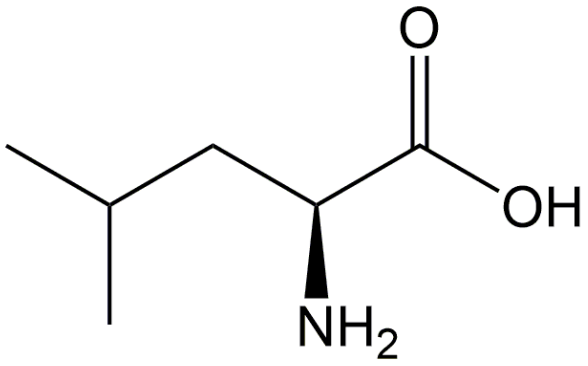
Proteins

Learning Outcomes

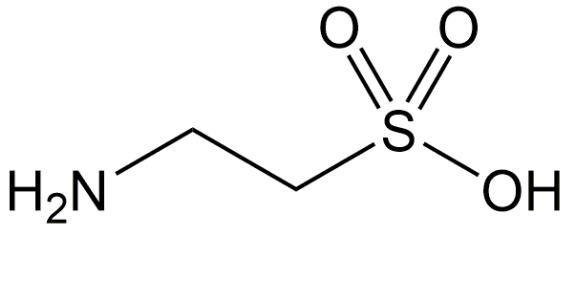
1. Identify an amino acid by structure
2. Understand that proteins are biopolymers of amino acids
3. Explain primary, secondary, tertiary, and quaternary structure
4. Correlate pH change at the molecular level to protein denaturation at the secondary and tertiary levels

Amino Acids



L-Leucine

2-Amino-4-methylpentanoic Acid



Taurine

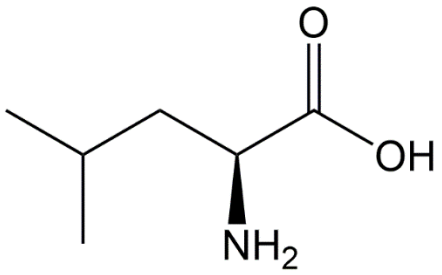
2-Aminoethanesulfonic Acid

Essential vs. Nonessential Amino Acids

Essential Amino Acid: An amino acid that cannot be synthesized from simple molecules such as sugars and amino acids [*de novo* ("from scratch") synthesis].

Non-Essential Amino Acid: An amino acid that can be synthesized from *de novo*.

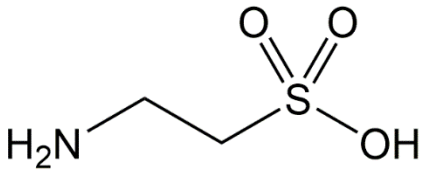
Amino Acids



L-Leucine

2-Amino-4-methylpentanoic Acid

Essential (Humans and Cats)



Taurine

2-Aminoethanesulfonic Acid

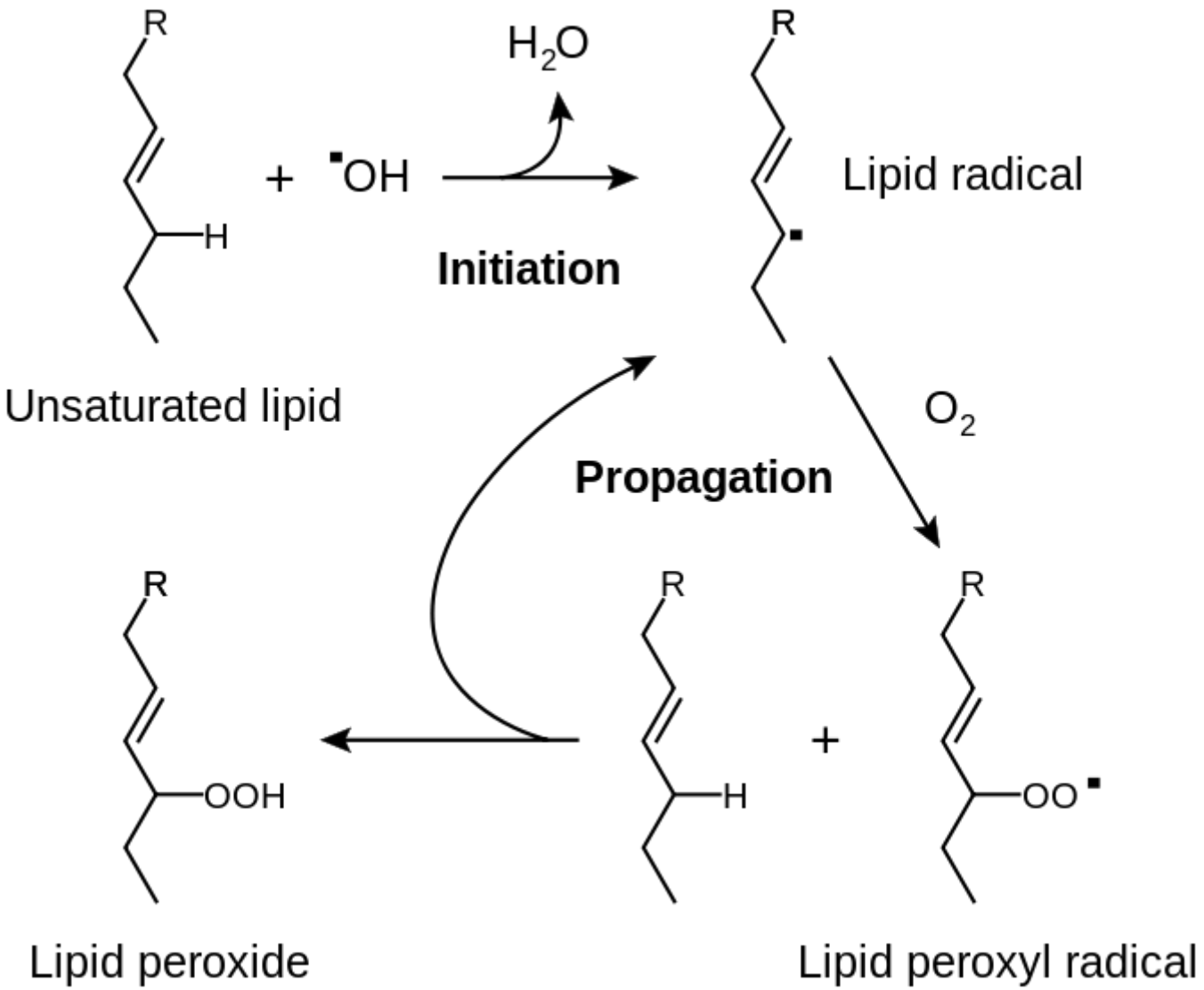
Essential (Cats)
Non-Essential (Humans)

Is Taurine Really Non-Essential?

Because it is one of the few amino acids not used in protein synthesis, taurine is often referred to as a "nonessential" amino acid, or more generously as a "conditionally essential" amino acid. Considering its broad distribution, its many cytoprotective attributes [29,30], and its functional significance in cell development, nutrition, and survival [31,32], these are clearly misnomers. Taurine is undoubtedly one of the most essential substances in the body. Moreover, there is ever-increasing evidence that taurine depletion leads to a wide range of pathological conditions, including severe cardiomyopathy [33], renal dysfunction [34], pancreatic β cell malfunction [35], and loss of retinal photoreceptors [36]. The close relationship between taurine levels and nutritionally induced degeneration is supported further in that taurine supplementation can inhibit light-induced lipid peroxidation, and thereby protect isolated rod outer segments from photic damage [37,38].

"Review: Taurine: A "Very Essential" Amino Acid", *Molecular Vision*, **18**, 2673-2686 **(2012)**.

Lipid Peroxidation



Is Taurine Really Non-Essential?

154

Brain Research, 330 (1985) 154–157
Elsevier

BRE 20669

Taurine and hypotaurine inhibit light-induced lipid peroxidation and protect rod outer segment structure

Exposure of isolated frog rod outer segments to light (5000 lux) induces membrane disorganization and swelling. An increase of about 50% on lipid peroxidation, measured by the extent of malonaldehyde formation, accompanied the light-induced damage. Taurine and hypotaurine (25 mM) prevented the increase in lipid peroxidation, and provided an entire protection of rod outer segment structure.

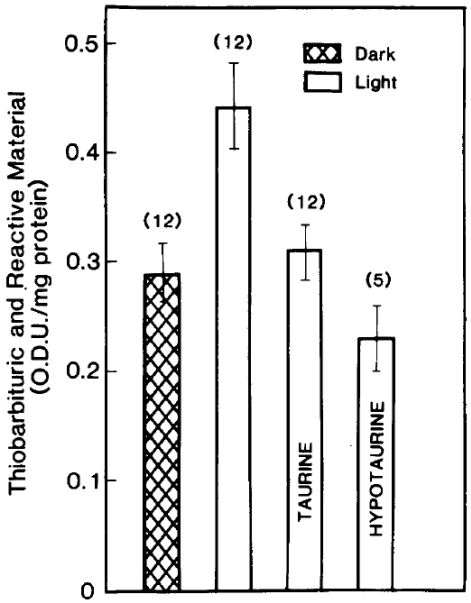


Fig. 1. Malonaldehyde formation by rod outer segments after 2 h of incubation in darkness or light in the presence or absence of taurine or hypotaurine (25 mM). Results are the means ± S.E.M. of the number of experiments indicated in parenthesis.

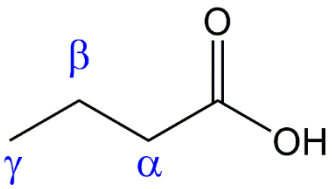
TABLE I

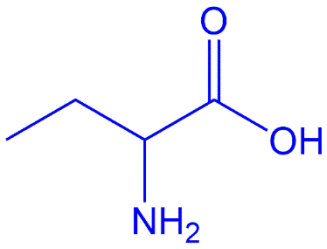
The effect of amino acids and taurine antagonists on the light-induced disruption of rod outer segments

*Compound**	*Disrupted ROS (%)*	
	Dark	*Light*
None	24.8	60.1
Taurine		22.0
Hypotaurine		20.0
GABA		39.0
Glycine		34.0
B-alanine		51.0
Glutamate		53.0
GES		59.0
Bicuculline		59.0
Strychnine		57.1

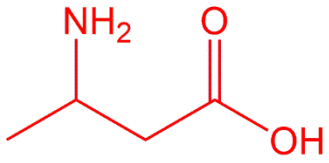
* Amino acids and GES were used at a concentration of 25 mM. Bicuculline and strychnine were 1 mM. Illumination time was 2 h. Results are the means of 4–10 separate experiments. S.E.M. were always lower than 10%.

Definition of Amino Acids

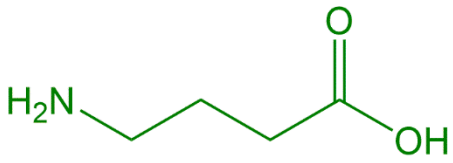




α-Amino Acid

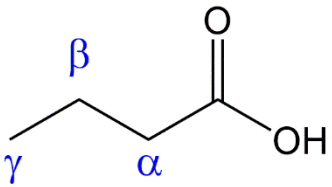


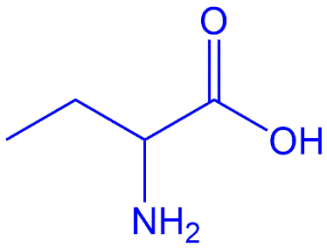
β-Amino Acid



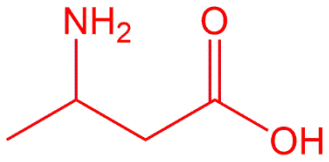
γ-Amino Acid

What We Colloquially Call "Amino Acids" Are Alpha-Amino Acids

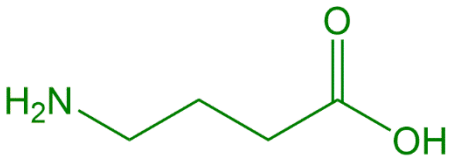




α-Amino Acid

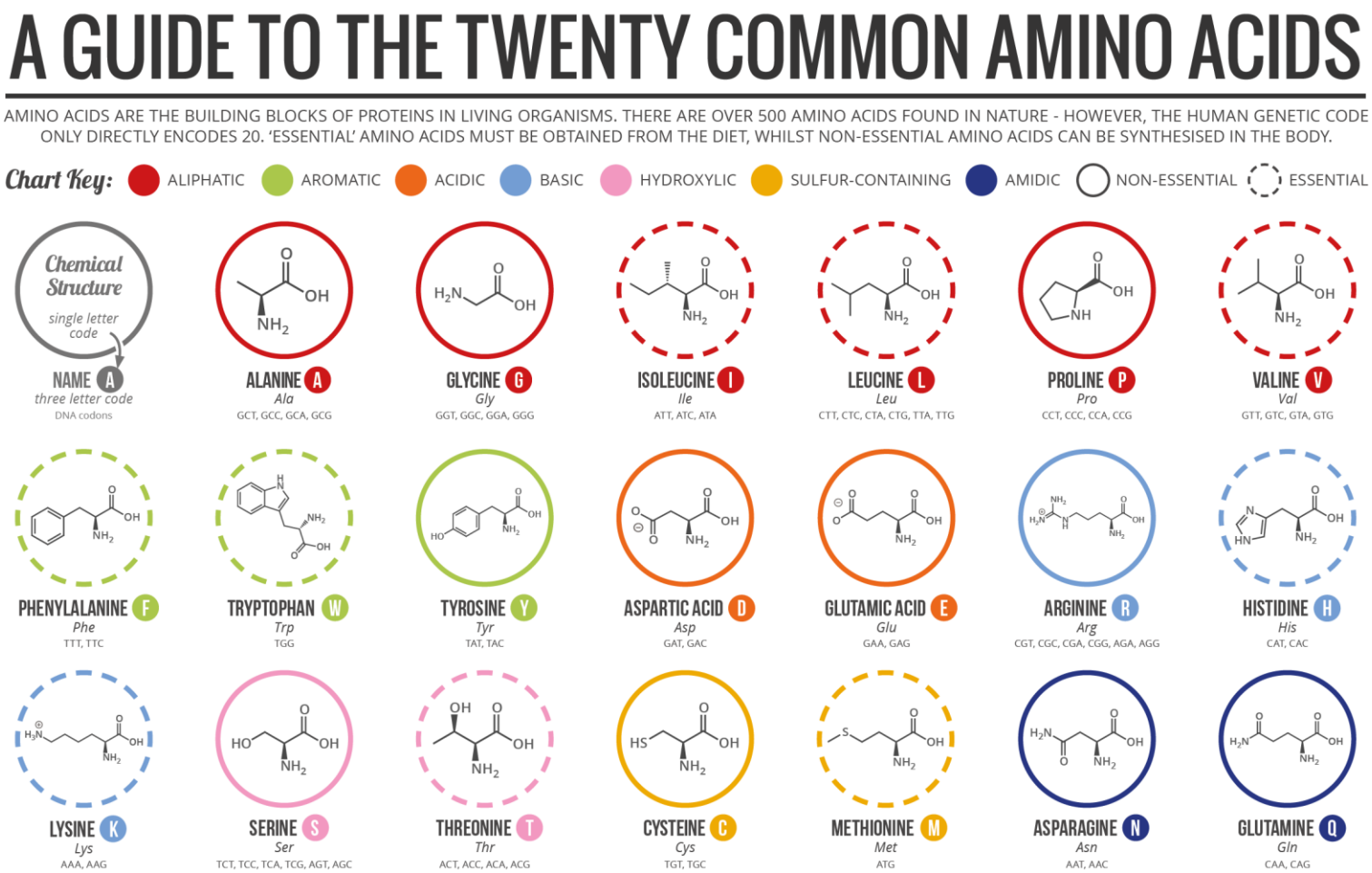


β-Amino Acid



γ-Amino Acid

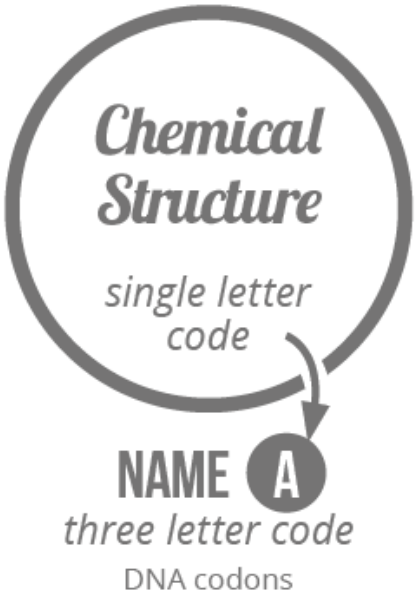
20 Common *Alpha*-Amino Acids

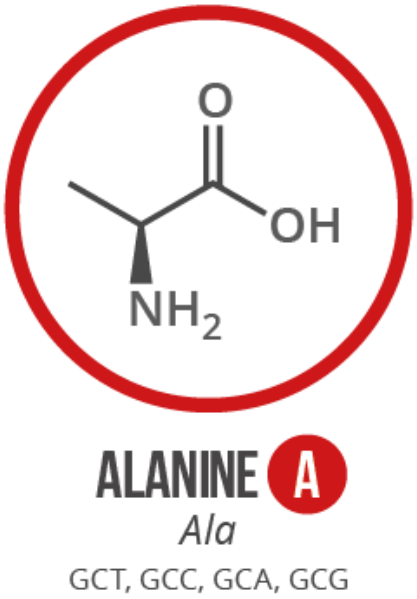


20 Common *Alpha*-Amino Acids

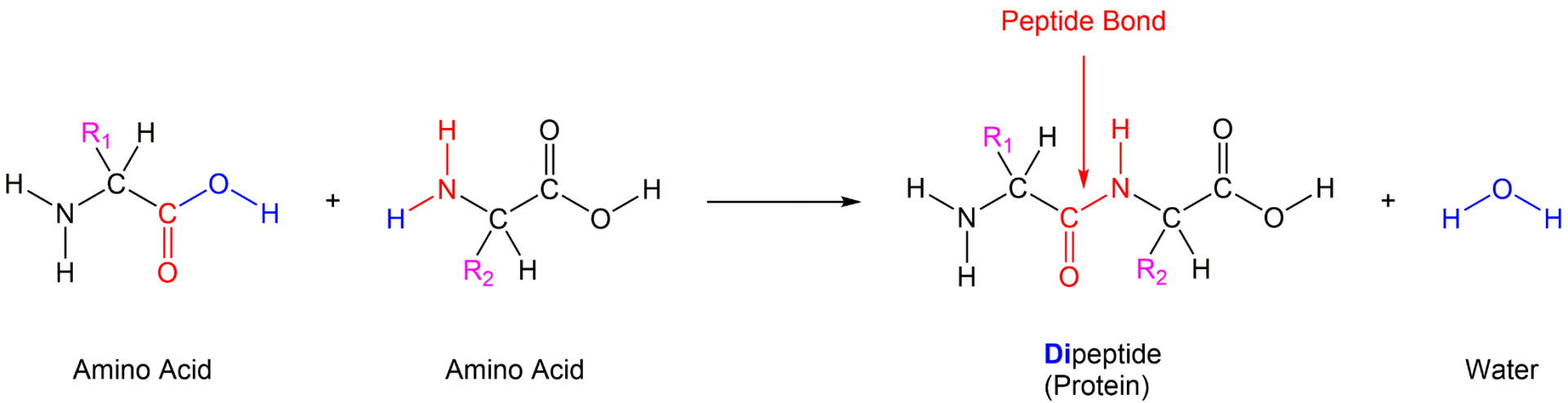
When you take biochemistry you will need to memorize the **1)** the chemical structure, **2)** single-letter code, and **3)** three-letter code for each of the 20 common amino acids.

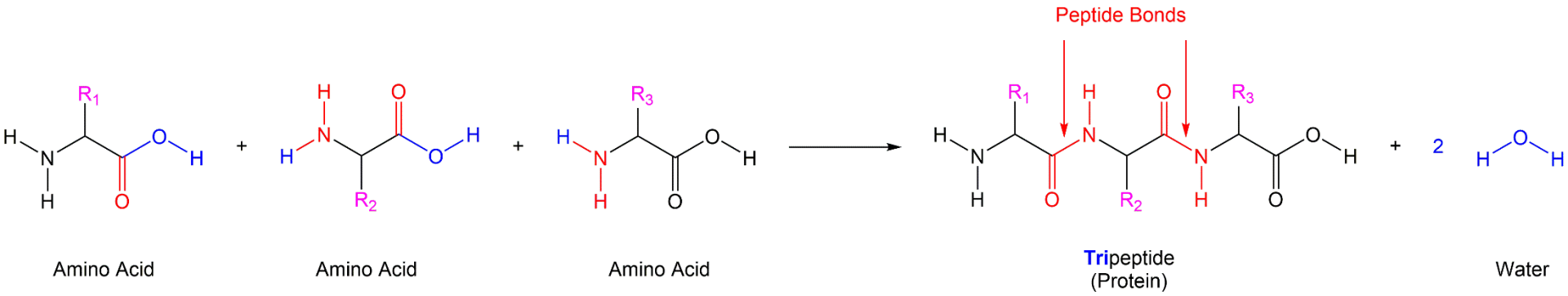
Not required for CHEM 60!!



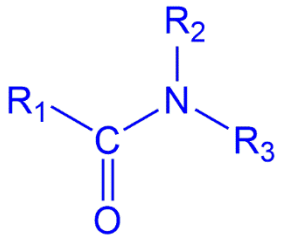


Proteins (Peptides) Are A Biopolymer of Amino Acids

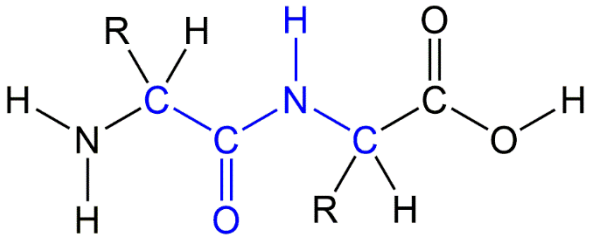




A Peptide Bond is an Amide Bond

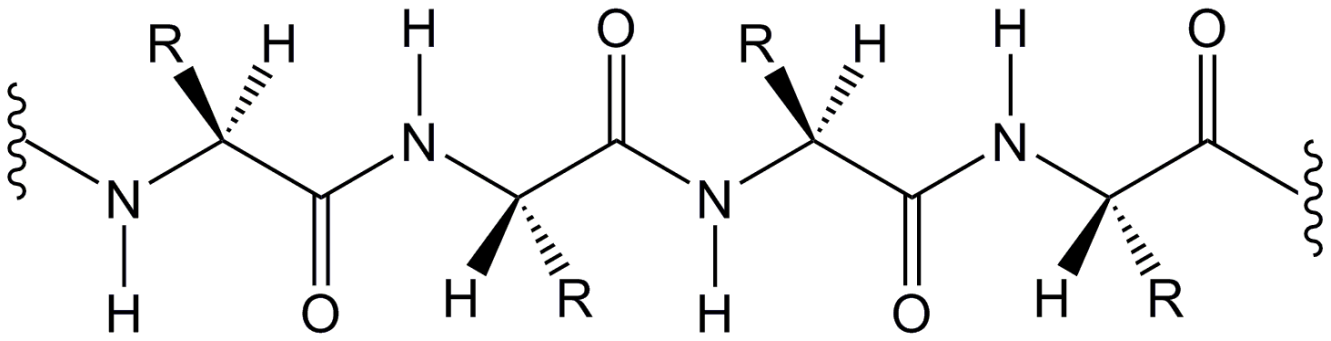


Amide



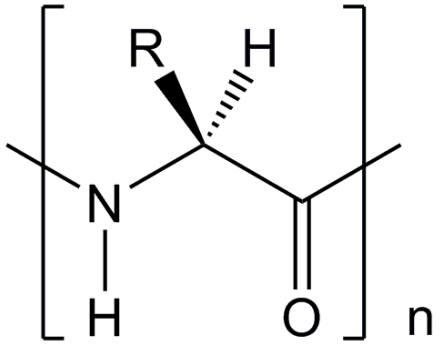
Protein

A Protein is a Biopolymer



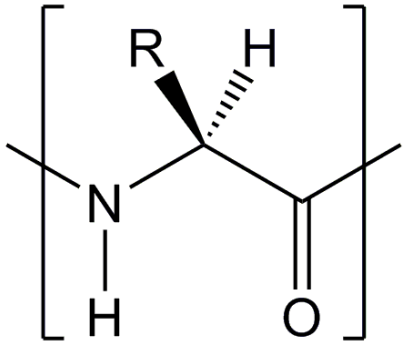
N-terminus

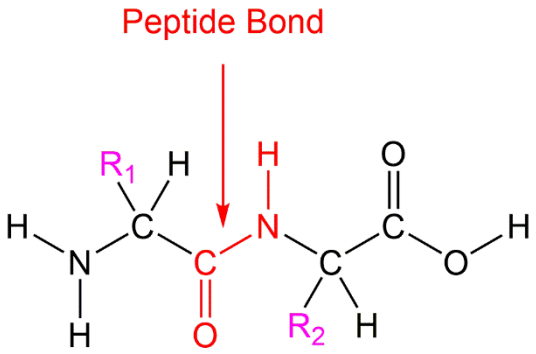
C-terminus



N, C-alpha-R, CO and then REPEAT!!!

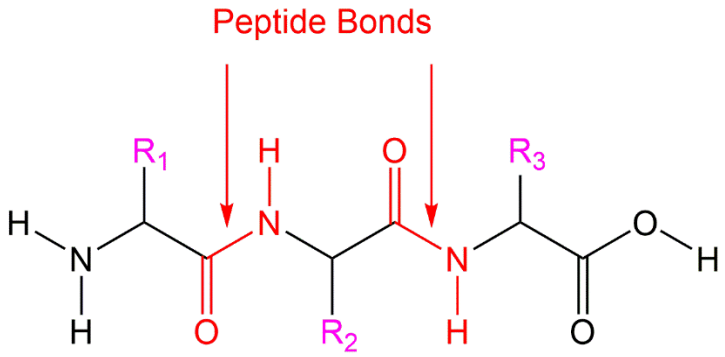
A Protein is a Biopolymer





Dipeptide
(Protein)

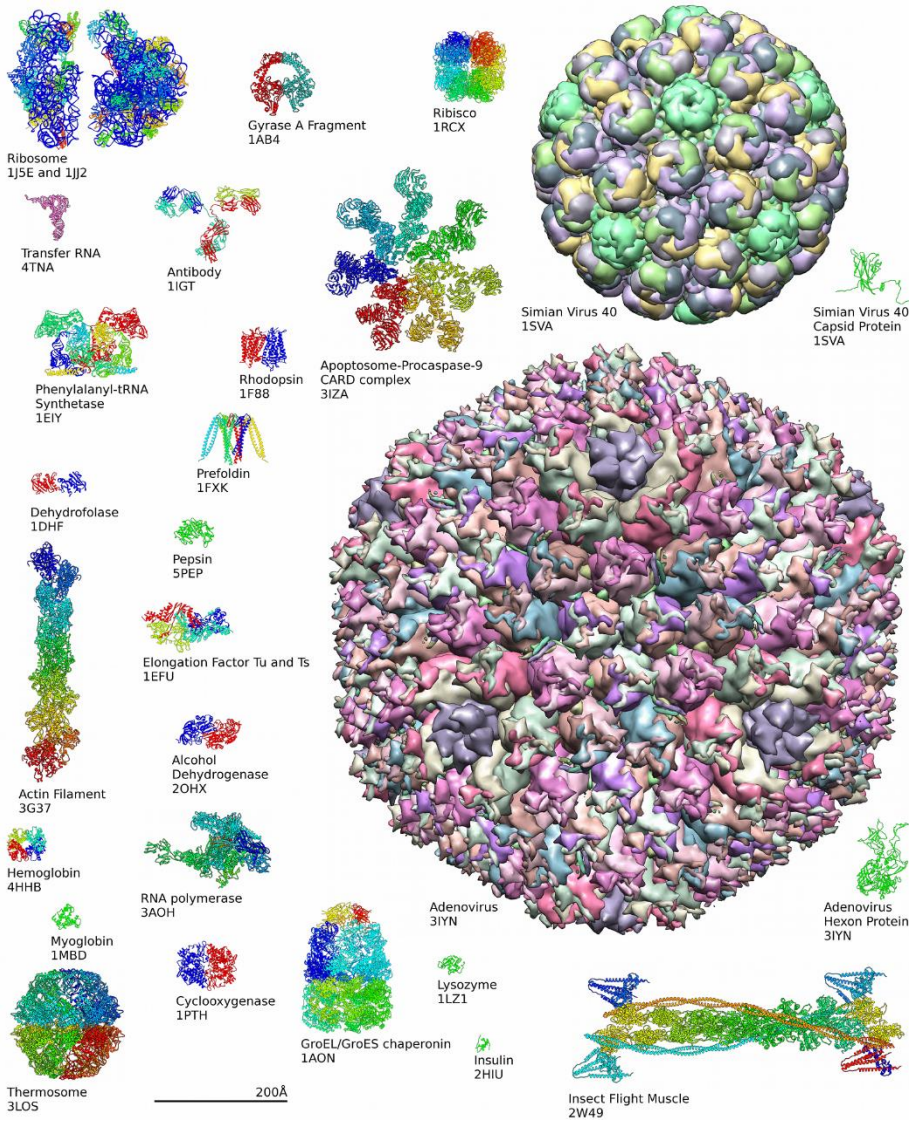
$n = 2$



Tripeptide
(Protein)

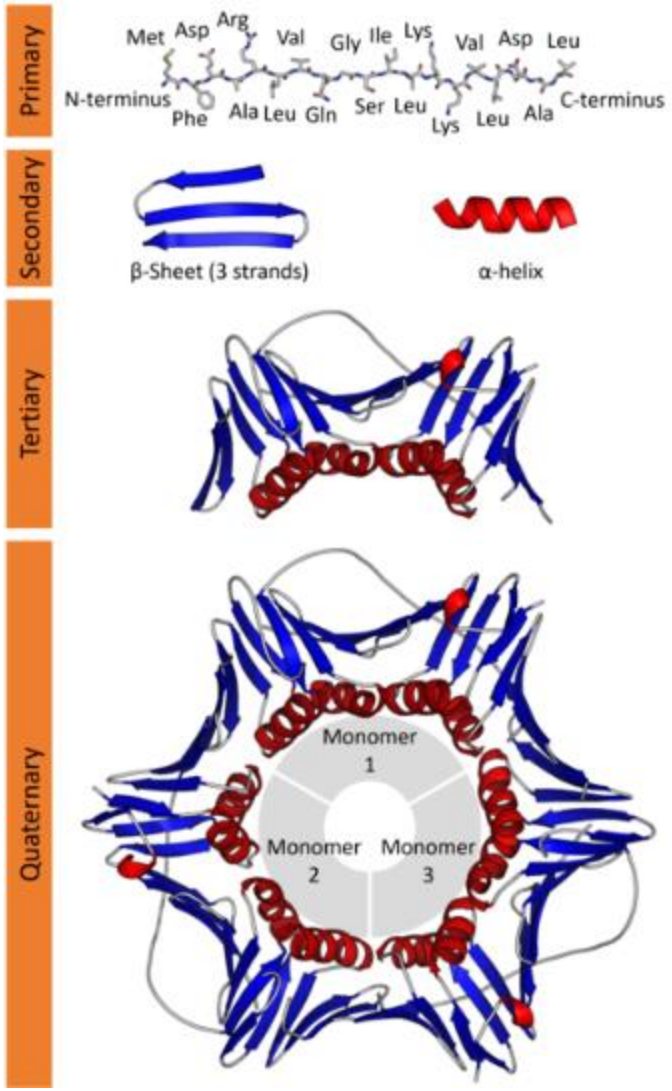
$n = 3$

Protein Structure is the 3-D Arrangement of Atoms in an Amino Acid Chain Molecule



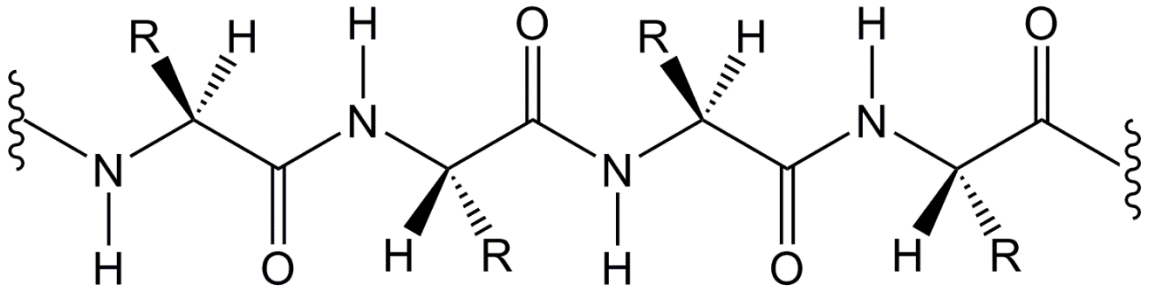
Types of Protein Structure

1. Primary
2. Secondary
3. Tertiary
4. Quaternary



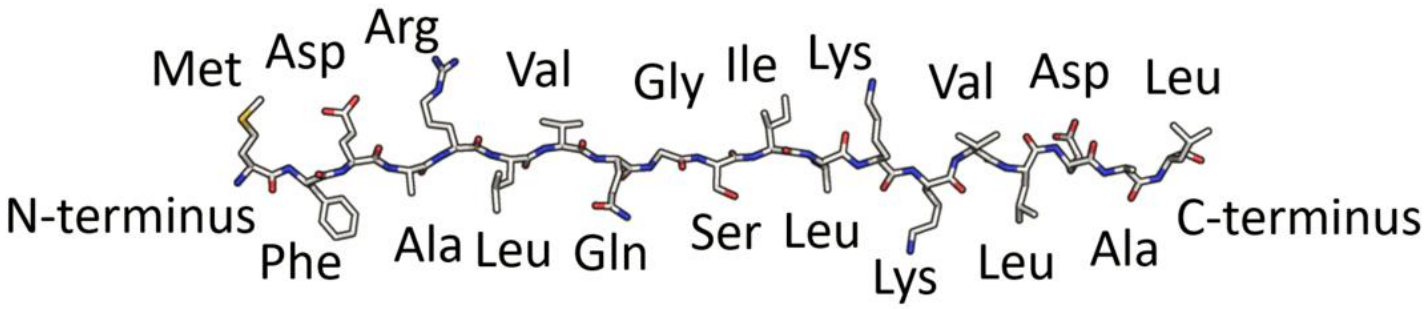
Primary Structure

Primary Structure: The local sequence of amino acids.



N-terminus C-terminus

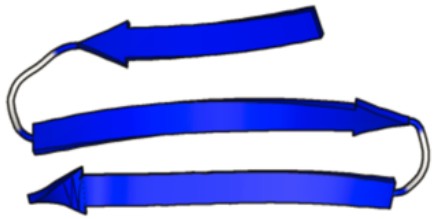
Primary



Secondary Structure

Secondary Structure: The 3-D structure of biopolymer (polypeptide) segments. In a protein the secondary structure is one of three motifs: helix, sheet, or coil.

Secondary

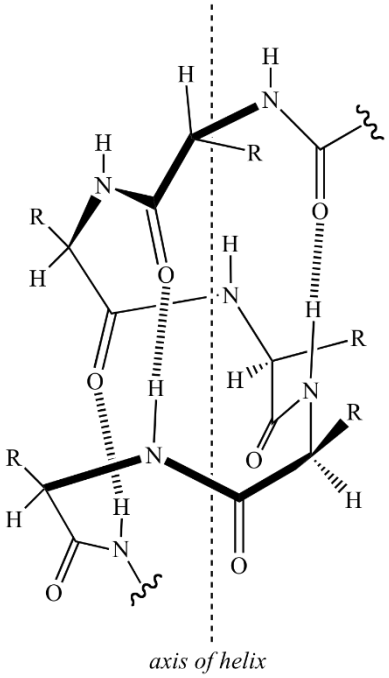


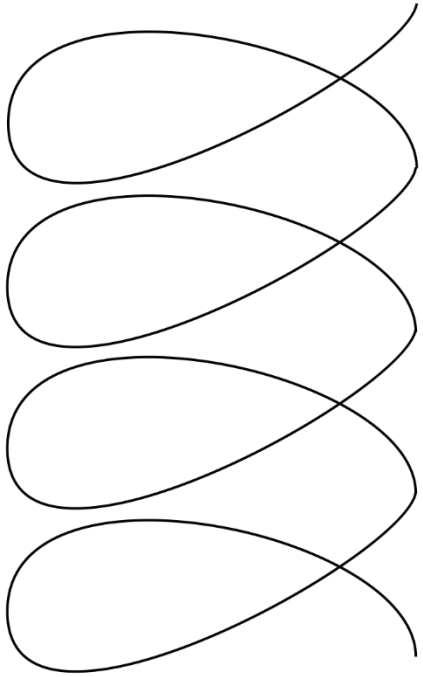
β-Sheet (3 strands)

α-helix

Secondary Structure: Helix

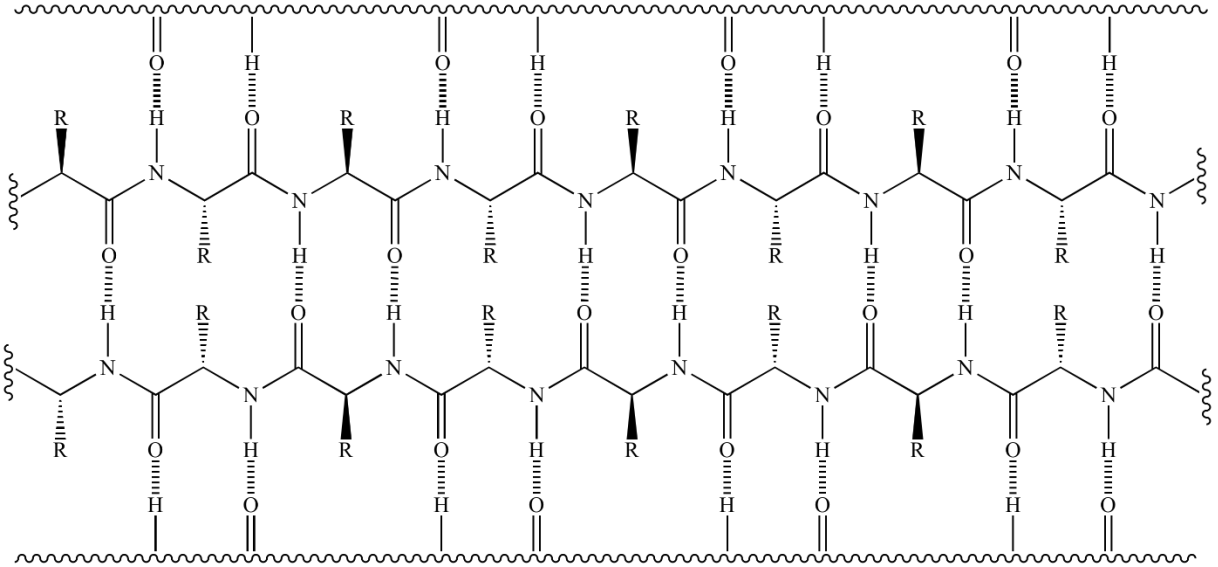
Alpha-Helix: A motif in the secondary structure of peptides and proteins characterized by a right-handed coiled conformation in which each backbone N-H group forms a hydrogen bond with the backbone C=O group of the amino acid four residues earlier in the amino acid sequence.

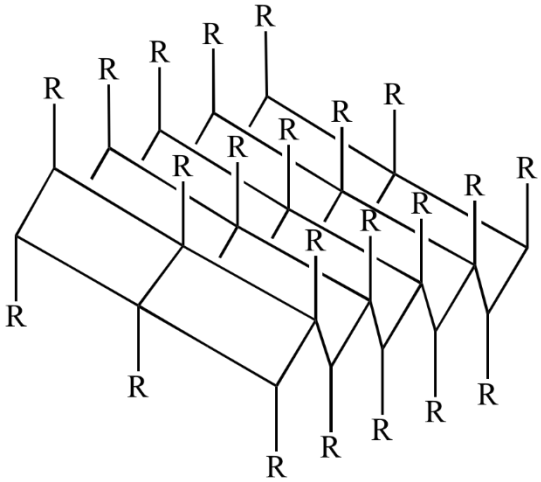




Secondary Structure: Sheet

Beta-Sheet: A motif in the secondary structure of peptides and proteins characterized by two or more amino acid strands connected laterally by two or more hydrogen bonds between a peptide bond N-H in one strand and a peptide bond C=O in the adjacent strand. The resultant structure has a twisted, ‘pleated sheet’ topography.





Silk is a Protein: The Beta-Sheets of the Protein Fibroin Is Responsible for its Structure

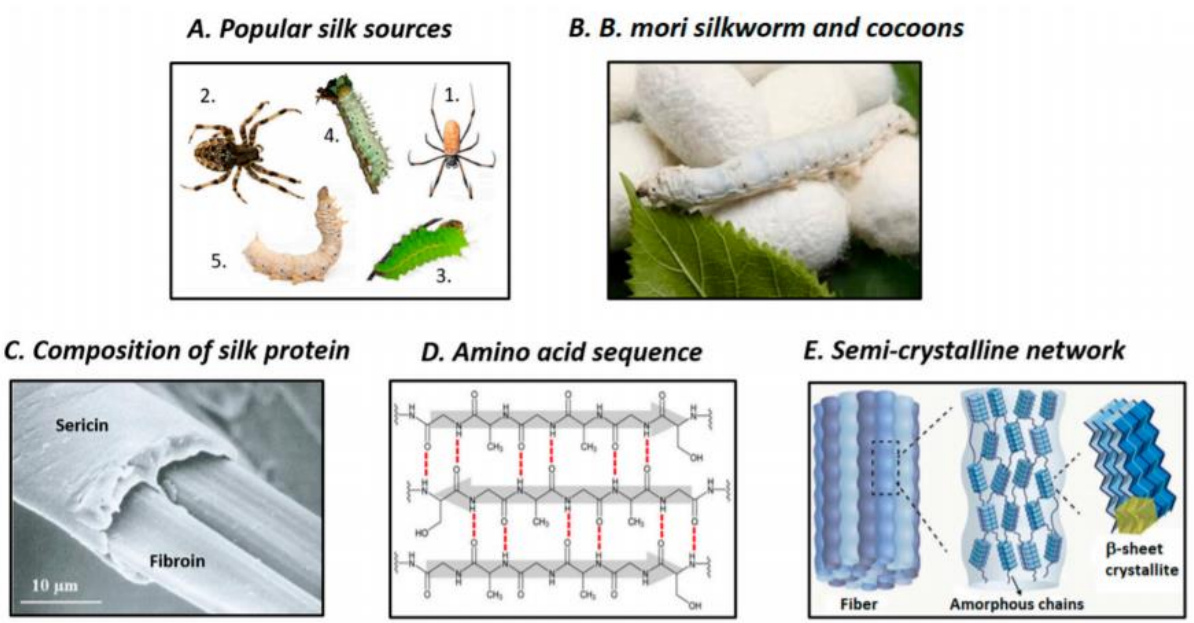
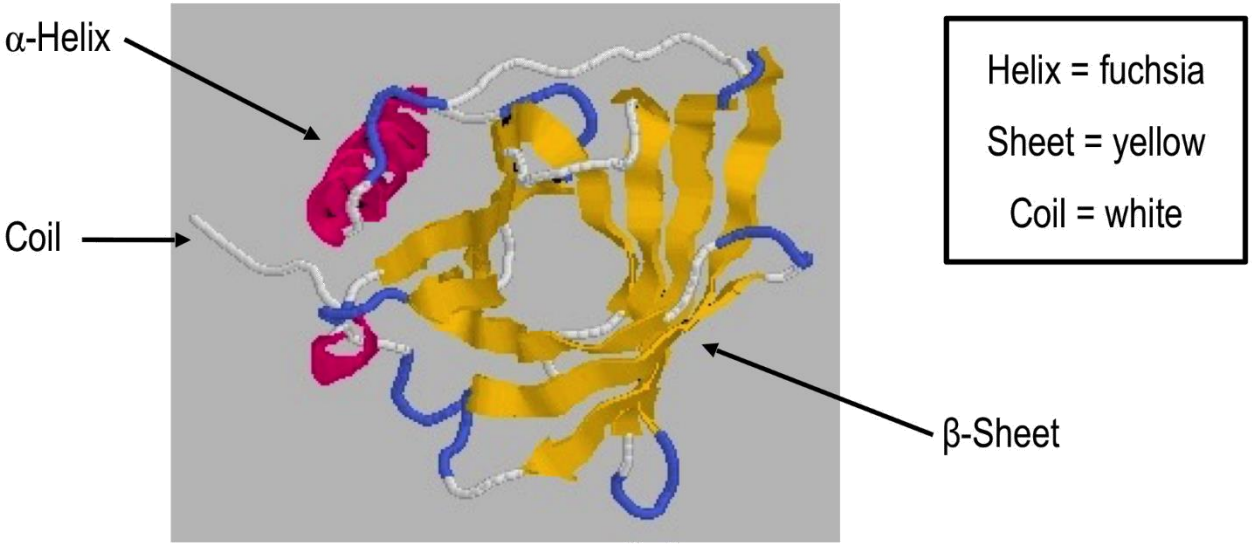


Figure 1. Overview of the origin and structures of silk fibroin. (**A**) Popular silk sources include *Nephila clavipes* (1.) and *Araneus diadematus* (2.) spiders, *Antheraea pernyi* (3.) and *Samia cynthia ricini* (4.) wild silkworms, and *Bombyx Mori* (5.) domestic silkworms. (**B**) Among them, *B. mori* silkworm is the most dominant source for silk fibers production. (**C**) Main proteins of silkworm silk fibers are fibroin and sericin (reproduced with permission [15]). (**D**) Hydrogen bonds between primary amino acid sequence of fibroin contribute to the generation of β-sheet crystallites (reproduced with permission [16]). (**E**) Fibroin is assembled from nanofibril units which crystal network consists of β-sheet crystallites dispersed within an amorphous matrix (reproduced with permission [17]).

Secondary Structure: Coil

Coil: A motif in the secondary structure of peptides that is not a helix or sheet. Also called a statistical coil or by the misleading term ‘random coil’.

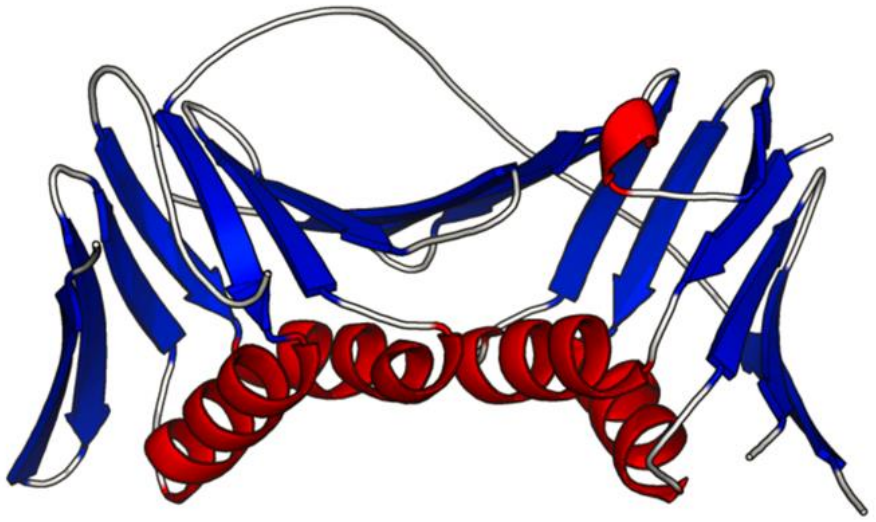


Secondary structures in retinol binding protein (a carrier protein that binds retinol)

Tertiary Structure

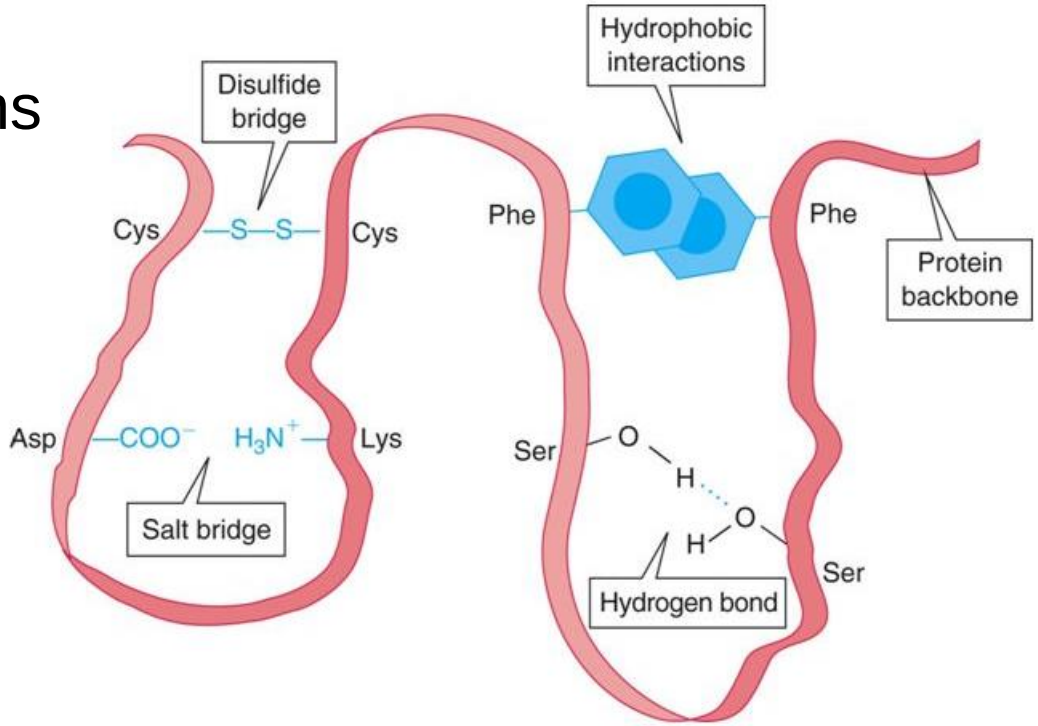
Tertiary Structure: The three-dimensional structure of a biopolymer, as defined by the spatial coordinates of all the atoms. In a protein, tertiary structure is controlled by disulfide bridges and folding in response to the environment.

Tertiary



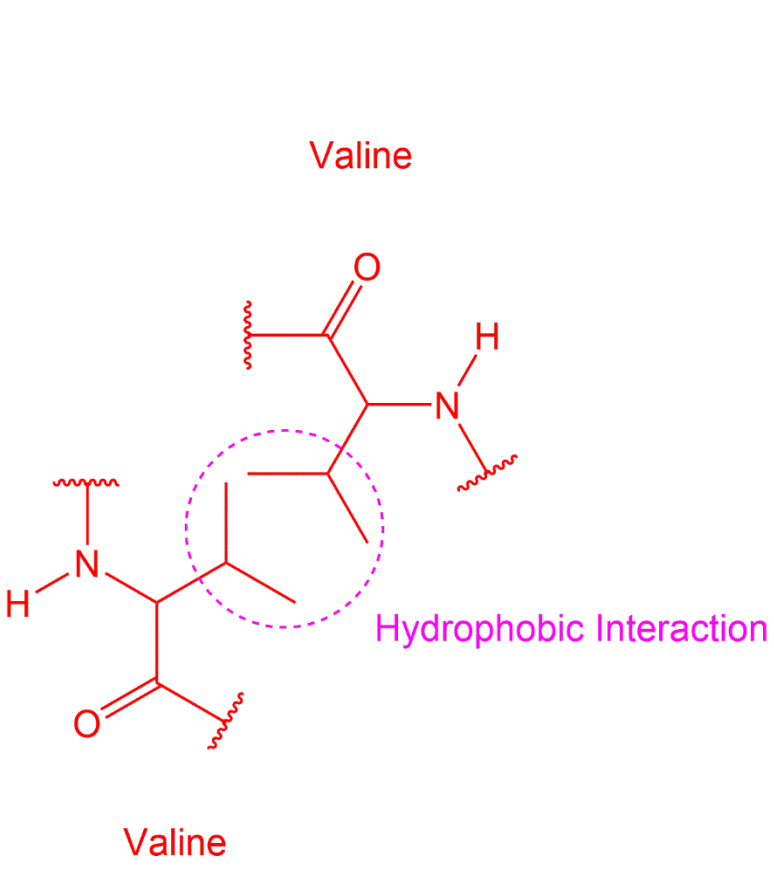
Residue Interactions are Responsible for Tertiary Structure

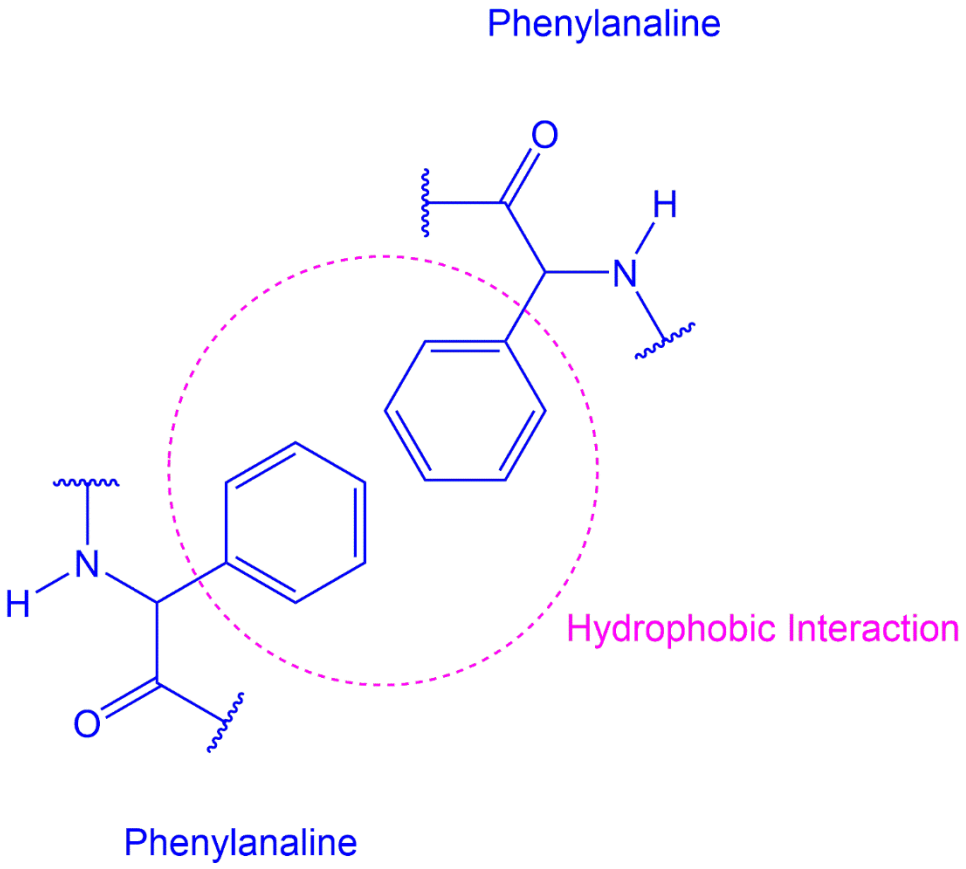
1. Hydrophobic Interactions
2. Hydrogen Bonding
3. Salt Bridges
4. Disulfide Bridges



Hydrophobic Interactions

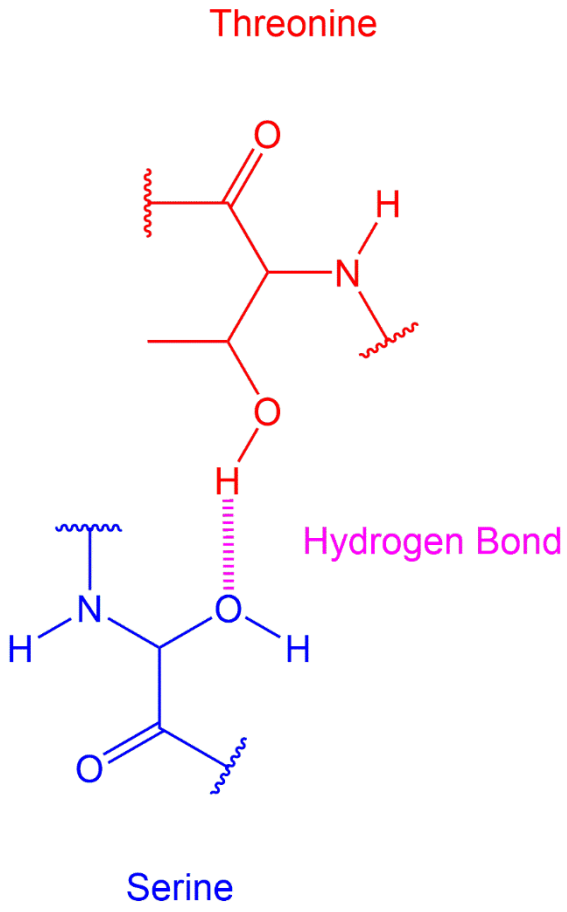
Hydrophobic interactions are the interactions between the nonpolar side chains.





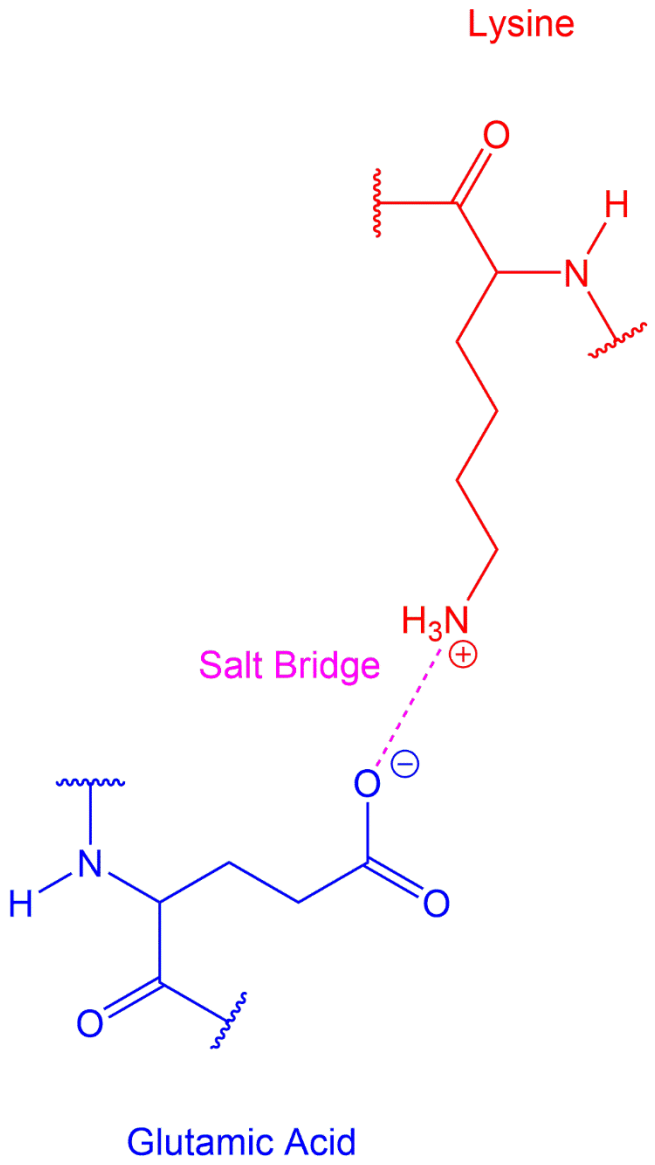
Hydrogen Bonding

Hydrogen bonding between side chains



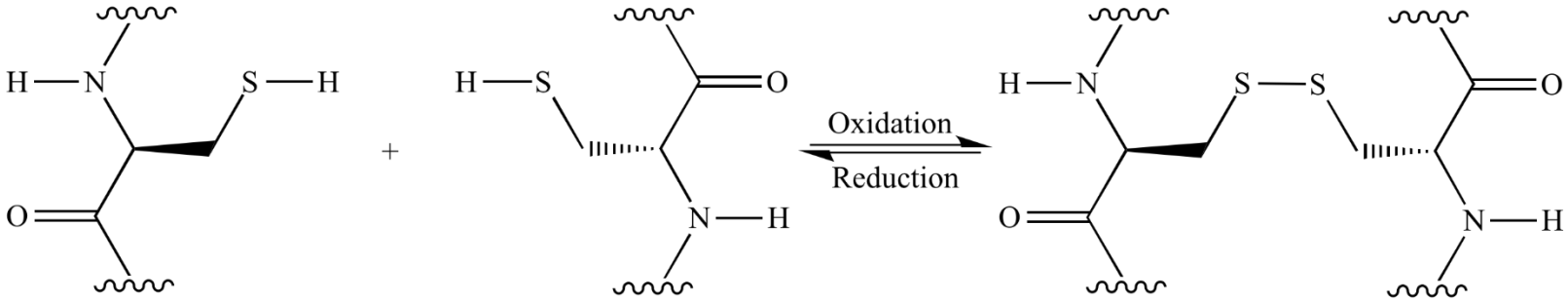
Salt Bridges

Salt bridges form between the charged acidic and basic groups of amino acids residues.



Disulfide Bridges

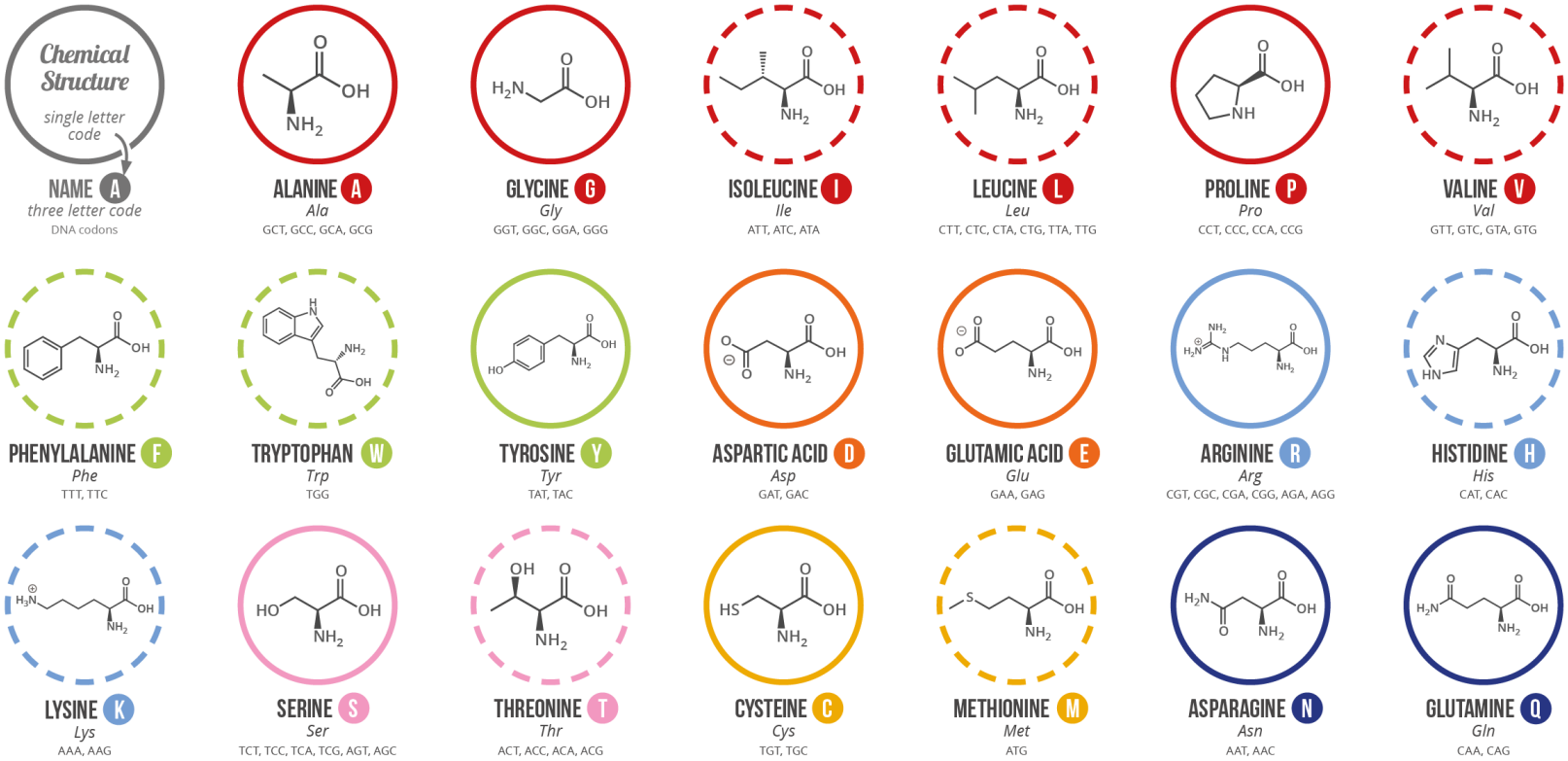
Disulfide bridges form between the thiol (sulfur analog of alcohol) functional groups.



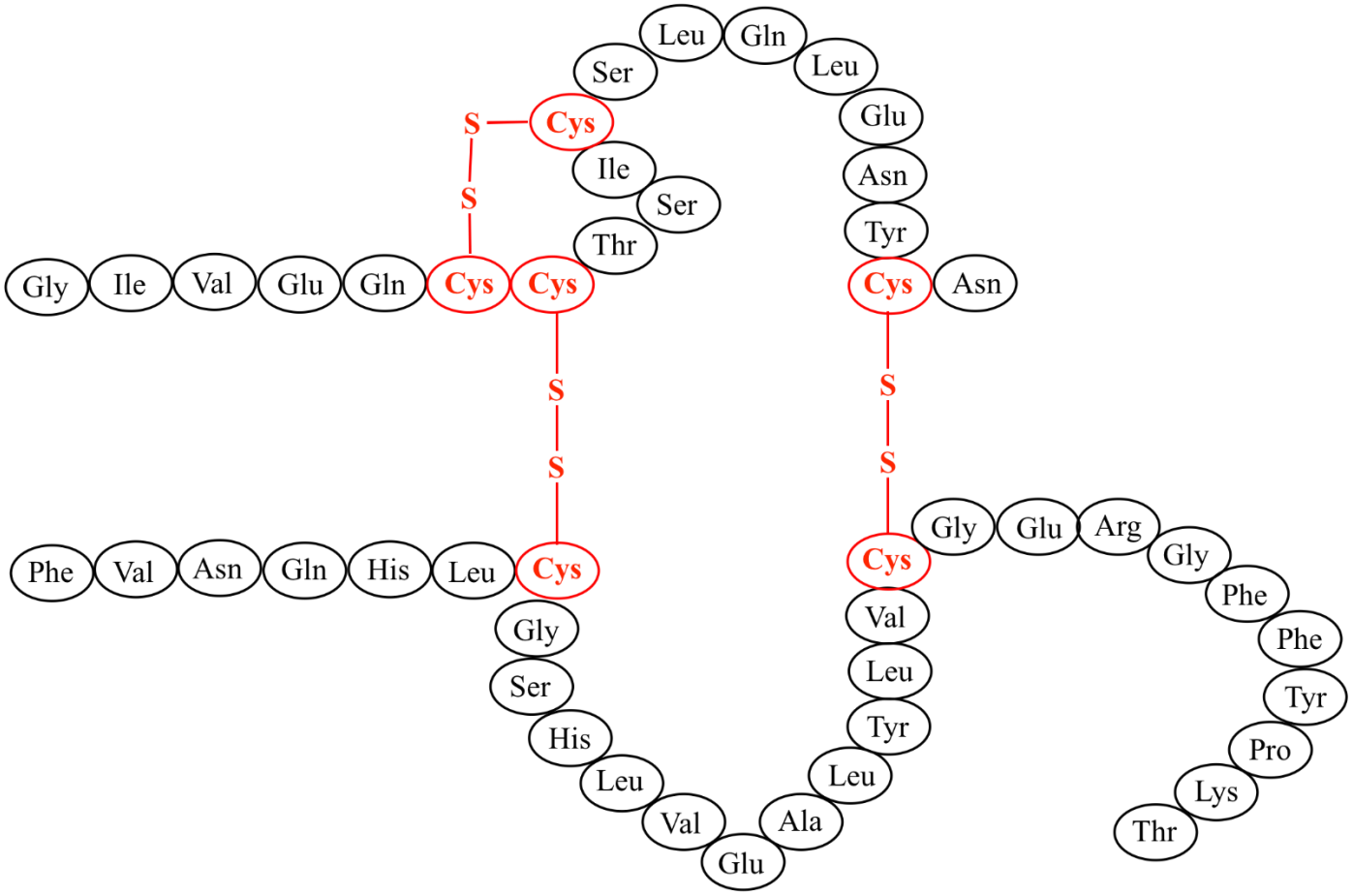
Disulfide Bridge

Problem 1

Which amino acid(s) form disulfide bridges?

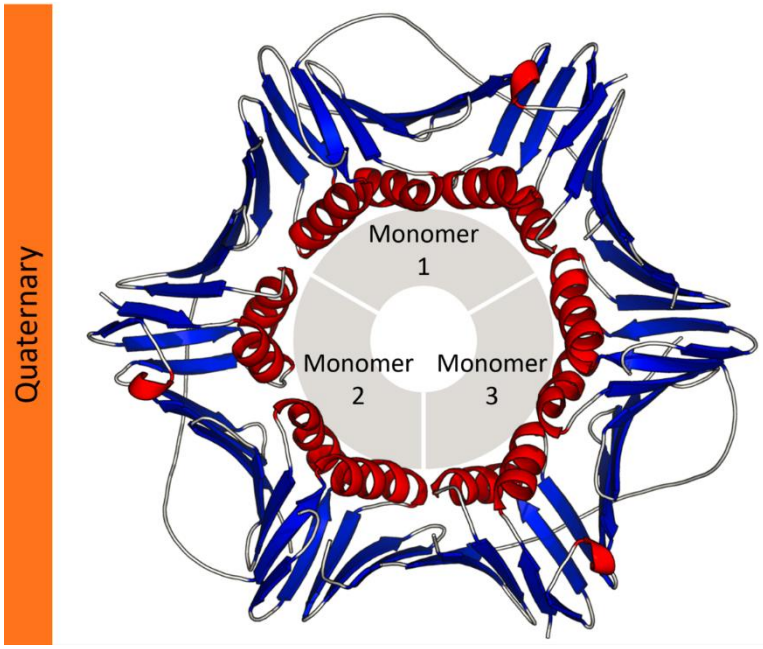


Disulfide Bridge



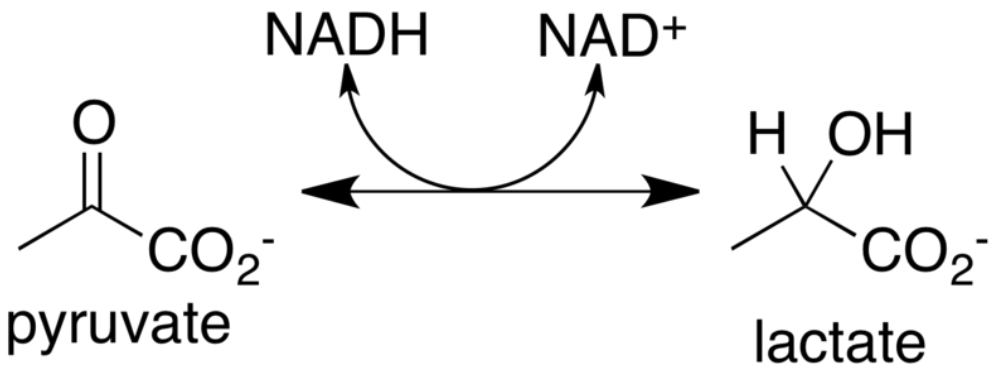
Quaternary Structure

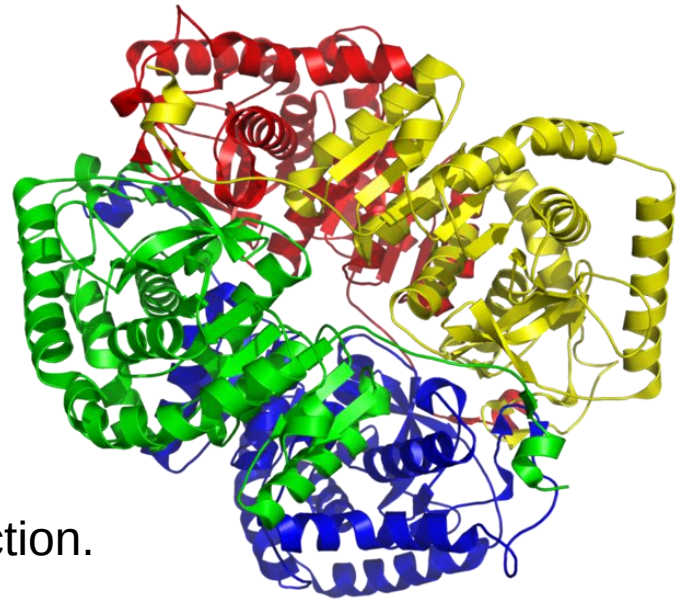
Quaternary Structure: Noncovalent association of subunits (proteins) into a single, larger protein structure.



Quaternary Structure

Lactate dehydrogenase a: A tetramer of four identical monomers with the subunit M. Note, the color is different to highlight the four subunits, although they are all identical.

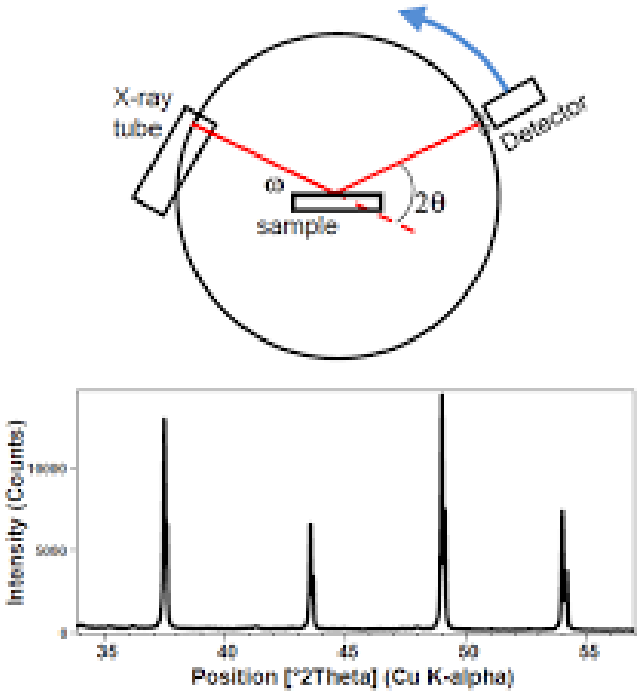




Lactate dehydrogenase a catalyzes the following reaction.

X-Ray Diffraction (XRD)

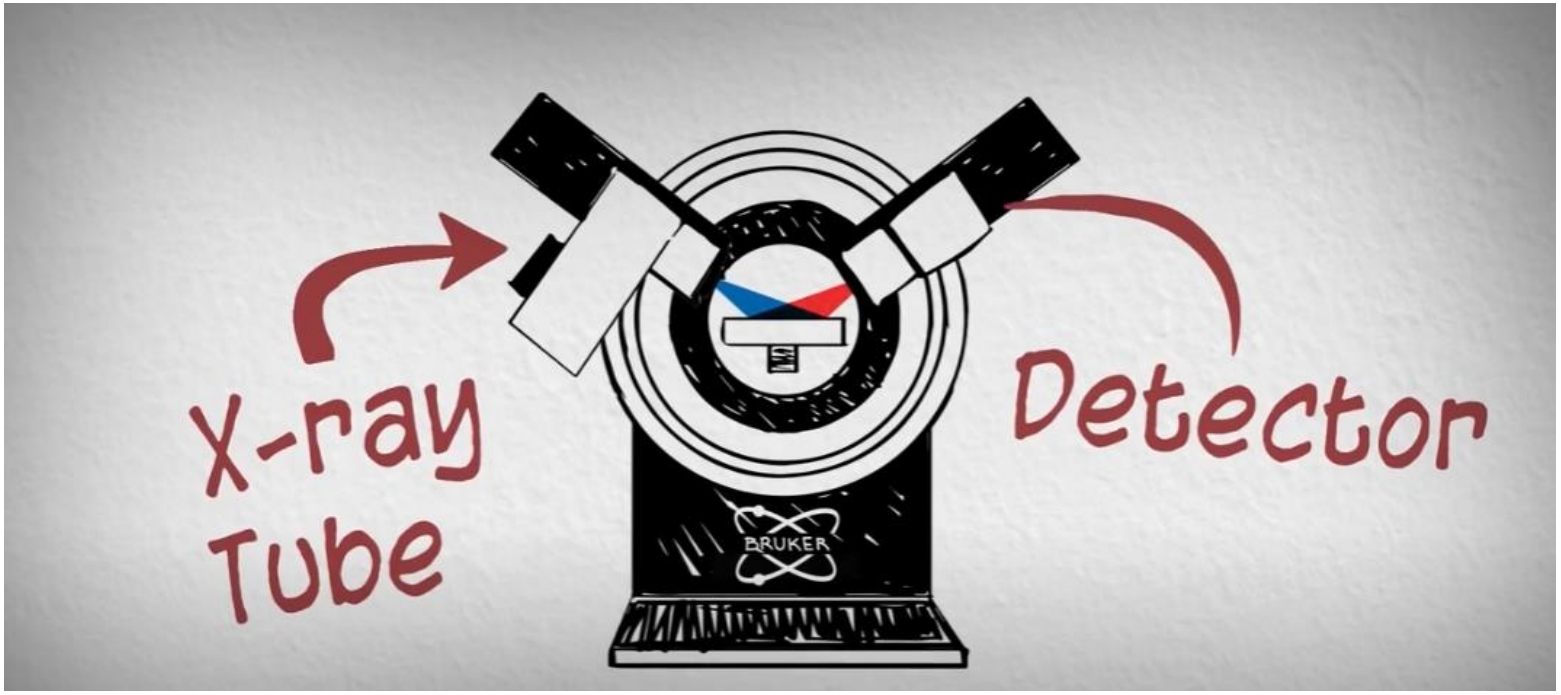
X-Ray Diffraction: The study of crystal structure and the structure of the atoms, molecules, or ions which compose the crystal, based on diffraction of X-rays.



XRD Explanation and Video

https://www.youtube.com/watch?v=QHMzFUo0NL8

4 minutes and 7 seconds

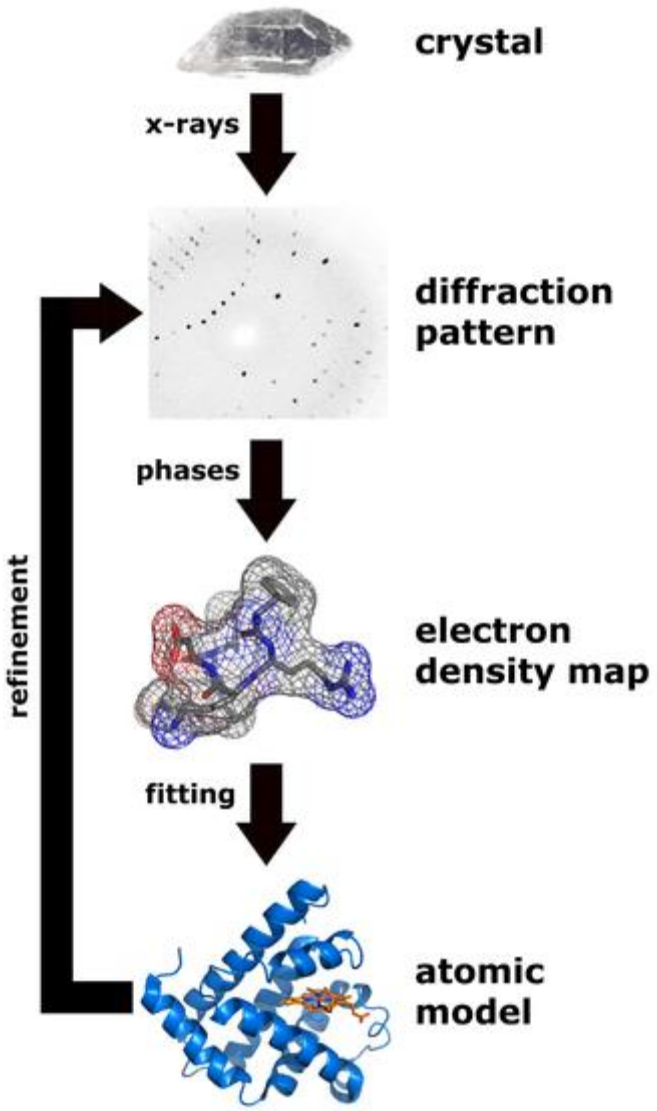


XRD Explanation and Video

https://www.youtube.com/watch?v=gLsC4wlrR2A

7 minutes and 47 seconds

Determining Protein Structure: X-Ray Crystallography



Protein Denaturation Results in a Loss of Structure AND Function

Quaternary Structure Denaturation: Disruption of the protein monomers (subunits)

Tertiary Structure Denaturation: Includes disruption of the following:

1. Covalent interaction between amino acid side-chains (disulfide bridges)

2. Non-covalent dipole-dipole interactions between polar amino acid side-chains

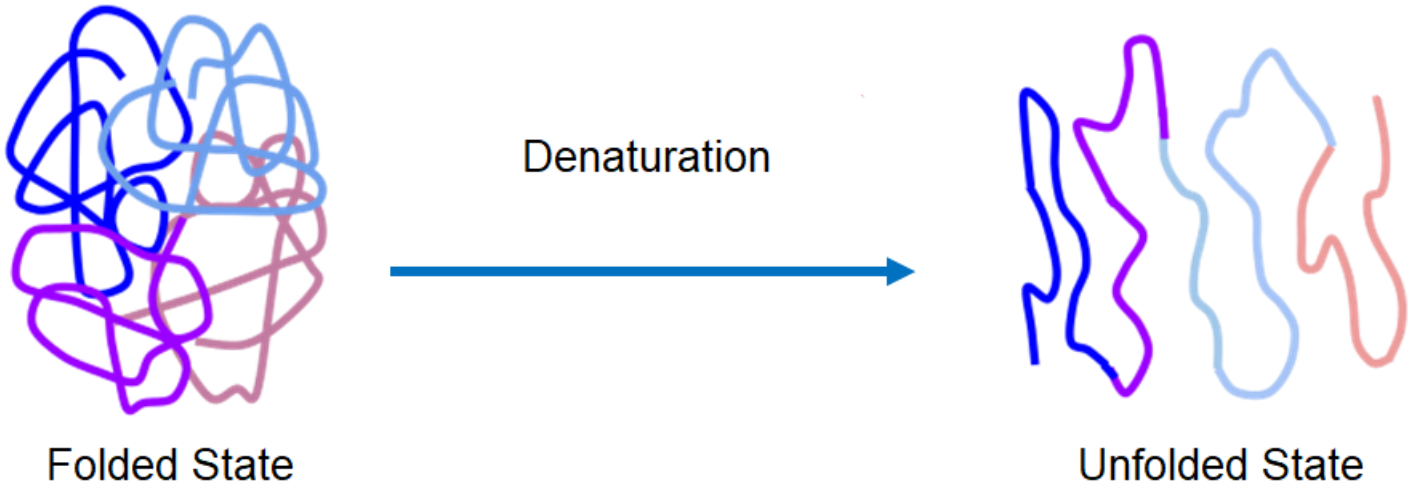
3. Instantaneous dipole-induced dipole between nonpolar amino acid side chains

Secondary Structure Denaturation: Loss of all regular repeating patterns such as alpha-helices, beta-sheets. Adoption of a random coil configuration

Primary Structure: Note, the primary structure is maintained by peptide bonds and is ***NOT*** affected by protein denaturation

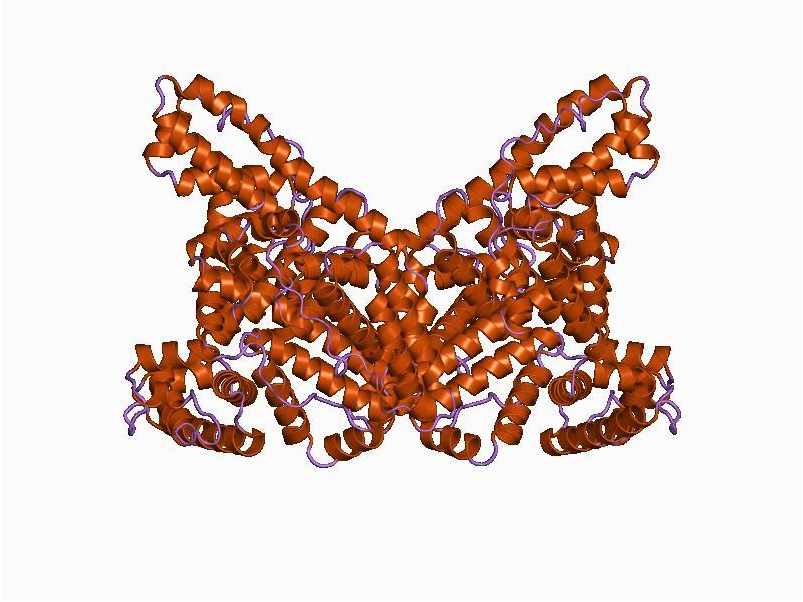
Avenues of Protein Denaturation

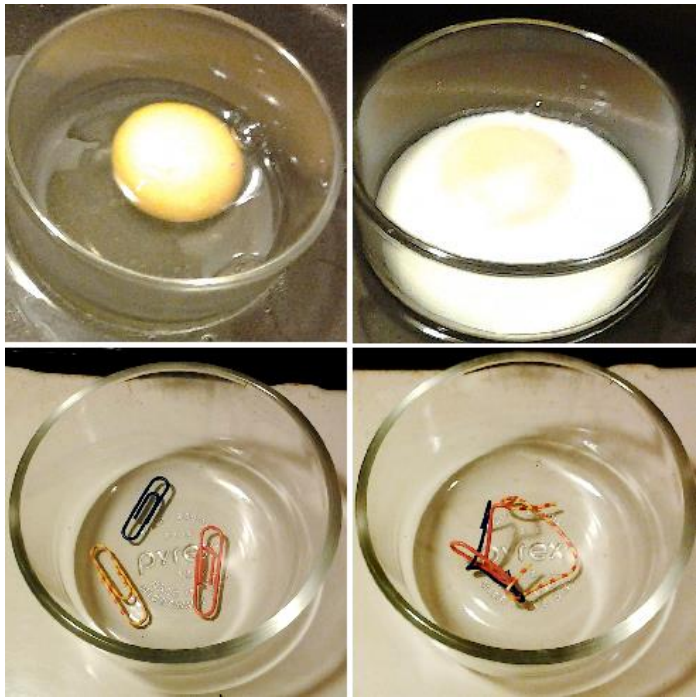
1. Heat Denaturation
2. pH Denaturation
3. Alcohol Denaturation



Heat Denaturation of Proteins

Denaturation of the egg white protein (*albumin*)





Unboiling an Egg!!!

https://www.youtube.com/watch?v=0msE39RgjgA

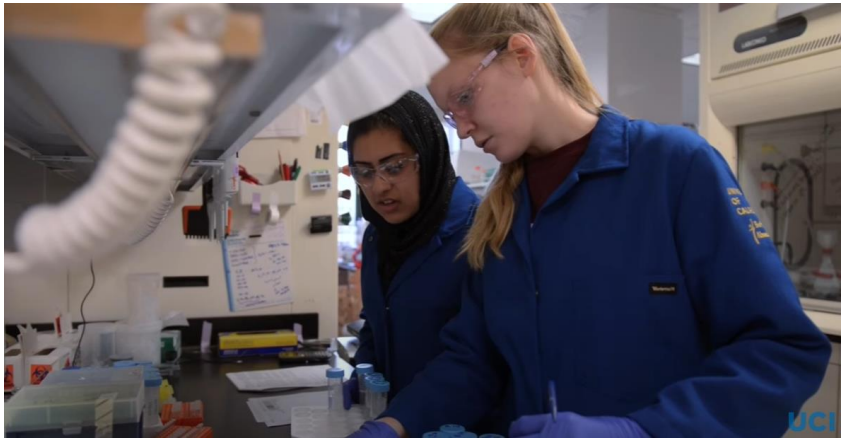
2 minutes and 48 seconds

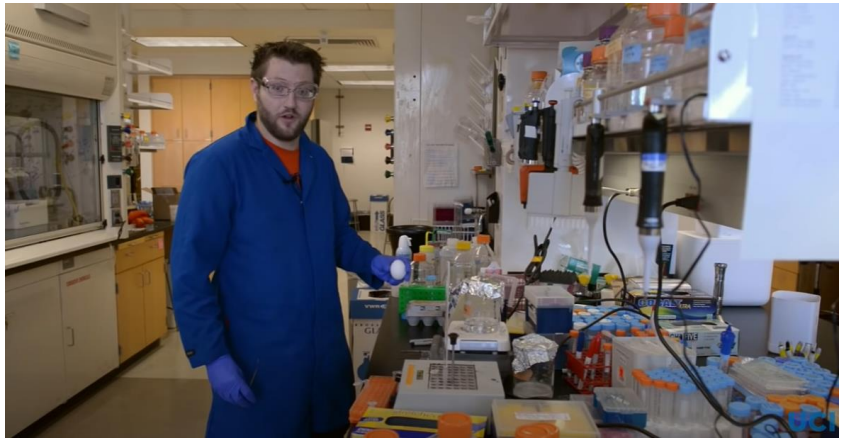
https://www.youtube.com/watch?v=3d1rNTWcDeo

2 minutes and 35 seconds

https://www.youtube.com/watch?v=CHMY4G9gTPA

4 minutes and 9 seconds





Unboiling an Egg Paper

Shear-Stress-Mediated Refolding of Proteins from Aggregates and Inclusion Bodies

Abstract

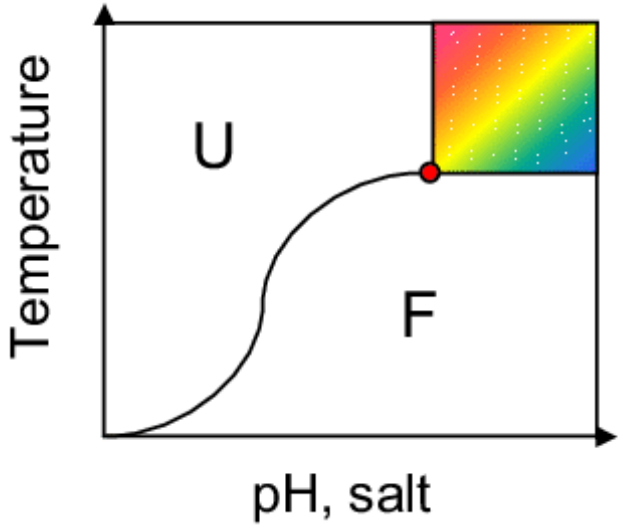
Recombinant protein overexpression of large proteins in bacteria often results in insoluble and misfolded proteins directed to inclusion bodies. We report the application of shear stress in micrometer-wide, thin fluid films to refold boiled hen egg white lysozyme, recombinant hen egg white lysozyme, and recombinant caveolin-1. Furthermore, the approach allowed refolding of a much larger protein, cAMP-dependent protein kinase A (PKA). The reported methods require only minutes, which is more than 100 times faster than conventional overnight dialysis. This rapid refolding technique could significantly shorten times, lower costs, and reduce waste streams associated with protein expression for a wide range of industrial and research applications.

pH Denaturation of Proteins

Acid Denaturation: pH 2-5

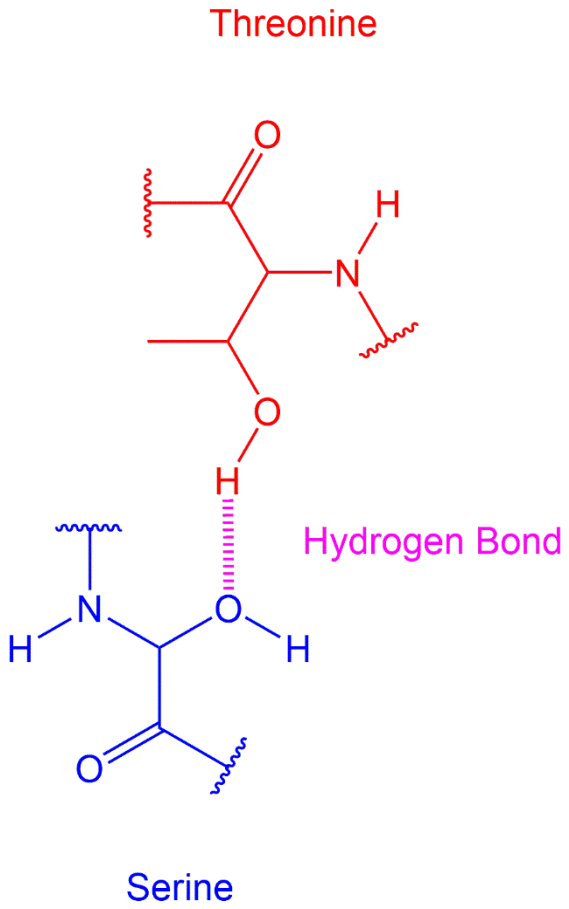
Base Denaturation: pH 10+

Red: Mostly Unfolded
Yellow: Partly Unfolded
Blue: Mostly Folded



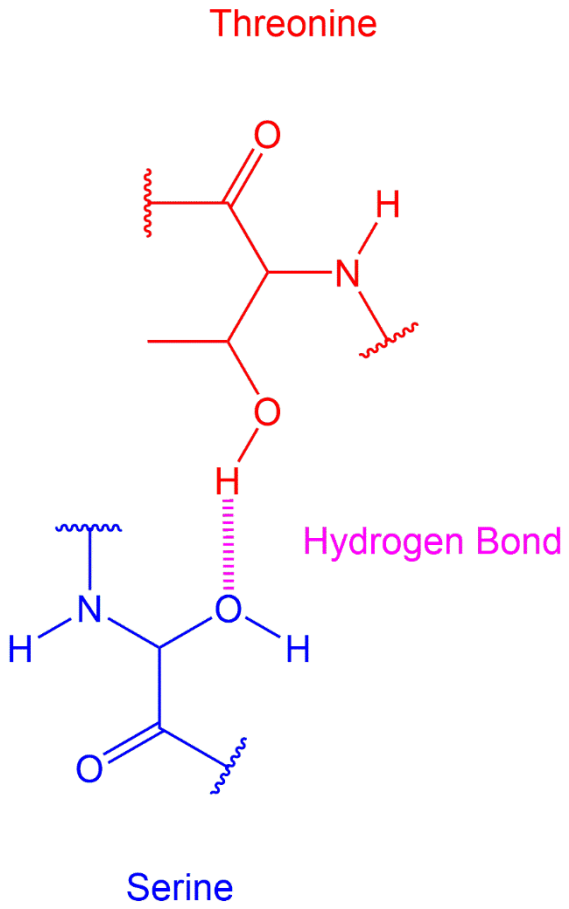
Problem 2

What do you expect to occur to occur to the hydrogen bond when the pH lowers to 2



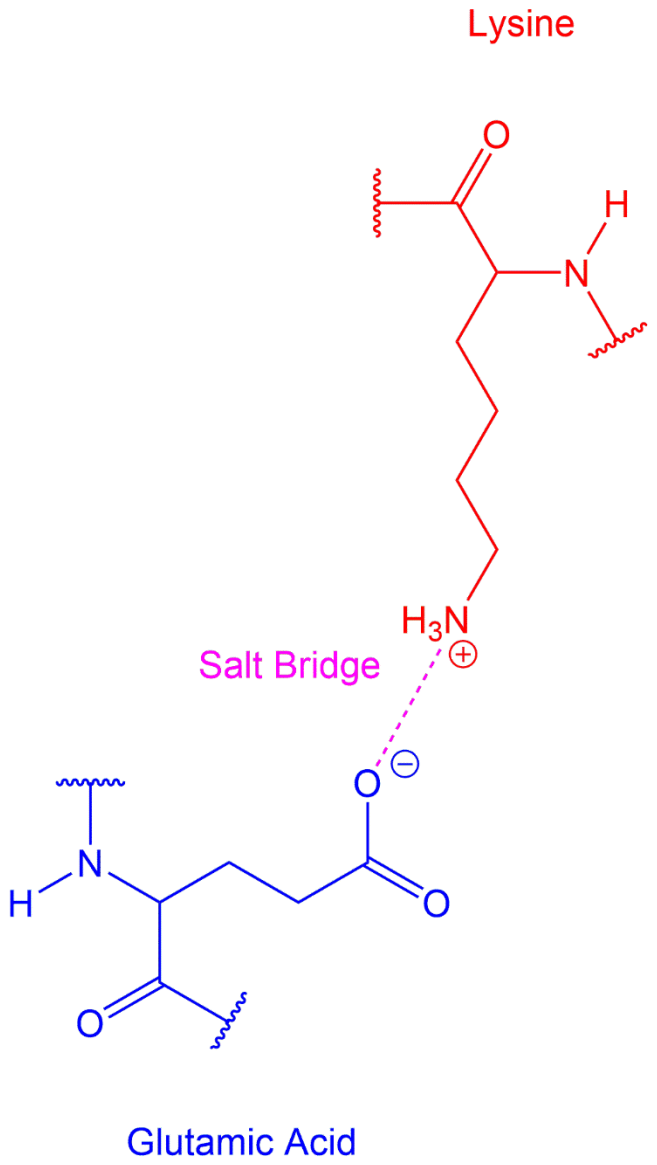
Problem 3

What do you expect to occur to occur to the hydrogen bond when the pH increases to 11



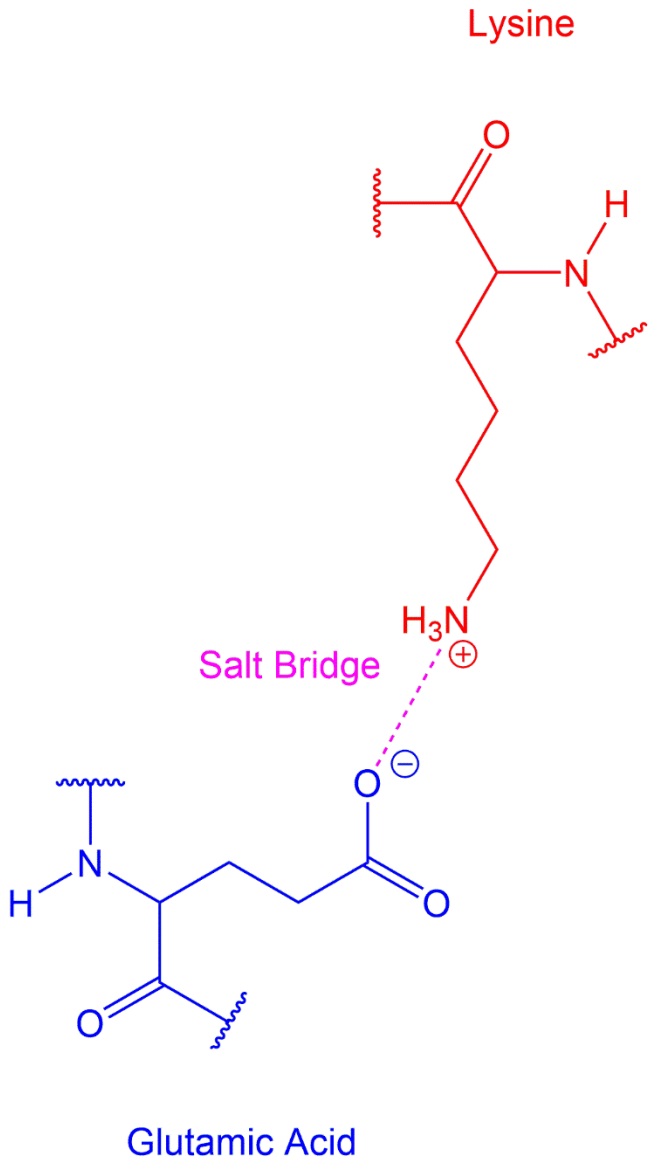
Problem 4

What do you expect to occur to occur to the salt bridge when the pH lowers to 2?



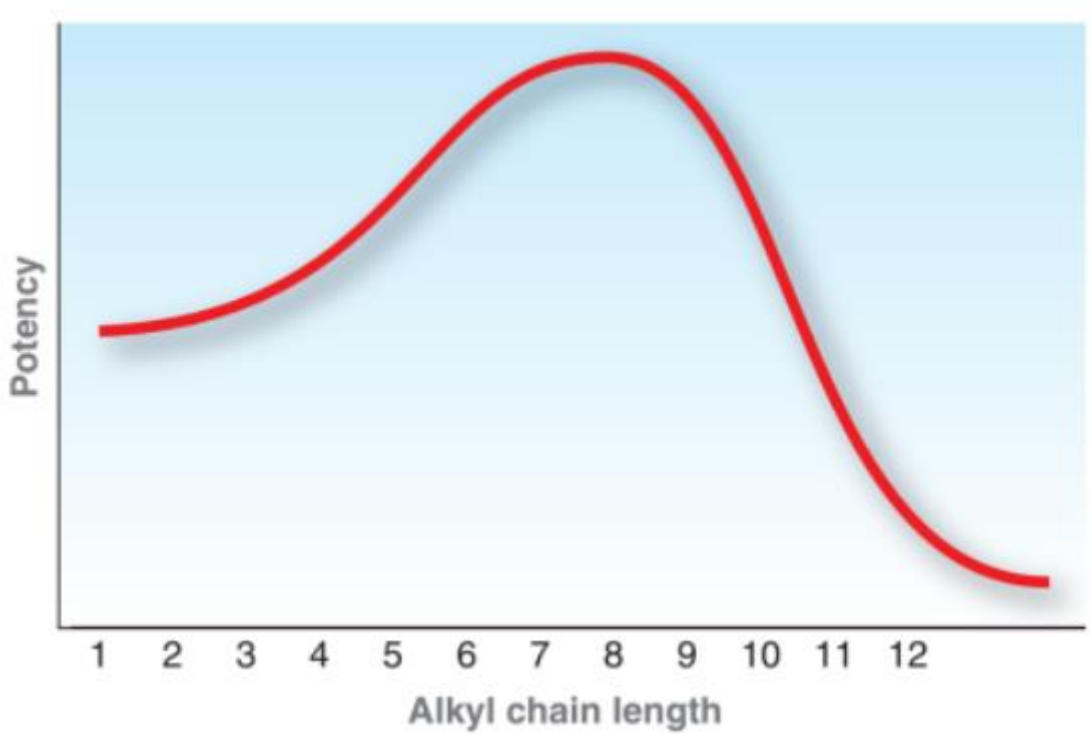
Problem 5

What do you expect to occur to occur to the salt bridge when the pH increases to 11?



Alcohol Denaturation of Proteins

Why are alcohols potent against bacterial and viral walls?



Organic Chemistry by Kline (3rd Edition)

Antimicrobial Properties of Alcohols

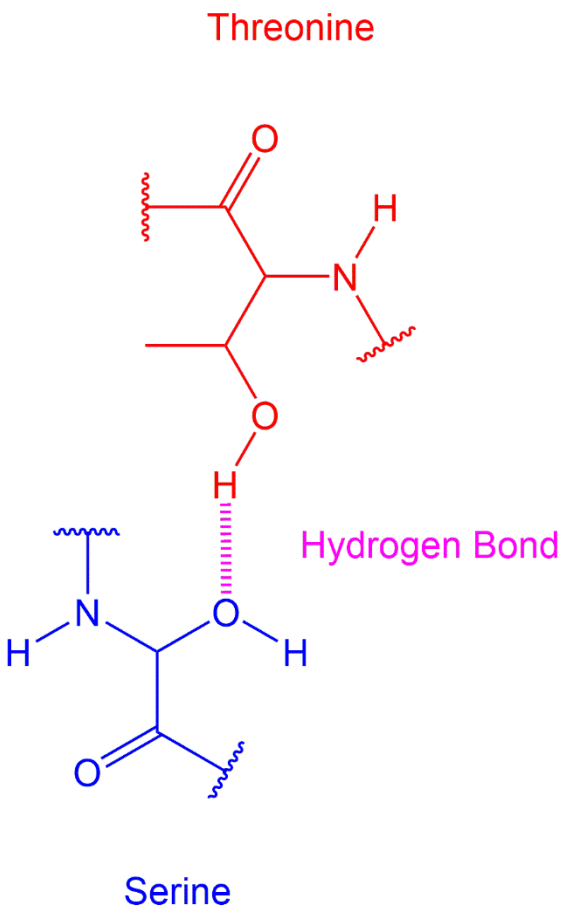
Alcohols

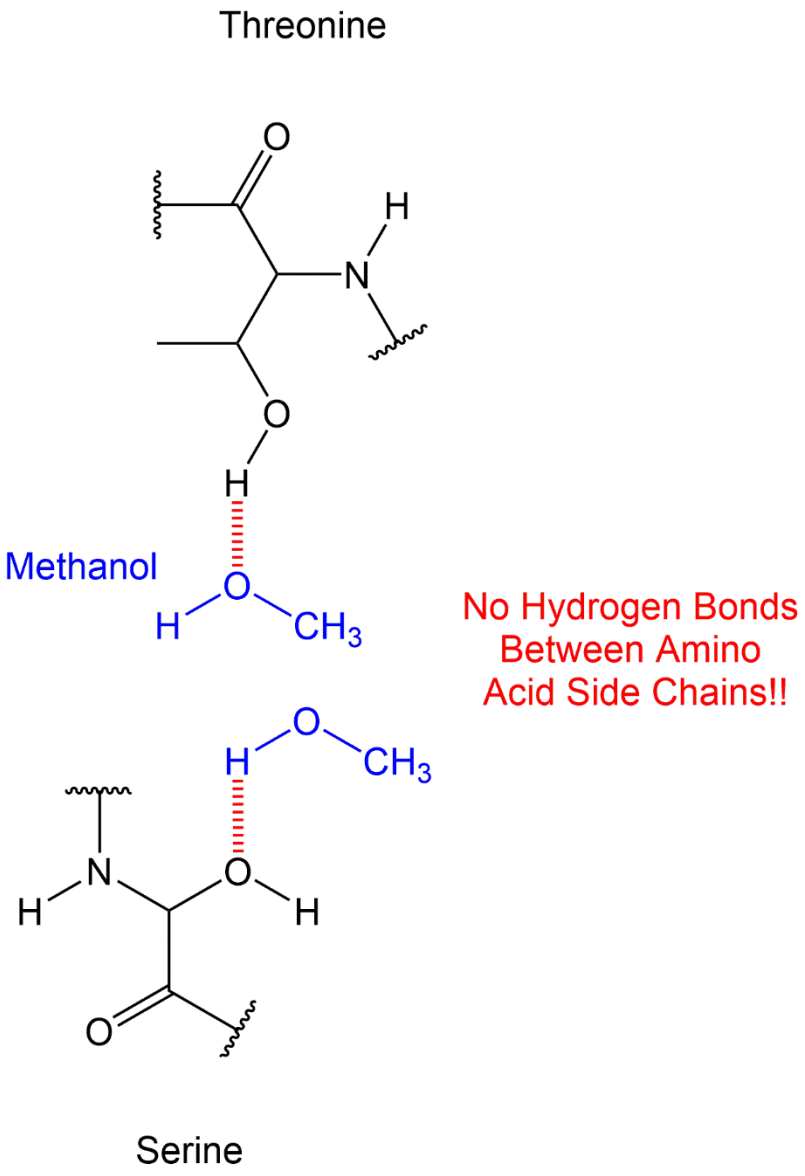
Although several alcohols have been shown to be effective antimicrobials, ethyl alcohol (ethanol, alcohol), isopropyl alcohol (isopropanol, propan-2-ol) and *n*-propanol (in particular in Europe) are the most widely used (337). Alcohols exhibit rapid broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), viruses, and fungi but are not sporicidal. They are, however, known to inhibit sporulation and spore germination (545), but this effect is reversible (513). Because of the lack of sporicidal activity, alcohols are not recommended for sterilization but are widely used for both hard-surface disinfection and skin antisepsis. Lower concentrations may also be used as preservatives and to potentiate the activity of other biocides. Many alcohol products include low levels of other biocides (in particular chlorhexidine), which remain on the skin following evaporation of the alcohol, or excipients (including emollients), which decrease the evaporation time of the alcohol and can significantly increase product efficacy (68). In general, isopropyl alcohol is considered slightly more efficacious against bacteria (95) and ethyl alcohol is more potent against viruses (259); however, this is dependent on the concentrations of both the active agent and the test microorganism. For example, isopropyl alcohol has greater lipophilic properties than ethyl alcohol and is less active against hydrophilic viruses (e.g., poliovirus) (259). Generally, the antimicrobial activity of alcohols is significantly lower at concentrations below 50% and is optimal in the 60 to 90% range.

Little is known about the specific mode of action of alcohols, but based on the increased efficacy in the presence of water, it is generally believed that they cause membrane damage and rapid denaturation of proteins, with subsequent interference with metabolism and cell lysis (278, 337). This is supported by specific reports of denaturation of *Escherichia coli* dehydrogenases (499) and an increased lag phase in *Enterobacter aerogenes*, speculated to be due to inhibition of metabolism required for rapid cell division (101).

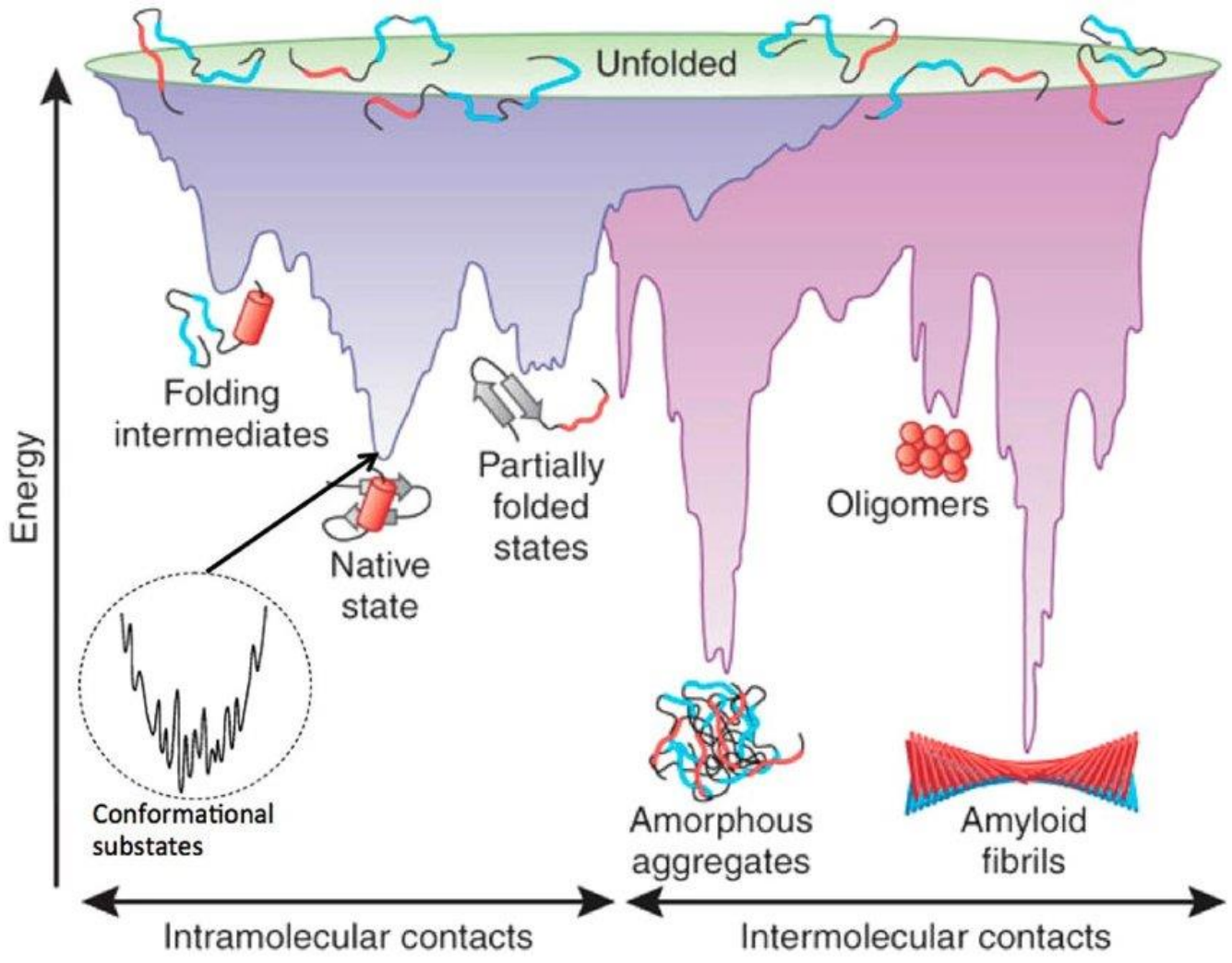
"Antiseptics and Disinfectants: Activity, Action, and Resistance", Clinical Microbiology Reviews, **12**(*1*) 147-179 (1999).

Alcohol Denaturation of Proteins



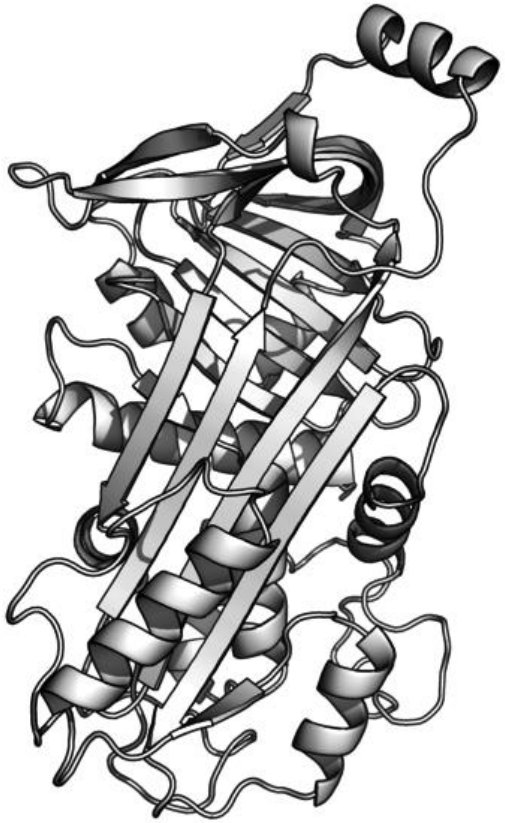


Energetics and Conformations of Protein Folding

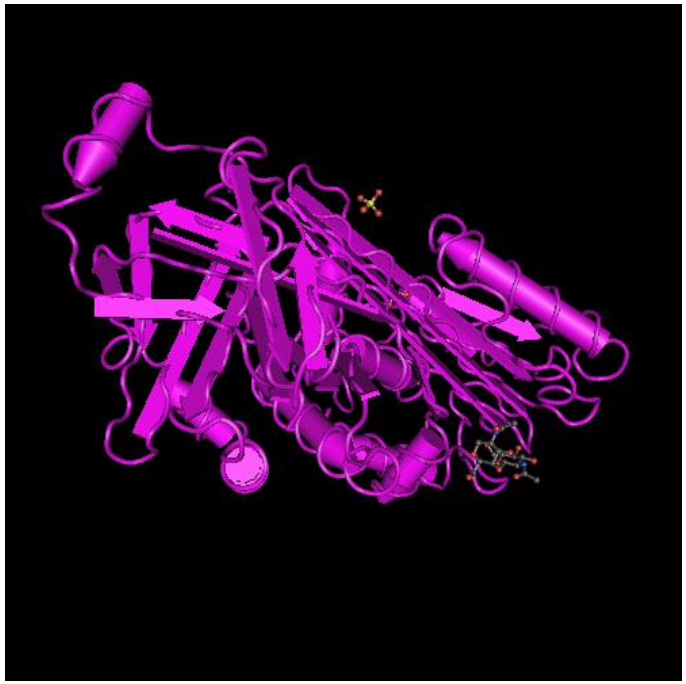


The Stability of the S-Ovalbumin Explains Why Fresh Egg Whites Cook More Readily

Ovalbumin

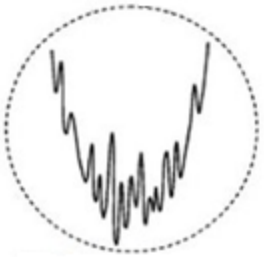


S-Ovalbumin



Why is S-Ovalbumin More Stable?

It is hypothesized that an inversion of configuration from L-serine to D-serine is responsible for the stability that facilitates the conformational changes.



Conformational States

THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Crystal Structure of S-ovalbumin as a Non-loop-inserted Thermostabilized Serpin Form*

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Ovalbumin, a non-inhibitory member of serine proteinase inhibitors (serpin), is transformed into a heat-stabilized form, S-ovalbumin, under elevated pH conditions. The structural mechanism for the S-ovalbumin formation has long been a puzzling question in food science and serpin structural biology. On the basis of the commonly observed serpin thermostabilization by insertion of the reactive center loop into the proximal β-sheet, the most widely accepted hypothetical model has included partial loop insertion. Here we demonstrate, for the first time, the crystal structure of S-ovalbumin at 1.9-Å resolution. This structure unequivocally excludes the partial loop insertion mechanism; the overall structure, including the reactive center loop structure, is almost the same as that of native ovalbumin, except for the significant motion of the preceding loop of strand 1A away from strand 2A. The most striking finding is that Ser-164, Ser-236, and Ser-320 take the D-amino acid residue configuration. These chemical inversions can be directly related to the irreversible and stepwise nature of the transformation from native ovalbumin to S-ovalbumin. As conformational changes of the side chains, significant alternations are found in the values of the χ_1 of Phe-99 and the χ_3 of Met-241. The former conformational change leads to the decreased solvent accessibility of the hydrophobic core around Phe-99, which includes Phe-180 and Phe-378, the highly conserved residues in serpin. This may give a thermodynamic advantage to the structural stability of S-ovalbumin.

pH due to the release of carbon dioxide through the eggshell, and it can be reproduced *in vitro* by an alkaline treatment (1, 4).

To understand the protein thermostabilization mechanism, the structural difference between native ovalbumin and S-ovalbumin has been extensively investigated by spectroscopic and biochemical approaches. The obtained data, however, have been consistent with a variety of models, including deamidation (6), surface hydrophobicity change (7), and secondary structure transition (8, 9) mechanisms. As for secondary structure change, studies using far UV CD and Raman spectroscopy have concluded that a small loss of α-helix content and a concomitant increase in β-structure participate in S-ovalbumin formation (8, 9), though a latter analysis from a different laboratory has been consistent with the absence of a secondary structure change (10). This conclusion about the secondary structure transition along with alternative evidence have led to the proposal of a widely accepted model for S-ovalbumin formation that includes a partial insertion of the reactive center loop into the proximal β-sheet (9, 11).

On the basis of structural similarity (12, 13), ovalbumin has been grouped as a member of the serine proteinase inhibitor (serpin) superfamily. Serpin is a protein with metastable conformation, which undergoes a unique conformational change upon exertion of the inhibitory activity (14–16); following the proteolytic cleavage at the P1-P1′ site, the reactive center loop is inserted into the proximal β-sheet, and this insertion accompanies large thermostabilization of the protein (14). Although ovalbumin is a non-inhibitory serpin that lacks a loop insertion

Why Do Egg Whites Foam?

https://www.exploratorium.edu/cooking/eggs/pavlova-pop.html