On the Precision of Experimentally Determined Protein Folding Rates and $\phi$ Values

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The $\phi$-value is defined as the ratio $\Delta\Delta G / \Delta\Delta G^u$.

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**Energy Profile**

- If the part of the protein that contains the variant amino acid is fully structured in the transition state, we have $\Delta\Delta G = \Delta\Delta G^u$ and hence $\phi \approx 1$.
- If the part of the protein that contains the variant amino acid is equal in denatured and the transition state, we have $\Delta\Delta G_2 \approx 0$, and hence $\phi \approx 0$.

At least this is the idea …

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**Phi-Value Estimation**

\[
\log(k_f) = \log \left( \exp \left( \log(k_o) + \frac{\Delta G}{RT} \right) + \exp \left( \log(k_o) + \frac{\Delta G}{RT} \right) \right)
\]

\[
\Delta\Delta G_2 = RT \times \left[ \log(k_o^{\text{den}}) - \log(k_o^{\text{trans}}) \right]
\]

\[
\Delta\Delta G_3 = RT \times \left[ \log(k_{f}^{\text{trans}}) - \log(k_{f}^{\text{den}}) - \log(k_o^{\text{den}}) - \log(k_o^{\text{trans}}) \right]
\]

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**Questions**

- What is the threshold for the difference in stability ($\Delta\Delta G^u$) between two variants to assure reliable estimates of $\phi$?

It depends.

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**Precision**

I28A WILDTYPE

\[
\phi = 0.60 \\
\Delta\Delta G^u = 3 \text{ kcal/mol}
\]
Precision

10 data points / chevron

Precision

20 data points / chevron

Precision

40 data points / chevron

Precision

Questions

- What is the threshold for the difference in stability ($\Delta\Delta G$) between two variants to assure reliable estimates of $\phi$?

- How can we construct valid standard errors for the estimates of $\phi$?
\[
\ln(k_{\text{obs}}) = \log\left( \exp\left[ \log(k_0) + m_1 \times \frac{\Delta G_1}{\Delta G_0} \right] + \exp\left[ \log(k_0) + m_2 \times \frac{\Delta G_2}{\Delta G_0} \right] \right)
\]

\[
\text{Se}(\hat{\theta}) = |\theta| \times \sqrt{\frac{\sigma^2_{\Delta G_1}}{\Delta G_1} + \frac{\sigma^2_{\Delta G_2}}{\Delta G_2} - 2 \rho_{\Delta G_1 \Delta G_2} \frac{\sigma_{\Delta G_1} \sigma_{\Delta G_2}}{\Delta G_1 \Delta G_2}}
\]

Web Server
Questions

- What is the threshold for the difference in stability ($\Delta \Delta G_u$) between two variants to assure reliable estimates of $\phi$?
- How can we construct valid standard errors for the estimates of $\phi$?
- How reproducible are $\phi$-value measurements?

Reproducibility?

Alternative Techniques

Dang...

Estimates of the speed of light, with "confidence intervals" (1895 - 1950).

Youden (Technometrics, 1972).

Questions

- What is the threshold for the difference in stability ($\Delta \Delta G_u$) between two variants to assure reliable estimates of $\phi$?
- How can we construct valid standard errors for the estimates of $\phi$?
- How reproducible are $\phi$-value measurements?
- What are the effects of other commonly employed techniques to calculate $\phi$ from kinetic data?

References

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