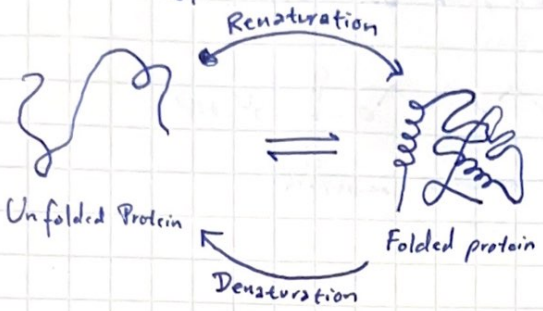
Protein Folding and Unfolding

Some terminology:



Protein Denaturation

→ The equilibrium of $K_{eq} = \frac{[folded]}{[unfolded]}$ is quite large, so under physiological conditions there are much more folded proteins than unfolded proteins.

→ For biochemists to study protein folding, the process of denaturation should be understood completely.

To perturb the equilibrium, there are three main methods which biochemists use:

- Heat
- Chemical denaturants (guanidinium ion, urea, detergents)
- Extreme pH (< 4, or > 12)

Heat as a denaturant

→ When heat is used in ~~these~~ denaturation, the $T\Delta S^\circ$ values are more and more negative:

Protein	T	ΔG°	ΔH° (kJ/mol)	$T\Delta S^\circ$	K_{eq}
RNaseA	25°C	-44	-280	-236	5.5×10^{7}
	100°C	+89.4	-715	-804.5	6.2×10^{-13}
Trypsin	25°C	-63.2	-275	-211	1.5×10^{11}
	100°C	+129.5	-1289	-1419	2×10^{-18}
Myoglobin	25°C	-43.4	-6.2	+37.2	4.2×10^{7}
	100°C	+75.8	-994	1069	4.5×10^{-11}

$\Delta G = \Delta H - T\Delta S$

Note how the K_{eq} is much smaller in a high temperature.

These are the values ~~for~~ for the K_{eq} when the temperature is 100°C.

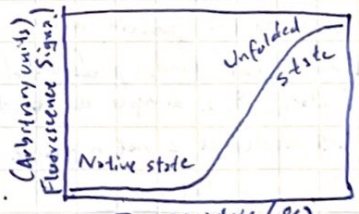
Experiment to observe heat denaturation of proteins:

→ Place protein in solution, with low temperature.

→ Measure A280, or fluorescence

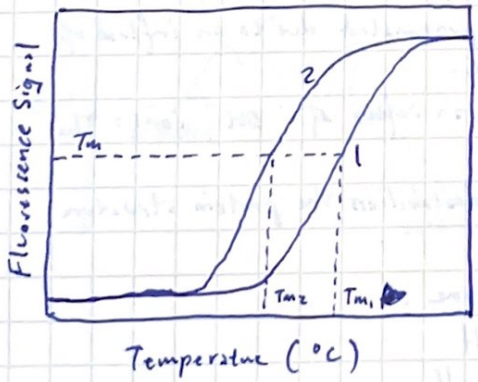
→ Raise the temperature

→ Measure A280, or fluorescence, at the new equilibrated sample.



This experiment ~~do~~ results in this graph.

Heat denaturation of proteins: melting temperatures (T_m)



1 = Under physiological conditions

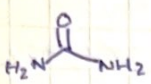
2 = Under nonphysiological conditions

→ (T_m), or the melting temperature of this protein is defined as the temperature at which ½ of the total signal change has occurred.

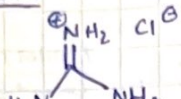
Typically the T_m for nonphysiological proteins is lower than that of pure proteins (think melting point analysis)

→ T_m can also be defined as the point at which 50% of the protein is folded, and 50% of the protein is unfolded.

Chemical Denaturants



Urea
(6-8 M)



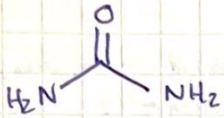
Guanidinium Hydrochloride
(5-6 M)

Detergents (SDS) → 0.1 – 1% w/w

Note that this molecule is amphipathic

Protein denaturation by urea and guanidinium

Urea:



Note that the structure of Urea contains 2 H-bond donor localities and one H-bond acceptor locality.

A high concentration of urea would disrupt structures like α-helices and β-pleated sheets due to their reliance on H-bonding

- Additionally, urea bonds via Vander ~~Waals~~ Waals bonds to most parts of the protein.
- Guanidinium has the same idea.
- In an unfolded protein, it is favorable for urea ~~to bind~~ or guanidinium to bind as the conformation is extended, with a large surface area than if the protein were compact.

Effects of detergents (SDS) on protein structure

→SDS binds to the protein main chain. One dodecyl sulfate binds for every 1-2 amino acid residues. SDS binds preferentially to the unfolded state of the protein.
- Similarly, SDS disrupts the ~~bonds of~~ H-bonds and VdW interactions that form a protein structure.
- SDS results in a net negative charge for the protein it interacts with.

Extreme pH as a denaturant

Consider the overall (net charge) on a protein. Typically proteins have net positive or negative charges scattered across the surface of the protein. ~~the base~~

→ At a low pH, ~~proteins~~ amino acids will begin to be protonated due to an influx of H^+ ions: The protein will carry a more positive charge.

→ At a high pH, amino acids will be deprotonated due to an influx of OH^- ions: The protein will carry a more negative charge.

At extreme pH values, there will be more charge repulsion which destabilizes the protein structure.

The biggest problem for internal (buried) protein structures are:
- Protonation of buried His residues at low pH
- Deprotonation of buried Tyr residues at high pH

Protonating a His residue results in more entropically favorable conformation of unfolded protein – trying to bury a charged residue is incredibly unstable and requires a lot of energy. Therefore the reaction shifts to the unfolded protein (lower K value)

The Anfinsen Experiment

→ Proved the existence of an equilibrium between folded and unfolded proteins. More specifically, the experiment proved that one could take a folded protein, denature it, and that the denatured protein could once again spontaneously form a folded protein (Spontaneous Anfinsen experiments)

RNAse A (Ribonuclease A) is isolated in its catalytically active, native state

+ Urea
+ Mercaptoethanol ↓ Step 1: Denature RNase A

RNase A is in unfolded, denatured state

− Urea
− Mercaptoethanol ↓ Step 2: Remove denaturants via Dialysis

RNase A returns to catalytically active form

Addendum on dialysis

- Dialysis bag ~~is~~ full of protein and mercaptoethanol is immersed in buffer containing no urea or mercaptoethanol
- Separates large molecules from smaller molecules via dilution

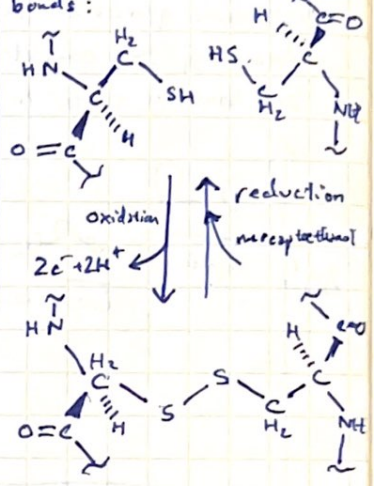
Anfinsen then did a second experiment in which the ~~RNase was first~~ denatured RNase A has ~~the~~ mercaptoethanol removed, but urea retained through dialysis. Then urea was removed after. After conducting this experiment, Anfinsen discovered that only 1% was active as the original enzyme.

Q- Why did this happen?

A- The wrong disulfide bonds formed in the protein, resulting in a constant structure in the protein which resulted in the protein becoming majority catalytically inactive. The 1% active proteins were the result of chance, that the correct disulfide bonds formed.

Addendum on Mercaptoethanol

- Mercaptoethanol is a reducing agent which breaks disulfide bonds:

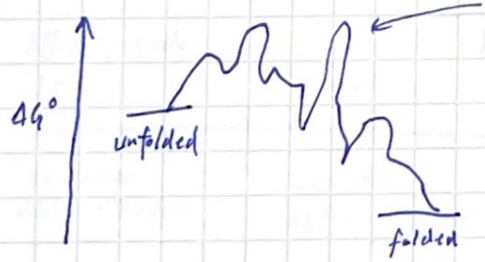


Note that 2 moles of mercaptoethanol are needed to reduce one disulfide bridge.

Significance of the experiment

1. Determined that the specific folded structures of proteins under physiological conditions is the structure which has the lowest Gibbs free energy at those conditions
2. Folded structure of a protein is determined by the amino acid sequence of a protein alone — no other factors contribute to the protein folding into that specific conformation.

The Anfinsen Experiment did not however address any mechanisms of protein folding:

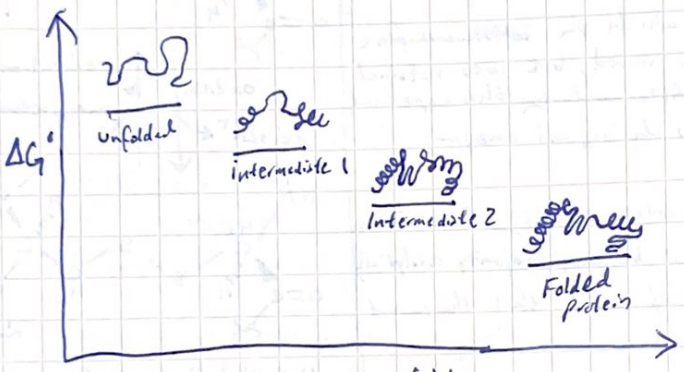


Mechanisms of Protein Folding

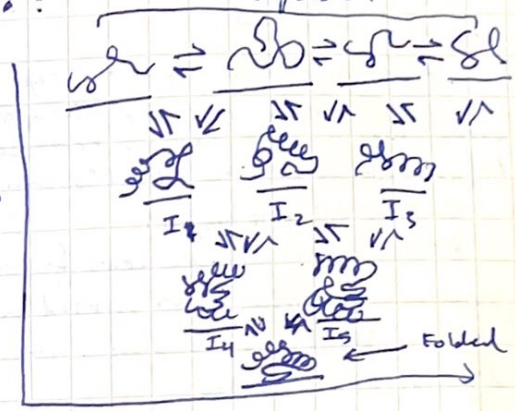
After Anfisen's experiment, many biochemists pondered what the pathway was between a folded and an unfolded protein was. Enter Levinthal's paradox:

—Proteins could do a random search of all possible conformation to ~~see~~ come across the conformation by chance. However, such a task should take forever, whereas proteins fold in < seconds...

Therefore this method of folding could not be a valid answer for the protein folding mechanism. A more reasonable guess would be that there must be a folding pathway: some conformations are at a lower free energy than completely unfolded proteins, and as such the protein follows a path of intermediates and transition states to become the folded protein in the end:



Old view of protein folding
- Did not distinguish between different unfolded proteins
- Very linear



New view of protein folding
- Differentiated between different unfolded proteins
- Included many different pathways with many intermediates,
- "Folding funnel"

Old view:

Either / or

Secondary structure forms first

New view:
Nucleation-Condensation

"Hydrophobic" collapse

Argued that both the top pathway and the bottom pathway occurred on the same time scale.