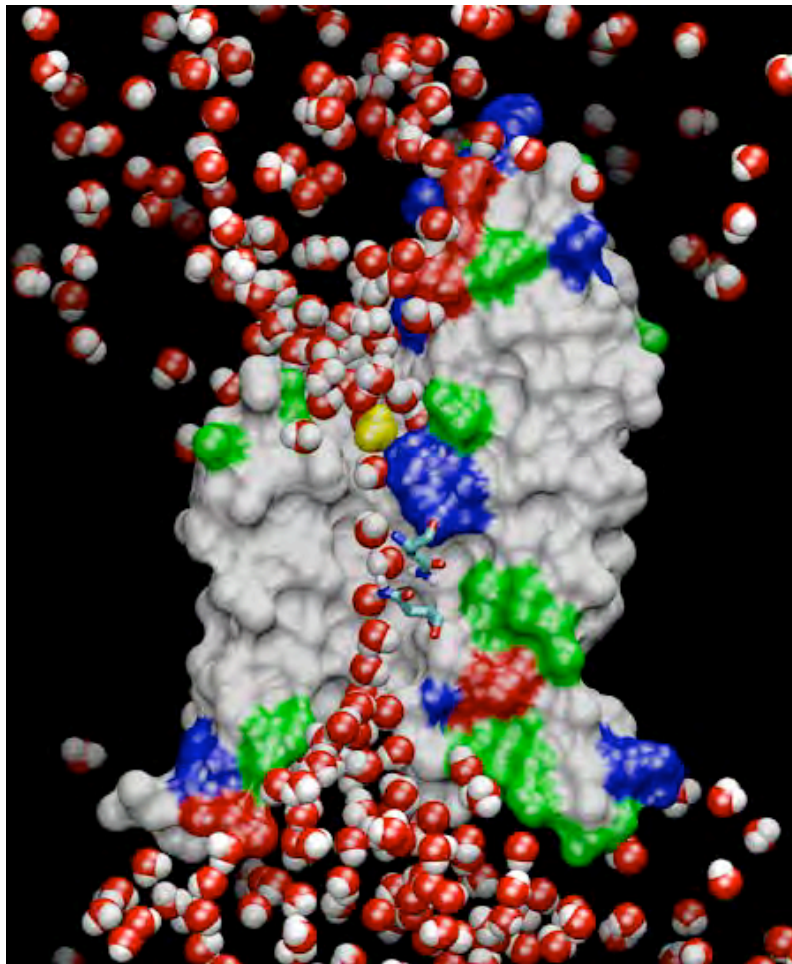
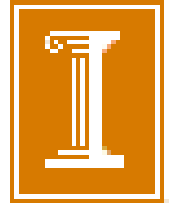
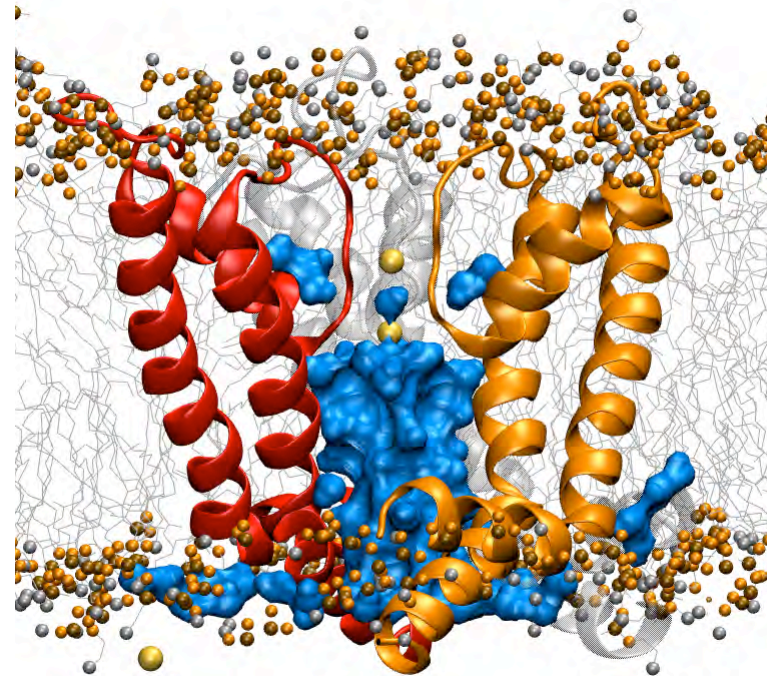


Modeling and Molecular Dynamics of Membrane Proteins



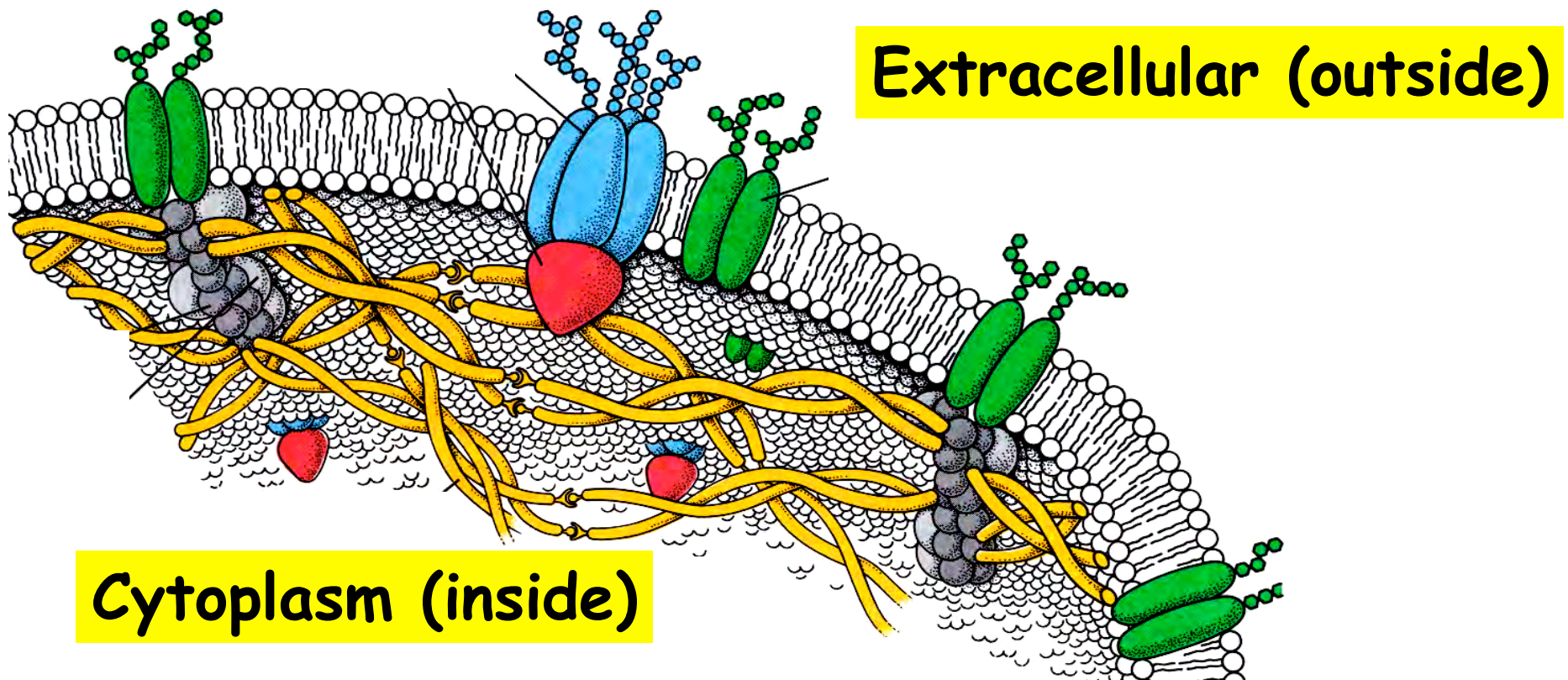
Emad Tajkhorshid
Department of Biochemistry, Center for
Biophysics and Computational Biology, and
Beckman Institute
University of Illinois at Urbana-Champaign



Why Do Living Cells Need Membrane Channels (Proteins)?

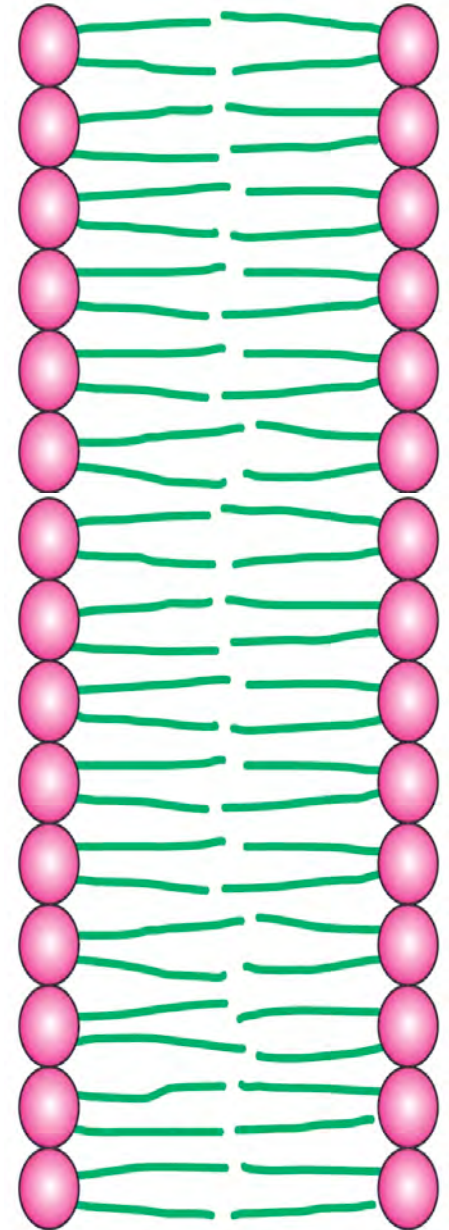
- Living cells also need to exchange materials and information with the outside world

... however, in a highly selective manner.

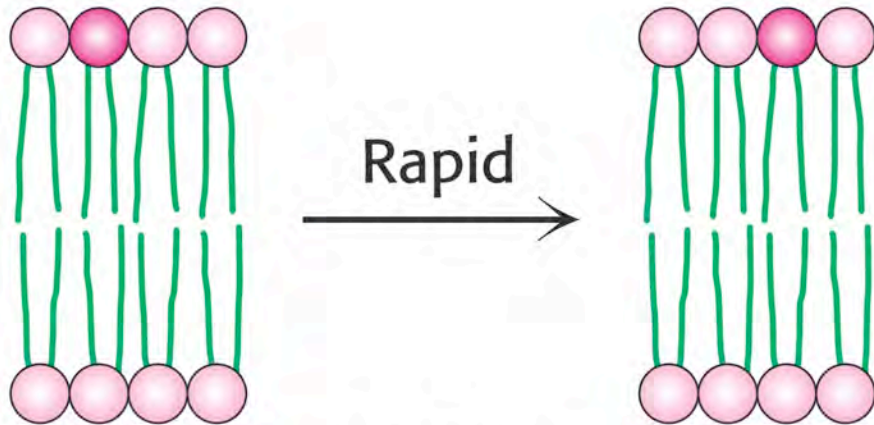


Lipid Bilayers Are Excellent For Cell Membranes

- 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

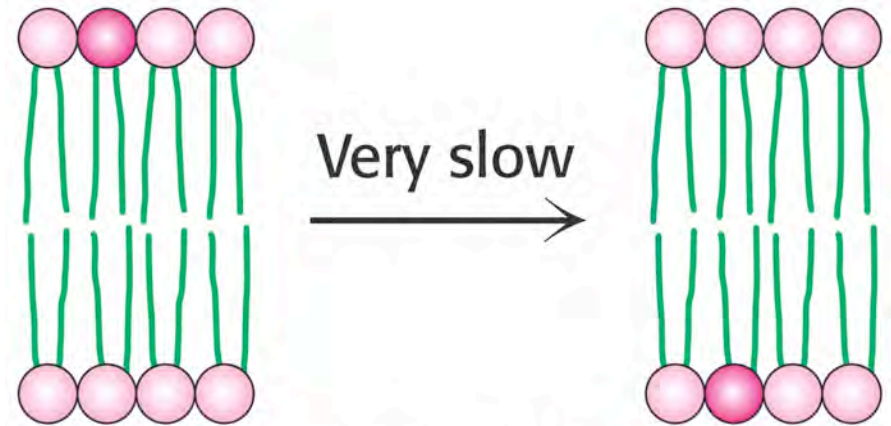


Lateral diffusion

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

$$50 \text{ \AA} \text{ in } \sim 2.5 \times 10^{-5} \text{ s}$$

$$D_{\text{lip}} = 10^{-8} \text{ cm}^2 \cdot \text{s}^{-1}$$
$$D_{\text{wat}} = 2.5 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$$

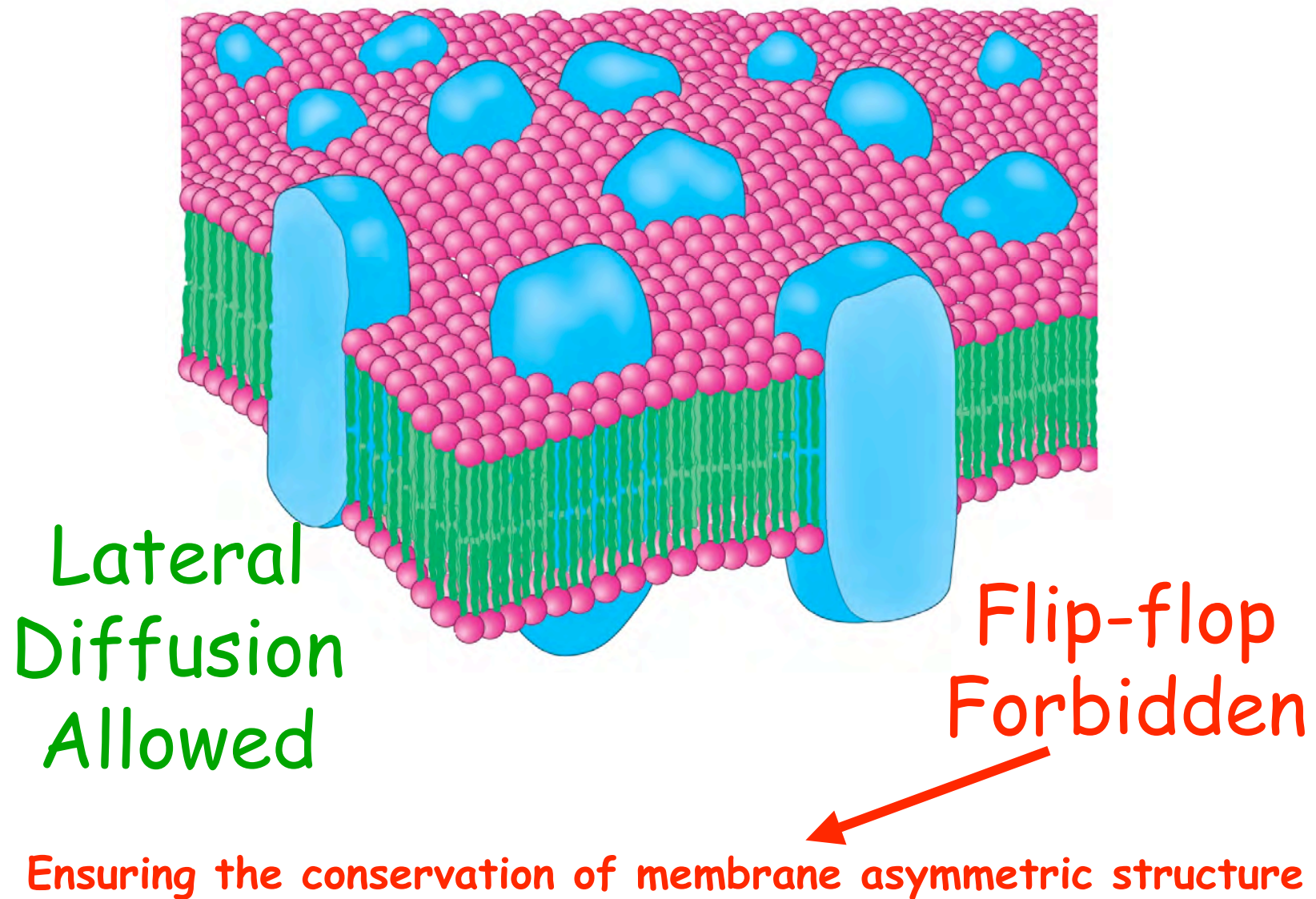


Transverse diffusion
(flip-flop)

Once in several hours!
(10^4 s)

**~9 orders of magnitude
difference**

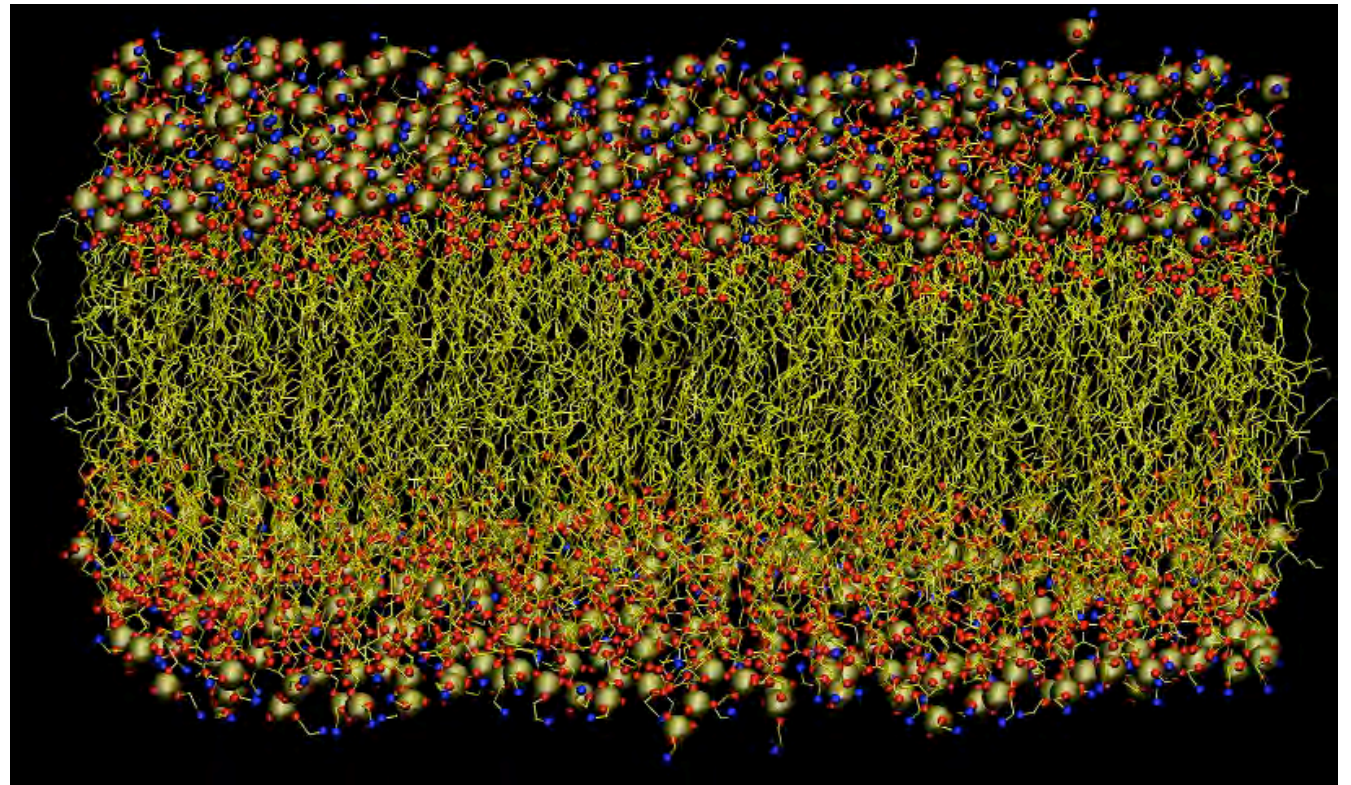
Fluid Mosaic Model of Membrane



Technical difficulties in Simulations of Biological Membranes

- Time scale
- Heterogeneity of biological membranes ☹️

60 x 60 Å
Pure POPE
5 ns
~100,000
atoms

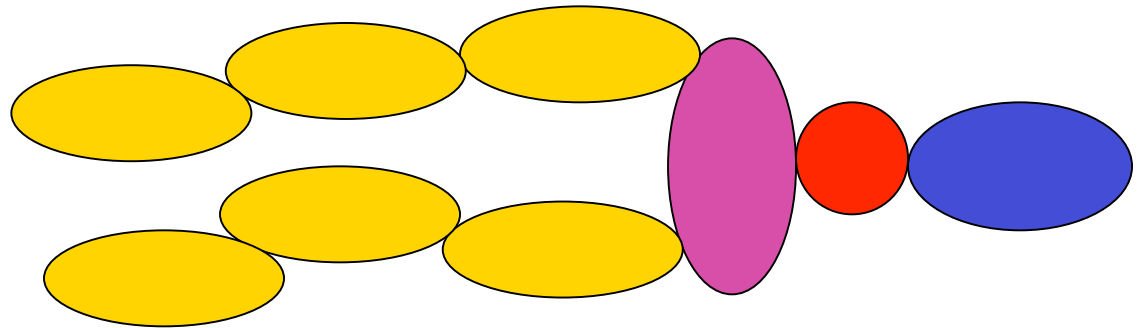
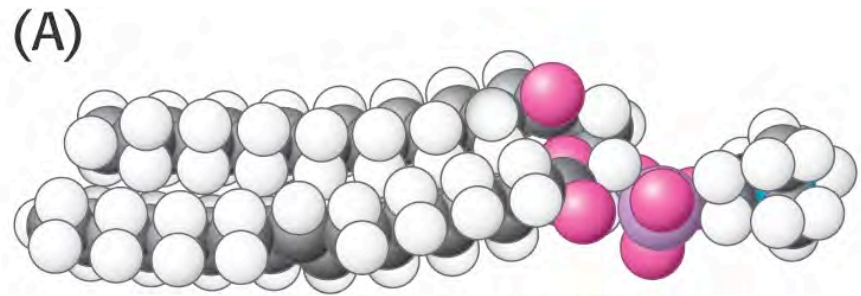


Coarse grain modeling of lipids

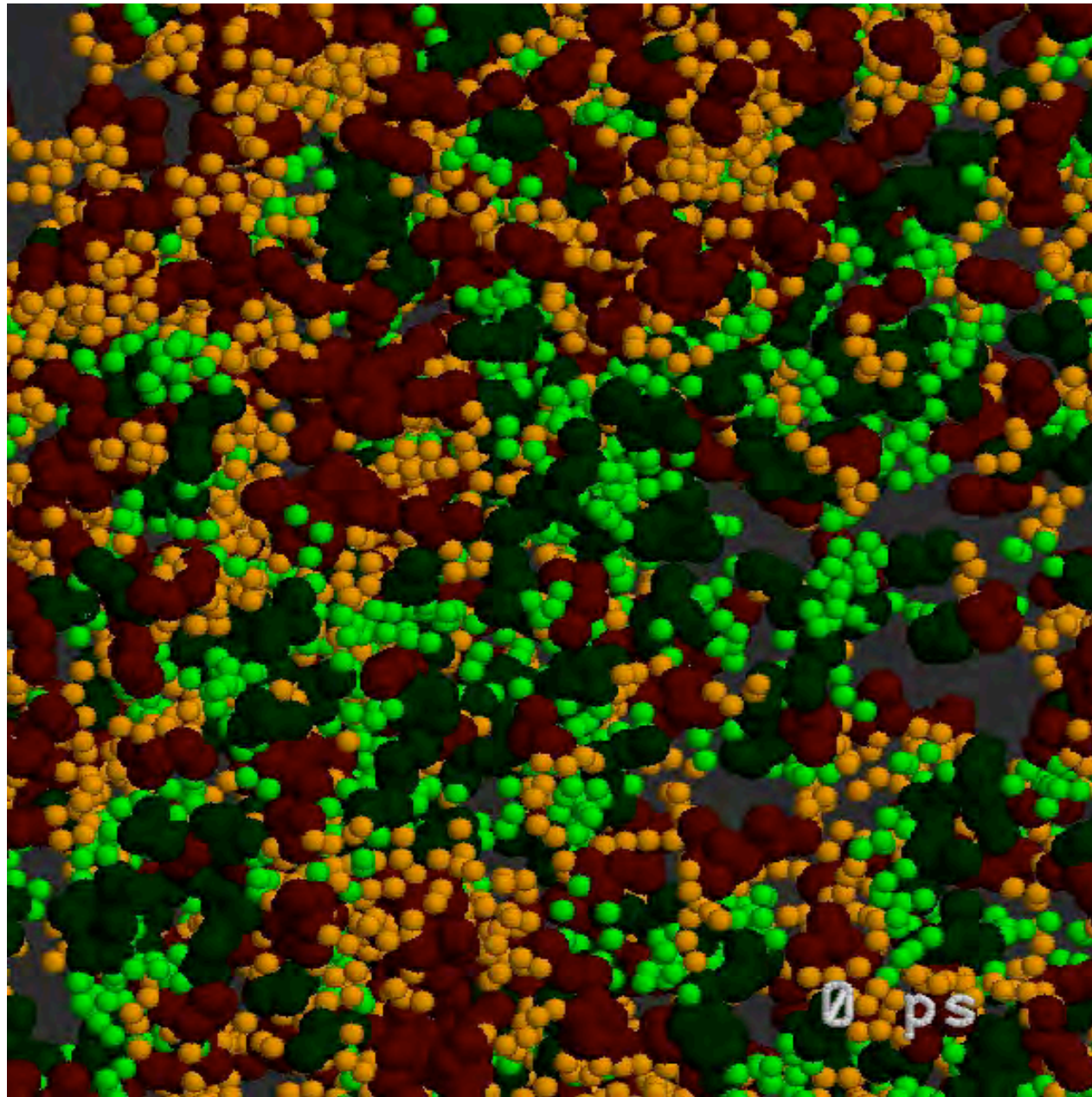
150 particles



9 particles!



Also, increasing the time step by orders of magnitude.

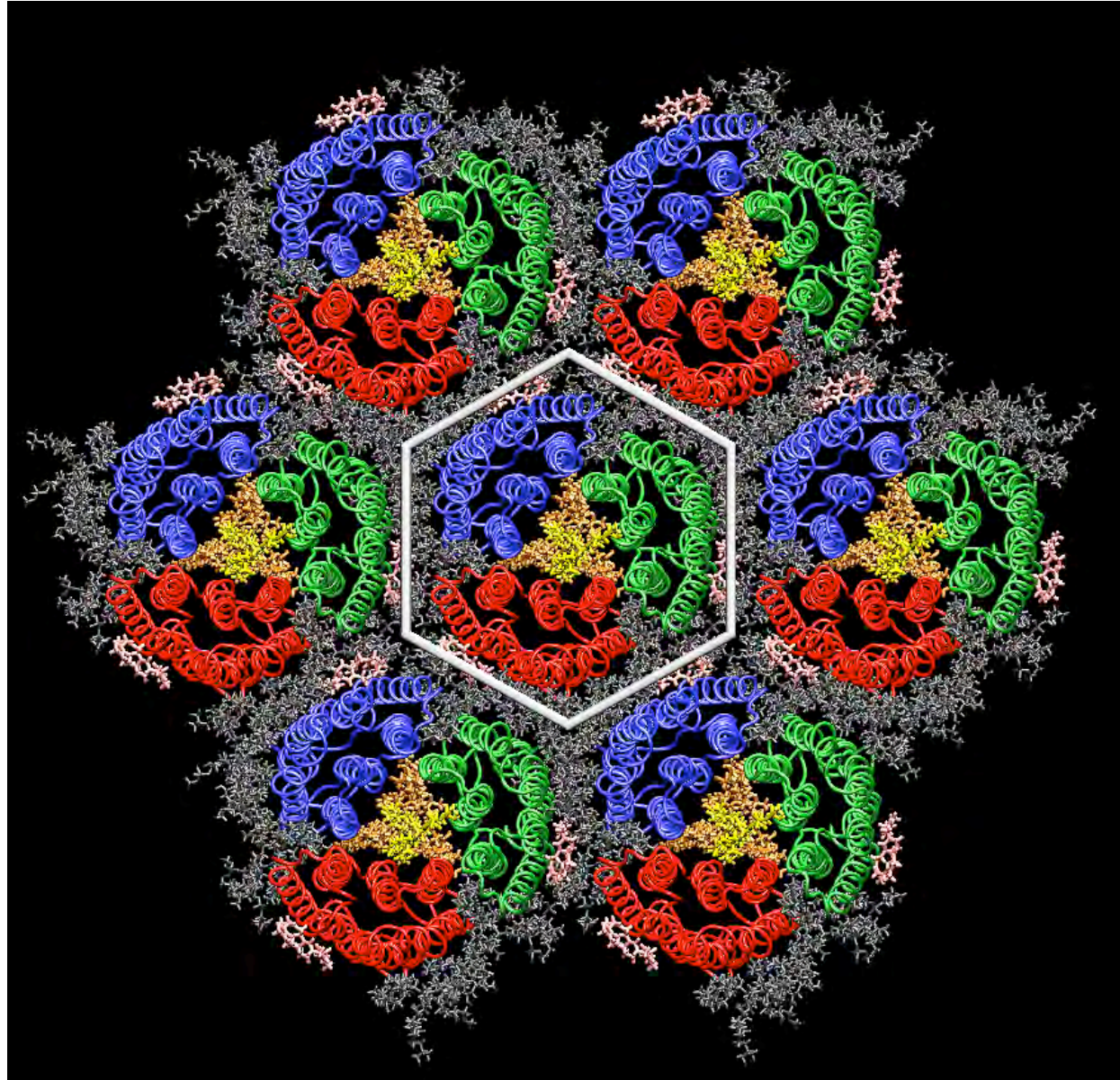


by: J. Siewert-Jan Marrink and Alan E. Mark, University of Groningen, The Netherlands

Protein/Lipid ratio

- Pure lipid: insulation (neuronal cells)
- Other membranes: on average 50%
- Energy transduction membranes (75%)
Membranes of mitochondria and chloroplast
Purple membrane of halobacteria
- Different functions = different protein composition

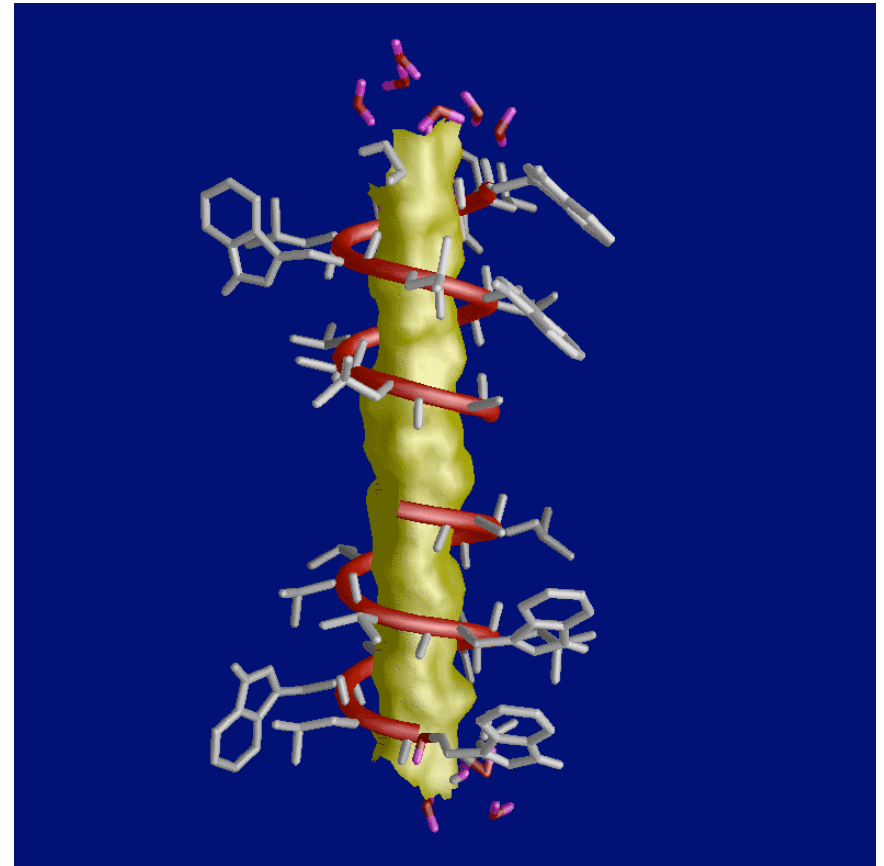
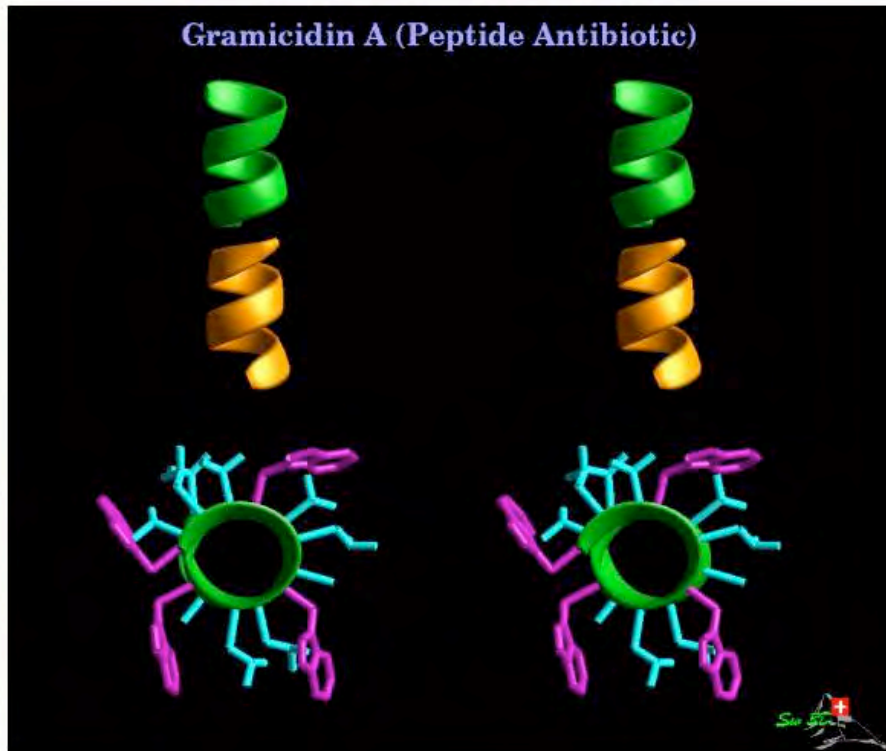
Protein / Lipid Composition



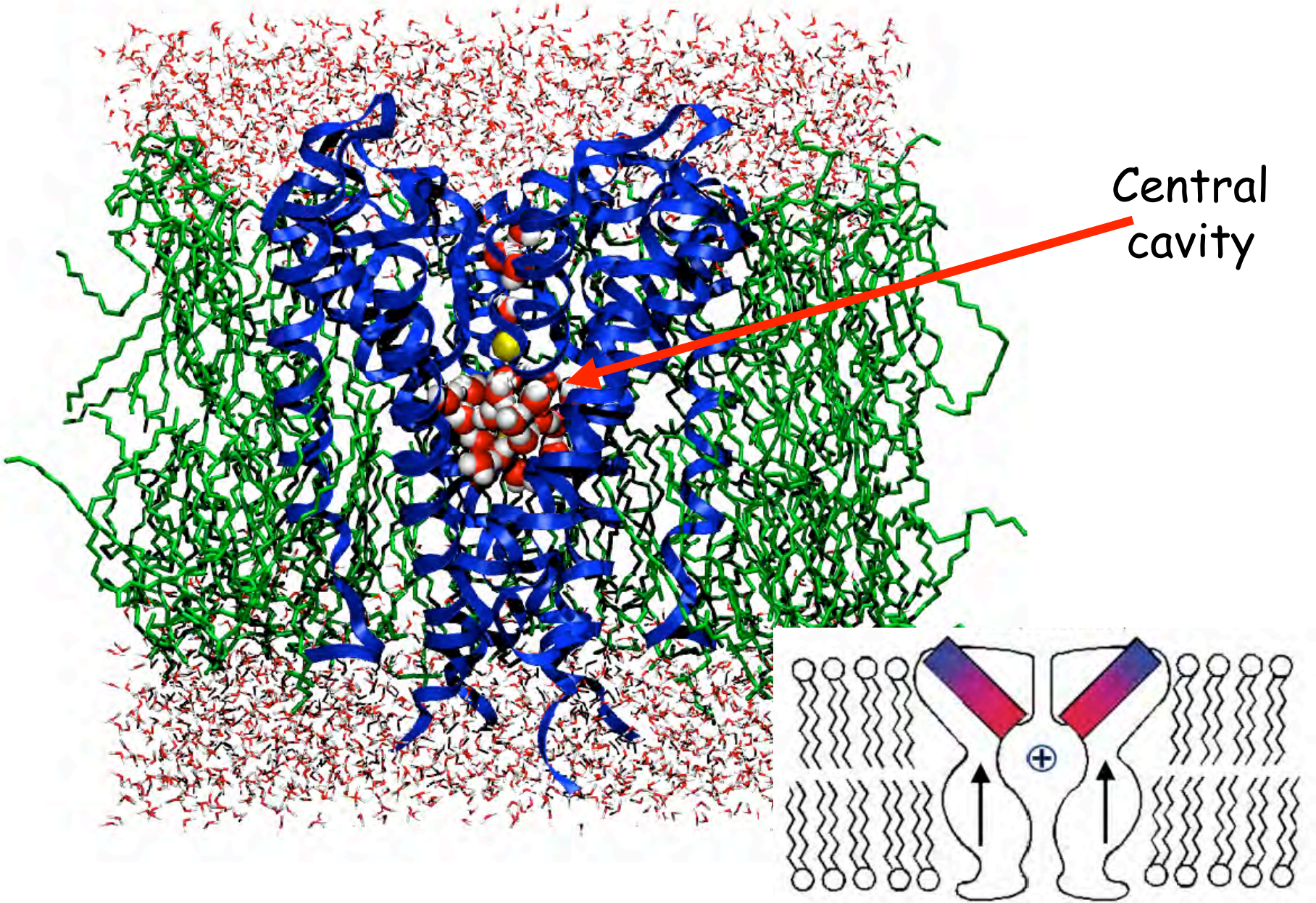
The purple membrane of halobacteria

Gramicidin A

an ion leak inside the membrane



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



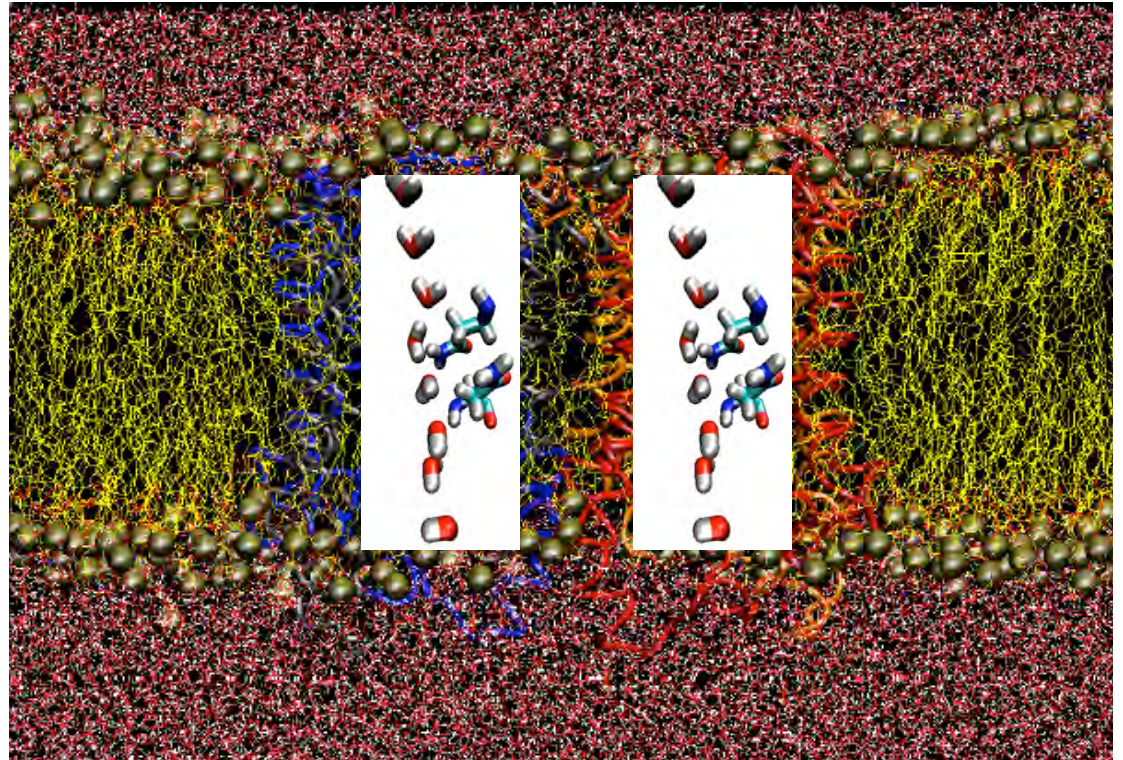
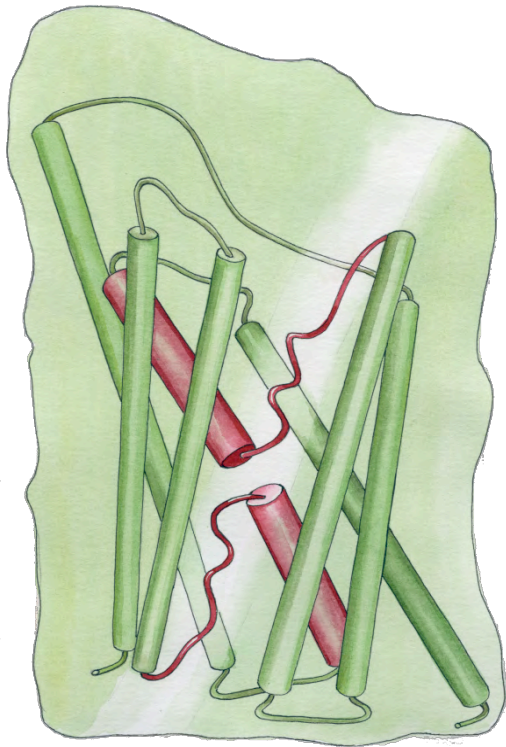
Analysis of Molecular Dynamics Simulations of Biomolecules

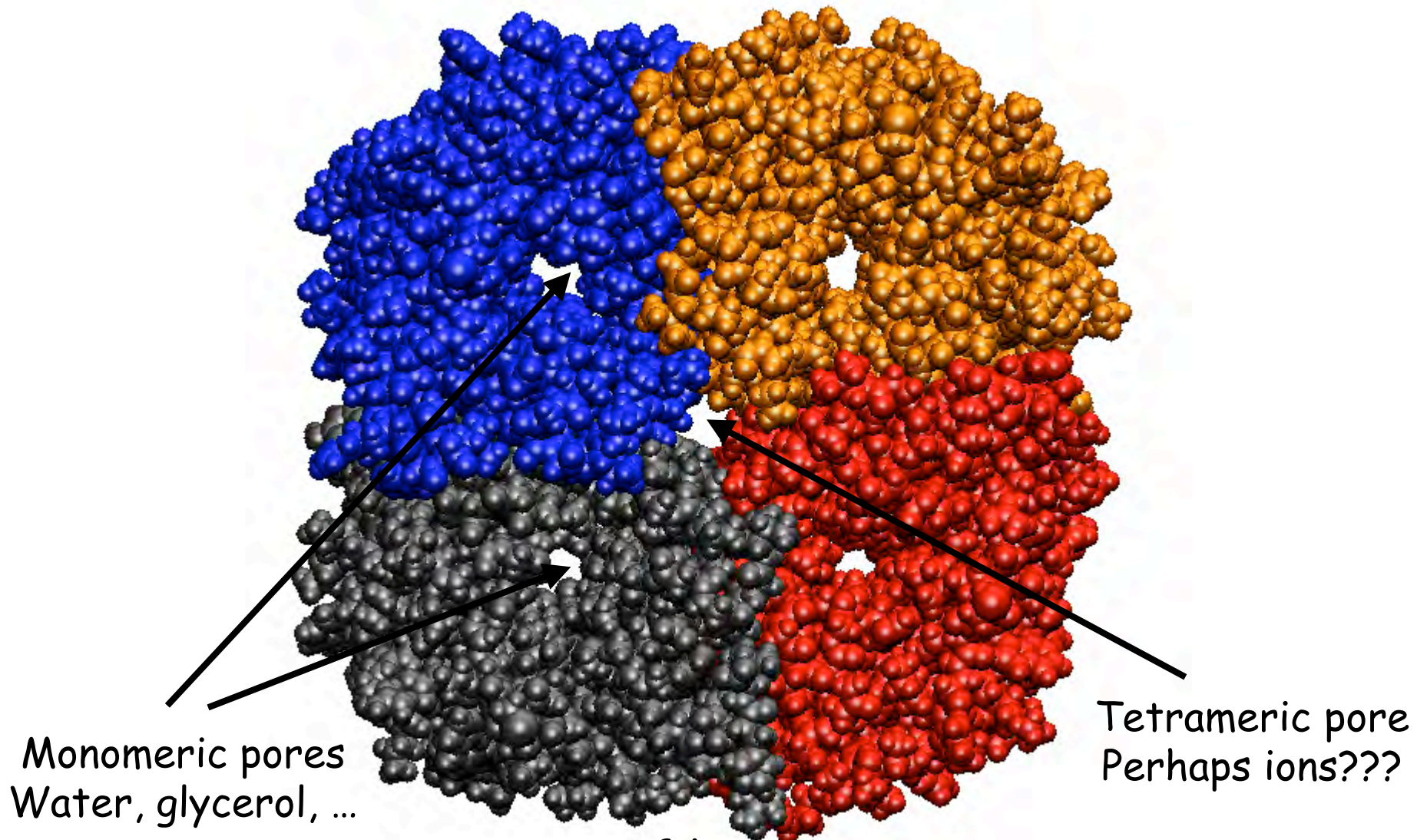
- A very complicated arrangement of hundreds of groups interacting with each other
- Where to start to look at?
- What to analyze?
- How much can we learn from simulations?

It is very important to get acquainted with your system

Aquaporins

Membrane water channels



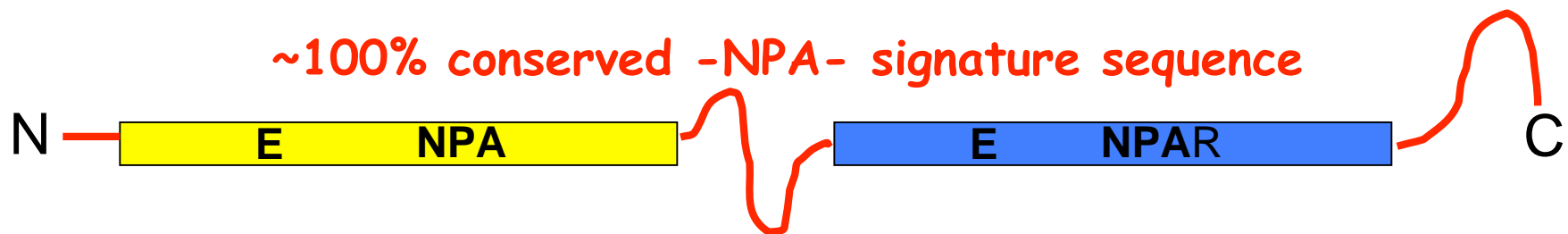
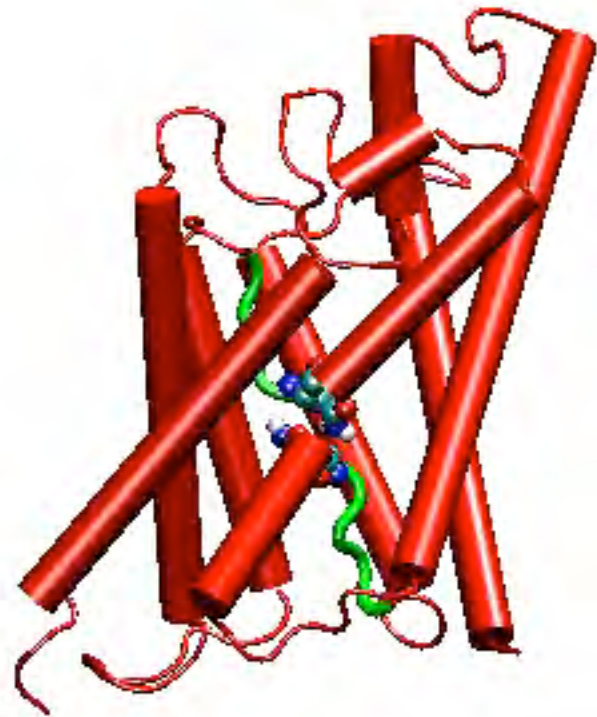


Aquaporins of known structure:

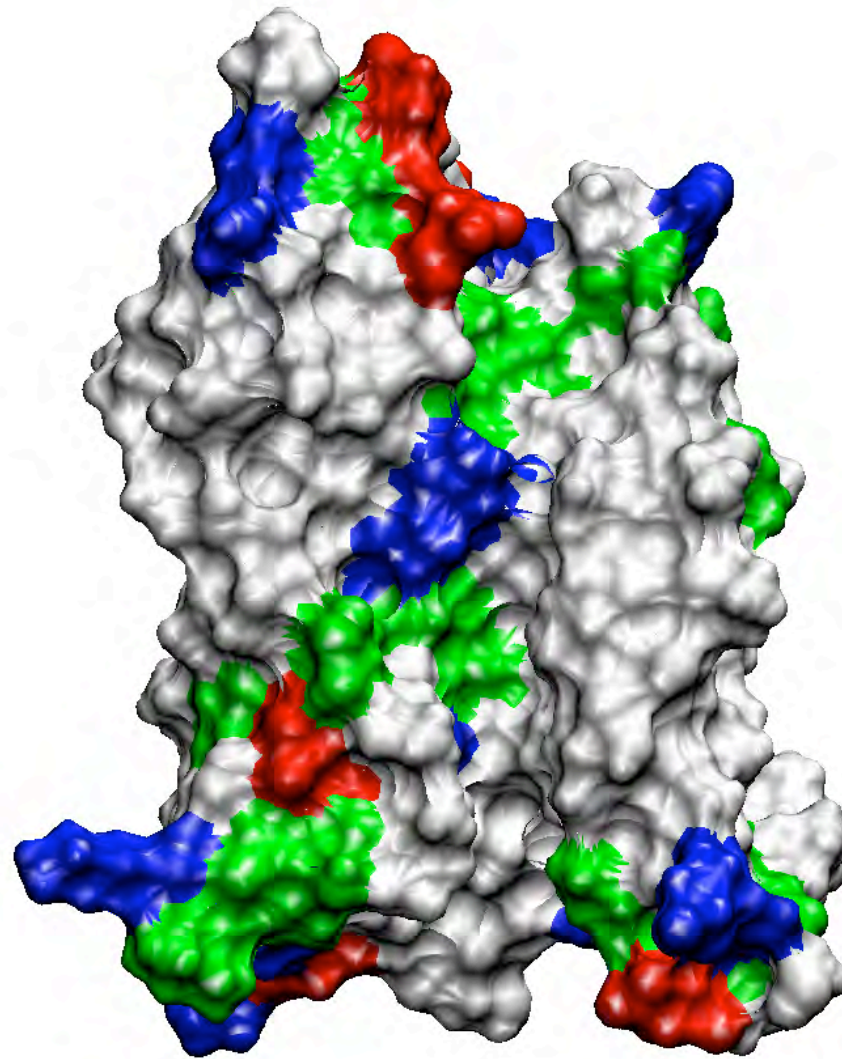
- GlpF** - E. coli glycerol channel (aquaglycerolporin)
- AQP1** - Mammalian aquaporin-1 (pure water channel)
- AqpZ and AQPO (2004)

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

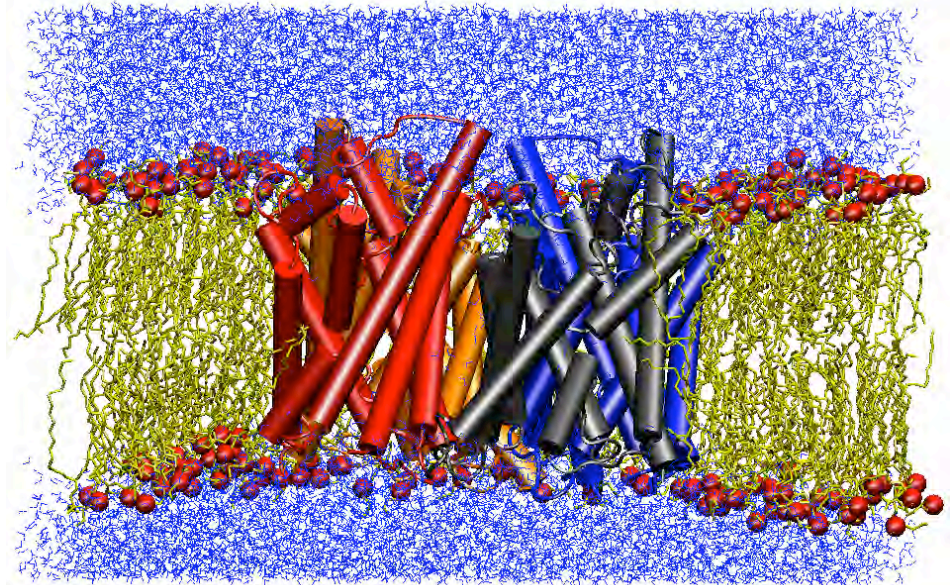
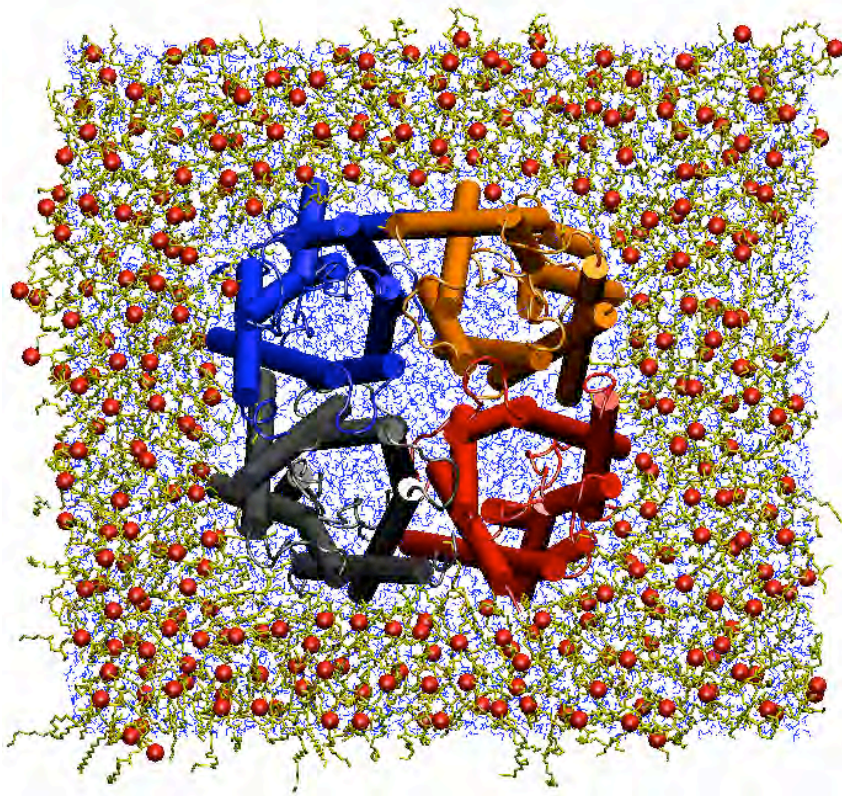


A Semi-hydrophobic channel



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

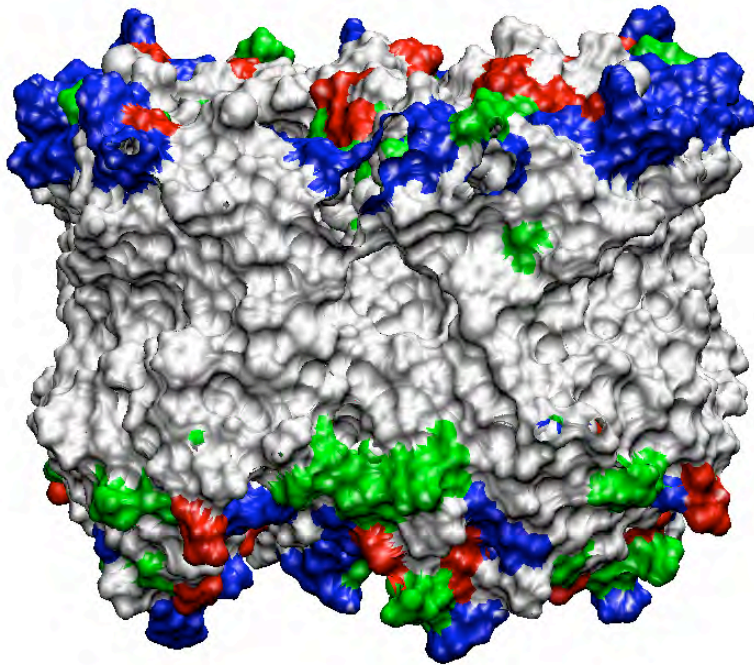
1ns equilibration, 4ns production

10 days /ns - 32-proc Linux cluster

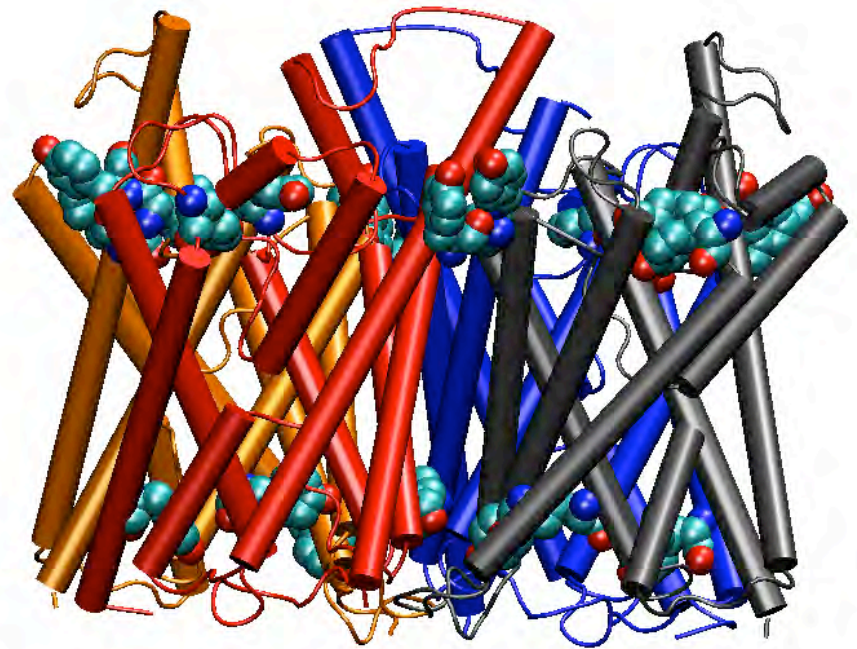
3.5 days/ns - 128 O2000 CPUs

0.35 days/ns - 512 LeMieux CPUs

Protein Embedding in Membrane

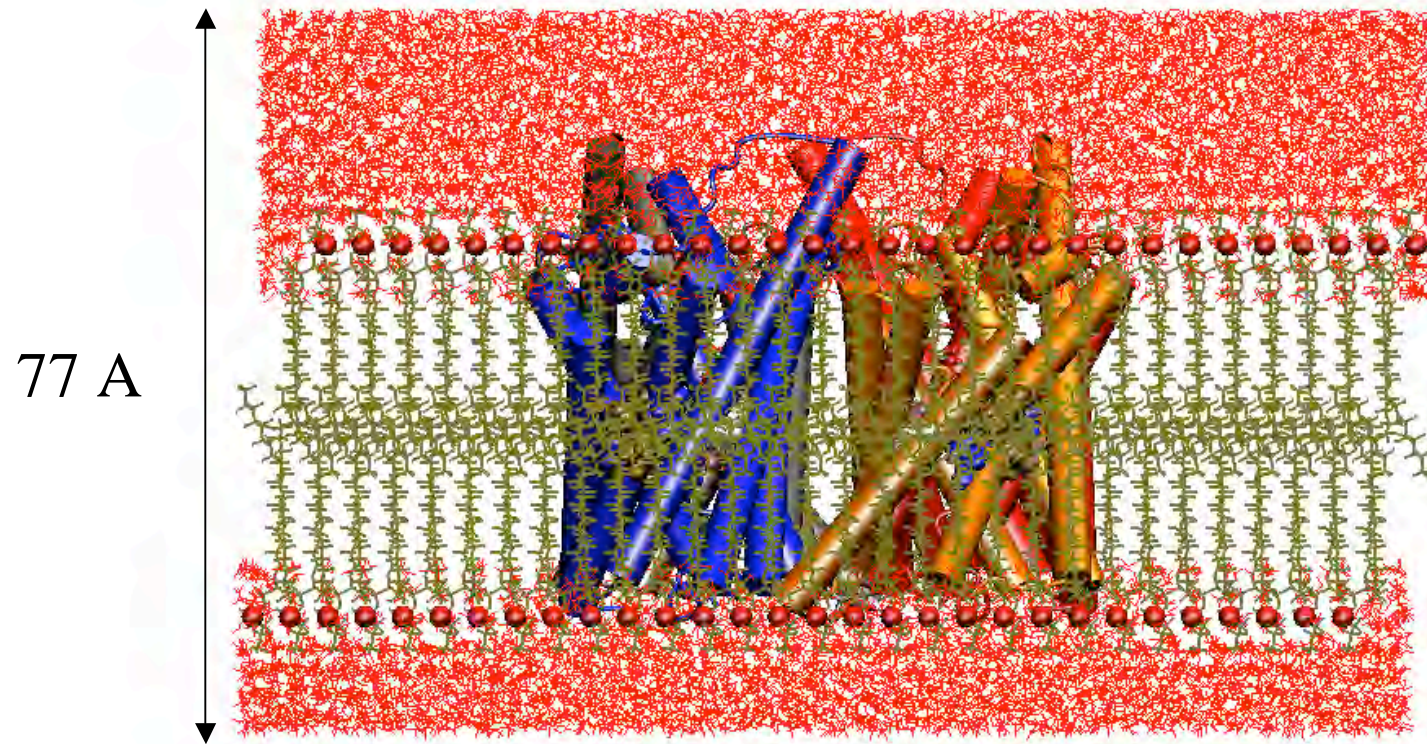


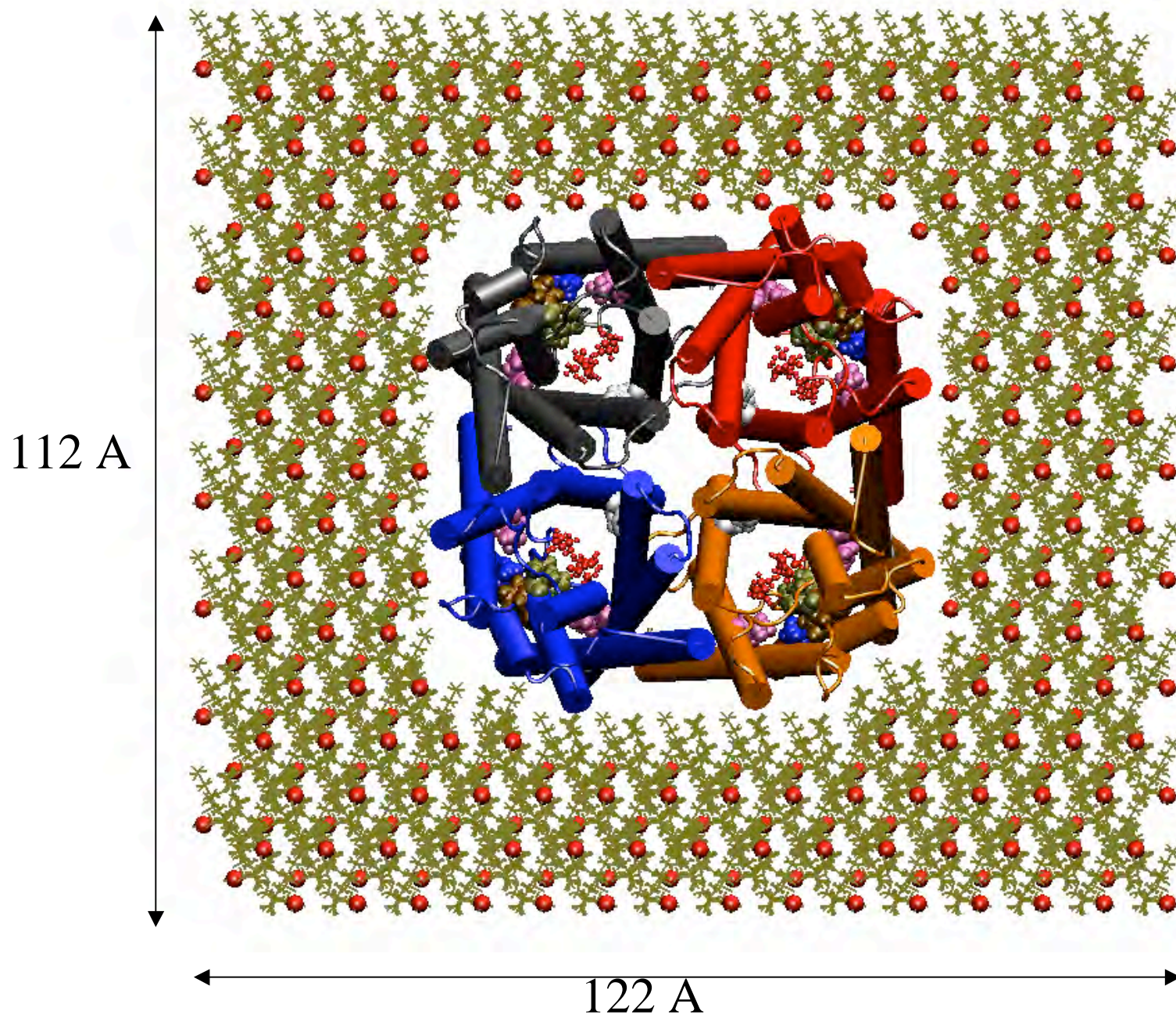
Hydrophobic surface
of the protein



Ring of
Tyr and Trp

Embedding GlpF in Membrane

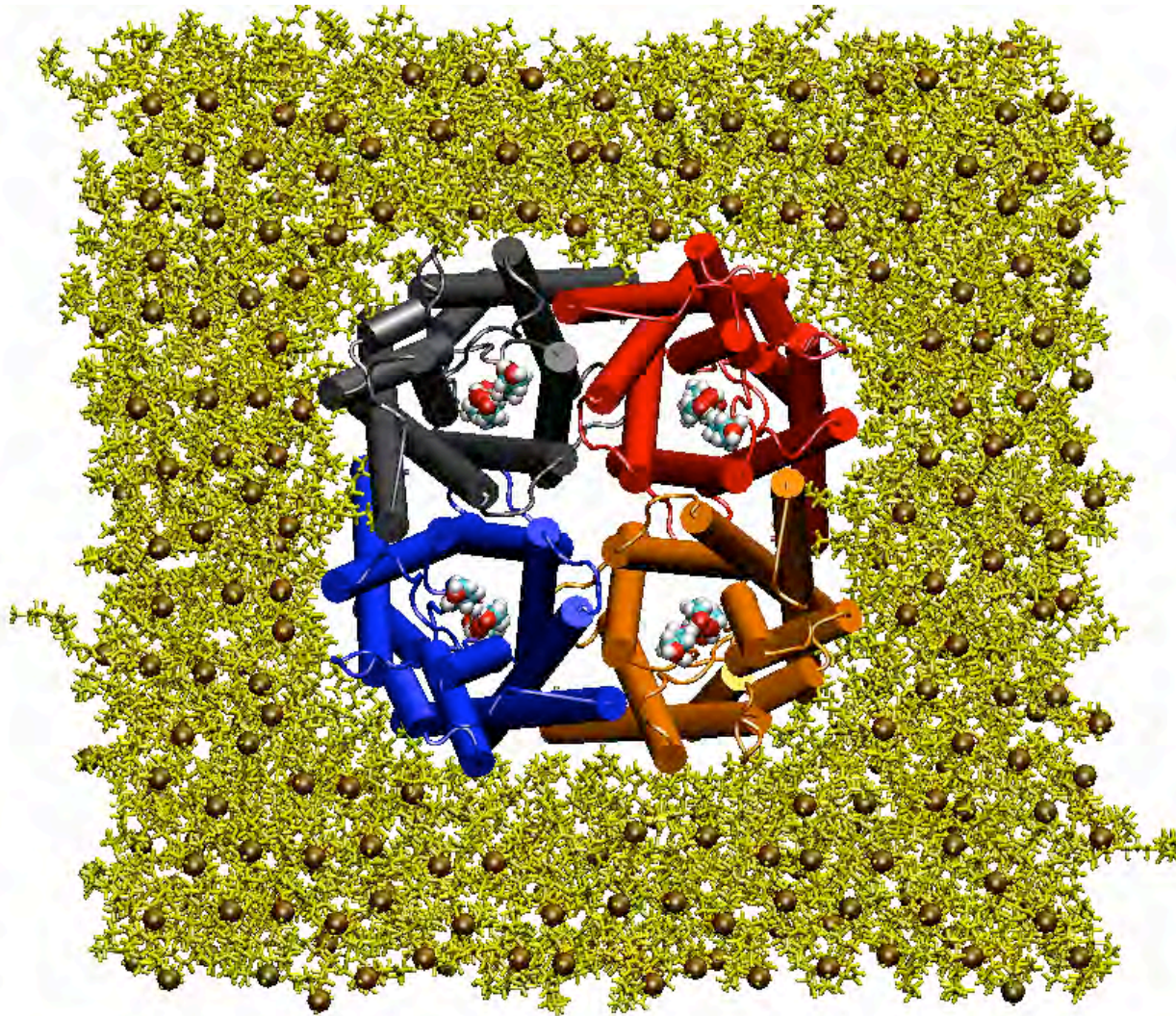




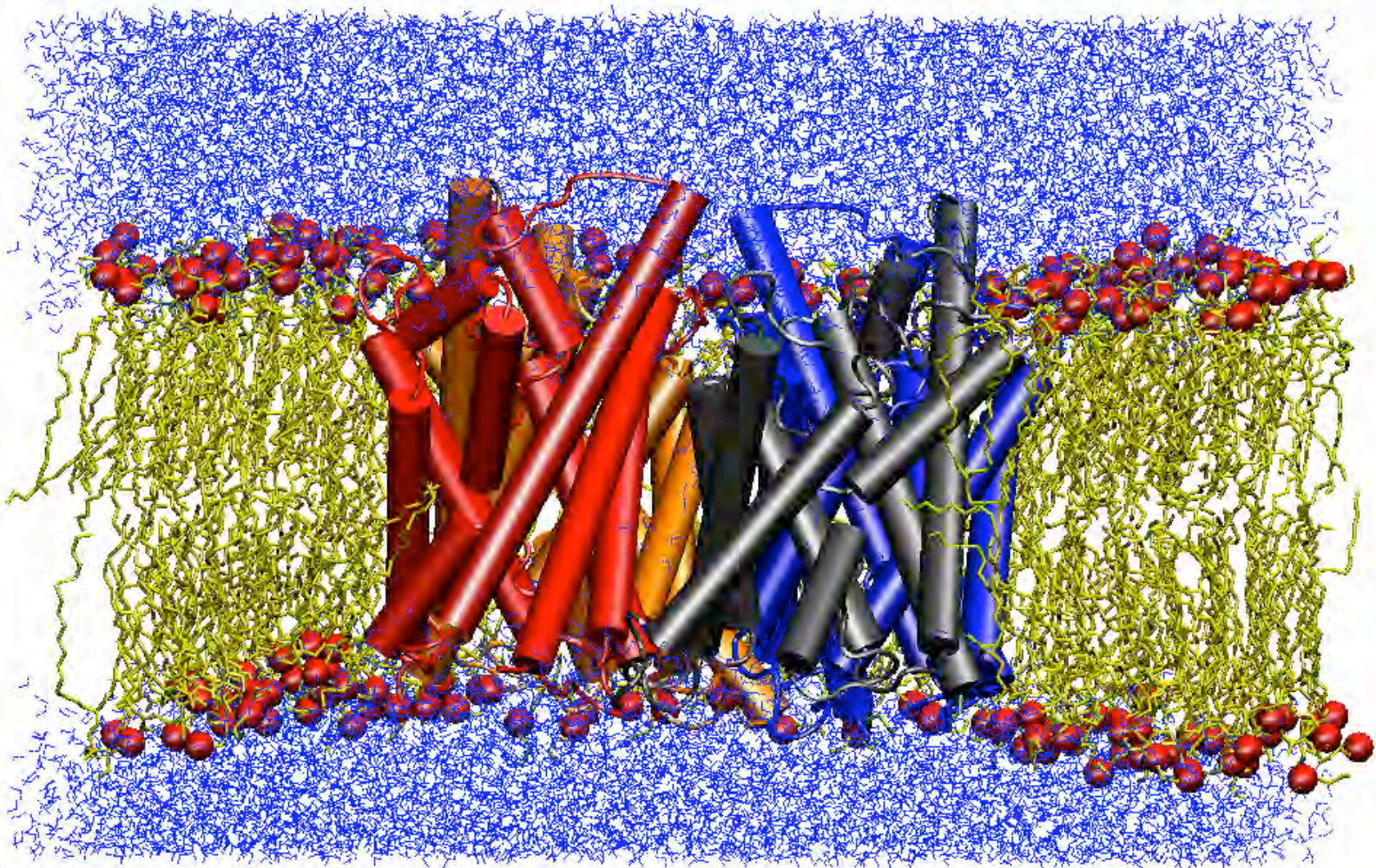
A Recipe for Membrane Protein Simulations

- 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.

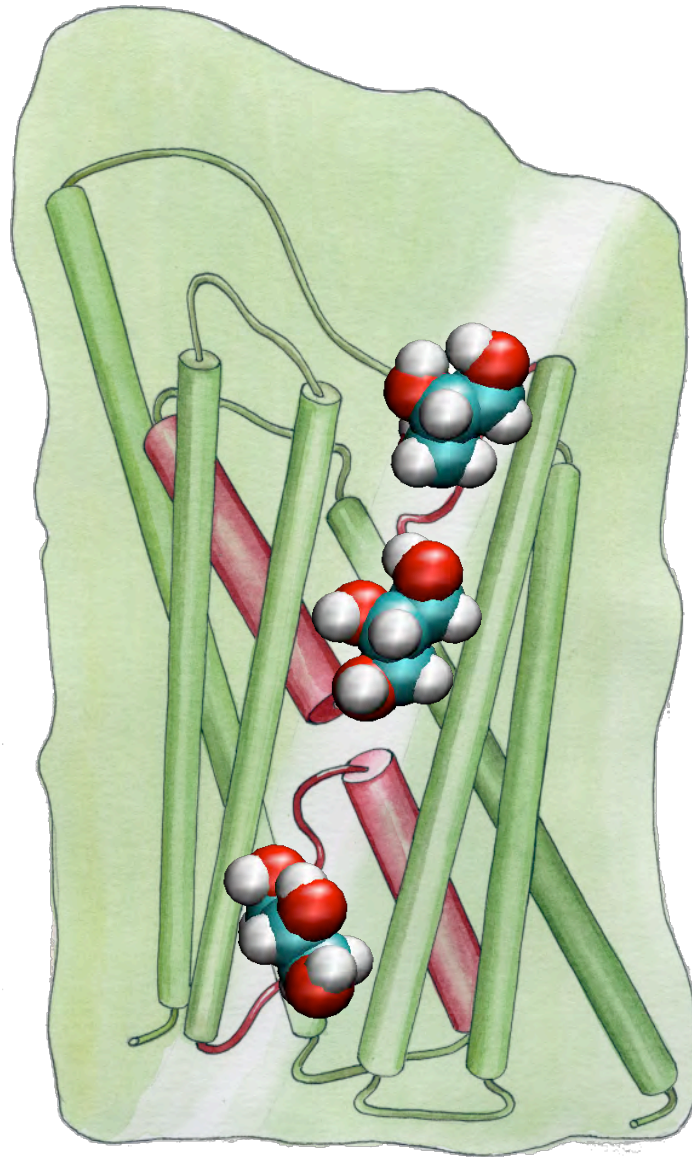
Lipid-Protein Packing During the Initial NpT Simulation

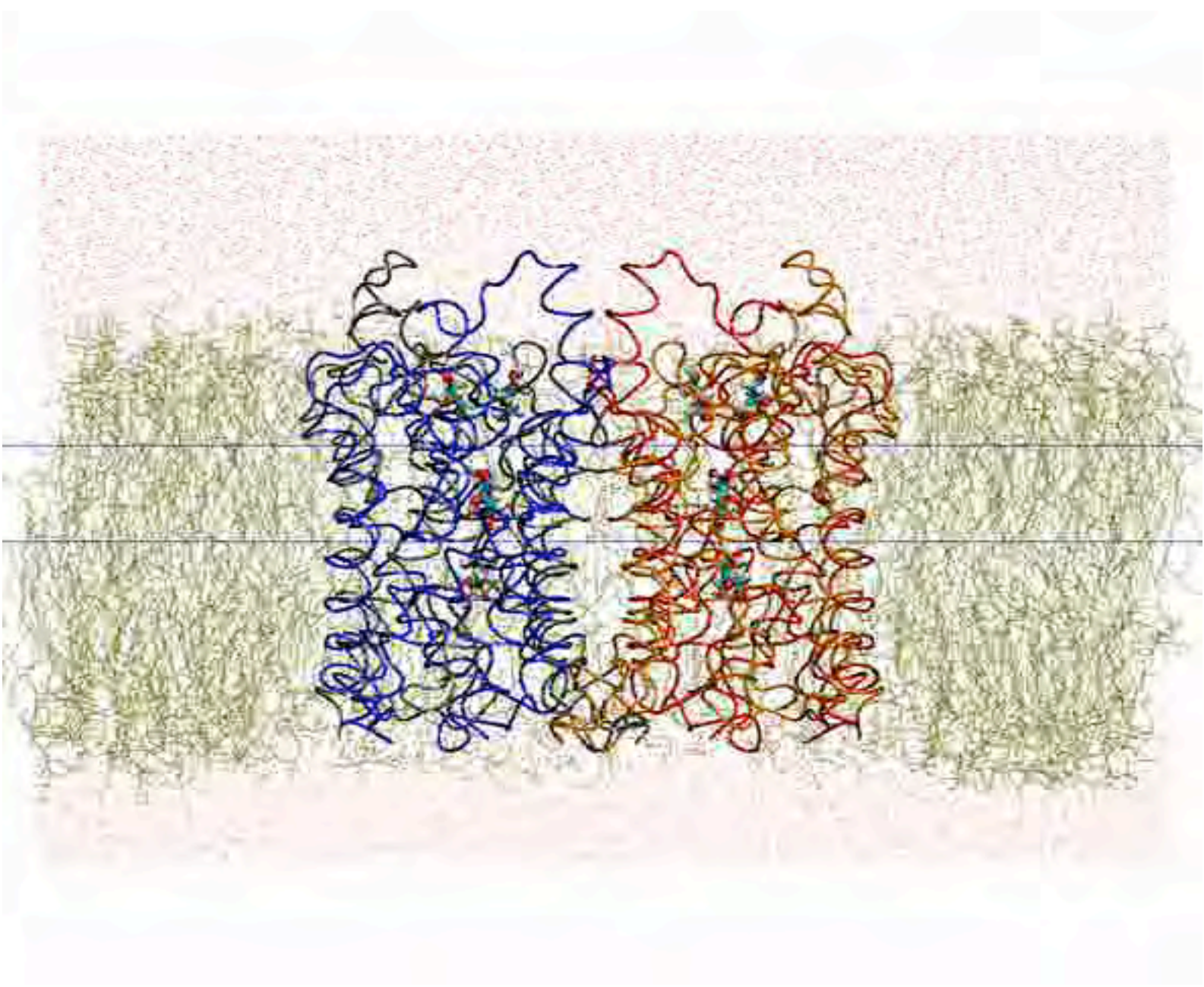


Adjustment of Membrane Thickness to the Protein Hydrophobic Surface

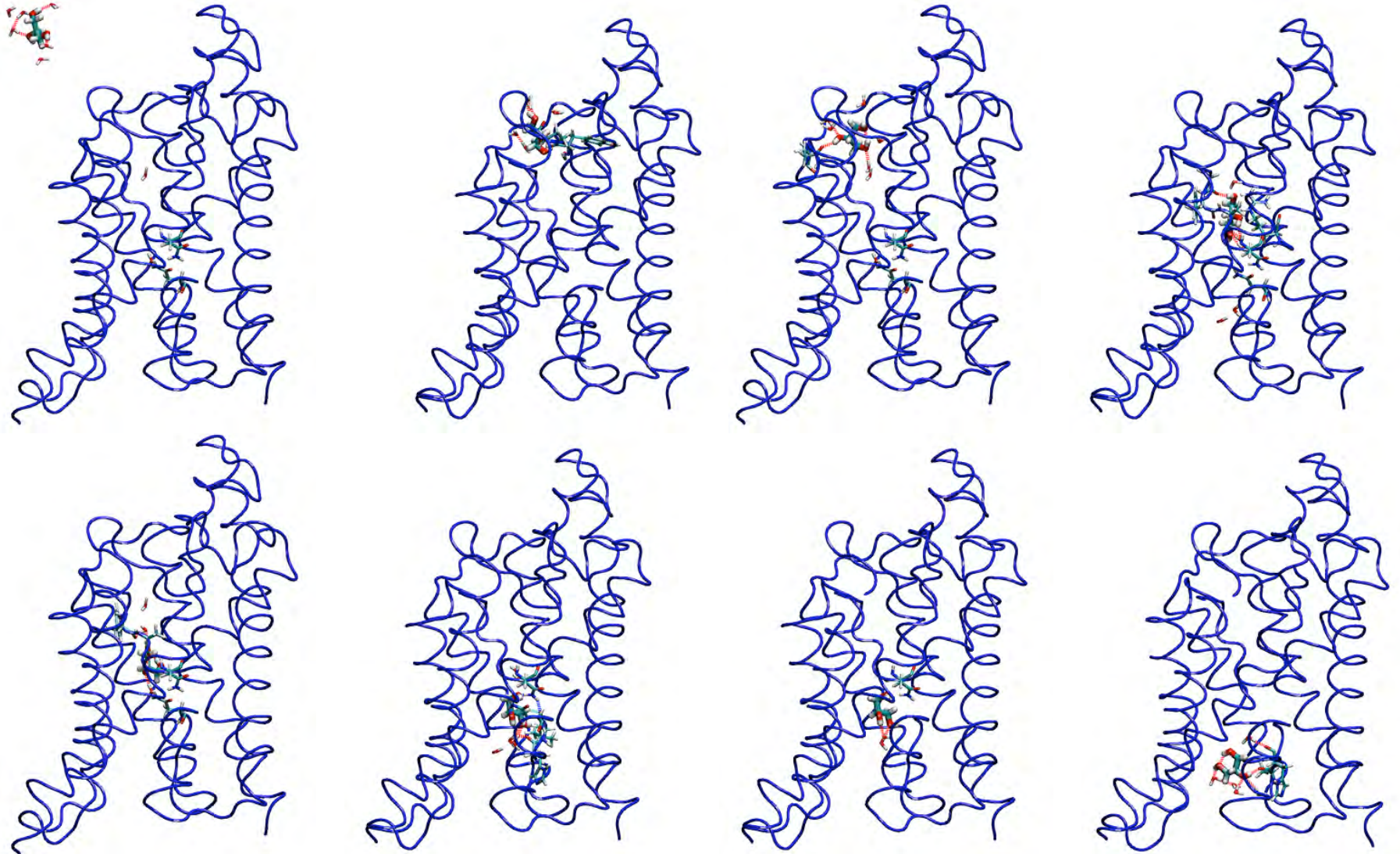


Glycerol-Saturated GlpF





Description of full conduction pathway



Complete description of the conduction pathway

Constriction region



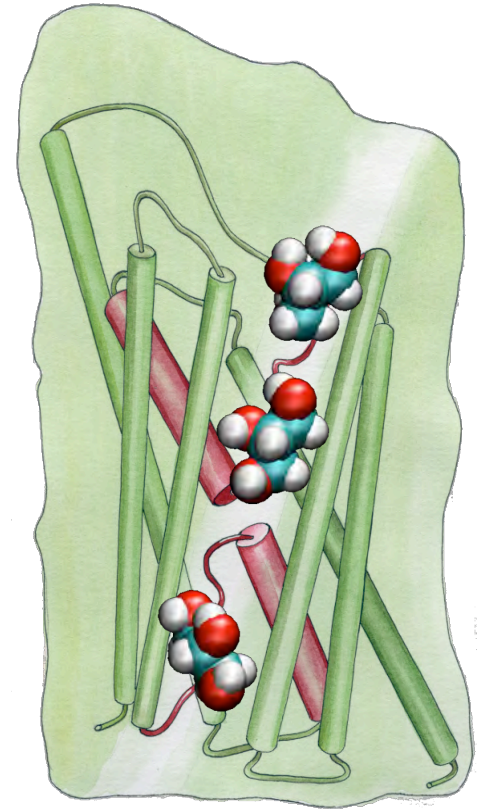
} Selectivity filter

Channel Hydrogen Bonding Sites

...

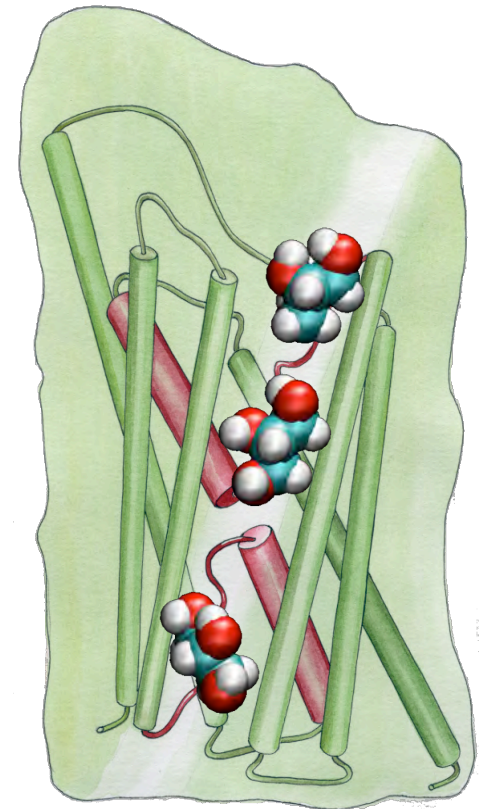
```
{set frame 0}{frame < 100}{incr frame}{  
  animate goto $frame  
  set donor [atomselect top  
    "name O N and within 2 of  
    (resname GCL and name HO)"]  
  lappend [$donor get index] list1  
  set acceptor [atomselect top  
    "resname GCL and name O and  
    within 2 of (protein and name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

...



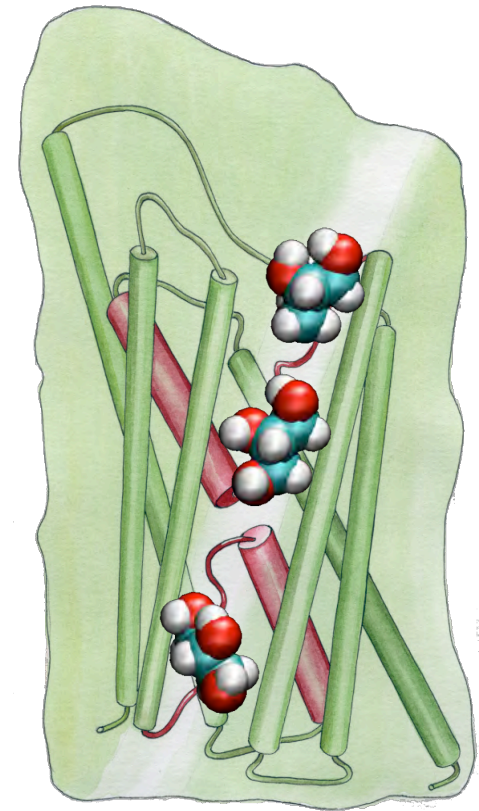
Channel Hydrogen Bonding Sites

GLN	41	OE1 NE2	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	<u>ASN</u>	68	HD22
ASN	140	OD1 ND2	<u>TYR</u>	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	<u>ASN</u>	203	HN HD21HD22
			<u>ARG</u>	206	HE HH21HH22

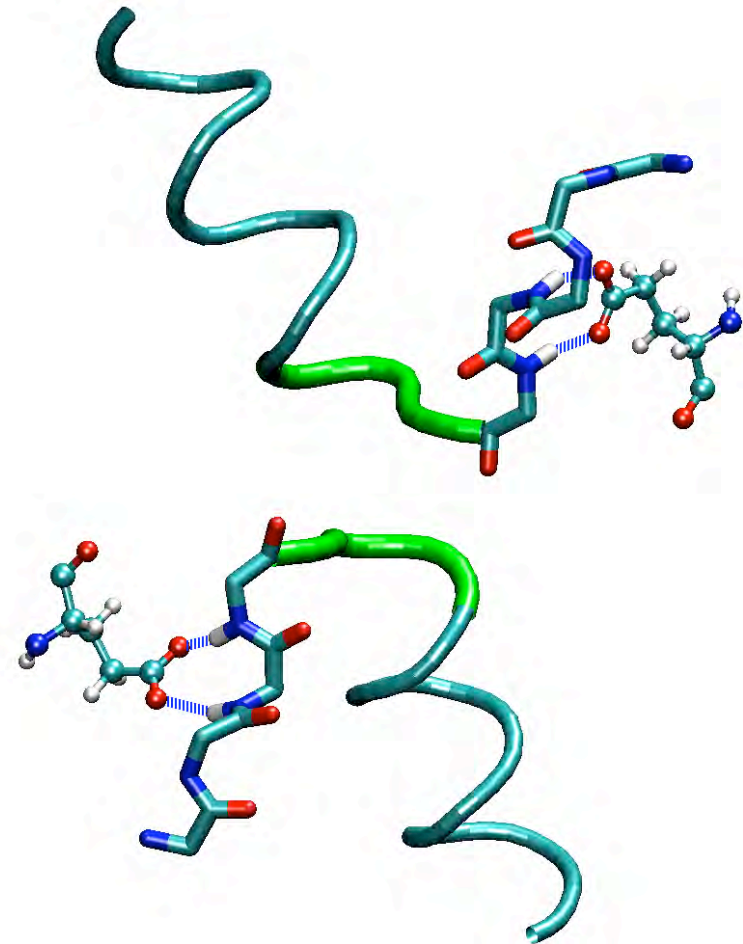
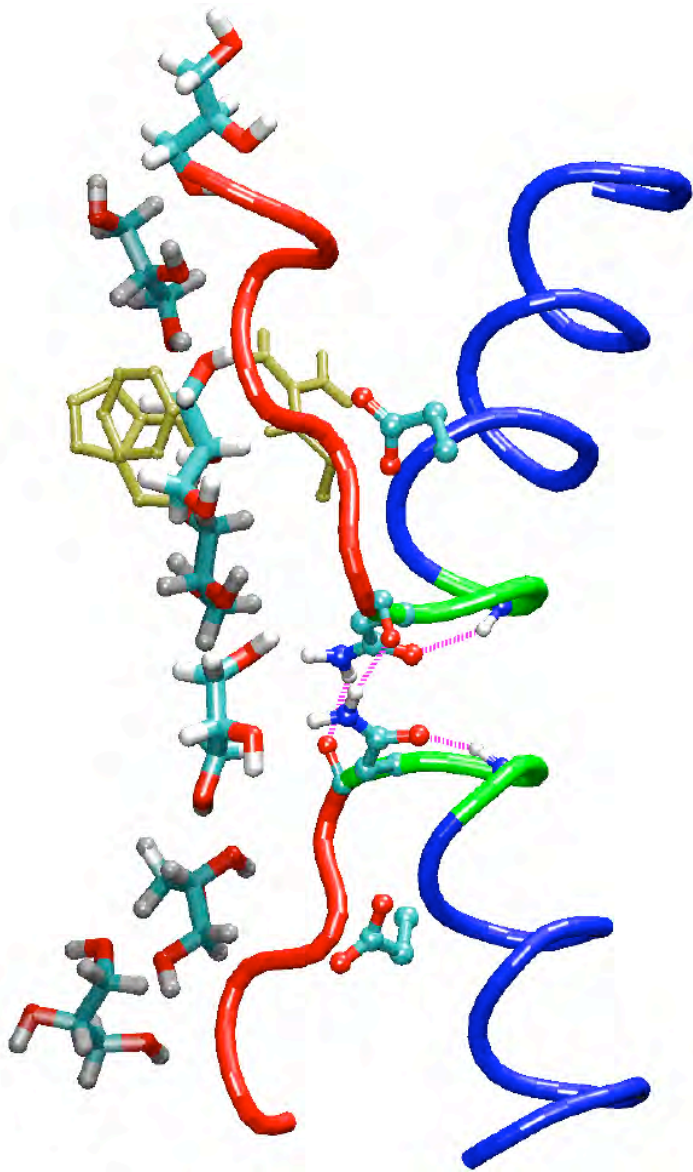


Channel Hydrogen Bonding Sites

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	<u>ASN</u>	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	<u>ASN</u>	203	HN HD21HD22
			<u>ARG</u>	206	HE HH21HH22

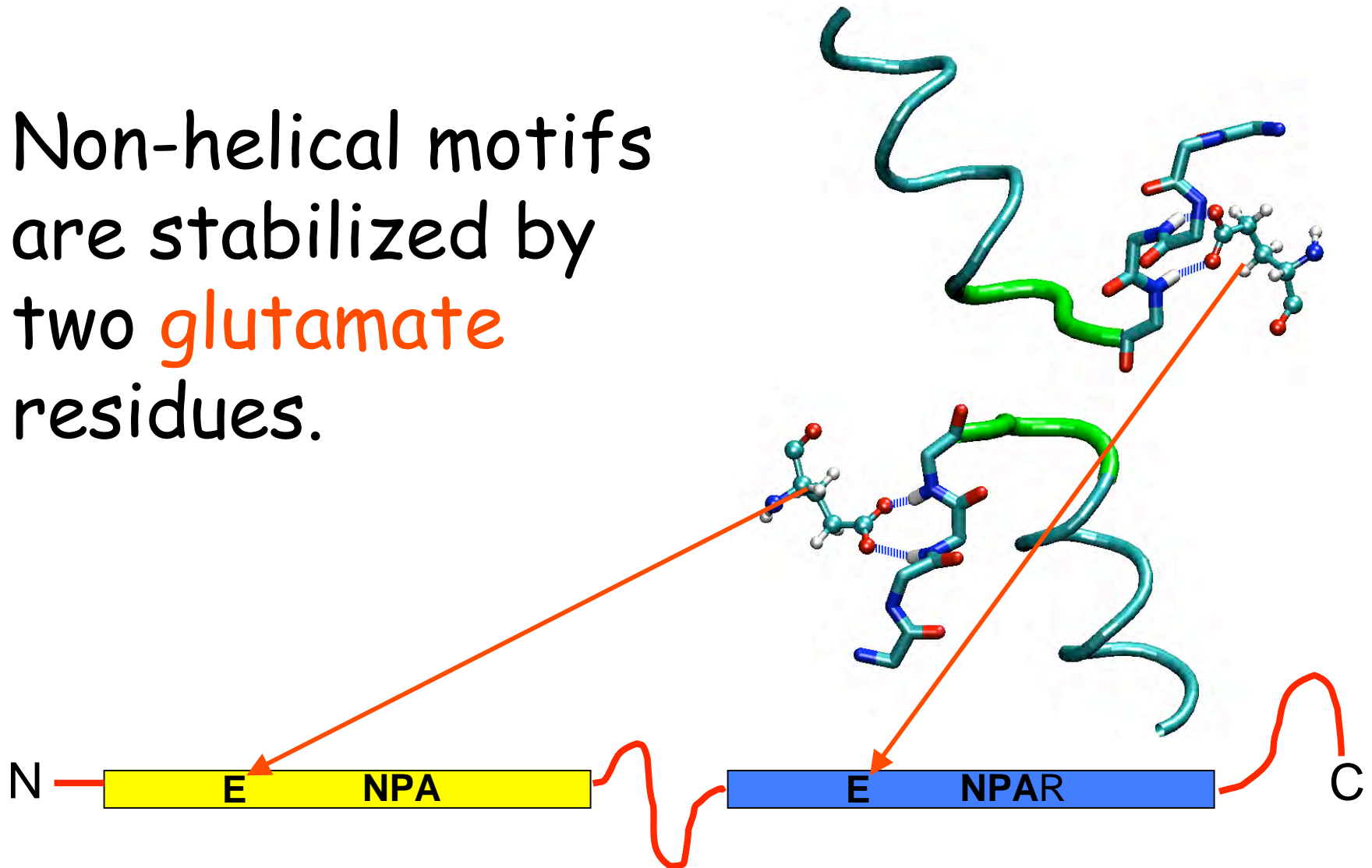


The Substrate Pathway
is formed by $C=O$ groups



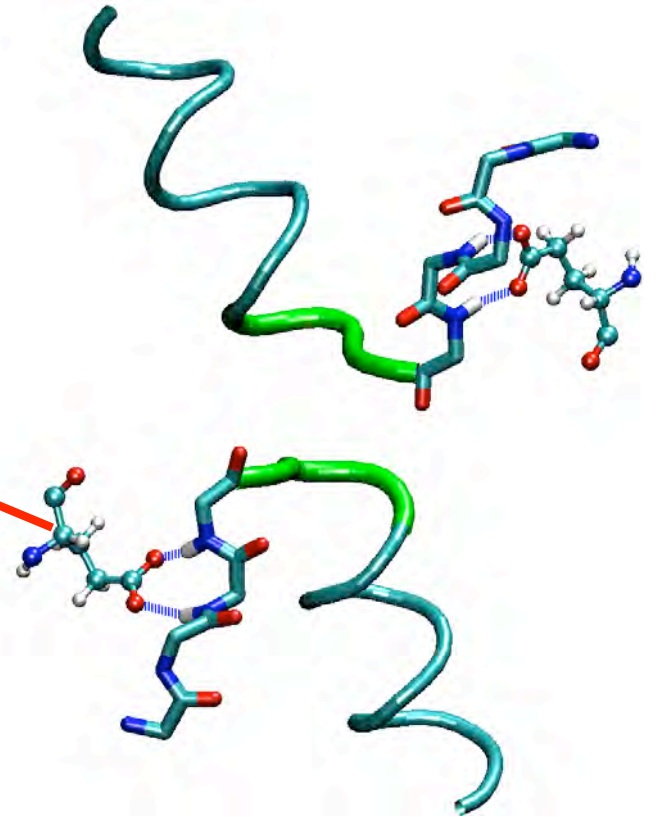
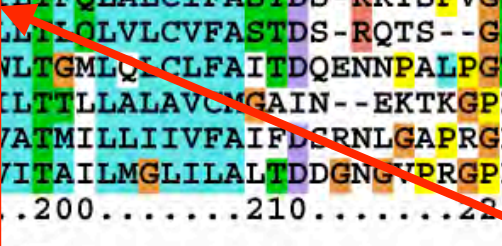
The Substrate Pathway is formed by $C=O$ groups

Non-helical motifs
are stabilized by
two **glutamate**
residues.

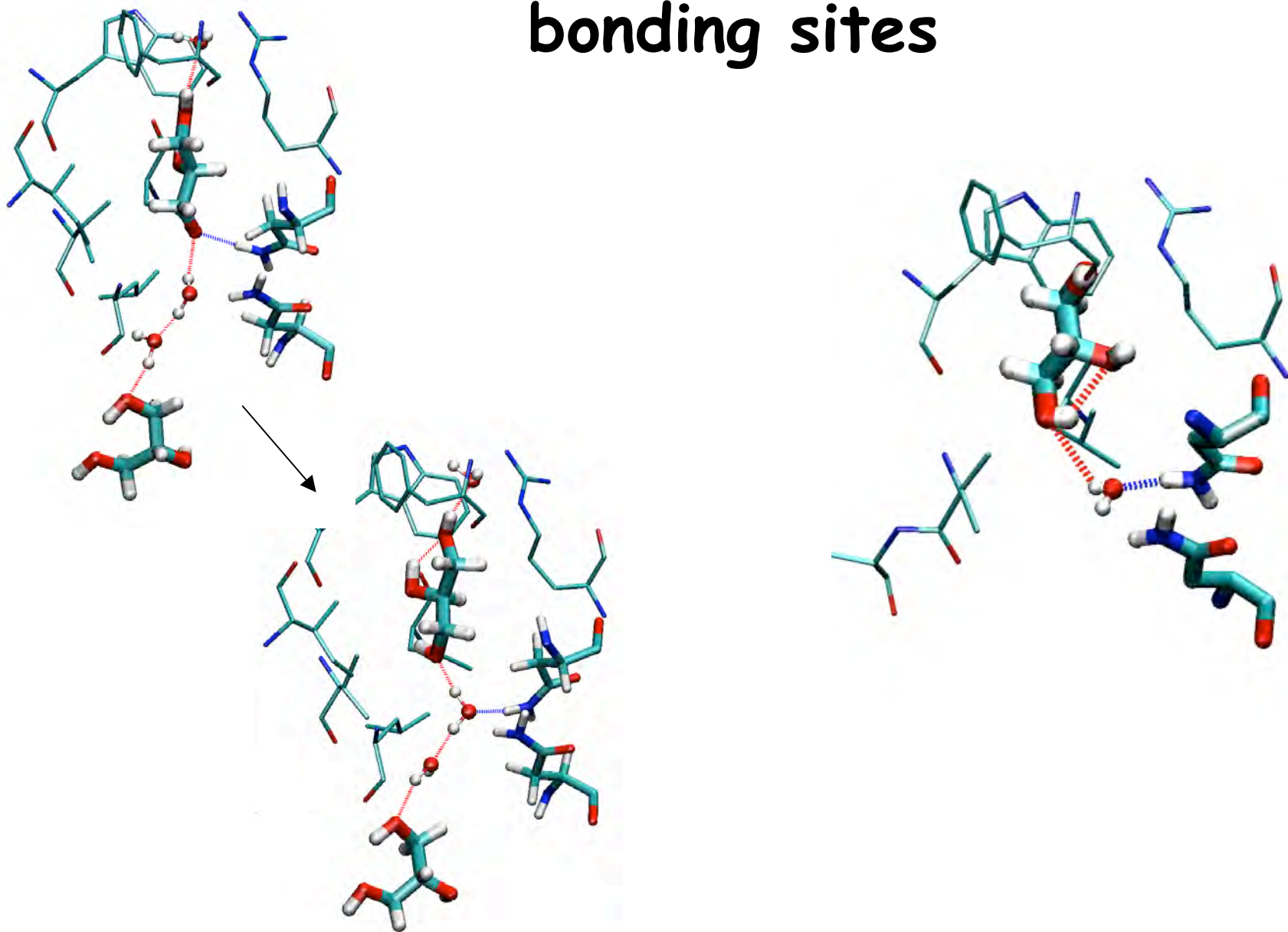


Conservation of Glutamate Residue in Human Aquaporins

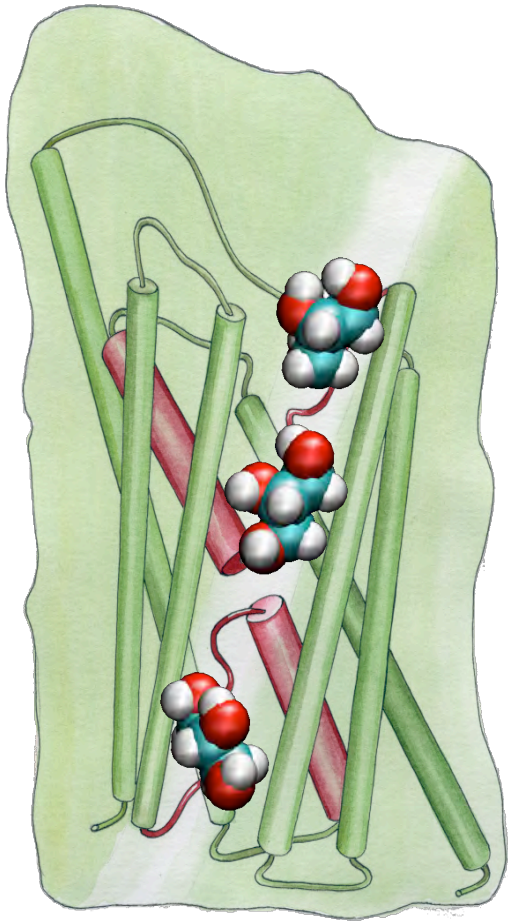
			.	:	*	:	:
AQP0_HUMAN	---	LNTLHPAVSVGQATTVEI	FLTLQFVLCIFATYDE	-	RRNGQLG		
AQP1_HUMAN	---	RNDLADGVNSGQGLGIEI	IGTLQLVLCVLATTD	-	RRRDLGG		
AQP2_HUMAN	---	VNALSNSSTTAGQAVTVEL	FLTLQLVLCIFASTDE	-	RRGENPG		
AQP3_HUMAN		GIFATYPSGHLDMINGFFD	QFIGTASLIVCVLAIVD		PYNNPVPRG		
AQP4_HUMAN	---	VTMVHGNTAGHGLIVELI	ITFQLVFTIFASCDS	-	KRTDVTG		
AQP5_HUMAN	---	VNALNNNTTQGGQAMVEL	ILTFQLALCIFASTDS	-	RRTSPVG		
AQP6_HUMAN	---	INVVRNSVSTGQAVAVEL	LLQLVLCVFASTDS	-	RQTS--G		
AQP7_HUMAN		GIFATYLPDHMTLWRGFINE	AVLTGMLQLCLFAITDQ		ENNPALPG		
AQP8_HUMAN	-	AAFVTVQEQGQVAGALVA	EILTLLALAVCMGAIN	-	EKTKGP		
AQP9_HUMAN		HIFATYPAPYLSLANAFAD	QVATMILLIIVFAIFD		SRNLGAPRG		
GLPF_ECOLI		GTFSTYPNPHINFVQAF	AVEMVITAILMGLILALT		DDGNGVPRGP		
ruler	...	180.....190.....200.....210.....220.....			



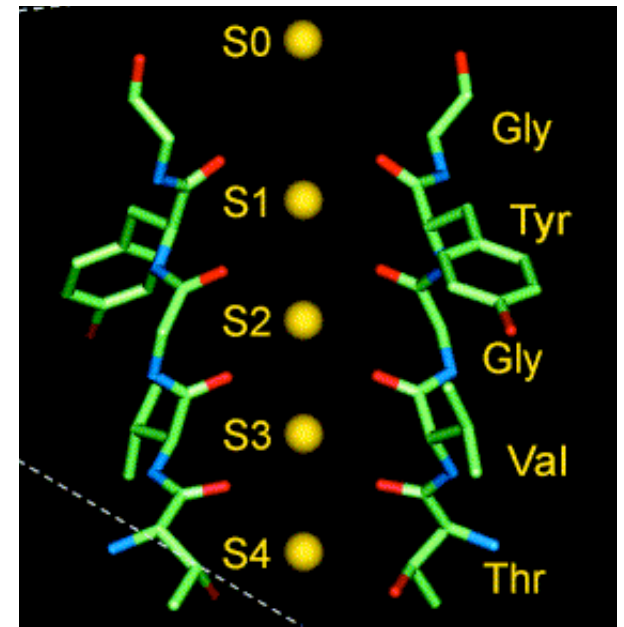
Glycerol - water competition for hydrogen bonding sites



Revealing the Functional Role of Reentrant Loops

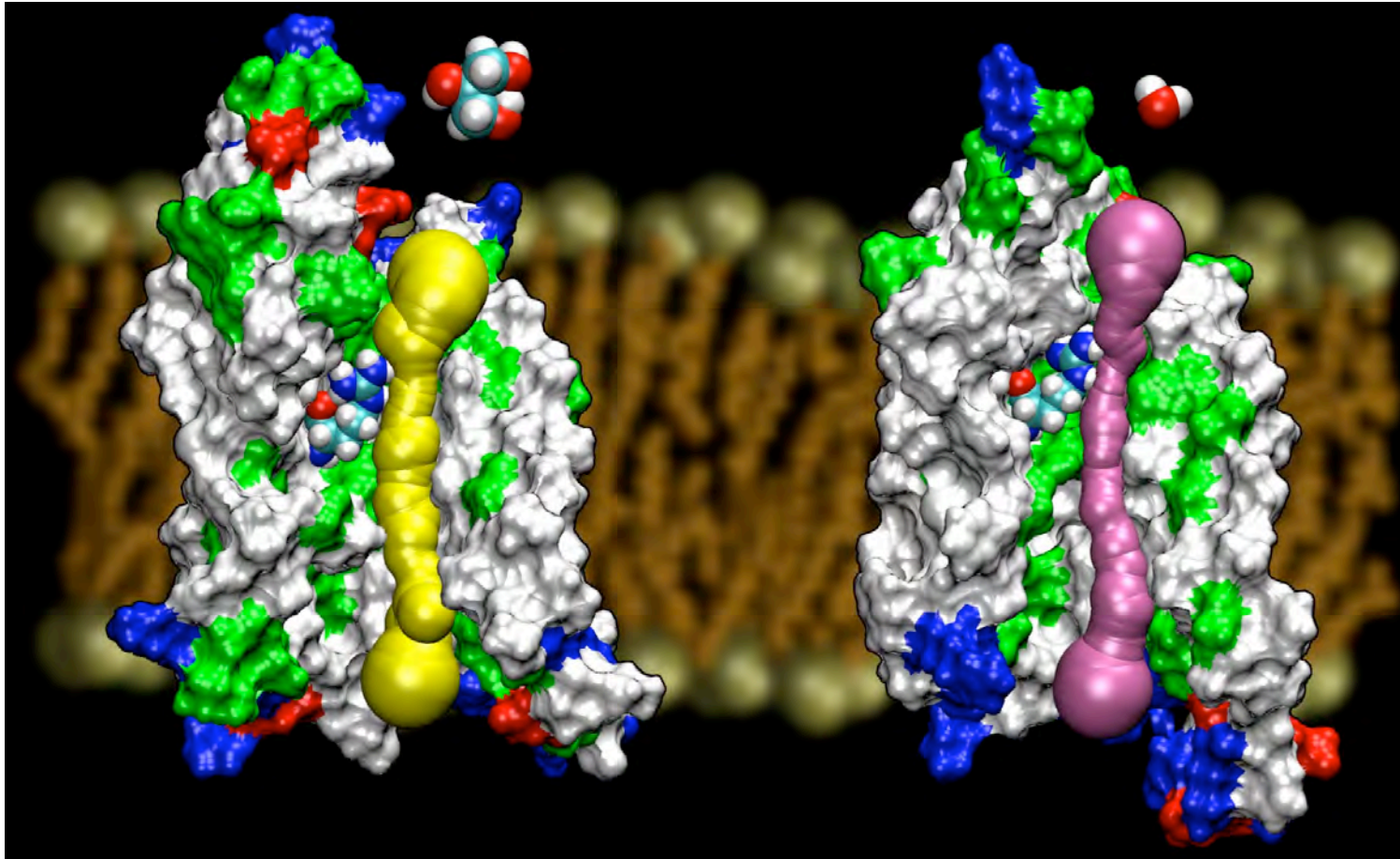


Potassium channel



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



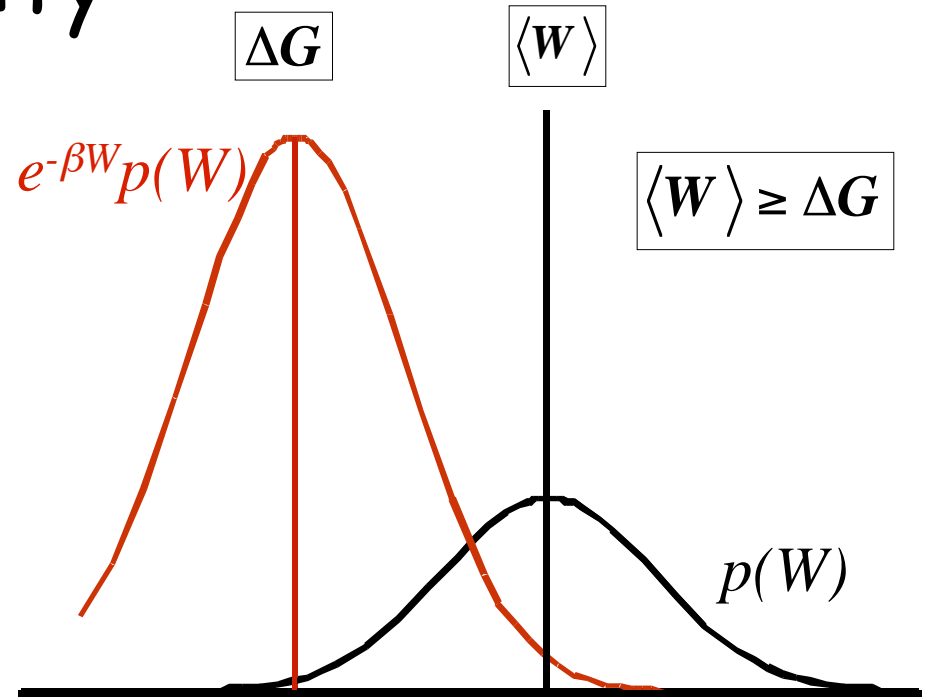
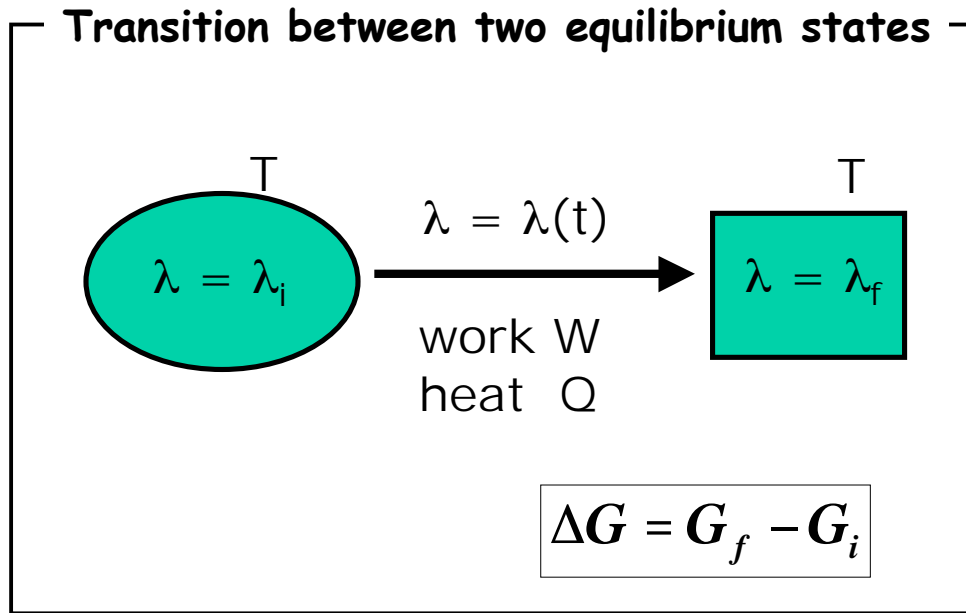
Steered Molecular Dynamics is a non-equilibrium method by nature

- 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



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

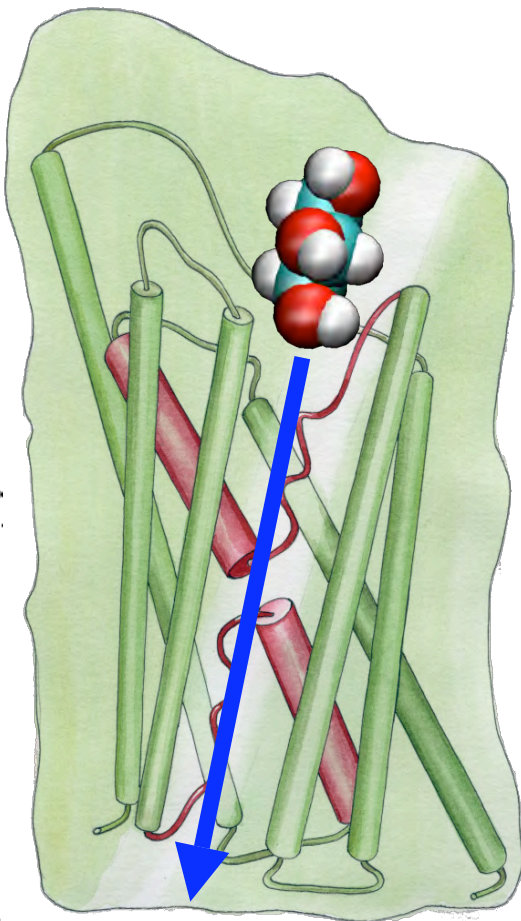
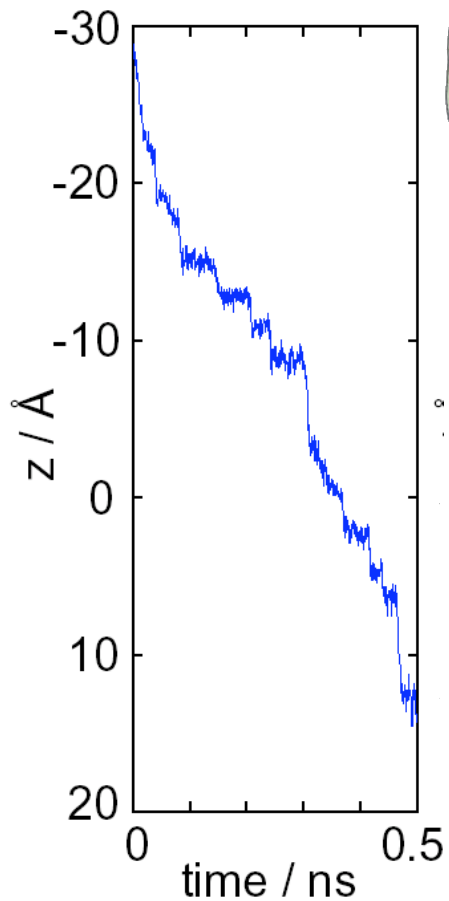
$$\langle e^{-\beta W} \rangle = e^{-\beta \Delta G}$$

$$\beta = 1/k_B T$$

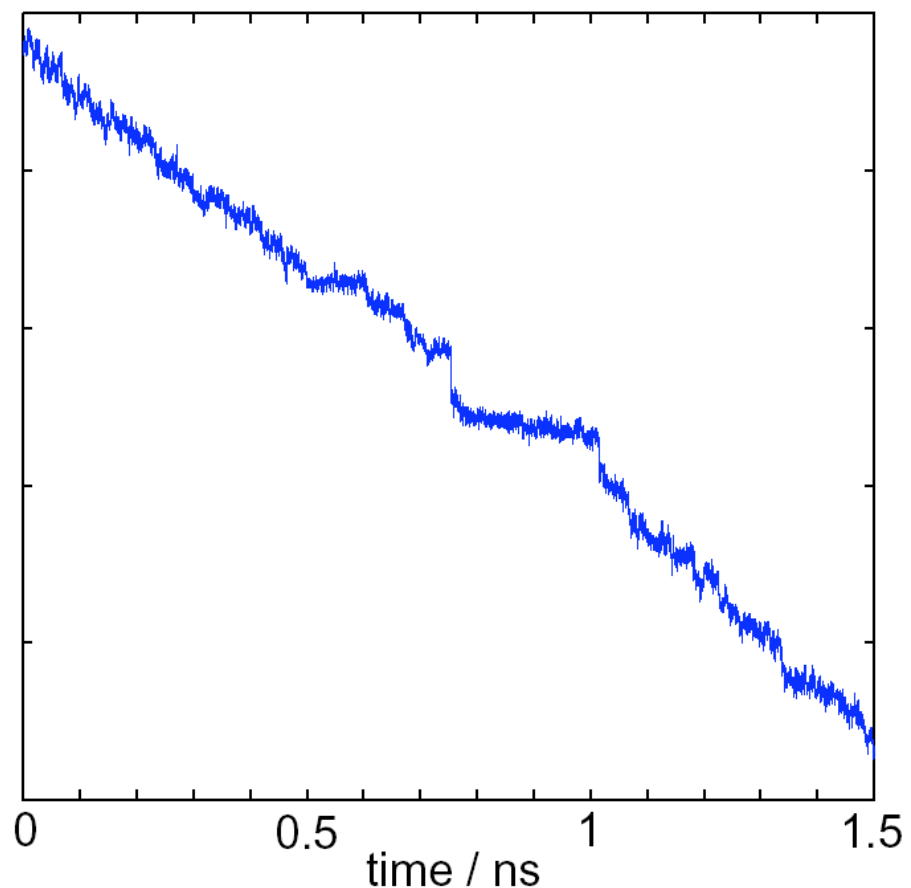
In principle, it is possible to obtain free energy surfaces from repeated *non-equilibrium* experiments.

Steered Molecular Dynamics

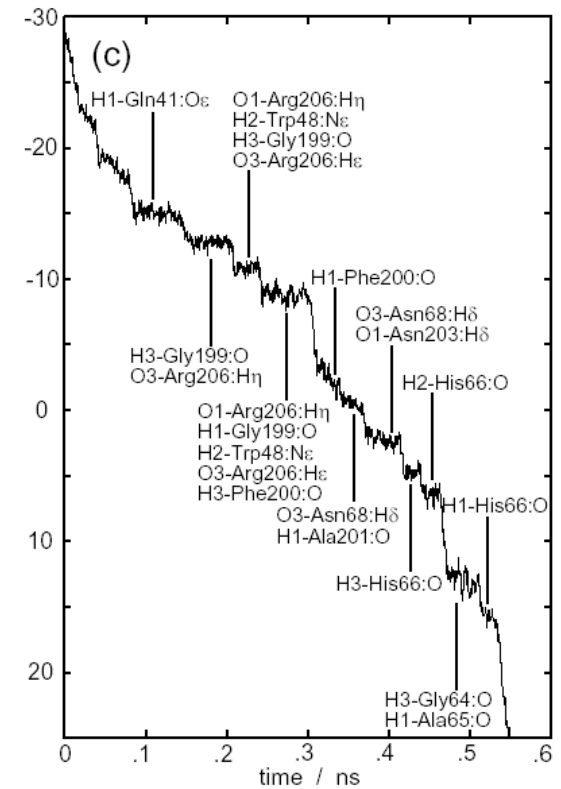
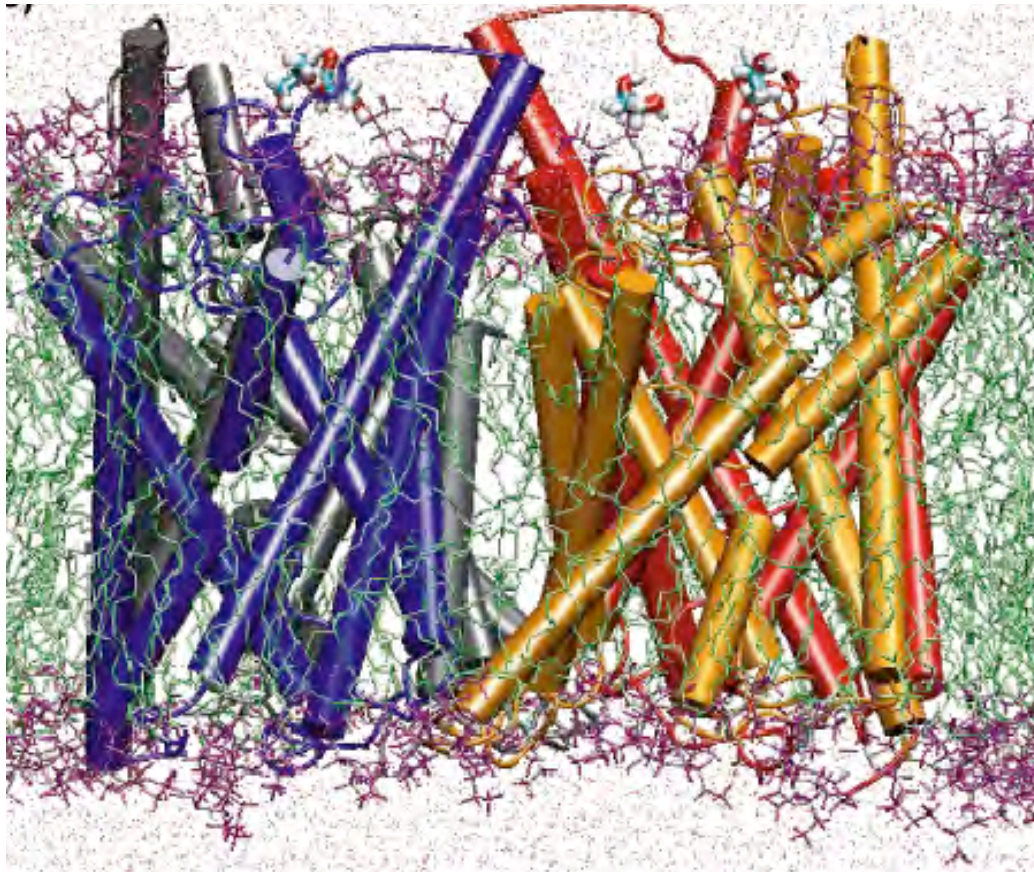
constant force
(250 pN)



constant velocity
(30 $\text{\AA}/\text{ns}$)



SMD Simulation of Glycerol Passage



Trajectory of glycerol pulled by **constant force**

Constructing the Potential of Mean Force

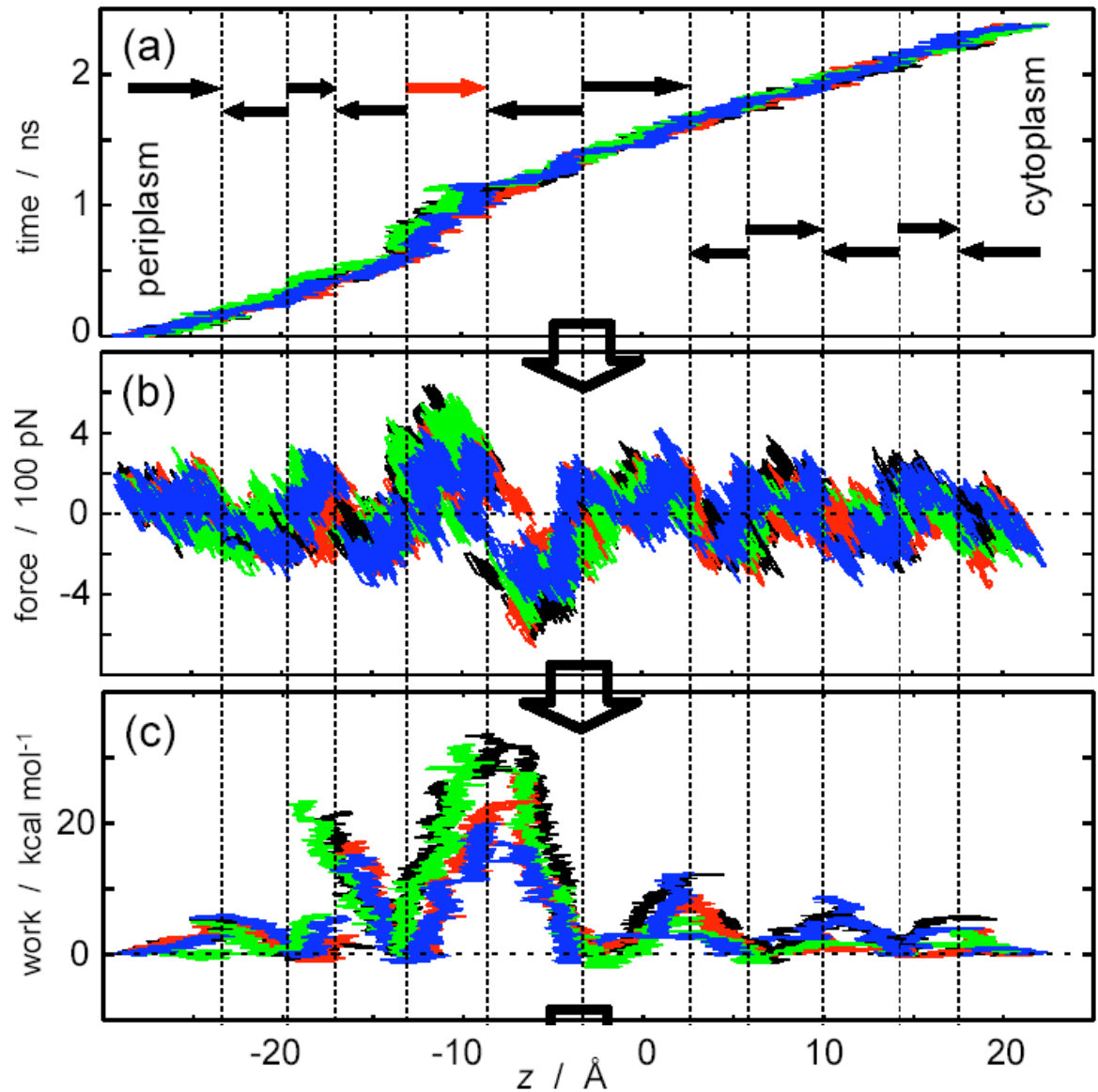
4 trajectories

$v = 0.03, 0.015 \text{ \AA/ps}$

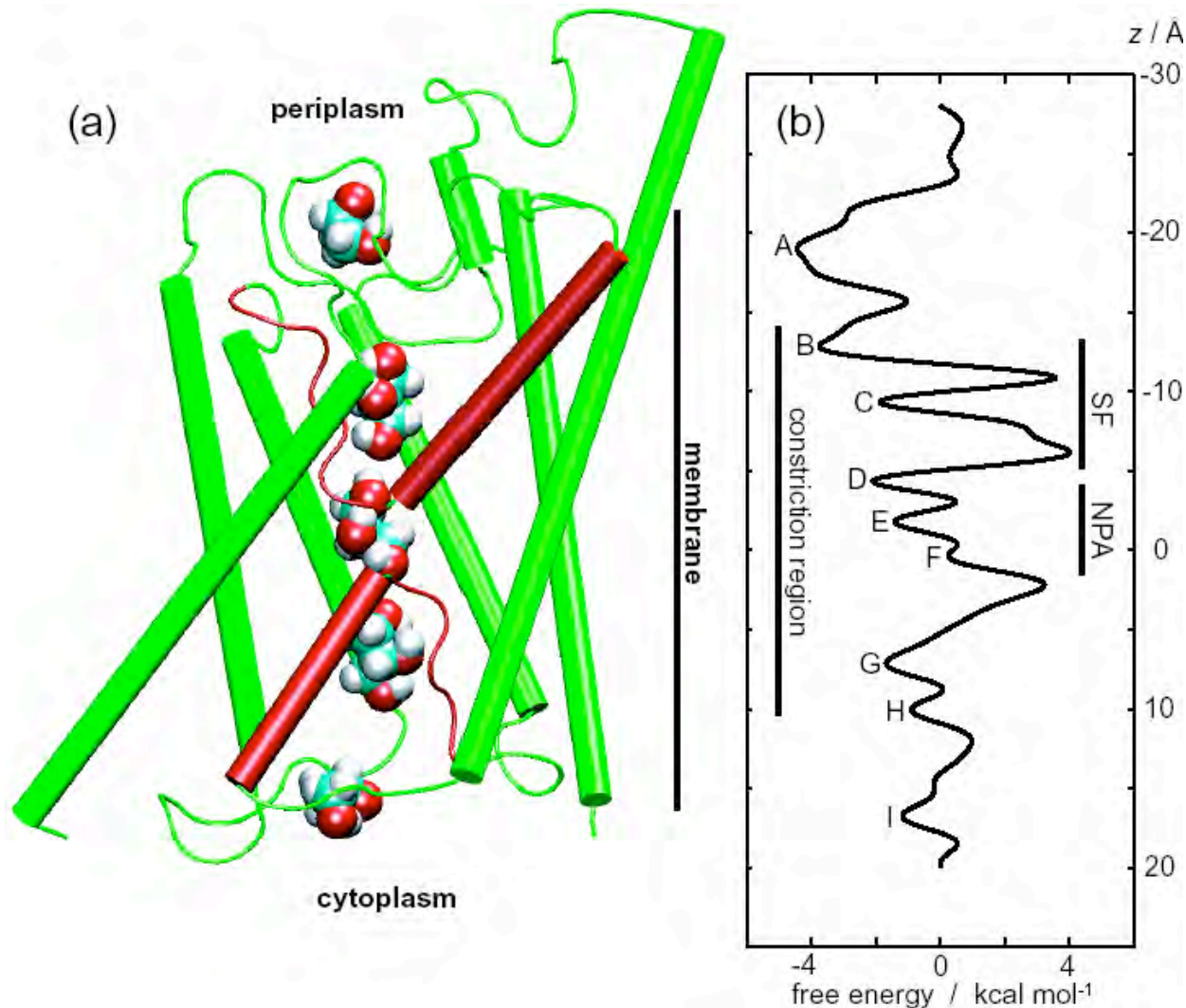
$k = 150 \text{ pN/\AA}$

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

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

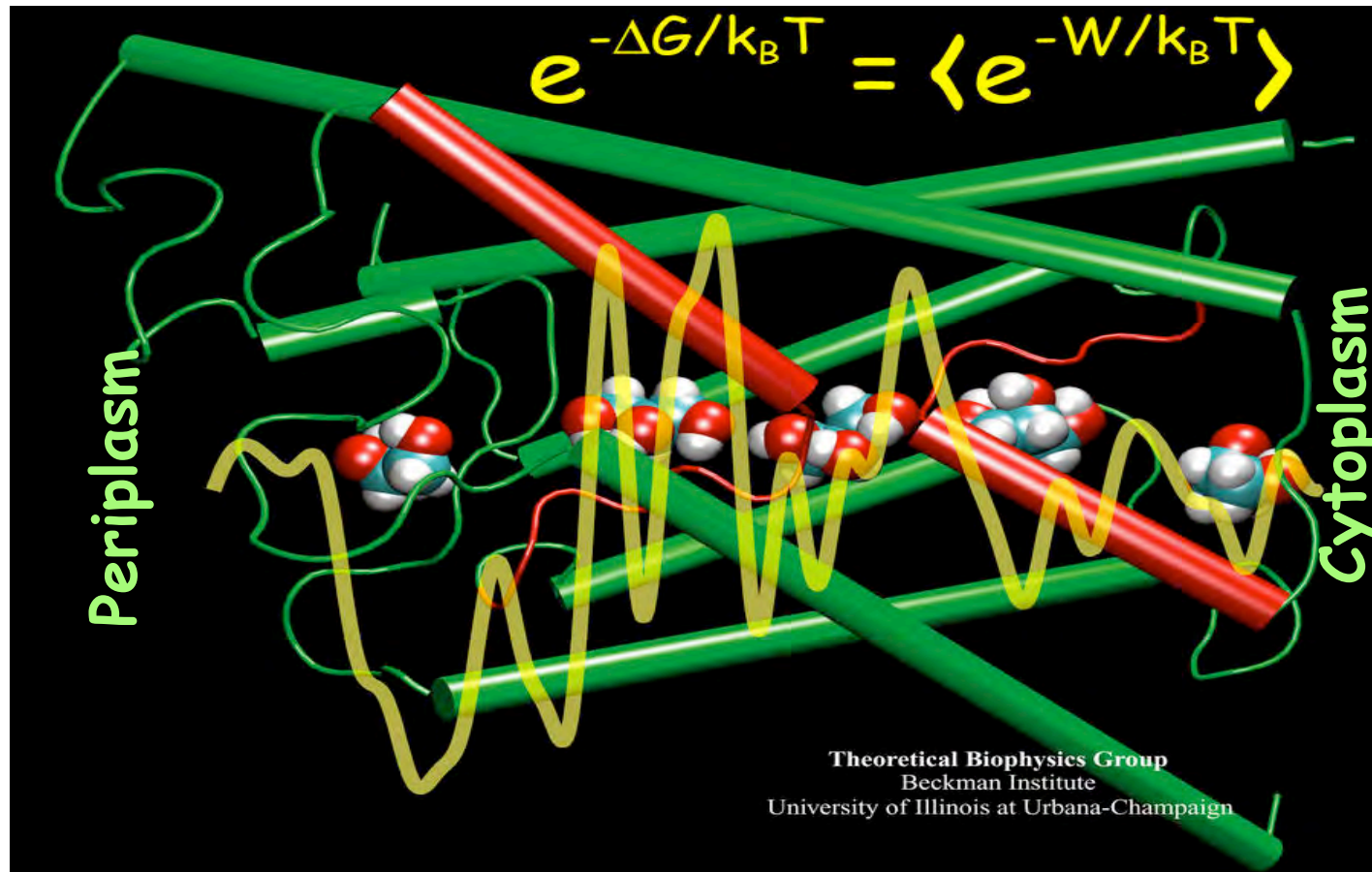


Features of the Potential of Mean Force



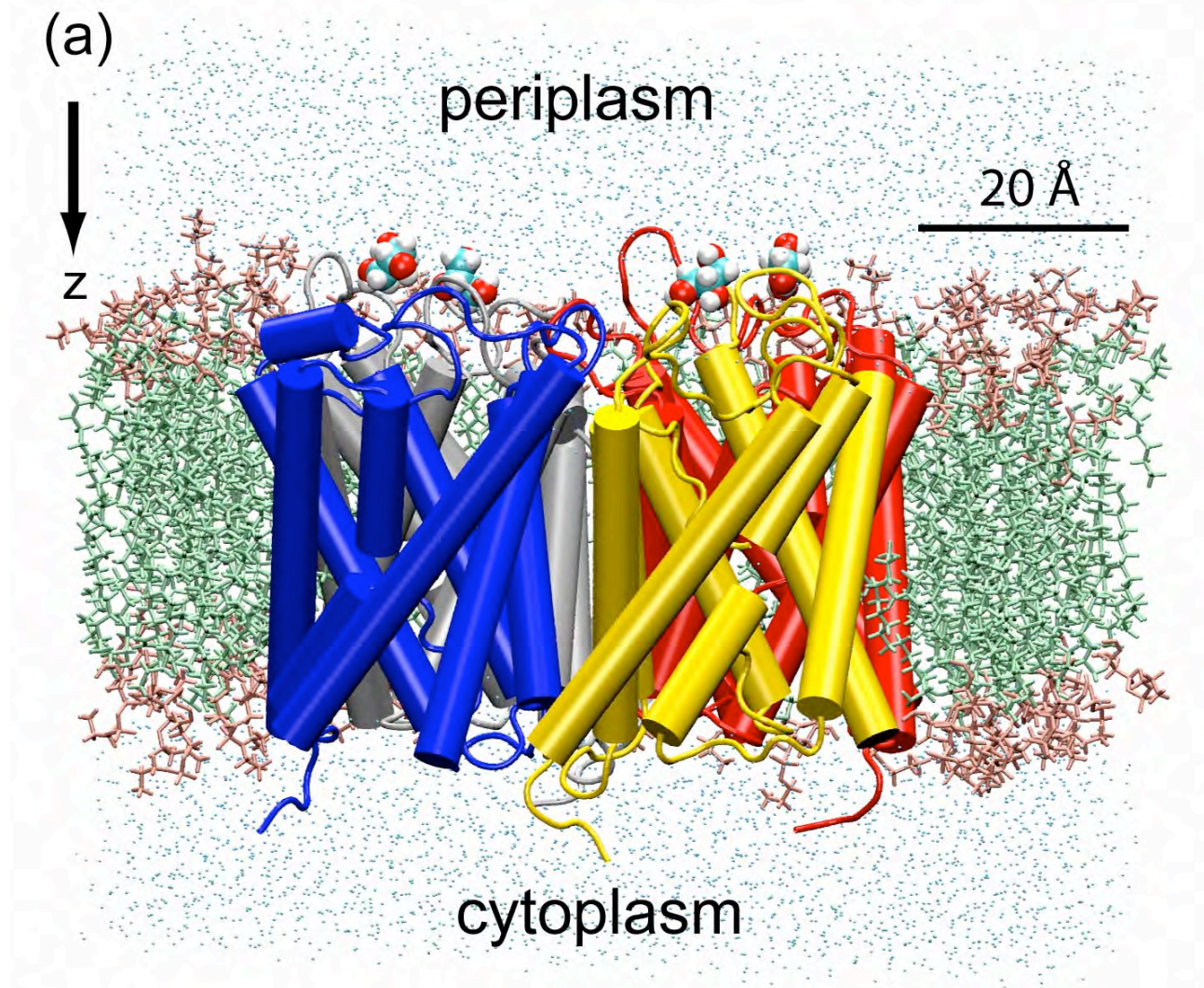
- Captures major features of the channel
- The largest barrier ≈ 7.3 kcal/mol; exp.: 9.6 ± 1.5 kcal/mol

Features of the Potential of Mean Force

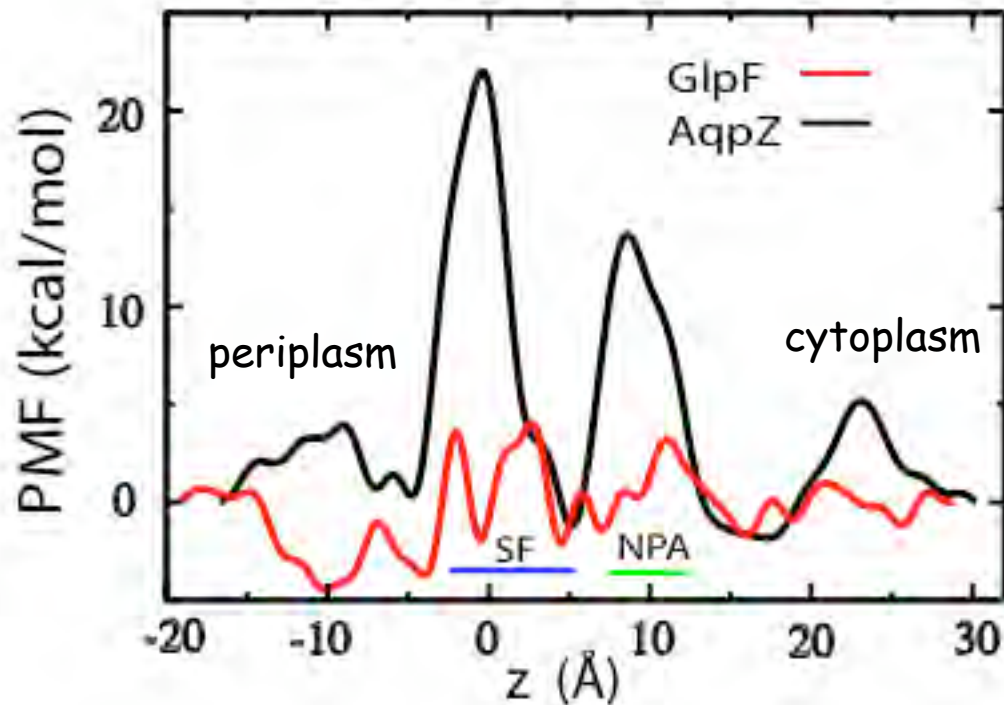


Asymmetric Profile in the Vestibules

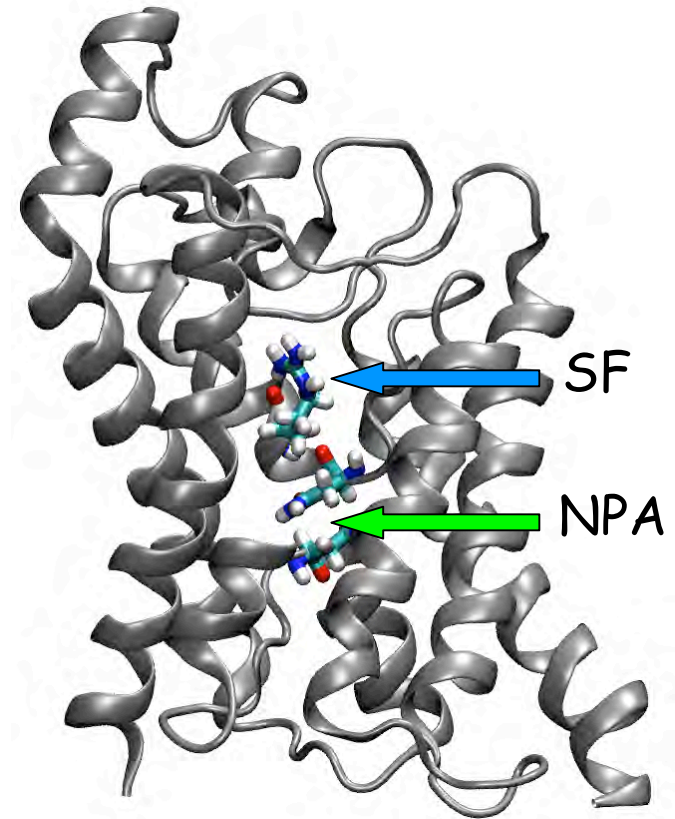
Artificial induction of glycerol conduction through AqpZ



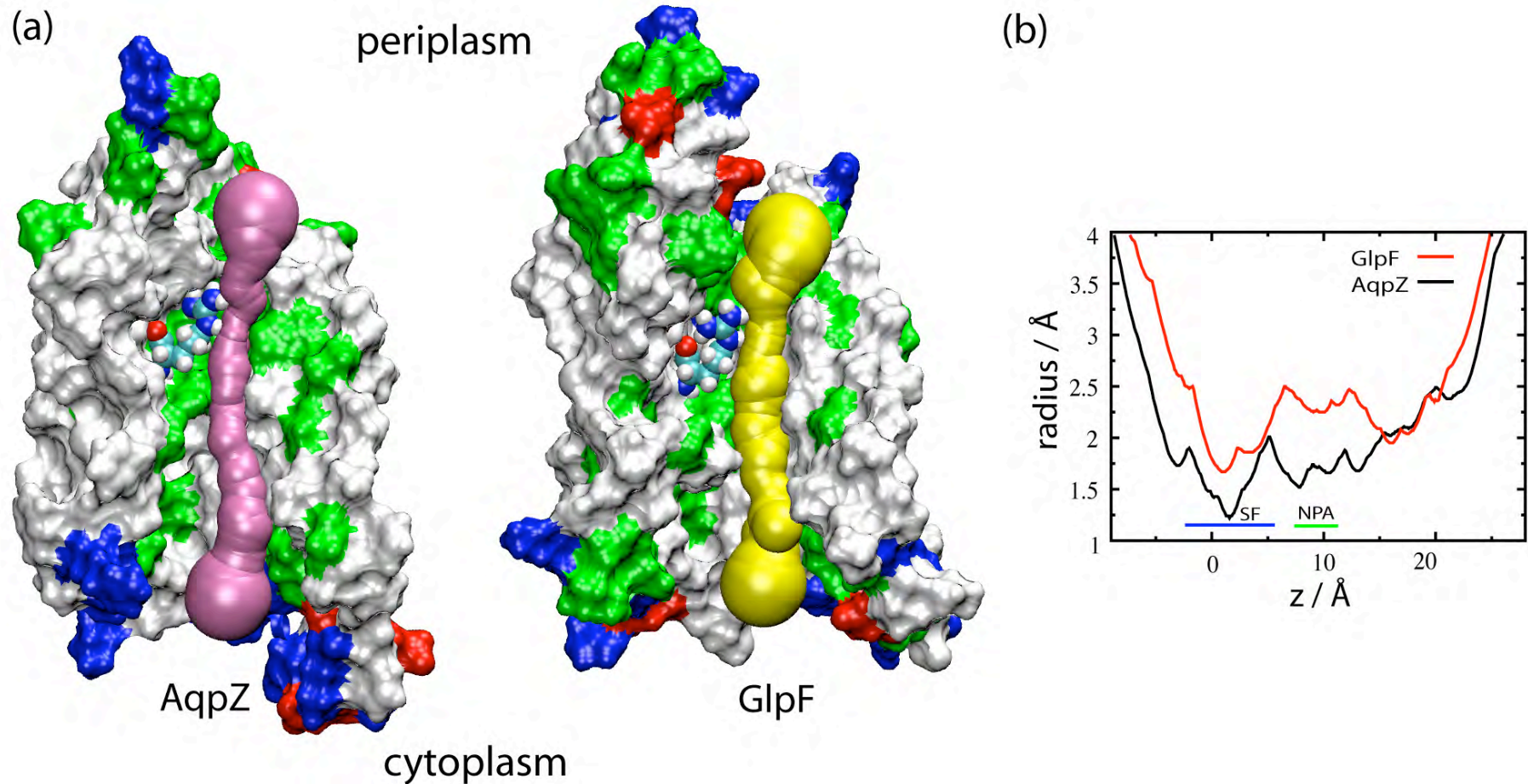
Three fold higher barriers



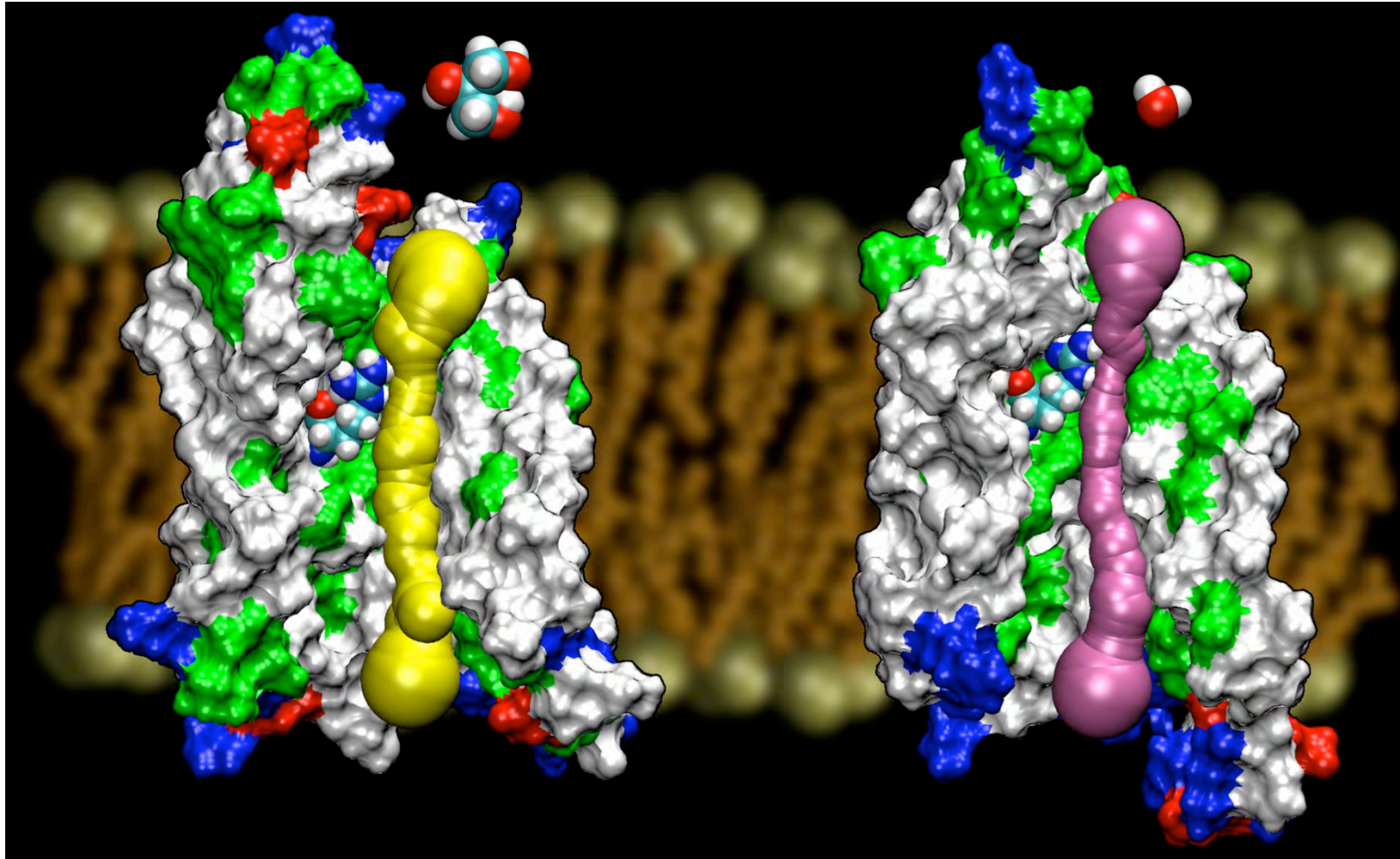
AqpZ 22.8 kcal/mol
GlpF 7.3 kcal/mol



Could it be simply the size?

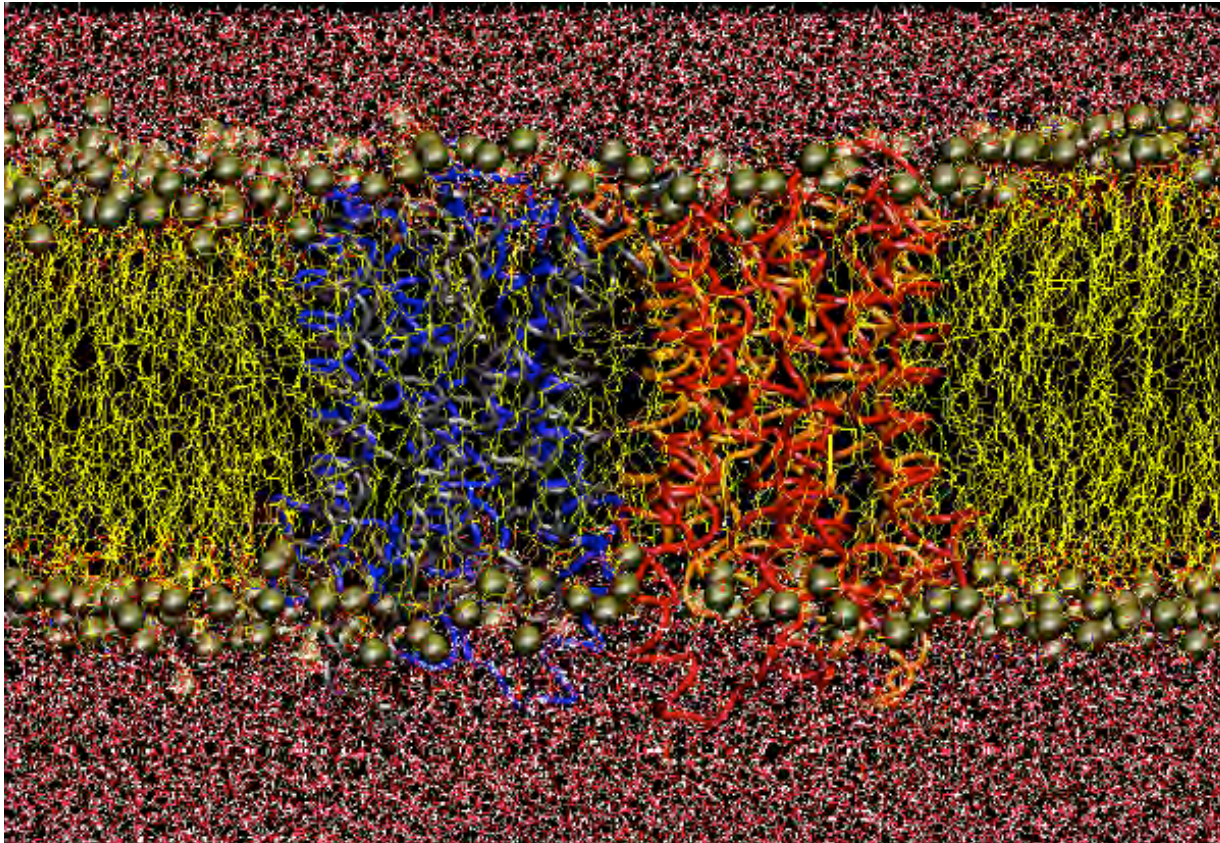


It is probably just the size that matters!



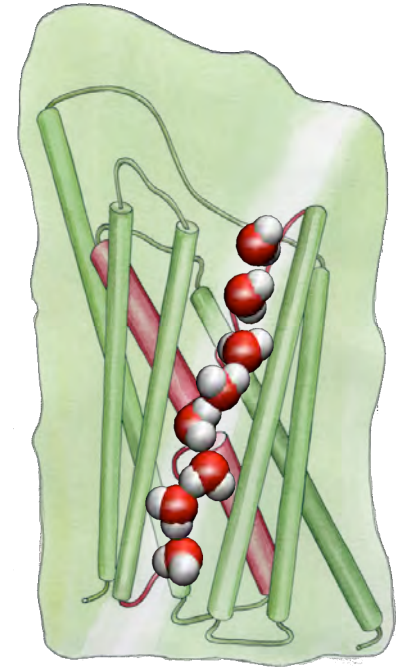
Y. Wang, K. Schulten, and E. Tajkhorshid *Structure* 13, 1107 (2005)

Water permeation

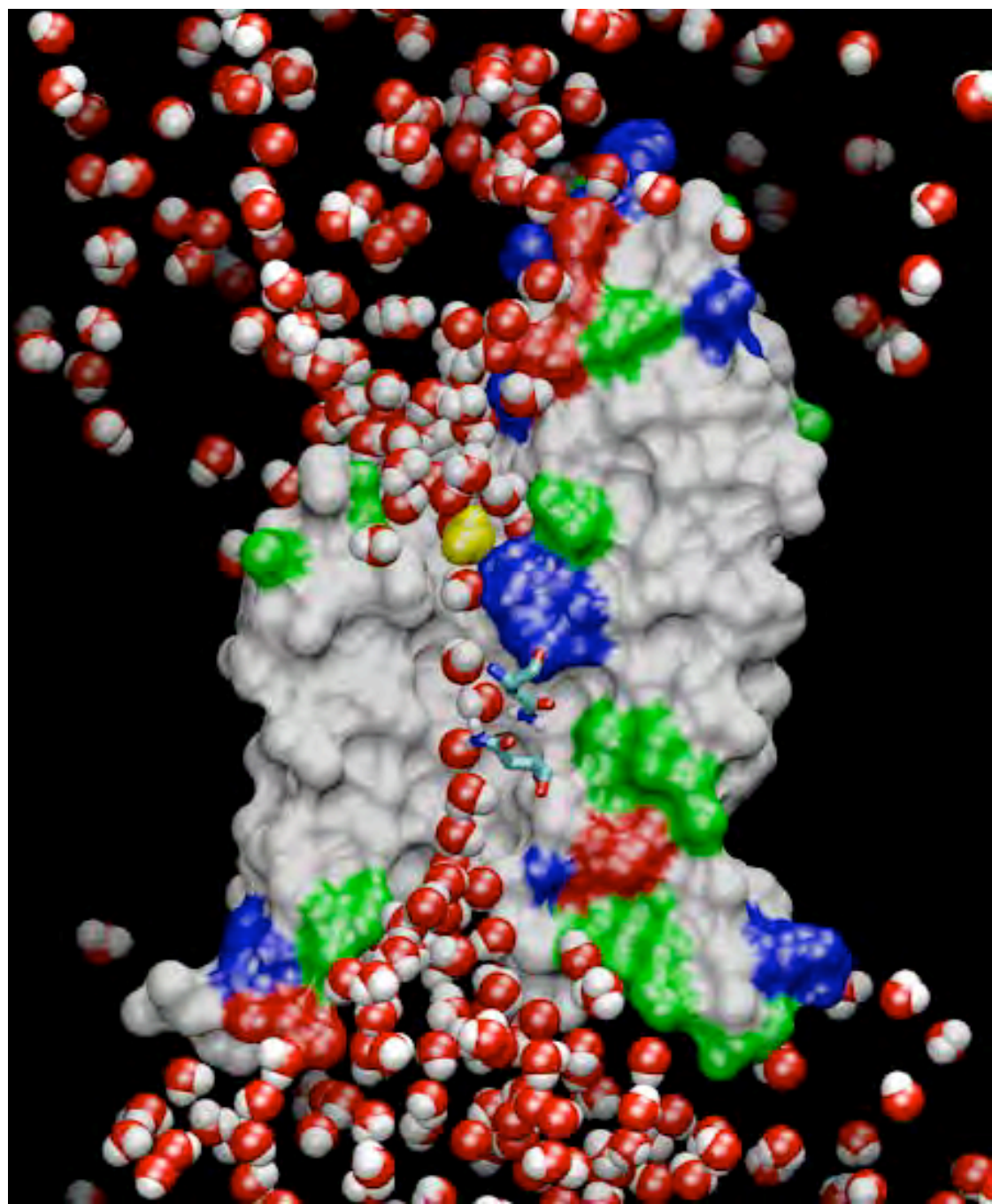


18 water conducted
In 4 monomers in 4 ns
1.125 water/monomer/ns
Exp. = $\sim 1-2$ /ns

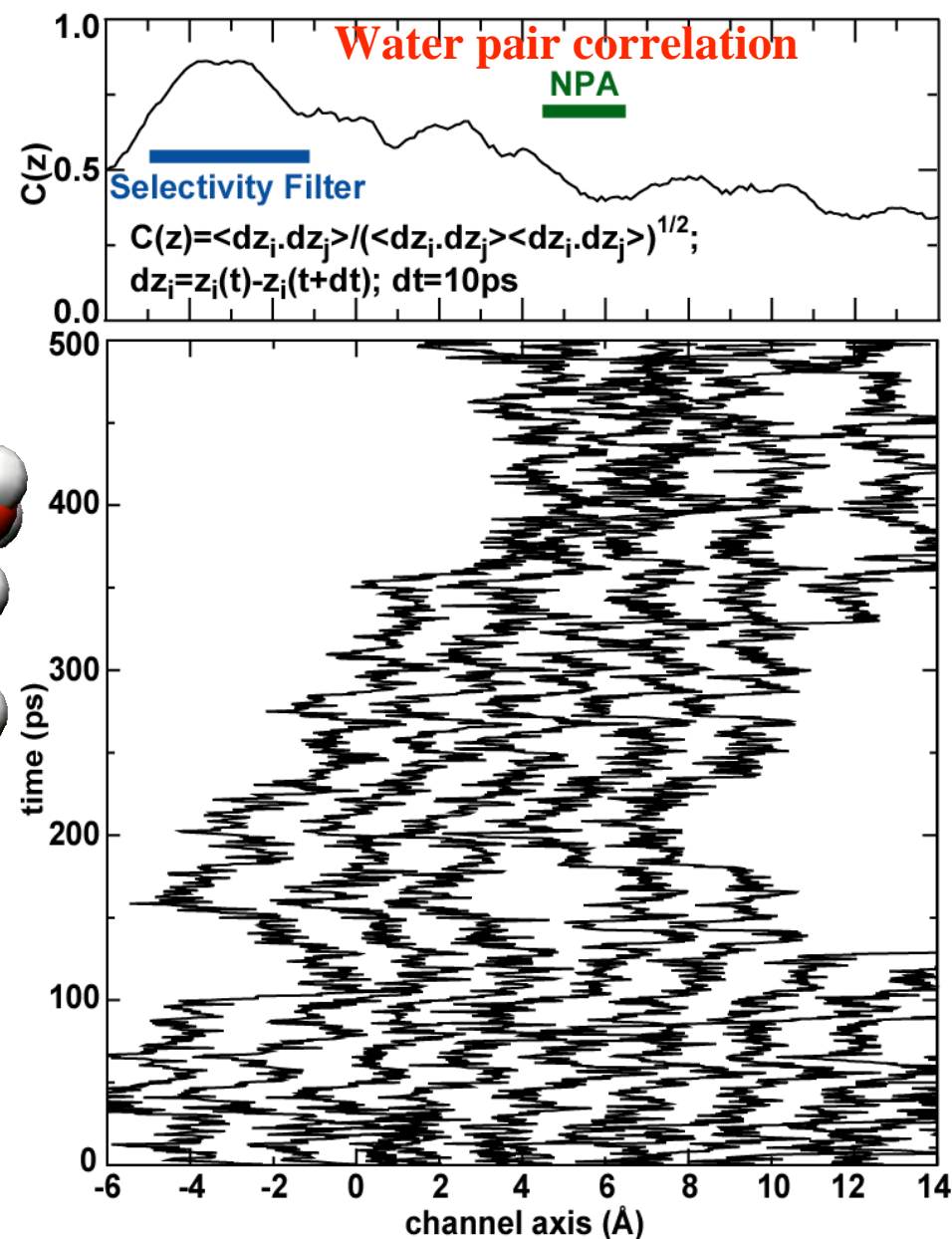
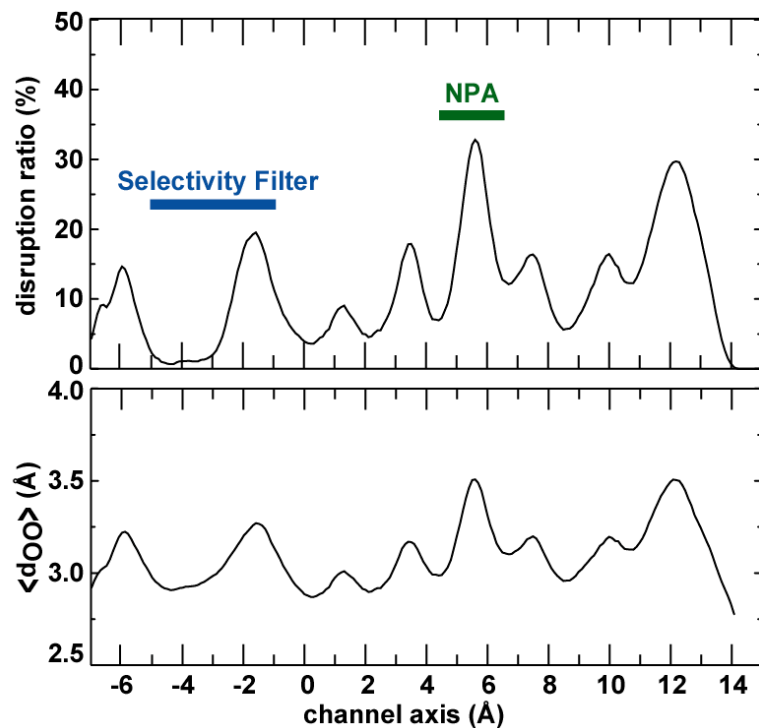
5 nanosecond
Simulation



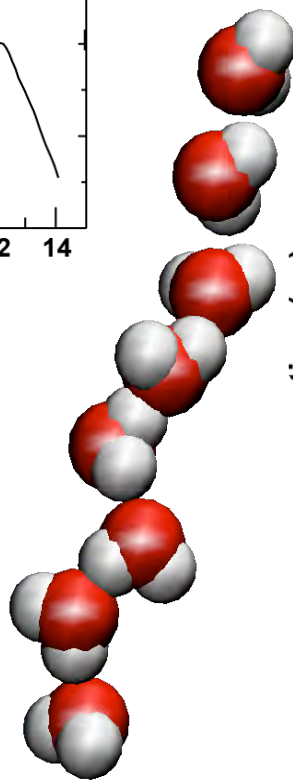
7-8 water
molecules in each
channel



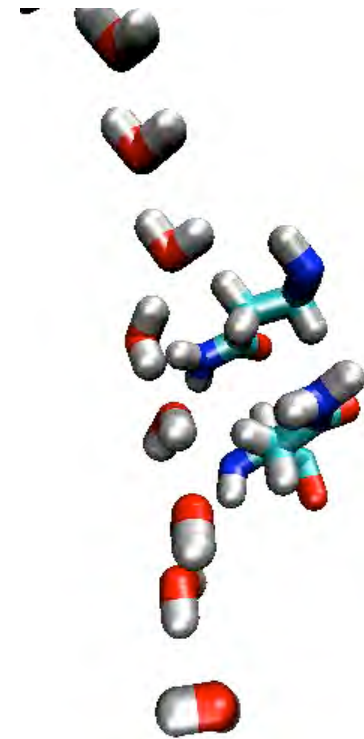
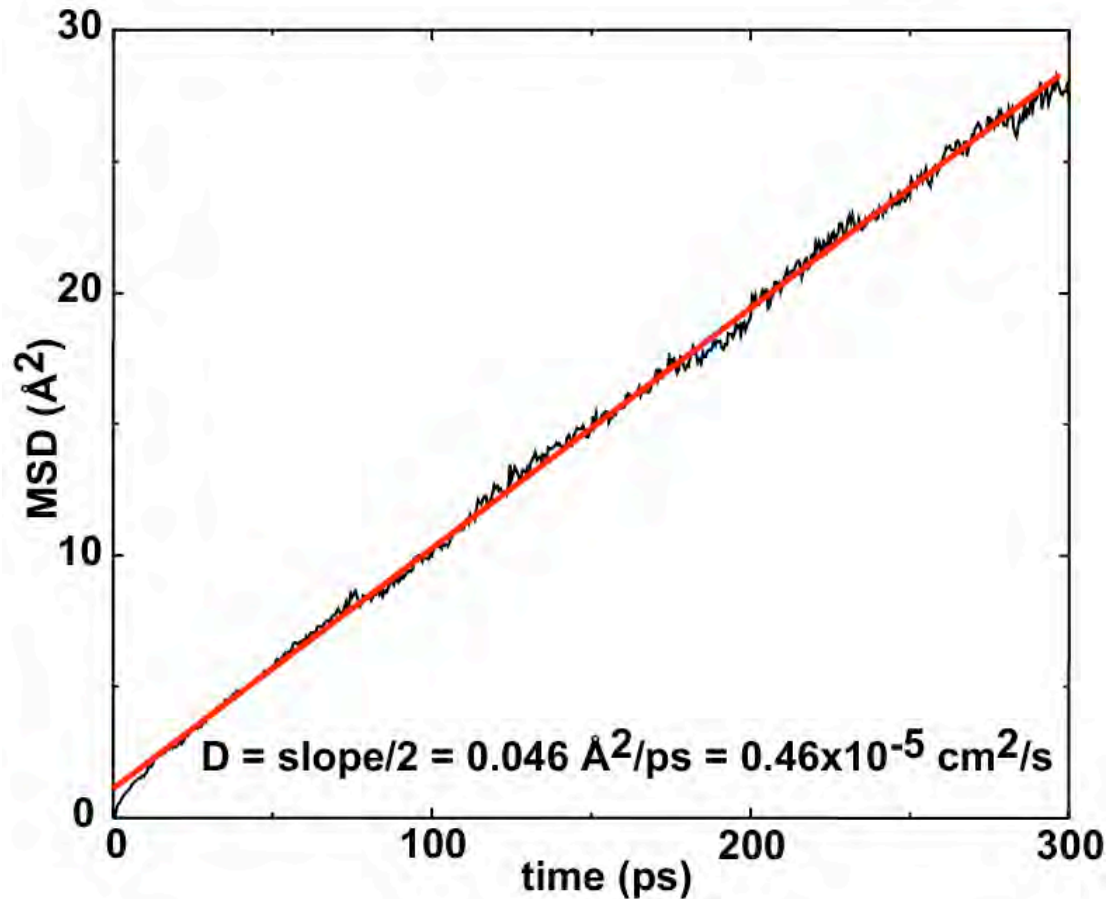
Correlated Motion of Water in the Channel



The single file of water molecules is maintained.



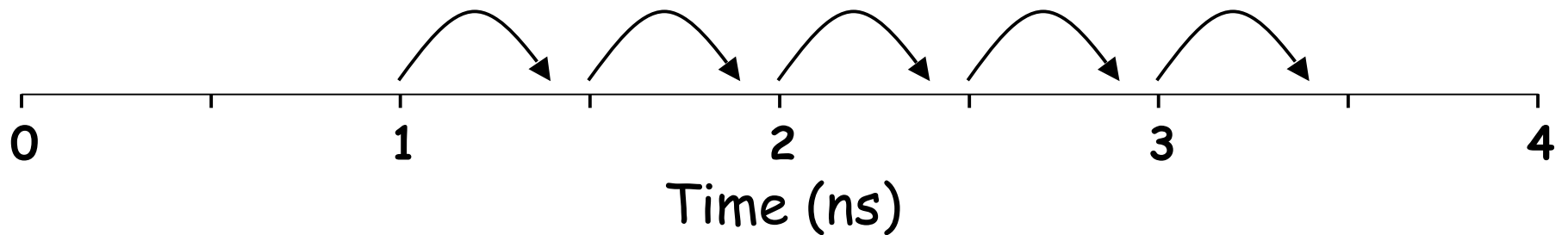
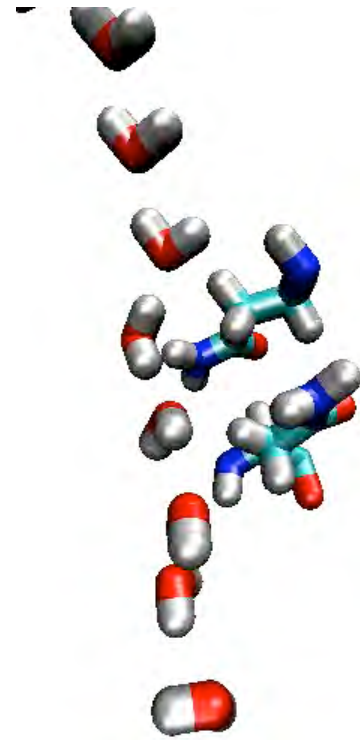
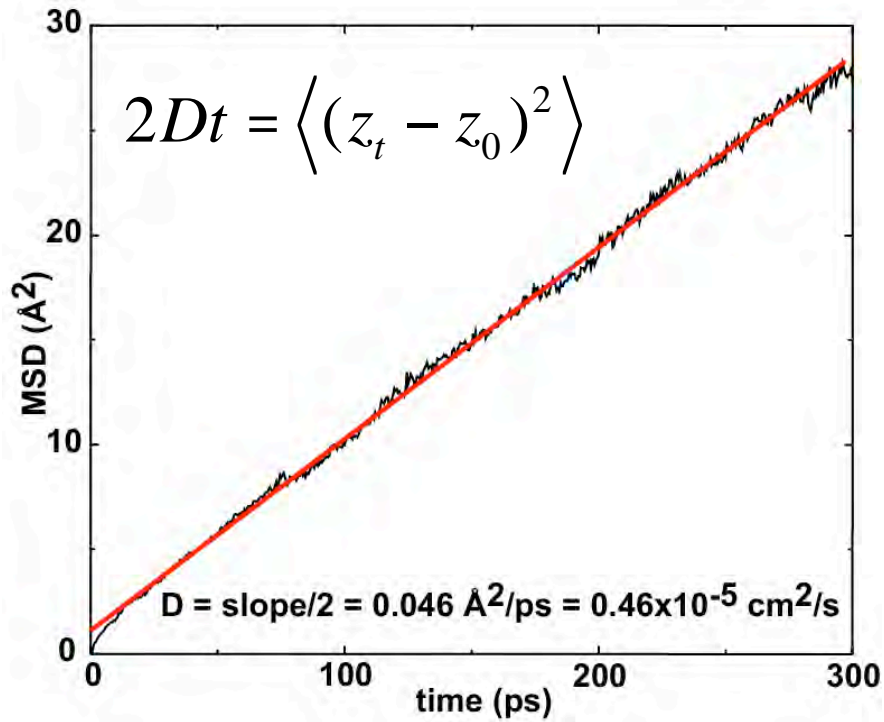
Diffusion of Water in the channel



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

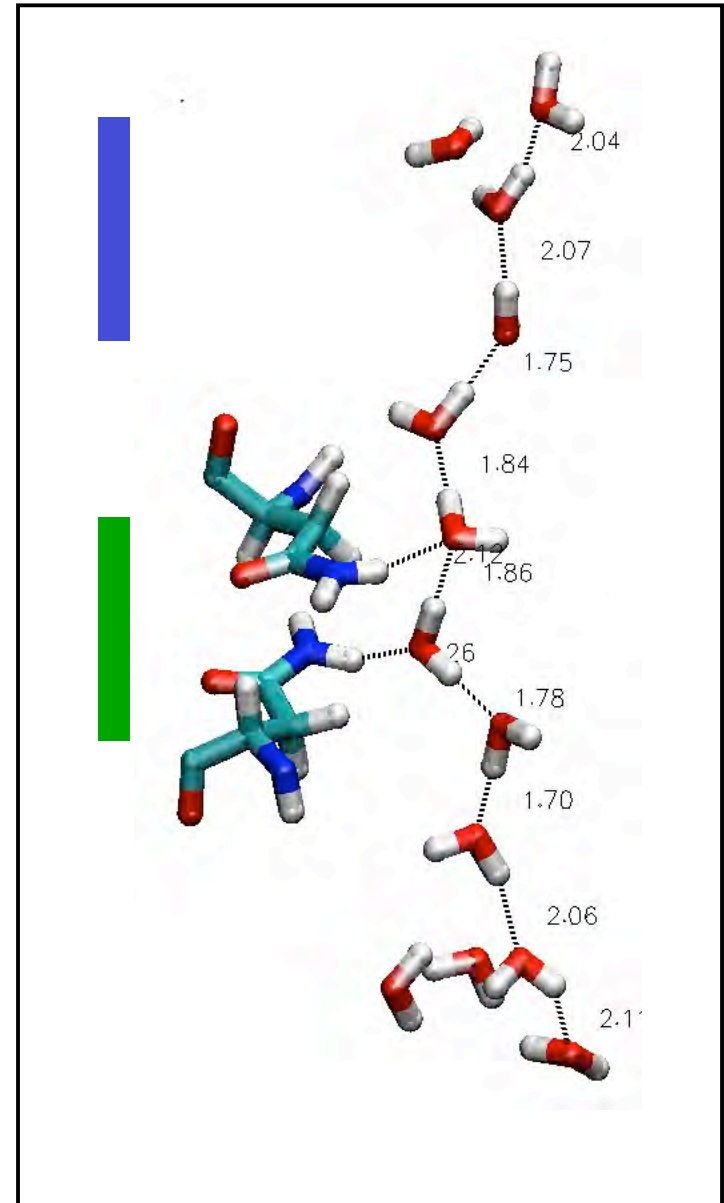
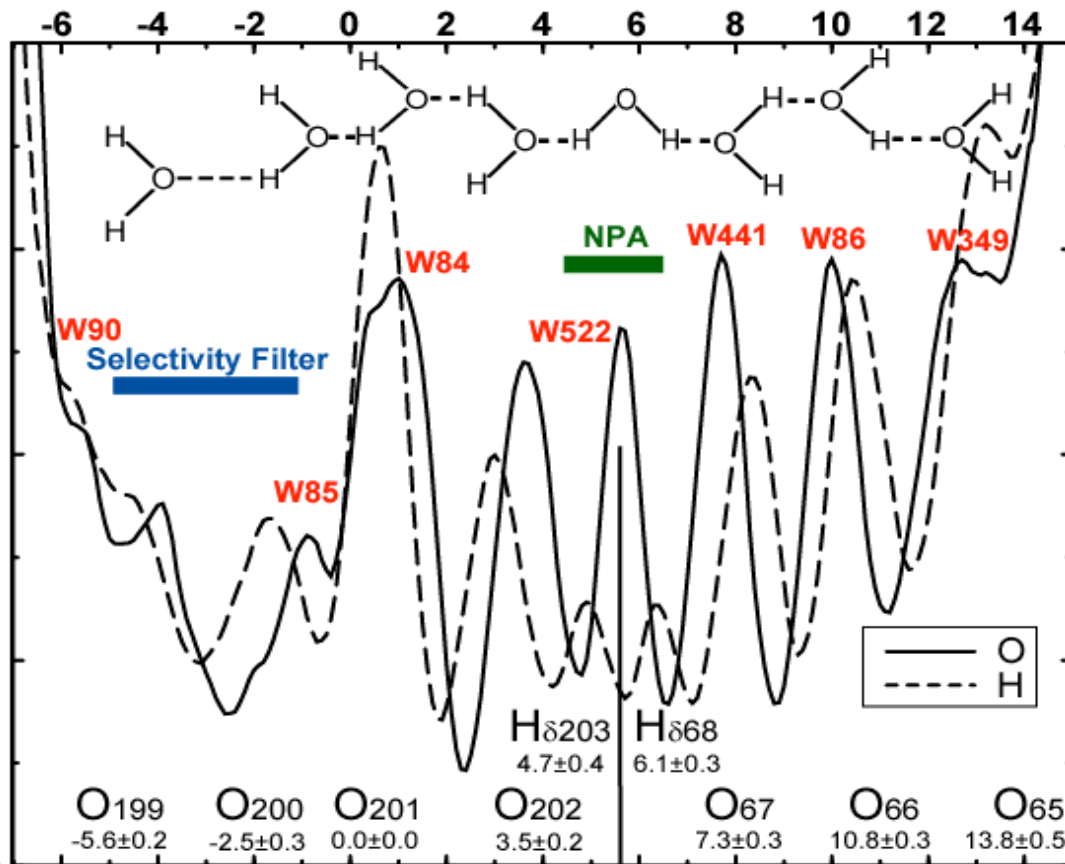
Experimental value for AQP1: $0.4-0.8 \text{ e-}5$

Diffusion of Water in the channel

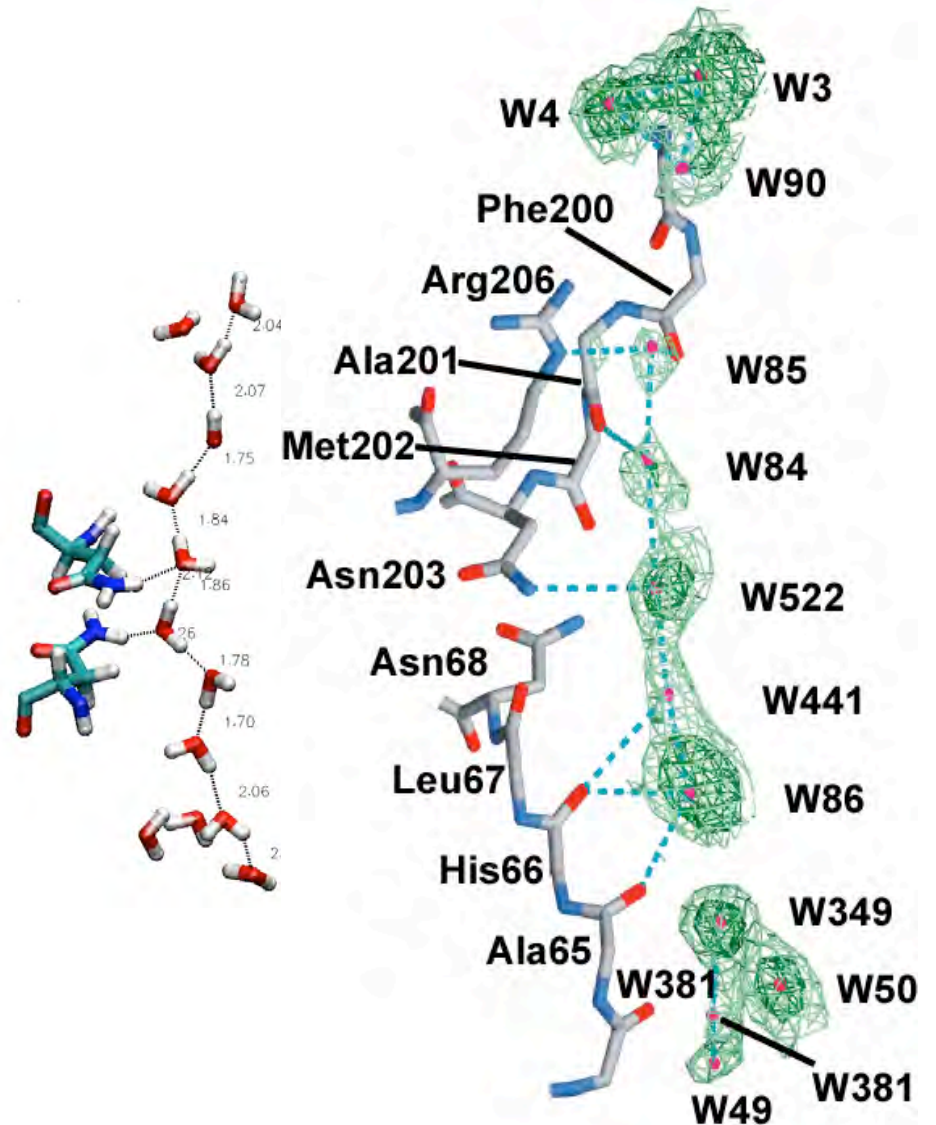
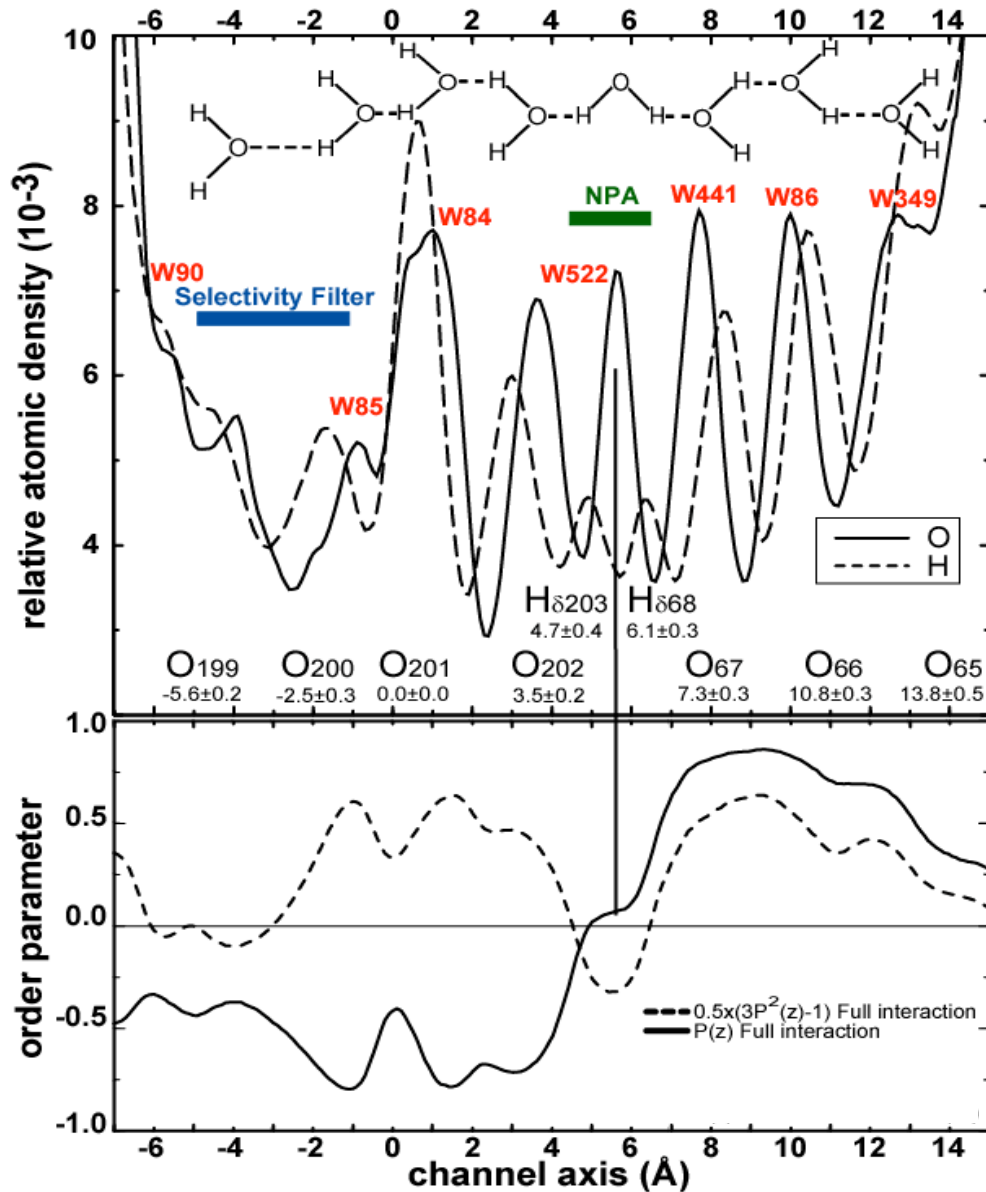


Improvement of statistics

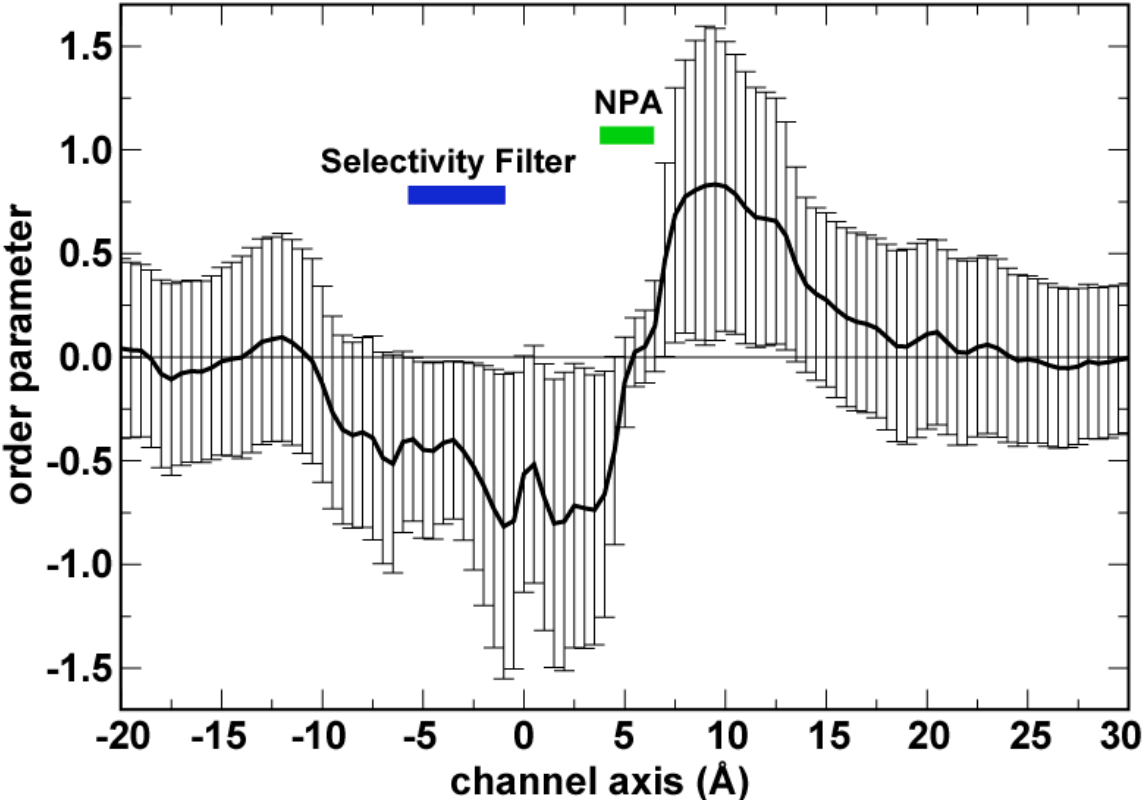
Water Bipolar Configuration in Aquaporins



Water Bipolar Configuration in Aquaporins



channel region (20 Å)

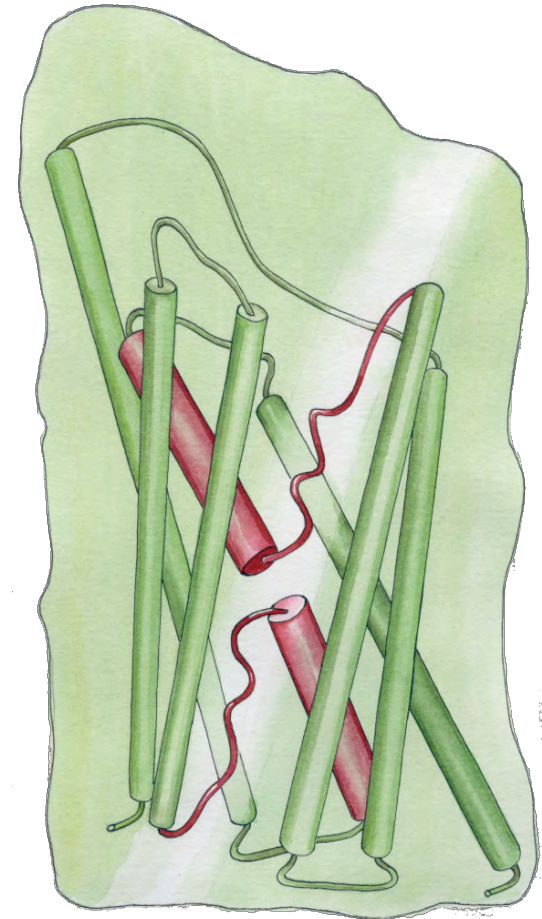
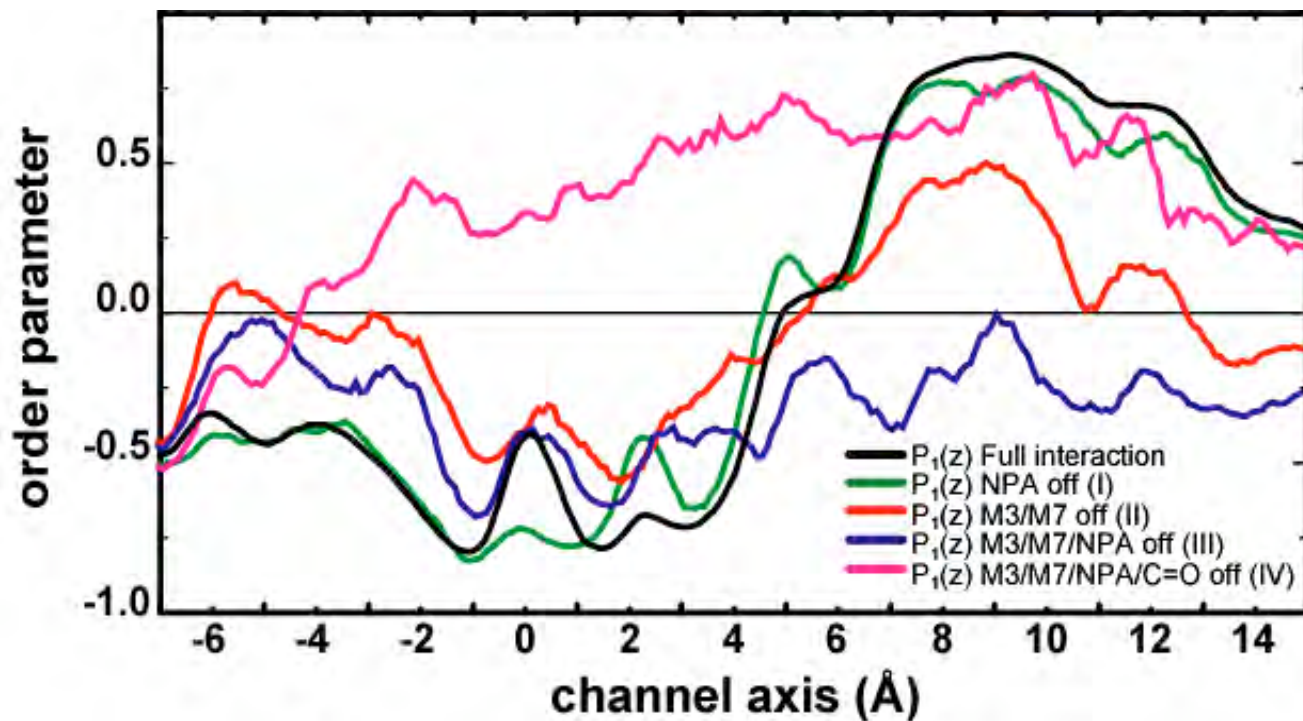


R E M E M B E R:

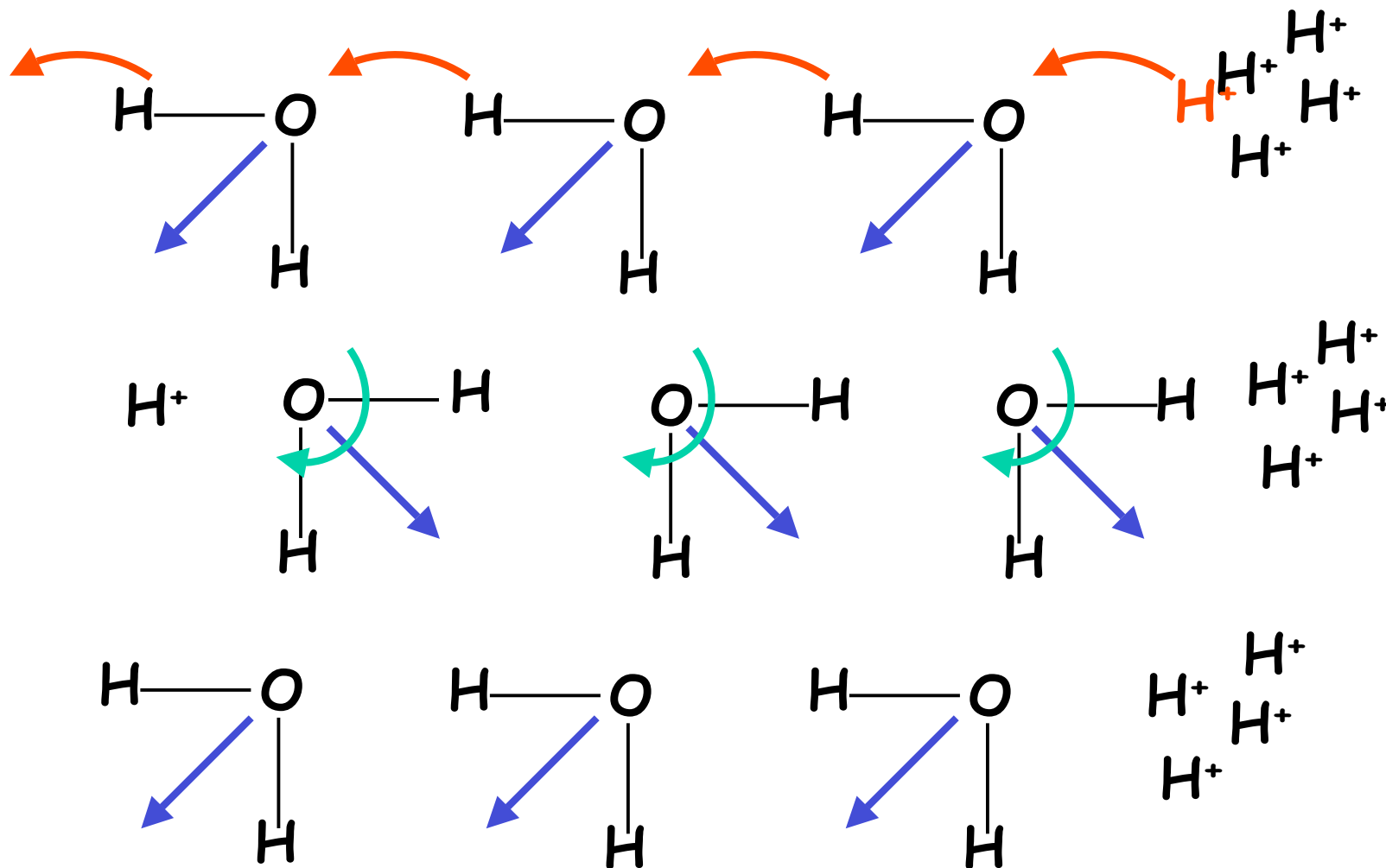
One of the most useful advantages of simulations over experiments is that you can modify the system as you wish: You can do modifications that are not even possible at all in reality!

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

Electrostatic Stabilization of Water Bipolar Arrangement

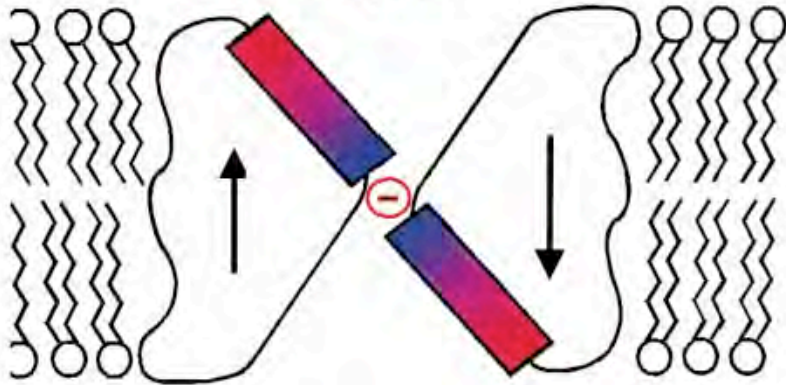


Proton transfer through water

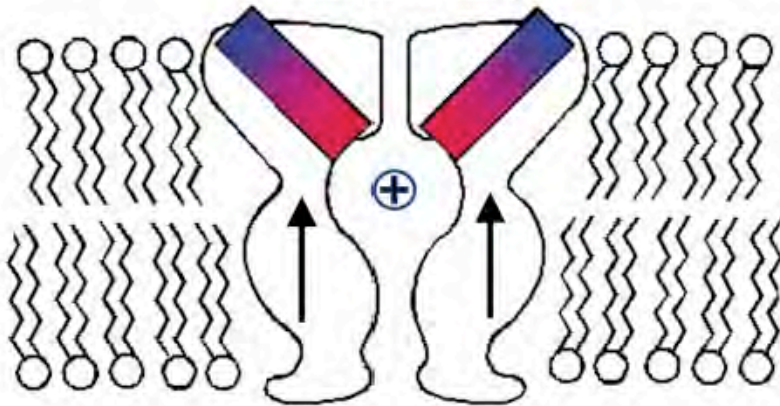


Cl⁻ channel

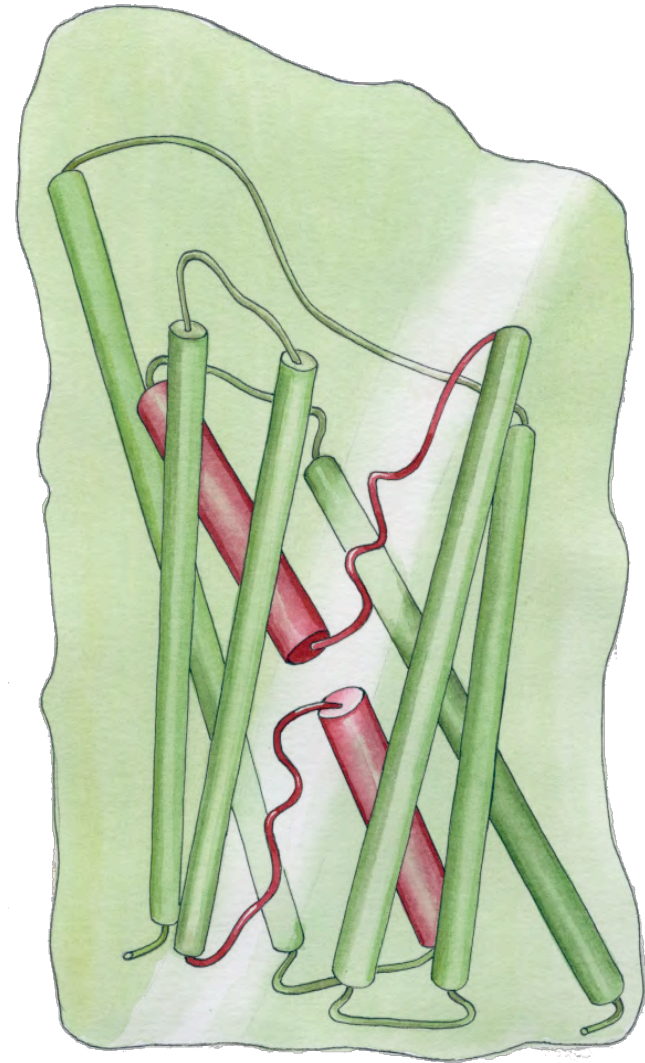
Anti-parallel



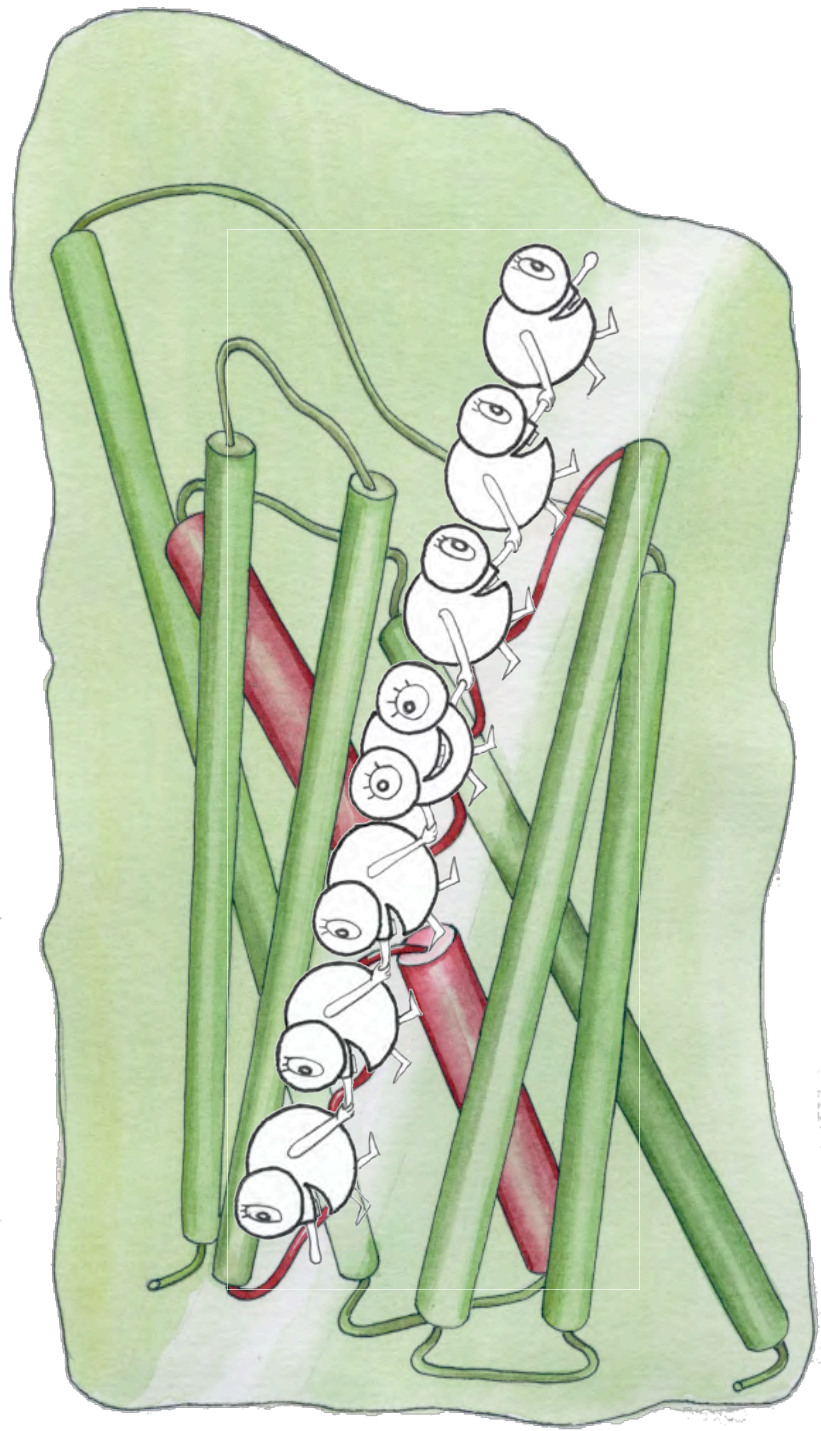
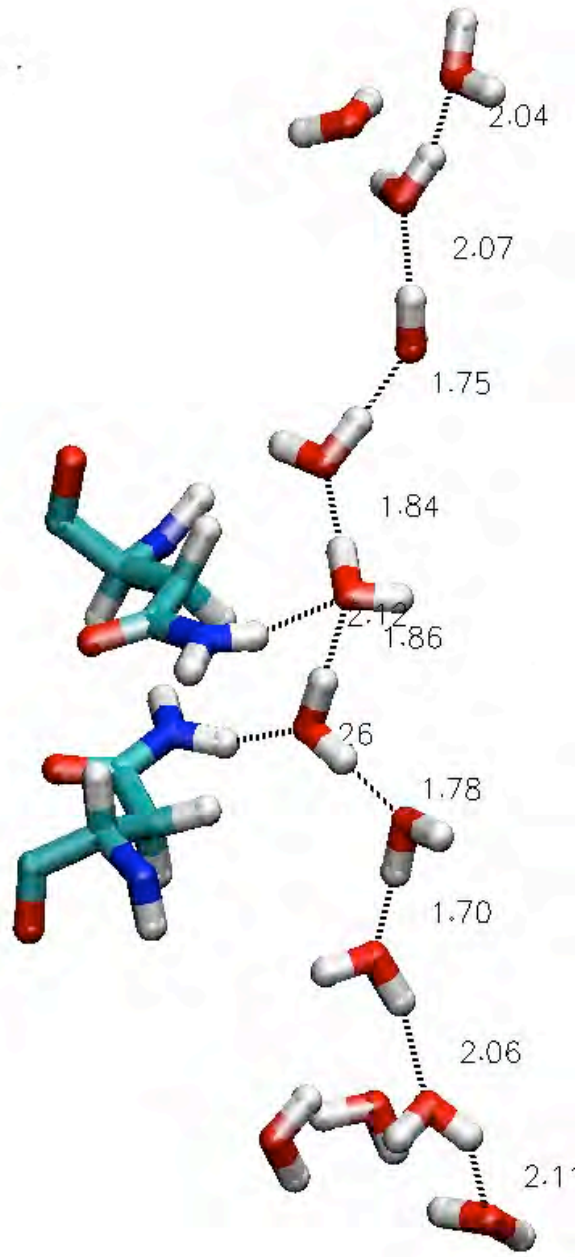
Parallel (barrel stave)



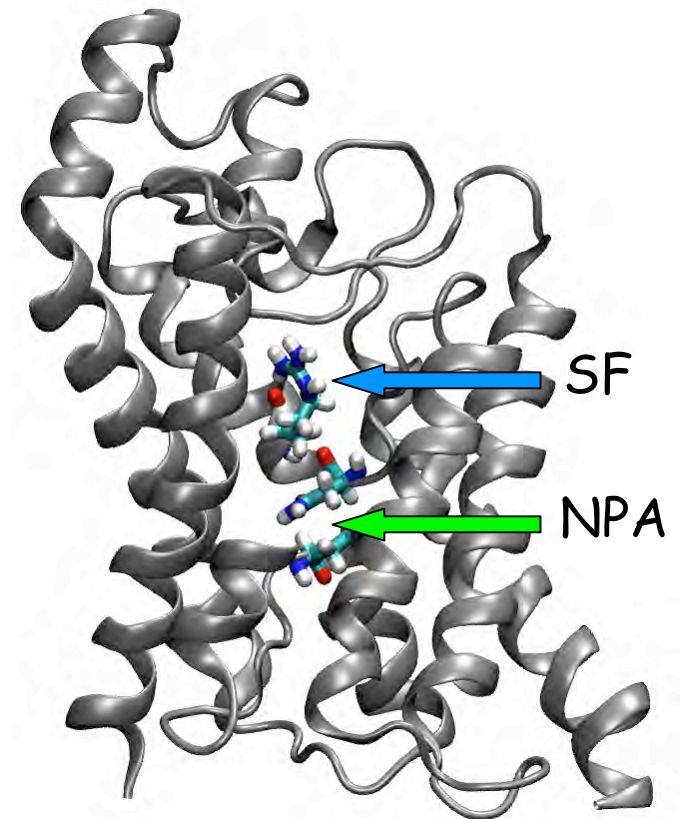
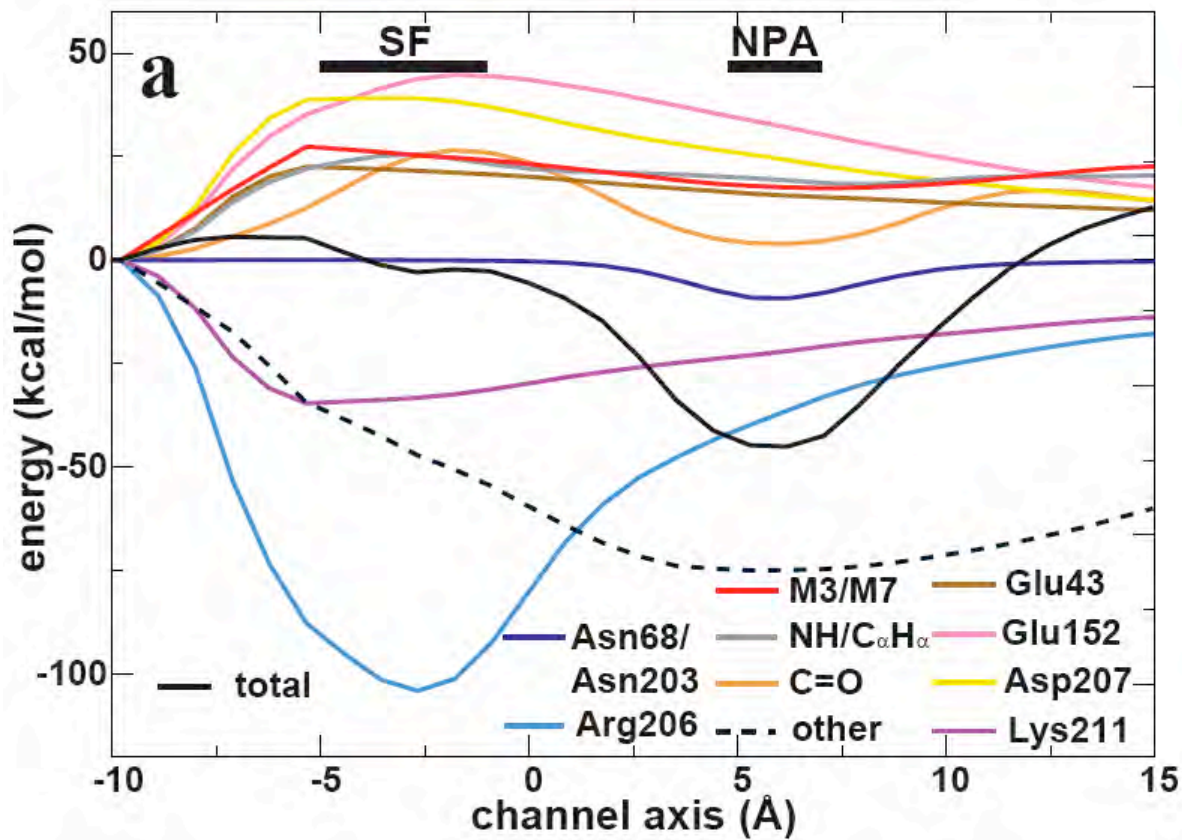
K⁺ channel



Aquaporins

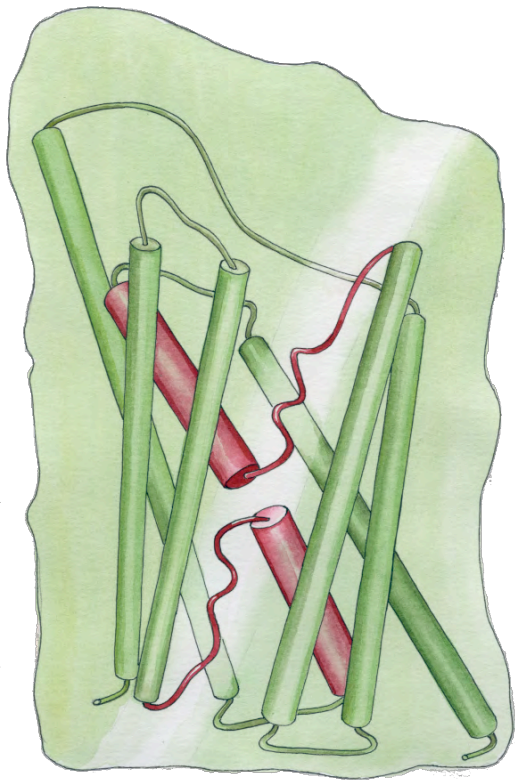


A Complex Electrostatic Interaction

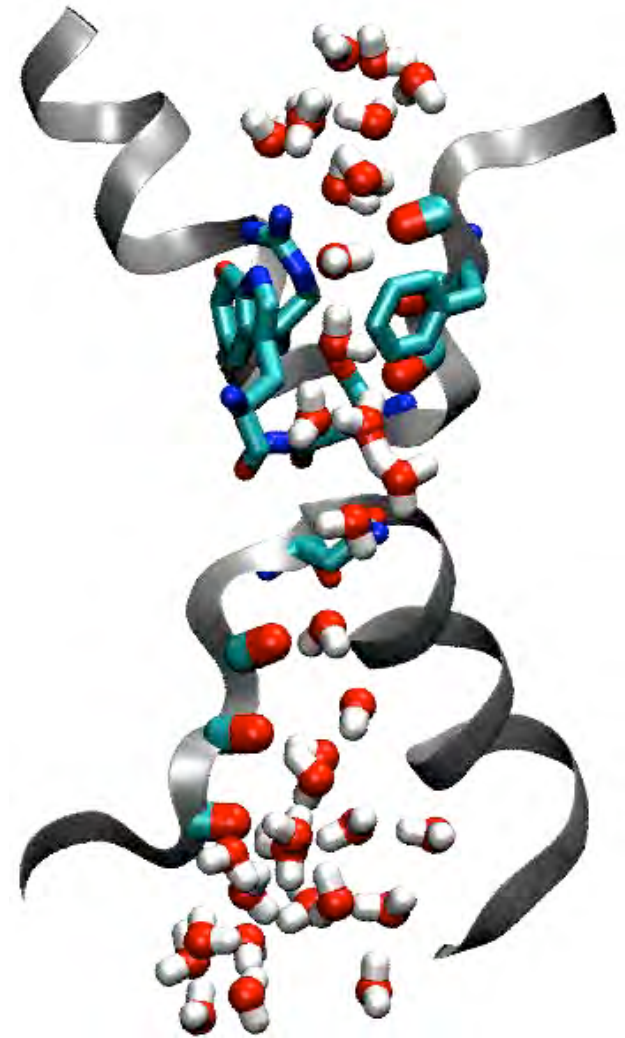


“Surprising and clearly not a hydrophobic channel”

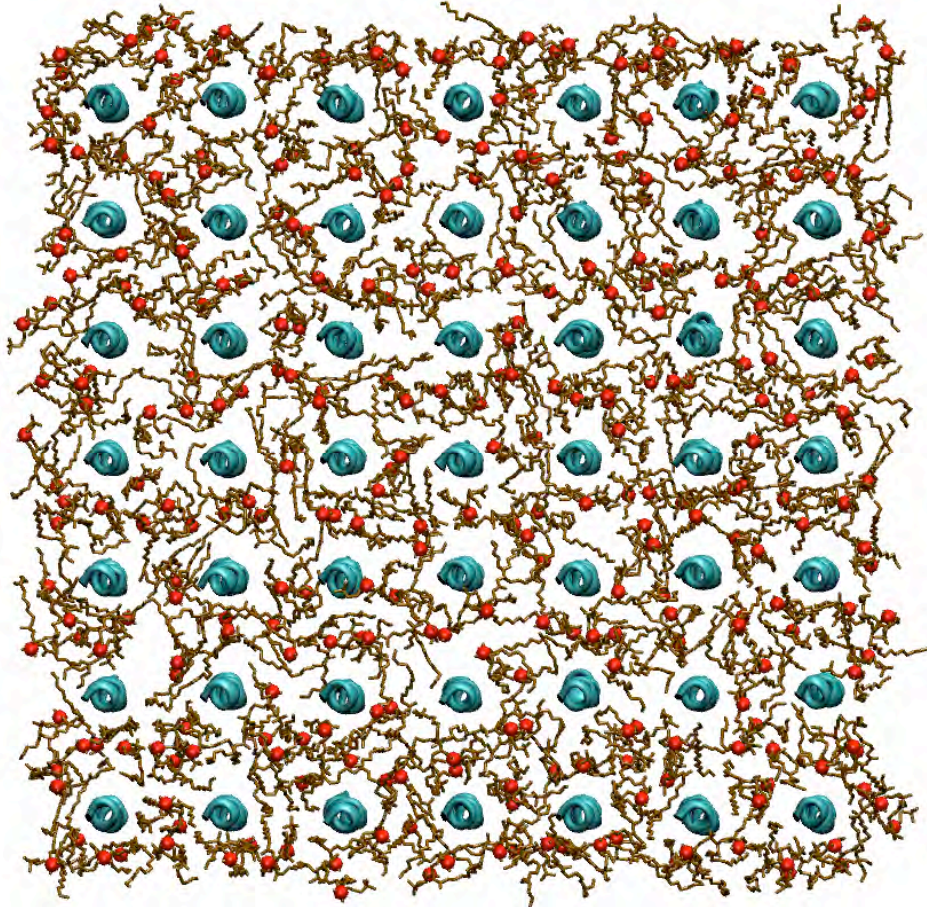
A Repulsive Electrostatic Force at the Center of the Channel



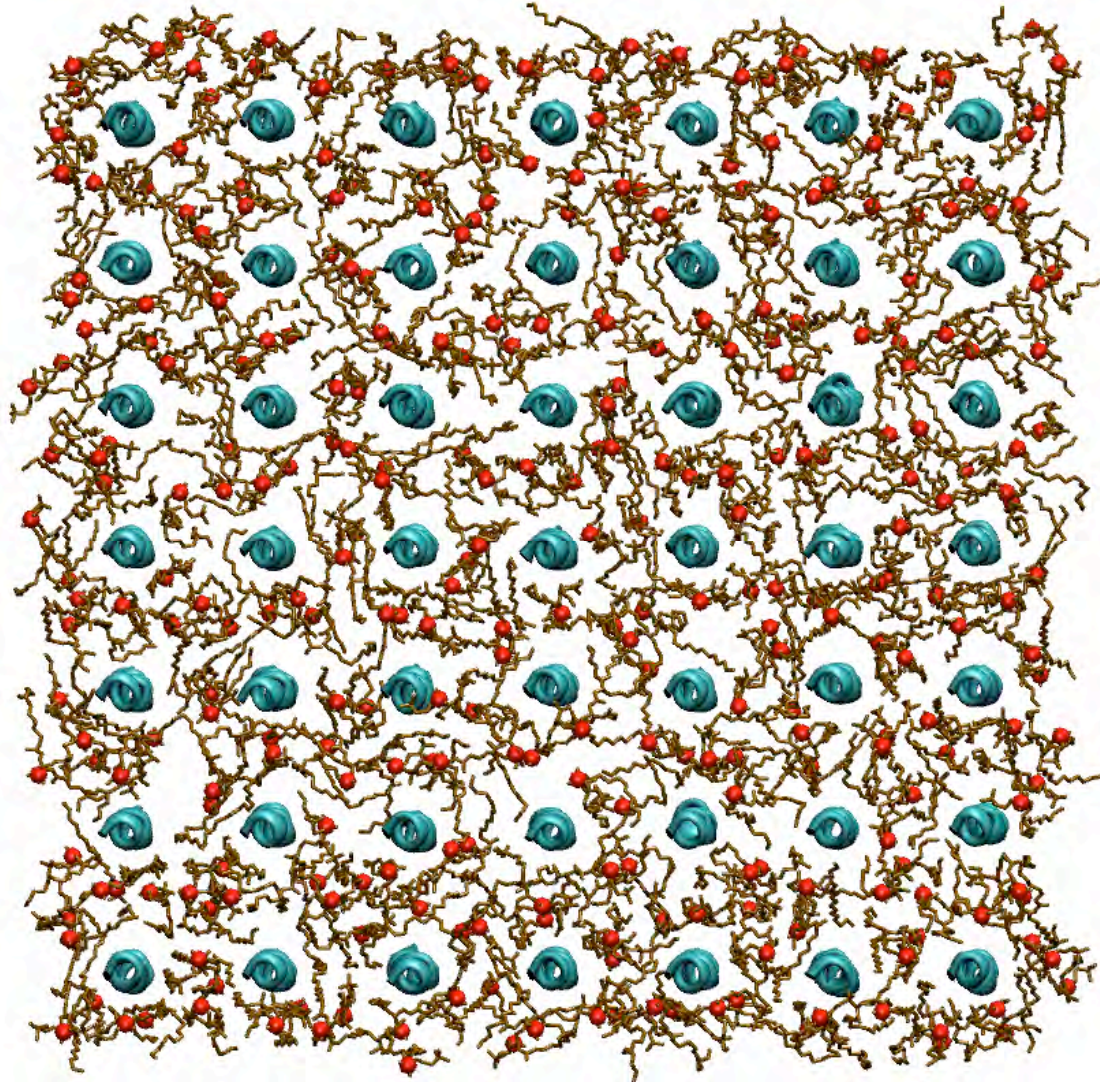
QM/MM MD of the behavior
of an excessive proton



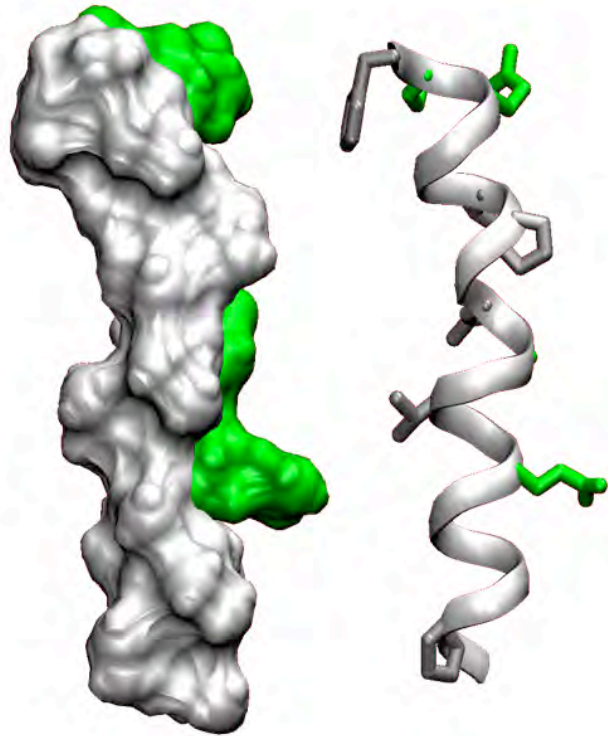
Combining all-atom and coarse-grained models to simulate transport across lipid bilayers



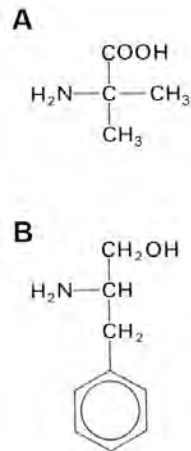
Peptide aggregation and "Pore" formation in lipid bilayers



Alamethicin



PhI20
Gln19
Glu18
Aib17
Aib16
Val15
Pro14
Aib13
Leu12
Gly11
Aib10
Val9
Aib8
Gln7
Ala6
Aib5
Ala4
Aib3
Pro2
Aib1



- 20-residue peptide
- No charge
- forming pores in the membrane

20 residue antimicrobial peptide

CG molecular systems allow for time scales of 3-4 orders of magnitude longer, because:

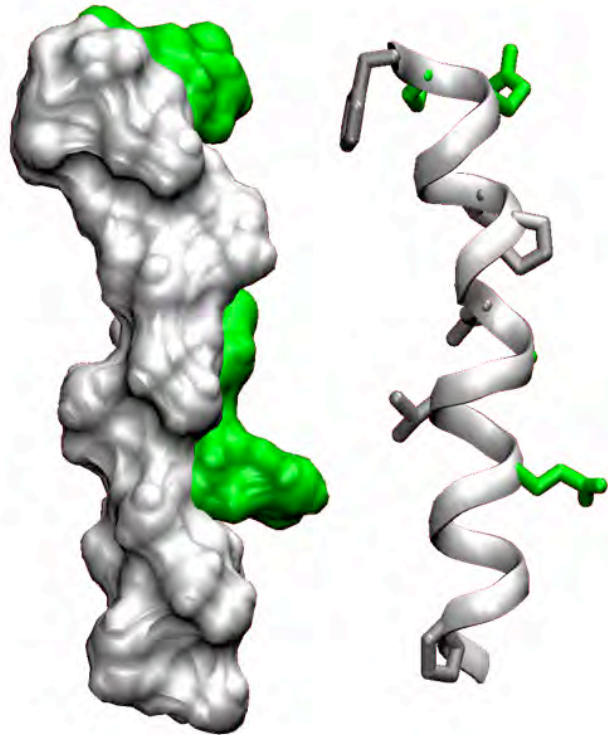
- Significant reduction of the degrees of freedom (or number of interacting particles/beads)
- Softer potentials allowing much longer time steps

μm length scale and μs time scale

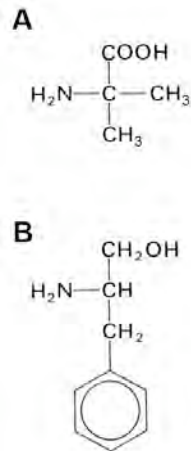
Bilayer, micelle, and vesicle formation

Fusion of bilayers and vesicles, ...

Alamethicin



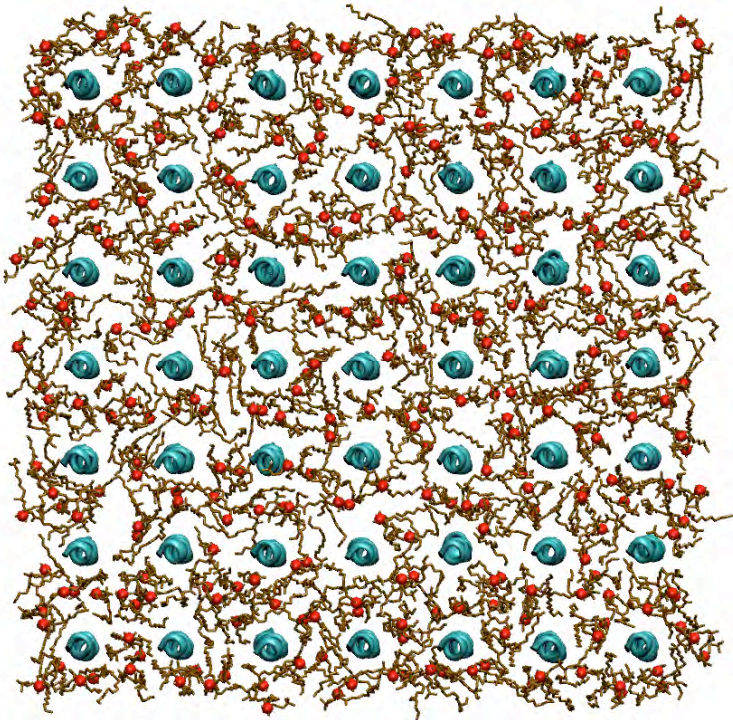
PhI20
Gln19
Glu18
Aib17
Aib16
Val15
Pro14
Aib13
Leu12
Gly11
Aib10
Val9
Aib8
Gln7
Ala6
Aib5
Ala4
Aib3
Pro2
Aib1



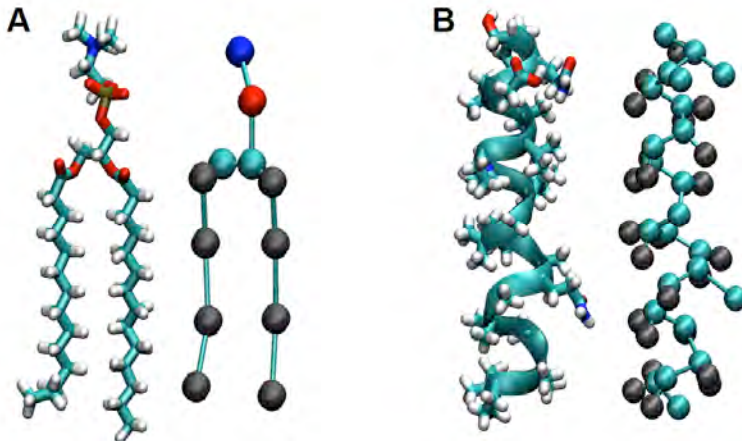
- 20-residue peptide
- No charge
- forming pores in the membrane

20 residue antimicrobial peptide

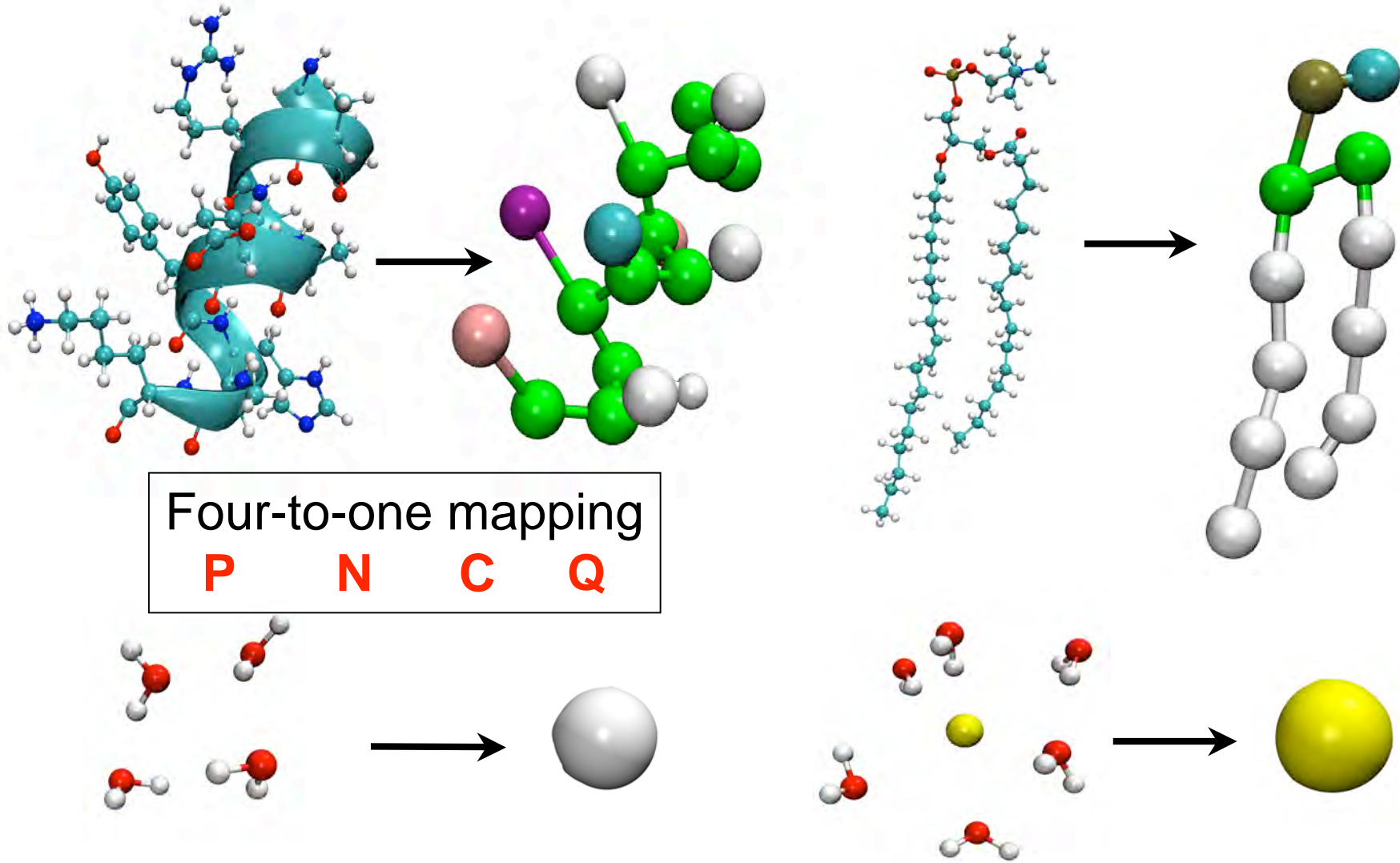
Simulation Setting



- 49 peptides in 288 DMPC
- All-atom model equilibrated 1ns
- Converted to a CG model
- Simulated for 1 μ s
- 0.5 μ s snapshot was reverse-coarse-grained to an all atom model
- All atom model simulated for 20 ns

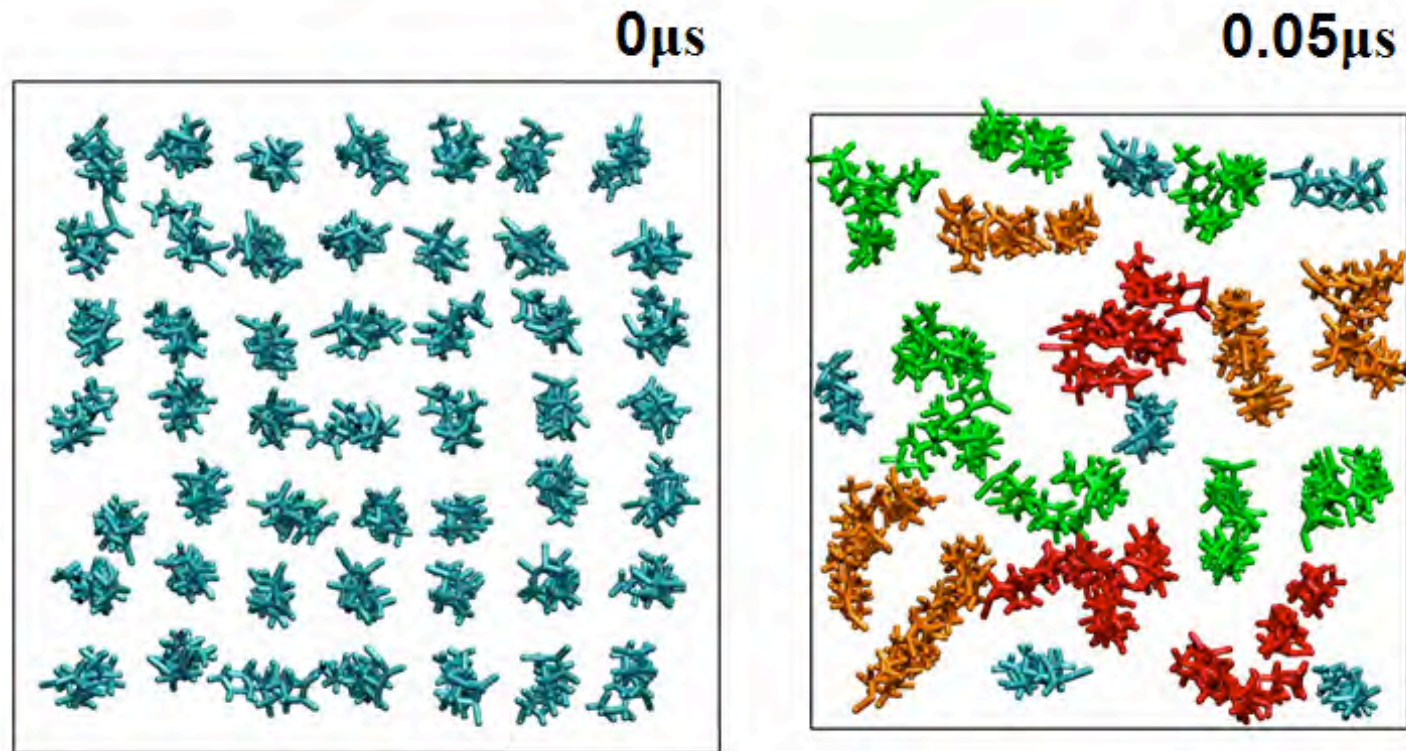


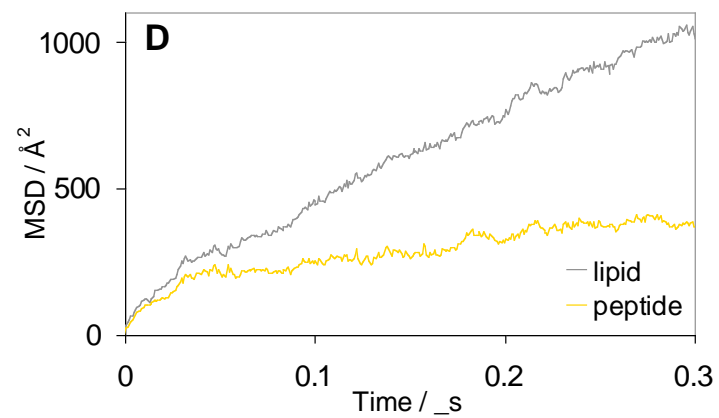
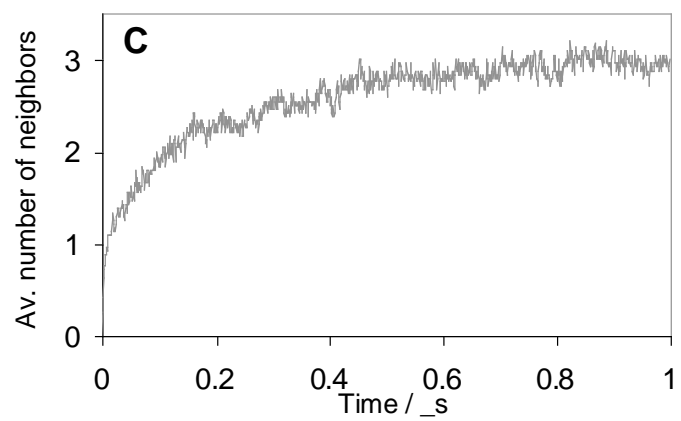
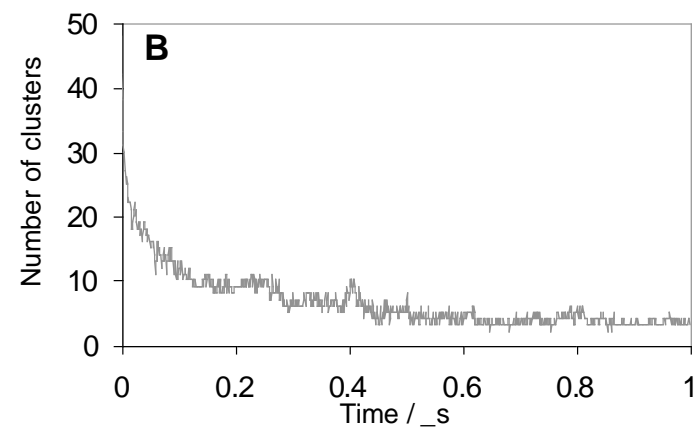
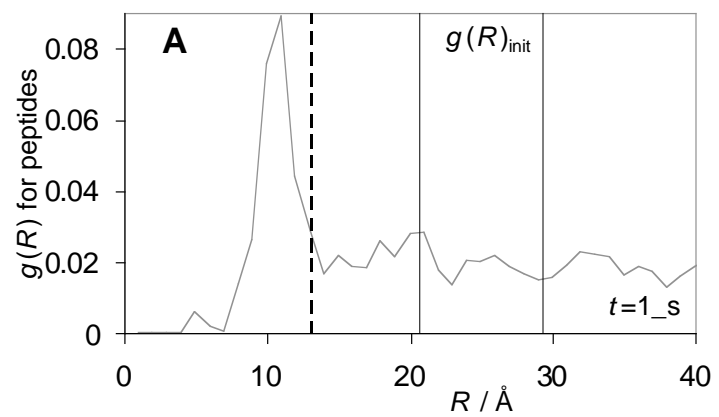
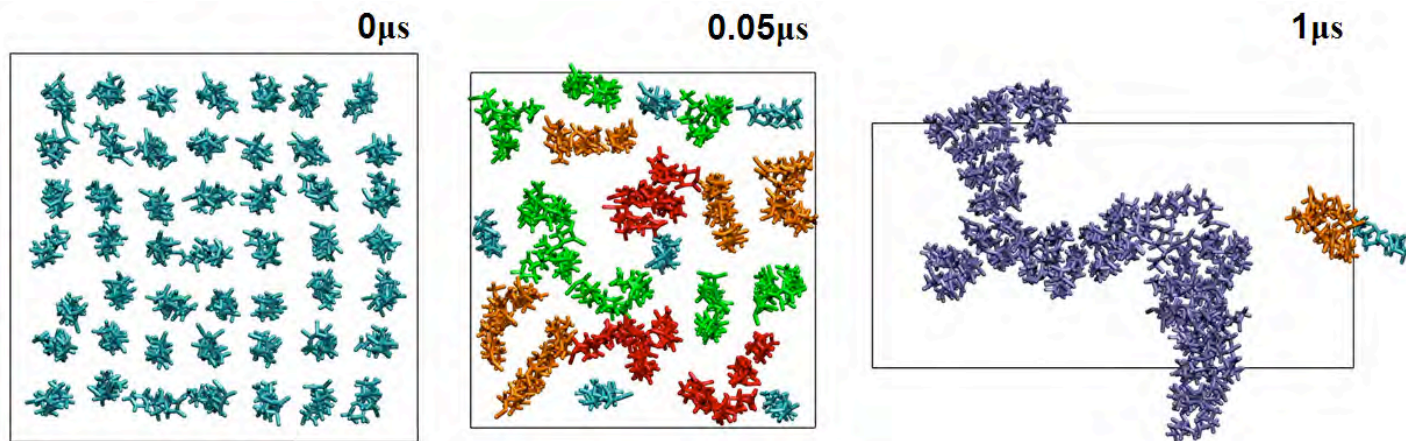
Coarse-Graining



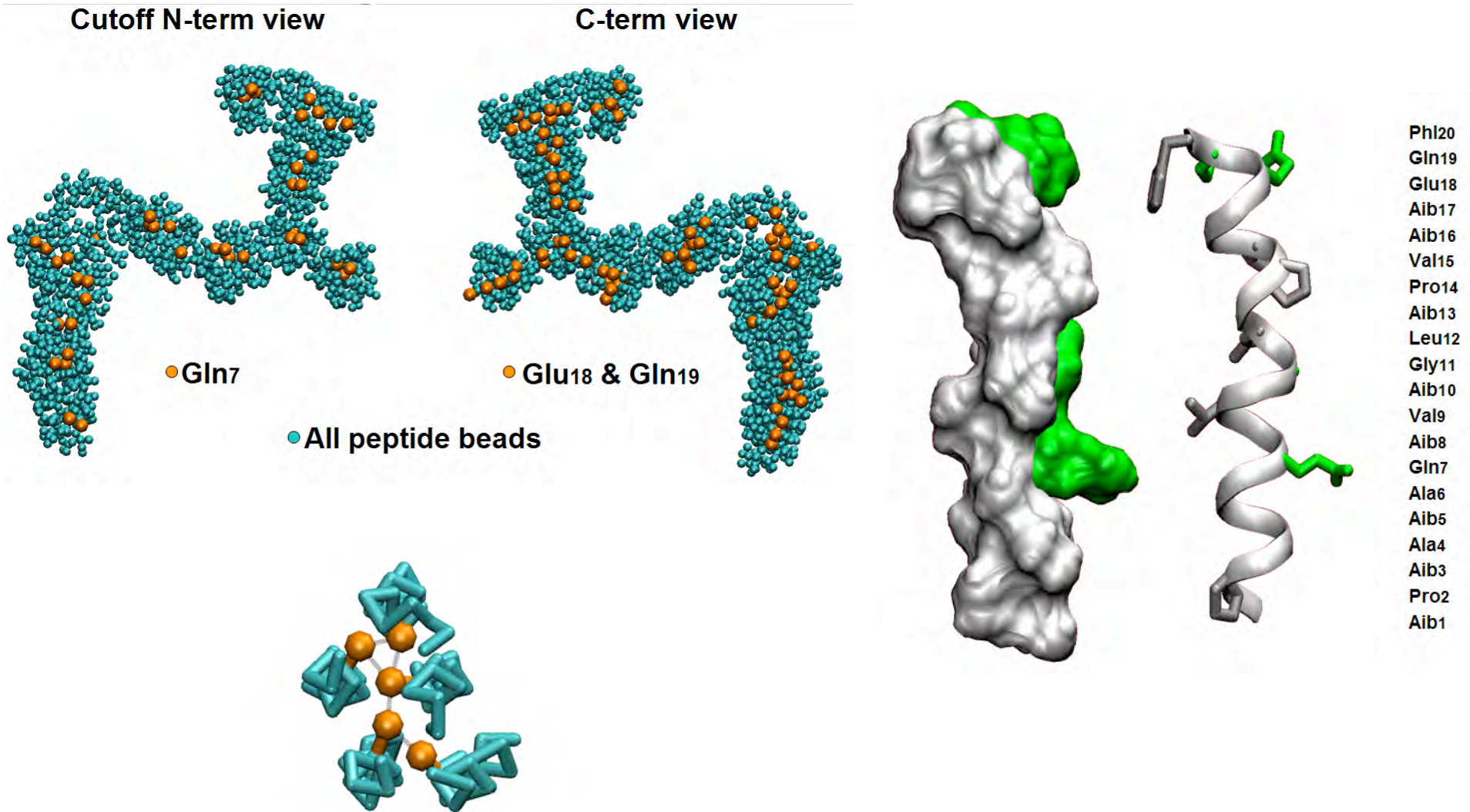
Employs CHARMM-like force fields
parameterized using all-atom simulations

Peptide Aggregation

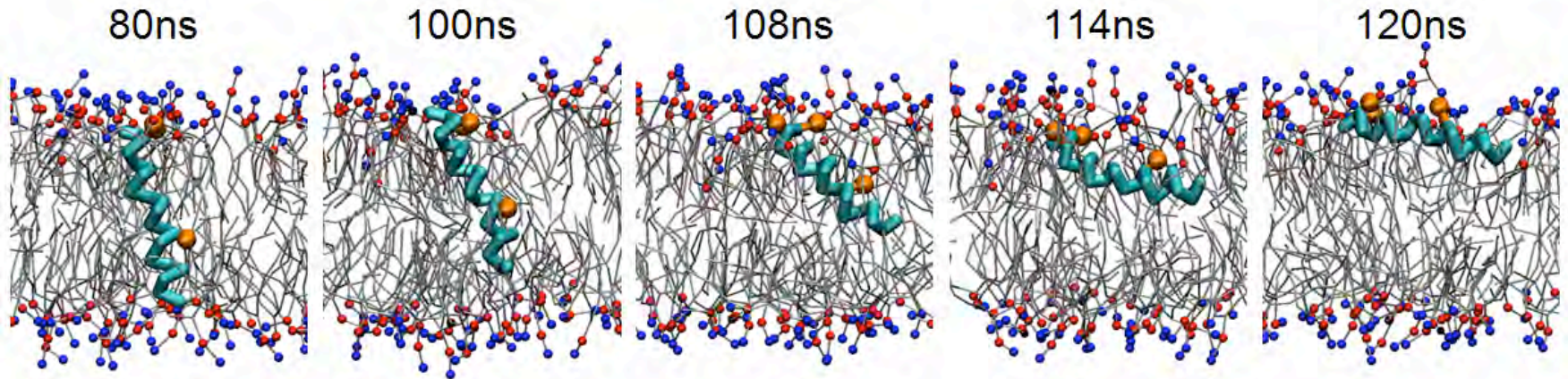




What brings the helices together?

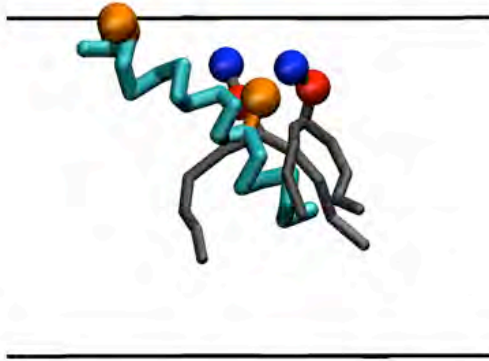


Peptide Insertion

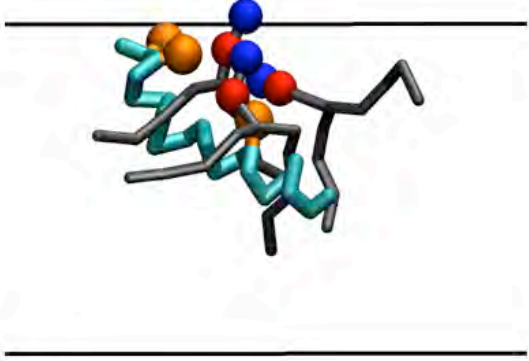


Strong perturbation of the bilayer structure

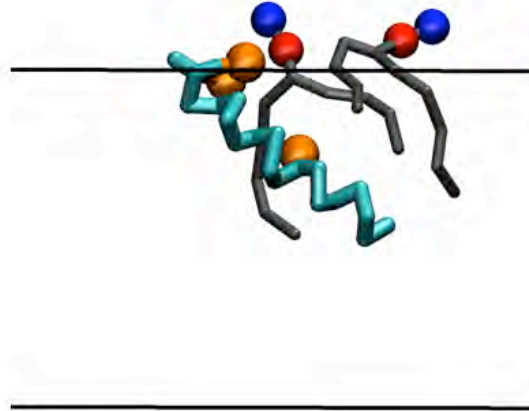
Pept1 108ns



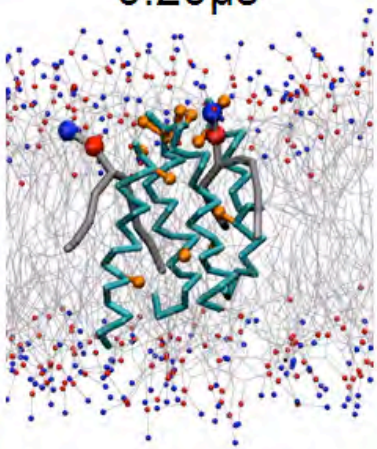
Pept2 146ns



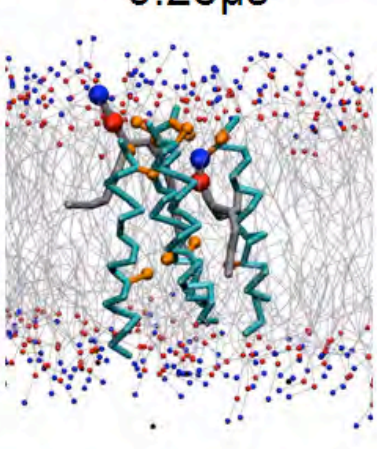
Pept3 927ns



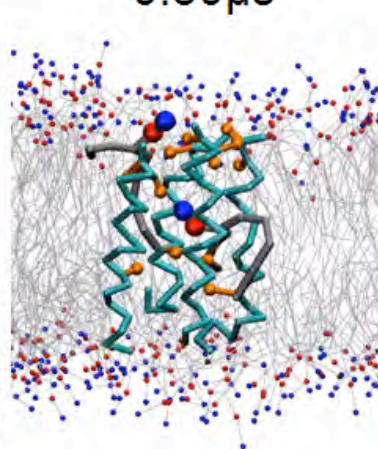
0.20 μ s



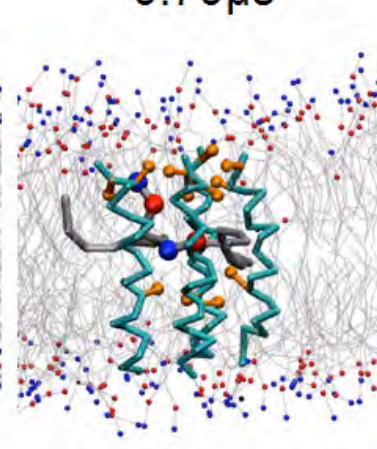
0.28 μ s



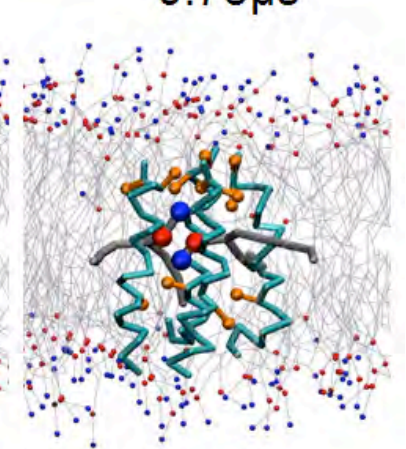
0.30 μ s



0.70 μ s

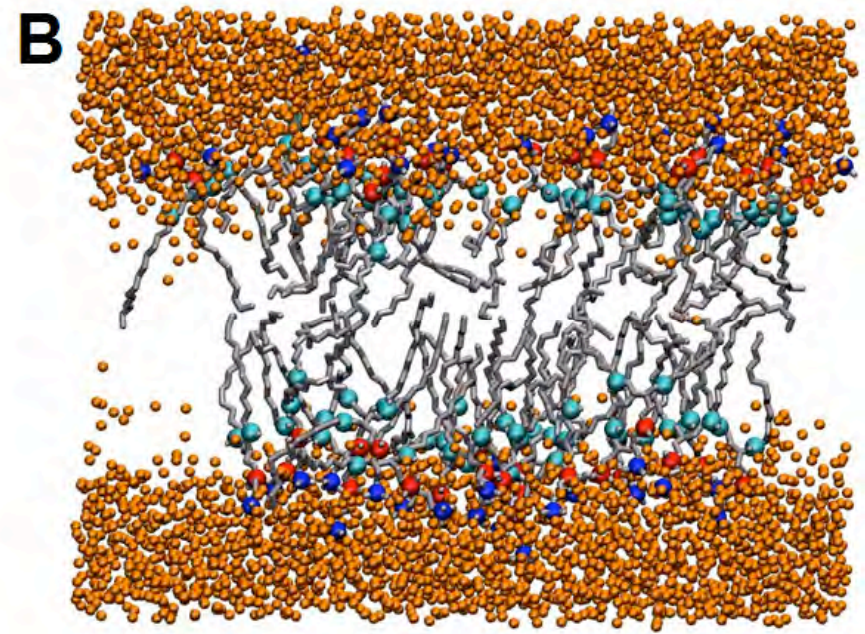
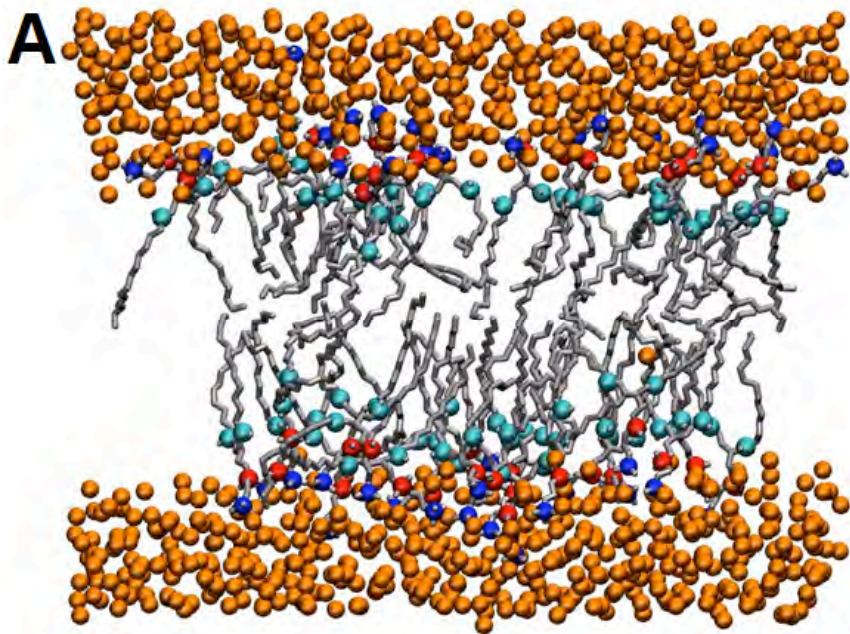


0.75 μ s

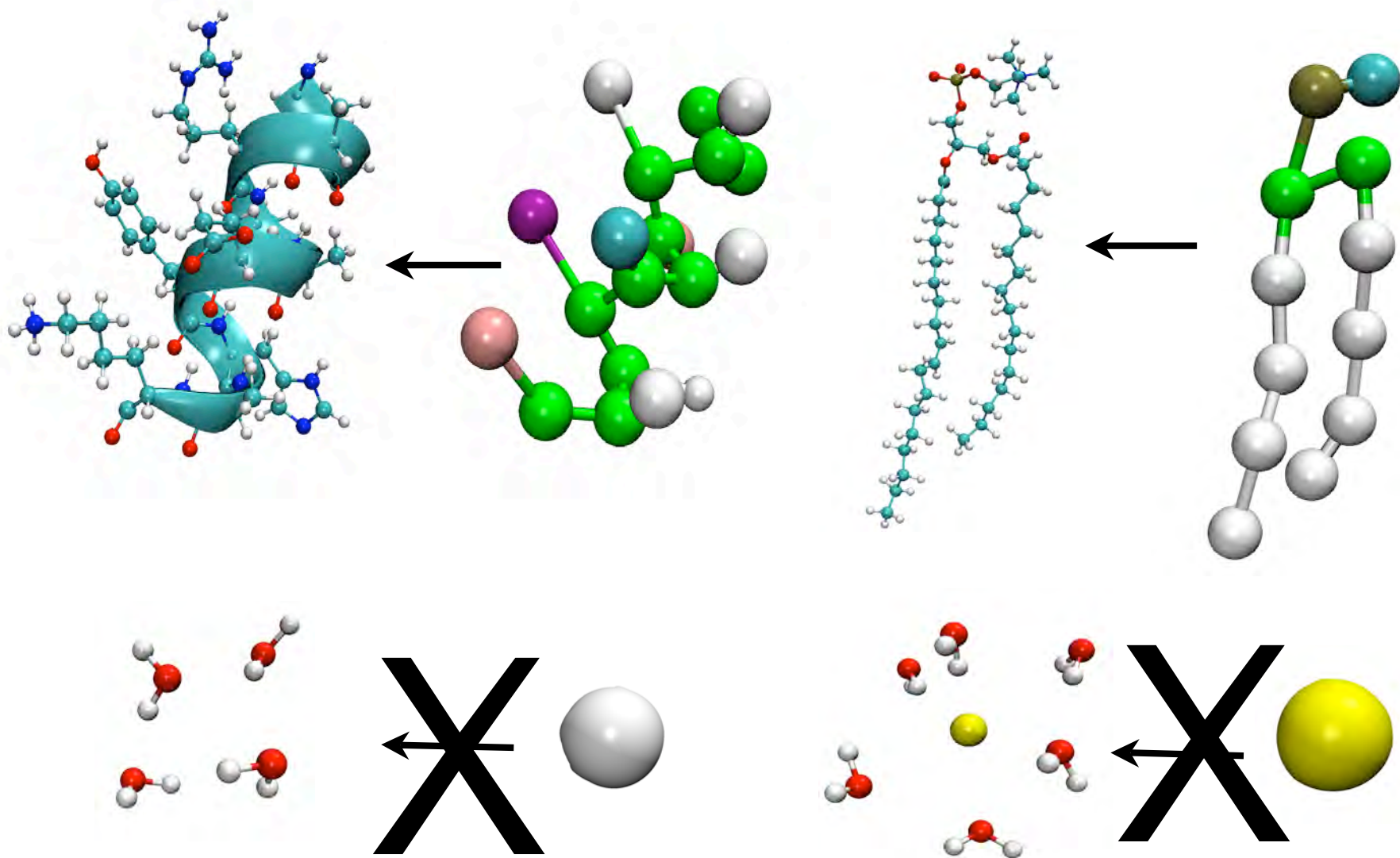


BUT no water permeation/pore formation!?

Hydration of the head group region in coarse-grained and all-atom models



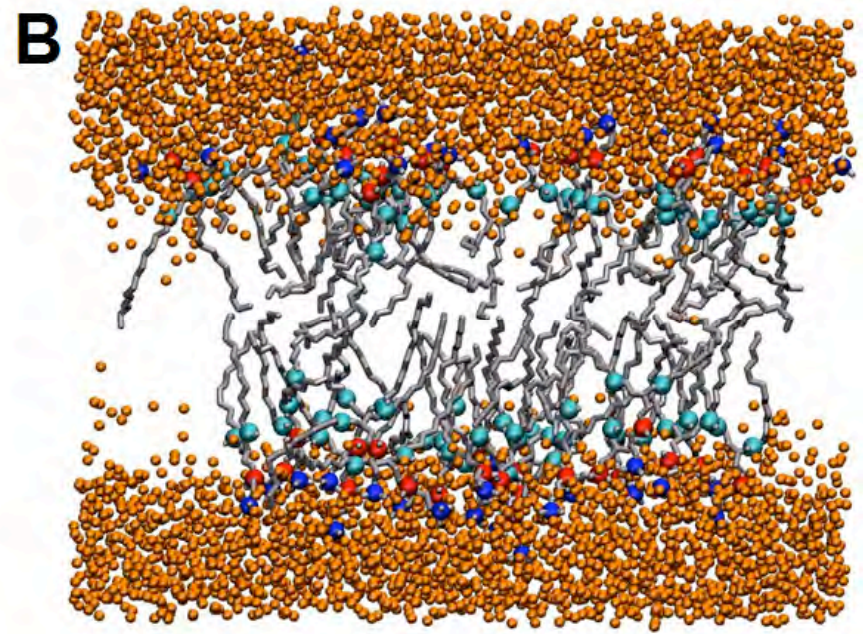
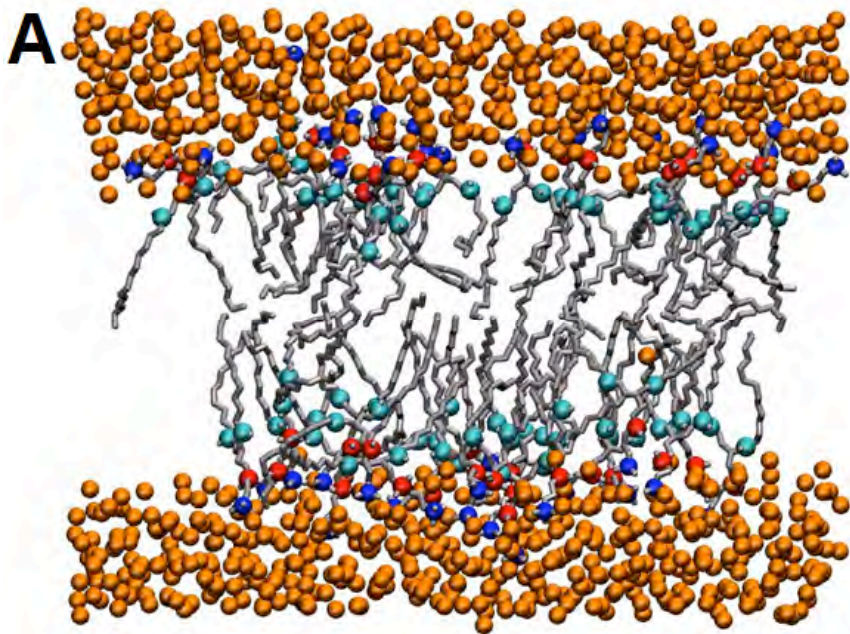
Reverse Coarse-Graining



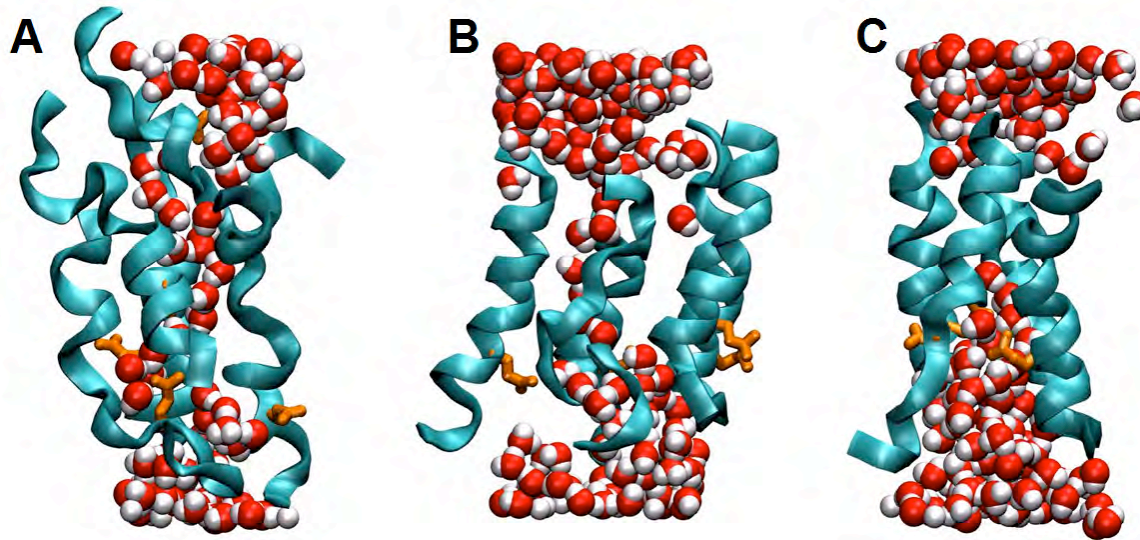
Reverse Coarse-Graining

- Mapping back CG beads to all-atom clusters
- *Re-solvating the system*
- 5000 steps of minimization
- Simulated annealing for 20 ps (T changing from 610K to 300K, $\Delta T = -10\text{K}$) while constraining atoms to the position of the corresponding CG beads

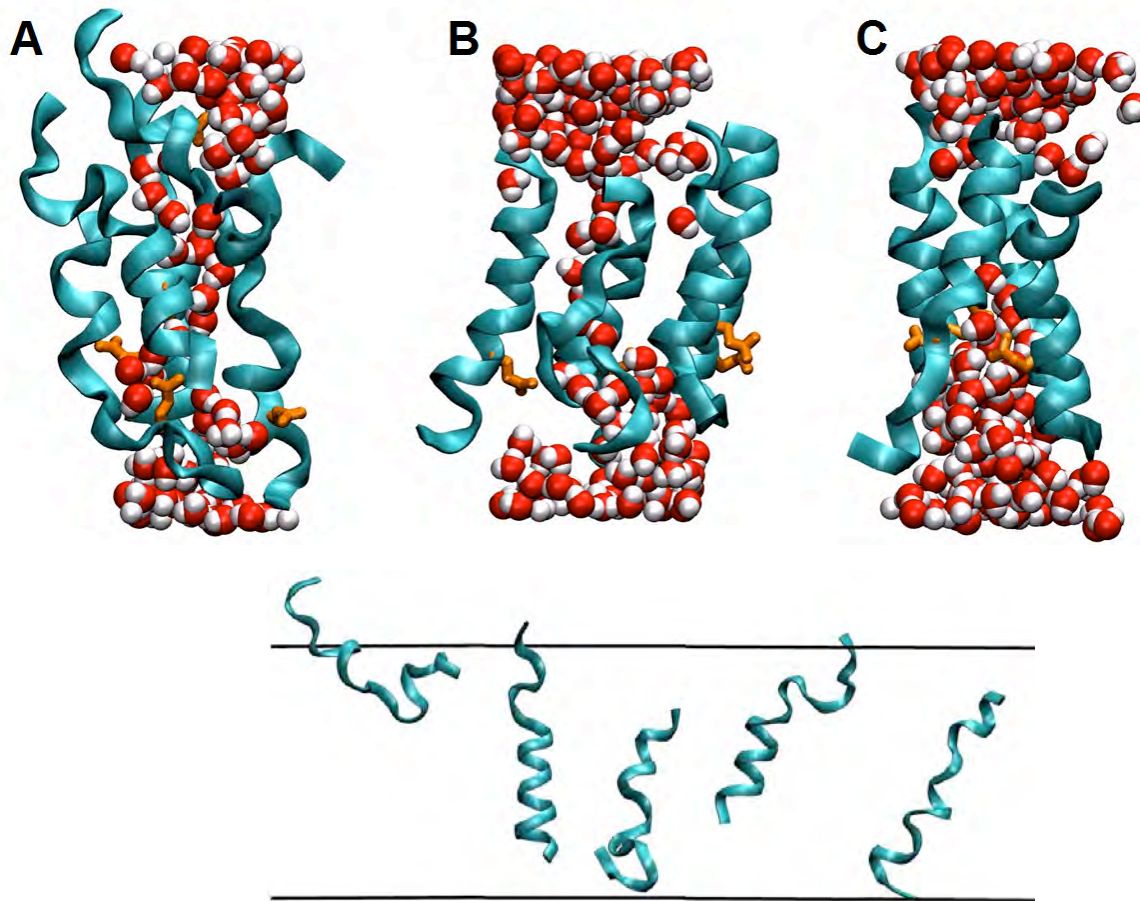
Hydration of the head group region in coarse-grained and all-atom models



Alemethicin-induced Membrane Poration

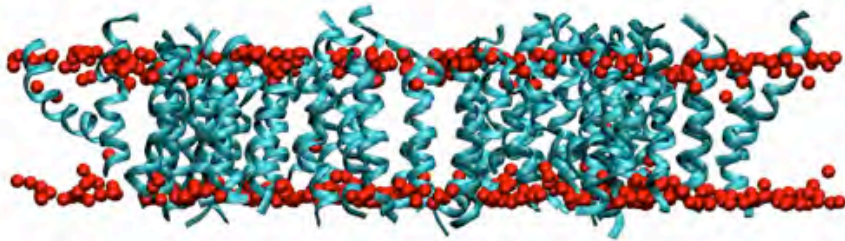


Alemethicin-induced Membrane Poration



Alemethicin-induced Membrane Poration

0ns



20ns

