Jamming Proteins with Slipknots and Their Free Energy Landscape

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Theoretical studies of stretching proteins with slipknots reveal a surprising growth of their unfolding times when the stretching force crosses an intermediate threshold. This behavior arises as a consequence of the existence of alternative unfolding routes that are dominant at different force ranges. The existence of an intermediate, metastable configuration where the slipknot is jammed is responsible for longer unfolding times at higher forces. Simulations are performed with a coarse-grained model with further quantification using a refined description of the geometry of the slipknots. The simulation data are used to determine the free energy landscape of the protein, which supports recent analytical predictions.

FIG. 1 (color online). Dependence of the unfolding times \( \tau \) on the stretching force \( F \) for 1E2I (solid line, in red). In this Letter we describe this mechanism as a superposition of two unfolding pathways: I for small forces [dashed (lower) line, in blue], and II for intermediate and large forces [dash-dotted (upper) line, green].
by realizing that unfolding is dominated by two distinct, alternative routes that are dominant at different force regimes. A routing switch occurs when threshold is crossed between weak and intermediate forces. At higher forces, mechanical unfolding is dominated by a route that involves a jammed slipknot. This jamming gives rise to the unexpected dependence of unfolding time on applied force. Characterizing this mechanism is the central goal of this Letter.

To describe the evolution of a slipknot quantitatively requires a refined description. A slipknot is characterized by the three points shown in Fig. 2. The first point \( k_1 \) is determined by eliminating amino acids consecutively from one terminus until the knot configuration is reached (which can be detected, e.g., by applying the Koniaris-Muthukumar-Taylor algorithm [20]). The two additional points, \( k_2 \) and \( k_3 \), correspond to the ends of this knot. In the native state the protein 1E2I contains a slipknot with \( k_1 = 10 \), \( k_2 = 128 \), \( k_3 = 298 \). These three points divide the slipknot into two loops, which are called the knotting loop \( \kappa \) and the threaded loop \( \ell \). The former one is the loop of the trefoil knot and the latter one is threaded through the knotting loop. Unfolding of the slipknot upon stretching depends on the relative shrinking velocity of these two loops (see Fig. 3). When the threaded loop shrinks faster than the knotting loop, the slipknot unties. In the opposite case the slipknot gets (temporarily) tightened or jammed, resulting in a metastable state associated with a local minimum in the protein’s FEL. Upon further stretching, this configuration eventually also unties. The evolution of both loops of the slipknot is encoded in the time dependence of the points \( k_1, k_2, k_3 \), see Fig. 3.

Before discussing the stretching of 1E2I, we explain why a slipknot formed by a uniformly elastic polymer should smoothly unfold under stretching. To simplify the discussion we approximate the threaded and knotting loops by circles of size \( R_k \) and \( R_{\ell} \). These two loops shrink during stretching and, when the threaded one eventually vanishes, the slipknot gets untied. If both loops have similar sizes, the slipknot is very unstable and unties immediately. When the threaded loop is much larger than the knotting one, \( R_{\ell} \gg R_k \), loosening can be explained as follows. The elastic energy associated to local bending is proportional to the square of the curvature. If the loop is approximated by a circle of radius \( R \), then its local curvature is constant and equals \( R^{-1} \). The total elastic energy is \( \frac{1}{2} ds R^{-2} \sim R^{-1} \) [21]. From the assumption \( R_{\ell} \gg R_k \) we conclude that upon stretching it is energetically favorable to decrease \( R_{\ell} \) rather than \( R_k \). This happens until both radii become equal and then, just as above, the slipknot gets very unstable and loosens. In this discussion we have not yet taken into account that when a slipknot is stretched some parts of a chain slide along each other. This effect could be incorporated by including the friction generated by the sliding [22]. But in the slipknot the sliding region associated with the knotting loop is much longer than the region associated to the threaded loop. Thus this effect results in a faster tightening of the threaded rather than the knotting loop, facilitating even more the loosening of the slipknot.

The above argument should apply to slipknots in biomolecules because they are characterized by a persistence length that in principle is simply related to their elasticity [23]. For DNA this effect is described by wormlike-chain models (WLC) [24] and it has been confirmed experimentally. Although WLC models are too simple to describe the protein general behavior, they are useful in some limited applications. Thus at first sight one might expect that slipknots in proteins should smoothly untie upon stretching. Proteins, however, are much more complicated than DNA or uniformly elastic polymers. The presence of stabilizing native tertiary contacts leads to a jumping charac-

FIG. 2 (color online). A slipknot (left) consists of a threaded loop \( (k_1 \rightarrow k_2, \text{in red or gray}) \) which is partially threaded through a knotting loop \( (k_2 \rightarrow k_3, \text{in blue or dark gray}) \). An example of a protein configuration with a tightened slipknot is shown in the right panel.
ter during stretching [12]. In addition their bending energy is not uniform along the chain due to the heterogeneity of the amino-acid sequence. As a consequence it turns out that the intuition obtained through the above analysis of polymers or WLC models is misleading.

Our analysis of the evolution of the endpoints $k_1$, $k_2$, $k_3$ (Fig. 3, bottom) reveals that for various stretching forces unfolding proceeds along two distinct pathways (Fig. 3, top). In pathway I the slipknot smoothly unties, which is observed for relatively weak forces. At intermediate forces pathway II starts to dominate and the knotting loop can shrink tightly before the threaded one vanishes. In this regime the protein gets temporarily jammed (Fig. 3, right), leading to much longer unfolding times (catch pathway). The probability of choosing pathway I at different forces is shown in Fig. 4. This pathway competition explains the nontrivial total unfolding time dependence observed in Fig. 1.

The two different pathways I and II arise from completely different unfolding mechanisms. Pathway I starts and continues mostly from the C-terminal side, along 16α, 15β, 14α, 13β, 12 (helices bundle), 11α (here the number denotes a consecutive secondary structure as counted from N terminal, and α or β specifies whether this is a helix or a β sheet; for more details about the structure of 1E21 see the PDB). This is followed by unfolding of helices 11α, 10α that allows breaking of the contacts inside the β sheet created by the N terminal, with unfolding proceeding also from the N terminal. Pathway II also starts from the C terminal but rapidly (as soon as helix 15 is unfolded) switches to the N terminal. In this case, differently from pathway I, the β sheet from the N terminal unfolds even before 13β. These scenarios indicate that the pathway I should be dominant at weak forces since they are not sufficient to break the β sheet during the first steps of unfolding. The jammed pathway is typical only if stretching forces are sufficiently strong for unfolding to proceed from the two terminals of the protein.

A similar phenomenon was firstly proposed in Ref. [25] and referred to as catch bonds. Experimental evidence suggesting this mechanism was first observed for adhesion complexes [26,27]. Using atomic force microscopy (AFM), at large forces the ligand-receptor pair becomes entangled and therefore expands the unfolding time. A theoretical description of this mechanism was given in Refs. [28–30].

The kinetic data can also be used to determine the associated free energy landscape (FEL) [7]. In an initial simplification we associate the barriers along the stretching coordinate as the kinetic bottlenecks during the mechanical unfolding event. Generalizing Bell’s model, a recent description of two-state mechanical unfolding in the presence of a single transition barrier has been developed in [19], with the rate equation

$$
\tau(F) = \tau_0 \left( 1 - \frac{\nu F x^+}{\Delta G} \right)^{1-1/\nu} e^{-\left( \Delta G/k_BT \right) \left( 1 - (1 - \nu F x^+ / \Delta G)^{1/\nu} \right)},
$$

where $\nu$ encodes the shape of the barrier. Here $x^+$ denotes the distance between the barrier and the unfolded basin (in a first approximation it can be regarded as $F$ independent) and lies on the reaction coordinate along the AFM pulling direction. It can be experimentally determined by measuring how the stretching force modulates the unfolding times $\tau$. The height of the barrier is denoted by $\Delta G$. Figure 1 (unfolding times are given by solid red line) shows that this single barrier theory is not sufficient for the full range of forces. As described before, in the higher force regime, additional basins have to be included in the energy landscape. Models with several metastable basins have been called multistate FEL models [31]. Evidence supporting the need of multistates FEL was confirmed by AFM experiments in different systems [32,33].

To construct a multistate FEL that incorporates two unfolding pathways I and II we use a linear combination of Eq. (1)-like expressions with different shapes and barrier heights. Each one of them essentially accounts for the distinct barrier along a relevant unfolding route. Fitting the stretching data to Eq. (1) with a cusplike $\nu = 1/2$ approximation (another possibility $\nu = 2/3$ for the cubic potential in general leads to similar results [19]) determines accurately the location and the height of the potential barriers. Pathway II involves two barriers: first until the moment of creation of the intermediate which is followed the untieing event. They are characterized by $(x_1, \Delta G_1)$ and $(x_2, \Delta G_2)$ arising, respectively, from the lower and upper fits in Fig. 5 (left). The superposition of these two fits gives the overall mean unfolding time for pathway II [dotted-dashed (upper) green curve in Fig. 1]. For the ordinary slipknot unfolding (pathway I), the results $x_i$ and $G_i$ arise from the dashed blue curve in Fig. 1. This analysis leads to the results

$$
x_1 = 2.3 \frac{k_BT}{\epsilon}, \quad x_2 = 0.7 \frac{k_BT}{\epsilon}, \quad x_f = 1.4 \frac{k_BT}{\epsilon},
$$
$$
\Delta G_1 = 8.0k_BT, \quad \Delta G_2 = 4.2k_BT, \quad \Delta G_f = 4.7k_BT.
$$

We conclude that the free energy landscape consists of two
elastic polymers. In this Letter we concentrated on protein forces. This phenomenon does not exist for uniformly corresponding free energy landscape. Its main feature is the presence of the slipknot in protein 1E2I and determined the valleys in Fig. 5 (right).

The force-dependent probability of choosing one of the valleys during stretching depends on the details of the protein structure. It is determined from our simulations as shown in Fig. 4. Using these probability values and the parameters above for $x$ and $\Delta G$, we can accurately represent the simulation data using a linear combination of equations of the form (1). This agreement supports our analysis and generalizes Eq. (1) for the full range of forces. In addition it demonstrates that structure-based models sufficiently capture the major geometrical properties of a slipknoted protein. A schematic representation of the free energy landscape for pathway II is shown in Fig. 5 (right).

Summarizing, we have analyzed the process of tightening of the slipknot in protein 1E2I and determined the corresponding free energy landscape. Its main feature is the presence of a metastable configuration with a tightened slipknot, which is observed for sufficiently large pulling forces. This phenomenon does not exist for uniformly elastic polymers. In this Letter we concentrated on protein 1E2I but similar behavior has also been observed for other proteins with slipknots, e.g., 1P6X. Our results provide testable predictions that can now be verified by AFM stretching experiments.

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