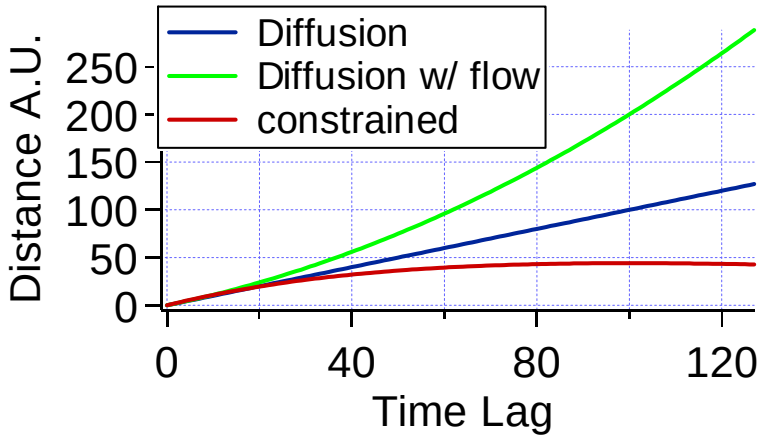
Particle tracking, cont..

Data Analysis Techniques

- MSD versus time
- Anomalous diffusion coefficient
- Temporary Confinement



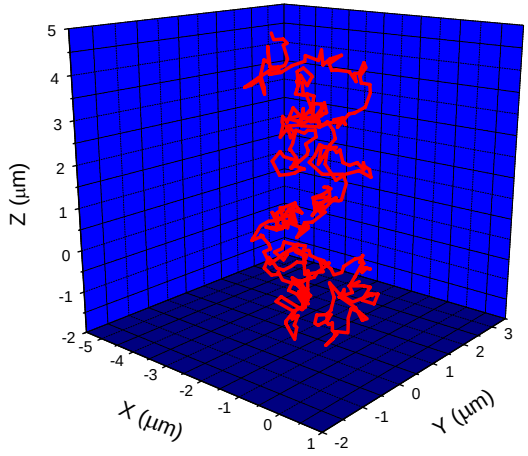
Pure Diffusion

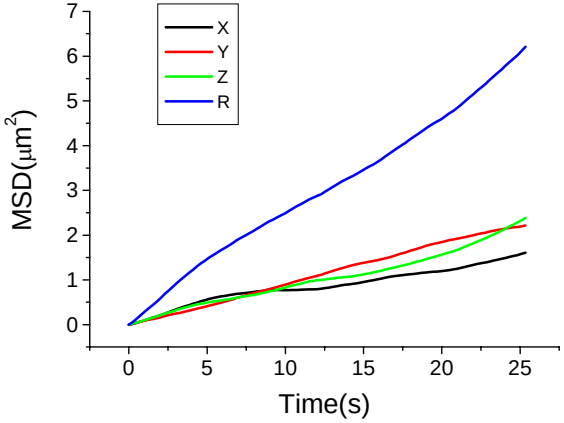
$$\left\langle r^2 \right\rangle = 6Dt$$

Diffusion w/ flow

$$\left\langle r^2 \right\rangle = 6Dt + (Vt)^2$$

Pure diffusion

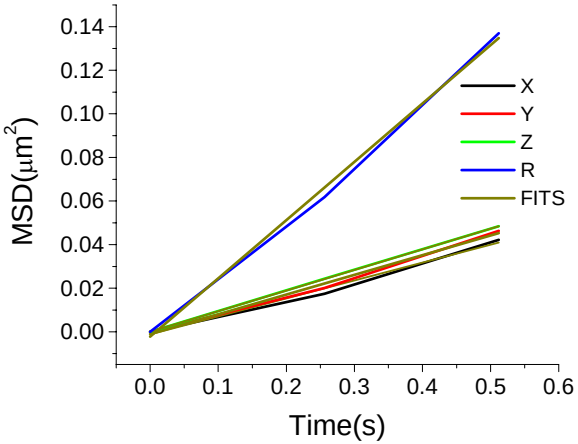




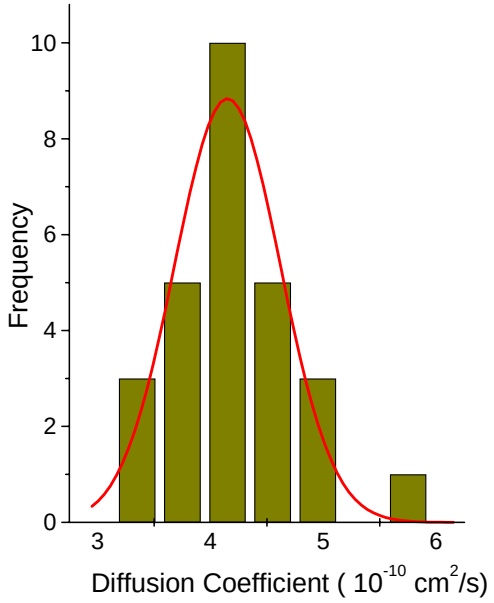
200 nm sphere in 60% sucrose

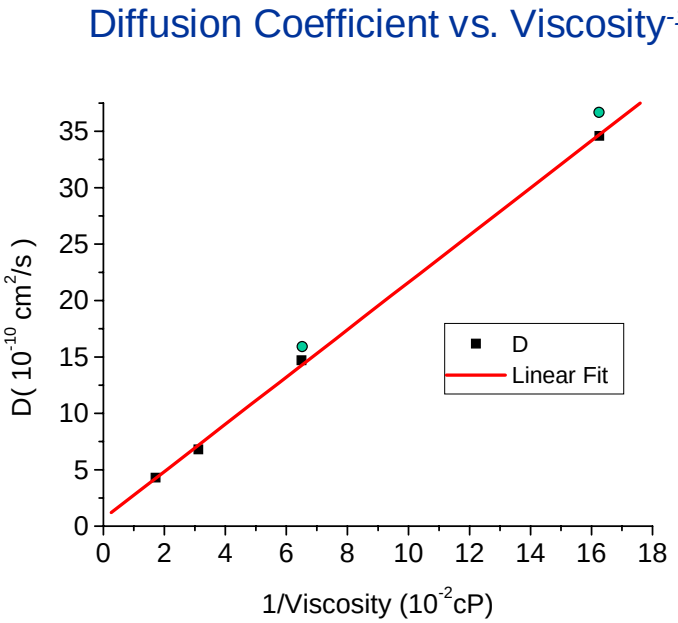
(a) 3D trajectory of sphere.
(b) MSD versus time plot
(c) Short term MSD versus time for lag times

Diffusion coefficient $4.0*10^{-10}$ cm^2s



Histogram of Diffusion Coefficients for 200 nm sphere in 60% sucrose





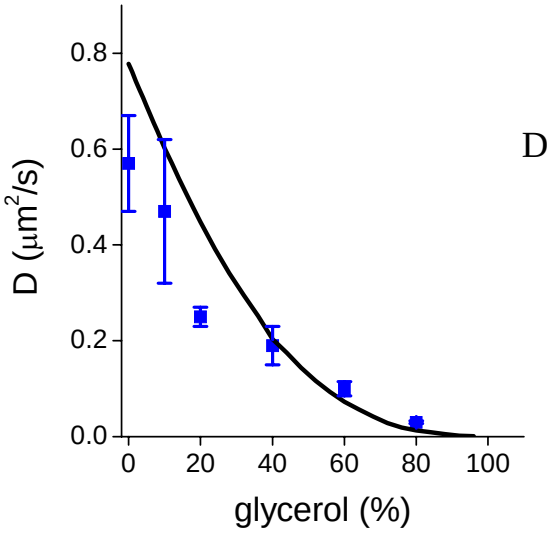
The histogram of diffusion coefficients is Gaussian and the mean is within 2% of the of the Stokes-Einstein value given by:

$$D = \frac{kT}{6\pi\eta R}.$$

k = Boltzmann's constant
T = Temperature
n - Viscosity
R = Radius of Particle

The diffusion coefficients for 200 nm spheres were plotted versus inverse viscosity and slope of the fit was within 3% of the value expected from the Stokes-Einstein relationship.

Diffusion coefficients of fluorescent beads



$$D = \frac{k\,T}{6\,\pi\,r\,\eta}$$

k = Bolztmann 's constant
T= temperature
r= particle radius
η = viscosity

	D ($\mu m^2/s$)	
virus	0.25 – 1.3	Seisenberger et al. *Nature* (2001)
MHC (membrane protein)	~ 10^{-4}	Smith et al. *Biophys J* (1997)
lipid-bead	0.08	Hicks et al. *J Memb Biol* (1995)
secretory vesicles (NT)	2.1 10^{-4} – 5.4 10^{-2}	Abney et al. *Biophys J* (1999)

Transient Trapping of a particle

- We wish to identify regions of Brownian versus non-Brownian motion
- Has been useful in 2D experiments on the plasma membrane
- Endocytosis, Intracellular Traffic, Chromatin Dynamics
- Not possible to do this by visually inspecting the trajectory

Statistical method: The probability that a particle with diffusion coefficient D stays within a region R for all times t.

Diffusion Equation $\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial^2 x}$

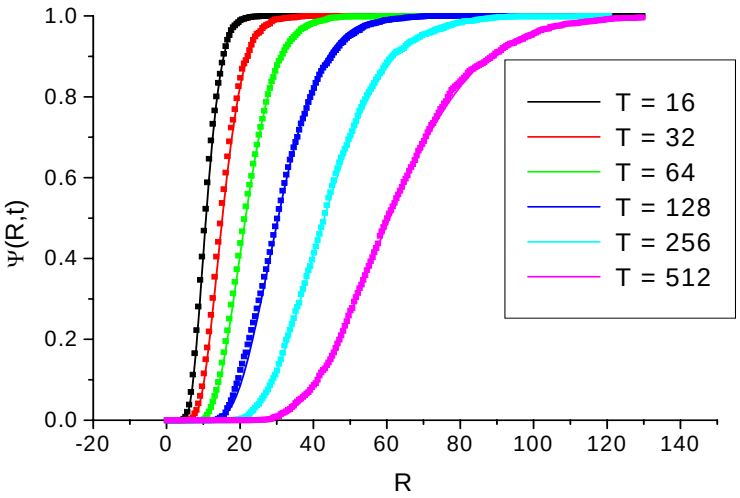
I.C. C(r,0) = δ(0)

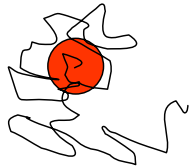
B.C. C(R,t) = 0

$$\Psi(R,t) = 2 \cdot \sum_{k=1}^{\infty} (-1)^k \exp(-\pi^2 k^2 Dt / R^2)$$

Rubin et al., 1976

$$\Psi(R,t) = 2 \cdot \sum_{k=1}^{\infty} (-1)^k \exp(-\pi^2 k^2 DT / R^2)$$

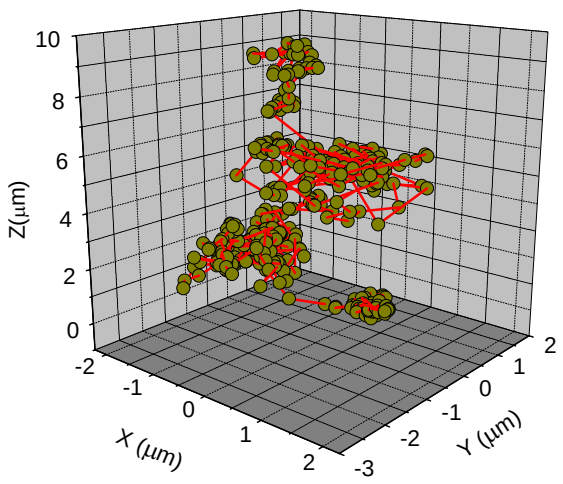


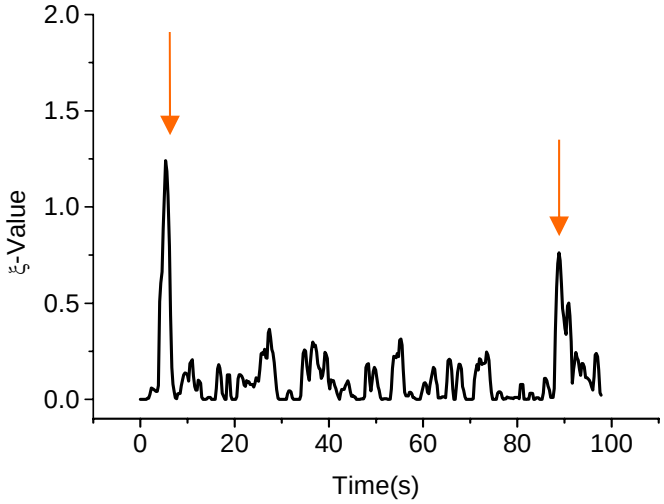


The probability to remain within R (the red trap) is small and it decreases as T (time) increases

200 nm spheres in 0.3 % agarose

Agarose gels are porous structures that can trap particles if the pore size if comparable to the particle size





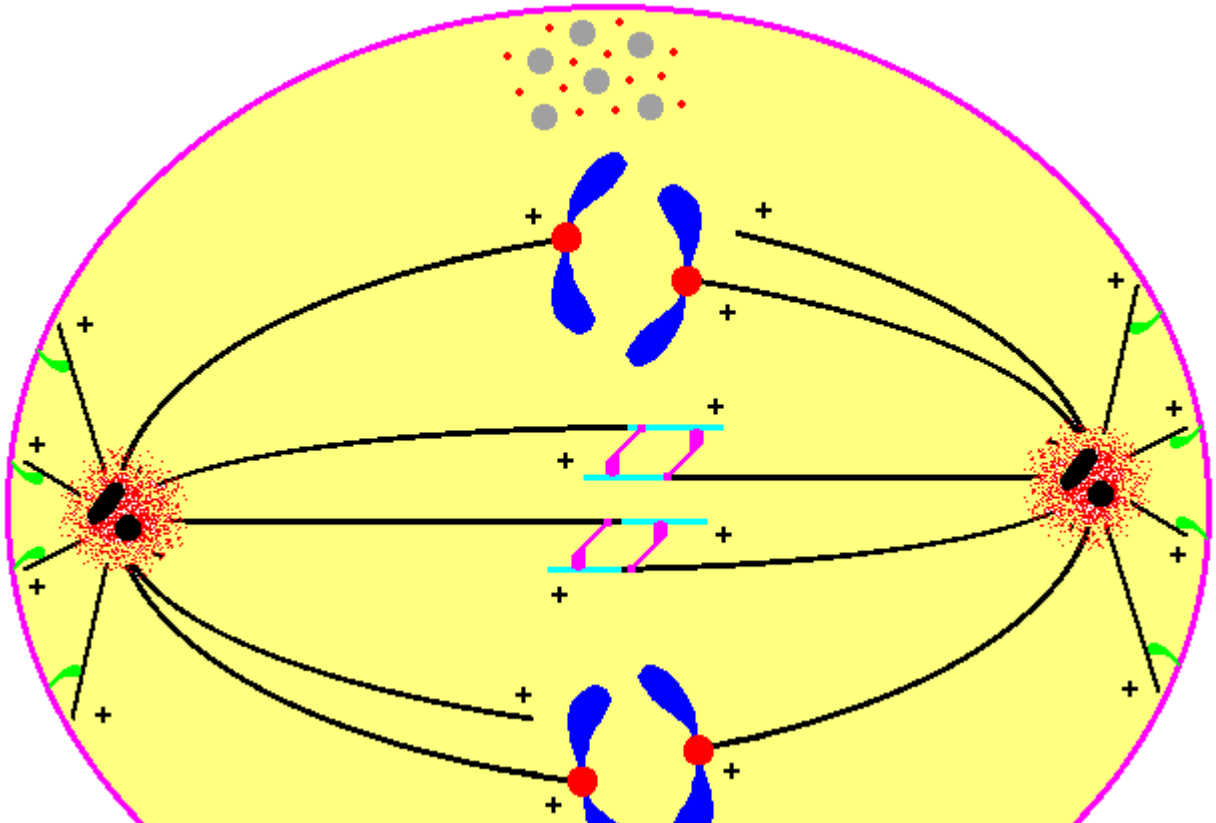
Cell Division (overview)

NOT TO SCALE!

Prophase: the spindle poles separate, the sister chromatids condense and the nuclear membrane disperses.

Metaphase: the sister chromatids are aligned at the middle of the cell.

Anaphase: the cell elongates, the daughter chromosomes separate and are



Telophase: the nuclear membranes reform and cytoplasmic division is completed.

Recent papers and reviews:

Brunet & Vernos (2001) *Chromosome motors on the move - From motion to spindle checkpoint activity* EMBO Reports 2(8), 669-673.

Doxsey (2001) *Re-evaluating centrosome function* Nature Reviews Molecular Cell Biology **2**(9), 688-698.

Endow, SA (1999) *Microtubule motors in spindle and chromosome motility.* Europ. J. Biochem. **262**(1), 12-18.

Faulkner et al (2000) *A role for the lissencephaly gene LIS1 in mitosis and cytoplasmic dynein function* Nature Cell Biology **2**(11), 784 - 791.

Glotzer (2001) *Animal Cell Cytokinesis* Annu. Rev. Cell Dev. Biol. **17**, 351-386.

Heald (2000) Cell **102**, 399 - 402 provides an excellent minireview of two full papers on the chromokinesin Xkid (by Antonio et al and Funabiki et al) in the same issue of this journal. The Xkid motor protein aligns the sister chromatids in the metaphase plate, and must be degraded before anaphase can commence.

Scholey et al (2001) *Mitosis, microtubules, and the matrix* J. Cell Biology **154**(2), 261-266.

Myosin

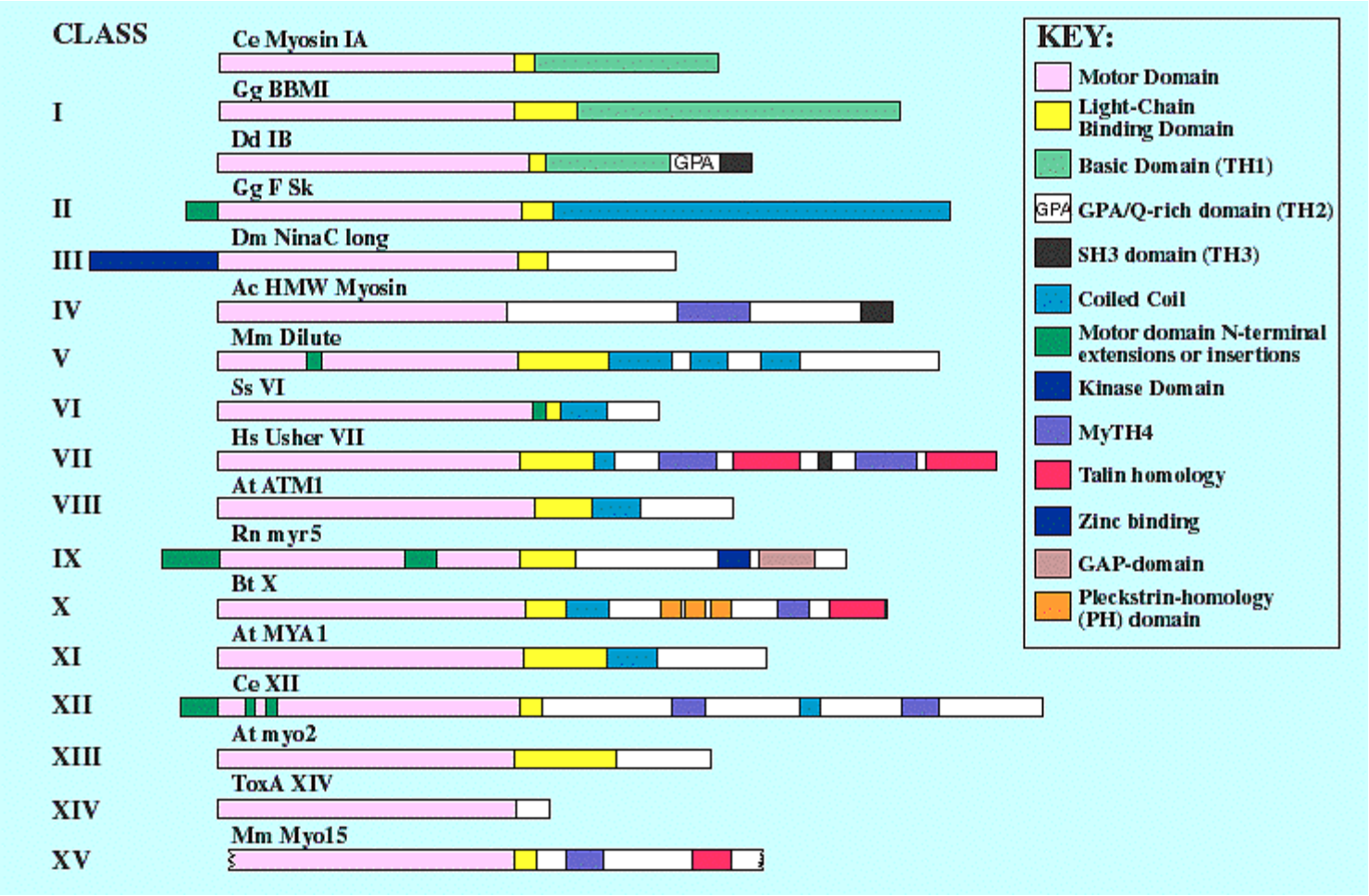
Duke (1999) *Molecular model of muscle contraction.* Proc. Natl. Acad. Sci. USA **96**(6), 2770-2775.

Geeves & Holmes (1999) *Structural mechanism of muscle contraction* Annu. Rev. Biochem. **68**, 687-728.

Ruff et al. (2001) *Single-molecule tracking of myosins with genetically engineered amplifier domains* Nature Structural Biology **8**(3), 226-229.

Spudich (2001) *The myosin swinging cross-bridge model* Nature Reviews in Molecular Cell Biology **2**(5), 387-392.

The motor domain is a relatively constant feature of the numerous myosin variants, but the remainder of these molecules shows enormous variation, in keeping with their differing physiological functions. The following figure derives from the Cambridge MRC muscle website, which specializes in clustal analysis of the myosin genes.



Non-muscle myosins are required for *cytokinesis* at the end of cell division, when a contractile ring near the plasmalemma divides the cytoplasm of the

daughter cells. They are involved in cytoplasmic streaming movements in many tissues, and especially in actively motile cells such as fibroblasts and macrophages. Special myosin variants are required for sensory processes involved in hearing and vision.

Bridgman & Elkin (2000) *Axonal myosins* J. Neurocytol. **29**, 831-841

Hearing and vision

The unexpected roles of myosin in hearing and vision have attracted considerable medical interest. It seems likely that myosins are involved in the correct assembly of melanosomes within the pigmented layer of the retina, and also in the correct differentiation of *hair cells* within the inner ear. These cells display actin-based *stereocilia* which transduce sound waves and the movement of fluid in the semicircular canals into nerve impulses. In addition to the roles of myosin isoforms in hair cell differentiation, myosin has a further function in the adaptation of the hearing transducer to cope with loud and faint noises. The adaptation motor is thought to be myosin 1 beta, which slides an actin-based "needle valve" to almost block an ion channel in the hair cell membrane. Sound vibrations displace the membrane, allowing ions to enter the cell, and this is thought to initiate the signaling process.

The central point is that **all** hair cells are motile, negative feedback systems, actively changing their shape in response to membrane potential, as well as changing their membrane potential in response to deformation. There is specialization, in that the inner hair cells are mostly sensory, whereas the more numerous outer hair cells have an obvious motor function. The main function of the cochlear amplifier is to select particular frequencies in the face of much louder background noises. There is also an overall volume control through the reflex contraction of the tensor tympani and stapedius muscles in the middle ear when the subject is exposed to loud noises, thereby reducing the movement of the auditory ossicles.

Usher syndromes *type IA* (dynein) and *type 1B* (myosin 7A) are autosomal recessive disorders characterized by profound congenital deafness, vestibular areflexia, and progressive retinitis pigmentosa. *Nonsyndromic* deafness results from mutations in myosin 15, myosin 7 and possibly in the myosin 9 and MYO1D genes. *Myosin 6* is also a strong candidate gene, which encodes the only known myosin motor directed toward the minus end of actin filaments. There is a recent review by Willems (2000) *Genetic Causes of Hearing Loss* New England J. Med. **342**(15) 1101 - 1109.

Mouse "dilute" mutations (myosin 5A) are associated with neurological deficits, although the analogous *Griscelli syndrome* in humans apparently has no neural component.

Ashmore & Mammano (2001) *Can you still see the cochlea for the molecules?* Current Opinion In Neurobiology **11**(4), 449-454.

Eatock (2000) *Adaptation in Hair Cells* Annu. Rev. Neurosci. **23**, 285-314.

Gillespie & Walker (2001) *Molecular basis of mechanosensory transduction* Nature **413**(6852), 194-202.

Holt & Corey (2000) *Two mechanisms for transducer adaptation in vertebrate hair cells* Proc. Natl. Acad. Sci. USA **97**(22), 11730-11735.

Hudspeth et al (2000) *Putting ion channels to work: Mechanoelectrical transduction, adaptation, and amplification by hair cells* Proc. Natl. Acad. Sci. USA **97**(22), 11765-11772.

Martin et al (2000) *Negative hair-bundle stiffness betrays a mechanism for mechanical amplification by the hair cell* Proc. Natl. Acad. Sci. USA **97**(22), 12026-12031.

Muller & Littlewood-Evans (2001) *Mechanisms that regulate mechanosensory hair cell differentiation* Trends In Cell Biology **11**(8), 334-342.

Petit (2001) *Usher syndrome: from genetics to pathogenesis* Annu. Rev. Genom. Human. Genet. **2**, 271-297.

De la Cruz et al. (2001) *Kinetic mechanism and regulation of myosin VI* J. Biol. Chem. **276**(34), 32373-32381.

Cell migration

Cells migrate by extending actin-stiffened "ruffles" (lamellipodia) at the front which form new focal adhesions to the substrate, then dragging the cell body forwards, and finally letting go of the old focal adhesions at the rear. Myosin I provides the motive power at the leading edge of the cell, and myosin II at the rear.

Geiger & Bershadsky (2001) *Assembly and mechanosensory function of focal contacts* Current Opinion In Cell Biology 13(5), 584-592.

Vesicular Motors

Myosin I and Myosin V are both involved in vesicular trafficking, particularly that involving the Golgi apparatus and the cell membrane. Trafficking is normally a two-way activity (you have to collect the "empties") but it is not clear at present which myosin variants are responsible for which stages of the process.

Karcher et al. (2001) *Cell cycle regulation of myosin-V by calcium/calmodulin-dependent protein kinase II* Science **293**(5533), 1317-1320.

Mehta (2001) *Myosin learns to walk* J. Cell Science 114(11), 1981-1998.

Veigel, C et al (1999) *The motor protein myosin-I produces its working stroke in two steps.* Nature **398**(6727), 530-533.

Circumferential belt

There is a circular actomyosin contractile bundle underlying the adherens junction between adjacent epithelial cells. This is important for closing any gaps in the sheet of cells and healing wounds.

Mandato & Bement (2001) *Contraction and polymerization cooperate to assemble and close actomyosin rings around Xenopus oocyte wounds* J. Cell Biol. **154**(4), 785-797.

Cell division motors (cytokinesis)

Myosin II powers the contractile band that forms around the equator of dividing cells after nuclear division is complete, and squeezes the cytoplasm into two daughter cells.

Komatsu et al. (2000) *Effects of the regulatory light chain phosphorylation of myosin II on mitosis and cytokinesis of mammalian cells* J. Biol. Chem. **275**(44), 34512-34520.

Poperechnaya et al.(2000) *Localization and activity of myosin light chain kinase isoforms during the cell cycle* J. Cell Biol. **151**(3), 697-707.

Kinesin and kinesin related proteins

This is the X-ray structure of rat kinesin, reported by Kozielski et al in 1997. [Brookhaven code 3KIN.] Note the two head groups and the long helical tail, by which the cargo is attached. Switch to ribbon view and colour in the four protein strands. Rotate the molecule and experiment with different views to study the mechanism.

Most kinesins and kinesin related proteins are + end directed motors, but - end directed variants are known in the cell division apparatus.

For much useful background information, and some nice movies of molecular motors moving things, visit the kinesin home page hosted by the Fred Hutchinson Cancer Research Center in Washington. It is worth taking some time to explore this site, which includes an illustrated account of organelle jams in the *Khc* mutants in *Drosophila.*

Goldstein (2001) *Kinesin molecular motors: Transport pathways, receptors, and human disease* Proc. Natl. Acad. Sci. USA **98**(13), 6999-7003.

Goldstein & Philp (1999) *Emerging Principles of Kinesin Motor Utilization* Annu. Rev. Cell Dev. Biol. 15, 141-183.

Kikkawa et al. (2001) *Switch-based mechanism of kinesin motors* Nature **411**(6836), 439-445.

Schnitzer et al (2000) *Force production by single kinesin motors* Nature Cell Biology **2**(10), 718 - 723.

Vision

The photoreceptors on the rod and cone cells are greatly modified cilia. In addition to the contractile protein dynein, which is responsible for bending, normal cilia also contain a kinesin-based transport system that moves internal components to the far end of each cilium. This mechanism is exploited in the photoreceptors for opsin transport, and mutations in this molecular motor lead to blindness.

Retinitis pigmentosa is a common cause of blindness. It is often caused by rhodopsin mutations, but may also result from mitochondrial defects and a failure of kinesin II to transport opsin into the photoreceptors. KIF1A gene

knockout produces motor and sensory defects in mice through a failure in synaptic vesicle transport. No human equivalent has yet been identified. There is an excellent review by Fernald (2000) *Evolution of eyes* Current Opinion in Neurobiology **10**(4) 444-450 which distinguishes the invertebrate photoreceptors based on microvilli from the vertebrate type which evolved from cilia.

- Nelson & Cox chapter 13 pages 458 - 463
- Tortora & Grabowski chapter 16 pages 512 - 529
- Zigmond et al chapter 24 pages 671 - 683
- Kandel et al chapter 26 pages 507 - 517

Recent papers:

Marszalek et al (2000) *Genetic evidence for selective transport of opsin and arrestin by kinesin-II in mammalian photoreceptors* CELL **102**,(2) 175-187. [no electronic copies]

There is a review on *Rhodopsin trafficking and its role in retinal dystrophies* by Sung & Tai (2000) International Review Of Cytology **195**, 215-267 in the Health Sciences Library, but no electronic copies are available.

Forward axonal transport

- Lodish et al chapter 19 pages 809 - 817
- Zigmond et al chapter 4 pages 94 - 104
- Kandel et al chapter 5

Recent papers:

Galbraith & Gallant (2000) *Axonal transport of tubulin and actin* J. Neurocytol. **29**, 889-911.

Kaether et al (2000) *Axonal Membrane Proteins Are Transported in Distinct Carriers: A Two-Color Video Microscopy Study in Cultured Hippocampal Neurons* Molecular Biology of the Cell **11**(4), 1213 - 1224.

Roy et al (2000) *Neurofilaments Are Transported Rapidly But Intermittently in Axons: Implications for Slow Axonal Transport* J. Neurosci. **20**(18), 6849 - 6861.

Cell division motors (spindle microtubules)

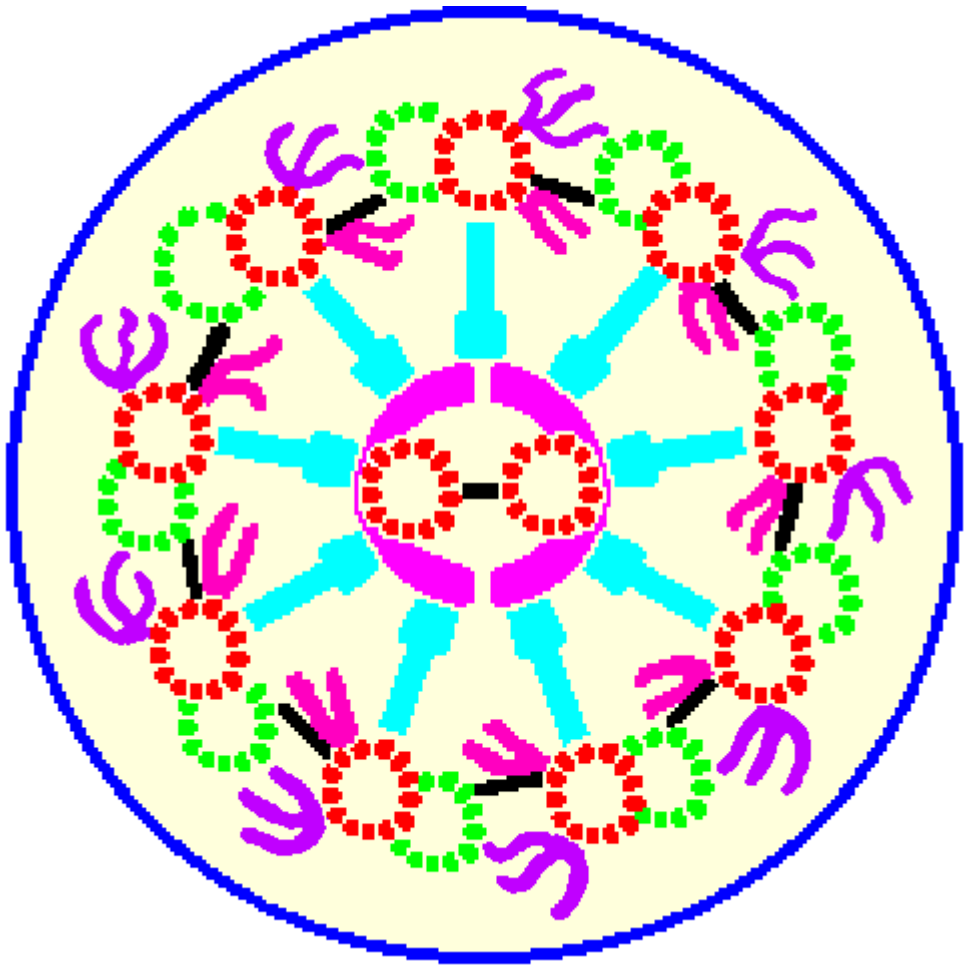
deCastro et al (2000) *Working strokes by single molecules of the kinesin-related microtubule motor ncd* Nature Cell Biology **2**(10), 724 - 729.

Dynein and cytoplasmic dynein

Dyneins are usually minus end-directed motors that pull on microtubules. There are two kinds of dynein: the protein found in cilia differs slightly from the cytosolic version responsible for pulling on the astral microtubules and reverse axonal transport. A rather complex multi-subunit protein called *dynactin* is required to attach the dynein molecules to their cytosolic cargoes.

King (2000) *AAA domains and organization of the dynein motor unit* J. Cell Science **113**(14), 2521-2526.

Vaughan et al (2001) *Cytoplasmic dynein intermediate chain phosphorylation regulates binding to dynactin* J. Biol. Chem. **276**(28), 26171-26179.



Cilia beating

Virtually all eukaryotic cilia and flagella have the same basic organization, based on the 9+2 arrangement of microtubules illustrated in the diagram. The two inner microtubules are singlet structures, but the outer ring consists of 9 doublet microtubules, each bearing hundreds of dynein molecules distributed along their length.

The individual doublet microtubules are cross-linked by nexin and connected to the central structure by radial spokes. Consequently, when the dynein molecules in one doublet exert a force on the neighbouring doublet, the whole structure bends instead of the microtubules sliding against each other. This also requires that the activity of individual dynein molecules is regulated in some way.

Dynein molecules towards the outside of the cilium have three functional head groups, but those on the inside have only two. In addition to the bending motility of the axonemes, there are separate anterograde and retrograde transport systems *inside* each cilium / flagellum to assemble and replace the protein components, and a third system responsible for surface motility on the plasmalemma surrounding the organelle. One of the dynein light chains is essential for the retrograde transport system.

For an introduction to pulmonary function, consult one of the physiology texts in the medical library, or visit the Cornell Medical Center website. Tracheal cilia beat about 20 times per second, and the activities of adjacent

cells are synchronised by travelling calcium waves. Shovelling mucus is hard work, but it is essential for normal life: *Kartagener syndrome* and *immotile cilia syndrome* are autosomal recessive disorders characterized by bronchiectasis, sinusitis, dextrocardia, and infertility. Patients with Kartagener syndrome have defective dynein arms and suffer from chronic respiratory disease, immotile sperm and left/right inversion of the viscera. Cilia are extremely complex structures, and contain many other components that might go wrong, in addition to the molecular motors.

Iomini et al (2001) *Protein particles in Chlamydomonas flagella undergo a transport cycle consisting of four phases* J. Cell Biol. **153**(1), 13-24.

Sakakibara, H et al (1999) *Inner-arm dynein c of Chlamydomonas flagella is a single-headed processive motor.* Nature **400**(6744), 586-590.

Yang et al (2001) *Localization of calmodulin and dynein light chain LC8 in flagellar radial spokes* J. Cell Biol. **153**(6), 1315-1325.

Reverse axonal transport

- Lodish et al chapter 19 pages 809 - 817
- Zigmond et al chapter 4 pages 94 - 104
- Kandel et al chapter 5

Recent papers:

Bearer et al (2000) *Retrograde axonal transport of herpes simplex virus: Evidence for a single mechanism and a role for tegument* Proc. Natl. Acad. Sci. USA **97**(14), 8146-8150.

Susalka & Pfister (2000) *Cytoplasmic dynein subunit heterogeneity: implications for axonal transport* J. Neurocytol. **29**, 819-829.

Shea (2000) *Microtubule motors, phosphorylation and axonal transport of neurofilaments* J. Neurocytol. **29**, 873-887.

Cell division motors (astral microtubules)

O'Connell & Wang (2000) *Mammalian Spindle Orientation and Position Respond to Changes in Cell Shape in a Dynein-dependent Fashion* Mol. Biol. Cell **11**, 1765-1774.

Dionne et al (2000) *ch-TOGp is required for microtubule aster formation in a mammalian mitotic extract* J. Biol. Chem. **275**(16), 12346-12352.