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Complete List of Authors:	Geeves, Michael; University of Kent School of Biosciences, School of Biosciences
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The ATPase mechanism of myosin and actomyosin.

Michael A Geeves

School of Biosciences, University of Kent, Canterbury, UK

Michael A Geeves
School of Biosciences,
University of Kent,
Canterbury, CT1 7NJ
UK

m.a.geeves@kent.ac.uk

Tel +44 1227 827597

Abstract

Myosins are a large family of molecular motors that use the common P-loop, Switch 1 and Switch 2 nucleotide binding motifs to recognise ATP, to create a catalytic site than can efficiently hydrolyse ATP and to communicate the state of the nucleotide pocket to other allosteric binding sties on myosin. The energy is of ATP binding is used to do work against an external load. In this short review I will outline current thinking on the mechanism of ATP hydrolysis and how the energy of ATP hydrolysis is coupled to a series of protein conformational changes that allow a myosin, with the cytoskeleton track actin, to operate as a molecular motor of distinct types.

Myosin was identified more than 100 years ago in muscle where it is the protein that drives muscle contraction using ATP as the energy source[1,2]. In the that last 25 years knowledge of the myosin family has grown explosively and now includes at least 35 different classes in the myosin superfamily found across all eukaryotic cells [3]. Myosins are a large family of ATP driven motor proteins which use the cytoskeleton protein actin as a track along which the myosin motor can move cargos or generate and sense mechanical forces in the cytoskeleton [4,5]. Mammalian cells will typically express more than a dozen myosins from at least 5 different classes and they are involved in a wide range of actin cytoskeleton based activities from organising the cytoskeleton, transporting material around the cell, generating forces and sensing forces in the cytoskeleton[6,7].

Myosins are defined by the presence of a conserved globular motor domain of about 90 kDa (Figure 1). The domain is roughly pear or tear-drop in shape and is distinguished by a large cleft that spits the broad end of the molecule and a single α-helix stabilised by light chains at the narrow end. The actin binding sites span either side of the large cleft and closing of the cleft is required for tight binding to actin. The binding sites for the ATP phosphate groups are near the bottom of the cleft where the familiar *P-loop* and *switch-1* and *switch-2* loops (SW1 & SW2) are found and identify myosins as part of the broader family of the P-Loop containing ATP and GTPases. ATP binding and ADP release does not occur via this large cleft but via a pocket on the side of the molecule. The Pi groups enter the pocket first with the nucleoside blocking the exit of hydrolysis product Pi via the pocket.

The C-terminus of the motor domain, consists of a long α-helix stabilised by calmodulin or calmodulin-like light chains (between 2 and 6 such light chains depending upon the isoform). This light chain binding domain (LCBD) is also known as the lever arm because it swings through and angle of ~60° during the ATP cycle thereby amplifying small domain movements within the motor domain to produce 5-20 nm movements of the tip of the lever relative to the actin binding sites.

The C-terminus of the motor domain extends into a further domain known as the cargo binding domain. This region is highly variable even within family groups and comprises a combination of protein and lipid binding motifs (the cargo binding motifs) and/or dimerization domains that can also allow assembly into higher order myosin filaments [4,8].

Myosin ATPase cycle

The normal physiological substrate of myosin is Mg·ATP but unusually myosin can be defined as a Mg^{2+} inhibited ATPase, since in the complete absence of Mg^{2+} myosins have an unusually high turnover rate (eg ~10 s^{-1} for muscle myosin 2) that is reduced to 0.05 s^{-1} when Mg^{2+} is present . This

is because the products of the ATPase reaction, Mg·ADP and Pi, are both tightly bound in the ATP site and an interaction with actin is normally required to release the products. The outline of the myosin ATPase (and actin.myosin) cycle has been known for 40 years (Bagshaw & Trentham 1976, Geeves 1991) but some details remain elusive in particular the catalytic mechanism of hydrolysis has been debated continually. The following outline is that appropriate for the muscle myosin II but the outline varies little between this and other myosin forms that have been studied. In its basic form the cycle begins when Mg·ATP binds myosin rapidly and almost irreversibly followed by rapid reversible hydrolysis of ATP to the tightly bound Mg·ADP·Pi. The low free energy change for the hydrolysis step and the slow release of ATP or Pi from this complex indicates the stability of the two forms, with reactant and products both tightly bound in the myosin pocket. In the absence of actin the bound Pi has an average lifetime of ~20 sec. Pi escape is followed by a more rapid release of Mg·ADP. The release of ADP is linked to Mg^{2+} release but whether they are release together or sequentially, Mg^{2+} escapes first then promoting ADP release, appears to vary between myosin isoforms ([9] [10] [11] and may be linked to the precise role of ADP release in different classes of myosin[12]. However, the rate of product release observed experimentally is often much faster than that occurring physiologically as there are additional regulation events that slow Pi release even further in the absence of available actin sites. These vary across the family, but for some types of myosin folding of two myosin heads together (e.g. smooth muscle myosin II[13]) or of motor and cargo domains together (myosin V [14]) or stabilising motor and filaments structures[15] to further trap/stabilise the M·ADP·Pi complex are common. This is one of the differences in the myosin compared to many G-proteins (or even kinesin). M·ADP·Pi is the stable complex that sits and waits for a signal to complete it biological role and that signal can come either via the actin (making actin site available) or via the myosin – relieving inhibition of the motor domain. There are exceptions (and across the 35 members of the myosin family there are plenty of exceptions to most rules) and for some myosin involved in mechanical signalling the mechanically loaded A·M·ADP complex is a long lived stable complex and the loss of load leads to rapid release of ADP [16] [17] [18].

The complexes of myosin with ATP and ADP.Pi are stable and therefore might be expected to crystalize but the rapid reversibility of the hydrolysis step (which is accompanied by a large change of conformation) makes this largely unsuccessful. However, crystals of myosin with putative transition state analogues, such as ADP·AlFl, ADP·BeF, and ADP·Vi have all been crystallised[19] [20] . In addition to complexes with inhibitors such as blebbistatin bound to the motor domain have given insights into the catalytic mechanism of ATP hydrolysis [21] and how the conformational changes accompanying ATP binding, hydrolysis and ordered product release couple the ATPase cycle to the biological role of different myosins. These are discussed in the section below.

The key point of relevance to the biological role is that ATP binds to myosin – and the combination of the binding energy and hydrolysis step primes the myosin into a conformation, which, while stabile, is ready to interact with actin to complete mechanical work or drive mechanical signals through the cytoskeleton.

Actin activated myosin ATPase and the mechanical cycle

A simple cartoon outline of the major events and conformation changes in the actin-myosin ATPase cycle is outlined in Figure 2 and described in detail in the legend. Here it can be seen that the role of ATP is to bind to myosin, force apart the major cleft and thereby reduce the affinity of myosin for actin by about 1000 fold, then hydrolyse ATP. These activities are linked at the simplest level with the closing of the two switch elements onto the Pi of ATP. On ATP binding SW1 closes first and is coupled with the opening of the major cleft and hence actin dissociation. SW2 closure follows and is linked to the *recovery stroke* in which the *converter* domain and *lever* are repositioned into the *pre-power* or *working-stroke* conformation. In addition to any structural constraint that require SW1 to close before SW2 the time of events also ensures the ordering of events. SW1 closure and actin dissociation takes about ~1 msec at saturating ATP for a fast muscle myosin and the hydrolysis step occurs about 10 times slower in ~10 msec. The resulting complex is stable for ~20 sec unless it encounters an actin binding site which results in the completion of the rest of the cycle in ~20 msec

The molecular details of the nucleotide-free actin·myosin complex, the detached M·ATP and the post-recovery M·ADP·Pi conformations are well defined from crystal structures [22,23] and high resolution EM images [24] and such images have been used in molecular dynamic simulations generate plausible models of the transitions between defined structural states[25,26,27]. The transition between the detached M·ADP·Pi state back to the A·M state remain more problematic and this, frustratingly, is of course the business end of the cycle where myosin can generate force and movement. As set out in the cartoon (Fig 2) actin binding is accompanied by Pi release, myosin cleft closure and force generation but which order these events take place in remains hotly debated. These are drawn as all occurring on a single step, step 3. But the order is important for understanding the way the energy of the event is efficiently coupled to force and movement. There are several possible scenarios but evidence is hard to generate because such structures are going to be short-lived, possibly unstable and the mechanical forces involved are large at the molecular scale and may well influence the conformation route taken between M·ADP·Pi and A·M. Most structural methods work on protein in the absence of any external load and under such conditions all of the molecular events consolidated in step 3 are instantaneously followed by transition via route 4a/5a to the stable A·M·D complex that can easily be formed by addition of ADP to the stable A·M complex. The more

interesting route is that via step 4 and 5 where the load on the motor domain (and the external work being done) slows the transition through the molecular events. The load-induced slowing of the cycle leads to the classical force-velocity relationship observed when the recording forces and movements generated by an ensemble of motor molecules working together against a load. That is the rate of the ATPase cycle slows as the opposing load increases to a point where the ensemble of motors continues to use ATP but can no longer move the load. A possible outline of events and some of the alternatives are described below

Actin rebinding to M.ADP.Pi initiates the process of product release and force generation and the current view favours the lower 50 kDa domain making the important hydrophobic inter face with actin– in particular the contact of the activation loop (Dictyostelium Myosin II residues ^{519}GRQPP$_{523}$) has been shown to have a role in promoting Pi release [28]. But does Pi release occur before or after cleft closure and forming the complete actin myosin interface? Two views clash here: crystal structures indicate the most straight forward route for Pi to be released from the pocket (since ADP blocks Pi from escaping back the way it came in when attached to ATP) is through a *backdoor* which allows a Pi route out via the cleft. When the cleft closes this route is no longer accessible the backdoor is closed [2229]. Recent mutational studies attempting to block this back door have supported such an idea[30]. The counter view is that other routes are possible and breathing motions of the structure, SW1 opening – and mechanical loading across the domains may aid such alternate exit routes [2331]. The argument against Pi release before cleft closure is that since Pi escape is linked to force generation it does not seem to make sense to generate force at the actin-myosin interface until the majority of the actin myosin interface is in place. A partially formed interface will be weaker and more prone to mechanical failure. Recent fluorescent probe studies from the Thomas lab [3233] [34]indicate that in the absence of actin the conformation of the myosin is dynamic and actin traps the post power stroke conformation and this precedes Pi release, a slower process. The events surrounding step 3 therefore remain under-defined and until there is more experimental evidence the differing viewpoints will remain strongly held by different experimental camps.

The one clear undisputed result is that once the myosin has completed its working stroke Pi release is irreversible but while force is being generated –and the energy not dissipated, Pi can bind reversibly into the site and the cycle is freely reversible back through steps 3 and 2 (see Fig 2) to form tightly bound ATP. This is illustrated in Figure 2. As the Pi is released in step 3 the blue circle rotates and generates a force against an external load attached to the tail (indicated by the bent tail) while the tail remains bent Pi can rebind into the pocket, but as soon as the tail moves the load forward and the tail straightens against the load, the energy has been dissipated and Pi can no

longer reverse the process. The domain rotates further in the absence of load, further preventing Pi rebinding and allowing ADP release.

The role of ADP release is much better understood and occurs after the Pi release and force generating events. The key role here is for ADP to remain in the pocket until the "working stroke" has been completed. That is, it remains in the pocket as long any force generated by or accompanying Pi release has been dissipated (step 4). This makes the efficiency of the system very high and prevents futile burning of ATP. Any myosin designed to have very long-lived force holding states have relatively slow rates of ADP release and or a strong load dependence of the rate constant of ADP release[12,18,35]. This aspect is discussed in more detail below where the adaptations of myosin to different mechanical functions is outlined. In essence ADP remains in the pocket while the energy from ATP breakdown can still be used. Once ADP is released, the high concentration of cellular ATP ensures very rapid rebinding of ATP and dissociation from actin losing any mechanical (force or strain sensing) contact with the actin cytoskeleton.

Catalytic Mechanism of Hydrolysis

The crystal structure of the ATP and ADP.Pi analogues revealed that the active site was not fully formed until the SW1 and SW2 elements closed down on to the γ-Pi of ATP. The structures then allow identification of residues likely to be involved in the catalytic mechanism. Despite several different crystal structures of putative transition state analogues and extensive kinetic studies of the hydrolysis step, two mechanism have remained possible: An associative (direct attack by water via a penta-covalent γ-Pi) or a dissociative mechanism (attack of water on a stabilised meta-phosphate intermediate) with opinion swinging in either direction over time. The most recent contribution to the debate has been from a series of quantum mechanical/ molecular mechanics calculations (QM/MM) of the hydrolysis pathway based on crystal structures of analogues of the M.ATP structure (predominantly an ADP vanadate structure)and the transition states using data from a range of different myosin types ([36,37]. A summary of this recent work view is presented here and the reader is invited to explore a recent accessible summary for a detailed evaluation of the evidence ([38].

In the following description residue numbers refer to those of Dictyostelium myosin II. The phosphates of Mg^{2+}ATP are tightly bound through interactions with the three loops. α- & β- Pi contact the P-loop (^{179}GESGAGKTEN$_{187}$ makes 8 H-bonds formed mainly through peptide backbone contacts), while the γ-Pi are stabilised via SW1 (^{236}SSR$_{238}$) and SW2 (^{455}ISGF$_{458}$) again primarily via peptide H-bonds (see Fig 3A). The closed position of SW1 and SW2 is further stabilised by salt bridge between Glu459 and Arg238. The primary catalytic side chains are Glu-459, Ser-181 and Ser236/7

and involves 3 water molecules. There are three elements to the mechanism envisaged (outlined in Fig 3B)

– breaking of the β-γ bond phosphate bond between the γ-phosphorus and the β- γ bridging oxygen and stabilisation of the resulting meta-phosphate group. A wide range of interaction with the peptide backbone of SW1, SW2 and the P-loop contribute to this stabilisation of the metaphosphate (listed above) and results in almost zero change in energy between the ATP and metaphosphate states.

- a combination of Glu459 and Ser237 polarize an attacking water molecule allowing formation of the OH^- anion which can more easily attack the stabilised metaphosphate.

- creation of a low energy pathway to transfer the abstracted proton to the γ-phosphate group via so called *proton wires*. The contribution of Glu459 to the catalytic mechanism and the role of protein wires have only been appreciated in the most recent simulations.

The combination of the three elements generates a pathway with small intermediate energy levels (+2.7 to -3 kcal/mol) allowing reversibility and relatively low energy barriers between states (8.7-10.3 kcal/mol) allowing high rates of transition consistent with experimental observations.

Overview of the ATPase & mechanical cycles

With the above outline of the actin-myosin ATPase mechanism the role of ATP in the overall mechanism can be considered. Two viewpoints are essential – the thermodynamic and the kinetic. How the binding energies and the energy of ATP hydrolysis is used to drive the work cycle - and how the timing of events contributes the efficiency of the cycle.

The energy associated with ATP binding is essential to first of all displace the actin from the myosin, itself very tightly bound with an affinity of ~10 nM. This is associated with SW1 closing and opening the myosin cleft. This is followed by the re-priming or recovery-stroke returning the myosin tail into the *up* position ready to complete the next working-stroke. This is associated with SW2 closing. These two major conformational changes are triggered by SW1 then SW2 closing on to the γ-Pi and in doing so create the active site that can stabilise the metaphosphate and attacking OH^-. The whole process is accompanied by very small free energy changes allowing each of these events to be readily reversible – except actin rebinding to the M·ATP form. As stated above the actin dissociation event is faster than the recovery-stroke/hydrolysis step ensuring the recovery stroke does not occur while bound to actin which could result in a futile mechanical cycle.

The timing of the hydrolysis step is important for another reason. This sets the time during which the myosin is unable (or unlikely) to rebind to an actin site – once M·ADP·Pi is formed it can rebind an actin site and complete the ATPase cycle. Thus the lifetime of the M·ATP is important to ensure the myosin can diffuse to its next productive actin site (reattaching to the site just detached from would not produce net forward movement) and this varies for different types of myosin. It is noticeable that myosins that spend most of their ATP cycle time attached to actin can have very fast ATP hydrolysis steps ([39]). The lifetime of M·ATP can partially control the *duty ratio* – the fraction of time a myosin remains tightly bound to actin during a single ATPase cycle.

Like the ATP hydrolysis, the Pi release step and force generation occur with little change in free energy and the step remains freely reversible. Only if the free energy is dissipated through the completion of the working stroke does the Pi step become essentially irreversible and the system is committed to completion of the ATP hydrolysis. Exactly how the energy of the system is stored, in for example, a bending or stretching of an elastic component remains to be defined. Current thinking focusses on some combination of the lever-arm bending and to central β-sheet distortion. One issue is to understand if such a mechanically distorted state appears different at the single molecule level vs an ensemble of myosin motors. Certainly in a sophisticated structure such as the muscle sarcomere any mechanical force generated by a single myosin will rapidly dissipate into various compliant elements of the super structure.

The ADP release step varies for different type of actin-myosin motor and can occur with zero, positive or negative free energy changes. Bloemink & Geeves (2011)[12] outlined how the free energy of ADP release can be modified in different myosins to generate motors of different types. A fast ADP release event with a significant negative free energy change is characteristic of a motor generating fast movement even under load. A slower motor with high efficiency for both movement and force-holding actions has a slower ADP release and the rate of ADP release is readily inhibited by load on the motor. A myosin designed for generation tension over long periods and sensing tension changes in the cytoskeleton has a small or zero free energy associated with ADP release and effectively ADP release is blocked by any small load on the motor. Finally if ADP release occurs with a positive free energy change then ADP is retained by the motor unless it receives additional information. This is similar to many G-proteins that require an exchange factor to release GDP, and is observed in a two-headed processive motor such as myosin V; the ADP on the attached head that has completed its working stroke remains in the trapped in nucleotide pocket. If he second head attaches to actin and goes through its force generation step then it pulls on the rear head opening up the exit route for ADP allowing ADP to be exchanged for ATP, the head then

detaches and the ATPase cycle restarts. Such a process ensures the heads work alternately and allows the motor to *walk* hand-over-hand along an actin filament.

This flexible ADP release mechanism allows quite small changes in the free energy of ADP release to tune the myosin for different types of mechanical function. Of course optimising each myosin for its own function involves more than just changing the free energy of ADP release. Other factors that are altered between myosin isoforms include the length of the lever arm (varying from 2 to 6 calmodulin binding domains) which can alter the size of the step – and thus on simple lever arm theory adjusting the distance moved per ATP hydrolysed and the force developed in each working stroke[4041]. The overall speed of the cycle also allows for different speeds of movement and different response times to different mechanical or chemical signals. And the duty ratio – the fraction of each ATPase cycle time the motor spends strongly attached to actin –can be altered to change between efficient force holders/sensors where the long lived A·M·D complexes can maintain force with little ATP turnover but by reducing the response time to changes in force and being very slow transport systems[3912].

There remain many molecular details we do not fully understand (such as the exact details of Pi release and force generation) but we are beginning to understand how the *design* of the myosin motor has allowed the development of a range of motors tuned for different roles within the cell. For each motor the basic ATPase cycle remains the same; the mechanical cycle is largely the same and the mechanism of catalysis remains the same. What differs is how the how the cycle is tweaked to alter the cycle time, to alter where in each cycle myosin spends most of its time, and to alter how the energy from ATP hydrolysis is coupled to generate different types of mechanical activity. These we are beginning to understand. Coupled to this is the question of how myosin activities are regulated and integrated with the rest of cellular responses - but that as they say is a different story.

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Fig 1 Structure of the myosin motor domain

The post-rigor structure of the myosin motor domain [42]. The structure of the myosin cross-bridge shown as a ribbon diagram in the orientation it would take on binding to actin viewed from the pointed (-) end of the actin filament. The N-terminus is shown green and the nucleotide binding P-loop and adjoining helix are shown yellow; the upper 50K is red; the lower 50K domain is grey. Note the cleft separating the upper and lower 50K domains. The lower 50K domain appears to be the primary actin-binding site. The N-terminal boundary of the upper 50K domain comprises the disordered loop 1 (between the points marked A and B). The upper and lower 50K domains are also connected by a disordered loop (loop 2 between C and D). The C-terminal long helix (dark blue) carries two calmodulin-like light chains and joins onto the thick filament. The relay helix and converter domain are shown. In this conformation of the cross-bridge (post-rigor state) the lever arm is in the post-power stroke position or "DOWN", as in the rigor state. The colouring corresponds with sub-domain boundaries. For clarity, the proximal end of the relay helix is shown light blue although it is actually part of the lower 50K domain. The distal end (beyond the kink) is firmly attached to the converter domain. In the post-rigor structure the relay helix is straight (no kink). Reproduced from Geeves & Holmes 2005 Advances in Protein Chemistry **71**, 161-193 (2005)[23]

Fig 2 The ATP driven actin-myosin mechanical cycle

The myosin cross-bridge is shown as consisting of 3 major components. 1. The central core of the myosin head is shown in red (filled circle) with the lower 50k domain projecting up to make contact with one actin monomer (grey circles). The lower 50k domain is one half of the major cleft that splits the actin binding site. The P-loop that binds to the γ-Pi of ATP is part of the central core. This red segment is drawn as a fixed reference point during the cycle. 2. The upper 50 K domain with SW-1 (switch 1) is shown in yellow. This is drawn as two parts; the upper jaw of the major cleft that splits the actin binding site and a yellow ring with SW-1 projecting into the center. 3. A blue ring represents the relay loop with SW-2 projecting to the center while the converter domain and the lever arm (shown with 2 light chains attached) is projected out from the ring. The nucleotide pocket is shown as lying on top of the central core (red) bounded by two circles one part of the upper 50 K domain (yellow) the other part of the relay/converter domain (blue). Each ring has a segment missing to represent the entrance to the nucleotide pocket.

A·M at the top left represents the rigor actin-myosin complex with the upper 50kDa domain cleft closed to allow both sides of the cleft to make contact with actin. The nucleotide pocket entrance is open with both SW1 & 2 open.

Step 1 ATP binds to myosin and myosin dissociates from actin. In detail ATP binds into the nucleotide pocket and the yellow segment rotates to close SW-1 onto the ATP in doing so the major cleft opens destroying the actin binding site leading to dissociation from actin.

Step 2. The recovery stroke and ATP-hydrolysis. The blue segment rotates to bring SW-2 into contact with ATP thus rotating the converter/light-chain binding domain to complete the recovery stroke or re-priming of the motor while detached from actin. Only after both SW1 and SW2 are closed is ATP hydrolysed to form the stable M·ADP·Pi complex.

Step 3 Actin rebinding and the power stroke. After hydrolysis the lower 50kDa part of the cleft rebinds to actin, the cleft closes and both upper and lower 50kDa domains bind actin, this involves a rotation of the yellow segment which triggers both Pi release and the rotation of the blue segment generating a force – represented as a distortion of the converter domain. The exact order of cleft closure, Pi release and power stroke remains under debate. The location of the "elasticity" within the cross bridge is not defined.

Step 4 "Sliding". Provided the force generated in step 3 is large enough the load is moved 5-10 nm by the cross bridge working-stroke. This is shown as a relative displacement of the end of the lever relative to the actin.

Step 5 Opening of the nucleotide pocket. Once the strain in the converter is dissipated by the sliding movement, the blue segment rotates further to open the nucleotide pocket and allows ADP to escape in step 6. An efficient motor requires that ADP release be limited until the cross-bridge has completed its movement or working stroke.

Step 4a/5a. The strain holding cross-bridge. If the force generated in step 3 is too small to move the load no sliding takes place. The further movement of the blue segment against the load is strongly inhibited (~5-100 fold for different myosins). The degree to which this rotation is inhibited in a load bearing myosin defines the load sensitivity of the myosin. This is a function of the size of the rotation, the length of the lever arm and the stiffness of the "spring" in the structure.

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Fig 3 A Stabilisation of the 3 phosphates in the myosin nucleotide pocket.

The ATP binding site in the catalytically active conformation, i.e. switch 1 and switch 2 closed and the stabilizing salt bridge formed between Ag 238 (of SW1) and Glu 459 (of SW2). ATP has been modelled into the 1VOM crystal structure (Dyctyostelium myosin II with Mg^{2+} .ADP and vanadate in the active site). Only the three phosphates are depicted. Dotted lines show hydrogen bonds shorter than 2.8 A between the heavy atoms. The Mg^{2+} coordination sphere is represented with thick grey lines. Taken from Kiani & Fischer PNAS 2014 11, E2947-E2956[36].

Fig 3B The catalysis of the ATP hydrolysis step and the role of Glu459.

Reactions on the left: during the breaking of water W_a, a proton is first transferred onto Glu459 before being transferred to the final γ-Pi. Reaction on the right: the proton is transferred onto the γ-Pi without transiting Glu459. a) ATP reactant state **R**. Arrows with black heads show covalent rearrangements. b & c) Metaphosphate intermediate **m**. d) Stable intermediate **e** with protonated Glu459. E) Product precursor **g**, with proton on γ-Pi. Arrows with while heads indicate rearrangements of the H-bond network. f) Final ADP·Pi product **P**.

Numbers in parentheses indicate the energy (kcal/mol, relative to the reaction state R) of the stable intermediates. Numbers on the large arrows between boxes indicate the energy barriers between states. Taken from Kiani & Fischer Current Opinion in Structural Biology Volume 31, 1-140[38]

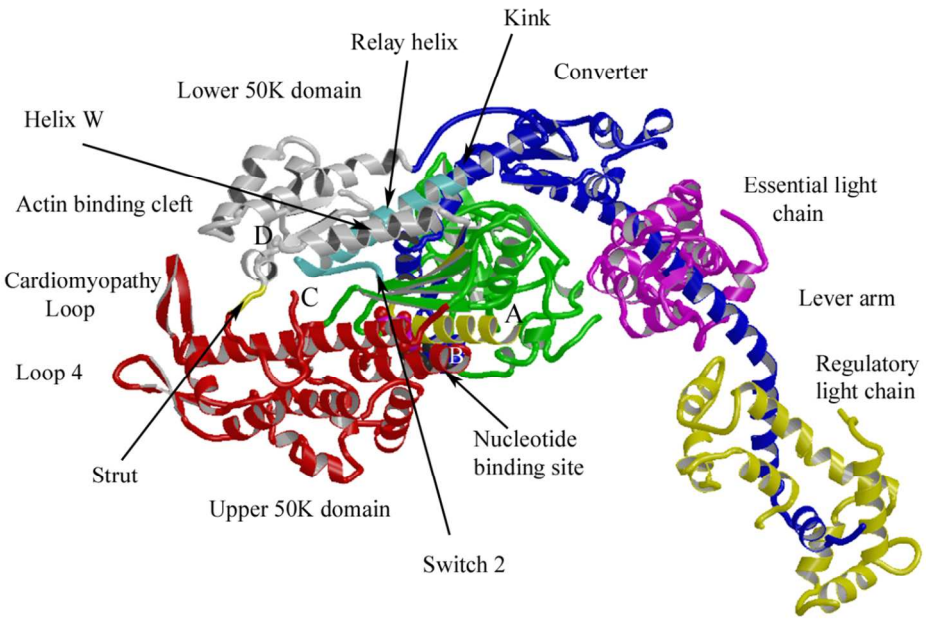

Figure 1
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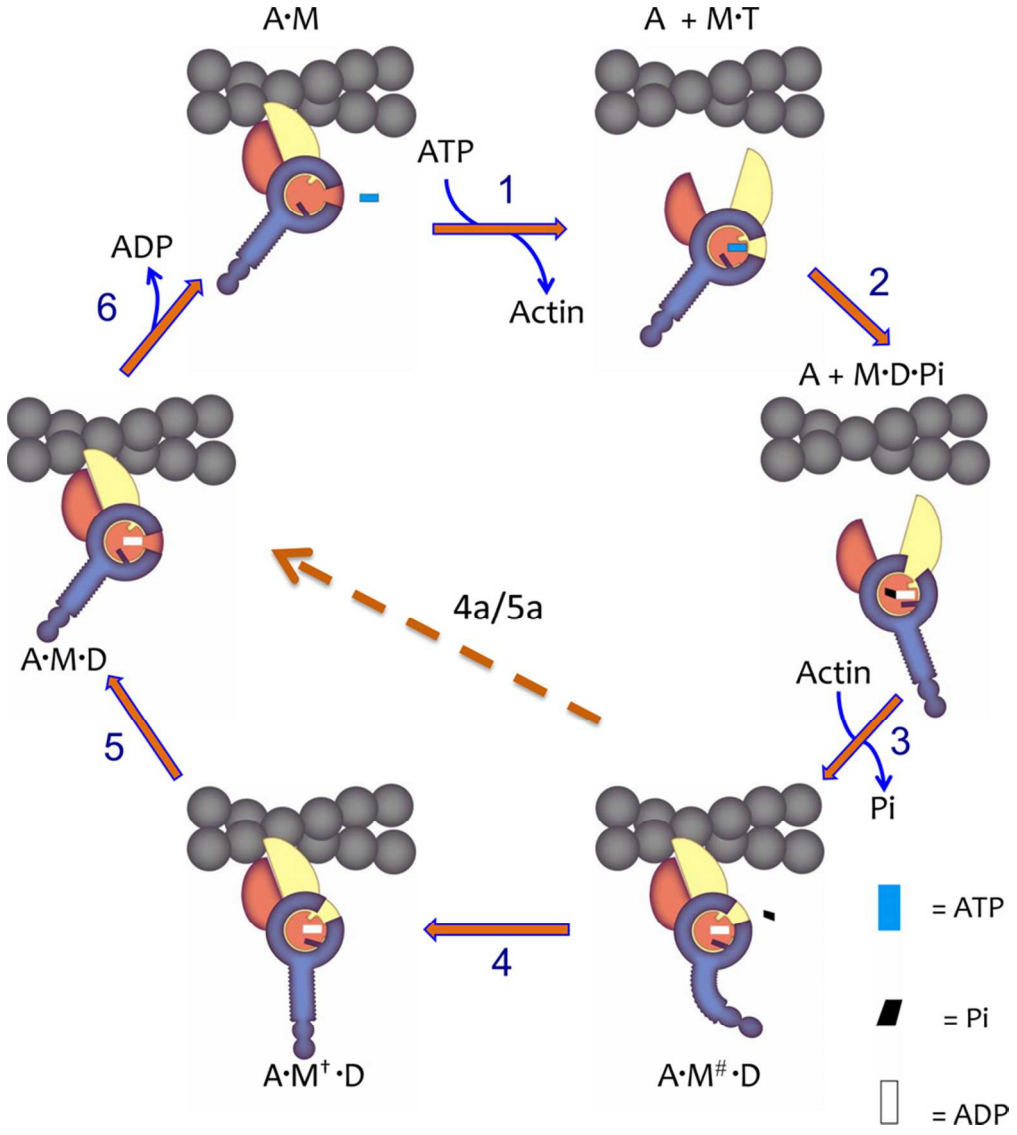

Figure 2
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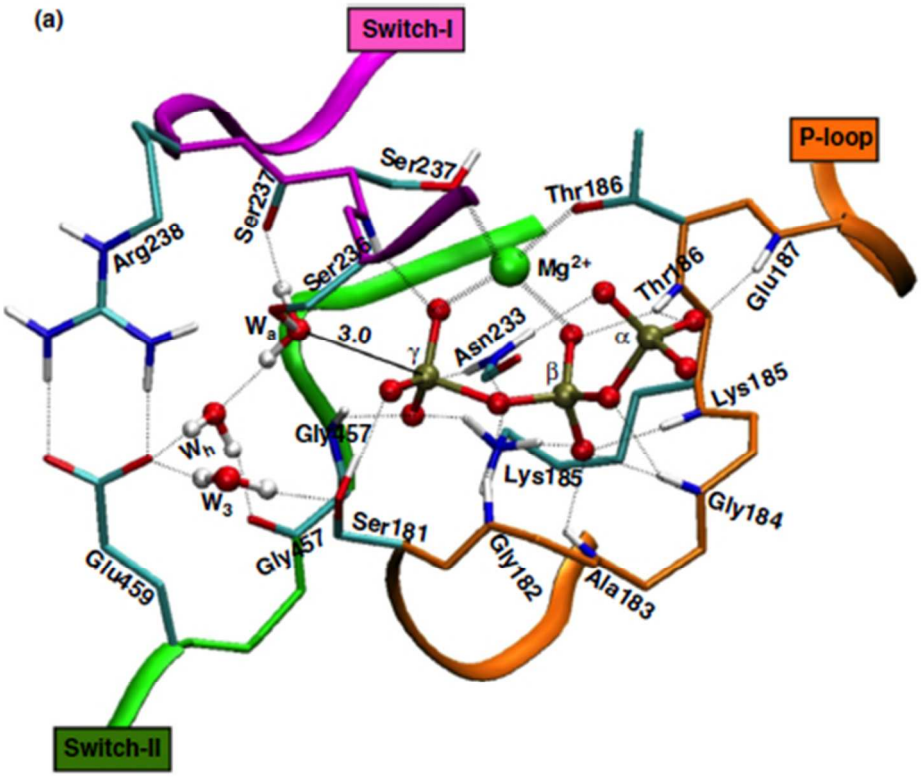

Figure 3A
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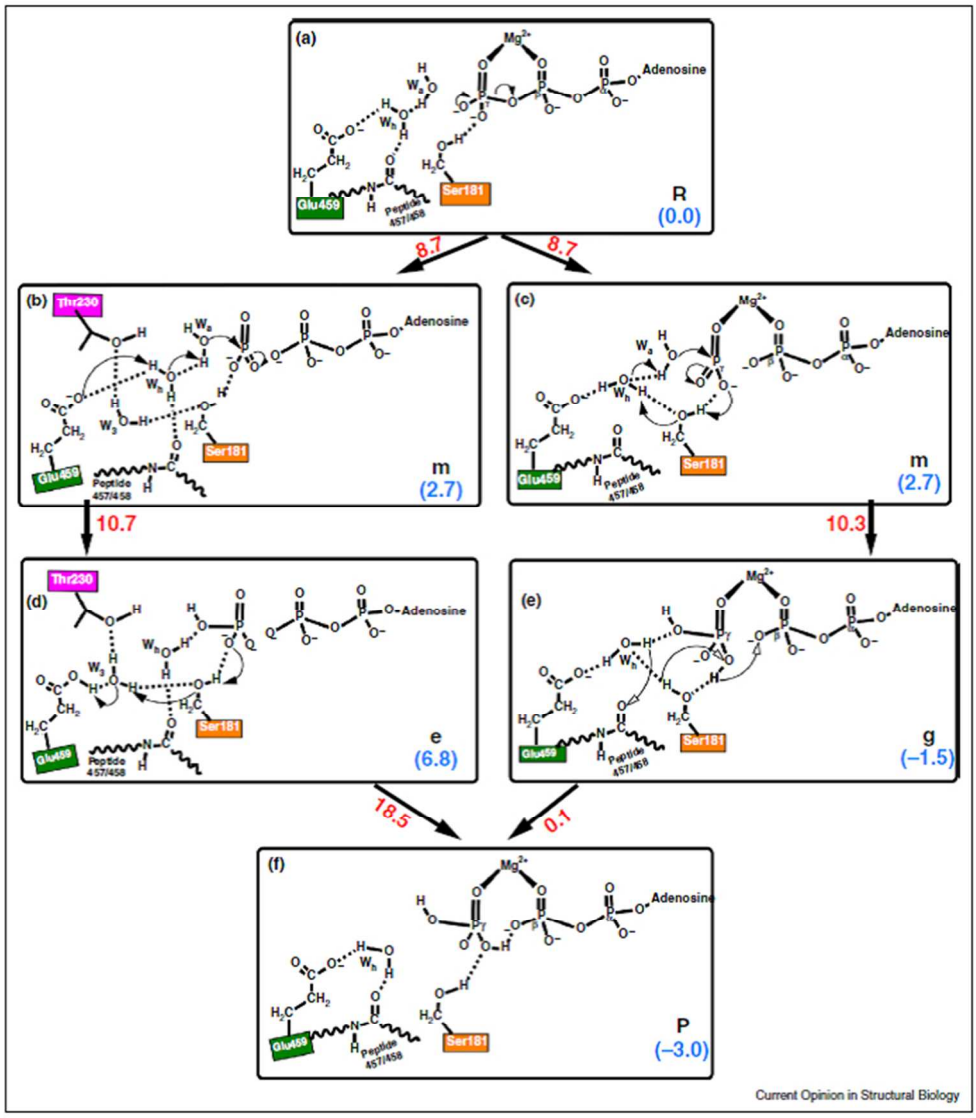

Figure 3B
188x213mm (96 x 96 DPI)