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in the name of god

Three-Dimensional Structure

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PROTEIN FOLDING AND STABILITY

Incredible as it may seem, thermodynamic measurements indicate that *native proteins are only marginally stable under physiological conditions*. The free energy required to denature them is **~0.4 kJ.mol^{-1} per amino acid residue**, so a fully folded 100-residue protein is only about 40 kJ.mol^{-1} more stable than its unfolded form ***(for comparison, the energy required to break a typical hydrogen bond is ~20 kJ .mol^{-1}).***

Forces That Stabilize Protein Structure

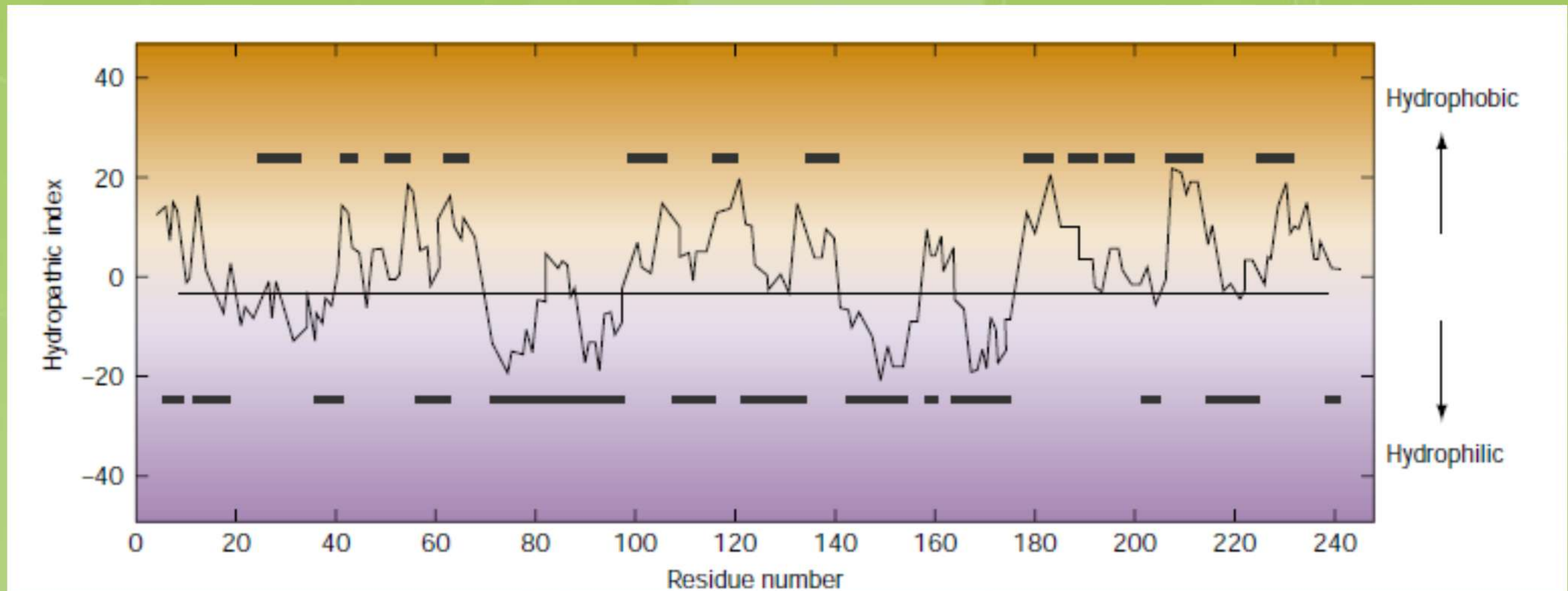
- The Hydrophobic Effect
  - The *aggregation of nonpolar side chains* in the interior of a protein is favored by the *increase in entropy of the water* molecules that would otherwise form ordered “cages” around the hydrophobic groups.
  - Hydropathy index
    - It is a number representing the hydrophobic or hydrophilic properties of side-chains of protein residues -proposed in 1982 by *Jack Kyte* and Russell *F. Doolittle*.

Hydropathy Scale for Amino Acid Side Chains

Side Chain	*Hydropathy*
Ile	4.5
Val	4.2
Leu	3.8
Phe	2.8
Cys	2.5
Met	1.9
Ala	1.8
Gly	−0.4
Thr	−0.7
Ser	−0.8
Trp	−0.9
Tyr	−1.3
Pro	−1.6
His	−3.2
Glu	−3.5
Gln	−3.5
Asp	−3.5
Asn	−3.5
Lys	−3.9
Arg	−4.5

Source: Kyte, J. and Doolittle, R.F., *J. Mol. Biol.* 157, 110 (1982).

Hydropathy plot



A hydropathic index plot for bovine chymotrypsinogen. The sum of the hydropathies of *nine consecutive residues* is plotted versus residue sequence number

Forces That Stabilize Protein Structure

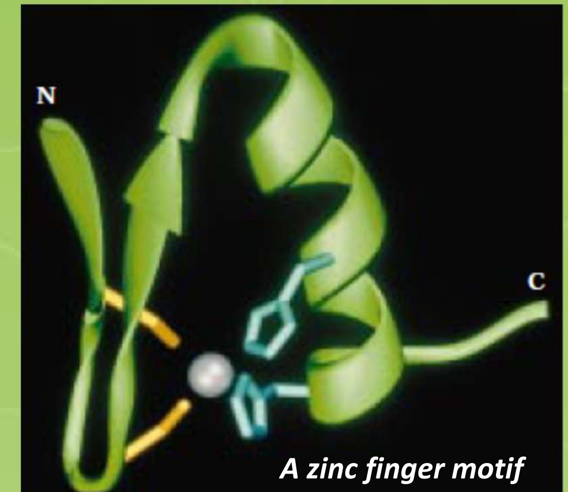
- *The Hydrophobic Effect*
- Electrostatic Interactions
  - van der Waals forces
  - hydrogen bonds
    - Hydrogen bonding fine-tunes tertiary structure by "**selecting**" the unique native structure of a protein from among a relatively small number of hydrophobically stabilized conformations
  - ion pair or salt bridge
    - About 75% of the charged residues in proteins are members of ion pairs that are located mostly on the protein surface

Forces That Stabilize Protein Structure

- *The Hydrophobic Effect*
- *Electrostatic Interactions*
- Chemical Cross-links
  - Disulfide bonds
    - maybe important for "locking in" a particular backbone folding pattern as the protein proceeds from its fully extended state to its mature form
    - Disulfide bonds are rare in intracellular proteins (***why?***)

Forces That Stabilize Protein Structure

- *The Hydrophobic Effect*
- *Electrostatic Interactions*
- Chemical Cross-links
  - Disulfide bonds
  - Metal ions cross-link proteins!
    - **ten** motifs collectively known as *zinc fingers* have been described in nucleic acid–binding proteins
    - These structures contain about 25–60 residues arranged around one or two Zn^{2+} ions that are tetrahedrally coordinated by the side chains of **Cys**, **His**, and occasionally Asp or Glu



A zinc finger motif

Protein Denaturation and Renaturation

- Heating
  - causes a protein's conformationally sensitive properties, such as optical rotation, viscosity, and UV absorption, to change abruptly over a narrow temperature range.
  - Such a sharp transition indicates that the entire polypeptide unfolds or "melts" ***cooperatively***, that is, nearly simultaneously.

Protein Denaturation and Renaturation

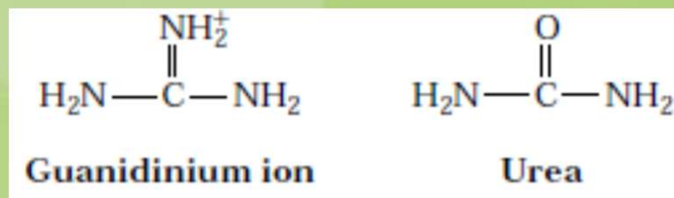
- *Heating*
- pH variations
  - alter the ionization states of amino acid side chains, thereby changing protein charge distributions and hydrogen bonding requirements.

Protein Denaturation and Renaturation

- *Heating*
- *pH variations*
- Detergents
  - associate with the nonpolar residues of a protein, thereby interfering with the hydrophobic interactions responsible for the protein's native structure.

Protein Denaturation and Renaturation

- *Heating*
- *pH variations*
- *Detergents*
- Chaotropic agents
  - They are ions or small organic molecules *that increase the solubility of nonpolar substances in water*
  - Their effectiveness as denaturants stems from their ability to disrupt hydrophobic interactions, although their mechanism of action is not well understood.



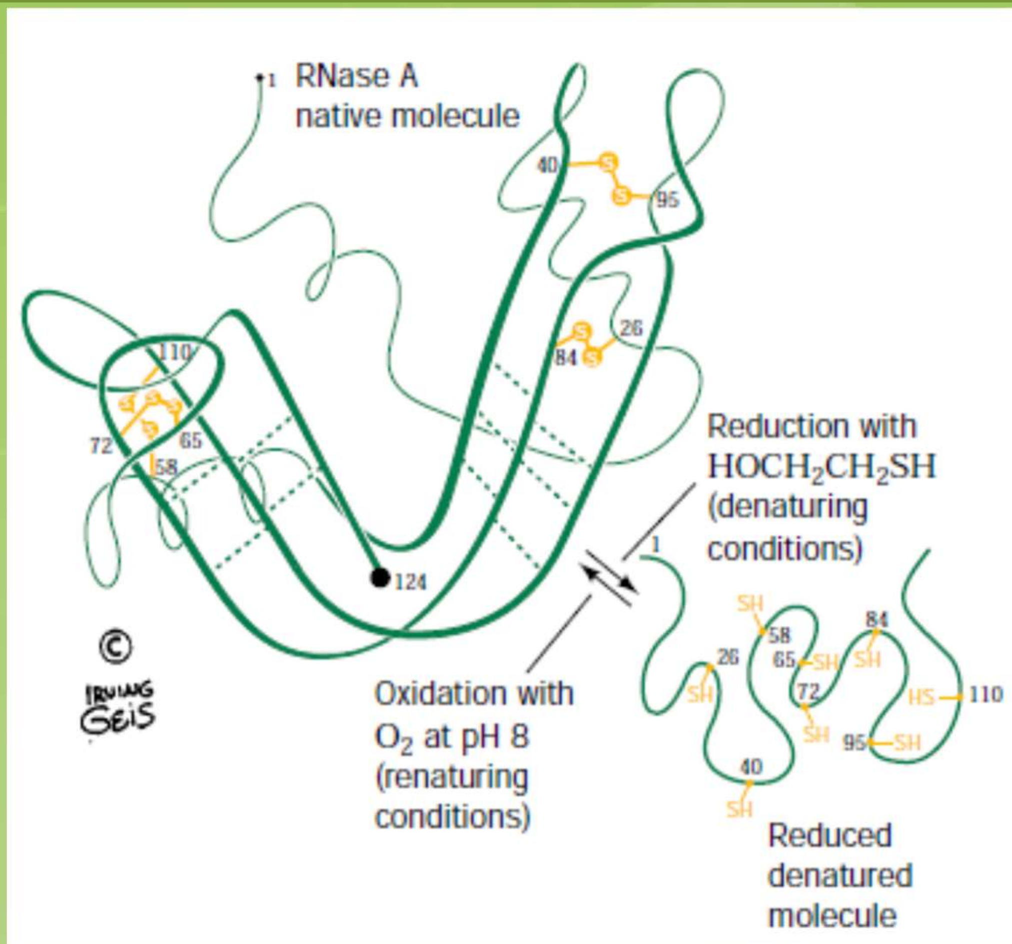
concentrations in the range 4 to 10 M

Denatured proteins can be Renatured

- In 1957, the elegant experiments of Christian Anfinsen on ribonuclease A (RNase A) showed that proteins can be denatured reversibly.
  - Denaturing condition: (*8M urea solution containing 2-mercaptoethanol*)
  - Renaturing condition: (*Dialyzing away the urea and reductant and exposing the resulting solution to O_2 at pH 8*)

Efficiency 100 % !!!

RNase A



The overall probability of RNase A reforming its four native disulfide links at random is:

$$\frac{1}{7} \times \frac{1}{5} \times \frac{1}{3} \times \frac{1}{1} = \frac{1}{105}$$

A 124-residue single-chain protein with 4 disulfide bond seems renature spontaneously !!!

- Anfinsen's work demonstrated that *proteins can fold spontaneously into their native conformations under physiological conditions*.
- This implies that a *protein's primary structure dictates its three-dimensional structure*.

Protein Folding Pathways

- *how* does a protein fold to its native conformation?
  - random exploration of all available conformations!

Protein Folding Pathways

- random exploration of all available conformations?
  - Assumptions:
    - an *n*-residue protein have 2^n torsion angles, ϕ and ψ
    - Each angel has three stable conformations

    $\therefore$ We will have $3^{2n} \cong 10^n$ possible conformations for the protein!

    $\therefore$ if the protein could explore a new conformation every 10^{-13} S (the rate at which single bonds reorient), the time t, in seconds, required for the protein to explore all the conformations available to it is:

$$t = \frac{10^n}{10^{13}}$$

- For a small protein of 100 residues, $t=10^{87}$ S, which is immensely greater than the apparent age of the universe *(20 billion years, or 6 X 10^{17} S)* !!!

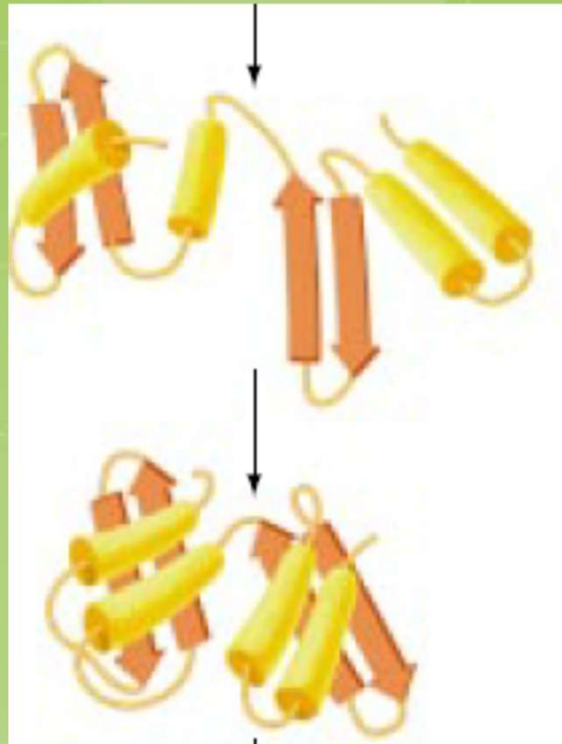
how does a protein fold to its native conformation?

1. Experimental observations indicate that protein folding begins with the formation of local segments of secondary structure (α helices and β sheets). This early stage of protein folding is extremely rapid, with much of the native secondary structure in small proteins appearing within *5 ms* of the initiation of folding.
2. Since native proteins contain compact hydrophobic cores, it is likely that the driving force in protein folding is what has been termed a hydrophobic collapse. The collapsed state is known as a **molten globule**, a species that has much of the secondary structure of the native protein but little of its tertiary structure.

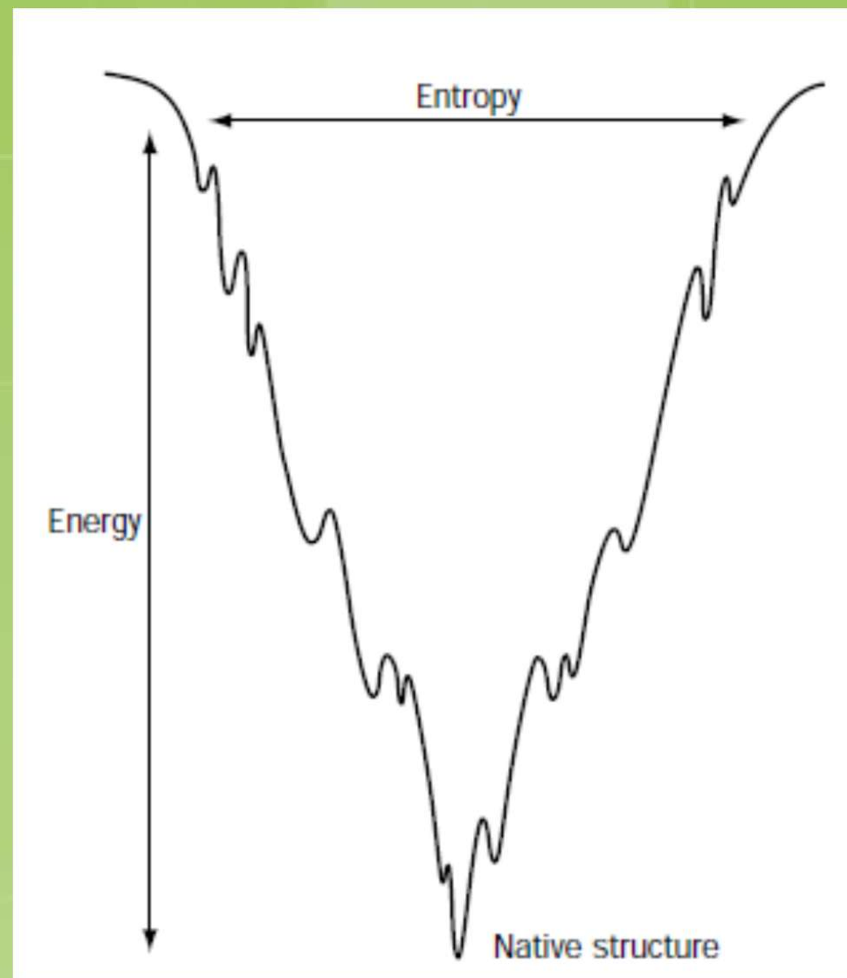
how does a protein fold to its native conformation?

3. Over the next 5 to 1000 ms, the secondary structure becomes stabilized and tertiary structure begins to form.
4. In the final stage of folding, which for small single-domain proteins occurs over the next few seconds, the protein undergoes a series of complex motions in which it attains its relatively rigid internal side chain packing and hydrogen bonding while it *expels the remaining water molecules from its hydrophobic core*.

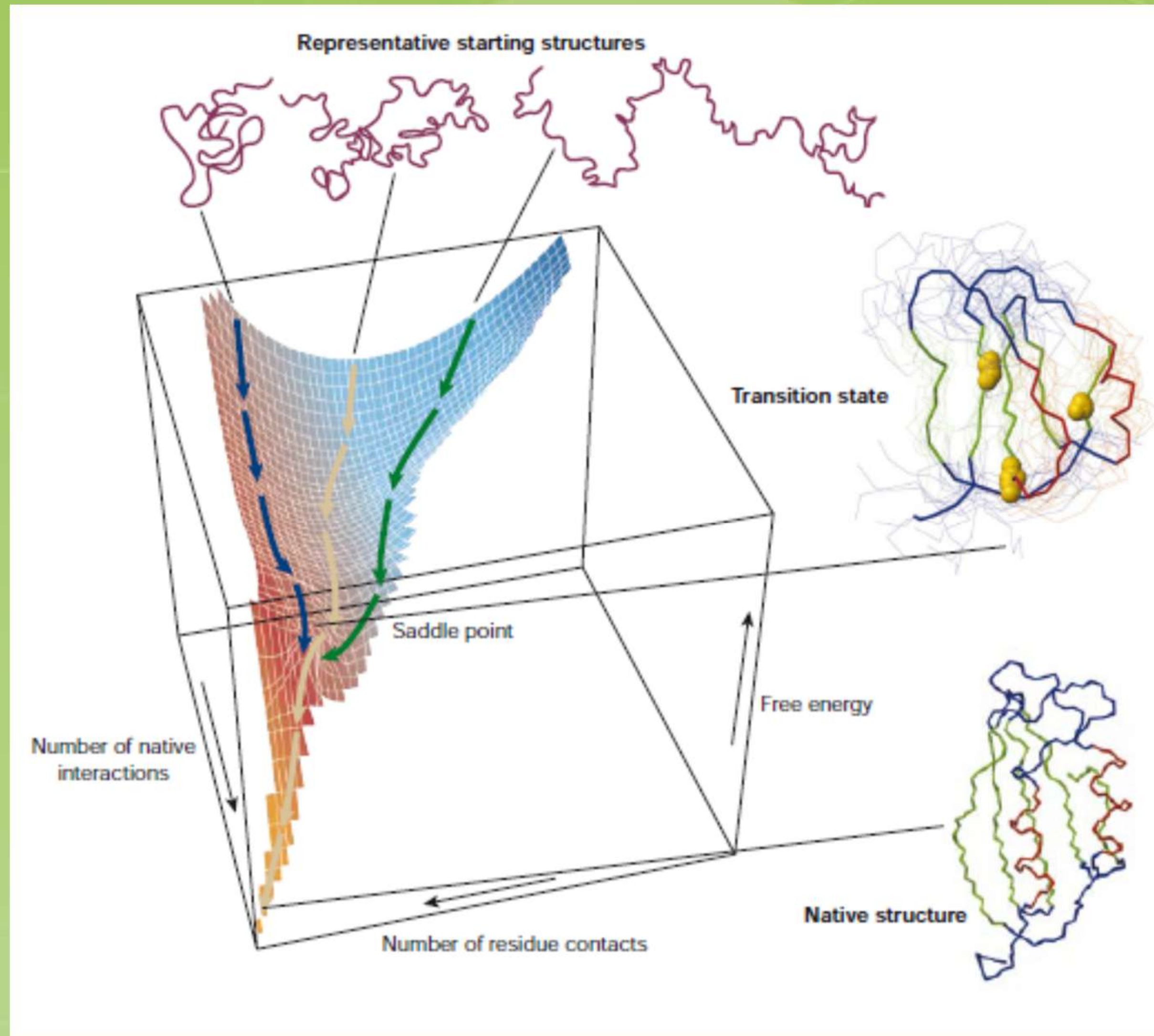
Hypothetical protein folding pathway



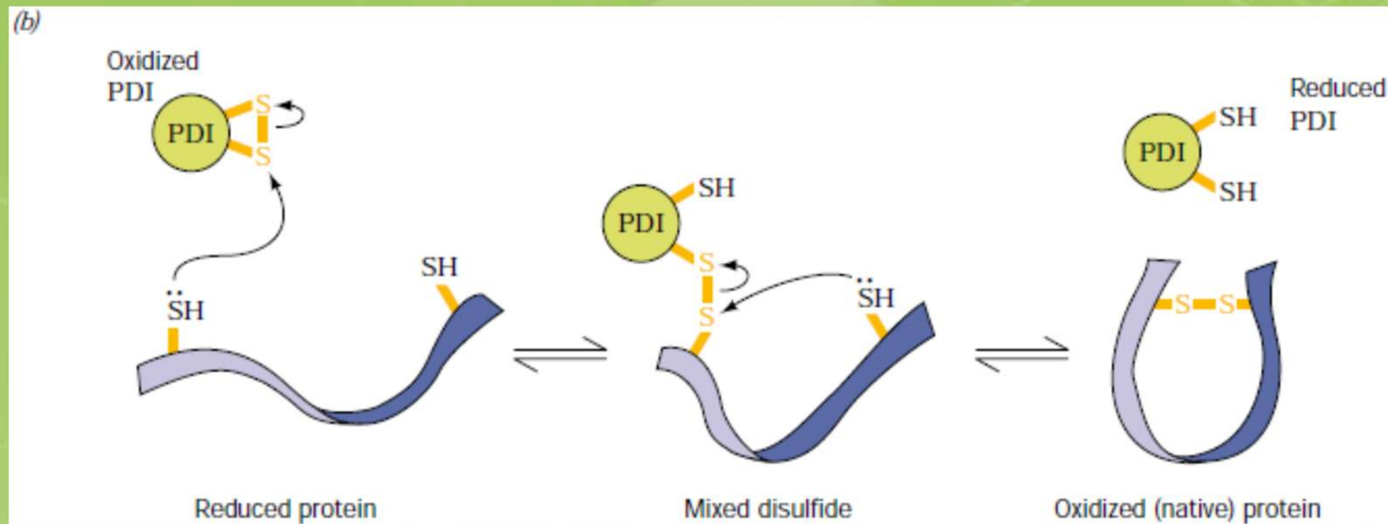
Energy–entropy diagram for protein folding.

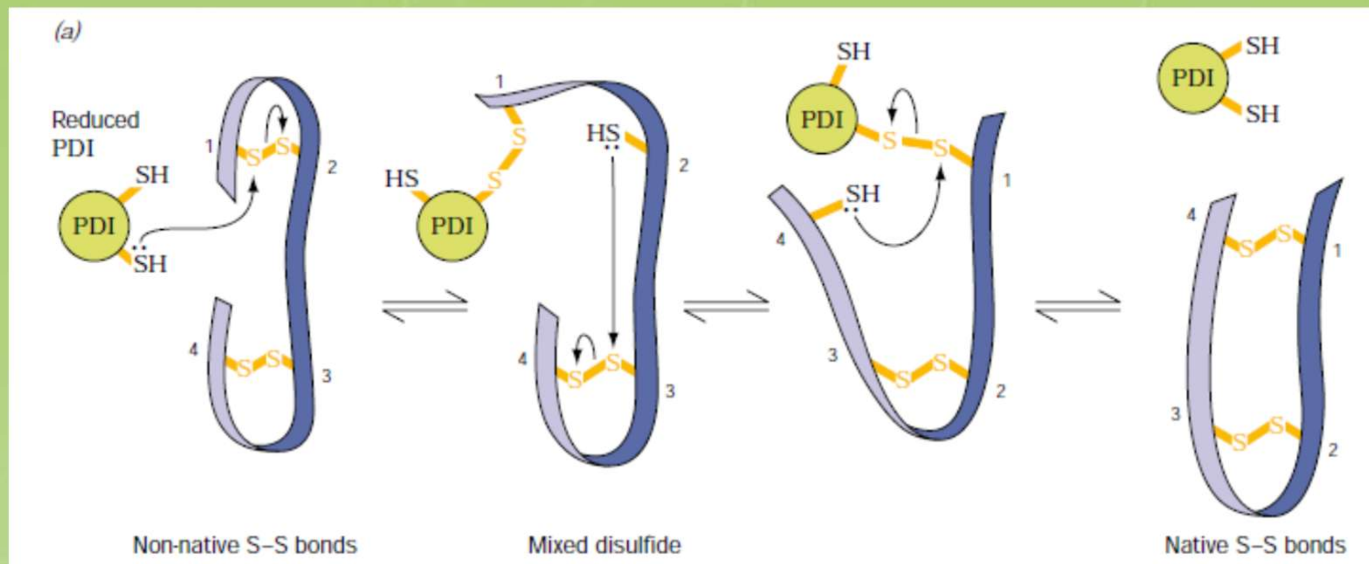


schematic energy landscape for protein folding



Protein Disulfide Isomerase (PDI)





GOOD LUCK ;)