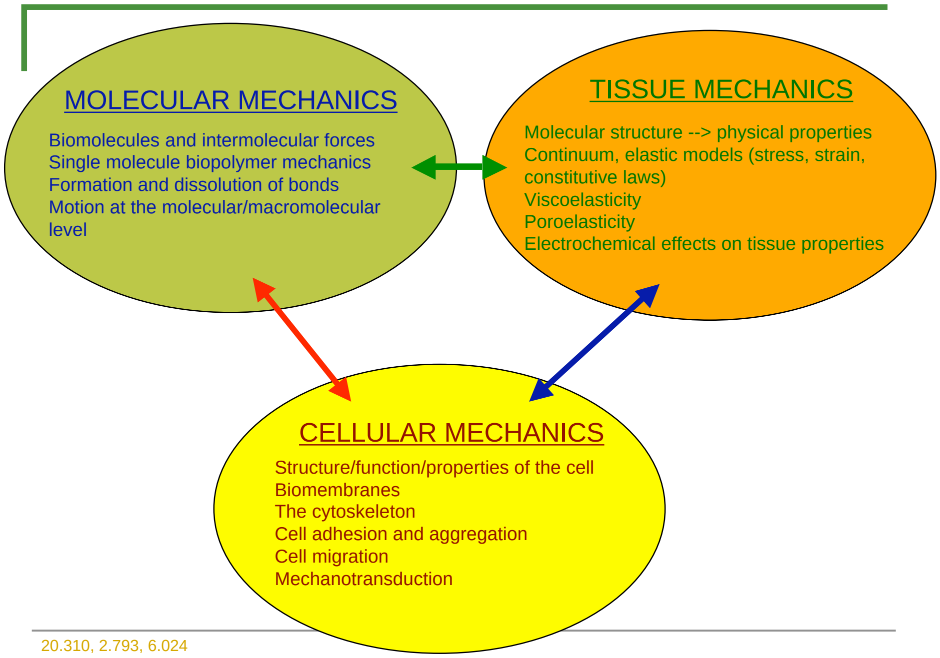
Molecular, Cellular & Tissue Biomechanics

Goal: Develop a *fundamental* understanding of biomechanics over a wide range of length scales.



Some Learning Objectives

1. To understand the fundamental concepts of mechanics and be able to apply them to simple problems in the deformation of continuous media
2. To understand the underlying basis for the mechanical properties of molecules, cells and tissues
3. To be able to model biological materials using methods appropriate over diverse length scales
4. To be familiar with the wide spectrum of measurement techniques that are currently used to determine mechanical properties
5. To appreciate the close interconnections between mechanics and biology/chemistry of living systems

Biomechanics of tissues

Mechanics

I. Linear elastic behavior
II. Viscoelasticity
III. Poroelasticity
IV. Electrochemical and physicochemical properties

Biology

I. Biochemical and molecular biology of ECM molecules
   A. Collagen superfamily
   B. Proteoglycan superfamily
   C. Other glycoproteins
II. Nanomolecular structures <--> tissue
III. Mechanobiology

Some preliminaries

Equilibrium -- balance of forces

concept of a stress tensor

Compatibility -- relations between displacements and strains or deformation

normal strains; shear strains; strain tensor

Constitutive laws

stress -- strain

Young's modulus; Poisson's ratio, shear modulus

linear, isotropic, elastic materials

Force balance in a single cell

Desprat et al.,
Biophys J., 2005.

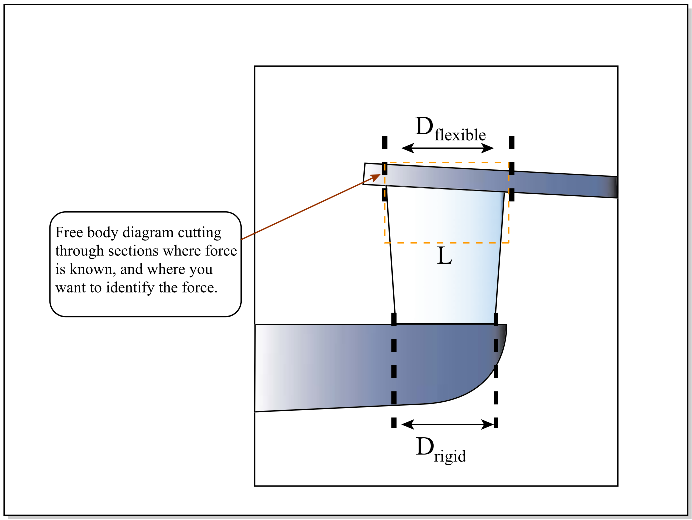


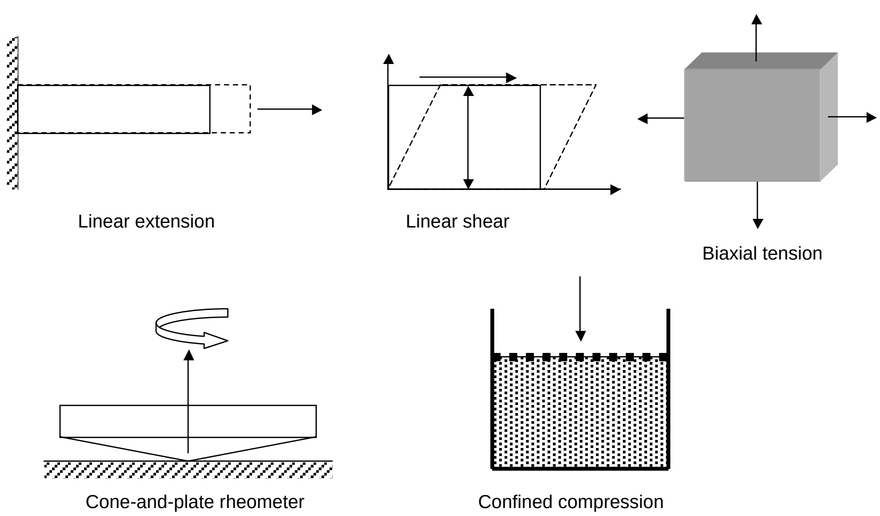
Figure by MIT OCW.

Stress distributions along the basal surface of a resting cell

Graphical image of stress distributions on a cell surface removed due to copyright restrictions.

Hu, et al., AJP Cell, 2003

Several material testing methods



Tissue properties: common simplifying assumptions

Linear -- the elastic modulus is constant, indpendent of strain amplitude

Homogeneous -- the material is spatially uniform

Isotropic -- the material exhibits the same elastic properties in all directions

Time-independent -- stresses and strains are uniquely related, independent of rate of strain

Constitutive laws for a linear elastic, isotropic material

$$\varepsilon_{11} = \frac{1}{E}\left[\sigma_{11} - \nu\left(\sigma_{22} + \sigma_{33}\right)\right]$$
$$\varepsilon_{22} = \frac{1}{E}\left[\sigma_{22} - \nu\left(\sigma_{11} + \sigma_{33}\right)\right]$$
$$\varepsilon_{33} = \frac{1}{E}\left[\sigma_{33} - \nu\left(\sigma_{11} + \sigma_{22}\right)\right]$$
$$\varepsilon_{12} = \frac{\sigma_{12}}{2G} = \frac{(1+\nu)\sigma_{12}}{E}$$
$$\varepsilon_{13} = \frac{\sigma_{13}}{2G} = \frac{(1+\nu)\sigma_{13}}{E}$$
$$\varepsilon_{23} = \frac{\sigma_{23}}{2G} = \frac{(1+\nu)\sigma_{23}}{E}$$

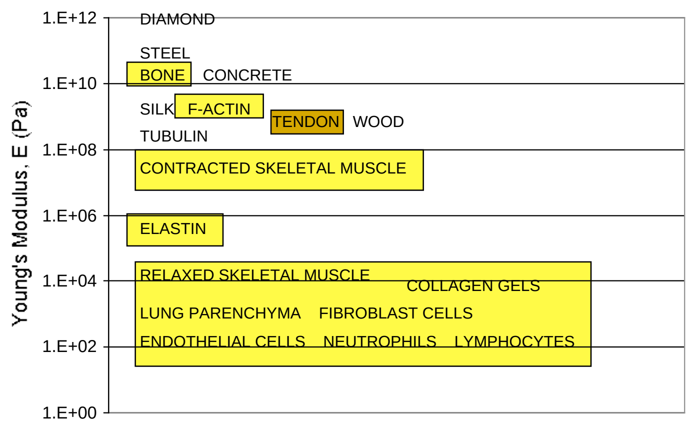
$$\sigma_{11} = \lambda\left(\varepsilon_{11} + \varepsilon_{22} + \varepsilon_{33}\right) + 2G\varepsilon_{11}$$
$$\sigma_{22} = \lambda\left(\varepsilon_{11} + \varepsilon_{22} + \varepsilon_{33}\right) + 2G\varepsilon_{22}$$
$$\sigma_{33} = \lambda\left(\varepsilon_{11} + \varepsilon_{22} + \varepsilon_{33}\right) + 2G\varepsilon_{33}$$
$$\sigma_{12} = 2G\varepsilon_{12}$$
$$\sigma_{13} = 2G\varepsilon_{13}$$
$$\sigma_{23} = 2G\varepsilon_{23}$$

$$\lambda = \frac{2G\nu}{1-2\nu} = \frac{E\nu}{\left[(1+\nu)(1-2\nu)\right]}$$

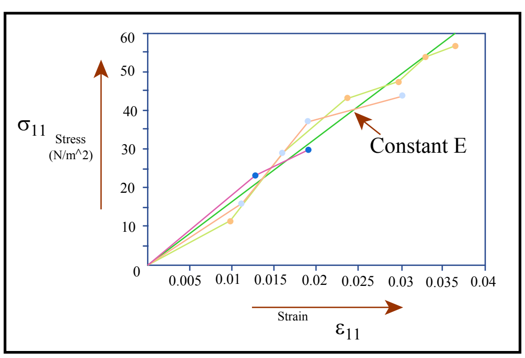
E = Young's modulus λ = Lame' constant

ν = Poisson's ratio G = shear modulus

Values of the elastic or Young's modulus (E) for various materials



Linear? Unidirectional tensile tests
Stress-strain behavior of a peptide hydrogel



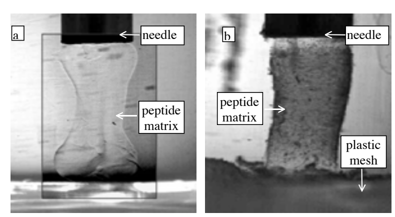


Figure by MIT OCW. Adapted from Leon, et. al., 1998

Linear behavior up to fracture

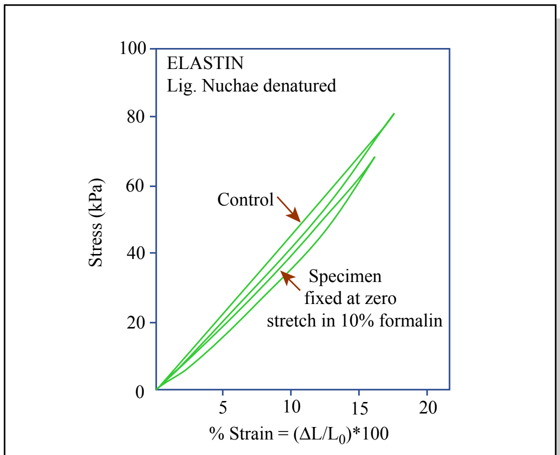
Relatively low toughness due to small fracture strain

Linear? Elastin is one of main structural components of tissue

Linear; little hysteresis.

Provides the "stretchiness" of tissues.

Combination of single-molecule characteristics and microscale structure.



The stress-strain curve of elastin. Data from Fung and Sobin (1981).

Figure by MIT OCW.

Linear? ACL -- different strain rates

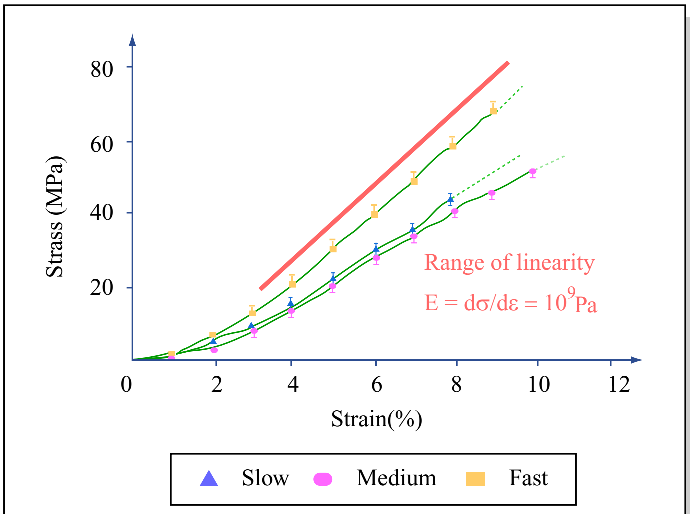


Figure by MIT OCW.

Linear and Isotropic? Right tibia

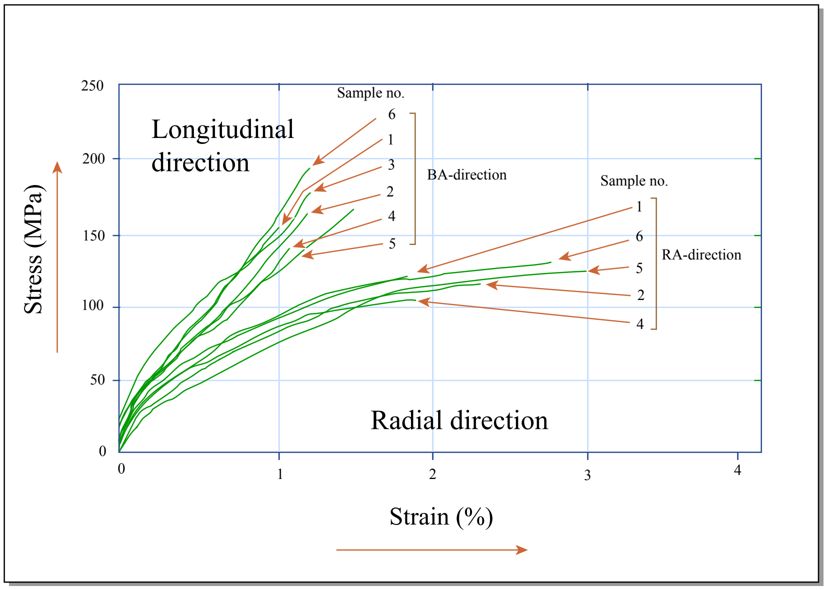


Figure by MIT OCW.

Canine aorta showing elastic fiber content

Scanning electron micrographs showing a low-power view of dog's aorta and a high-power view of the dense network of longitudinally oriented elastic fibers in the outer layer of the same blood vessel. Images removed due to copyright restrictions. See Haas, K. S., S. J. Phillips, A. J. Comerota, and J. W. White. *Anat. Rec.* 230 (1991): 86-96.

Collagen fiber arrangement in skin and cornea with alternating directions

Electron micrograph of a cross-section of tadpole skin. The arrangement of collagen fibrils is plywoodlike, with successive layers of fibrils laid down nearly at right angles to each other. Image removed due to copyright restrictions.

Electron micrographs of parallel collagen fibrils in a tendon and the mesh work of fibrils in skin removed due to copyright restrictions.

Homogeneous? Only rarely

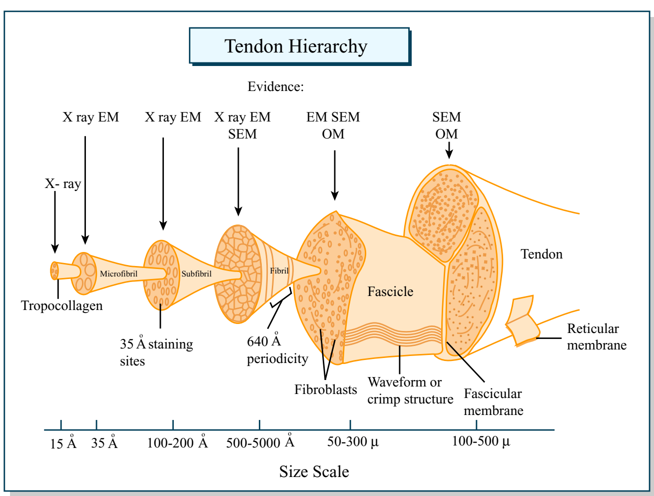
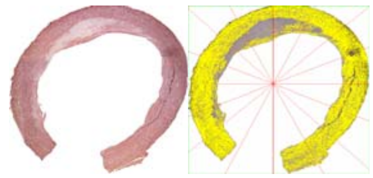


Figure by MIT OCW.

Histological cross-section of a diseased carotid artery stained for smooth muscle cells.



Elastic response initially, then stiff, collagen response at high degrees of extension.

H = hypertensive

High wall stress leads to functional remodeling.

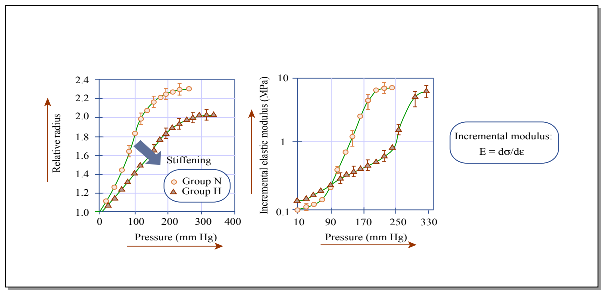
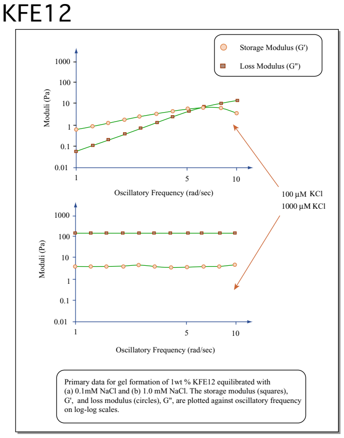
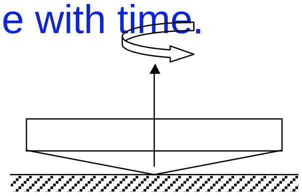


Figure by MIT OCW.

Time-dependent? Peptide gels Pre- and Post-Gelation. Properties can change with time.

1%wt KFE12





100 µM KCl

1000 µM KCl

- ● Storage Modulus (G')
- ■ Loss Modulus (G'')

Figure by MIT OCW.

Shear Modulus of cartilage. Modulus can be frequency-dependent (Dynamic @ 0.5Hz, 0.8% strain)

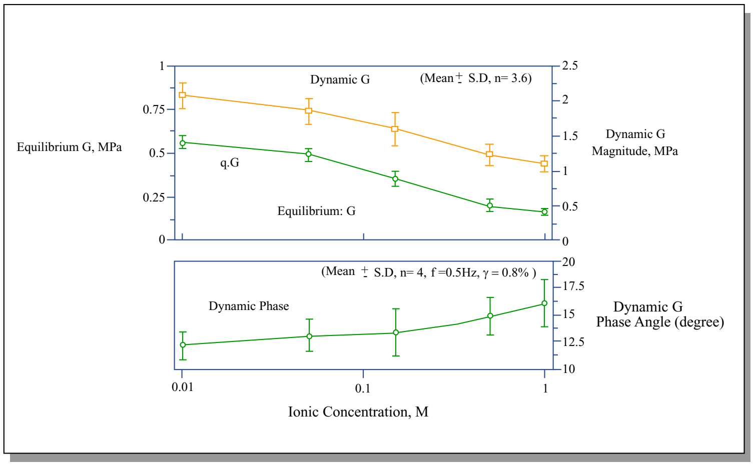


Figure by MIT OCW.

Other complications: Surface tension in lung parenchyma

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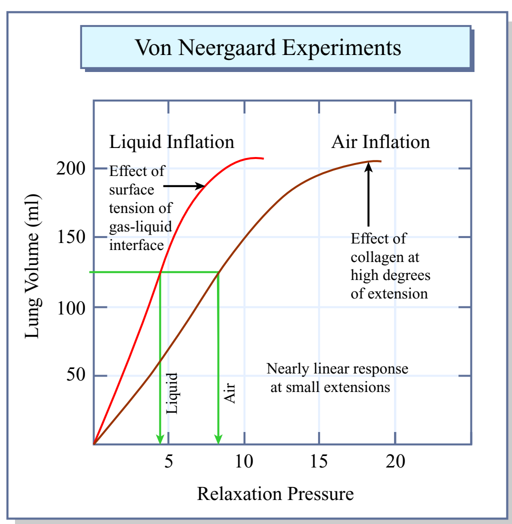
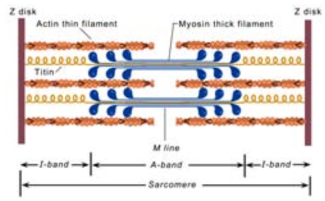


Surface tension effects

Figure by MIT OCW.

Other complications: Active contraction

Striated



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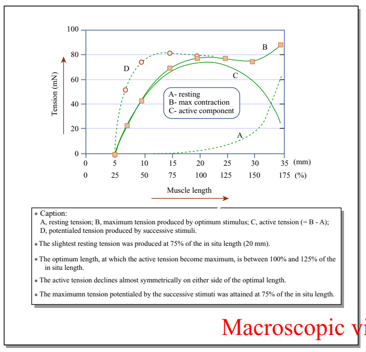
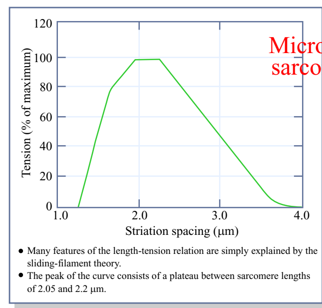


Macroscopic view



Microscopic - sarcomere level

Figure by MIT OCW.

Figure by MIT OCW.

Underlying basis for mechanical properties

Extracellular matrix, cartilage, and tendon are largely comprised of:

collagen

elastin

proteoglycan

(role of constituent cells??)

Typical elastic behavior for tissues containing collagen and elastin

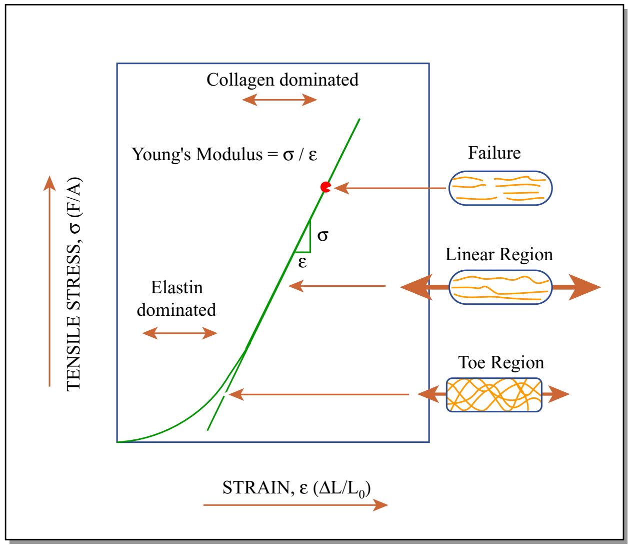
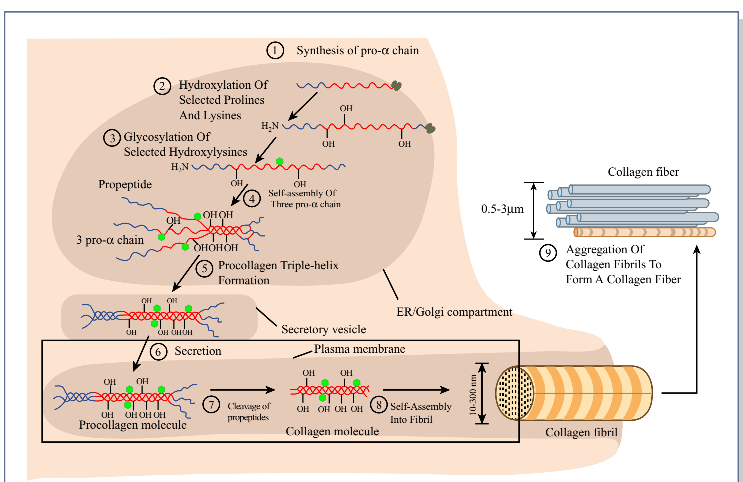


Figure by MIT OCW.

Collagen fibril formation

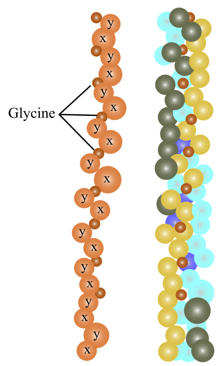


The Intracellular and Extracellular Events Involved in the Formation of a Collagen Fibril

Figure by MIT OCW.

Collagen -- single molecule characteristics

The Structure of a Typical Collagen Molecule



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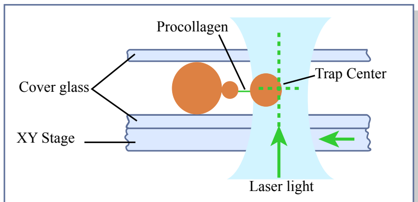
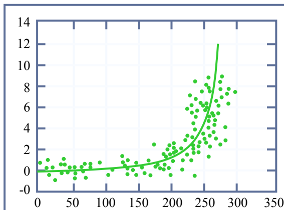


Stretching a procollagen II molecule with optical tweezers.

Figure by MIT OCW.



The force-extension curve of a single collagen II.

Figure by MIT OCW.

Major Collagen Molecules			
Type	Molecule composition	Structural features	Representative tissues
Fibrillar collagens			
I	[α 1(I)]$_2$ [α 2(I)]	300-nm-long fibrils	Skin, tendon, bone, ligaments, dentin, interstitial tissues
II	[α 1(II)]$_3$	300-nm-long fibrils	Cartilage, vitreous humor
III	[α 1(III)]$_3$	300-nm-long fibrils; often with type I	Skin, muscle, blood vessels
V	[α 1(V)]$_3$	390-nm-long fibrils with globular N-terminal domain; often with type I	Similar to type I; also cell cultures, fetal tissues
Fibril-associated collagens			
VI	[α 1(VI)][α 2(VI)]	Lateral association with type I; periodic globular domains	Most interstitial tissues
IX	[α 1(IX)][α 2(IX)][α 3(IX)]	Lateral association with type II; N-terminal globular domain; bound glycosami-noglycan	Cartilage, vitreous humor;
Sheet-forming collagens			
IV	[α 1(IV)]$_2$ [α 2(IV)]	Two-dimensional network	All basal laminaes

Figure by MIT OCW.

As of 2003, there were 28 (!) different types of collagen identified.

Type IX collagen decorated with type II collagen

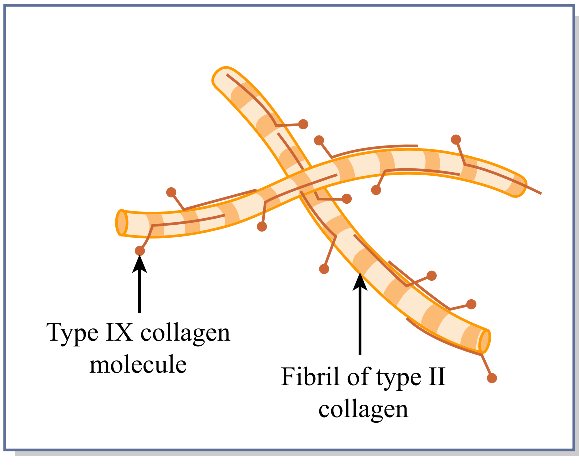


Figure by MIT OCW.

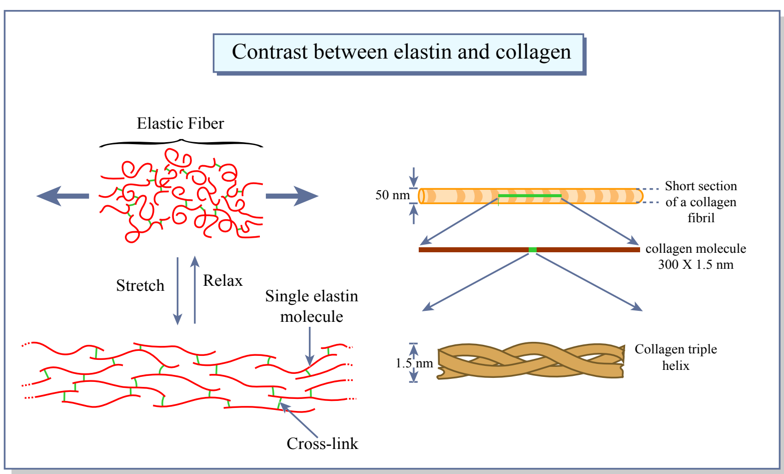


Figure by MIT OCW.

Entropic elasticity

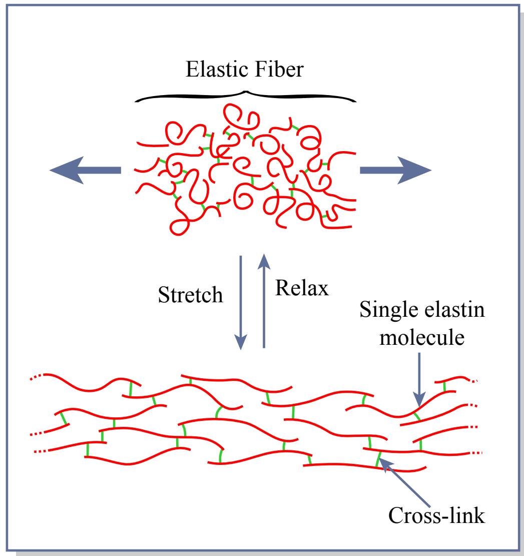


Figure by MIT OCW.

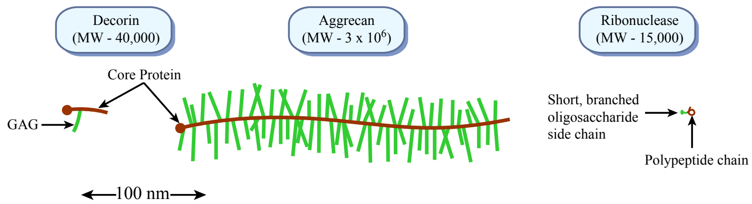
Proteoglycans (PGs) and glycosaminoglycans (GAGs)

a) GLYCOSAMINOGLYCANS (GAGs) form gels
- i) polysaccharide chains of disaccharide units
- ii) too inflexible and highly charged to fold in a compact way
- iii) strongly hydrophilic
- iv) form extended conformations and gels
- v) osmotic swelling (charge repulsion)
- vi) usually make up less than 10% of ECM by weight
- vii) fill most of the ECM space
- viii) four main groups
  - a. hyaluronan
  - b. chondroitin sulfate and dermatin sulfate
  - c. heparin sulfate and heparin
  - d. keratin sulfate

b) Proteoglycans (PGs)

i) form large aggregates
ii) aggrecan is a large proteoglycan in cartilage
iii) decorin is secreted by fibroblasts
iv) PGs have varying amounts of GAGs.
v) PGs are very diverse in structure and content
vi) PGs and GAGs can also complex with collagen
vii) secreted proteoglycans have multiple functions
viii) PG/GAGs have important roles in cell-cell signaling

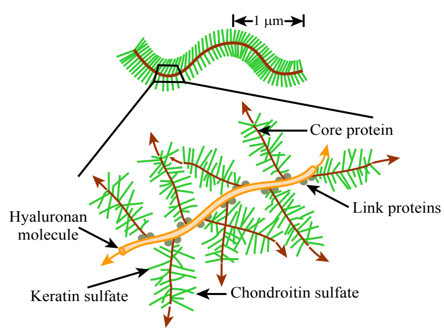
Electron micrograph of proteoglycans in the extracellular matrix of rat cartillage removed due to copyright restrictions.
See Hunziker, E. B., and R. K. Schenk. *J Cell Biol* 98 (1985): 277-282.



Examples of a large (aggrecan) and a small (decorin) proteoglycan found in the extracellular matrix. They are compared to a typical secreted glycoprotein molecule (pancreatic ribonuclease B). All are drawn to scale. The core proteins of both aggrecan and decorin contain oligosaccharide chains as well as the GAG chains, but these are not shown. Aggrecan typically consists of about 100 chondroitin sulfate chains and about 30 keratan sulfate chains linked to a serine-rich core protein of almost 3000 amino acids. Decorin "decorates" the surface of collagen fibrils, hence its name.

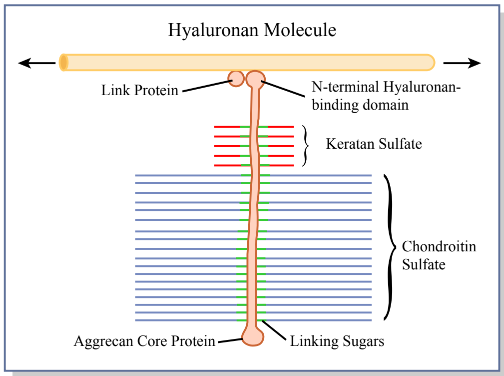
Figure by MIT OCW.

Aggrecan Aggregate



Schematic drawing of an aggrecan aggregate. It is composed of 100 aggrecan monomers noncovalently bound to a single hyaluronan chain through two link proteins that bind to both the core protein of the proteoglycan and to the hyaluronan chain, thus stabilizing the aggregate.

Figure by MIT OCW.



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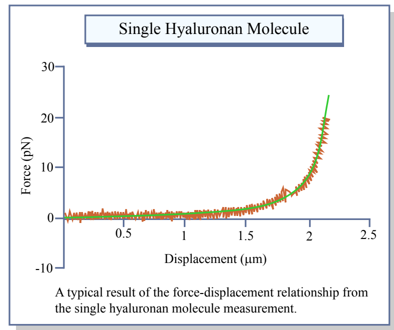


Figure by MIT OCW.

Electron micrograph of an aggrecan aggregate removed due to copyright restrictions.

Atomic Force Microscopy of Aggrecan

Images removed due to copyright restrictions.

Aggrecan Chondroitin sulfate GAG Chains
(Fetal Bovine)

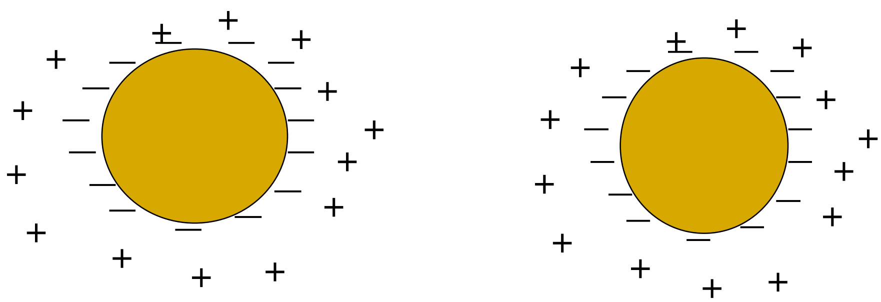
Ave. GAG length: ~36 nm

Inter-GAG spacing: 4-5 nm

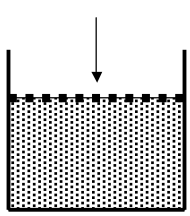
(L. Ng, C. Ortiz, A. Grodzinsky)

Like charge repulsion accounts for a large fraction (~50%) of the stiffness in tissues with high GAG content.

These effects can be eliminated either by shielding with counter-ions or neutralization by changing pH.



Confined compression experiments



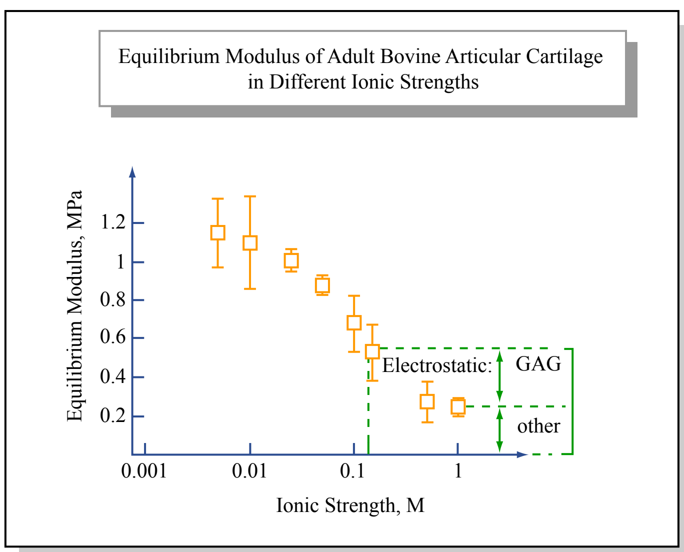


Figure by MIT OCW.

Some common proteoglycans					
Proteoglycan	Approximate molecular weight of core protein	Type of GAG chains	Number of GAG chains	Location	Functions
Aggrecan	210,000	Chondroitin Sulfate + Keratan Sulfate	~130	Cartilage	Mechanical support; forms large aggregates with hyaluronan
Betaglycan	36,000	Chondroitin Sulfate/ Dermatan Sulfate	1	Cell surface and matrix	Binds TGF - β
Decorin	40,000	Chondroitin Sulfate/ Dermatan Sulfate	1	Widespread in connective tissue	Binds to type 1 collagen fibrils and binds TGF - β
Perlecan	600,000	Heparan Sulfate	2-15	Basal laminae	Structural and filtering function in basal lamina
Serglycin	20,000	Chondroitin Sulfate/ Dermatan Sulfate	10-15	Secretory vesicles in white blood cells	Helps to package and store secretory molecules
Syndecan-1	32,000	Chondroitin Sulfate + Heparan Sulfate	1-3	Fibroblast and epithelial cell surface	Cell adhesion; binds FGF

Figure by MIT OCW.

ADHESION PROTEINS

a) fibronectin
- i) principal adhesion protein of connective tissues
- ii) fibronectin is a dimeric glycoprotein
- iii) fibronectin interacts with other molecules

b) laminin
- i) found in basal laminae
- ii) form mesh-like polymers
- iii) has various binding sites
- iv) assembles networks of crosslinked proteins

c) integrins
- i) cell surface receptor, for attachment of cells to ECM
- ii) family of transmembrane proteins
- iii) two subunits, alpha and beta
- iv) about 20 different integrins
- v) binding sites for ECM components
- vi) binding sites for the cytoskeleton and linkage to ECM

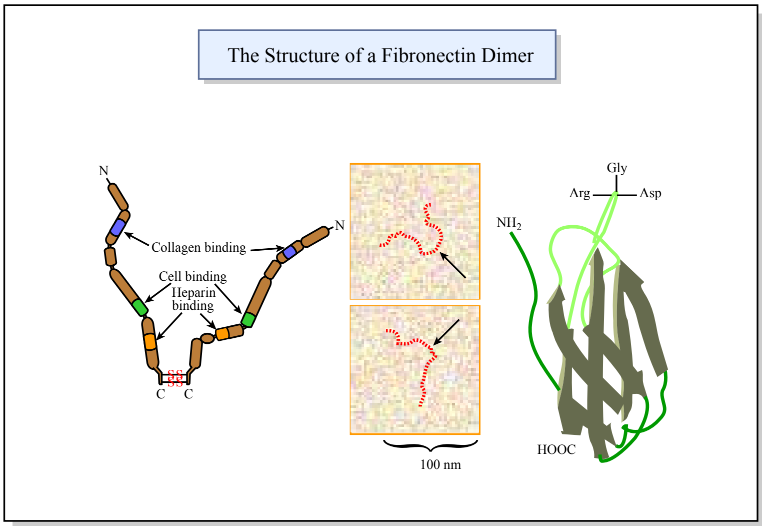


Figure by MIT OCW.

The bundle thickness, mesh size, elastic modulus, and critical strain as a function of R at cA = 11.9 µM

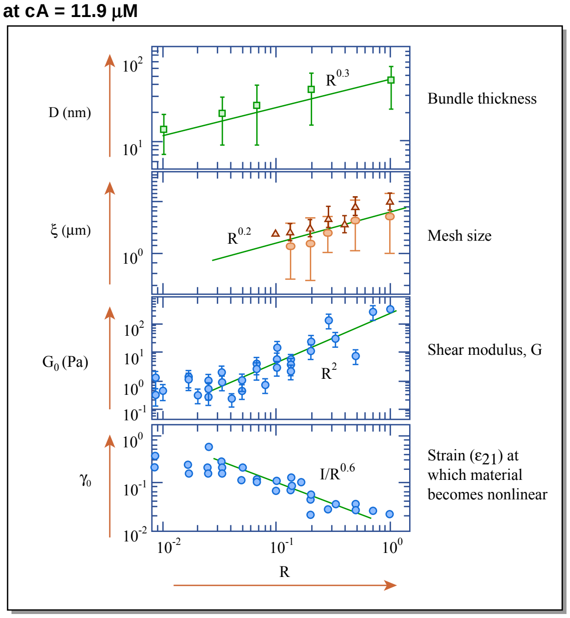


Figure by MIT OCW. Adapted from Shin, J. H. et al. *Proc Natl Acad Sci USA* 101 (2004): 9636-9641.

The stress-strain relationships of F-actin networks formed with different FLN mutants

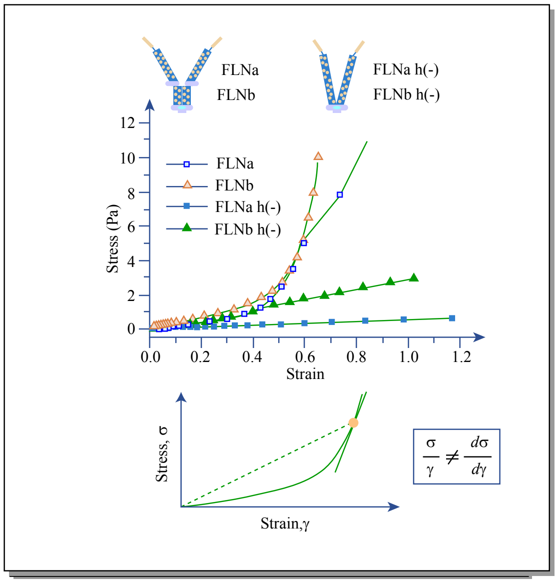


Figure by MIT OCW. Adapted from Gardel, M. L. et. al. *Proc Natl Acad Sci USA* 103 (2006): 1762-1767.

We apply a prestress to the network (Inset, single-headed filled arrow) and measure the deformation (Inset, dashed arrow) in response to an additional oscillatory stress (Inset, double-headed filled arrow)

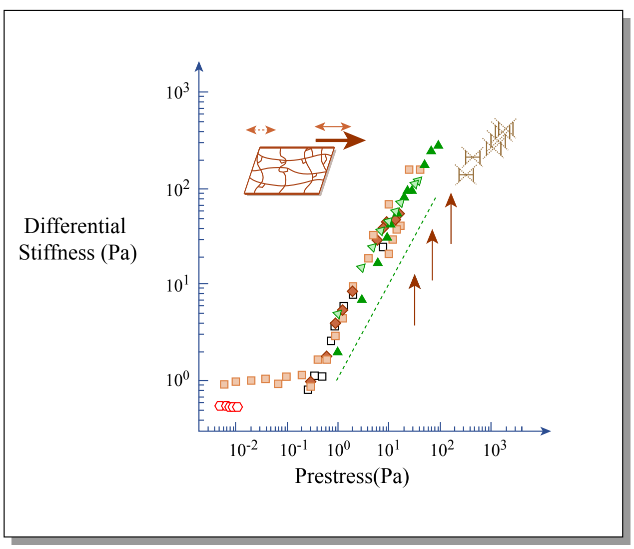


Figure by MIT OCW. Adapted from Gardel, M. L. et. al. *Proc Natl Acad Sci USA* 103 (2006): 1762-1767.

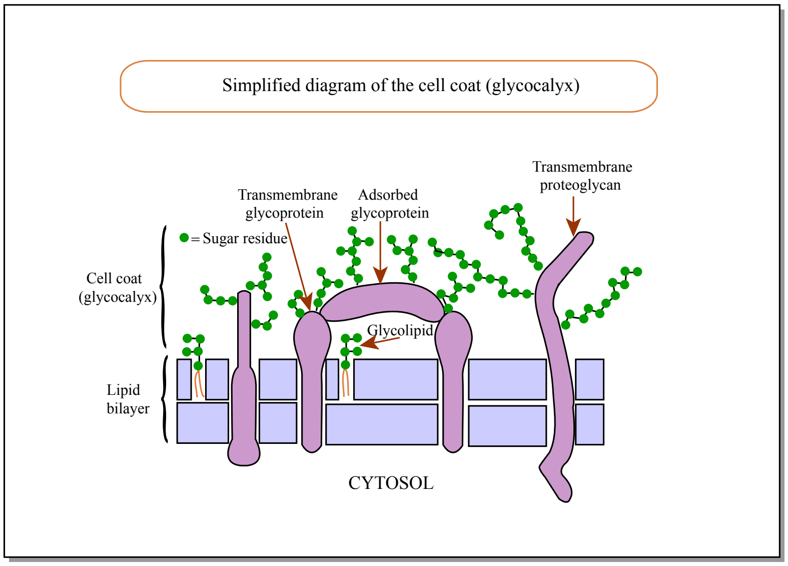


Figure by MIT OCW.

Focal adhesion complex

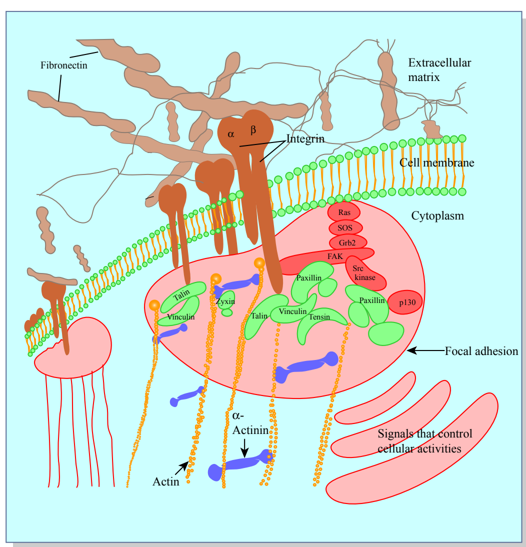


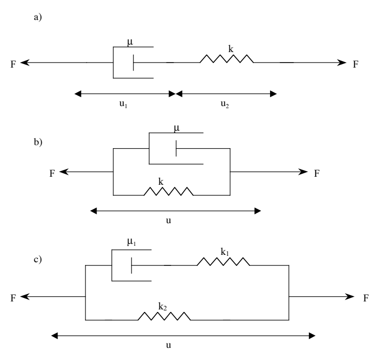
Figure by MIT OCW.

Viscoelastic models

Maxwell

Voigt

Standard linear solid



Stress relaxation

$$F(t) = ku_0 \exp(-t/\tau)$$

$$\tau = \mu / k$$

Creep

$$u(t) = \frac{F}{k}\left[1 - \exp\left(-t/\tau\right)\right]$$

Steady-state response to oscillatory displacement

Concept of a complex elastic or shear modulus	$E^*(\omega) = E' + iE''$

20.310, 2.793, 6.024
Fall, 2006

Poroelastic materials

Governing equations:

1. Constitutive law

$$\sigma_{ij}^{tot} = 2G\varepsilon_{ij} + \lambda\varepsilon_{ij}\delta_{ij} - p\delta_{ij}$$

2. Fluid-solid viscous interactions (Darcy's Law)

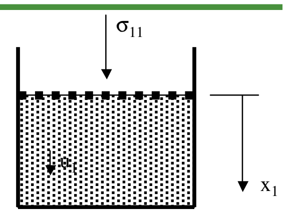
$$\vec{U} = -k\nabla p$$

3. Conservation of mass

$$\vec{U} = \phi\left(\vec{v}_f - \vec{v}_s\right) = \phi\vec{v}_{rel} \qquad \vec{v}_s = \frac{\partial\vec{u}}{\partial t}$$

4. Conservation of momentum

$$\nabla \cdot \bar{\bar{\sigma}} = 0$$



1D forms

$$\sigma_{11}^{tot} = (2G + \lambda)\varepsilon_{11} - p$$

$$U_1 = -k\frac{\partial p}{\partial x_1}$$

$$U_1 = -\frac{\partial u_1}{\partial t} + U_0$$

$$\frac{\partial \sigma_{11}}{\partial x_1} = 0$$

$$\frac{\partial u_1}{\partial t} = Hk\frac{\partial^2 u_1}{\partial x_1}$$

20.310, 2.793, 6.024
Fall, 2006

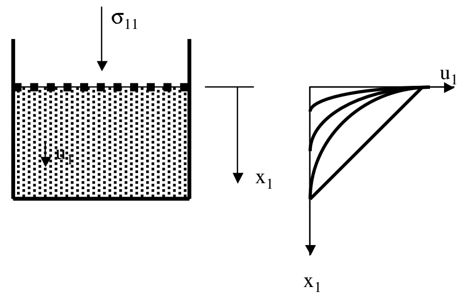
Poroelasticity -- confined compression

Impose displacements at boundaries:

$u_1(x_1, t=0) = 0$

$u_1(x_1=L, t>0) = 0$

$u_1(x_1=0, t>0) = u_0$



$$\frac{\partial u_1}{\partial t} = Hk \frac{\partial^2 u_1}{\partial x_1}$$

Characteristic time ~ L²/Hk

Solution (Fourier series)

$$u_1(x_1,t) = u_0\left(1 - \frac{x_1}{L}\right) - \sum_n A_n \sin\left(\frac{n\pi x_1}{L}\right)\exp\left(-\frac{t}{\tau_n}\right)$$

$$\tau_n = \frac{L^2}{n^2\pi^2 Hk}$$

Finite rates of protein folding and unfolding can also contribute to time-dependent behavior

- AFM can be used to measure both the elastic (k) and viscous (z) properties of a single molecule as a function of extension
- Sequential unfolding of the immunoglobulin (Ig) domains of titin during oscillations to measure viscoelasticity
- Siingle molecule elastic and viscous properties appear to scale with each other

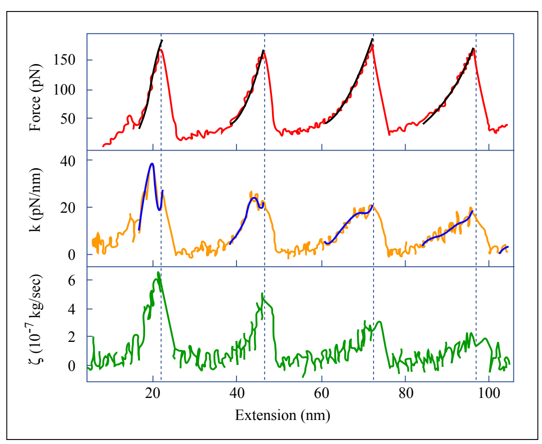
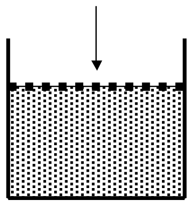
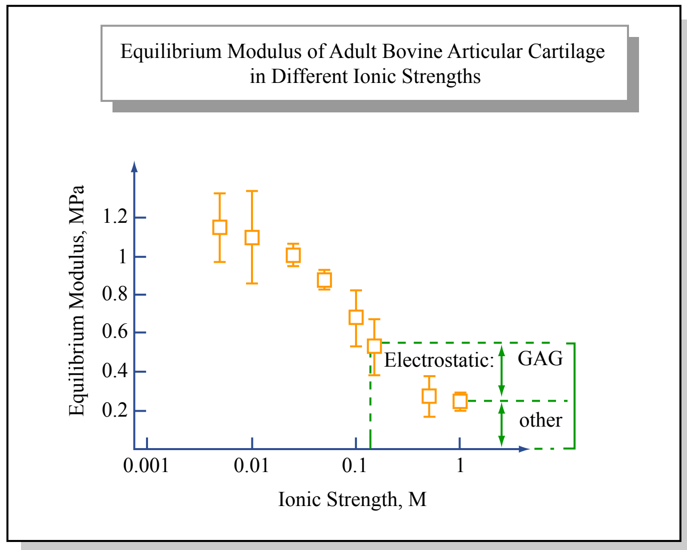


Figure by MIT OCW.

Kawakami et al., BJ, 2006

Additional factors: Ionic strength





$$\lambda + 2G$$

Figure by MIT OCW.