# To fold or expand—a charged question

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It is no secret anymore that many proteins "defy" the common paradigm and do not fold to a well-defined 3D structure under native conditions. These are the so-called intrinsically disordered proteins (IDPs) (1). Some of these proteins do fold upon binding to a target (2), whereas others do not seem to fold under any known conditions. Much has been written in recent years about the connection between the folding behavior of IDPs and their activity. Furthermore, it was recognized that proteins belonging to this group are in general characterized by low hydrophobicity and high charge density (3); but are there any structural characteristics that might help us to understand the differences among various IDPs? An article by Müller-Späth, Soranno et al. (4) in PNAS proposes a correlation between the charge density and the overall dimensions of IDPs. The authors perform single-molecule fluorescence resonance energy transfer (FRET) spectroscopy on diffusing molecules. From the measured mean FRET efficiency they are able to compute the radius of gyration ($R_g$) of the molecules as a function of chemical denaturant concentration. The proteins studied include one stably folded protein (the globular cold shock protein Csp*Tm*) and two IDPs (the N-terminal domain of HIV-1 integrase, which folds upon binding of a zinc ion, and human prothymosin α). As is now well established (5), all three proteins gradually collapse when denaturant concentration is lowered. Surprisingly, the authors find that in the case of the two IDPs, $R_g$ grows again as the concentration of the ionic denaturant guanidinium chloride is lowered below 1 M. They conclude that this is due to "release" of the proteins from electrostatic screening, because the extent of the observed expansion in buffer correlates well with the mean net charge on each chain.

This article is consistent with another recent study published in PNAS, in which Mao et al. (6) used experiment and simulation to obtain the sizes of 21 members of the protamine family of IDPs. They found a monotonic dependence of $R_g$ on the mean net charge. Thus, whereas Uversky et al. (7) made the important point that highly charged proteins with low mean hydrophobicity are likely to belong to the IDP group, the new work now provides a rationale for this correlation: these proteins tend to be more expanded under native conditions than foldable proteins, which confers extra stability on their disordered state and prevents them from folding.

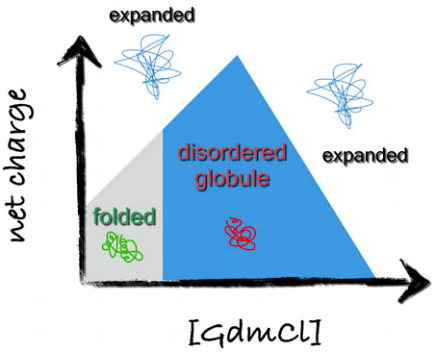


**Fig. 1.** Schematic phase diagram for proteins as a function of their net charge and the concentration of the ionic denaturant guanidinium chloride. With increasing denaturant concentrations a folded protein first denatures into a disordered globular phase, which then expands to form a random coil. Many IDPs are already expanded under native conditions because of their high net charge and cannot fold. With increasing guanidinium chloride concentrations, they first incur collapse due to electrostatic screening and then expand again.

The relation between denatured state size and stability against folding has only recently become clear. As already noted above, proteins tend to expand as the concentration of chemical denaturants is increased. This has been demonstrated by equilibrium experiments performed in many laboratories, using small-angle x-ray scattering (8), ensemble FRET experiments (9), and single-molecule FRET spectroscopy (see, e.g., refs. 10–12; for a more complete list of such experiments, see ref. 13). In addition, multiple experiments demonstrated a time-dependent collapse of denatured proteins upon transfer into buffer solution (see, e.g., refs. 14 and 15). Both of these are manifestations of the same phenomenon, the globule–coil transition that every polymer undergoes when transferred from a poor to a good solvent, or vice versa. The collapse of foldable proteins in buffer is likely to be driven mostly (but not only) by hydrophobic interactions, which can be disrupted by chemical denaturants.

The size of polymers is governed by universal scaling laws (16). Thus, in a poor solvent, the effective intrachain interaction is attractive, and a polymer is globular, meaning its $R_g$ scales like $N^{1/3}$, where $N$ is the number of monomers. In a good solvent, on the other hand, the intrachain interaction is repulsive, and a polymer explores an expanded ensemble of random coil states, with $R_g$ scaling like $N^{3/5}$. The transition between these two phases is a continuous, second-order one, which is theoretically well-understood (17). Ziv and Haran (18) used the theory of the globule–coil transition to analyze a large number of single-molecule FRET datasets showing chain expansion. They extracted from their analysis the equilibrium change in conformational free energy accompanying the globule–coil transition. Surprisingly, the free energy change due to expansion was very similar to the overall free energy change due to protein unfolding. It was therefore concluded that chain expansion stabilizes the denatured state and is responsible for the increased propensity for unfolding in high denaturant concentration. Thus, to fold, a protein has to be able to collapse first to a globular state. Many IDPs are polar and charged molecules, and they cannot collapse, unless their charges are screened by electrolytes (4). This property keeps them disordered unless they are forced to fold, for example, by binding to a partner protein. However, some IDPs have low charge density but are polar enough to form a loose globular state in the absence of denaturants (6, 19). Interestingly, as noted by Müller-Späth et al., even when a protein's chain is electrically neutral overall, a larger number of charged residues may increase its self-attraction and lead to an even more collapsed configuration than dictated by hydrophobic interactions alone. This behavior should then strongly promote folding.

The current experiments pose interesting challenges for theoretical biophysicists. Müller-Späth et al. (4) fit their experimental results using a hybrid theory, combining polyampholyte theory with a binding model for denaturant molecules. Their approach thus introduces the effect of the denaturant through two factors, an electrostatic screening length and a binding constant. The globule–coil transition theory, on the other hand, typically lumps all solution effects into a single mean-field parameter, which, in principle, can be computed from a more

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detailed model of the molecular interactions (20). A folding theory is required that includes, in a self-consistent way, both hydrophobic and electrostatic interactions and correctly reproduces the scaling laws in poor and good solvents, as well as the various possible states on the phase diagram of proteins (Fig. 1).

This task is particularly challenging because of the subtle combination of effects that typical modulations of the protein solvent environment bring about. For example, guanidinium cations are thought to weaken the hydrophobic effect, making water a better solvent for nonpolar side-chains and parts of the protein backbone. At the same time, guanidinium chloride is also a salt solution, which provides increased screening of a chain's electrostatic self-interactions. Other salts that also possess this screening ability may meanwhile have the opposite effect from guanidinium on hydrophobic interactions, driving the "salting-out" of proteins by increasing the surface tension of water (21). Thus, a full account of any given protein's propensity to expand or collapse would seem to require careful attention not only to the details of the chain's net charge and average hydrophobicity but also to the ionic strength and concentration of denaturant cosolvent molecules in the surrounding solution. Students of folding should certainly be grateful to Müller-Späth and coauthors for bringing to the fore an aspect of folding physics that is too often neglected.

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1. Uversky VN, Oldfield CJ, Dunker AK (2008) Intrinsically disordered proteins in human diseases: Introducing the D2 concept. *Annu Rev Biophys* 37:215–246.
2. Wright PE, Dyson HJ (2009) Linking folding and binding. *Curr Opin Struct Biol* 19:31–38.
3. Uversky VN (2002) Natively unfolded proteins: A point where biology waits for physics. *Protein Sci* 11: 739–756.
4. Müller-Späth S, et al. (2010) Charge interactions can dominate the dimensions of intrinsically disordered proteins. *Proc Natl Acad Sci USA* 107:14609–14614.
5. Schuler B, Eaton WA (2008) Protein folding studied by single-molecule FRET. *Curr Opin Struct Biol* 18: 16–26.
6. Mao AH, Crick SL, Vitalis A, Chicoine CL, Pappu RV (2010) Net charge per residue modulates conformational ensembles of intrinsically disordered proteins. *Proc Natl Acad Sci USA* 107:8183–8188.
7. Uversky VN, Gillespie JR, Fink AL (2000) Why are "natively unfolded" proteins unstructured under physiologic conditions? *Proteins* 41:415–427.
8. Smith CK, et al. (1996) Surface point mutations that significantly alter the structure and stability of a protein's denatured state. *Protein Sci* 5:2009–2019.
9. Lakshmikanth GS, Sridevi K, Krishnamoorthy G, Udgaonkar JB (2001) Structure is lost incrementally during the unfolding of barstar. *Nat Struct Biol* 8: 799–804.
10. Schuler B, Lipman EA, Eaton WA (2002) Probing the free-energy surface for protein folding with single-molecule fluorescence spectroscopy. *Nature* 419:743–747.
11. Kuzmenkina EV, Heyes CD, Nienhaus GU (2006) Single-molecule FRET study of denaturant induced unfolding of RNase H. *J Mol Biol* 357:313–324.
12. Tezuka-Kawakami T, Gell C, Brockwell DJ, Radford SE, Smith DA (2006) Urea-induced unfolding of the immunity protein Im9 monitored by spFRET. *Biophys J* 91: L42–L44.
13. Ziv G, Thirumalai D, Haran G (2009) Collapse transition in proteins. *Phys Chem Chem Phys* 11:83–93.
14. Kimura T, et al. (2005) Specific collapse followed by slow hydrogen-bond formation of beta-sheet in the folding of single-chain monellin. *Proc Natl Acad Sci USA* 102:2748–2753.
15. Arai M, et al. (2007) Microsecond hydrophobic collapse in the folding of *Escherichia coli* dihydrofolate reductase, an alpha/beta-type protein. *J Mol Biol* 368:219–229.
16. de Gennes P-G (1979) *Scaling Concepts in Polymer Physics* (Cornell Univ Press, Ithaca, NY).
17. Grosberg AY, Kokhlov AR (1994) *Statistical Physics of Macromolecules* (AIP Press, New York).
18. Ziv G, Haran G (2009) Protein folding, protein collapse, and tanford's transfer model: Lessons from single-molecule FRET. *J Am Chem Soc* 131:2942–2947.
19. Mukhopadhyay S, Krishnan R, Lemke EA, Lindquist S, Deniz AA (2007) A natively unfolded yeast prion monomer adopts an ensemble of collapsed and rapidly fluctuating structures. *Proc Natl Acad Sci USA* 104:2649–2654.
20. Alonso DO, Dill KA (1991) Solvent denaturation and stabilization of globular proteins. *Biochemistry* 30: 5974–5985.
21. England JL, Pande VS, Haran G (2008) Chemical denaturants inhibit the onset of dewetting. *J Am Chem Soc* 130:11854–11855.