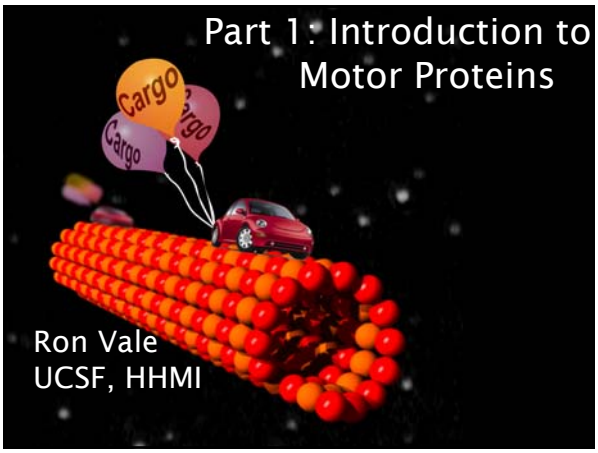
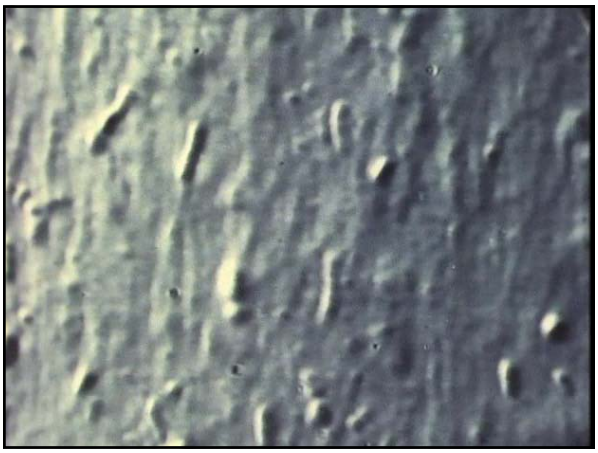


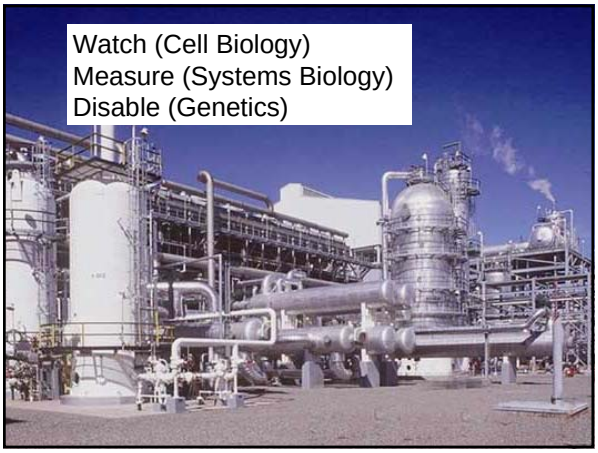



Chromosome Segregation in Drosophila Embryos

MOVIE

G. Rogers
And D. Sharp

Xvivo Movie



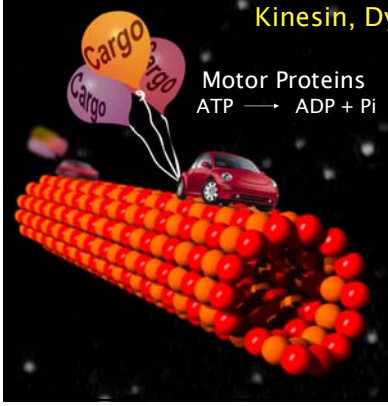
MOVIE

Part 3 will describe efforts to understand the assembly of a complex structure, the mitotic spindle

## Understanding the Parts

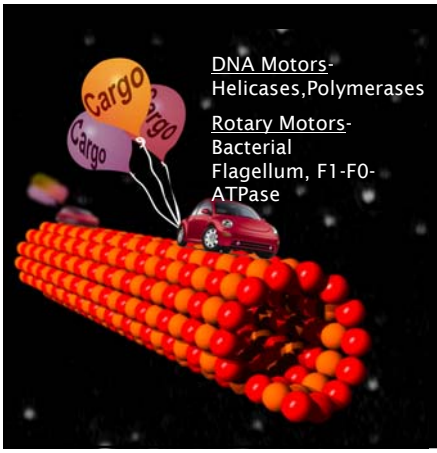
Part II: Effort to understand the molecular mechanism of a motor- cytoplasmic dynein

## Kinesin, Dynein, Myosin




Motor Proteins

ATP $\longrightarrow$ ADP + Pi



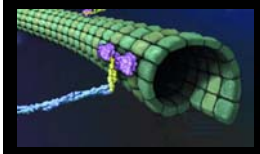

DNA Motors-
Helicases,Polymerases

Rotary Motors-
Bacterial
Flagellum, F1-F0-
ATPase

| | Kinesin | Automobile Engine |
|---|---|---|
| Size | $10^{-8}$ m | 1 m |

| | Kinesin | Automobile Engine |
|---|---|---|
| Size | $10^{-8}$ m | 1 m |


| | Kinesin | Automobile Engine |
|---|---|---|
| Size | $10^{-8}$ m | 1 m |




| | Kinesin | Automobile Engine |
|---|---|---|
| Size | $10^{-8}$ m | 1 m |
| Fuel | ATP | Hydrocarbons |

| | Kinesin | Automobile Engine |
|---|---|---|
| Size | $10^{-8}$ m | 1 m |
| Fuel | ATP | Hydrocarbons |
| Speed | $4 \times 10^{-3}$ m/hr | $10^5$ m/hr |
| | $4 \times 10^5$ lengths/hr | $10^5$ lengths/hr |

| | Kinesin | Automobile Engine |
|---|---|---|
| Size | $10^{-8}$ m | 1 m |
| Fuel | ATP | Hydrocarbons |
| Speed | $4 \times 10^{-3}$ m/hr | $10^5$ m/hr |
| | $4 \times 10^5$ lengths/hr | $10^5$ lengths/hr |
| Work Efficiency | ~60% | ~10% |

## Why Study Cytoskeletal Motor Proteins?

Understanding how living organisms create motion has intrigued scientists for thousands of years.

## Why Study Cytoskeletal Motor Proteins?

Cytoskeletal motor proteins intersect with almost every facet of cell biology.

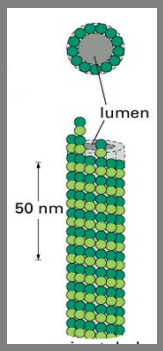
## Why Study Cytoskeletal Motor Proteins?

Relevance to medicine:

Transport defects can cause disease.

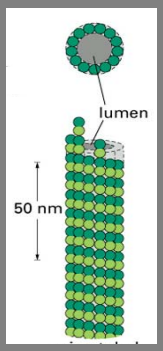
Inhibition or enhancement of motor protein activity may have therapeutic benefit.

## The Tracks

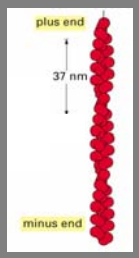



Microtubule

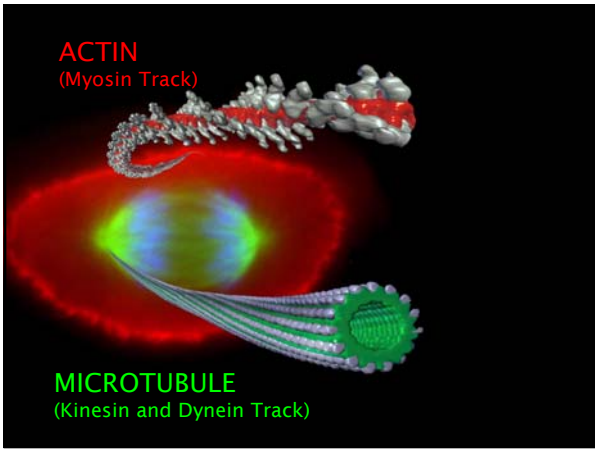
## The Tracks

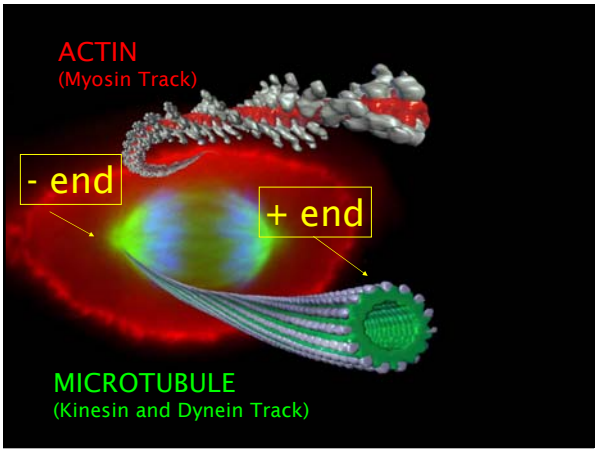


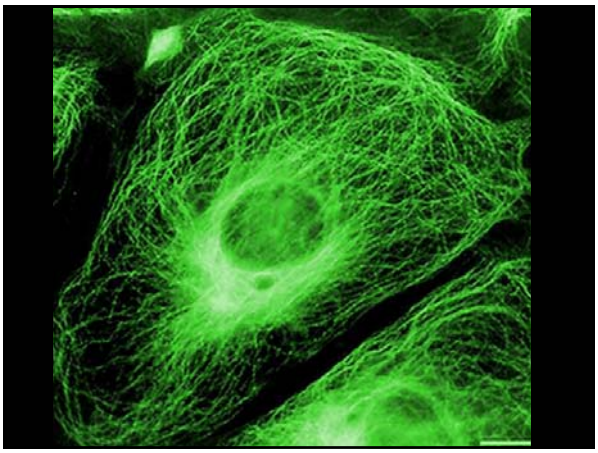

Microtubule

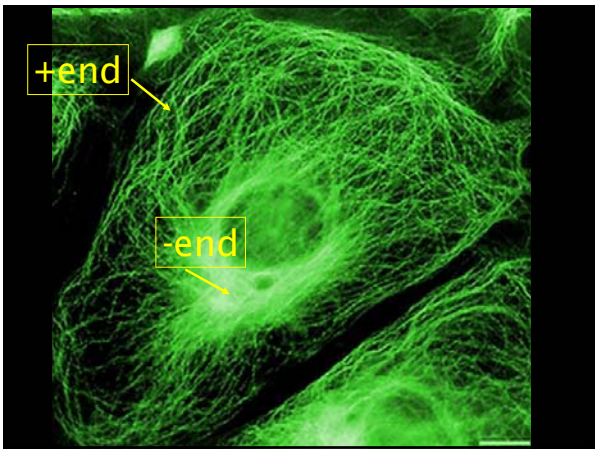


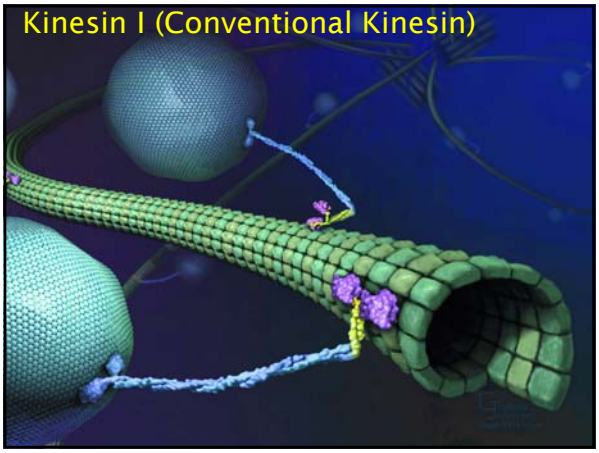

Actin Filament







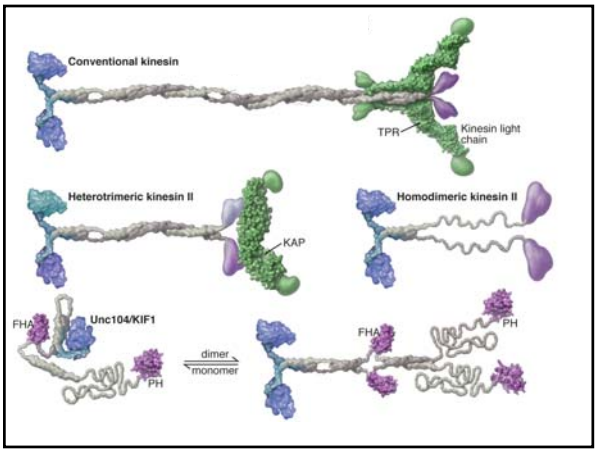

## Many Motors, Not Just One

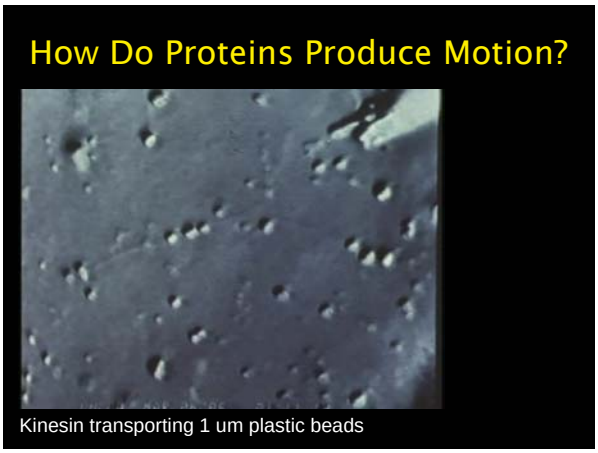
- 45 human kinesin genes
- The different kinesins are specialized for different transport activities

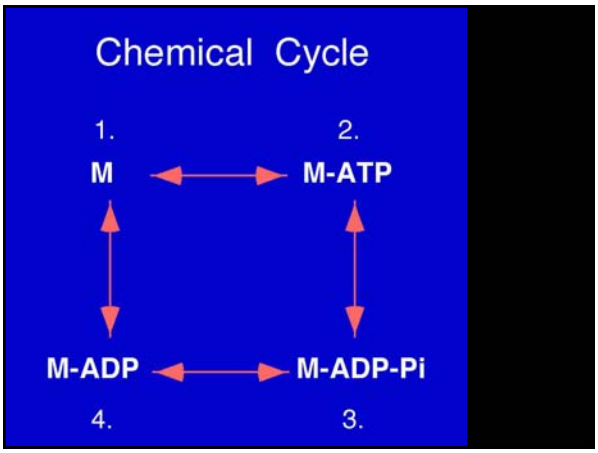
## Many Motors, Not Just One

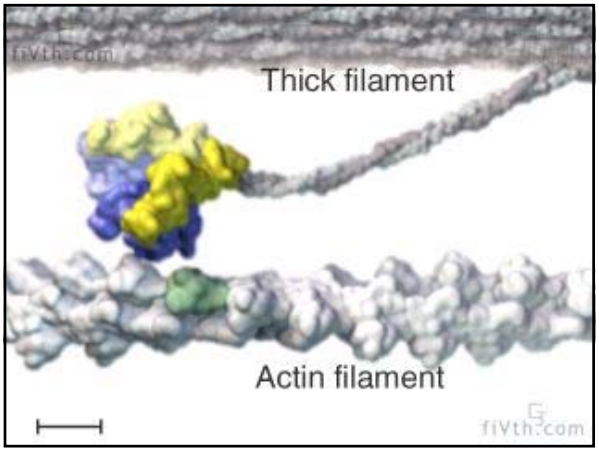
- Kinesin Functions

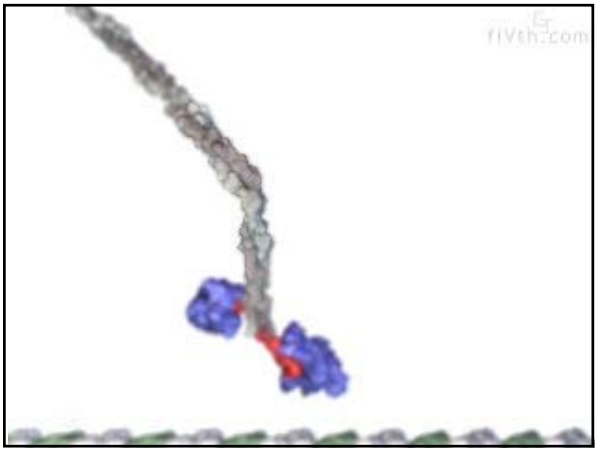
- Organelle movement
- Transport of RNAs and proteins
- Assembly of cilia/flagella
- Signaling pathways
- Mitotic spindle formation and chromosome movement



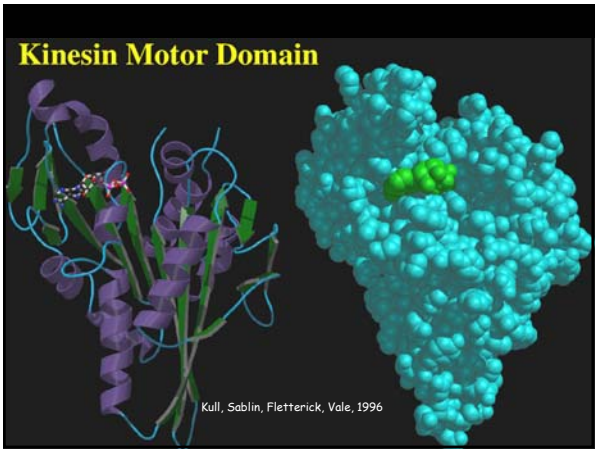


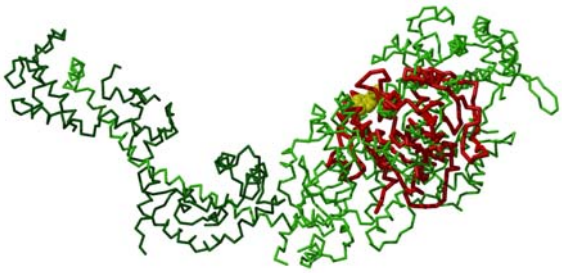



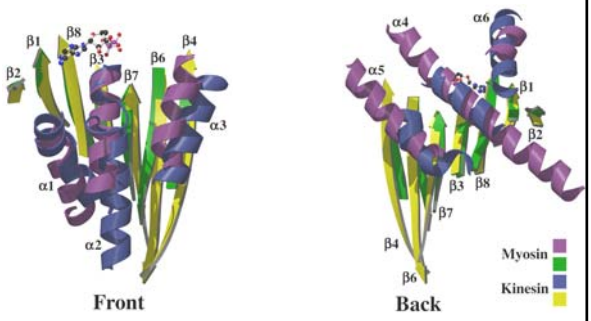
How do you study the mechanism of a molecular motor?



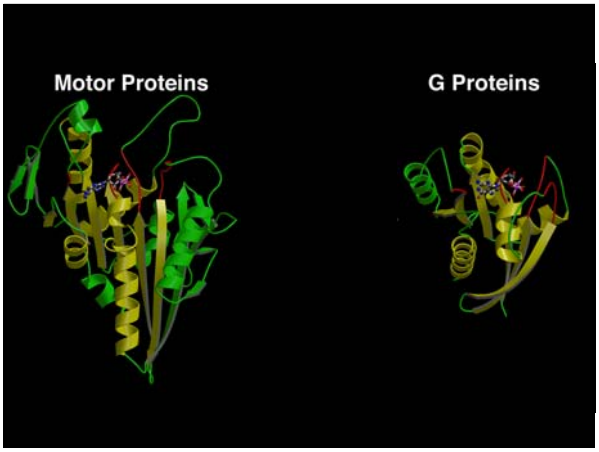
Kinesin (Red) and Myosin (Green)
(Microtubule motor) (Actin Motor)



Similarity in Atomic Structure Reveals that
Kinesin and Myosin are Long Lost Relatives




Kull, Sablin, Fletterick, Vale, 1996




THE EVOLUTION OF MOTOR PROTEINS

Nucleotide Switch

Motor Precursor G Proteins

THE EVOLUTION OF MOTOR PROTEINS

Nucleotide Switch

Motor Precursor G Proteins

Kinesin  Myosin 

THE EVOLUTION OF MOTOR PROTEINS

Nucleotide Switch

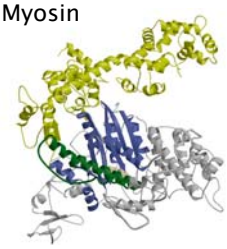
Motor Precursor G Proteins

Kinesin  Myosin 

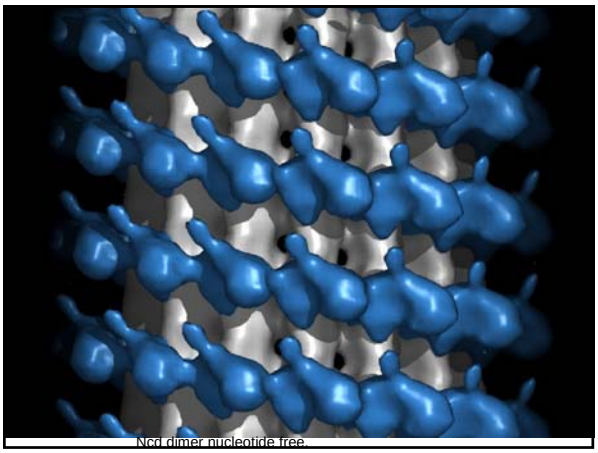
Kinesin Subfamilies Myosin Subfamilies

Nucleotide Chemistry is Similar
Mechanical Elements Differ

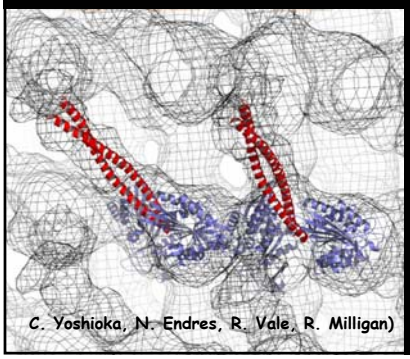
Myosin



Kinesin




**Cryo-EM helps to understand how motors interact with their tracks**




In Vitro Motility Assays:
Studying Motion in a Test Tube with Purified Kinesin and Tubulin (Microtubules)

Vale, Schnapp, Reese, Sheetz
Cell 1985

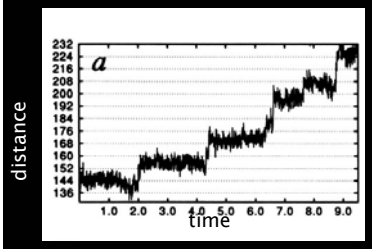
MOVIE
In Vitro Motility Assays:
Studying Motion in a Test Tube with Purified Kinesin and Tubulin (Microtubules)

MOVIE
One Can Study the Motion of Single Motor Proteins!

Low Kinesin Density on Glass

J. Howard, J. Hudspeth, R. Vale Nature, 1989

Single Molecule Fluorescence and Optical Trap Microscope Detect the Motion of Single Molecules!




K. Svoboda, S. Block et al.
Nature, 1993

MOVIE
Single Molecule Fluorescence and Optical Trap Microscope Detect the Motion of Single Molecules!

Vale, Yanagida et al.
Nature 1996

What I cannot create,
I do not understand

-Richard Feynman

Testing Ideas on How Motors Work

Sequence/Structure
↓
Hypothesis
↓
Protein Engineering
↓
Battery of Assays

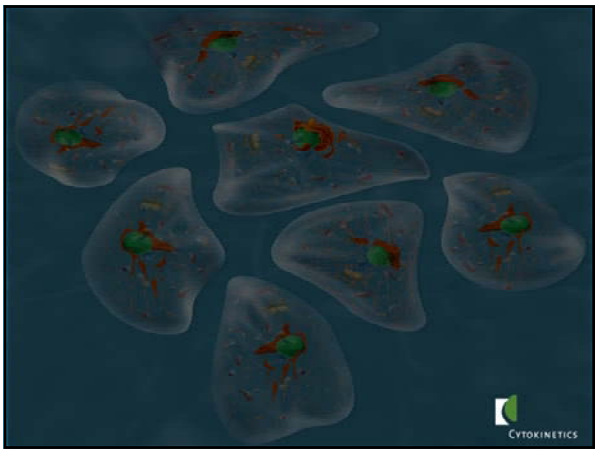
Testing Ideas on How Motors Work

Sequence/Structure
↓
Hypothesis
↓
Protein Engineering
↓
Battery of Assays

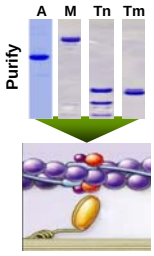
Apply our knowledge of motor proteins to practical outcomes?

Small molecule drugs with therapeutic benefit?

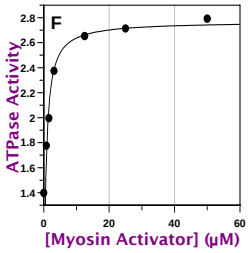
Engineer motors for cells or nanotechnology?




## Reconstituting the Sarcomere for Drug Discovery



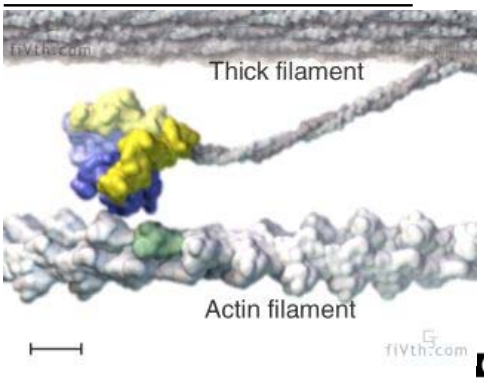

## Reconstituting the Sarcomere for Drug Discovery




**High Throughput Screen**
**PUMA™**

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

Myosin Activating Drugs Accelerate Phosphate Release




MOVIE

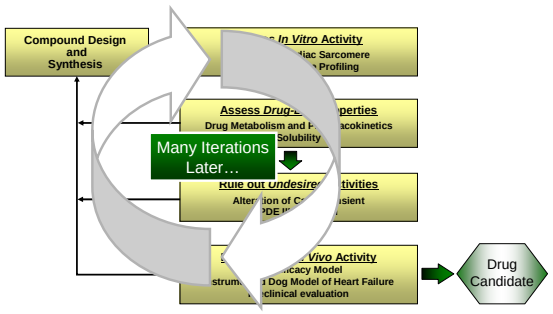
CK-1827452: Activator of Cardiac Myosin Increases Cardiac Output and is in Phase II Clinic Trials for Heart Failure

Before dosing After dosing

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

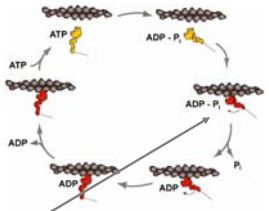
CK-1827452 : Infusion in a dog model

## Lead Optimization of Myosin Activators



## How might a myosin activator work?

The Chemical and Mechanical Cycles are Linked




Chemical Cycle
Accelerate the rate limiting step in ATP hydrolysis cycle

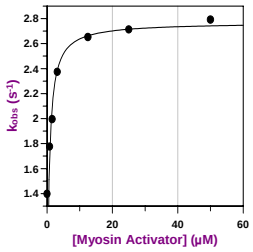
Mechanical Cycle
Increase the number of myosin heads interacting with the actin filament during the cardiac cycle

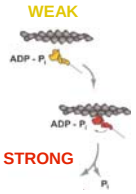
= strongly bound state

CYTOKINETICS

How do cytoskeletal motors produce motion?

## Myosin activators increase productive ATP hydrolysis




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

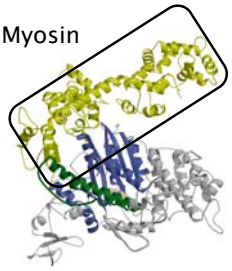
CYTOKINETICS

What are we trying to learn?

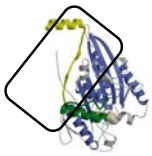
How do motors contribute to cell biological processes?

## Mechanical Elements are Modular

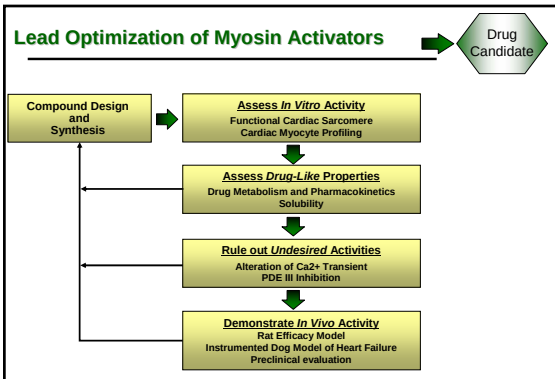
Myosin



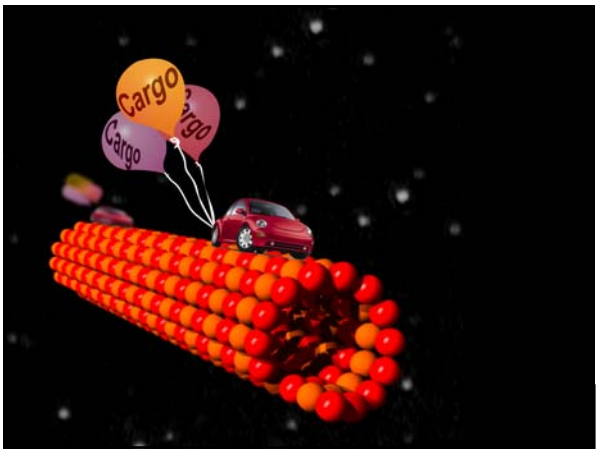
Kinesin



## Lead Optimization of Myosin Activators




CYTOKINETICS


Cargo
Cargo
Cargo