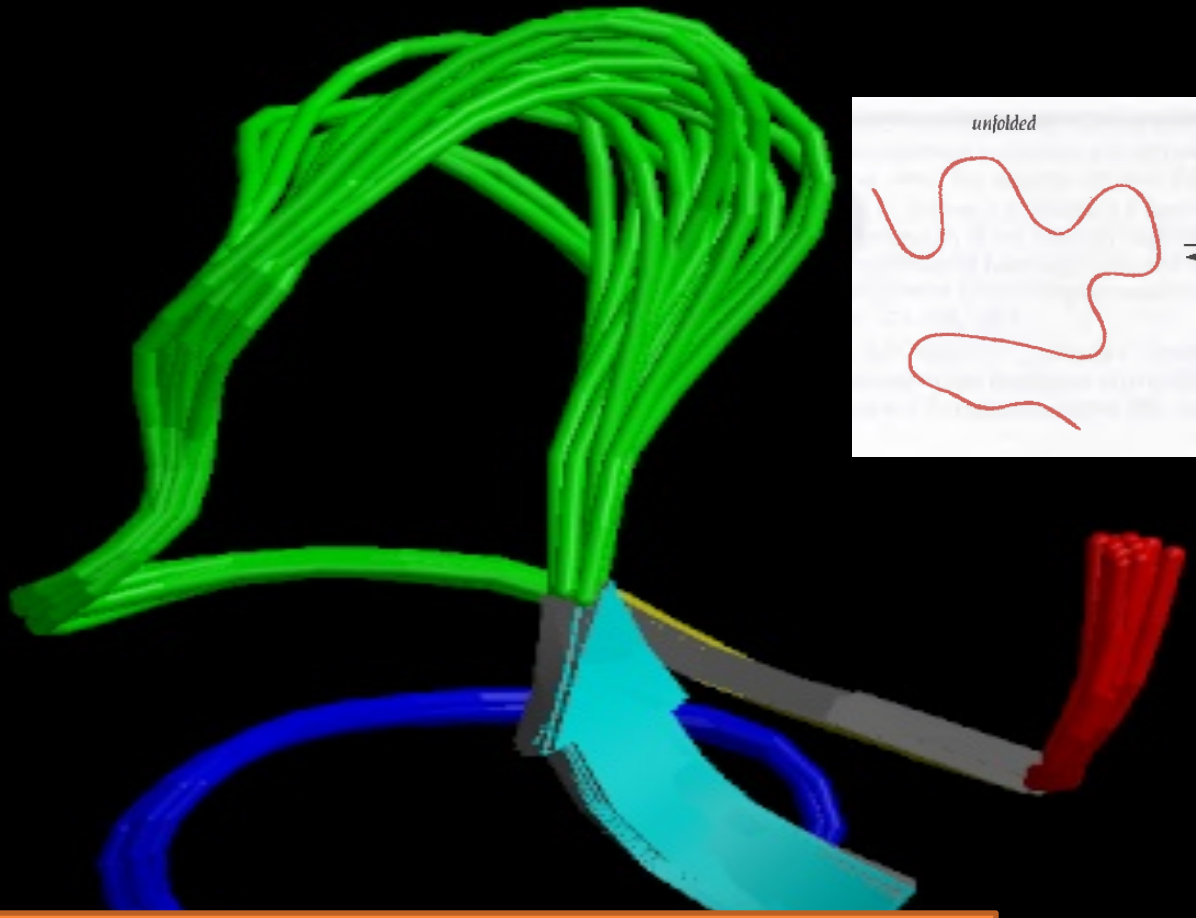
Course: Protein Structure, Function and Engineering



Lecture 7

Levels of Organization in Proteins

Tertiary Structures Stability

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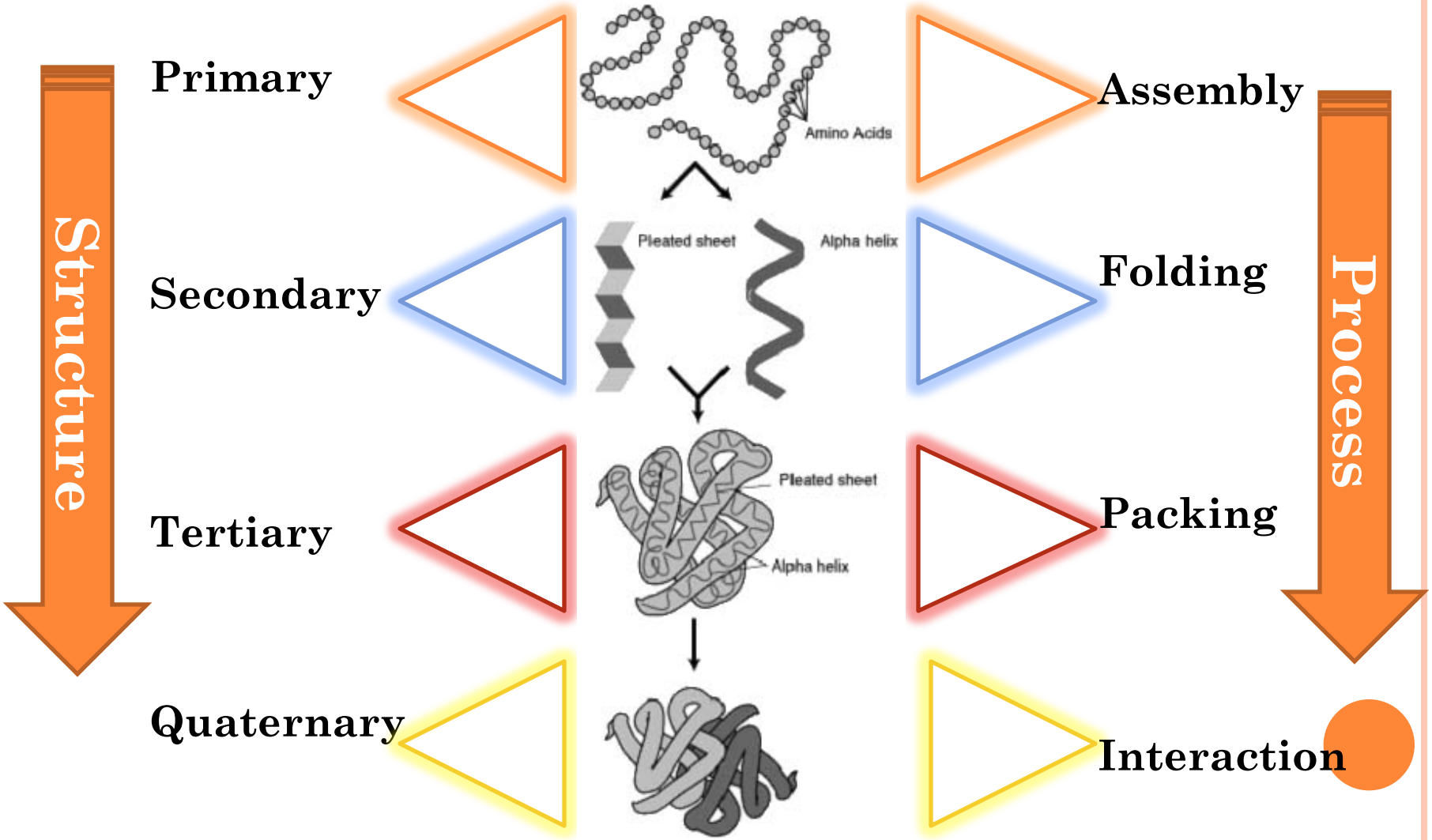
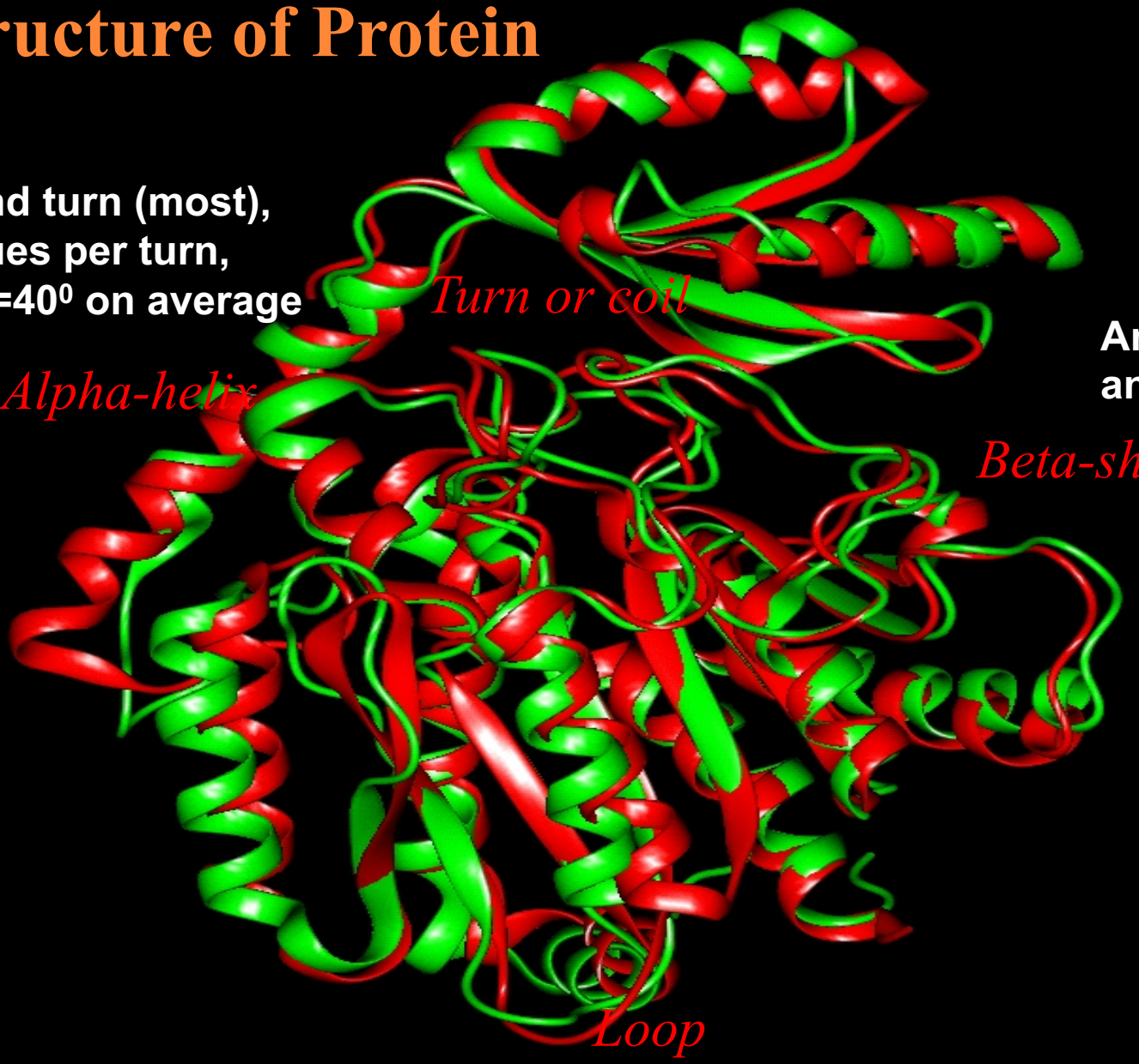
Last Lecture

- Protein Tertiary Structure
  - surface topography
  - Random coil
- Surface Water Molecules
- Protein Tertiary Structure
  - H bonding
  - Ionic Interaction
  - Disulfide Bonding
  - Hydrophobic Interaction
- Packing of atoms in the protein interior
- Integral Membrane Proteins

Protein Structures



3-D Structure of Protein



STABILITY OF PROTEIN TERTIARY STRUCTURE

- Above absolute zero, all chemical bonds have some flexibility: atoms vibrate and chemical groups can rotate relative to each other.
- Protein Stabilizing Forces
  - Non-Covalent
- Protein structures continuously fluctuate about the equilibrium conformation
- These fluctuations are large enough to allow small molecules such as water to penetrate into the interior of the protein
- ligand binding and catalysis, for they allow the structure to adjust to the binding of another molecule or to changes in the structure of a substrate as a reaction proceeds.

Covalent bonds can add stability to tertiary structure

- cross-linking between segments of secondary structure in the native state
  - disulfide bridge
    - Not present in intracellular Protein
    - Present in Secretary Proteins
  - coordination of a metal ion (coordinate covalent bonds)
    - more than one stabilizing metal ion binding site
    - very loose to very tight
    - calcium (Ca^{2+}) and zinc (Zn^{2+}), Na, K
    - removed by chelating agents such as EDTA
      - Cause denaturation or less stability

Covalent bonds can add stability to tertiary structure

- dissociable organic or organometallic **cofactor**
  - Always present at the active site
  - Formed with the organic part of some cofactors
    - D-amino acid aminotransferase
  - Formed with a metal ion that is an integral part of some cofactors
    - Vitamin B12, chlorophyll, and the heme group
  - Formed with both
    - Heme group in cytochrome c
- in some cases the cofactor is not a separable molecule, but is created by the chemical cross-linking of two amino-acid side chains
  - redox active cofactor PQQ
  - green fluorescent protein

Post-translational modification

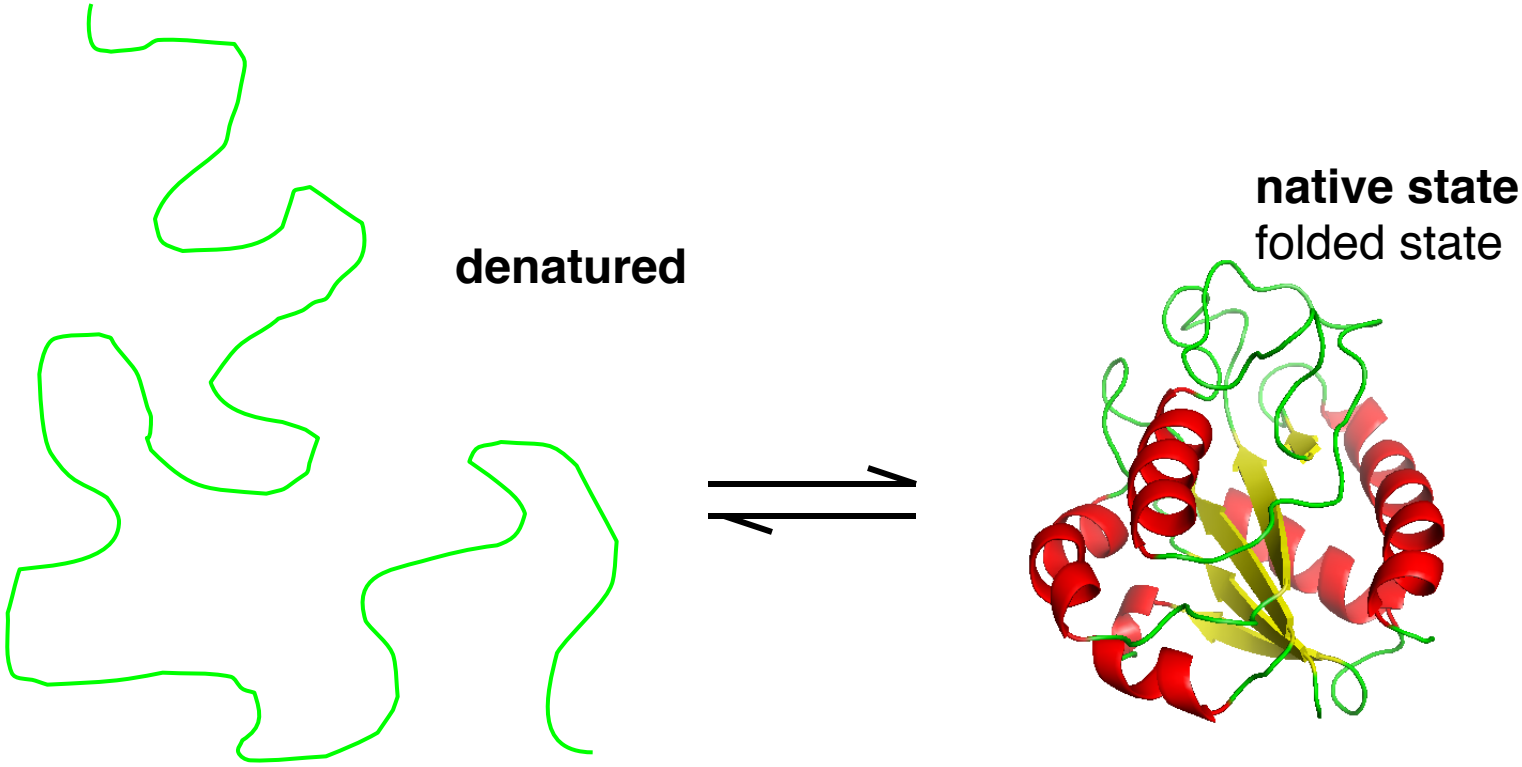
- Structure of Protein
- Stability of Protein

Most Common Post-translational Modifications	
Reversible	**Irreversible**
disulfide bridge	cofactor binding
cofactor binding	proteolysis
glycosylation	ubiquitination
phosphorylation	peptide tagging
acylation	lysine hydroxylation
ADP-ribosylation	methylation
carbamylation	
N-acetylation	

Protein Denaturation

- Denaturation changes the physical and chemical properties of a protein
- Denaturation results from the disruption of the bonds holding the protein structure together
  - Once bonds disrupted the protein can uncoil and form bonds other than those which provided it's original configuration
  - Protein cannot then regain original configuration
- The denatured proteins tend to
  - decrease in solubility;
  - increase the viscosity;
  - lose the biological activity;
  - lose crystalizability;
  - be susceptible to enzymatic digestion.

Native and denatured states



many different structures fluctuating;
not usually very compact;
disordered but not a “random coil”

single structure or ensemble
of very similar structures;
compact

For some proteins, **but not all**, this process is readily reversible and occurs without populated intermediate forms--> **“two-state” folding**

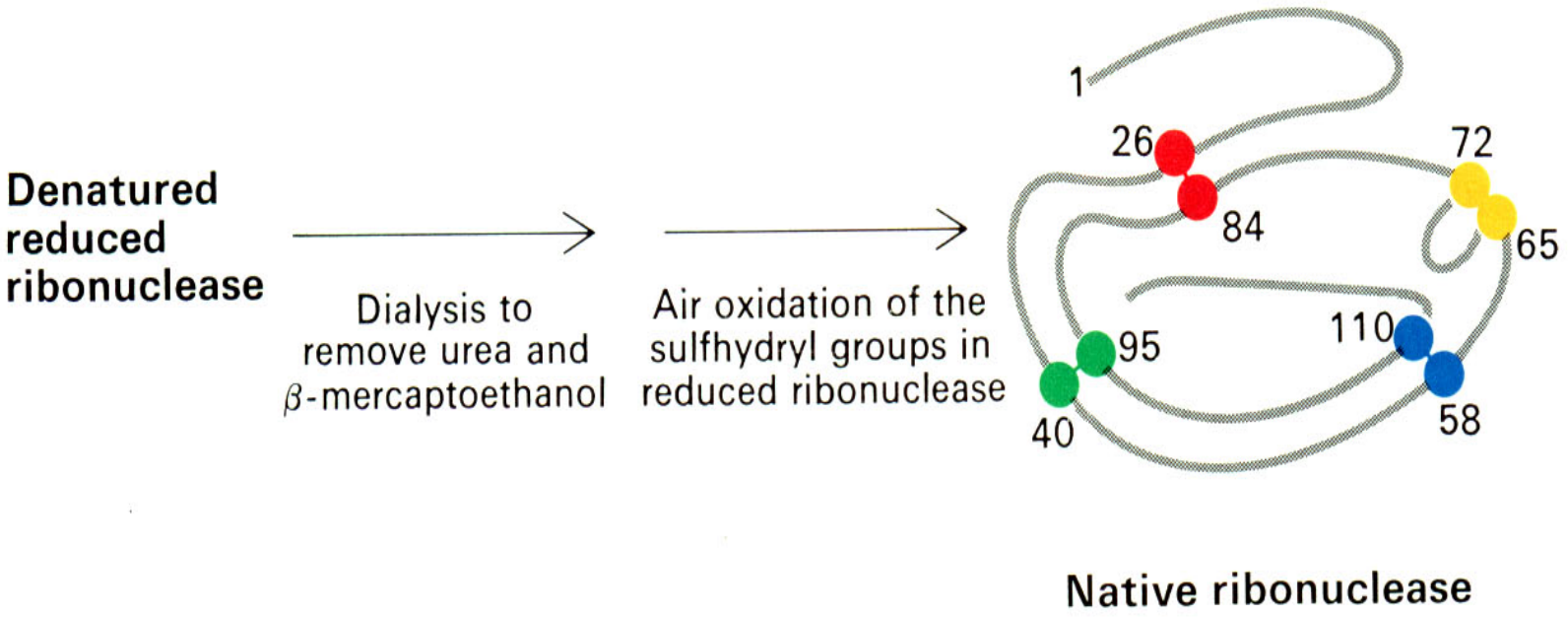
- Cause of Denaturation
  the disruption of hydration shell and electric repulsion
- Denaturants
  physical: heat, ultraviolet light, violent shaking, …
  chemical: strong acids, bases, organic solvents, detergents, …
- Applications
  sterilization, Lyophilization

Renaturation

- Once the denaturants are removed, the denatured proteins tend to fold back to their native conformations partially or fully.

- The renatured proteins can restore their biological functions.

Renaturation

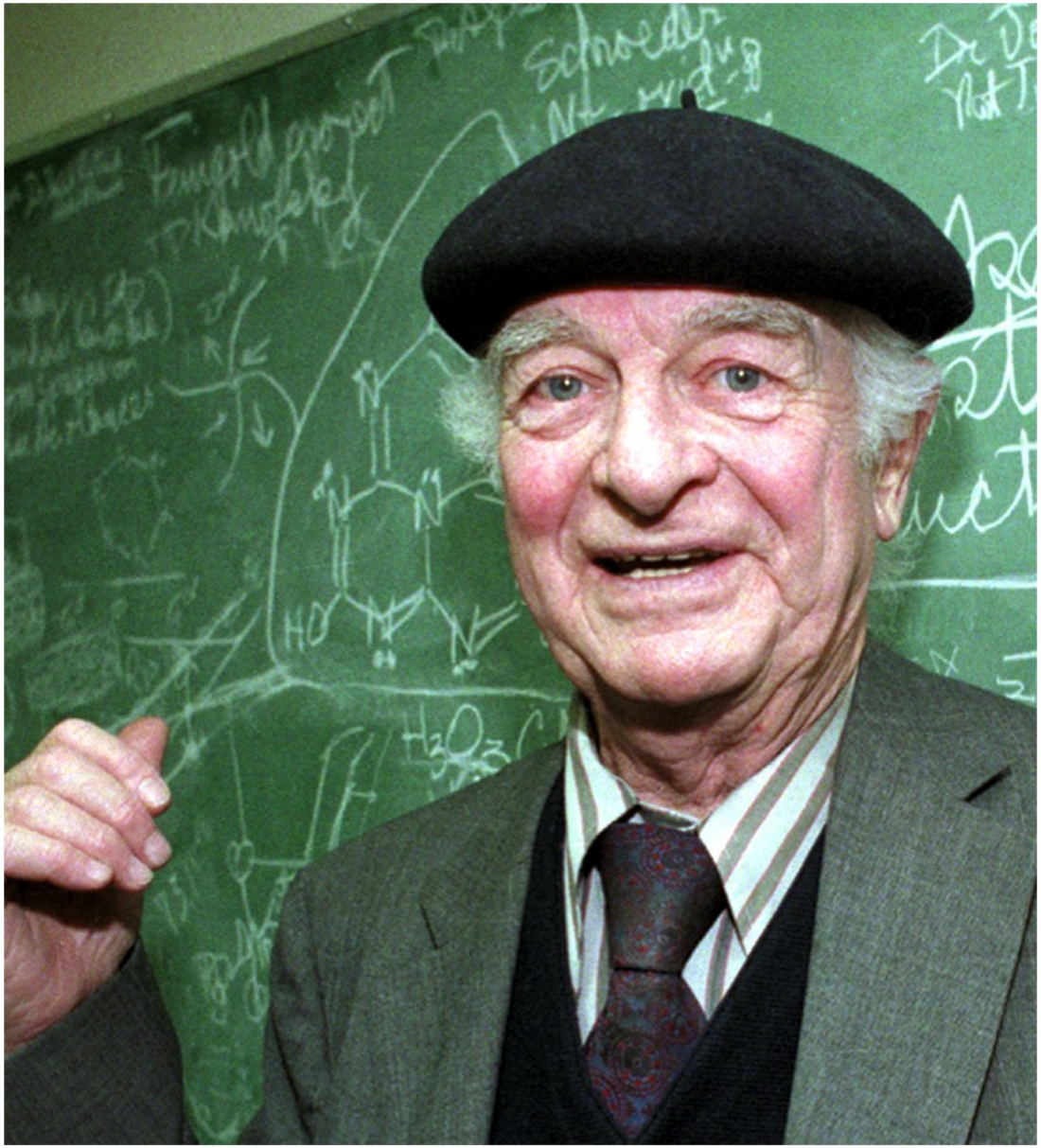


Protein Denaturants

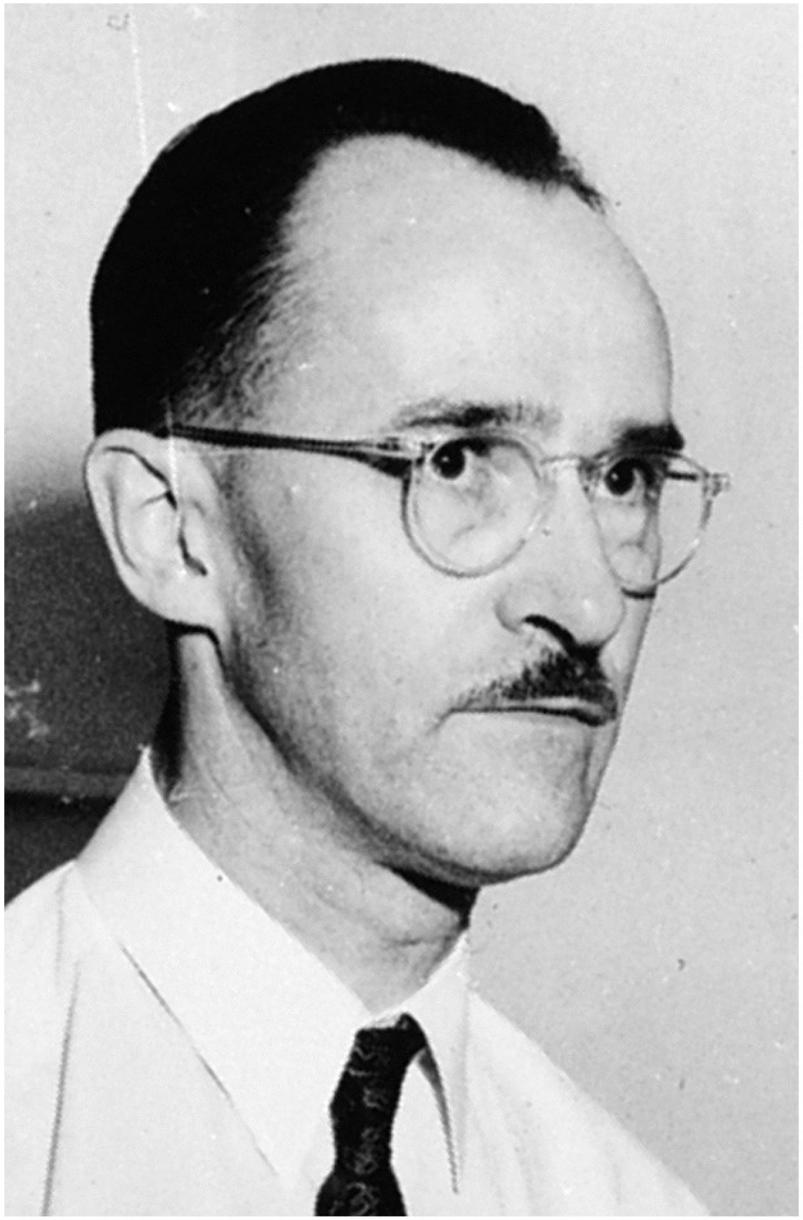
- High temperatures break the weak interactions
- Denatured State
  - loss of biological or biochemical activity, or by spectroscopic signals characteristic of an unfolded polypeptide
- Temperature-sensitive (ts) mutation
  - More or Less Stable
- Chemical denaturants
  - Urea or guanidinium hydrochloride, or detergents like SDS
- Thermostable Proteins
  - Thermophilic proteins have more salt bridges, while others appear to have more hydrophobic interactions and shorter protruding loops.

PROTEIN DENATURANTS

- Protein Precipitation
  - The denatured proteins expose their side chains or the inner part to the aqueous environment, which causes the proteins aggregated and separated out from the aqueous solution.
- Protein Coagulation
  - When the denatured proteins become insoluble fluffy materials, heating denatured proteins will turn them into a hard solid which are not soluble even strong acids and bases are applied.
  - Coagulation is an irreversible process.



Linus Pauling, 1901–1994



Robert Corey, 1897–1971

Robert G. Edwards
The Nobel Prize in Physiology or Medicine 2010 was awarded to Robert G. Edwards *"for the development of in vitro fertilization"*.