A brief beginners guide to kinetic analyses and chevron plots

This very brief guide is written at the suggestion of one of the referees to make our manuscript more accessible to the non-specialist reader. Our intention is to explain what a chevron plot means, why it is the shape it is, and most importantly to show how differences in the chevron plot of a protein can be interpreted. We hope it is useful.

(1) An experimental protein folding landscape (for a 2-state protein with no intermediates):

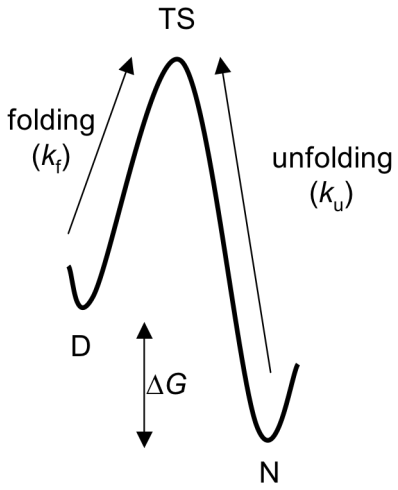

Figure S1: Proteins fold on a complex multidimensional, funnel-like energy landscape. This simple, experimental free energy landscape shows the important species when studying the folding of a simple, 2-state protein. The stability of the protein (ΔG) is the difference in free energy between the denatured state (D) and the native state (N). The rate constant of folding (k_f) is determined by the difference in free energy between D and the rate limiting transition state (TS). Likewise the rate constant of unfolding (k_u) is determined by the difference in free energy between N and TS.

(2) What is a chevron plot?

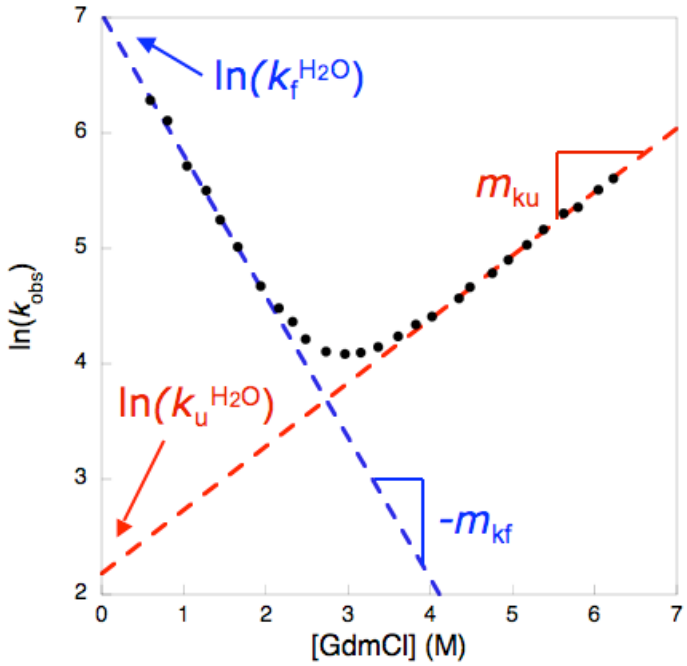

Figure S2: A sample chevron plot where each black dot represents a separate kinetic experiment.

- For unfolding experiments, (those on the red dashed line), a sample of folded protein in buffer is rapidly mixed with an excess of denaturant solution, (typically 1:10), which results in a high overall concentration of denaturant. The unfolding of the protein is monitored by following changes in a structural probe e.g. CD or intrinsic fluorescence. From these data the unfolding rate constant (k_u) can be determined and this is plotted against the concentration of denaturant at which it occurred.
- For folding experiments, (those on the blue dashed line), a sample of unfolded protein in high denaturant solution is rapidly mixed with an excess of buffer, resulting in a low overall concentration of denaturant. The same optical probes are used to monitor the protein folding, and the calculated folding rate constant (k_f) is again plotted against the concentration of denaturant at which it occurred.
- The protein folds more slowly in the presence of denaturant than in pure buffer. Likewise, the higher the concentration of denaturant, the faster the protein unfolds. Thus the resultant plot has a characteristic V-shape – hence it is called a chevron plot.
- Provided that there is no change in the structure of the transition state, the logarithm of the folding and unfolding rate constants ($\ln k_f$ and $\ln k_u$ respectively) will be directly proportional to the concentration of denaturant. These rate constants are shown as dashed lines in **Figure S2**, ($\ln k_f$ in blue, $\ln k_u$ in red), and can be extrapolated back to zero molar denaturant to give the folding rate constant in water ($\ln k_f^{H2O}$) and the unfolding rate constant in water ($\ln k_u^{H2O}$). These values can then be used to determine the stability (the free energy of unfolding) of the protein in the absence of denaturant ($\Delta G = RT\ln(k_f^{H2O}/k_u^{H2O})$).
- Note that if an intermediate is populated (i.e. the protein is not 2-state) then the chevron plot may be curved.

(3) How kinetic data report on folding in a multidomain protein

Where protein domains exist in a multidomain construct, a single domain can be stabilised by its neighbour(s) in one of three ways.

(A) **If the neighbour provides favourable interactions in the native state only**, then the kinetic barrier to unfolding is raised but the kinetic barrier to folding is unchanged. This results in a change in the chevron plot, as shown in **Figure S3**. Notice that the minimum of the chevron plot has shifted to the right, indicating a higher stability.

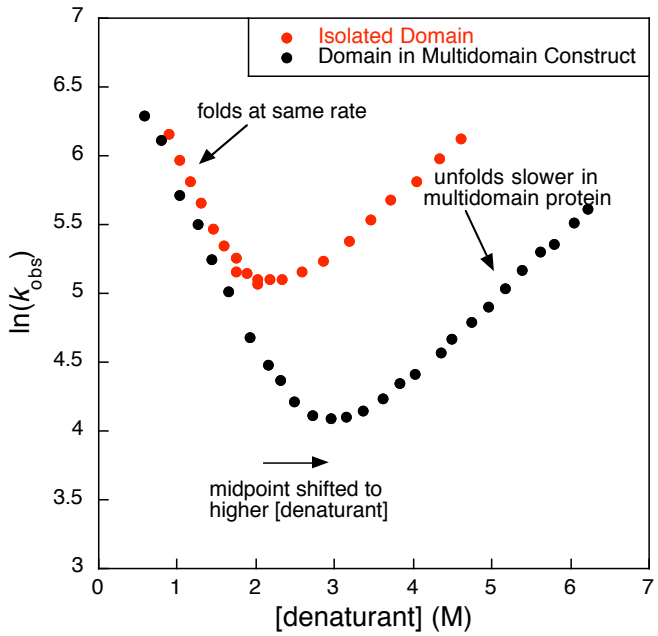

Figure S3: A domain folds at the same rate in a multidomain context but unfolds more slowly.

(B) **If the neighbour provides favourable interactions to both the native state and the transition state, and these are comparable**, then the kinetic barrier to unfolding is unchanged, but the kinetic barrier to folding is lowered. This is shown in **Figure S4**. Again, notice that the minimum of the chevron plot has shifted to a higher concentration of denaturant.

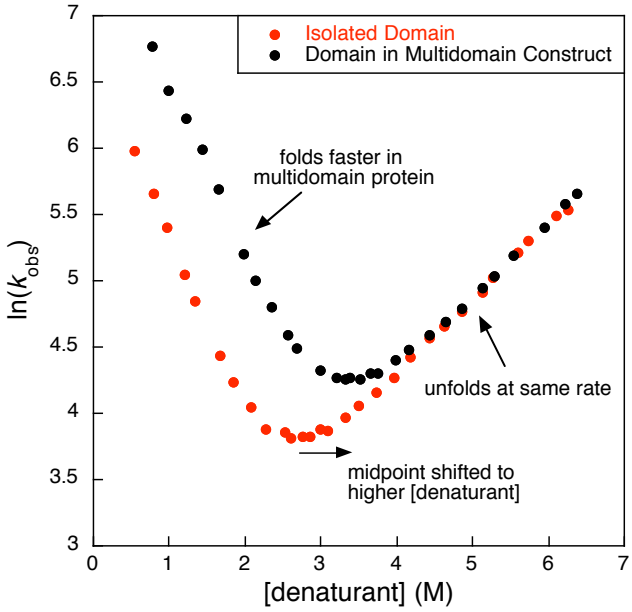

Figure S4: A domain unfolds at the same rate in a multidomain context but folds more quickly.

(C) **If the neighbour provides favourable interactions to both the native state and the transition state, but those to the native state are stronger**, then the kinetic barrier to unfolding is raised and the kinetic barrier to folding is lowered. This is shown in **Figure S5**. Again, notice the increase in stability.

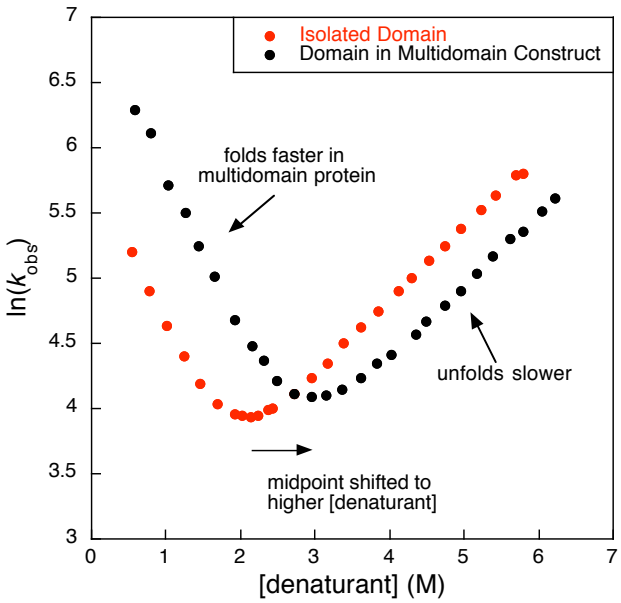

Figure S5: Interactions with a neighbouring domain stabilise the protein by both speeding folding and slowing unfolding.

In this brief tutorial we have not considered any situation where the neighbour stabilises the denatured state. Provided that the native state and transition state are stabilised more than the denatured state, then the situation will resemble one of those listed above. We have not yet identified a construct where the denatured state is stabilised to a greater extent than either the transition state or the native state, and such situations are likely to be very rare.

Recommended text:

A.R. Fersht, Structure and Mechanism in Protein Science. (Freeman)