LECTURE 6

BB 101

MODULE: *PHYSICAL BIOLOGY*

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Review of Lecture 5

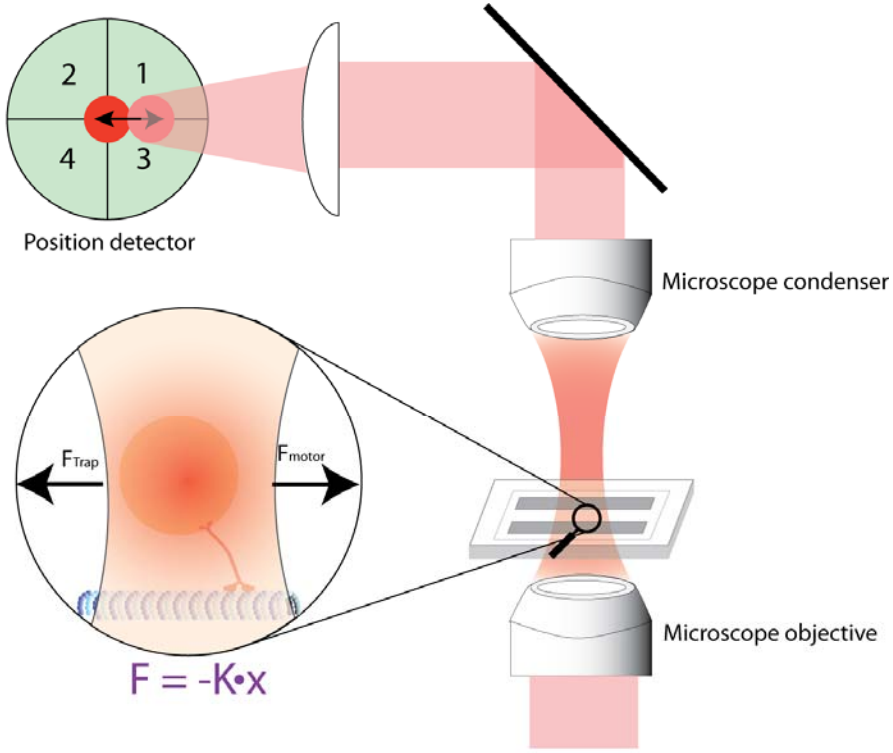
- Persistence Length and its significance
- Microtubules and Actin filaments are polymers of tubulin and actin monomers
- Dynamics of Microtubule and Actin Filaments
- Example of force generation by microtubule and actin filament
- Measurement of force generated by microtubule and actin filament

Measurement of force generated by microtubule and actin filament

- Forces generated by biofilaments are typically of the order of pN

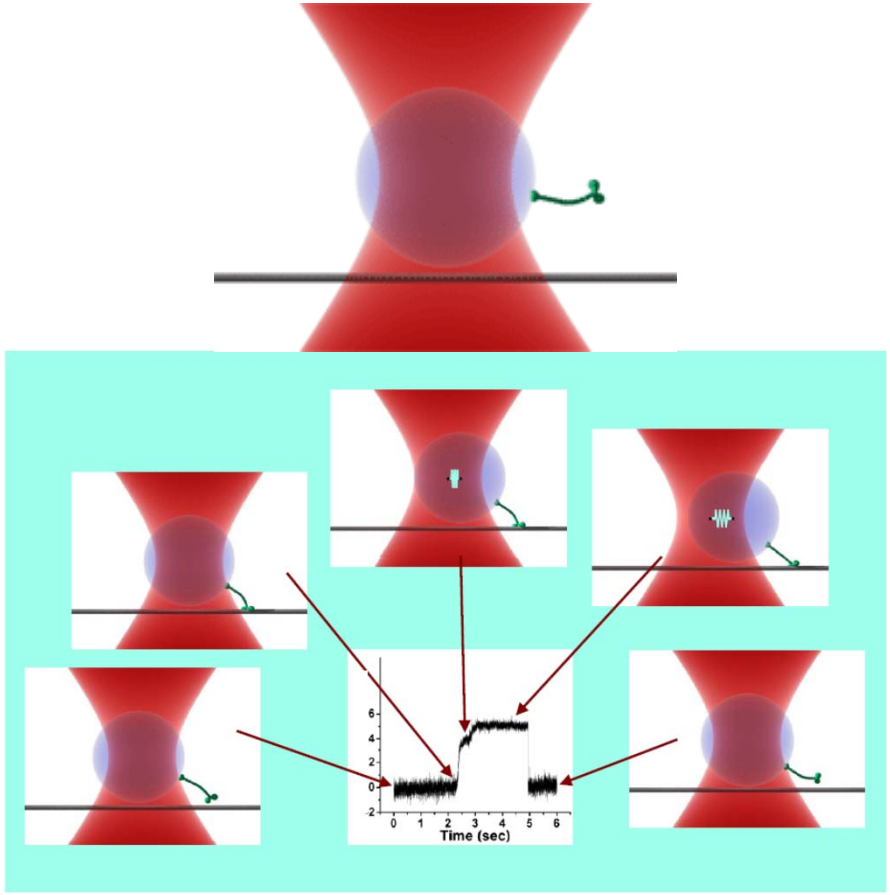
- Modern Techniques such as Optical Tweezers and AFM are used for measurement of such forces

Measurement of Forces using Optical Tweezers

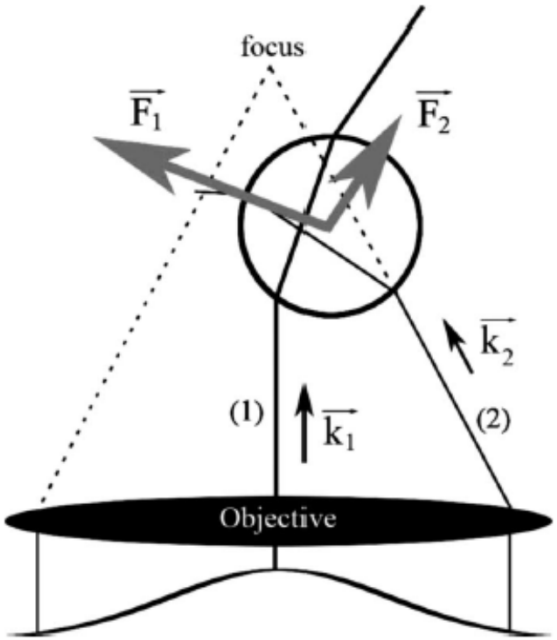


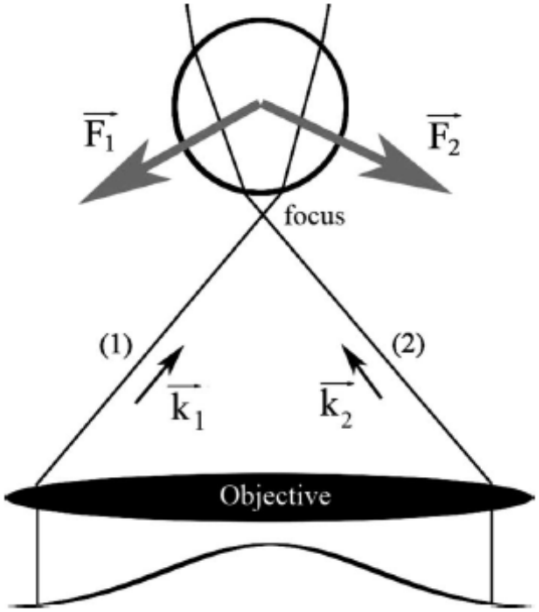
Force on the bead is proportional to its displacement inside an optical trap

© Ambarish Kunwar



Optional Reading Material on Optical Tweezers





Optical tweezers for undergraduates: Theoretical analysis and experiments, M. S. Rocha, Am. J. Phys. 77, 704 (2009)

http://dx.doi.org/10.1119/1.3138698

Measuring force exerted by microtubule

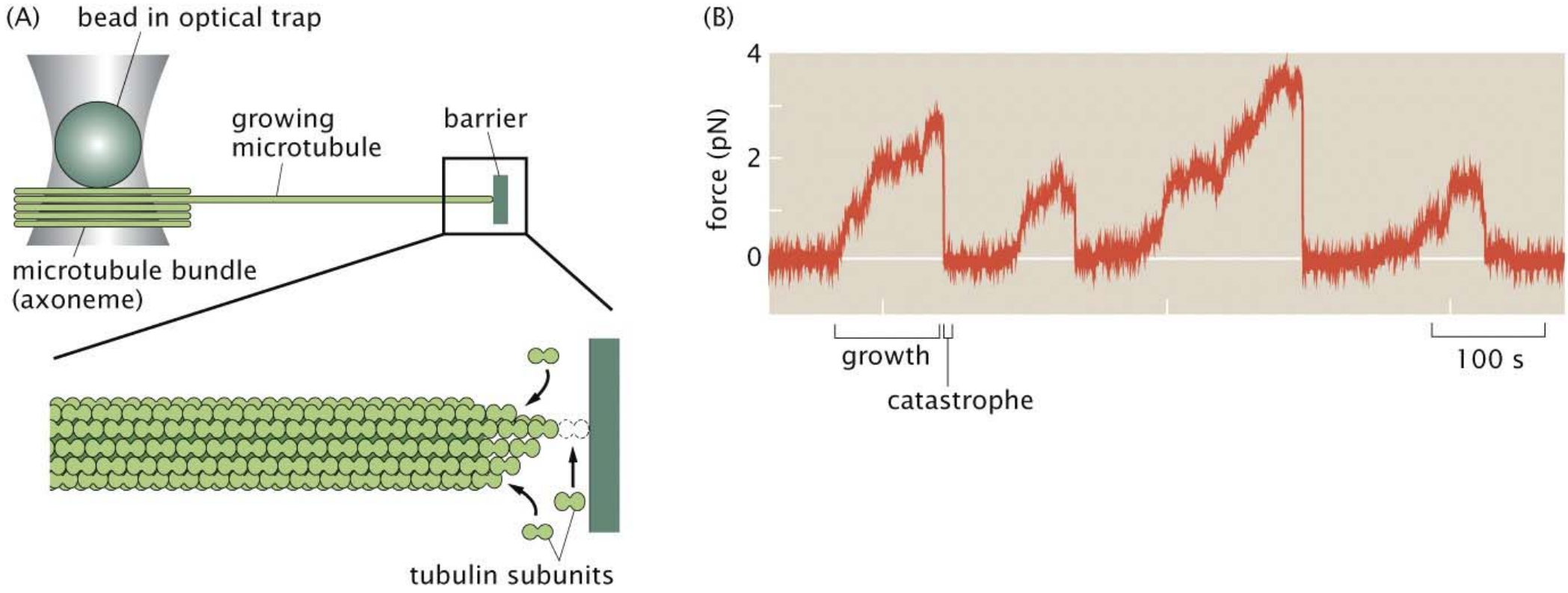


Figure 16.49 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Optical trap essentially behaves like a linear spring. If you know the displacement, you can calculate the force

Measuring force exerted by actin network

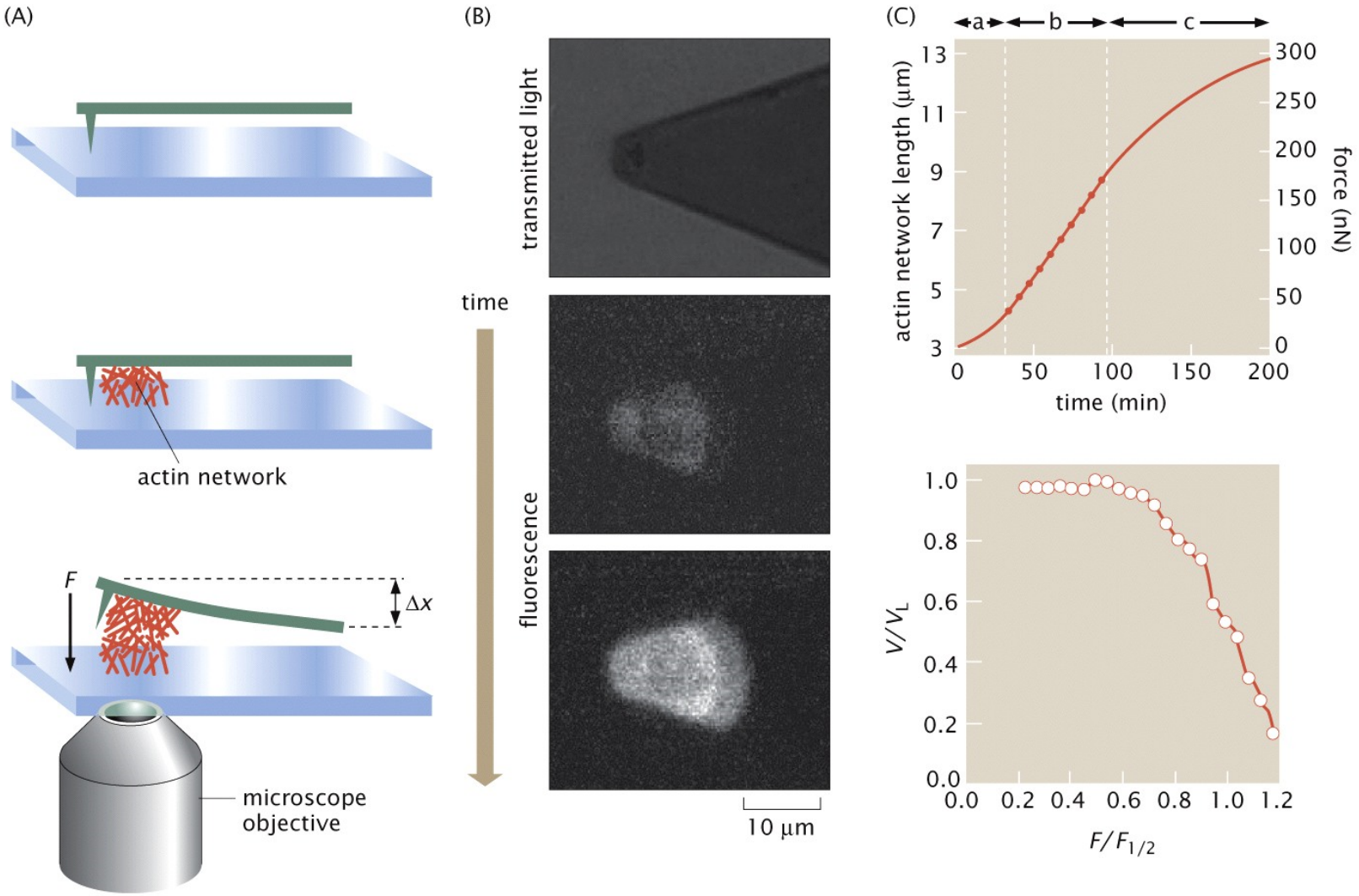
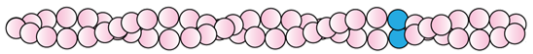


Figure 16.50 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Proteins and their structures

- Tubulin and actin monomers are protein molecules



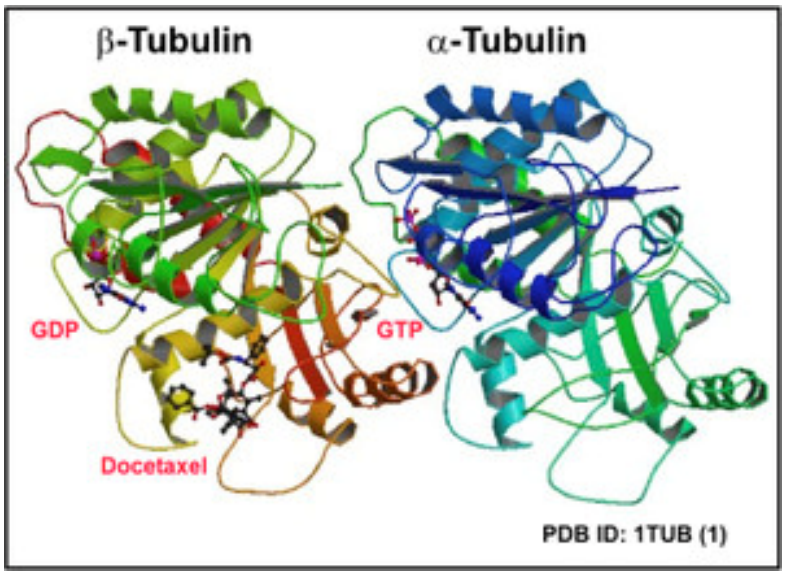


Figure Source: http://www.mastcell-basophil.net/wiki/wiki-start/microtubules-and-mast-cell-signaling/

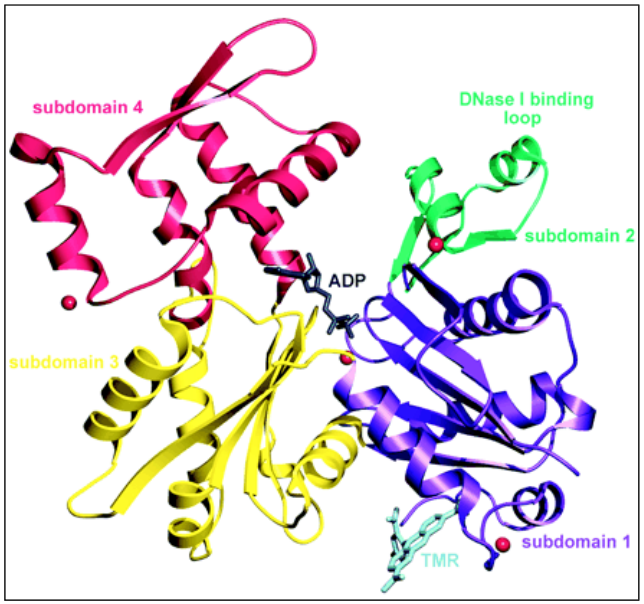


Figure Source: Otterbein et al., Vol. 293, 708-711 (2001)

Proteins and their structures

- Proteins perform their function by folding into different shapes
- Proteins are sequence of amino acids
- Given a sequence of amino acids can be predict the structure of the protein?

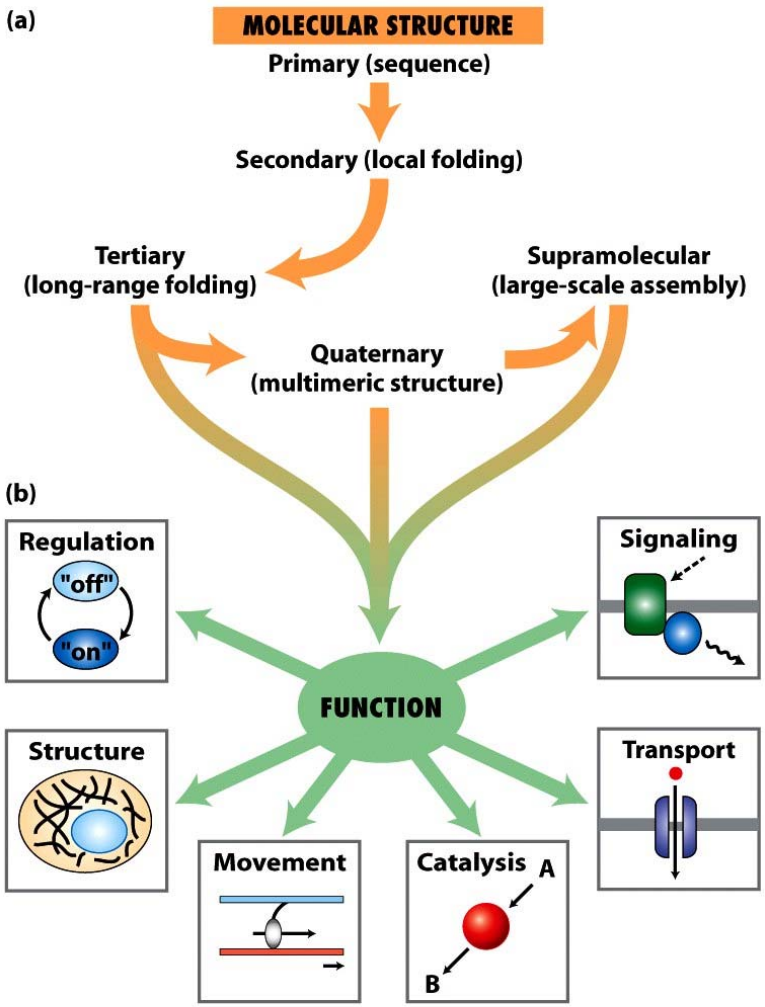


Figure 3-1
Molecular Cell Biology, Sixth Edition
© 2008 W. H. Freeman and Company

Figure Source: http://www.mastcell-basophil.net/wiki/wiki-start/microtubules-and-mast-cell-signaling/

Figure Source: Otterbein et al., Vol. 293, 708-711 (2001)

Proteins and their structures

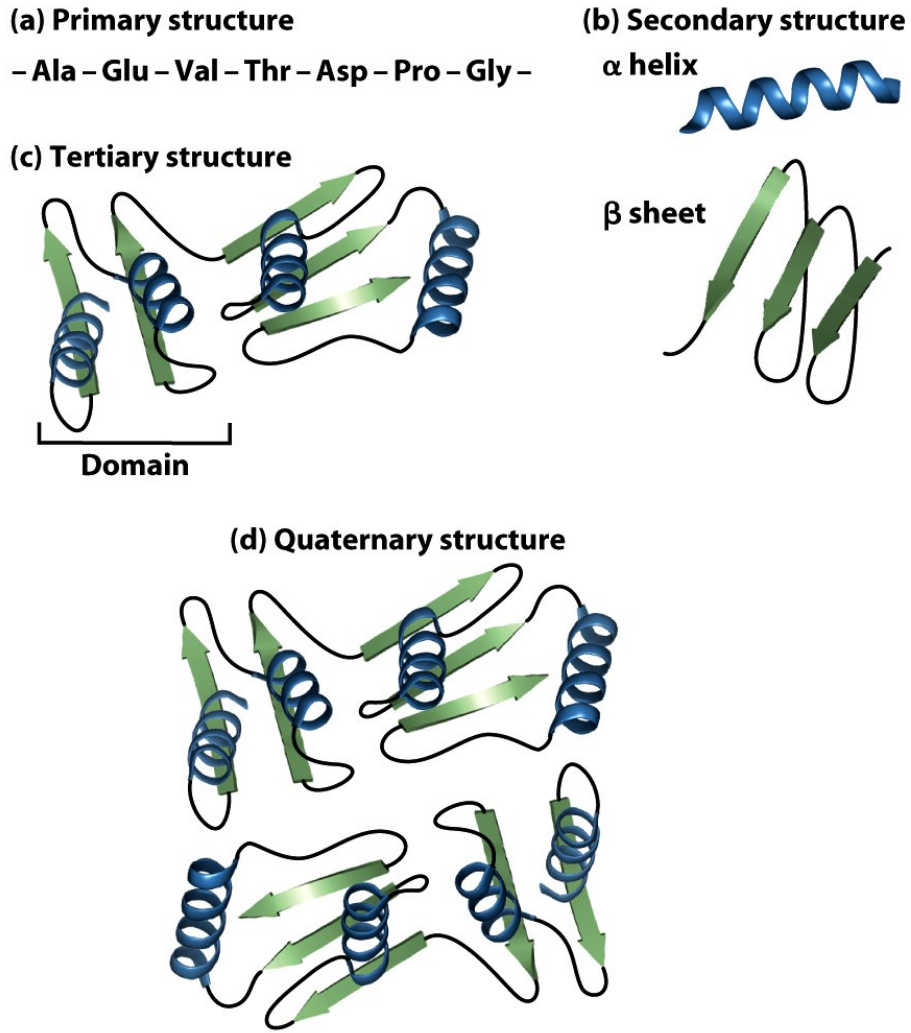


Figure 3-2
Molecular Cell Biology, Sixth Edition
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Proteins and their structures

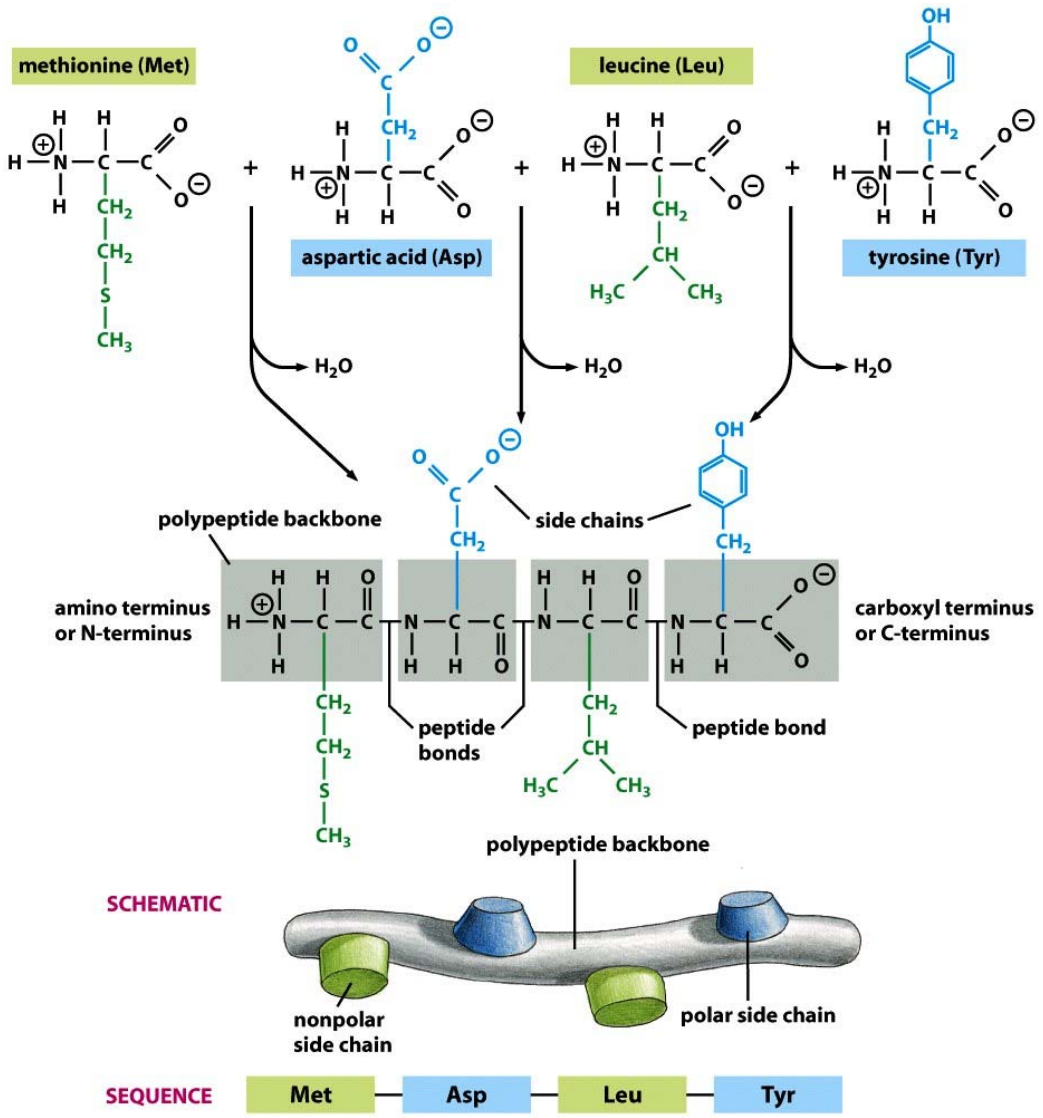


Figure 3-1 *Molecular Biology of the Cell* (© Garland Science 2008)

Proteins and their structures

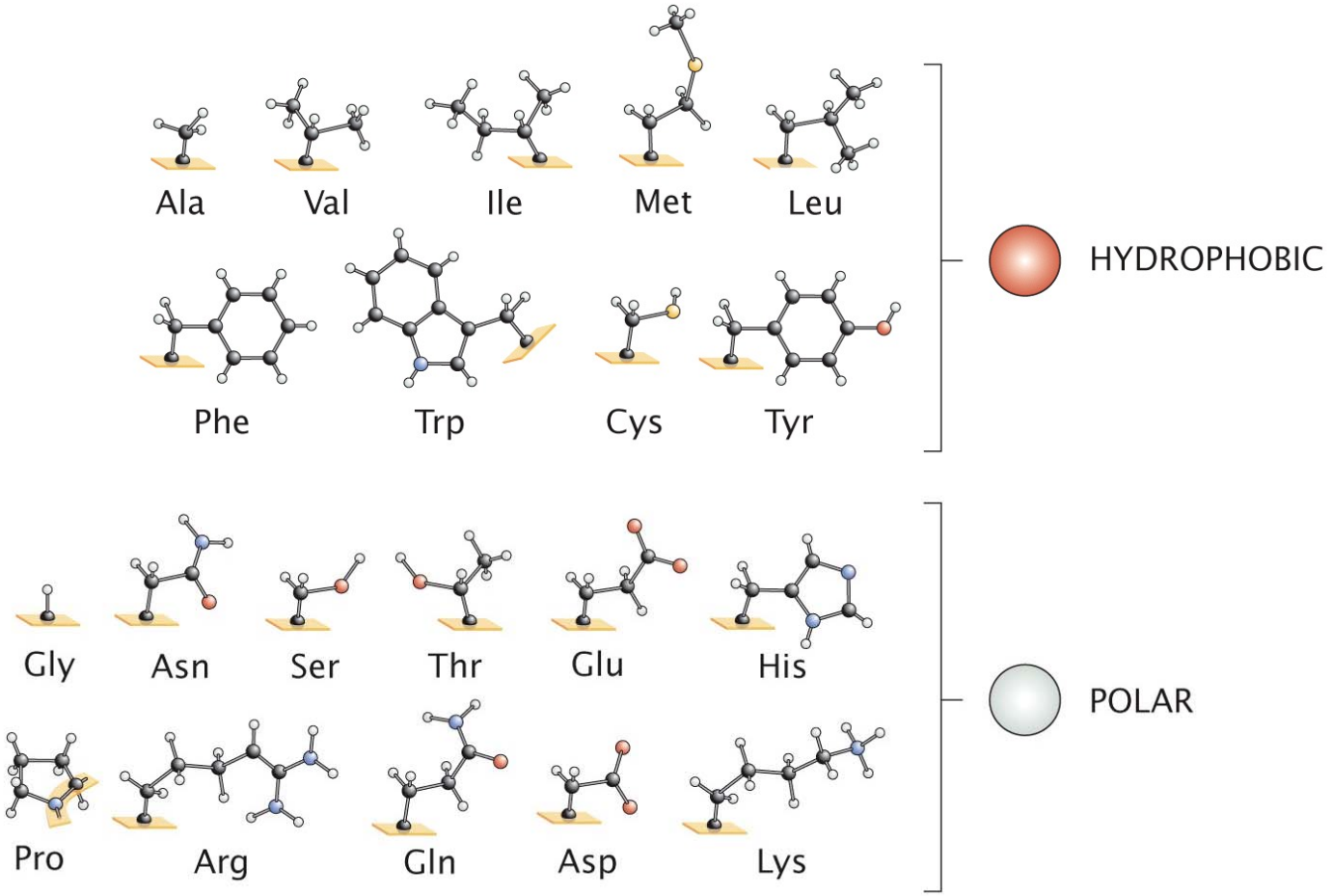


Figure 8.28 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Figure 3-2 *Molecular Biology of the Cell* (© Garland Science 2008)

Protein Structures and Folding

- Proteins are sequence of amino acids
- **Given a sequence of amino acids can be predict the structure of the protein?**
- Classical mechanics tell us that protein would prefer the structure/conformation which minimizes energy
- However, this is not true when thermal fluctuations are present

Protein Structures are free energy minimizers

- In presence of thermal fluctuations, a protein folds into the structure which minimizes free energy, out of all the possible ways that a particular chain of amino acids can fold up

- What is free energy?

- Helmholtz free energy

$$A = U - TS$$

- Gibbs free energy

$$G = H - TS$$

$$H = U + pV$$

Free Energy

- Free energy=Energy- Temperature x Entropy
- Free energy can be minimized by either decreasing energy or increasing entropy
- In order to calculate free energy we have to calculate entropy
- **What is entropy? How to calculate entropy?**

Entropy

- Entropy is a measure of the microscopic degeneracy of a macroscopic state ("macro-state"

- In other words, entropy can be computed by counting the number of possible microscopic arrangements/states ("micro-states") for a given macroscopic state ("macro-state")

$$S = k_B \ln W$$

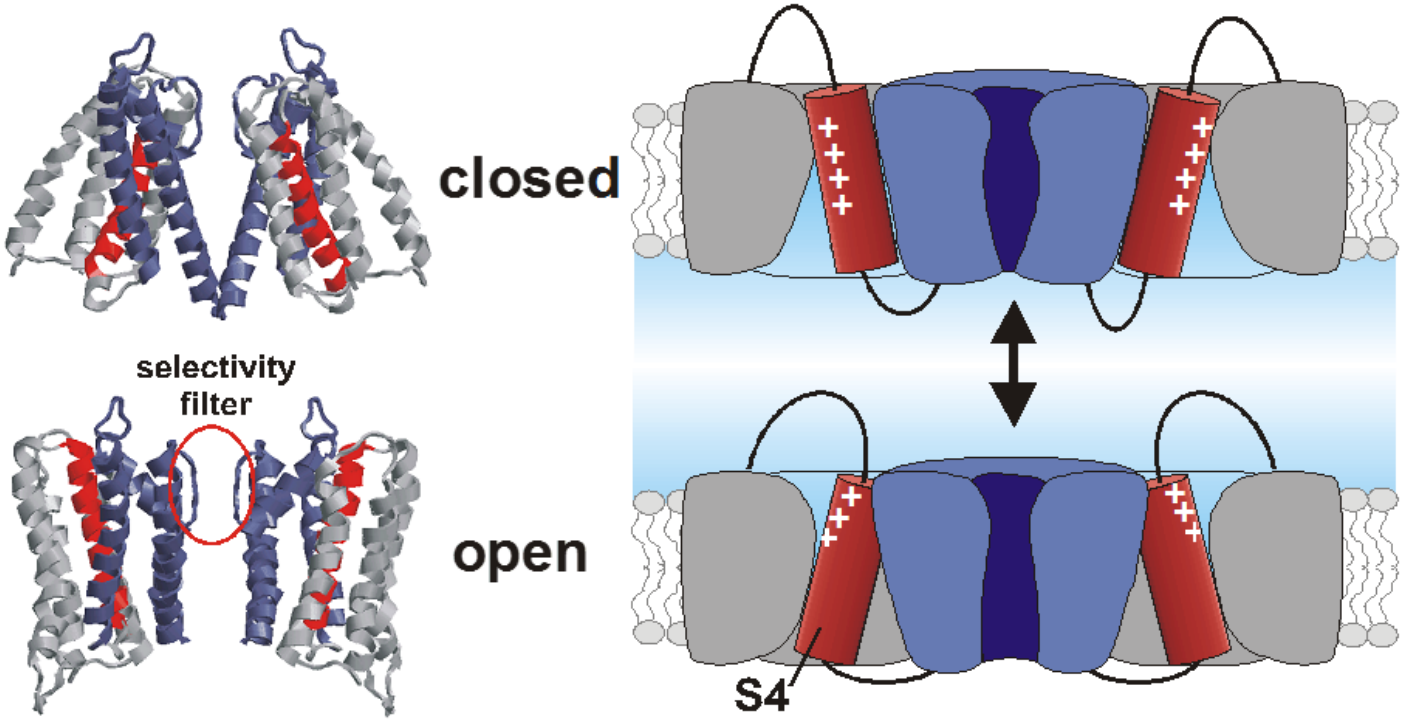
***W* is the number of possible microscopic arrangements (or "micro-states")**

Entropy

Figure Sources: http://en.wikipedia.org/wiki/Boltzmann%27s_entropy_formula#/media/File:Zentralfriedhof_Vienna_-_Boltzmann.JPG

Micro-state and Macro-state

- Consider a protein that can exist in two **structural states.** For example open (1) and closed (2)



http://www.biophys.umontreal.ca/Bluncklab/research.html

Micro-state and Macro-state

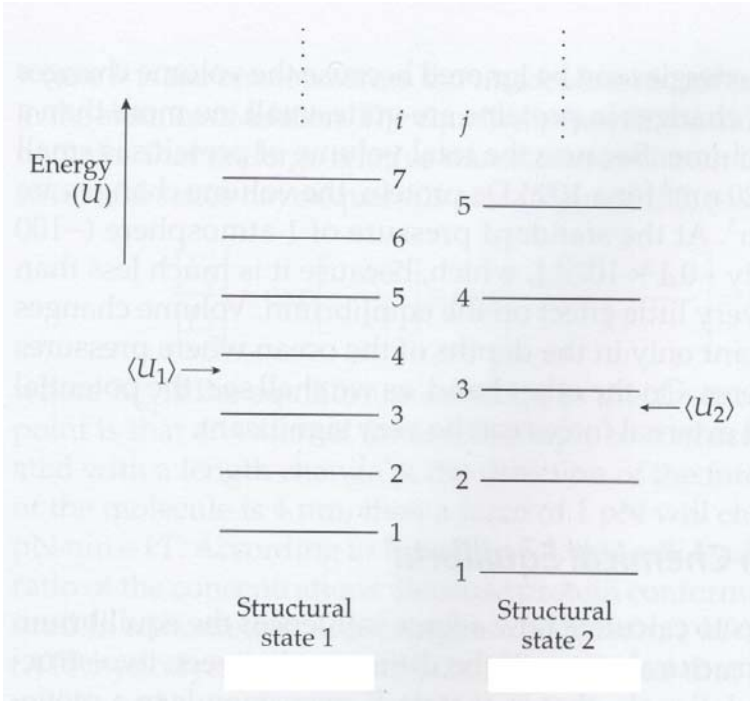
- However, due to thermal fluctuations, these structural states consists of enormous number of **conformations states**, where a conformational state is a set of positions of all the atoms

- Structural states $\Rightarrow$ “Macro-states”

- Conformational states $\Rightarrow$ “Micro-states”

Micro-state and Macro-state

- We know that probability of finding protein any microstate i or j is given by Boltzmann law



$$p_i = \frac{1}{Z} e^{-\frac{U_i}{k_B T}} \qquad p_j = \frac{1}{Z} e^{-\frac{U_j}{k_B T}}$$

- What is the probability of finding protein in a given macrostate?

- The probability of finding protein in a given macrostate is given sum of all p_i or p_j

Micro-state and Macro-state

- Thus, Probability of finding in a macrostate X is given by

$$p(X) = \sum_{iX} p_i = \sum_{iX} \frac{1}{Z} e^{-\frac{U_i}{k_B T}}$$

- Using relation $G = -k_B T ln Z$ between partition function and free energy it can be shown that

$$\boxed{p(X) = \frac{1}{Z} e^{-\frac{G(X)}{k_B T}}}$$

- Similar to Boltzmann formula for microstate, energy replaced by free energy of macrostate

Proof of $G = -k_B T ln Z$

$$\left.\begin{aligned} G &\equiv < U > -TS \\ < U > &= \sum_{i=1}^{N} U_i P_i \\ S &= -k \sum_{i=1}^{N} p_i \ln p_i \end{aligned}\right\} \Rightarrow G = \sum_{i=1}^{N} U_i P_i + kT \sum_{i=1}^{N} p_i \ln p_i = \sum_{i=1}^{N} P_i \left\{ U_i + kT \ln p_i \right\}$$

$$P_i = \frac{1}{Z} \exp\left[-\frac{U_i}{kT} \right]$$

$$\Rightarrow G = \sum_{i=1}^{N} \frac{1}{Z} \exp\left[-\frac{U_i}{kT} \right] \left\{ U_i + kT \ln\left(\frac{1}{Z} \exp\left[-\frac{U_i}{kT} \right] \right) \right\} = \frac{1}{Z} \sum_{i=1}^{N} \exp\left[-\frac{U_i}{kT} \right] \left\{ U_i - kT \ln Z - U_i \right\}$$

$$Z = \sum_{i=1}^{N} \exp\left[-\frac{U_i}{kT} \right]$$

$$\Rightarrow G = -kT \ln Z \Rightarrow Z = \exp\left(-\frac{G}{kT} \right)$$

So far…

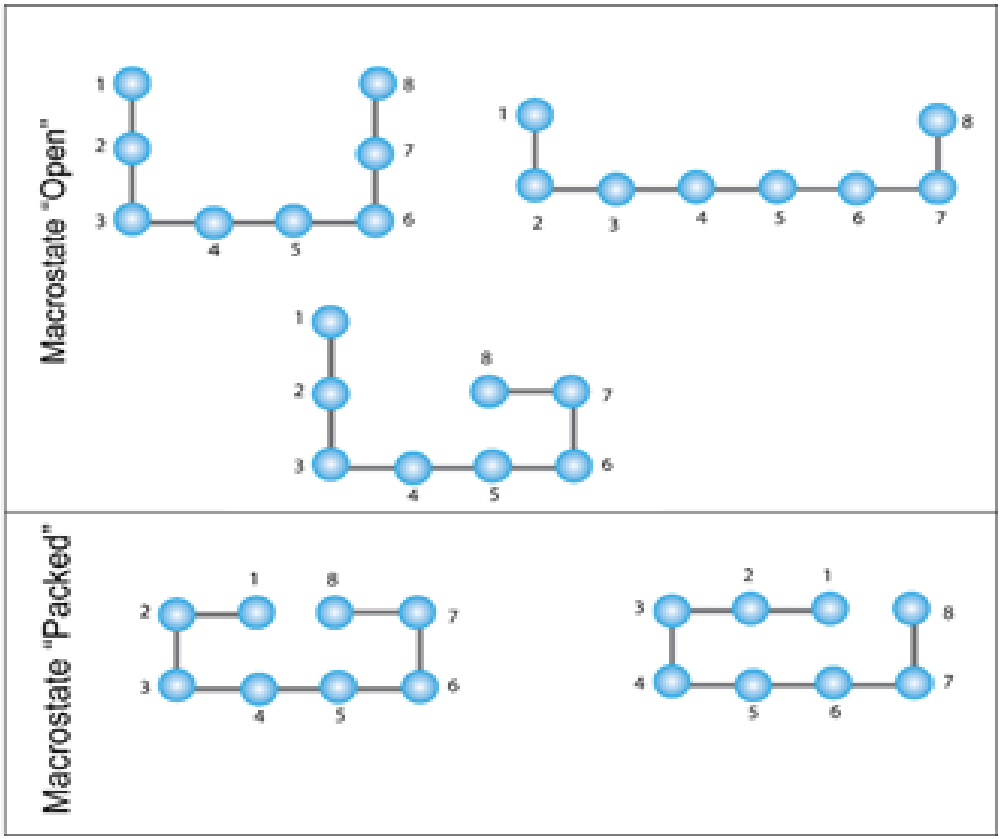
- Proteins can exist in many structural states "macrostates"
- Each "macrostate" consists of many "microstates"
- "macrostate" with minimum free energy is preferred
- $G = H - TS$, $G = -k_B T ln Z$ and $S = k_B \ln W$
- Probability of finding a macrostate X is given by

$$p(X) = \frac{1}{Z} e^{-\frac{G(X)}{k_B T}}$$

HP models of protein folding

- Consider a protein consisting of eight amino acids consisting of H and P
- The sequence of the amino acids is HPHHHHHP
- Suppose that this protein can fold into five possible structures such that energy increases by ε for every contact of H with either P or solvent molecule
- Suppose these structures can be broadly classified into two macroscopic states “Open” and “Packed”

HP models of protein folding



- What will be the structural state of the protein at temperature T i.e. Open or Packed?

HP Models of Protein Folding

$$G_{Open} = 13\varepsilon - k_B T \ ln\ 3$$

$$G_{Packed} = 11\varepsilon - k_B T \ ln\ 2$$

Open will be preferred as long as $G_{Open} < G_{Packed}$

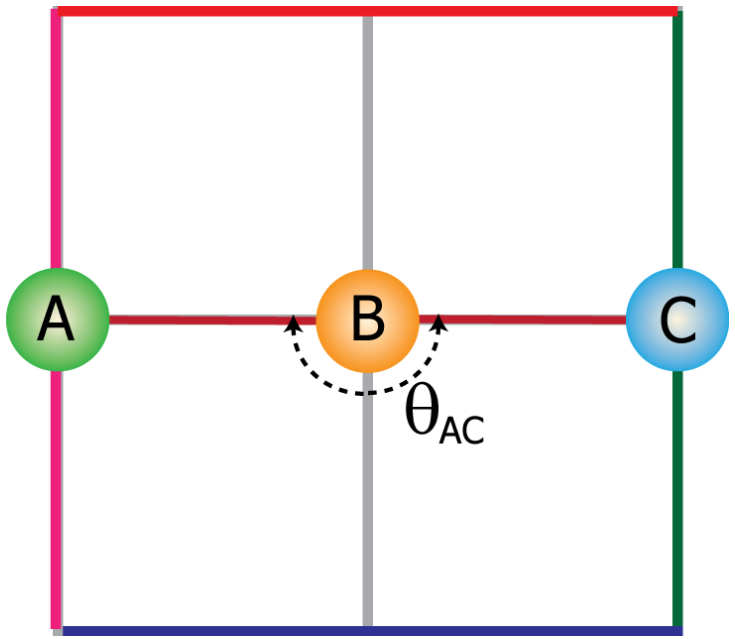
Or, $13\varepsilon - k_B T \ ln\ 3 < 11\varepsilon - k_B T \ ln\ 2$

Or, $$T < \left(\frac{\varepsilon}{k_B}\right)\left(\frac{2}{\ln(\frac{3}{2})}\right)$$

Another Toy Models of Protein Folding

- Consider a protein consisting of three amino acids A, B and C connected with bonds of equal length

- This protein is placed on a square grid such that only A and C are free to rotate in a plane such that the bonds are always aligned along the grid lines



Colored lines denote that space is inhomogeneous

Another Toy Models of Protein Folding

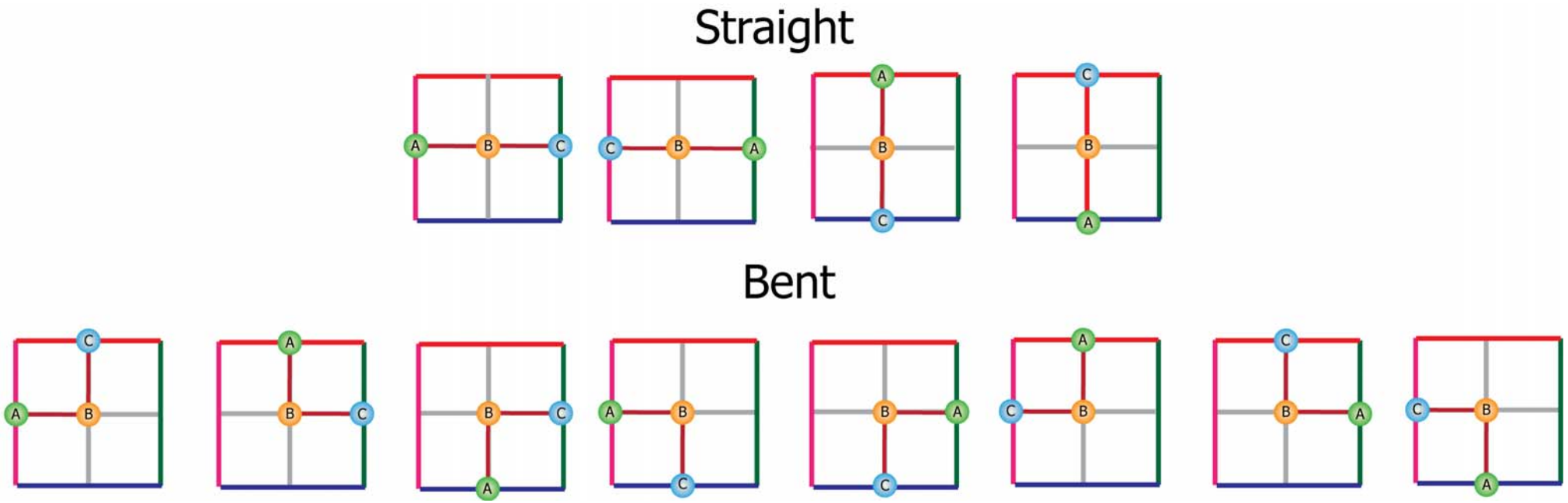
- Bending energy of the protein is given by

$$\epsilon = A(1 + cos\theta_{AC})$$

Whether the structural state of the protein will be bent or straight?

- What is the energy of the straight conformation?
- What is the energy of the bent conformations?
- Classical Mechanics: It should be in straight state

Number of Straight and Bent conformations

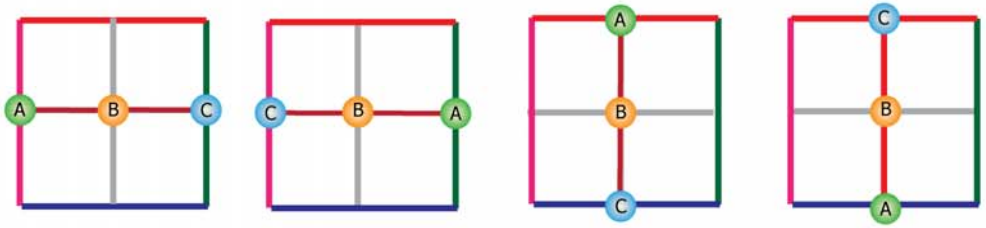


- No. of straight conformations=4
- No. of bent conformations=8

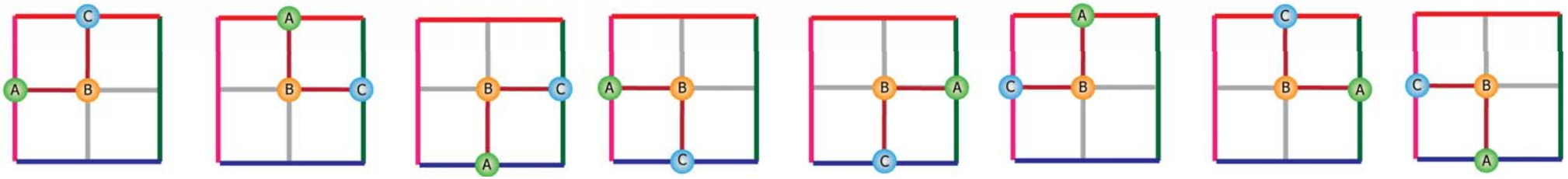
Calculate Average Bending Energy

$$\epsilon = A(1 + cos\theta_{AC})$$

Straight



Bent



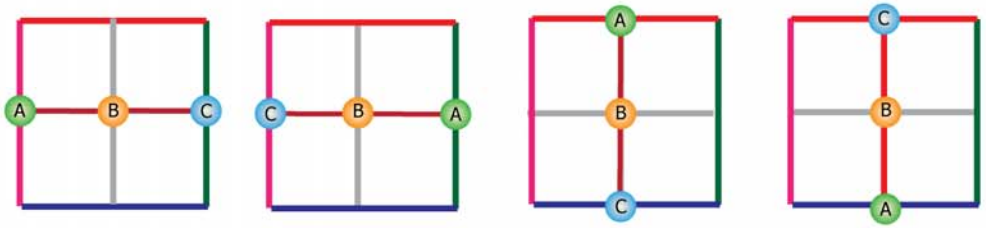
$$\epsilon_{Straight} = 0$$

$$\epsilon_{bent} = A$$

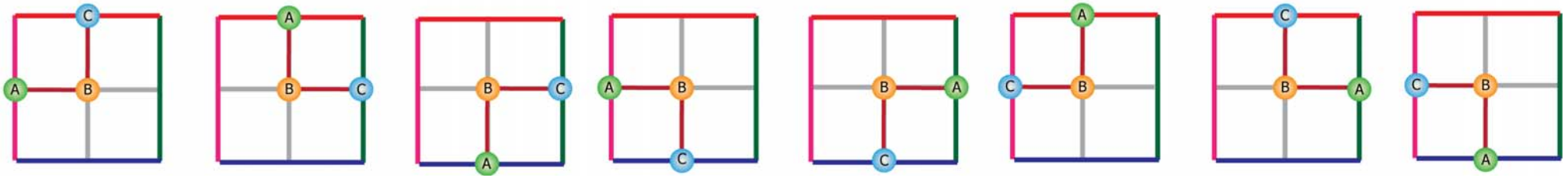
Calculate Entropy

$$S = k_B \ln W$$

Straight



Bent



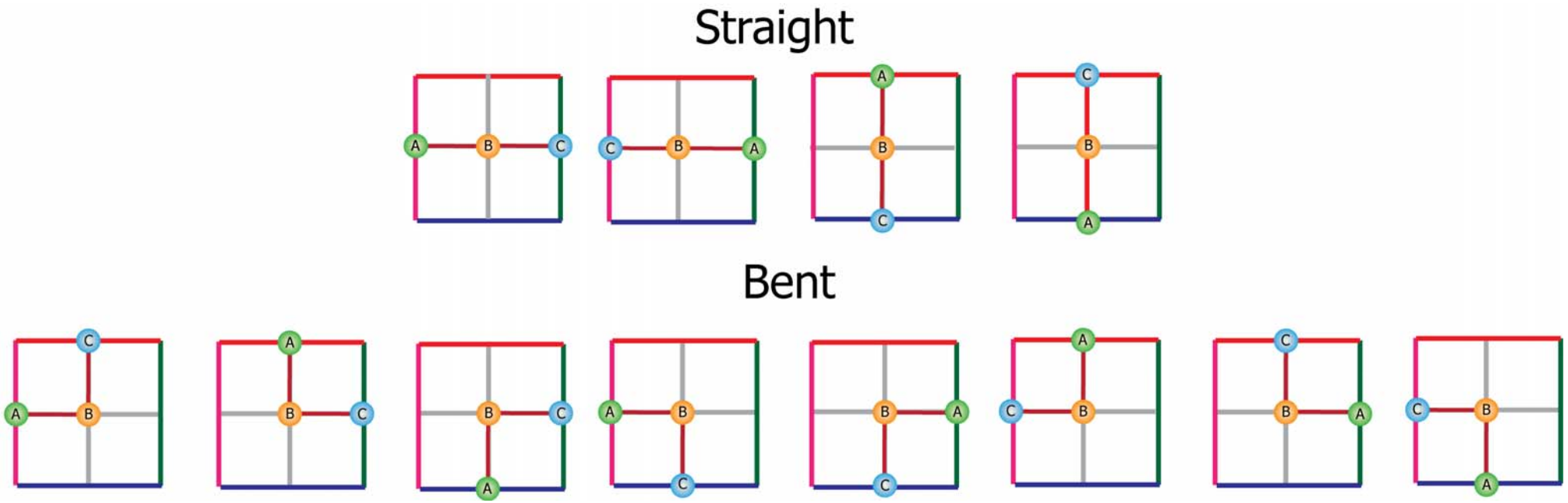
$$W_{Straight} = 4$$

$$S_{Straight} = k_B \ln 4 = 1.4\, k_B$$

$$W_{bent} = 8$$

$$S_{bent} = k_B \ln 8 = 2.1\, k_B$$

Calculate Free Energy



$$G_{Straight} = 0 - 1.4\, k_B\, T$$

$$G_{bent} = \mathrm{A} - 2.1\, k_B\, \mathrm{T}$$

Bent or Straight

$$G_{Straight} = 0 - 1.4\, k_B\, T$$

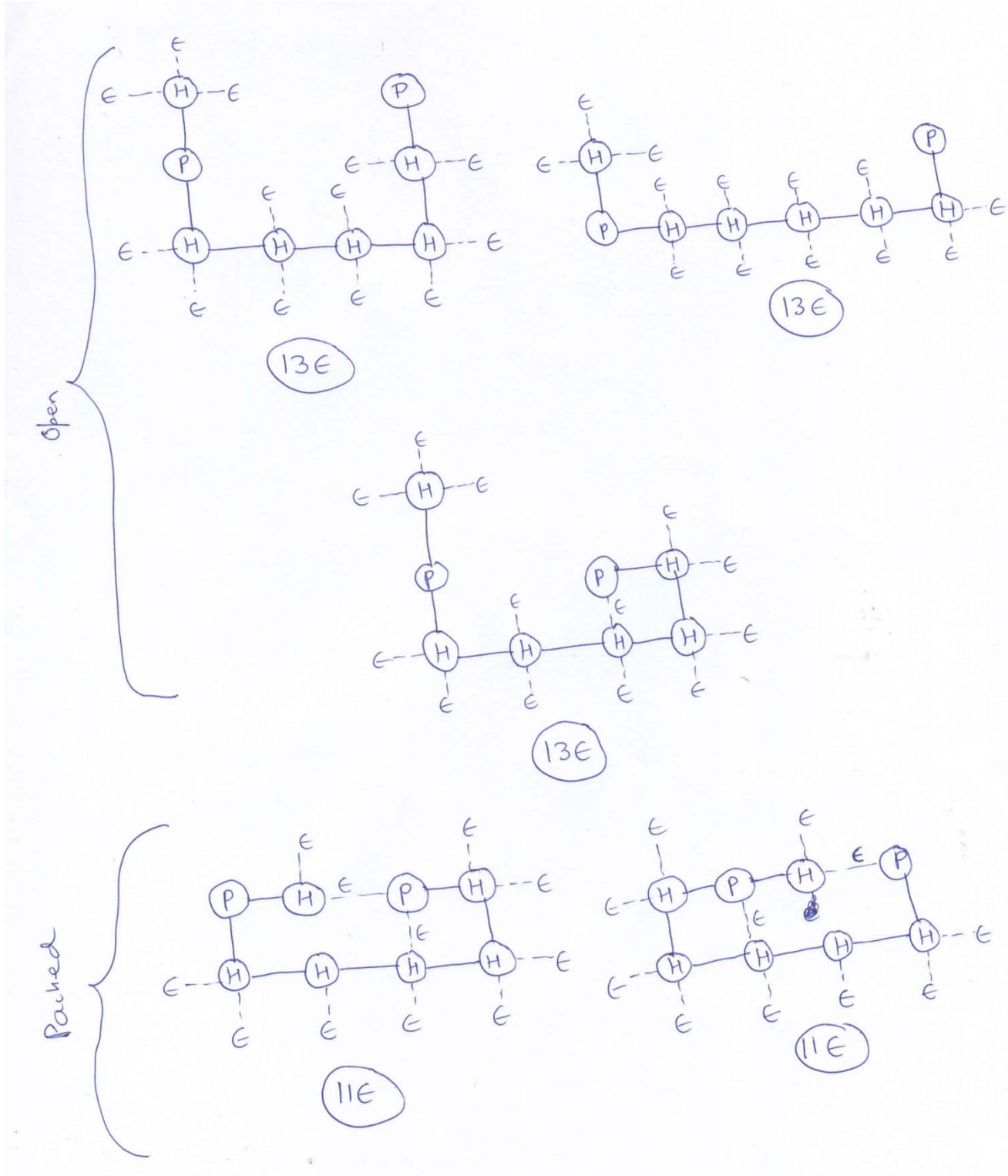
$$G_{bent} = \mathrm{A} - 2.1\, k_B\, \mathrm{T}$$

- When $A = 0.1\, k_B\, \mathrm{T}$ bent structure is preferred

- However, when $A = 1.5\, k_B\, \mathrm{T}$, straight structure is preferred

Summary

- Proteins and their structures
- Proteins are free energy minimizers
- Microstate and Macrostate
- Relations $G = H - TS$ and $G = -k_B T ln Z$
- $\mathrm{S} = k_B \ln W$
- HP model and a Toy model of protein folding


Open
13ϵ
13ϵ
13ϵ
Packed
11ϵ
11ϵ