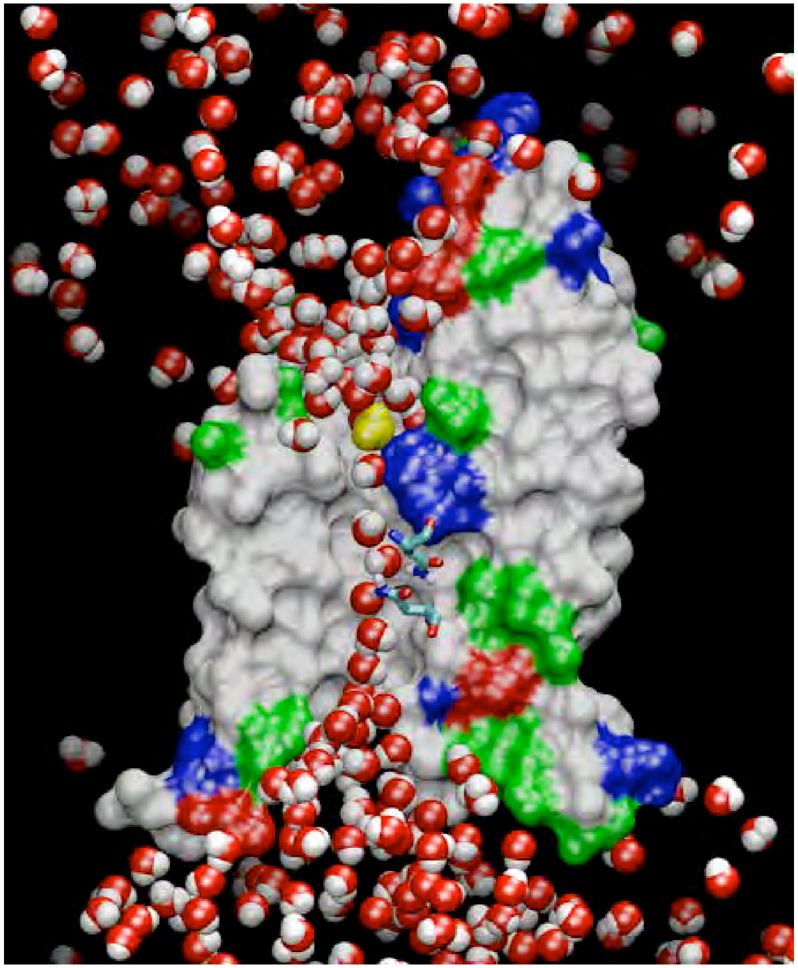
Molecular Dynamics Studies of Mechanisms of Permeation, Selectivity, and Gating in Membrane Channels



Emad Tajkhorshid
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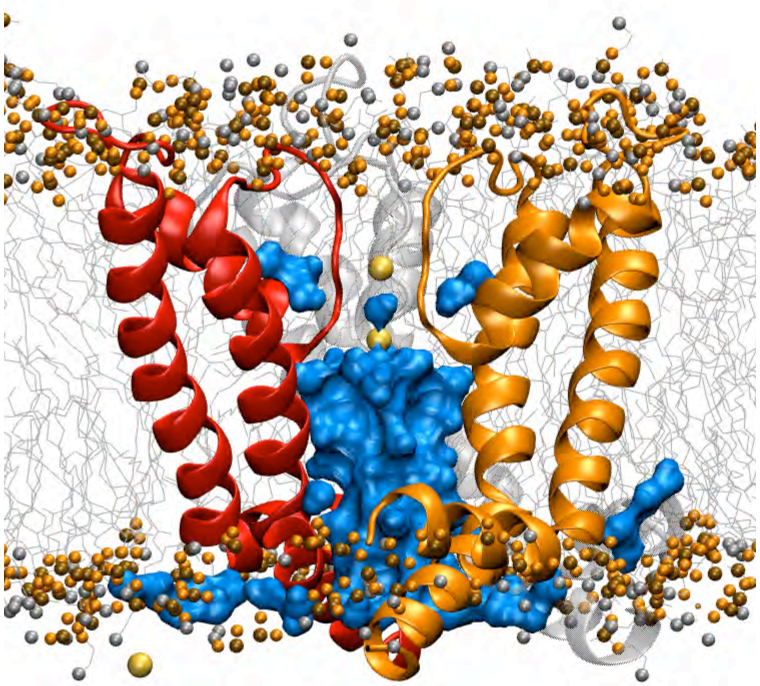
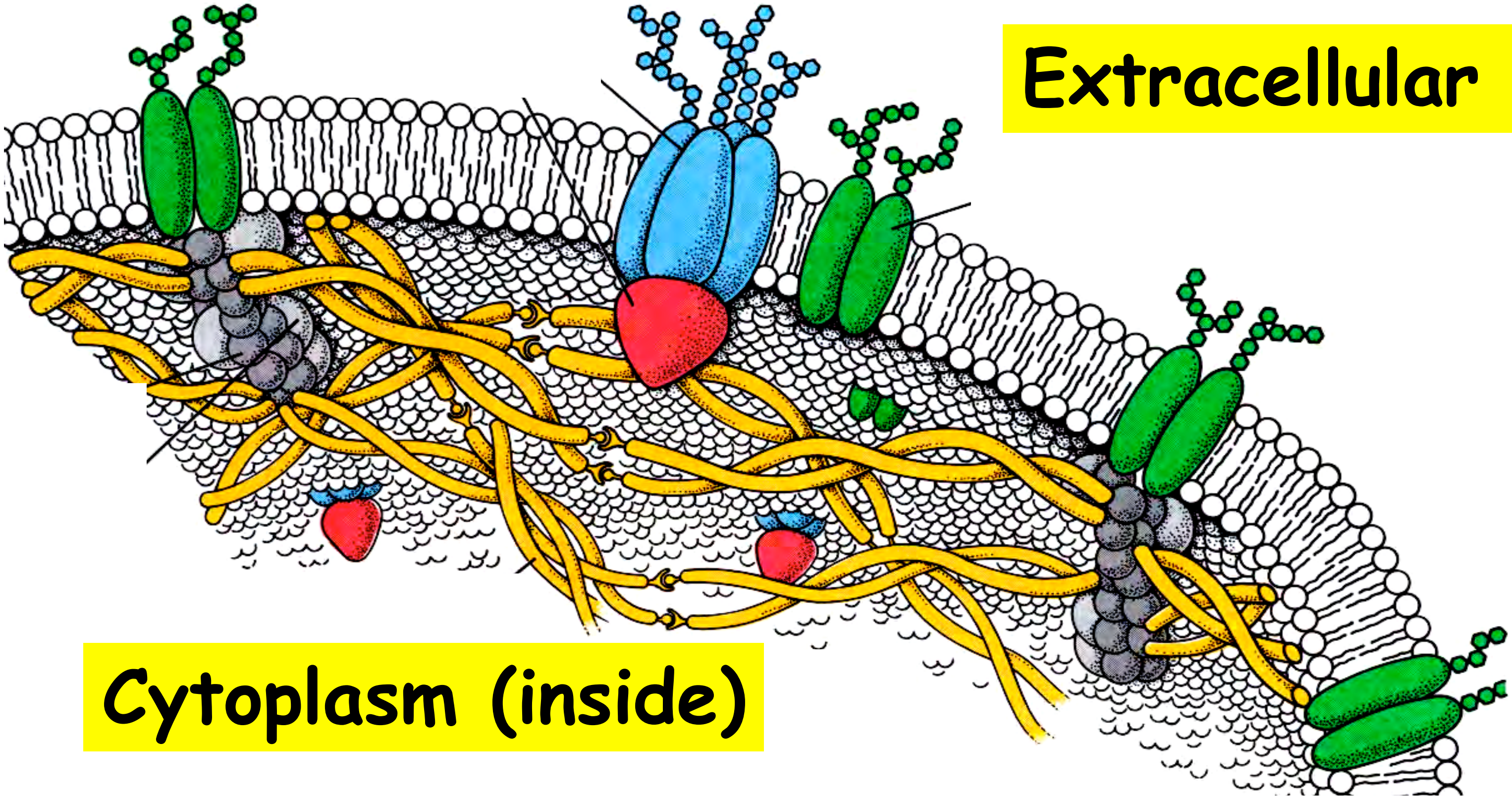
University of Illinois at Urbana-Champagin



Why Do Living Cells Need Membrane Channels (Proteins)?

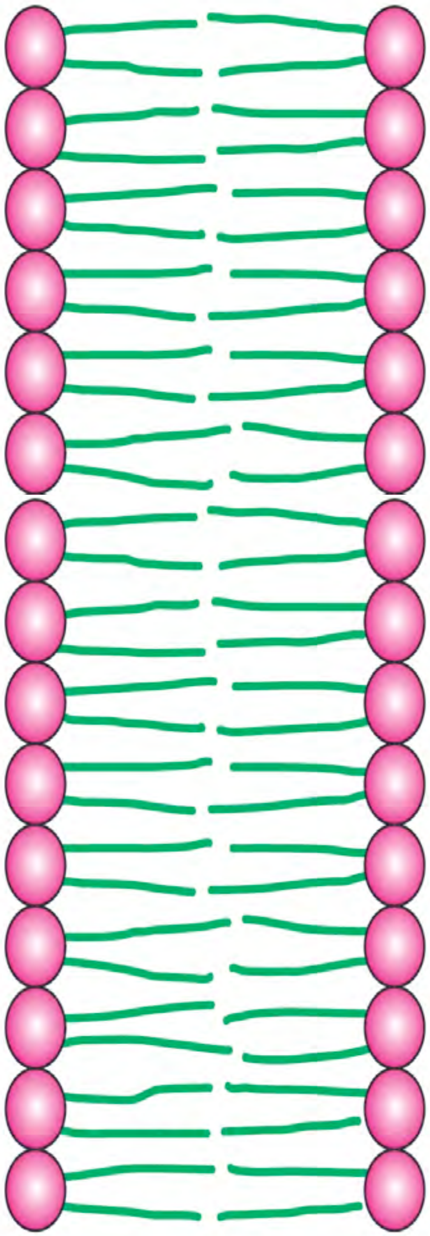
- Living cells also need to exchange materials and information with the outside world

… however, in a highly selective manner.

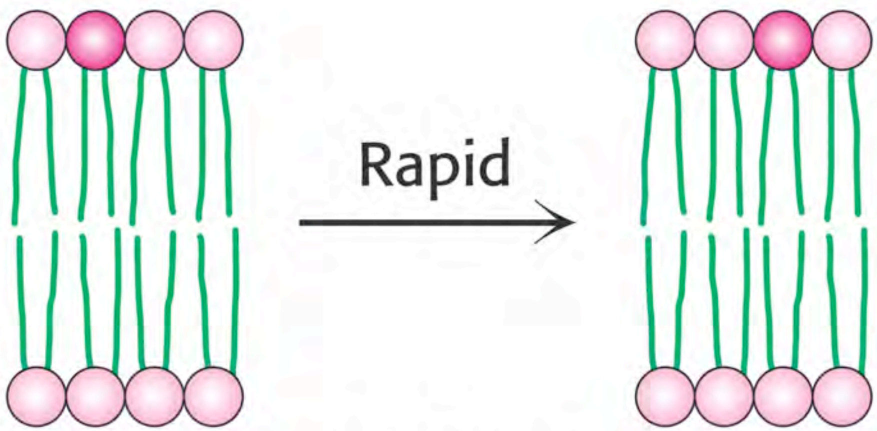


Lipid Bilayers Are Excellent For Cell Membranes

- 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 Membrane



Lateral diffusion

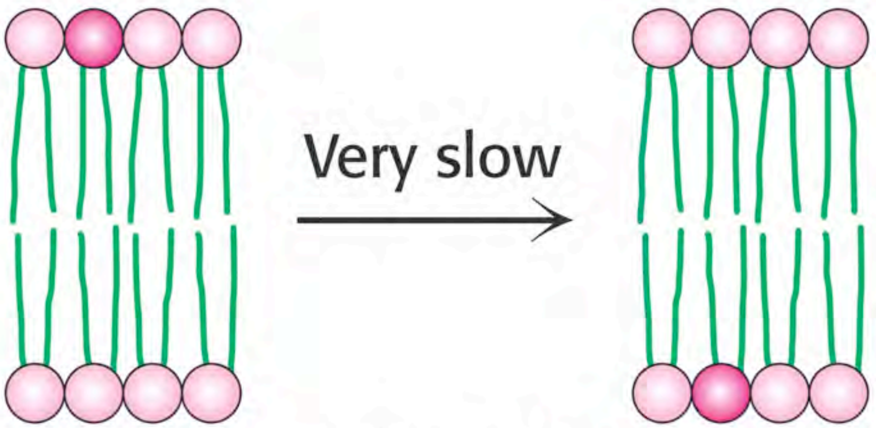
$D = 1\ \mu m^2.s^{-1}$

$50\ Å$ in ~ 2.5×10^{-5} s

~9 orders of magnitude difference

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

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

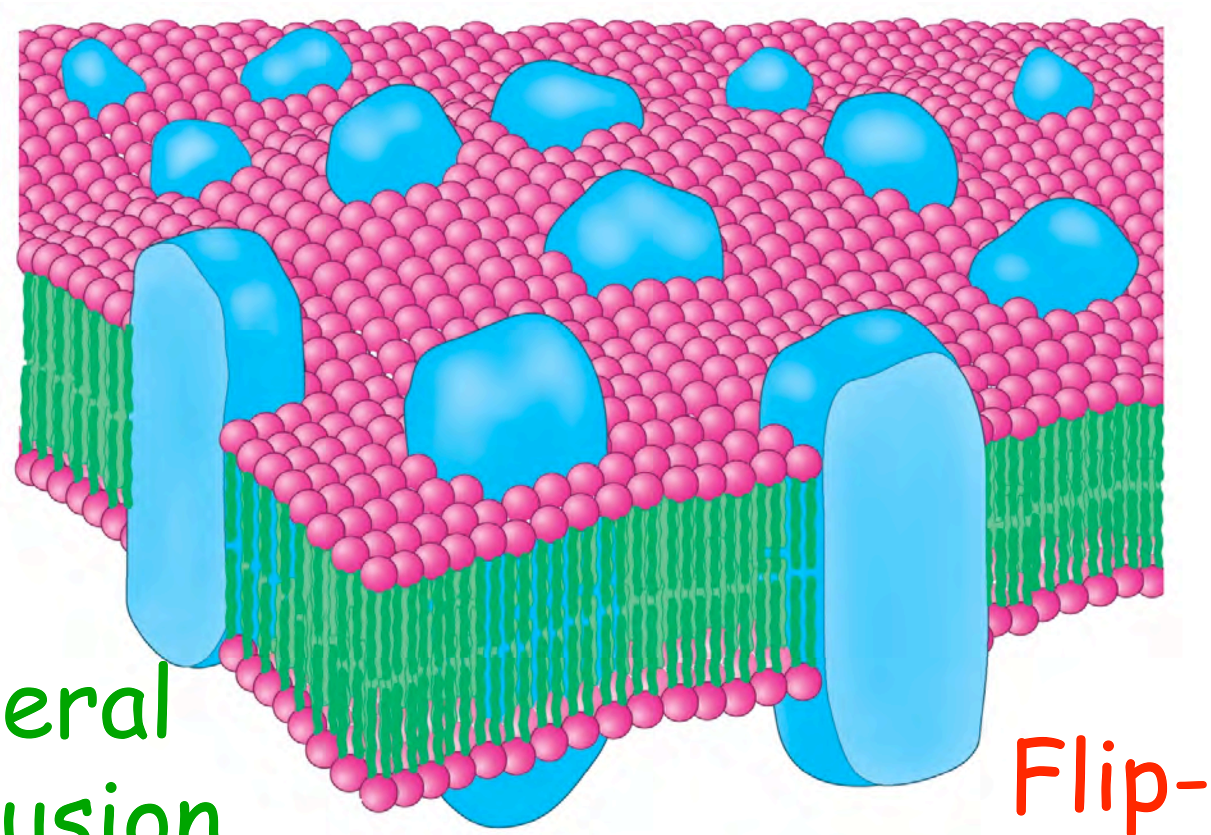


Tranverse diffusion (flip-flop)

Once in several hours!

(10^4 s)

Fluid Mosaic Model of Membrane



Lateral Diffusion Allowed

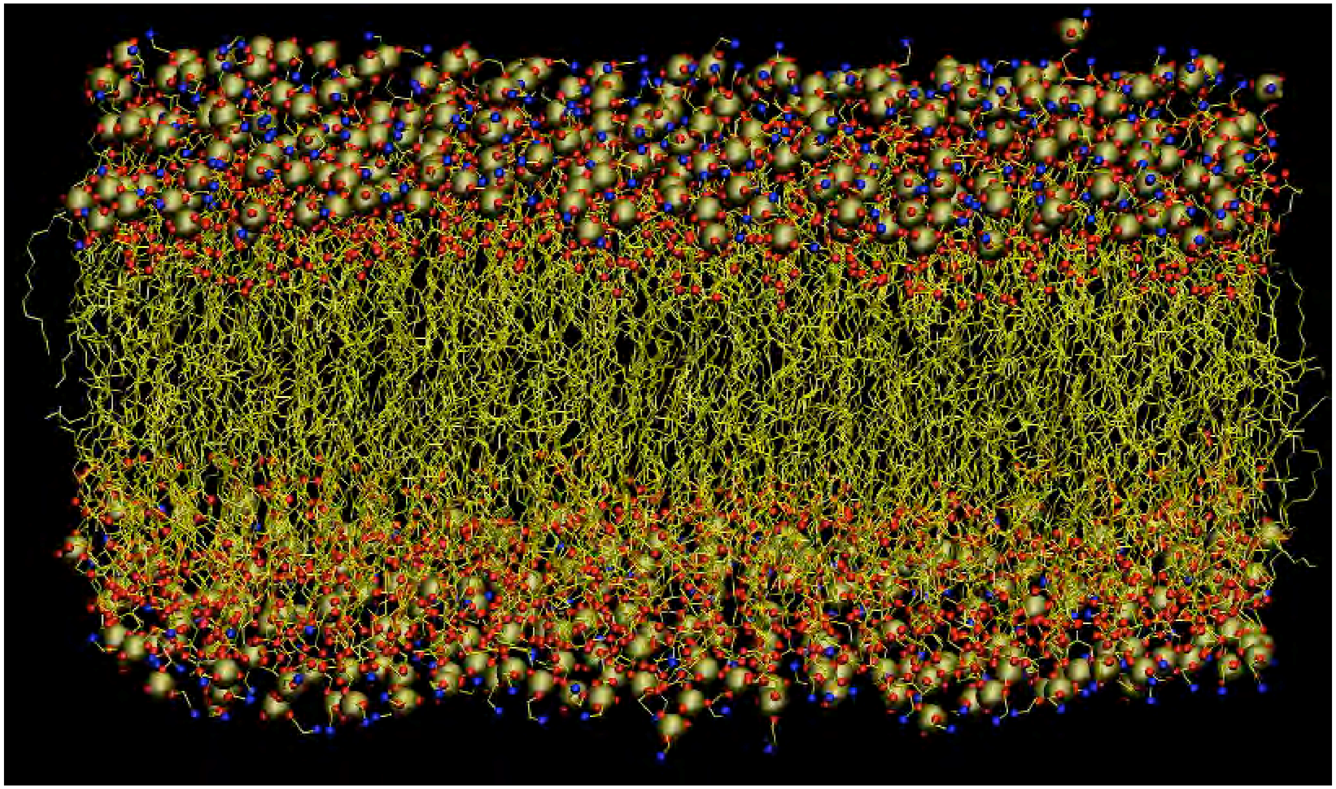
Flip-flop Forbidden

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

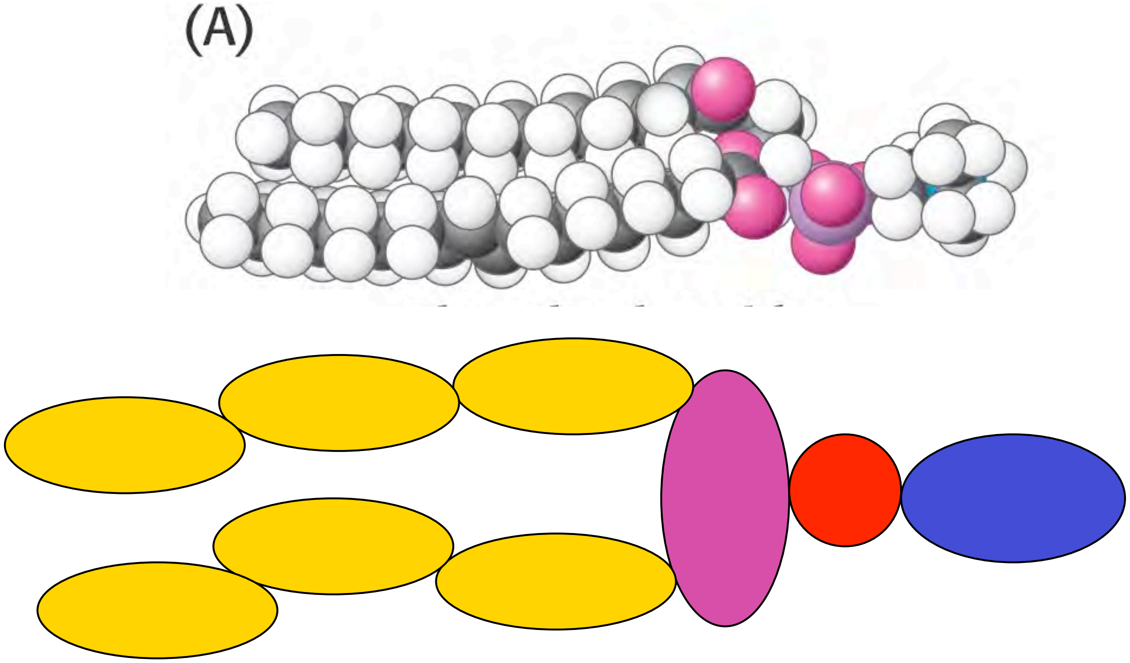


Coarse grain modeling of lipids

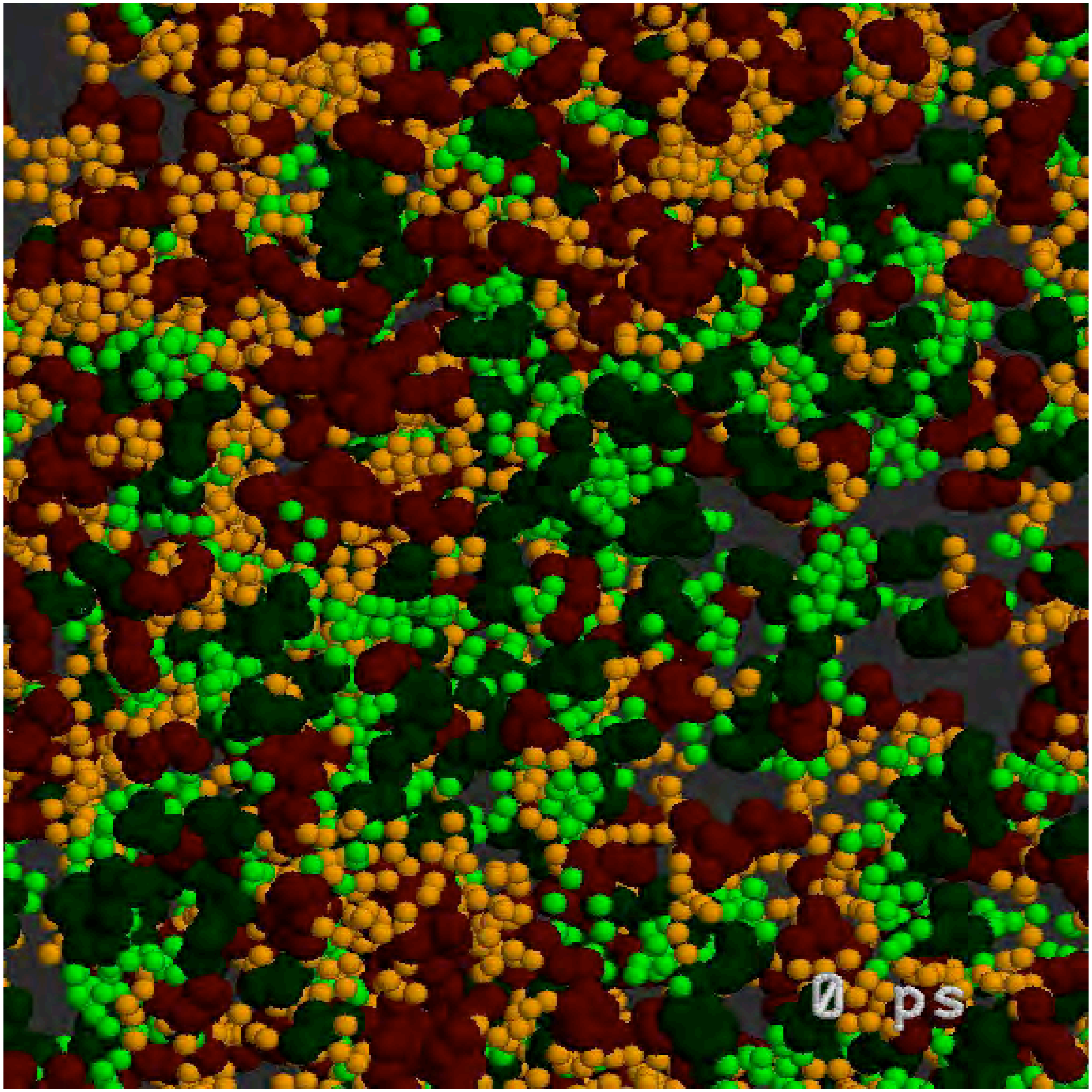
150 particles

↓

9 particles!



Also, increasing the time step by orders of magnitude.



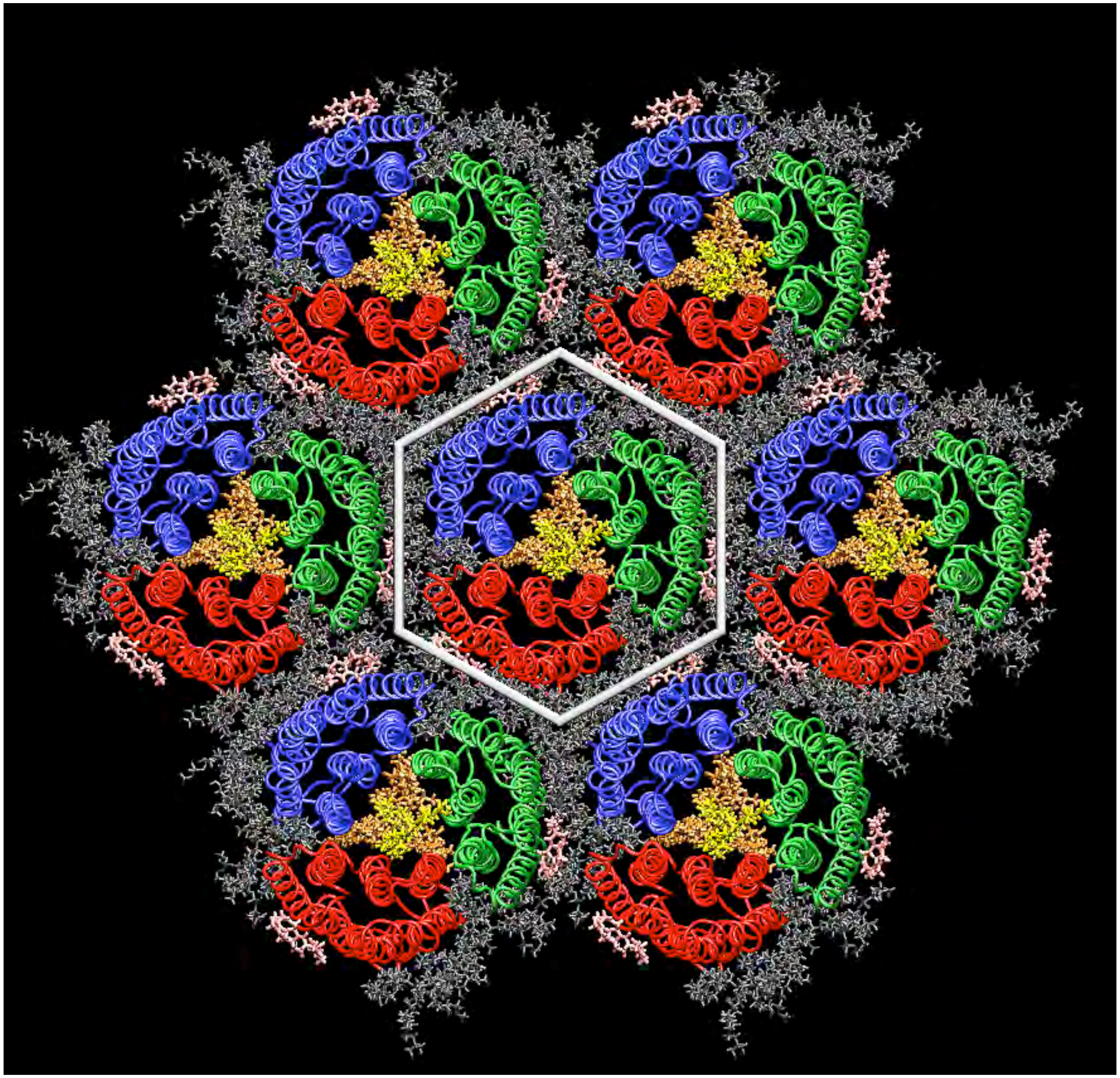
by: J. Siewert-Jan Marrink and Alan E. Mark, University of Groningen, The Netherlands

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

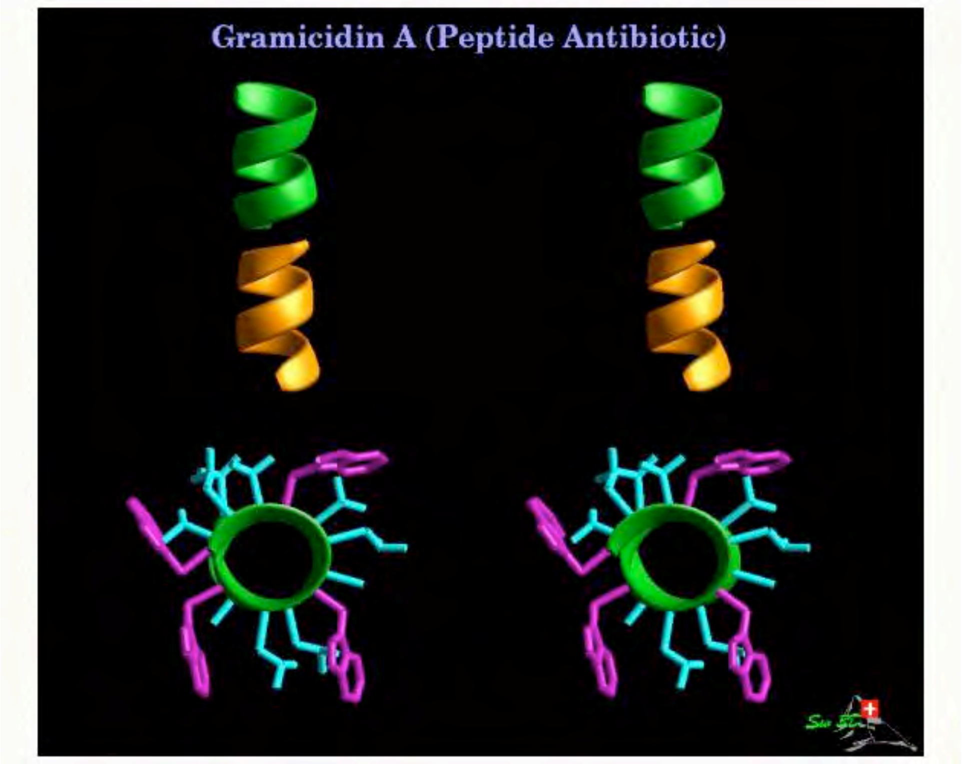
- Different functions = different protein composition

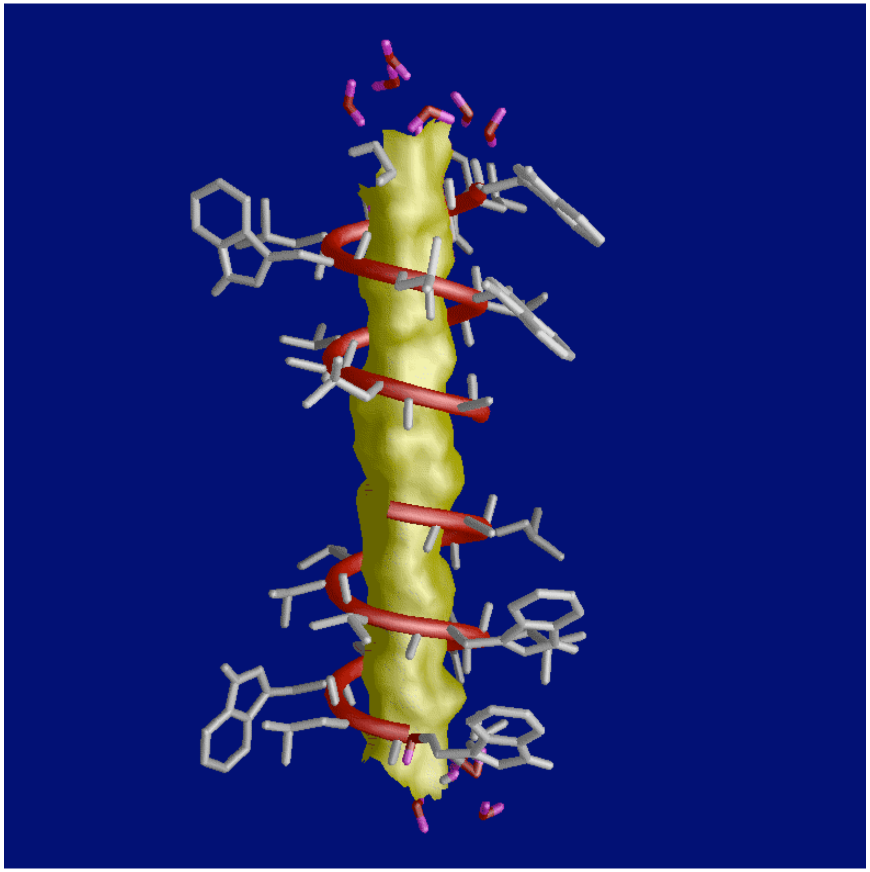
Protein / Lipid Composition



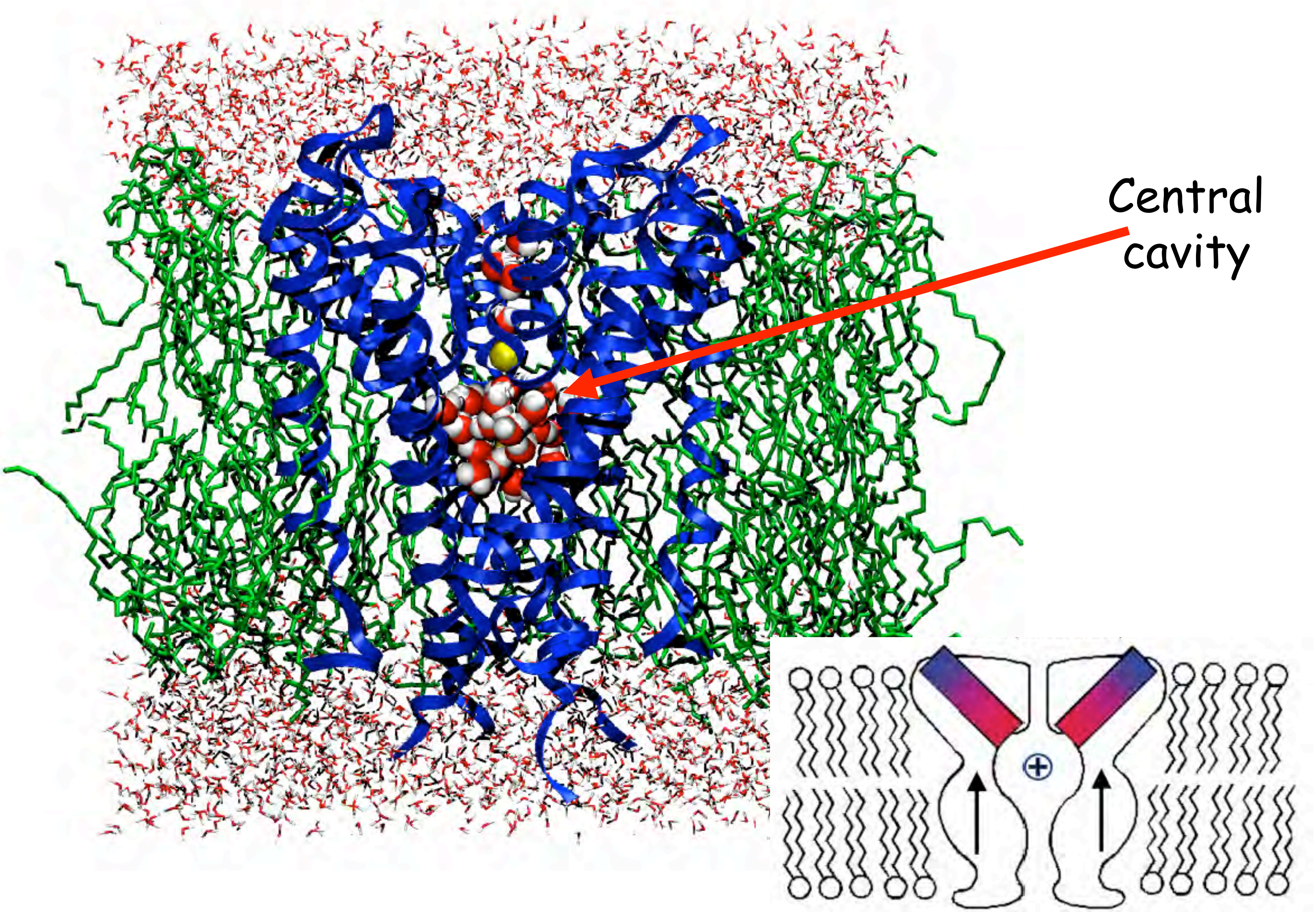
The purple membrane of halobacteria

Gramicidin A
an ion leak inside the membrane





Through dissipating the electrochecmical potential of membrane, gramicidin A acts as an antibiotic.

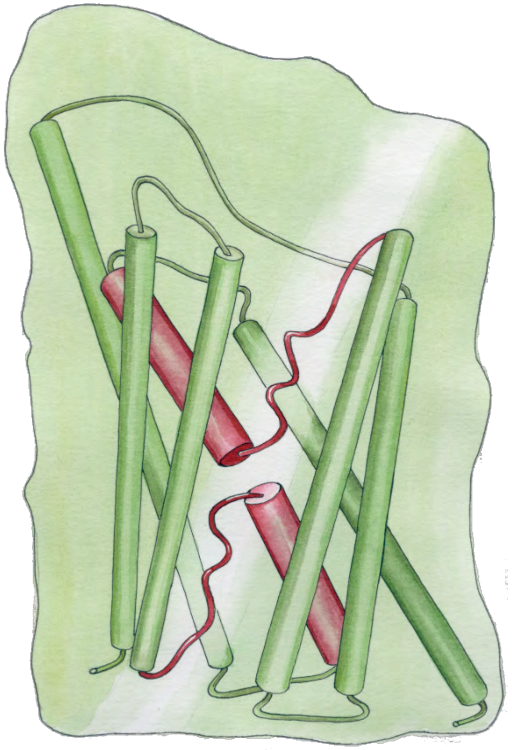

Central cavity

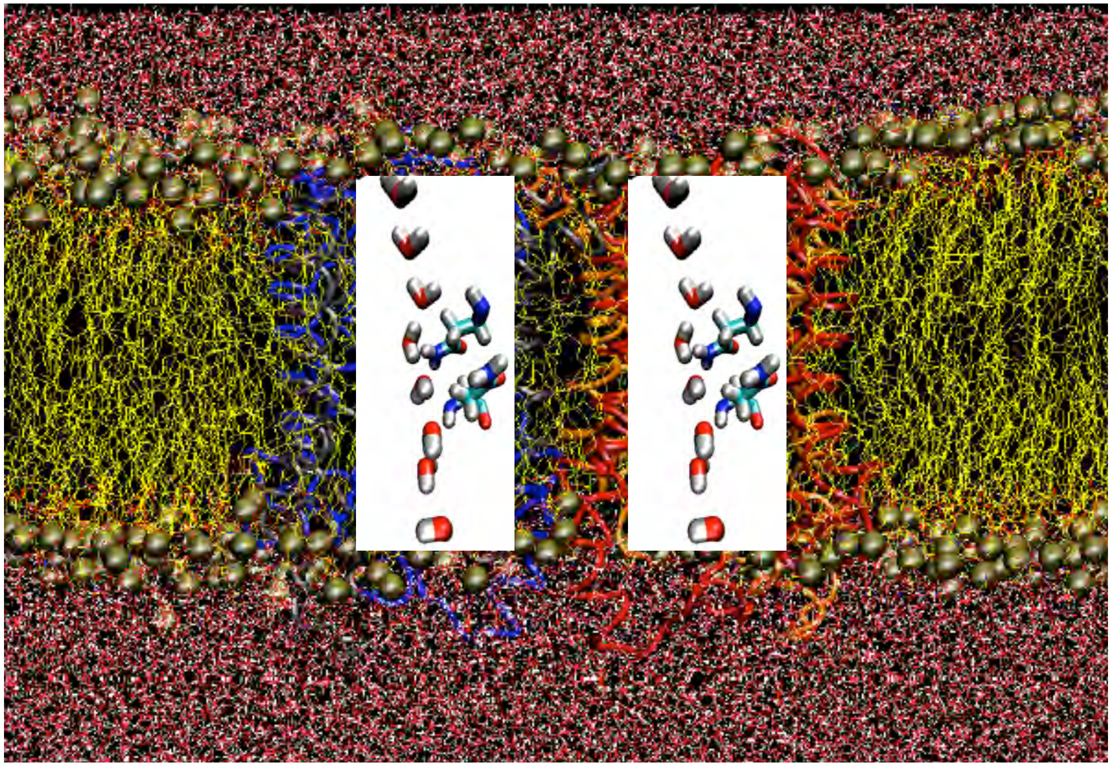
Analysis of Molecular Dynamics Simulations of Biomolecules

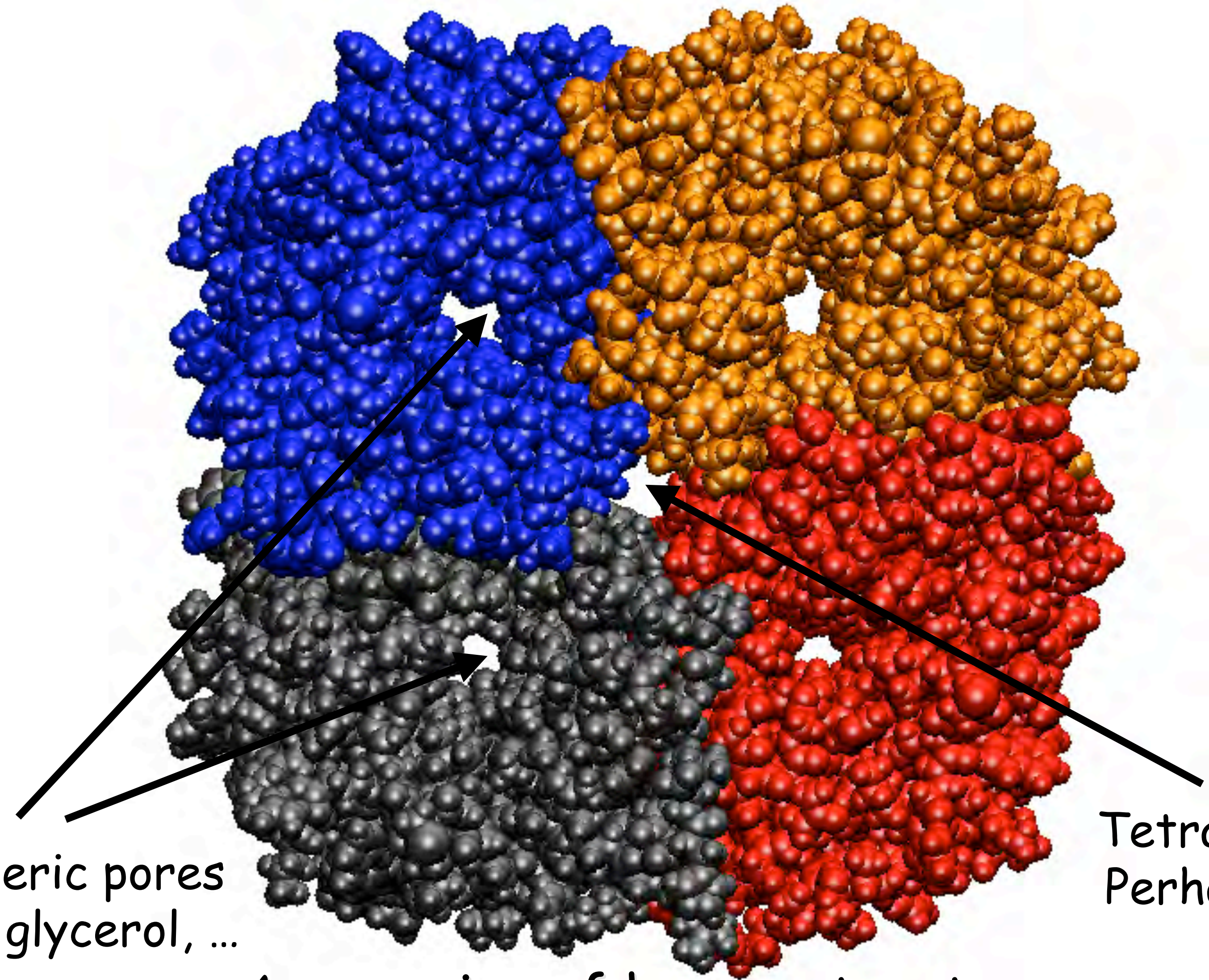
- A very complicated arrangement of hundreds of groups interacting with each other
- Where to start to look at?
- What to analyze?
- How much can we learn from simulations?

It is very important to get acquainted with your system

Aquaporins
Membrane water channels







Monomeric pores
Water, glycerol, …

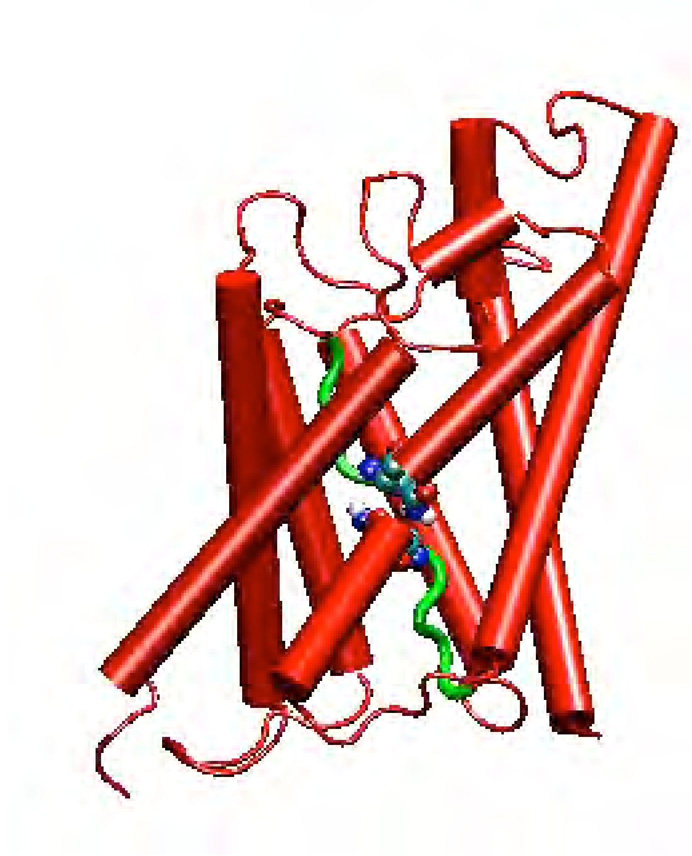
Tetrameric pore
Perhaps ions???

Aquaporins of known structure:

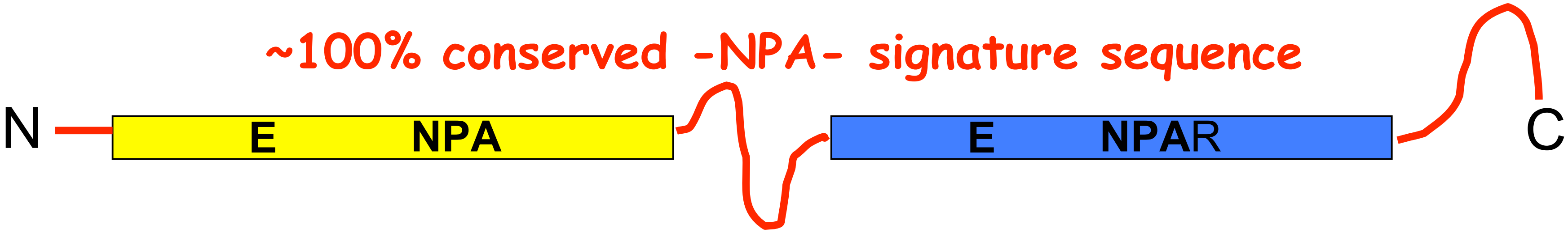
GlpF – E. coli glycerol channel (aquaglycerolporin)
AQP1 – Mammalian aquaporin-1 (pure water channel)
AqpZ and AQP0 (2004)

Functionally Important Features

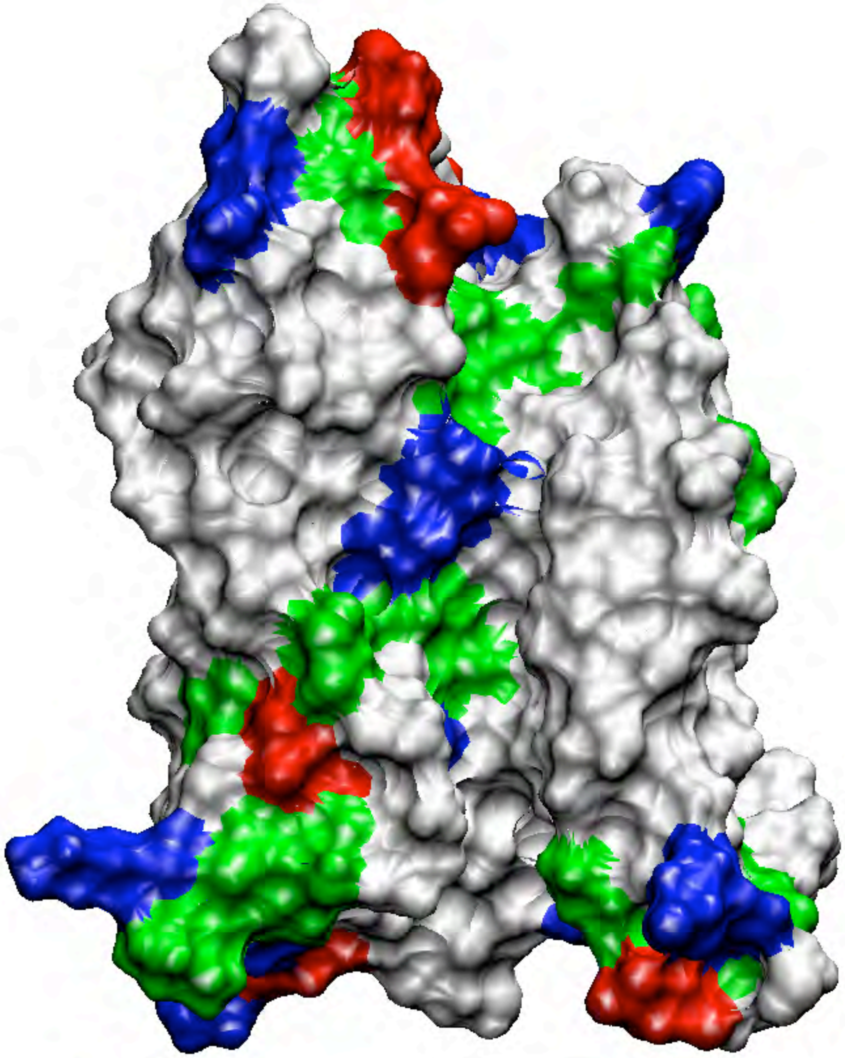
- Tetrameric architecture
- Amphipatic channel interior
- Water and glycerol transport
- Protons, and other ions are excluded
- Conserved asparagine-proline-alanine residues; NPA motif
- Characteristic half-membrane spanning structure



~100% conserved -NPA- signature sequence

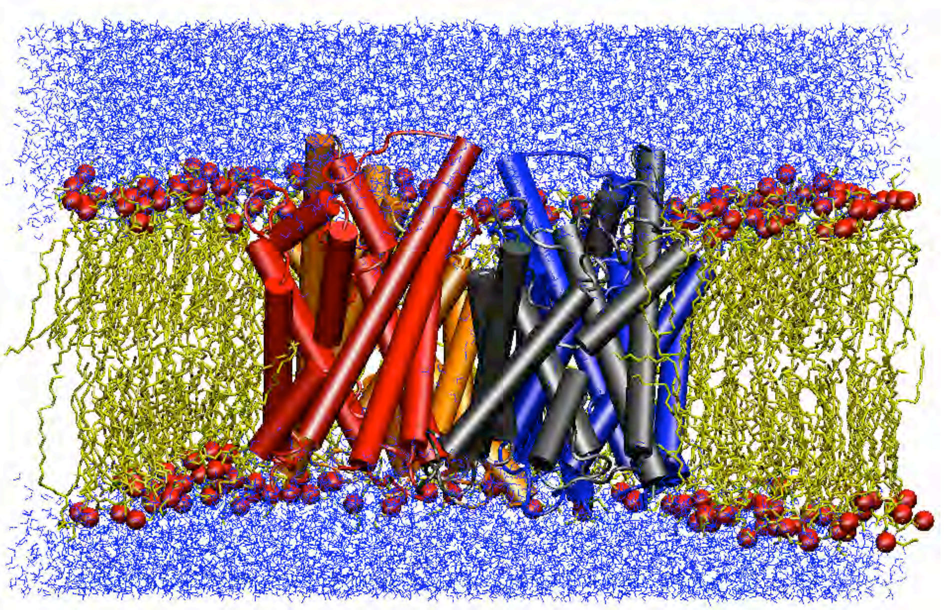


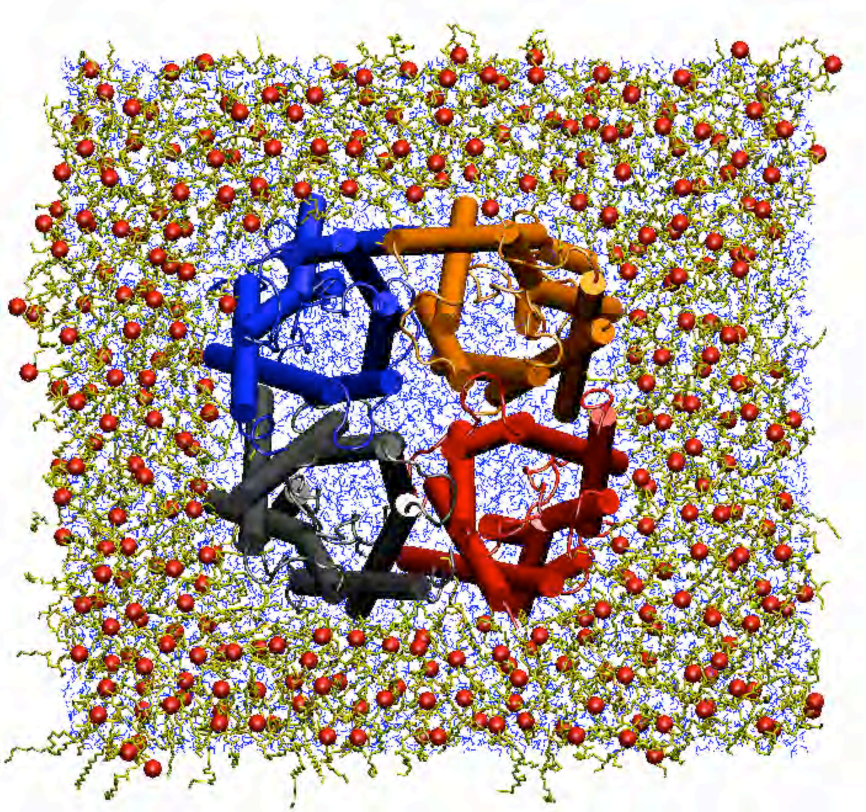
A Semi-hydrophobic channel



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

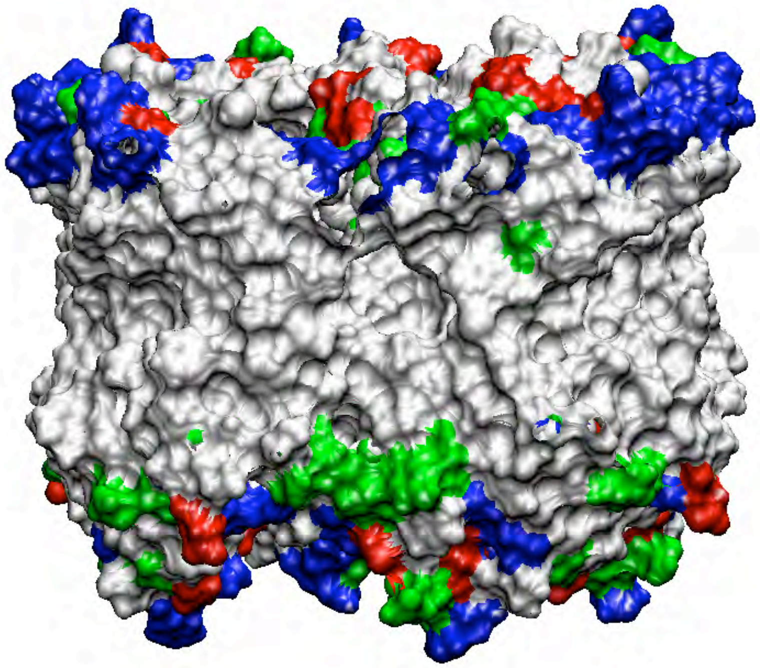
1ns equilibration, 4ns production

10 days /ns – 32-proc Linux cluster

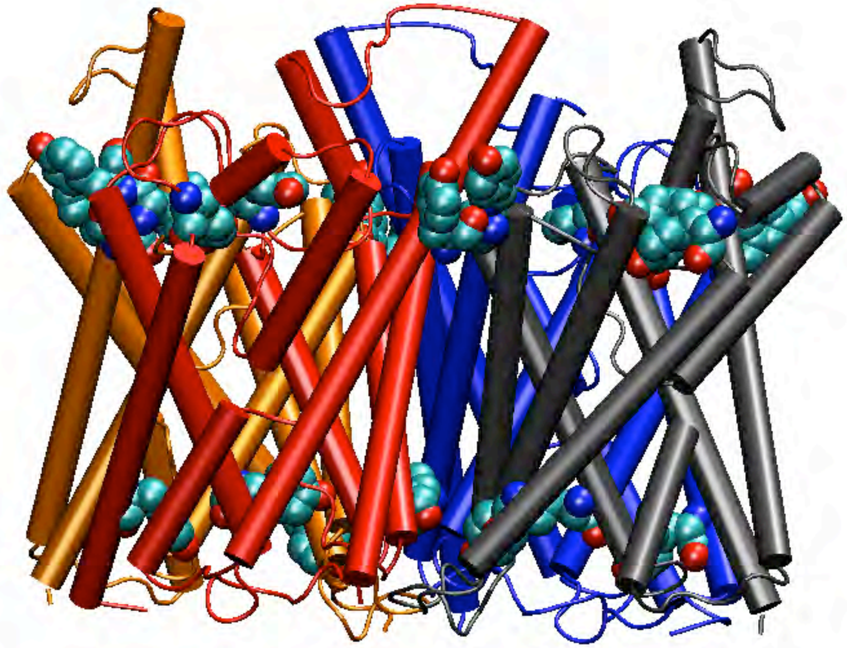
3.5 days/ns - 128 O2000 CPUs

0.35 days/ns – 512 LeMieux CPUs

Protein Embedding in Membrane

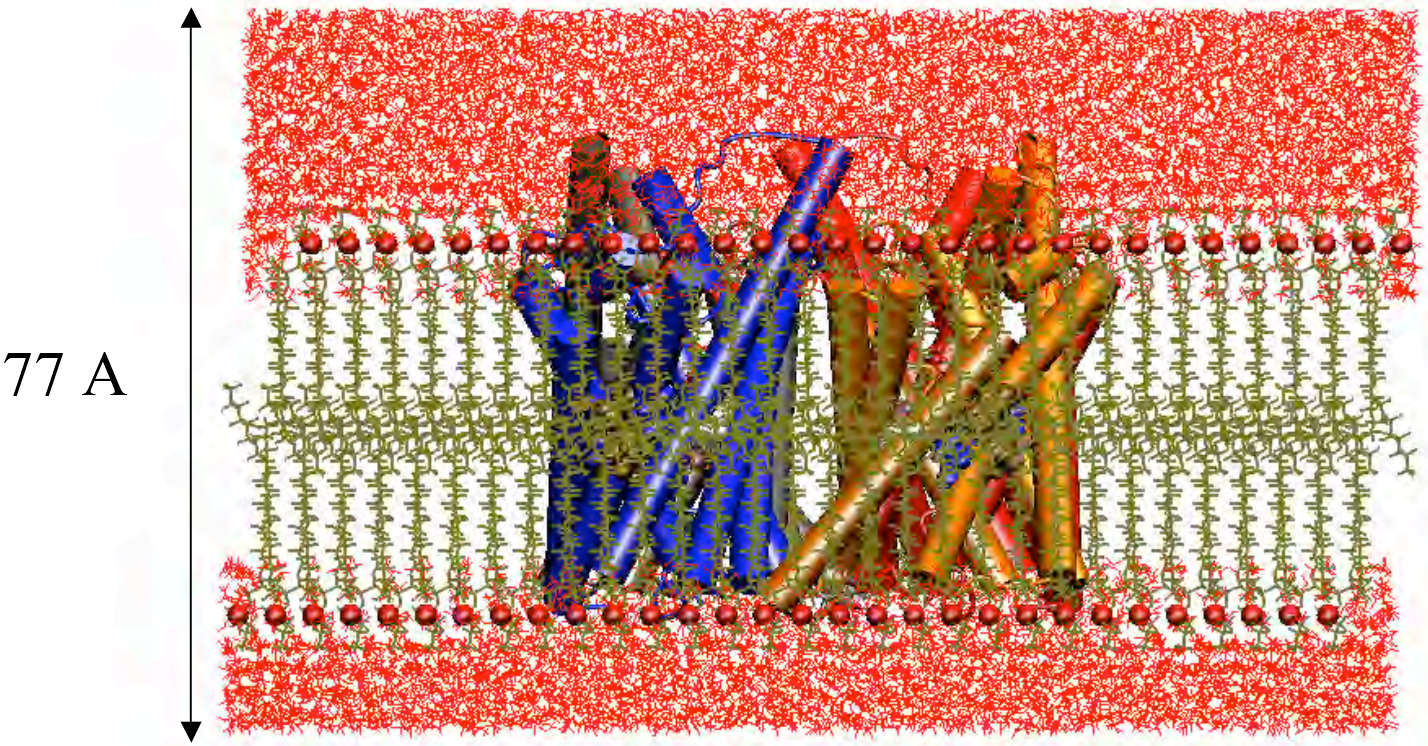


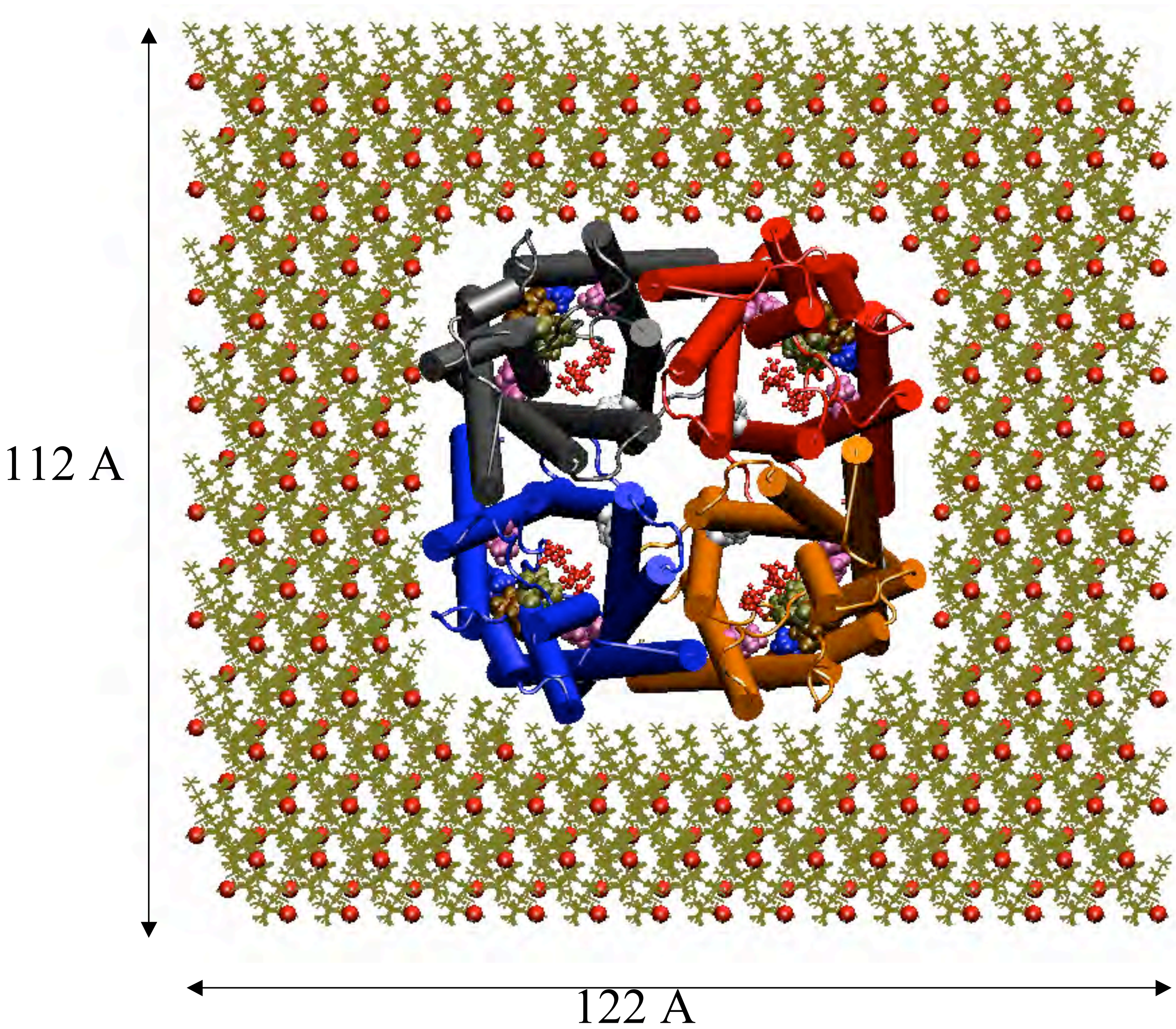
Hydrophobic surface
of the protein



Ring of
Tyr and Trp

Embedding GlpF in Membrane

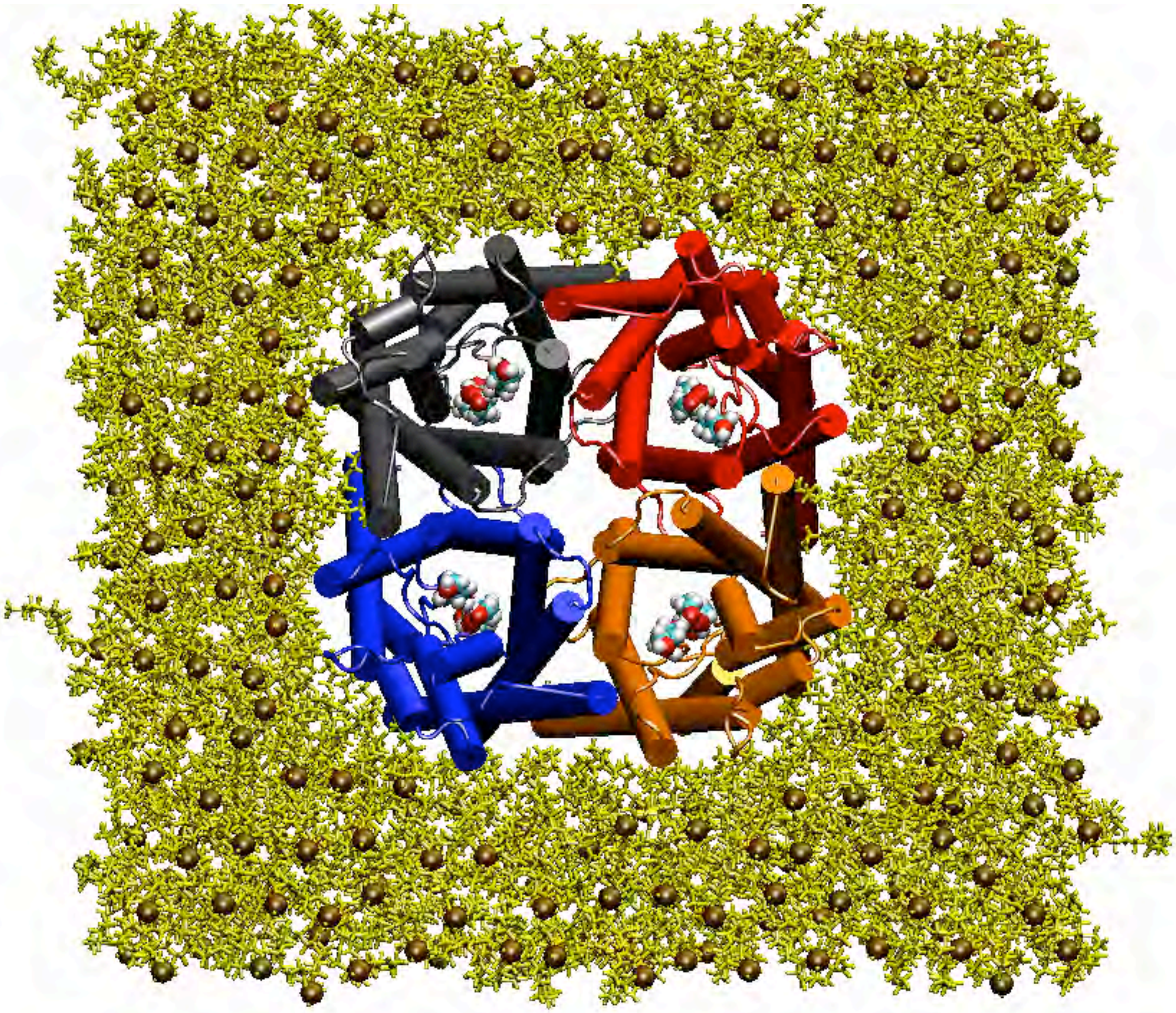



112 A
122 A

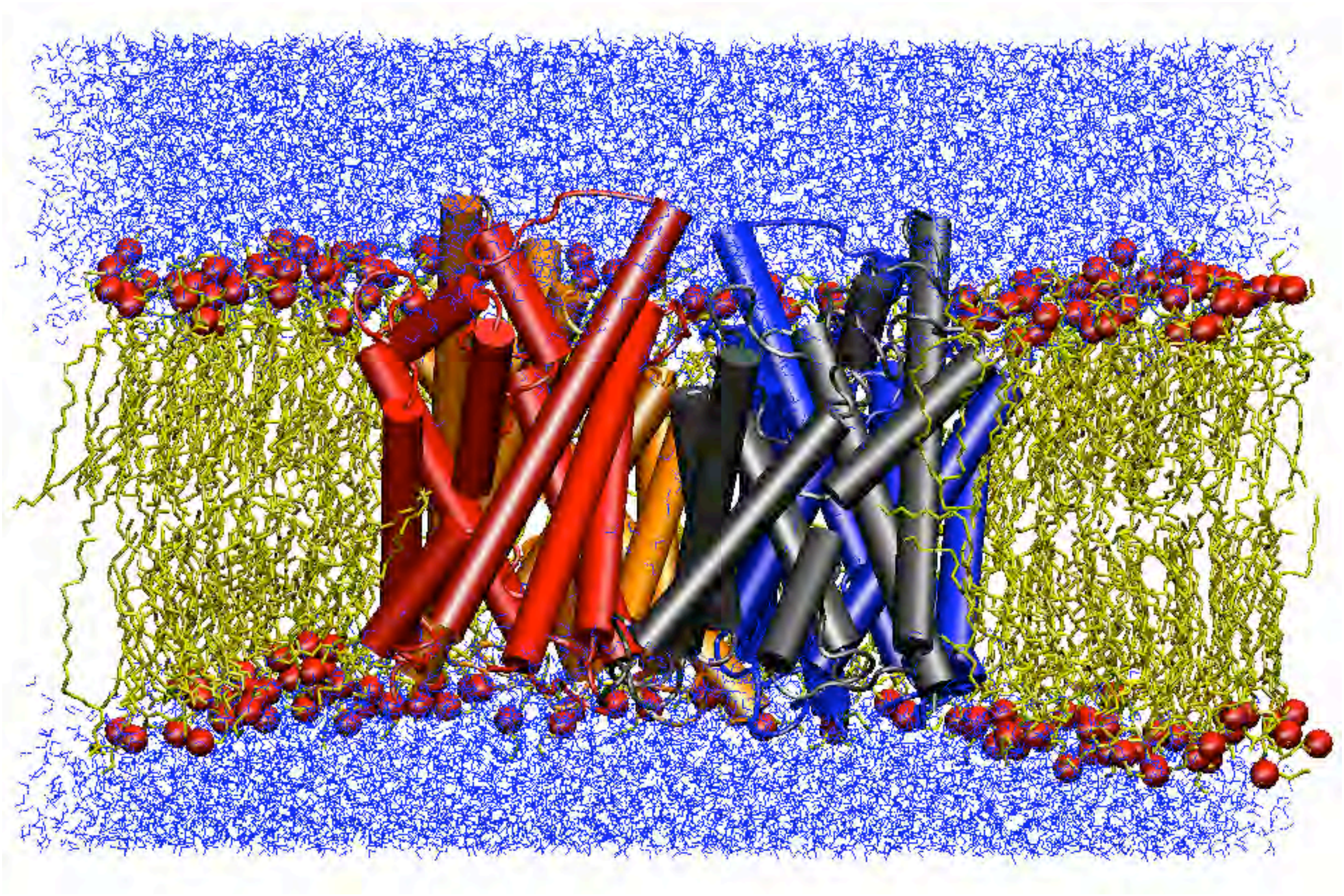
A Recipe for Membrane Protein Simulations

- 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.
- If desired, you may switch to an NVT simulation, when the system reaches a stable volume.

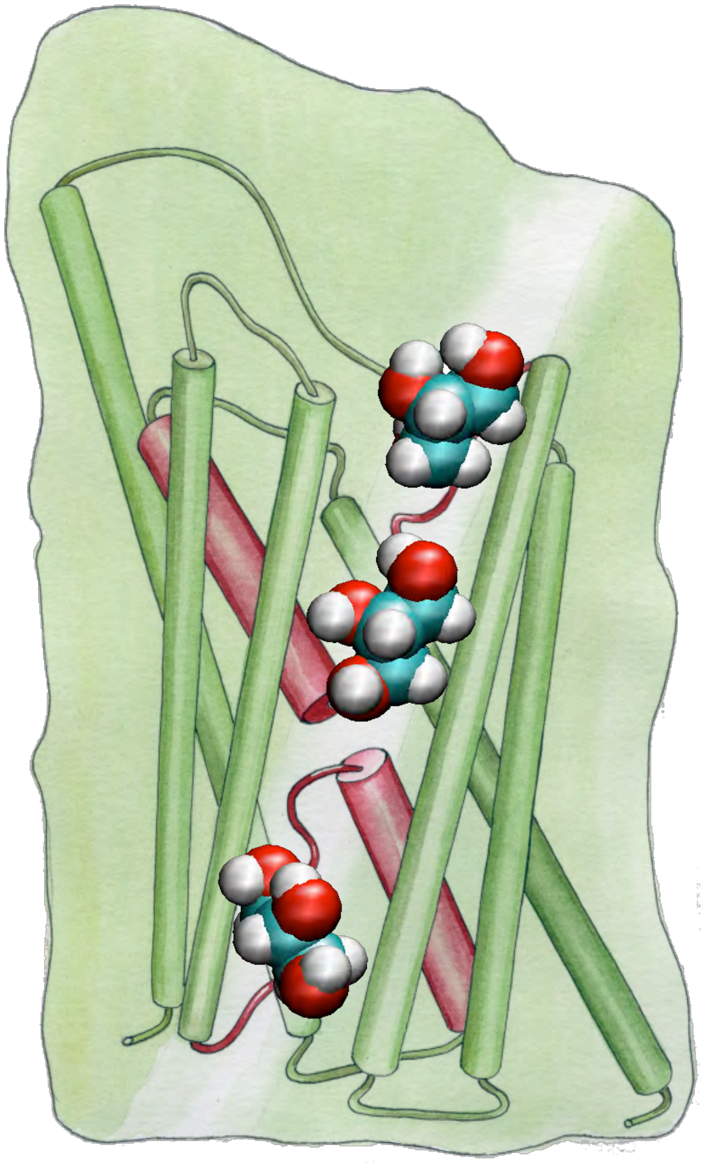
Lipid-Protein Packing During the Initial NpT Simulation

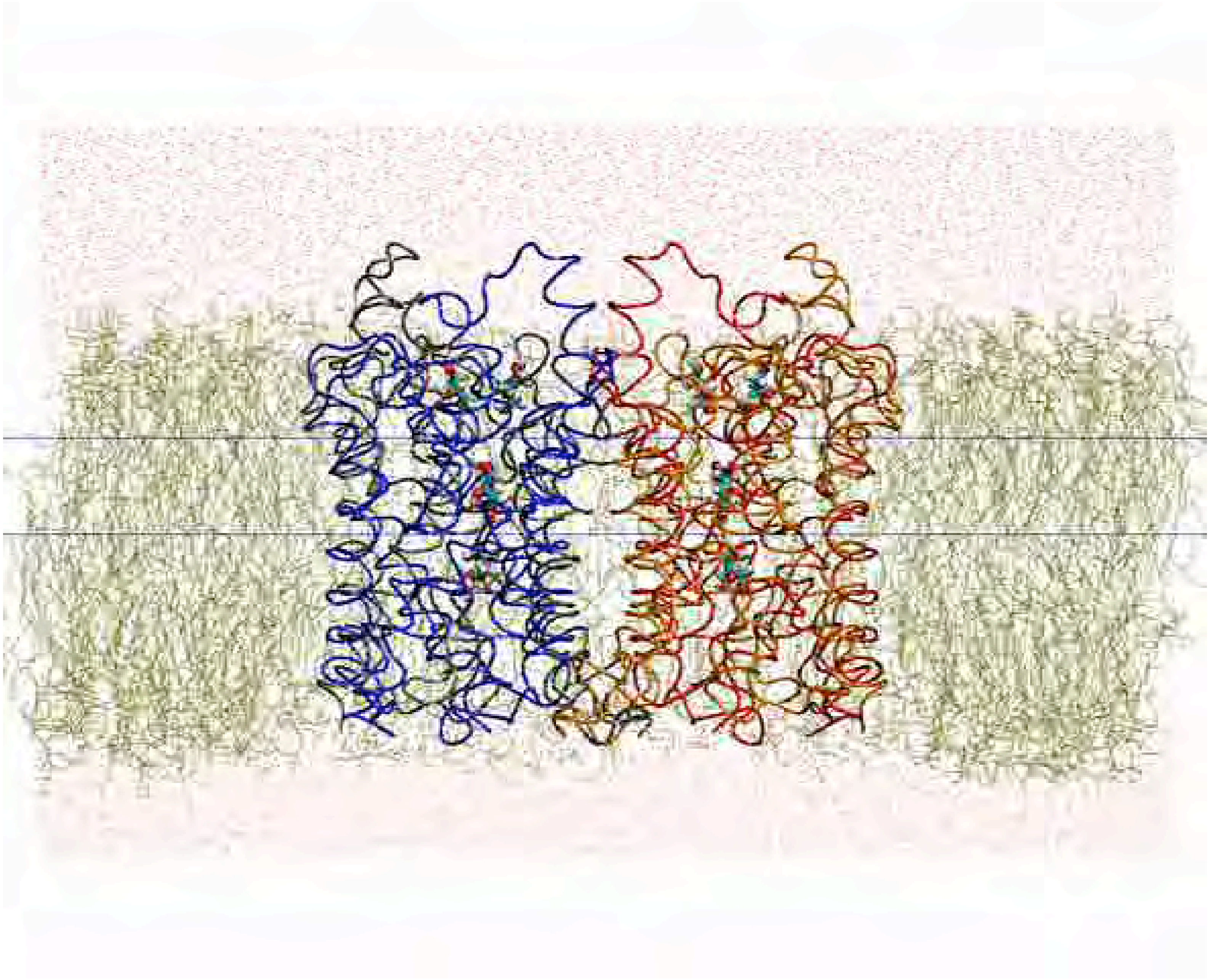


Adjustment of Membrane Thickness to the Protein Hydrophobic Surface

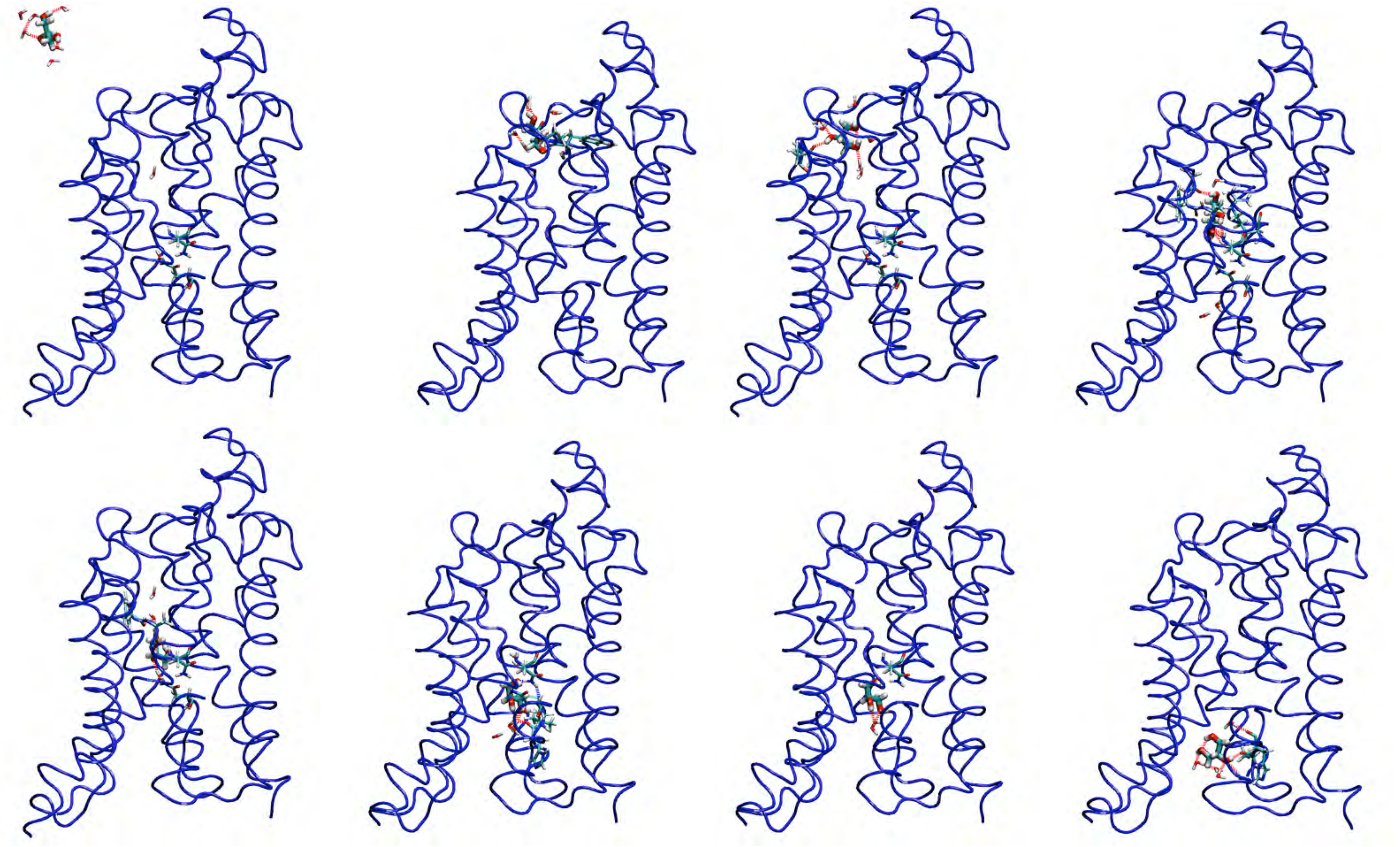


Glycerol-Saturated GlpF





Description of full conduction pathway



Complete description of the conduction pathway

Constriction region

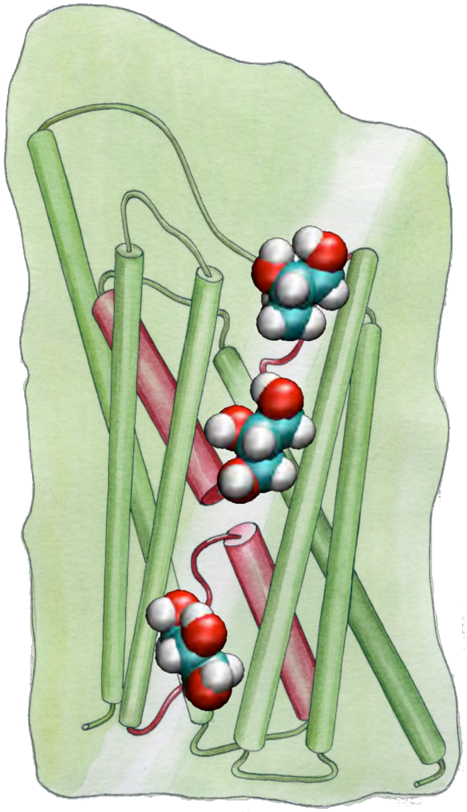
Selectivity filter

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
}
```

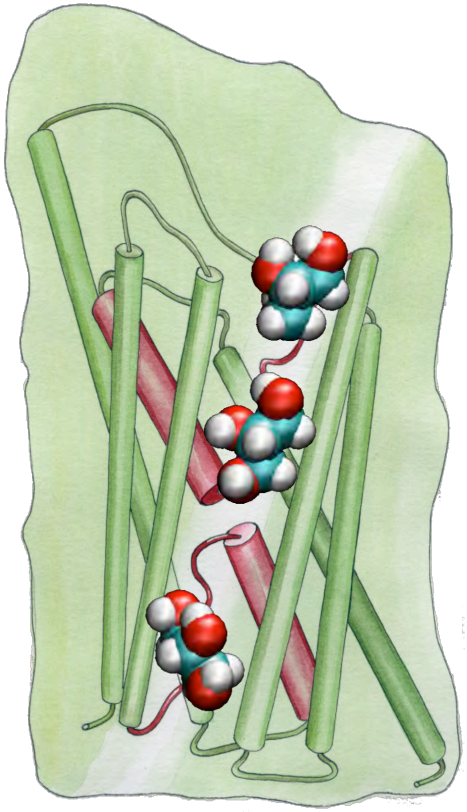
…



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



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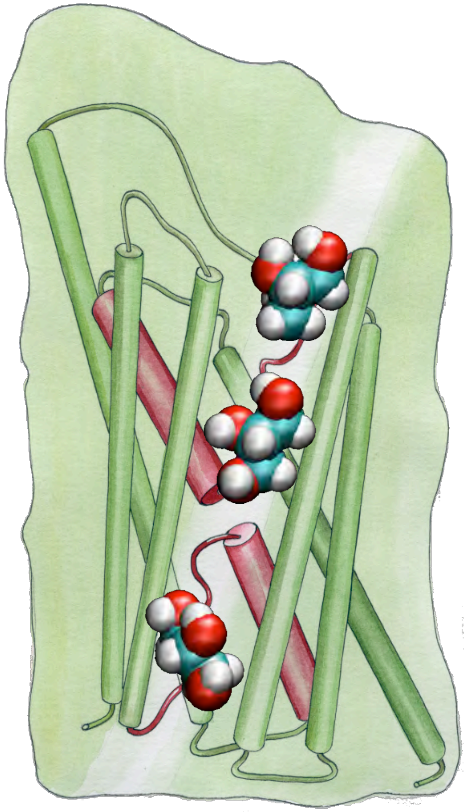
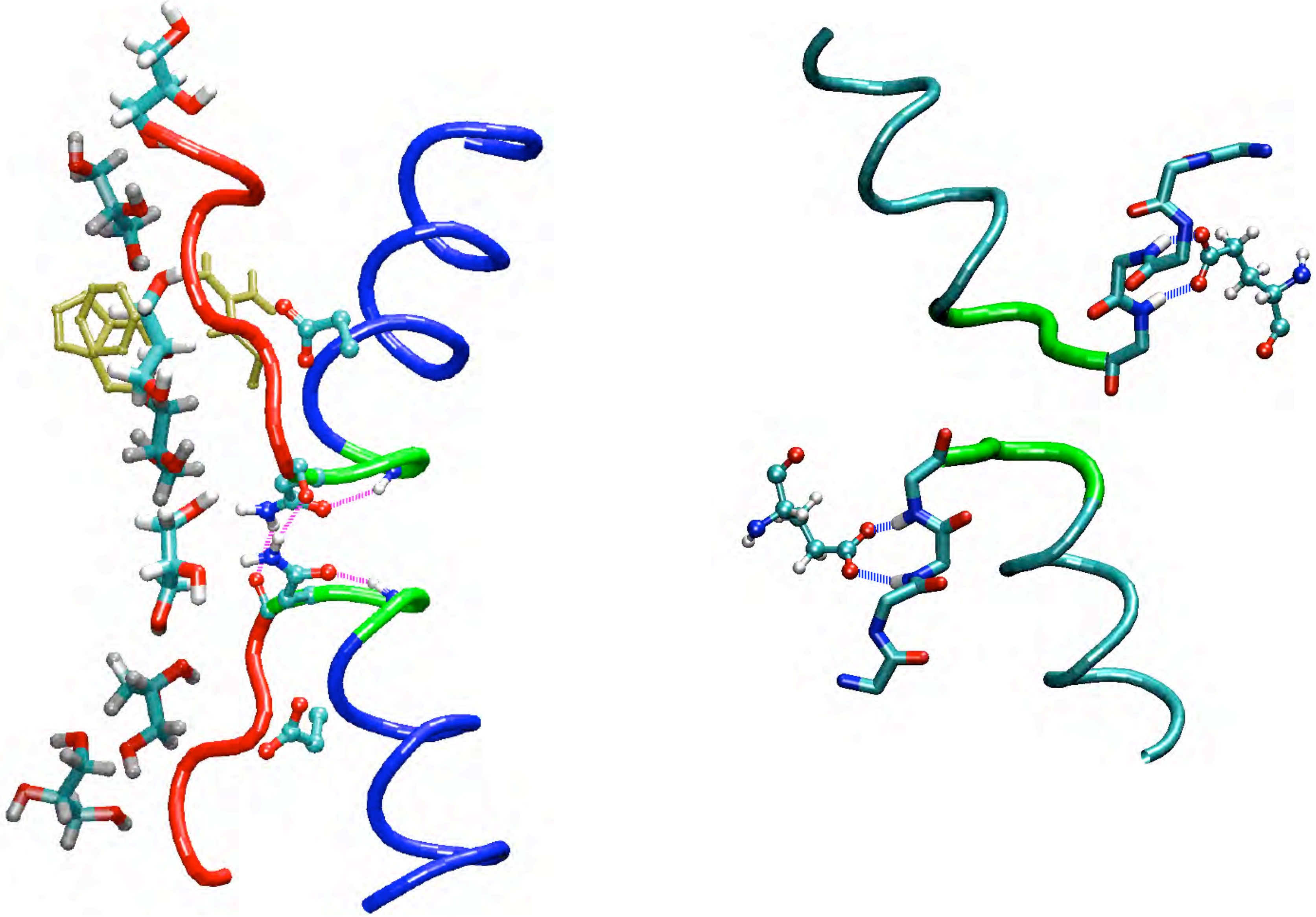
GLY	133	O
SER	136	O
TYR	138	O
PRO	139	O N
ASN	140	OD1 ND2
HIS	142	ND1
THR	167	OG1
GLY	195	O
PRO	196	O

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

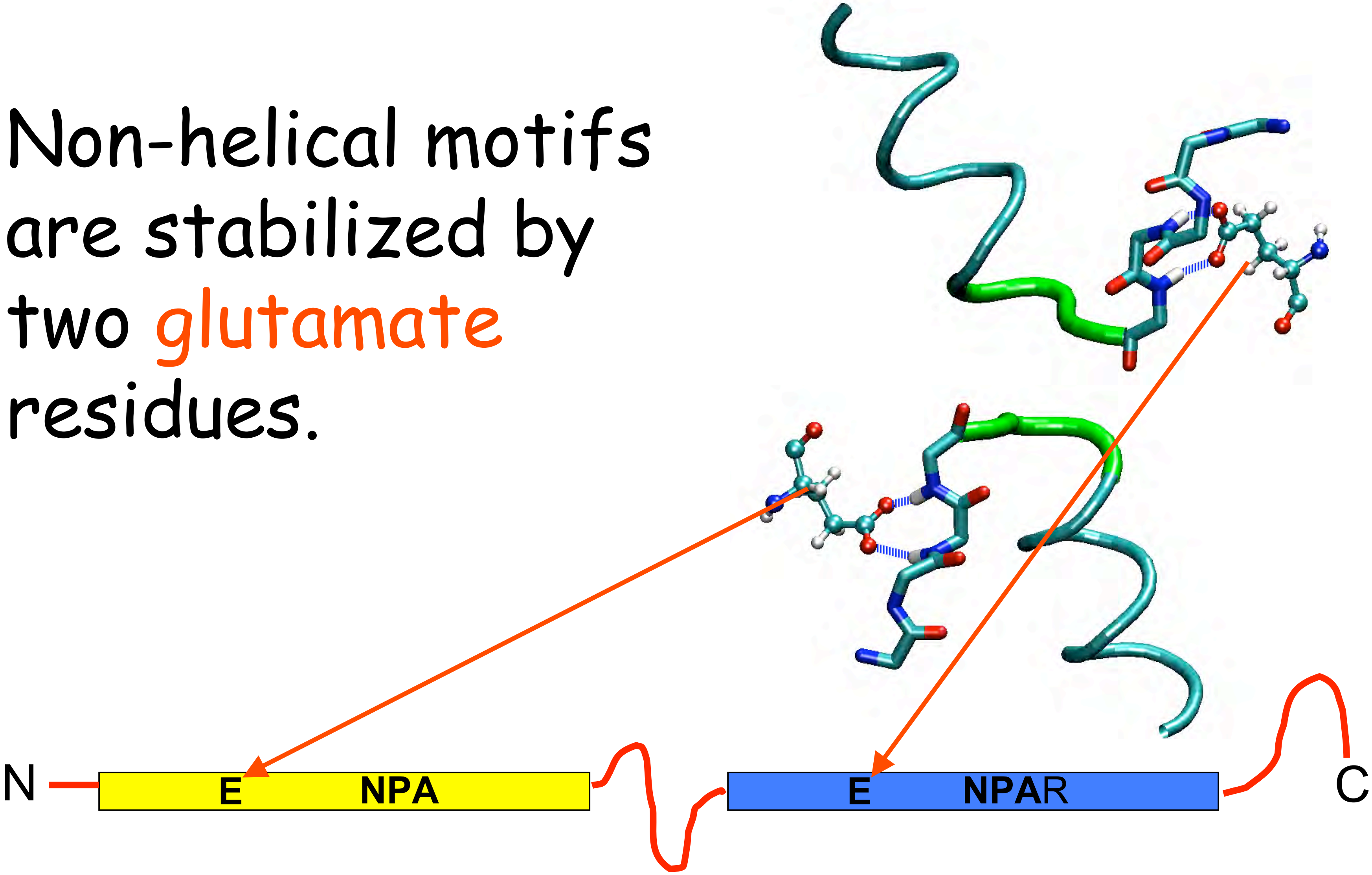


The Substrate Pathway
is formed by C=O groups

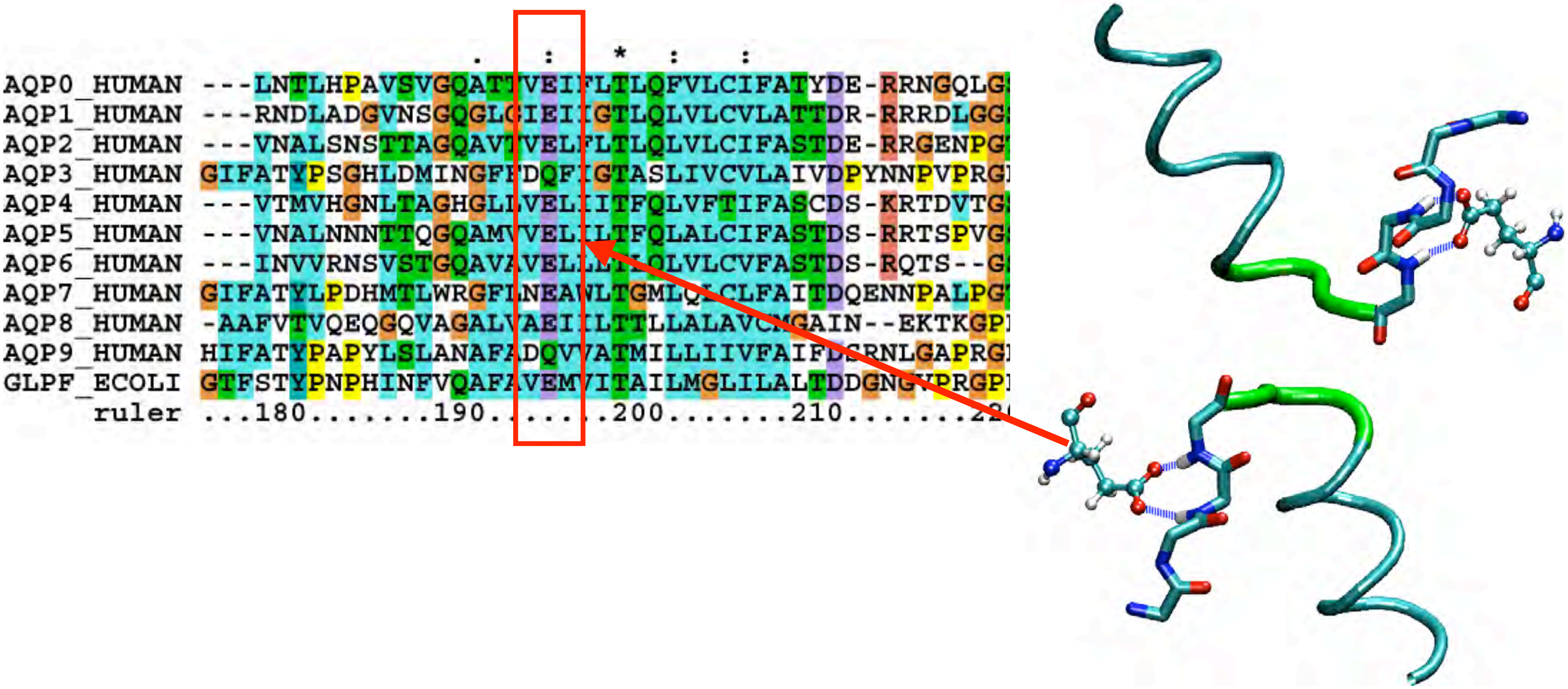


The Substrate Pathway
is formed by C=O groups

Non-helical motifs are stabilized by two glutamate residues.

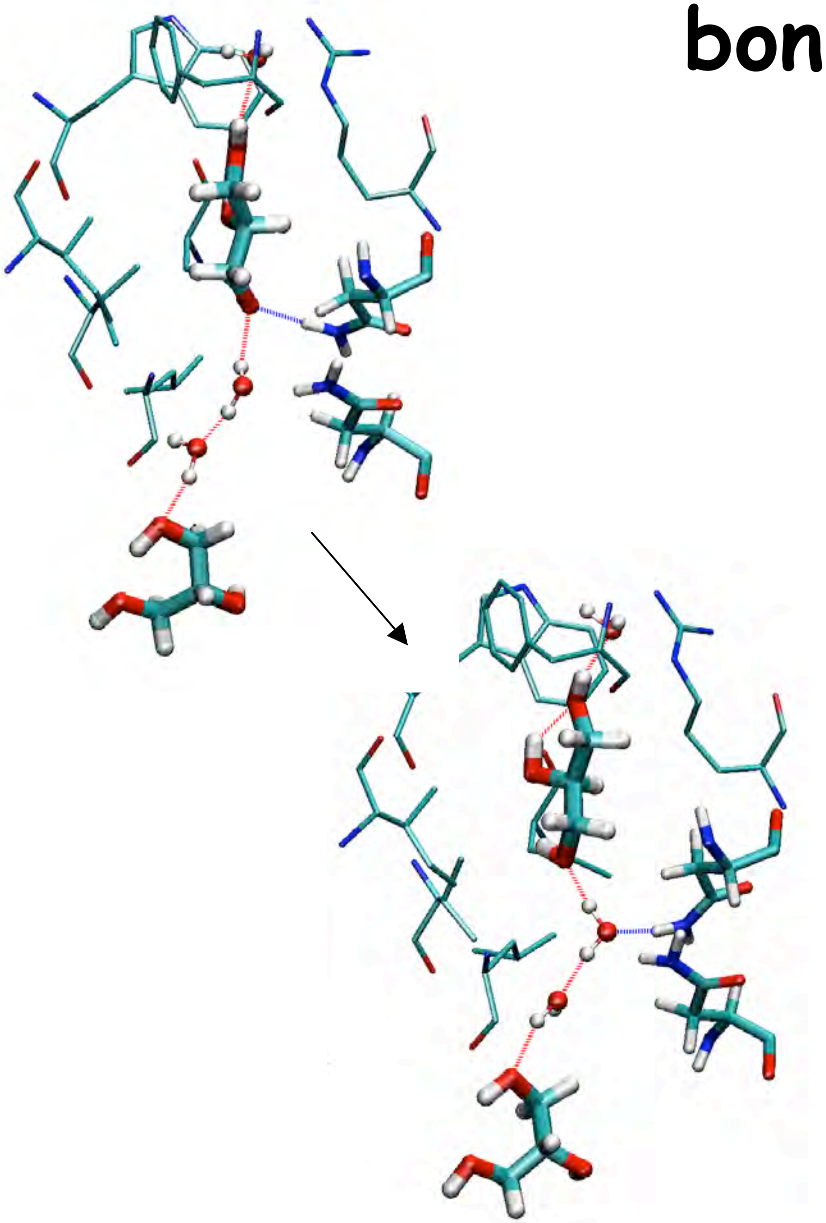


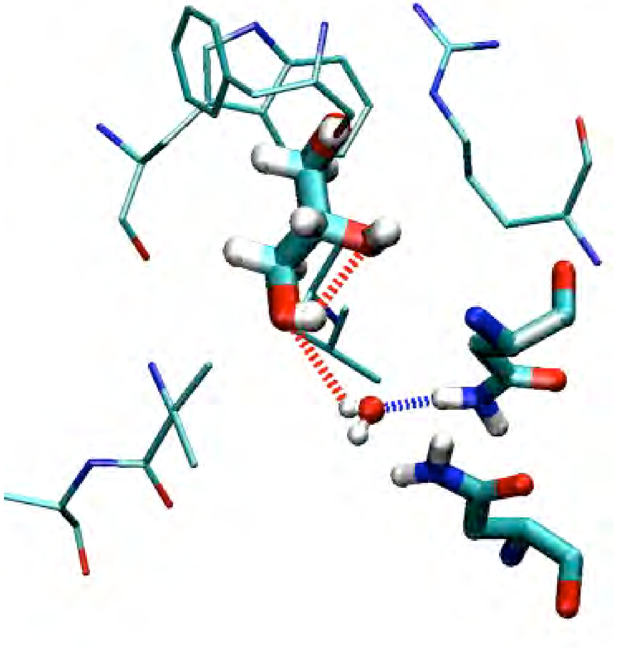
Conservation of Glutamate Residue in Human Aquaporins



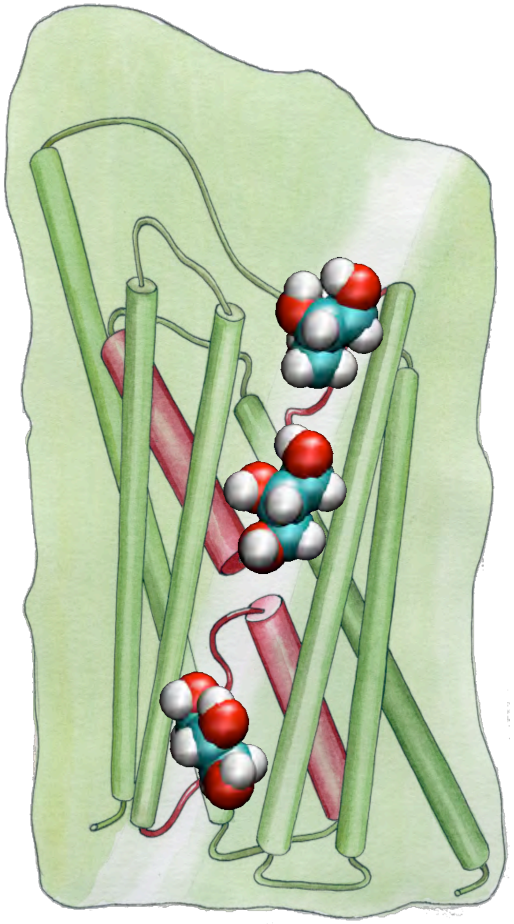
```
                          .   :   *  :   :
AQP0_HUMAN ---LNTLHPAVSVGQATTVEIFLTLQFVLCIFATYDE-RRNGQLG
AQP1_HUMAN ---RNDLADGVNSGQGLGIEIIGTLQLVLCVLATTDR-RRRDLGG
AQP2_HUMAN ---VNALSNSTTAGQAVTVELFLTLQLVLCIFASTDE-RRGENPG
AQP3_HUMAN GIFATYPSGHLDMINGFFDQFIGTASLIVCVLAIVDPYNNPVPRG
AQP4_HUMAN ---VTMVHGNLTAGHGLLVELIITFQLVFTIFASCDS-KRTDVTG
AQP5_HUMAN ---VNALNNNTTQGQAMVVELLLTFQLALCIFASTDS-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
```

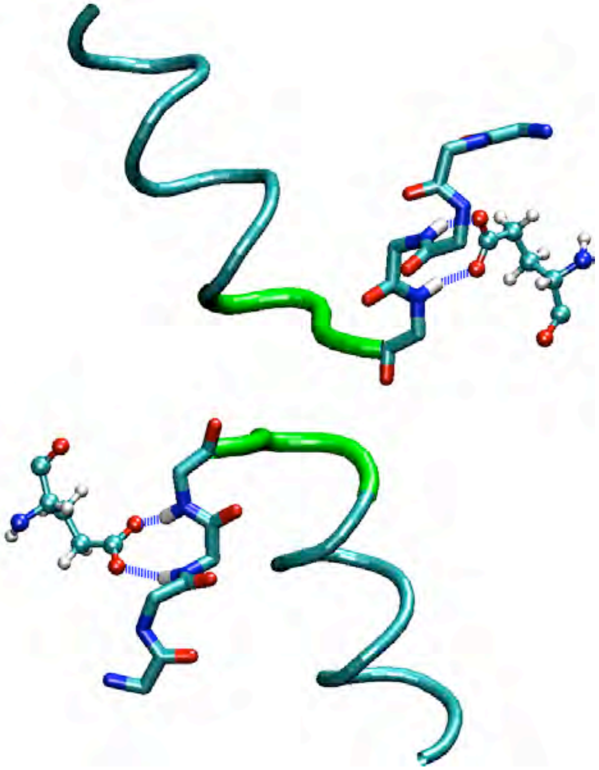
Glycerol – water competition for hydrogen bonding sites



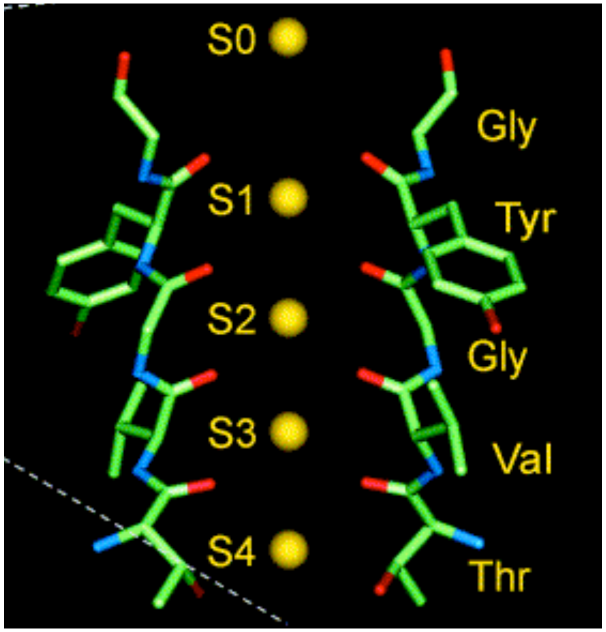


Revealing the Functional Role of Reentrant Loops



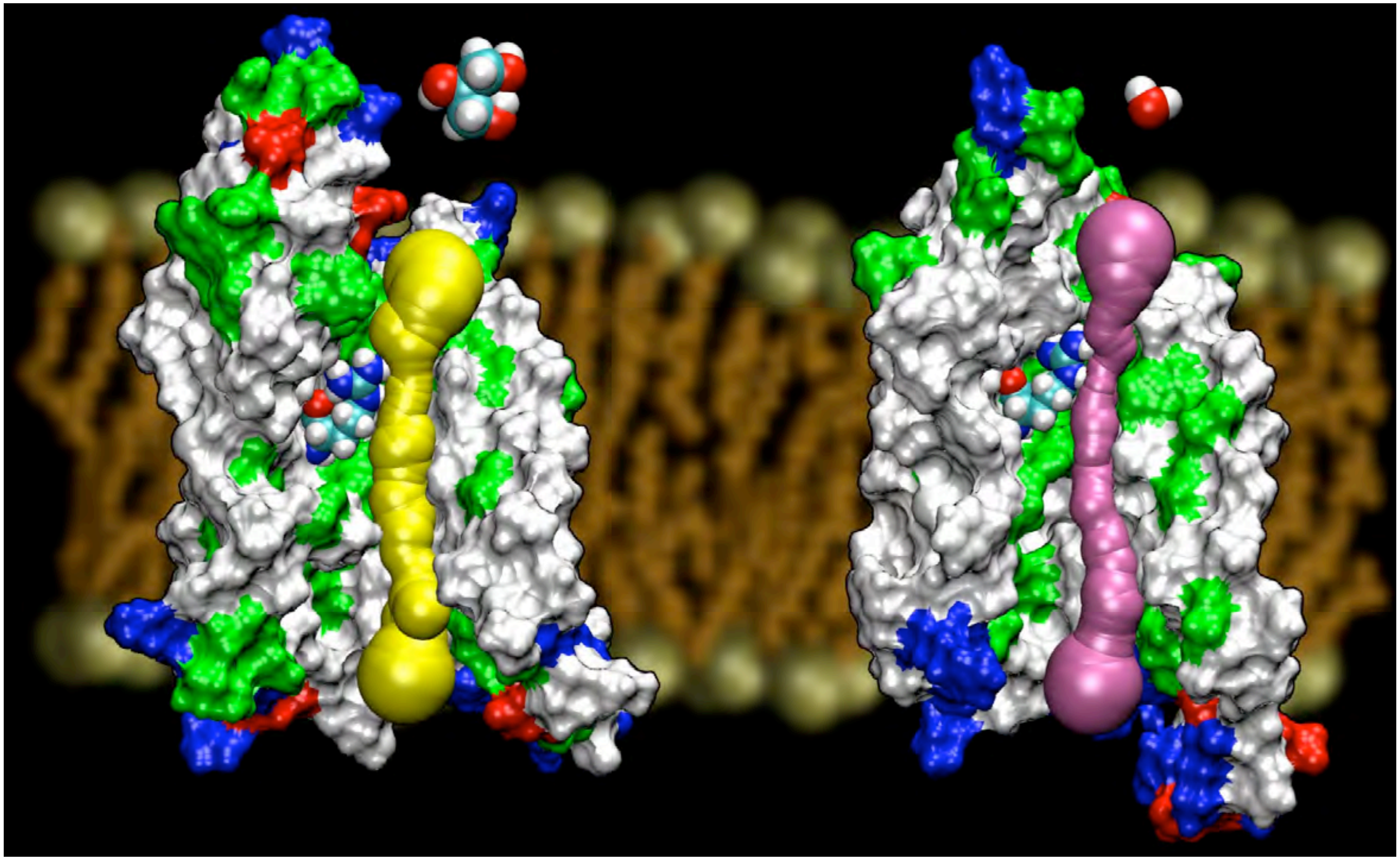


Potassium channel



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

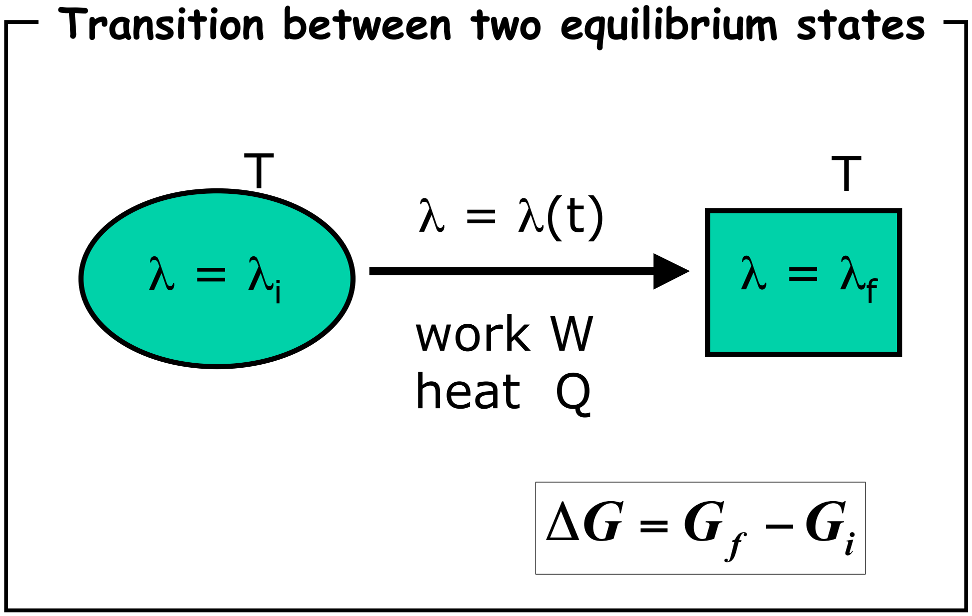


Steered Molecular Dynamics is a non-equilibrium method by nature

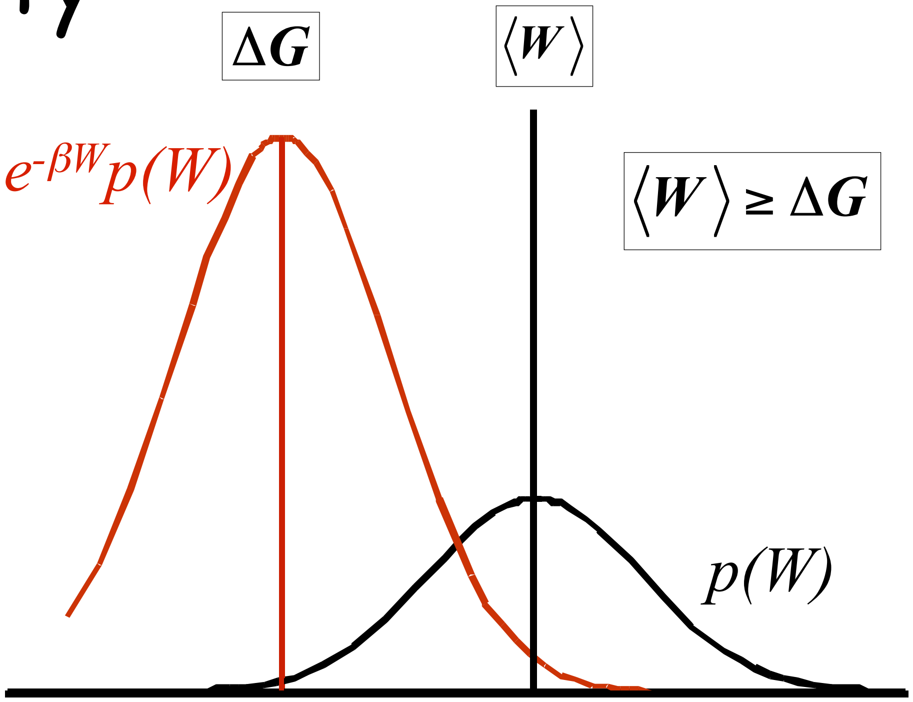
- A wide variety of events that are inaccessible to conventional molecular dynamics simulations can be probed.
- The system will be driven, however, away from equilibrium, resulting in problems in describing the energy landscape associated with the event of interest.

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

Jarzynski's Equality



$$\Delta G = G_f - G_i$$



$$\langle W \rangle \geq \Delta G$$

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

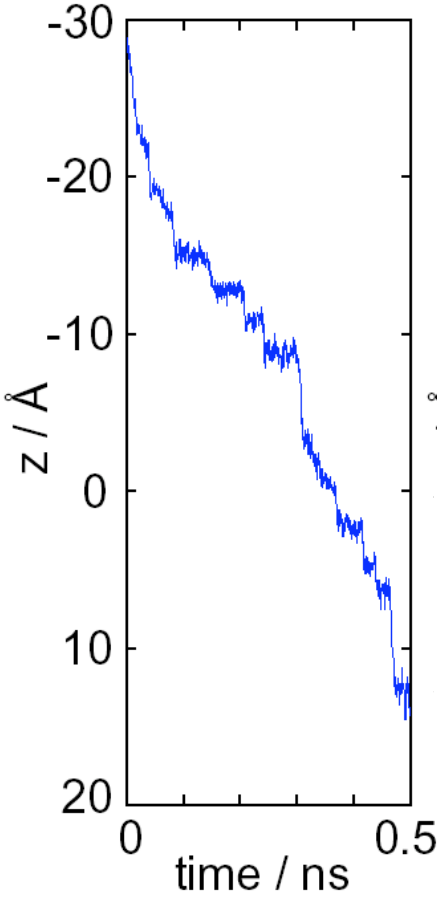
$$\left\langle e^{-\beta W} \right\rangle = e^{-\beta \Delta G}$$

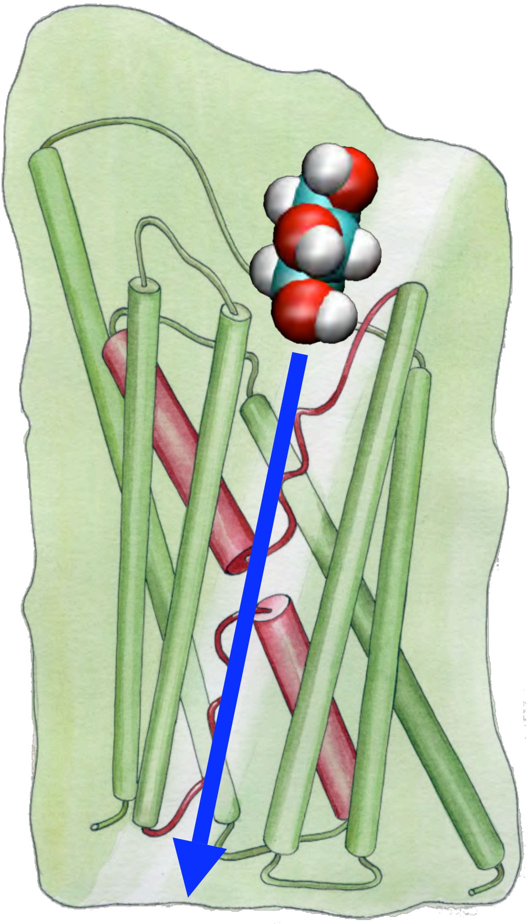
$$\beta = {}^{1}\!/_{k_B T}$$

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

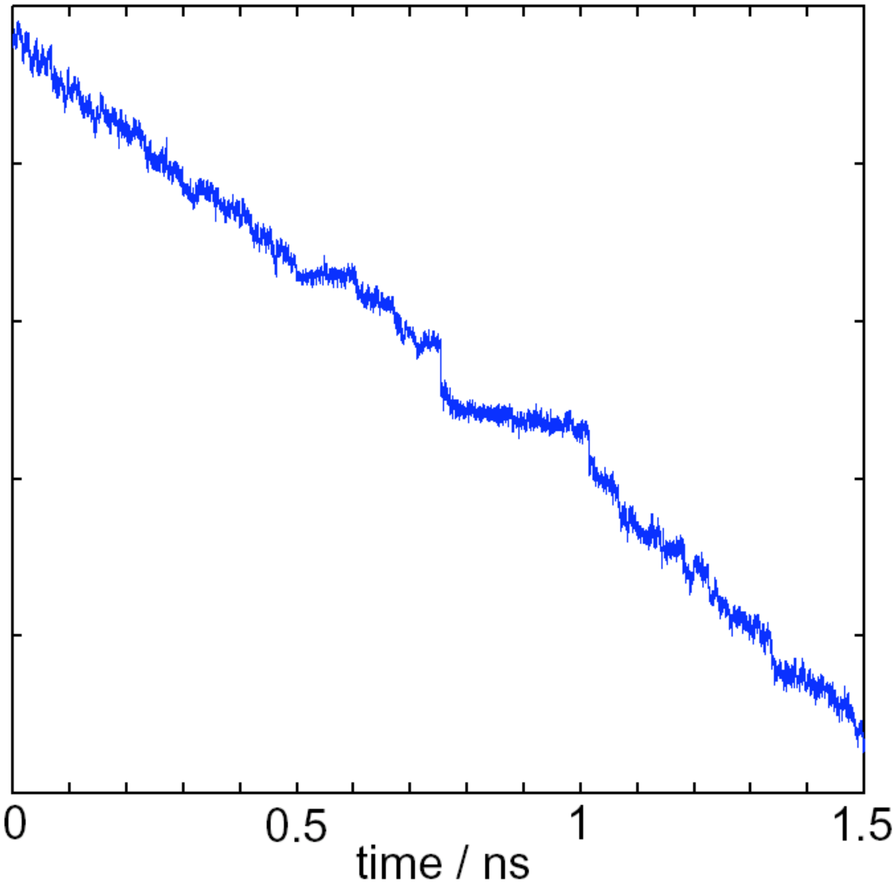
Steered Molecular Dynamics

constant force
(250 pN)

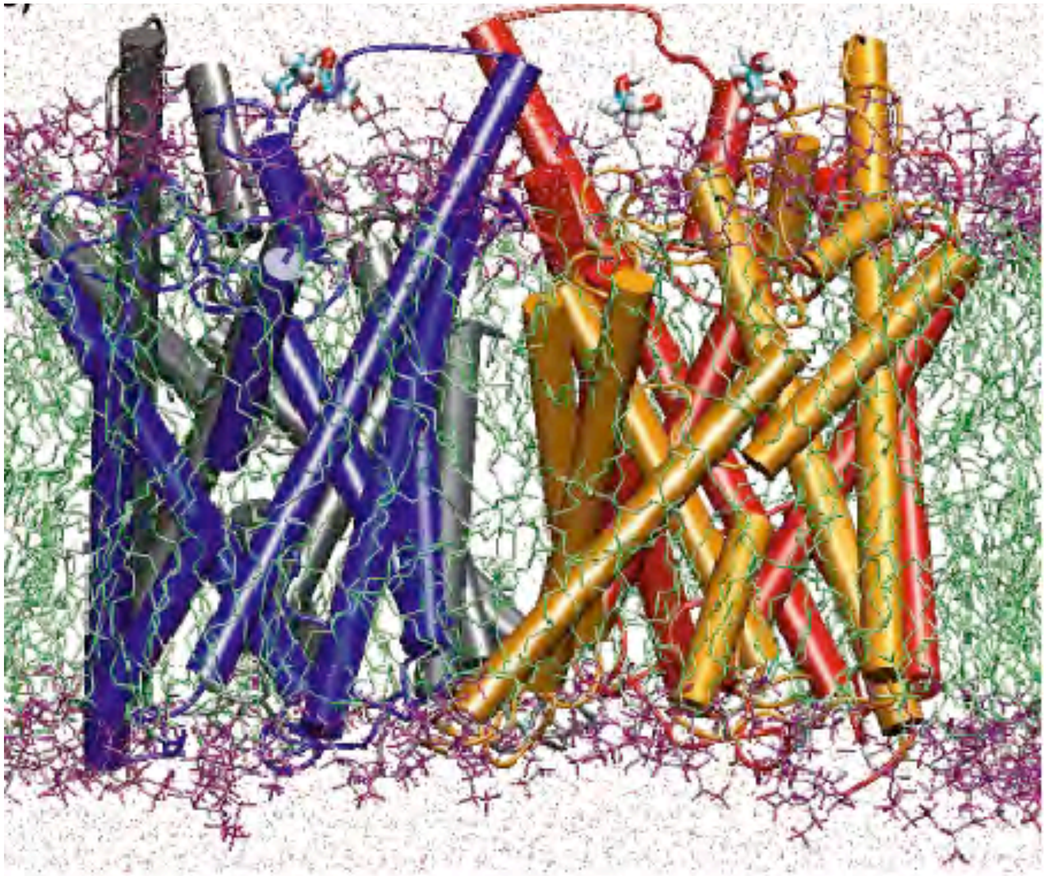


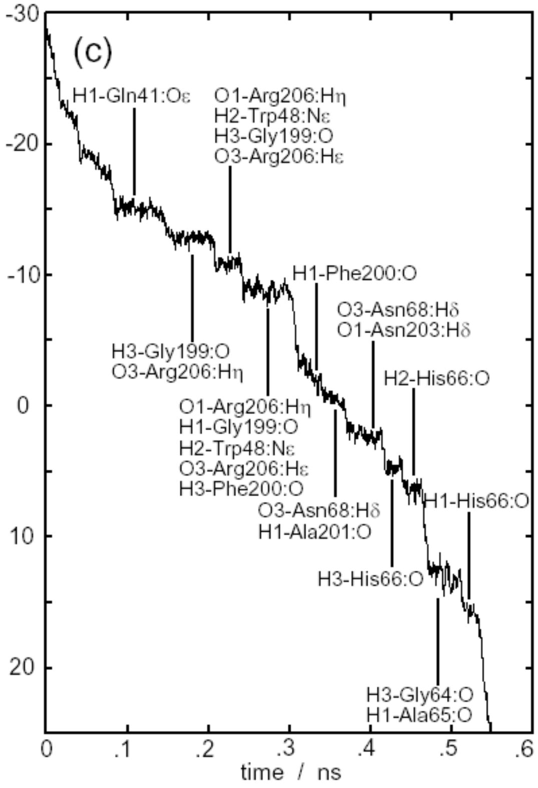


constant velocity
(30 Å/ns)



SMD Simulation of Glycerol Passage





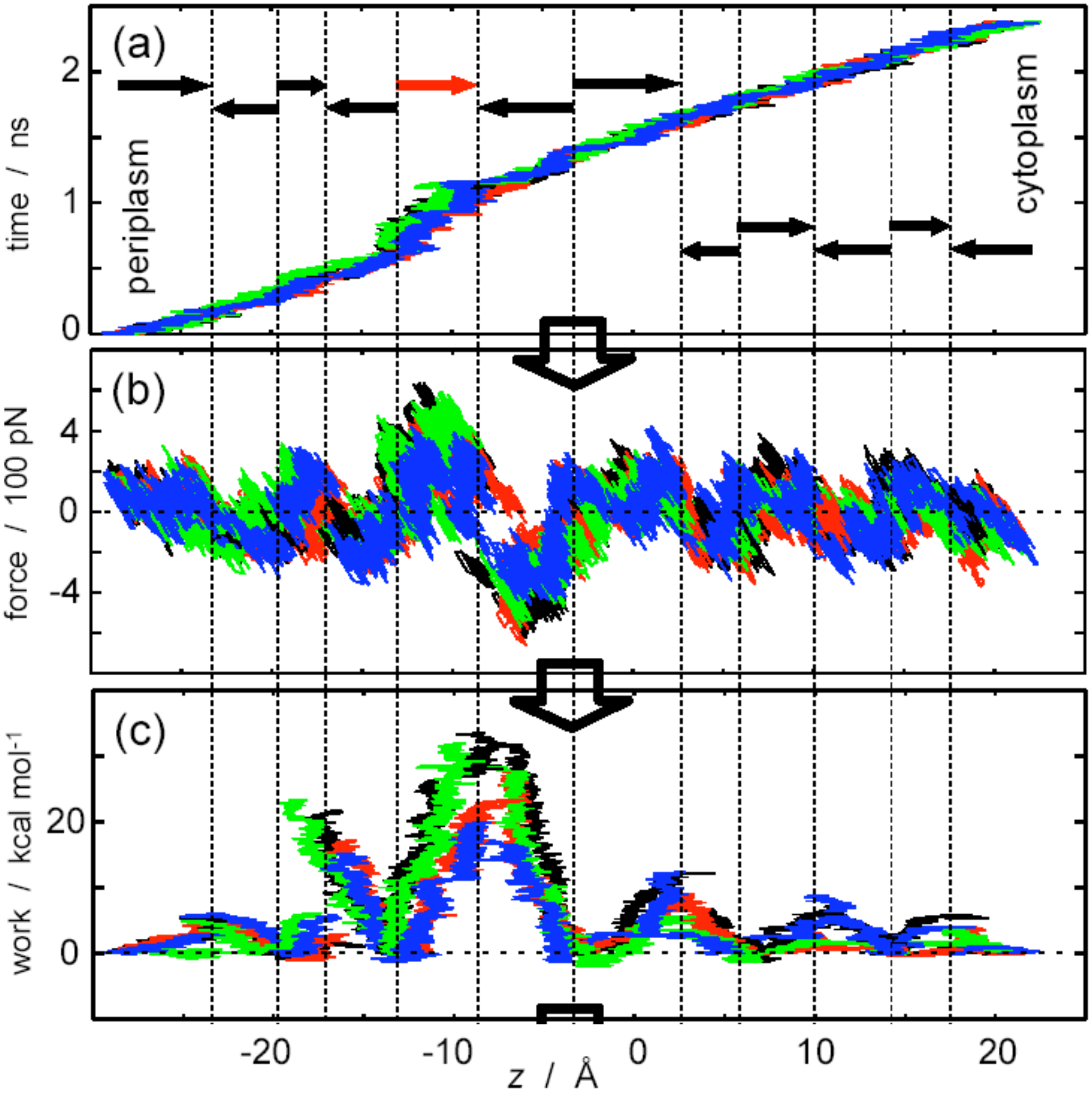
Trajectory of glycerol pulled by **constant force**

Constructing the Potential of Mean Force

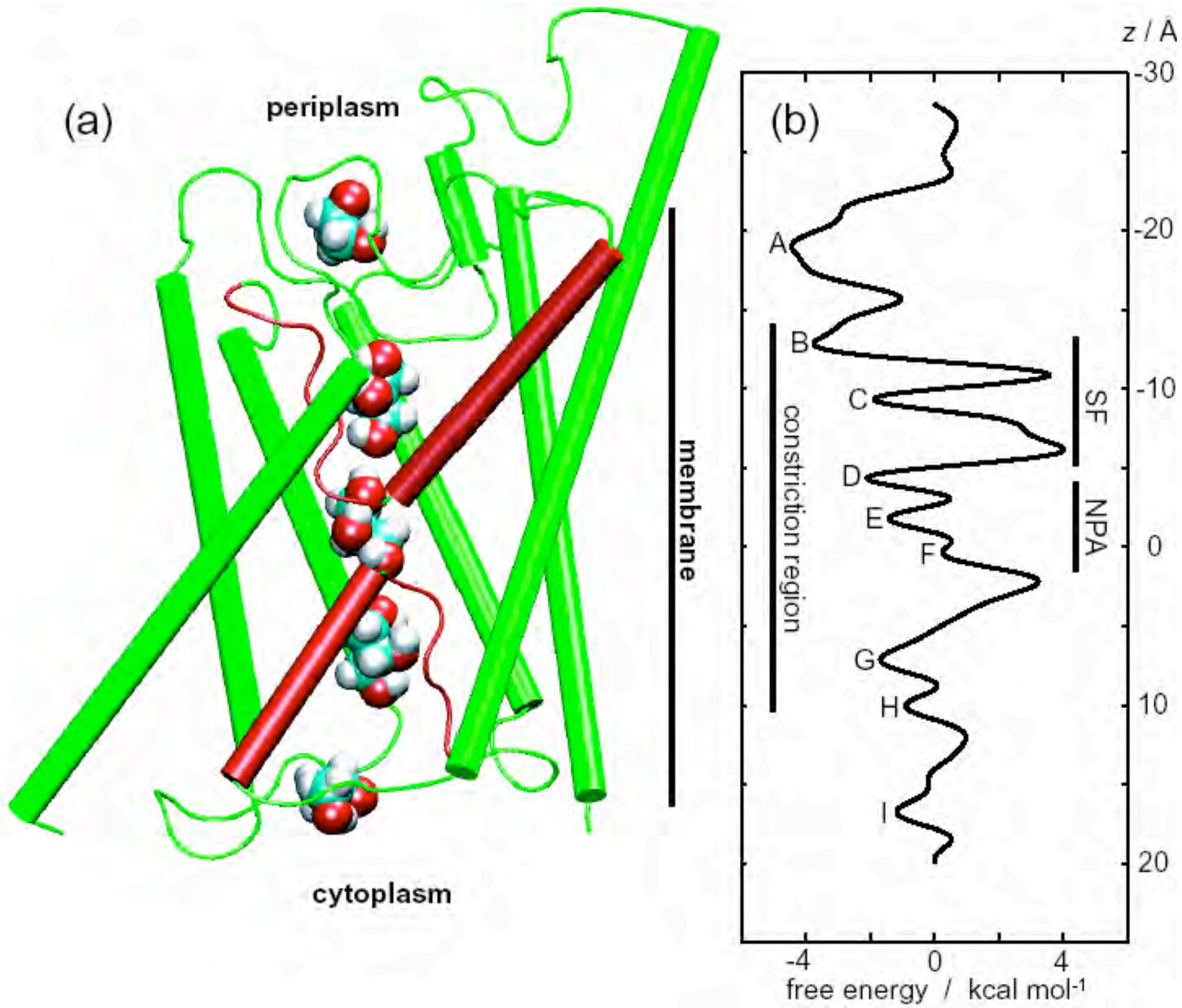
4 trajectories
v = 0.03, 0.015 Å/ps
k = 150 pN/Å

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

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



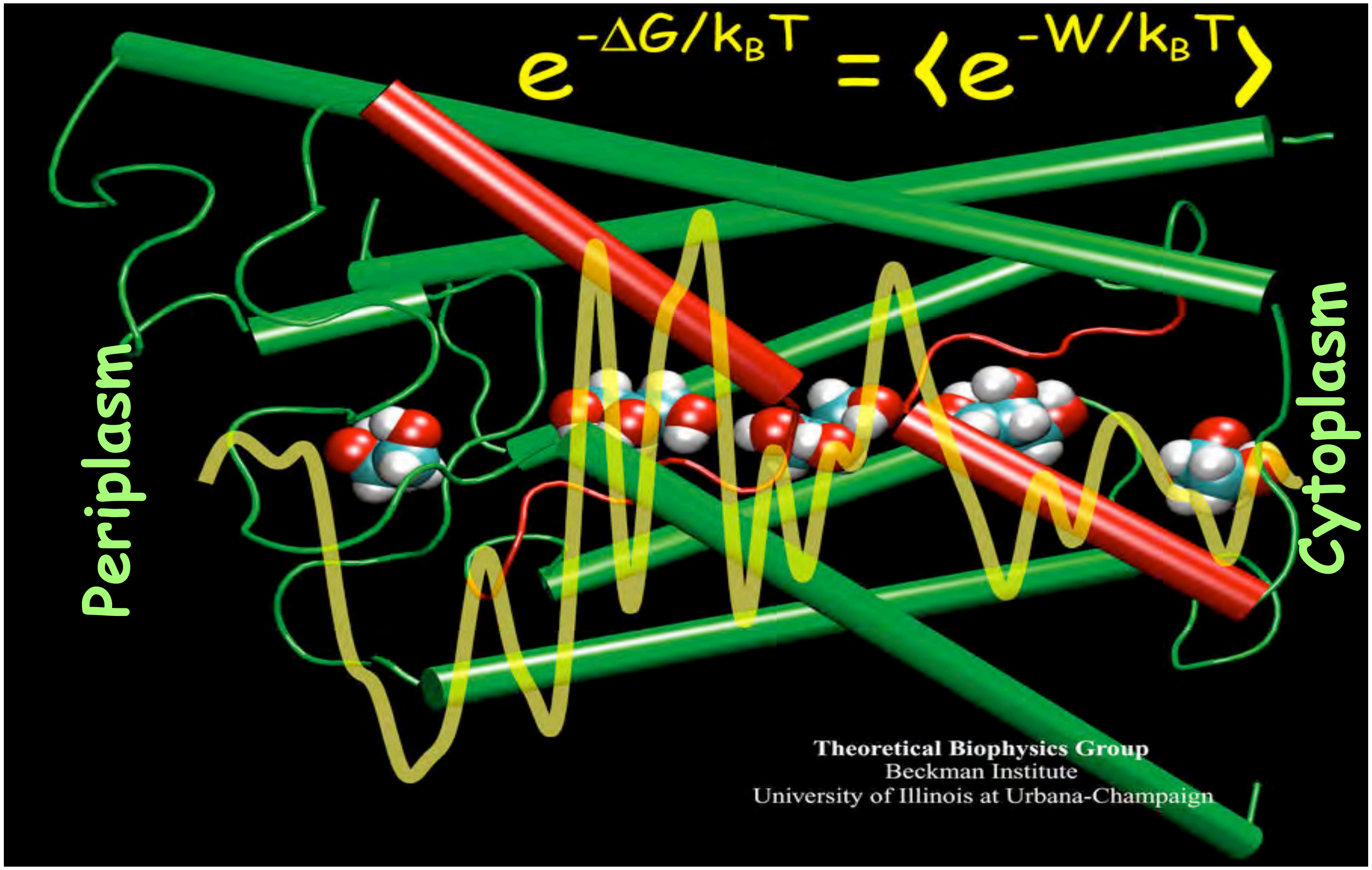
Features of the Potential of Mean Force



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

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

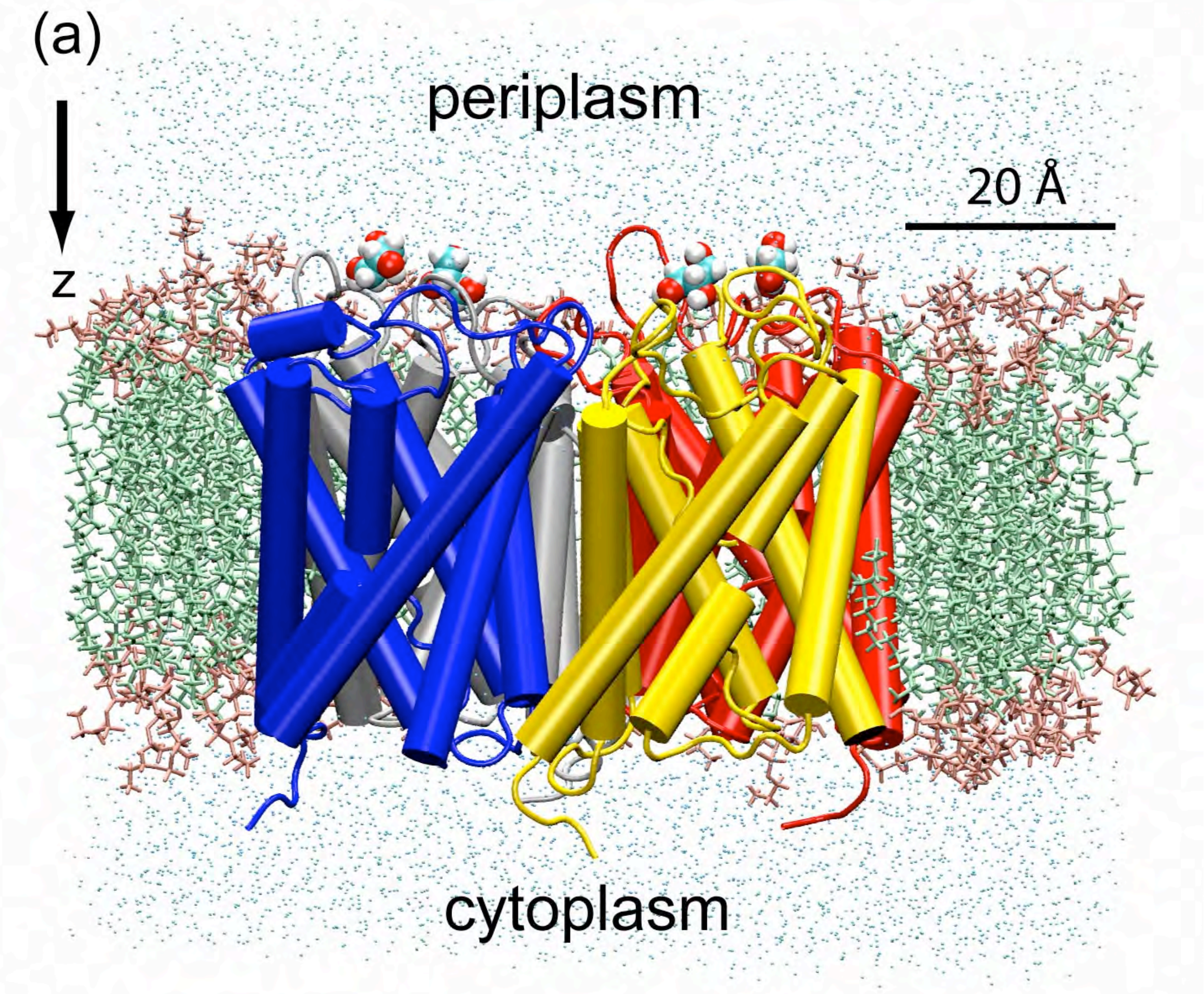
Features of the Potential of Mean Force



Asymmetric Profile in the Vestibules

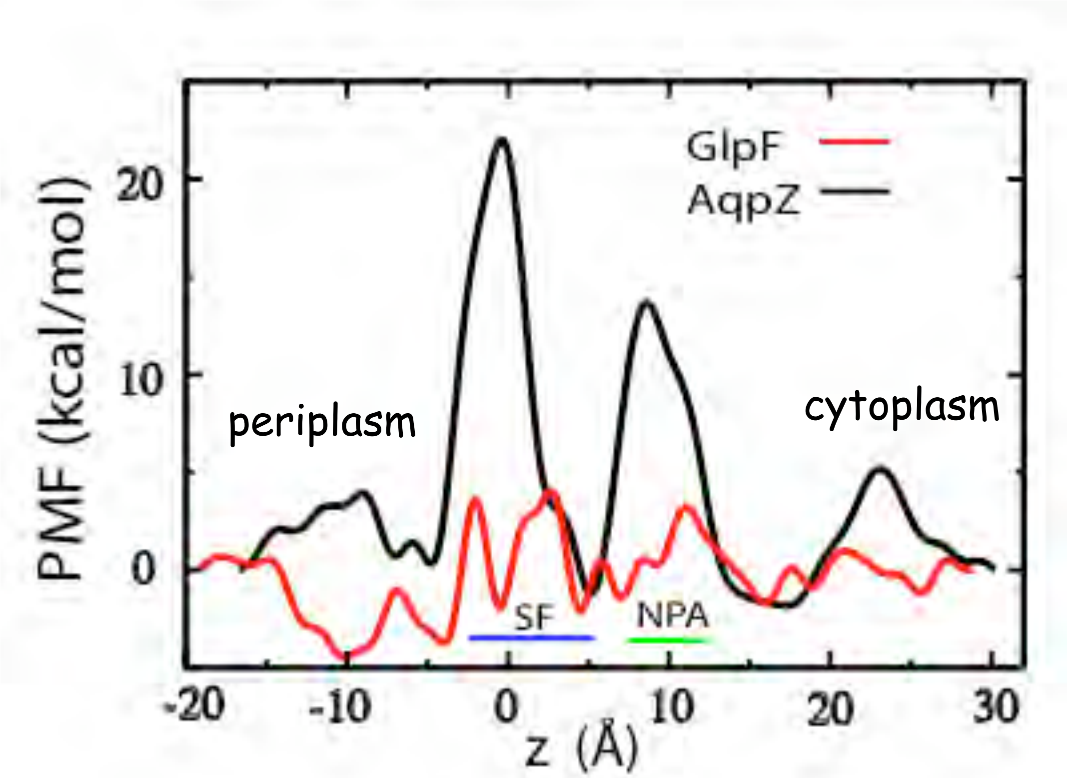
Jensen et al., *PNAS*, 99:6731-6736, 2002.

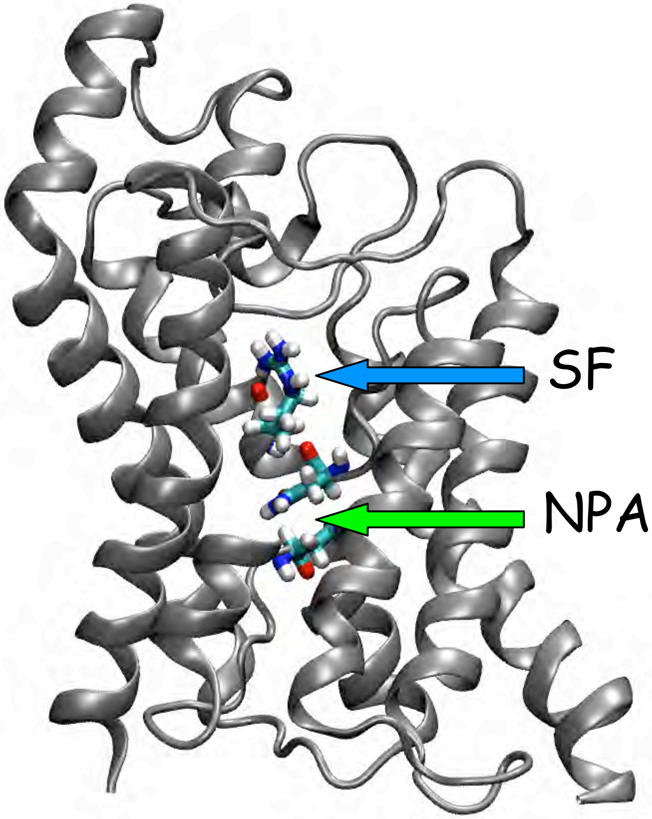
Artificial induction of glycerol conduction through AqpZ



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

Three fold higher barriers

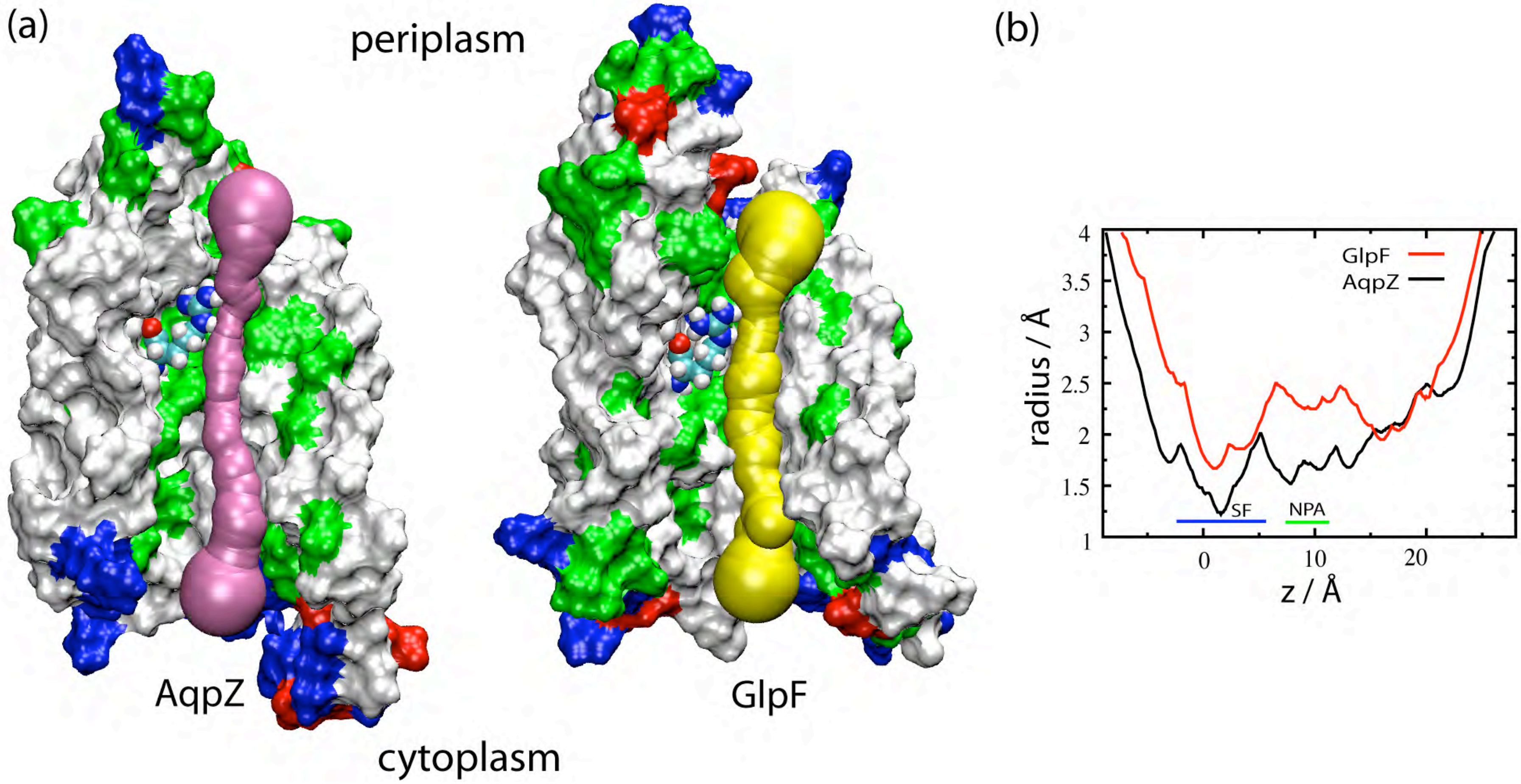




AqpZ 22.8 kcal/mol
GlpF 7.3 kcal/mol

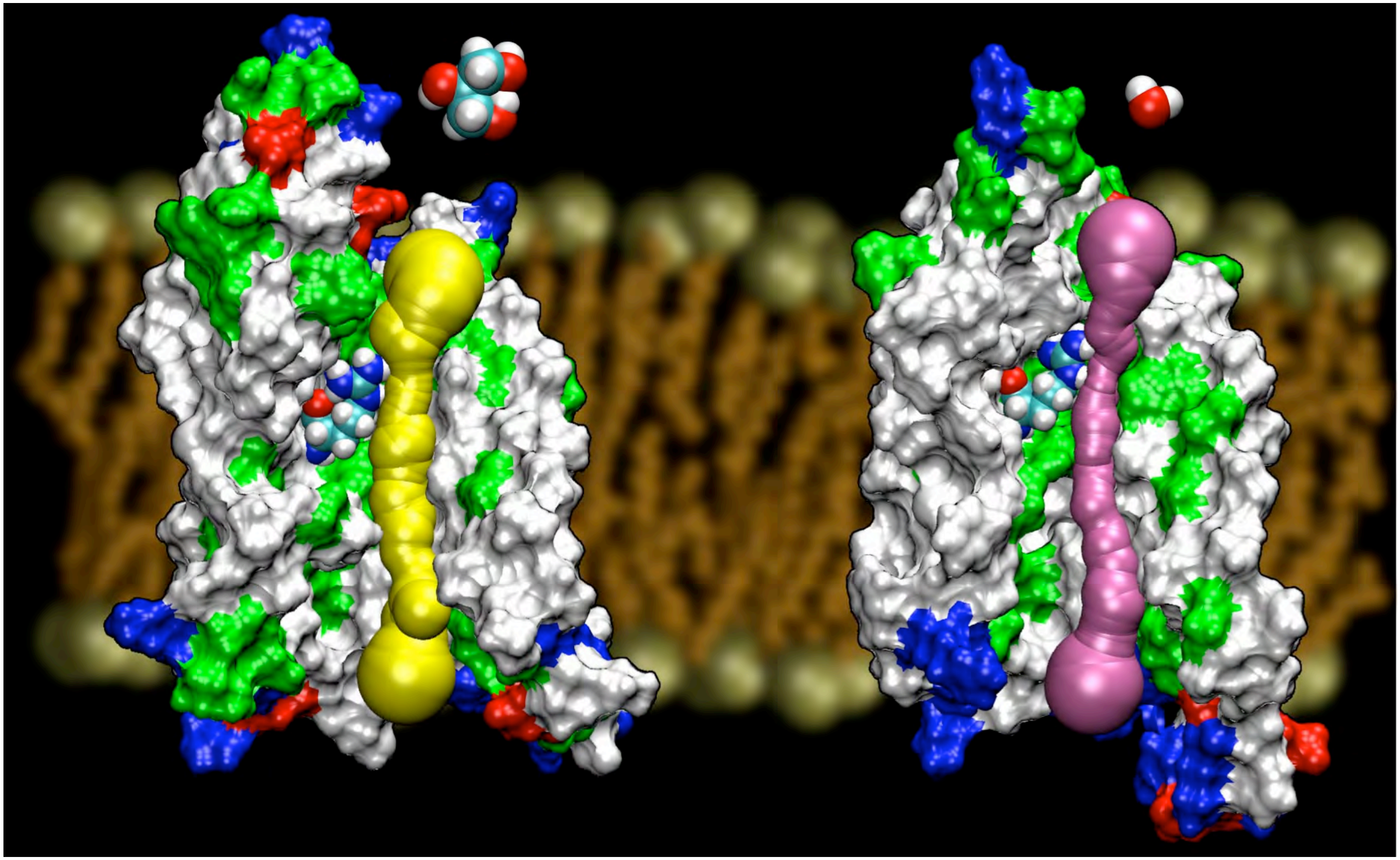
Y. Wang, K. Schulten, and E. Tajkhorshid ***Structure*** 13, 1107 ***(***2005)

Could it be simply the size?



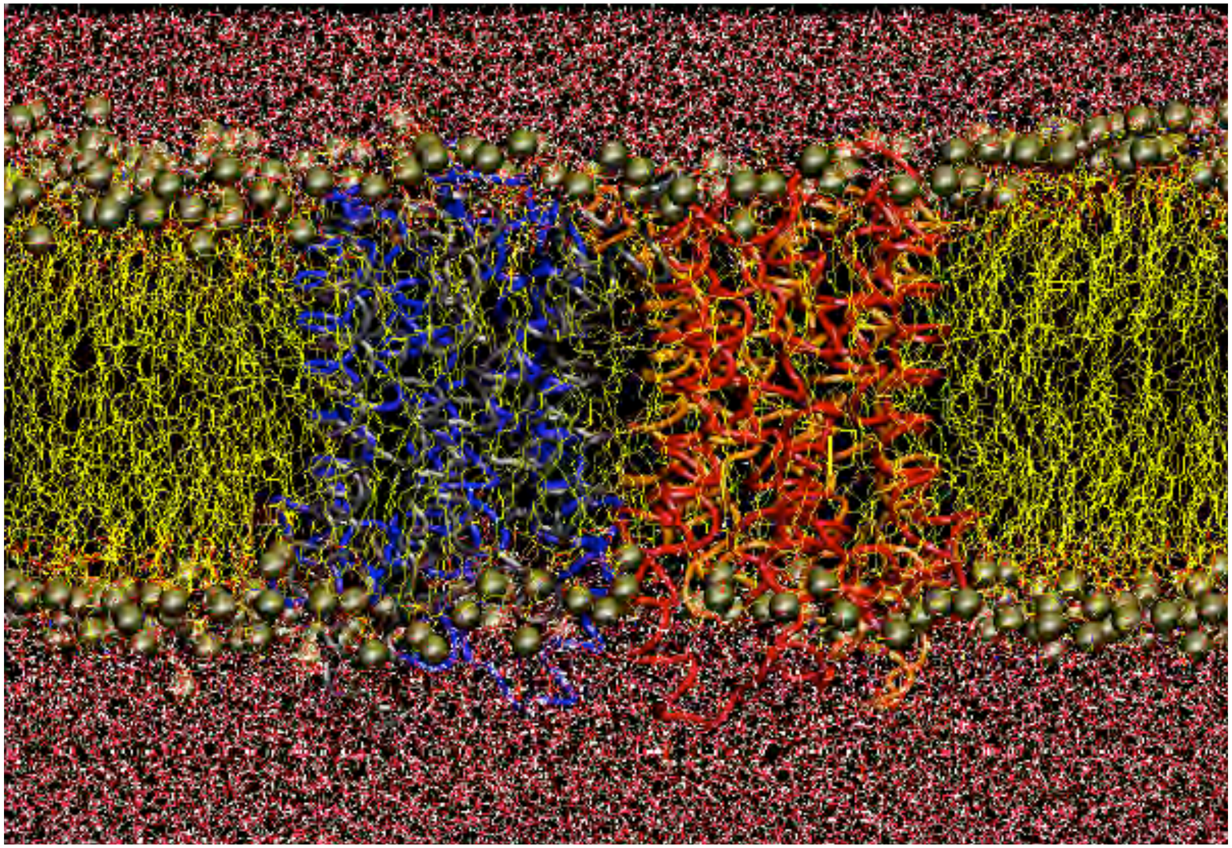
Y. Wang, K. Schulten, and E. Tajkhorshid ***Structure*** 13, 1107 (2005)

It is probably just the size that matters!



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

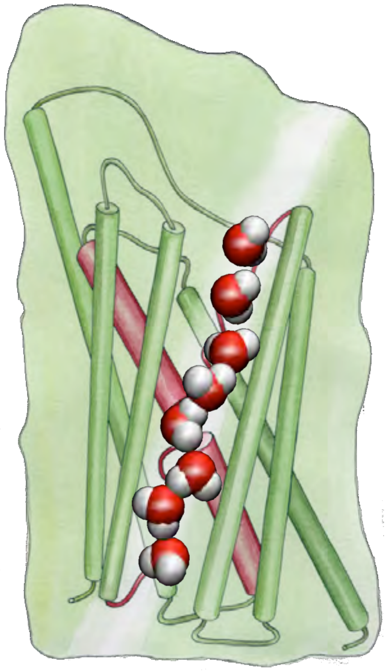
Water permeation



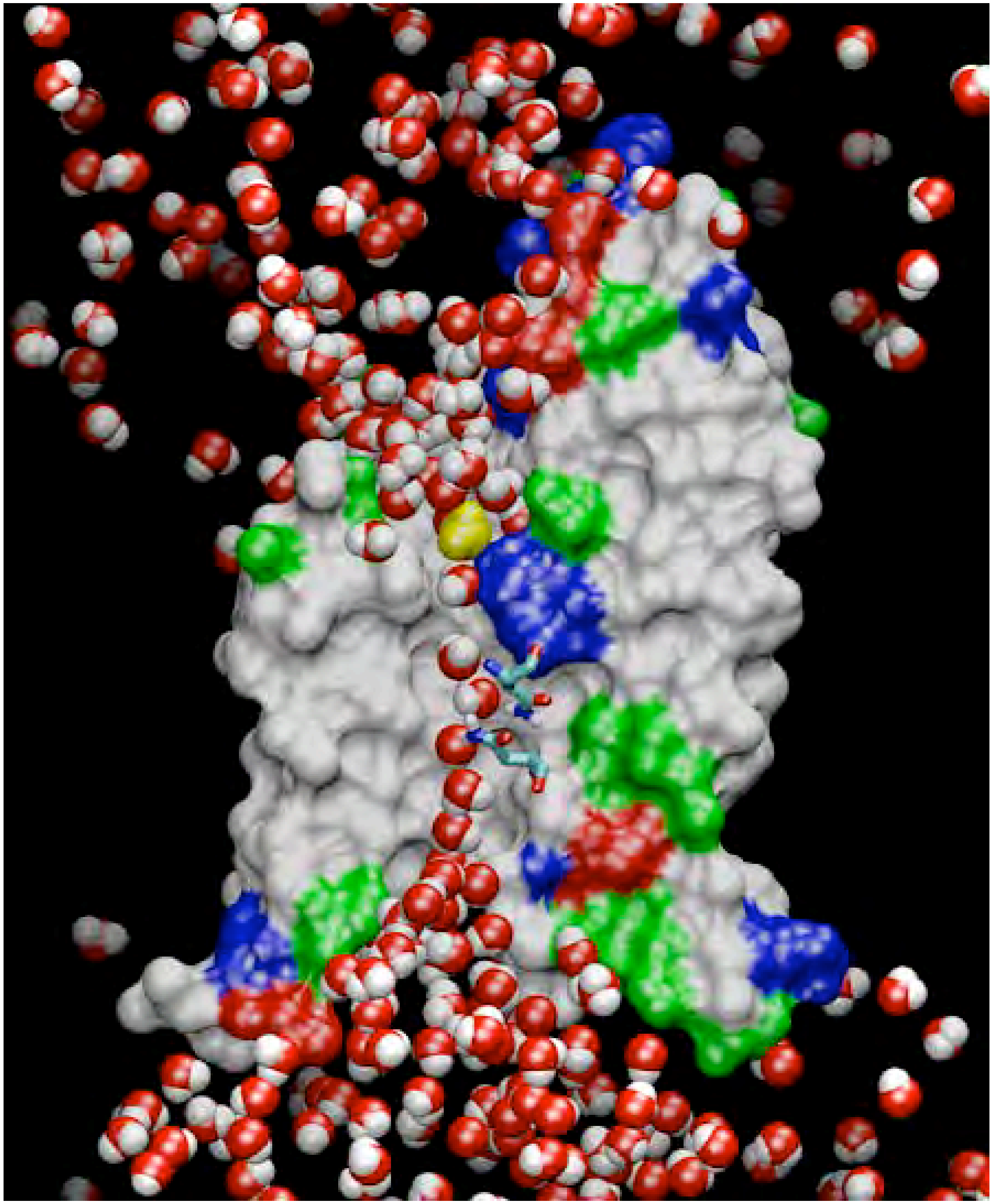
18 water conducted
In 4 monomers in 4 ns
1.125 water/monomer/ns

Exp. = ~ 1-2 /ns

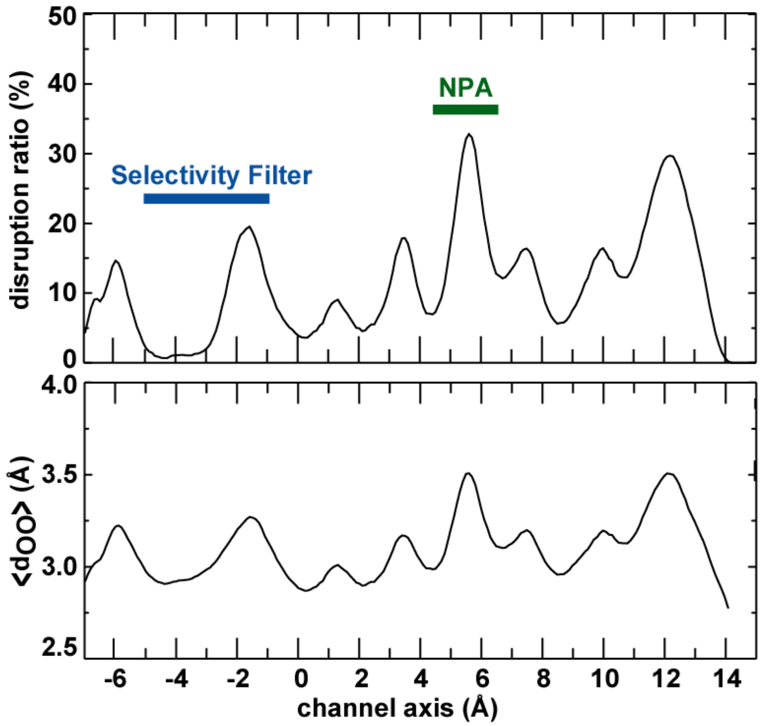
5 nanosecond
Simulation

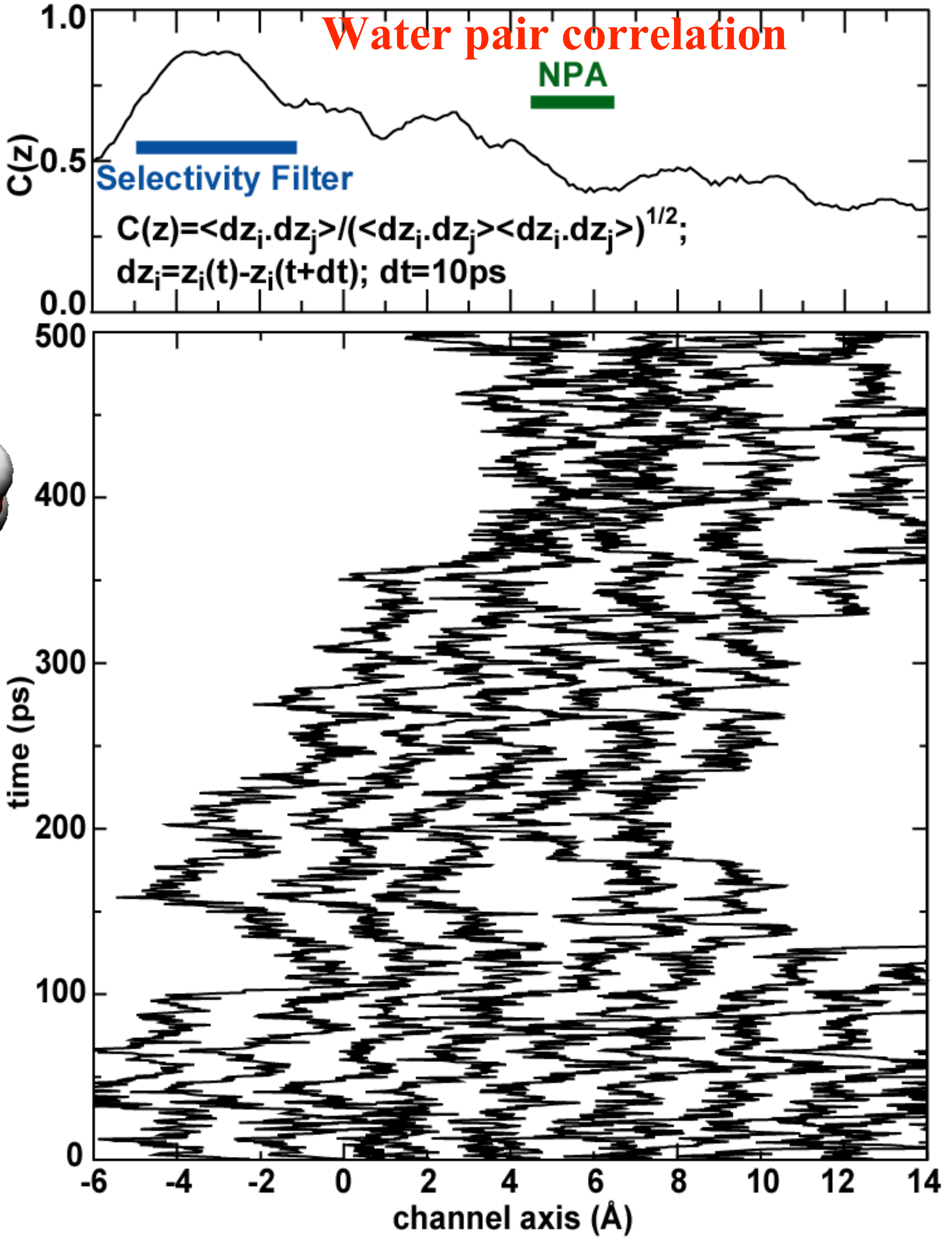


7-8 water
molecules in each
channel

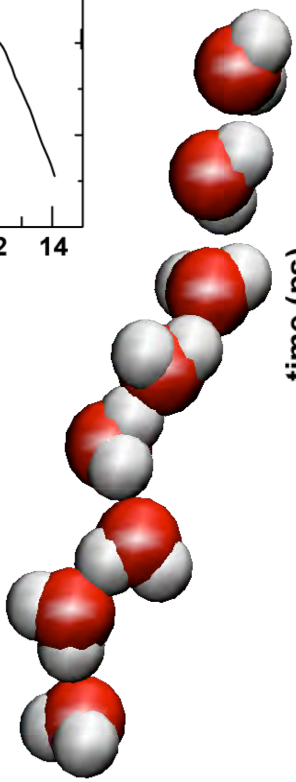


Correlated Motion of Water in the Channel

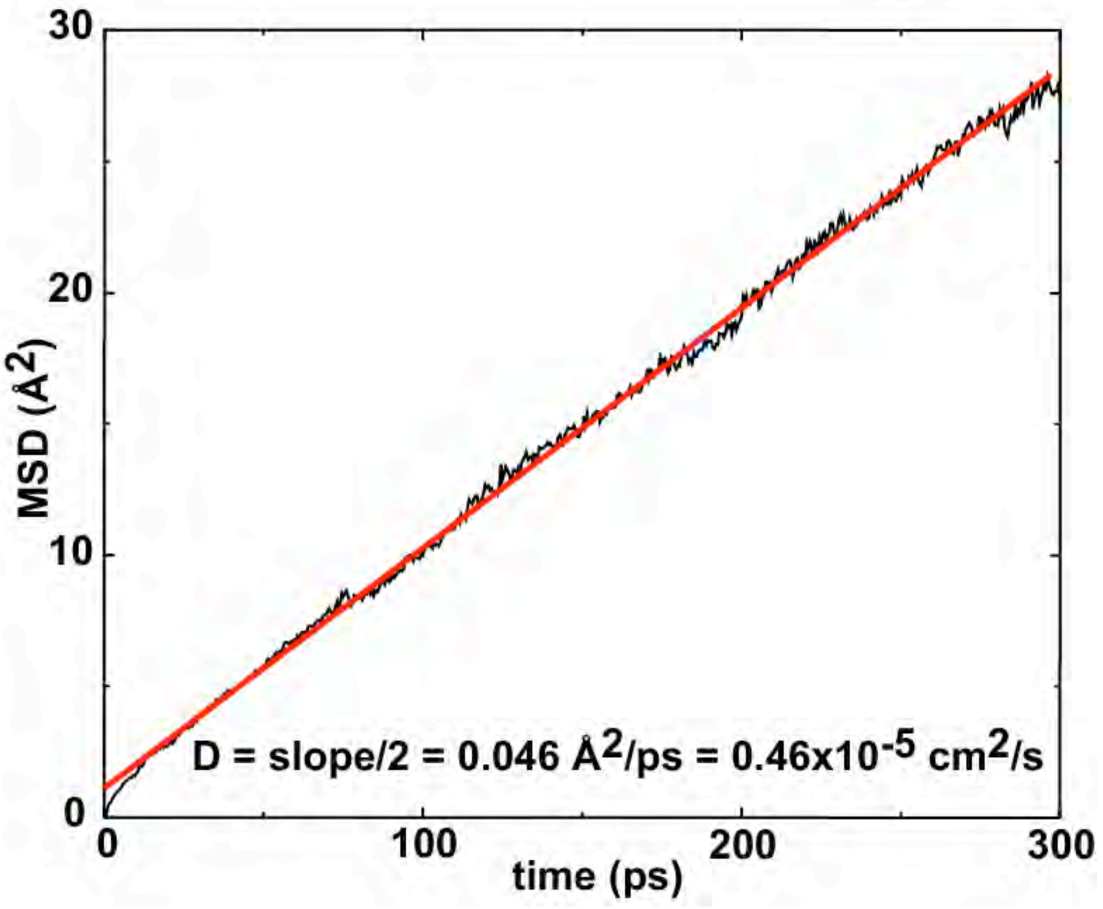


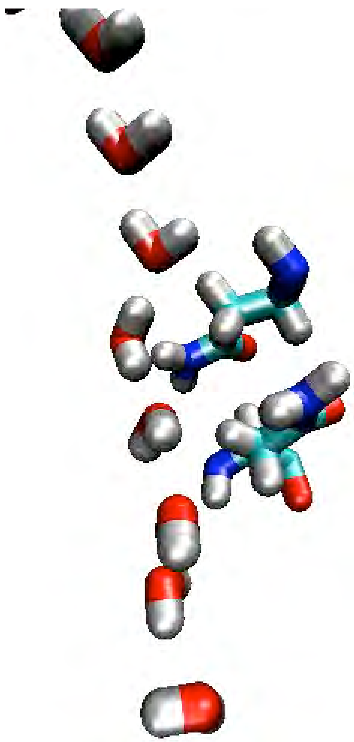


The single file of water molecules is maintained.



Diffusion of Water in the channel

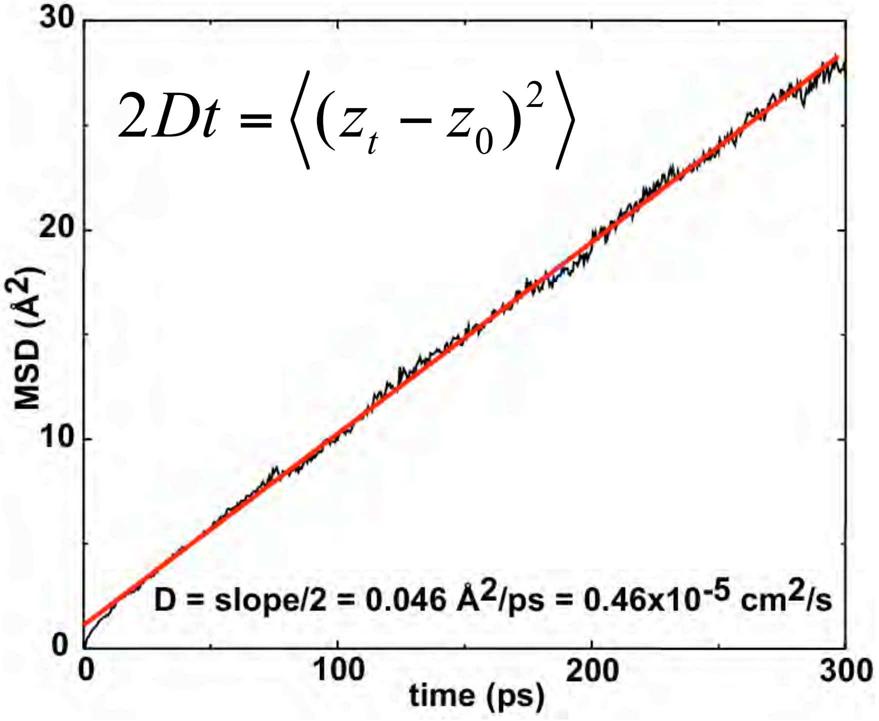


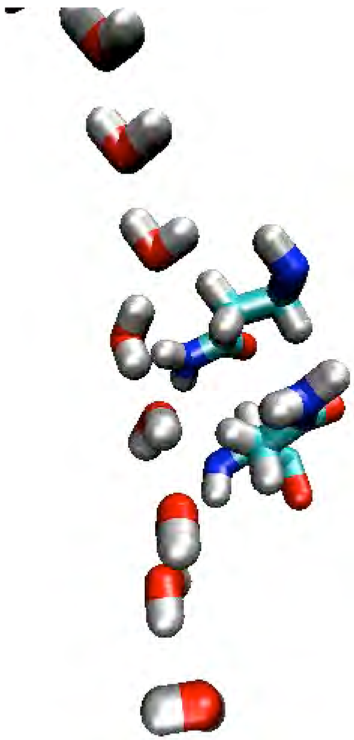


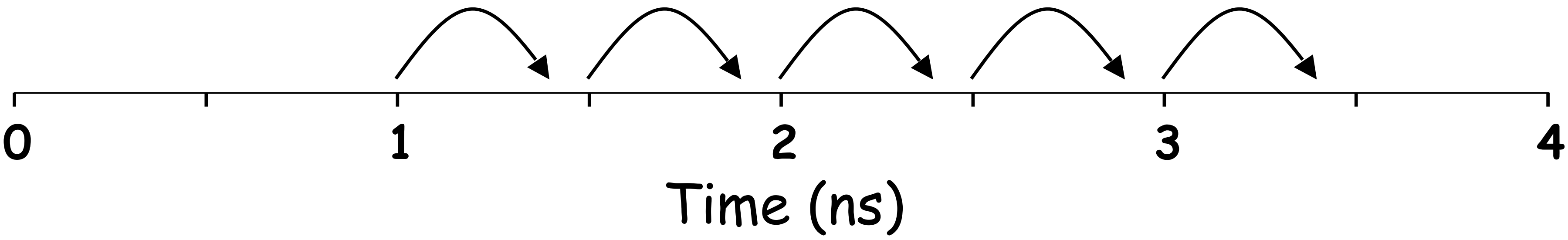
One dimensional diffusion: $2Dt = \left\langle (z_t - z_0)^2 \right\rangle$

Experimental value for AQP1: 0.4-0.8 e-5

Diffusion of Water in the channel

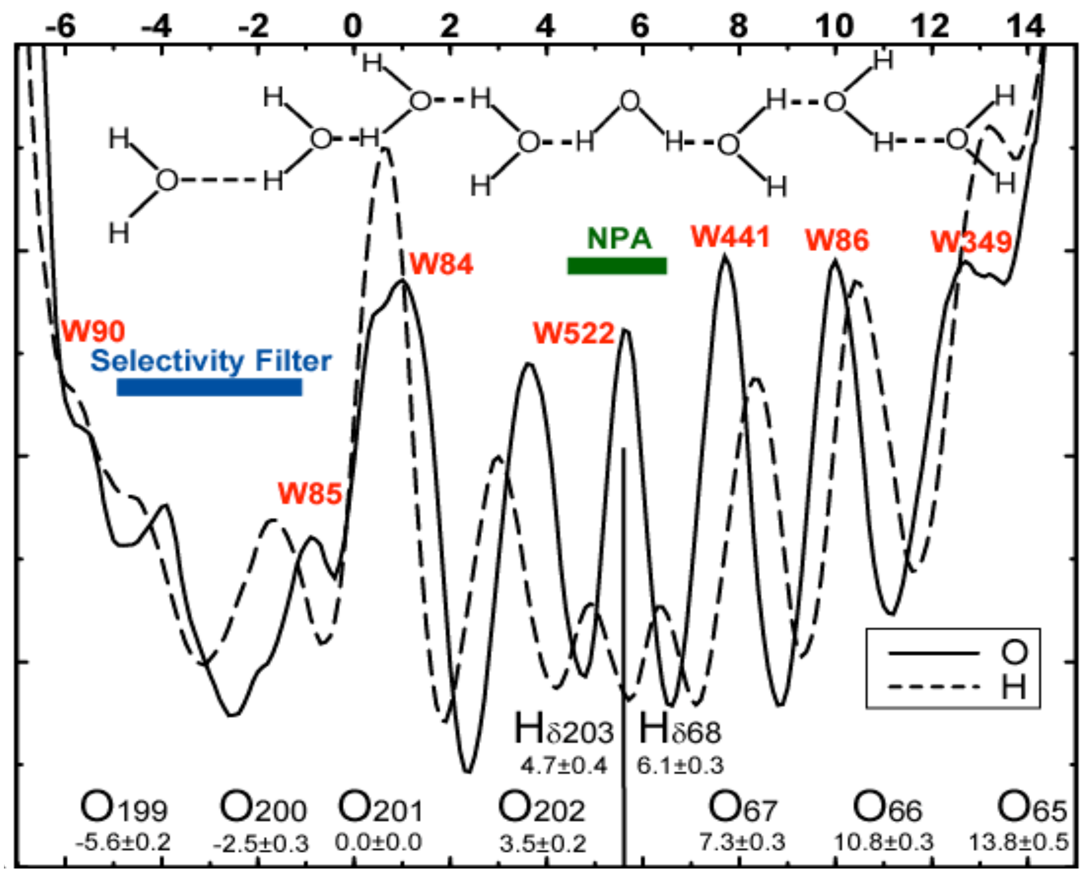


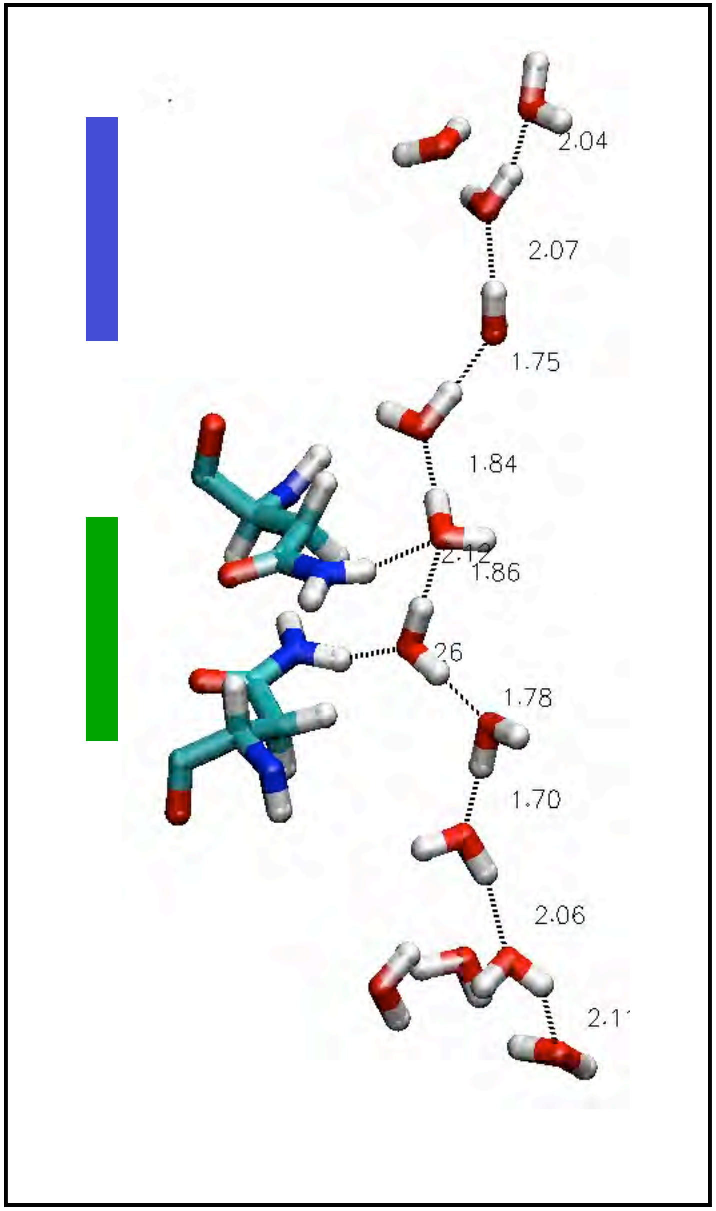




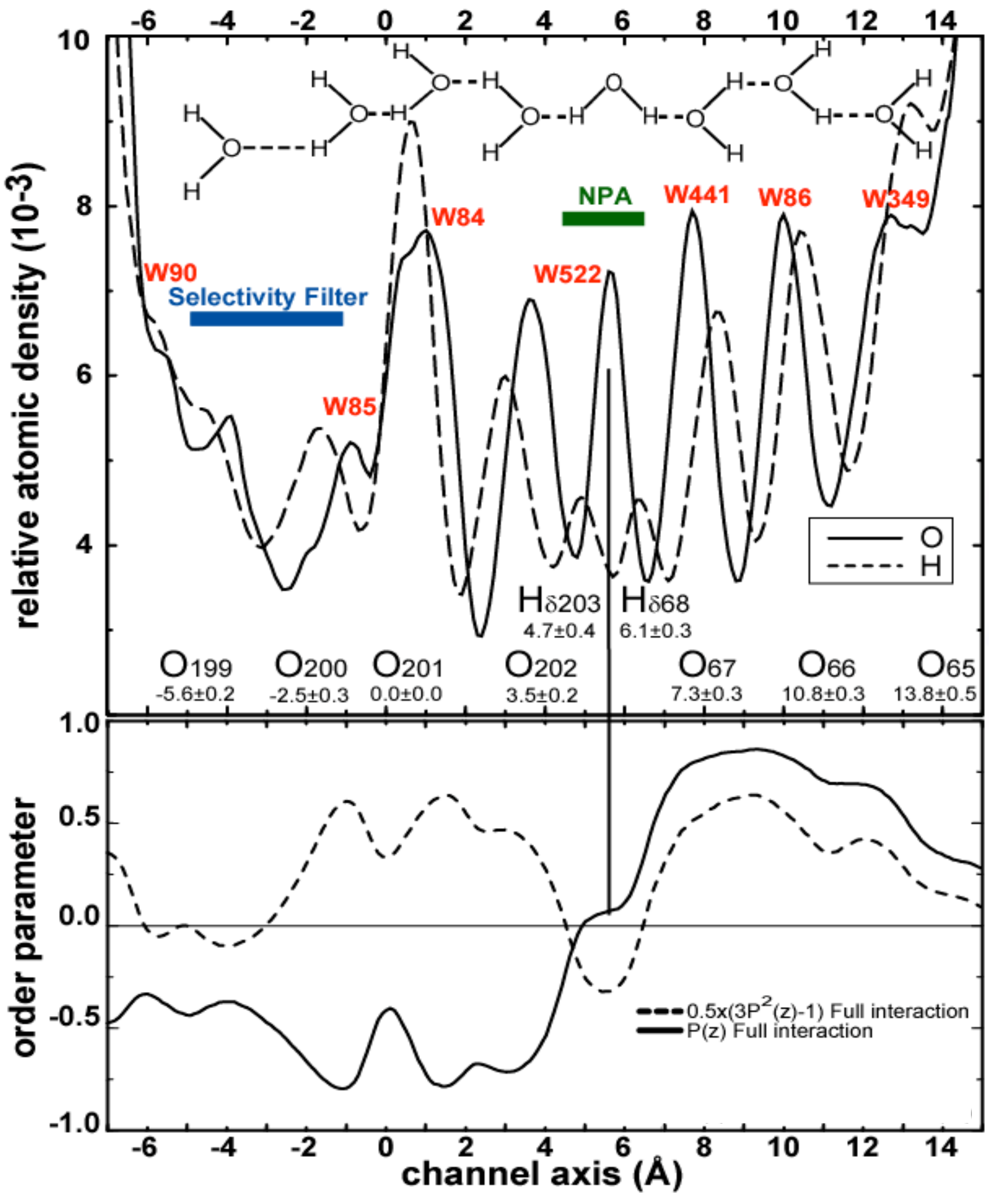
Improvement of statistics

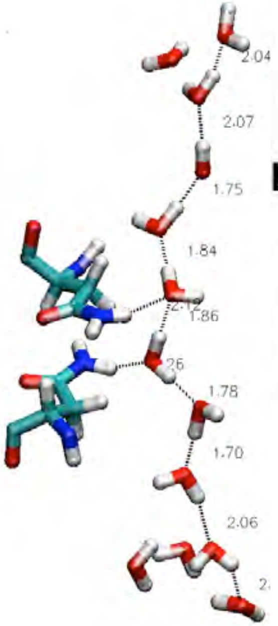
Water Bipolar Configuration in Aquaporins

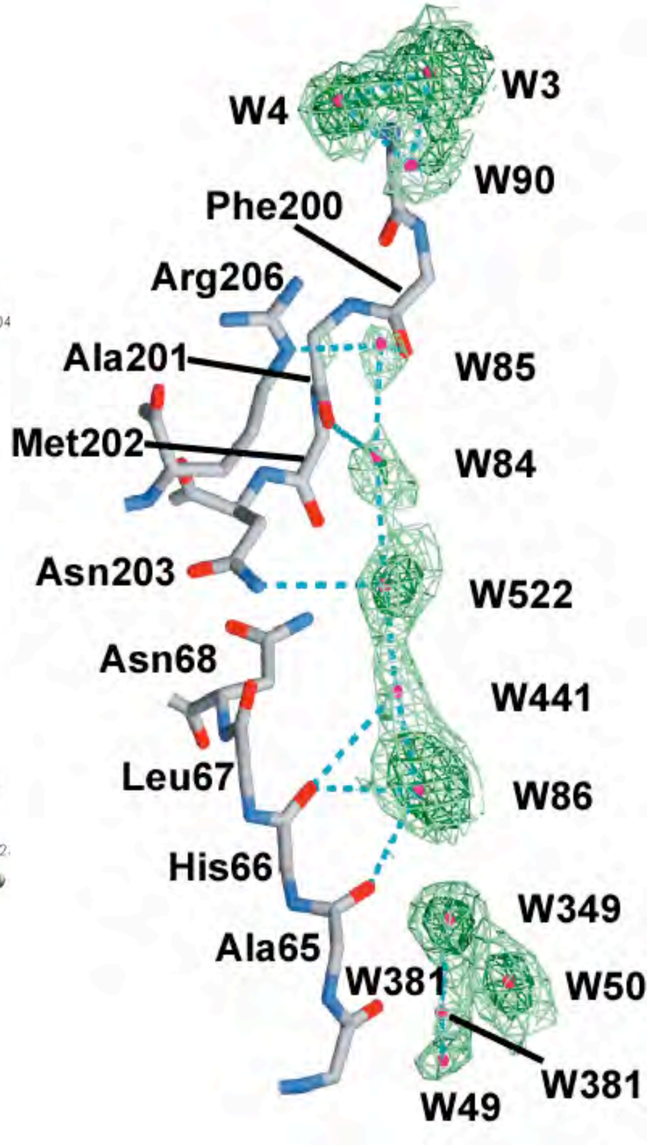


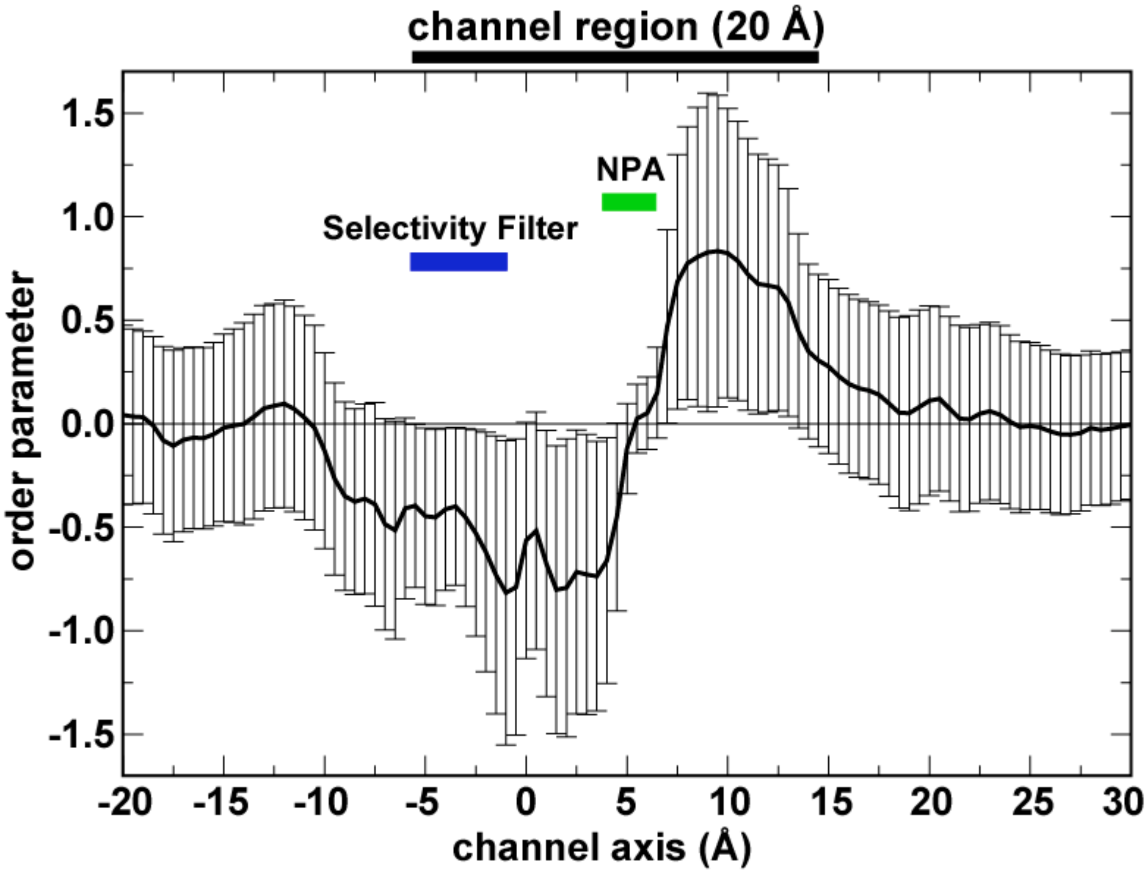


Water Bipolar Configuration in Aquaporins







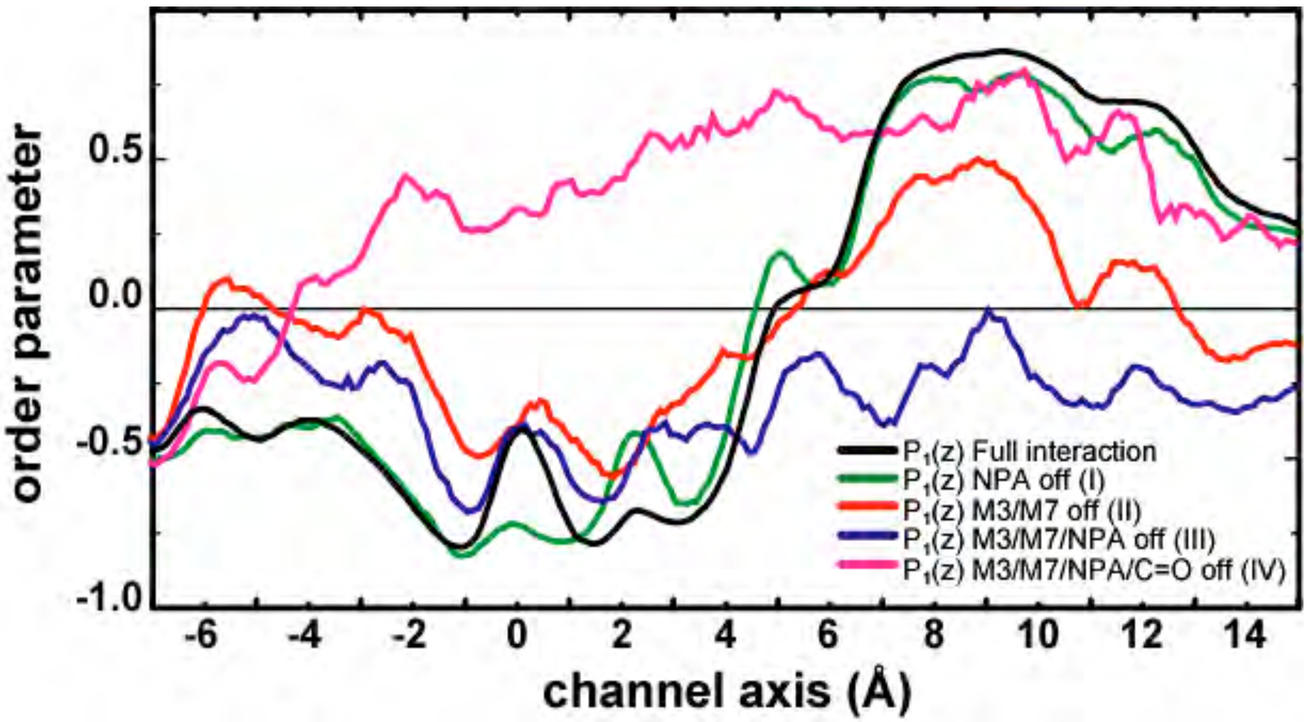

channel region (20 Å)
NPA
Selectivity Filter
order parameter
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-20
-15
-10
-5
0
5
10
15
20
25
30
channel axis (Å)

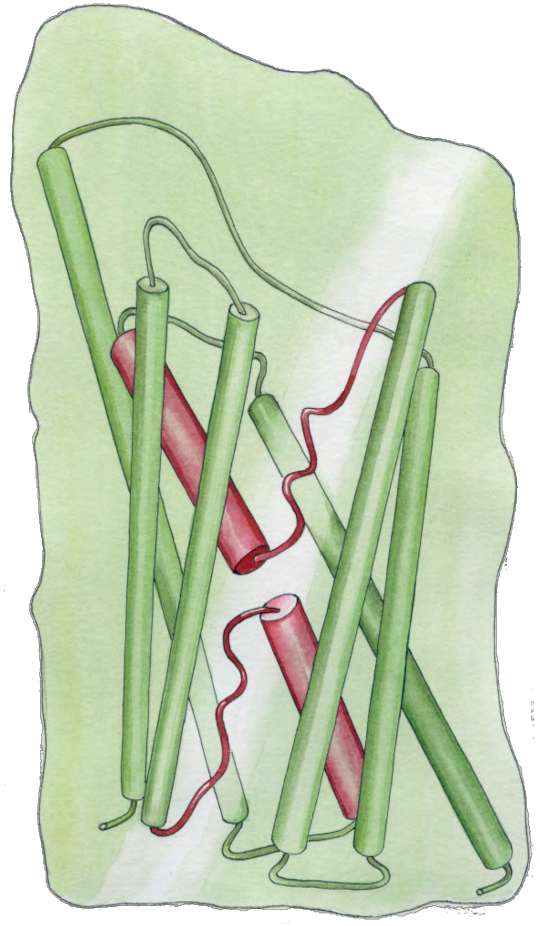
REMEMBER:

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!

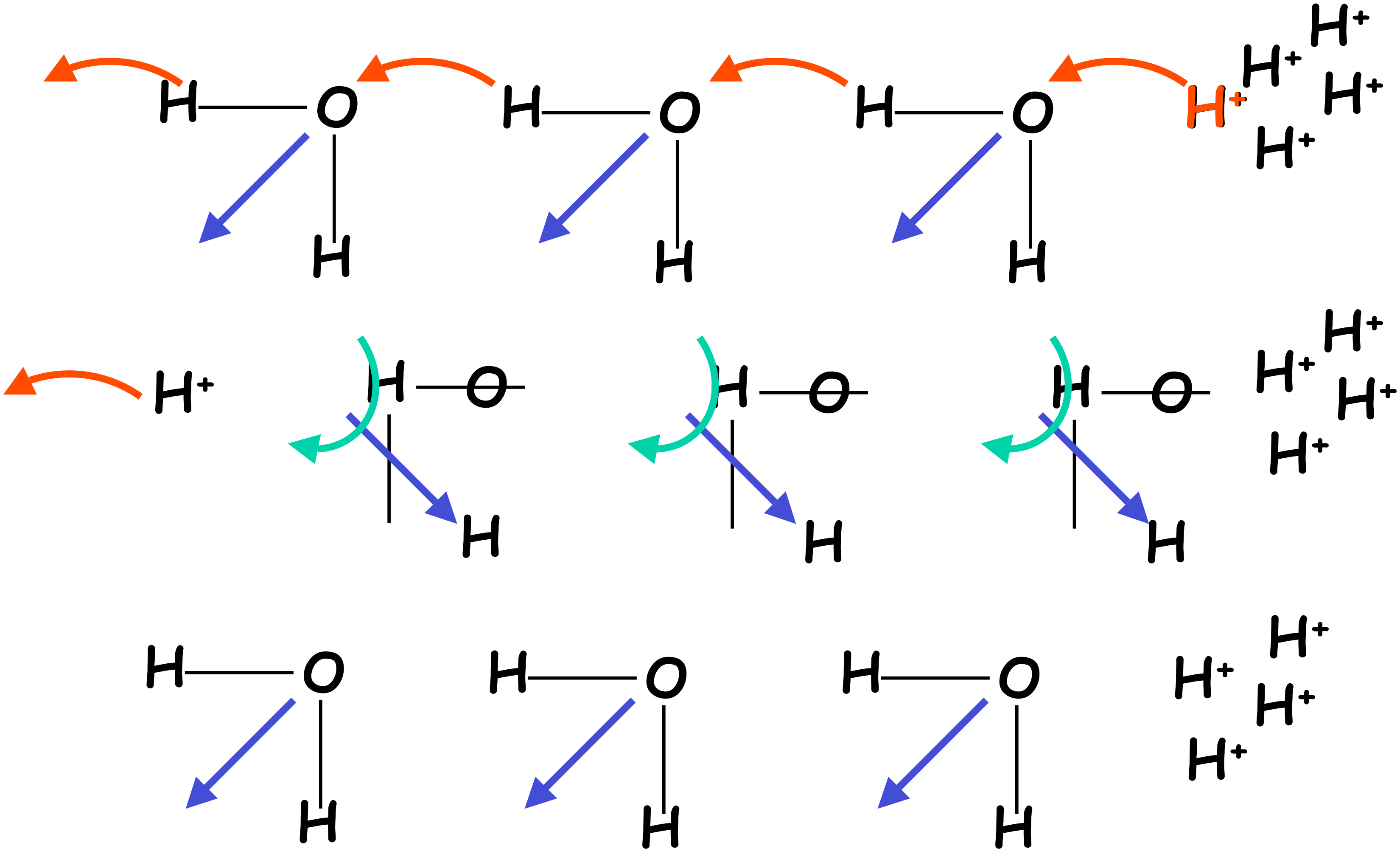
This is a powerful technique to test hypotheses developed during your simulations. Use it!

Electrostatic Stabilization of Water Bipolar Arrangement

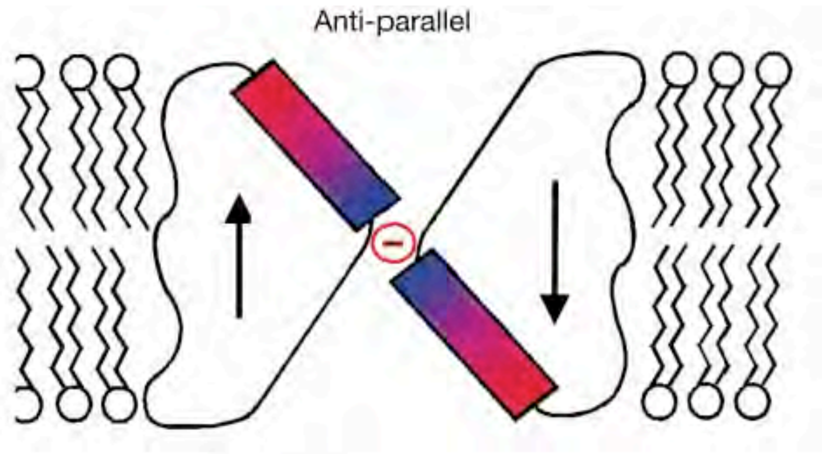


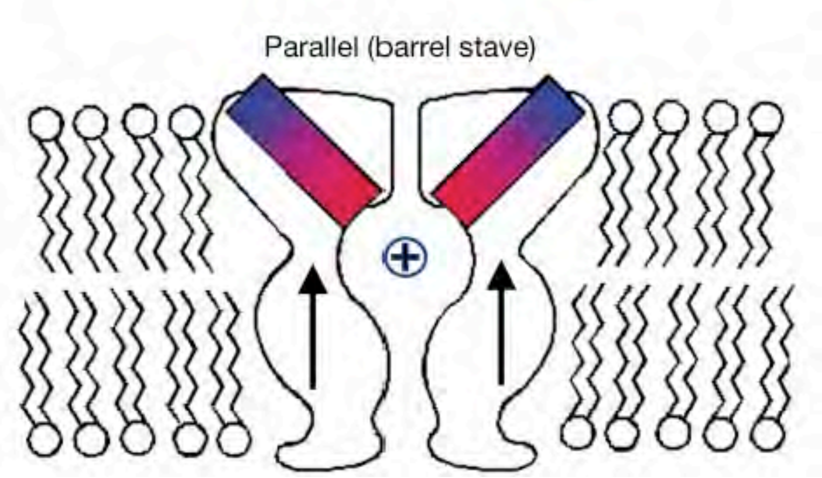


Proton transfer through water

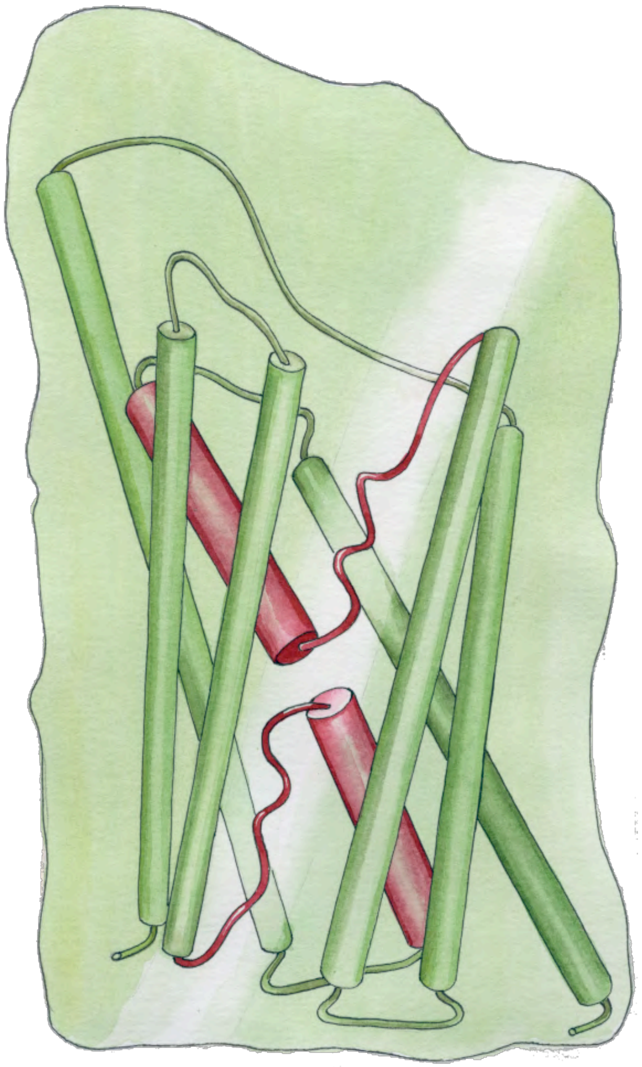


Cl^- channel

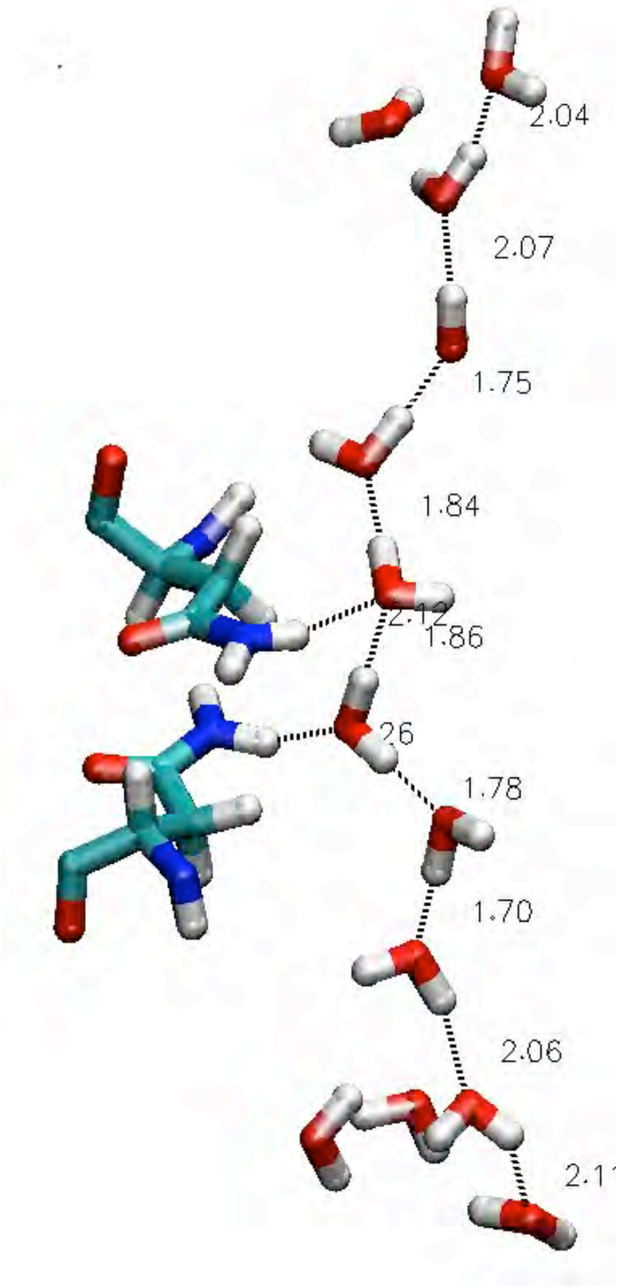




K^+ channel



Aquaporins


2.04
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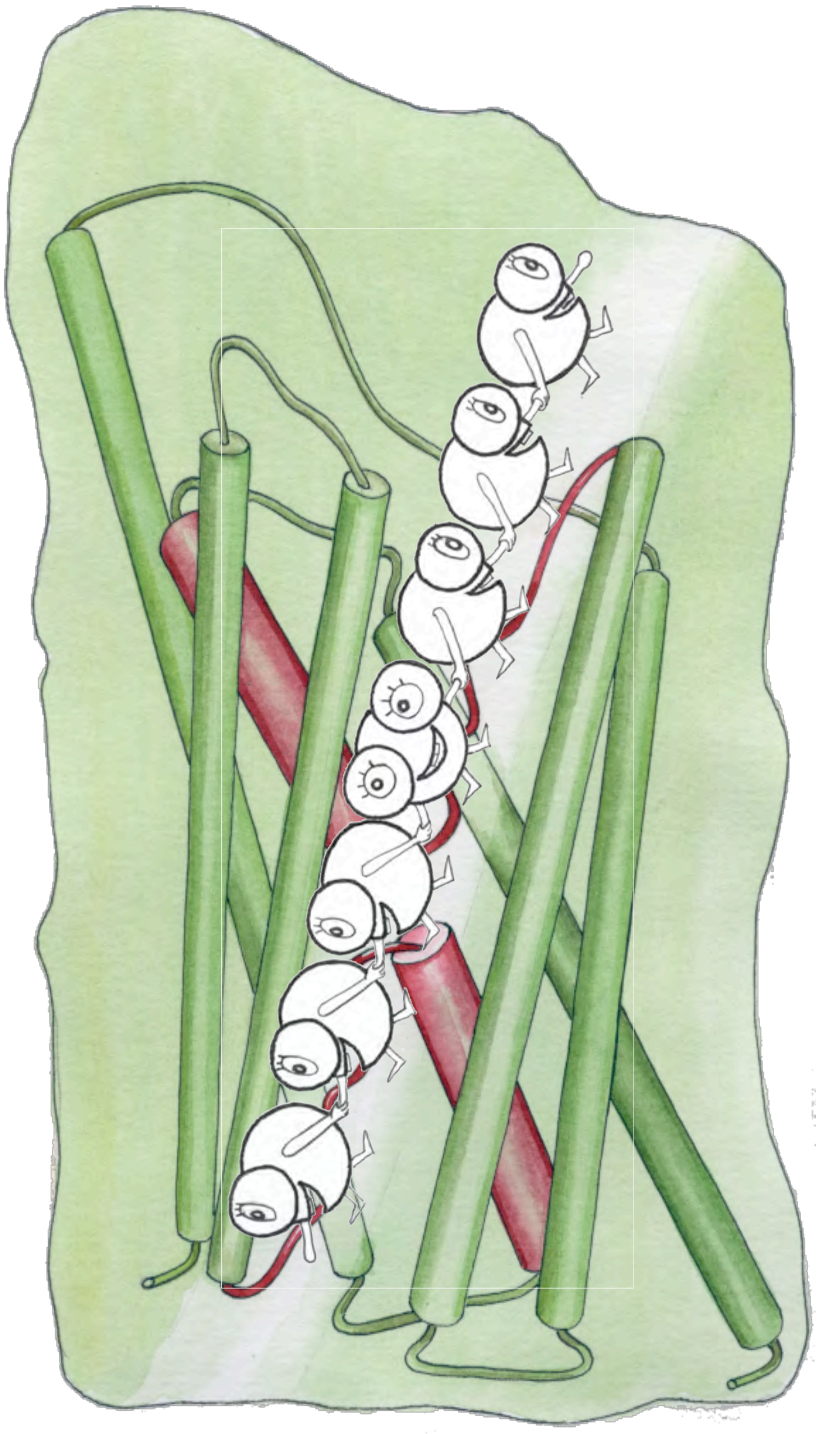
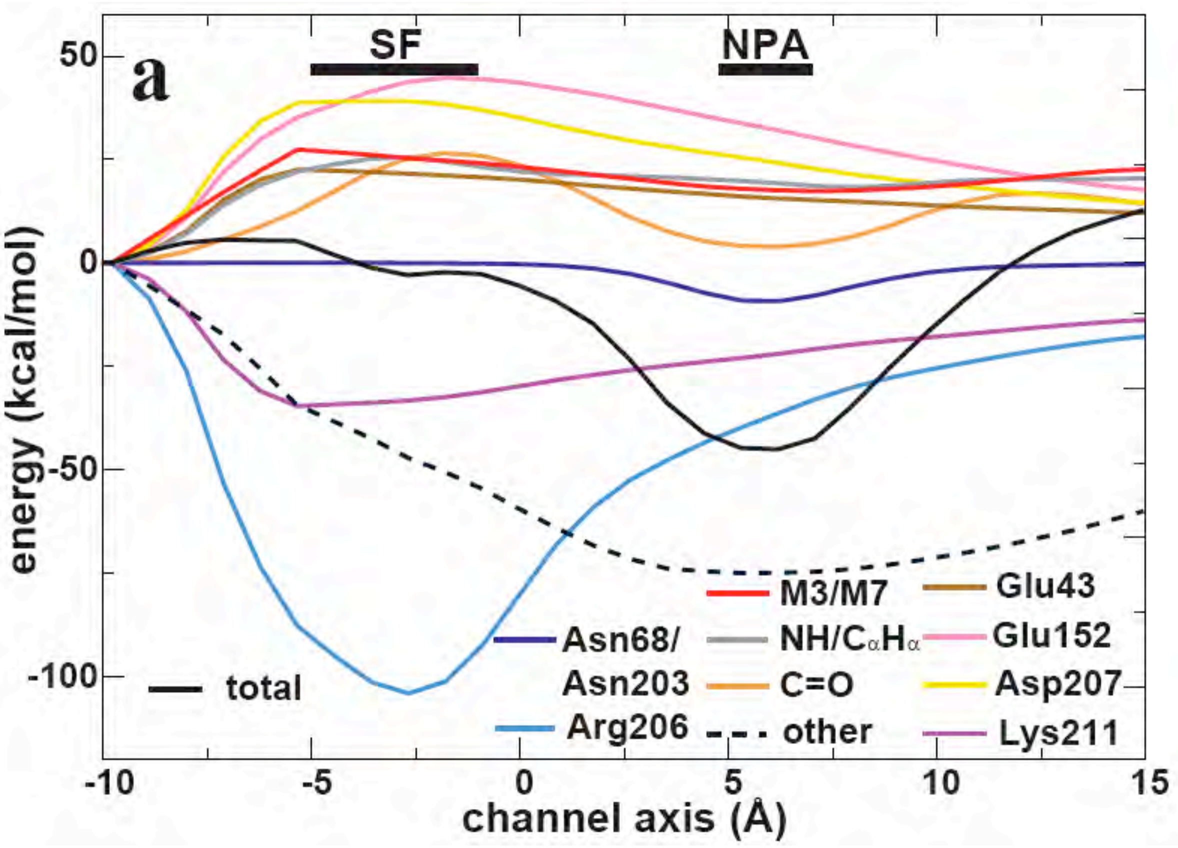
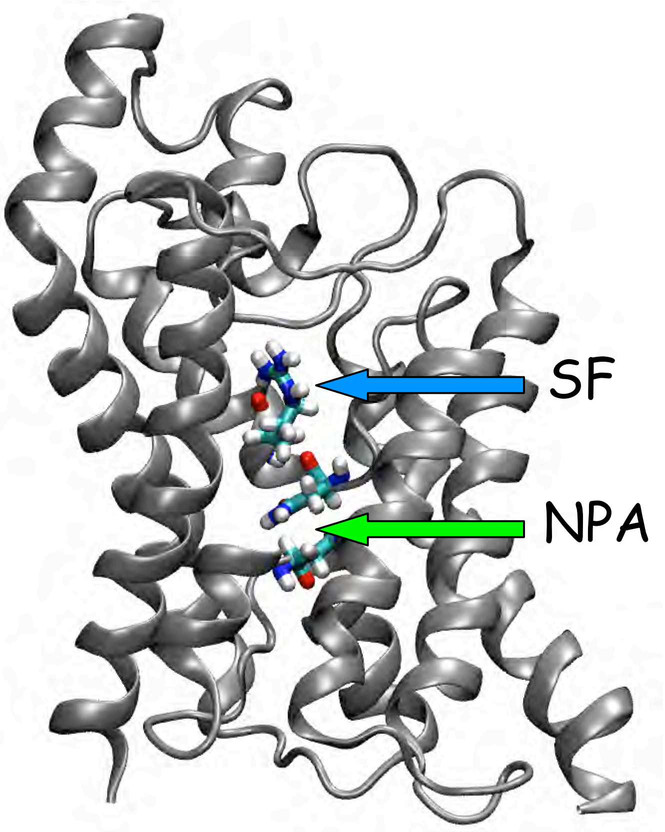
1.84
2.12
1.86
26
1.78
1.70
2.06
2.1



A Complex Electrostatic Interaction

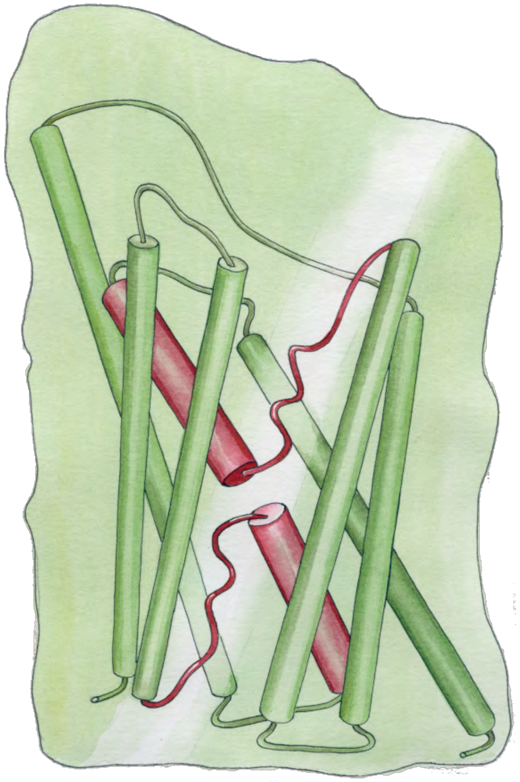




"Surprising and clearly not a hydrophobic channel"

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