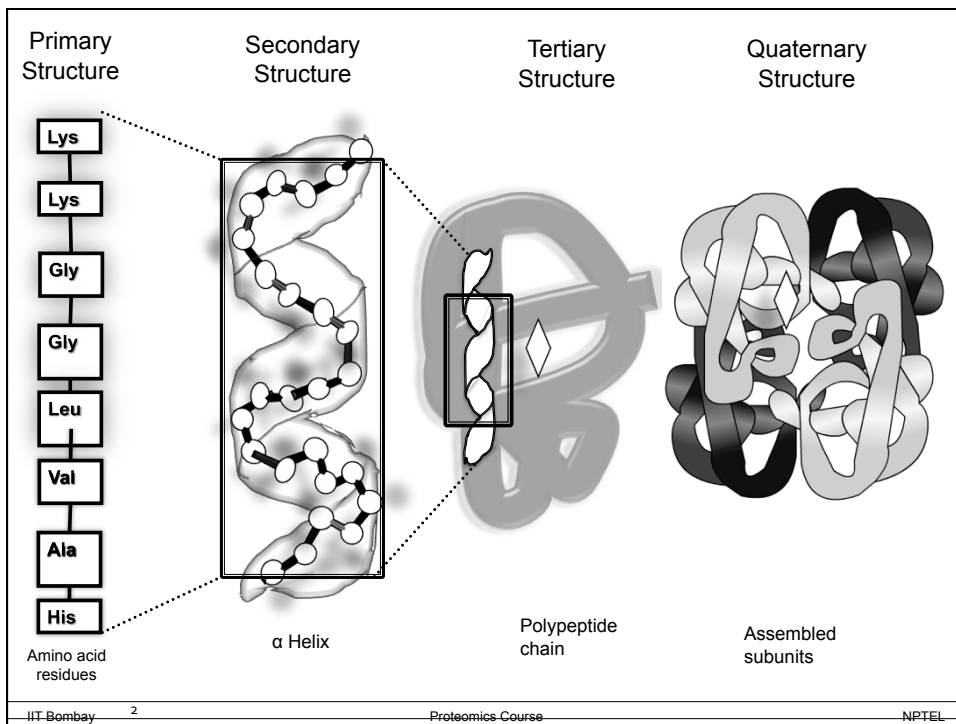


Proteomics Course

LECTURE-5 Proteins: Folding and misfolding



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IIT Bombay



Lecture outline

- Amino acid sequence determines 3-D structure
- Protein folding
- Thermodynamics of protein folding
- Molecular chaperone for protein folding
- Protein misfolding; diseases

Amino acid sequence determines 3-D structure

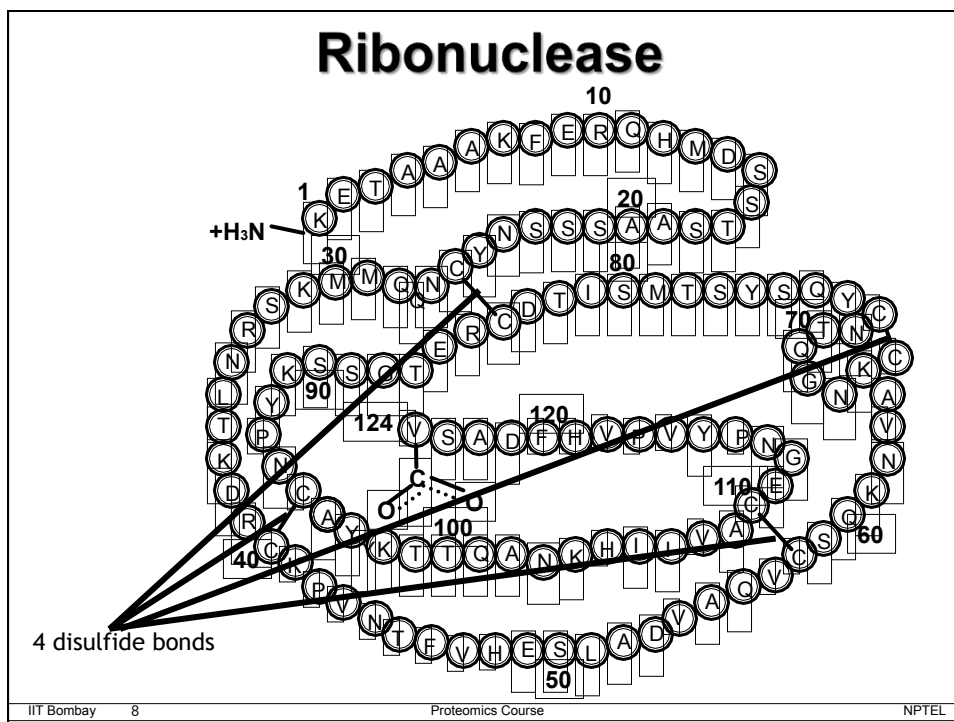
Anfinsen experiment

Proof for relation between amino acid sequence and its conformation

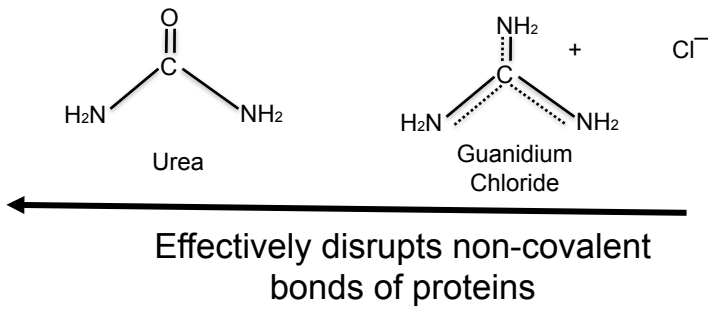
- Anfinsen's experiment
 - Denaturation
 - Refolding

Anfinsen's experiment

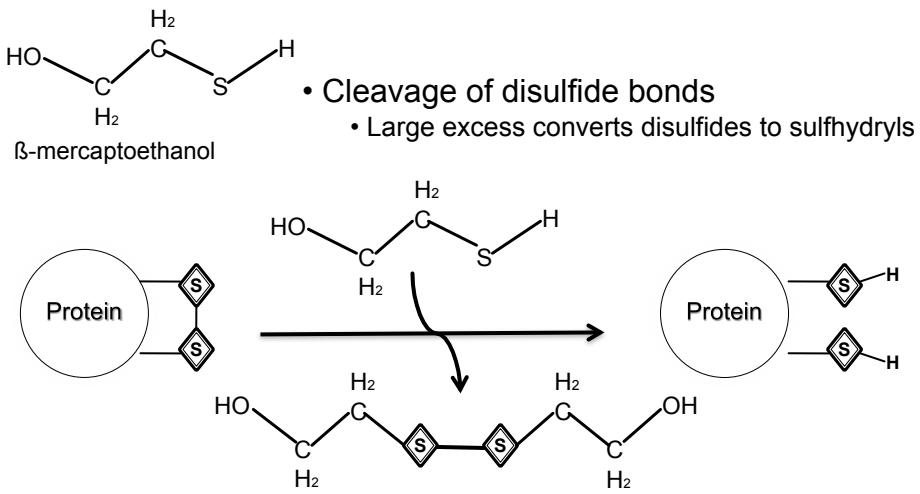
- Ribonuclease A
- Urea, guanidine HCl
 - denaturants
- β -mercaptoethanol
 - breaks disulfide bonds



Role of Urea and Guanidinium Chloride



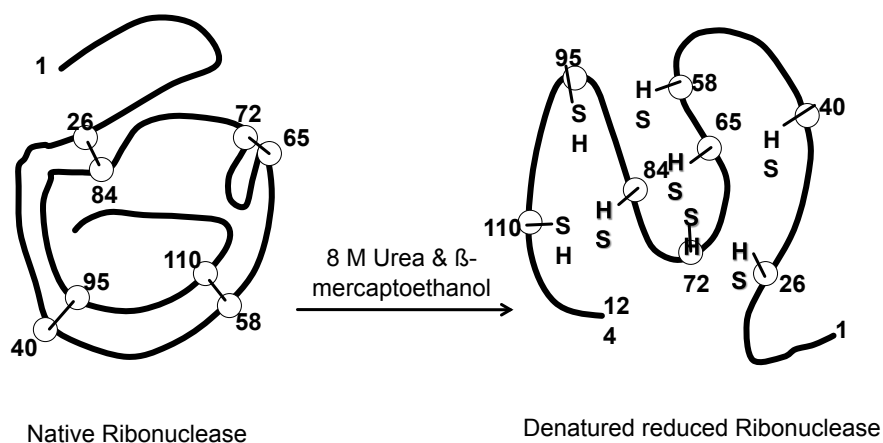
Role of β -mercaptoethanol



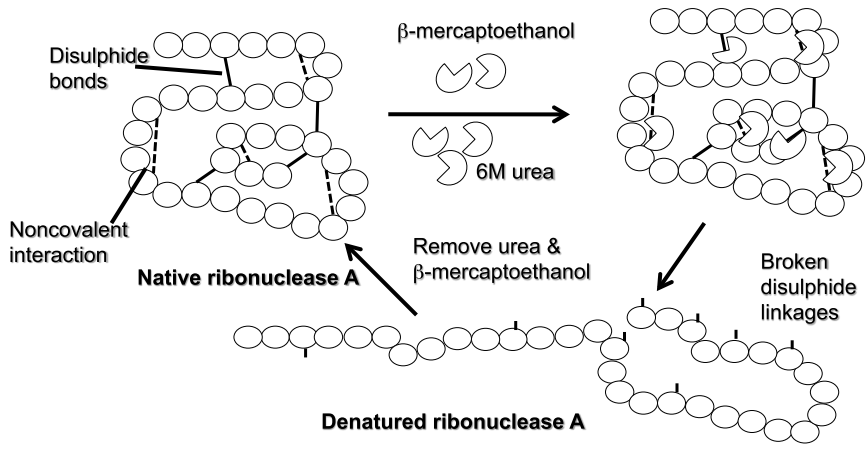
Anfinsen's experiment

- 8 M urea and β -mercaptoethanol treatment
 - converted native protein to fully reduced, randomly coiled polypeptide "denatured"
 - lacked enzymatic activity

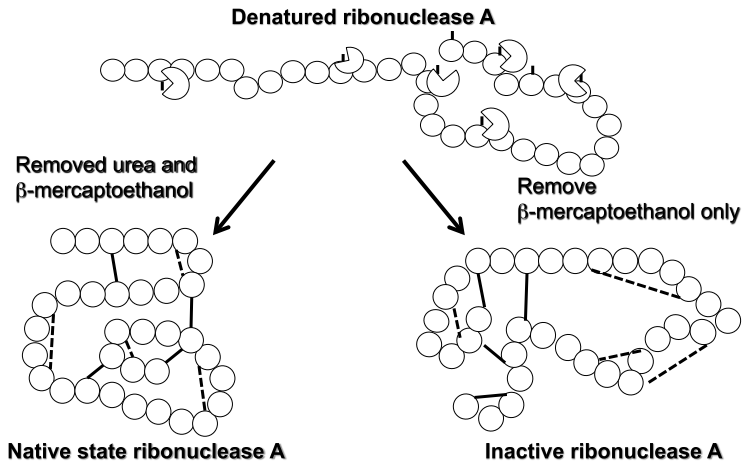
Anfinsen's experiment (2)



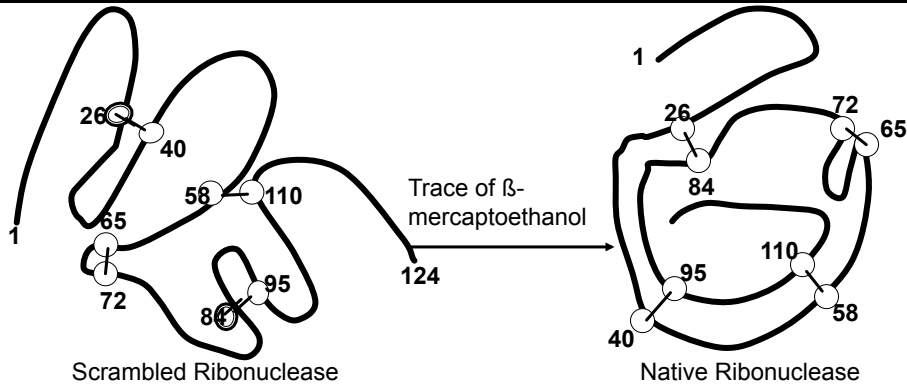
Anfinsen's experiment (3)



Anfinsen's experiment (4)



Anfinsen's experiment (5)



- Scrambled – wrong pairings, 104
- Trace amount of β -mercaptoethanol catalyzed rearrangement of disulfide pairing

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Anfinsen's experiment (6)

- Urea and β -mercaptoethanol removed by dialysis
- Denatured ribonuclease regained activity
- Enzyme refolded into active form
- Sulfhydryl groups became oxidized by air

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Protein folding

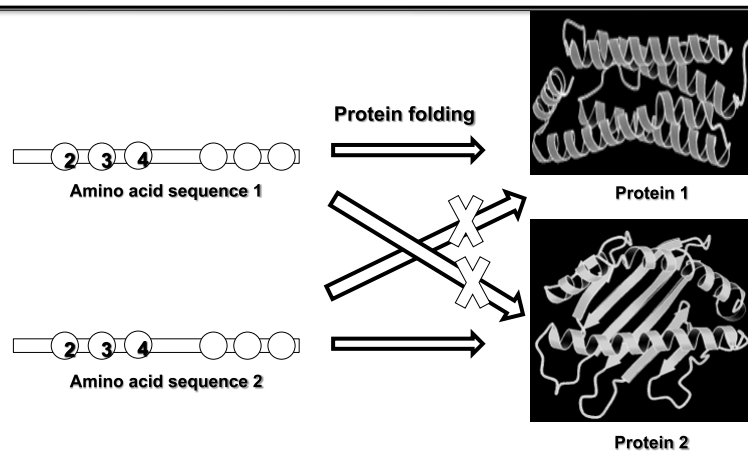
Horwich et al. 2002

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Protein folding



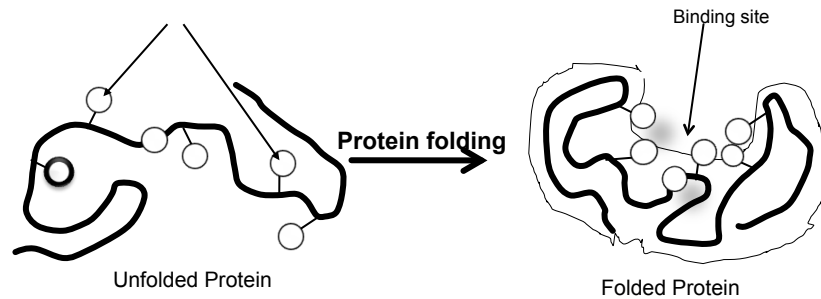
- Amino acid sequence determines 3D structure

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Protein folding



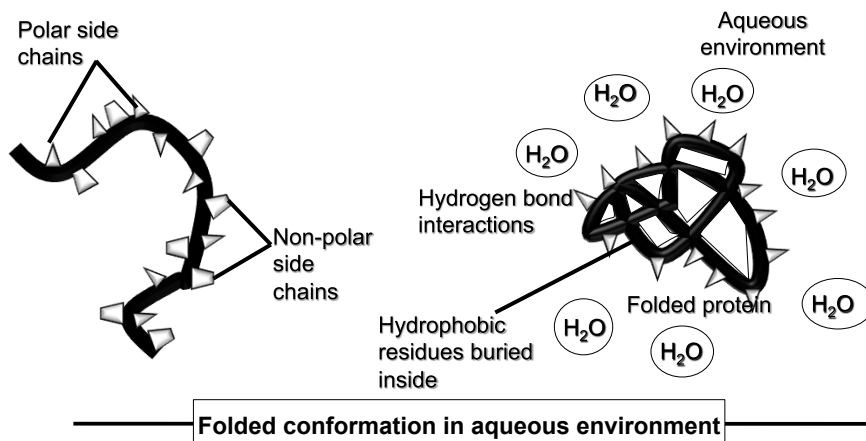
- Polar amino acid side chains tend to gather on outside of the protein
- Non-polar amino acid side chains are buried inside

Polar and non-polar amino acids

Amino Acid	Abbreviation	Symbol	Hydrophobicity/ Charge
Aspartic acid	Asp	D	Negative
Glutamic acid	Glu	E	Negative
Arginine	Arg	R	Positive
Lysine	Lys	K	Positive
Histidine	His	H	Positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar
Cysteine	Cys	C	non-polar
Glycine	Gly	G	non-polar
Isoleucine	Ile	I	non-polar
Leucine	Leu	L	non-polar
Methionine	Met	M	non-polar
Phenylalanine	Phe	F	non-polar
Proline	Pro	P	non-polar
Tryptophan	Trp	W	non-polar
Valine	Val	V	non-polar
Alanine	Ala	A	non-polar

Protein folding

Overall increase in an entropy drives the folding process



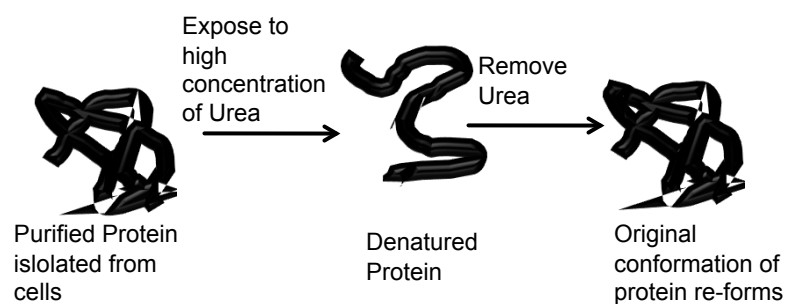
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Protein unfolding by denaturation

- Protein denaturation by chemical and heat

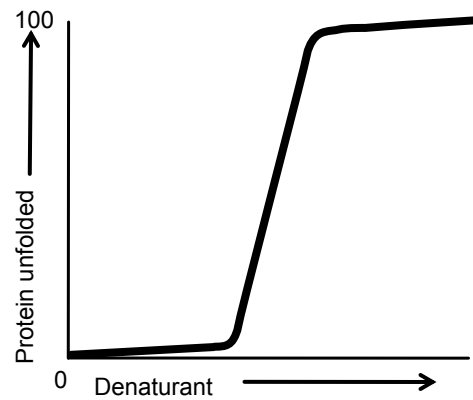


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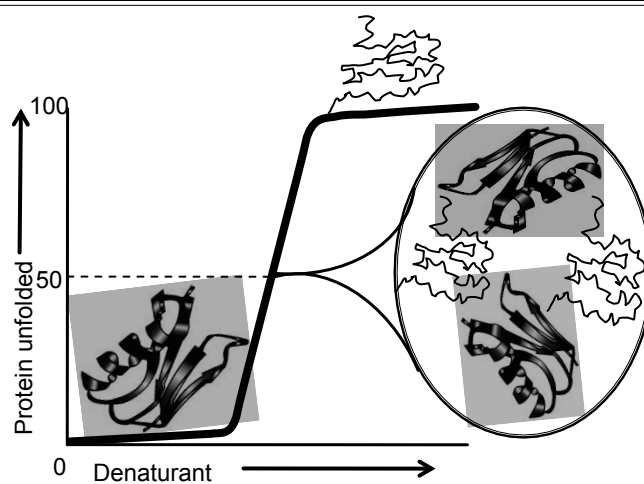
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Cooperative transition from folding to unfolding

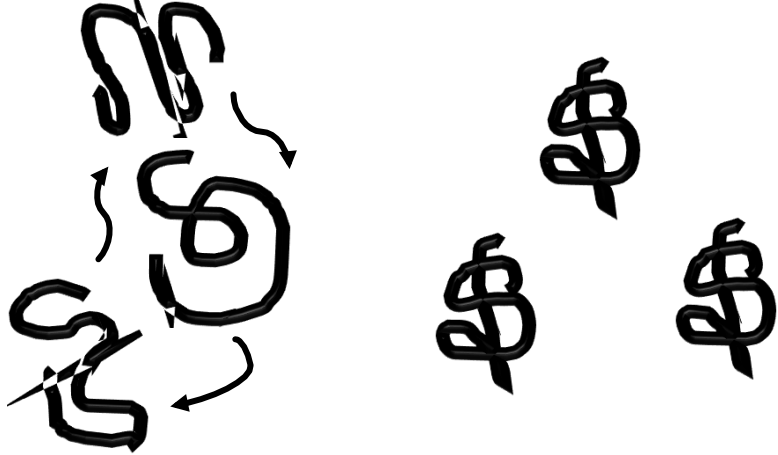


- A sharp transition from native (folded) to denatured (unfolded) form

Components of partially denatured protein solution



Folding from many conformations to just one?



The diagram shows a transition from multiple unstable states to a single stable state. On the left, several tangled, irregular loops represent 'Many unstable conformations'. Arrows indicate the progression towards a single, well-defined, compact structure on the right, labeled 'One stable conformation'. The stable structure is a complex, folded loop with a specific topology.

Many unstable conformations

One stable conformation

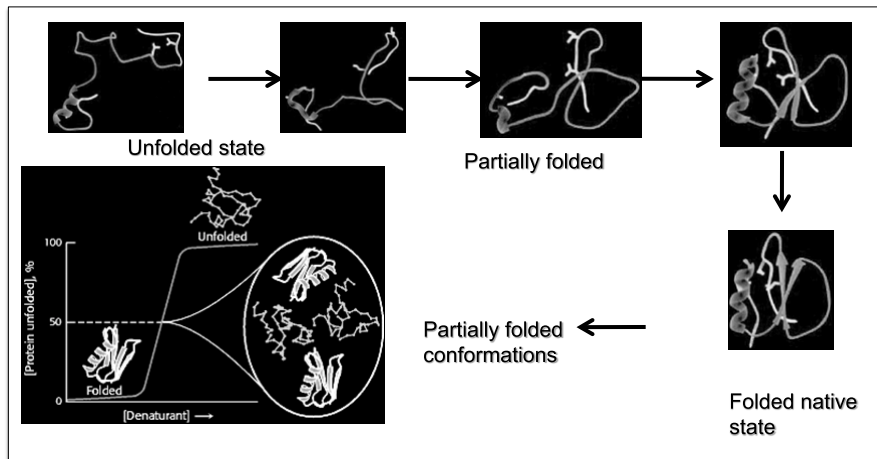
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Progressive stabilization of intermediates

- Folding is a cooperative process
- In general, any protein adopts only one conformation
- Or, few very closely related characteristic functional conformations "*native state*"

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Cooperative transition from folding to unfolding



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Prediction of protein structure

- Amino acid sequence dictates protein structure
- knowledge-based and *Ab initio* “from the beginning” prediction to predict protein structure

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Thermodynamics of protein folding

Folding of proteins into their native conformations occurs spontaneously under physiological conditions and is dictated by the primary structure of the protein.

Thermodynamics of protein folding

- Hydrophobic amino acids are driven to associate- hydrophobic collapse
- Thus, overall increase in an entropy drives the folding process

Thermodynamics of protein folding

Unfolded polypeptide chain

Partially folded polypeptide

Native state α -helix

Molten globule state

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Thermodynamics of protein folding

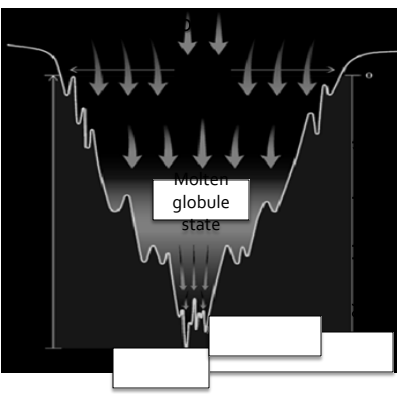
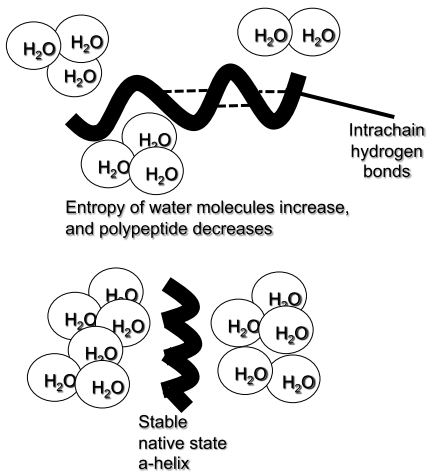
Unfolded polypeptide chain, high free energy & entropy

Helix formation commences, free energy & entropy decrease

Beginning of helix formation

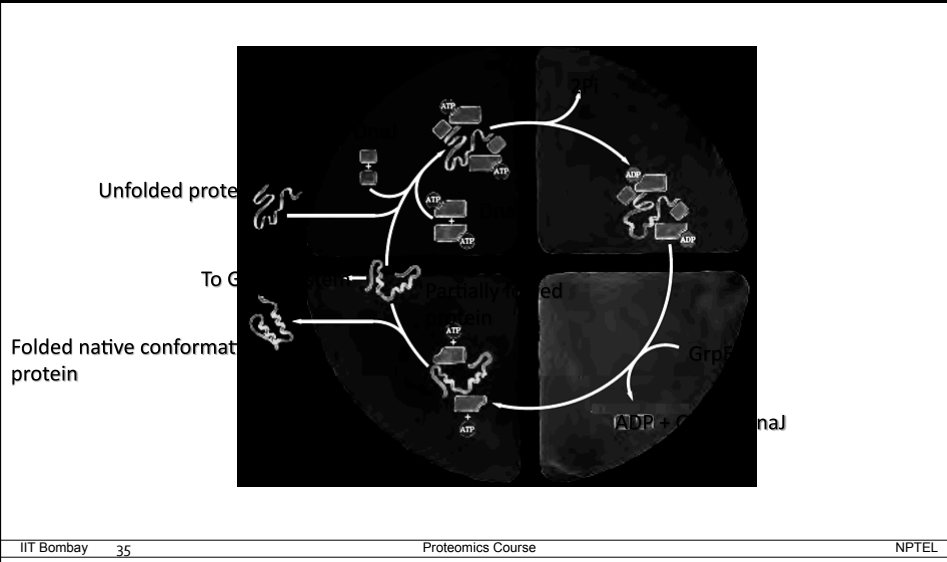
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Thermodynamics of protein folding



Molecular chaperone for protein folding

Molecular chaperone systems

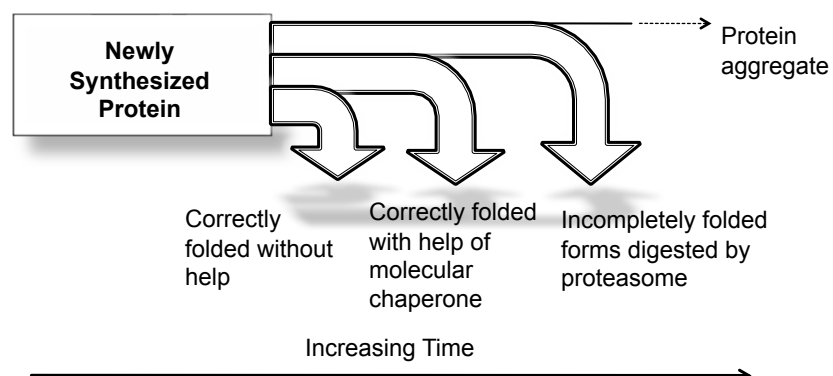


Protein misfolding & diseases

Protein misfolding

- Protein folds into a single, energetically favorable conformation, specified by its amino acid sequence
- A protein may fold into alternative 3D structure due to mutations, inappropriate covalent modifications

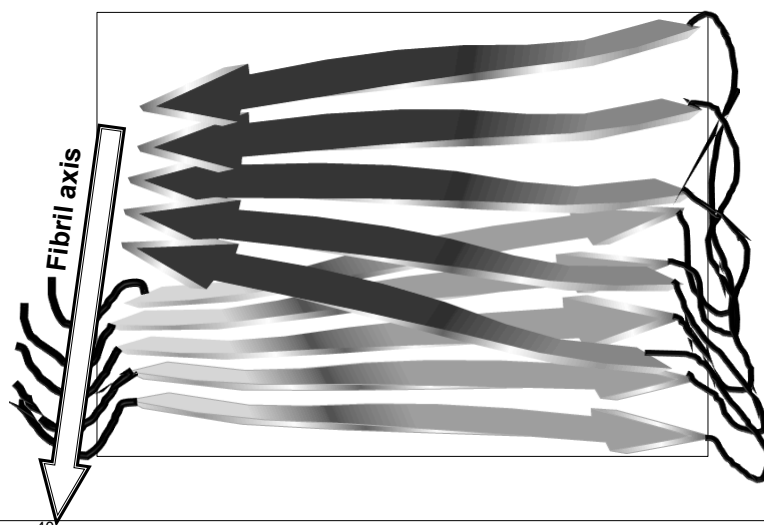
Protein misfolding



Protein misfolding

- Accumulation of misfolded protein or proteolytic fragments results into few degenerative diseases
- Characterized by presence of insoluble protein plaques in organs such as brain and liver

Protein misfolding



Summary

- Anfinsen experiment
- Protein folding
- Thermodynamics of protein folding
- Molecular chaperons
- Protein mis-folding and diseases

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