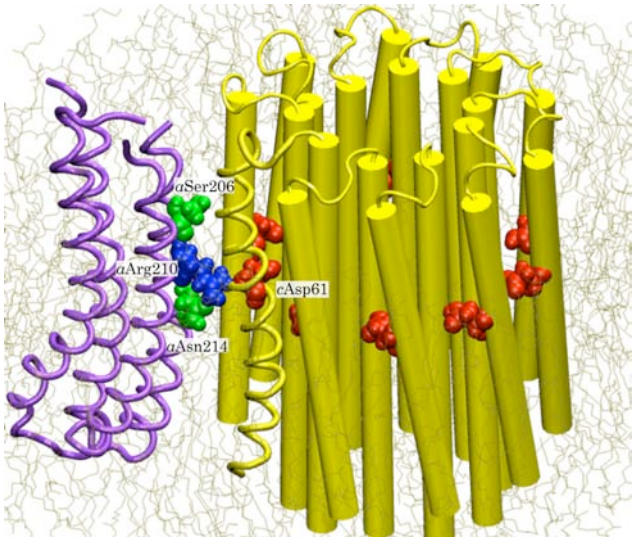
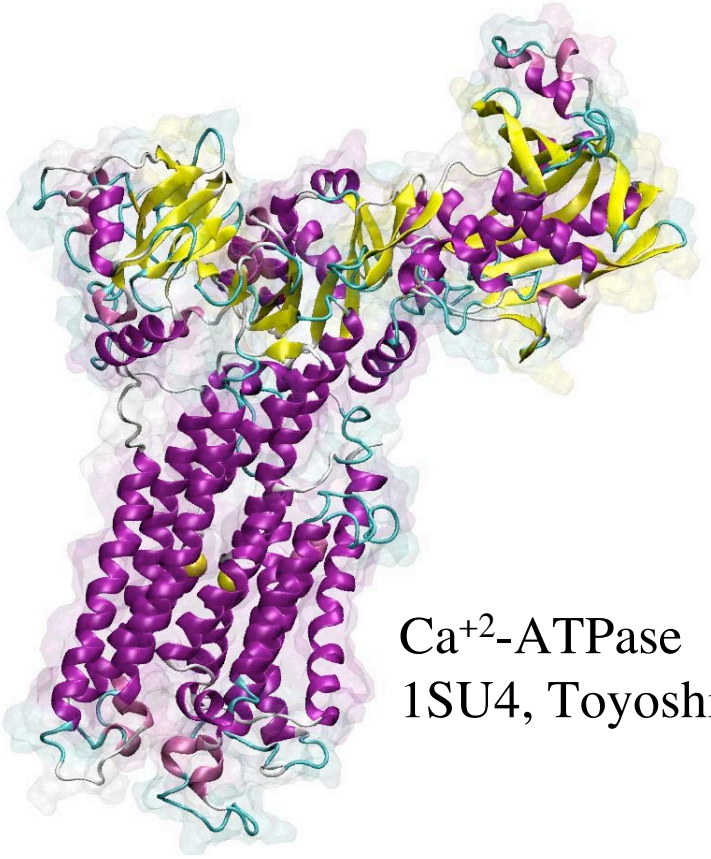
# MD Simulations of Membrane Channels

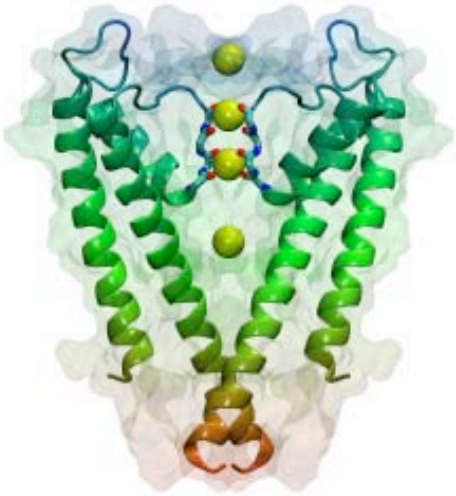



Fo-ATP Synthase
NMR, Fillingame, *E. coli*



$Ca^{+2}$-ATPase
1SU4, Toyoshima

# Membrane Proteins Tutorial



Alek Aksimentiev

Marcos Sotomayor

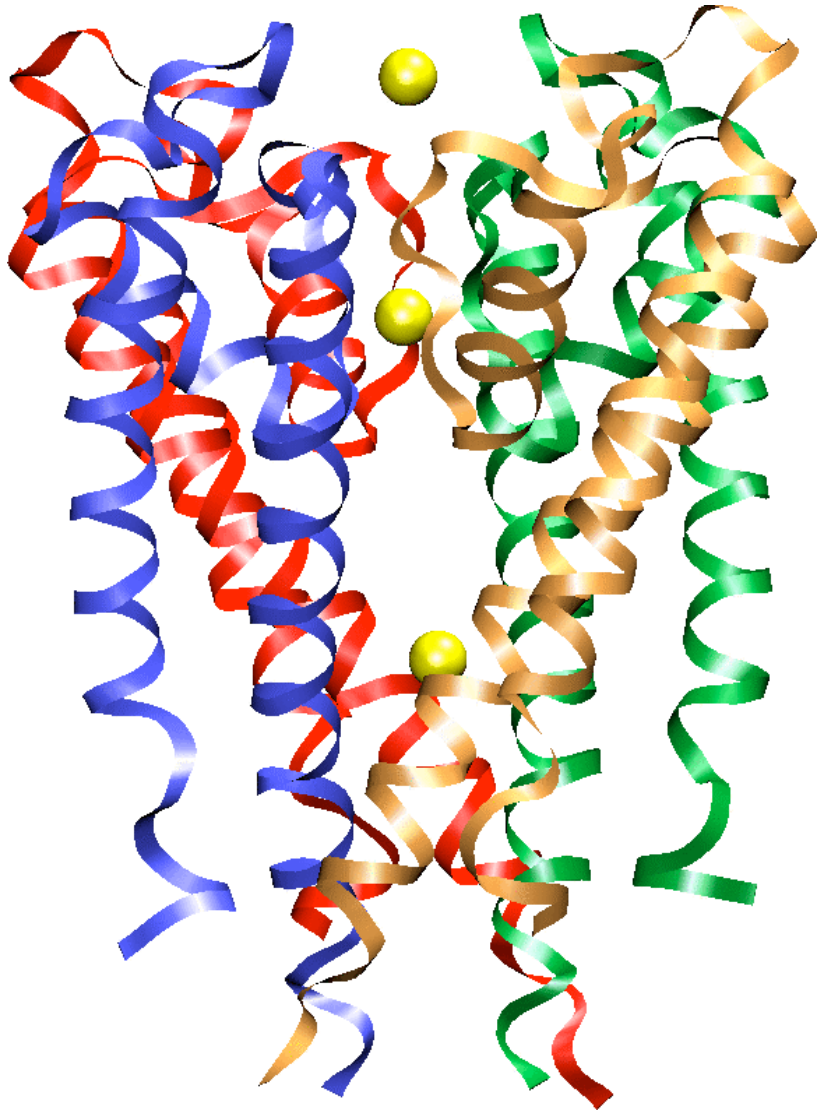
David Wells

October 2006

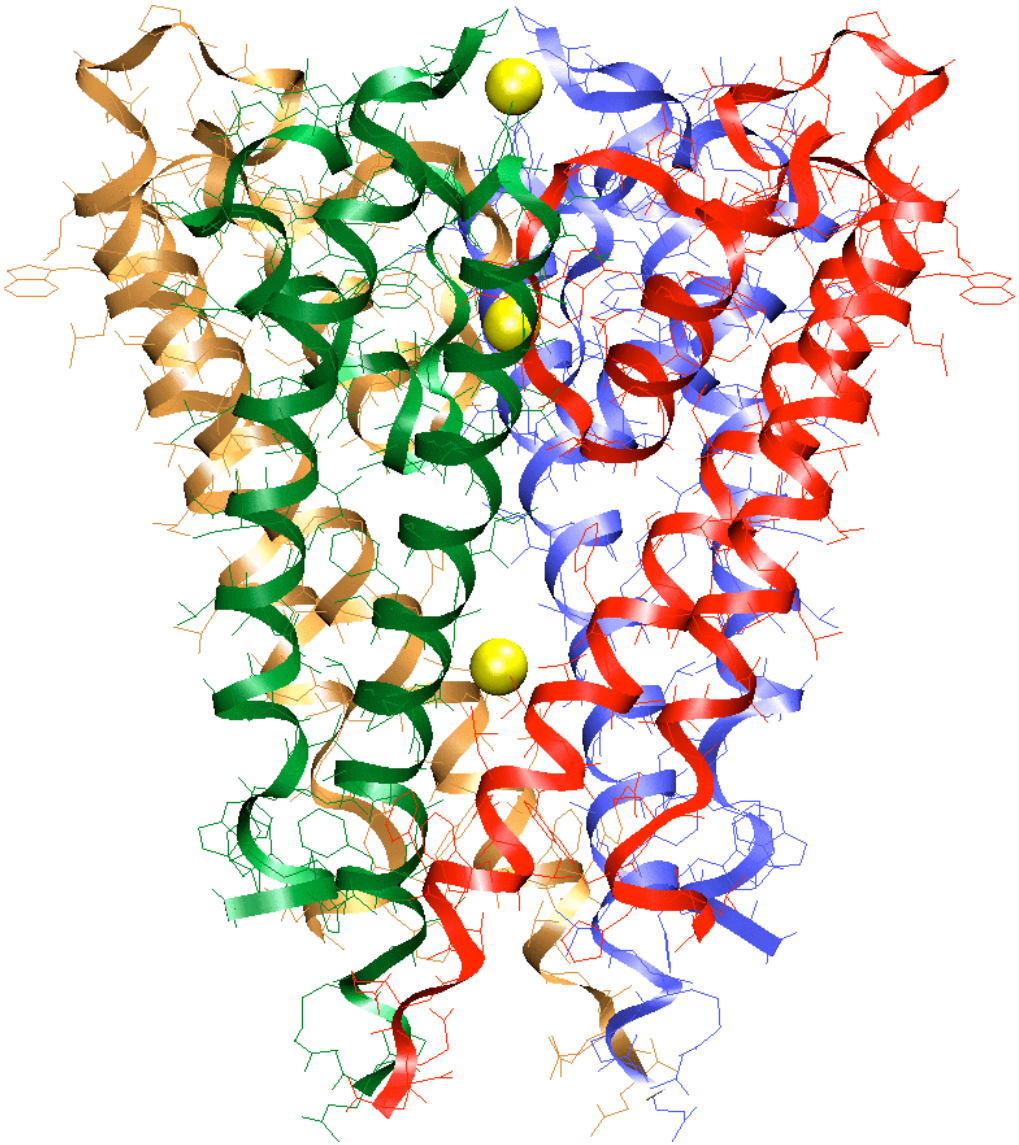
# Example: MD Simulations of the $K^+$ Channel Protein

Ion channels are membrane - spanning proteins that form a pathway for the flux of inorganic ions across cell membranes.

Potassium channels are a particularly interesting class of ion channels, managing to distinguish with impressive fidelity between $K^+$ and $Na^+$ ions while maintaining a very high throughput of $K^+$ ions when gated.

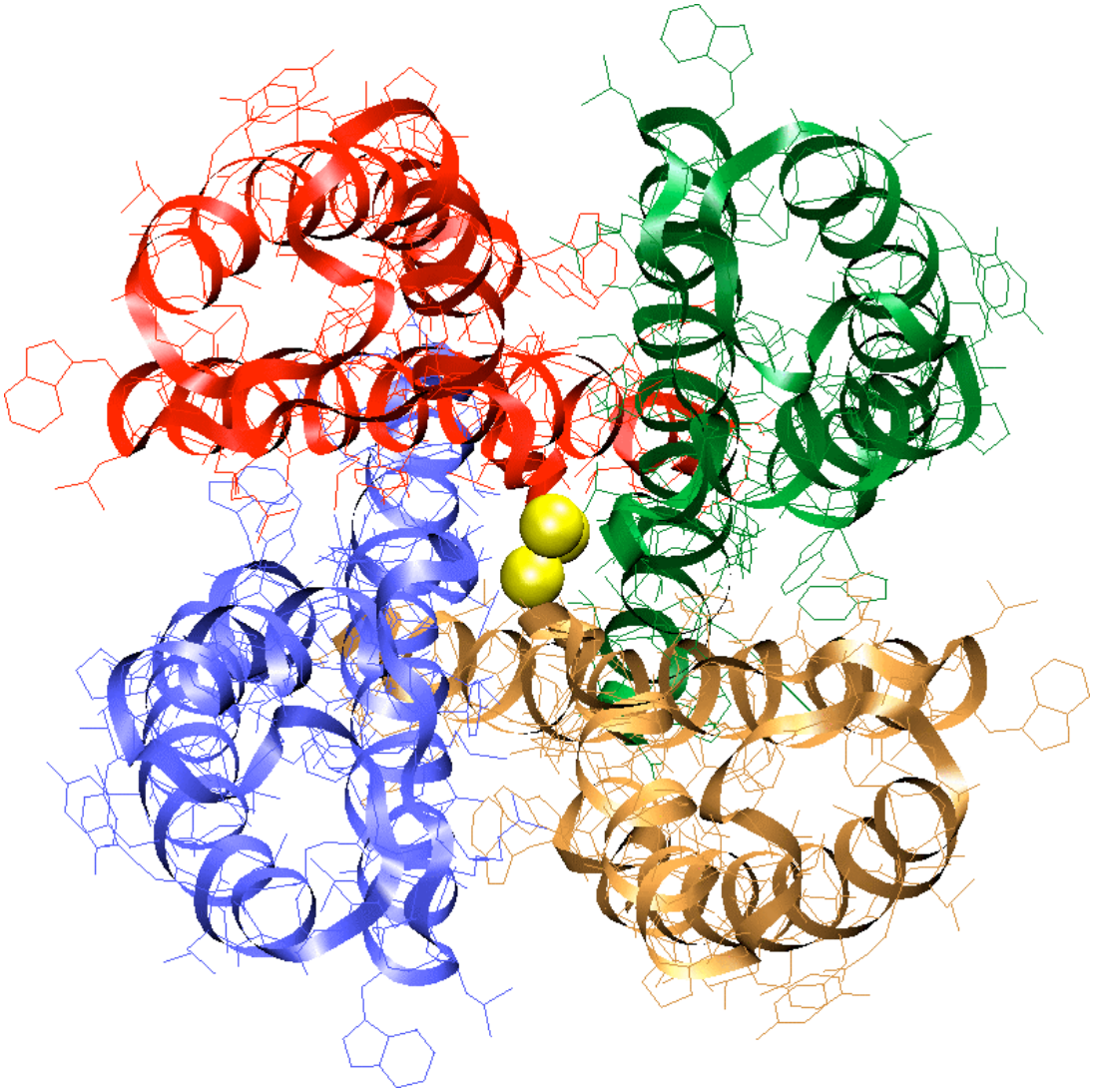


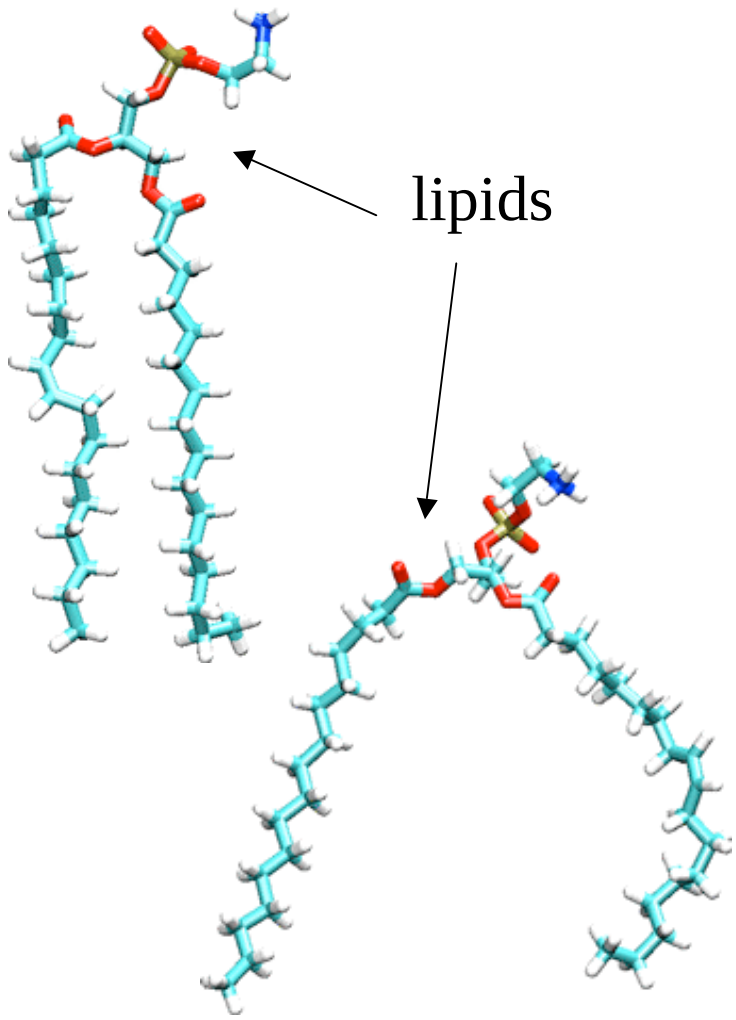
# Setting up the system (1)



- retrieve the PDB (coordinates) file from the Protein Data Bank
- add missing atoms using PSFGEN
- Inspect X-ray waters with Dowser
- Build a tetramer
- Examine protonation states
- Add internal waters with Dowser

# Setting up the system (2)

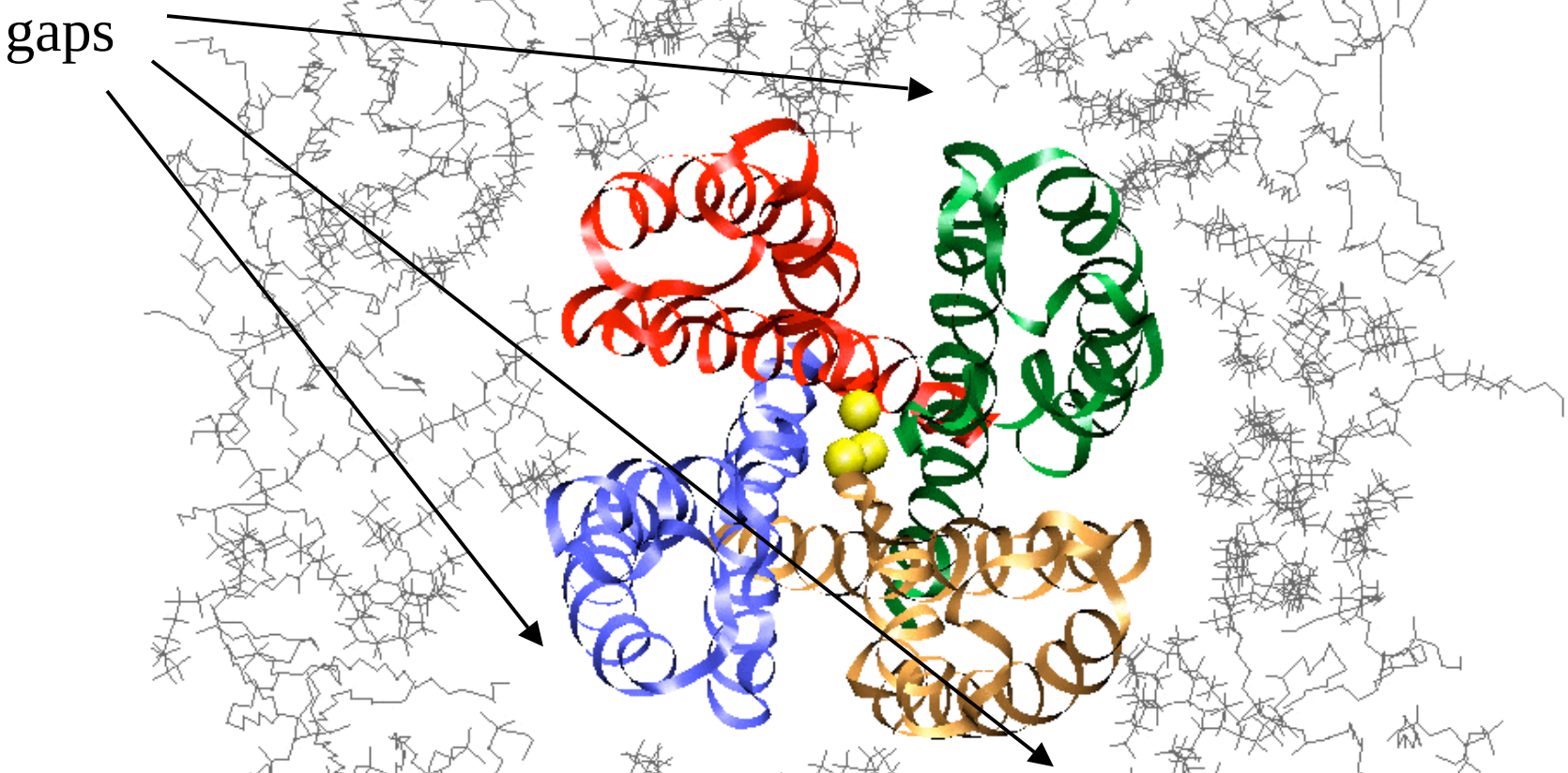





Simulate the protein in its natural environment: solvated lipid bilayer

# Setting up the system (3)

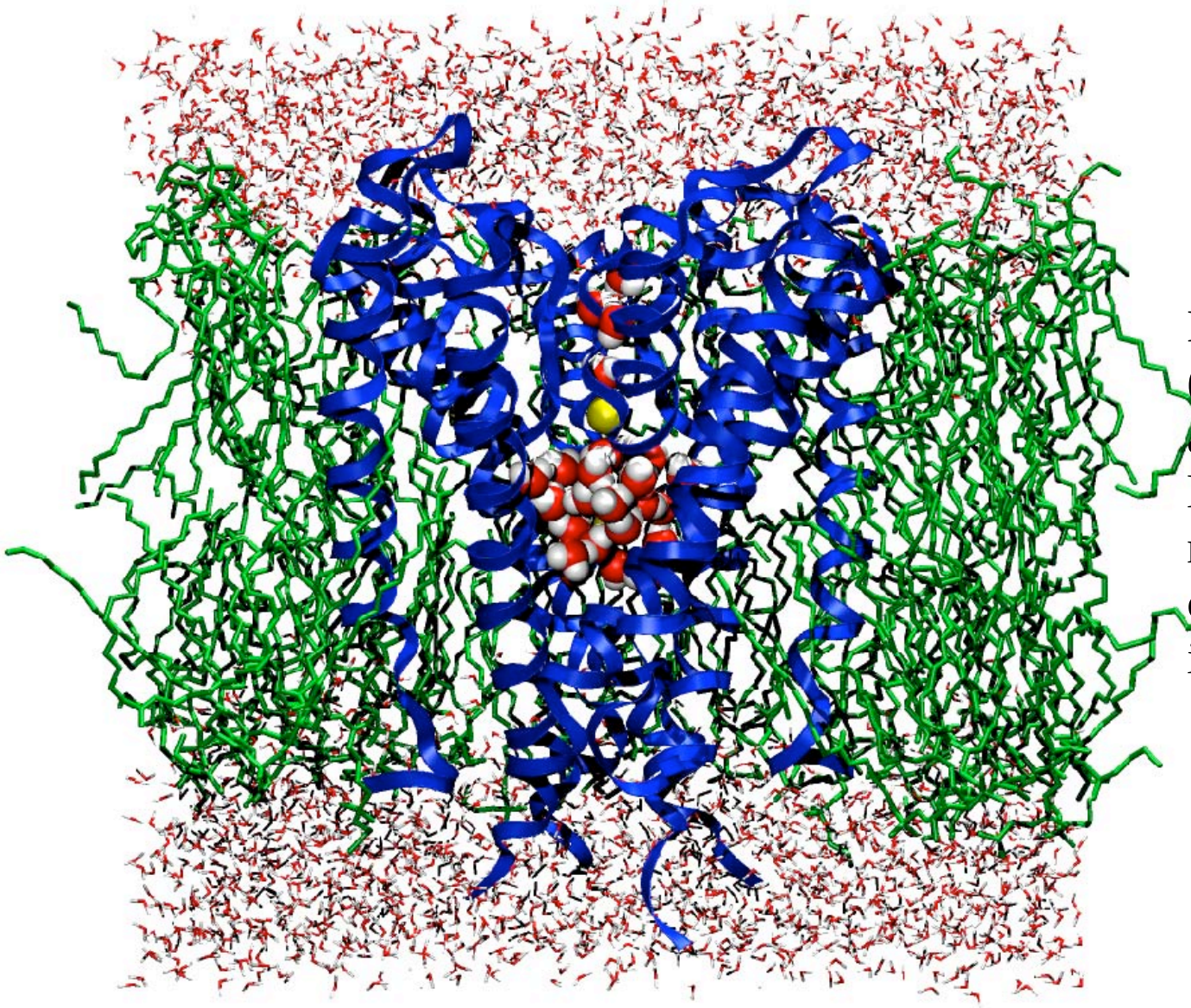
## Inserting the protein in the lipid bilayer




Automatic insertion into the lipid bilayer leads to big gaps between the protein and the membrane => long equilibration time required to fill the gaps.

Solution: manually adjust the position of lipids around the protein

# The system

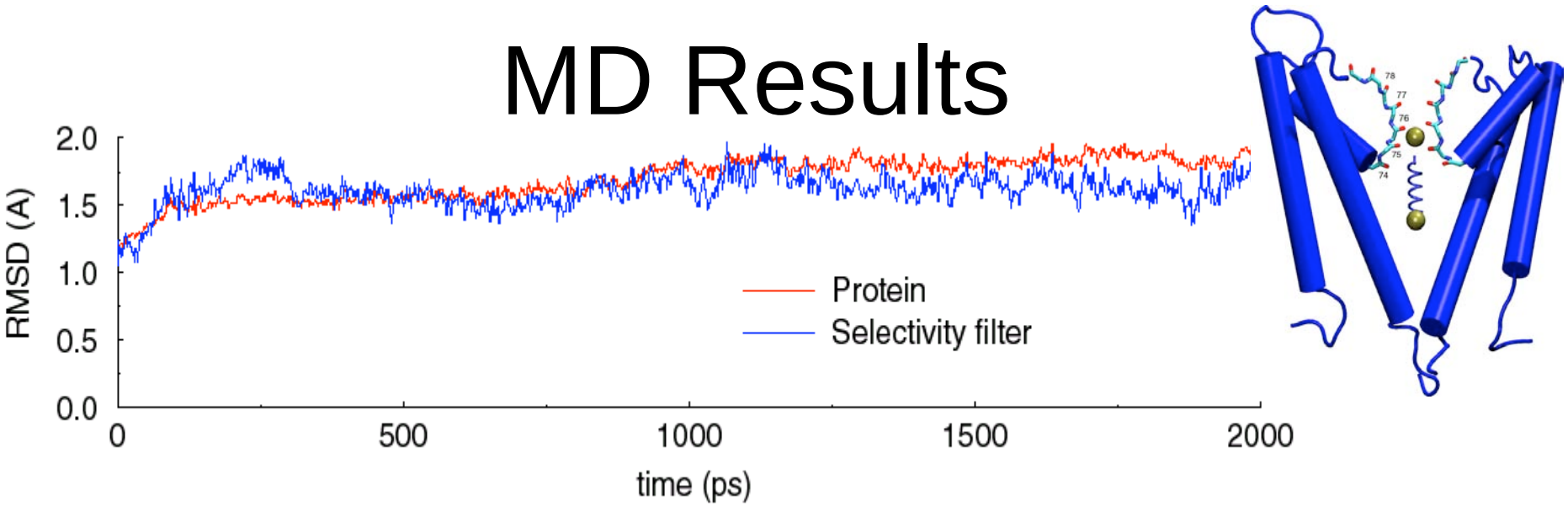


solvent

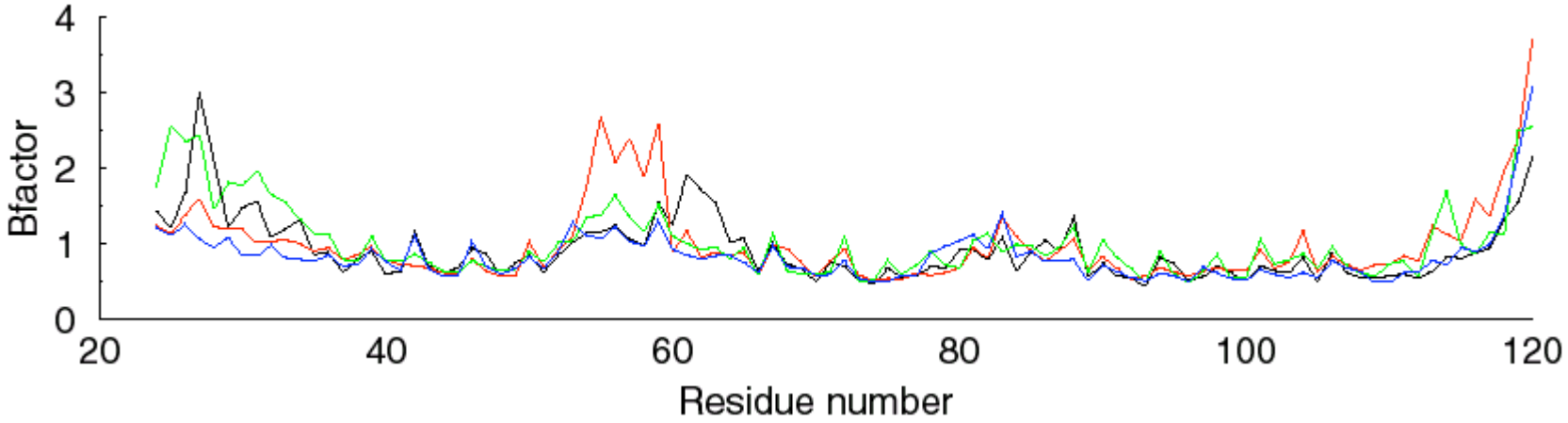
Kcsa channel protein (in blue) embedded in a (3:1) POPE/POPG lipid bilayer. Water molecules inside the channel are shown in vdW representation.

solvent

# MD Results

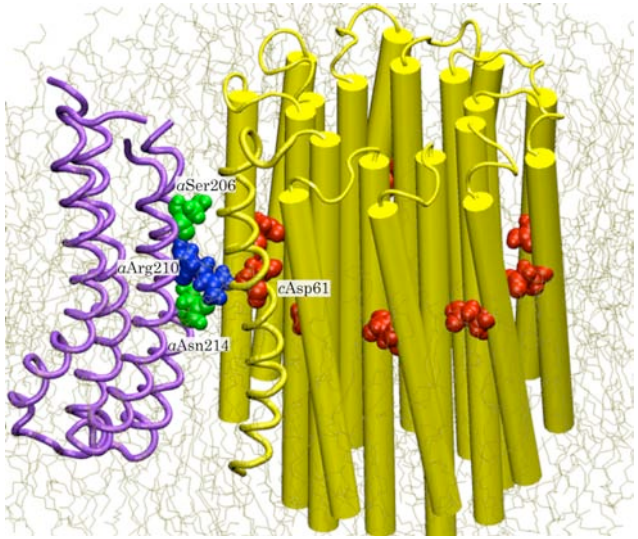



RMS deviations for the KcsA protein and its selectivity filer indicate that the protein is stable during the simulation with the selectivity filter the most stable part of the system.

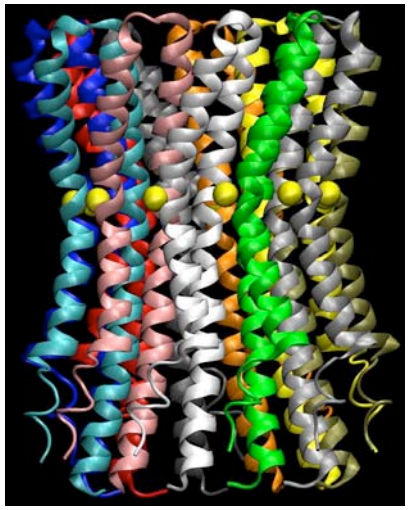



Temperature factors for individual residues in the four monomers of the KcsA channel protein indicate that the most flexible parts of the protein are the N and C terminal ends, residues 52-60 and residues 84-90. Residues 74-80 in the selectivity filter have low temperature factors and are very stable during the simulation.

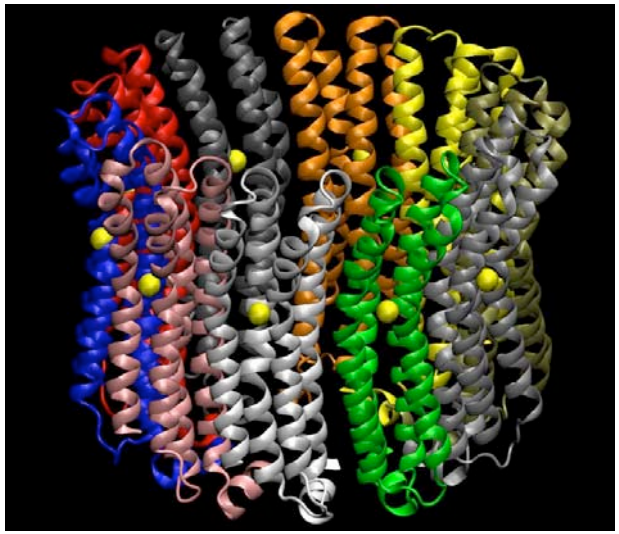
# Systems



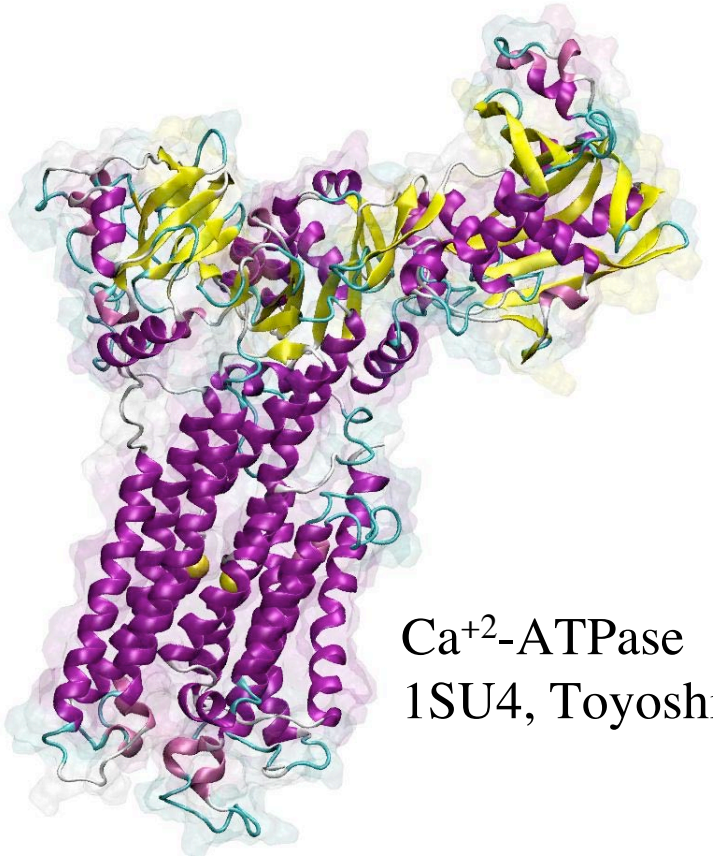

Fo-ATP Synthase
NMR, Fillingame, *E. coli*



$Na^{+}$-ATP Synthase
Meier et al, 1YCE
*Ilyobacter tartaricus*

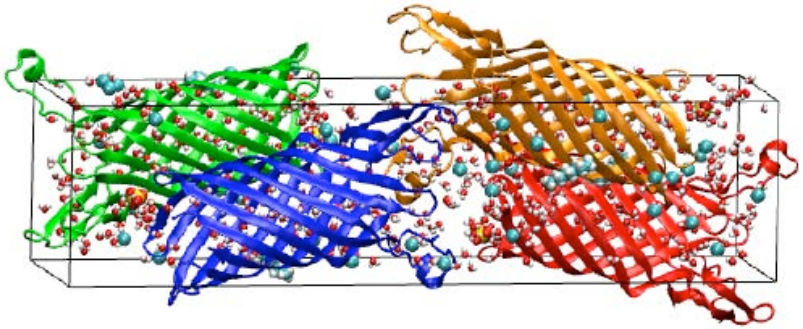


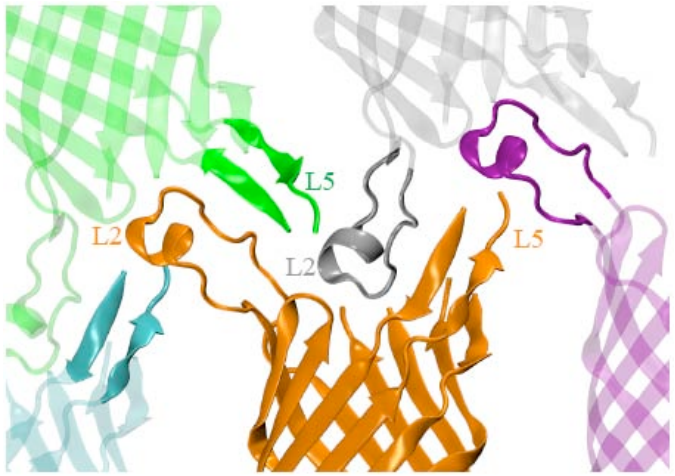
V-type $Na^{+}$-ATPase, 2BL2
X-Ray, Murata et al, *Entericoccus hirae*

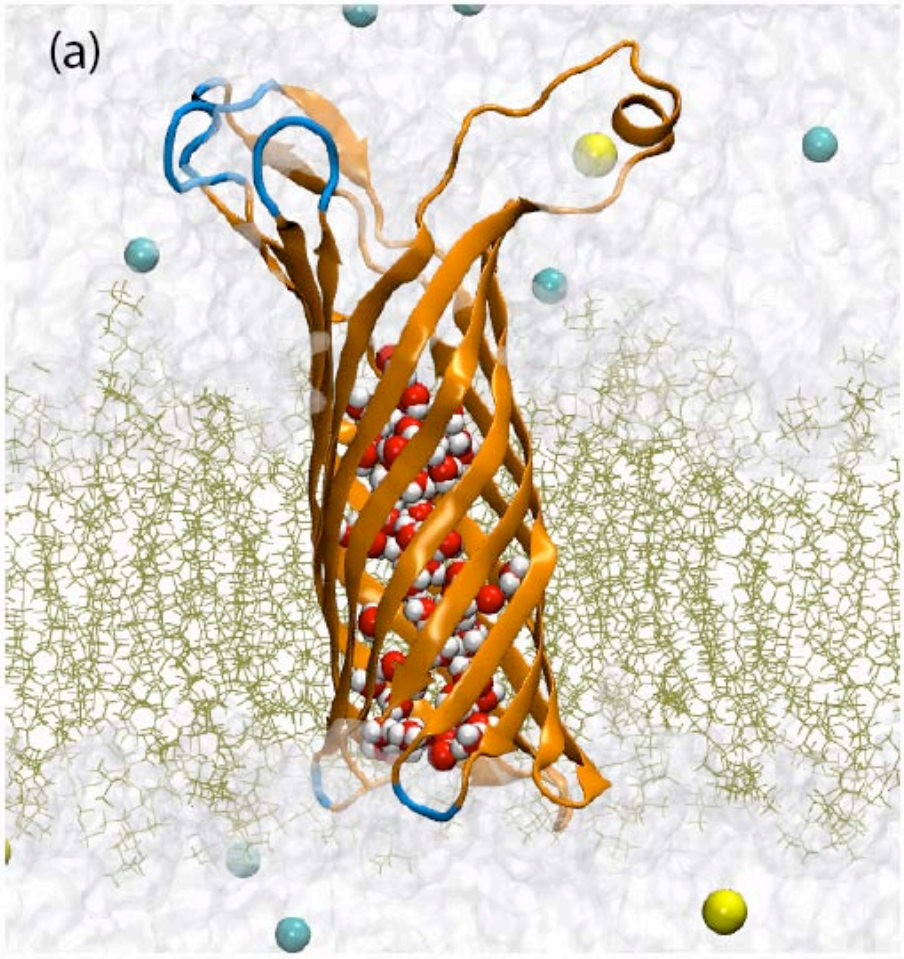


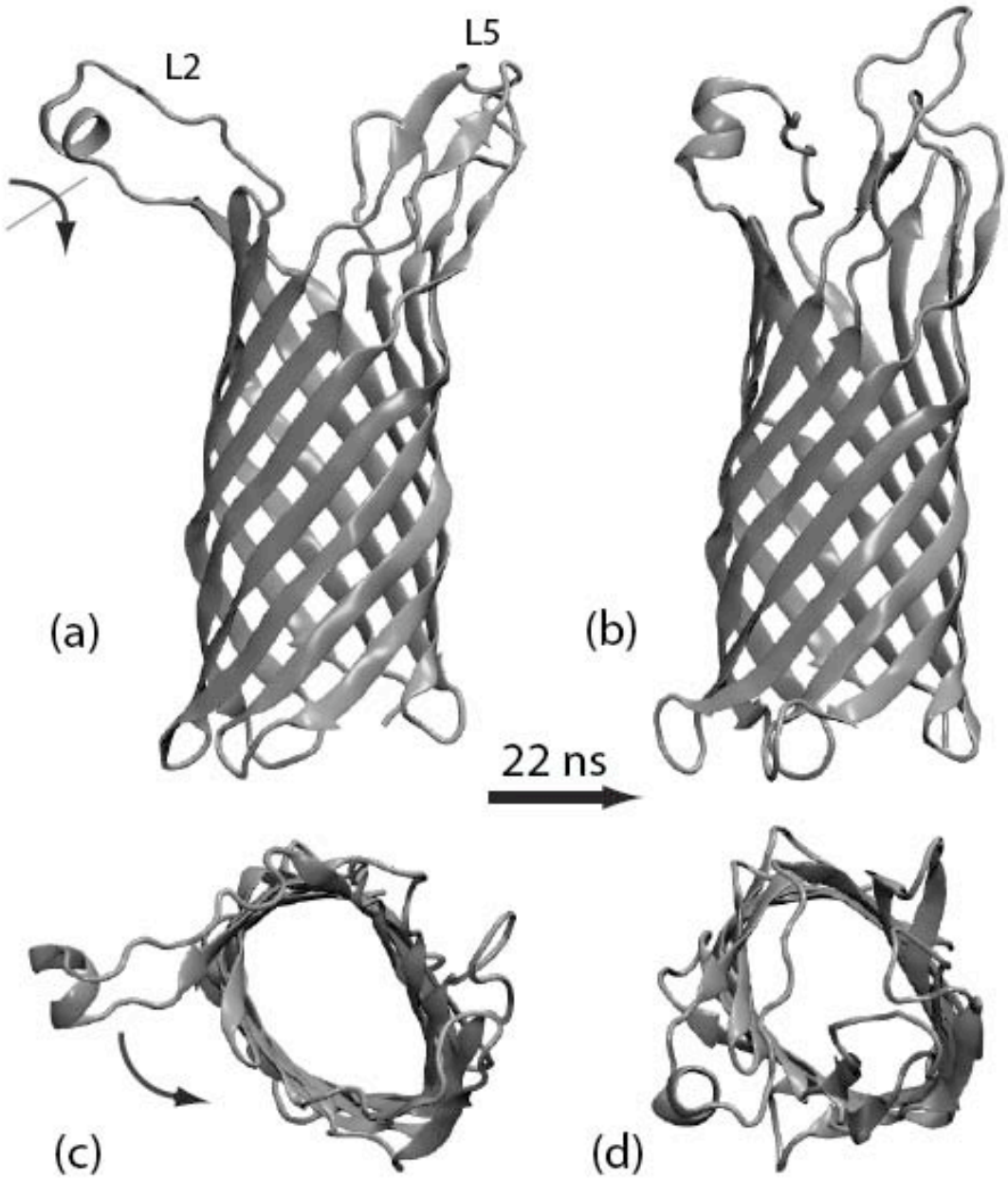
$Ca^{+2}$-ATPase
1SU4, Toyoshima

# OpcA adhesin

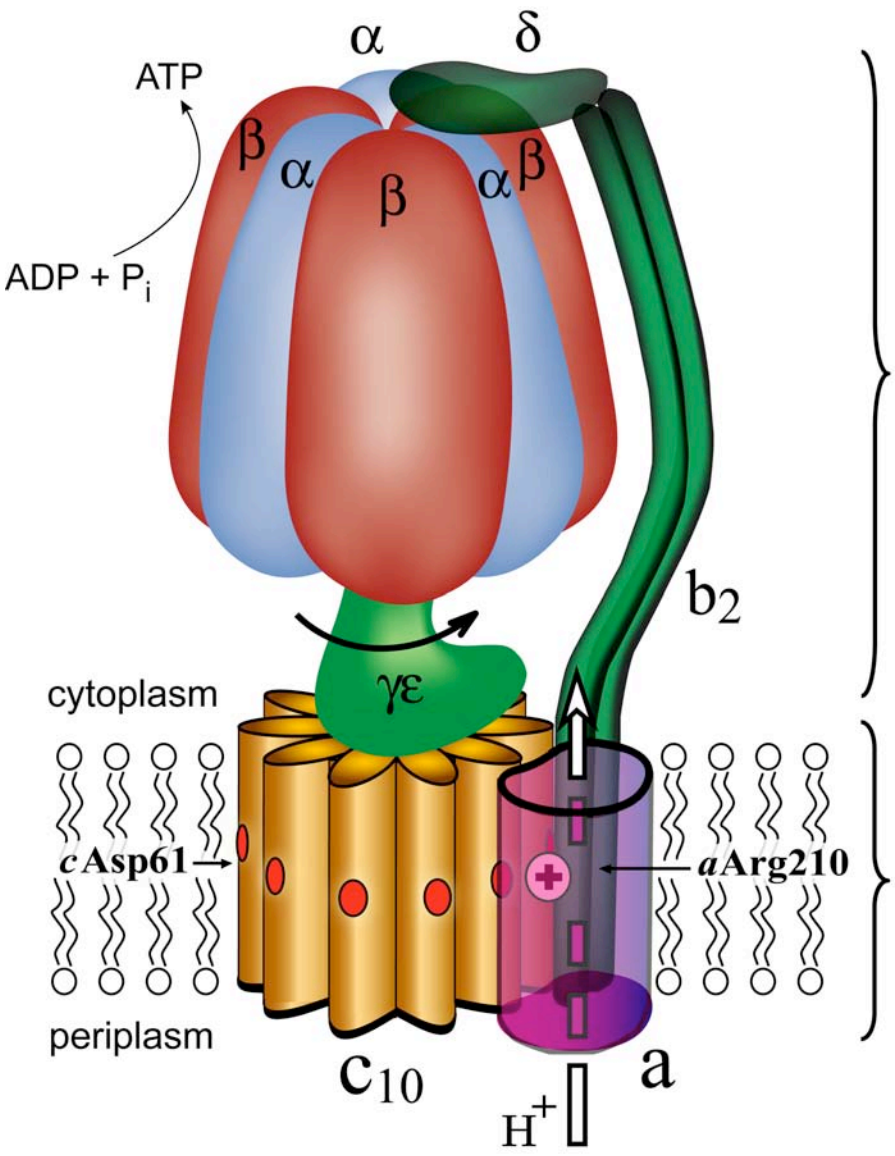





L2
L5
(a)
(b)
22 ns
(c)
(d)

# F-ATP synthase

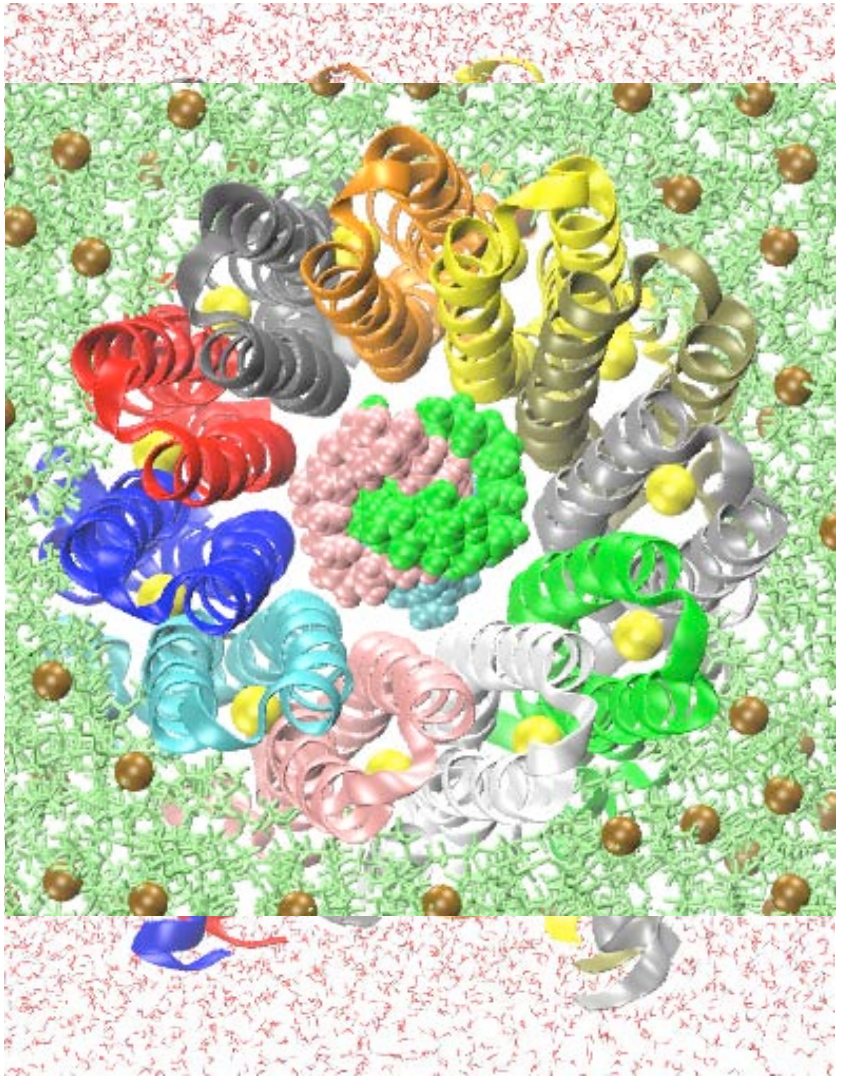


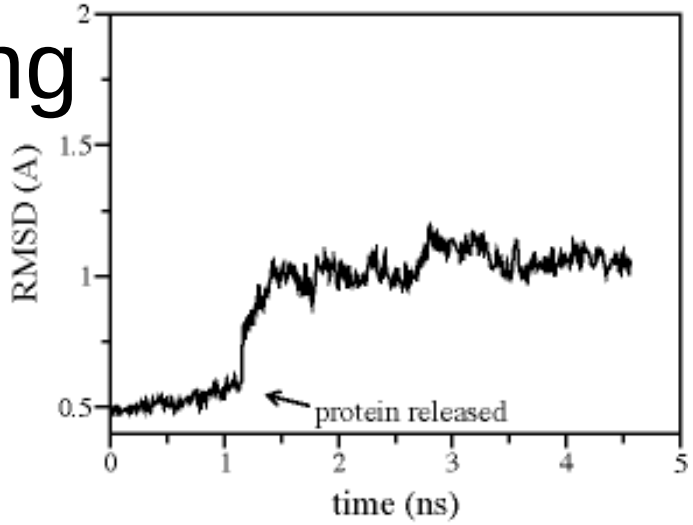

Rotary catalysis: Two protein motors coupled via common central stalk γδ

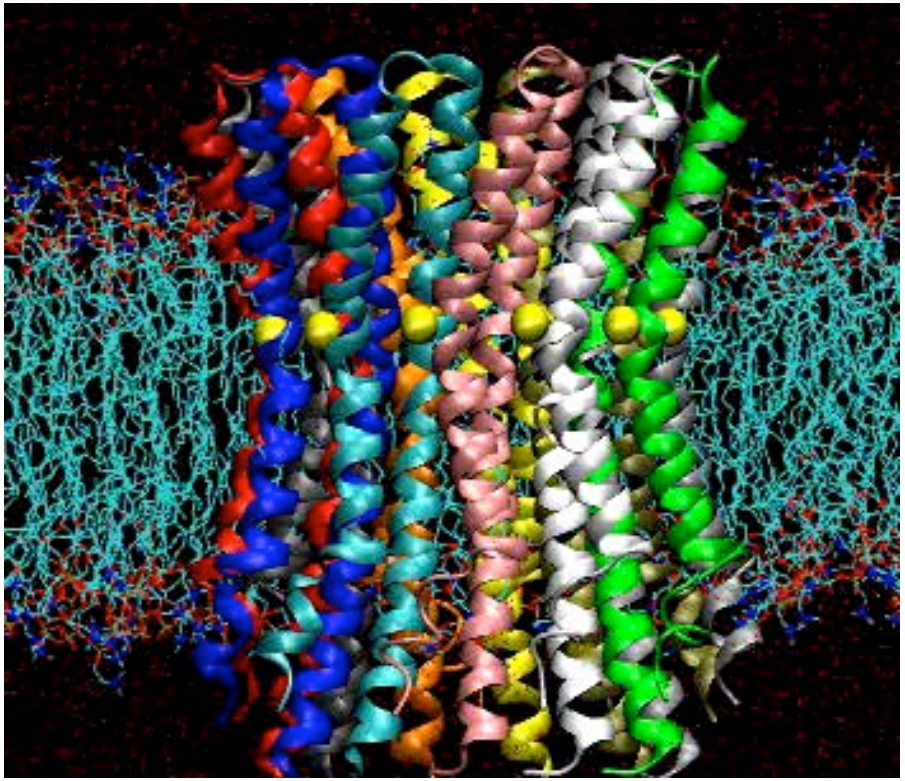
Solvent exposed $F_1$ unit ($\alpha_3\beta_3\gamma\delta\varepsilon$): central stalk rotation causes conformational changes in catalytic sites, driving ATP synthesis

Transmembrane $F_o$ unit ($ab_2c_{10}$): converts proton motive force into mechanical rotation of central stalk

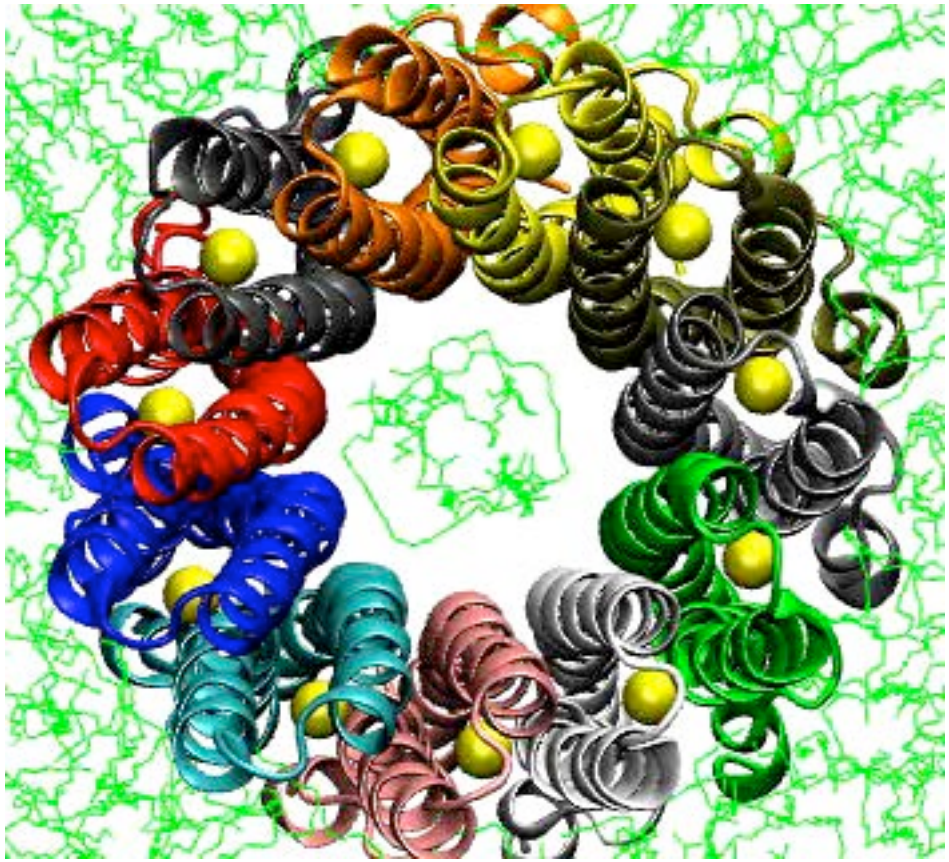
# Equilibration of the $c_{11}$-ring of $Na^+$-ATPase



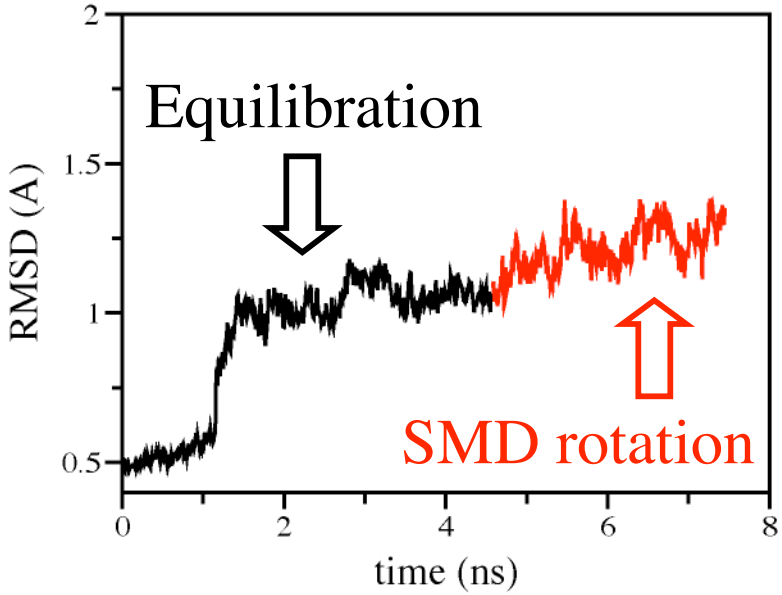


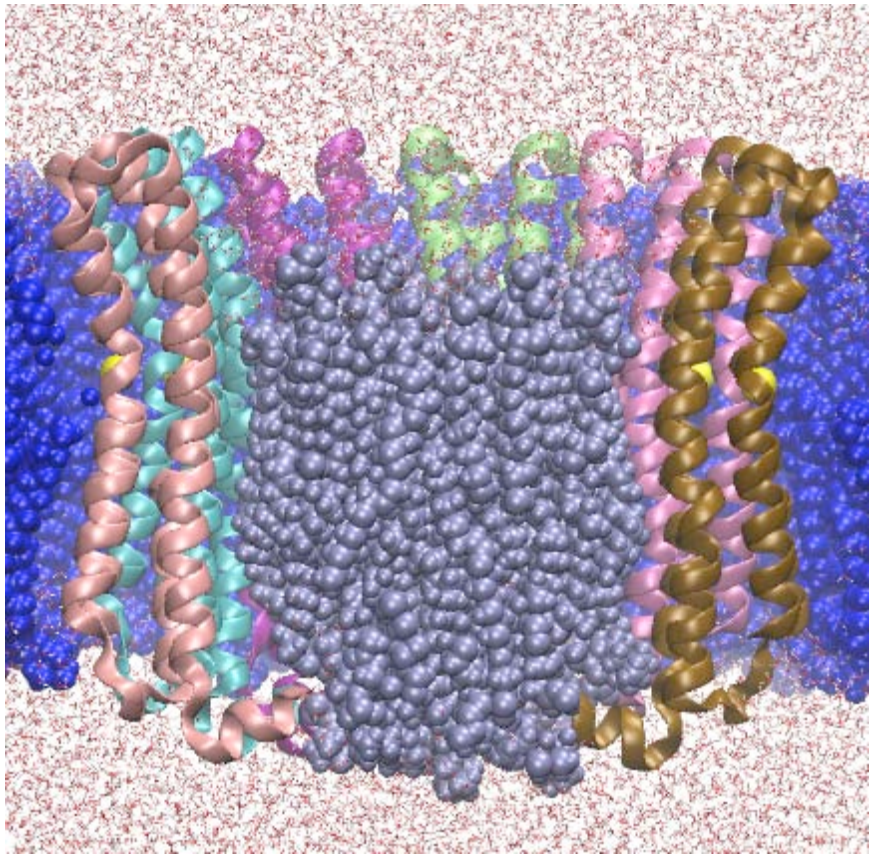
# Forced Rotation of $c_{11}$ in $Na^+$-ATP Synthase



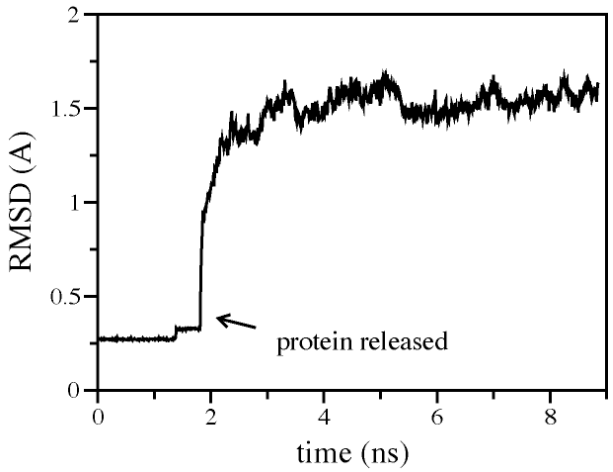
3-ns SMD simulation
torque: 1000 kcal/mol

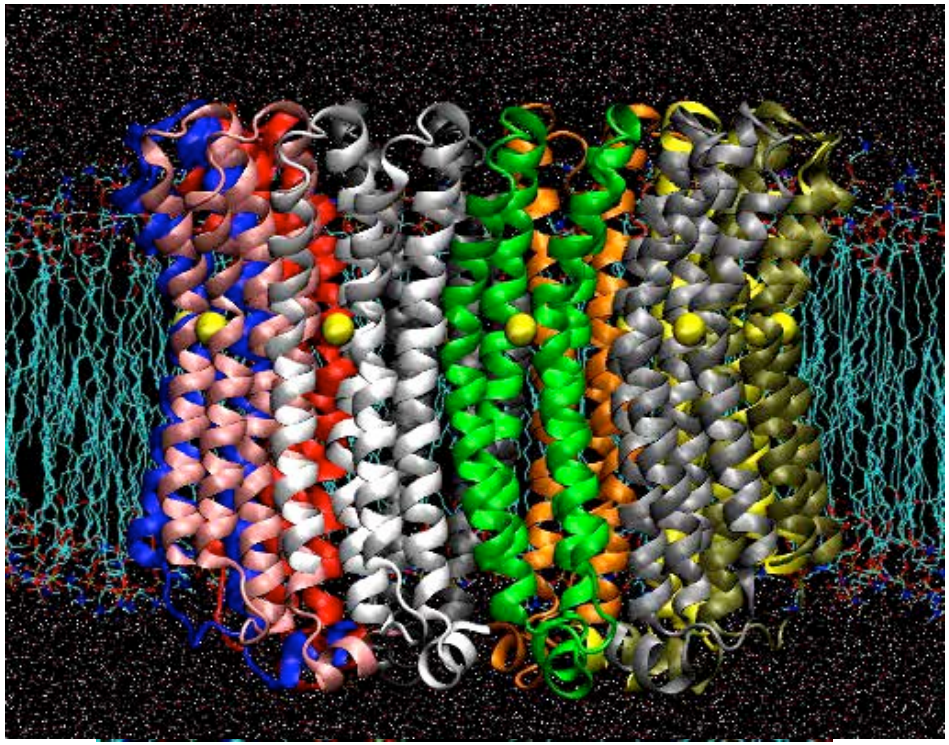


# V-type ATPase



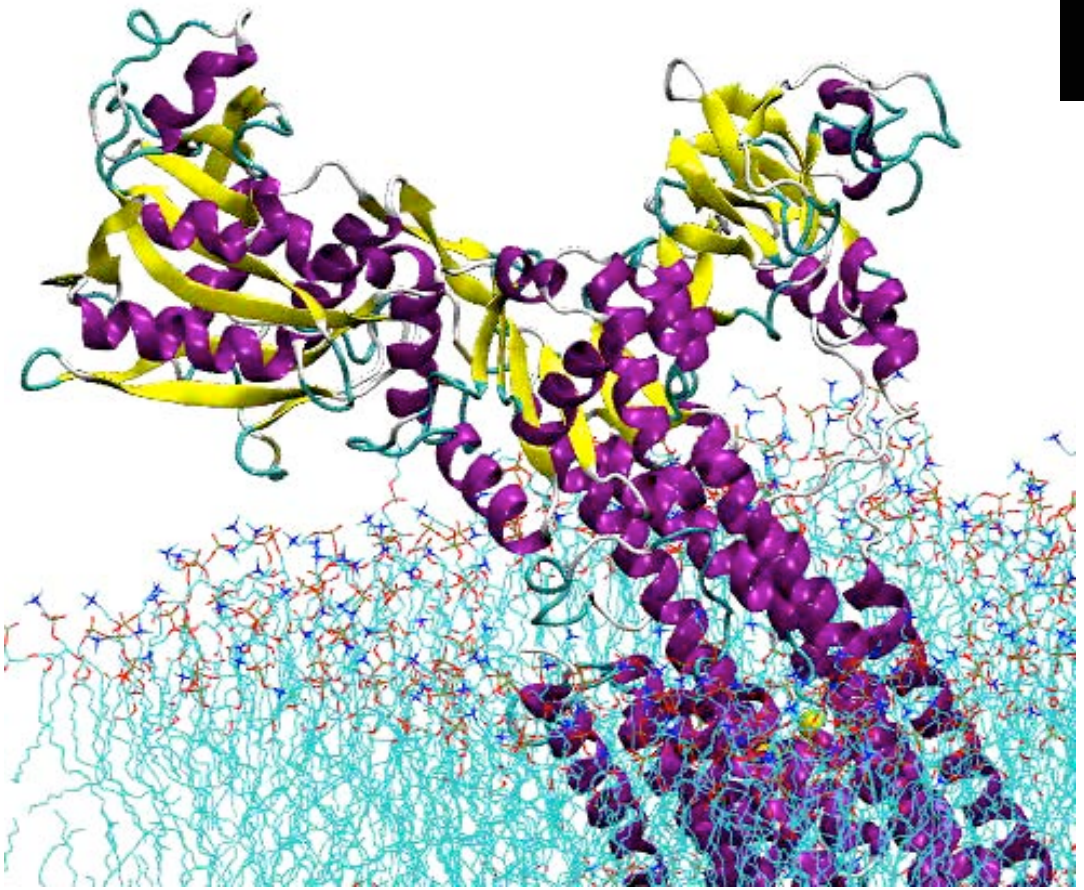
~200,000 atoms



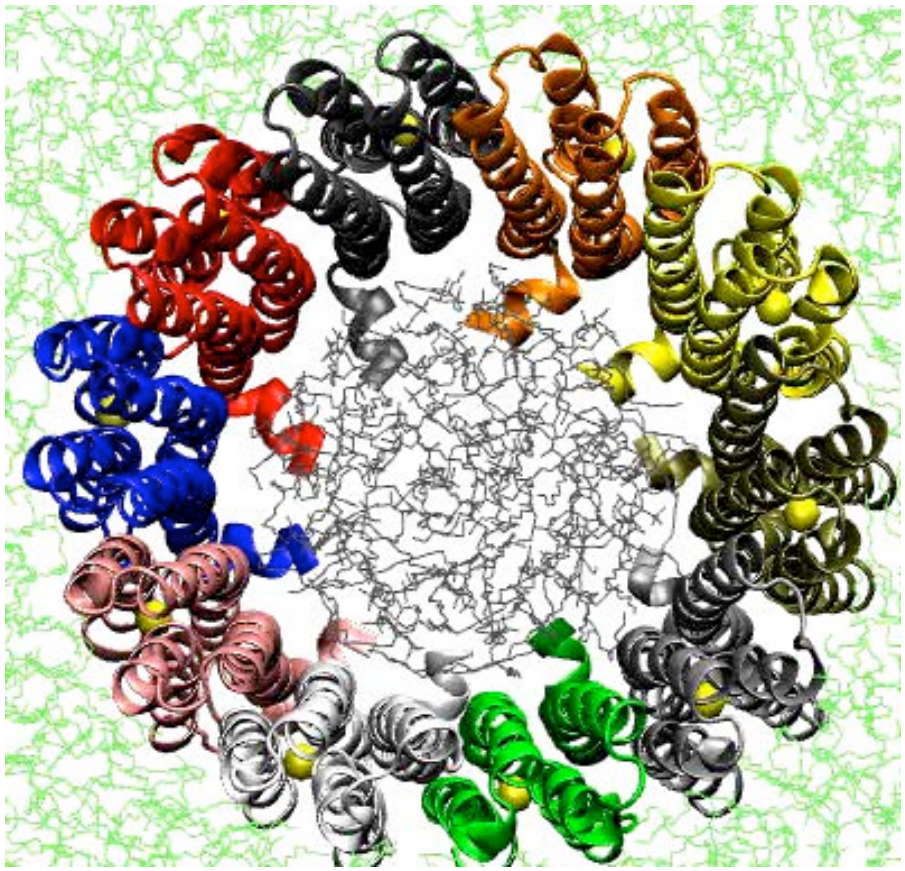
# $Ca^{+2}$-ATPase

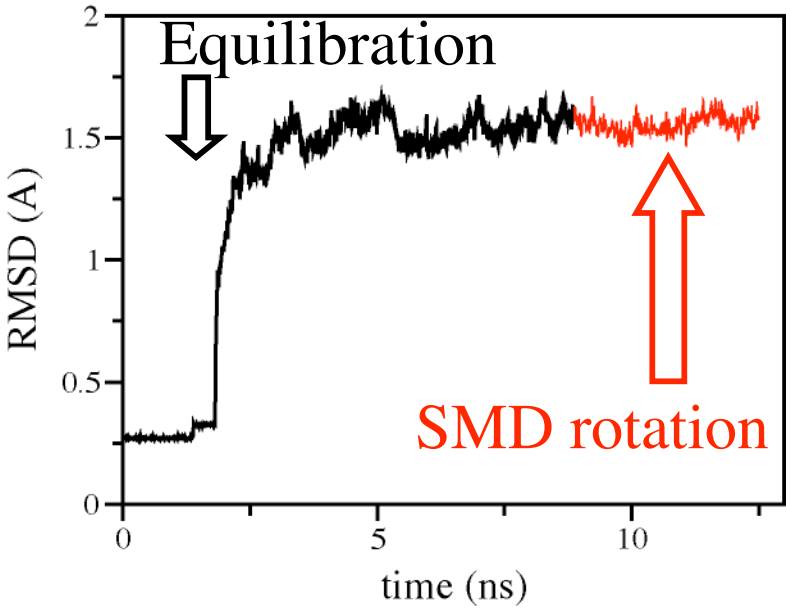

Chikashi Toyoshima's Lab



~215,000 atoms, 1SU4

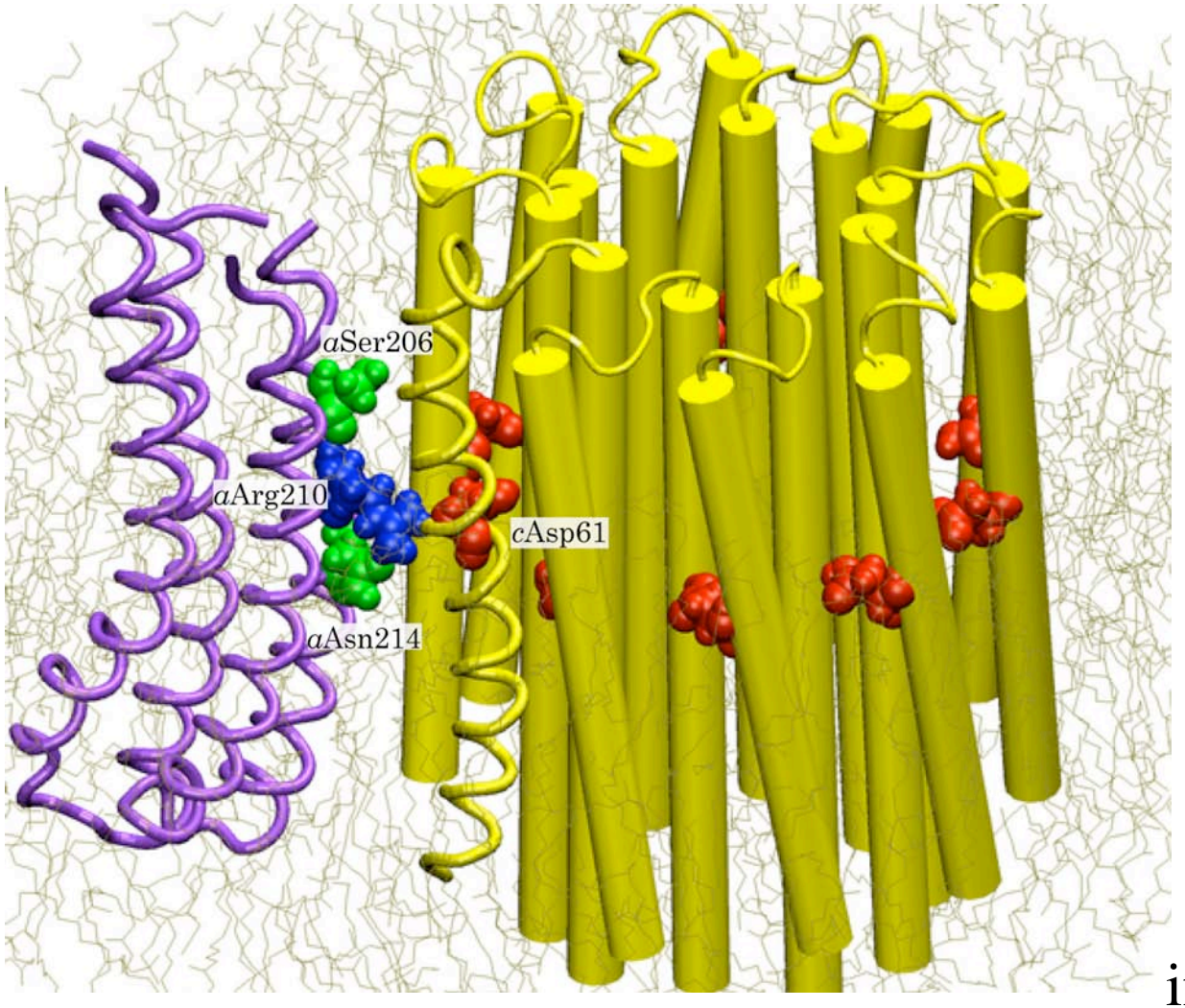
# Forced Rotation of $K_{10}$-Ring of V-ATPase

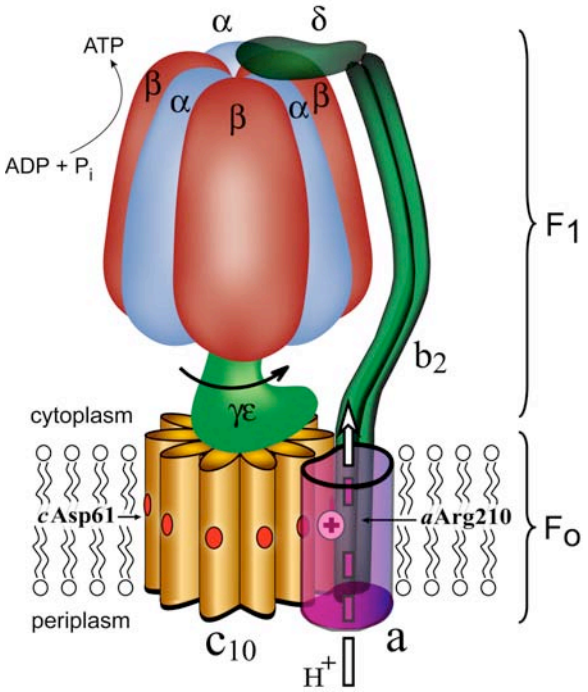





3.6-ns SMD simulation
torque: 1560 kcal/mol

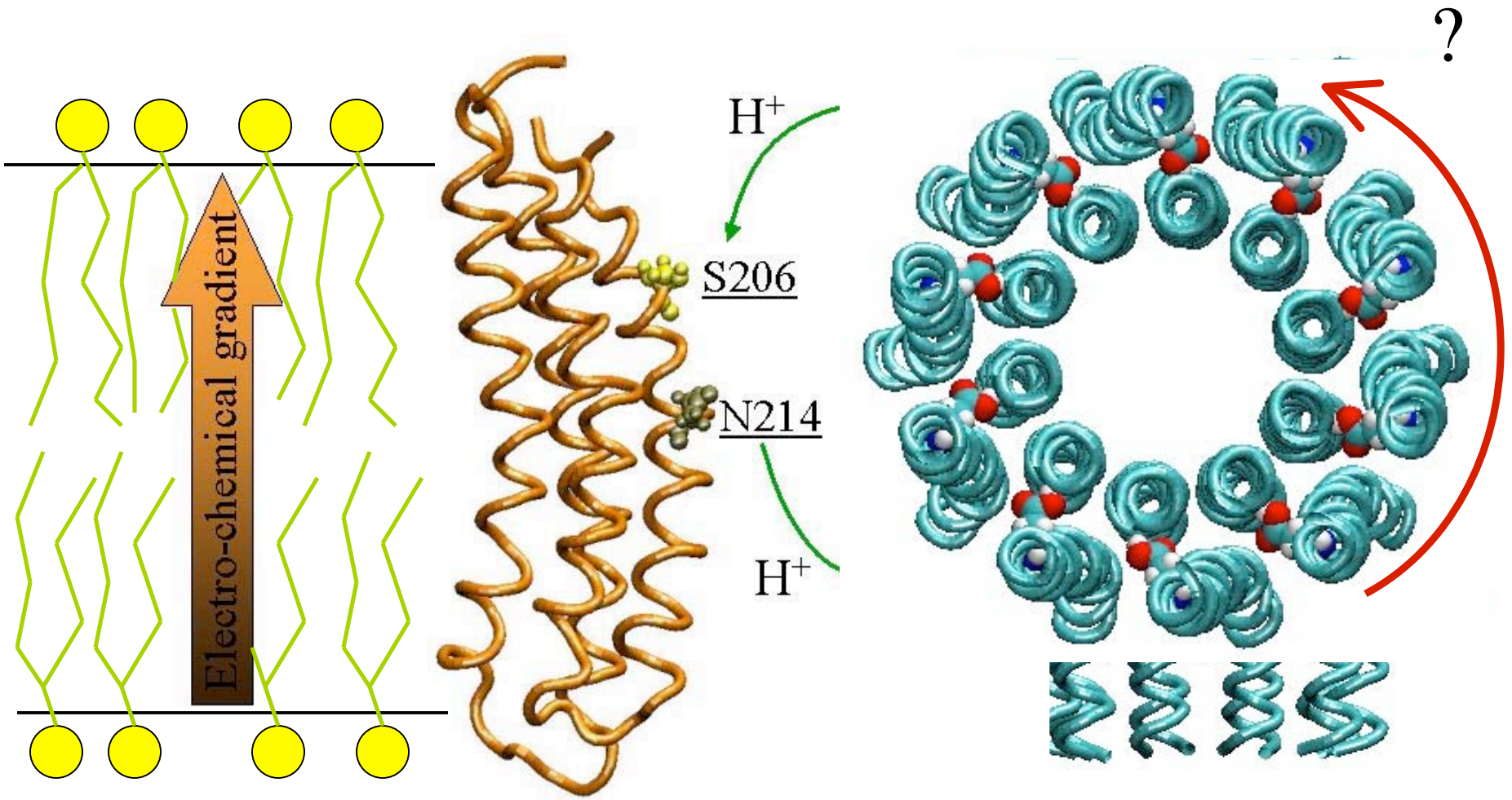
# What are the problems




How proton flux induces rotation of $c_{10}$?

# Suggested Mechanism of Proton Translocation




(R.H. Fillingame, 2002)

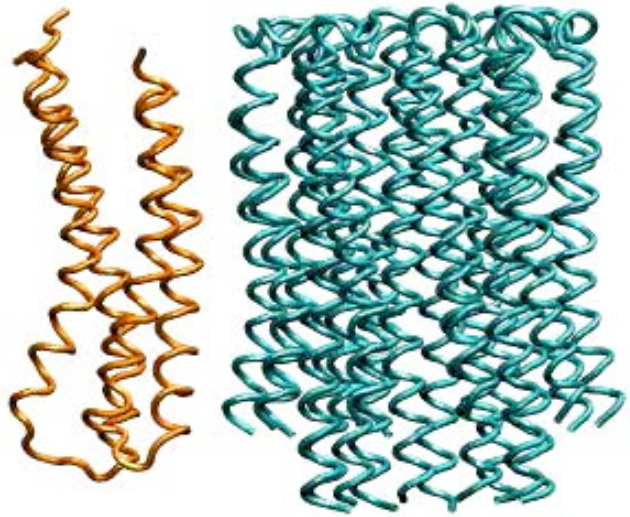
# Structural Model of *E. coli* $F_o$

$a_1c_{12}$ (Rastogi & Girvin, 1999, NMR)

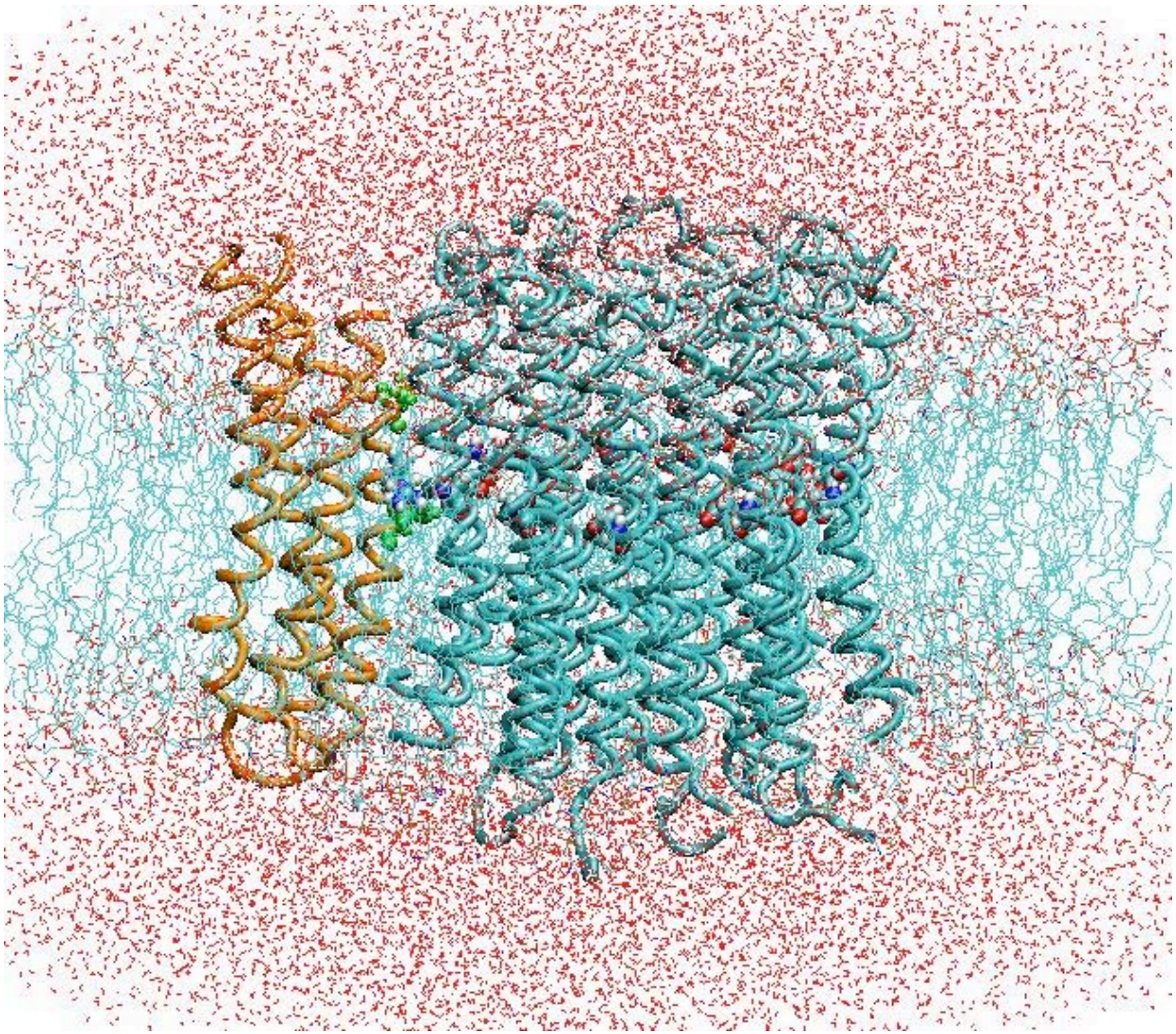
+

$c_{10}$ (Fillingame et al, 1999, NMR)

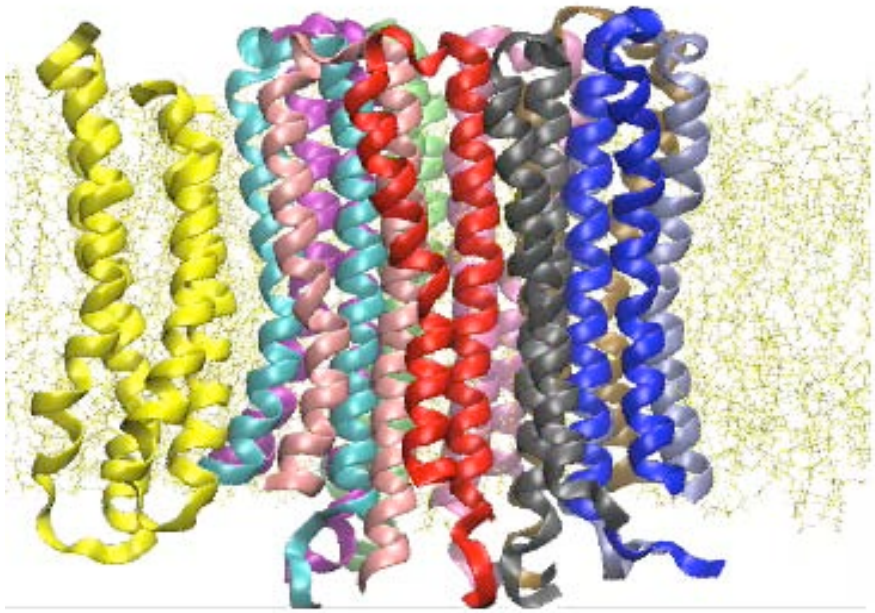
=



$a_1c_{10}$ (2001-2002, modeling)

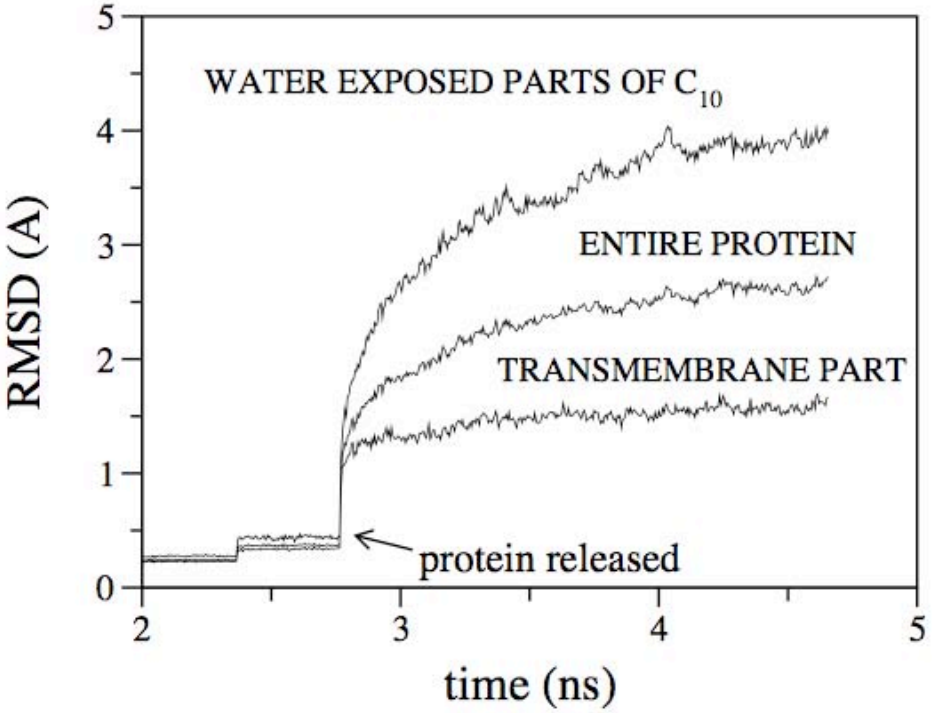


# Simulated Systems

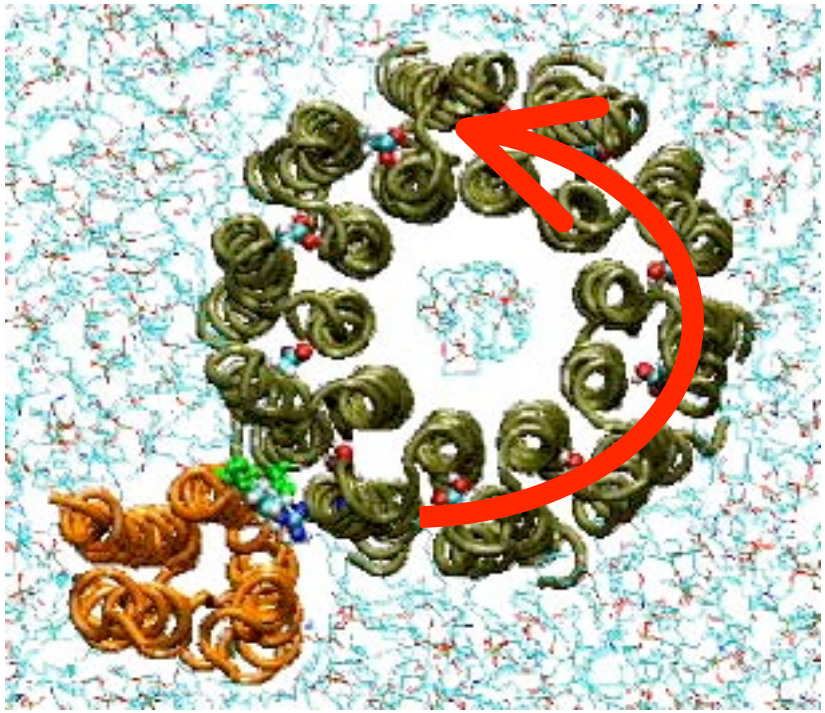


$a_1c_{10}$ + POPE + water
~112,000 atoms

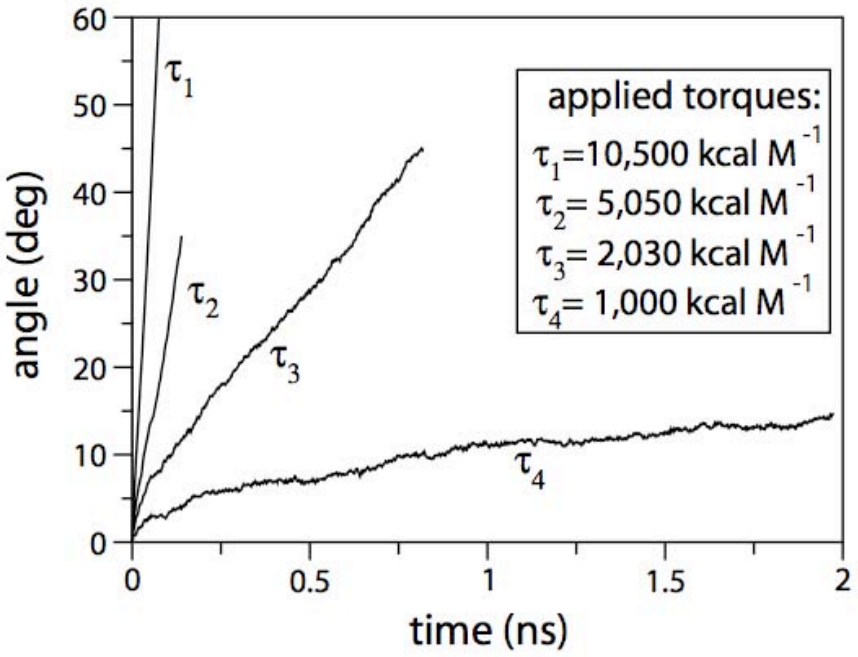
Water exposed parts of the $c_{10}$ oligomer alpha helices begun to unwind



# Forced Rotation of the $c_{10}$ Subunit



Forces were applied to all backbone atoms of $c_{10}$

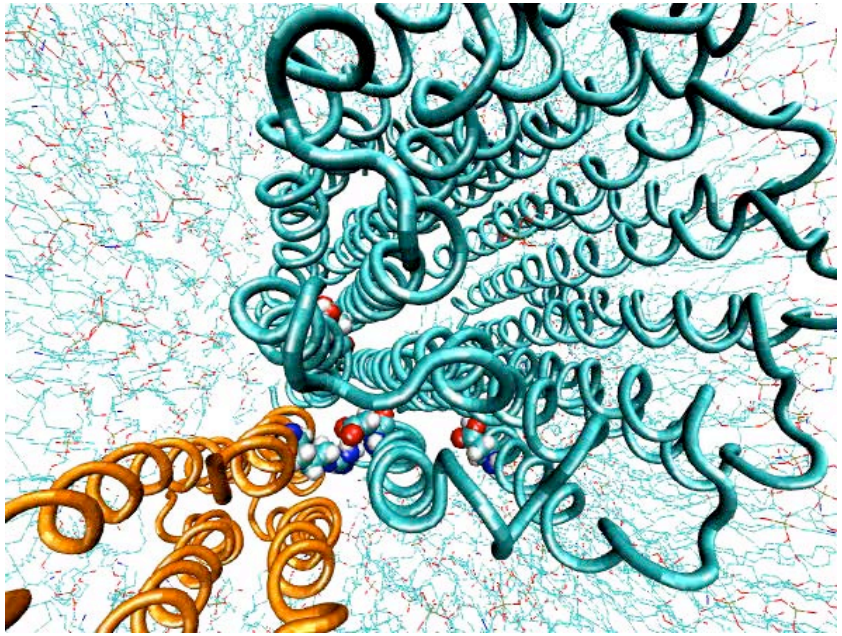



Estimated friction coefficient $\zeta \sim 10^5$ kcal/(M sec)

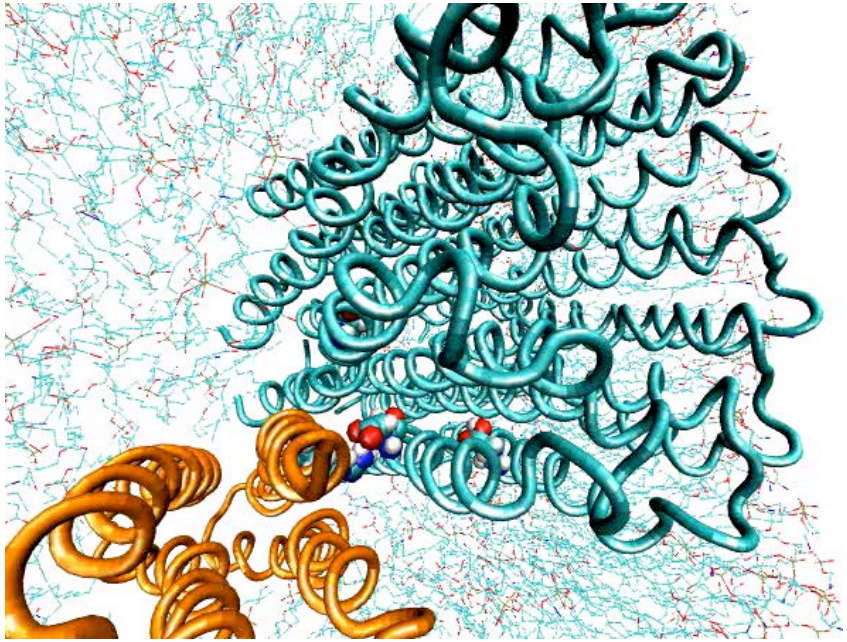
# Salt Bridge $Arg_{210}$-$Asp_{61}$ is Formed

**With only one $Asp_{61}$ residue deprotonated, SMD rotation of $c_{10}$ breaks the structure apart.**

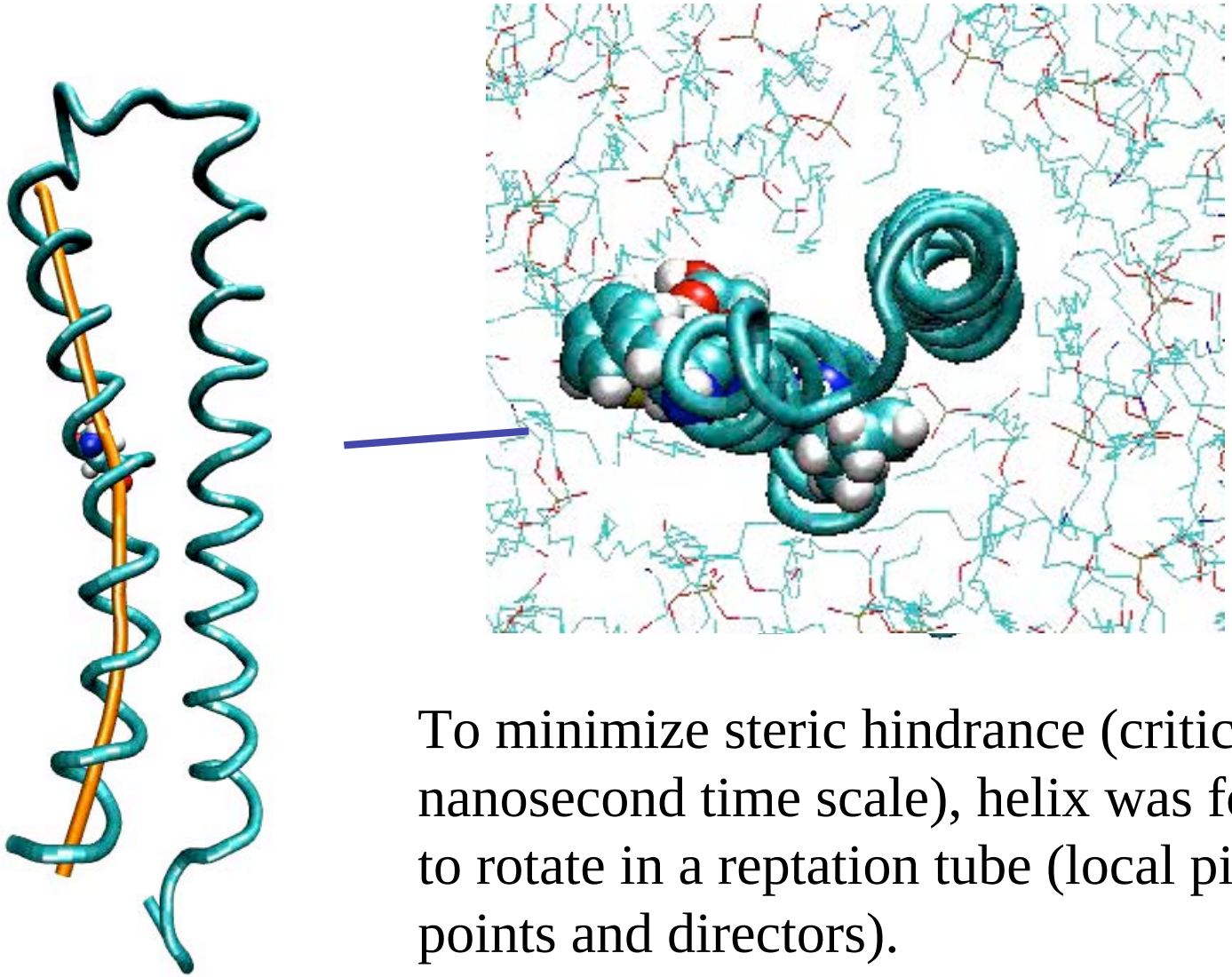
No restraints



Subunit *a* is restrained

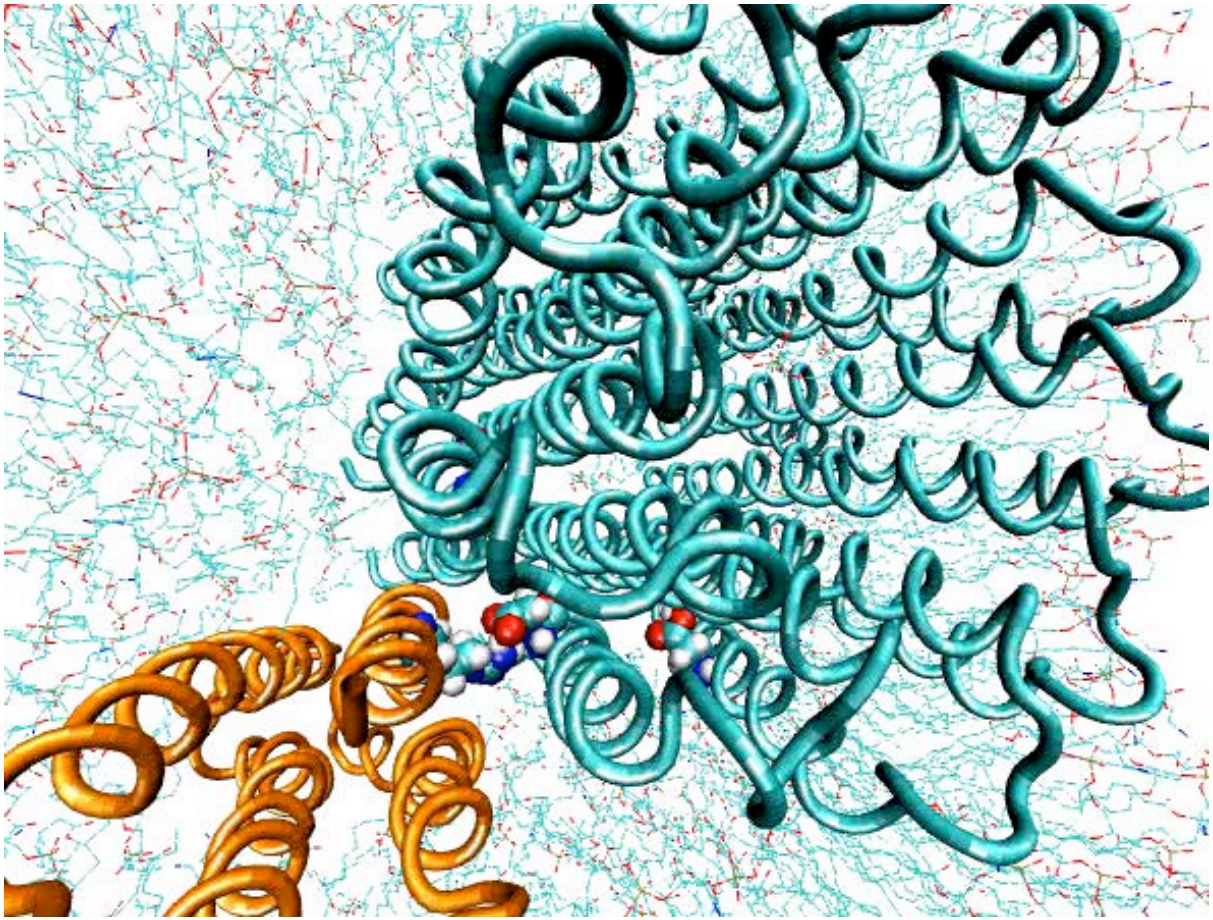


# Single Helix Rotation



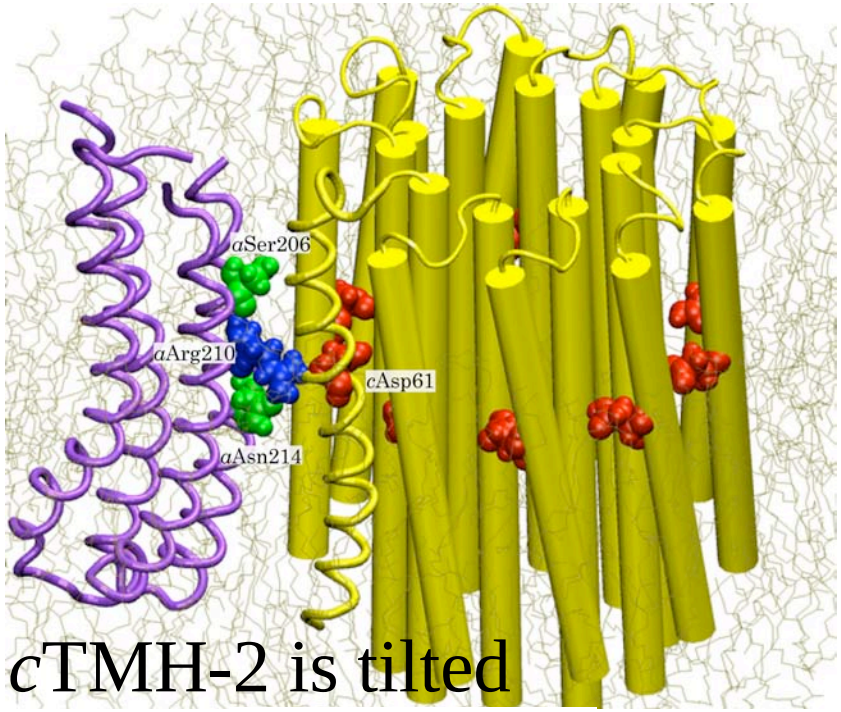
To minimize steric hindrance (critical on nanosecond time scale), helix was forced to rotate in a reptation tube (local pivot points and directors).

# Salt Bridge Can Be Transfered

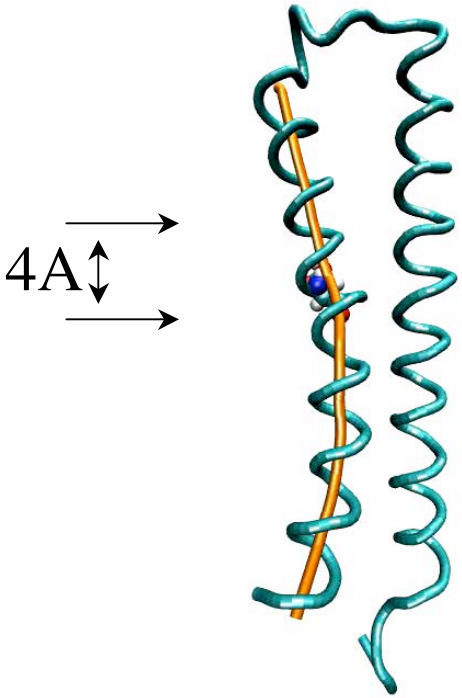


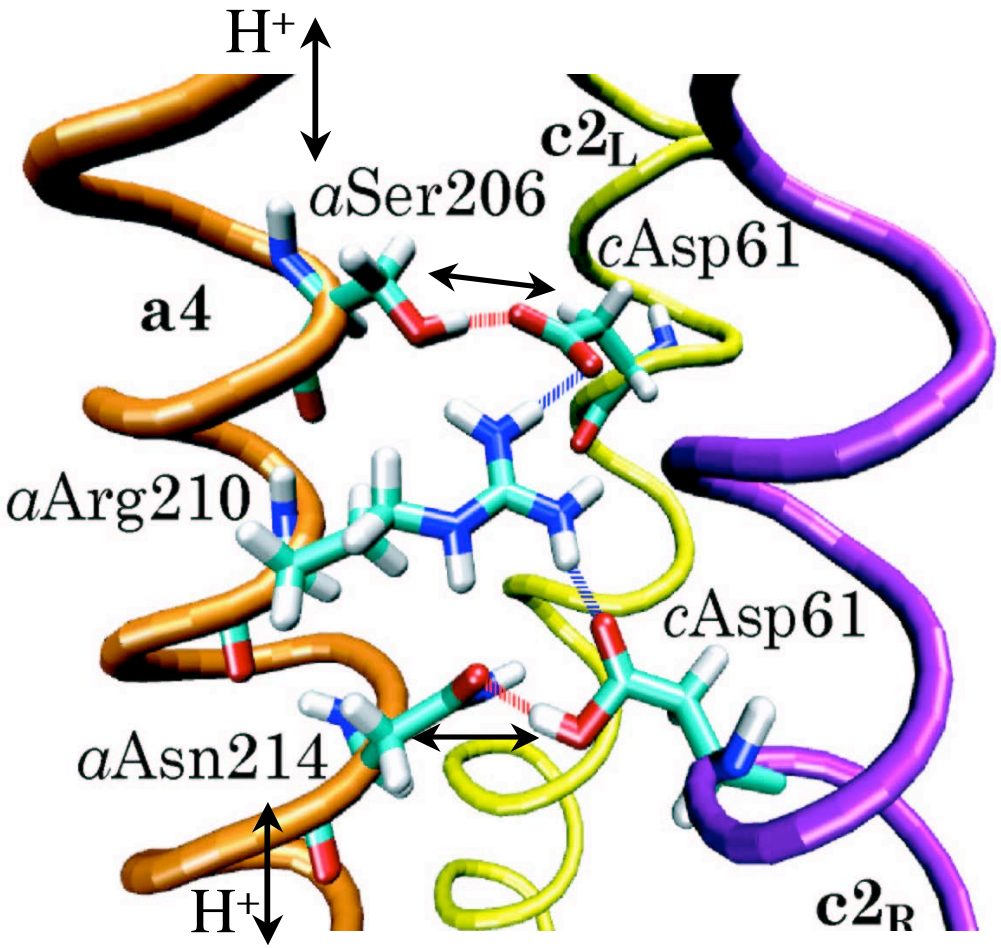
The salt bridge can be transferred by the concerted rotation of the $c_{10}$ complex and the outer TMH of subunit *c*

# Molecular mechanism for unidirectional rotation

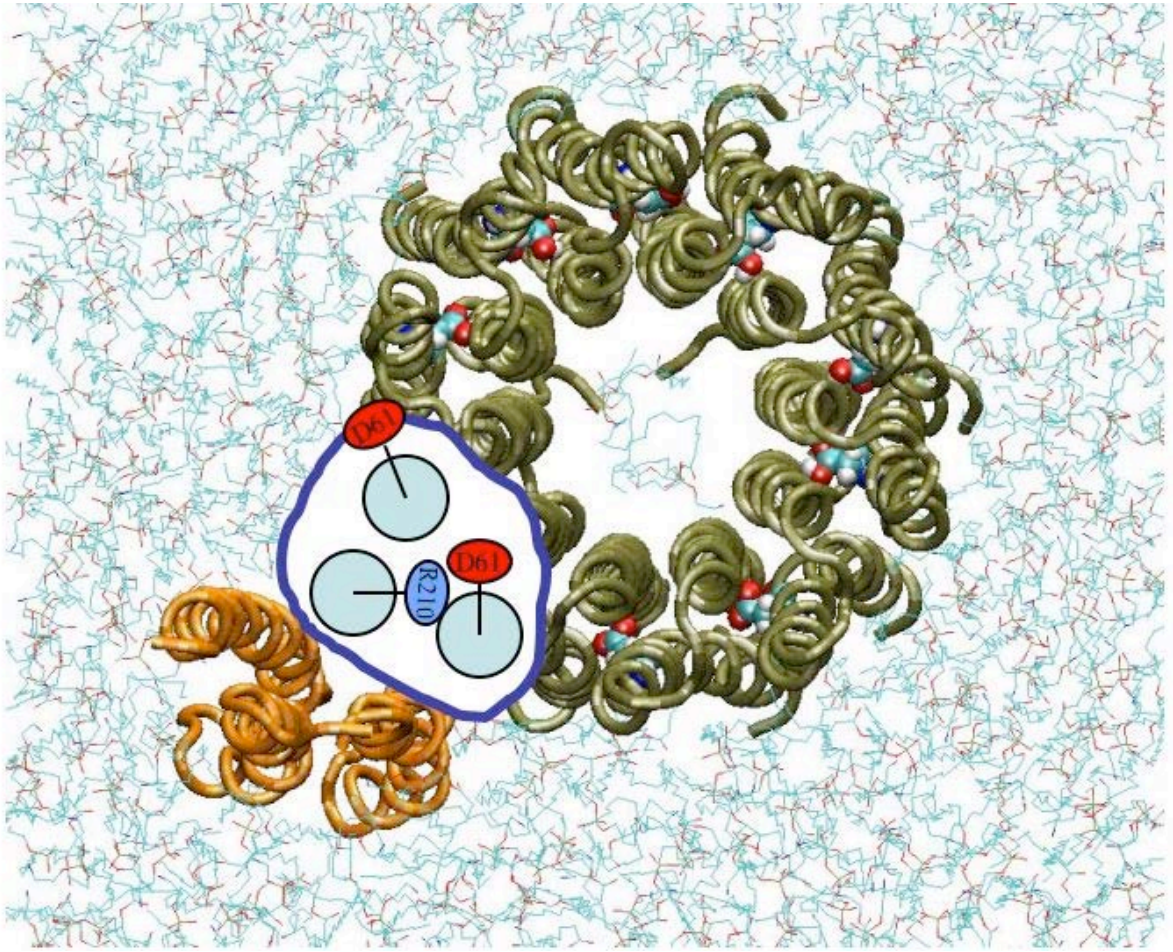



*c*TMH-2 is tilted



# Stochastic model




Microscopic model

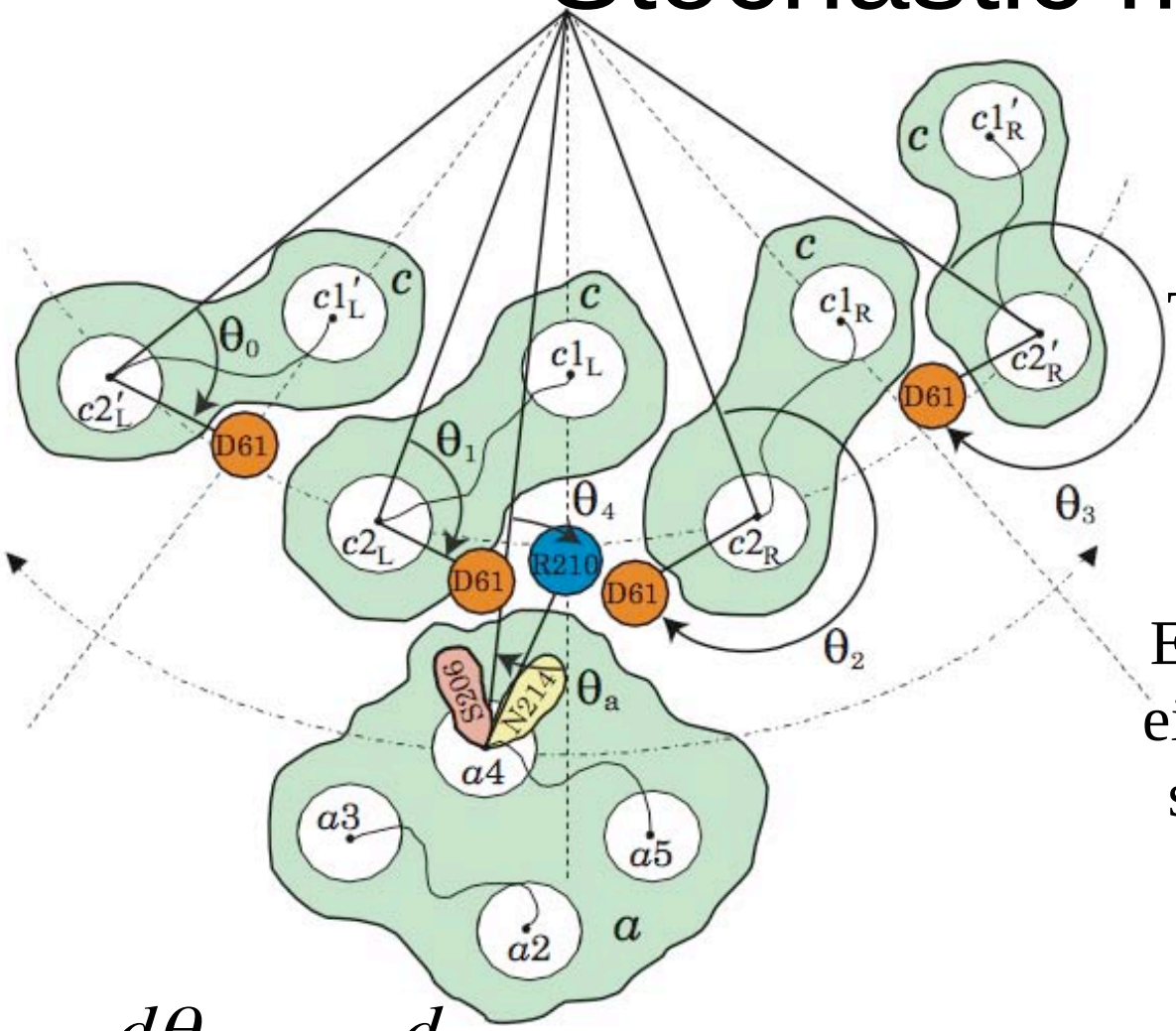
↓

Reduced system

↓

Principle degrees of freedom

# Stochastic model



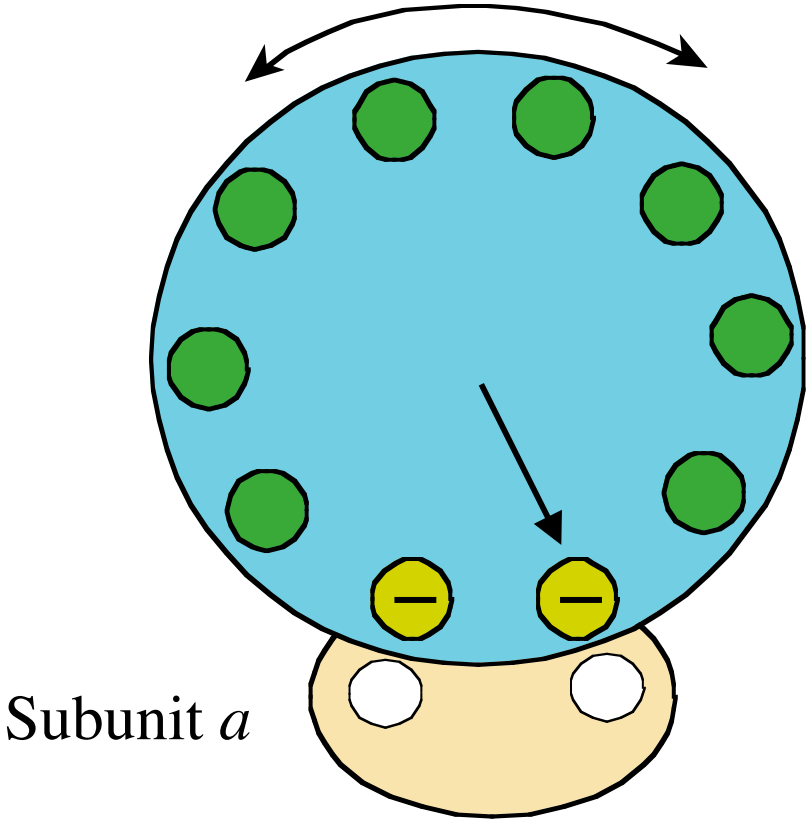

6 degrees of freedom: $\theta_0$, $\theta_1$, $\theta_2$, $\theta_3$, $\theta_4$ are TMH rotation angles; $\theta_A$ - position of the *a* subunit

Each Asp61 can be in either of two chemical states (protonated or deprotonated).

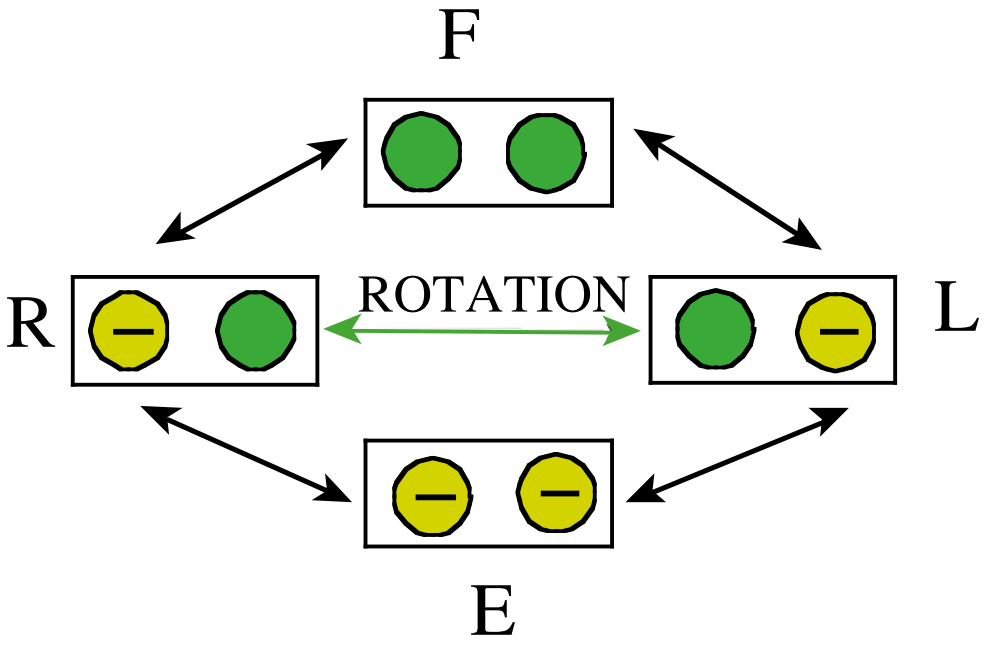
$$\zeta_i \frac{d\theta_i}{dt} = -\frac{d}{d\theta_i}[\mathrm{U}_{\mathrm{group}} + \mathrm{U}_{\mathrm{hydroph.}} + \mathrm{U}_{\mathrm{internal}} - \tau\theta_a] + \eta_i(t)$$

Langevin formulation:

$$\begin{cases} \zeta_i \dfrac{d\theta_i}{dt} = -\dfrac{d\Psi(\theta;s)}{d\theta_i} + f_i(t), \qquad s = R,L,F,E \\ \text{Chemical reaction is simulated as a Markov process} \end{cases}$$



Subunit *a*




4 states: R,L,F,E

Langevin formulation:

$$\begin{cases} \varsigma \dfrac{d\theta}{dt} = -\dfrac{d\Psi(\theta;s)}{d\theta} - \tau + f(t), \qquad s = R, L, F, E \\ \text{Chemical reaction is simulated as a Markov process} \end{cases}$$

Fokker Planck formulation:

$$\vec{\rho} = [\rho_E(\theta), \rho_R(\theta), \rho_F(\theta), \rho_L(\theta)]$$

$$\begin{cases} \dfrac{\partial \vec{\rho}}{\partial t} = \dfrac{1}{\varsigma} \dfrac{\partial}{\partial \theta} \left\{ \left( \dfrac{d\hat{\Psi}}{d\theta} + \tau \right) \vec{\rho} \right\} + D \dfrac{\partial^2 \vec{\rho}}{\partial \theta^2} + \hat{K}\vec{\rho} \\ \text{Boundary conditions} \end{cases}$$

rate constant matrix

diffusion constant

# Stochastic Simulations of $F_o$ Operation