“Computational study of the degradation mechanisms of knotted proteins mediated by Clp biological nanomachine”

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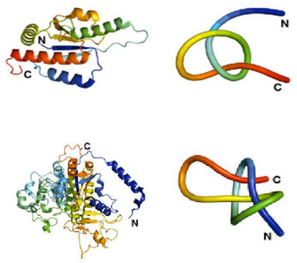
Abstract: Molecular knots can occur in biological macromolecules such as DNA, RNA and proteins. It has already been shown that difficulties in degrading knotted proteins can result in a variety of neurodegenerative diseases. However, the details of unfolding and translocation of knotted substrate proteins (SP) by ATP-dependent proteases (ATPase) nanomachines are not fully understood. The central focus of this computational study is to investigate the mechanism of translocation and unfolding of knotted SP by bacterial caseinolytic protease (Clp) ATPases. With this goal, we use an implicit solvent model to perform Langevin dynamics simulations of unfolding and translocation of knotted substrate proteins (SPs), with knots of three different types, through the narrow central pore of the ATPase ClpY. By conducting an analysis of the time evolution of native and non-native contacts, we find that the non-native contacts play a fundamental role in determining knot size and controlling knot diffusion. We also observe, in good agreement with results from knotted biopolymers, that the translocation of knotted proteins occurs in two main regimes, tension propagation and tail retraction.

Knotted Proteins



- Molecular knots are topological structures present in long polymers chains.[1]
- In the early 1990s, the hypothesis of knotted proteins was confirmed.
- 6% of proteins in the protein data bank are entangled and have knots.
- Knots could play a role in thermodynamic stability of proteins and also promote some molecular motions required for efficient catalysis.
- Neurodegenerative diseases may lead to some difficulties in degrading proteins .[2]
- Clp biological nanomachines perform unfolding and translocating a wide variety of substrate protein (SPs).

Details of the simulations performed

$$Q_N = (1/N_C) \sum_{i \neq j, j \pm 1} \theta\left[\eta - |r_{ij}(t) - r_{ij}^0|\right]$$

$r_{ij}(t)$ is the minimum distance between any two heavy atoms of residues i and j

$\theta(x)$ is the Heaviside step function for which $\theta(x) = 1$ if $x \geq 0$ and $\theta(x) = 0$ if $x < 0$, with tolerance $\eta = 2$ Å.

$Q_{NN} = N_{NC}/N_C$, where N_{NC} is the number of non-native heavy atom

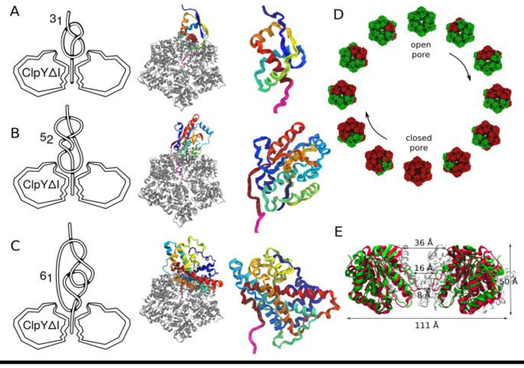
Fraction of native contacts (Q_N) and non-native contacts (Q_{NN}):

SP	Knot Type	Chain Length (residues)	N_{trajs}[a]
PDB ID 2EFV	3_1	82	38
Poly-glycine	3_1	82	36
PDB ID 2LEN	5_2	231	18
PDB ID 3BJX	6_1	295	20

[a]The number of simulation trajectories performed. The duration of each trajectory corresponds to 1000 Clp ATPase cycles of $\tau = 120$ ps for a total of 120 ns.

Systems Setups

Schematic view and molecular representations of the simulation setup:



Left and middle panels:

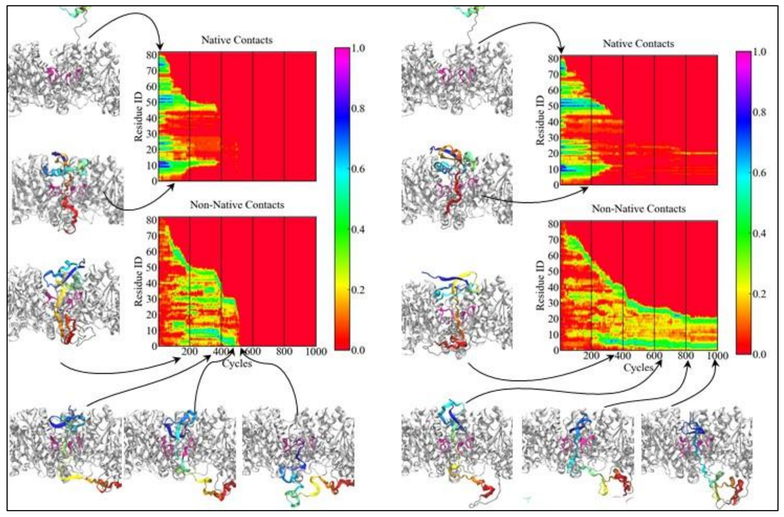
A) 3_1 knotted protein

B) 5_2 knotted protein

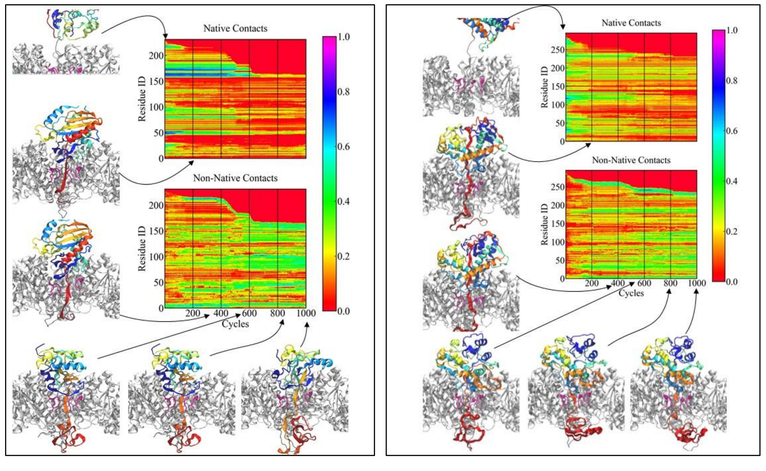
C) 6_1 knotted protein

Results

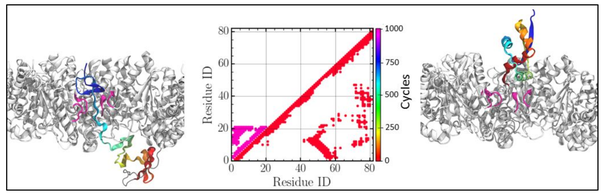
Q_N and Q_{NN} for the SP with 3_1 knot-type for low (A) and high (B) energy pathways

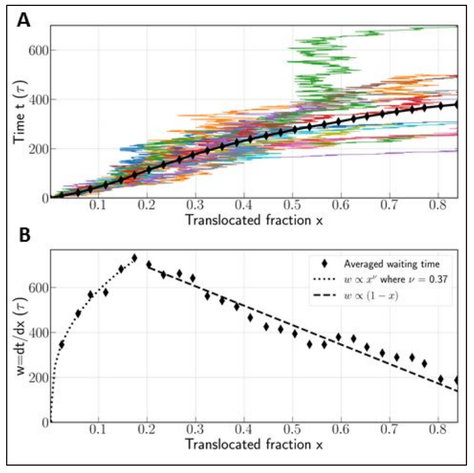


Q_N and Q_{NN} time evolution of the SPs with 5_2 (left) and 6_1 (right) knots



Time evolution of contact map for the ClpYΔI- 3_1 system with high energy barrier





(A) Polyglycine SP’s average translocated fraction time evolution

(B) Tension propagation and tail retraction regimes

Conclusions

- Based on the three knotted proteins we selected, this study reveals that the knots in the backbone of proteins reduce the translocation speed significantly when compared with typical substrate proteins such as Titin I27 or GFP
- The 3_1 cases are in good agreement with experimental results, for 5_2 and 6_1 the degradation is stalled at short timescales. To the best of our knowledge there is no experimental investigation which would allow us to make a direct comparison.
- In about half of the simulated trajectories, the 3_1 knot slowly moves toward the N-terminal and slides off the chain. For the remaining trajectories after the translocation of the knot towards the N-terminal, the knots get tightened and the translocation process stops

(1) Molecular knots in biology and chemistry. N. Lim and S. Jackson J. of Physics: Cond. Matter 27,35 **2015.**

(2) An exploration of the universe of polyglutamine structures. A. Gomez- Sicilia, M. Sikora, and M. Cieplak, and M. Carrion-Vazquez. PLoS Comp. Biology 11, 10 **2015**.

(3) Posttranslational quality control: folding, refolding, and degrading protein. S. Wickner, M. R. Maurizi, S. Gottesman. Science 286, 5446 **1999.**

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