Protein folding rates correlate with heterogeneity of folding mechanism

B. Öztop‡, M. R. Ejtehadi‡†, and S. S. Plotkin‡∗

† Department of Physics and Astronomy, University of British Columbia, Vancouver, BC V6T-1Z1, Canada
‡ Department of Physics, Sharif University of Technology, Tehran 11365-9161, Iran

(Dated: February 9, 2008)

Abstract

By observing trends in the folding kinetics of experimental 2-state proteins at their transition midpoints, and by observing trends in the barrier heights of numerous simulations of coarse grained, Cα model, Gō proteins, we show that folding rates correlate with the degree of heterogeneity in the formation of native contacts. Statistically significant correlations are observed between folding rates and measures of heterogeneity inherent in the native topology, as well as between rates and the variance in the distribution of either experimentally measured or simulated φ-values.

PACS numbers: 87.15.Aa, 87.15.Ya, 87.15.He, 87.14.Ee

∗Electronic address: steve@physics.ubc.ca
Protein folding is a relaxation process driven by a first order like fluctuation of a critical nucleus \[1\]. Because proteins are evolutionarily designed to fold to a particular structure, frustrating interactions are minimized and the folding process can be projected onto one or a few reaction coordinates without too much loss of information \[2\]. This projection yields a free energy surface whose structure is subject to much interest. Different proteins have different free energy surfaces with different barrier heights.

What factors determine the height of the folding free energy barrier for the various proteins? As one would expect, the barrier decreases as the energetic stability of the folded structure increases \[3\]. Moreover folding rates tend to increase with energetic discrimination measures between the folded state and unfolded or misfolded decoys \[4\]. As one might also expect, the barrier increases for native structures that have longer polymer loops formed during folding. A property capturing this effect, dubbed absolute contact order (ACO), measures the mean sequence separation between amino acids in close proximity (and thus interacting) in the native structure \[5\]:

\[
ACO \equiv \bar{\ell} = \frac{1}{M} \sum_{i<j} |i-j| \Delta_{ij} \quad \text{where } i \text{ and } j \text{ label amino acid index, } \Delta_{ij} = 1 \text{ (or 0) if amino acids } i \text{ and } j \text{ are (or are not) interacting in the native structure, and } M \text{ is the total number of contacts in the native structure determined by either heavy side chain atoms or } C_{\alpha} \text{ atoms within a cut-off distance of } 4.8 \ \text{Å} \[6\].
\]

In what follows we first re-examine the trend of rates with \(\bar{\ell}\) in light of theoretical predictions \[7, 8, 9\], then we will go on to further examine higher-order aspects of native topology (and energetics) that act as predictors of folding rate.

If we take data that first corrects for the effects of differing native stabilities for different proteins by adjusting denaturant concentration to conditions at the transition midpoint, and then plot the log folding rate \(\text{vs } \bar{\ell}\), we find a statistically significant correlation for a representative set of 19 2-state proteins (and P\(^{13–14}\) circular permutant of S6) (Fig. 1A) \[10\]. Observations similar to this led the folding community to accept the idea that properties of native topology strongly determine folding rate \[11\]. Moreover if one simulates off-lattice \(C_{\alpha}\) Gō models \[6\] to 18 structures of known 2-state folders \[12\], one also finds a statistically significant correlation between barrier height and absolute contact order (Fig. 1B). One also notices from Fig. 1 that there must be more to the story then absolute contact order in determining folding rates, since the fluctuations around the best fit line are significant.

The effects of native topology (and energetics) should be describable analytically as well. To this end a free energy functional approach was developed \[7, 8, 9\] within which it was
FIG. 1: (A) Logarithm of experimental folding rate (in sec$^{-1}$) at the transition midpoint $\ell$ vs absolute contact order or mean sequence separation between interacting residues in the native structure. Wild type protein S6 is shown by an open square and P$^{13-14}$ circular permutant of S6 is shown by an open circle. (B) The equivalent measure in Gō simulations is $-\Delta F_{\text{sim}}^\dagger/T_f$, again plotted $\ell$. Both show a statistically significant anti-correlation: $r$ (or $\tau$) is the correlation coefficient (or Kendall’s Tau). Statistical significance is defined here by the probability $P(r)$ ($P(\tau)$) to observe a given correlation coefficient or greater by chance. If $P(r)$ ($P(\tau)$) $< 0.05$, the dependence is typically deemed statistically significant. Shown in (A) are 19 proteins (and P$^{13-14}$ circular permutant of S6) for which experimental rate data are available at various denaturant concentrations, and in (B) 18 simulated Gō model proteins.

It is shown that the free energy barrier may be written in terms of an expansion involving moments of distributions of native contact interaction energies $\{\epsilon_{ij}\}$, and native contact sequence separations $\{\ell_{ij}\} \equiv \{|i-j|\}$. The lowest order corrections to the mean-field barrier are:

$$\frac{\Delta F^\dagger}{MT}((\epsilon_{ij}), (\ell_{ij})) = \frac{\overline{\Delta F^\dagger}}{MT} - A \frac{\delta \epsilon^2}{T^2} - B \frac{\delta \epsilon \delta \ell}{\ell T} - C \frac{\delta \ell^2}{\ell}$$

where $A$, $B$, $C$ are all positive and of order unity. The lowest order mean field term $\overline{\Delta F^\dagger} \equiv$
$\Delta F^\dagger(\tau, \bar{t})$, where $\tau, \bar{t}$ are the first moments (mean) of the distributions, indeed increases as $\bar{t}$ increases, consistent with the observed trend. The theory gives the slope $m_{MF}$ of the mean field barrier vs $\bar{t}$ as

$$m_{MF} \equiv \partial(-\Delta F^\dagger/T)/\partial \bar{t} \approx -(3/2)(M/\bar{t}^2) \ln(\bar{t}^1/2)/2.$$ (2)

Calculating Eq. (2) for all proteins used in Fig. 1A, $m_{MF} = -0.41 \pm 0.09$, which is consistent with the slope of the best fit line $-0.36$. The mean field slope for the proteins in Fig. 1B is $-0.42\pm0.08$ which is almost twice the slope of the best fit line $-0.19$. There may be several reasons for this, including the fact that the theory used the mean field approximation, while the nucleus may be better approximated by a capillary model, and the Gaussian approximation for polymer loops used in the theory may be poor for many contacts. There may also be a cancellation of errors in Fig. 1A due to the presence of a capillary nucleus with many-body interactions present [15], which would result in unexpectedly good agreement.

Second order terms in Eq. (1) involving the fluctuations of native energies and loop lengths contact to contact all tend to decrease the barrier, leading to the notion that proteins with more heterogeneous folding mechanisms should fold faster [8, 9]. We note that here a more heterogeneous folding mechanism corresponds to a more specific, polarized folding nucleus, i.e. the heterogeneity here refers to contact formation probability, not conformational diversity of the transition state. Earlier lattice-simulation studies [16] as well as more recent experimental studies of circular permutants [13] support the notion that a more polarized nucleus results in a faster folding protein.

We can readily check if the second moment of the loop length distribution has an observable effect on rates, even if we ignore variations due to different $\bar{t}$ values protein to protein, as well as the terms with coefficients $A$ and $B$ in Eq. (1). The functional theory gives coefficient $C \approx Q^\dagger$ in Eq. (1) [9], so the change in barrier height due to the presence of structural variance is:

$$\frac{(\Delta F^\dagger - \overline{\Delta F}^\dagger)/MT \equiv \delta \Delta F^\dagger/MT \approx -Q^\dagger \delta \bar{t}^2/\bar{t}^2.}{}$$ (3)

Here, $Q$ is the overall fraction of native contacts, and $Q^\dagger$ is the value of $Q$ at the barrier peak.

Plots of experimental log folding rate and simulated barrier heights (over $MT$) both show statistically significant correlation with $\delta \bar{t}^2/\bar{t}^2$ (Fig. 2).
FIG. 2: Plotted in (A) are log experimental rate data (at the transition midpoints) and in (B), simulated barriers (at $T_f$), as a function of the measure of structural heterogeneity that appears in the functional theory in Eq.s (1) and (3). Both show a moderate, but statistically significant correlation with structural variance. Three $\alpha/\beta$ proteins ($\lambda$-repressor chain 3, cytochrome c, yeast iso-1-cytochrome c) tend to have both large structural variance and fast folding rates. However there are large fluctuations present, and the slope of the best fit line is only about a tenth the theoretical prediction. Neglecting trends due to contact order and energetic variance introduces errors in the plots.

Experimentally measured $\phi$-values involve both energetics and entropics and should better capture the effects of heterogeneity in folding mechanism. The variance in $\phi$-values couples together the last 3 terms in Eq. (1). To facilitate a comparison of rates with $\phi$-variance, the free energy barrier maybe recast in terms of the variance in native contact formation probabilities ($Q_{ij}$)

$$\delta \Delta F^\| / MT \approx -\delta Q^2 / 2Q^\| .$$  

Eq. (4) only includes the effects of heterogeneity in polymer loop length, however energetic heterogeneity can be incorporated as well, which only changes the coefficient $(1/2Q^\|)$ in
FIG. 3: Plots of log experimental folding rate (over $M$) for a subset of proteins in Fig. 1A for which experimental $\phi$ values are available and minus free energy barrier (over $MT$) for simulated proteins vs $\phi$-variance. Both show strong statistically significant correlation. In particular the trend in experimental data is strong even though the number of proteins with available data for both $\phi$-variance and transition midpoint rate is not large. Experimental data for wild type S6 is shown by an open square and P$_{13-14}$ circular permutant of S6 is shown by an open circle which fits very well to the rest of the data and increases correlation. The strong correlation remains upon dividing by chain length $N$ instead of total number of contacts $M$.

Eq. (4) to $(3/2Q^\dagger)$. The simulations have no variance in native contact energies, moreover statistics arguments suggest that this native variance may be significantly reduced with respect to the variance in collapsed random structures.

$\phi$-values may be defined analytically as

$$\phi_i = \frac{\sum_{j \neq i} (Q^\dagger_{ij} - Q^U_{ij}) \Delta^N_{ij}}{\sum_{j \neq i} (Q^F_{ij} - Q^U_{ij}) \Delta^N_{ij}}$$

(5)

where $Q^U_{ij}$, $Q^\dagger_{ij}$ and $Q^F_{ij}$ are the probabilities of native contact formation between residues $i$ and $j$ in the unfolded, transition and folded states respectively.
In the approximation that all contacts are fully formed in the native structure \((Q_F = 1)\), and unformed in the unfolded structures \((Q_U = 0)\), the \(\phi\)-value for residue \(i\) is the mean of \(Q_{ij}\) values in the transition state (c.f. Eq. (5)). Further approximating the same number of nearest neighbors \(z\) for all residues, the variances are related by \(\delta \Phi^2 \approx (1/z) \delta Q^2\). If we make no approximations and simply plot \(\overline{\delta Q^2}\) vs. \(\overline{\delta \phi^2}\) (for the simulation data), the quantities correlate extremely well (see Table I) with a slope of \(\approx 1.2\) and an intercept \(-0.04\). The intercept may be non-zero since other fluctuating quantities (e.g. \(Q_U\), \(z\)) contribute to the variance of \(\phi\)-values.

The above arguments indicate \(\overline{\delta Q^2}\) and \(\overline{\delta \phi^2}\) are within a factor of approximately unity, so we rewrite Eq. (4) in the form

\[
\delta \Delta F^\dagger / MT \approx -D \overline{\delta \phi^2}
\]

with \(D\) a parameter of order unity.

According to Eq. (6) more polarized nuclei have lower free energy barriers. Plots of \(-\Delta F^\dagger / MT \) vs \(\overline{\delta \phi^2}\) for experiments and simulations are shown in Fig. 3. Here we see a strong statistically significant correlation of both rates and barriers with \(\phi\) variance. Moreover the slopes of the best fit lines \((\approx 0.3)\) compare somewhat more favorably with the theoretically predicted values \((\approx 0.8)\) than was the case for structural variance. A precise comparison with experimental data is more difficult since the coordination number \(z\) as well as the numbers \(Q_U\) and \(Q_F\) are not accurately known for all proteins. Taking the slope from Fig. 3A and using the approximations mentioned above allows us to infer the residue-residue coordination number: \(z \approx 4\) if energetic heterogeneity is negligible (Eq. (4)), \(z \approx 11\) if it is substantial (Eq. (4) with coefficient \(3/2Q^\dagger\)).

The residuals of \(-\Delta F^\dagger / MT \) vs \(\overline{\ell^2}\), when plotted against \(\overline{\delta \ell^2}/\overline{\ell^2}\) and \(\overline{\delta \phi^2}\), show comparable but typically slightly less significant correlation (within 10%) to those in Fig. 2 and Fig. 3. The term \(\delta \Delta F^\dagger / MT\) can be thought of as a measure of these residuals. We have plotted absolute rates, which are easily measurable from experiments or simulations, while the mean-field barrier is not.

We note that \(\overline{\delta \phi^2}_{exp}\) has errors due both to experimental measurement as well as the small set of \(\phi\)-values for each protein. Moreover the experimental rates at the transition midpoint are compared to the variance in \(\phi\)'s typically measured in water or stabilizing conditions. Interestingly, experimental folding mechanisms tend to be more polarized than uniform Gō
models.

In the case of the simulations, the correlation between $\delta \phi^2$ vs $\delta \ell^2/\ell^2$ is strong as expected, since there is no variance in native contact energies, by construction of the model. For experimental data however the correlation is poor, which implies that there may be substantial energetic heterogeneity present in native contact energies of real proteins. It is not too surprising then that there is no correlation between the variance of experimental $\phi$-values and simulation $\phi$-values (see Table I). In the analysis then, simulated barriers were plotted against simulated $\phi$-variance, and experimental rates were plotted against experimental $\phi$-variance.

We did not find any significant correlation between rates and structural variance $\delta \ell^2/\ell^2$ for 3-state folders. Here there is the intriguing picture that (on-pathway) intermediates in 3-state folders are in fact induced by structural or energetic heterogeneity, so that there is no a priori reason for folding rates to continue to increase with increasing heterogeneity.

S6 displays significant correlation between native contact energies and native loop lengths [13]. For this reason we did not include it in Fig. 2A, which only includes a structural measure of heterogeneity- if it is included the correlation decreases to $r = 0.57$, $P(r) = 9.6 \times 10^{-3}$. We note that the inclusion of the two data points corresponding to S6 does not change the correlation in Fig. 3A and decreases the correlation in Fig. 1A by 8%.

We showed here that both experimental rates and simulated free energy barriers for 2-state proteins depend on the degree of heterogeneity present in the folding process. The results compared quite well with the predictions of the free energy functional theory [8, 9]. Heterogeneity due to variance in the distribution of native loop lengths, as well as variance in the distribution of $\phi$-values, were both seen to increase folding rates and reduce folding barriers. The observed effect due $\phi$-variance was the most statistically significant (as expected), because $\phi$-variance captures both heterogeneity arising from native topology as well as that arising from energetics.

Acknowledgments

S. S. P. acknowledges support from the Natural Sciences and Engineering Research Council and the Canada Research Chairs program. We thank Kevin Plaxco and Mikael Oliveberg for helpful discussions.
### TABLE I: Correlation coefficient and statistical significance for various quantities.

| $y$ vs $x$ | $r$ | $P(r)^a$ | $\tau$ | $P(\tau)^a$ |
|-----------|-----|---------|--------|-------------|
| $\ln(k_f)$ | -0.69 | $9 \times 10^{-4}$ | -0.46 | $5.3 \times 10^{-3}$ |
| $-\Delta F_{\text{sim}}^\dagger/T_f$ | -0.71 | $10^{-3}$ | -0.61 | $4 \times 10^{-4}$ |
| $\ln(k_f)/M^{b}$ | 0.78 | $2.8 \times 10^{-3}$ | 0.52 | $2 \times 10^{-2}$ |
| $-\Delta F_{\text{sim}}^\dagger/MT_f$ | 0.67 | $2.3 \times 10^{-3}$ | 0.47 | $7.2 \times 10^{-3}$ |
| $\ln(k_f)/M$ | 0.62 | $6.6 \times 10^{-3}$ | 0.48 | $5.7 \times 10^{-3}$ |
| $-\Delta F_{\text{sim}}^\dagger/MT_f$ | 0.53 | $2.7 \times 10^{-2}$ | 0.36 | $3.7 \times 10^{-2}$ |
| $\ell$ | -0.14 | 0.52 | -0.07 | 0.7 |
| $\ell$ | -0.64 | $2.5 \times 10^{-2}$ | -0.43 | $5.5 \times 10^{-2}$ |
| $\ell$ | 0.16 | 0.52 | 0.15 | 0.38 |
| $\delta \phi_{\text{sim}}^2$ | 0.71 | $10^{-3}$ | 0.32 | $6.4 \times 10^{-2}$ |
| $\delta \phi_{\text{exp}}^2$ | 0.29 | 0.37 | 0.18 | 0.41 |
| $\delta \phi_{\text{exp}}^2$ | -0.16 | 0.8 | 0.2 | 0.63 |
| $\delta \phi_{\text{sim}}^2$ | 0.94 | $< 10^{-6}$ | 0.77 | $9 \times 10^{-6}$ |

*a*2-sided statistical significance has been used.

*b*Here we divide by the number of native contacts $M$. Dividing instead by chain length $N$ gives correlations within 10%. $M$ and $N$ correlate very strongly ($r = 0.94$).

*c*Data from both simulated and experimental proteins used.

---

[1] D. B. Wetlaufer, Proc Nat Acad Sci USA 70, 697 (1973), R. R. Matheson and H. A. Scheraga, Macromolecules 11, 819 (1978), V. I. Abkevich, A. M. Gutin and E. I. Shakhnovich, Biochemistry 33, 10026 (1994), A. R. Fersht, Proc Nat Acad Sci USA 92, 10869 (1995), P. G. Wolynes, Proc Nat Acad Sci USA 94, 6170 (1997), D. K. Klimov and D. Thirumalai, J Mol Biol 282, 471 (1998), O. V. Galzitskaya, D. N.Ivankov and A. V. Finkelstein, FEBS Lett 489, 113 (2001).

[2] S. S. Plotkin and P. G. Wolynes, Phys Rev Lett 80, 5015 (1998), S. S. Plotkin and J. N. Onuchic, Quart. Rev. Biophys. 35, 111 (2002), S. S. Plotkin and J. N. Onuchic, Quart. Rev. Biophys. 35, 205 (2002).
Initially relative contact order (RCO ≡ ℓ/N) was used as a predictor of rates in water (K. W. Plaxco, K. T. Simons, I. Ruczinski and D. Baker, Biochemistry, 39, 11177 (2000)). Later it was found ℓ was a better predictor for both 2 and 3-state proteins (D. N. Ivankov, S. O. Garbuzynskiy, E. Alm, K. W. Plaxco, D. Baker and A. V. Finkelstein, Protein Sci 12, 2057 (2003)). Here we control for the effects due to varying chain length N and stability by considering only rates at the transition midpoint vs ℓ. Stability in fact correlates with chain length for 2-state proteins in water (r = 0.58, P(r) = 0.012). This may be in part why RCO acts as a better predictor of rates than ℓ under these conditions. A Kramers analysis of folding times predicts a speed limit of about N/100µs (J. Kubelka, J. Hofrichter and W. A. Eaton, Curr. Opin. Struct. Biol. 14, 76 (2004)), however here we are considering rates at the fixed stability of the transition midpoint. Other topological measures such as cliquishness (C. Micheletti, Proteins 51, 74 (2003)), which measure correlations between native pair contacts, were also seen to correlate well with rate. However these measures also tend to correlate with contact order for PDB structures.

For a detailed description of the model see for example Z. Guo, D. Thirumalai, and J. D. Honeycutt, J Chem Phys 97, 525 (1992), J. E. Shea, Y. D. Nochomivitz, Z. Guo and C. L. Brooks III, J Chem Phys 109, 2895 (1998), or C. Clementi, H. Nymeyer, and J. N. Onuchic, J Mol Biol 298, 937 (2000).

B. A. Shoemaker, J. Wang, P. G. Wolynes, J Mol Biol 287, 675 (1999), B. A. Shoemaker, J. Wang, P. G. Wolynes, Proc Nat Acad Sci USA 94, 777 (1997).

S. S. Plotkin and J. N. Onuchic, Proc Nat Acad Sci USA 97, 6509 (2000).

S. S. Plotkin and J. N. Onuchic, J Chem Phys 116, 5263 (2002).

The PDB codes of these proteins are: 1AEY, 1APS, 1BF4, 1FKB, 1HRC, 1LMB, 1MJC, 1NYF, 1PGB, 1RIS, 1SRL, 1TEN, 1TIT, 1UBQ, 1YCC, 2AIT, 2C12, 2PTL, 2VIK. The various references containing experimental data for rates and φ-values for these proteins can be found at www.physics.ubc.ca/~steve/expt1.html.

S. Takada, Proc Nat Acad Sci USA 96, 11698 (1999), D. Baker, Nature 405, 39 (2000).

PDB codes: 1AB7, 1AEY, 1APS, 1CSP, 1FKB, 1HRC, 1LMB, 1MJC, 1NMG, 1NYF, 1SHG, 1SRL, 1UBQ, 1YCC, 2AIT, 2C12, 2PTL, 2U1A.
[13] M. Lindberg, J. Tangrot, and M. Oliveberg (2002), preprint.

[14] R. von Mises, *Mathematical Theory of Probability and Statistics* (Academic Press, New York, 1964).

[15] M. R. Ejtehadi, S. P. Avall and S. S. Plotkin, Proc. Natl. Acad. Sci. USA submitted.

[16] V. I. Abkevich, A. M. Gutin, and E. I. Shakhnovich, Biochemistry 33, 10026 (1994).

[17] A. R. Fersht, *Structure and mechanism in protein science* (W. H. Freeman and Co., New York, 1999), 1st ed.

[18] J. N. Onuchic, N. D. Socci, Z. Luthey-Schulten, and P. G. Wolynes, Folding and Design 1, 441 (1996).