Protein Folding and Denaturation

Protein folding

Protein folding is driven by the aqueous environment, particularly the hydrophobic interactions, due to the unfavorable entropy decrease (mostly translational [686a, 1577]) forming a large surface area of non-polar groups with water. Consider a water molecule next to a surface to which it cannot hydrogen bond. The incompatibility of this surface with the low-density water that forms over such a surface [29] encourages the surface minimization that drives the proteins' tertiary structure formation (for example, see [1312]). An alternative view of this process is that the protein conformation tends to minimize the disruption of the water matrix [2037]. Such hydrophobic collapse is necessarily accompanied and guided by (secondary) structural hydrogen-bond formation between favorable peptide linkages in parallel with their desolvation [467].[a] A driving force for this, in crowded intracellular environments, is the release of water to be available for the hydration of other solutes and maximizing its entropy [686b]. The folding route is controlled by the desolvation barriers [676] and aided and directed by water-mediated contacts zipping up neighboring residues [1417]. Similar factors help organize proteins involved in quaternary and equilibrium cluster formation, where each water-mediated interaction has been estimated to contribute an average of 4.4 kJ mol^{-1} to protein-protein interface stabilization [688]. Water is thus intimately involved in guiding protein folding and needs to be involved in protein structural prediction studies [643]. The importance of subtle hydration forces is shown in the α-helix to β-sheet conformational transition that accompanies the racemic self-assembly of polylysine [727].
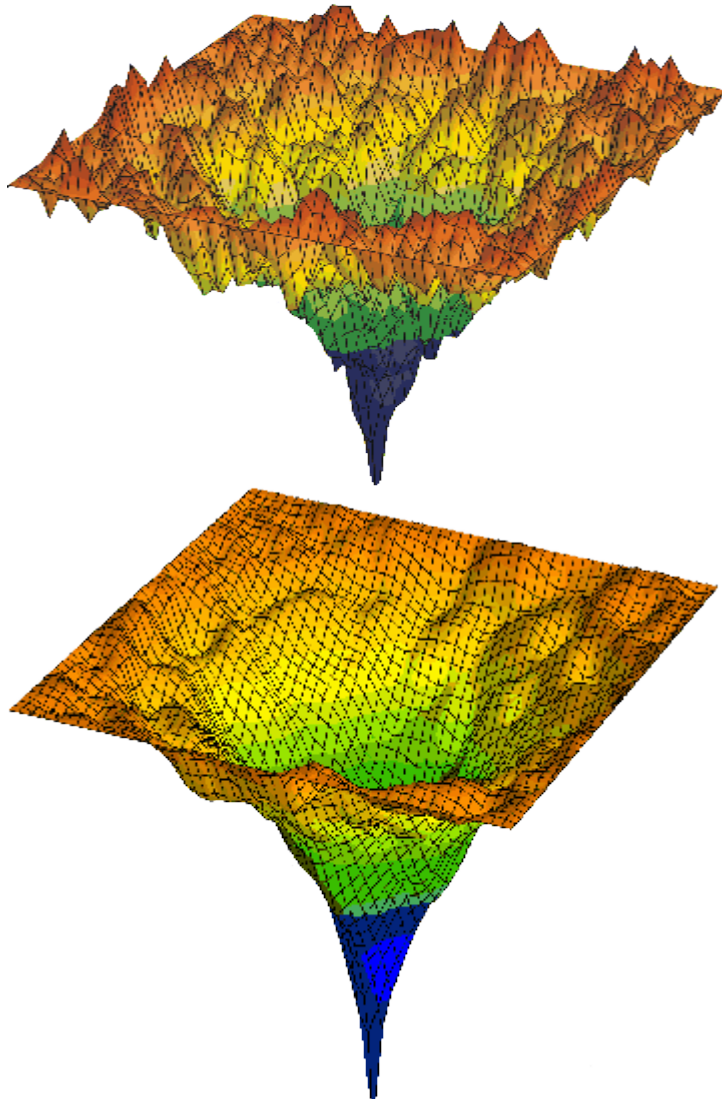

Opposite is shown schematic potential energy funnel for the folding of proteins without sufficient water present. it highlights the many barriers to the preferred minimum energy structure on the folding pathway. There are numerous local minima that might trap the protein in an inactive three-dimensional molecular conformation.The top rim represents the high energy of the unfolded protein with folding lowering

the energy towards a minimum energy structure that is at the bottom of the funnel. It should be noted that these funnels represent three-dimensional landscapes, whereas the actual energy landscapes are multidimensional.

When a protein is fully hydrated, the potential energy landscape is seen to be considerably smoothed.[a] Under conditions of sufficient hydration, this allows proteins to attain their active minimum-energy conformation in a straightforward and rapid manner. The potential energy barriers are lowered and smoothed due to the ease with which water molecules can lubricate the movement of the amino acid backbone and side groups by the rapid formation and exchange of hydrogen bonds. Similar effects may be seen on the activity of enzymes with hydration [822] although complete hydration is unnecessary for some activity to be evident. Under physiological conditions inside cells the water is known to be more ordered (see intracellular water). Such water promotes both the folding rate and stability of the protein [1250] even further.

Although indicated as such in the cartoons, there is not one 'minimum' structure but a collection of substates with small energetic differences. Jumps between these substates, eased by hydration, allows and determines the flexibility that the protein needs for its biological actions.
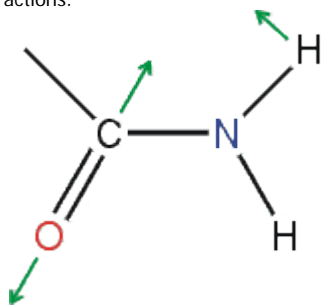
As the amide I stretch vibration (~1680 cm^{-1}) is similar to liquid water's bend vibration(v_2, ~1645 cm^{-1}), transfer of energy from water hydrogen-bonded to protein asparagine and glutamine groups is facilitated [913]. This explains the increased structural instability of proteins containing greater numbers of surface asparagine and glutamine residues and, in particular, is of relevance to the α-helix - β-sheet structural instability in prions [913].[b]

Although the native state of a protein resides at a minimum on the potential energy surface, there is no reason to suppose that this structure is the global minimum free energy structure as its folding route is a guided, rather than random, process. It is clear that other structures with lower minima exist, such as those often irreversibly produced on denaturation using intermolecular interactions [1152].

Compatible solutes (osmolytes, for example, betaine), that stabilize the surface low-density water and increase the surface tension, will also stabilize the protein's structure (see also the Hofmeister effect and the solubility of non-polar gases).

Many proteins are glycosylated with increased solubility. The role of the carbohydrate groups has been debated for many years. It now appears that this increased solubility is mainly as the low intermolecular interaction between surface glycans reduces the tendency for aggregation (and crystallization) rather than the glycan groups increasing the interactions with water [1164, 2104]. Rather unexpectedly, deglycosylated proteins appear to have stronger interaction with water (by weight) and more extensive water binding (by molecule) than their glycoproteins [1164]; perhaps because some carbohydrate hydroxyl groups replace several of the polypeptide surface water interactions.

Protein crystallization

Proteins may form crystals when precipitated slowly from an aqueous solution (e.g. of ammonium sulfate). Slow precipitation is required to produce small numbers of larger crystals rather than very large numbers of small crystals. Crystals of undenatured proteins for structural analysis are best formed with water molecules retained within the crystal lattice. Crystallisation of native proteins appears to have a three-step mechanism involving nucleation, in which mesoscopic metastable protein clusters of dense liquid serve as precursors to the ordered crystal nuclei followed by crystal growth [1800]. This processseems to involve an aqueous biphasic separation and fits nicely with the two-state structuring in liquid water, where the crystallisation takes place within the dense phase.

Protein denaturation

Protein denaturation involves a change in the protein structure (generally an unfolding) with the loss of activity. Water is critical, not only for the correct folding of proteins but also for the maintenance of this structure. Heat denaturation and loss of biological activity has been linked to the breakup of the 2-D-spanning water network (see above) around the protein [1215] (due to increasing hydrogen bond breakage with temperature), which otherwise acts restrictively on protein vibrational dynamics [976]. The free energy change on folding or unfolding is due to the combined effects of both protein folding/unfolding and hydration changes. These compensate to such a large extent that the free energy of stability of a typical protein is only 40-90 kJ mol^{-1} (equivalent to very few hydrogen bonds), whereas the enthalpy change (and temperature times the entropy change) may be greater than ±500 kJ mol^{-1} different. There are both enthalpic and entropic contributions to this free energy that change with temperature and so give rise to heat denaturation and, in some cases, cold denaturation. Protein unfolding at higher temperatures (heat denaturation) is easily understood but the widespread existence of protein unfolding at low temperatures is surprising, particularly as it is unexpectedly accompanied by a decrease in entropy [416]. Heat denaturation is endothermic (on heating) but cold denaturation is exothermic (on cooling) [1548].
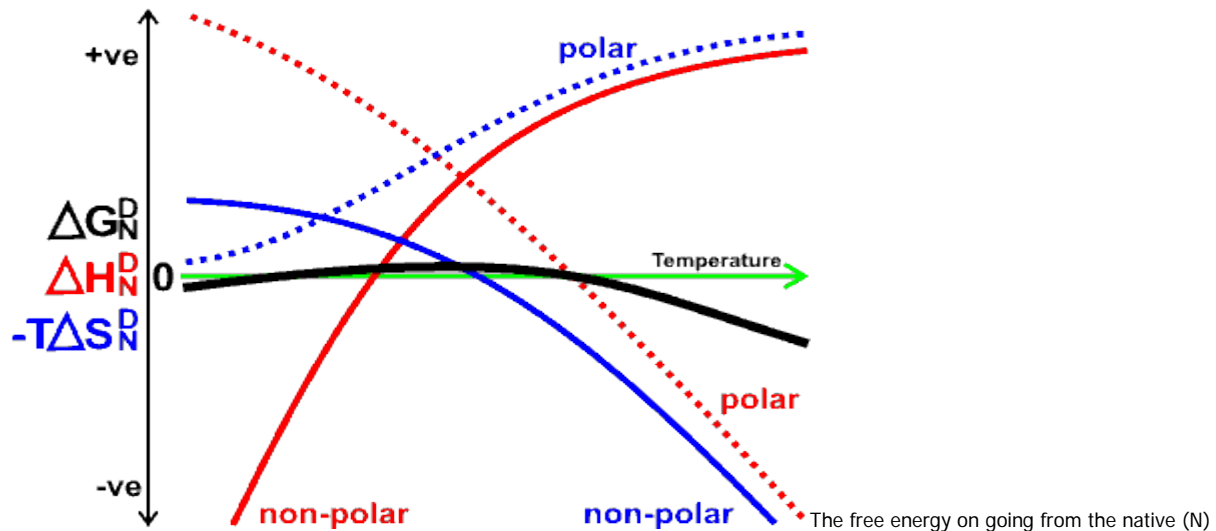
The free energy on going from the native (N) state to the denatured (D) state is given by $\Delta G_N^D = \Delta H_N^D - T\Delta S_N^D$. The overall free energy change (ΔG_N^D) depends on the combined effects of the exposure of the interior polar and non-polar groups and their interaction with water together with the consequential changes in the water-water interactions on ΔG_N^D, ΔS_N^D and ΔH_N^D.

The graph is meant to be indicative only. Denaturation is only allowed when ΔG_N^D is negative; its rate is then dependent circumstances and may be fast or immeasurably slow. On heat denaturation ΔH_N^D and ΔS_N^D are generally both positive but on cold denaturation they are both negative.

The midpoint temperatures of both heat and cold denaturation may be determined from peaks in the temperature dependence of the heat capacity, where additional heat is being absorbed by the intermediate structures.

The enthalpy of transfer of polar groups from the protein interior into water is positive at low temperatures and negative at higher temperatures [150]. This is due to the polar groups creating their own ordered water, which generates a negative enthalpy change due to the increased molecular interactions. Balanced against this is the positive enthalpy change as the pre-existing water structure and the polar interactions within the protein both have to be broken. As water naturally has more structure at lower temperatures, the breakdown of the water structure makes a greater positive contribution to the overall enthalpy at lower temperatures.

In contrast, the enthalpy of transfer of non-polar groups from the protein interior into water is negative below about 25 °C and positive above [150]. At lower temperatures, non-polar groups enhance pre-existing order such as the clathrate-related ES structure [270], discussed elsewhere, generating enthalpy but this effect is lost with increasing temperature, as any pre-existing order is also lost. At higher temperatures, the creation of these clathrate structures requires an enthalpic input. Thus, there is an overall positive enthalpy of unfolding at higher temperatures. An equivalent but alternative way of describing this process is that at lower temperatures the clathrate-type structure optimizes multiple van der Waals molecular interactions whereas at higher temperatures such favorable structuring is no longer available.

At ambient temperature, the entropies of hydration of both non-polar and polar groups are negative [151] indicating that both create order in the aqueous environment. However these entropies differ with respect to how they change with increasing temperature. The entropy of hydration of non-polar groups increases through zero with increasing temperature, indicating that they are less able to order the water at higher temperatures and may, indeed, contribute to its disorder by interfering with the extent of the hydrogen-bonded network and allowing an easier molecular rotation of water. Also, there is an entropy gain from the greater freedom of the non-polar groups when the protein is unfolded. In contrast, the entropy of hydration of polar groups decreases, becoming more negative with increasing temperature, as they are able to create ordered hydration shells even from the more disordered water that exists at higher temperatures. A consequence is that the water is more ordered around hydrophilic groups, compared with just water, as the temperature is raised and that this hydrophilic hydration has negative heat capacity [1578].

Overall, protein stability depends on the balance between these enthalpic and entropic changes. For globular proteins, the ΔG of unfolding has a maximum 10-30 °C, decreasing both colder and hotter through zero with the thermodynamic consequences of both cold and heat denaturation. The hydration of the internal non-polar groups is mainly responsible for cold denaturation as their energy of hydration is greatest when cold. Thus, it is the increased natural structuring of water at lower temperatures that causes cold destabilization of proteins in solution (that is, the entropic cost of denaturation, due to the structuring of the water molecules around the exposed groups, is reduced) [1371]. An equivalent alternative view is that the hydrophobic interactions increase as the temperature is raised from a low value, such that the extended polypeptide chain, present at very low temperatures, folds up to produce an active globular protein so releasing water molecules to the bulk environment.

Heat denaturation is primarily due to the increased entropic effects of the non-polar residues (that is, the increased entropy gain of the unfolded chain is not much reduced by the small amount of entropy loss caused to the solute). Although both processes have been reported to lead to irreversible changes, which often occur cooperatively, cold inactivation in supercooled water is usually likely to be reversible and it is any ice crystal formation that leads to observed irreversible effects. Interestingly, proteins from thermophilic organisms tend to have

higher amounts of non-polar residues and lower amounts of polar residues when compared to comparable proteins from mesophilic organisms [982]. This is related to decreased bound water around thermophilic enzymes in crystals and solution [1291] as part of their strategy for stability (other factors being increased salt bridges and main chain hydrogen bonds) [982].
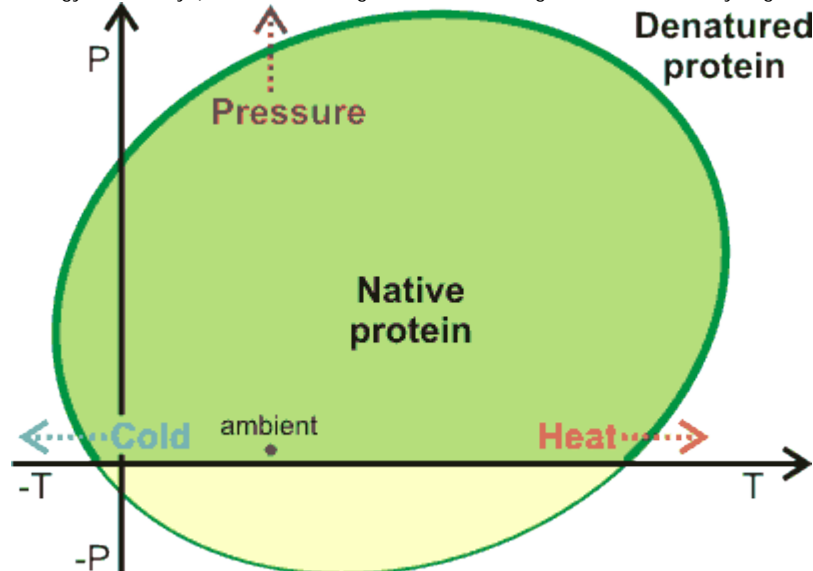
Protein stability has been directly tied to the equilibrium structuring of water between low-density and higher density forms [210, 416, 1481] (see also). This provides an equivalent but alternative way of looking at the above analysis. Opposite shows a representation of the pressure-temperature (P/T) phase diagram for proteins showing heat-, cold- and pressure-denaturation [1480]. The diagram is also representative of the solubility of other polymers, such as starch, the aggregation of non-polar solutes [1959] and is related to the pressure-temperature relationship of the thermal expansion of water [1481].

Denaturation may be effectively treated as increased solubility of the unfolded form in a manner similar to that given in the treatment of the anomalous solubility behavior of non-polar gases. Thus, protein aggregates and amyloid fibrils (such as are found in prion and Alzeimer's diseases) may be dissolved on cooling or under high pressure [1490].

The effect of pH on denaturation (for example, low pH causing easier heat denaturation [846]) may be understood by recognizing that extremes of pH cause an increase in higher density clustering which may be partially reversed by the presence of non-ionic kosmotropes [846].

There is a change in volume with denaturation (ΔV_N^D) varying with the protein concerned but typically from negative (that is, overall volume of water and protein is smaller on denaturation) at low temperatures to slightly positive at high temperatures. This is due to the released nonpolar residues producing less-dense water (for example, ES) at low temperatures but less able to do this at high temperature whereas the released polar groups cause a greater increase in density at low temperatures, due to their destruction of the low-density water, than at high temperatures, where there is less destroyable low-density water. A small pressure increase may stabilize the protein against both cold and heat denaturation. At low temperatures, a small pressure increase reduces the size of the enthalpic contribution of non-polar group hydration due to the reduced aqueous structuring. At high temperatures the enthalpic cost of hydrating these non-polar groups is increased when under a small pressure increase, and this may help the increased thermal stability of proteins seen in crowded environments [1052]. Under higher pressure, proteins take up water into empty cavities [1940]. This penetrating water eases the process of denaturation by destabilizing the internal links. The negative volume change at higher pressures, due to cavity filling, helps shift the enthalpy change in favor of denaturation across the temperature range but has been particularly noted at low temperatures, in line with the Figure above (for further discussion see [847]).

Elastin is an important protein with properties governed by its interactions with water. It consists of a high proline, high glycine hydrophobic chain (for example, typical section PGVGV) that cannot form regular α-helices or β-sheets but does form an extended structure probably containing β-spiral sections with most of the peptide links hydrogen bonded to water [1149]. On warming the structure undergoes a structural collapse due to the difficulty in maintaining the low-density clathrate structures around its hydrophobic groups.

Footnotes

Correct folding is also aided by the molecular chaperones (chaperonins) [1047], where the chaperonin increases the density and hydration of the water surrounding the misfolded protein to drive correct re-folding [1483]. [Back]

Prion fibril formation has also been explained as being due to the increase of water trimers and hydrophobicity of the Mn-linked (as opposed to the normal Cu-linked) protein in solution. [1614]. Water release has an important effect on the rate of initial fibril formation, due to the slow rate with which it is expelled from the hydrophilic amide side chains [1838].

Source: http://www1.lsbu.ac.uk/water/protein2.html