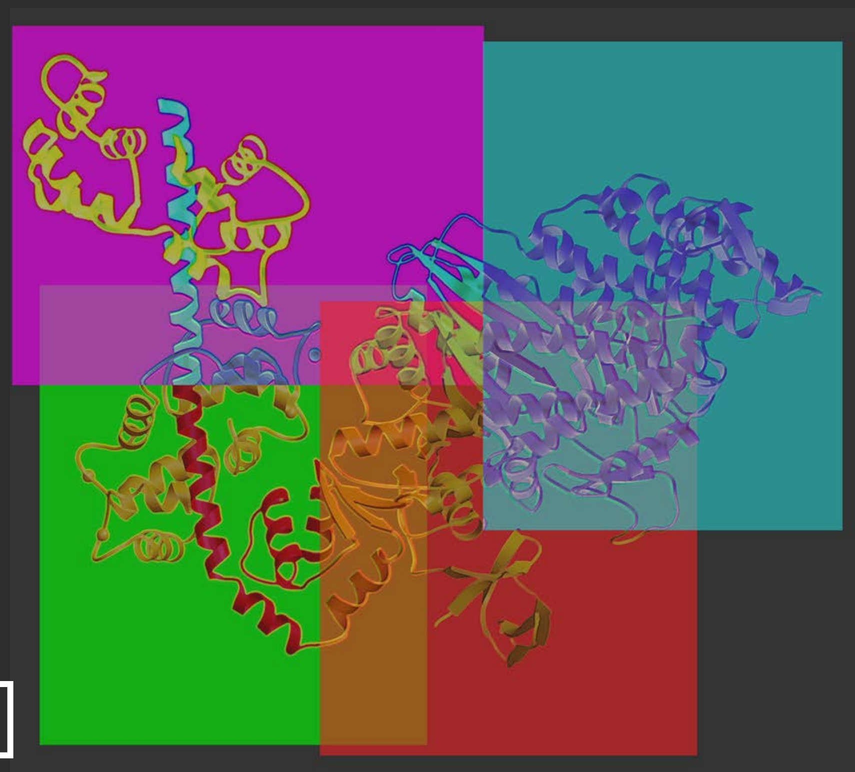
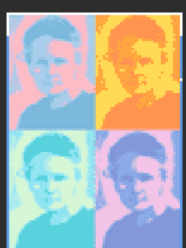
Défis et succès dans l’étude de la production de force et du rôle cellulaire des nanomoteurs myosines.





The Structural Motility Group

Colloque annuel - ITMO Bases moléculaires et structurales du vivant, Paris, France.
Anne Houdusse – 24th of May 2013

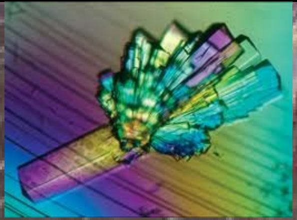
The cytoskeleton, a dynamic city plan for the cell

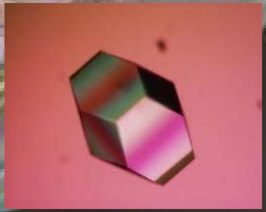
Molecular motors are essential for cell polarity and division

Cellular traffic – cell division – cell migration

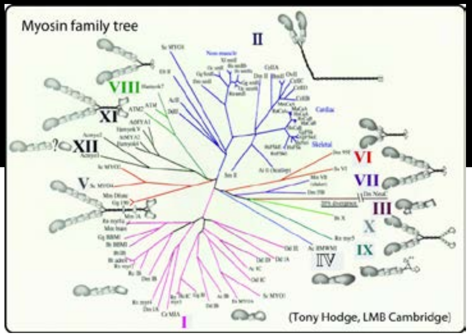
« Seeing is necessary for understanding »

Our goal : Visualize the motor <u>conformational changes</u> necessary for function and regulation





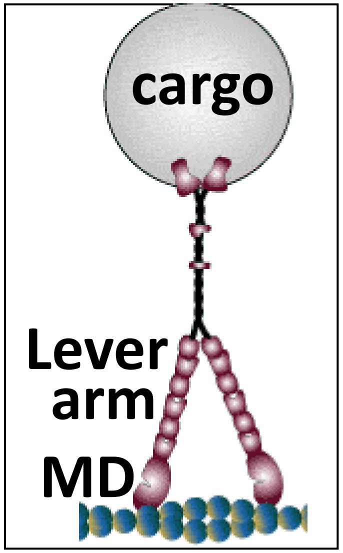
Molecular Understanding of Transport and Tension Generation by Molecular Motors



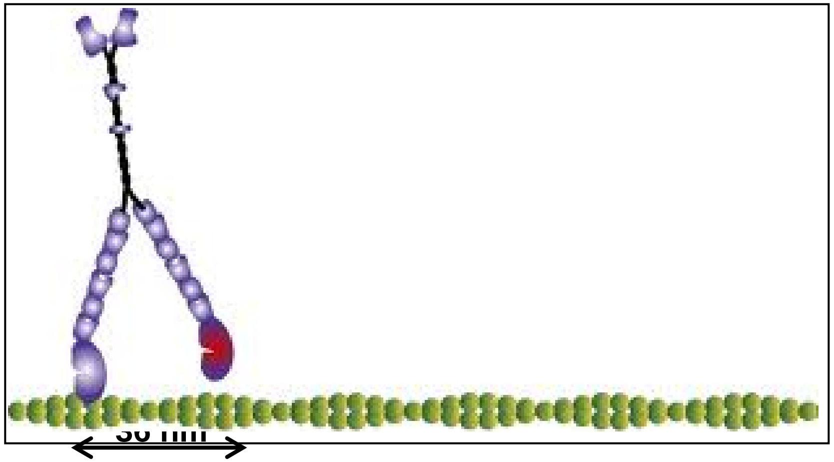
Myosin motors

Actin-based molecular motors

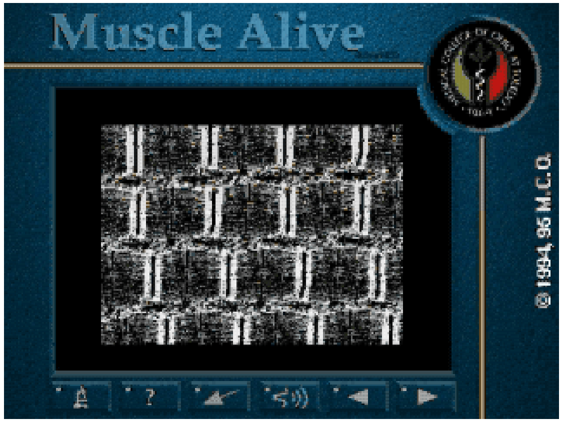
How do they convert chemical energy into force production

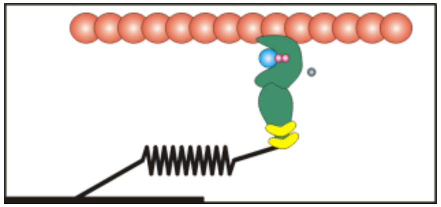


Myosin V steps on actin

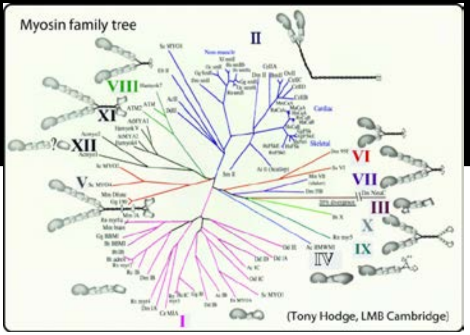


Powerstroke





The myosin superfamily

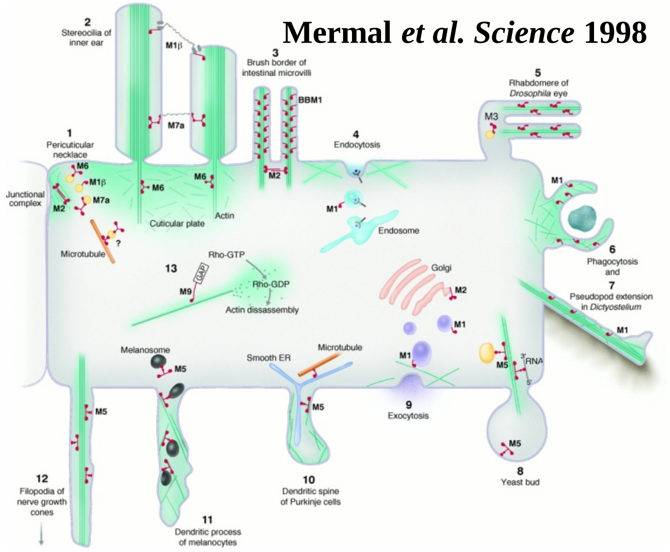


Myosin motors

Actin-based molecular motors

How do they convert chemical energy into force production

How do they tune their activity for different cellular functions



Various myosins can either contract rapidly or hold force

Actin filament contraction

Molecular transporters of cargoes in the cell

Tension sensors

Anchoring function - Dynamic tethers

Role in membrane deformation and fission

Sequence differences can tune motor activity – or impair the motor

OUTLINE

Myosin motors - force production, regulation, cellular roles :

What is currently known for force production by the myosin superfamily.

Testing the theory with a reverse motor

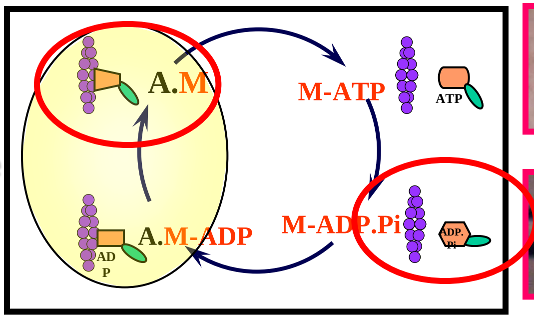
Current challenges

Allostery, basis to convert chemical energy into force production

Myosin motors

Nucleotide Free (rigor)

Actin-activated ATPase cycle



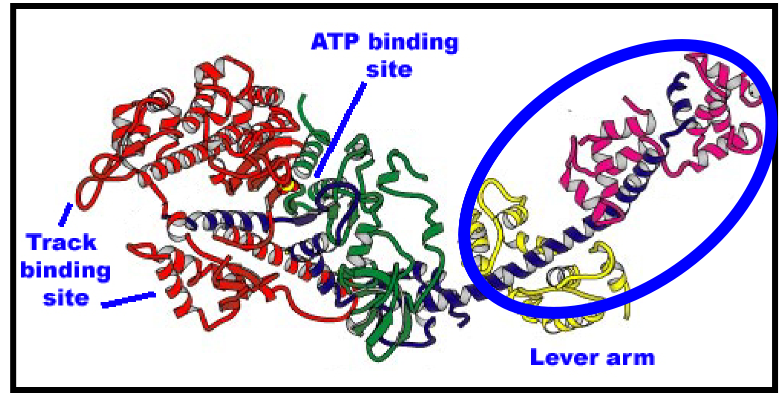
Lymn and Taylor *Biochemistry* 1971

Cohen – Rayment – Houdusse

AMPPNP

ADP.VO4

Myosin II MD + lever arm = 125 kDa



Rayment et al. *Science* 1993

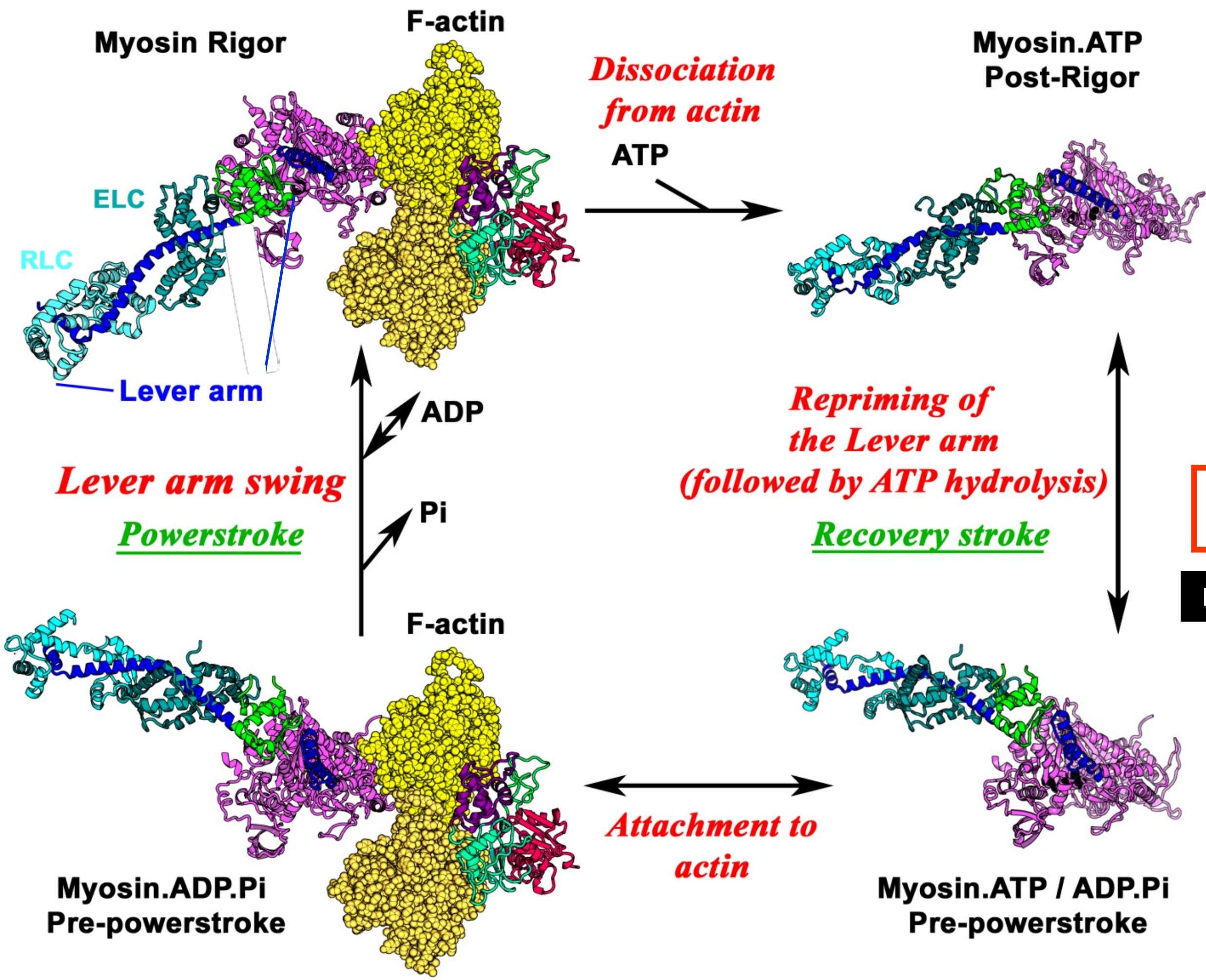
– Allostery –

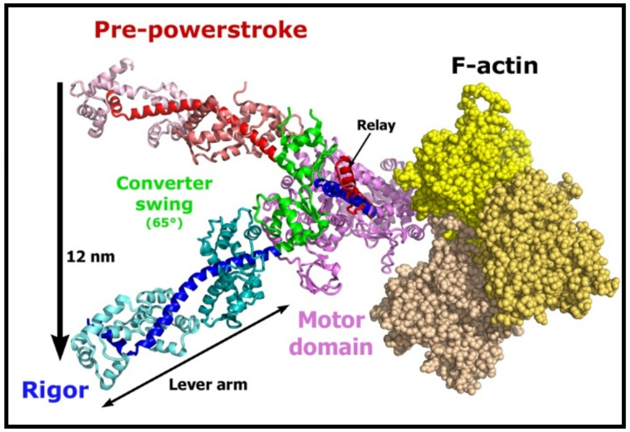
Communication across the enzyme at several nm distances

6 nm actin binding site / lever arm – 3.5 nm active site / lever arm

Changes in the three allosteric sites must be precisely synchronized

The simplified actomyosin cycle
How ATP hydrolysis is converted in mechanical energy





Swinging lever arm theory

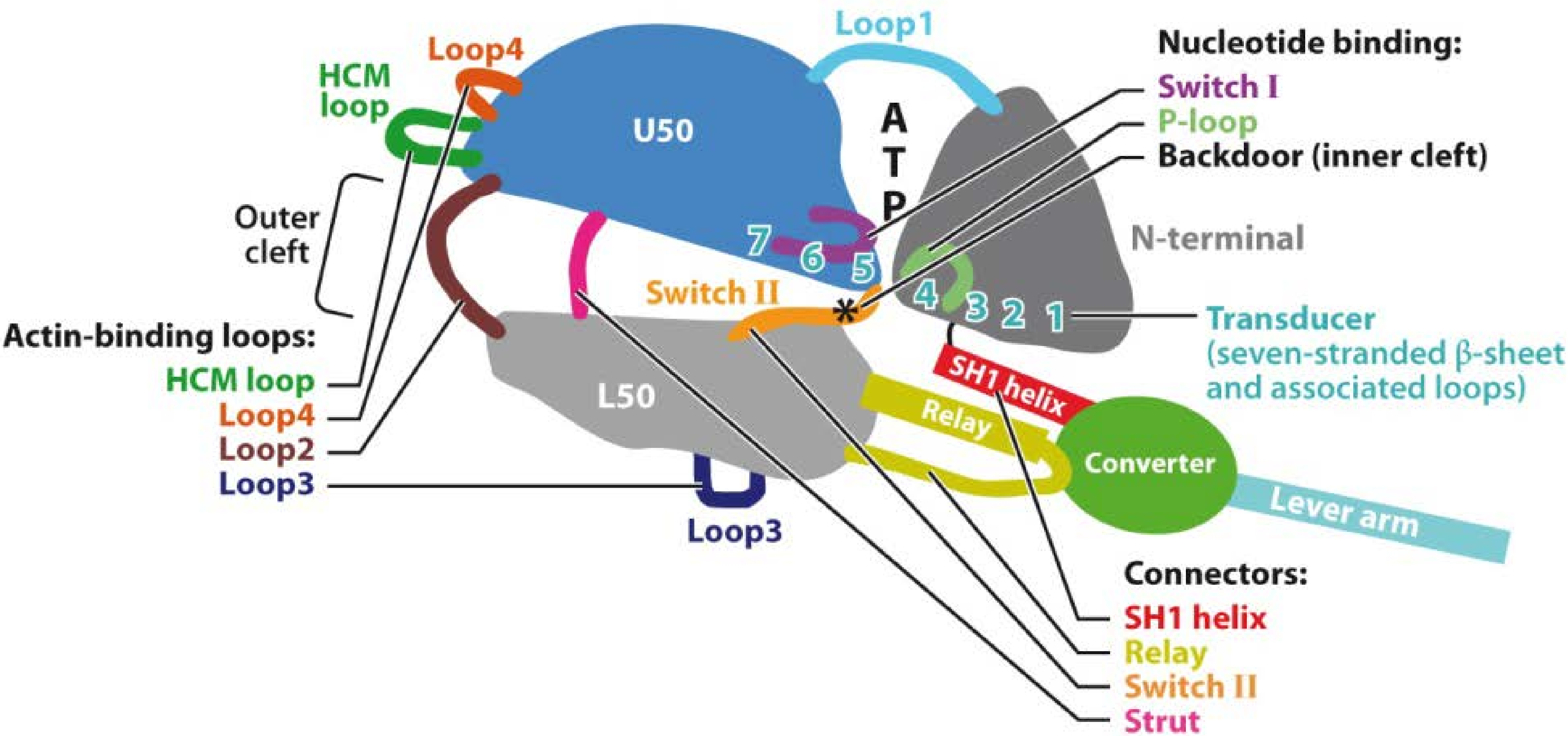
Holmes & Geeves 1999 *Ann Rev Biochem 68:687*

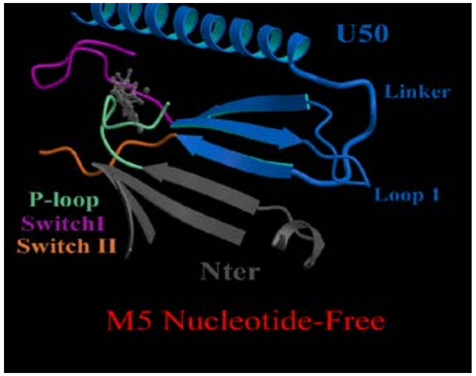
Rotation of the converter/lever arm

Principle of force production:
an extended lever arm amplifies the conformational changes of the MD

Long range pathways of communication
Within the motor

Connectors and subdomains in the myosin motor





Twisting of the central beta sheet controls cleft closure and actin affinity

Transducer

Coupling between nucleotide- and actin- binding sites

Detachment from the actin track upon ATP binding

Coureux et al. 2003 *Nature* 425:419-423.
Coureux et al. 2004 *Embo J.* 23:4527-37.

Cecchini, Houdusse, Karplus. *Plos Comput Biol* 2008.

N.F ➡ ATP

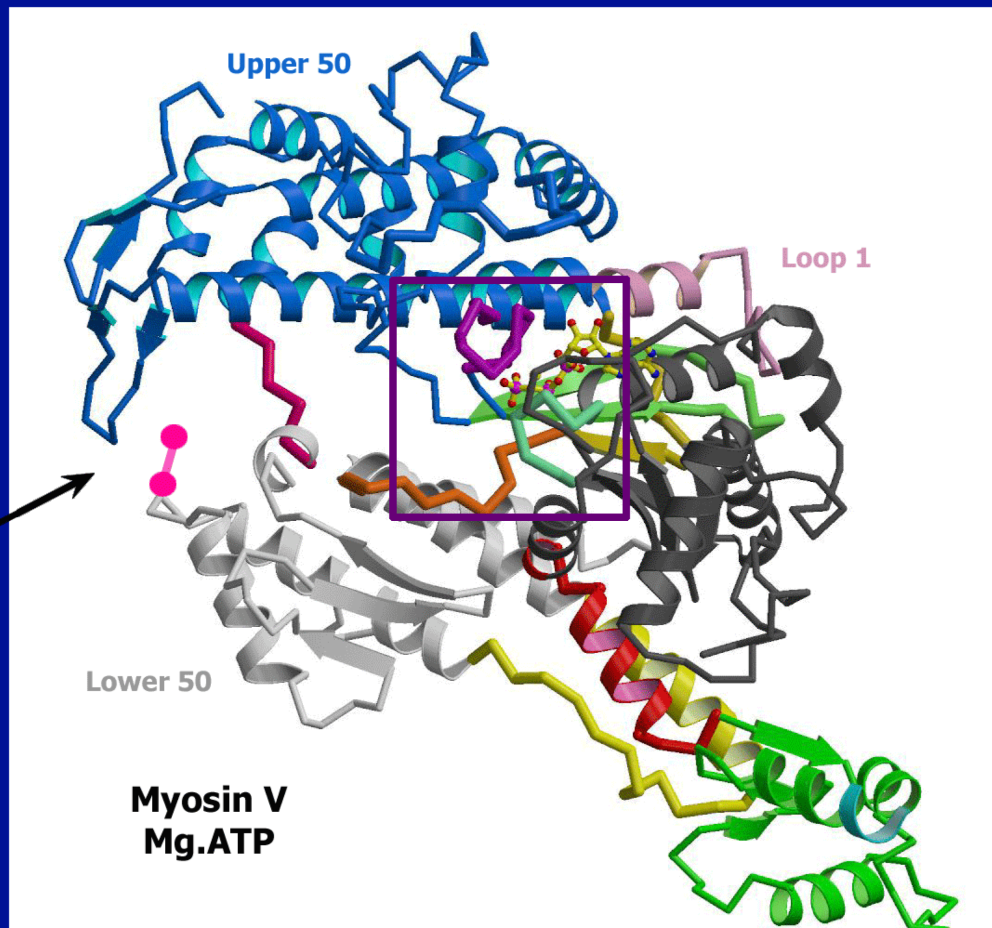
2-10 ms

Weak Actin binding Interface

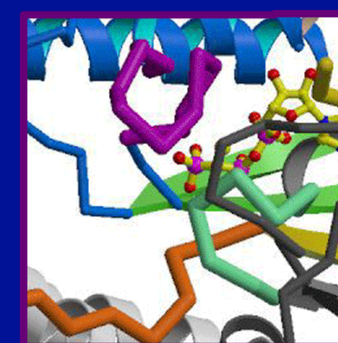
50 kDa Cleft

Open

Total cleft closure in the rigor state



Active Site



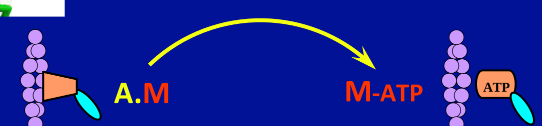
Switch I

near

Switch II

P-loop

These large scale rearrangements are largely encoded in the low-frequency normal modes of the motor. The transition requires local rearrangements combined with these vibration modes.

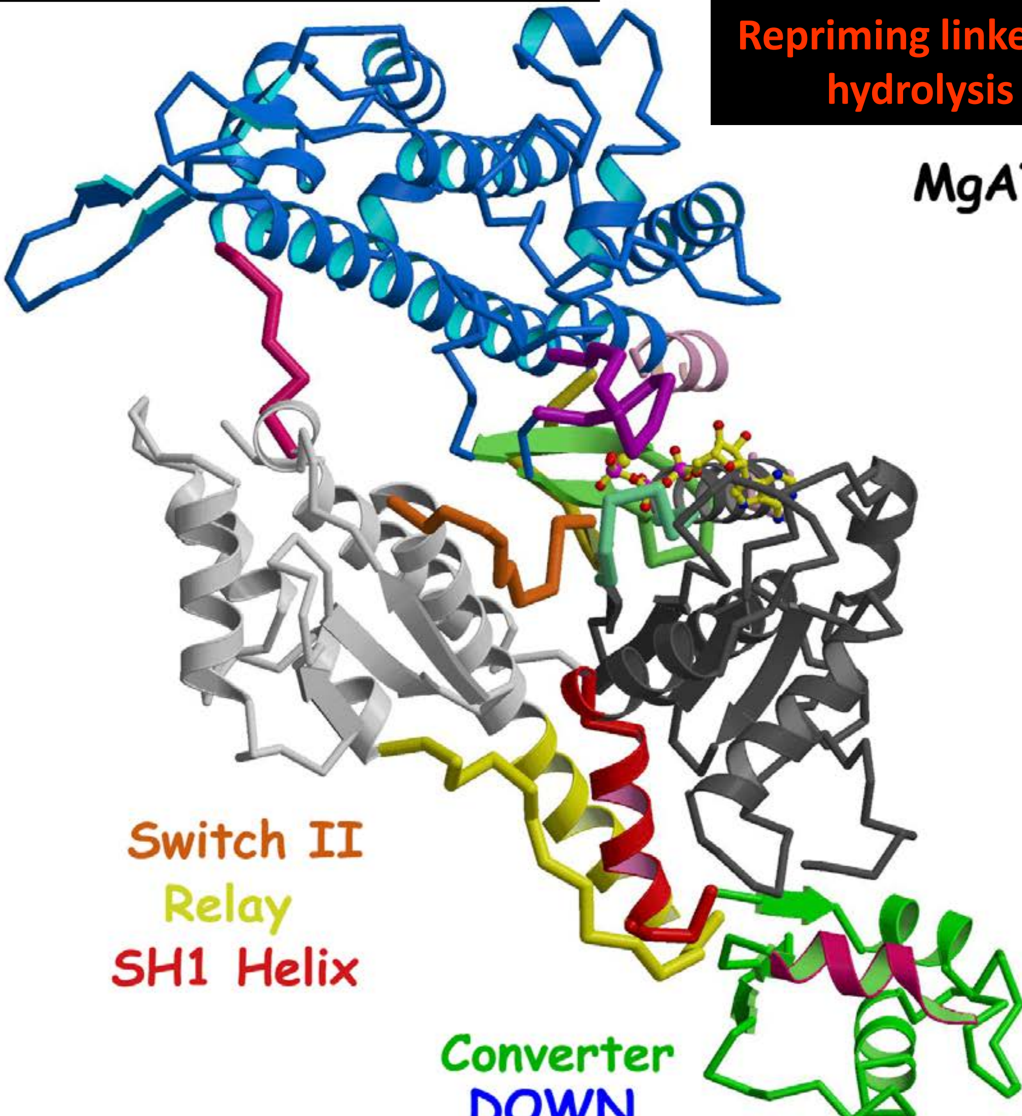


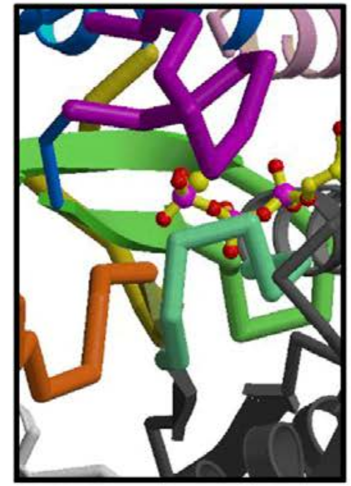
The recovery stroke promoting ATP hydrolysis

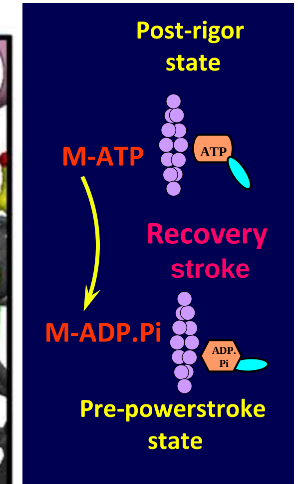
ATP ↔ ATP ↔ ADP.Pi

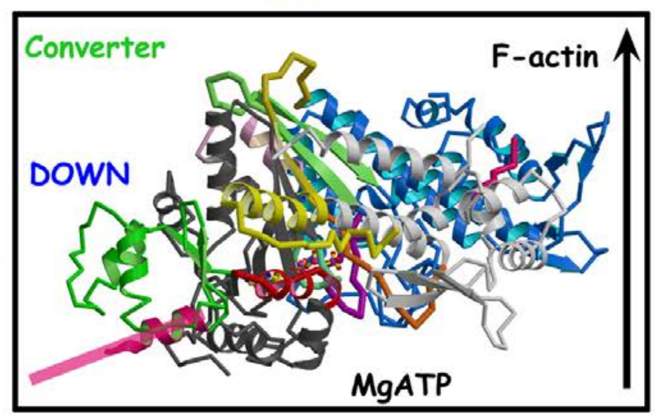
1-60 ms

Repriming linked to hydrolysis









OUTLINE

Myosin motors - force production, regulation, cellular roles :

What is currently known
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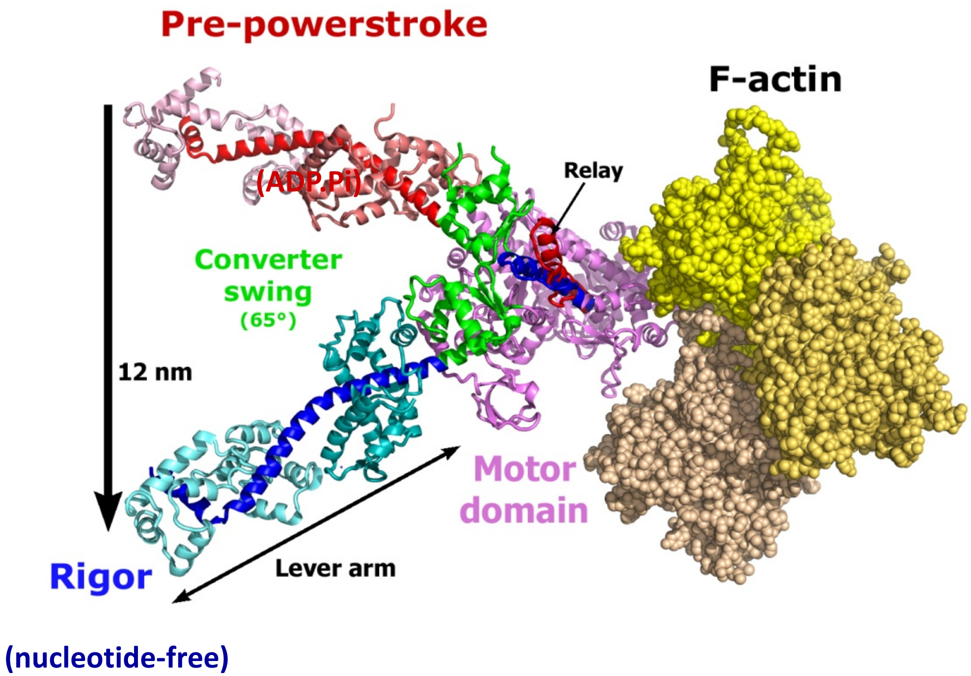
Testing the theory with a reverse motor

Current challenges

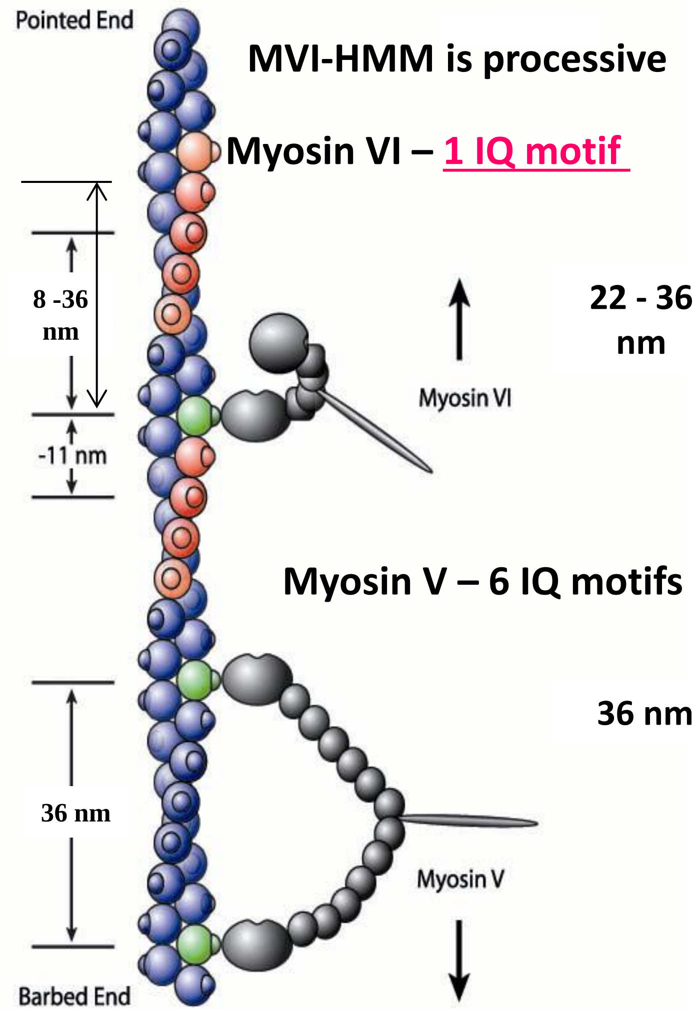
Directionality and step size

Holmes and Geeves Annu. Rev. Biochem. 1999



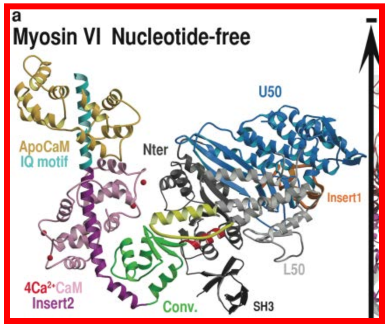
The swinging lever arm theory does not seem compatible with the step size of myosin VI

Rock *et al.* (2001) *PNAS USA* 98, 13655-9.



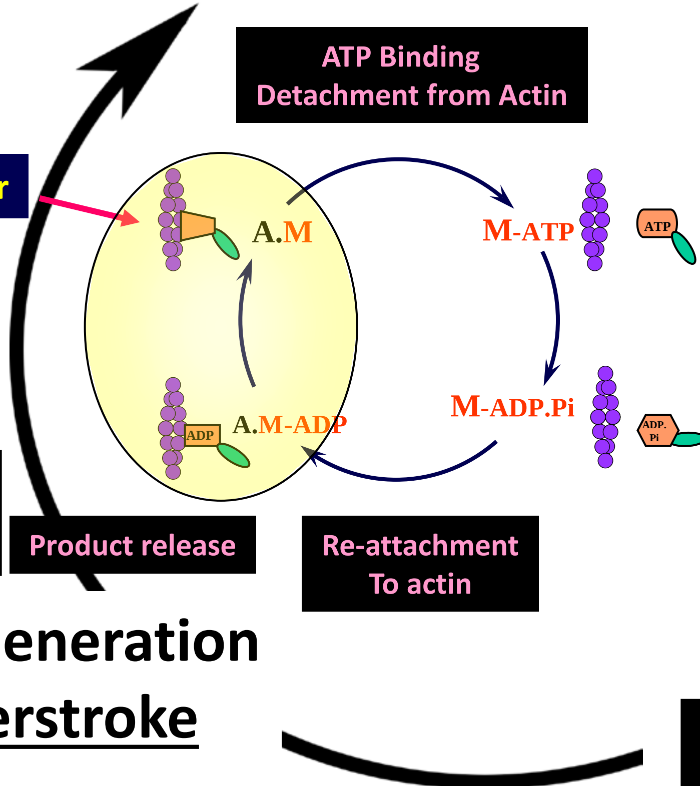
What are the structural elements that define the directionality of the force produced ?
What are the adaptations of the motor that allow to generate so big steps ?

Direct visualization of the M6 powerstroke



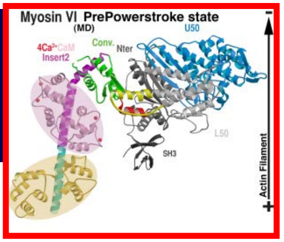
Ménétrey*, Bahloul* et al, *Nature* 2005

Post-stroke: nucleotide-free
2.4 Å resolution



ATP hydrolysis
Repriming of the Lever arm

Pre-Powerstroke (PPS)



Ménétrey*, Llinas* et al, *Cell* 2007

Pre-stroke: ADP.VO4
1.75 Å resolution

Identify the structural features that control directionality in this reverse motor.

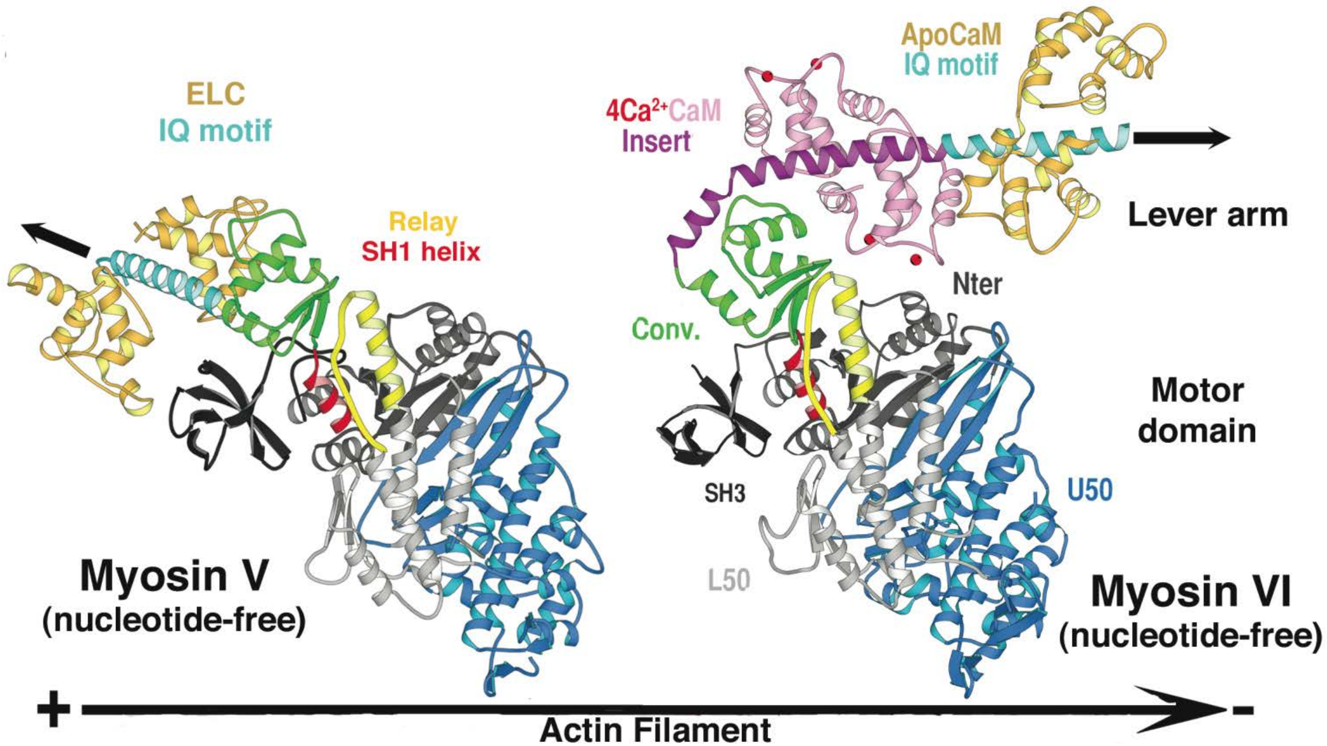
Understanding how this motor can produce a big stroke.

Directionality in myosin motors

Visualization of the conformation at the end of the stroke

The structure of the nucleotide-free state of the myosin VI motor reveals the mechanism of directionality reversal, Ménétrey J.*, Bahloul A.*, Wells A., Yengo C., Morris C., Sweeney H.L. and Houdusse A.

(Nature, 2005, 435 :779-85) * co-authors



Myosin V and Myosin VI at the end of Force Production

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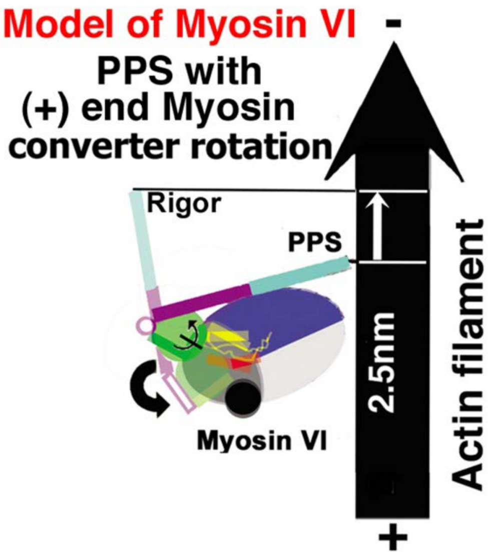
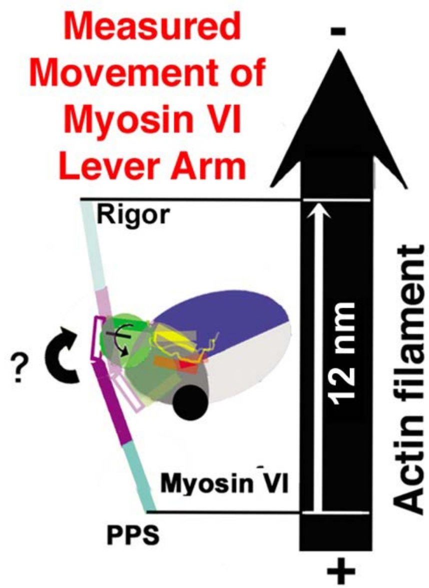
Ensemble, prenons le cancer de vitesse.

How myosin VI generates a big stroke

Myosin VI at the beginning of Force Production





Measured working stroke of 12 nm from single molecule studies

Rock et al. (2005) *Mol Cell* 17:603-9.

Only 2.5 nm predicted from modeling

Model based on the pre-powerstroke state observed for plus-end motors – just adding the insert/CaM after the converter

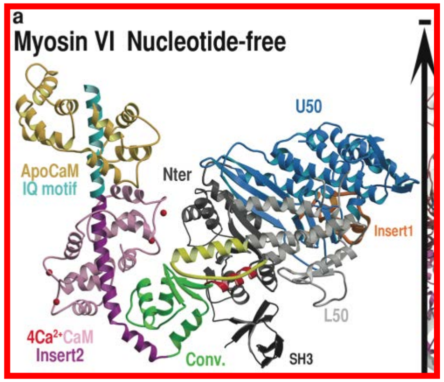
The Myosin VI Pre-power stroke must be different from that of other myosins !

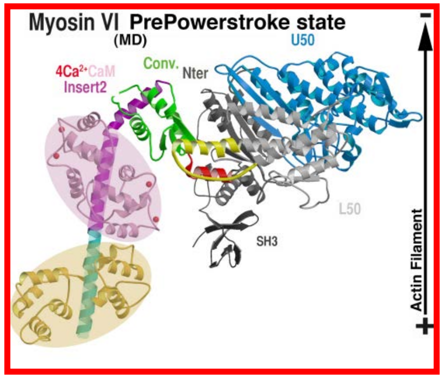
How myosin VI generates a big stroke

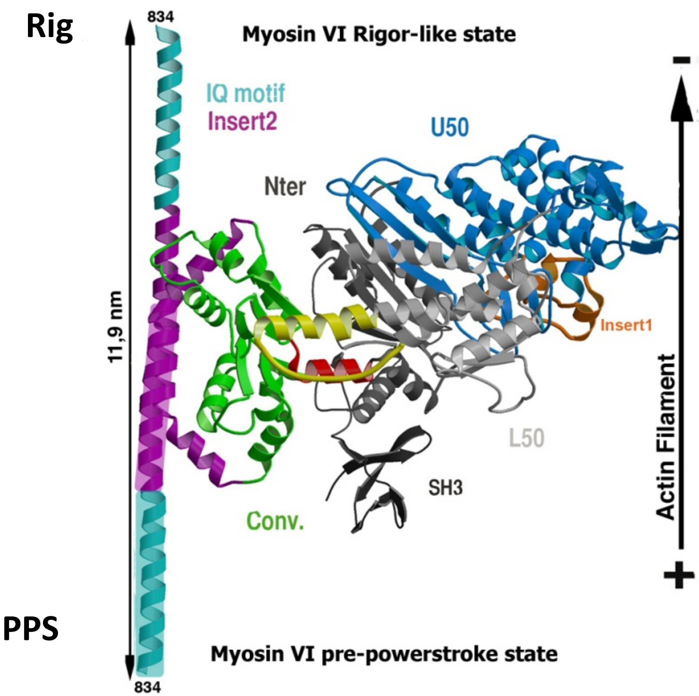
The structural basis for the large powerstroke of myosin VI.

Ménétrey J.*, Llinas P.*, Mukherjea M., Sweeney H. L. and Houdusse A.

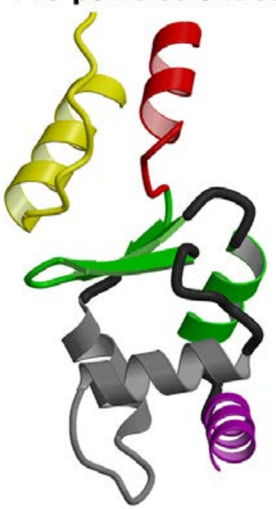
(Cell, 2007, 131:300-308) * co-authors







Pre-powerstroke state



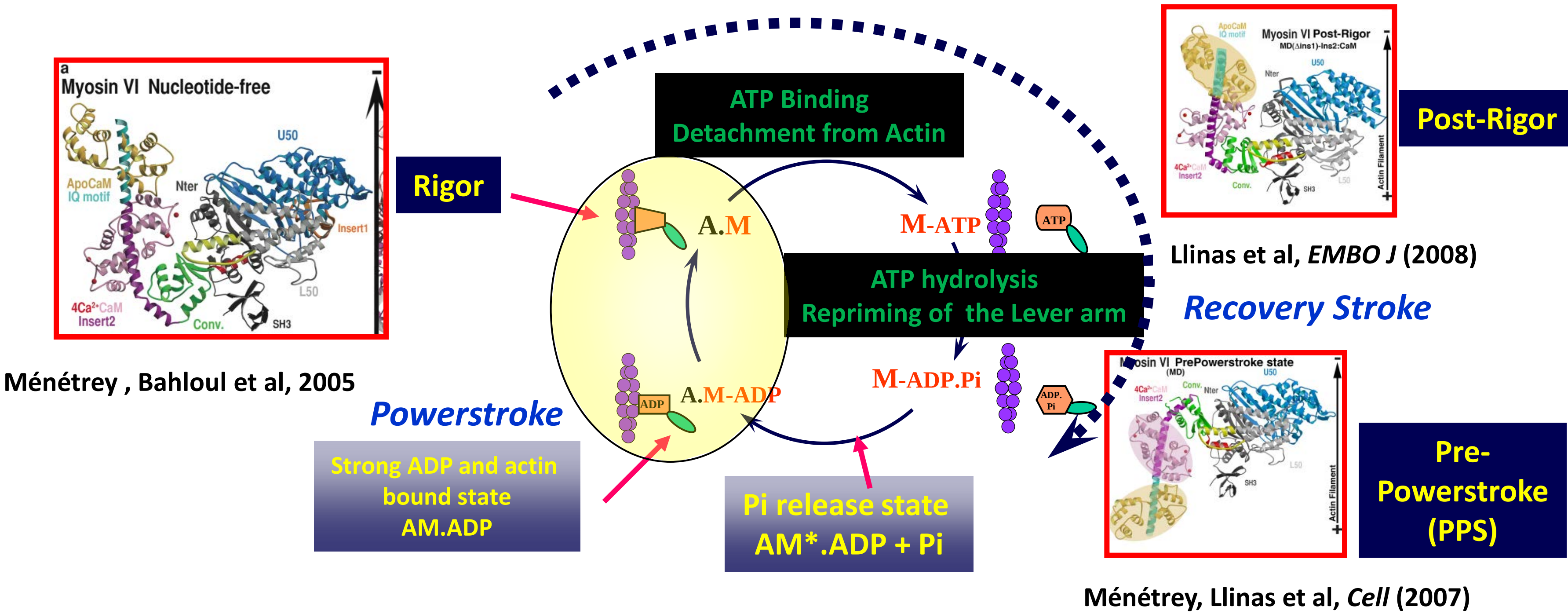
These two M6 structures predict a 180° reorientation of the lever arm parallel to the actin filament and a 12 nm stroke for the S1 fragment

cnrs dépasser les frontières

Penn University of Pennsylvania

institut Curie Ensemble, prenons le cancer de vitesse.

Three structural states of the M6 motor



These M6 structures show that the <u>swinging lever arm theory applies</u> to correctly predict the beginning and the end of the stroke for M6!

The communication pathway between the active site and the converter is conserved in minus-end motors.

OUTLINE

Myosin motors - force production, regulation, cellular roles :

What is currently known
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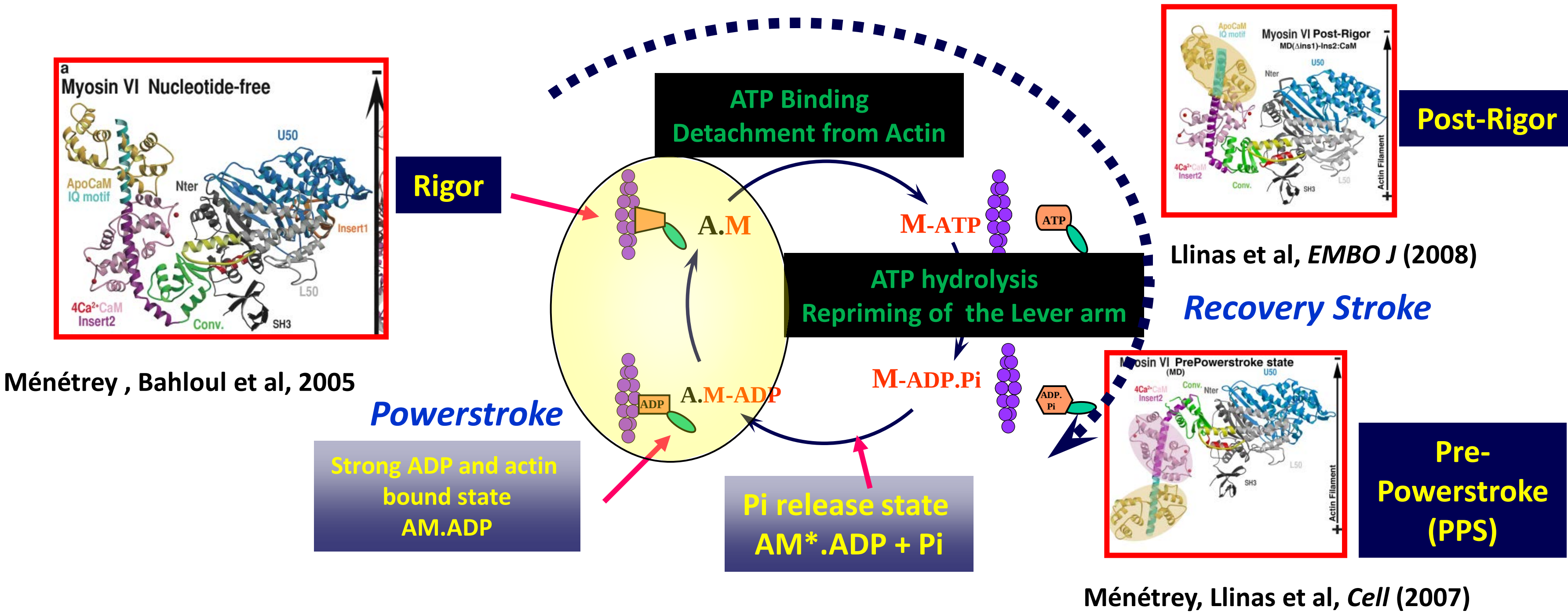
Current challenges

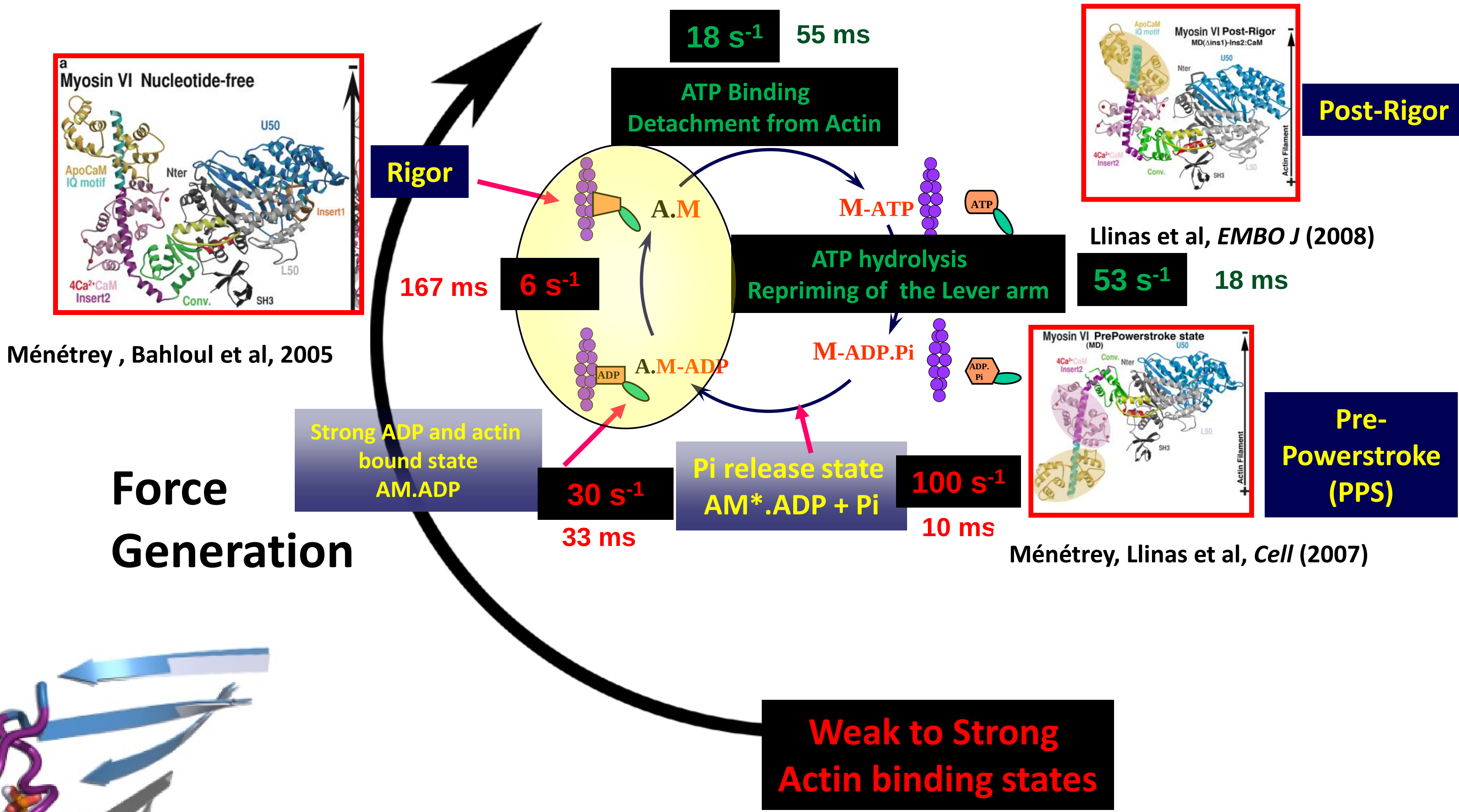
Three structural states of the M6 motor

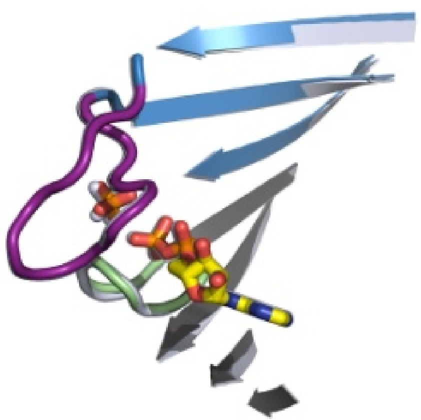


To be a motor, myosin must trap its hydrolysis products. Interestingly, the recovery stroke is reversible with ATP bound, but not with ADP.Pi bound. The dynamics in the myosin head is highly sensitive to the content of the active site.

Actin promotes a different type of rearrangement to release Pi.
The powerstroke is NOT a reversal of the recovery stroke !

How does myosin produce force





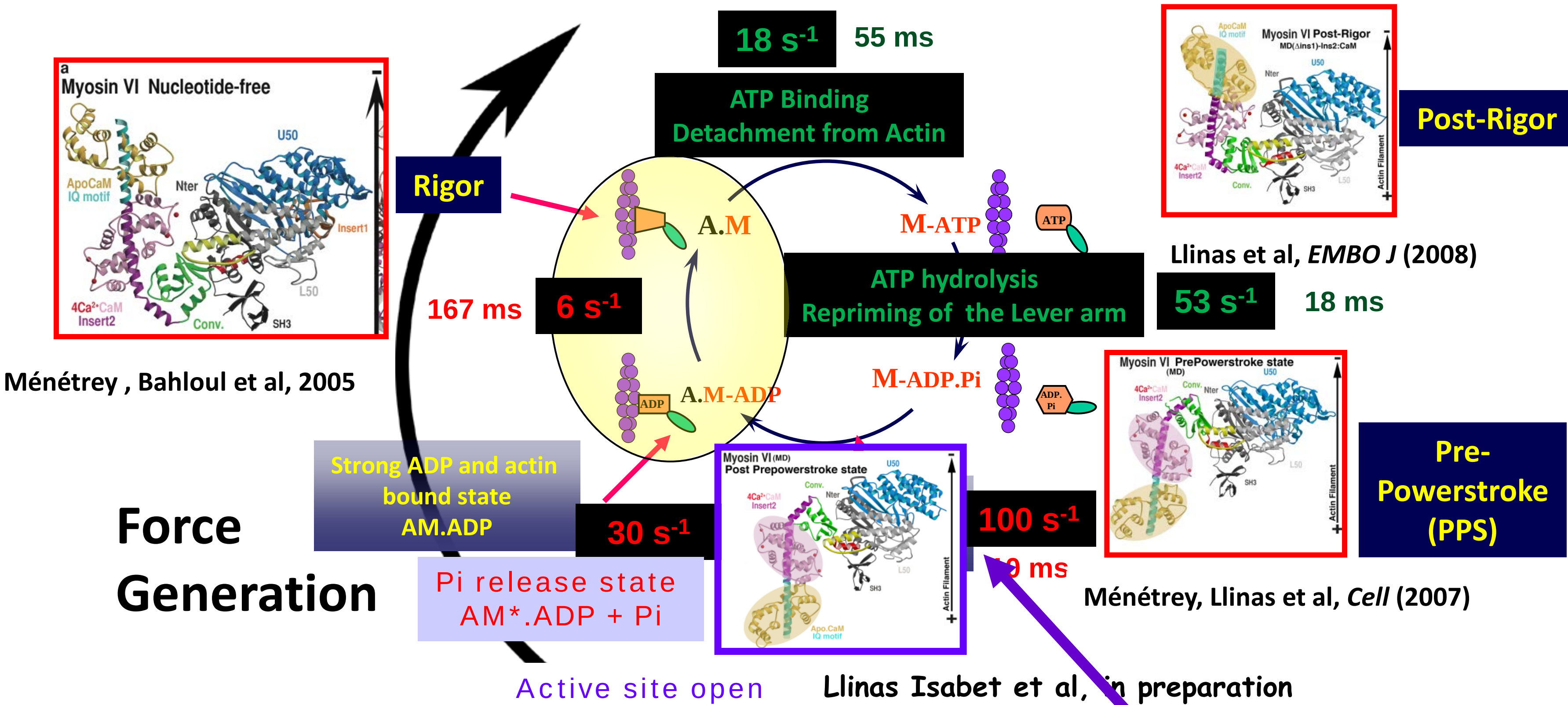
When does the cleft close and the transducer rearrange ?

When does the lever arm swing ?

Which transitions promote product release ?

How does myosin produce force

Pi release on actin



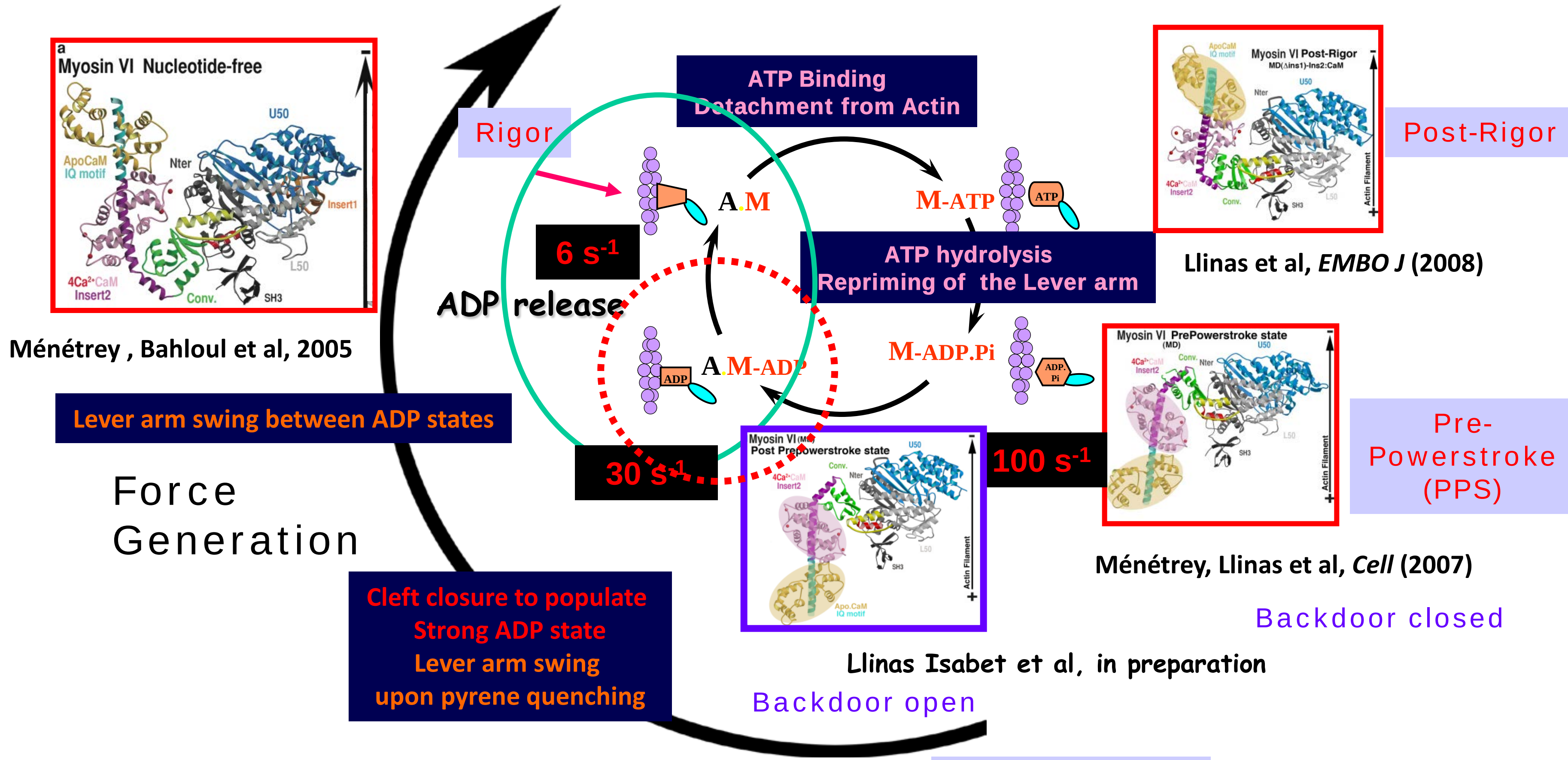
How myosin releases its Pi at the beginning of force production

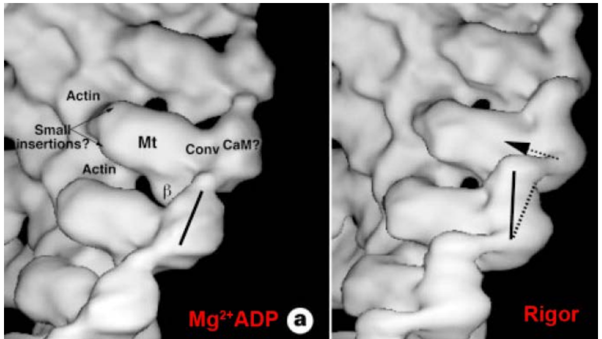
The Pi release state should have the active site open and the lever arm primed

This state should have a higher affinity for actin than PPS but not as high as AM.ADP or AM

This state should promote commitment of the motor on actin

The mechanism of Force Generation by myosin motors





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Structural Motility Group

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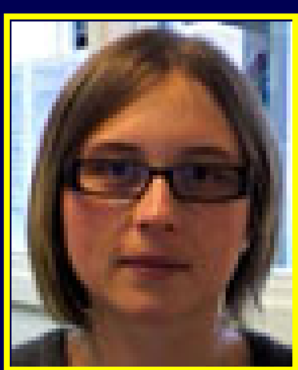
Hannah Benisty
Anne Houdusse



ANR AGENCE NATIONALE DE LA RECHERCHE

Special Thanks

ESRF

SOLEIL SYNCHROTRON

Protein Expression Functional Assays

Allan B. Zhong
Xiaoyan Liu
Li-Qiong Chen
Dan Safer
Lee Sweeney

Computational Studies

Marco Cecchini
Martin Karplus