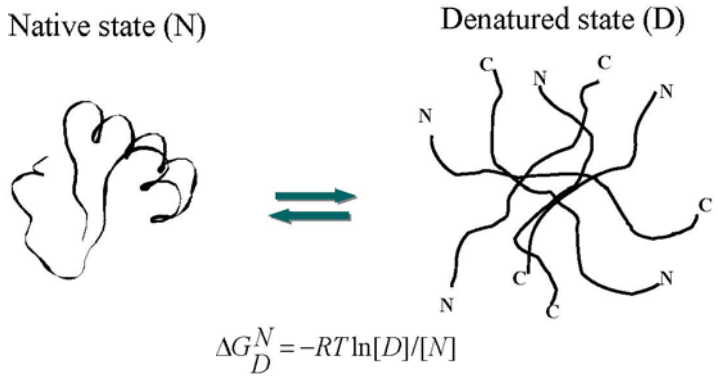
Experiment No. 3

Protein Denaturation Studies

Disruption of the noncovalent interactions will lead to unfolding of the protein with reversible or irreversible denaturation. It is believed that hydrophobic collapse is a key driving force for protein folding resulting in the formation of a hydrophobic core with the polar surface interacting with the solvent.



Fundamental constraints of protein structures, φ and ψ angles dictate possible polypeptide backbone conformations. Both local and nonlocal interactions play a role in protein folding. Apart from these hydrophobic interactions and hydrogen bonding play an important role.

Factors that cause denaturation are (i) Temperature and (ii) Denaturants such as acid, urea and Guanidinium Hydrochloride (GuHCl)

In this experiment you will follow the effect of urea (up to 8 M) on protein unfolding by viscosity and fluorescence measurements. Unfolding may also follow a two-state mechanism.
You will use two different proteins for the two sets of experiments.

For viscosity measurements use the viscometer (5ml/10ml) and add the denaturant – mix thoroughly and then measure the time taken. Calculate the viscosity by comparing with the viscosity for water.
For fluorescence measurements, you need a much smaller volume of sample – you will do this experiment with a different protein. The excitation wavelength used will be 295 nm with emission set at 350nm for Tryptophan residues in proteins.
Mix specific proportions of solutions indicated and record your measurements.

A sample table is given below – note that the volumes used are the same in each case (the x and y values will differ based on the experiment being performed)

	Protein soln	Denaturant solution	Viscosity/fluorescence
1	Water (for blank reading)		
2	x ml	y ml of 0 M	
3	x ml	y ml of 1 M	
4	x ml	y ml of 2 M	
.	…	…	

Determine the ΔG for unfolding from the equation:

$\Delta G = -RT\ln K = -RT\ln[(yN - y)/(y - yD)]$
where K is the equilibrium constant, y is the observed value of the parameter used to follow unfolding, yN and yD are the values of y characteristic of the native and denatured conformations respectively (these are functions of urea concentration: y = a + b[urea]).

Plot ΔG as a function of denaturant concentration and extrapolate to 0M urea concentration to determine the ΔG of unfolding in water.