Cellular Biophysics
Lecture 2: Extracellular Matrix

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Cellular Mechanotransduction

Contents

1. Recap from previous lecture
2. Extracellular matrix
3. Collagens
4. Elastic fibers and proteoglycans
5. Cell adhesion mediating proteins

Cellular Mechanotransduction

Proteins and protein folding

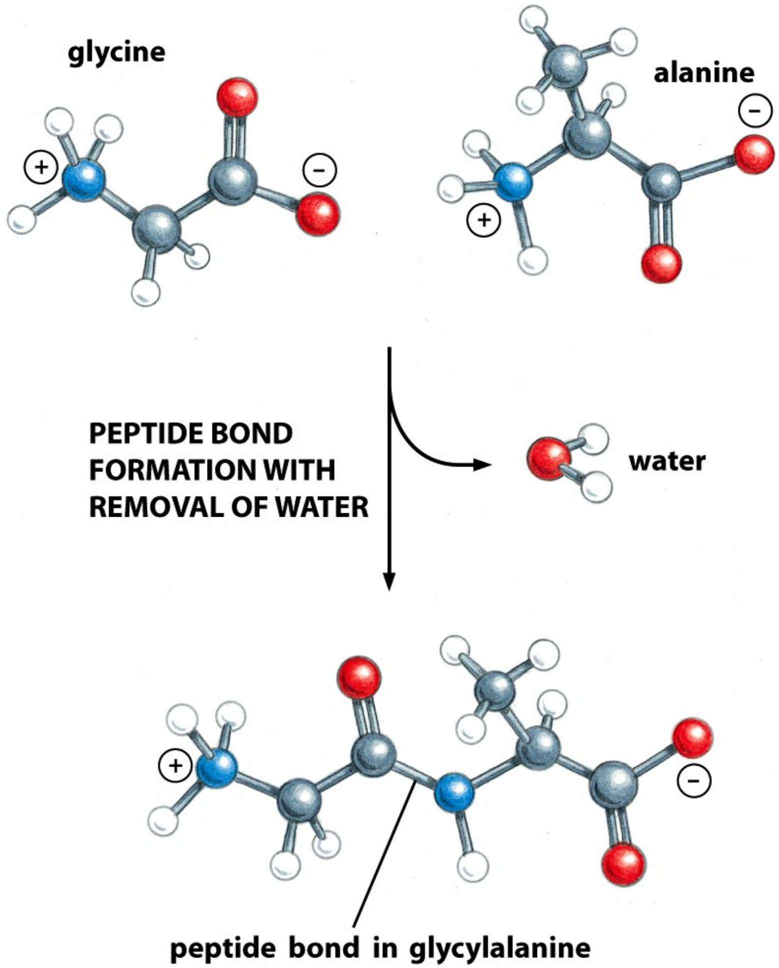
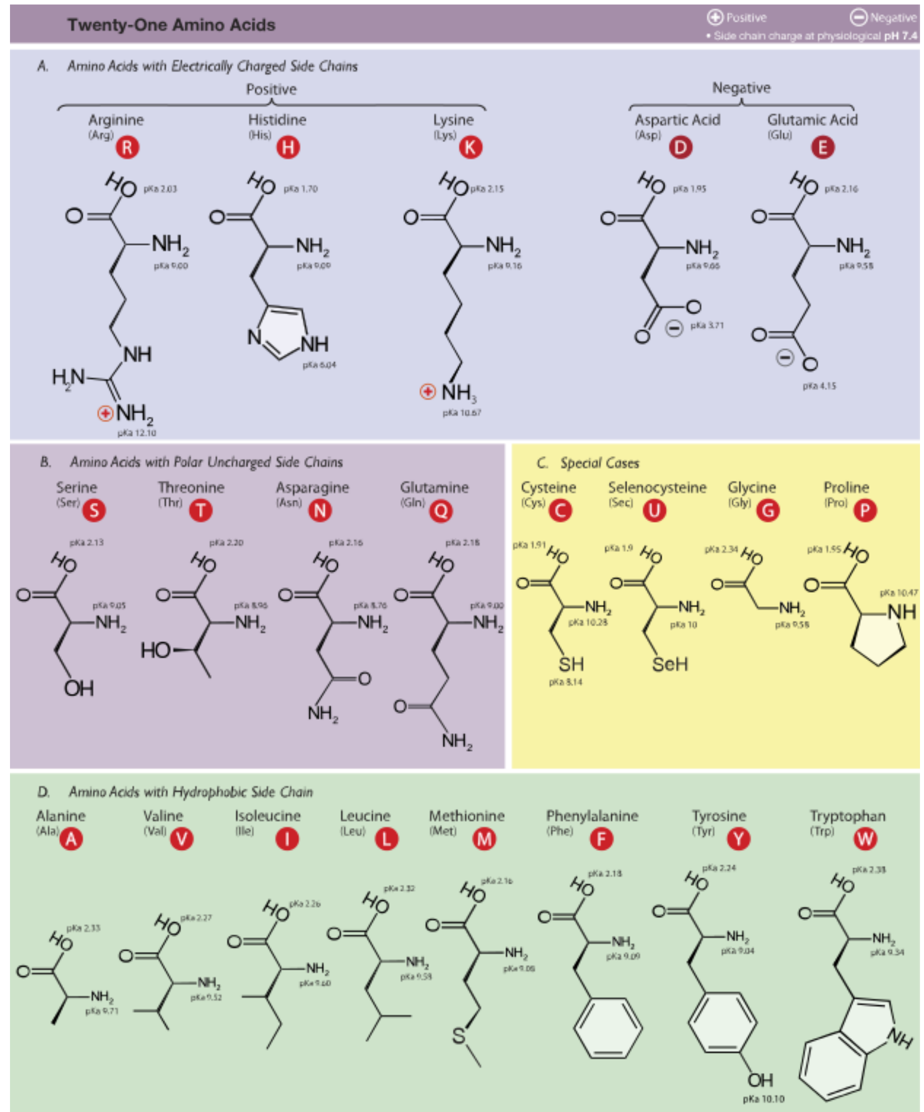
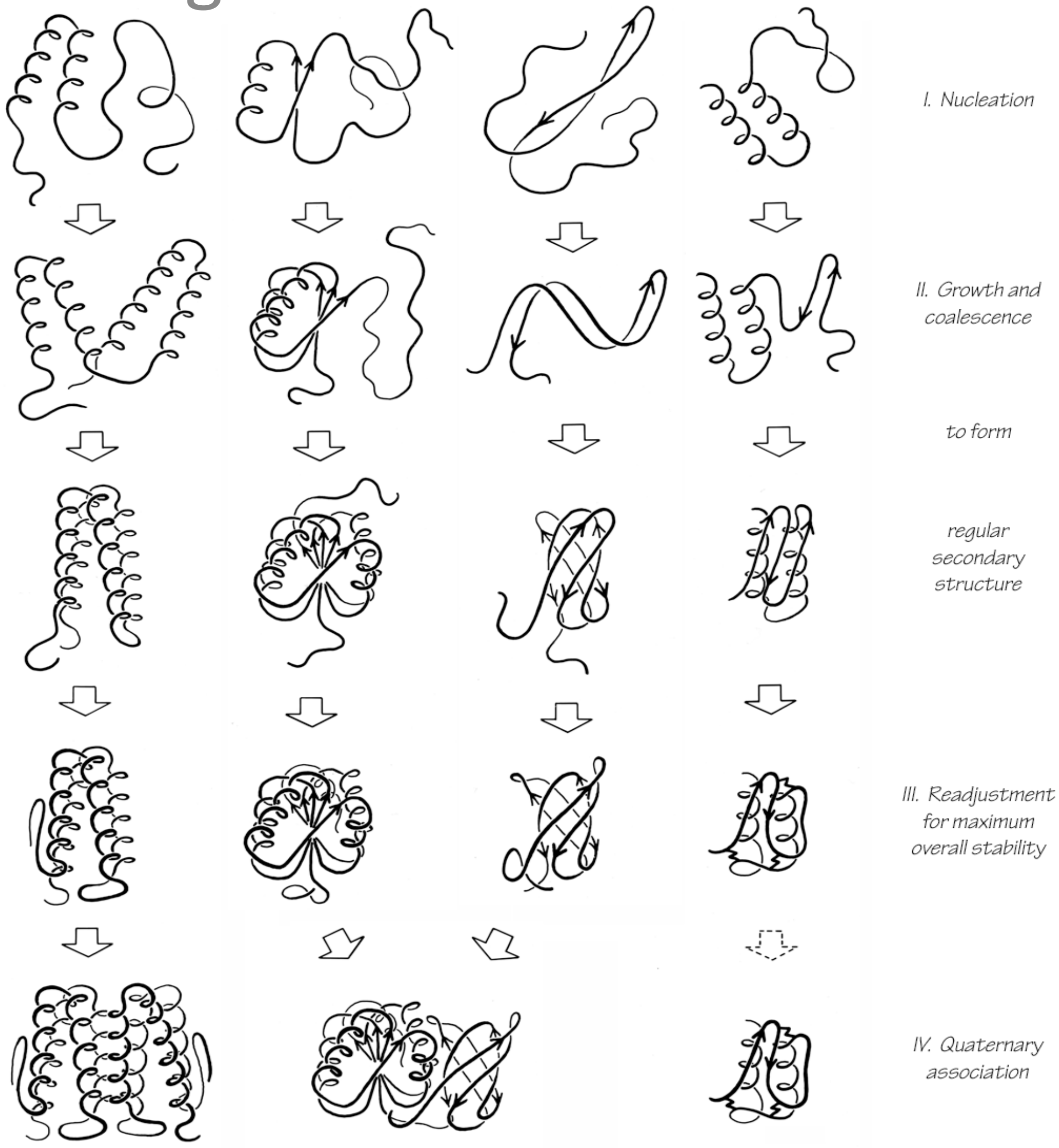


Figure 4-1 Essential Cell Biology 3/e (© Garland Science 2010)



Cellular Mechanotransduction

Protein folding



Cellular Mechanotransduction

Protein folding

$$\Delta U = -(U_c + U_e + U_d) + E_t + E_r$$

For protein structure stability, the term U_e is important (hydrogen bonds)

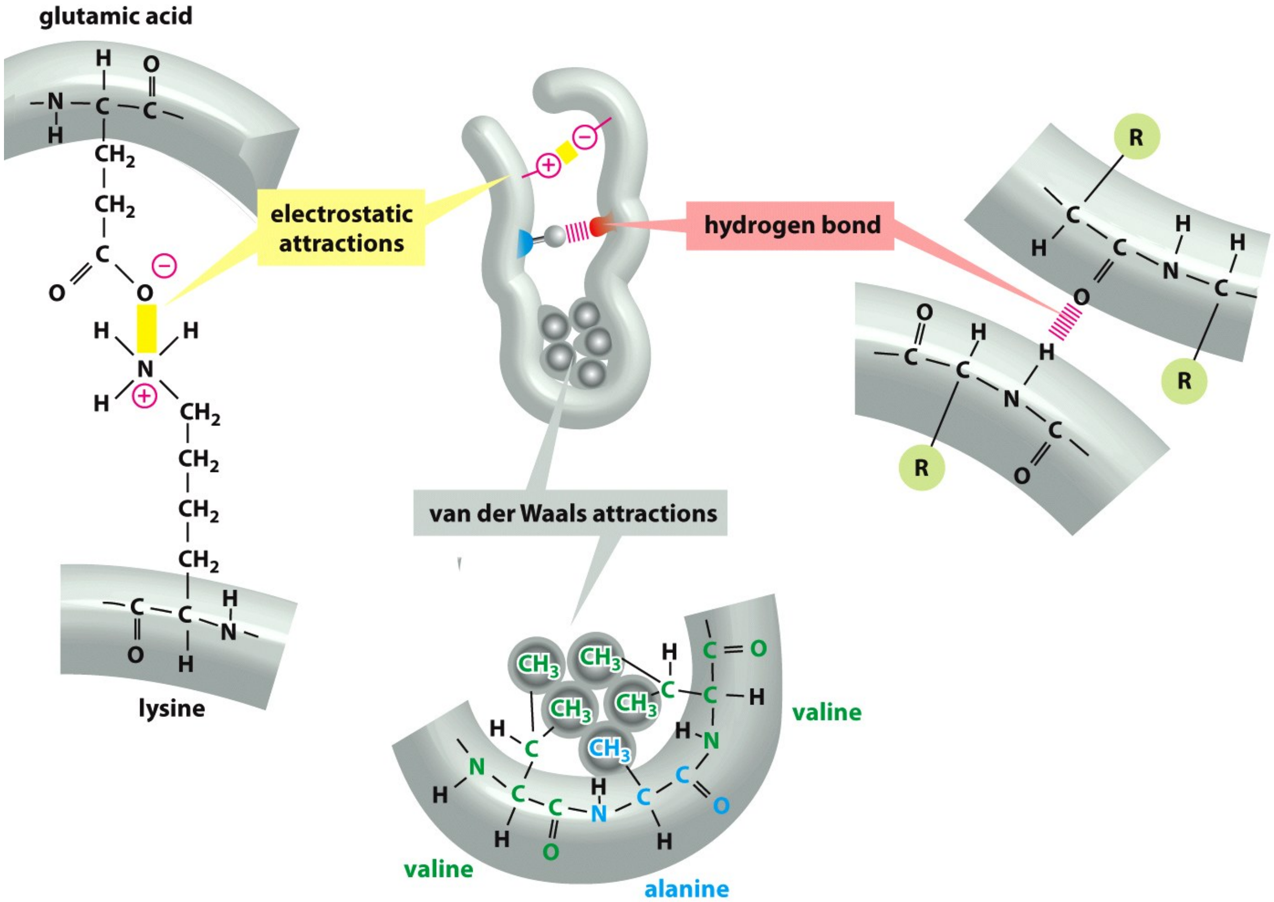


Figure 4-4 Essential Cell Biology 3/e (© Garland Science 2010)

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Protein folding

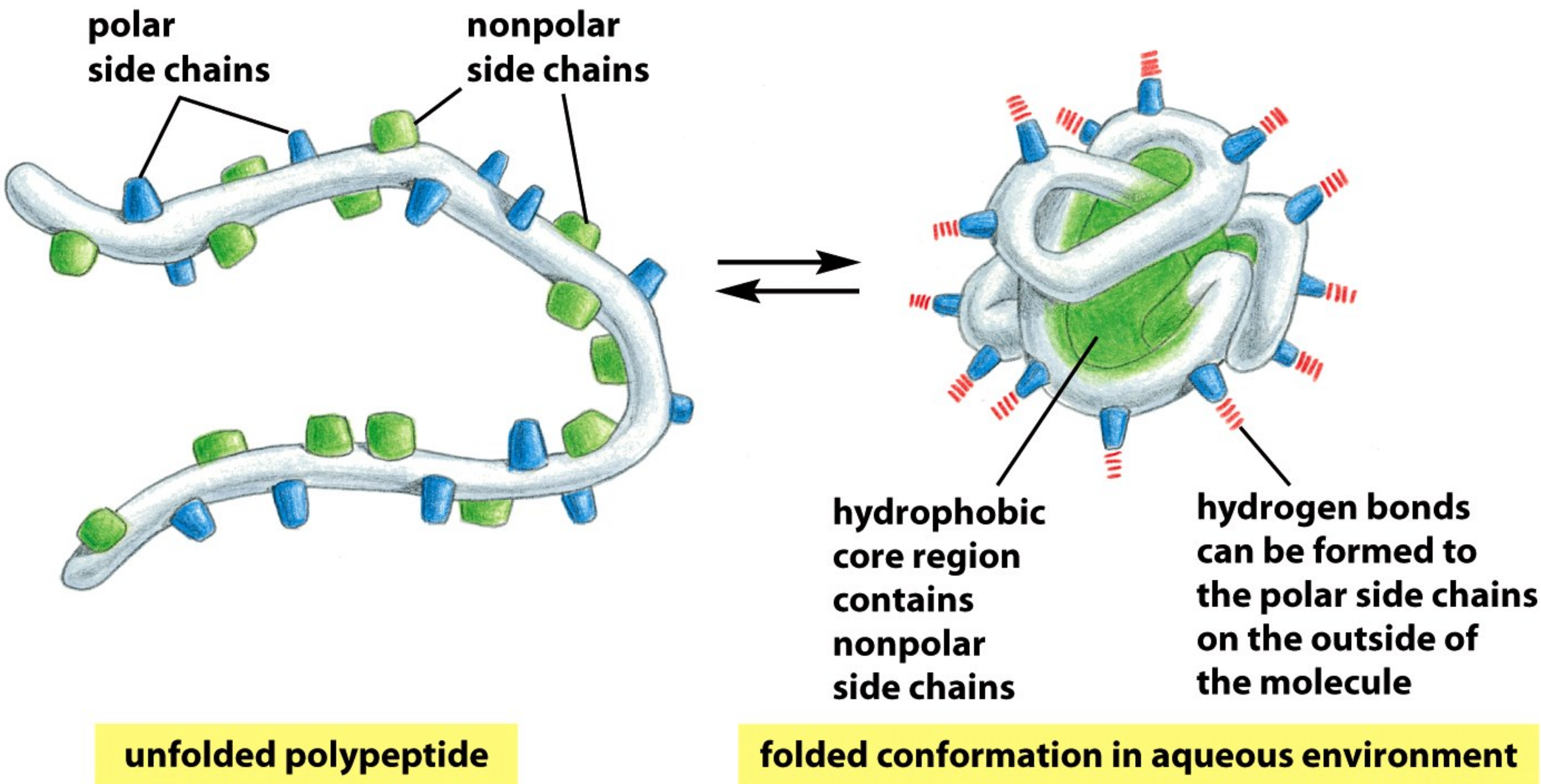

Figure 4-5 Essential Cell Biology 3/e (© Garland Science 2010)

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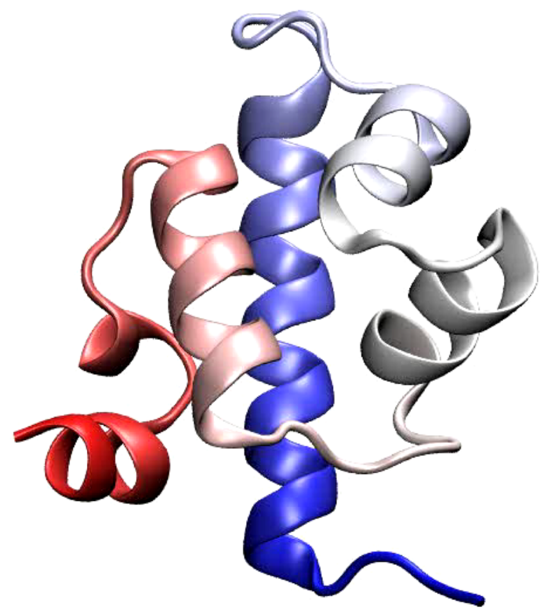
Protein folding and chemical equilibrium

In order to understand how external force might influence the protein structure and function, we need to take a closer look on the protein folding

Folding of λ-repressor protein, 100μs

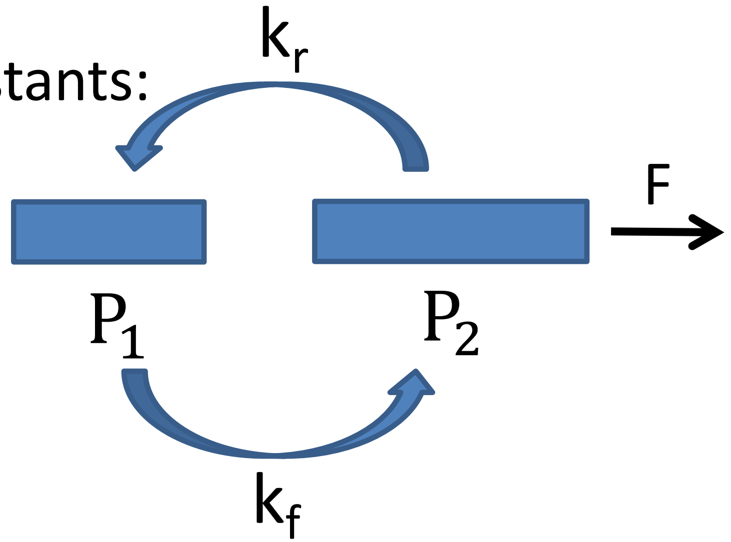


http://www.ks.uiuc.edu/Highlights/index.cgi?section=2012

Cellular Mechanotransduction

Protein folding, influence of force

$K_{eq.}$ can be written also using the rate constants:



$$\frac{[\text{Species}_2]}{[\text{Species}_1]} = \frac{k_f}{k_r} = K_{eq.}$$

k_f = forward rate constant

k_r =reverse rate constant

Force brings more energy to the system:

$\Delta U(x) = F\Delta X$

Then,

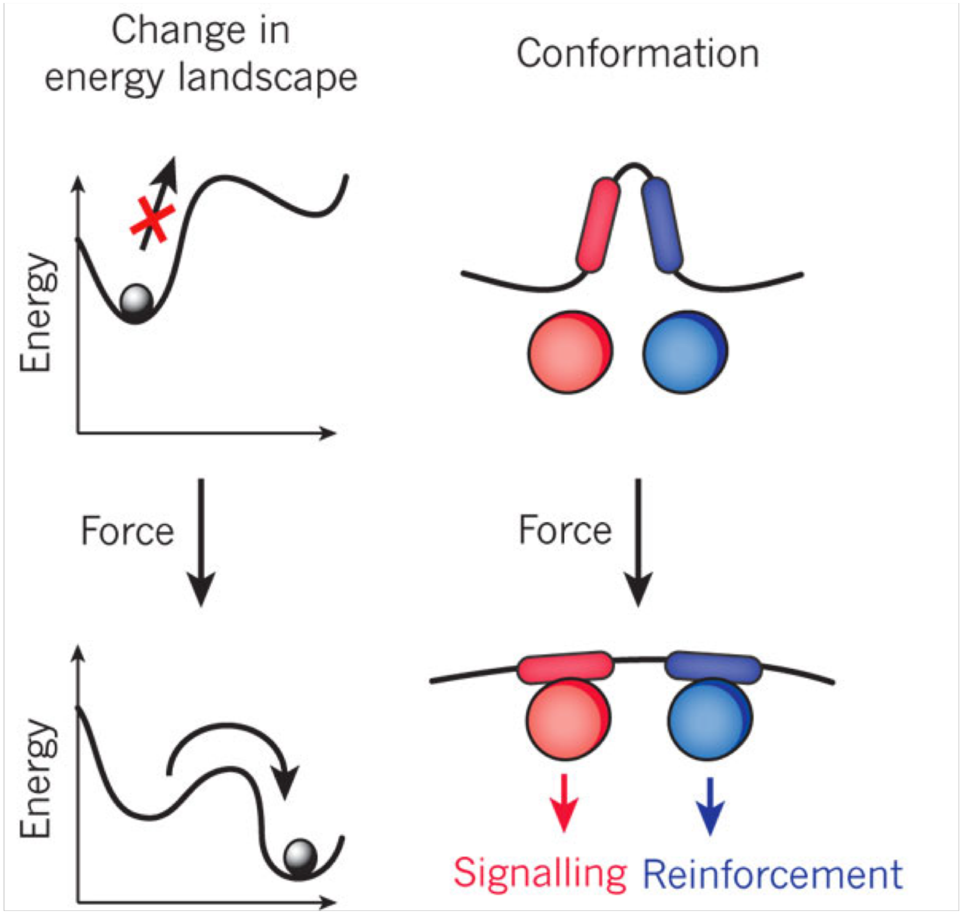
$$\frac{k_f}{k_r} = e^{-\frac{\Delta E + F\Delta X}{kT}} = K_{eq.} e^{\frac{F\Delta X}{kT}}$$

The force changes the equilibrium state!

Cellular Mechanotransduction

Force sensing

Force alters the "energy landscape" of the protein
→ new equilibrium state
→ changes in structure and function



Cellular Mechanotransduction

Force induced unfolding/structural change

Force induced unfolding changes the equilibrium state

→ protein structure changes

Mechanical force can change the structure of the proteins

~~→ Denaturation???~~

→ Exposure of new cryptic binding sites

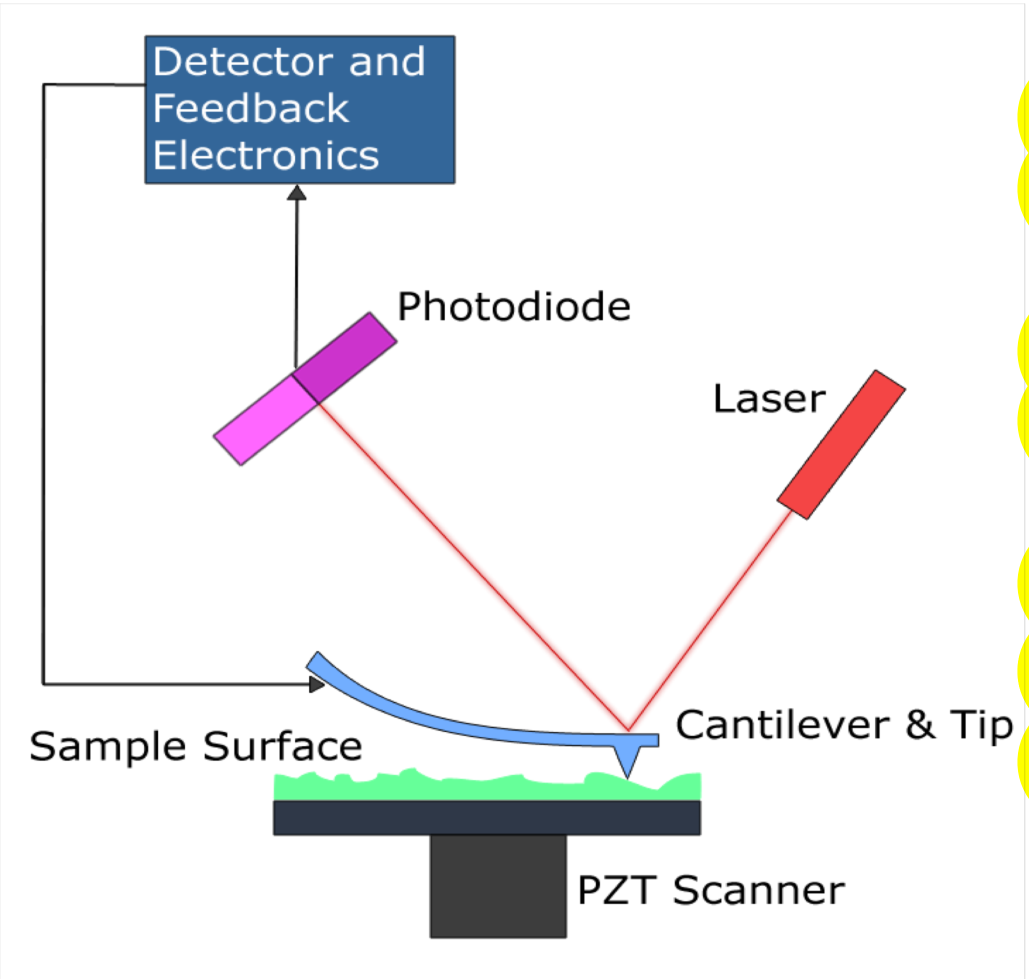
→ Release of bound factors

→ Changed function of the protein

Cellular Mechanotransduction

Atomic Force Microscopy (AFM)

Scanning Probe Microscopy (SPM) allows the manipulation and imaging in nanometer scale.



Tip is brought to contact with the sample surface

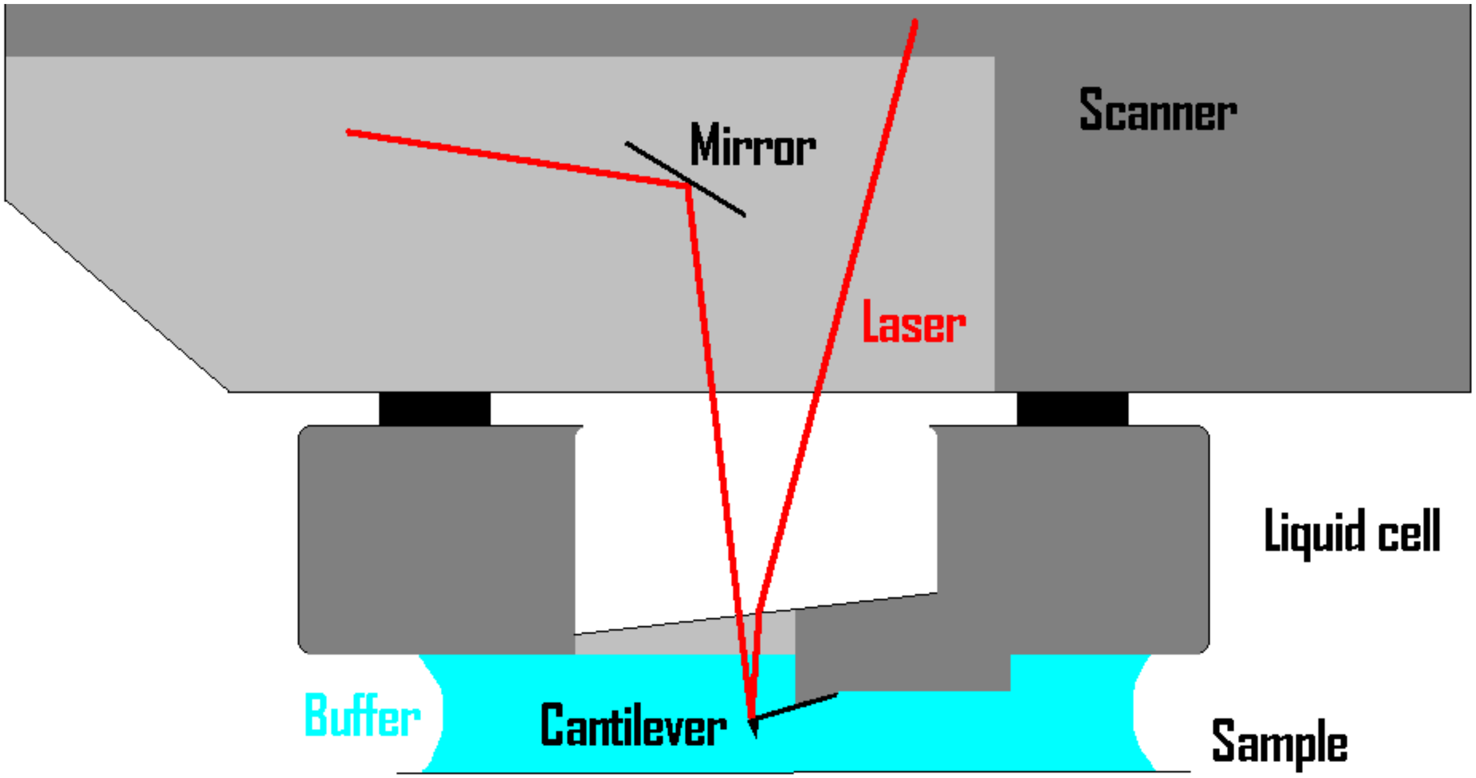
An interatomic forces bends the cantilever beam.

A detector measures the deflection as the tip scans over the sample.

Cellular Mechanotransduction

Atomic Force Microscopy (AFM)

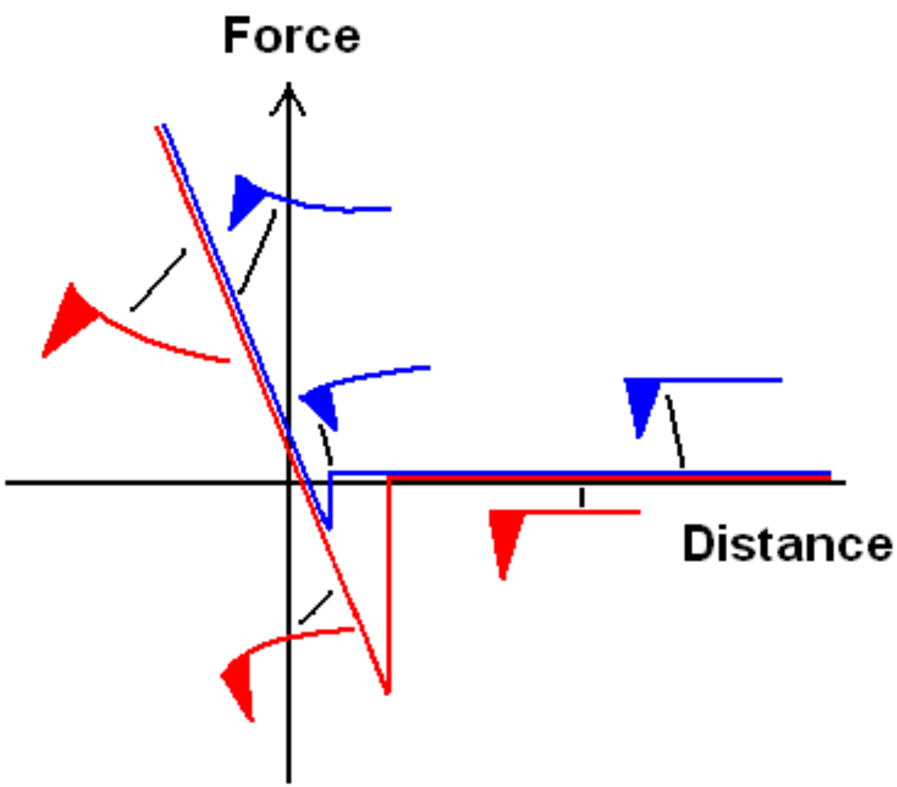
AFM can be conducted also under liquid



Cellular Mechanotransduction

Atomic Force Microscopy (AFM)

AFM senses forces between the tip and the sample
→ extremely sensitive force probe (sensitivity < 5pN)



Hooke's law F=-kx

k=spring constant

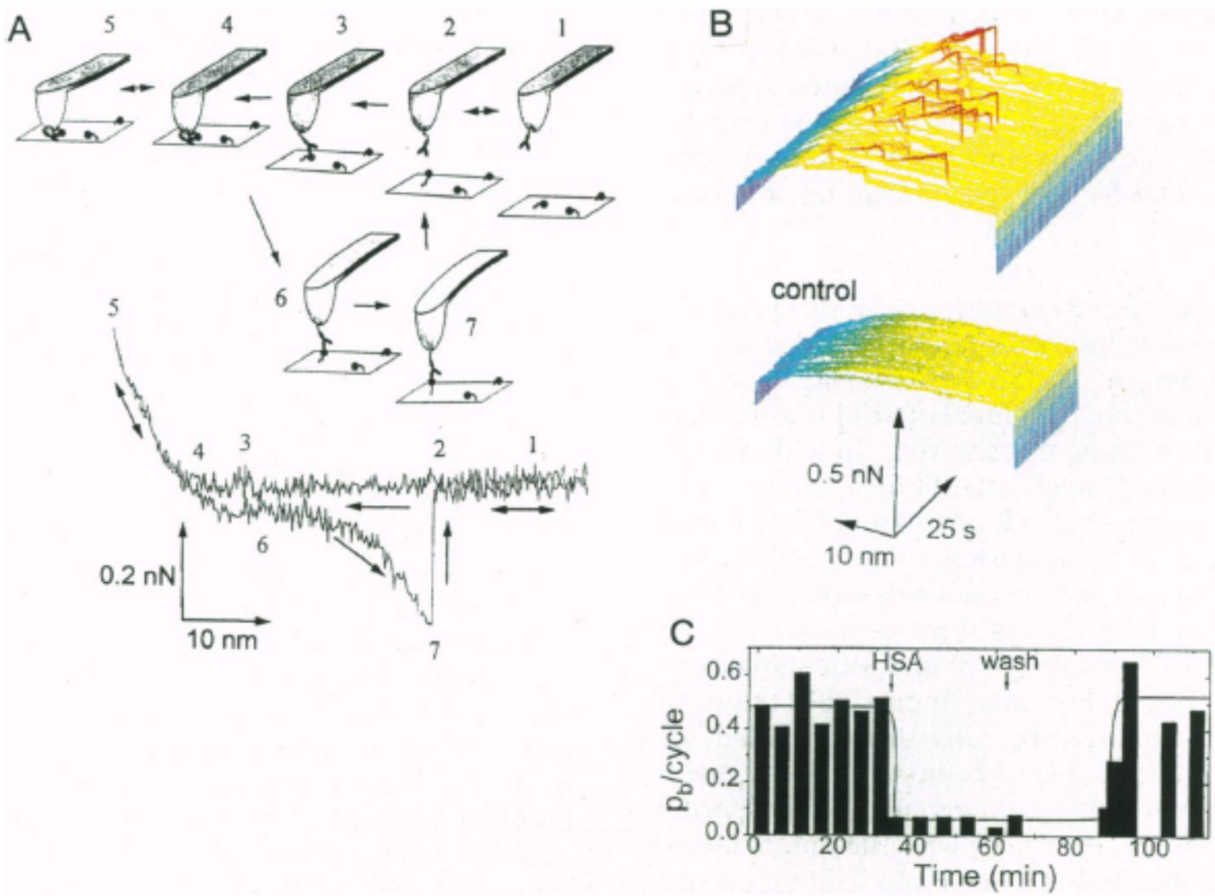
x=deflection

Cantilever k = 1-100 pN/nm

Cellular Mechanotransduction

Atomic Force Microscopy (AFM)

Binding force between human serum albumin and HSA antibody

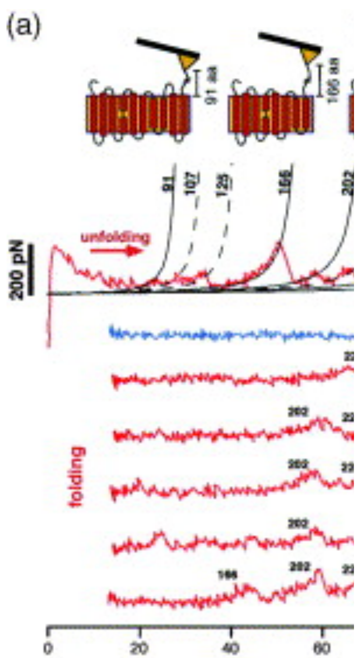


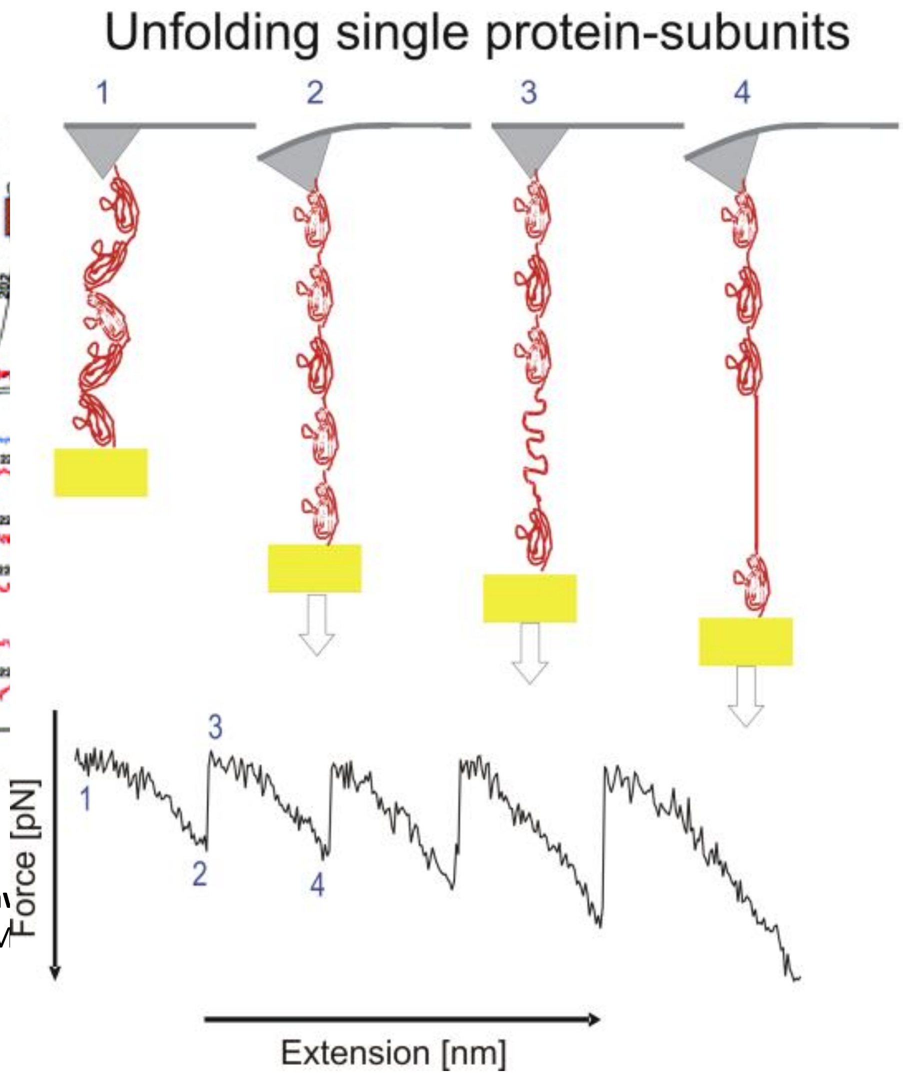
"Detection and localization of individual antibody-antigen recognition events by atomic force microscopy"
Hinterdorfer *et al.* PNAS, 1996 Apr 16;93(8):3477-81.

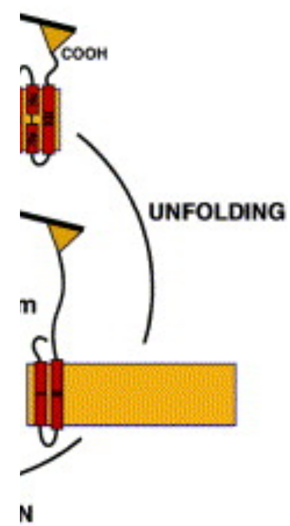
Cellular Mechanotransduction

Atomic Force Microscopy (AFM)

Mechanically forced unfolding





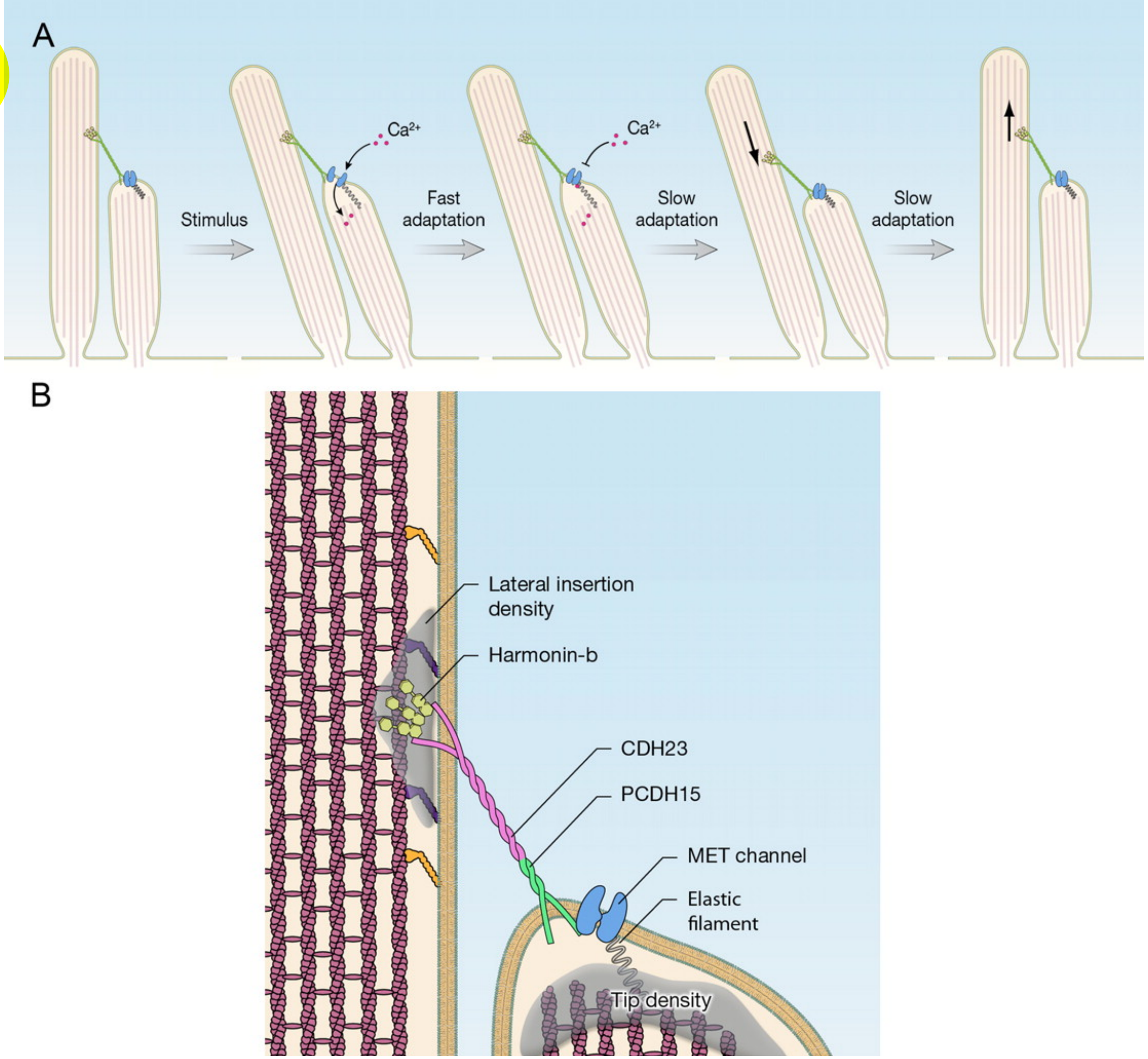


“Observing Folding Pathw ... **a coli”**
Kedrov *et al.* Journal of M

Cellular Mechanotransduction

Mechanotransduction

Ion channel activation and adaptation



Molecular components of hearing

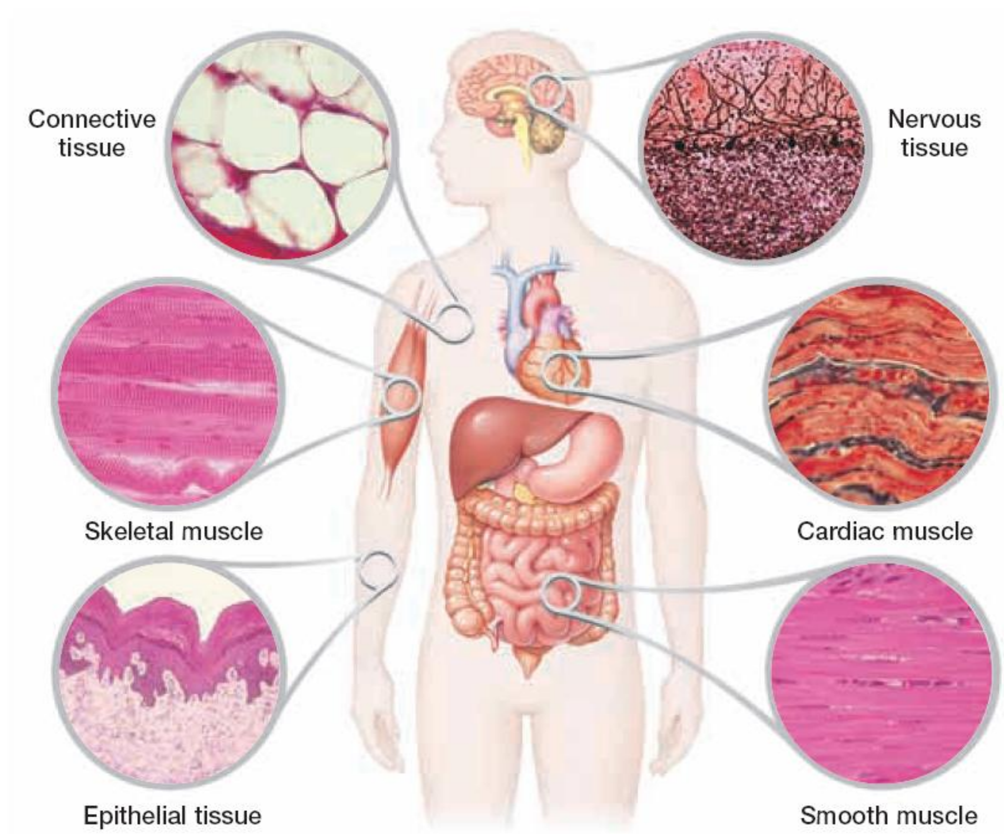
Schwander M et al. J Cell Biol 2010;190:9-20

Cellular Mechanotransduction

Tissues

Different tissues have different functions

Human Body Tissues



Cellular Mechanotransduction

Tissues

Animals tissues have been traditionally divided into 4 categories:

1. Connective tissue
2. Epithelial tissue
3. Nervous tissue
4. Muscular tissue

However, the division can be made just between "connective tissue and the rest"

- All the tissues have extracellular matrix (ECM) component, i.e. tissue is made from cells + ECM

- Connective tissue has a lot of ECM compared to the other tissues

Cellular Mechanotransduction

Extracellular Matrix

The ECM

Composed from proteins and polysaccharides

Plays an important role in regulating cell function.
- ECM proteins bind to cell surface receptors

- Activate signaling pathways, influencing
  - Cellular morphology
  - Cell adhesion
  - Cell migration
  - Cell proliferation
  - Apoptosis

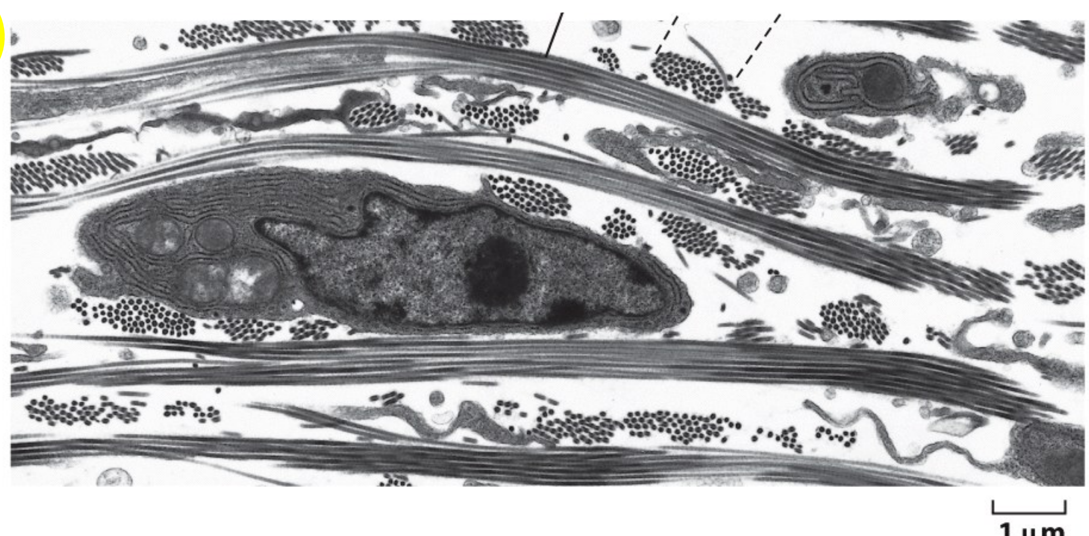


Figure 20-9 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Extracellular Matrix

The ECM

A gel containing fiber like proteins, sugars and fluid

Extracellular fluid:

Isotonic with the cytoplasm
Doesnt contain much proteins

Electrolyte	Plasma [mM]	Interstitial Fluid [mM]
Cations		
Sodium	142	**145**
Potassium	4	4
Calcium	5	5
Magnesium	2	2
Total Cations:	153	156
Anions		
Chloride	101	**114**
Bicarbonate	27	**31**
Phosphate	2	2
Sulphate	1	1
Organic Acid	6	7
Protein	**16**	**1**
Total Anions:	153	156

Cellular Mechanotransduction

Extracellular Matrix

The ECM components are secreted by the cells. The major ECM modifying cells are:

- Fibroblasts (skin, tendon, etc.)
- Osteoblasts (bone)

Fibroblast cells and collagen fibers in the rat cornea

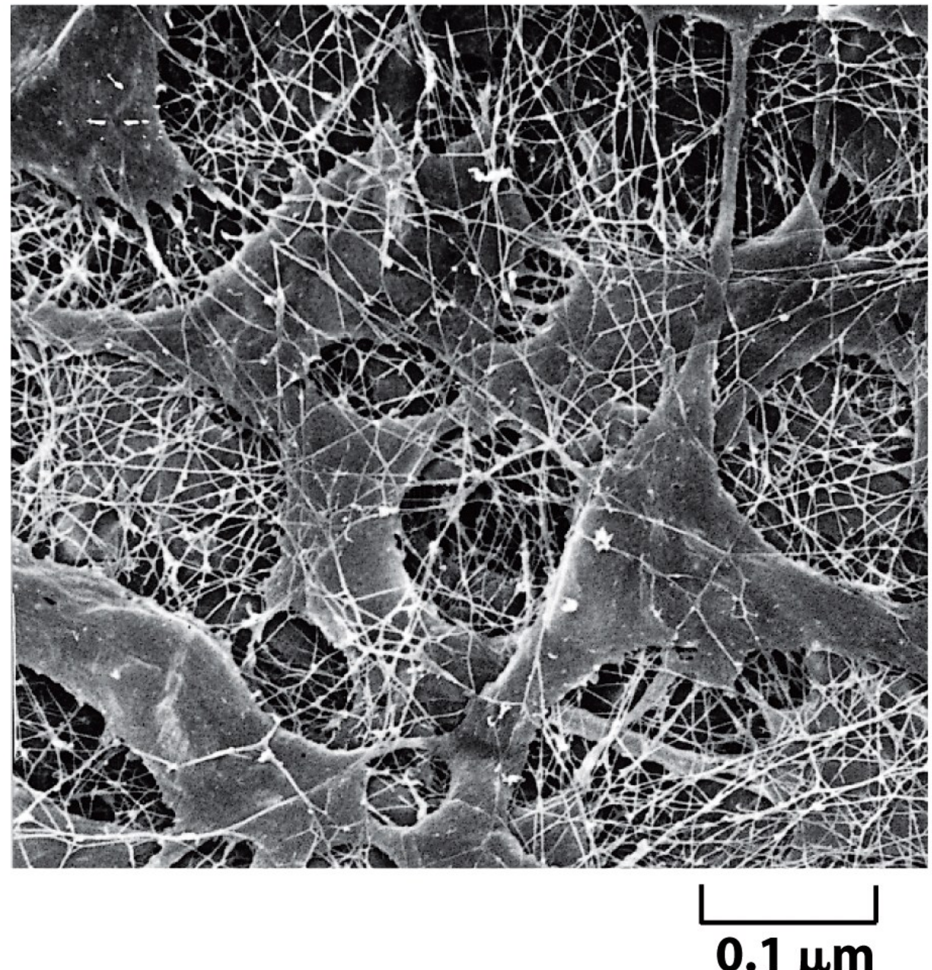


Figure 20-10 Essential Cell Biology 3/e (© Garland Science 2010)

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Extracellular Matrix

Different ECM components and their locations in the ECM:

Collagens	Thick and thin fibers
Elastin & Microfibrillar proteins	Elastic fibers of skin, lungs, blood vessels
Proteoglycans	Spaces between the proteins
Fibronectin & Laminins	Cell-ECM contacts

Tensile strength | Elasticity | "Shock absorber" | Cell attachment

Cellular Mechanotransduction

Extracellular Matrix

Mechanical properties isotropic material

Mechanical **stress** is defined as a measure of internal forces as object is deformed, $\sigma = \frac{F}{A}$

F= force (Newtons)

A = cross-section area (m^2)

Mechanical **strain** is the amount of deformation compared to the original shape, $\varepsilon = \frac{\Delta L}{L_0}$

ΔL = change in length

L_0 = original length

For isotropic material, the ratio between the stress and strain is material dependent constant, **Youngs Modulus, E**

$$\mathbf{E} = \frac{\boldsymbol{\sigma}}{\boldsymbol{\varepsilon}}$$

[E] = Pascals

Cellular Mechanotransduction

Extracellular Matrix

Mechanical properties isotropic material

When stress is applied we usually have first linear stress-strain behavior

After certain strain,
→ "strain hardening" and fracture

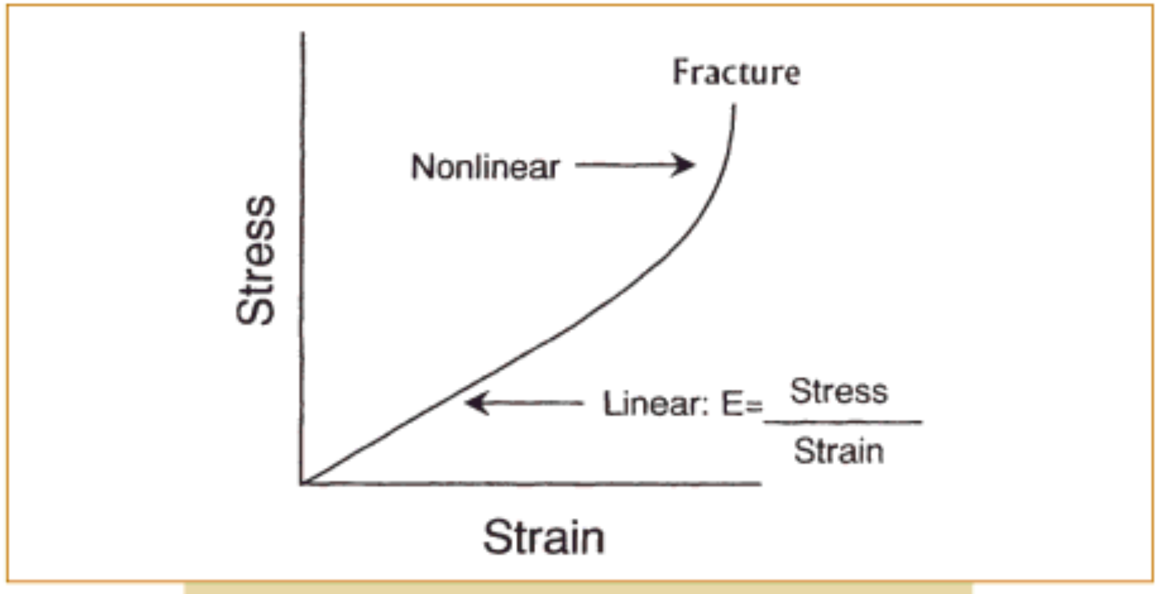


FIG. 18.4. Plot of stress (σ) vs. strain (ε), showing an initial linear region, in which the Young's modulus (E) is constant, and a nonlinear region, with increasing E, leading ultimately to fracture of the specimen.

Cellular Mechanotransduction

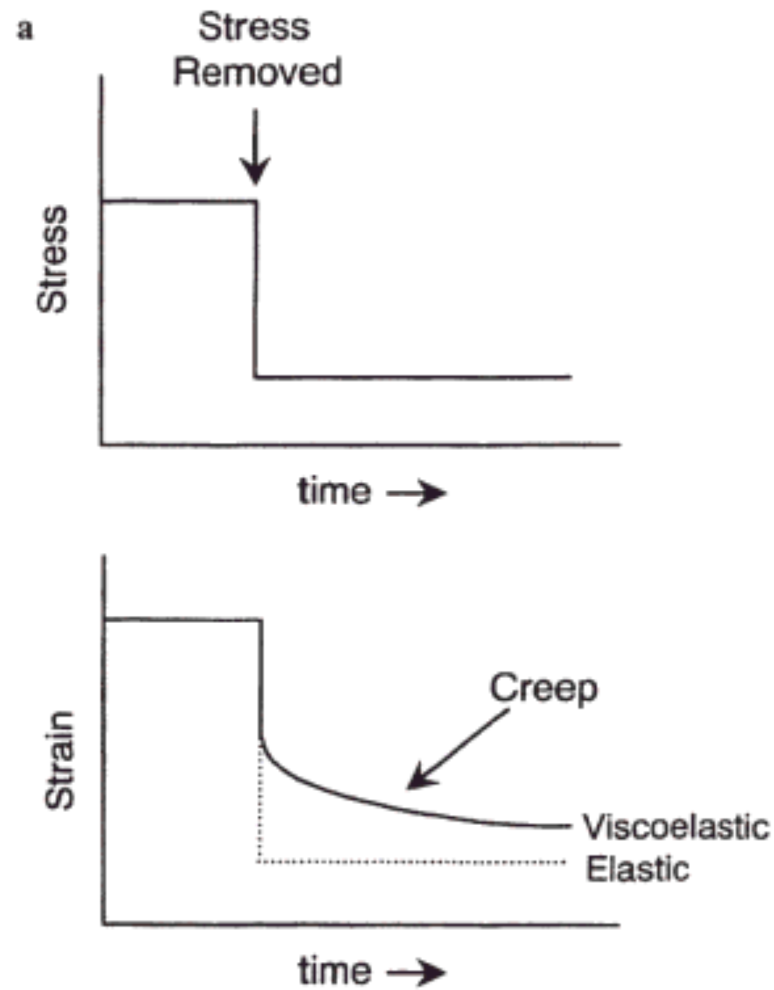
Extracellular Matrix

Mechanical properties isotropic material

Collagen and elastic fiber stress-strain behavior is **time independent**

However, tissues show **time dependent** behavior

- initial elastic response
- creep



Cellular Mechanotransduction

Extracellular Matrix

Collagens:

- over 25 different collagens have been found
- characteristically triple stranded helical structure

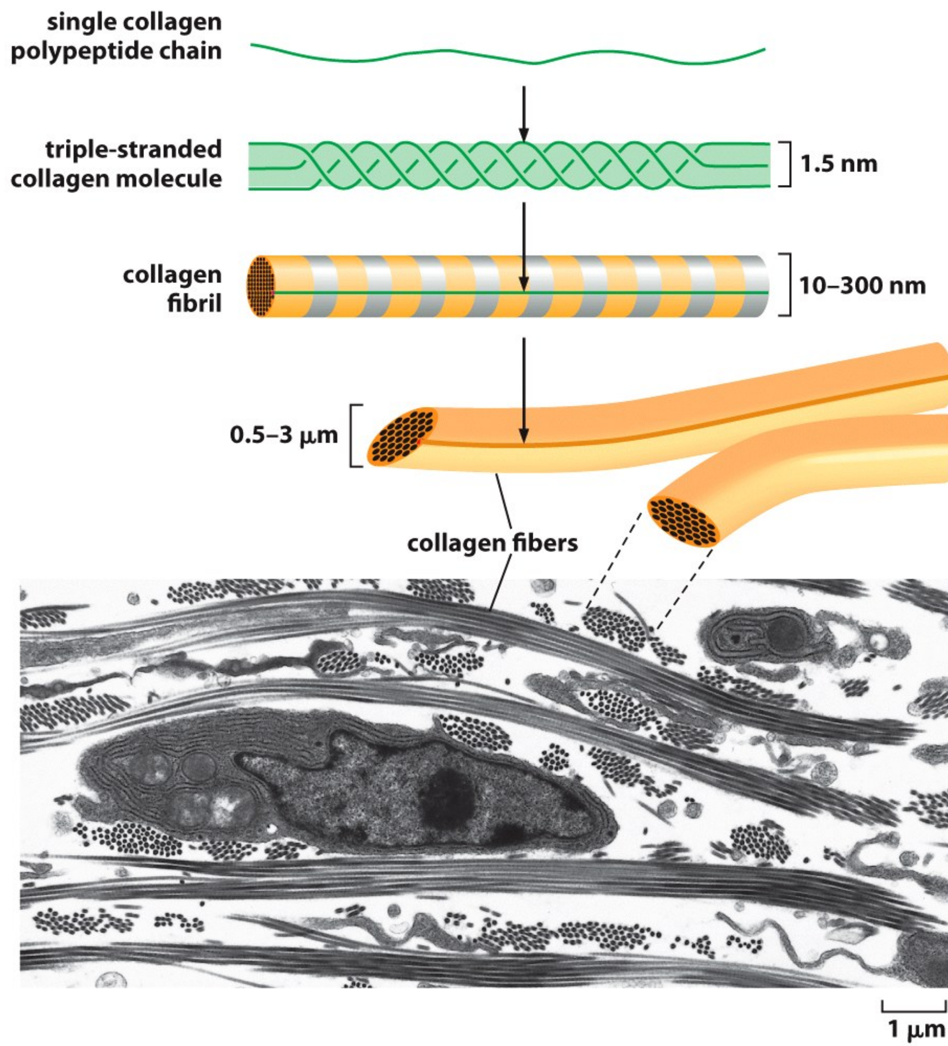


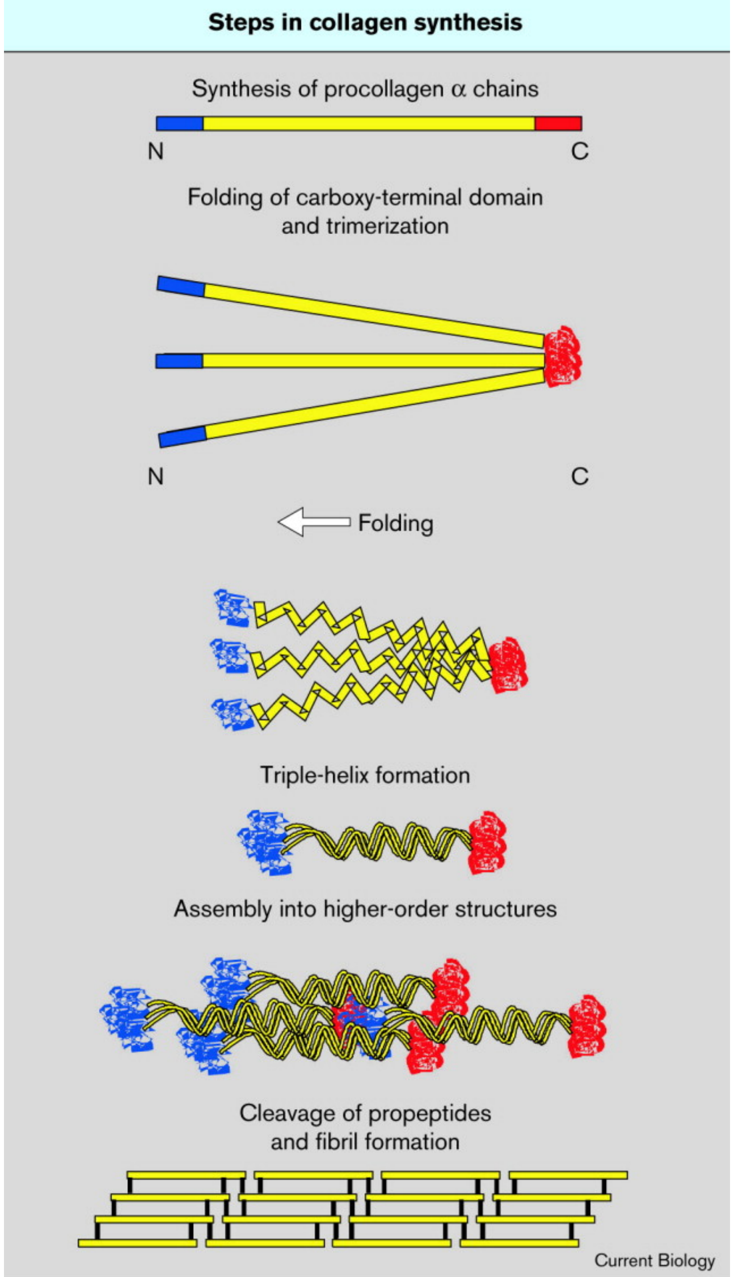
Figure 20-9 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Extracellular Matrix

Collagen assembly

i) Single collagen polypeptides are produced by the cells and modified
ii) C-termini interact (cysteines) and form disulphide bonds
iii) Folding into triple helix
iv) Export of procollagen
v) Cleavage of globular N- and C-terminal tails (outside of the cells)
vi) Assembly into fibrils



Current Biology (2000)
Vol10, Is24, 14 p. R912–R915

Cellular Mechanotransduction

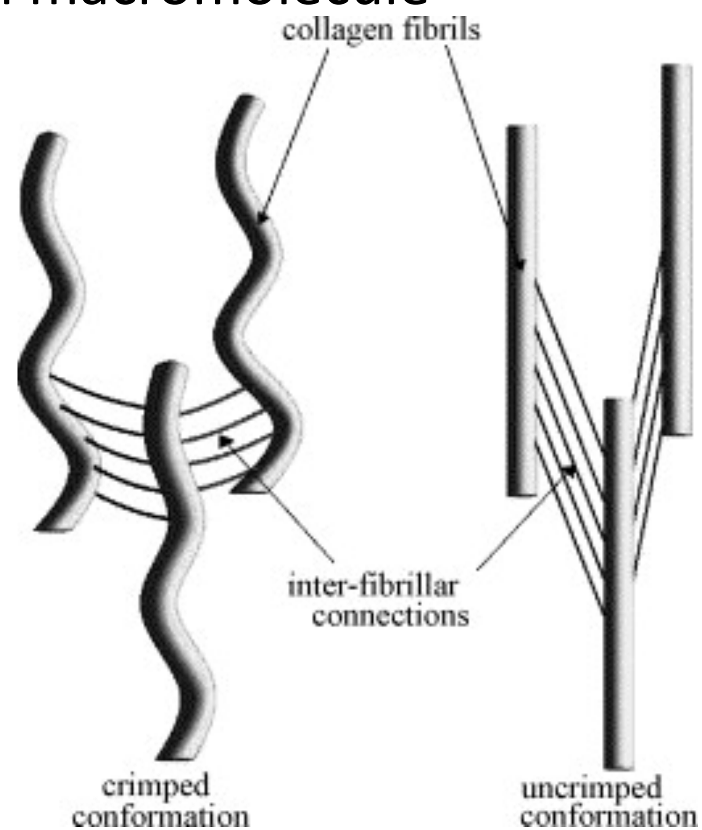
Extracellular Matrix

Mechanical properties of collagenous tissue

Linear stress-strain curve indicates conformational change either by uncoiling a polymer or stretching a helical macromolecule

In tissues we have complex behavior
- not completely aligned fibers (anisotropic response)
- different fiber components, etc.

→ non linear stress-strain behavior (+ time dependency i.e. viscoelasticity)

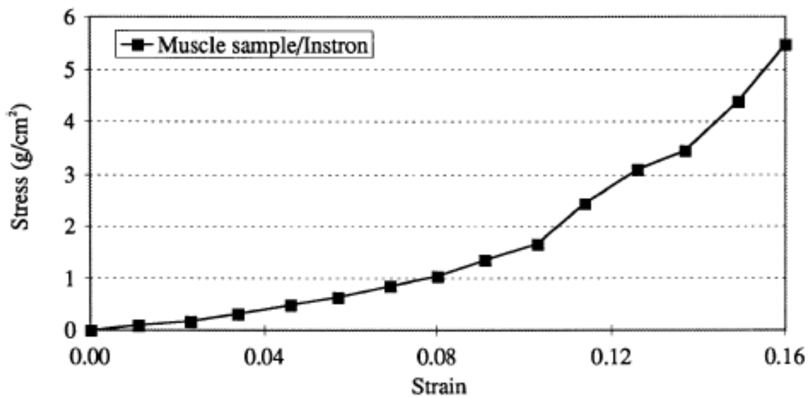


Medical Engineering & Physics
Volume 27, Issue 3, April 2005, Pages 209–214

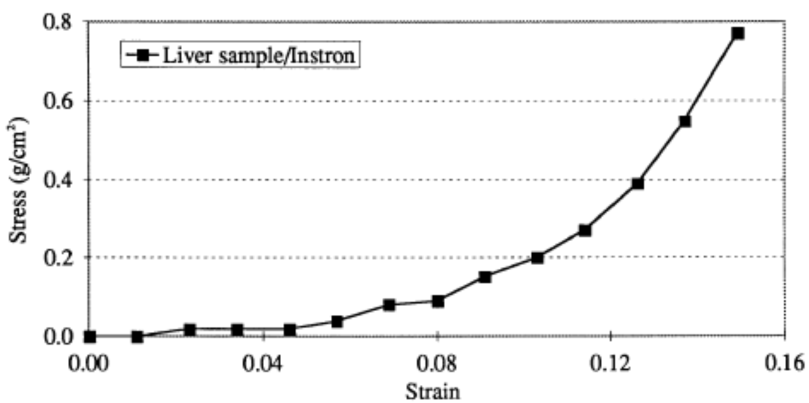
Cellular Mechanotransduction

Extracellular Matrix

Mechanical properties of muscle and liver tissue



(a)



Cellular Mechanotransduction

Extracellular Matrix

Young's moduli of different tissues and ECM:

Table 18.1. Elastic constants for a variety of biologic materials[a]

Material	*Elastic modulus*	*Yield stress*	*Max. strain*	*Ref.*
Cortical bone	6–30 GPa	50–200 MPa	—	Cowin *et al.* (1987)
Collagen fibers	500 MPa	50 MPa	0.1	Kato *et al.* (1989)
Elastin	100 kPa	300 kPa	3.0	Mulcherjee *et al.* (1976)
Cartilage	10 MPa	8–20 MPa	0.7–1.2	Woo *et al.* (1987)
Skin	35 MPa	15 MPa	1.1	Yamada (1970)
Muscle facia	340 MPa	15 MPa	1.17	Yamada (1970)
Tendon	700 MPa	60 MPa	0.10	Yamada (1970)

[a]Note that in many instances, the stress–strain relationship is highly nonlinear; the elastic moduli in those cases represent an approximate, characteristic value.

Cellular Mechanotransduction

Extracellular Matrix

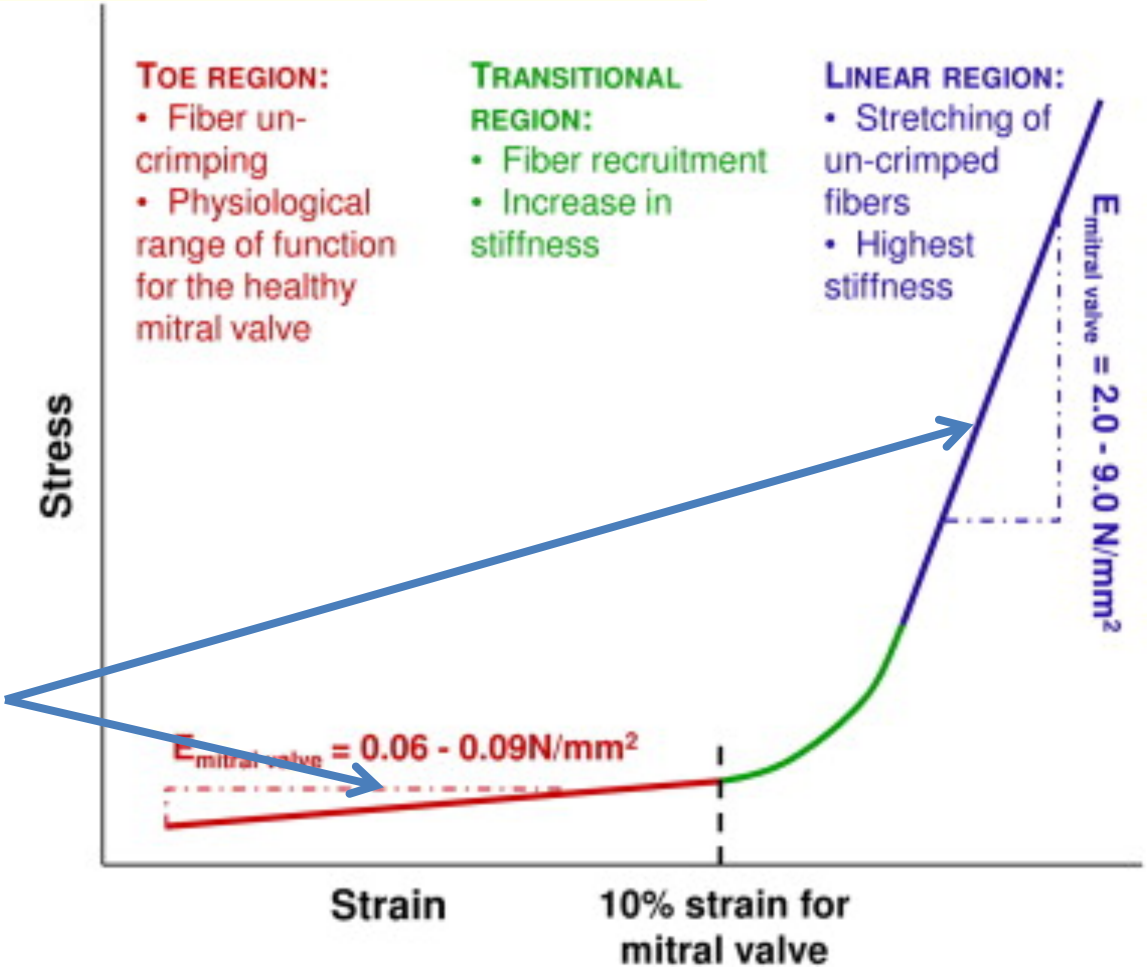
Mechanical properties of collagenous tissue

Stress-strain curve of the heart mitral valve

E = 60 kPa

E = 2000 kPa

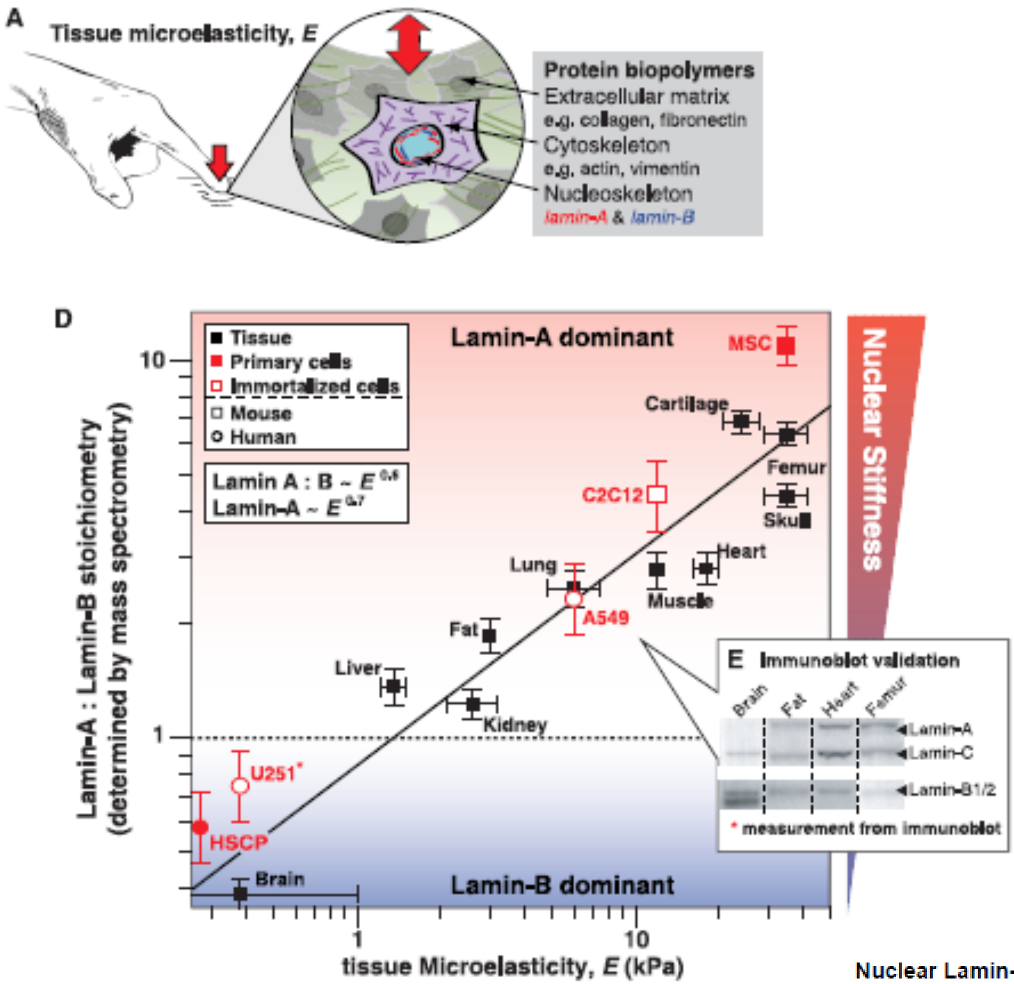
2 different regions



Cellular Mechanotransduction

Extracellular Matrix

Mechanical properties of cells and tissues



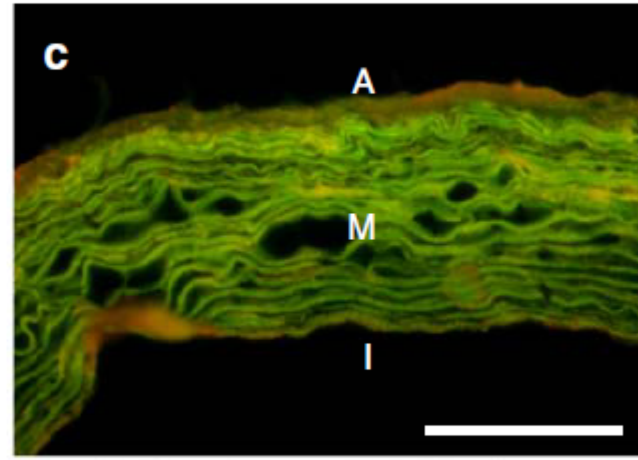
Nuclear Lamin-A Scales with Tissue Stiffness and Enhances Matrix-Directed Differentiation
Joe Swift *et al.*
Science **341**, (2013);

Cellular Mechanotransduction

Extracellular Matrix

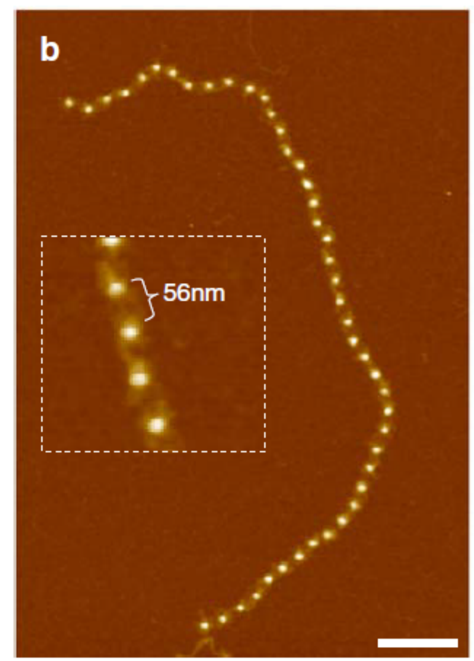
Elastic fibers

Elastin core (90%) which is surrounded by microfibrillar (10%) fibers



Isolated fibrillin fibers have beaded periodicity

Structure and function has not been resolved in detail



Cellular Mechanotransduction

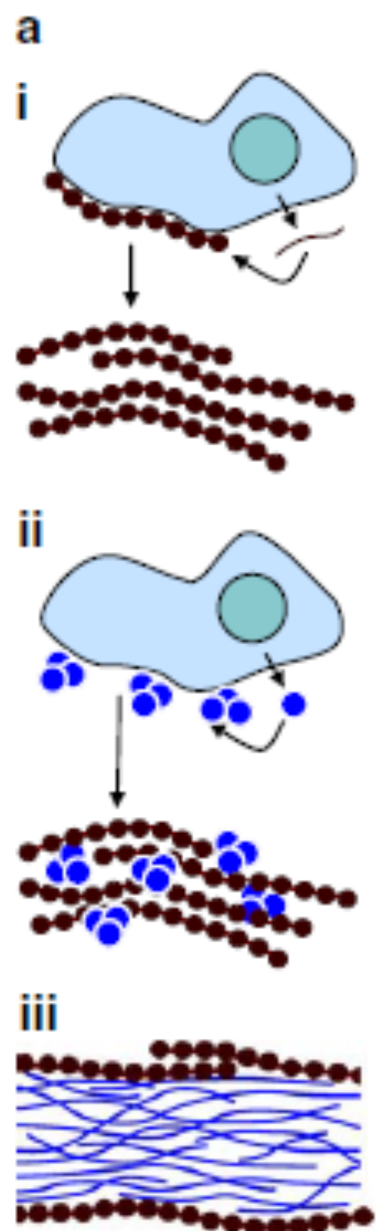
Extracellular Matrix

Elastic fibers assembly

i) Secreted profibrillin is processed and assembled into microfibrils

ii) Elastin globules which have assembled at the cell surface coalesce on the microfibril scaffold

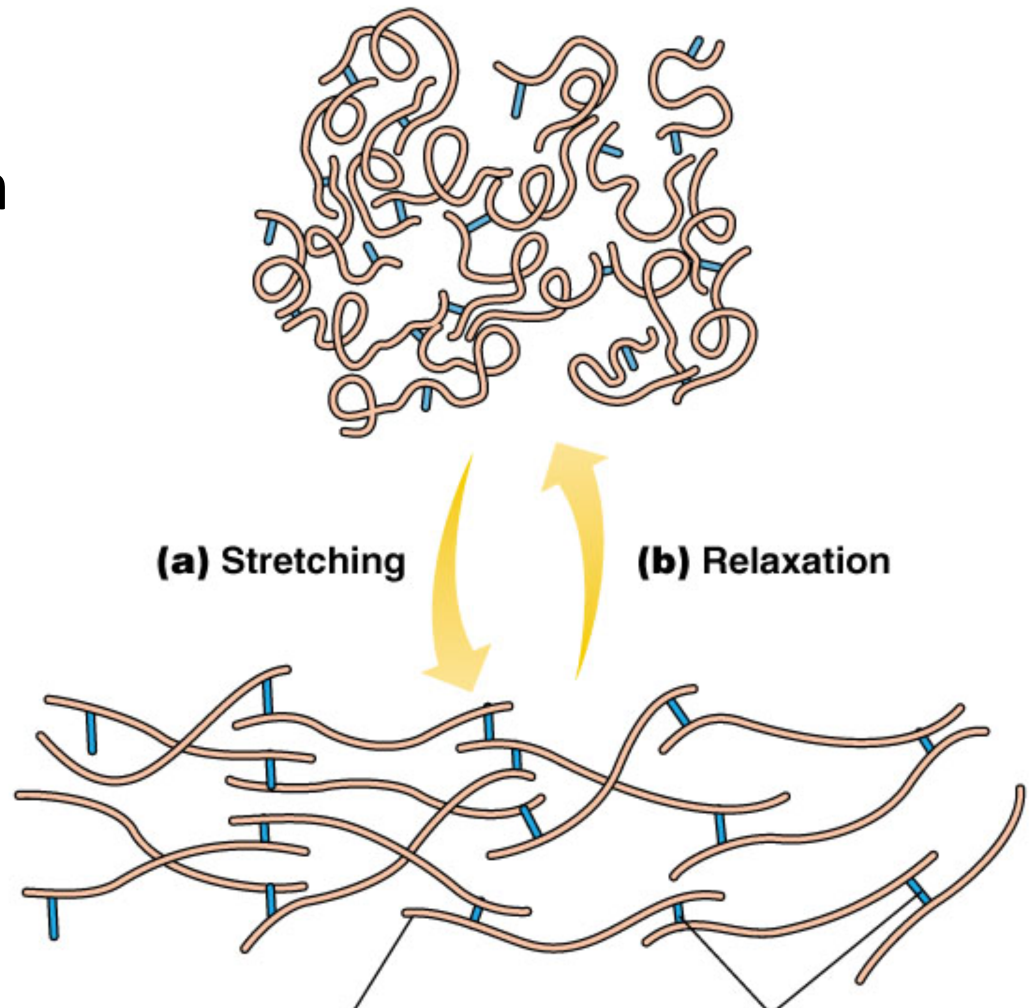
iii) Elasic fibers are formed from cross-linked elastin and fibrillin fibers



Cellular Mechanotransduction

Extracellular Matrix

- Elastin
  - Amorphous
  - Lower E than collagen



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Cellular Mechanotransduction

Extracellular Matrix

Elastin

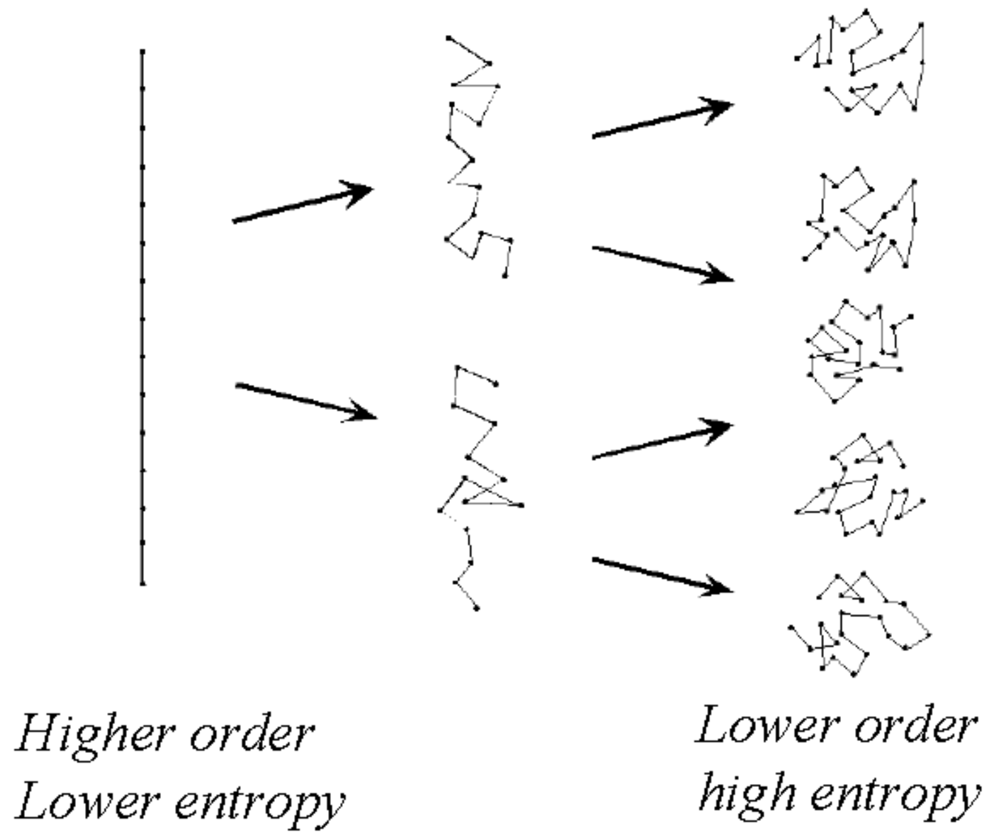
Behaves as an entopic spring

$$\Delta G = \Delta U - T\Delta S$$

ΔU can be approximated to 0

During stretching $\Delta S < 0$ → $\Delta G > 0$

→ non-spontaneous process



Cellular Mechanotransduction

Extracellular Matrix

Elastin

- Elastic fiber spring stores the energy of the deformation
  - → restoring force
    - → important in the skin, blood vessels etc.

Cellular Mechanotransduction

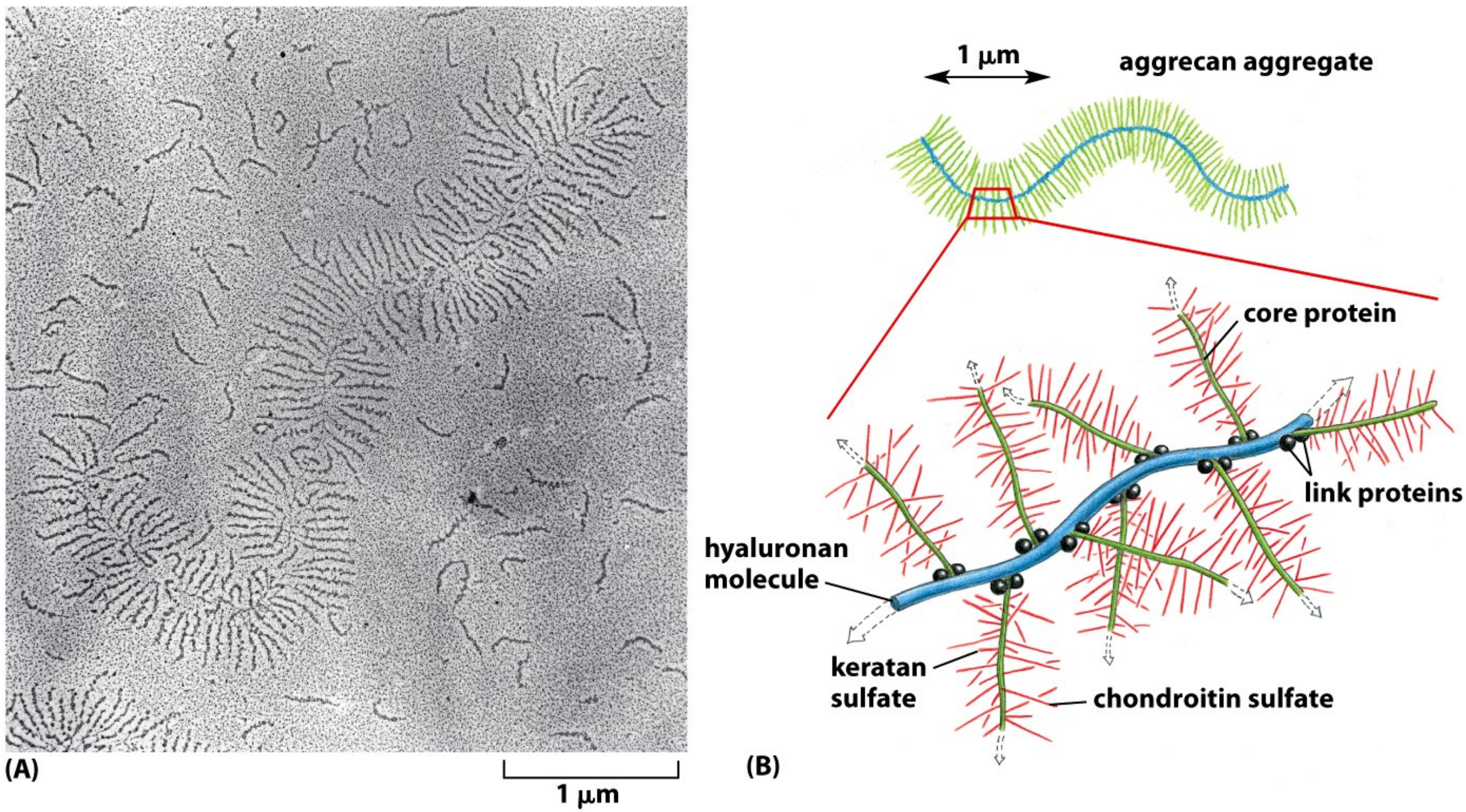
Extracellular Matrix

Proteoglycans

- Composed of proteins linked to negatively charged sugars
- Usually have a “core” where sugars are attached via proteins
- Huge molecules (MDa)
- Relative amount depends on the location
  - low: tendon, bone
  - high: interior of the eye

Cellular Mechanotransduction

Extracellular Matrix



Aggregan molecule from cartilage
Sugars are red and blue, proteins green and black

Cellular Mechanotransduction

Extracellular Matrix

Proteoglycans

Proteoglycans are highly negatively charged

- Form gels even at low concentrations

High charge leads into high osmotic activity

→ Swelling pressure

ECM of collagens + elastic fibers + proteoglycans

- Gel which is “tough” (proteoglycans), “tensed” (collagens) and “elastic” (elastic fibers)

Cellular Mechanotransduction

Cell Attachment to Extracellular Matrix

Cells can attach directly to the “structural” ECM proteins

- -Collagens
- -Elastic fibers

But often cell attachment is mediated by liker proteins which bind the ECM and cells

Two major proteins:

- - Fibronectin
- - Laminins

Cellular Mechanotransduction

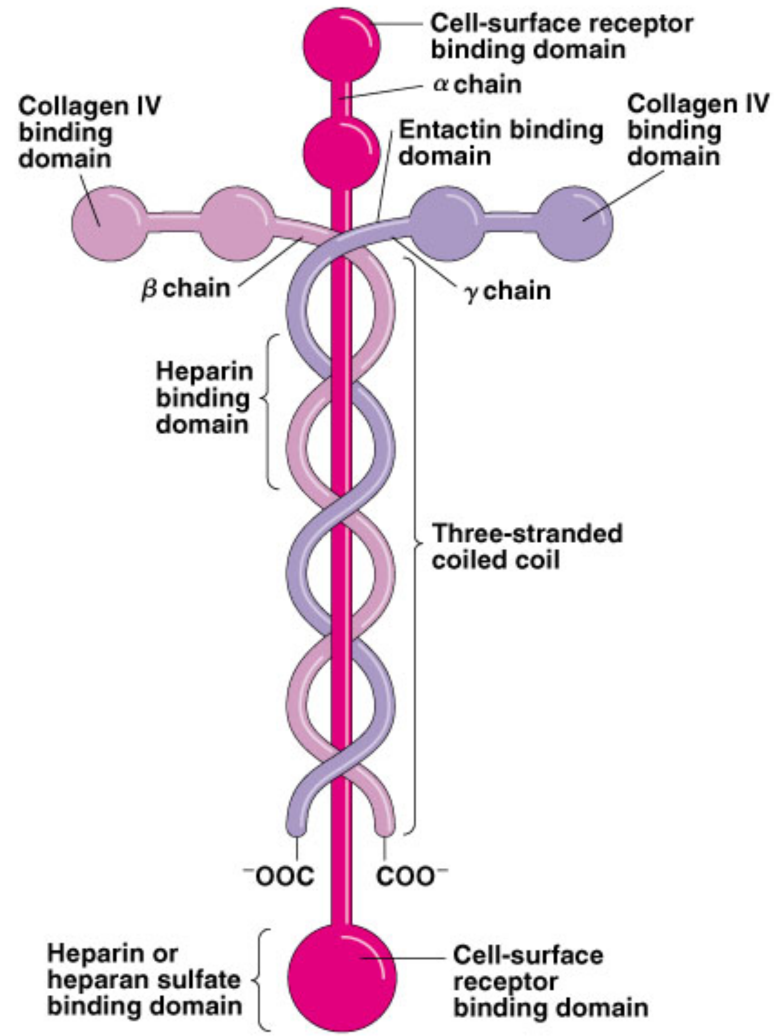
Cell Attachment to Extracellular Matrix

Laminin

-Found in basal lamina and basement membrane

- Binds heparin, Type IV collagen, entactin

- Offers binding sites for cells



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Cellular Mechanotransduction

Cell Attachment to Extracellular Matrix

Laminin &
Basal lamina

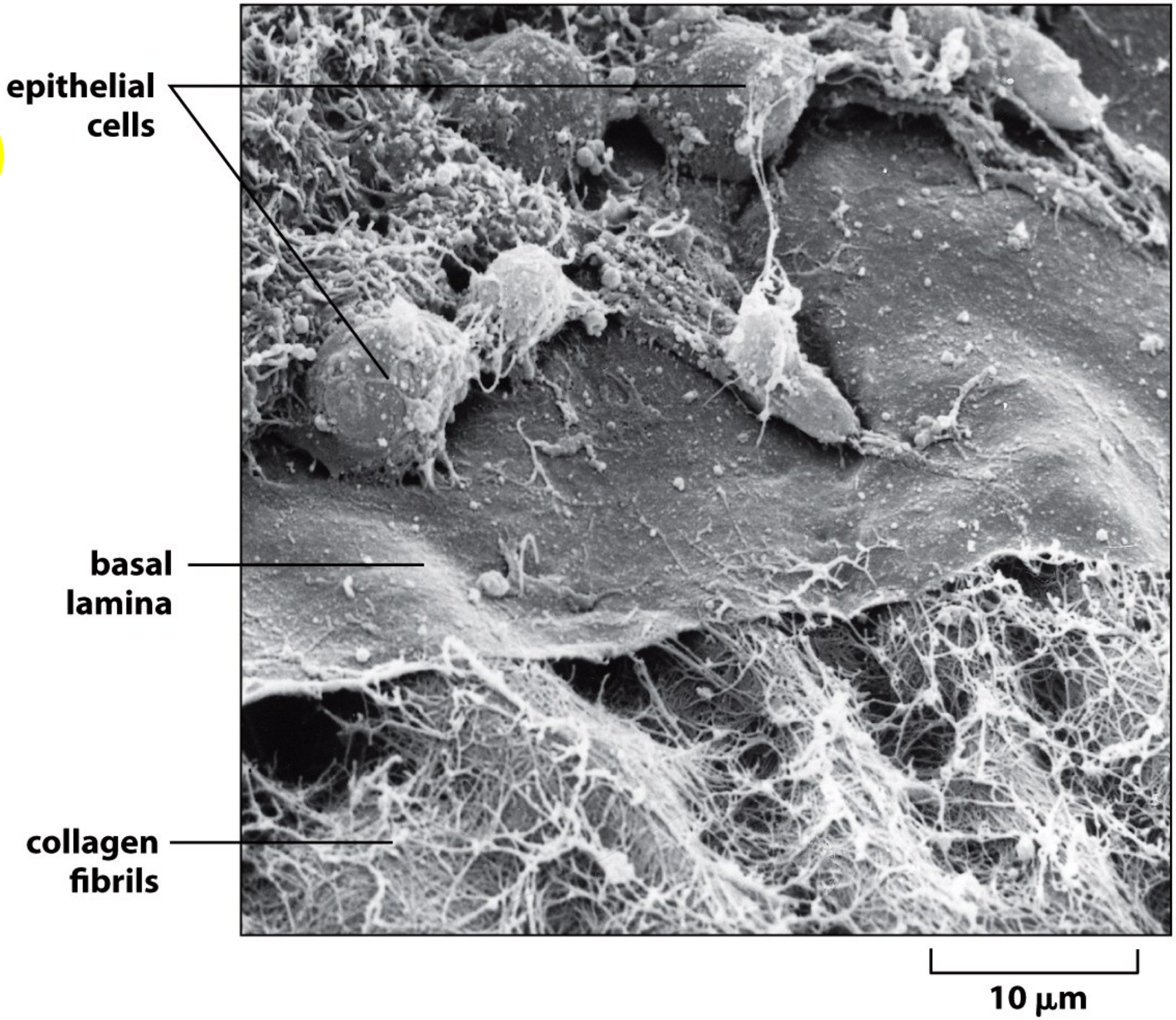

Figure 20-20 Essential Cell Biology 3/e (© Garland Science 2010)

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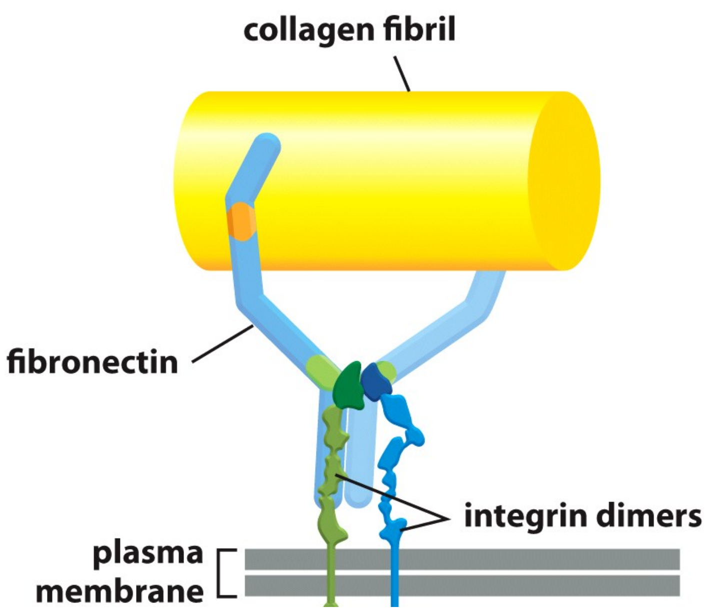
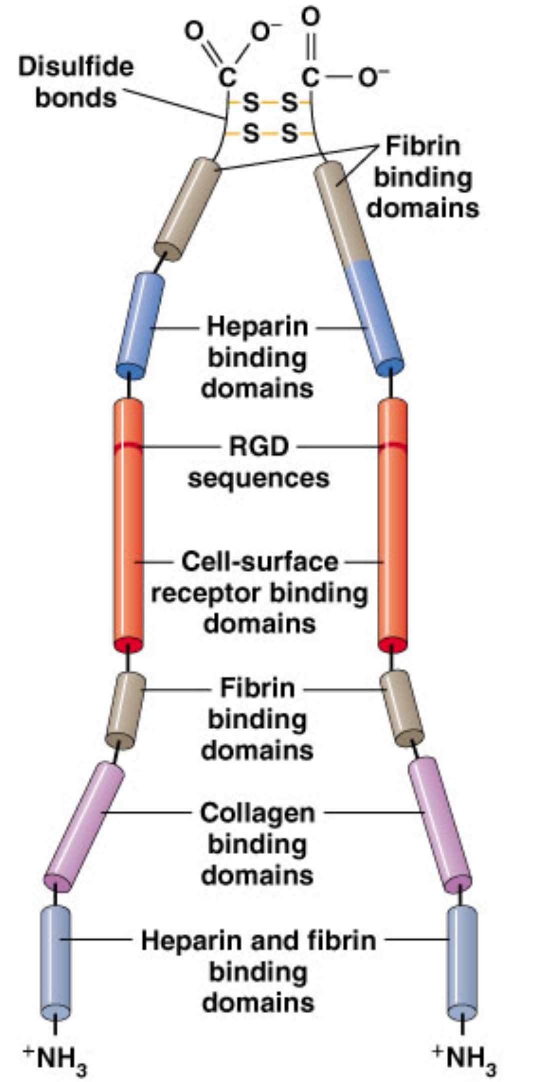
Cell Attachment to Extracellular Matrix

Fibronectin

- Dimeric protein, forms fibers
- Can bind collagen, fibrin (converted from fibrinogen by thrombin), proteoglycans
- Offers binding sites for cells





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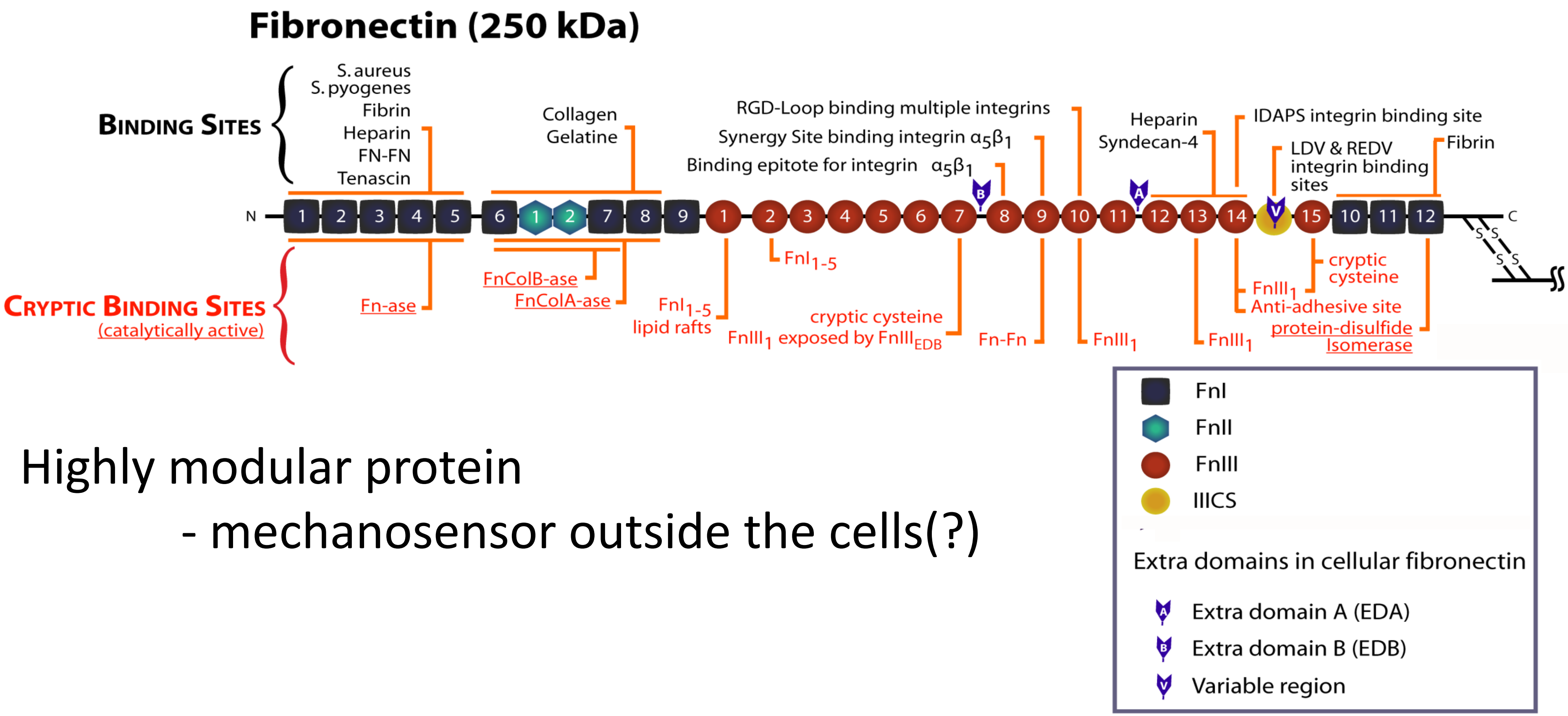
Cell Attachment to Extracellular Matrix



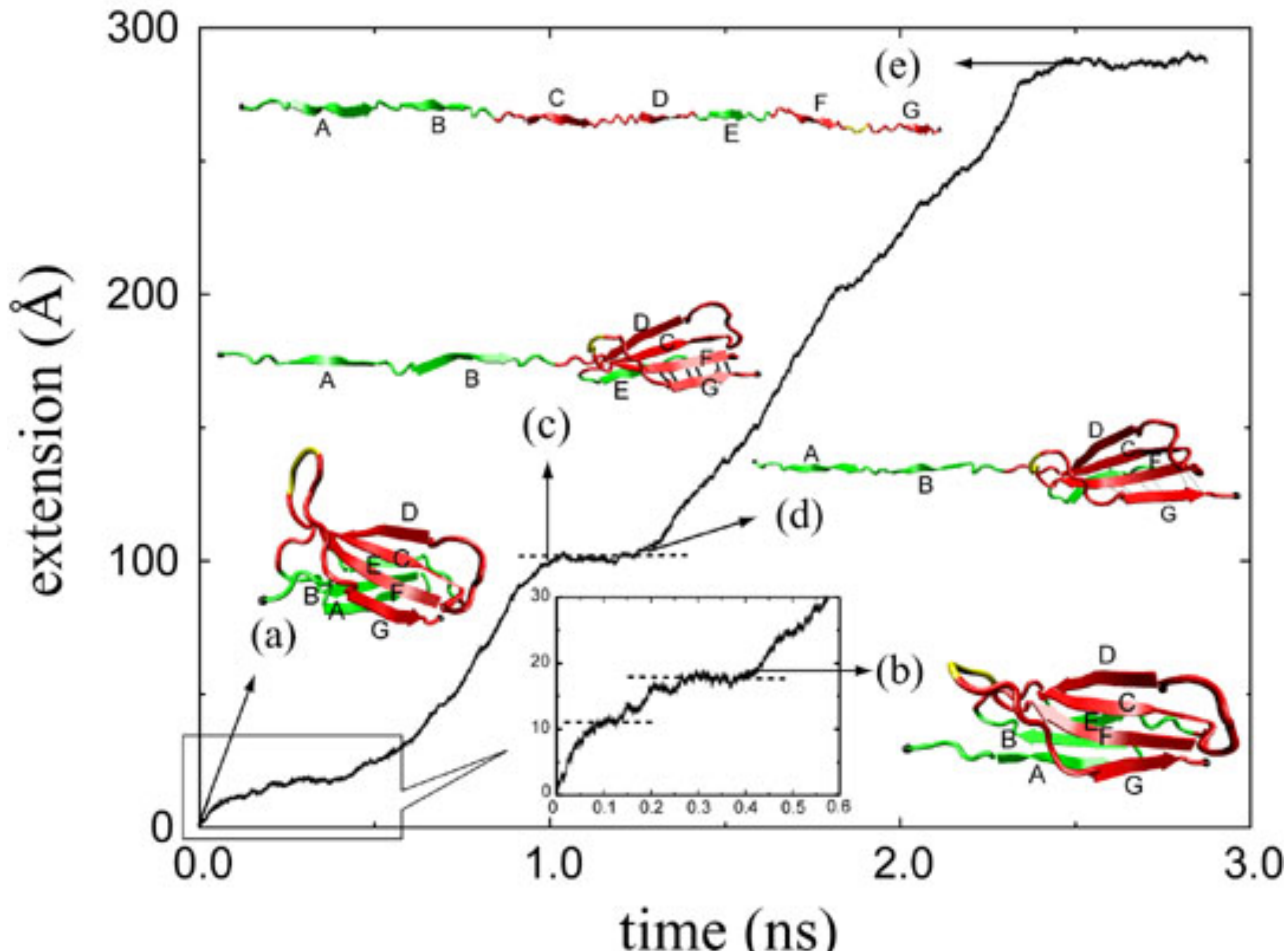
Highly modular protein

- mechanosensor outside the cells(?)

Cellular Mechanotransduction

Cell Attachment to Extracellular Matrix

Typical extension force profile from a constant force SMD stretching of FN-III-10 domain



http://www.ks.uiuc.edu/Research/fibronectin/index.old.html

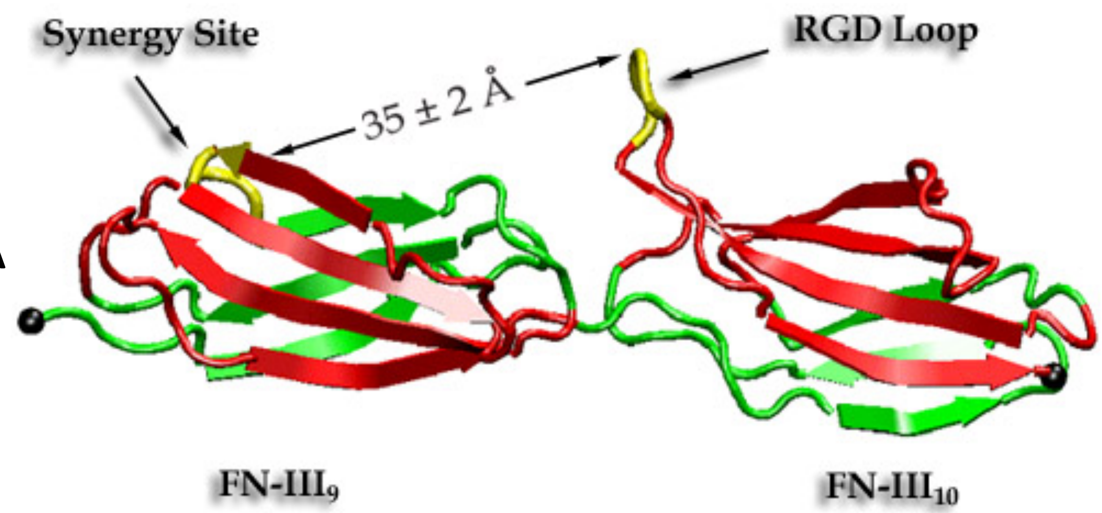
Cellular Mechanotransduction

Cell Attachment to Extracellular Matrix

FN-III-9 has synergy site which enhance the cell binding on RGD loop on FN-III-10

External force increases the synergy-RGD distance to 55 Å (conformations of both sites remained unperturbed)

Experimentally it has been found that longer linker chain between FN-III-9 and FN-III-10 reduces cell binding

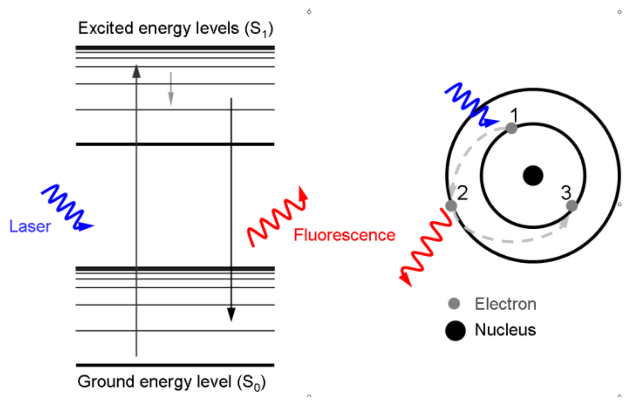


http://www.ks.uiuc.edu/Research/fibronectin/index.old.html

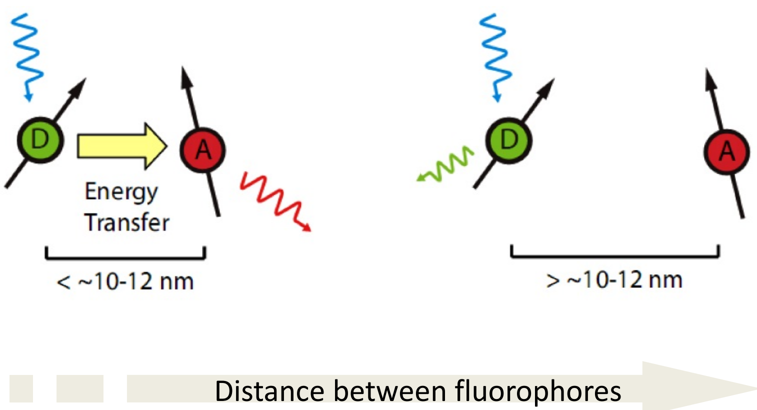
Cellular Mechanotransduction

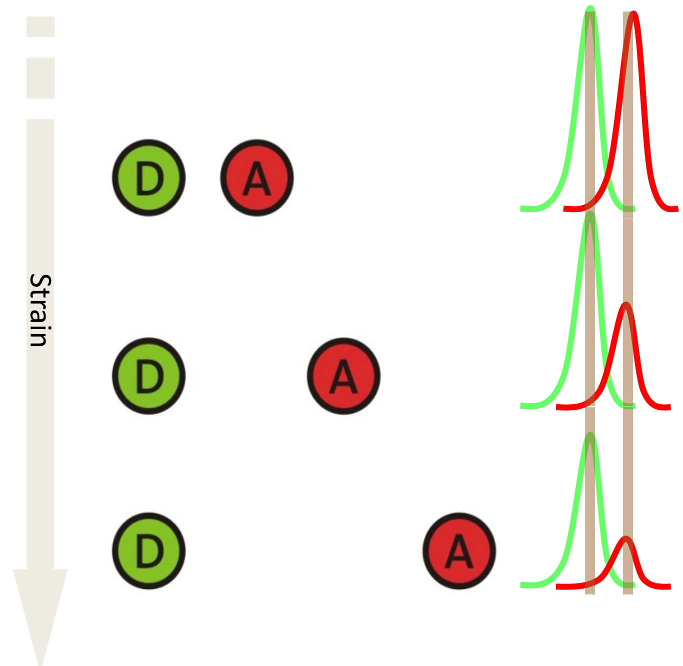
Cell Attachment to Extracellular Matrix

Fluorescence



Energy transfer



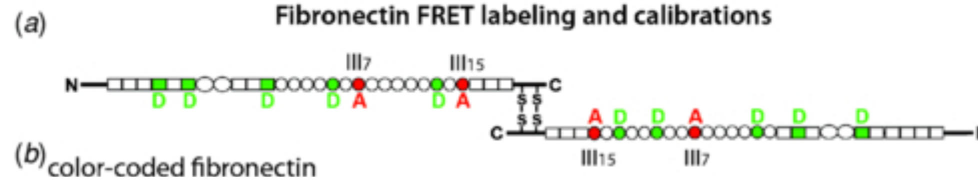


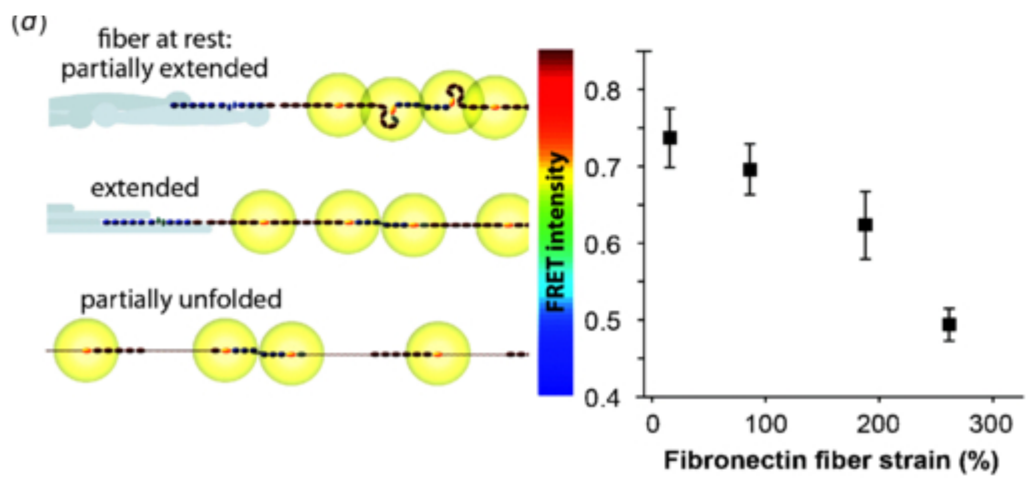
$$\text{Intensity Ratio} = \frac{I_{acceptor}}{I_{donor}}$$

Cellular Mechanotransduction

Cell Attachment to Extracellular Matrix

FRET labeling of Fibronectin and effect of stretching on the FRET ratio



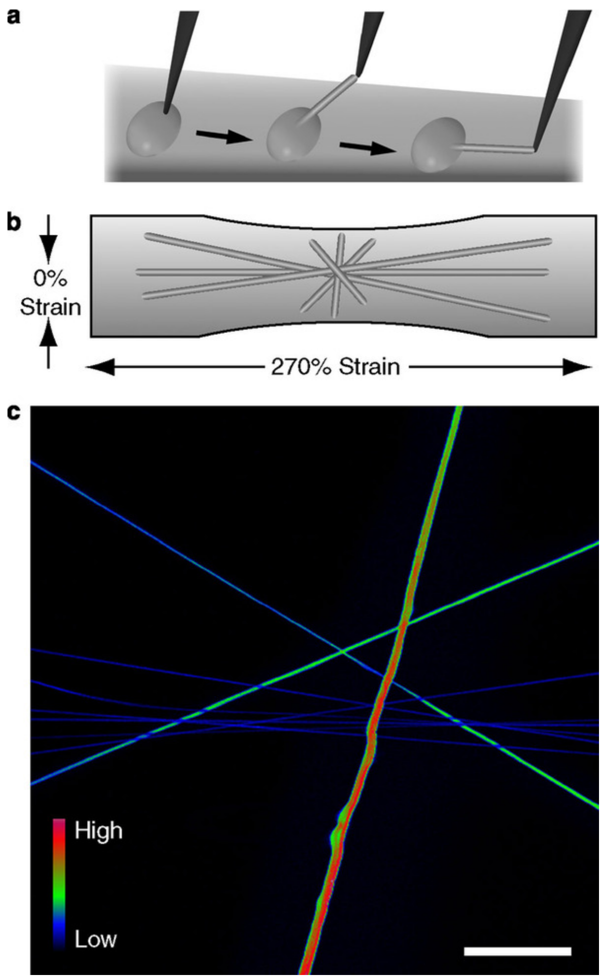


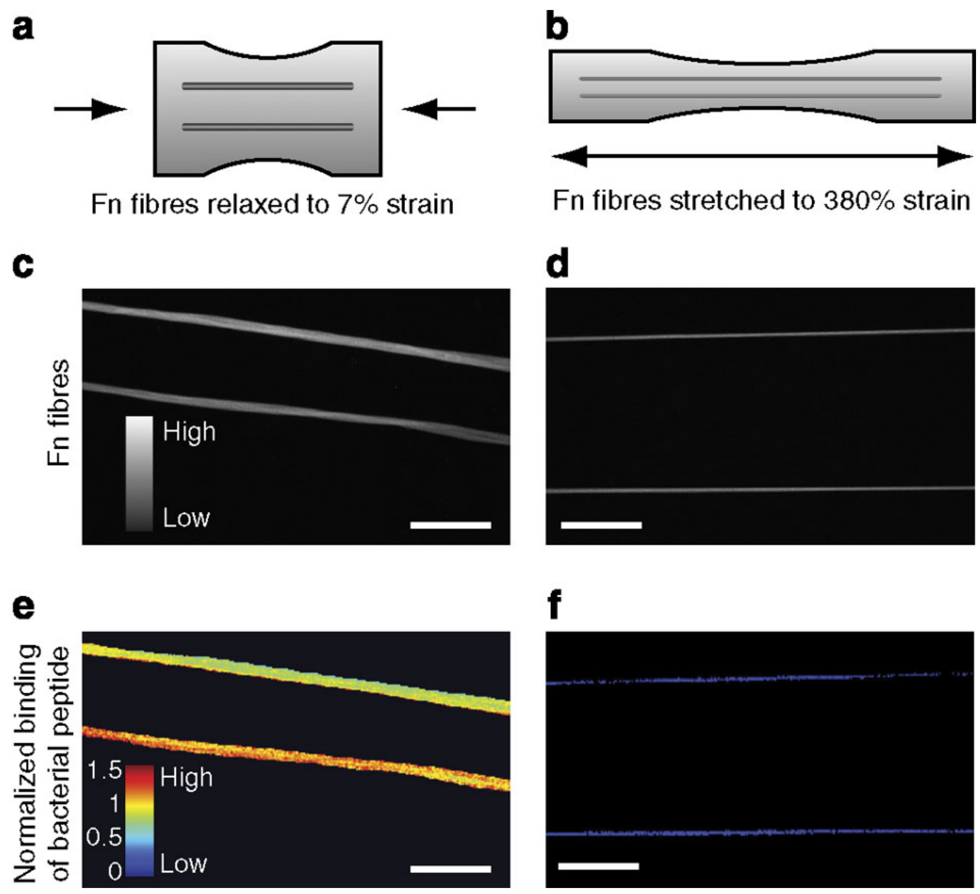
"Adipose progenitor cells increase fibronectin matrix strain and unfolding in breast tumors"

Cellular Mechanotransduction

Cell Attachment to Extracellular Matrix

Stretching of manually pulled fibronectin fibers





"Stretching fibronectin fibres disrupts binding of bacterial adhesins by physically destroying an epitope"

Cellular Mechanotransduction

Cell Attachment to Extracellular Matrix

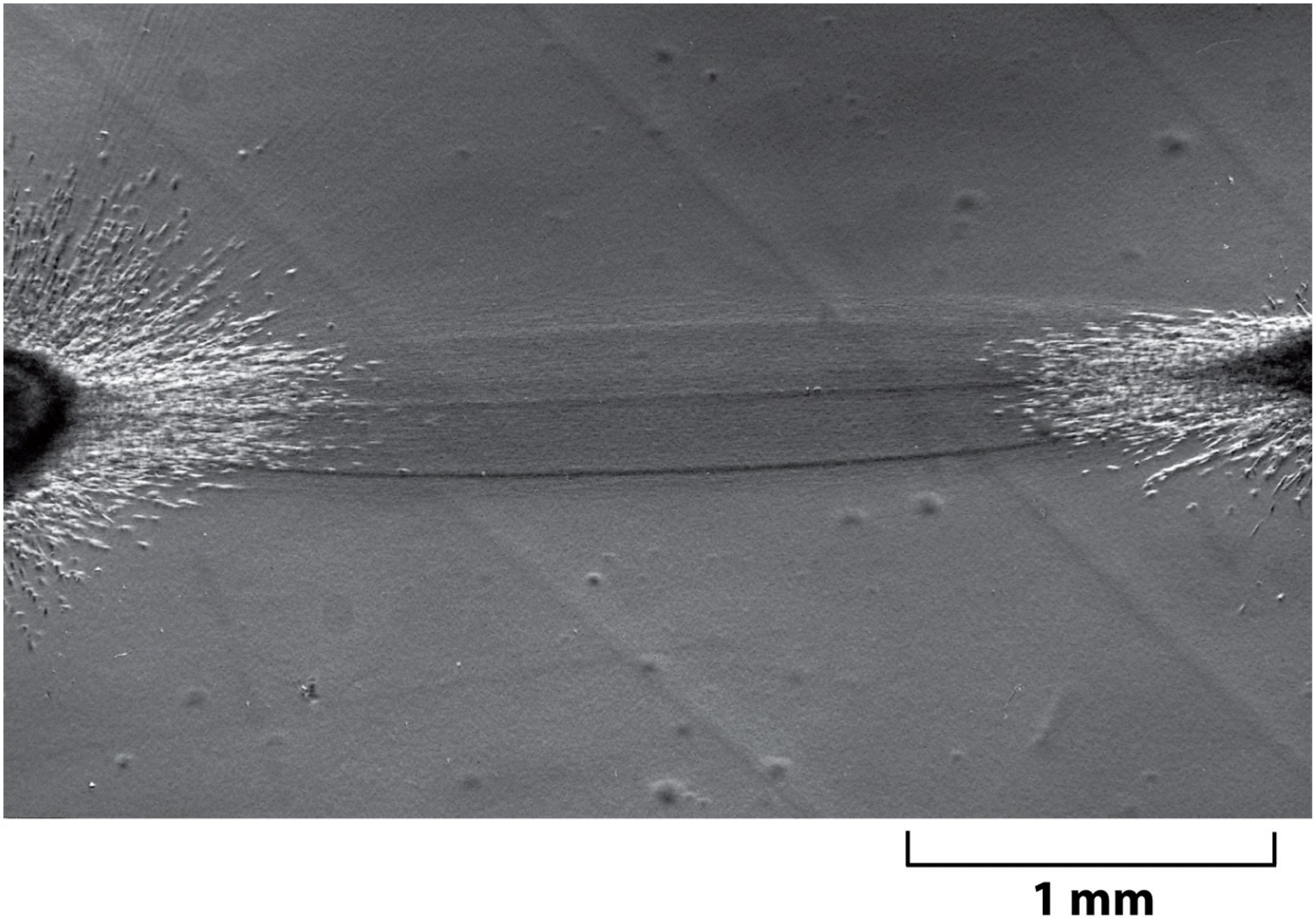
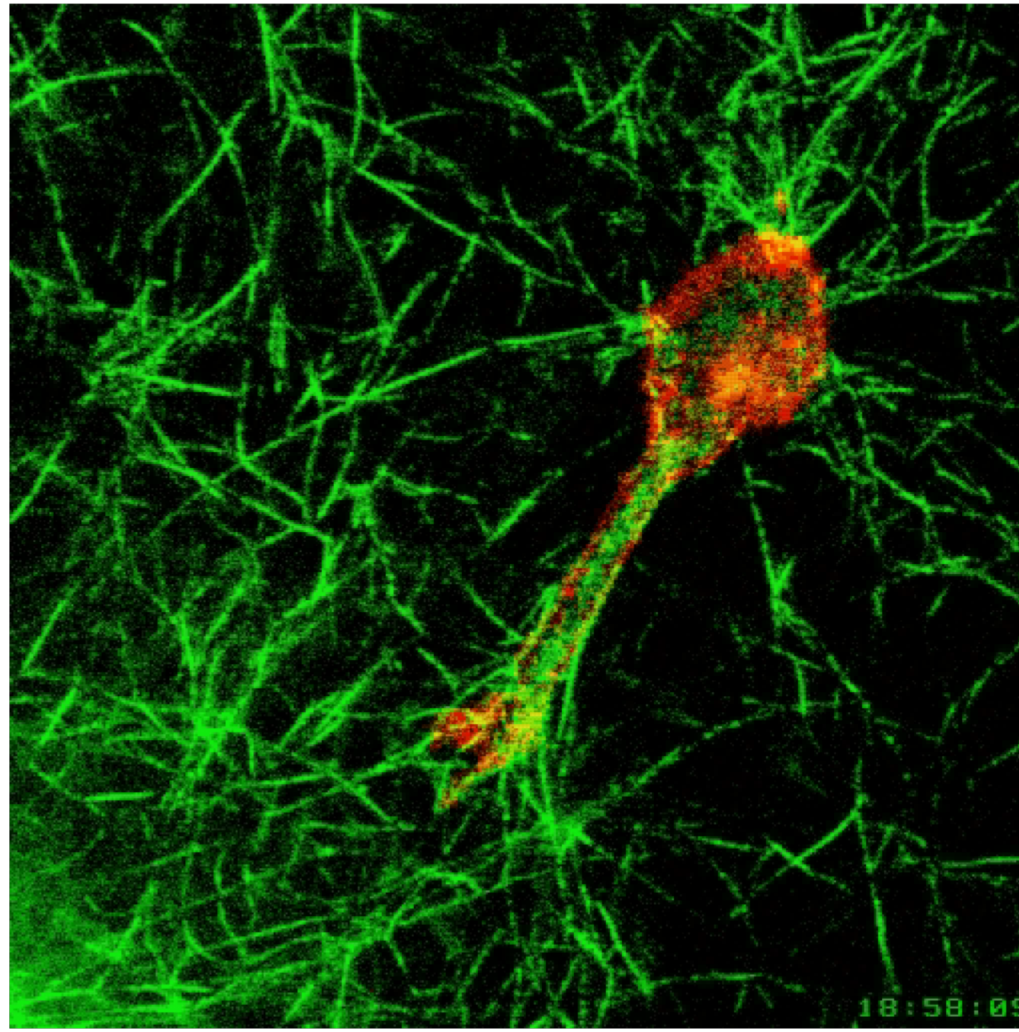


Figure 20-13 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Cell Attachment to Extracellular Matrix

Time-resolved confocal reflection and fluorescence imaging of MV3 melanoma cell migrating within 3D collagen lattice.



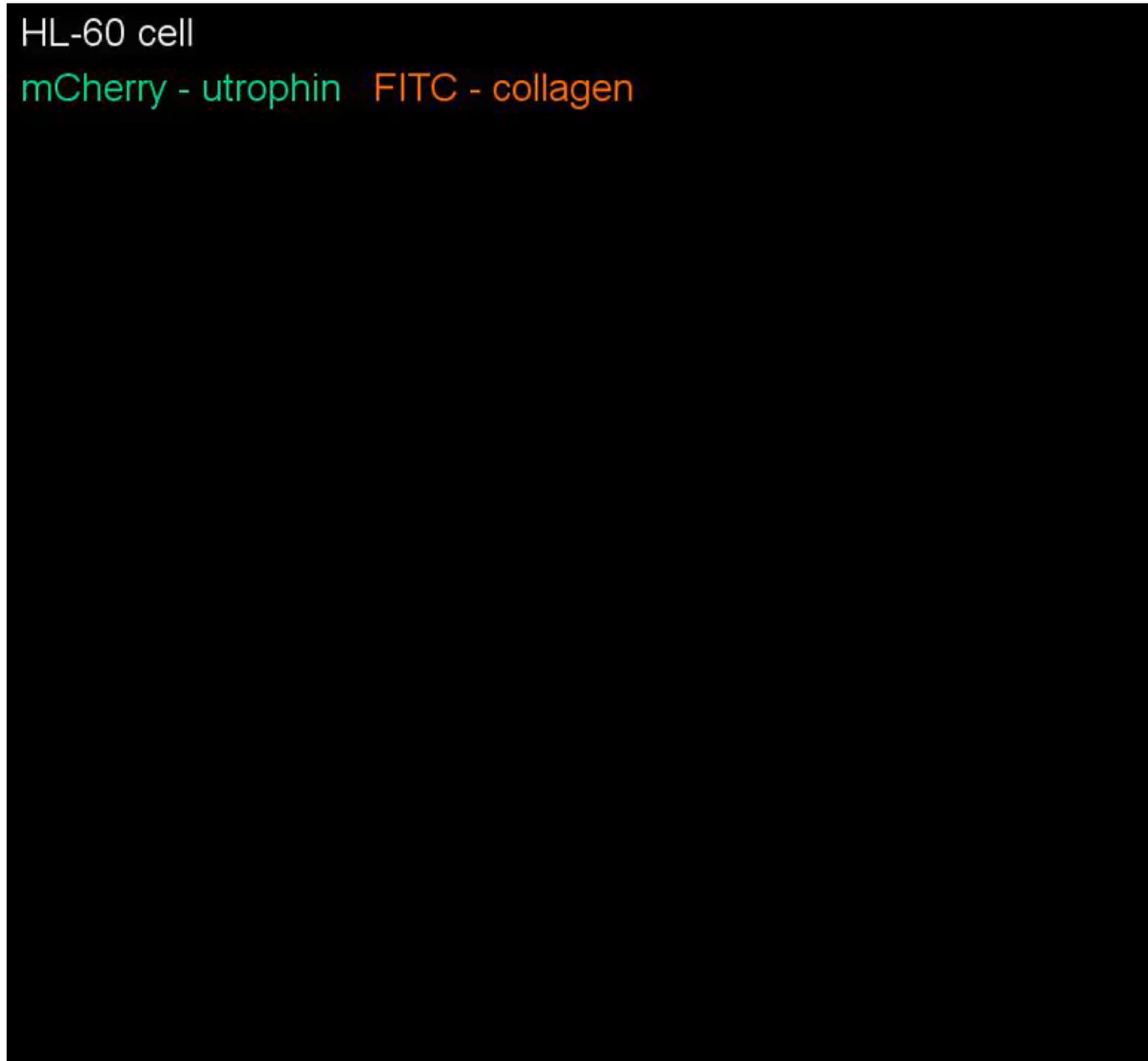
“Dynamic imaging of cellular interactions with extracellular matrix”

Cellular Mechanotransduction

- Cell Attachment to Extracellular Matrix

Two-color volume rendering of a neutrophilic HL-60 cell expressing mCherry-utrophin migrating through a 3D collagen matrix labeled with FITC over 250 time points at 1.3 sec intervals

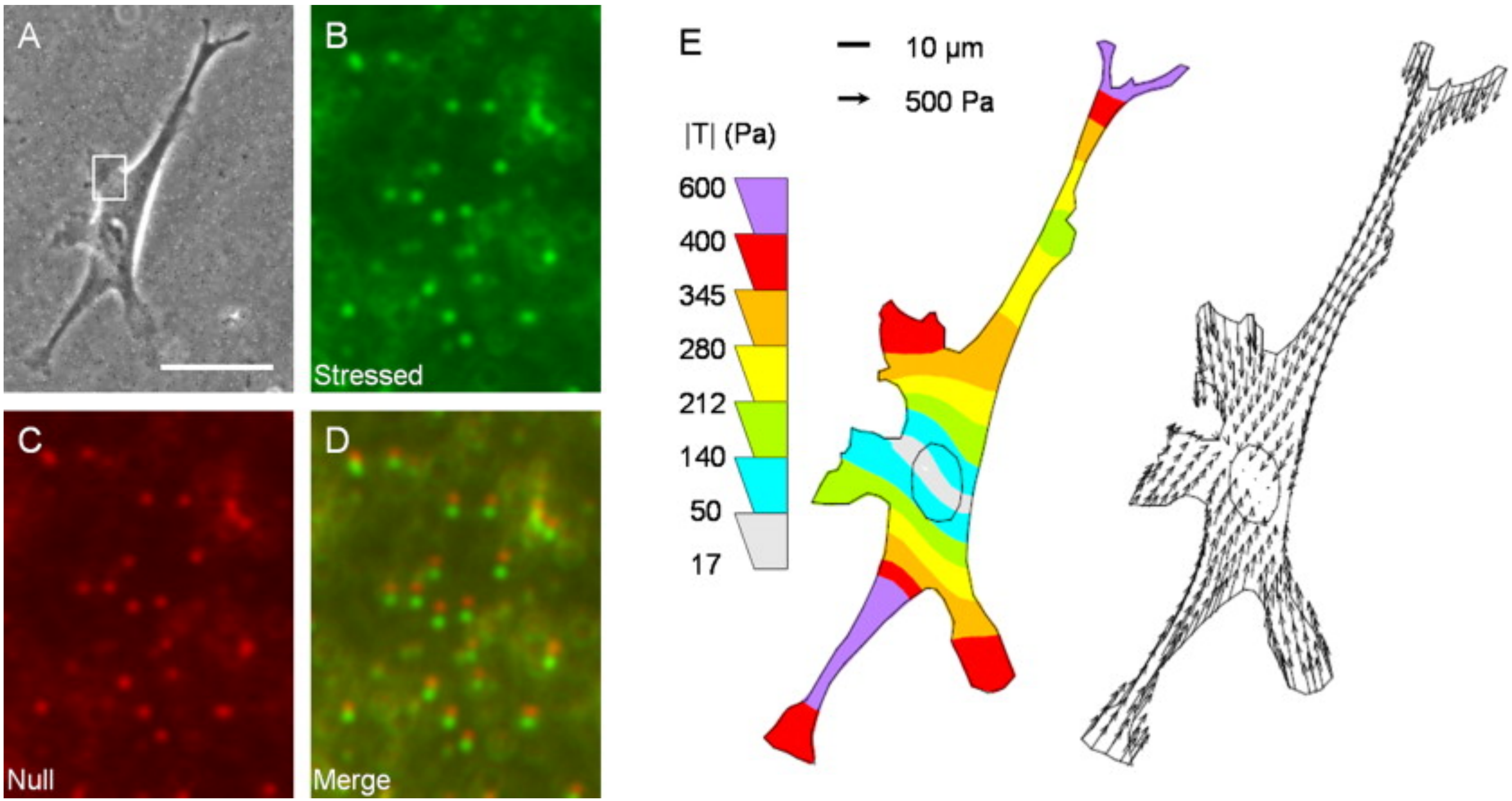
Betzig lab



Cellular Mechanotransduction

Cell Attachment to Extracellular Matrix

Traction force microscopy (TFM)
- method to study cell contractility

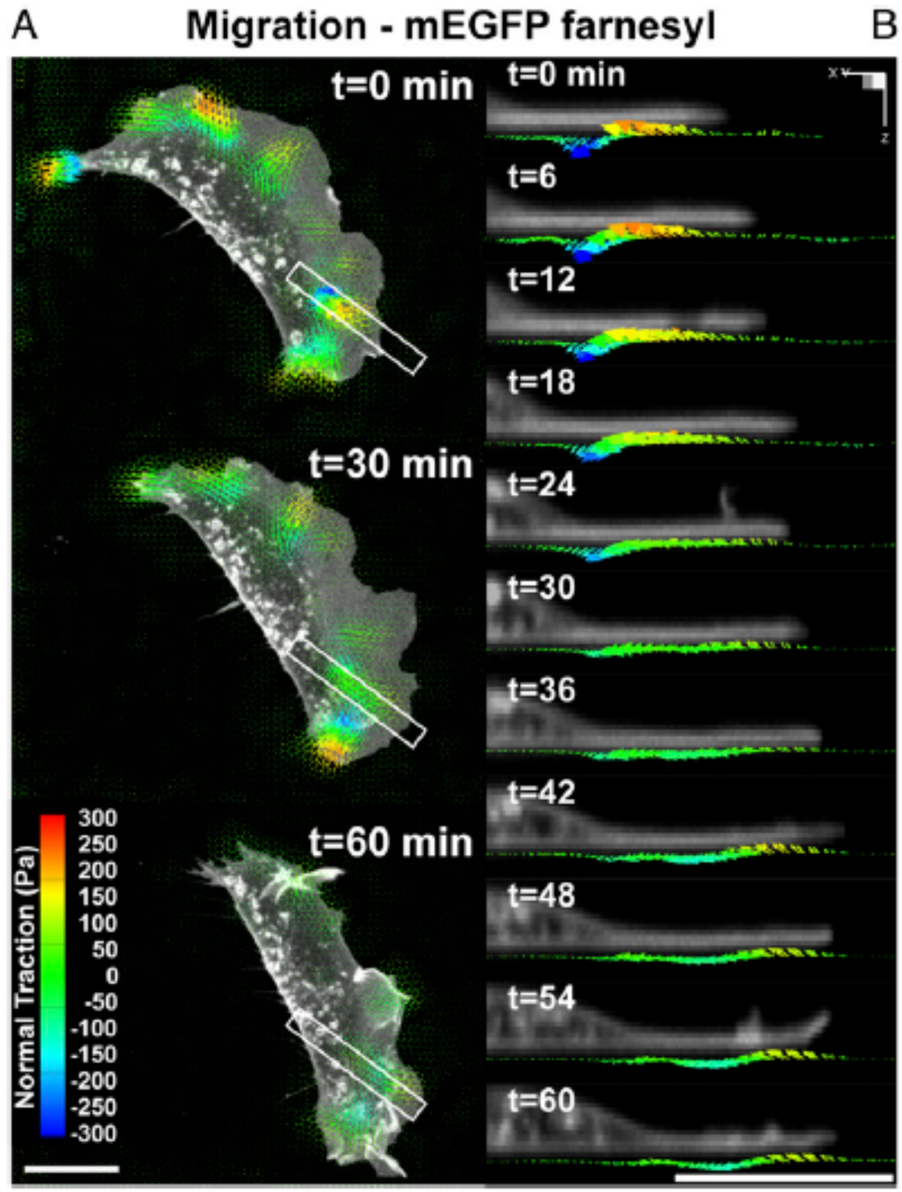


"Exogenous and endogenous force regulation of endothelial cell behavior"

Cellular Mechanotransduction

Cell Attachment to Extracellular Matrix

TFM can also be done in 3D



“Multidimensional traction force microscopy reveals out-of-plane rotational moments about focal adhesions”

Cellular Mechanotransduction

Summary

The ECM is composed from various proteins serving different functions:

- Collagen → tensile strength
- Proteoglycans → rigidity
- Elatic fibers → elasticity
- Fibronectin and Laminin → cell attachment

Cellular Mechanotransduction

Summary

Cells pull and push extracellular matrix

- Cell contractility and migration control the forces inside the ECM

- The tension in the ECM can lead into force induced unfolding or new structures in the proteins, e.g. in fibronectin

→ Release of growth factors
→ Exposure of new binding sites
→ Destruction of existing binding sites
→ Transduction of tension to the neighboring cells