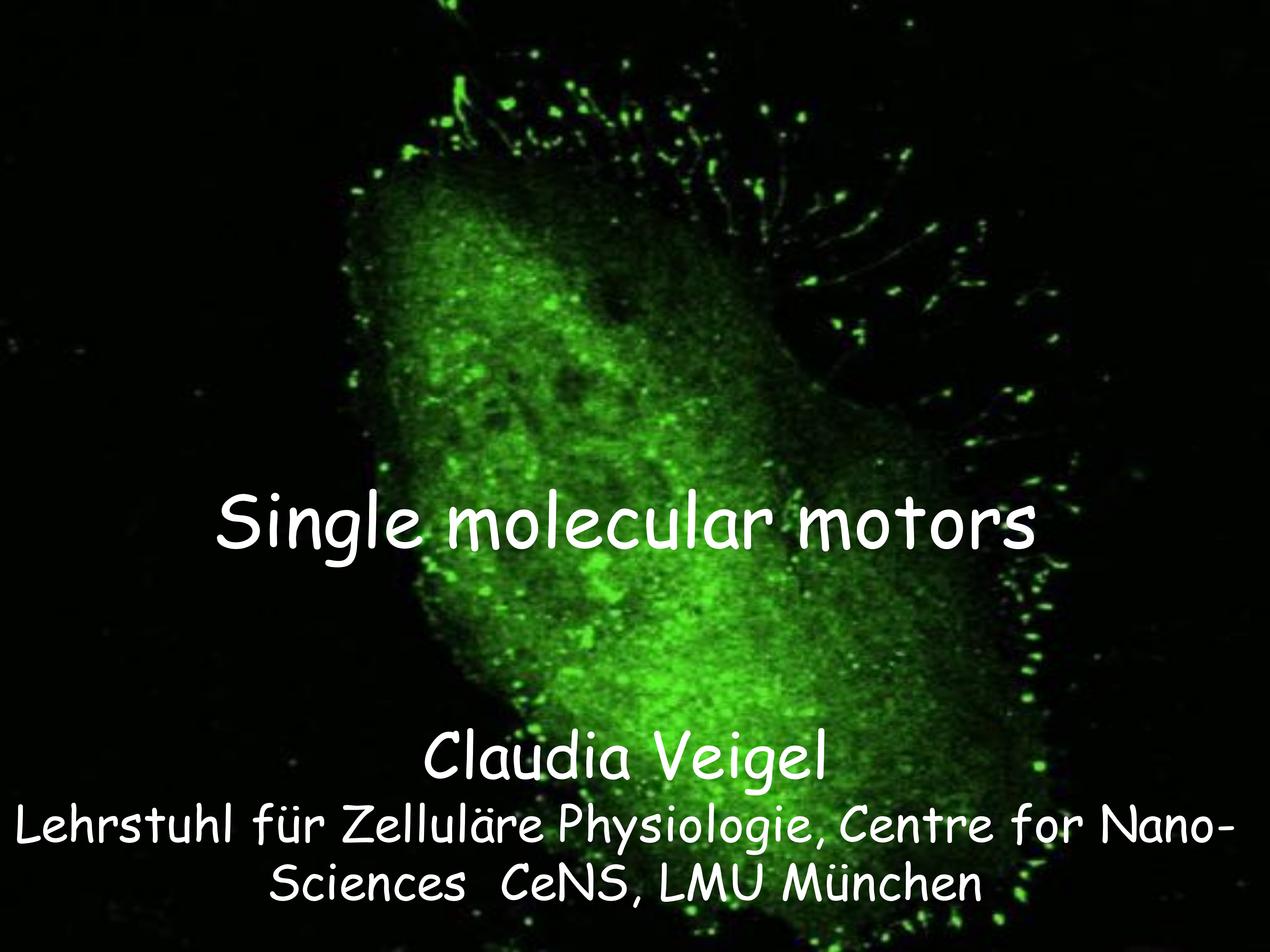


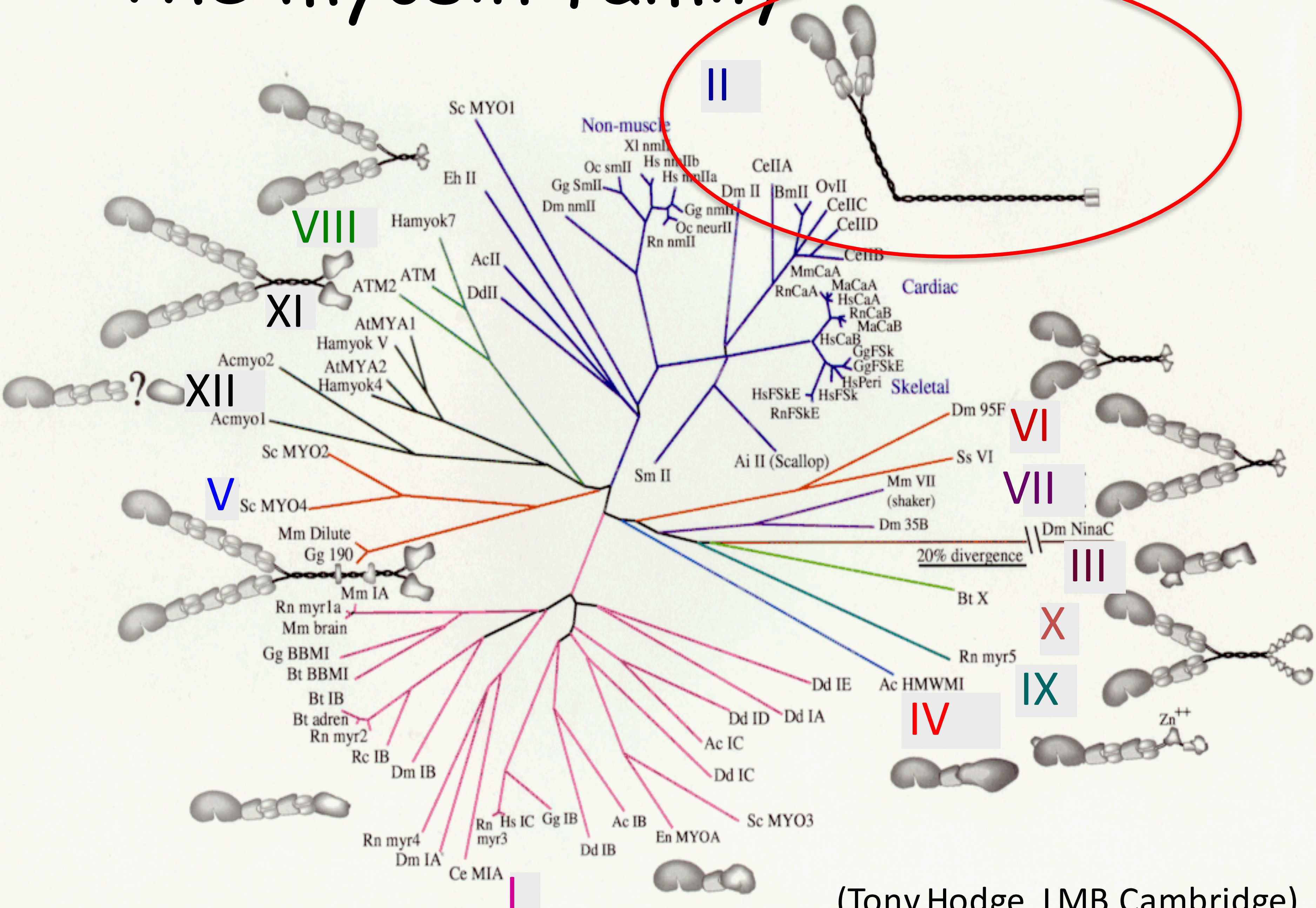
Single molecular motors

Claudia Veigel
Lehrstuhl für Zelluläre Physiologie, Centre for Nano-Sciences CeNS, LMU München

Many types of cellular motility are driven by cytoskeletal stepping motor proteins

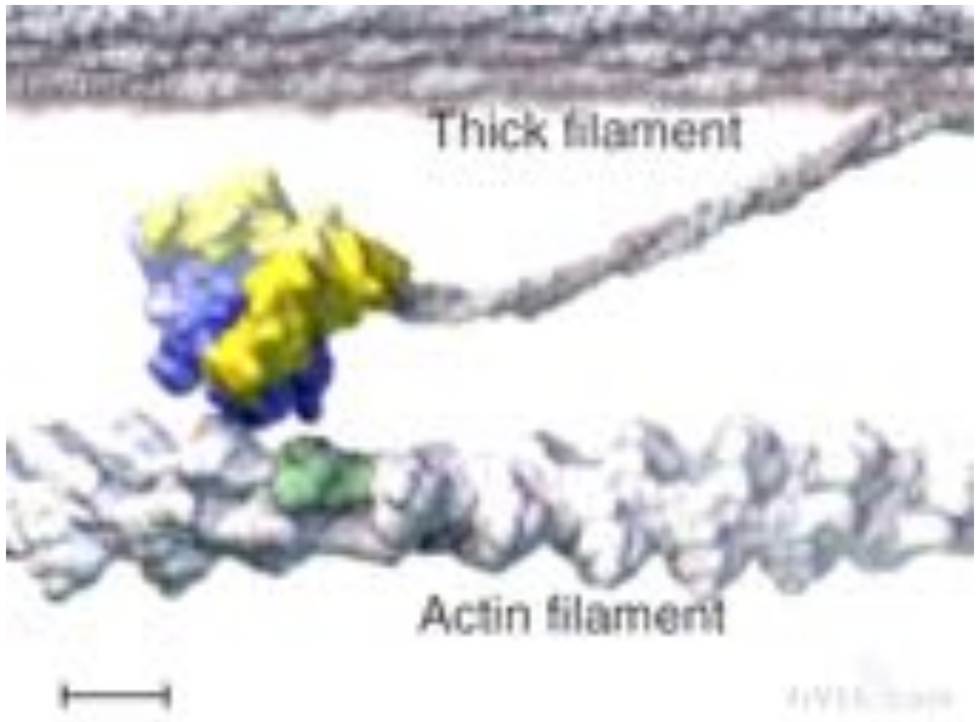
- Cell locomotion
- Cell division
- Intracellular transport processes and membrane trafficking
- Endo-and exocytosis
- ……

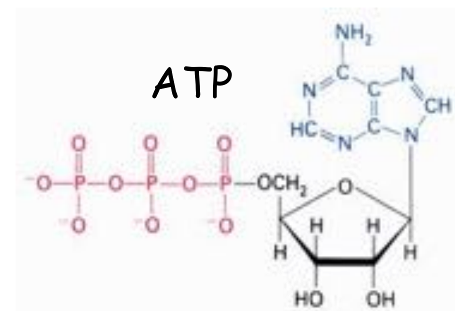
The myosin family



(Tony Hodge, LMB Cambridge)

Myosin motors: current model





- Movement and conformational changes are driven by thermal motion
- Thermal energy (kT) at room temp ~ 4×10^{-21}J = 4 pNnm

  1 ATP molecule   ADP + Pi; $\Delta G \sim 10^{-19}$ J = 100 pNnm

Movie by R. Vale-lab, UCSF

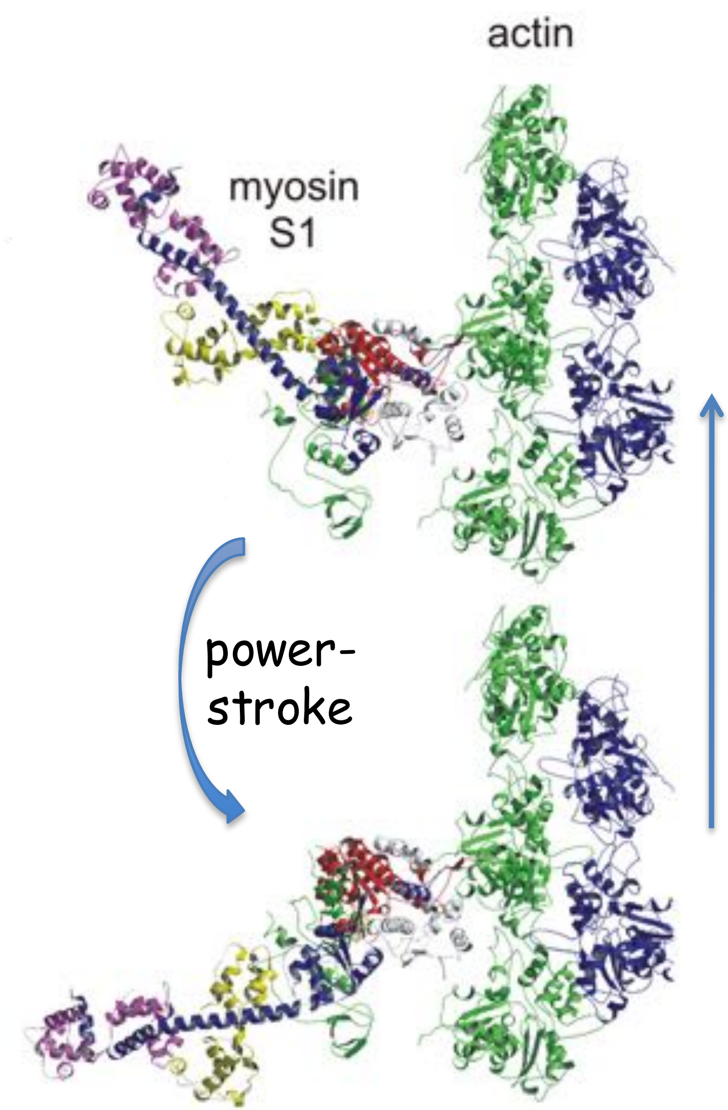
Single molecule studies of myosins: Aims

1. Basic mechanisms of chemo-mechanical energy transduction

- correlate structural, biochemical and mechanical states
- role of various parts of structure for the production of force and movement
- mechanics under load

2. Properties of ensembles of motors in the cellular context, regulation

- Monomers / dimers / oligomers
- Targeting
- Activation / inactivation



(Geeves and Holmes, Annual Reviews)

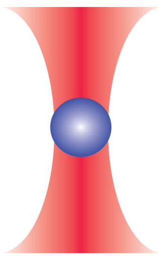
Optical tweezers:

- Mechanical energy differences on the order of thermal energy, i.e. **4 pN.nm**, can be measured: e.g. ~1/25 of energy of a single ATP molecule

- Resolution limited not by detection electronics but by thermal motion.

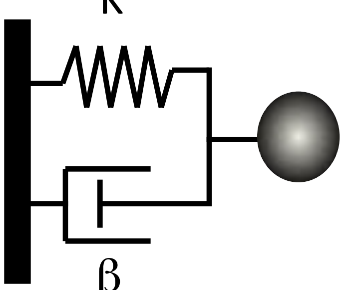
- The transducer is usually a 1μm diameter plastic bead
  - damping constant $\beta = 6\pi\eta r = 10^{-8}$ Nsm^{-1}
  - Stiffness $\kappa \leq 0.1$ pNnm^{-1}
  - Movement of bead in trap: equipartition principle

    $\frac{1}{2} kT = \frac{1}{2} \kappa \langle x^2 \rangle$

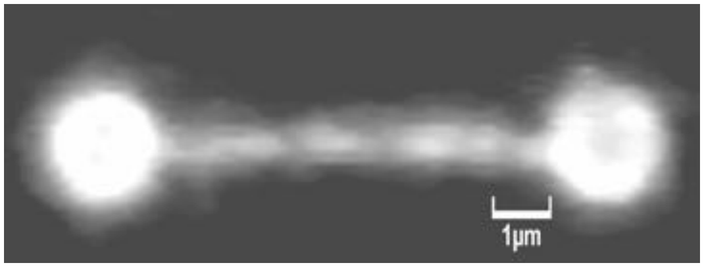


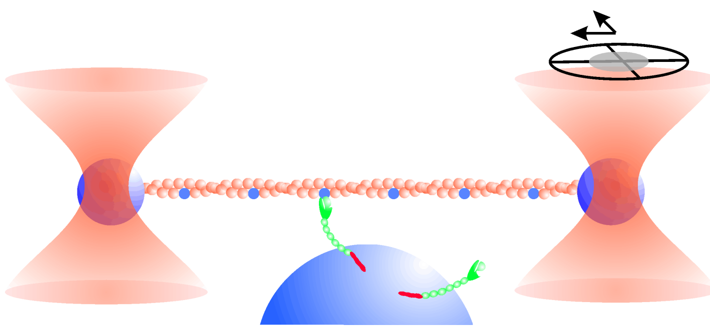
Therefore:

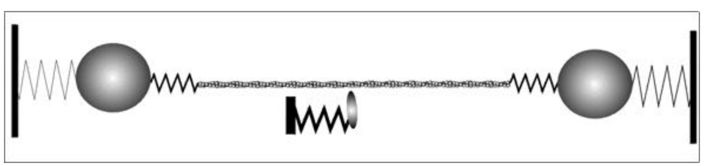
- **Positional noise is 6 nm** (r.m.s. $x = (kT/\kappa)^{0.5}$),
- **Force noise is 0.6 pN** ($=(kT\kappa)^{0.5}$) and
- **Bandwidth is 1.5 kHz** ($f_c = \kappa/2\pi\beta$) (Lorentzian).



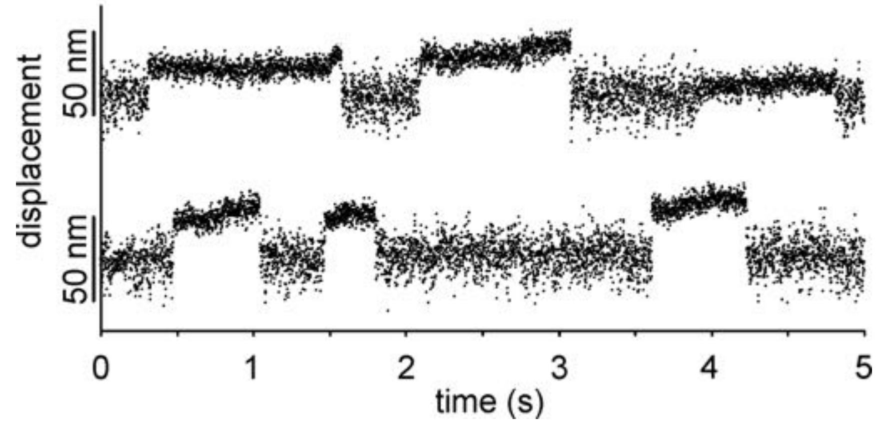
'Three-Bead-Assay' to study myosin motors







$$1/2\ kT = 1/2\ \kappa \langle x^2 \rangle$$

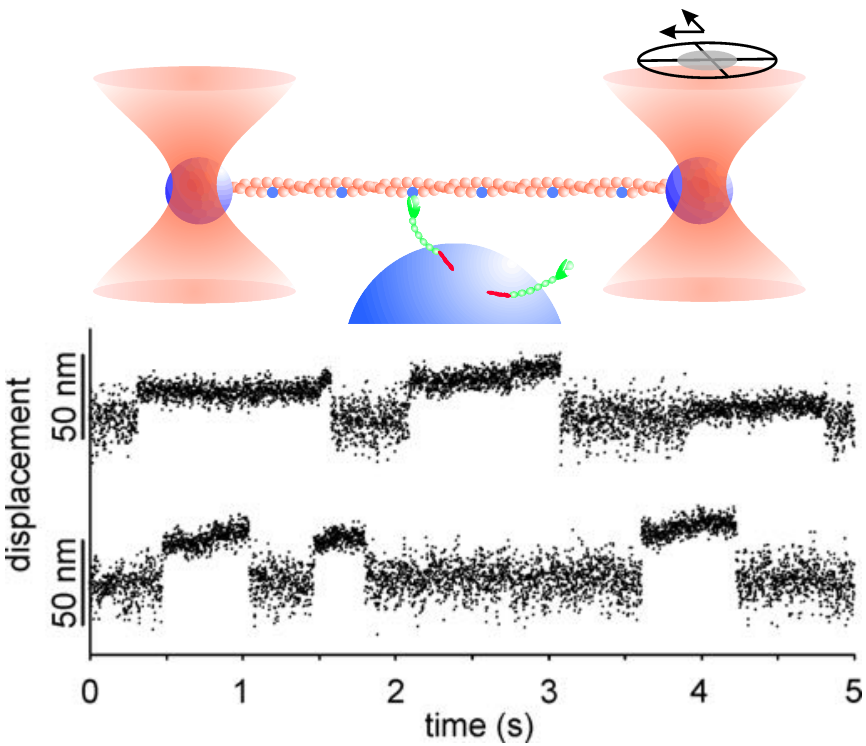


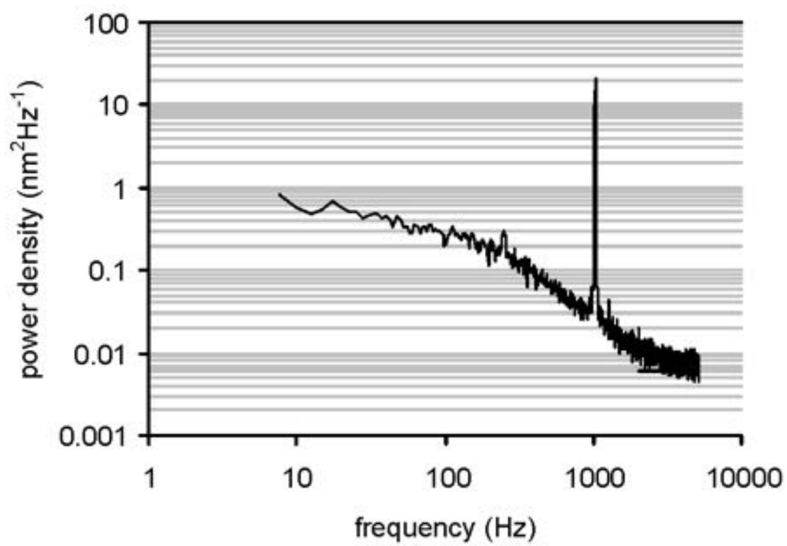
Identification of myosin binding events from the change in thermal noise

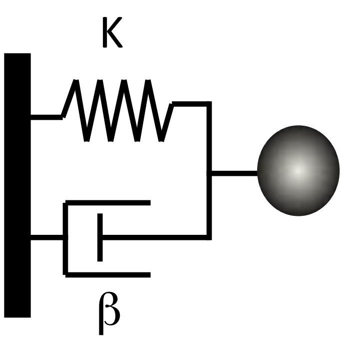
'Three-Bead – Assay':
Finer *et al.* (1994) Nature

Data analysis:
Molloy *et al.* (1995) Nature

'Three-Bead-Assay' to study myosin motors







$1/2\ kT = 1/2\ \kappa \langle x^2 \rangle$

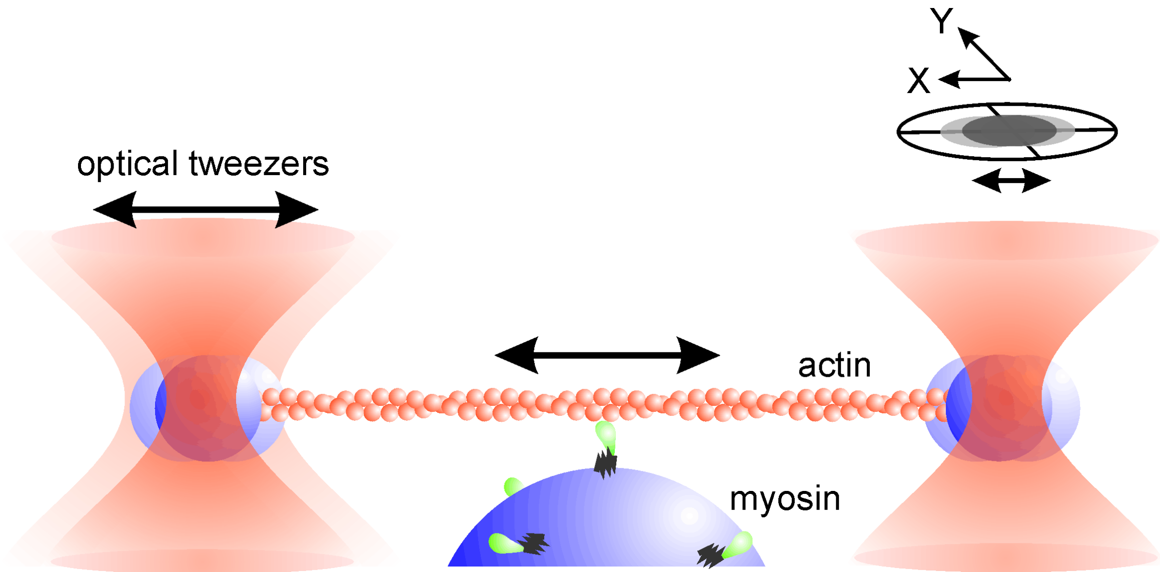
$f_c = \kappa / 2\pi\beta$

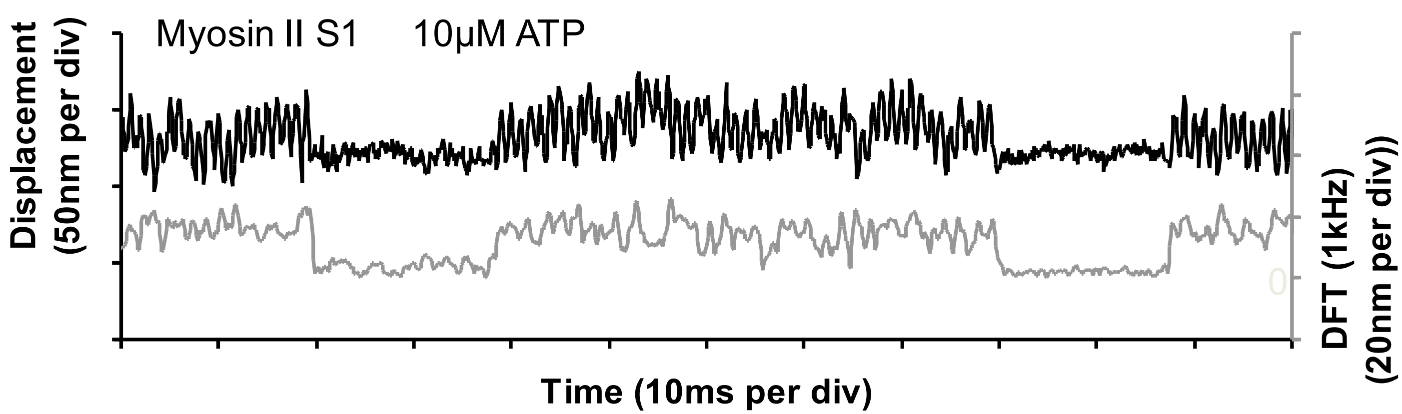
time resolution

Methods to improve time resolution with different advantages and disadvantages:

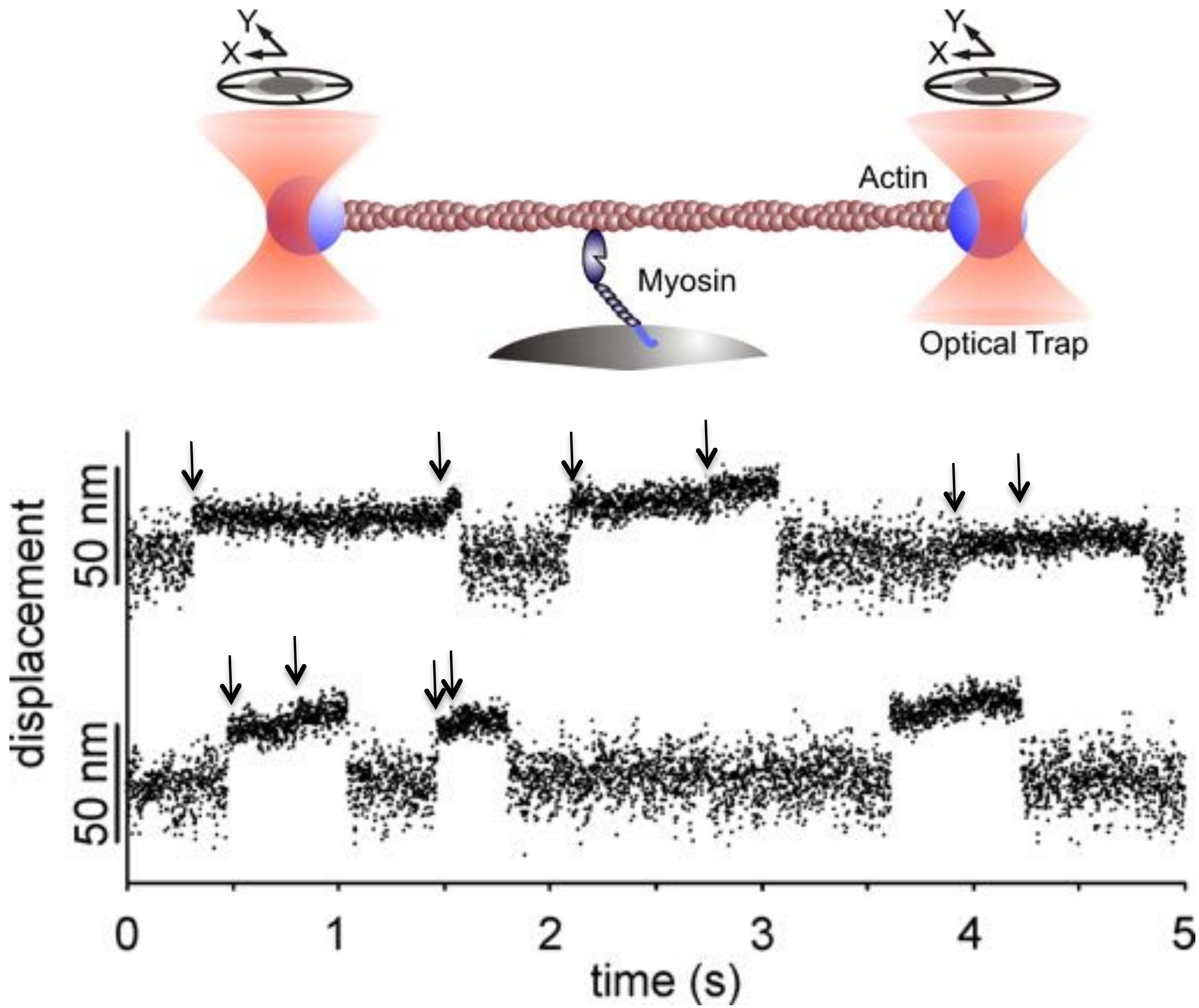
Veigel and Schmidt (Nat Rev Mol Cell Biol)

Using kHz carrier signals to achieve sub-millisecond time resolution



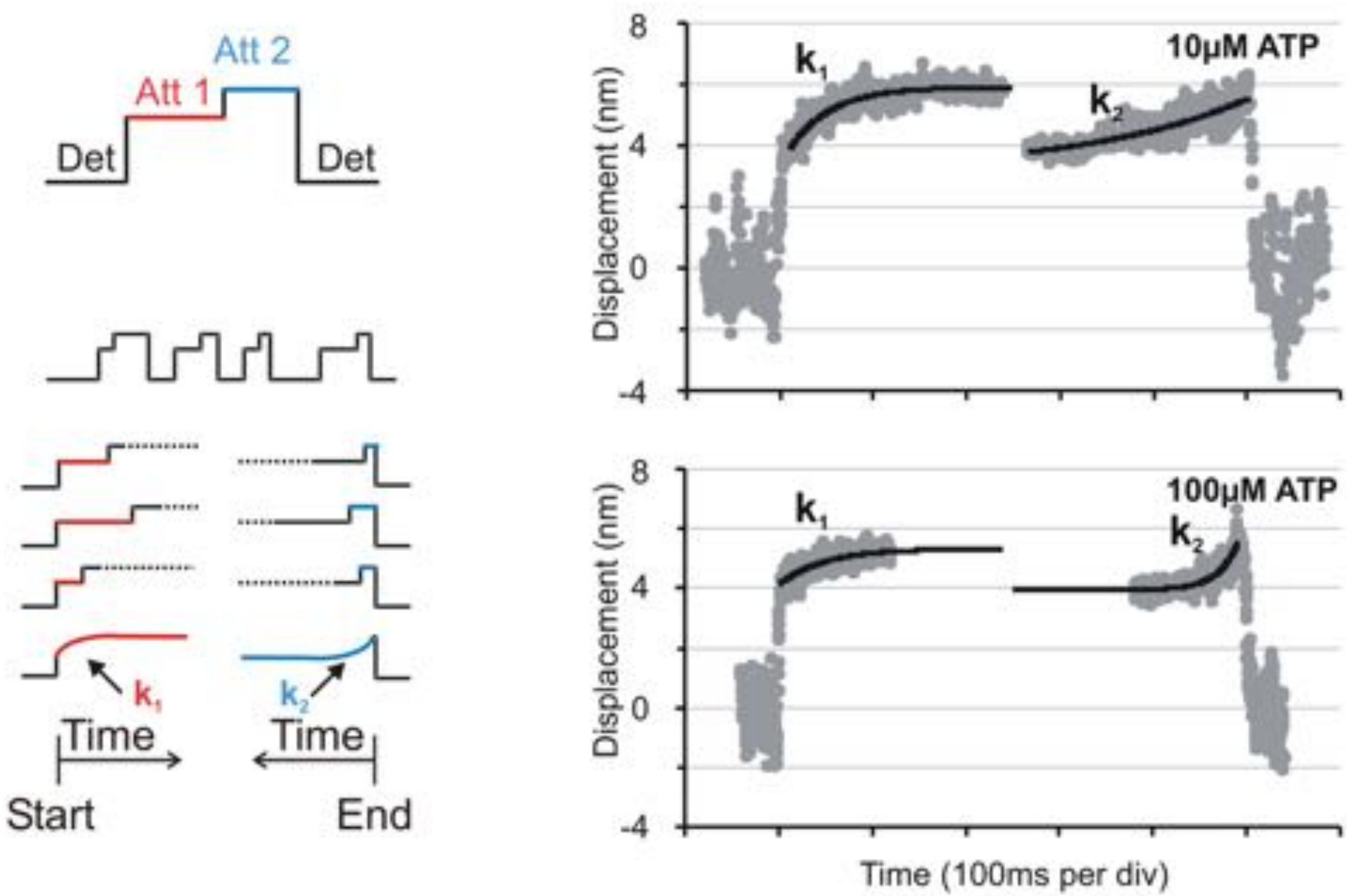


Myosin working stroke produced in two phases



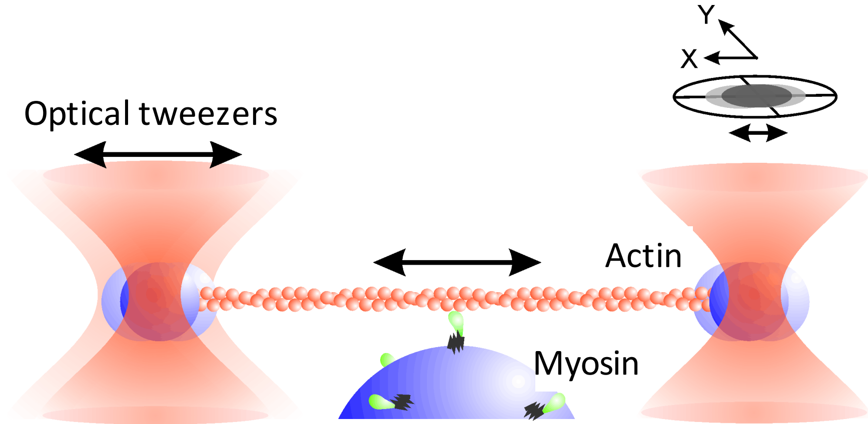
Veigel *et al.* (1999) Nature

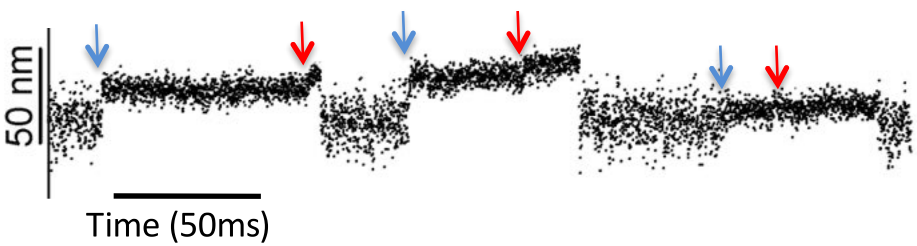
Myosin working stroke is produced in two phases

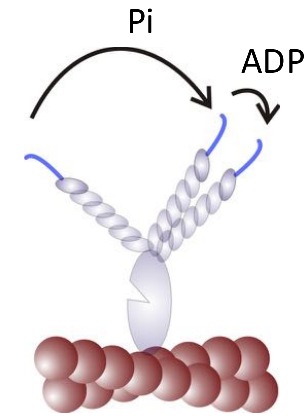


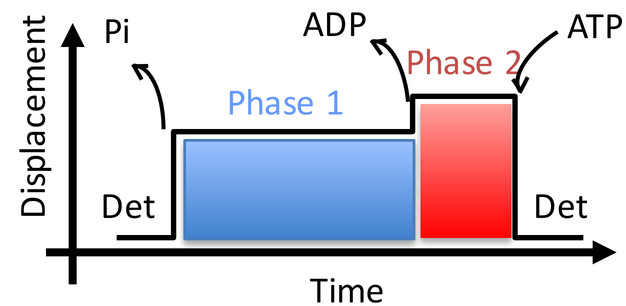
Veigel *et al.* (2003) Nature Cell Biol

The basic mechanics of single myosins







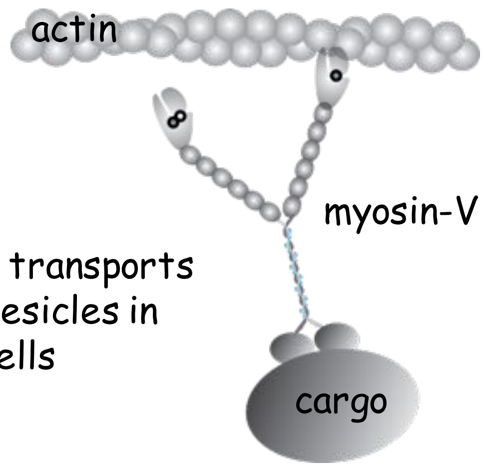


Mechanism 1:

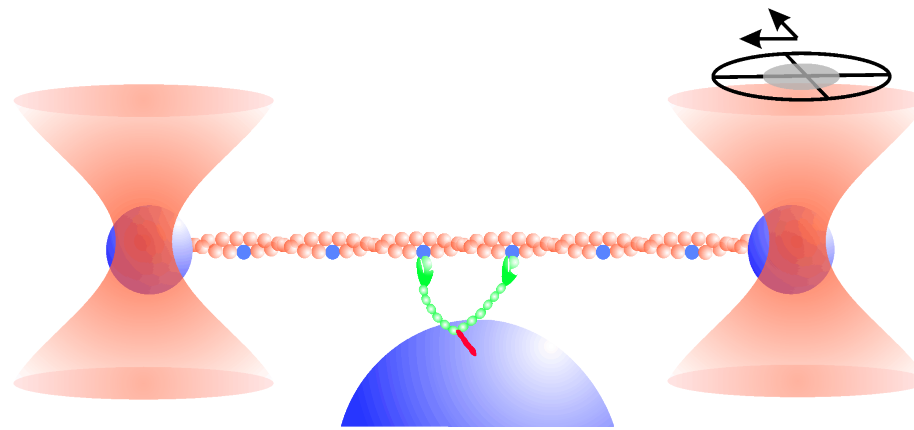
- working stroke in 2 phases
- w/s proportional to lever arm length

Myosin **I**: Veigel *et al.* (1999) *Nature;* Laakso *et al.* (2008) *Science,*
Myosin **II**: Veigel *et al.* (2003) *Nat Cell Biol,*; Capitanio *et al* (2006) PNAS; Non-muscle myosin **IIa**: Veigel –lab (unpub)
Myosin **V**: Veigel *et al. Nat Cell Biol; Nat Struct Mol Biol (2002, 2005, 2010)*
Myosin **VI**: Lister *et al.* (2004) *EMBO J.;* Myosin **X**: Takagi *et al* (2014) *PNAS*

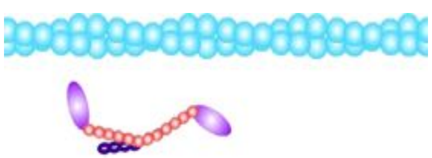
Processive movement of **dimeric myosins**: e.g. Myosin-V



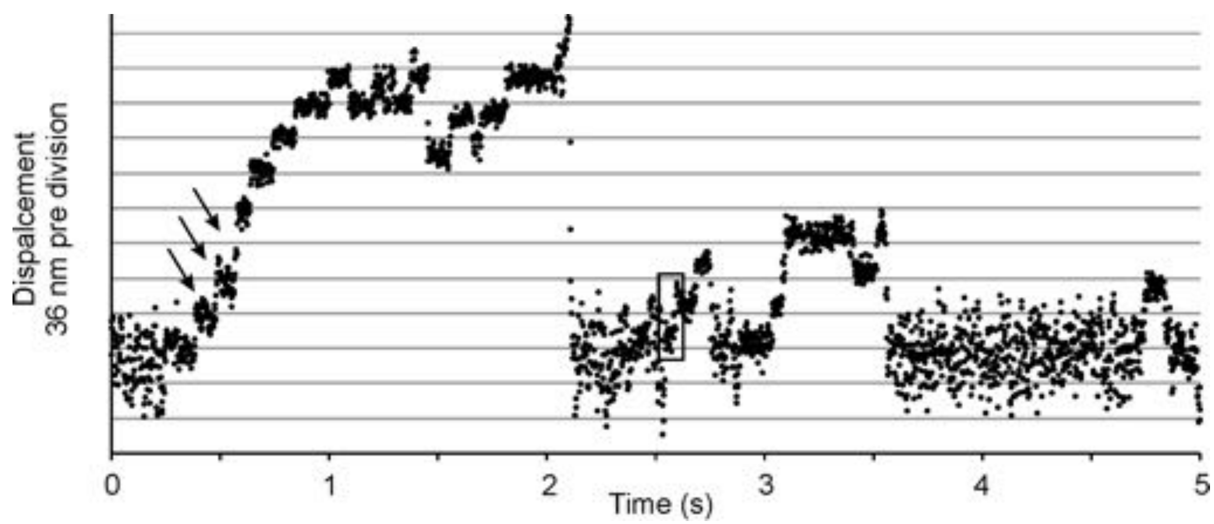
Myosin-V transports cargo vesicles in cells



Uncoordinated heads

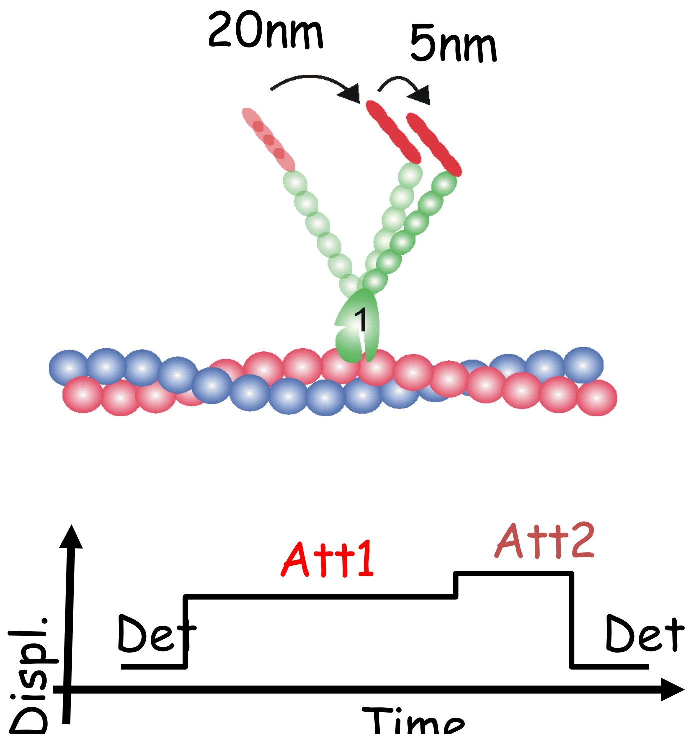


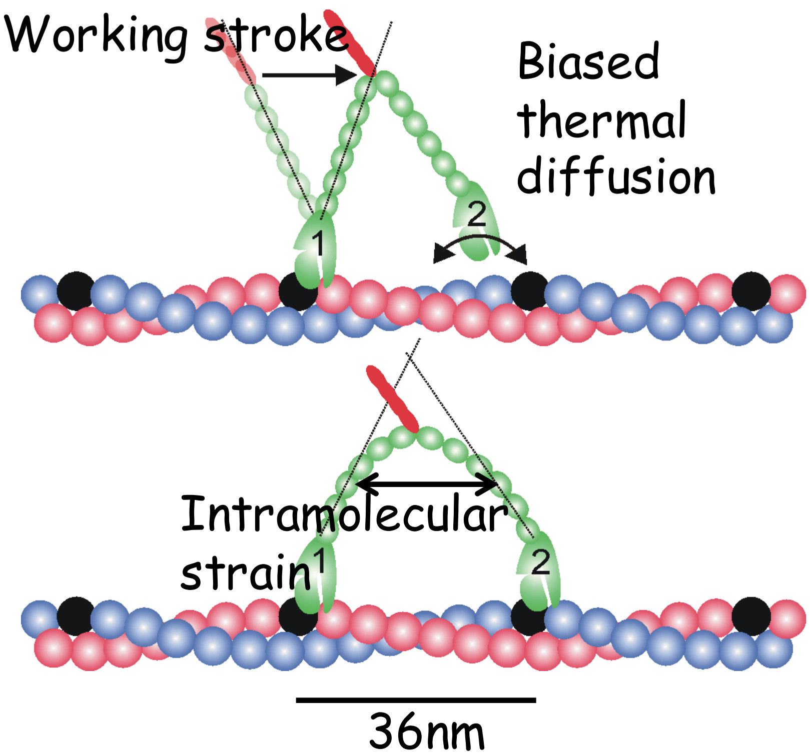
Coordinated heads



Veigel *et al.* (2002) Nature Cell Biol

Processivity and strain dependent kinetics





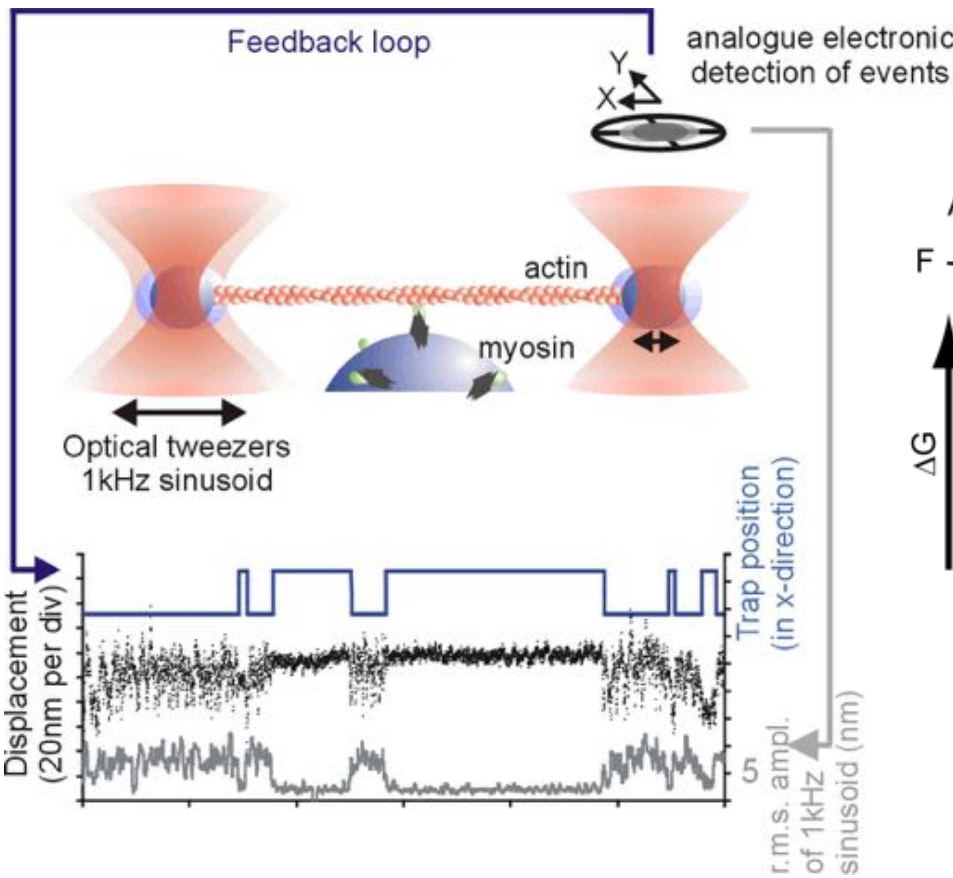
Questions:

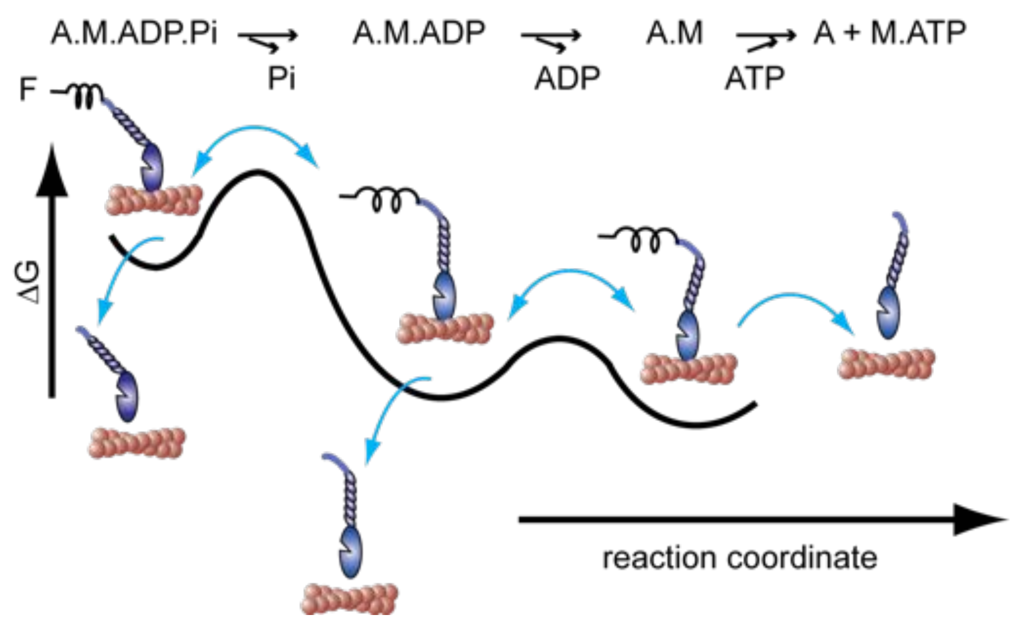
Intramolecular strain: Cooperativity between the heads?

Kinetics gated by intramolecular strain?

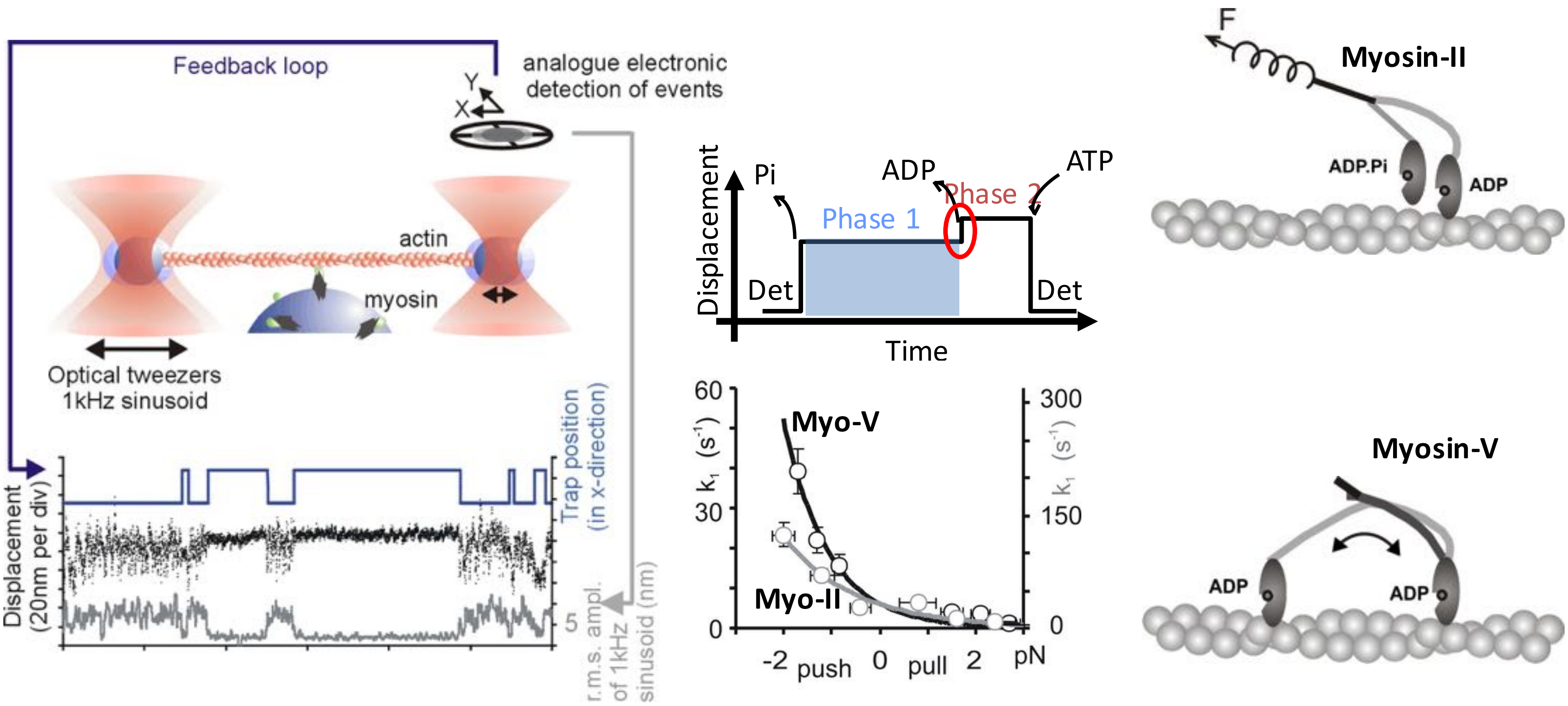
Are the kinetics of a single head load dependent?

Use kHz 'carrier signal' to obtain sub-ms time resolution and fast application of load





Veigel *et al.* (2003) Nature Cell Biol



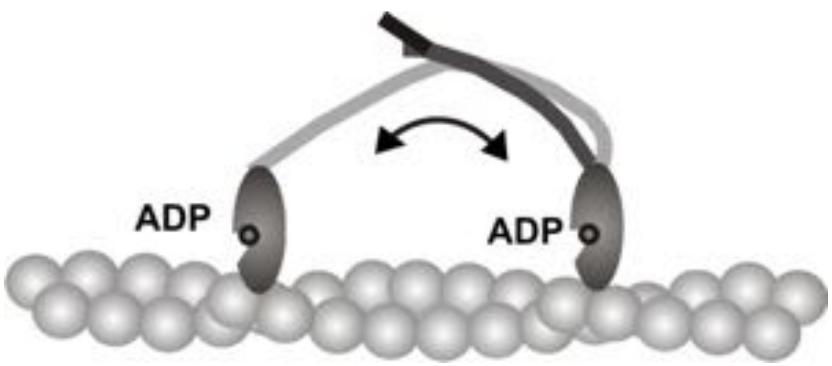
Mechanism 2:

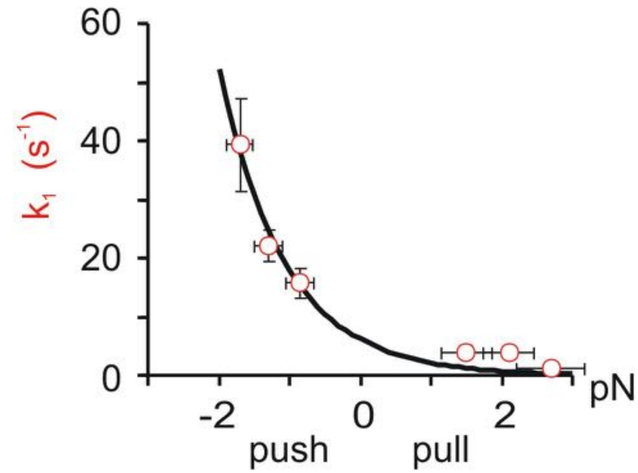
- ADP release strongly load dependent
- ATP binding little load dependent

Myosin **II**: Veigel *et al.* (2003) *Nat Cell Biol*,; Non-muscle myosin **IIa**: Veigel -lab (unpub)
Myosin **V**: Veigel *et al. Nat Cell Biol; Sellers & Veigel Nat Struct Mol Biol (2002, 2005, 2010)*
Myosin **I**: Laakso *et al.* (2008) *Science*
Myosin **VI**: Veigel-lab (unpub)

Gated processive movement for myosin-V

Intramolecular strain



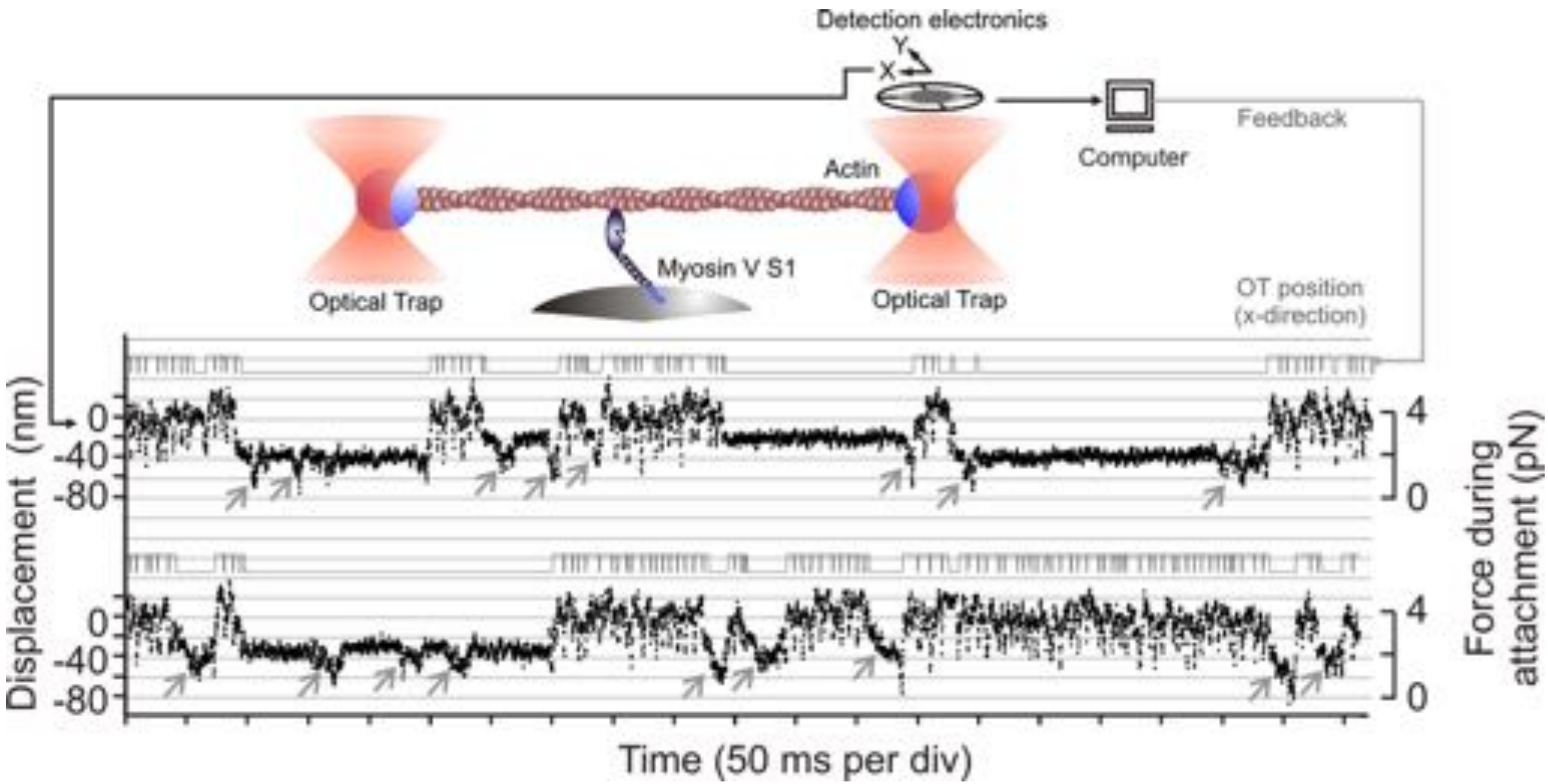


Force during attachment: ~1.5 to 3.6pN; (average stiffness during attachment ~ 0.2 pN.nm^{-1} per head)

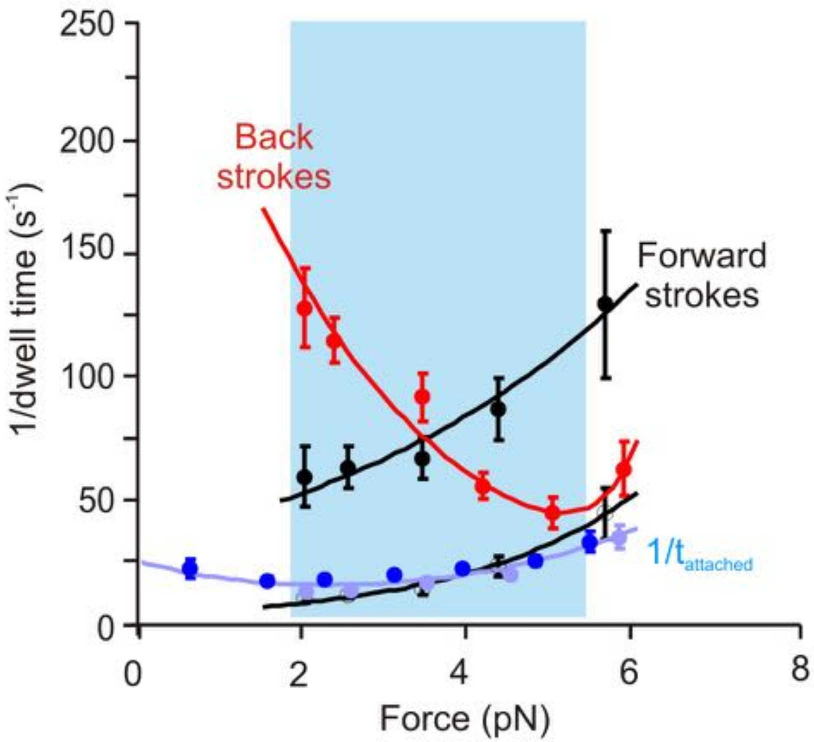
k_1 (head1) > 40-60 x k_1 (head2); >40-60 steps per diffusional encounter

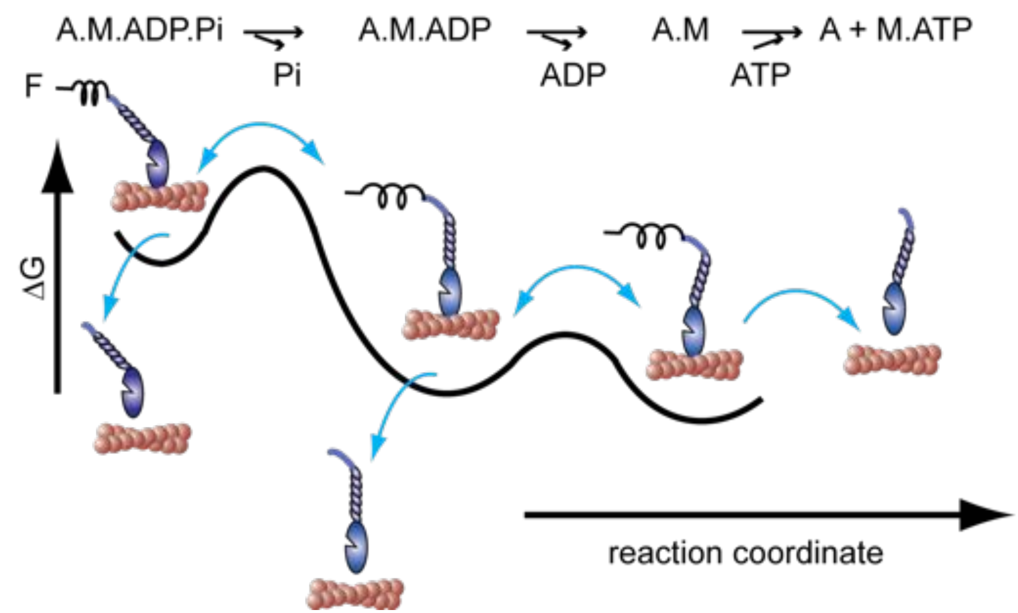
Veigel *et al.* (2005) Nat Cell Biol

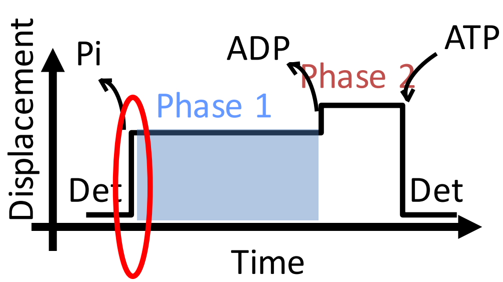
Reversal of the myosin power stroke at forces near stall



Sellers and Veigel (2010) Nat Struct Mol Biol







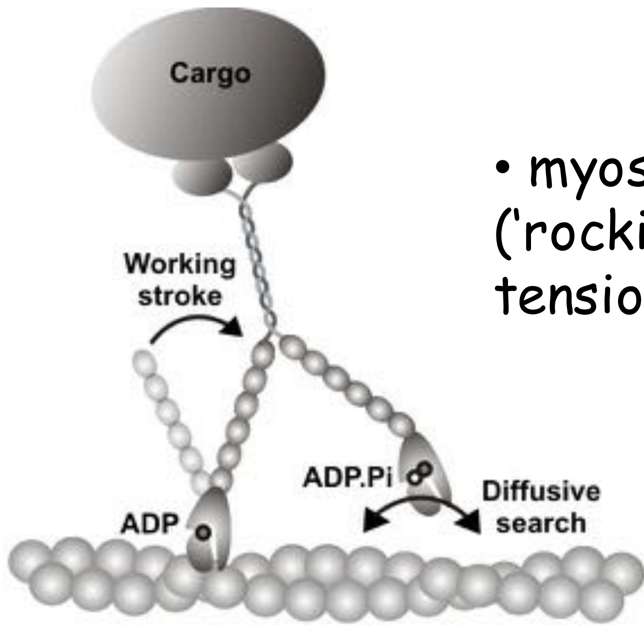
Mechanism 3:

- Power stroke is reversible

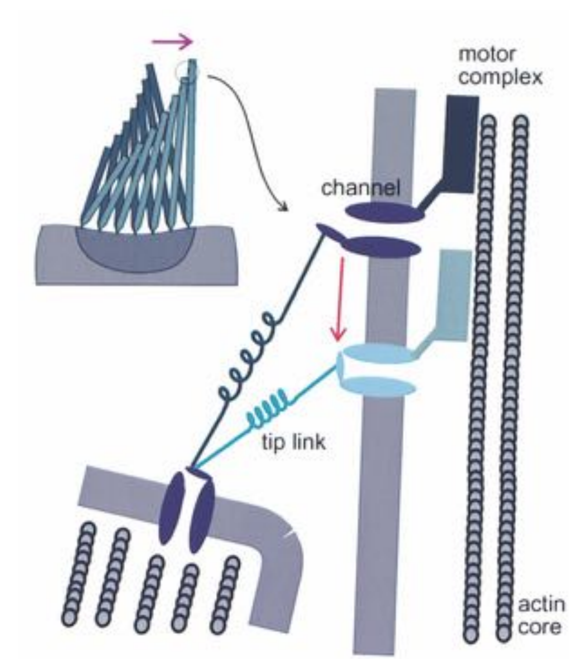
Myosin V: Sellers and Veigel (2010) *Nat Struct Mol Biol*

Power stroke reversibility:

• myosin **V**: change in directionality of processive movement under load



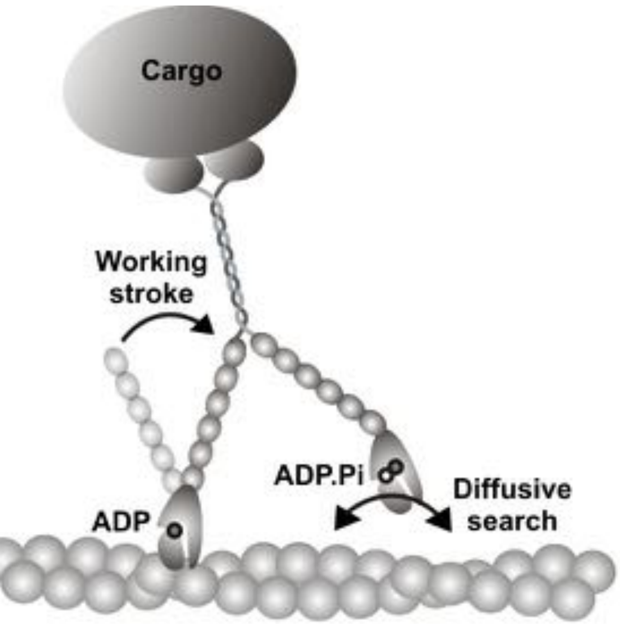
• myosin **Ic**: fast adaptation in hearing? ('rocking crossbridge' might regulate tip link tension, hair cells, inner ear)



From: Gillespie and Corey, Neuron 1997; Gillespie and Walker, Nature 2001, Batters *et al* (2004) EMBO J

Conclusion:

basic mechanisms can be tuned for diverse myosin functions



Mechanisms 1 and 2

Two-step w/s and load dependent ADP release:

- myosin II: sustain tension at low cost
- myosin V: head coordination, processivity
- myosin I: force sensing
- ...

Mechanism 3

Working stroke reversibility:

- myosin V: change in directionality
- myosin Ic: fast adaptation in hearing?
- ...

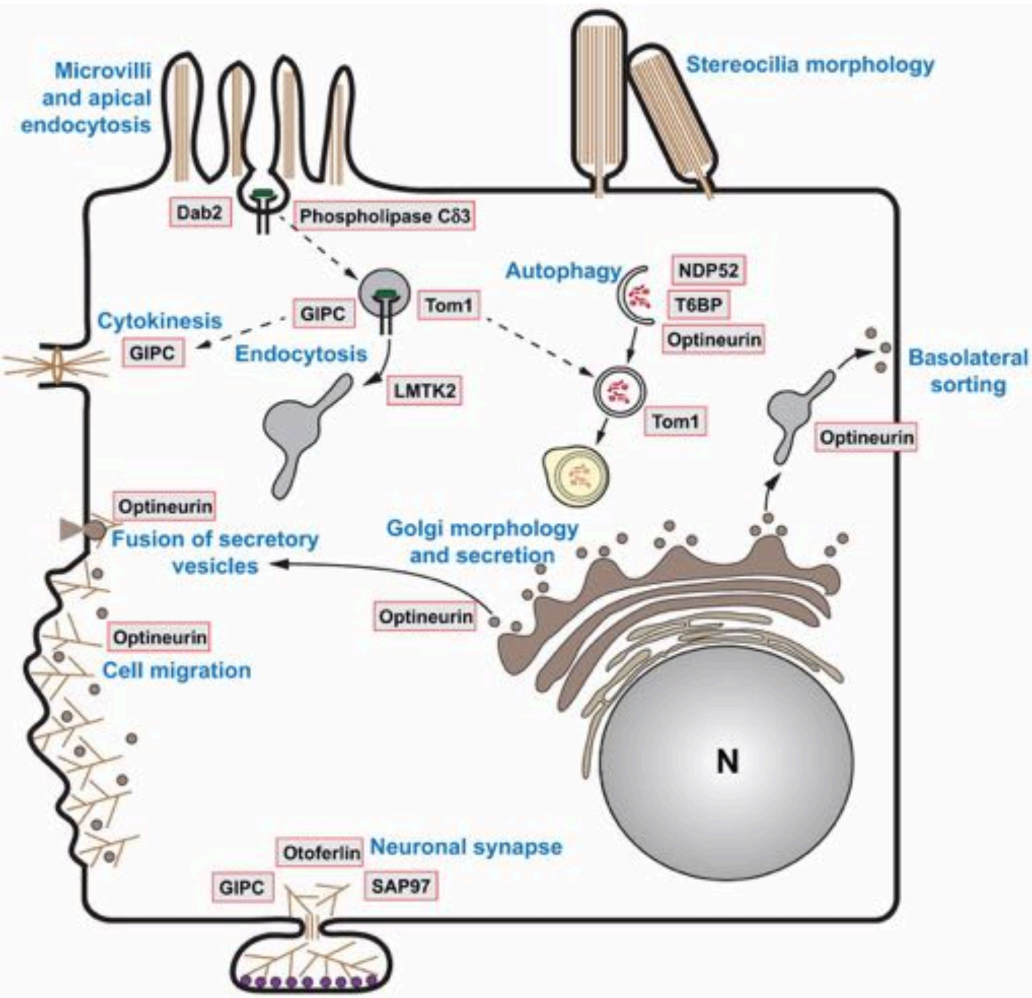
These are all mechanisms of the activated motors.

Motor mechanics in the cell biological context?

Regulation of motor targeting and motor activation

2. Regulation of myosin motors

Example 1: targeting and activation of myosin-VI



Tumbarello, Kendrick-Jones and Buss (2013)

- only myosin to move towards (-) end of actin filaments
- monomer or dimer? maybe both?
- involved in a myriad of cellular motile functions including:
  - formation of stereocilia in hair cells
  - endo- and exocytosis
  - membrane delivery to leading edge in migrating cells
  - …
- upregulated in migrating carcinoma cells (used as a marker)

Migrating cells

• localised calcium transients play a multifunctional role:

⇒ steering directional movement of the cell

⇒ cytoskeleton redistribution, relocation of focal adhesions

Effects of calcium transients on the mechanics of myosin-VI?

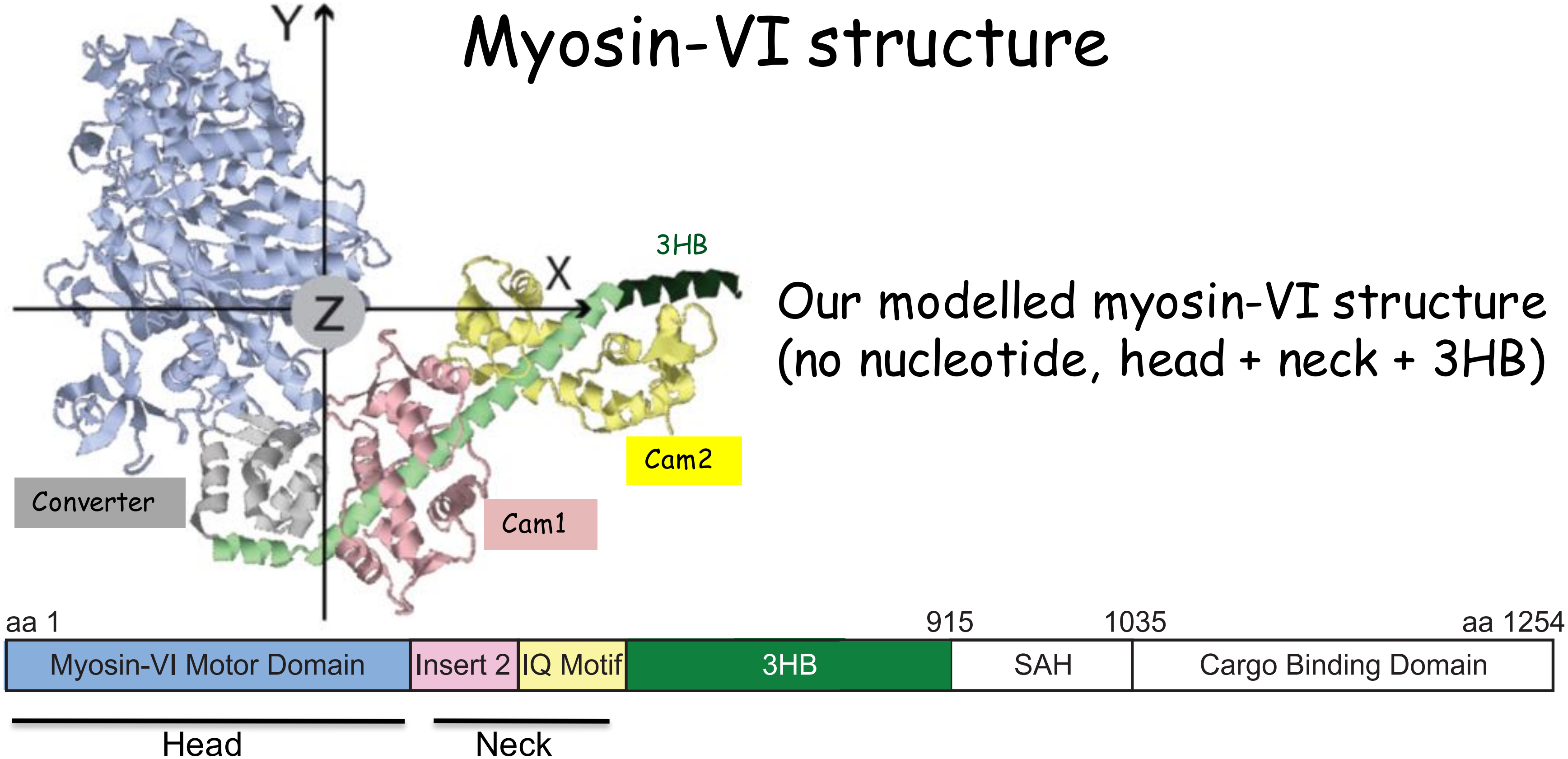
Required background information before designing mechanical experiments:

effect of calcium on the myosin-VI structure (conformation)

⇒ effect on the calmodulin (Cam) binding neck (leverarm)

⇒ effect on the myosin-VI target binding tail

Myosin-VI structure



Our modelled myosin-VI structure (no nucleotide, head + neck + 3HB)

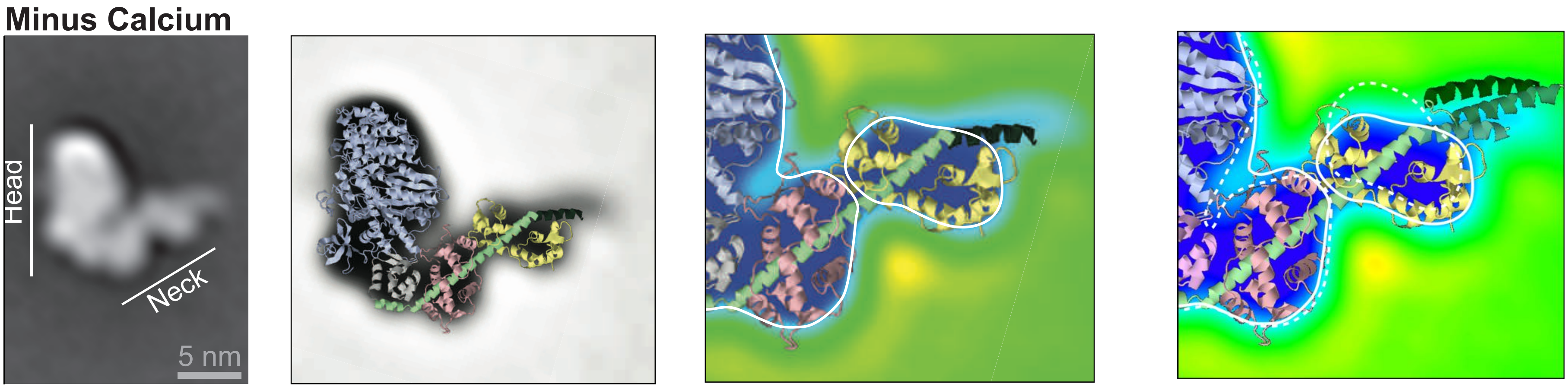
crystal structure: parts of the molecule, no structures of the whole tail

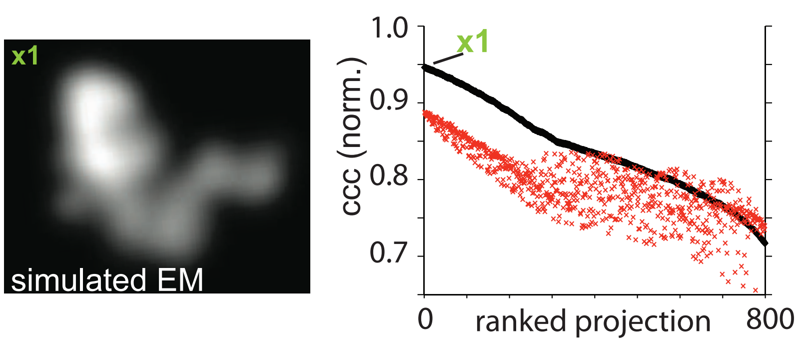
Electron microscopy:

Cryo: Head + Neck bound to actin, no tail

Negative stain: Head + Neck without actin, no tail

Full-length Myosin-VI at low Ca^{2+} (negative stain EM)



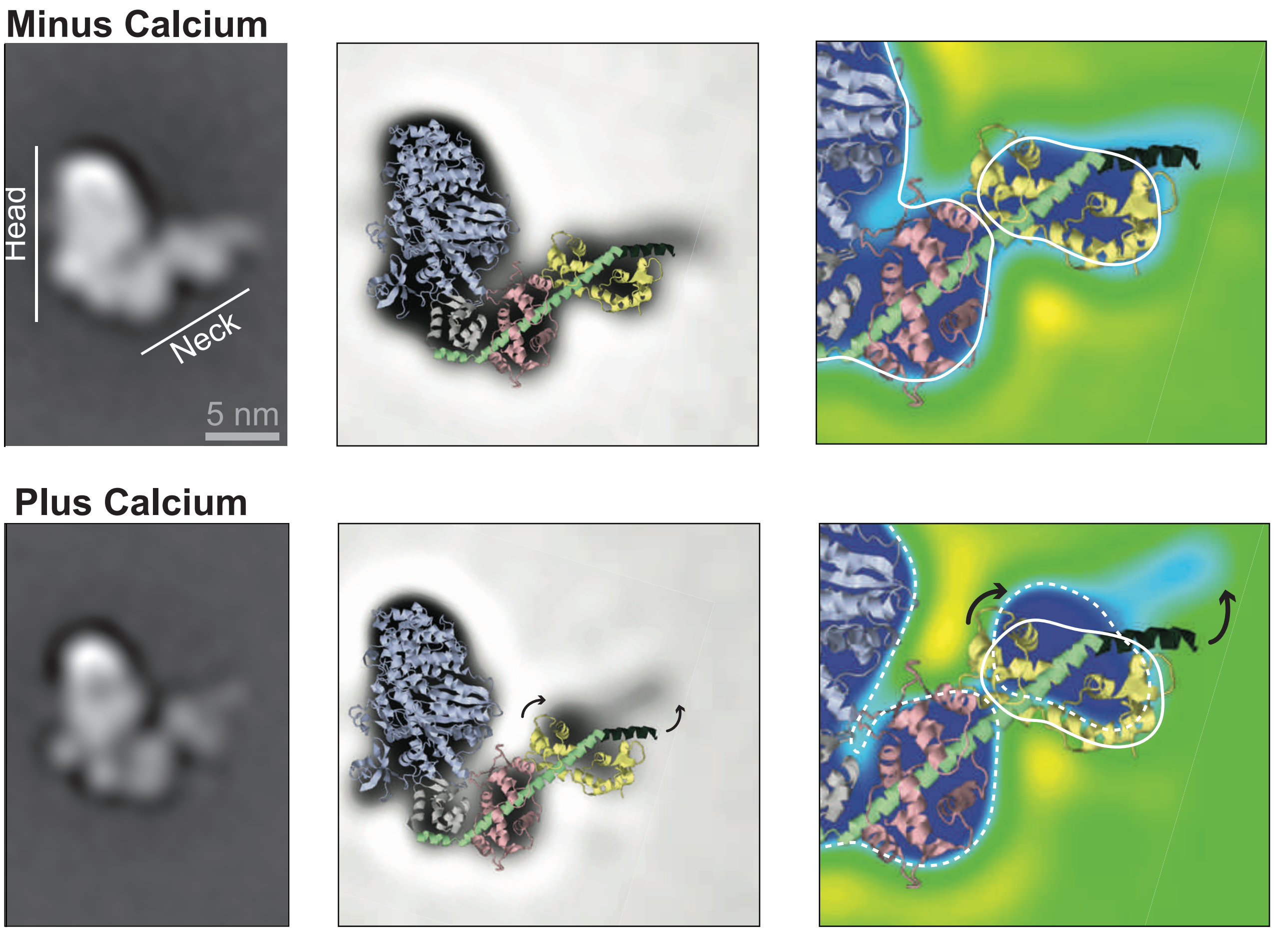


Low calcium:

Modelled structure fits nicely to negative stain EM of full-length myoVI

=> some additional mass between 1st calmodulin, converter, catalytic domain

Full-length Myosin-VI at high Ca^{2+} (negative stain)



High calcium:

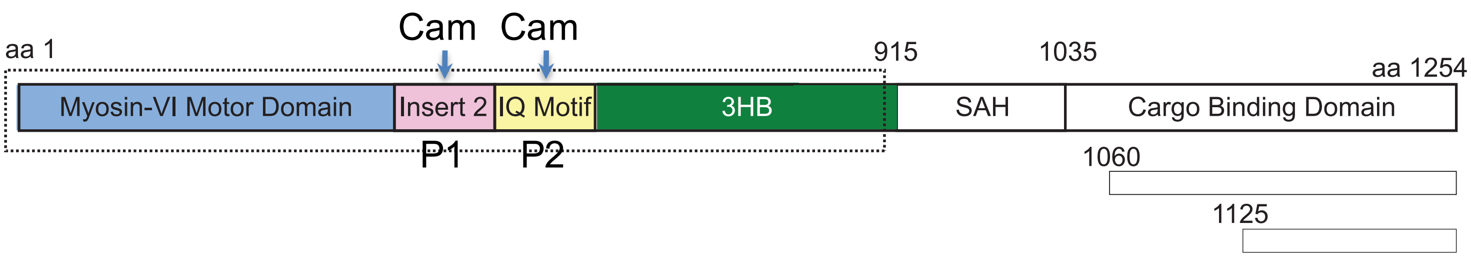
⇒ catalytic domain of the model fits the EM even better (no extra mass between 1st Cam, converter, catalytic domain, i.e. no tail bound to the head?)

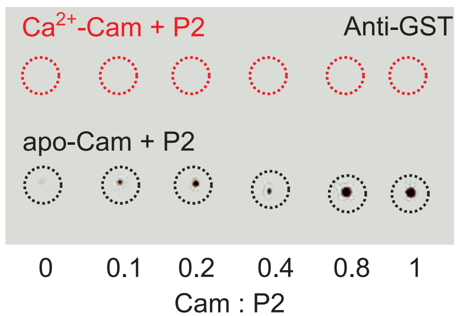
⇒ conformational change of the 2nd calmodulin

⇒ conformational change of the 3HB (i.e. tail)

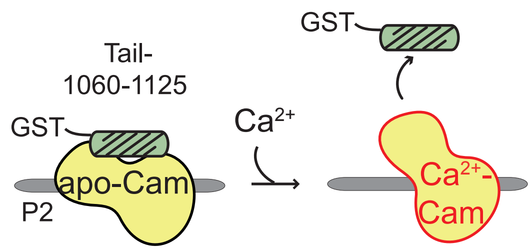
Batters *et al.* (2016) PNAS

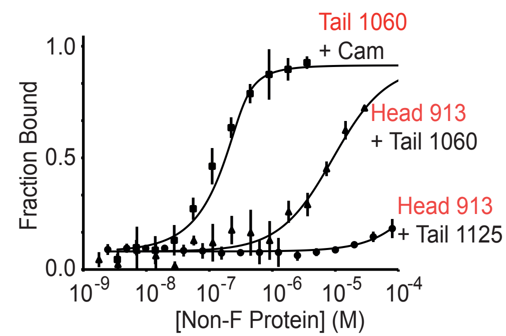
Myosin-VI tail (1060-1125) backfolds onto apo-calmodulin





Dot far Western blot





Fluorescence based binding study (MST)

Conclusion of binding studies:

⇒ Tail 1060-1125 binds to apo-calmodulin, not to Ca^{2+}-calmodulin

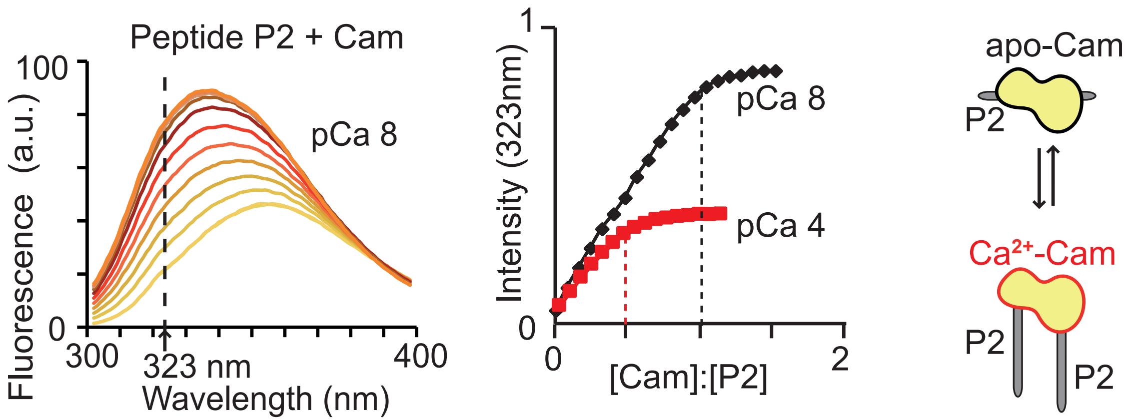
⇒ Backfolding of the tail onto calmodulin at low calcium

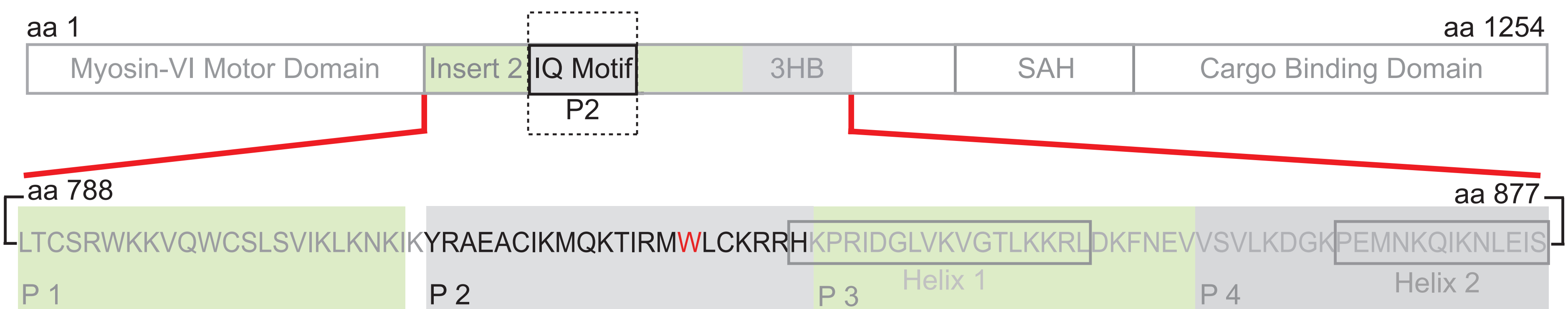
(apo calmodulin = calmodulin without calcium ions bound)

Batters *et al.* (2016) PNAS

Calmodulin binding to P2

Tryptophan fluorescence studies



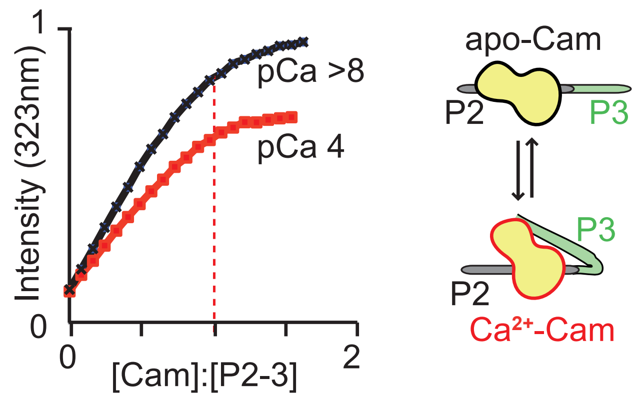


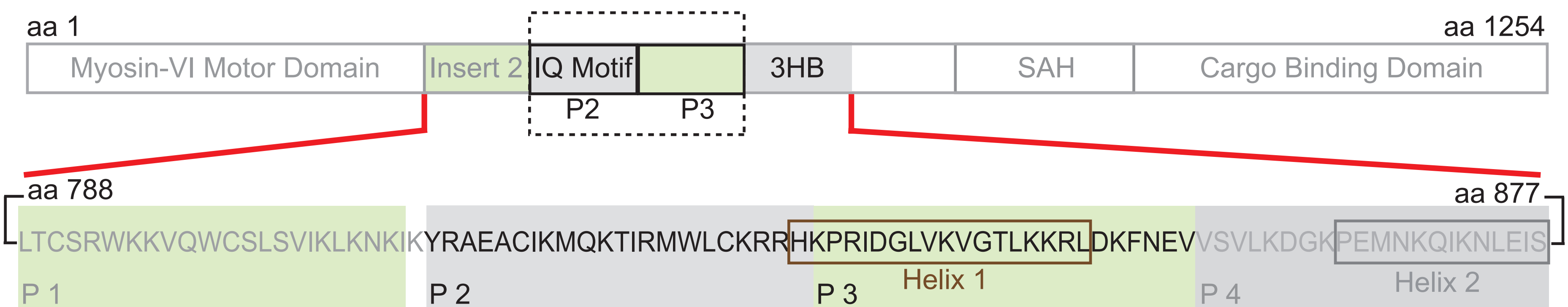
Result:

⇒ Low calcium: binding stoichiometry 1 Cam : 1 P2

⇒ High calcium: 1 Cam : 2 P2

Calmodulin binding to P2-3



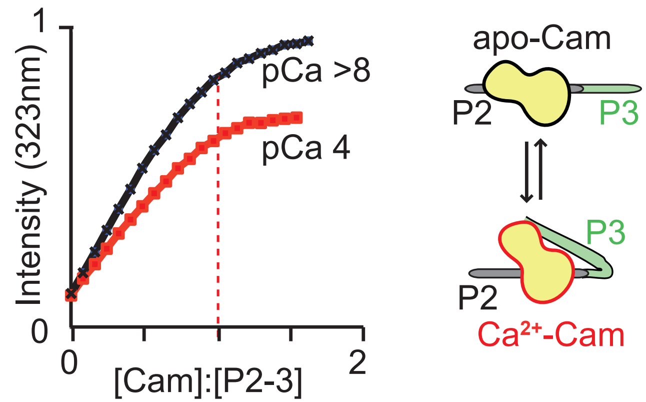


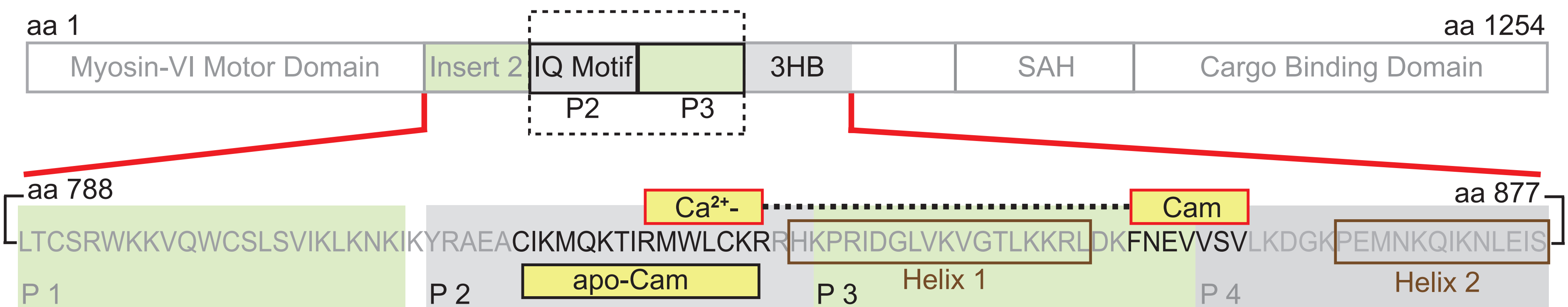
Result:

⇒ Low calcium: binding stoichiometry 1 Cam : 1 P2-3

⇒ High calcium: 1 Cam : 1 P2-3

Bi-partite calmodulin binding site at high calcium



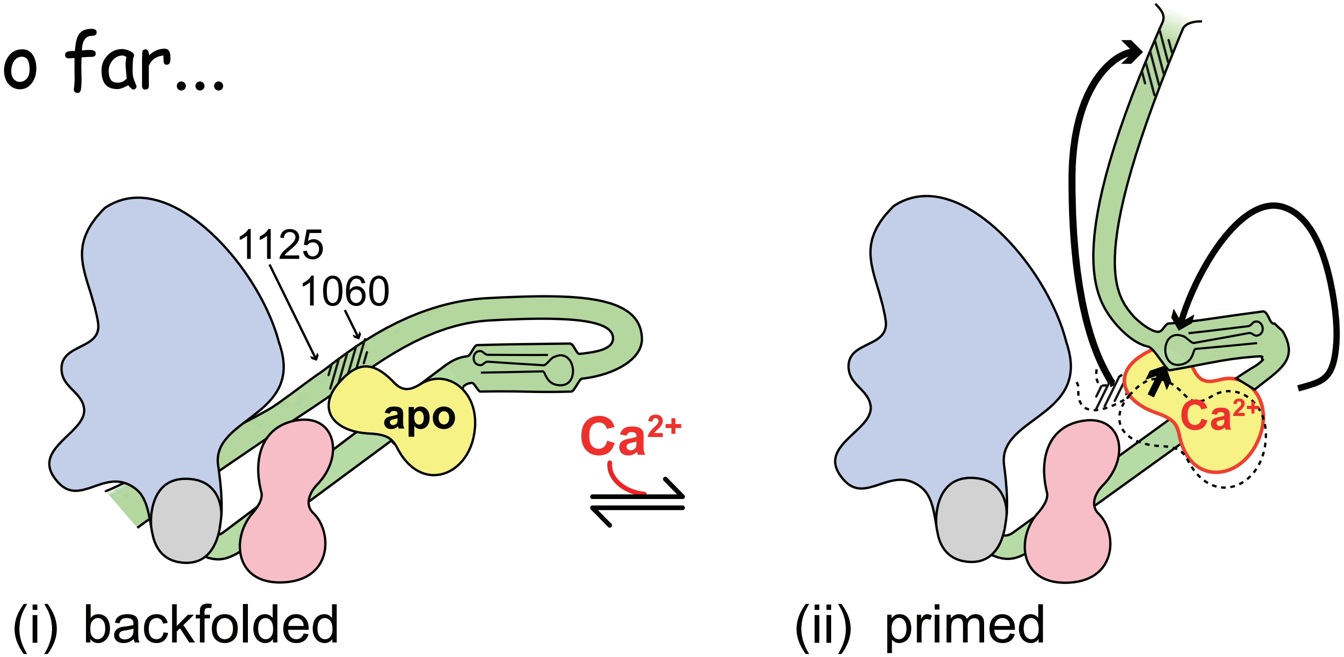


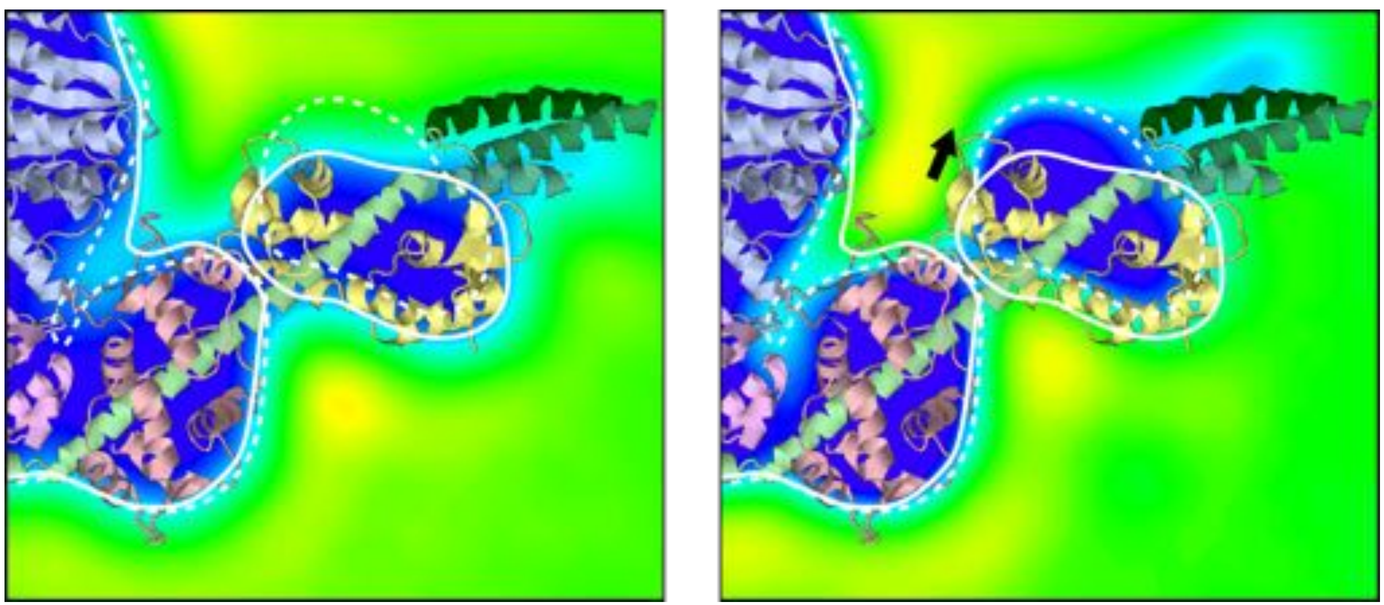
<u>Conclusion from 16 different peptides</u>:

⇒ Low calcium: binding stoichiometry 1 Cam : 1 P2

⇒ High calcium: 1 Cam : 1 P2-3, bi-partite site

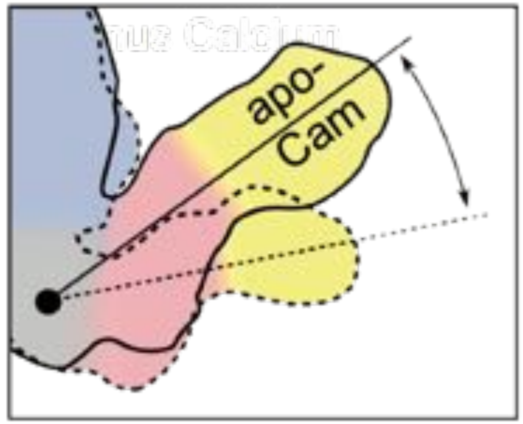
Model so far...



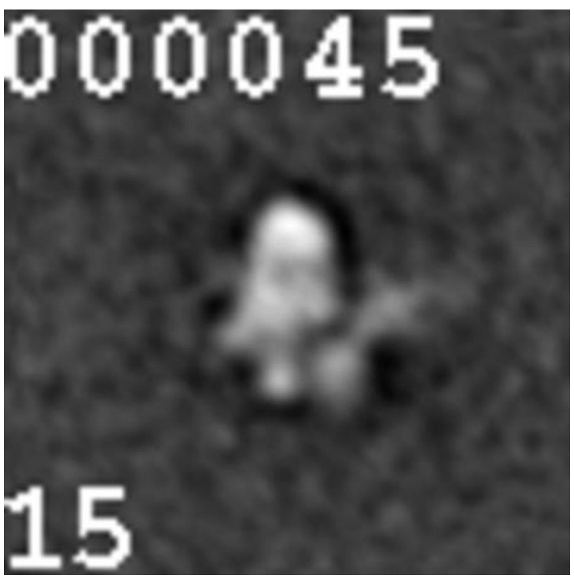


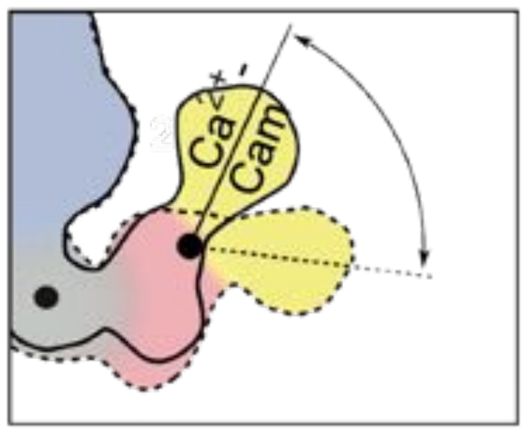
But the mechanical properties ...? Destabilised leverarm..?

Flexibility of the neck region at low and high Calcium



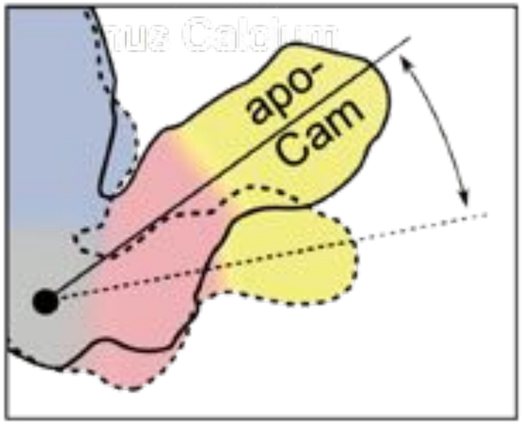
Low calcium:
- single pivot
  => at the converter

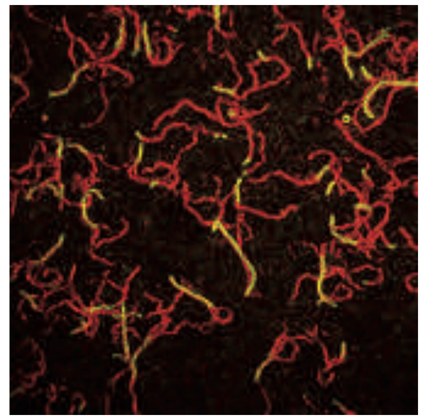


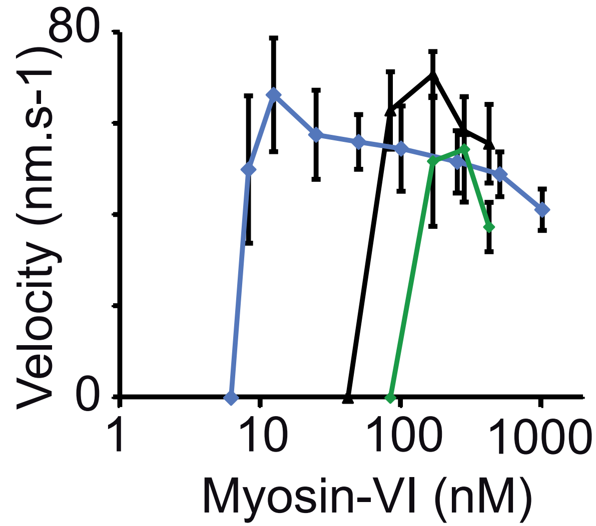


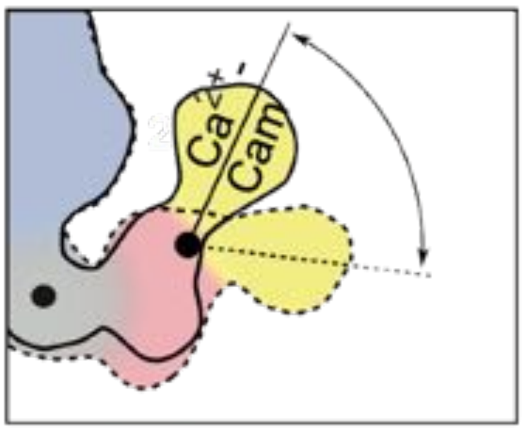
High calcium:
- two pivots
  => at the converter
  => between the two calmodulins

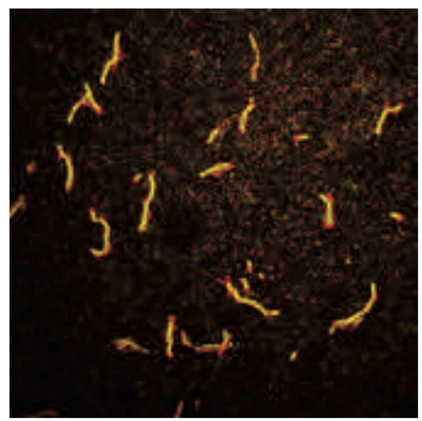
In vitro motility requires low Calcium




motile



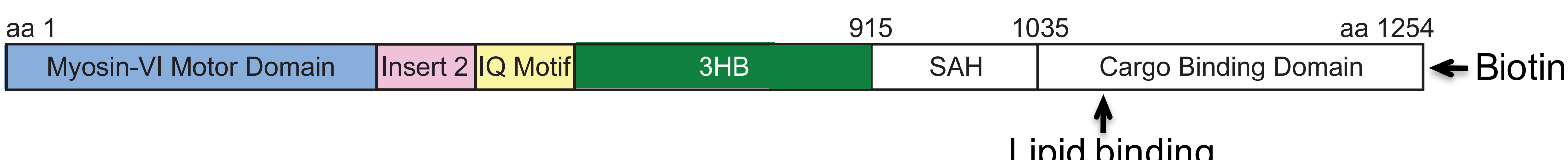



non-motile

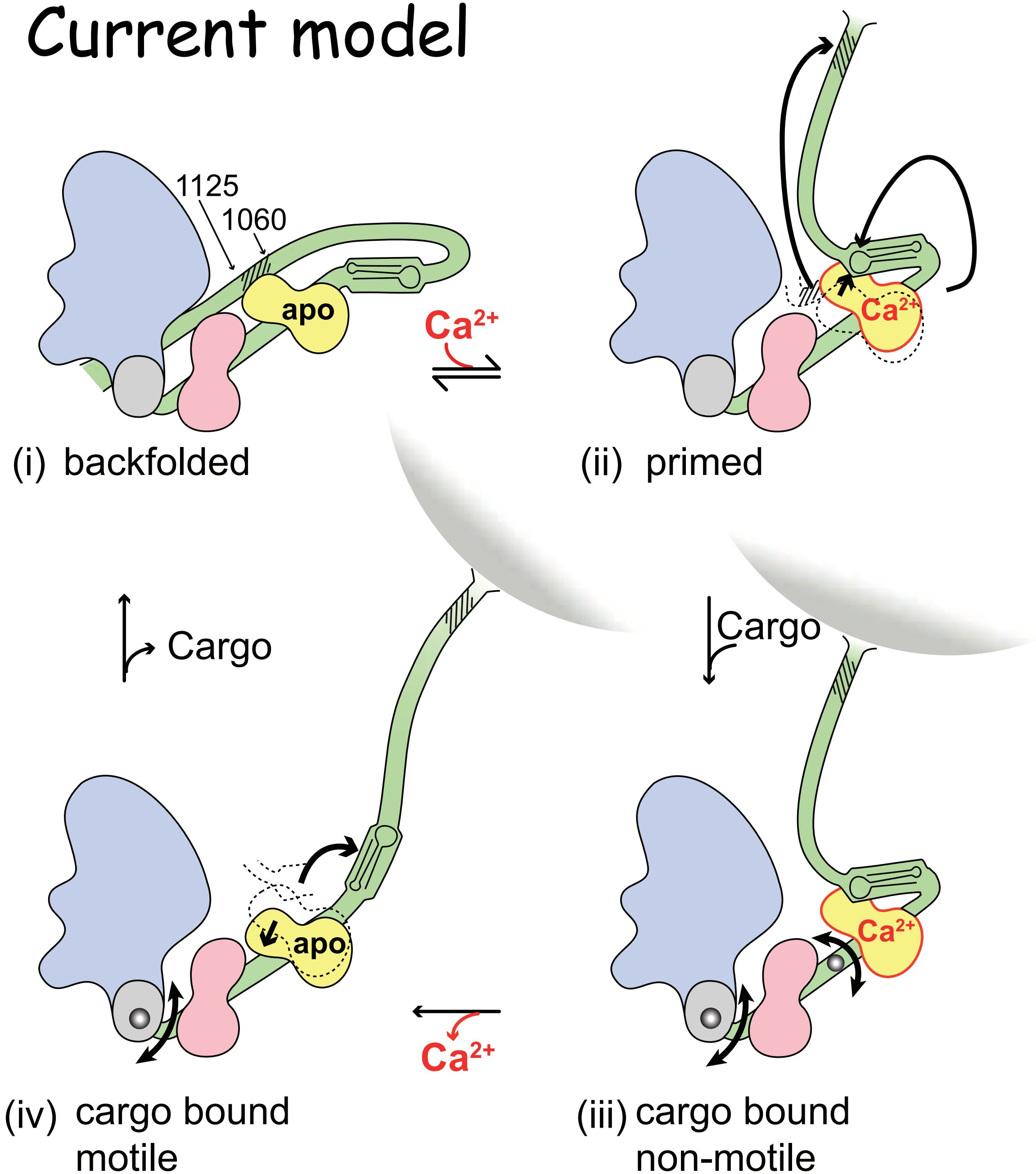
Folch lipid bilayer

GT-tail antibody

C-terminal biotin
(Streptavidin surface)



Current model



Batters et al. (2016) PNAS

Calcium regulation of myosin-VI is a two-stage process:

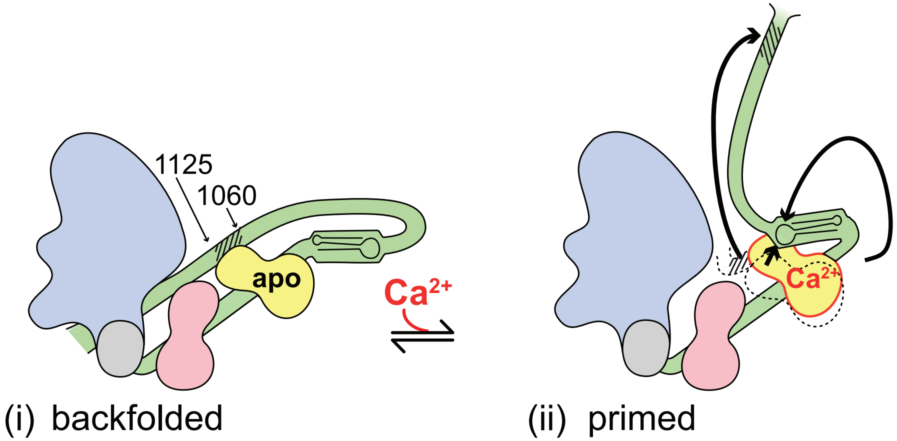
1. Priming -> target binding
2. Mechanical activity

Next:

- effect of the nucleotide state?
- di-/oligomerisation when target binding?
- single molecule mechanics

Single molecule mechanics, regulation, targeting and activation of myosin motors

movie



Our group:

Masters-students, PhD-Students, and Postdocs from Physics und Biochemistry

(international, from England, France, Italy, Ukraine, and Germany)

New Masters- and PhD-Projects in electro-microscopy and single molecule mechanics

claudia.veigel@med.uni-muenchen.de

Division of Cellular Physiology and CeNS LMU Munich