November 3, 2006 @ IMMBI Annual Retreat

Statistics Schmatistics

On the folded, the unfolded, and the transition state

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Biostatistics for Laboratory Scientists

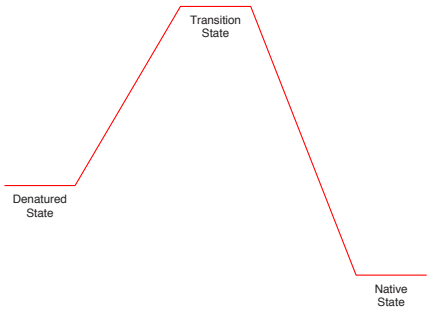
140.615 Introduction to Biostatistics for Laboratory Scientists I
3rd term (January 16 - March 9, 2007).

140.616 Introduction to Biostatistics for Laboratory Scientists II
4th term (March 19 - May 11, 2007).

MWF 10:30 - 11:50.

East Baltimore Campus
Bloomberg School of Public Health
615 N Wolfe St

Energy Profile



Radius of Gyration of Denatured Proteins

Do chemically denatured proteins behave like random coils?

- The radius of gyration R_g of a protein is defined as the root mean square distance from each atom of the protein to their centroid.
- For an ideal (infinitely thin) random-coil chain in a solvent, the average radius of gyration of a random coil is a simple function of its length n:

$$R_g \propto n^{0.5}$$

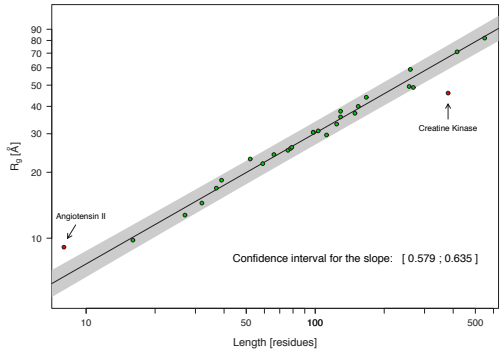
- For an excluded volume polymer in a solvent, the average radius of gyration of a random coil is given by

$$R_g \propto n^{0.588}$$

$\longrightarrow$ The radius of gyration can be measured using small angle x-ray scattering.

Reference:
Flory PJ (1953). Principles of Polymer Chemistry, *Cornell University Press.*

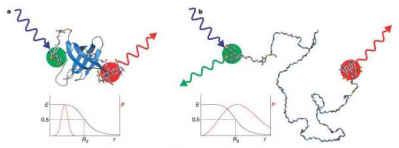
Radius of Gyration of Denatured Proteins



Deviations from Random Coil Behavior

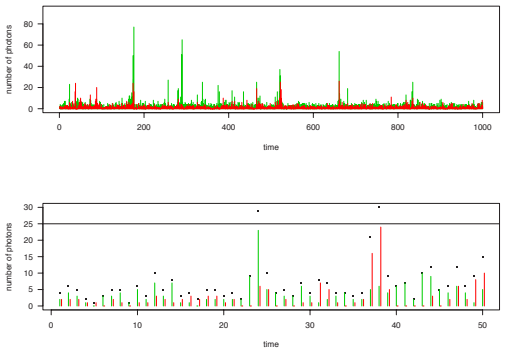
Are there site-specific deviations from random coil dimensions?

Förster Resonance Energy Transfer enables us to measure the distance between two dye molecules within a certain range. This can be used to study site-specific deviations from random coil dimensions in denatured peptides.

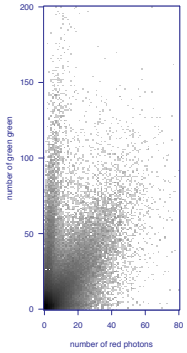


Reference:
Schuler B, Lipman EA, Eaton WA (2002). Abstract Probing the free-energy surface for protein folding with single-molecule fluorescence spectroscopy. *Nature*, 419 (6908): 743-7.

Deviations from Random Coil Behavior



Deviations from Random Coil Behavior

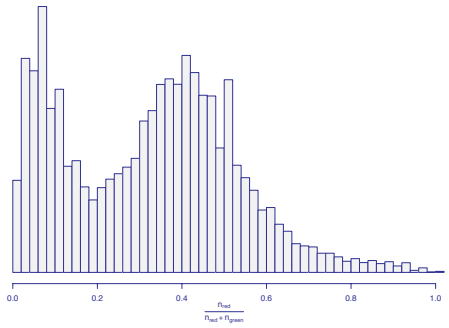


We have two underlying distributions for the green and red photons:

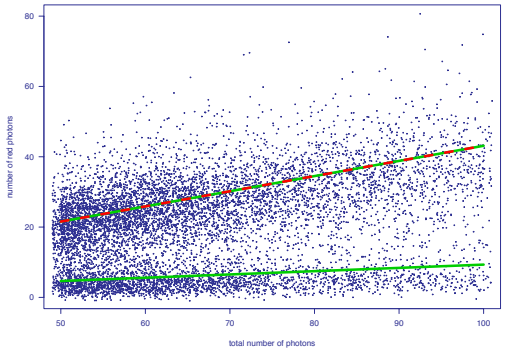
→ One stemming from a peptide only having a donor dye.

→ One stemming from a peptide being properly tagged with a donor and an acceptor dye.

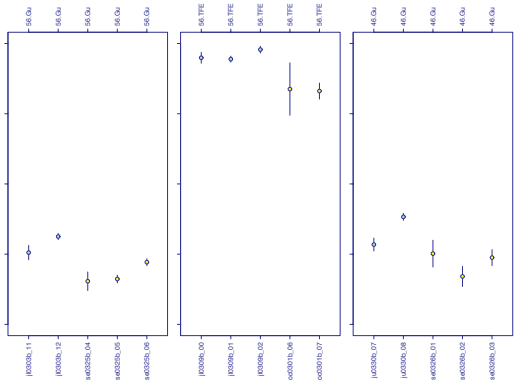
Deviations from Random Coil Behavior



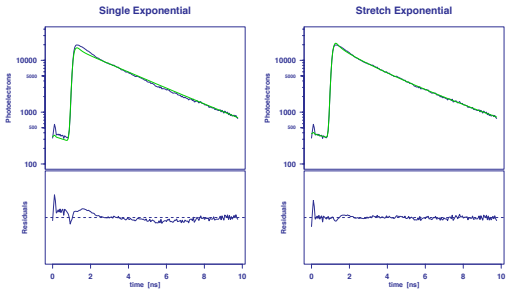
Deviations from Random Coil Behavior



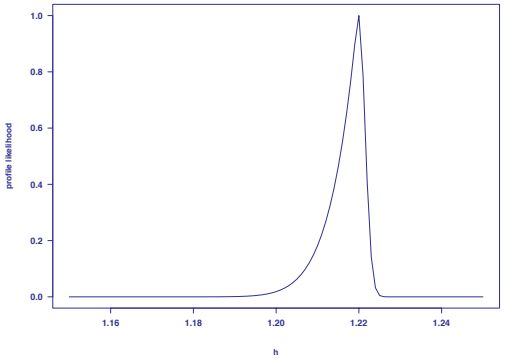
Replication



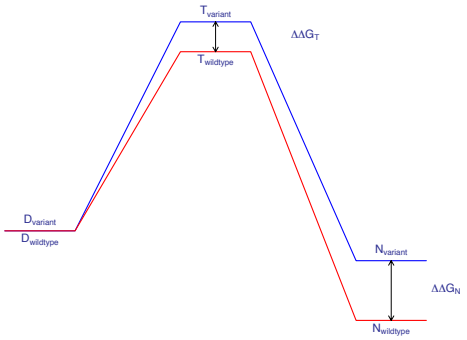
Photon Lifetime



Photon Lifetime

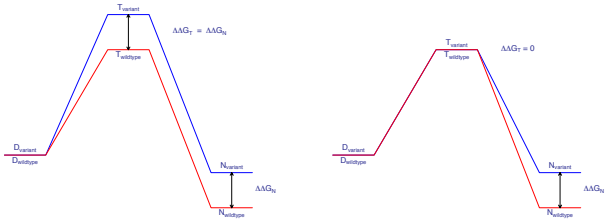


Energy Profile



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

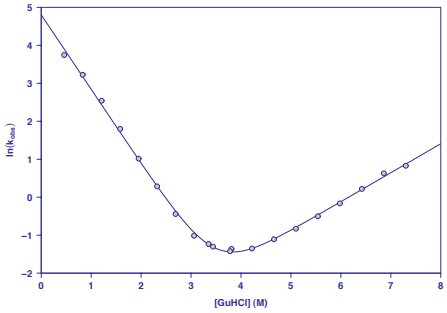
Energy Profile



- 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 $\Phi \approx 0$.

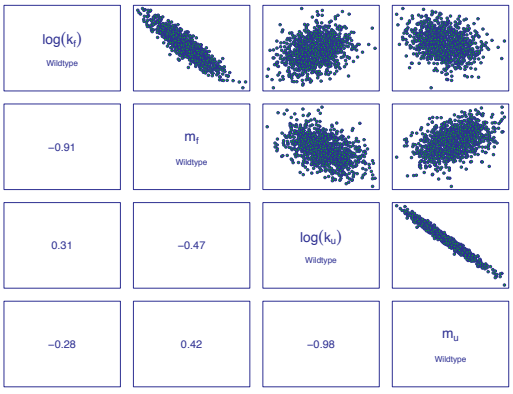
At least this is the idea . . .

Standard Error

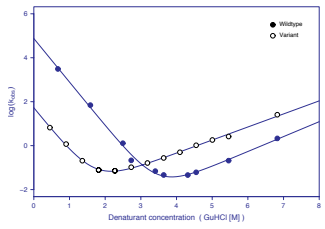


$$\ln(k_{obs}) = \log\Big(\exp\Big[\log(k_f) + m_f \times \frac{[GuHCl]}{RT}\Big] + \exp\Big[\log(k_u) + m_u \times \frac{[GuHCl]}{RT}\Big] \Big)$$

Standard Errors



Phi-Value Estimation



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

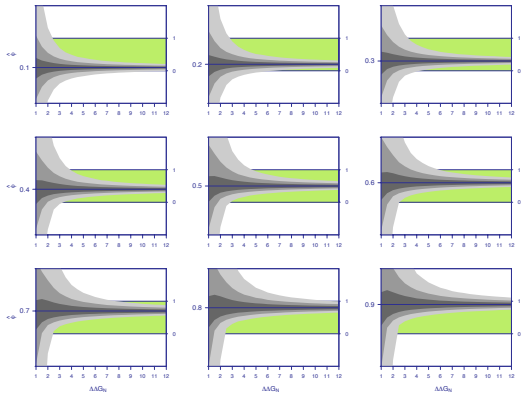
$$\Delta\Delta G_T = RT \times \Big[\log(k_f^{wildtype}) - \log(k_f^{variant}) \Big]$$

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

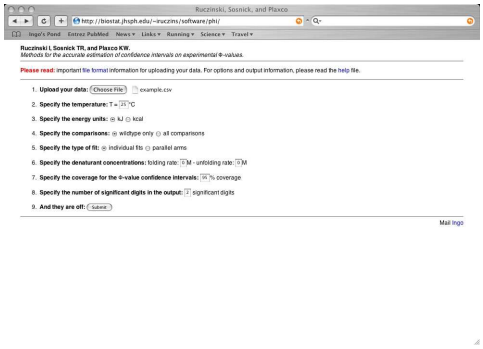
Standard Error

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

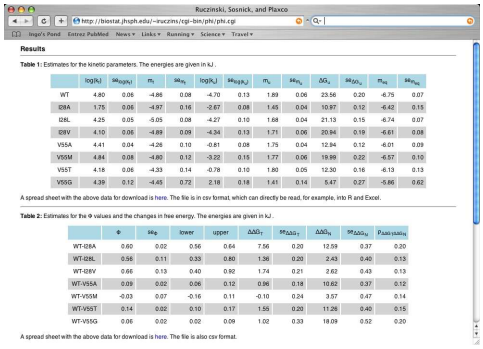
Standard Error



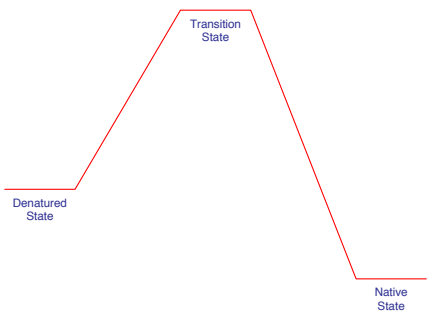
Web Server



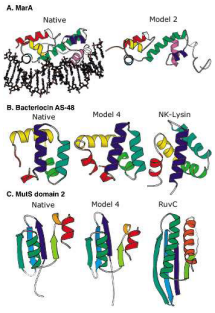
Web Server



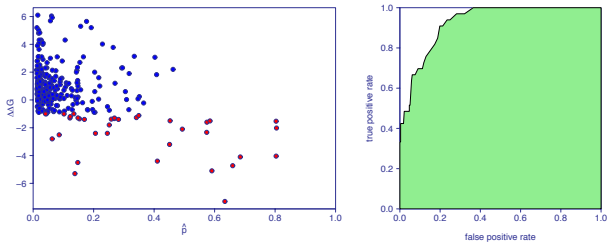
Energy Profile



Structure Prediction / Functional Annotation



Stability / Affinity



http://biostat.jhsph.edu/∼iruczins

Acknowledgments

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