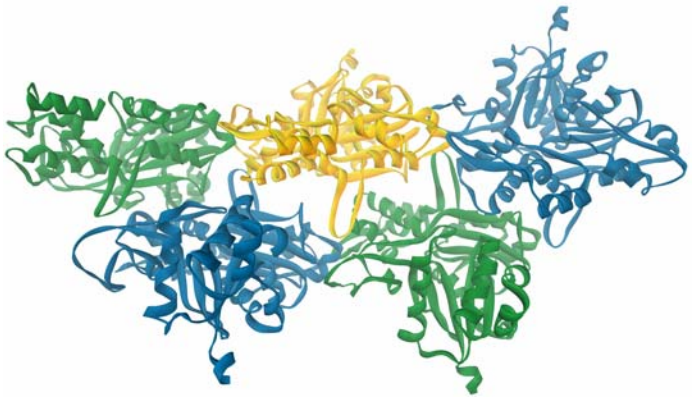
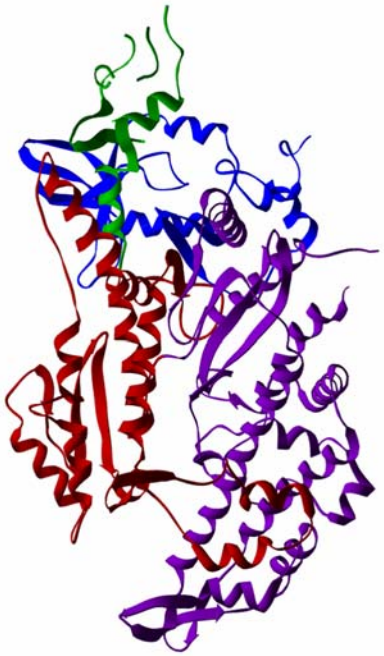
Introduction: actin and myosin

Actin



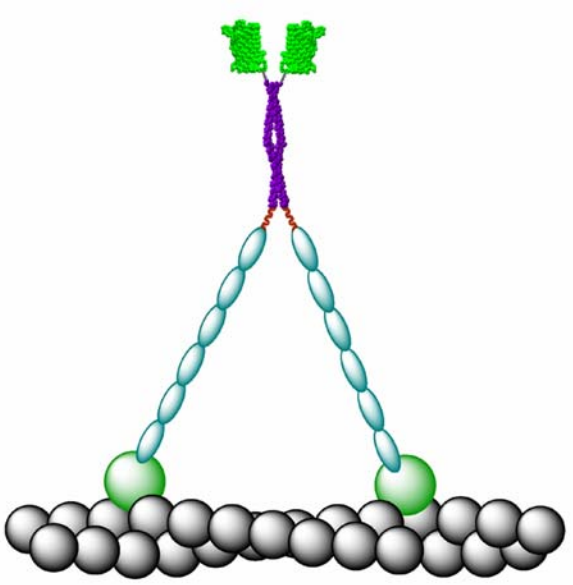
- 375 residues
- Found in all eukaryotes
- Polymeric
- Forms track for myosin
- Many other cellular functions
- 36 nm pseudo-helical repeat

Myosin



- Catalytic domain: ~750 res.
- Entire protein: ~1800 res.
- Binds 2-6 light chains (calmodulin)
- Found in *almost* all eukaryotes
- Hydrolyzes ATP to generate motion

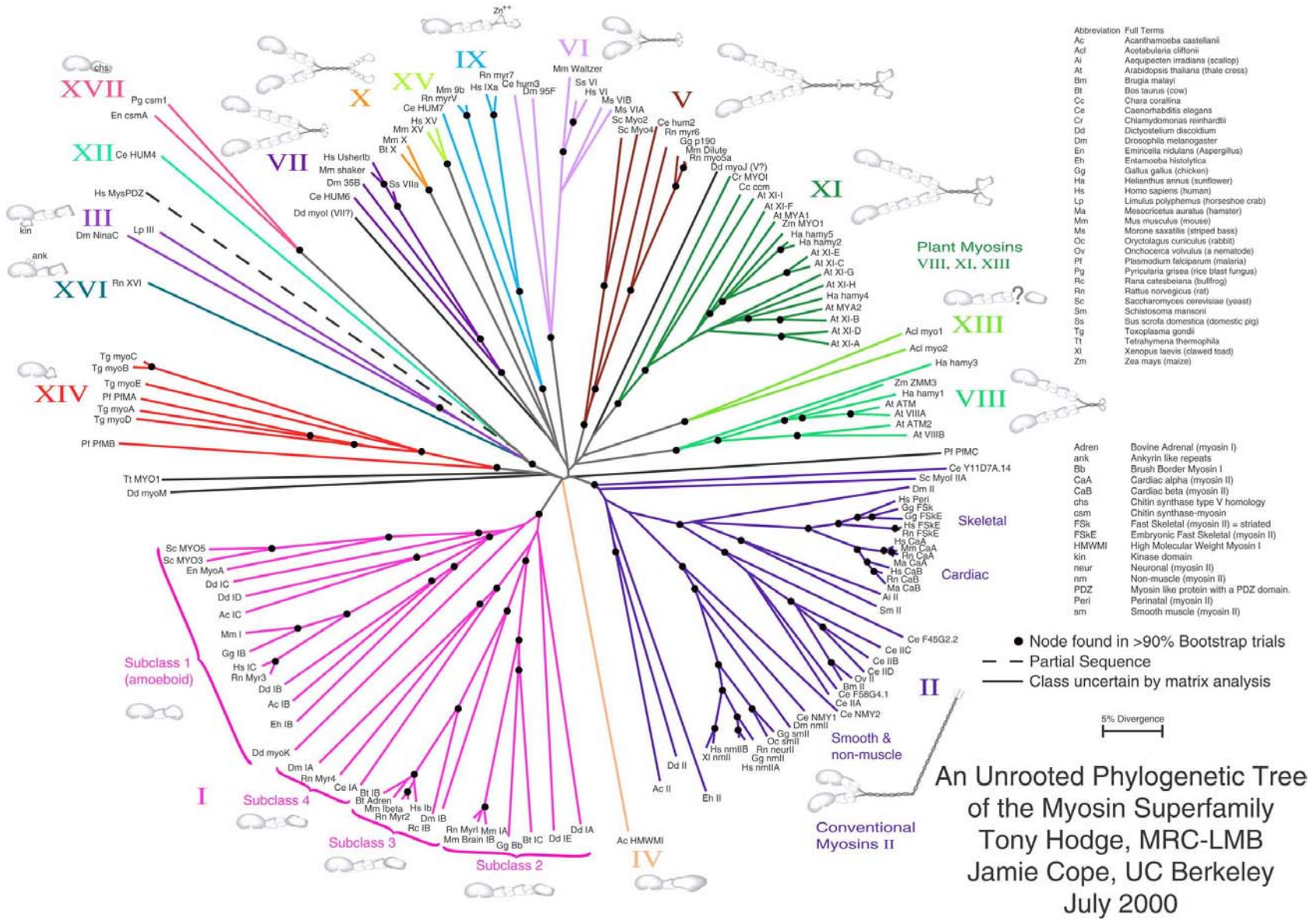
Myosin V and actin



- Myosin V: 6 light chains, coiled-coil domain, and globular tail domains
- Lever arm swing generates motion
- Other myosins function as monomers, dimers, or filaments

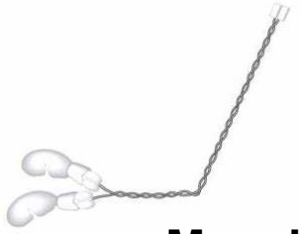
Alex Dunn, Sept. 2005

Myosin evolution



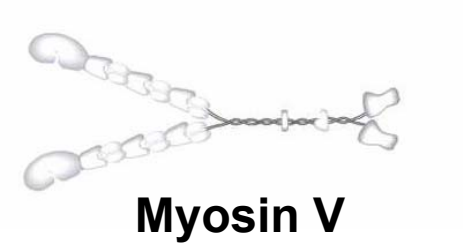
Hodge *et al. J. Cell Sci.* **2000**, *113*, 3353. Richards *et al. Nature*, **2005**, *436*, 1113.

Characterized myosins



Myosin II

- "Conventional" myosin by default.
- The myosin in skeletal muscle.
- Also responsible for pinching off cell during cytokinesis.
- Commonly studied myosin II varieties polymerize into filaments with thousands of heads.
- Binds 2 light chains.
- Cardiomyopathy mutations, also congestive heart failure, cancer.



Myosin V

- Moves cargo around cell: vesicles, RNA.
- Binds 6 light chains, and forms a tight dimer.
- Walks hand-over-hand, with many consecutive steps before detaching.
- 36 nm step: 1 actin pseudo-repeat.
- Rare hereditary disease.

Myosin VI

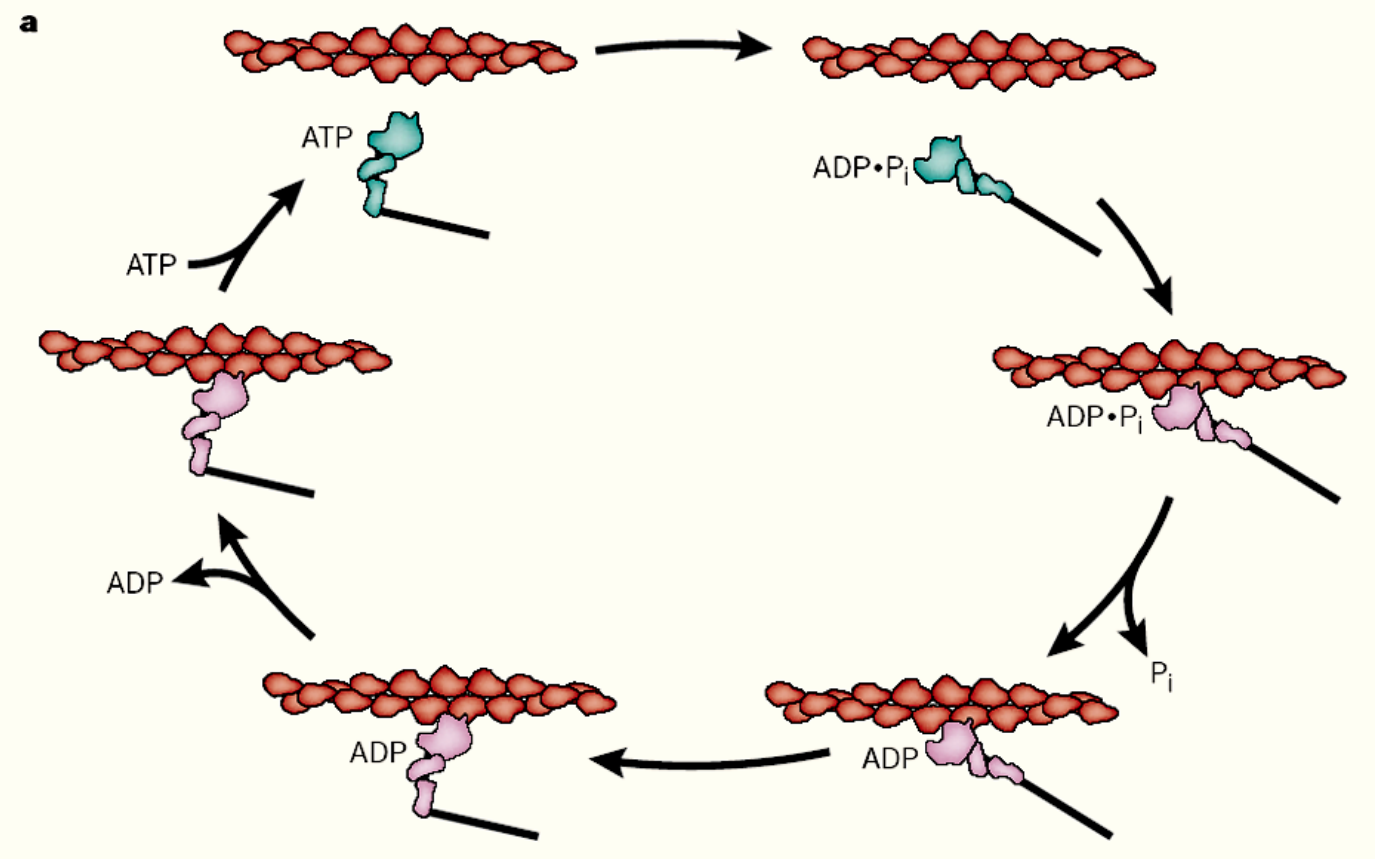
- Moves cargo around cell.
- Important in inner ear hair cell formation.
- Walks in the opposite direction of other myosins, toward (-) or pointed end of the actin filament.
- *Probably* functions as a dimer, but dimerizes weakly by itself.
- Binds only 2 calmodulins, but dimeric constructs take 36 nm steps.
- Rare hereditary deafness

Lever arm hypothesis

Proposed by Hugh Huxley, 1969

Supported by single molecule spectroscopy, x-ray crystallography, FRET, electron microscopy

Consensus model. Dissenting model proposed by Yanagida: Tanaka, *Nature*. **2002,** *415,* 192.

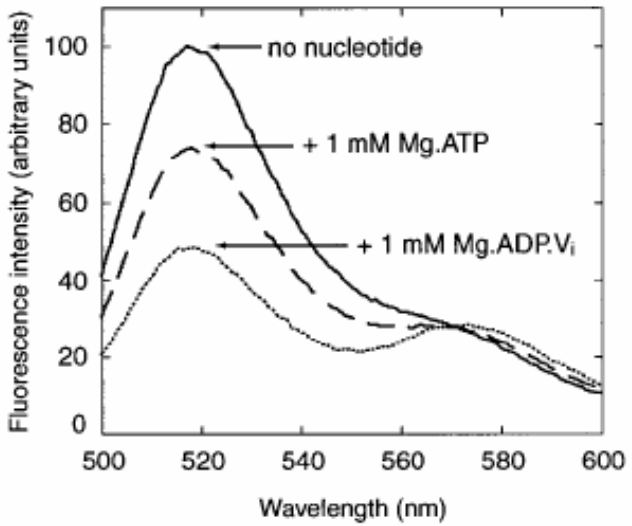


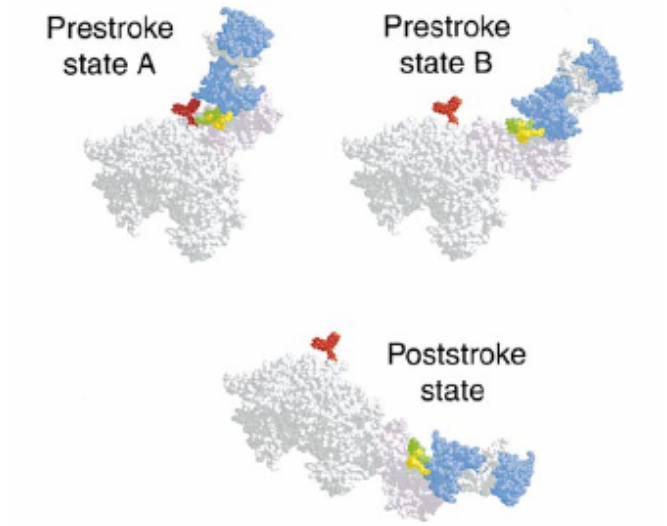
Spudich, J.A. *Nature Rev. Mol. Cell Biol.* **2001**, *2*, 387.

Purcell, *et al. Proc. Natl. Acad. Sci. USA* **2002**, *99*, 14159.

Example experimental data

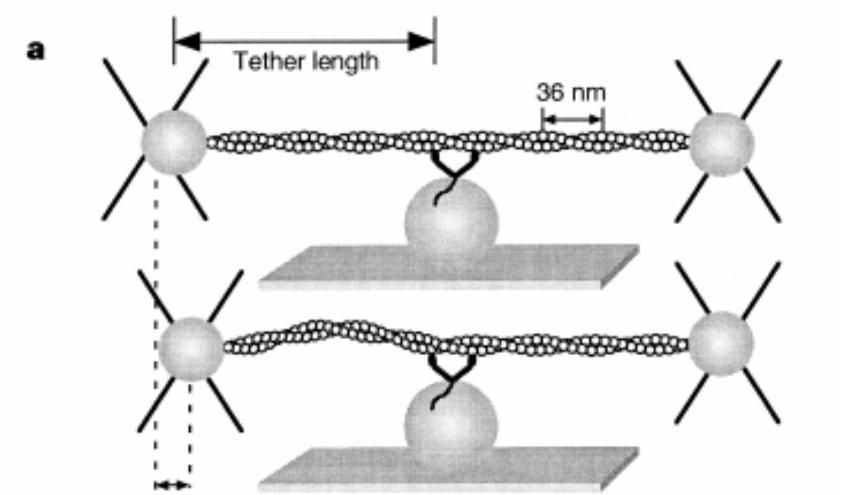
FRET

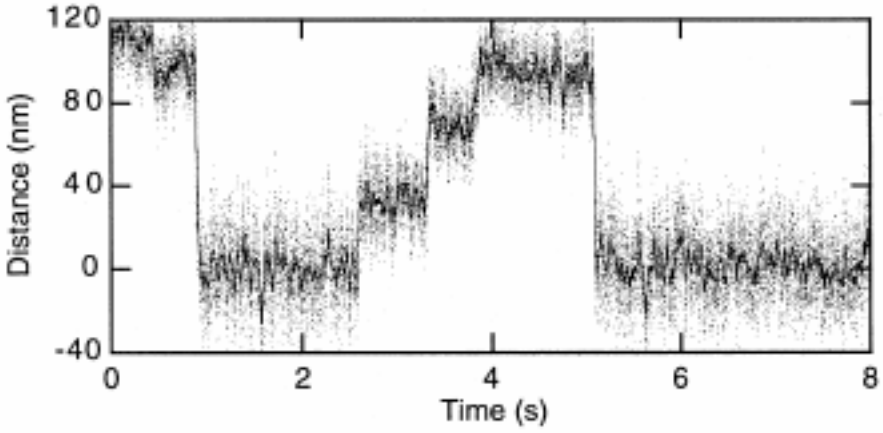




Shih, *et al. Cell*, **2000**, *102*, 683.

Optical Trapping

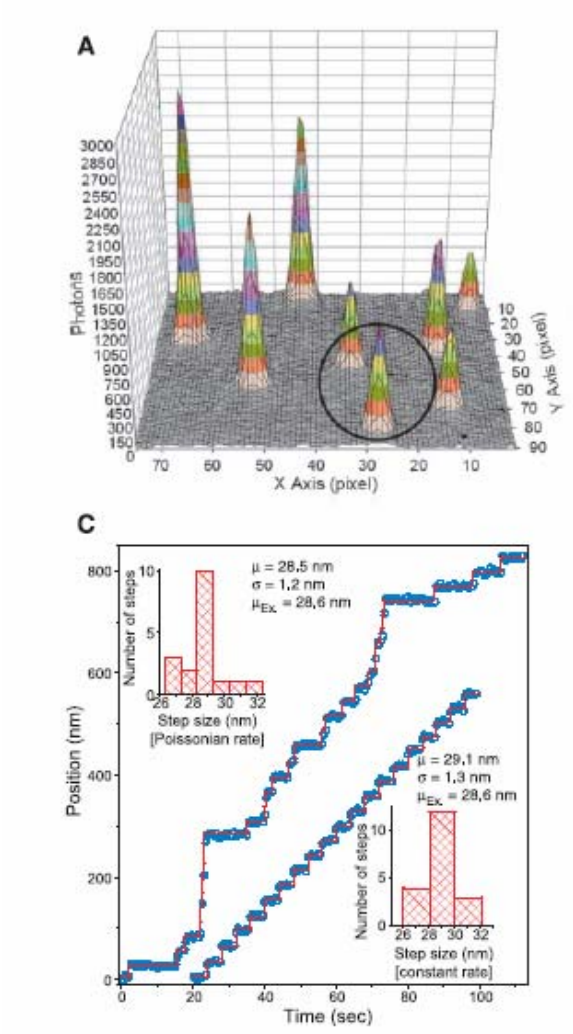




Mehta *et al. Nature*, **1999**, *400*, 590.

Example experimental data

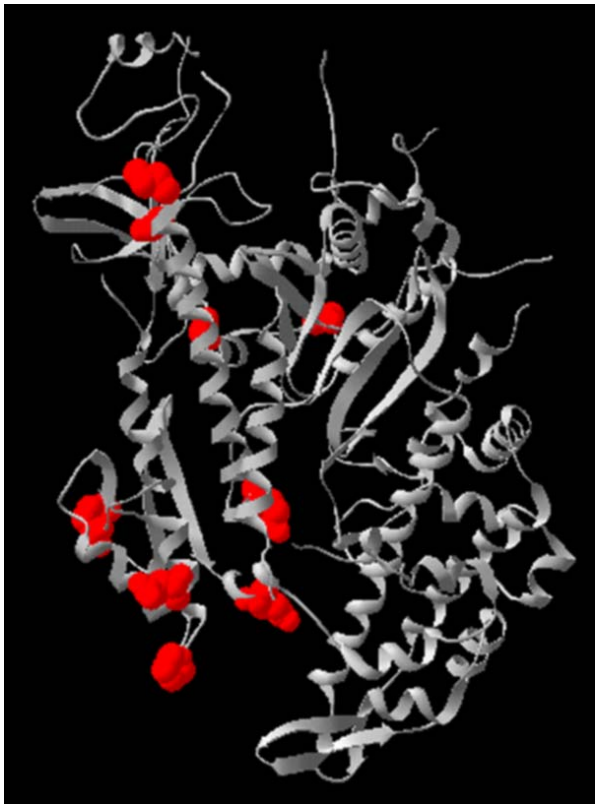
Single molecule Fluorescence



Yildiz *et al. Science* **2003**, *300*, 2061.
Ökten *et al. Nature Struct. Mol. Biol.* **2004**, *11*, 884.

Random or directed mutations

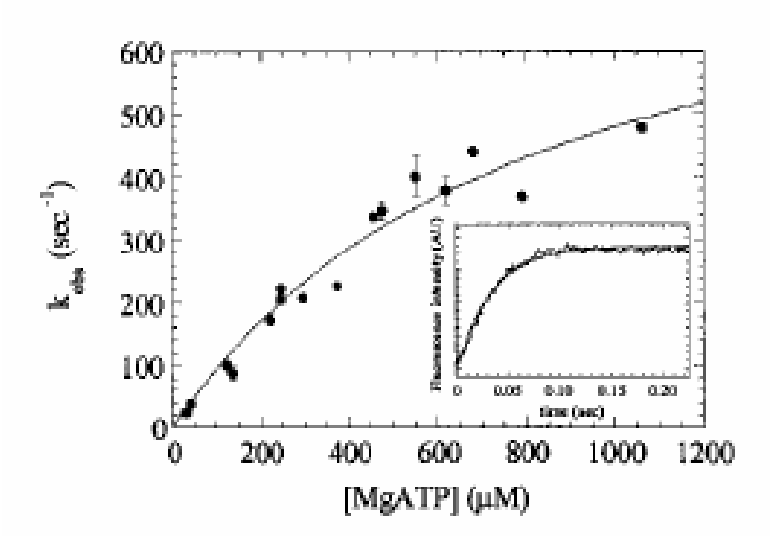
Below: result of a screen for *Dicty* mutants with temperature-sensitive myosin function.



Patterson, *et al. Genetics* **1996**, *143*, 801.
Sasaki *et al. Biochemistry* **2003,** *42,* 90.

Example experimental data

Bulk kinetics



Steady-state		ATP binding and hydrolysis	
V_{max} (+ actin)	15 s^{-1}[b]	$K_1' k_2'$	0.9 $\mu M^{-1} \cdot s^{-1}$[f,g]
V_{max} (+ actin)	12 s^{-1}[c]	k_2'	870 s^{-1}[f]
v_0 (− actin)	0.03 s^{-1}[b]	$K_1 k_2$	1.6 $\mu M^{-1} \cdot s^{-1}$[g,h]
v_0 (− actin)	0.02 s^{-1}[d]	k_2	≥750 s^{-1}[h]
K_{ATPase}	1.4 μM[b,e]	$k_{+3} + k_{-3}$	>250 s^{-1}[i]
		$k_{+3} + k_{-3}$	750 s^{-1}[h]
		k_4'	>250 s^{-1}[i]

Other techniques

- Cryo-electron microscopy
- Muscle fiber pulling
- Fiber x-ray diffraction
- EPR spin labels
- Mass spectrometry
- Crystallography (next slides)

De La Cruz, *et al. Proc Natl. Acad. Sci. USA* **1999**, *96*, 13726.
and many, many others…

Known myosin conformations

1) Pre-stroke

Smooth muscle ADP BeF_x, AlF_x: 1BR2

Dicty ADP VO_4: 1VOM

Scallop ADP VO_4: 1QVI

- Lever arm cocked, pre-stroke
- Cleft Open

2) Rigor-like

Myosin V no nucleotide, ADP: 1OE9

- Lever arm post-stroke
- Closed cleft consistent with cryo-EM structures of Actin-myosin complex.

Dicty-GTPase fusion*, no nuc.: 2AKA

- Not as “closed” as 1OE9

3) Post-Rigor

Scallop no nucleotide: 1KK7

Dicty ADP: 1MMA

Myosin V ADP BeF_x: 1W7J

- Lever arm post-stroke
- Cleft open: after release from actin, but before ATP hydrolysis

Post-post-stroke state (?)

Scallop ADP extended “past” rigor: 1B7T

- Lever arm swung past post-stroke angle.
- Cleft open
- Significance uncertain.

* Dicty myosin fused to a GTPase to get the GTPase to crystallize. Novel myosin structure was fortuitous, and is only observed with the fusion. However, it is structurally similar to 1OE9.

Myosin parts list

Large Domains

N-terminal

Contacts coverter in post-stroke state (?)

Upper 50 kD domain

Binds, hydrolyzes ATP

Lower 50 kD domain

Binds actin

Converter

Fulcrum, connects to lever arm

Lever Arm

Amplifies motion, binds light chains

Smaller Elements

Switch I loop

Communicates actin binding state to nucleotide pocket (?)

Switch II loop

Communicates nucleotide state to lever arm

P-loop

ATP binding and hydrolysis (?)

Relay Helix

Undergoes large conformational shifts between pre, post-stroke states

SH1 and SH2 helices

Communicate nucleotide state to lever arm

Switch I and Switch II

Switch I

Closed = bound nucleotide, Mg^{2+}

Open = no nucleotide

- Alanine scanning shows switch I is essential for in vitro function

Shimada *Biochemistry* **1997,** *36,* 14037.

- Structures of apo Dicty and chicken myosin V *cited as* showing switch I in an open conformation.

Reubold, *Nature Struct. Biol.* **2003**, *10* 827.
Coureux *EMBO J.* **2004,** *23*, 4527.

In the manuscript Coureux *et al.* say that active site changes due to P loop, not switch I.

- Communication between switch I and the upper 50 kD (actin binding) domain (?)

Switch II

Closed = pre-stroke and rigor

Open = post-rigor

- Mutagenesis shows switch II is essential for both ATP hydrolysis and communication with the lever arm.

Sasaki, *J. Biol. Chem.***1998**, *273*, 20334.
Murphy *Nature Cell Biol.* **2001**, *3*, 331.

- Best example: scallop structures show switch II in open, closed conformations.

Gourinath *Structure* **2003**, *11*, 1621.
Houdusse *Cell* **1999**, *97*, 459.

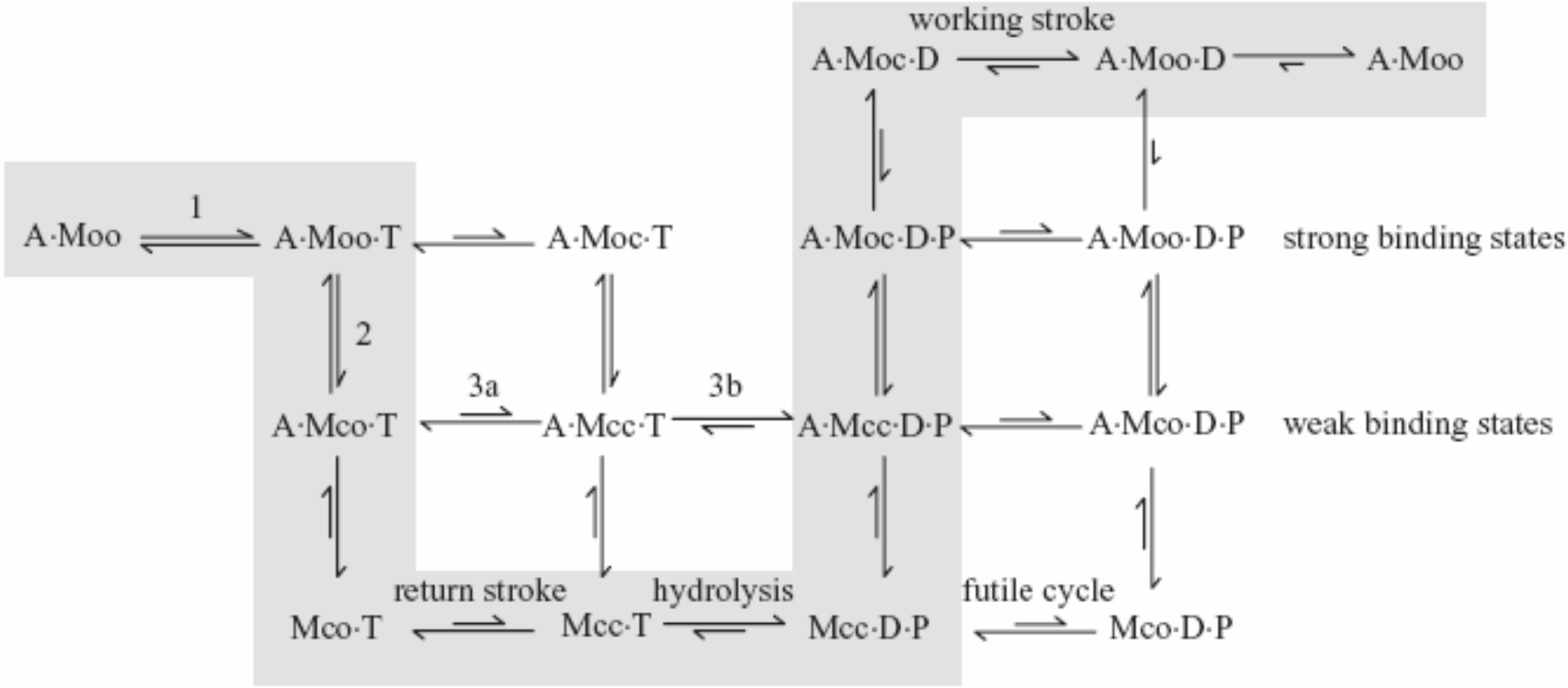
Bagshaw group tryptophan fluorescence papers (Switch I, II)

Zeng *Phil. Trans. R. Soc. B* **2004** *359*, 1843.
Malanasi-Csizmadia *J. Mus. Res. Cell Motil.* **2005** online ed.

One detailed (among several) myosin mechanism

A = Actin
M = Myosin
o = open (switch I, switch II)

T = ATP
D = ADP
P = phosphate



Zeng *Phil. Trans. R. Soc. B* **2004** *359*, 1843.

The order of the power stroke, Pi release, and Mg^{2+} release

1) Multiple myosin ADP/actin states can be kinetically distinguished

   - Rosenfeld, *J. Biol. Chem.* **2000**, *275*, 25418.

2) Pi diminishes force generation

   - (Millar & Homsher 1990; Dantzig *et al.* 1992; Walker et al. 1992)

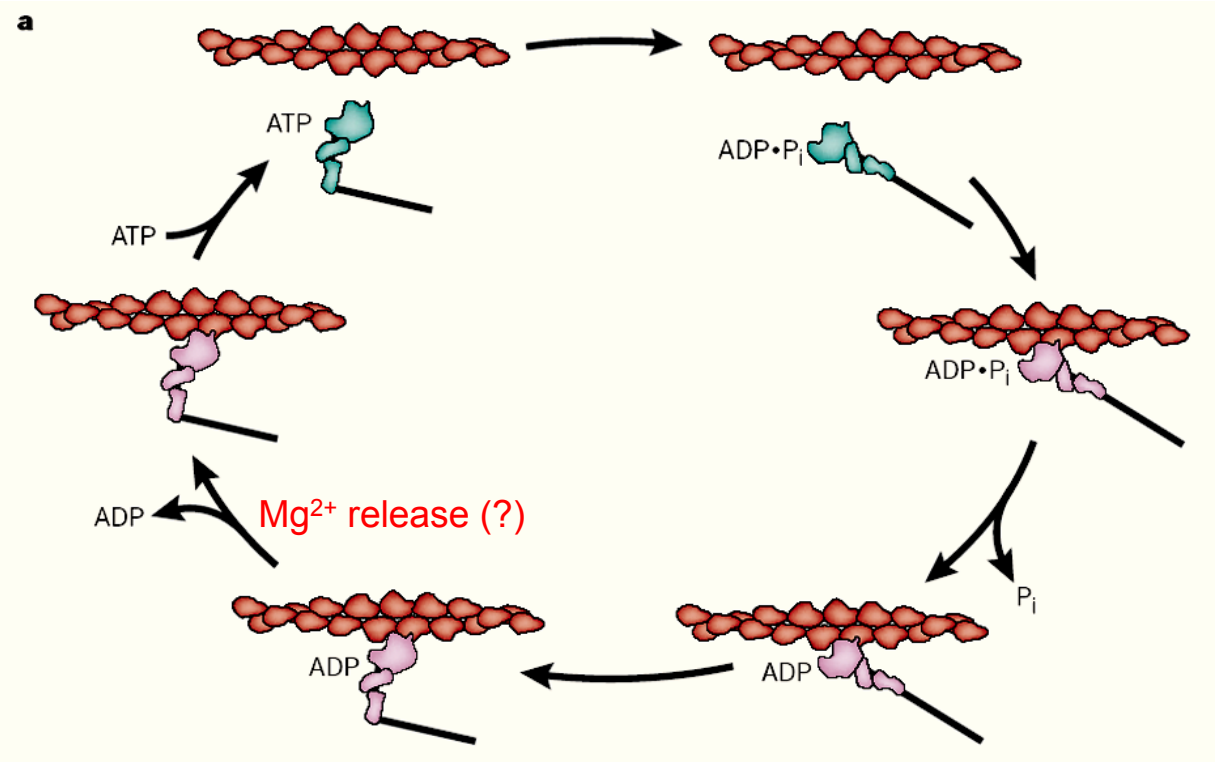
3) The initial interaction between myosin and actin is weak and salt sensitive

4) ADP release is rate limiting

   - Siemankowski et al. 1985

5) Recent results: Mg^{2+} release weakens the myosin ADP complex.

   - Rosenfeld *J Biol. Chem.* **2005**, *280*, 6072.



power stoke *may* begin with Pi release

Refs. in parentheses from: Takagi *et al. Phil. Trans. R. Soc. B 2004 359*, 1913.

Myosin thermodynamics

1) ATP binding is a large source of potential energy: $K_d \sim 10^{-10}$ M, ~60 kJ/mol.

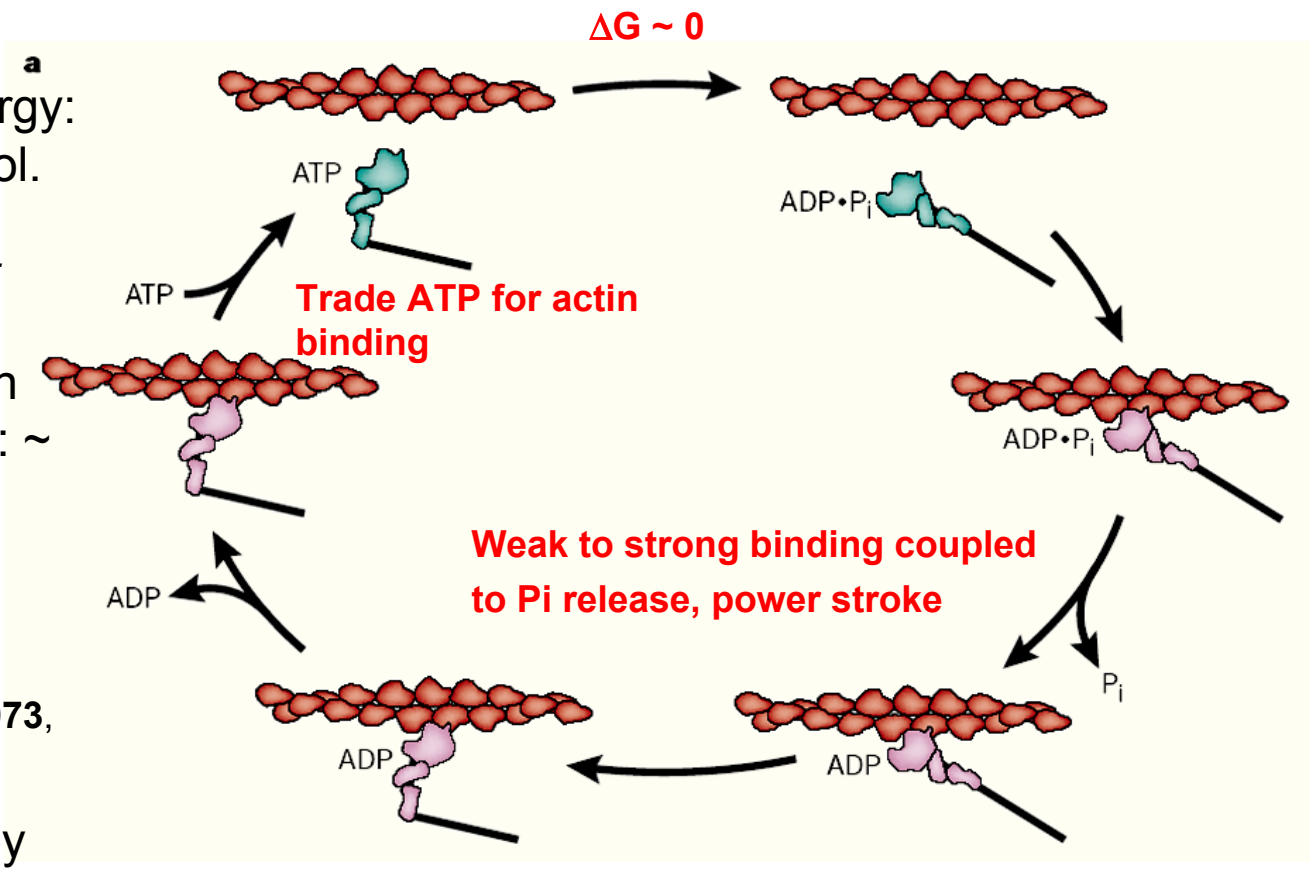
Goody *et al. Eur. J. Biochem.* **1977,** *78,* 317.

2) The post-stroke myosin binds actin quite tightly: ~ 30 kJ/mol.

3) ATP hydrolysis is reversible: ~ 0 kJ/mol

Bagshaw *et al. Biochem J.* **1973**, *133*, 323.

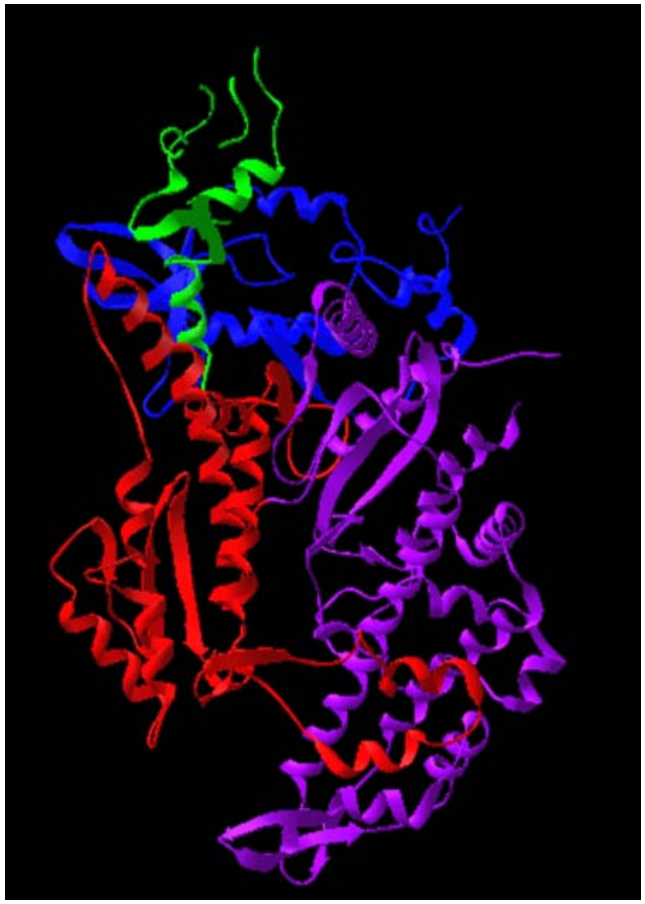
4) Maximum work done by myosin: 30-40 kJ/mol.

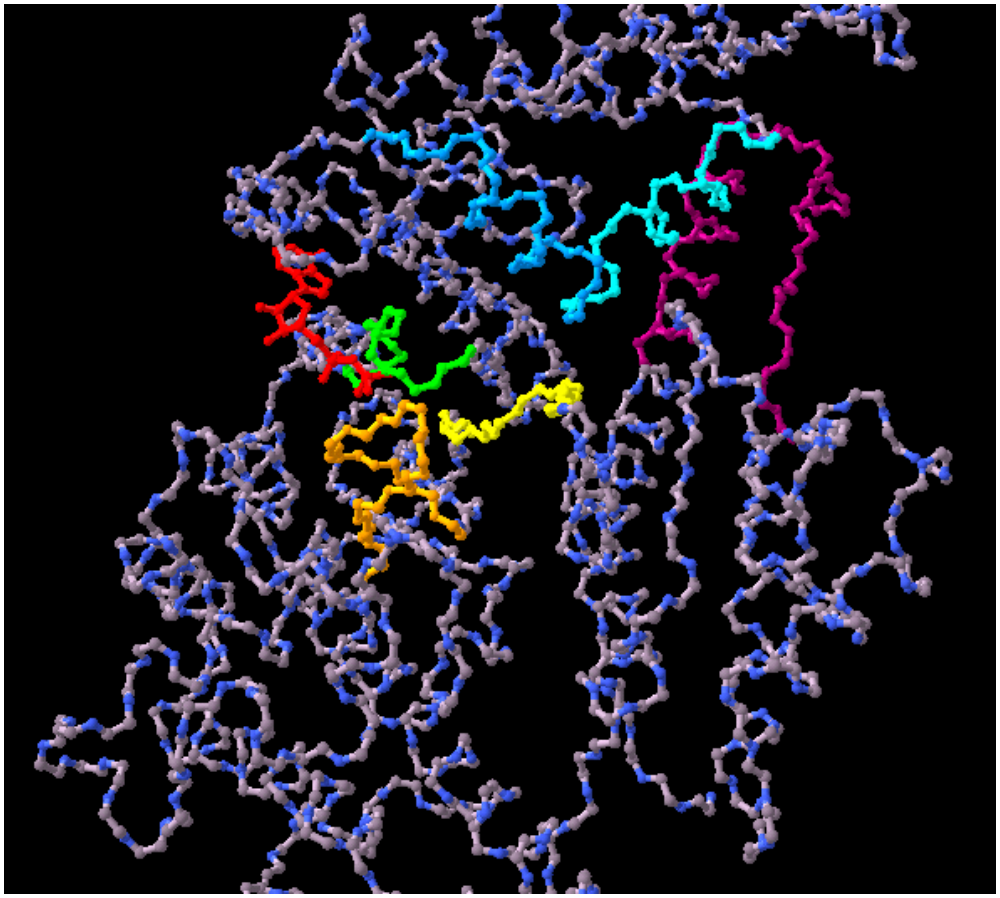


Karatzaferi, *et al. Biophysical J.* **2004**, *87,* 2532.

Cooke, *J. Gen. Physiol.* **2004**, *123*, 643.

Supplemental: myosin screen shots





blue: N-terminal domain
purple: upper 50 kD domain
red: lower 50 kD domain
green: converter

red: nucleotide
green: P-loop
orange: switch I
yellow: switch II

cyan: SH1 helix
light blue: SH2 helix
purple: relay helix/loop

Note: Pictures are rotated 180 degrees about the vertical with respect to each other