New and Notable

A Sticky Cage can Slow Down Folding

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The interplay between experimental and computational research has become increasingly important in recent years, with each side benefiting from the other's input. This notion is perhaps most widely accepted in the simulation community, but the experimental side is increasingly appreciative of this symbiosis. In particular, many types of experiments produce data of a complexity—and sometimes size—that may make direct interpretation extremely difficult, and conclusions are thus frequently based on some form of computational analysis. One common scenario is that experiments provide an overall picture of a biological system, which can then be further refined and interpreted through simulations.

An illustrative example of this approach is the study by Sirur and Best published in this issue of the *Biophysical Journal* (1). The authors are motivated by a debate in the literature on the molecular mechanism by which the chaperonin GroEL facilitates the folding of proteins. Chaperonins such as GroEL are made up of ringlike structures with a central cavity in which folding takes place. An important question is whether the chaperone acts mainly as an inert cage protecting the protein from aggregation, or whether substrate-chaperone interactions play a more active role in chaperone-assisted folding (2).

The effect of confinement on protein folding has been studied extensively in the literature. At first sight, encapsulation has the consequence of reducing the available volume for, and thereby the entropy of, the unfolded state. This should lead to a significant increase in the stability of the folded state, and an increase in folding rate (3). This explanation does not, however, seem to capture the entire picture, and more complex models suggest the possibility of a decrease in folding rate due to strong interactions between the substrate and the cavity wall (4,5).

Experimental studies have shown that the interaction forces between substrate and cavity may depend strongly on the substrate (6). This observation suggests that the relative roles of encapsulation (steric hindrance) and other substrate-chaperonin interactions may be system-specific (7). A similar view is put forward in recent experimental studies. One important example is the work of Hofmann et al. (8), who used single molecule spectroscopy and microfluidic mixing to directly compare rates of GroEL-mediated folding with folding in solution. They demonstrated a significant decrease in folding rate for the slow-folding substrate, Rhodanese, and suggested that the effective folding rates inside the chaperonin are the result of a complex balance between inter- and intramolecular interactions, rather than a universal chaperonin mechanism. As to the origin of the observed slowdown, they found no evidence for an increased enthalpic contribution to the folding barrier or confined water molecules. Instead, they suggested the cause to be either a decreased intramolecular diffusion rate or the unfolded substrate "sticking" onto the chaperonin cage.

This brings us back to the article by Sirur and Best. In the absence of a general mechanism, further progress in the understanding of the GroEL system requires that the details of a specific substrate be taken into account. From a simulation perspective, this is no trivial task. The size of the GroEL system alone is intimidating. More importantly, the Rhodanese substrate, which was the subject of the experimental studies by Hofmann et al. (8), has folding times of up to 40 min. Although significant progress has been made in recent years both in simulation software and hardware, these timescales are still many orders of magnitude larger than anything that can be simulated using all-atom molecular dynamics simulations. It therefore requires some creativity in the design of the simulation, striking a balance between computational demands and a level of detail that still makes it possible to make nontrivial observations about the interactions between substrate and cavity. The authors address this problem using a hierarchy of models, gradually including an increasing level of detail. Folding of Rhodanese in the absence of chaperone is studied using a native-centric, Gō-type model. Such models have been shown to provide a computationally efficient, yet relatively accurate description of the folding mechanisms of a range of proteins. However, as this type of model focuses only on intramolecular interactions that are present in the native state, it is not sufficient to study complex interactions between chaperone and substrate. As a supplement to the Gō model, the authors represent the interaction with the chaperone in two different ways: The simplest model, assuming a strictly repulsive cavity wall to represent the effect of confinement, results in an increase in folding rate of several orders of magnitude, in line with predictions from excluded-volume based theoretical models. In the more complex representation, which includes a coarse-grained potential (9) for sequence-dependent residue-residue interactions between substrate and chaperone (which by their very nature are nonnative), the effect is, however, reversed, producing folding rates below that of the unconfined case.

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The simulations are conducted using a setup similar to that of the study of Hofmann et al., allowing for a direct comparison between simulation and experiment. Although some discrepancies are observed both in the magnitude of the changes in folding rates and in the relative order in which the two domains in Rhodanese form, the overall correspondence is remarkable. More importantly, however, by decomposing the problem into the different types of physical interactions, the authors manage to isolate the important ingredients in their model, and thus provide a tool for understanding the range of seemingly conflicting results reported in the literature. This illustrates a particularly strong advantage for computational methods, where different kinds of interactions can be tuned and switched on and off in ways that are extremely difficult to do experimentally. While suspending or manipulating the forces of nature in this manner does not always produce physically realistic results, it can act as a catalyst to generate new hypotheses and ideas for further experimental studies.

Both the experiments by Hofmann et al. and the simulations by Sirur and Best involve a single-ring variant of GroEL:GroES. The functional cycle of the complete GroEL:GroES machinery, however, includes a complex set of conformational changes that involve ATP binding and hydrolysis. With increasing computational power and creative modeling it will, we hope, become possible to study the full complex, making it also possible to include allosteric effects and to test hypotheses on more active mechanisms of GroEL-mediated folding, such as the previously proposed iterative annealing model (10,11). However, it is encouraging to see how much can already be done with the current state of the art. The observation that the dramatic speedup caused by entropy reduction is cancelled by an equally powerful effect in the opposite direction is fascinating. From a computational perspective, the fact that molecular systems are in some cases governed by such a delicate balance of large and opposing effects also points to the importance of finding the correct balance between various types of interactions when designing a force field. Obtaining such subtle balances in molecular energy functions can be difficult from theory alone and must therefore typically be verified against (12)—or parameterized using (13)—experiments, thus closing the circle of reciprocity.

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