

Physics of Molecular Machines
Les Houches 2006

**Chemomechanical Coupling in
Myosin II :
Experiment vs. Theory**

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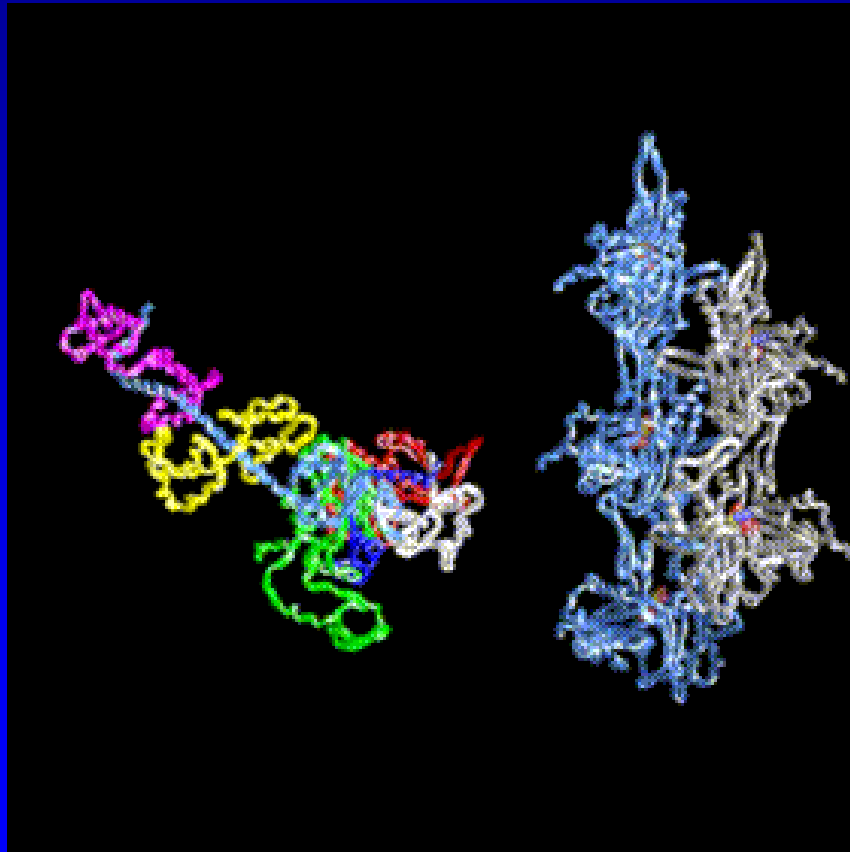
<http://www.le.ac.uk/biochem>



Topics: Myosin II

- States identified from crystal structures
- Switch movements probed by tryptophan fluorescence
- Domain movements probes by other fluorophores
- Coupling of movements to Pi release

Crossbridge cycle based on crystal structures



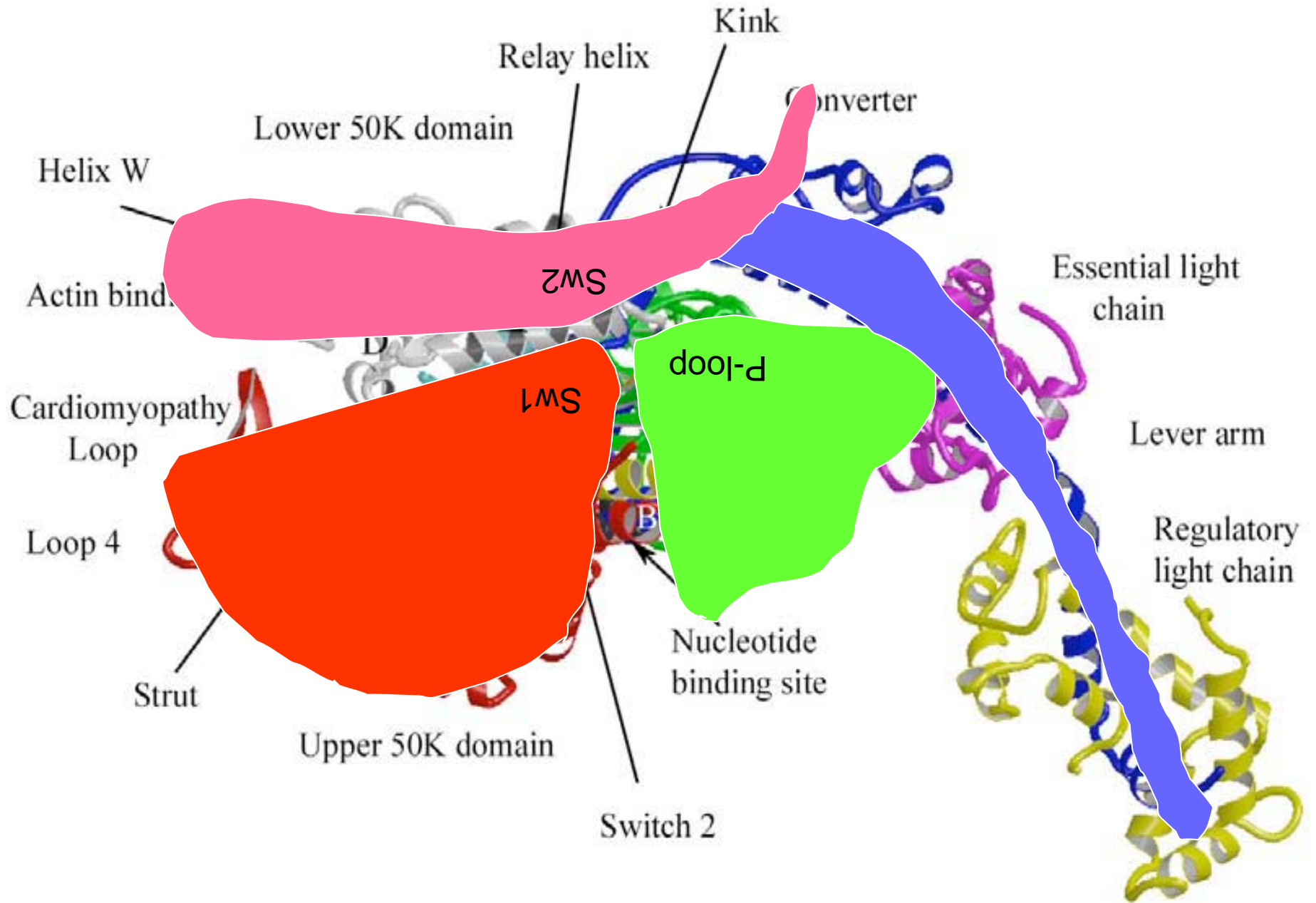
Animation by Ken Holmes

Model based on two (now three) crystal structures of myosin and em of decorated filaments

Does the neck really swing?

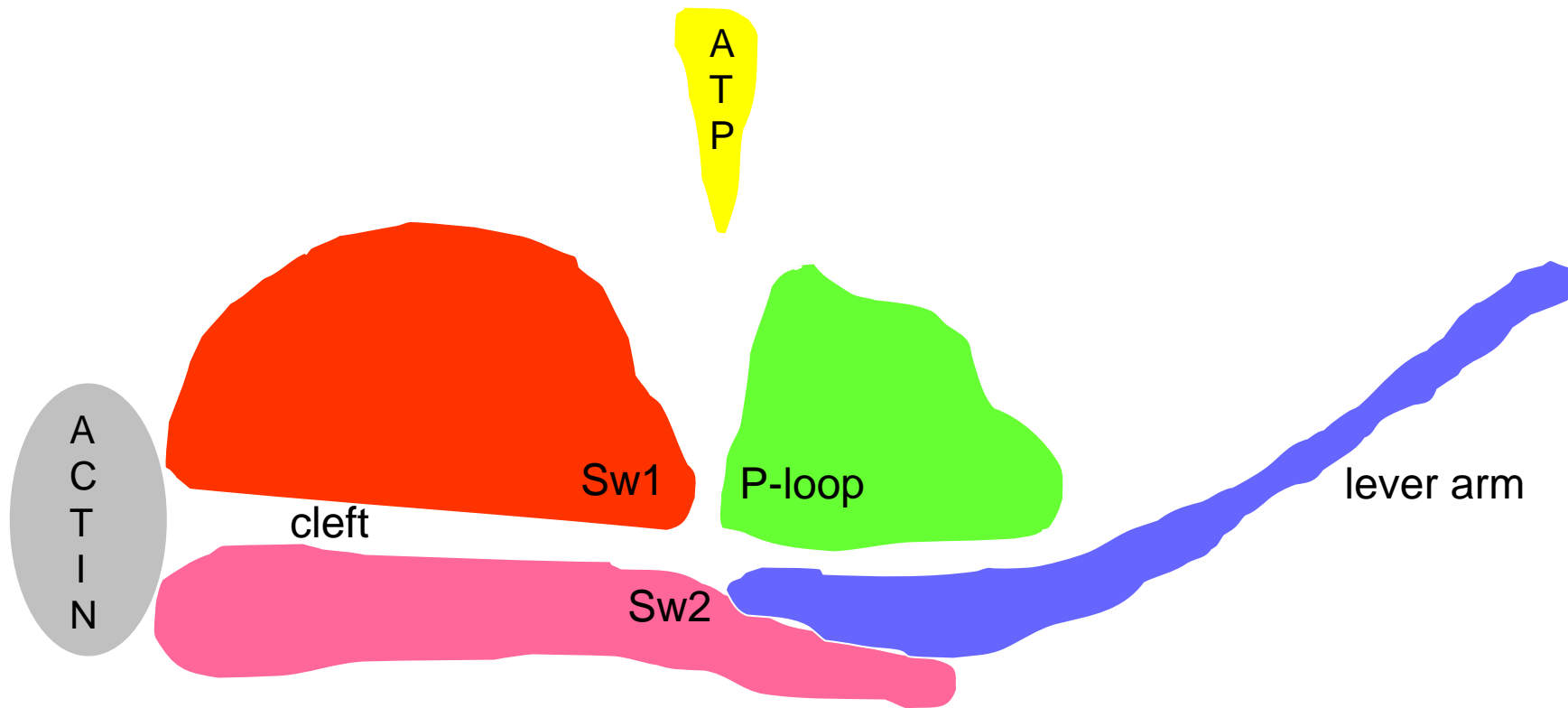
How is tail swing reversed by actin?

Is the lever arm swing the only mechanism contributing to sliding?



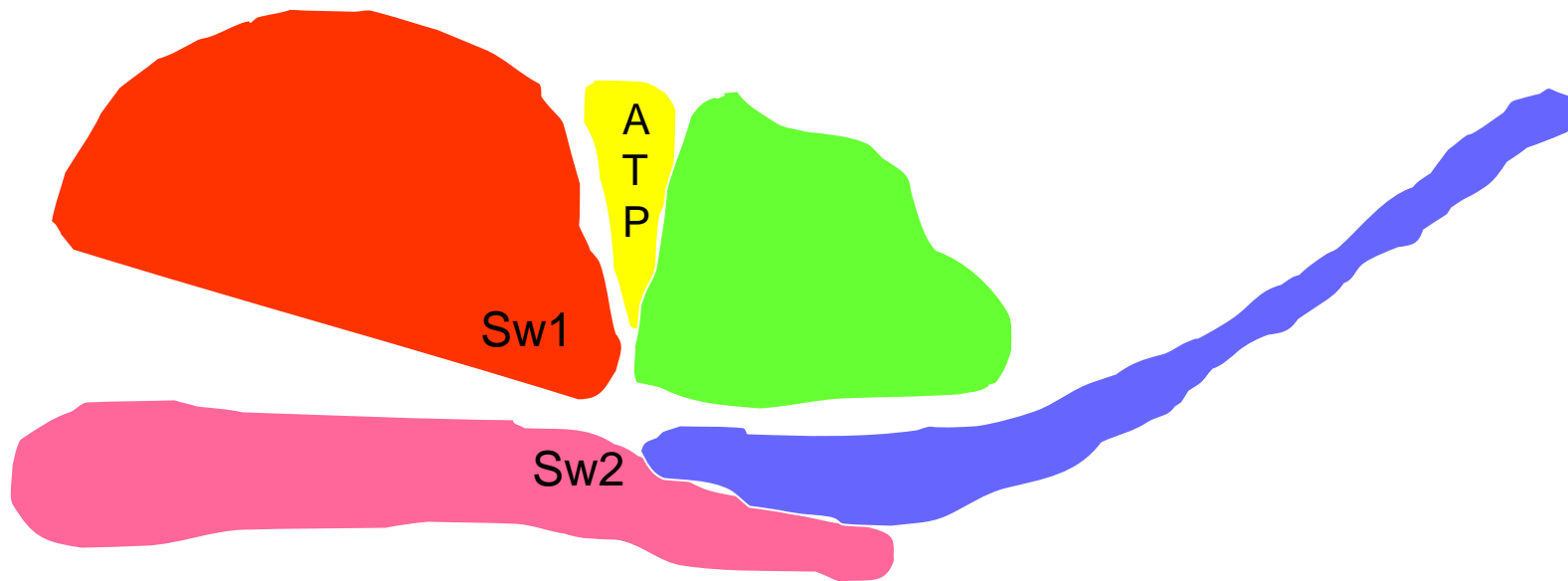
From Geeves & Holmes 2005

A.M (actomyosin)



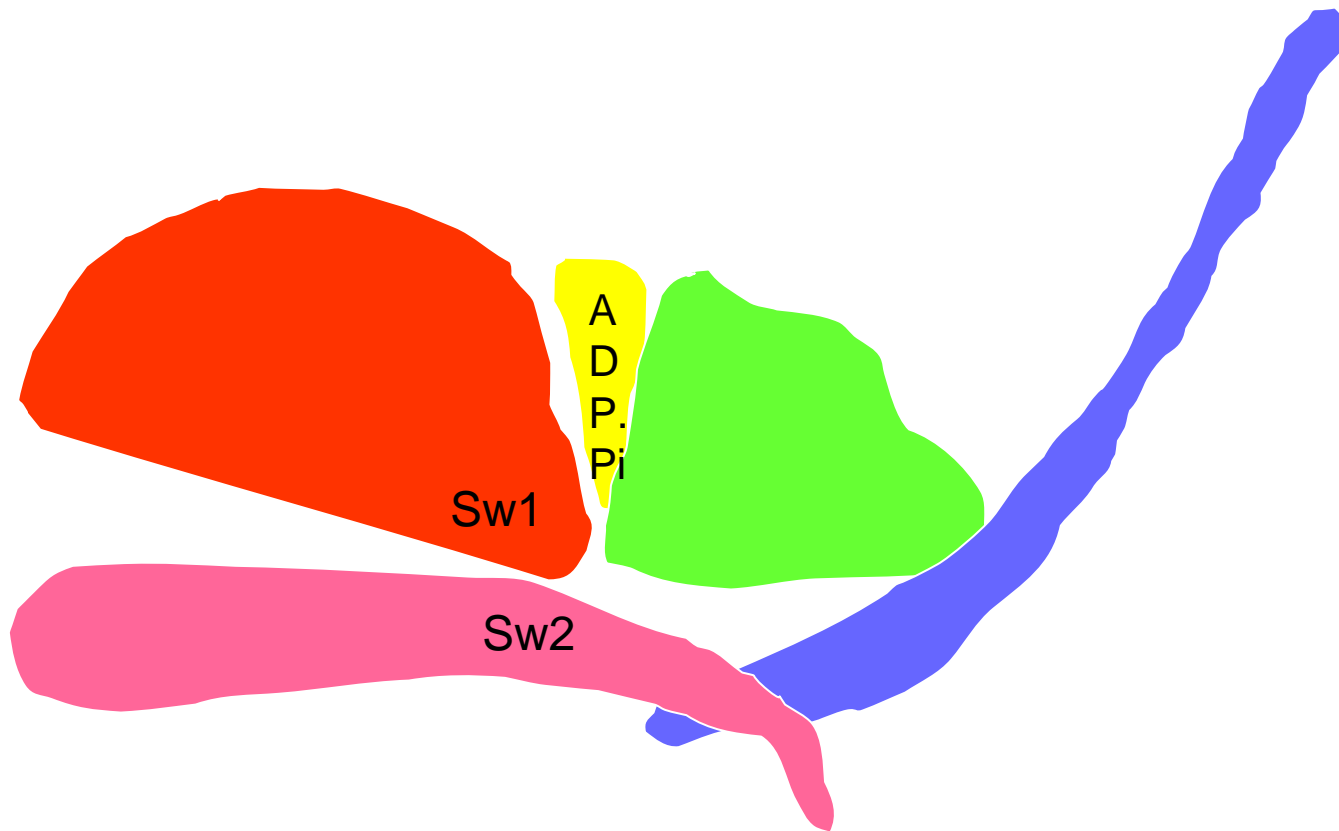
Cleft closed, Switch 1 and Switch 2 open

M⁺.ATP



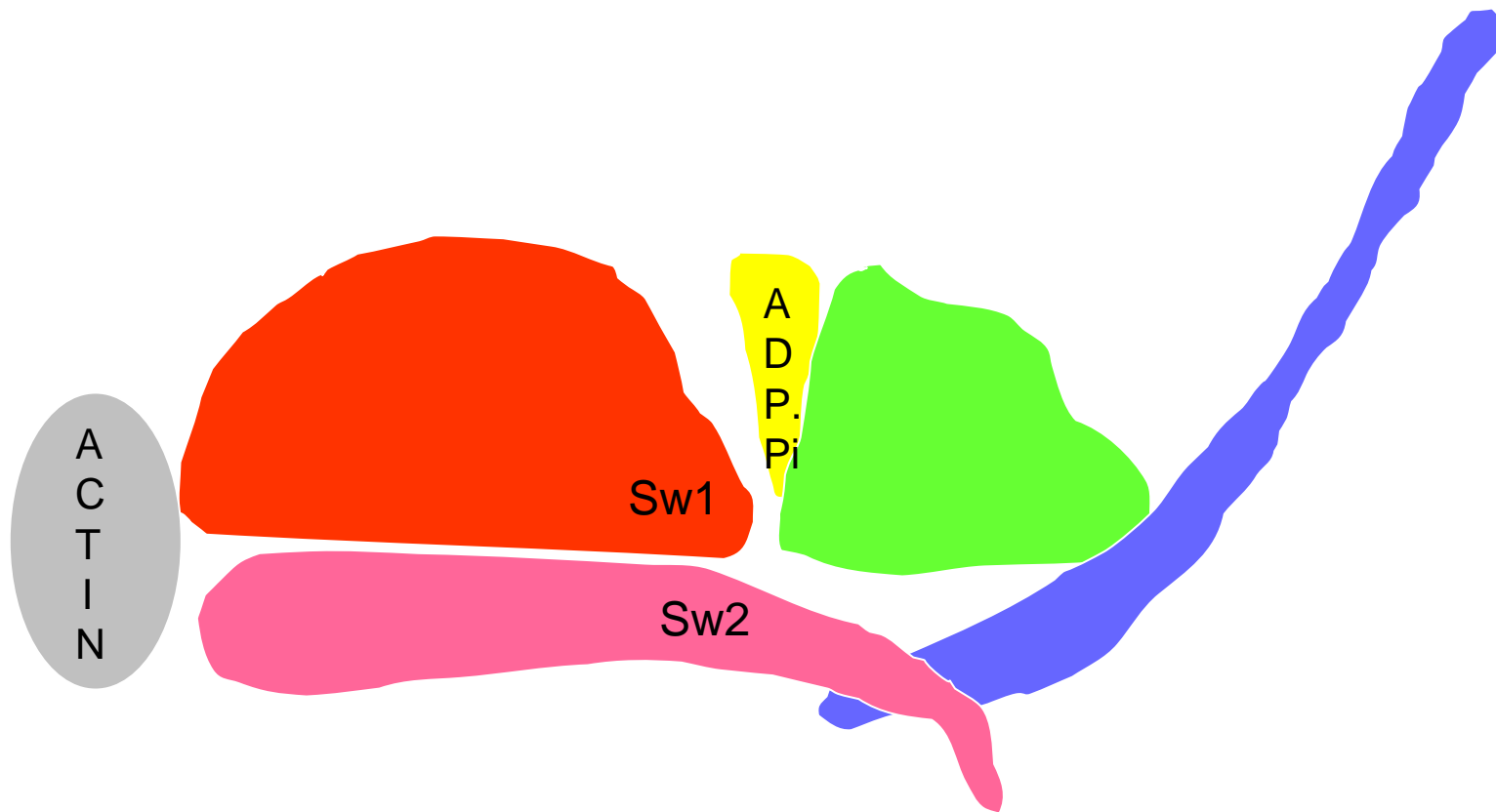
Switch 1 closes, Cleft opens, actin dissociates

M*.ADP.Pi



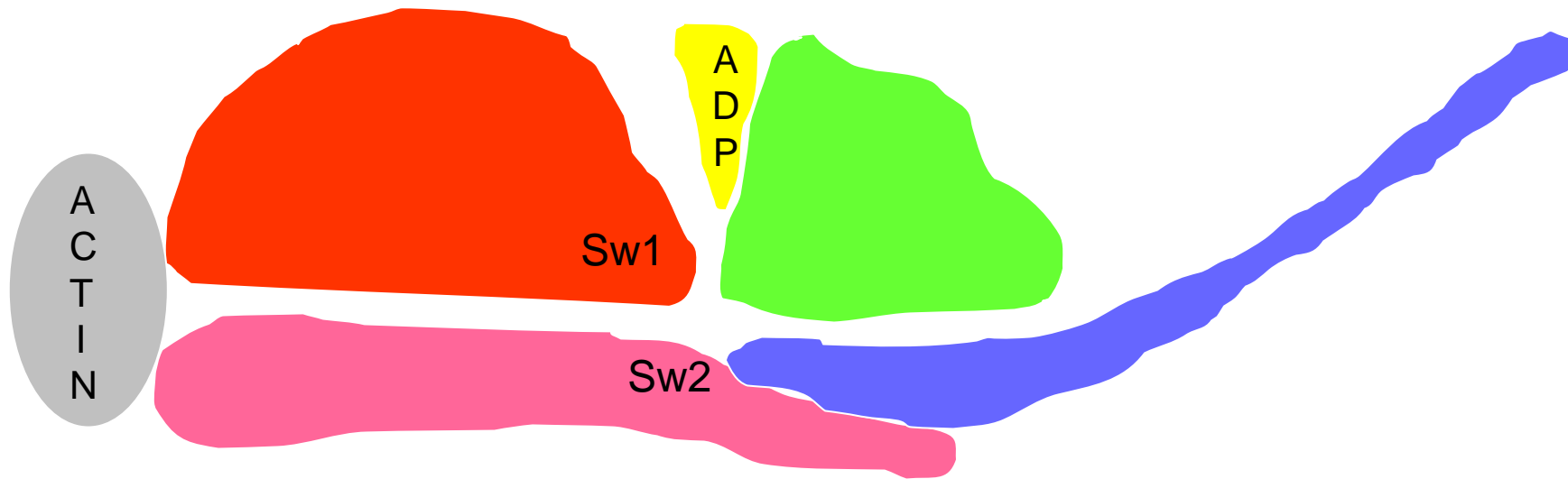
Switch 2 closes, ATP hydrolysis occurs

A.M*.ADP.Pi



Actin binds, cleft closes, Switch 1 opens

A.M*.ADP



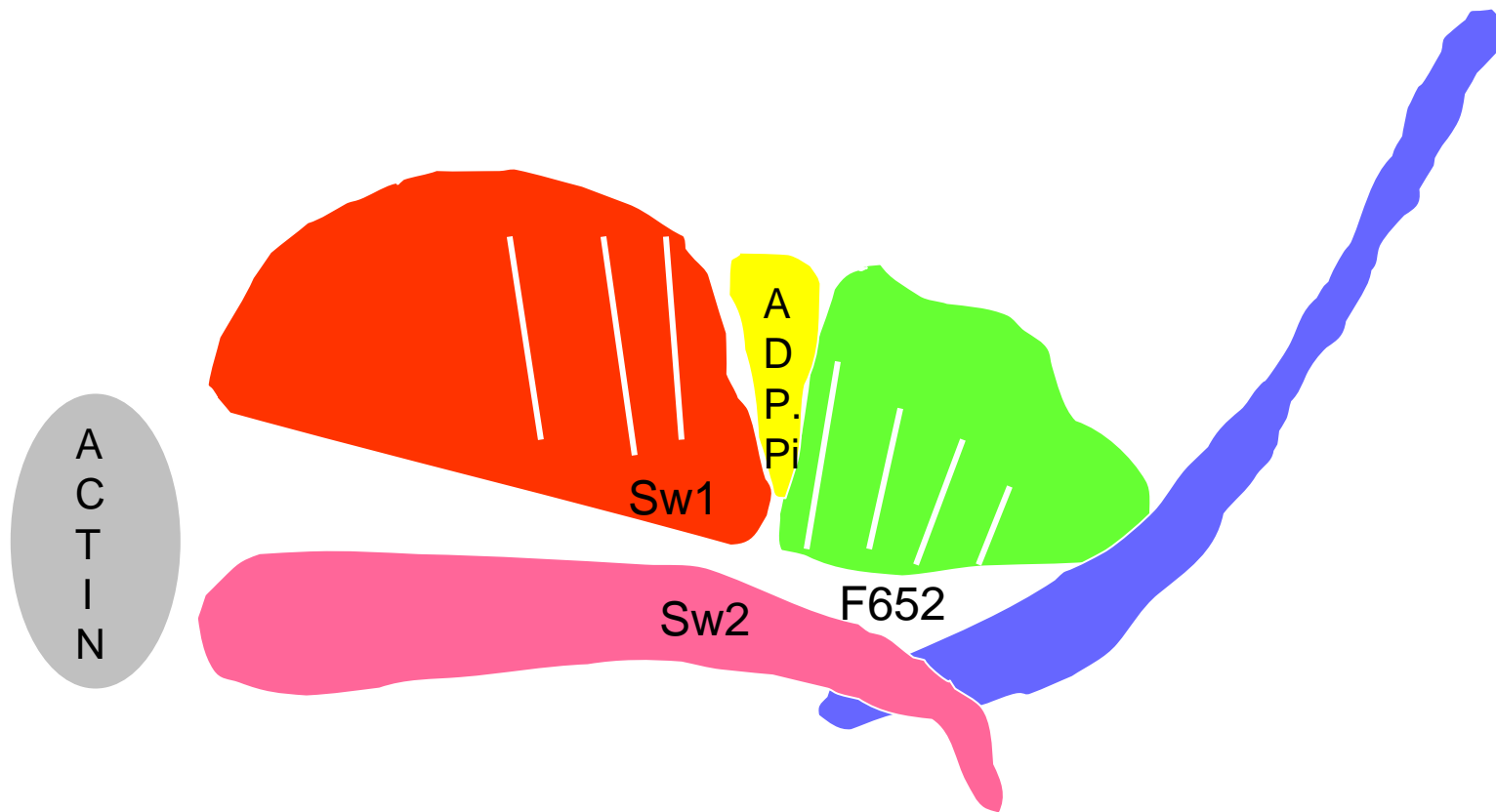
Pi released and Switch 2 opens = stroke

But

Holmes, Schröder, Sweeney & Houdusse (2004)
Phil. Trans. R. Soc. B **359**, 1819-1828

They proposed that relay helix could unkink without switch 2 having to open. This was brought about by the twisting of the core 7-strand β -sheet which moved F652 (the proposed cause of the relay helix kinking)

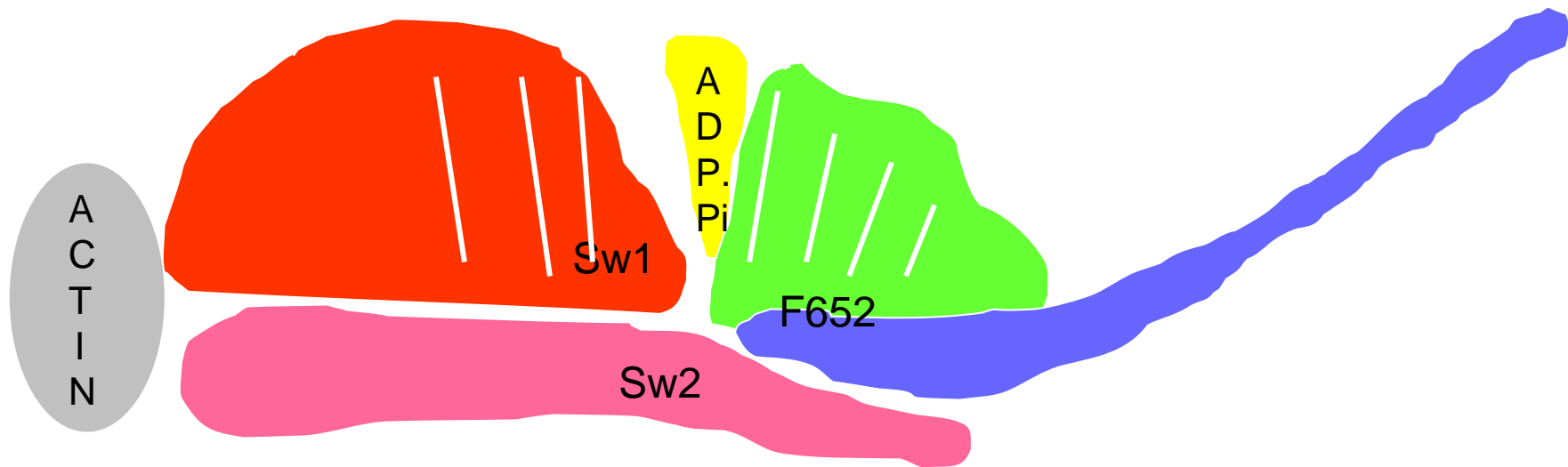
A.M*.ADP.Pi
(weakly bound)



Cleft closes, Switch 1 opens

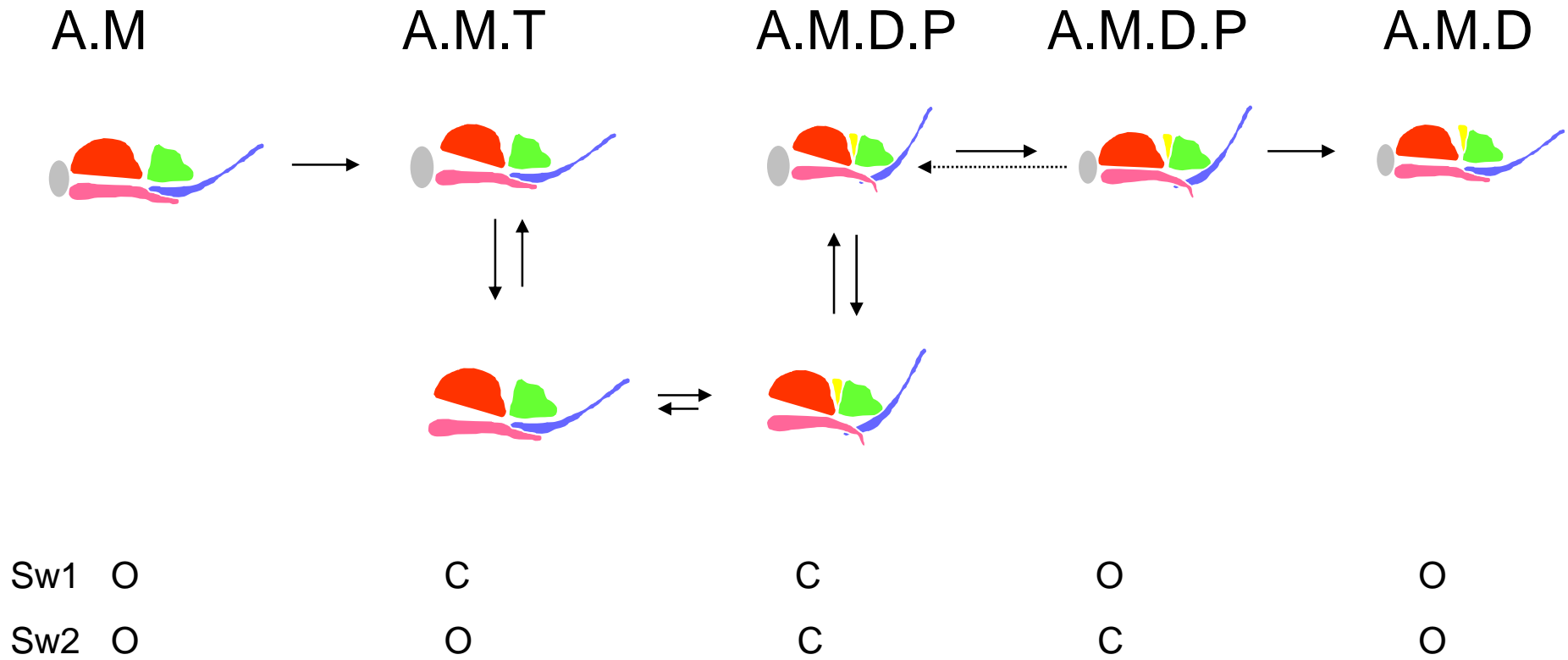
A.M*.ADP.Pi

The β -sheet twist model

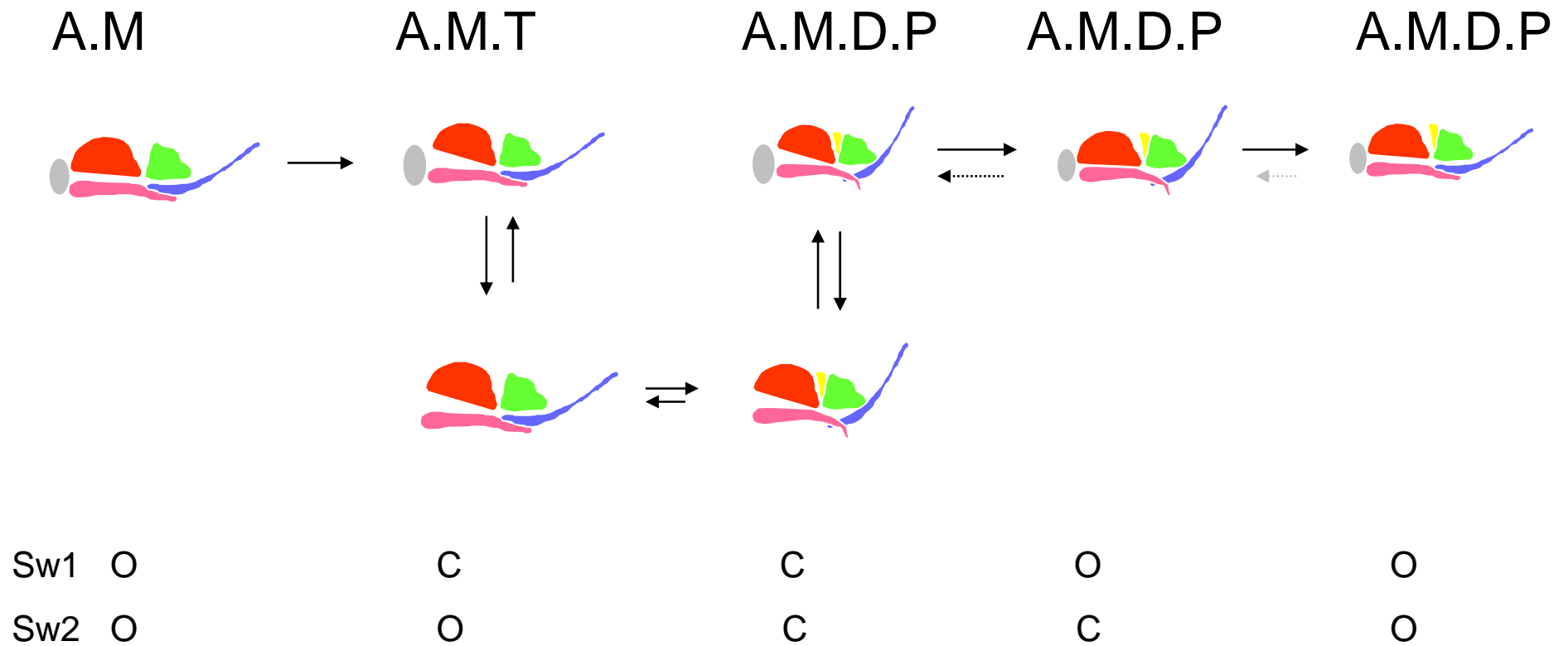


Switch 2 remains closed during lever arm swing

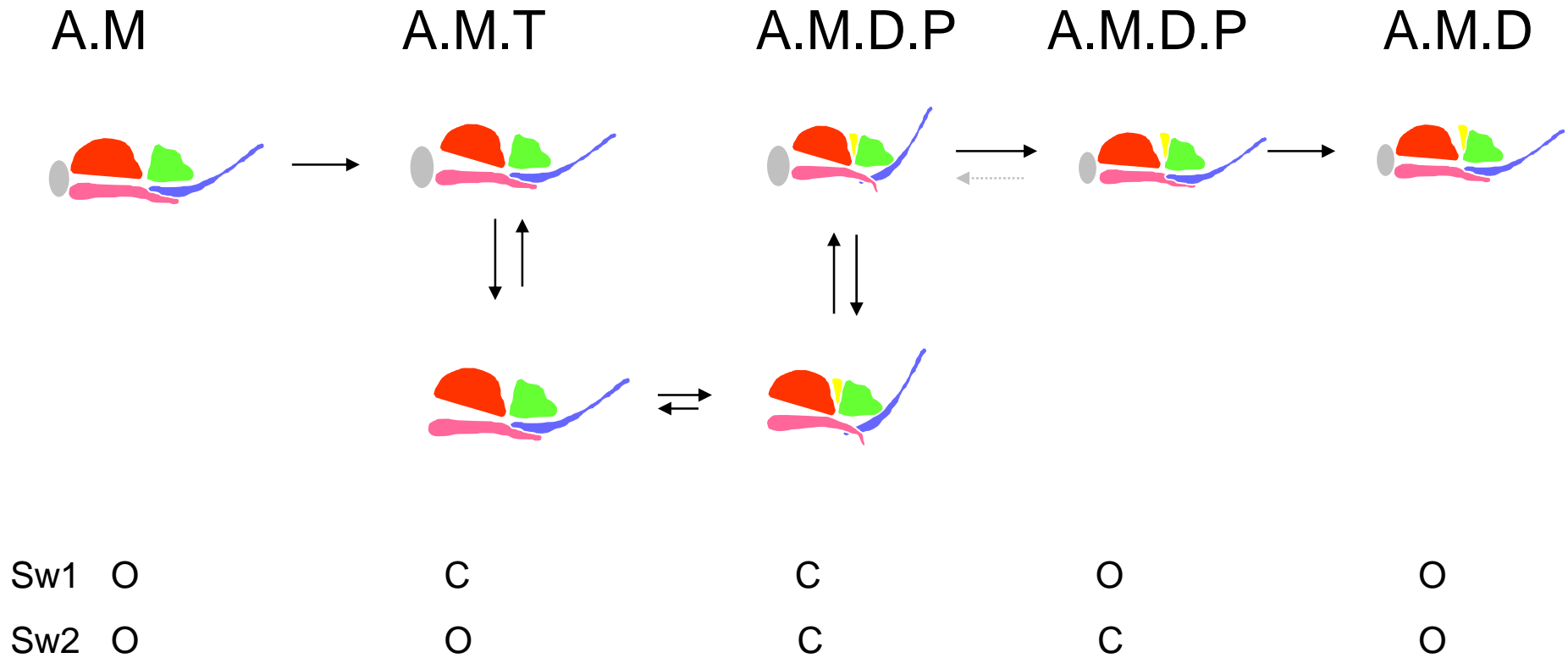
Stroke = Sw2 opening + Pi release



Stroke = Sw2 opening before Pi release

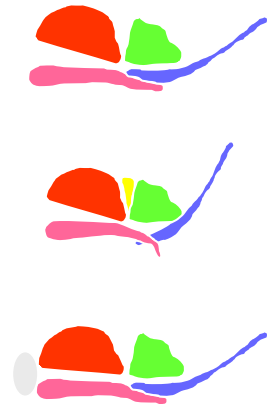


Stroke = relay helix unkinking due to β sheet twist



Key crystal states

Author	myosin	state	50K actin cleft	switch 1	switch 2	β sheet	lever arm	jargon
Rayment	Dicty 1MMD	ADP.BeFx	open	closed	open	straight	down	post rigor
Rayment	Dicty 1MND	ADP.AIF4	open	closed	closed	straight	up	pre power
Courreux	myosinV 10E9	apo	closed	open	different ¹ closed ² open ³	twist	down	rigor-like



- 1 = Houdusse
- 2 = Holmes (but not quite the same closed)
- 3 = Kull (P loop as ref)

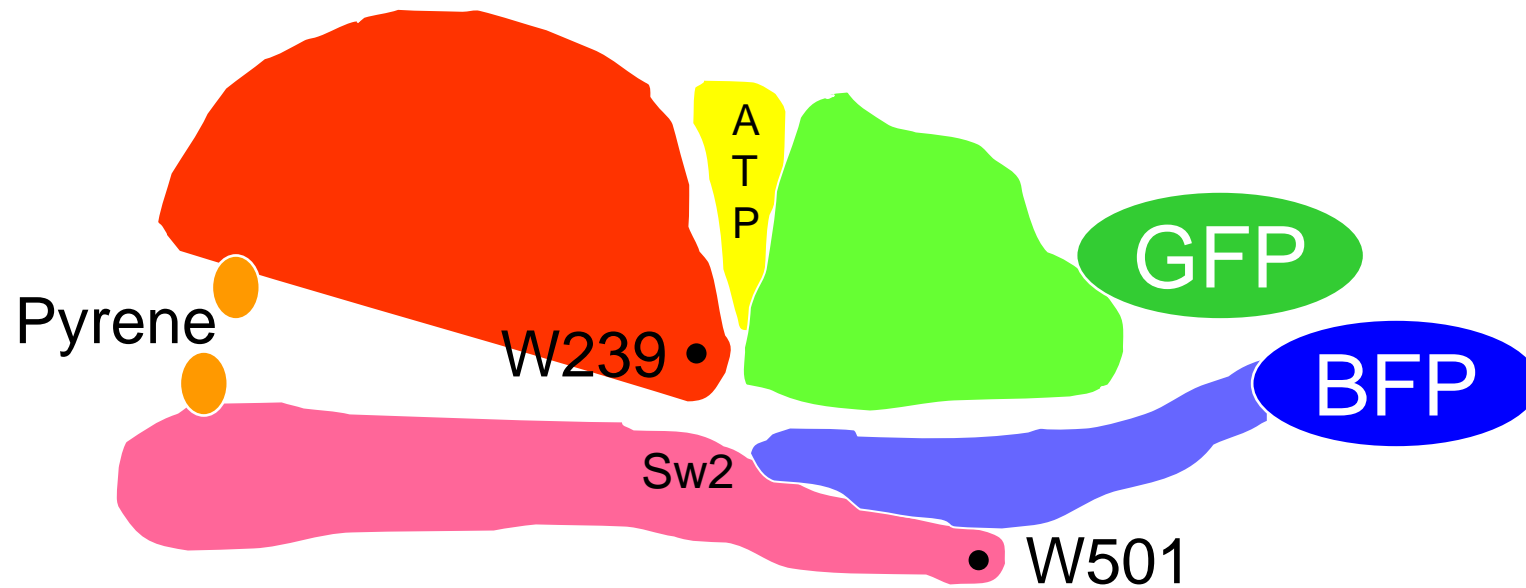
Author	myosin	state	50K actin cleft	cleft base	switch 1	switch 2	β sheet	lever arm	jargon
Rayment	skeletal 1mys	apo β S04	open	open	closed	open		down	post rigor
Rayment	Dicty 1MMD	ADP.BeFx	open	open	closed	open		down	post rigor
Holmes/Kull unpublished	Dicty	ADP.BeFx	open	closed	closed	closed		up	pre- power
Rayment	Dicty 1MND	ADP.AIF4	open	closed	closed	closed		up	pre power stroke
Houdusse	Scallop B7T	ADP	open		closed	open		more down	
Courreux	myosinV 10E9	apo	closed	open	open	different ¹ closed ²	twist	down	rigor-like
Reubold	Dicty 1Q5G	apo	intermedi ate		open	open ³ closed ²	twist	down	on the way to rigor

1 = Houdusse

2 = Holmes

3 = Kull (P loop as ref)

Fluorescence Probes of domain movement



Computational Chemistry

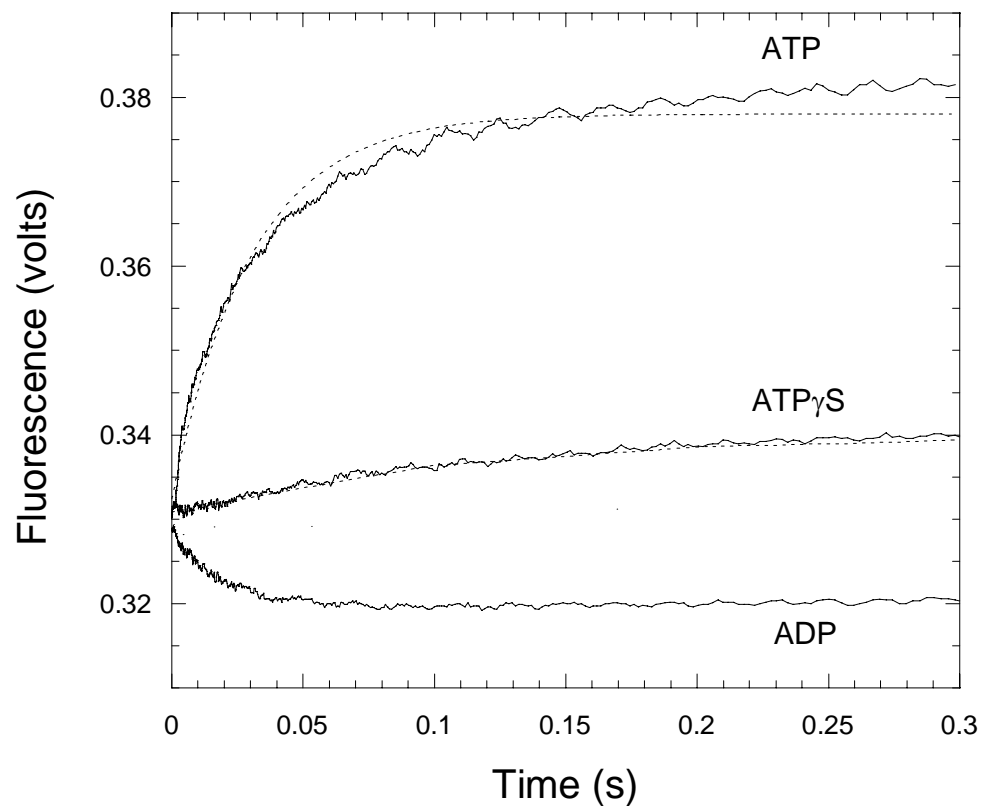
G. Li, Q. Cui, (2004) Mechanochemical coupling in myosin. A theoretical analysis of ATP hydrolysis with molecular dynamics and combined QM/MM reaction path calculations, *J. Phys. Chem. B* 108, 3342-3357.

G. Li, Q. Cui, (2004) Analysis of functional motions in "Brownian molecular machines" with an efficient block normal mode approach. Myosin-II and Ca²⁺-ATPase *Biophys. J.*, 86, 743-763

Schwarzl, S. M., J. C. Smith, and Fischer, S. (2006). "Insights into the Chemomechanical Coupling of the Myosin Motor from Simulation of Its ATP Hydrolysis Mechanism." *Biochemistry* 45(18): 5830-5847

Nucleotide binding to W501+

(saturating [nucleotide] at 20°C)

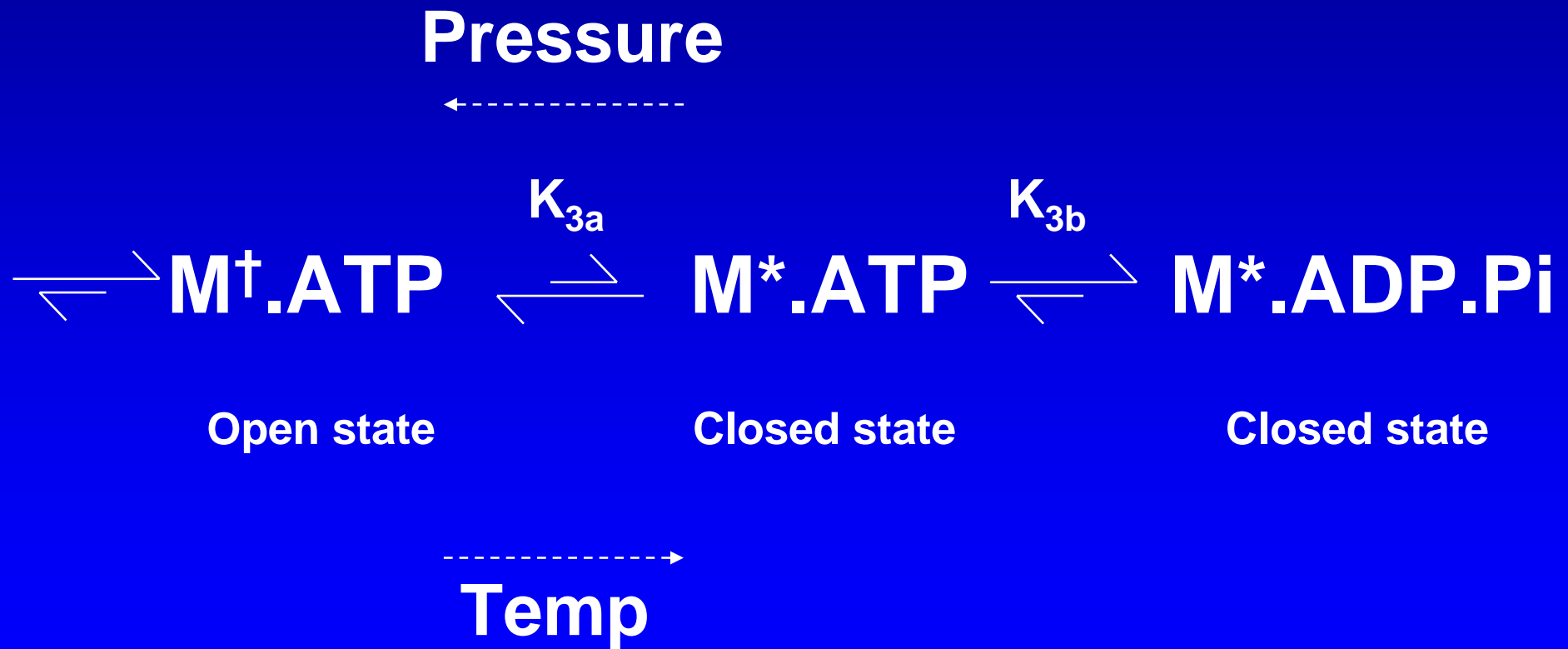


$M^*.ADP.Pi$

$M^\dagger.ATP_\gamma S \leftrightarrow M^*.ATP_\gamma S$

$M^\dagger.ADP$

ATP Hydrolysis Step has been Resolved into Two Steps



Conclusions from W501 fluorescence

- Model independent
 - Relay helix and/or converter domain moves in response to ATP binding, but hydrolysis is required to make equilibrium favorable
 - Reaction is freely reversible, even when hydrolysis occurs
 - Actin has no direct effect on this reaction
- Model dependent
 - W501 senses switch 2 and lever arm movement (consistent with crystal structures)
 - Actin influences lever arm movement through Switch 1

Key Question

What is the difference between the lever arm swing in the absence and presence of actin?

Depends on Switch 1 position ?

Coupling between actin binding and switch 1

- Is there a 1:1 coupling between cleft closure and switch 1 opening?
- Answer: not simple as there multiple states of switch 1 conformation
- but there is evidence of some reciprocity between 50k cleft closure and switch 1 opening.

Conclusions from W239+

- Apo and +MgADP states comprise a mixture of conformers
- MgATP and actin lock the W239 in a “single” state with respect to W239 with low and high fluorescence respectively.
- This is consistent with structural ideas about switch 1, but there are more than two states

Comparison of W501 and W239

W501

MgADP and apo single states

MgATP and MgATP γ S two states

Little sensitivity to actin binding

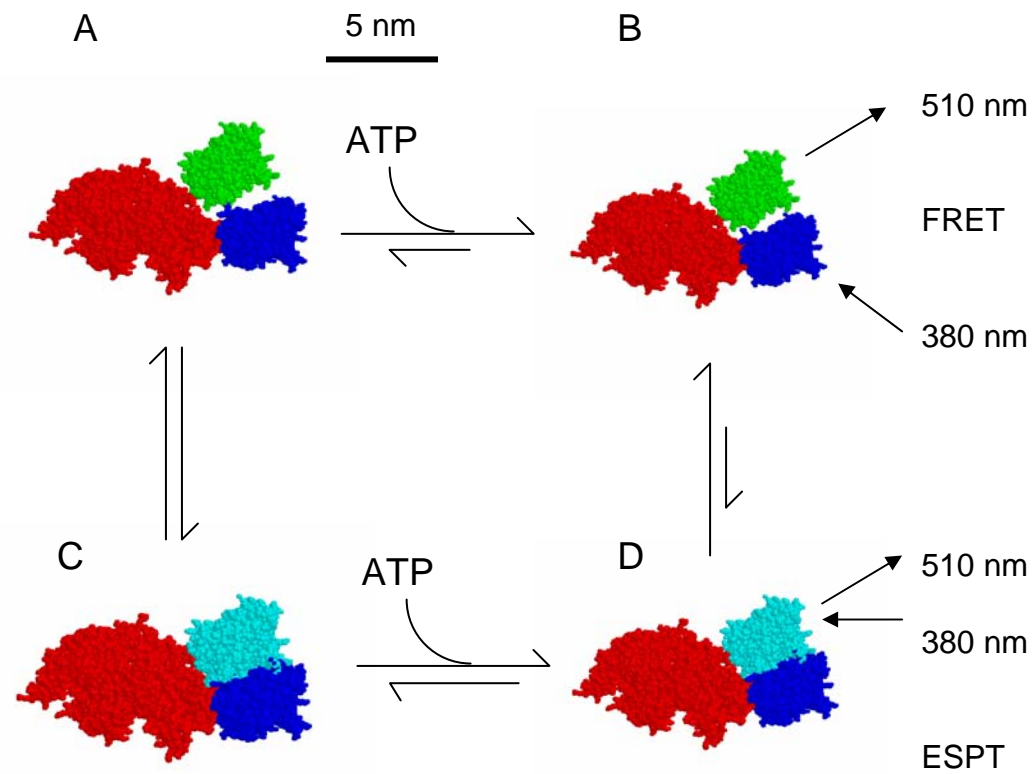
W239

MgADP two states

MgATP and MgATP γ S single states

Sensitive to actin binding

Effect of intramolecular GFP-BFP association



Conclusions from FRET experiments

Author	Construct	FRET efficiency	Distance change	Uncertainty
Suzuki et al Nature 1998	Apo GFP-M-BFP + ATP	0.439 0.082	+ 15 Å	± 1.4 Å (S.D.)
Zeng et al 2006	Apo GFP-M-BFP + ATP	0.70 0.66	+ 1.5 Å	± 13 Å (κ^2)
Zeng et al 2006	Apo YFP-M-CFP + ATP	0.58 0.61	- 1.5 Å	± 10 Å (κ^2)