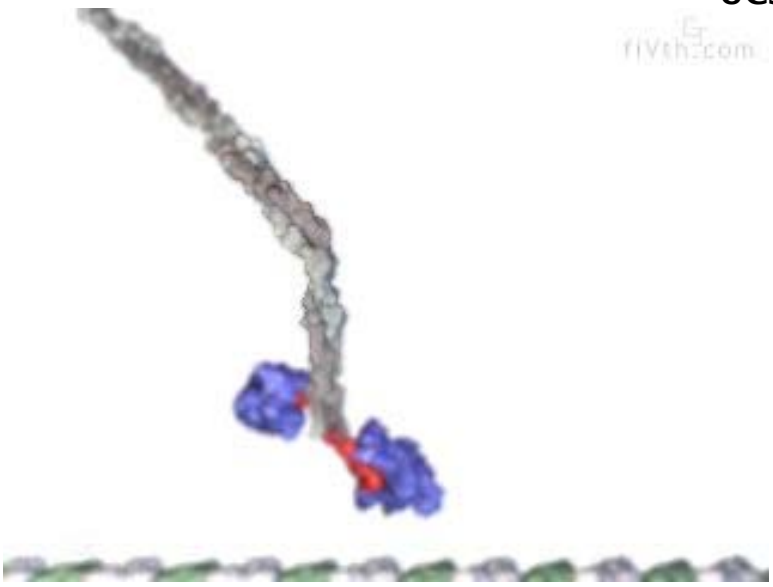
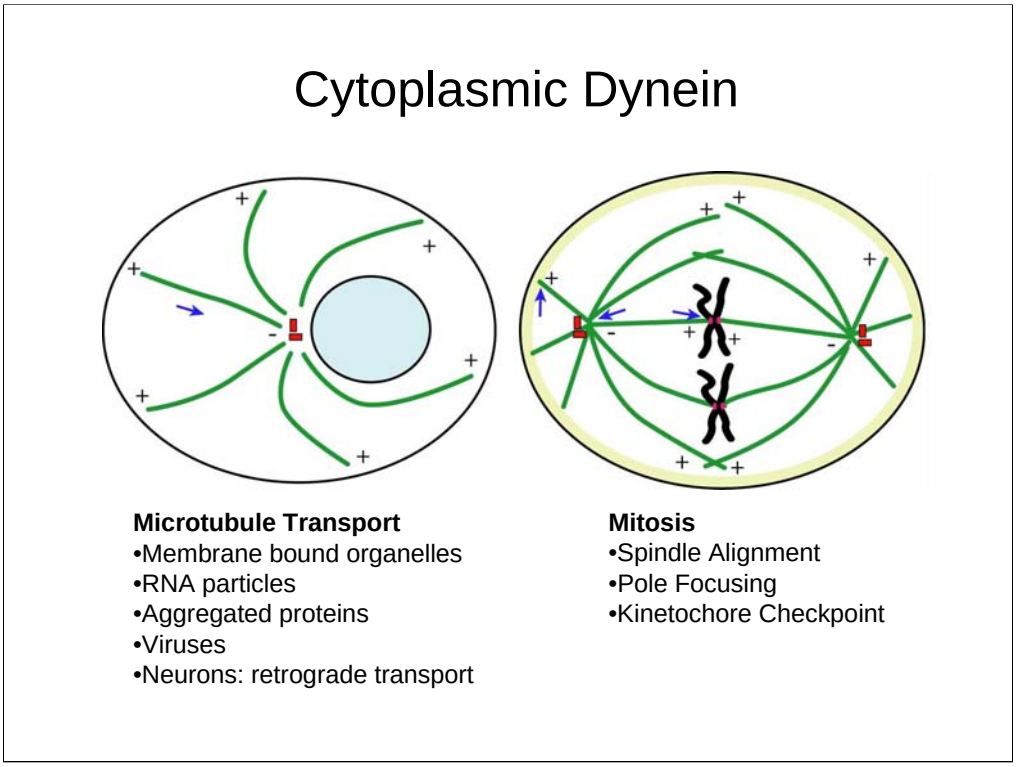
Part II: Single Molecule Approaches for Understanding Molecular Motors

Ron Vale
UCSF, HHMI



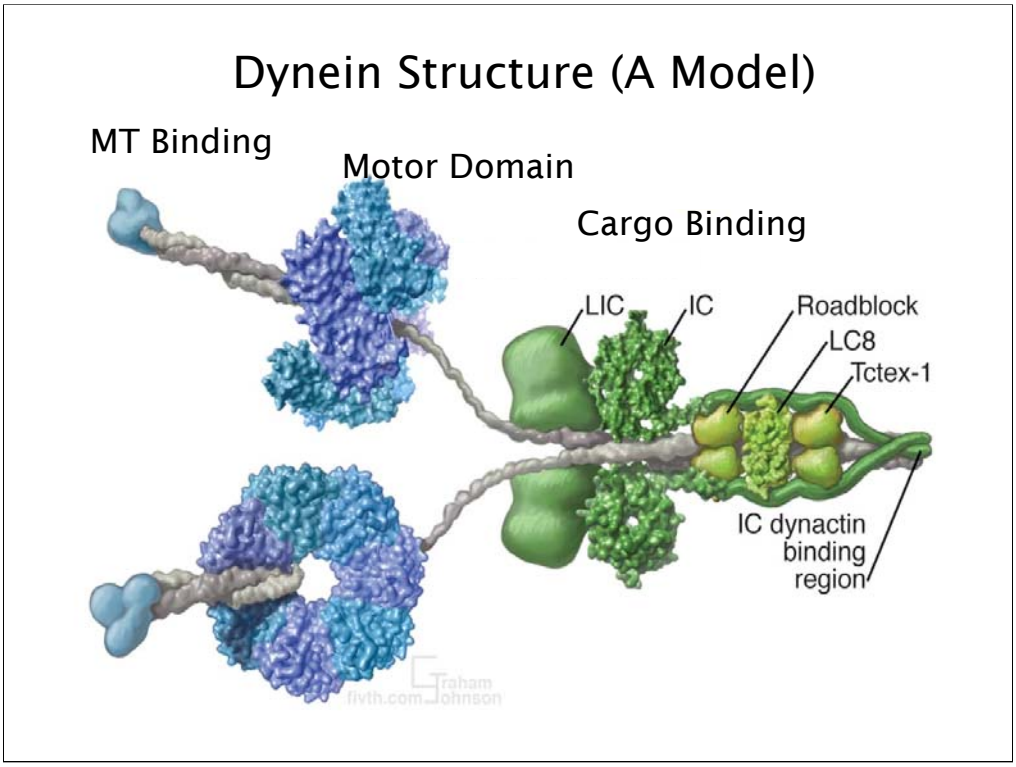


In interphase cells cytoplasmic dynein is responsible for carrying a diverse range of cargos back towards the nucleus (Figure 1A). These include membrane bound organelles (components of the endosome pathway [2], golgi vesicles [3] and peroxisomes [4]), viruses [5], transcription factors [6], aggregated proteins [7] and mRNA containing particles [8]. In neurons dynein drives retrograde transport back along axons towards the cell body [1, 9].

Cytoplasmic dynein also plays a fundamental role in mitosis (Figure 1B) [10-12]. It has been found at the cortex (where it pulls on microtubules attached to the spindle poles [13-15]) and at the spindle pole (where it accumulates after transporting factors required for focusing of the poles [16, 17]). It has also recently been localized to the kinetochore where it appears to play a role in the checkpoint that monitors correct attachment of the spindle to the chromosome [18, 19].

Dynein is Larger than Other Cytoskeletal Motors

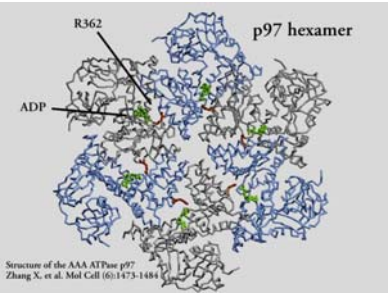
Motor	Size (KDa)
Dynein	~500
Myosin	~100-200
Kinesin:	~100



Before I presented summaries of motor proteins, kinesin and myosin, where we do have reasonable knowledge about their mechanism. Here I would like to discuss current work going on in our laboratory to study dynein, a minus end directed microtubule motor protein. This is a motor that we know relatively about, and I will not be able to provide for you a completely satisfying model in the end. Rather, you will get a very much in-progress view of how we are trying to decipher how this motor works.

Dynein is a very large and complex motor, about an order of magnitude bigger than kinesin. The motor domain is found in the ring and unlike kinesin and myosin, the microtubule binding domain extends from the ring at the tip of this anti-paralllel coiled coil stalk. The tail domain is contained at this regions, where numerous dynein associated proteins are found.

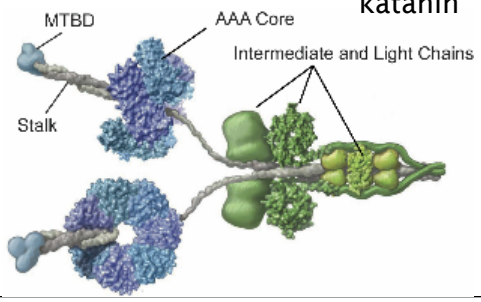
Dynein Emerged from the AAA ATPase Lineage, Not from G proteins/Kinesin/Myosin



Some well known AAA proteins include:

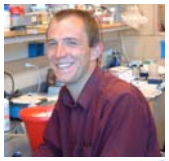
Protease-Associated AAAs
- ClpX (bacteria).
- proteosome lid)

Destablizing AAAs
- p97,
- NSF,
- katanin



How Does Dynein Work?

Andrew Carter



Sam Reck-Peterson

Ahmet Yildiz

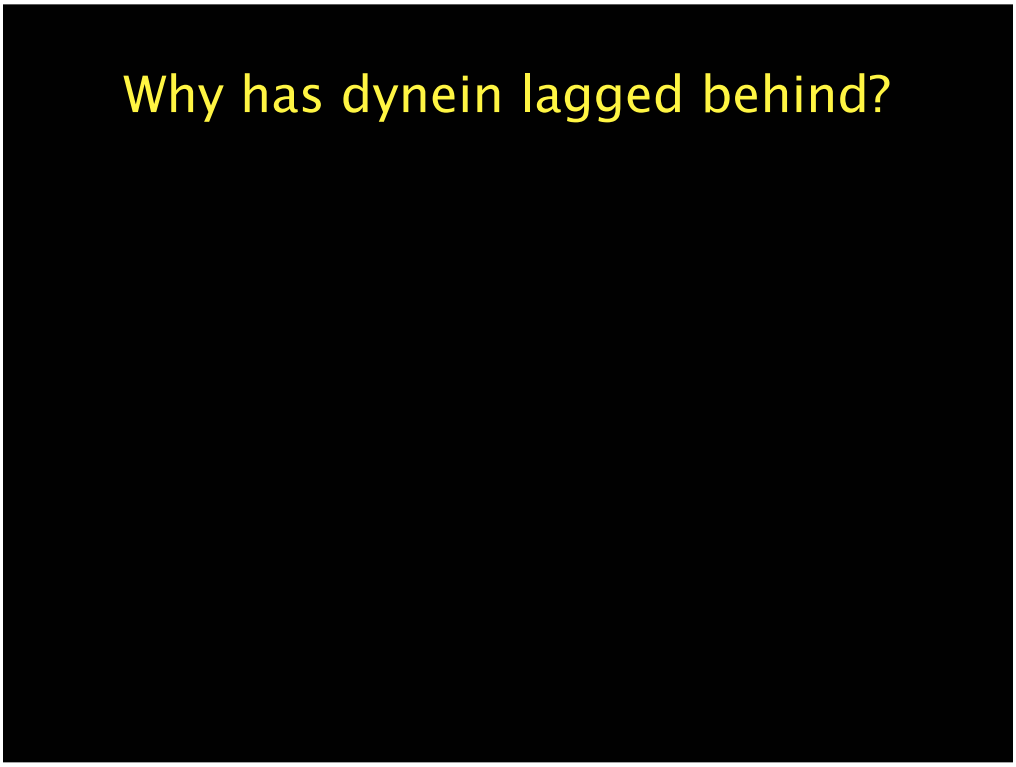
Arne Gennerich

Why has dynein lagged behind?

Big = 10-fold larger than kinesin; difficult to express

Complex- many associated proteins

Little structural information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Simplified motor constructs
that are easier to study

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In vitro assays

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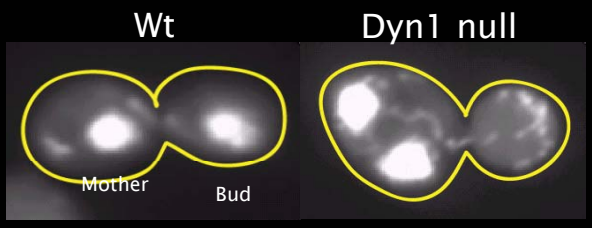
Crystal structures

Producing Recombinant Dynein in Yeast



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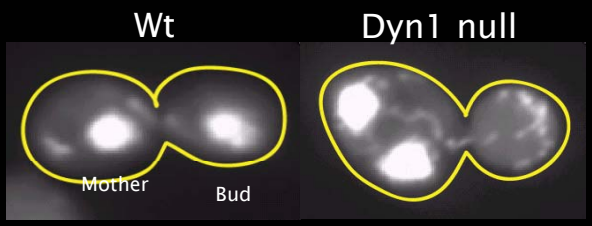
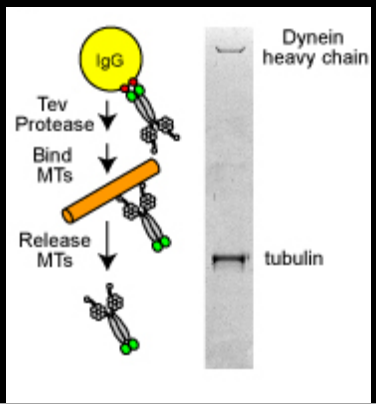
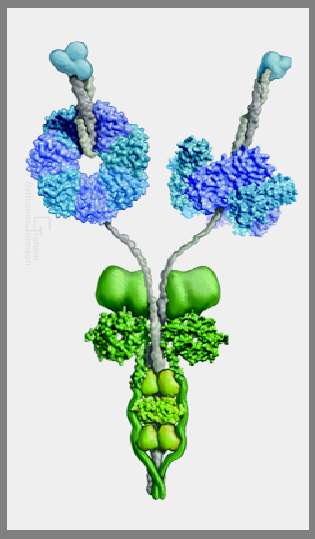
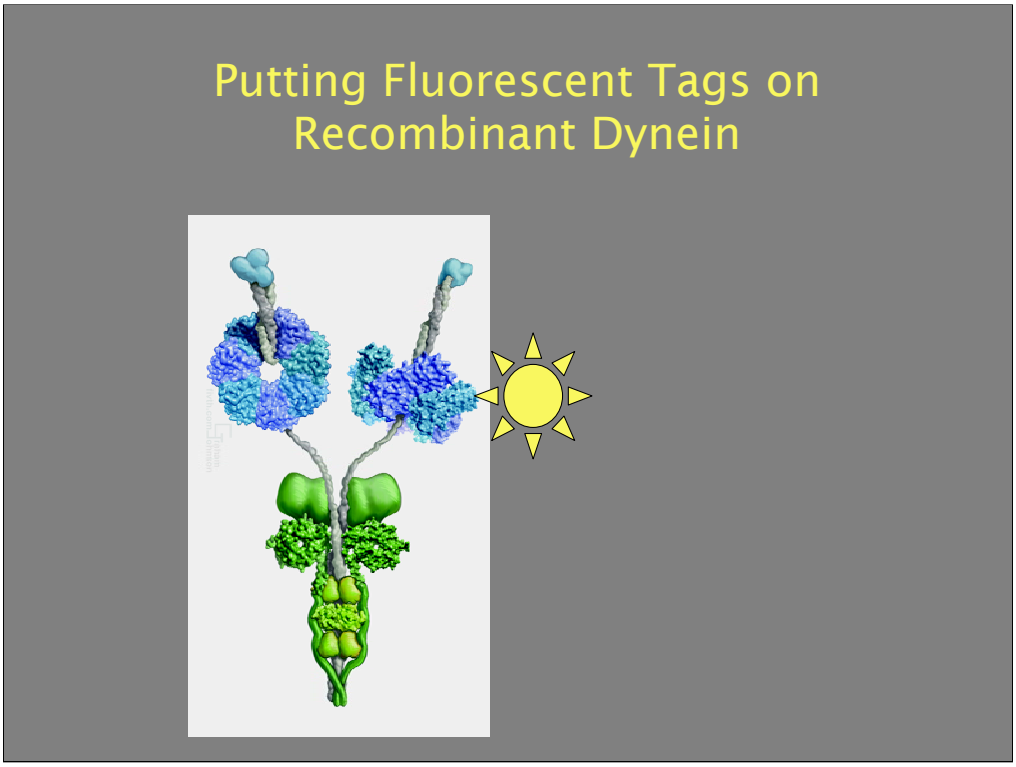




Putting Fluorescent Tags on Recombinant Dynein



Other tags can be added to other regions of site specific covalent labeling. For example, I will show experiments where we have been able to place a Qdot (explain)



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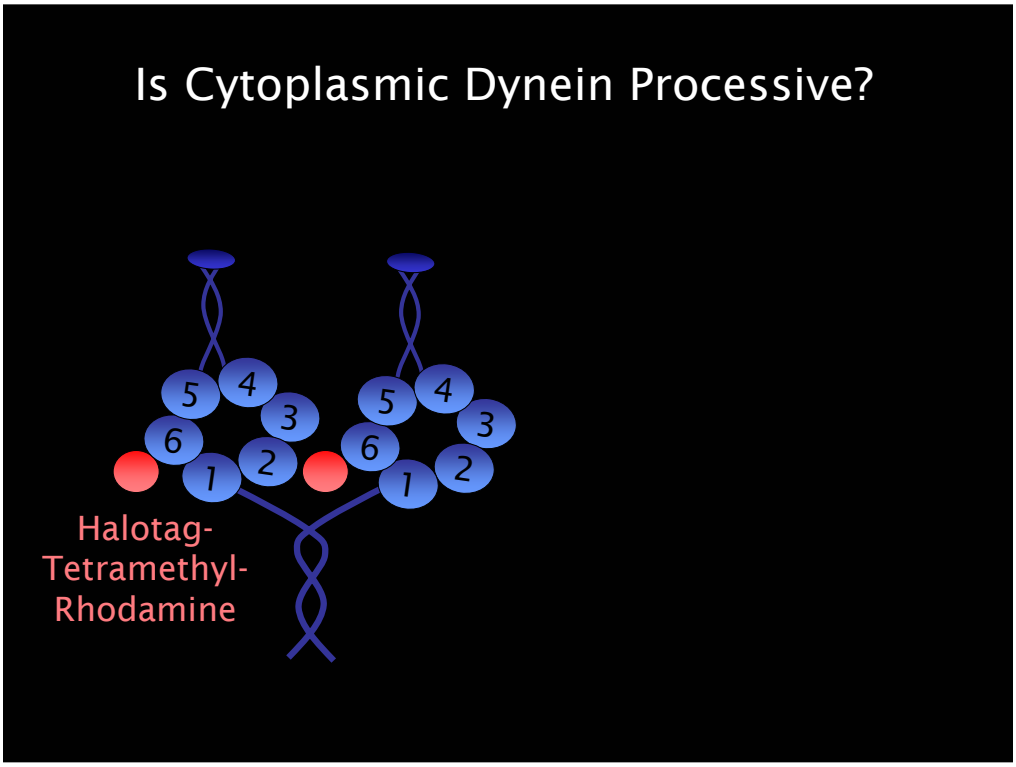
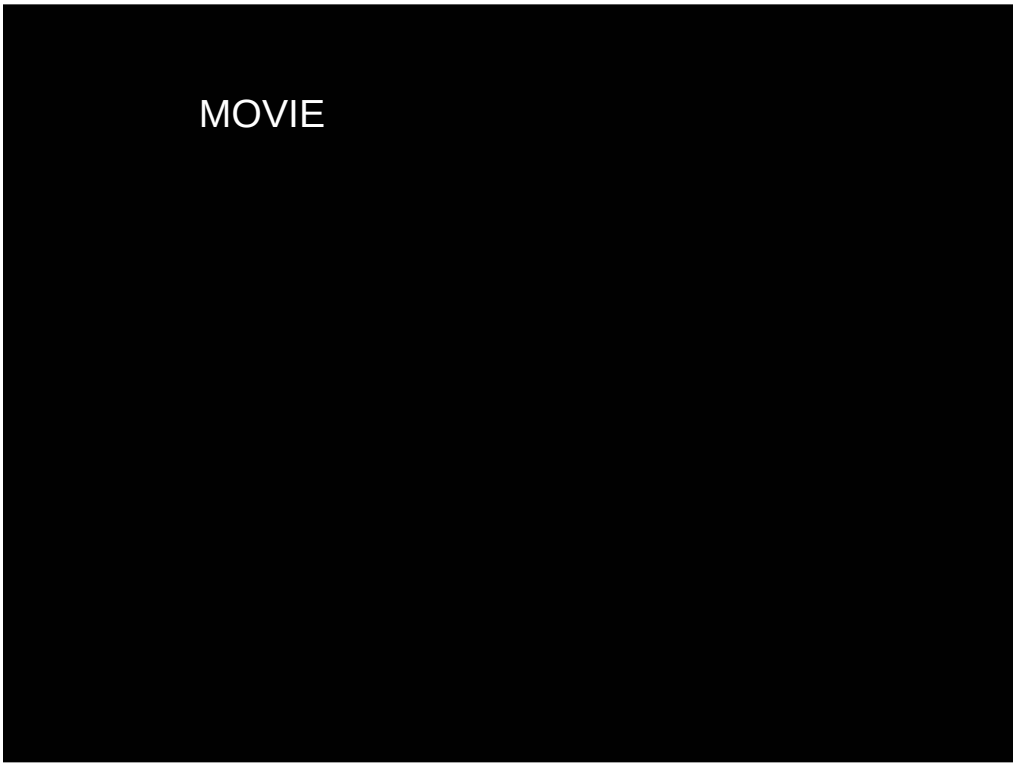


TIRF microscopy: only the glass/ water interface is illuminated (~100nm). Conventional microscopes illuminate a nuch larger region. Using TIRF cuts down on the background flourescence.



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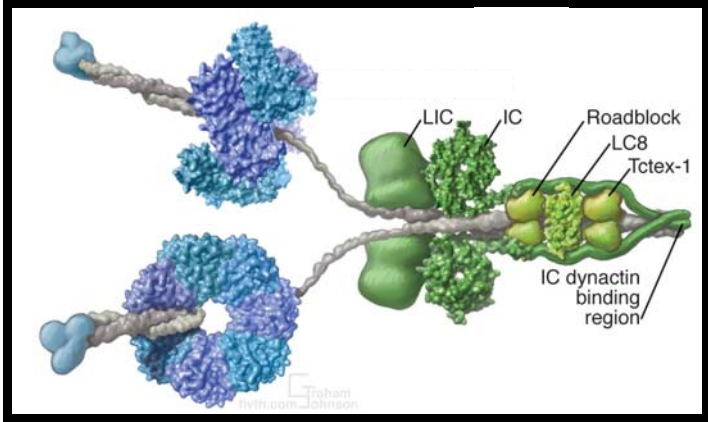
What are the requirements for dynein processivity?

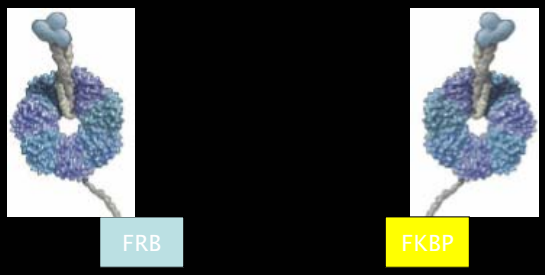


We wanted to know whether a cytoplasmic dynein monomer was processive. So we made a monomeric dynein which contains about 600 aa of the dynein tail domain. This protein is a monomer based on sucrose gradient and gel filtration experiments.

Monomeric dynein is non-processive, but an ensemble of monomeric motors can induce movement

In vitro gliding assay of monomeric dynein

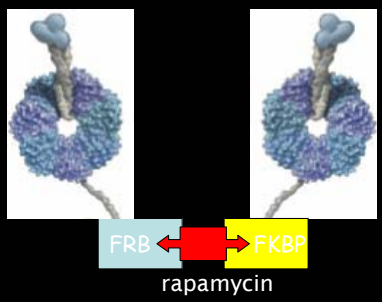
Are Two Heads are Better than One?



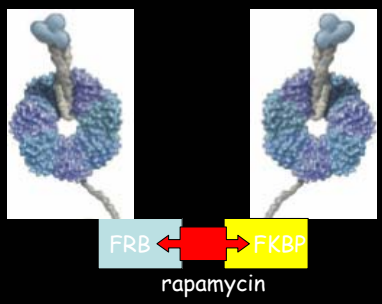
No Processive Movement

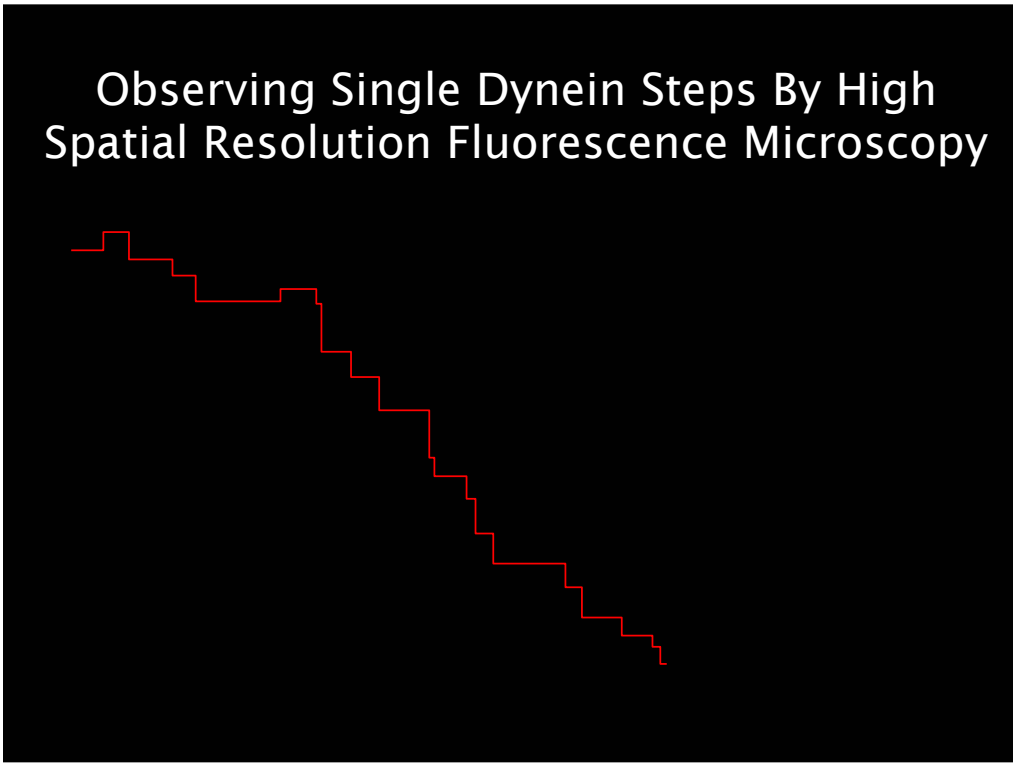

Are Two Heads are Better than One?
FRB
FKBP
rapamycin

Are Two Heads are Better than One?

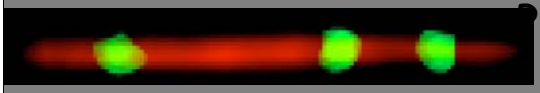


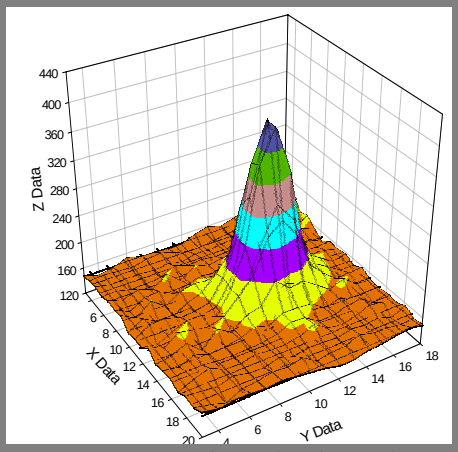
Are Two Heads are Better than One?




Observing Single Dynein Steps By High
Spatial Resolution Fluorescence Microscopy

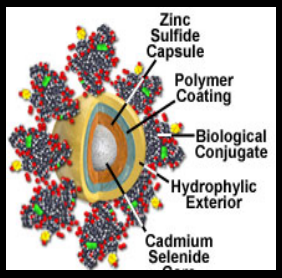
Tracking Fluorescent-Dynein with ~2 nm Precision





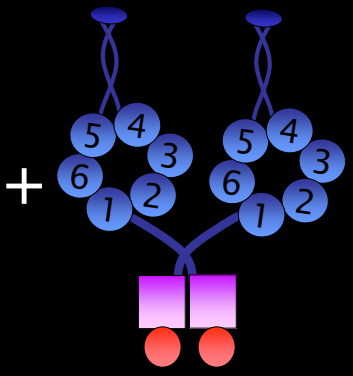
2 D Gaussian of Single Fluorophore
A. Yildiz and P. Selvin, U. Illinois

Quantum dot- labeled dynein



Streptavidin-Qdot

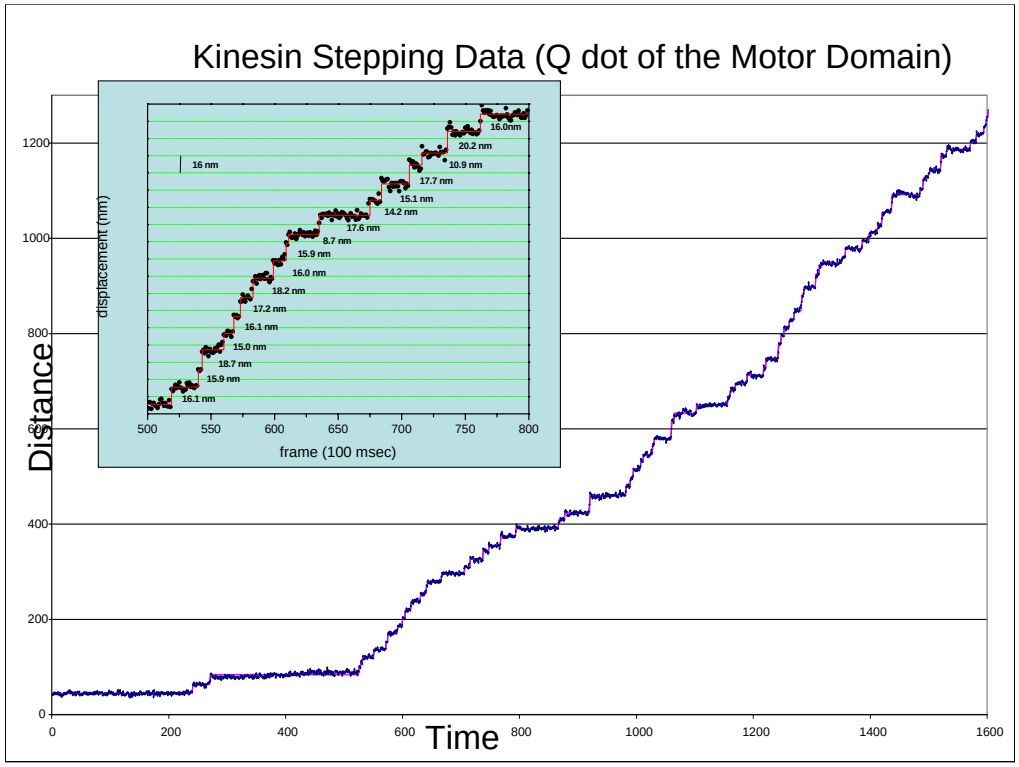
+



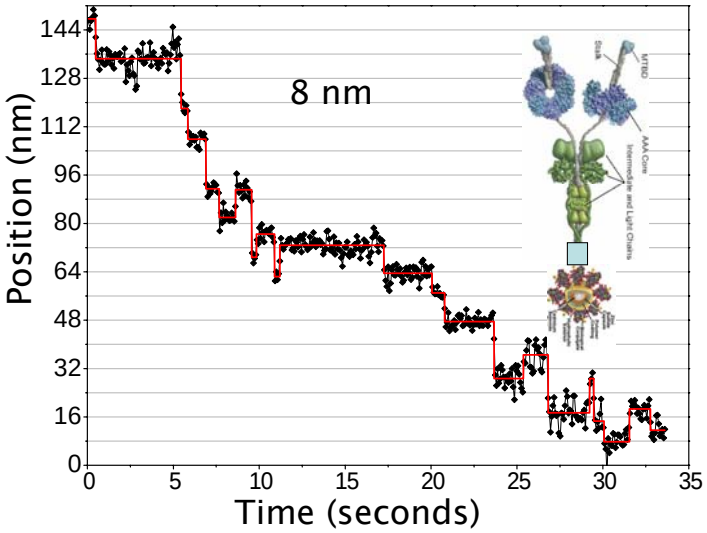
Biotin-Tail

MOVIE

QuickTime™ and a
H.263 decompressor
are needed to see this picture.

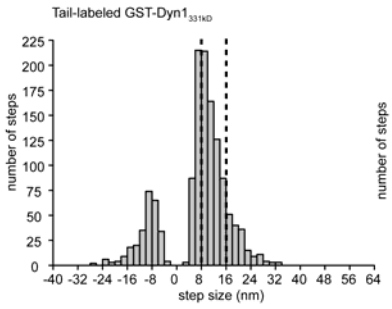

Kinesin Stepping Data (Q dot of the Motor Domain)
Distance
Time
displacement (nm)
frame (100 msec)
16 nm
16.0nm
20.2 nm
10.9 nm
17.7 nm
15.1 nm
14.2 nm
17.6 nm
8.7 nm
15.9 nm
16.0 nm
18.2 nm
17.2 nm
16.1 nm
15.0 nm
18.7 nm
15.9 nm
16.1 nm

Tail-labeled dynein takes ~8 nm steps as well as larger and backward steps



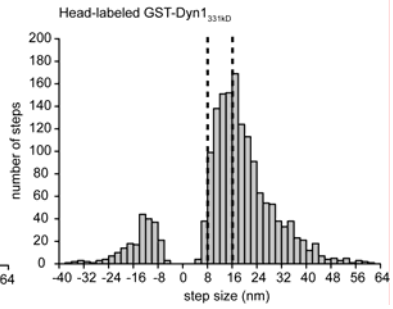
Step Size Data for Cytoplasmic Dynein

Peak at 8 nm

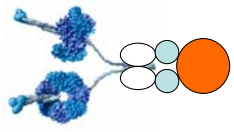


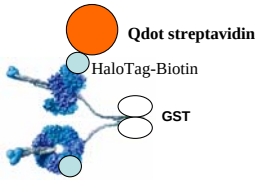
Tail Labeled Dynein

Peak at 16 nm

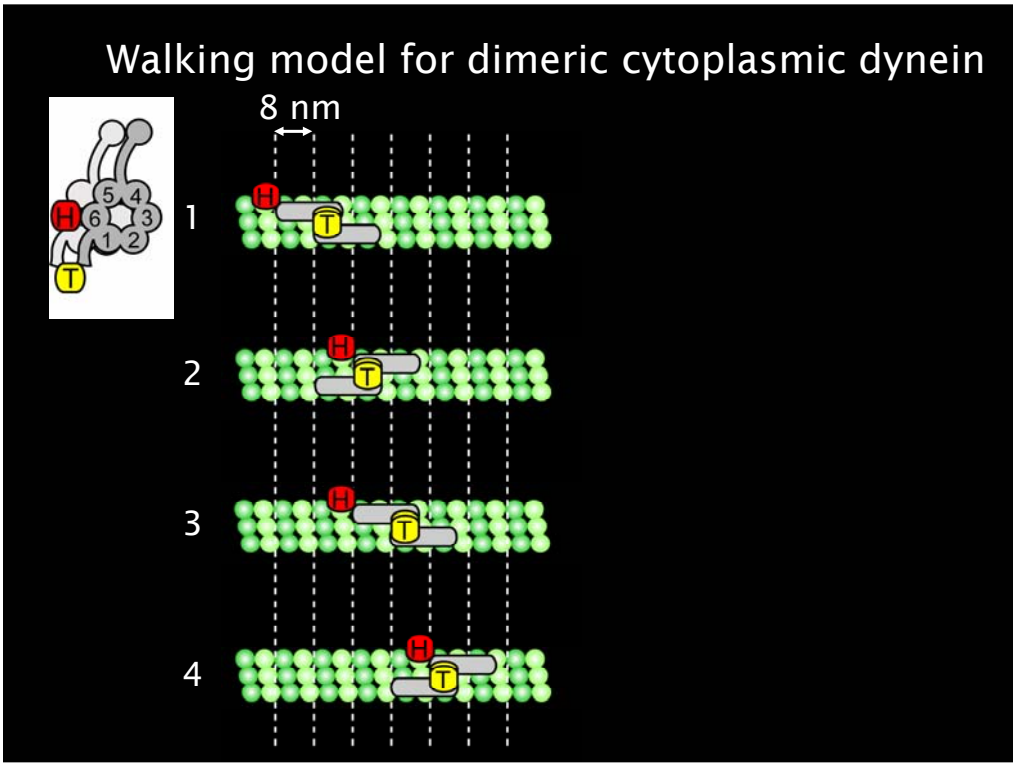


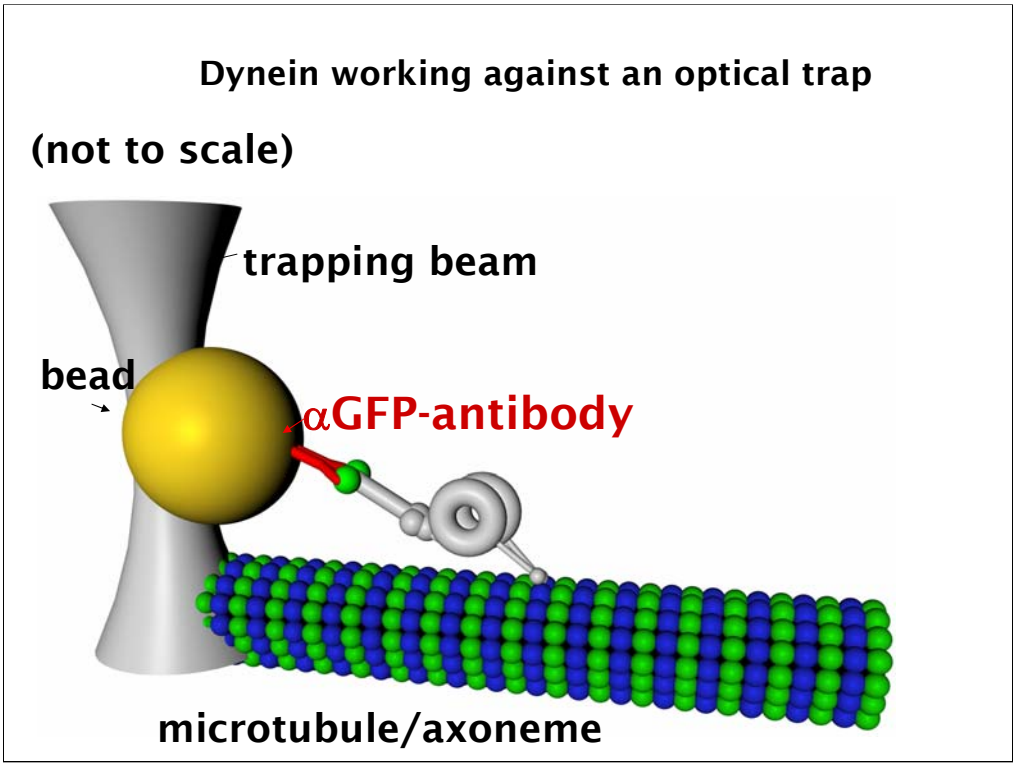
Head Labeled Dynein





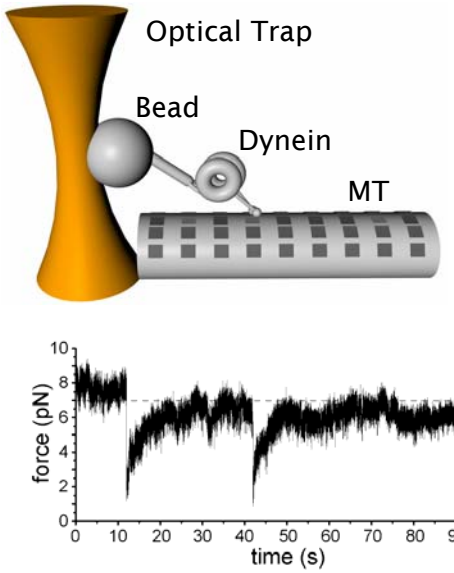
Reck Peterson et al (2006)


Walking model for dimeric cytoplasmic dynein
8 nm
H
T
1
2
3
4
5
6

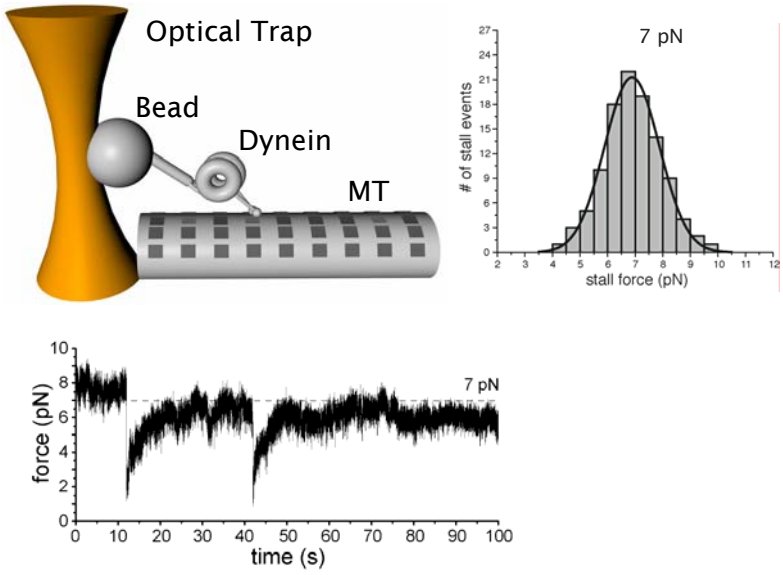

Dynein working against an optical trap
(not to scale)
trapping beam
bead
αGFP-antibody
microtubule/axoneme

MOVIE

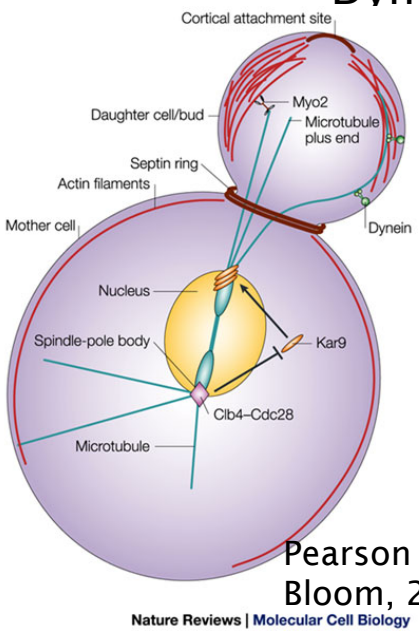
Dynein working against an optical trap



Dynein working against an optical trap



Biological Role of Yeast Cytoplasmic Dynein

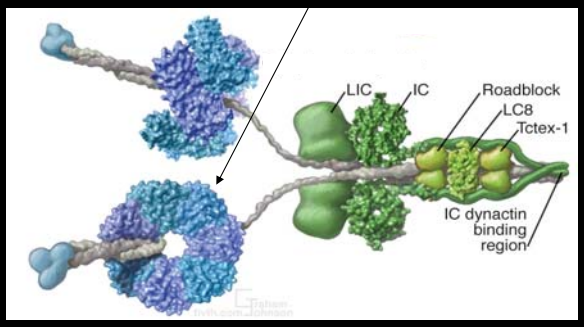


Pearson and Bloom, 2004

Nature Reviews | Molecular Cell Biology

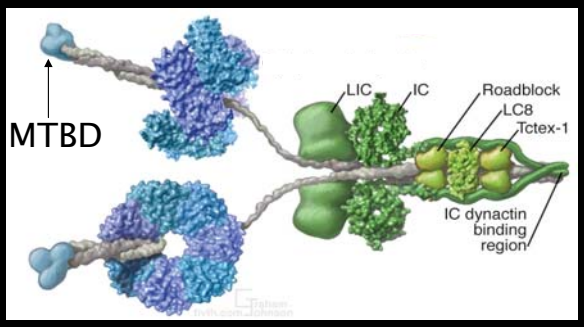
Dynein- What Next?

4 ATP binding sites



What the four ATP binding sites in dynein doing?

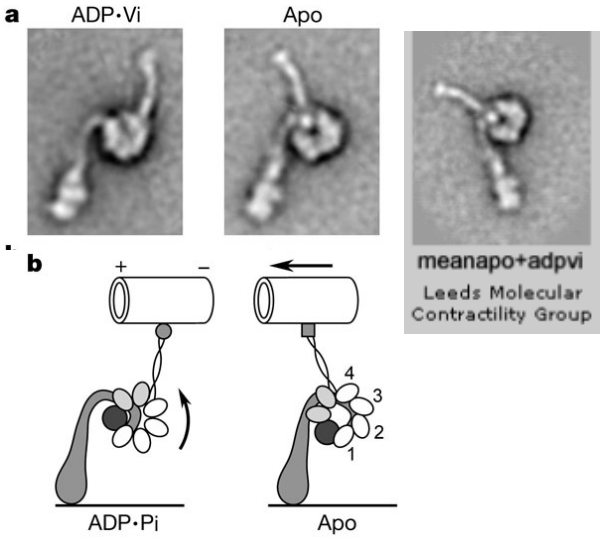
Dynein- What Next?

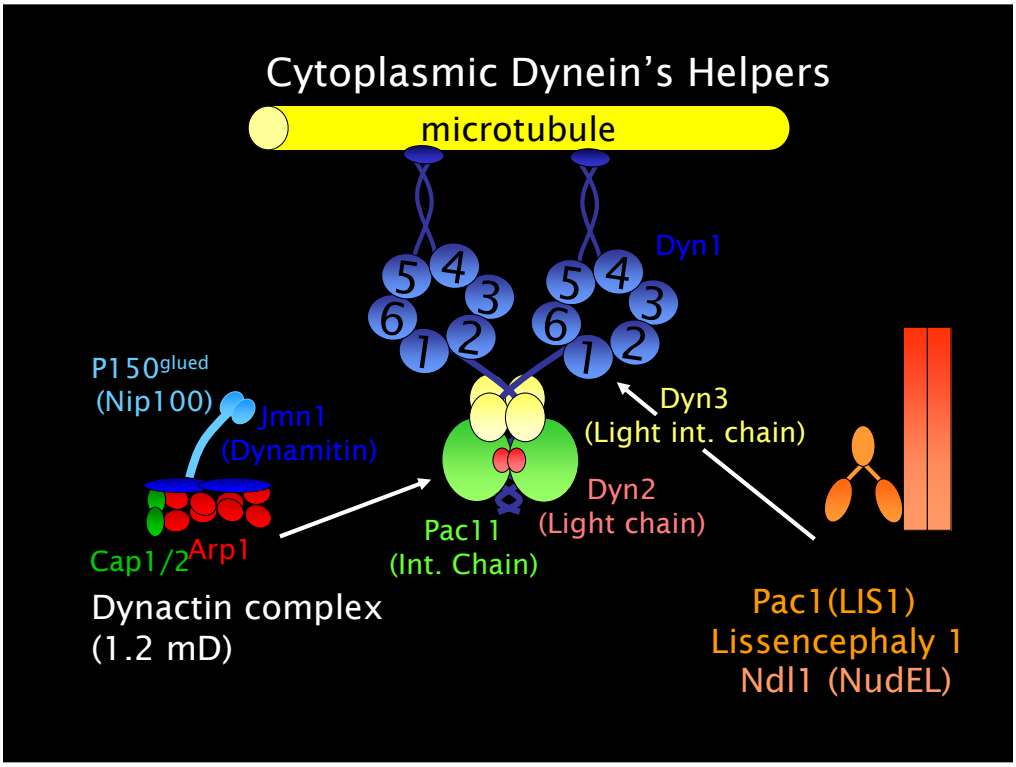


How does the microtubule binding domain (MTBD) communicate with the ATPase ring?

Dynein- What Next?

EM studies by Burgess, Knight, Oiwa et al.



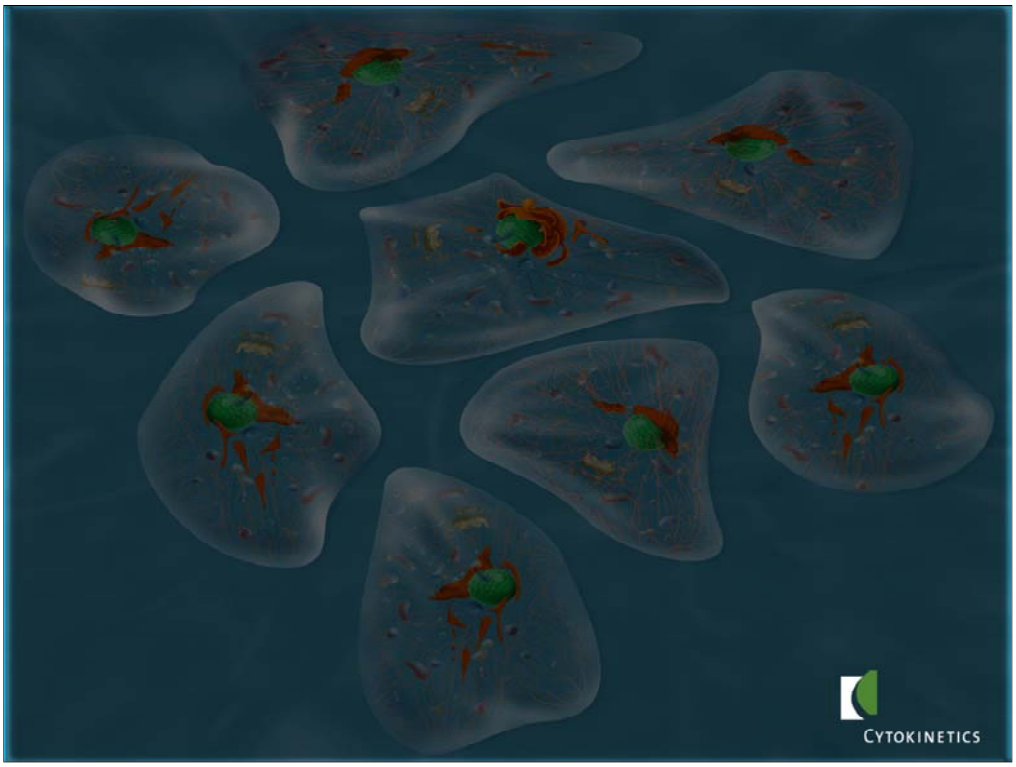


We can go back and embrace its complexity

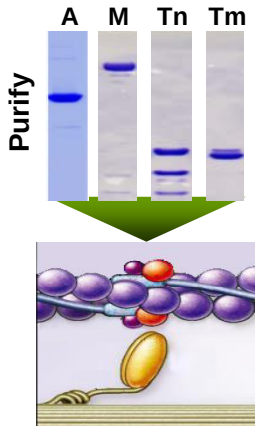
Apply our knowledge of motor proteins to practical outcomes?

Small molecule drugs with therapeutic benefit?

Engineer motors for cells or nanotechnology?


CYTOKINETICS

Reconstituting the Sarcomere for Drug Discovery



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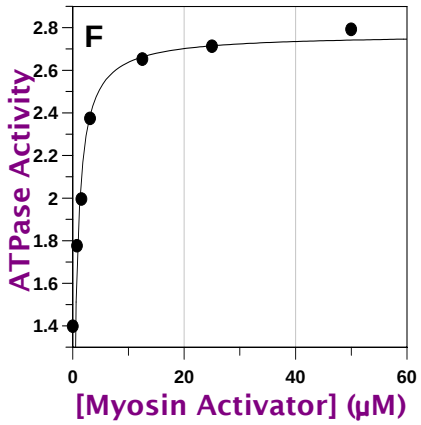

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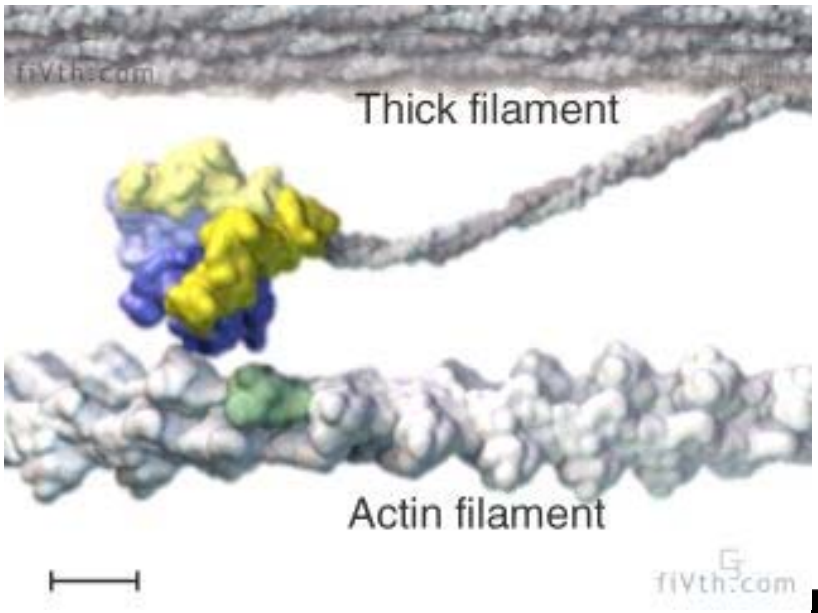
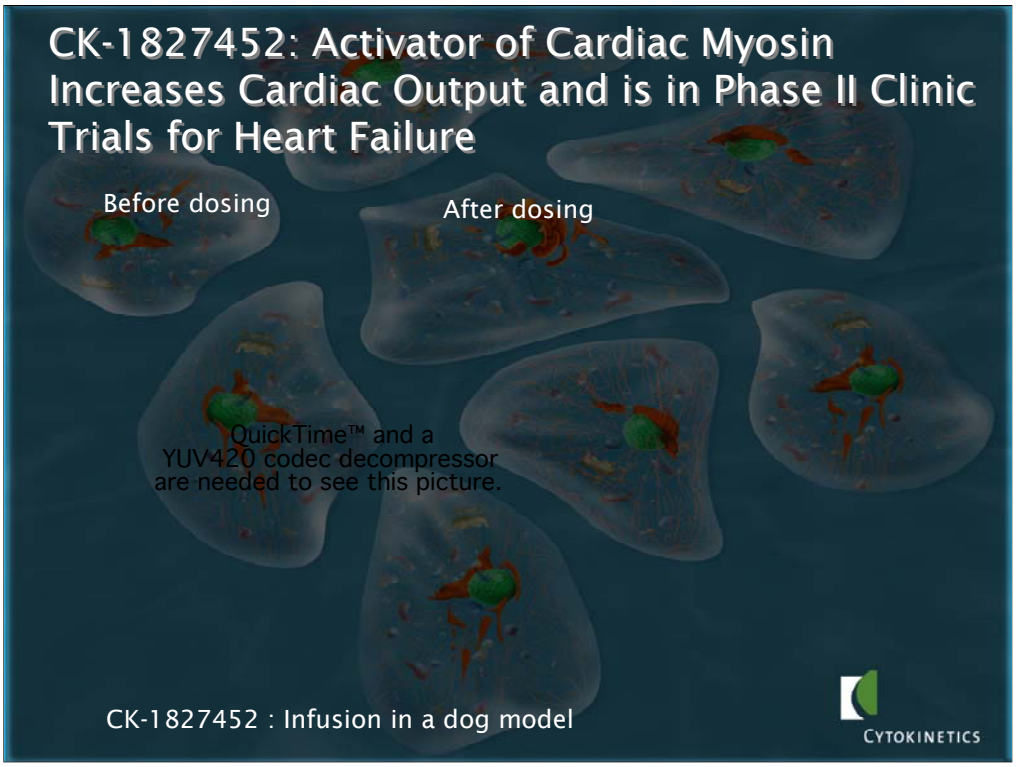
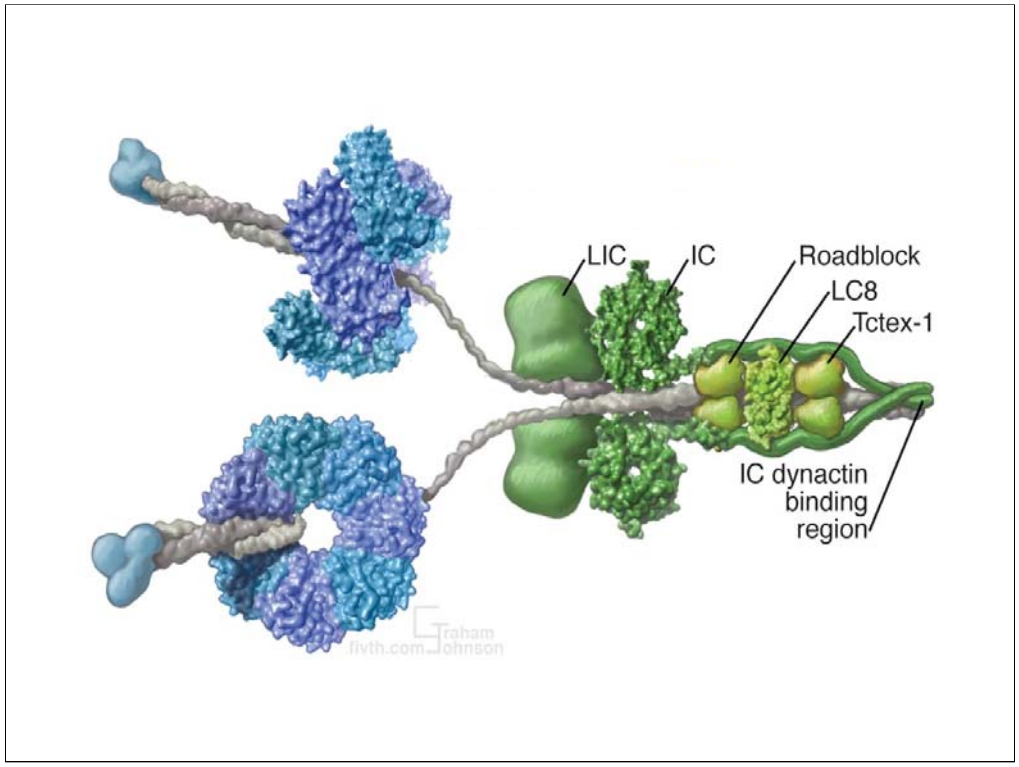
CYTOKINETICS

Myosin Activating Drugs Accelerate Phosphate Release




CK-1827452: Activator of Cardiac Myosin Increases Cardiac Output and is in Phase II Clinic Trials for Heart Failure
Before dosing
After dosing
QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.
CK-1827452 : Infusion in a dog model
CYTOKINETICS



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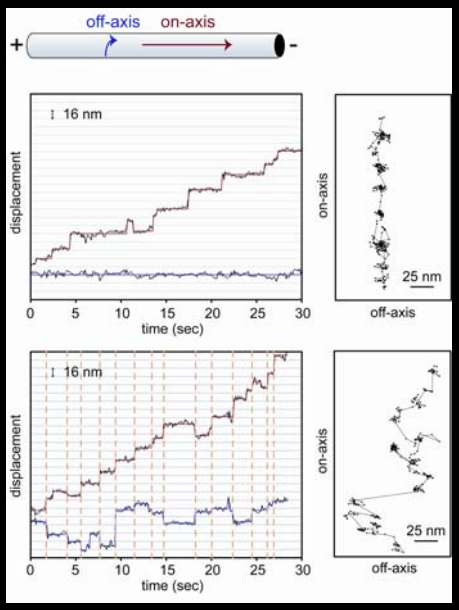
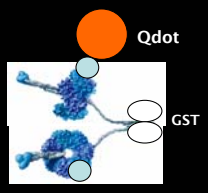
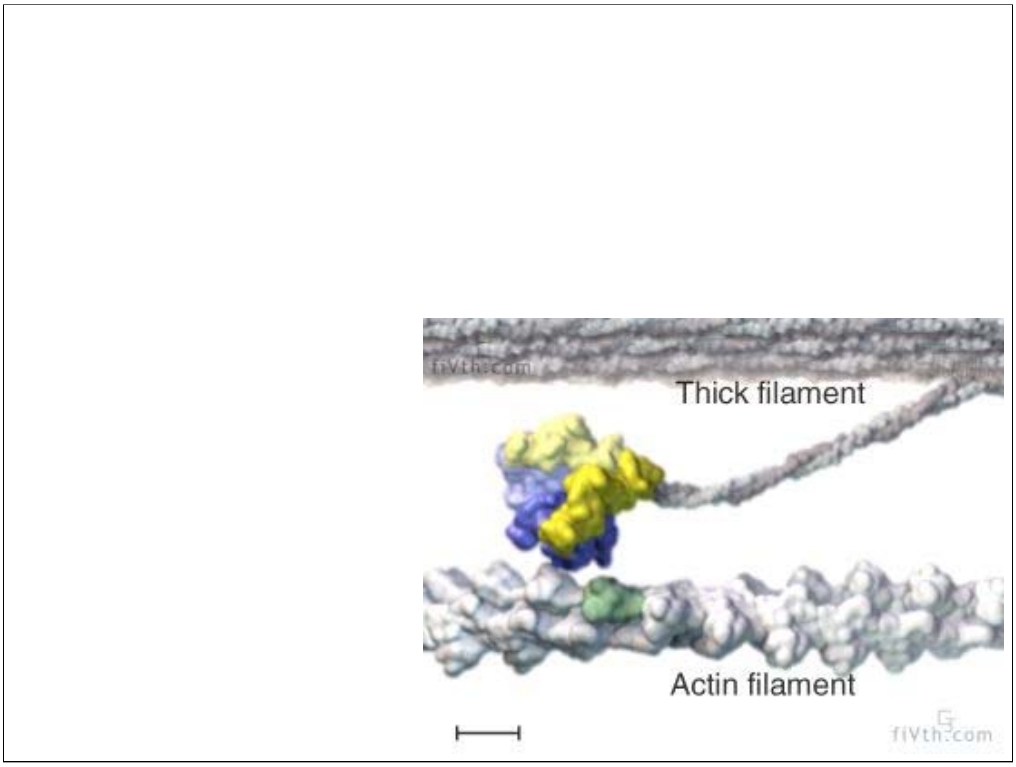
Cytoplasmic Dynein Stepping



Head Labeled Dynein

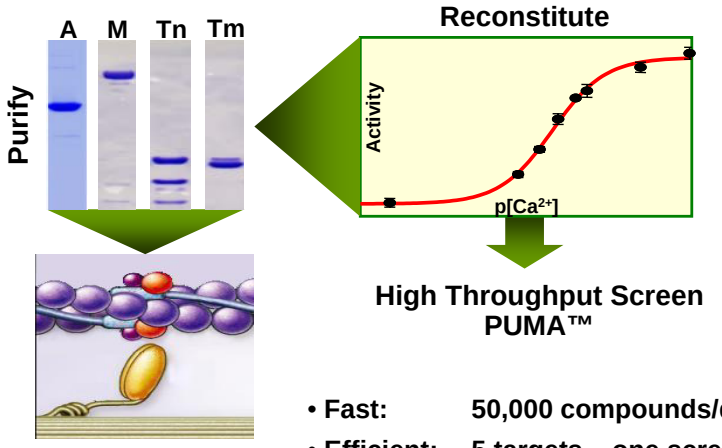


Reck Peterson et al (2006)



These movie animations, made by Graham Johnson, and based upon actual crystal structures and decades of research on myosin illustrate howthe force generating cycle of myosin is thought to work.

Reconstituting the Sarcomere for Drug Discovery



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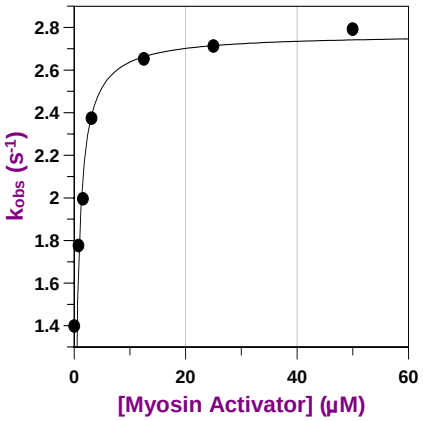
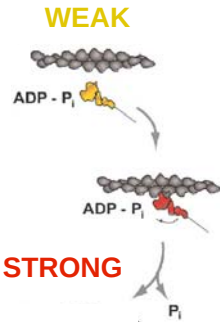
CYTOKINETICS

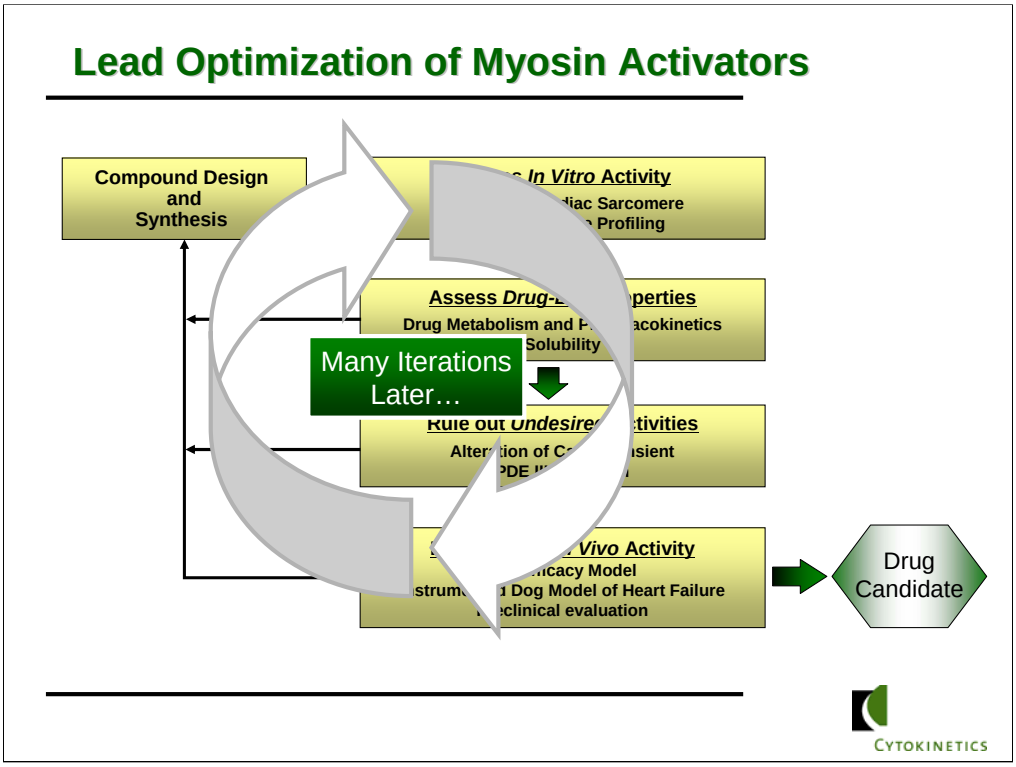
Myosin activators increase productive ATP hydrolysis

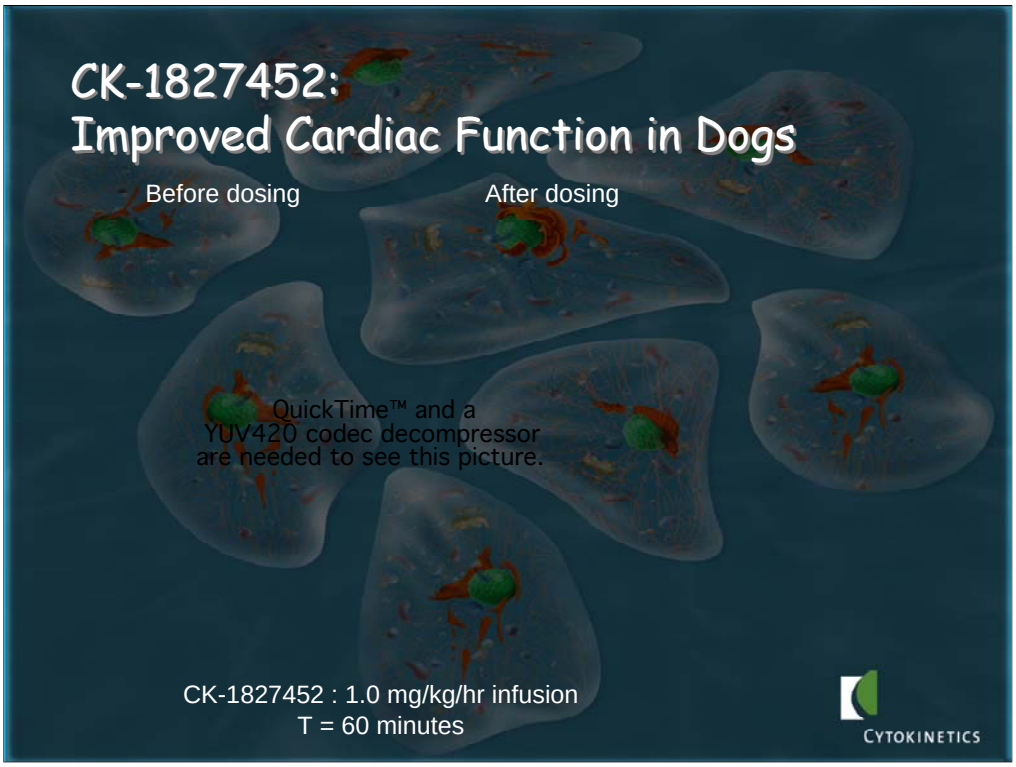




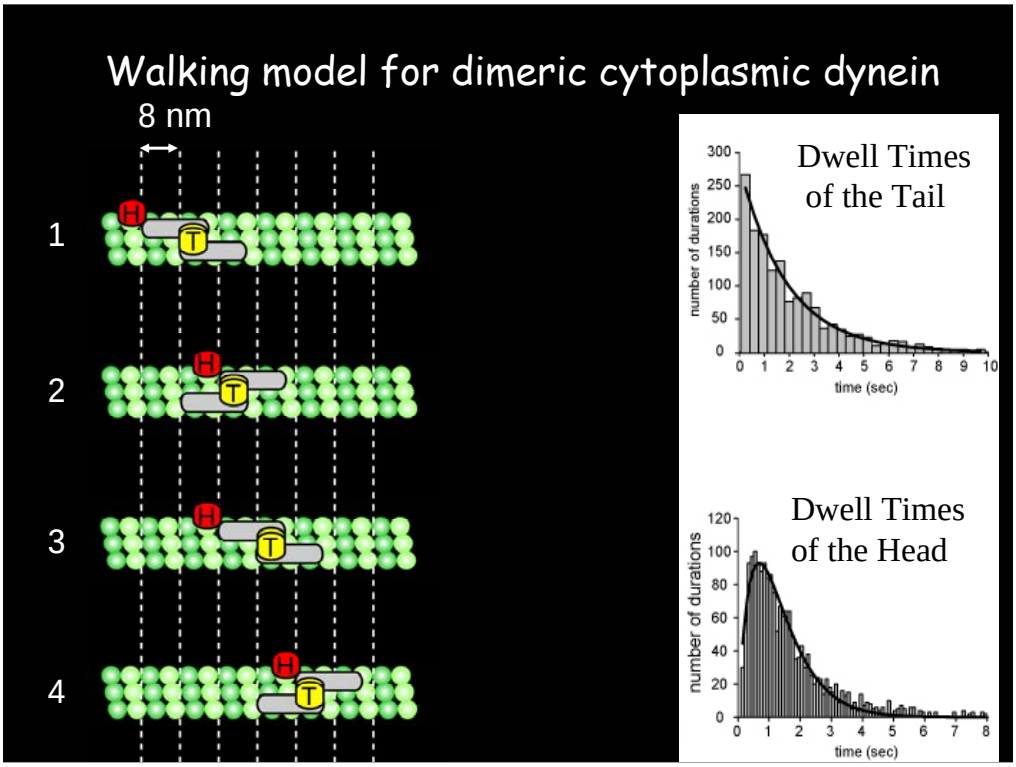
In the <u>presence</u> of actin, the myosin activator *accelerates* phosphate release and thus productive ATP hydrolysis

CYTOKINETICS


Lead Optimization of Myosin Activators
Compound Design and Synthesis
In Vitro Activity
Profiling
Many Iterations Later…
Drug Candidate
CYTOKINETICS


CK-1827452:
Improved Cardiac Function in Dogs
Before dosing
After dosing
QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.
CK-1827452 : 1.0 mg/kg/hr infusion
T = 60 minutes
CYTOKINETICS

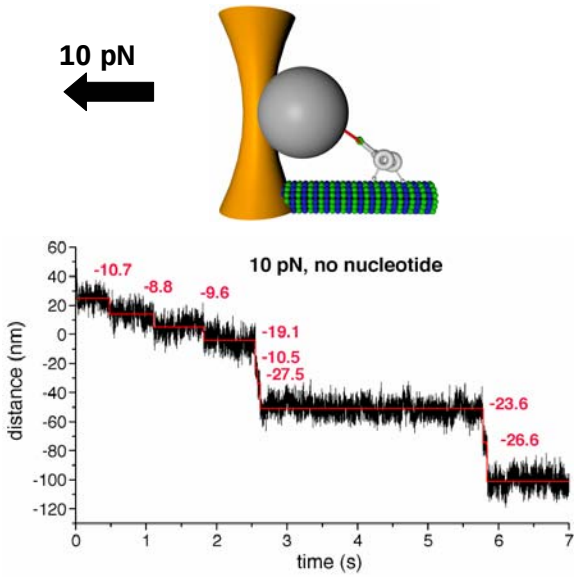

Myosin Activators Now in Phase II Clinical Trials
Before dosing
After dosing
QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.
CK-1827452 : 1.0 mg/kg/hr infusion
T = 60 minutes
CYTOKINETICS



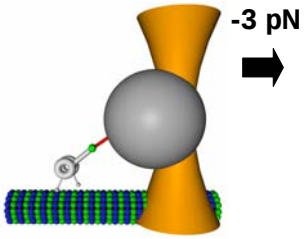
Dimension of molecule and our stepping data: we favor the rings being stacked. Other AAA proteins rings are stacked.

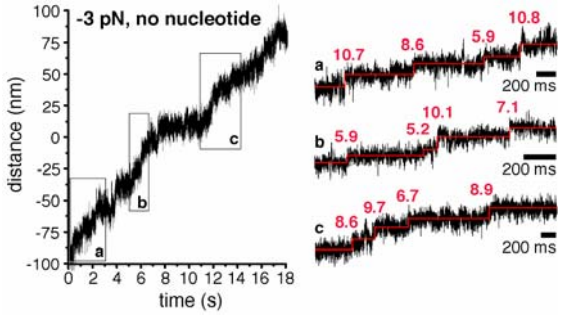
Open questions: what is the structural basis for movement? Is the stem involved?

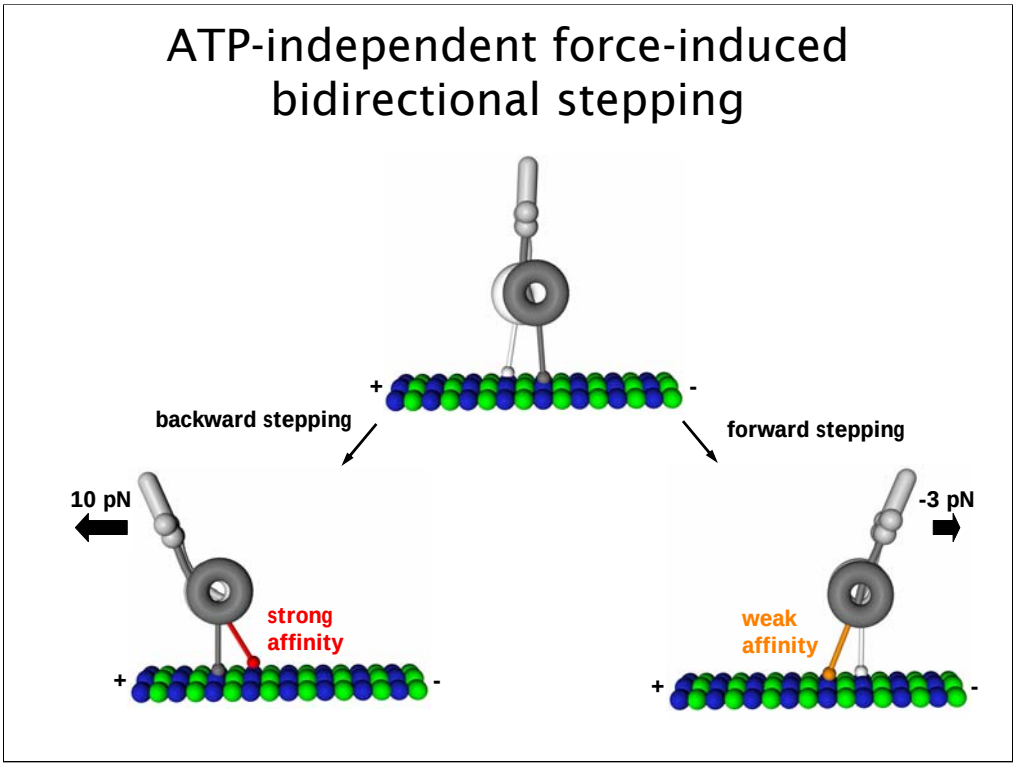
Force can induce dynein stepping without nucleotide

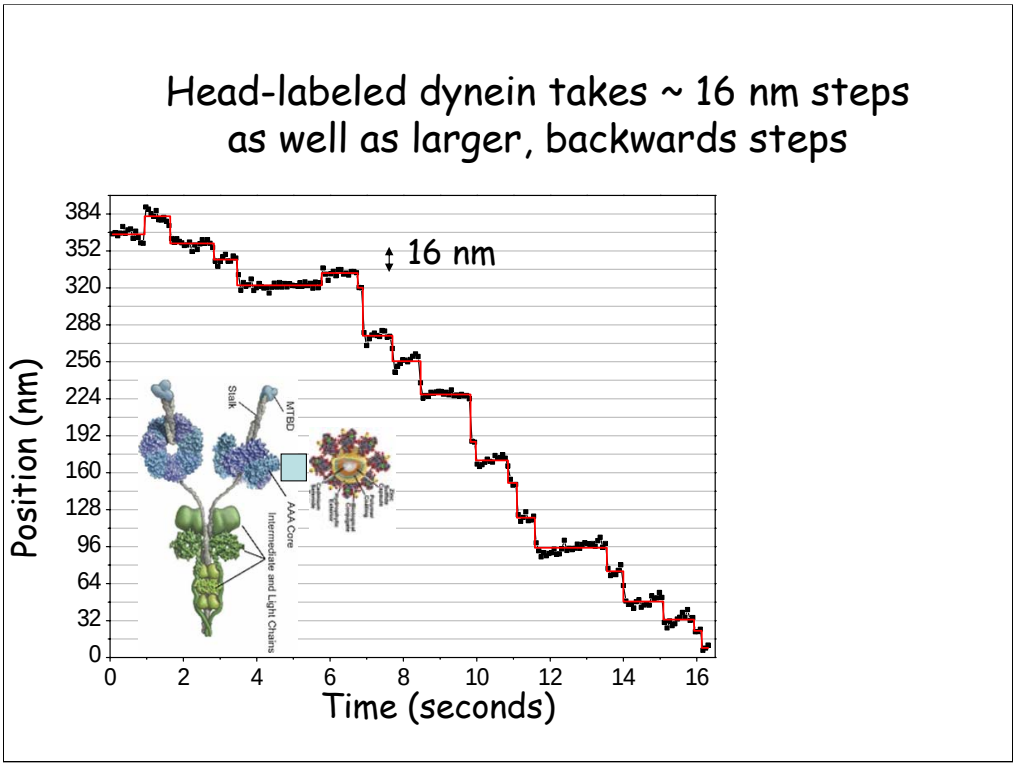


Substall forward loads suffice to induce forward stepping in the absence of ATP

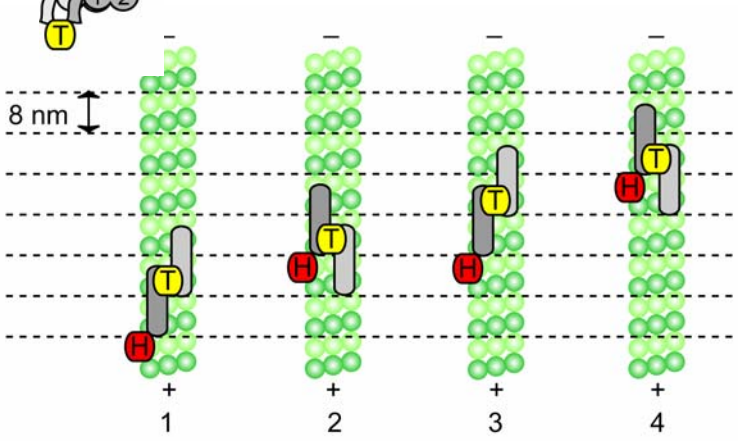




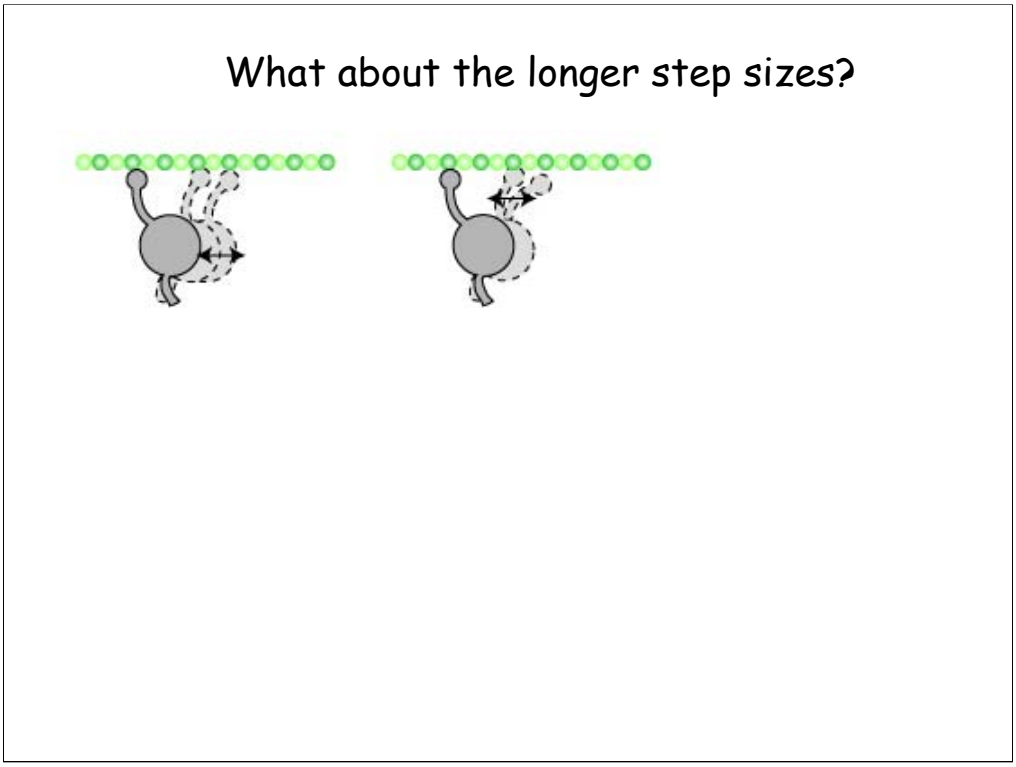

ATP-independent force-induced bidirectional stepping
+
-
backward stepping
forward stepping
10 pN
-3 pN
strong affinity
weak affinity
+
-
+
-


Head-labeled dynein takes ~ 16 nm steps
as well as larger, backwards steps
16 nm
Position (nm)
Time (seconds)
384
352
320
288
256
224
192
160
128
96
64
32
0
0
2
4
6
8
10
12
14
16
Stalk
MTBD
AAA Core
Intermediate and Light Chains

Walking Model for Cytoplasmic Dynein

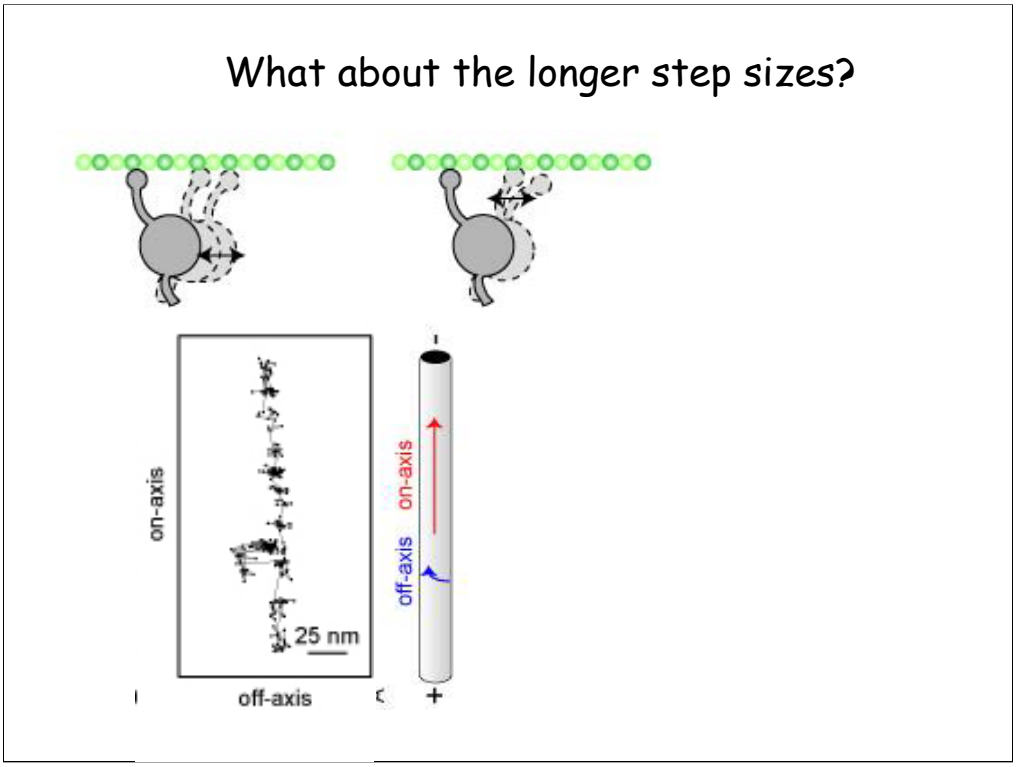


Reck Peterson et al (2006)



Dimension of molecule and our stepping data: we favor the rings being stacked. Other AAA proteins rings are stacked.

Open questions: what is the structural basis for movement? Is the stem involved?



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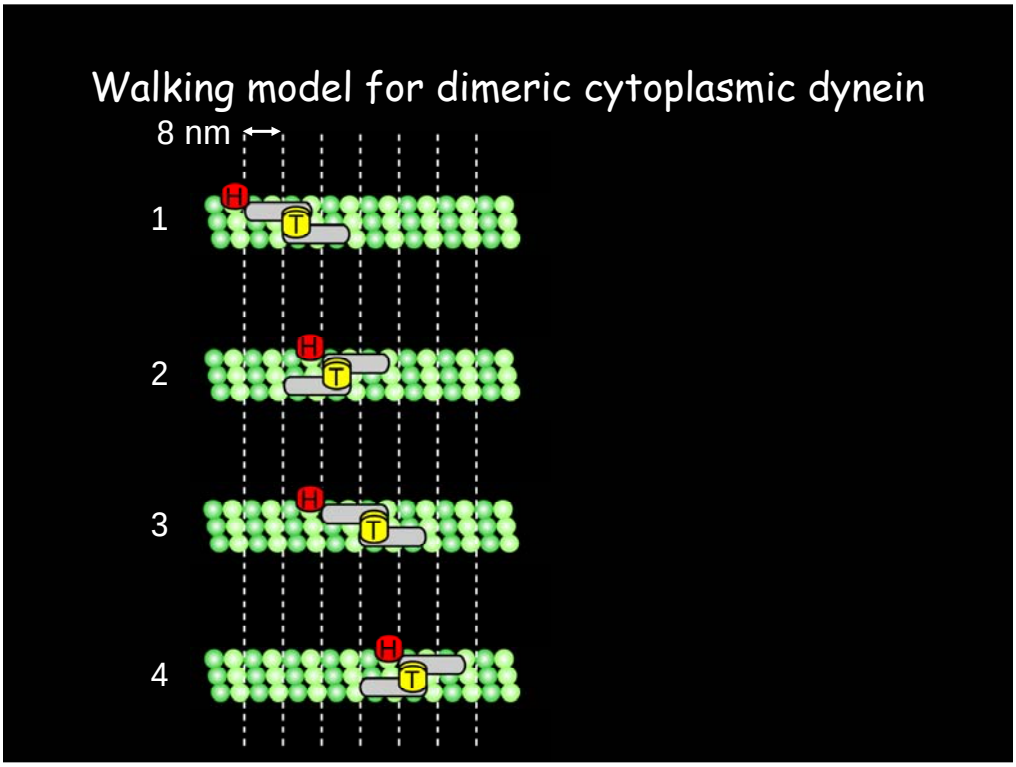

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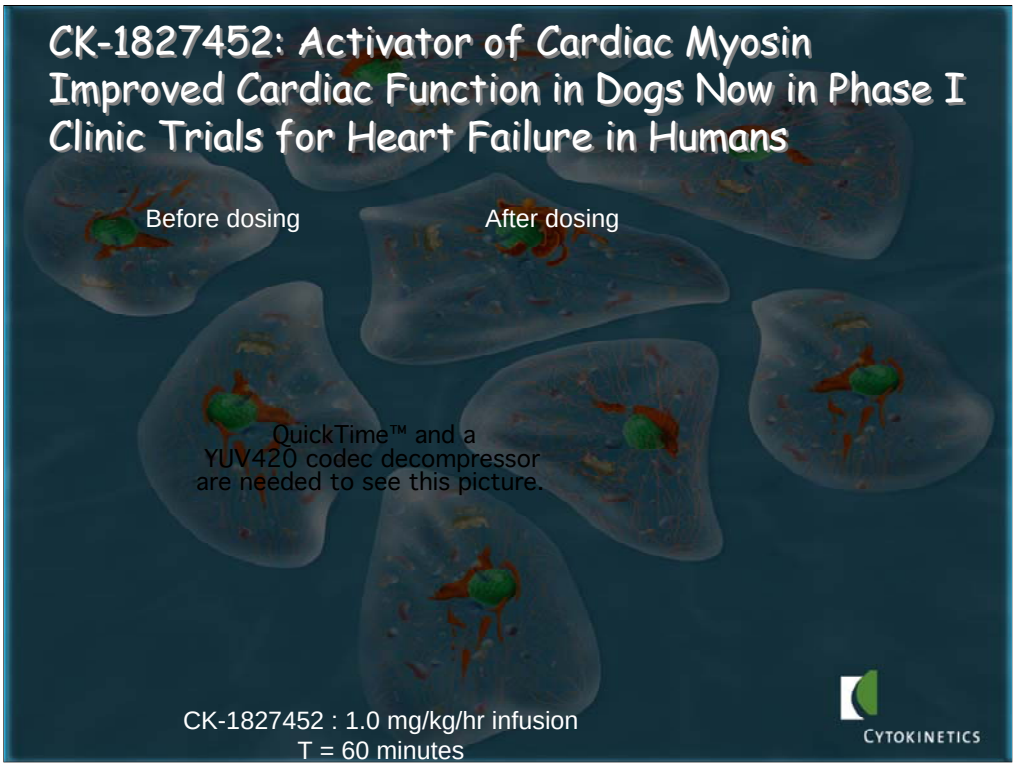
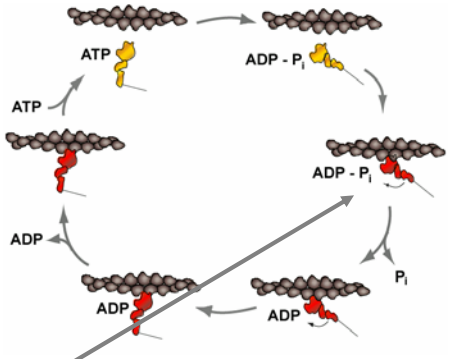

CK-1827452: Activator of Cardiac Myosin Improved Cardiac Function in Dogs Now in Phase I Clinic Trials for Heart Failure in Humans
Before dosing
After dosing
QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.
CK-1827452 : 1.0 mg/kg/hr infusion
T = 60 minutes
CYTOKINETICS

How might a myosin activator work?

The Chemical and Mechanical Cycles are Linked

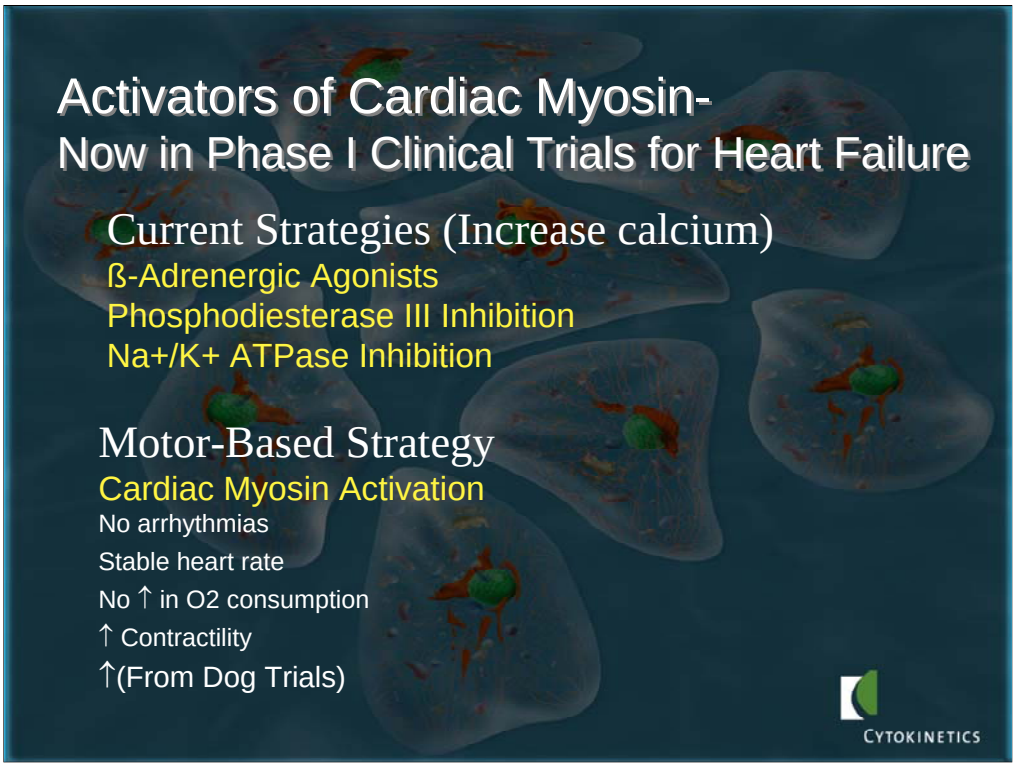


Chemical Cycle
Accelerate the rate limiting step in ATP hydrolysis cycle

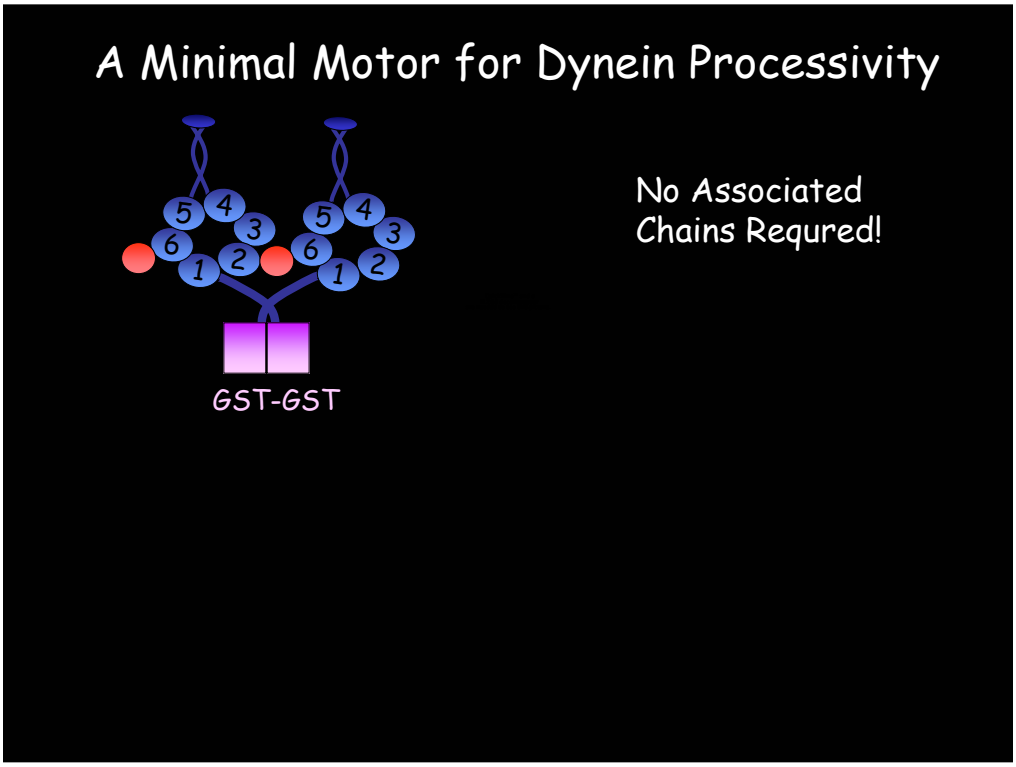
Mechanical Cycle
Increase the number of myosin heads interacting with the actin filament during the cardiac cycle

= strongly bound state

CYTOKINETICS



1. Effective in a well understood myocardial infarction-pacing model ofHF.2. Unlike PDE inhibitors and sympathomimetics, they do not causearrythmias, increased heart rate and increased oxygen consumption.3. In fact, in HF dogs, they cause and increase in Stroke volume,cardiac output and a reflex decrease in heart rate and systemic vascularresistance all with little or no increase in myocardial oxygenconsumption.



We could induce processivity of the dynein monomers by artificially dimerization with GST. Here is an example of a TIRF movie that I made.

Questions?

1. Is cytoplasmic dynein processive?

2. If so, what are the structural requirements for processivity?

3. How does dynein take steps along a microtubule?

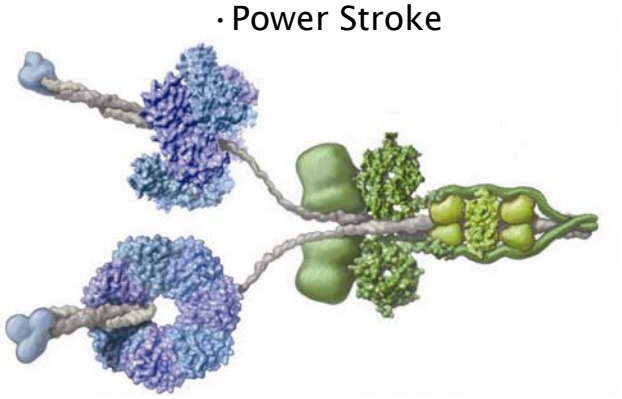
4. How much force can dynein produce and how does dynein perform when working against a load?

5. Can we derive structural/kinetic mechanism for motility?

How Does Dynein Work?

Questions:

- Walking Model
- ATP Sites
- Stalk Communication
- Power Stroke



Sign Post at the End

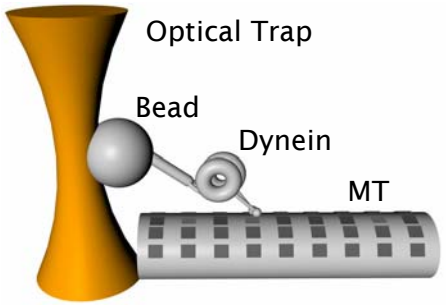
Expression System

Use expression system and biophysical techniques to answer first question

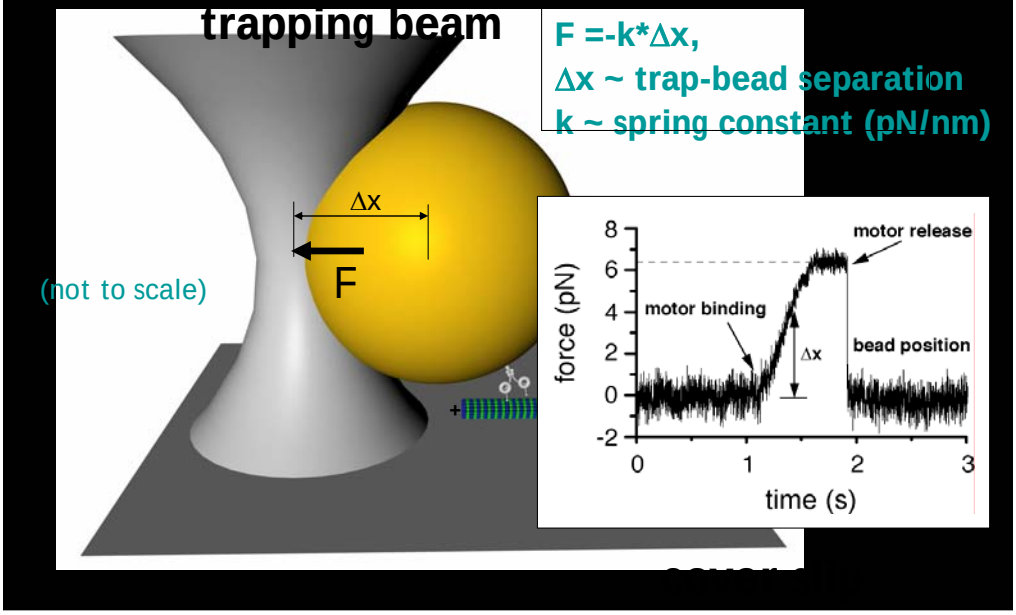
Use of expression to make protein for crystal trials

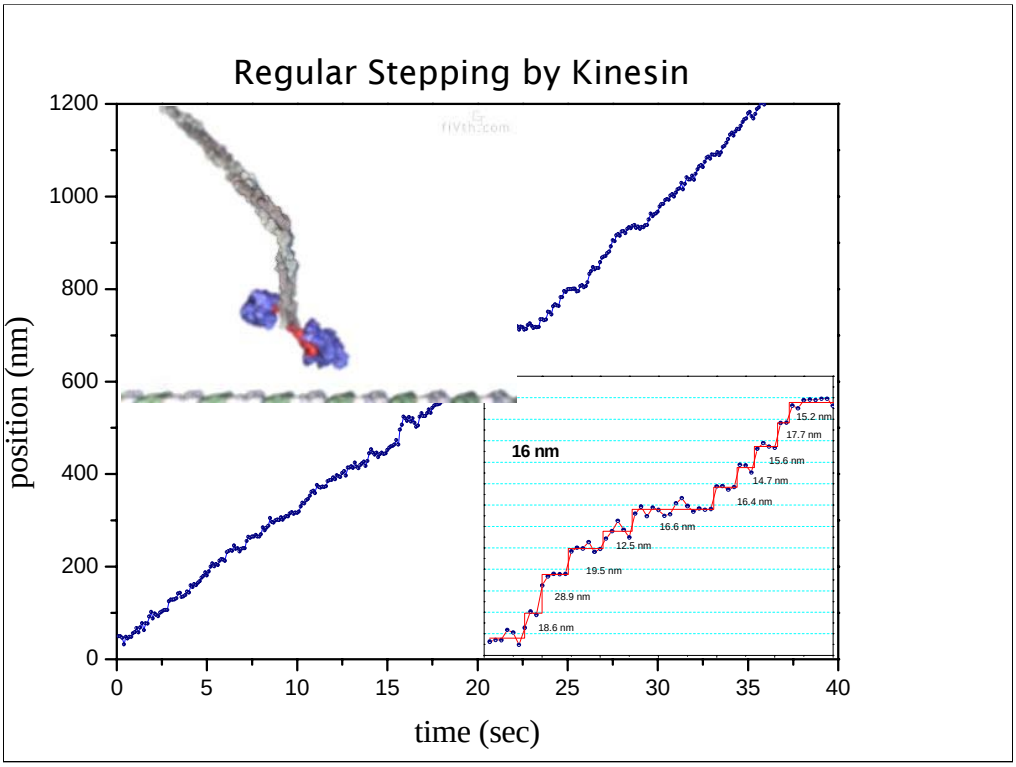
Future directions for crystallography project

Dynein working against an optical trap

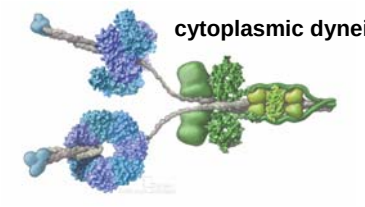


Mimicking cargo transport in an optical trapping assay



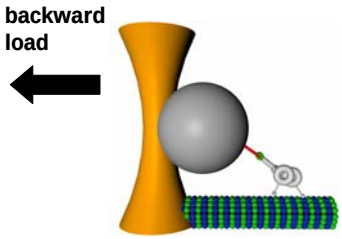

Regular Stepping by Kinesin
position (nm)
time (sec)
16 nm
15.2 nm
17.7 nm
15.6 nm
14.7 nm
16.4 nm
16.6 nm
12.5 nm
19.5 nm
28.9 nm
18.6 nm

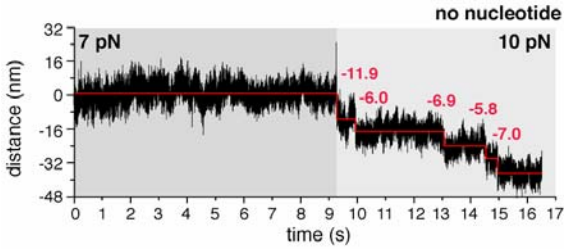
Superstall rearward load induces backward stepping of dynein



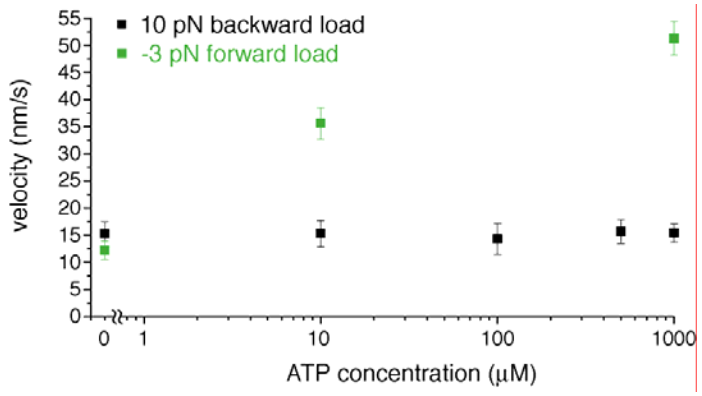
7 pN: ~52% of dynein-coated beads exhibited motion within a ~10 s window of applied rearward load

10 pN: ~90% of dynein-coated beads exhibited motion within a ~10 s window of applied rearward load





Dynein exhibits a mechanical asymmetry



ATP-dependent forward stepping at zero load