Pincus blob elasticity in an intrinsically disordered protein

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Abstract

Understanding the dynamic structure of intrinsically disordered proteins (IDPs) is important to deciphering their biological functions. Here, we exploit precision entropic elasticity measurements to infer the conformational behavior of a model IDP construct formed from the disordered tail of the neurofilament low-molecular weight protein. The IDP construct notably displays a low-force power-law elastic regime, consistent with the Pincus blob model, which allows direct extraction of the Flory exponent, $\boldsymbol{\nu}$, from the force-extension relationship. We find $\boldsymbol{\nu}$ increases with added denaturant, transitioning from a nearly ideal chain to a swollen chain in a manner quantitatively consistent with measurements of IDP dimensions from other experimental techniques. We suggest that measurements of entropic elasticity could be broadly useful in the study of IDP structure.

Keywords: intrinsically disordered proteins, magnetic tweezers, entropic elasticity, Pincus blobs

1 Introduction

Intrinsically disordered proteins (IDPs) do not fold into a well-defined three-dimensional structure in their native state, yet they remain biologically active [1, 2]. The existence of IDPs challenges the classic structure-function paradigm in which the higher-order structure of a protein dictates its functionality [3]. To fully understand the role of IDPs in various biological processes, detailed knowledge of their conformations would be valuable. In the case of folded proteins, their structures can be characterized experimentally with great precision, such as with crystallographic analyses. However, IDPs are dynamic and fluctuate across a broad conformational ensemble [4, 5], thus disallowing traditional approaches to measuring and classifying protein structure.

As an alternative, prior studies have modeled IDPs as random-walk polypeptide chains and used structural classifications derived from polymer physics scaling laws[6–8]. For example, Marsh and Forman-Kay compiled measurements of the hydrodynamic radii, R_h, of IDPs of various residue length N, and demonstrated that they followed a rough power-law scaling, $R_h \sim N^{\nu}$, with a near ideal-chain Flory exponent of $\nu \approx 1/2$ [6]. Later authors carried out direct high-precision measurements using single-molecule Forster energy transfer (smFRET) [7, 8] or small angle X-ray scattering (SAXS) [9–11], and employed a polymer physics framework to interpret the data, e.g. in terms of the scaling behavior of ensemble average quantities (such as mean-squared end-to-end distance), or of the probability distributions governing the full conformational ensemble. Generally, some caution is needed in applying simple polymer scaling models to IDPs, as such models are formulated for long, homopolymeric chains, whereas IDPs can be somewhat short and are always heteropolymeric. Thus, IDPs can have heterogeneous interactions along their contour that are not described by simple scaling theories; indeed, several prior works have observed evidence of such heterogeneity [12–17]. Yet, the examples above demonstrate the utility of polymer scaling models in providing a framework for interpreting the dynamic structure of IDPs [18].

A hallmark of polymers is the entropic elastic response, in which the internal conformational freedom of the chain provides an entropic force that resists increases in extension. The advent of single-molecule force spectroscopy enabled direct measurement of the elastic response of biopolymers [19], including IDPs [20]. Such measurements are typically carried out in the high force regime where the chain is nearly aligned in the direction of applied force, and the elastic response arises from a mix of enthalpic contributions and entropic contributions arising from small fluctuations controlled by the chain's local configurational freedom[21, 22]. However, as initially pointed out by Pincus [23], the single-chain entropic elastic response is quite rich at lower forces (i.e. forces, f, less than a few picoNewtons). At such forces, chains can exhibit featuring multiple distinct elastic regimes that are sensitive to other polymeric structural parameters. Of particular interest here is the intermediate-force regime, in which, from a scaling perspective, the chain can be modeled as a series of tensile (or Pincus) blobs of size given by the tensile screening length, k_BT/f, where k_BT is the thermal energy and f is the applied force. In this regime, the force-extension response follows a power-law scaling sensitive to the Flory exponent, $L \sim f^{1/\nu-1}$[23]. Previous single-molecule stretching studies have exploited the low-force capabilities of

magnetic tweezers to observe the Pincus blob elasticity regime in various biopolymers, using it to analyze solvent-quality and polyelectrolyte effects [24–27].

Here, we demonstrate the utility of measurements of entropic elasticity to quantify the structure of a disordered protein. Specifically, we use magnetic tweezers to observe Pincus blob elasticity within a model IDP construct, and, using the Flory exponent inferred from elasticity, we study the structure of the chain in various solvent conditions. The model chain is a construct formed from the disordered tail of the neurofilament low-molecular-weight (NFL) subunit protein. Neurofilaments are important constituents of the axonal cytoskeleton, and their disordered tail domains control the spacing and mechanical stability of the cytoskeletal network [28–30]. The NFL tail (NFLt) is a relatively highly charged protein (net charge per residue -0.23), yet our stretching experiments show that the construct, in low-ionic-strength aqueous solvent, is more compact than the sequence's net charge would suggest. We find that the Flory exponent, ν, increases upon adding the denaturant guanidinium hydrochloride (GuHCl); at the highest denaturant concentration, our estimate of ν is comparable to that expected for a polymer in good solvent. This observation is consistent with other studies on disordered or denatured proteins [7, 11], as well as recent FRET and SAXS measurements on segments of the NFLt [17]. Finally, the generality of the entropic-elastic response means that the single-molecule stretching method and analysis used here could be usefully applied to probing the structure of other IDPs.

2 Experimental Method

A recombinant NFLt construct was expressed in *E. coli* and purified by chromatography to >95% purity. The 163-residue NFLt sequence (available in Supplementary Information) was designed to include terminal cysteine residues. This allowed the linking of individual NFLt units through end-to-end disulfide bridges; the resulting polyprotein chains typically contained 10-30 units. Given the 62 nm contour length of a single 163-residue sequence, the resulting polyprotein chains were thus long enough ($>$ 500 nm) for magnetic tweezer analysis. The disulfide-linked polyproteins were labeled at each terminus with, respectively, azide and biotin groups, permitting specific attachments to a functionalized glass surface and 1 μm-diameter magnetic beads as needed for mechanical manipulation. Details on the synthesis and characterization of the polyprotein construct are described in a previous study [15].

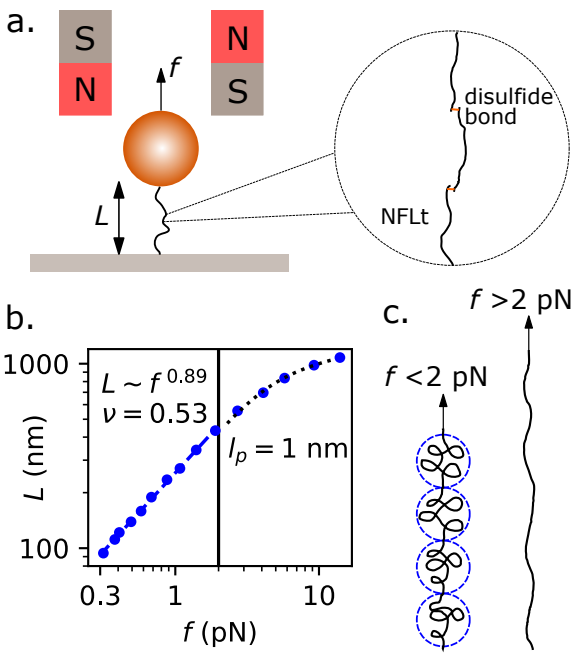

Fig. 1 (a) Schematics of a magnetic tweezers experiment. A polyprotein is immobilized on a glass surface and attached to a magnetic bead. A pair of magnets attracts the bead and stretches the polymer with a force f, causing the polyprotein to have an extension L. The NFLt polyprotein construct is made from individual proteins linked by disulfide bonds. (b) A force-extension curve of an individual polyprotein in aqueous (10 mM MES buffer pH 6.8), showing two distinct elastic regimes: a low force power-law scaling (blue dashed line), and a high-force asymptotic elasticity consistent with the WLC model (black dotted line). (c) Schematics of polymer configurations under different applied forces. At low forces ($f < 2$pN), the polymer has significant looping, and behaves like a chain of Pincus blobs. At higher forces ($f > 2$pN), the loops are pulled out, and the polymer extension approaches its contour length.

Magnetic tweezer experiments were carried out by, first, constructing a glass flow cell using an NHS-functionalized, PEG-coated glass surfaces (Microsurfaces, Inc.) that were treated with 5 mg/mL dibenzocyclooctyne-amine (DBCO-amine) in 1:1 DMSO:phosphate buffered saline pH 8.3 for 1 hour. Azide-labeled polyproteins (~1 μM) in 10 mM tris buffer pH 7.4 with 0.1% Tween-20 were incubated in the flowcell for 24 hours, allowing coupling of the azide on the polyprotein to the DBCO on the surface. Unbound polyproteins were washed away with the same buffer solution. Streptavidin-coated, 1 μm-diameter magnetic beads (Dynabeads) were incubated for 10 minutes for attachment to the polyproteins, thus forming stretchable tethers; excess beads were then rinsed away. Elastic measurements were then performed in a custom-built magnetic tweezers setup that applies force to the tethers through an externally-applied magnetic field. The applied force and extension were quantified using image-based bead tracking and analysis of the bead's thermal fluctuations, as previously described [31, 32]. Experiments were performed in 10 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer at pH 6.8 with 0.1% Tween-20.

3 Results and Discussions

We polymerized the NFLt constructs, attached their termini to a glass surface and a magnetic bead, and used magnetic tweezers to carry out elastic measurements over a range of forces as depicted in Fig. 1a. A typical force-extension measurement, carried out in low-ionic-strength aqueous solution, is shown in Fig. 1b. As seen there, the elastic response of a single chain shows a low force (subpicoNewton) power-law regime along with a high force regime where the chain becomes less compliant. This curve shape is similar to that found previously for other biopolymers [24–27], and is indicative of Pincus-blob elasticity at low forces. In this regime, the chain displays significant loop formation, and long-range monomer interactions are allowed. As the force increases beyond a few picoNewtons, the loops are pulled out, and the compliance of the chain accordingly decreases. In this regime, the chain asymptotically approaches its contour length, L_c. The crossover force, f_c between the regimes is expected to occur when the tensile screening length, $\xi \equiv k_B T/f$, is roughly equal to the chain's persistence length, l_p [23, 27]. Thus, the picoNewton scale of f_c indicates that l_p is of nanometer scale.

Estimates of l_p are found by analyzing the asymptotic regime at high force, which we find to be well-described by the Marko-Siggia worm-like chain (WLC) model [19] (dotted line, Fig. 1b). As Schwarzl et al. noted, the WLC model is inexact in its application to polypeptides; they suggested it is superseded by a modified freely-rotating chain model that is more accurate when the chain contour length is known [21]. However, the polydispersity that results from our synthesis scheme does not allow *a priori* knowledge of the contour length; further, we note that detailed analysis of the local stiffness is not a focus of this study. We thus proceeded to fit the force-extension data to an analytical approximation of the WLC model derived by Bouchiat [33]. Because the WLC model does not consider long-loop, excluded-volume interactions, we only fit it to the data at forces above f_c, here estimated to be 2 pN. The fitted parameters are not sensitive to small changes in the value of f_c used as a cutoff. We found the average l_p from 11 individual polyprotein molecules to be 1.07 ± 0.07 nm (error reported is the standard deviation). This value is within the broad range of $0.4 - 2$ nm found in previous studies on polypeptides [34–36], disordered proteins [37], and denatured proteins [38–40]. Thus, the measured local flexibility is consistent with the polyprotein construct acting as an IDP without significant secondary structures.

We further directly extract the Flory exponent of the polyprotein from the force-extension curve by analyzing the Pincus elasticity regime ($f < 2$ pN). The Pincus blob model indicates that the expected force-extension scaling relationship is $L \sim f^{1/\nu - 1}$ in this regime [23]. By fitting a power-law to the $f < 2$ pN data and applying this scaling relation, we find the Flory exponent of the NF tail in low-ionic-strength aqueous solution to be 0.52 ± 0.01. This value is distinctly lower than the value $\nu = 0.6$ expected of a swollen, self-avoiding chain, i.e. a polymer in good solvent. Such a swollen-chain exponent might have been expected given the highly negatively charged nature of the sequence. Prior studies on homogeneously negatively charged biopolymers, specifically nucleic acids and polysaccharides, observed elastic responses consistent with swollen-chain exponents in low salt conditions [24–26]. Instead, the Flory exponent of the NFLt construct is more consistent with that of an ideal chain. Because the ideal

chain model ignores interactions between monomers, this finding suggests that the repulsive electrostatic monomer interactions are counterbalanced by an attraction in these conditions.

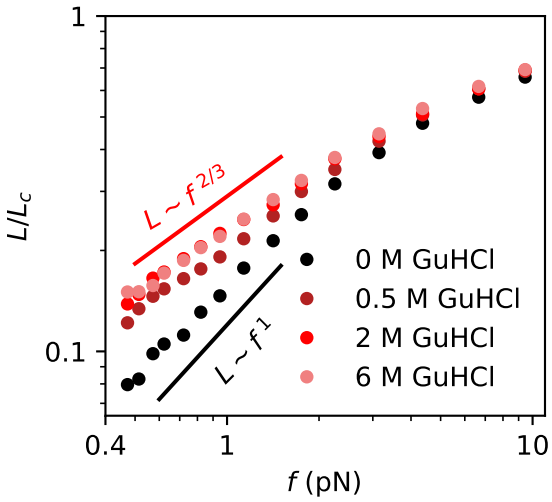

Fig. 2 Force-extension data on an individual NFLt polyprotein in various GuHCl concentrations. The extension is normalized by the contour length. All solutions contain 10 mM MES buffer at pH 6.8, along with the indicated GuHCl concentration.

To probe the nature of the self-attractions within the NFLt construct, we measured its elastic response in a range of concentrations of the denaturant, guanidinium hydrochloride (GuHCl). GuHCl is known to disrupt the globular state of folded proteins and promote polymer-solvent interactions, thus high GuHCl concentrations are effectively a good solvent condition for the protein backbone [41]. We found the power-law exponent of the Pincus elastic regime to monotonically decrease with increasing denaturant (Fig. 2), indicating an improvement in solvent quality. Indeed, in the highest denaturant concentration (6 M GuHCl), the force-extension power law exponent changed from ≈ 1, characteristic of an ideal chain, to near $2/3$, which is expected of a swollen chain in good solvent.

We compare our findings on the variation with denaturant of the Flory exponent, ν, to previous studies on other disordered proteins in Fig. 3 [7, 11]. Our results are quantitatively nearly identical to those of several proteins analyzed in prior works, including the foldable spectrin domain R17 (net charge per residue -0.09) [7], the IDP HIV integrase (net charge per residue -0.07) [7], and the disordered yet hydrophobic and low charge N-terminal domain of the protein pertactin (net charge per residue -0.01) [11]. While Fig. 3 demonstrates a clear agreement between our results and the prior works, two distinct differences are also observable: the highly-charged IDP prothymosin-α (net charge per residue -0.38) has a significantly higher ν at all [GuHCl], an effect attributed to large electrostatic repulsion that drives that chain beyond the swollen-chain exponent and towards that of a rod [7]. The second difference is that, upon increasing denaturant concentration from zero, prothymosin-α and HIV integrase were

observed to initially become more compact, i.e. the Flory exponents decreased [7]. This phenomenon was observed in more than one experimental method [7, 10, 42] and was attributed to an electrostatic screening effect of the ionic denaturant. It is perhaps surprising that the NFLt, which has a considerable negative charge (net charge per residue -0.23), does not display either of the electrostatic phenomena observed in charged proteins in other works.

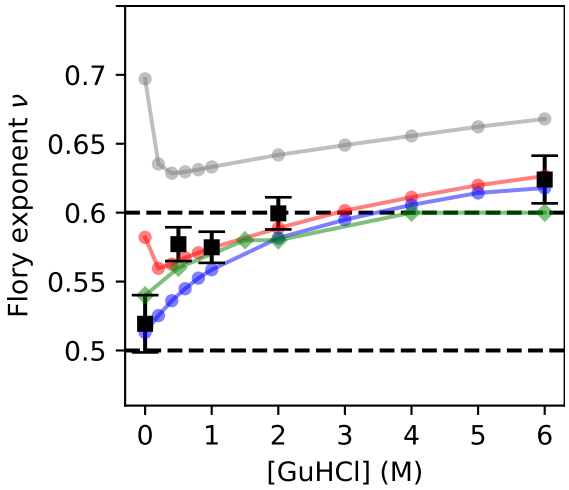

Fig. 3 Changes in the Flory exponent, ν, of the NFLt construct with [GuHCl], as inferred from our elastic measurements (black squares). Each point represents 1-11 measurements from individual polyproteins, with errors reported as standard deviations. Also plotted are estimates of ν vs. [GuHCl] from Hofmann et al. [7] on different proteins measured with single-molecule FRET, including prothymosin-α (ProTα, grey circles), HIV integrase (HIV-IN, red circles), and spectrin domain R17 (R17, blue circles); and from Riback et al. [11] on pertactin N-terminus (PNt, green diamonds) measured with SAXS.

It is possible that the two differences between our measurements of the Flory exponent of the NFLt construct and those of other negatively-charged IDPs are due to differences in the propensity for self-interactions of the various chains. A prior work on a similar NFLt construct found evidence for glassy relaxation dynamics [15], which was attributed to electrostatic or hydrophobic interactions within the chain. The data presented here also indicates a propensity for intramolecular attractions in this NFLt construct. Such self-attractions would cause the chain to be more compact, with a smaller ν, than a chain with similar charge density but lacking such interactions, in line with the data shown in Fig. 3. Generally, the Flory exponent measured from stretching experiments is a global estimate of chain conformation that effectively averages over any intrachain configurational heterogeneity; for the NFLt, the combination of the highly charged C-terminus and more neutral and hydrophobic N-terminus might lead to the a ν value lower than expected for a sequence with a more homogeneously distributed net charge.

Alternatively, or in addition, the differences in the behavior of the Flory exponents in Fig. 3 could be caused by the differing techniques and constructs used in the various studies. Our work relies on low-force elasticity measurements of long polyprotein constructs and estimates ν using a very general, model-independent scaling relation. Meanwhile, smFRET [7, 8] and SAXS [11] studies must convert the radii of gyration, R_g, of individual proteins, as estimated from experimental measurements, into Flory exponents, which is a nontrivial task that requires making assumptions about chain behavior [8, 10]. In comparison, the model independence of the elastic approach is an advantage; however, its use of long polyprotein constructs is a disadvantage. A more subtle issue with the elastic approach lies in the possible bias of ν by the applied tension: the Pincus blob picture assumes that the intra-blob polymer structure is completely dominated by thermal fluctuations, with tension being negligible. Yet, some small effects of tension should be present, and indeed, prior simulations indicated a slight stretching within the blobs by the applied force [43].

4 Conclusions

We demonstrate the use of single-molecule force experiments as a means to investigate the conformations of IDPs, specifically by allowing, from a single curve, estimates of both the Flory exponent (through analysis of the Pincus blob elastic regime at relative low force) and the persistence length (through analysis of the high-force elastic regime). Our specific results for the NFLt construct show that, despite a relatively high net charge, the chain shows a tendency towards compactness in aqueous buffer, as indicated by a Flory exponent that is comparable to that expected for an ideal chain. This compactness is removed with added denaturant, in good agreement with other studies, indicating the likely role of sequence-dependent self-attractions on chain conformation.

Overall, we suggest that the elastic analysis framework presented here provides a new tool for the study of IDPs and their interactions with the solution environment. There is significant literature that analyzes the dimensions of disordered or denatured proteins, with some opposing evidence that has been attributed to specific experimental differences [12, 44]. The elastic approach could thus be useful in enabling an alternative perspective and methodology in approaching this problem.

5 Acknowledgements

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6 Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

7 Author Contribution Statement

OAS and RB conceived the project. GK and RA synthesized the NFLt construct. HPT conducted single-molecule experiments and performed data analysis. HPT and OAS drafted the manuscript, with assistance and commentary from GK and RB.

References

[1] Van Der Lee, R., Buljan, M., Lang, B., Weatheritt, R.J., Daughdrill, G.W., Dunker, A.K., Fuxreiter, M., Gough, J., Gsponer, J., Jones, D.T., *et al.*: Classification of intrinsically disordered regions and proteins. Chemical reviews **114**(13), 6589–6631 (2014)

[2] Uversky, V.N., Oldfield, C.J., Dunker, A.K.: Intrinsically disordered proteins in human diseases: introducing the d2 concept. Annu. Rev. Biophys. **37**, 215–246 (2008)

[3] Wright, P.E., Dyson, H.J.: Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. Journal of molecular biology **293**(2), 321–331 (1999)

[4] Chong, S.-H., Ham, S.: Folding free energy landscape of ordered and intrinsically disordered proteins. Scientific reports **9**(1), 14927 (2019)

[5] Papoian, G.A.: Proteins with weakly funneled energy landscapes challenge the classical structure–function paradigm. Proceedings of the National Academy of Sciences **105**(38), 14237–14238 (2008)

[6] Marsh, J.A., Forman-Kay, J.D.: Sequence determinants of compaction in intrinsically disordered proteins. Biophysical journal **98**(10), 2383–2390 (2010)

[7] Hofmann, H., Soranno, A., Borgia, A., Gast, K., Nettels, D., Schuler, B.: Polymer scaling laws of unfolded and intrinsically disordered proteins quantified with single-molecule spectroscopy. Proceedings of the National Academy of Sciences **109**(40), 16155–16160 (2012)

[8] Schuler, B., Soranno, A., Hofmann, H., Nettels, D.: Single-molecule fret spectroscopy and the polymer physics of unfolded and intrinsically disordered proteins. Annual Review of Biophysics **45**, 207–231 (2016)

[9] Bernado, P., Svergun, D.I.: Structural analysis of intrinsically disordered proteins by small-angle x-ray scattering. Molecular biosystems **8**(1), 151–167 (2012)

[10] Maity, H., Baidya, L., Reddy, G.: Salt-induced transitions in the conformational ensembles of intrinsically disordered proteins. The Journal of Physical Chemistry B **126**(32), 5959–5971 (2022)

[11] Riback, J.A., Bowman, M.A., Zmyslowski, A.M., Knoverek, C.R., Jumper, J.M., Hinshaw, J.R., Kaye, E.B., Freed, K.F., Clark, P.L., Sosnick, T.R.: Innovative scattering analysis shows that hydrophobic disordered proteins are expanded in water. Science **358**(6360), 238–241 (2017)

[12] Fuertes, G., Banterle, N., Ruff, K.M., Chowdhury, A., Mercadante, D., Koehler, C., Kachala, M., Estrada Girona, G., Milles, S., Mishra, A., *et al.*: Decoupling of size and shape fluctuations in heteropolymeric sequences reconciles discrepancies in saxs vs. fret measurements. Proceedings of the National Academy of Sciences **114**(31), 6342–6351 (2017)

[13] Song, J., Gomes, G.-N., Shi, T., Gradinaru, C.C., Chan, H.S.: Conformational heterogeneity and fret data interpretation for dimensions of unfolded proteins. Biophysical journal **113**(5), 1012–1024 (2017)

[14] Baul, U., Chakraborty, D., Mugnai, M.L., Straub, J.E., Thirumalai, D.: Sequence effects on size, shape, and structural heterogeneity in intrinsically disordered proteins. The Journal of Physical Chemistry B **123**(16), 3462–3474 (2019)

[15] Morgan, I.L., Avinery, R., Rahamim, G., Beck, R., Saleh, O.A.: Glassy dynamics and memory effects in an intrinsically disordered protein construct. Physical Review Letters **125**(5), 058001 (2020)

[16] Kornreich, M., Malka-Gibor, E., Zuker, B., Laser-Azogui, A., Beck, R.: Neurofilaments function as shock absorbers: compression response arising from disordered proteins. Physical review letters **117**(14), 148101 (2016)

[17] Koren, G., Meir, S., Holschuh, L., Mertens, H.D., Ehm, T., Yahalom, N., Golombek, A., Schwartz, T., Svergun, D.I., Saleh, O.A., et al.: Intramolecular structural heterogeneity altered by long-range contacts in an intrinsically disordered protein. arXiv preprint arXiv:2212.01894 (2022)

[18] Zheng, W., Dignon, G., Brown, M., Kim, Y.C., Mittal, J.: Hydropathy patterning complements charge patterning to describe conformational preferences of disordered proteins. The journal of physical chemistry letters **11**(9), 3408–3415 (2020)

[19] Marko, J.F., Siggia, E.D.: Stretching dna. Macromolecules **28**(26), 8759–8770 (1995)

[20] Solanki, A., Neupane, K., Woodside, M.T.: Single-molecule force spectroscopy of rapidly fluctuating, marginally stable structures in the intrinsically disordered protein α-synuclein. Physical review letters **112**(15), 158103 (2014)

[21] Schwarzl, R., Liese, S., Brünig, F.N., Laudisio, F., Netz, R.R.: Force response of polypeptide chains from water-explicit md simulations. Macromolecules **53**(12), 4618–4629 (2020)

[22] Liese, S., Gensler, M., Krysiak, S., Schwarzl, R., Achazi, A., Paulus, B., Hugel, T., Rabe, J.P., Netz, R.R.: Hydration effects turn a highly stretched polymer from an entropic into an energetic spring. ACS nano **11**(1), 702–712 (2017)

[23] Pincus, P.: Excluded volume effects and stretched polymer chains. Macromolecules **9**(3), 386–388 (1976)

[24] McIntosh, D.B., Duggan, G., Gouil, Q., Saleh, O.A.: Sequence-dependent elasticity and electrostatics of single-stranded dna: signatures of base-stacking. Biophysical journal **106**(3), 659–666 (2014)

[25] Saleh, O.A., McIntosh, D., Pincus, P., Ribeck, N.: Nonlinear low-force elasticity of single-stranded dna molecules. Physical review letters **102**(6), 068301 (2009)

[26] Berezney, J.P., Saleh, O.A.: Electrostatic effects on the conformation and elasticity of hyaluronic acid, a moderately flexible polyelectrolyte. Macromolecules **50**(3), 1085–1089 (2017)

[27] Saleh, O.A.: Perspective: Single polymer mechanics across the force regimes. The Journal of chemical physics **142**(19), 194902 (2015)

[28] Beck, R., Deek, J., Jones, J.B., Safinya, C.R.: Gel-expanded to gel-condensed transition in neurofilament networks revealed by direct force measurements. Nature materials **9**(1), 40–46 (2010)

[29] Laser-Azogui, A., Kornreich, M., Malka-Gibor, E., Beck, R.: Neurofilament assembly and function during neuronal development. Current opinion in cell biology **32**, 92–101 (2015)

[30] Kornreich, M., Avinery, R., Malka-Gibor, E., Laser-Azogui, A., Beck, R.: Order and disorder in intermediate filament proteins. FEBS letters **589**(19), 2464–2476 (2015)

[31] Ribeck, N., Saleh, O.A.: Multiplexed single-molecule measurements with magnetic tweezers. Review of Scientific Instruments **79**(9), 094301 (2008)

[32] Morgan, I.L., Saleh, O.A.: Tweezepy: A python package for calibrating forces in single-molecule video-tracking experiments. Plos one **16**(12), 0262028 (2021)

[33] Bouchiat, C., Wang, M.D., Allemand, J.-F., Strick, T., Block, S., Croquette, V.: Estimating the persistence length of a worm-like chain molecule from force-extension measurements. Biophysical journal **76**(1), 409–413 (1999)

[34] Buscaglia, M., Lapidus, L.J., Eaton, W.A., Hofrichter, J.: Effects of denaturants on the dynamics of loop formation in polypeptides. Biophysical Journal **91**(1), 276–288 (2006) https://doi.org/10.1529/biophysj.105.071167

[35] Bustamante, A., Sotelo-Campos, J., Guerra, D.G., Floor, M., Wilson, C.A.M.,

Bustamante, C., Báez, M.: The energy cost of polypeptide knot formation and its folding consequences. Nature Communications **8**(1), 1581 (2017)

[36] Singh, V.R., Lapidus, L.J.: The intrinsic stiffness of polyglutamine peptides. The Journal of Physical Chemistry B **112**(42), 13172–13176 (2008)

[37] Chin, A.F., Toptygin, D., Elam, W.A., Schrank, T.P., Hilser, V.J.: Phosphorylation increases persistence length and end-to-end distance of a segment of tau protein. Biophysical Journal **110**(2), 362–371 (2016)

[38] Singh, V.R., Kopka, M., Chen, Y., Wedemeyer, W.J., Lapidus, L.J.: Dynamic similarity of the unfolded states of proteins l and g,. Biochemistry **46**(35), 10046–10054 (2007)

[39] Schwalbe, H., Fiebig, K.M., Buck, M., Jones, J.A., Grimshaw, S.B., Spencer, A., Glaser, S.J., Smith, L.J., Dobson, C.M.: Structural and dynamical properties of a denatured protein. heteronuclear 3d nmr experiments and theoretical simulations of lysozyme in 8 m urea. Biochemistry **36**(29), 8977–8991 (1997)

[40] Damaschun, G., Damaschun, H., Gast, K., Gernat, C., Zirwer, D.: Acid denatured apo-cytochrome c is a random coil: Evidence from small-angle x-ray scattering and dynamic light scattering. Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology **1078**(2), 289–295 (1991)

[41] O'Brien, E.P., Dima, R.I., Brooks, B., Thirumalai, D.: Interactions between hydrophobic and ionic solutes in aqueous guanidinium chloride and urea solutions: lessons for protein denaturation mechanism. Journal of the American Chemical Society **129**(23), 7346–7353 (2007)

[42] Müller-Späth, S., Soranno, A., Hirschfeld, V., Hofmann, H., Rüegger, S., Reymond, L., Nettels, D., Schuler, B.: Charge interactions can dominate the dimensions of intrinsically disordered proteins. Proceedings of the National Academy of Sciences **107**(33), 14609–14614 (2010)

[43] Morrison, G., Hyeon, C., Toan, N.M., Ha, B.-Y., Thirumalai, D.: Stretching homopolymers. Macromolecules **40**(20), 7343–7353 (2007)

[44] Borgia, A., Zheng, W., Buholzer, K., Borgia, M.B., Schüler, A., Hofmann, H., Soranno, A., Nettels, D., Gast, K., Grishaev, A., *et al.*: Consistent view of polypeptide chain expansion in chemical denaturants from multiple experimental methods. Journal of the American Chemical Society **138**(36), 11714–11726 (2016)