Got Data?

On Biostatistics in Public Health Research

Some Selected, Non-Random Examples

Ingo Ruczinski

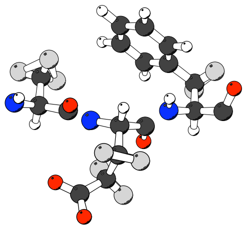
Department of Biostatistics, Johns Hopkins University

Outline

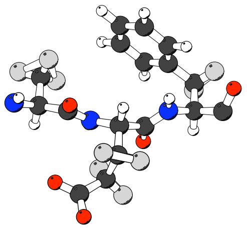
- Ab Initio Protein Structure Prediction
- Proteomics: 2D Gel Electrophoresis
- Chromosomal Abnormalities in Disease
- Protein Folding and Folding Kinetics

Proteins

Amino acids without peptide bonds.

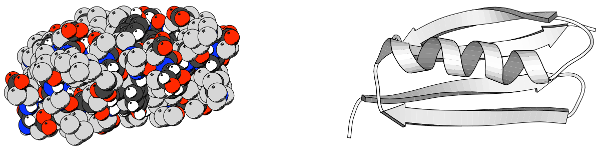


Amino acids with peptide bonds.



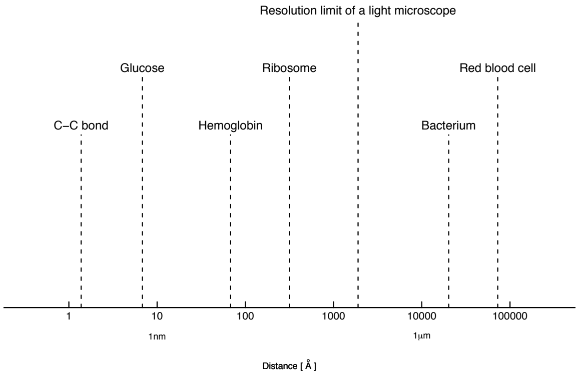
⟶ Amino acids are the building blocks of proteins.

Proteins

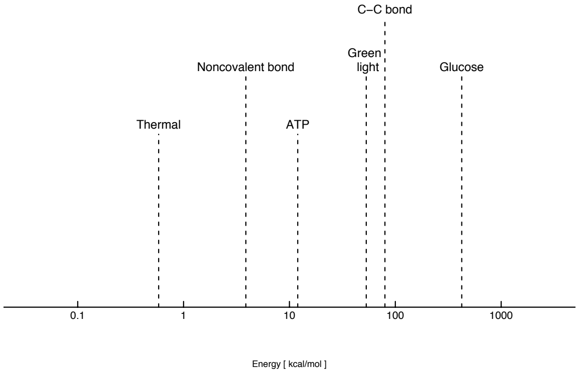


Both figures show the same protein (the bacterial protein L). The right figure also highlights the secondary structure elements.

Space



Energy



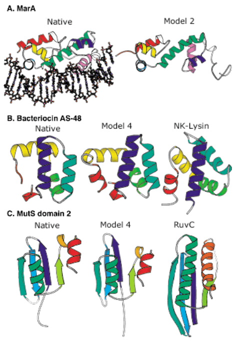
Non-Bonding Interactions

Amino acids of a protein are joined by covalent bonding interactions. The polypeptide is folded in three dimension by non-bonding interactions. These interactions, which can easily be disrupted by extreme pH, temperature, pressure, and denaturants, are:

- Electrostatic Interactions (5 kcal/mol)
- Hydrogen-bond Interactions (3-7 kcal/mol)
- Van Der Waals Interactions (1 kcal/mol)
- Hydrophobic Interactions ($<$ 10 kcal/mol)

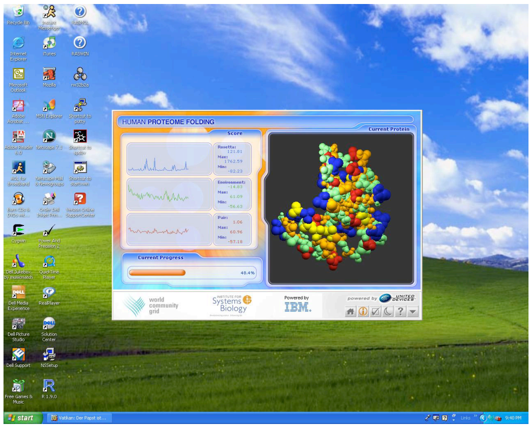
The total inter-atomic force acting between two atoms is the sum of all the forces they exert on each other.

Functional Annotation



⟶ ROSETTA is used for functional annotation of genes.

Genome Wide Annotation



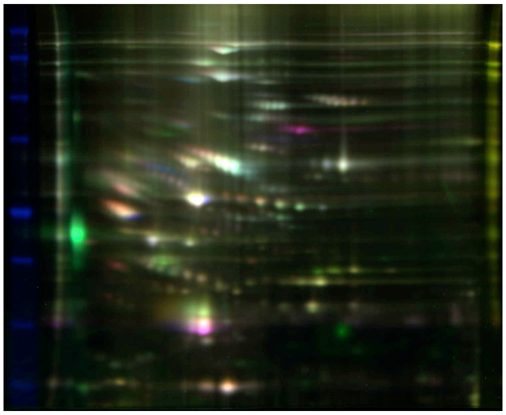
Statistical Software

```
> read.pdb("1amu",id="A")
   nat  at  aa id naa      x      y      z
1    1 N   GLY  A  17 10.929 62.747 30.169
2    2 CA  GLY  A  17 12.121 63.555 30.349
3    3 C   GLY  A  17 11.903 64.708 31.310
4    4 O   GLY  A  17 10.812 65.281 31.365
5    5 N   THR  A  18 12.968 65.107 31.999
6    6 CA  THR  A  18 12.892 66.160 33.009
7    7 C   THR  A  18 13.464 67.514 32.561
8    8 O   THR  A  18 13.206 68.542 33.189
...

> read.pdb("1amu",id="A",atms="CA")

   nat  at  aa id naa      x      y      z
2    2 CA  GLY  A  17 12.121 63.555 30.349
6    6 CA  THR  A  18 12.892 66.160 33.009
13  13 CA  HIS  A  19 14.765 68.754 30.893
23  23 CA  GLU  A  20 17.327 69.446 33.609
32  32 CA  GLU  A  21 19.913 71.318 31.511
41  41 CA  GLU  A  22 17.278 73.664 30.123
50  50 CA  GLN  A  23 15.880 74.276 33.602
...
```

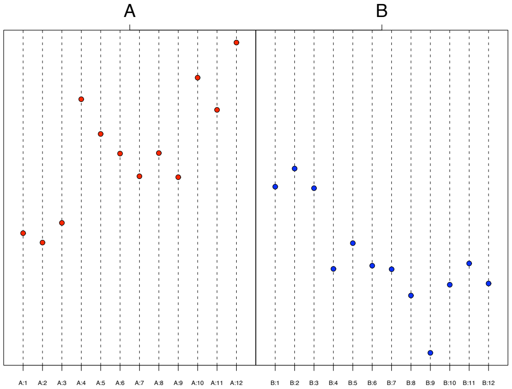
Outline

- Ab Initio Protein Structure Prediction
- Proteomics: 2D Gel Electrophoresis
- Chromosomal Abnormalities in Disease
- Protein Folding and Folding Kinetics

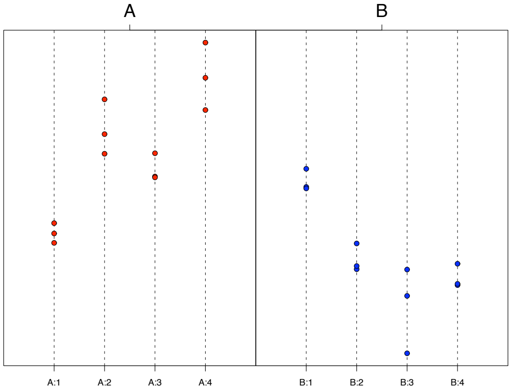
2D Gel Electrophoresis



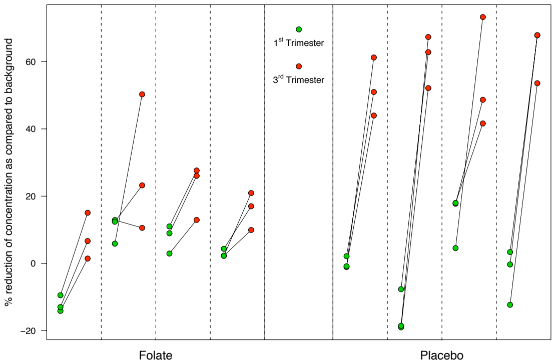
2D Gel Electrophoresis



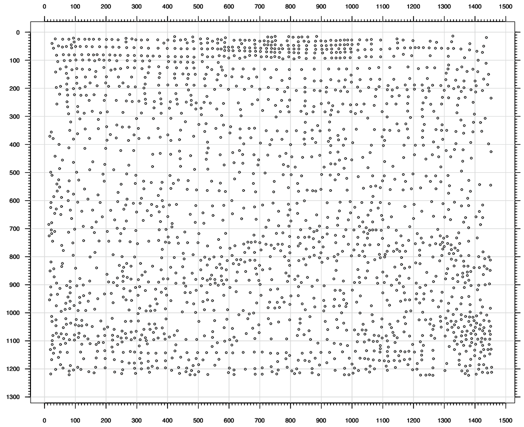
2D Gel Electrophoresis



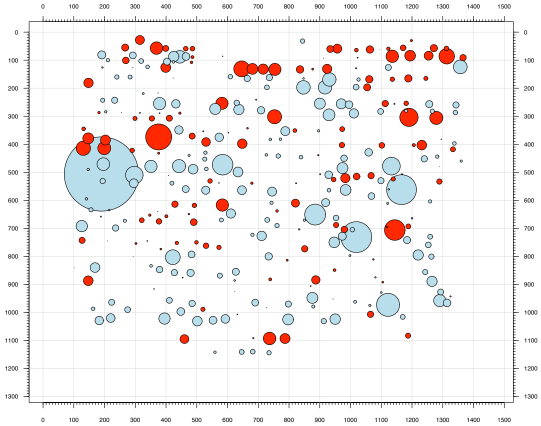
2D Gel Electrophoresis



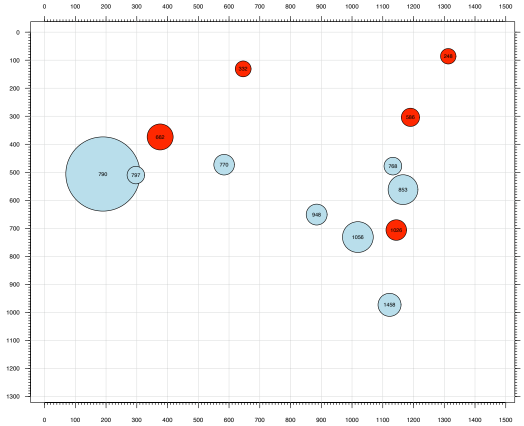
2D Gel Electrophoresis



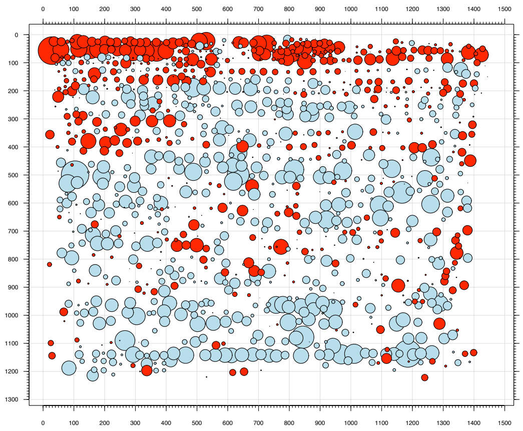
2D Gel Electrophoresis



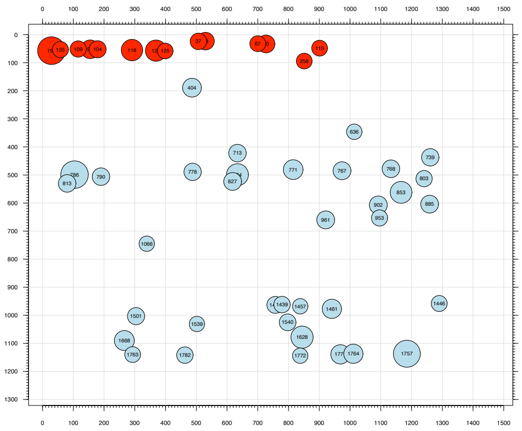
2D Gel Electrophoresis



2D Gel Electrophoresis



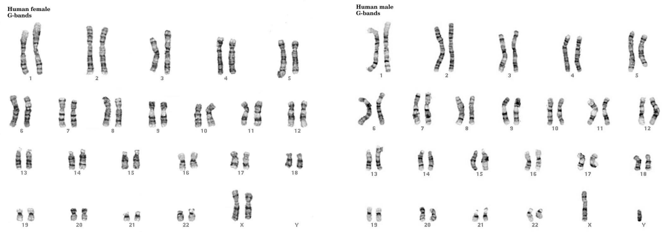
2D Gel Electrophoresis



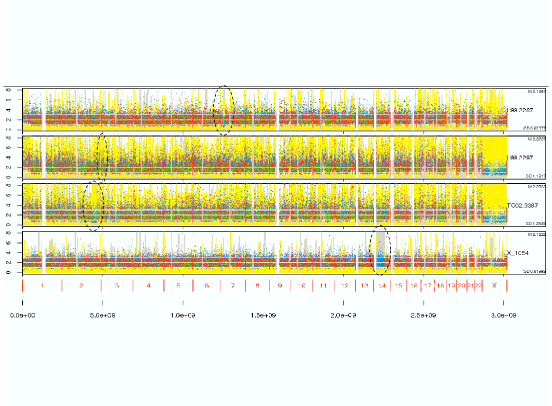
Outline

- Ab Initio Protein Structure Prediction
- Proteomics: 2D Gel Electrophoresis
- Chromosomal Abnormalities in Disease
- Protein Folding and Folding Kinetics

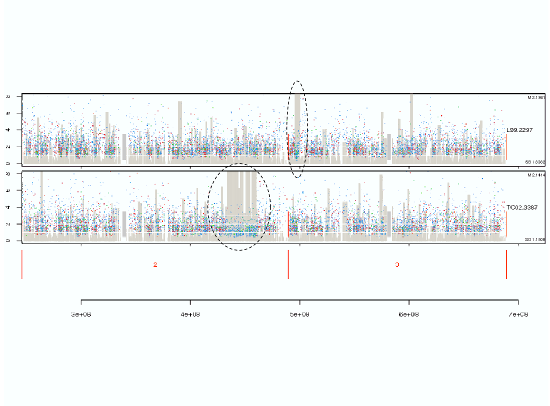
Karyotype



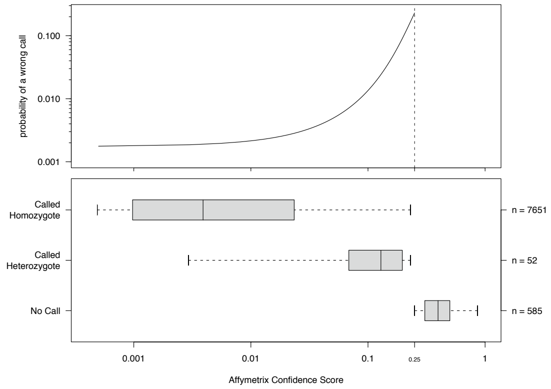
SNPscan



SNPscan



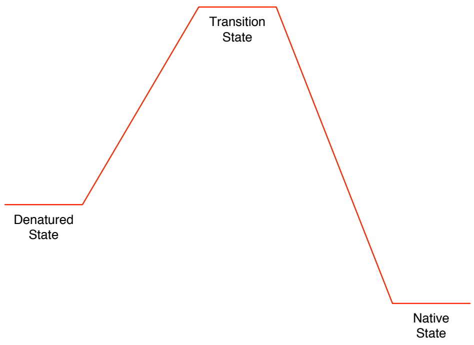
Additional Information



Outline

- Ab Initio Protein Structure Prediction
- Proteomics: 2D Gel Electrophoresis
- Chromosomal Abnormalities in Disease
- Protein Folding and Folding Kinetics

Energy Profile



Radius of Gyration of Denatured Proteins

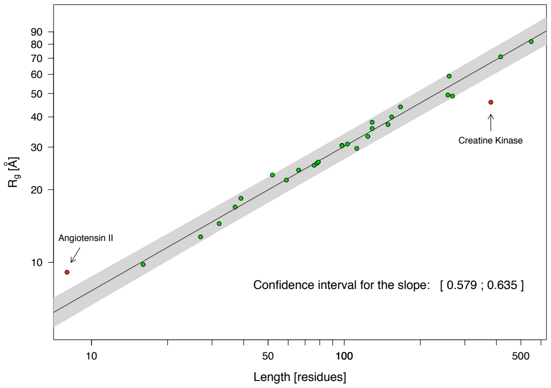
Do chemically denatured proteins behave as random coils?

- 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 (a polymer with non-zero thickness and non-trivial interactions between monomers) in a solvent, the average radius of gyration, we have $R_g \propto n^{0.588}$ (Flory 1953).
- This can easily be written as a simple linear regression model:

$\log_{10}(R_g) = c + 0.588 \times \log_{10}(n)$

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

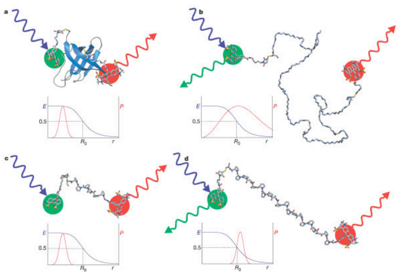
Radius of Gyration of Denatured Proteins



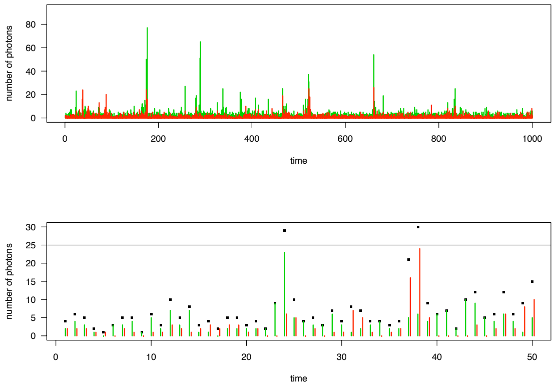
Deviations from Random Coil Behaviour

Are there site-specific deviations from random coil dimensions?

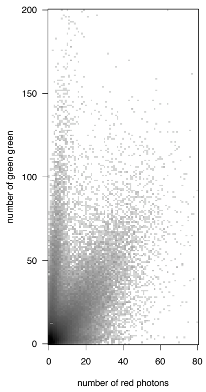
Förster Resonance Energy Transfer enables us to measure the distance between two dye molecules within a certain range. This can be used to study site-specific deviations from random coil dimensions in highly denatured peptides.



Deviations from Random Coil Behaviour



Deviations from Random Coil Behaviour

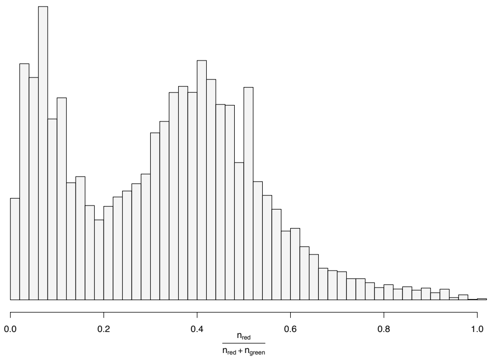


We have two underlying distributions for the green and red photons:

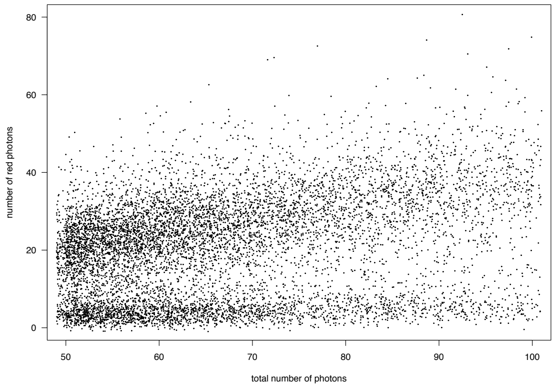
- One stemming from a peptide only having a donor dye.
- One stemming from a peptide being properly tagged with a donor and an acceptor dye.

Assume a photon has probability p_0 of being red in the former situation, and p_1 in the latter.

Deviations from Random Coil Behaviour



Deviations from Random Coil Behaviour



Deviations from Random Coil Behaviour

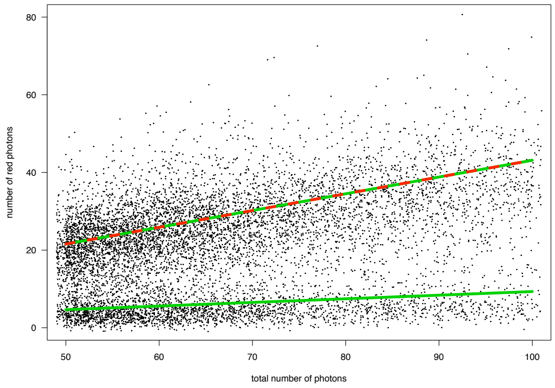
Assume we observe n_i photons at time point i. Then the number of red photons is simply Bernoulli(n_i, p_i), where p_i is either p_0 or p_1 . Assume that the probability of observing photons from a peptide without an acceptor dye at any time is p, independent of the total number of photons observed. Let X be the number of red photons. Then

$$\begin{aligned} P(X = x_i | n_i) &= P(X = x_i | n_i, p_0) \times p + P(X = x_i | n_i, p_1) \times (1-p) \\ &= \binom{n_i}{x_i} p_0^{x_i}(1-p_0)^{n_i - x_i} \times p + \binom{n_i}{x_i} p_1^{x_i}(1-p_1)^{n_i - x_i} \times (1-p), \end{aligned}$$

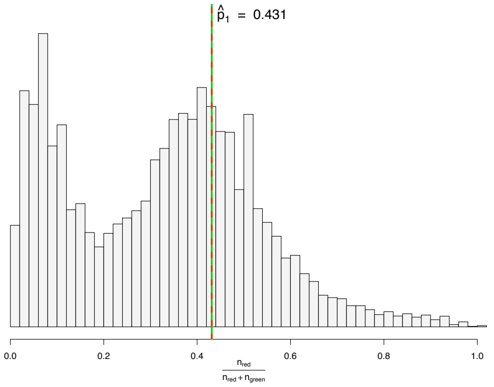
and hence

$$L(p, p_0, p_1) = \prod_{i=1}^{N} \left[\binom{n_i}{x_i} p_0^{x_i}(1-p_0)^{n_i - x_i} \times p + \binom{n_i}{x_i} p_1^{x_i}(1-p_1)^{n_i - x_i} \times (1-p) \right].$$

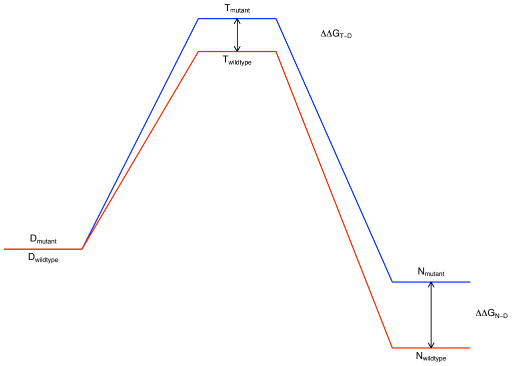
Deviations from Random Coil Behaviour



Deviations from Random Coil Behaviour

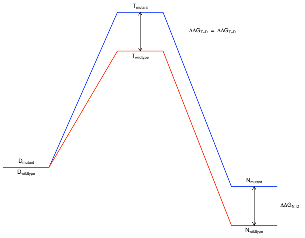


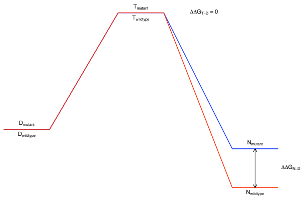
Energy Profile



$\longrightarrow$ The Φ-value is defined as the ratio $\Delta\Delta G_{T\text{-}D}/\Delta\Delta G_{N\text{-}D}$.

Energy Profile

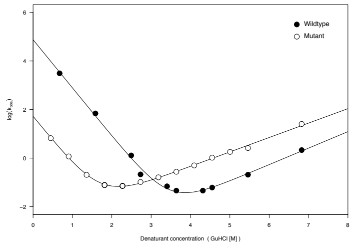




- If the part of the protein that contains the mutant amino acid is fully structured in the transition state, we have $\Delta\Delta G_{T\text{-}D} \approx \Delta\Delta G_{N\text{-}D}$, and hence $\Phi \approx 1$.
- If the part of the protein that contains the mutant amino acid is equal in denatured and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

At least this is the idea …

Phi-Value Estimation

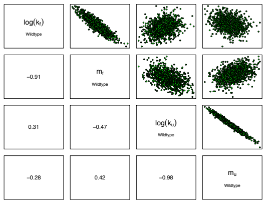


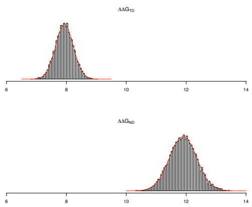
$$\log(k_{obs}) = \log\left(\exp\left[\log(k_f) + m_f \times \frac{C_{GuHCl}}{RT}\right] + \exp\left[\log(k_u) + m_u \times \frac{C_{GuHCl}}{RT}\right] \right)$$

$$\Delta\Delta G_{T\text{-}D} = RT \times \left[\log(k_f^{wildtype}) - \log(k_f^{mutant}) \right]$$

$$\Delta\Delta G_{N\text{-}D} = RT \times \left[\log(k_f^{wildtype}) - \log(k_u^{wildtype}) - \log(k_f^{mutant}) + \log(k_u^{mutant}) \right]$$

Confidence Intervals





$$\begin{bmatrix} \widehat{\Delta\Delta G}_{\text{TD}} \\ \widehat{\Delta\Delta G}_{\text{ND}} \end{bmatrix} \sim N\left(\begin{bmatrix} \Delta\Delta G_{\text{TD}} \\ \Delta\Delta G_{\text{ND}} \end{bmatrix}, \begin{bmatrix} \sigma_1^2 & \sigma_3^2 \\ \sigma_3^2 & \sigma_2^2 \end{bmatrix} \right)$$

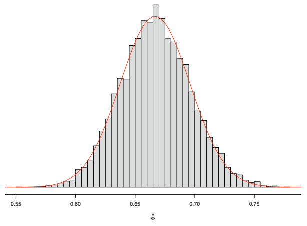
$$\begin{aligned} \sigma_1^2 &= \sigma_{\mathsf{F}_W}^2 + \sigma_{\mathsf{F}_M}^2 \\ \sigma_2^2 &= \sigma_{\mathsf{F}_W}^2 + \sigma_{\mathsf{F}_M}^2 + \sigma_{\mathsf{U}_W}^2 + \sigma_{\mathsf{U}_M}^2 - 2\rho_{\mathsf{W}}\sigma_{\mathsf{F}_W}\sigma_{\mathsf{U}_W} - 2\rho_{\mathsf{M}}\sigma_{\mathsf{F}_M}\sigma_{\mathsf{U}_M} \\ \sigma_3^2 &= \sigma_{\mathsf{F}_W}^2 + \sigma_{\mathsf{F}_M}^2 - \rho_{\mathsf{W}}\sigma_{\mathsf{F}_W}\sigma_{\mathsf{U}_W} - \rho_{\mathsf{M}}\sigma_{\mathsf{F}_M}\sigma_{\mathsf{U}_M} \end{aligned}$$

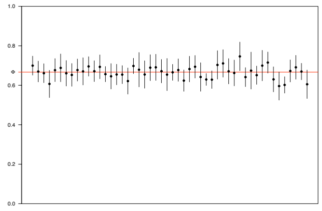
For sufficiently large $\Delta\Delta G_{N\text{-}D}$, some more math shows that the estimate for Φ is approximately normal (there is some slight abuse of the “delta method” involved).

$$\widehat{\Phi} = \frac{\widehat{\Delta\Delta G}_{\text{TD}}}{\widehat{\Delta\Delta G}_{\text{ND}}} \approx N(\Phi, B) \qquad B = \frac{1}{(\widehat{\Delta\Delta G}_{\text{ND}})^4} \times (\sigma_1^2(\widehat{\Delta\Delta G}_{\text{ND}})^2 - 2\sigma_3^2\widehat{\Delta\Delta G}_{\text{TD}}\widehat{\Delta\Delta G}_{\text{ND}} + \sigma_2^2(\widehat{\Delta\Delta G}_{\text{TD}})^2).$$

Confidence Intervals

Confidence intervals for the Φ-value: $I = \left[\hat{\Phi} - t_{n_1+n_2-10}^{0.975} \times \sqrt{B}\ ;\ \hat{\Phi} + t_{n_1+n_2-10}^{0.975} \times \sqrt{B}\right]$

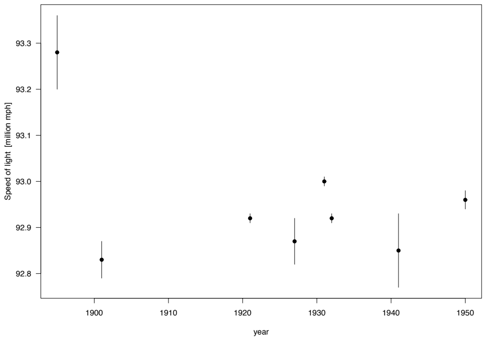




$\longrightarrow$ It is not a priori clear what the degrees of freedom in the t-quantile should be. Adding the number of data points used to fit the two chevron curves (n_1 and n_2) and subtracting the number of parameters estimated in the fitting procedure (a total of 10) however gave 95% coverage for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

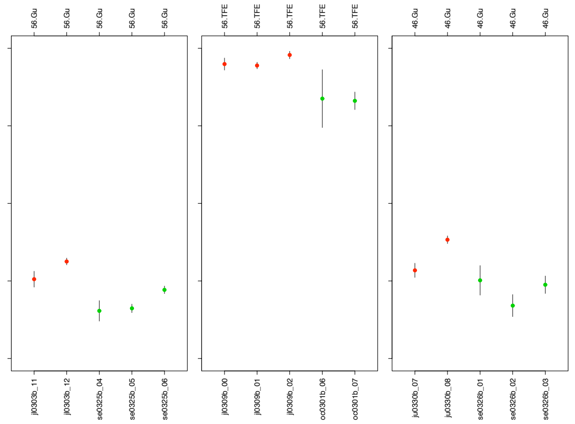
Dang...

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

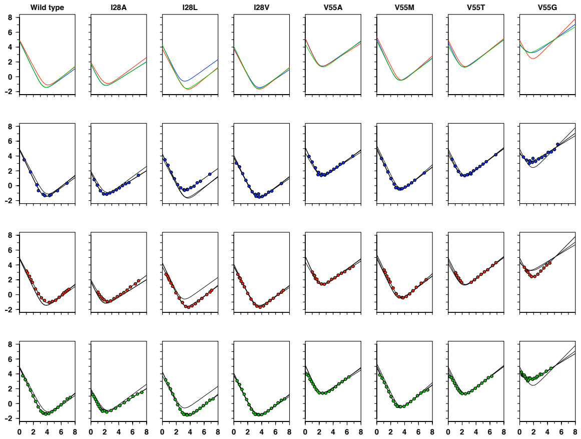


Youden (Technometrics, 1972).

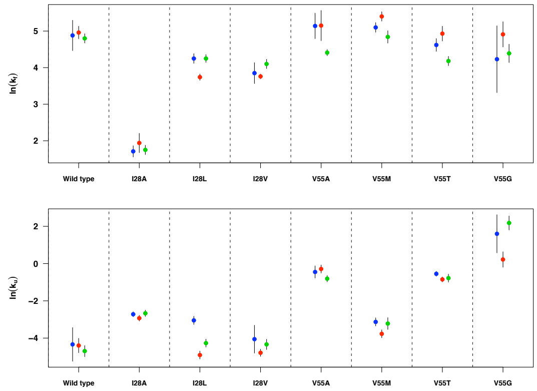
Variance Components



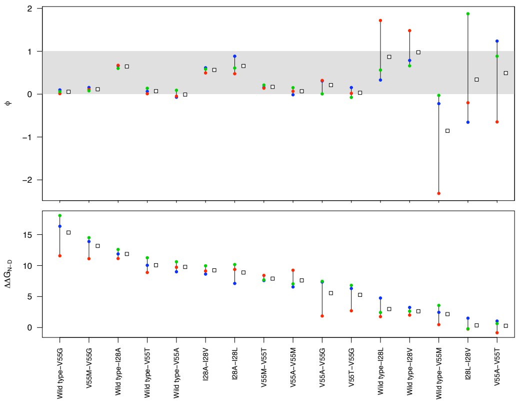
Chevron Plots



Variability



Variability



Evolution and Folding Kinetics

Are amino acids in proteins conserved because of folding kinetics?

To what extent does natural selection act to optimize the details of protein folding kinetics? Is there a relationship between an amino acid's evolutionary conservation and its role in protein folding kinetics?

Some comments:

- Our studies of sequence conservation among residues known to participate in the folding nuclei of all of the appropriately characterized proteins reported to date have not provided any evidence that highly conserved residues are more likely to participate in the protein folding nucleus than poorly conserved residues.
- This is in contrasts to some of the beliefs stemming from theoretical considerations (good science, good people).
- This is also in contrast to the conclusions certain people drew from experimental data (really aweful statistics).
- The latter people do not like us.