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Uncertainty about Phi-Values

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Energy Profile

→ The φ-value is defined as the ratio $\Delta \Delta G_{1,0} / \Delta \Delta G_{\text{eq}}$.

Energy Profile

- If the part of the protein that contains the mutant amino acid is fully structured in the transition state, we have $\Delta \Delta G_{1,0} \approx \Delta \Delta G_{\text{eq}}$, and hence $\phi \approx 1$.
- If the part of the protein that contains the mutant amino acid is equal in denatured and the transition state, we have $\Delta \Delta G_{1,0} \approx 0$, and hence $\phi \approx 0$.

At least this is the idea ...

Phi-Value Estimation

$\log(k_{\text{off}}) = \log\left( \exp\left[ \log(k_{\text{on}}) + m_1 \times \frac{\Delta \Delta G_{1,0}}{RT} \right] + \exp\left[ \log(k_{\text{on}}) + m_2 \times \frac{\Delta \Delta G_{\text{eq}}}{RT} \right] \right)$

$\Delta \Delta G_{1,0} = RT \times \left[ \log(k_{\text{off} \text{mut}}) - \log(k_{\text{off} \text{wt}}) \right]$

$\Delta \Delta G_{\text{eq}} = RT \times \left[ \log(k_{\text{on} \text{mut}}) - \log(k_{\text{on} \text{wt}}) - \log(k_{\text{off} \text{mut}}) + \log(k_{\text{off} \text{wt}}) \right]$

Questions

- What is the threshold for the difference in stability ($\Delta \Delta G_{\text{eq}}$) between two variants to assure reliable estimates of $\phi$?
- How can we construct valid confidence intervals for the estimates of $\phi$?
- How reproducible are $\phi$-value measurements?
Questions

- What is the threshold for the difference in stability (∆∆G_{m,m}) between two variants to assure reliable estimates of ϕ?

It depends.

Precision

![Graph showing I28A and Wildtype](image)

- What is the threshold for the difference in stability (∆∆G_{m,m}) between two variants to assure reliable estimates of ϕ?

- How can we construct valid confidence intervals for the estimates of ϕ?

- How reproducible are ϕ-value measurements?
Error Propagation

Towards Confidence Intervals

\[ \ln(K_w) = \log \left( \exp \left[ \log(k_0) + m_1 \times \frac{[H_2O]}{[H]} \right] + \exp \left[ \log(k_1) + m_2 \times \frac{[H_2O]}{[H]} \right] \right) \]
Towards Confidence Intervals

Confidence Intervals

Confidence intervals for the $\phi$-value: 

\[ I = \left[ \phi - \frac{F_{(n-1),\alpha}}{\sqrt{n}}, \phi + \frac{F_{(n-1),\alpha}}{\sqrt{n}} \right] \]

It is not a priori clear what the degrees of freedom in the t-quantile should be. Adding the number of data points used to fit the two chevron curves ($n_1$ and $n_2$) and subtracting the number of parameters estimated in the fitting procedure (a total of 10) however gave 95% coverage for the confidence intervals in simulation studies.

Questions

- What is the threshold for the difference in stability ($\Delta\DeltaG_{\text{m}}$) between two variants to assure reliable estimates of $\phi$?
- How can we construct valid confidence intervals for the estimates of $\phi$?
- How reproducible are $\phi$-value measurements?

Reproducibility

We performed independent, triplicate measurements of the folding kinetics of the wild-type and seven point mutations at two sites of the FynSH3 domain.

The relevant proteins were expressed and purified in one laboratory and provided to the other two laboratories.

Each laboratory collected chevron curves for each protein (under previously defined conditions) using the methods traditionally employed in that laboratory.

- **Group 1** monitored folding using a pneumatically-driven Applied Photophysics SX18MV stopped-flow fluorimeter in pressure-hold mode.
- **Group 2** performed kinetic measurements of unfolding and folding using a N-Star pneumatically-driven stopped-flow reaction analyzer (Applied Photophysics, Leatherhead, UK) in fluorescence mode.
- **Group 3** measured folding and unfolding rates using a Biologic SFM 4 stopped-flow device coupled to a Fluoromax 3 fluorimeter.

Chevron Plots