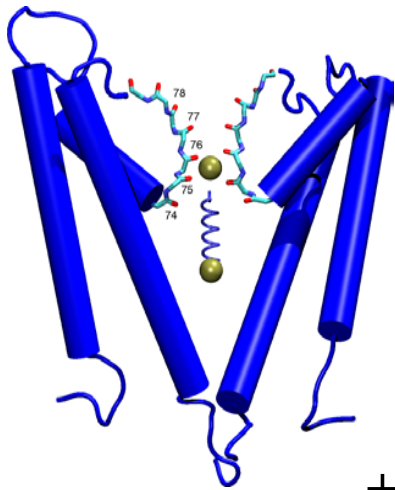
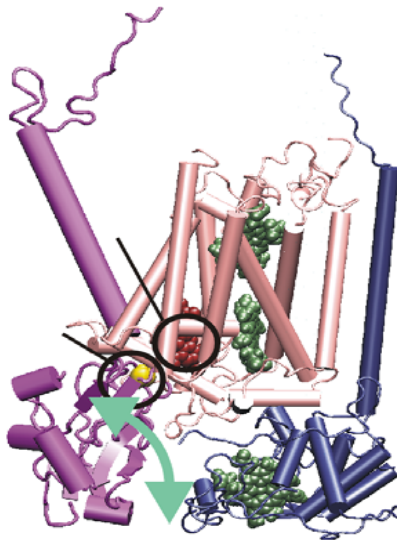


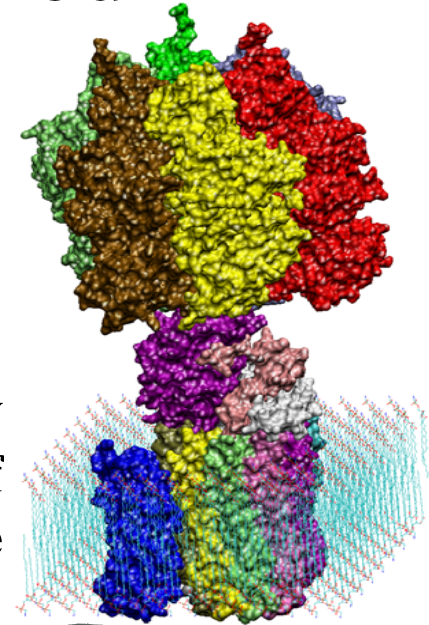
Accelerating Processes via Steered MD



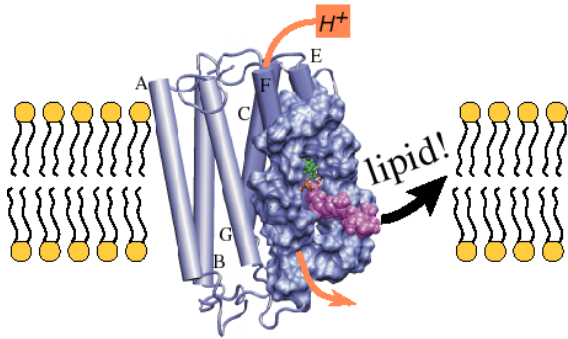
Conduction of K^+



Rotatory mechanism of bc1 complex



Rotatory mechanism of ATPase

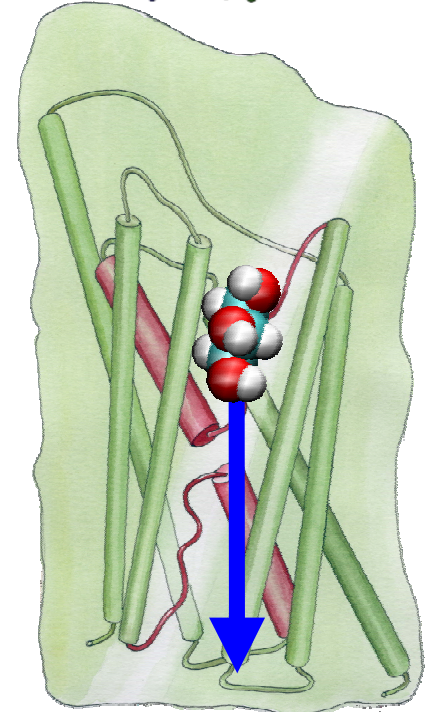


Binding pathway of retinal in bacteriorhodopsin

Gating of mechanosensitive channel

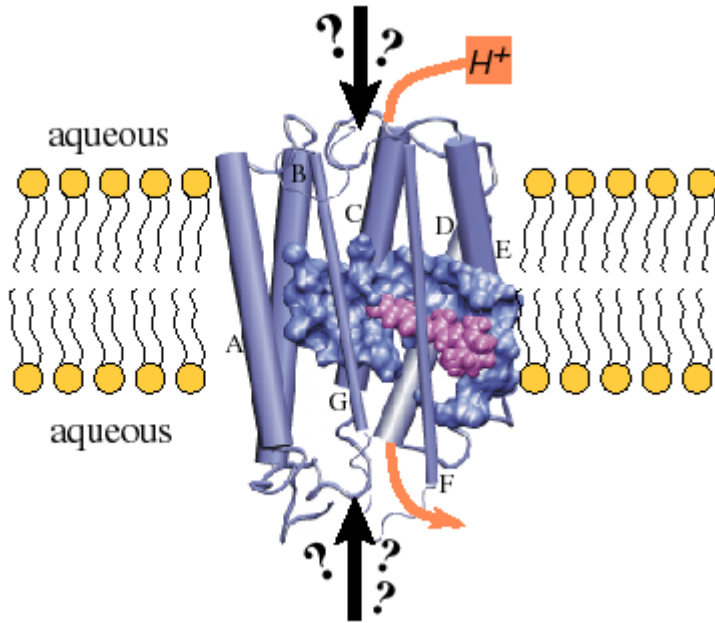


Pulling glycerol through a membrane channel



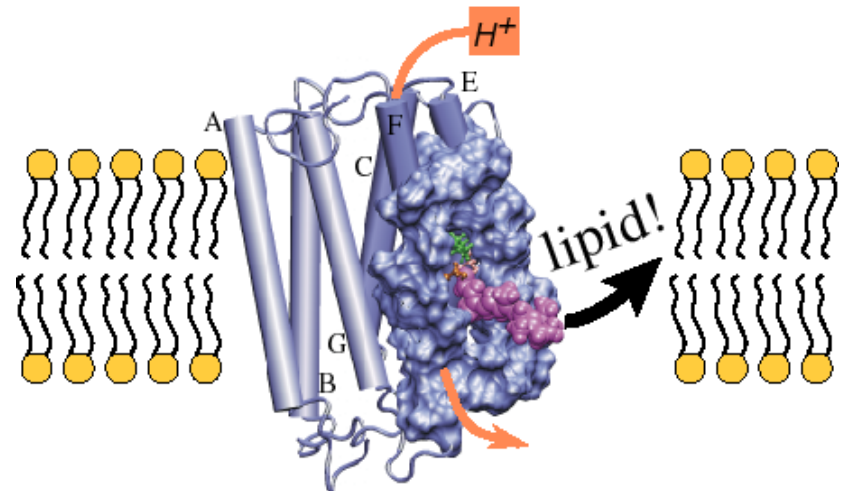
Interactive Modeling

Binding path of retinal to bacterio-opsin (1)

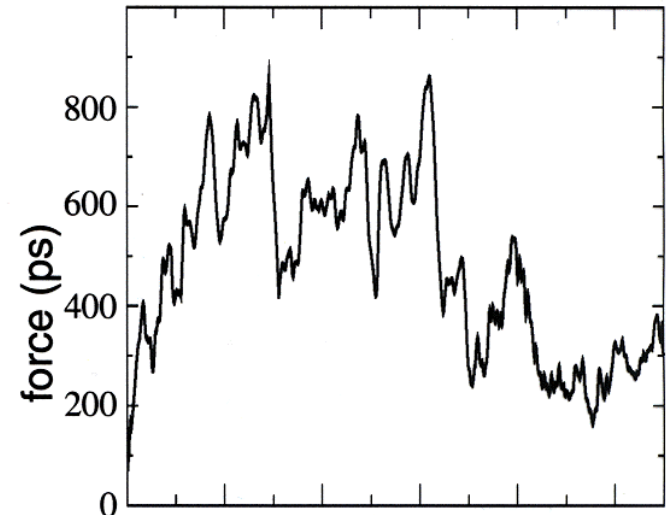
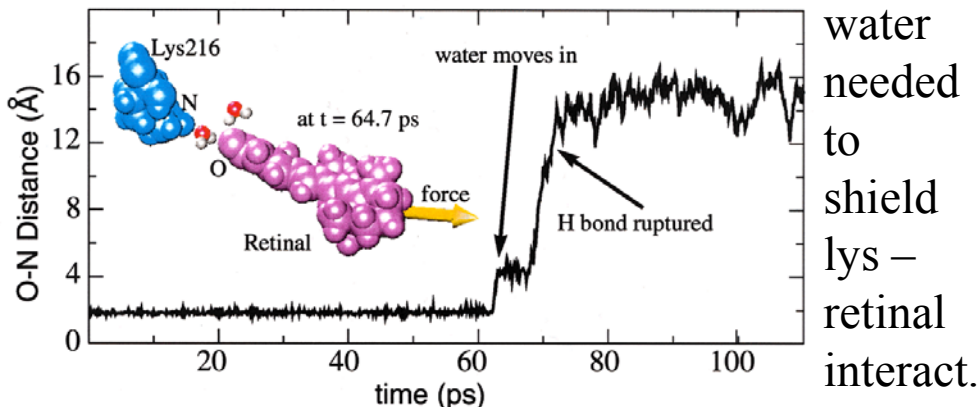


- Retinal deep in bacterio-opsin binding cleft
- How does it get in?
- Use batch mode interactive steered molecular dynamics to pull retinal out of cleft, find possible binding path

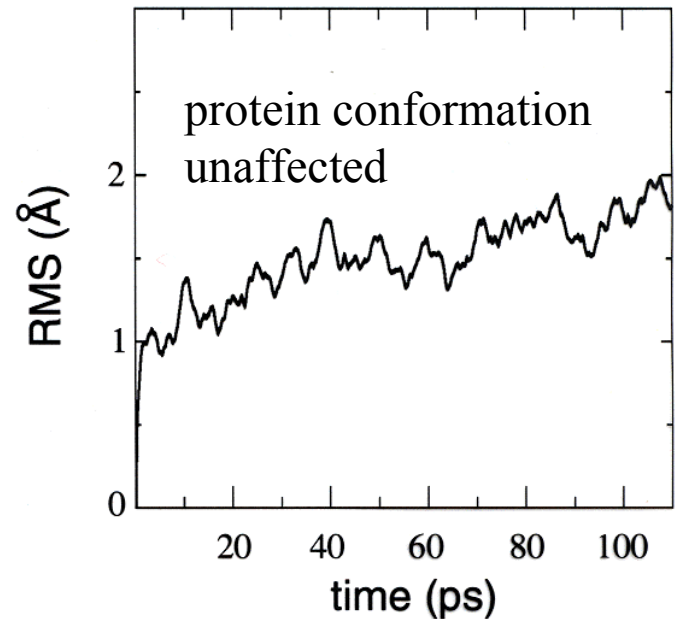
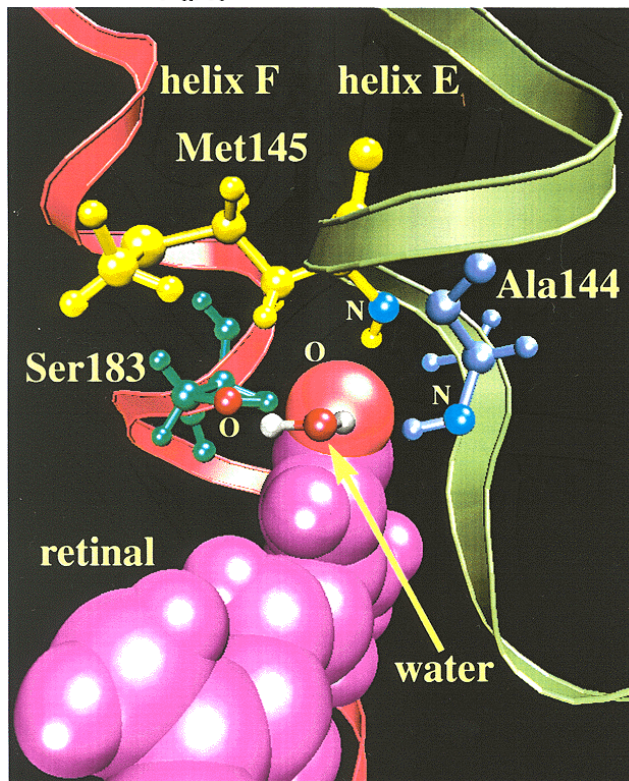
- 10 path segments, 3 attempts each
- Choose best attempt at 9 points during pull
- Found path through membrane, and electrostatically attractive entrance window



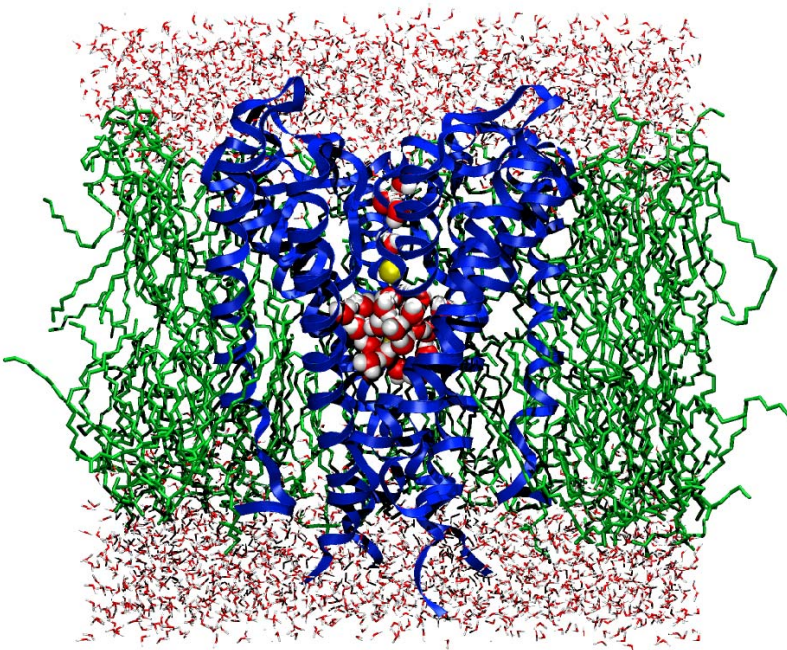
Stepwise Unbinding of Retinal from bR



Retinal's exit and entrance "door" attracts its aldehyde group



Steered Molecular Dynamics of the KcsA ion channel



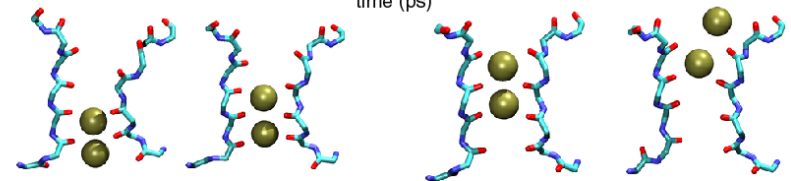
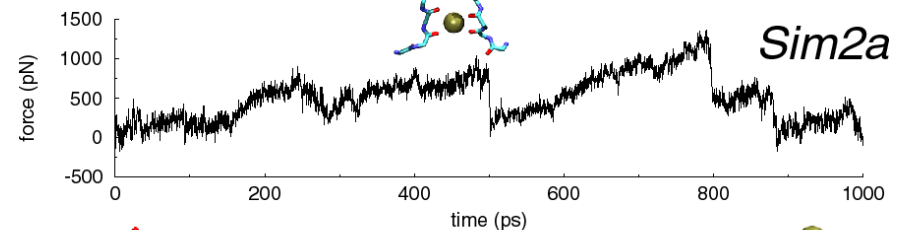
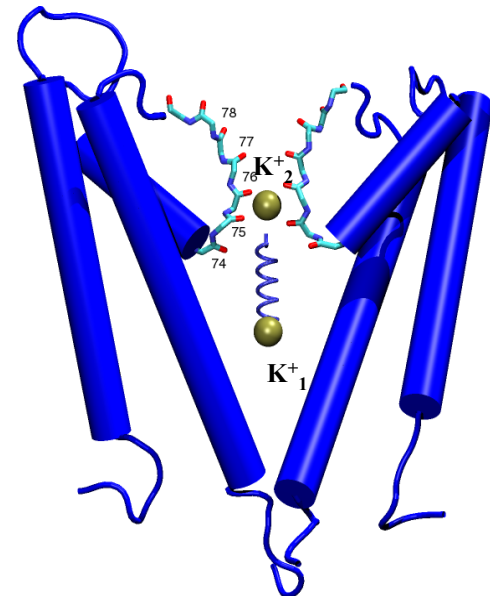
solvent

Kcsa channel protein (in blue) embedded in a (3:1) POPE/POPG lipid bilayer. Water molecules inside the channel are shown in vdW representation.

solvent

Summary of simulations:

- protein/membrane system contains 38,112 atoms, including 5117 water molecules, 100 POPE and 34 POPG lipids, plus K⁺ counterions;
- NAMD2, periodic boundary conditions, PME electrostatics;
- 1 ns equilibration, NpT
- 1 ns SMD, constant volume



Force on K⁺₁

Understanding Aquaporins

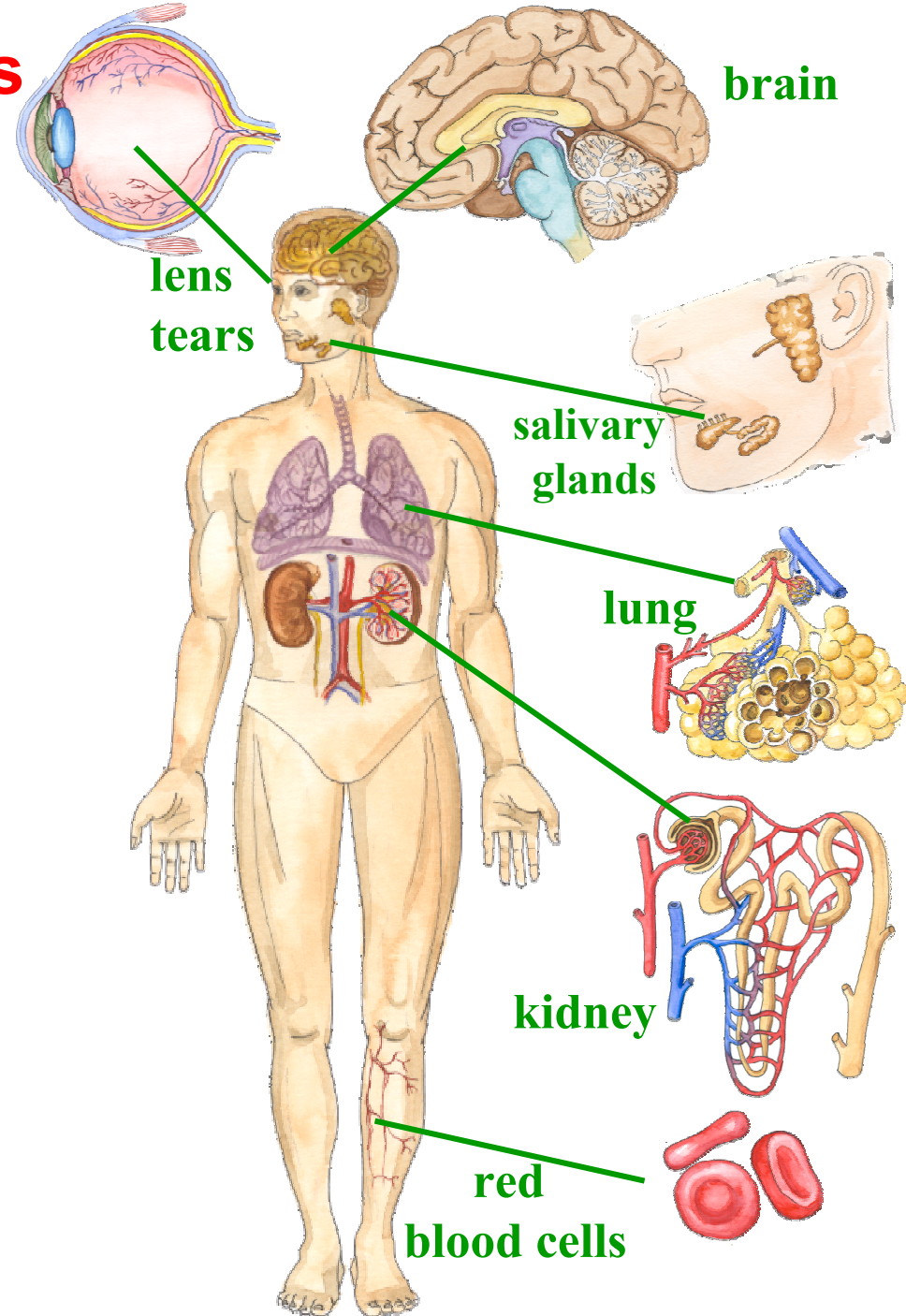
•**Global orientational tuning controls the selectivity of the aquaporin water channel family.** E. Tajkhorshid, P. Nollert, M. Ø. Jensen, L. J. W. Miercke, J. O'Connell, R. M. Stroud, and K. Schulten. *Science*. In press

•**Energetics of Glycerol Conduction through Aquaglyceroporin GlpF.** M. Ø. Jensen, S. Park, E. Tajkhorshid, and K. Schulten. *Proc. Natl. Acad. Sci., USA*. In press.

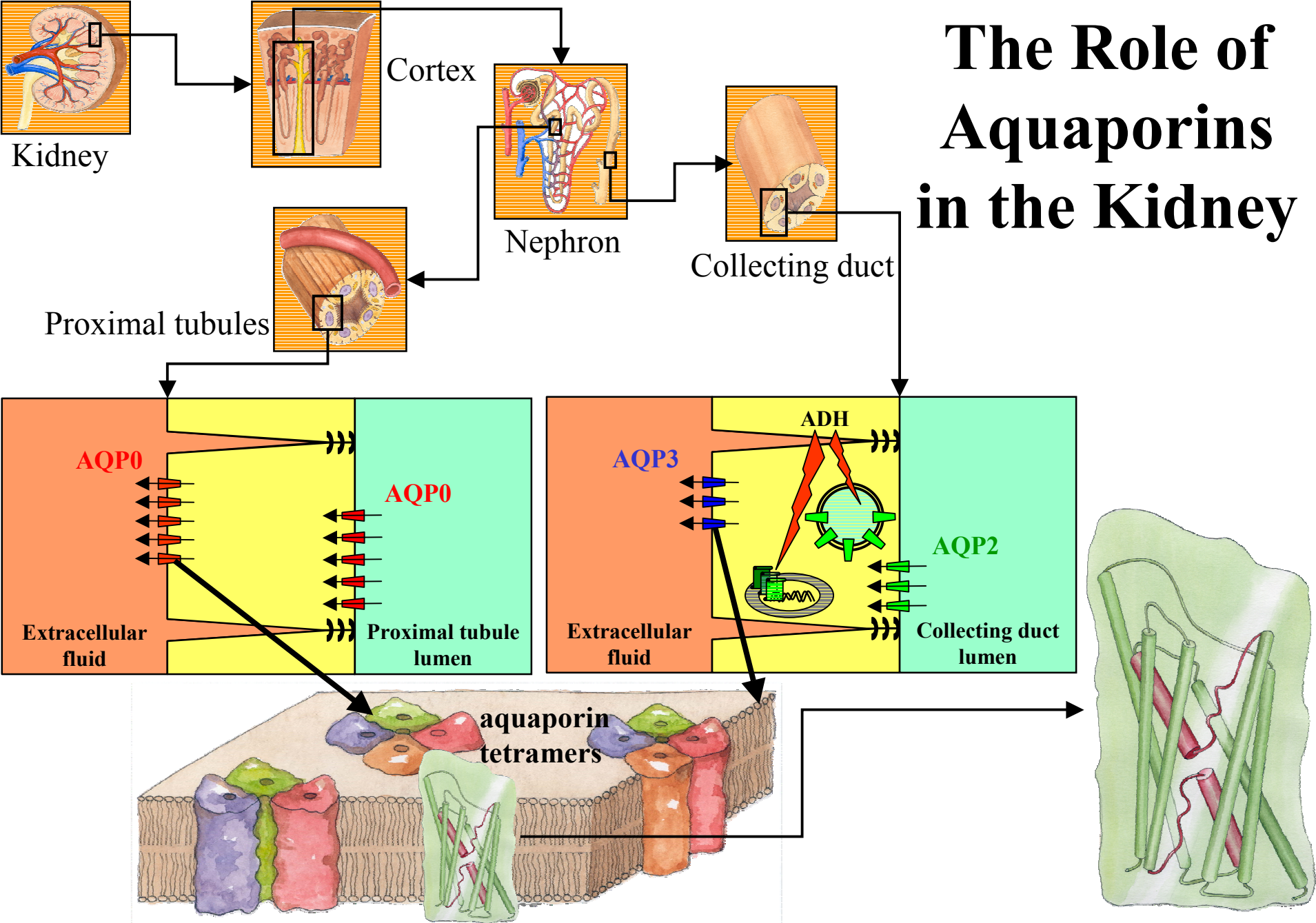
•**Pressure-induced water transport in membrane channels studied by molecular dynamics.** F. Zhu, E. Tajkhorshid, and K. Schulten. *Biophys. J.*, 2002. In press.

•**The mechanism of glycerol conduction in aquaglyceroporins.** M. Ø. Jensen, E. Tajkhorshid, and K. Schulten. *Structure*, 9:1083-1093, 2001.

•**Molecular dynamics study of aquaporin-1 water channel in a lipid bilayer.** F. Zhu, E. Tajkhorshid, and K. Schulten. *FEBS Lett.*, 504:212-218, 2001.

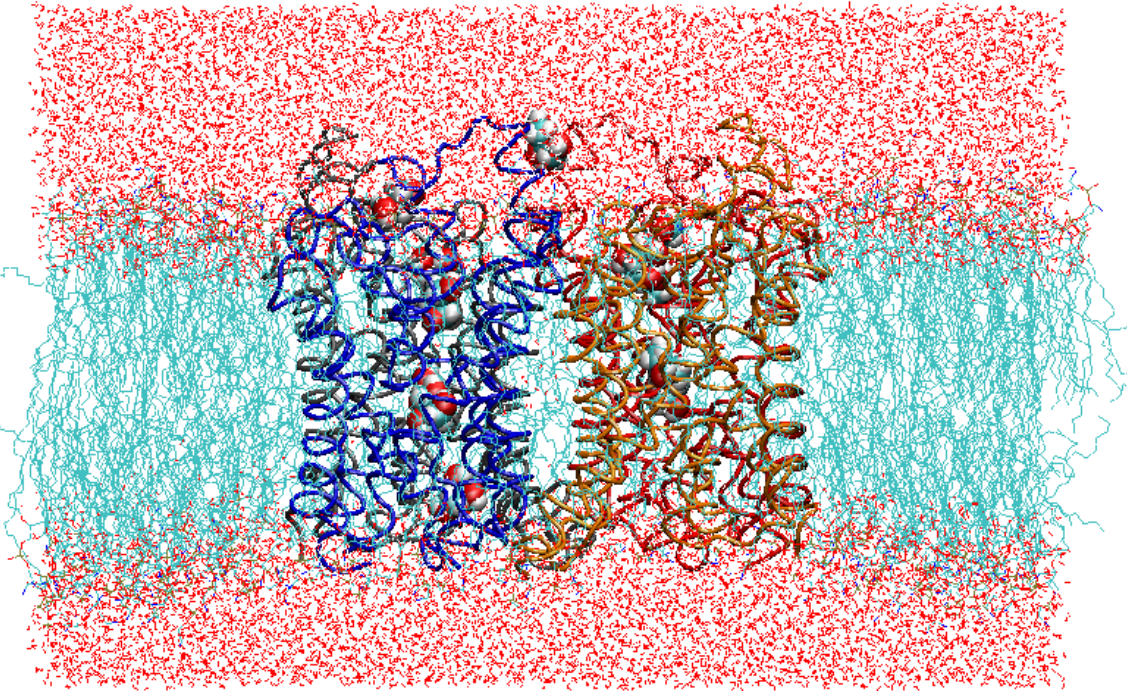


The Role of Aquaporins in the Kidney



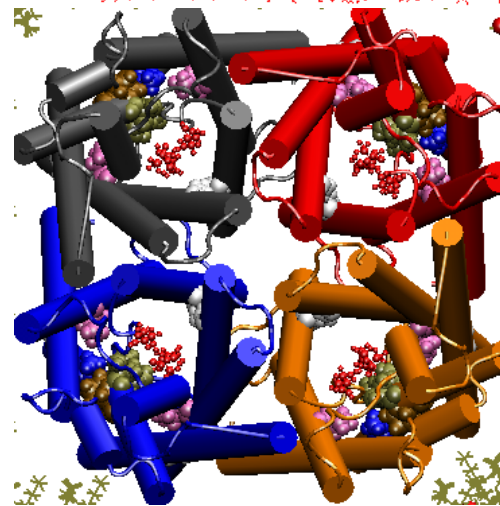
GlpF Simulation: Very Advanced and Large Scale

NAMD with full electrostatic (PME)
Periodic boundary conditions
NpT ensemble at 310 K
1ns equilibration



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

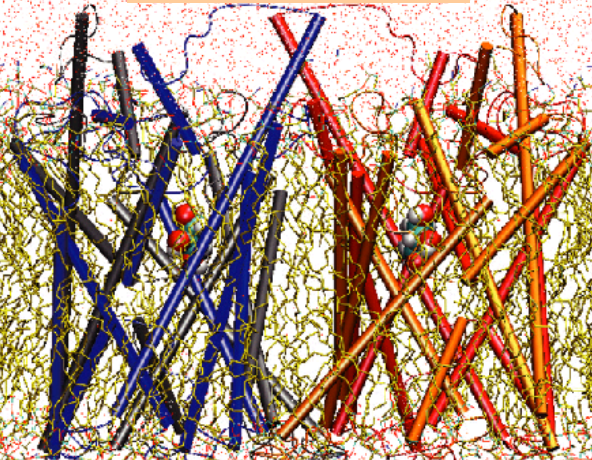
3.5 days / ns - 128 O2000 CPUs



GlpF
tetramer

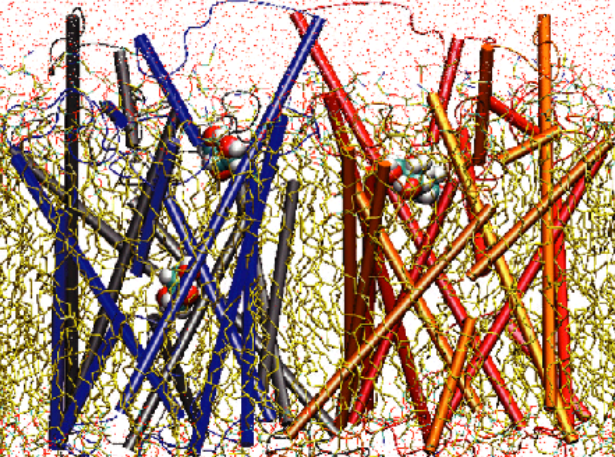
0.35 days / ns – 512 LeMieux CPUs

initial state

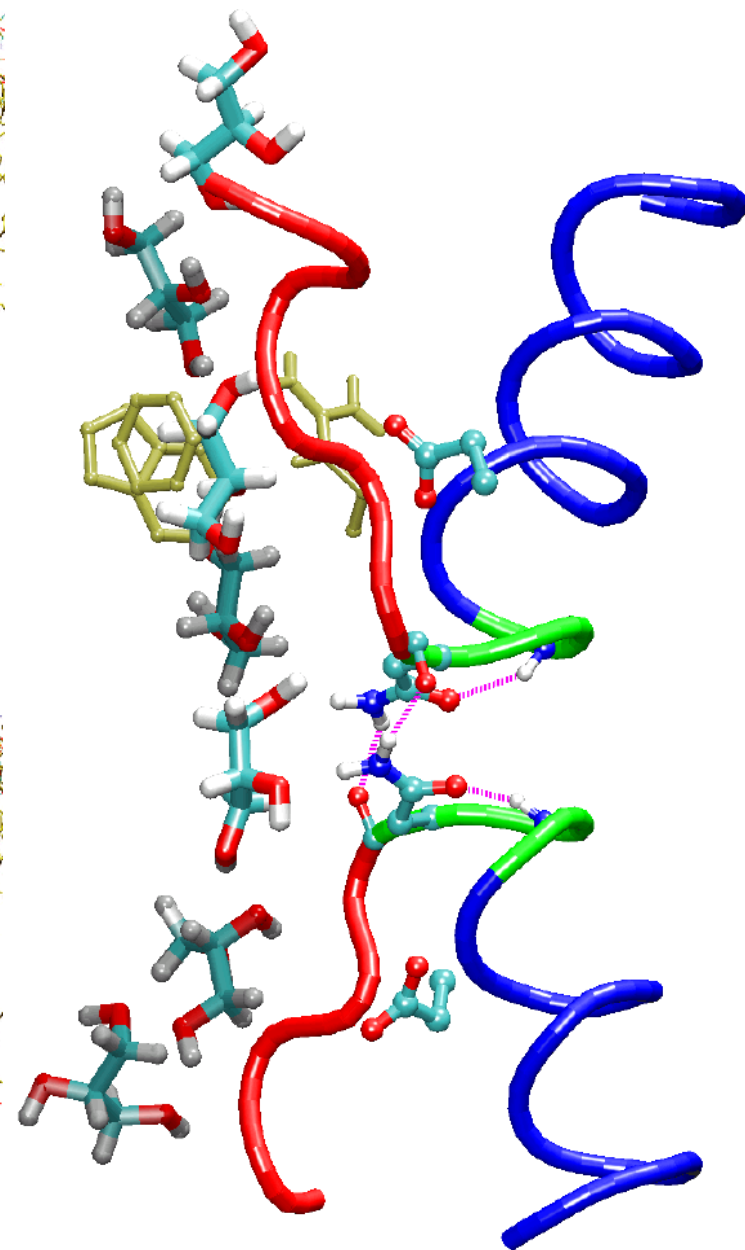


Note that glycerols moved, but not as extensively as earlier!

final (1ns) state



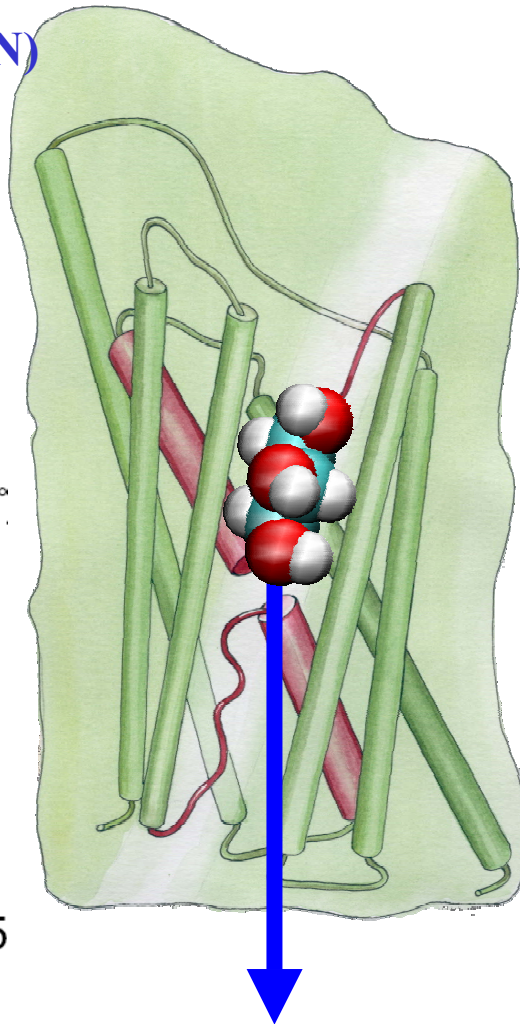
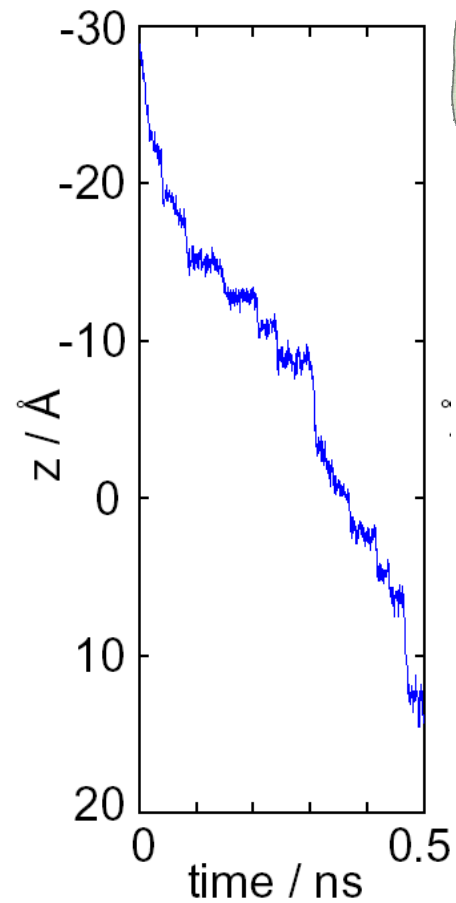
Glycerol Pathway



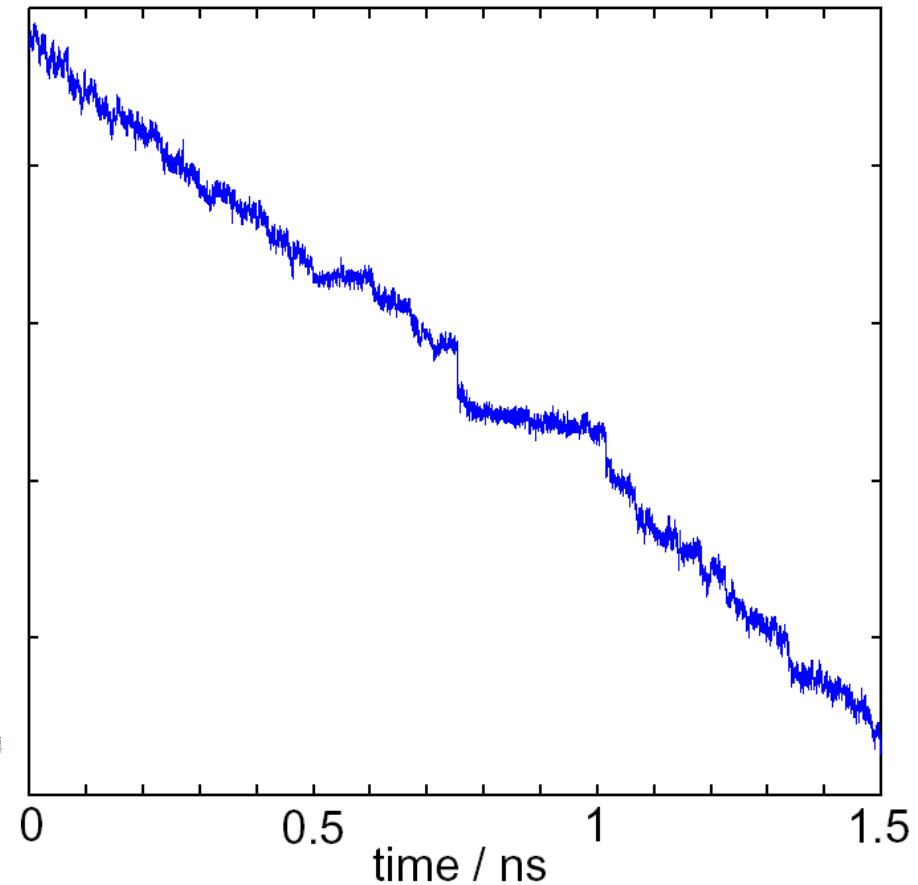
Steered Molecular Dynamics

Simulation of Glycerol Passage through GlpF

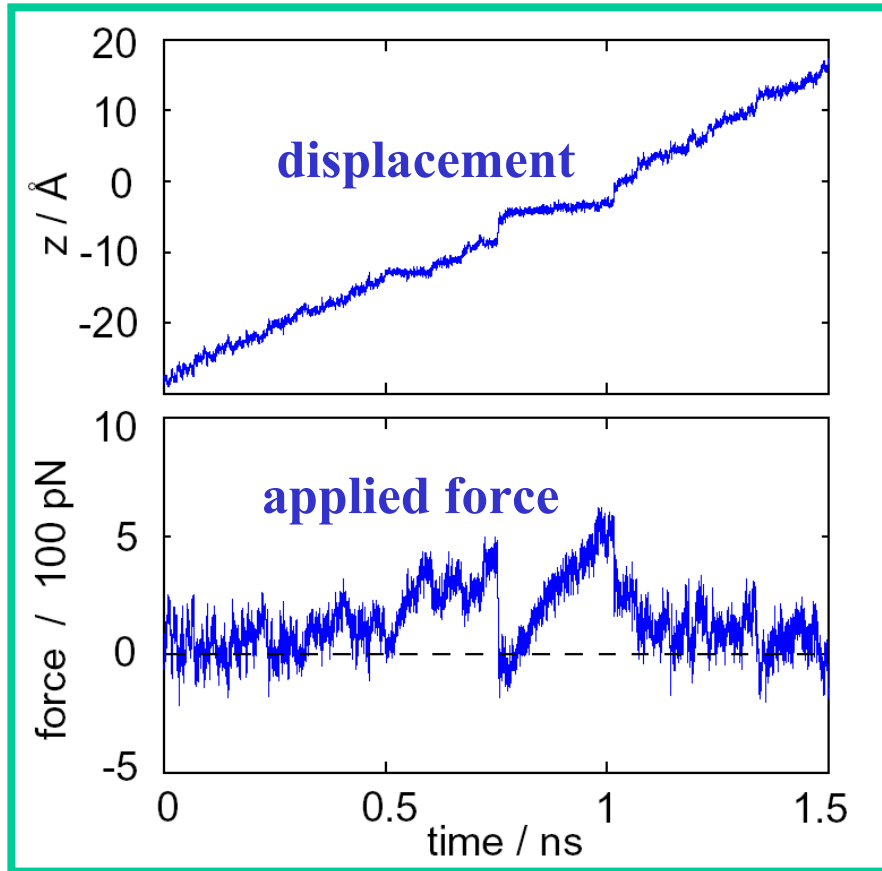
constant force (250 pN)



constant velocity (30 Å/ns)



Quantitative Analysis of SMD – Grand Challenge



time series analysis

Free energy

SMD simulation
is a **non-equilibrium** process.

a non-equilibrium process
(state $A \rightarrow$ state B)

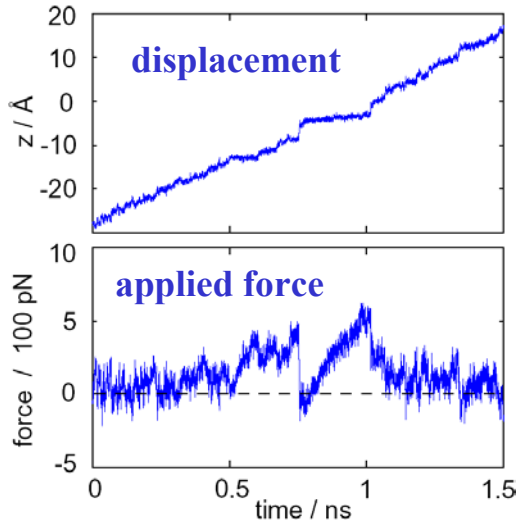
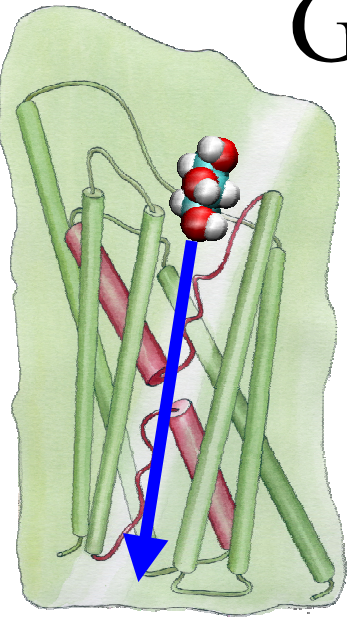
Thermodynamics: $\Delta G \leq \langle W \rangle$

Free energy difference ΔG
external work W

**One needs to discount
irreversible work !**

Jarzynski (1997): $e^{-\Delta G/k_B T} = \langle e^{-W/k_B T} \rangle$

Glycerol Passage Through GlpF

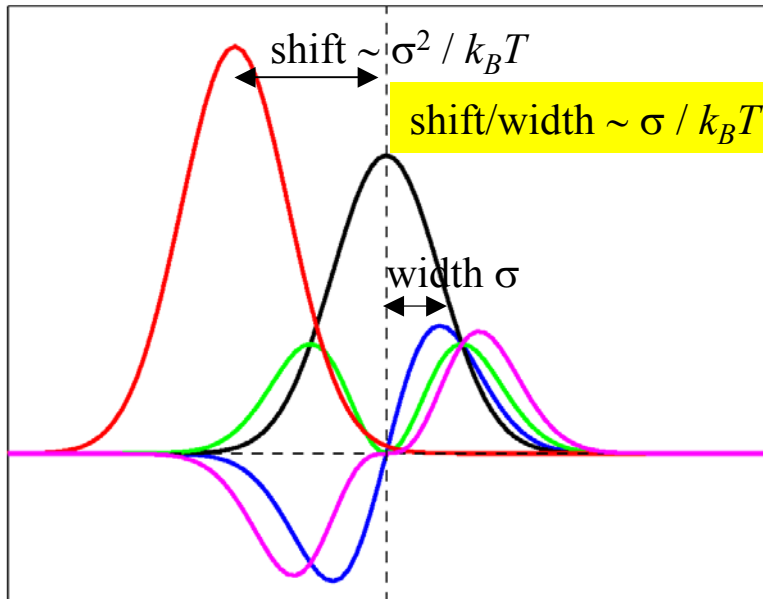


Thermodynamics: $\Delta G \leq \langle W \rangle$

Jarzynski (1997): $e^{-\Delta G/k_B T} = \langle e^{-W/k_B T} \rangle$

cumulant expansion:

$$\Delta G = \langle W \rangle - \frac{1}{2k_B T} \left(\langle W^2 \rangle - \langle W \rangle^2 \right) + \Lambda$$



$$p(W)$$

$$e^{-W/k_B T} \times p(W)$$

$$W \times p(W)$$

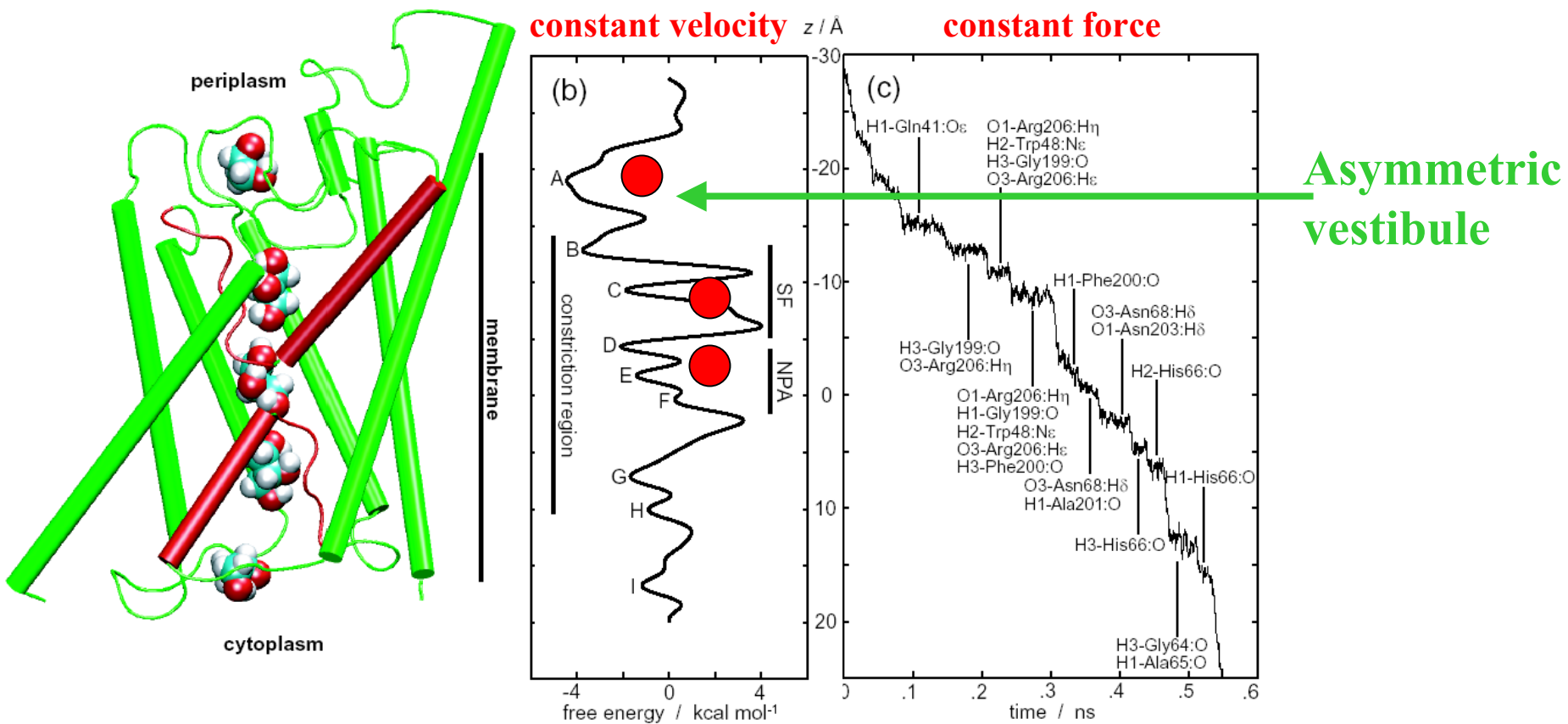
$$W^2 \times p(W)$$

$$W^3 \times p(W)$$

in case of strong non-equilibrium
 $\Rightarrow \sigma \gg k_B T$

Higher order moments are more difficult to estimate.

Features of the Potential of Mean Force



- The largest free energy barrier \approx **7.3 kcal/mol**
cf. Arrhenius activation energy measured:
9.6 \pm 1.5 kcal/mol, Borgnia and Agre (2001)

● crystallographic glycerols

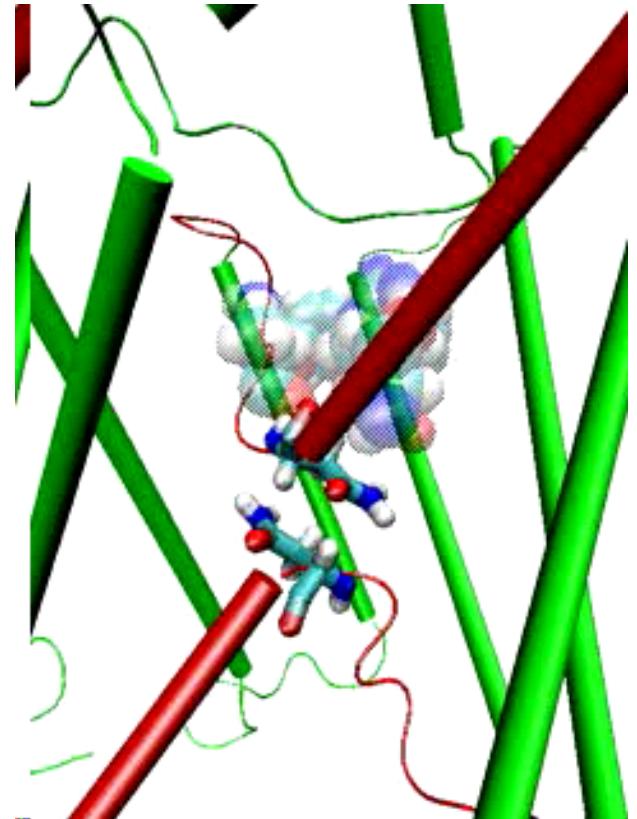
Interactive Molecular Dynamics

VMD ←-----→ **NAMD**

- Any PC/Workstation
- Supports 3D force-feedback devices for interaction

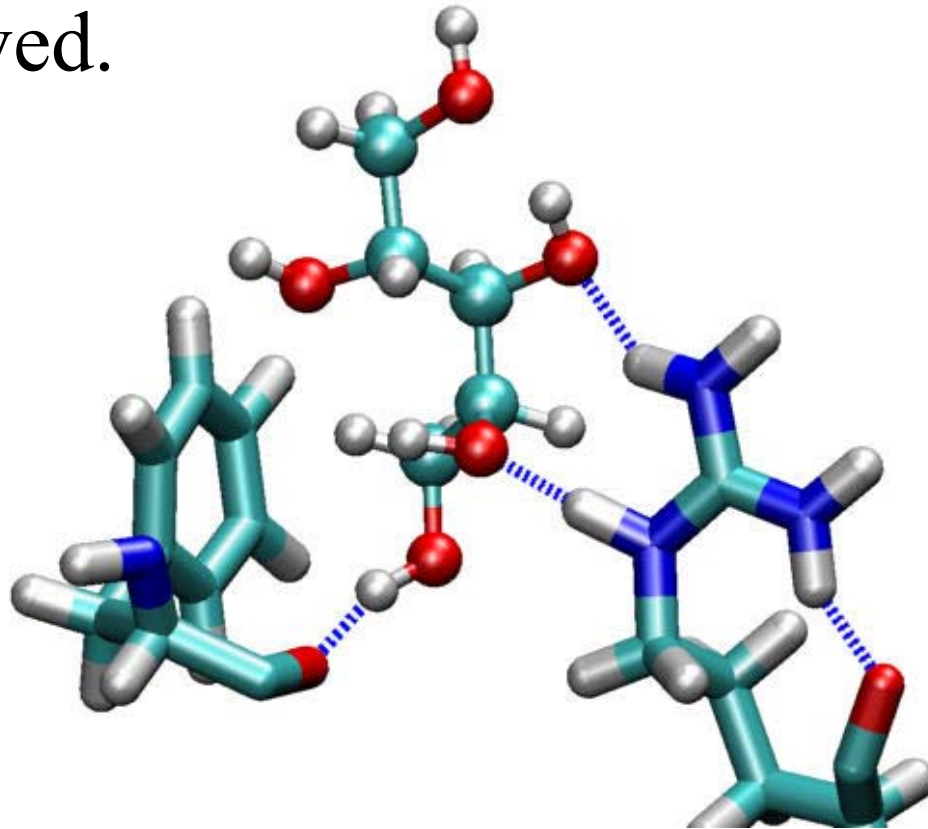


John Stone, Justin Gullingsrud, Klaus Schulten, and Paul Grayson. *A System for Interactive Molecular Dynamics Simulation*. 2001 ACM Symposium on Interactive 3D Graphics, pp.191-194, ACM SIGGRAPH



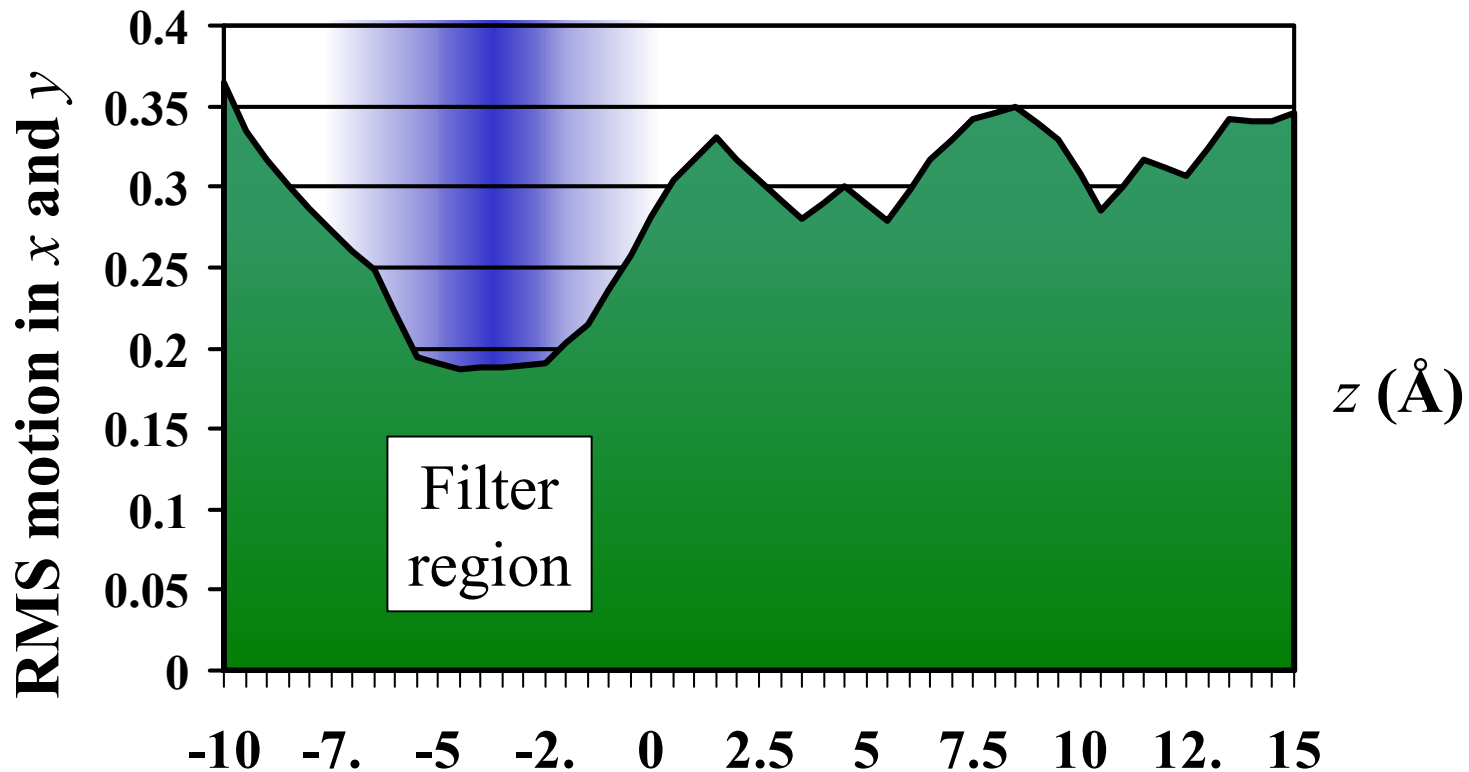
Observed Binding in Filter

- Selection by concerted H-bonding
- Locking mechanism of Fu, Stroud, et al. observed.



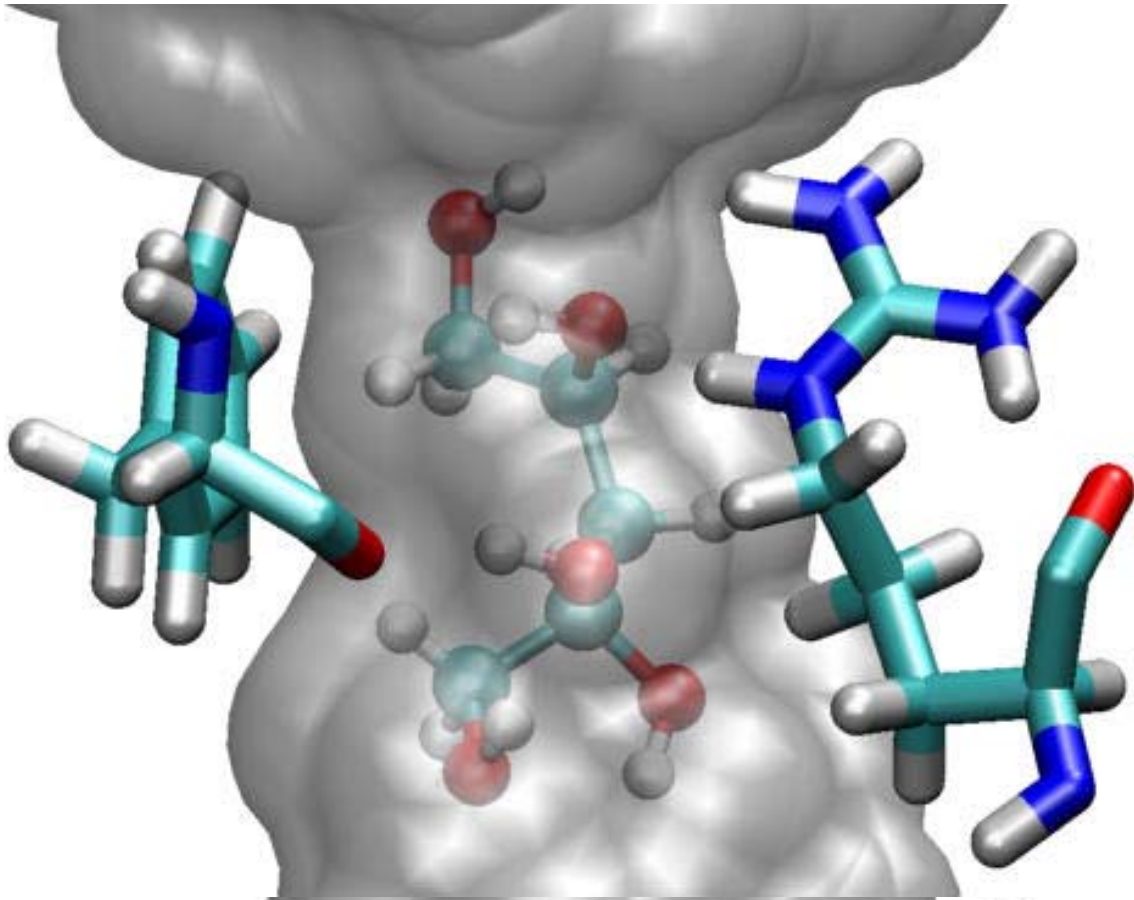
Confinement in Filter

- Selection occurs in most constrained region.
- Caused by the locking mechanism.



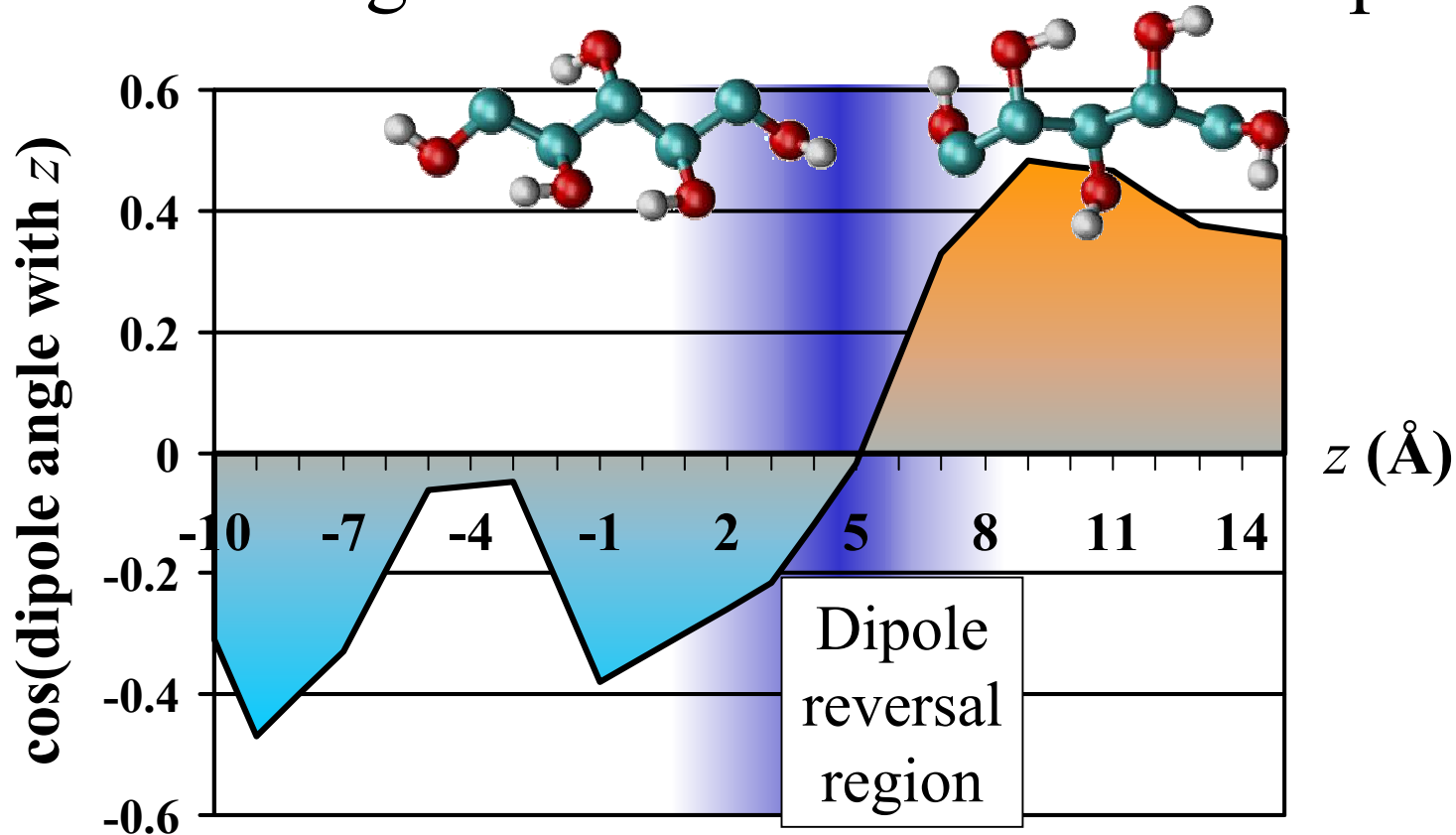
Observed Induced Fit in Filter

- Channel expands to select sugar.



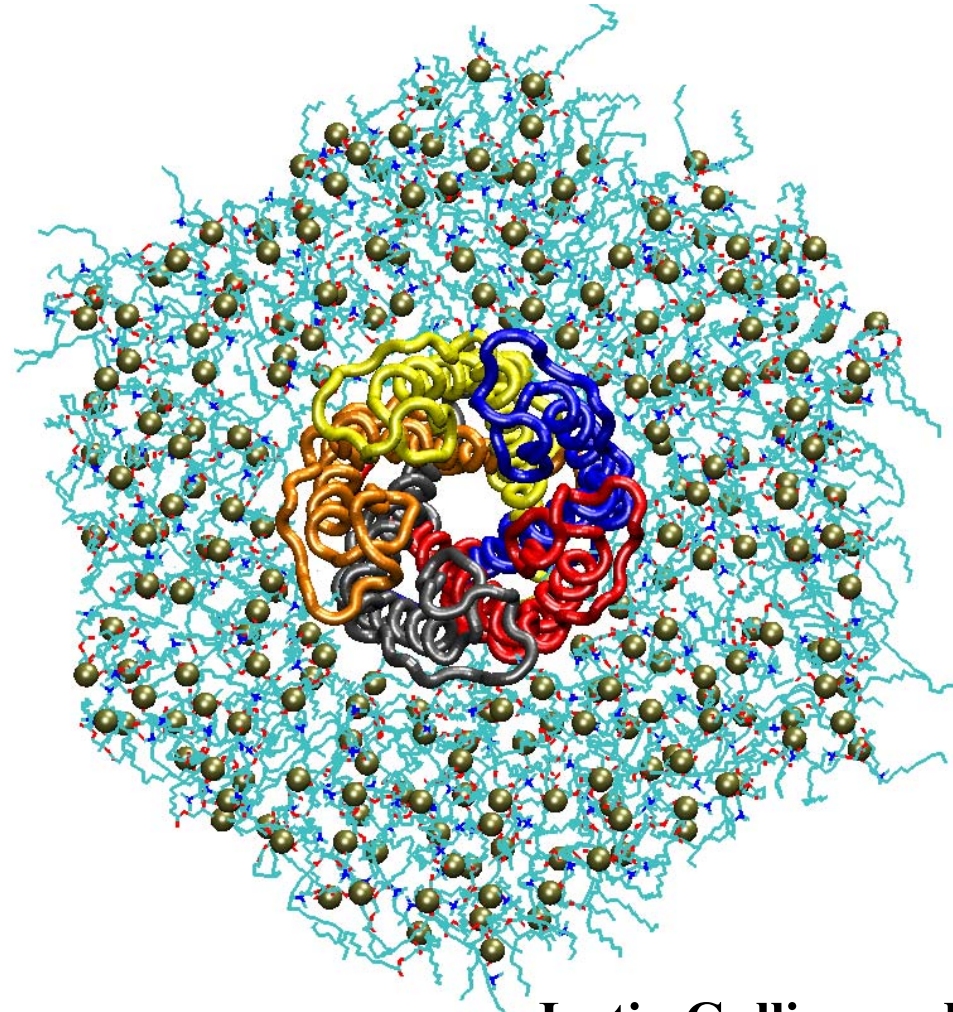
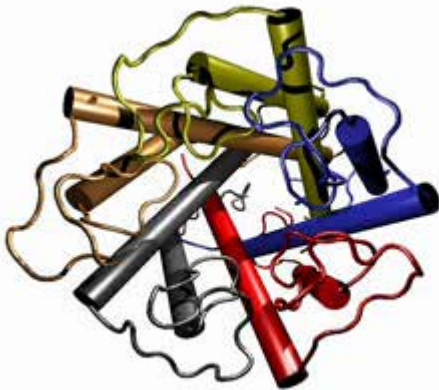
Dipole Reversal in Channel

- Dipole reversal pattern matches water.
- Selects large molecules with flexible dipole.



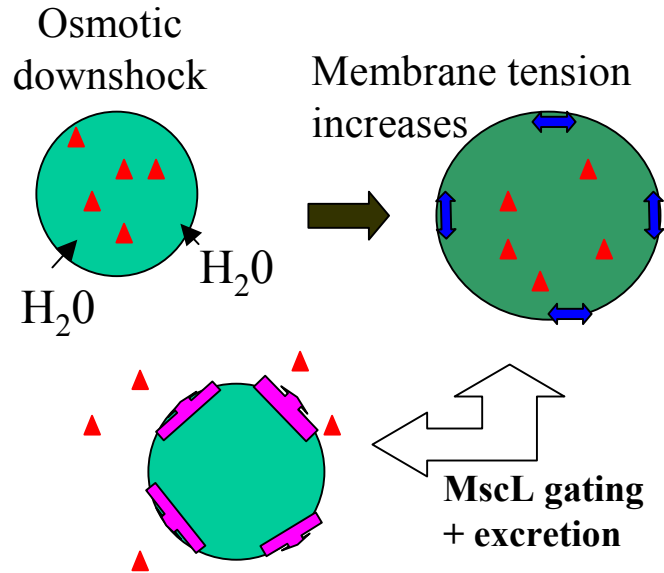
Gating Mechanism of a Mechanosensitive Channel

- Inserted MscL protein from crystal structure into equilibrated POPC membrane – 242 lipids, 16,148 water molecules, **88,097 atoms**
- Program NAMD, periodic boundary conditions, full electrostatics (PME), NpT ensemble, anisotropic pressure to describe surface tension, **2.4 days on 128 T3E CPUs**



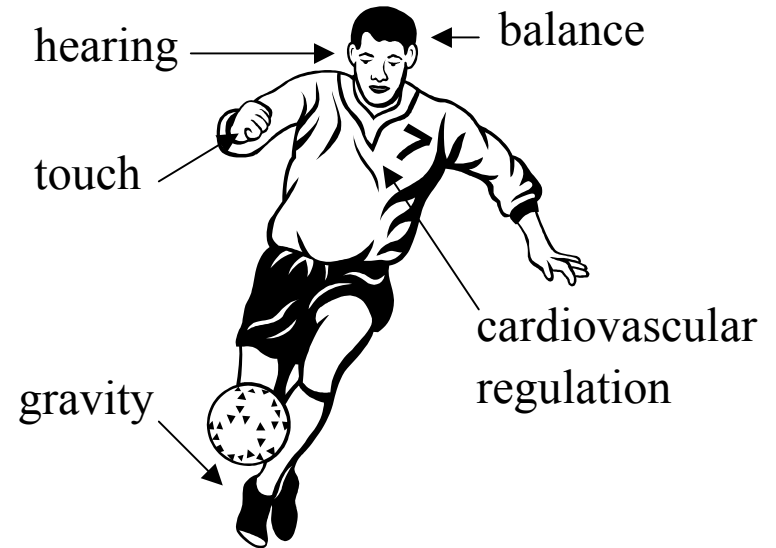
Ubiquitous Mechanosensitive Channels

MscL is a bacterial safety valve



Bacterial MscL is functional in reconstituted lipid bilayers (Sukharev et al., 1994).

Roles in Higher Organisms

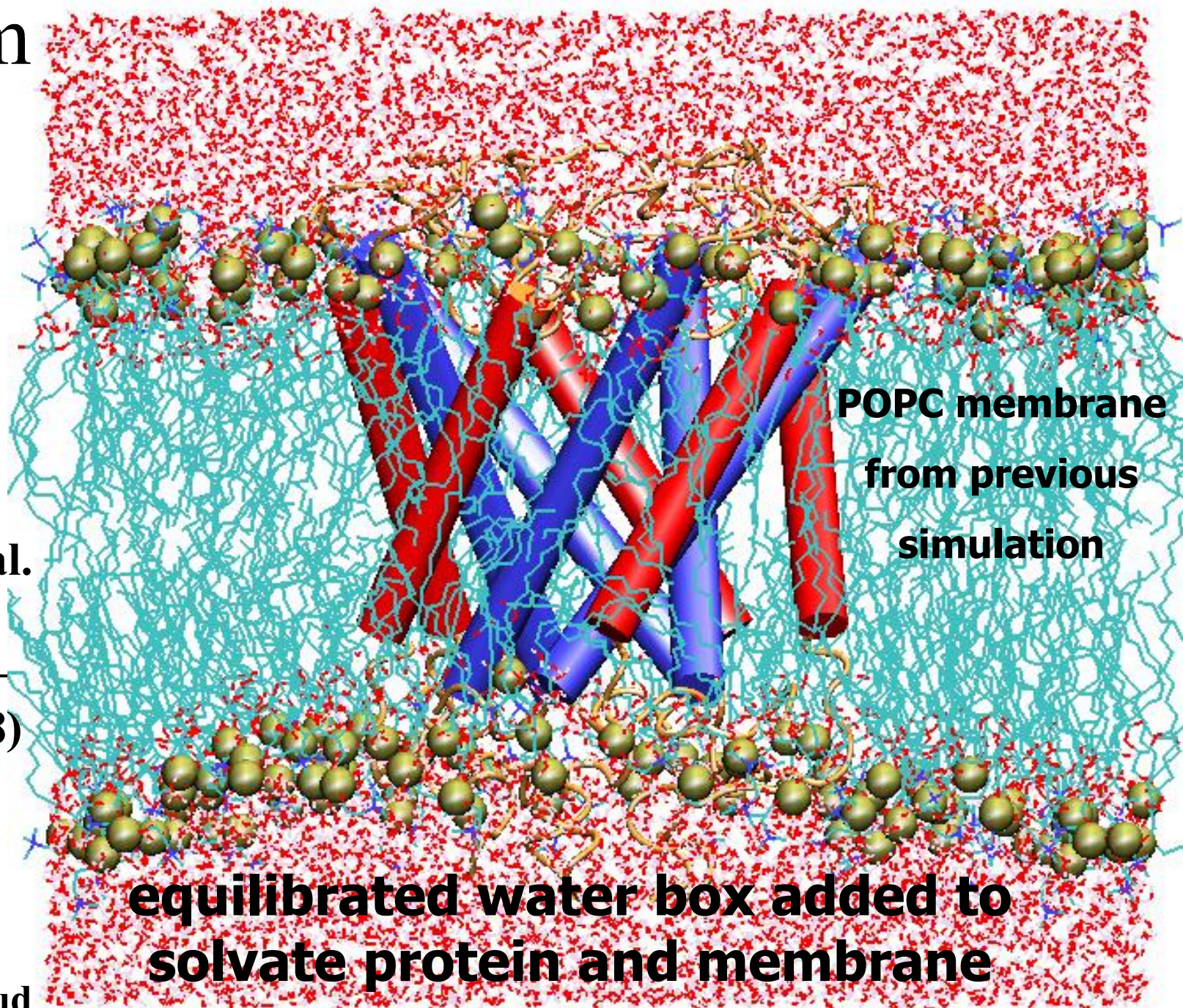


Most eukaryotic MS channels require coupling to the cytoskeleton and/or the extracellular matrix (Sachs and Morris, 1998).

- Mammals: TRAAK (Maingret, JBC 274, 1999).
- *Haloferax volcanii*, a halophilic archaeon.
- Prokaryotes: MscL in *E. coli*, *Mycobacterium tuberculosis*, many others.
- Eukaryotes: Mid1 gene in yeast (Kanzaki et al, Science (1999), **285**, 882-886).

System Setup

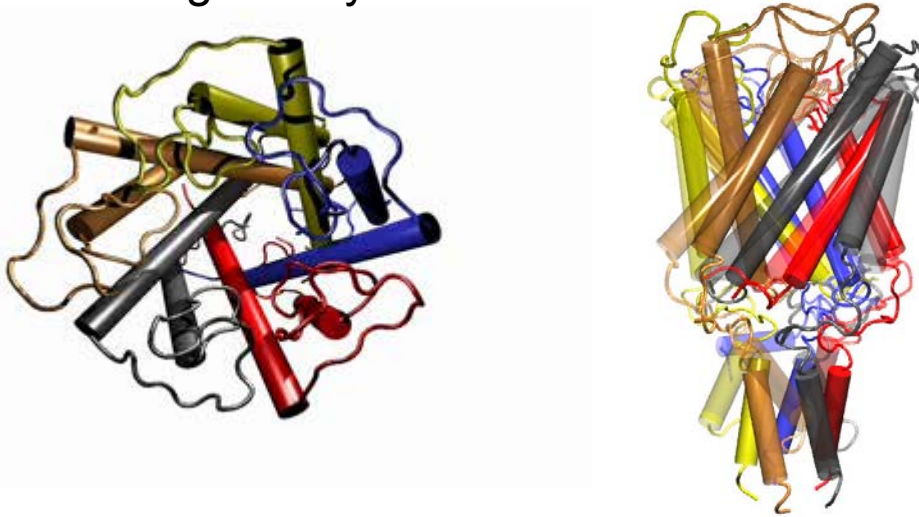
Protein structure from PDB entry 1msl
Chang et al.
Science
282:2220 – 2226 (1998)



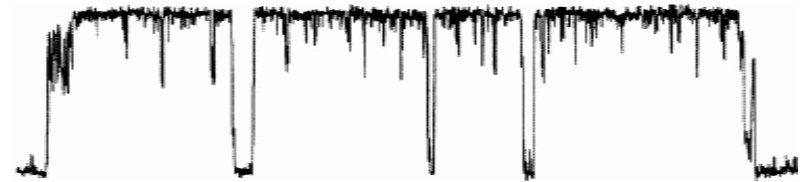
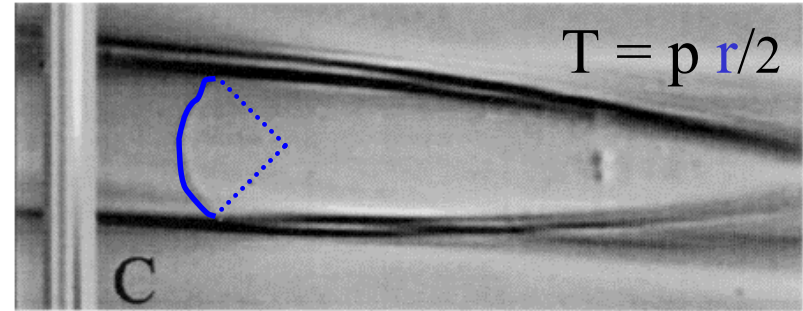
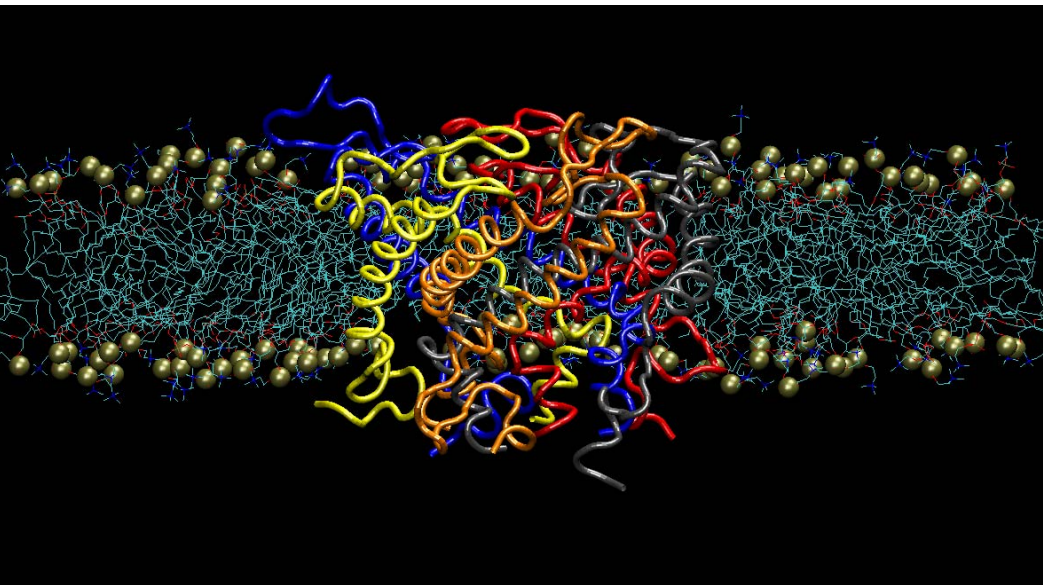
J. Gullingsrud

Mechanosensitive Ion Channel

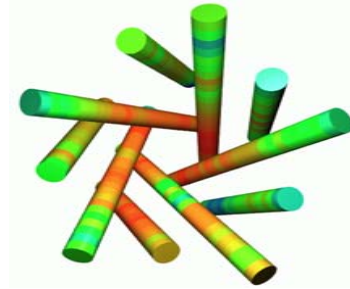
MscL gates by membrane tension



Pore expands to 30 Å as helices flatten out



Patch-clamp measurements
relate membrane tension to
channel gating

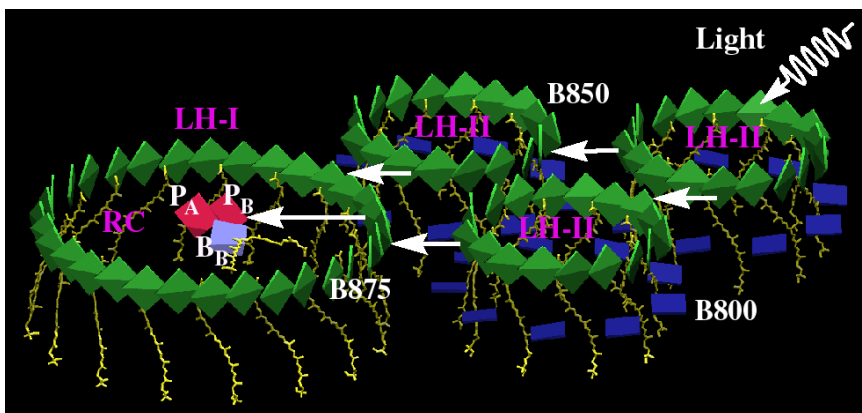
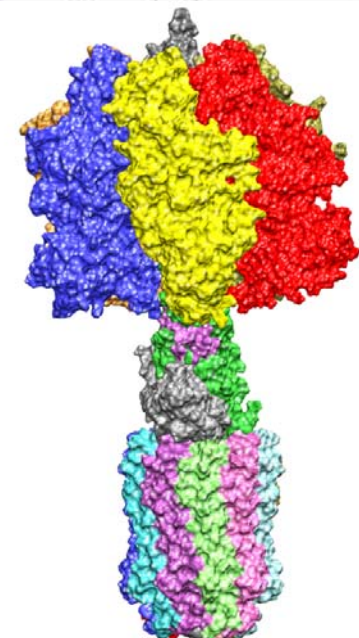
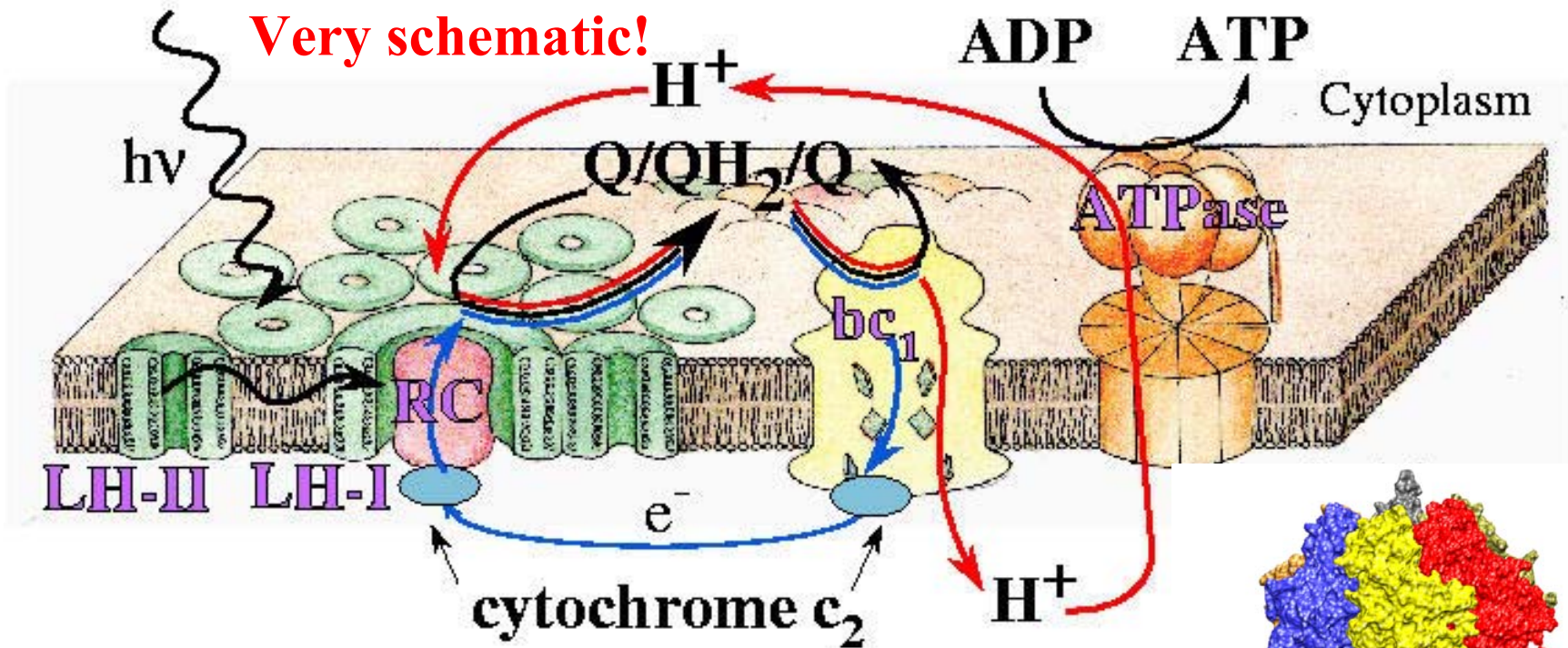


The protein is stiffest in the pinched
gating region, in agreement with EPR
measurements (Martinac et al,
unpublished results)

Biophys. J. 80:2074-2081, 2001.

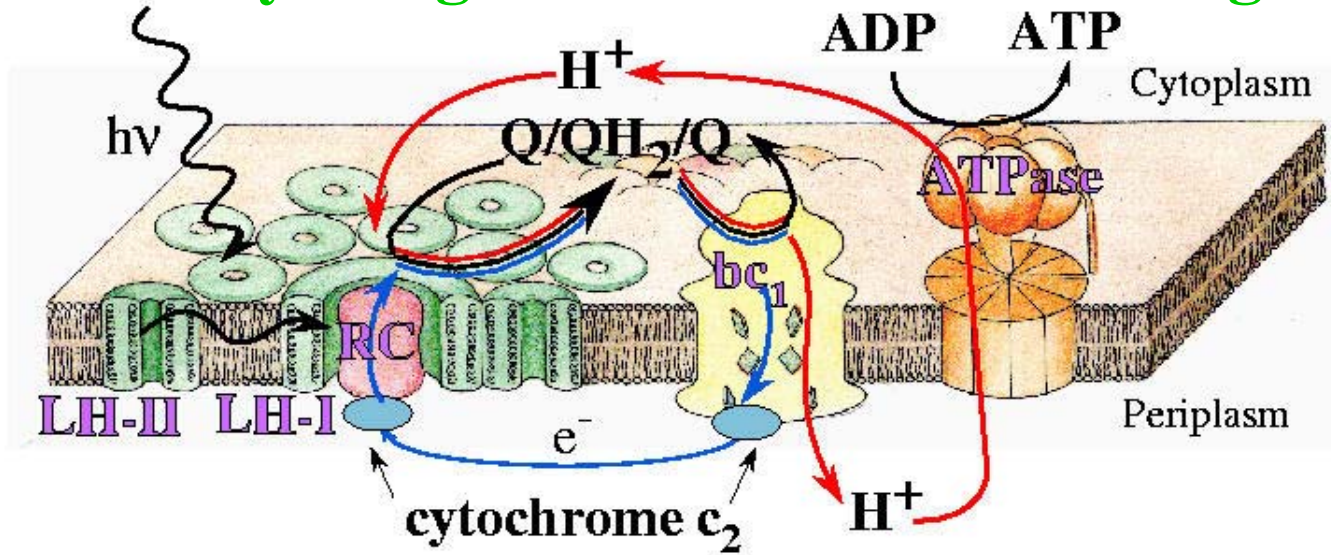
Photosynthetic Apparatus of Purple Bacteria

Function Achieved Through Very Large Structures

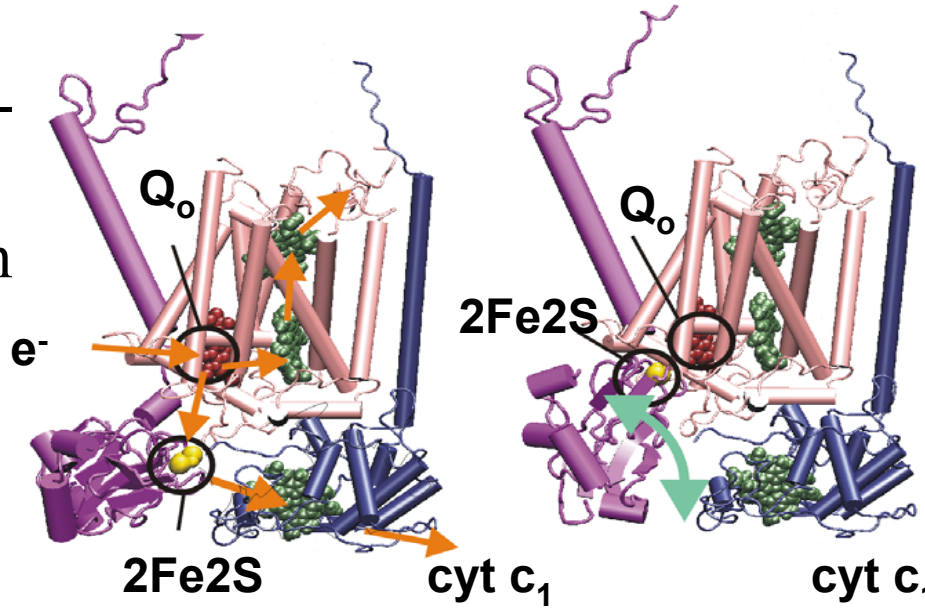


Photosynthetic Apparatus of Purple Bacteria

Very Large Conformational Changes



two pathways for oxidation of Q_0 site



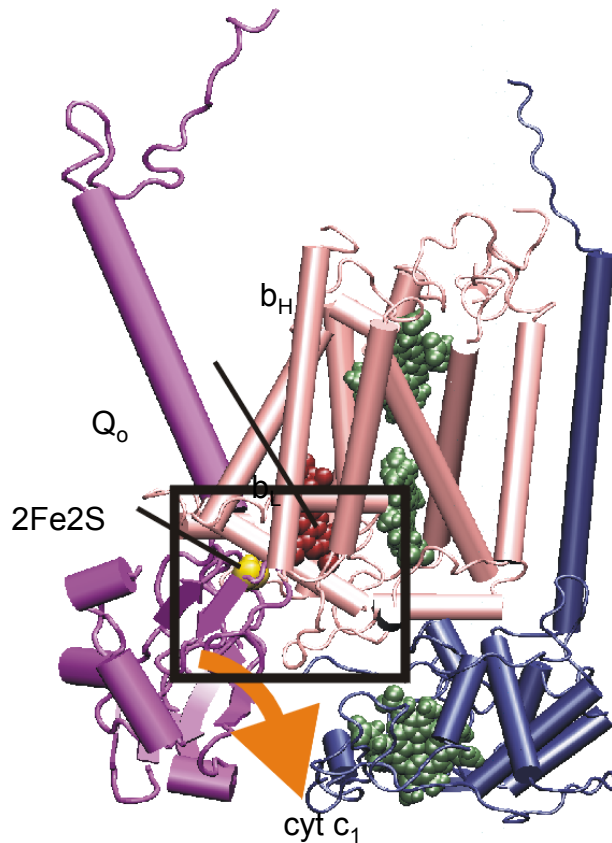
Iron Sulfur Protein head rotation can redirect 2nd electron

Sergei Izrailev

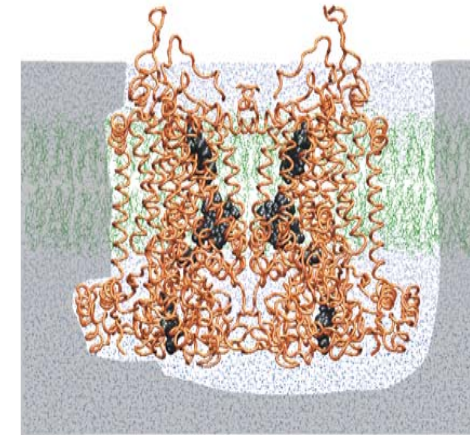
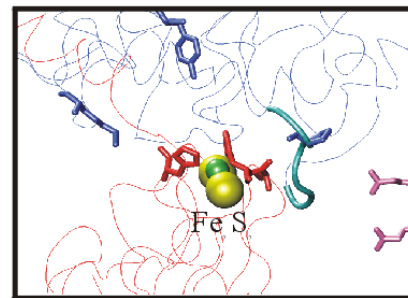
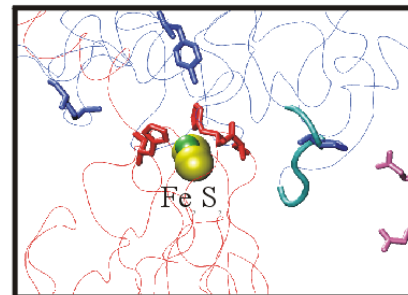
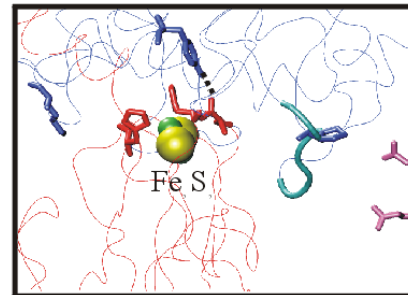
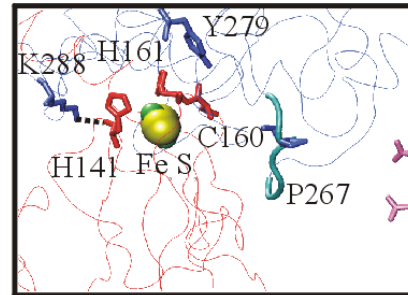
Enforcing domain rotation in the bc₁ complex (3)

Events during torque application to ISP head

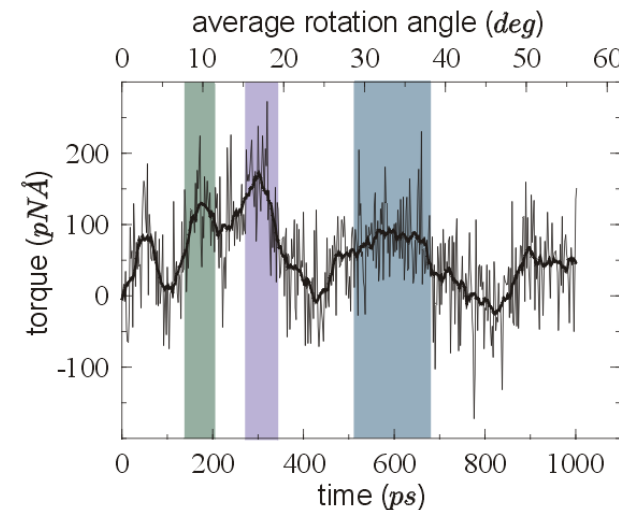
Izrailev et al., Biophys J.,
77:1753-1768 (1999)



Torque applied
to 126 C _{α} atoms
K = 70 pN/Å
 ω = 0.0561 rad/s

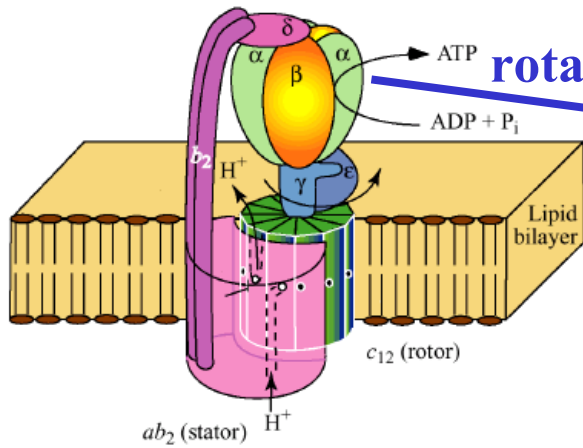


206,720 atoms



Photosynthetic Apparatus of Purple Bacteria

Very Large Conformational Changes



ATP rotatory catalysis

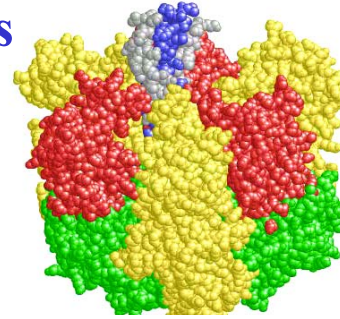
ADP + P_i

Lipid bilayer

c_{12} (rotor)

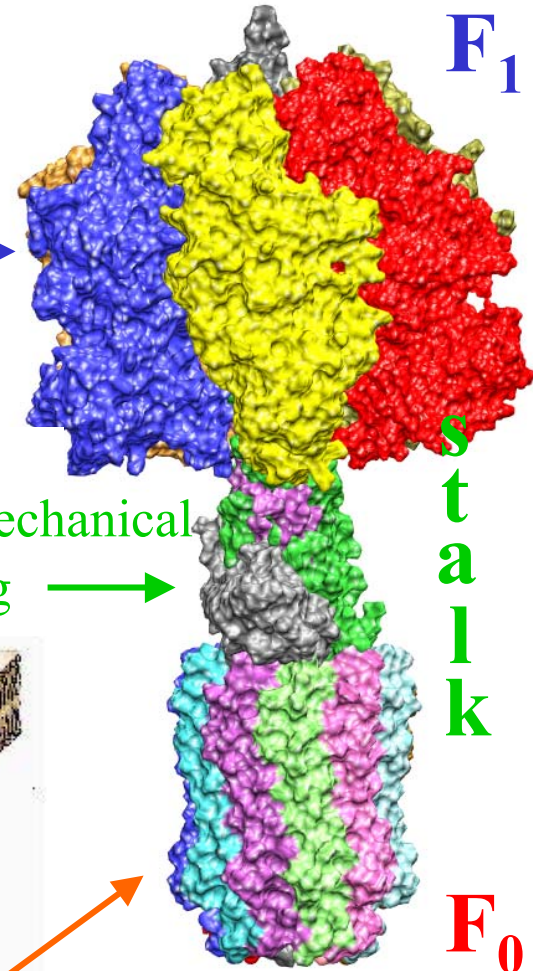
ab_2 (stator)

H^+



(from Wang & Oster)

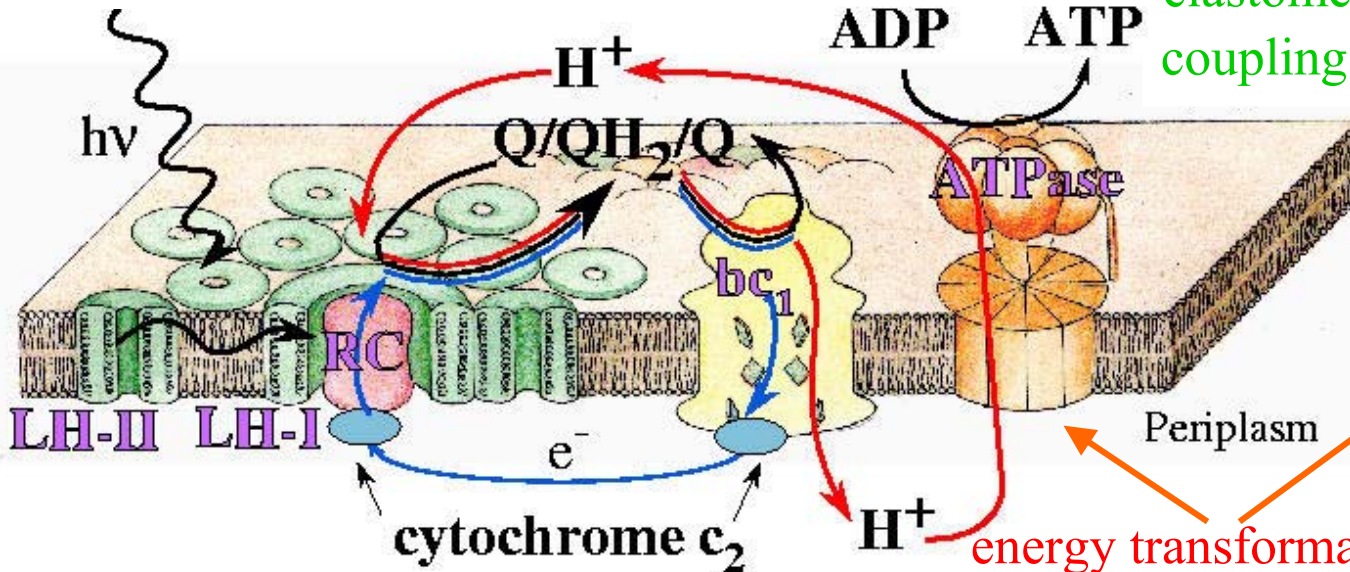
elastomechanical coupling



F₁

stalk

F₀



$h\nu$

H^+

Q/QH₂/Q

ADP

ATP

ATPase

b_6

c_1

LH-II

LH-I

RC

cytochrome c_2

H^+

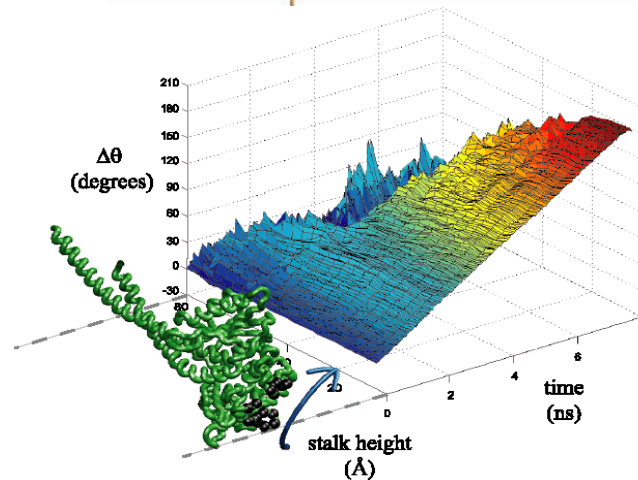
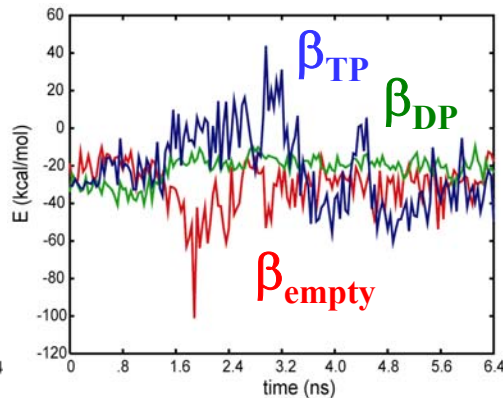
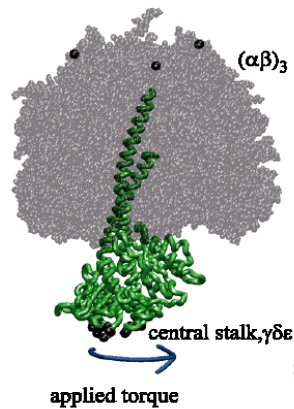
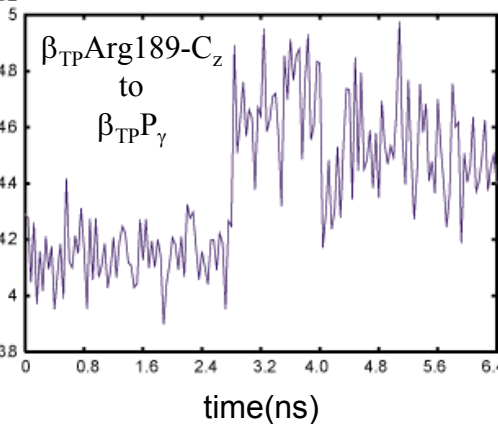
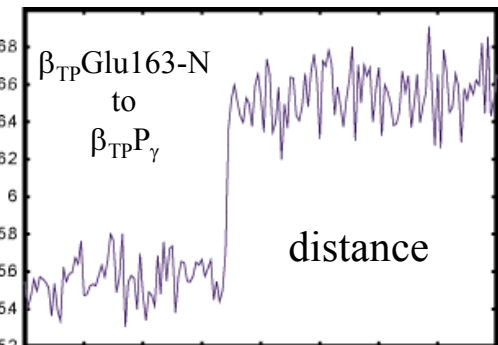
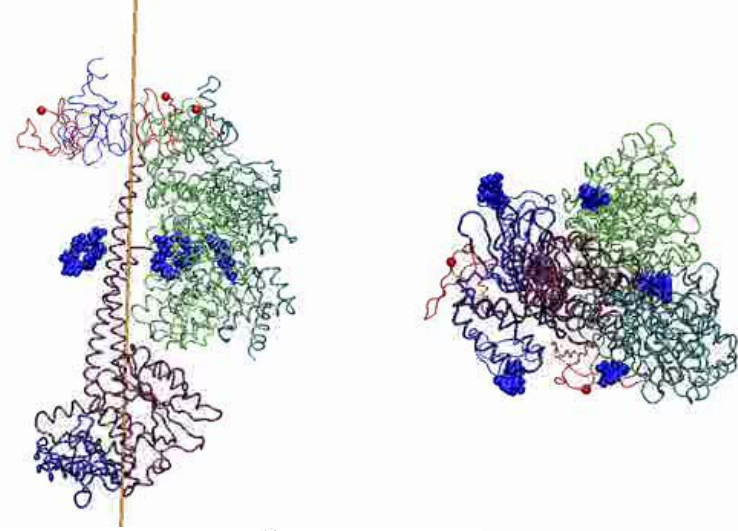
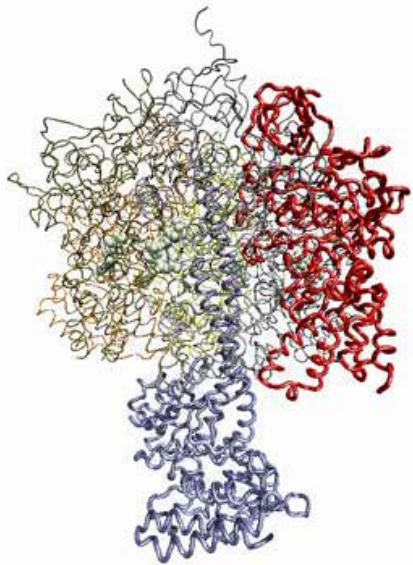
Periplasm

energy transformation
(proton motive force
↔ rotation)

ATP synthase

(Walker, Whilce,
Fillingame)

Rotation of ATPase Stalk in F1 ATPase



- **327,000 atoms**
- 12 ns simulation on NCSA Platinum and PSC Lemieux, 1024 processors

SMD Publications 1997-2002

1. **Extraction of lipids** from phospholipid membranes by steered molecular dynamics. S. Stepaniants, S. Izrailev, and K. Schulten. *J. Mol. Model.*, 3:473-475, 1997.
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