A topological study of protein folding kinetics

Eleni Panagiotou# and Kevin W. Plaxco§ *

#Department of Mathematics, University of Tennessee at Chattanooga, TN 37403
§Department of Chemistry and Biochemistry, University of California Santa Barbara, CA 93106-3080
* Center for Bioengineering, University of California Santa Barbara, CA 93106-3080

December 21, 2018

Focusing on a small set of proteins that i) fold in a concerted, all-or-none fashion and ii) do not contain knots or slipknots, we show that the Gauss linking integral, the torsion and the number of sequence-distant contacts provide information regarding the folding rate. Our results suggest that the global topology/geometry of the proteins shifts from right-handed to left-handed with decreasing folding rate, and that this requires more sequence-distant contacts.

1 Introduction

Proteins are macromolecules composed by a varying sequence of amino acids and are important parts of living organisms [1]. In order for proteins to function they must attain conformations that belong in a small subset of the possible 3-dimensional conformations a macromolecule can attain, called native state. Protein folding is the process by which a protein molecule folds into its unique, three-dimensional conformation. Understanding protein folding is important in predicting of the native structure of a protein from its amino acid sequence which could lead to a better understanding of protein function as well as to the engineering of biopolymers with desired functions. In this study we use topological tools to understand how the topology of the protein relates to its folding rate.

The native structure is determined by the protein sequence (called primary structure) to a high extend, even though it has been shown that there exist small changes in the sequence that do not affect the 3-dimensional conformation (also known as tertiary structure) [11]. Some patterns of 3-dimensional structure that appear often in proteins are α-helices and β-strands. The sequence of such patterns is called the secondary structure of the protein. Structure is a very important clue to understanding and manipulating biological function. However, protein folding is very complex and it has been very difficult to provide a model for it. An interesting fact is that the rate at which proteins fold varies significantly from one protein to the other. At first, this might seem intuitive, since the length of the proteins also varies and one can expect a longer macromolecule to take longer time to organize itself into a particular 3-dimensional structure. However, studies have shown that the length of the proteins correlates only weakly with the protein folding rate [27]. This lead to the study of the topological complexity of the native state [5, 12, 14, 15, 26, 27]. In [26], a measure of topological complexity, the relative contact order, was introduced and showed a very strong correlation with protein folding rates. Moreover, a simpler measure, the number of contacts, which depicts the sequence distant monomers that are close in 3-space, provided an even stronger correlation with the protein folding rate. The number of sequence distant contacts is a global measure of structure which captures features beyond the secondary structure motifs. Its highly significant correlation with experimental folding rates shows the extent to which the topology of the
native state can influence the folding process. The number of contacts was also successfully used to provide a model for protein folding [12]. The underlying idea is that a high number of sequence distant contacts corresponds to few local interactions, suggesting that the route from the unfolded conformation to the native state is slow, being hindered by the overcoming of several barriers due to spatial restraints. However, it is still unclear what is the reason behind the change in the number of sequence distant contacts and whether it is a proxy for a more meaningful topological measure, which could help us improve our understanding of protein folding.

In the present study, we investigate how the topology of the native state can be further analyzed to help understanding protein folding. Looking at the sequence of CA atoms of the proteins as vertices connected by edges, we can represent the protein as a polygonal curve. The complexity of curves in space is studied in knot theory. A knot is a simple closed curve in space which does not intersect itself anywhere. We say that two knots are equivalent if we can deform one to the other without allowing crossings. Topological invariants are functions which, for any conformation of a knot in space give a number (or a polynomial) which is the same for all equivalent knots and is different for any pair of non-equivalent knots. The problem with using tools from knot theory to characterize protein structure is that proteins are linear chains. Then one has to deal with a statistical notion of knottedness which relies on closing the open chain and calculating the resulting knot type, with a spectrum of possible knot types arising from repeating this process over different closures [18]. This method revealed the existence of knots in proteins and it was extended by assigning a knot fingerprint to each protein, which depicts the knottedness of any part of the protein [17, 30, 31]. The knot fingerprints for the proteins whose native state is known can be found in KnotProt, a databank that provides a rich topological analysis of proteins [10].

However, it is still unknown how this information can be used in practice and if there is a connection of protein folding kinetics with the knot fingerprint of proteins. A measure of entanglement that can be directly applied to open chains and is a continuous function of the chain coordinates is the Gauss linking integral. This measure has been successfully applied to polymers in order to study polymer entanglement and it has been shown that it correlates with polymeric material properties [22–25]. The Gauss linking integral has been used to classify protein conformations successfully [3, 28] and it was shown that the maximum linking between any two parts of a proteins correlates with its folding rate [4]. In this study we further use the Gauss linking integral to reveal new characteristics of protein entanglement relevant to protein folding kinetics that can be useful in future models of protein folding. More precisely, we study the correlation between folding rate and the geometry/topology of the entire protein and also that of the secondary structures that compose it. We also examine if the number of contacts is related to our topological parameters.

The paper is organized as follows: in Section 2 we define the measures of geometrical/topological complexity we use to characterize protein structure and Section 3 presents our results.

2 Characterization of protein structure

2.1 The number of contacts

A way in which a straight conformation of a chain differs by a more complex conformation is that in the straight conformation, the 3-space distance of the $i$-th and $j$-th monomer of the molecule is $|i - j|$ times the bond length. This is not the case for any other conformation, where chains whose
sequential distance is $|i - j|$ may have been brought closer in 3-space.

Therefore, a way to measure the topological complexity of the native state of a protein is to measure how much this property deviates from that of the straight configuration, by accounting for the number of sequence distant contacts [26].

We say that two monomers form a contact if their real distance is less than 6 Å and their sequence distance greater than 12 amino acids. We denote by $Q_D$ the number of contacts in a protein. It has been shown that the number of contacts and measures related to that (the absolute and relative contact order) correlate well with the protein folding rate [5, 26]. Moreover, in [12] a model for protein folding, the topomer search model was introduced which showed a great correlation between the normalized number of contacts by the length of the proteins and $\log(k_f/Q_D)$. In this paper we will not provide a model for protein folding, but we will examine the correlations of several topological parameters with folding rates and their relation to the number of contacts.

2.2 Gauss linking number and the Total Torsion

A measure of the degree to which polymer chains interwind and attain complex configurations is given by the Gauss linking integral:

**Definition 2.1.** (Gauss Linking Number). The Gauss Linking Number of two disjoint (closed or open) oriented curves $l_1$ and $l_2$, whose arc-length parametrizations are $\gamma_1(t), \gamma_2(s)$ respectively, is defined as the following double integral over $l_1$ and $l_2$ [9]:

$$L(l_1, l_2) = \frac{1}{4\pi} \int_{[0,1]} \int_{[0,1]} \frac{\langle \dot{\gamma}_1(t), \dot{\gamma}_2(s), \gamma_1(t) - \gamma_2(s) \rangle}{||\gamma_1(t) - \gamma_2(s)||^3} dtds,$$

(1)

where $\langle \dot{\gamma}_1(t), \dot{\gamma}_2(s), \gamma_1(t) - \gamma_2(s) \rangle$ is the scalar triple product of $\dot{\gamma}_1(t), \dot{\gamma}_2(s)$ and $\gamma_1(t) - \gamma_2(s)$.

The Gauss Linking Number is a topological invariant for closed chains and a continuous function of the chain coordinates for open chains.

We also define a one chain measure for the degree of intertwining of the chain around itself:

**Definition 2.2.** (Writhe). For a curve $\ell$ with arc-length parameterization $\gamma(t)$ is the double integral over $\ell$:

$$W_{\ell}(\ell) = \frac{1}{4\pi} \int_{[0,1]} \int_{[0,1]} \frac{\langle \dot{\gamma}(t), \gamma(s), \gamma(t) - \gamma(s) \rangle}{||\gamma(t) - \gamma(s)||^3} dtds.$$

(2)

The Writhe is a continuous function of the chain coordinates for both open and closed chains. The Average Crossing Number (ACN) is obtained when we consider the absolute value of the integrand in the Writhe. ACN measures the expected number of crossings of a chain with itself in a random projection, while the writhe gives the expected number of signed crossings, which accounts for which strands come over and under. Similarly, the linking number of two chains, measures the expected number of signed inter-crossings of two arcs with each other accounting for which comes over and under in a random projection.

The total torsion of a chain, describes how much it deviates from being planar and is defined as:

**Definition 2.3.** The torsion of a curve $\ell$ with arc-length parameterization $\gamma(t)$ is the double integral over $\ell$:
\[ T(l) = \frac{1}{2\pi} \int_{[0,1]} \frac{(\gamma'(t) \times \gamma''(t)) \cdot \gamma'''(t)}{||\gamma'(t) \times \gamma''(t)||^2} \, dt. \] (3)

Notice that it is possible to construct a random walk with high writhe or torsion that does not contain a knot, however, the mean absolute value of the writhe and torsion increases with knot complexity [2,6,7,16,25]. In the same way, one can construct a configuration of a non-flat, polygonal chain with zero writhe. However, the writhe of a random walk is non-zero with probability one.

### 3 Results

We analyze a set of simple, single domain, non-disulfide-bonded proteins that have been reported to fold via two-state kinetics under at least some conditions [27]. These are 2-state proteins (as opposed to multi-state proteins), which means that these are proteins which fold directly to their native state and do not remain in other folding states (intermediates) for a long period of time. Despite their simplicity compared to other proteins, the slowest folding rate of the proteins in the set is a million times smaller than the largest folding rate in the same set. Therefore, this provides a homogeneous set with respect to folding behavior with varying folding rates, and could be such that geometrical and topological features important in protein folding may become apparent.

#### 3.1 The number of contacts

Figure 1 shows the folding rate as a function of the number of sequence-distant contacts, \( Q_D \), in a protein. We see that the number of contacts increases with decreasing folding rate. This suggests that more and more global interactions are required to form the native state. The correlation coefficient is \( R^2 = 0.619 \).

#### 3.2 The writhe and total torsion of the proteins

The set of proteins we analyze in this study do not contain knots or slipknots and their length is in the range of 50-150 amino acids. Therefore, we can expect the absolute value of the writhe of the proteins to be small [7,8,22,25].

Figure 2 shows the folding rate as a function of the writhe and torsion of the proteins. The writhe and the torsion take mostly positive values and are decreasing with decreasing folding rate with correlation coefficients \( R^2 = 0.473 \) and \( R^2 = 0.449 \), respectively. The values of the writhe exceed by a lot those of a random walk of that length.

At first the fact that the writhe and the torsion decrease with decreasing folding rate might seem counterintuitive, since one might expect the topological complexity to be increasing with decreasing folding rate. However, we notice that the writhe is a quantity that is influenced by the presence of secondary structures. Helices are right-handed and contribute a large, positive amount of writhe and \( \beta \)-strands have particular configurations that have a small in absolute value, but negative writhe (see Section 3.3). It is also known that the number of helices decreases with decreasing folding rate, while the number of \( \beta \)-strands increases [13]. Therefore, the observed behavior of the writhe and torsion of the proteins may be reflecting exactly this change in secondary structure. Notice that this change is local and may be hiding the underlying global topology/geometry of the protein.
Figure 1: The number of sequential distant contacts is increasing with decreasing folding rate suggesting the presence of more global organization.

Figure 2: The writhe and torsion of the proteins. We notice that the writhe and the torsion may be influenced by the number of helices which is decreasing with decreasing folding rate.
In order to extract information about the global geometry of a protein we propose to study the
conformation of its primitive path. We define the primitive path (PP) of the protein (inspired by
the tube model for polymer melts [29]) to be the axis of the thinnest tube that surrounds the chain,
with no self-intersections and whose diameter is that of a helix.

To compute the writhe of the PP in practice, we obtain a semi-analytical formula for the writhe
of the PP of the protein using the Gauss linking integral only, without constructing the tube.

First, we notice that the writhe of the protein can be expressed as:

$$ \text{Wr}(\text{protein}) = \sum_{i \in S} \text{Wr}(s_i) + \sum_{j \in \text{coils}} \text{Wr}(\text{coil } j) + \sum_{i,j \in S} \text{Lk}(s_i, s_j) + \sum_{i,j \in \text{coils}} \text{Lk}(\text{coil } i, \text{coil } j) $$

where \( S \) denotes the set of secondary structure elements of the protein.

The protein PP is formed by replacing the protein secondary structure elements by their axis,
giving \( \text{PP} = \{ e_i | e_i \text{ axis of secondary element } i \} \cup \{ \text{coils} \} \). Notice that \( \text{Wr}(e_i) = 0 \) for all axes \( e_i \).

Thus,

$$ \text{Wr}(\text{PP}) = \sum_{i,j \in S'} \text{Lk}(e_i, e_j) + \sum_{i,j \in S'} \text{Lk}(e_i, \text{coil } j) + \sum_{j \in \text{coils}} \text{Wr}(\text{coil } j) + \sum_{i,j \in \text{coils}} \text{Lk}(\text{coil } i, \text{coil } j) $$

where \( S' = \{ e_i | e_i \text{ axis of secondary element } i \} \).

We notice that, since the secondary structure elements lie in disjoint cells (disjoint convex
hulls) from coils and from each other and due to sign cancellations, we can make the following
approximations:

$$ \text{Lk}(e_i, \text{coil } j) \approx \text{Lk}(s_i, \text{coil } j) $$

and

$$ \text{Lk}(e_i, e_j) \approx \text{Lk}(s_i, s_j) $$

Using Eq. 6 and 7 in Eq. 5 and comparing it with Eq. 4, the writhe of the PP can be obtained
by the writhe of the protein and its secondary structure elements as:

$$ \text{Wr}(\text{PP}) \approx \text{Wr}(\text{protein}) - \sum_{\alpha - \text{helices}} \text{Wr}_\alpha - \sum_{\beta - \text{strands}} \text{Wr}_\beta $$

Similarly, we define the ACN and the torsion of the PP to be:

$$ \text{ACN}(\text{PP}) = \text{ACN}(\text{protein}) - \sum_{\alpha - \text{helices}} \text{ACN}_\alpha - \sum_{\beta - \text{strands}} \text{ACN}_\beta $$
Figure 3: Writhe and torsion of the primitive path. The writhe and the torsion of the PP of the protein change from positive to negative values with decreasing folding rate.

\[ T(PP) = T(\text{protein}) - \sum_{\alpha-\text{helices}} T_{\alpha-\text{helix}} - \sum_{\beta-\text{strands}} T_{\beta-\text{strand}} \]  

Figure 3 shows the folding rate as a function of the writhe and the torsion of the PP. The range of values is now comparable to those for random walks of that length. We see that the writhe and the torsion of the PP are decreasing with decreasing folding rate starting from positive values and attaining negative values with correlation coefficients \( R^2 = 0.483 \) and \( R^2 = 0.456 \), respectively. This reveals a significant change in the global conformation of proteins with folding rate: the proteins fold more slowly when they need to attain a configuration with negative global writhe and torsion. This may suggest a role of handedness of proteins not only in local organization but also in global structure.

Figure 4 shows the ACN of the protein and its PP. The ACN shows a small correlation with folding rate and its values are larger than those for similar length random walks [8]. This is expected due to the high ACN values of helices. The ACN of the PP reveals that the conformation of the PP remains large, even when the writhe is decreasing, confirming that the decrease of the writhe is not reflecting a decrease in complexity but a preference for left-handed global conformations with decreasing folding rate. This suggests that the left-handed conformations have lower energy but they are more difficult to attain [21].

3.3 The topology and geometry of secondary structures

It has been shown that proteins whose native state has only \( \alpha \)-helices fold faster than proteins who also have \( \beta \)-strands, while the proteins with only \( \beta \)-strands fold even slower [19,20]. Our results so far suggest that the folding rate is related to the global rearrangement of the secondary structure elements in space and not on the local structure of \( \alpha \)-helices and \( \beta \)-strands. In this section, we study how the different secondary structure elements (\( \alpha \)-helices, \( \beta \)-strands and coils) link with each other and how this correlates with the folding rate.

When examining the linking of \( \alpha \)-helices with coils in a protein we notice that the linking number is rather random in sign and we find only a small correlation with folding rate, \( R^2 = 0.077 \). However,
we find that the linking of a helix with the neighboring (in secondary structure) coils is mostly positive and it tends to decrease. The maximum absolute linking number relative to the length of the coil and helix involved is shown in Figure 5. We see that the maximum linking number of a helix with a neighboring coil is decreasing with decreasing folding rate, with $R^2 = 0.165$. The fact that it is positive shows a right-handed preference for the conformation helix-neighboring coil. The tendency of the maximum linking between helices and coils to decrease may indicate an increasing difficulty of the protein to keep right-handed conformations.

Figure 5 also shows the average linking number between helices in each protein of the dataset that contains at least two helices. The average linking number between helices becomes more and more negative with $R^2 = 0.556$, confirming our results in the previous section which suggest a change in the preferred handedness of the global linking of the protein.

Our results reveal a change in the sign of the linking between secondary structures with decreasing folding rate. Next, we examine if the number of occurrences of pairs of secondary structure elements which have negative linking correlates with the folding rate.

Figure 6 shows the folding rate as a function of the number of pairs of helices or pairs of helices-coils with negative linking number over the total number of pairs. Figure also 6 shows the number of contacts as a function of the number of pairs of helices and helices coils with negative linking. The results suggest that more contacts are required for increasing number of negative linking of helices with other helices and helices with coils and that the number of such negative linking pairs increases with folding rate.

The sign of the linking number between $\alpha$-helices and $\beta$-strands is rather random with $R^2 = 0.089$. However, the relative number of pairs of $\alpha$-helices and $\beta$-strands with negative linking is decreasing with decreasing folding rate with $R^2 = 0.195$ (see Figure 7). We notice a large set of proteins with exactly 0.5 ratio of pairs of helices-strands with negative linking over the total number of pairs. This is because of the particular conformations of sheets, with antiparallel $\beta$-strands, which contribute an opposite sign to the average.

Our results suggest a dependence of the folding rate on the number of negative linking occurrences involving secondary structure elements. Since the secondary structure elements follow an almost
Figure 5: *Left:* The folding rate as a function of the maximum absolute linking between helices with neighboring coils. *Right:* The folding rate as a function of the average linking number of helices in a protein. The linking number is decreasing suggesting that the proteins fold slowly when a left-handed conformation of helices is required.

Figure 6: The folding rate and the number of contacts as a function of the relative number of pairs of α-helices or pairs of α-helices and coils with negative linking number.
Figure 7: The folding rate and the number of contacts as a function of the relative number of pairs of α-helices and β-strands with negative linking number. The 0.5 ratios indicate the presence of antiparallel β-strands which contribute a negative and positive linking number.

straight axis, the source of change in their relative orientation stems from the coils in-between. Indeed, the coils can attain any random configuration. Out results however show that the writhe of the coils is on average positive. However, negative writhe coils also exist and our results suggest that the number of coils with negative writhe over the number of all coils in a protein is increasing as the folding rate decreases. In Figure 8 we see that the number of coils with negative writhe and the number of coils with negative torsion over the total number of coils in a protein is increasing with decreasing folding rate with $R^2 = 0.138$.

Figure 8 shows also the number of contacts as a function of the number of coils with negative writhe and as a function of coils with negative torsion. The results suggest that more contacts are required for increasing number of negative writhe and negative torsion coils. However, the results show that the folding rate is more sensitive to that number than the number of contacts. Therefore, this may be a feature that is only weakly captured by the number of contacts but influences the folding rate.

4 Conclusions

Studies have shown that the number of sequentially distant contacts correlates very well with protein folding rates [12]. By studying a set of two-state proteins which do not contain knots, we showed that their writhe, the ACN and torsion of the proteins correlates with their folding rate and with their number of sequence distant contacts. By examining the global entanglement of the proteins, ignoring the local secondary structure, we showed that the global writhe and torsion of the protein becomes more and more negative. We saw that the folding rate is related to the relative orientation of helices and strands in space and that negative linking conformations require more sequence distant contacts. We also see that coils with negative writhe or torsion slow down the folding process even if they do not require many contacts. Our results confirm that the highly organized native structure is too complex to be captured by a single parameter, and suggest that the combination of the topological and geometrical tools, such as the number of sequence-distant...
Figure 8: The folding rate and the number of contacts as a function of the relative number of coils with negative writhe (above) and torsion (below). The folding rate is related to the number of coils with negative torsion, even if their presence does not correlate with the number of contacts.
contacts and the Gauss linking integral, could provide complementary information useful in the search of a model for protein folding.

References

[1] B Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. *Molecular Biology of the Cell*. New York: Garland Science, 2002.

[2] J. Arsuaga, T. Blackstone, Y. Diao, E. Karadayi, and M. Saito. The linking of uniform random polygons in confined spaces. *J. Phys. A: Math. Theor.*, 40:1925–36, 2007.

[3] G. A. Arteca and O. Tapia. Relative measure of geometrical entanglement to study folding-unfolding transitions. *Int. J. Quantum Chem.*, 80:848–855, 2000.

[4] M. Baiesi, E. Orlandini, F. Seno, and A. Trovato. Exploring the correlation between the folding rates of proteins and the entanglement of their native state. *J. Phys. A: Math. Theor.*, 50:504001, 2017.

[5] A. Broom, S. Gosavi, and E. A. Meiering. Protein unfolding rates correlate as strongly as folding rates with native structure. *Prot. Sci.*, 24:580–7, 2015.

[6] Y. Diao, A. Dobay, and A. Stasiak. The average inter-crossing number of equilateral random walks and polygons. *J. Phys. A: Math. Gen.*, 38:7601–7616, 2005.

[7] Y. Diao, C. Ernst, K. Hinson, and U. Ziegler. The mean-squared writh of alternating random knot diagrams. *J. Phys. A: Math. Theor.*, 43:495202, 2010.

[8] Y. Diao, R. N. Kushner, K. C. Millett, and A. Stasiak. The average crossing number of equilateral random polygons. *J. Phys. A: Math. Gen.*, 36:11561–11574, 2003.

[9] K. F. Gauss. *Werke*. Kgl. Gesellsch. Wiss. Göttingen, 1877.

[10] M. Jamroz, W. Niemyska, E. Rawdon, A. Stasiak, K. C. Millett, P. Sulkowski, and J. I. Sulkowska. Knotprot: a database of proteins with knots and slipknots. *Nuc. Ac. R.*, 43:D306–D314, 2015.

[11] C. Lawrence, J. Kuge, K. Ahmad, and K. W. Plaxco. Investigation of an anomalously accelerating substitution in the folding of a prototypical two-state protein. *J. Mol. Biol.*, 403:446–458, 2010.

[12] D. E. Makarov and K. W. Plaxco. The topomer search model: a simple, quantitative theory of two-state protein folding kinetics. *Protein Science, 12*:17–26, 2003.

[13] S. Malik, T. Ray, and S. Kundu. Transiently disordered tails accelerate folding of globular proteins. *FEBS Lett*, 591:2180–2191, 2017.
[14] K. L. Maxwell, D. Wildes, A. Zarrine-Afsar, M. A. De Los Rios, A. G. Brown, C. T. Friel, L. Hedberg, J. Horng, D. Bona, E. J. Miller, A. Vallée-Bélisle, E. R. G. Main, F. Bemporad, L. Qiu, K. Teilum, N. Vu, A. M. Edwards, I. Ruczinski, F. M. Poulsen, B. B. Kragelund, S. W. Michnick, F. Chiti, Y. Bai, S. J. Hagen, L. Serrano, M. Oliveberg, D. P. Raleigh, P. Wittung-Stafshede, S. E. Radford, S. Jackson, T. R. Sosnick, S. Marqusee, A. R. Davidson, and K. Plaxco. Protein folding: defining a “standard” set of experimental conditions and a preliminary kinetic data set of two-state proteins. *Protein Science*, 14:602–616, 2005.

[15] C. Micheletti. Prediction of folding rates and transition-state placement from native-state geometry. *Proteins*, 51:74–84, 2003.

[16] K. C. Millett, A. Dobay, and A. Stasiak. Linear random knots and their scaling behavior. *Macromolecules*, 38:601–606, 2005.

[17] K. C. Millett, A. S. Rawdon, and J. I. Sulkowska. Identifying knots in proteins. *Biochem. Soc. Trans.*, 41:533–537, 2013.

[18] K. C. Millett and B. Sheldon. Tying down open knots. *Series of Knots and Everything*, 36:203–217, 2005.

[19] V. Munoz and M. Cerminara. When fast is better: protein folding fundamentals and mechanisms from ultrafast approaches. *Biochem. J.*, 473:2545–2559, 2016.

[20] H. Nguyen, M. Jäger, J. W. Kelly, and M. Gruebele. Engineering a β-sheet toward the folding speed limit. *J. Phys. Chem. B Lett.*, 109:15182–15186, 2005.

[21] J. N. Onuchic, P. G. Wolynes, Z. Luthey-Schulten, and N. D. Socci. Toward an outline of the topography of a realistic protein-folding funnel. *PNAS*, 92:3626–3630, 1995.

[22] E. Panagiotou and M. Kröger. Pulling-force-induced elongation and alignment effects on entanglement and knotting characteristics of linear polymers in a melt. *Phys. Rev. E*, 90:042602, 2014.

[23] E. Panagiotou, M. Kröger, and K. C. Millett. Writhe and mutual entanglement combine to give the entanglement length. *Phys. Rev. E*, 88:062604, 2013.

[24] E. Panagiotou, K. C. Millett, and P. Atzberger. Topological methods for polymeric materials: Characterizing the relationship between polymer entanglement and viscoelasticity. (submitted), 2017.

[25] E. Panagiotou, K. C. Millett, and S. Lambropoulou. The linking number and the writhe of uniform random walks and polygons in confined space. *J. Phys. A*, 43:045208–30, 2010.

[26] K. W. Plaxco, K. T. Simons, and D. Baker. Contact order, transition state placement and the refolding rates of single domain proteins. *J. Mol. Biol.*, 277:985–994, 1998.

[27] K. W. Plaxco, K. T. Simons, I. Ruczinski, and D. Baker. Topology, stability, sequence and length: defining the determinants of two-state protein folding kinetics. *Biochemistry*, 39:11177–11183, 2000.
[28] P. Rogen and B. Fain. Automatic classification of protein structure by using gauss integrals. *Proc. Natl Acad. Sci.*, 100:119–24, 2003.

[29] M. Rubinstein and R. Colby. *Polymer Physics*. Oxford University Press, 2003.

[30] J. I. Sulkowska, J. K. Noel, C. A. Ramirez-Sarmiento, E. J. Rawdon, K. C. Millett, and J. N. Onuchic. Knotting pathways in proteins. *Biochem. Soc. Trans.*, 41:523–527, 2013.

[31] J. I. Sulkowska, E. J. Rawdon, K. C. Millett, J. N. Onuchic, and A. Stasiak. Conservation of complex knotting and slpiknotting in patterns in proteins. *PNAS*, 109:E1715, 2012.