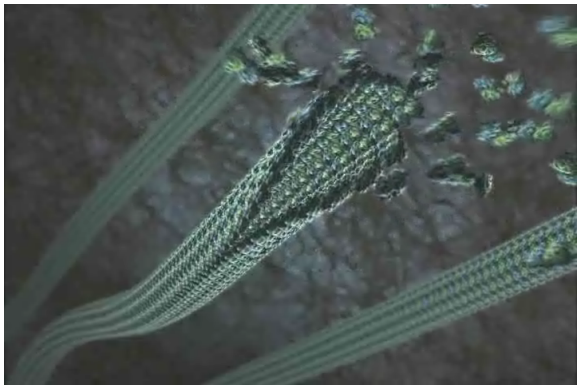
3. biopolymers



the inner life of a cell, viel & lue, harvard [2006]

markus buhler, MIT, thursday@4:15, thornton 110

Turning weakness into strength

How protein materials balance strength, robustness and adaptability

Biology exquisitely creates hierarchical structures, where initiated at nano scales, are exhibited in macro or physiological multifunctional materials to provide structural support, force generation, catalytic properties or energy conversion. This is exemplified in a wide range of biological materials such as hair, skin, bone, spider silk or cells, which play important roles in providing key functions to biological systems. This talk focuses on multiscale studies of deformation and failure of biological protein materials, used here to elucidate fundamental design concepts in order to understand physiological functions, disease mechanisms as well as to translate new material paradigms towards engineered materials. Based on a multiscale simulation approach validated through multiscale experiments, we explicitly consider the architecture of proteins across multiple scales, including the details of chemical bonding and explain how complex multifunctional properties of protein materials emerge. I will present a survey of recent studies of major classes of protein materials, including cellular protein networks, beta-sheet structures as found in spider silk and Alzheimer's disease, as well as collagenous tissues that form the structure of tendon and bone. Case studies will be presented that illustrate size effects in protein materials, flaw-tolerance mechanisms, and applications of materials science to genetic diseases, showing how structural defects at the molecular level can have profound effects at the material behavior at larger scales. Our work explains a universal materials design paradigm found in biology, where the formation of hierarchical structures at multiple scales is used to overcome the intrinsic limitations of inferior building blocks such as weak H-bonds. We show that through this paradigm, it is possible to create highly functional, tunable and changeable materials out of simple, abundant and inexpensive constituents.

biopolymers

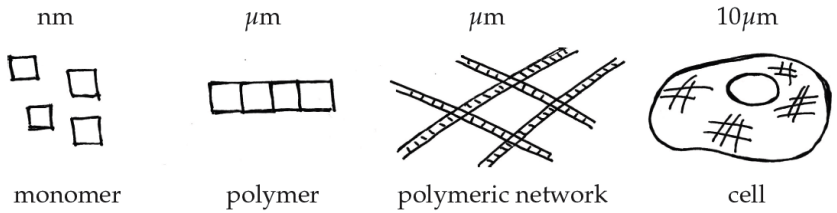


Figure 3.1. Biopolymers. Characteristic length scales on the cellular and sucellular level..

biopolymers

biopolymers are made up of **monomers** and **polymers**. monomers are smaller micromolecules such as nucleic acids, amino acids, fatty acid, and sugar. assembled together as repeating subunits, monomers form long macromolecules which are referred to as polymers.

typical examples of biopolymers

- genes: RNA and DNA
- gene products: peptides and proteins
- biopolymers not coded by genes: lipids, polysaccharides, and carbohydrates

biopolymers are **extremely flexible**. upon **thermal fluctuations**, they may bend from side to side and jiggle around. this is the nature of **soft matter** related to the notion of **entropy**.

the cytoskeleton

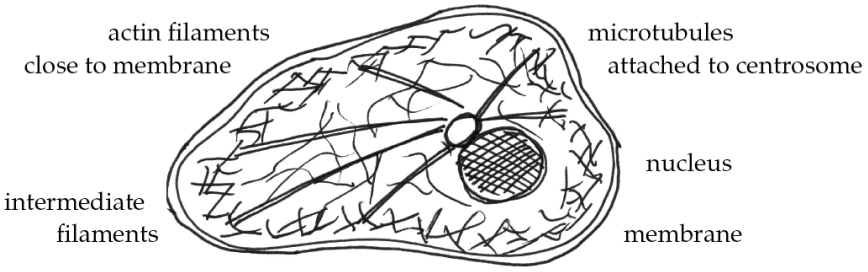


Figure 1.3. Eukaryotic cytoskeleton. The cytoskeleton provides structural stability and is responsible for force transmission during cell locomotion. Microtubules are thick hollow cylinders reaching out from the nucleus to the membrane, intermediate filaments can be found anywhere in the cytosol, and actin filaments are usually concentrated close to the cell membrane.

actin filaments

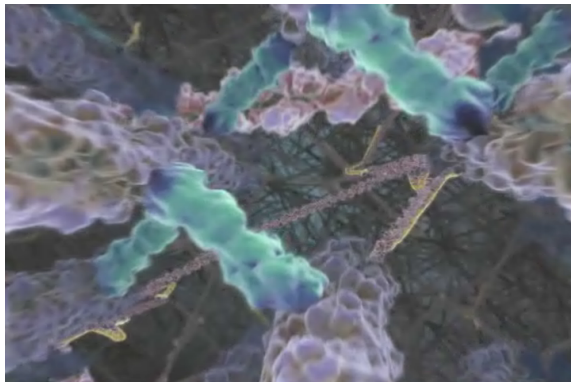


Figure 1.4.1 Actin filaments form tight parallel bundles which are stabilized by cross-linking proteins. Deeper in the cystol the actin network adopts a gel-like structure, stabilized by a variety of actin binding proteins.

the inner life of a cell, viel & lue, harvard [2006]

microtubules

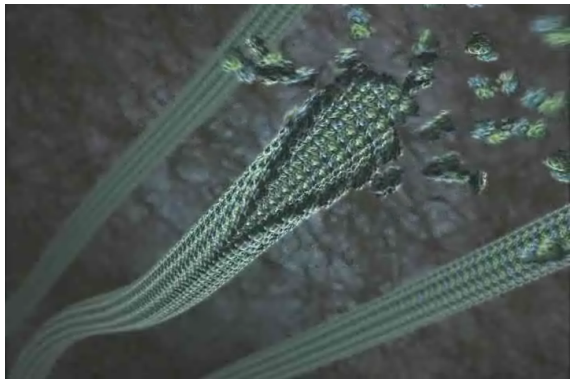


Figure 1.4.3 The cytoskeleton includes a network of microtubules created by the lateral association of protofilaments formed by the polymerization of tubulin dimers.

the inner life of a cell, viel & lue, harvard [2006]

the cytoskeleton

actin filaments are 7nm in diameter and consist of two intertwined actin chains. they are tension bearing members of the cell. being located close to the cell membrane, they are responsible for inter- and intracellular transduction. together with myosin, they from the contraction apparatus to generate muscular contraction of skeletal and cardiac muscle.

intermediate filaments are 8-12nm in diameter and thus more stable than actin filaments. they are also tension bearing within a cell. anchoring at organelles, they organize and maintain the three dimensional structure of the cell.

microtubules are hollow cylinders, 25nm in diameter with a 15nm lumen. they are comprised of 13 protofilaments consisting of α and β tubulin. microtubules are organized by the centrosome, but reassemble dynamically. unlike actin and intermediate filaments, microtubules can also bear compression. in addition, they form a highway for intracellular transport.

axial deformation - tension

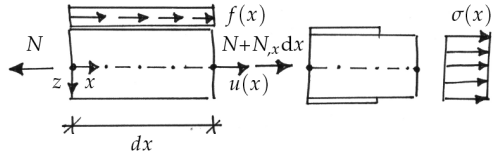
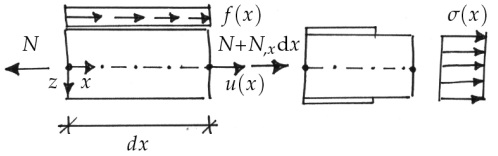


Figure 3.1: Axial loading of one dimensional structure ◦ Stresses σ are constant across the cross section

- ◦ kinematics $\varepsilon = \lim_{x\to 0} \frac{u}{x} = \frac{\mathrm{d}u}{\mathrm{d}x} = u_{,x}$ homogeneous $\varepsilon = \frac{\Delta l}{l}$
- ◦ constitutive equation $\sigma = \sigma(\varepsilon)$ linear elastic $\sigma = E\,\varepsilon$
- ◦ stress resultant $N = \iint \sigma \,\mathrm{d}y\mathrm{d}z$ homogeneous $\sigma = \frac{N}{A}$
- ◦ equilibrium $\sum f \doteq 0$ in axial direction $N_{,x} + f = 0$

axial deformation - tension



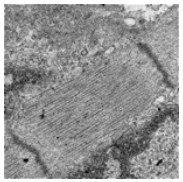
$EA\,u_{,xx} + f = 0$ with EA ... axial stiffness

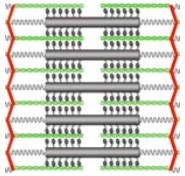
cross section area $A = \pi\, r^2$

	r	A	E	EA
microtubule	12.5 nm	491 nm^2	1.9·10^9 N/m^2	93·10^{-8} N
intermediate filament	5.0 nm	79 nm^2	2.0·10^9 N/m^2	15·10^{-8} N
actin filament	3.5 nm	39 nm^2	1.9·10^9 N/m^2	7·10^{-8} N

Table 3.1: Axial stiffness EA of major consituents of cytoskeleton: microtubules, intermediate filaments and actin filaments

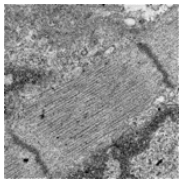
axial deformation - tension

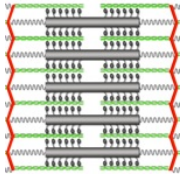




Example Determine the elongation of an active muscle with Young's modulus $E = 40\text{MPa} = 4 \cdot 10^7\text{N/m}^2$, a cross section of $A = 1000\text{mm}^2 = 10^{-3}\text{m}^2$ and a total length $l = 10\text{mm} = 0.01\text{m}$! Assume that the muscle is loaded by a weight of $m = 10\text{kg}$. What is its elongation Δl and its its strain ε?

axial deformation - tension





Example Determine the elongation of an active muscle with Young's modulus $E = 40\text{MPa} = 4 \cdot 10^7\text{N/m}^2$, a cross section of $A = 1000\text{mm}^2 = 10^{-3}\text{m}^2$ and a total length $l = 10\text{mm} = 0.01\text{m}$! Assume that the muscle is loaded by a weight of $m = 10\text{kg}$. What is its elongation Δl and its its strain ε? ◦ The force acting on the muscle is $N = m\,g$ with the acceleration due to gravity $g = 10\text{m/s}^2 = 10\text{N/kg}$, thus $N = 10\text{kg}{\cdot}10\text{N/kg} = 100\text{N}$. The elongation Δl then follows as $\Delta l = \varepsilon\, l = \sigma\, l \,/\, E = N\, l \,/\, [EA] = 100\text{N} \cdot 0.01\text{m} \,/\, [4 \cdot 10^7\text{N/m}^2 \cdot 10^{-3}\text{m}^2] = 2.5 \cdot 10^{-2}\text{mm}$. The strain simply follows as $\varepsilon = \Delta l \,/\, l = 2.5 \cdot 10\text{mm} \;/\; 10\text{ mm} = 0.0025 = 0.25\%$.

tension vs bending - trusses vs beams

	dimension	geometry	loading	deformation	gov eqn
truss	1d straight	$w, h \ll l$	axial	tension	2^{nd} order
beam	1d straight	$w, h \ll l$	transverse	bending	4^{th} order
wall	2d flat	$h \ll w, l$	in plane	tension/shear	2^{nd} order
plate	2d flat	$h \ll w, l$	transverse	bending	4^{th} order
membrane	3d curved	$h \ll w, l$	in plane	tension/shear	2^{nd} order
shell	3d curved	$h \ll w, l$	transverse	bending	4^{th} order

Table 2.1: Classification of structural elements based on dimension, geometry and loading

tension vs bending - trusses vs beams

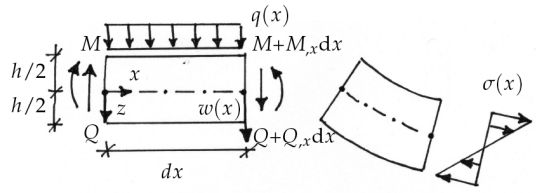


Figure 3.2: Transverse loading of one dimensional structure ∘ stresses σ vary linearly across the cross section

overall deformation = axial + transverse deformation

$$u^{\text{tot}}(x,z) = u(x) - z\,w(x)_{,x}$$

$$\varepsilon = u^{\text{tot}}_{,x} = u_{,x} - z\,w_{,xx}$$

- axial deformation $u(x)$
- transverse deformation, scaled rotation of beam axis $z\,w(x)_{,x}$

transverse deformation - bending

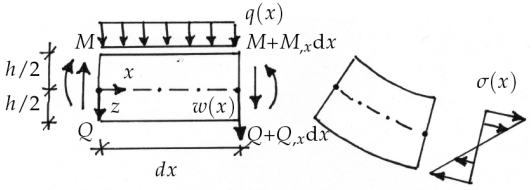


Figure 3.2: Transverse loading of one dimensional structure ∘ stresses σ vary linearly across the cross section

euler bernoulli beam theory

- normals remain straight (they do not bend)
- normals remain unstretched (they keep the same length)
- normals remain normal (they remain orthogonal to the beam axis)

transverse deformation - bending

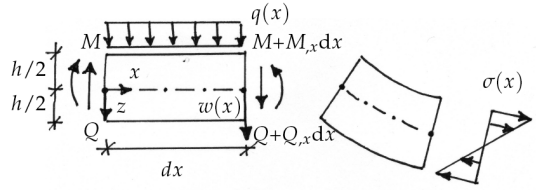


Figure 3.2: Transverse loading of one dimensional structure ∘ stresses σ vary linearly across the cross section

transverse deformation - bending

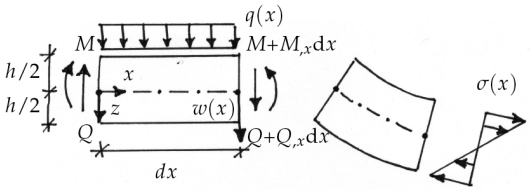


Figure 3.2: Transverse loading of one dimensional structure ○ stresses σ vary linearly across the cross section

- kinematics
- constitutive equation
- stress resultants
- equilibrium

$$\varepsilon = -w_{,xx}\, z = \kappa\, z$$

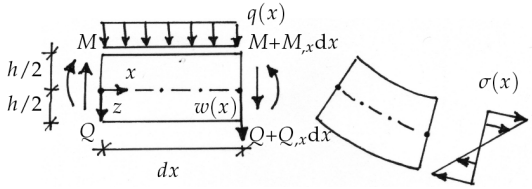
$$\sigma = E\,\varepsilon = -E\, w_{,xx}\, z = E\,\kappa\, z$$

$$M = \int_{-h/2}^{+h/2} \sigma\, z\, \mathrm{d}z = \int_{-h/2}^{+h/2} E\,\kappa\, z^2\, \mathrm{d}z = EI\,\kappa$$

$$\sum f_z \doteq 0 \qquad Q_{,x} + q = 0$$

$$\sum m \doteq 0 \qquad M_{,x} - Q = 0$$

transverse deformation - bending



$q = EI\, w_{,xxxx}$ with EI ... bending stiffness

for circular cross sections $I = \pi\, r^4 / 4$

	r	I	E	EI
microtubule	12.5 nm	19,175 nm^4	1.9·10^{9}N/m^2	364·10^{-25}Nm^2
intermediate filament	5.0 nm	491 nm^4	2·10^{9}N/m^2	10·10^{-25}Nm^2
actin filament	3.5 nm	118 nm^4	1.9·10^{9}N/m^2	2·10^{-25}Nm^2

Table 3.2: Bending stiffness of major consituents of cytoskeleton: microtubules, intermediate filaments and actin filaments

Citation	Tubulin source	Temp (°C)	Variation	EI (× 1024 Nm^2)	Lp (mm)	Measurement technique
Mizushima-Sugano et al. (1983)	Porcine	25	GDP tubulin	0.45	0.74	Affixed to cover slip
Gittes (1993)		37	With Taxol Rhodamine tubulin with Taxol	22 ± 1.5 21 ± 1	5.1 5.6	Thermal fluctuations
Dye et al. (1993)		37	GDP tubulin with Taxol with MAP mix	1.36 0.13 0.30	0.33 ± 0.09 0.031 ± 0.008 0.074 ± 0.009	Calibrated flow
Venier et al. (1994)	Porcine	37	GDP tubulin with Taxol with Taxotere GDP–BeF3 tubulin GDP tubulin GDP–BeF3 tubulin GDP–AlF4 tubulin	9.2 ± 0.9 4.7 ± 0.4 4.8 ± 0.4 26 ± 2.7 8.5 ± 2.9 ± 5 25 ± 5	2.2 1.1 1.3 6.1 2.0 6.8 5.8	Thermal fluctuations one end calibrated flow
Mickey and Howard (1995)	Bovine	37 25	GMPCPP tubulin with Tau with Taxol GDP capped with GMPCPP tubulin with Taxol	63 ± 9 34 ± 3 32 ± 2 26 ± 2 21 ± 1	14 5.7 9.7 5.1 6.1 5.1	Thermal fluctuations
Kurz and Williams (1995)	Bovine	37	GDP tubulin with MAP mix GDP tubulin with MAP mix	35.8 ± 0.95 39.5 ± 1.25 26.4 27.3	8.4 ± 2.2 9.4 ± 2.7 6.2 ± 0.8 6.5 ± 0.8	Calibrated flow thermal fluctuations
Kurachi et al. (1995)	Bovine	37	With MAPs (10 um length) with MAPs (30 um length) with Taxol (5 um length) with Taxol (20 um length)	34 ± 17 200 ± 60 1 ± 0.65 20 ± 6	7.9 46.8 0.2 4.7	Optical trap buckling
Elbaum et al. (1996)	Bovine	27	GDP tubulin	26 ± 10	6.3 ± 2.4	Vesicle deformation
Felgner et al. (1996)	Porcine	22–25	GDP tubulin with Taxol with MAPs GDP tubulin with Taxol with MAPs	3.7 ± 0.8 1 ± 0.3 16 ± 3 4.7 ± 0.4 1.9 ± 0.1 18 ± 3	0.9 0.2 39.2 1.2 0.5 4.4	Optical trap RELAX method optical trap WIGGLE method
Felgner et al. (1997)	Porcine	24–27	GDP tubulin with Taxol with 2% full-length Tau with 18% full-length Tau with 48% full-length Tau with 85% full-length Tau with tau binding repeat constructs with tau projection domain constructs with double tau binding domains with MAP 2c2 with MAP 2d2 with MAP 2c3 with MAP mix	3.8 ± 0.9 1.0 ± 0.3 4.5 ± 1.5 8.9 ± 1.3 9.4 ± 2.6 10.4 ± 3.1 5.8 ± 1.5 to 8.5 ± 1.6 6.1 ± 2 to 9.2 ± 1.4 29.6 ± 9.3 15.1 ± 3.3 16.1 ± 2.7 14.5 ± 3.8 16.0 ± 3.0	0.9 0.2 1.1 2.2 2.3 2.5 1.4 2.2 7.2 3.7 3.9 3.5 3.9	Optical trap RELAX method
Dogterom and Yurke (1997)	Bovine	22	GDP tubulin	34 ± 7	8.4	Thermal fluctuations
Cassimeris et al. (2001)	Porcine		GDP tubulin with XMAP215	18.5 ± 2.0 17.5 ± 2.2	7.1 4.4	Thermal fluctuations one end
Janson and Dogterom (2004)	Bovine	23	Fast polymerization slow polymerization	18 28	4.2 ± 0.3 6.6 ± 0.9	Thermal fluctations of shape
Pampaloni et al. (2006)	Porcine		With Taxol (2.6 um length) with Taxol (47.5 um length)	0.45 21	0.11 ± 0.05 5.035 ± 0.8	Thermal fluctuations one end
Kikumoto et al. (2006)	Bovine	33	GDP tubulin with Taxol	7.9 ± 0.7 2.0 ± 0.8	1.9 0.5	Optical trap buckling
Brangwynne et al. (2007)	Bovine		With Taxol (25–66 um length) with Taxol (18–25 um length)	12 6.2	2.8 ± 1 1.5 ± 0.7	Thermal fluctuations
van den Heuvel et al. (2007)	Bovine	37	With Taxol	1 ± 0.1	0.24 ± 0.03	Microtubule trajectories
Kawaguchi et al. (2008)	Porcine	20–35	With Taxol with Taxol	2.5 ± 0.5 2.7 ± 0.4	0.6 0.6	Thermal fluctuations one end kinesin forced buckling
van den Heuvel et al. (2008)	Bovine	37	With Taxol (short length) with Taxol (long length)	0.34 ± 0.086 15 ± 1.28	0.08 ± 0.02 3.6 ± 0.3	Microtubule trajectories
Van Mameren et al. (2009)	Porcine		With Taxol	6.1 ± 1.3	1.4	Optical trap buckling

bending stiffness of microtubules
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mechanics of microtubules

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Mechanics of microtubules

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ABSTRACT

Microtubules are rigid cytoskeletal filaments, and their mechanics affect cell morphology and cellular processes. For instance, microtubules for the support structures for extended morphologies, such as axons and cilia. Further, microtubules act as tension rods to pull apart chromosomes during cellular division. Unlike other cytoskeletal filaments (e.g., actin) that work as large networks, microtubules work individually or in small groups, so their individual mechanical properties are quite important to their cellular function. In this review, we explore the past work on the mechanics of individual microtubules, which have been studied for over a quarter of a century. We also present some prospective on future endeavors to determine the molecular mechanisms that control microtubule rigidity.

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mechanics of microtubules

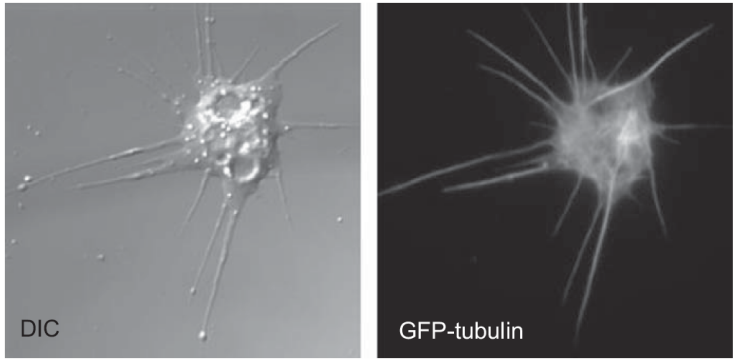

Figure 1. Microtubules as cellular supports. Microtubules are support structures for the cell. When S2 cells are depleted of actin filaments, long, mircotubule-filled processes remain. The outline of the cell is clearly seen in differential interference contrast microscopy (DIC) on the left. Fluorescence imaging of GFP-tubulin reveals that long extensions are supported by microtubules. Before actin was depleted, the cells were almost perfectly round.

hawkins, mirigian, yasar, ross [2010]

mechanics of microtubules

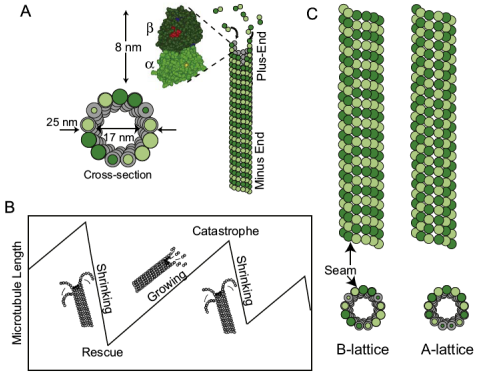

Figure 2. Microtubule structure and dynamics. Microtubules are polymer filaments made from tubulin dimers. The tubulin heterodimer is made of a beta (dark) and alpha (light) subunit. A few hundred dimers bind together to nucleate the polymer, and individual dimers add on to the ends to growt the microtubule. The plus end is the more dynamic and rapidly growthing and shrinking end, the minus end is less dynamic.

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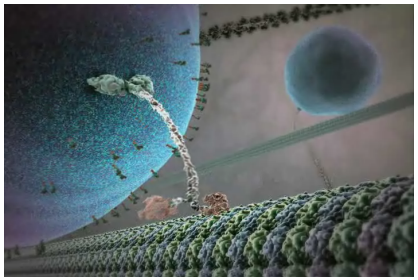
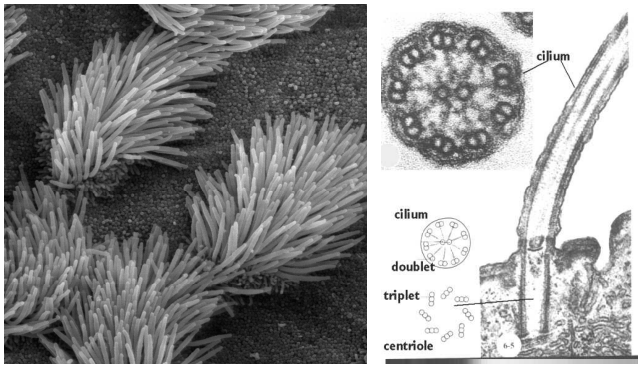
mechanics of microtubules



- essential structural elements, outline cell shape
- serve as support for extended morphologies
  axons, dendrites, and cilia
- enable efficient, long-range transport
- work individually instead of as a network

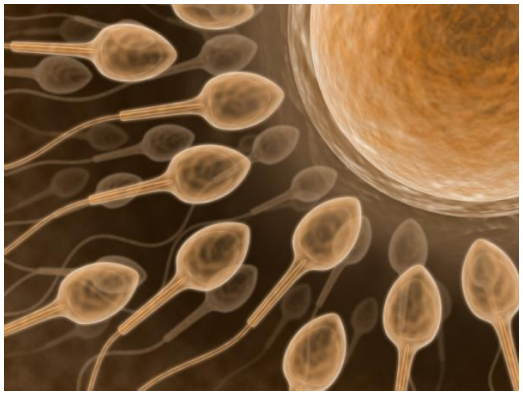
mechanics of microtubules - cilia



Scanning electron microscope image of lung trachea epithelium. Inside cilia and flagella is a microtubule-based cytoskeleton called the axoneme. The axoneme of primary cilia typically has a ring of nine outer microtubule doublets, and the axoneme of a motile cilium has two central microtubule doublets in addition to the nine outer doublets. The axonemal cytoskeleton acts as a scaffolding for various protein complexes and provides binding sites for molecular motor proteins such as kinesin II, that help carry proteins up and down the microtubules.

daghlian [2006]

mechanics of microtubules - flagella



Though eukaryotic flagella and motile cilia are ultrastructurally identical, the beating pattern of the two organelles can be different. In the case of flagella, e.g., the tail of a sperm, the motion is propeller-like. In contrast, beating of motile cilia consists of coordinated back-and-forth cycling of many cilia on the cell surface.

markus buhler, MIT, thursday@4:15, thornton 110

Turning weakness into strength

How protein materials balance strength, robustness and adaptability

Biology exquisitely creates hierarchical structures, where initiated at nano scales, are exhibited in macro or physiological multifunctional materials to provide structural support, force generation, catalytic properties or energy conversion. This is exemplified in a wide range of biological materials such as hair, skin, bone, spider silk or cells, which play important roles in providing key functions to biological systems. This talk focuses on multiscale studies of deformation and failure of biological protein materials, used here to elucidate fundamental design concepts in order to understand physiological functions, disease mechanisms as well as to translate new material paradigms towards engineered materials. Based on a multiscale simulation approach validated through multiscale experiments, we explicitly consider the architecture of proteins across multiple scales, including the details of chemical bonding and explain how complex multifunctional properties of protein materials emerge. I will present a survey of recent studies of major classes of protein materials, including cellular protein networks, beta-sheet structures as found in spider silk and Alzheimer's disease, as well as collagenous tissues that form the structure of tendon and bone. Case studies will be presented that illustrate size effects in protein materials, flaw-tolerance mechanisms, and applications of materials science to genetic diseases, showing how structural defects at the molecular level can have profound effects at the material behavior at larger scales. Our work explains a universal materials design paradigm found in biology, where the formation of hierarchical structures at multiple scales is used to overcome the intrinsic limitations of inferior building blocks such as weak H-bonds. We show that through this paradigm, it is possible to create highly functional, tunable and changeable materials out of simple, abundant and inexpensive constituents.