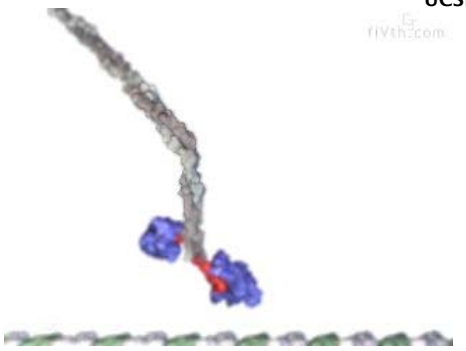
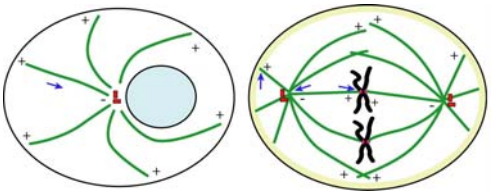
Part II: Single Molecule Approaches for Understanding Molecular Motors

Ron Vale
UCSF, HHMI



Cytoplasmic Dynein



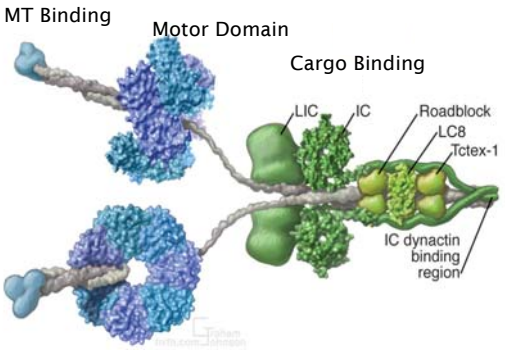
Microtubule Transport
- Membrane bound organelles
- RNA particles
- Aggregated proteins
- Viruses
- Neurons: retrograde transport

Mitosis
- Spindle Alignment
- Pole Focusing
- Kinetochore Checkpoint

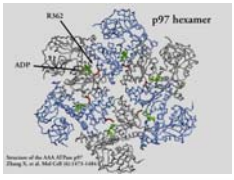
Dynein is Larger than Other Cytoskeletal Motors

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

Dynein Structure (A Model)



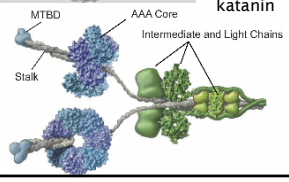
Dynein Emerged from the AAA ATPase Lineage, Not from G proteins/Kinesin/Myosin



Some well known AAA proteins include:

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

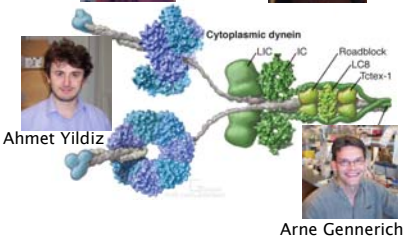
Destablizing AAAs
- p97,
- NSF,
- katanin



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

Why has dynein lagged behind?

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Need expression systems

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Simplified motor constructs that are easier to study

Why has dynein lagged behind?

Need expression systems

Simplified motor constructs that are easier to study

In vitro assays

Why has dynein lagged behind?

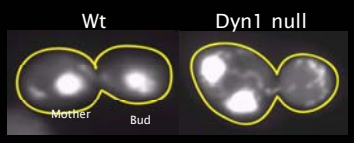
Need expression systems

Simplified motor constructs that are easier to study

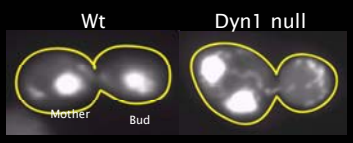
In vitro assays

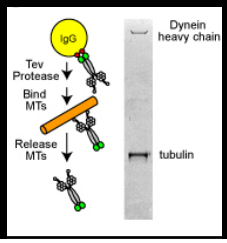
Crystal structures

Producing Recombinant Dynein in Yeast

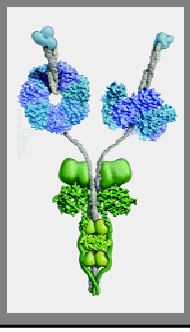


Producing Recombinant Dynein in Yeast

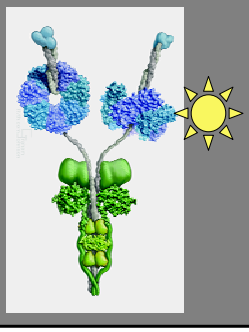




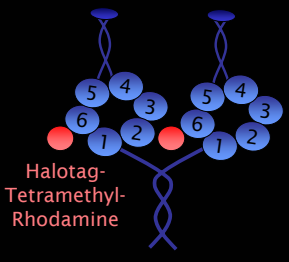
Putting Fluorescent Tags on Recombinant Dynein



Putting Fluorescent Tags on Recombinant Dynein

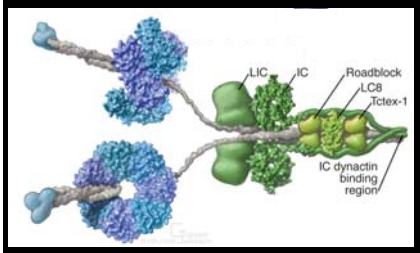


Is Cytoplasmic Dynein Processive?



MOVIE

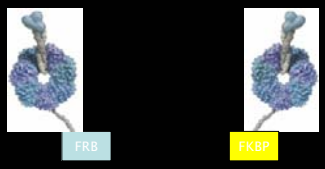
What are the requirements for dynein processivity?



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

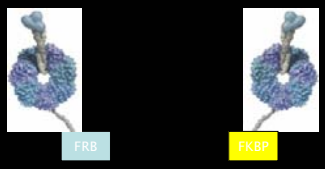
In vitro gliding assay of monomeric dynein

Are Two Heads are Better than One?



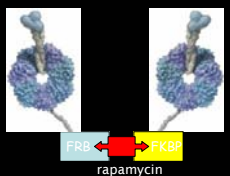
No Processive Movement

Are Two Heads are Better than One?



rapamycin

Are Two Heads are Better than One?



rapamycin

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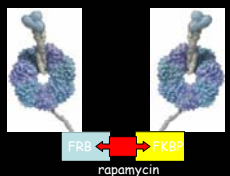
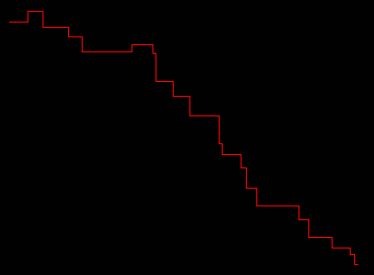
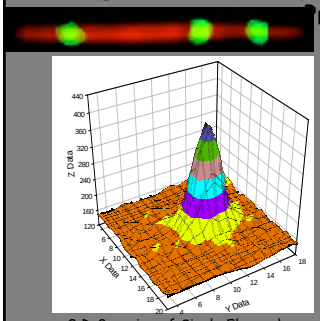


rapamycin

Observing Single Dynein Steps By High Spatial Resolution Fluorescence Microscopy

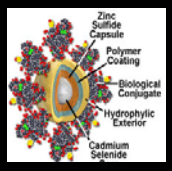


Tracking Fluorescent-Dynein with ~2 nm Precision

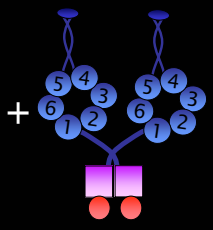


2 D Gaussian of Single Fluorophore
A. Yildiz and P. Selvin, U. Illinois

Quantum dot- labeled dynein



Streptavidin-Qdot

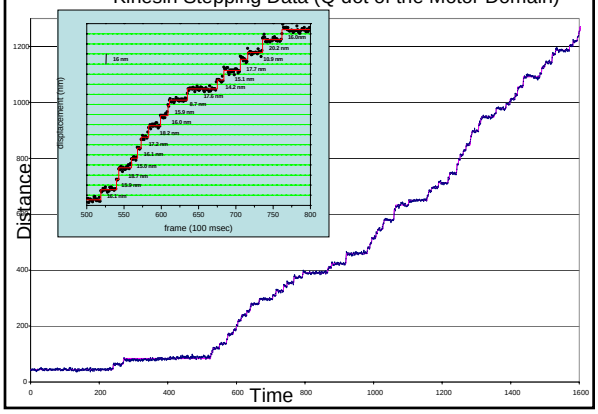


Biotin-Tail

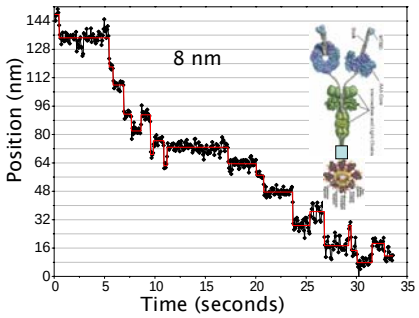
MOVIE

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

Kinesin Stepping Data (Q dot of the Motor Domain)

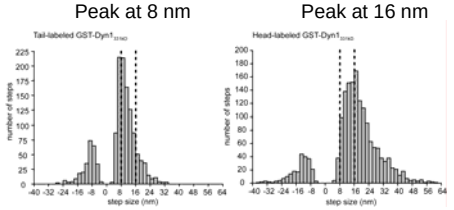


Tail-labeled dynein takes ~8 nm steps as well as larger and backward steps



Step Size Data for Cytoplasmic Dynein

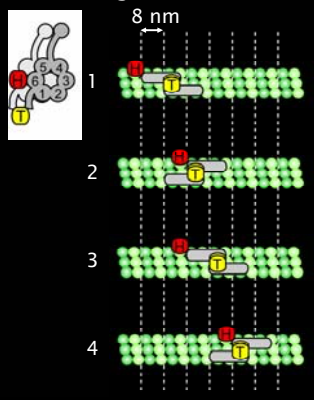
Peak at 8 nm Peak at 16 nm



Tail Labeled Dynein Head Labeled Dynein

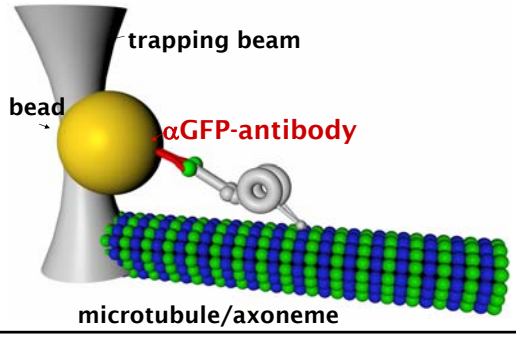
Reck Peterson et al (2006)

Walking model for dimeric cytoplasmic dynein



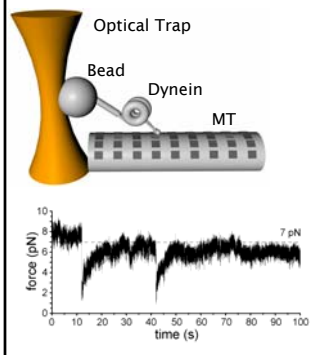
Dynein working against an optical trap

(not to scale)

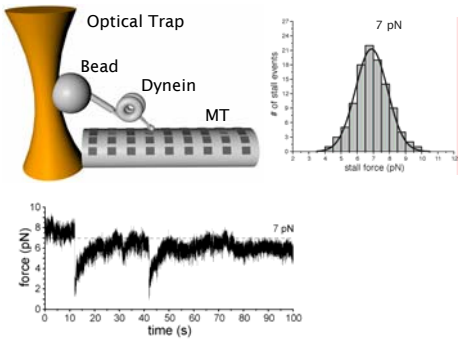


MOVIE

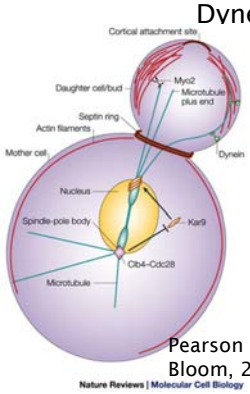
Dynein working against an optical trap



Dynein working against an optical trap



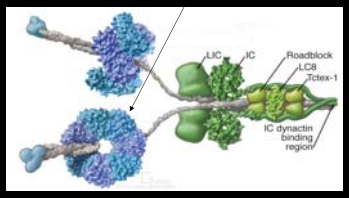
Biological Role of Yeast Cytoplasmic Dynein



Pearson and Bloom, 2004

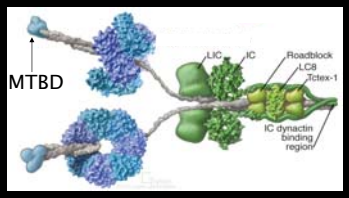
Dynein- What Next?

4 ATP binding sites



What the four ATP binding sites in dynein doing?

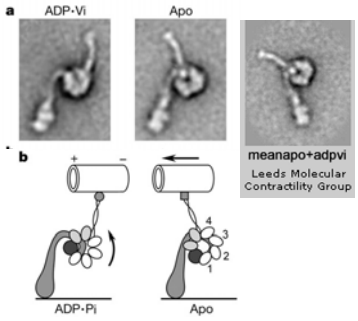
Dynein- What Next?



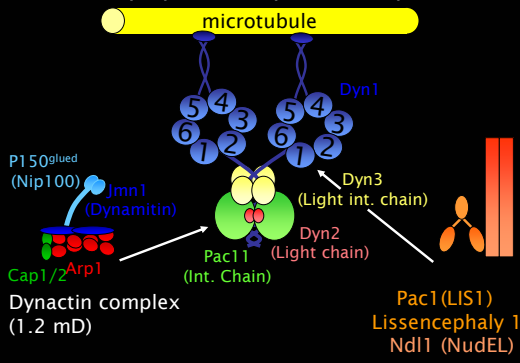
How does the microtubule binding domain (MTBD) communicate with the ATPase ring?

Dynein- What Next?

EM studies by Burgess, Knight, Oiwa et al.



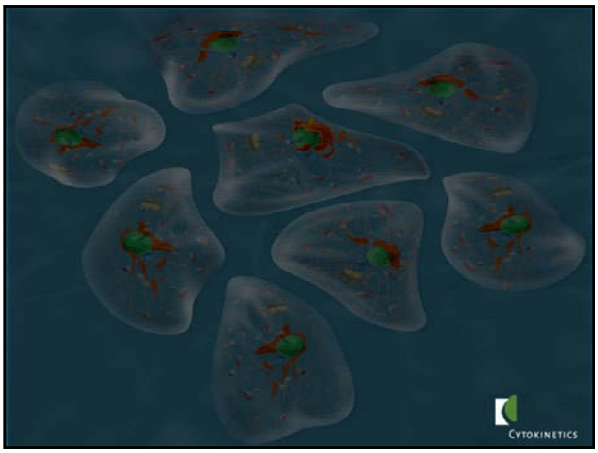
Cytoplasmic Dynein's Helpers



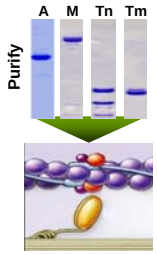
Apply our knowledge of motor proteins to practical outcomes?

Small molecule drugs with therapeutic benefit?

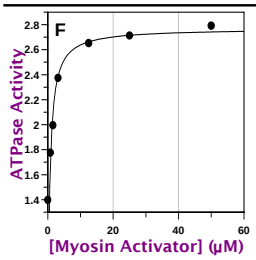
Engineer motors for cells or nanotechnology?



Reconstituting the Sarcomere for Drug Discovery



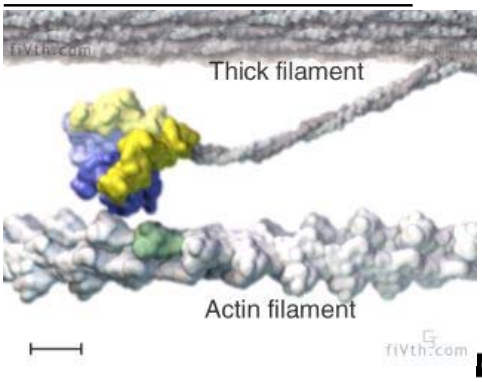
Reconstituting the Sarcomere for Drug Discovery



High Throughput Screen
PUMA™

Fast: **50,000 cmpounds/day**

Myosin Activating Drugs Accelerate Phosphate Release



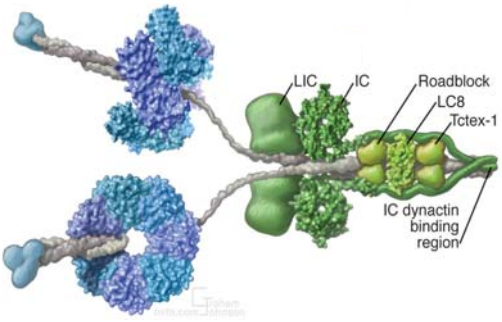
CK-1827452: Activator of Cardiac Myosin Increases Cardiac Output and is in Phase II Clinic Trials for Heart Failure

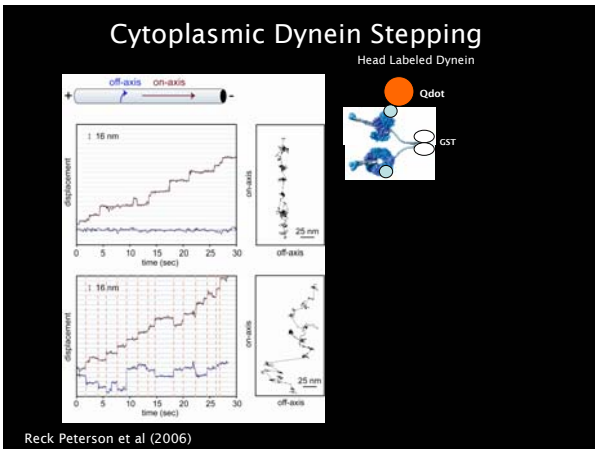
Before dosing

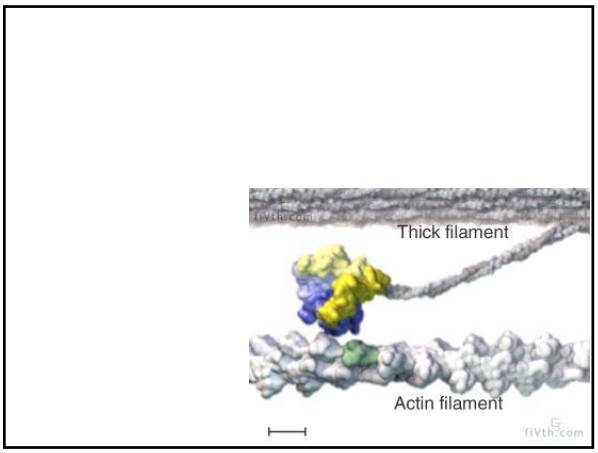
After dosing

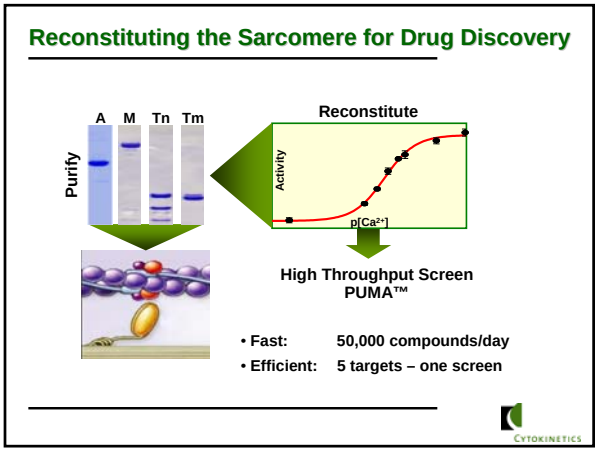
QuickTime™ and a YUV420 codec decompressor are needed to see this picture.

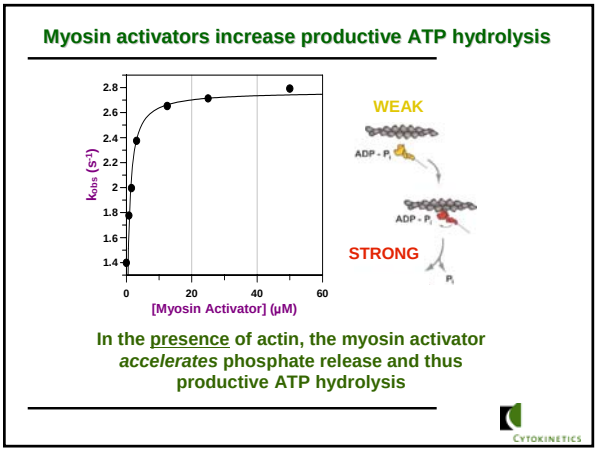
CK-1827452 : Infusion in a dog model

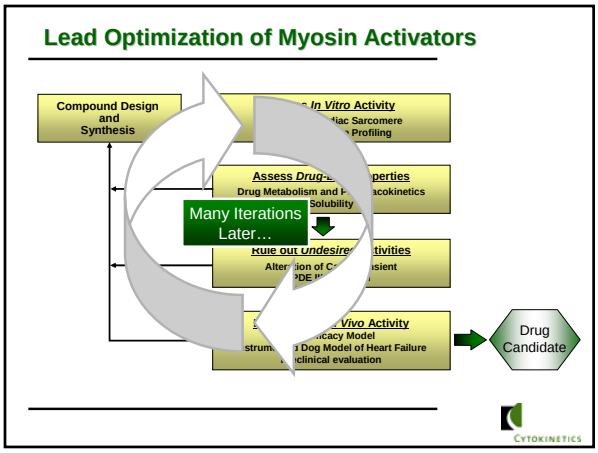


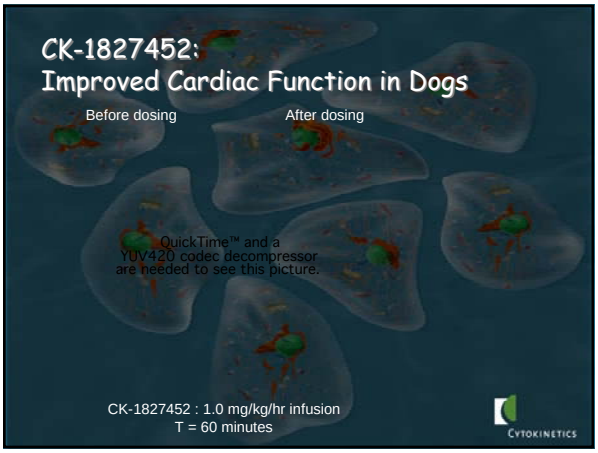

Cytoplasmic Dynein Stepping
Head Labeled Dynein
Qdot
GST
off-axis
on-axis
displacement
time (sec)
16 nm
25 nm
Reck Peterson et al (2006)


Thick filament
Actin filament

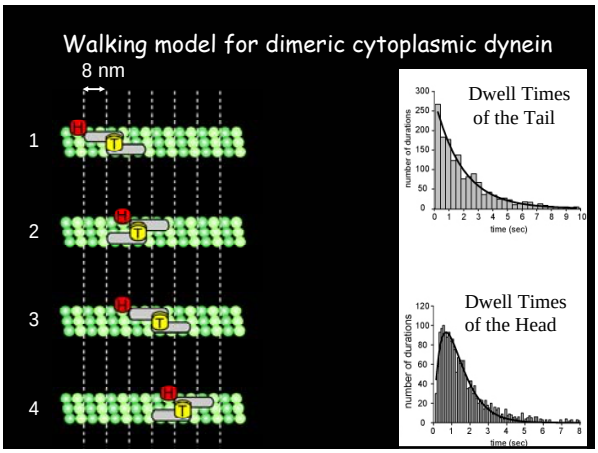

Reconstituting the Sarcomere for Drug Discovery
A
M
Tn
Tm
Purify
Reconstitute
Activity
p[Ca2+]
High Throughput Screen
PUMA™
• Fast: 50,000 compounds/day
• Efficient: 5 targets – one screen
CYTOKINETICS

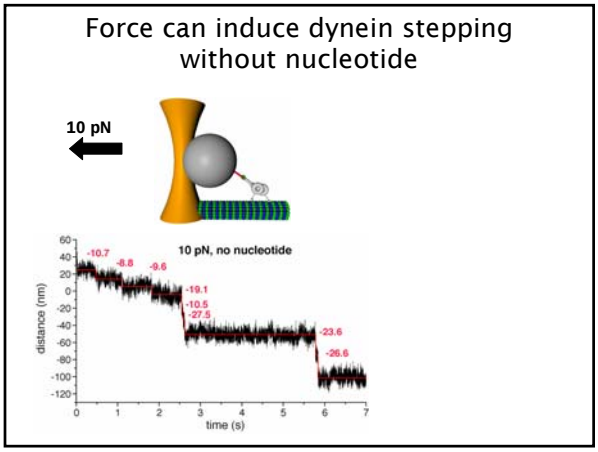

Myosin activators increase productive ATP hydrolysis
kobs (s-1)
[Myosin Activator] (μM)
WEAK
ADP - Pi
STRONG
In the presence of actin, the myosin activator accelerates phosphate release and thus productive ATP hydrolysis
CYTOKINETICS

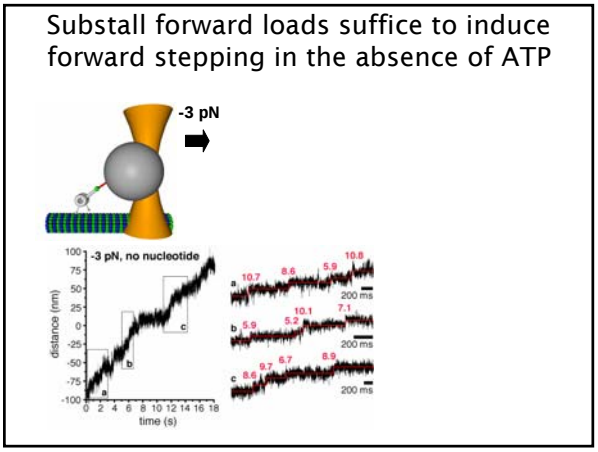

Lead Optimization of Myosin Activators
Compound Design and Synthesis
In Vitro Activity
Assess Drug-
Many Iterations Later…
Rule out Undesire
In Vivo Activity
Drug Candidate
CYTOKINETICS

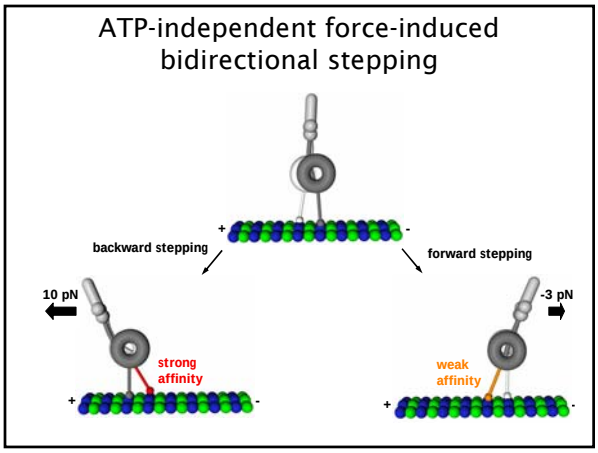

CK-1827452:
Improved Cardiac Function in Dogs
Before dosing
After dosing
QuickTime™ and a
YUV420 codec decompressor
are needed to see this picture.
CK-1827452 : 1.0 mg/kg/hr infusion
T = 60 minutes
CYTOKINETICS

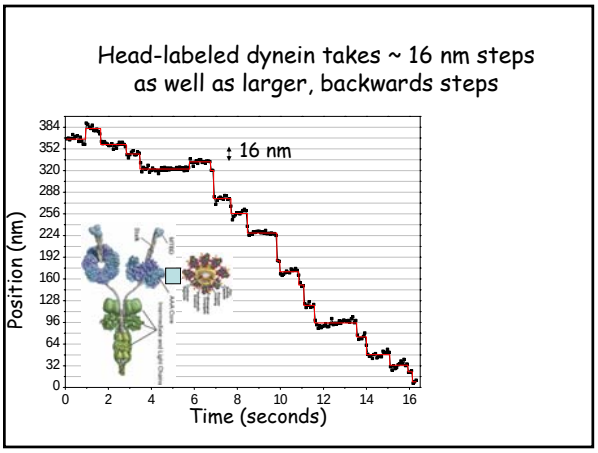

Myosin Activators Now in Phase II Clinical Trials
Before dosing
After dosing
QuickTime™ and a YUV420 codec decompressor are needed to see this picture.
CK-1827452 : 1.0 mg/kg/hr infusion
T = 60 minutes
CYTOKINETICS


Walking model for dimeric cytoplasmic dynein
8 nm
1
2
3
4
Dwell Times of the Tail
number of durations
time (sec)
Dwell Times of the Head
number of durations
time (sec)

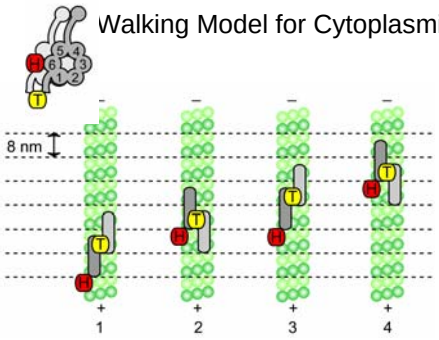

Force can induce dynein stepping without nucleotide
10 pN
10 pN, no nucleotide
distance (nm)
time (s)


Substall forward loads suffice to induce forward stepping in the absence of ATP
-3 pN
-3 pN, no nucleotide
distance (nm)
time (s)
200 ms


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


Head-labeled dynein takes ~ 16 nm steps as well as larger, backwards steps
16 nm
Position (nm)
Time (seconds)

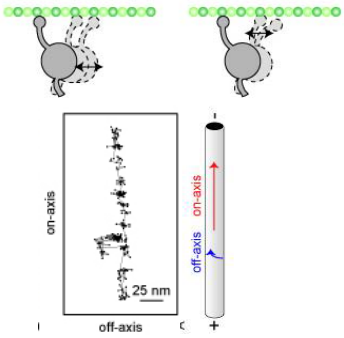
Walking Model for Cytoplasmic Dynein



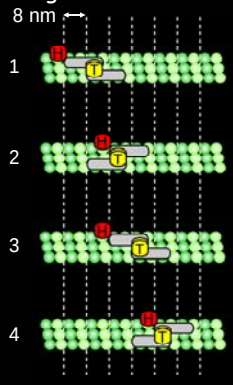
Reck Peterson et al (2006)

What about the longer step sizes?

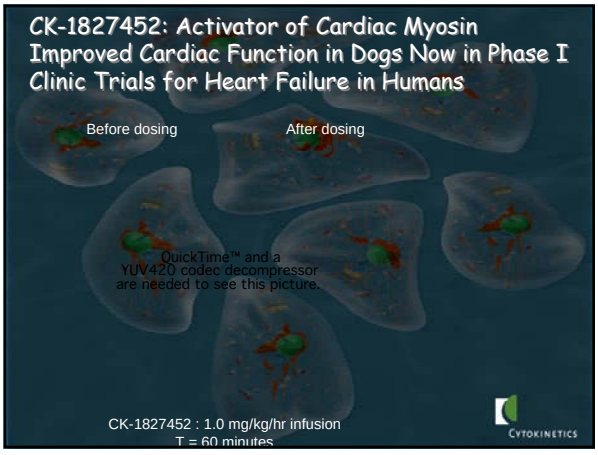
What about the longer step sizes?



Walking model for dimeric cytoplasmic dynein

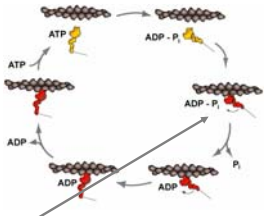


CK-1827452: Activator of Cardiac Myosin Improved Cardiac Function in Dogs Now in Phase I Clinic Trials for Heart Failure in Humans



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

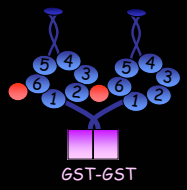
Activators of Cardiac Myosin-
Now in Phase I Clinical Trials for Heart Failure

Current Strategies (Increase calcium)
- ß-Adrenergic Agonists
- Phosphodiesterase III Inhibition
- Na+/K+ ATPase Inhibition

Motor-Based Strategy
- Cardiac Myosin Activation
- No arrhythmias
- Stable heart rate
- No ↑ in O2 consumption
- ↑ Contractility
- ↑(From Dog Trials)

CYTOKINETICS

A Minimal Motor for Dynein Processivity



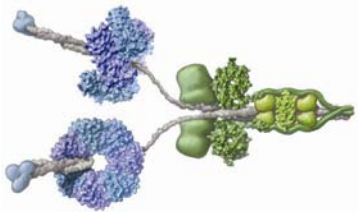
No Associated Chains Requred!

Questions?

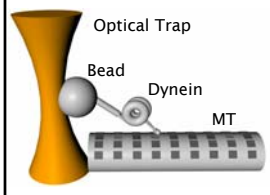
1. Is cytoplasmic dynein processive?
2. If so, what are the structural requirements for processivity?
3. How does dynein take steps along a microtubule?
4. How much force can dynein produce and how does dynein perform when working against a load?
5. Can we derive structural/kinetic mechanism for motility?

How Does Dynein Work?

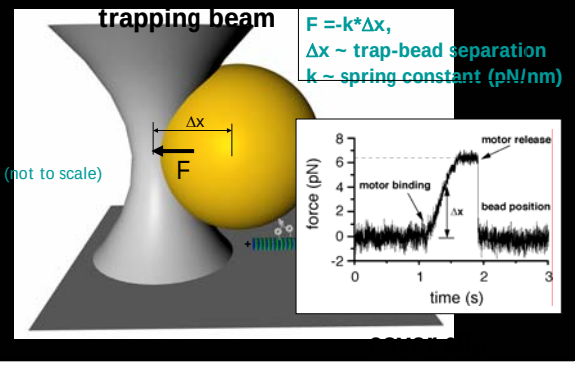
Questions:
- Walking Model
- ATP Sites
- Stalk Communication
- Power Stroke

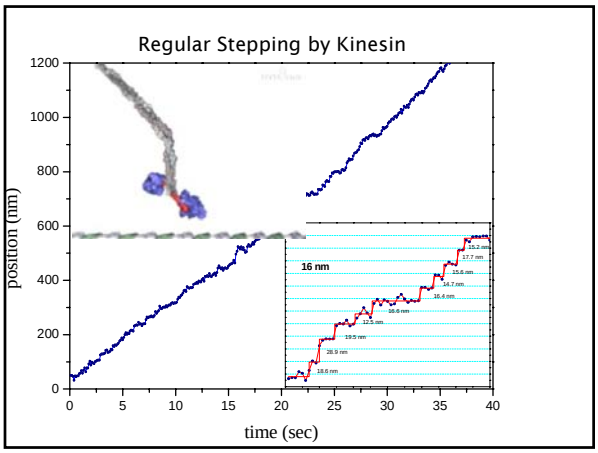


Dynein working against an optical trap

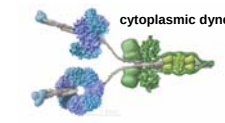


Mimicking cargo transport in an optical trapping assay



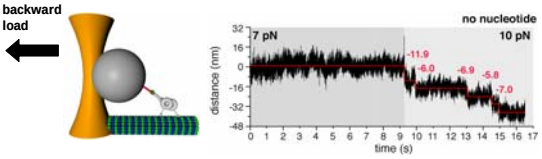


Superstall rearward load induces backward stepping of dynein

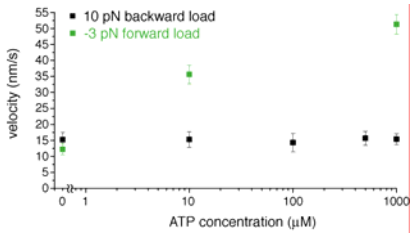


7 pN: ~52% of dynein-coated beads exhibited motion within a ~10 s window of applied rearward load

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