LECTURE 8

BB 101: MODULE II

PHYSICAL BIOLOGY

Review of Lecture 7

- Microtubules and Actin filaments are polymers of tubulin and actin monomers
- Dynamics of Microtubule and Actin Filaments
- Treadmilling of microtubule and actin filament
- A simple model for cytoskeletal filament polymerization, treadmilling and dynamic instability

Proteins and their structures

- Tubulin and actin monomers are protein molecules
- Proteins can be of different shapes

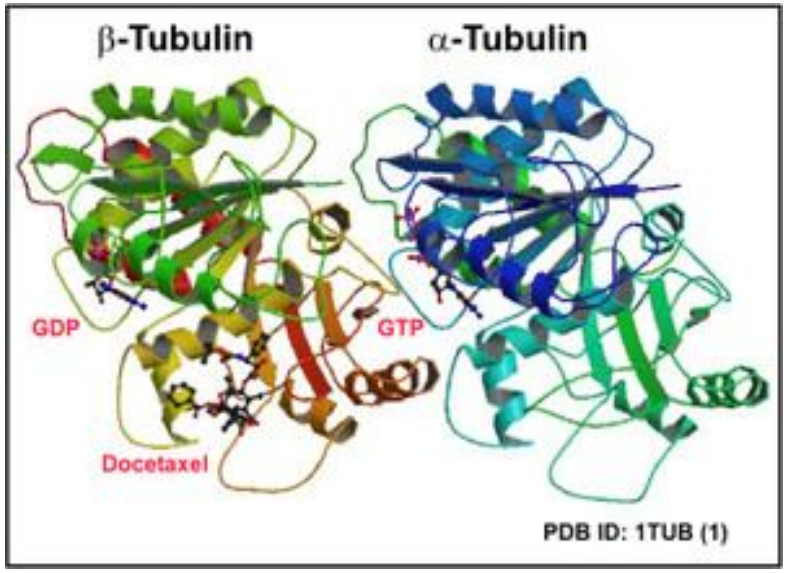


Figure Source: http://www.mastcell-basophil.net/wiki/wiki-start/microtubules-and-mast-cell-signaling/

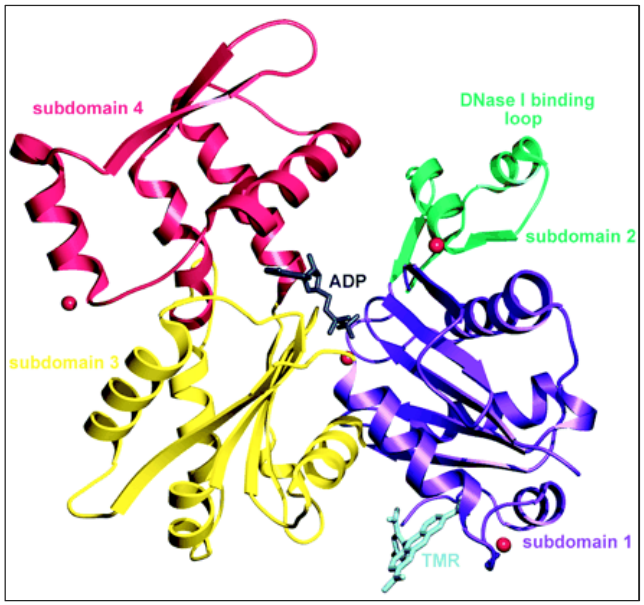


Figure Source: Otterbein et al., Vol. 293, 708-711 (2001)

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?

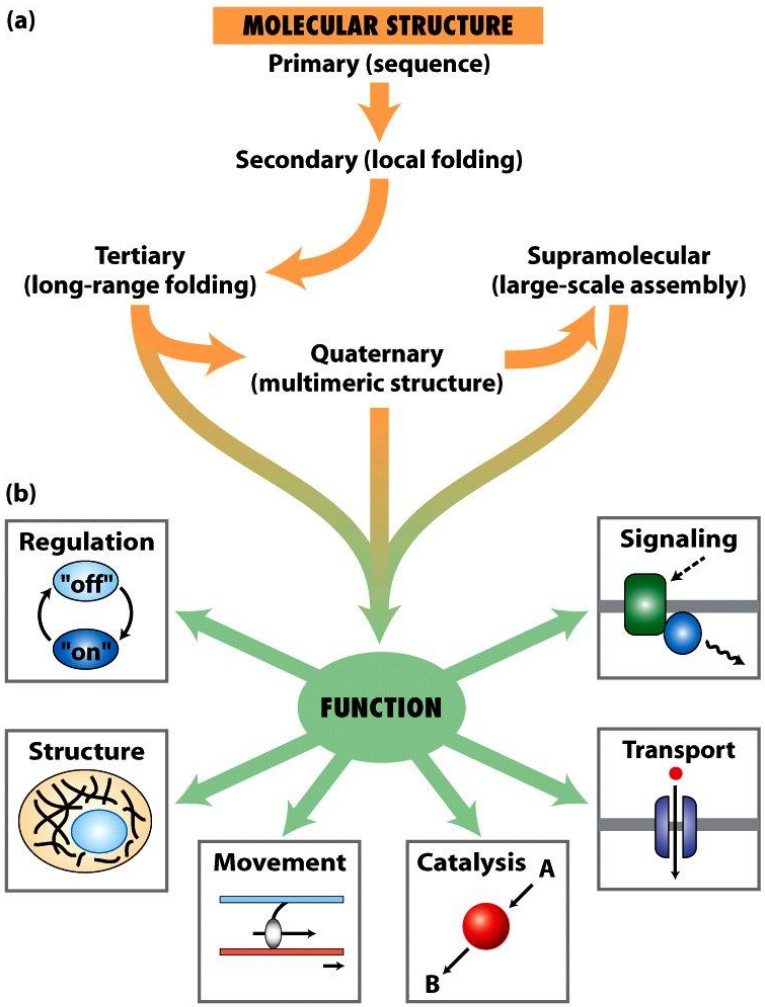


Figure 3-1
Molecular Cell Biology, Sixth Edition

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

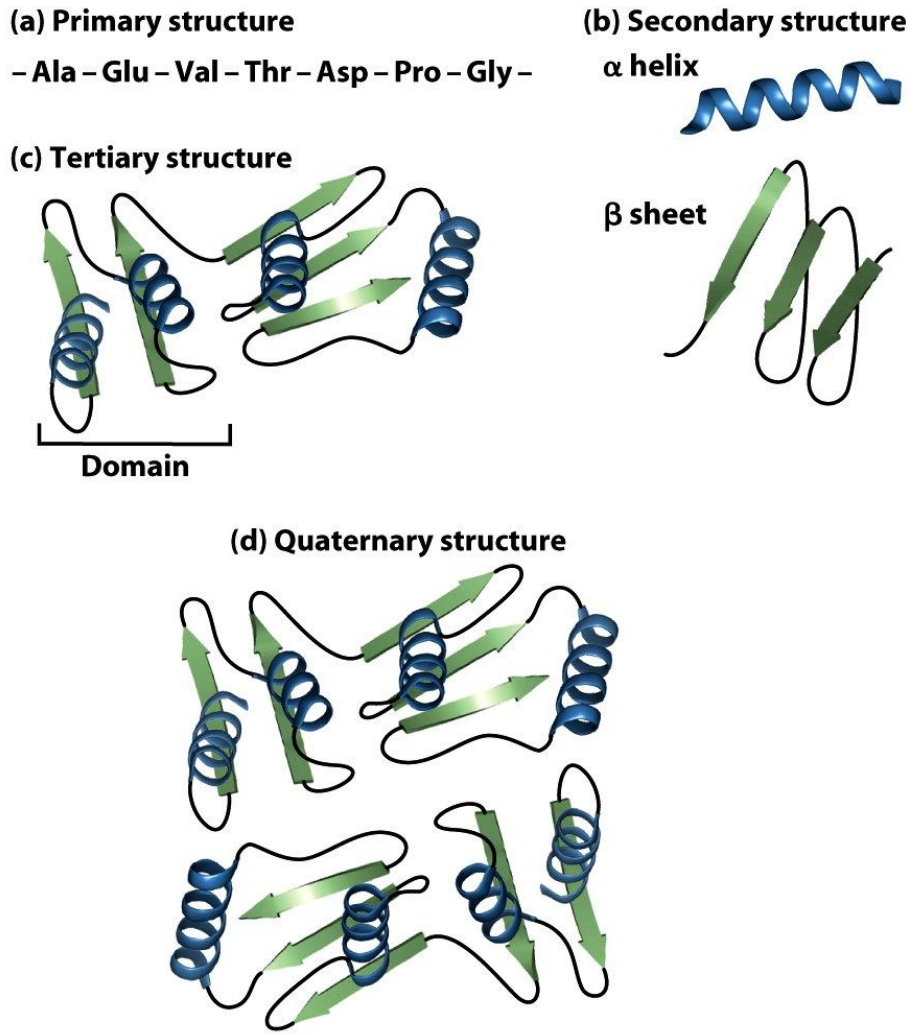


Figure 3-2
Molecular Cell Biology, Sixth Edition

Proteins and their structures

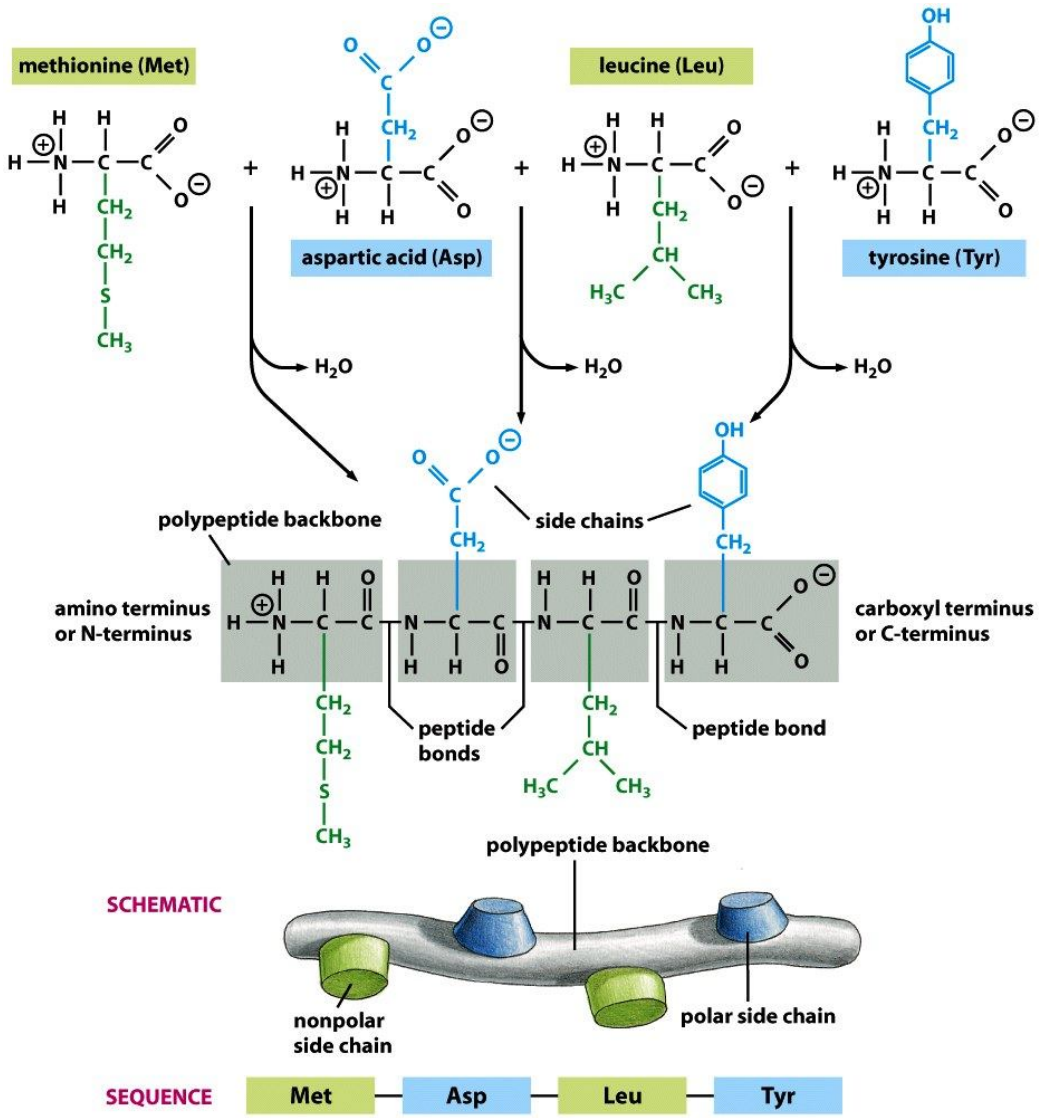


Figure 3-1 *Molecular Biology of the Cell* (© Garland Science 2008)

Proteins and their structures

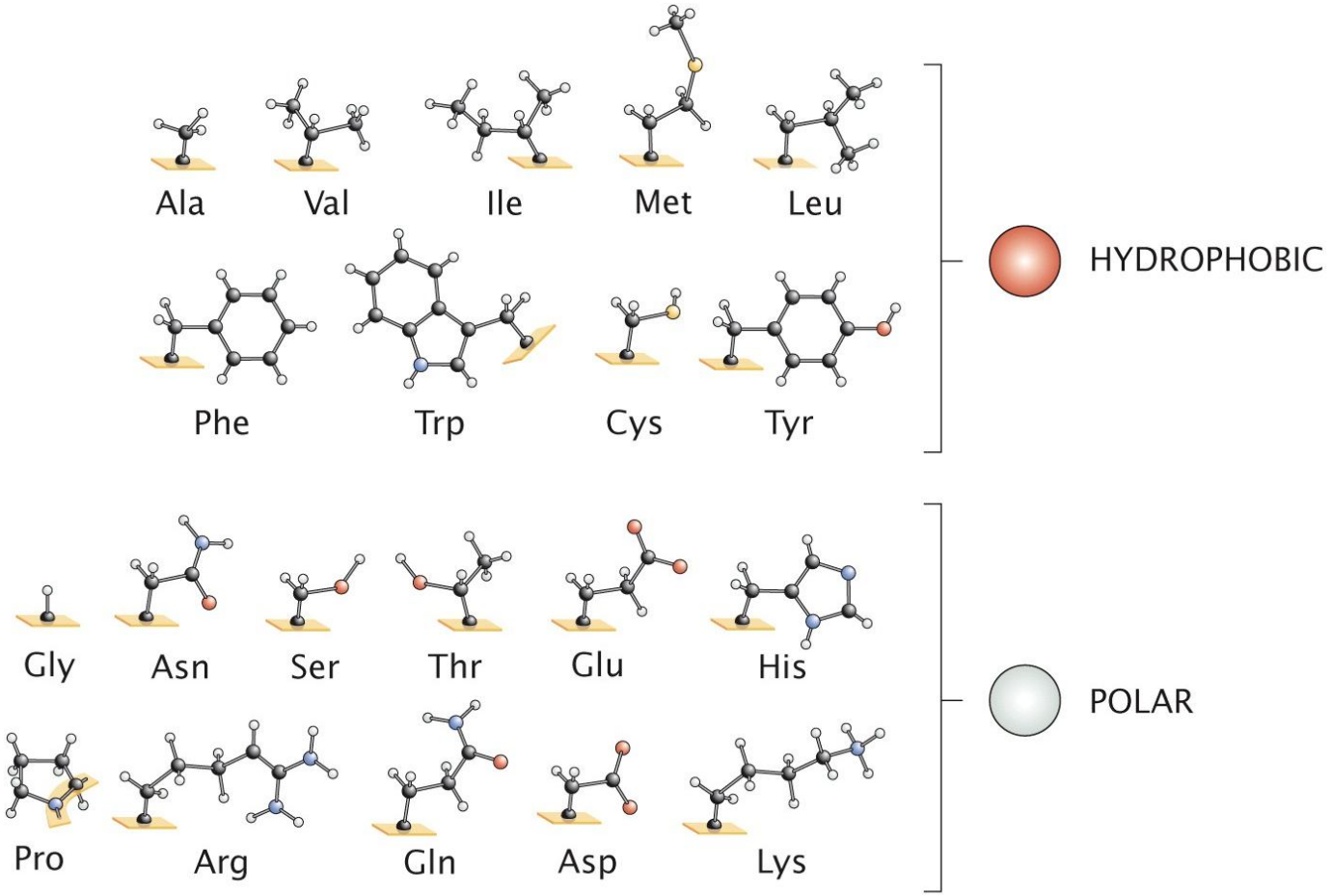


Figure 8.28 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

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

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

Micro-state and Macro-state

- 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")**

Micro-state and Macro-state

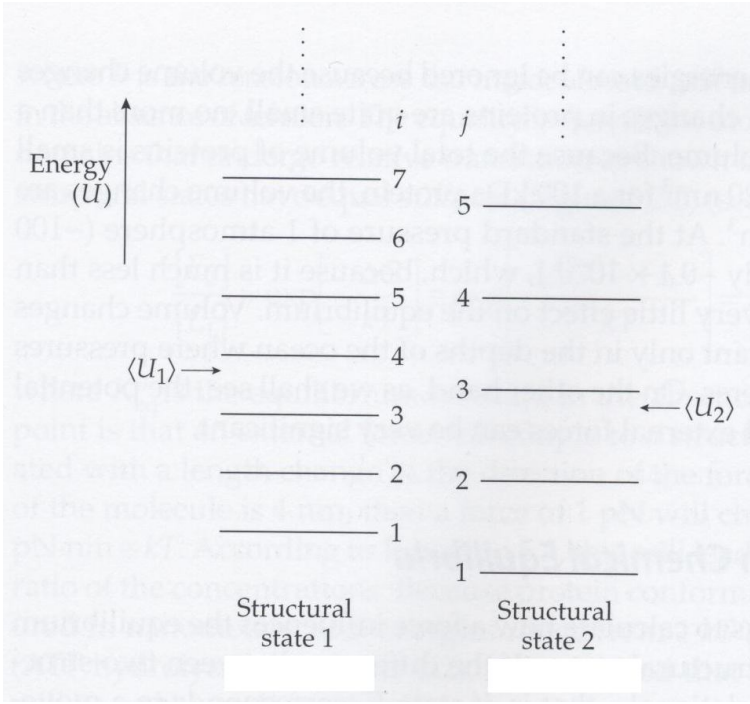
- Consider a protein that can exist in two **structural states.** For example open (1) and closed (2) ion channel protein.

- 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

$$\boxed{p(X) = \frac{1}{Z} e^{-\frac{G(X)}{k_B T}}} \qquad G(X) = -k_B T ln \left(\sum_{iX} e^{-\frac{U_i}{k_B T}} \right)$$

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

Proof of $G = -k_B T ln Z$

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

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

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

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

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

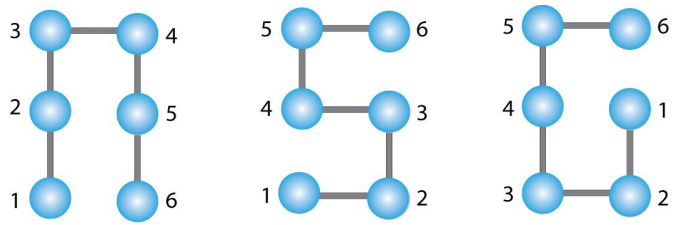
So far…

- 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 microstate X is given by

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

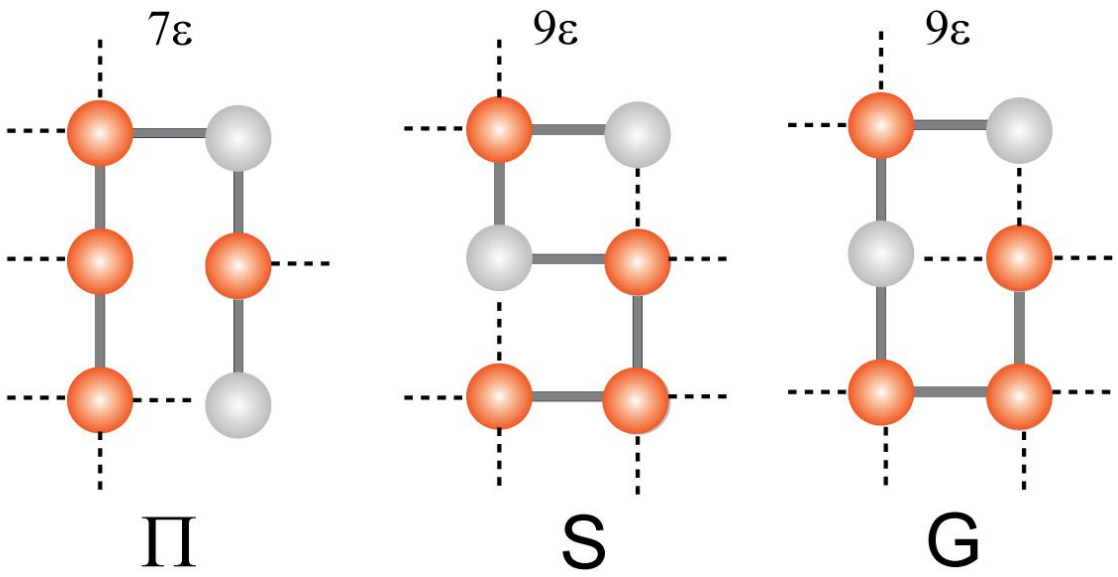
HP models of protein folding

- Consider a protein consisting of six amino acids consisting of H and P
- The sequence of the amino acids is HHHPHP
- Suppose that can fold into three possible structures Π, S and G such that energy increases by ε for every contact of H with either P or solvent molecule
- What will be the structural state of the protein?



Π S G

HP Models of Protein Folding



$$G_{\prod} = 7\varepsilon - k_B T \ ln\ 1$$

$$G_{others(S\ or\ G)} = 9\varepsilon - k_B T \ ln\ 2$$

Π Structure will be preferred as long as $G_{\prod} < G_{others}$

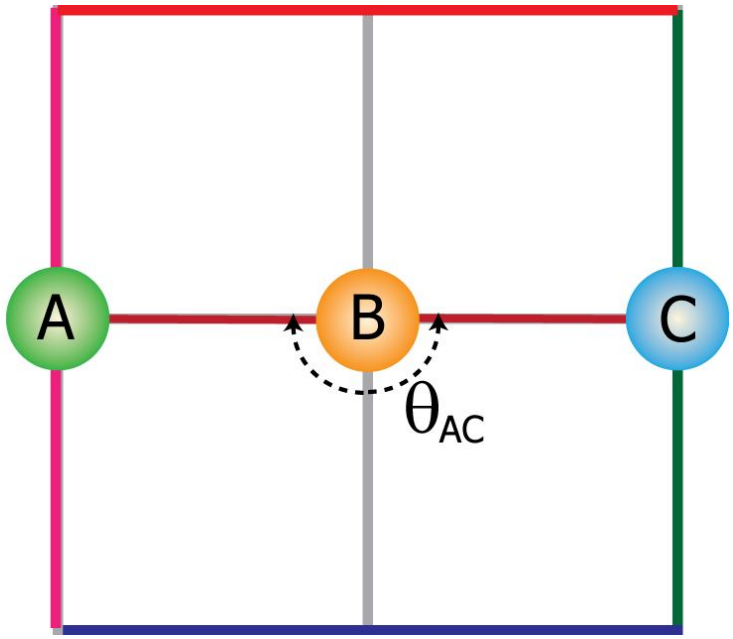
Or, $7\varepsilon - k_B T \ ln\ 1 < 9\varepsilon - k_B T \ ln\ 2$

Or, $T < \left(\frac{\varepsilon}{k_B}\right)\left(\frac{2}{ln2}\right)$

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



Another Toy Models of Protein Folding

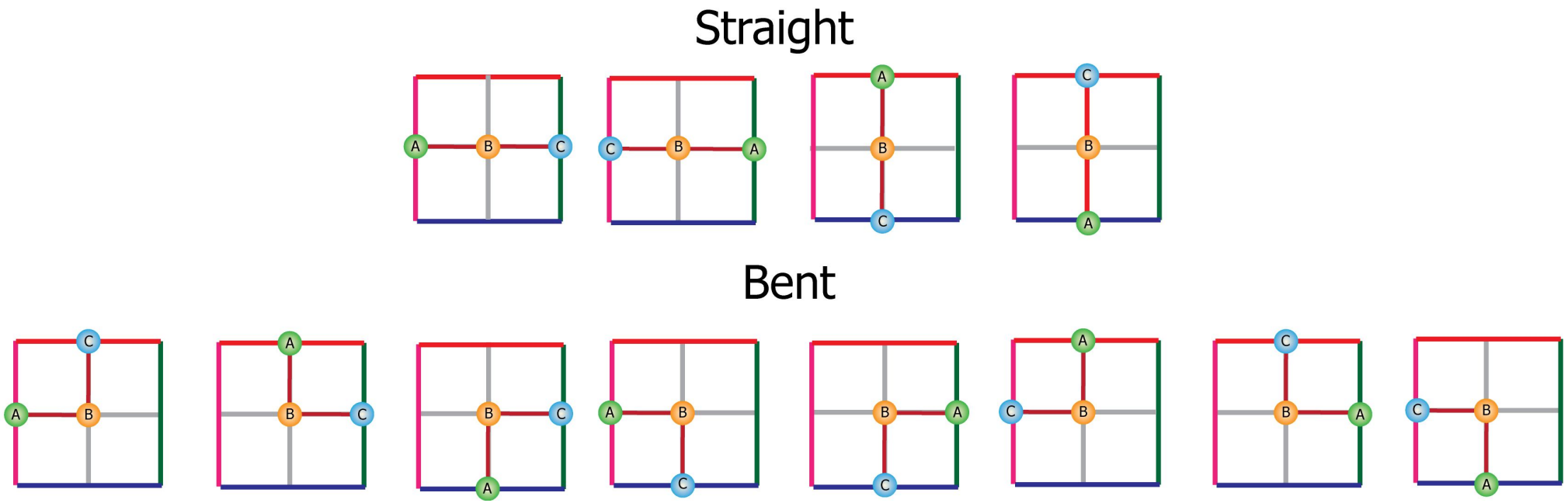
- Bending energy of the protein is given by

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

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

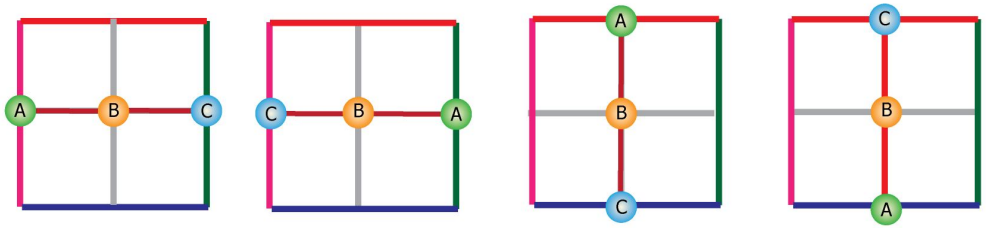


- No. of straight conformations=4
- No. of bent conformations=8

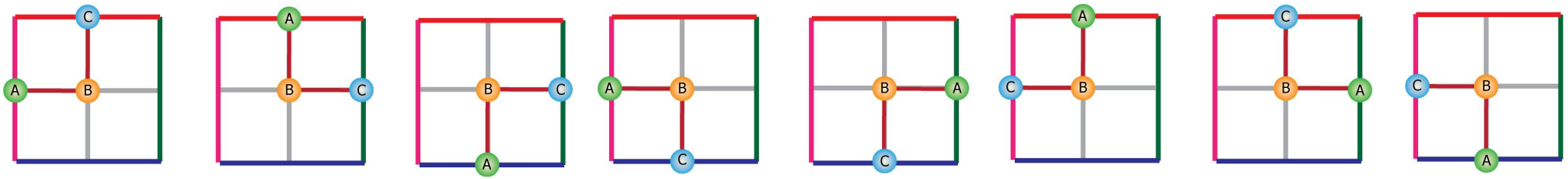
Calculate Average Bending Energy

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

Straight



Bent



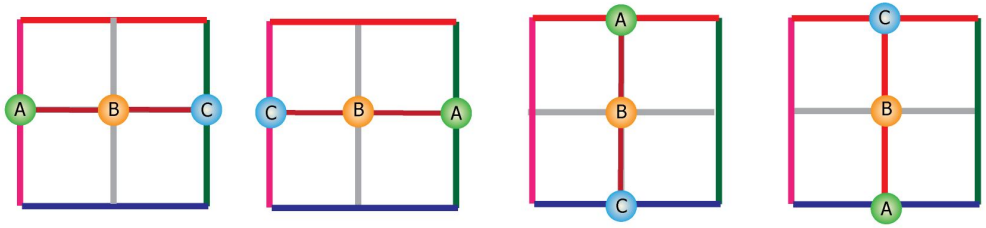
$$\epsilon_{Straight} = 0$$

$$\epsilon_{bent} = A$$

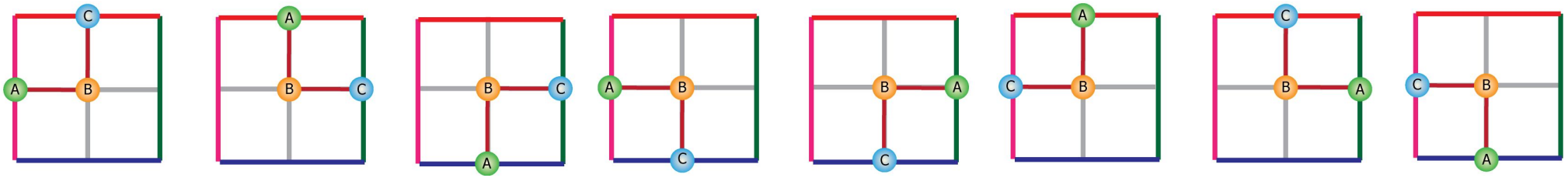
Calculate Entropy

$$S = k_B \ln W$$

Straight



Bent



$$W_{Straight} = 4$$

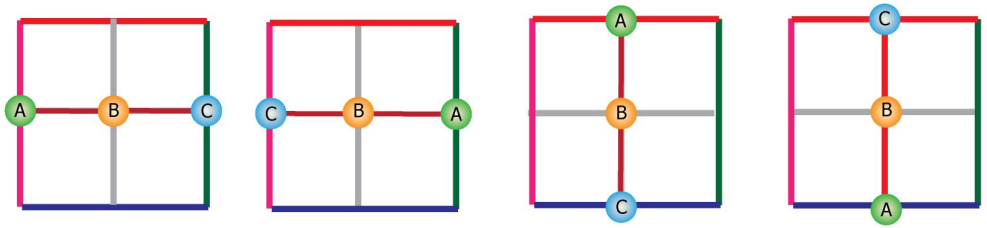
$$S_{Straight} = k_B \ln 4 = 1.4\, k_B$$

$$W_{bent} = 8$$

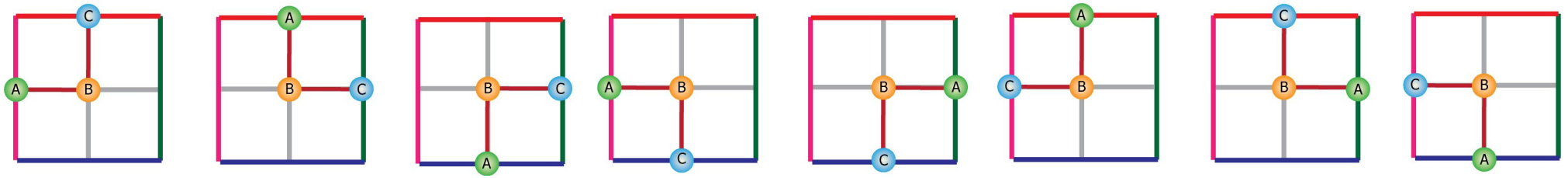
$$S_{bent} = k_B \ln 8 = 2.1\, k_B$$

Calculate Free Energy

Straight



Bent



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

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

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

Protein folding in reality

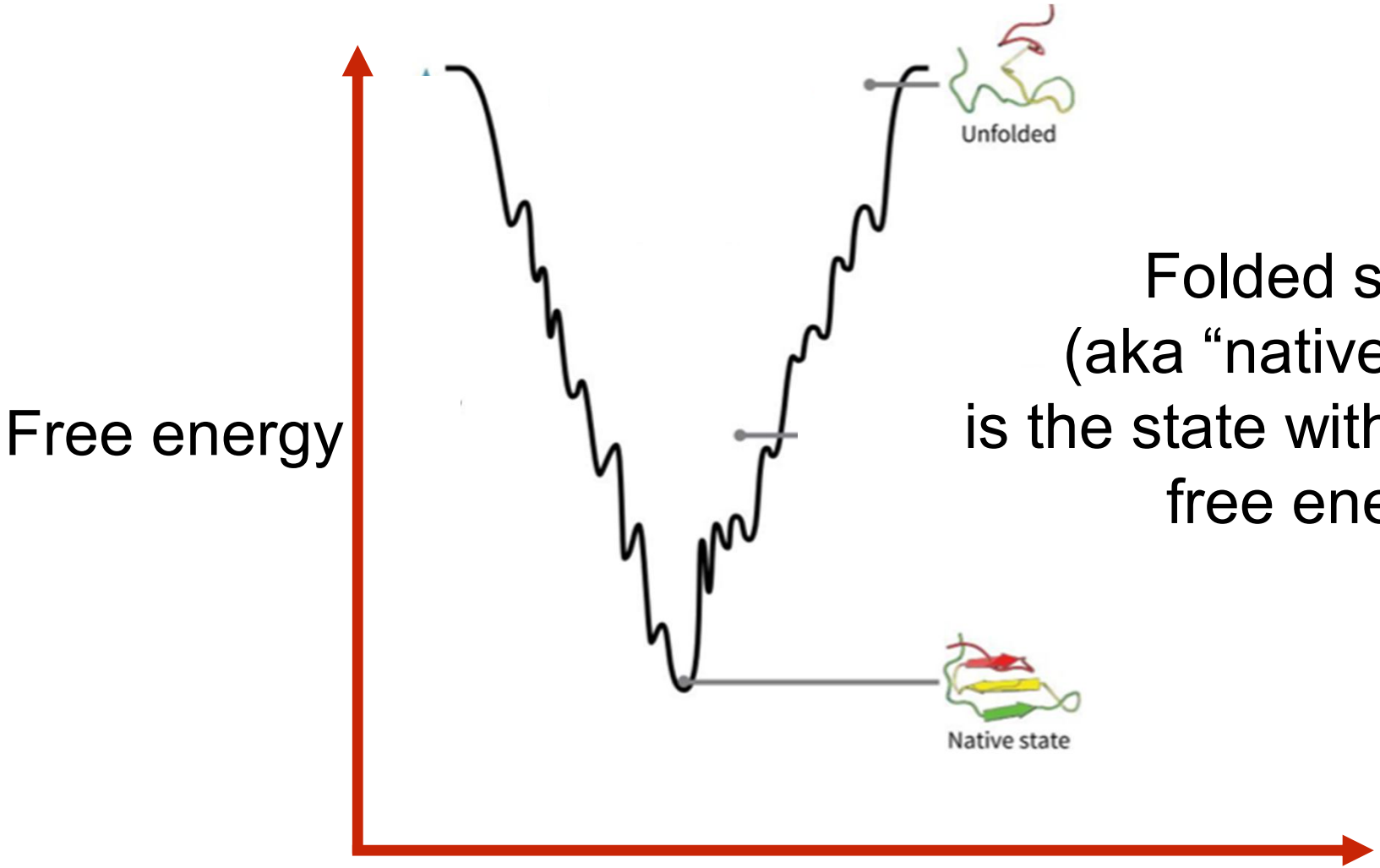
Here we considered only toy models for protein folding

However, in reality

- Protein monomers have many types of interaction: electrostatic, bending, Van der Waals etc
- Protein monomers interact with water (hydrophobic/hydrophilic)
- Energy/Enthalpy is more complicated than simple bending example we discussed
- One has to worry about entropy of the whole system (protein monomers+water+other ions like Na+ and Cl-)

Protein folding in reality

Typical proteins “see” such a free energy landscape

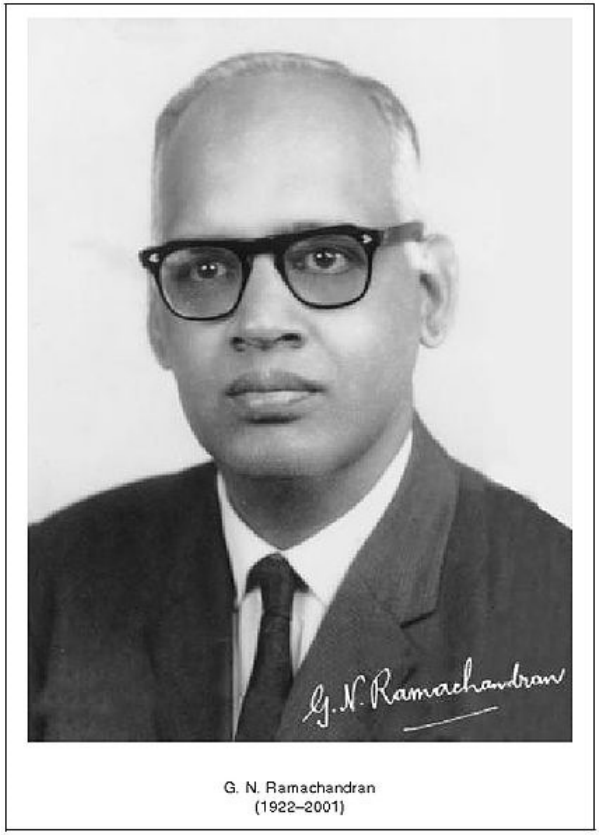


Folded state
(aka “native state”)
is the state with minimum
free energy

Ramachandran Plot

About 50 years ago, G. N. Ramachandran, an Indian Physicist, made a famous discovery on proteins

Ramachandran and his colleagues said that, due to various constraints of arrangements of atoms in 3D, neighboring amino acids (protein monomer) in a protein can't fold into any shape — there are some constraints that their arrangements have to satisfy

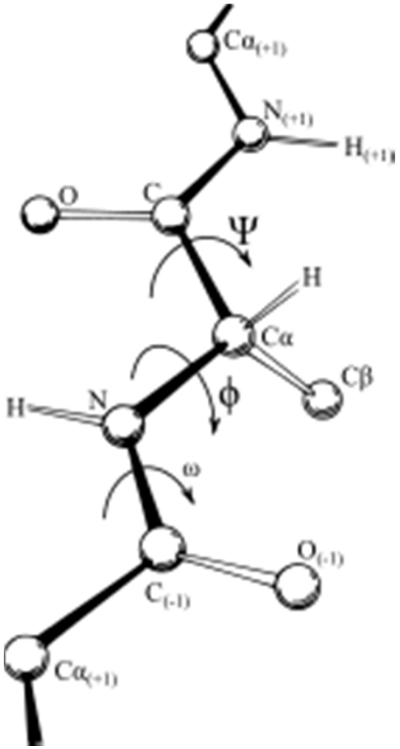


G. N. Ramachandran
(1922–2001)

Figure Sources: http://en.wikipedia.org/wiki/Ramachandran_plot

Ramachandran Plot

The set of “allowed” angles can be plotted: This plot is called the “Ramachandran Plot”



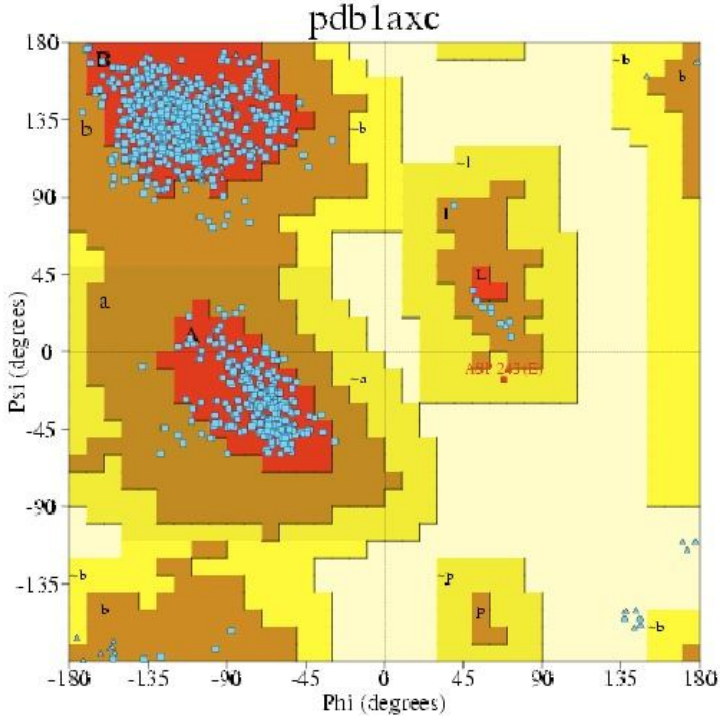


Figure Sources: http://en.wikipedia.org/wiki/Ramachandran_plot

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
- Some aspects of real protein folding