Proteins are synthesized by plants and animals to play a role in their biology. The functions a protein has from the organism’s point of view include communication (e.g., insulin), structural (e.g., collagen in skin or keratin in hair), biochemical catalysts (e.g., enzymes), transportation (e.g., hemoglobin to transport oxygen in the blood), defense (e.g., antibodies), and storage (e.g., globulins in seeds). Very rarely do our needs of the protein match those of the plant/animal we are going to eat. Our needs are firstly nutritional – of the 20 amino acids common in nature we can synthesize 11 of them and must gain the rest from dietary sources and secondly functional – we rely on proteins to change the texture of our food for example by forming gels, or by stabilizing foams and emulsions. The only area where the proteins’ evolved function overlaps with our needs is when we use proteins as enzymes in bioprocessing (e.g., making corn syrup from the action of amylase enzymes on starch). We are also interested in how proteins interact with other food components (e.g., flavor binding) or have toxic or antinutritional properties (e.g., botulinum toxin is a protein).

In this section we will start by briefly reviewing the biochemical structure of proteins. We will then examine how they can be modified post-mortem by processing and through interactions with other ingredients and how this leads to some functional roles. We will finally look at some examples of the uses of enzymes in food processing.

1. Protein structure

A protein is a linear sequence of amino acids linked together by peptide bonds. There are 20 amino acids prevalent in nature and the sequence in which the cell assembles them is set in the DNA code (i.e., the primary structure of the protein). The 20 amino acids all have distinct structures and unique properties but with a couple of important exceptions we can classify them as:

- Charged/uncharged: Some amino acids have functional groups (e.g., carboxylic acid, amine, phenol, thiol) that can carry a charge depending on the pH.
- Hydrophobic/hydrophilic: Amino acids may be more or less water-soluble depending on the polarity of their structure. Charged amino acids tend to be more water-soluble.

Some important example amino acids include:

- Glutamic acid is a very polar.
- Phenylalanine has a bulky non-polar benzene ring as part of its side.
carboxylic acid which may carry a negative charge depending on the pH

Cysteine contains a functional thiol group. Thiols can dimerize under oxidizing conditions to form disulfide bonds (a dicysteine is called cystine).

Lysine contains an amine group that is an unusually strong nucleophile. The reactivity of this amine with reducing sugars in the Maillard reaction is particularly strong and so lysine is quickly destroyed during nonenzymatic browning.

During their RNA-mediated polymerization process, the amine group of one amino acid reacts with the carboxylic acid group of another and water is eliminated. The dipeptide formed still has a reactive amino and carboxylic acid group, which can continue to react to form sequentially larger polypeptides and eventually a complete protein. The number of amino acids required to form a protein varies widely (e.g., lysozyme is egg white is a simple globular protein with 129 residues while collagen is a triple helix of chains each containing more than 1400 residues).

A freshly synthesized polypeptide is no real use to the organism. It must first spontaneously fold into a characteristic shape necessary for it to function properly. Protein folding is a spontaneous process but incredibly precise process that remains one of the wonders of the natural world. The first step in understanding protein folding is to think about the preferred conformation of a polymer. A flexible chain will not stand straight in solution, the random conformation of the movable bond angles means instead it will take on a random coil conformation. In the case of a protein there are a number of hydrophobic groups on the chain that would prefer to remove themselves from the aqueous cell matrix by being buried in the core of the protein. This hydrophobic effect will tend to fold the random coil somewhat more tightly, however folding too tightly would have an entropy cost:
Another way of looking at protein folding is as a competition between the chain entropy and the solvent entropy. The highest entropy a chain can take is to form a random coil. Each bond angle is set randomly and can flex randomly. (Note that a random coil does not imply a single structure for the protein, instead a number of interchanging shapes all based on the principle that the bond angles are random). However, a random coil protein still has many hydrophobic amino acid residues exposed to the aqueous solvent. Water molecules organize themselves into a structured clathrate cage around hydrophobic groups, i.e., lowering their entropy. The lowered solvent entropy is a problem that can be overcome by more tightly folding the protein but that would create a problem by increasing the degree of ordering of the chain. The basis of protein folding then is a balance of forces argument between chain entropy (seek to unfold to maximize the disorder of the polymer itself) and solvent entropy (seek to fold up to remove the ordering effects of hydrophobic amino acid residues on water).

This simple argument allows us to image a protein in solution spontaneously taking on a tightly folded conformation, but the details distinguishing proteins lead to other structural intricacies. A whole range of other noncovalent interactions are important in supporting and refining the shapes taken. Important amongst these are (i) steric restraints – the chains flexibility is restricted to movement about the a-carbon and may be further restricted by bulky side chains, (ii) strong interchain hydrogen bonding may strengthen certain configurations, and (iii) permanent charges on the chain will lead to interchain electrostatic repulsion or attraction; these forces will be strongly pH dependent. Finally covalent disulfide bonds between two cysteine residues are very strong and can readily stabilize structure.

There are some common structural features that occur across a wide range of protein types. These are the secondary structures – a local folding of the polypeptide chain over
a part of its length. A protein may contain several types of different secondary structure or it may contain none. Examples of secondary structure include the $\alpha$-helix and $\beta$-sheet:

An $\alpha$-helix is a coil formed over a region of the polypeptide chain. There are strong hydrogen bonds between the nitrogen on the chain and the oxygen on the forth proceeding amino acid in the helix. However, probably more important is the radial positioning of the side chains sticking out at angles from the helix. One side of the helix tends to accumulate hydrophobic residues and one side hydrophilic. The overall helix is therefore amphiphillic and can easily be used to build tertiary structure by hiding the hydrophobic half in the core of the protein.

In a $\beta$-sheet polypeptides line up either parallel or antiparallel with one another. There are strong hydrogen bonds between the nitrogens on one chain and the oxygen on a second chain. However probably a more important driving force is half the side chains line up above the plane of the sheet and half below. The half above tend to be hydrophobic and the half below hydrophilic. The overall structure is amphipillic and held together by hydrophobicity.

Tertiary structure is the bulk folding of the chain to make a defined three-dimensional structure. As we have seen the primary driving force for protein folding is solvent entropy. Interchain hydrogen bonding, electrostatic interactions, and disulfide bonds may reinforce the structure formed. Most proteins are at least approximately spherical blobs in solution as this allows them to minimize their surface to volume ratio and hide more hydrophobic amino acids in the core away from water. More hydrophilic polymers will tend to be more open and more hydrophobic more densely packed. Non-spherical proteins (e.g., collagen) need a strong secondary structure to maintain an extended shape; normally for structural reasons.
2. **Protein Denaturation**

The native structure of a protein is the energetic minimum under physiological protein. Any change in conformation away from this shape will represent an energy cost. The protein is not completely static – it will flex and bend in response to thermal energy or binding ligands but will always tend to return to the same shape. When the protein is moved out of the physiological state the balance of forces acting are changed and the protein may respond to change shape to minimize its energy under the new conditions. For example a globular protein folds up tightly in water to hide its hydrophobic residues. If the same molecule was moved into an organic solvent it would “want” to unfold to hide its more hydrophilic groups in the core away from the nonpolar solvent.

The most common way to denature proteins is through heat. Consider how the energy balance holding the protein together change with temperature; for the reaction of native protein transforming to denatured protein: $P_N \leftrightarrow P_D$. The reaction will proceed spontaneously if the Gibbs free energy for the reaction is negative. The two major contributions to Gibbs free energy here are solvent entropy (opposes unfolding) and chain entropy (favors unfolding).

$$\Delta G = \Delta G_{\text{solvent}} + \Delta G_{\text{chain}}$$

If a protein unfolds, the chain becomes less organized, so its entropy increases, so $-T\Delta S$ becomes negative and the reaction is favored. On the other hand if the chain unfolds, more clathrate water is formed, the solvent becomes more organized, so its entropy ($-T\Delta S$) decreases, so $-T\Delta S$ becomes positive and the reaction is opposed. Under physiological conditions these factors balance each other with $\Delta G_{\text{total}}$ slightly positive and the reaction stays in the native state. As temperature increases the “$-T$” multiplier on both entropy terms increases and the value of $\Delta G_{\text{solvent}}$ and $\Delta G_{\text{chain}}$ increase in parallel. $\Delta G_{\text{solvent}}$ becomes increasingly positive and $\Delta G_{\text{chain}}$ increasingly negative. Their sum is unchanged and the protein remains stable. However at about 70oC the structure of the clathrate water starts to break down and the hydrophobic opposition to chain entropy stops being able to keep up and their sum (the free energy for denaturation) becomes negative and the protein unfolds. In practice the various other interactions (e.g., interchain hydrogen bonds, electrostatic interactions, disulfide bonds etc) will oppose or favor denaturation to different extents depending on their prevalence and strength in a given protein and the denaturation temperature will vary between types of protein.

Other factors can also cause denaturation. Any factor or combination of factors that increase the forces forcing a protein apart or reduces those holding them together may cause denaturation. Examples include:

- Changing the pH so the protein is highly charged (positive below the pI or negative above) can favor protein denaturation because the like charges on the chain repel one another and favor
expansion of the folded structure.

- Making the solvent more non-polar (e.g., by adding certain alcohols) reduces the energy cost of exposing the hydrophobic amino acids to solvent and can favor denaturation.
- When there is a surface present (e.g., a freshly prepared foam or emulsion) (1) an aqueous protein will (1) adsorb at the interface to allow any hydrophobic surface patches to move out of the aqueous environment. The surface protein may then (3) unfold at the surface to allow more of the hydrophobic amino acids to move into the non-polar environment.

Protein denaturation is, in principle reversible. Once the denaturing stress is removed the protein will “try” to get back into its energy minimum, which, under physiological conditions is the native state. In practice protein regeneration is difficult because the precise sequence of steps needed to fold the protein is hard to repeat. It is very easy for the protein to try to minimize its energy by misfolding. The misfolded structure is not ideal (native) but in order to get out of this state it must unfold again before it can have another try at refolding correctly. Getting out of the misfolded state is a cost and the protein is frequently stuck secondary energy minimum and never regenerates. Additionally denaturation leads to the exposure of hydrophobic amino acids at the protein surface. These amino acids would ideally repack into the core of the protein, but frequently can be left “stranded” at the surface. The surface hydrophobic patches can lead to proteins aggregating to hide from water.

**Summary of Protein Denaturation**

(a) The native protein is the most stable (lowest energy) state under physiological conditions. When the conditions are changed, the balance of forces changes and (b) the protein denatures. When the denaturing conditions are removed the protein could (c) regenerate but this can be quite difficult in practice as the many (d) denatured forms must refold in precisely the same sequence to rebuild the ideal native structure.

In many cases the proteins will move their hydrophobic amino acids away from water by (e) aggregating. Aggregated proteins (f) cannot regenerate and may either precipitate or gel.
3. **Protein Functionality**

Protein structure describes the chemical and physical shape of a protein. Functionality describes what the protein does from our point of view.

As alluded to above, an important functional role of protein that depends on their native state functionality (i.e., biological functionality) depends on their behavior as enzymes. Enzymes are biological catalysts that increase the rate of a chemical reaction by providing a lower-energy pathway between starting conditions and end point. Frequently the reaction accelerated by the enzyme may be not apparent in its absence and it may seem as if a completely new pathway is being opened up but in fact the only effect is catalytic. Enzymes are unusually specific catalysts in that they will work on a very defined starting material to make a single, often chirally pure, form. Because the catalyst is an enzyme, any factor leading to denaturation may quickly destroy its activity.

A detailed treatment of enzyme structure/functionality, kinetics and associated biotechnology is beyond the scope of this course and we shall instead focus on some illustrative examples.

- **Polyphenoloxidase** catalyzes the oxidation of a wide range of phenolic compounds to diphenols and hence to quinonones. Quinonones spontaneously (i.e., nonenzymically) polymerize to brown melanin pigments. Polyphenoloxidase needs oxygen and a copper cofactor to function.

  ![Polyphenoloxidase Reaction](image)

  Polyphenoloxidase activity is a plant defense mechanism designed to turn natural plant phenols into antimicrobial products in the case of tissue injury. In plant processing this is a problem because freshly cut fruit and vegetables will spontaneously brown quickly unless steps are taken to (i) denature the enzyme (i.e., blanching), (ii) remove the oxygen (e.g., store the vegetables under water or add ascorbic acid which reacts preferentially with the oxygen) or (iii) inhibit the enzyme (e.g., drop the surface pH with acid. Sulfur dioxide is a very effective inhibitor of enzymatic browning by binding to the quinonones and preventing the nonenzymative polymerization step that leads to melanins). The products of polyphenoloxidase are nontoxic and flavorless but extensive browning quickly leads to perceived spoilage.

- **Lipoxygenase** catalyzes the formation of lipid peroxides from polyunsaturated fatty acids. Lipid peroxides are a crucial step in the oxidation of fats to form rancid flavors and peroxide activity can quickly lead to off-flavors (e.g., if the germ is not adequately separated before milling grain). In other cases lipoxygenase is added to facilitate flavor formation. The radicals forms as the
peroxides break down radicals are formed that can co-oxidise pigments and destroy vitamins. Soy lipoxygenase is often added to bleach flour.

- **Amylase** is an important starch-degrading enzyme. Amylase enzymes are used to produce dextrins (small glucose oligimers) and glucose from suspensions of starch. Initially a heat-sensitive endo-amylase (α-amylase) is added to a hot suspension of starch to rapidly reduce the average molecular weight. The starch is then cooled (but is less viscous because of the lower MW) and a more heat sensitive exo-amylase (glucoamylase) is added to further reduce the starch polymers to smaller units that can be partially purified and used as food ingredients (see carbohydrates section below).

The other major functional roles of proteins depend more on their properties as polymers rather than their evolved biological functionality and frequently denatured proteins can be as or more functional than the native form. While there is a wide range of these functions we will categorize them as hydrodynamic and surface properties.

Hydrodynamic functionality depends on the physical size and shape of the protein as an object suspended in a fluid. In this case the protein can be seen as a very small colloidal particle or neutral buoyancy. It will build viscosity by forcing fluid streamlines to deflect around it and may aggregate to form a gel. The analogy between a polymer molecule and a colloidal particle is pursued further in the polysaccharides section (below) and at this stage we can settle for the general rule that inter-protein cross links (c.f. flocculation in emulsions) tend to lead to first an increase in viscosity and second the formation of a gel. The mechanisms of interchain bond formation include disulfide bonds but the most common are the attractions between the hydrophobic groups on the surface of denatured proteins. Hydrophobic attraction can be mediated by strong electrostatic repulsion (c.f., the DVLO potential) if the protein is far from its isoelectric point and the salt concentration not too high. Another important interaction is the strong affinity between certain amino acid residues and calcium. If two separate proteins attempt to bind the same calcium, it can form a strong link between the. Calcium-mediated aggregation is particularly important in forming a tofu gel from soy proteins or setting caseinate gels.

The second group of functionalities important in proteins depends on their surface hydrophobicity. Because a native protein can often not fold adequately to protect all of their hydrophobic amino acids there are frequently hydrophobic regions on their surfaces. A denatured protein has even more exposed surface hydrophobicity. Either of these cases can allow a protein to adsorb at the surface of an emulsion droplet or a foam bubble and protect them against flocculation and coalescence. In addition, small hydrophobic molecules can bind to the hydrophobic regions on the protein. Importantly a protein can bind up flavor molecules and stop them becoming volatile and thus perceived. We will return to the amphiphilic properties of proteins in the lipids section when we consider emulsions as an example of dispersions and here we will consider in some more detail the ways proteins can be functional as aqueous polymers.
4. Food Polymer Functionality

Many food ingredients owe their interesting properties to the fact they are polymer more than any specific chemical structure or reactivity. In this section we will investigate the underlying molecular basis by which food polymers can build viscosity or gel foods and later see how specific examples behave. Many of the same arguments used here in the context of polysaccharides could equally apply to proteins (see above).

In the absence or any organizing factor (e.g., the hydrophobic effect in proteins) a polymer will take on an extended random coil configuration in solution. The greater the length of the chain, the larger the coil radius. Any feature that favors polymer-polymer interactions (e.g., a hydrophobic backbone) will tend to collapse the coil to a smaller hydrodynamic radius. Any feature that favors polymer-polymer repulsion (e.g., many strong similar charges on the backbone will repel one another) or favors polymer-solvent interactions (e.g., a very hydrophilic chain) will favor a more expanded coil.

In very dilute polymer solutions, each coil behaves as an isolated sphere and will add to the viscosity of a solution in a similar manner to small emulsion droplets (i.e., \( \eta = \eta_0 + 2.5\phi \) – see Dispersions section). The same molecular weight of polymer in a more open coil will have a larger hydrodynamic radius so a larger effective volume fraction. Bear in mind that the spheres we are now considering as the structural elements responsible for viscosity are almost all water and a very small mass fraction of gum (<<1%) can provide a large dispersed phase volume fraction and viscosity.

The simple Stokes-Einstein approach provides some measure of the viscosity of the most dilute polymer solutions, but as the concentration is increased the polymer coils will quickly start to overlap and interact. The interactions build viscosity even more rapidly than the isolated polymer coils. Eventually, even at a very low mass concentration, the concept of polymer coils becomes redundant and the solution is better imagined as a plate of spaghetti in sauce. The interactions between polymer chains lead to a very rapid increase in viscosity. Note that even in one of these concentrated polymer solutions we may still have less than one percent of the mass of the system as solids. In the water section earlier in this course we investigated the glassy and rubbery behavior of very concentrated polymer systems where water was only present in a few percent. In this case there is always enough
solvent present to allow the polymer to be considered a solution and not an amorphous solid.

A viscous polymer solution will still flow, albeit slowly. If a force is applied to it, for example by tipping a container, the fluid will immediately respond by flowing at a constant rate. The higher the viscosity the lower the rate of flow. To gel it must form some form of interchain bonds between polymer molecules. The interactions between molecules, if they extend over the entire container, allow it to instantaneously transmit mechanical forces and behave as a solid.

The two basic pictures of a gel are a particle gel (left) where the structural objects (e.g., globular proteins, fat crystals, emulsion droplets) are linked together in a network of discrete pieces and a polymer gel (right) (e.g., mostly polysaccharides) where local regions of the chains interact to form cross links but other regions do not.

The more cross links and the stronger they are, the more elastic and less viscous will be a given polymer system. In proteins we have seen cross-links form in the form of disulfide bonds and hydrophobic interactions. In polysaccharides the cross linking is more often the formation of multi-chain helices of polymer (supported by hydrogen bonding and/or hydrophobic interactions) or simultaneous binding of specific ions (usually calcium) by two different chains.