

It appears there was a somewhat general confusion with the definition of the m-value coming from the lecture slides:(

uration. An *m*-value is defined as the magnitude of the derivative of the standard free energy change for protein unfolding (ΔG_{obs}°) with respect to the molar concentration (C_3) of denaturant:

$$m\text{-value} \equiv -d\Delta G_{obs}^{\circ}/dC_3 = RT d \ln K_{obs}/dC_3 \quad (1)$$

So this is the official definition of the m-value. It is a minus derivative (or, it's equivalent to the absolute value, always positive) of the protein unfolding energy with the respect to denaturant concentration. Now, when we're talking about the unfolding energy it typically should be positive. But when we're talking about protein stability, we would indicate the protein's folding Gibbs energy, which should be negative. Therefore, apparently in this slide from the lecture

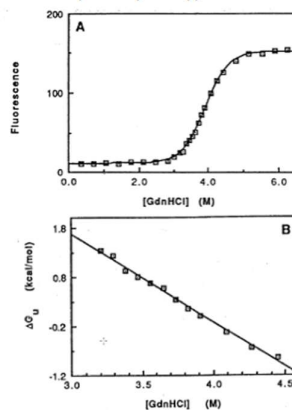
Protein denaturation with denaturants

The **effect of denaturants** on the free energy is linear (empirical finding)

$$\Delta G^{\circ} = \Delta G_{H_2O}^{\circ} + m [\text{denaturant}]$$

The **free energy of unfolding** can thus be determined by an extrapolation to **0 M denaturant**

Example: Chymotrypsin inhibitor 2 (CI2)



There's a small confusion. Here we are talking about the free energy of unfolding - thus it should be positive (which we also see in the graph at the bottom. And we have a negative slope; but by definition, m-value should be positive, therefore, the equation in the box should look like this: $\Delta G^{\circ} = \Delta G^{\circ}(H_2O) - m[\text{denaturant}]$ (with the - sign to get a final positive m-value).

Also, there's another confusion regarding whether the amino acids fluoresce in proteins should increase or decrease upon unfolding. Apparently, it can be both cases, depending on the protein and also fluoresce of which amino acids you are measuring (as can be seen in the paper I attached, in Fig. 5a). But whether fluorescence increases or decreases it should not affect the calculations of ΔG and m-values.