On the Precision of Experimentally Determined Protein Folding Rates and Φ Values

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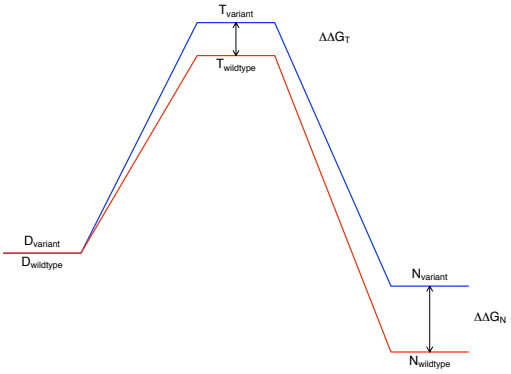
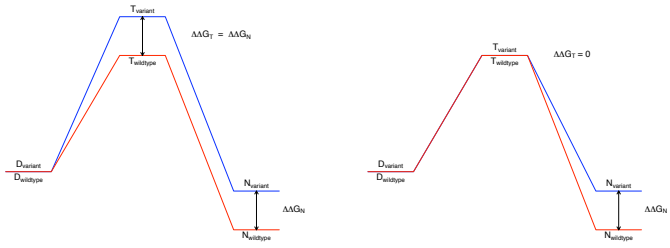
Energy Profile



$\longrightarrow$ The Φ-value is defined as the ratio $\Delta\Delta G_T / \Delta\Delta G_N$.

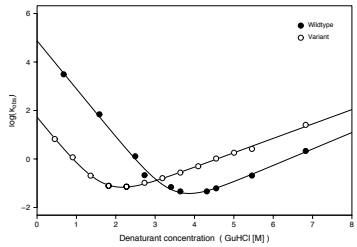
Energy Profile



- If the part of the protein that contains the variant amino acid is fully structured in the transition state, we have $\Delta\Delta G_T \approx \Delta\Delta G_N$, 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 $\Delta\Delta G_T \approx 0$, and hence $\Phi \approx 0$.

At least this is the idea ...

Phi-Value Estimation



$$\log(k_{obs}) = \log\left(\exp\left[\log(k_f) + m_f \times \frac{C_{GuHCl}}{RT}\right] + \exp\left[\log(k_u) + m_u \times \frac{C_{GuHCl}}{RT}\right] \right)$$

$$\Delta\Delta G_T = RT \times \left[\log(k_f^{wildtype}) - \log(k_f^{variant}) \right]$$

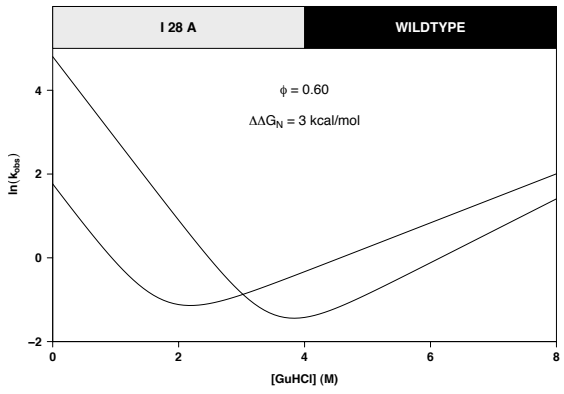
$$\Delta\Delta G_N = RT \times \left[\log(k_f^{wildtype}) - \log(k_u^{wildtype}) - \log(k_f^{variant}) + \log(k_u^{variant}) \right]$$

Questions

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

It depends.

Precision



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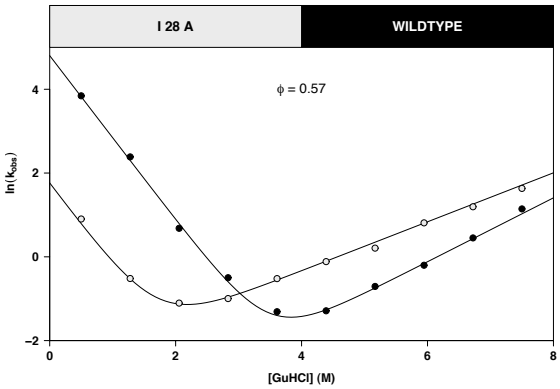
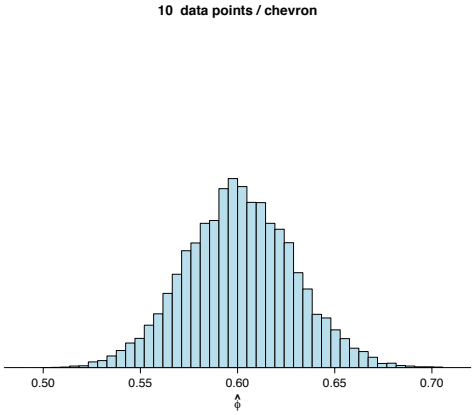


Precision



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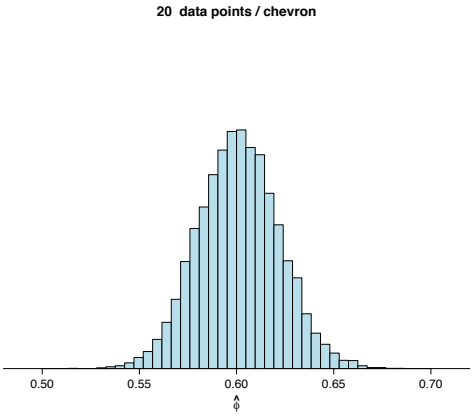

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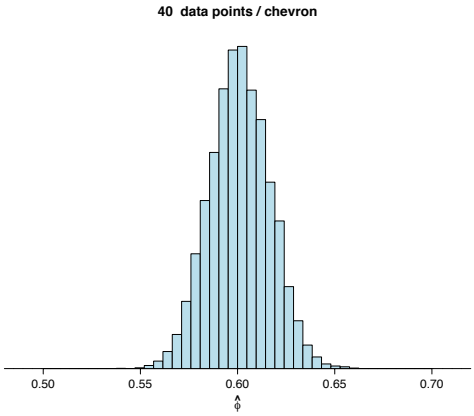

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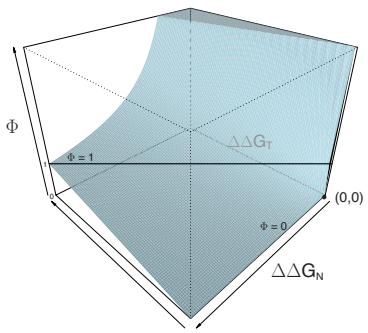
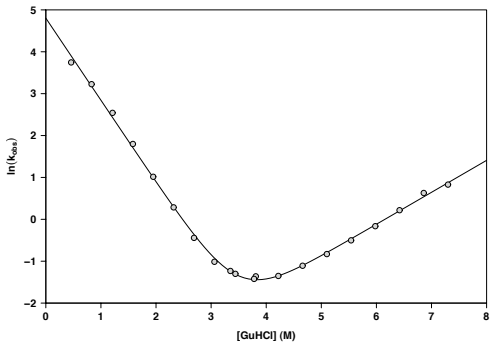


Questions

- What is the threshold for the difference in stability ($\Delta\Delta G_N$) between two variants to assure reliable estimates of Φ?
- How can we construct valid standard errors for the estimates of Φ?

Standard Error

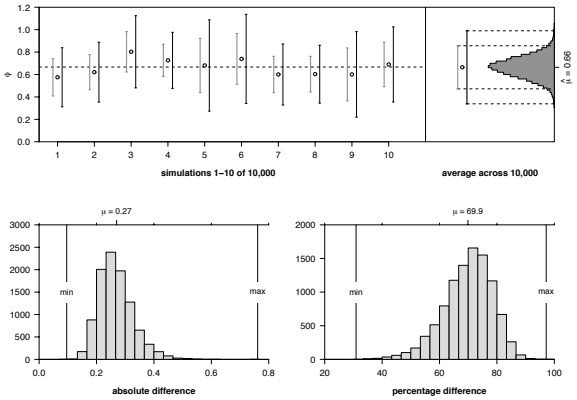


$$\ln(k_{obs}) = \log\left(\exp\left[\log(k_f) + m_f \times \frac{[GuHCl]}{RT}\right] + \exp\left[\log(k_u) + m_u \times \frac{[GuHCl]}{RT}\right] \right)$$

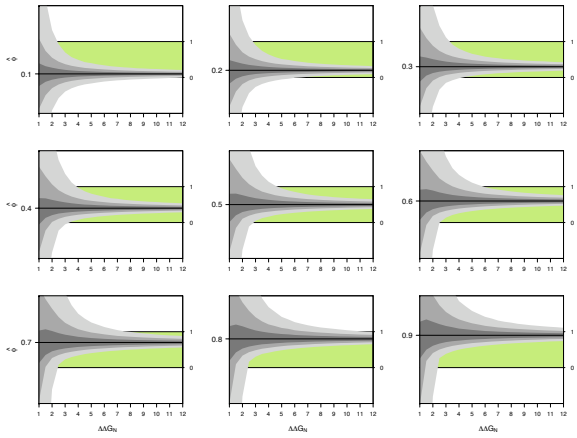
Standard Error

$$\mathrm{se}\left(\widehat{\Phi}\right) = |\Phi| \times \sqrt{\left(\frac{\sigma_T}{\Delta\Delta G_T}\right)^2 - 2\rho_{\Delta\Delta G}\left(\frac{\sigma_T}{\Delta\Delta G_T}\right)\left(\frac{\sigma_N}{\Delta\Delta G_N}\right) + \left(\frac{\sigma_N}{\Delta\Delta G_N}\right)^2}$$

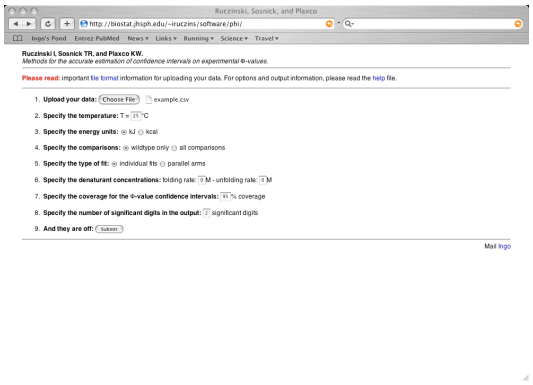
Standard Error



Standard Error



Web Server



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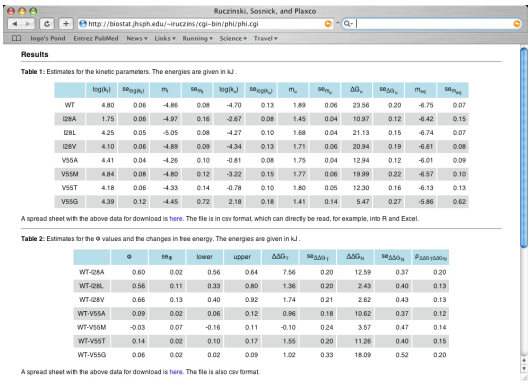
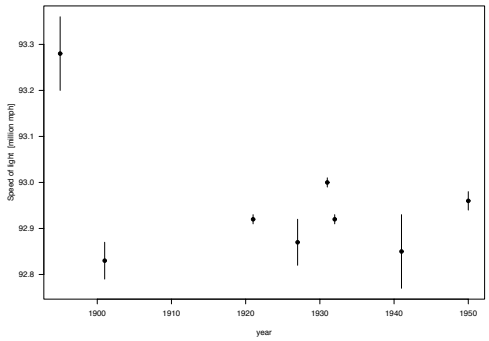


Questions

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

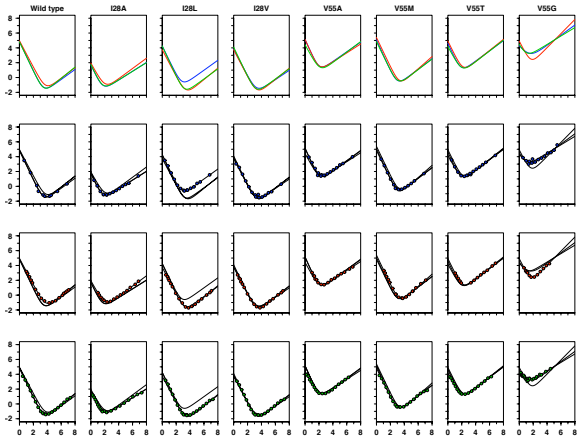
Dang...

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



Youden (Technometrics, 1972).

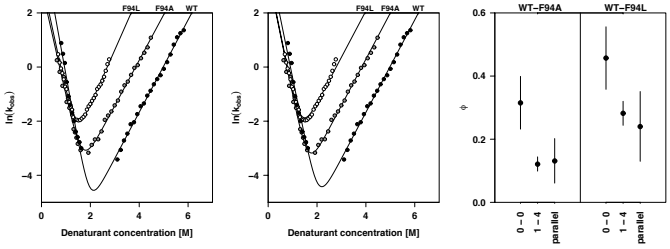
Reproducibility?



Questions

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

Alternative Techniques



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Acknowledgments

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