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Exploring the correlation between the folding rates of proteins and the entanglement of their native states

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Abstract

The folding of a protein towards its native state is a rather complicated process. However, there is empirical evidence that the folding time correlates with the contact order, a simple measure of the spatial organization of the native state of the protein. Contact order is related to the average length of the main chain loops formed by amino acids that are in contact. Here we argue that folding kinetics can also be influenced by the entanglement that loops may undergo within the overall three-dimensional protein structure. In order to explore such a possibility, 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_α atoms. By analyzing experimental data sets of two-state and multi-state folders, we show that the new index is also 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.

Keywords: Protein native structure, folding rates, topology, linking number

(Some figures may appear in colour only in the online journal)

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1. Introduction

Simple paradigms very often play an invaluable role in helping to understanding complex systems. A well known example is given by protein folding. Protein folding is the physical process by which a protein chain acquires its final three-dimensional structure, the native state, which is usually biologically functional, in a reproducible manner. The characteristic time of this process is named the folding time. Protein folding 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 [3–6]. Other 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 a set of competing decoys [7–11] and 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 [12–14].

Further evidence of this emerging simplicity is the empirical result of Plaxco and coworkers [15, 16], who found a significant correlation between experimental folding rates of proteins, e.g. the inverse folding times, and a simple descriptor of the native state organization, 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 that are in contact. This results is somewhat surprising because folding inevitably involves states other than the native one and these conformations might affect the kinetic process. Despite some evidence that this correlation is weak for proteins belonging to the all- β structural class [17], later studies confirmed correlations between folding rates and other descriptors of the native state organization. These descriptors are *long range order* [18], the *number of native contacts* [19, 20], the *total contact distance* [21], the *cliquishness* [22], the *local secondary structure content* [23] and the *chain crosslinks contact order* [24].

The contact order and all its possible variants are descriptors that focus on the network of pairs of residues that are nearby in space regardless of the full spatial arrangement of the protein conformation. More realistically, 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 [25]. In protein physics, the writhing number was first used in [26] to quantify the amount of self-threading in the native state and it was later extended to perform a systematic classification of existing protein folds [27, 28].

After the seminal observation that the backbone of a protein may self-entangle into physical knots [29–36], growing attention has been devoted to finding 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 [37–40] or to detect specific topological knots, links and lassos within a single chain [40–42].

The aim of this study is to explore the correlation between protein folding rates and a novel topological descriptor of protein 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 [43]. Being quite easy to compute, the Gauss linking number has been extensively used in the past to characterize the mutual entanglement of diluted and concentrated solutions of linear polymers [44–47], to estimate the linking probability and link complexity of a pair of loops under geometrical constraints [48–51] as well as to identify threadings in dense solutions of unlinked loops diffusing in a gel [52].

By exploring a data set of 48 proteins [22, 24, 53] 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

$$G \equiv \frac{1}{4\pi} \oint_{\gamma_1} \oint_{\gamma_2} \frac{\mathbf{r}^{(1)} - \mathbf{r}^{(2)}}{|\mathbf{r}^{(1)} - \mathbf{r}^{(2)}|^3} \cdot (\mathbf{dr}^{(1)} \times \mathbf{dr}^{(2)}) \quad (1)$$

between two closed curves γ_1 and γ_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 also provides a meaningful assessment of the mutual entanglement if either one or both curves are open [37, 44, 47].

Our strategy here is to consider as γ_1 and γ_2 any pair (γ_i, γ_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 ($i = 1, \dots, N$) placed at the position \mathbf{r}_i of the C_α atoms, it is natural to define the average positions

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

and the bond vectors

$$\mathbf{dR}_i = \mathbf{r}_{i+1} - \mathbf{r}_i. \quad (3)$$

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

$$G'_{ij} \equiv \frac{1}{4\pi} \sum_{i=i_1}^{i_2-1} \sum_{j=j_1}^{j_2-1} \frac{\mathbf{R}_i - \mathbf{R}_j}{|\mathbf{R}_i - \mathbf{R}_j|^3} \cdot (\mathbf{dR}_i \times \mathbf{dR}_j) \quad (4)$$

where the prime in G' highlights the fact that the measure is for open chains. Note that, unlike in our previous study [37] where the entanglement was estimated between two different protein backbones, here the pairs of subchains (γ_i, γ_j) are extracted from the same protein backbone.

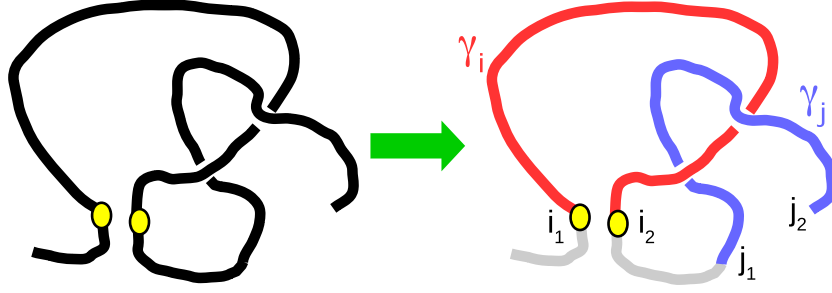


Figure 1. Sketch of the procedure we used for computing G'_{ij} . First, a looped portion γ_i ($i_1 \rightarrow i_2$, red) was identified when the C_α coordinates of the amino acids i_1 and i_2 (yellow ovals) were closer than $d = 9$ Å. Then, the double sum (4) was computed for any other portion γ_j ($j_1 \rightarrow j_2$, blue) preceding or following γ_i .

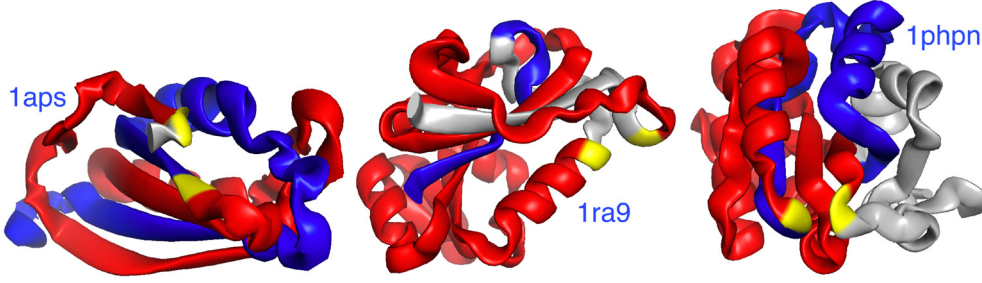


Figure 2. Following the color code of figure 1, examples of protein native structures in which we identified the subchains γ_i ($i_1 \rightarrow i_2$, yellow-red-yellow) and γ_j ($j_1 \rightarrow j_2$, blue) yielding the maximum intrachain contact entanglement.

The definition (4) is rather generic and can be applied to any pair (γ_i, γ_j) of non-overlapping portions of the protein backbone. Here we focus the analysis on the subset of (γ_i, γ_j) where the subchain γ_i has its first (i_1) and last (i_2) residues forming a *contact* ($i_1 \div i_2$), i.e. when $|\mathbf{r}_{i_1} - \mathbf{r}_{i_2}| < d$, with $d = 9$ Å. With this restriction γ_i is essentially a loop. The same restriction is not applied to γ_j , which can either precede or follow γ_i along the protein backbone.

This way of detecting entangled configurations is sketched in figure 1. It is similar to that leading to the definition of ‘lassos’ [42], although here we do not restrict the contacts to chemically strong bonds as in cysteine pairs.

A preliminary analysis of our data set showed that such entangled configurations are not rare. Some examples can be seen in figure 2. Given their topological complexity, it is reasonable to think that these native states host proteins that might fold slowly, especially when the mutual entanglement between γ_i and γ_j is considerable.

Motivated by the considerations above, we performed a statistical analysis to test the existence of a negative correlation between folding rates and a quantitative measure of the intrachain entanglement present in a protein native structure. We define this measure to be the largest absolute value of the mutual entanglement found for all possible pairs (γ_i, γ_j) having the lasso structure discussed above

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

Table 1. Data set for two-state folders. N is the number of C_α atoms with available coordinates used in the computation of $|G'|_c$, ACO and RCO. 1bnza refers to chain A in the 1bnz protein-DNA complex. 1div.n refers to the N-terminal domain of protein 1div. 1hz6a refers to chain A in the 1hz6 protein complex. 1lmb3 refers to chain 3 in the 1lmb protein-DNA complex. 1urna refers to chain A in the 1urn protein-RNA complex.

PDB code	$\ln(\text{rate})$	N	$ G' _c$	ACO	RCO
1afi	0.6	72	0.77	22.99	0.32
1aps	-1.47	98	1.62	34.1	0.35
1aye	6.63	80	0.27	13.83	0.17
1bnza	6.95	64	0.27	16.39	0.26
1bzp	11.12	153	0.47	24.28	0.16
1csp	6.54	67	0.4	19.65	0.29
1div.n	6.61	56	0.84	13.	0.23
1fkb	1.38	107	0.96	32.4	0.3
1hrc	8.75	104	0.56	23.56	0.23
1hz6a	4.1	62	0.54	17.36	0.28
1imq	7.28	86	0.5	18.24	0.21
1lmb3	11.01	80	0.3	15.14	0.19
1pgb	5.66	56	0.39	17.2	0.31
1poh	2.69	85	0.49	27.04	0.32
1psf	1.17	69	0.47	20.86	0.3
1shf	4.54	59	0.71	18.12	0.31
1ten	1.06	89	0.67	28.84	0.32
1tit	3.48	89	0.61	30.98	0.35
1ubq	7.35	76	0.47	21.82	0.29
1urna	2.5	96	1.15	28.34	0.3
1wit	0.41	93	0.72	33.35	0.36
256b	12.2	106	0.33	15.53	0.15
2abd	6.56	86	0.6	21.56	0.25
2ci2	4.03	64	0.68	20.23	0.32
2pdd	9.67	41	0.3	9.04	0.22
2vik	7.48	126	0.86	27.02	0.21

The index ‘c’ indicates that this maximum intrachain *contact* entanglement is subject to the loop constraint of having the ends of γ_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

$$\text{ACO} \equiv \frac{1}{n_c} \sum_{i \div j} |j - i|. \quad (6)$$

The relative contact order (RCO) is simply the ACO divided by the chain length N , which is the average of the 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 [54]. We use folding rates (in 1/s) as reported previously [24, 54]; see table 1.

Table 2. Data set for multi-state folders. N is the number of C_α atoms with available coordinates used in the computation of $|G'|_c$, ACO and 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	$\ln(\text{rate})$	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
1ra9	-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

The second data set, for multi-state folders, is summarized in table 2 and includes proteins that exhibit one or more folding intermediates in water, the entries 34-57 in table 1 from [53]. We used folding rates from that table with two exceptions. For 1ra9, we used the folding rate reported instead in [55, 56]. We then removed 1cbi and 1lfc 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 multi-state 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 intrachain contact entanglement ($|G'|_c$).

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 and 2). The largest value in our study is $|G'|_c = 1.65$ for the multi-state protein 1ra9 (one of the examples shown in figure 2).

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 a two-state folder together with the corresponding Pearson correlation coefficient r . As already known [24], the 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 achieved by the 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 the RCO, the correlation of the 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 intra-chain contact entanglement $|G'|_c$, the correlation is essentially as good ($r = -0.64$) as for the 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 $k = 1, \dots, N_p$ proteins, we rescale linearly any descriptor X_k as

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

where $X_m = \min_k \{X_k\}$ and $X_M = \max_k \{X_k\}$. The Pearson correlation coefficient is then considered for the linear combination

$$(1 - \alpha) \text{Resc}(|G'|_c) + \alpha \text{Resc}(\text{ACO}) \quad (8)$$

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

Results for the values of α that yield the higher quality correlations are shown in figure 3(e) for the ACO ($r = -0.71$) and in figure 3(f) for the 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 α to be used in the mixing, $\alpha = 0.49$ for the ACO and $\alpha = 0.67$ for the 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 descriptor, 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) and (b) we show the correlation of $|G'|_c$ with, respectively, the chain length and the 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 the ACO should have been expected, since both quantities correlate well with the experimental results (see the discussion section).

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 the 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 the ACO. As expected for a not so large number of data,

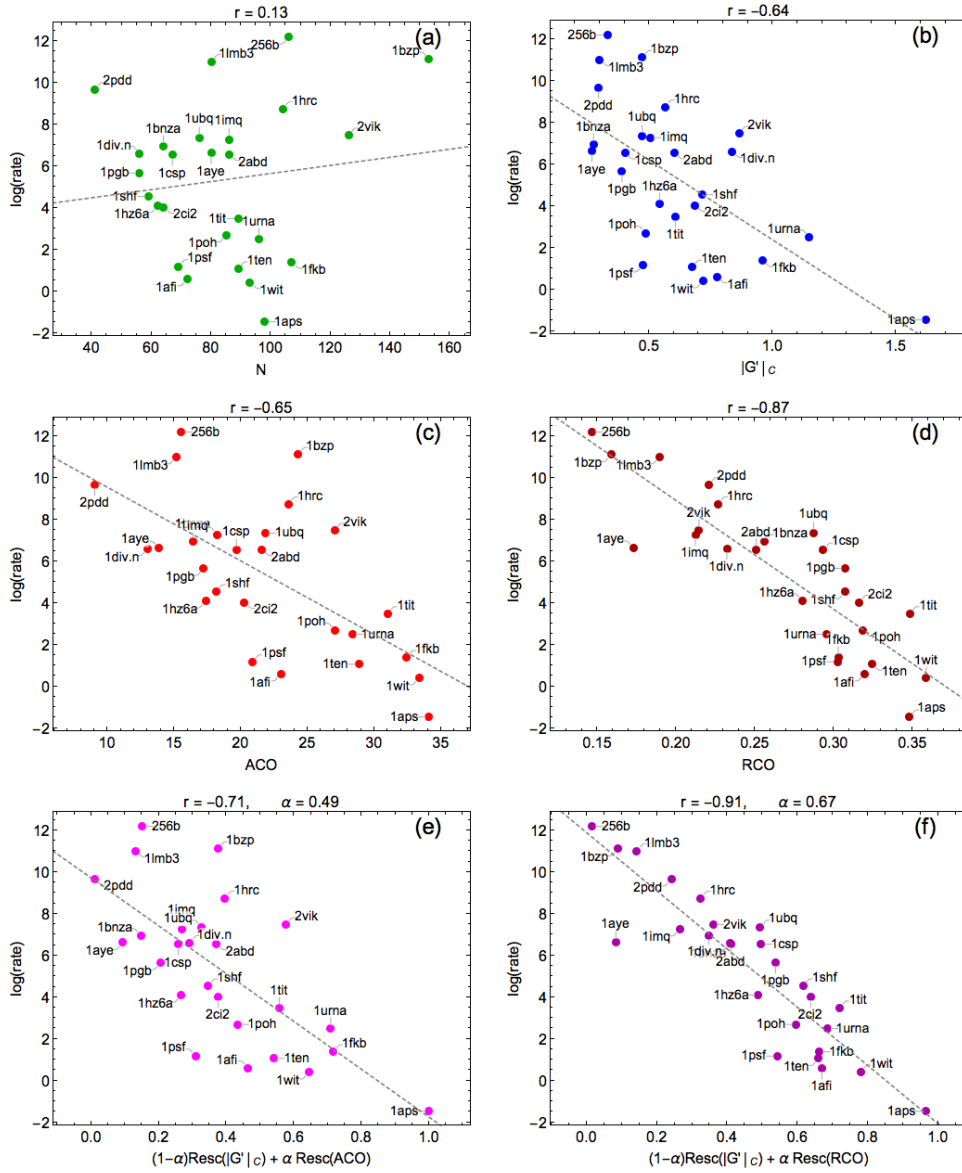


Figure 3. For two-state folders, correlation between the natural logarithm of the folding rate and (a) chain length, (b) the indicator of entanglement $|G'|_c$ proposed in this work, (c) ACO, (d) RCO, (e) a linear combination of the rescaled ACO and $|G'|_c$ (see the text), and (f) a similar linear combination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

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 performance of both descriptors, much more so for $|G'|_c$, whereas the removal of 1bzip greatly boosts the performance of the ACO.

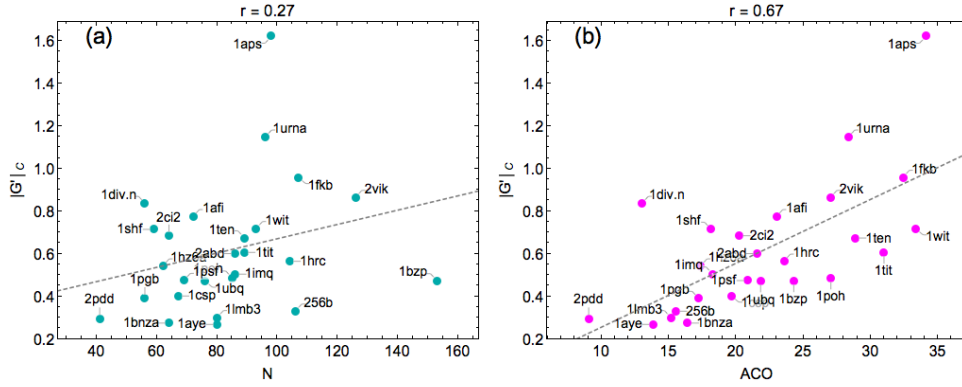


Figure 4. For two-state folders, correlation between $|G'|_c$ and (a) chain length and (b) the ACO.

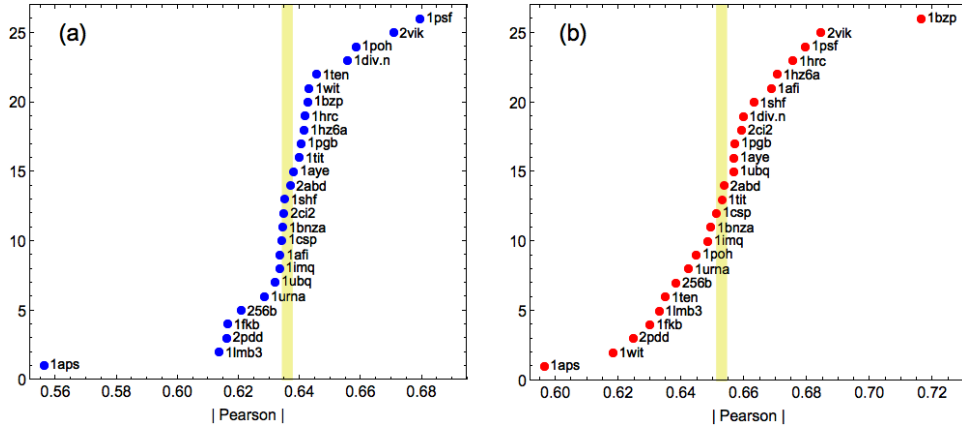


Figure 5. For two-state folders: (a) absolute value of the Pearson correlation coefficient of log-rate versus $|G'|_c$ obtained by removing one protein from the database, ranked from the lowest to the highest value. The vertical yellow line indicates the coefficient obtained with the full database. (b) The same for the 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 that spoils the global value the most.

3.2. Multi-state proteins

In analogy with figure 3, for multi-state folders we plot in figure 6 the correlations between several quantities and the natural logarithm of the experimentally measured folding rate. As already known [57], the correlation of the ACO with folding rate is good (Pearson correlation coefficient $r = -0.78$) for multi-state 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 the RCO with the folding rate of multi-state 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 the ACO ($r = -0.74$, see figure 6(b)).

We next consider how much the linear combination of $|G'|_c$ with either the ACO or RCO can improve the correlation with folding rates of the contact-order descriptors. In all cases,

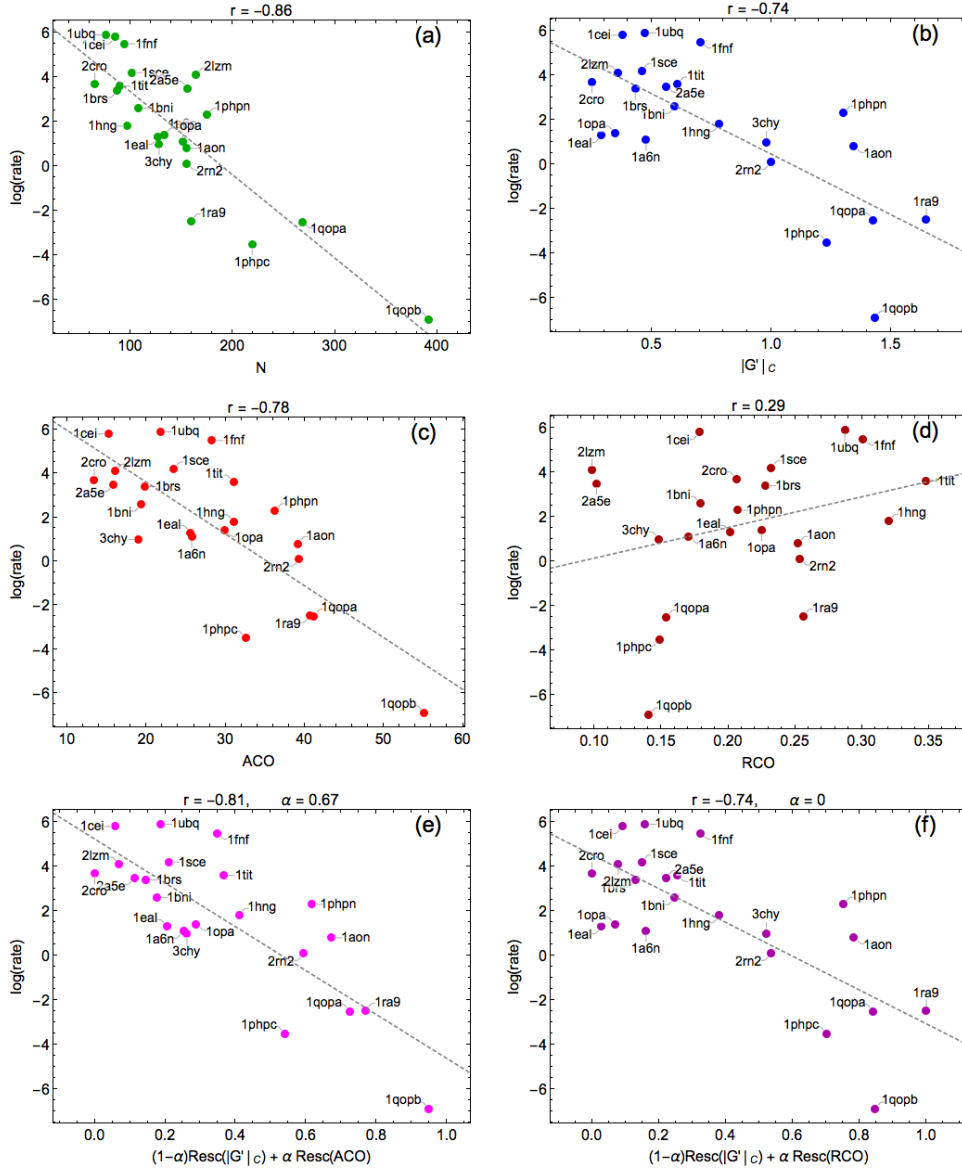


Figure 6. As in figure 3, but for multi-state folders.

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 α that yield the higher quality correlations are shown in figure 6(e) for the ACO ($r = -0.81$) and in figure 6(f) for the 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 value of α to be used in the linear combination is $\alpha = 0.67$ for the 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 multi-state folders. The linear combination of $|G'|_c$ with the RCO is instead illustrative

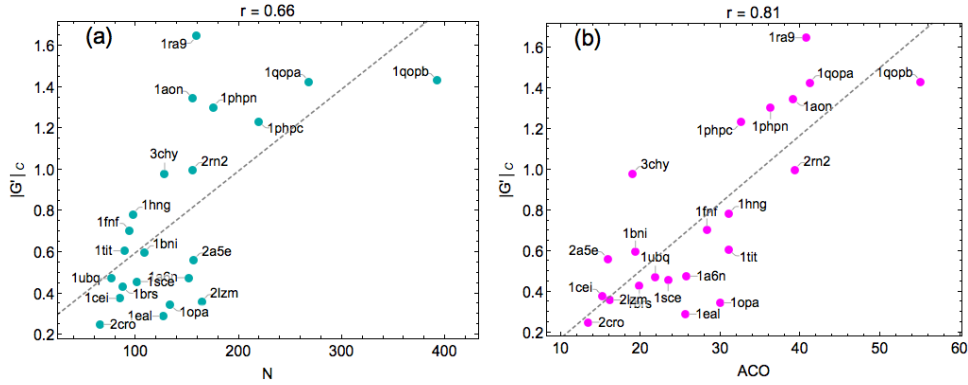


Figure 7. For multi-state folders, correlation between $|G'|_c$ and (a) chain length and (b) the ACO.

of the case when one of the combined predictors ($|G'|_c$) is much more informative than the other, as evident from the optimal value $\alpha = 0$.

We finally show the correlation of the novel descriptor $|G'|_c$ with other descriptors, such as chain length (figure 7(a)) and the 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 the ACO, which is very sensitive 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 have typically considered different data sets [57]. 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 to the presence or absence of even single proteins in the data set.

As a matter of fact, several, yet not all, authors have considered separate data sets for two-state and multi-state protein folders [57]. 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. Multi-state proteins exhibit one or more intermediate states in the folding process, resulting in multiple relaxation times being measured. The folding rate of multi-state proteins is associated with the final relaxation to the native state [55]. A given protein may switch from two-state to multi-state folding behavior upon changing experimental conditions, so that it can be found in data sets for both categories (see section 2). Other authors [53, 58, 59] considered merged data sets with both two-state and multi-state 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 the

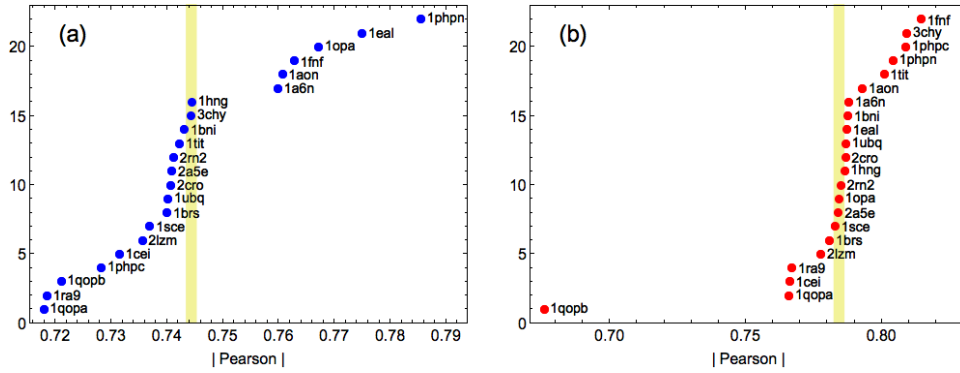


Figure 8. For multi-state folders: (a) absolute value of the Pearson correlation coefficient of log rate versus $|G'|_c$ obtained by removing one protein from the database, ranked from the lowest to the highest value. The vertical yellow line indicates the coefficient obtained with the full database. (b) The same for the ACO.

RCO, is in fact very different in the two classes, see figures 3(a) and (d) versus figures 6(a) and (d). We are not aware of any effective explanation for this puzzling behavior.

More generally, the data shown in figures 3 and 6 confirm what was found in a previous study [57]. The RCO is a very good predictor for the folding rate of two-state folders but is very poor for multi-state ones, to the extent of reversing the sign of the correlation coefficient in the latter case. The converse is true for chain length, which is a very good predictor for the folding rate of multi-state folders but essentially does not correlate with the folding rate of two-state folders. The ACO is instead a more robust predictor that performs 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 the ACO by the average sequence separation of contacting residue pairs, the longer it takes to fold to that structure.

Several other descriptors have been introduced in the past to capture the topological complexity of the network of contacts in the native structures better than the ACO. These include long range order, total contact distance, cliquishness, logCO, number of non-local contacts, and number of geometric contacts [18, 19, 21–24, 56]. All such descriptors are based on the notion of pairwise residue contacts. The performance of the different descriptors in predicting folding rates vary somewhat depending on the considered data sets [57]. It is fair to state that most of the cited predictors, including the ACO, exhibit overall similar performances.

In this work, in fact, our main focus was not to establish 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 with contact formation. We then showed that $|G'|_c$ can be used to predict folding rates with a performance comparable to the one achieved by the ACO, for both data sets of two-state and multi-state folders, as shown in figures 3(b) and (c) and in figures 6(b) and (c). Figure 5 further shows that the ACO performance is slightly more robust for two-state folders, whereas figure 8 shows that $|G'|_c$ performance is instead more robust for multi-state folders.

Our main message is related to the complementary nature of the $|G'|_c$ and the ACO descriptors in capturing the topological complexity of protein native structures at two different 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. Note that the former feature refers to the topological complexity of the network of native contacts, whereas the latter relates to the topology of the protein chain as a curve in three-dimensional space. The explicit consideration of the three-dimensional topological properties of the native structures represents one of the main novelties of the present paper.

The two descriptors $|G'|_c$ and the ACO are indeed correlated for both data sets of two-state and multi-state folders, as shown in figure 4(b) and in figure 7(b). However, a linear combination of $|G'|_c$ and the ACO, after proper rescaling of the two quantities, achieves a better performance than the 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 the RCO, after proper rescaling of the two quantities, achieves a better performance than the RCO alone for two-state folders, as shown in figure 4(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 characterizations of the topological complexity of single proteins, which may also find applications in contexts different from the prediction of folding rates considered in this work.

As an illustrative example, we conclude our discussion by reporting that $|G'|_c = 1.21$ for the human single-domain protein K-Ras (167 residues as a single chain in the PDB ID 3GFT). K-Ras fluctuations in its native ensemble were recently shown by atomistic simulations to exhibit anomalous non-ergodic kinetics over several decades [60]. The same behavior was reported for two larger multi-domain proteins. The observed kinetics was quantitatively well described by a continuous time random walk with heavy-tailed waiting time distributions [61].

The maximum intrachain contact entanglement found for K-Ras is higher than the one expected for single-domain proteins with similar length based on a linear interpolation for the data sets considered in this paper (see figures 4(a) and 7(a)). It may be then appealing to speculate whether the locking of the protein chain into conformations that are entangled, according to the $|G'|_c$ descriptor, or to similar ones, could play some role in shaping the non-ergodic kinetics mentioned above. Highly entangled conformations could in fact explain the presence of deep traps in the energy landscape where the protein chain would remain stuck for extended periods of time. Clearly, such a hypothesis needs to be thoroughly validated by further studies. The generalization of the maximum intrachain contact entanglement indicator to the case of multi-domain proteins should also be considered in this respect.

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