Cytoskeletal Transport Systems

An Introduction to Molecular Motors

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Cellular Motility is Essential for Life


Fertilization


Axonal Transport


Muscle Contraction

Just three families of motor proteins power eukaryotic cellular movement

myosin kinesin dynein

Cytoskeletal Transport Systems

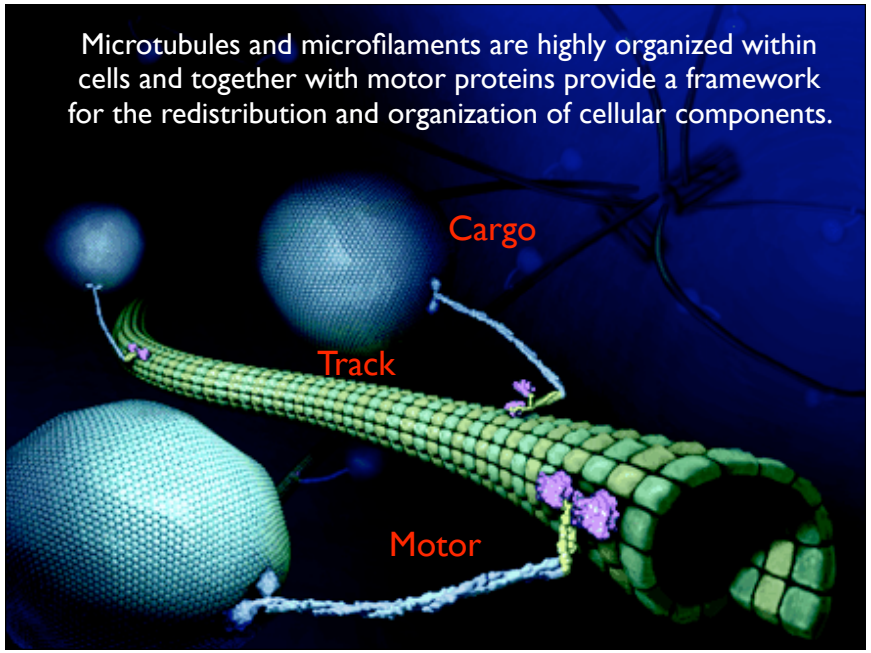
At the molecular level cytoskeletal transport systems consist of four basic components.

Motor
myosin, kinesin and dynein

Track
microfilaments and microtubules

Cargo
organelles, vesicles, chromosomes, etc.

Fuel
ATP and GTP



Why Study Cytoskeletal Motor Proteins?

Relevance to biology:
- Motor systems intersect with almost every facet of cell biology.

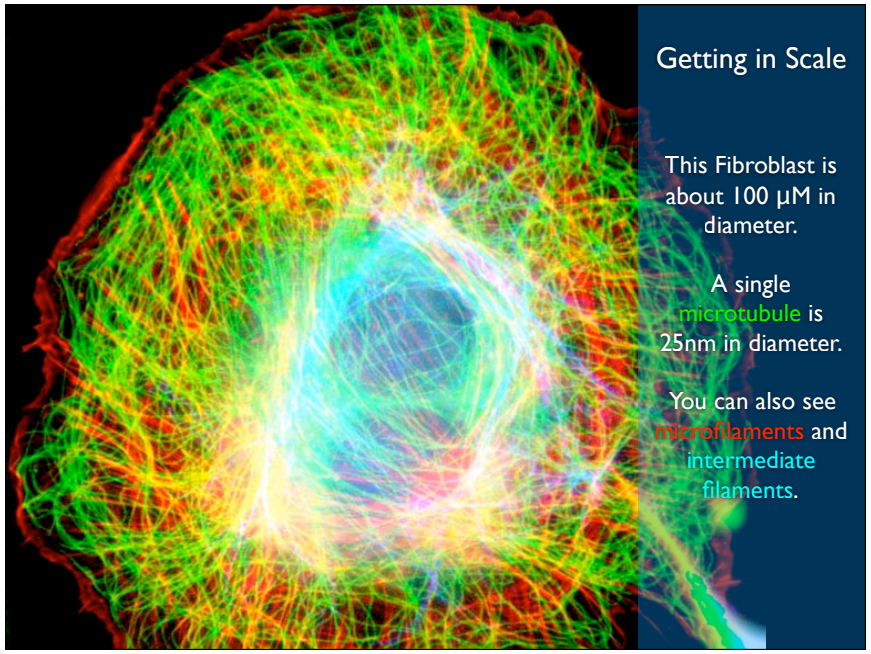
Relevance to medicine:
- Transport defects can cause disease.
- Inhibition or enhancement of motor protein activity has therapeutic benefits.

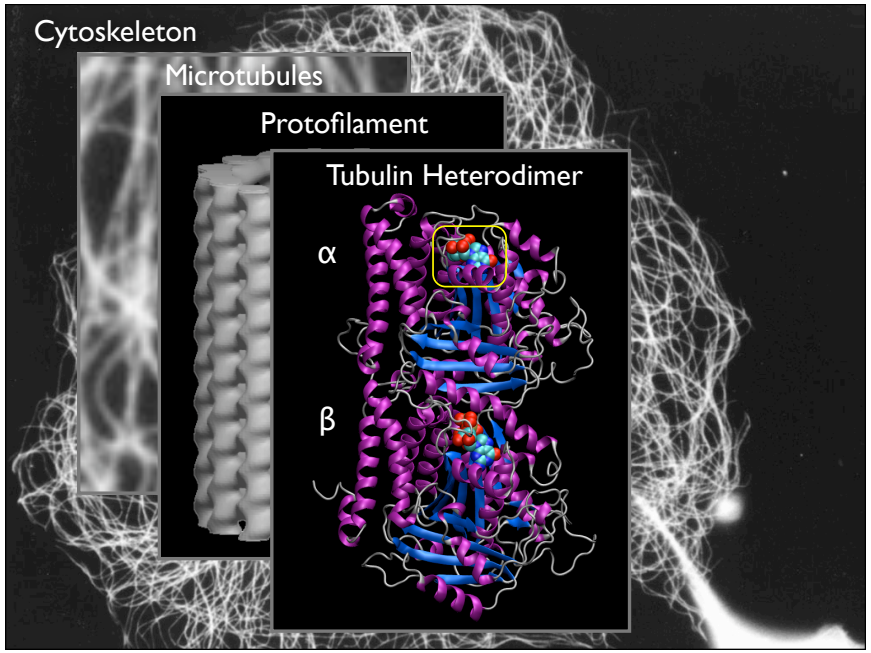
Relevance to engineering:
- Understanding the design principles of molecular motors will inform efforts to construct efficient nanoscale machines.

Motors Are Efficient Nanoscale Machines

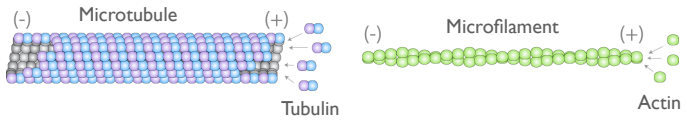
	Kinesin	Automobile
Size	10^{-8} m	1m
Speed	4×10^{-3} m/hr 4×10^{5} lengths/hr	10^{5} m/hr 10^{5} lengths/hr
Efficiency	~70%	~10%

Other non-cytoskeletal motors
DNA motors: Helicases, Polymerases
Rotary motors: Bacterial Flagellum, F1-F0-ATPase


Getting in Scale
This Fibroblast is about 100 μM in diameter.
A single microtubule is 25nm in diameter.
You can also see microfilaments and intermediate filaments.


Cytoskeleton
Microtubules
Protofilament
Tubulin Heterodimer
α
β

Key Concept: Directionality



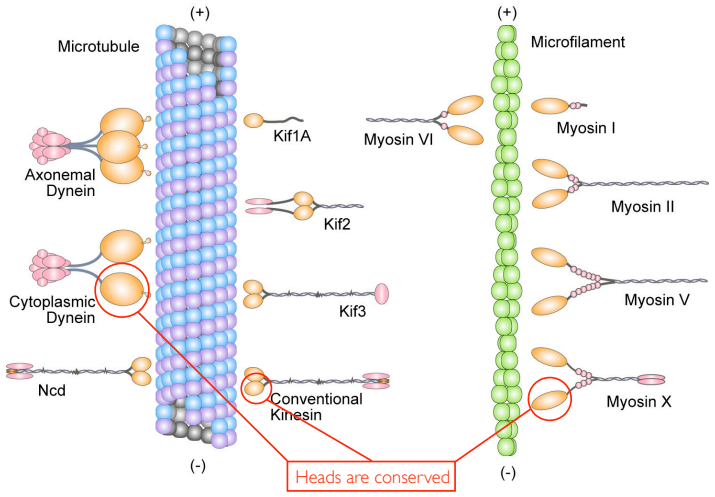
Track Polarity

- Due to ordered arrangement of asymmetric constituent proteins that polymerize in a head-to-tail manor.
- Polymerization occurs preferentially at the + end.
- Organized with a uniform polarity in the cell.

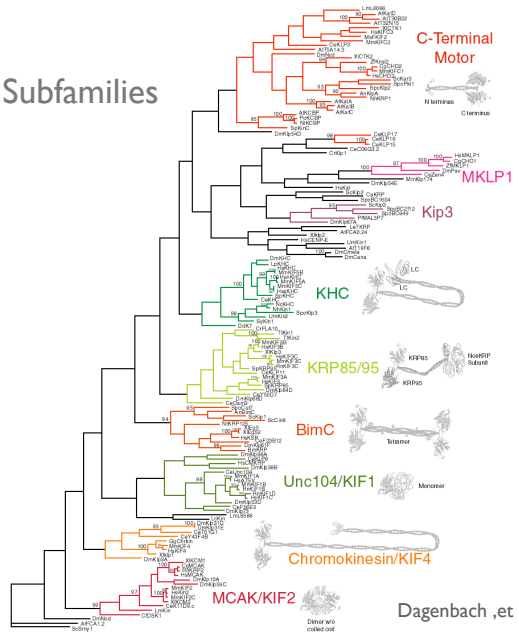
Motors recognize track polarity and move unidirectionally

- Kinesin moves to the + end of microtubules
- Dynein moves to the - end of microtubules
- Myosin moves to the + end of microfilaments

Cytoskeletal Motors are Modular



Kinesin Subfamilies



Dagenbach ,et al. J. Cell. Sci. (2004)

Kinesins and microtubules allow cells to self-organize

Organelle movement

Assembly of cilia/flagella

Signaling pathways

Mitotic spindle formation and chromosome movement

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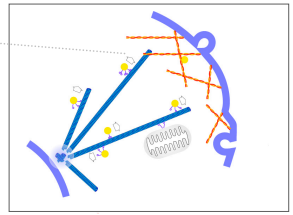
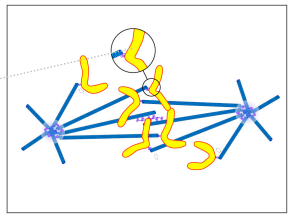
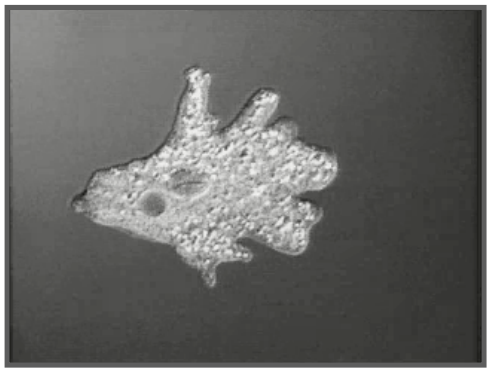




How Do Cytoskeletal Motors Work?



How are motors able to convert chemical energy into this remarkable motion?

Alberts ,et al."Molecular Biology of the Cell" (2002)

Key Concept: Mechanochemical Coupling

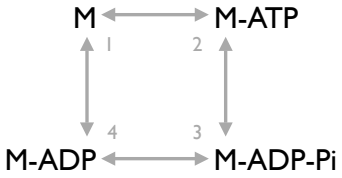
Molecular machines have moving parts and a mechanical mechanism.

The mechanical action is driven by chemistry. The chemistry can be altered by applied force.

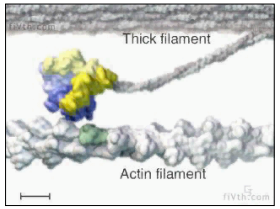
This is called mechanochemical coupling

Mechanochemical Coupling

Chemical cycle
ATP hydrolysis cycles result in conformational changes that are coupled with track binding and release events.



Conformational cycle
Characterizing the complete sequence of conformational changes is essential for understanding motor mechanisms.



Vale & Milligan, Science (2000)

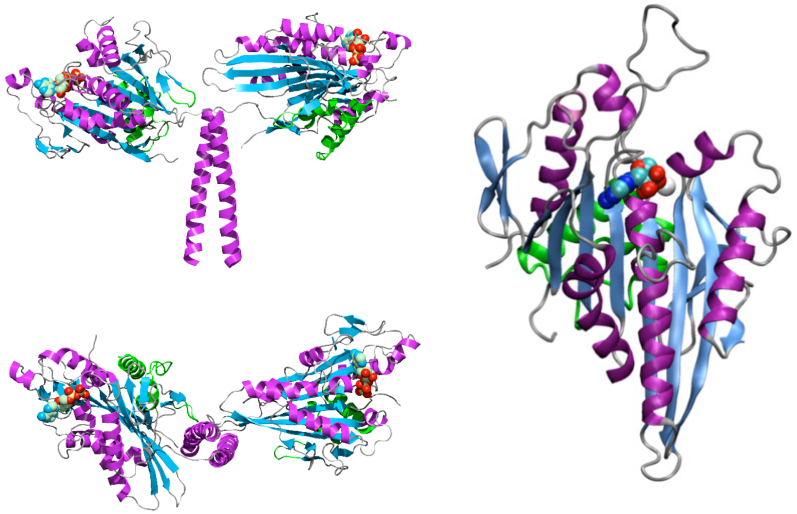
How do you study the mechanism of a molecular motor?

Structural Data
Crystallography, cryoEM and modeling

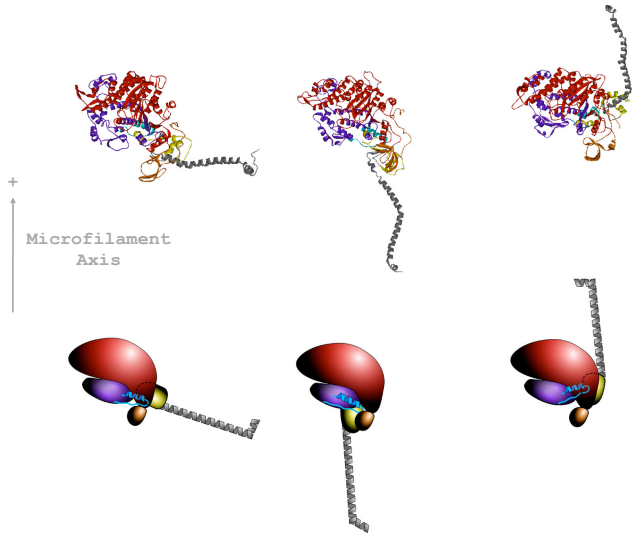
Kinetic Data
Kinetic studies, mutagenesis

Mechanical Data
Single molecule biophysical studies

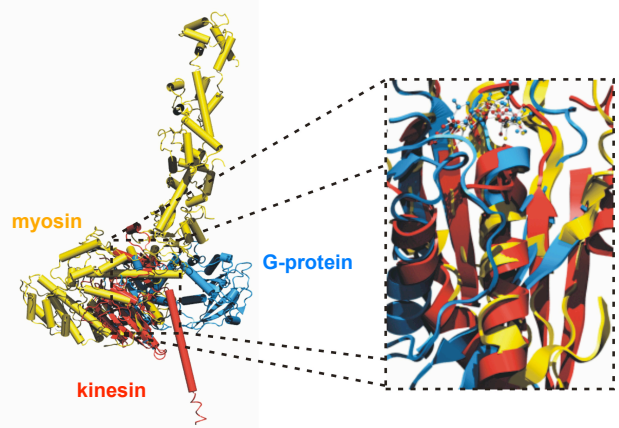
Kinesin Motor Domain Structure



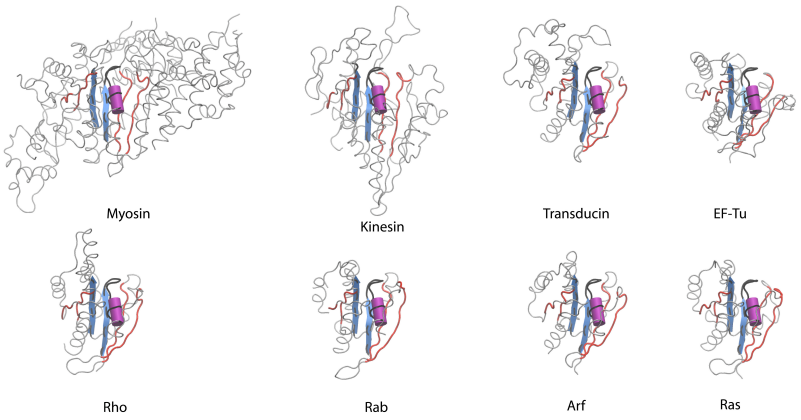
Structural States of Myosin: Swinging Lever



Kinesin, Myosin and G-proteins are Related

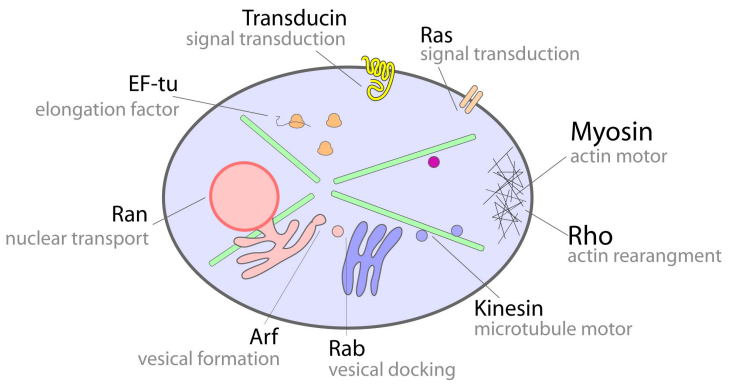


Nucleotide Dependent Conformational Switches



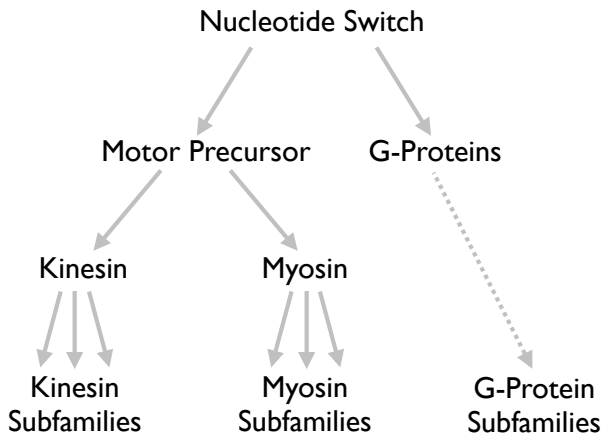
Myosin, kinesin and G-proteins are distant relatives

Nucleotide Dependent Conformational Switches

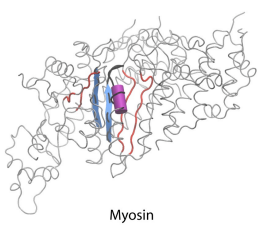


Participate in diverse cellular functions but share the ability to switch between nucleotide dependent conformations.

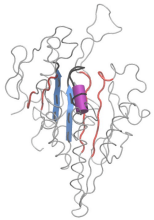
Evolution of Motor Proteins



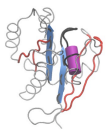
Nucleotide Chemistry is Similar Mechanical Elements Differ



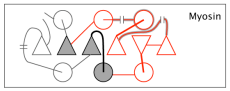
Myosin

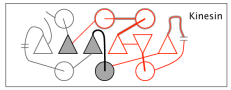


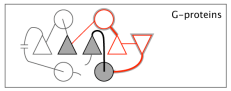
Kinesin



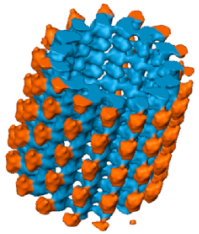
Ras



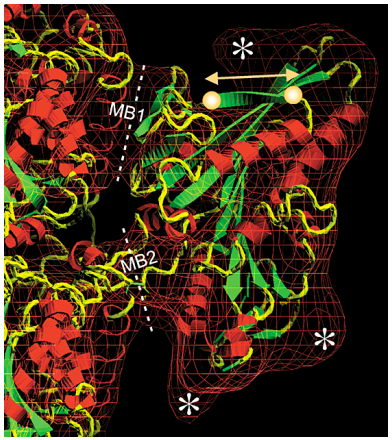




Cryo-EM: Motor Track Interactions

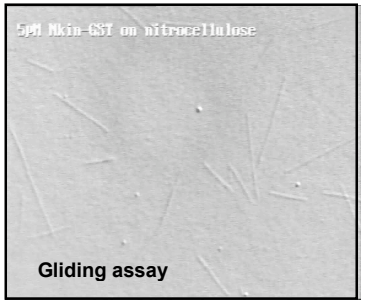


Neumann, Ibs (2006)

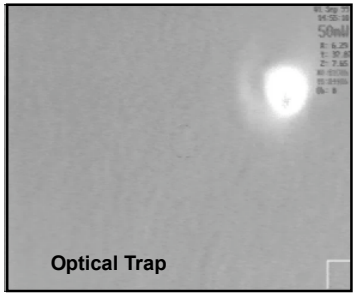


Kikkawa ,et al. Cell (2000)

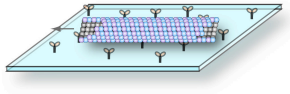
Motility Assays

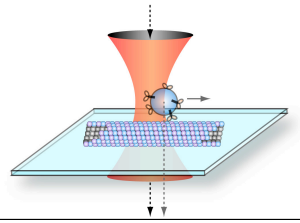


Gliding assay

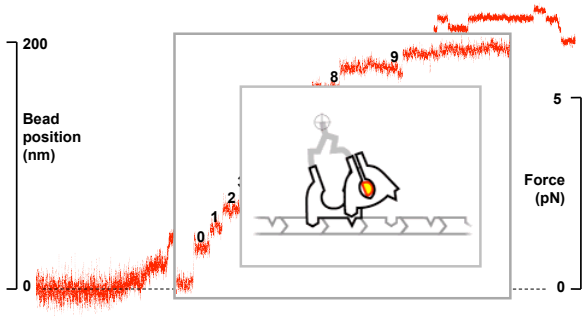


Optical Trap





Single Molecule Stepping



Kinesin is processive:
Walks with 8 nm strides (the distance between tubulin dimers) consuming 1 ATP per step.

Carter & Cross (communication)

Key Concept: Processivity

A single processive motor can move continuously along its track for several microns.

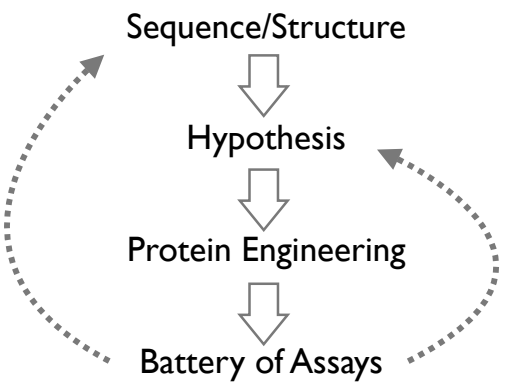
Porters and Rowers:

- Processive motors operate alone or in small numbers.
- Non-processive motors operate in large arrays.

Some kinesins & most dyneins are processive
Most myosins are non-processive

"What I cannot create, I do not understand"
-Richard Feynman

Testing How Motors Work?



Knowledge of detailed molecular mechanisms can lead to potential new therapeutic routes - drug the track & motor!

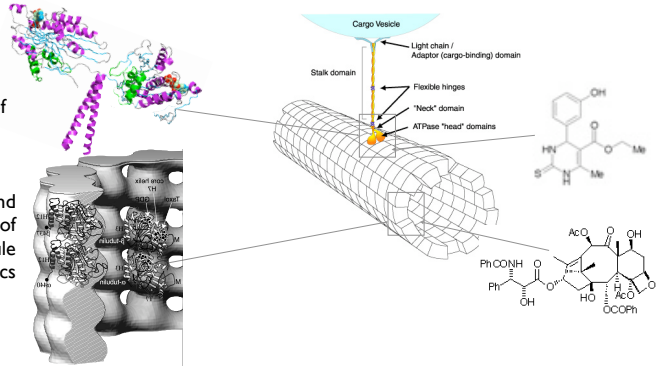
Apply Our Knowledge For Practical Outcomes

Small molecule drugs for the track and motor

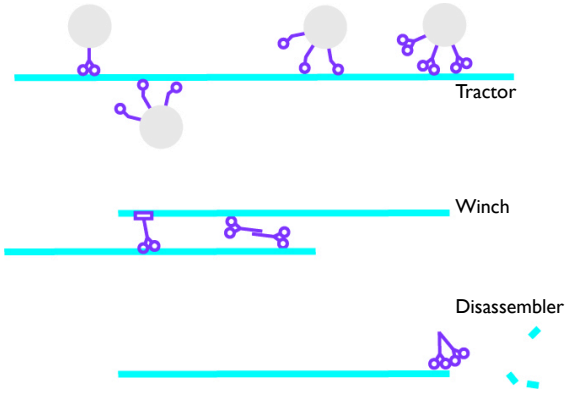
Inhibition or enhancement of motor protein activity.

Stepping mechanism of kinesin

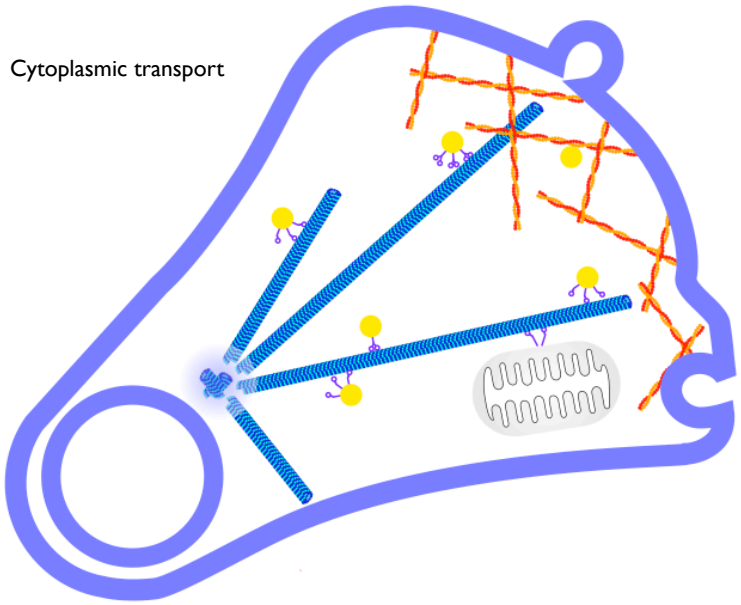
Mechanism and regulation of microtubule dynamics



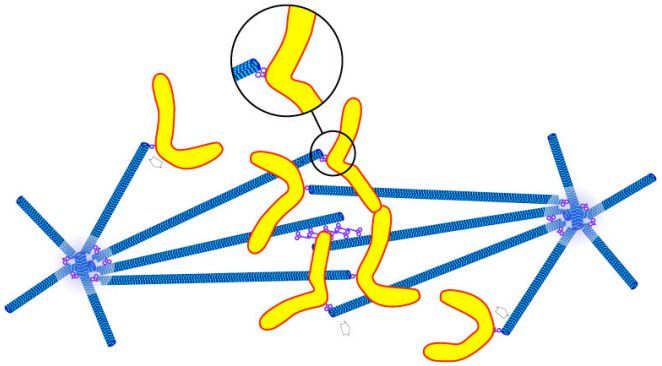
Operational Modes of Kinesin



Cytoplasmic transport



Mitosis



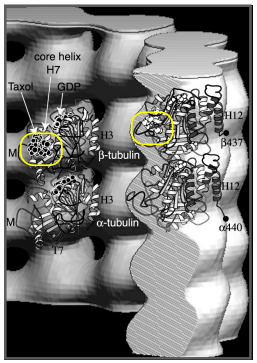
Drug The Track

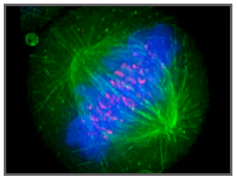
Drugs that interfere with mitotic spindle function have proved effective as anti-cancer agents

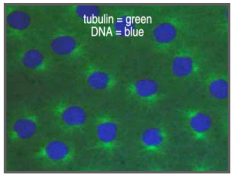
Paclitaxel
Docetaxel

Vincristine
Vinblastine
Vinorelbine

Side effects due to their action on all microtubules







Drug The Motor

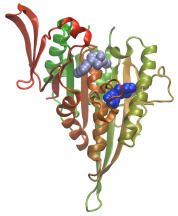
Mitosis specific kinesin 5 is essential for bipolar spindles.

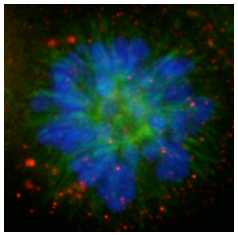
Inhibitors of kinesin 5 result in monopolar spindles and inhibited tumor growth in animals.

Ispinesib

Monastral

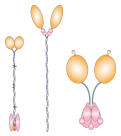
Potentially less side effects due to their specificity for dividing cells

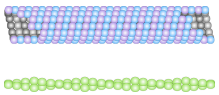


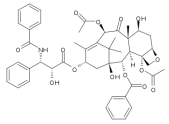


Currently in phase II clinical trials in humans

Summary of Key Points







Kinesin, myosin and dynein: cytoskeletal motor proteins with diverse functions.

Microtubules and actin filaments: polar cytoskeletal tracks upon which motors operate.

Directionality: motor subfamilies move in only one direction.

Mechanochemical transduction: conversion of chemical energy into molecular motion.

Conformational changes: changes in structure are linked to force production.

Stepping: motors can be thought of as stepping machines.

Processivity: the ability to move continuously for many hundreds of steps.

Drug development: small molecules that affect motors or their tracks.

Further Reading: Alberts, Molecular Biology of the Cell. Ch 16