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Towards developing principles of protein folding and dynamics in the cell

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Abstract

Proteins must fold and function in the immensely complex environment of a cell, i.e. the cytoplasm—this is far from the ideal test-tube setting of a dilute solution. Here we review the advances in protein folding and dynamics inside the cell. In developing principles of protein behavior *in vivo,* we also begin to understand the organization and dynamics of the cytoplasm, unifying the single protein scale with the many-protein architectures at the subcellular scale. Our group has significantly contributed to this frontier by characterizing the effect of macromolecular crowding on the distribution of protein conformations. Additionally, we provide a personal perspective on becoming a theoretical biological physicist in the era of interdisciplinary research that has been greatly influenced by Dr Kamal Shukla. We also share our view on the future direction of protein folding inside a cell.

Keywords

protein folding inside a cell; macromolecular crowding effect; coarse-grained molecular simulation

Introduction

The field of protein folding and dynamics is expanding towards cellular biological physics. An understanding of the physical principles of macromolecules inside the cell is needed to bridge the scale of single proteins to the scales of subcellular organizations, such as supramolecular assemblies [1], aggregation [2], or phase separation and cellular compartmentalization [3]. As macromolecules and cytosolic scaffolds crowd the interior of a cell, robust networks of smart matter form that are capable of making collective decisions for cellular survival. The structure and dynamics of these crowded spatial networks of macromolecules manifest from a multitude of weak enthalpic interactions, called ‘quinary’ interactions [4], and collective entropic forces. Since subcellular systems are held together through heterogenous weak interactions and entropic effects, the macroscopic degrees of freedom are often interwoven with the microscopic. One such example is the non-

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equilibrium dynamics of the cytoskeletal network [5]. This is strikingly different from conventional condensed matter physics systems where a key triumph of the past century came from averaging out the weak microscopic interactions (i.e. renormalization group [6]). Moreover, the cytoplasm allows for signals (mechanical or chemical) to propagate over many lengths of a single protein. The challenge in understanding signal transduction lies in the unknown complexity of molecular interactions in a jammed-packed space. It is our group's long-term research goal to understand how signals from meaningful external stimuli passes through networks of proteins across the noisy environment of the cell. In order to reach that goal, both theory and experiment are needed to link the relationship between the cellular environment and protein structure and function.

In addition, we owe much of the shaping of the field of biological physics and its current direction to the late Dr Kamal Shukla. His program at the National Science Foundation (NSF) supported the professional growth and training of scientists by fostering the infrastructure of the science community so that Cheung and other biological physics researchers can be sufficiently supported to reach each milestone along their career paths. Cheung began as a graduate student of Professor Jose Onuchic at the University of California, San Diego. She benefited scientifically from the education and training program provided by the Center for Theoretical Biological Physics, which was funded by the NSF. Cheung learned about the beauty of using minimalist models to understand desolvation effects in protein folding [7, 8]. She met her postdoctoral advisor, Professor Dev Thirumalai, at the first Gordon Research Conference on Protein Folding Dynamics, which was also funded by Dr Shukla's Molecular Biophysics program at the NSF. Working with Professor Thirumalai, she completed the first coarse-grained molecular simulations of protein folding in a crowded medium [9]. After Cheung accepted a faculty position as an assistant professor at the University of Houston, she communicated to Dr Shukla her scientific vision of growing a new community, within the Biophysical Society, that is dedicated to researching *in vivo* environments; he encouragingly supported the idea by funding its Inaugural Symposium. Since the inception of the 'Biopolymers *in vivo*' subgroup in 2011, we have attracted attention from hundreds of junior and senior scientists alike. Dr Krastan Blagoev also supported the community through the Physics of Living Systems program at the NSF. We are overcoming the challenges of understanding how macromolecules work inside the cell by forming a critical mass that collectively advances the science of this important field experimentally, computationally, and theoretically.

A unique aspect of our group's research has been the forging of close collaborations with experimentalists who employ a wide range of instrumentation that interrogates the dominant driving forces of macromolecular interactions in cell-like environments. By developing coarse-grained models of proteins in cell-like media [9] and efficiently using high-performance computation, our group has developed statistical-mechanical approaches to understanding a general outcome of several forces competing to determine the dynamics of proteins. This investigation is complementary to the experiments that often measure the protein dynamics under specific conditions, allowing us to make experimentally testable predictions. We were often motivated to develop new hypotheses and ideas of modeling when predictions failed. Such a persistent quest for truth leads to discoveries of new environment–structure–function relationships for complex-structured proteins in the cell.

Each milestone brings us a step closer to revealing the molecular underpinning of a protein network.

Protein folding in the cell

The theory of protein folding *in vitro* is an idealized view of protein folding in the cell [10]; namely, we expect the general principles to be the same, but new behavior will arise with added complexity of the cellular environment [11]. The cellular environment differs from the test tube by two main forms (see figure 1): (i) entropic effects from crowding, packing shapes, and solvent dynamics, and (ii) enthalpic effects from electrostatics, quinary van der Waals interactions, and chemical perturbants. Since cells are highly crowded by macromolecules, non-trivial collective effects arise. Additionally, protein sequence information can contain quinary interactions [4] that guide proteins towards self-assembly of transiently stable complexes to perform various functions. In order to bridge the gap from single protein dynamics to subcellular network dynamics, we first need to understand the behavior of single (or few) molecule(s) placed in the cellular environment.

Several *in vivo* experiments have begun to scratch the surface of understanding protein folding and dynamics *in vivo.* By studying proteins in an actual cellular environment with all of its complexity, we gain key phenomenology. Motivated by the possibility of living cells controlling the function and dynamics of their proteome through modulating their proteins' landscapes, these studies investigate protein stability inside living cells. Studies in [12–16] focus on the effects of the environmental perturbations, on the order of a k_BT, to proteins. These weak interactions produce mixed results, shifting the free energy, positively, negatively or have little effect compared to *in vitro* folding. Other *in vivo* experiments [17–19] focus on the quinary interactions encoded in the protein sequence in order to understand the protein folding stability changes. These *in vivo* results are also corroborated by all-atom simulations of the cytoplasm of a bacterium [20].

The development of a theory is needed for the in-living-cells protein folding experiments; however, it is difficult to develop principles of *in vivo* folding and dynamics without a firm grounding of the interactions and entropic effects. Macromolecular crowding is arguably the most universal effect, since all molecules occupy volume. During the three decades since the 'macromolecular crowding effect [21]' was coined in 1981, the native state of proteins has been modeled as incompressible hard cores in theories that evaluate the impact of volume exclusion on protein stability and dynamics. This view has been critically challenged by collaborative work between the experimentalist group of Pernilla Wittung-Stafshede and our own group. Using computer simulations and theories of statistical physics, we predicted that a structurally complex protein resembles a 'pom–pom' instead of a hard core in solutions. When the macromolecular crowding effects are considered, the stability of the native state is enhanced by the mechanistic interactions between surrounding macromolecules and the native state of a protein. This prediction has been validated experimentally in studies of a globular apoflavodoxin protein in the presence of the synthetic macromolecule Ficoll 70, which acts as a crowding agent [22]. Our group further predicted the change in folding routes of apoflavodoxin caused by different shapes and sizes of crowders [23]. The folding

routes in the bulk solutions experience a higher percentage of unproductive folding intermediates than in the crowded environment [23].

The macromolecular crowding effect is a result of volume exclusion by surrounding macromolecules. It has been known since the 1950s that density fluctuations of macromolecules create a void where a compact protein resides and compact conformations are statistically favored over extended conformations [24]. However, the determination of the 'native' state becomes non-trivial when the conformation of a compact protein is malleable and can be easily changed by interactions with other objects. In collaboration with Professor Stafshede-Wittung's group, we showed that the shape of aspherical VlsE proteins could be changed under cell-like conditions, such as those generated by chemical and thermal denaturation [25]. In crowded milieus, distinct conformational changes from an olive shape to a sphere in VlsE are accompanied by secondary structure alterations that lead to exposure of a hidden antigenic region. This work demonstrates the unprecedented malleability of 'native' proteins and implies that crowding-induced shape changes may be important for protein function and malfunction *in vivo.*

The idea of a protein' s 'native' state in a crowded cell has established an important milestone towards protein folding inside the cell. In collaboration with Professor Martin Gruebele, we identified the enzymatic mechanism of a protein, phosphoglycerate kinase (PGK), with the shape of a 'pac-man' in cell-like conditions [26]. Our coarse-grained models showed that PGK adopts a closed 'pac-man' conformation in a crowded cell, bringing the two lobes together to react. Indeed, experiments have shown that the enzymatic activity of PGK is a remarkable 15-fold higher in a crowded environment.

Further theoretical developments of the macromolecular crowding effect by scaling relationships [27] allows us to understand the relationship between the ration of crowder size to a generic biopolymer radius of gyration and the collapse of that generic biopolymer. This was also validated experimentally in a general colloid polymer solution [28] and with intrinsically disordered proteins (IDPs) in a crowded environment [29]. In addition to the size of crowders, the shape also has a significant entropic effect [30, 31]. Even though much progress has been made in understanding the macromolecular crowding effect, there is still much we do not fully understand such as the effects of heterogeneous sizes and shapes as is found in the cytoplasm.

The last entropic effect that is necessary for a complete understanding of *in vivo* folding deals with the behavior of water in the cell. As shown for protein folding in the test tubes, the dynamical fluctuations of water at the protein' s surface [32, 33], desolvation [7] between residues, and hydrodynamic interactions [34, 35] will also play an important role in the cell. When departing from the dilute solution limit to high volume fraction of macromolecules, the behavior of water will change entropically [36, 37], kinetically [38, 39], and possibly even quantum mechanically [40].

A great challenge is to understand how weak, seemingly random, quinary interactions [4] can lead to non-random arrangements of protein organization. These interactions can alter the thermodynamic folding barriers leaving the protein marginally stable. Both theoretical

and experimental developments have been made to understand macromolecular crowding with enthalpic contributions [41–45]. Being able to separate out the entropic from enthalpic effects is a vital step towards the development of general principles of *in vivo* protein folding.

We further extended these research directions to investigate the effects of chemical interference on proteins, which is another important aspect of proteins in a crowded environment. We refer readers to a review article by Gruebele *et al* [10] on the experimental aspect of the chemical interference on protein folding and another review article by García *et al* [46] on the theoretical and computational aspect of it. This step is necessary because experimentalists use chemical denaturation to investigate isothermal folding/unfolding in a laboratory setting, while our group often compared these outcomes with computer simulations under thermal denaturation conditions. We investigated chemical denaturation of proteins by developing statistical mechanical models from coarse-grained and atomistically detailed simulations. One of the main focuses has been the development of multiscale methods (MultiSCAAL) that integrate the mean force potential obtained from all-atomistic molecular dynamics simulations with a knowledge-based energy function for coarse-grained molecular simulations [47, 48]. The Cheung group tested the performance of MultiSCAAL by studying the folding energy landscape of a Trp-cage protein. That involved the movement of a side-chain in a tryptophan that flips in and out of the hydrophobic pocket. By improved sampling of confirmations, their simulations agree with the results from the NMR experiments better than other current computational methods. This computational approach advanced the next step of research in understanding protein folding in cytoplasmic media [49].

Conclusion

In summary, the combination of crowding, solvent fluctuations, quinary interaction and chemical changes can greatly change the dynamics and stability of a protein, and in turn, its function. Our understanding of relationships between the cellular environment and protein folding and dynamics will unify the single molecule scale to the subcellular scale. More theory and experiment will continue to rely on each other to push this goal forward. As we rebuild the complexity back into the ideal solution (i.e. *in vitro*) we can begin to develop *in vivo* principles. At the other end of the complexity spectrum, *in vivo* experiments and all-atom simulations of cellular cytoplasm will give us a phenomenological understanding. The challenges in understanding protein folding and dynamics in the cell are daunting, but the drive to discover is greater. Moving forward to mesoscopic assembles and supramolecular machine dynamics, further bridging the length and time scales, we will elucidate the stunning complexity of subcellular organization and dynamics, and continue to realize Dr Kamal Shukla's vision of interdisciplinary science at the interface of physics and biology.

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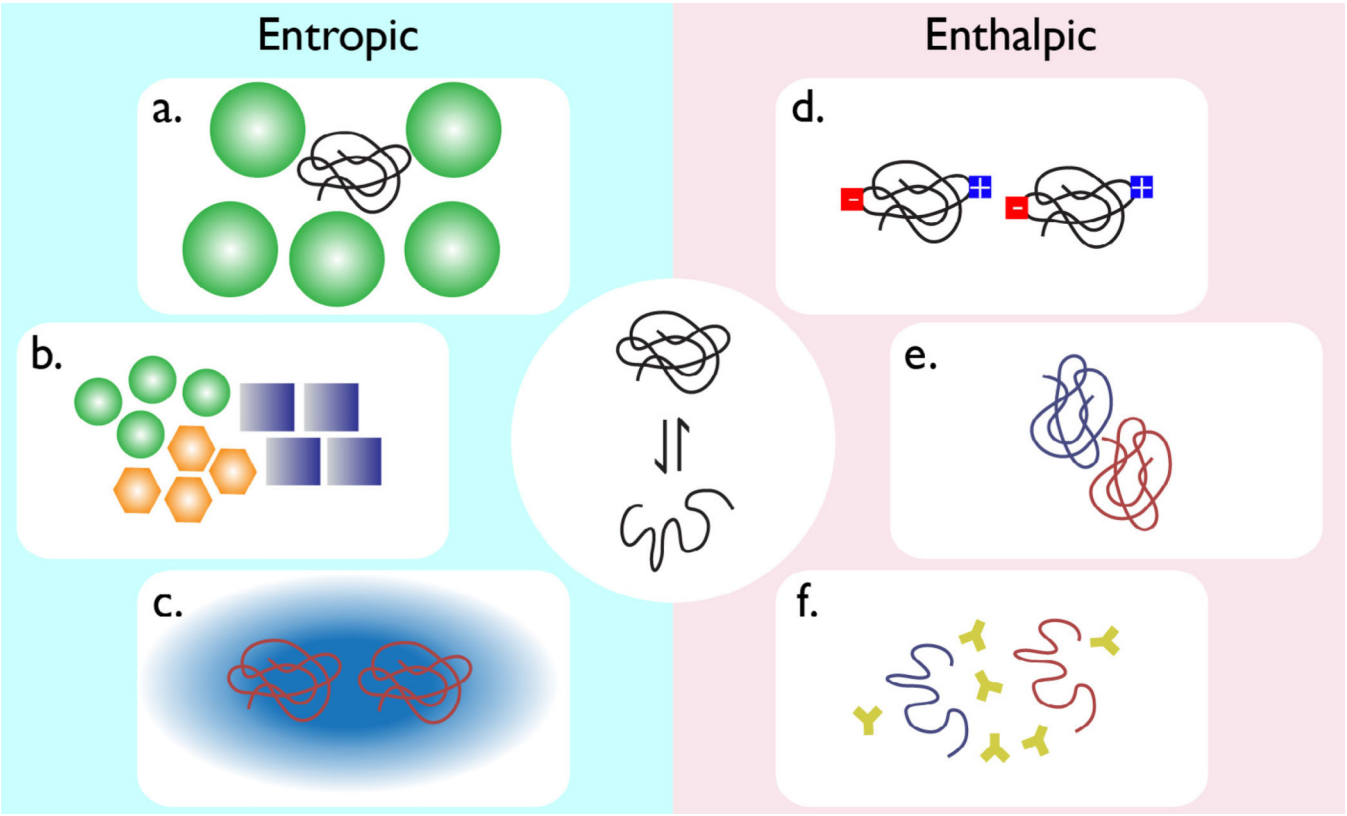

Figure 1.
Protein folding and dynamics under the influence of the entropic and enthalpic contributions of the cellular environment. (a) Macromolecular crowding effect, (b) packing shape, (c) hydration or solvent fluctuations, (d) electrostatics, (e) quinary van der Waals interactions, and (f) chemical perturbants.