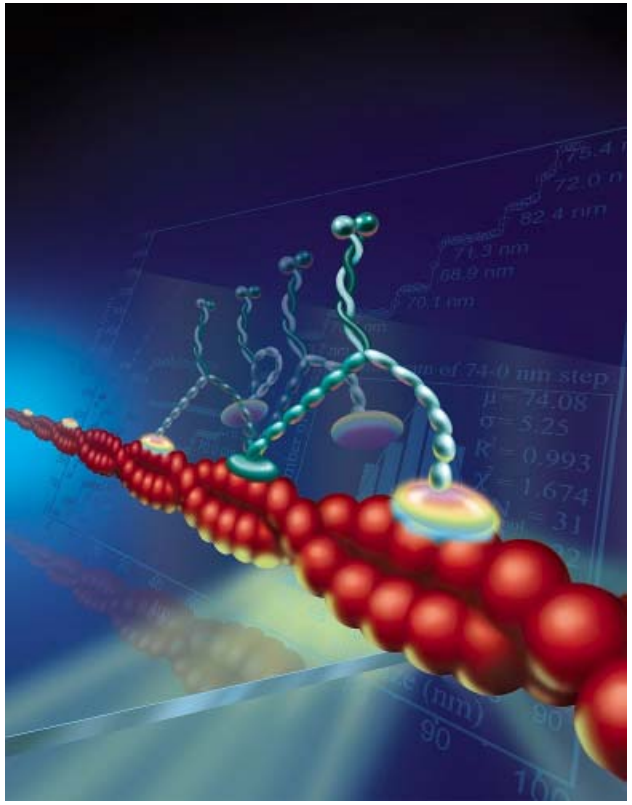
From Paul Selvin's lab at UIUC

Fluorescence imaging with one nanometer accuracy

FIONA



Myosin V (green), a biomolecular motor that moves in nanometer-size steps on actin (red), transports cargo within cells. By placing a fluorophore near one foot (rainbow colored oval), and following the motion a single myosin V, Yildiz and others were able to determine that myosin V "walks," placing one foot over the other, and does not "crawl."

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Myosin V

Myosin V is a processive motor involved in transporting vesicles and organelles in the cell .

Myosin V It contains two “heads” held together by a coiled-coiled cargo-carrying stalk. Each head contains a globular domain responsible for actin-binding and ATP hydrolysis, and a $<$24 nm long α-helix bound to six calmodulins that likely

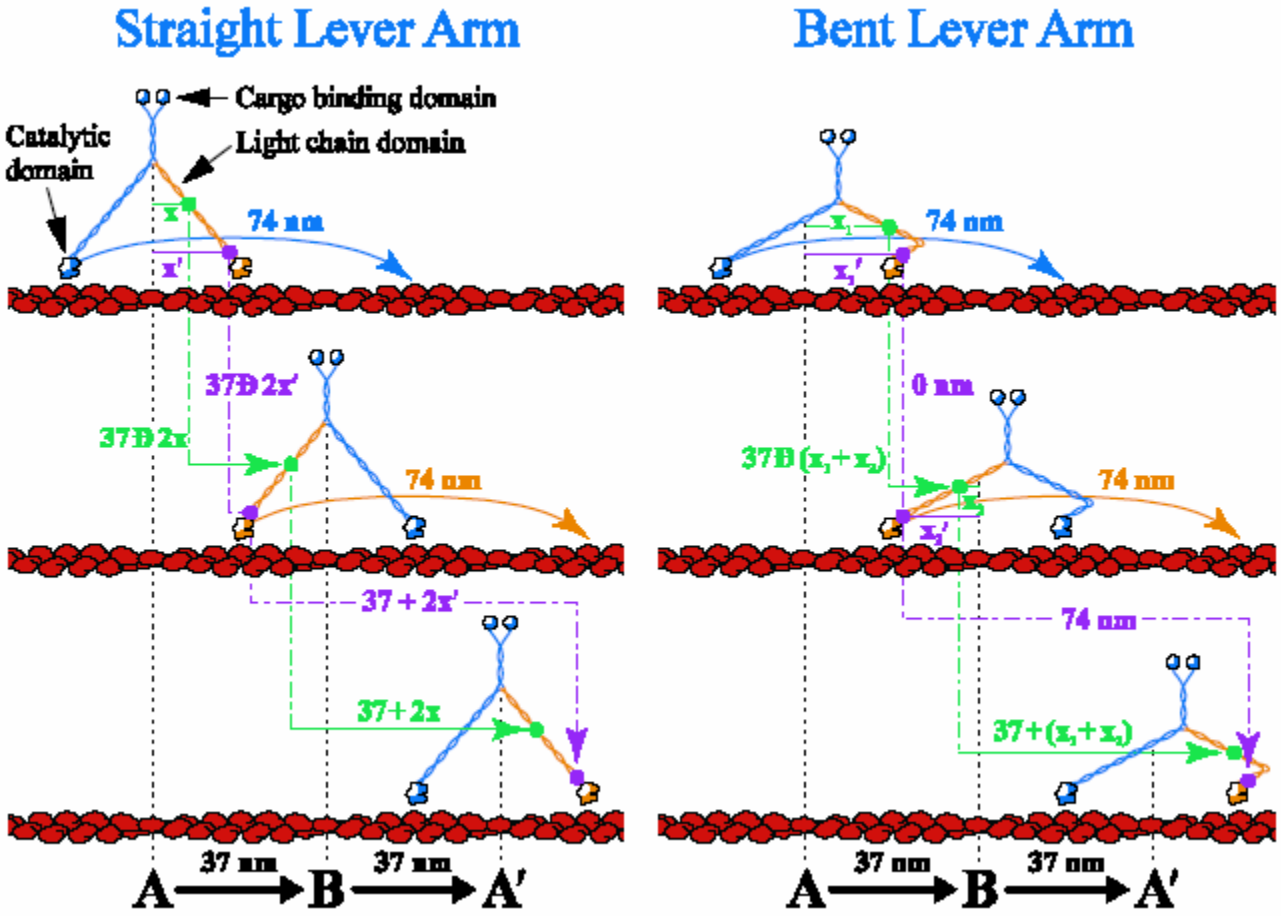
act as a lever arm to amplify small nucleotide-dependent conformational changes in the globular domain.

The cargo, or center-of-mass, moves 35-40 nm for each ATP hydrolyzed.

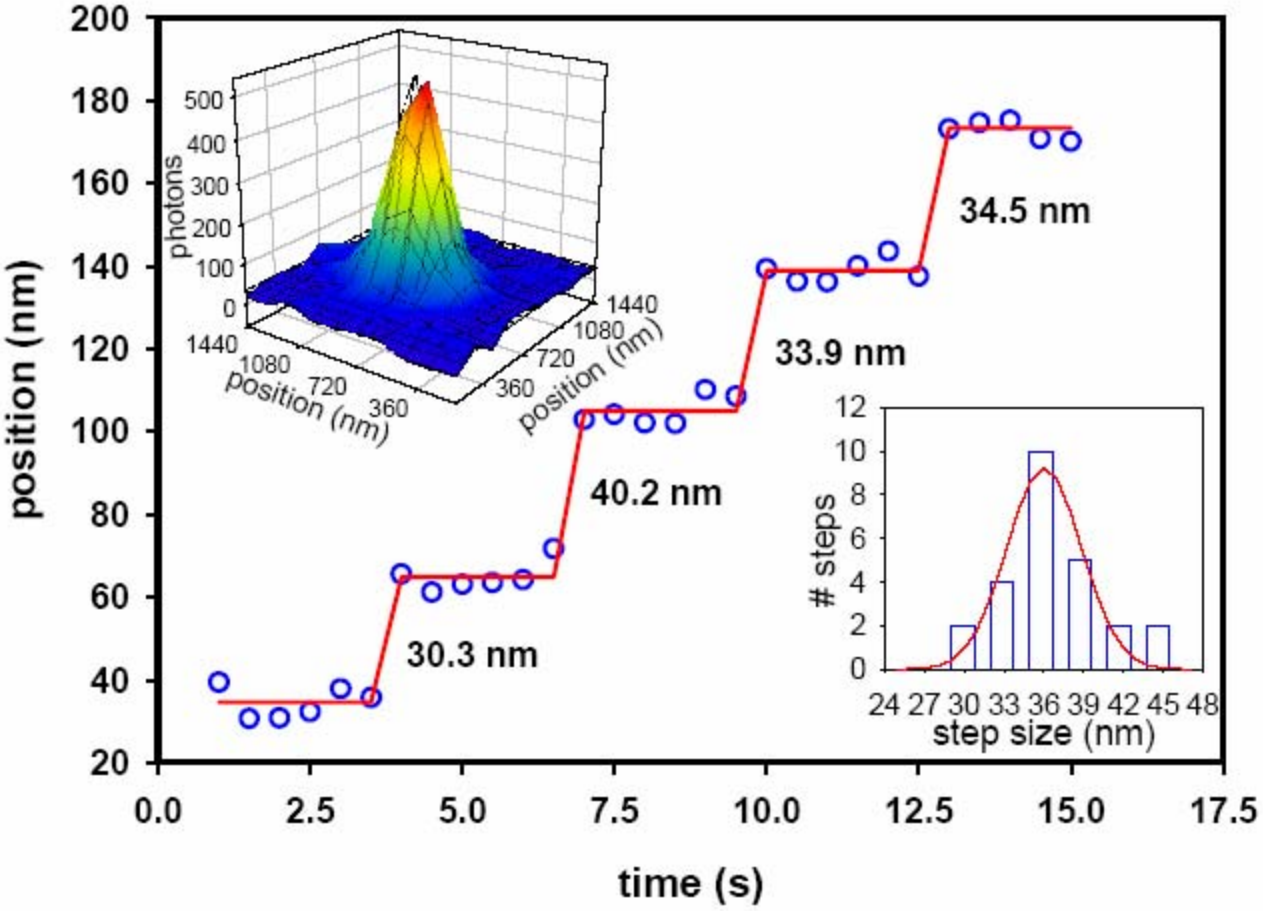
Recent work has shown that myosin V moves processively along actin in a "hand-over-hand" or "walking" manner in which each head takes turn in the lead (Forkey et al. 2003; Yildiz et al. 2003).

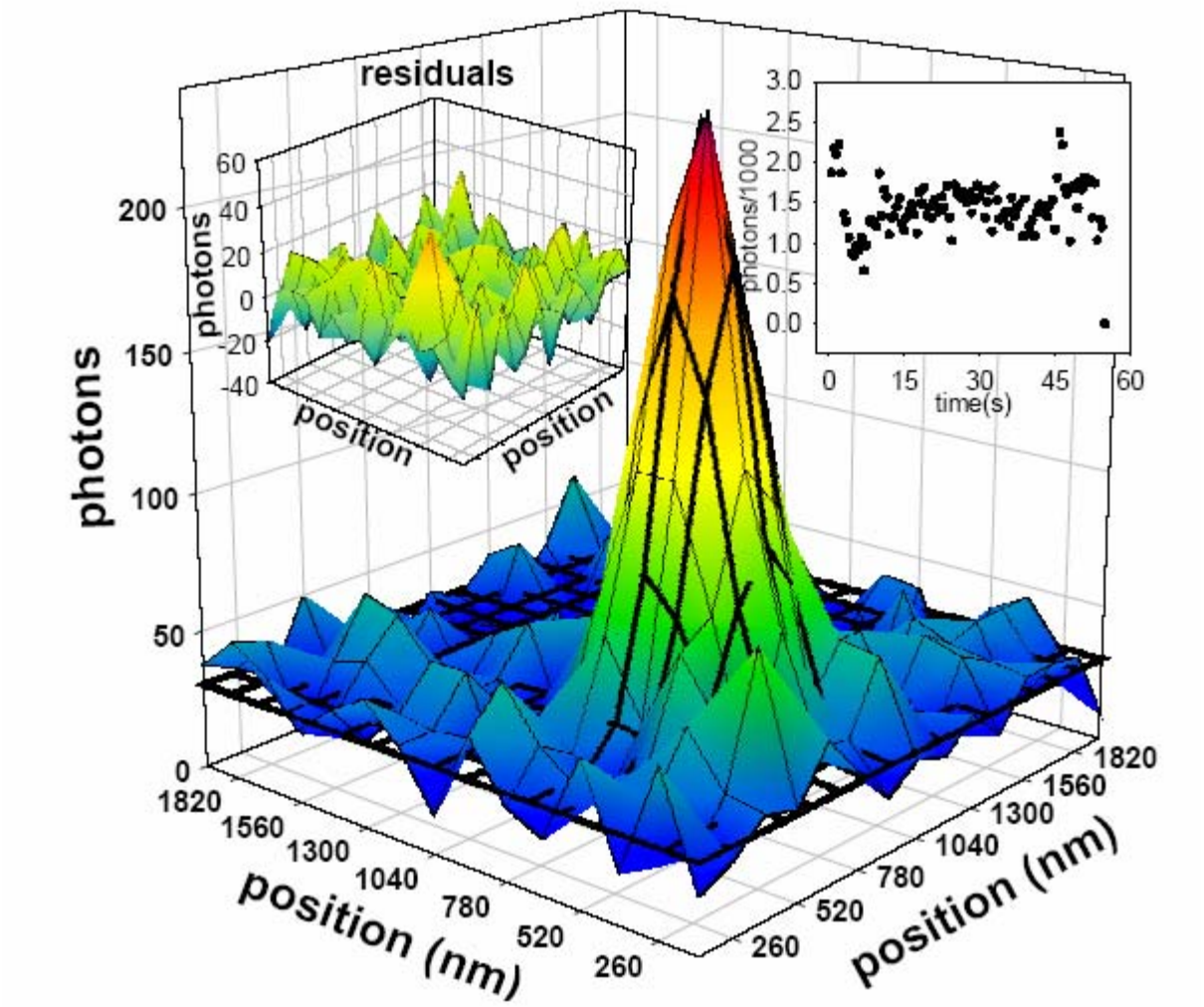
The rear head translates 74 nm, moving past the front head, which remains stationary, and the cargo is moved $<$37 nm for each step (Fig. 1).

This is in contrast to an **inchworm** model, which has, for example, been postulated for kinesin (Hua et al. 2002). In an inchworm model, all parts of the motor protein translate uniformly – for myosin V, this would be 37 nm.


Straight Lever Arm
Bent Lever Arm
Cargo binding domain
Catalytic domain
Light chain domain
74 nm
37Đ 2x'
37Đ 2x
37 + 2x'
37+2x
0 nm
37Đ (x1+x2)
37+(x1+x2)
37 nm
A
B
A'

Methods of center of mass determination


position (nm)
time (s)
30.3 nm
40.2 nm
33.9 nm
34.5 nm
photons
position (nm)
position (nm)
steps
step size (nm)

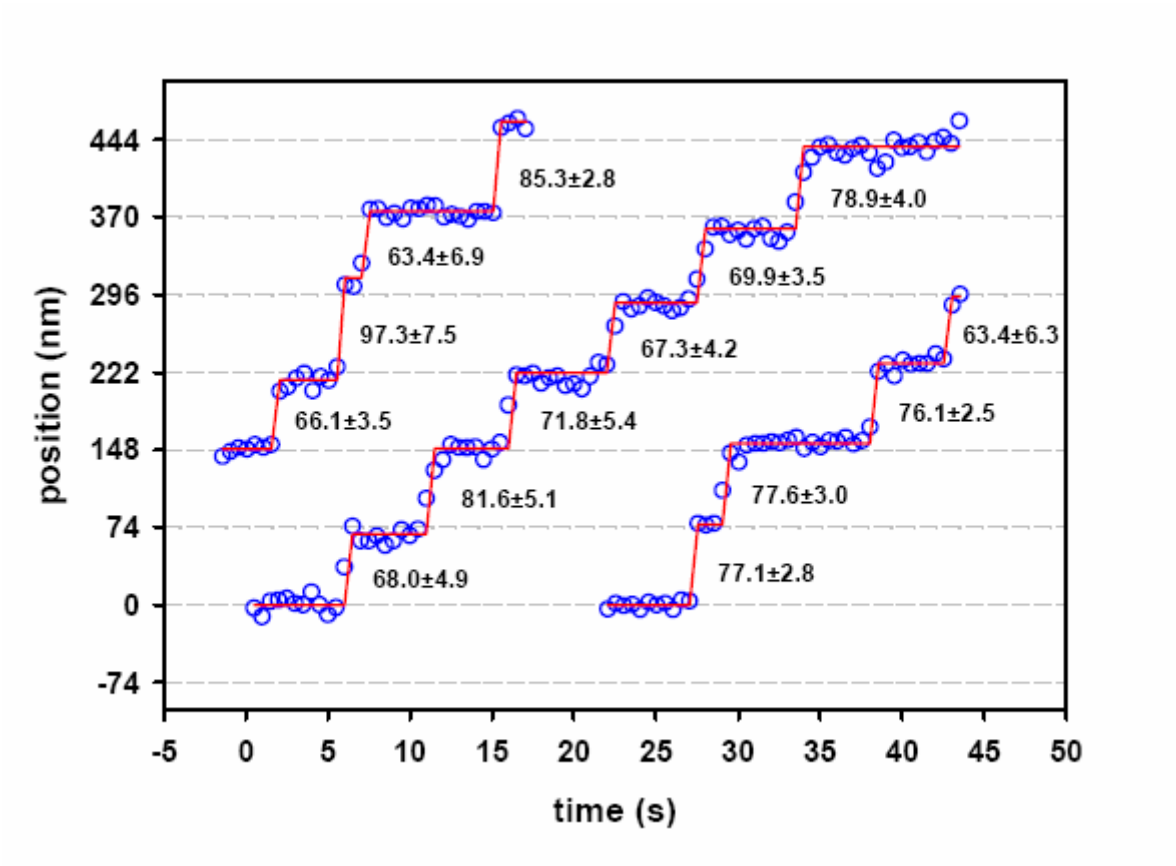

residuals
photons
200
150
100
50
0
1820
1560
1300
1040
780
520
260
position (nm)
60
40
20
0
-20
-40
position
position
3.0
2.5
2.0
1.5
1.0
0.5
0.0
photons/1000
0
15
30
45
60
time(s)

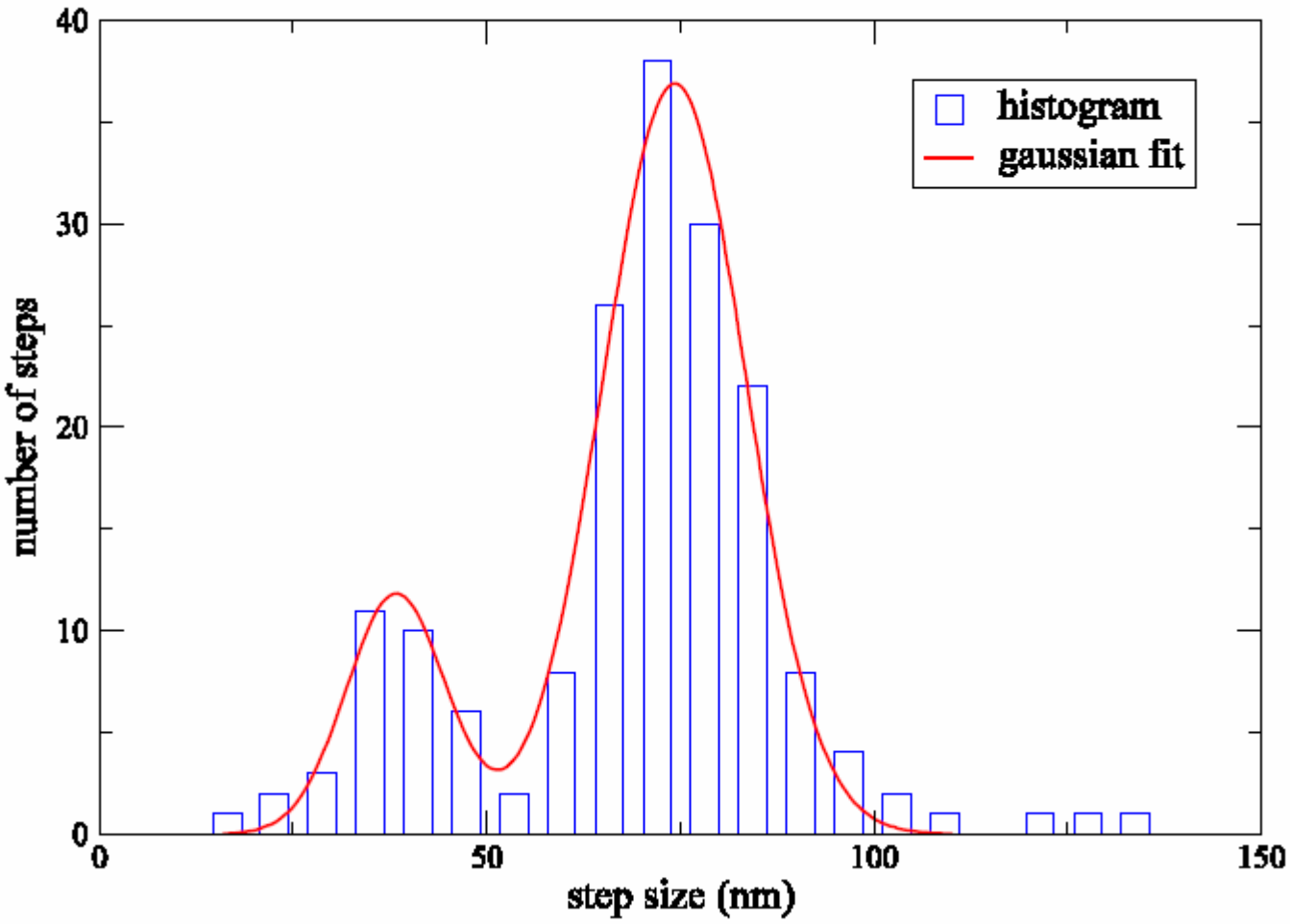
How the two heads are coordinated in the hand-over-hand mechanism is a central unresolved question. Nucleotide-hydrolysis, actin-binding, and lever arm position are likely tightly coupled.

Unidirectional motion implies functional asymmetry between the heads to create out-of-phase binding and hydrolysis. For example, to create a forward step, the front head should be tightly bound while the rear head is not bound.

One model postulates that ATP binding to the rear head releases that head, which is then thrown forward by strain created previously in the myosin.

(To prevent binding of ATP and release of the front head, strain-induced inhibition of ATP binding to the front head has been postulated in the kinesin motor)

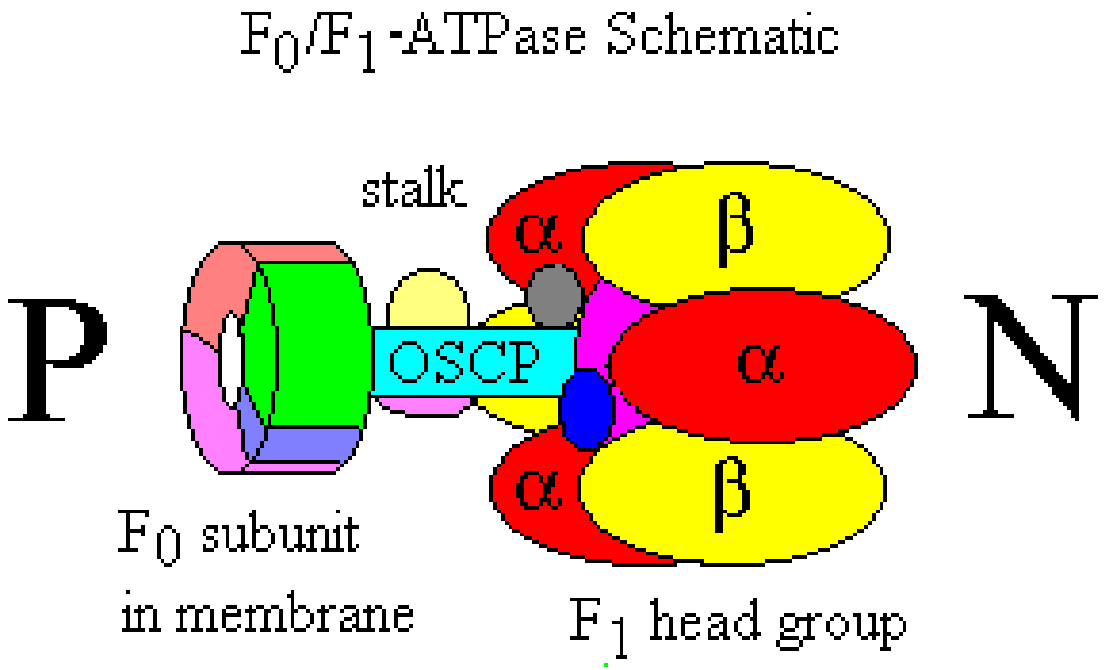

position (nm)
444
370
296
222
148
74
0
-74
-5
0
5
10
15
20
25
30
35
40
45
50
time (s)
85.3±2.8
63.4±6.9
97.3±7.5
66.1±3.5
78.9±4.0
69.9±3.5
67.3±4.2
63.4±6.3
71.8±5.4
76.1±2.5
81.6±5.1
77.6±3.0
68.0±4.9
77.1±2.8


histogram
gaussian fit
number of steps
step size (nm)
0
10
20
30
40
0
50
100
150

The 74 nm steps observed with individual eGFP-myosin V are further support of a hand-over-hand model for myosin V, consistent with our earlier studies using exogenous fluorophores placed on the light-chain domain of myosin V (Yildiz et al. 2003).

In addition, the 74.3 nm steps of the GFP on the globular domain are essentially identical to the 73.8 nm found previously for a dye on the light-chain domain, 18.5 nm from the midpoint along the direction of motion (Yildiz et al. 2003).

This in turn implies there is a kink in the lever arm domain above the dye, creating a telemark skier configuration in the waiting state between steps (Fig. 1). The exact position of the kink is unknown, although simple geometric arguments indicate the kink is likely between the first and second IQ domains.



The F0F1-ATPase

This substantial enzyme (Mr approximately 500,000) is readily visible in electron micrographs as 8.5 nm spheres attached to the matrix side of the mitochondrial inner membrane. The spheres can be detached by a variety of methods, after which they act as an ATPase. The physiological function of this enzyme is the synthesis of ATP, using the energy stored in the transmembrane pH and potential gradients.

The complete assembly contains at least 12 different types of polypeptide chain, several of which are present in multiple copies. The catalytic head group is connected by an oligomycin sensitive stalk to a proton conducting baseplate in the mitochondrial inner membrane. Three protons are thought to pass through

the membrane from the external P phase to the internal N phase for each molecule of ATP manufactured by the complex.

The F1 head group contains three nucleotide binding sites, and the enzyme probably performs a three-phase catalytic cycle. In the first phase, ADP and phosphate bind to one active centre, which catalyses the formation of *bound* ATP. This step is energetically possible because the free energy released by tightly binding the ATP to the active centre compensates for the instability of the new phospho anhydride bond. The energy from the proton motive force is required to prise the ATP from the active centre.

The F0 base piece embedded in the mitochondrial inner membrane is a molecular turbine driven by the trans-membrane proton gradient. Proton entry forces a central camshaft to rotate within the F0 baseplate and the F1 head group, altering the subunit conformation as this movement takes place. A second, off-centre protein tether connects the head group to the base piece and prevents the head piece spinning uselessly as the central shaft rotates. Energy is transmitted to the catalytic subunits in the ATP synthase F1 headpiece by the rotation of the camshaft. The "cam" distorts the protein subunits, destroying their ability to bind ATP. The energy input is used to drive ATP release, not for bond formation.

It is presumably necessary to disable the catalytic mechanism on the centre which has just formed ATP (to stop this centre hydrolyzing its own product) before destroying its ability to bind ATP. This allows the product to be released. Meanwhile, the two other active centers are performing their own parts of the catalytic cycle. The three active centers operate simultaneously, but 120^{o} out of phase. It takes at least 9 protons (possibly as many as 12) to drive one revolution of the camshaft and produce 3 ATP molecules.

Remember that the whole complex is reversible. Normally the energy from the proton gradient is used to manufacture ATP, but it is equally possible *in vitro* to do things the other way round, and use the hydrolysis of ATP to drive the camshaft, and ultimately pump protons back through the turbine and into the extramitochondrial compartment. If the F0 base piece is not attached to a membrane nothing useful will be accomplished, and the complex will simply act as an ATPase, as was originally observed.

It is possible to directly observe the rotation of the F1ATPase cam shaft using a fluorescence microscope, although considerable ingenuity is required. Noji et al (1997) Nature **386,** 299-302 used a genetically modified F1ATPase from a thermophilic bacterium expressed in E. coli. They discarded the F0 basepiece and tethered the F1 motor head groups to a glass plate using polyhistidine tags

attached to the N-termini of all three beta subunits. The glass plate had been pre-treated with horseradish peroxidase conjugated with the nickel complex of nitrilotriacetic acid, to which polyhistidine binds with high affinity. [Nitrilotriacetic acid looks like *half* an EDTA molecule, so it leaves un-coordinated nickel positions available for external ligands.]

The motors were glued down by their large catalytic subunits, leaving the motor shafts exposed, and facing away from the glass. The gamma subunits which form the shaft were modified by site directed mutagenesis to remove the original Cys193 (which is inconveniently far down the shaft) and replace it with serine. These workers also replaced Ser107 in the stalk region with cysteine.

This single cysteine residue (the only one in the molecule) could then be biotinylated, and linked using streptavidin to fluorescently labelled, biotinylated actin filaments. [Streptavidin has four biotin binding sites.] The fluorescent actin filaments were many times larger than the tethered motors and could be visualised in a light microscope.

f1atpase.mov

Addition of 2mM ATP caused a small number of the motor shafts marked by the actin filaments to rotate in a counter- clockwise direction. The movie shows the results they obtained.

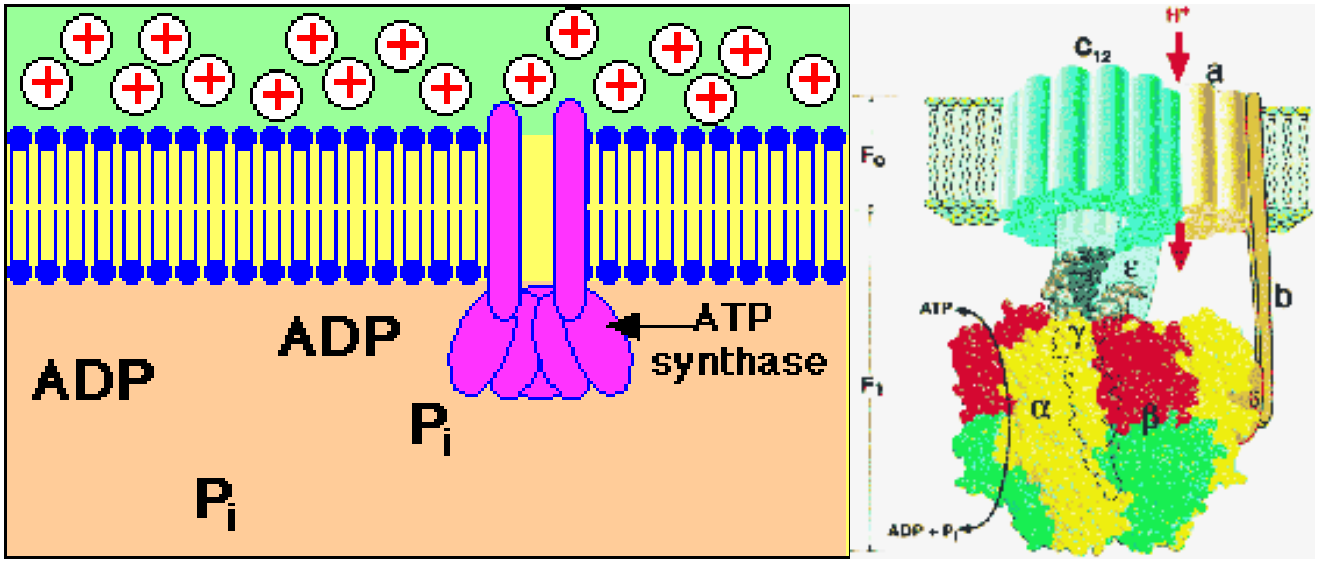
Circular motion also occurs in the proteins which rotate bacterial flagellae, another important enzyme system which is driven by the proton motive force. It is apparent that the wheel has been in continuous use for at least 2000 million years.

The Life Fuel Maker - F0F1 ATPase

Function:

ATP synthesis in the membranes of mitochondria, chloroplasts or bacteria is performed by ATPase, and according to Mitchell chemiosmotic theory, is driven by the energy of a transmembrane proton electrochemical gradient generated by

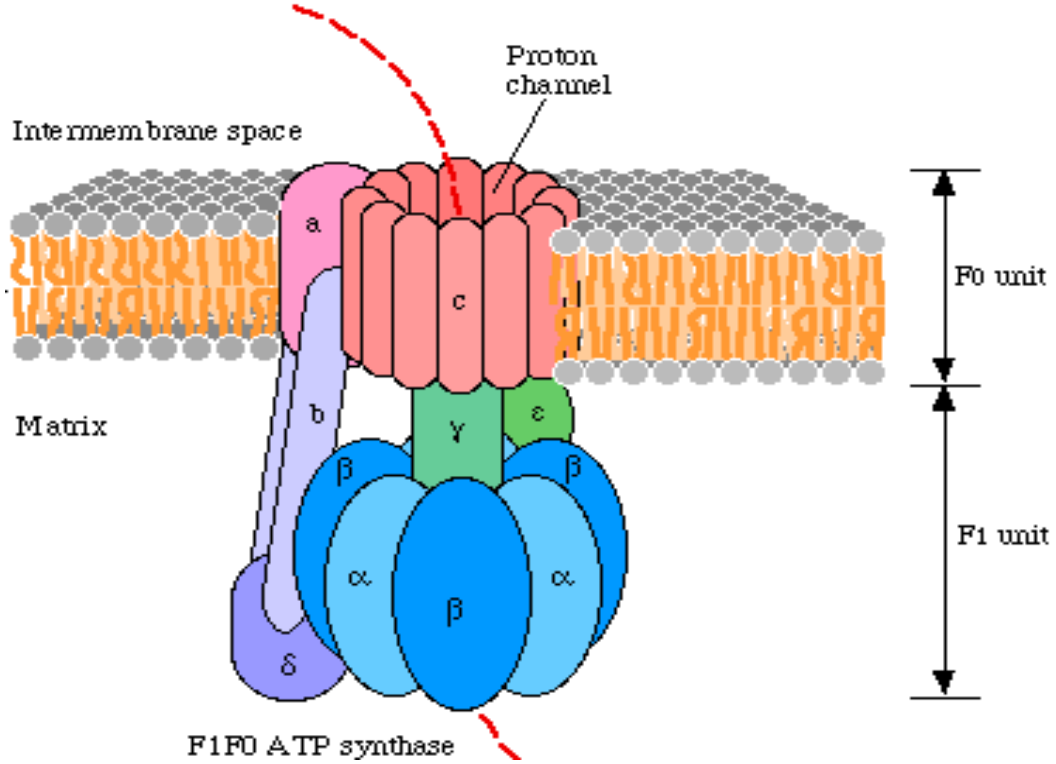
electron transfer. The flux goes from the protochemically positive (P) side (high proton electrochemical potential) to the protochemically negative (N) side. The reaction catalyzed by ATPase is fully reversible, so ATP hydrolysis generates a proton gradient by a reversal of this flux. In some bacteria, ATPase is to operate in the ATP hydrolysis direction, using ATP generated by fermentative metabolism to provide a proton gradient to drive substrate accumulation, and maintain ionic balance.



Structure:

The enzyme coming from thermophilic bacterium PS3 (TF0F1-ATPase) is very stable without ATP-Mg and highly reconstitutable. It consists of two sectors, hydrophobic part F0 and hydrophilic part F1, with a stalk connecting the two parts. F0 consists of three subunits with relative molecular masses of 23 500(a), 17 300(b), 7300(c) in a stoichiometry ab2c9-12. The membrane-embedded F0 provides a pathway of protons down the electrochemical gradient through membrane. The subunit α and two subunits β are located outside of the subunit χ oligomer. The subunit α interacts with subunits χ directly and also interact with subunits β. A nonpolar segment of about 30 amino acids at the N-terminus of subunit β forms a transmembrane helix, which interacts with subunits α and χ, and acts as a membrane anchor. The remaining 80% of the molecule is hydrophilic and predicted to protrude from the membrane. The extra-membranous segment is required for F1 binding and interacts with one beta subunit in F1. Subunit χ is a small, hydrophobic protein and forms a hairpin

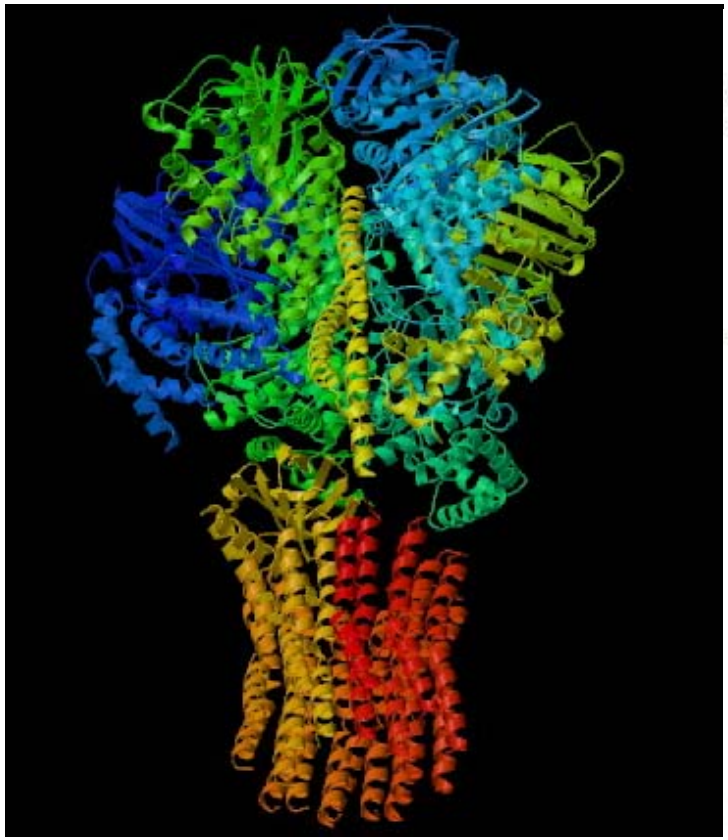
consisting of two transmembrane helices connected by a polar loop, which points towards F1. The subunit c contains DCCD-reactive carboxyl residue.



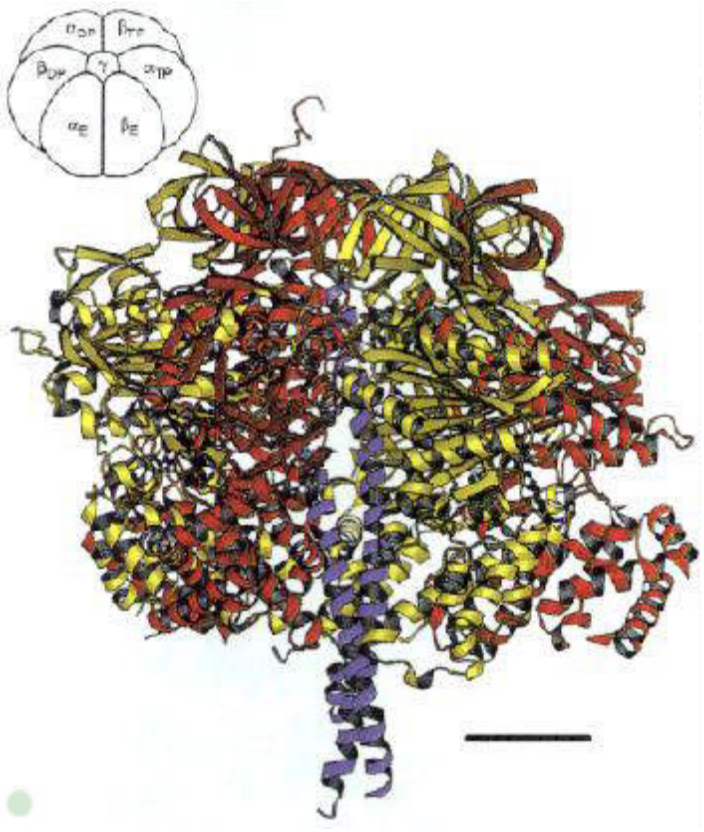
The Cartoon showing the structure of ATPase

The entirely membrane-extrinsic F1 consists of five different subunits with relative molecular weight 54 600(alpha), 51 900(beta), 31 800(gama), 19

700(epsilon), 14 300(delta) in a stoichiometry (alpha)3(beta)3(gama)(epsilon)(delta). alpha, beta and gamma is minimal requirement for reconstituting ATP hydrolysis activity while delta and epsilon connect F1 to F0. There are six nucleotide-binding domains in F1, three on the alpha and three on the beta subunits. The three nucleotide binding sites formed by α subunits are noncatalytic while the three formed by beta subunits are catalytic. The gamma and epsilon subunits contact with the polar loop region of the c subunit and span the full-length of the stalk. The delta subunit extends from the center of F1 through the stalk to F0 and make contact with alpha and beta, and with F0 subunit b. The tentative structure diagram is showed in figure (Weber J. et al 1997 Fig 2.) All eight subunits are required for complete functional ATP synthase. (Weber J. et al 1997, Ohta S. et al 1988, Boyer PD 1997)



The three dimensional structure by X-ray diffraction

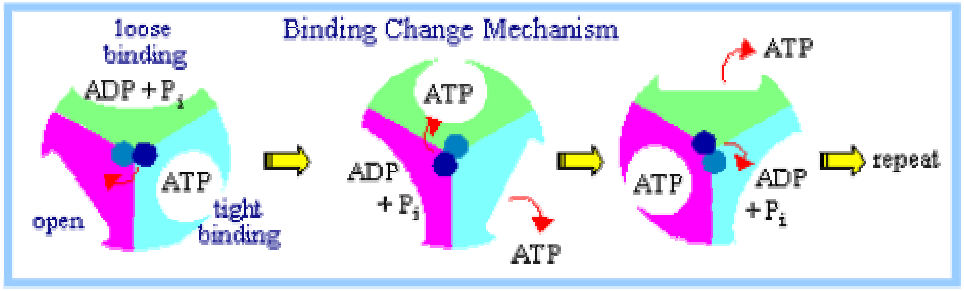


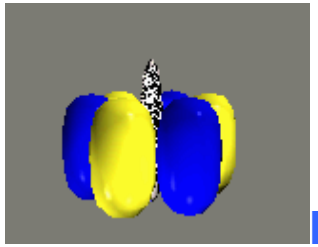
The structure of the F1 ATP-ase, viewed from the side

Catalytic Mechanism:

The catalytic mechanism of F0F1 ATPase has been a central topic in biochemistry for over a century. The 1997 Nobel Prize in Chemistry was awarded to Paul D. Boyer due to his outstanding work - “binding change mechanism” for catalysis, in which the energy from protons down the electrochemical gradient in F0 portion serves primarily for the release of a tightly bound ATP and drives an internal rotation of the γ subunit causing conformational changes that facilitate the binding, interconversion, and release steps, which cooperatively happen in the three beta subunits. The functional conformation of each β subunit is different at any time though all three beta subunits are all structurally identical. The “open” site has very low affinity for substrates. The “loose” site loosely binds ADP and Pi, but is catalyticall inactive. The tight” site catalyzes the formation of ATP. The proton translocation in F0 portion triggers the “open” site to “loose” site, the “loose” site to the “tight” site and the “tight” site to the “open” site, which causes the ATP release from the enzyme (Boyer PD 1993). The active site was crystallized in the presence of ADP and 5’adenylyl-b, g-imido-diphosphate (AMP-

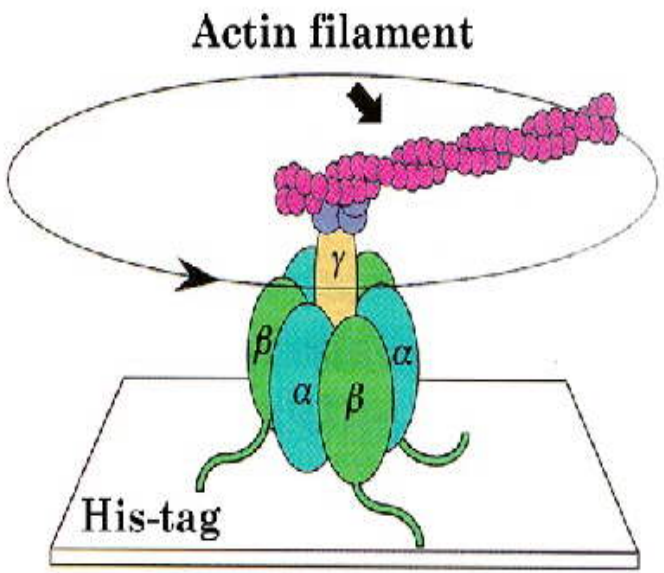
PNP), a non-hydrolyzable analogue of ATP by Abrahams et al (Abrahams et al. 1994). The beta subunit containing Mg2+-AMP-PNP interacted with the short a helix of the γ subunit. The subunit with an empty catalytic site interacted with the two long α helices of the gama subunit. The third β subunit contained bound Mg2+-ADP. These three sites could correspond to the tight, open and loose states of the “binding change mechanism”. The interconversion of the catalytic site conformations could be explained if (alpha)3(beta)3 portion of F0F1 ATPase rotates with respect to the g subunit. The asymmetric positioning of the gamma subunit is related to the difference in the three beta subunit active sites. Click here for animation movies of the F1 ATPase mechanism in action.



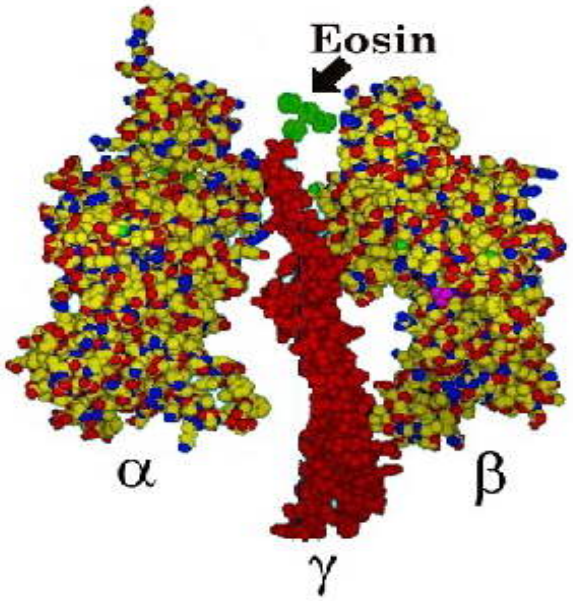


Biomotor:

ATPase is believed to be a rotatory engine driven by proton-motive force and thus the smallest molecular motor known. The energy transfer from the F0 portion to the active sites in the F1 portion is thought to be mediated by the gamma subunit, an asymmetric, coiled-coil shaft. This subunit acts like a crankshaft within the trigonal (alpha)3(beta)3 substructure of the F1 headpiece (Sabbert D 1996). It has been shown that translocation of 3-4 protons through the cell membrane is required for the synthesis and release of one ATP in an active site of the F1 headpiece (Boyer PD 1997). This rotational motion has been captured by Noji H et al (Noji H et al 1997). The F1-ATPase was bound to a glass surface by the beta-subunit, using a His-tag engineered into the protein at the N-terminus, and NTA-ligand on the glass. An alternative approach using photometric methods has been explored by Sabbert D and Junge W (Sabbert D and Junge W 1997).The mechanical force generated by F1-ATPase was utilized by Carlo Motemagno (Soong Rk et al 2000) to power a nanodevice.



W. Junge, H. Lill & S. Engelbrecht
TIBS 22, 420 - 423 (1997)



W. Junge, H. Lill & S. Engelbrecht
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