

# Stretching of the knotted protein YibK and its unknotted constructs

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## Abstract

We review the status of theoretical studies on stretching of proteins with knots within a coarse grained model. We discuss the effect of knots on mechanical properties of proteins and show that may depend on the particular protein studied.

## 1 Introduction

General mechanical properties of proteins with trivial topology are well established due to the joint experimental and theoretical efforts. In contrast, much less is known about properties of proteins which have non-trivial topology and contain knots. This limited knowledge is partly a consequence of the fact that knotted proteins were discovered only very recently. Knots in the native conformation of protein were found first by Mansfield [1], while the most up-to-date list of the knotted proteins in Protein Data Bank (PDB) was provided by Virnau [2]. These results show that knotted proteins are not so exceptional as once thought, and they comprise around 1% of the entire PDB. However, only the simplest types of (open) knots have been found in proteins, such as  $3_1, 4_1, 5_2$  [1, 2, 3, 4].

While the analysis of the topological aspects of the structure is becoming increasingly available, there are few studies describing mechanical properties of the knotted proteins. The first such work, including both theoretical and experimental aspects, concerned the bovine carbonic anhydrase and involved manipulations which result in unwinding the knot in its native structure [7]. The following theoretical analysis of pulling knotted proteins by terminals showed that during tightening, the knot moves along the protein backbone in a series of jumps, before arriving at the final fully tightened conformation [5]. These results are in direct contrast to the well studied case of knots in homopolymers, which tend to diffuse smoothly along the chain and to slide off eventually [8].

Direct influence of knots on mechanical properties of proteins have been recently investigated theoretically in [6]. This work relies on a direct comparison of two very similar proteins – one knotted and the other unknotted – the backbone structure of which differ only in the way one crossing is accomplished. This analysis has shown that the presence of a knot results in generation of an additional mechanical resistance to pulling of the protein, and it modifies the mechanical and thermal unfolding pathways. However, up to now, only one such pair of proteins has been

found in nature, so this is difficult to make general statements about influence of knotting on mechanical properties of proteins.

In this work, using structure-based theoretical coarse-grained models [11, 12], we continue the investigation of influence of knots on mechanical properties of protein. In Ref. [6] we have considered relatively large and complex proteins. Here, we focus on a pair of shorter and simpler proteins. One is the Haemophilus influenzae YibK of the PDB code 1j85, which is a homodimeric  $\alpha/\beta$ -knot methyltransferase (MTase). As no similar natural unknotted structure is known, we engineer theoretically two different (though similar) rebuilt proteins, by rebuilding one of the backbone crossings, between amino acids 80 and 87, Fig. 1. One reason for such an analysis is that focusing on a simple example often allows one to capture the most important features of a given physical situation. Secondly, the effect of a knot in YibK was also analyzed experimentally in [10]. The results reported here are in agreement with those experimental observations and suggest that the knot does not influence the mechanical stability of YibK in a significant way. On the other hand, in the case of proteins considered in Ref. [6], the knot has been shown to enhance the mechanical stability. Our main result is that the role of the secondary structures (e.g. that of the length of the  $\beta$  strands) and the overall tertiary structure can be as important as the topological configuration of a protein, and mechanical properties of knotted proteins are a delicate interplay of both factors, which depend to much extent on the particular protein.

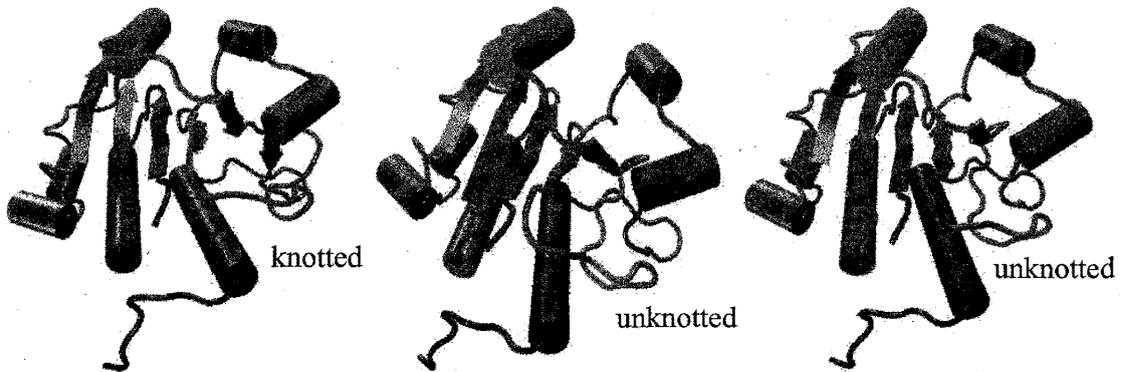


Figure 1: 1j85 native structure (left) and its two rebuilt forms (middle, right).

## 2 Results – stretching knotted proteins

The mechanical properties of proteins are investigated within the coarse-grained molecular dynamics model described in detail in ref. [12]. We consider the case in which the energy parameter is uniform [13] and simulation is performed at  $k_B T/\epsilon$  of 0.3 which corresponds approximately to the room temperature. We consider stretching at constant speed and attach the two termini to elastic springs. The N-terminal spring is anchored to a substrate and the C-terminal is pulled at a constant velocity  $v_p$ . This allows to monitor the resistance force,  $F$ , as a function of the pulling spring displacement  $d$ . Here  $v_p=0.005$  A/ $\tau$  which is roughly about 100 times faster than usual experimental speeds.

The analysis of the knot-related characteristics is made along the lines described in [5]. At each instant, the ends of the knot are found using the KMT algorithm [9]. In that way we obtain the trajectories of knot's ends, an example of which is shown in Fig. 2. They are characterized by sudden jumps, either to the entirely tightened configuration, or to the metastable locations.

As discussed in [5], such metastable locations are associated with the sharp turns of the protein backbone in which amino acids Pro and Gly often reside.

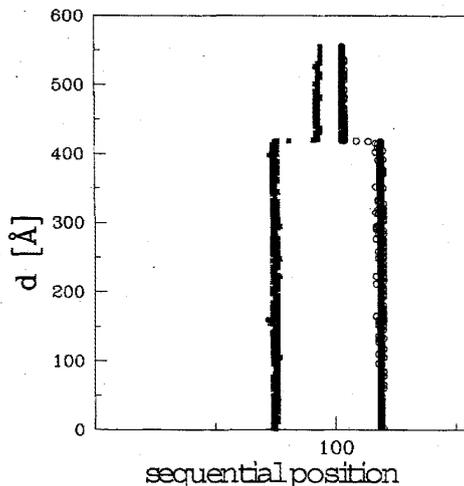


Figure 2: Evolution of knot's ends during stretching of 1j85. The two lines denote sequential positions of those ends. In this case, the tightening of the knot involves a single jump in the location of the knot ends.

In our analysis, the YibK superfamily is represented by a protein with the PDB code 1j85. This protein contains a knot of type  $3_1$  between amino acids 72 and 120 in the native conformation. We have shown previously [6] that the biggest force of resistance to stretching,  $F_{max}$ , originates from shear-breaking secondary structure inside the knotted core. Two alternative unfolding pathways have been observed, and the corresponding unfolding curves are shown in the two left panels of Fig. 3. These pathways are characterized by the presence of two main peaks, at  $d \approx 300\text{\AA}$  and  $d \approx 400\text{\AA}$ . In each pathway, the higher peak corresponds to tightening of the knot. The main difference between both pathways is observed in the ordering of the peaks: in pathway I tightening of the knot corresponds to the last event during stretching, while in pathway II the order of these two peaks is reversed.

Subsequently, we have analyzed the protein rebuilt in two ways. We find that in both those cases there are also two similar looking unfolding pathways, as shown in the right panels of Fig. 3. Similarly to the knotted case, those pathways are also characterized by two main peaks, the order of which is different in both trajectories. This shows that in the case of 1j85 and for the particular way of building the unknotted constructs, the presence of the knot does not influence the maximal resistance to stretching of the protein in a significant way. However, it does affect the force-displacement pattern.

### 3 Conclusions

In this note, we summarized how one can describe and analyze the process of knot tightening in proteins, and discussed how presence of knots can influence mechanical properties of proteins. We focused on a rather small knotted protein 1j85 from YibK family and compared its mechanical properties with those of a synthetic, unknotted construct obtained by reversing the order of one of the backbone crossings in 1j85. We found that the original and rebuilt proteins behave alike – in particular they have two characteristic unfolding pathways with the highest peak representing

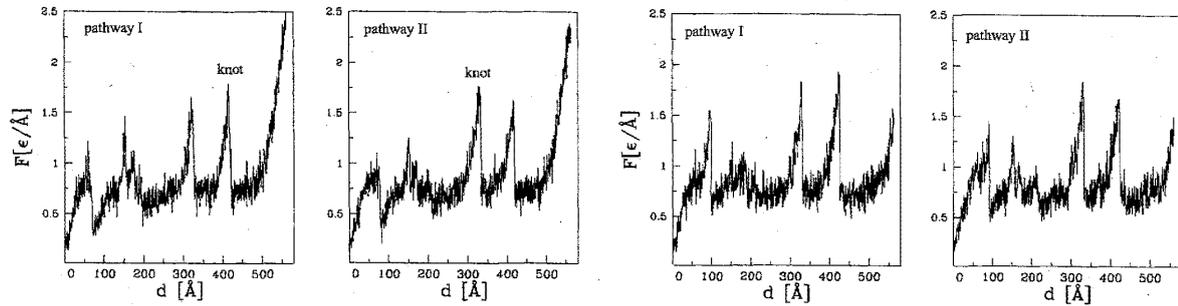


Figure 3: Force ( $F$ )–displacement ( $d$ ) curves for two possible pathways of the unfolding of 1j85 (left panels) and its rebuilt knotless synthetic construct (right panels).

knot tightening. Therefore the presence of a knot does not enhance the mechanical resistance of 1j85. These results are in agreement with [10], but are a little opposite to the findings of Ref. [6]. This proves that the analysis of a single case is not sufficient to determine general impact of knots on proteins. Nonetheless, even though  $F_{max}$  is similar in both pathways, the details of other peaks are different. This is a manifestation of the presence of the knot on the dynamical behavior of the protein. It is also likely that yet another way of rebuilding 1j85 could lead to a protein which is indeed much less resistant than the original knotted structure. Finally, the knot in 1j85 is not as deep as in Ref. [6], which also may be a reason why its impact is not so overwhelming. To summarize, the mechanical role of a knot in a protein appears to depend on the protein and remains to be explored further.

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