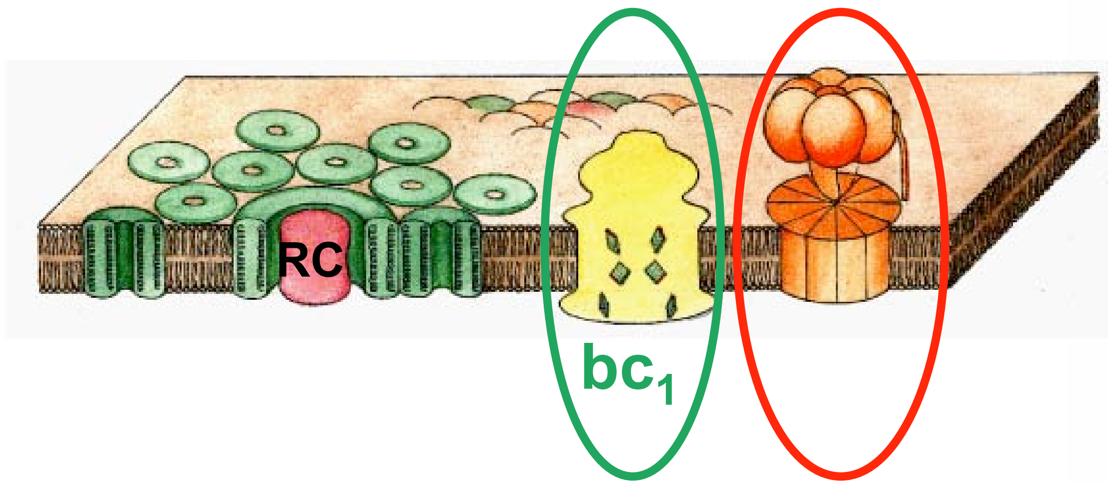
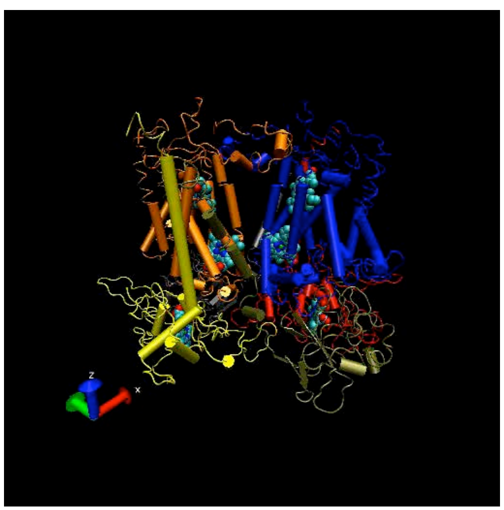
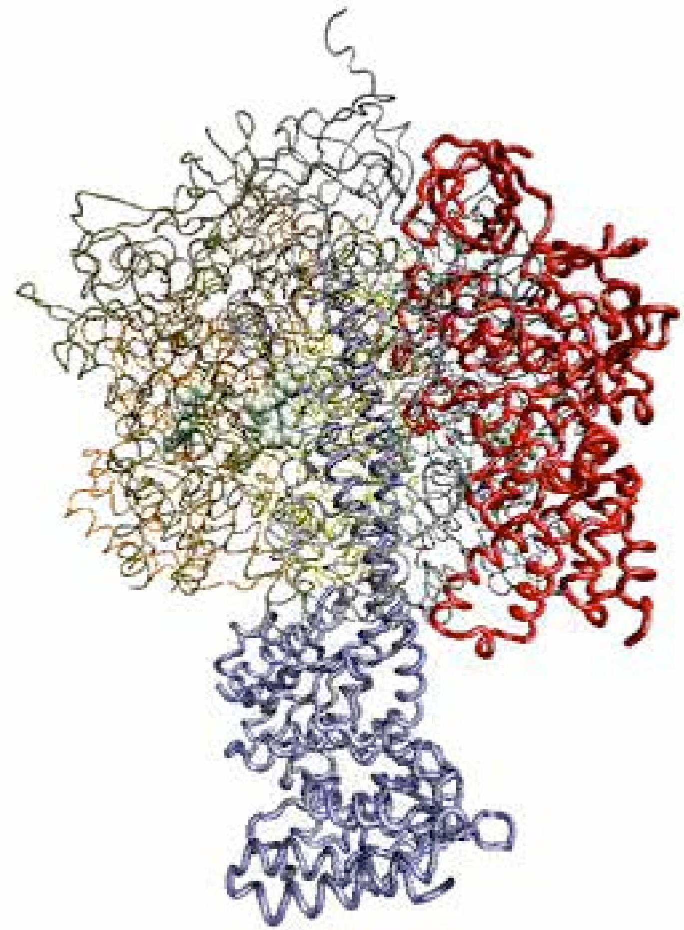
ATPase Synthase - A Molecular Double Motor



bc$_1$

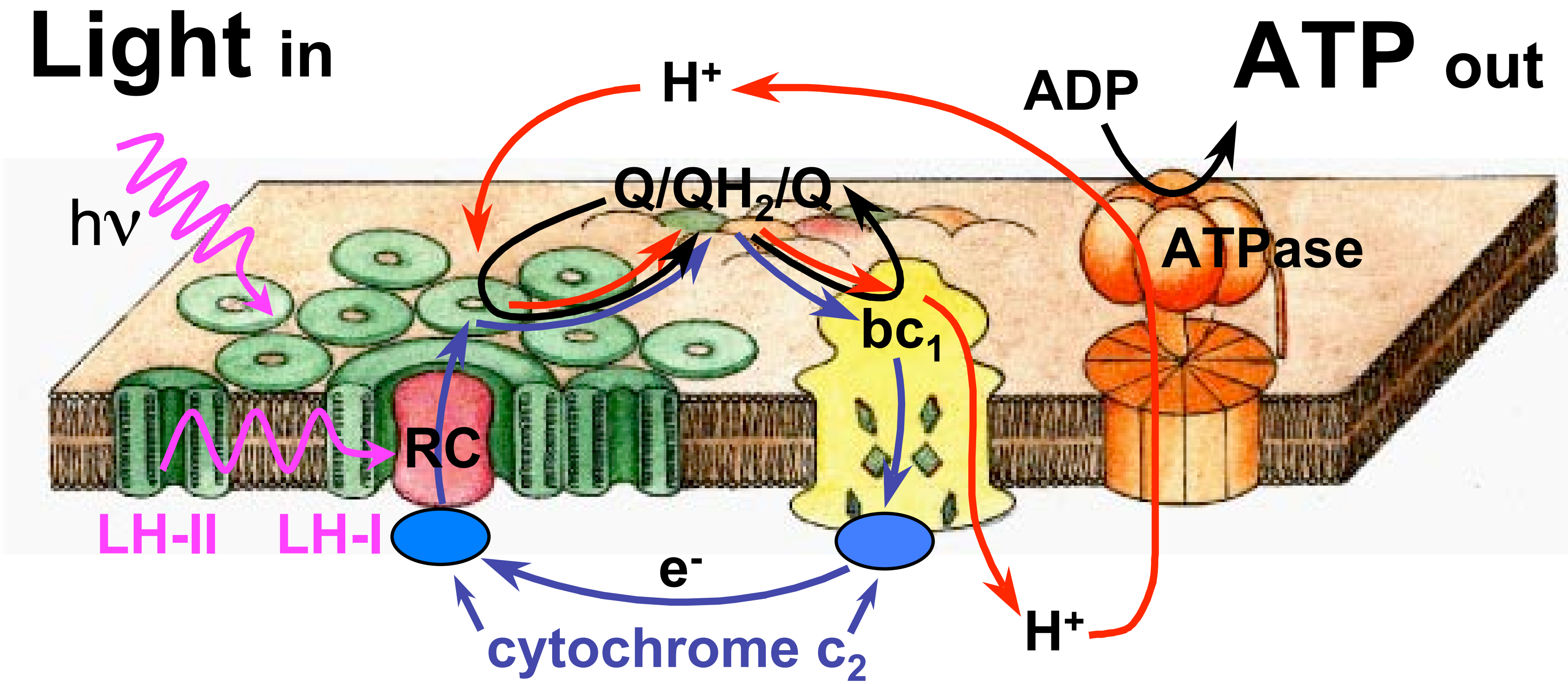


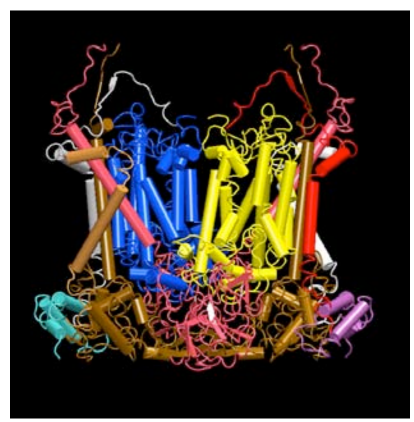


ATPase

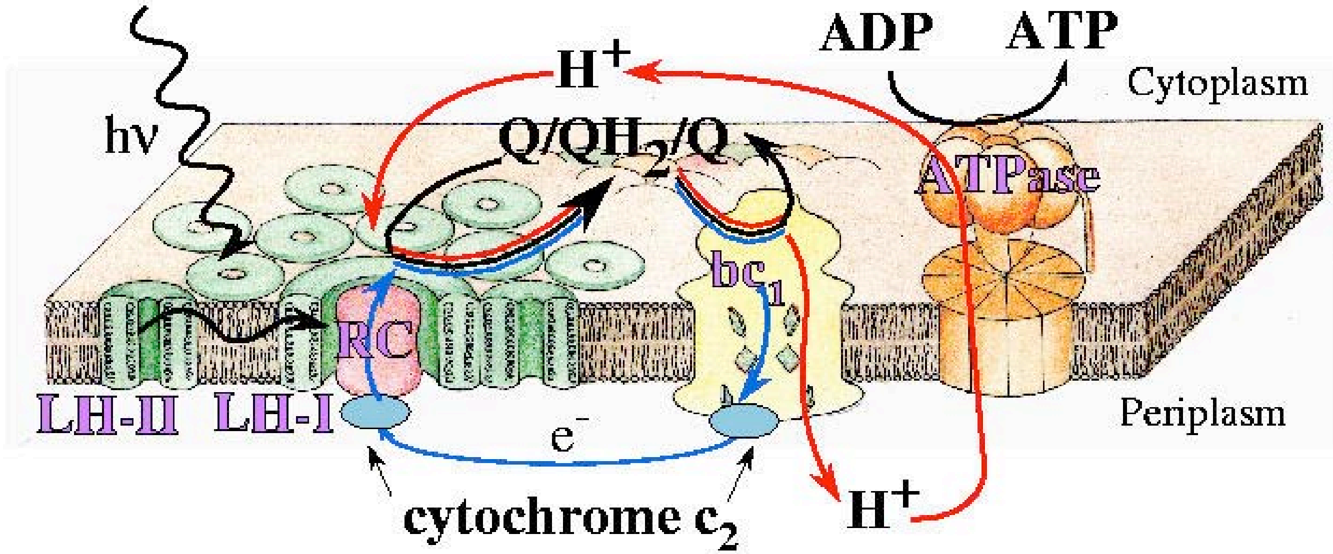
Photosynthesic Unit of Purple Bacteria

Module that converts sun light into chemical energy (ATP)

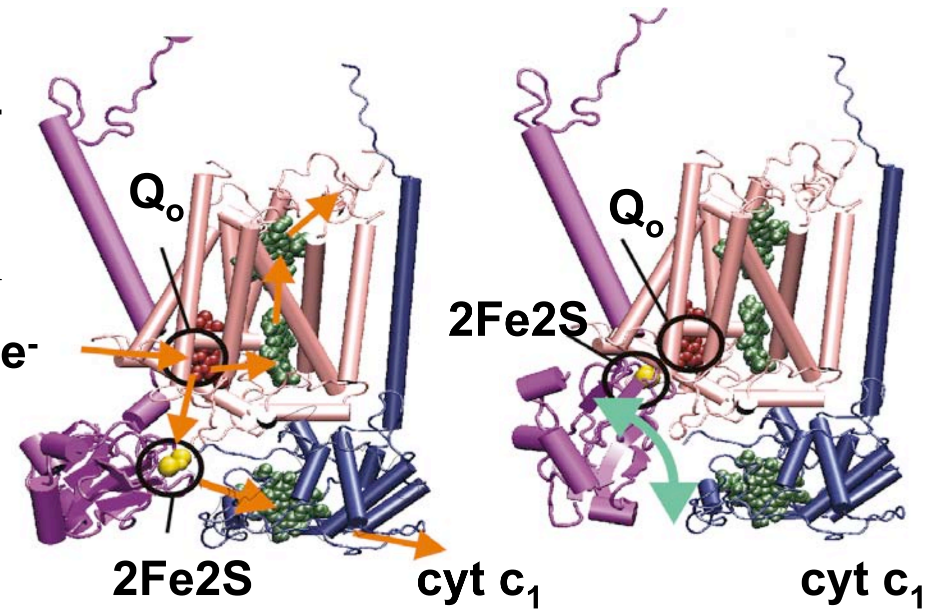




Mechanism of the bc1 Complex in the Photosyntehtic Unit



two path-ways for oxidation of Q_o site

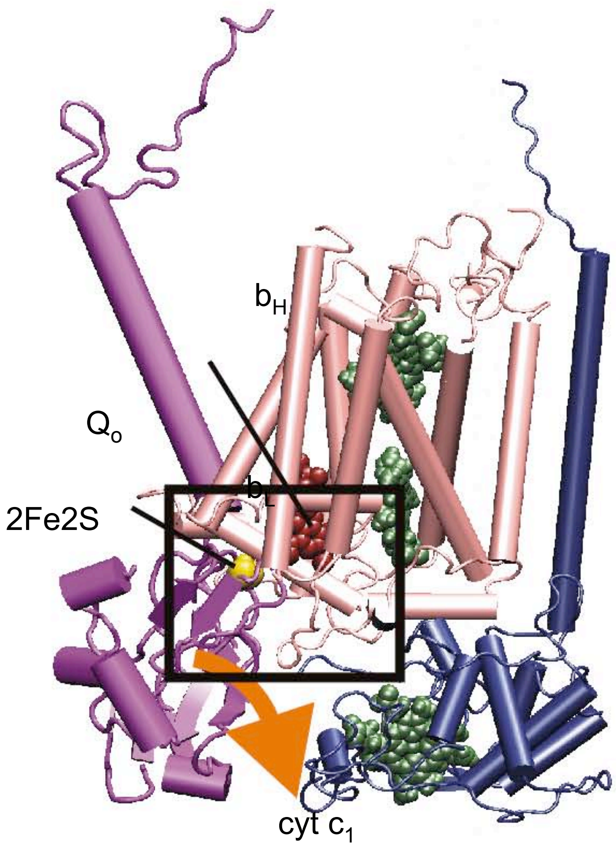


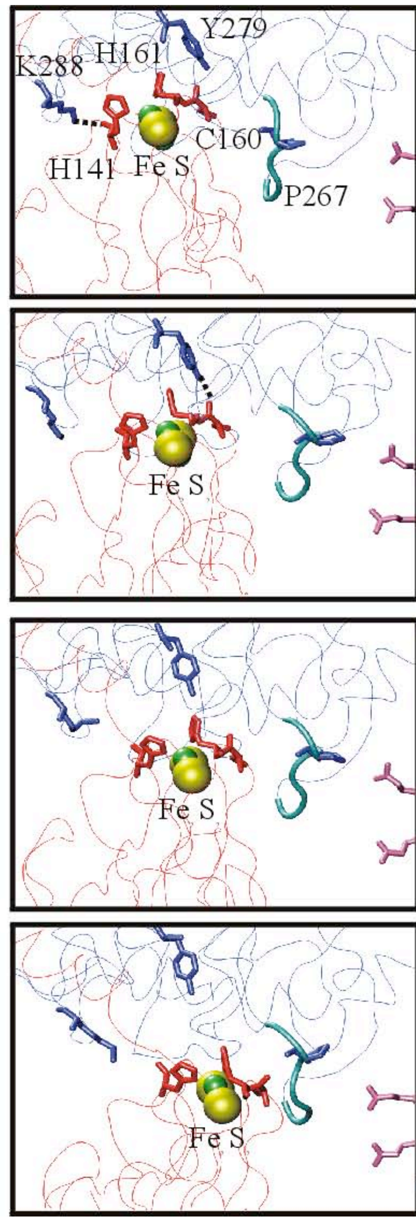
Iron Sulfur Protein (ISP) head rotation can redirect 2nd electron

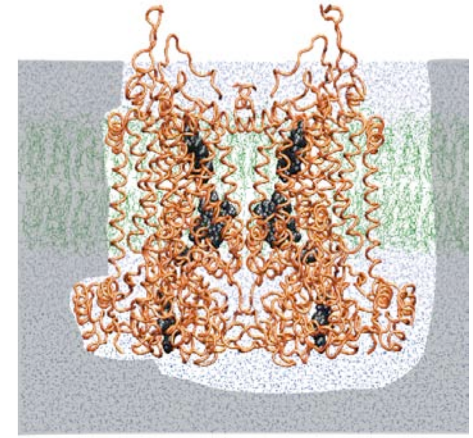
Enforcing domain rotation in the bc_1 complex

Events during torque application to ISP head

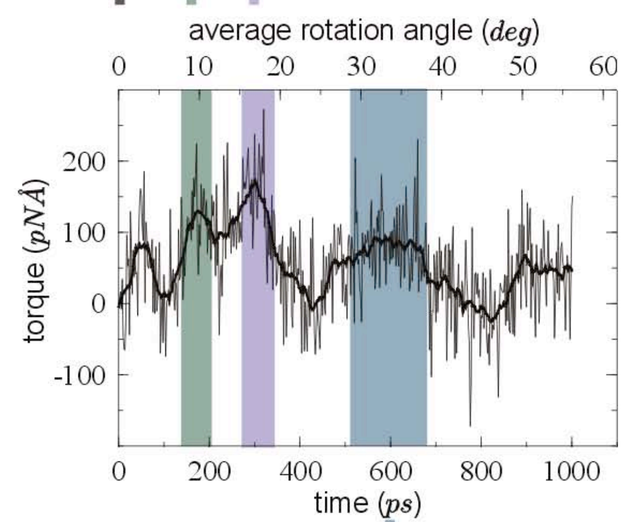
Izrailev et al., Biophys J., 77:1753-1768 (1999)





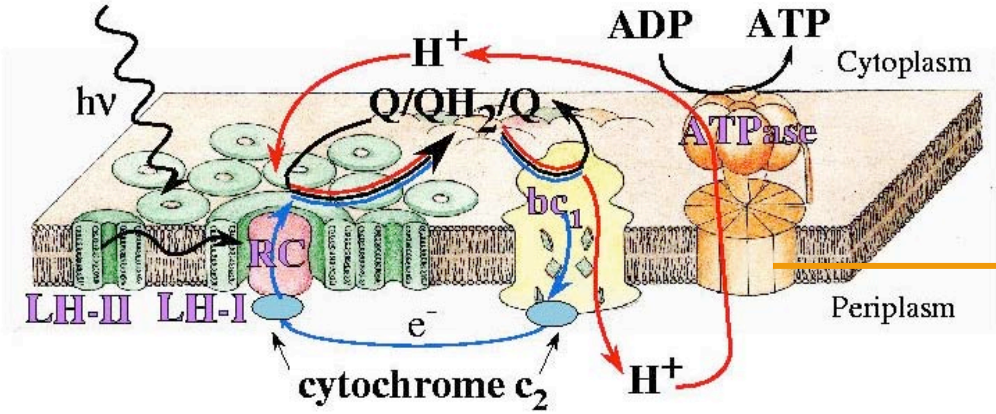


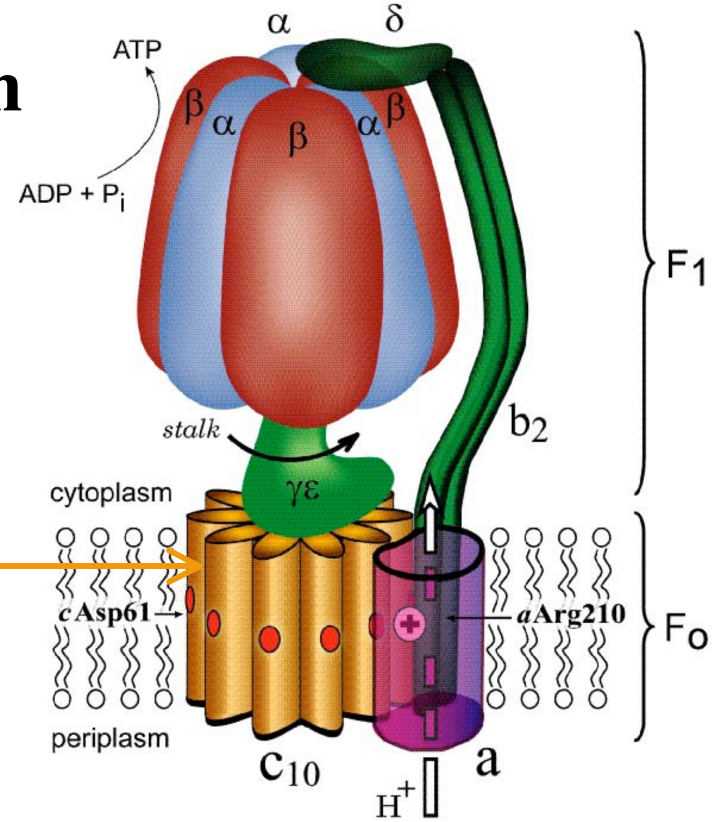
206,720 atoms

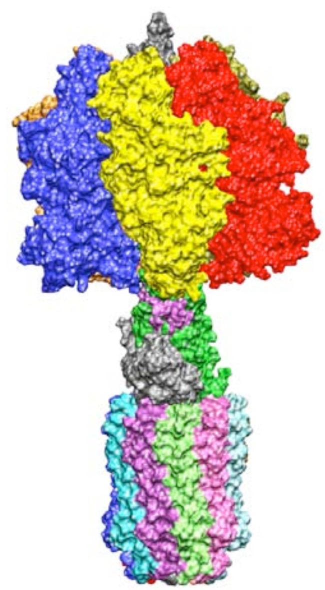


Torque applied
to 126 C_α atoms
$K = 70$ pN/Å
$\omega = 0.0561$ rad/s

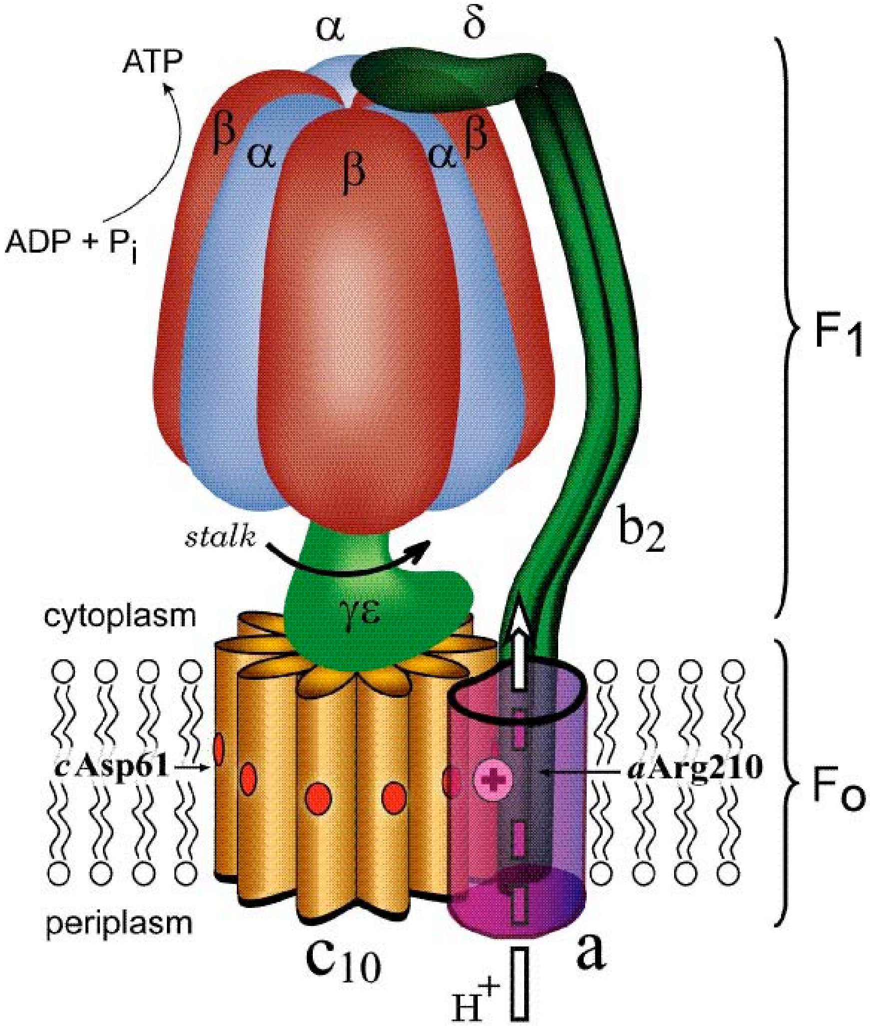
Mechanisms of Rotatory Molecular Motor that Converts Voltage (proton gradient) into ATP Synthesis







Adenosine Triphosphate (ATP) Synthase

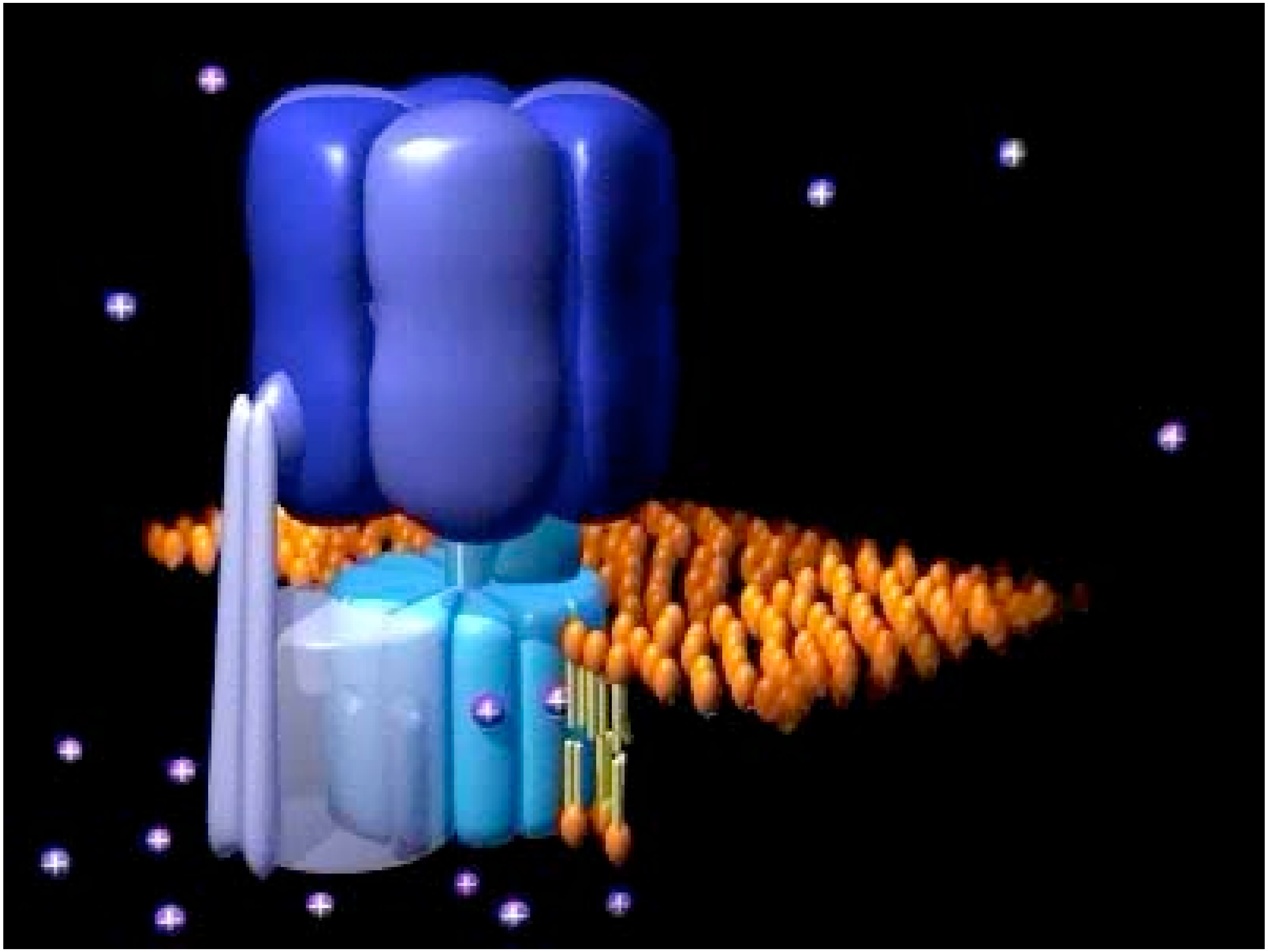


Rotary catalysis: Two protein motors coupled via common central stalk γδ

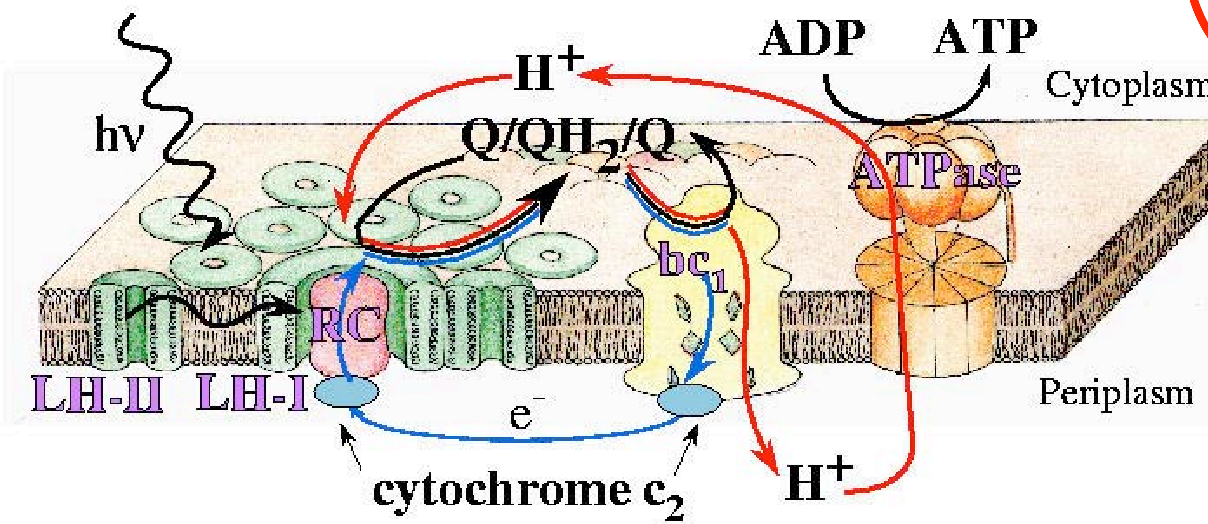
Solvent exposed F_1 unit ($\alpha_3\beta_3\gamma\delta\varepsilon$): central stalk rotation causes conformational changes in catalytic sites, driving ATP synthesis

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

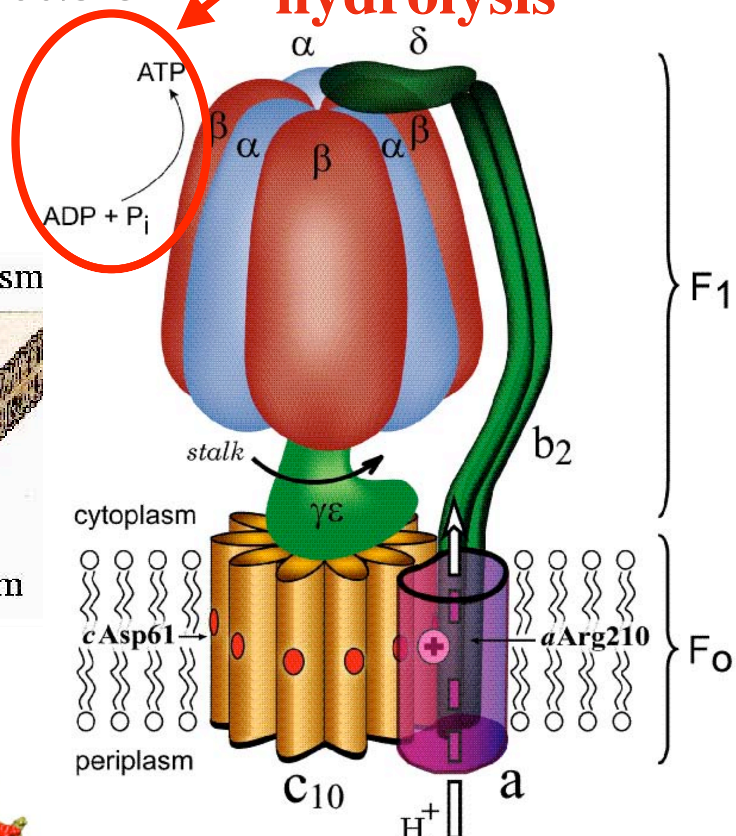
Animation of the ATP Synthase

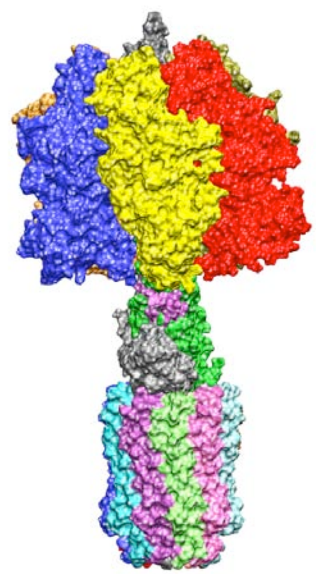


Mechanism of ATP synthase

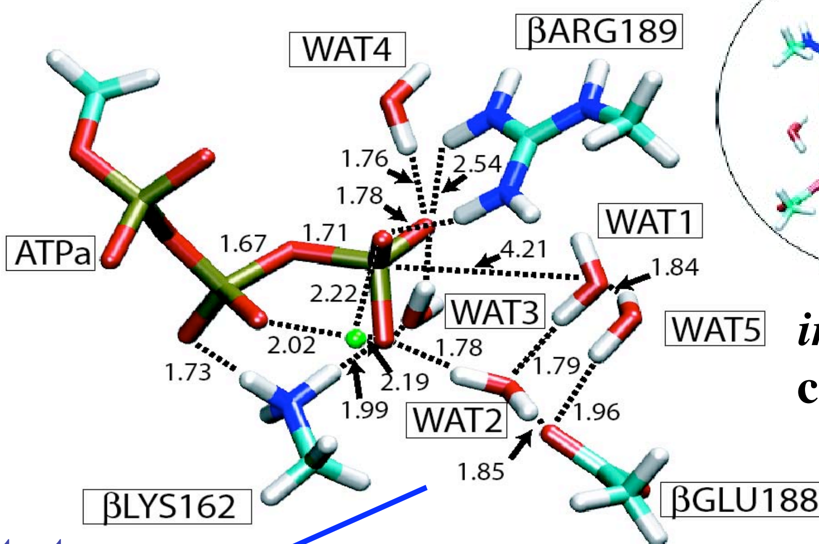


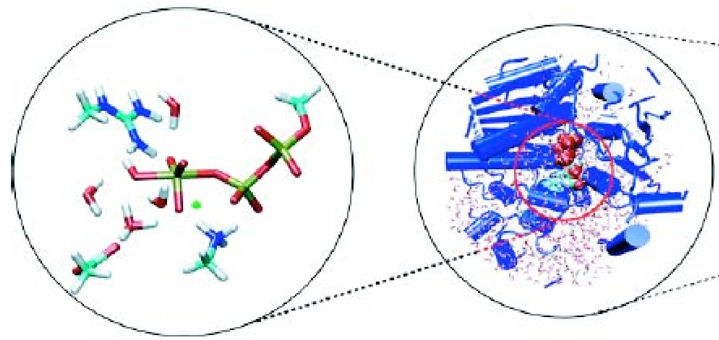
ATP synthesis / hydrolysis

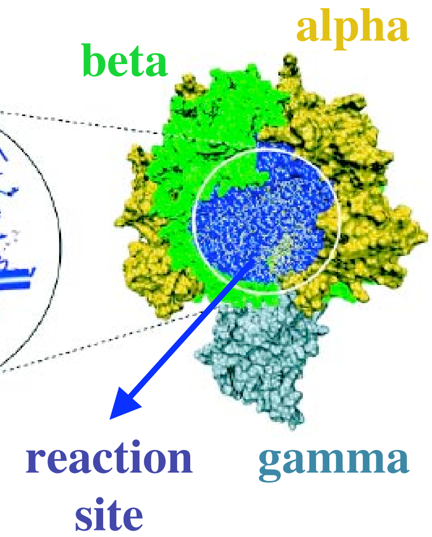




Reaction Mechanism of ATP Hydrolysis

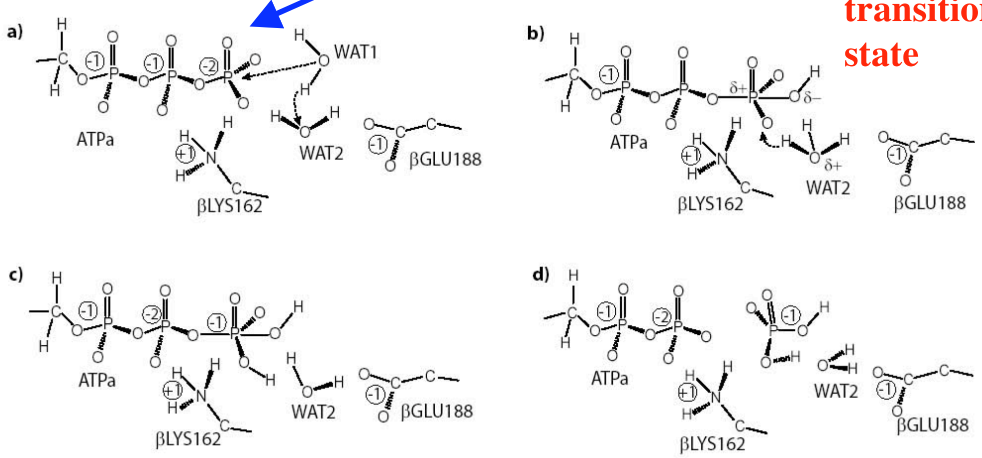




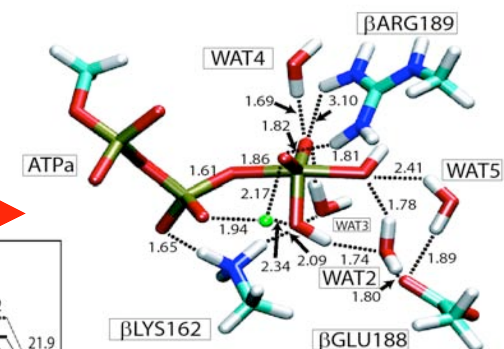


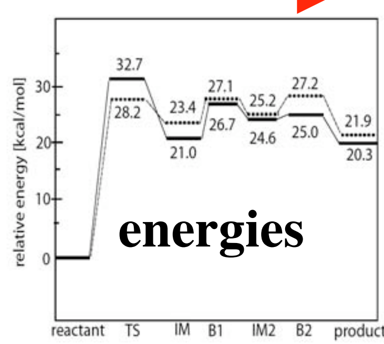
in situ quantum (QM/MM) chemistry calculation

reactant



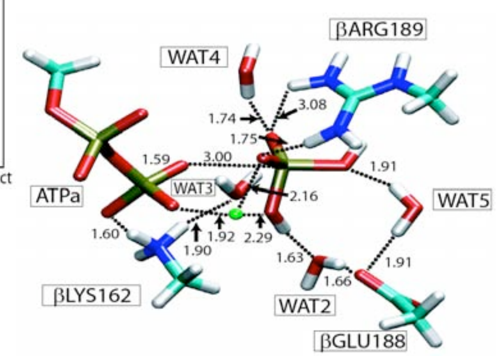
transition state



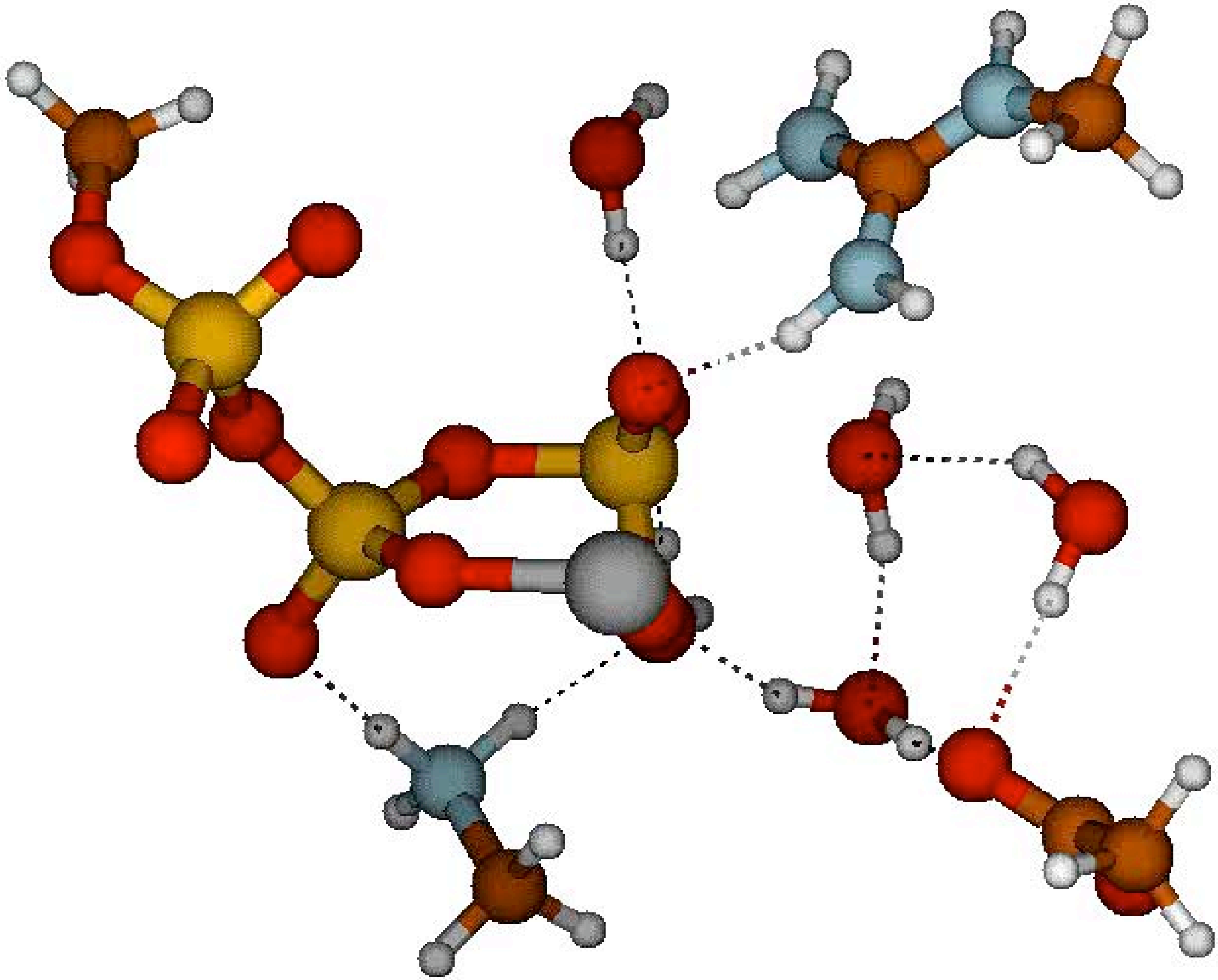


energies

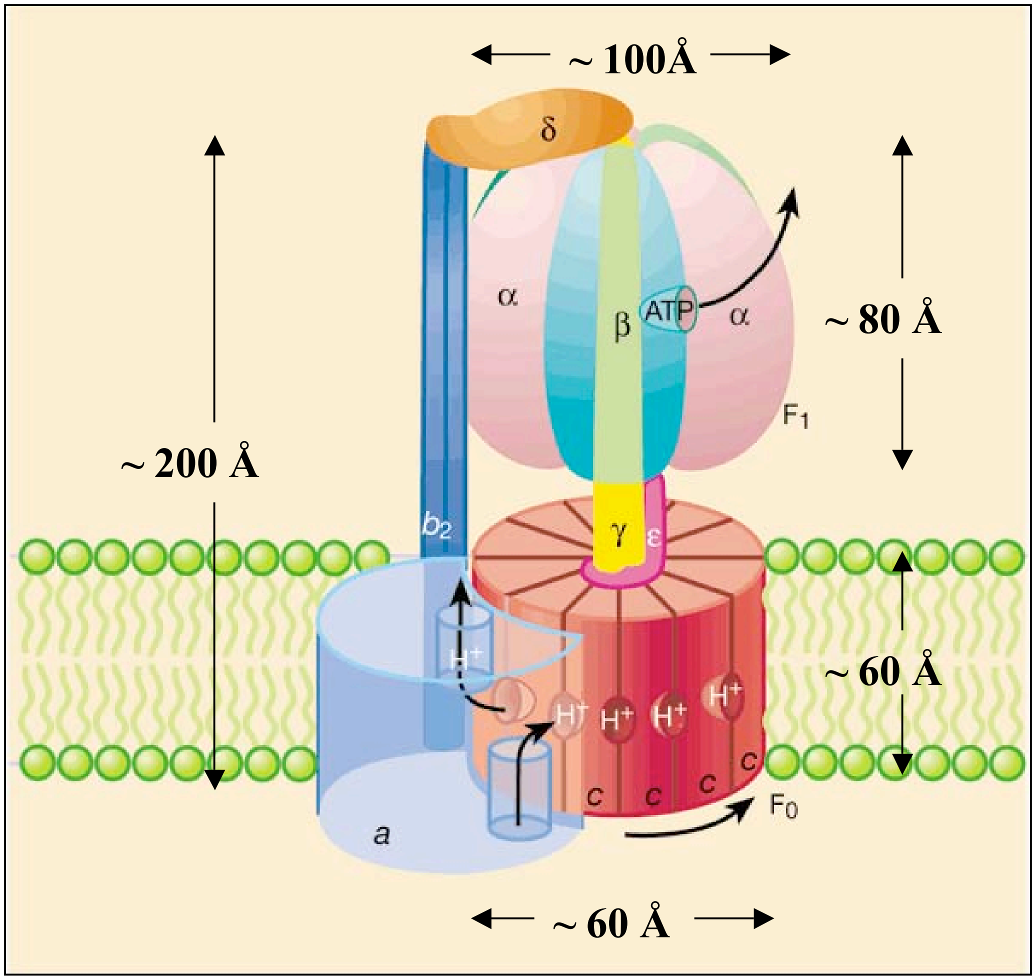
product

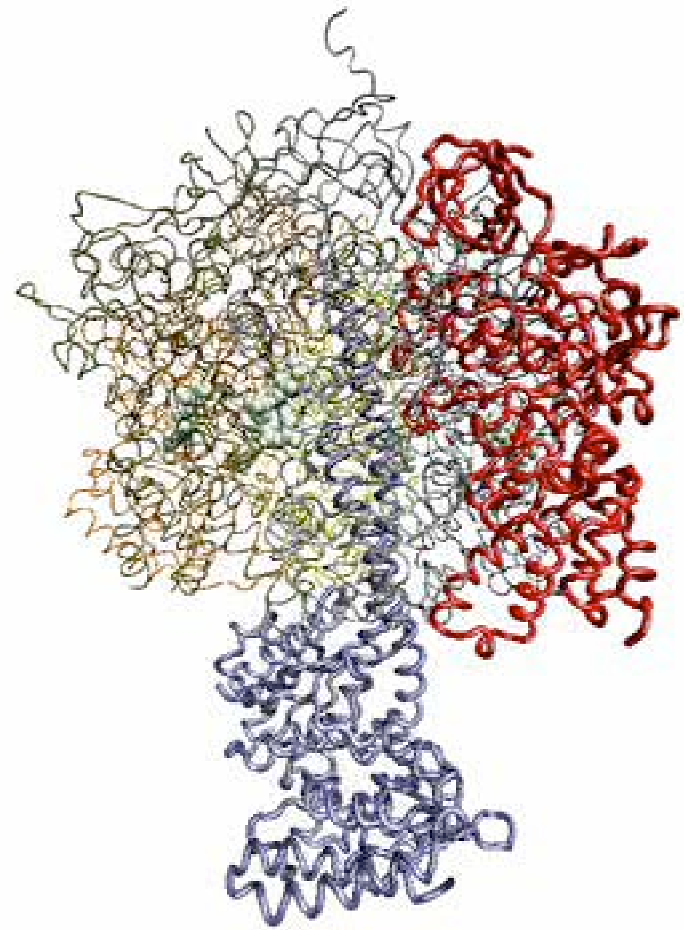


Mechanism of ATP Hydrolysis in F1 ATPase



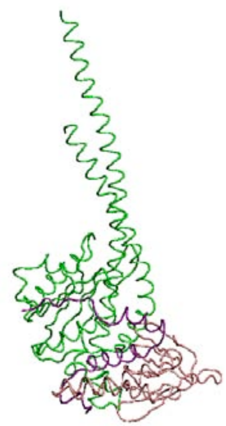
Let’s look at F1





Torque is transmitted between the motors via the central stalk.

Assembling ATP Synthase F_1



- Start with DCCD-inhibited structure, has near-complete stalk. (Gibbons 2000, PDB code 1E79)
- Total **327,000 atoms** (3325 residues, 92,000 water molecules, nucleotides, and ions).
- The 1.2 ns equilibration + 10.5 ns torque application were performed on NCSA Platinum and PSC Lemieux as parallel NAMD jobs using up to 512 processors.

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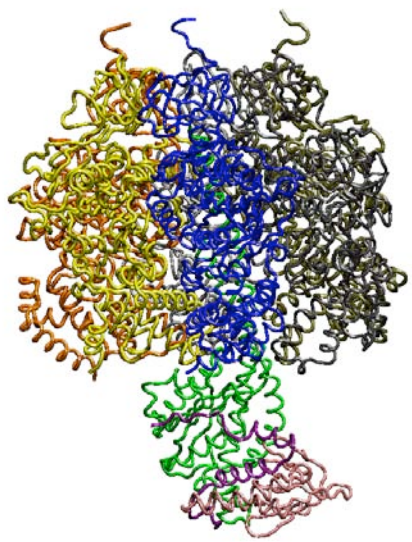

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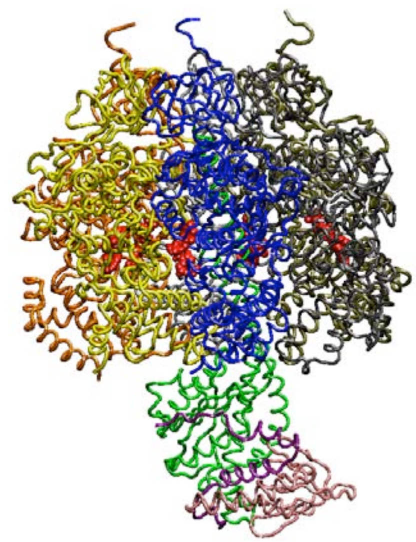

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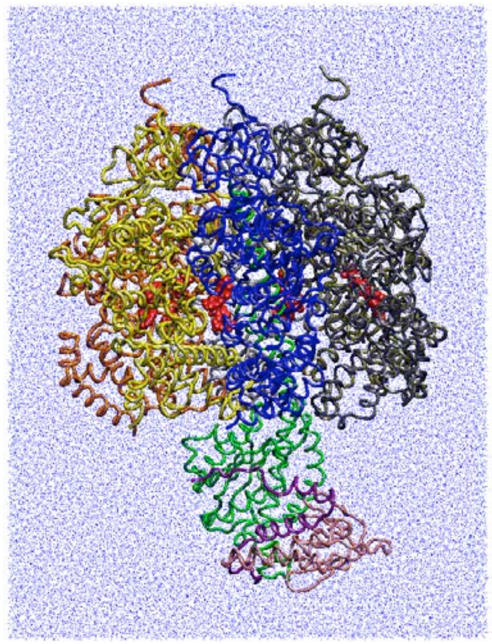

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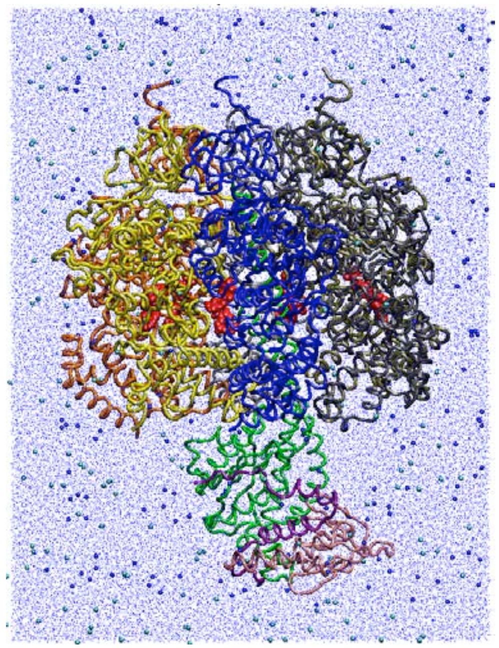

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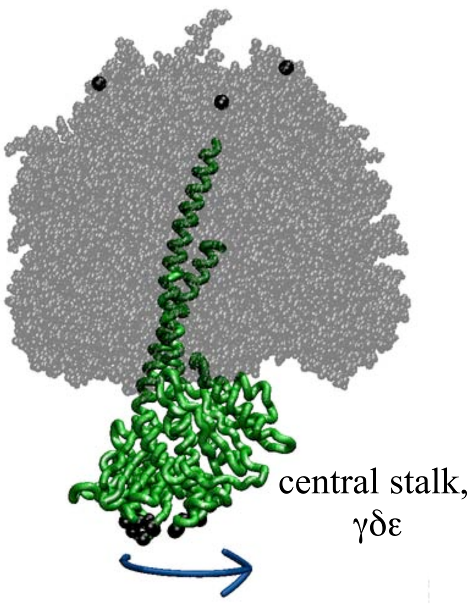
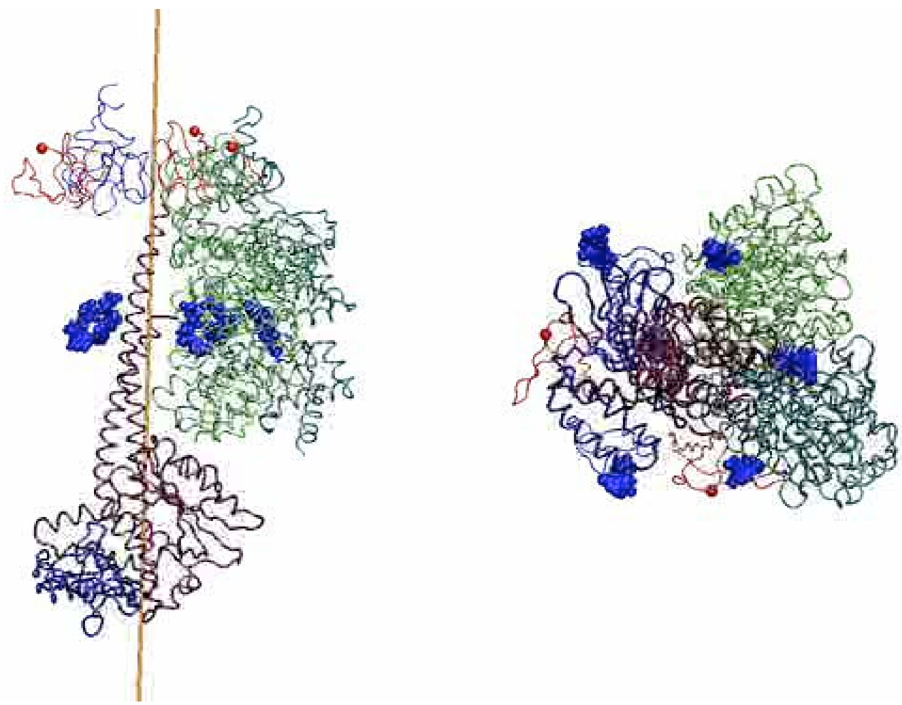
Torque application to F_1

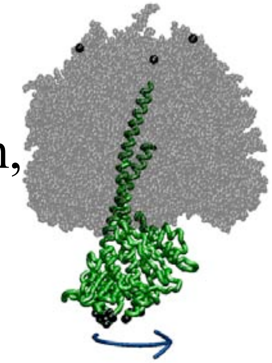
Torque is applied to the central stalk atoms at the F_1-F_o interface to constrain their rotation to constant angular velocity ω = 24 deg/ns.





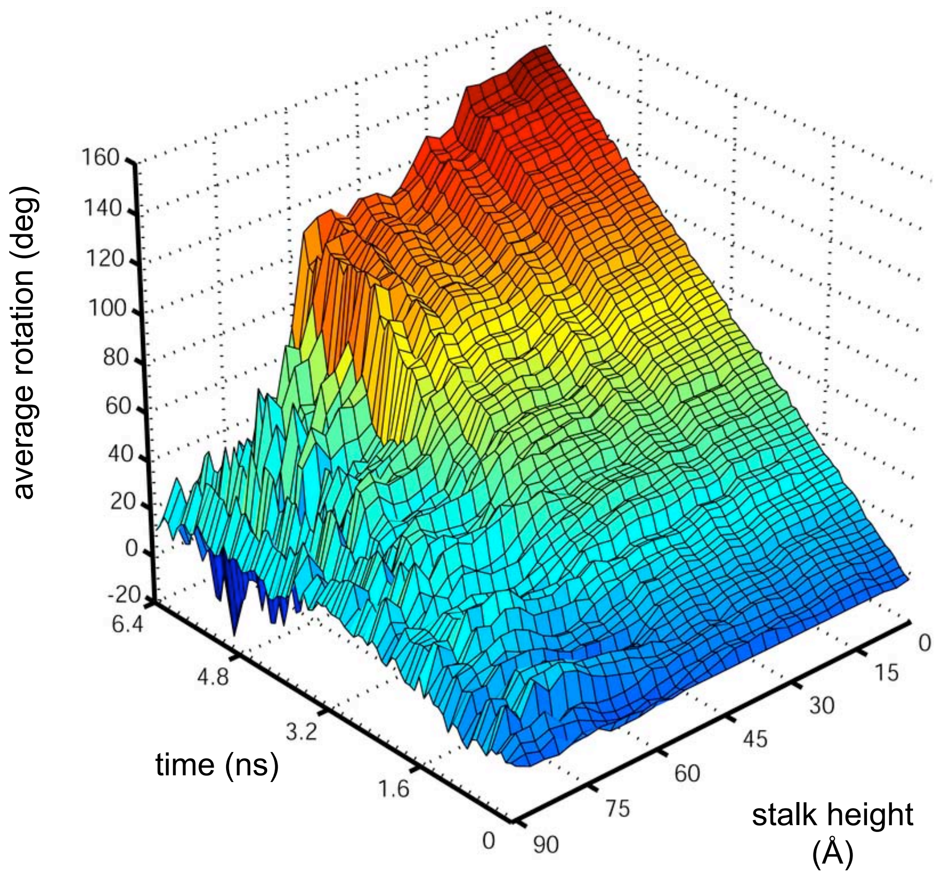
0.0 to 5.0 ns (0 to 120 deg) of torqued F_1 rotation, ω = 24 deg/ns.

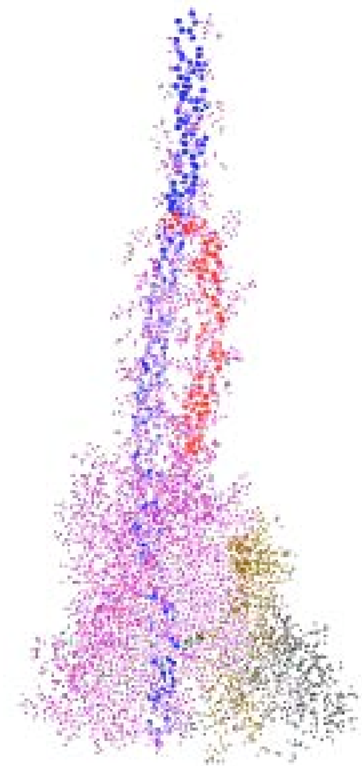
Stalk analysis



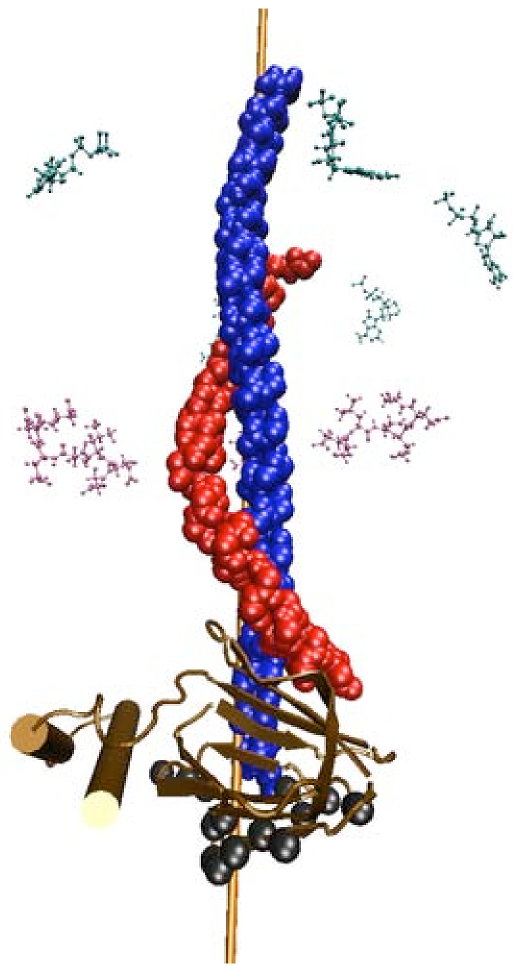
Using best RMSD rotation fit for stalk sections binned along axis direction, at 3.0 ns (72 deg) of rotation, we observe:

- slowed torque transmission along central stalk

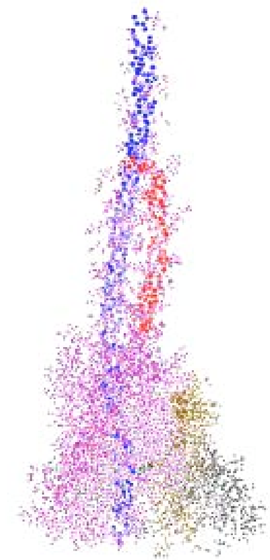




Winding of γ coiled-coil



t= 3.0 ns
θ= 72°

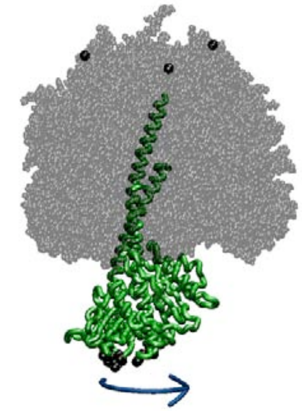


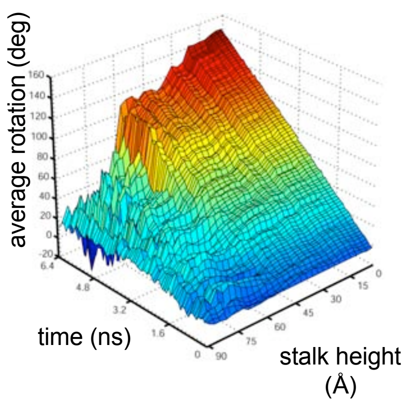
Different coupling for the two γ helices:
1—50, partially via δ subunit
197—272, directly to F_o

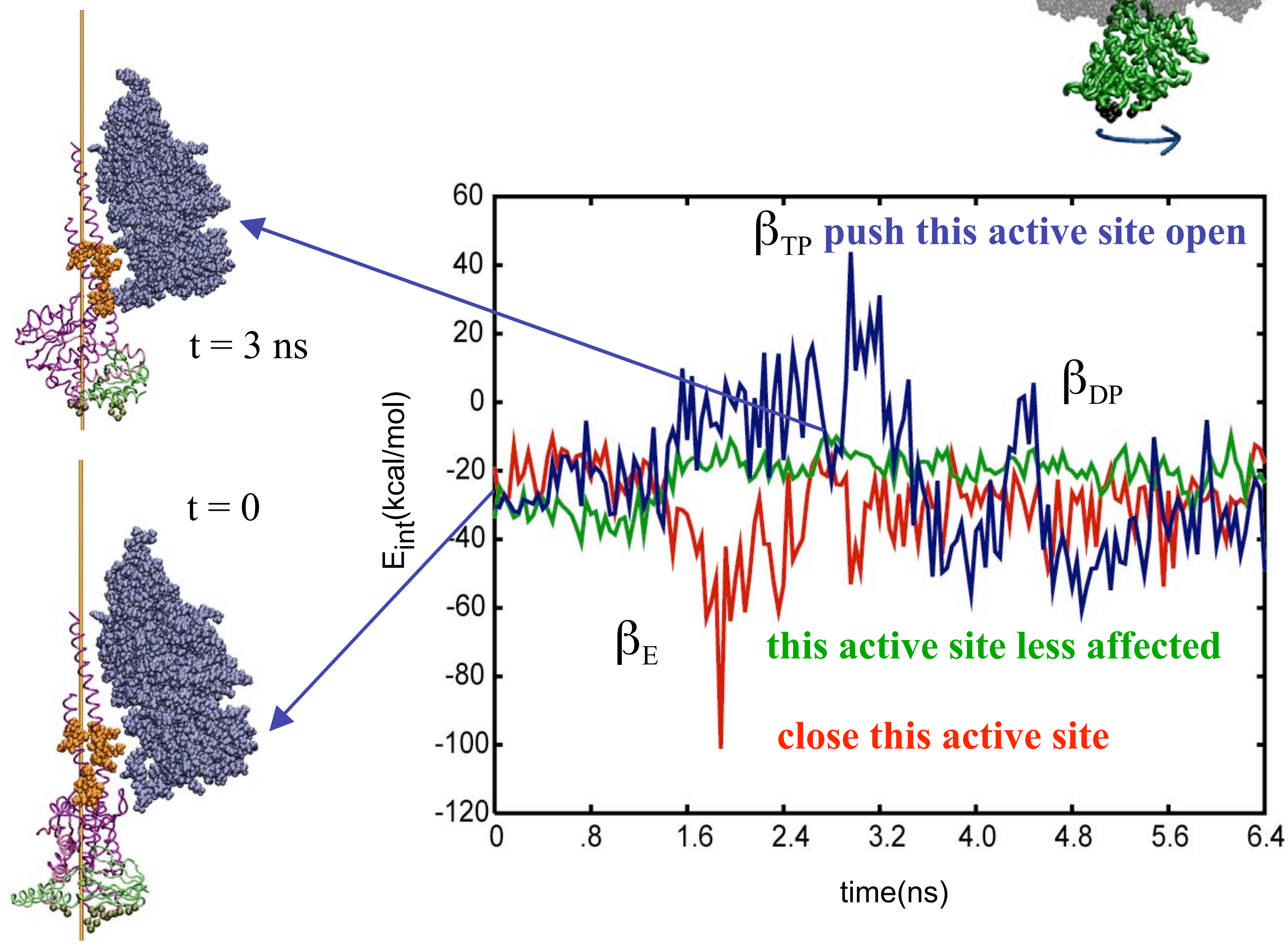
Rotation Produces Synthesis-like Events (1)

Around 3 ns (72 deg) of rotation, we observe:

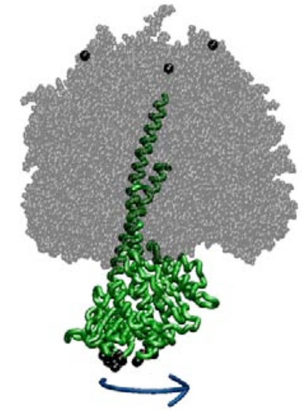
- slowed torque transmission along central stalk
- cooperative interactions at stalk - β subunit interfaces







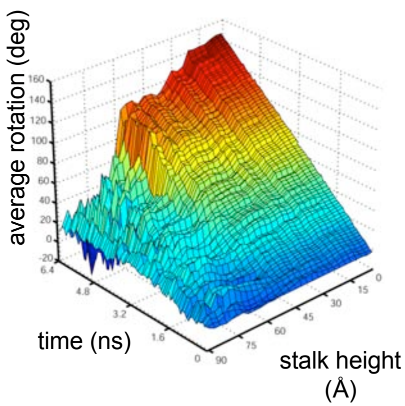
Rotation Produces Synthesis-like Events (3)

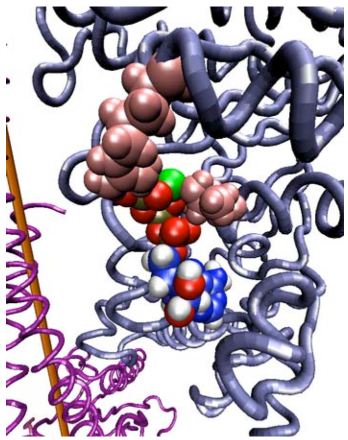


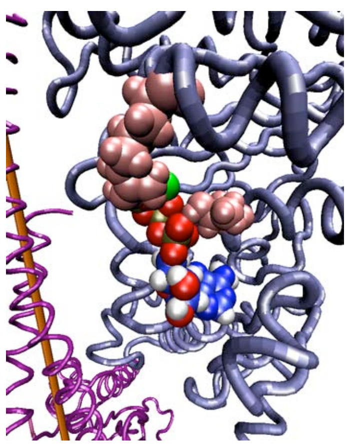
At 3.0 ns (72 deg) of rotation, we observe:

- slowed torque transmission along central stalk
- unbinding from ATP at the β_{TP} catalytic site

0 ns: active site closed

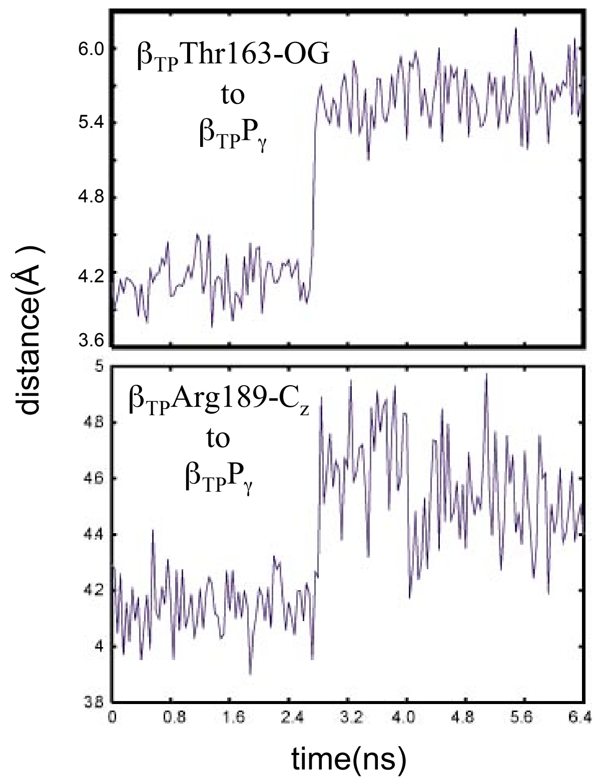




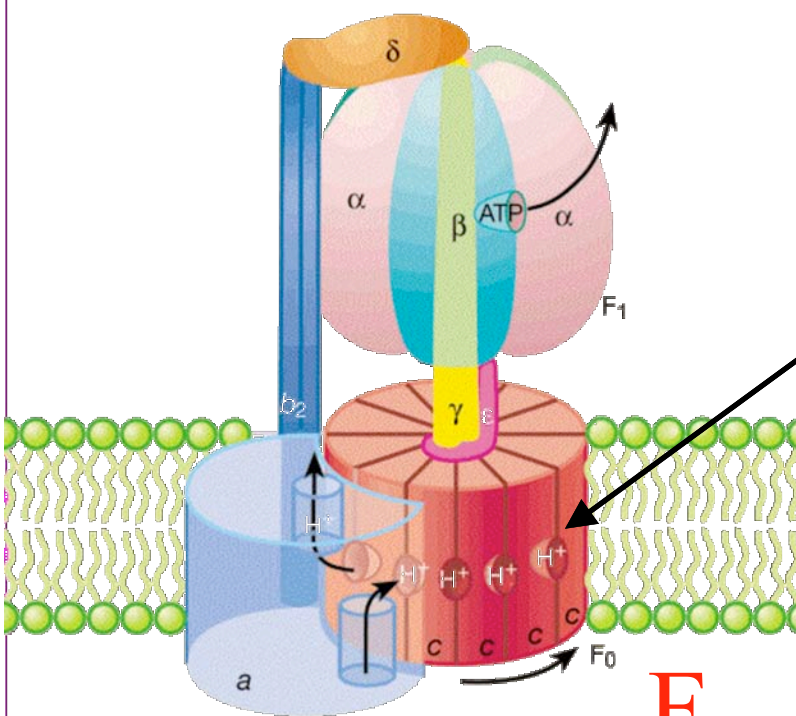


3 ns: active site open

ATP separates from active site residues



Let's Look at Fo ATP Motor

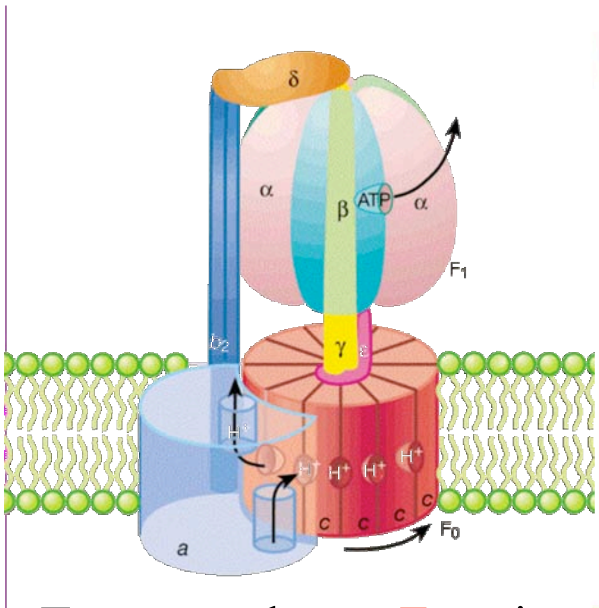


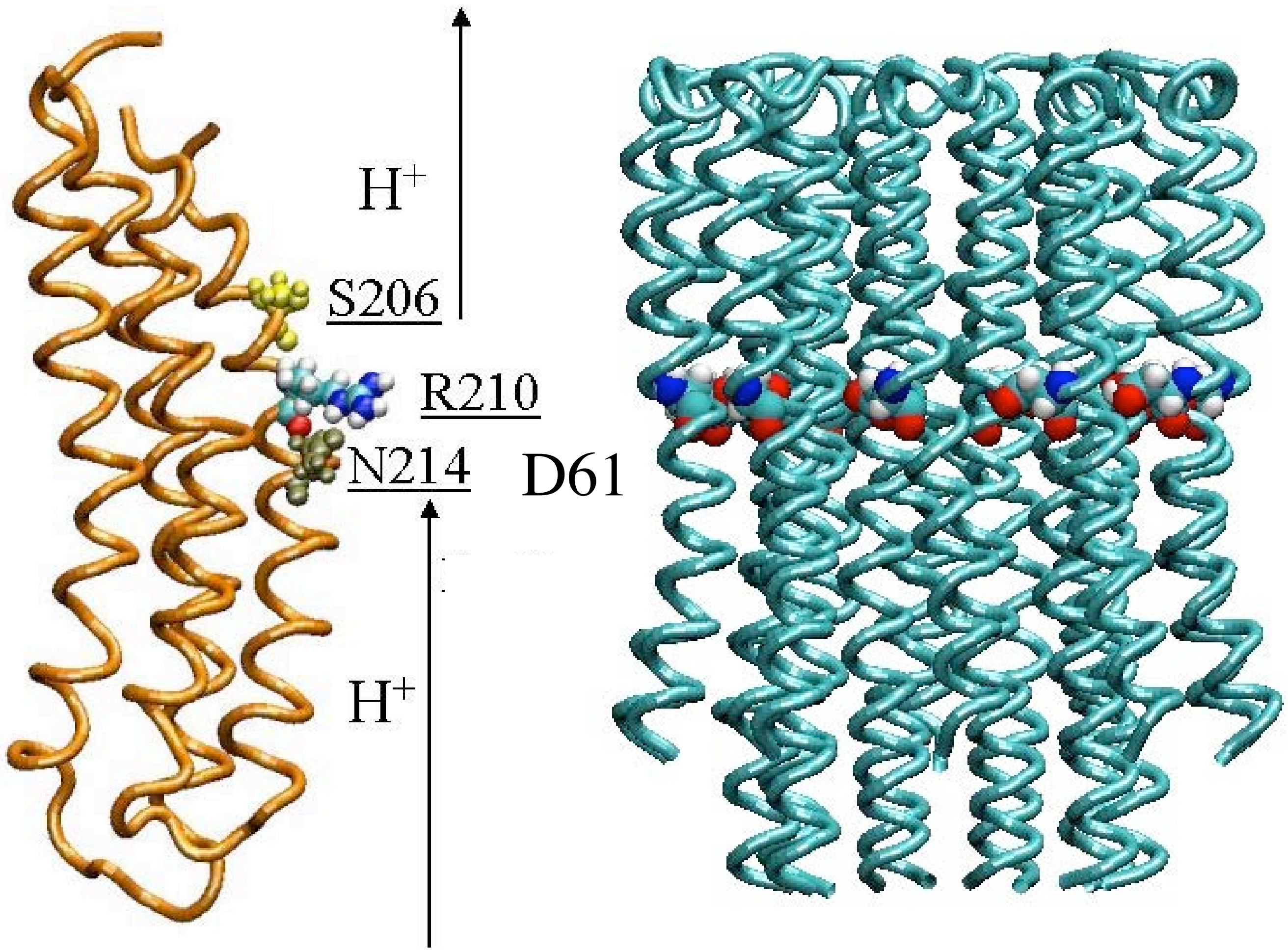
Asp 61 (D61) side groups take protons

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

F_0

Key Amino Acids Participating in Electro-Mechanical Motor





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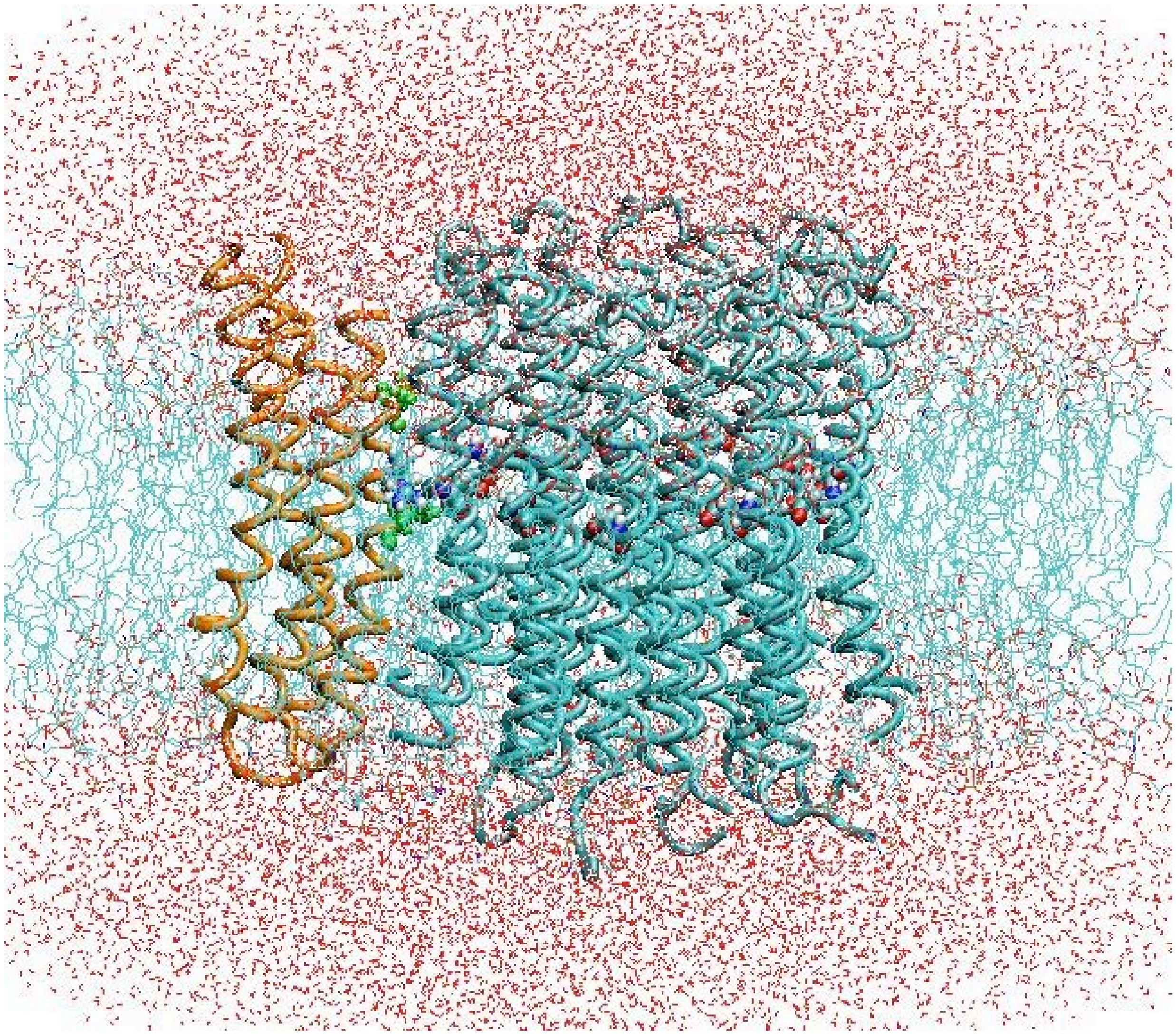
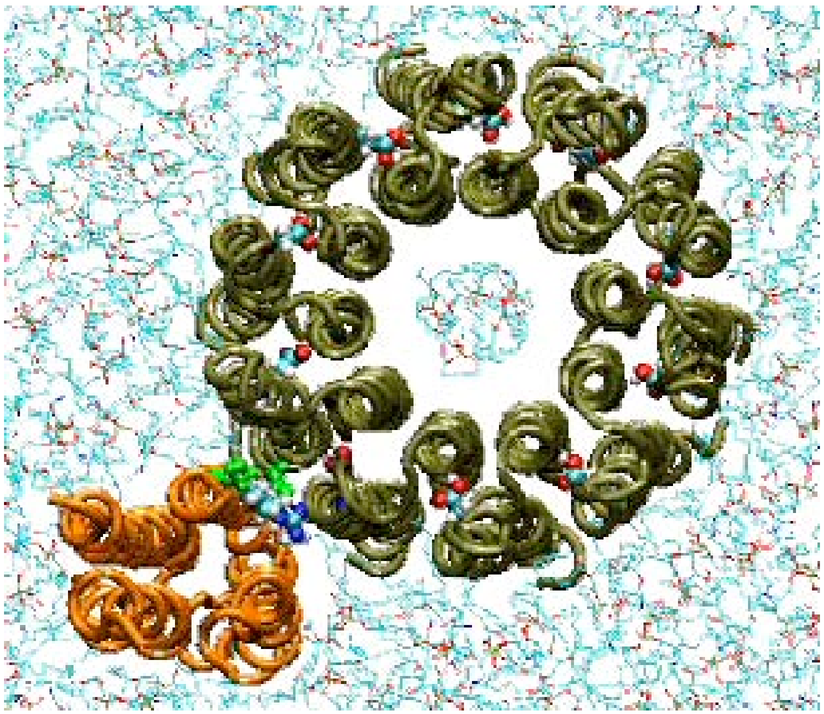
A. Aksimentiev, I. Balabin, R. Fillingame, K. Schulten, *Biophys. J.* 86: 1332-1344 (2004)

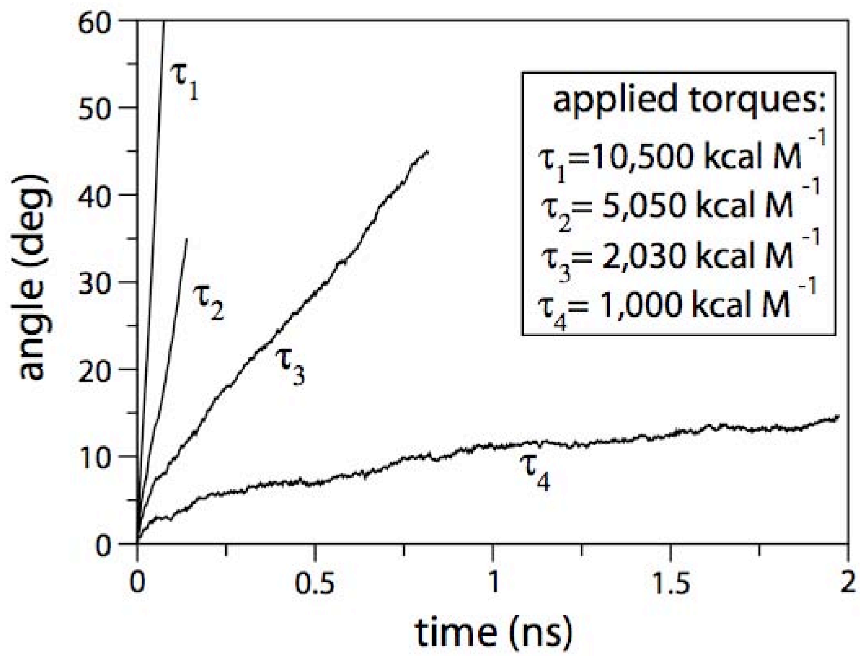
System Simulated



A. Aksimentiev, I. Balabin, R. Fillingame, K. Schulten, *Biophys. J.* (in press)

Forced Rotation of the c10 Subunit





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

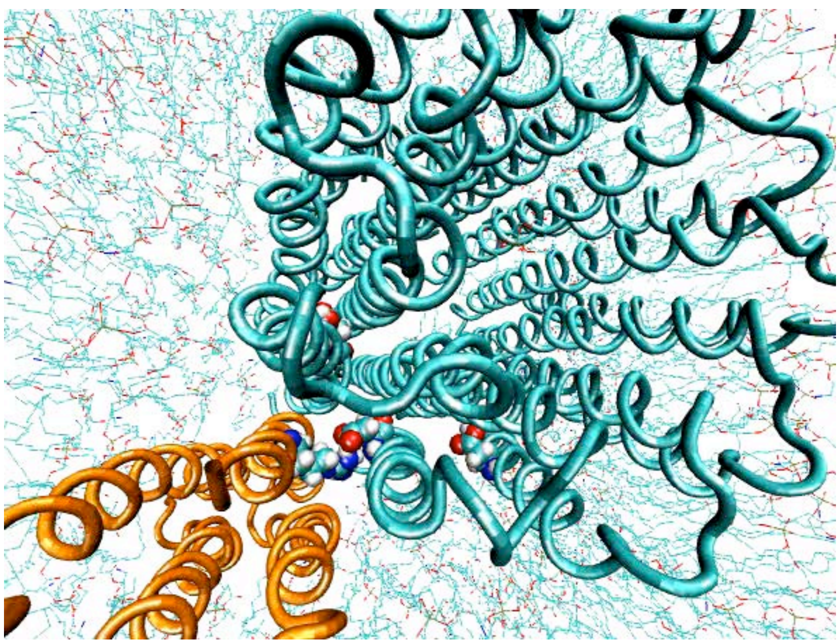
Estimated friction coefficient $\zeta \sim 10^5$ kcal/(M sec)

A. Aksimentiev, I. Balabin, R. Fillingame, K. Schulten, *Biophys. J.* (in press)

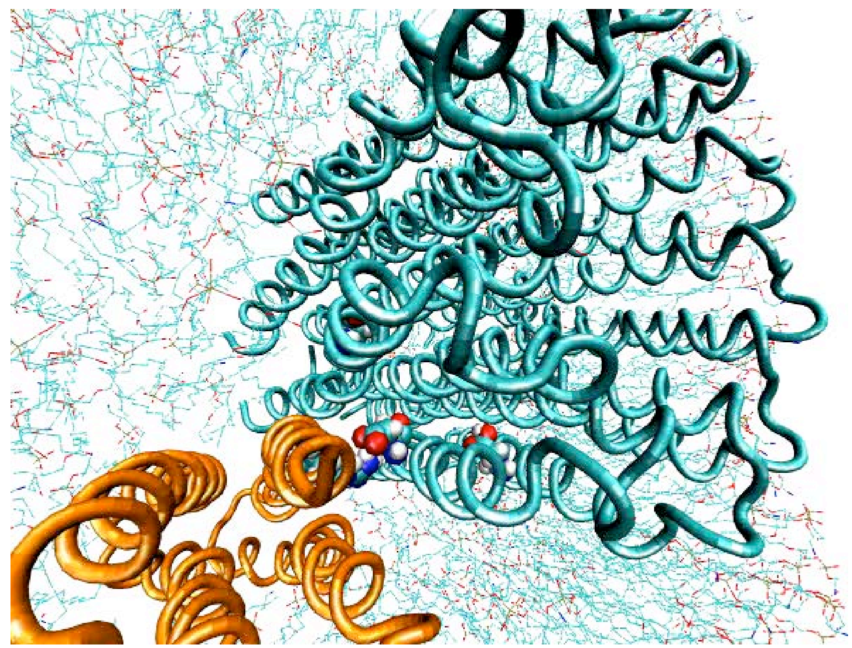
Salt Bridge Arg_{210}-Asp_{61} is Formed

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

No restraints

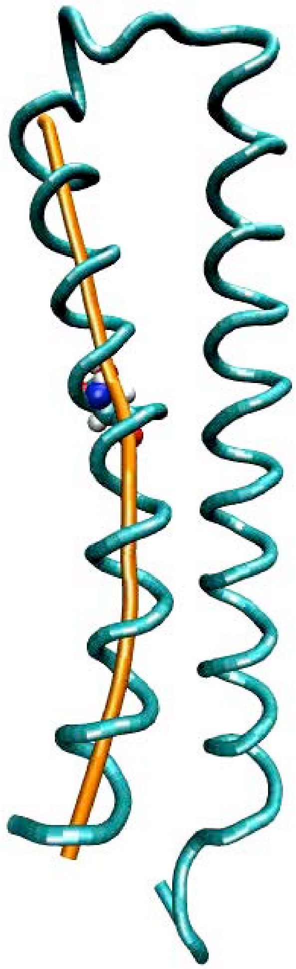


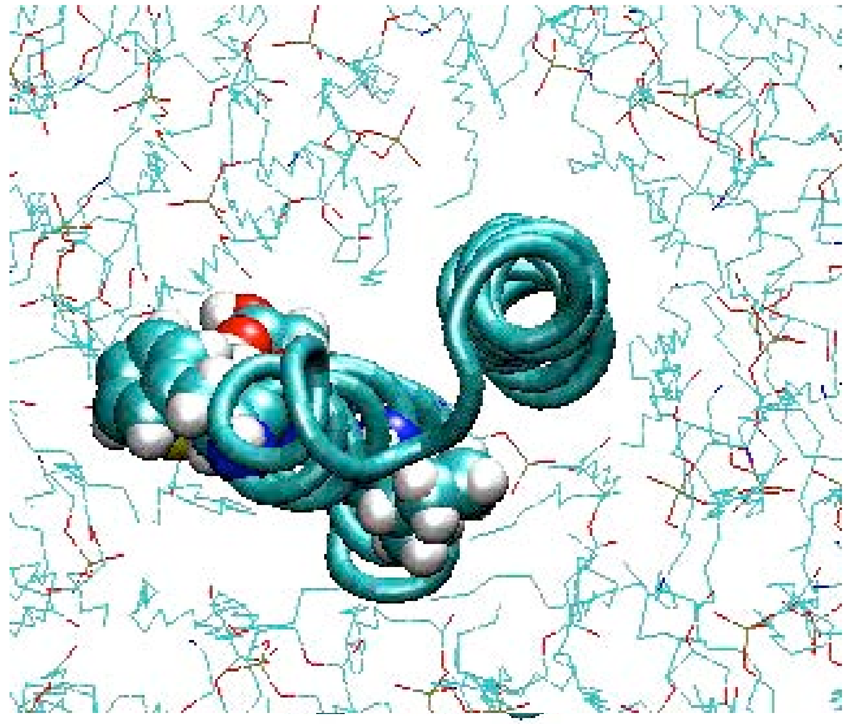
Subunit *a* is restrained



A. Aksimentiev, I. Balabin, R. Fillingame, K. Schulten, *Biophys. J.* (in press)

Single Helix Rotation is Feasible

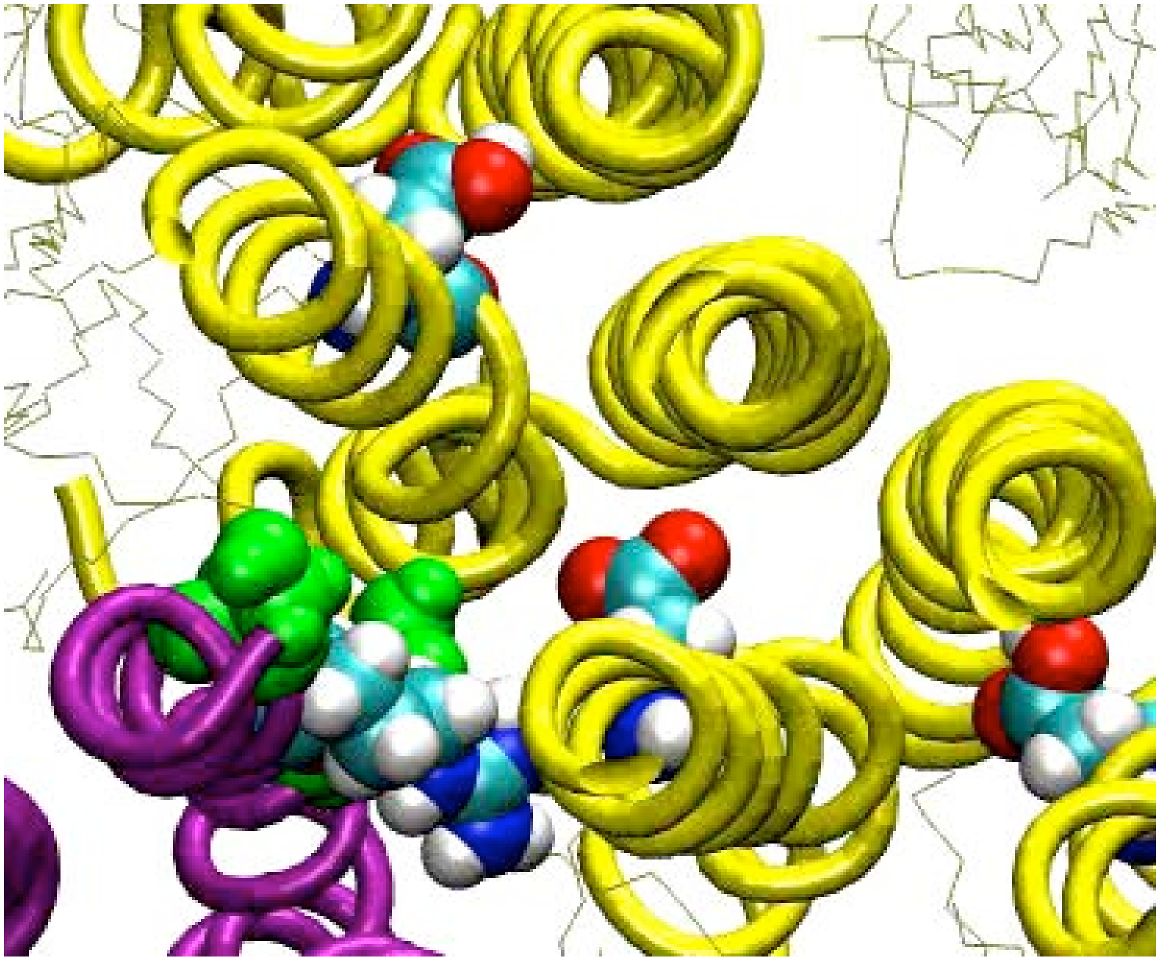




To minimize steric hindrance (critical on nanosecond time scale), helix was forced to rotate in a reptation tube (local pivot points and directors).

A. Aksimentiev, I. Balabin, R. Fillingame, K. Schulten, *Biophys. J.* (in press)

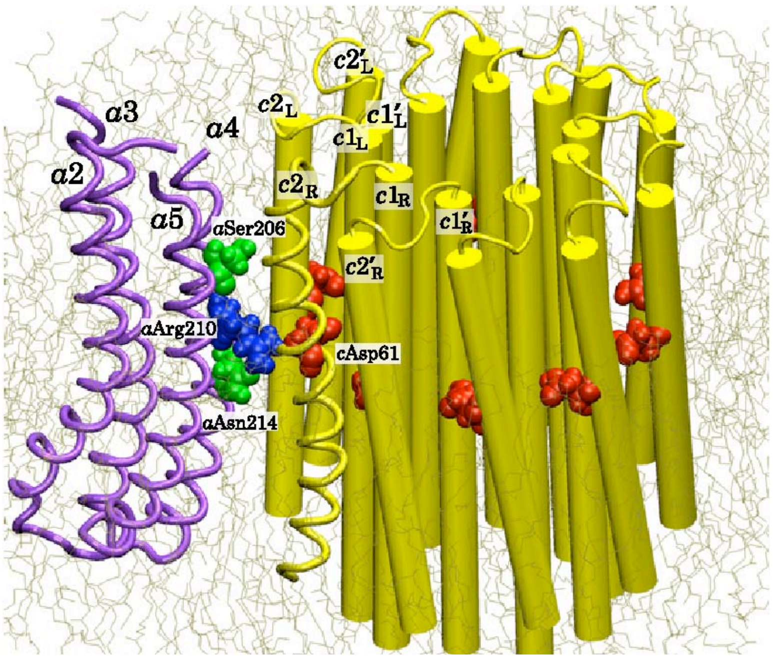
Salt Bridge Cannot be Broken, but Transfered

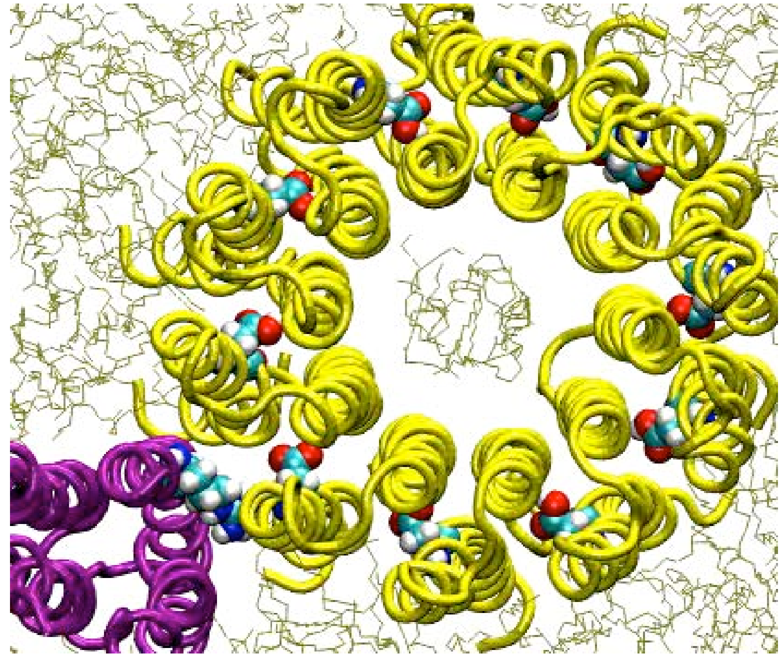


The salt bridge can be transferred by the concerted rotation of the c_{10} complex and the outer TMH of subunit *c*

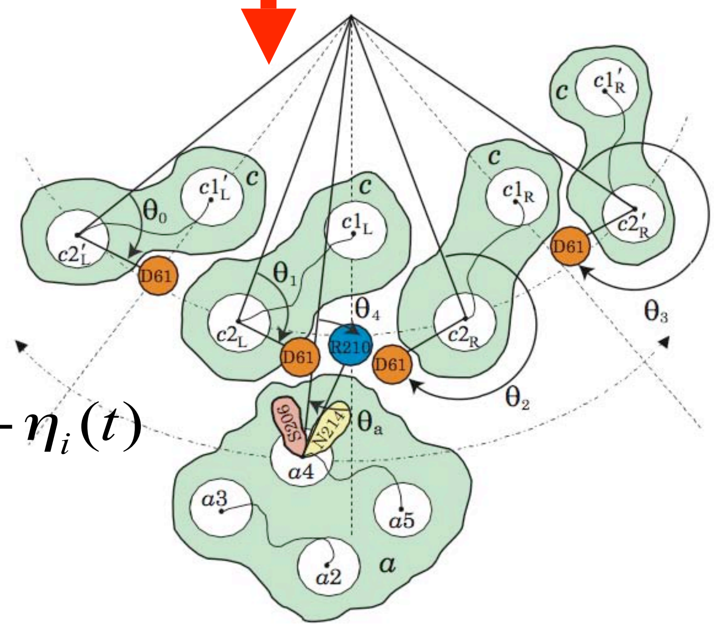
A. Aksimentiev, I. Balabin, R. Fillingame, K. Schulten, *Biophys. J.* (in press)

Overall Mechanism: Theoretical Challenge



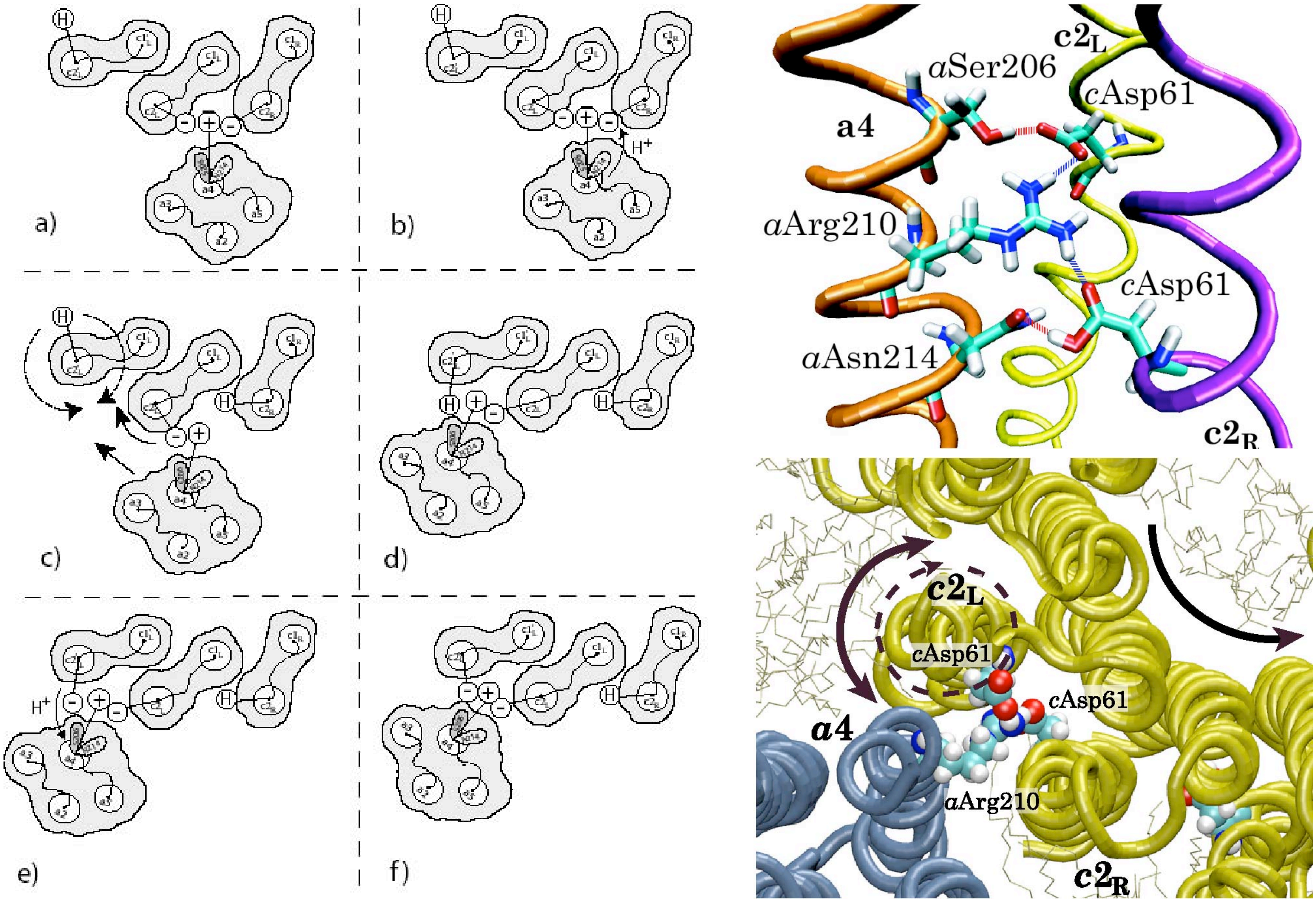


From ns simulation to ms model!

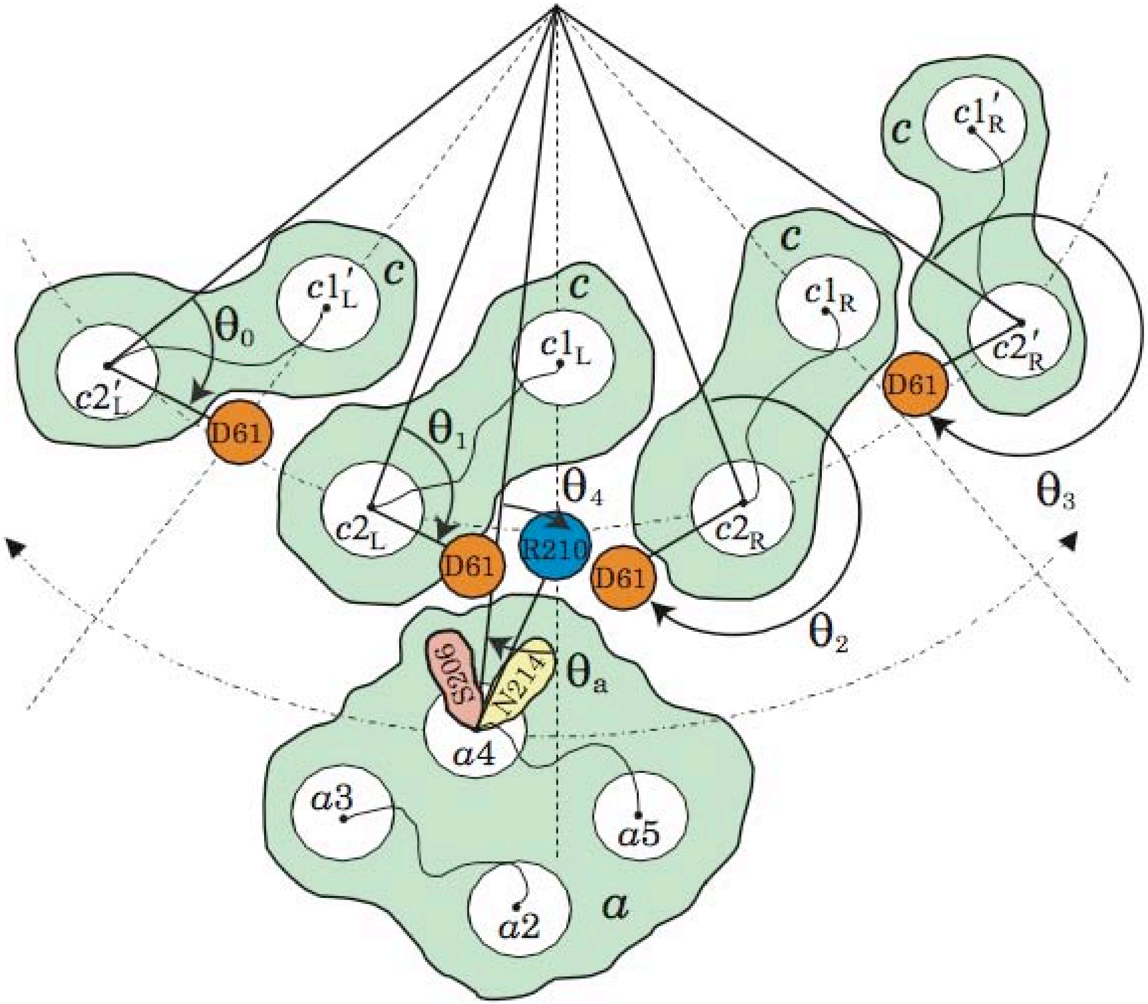


$$\zeta_i \frac{d\theta_i}{dt} = -\frac{d}{d\theta_i}[U_{group} + U_{hydroph.} + U_{internal}] + \eta_i(t)$$

Key Steps in the Mechanism of the Fo Motor



Stochastic Model
Extends Simulation to ms Time Scale



6 degrees of freedom: θ_0, θ_1, θ_2, θ_3, θ_4 are TMH rotation angles; θ_A - position of the *a* subunit.

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

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

Stochastic Simulations of F_o Operation

Time evolution of rotation angles θ_1 (black), θ_2 (red), θ_4 (green), and θ_A (blue). Motor rotation speed is close to physiological.