Knots And Swelling in Protein Folding

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Abstract

Proteins can sometimes be knotted, and for many reasons the study of knotted proteins is rapidly becoming very important. For example, it has been proposed that a knot increases the stability of a protein. Knots may also alter enzymatic activities and enhance binding. Moreover, knotted proteins may even have some substantial biomedical significance in relation to illnesses such as Parkinson’s disease. But to a large extent the biological role of knots remains a conundrum. In particular, there is no explanation why knotted proteins are so scarce. Here we argue that knots are relatively rare because they tend to cause swelling in proteins that are too short, and presently short proteins are over-represented in the Protein Data Bank (PDB). Using Monte Carlo simulations we predict that the figure-8 knot leads to the most compact protein configuration when the number of amino acids is in the range of 200 – 600. For the existence of the simplest knot, the trefoil, we estimate a theoretical upper bound of 300 – 400 amino acids, in line with the available PDB data.
1. INTRODUCTION

There are currently only some 300 known knotted proteins [1]-[7] that are listed in the Protein Data Bank PDB [8]. Furthermore, most of them correspond to the same protein but in a different species. Alternatively, they appear in multiple domain proteins, and often with the same knot repeated in each of the domains. When we only consider proteins in a single domain, there are no more than 17 different known knotted proteins [6]. With one single exception of a figure-8 knot, these are all trefoil knots with the value of central carbons $N$ in the range of $N \sim 82 - 380$. Even though there are examples of other knots such as the twist-3 knot, these have only been found in multiple domain proteins [6]. In the present article our goal is to identify some universal characteristics of knotted proteins and to try and employ these to make predictions on the existence of knots. In particular, we look for explanations why knots are so rare in the PDB data.

Biologically active proteins are compact objects, and one might expect that compactness is in some (yet unknown) manner important for their function. Consequently we propose that a study of the relationship between knottedness and compactness could help to understand why knotted proteins are rare. Compactness can be measured by the Hausdorff dimension $d_H$ of the protein backbone, that can be determined from the scaling properties of the radius of gyration $R_g$ [9]. In the limit where the number of central carbon atoms $N$ becomes very large $R_g$ obeys the scaling law

$$R_g = \frac{1}{N+1} \sqrt{\frac{1}{2} \sum_{i,j} (r_i - r_j)^2} \propto L \cdot N^{1/d_H}$$

(1.1)

with $r_i (i = 1, 2, ..., N)$ the space coordinates of the central carbons. Here $L$ is a dimensionfull swelling factor that sets the scale for the size of the protein, and the inverse Hausdorff dimension $\nu = 1/d_H$ is called the compactness index. The swelling factor $L$ is not a universal quantity. But $\nu$ is universal: Different values of $\nu$ characterize different universality classes (phases) of proteins. Biologically active proteins that have
2. ANALYSIS OF PDB DATA

In Figure 1 we display the radius of gyration for the 17 known single domain knotted proteins that are presently listed in PDB, versus the number of their central carbons \( N \). When we perform a least square linear fit to this data we find that the result is distorted by the shortest known knotted protein, the 2efv for which \( N=82 \) [8]. In order to have a meaningful fit we proceed by leaving out 2efv. We also leave out 1ztu since it is (the only known) figure-8 protein.

We are then left with the 15 trefoil proteins presently in PDB, and for these \( N \) is in the range of \( N \in [147, 380] \). For these trefoils we obtain the following least square linear

\[
\text{Radius of gyration } R_g \text{ versus } N \text{ for the 15 single domain knotted proteins in PDB, as described in the text.}
\]

\( N \) in the range of \( 100 \leq N \leq 1.000 \) obey the scaling law (1.1) with \( \nu \approx 0.378 \) [10]. This is very close to the value \( \nu = 1/3 \) that determines the universality class of fully collapsed protein (solid matter), the difference is presumably due to some yet to be understood finite scaling effects [11].
fit for the radius of gyration,

\[ R_{\text{trefoil}}^g \sim L \cdot N^{\nu} \sim (2.499 \pm 0.661) \cdot N^{0.361 \pm 0.120} \]  

(2.1)

We wish to compare this to the least square linear fit of \( R_g \) to all proteins presently in PDB. However, in order to ensure compatibility with (2.1), we choose only those proteins for which \( N > 125 \). For these we get

\[ R_{\text{all}}^g \sim L \cdot N^{\nu} \sim (2.142 \pm 0.03) \cdot N^{0.387 \pm 0.003} \]  

(2.2)

but we note that if we include all proteins in PDB we obtain the estimate

\[ R_{\text{all}}^g \sim L \cdot N^{\nu} \sim (2.254 \pm 0.021) \cdot N^{0.378 \pm 0.002} \]

Despite slight differences in the numerical values which is due to finite scaling effects, these two fits for \( R_{\text{all}}^g \) are very close to each other over the range of interest \( N \in [125, 400] \).

In our analysis we shall use both of them, and in this way we hope to control finite scaling corrections that are due to proteins with small values of \( N \).

Since the quality of data that underlies (2.1) is poor, one should be careful in drawing conclusions and with this in our mind we observe the following:

Unknotted proteins have a clearly smaller value of \( L \) than trefoils. Thus for small \( N \) the unknotted proteins have a tendency to be more compact (smaller) than trefoil proteins. When size matters this could explain why there are so few trefoils for small values of \( N \) in the PDB data: The trefoil knot causes increased swelling in small proteins.

But when \( N \) grows, the swelling caused by the trefoil knot starts to diminish. When \( N \) reaches a value \( N_c \approx 400 - 500 \) i.e. close to the upper bound \( N = 400 \) of our range, comparison between (2.1) and (2.2) predicts that the trefoil proteins become equally compact than the unknotted ones. (The exact value of \( N_c \) varies slightly depending on how we account for the finite scaling effects due to short proteins.) For \( N > N_c \approx 400 - 500 \) our data for trefoil proteins is unreliable. But if the tendency continues
FIG. 2: Probability distributions of the generalized gamma form $p(N) \propto N^a \exp(-bN^c)$, fitted to the number density of all proteins (red line) and single domain trefoil proteins (blue line); The latter are displayed in Figure 1.

there should be a range of values $N$ above $N_c$ where the presence of a trefoil improves compactness over proteins without knots. In this range we expect that the relative number of trefoil proteins increases.

Note that asymptotically, for very large values of $N$, the scaling law (1.1) should be insensitive to the presence of a single localized knot.

In order to verify the reasonableness of the previous conclusion and in particular whether there are additional reasons, we consider Figure 2 where we display the (probability) density function for the number of proteins in PDB as a function of the length $N$, both for all proteins and for the single domain trefoil proteins. For all proteins the probability density peaks at around $N = 90$ while for trefoil proteins the peak is near $N = 250$ where the total number of resolved protein structures in PDB is already relatively small. In particular, we observe that there are relatively few resolved protein structures with $N$ larger than $N_c \approx 400 - 500$ where our estimates predict that trefoil proteins might become less swollen than unknotted ones. Consequently the small number of trefoil knots could be partly due to the scarcity of data in PDB. This is in line
with our observation on the behaviour of (2.1), (2.2) for \( N > 400 \).

In addition, since the probability density of trefoils is sharply peaked within the range \( N \approx 150 - 400 \) a partial explanation could also be that there is some yet unknown reason why proteins with trefoil knots prefer these values of central carbons.

3. THEORETICAL ESTIMATES

In order to better understand the effect of knots on protein swelling, and in particular to clarify whether the presence of trefoil knots tends to decrease or increase the compactness of proteins when \( N > 400 \) we have theoretically investigated how different knots influence the radius of gyration of native state proteins when \( N \) is within the range \( 125 \leq N \leq 800 \). For this we have employed the Landau-Ginsburg model of protein folding that we have described in [10]. Since the model provides a good description of the universal aspects of protein folding in particular for the mostly-\( \alpha \) and \( \alpha/\beta \) family of proteins that have been found to support knots [6], we expect it to have good predictive power on the effects of knots on protein swelling.

We have performed extensive Monte Carlo simulations with two different sets of knotted proteins. We summarize the results in Table 1. The first set consists of chains that have either one, three or five distinct trefoil knots along the backbone. The second set consists of chains where the proteins have either the trefoil knot \((3_1)\), the figure-8 knot \((4_1)\), or the twist-3 knot \((5_2)\) along their backbone. The simulations have been performed by selecting up to 10 different values of \( N \) in the ranges displayed in Table 1, with the exact values depending on the knot complexity (see Table 1), and then performing around 80 independent runs at each of these value \( N \) to compute the radius of gyration; See Figure 3. The initial configuration is a (relatively tight) knot located deep inside the protein structure. In each of the cases we find that the least square linear
FIG. 3: Least square linear fits of $R_g$ for the trefoil ($3_1$), figure-8 ($4_1$) and twist-3 ($5_2$) proteins.

fit of (1.1) provides an excellent match for the data, we have not been able to identify any kind of systematic nonlinear corrections. But in order to eliminate the influence of finite scaling effects that are due to short unknotted backbones, following (2.1), (2.2) we compare the knotted proteins to unknotted ones using a set of different ranges of values $N$ for the latter. These are given in Table 2.

We observe from Table 2 that the small $N$ finite scaling corrections tend to systematically decrease the value of $L$ and increase the value of $\nu$. Already for the range $250 < N < 1,000$ we find that $\nu$ is very close to its theoretical value $\nu = 1/3$ corresponding to totally collapsed proteins.

In the case of the first set we compare the unknotted proteins with proteins that have either one, three or five trefoil knots along their backbone.

For proteins with a single trefoil ($3_1$), we find that there is a tendency for long trefoil proteins to be more swollen than the unknotted ones. We estimate that a transition occurs at around $N_c \approx 300$, and for $N < N_c$ our simulations predict that trefoils are (slightly) more compact than unknots. Since we estimate that there is a minimum length of about 50-70 central carbons for a trefoil knot to form, our simulations suggest
| knot type | $L$ | $\nu$ | $\Delta L$ | $\Delta \nu$ | $N_{knot}$ | $N_{range}$ |
|-----------|-----|------|-----------|------------|-----------|-----------|
| single $3_1$ | 2.719 | 0.378 | $\pm 0.072$ | $\pm 0.013$ | 50-70 | 125-550 |
| three $3_1$ | 2.552 | 0.395 | $\pm 0.109$ | $\pm 0.019$ | 225-550 |
| five $3_1$ | 2.500 | 0.403 | $\pm 0.167$ | $\pm 0.027$ | 350-800 |
| $4_1$ | 2.729 | 0.373 | $\pm 0.109$ | $\pm 0.018$ | 70-100 | 225-625 |
| $5_2$ | 2.764 | 0.372 | $\pm 0.084$ | $\pm 0.014$ | 90-110 | 225-625 |

TABLE I: Swelling factor $L$ and compactness index $\nu$, with corresponding standard errors $\Delta L$ and $\Delta \nu$ and an estimate for the average length of knots in the number of central carbons $N_{knot}$, for different knot types simulated using the model described in [10]. The last column gives the range of values of $N$ for which the simulations have been performed. The lower bound is selected to accommodate a deep knot.

that deep trefoil knots should predominantly be present for values $N \approx 100 – 300$. This conclusion is in line with the PDB data in Figure 1 over its range of validity, and consistent with the probability density displayed in Figure 2.

We find that the presence of several trefoils along the backbone clearly increases swelling for all values of $N$ we have studied. Consequently our simulations suggest that these configurations should be very rare. Indeed, in PDB data multiple trefoil knots have until now only been observed in multiple domain proteins, with only a single knot in the independent domains.

In the case of our second set we compare the unknotted proteins to proteins with two more complex knots, the figure-8 ($4_1$) knot and the twist-3 ($5_2$) knot along the protein backbone.
TABLE II: Least square linear fits for $L$ and $\nu$ with standard errors $\Delta L$ and $\Delta \nu$, computed for unknotted proteins with varying range for $N$.

| range of $N$ | $L$ | $\nu$ | $\Delta L$ | $\Delta \nu$ |
|-------------|-----|-------|------------|------------|
| $75 < N < 1.000$ | 2.656 | 0.379 | $\pm 0.049$ | $\pm 0.008$ |
| $125 < N < 1.000$ | 3.103 | 0.353 | $\pm 0.079$ | $\pm 0.013$ |
| $175 < N < 1.000$ | 3.411 | 0.338 | $\pm 0.093$ | $\pm 0.015$ |
| $250 < N < 1.000$ | 3.522 | 0.333 | $\pm 0.128$ | $\pm 0.02$ |

For the figure-8 knot in our range of $N$ we find that the ensuing proteins are slightly more compact than the unknotted ones. We find an upper bound $N_c \approx 600$ beyond which the figure-8 proteins start to become more swollen than the unknotted ones. Since we estimate that the lower length of a figure-8 knot is close to 100 central carbons, we propose that these knots should be present in PDB data as deep knots, predominantly with $N$ in the range between 150 and 600. Thus far only one has been found, 1ztu with $N = 320$ [6]. But as visible in Figure 2 there are also relatively very few protein structures that have been resolved within this range of $N$.

In the case of the twist-3 knot, we find that proteins with this knot are slightly more swollen than the figure-8 knots. But they appear more compact than unknots at least until $N$ reaches a value $N_c \approx 500$ beyond which they appear to swell more than unknots. Furthermore, our estimates suggest that there is a lower bound at around $N \approx 300$ below which these protein knots begin to be more swollen than unknots. However, this estimate is to some extent plagued by finite scaling effects due to the presence of short unknotted proteins in the analysis. We note that the two known $5_2$ knots [3] appear in multiple domain proteins, one (1xd3) in a domain with $N = 228$ and the other (2etl) in a domain
with $N = 223$ central carbons.

Finally, in the theoretically important $N \to \infty$ limit the effect of a (localized) knot to the scaling law (1.1) must vanish: Thus the presence of a knot leads to an intricate non-linear finite scaling effect that remains to be understood in detail.

4. CONCLUSIONS

In summary, knots are examples of topologically nontrivial structures in proteins with potentially very high biomedical relevance. However, until now knots have been identified in only relatively few single domain proteins. In order to understand the reason we have analysed the data in PDB to conclude, that in the case of short proteins the (trefoil) knots have a tendency to increase the swelling of the folded protein backbone. But when the backbone length increases, the swelling due to trefoil seems to decrease and from the PDB data we estimate that for backbones with $N \approx 400 - 500$ central carbons the swelling due to the trefoil disappears. The reason why knots are so rare in PDB data would then be partially explained by the fact that until now the structure of only relatively short proteins have been reliably resolved.

But in addition, it could be that for some reason trefoil knots only appear for $N \approx 100 - 400$. In order to resolve this and clarify what effect knots have on protein swelling, we have performed extensive Monte-Carlo simulations using a Landau-Ginsburg model of protein folding. We find that for trefoil knots the swelling is minimal when $N$ is within the range of $100 - 300$. But in contrast to the interpolated PDB data, beyond $N_c \approx 300$ we predict that trefoils tend to increase swelling. This prediction is consistent with Figure 2 that shows a clear peak of trefoils near $N \approx 250$. In the case of multiple trefoil knots, we find increased swelling in all cases and consequently these configurations should remain quite rare unless the protein chains become much longer.
But when we increase the knot complexity, we find that it leads to an improved compactness for a range of values of $N$. We note that by using a very different method, the authors of [12] arrived at a very similar conclusion. The effect appears to be most profound for figure-8 knot for which we predict an eventual relative increase in their number in PDB data as long as $N$ is less than $\sim 600$ but large enough to support a deep knot. For twist-3 we predict an eventual relative increase in their number in PDB data, until $N$ reaches a value $\sim 500$. However, since the twist-3 appears to be (slightly) more swollen than figure-8, it should remain more rare. Since the numerical differences in swelling that are revealed in our simulations are quite small and our conclusions are based more on tendencies than clear numerical differences, the effects of various biological and evolutionary factors may eventually turn out to be more dominant than swelling. More exhaustive numerical simulations that in particular take into account the detailed amino acid structures of the proteins are thus needed before more detailed predictions on swelling can be made. Furthermore, it remains a theoretical challenge to understand the universal structure of finite scaling corrections to the radius of gyration in the case of collapsed protein chains.

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