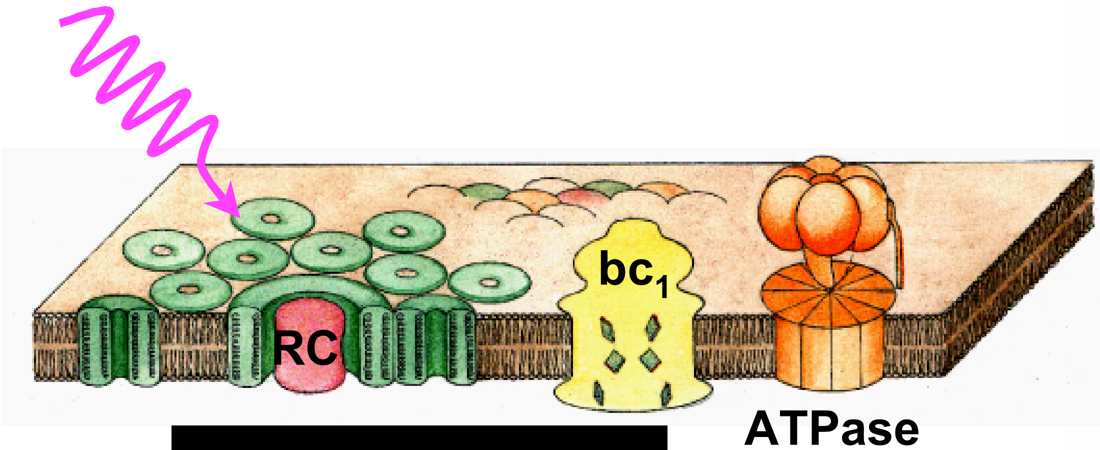
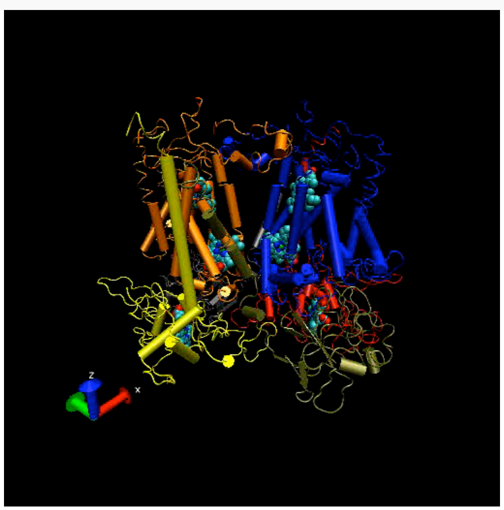
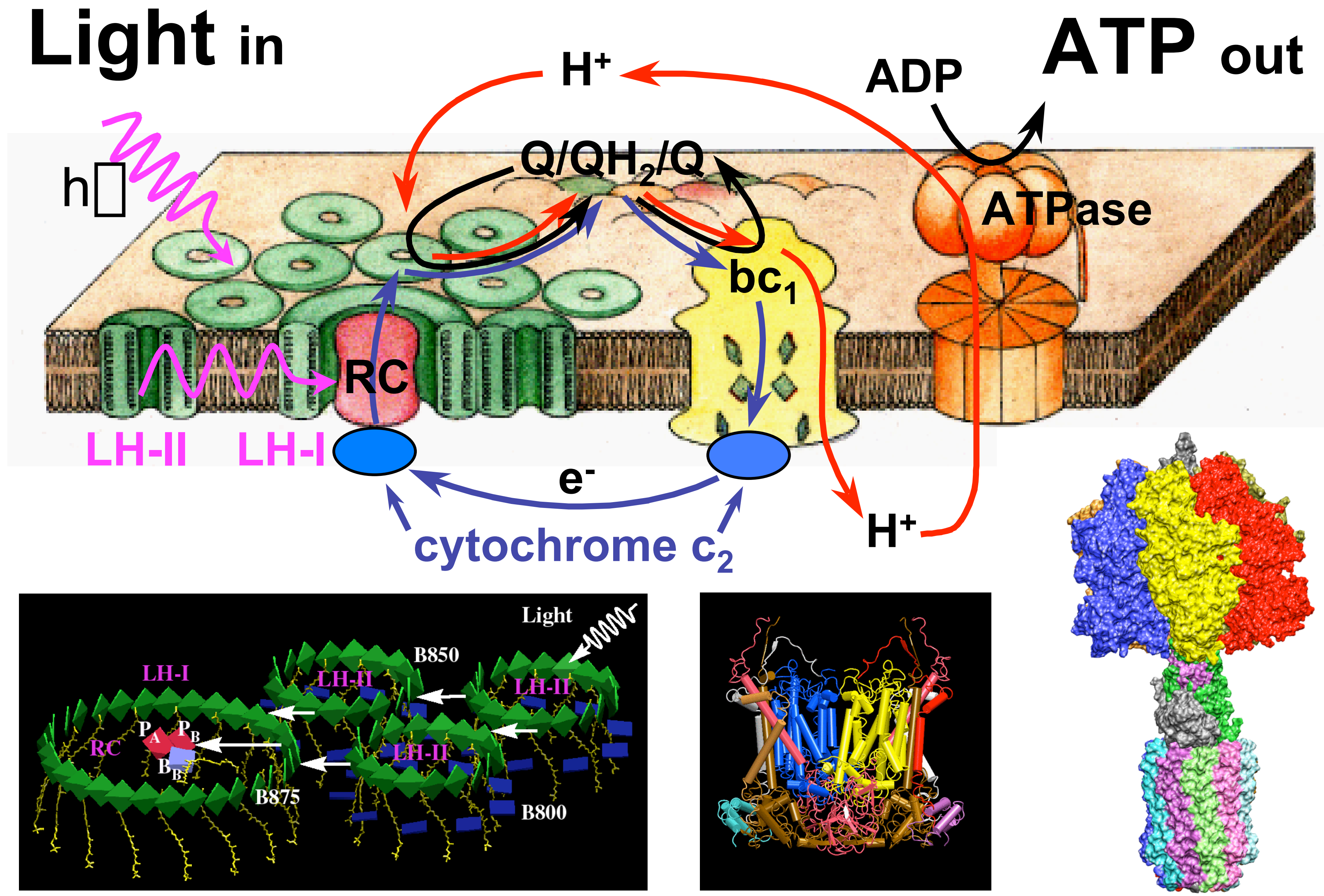
ATPase Synthase - A Molecular Double Motor



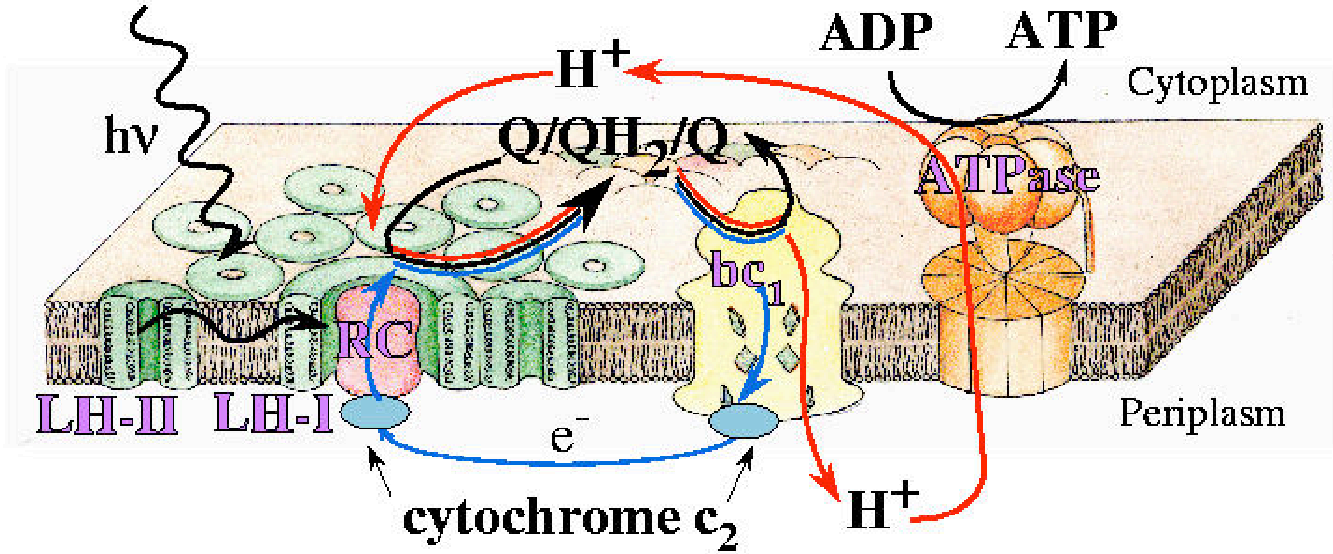


Photosynthesic Unit of Purple Bacteria

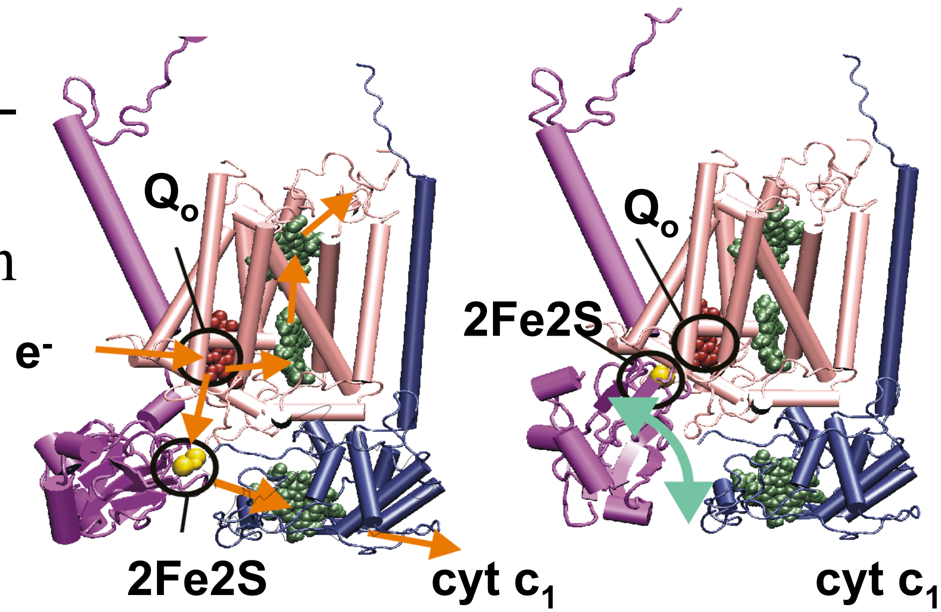
Module that converts sun light into chemical energy (ATP)



Mechanism of the bc1 Complex in the Photosyntehtic Unit



two path-ways for oxidation of Q_o site



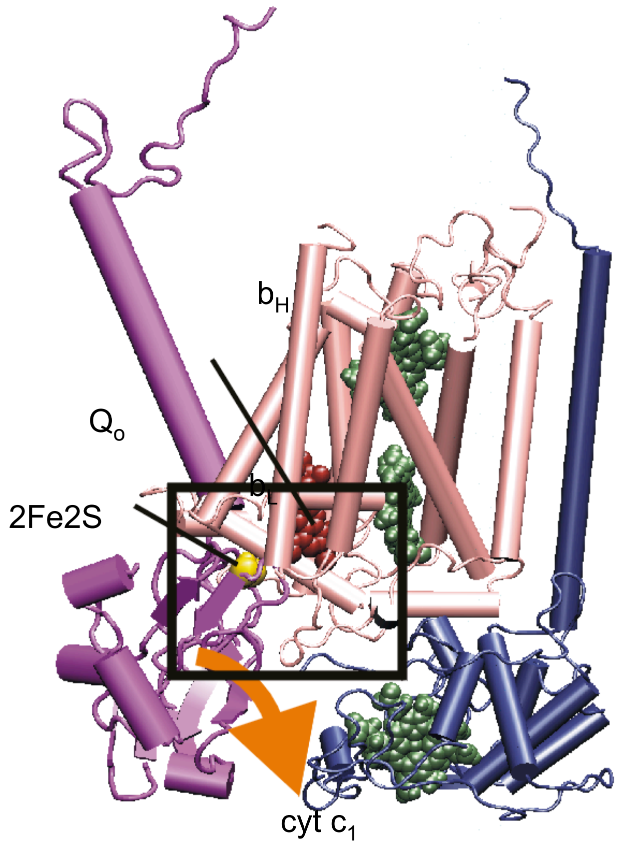
Iron Sulfur Protein (ISP) head rotation can redirect 2nd electron

www.ks.uiuc.edu

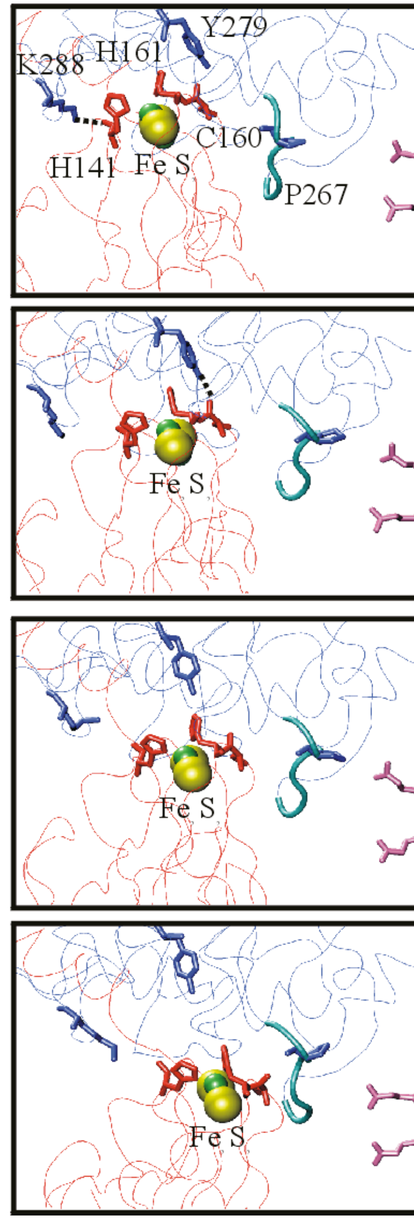
Enforcing domain rotation in the bc_1 complex

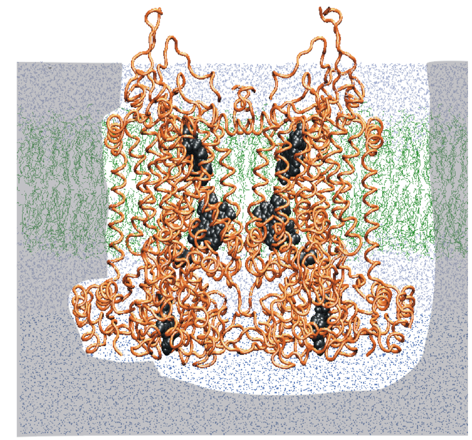
Events during torque application to ISP head

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

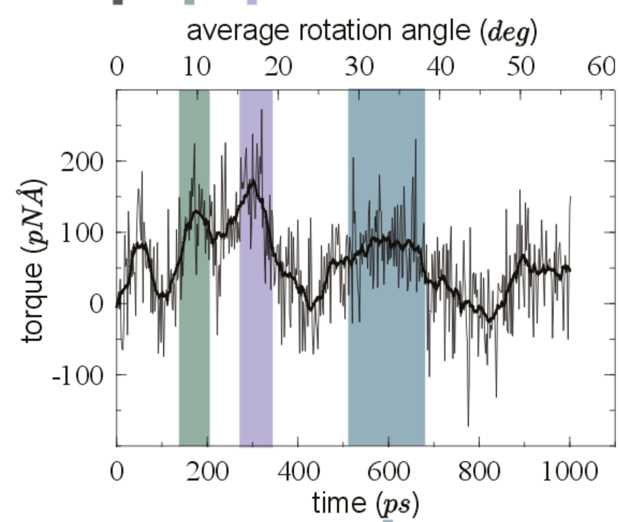


Torque applied
to 126 C□ atoms
K = 70 pN/Å
□ = 0.0561 rad/s

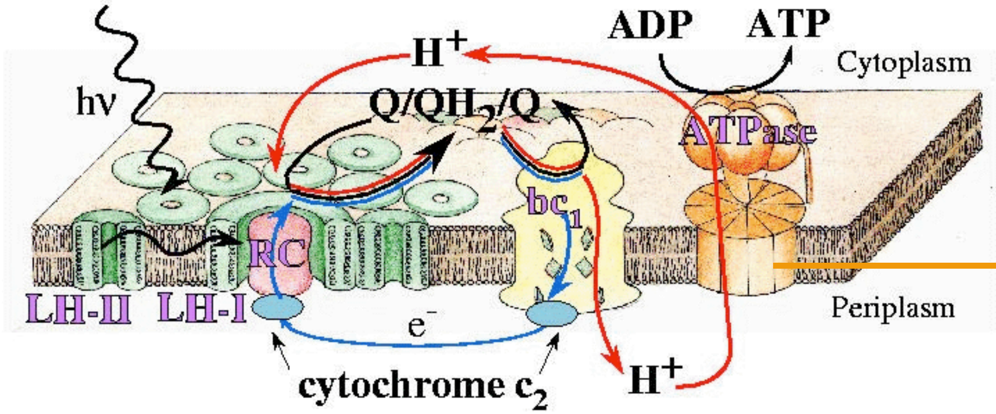


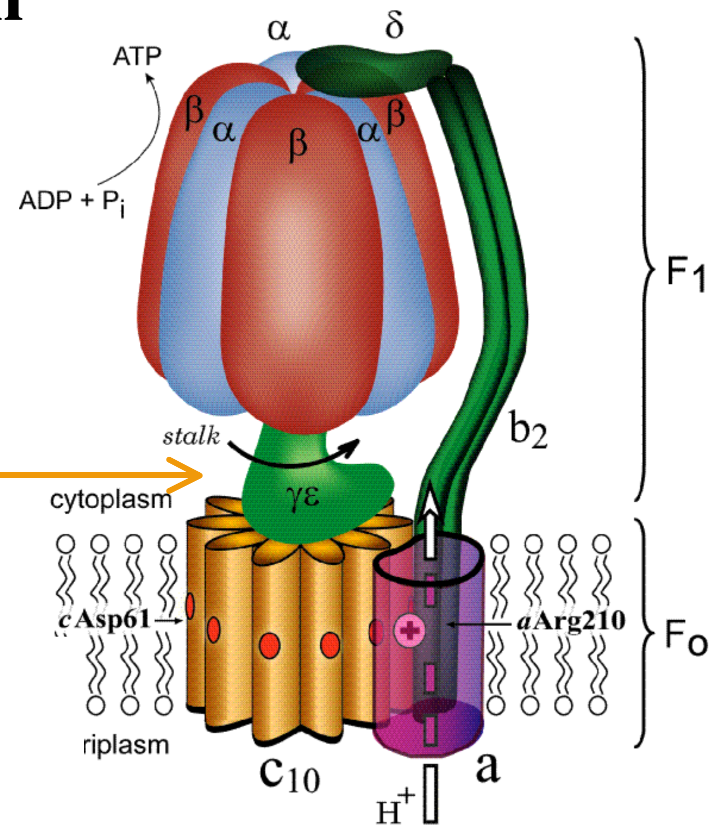


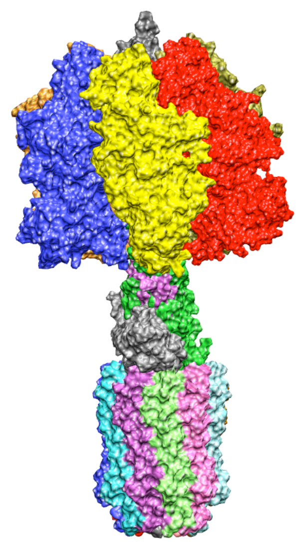
206,720 atoms



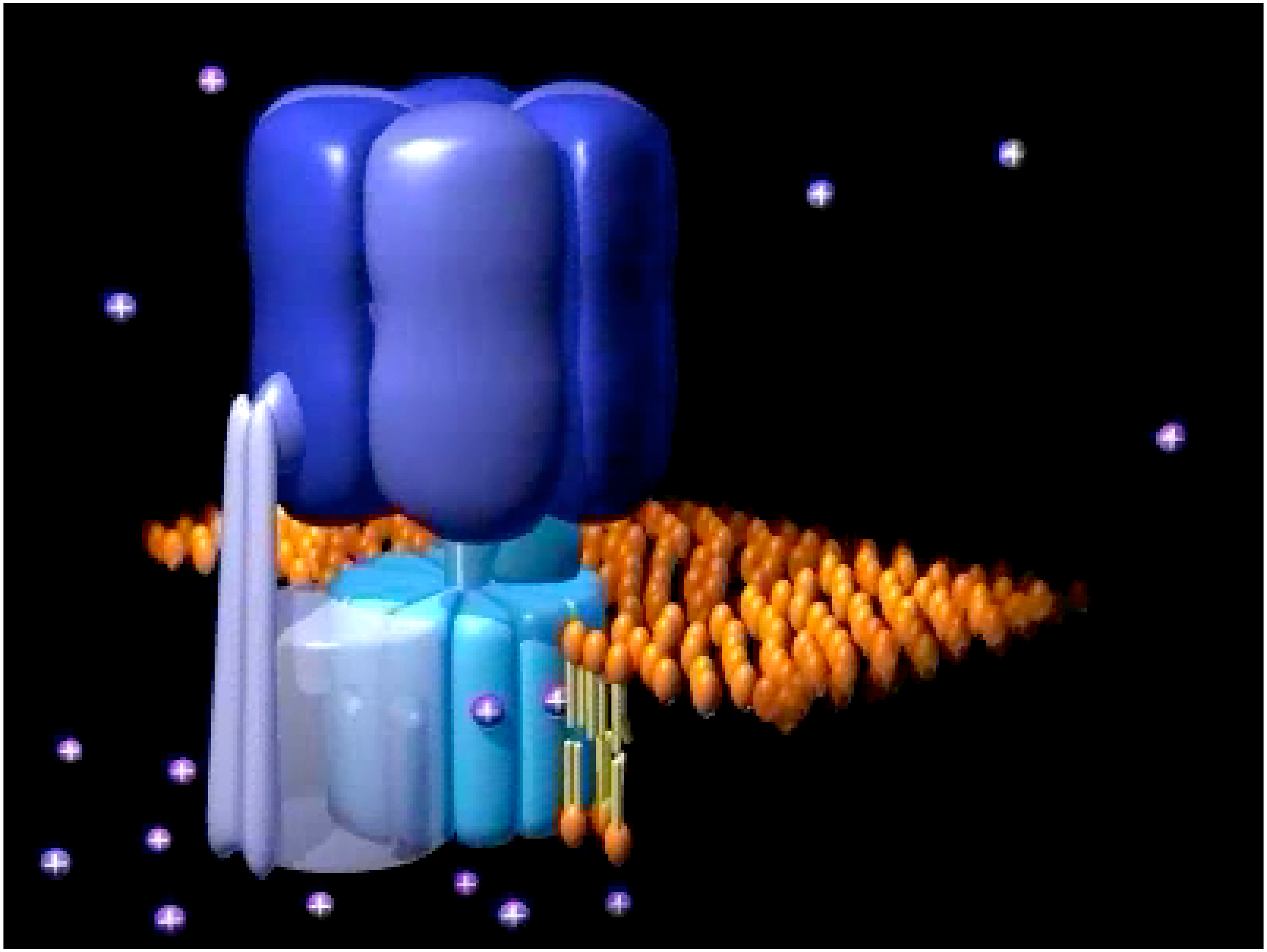
Mechanisms of Rotatory Molecular Motor that Converts Voltage (proton gradient) into ATP Synthesis



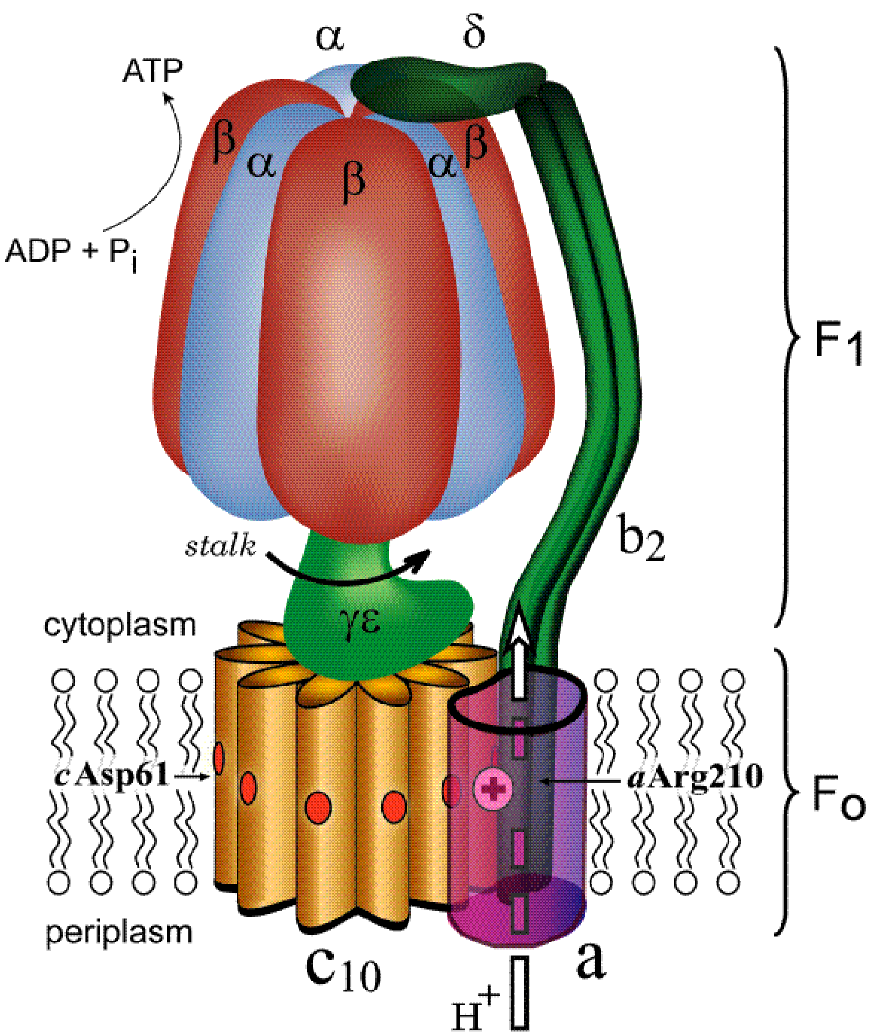




Animation of the ATP Synthase



Adenosine Triphosphate (ATP) Synthase

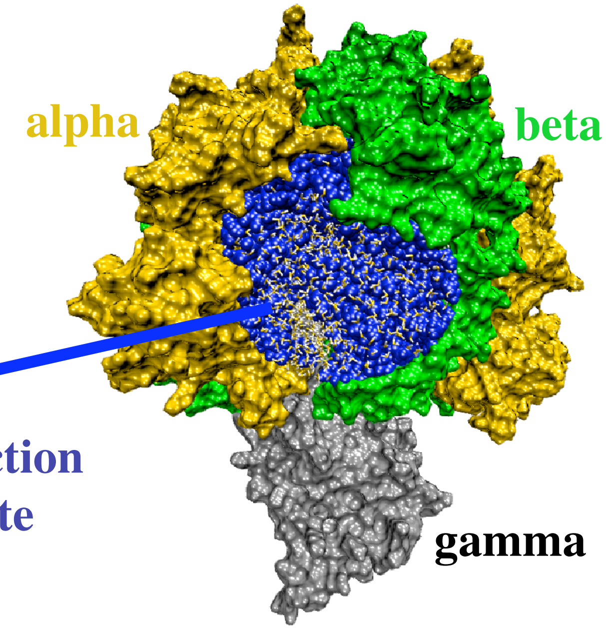


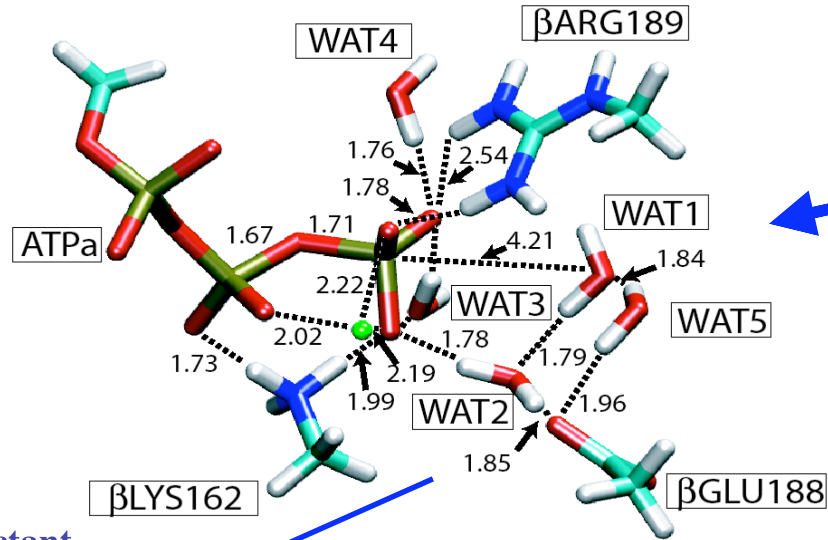
Rotary catalysis: Two protein motors coupled via common central stalk □□

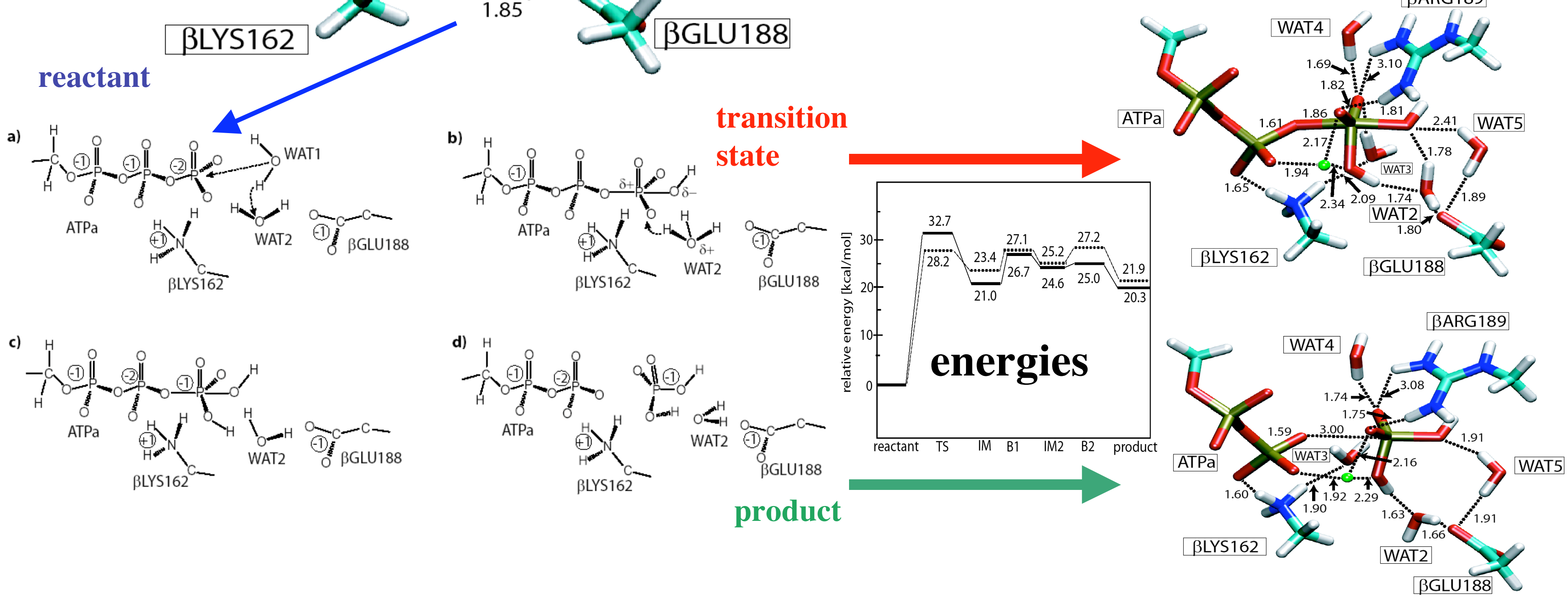
Solvent exposed F_1 unit ($\square_3\square_3\square\square\square$): central stalk rotation causes conformational changes in catalytic sites, driving ATP synthesis

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

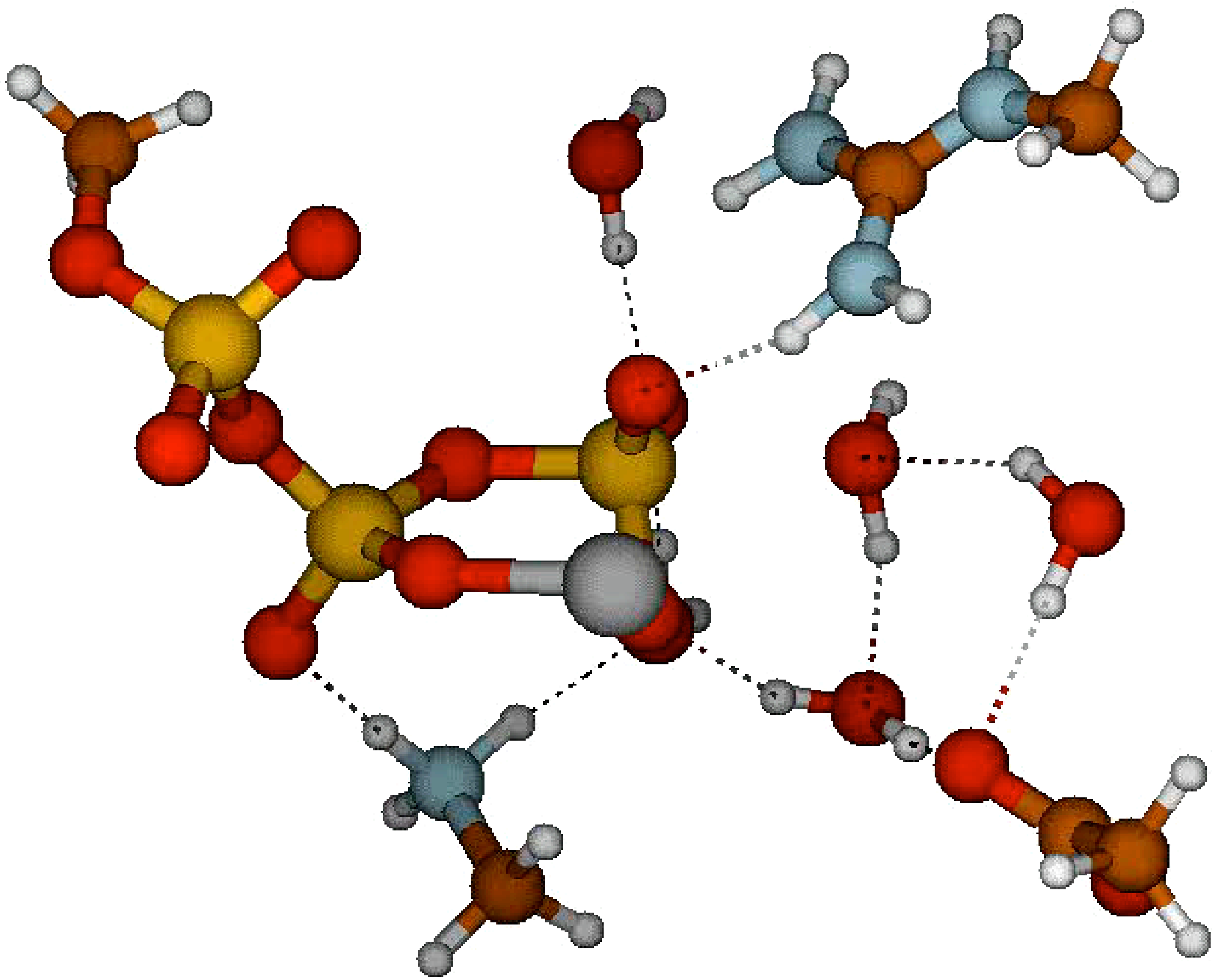
Reaction Mechanism of ATP Hydrolysis



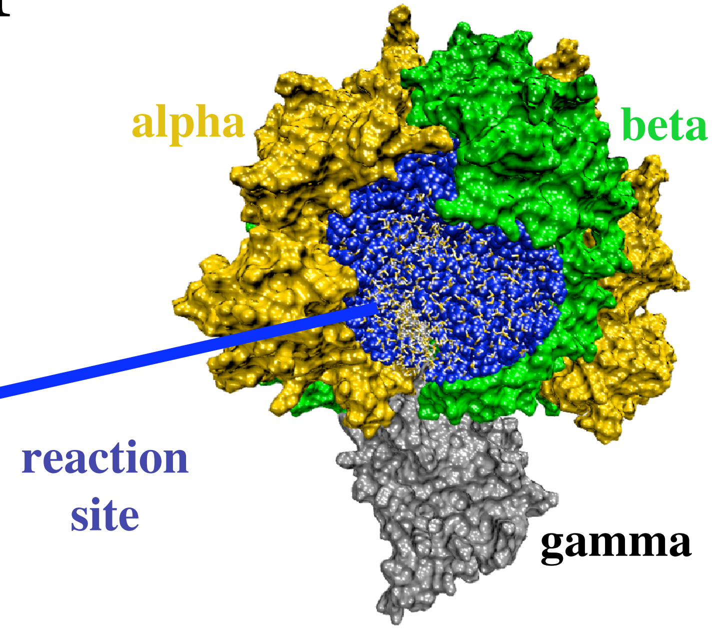


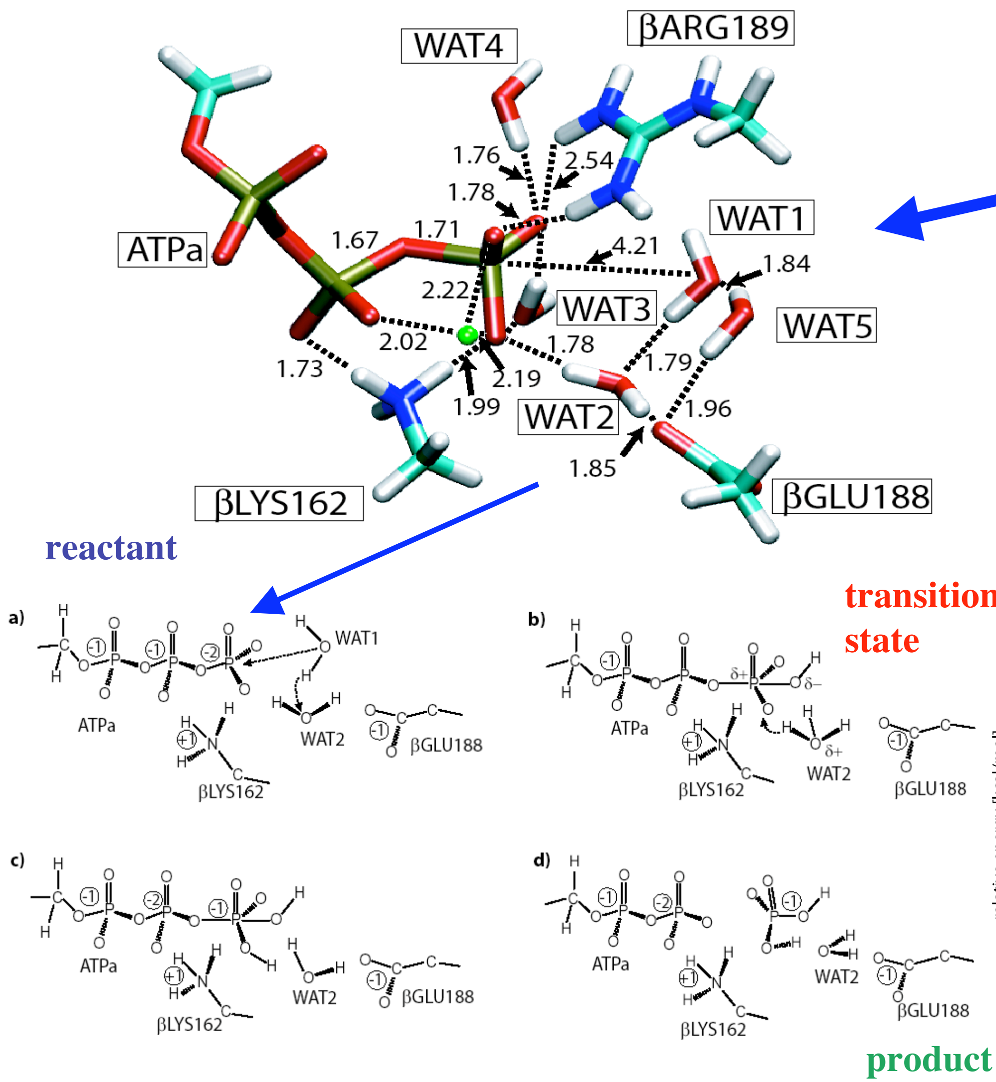


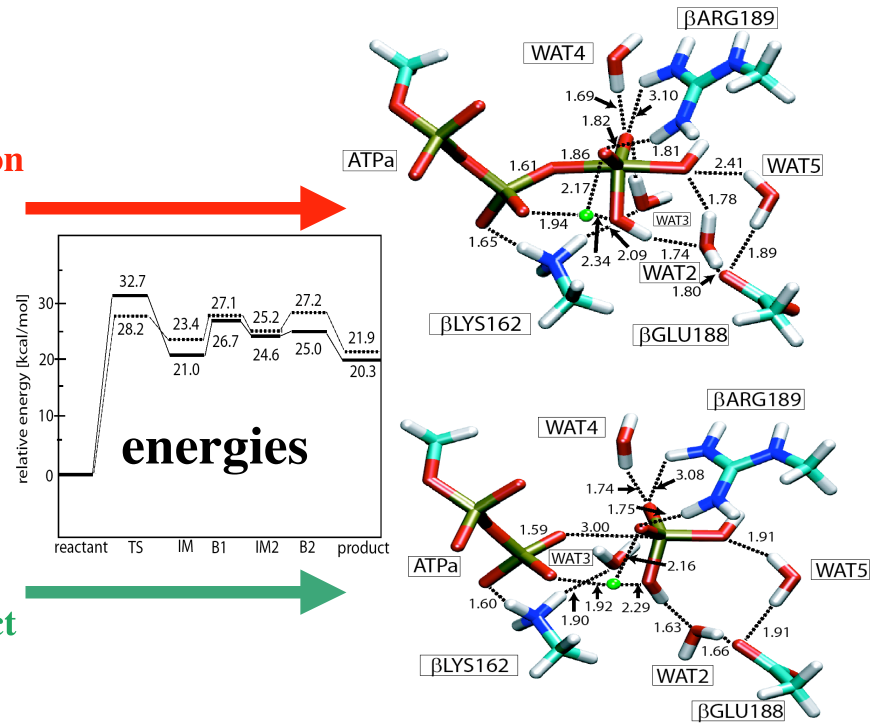
Mechanism of ATP Hydrolysis in F1 ATPase



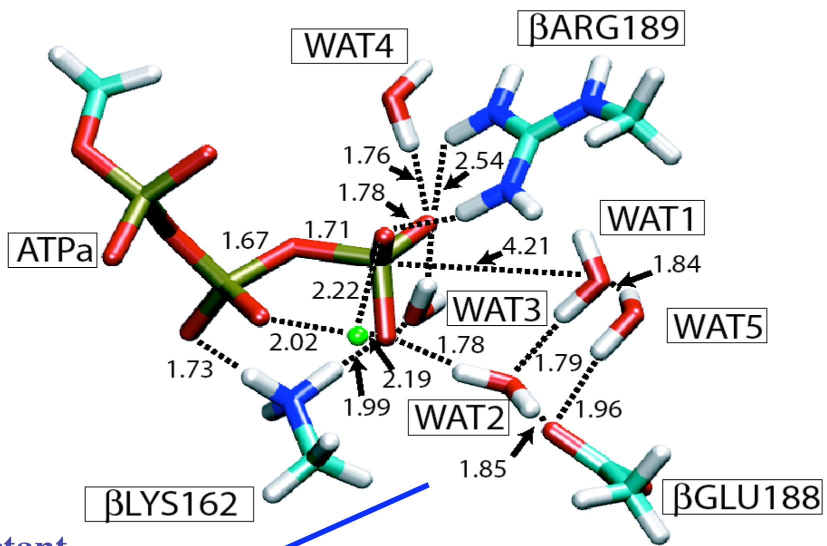
Reaction Mechanism of ATP Hydrolysis



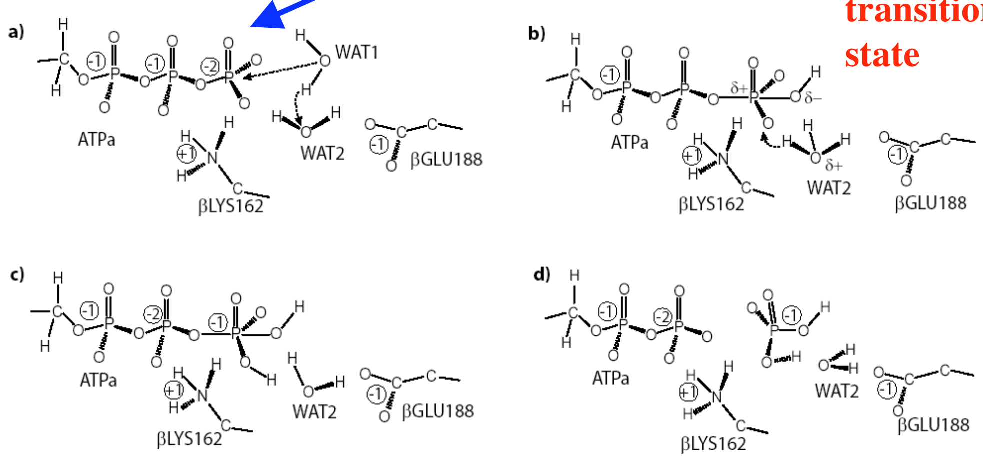




Reaction Mechanism of ATP Hydrolysis

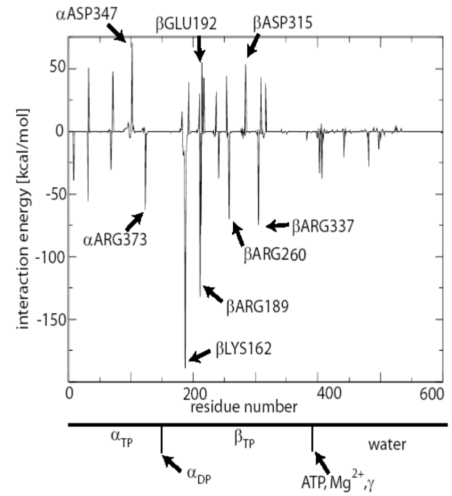


reactant

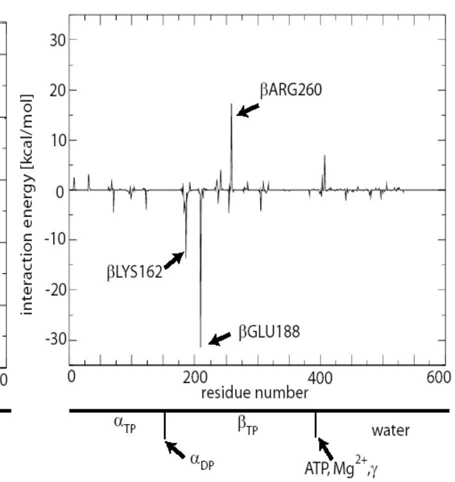


Role of protein side groups

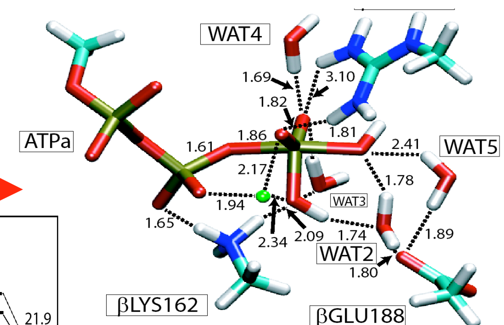
Interaction with reactant

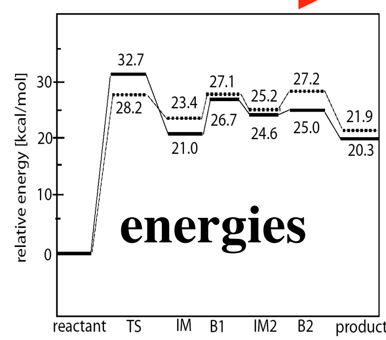


Control of TS barrier

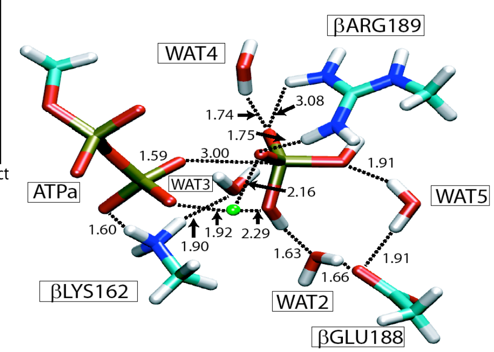


transition state

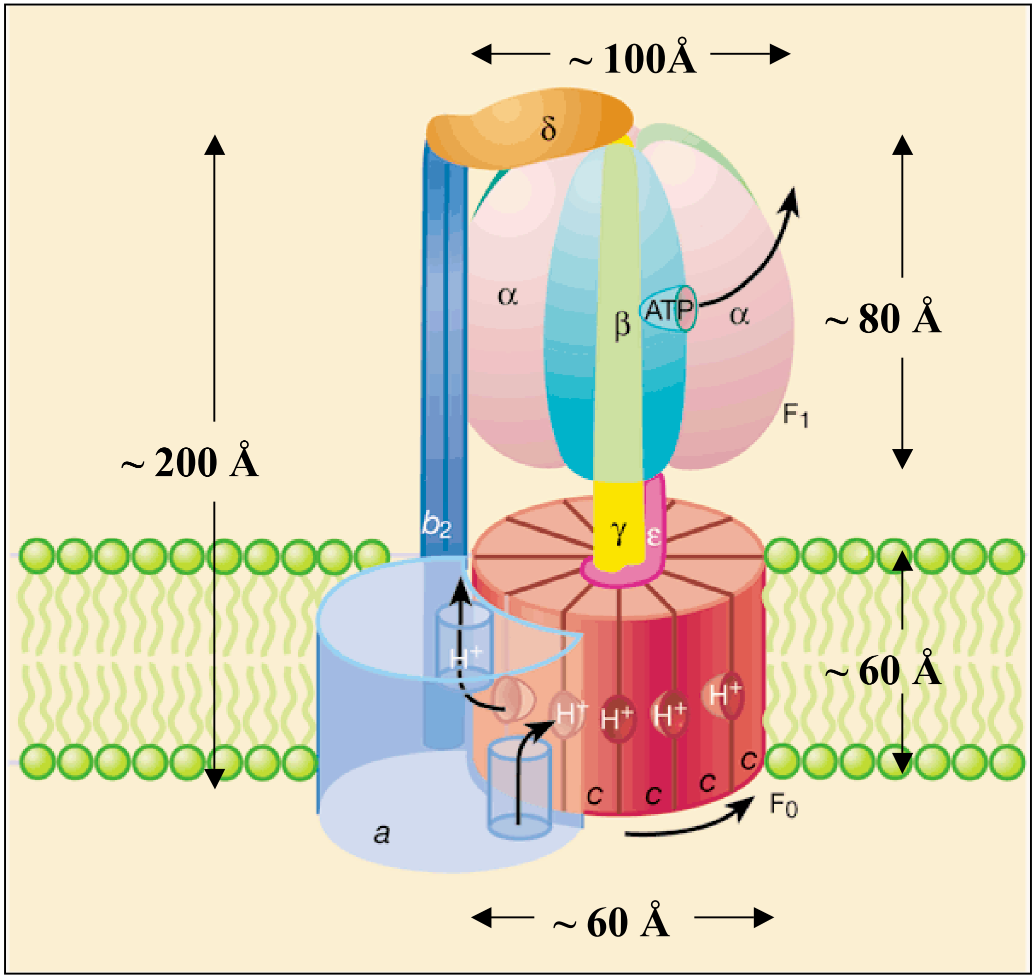




product



One shaft, two motors: Let's look at F1



Soluble part, F_1-ATPase

-Synthesizes ATP when torque is applied to it *(main function of this unit)*

-Produces torque when it hydrolyzes ATP *(not main function)*

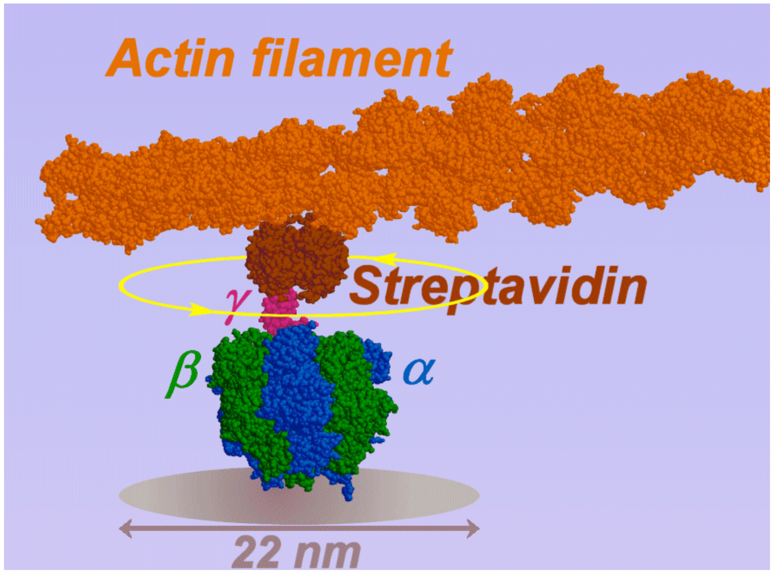
Membrane-bound part, F_0 Complex

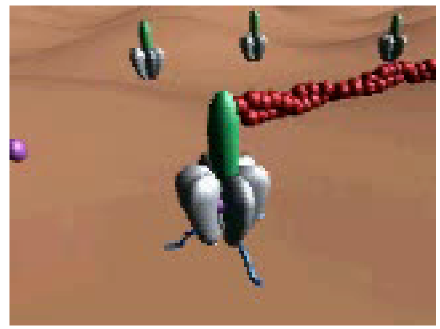
- Produces torque when positive proton gradient across membrane*(main function of this unit)*
- Pumps protons when torque is applied *(not main function)*

Torque is transmitted between the motors via the central stalk.

F1-ATPase: A Rotary Motor Made of a Single Molecule

http://www.k2.ims.ac.jp/F1movies/F1Prop.htm





To observe rotation, the three beta ***subunits were fixed on a glass surface through histidine tags engineered at the N terminus. To the putative rotor subunit*** gamma***, a micrometer-sized actin filament was attached through streptavidin. When ATP was added, the actin filament rotated continuously clockwise (movie). Note that, in this movie, the rotation occurs around the middle of the filament. If you hold an end of a long rod, you could make a fake rotation by twisting your wrist. If you hold the middle, however, you have to rotate yourself to keep the rod rotating. Thus, the propeller rotation in this movie shows that the __ subunit really slides against the surrounding alpha$_3$beta$_3$ subunits over finite angles.***

Noji, H. et al., *Nature* **386,** 299-302 (1997).

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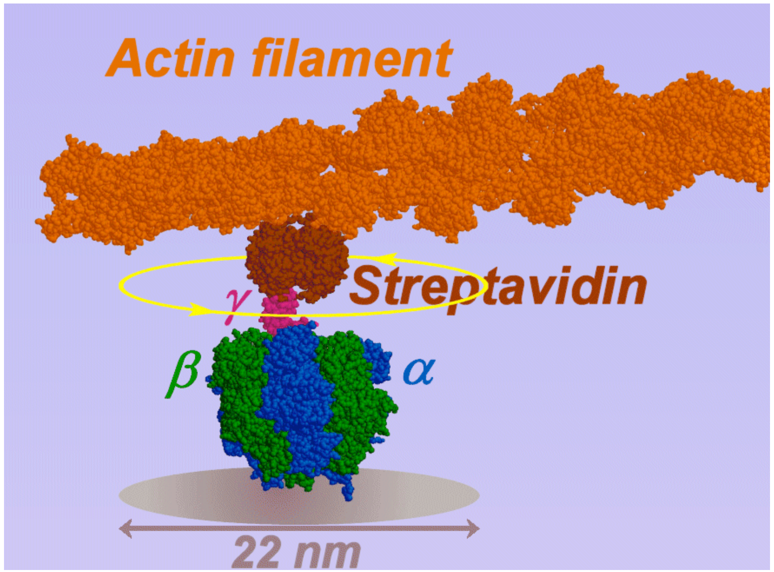
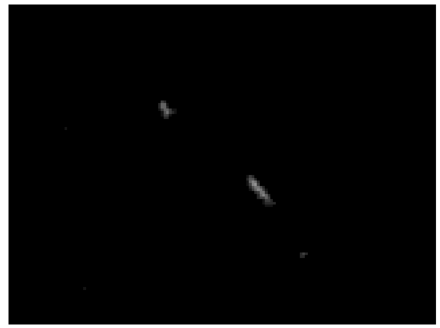


From Yoshida web site



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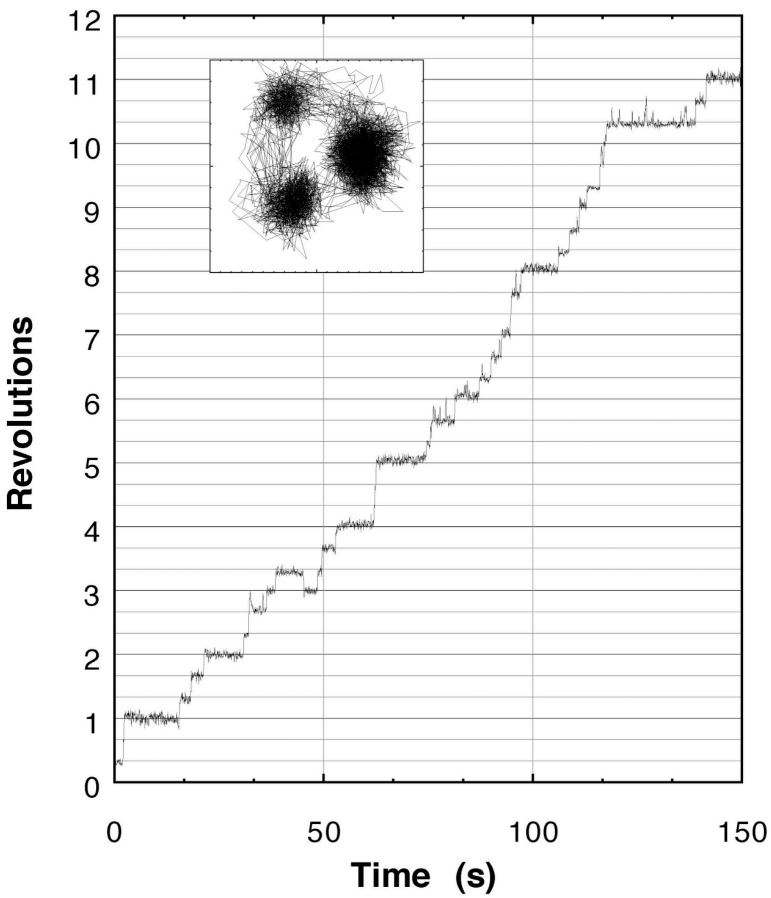
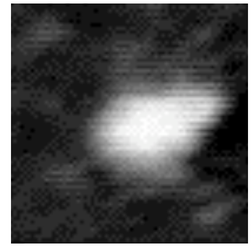
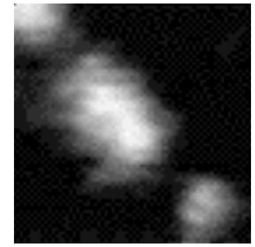
Stepping Rotation of F1-ATPase at Low ATP Concentrations

http://www.k2.ims.ac.jp/F1movies/F1Step.htm





[ATP] = 20 nM



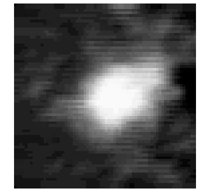
[ATP] = 200 nM

At low ATP concentrations, F_1 rotates in discrete 120° steps. The stepping rate is proportional to the ATP concentration, indicating that each step is driven by one and only one ATP molecule. In the movie at 20 nM ATP, there is an instant where the F_1 motor makes a mistake and steps backward (clockwise). A molecular machine occasionally makes mistakes, and its operation is always stochastic as seen in the figure at left. Because of the stochasic nature, one can never synchronize multiple molecular machines. To analyze their mechanism, therefore, one needs to observe individual molecules closely.

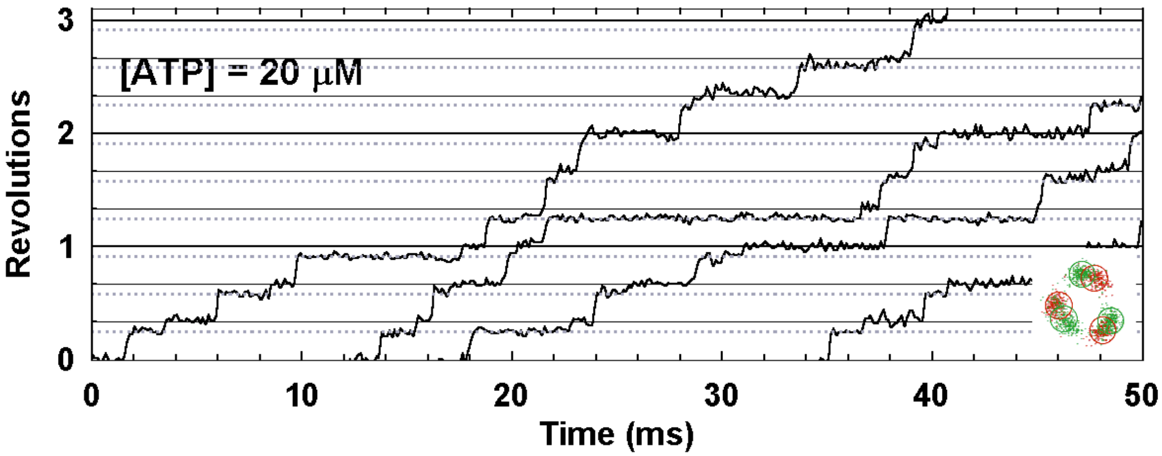
Yasuda, R et al. *Cell* **93,** 1117-1124 (1998).

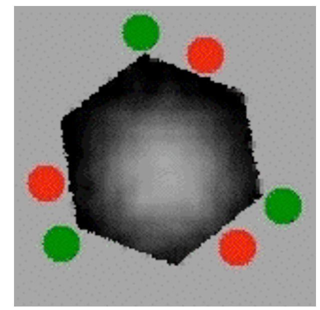
Substeps in F1 Rotation

From Yoshida web site



http://www.k2.ims.ac.jp/F1movies/F1Substp.htm

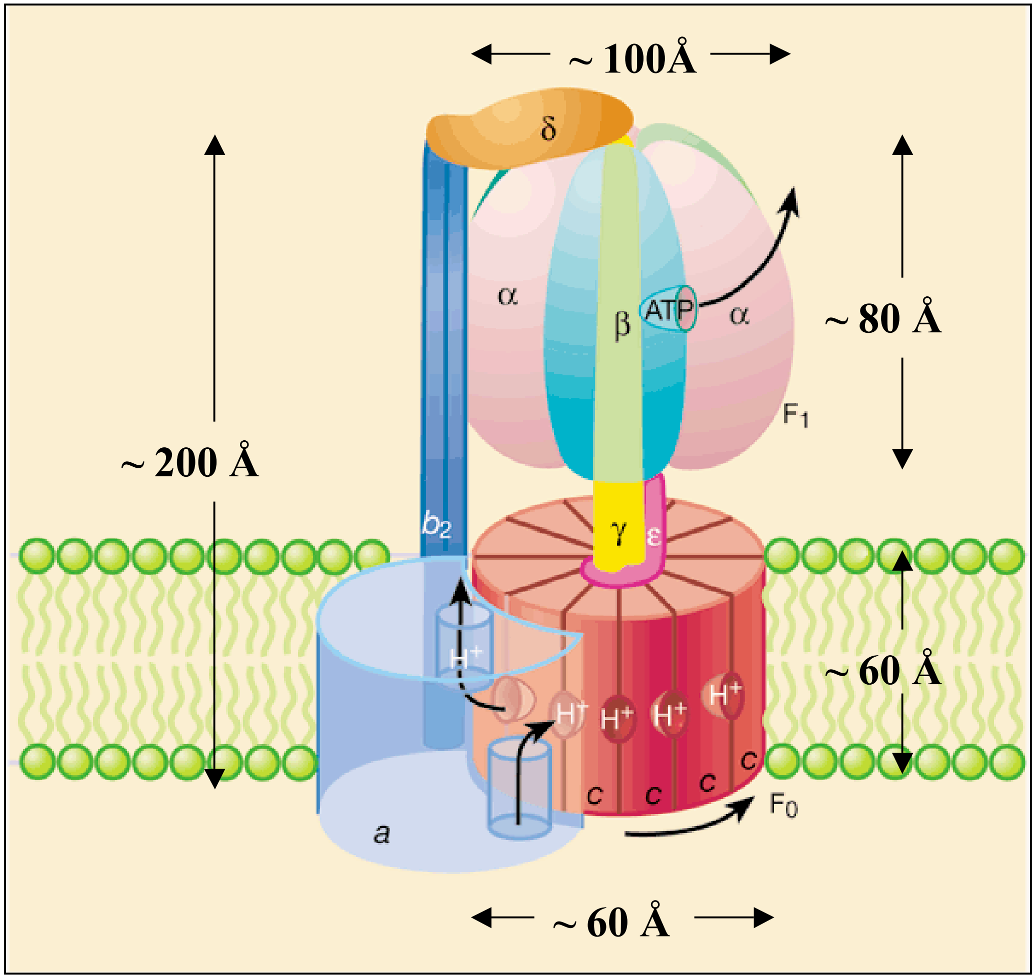




At speeds below the maximal, we were able to resolve substeps with an amplitude of 90° and 30° in the 120° step powered by the hydrolysis of one ATP molecule (see figure at left). If you have very good eyes, you may be able to detect some of the substeps in the actual images on the right. The 90° substep turned out to be driven by binding of ATP to a catalytic site on F_1, and the 30° substep by the release of a hydrolysis product(s). The hydrolysis reaction per se appeared to be mechanically almost silent.

Yasuda, R. et al., *Nature* **410,** 898-904 (2001).

Let’s look at F1

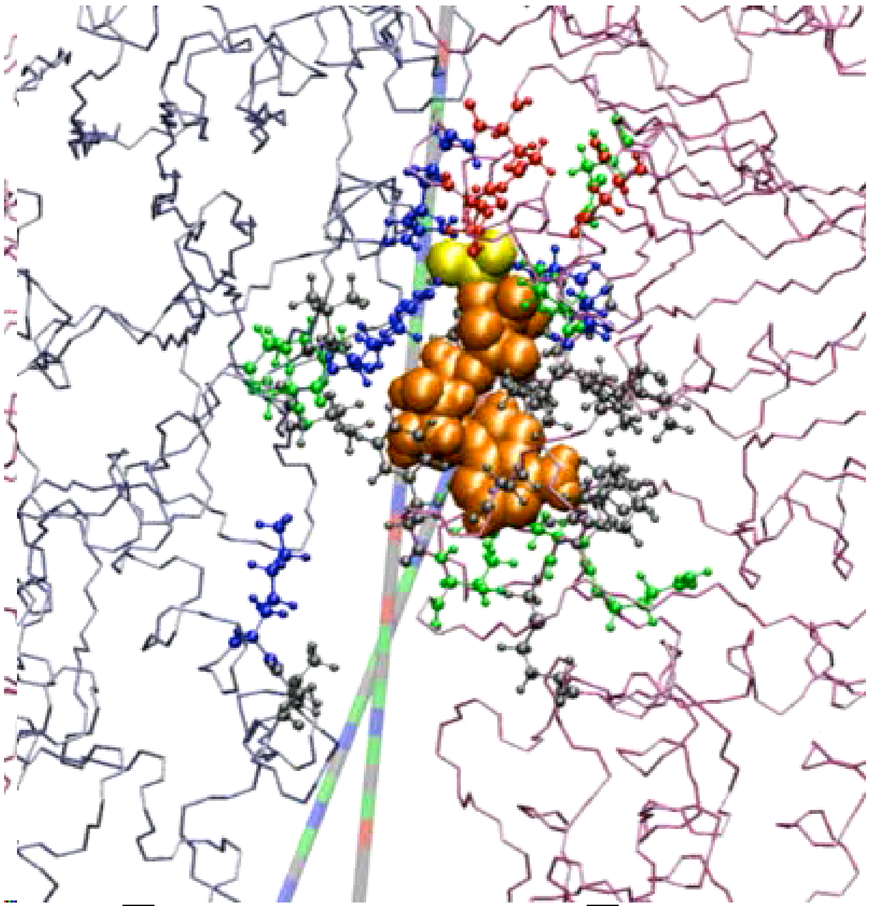


F1

Torque is transmitted between the motors via the central stalk.

A rough idea of central stalk's tasks:

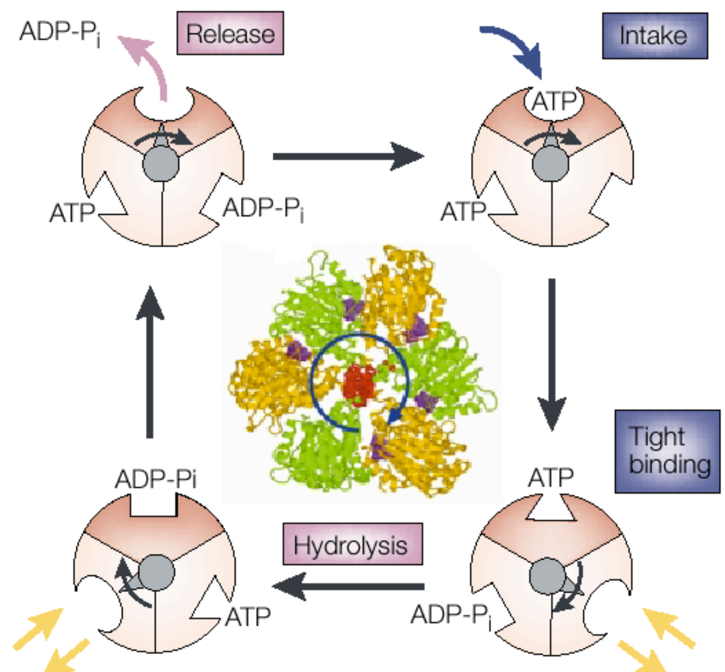
TP -> E -> DP -> TP (1994 Walker,1BMF)



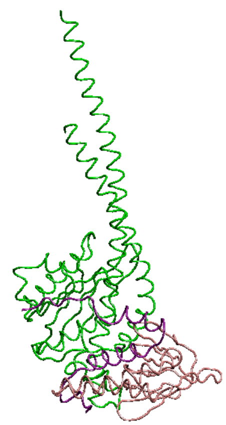
$\square_{TP}$ $\square_{TP}$

Interpolation of observed states

□-phosphate / orthophosphate is fixed at TP position



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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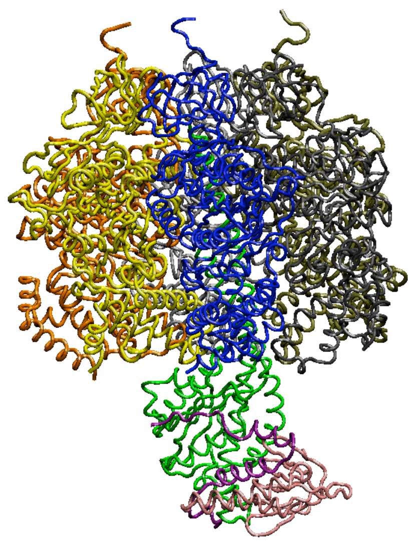

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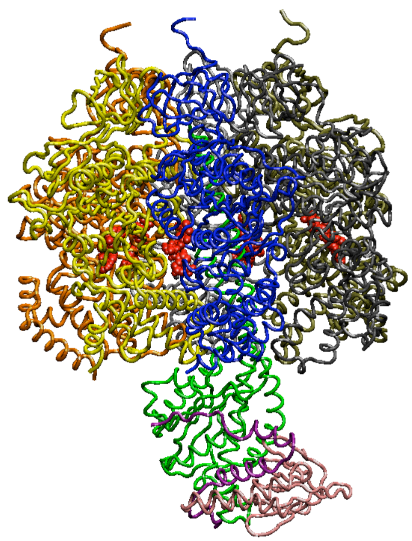

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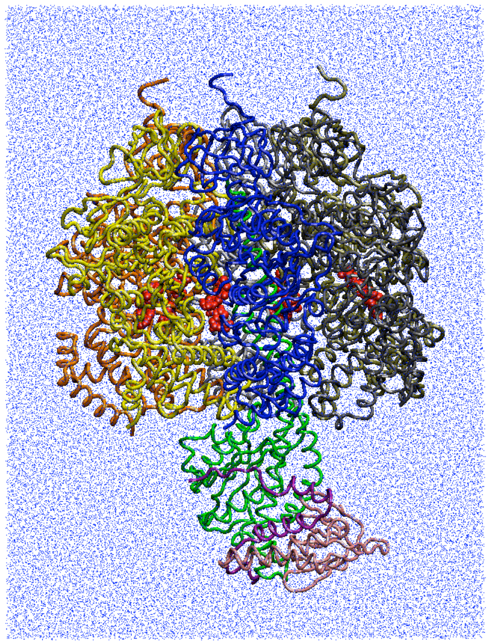

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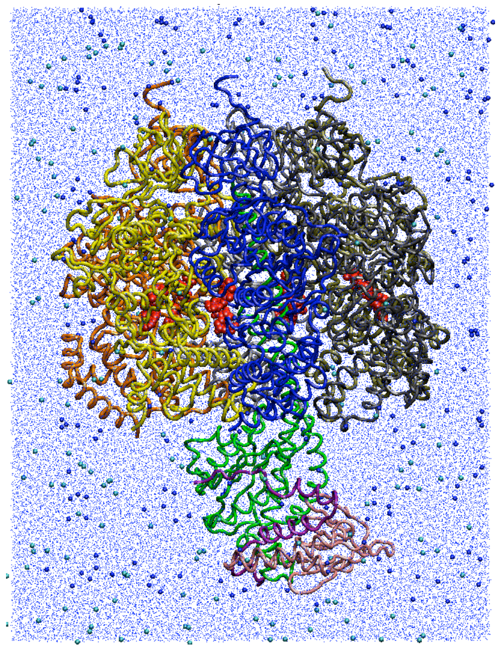

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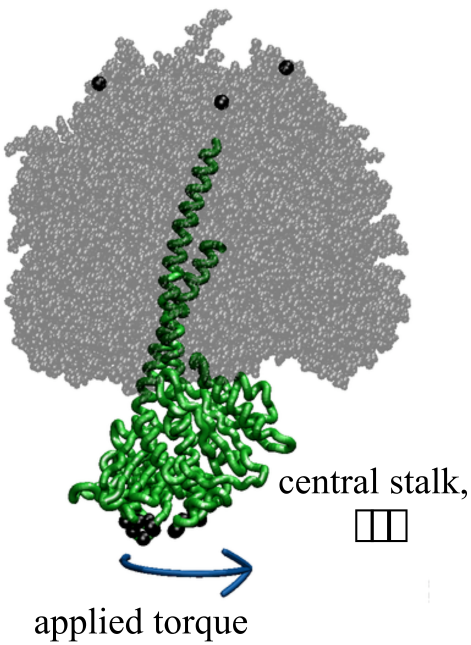
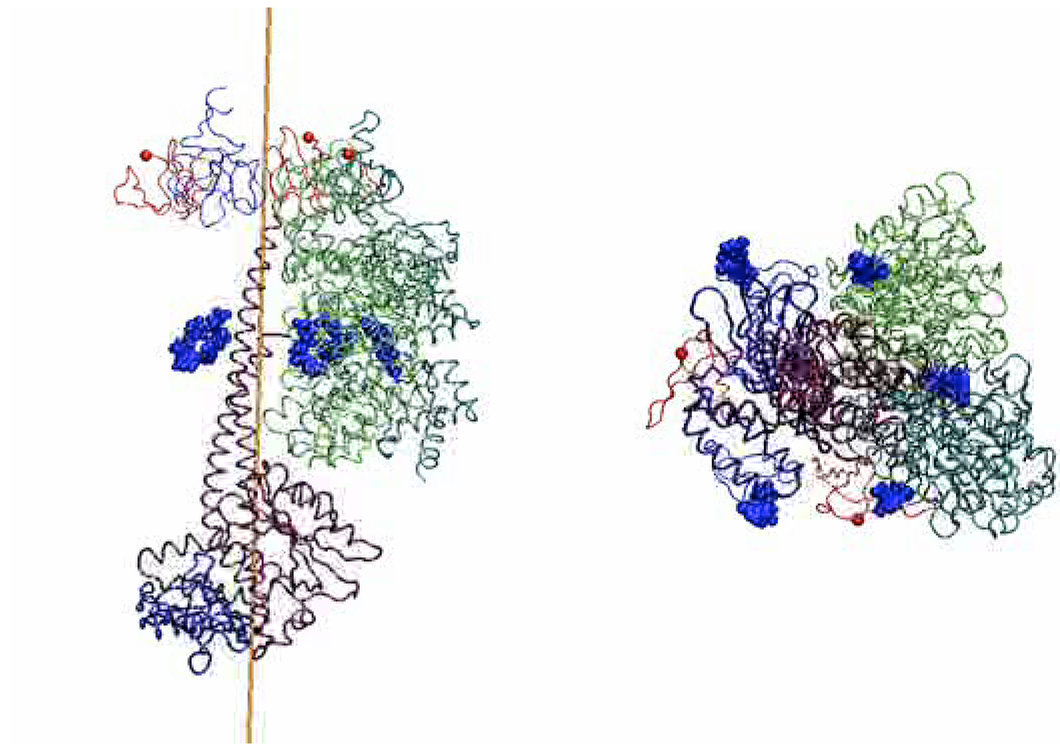
Torque application to F_1

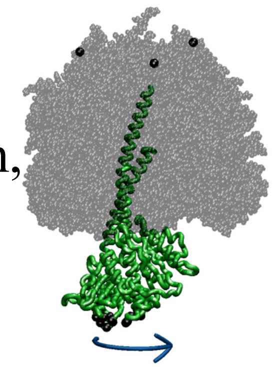
Torque is applied to the central stalk atoms at the F_1-F_o interface to constrain their rotation to constant angular velocity □ = 24 deg/ns.





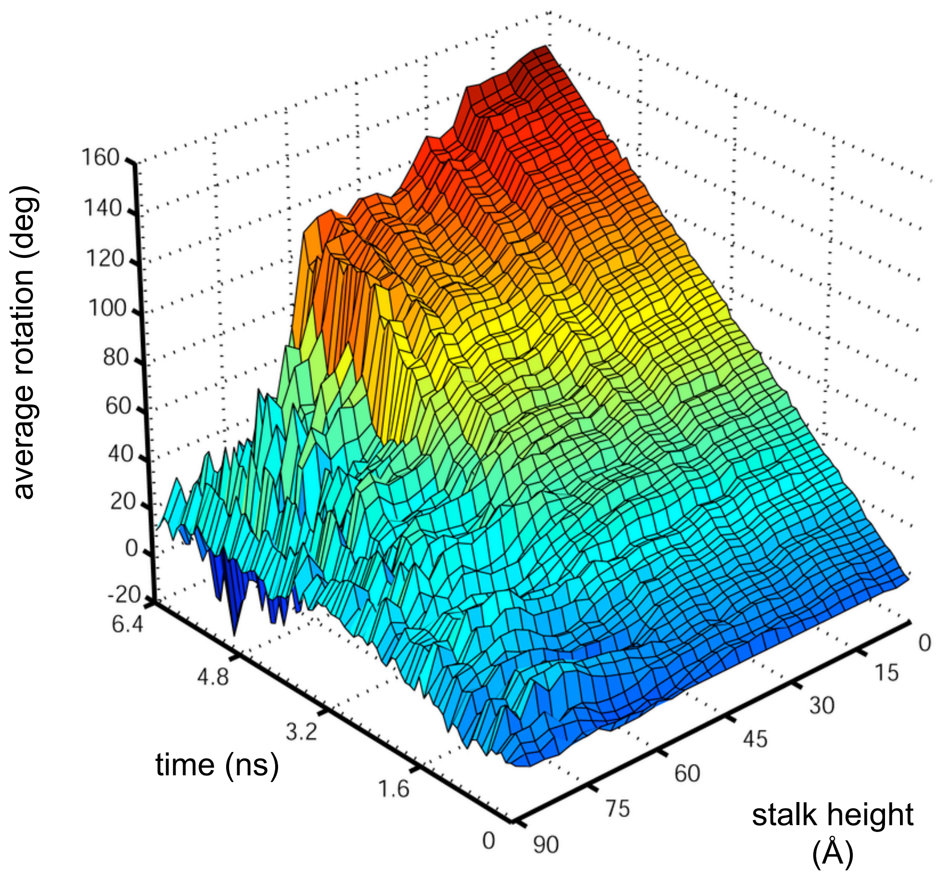
0.0 to 5.0 ns (0 to 120 deg) of torqued F_1 rotation, □ = 24 deg/ns.

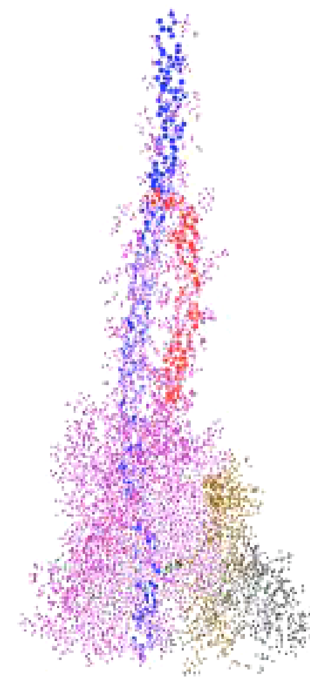
Stalk analysis



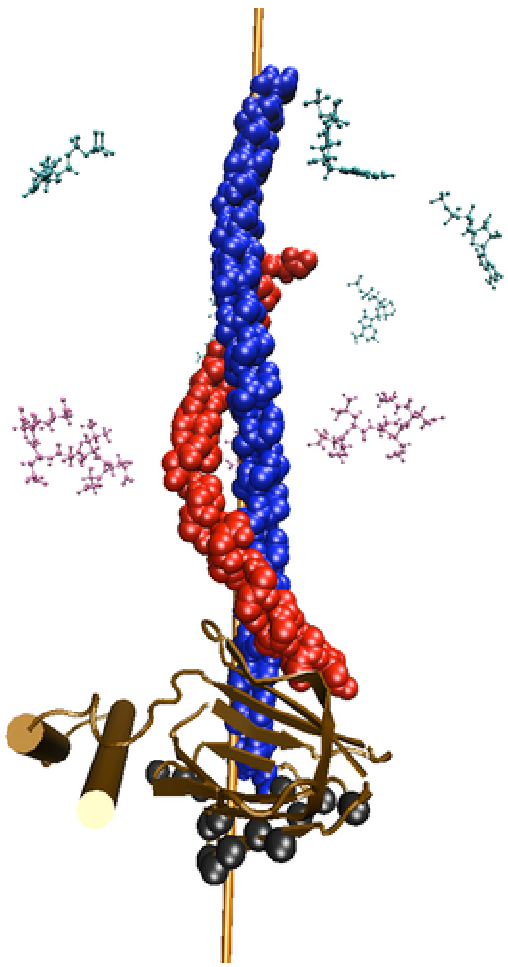
Using best RMSD rotation fit for stalk sections binned along axis direction, at 3.0 ns (72 deg) of rotation, we observe:

- slowed torque transmission along central stalk

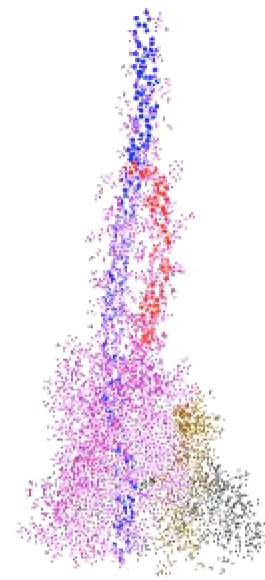




Winding of □coiled-coil

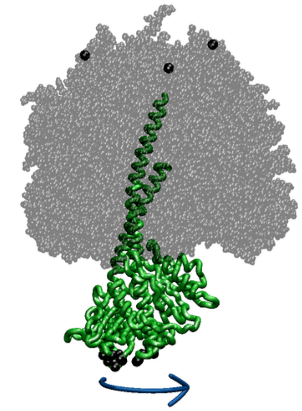


t= 3.0 ns
□= 72°



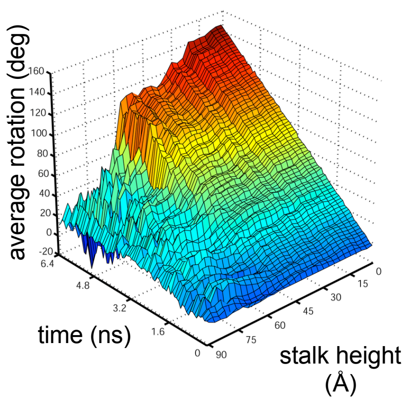
Different coupling for the two □helices:
1—50, partially via □ subunit
197—272, directly to F_o

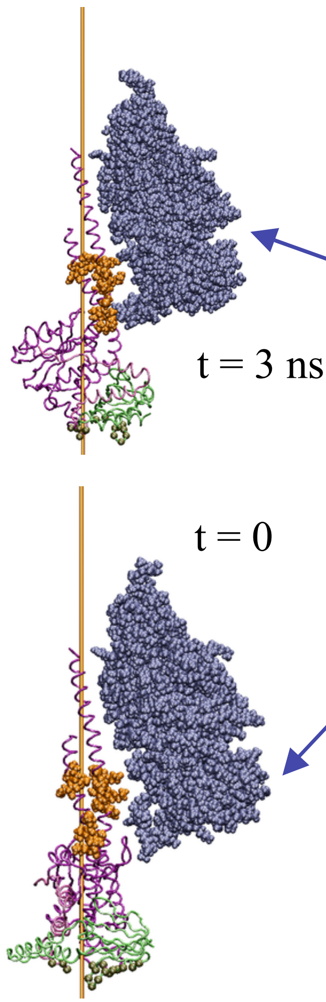
Rotation Produces Synthesis-like Events (1)

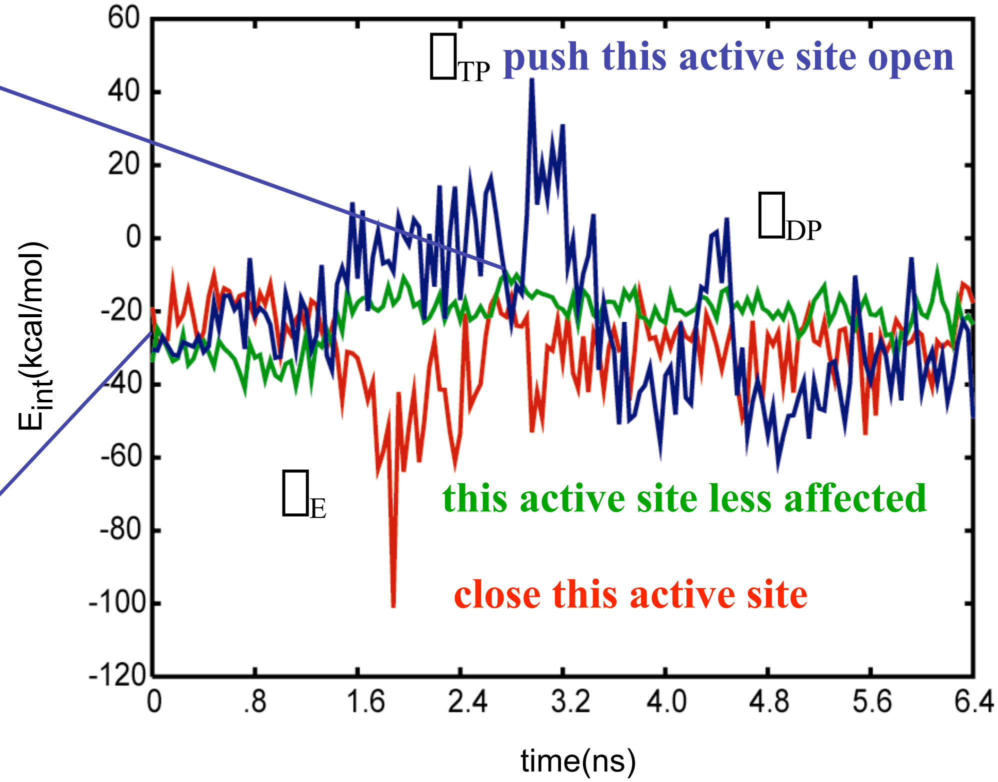


Around 3 ns (72 deg) of rotation, we observe:

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



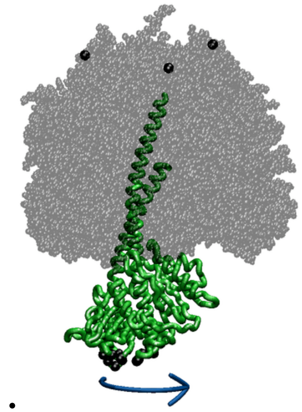




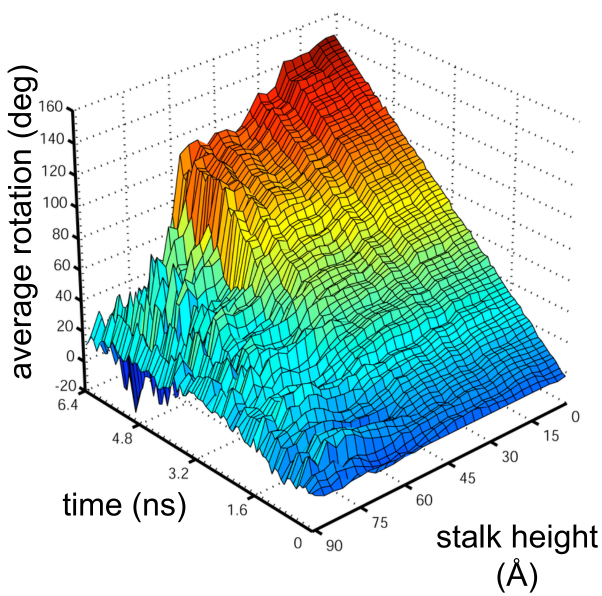
Rotation Produces Synthesis-like events (2)

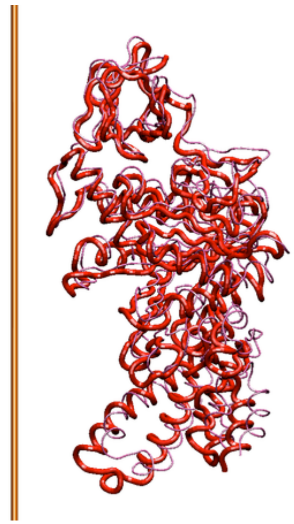
Around 3.0 – 3.5 ns (72 – 84 deg) of rotation, we observe:

- slowed torque transmission along central stalk
- opening and closing motions as expected

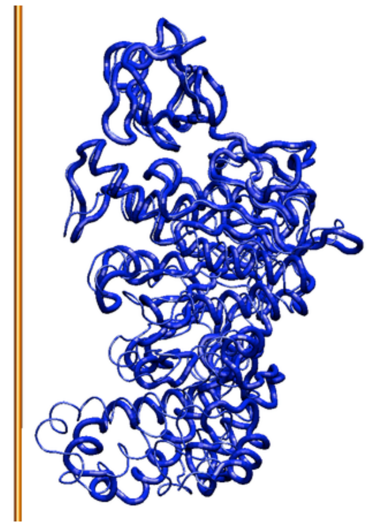


At 3.5 ns (84° rotation)…





β_E closes



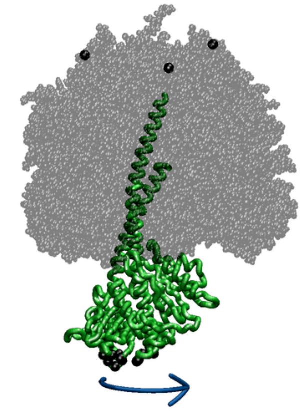
β_{TP} opens

β_{DP} does neither

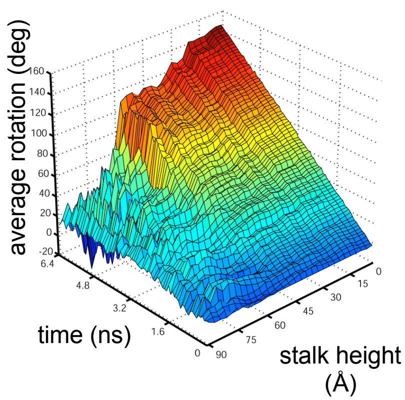
Rotation Produces Synthesis-like Events (3)

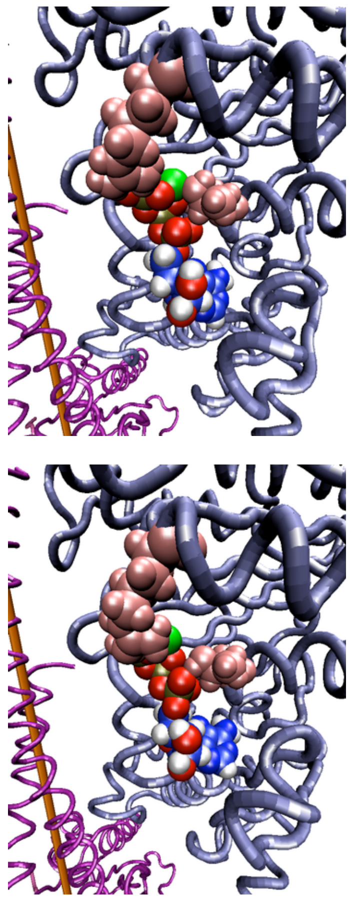
At 3.0 ns (72 deg) of rotation, we observe:

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



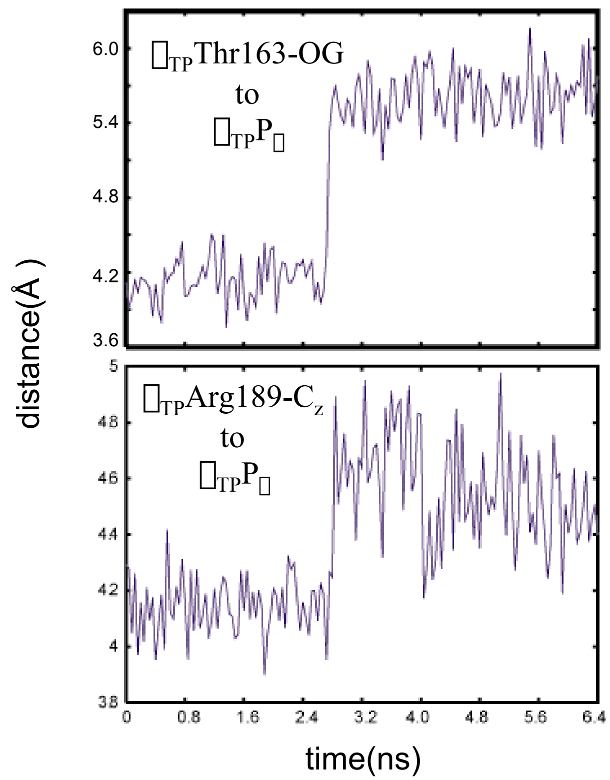
0 ns: active site closed



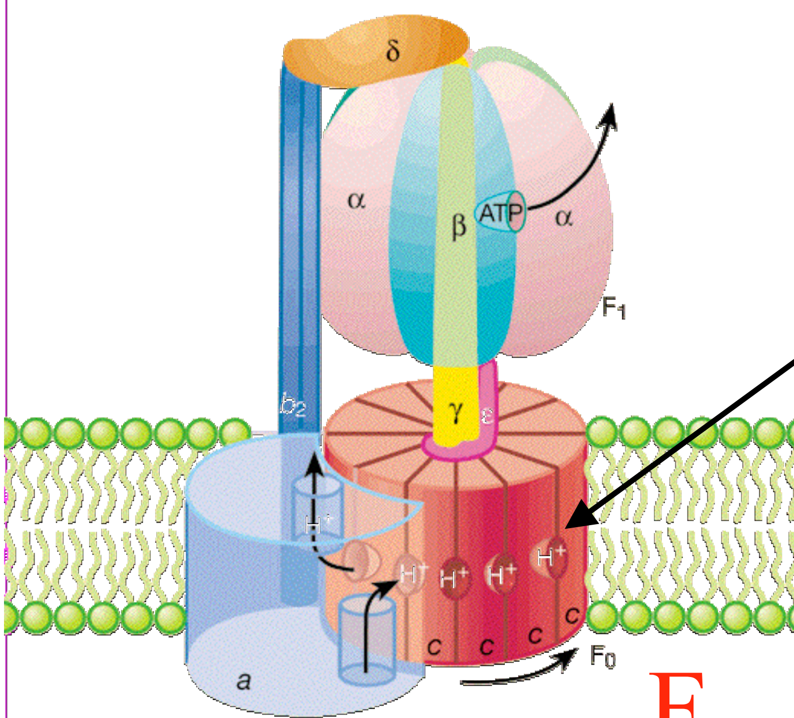


3 ns: active site open

ATP separates from active site residues



Fo ATP synthase

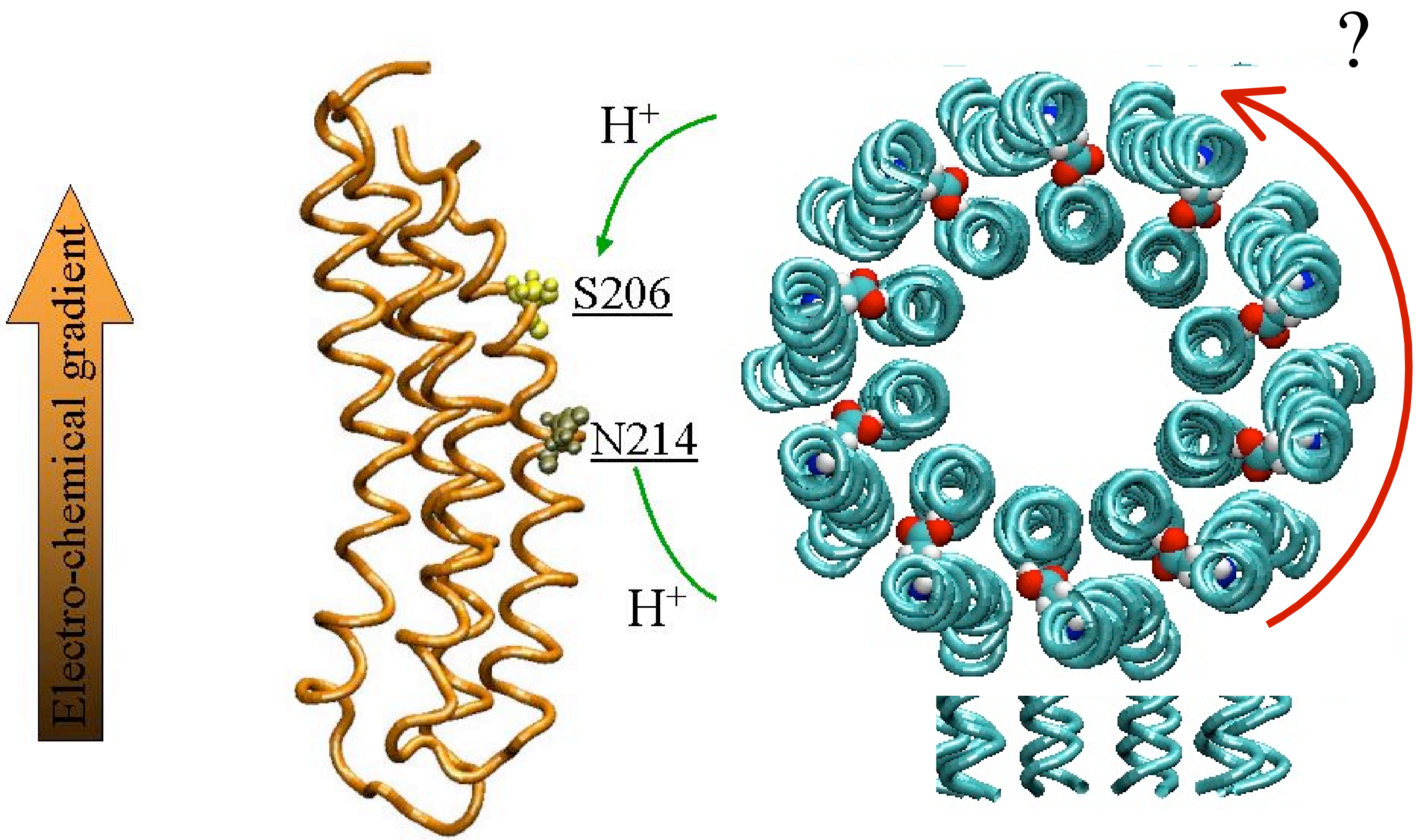


Asp 61 (D61) side groups take protons

F_0

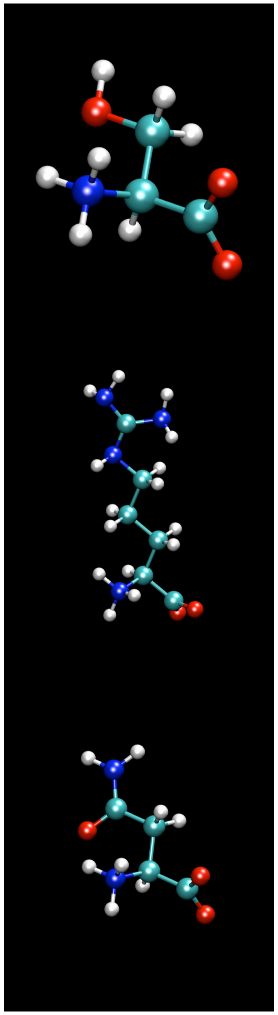
Transmembrane F_o unit (ab_2c_{10}): converts proton motive force into mechanical rotation of central stalk

Suggested Mechanism of Proton Translocation



(R.H. Fillingame, 2002)

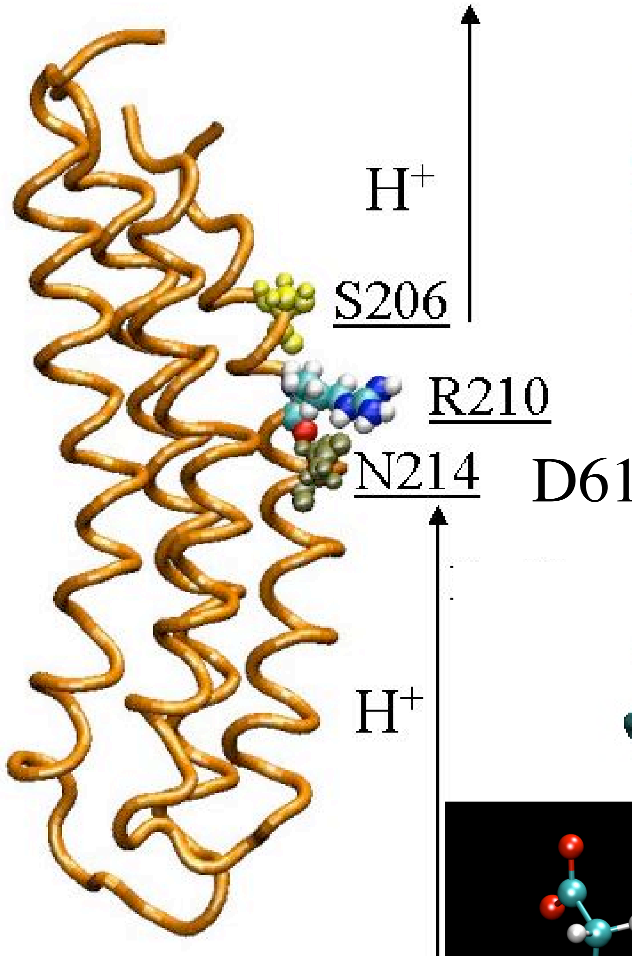
Key Amino Acids Participating in Electro-Mechanical Motor

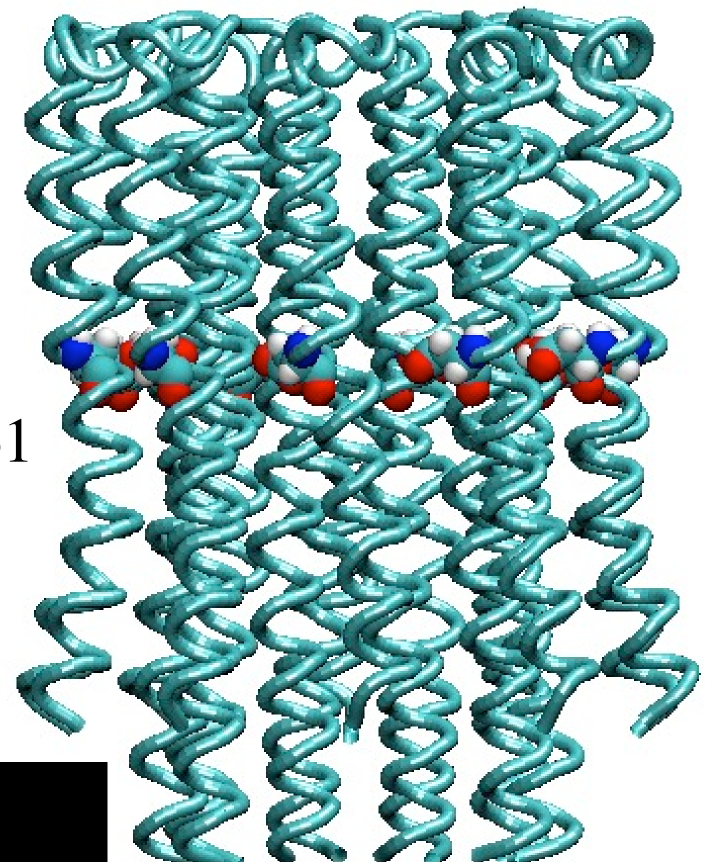


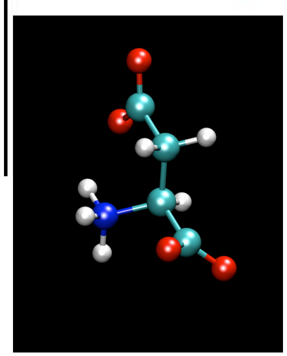
Serine
Ser = S

arginine
Arg = R

asparagine
Asn = N

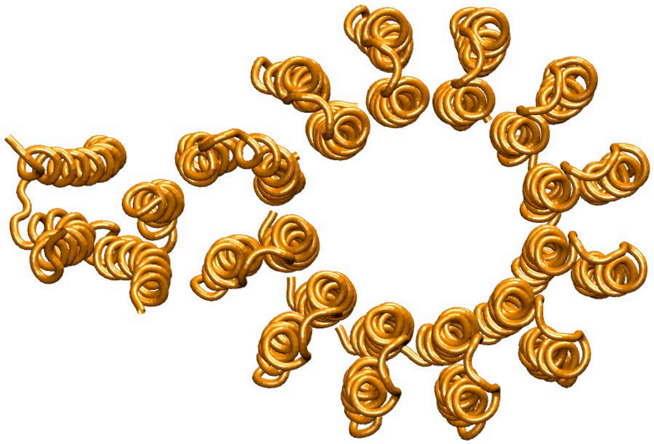






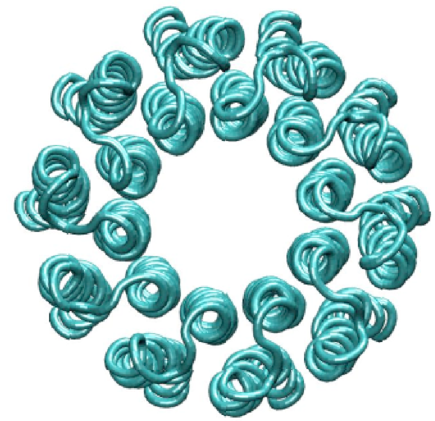
aspartate
Asp = D

Structural Model of *E. coli* F_o



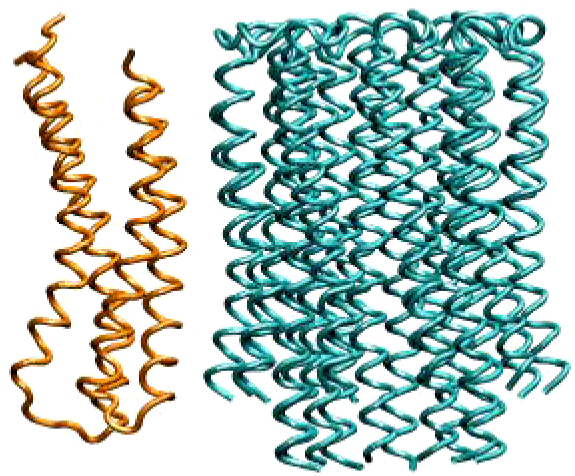
a_1c_{12} (Rastogi & Girvin, 1999, NMR)

+

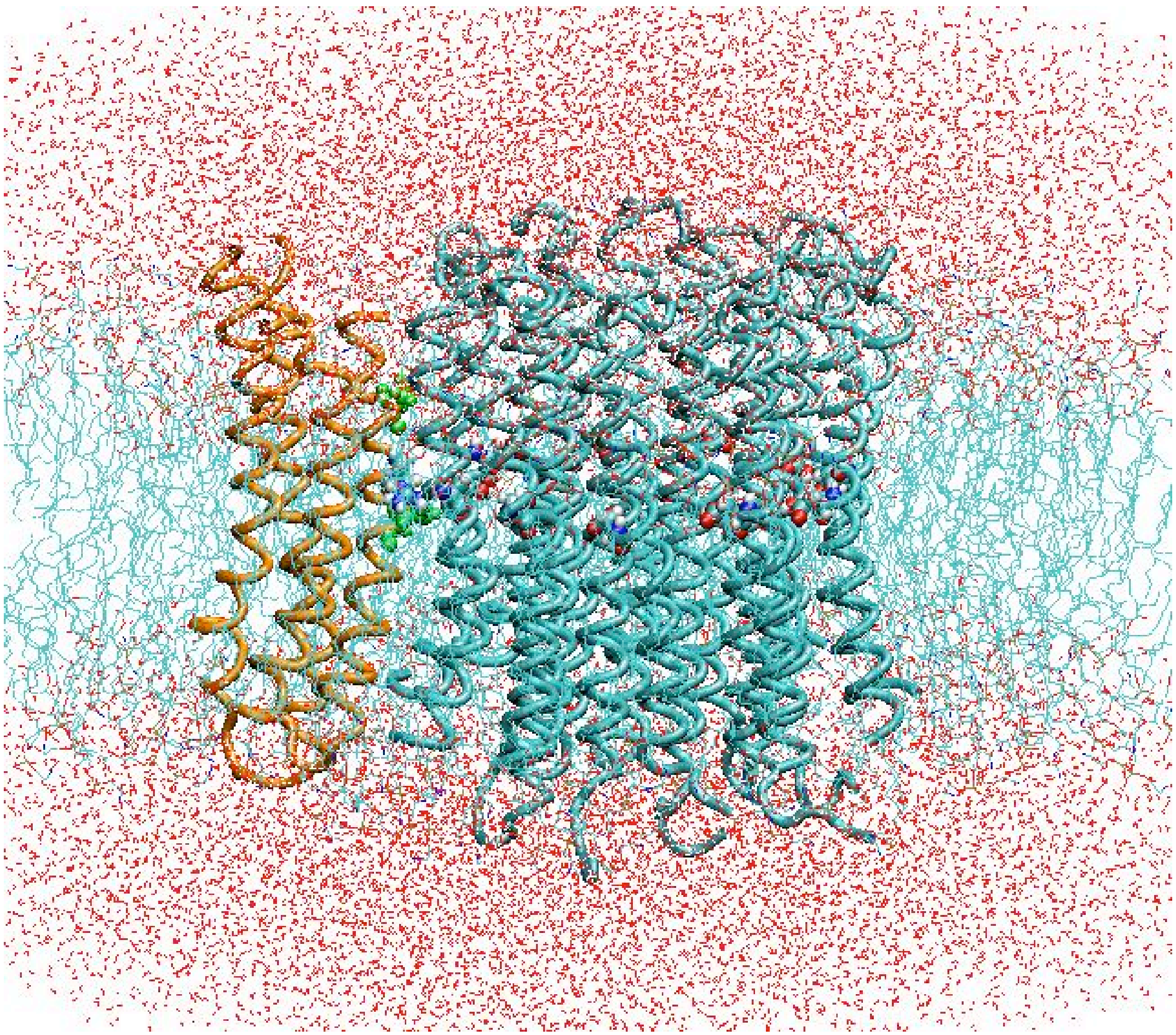


c_{10} (Fillingame et al, 1999, NMR)

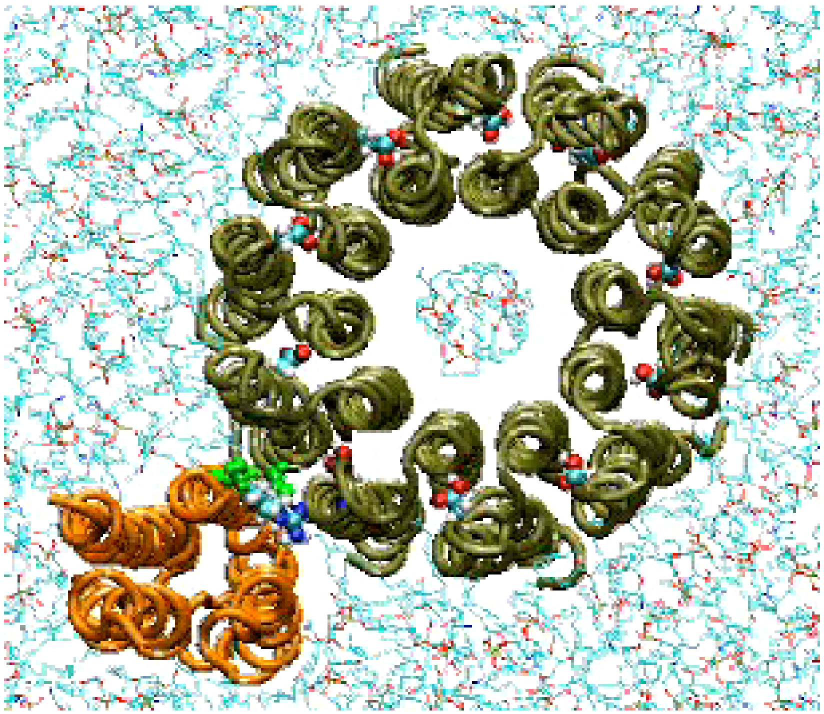
=



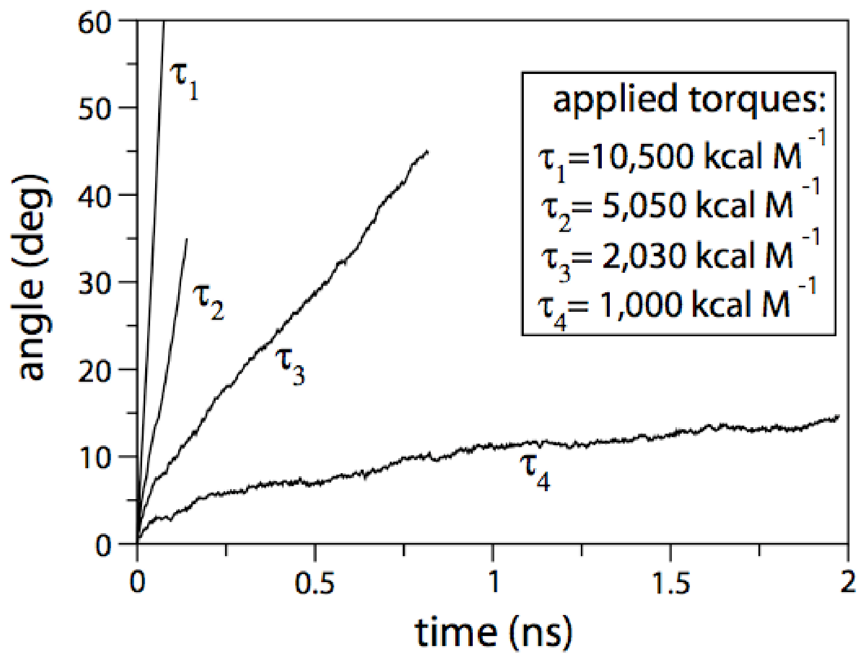
a_1c_{10} (2001-2002, modeling)



Forced Rotation of the c10 Subunit



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



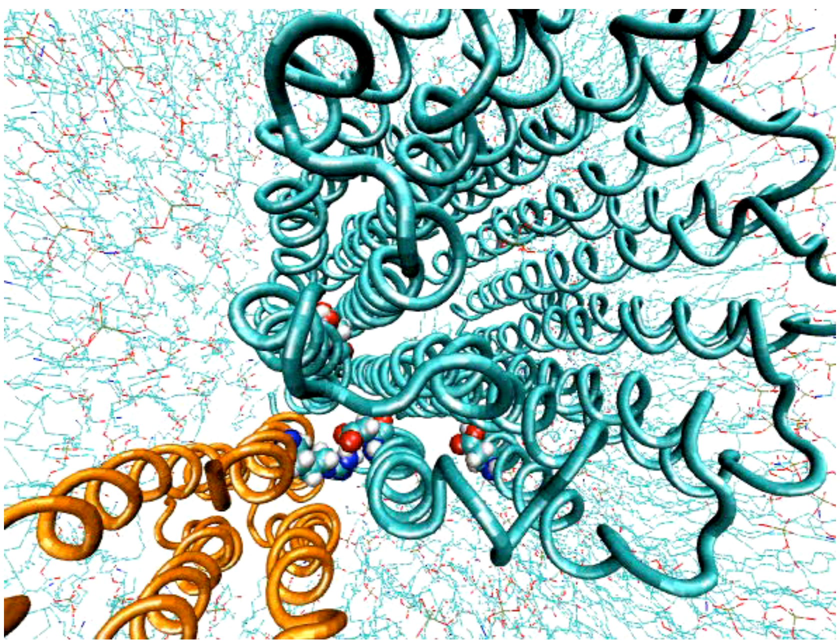
Estimated friction coefficient

□ $\sim 10^5$ kcal/(M sec)

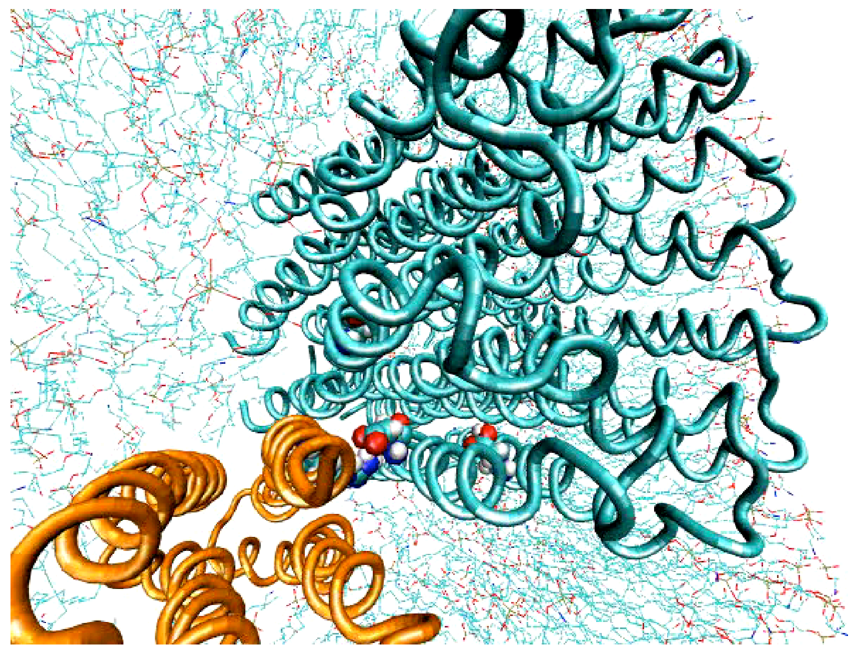
Salt Bridge Arg_{210}-Asp_{61} is Formed

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

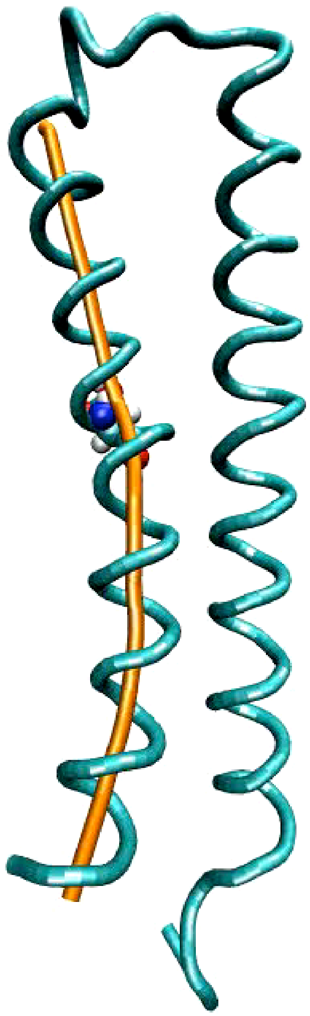
No restraints

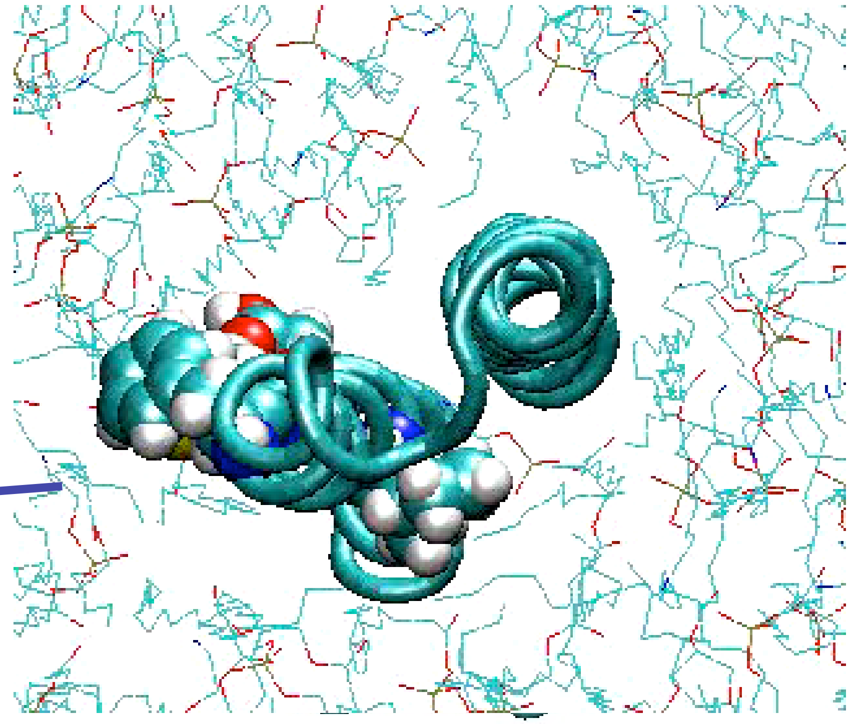


Subunit *a* is restrained



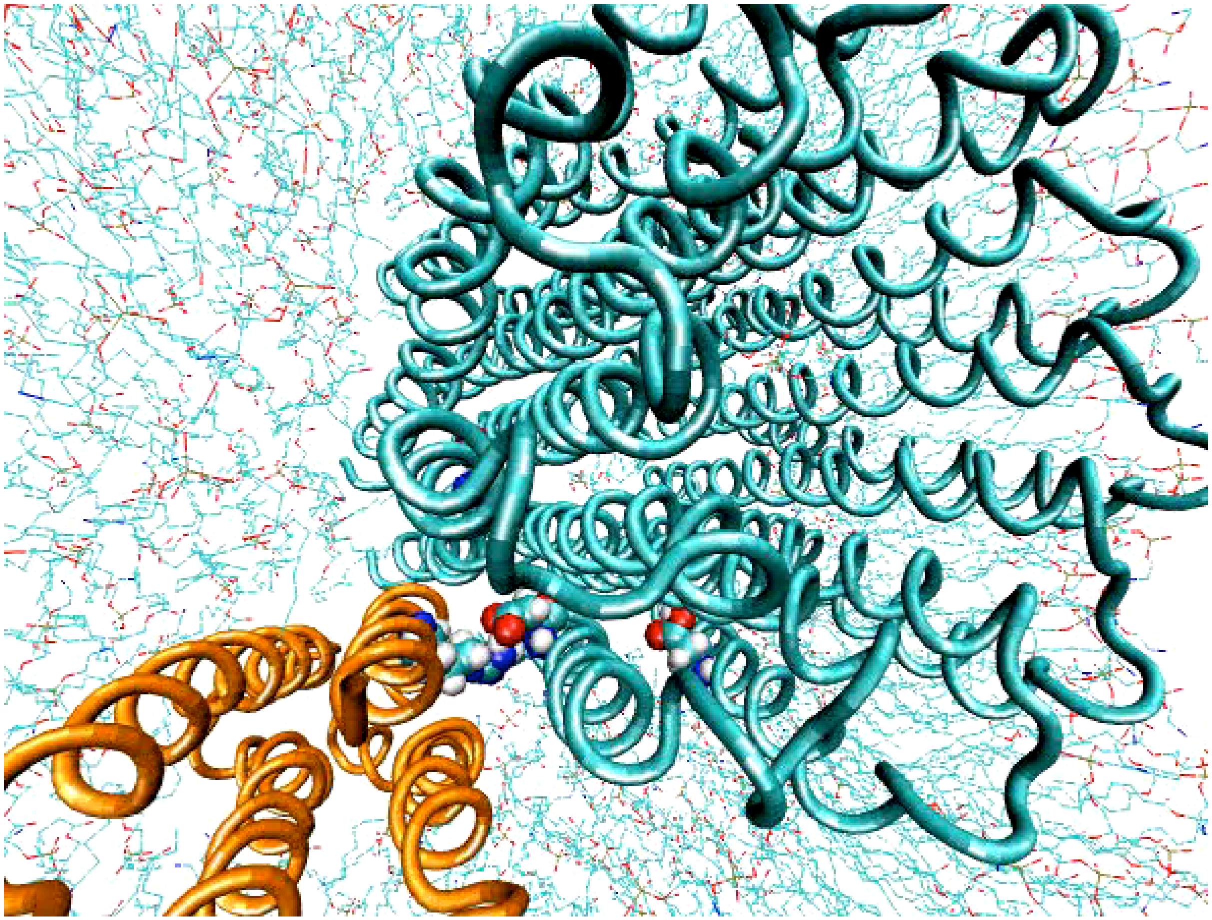
Single Helix Rotation





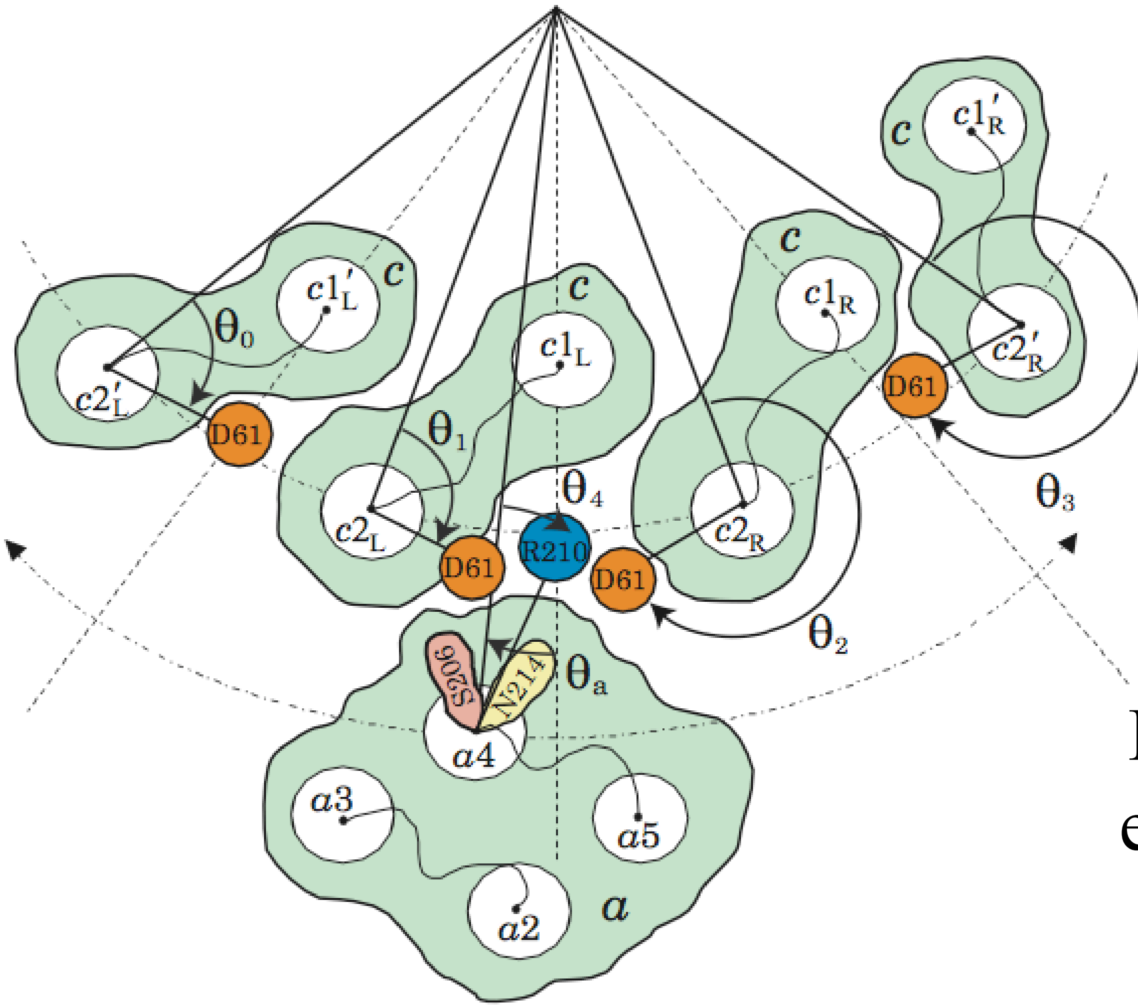
To minimize steric hindrance (critical on nanosecond time scale), helix was forced to rotate in a reptation tube (local pivot points and directors).

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*

Stochastic model

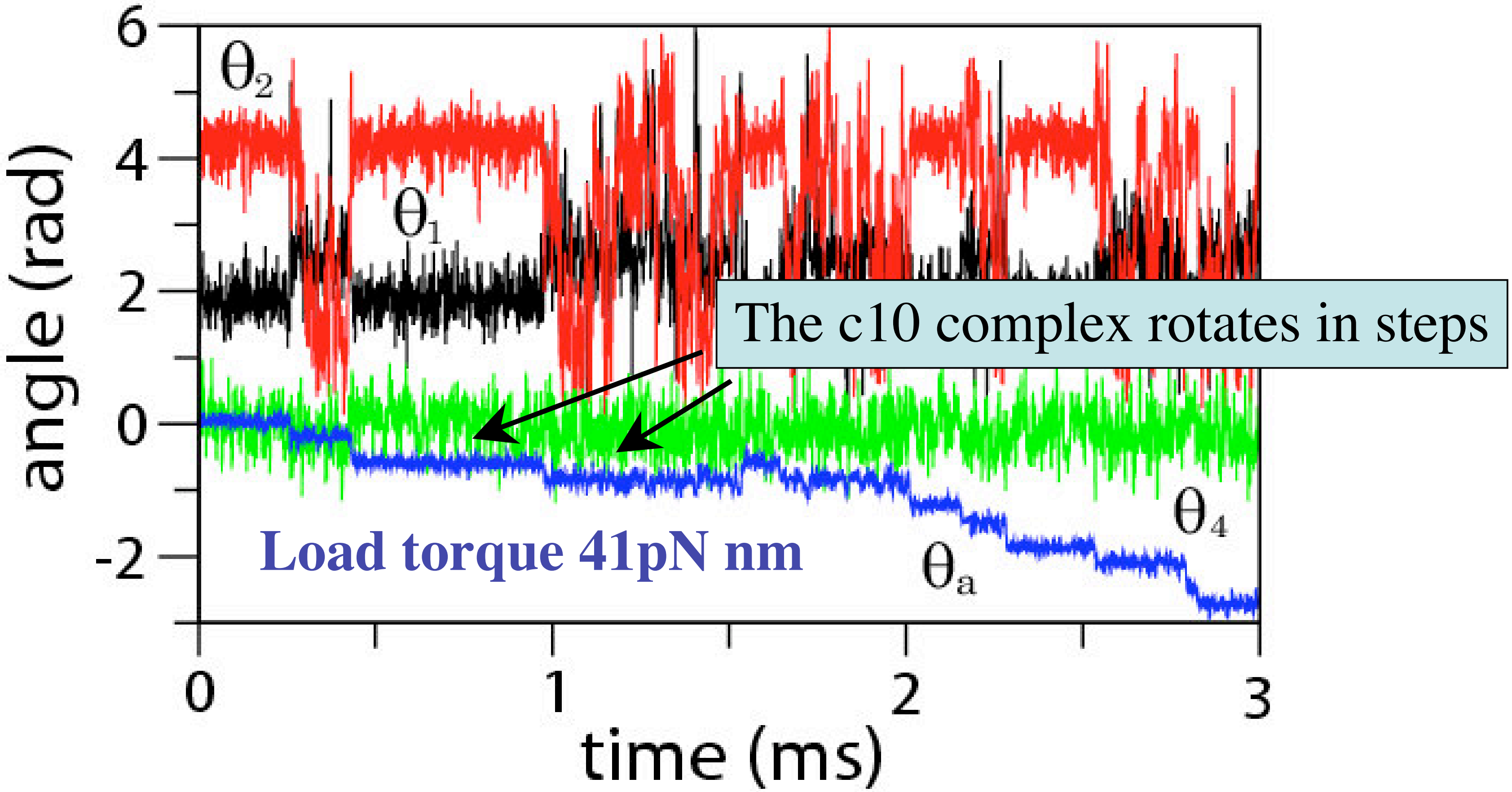


6 degrees of freedom: θ_0, θ_1, θ_2, θ_3, θ_4 are TMH rotation angles; θ_A - position of the *a* subunit

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

$$\square_i \frac{d\theta_i}{dt} = \square \frac{d}{d\theta_i}[\mathrm{U}_{\mathrm{group}} + \mathrm{U}_{\mathrm{hydroph.}} + \mathrm{U}_{\mathrm{internal}}] + \square_i(t)$$

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.