An Introduction to Molecular Motors

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UCSD

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.

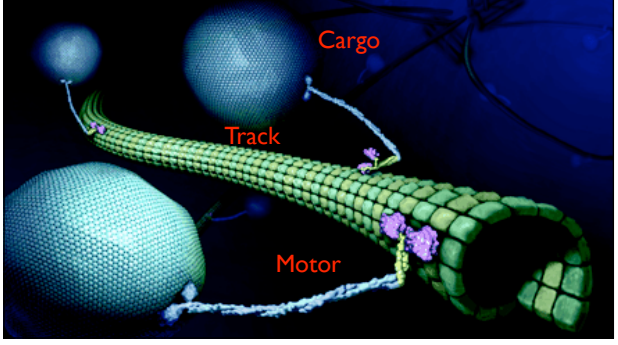
Motor
myosin, kinesin and dynein

Track
microfilaments and microtubules

Cargo
organelles, vesicles, chromosomes, etc.

Fuel
ATP and GTP

Microtubules and microfilaments are highly organized within cells and together with motor proteins provide a framework for the redistribution and organization of cellular components.



Why Study Cytoskeletal Motor Proteins?

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

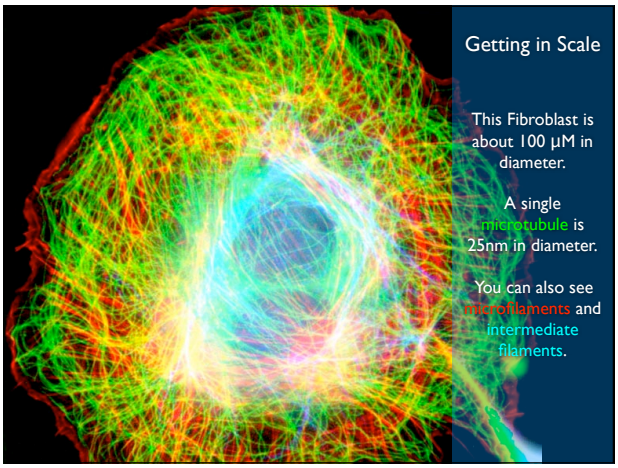
Relevance to medicine:
- Transport defects can cause disease.
- Inhibition or enhancement of motor protein activity has therapeutic benefits.

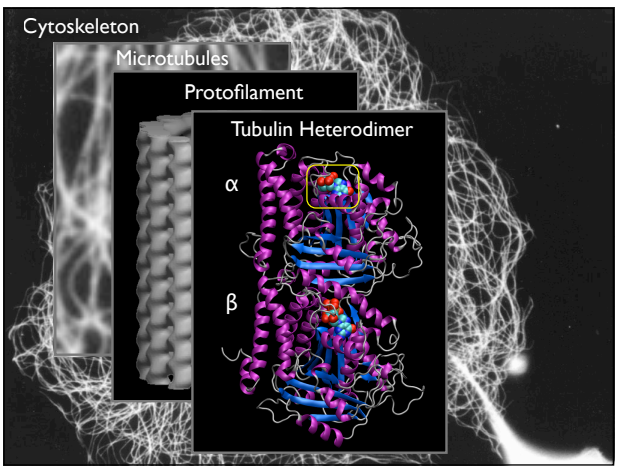
Relevance to engineering:
- Understanding the design principles of molecular motors will inform efforts to construct efficient nanoscale machines.

Motors Are Efficient Nanoscale Machines

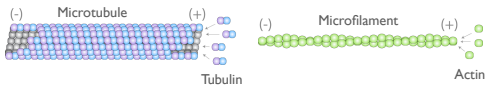
	Kinesin	Automobile
Size	10^{-8} m	1m
Speed	4×10^{-3} m/hr 4×10^{5} lengths/hr	10^{5} m/hr 10^{5} lengths/hr
Efficiency	~70%	~10%

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





Key Concept: Directionality



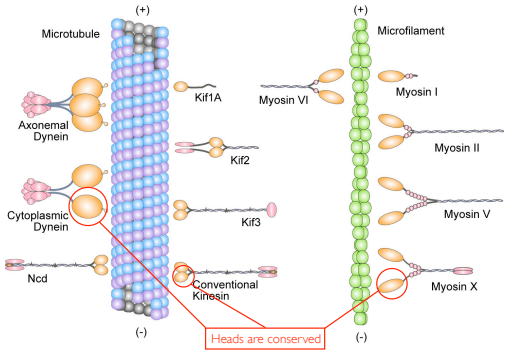
Track Polarity

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

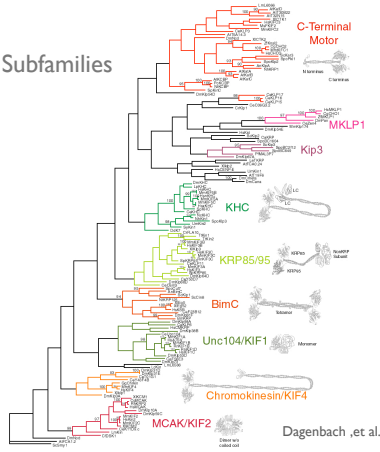
Motors recognize track polarity and move unidirectionally

- 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



Kinesins and microtubules allow cells to self-organize

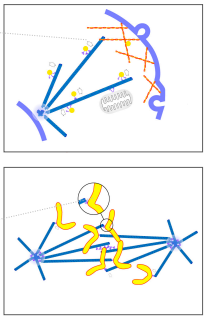
Organelle movement

Assembly of cilia/flagella

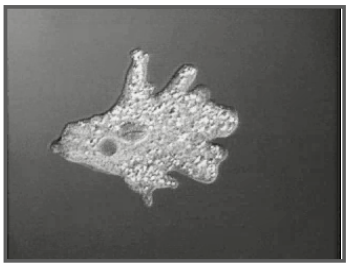
Signaling pathways

Mitotic spindle formation and chromosome movement

...



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

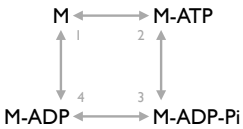
Molecular machines have moving parts and a mechanical mechanism.

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

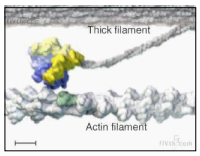
This is called mechanochemical coupling

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)

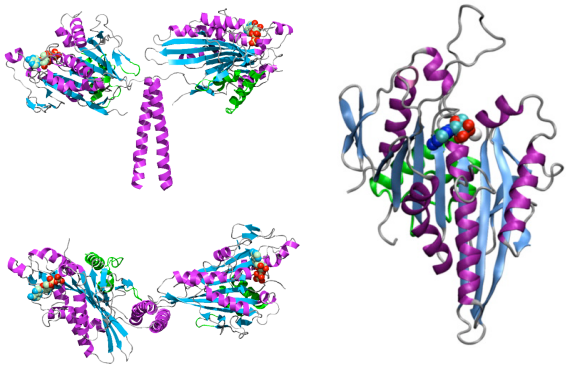
How do you study the mechanism of a molecular motor?

Structural Data
Crystallography, cryoEM and modeling

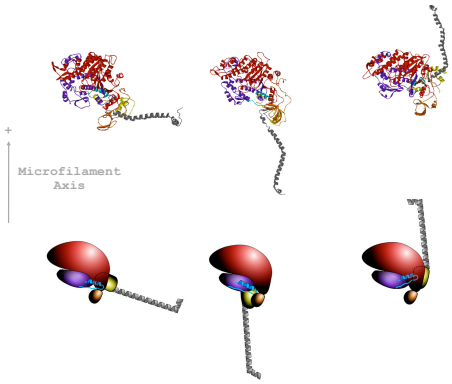
Kinetic Data
Kinetic studies, mutagenesis

Mechanical Data
Single molecule biophysical studies

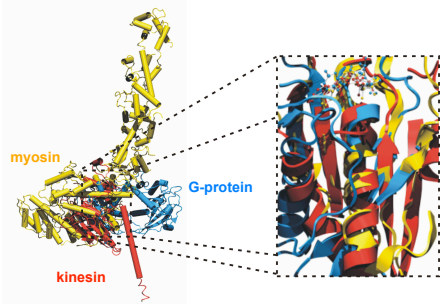
Kinesin Motor Domain Structure



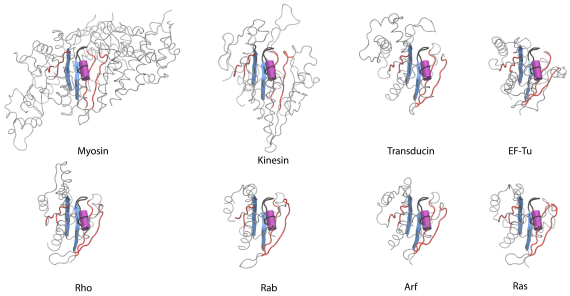
Structural States of Myosin: Swinging Lever



Kinesin, Myosin and G-proteins are Related

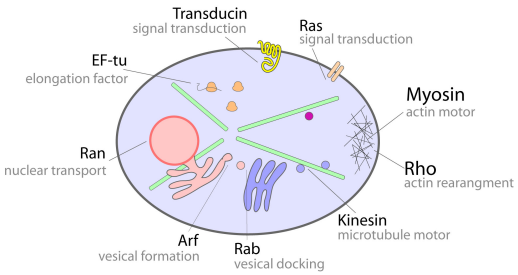


Nucleotide Dependent Conformational Switches



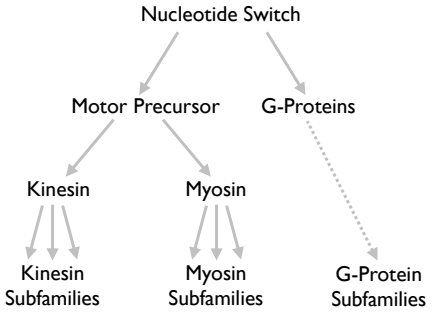
Myosin, kinesin and G-proteins are distant relatives

Nucleotide Dependent Conformational Switches

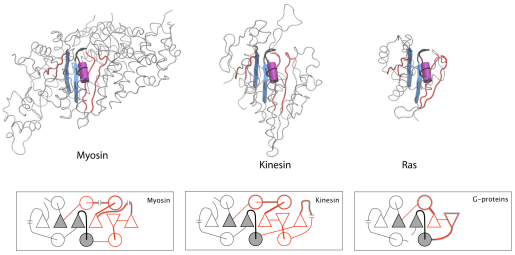


Participate in diverse cellular functions but share the ability to switch between nucleotide dependent conformations.

Evolution of Motor Proteins

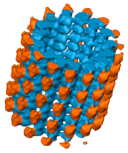


Nucleotide Chemistry is Similar Mechanical Elements Differ

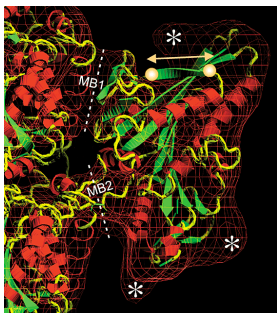


Cryo-EM: Motor Track Interactions

- There is no high resolution complex structure available
- CryoEM provides significant insight but at low resolution
  - highlight a potentially prominent role for charged residues

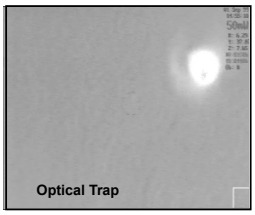


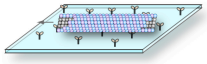
Neumann, Ibs (2006)

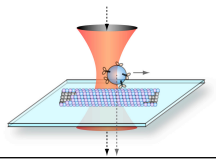


Kikkawa, *et al.* Cell (2000)

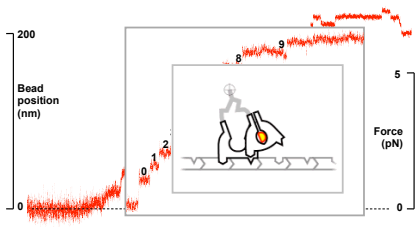
Motility Assays







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.

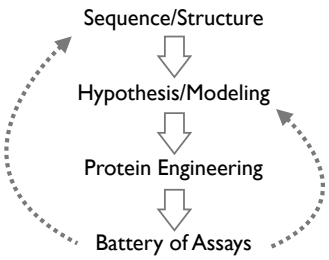
Porters and Rowers:
- Processive motors operate alone or in small numbers.
- Non-processive motors operate in large arrays.

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

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

Aim: To go beyond characterization and manipulate motors for our own benefit.

Testing How Motors Work?



Knowledge of detailed molecular mechanisms can inform the development of new drugs and new motors with tailored properties.

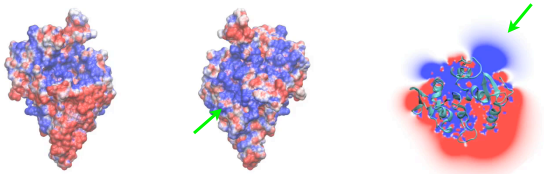
Probing The Association Mechanism: Ongoing Unpublished Work...

Q. What factors govern the association of kinesin to microtubules?

Our objective is to learn more about the design principles and functional mechanisms of molecular motors and use this knowledge to design motors with tailored properties

Electrostatic diversity of the kinesin family

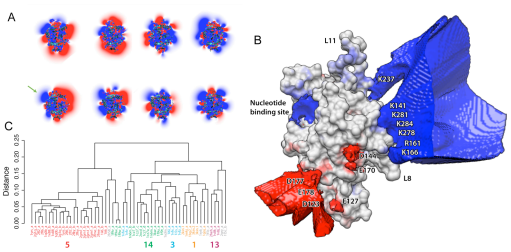
Available kinesin structures have diverse charge distributions. There are however consistent patches of positive potential.



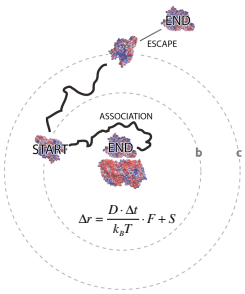
Front Back Top

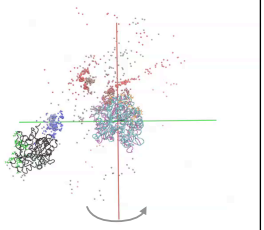
Kinesins have a common asymmetric charge distribution

Comparative electrostatic analysis highlights subfamily differences built on top a common underlying asymmetric charge distribution.



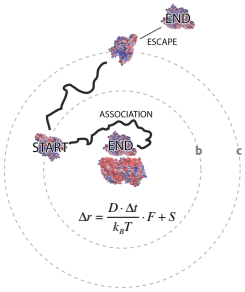
Brownian dynamics model of kinesin-tubulin association

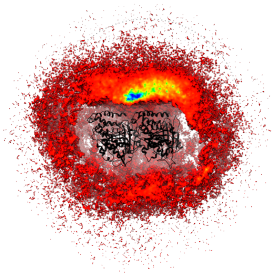




Single Trajectory

Brownian dynamics model of kinesin-tubulin association

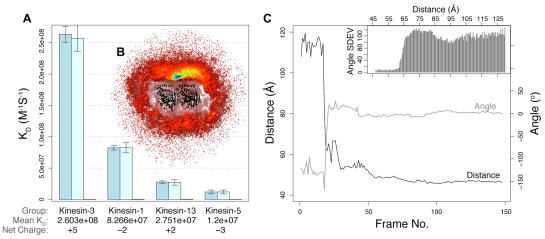




200k Trajectories

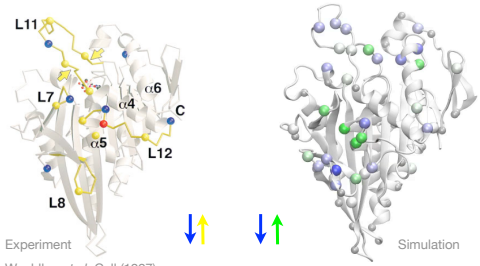
Electrostatically enhanced association rates

Long range electrostatic interactions enhance association rates with different subfamilies displaying distinct association rates.



Mutants dissect the contribution of key residues

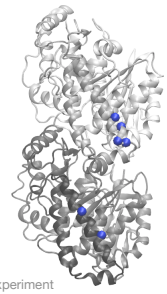
Mutational analysis of surface exposed charged sites
In agreement with experimental alanine scanning analysis



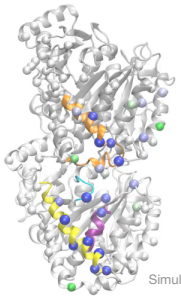
Woehlke, *et al.* Cell (1997)

Both tubulin subunits contribute to binding

Predictions for tubulin currently being tested



Experiment



Simulation

Uchimura, *et al.* EMBO (2006, 2010)

R326: towards rational alteration of kinesin velocity

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      * *
K...K.H.
K..ER.R.
....R.R.
R.......
R...R.K.
R...R...

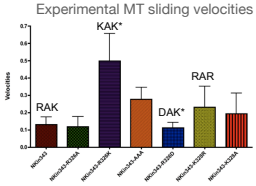
R..E...E

K.......
```

Computational predictions

	ΔG	ΔΔG (vs WT)
KAK*	-9.813	-2.200
RAR	-9.220	-1.607
RAE	-6.819	0.794
RAD	-6.646	0.967
RAA	-6.506	1.107
AAK	-3.677	3.936
DAK*	-1.135	6.478

Experimental MT sliding velocities



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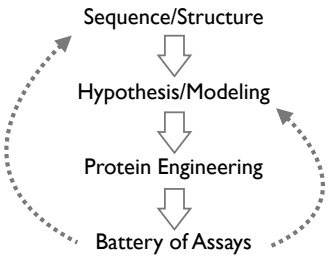


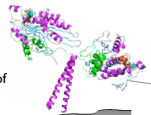
Rational charge perturbation via mutation of key positions can yield faster or slower velocity motors in the laboratory

Apply Our Knowledge For Practical Outcomes

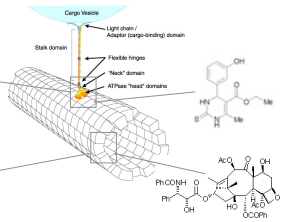
Small molecule drugs for the track and motor

Inhibition or enhancement of motor protein activity.

Stepping mechanism of kinesin



Mechanism and regulation of microtubule dynamics



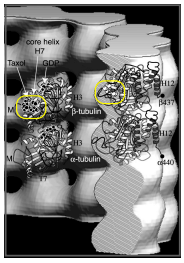
Existing Drugs Target The Track

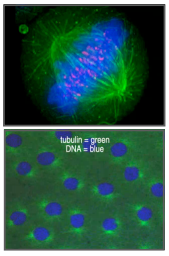
Drugs that interfere with mitotic spindle function have proved effective as anti-cancer agents

Paclitaxel
Docetaxel

Vincristine
Vinblastine
Vinorelbine

Side effects due to their action on all microtubules





Drug The Motor

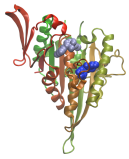
Mitosis specific kinesin 5 is essential for bipolar spindles.

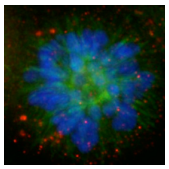
Inhibitors of kinesin 5 result in monopolar spindles and inhibited tumor growth in animals.

Ispinesib

Monastral

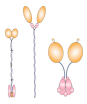
Potentially less side effects due to their specificity for dividing cells

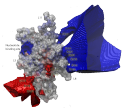


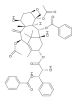


Currently in phase II clinical trials in humans

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.

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

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

Electrostatic interactions: can be tailored to yield altered velocity motors.

Drug development: therapeutic benefit of small molecules that affect motors & tracks.

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