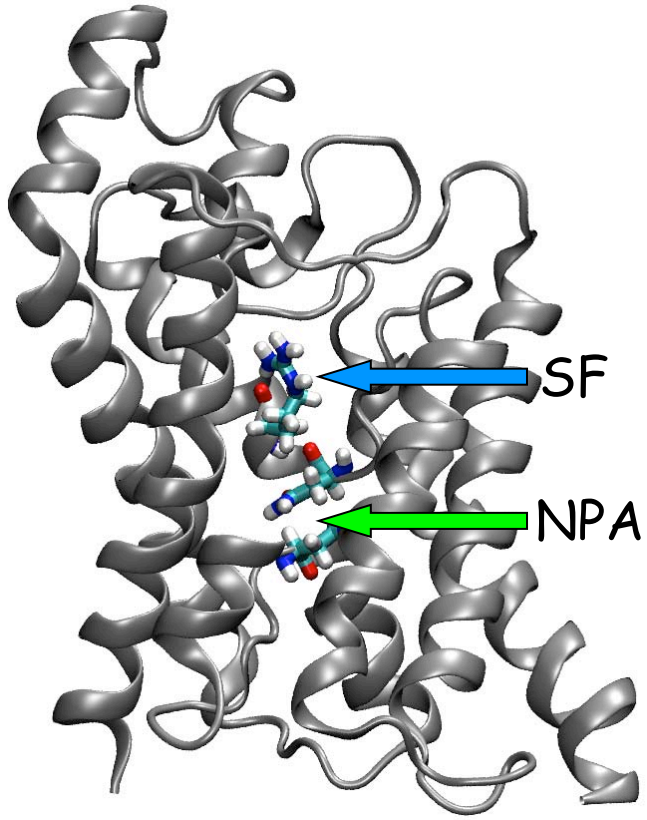
Artificial induction of glycerol conduction through AqpZ



Y. Wang, K. Schulten, and E. Tajkhorshid ***Structure*** 13, 1107 ***(***2005)

Three fold higher barriers



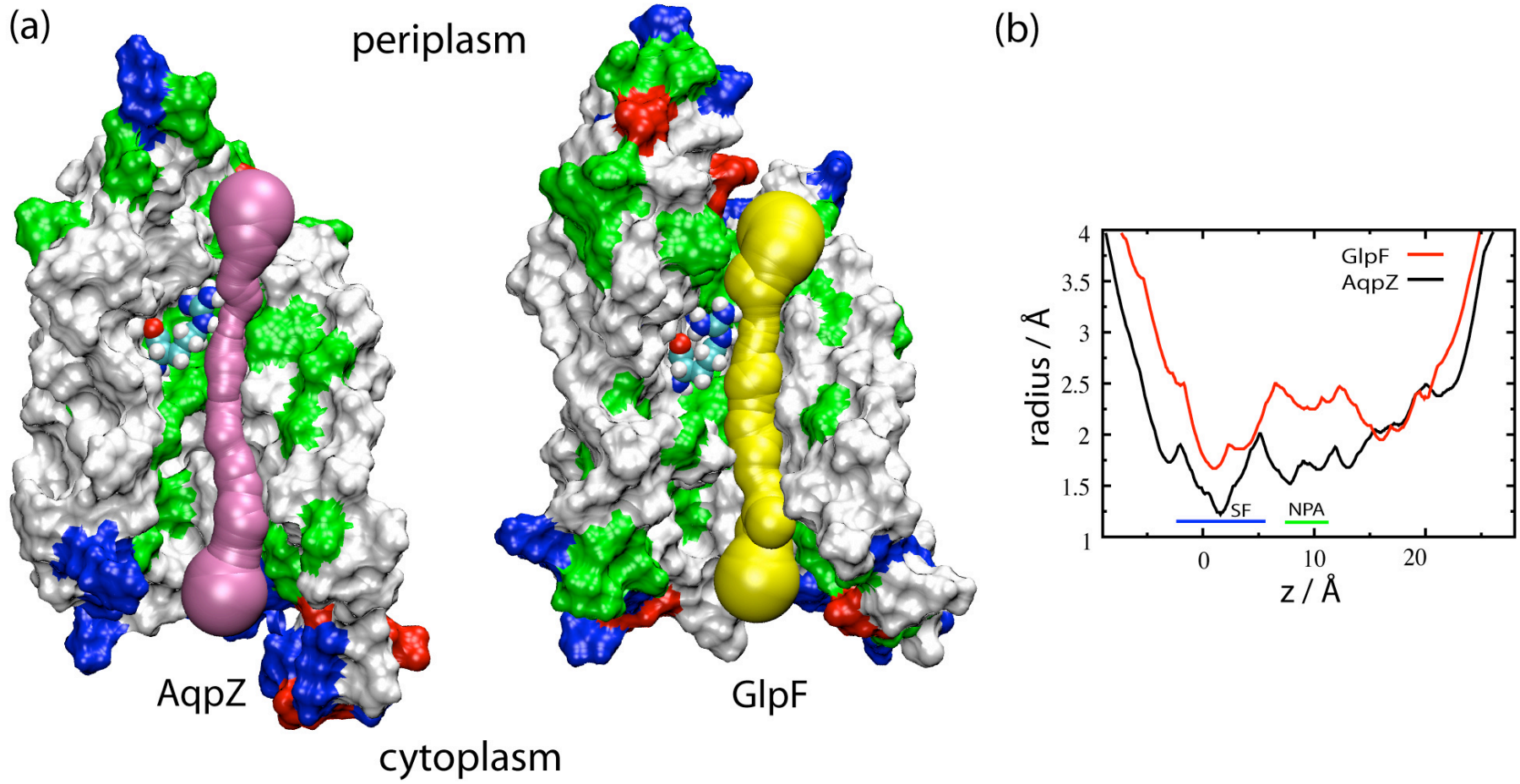


AqpZ 22.8 kcal/mol

GlpF 7.3 kcal/mol

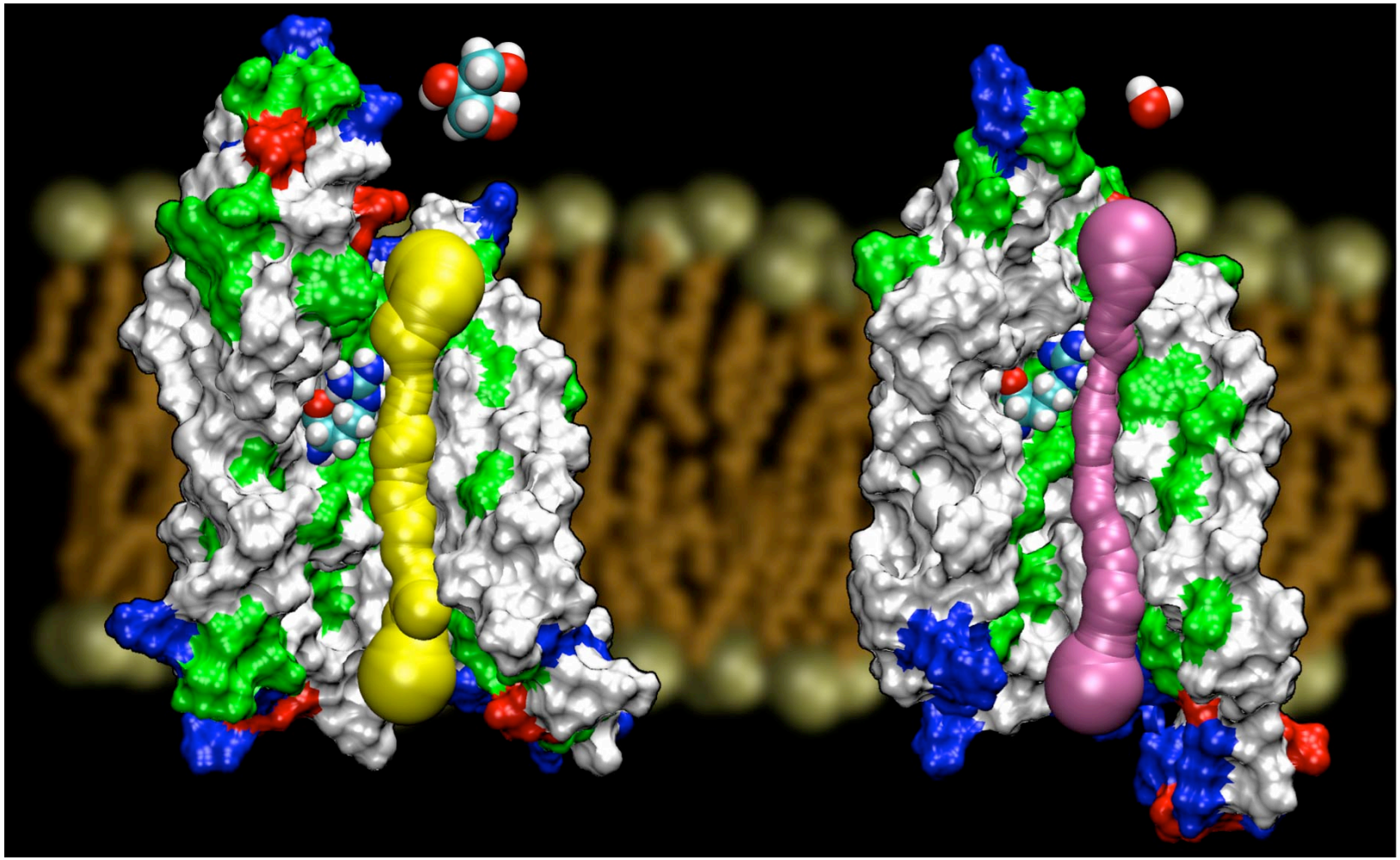
Y. Wang, K. Schulten, and E. Tajkhorshid ***Structure*** 13, 1107 ***(***2005)

Could it be simply the size?



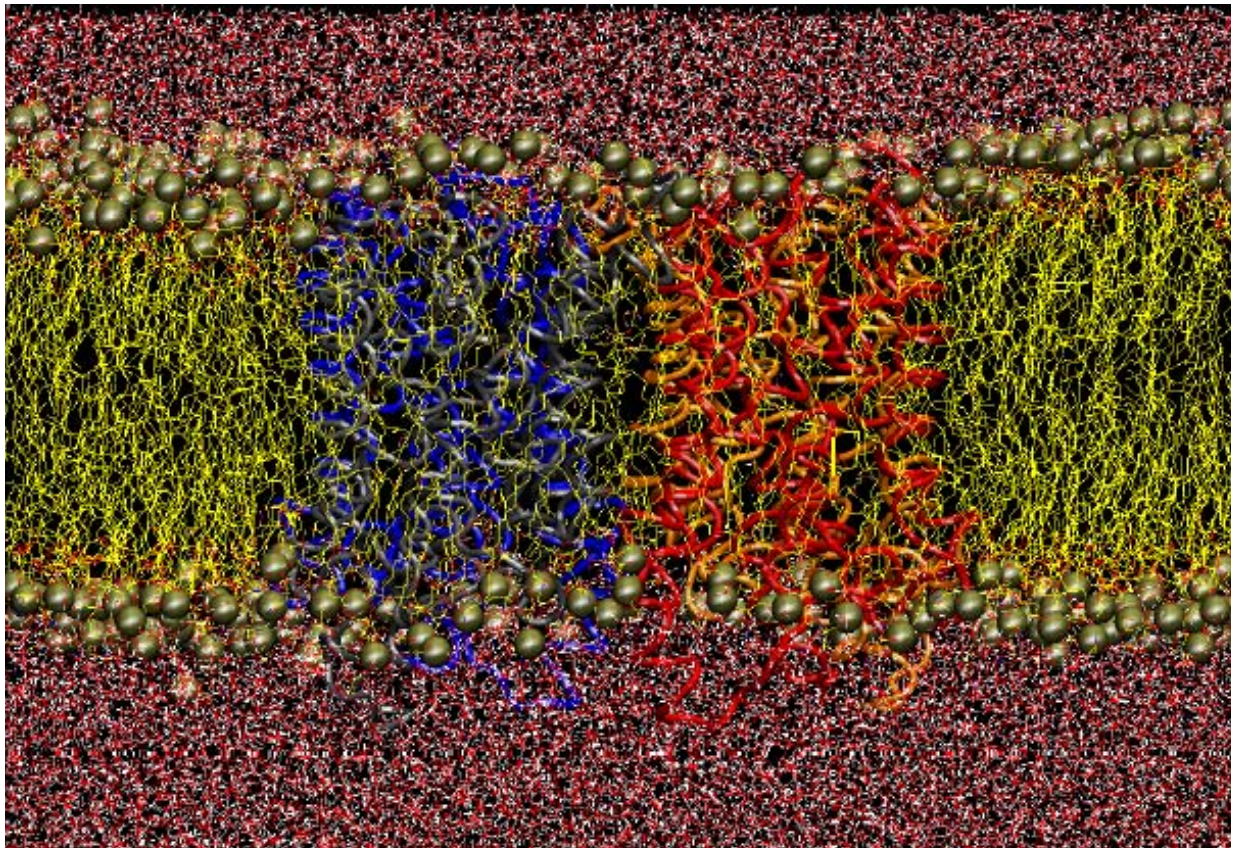
Y. Wang, K. Schulten, and E. Tajkhorshid ***Structure*** 13, 1107 ***(***2005)

It is probably just the size that matters!



Y. Wang, K. Schulten, and E. Tajkhorshid ***Structure*** 13, 1107 ***(***2005)

Water permeation



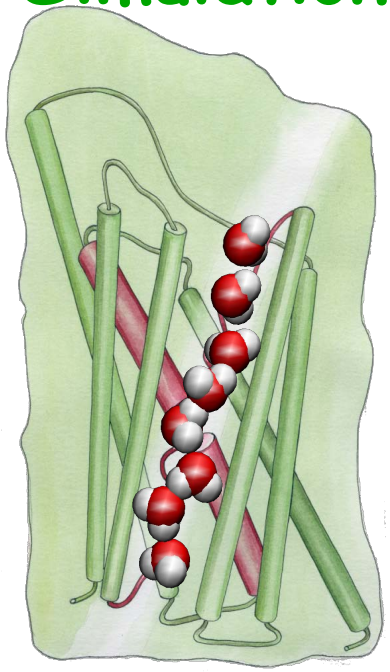
18 water conducted

In 4 monomers in 4 ns

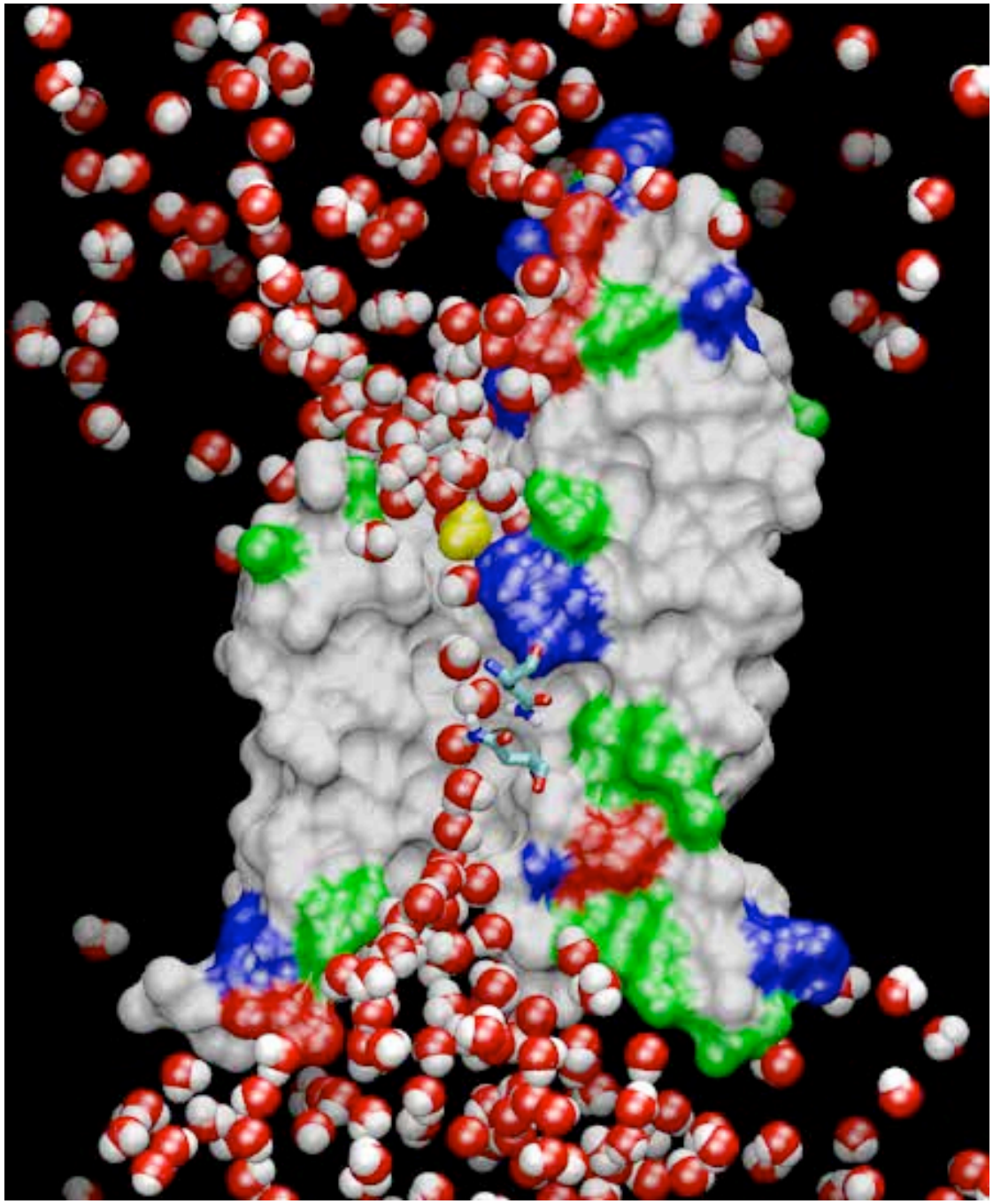
1.125 water/monomer/ns

Exp. = ~ 1-2 /ns

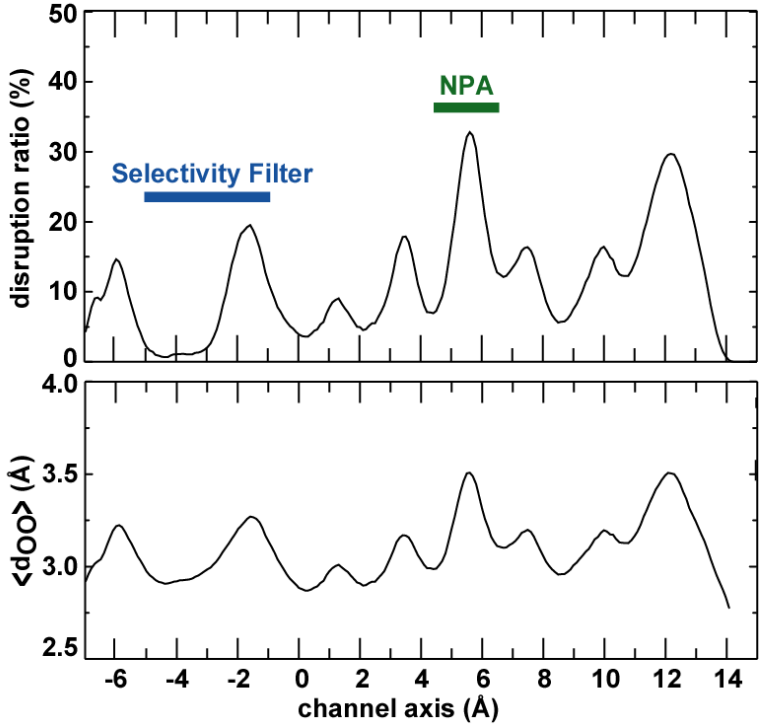
5 nanosecond Simulation

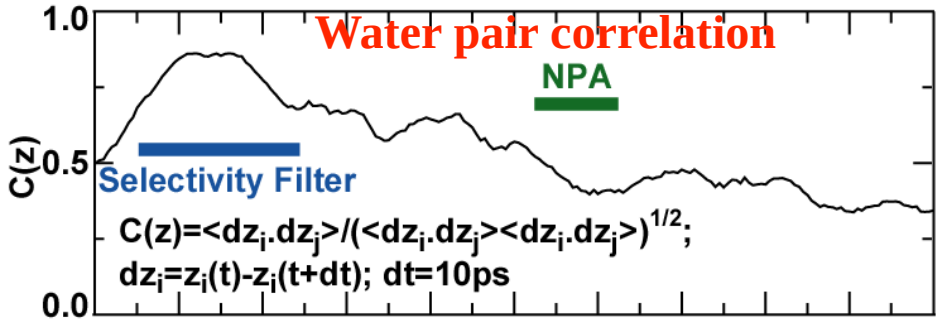


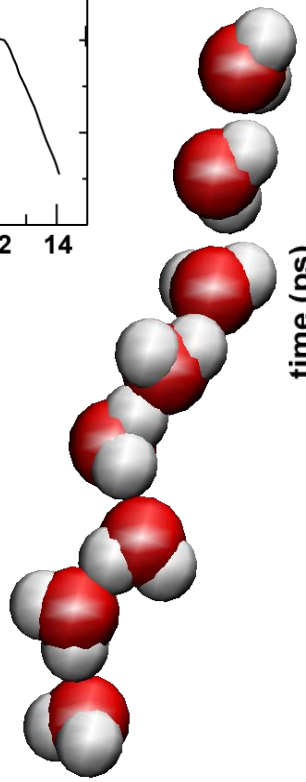
7-8 water molecules in each channel

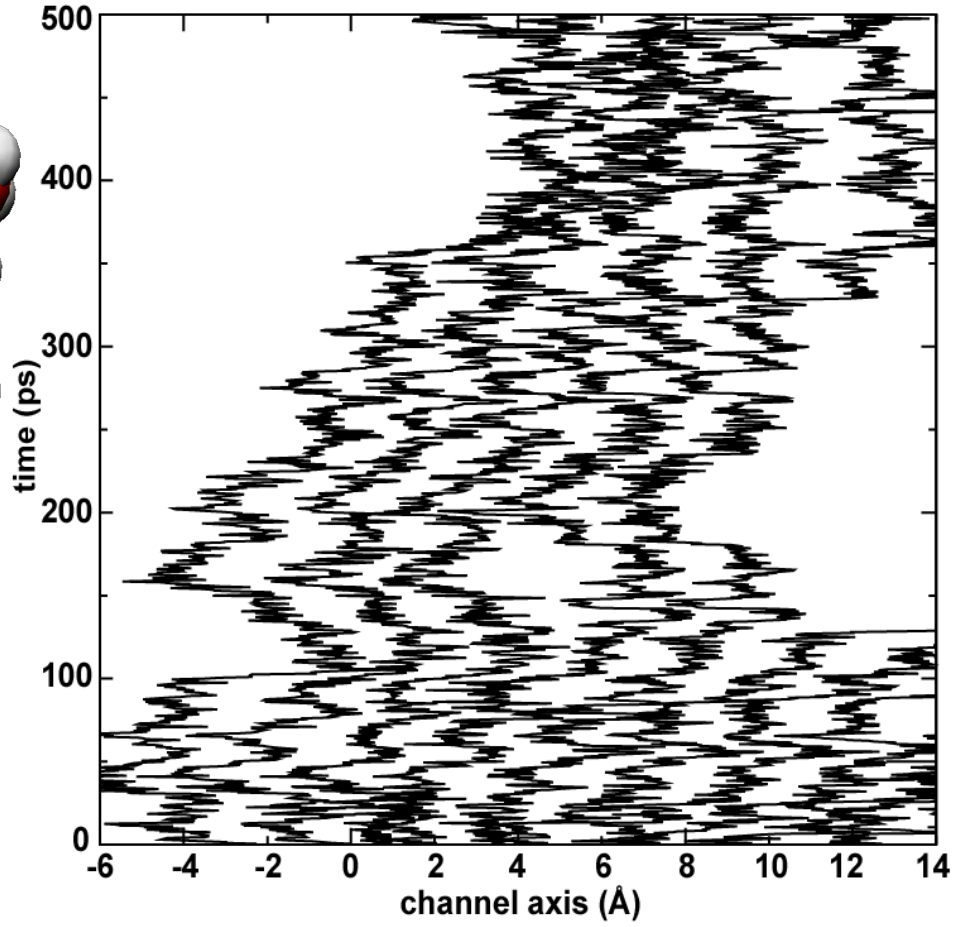


Correlated Motion of Water in the Channel



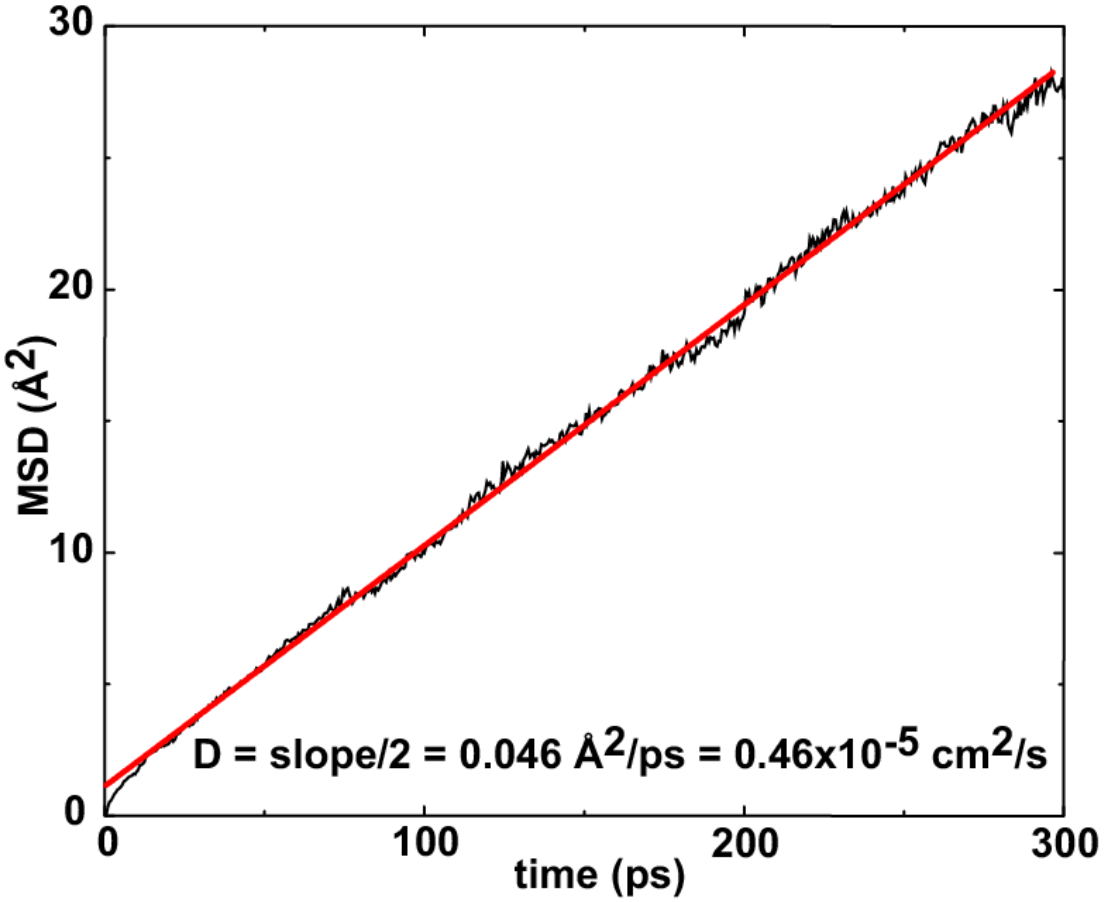


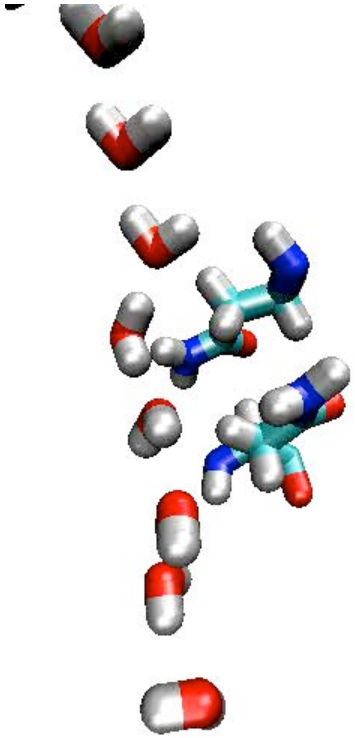




The single file of water molecules is maintained.

Diffusion of Water in the channel

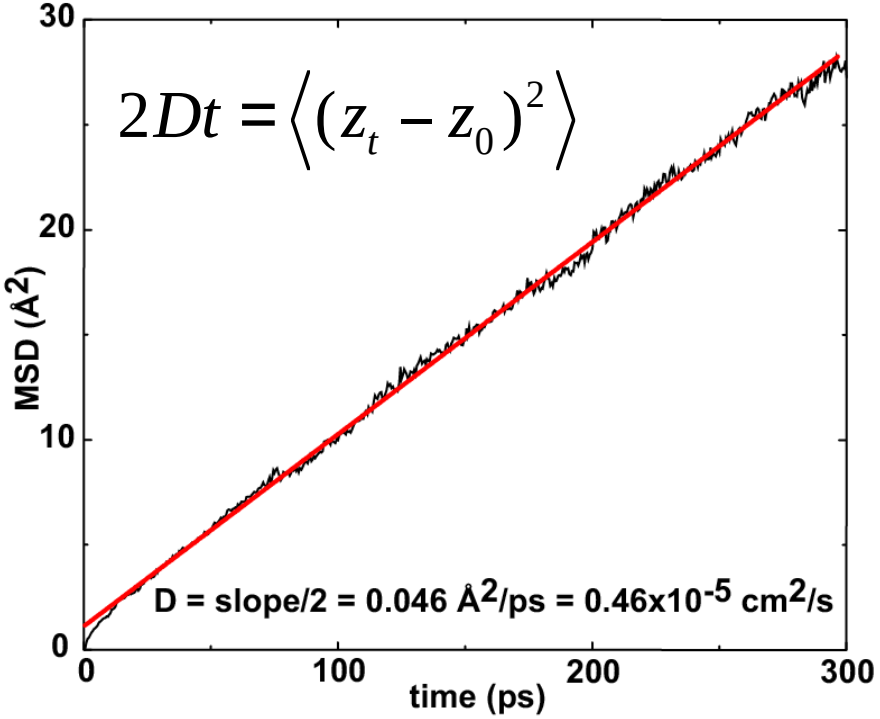


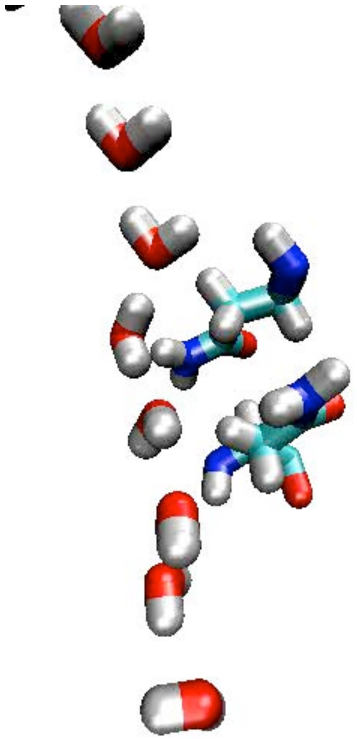


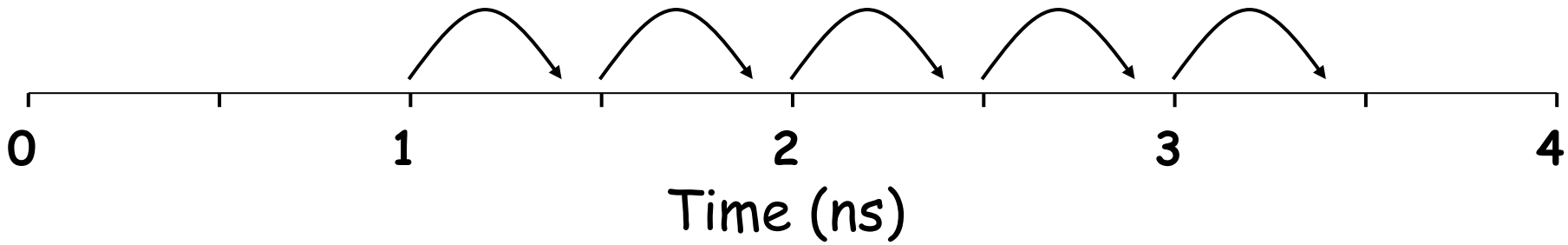
One dimensional diffusion: $2Dt = \left\langle (z_t - z_0)^2 \right\rangle$

Experimental value for AQP1: 0.4-0.8 e-5

Diffusion of Water in the channel

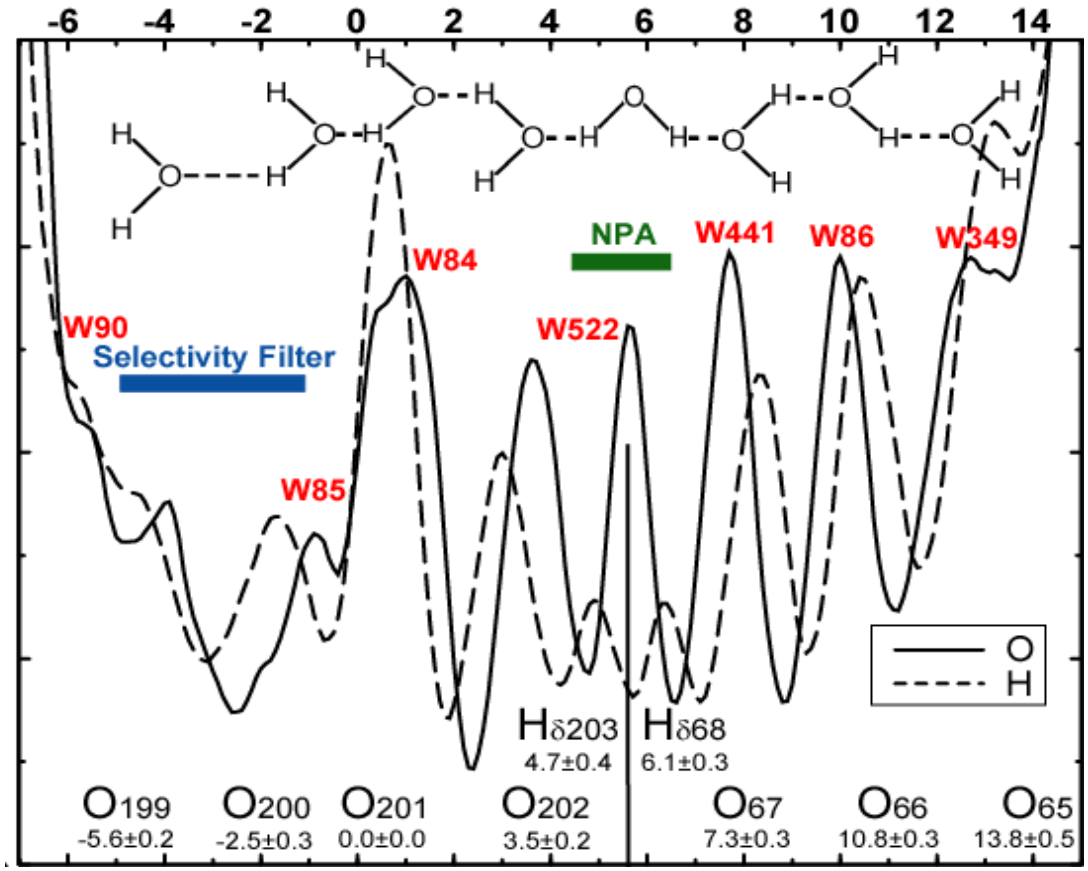


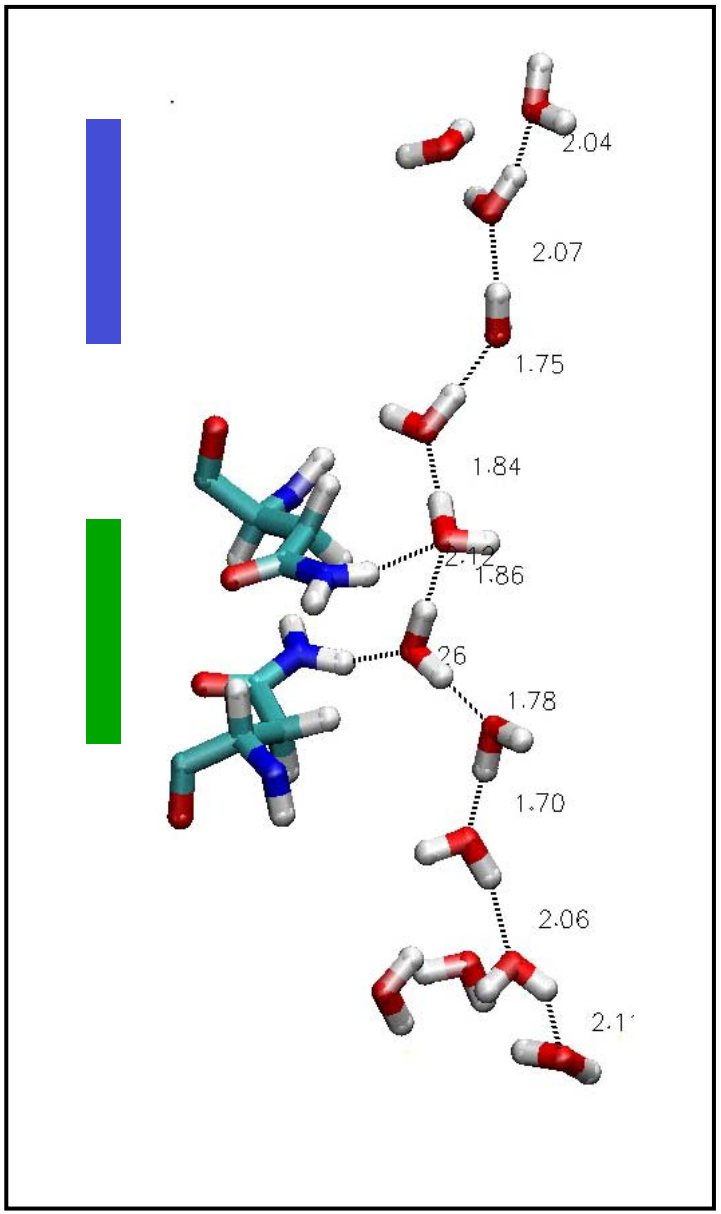




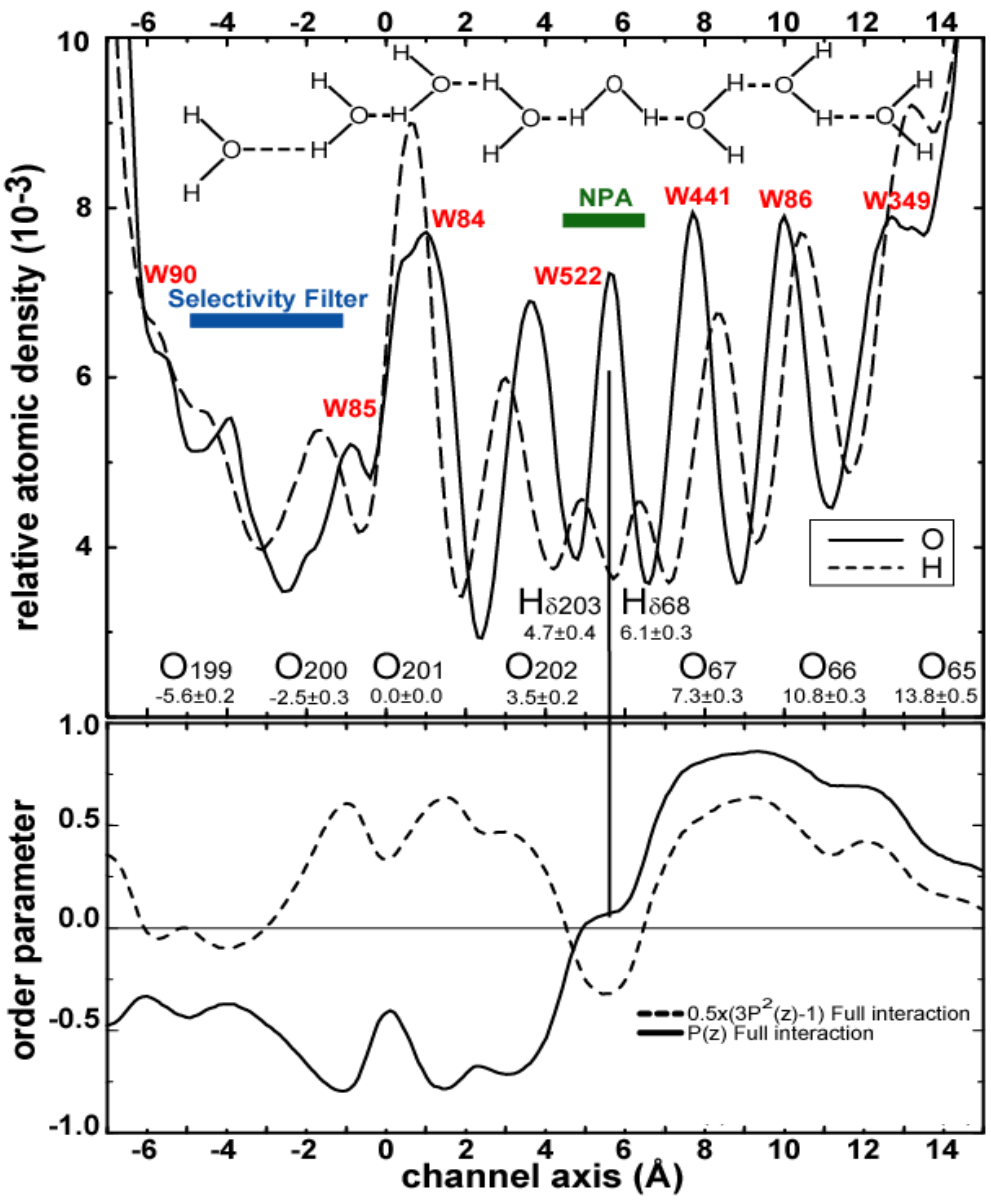
Improvement of statistics

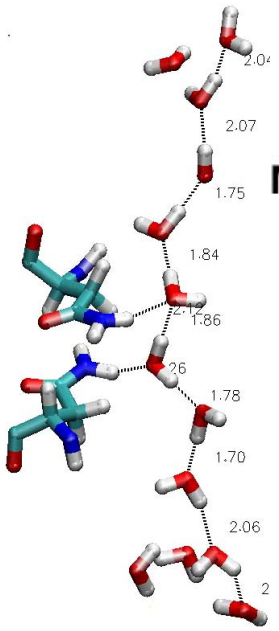
Water Bipolar Configuration in Aquaporins

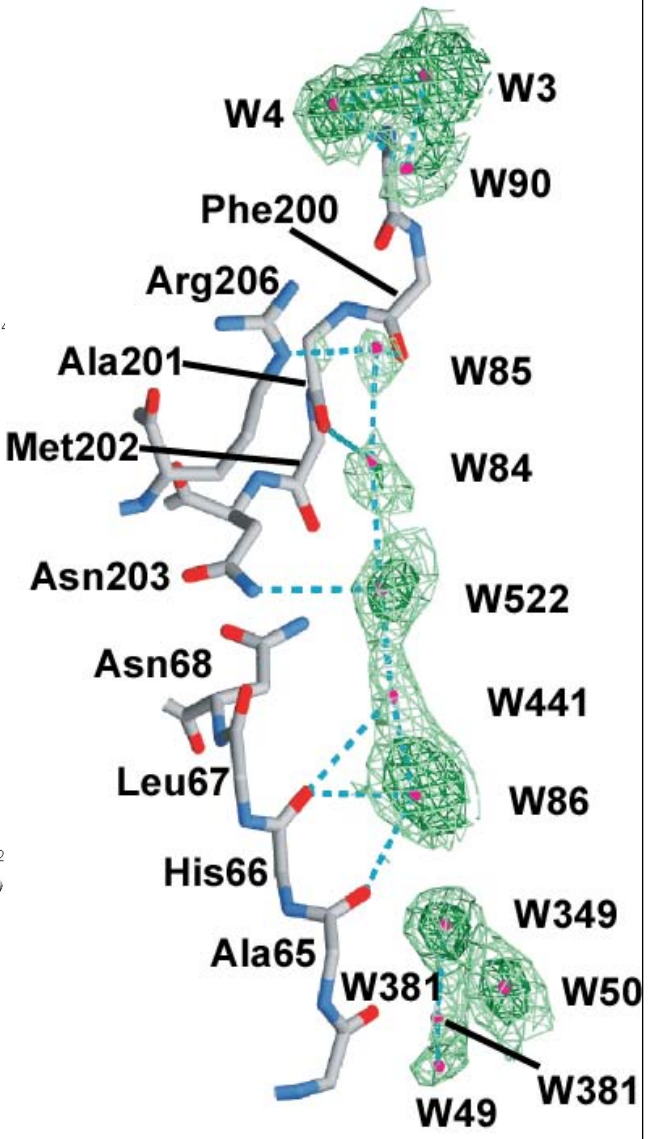


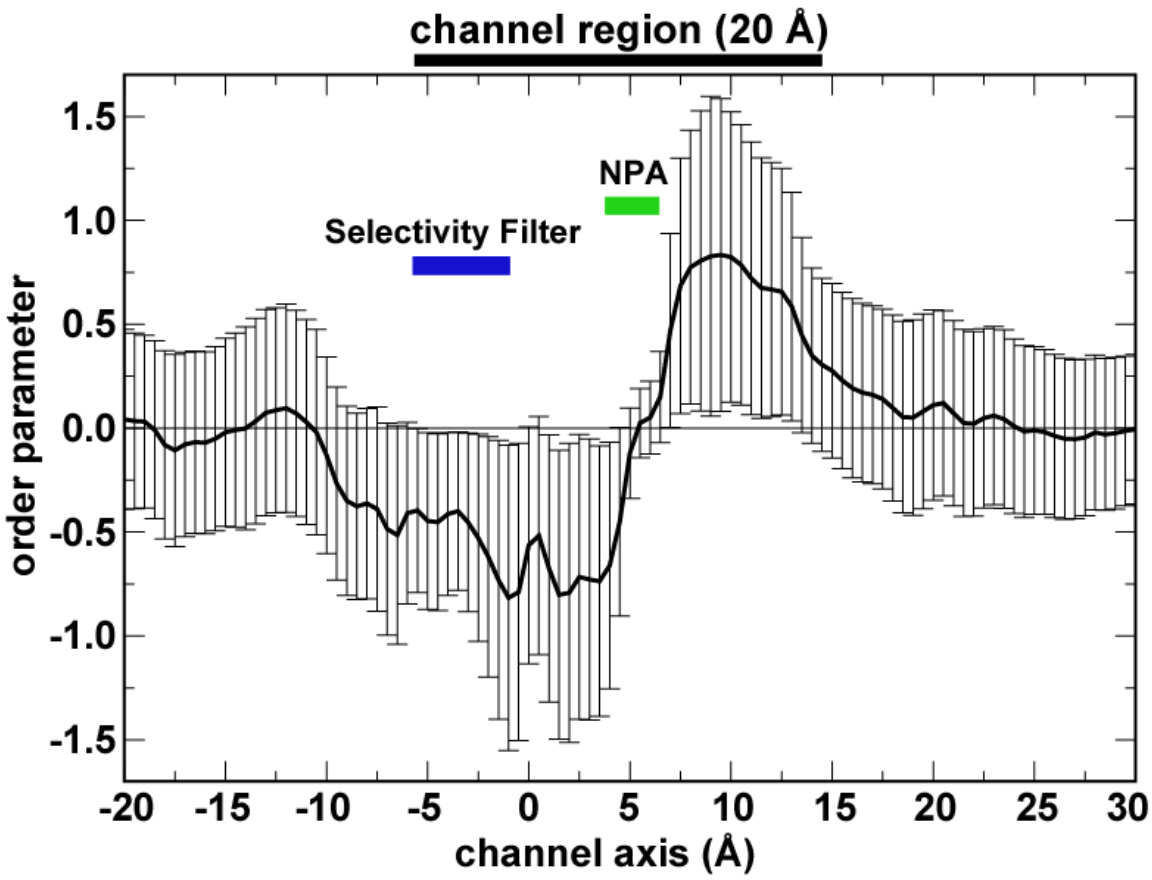


Water Bipolar Configuration in Aquaporins







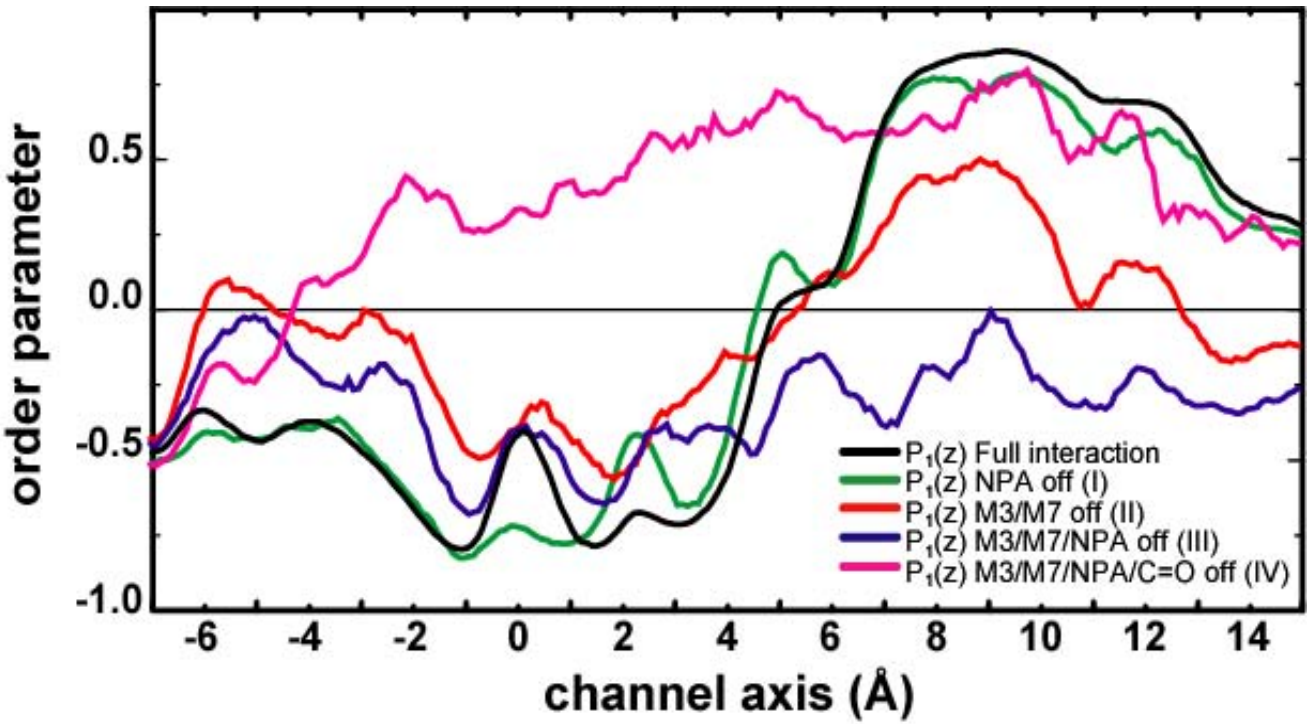

channel region (20 Å)
NPA
Selectivity Filter
order parameter
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-20
-15
-10
-5
0
5
10
15
20
25
30
channel axis (Å)

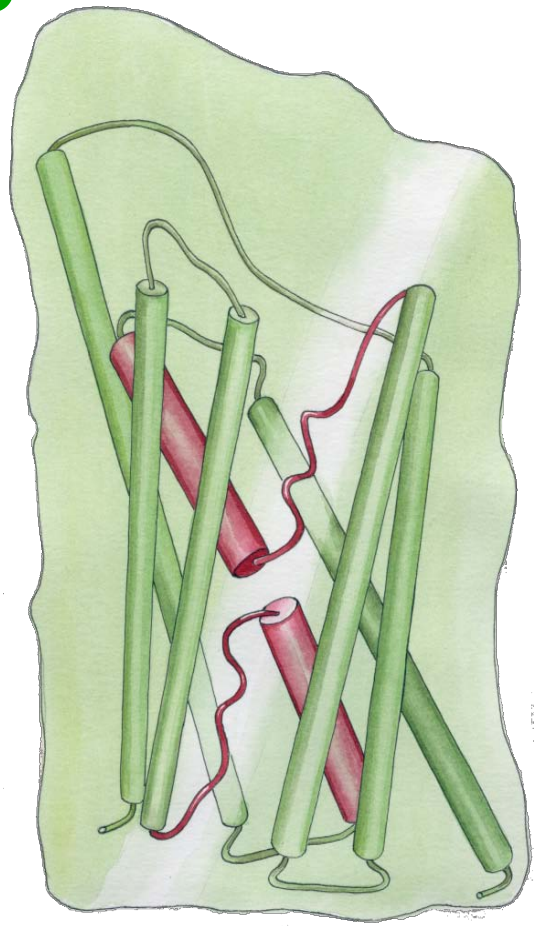
R E M E M B E R:

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!

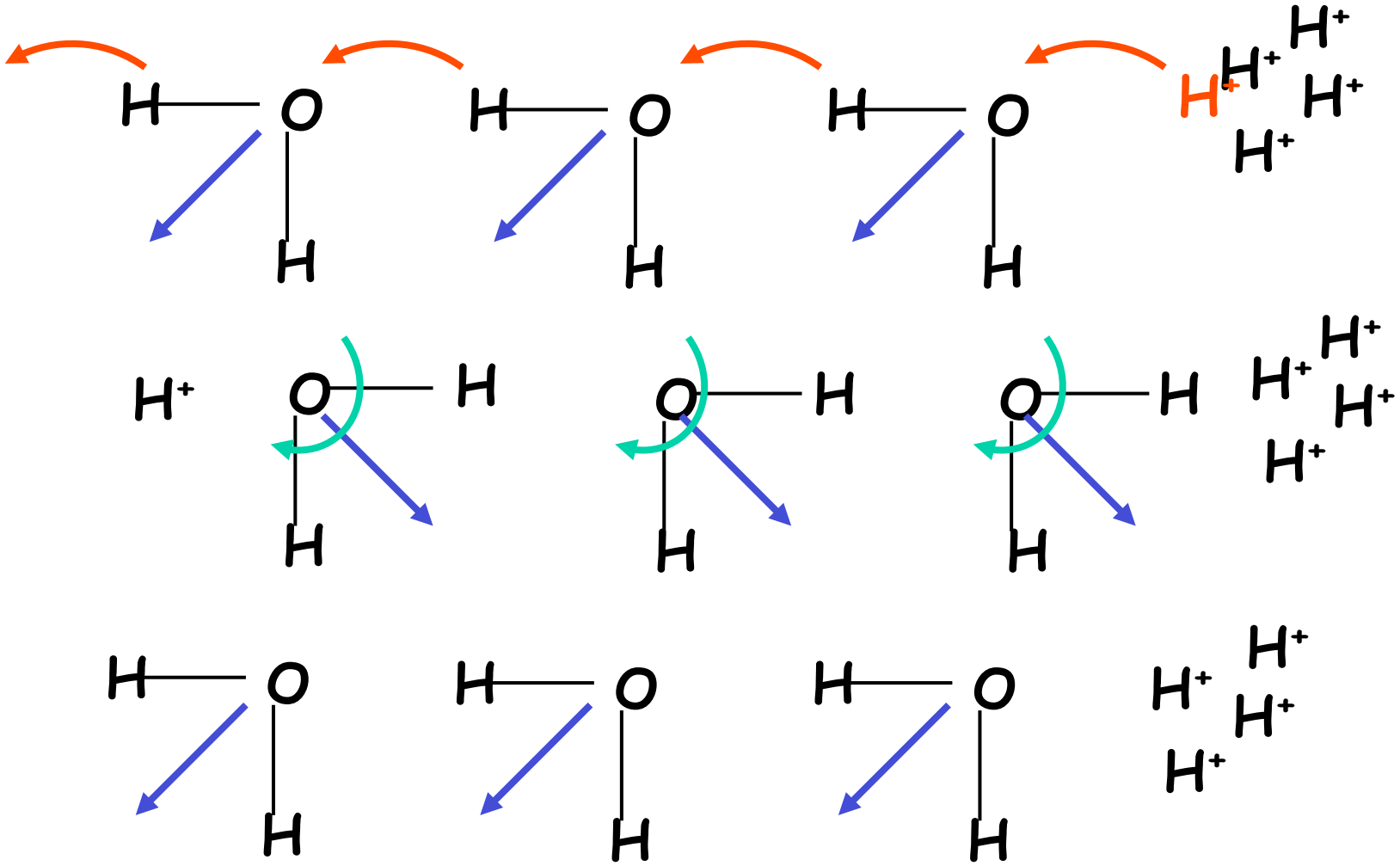
This is a powerful technique to test hypotheses developed during your simulations. Use it!

Electrostatic Stabilization of Water Bipolar Arrangement

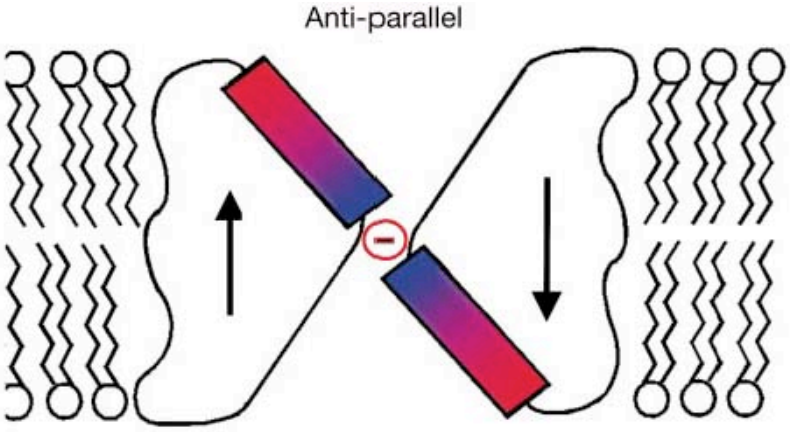


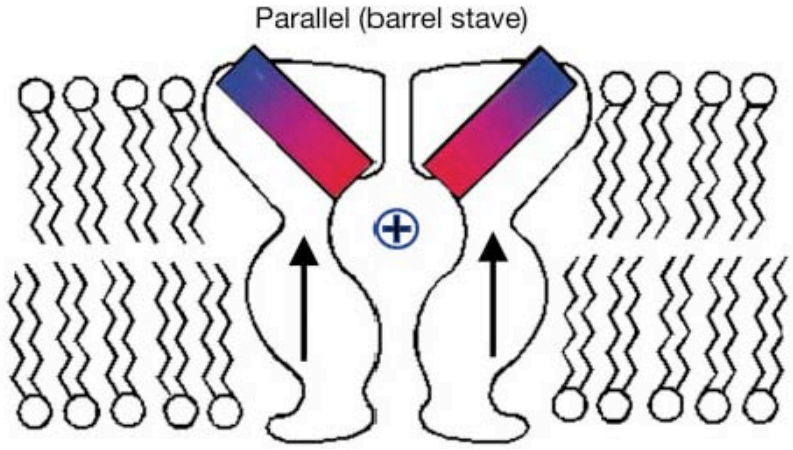


Proton transfer through water

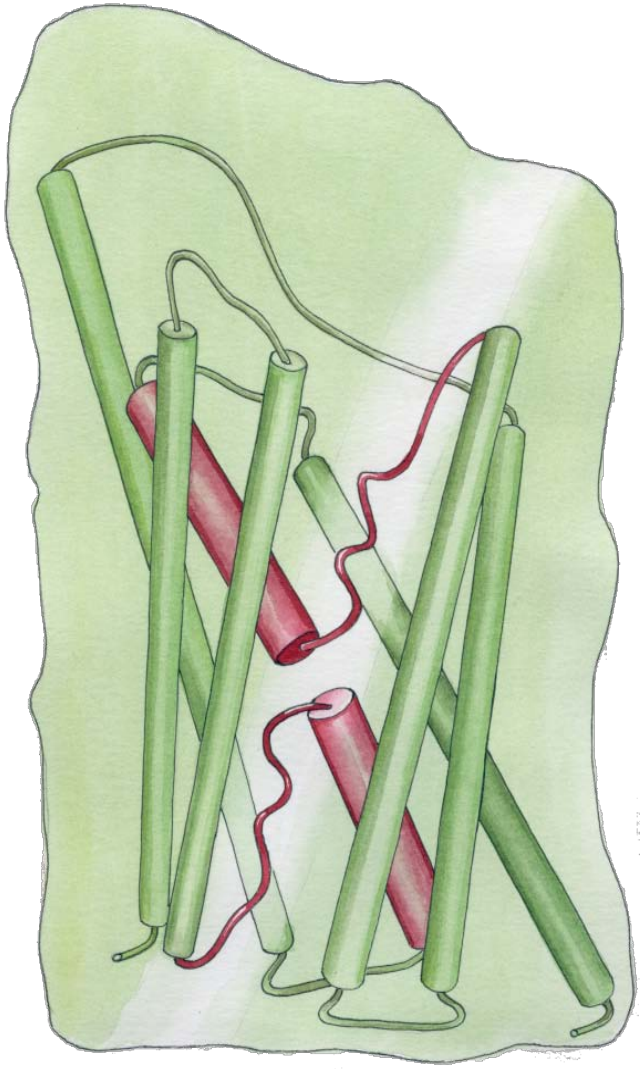


Cl^- channel

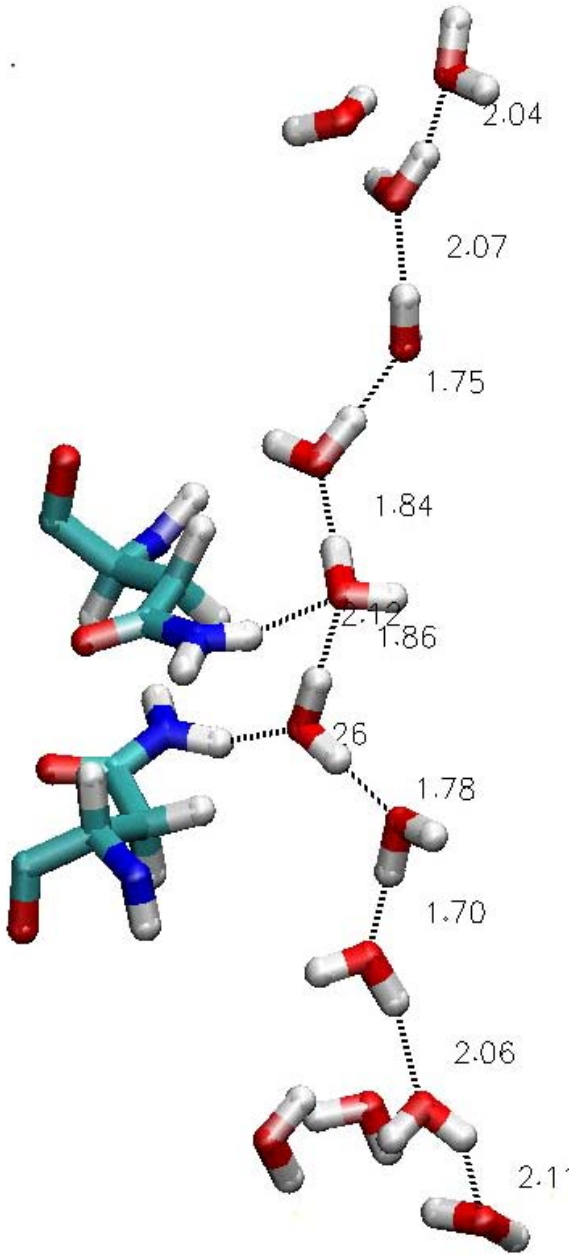


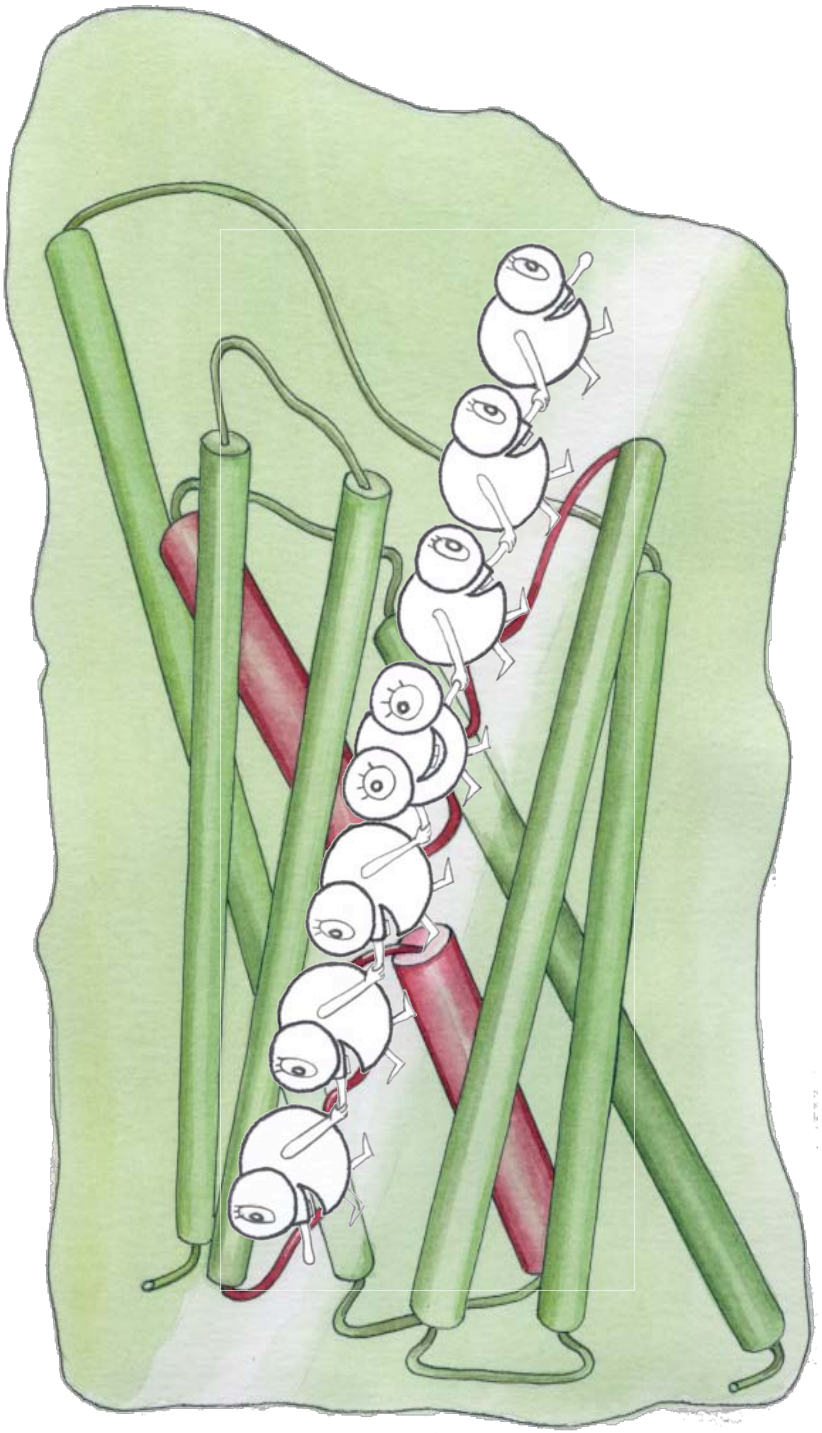


K^+ channel

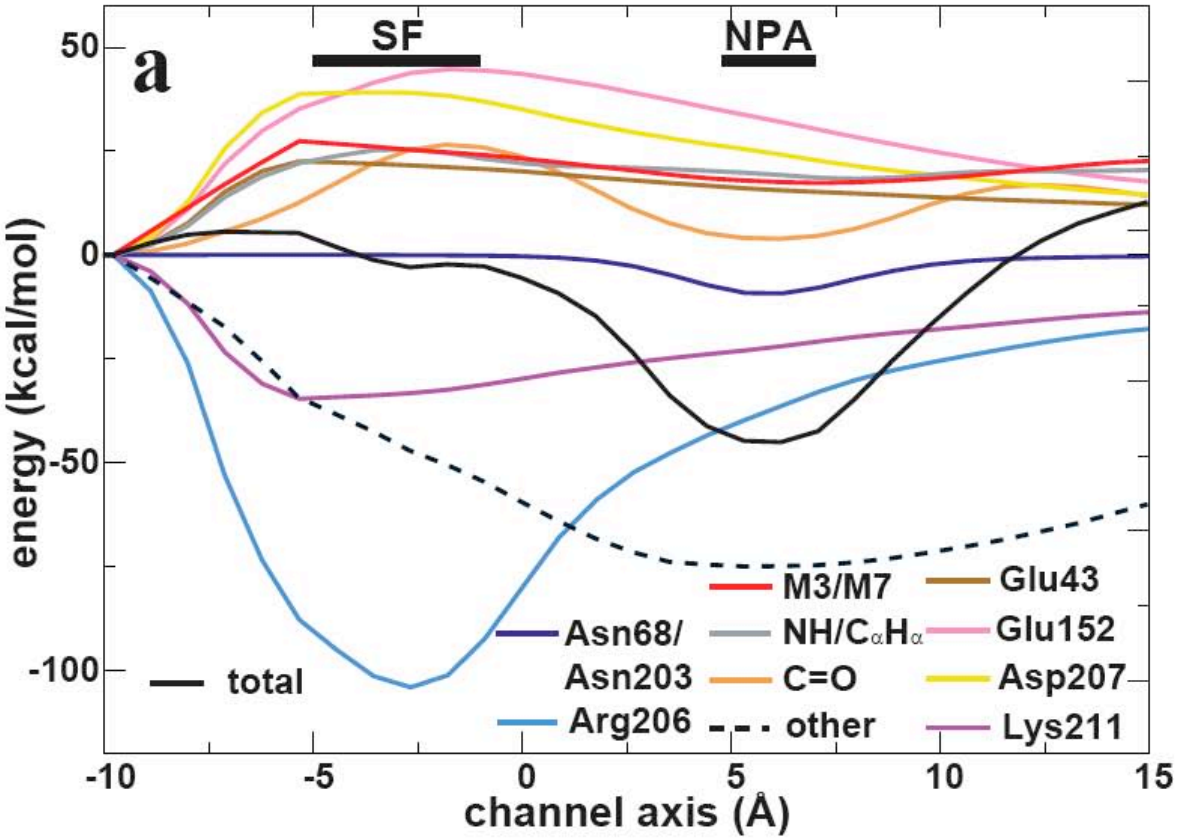


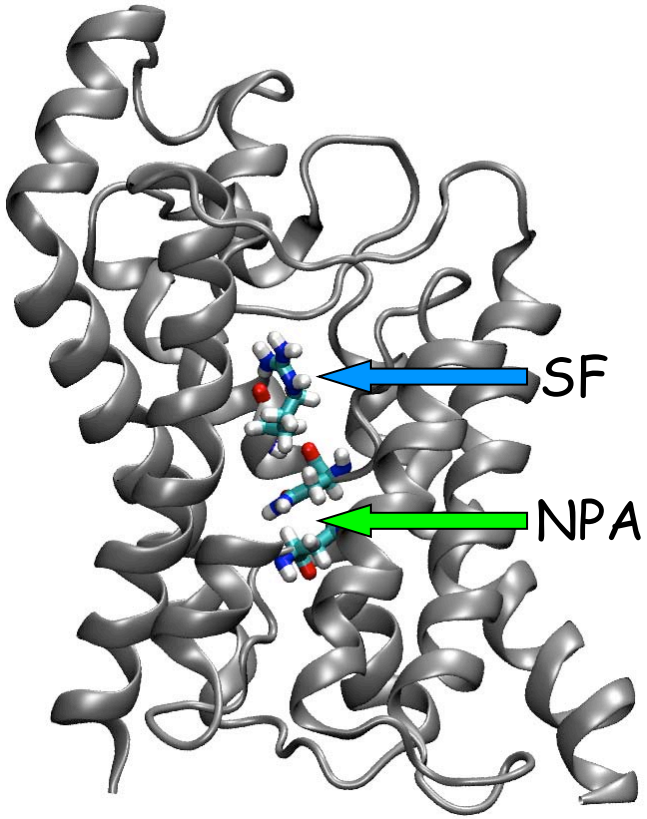
Aquaporins


2.04
2.07
1.75
1.84
2.12
1.86
26
1.78
1.70
2.06
2.1



A Complex Electrostatic Interaction

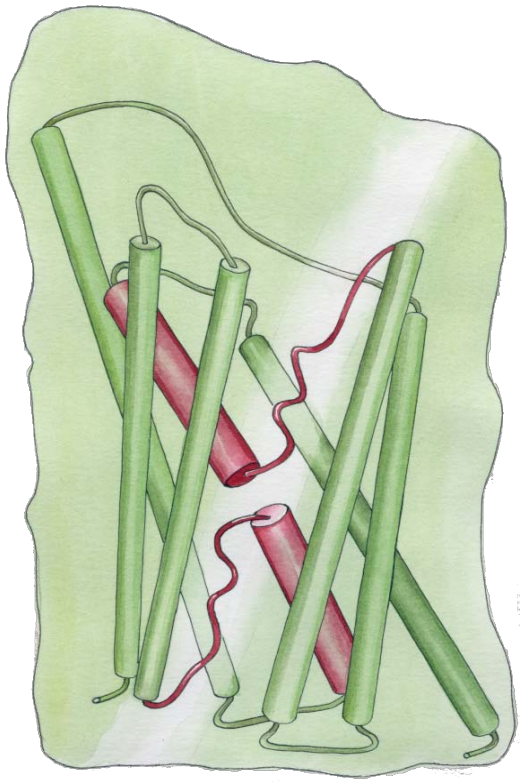


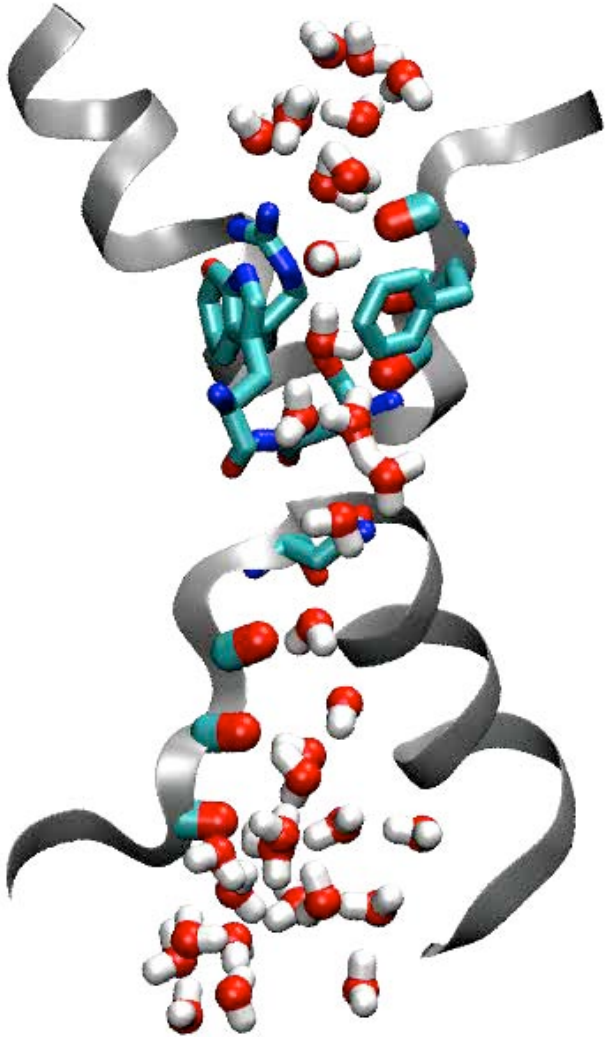


"Surprising and clearly not a hydrophobic channel"

M. Jensen, E. Tajkhorshid, K. Schulten, *Biophys. J.* **85**, 2884 (2003)

A Repulsive Electrostatic Force at the Center of the Channel





QM/MM MD of the behavior of an excessive proton