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Unfolding knots by the proteasome: behaviour of folded and neurotoxic proteins – Electronic Supplementary Information

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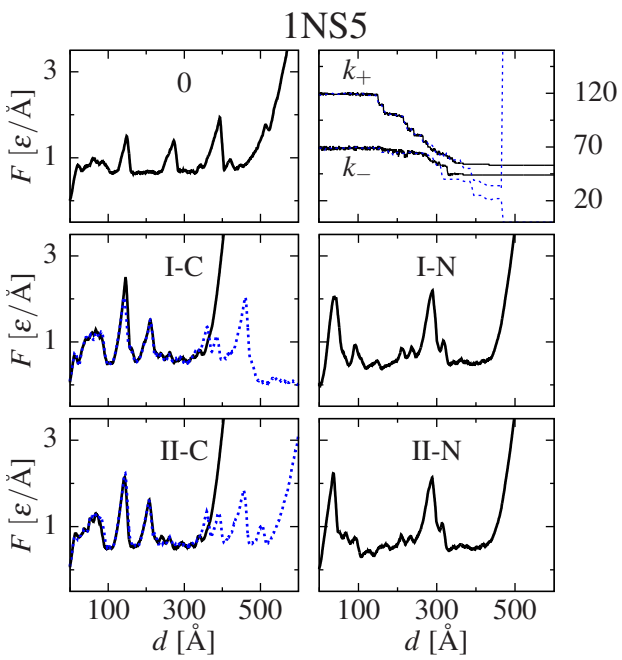

Fig. S1 Example F–d traces of the deeply knotted protein 1NS5 pulled at constant speed. The top-right panel shows the locations of the knot ends when pulled by the proteasome in protocol I-C. Two trajectories are shown. In one trajectory (dotted lines), the knot unties and the protein translocates. In the other (solid lines), the protein jams the channel and the tightened knot arrives at a permanent location. The remaining panels show examples of the F–d profiles at constant speed for 1NS5 for the protocols indicated. The profiles have been selected out of 100 trajectories for each protocol. The percentages of the jamming situations are 68, 96, 71, and 100% for the protocols I-C, I-N, II-C, and II-N respectively. The profiles shown in the middle-left panel correspond to the trajectories shown in the top-right panel.

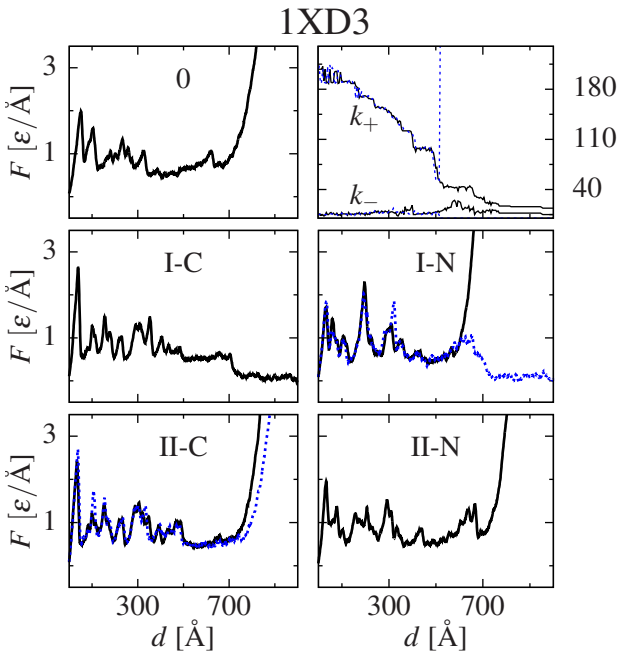

Fig. S2 Similar to S1 Fig. but for protein 1XD3. The top-right panel with the knot ends corresponds to the trajectories shown the bottom-left panel, *i.e.* in protocol II-C. The profiles have been selected out of 100 trajectories for each protocol. The percentages of the jamming situations are 0, 65, 6, and 100% for the protocols I-C, I-N, II-C, and II-N respectively.

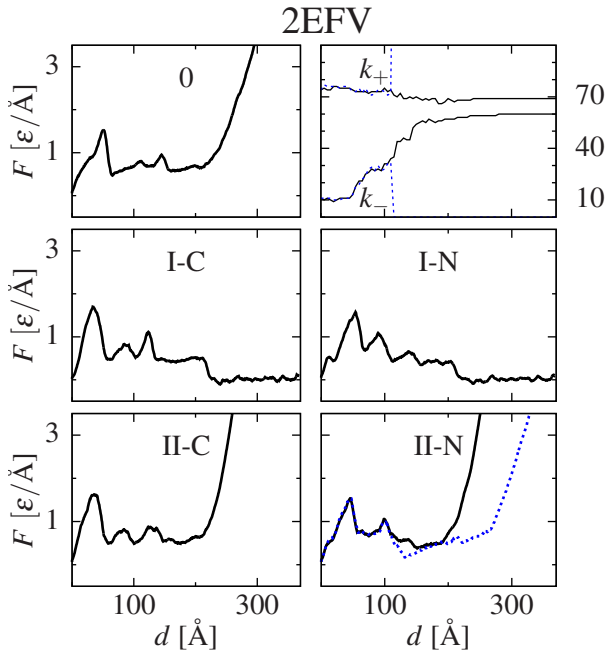

Fig. S3 Similar to S1 Fig. but for protein 2EFV. The top-right panel with the knot ends corresponds to the trajectories shown the bottom-right panel, i.e. in protocol II-N. The profiles have been selected out of 100 trajectories for each protocol. The percentages of the jamming situations are 0, 0, 100, and 70% for the protocols I-C, I-N, II-C, and II-N respectively.

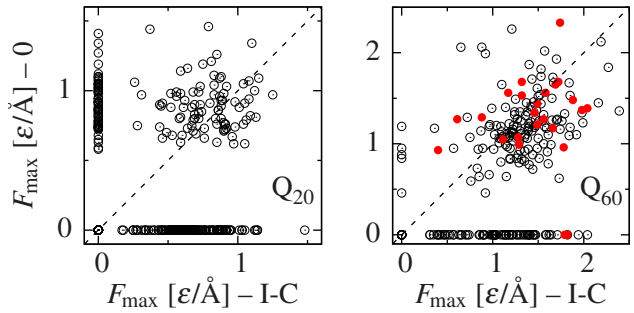

Fig. S4 Scatter plot of the mechanical stabilites of each polyQ confromer when pulled in protocol 0 (Y-axis) *vs*. protocol I-C (X-axis). Each point corresponds to one conformation. The knotted conformations of Q_{60} are highlighted with filled red dots. As previously observed for structured proteins[32], mechanical stability does not show any relation in these two pulling modes.

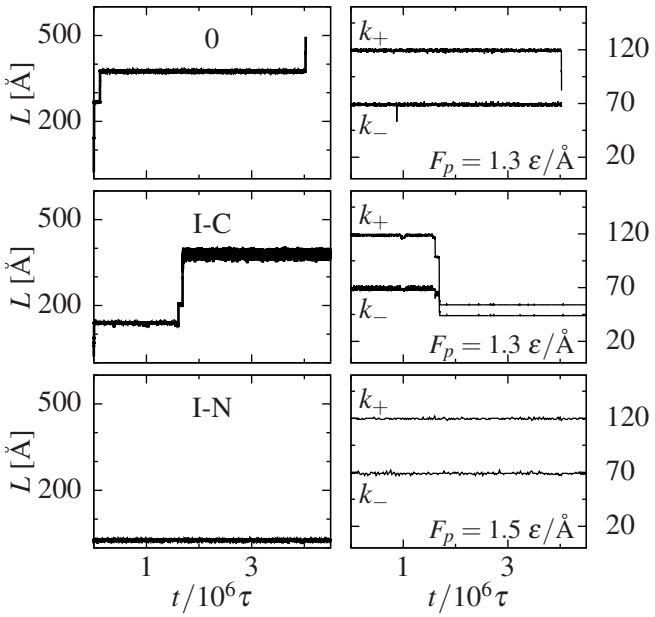

Fig. S5 Examples of the trajectories for 1NS5 when pulled with a constant force. For this protein, the knot can untie (top), tighten (middle) or stay put (bottom). Translocation is only achieved in the first case. 10 trajectories were obtained for each of the three protocols. In protocol 0, the median unfolding time is 980 530, 136 706, 40 817, 2801, and 1720 τ for F_p equal to 1.3, 1.4, 1.5, 2.0, and 2.5 ε/Å respectively. In each of the trajectories, the knot gets tightened. The native values of k_- and k_+ are 69 and 119. On tightening, the knot ends move to 44 and 54 respectively. In protocol I-C, the knot gets tightened but there is no translocation within the cutoff time of 10^7 τ for F_p of 1.3, 1.5, 2.0, and 2.5 ε/Å. In protocol I-N, there is no translocation either, but for F_p of 1.3 and 1.5 ε/Å the knot does not move sequentially whereas it gets tightened for F_p of 2.0 and 2.5 ε/Å.

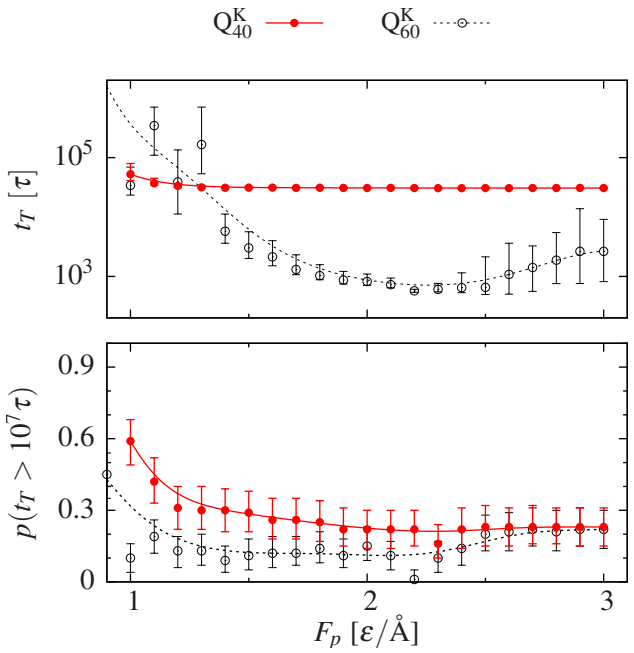

Fig. S6 Translocation time (top) and stalling probability (bottom) for Q_{40}. Our previous results did not yield knotted conformations for Q_{40}, probably due to the small statistics. Nonetheless, Q_{40} is above the threshold of most polyglutaminopathies (~35) and so the presence of knots in Q_{40} should prove difficult to translocate, as with Q_{60}. In order to model this effect, The knotted conformations of Q_{60} were cut from both ends in such a way that the total length became 40 residues and the knot was centered, i.e. there are the same number of residues between k_+ and the C-terminus and between k_- and the N-terminus. As expected, the behaviour at low, biologically relevant forces is qualitatively similar to that of Q_{60}, although both t_T and stalling probability are smaller for each particular force due to the fact that upon reducing the length, the knots are shallower. This is consistent with the fact that Q_{40} expansions, although prone to induce disease, result in less severe symptoms than Q_{60}. At medium and high forces the behaviour is much sturdier due to the faster tightening of the knot.

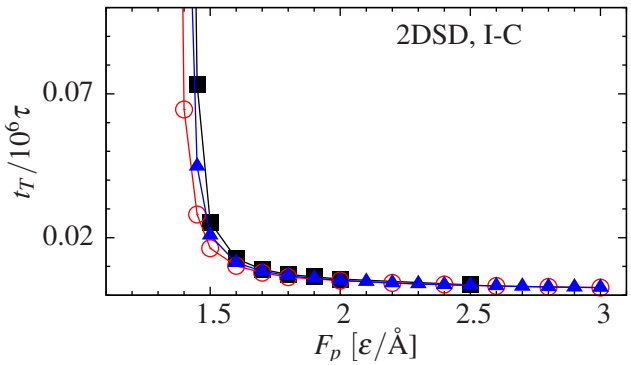

Fig. S7 Median translocation time as a function of F_p in the model in which the torus is replaced by 12 spheres. 100 trajectories are considered for each F_p. The black solid data points are for our standard torus-and-cylinder model. The blue triangles are for the model in which the torus is replaced by 12 identical spheres and the cylinder has a radius of 12 Å. The red open circles are for the model in which the radii of three consecutive spheres shrink periodically.