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How myosin generates force on actin filaments

Anne Houdusse^{†||} and H. Lee Sweeney[§]

[†] Structural Motility, Institut Curie, PSL Research University, CNRS, UMR 144, F-75005, Paris, France.

^{||} Sorbonne Universités, UPMC Univ Paris06, Sorbonne Universités, IFD, 4 Place Jussieu, 75252 PARIS cedex 05

[§] Department of Pharmacology & Therapeutics and the Myology Institute, University of Florida College of Medicine, PO Box 100267, Gainesville, FL 32610-0267, USA.

H. Lee Sweeney, Department of Pharmacology & Therapeutics, University of Florida College of Medicine, PO Box 100267, Gainesville, FL 32610-0267, USA.

Tel: 352-273-9416 – Fax: 352-392-3558 – *E-mail*: Lsweeney@ufl.edu

or

Anne Houdusse, Institut Curie CNRS, UMR144, 26 rue d’Ulm, 75248 Paris cedex 05, France.

Tel : 33-(0)1-56-24-63-95 – Fax: 33-(0)1-56-24-63-82 – *E-mail*: anne.houdusse@curie.fr

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Abstract

The mechanism of myosin interact with actin to generate force is a subject of considerable controversy. The major debate centers on understanding at what point in force generation the inorganic phosphate is released with respect to the lever arm swing, or powerstroke. Resolving the controversy is essential for understanding how force is produced as well as the mechanisms underlying disease-causing mutations in myosin. Recent structural insights on the powerstroke have come from a high-resolution structure of myosin in a previously unseen state and from a cryo-EM 3D reconstruction of the actin-myosin-MgADP complex. We argue that seemingly contradictory sets of data from time-resolved FRET studies can be reconciled and we put forward a model for force generation by myosin on actin.

Molecular motors generate movements and forces in cells. Understanding how they work as chemo-mechanical transduction machines is central to understanding how force generation powers critical and diverse cellular processes from DNA transcription to contractile ring during cell division, long and short-distance vesicle trafficking, organelle morphology and translocation, membrane deformation and formation of actin protrusions important for migration, nerve growth and specialized membrane protrusions such as microvilli and hair cells [1-4].

Among the cytoskeleton molecular motors, myosin is perhaps the molecular motor for which principles on force production are the best documented. However despite this knowledge, the force generation mechanism triggered by F-actin binding, which includes the myosin powerstroke, is still poorly understood. In particular, the mechanism of force production cannot be described without gaining insights into the

force producing states that control P_i release, ADP release, and movements of the myosin lever arm. There is considerable debate surrounding the interpretation of existing data that has led to conflicting models of myosin force generation. We describe how recent structural and functional characterization of the powerstroke can be reconciled to provide a framework for understanding myosin force generation.

The Product Release Steps on Actin

The key initial steps in the production of force by myosin when it interacts with actin are the release of inorganic phosphate (P_i) and the movement of the myosin lever arm. These steps are followed by the release of MgADP and an additional lever arm movement. New structural insights into how actin triggers the product release steps from myosin have come from two new myosin structures. The first is a high-resolution (1.75Å) structure of myosin VI in a previously unseen state [5]. The second structure is a cryo-EM structure of the actin-myosin-MgADP complex at 8Å [6]. A controversy in the interpretation of the significance of these structures centers on understanding at what point in force generation the inorganic phosphate is released with respect to the lever arm swing, or powerstroke. The new X-ray structure was interpreted as the state that actin stabilizes to release phosphate, which was supported by mutagenesis and kinetic studies. Other kinetic studies have been performed in combination with observing the rates of the movement of the lever arm with FRET probes, and have been interpreted as demonstrating that the lever arm movement, or powerstroke, precedes the release of phosphate [7-9].

Actin-myosin Kinetic Cycle

Myosin readily hydrolyzes ATP in the absence of actin, but rapid product release requires interaction with actin. Once P_i and MgADP have been released, ATP rapidly rebinds to the actin-bound myosin, causing fast dissociation from actin ([10]; Fig.1).

While all forms of myosin have the same basic kinetic cycle, the rates of transition between the states are highly variable. This allows myosin to be “kinetically tuned” for a variety of cellular functions by not only altering the rate that it proceeds through the ATPase cycle, but also by changing the relative amount of the cycle that myosin spends in strong actin-binding (force generating) states (known as the duty ratio). Filamentous myosins, such as those that power muscle contraction, function in large ensembles. Thus to maximize speed of shortening and power output, these types of myosin have a low duty ratio (i.e. the cross-bridges spend most of the cycle detached or weakly attached). Rapid detachment from the strongly bound states prevents drag on moving cross-bridges. Although more rapid detachment leads to higher shortening velocities, it also causes the economy of isometric force maintenance (i.e. the ratio of isometric mechanical response to energetic cost) to be lower. The strain dependence of transitions that involve lever arm motions (e.g. the release of MgADP) will slow the transitions necessary for detachment under isometric conditions, thus reducing the energetic cost.

Phosphate release

The initial interactions of myosin with actin trigger the release of inorganic phosphate (P_i) and the generation of force. In the absence of actin, phosphate release is quite slow since myosin traps the P_i in the pre-powerstroke state. Soon after the publication of the initial myosin structure [11], it was noted that in order for actin-activated phosphate release to precede ADP release, actin would likely create an escape route for phosphate that was an alternative to the normal exit to the nucleotide-binding pocket [10,12], which is blocked by MgADP. This “back door” mechanism is widely accepted, and there has been much speculation as to the nature of the back door [10,13]. What is clear is that in order for the phosphate to dissociate, there must be some rearrangement in either switch I or switch II. These elements, along with MgADP, block any possible dissociation of P_i from the pre-powerstroke state (PPS), which is the state that primes

the lever arm position for the powerstroke on actin. This PPS state does not bind strongly or with stereo-specificity to actin, and thus is not a force-generating state.

For many myosins, the initial actin interactions of the PPS state that lead to P_i release is highly dependent on ionic strength. This likely reflects the need for electrostatic steering, involving charged loops on the myosin surface that orient the myosin with respect to the highly charged surface of actin. Unconventional myosins, such as myosin V and VI, are much less sensitive to ionic strength than are sarcomeric myosins [14]. However, after stereospecific binding to F-actin, the rate of P_i release per se is likely to be fast for all myosins [5]. It is thought that the weak, non-specific electrostatic binding of the PPS state by surface loops to actin allows the myosin to explore the actin surface until stronger, stereo-specific interactions can be made, and in doing so, actin promotes a myosin transition to a state that binds to actin with an altered actin interface and force generation is initiated [10].

A putative phosphate release (P_iR) state structure was published that has all of the hallmarks of what is needed for the first force-generating myosin state [5]. A switch II movement has occurred that opens a tunnel (phosphate release tunnel) from which P_i can leave the nucleotide-binding pocket. However, this switch II movement occurs with minimal movement of the lever arm, and thus the initial rate of interaction would have minimal dependence on strain. Importantly, while the actin-binding cleft and interface have been altered as compared to PPS, presumably to facilitate stereo-specific binding to actin, the cleft is still open. This would allow the major lever arm swing to be coupled to closure of the actin-binding cleft to follow formation of the P_iR state [10]. Once P_i leaves the nucleotide pocket, rapid closure of the actin-binding cleft, coupled to a major component of the powerstroke (lever arm swing) could occur. A series of mutagenesis experiments with myosins II, V, and VI, coupled with kinetic assays, provided results that were consistent with this P_iR state being populated by actin to allow release via the observed phosphate tunnel prior to the structural transition that is sensed by pyrene-

actin quenching. Pyrene-actin quenching [15] has been postulated to monitor the closure of the actin-binding cleft [16].

By soaking crystals of this proposed phosphate release state (P_iR state) with high concentrations of phosphate it was shown that phosphate can reenter and bind within the tunnel. With prolonged soaking the phosphate can reach the nucleotide-binding pocket, where it promotes the formation of the pre-powerstroke state (PPS) in the absence of actin. Thus it was proposed, based on these P_iR structures in combination with mutagenesis/kinetic studies, that the new structural state represents the phosphate release state (P_iR) that is formed on actin, and that actin triggers the transition from the PPS state to this P_iR state to initiate force generation and P_i release. The P_iR state would then be followed by actin-binding cleft closure, and the powerstroke on actin [5]. A key observation of these studies is that once the P_i leaves the nucleotide-binding pocket, it can transiently well at the mouth of the phosphate release tunnel (coordinated by R203 and X). This implies that there may be a delay between the time that phosphate leaves the active site, allowing the cleft to close, and when the phosphate appears in solution.

Rapid Lever arm swing in the absence of load

However, interpretation of this structure as the true phosphate release state has been called into question by a series of FRET studies that monitor the movement of the lever arm following myosin attachment to actin [7-9]. The debate centers on whether the powerstroke gates phosphate release or whether phosphate release gates the powerstroke. The observations from the FRET studies demonstrate that there is a rapid transition within the motor domain that affects the lever arm position. This transition is faster than the measured rate of phosphate release, and is followed by a slow lever arm movement that precedes ADP release. These data have been interpreted as showing that the large component of the lever arm swing occurs in the first rapid transition and

precedes P_i release. FRET studies are in discrepancy with the published P_i release (P_iR) structure. In the structure, the lever arm is in a primed position, close to the position observed in the PPS state. In contrast to what has been proposed to interpret the FRET studies, the P_iR structure implies that P_i release would precede the powerstroke.

In a FRET study with myosin V [8], the results were similar to those earlier seen with myosin II [9]. Upon actin binding, there was a rapid lever arm movement ($>300/\text{sec}$) that was followed by a slower lever arm movement ($\sim 20/\text{sec}$), while phosphate release was observed at $\sim 200/\text{sec}$. As discussed below, the second, slower lever arm movement (interpreted as the ADP releasing transition) may have been comprised of two separate transitions (one associated with pyrene actin quenching and one with MgADP release) that could not be resolved. Again, since the measured rate of phosphate release was slower than the initial phase of the powerstroke, it was suggested that the powerstroke gates P_i release.

As noted above, P_iR structures were obtained in which phosphate has moved away from the nucleotide-binding pocket, but is found bound at the mouth of the proposed phosphate release tunnel [5]. In this position, the phosphate likely will not restrict structural rearrangements that may be necessary to close the actin-binding cleft and initiate the powerstroke, but the phosphate would not yet be detectable in solution. Since the transition from PPS to P_iR and the next transition that is coupled to the initial phase of the powerstroke may both be extremely rapid in the absence of load, phosphate may not be detected in solution prior to when the rapid component of the powerstroke has been completed. This apparent lag in P_i release would be consistent with what has been observed [7-9]. In other words, it is formation of the phosphate release state and phosphate leaving the active site that gates the powerstroke, not the entry of phosphate into solution.

Lever arm swing follows P_i translocation

A drug that could stabilize the phosphate release state on F-actin and slow the next transition associated with the initial phase of powerstroke, would provide further evidence for this hypothesis. We propose that omecamtiv mecarbil (OM), which is a small molecule that has been developed to increase cardiac force production [17], is such a drug. Kinetic analyses [18] reveal that this drug, which accelerates phosphate release upon cardiac myosin rebinding to F-actin, does so by stabilizing a force generating state that is prior to the ADP release step. The drug has no effect on the ADP releasing transition, which is the last transition in the powerstroke. OM was shown to stabilize the pre-powerstroke state, increasing the equilibrium constant of the hydrolysis step [18]. Given the structural similarities between the PPS state and the P_iR state, it is likely that OM can bind both with similar affinities and will likely stabilize both states. This would lead to the observed accelerated phosphate release in the presence of actin, since the drug would cooperate with actin in promoting and stabilizing the P_iR state. The transition from the P_iR state to the ADP state formed in the initial phase(s) of the power stroke would thus be slowed by the stabilization of the P_iR state if the region where OM binds must rearrange as the actin-binding cleft closes. Thus the rate of the large movement associated with the initial phase of the powerstroke would be slowed by this mechanism, potentially to a rate slower than P_i release, even though the rate of phosphate release is increased.

Evidence that OM has this effect on cardiac myosin has recently been presented [19]. The authors, using a similar FRET approach as in their earlier work described above, observed a slowing of the powerstroke movement in the presence of OM, to the extent that it was slower than the rate of phosphate release, which was increased in rate. These results provide compelling support for the proposal that formation of the published P_iR state precedes the powerstroke.

This generalized concept of the lever arm swing following phosphate release fits well with fiber studies that suggest that the phosphate release step is only reversible if the myosin lever arms are pulled back, or held at, a position near the beginning of the powerstroke [20]. Likewise, single molecule studies with skeletal myosin II and myosin V undergoing rapid feedback to counter movement led to the same conclusion [21-22]. The rapidity of the transition from P_iR to the powerstroke in the absence of load may essentially rectify the transition, by closing the phosphate tunnel so that phosphate cannot reenter the nucleotide-binding pocket.

Generation of Force

It is important to understand the distinction between the major movement of the lever arm, or powerstroke, and the generation of force. While the lever arm movement is a major part of the force generating mechanism, force generation should be initiated before the lever arm moves, as a result of the first stereo-specific binding of myosin to actin. As first proposed by A.F. Huxley in 1957 [23], such binding constitutes a Brownian ratchet [24], and generates an immediate force upon attachment of myosin to actin, since the myosin attachment rectifies thermal motion. However, as Huxley and Simmons later appreciated, such a model cannot explain the force transients generated by muscle fibers subjected to small but rapid changes in fiber length [25], unless there are additional movements of the myosin cross-bridges once they are tightly bound to actin. Based on our current structural knowledge, these additional movements can be interpreted as the powerstroke of myosin on actin. Interestingly, Huxley and Simmons noted that the movement that follows the attachment of myosin to actin must be comprised of multiple components. They suggested 2 or 3 additional components (most likely 3 additional), for a total of either 3 or 4 force generating states in order to explain both the force transients and the force and work that is generated by cross-bridges. Recent cross-bridge models that have been proposed to provide the best recapitulation of the properties of contracting muscle require four distinct force-generating states [26].

There is current structural evidence that the powerstroke itself does involve at least two components, with the release of MgADP following the final component of the powerstroke [27-28]. In the past, the signal coming from pyrene actin has been interpreted as reporting cleft closure, which has been postulated to be coupled with movement of the lever arm. It also indicates that myosin must alter the conformation of F-actin, perhaps as the cleft fully closes while maintaining strong interactions at the actin interface, which is then transmitted to the C-terminal sequence of actin to which the pyrene probe is attached. However, the primary lever arm movement sensed by FRET experiments is much faster than the movement sensed by pyrene-actin, and furthermore there is not a large lever arm movement observed at the rate of pyrene quenching in optical trap experiments [29] or in the FRET experiments [7-9]. Thus the initial powerstroke is rapid in the absence of load, and is followed by a slower structural transition that is sensed by the changes in pyrene actin. This may imply that the initial phase of the powerstroke is coupled to a partial cleft closure, and that additional cleft closure requires actin structural alterations in order to maintain the myosin interactions at the interface in the Strong ADP state (Fig.1). This final cleft closure may be coupled to a small lever arm swing. Whatever these actin changes are that are sensed by pyrene-actin during formation of the strongly bound ADP state on actin, they appear to be associated with a small alteration in the actin helical twist, at least in the case of myosin V, as was seen in cryo-EM reconstructions [6].

MgADP release

There is no mystery as to how MgADP coordination is lost during the transition to the final force generating state of myosin on actin (rigor). The final nucleotide-free, rigor conformation is formed by relieving the strain in the seven-stranded beta sheet, allowing switch I and the P-loop to be sufficiently separated to lose MgADP coordination [30-31]. The Mg²⁺ dissociates first [32-33], while the ADP can transiently

remain bound to the P-loop before its dissociation from the rigor state [34]. What is unclear is the nature of the structural state immediately prior to MgADP release. This is a state that binds MgADP strongly and simultaneously binds strongly to actin. The existence of this state was unsuspected until it was seen using cryoEM for both smooth muscle myosin II and for brush border myosin I [27-28].

The structural details of this state are not yet available at high-resolution, but a recent EM structure has provided some new insights [6]. The EM structure demonstrated a different conformation of the beta-sheet, and elements of what is known as the transducer [34], as compared to earlier structures. The P-loop has moved as a result of this, and the MgADP would appear to be coordinated in a somewhat different manner than is seen for either MgATP or MgADP in the myosin post-rigor state, populated when nucleotide is bound in the absence of actin [6]. This may result in weakening of the Mg²⁺ coordination, as Mg²⁺ has been shown to dissociate prior to ADP, and increasing [Mg²⁺] can slow ADP release [32-33]. The movement of the lever arm appears to be accomplished by novel positions of switch II, the relay and the SH1 helix (linked to the transducer conformation), which specify the converter and lever arm positions.

Model for force generation

We propose a model in which the first step in force generation by myosin is the stabilization of the P_iR state by actin binding, which produces force and allows P_i to leave the active site of myosin. Once the P_i moves away from the active site, the next structural transition involves the closure of the actin-binding cleft (to increase the interactions with actin), which is coupled to the large movement (powerstroke) of the myosin lever arm. The P_i leaves the active site, moves into the phosphate release tunnel, where it is bound at the mouth of the tunnel. From this position it can either move back to the nucleotide pocket if the tunnel remains open, or be released into solution. A rapid closure of the cleft (coupled to lever arm movement) that also closes access to the nucleotide binding pocket would drive the reaction forward into the next force

generating state. Thus it is not the release of phosphate into solution that gates the powerstroke, rather the gating event is the phosphate leaving the active site. After the initial phase of the powerstroke, there is a transition to the MgADP bound state that has been seen in EM reconstructions [6,27-28,35], which is coupled to a change in F-actin sensed by pyrene quenching. This state is followed by the final component of the lever arm swing, which releases MgADP and which is highly strain sensitive. Rebinding of MgATP terminates force generation by reopening the actin-binding cleft and allowing myosin to detach from actin.

Thus force is generated by sequential transitions through at least four discreet states on actin: (1) formation of the initial binding state (P_iR state); (2) rapid transition (in the absence of load) to the state that is coupled to a large movement of the lever arm (intermediate ADP state); (3) completion of cleft closure linked to the stabilization of the C-terminal region of F-actin (formation of the strongly bound ADP state); and (4) formation of the rigor state followed with the release of ADP. **These sequential transitions highlight how events at the actin interface trigger events at the myosin active site and vice versa to control force generation by myosin motors.** By tuning the kinetic constants in these transitions [36], the strain-sensitivity, and the stiffness of the different states of the cross-bridges, myosin motors have been selectively tuned for a wide range of cellular functions.

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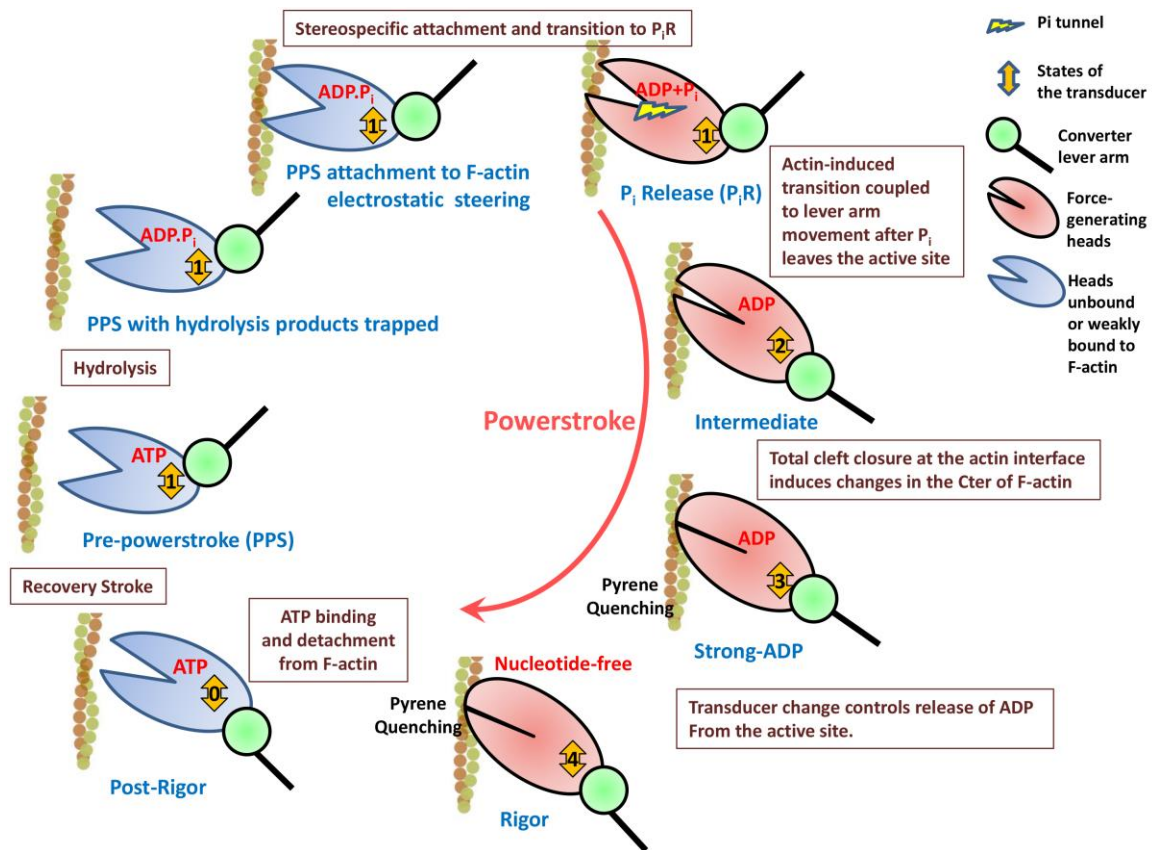


Figure 1. Myosin force generation on actin filaments. Several structural states of the motor allow this molecular machine to generate force upon ATP hydrolysis. This scheme provides a unifying view of the current available structural and functional data available for this motor. In red are the states of the motor that produce force. This cycle describes the structural transitions during the powerstroke that actin promotes in the

motor in order to sequentially release the hydrolysis products while directing a nm-long conformational change in the lever arm coupled to the swing of the converter (green).

Glossary

Lever Arm: the extension of the myosin motor that includes the converter subdomain and the extended alpha-helix to which calmodulin (CaM) or CaM-like light chains bind. Some classes of myosins have other structural elements that can further extend this mechanical lever arm. The role of the lever arm is to amplify the sequential conformational changes within the motor domain into larger, directed movements.

Powerstroke: the swing of the myosin lever arm on actin that generates force and movement.

Force generation: Force generation begins when myosin first bind to actin (with a lever arm primed) to initiate the powerstroke. Stereospecific binding of myosin to actin is indeed sufficient to generate force, by rectification of Brownian motion.

Transducer: Structural elements at the heart of the motor domain comprising the 7-stranded beta-sheet and connectors that control its conformation within the myosin motor. The transducer must rearrange to coordinate binding or release of MgATP and MgADP from the nucleotide-binding pocket. The 7-stranded beta-sheet conformation is controlled in large part by the nucleotide bound in the active site of the motor. It is flat and strained when MgATP binds and it is curved and relaxed when no nucleotide is bound in the motor. An intermediate position has been seen in the state that binds both actin and MgADP with high affinity, which is a major force-generating state on actin.

Trends Box

- A new high-resolution structure reveals rearrangements in the myosin motor that promote release of inorganic phosphate (following ATP hydrolysis).
- The new structure alters the actin-binding interface without closing the major cleft that must close during the force-generating powerstroke on actin.
- A series of FRET experiments reveal that, following binding to actin, the movement of the myosin lever arm (powerstroke) is extremely rapid and is faster than the rate of inorganic phosphate into solution.
- Mutagenesis experiments suggest that phosphate release can occur prior to closure of the actin-binding cleft.
- A controversy thus exists as to whether the myosin lever arm is coupled to closure of the actin-binding cleft and precedes or follows the release of inorganic phosphate, which is central to understanding chemo-mechanical force transduction by the myosin motor on actin.

Outstanding Questions Box (2000 characters, including spaces, required)

- Structural and functional studies are required to better describe the fundamentals of force generation.
- The specific interactions between myosin and actin that drive the sequential rearrangements of the actin-myosin complex must be determined to gain insights on how myosin members are tuned for different functions and how a single mutation can impair the motor and lead to pathology.
- Direct evidence is needed to demonstrate that formation of the putative phosphate release state of myosin precedes the initial large movement of the myosin lever arm.
- Is the initial, rapid movement of the myosin lever arm coupled to closure of the actin-binding cleft?
- Is there closure of the actin-binding cleft and movement of the myosin lever arm associated with the changes in actin that are detected by the changes in the pyrene probe on actin?
- What is the impact of strain on closure of the actin-binding cleft and on the movement of the myosin lever arm? Does compliance in the myosin lever arm allow strain to decouple these two rearrangements?
- Is the initial closure of the actin-binding cleft coupled to rearrangements in the myosin transducer region that are intermediate between the conformations seen in the pre-powerstroke state and the strong ADP state on actin ?
- What is the mechanism that accounts for the fact that some myosins are able to reverse their powerstroke easily and strain promote their detachment, while others act as anchors under load.

