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Research Highlights

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Proteins: Disorder, Folding, and Crowding

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The protein side of the central dogma of molecular biology holds that the native state of a protein is encoded by its sequence of amino acids. For more than 50 years, this principle has been largely applied to the folding of globular proteins. Both experimental and computational biophysicists have worked to determine the thermodynamic and kinetic parameters that yield underlying principles of protein folding, as well as to develop algorithms to successfully predict structure from sequence. More recently, the biophysical community has recognized that the native states of many proteins do not involve stable globular structures, but rather disordered, dynamic conformational ensembles. These intrinsically disordered proteins (IDPs) are involved in a broad range of cellular functions and assemblies, as well as having causal roles in a number of devastating human diseases. Disordered proteins and regions can be predicted by their amino sequences and even a single point mutation can disrupt their normal function. In other words, even when the native state of a protein does not involve stable tertiary contacts and canonical secondary structure, the central dogma still applies. Here, I highlight recent articles in the *Biophysical Journal* that represent advances and biophysical insights focusing on protein folding and IDPs.

Coupled folding and binding of some IDPs upon interaction is at the intersection of the protein folding and IDP fields. This phenomenon is investigated in two recent papers in the journal. In the first (1), co-translational interactions between two IDPs of opposite charges are investigated by fluorescence correlation spectroscopy and force measurements. The second paper (2) seeks to tease apart the recognition and folding steps of an IDP for its heterogeneously structured partner, by systematic mutation of the IDP.

While many small, globular proteins fold spontaneously in vitro, in the complex environment of the cell, proteins interact with a variety of biomolecules and many rely on a variety of chaperone molecules in order to fold properly. Two papers address protein folding in the presence of molecular chaperones. One of these (3) made circularly permuted variants of GFP which changed its contact order and characterized the interaction and folding of these variants with the chaperonin, GroEL. Fundamental aspects of surfactant mediated protein unfolding were studied in the second paper (4), where both ionic and hydrophobic properties of the surfactant were found to be important for unfolding an uncharged protein.

Two papers address fundamental biophysical properties of IDPs. Mukhopadhyay and coworkers (5) used a combination of experiments and simulations to create a hydration map of a model IDP, finding dynamically distinct groups of water molecules associated with the protein. A computational study (6) looked at different types of molecular crowders on the conformational ensembles sampled by an IDP, observing both compact and extended conformations that interact differentially with the crowding macromolecules.

One manuscript addresses the phenomenon of liquid-liquid phase separation by IDPs, a topic of growing interest in the past few years, as many IDPs have been observed drive the formation of biomolecular condensates both in vitro and in vivo. This study (7) used NMR to identify regions of secondary structure propensity in an IDP critical to intermolecular interactions in the early stages of phase separation.

The largest group of papers I would like to highlight has the broad goal of understanding functional mechanisms of IDPs, although in very different systems. Two focus on the IDPs which line the nuclear pore complex, the FG nups. The first (8) used polymer theory to investigate the relationship between cohesiveness and FG nup density as these parameters relate to permeability of the nuclear pore. Hough and colleagues (9) demonstrated in cell NMR in *Saccharomyces cerevisiae* to measure the dynamics of an FG nup, finding evidence of interactions between the protein and its intracellular environment. In the third paper in this group (10), a single phosphorylation modification in the disordered region of the protein synaptotagmin was found to disrupt transient helical structure in this region,

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providing mechanistic insight into the function of synaptotagmin in neuronal exocytosis. Dehydrins are a group of IDPs found in plants which protect against damage under extreme conditions. Graether and colleagues (11) find that dehydrins may be able to modulate the hydrogen bonding of bulk water, protecting plant enzymes from denaturation in times of stress. The final paper looked at the role of an IDP in the complex cellular machine, the spliceosome. The authors (12) used a variety of experimental approaches to demonstrate that Ntr2 remains disordered upon binding with its helicase partner and downregulates the function of the helicase.

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