Time and again, objects of non-trivial topology turn out to be relevant in physics. Polymers provide examples of such a relevance as they may acquire topologically non-trivial configurations known as knots [1,2,3]. In DNA’s – polymers which are nearly homogeneous – knots arise spontaneously and abundantly [3,4]. In proteins, however, they are a rarity. Knots in the native states of proteins were first discovered by Mansfield in 1994 [3]. Further research [5,6,7] and especially a survey by Virnau et al. [8], has led to an identification of 273 examples of proteins with knots which constitutes less than 1% of the structures deposited in the Protein Data Bank. The biological function of knots in proteins remains to be elucidated, but it is likely that such shapes are not accidental. It should also be noted that these 273 proteins correspond to only three different topologies denoted as $\text{3}_1$ (the trefoil knot), $\text{4}_1$ and $\text{5}_2$ where the main integer indicates the number of crossings and the subscript – a particular shape. (When identifying a knot, it is assumed implicitly that the protein terminals are connected by an outside segment that transforms a two-ended chain into a closed loop).

In this Letter, we explore the dynamical behavior of a knot when a protein is stretched, for example by a tip of an atomic force microscope. Experiments on knot-tightening have been performed recently [9] for the bovine carbonic anhydrase protein (coded 1v9e), which was also studied within all-atom simulations [10]. Our study is based on molecular dynamics simulations in a coarse-grained model that represents a protein as a chain of the C$\alpha$ atoms with effective attractive contact interactions [11]. In contrast to the all-atom simulations, a coarse grained approach allows for a survey of many proteins, incorporation of much larger statistics, slower rates of pulling, and extensive variation of parameters.

We observe that knot tightening process in a stretched protein is dominated by jumps, i.e. sudden displacements of positions of knot’s ends along the sequence towards each other. These jumps have definite lengths and together with the final location of a tightened knot they are specified by a local geometry of a protein chain. The larger the size of a knot, or its level of topological complication, the larger the number of jumps is observed before its final tightening. However, such jumps are not observed in the dynamics of knot motion on stretched polymers. In this case, the motion is of a diffusive character [4,5,12].

In order to define the knotted core, i.e. a minimal segment of amino acids that can be identified as a knot, we use the KMT algorithm [1,8]. It involves removing the C$\alpha$ atoms, one at a time, as long as the backbone does not intersect a triangle set by the atom under consideration and its two immediate sequential neighbors. As a result of this procedure, two end points of the knot are identified. The knot’s ends depend on the conformation and, as the protein gets stretched, they may depin and come closer together. We have studied 18 proteins with the trefoil knot $\text{3}_1$ (1j85, 1o6d, 1dmx, 1jd0, 1j86, 1ipa, 1js1, 1k3r, 1k0p, 1nxz, 1v9e, 1x7p, 1v2x, 1fug, 1vho, 1v9e, 1zrj, 1hcb, 1keq) and two $\text{5}_2$ proteins (2etl and 1xd3) [11]. We have found that once the knot shrinks from its native size, one end of a knot invariably lands in a sharp turn of a protein backbone. Then it moves again until a final position corresponding to the tightest knot is reached. In most cases, such turns contain proline which stiffens a backbone through a ring structure that forms a backbone angle $\sim 75$ deg. The second frequent knot-stopping turn contains glycine (in 1o6d, 1fug, 1vho, 1zrj, 1keq, 1v9e – the latter also has a turn with proline) which, due to the lack of the side chain, leads to strongly sinuous local conformations of the backbone. In one case (1hcb), the knot-stopping turn involved alanine. In the absence of a sharp turn in a protein backbone, the knot is stopped at the beginning of a helix. It should be noted that proteins with knots have a shorter effective end-to-end length available for stretching, which is similar to the case of proteins with covalent disulfide bonds between cysteins (not present in the proteins considered here). However, there are also

**Tightening of knots in proteins**

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(Dated: February 1, 2008)

We perform theoretical studies of stretching of 20 proteins with knots within a coarse grained model. The knot’s ends are found to jump to well defined sequential locations that are associated with sharp turns whereas in homopolymers they diffuse around and eventually slide off. The waiting times of the jumps are increasingly stochastic as the temperature is raised. Larger knots do not return to their native locations when a protein is released after stretching.

PACS numbers: 87.15.Aa, 87.14.Ee, 87.15.La, 82.37.Gk, 87.10.+e
important differences between the two: disulfide bonds stay in place whereas knots may move.

The details of our modeling of stretching are described in refs. [17, 18]. Native contacts are defined through heavy atom overlaps and are assigned the Lennard-Jones potentials with an amplitude $\epsilon$ and length parameters tuned in such a way as to guarantee that the native conformation of a protein corresponds to the global minimum of potential energy. The remaining non-native contacts are repulsive. We take $\epsilon/k_B = 900K$, which correlates well with the experimental data on protein unfolding ($T = k_BT/\epsilon \sim 0.3$ corresponds to the room temperature). Unlike Wallin et al. [19] who consider folding of protein 1j85, we do not need to introduce additional non-native attractive contacts leading to a knot formation, since our configurations are already knotted.

The presence of a solvent is mimicked by velocity dependent friction and fluctuational forces corresponding to a temperature $T$. The stretching was accomplished by attaching the protein to a pulling spring which moves with the velocity $v_p$ of 0.005 Å/ns. Our approach and its variants has passed many benchmark tests for protein stretching and agrees favorably with the experimental results [18] and all-atom molecular dynamics simulations [20]. In particular, our model predicts existence of three peaks in the force–displacement curves (at 130, 370, and 490 Å) for 1v9e as found in experiment [12, 13] and all-atom simulations [13] and a similar order of contact breaking events.

In order to represent motion of the ends of a knot we use diagrams such as one shown in the middle panel of Figure 1. The panel corresponds to protein 1j85 which contains $N=156$ amino acids that make a simple trefoil knot with the ends set at amino acids numbered $n_1=75$ and $n_2=119$ in the native state. The diagram shows what happens to the values of $n_i$ when the protein gets stretched as the pulling tip moves by a distance $d$ and the corresponding force ($F$) – displacement ($d$) curve for a protein (the right panel in Figure 1). It is seen that despite the presence of several force peaks the ends of the knot stay put for most of the stretching trajectory. However, at the final force peak, i.e. around $d=400$ Å, both ends jump towards each other along the sequence and then undergo another jump about 50 Å later. This jumpy behavior is not found when the protein is heated up or replaced by a homopolymer with purely repulsive contact interactions (the left panel in Figure 1). In the homopolymeric case, we start with the native conformation of a protein, but remove attractive contacts. Another possibility of observing homopolymer-like behavior in a protein is to increase the temperature of the system above that of the specific-heat maximum. In homopolymer, the positions of the knot ends diffuse around and, particularly in the initial stages, the distance between them may increase considerably which corresponds to swelling of the knot. Eventually, however, they come closer together but remain mobile and, in most cases, slide off the polymer chain. These results agree with earlier studies on the dynamics of knots in polymers and DNA, in which the diffusive character of knot motion was analyzed both experimentally [5] and theoretically [4, 10].

Both for the homopolymer and the protein, the motion of the knot’s ends depends on the particular trajectory even if the $F(d)$ curves look nearly the same. In particular, the ends may sometimes depin on an earlier force peak. The stretching process affects the knotted core of a protein much less than the outside region and thus leaves the geometry inside the knotted core and its secondary structures nearly native-like. For instance, a well tightened knot in 1o6d contains an entire $\alpha$-helix in its nearly native conformation.

The description of a knot dynamics is reduced and involves only the movement of its end points $n_i$ along the sequence. We have found, however, that the real space distances between the residues in the knotted core turn out to be mostly unchanged in between the knot jumps and undergo rapid changes as the knot ends jump. This indicates the existence of a coupling of the real space dynamics of a knot to its motion in the sequence space.

The final and metastable locations of the knot ends coincide with the sharp turns in the protein backbone (and/or the endpoints of a helix), as seen in Figures 2 and 3. The stopping points correspond to the deep local minima of the angle $\theta$ between every second vector.

FIG. 1: Motion and tightening of a knot on a homopolymer (left) and on protein 1j85 (middle) during stretching with constant velocity. Squares and circles indicate positions of the ends of the knot along the chain. Knots typically slide off homopolymeric chains. Here, however, we have chosen an example in which a knot tightens close to one end of the chain. In contrast, knots in proteins always tighten in a specific position inside its initial configuration, after making a series of jumps. Each jump corresponds to a definite force peak in the force–displacement curve shown in the panel on the right hand side.
FIG. 2: The ends of a knot in 1j85 protein in the native state are located at amino acids \( n_1 = 75 \) and \( n_2 = 119 \). In a tightened configuration, the ends of the knot are located between \( n_1 \) and \( n_2 \), with one end either in a sharp turn or at the end of a helix. The arrows indicate these characteristic places. The numbers show percentages of situations (based on 700 trajectories) in which a knot’s end is pinned at the feature after moving from the native state. The innermost features correspond to the tightest knot.

Along the \( C_\alpha \) backbone (i.e between the vector \( C_\alpha,iC_\alpha,i+1 \) and \( C_\alpha,i+2C_\alpha,i+3 \)), which coincides with Kuntz’s criterion \([21]\) for detection of turns (and is also satisfied at the end points of a helix). Such turns are usually stabilized by hydrogen bonds and are thus harder to break. At high temperatures \((kT > 0.5\epsilon)\), the motion of a knot gradually becomes less predictable, and the final position of the knot ends is no longer always connected to the turn in the native structure. Additionally, the knot may wander outside the initial knotted core. Finally, for \( kT \gg \epsilon \), a homopolymeric behavior is observed, with the knot freely diffusing along the backbone.

A protein typically contains several sharp turns in the native state. Thus there are several pinning centers on which the knot’s ends may settle during stretching. This is illustrated in Figure 2 for 1j85 protein. Another example is given in Figure 3 for the 2etl protein which supports a 5_2 knot spanning 174 (out of all 223) sites in the native state. In this case, there are two characteristic pinning centers leading to the final knot tightening either between sites 110–126 or 101–119 for a range of temperatures. It should be noted that the preference for a knot to begin or end on a turn does not appear to apply to the native conformation. It arises only during stretching.

In addition to the simple stretching (whether at a constant speed or at a constant force), we have also studied processes in which one pulls a protein to a certain extension and then releases it abruptly. If the stretching stage lasts sufficiently long (so that several force peaks are observed and the knot gets tightened substantially) then the protein misfolds on releasing and the knot ends continue to reside at the metastable locations. We have observed such irreversibility effects in 2etl, 1vho, and 1v2x and in 80% of trajectories for 1o6d. However, apart from a few trajectories (such as the one shown in Figure 4), the knot in protein 1j85 is usually found to return to its native location. The different behavior of 1j85 compared to the other four proteins may be due to the fact that 1j85 easily unfolds (and unties itself) through heating \([19]\) as well in equilibrium condition in the experiment \([22]\) and is thus less stable.

We now consider a distribution of waiting times, \( \delta t \), between the jumps. In fact, it is convenient to measure these times in terms of a respective displacement of the pulling tip \( \delta d = v_p \delta t \). We may separate jumps corresponding to \( v_p \) at \( T = 0 \), the process is deterministic, lasts for a relatively long time, with \( \delta d \) reaching 400 Å before the first (and only) jump is made. At the time of the jump, the knotted core constitutes the only portion of the original protein structure that has not been unfolded yet. This
FIG. 5: Distribution of waiting distances $\delta d$ of the left end of a knot in protein 1j85 at various temperatures $T$. Pathways 1 and 2 are indicated by symbols in circles. The panels are explained in the text.

The unfolding route is denoted as pathway 1 and corresponds to the rightmost peak in the top and middle panels of Figure 5. As the temperature is increased an alternative pathway 2 becomes stochastically available. In this pathway the knot is tightened at $\delta d \sim 340 \text{ Å}$, which is before the protein gets fully unfolded. The ratio of probabilities of choosing these pathways can be then described as

$$\frac{p_1}{p_2} = \exp \left( - \frac{\Delta F}{k_B T} \right),$$

where $\Delta F$ is the free energy barrier associated with the transition between pathway 1 and pathway 2. The data points shown in the inset of the top panel of Figure 5 suggest $k_B/T \Delta F \approx 1.6$. As the temperature increases, the jumps on each pathway gets shorter and are usually followed by another jump with much shorter jumping distance ($d < 100 \text{ Å}$ in the middle and bottom panel). Above $k_B T/\epsilon = 0.5$ the peaks corresponding to pathways 1 and 2 merge. At this stage, the short distance part of the distribution may be approximated by the exponential distribution $P(d) = \alpha^{-1} \exp(\alpha d)$, as shown in the bottom panel for $k_B T/\epsilon = 0.7$. In the inset in the bottom panel log $P(d)$ is fitted to a line whose slope yields $\alpha \approx 0.027$. In summary, we have found that the process of knot tightening in proteins is qualitatively distinct from that occurring in homopolymers. The proteinic knots shrink in size and one of their ends gets pinned on a sharp turn. The movement of knot ends in the protein along the sequence is characterized by sudden jumps, whereas in polymers knots perform a diffusive motion and, in most cases, slide off the chain. It would be interesting to devise stretching experiments that would monitor knot tightening and end-jumping in proteins, analogous to those reported for nucleic acids [5].

We appreciate useful comments of R. Kutner and R. Stolarski. This work was funded by the MNiSW grant N202 021 31/0739.

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