Contact order revisited: the importance of being entangled

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Abstract. It is well known that the folding time of a protein correlates with the contact order, a simple measure of the spatial organization of a protein. This is related to the average length of the main chain loops formed by amino acids which are in contact in the native state. Here we argue that folding kinetics can be influenced also by the entanglement that loops may undergo within the overall three dimensional protein structure. In order to explore such possibility, here we introduce a novel descriptor, which we call “maximum intrachain contact entanglement”. Specifically, we measure the maximum Gaussian entanglement between any looped portion of a protein and any other non-overlapping subchain of the same protein, which is easily computed by discretized line integrals on the coordinates of the $C_\alpha$ atoms. By analyzing experimental data sets of two-state and multistate folders, we show that also the new index is a good predictor of the folding rate. Moreover, being only partially correlated with previous methods, it can be integrated with them to yield more accurate predictions.

PACS numbers:

Submitted to: J. Phys. A: Math. Gen.
Keywords: Protein native structure, folding rates, topology, linking number
1. Introduction

Simple paradigms very often play an invaluable role to help understanding complex systems. A well known example is given by protein folding, which is complex because of the sheer size of protein molecules, the twenty types of constituent amino acids with distinct side chains, and the essential role played by the environment. Nevertheless, it is by now widely accepted that several aspects of the process driving a sequence of amino-acids to the corresponding native structure can be inferred by simple descriptors of the native-state geometry \[1, 2\]. For instance, it has been shown that the folding nucleus of a protein, including the residues whose interactions are essential for the folding to the native state, can be predicted through simulations of homopolymer models based on the mere knowledge of the native contact map, that is of the whole list of residue pairs in contact with each other \[3, 4, 5, 6\]. Another evidence of this simplicity is the ability of effective energy scores, derived by a statistical analysis of folded protein structures, to discriminate real native states among set of competing decoys \[7, 8, 9, 10, 11\]. A further example is the finding that the universe of possible proteins folds can be derived by simple coarse-grained models of polymers, which capture few universal properties typical of all amino-acids \[12, 13, 14\].

Possibly, the most striking and surprising evidence of the emerging simplicity in protein folding is the result of Plaxco and coworkers \[15, 16\]. They found that the experimental folding rates of proteins, e.g. the inverse folding times, correlate with simple descriptors of the native state organisation, such as the contact order; that is, the average chemical length (in terms of the number of amino-acids) of the loop formed between residues which are in contact. In later studies, almost equivalent correlations have been found also for similar descriptors of the native state organization such as the long range order \[17\], the number of native contacts \[18, 19\], the total contact distance \[20\], the cliquishness \[21\], the local secondary structure content \[22\] and the chain crosslinks contact order \[23\].

The contact order and all its possible variants are descriptors that focus on the network of pairs of residues that are nearby in space. One can however think of non-local descriptors that capture the degree of self-entanglement of the whole protein backbone, seen as a curve in a three dimensional space. An example is the writhing number, a measure of how a curve winds around itself in space \[24\]. In protein physics, the writhing number was first used in \[25\] to quantify the amount of self-threading in native state and it was later extended to perform a systematic classification of existing protein folds \[26\, 27\].

After the seminal observation that the backbone of a protein may self entangle into physical knots \[28, 29, 30, 31, 32, 33, 34, 35\], a growing attention has been devoted to find new, topologically inspired, descriptors for quantifying accurately the winding of a protein with itself or with other molecules. Specific descriptors have been proposed to measure the amount and location of mutual entanglement between protein complexes \[36, 39, 38, 37\] or to detect specific topological knots, links and lassos within
a single chain \cite{39, 40, 41}.

The aim of this study is to explore the correlation between protein folding rates and a novel topological descriptor of proteins three dimensional entanglement, which we name maximum intrachain contact entanglement. This indicator is the maximum value of the mutual entanglement measured between any looped portion of a protein and any other non-overlapping subchain extracted from the same protein. As a measure of the mutual entanglement, we consider the Gaussian double integral of two oriented curves. For closed curves this measure reduces to the Gauss linking number, a topological invariant that quantifies how pairs of loops are (homologically) linked \cite{42}. Being quite easy to compute, the Gauss linking number has been extensively used in the past to characterise the mutual entanglement of diluted and concentrated solutions of linear polymers \cite{43, 44, 45, 46}, to estimate the linking probability and link complexity of pair of loops under geometrical constraints \cite{47, 48, 49, 50} as well as to identify threadings in dense solutions of unlinked loops diffusing in a gel \cite{51}.

By exploring a data set of 48 proteins \cite{21, 23, 52} for which the folding time is known experimentally, we compute the linear correlation coefficient between the values of our descriptor and the experimental folding rates. We show that the maximum intrachain contact entanglement captures aspects that are different from those highlighted by the contact order, and we describe how the two descriptors can be combined to improve the predictions of folding rates.

2. Methods

2.1. A topologically inspired descriptor

It is well known that the Gauss double integral

\[ G \equiv \frac{1}{4\pi} \oint_{\gamma_1} \oint_{\gamma_2} \frac{\mathbf{r}^{(1)} - \mathbf{r}^{(2)}}{|\mathbf{r}^{(1)} - \mathbf{r}^{(2)}|^3} \cdot (d\mathbf{r}^{(1)} \times d\mathbf{r}^{(2)}) \]  

between two closed curves \( \gamma_1 \) and \( \gamma_2 \) in \( \mathbb{R}^3 \) gives an integer number, known as the linking number, whose value is a topological invariant. A nice feature of this measure, however, is that it provides a meaningful assessment of the mutual entanglement also if either one or both curves are open \cite{43, 46, 36}.

Our strategy here is to consider as \( \gamma_1 \) and \( \gamma_2 \) any pair \( (\gamma_i, \gamma_j) \) of non-overlapping subchains extracted from the same protein backbone and to compute their Gaussian entanglement \( G_{ij} \). Since the backbone of a native protein structure with \( N \) residues can be described as a discrete chain of monomers \( (i = 1, \ldots, N) \) placed at the positions \( \mathbf{r}_i \) of the \( \text{C}_\alpha \) atoms, it is natural to define the average positions

\[ \mathbf{R}_i \equiv \frac{1}{2}(\mathbf{r}_i + \mathbf{r}_{i+1}), \]  

and the bond vectors

\[ d\mathbf{R}_i = \mathbf{r}_{i+1} - \mathbf{r}_i. \]
Figure 1. Sketch of the procedure we use for computing $G'_{ij}$. First, a looped portion $\gamma_i$ ($i_1 \rightarrow i_2$, red) is identified when $C_{\alpha}$ coordinates of amino acids $i_1$ and $i_2$ (yellow ovals) are closer than $d = 9\text{Å}$. Then, the double sum (4) is computed for any other portion $\gamma_j$ ($j_1 \rightarrow j_2$, blue) preceding or following $\gamma_i$.

Figure 2. Following the color code of figure 1 examples of protein native structures in which we identified the subchains $\gamma_i$ ($i_1 \rightarrow i_2$, yellow-red-yellow) and $\gamma_j$ ($j_1 \rightarrow j_2$, blue) yielding the maximum intrachain contact entanglement.

Hence, for a given subchain $\gamma_i$ with monomers from index $i_1$ to $i_2$, and a subchain $\gamma_j$ with monomers from index $j_1$ to $j_2$ such that $\gamma_j \cap \gamma_i = \emptyset$, their Gaussian entanglement is given by

$$G'_{ij} = \frac{1}{4\pi} \sum_{i=i_1}^{i_2-1} \sum_{j=j_1}^{j_2-1} \frac{R_i - R_j}{|R_i - R_j|^3} \cdot (dR_i \times dR_j)$$  \hspace{1cm} (4)$$

where the prime in $G'$ highlights the fact that the measure is for open chains. Note that, unlike in our previous study [36] where the entanglement was estimated between two different protein backbones, here the pairs of subchains $(\gamma_i, \gamma_j)$ are extracted from the same protein backbone.

The definition (4) is rather generic and can be applied to any pair $(\gamma_i, \gamma_j)$ of not overlapping portions of the protein backbone. Here we specialize the analysis to the subset of $(\gamma_i, \gamma_j)$ where the subchain $\gamma_i$ has its first ($i_1$) and last ($i_2$) residues forming a contact ($i_1 \div i_2$), i.e. when $|r_{i_1} - r_{i_2}| < d$, with $d = 9\text{Å}$. With this restriction $\gamma_i$ is essentially a loop. The same restriction is not applied to $\gamma_j$, which can either precede or follow $\gamma_i$ along the protein backbone. This choice is sketched in figure 1.

Configurations as in figure 1 are assumed to constitute bottlenecks in the folding dynamics when the mutual entanglement between $\gamma_i$ and $\gamma_j$ is considerable, being intimately connected with the formation of the native contact, $i_1 \div i_2$. The way of
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choosing these configurations is similar to that leading to the definition of “lassos” [11], although here we do not restrict the contacts to chemically strong bonds as in cystein pairs.

We make the ansatz that the maximum of the mutual entanglement, among all possible pairs \((\gamma_i, \gamma_j)\) having the lasso structure discussed above, should positively correlate with the maximum relaxation time to that native state. To quantify this largest mutual entanglement, we pick up the combination of \((\gamma_i, \gamma_j)\) which gives the largest absolute value,

\[
|G'(c)| = \max_{[i_1, i_2], [j_1, j_2]} |G'_{ij}|. \tag{5}
\]

The index “c” indicates that this maximum intrachain contact entanglement is subject to the loop constraint of having the ends of \(\gamma_i\) in contact with each other, \(i_1 \div i_2\).

With the same definition of contact between non consecutive residues we can introduce the absolute contact order (ACO). This is the average chemical distance \(|j - i|\) between monomers in contact. Supposing that there are \(n_c\) of these contacts in the native state of a protein, we have

\[
ACO \equiv \frac{1}{n_c} \sum_{i+j} |j - i| \tag{6}
\]

The relative contact order (RCO) is simply the ACO divided by the chain length \(N\), which is the average of normalized chemical distances \(|j - i|/N\) of residues in contact [15].

2.2. Data sets

We use two separate data sets. A first data set for two-state folders includes single-domain, non-disulfide-bonded proteins that have been reported to fold via two-state kinetics under at least some conditions [53]. We use folding rates as reported previously [23, 53], see table 1.

The second data set, for multistate folders, is summarised in table 2 and includes proteins that exhibit one or more folding intermediates in water, the entries 34-57 in table 1 from Ref. [52]. We use folding rates from that table with two exceptions. For 1ra9, we use the folding rate reported instead in Refs. [54, 55]. We then removed 1cbi and 1ifc from the data set, as they are both homologous to 1opa, thus sharing essentially the same native structure. We kept 1opa because it has the intermediate rate among the three.

Note that two proteins, 1tit and 1ubq, belong to both data sets, since they are multistate folders in water, while switching to two-state kinetics upon different conditions.

3. Results

For each protein structure in the data sets described in section 2, we consider four different descriptors: the chain length \(N\), the ACO, the RCO and the maximum
The values of $|G'|_c$ should be compared to the reference value of 1 found for two closed curves that form a standard Hopf link (the same as for two flat linked rings). We find $|G'|_c \geq 1$ for 9 out of the overall 46 proteins analyzed in this work (see tables 1, 2). The largest value in our study is $|G'|_c = 1.65$ for the multistate protein 1ra9.

### 3.1. Two-state proteins

The linear correlations of the descriptors we consider with the natural logarithm of the experimentally measured folding rate are shown in figure 3 for two-state folder together with the corresponding Pearson correlation coefficient $r$. As already known [23], chain length is essentially not correlated with the folding rate ($r = 0.13$) for two-state folders (see figure 3(a)), whereas the best performance (see figure 3(d)) is...
Table 2. Data set for multistate folders. $N$ is the number of $C_\alpha$ atoms with available coordinates used in the computation of $|G'|_c$. ACO, RCO. 1phpn and 1phpc refer to the N-terminal and, respectively, C-terminal domains of 1php. 1qopa and 1qopb refer to the chains A and, respectively, B of the 1qopa protein complex.

| PDB code | ln(rate) | $N$ | $|G'|_c$ | ACO | RCO |
|----------|----------|-----|---------|-----|-----|
| 1a6n     | 1.1      | 151 | 0.48    | 25.71 | 0.17 |
| 1aon     | 0.8      | 155 | 1.35    | 39.1  | 0.25 |
| 1bni     | 2.6      | 108 | 0.6     | 19.32 | 0.18 |
| 1brs     | 3.4      | 87  | 0.43    | 19.8  | 0.23 |
| 1cei     | 5.8      | 85  | 0.38    | 15.18 | 0.18 |
| 1eal     | 1.3      | 127 | 0.29    | 25.58 | 0.2  |
| 1fnf     | 5.5      | 94  | 0.7     | 28.27 | 0.3  |
| 1hng     | 1.8      | 97  | 0.78    | 31.04 | 0.32 |
| 1opa     | 1.4      | 133 | 0.34    | 29.92 | 0.22 |
| 1rn9     | -2.46    | 159 | 1.65    | 40.71 | 0.26 |
| 1sce     | 4.2      | 101 | 0.46    | 23.41 | 0.23 |
| 1tit     | 3.6      | 89  | 0.61    | 30.98 | 0.35 |
| 1ubq     | 5.9      | 76  | 0.47    | 21.82 | 0.29 |
| 2a5e     | 3.5      | 156 | 0.56    | 15.83 | 0.1  |
| 2cro     | 3.7      | 65  | 0.25    | 13.39 | 0.21 |
| 2lzm     | 4.1      | 164 | 0.36    | 16.07 | 0.1  |
| 2rn2     | 0.1      | 155 | 1.      | 39.29 | 0.25 |
| 3chy     | 1.       | 128 | 0.98    | 18.96 | 0.15 |
| 1phpc    | -3.5     | 219 | 1.23    | 32.51 | 0.15 |
| 1phpn    | 2.3      | 175 | 1.3     | 36.25 | 0.21 |
| 1qopa    | -2.5     | 268 | 1.43    | 41.16 | 0.15 |
| 1qopb    | -6.9     | 392 | 1.43    | 55.09 | 0.14 |

achieved by RCO ($r = -0.87$), with negative correlation implying that slow folders have native structures with contacting residues that are on average well separated along the sequence. Although with a lower quality with respect to RCO, the correlation of ACO with the folding rate of two-state folders is still significant ($r = -0.65$) and with the proper (negative) slope (see figure 3(c)).

For the novel topological descriptor that we introduce in this work, the maximum intrachain contact entanglement $|G'|_c$, the correlation is essentially as good ($r = -0.64$) as for ACO (see figure 3(b)).

We next consider how one can combine linearly the predicting power of $|G'|_c$ and the contact order descriptors to achieve correlations with experimental folding rates that are better than the individual cases. To work with homogeneous quantities, acquiring values between 0 and 1 in a data set with $k = 1, \ldots, N_p$ proteins, we rescale linearly any descriptor $X_k$ as

$$
\text{Resc} \left( X_k \right) = \frac{X_k - X_m}{X_M - X_m}, \tag{7}
$$

where $X_m = \min_k \{X_k\}$ and $X_M = \max_k \{X_k\}$. The Pearson correlation coefficient is
Figure 3. For two-state folders, correlation between the natural logarithm of the folding rate and (a) chain length, (b) the indicator of entanglement $|G'|_c$ proposed in this work, (c) ACO, (d) RCO, (e) a linear combination of rescaled ACO and $|G'|_c$ (see the text), and (f) a similar linear combination of rescaled RCO and $|G'|_c$. The Pearson correlation coefficient $r$ of data is specified in all panels.

Then considered for the linear combination

$$(1 - \alpha) \text{Resc}(|G'|_c) + \alpha \text{Resc}(\text{ACO})$$

(8)
Figure 4. For two-state folders, correlation between $|G'|_c$ and (a) chain length and (b) ACO.

as a function of the parameter $\alpha \in [0,1]$ (and similarly for RCO). Note that $\alpha = 0$ corresponds to consider only $|G'|_c$ while $\alpha = 1$ represents the ACO.

Results for the values of $\alpha$ that yield the higher quality correlations are shown in figure 3(e) for ACO ($r = -0.71$) and in figure 3(f) for RCO ($r = -0.91$). In both cases the performance is increased by combining the contact-order predictor with the novel entanglement-based predictor. The optimal values of $\alpha$ to be used in the mixing, $\alpha = 0.49$ for ACO and $\alpha = 0.67$ for RCO, closer to 0.5 than to 1, show that the structural properties captured by $|G'|_c$ are at least in part complementary to those captured by contact order in the task of predicting folding rates for two-state folders.

Since the increment in the correlation is related to the amount of independent information contained in either descriptors, it is important to measure the extent to which the novel descriptor $|G'|_c$ and the other descriptors are mutually correlated. In figures 4(a), 4(b) we show the correlation of $|G'|_c$ with respectively the chain length and ACO. Structural entanglement, as measured by $|G'|_c$, is only slightly correlated with chain length ($r = 0.27$), whereas it exhibits a stronger correlation with the ACO ($r = 0.67$). Some correlation between $|G'|_c$ and ACO should have been expected, since both quantities correlate well with the experimental results (see Discussion).

Finally, we investigate the robustness of the correlation with the folding rates of two-state folders for two of the considered descriptors, $|G'|_c$ and ACO. We perform a leave-one-out analysis by removing, in turn, each single entry from the data sets. The Pearson correlation coefficients computed in all such cases are ranked according to their absolute value in figure 5(a) for $|G'|_c$ and figure 5(b) for ACO. As expected for a not so large number of data, the correlation coefficient can be very sensitive to the removal of single entries from the data set. In particular, the presence of 1aps is found to be crucial for the good performances of both descriptors, much more so for $|G'|_c$, whereas the removal of 1bzp greatly boosts the performance of ACO.
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Figure 5. For two-state folders: (a) absolute value of the Pearson correlation coefficient of log-rate vs. $|G'|_C$ obtained by removing one protein from the database, ranked from the lowest to the highest value. The vertical yellow line indicates the coefficient obtained with the full database. (b) The same for ACO. In both plots, the protein with the lowest value is the most important for obtaining the Pearson coefficient of the full data set, while the protein with the highest value is the one which spoils mostly the global value.

3.2. Multistate proteins

In analogy with figure[3] for multistate folders we plot in figure[6] the correlations between several quantities and the natural logarithm of the experimentally measured folding rate. As already known [50], the correlation of ACO with folding rate is good (Pearson correlation coefficient $r = -0.78$) for multistate folders (see figure[6](c)), whereas the best performance is achieved by chain length ($r = -0.86$, figure[6](a)). Contrary to the case of two-state folders, the correlation of RCO with the folding rate of multistate folders is very poor, even reversing its sign ($r = 0.29$, see figure[6](d)). For $|G'|_c$ the correlation is again almost as good as for ACO ($r = -0.74$, see figure[6](b)).

We next consider how much the linear combination of $|G'|_c$ with either ACO or RCO can improve the correlation with folding rates of the contact-order descriptors. In all cases, we rescale linearly the descriptors $X$ according to (7) and the Pearson correlation coefficient is then again considered for the linear combination (8).

The results for the values of $\alpha$ that yield the higher quality correlations are shown in figure[6](e) for ACO ($r = -0.81$) and in figure[6](f) for RCO ($r = -0.74$). In both cases the performance is increased by combining the contact-order predictor with the novel entanglement-based predictor. The optimal values of $\alpha$ to be used in the linear combination is $\alpha = 0.67$ for ACO, confirming that the structural properties captured by $|G'|_c$ are complementary to those captured by contact order in the task of predicting folding rates, also in the case of multistate folders. The linear combination of $|G'|_c$ with RCO is instead illustrative of the case when one of the combined predictors ($|G'|_c$) is much more informative than the other, as evident from the optimal value $\alpha = 0$. 
Figure 6. As in figure 3, but for multistate folders.

We finally show the correlation of the novel descriptor $|G'|_c$ with other descriptors, such as chain length (figure 7(a)) and ACO (figure 7(b)). Structural entanglement, as measured by $|G'|_c$, is significantly correlated with chain length ($r = 0.66$), whereas it exhibits a good correlation with ACO ($r = 0.81$).

As for the two-state folders, we conclude with a leave-one-out analysis. Figure 8(a)
suggests that the performance of $|G'|_c$ is robust, in this case more than that of ACO, which is very sensible to the presence of the protein 1qopb in the data set, as shown in figure 8(b) (the removal of 1qopb from the data set would cause a drop to $r = 0.68$).

4. Discussion

Data sets and performance robustness are sensible issues in the context of folding rate predictions. Different authors typically considered different data sets \[56\]. Moreover, folding rates may have been measured for the same protein in different conditions. Importantly, given the small number of proteins for which an experimental measure of the folding rate is available, the performance of different predictors can be very sensitive on the presence or absence of even single proteins in the data set.
As a matter of fact, several, yet not all, authors considered separate data sets for two-state and multistate protein folders \[56\]. The folding of two-state proteins to the native state is a cooperative process characterized by a unique time scale, whose inverse is the folding rate. Multistate proteins exhibit one or more intermediate states in the folding process, resulting in multiple relaxation times being measured. The folding rate of multistate proteins is associated to the final relaxation to the native state \[54\]. A given protein may switch from two-state to multistate folding behaviour upon changing experimental conditions, so that it can be found in data sets for both categories (see section 2). Other authors \[52, 57, 58\] considered merged data sets with both two-state and multistate folders, with the goal of testing general theories of protein folding that predict how the folding rate would increase with the chain length, for single-domain proteins.

Our choice here is to keep separate the data sets corresponding to the two different protein classes. The dependence of folding rate on simple descriptors, such as chain length or RCO, is in fact very different in the two classes, see figure 3(a) and (d) vs. figure 6(a) and figure 6(d). We are not aware of any effective explanation of this puzzling behaviour.

More generally, the data shown in figure 3 and figure 6 confirm what found in a previous study \[56\]. The RCO is a very good predictor for the folding rate of two-state folders but is very poor for multistate ones, to the extent of reversing the sign of the correlation coefficient in the latter case. The converse is true for chain length that is a very good predictor for the folding rate of multistate folders but essentially does not correlate with the folding rate of two-state folders. The ACO is instead a more robust predictor that perform reasonably well for both data sets and it embodies the “surprising simplicity” that characterizes protein folding \[1\]. The more topologically complex the network of contacts in the native structure, as measured in the case of ACO by the average sequence separation of contacting residue pairs, the longer it takes to fold to that structure.

Several other descriptors were introduced in the past to capture the topological complexity of the network of contacts in the native structures better than ACO. These include long range order, total contact distance, cliquishness, logCO, number of non local contacts, and number of geometric contacts \[17, 18, 20, 21, 22, 23, 55\]. All such descriptors are based on the notion of pairwise residue contacts. The performances of the different descriptors in predicting folding rates vary somewhat depending on the considered data sets \[56\]. It is fair to state that most of the cited predictors, including ACO, exhibit overall similar performances.

In this work, in fact, our main focus was not to establish which is the best predictor of folding rates nor to build such an optimal algorithm. We instead introduced a novel descriptor, the maximum intrachain contact entanglement \(|G'|_{c}\), not directly related to the contact order. It is rather based on the concept of the mutual entanglement between two portions of a protein chain that is inherently associated to contact formation. We then showed that \(|G'|_{c}\) can be used to predict folding rates with a performance
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comparable to the one achieved by ACO, for both data sets of two-state and multistate folders, as shown in figure 3(b),(c) and in figure 6(b),(c). Figure 5 further shows that ACO performance is slightly more robust for two-state folders, whereas figure 8 shows that $|G'|_c$ performance is instead more robust for multistate folders.

Our main message is related to the complementary nature of $|G'|_c$ and ACO in capturing the topological complexity of protein native structures at two different description levels. Not only the separation along the sequence between pairs of contacting residues is important, but also the possible entanglement of other chain portions with the loop connecting two contacting residues (see figure 1) plays a relevant role. The former refers to the topological complexity of the network of native contacts, whereas the latter relates to the topological properties of the protein chain as a curve in the three-dimensional space.

The two properties are indeed correlated for both data sets of two-state and multistate folders, as shown in figure 4(b) and in figure 7(b). However, a linear combination of $|G'|_c$ and ACO, after proper rescaling of the two quantities achieves a better performance than ACO alone, or $|G'|_c$ alone, for both data sets, as shown in figure 4(e) and in figure 7(e). Similarly, a linear combination of $|G'|_c$ and RCO, after proper rescaling of the two quantities achieves a better performance than RCO alone for two-state folders, as shown in figure 1(f).

The definition we chose for the entanglement descriptor requires that one of the two subchains is looped, and hence that the structure identified by a high $|G'|_c$ resembles a lasso. This definition is in line with similar analyses in the literature. Nevertheless, as Gauss double integrals do not require the looping condition for any of the two subchains, more flexible descriptors may be put forward to assess the degree of entanglement. General Gauss double integrals thus constitute a method for future characterisations of the topological complexity of single proteins.

Acknowledgments

We thank Stu Whittington for having introduced us into the exciting subject of topology of random polymers and for having been an inspiring teacher and friend.

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