Cellular Biophysics
Lecture 1: Mechanotransduction

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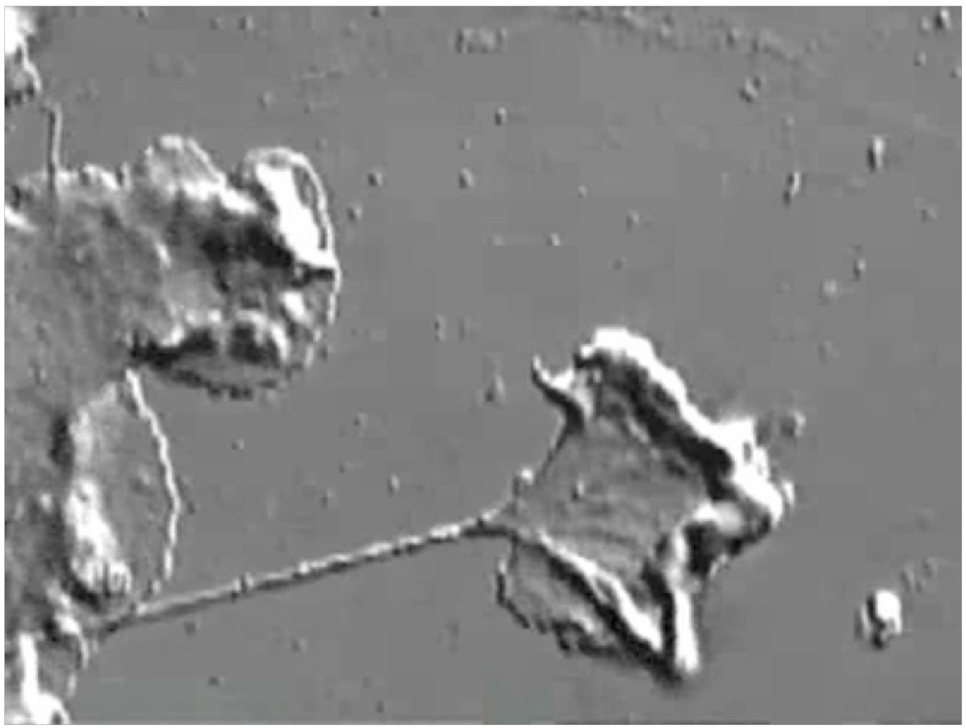
Cellular Mechanotransduction

Contents

Cellular Mechanotransduction

Force generation by tissues

Cells are able to convert chemical energy into mechanical force



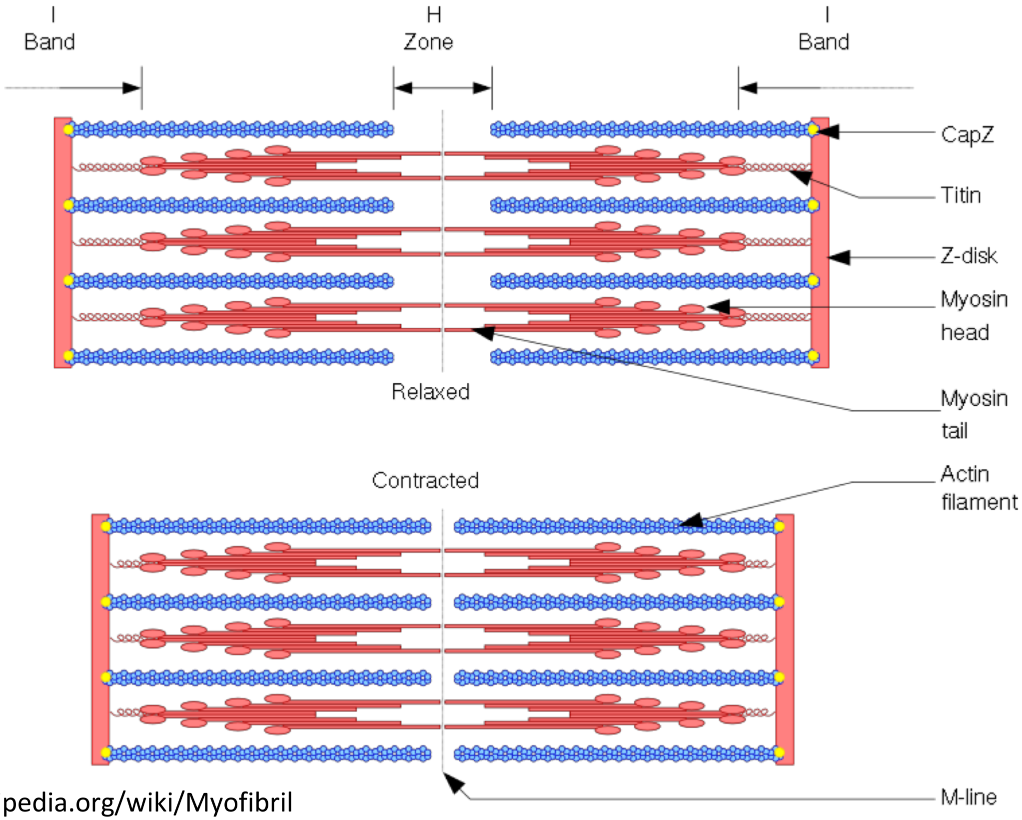
Cellular Mechanotransduction

Force generation by tissues

Cells are able to convert chemical energy into mechanical force

Example 2: muscle contraction, actin and myosin fibers

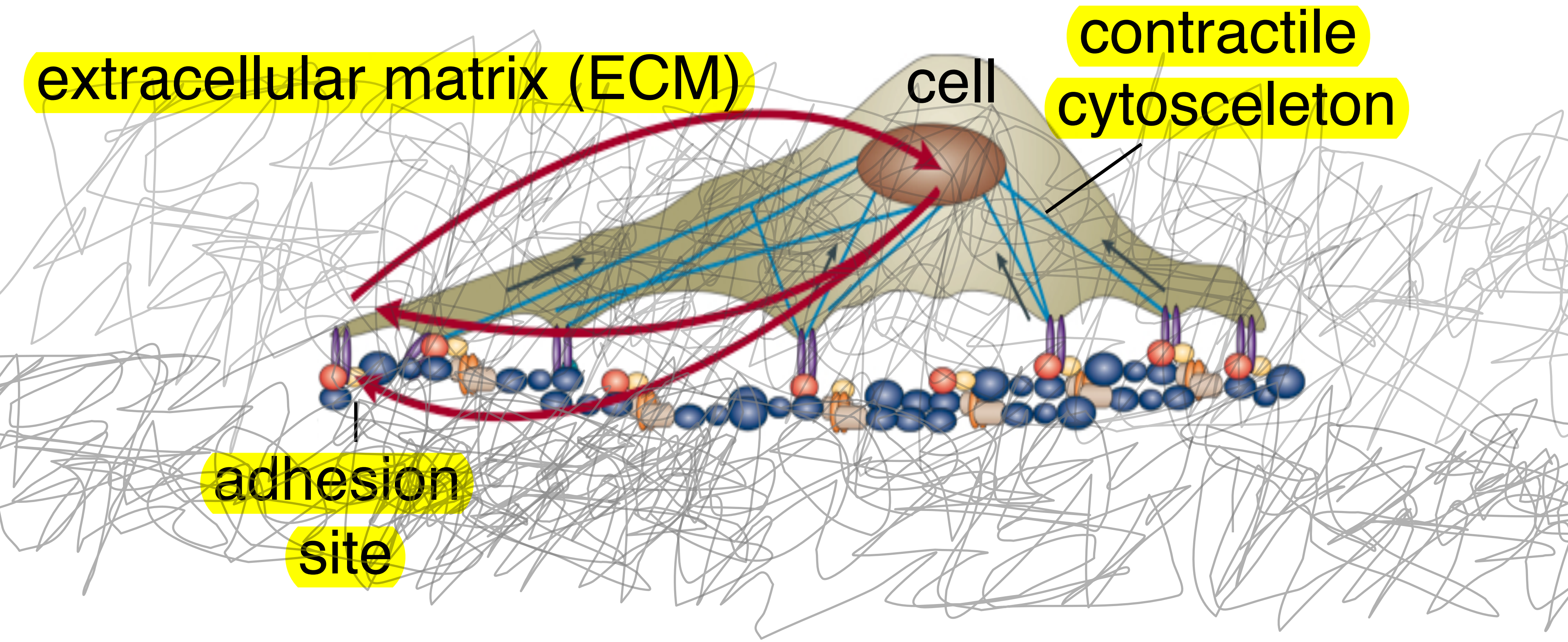
Sliding filament model of muscle contraction



http://en.wikipedia.org/wiki/Myofibril

Cellular Mechanotransduction

Mechanotransduction



Cells actively interact with the surrounding extracellular matrix

Cellular Mechanotransduction

Mechanotransduction

Nowadays it is well known that different cell types can sense mechanical force and properties of the surrounding tissue.

Conversion of mechanical stimuli into biochemical activity == Mechanotrasduction

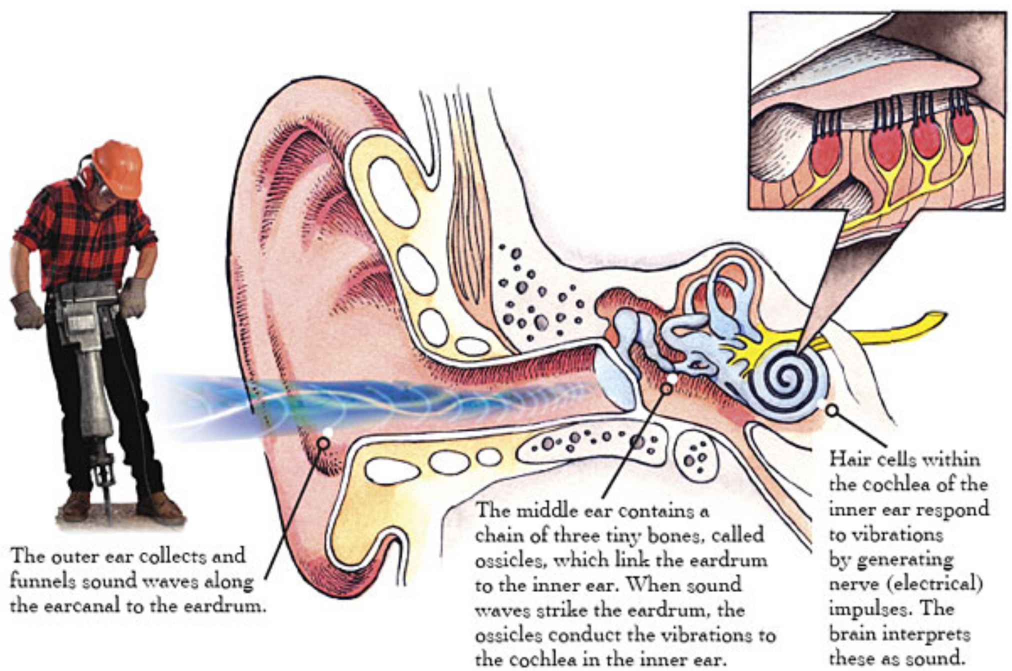
More general definition:
Mechanotrasnduction == process which allow cells to sense and alter the physical properties of the environment (rigidity, dimensionality, elasticity, etc.)

Cellular Mechanotransduction

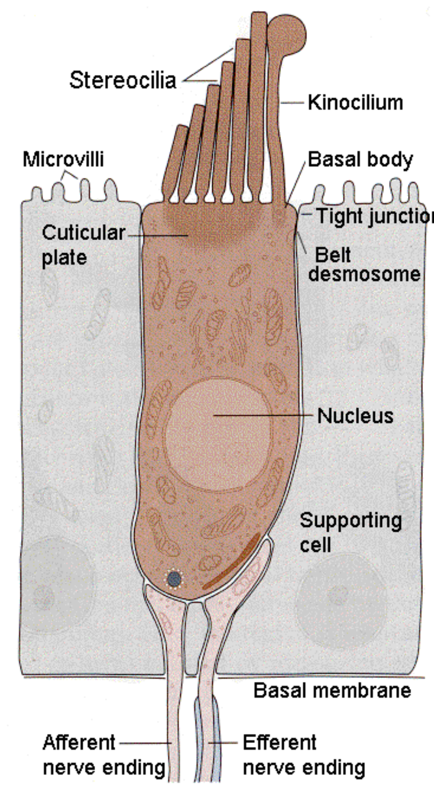
Mechanotransduction

Classical example of mechanotransduction: hearing

- Mechanical stimulus is transformed into electrical signal (voltage change) in the nerve cells



http://www.coopersafety.com/YourHearing.aspx



http://michaeldmann.net/pix_4b/hair_cell.gif

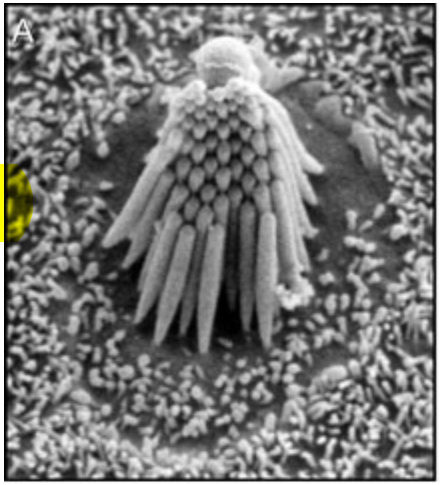
Cellular Mechanotransduction

Mechanotransduction

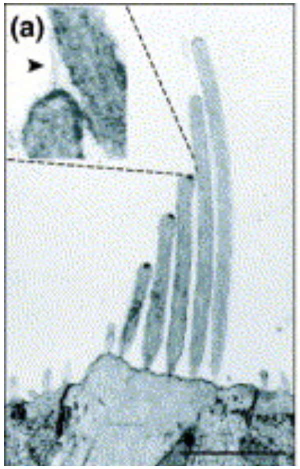
Mechanical stimuli bend the hair cells stereocilia

→ The bending opens mechanically gated transduction channels in the tips of stereocilia

→ Ion influx.



http://www.ks.uiuc.edu/Research/hearing/



Trends in Neuroscience, Vol 28, Issue 3, March 2005, Pages 140–144

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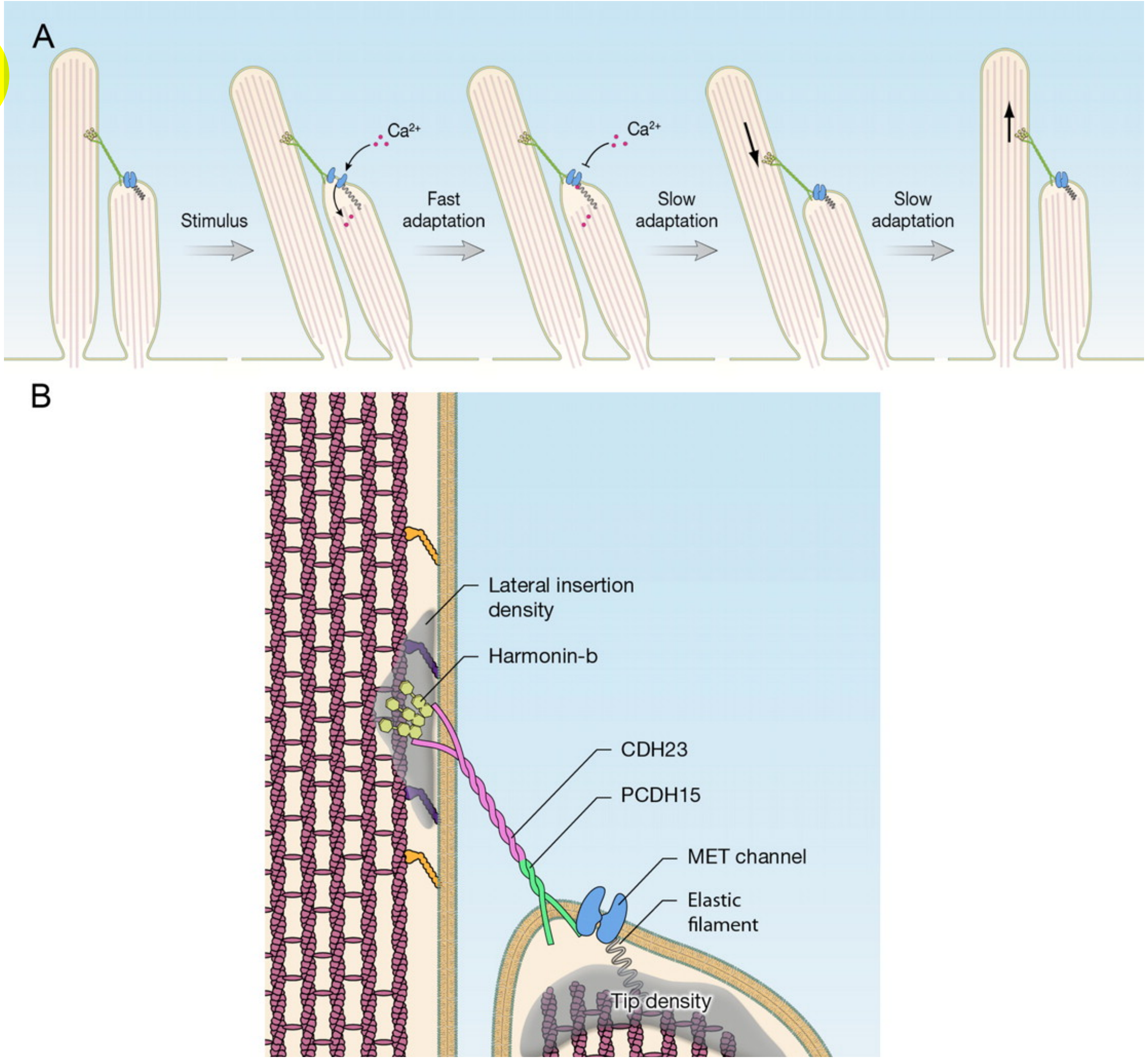
Mechanotransduction

Ion channel activation and adaptation



Molecular components of hearing

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

Cellular Mechanotransduction

Mechanotransduction

The mechanical properties and signals rising from the surrounding tissue can influence many processes:

- cell differentiation
- organogenesis
- tissue regeneration and homeostasis
- cancer and cancer metastasis

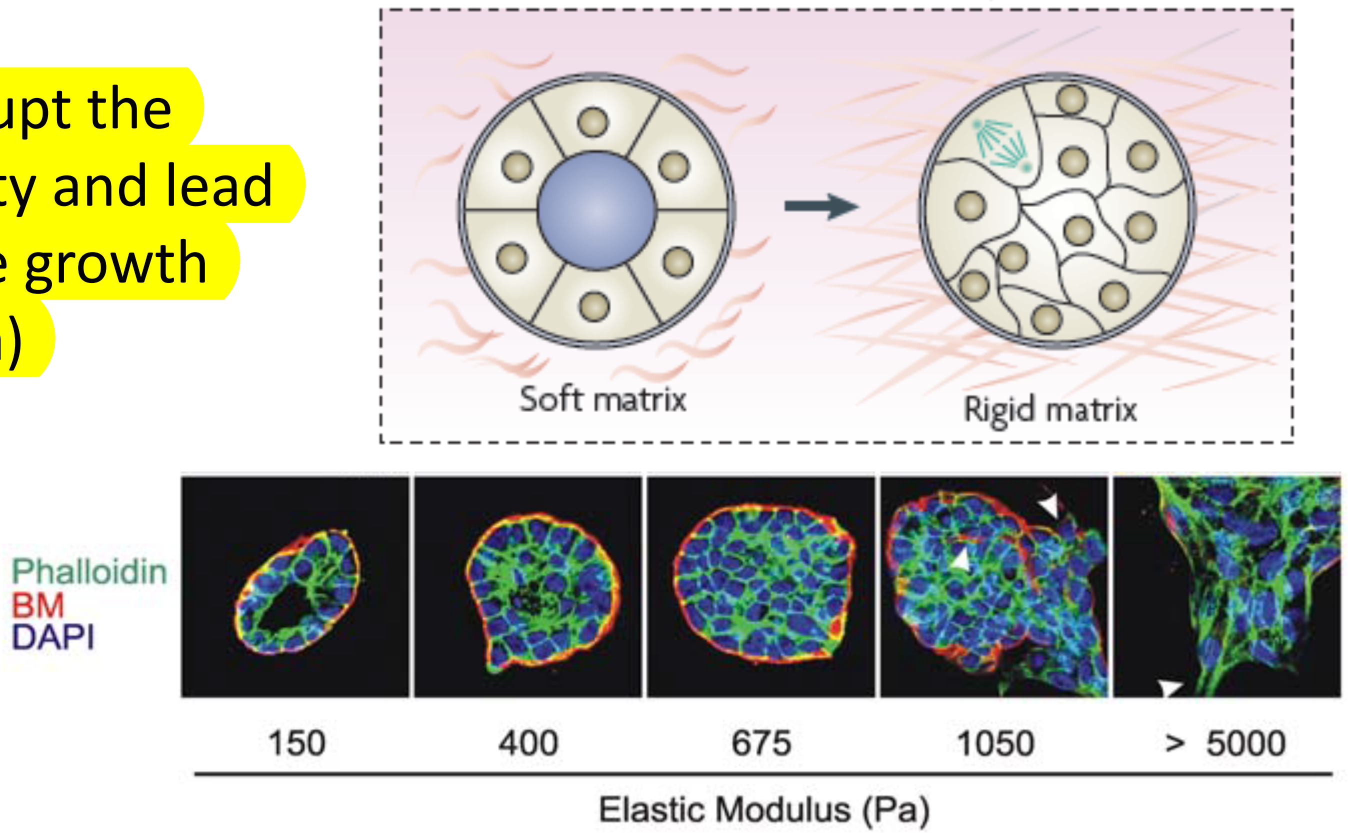
In many cases only **the phenomena** is well documented, but **the details** (molecular components and interactions) are not known

Cellular Mechanotransduction

Mechanotransduction

Example: Cancer
Tumors are usually stiffer than the surrounding tissue

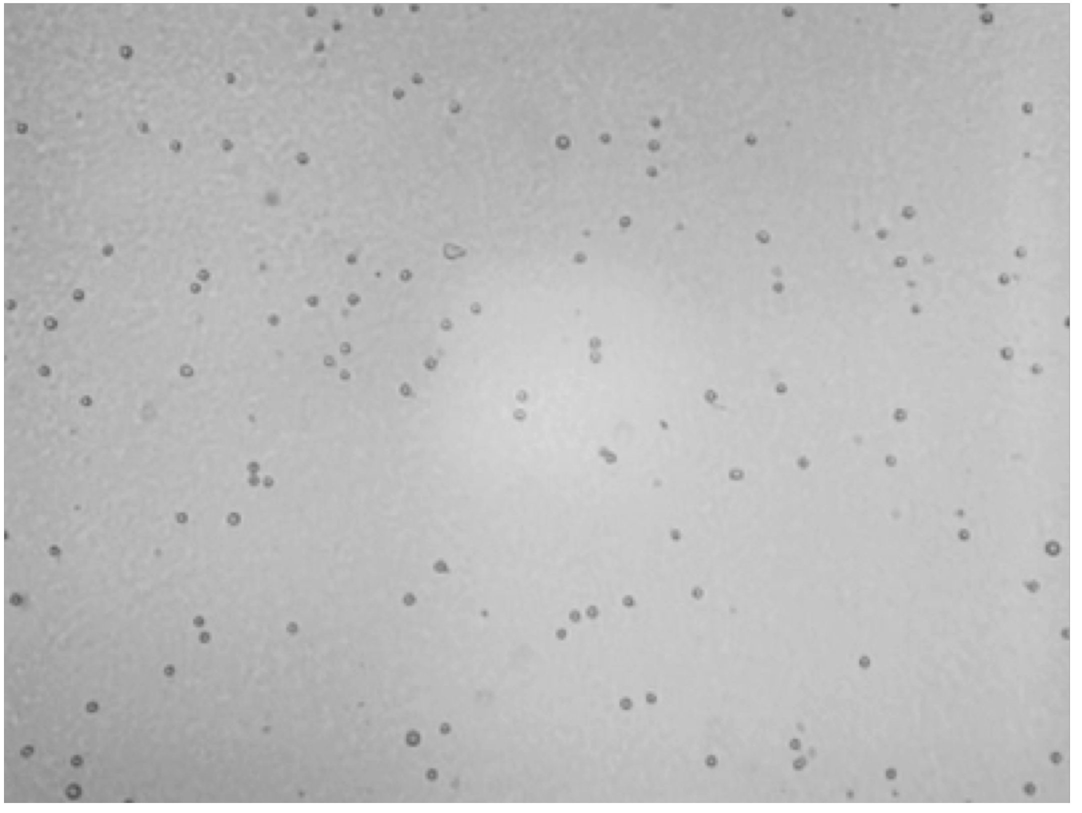
Stiffer ECM can disrupt the epithelial cell polarity and lead into small tumor like growth (mammary epithelia)



“Tensional homeostasis and the malignant phenotype”
CANCER CELL : SEPTEMBER 2005 · VOL. 8

Cellular Mechanotransduction

Mechanotransduction



Cellular Mechanotransduction

Mechanotransduction

1. Cells can generate forces and sense them

2. ”Physical properties” of the extracellular environment influence cell behavior

Cellular Mechanotransduction

Proteins

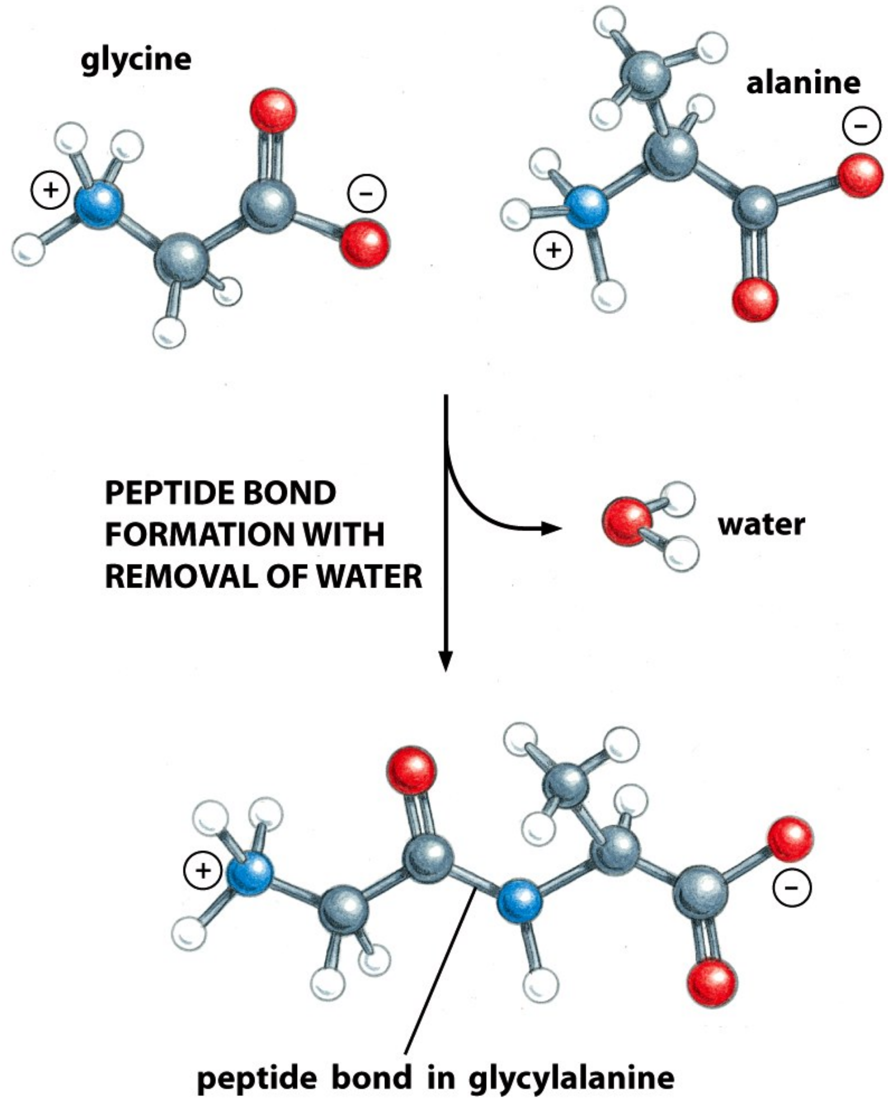


Figure 4-1 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

Proteins

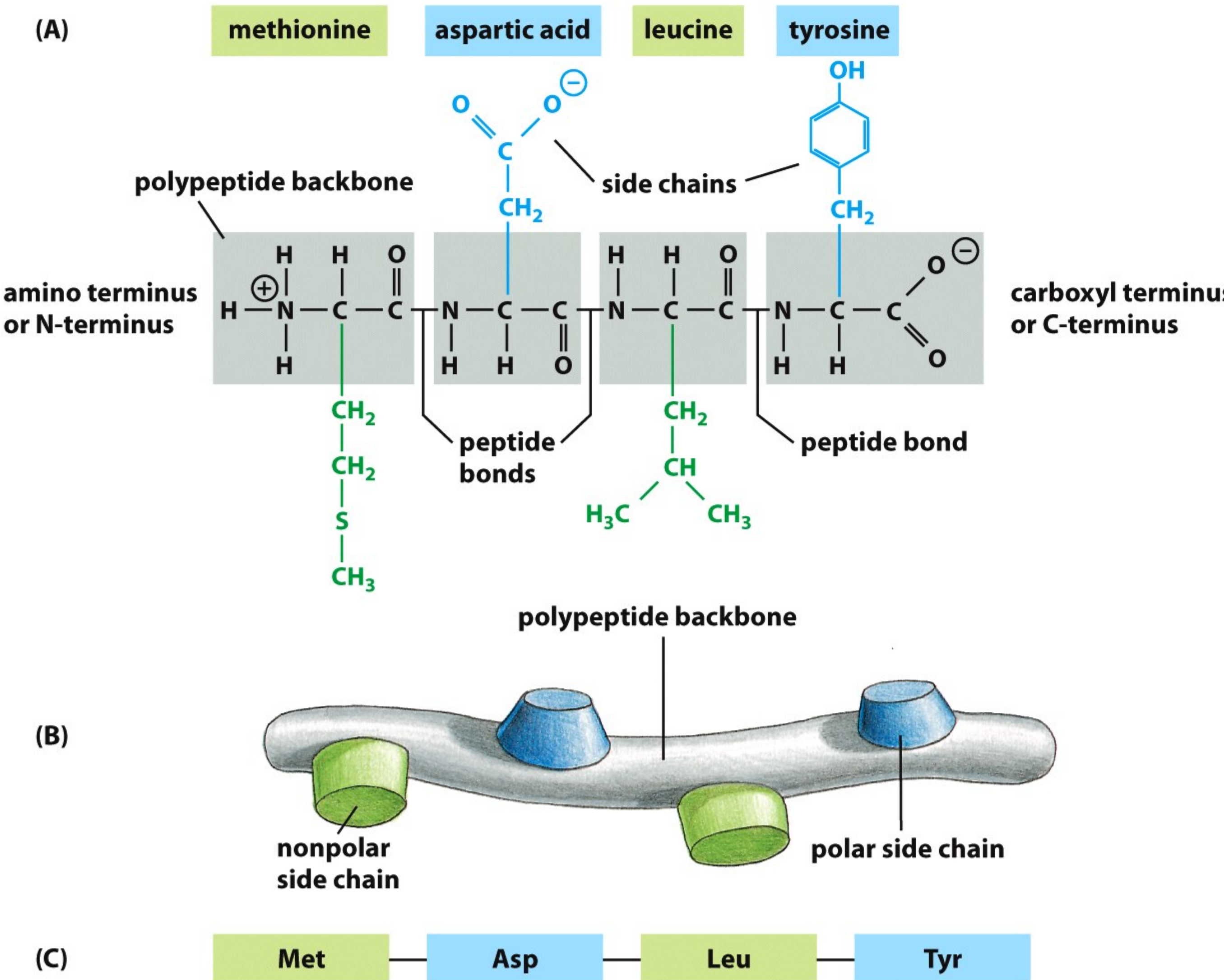
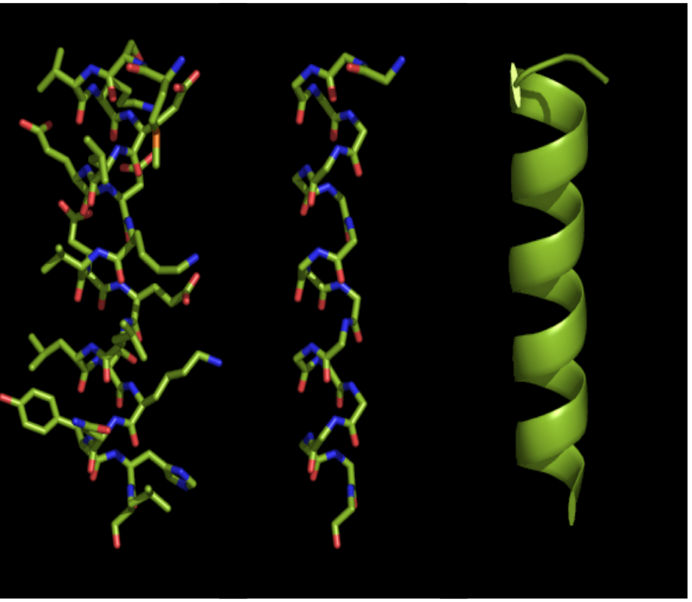


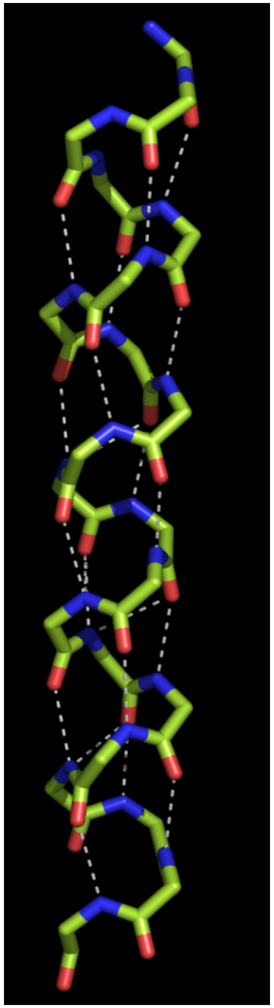
Figure 4-2 Essential Cell Biology 3/e (© Garland Science 2010)

Cellular Mechanotransduction

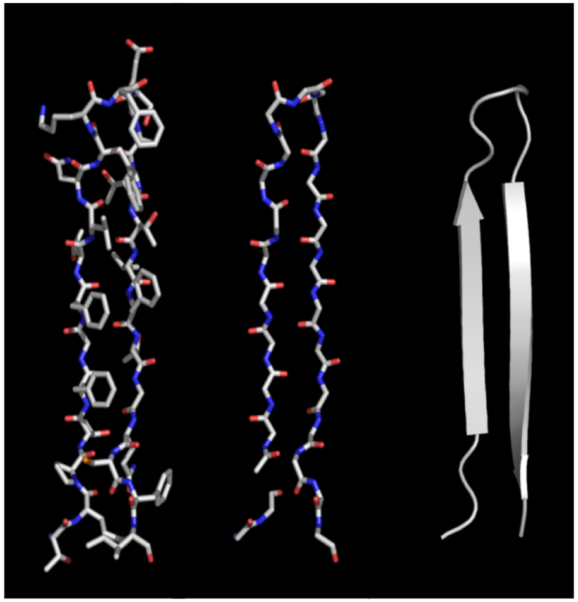
Protein secondary structures

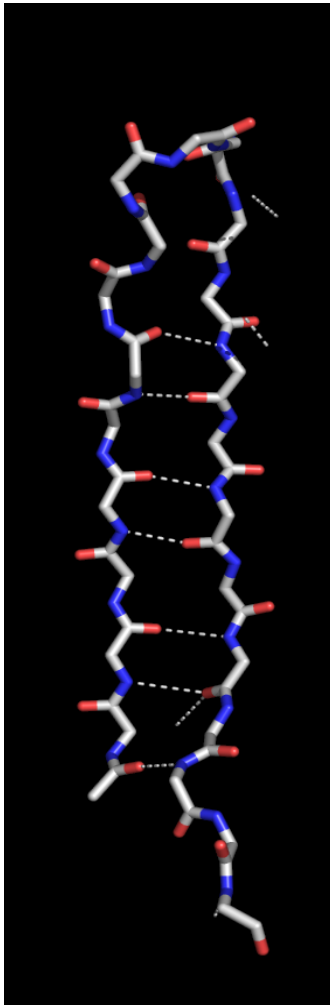
α-helix





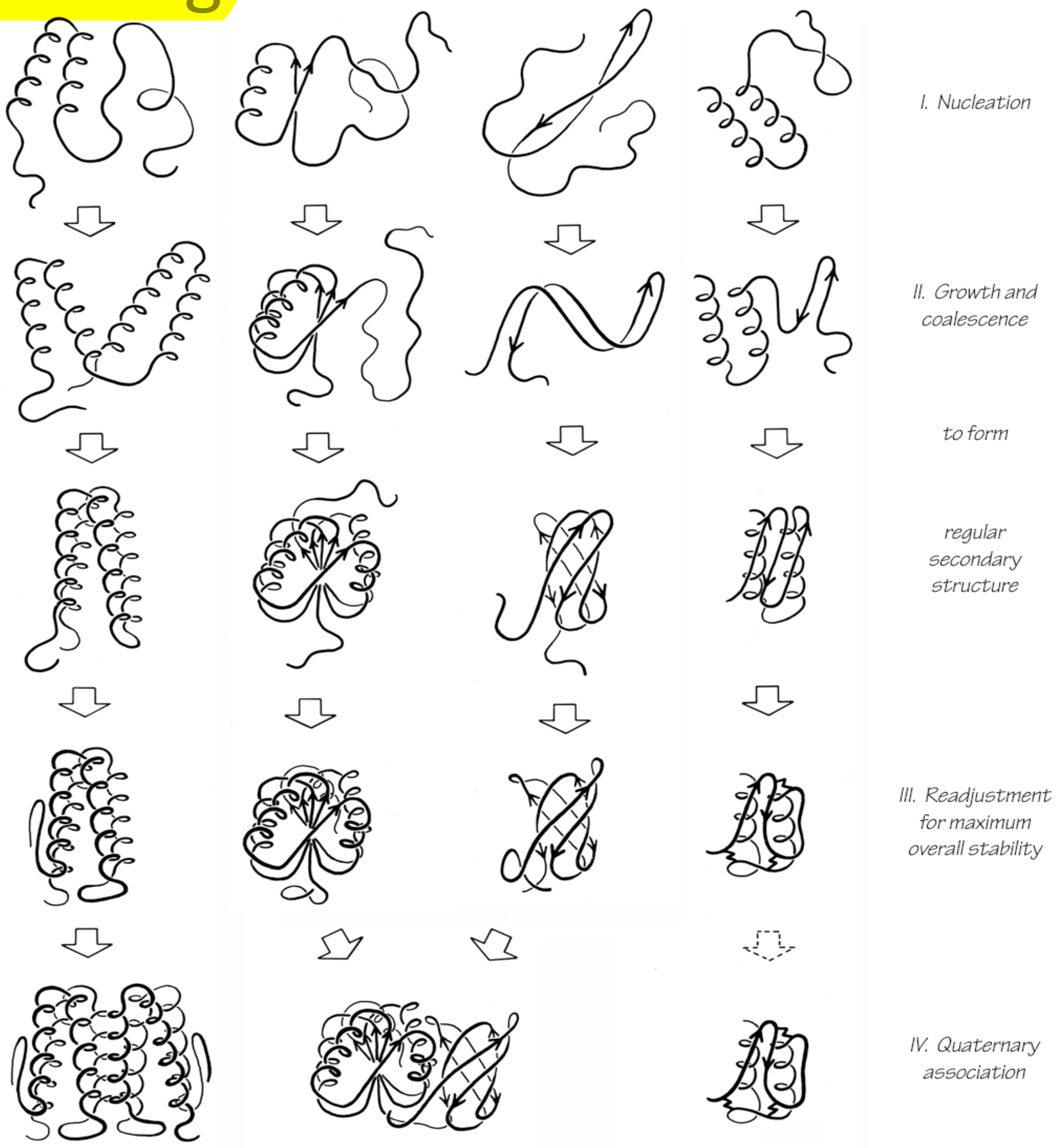
β-sheet





Cellular Mechanotransduction

Protein folding



Cellular Mechanotransduction

Proteins

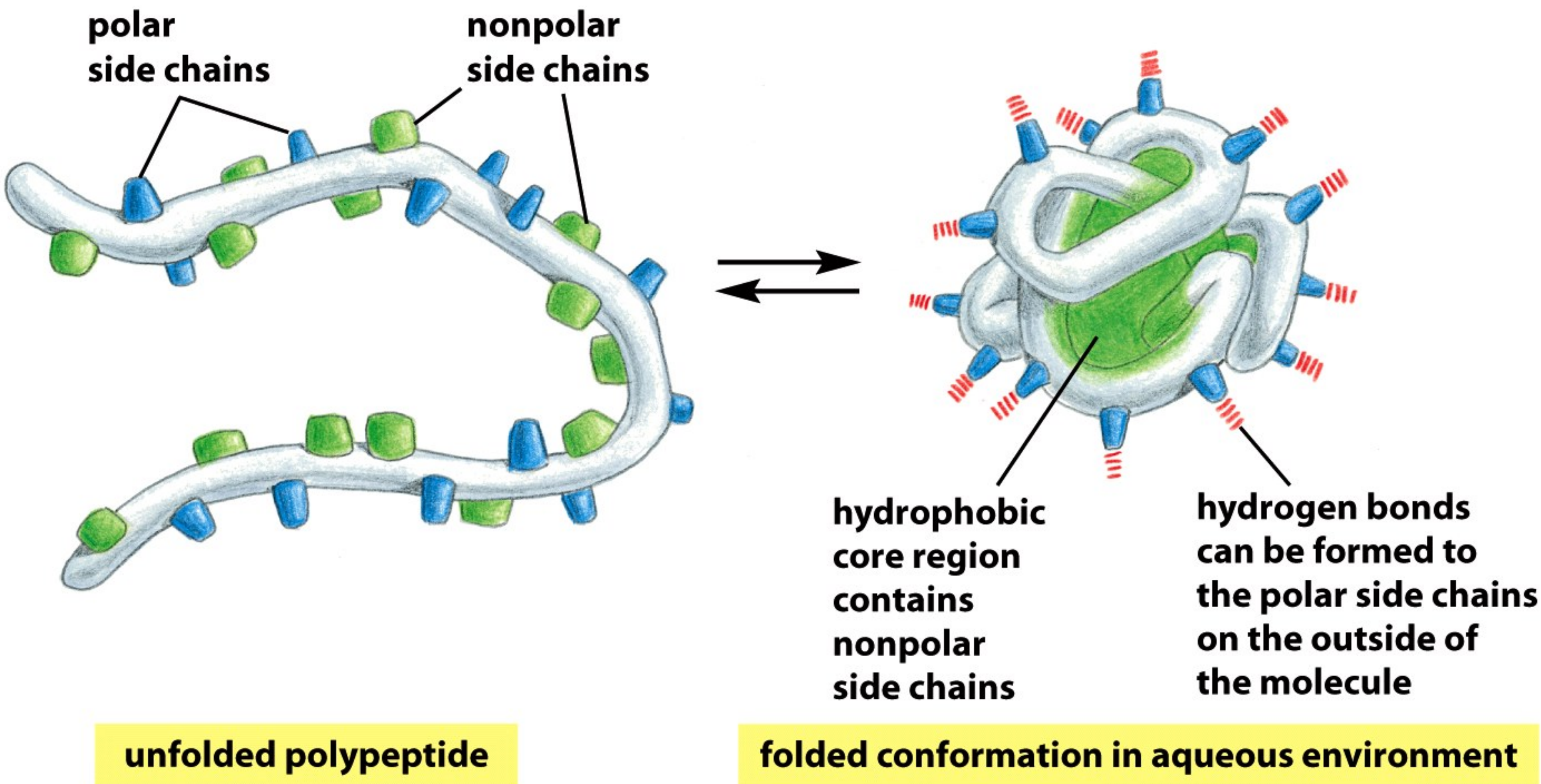


Figure 4-5 Essential Cell Biology 3/e (© Garland Science 2010)

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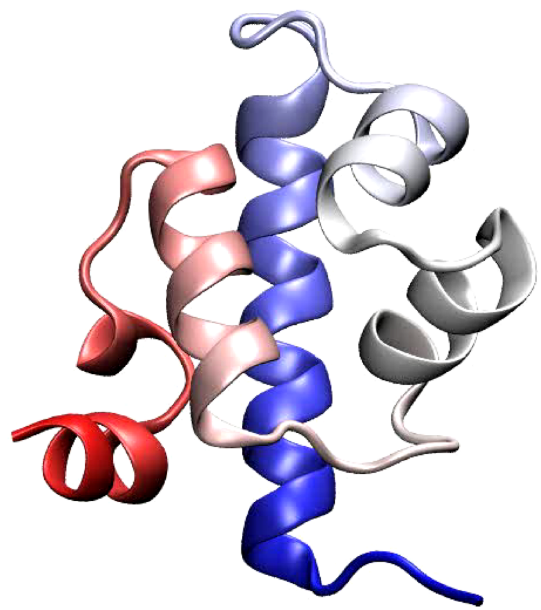
Protein folding and chemical equilibrium

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

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Chemical equilibrium

The process can be described using Gibb's (or Helmholtz) free energy:

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

Δ U = change in internal energy of the system

T = temperature

Δ S = change in entropy of the system

If Δ G < 0 reaction is spontaneous

if Δ G > 0 reaction is nonspontaneous

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Chemical equilibrium

The internal energy ΔU can be divided to different components:

I. U_c= energy from covalent bonds
II. U_e= energy from electrostatic bonds and hydrogen bonds
III. U_d= energy from dispersive bonds (van der Waals)
IV. E_t= translational kinetic energy
V. E_r= rotational kinetic energy

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

$U_c \sim 1000 - 5000$ eV per 1000 bonds
$U_e \sim 50 - 200$ eV per 1000 bonds
$U_d \sim 0.5 - 1$ eV per 1000 bonds

Cellular Mechanotransduction

Chemical equilibrium

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

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

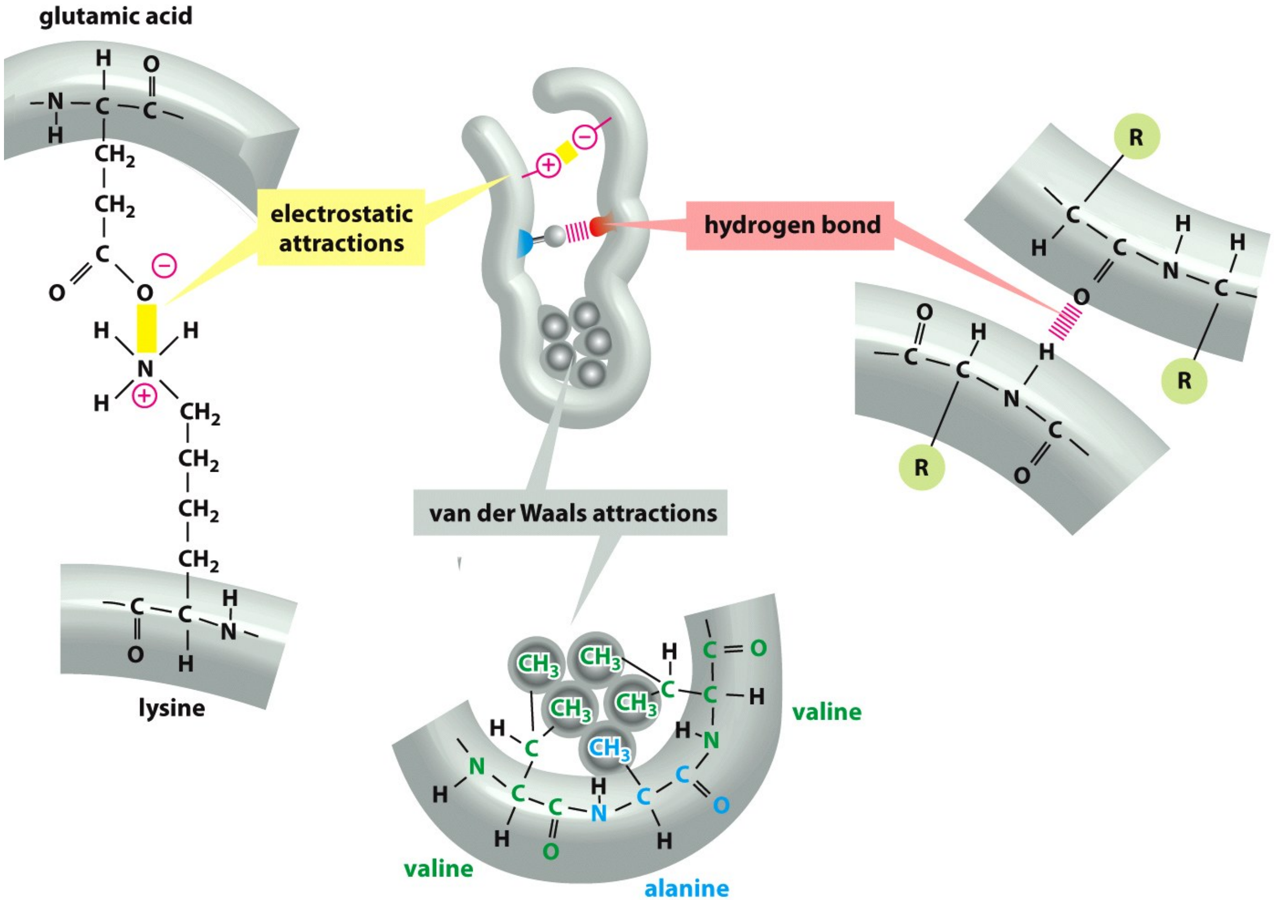

Figure 4-4 Essential Cell Biology 3/e (© Garland Science 2010)

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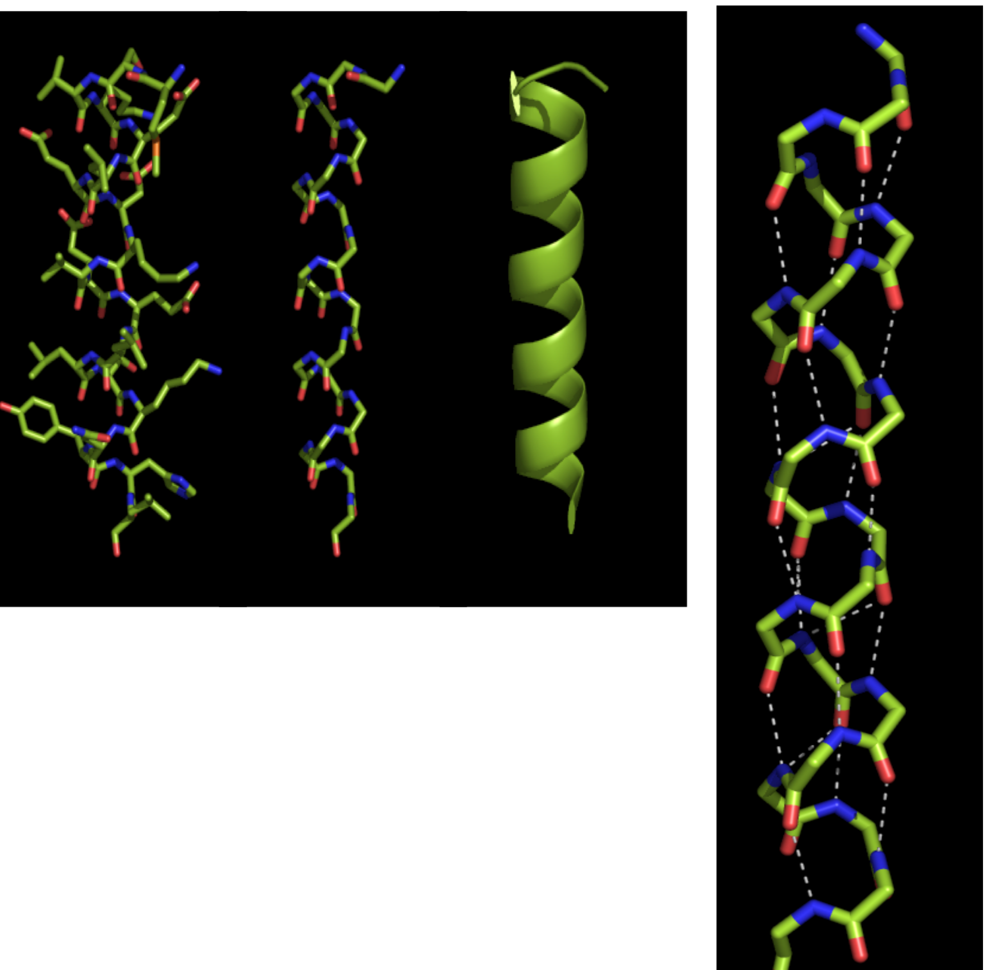
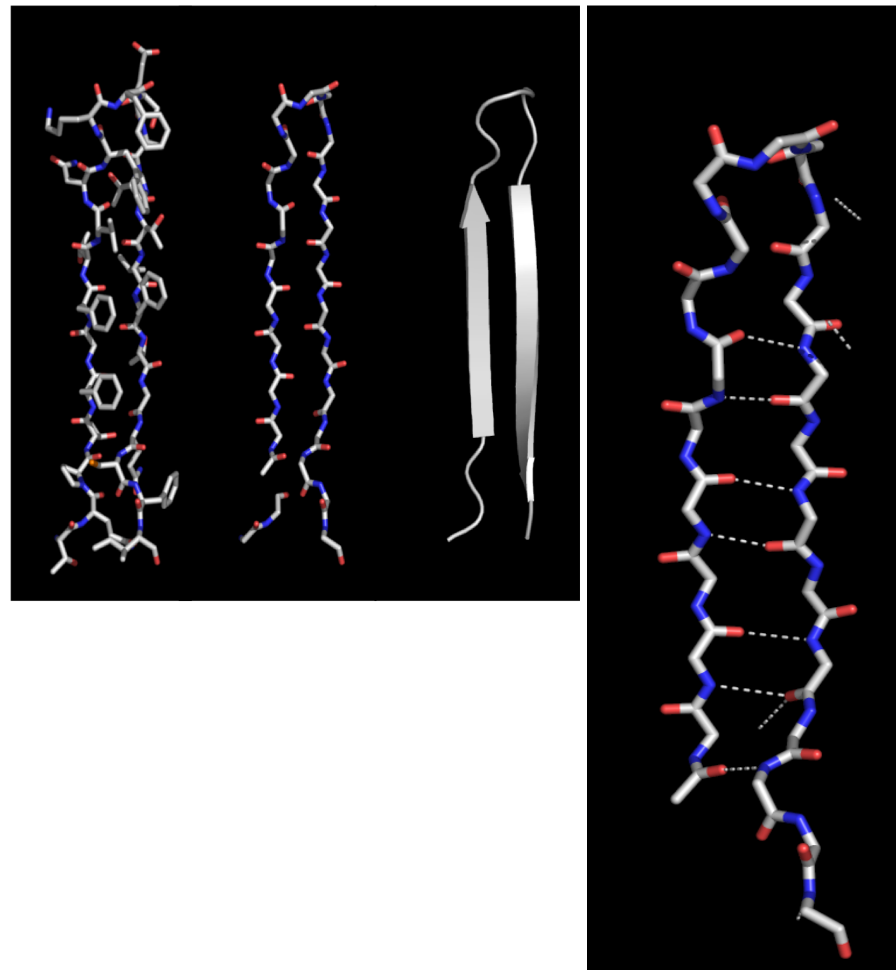
Chemical equilibrium

Hydrogen bonds of the secondary structure

α-helix



β-sheet



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Chemical equilibrium

Entropy of the system can be described with Boltzmann's entropy

$$S = k\ ln\Omega$$

k = Boltzmann's constant

Ω= number of allowed "microstates" giving certain "macrostate"

Cellular Mechanotransduction

Chemical equilibrium

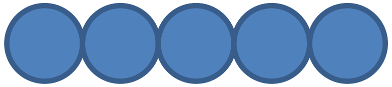
k = Boltzmann’s constant

$$S = k\, ln\Omega$$

Ω= number of allowed “microstates” giving certain “macrostate”

Example, macrostate = lenght of a polymer

Lets assume that bond angle between polymer subunits can be 0, +45deg or -45deg.

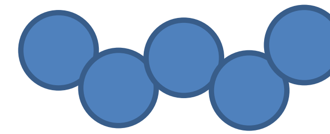


Probability for the molecule to be fully extended:

p= 1/3 * 1/3 * 1/3 * 1/3 = 1 / 81

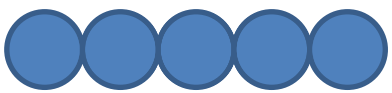
Cellular Mechanotransduction

Chemical equilibrium

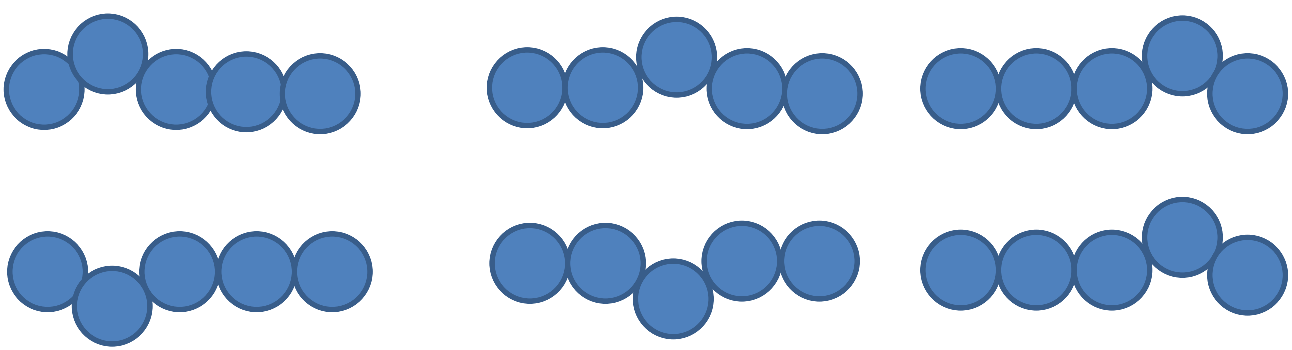


Example, macrostate = lenght of a polymer

Lets assume that bond angle between polymer subunits can be 0, +45deg or -45deg.



Fully extended: p= 1 / 81



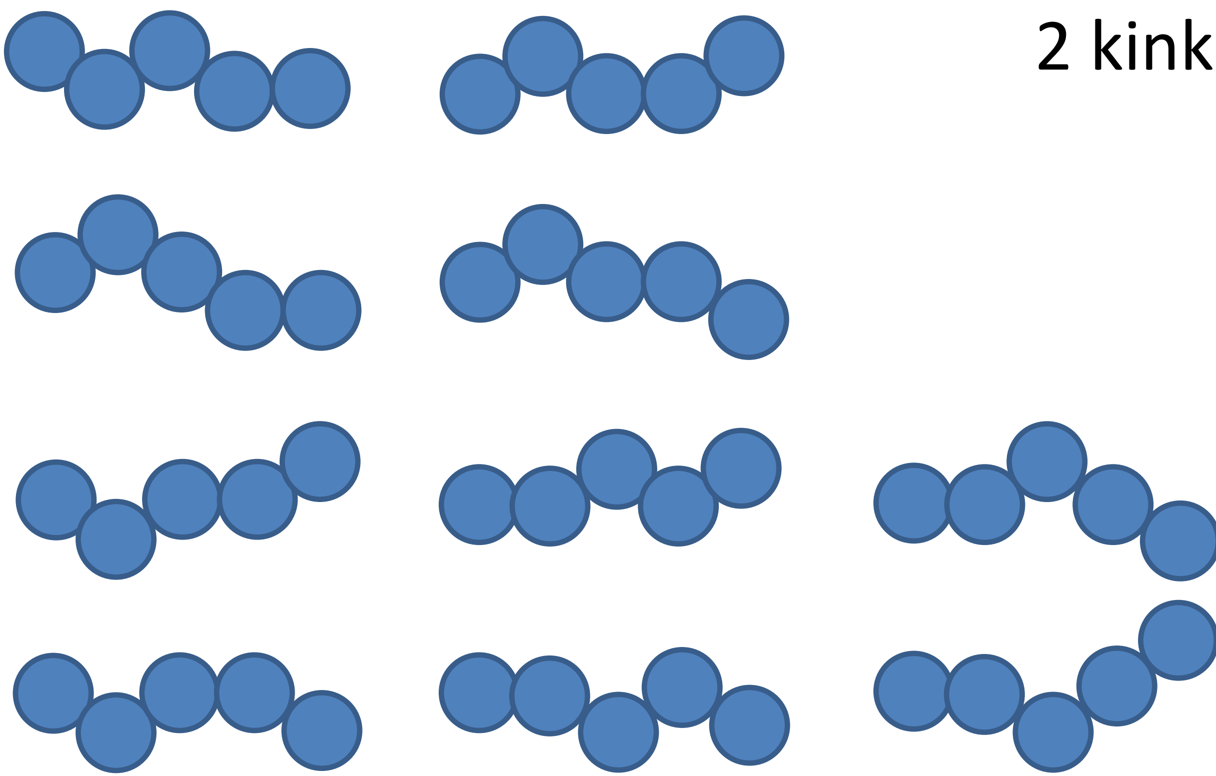
1 kink: p= 10 / 81

Cellular Mechanotransduction

Chemical equilibrium

Example, macrostate = lenght of a polymer

Lets assume that bond angle between polymer subunits can be 0, +45deg or -45deg.



2 kinks: p= 20 / 81

Cellular Mechanotransduction

Chemical equilibrium

Example, macrostate = lenght of a polymer

no kinks: p= 1 / 81

1 kink: p= 10 / 81

2 kinks: p= 20 / 81

.

.

.

$$S = k\, ln\Omega$$

Ω= number of allowed conformations leading into certain molecular length

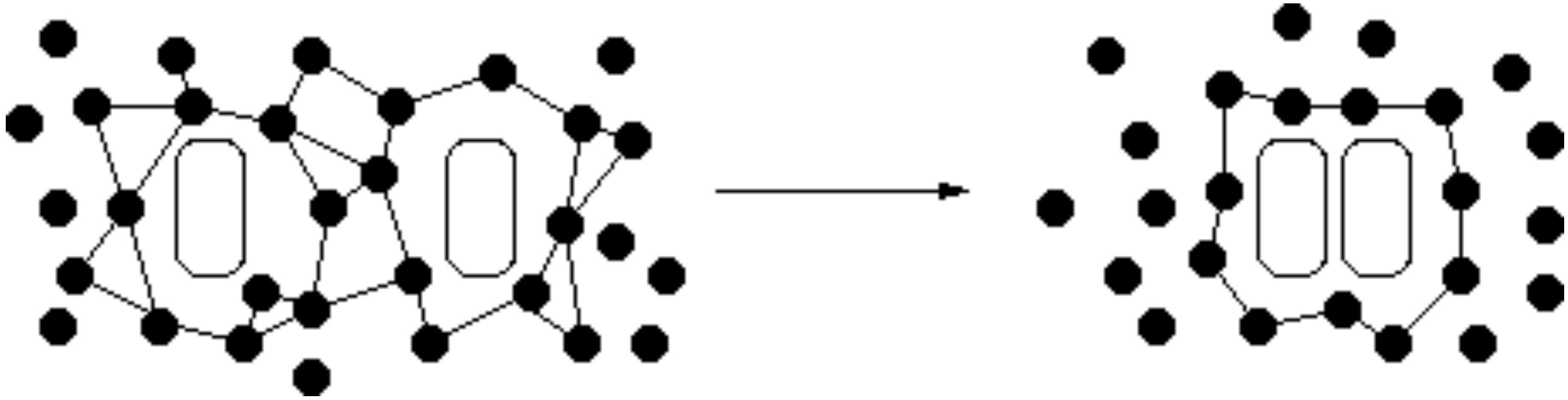
Thus, entropy favors more "disordered" polymer structure

Cellular Mechanotransduction

Chemical equilibrium

$$S = S_{protein} + S_{solvent}$$

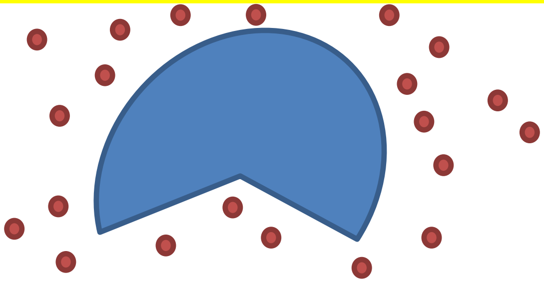
Hydrophobicity and water entropy



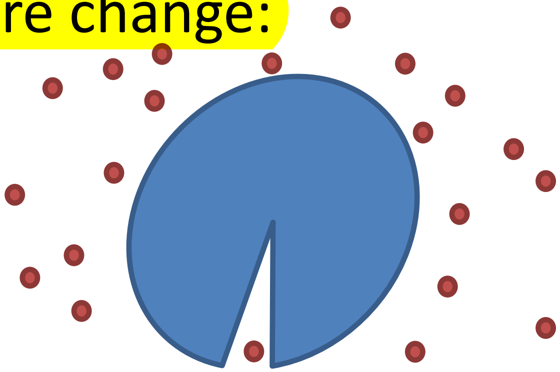
Cellular Mechanotransduction

Chemical equilibrium

Simplification of the protein structure change:



$G_1 = U_1 - TS_1$



$G_2 = U_2 - TS_2$

$$\Delta G = \Delta U - T\,\Delta S = \Delta U - T(S_2 - S_1)$$

$$\Delta G - \Delta U = -kT(ln\Omega_2 - ln\Omega_1) = -kT\ln\frac{\Omega_2}{\Omega_1}$$

$$\Delta E = -kT\ln\frac{\Omega_2}{\Omega_1}$$

Cellular Mechanotransduction

Chemical equilibrium

Simplification of the protein structure change:

$$\Delta E = -kT\ln\frac{\Omega_2}{\Omega_1} \quad \rightarrow \quad \ln\frac{\Omega_2}{\Omega_1} = -\frac{\Delta E}{kT}$$

$$\ln\frac{\Omega_2}{\Omega_1} = -\frac{\Delta E}{kT} \quad \rightarrow \quad \frac{\Omega_2}{\Omega_1} = e^{-\frac{\Delta E}{kT}}$$

If we assume that all the microstates are equally probable, then probability to be in a macrostate P_x:

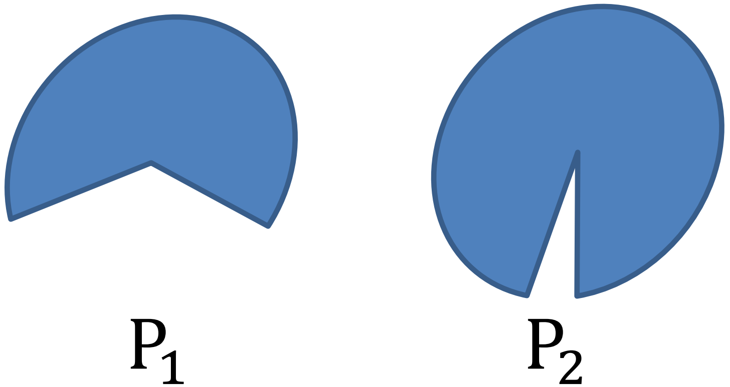
$$\mathrm{P}_x = \frac{\sum microstates\ giving\ P_x}{\sum all\ microstates}$$

Cellular Mechanotransduction

Chemical equilibrium

Thus, we can rewrite:

$$\frac{\Omega_2}{\Omega_1} = e^{-\frac{\Delta E}{kT}} \quad \rightarrow \frac{\mathrm{P}_2}{\mathrm{P}_1} = e^{-\frac{\Delta E}{kT}}$$



$$\frac{\mathrm{P}_2}{\mathrm{P}_1} = e^{-\frac{\Delta E}{kT}} = \frac{[\mathrm{Species}_2]}{[\mathrm{Species}_1]} = K_{eq.}$$

$K_{eq.}$= equilibrium constant

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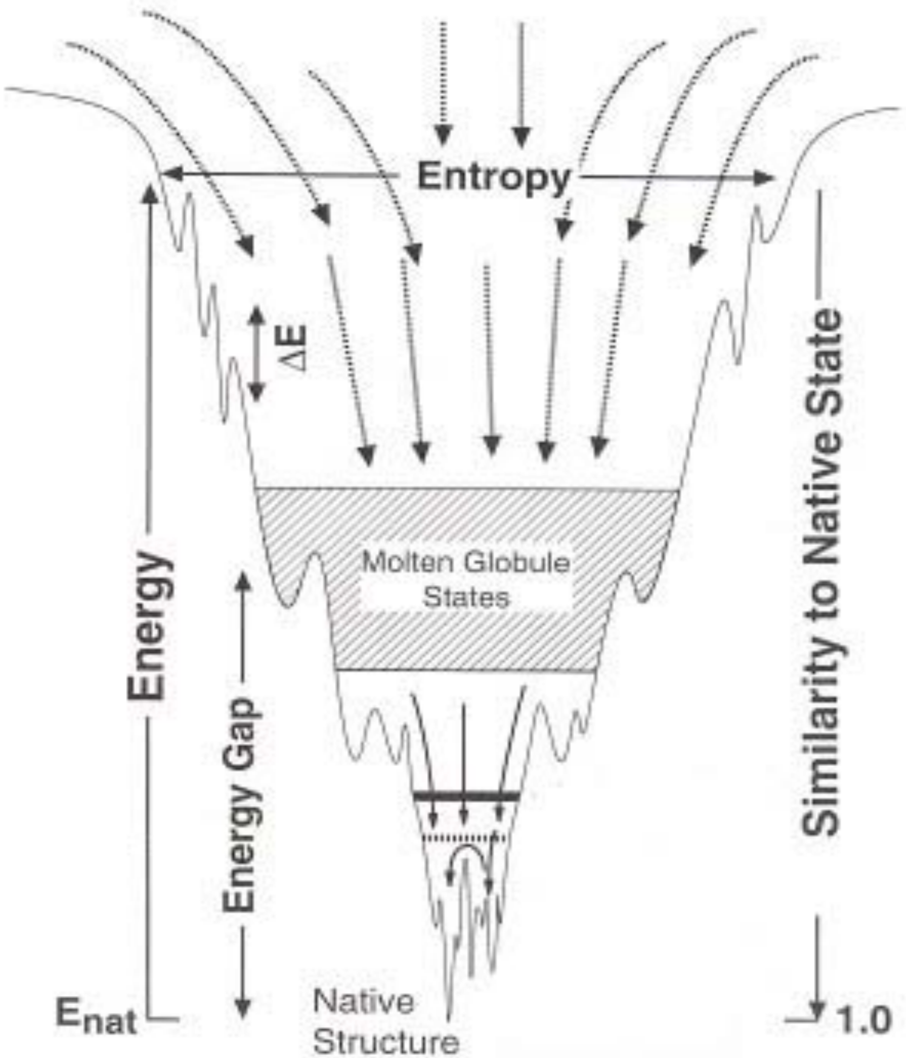
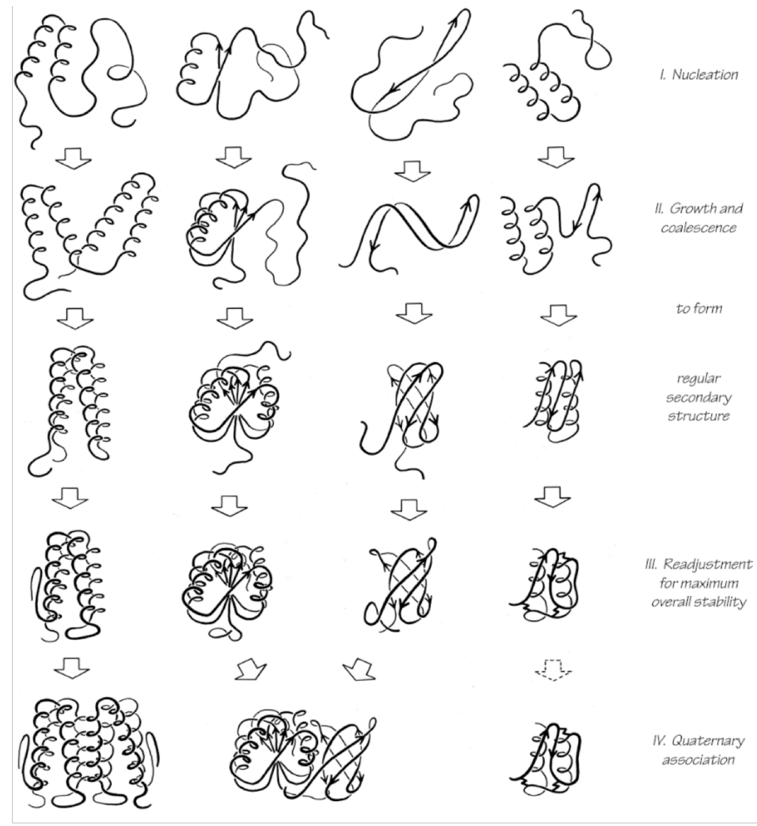
Protein folding





Cellular Mechanotransduction

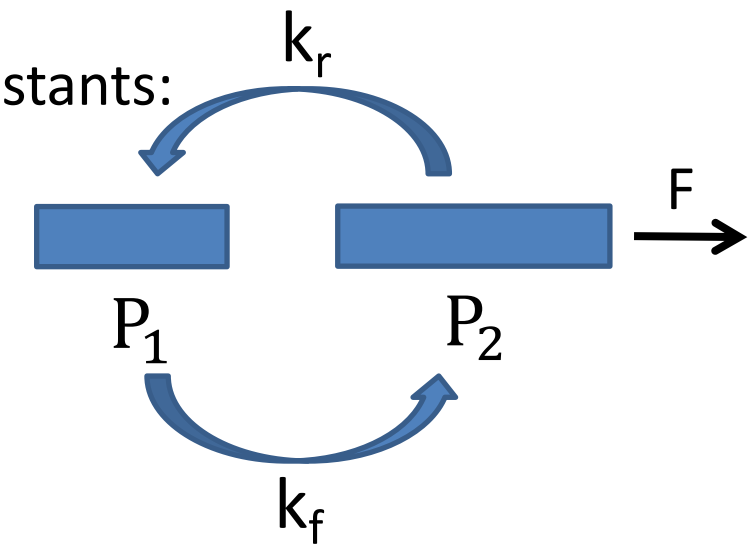
Protein folding, influence of force

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

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

k_f = forward rate constant

k_r =reverse rate constant



Force brings more energy to the system:

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

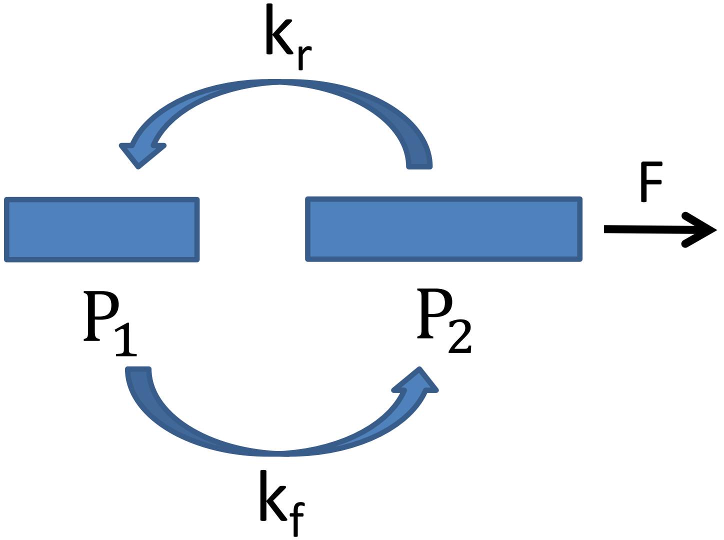
Then,

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

The force changes the equilibrium state!

Cellular Mechanotransduction

Protein folding, influence of force



In equilibrium state the molecular states shift between different conformations even without force contribution

Protein shape change is influenced by two different forces:

1. “elasticity” of the protein
2. “viscosity” rising from of the protein and the environment

The rate how fast the force is applied influences the “maximum force” which the bond(s) can handle

Cellular Mechanotransduction

Protein folding, influence of force

Elasticity can be simplified as a spring:

$F_{elastic} = k\Delta X$

k = “spring constant”

ΔX = length change

Viscosity can be described as:

$F_{viscous} = \gamma \frac{dX}{dt} = \gamma v$

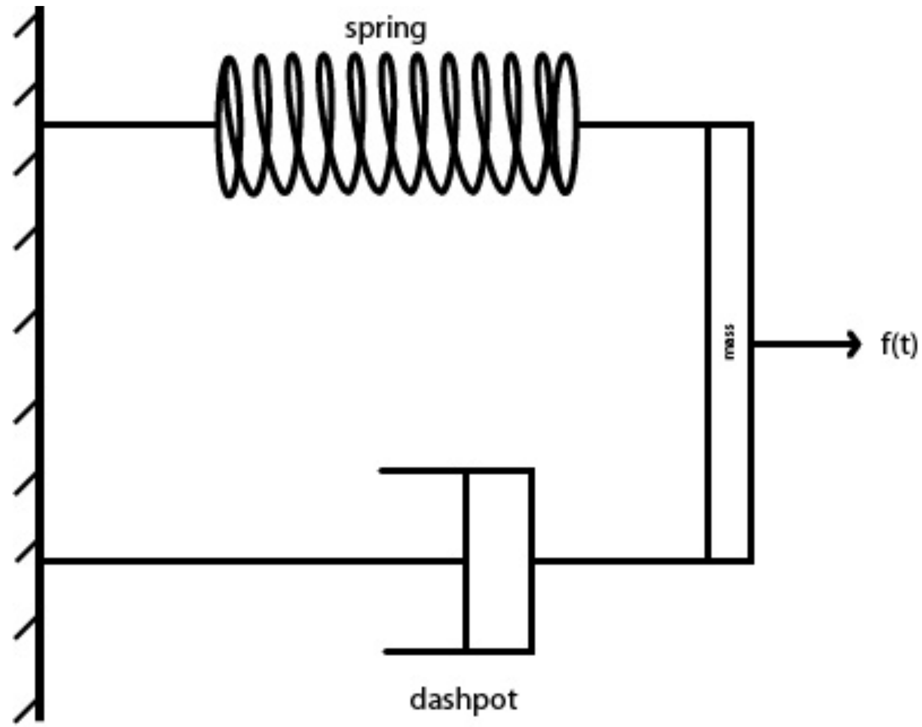
γ= drag coefficient

v=velocity

The sum of the forces:

$F = k\Delta X + \gamma v$

Kelvin-Voigt model of polymer behavior:



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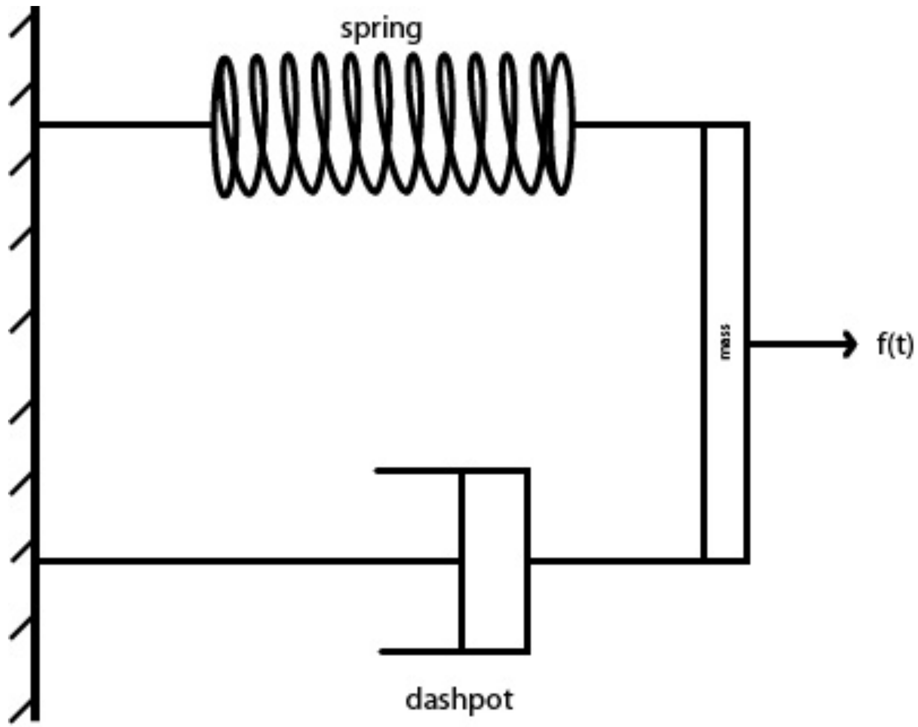
Protein folding, influence of force

The sum of the molecular forces **resisting** the structural change:

$$F = k\Delta X + \gamma v$$

This means that if we apply a force on the protein faster, **on average** it will withstand higher force before deformation.

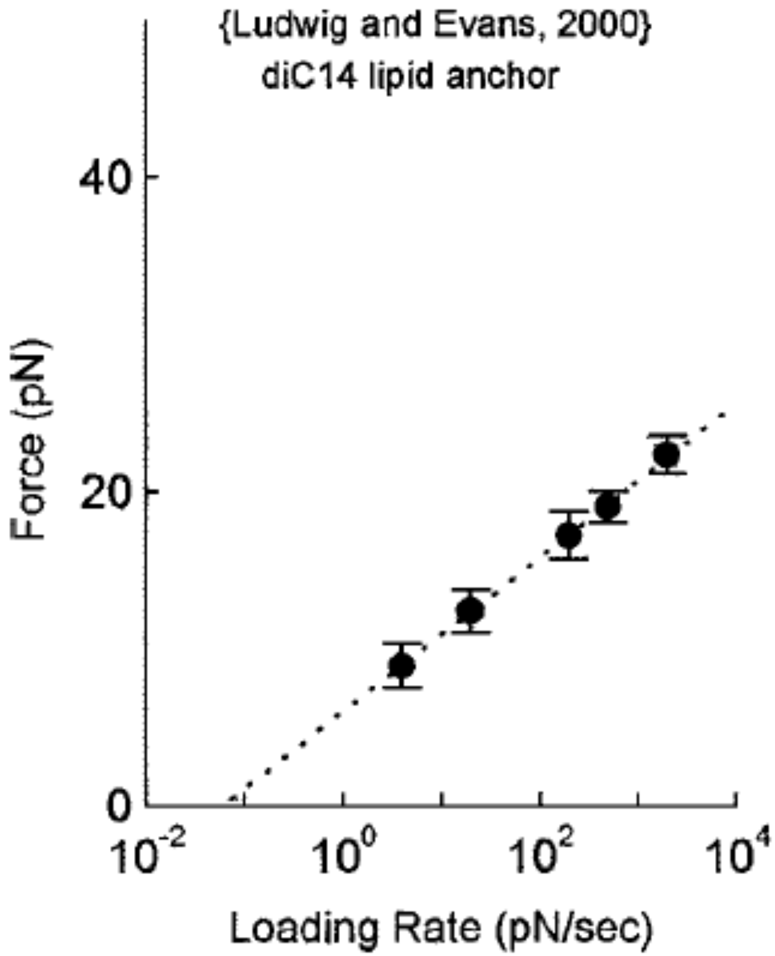
Kelvin-Voigt model of polymer behavior:



Cellular Mechanotransduction

Protein folding, influence of force

Mechanically forced unfolding, loading rate



The sum of the forces

$$F = k\Delta X + \gamma \mathrm{v}$$

Single lipid is pulled out from artificial lipid vesicle with different loading rates

Cellular Mechanotransduction

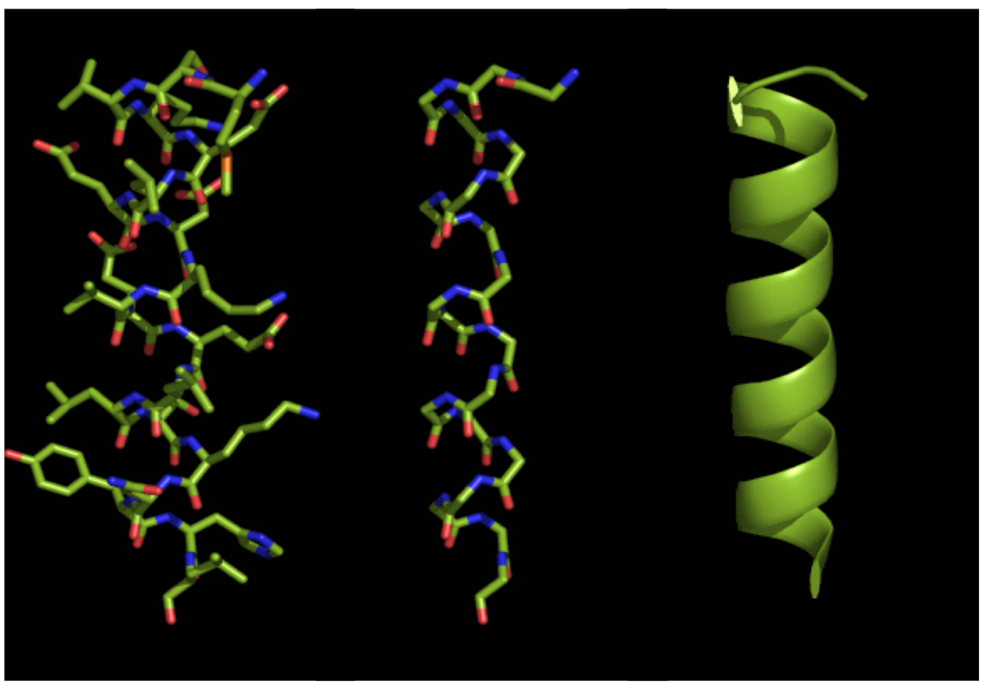
Protein unfolding under force

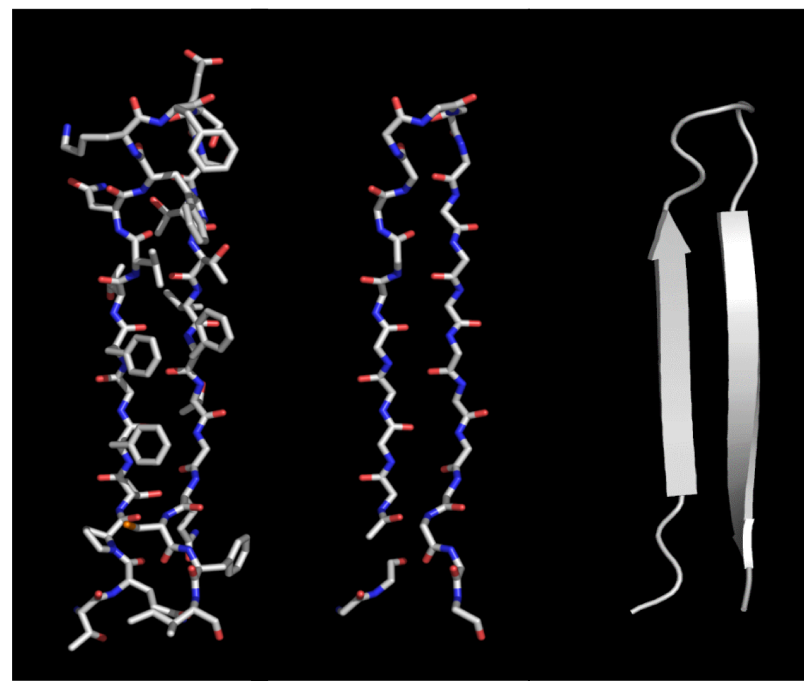
α-helix

Can be extended significantly

β-sheet

Almost completely extended polypeptide chain

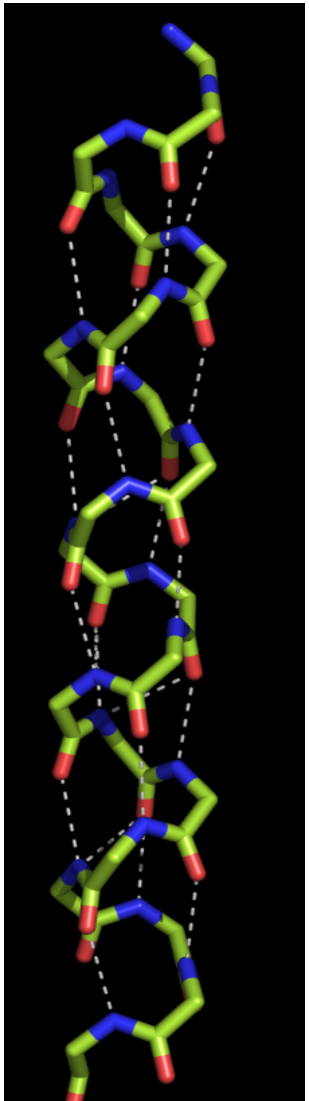




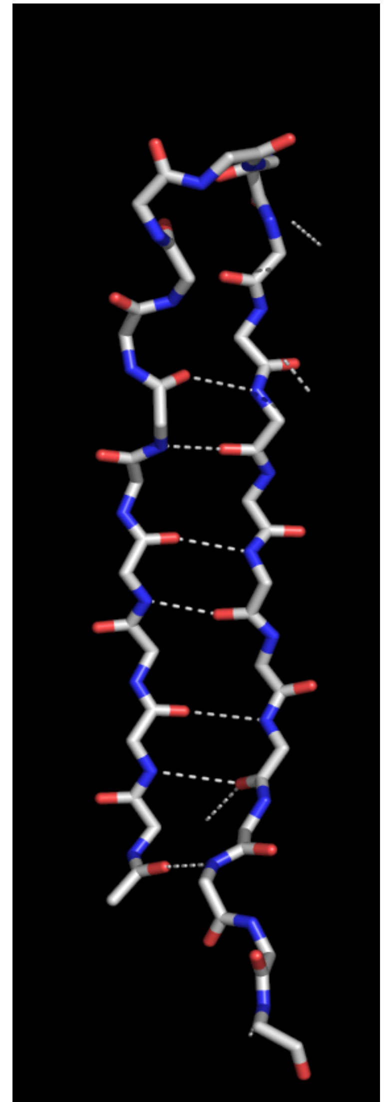
Cellular Mechanotransduction

Protein unfolding under force

α-helix



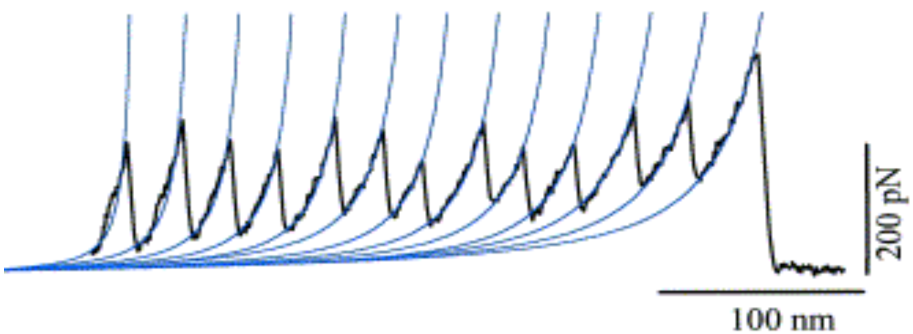
β-sheet

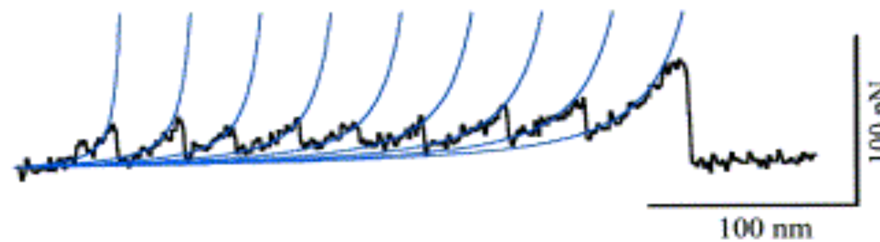


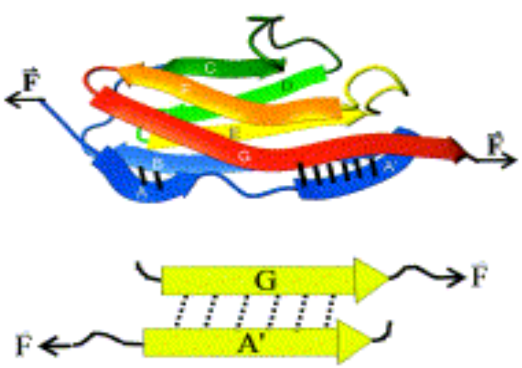
Cellular Mechanotransduction

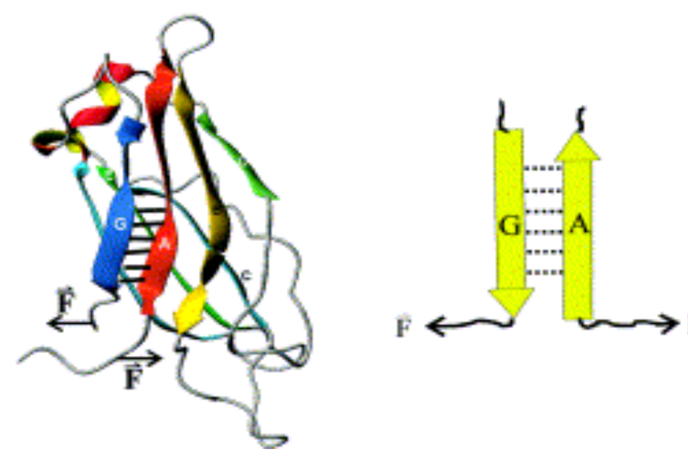
Protein unfolding under force

The orientation of the force-bearing bonds in relation to applied force largely determines the mechanical stability of the structure









Carrion-Vazquez et al. Prog. Biophys. Mol. Biol. 2000

Cellular Mechanotransduction

Protein unfolding under force

The orientation of the force-bearing bonds in relation to applied force largely determines the mechanical stability of the structure

Cellular Mechanotransduction

Forces on proteins

1. Mechanical force on protein can alter its structure
   → new functions???

2. Loading rate influences the maximum force what the bond can tolerate

3. Directionality of the force has a big impact on protein structure