Structural Ensemble Modulation upon Small-Molecule Binding to Disordered Proteins

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Abstract

Over the past decade, there has been a growing interest in investigating whether disordered proteins can be targeted for clinical purposes using small molecules [1–8]. While small-molecule binding to disordered proteins can be seen as unorthodox, examples of this phenomenon have been reported. In order to rationalize these observations, a variety of models are emerging, sometimes in apparent contradiction. Here, we offer a "structural ensemble modulation" view as an attempt to clarify the language, organize concepts, and facilitate the comparison of different studies. In doing so, we hope to promote the understanding of the general principles underlying this phenomenon toward the development of novel therapeutic compounds targeting disordered proteins, which are prevalent in a wide range of human diseases [1–8].

From the point of view of statistical mechanics, the most populated states of a protein correspond to the minima in its free energy landscape. For folded proteins, the free energy is often approximated by neglecting entropy, resulting in a description of the native state as an individual conformation (Fig. 1), as, for example, is typically the case in the Protein Data Bank [9]. However, as the community has long recognized the importance of conformational fluctuations in describing the behavior of folded proteins [10–13], it is becoming increasingly common to determine structural ensembles for folded proteins, for example, using molecular dynamics simulations or Monte Carlo approaches [14]. More generally, neglecting entropy is not appropriate in the case of a protein with large disordered regions. The conformationally heterogeneous nature of a protein of this type should always be represented by a structural ensemble, which is defined by providing a set of conformations and their corresponding statistical weights [14–19] (Fig. 1). In principle, one should also include the transition rates between conformations to have a more complete definition of a structural ensemble [19]. It is also important to note that some states within the ensemble may be undetectable because their populations remain below the sensitivity limit of the experiment and are thus effectively invisible.

With this view, one can provide a general characterization of the process of a small-molecule binding to a disordered protein as a structural ensemble modulation. A small molecule thus generates a shift in the populations of the states (Fig. 2). For a given experiment, this population shift may result in previously invisible states to become observable, or *vice versa* (Fig. 2). A continuum of models could be used to describe how the unbound ensemble of a disordered protein is modulated by the binding of a small molecule, which spans between two extreme cases, depending on whether the conformational entropy of the protein decreases or increases (Fig. 2). Using this conformational entropy as a ruler, it is possible to characterize the type of ensemble modulation caused by a small molecule. On one extreme, in the "entropic collapse" model, which is akin the "folding upon binding" model of protein–protein interactions [20], the bound state of the protein adopts a well-defined conformation. In this scenario, the small molecule induces a disorder-to-order transition and results in a shift in population

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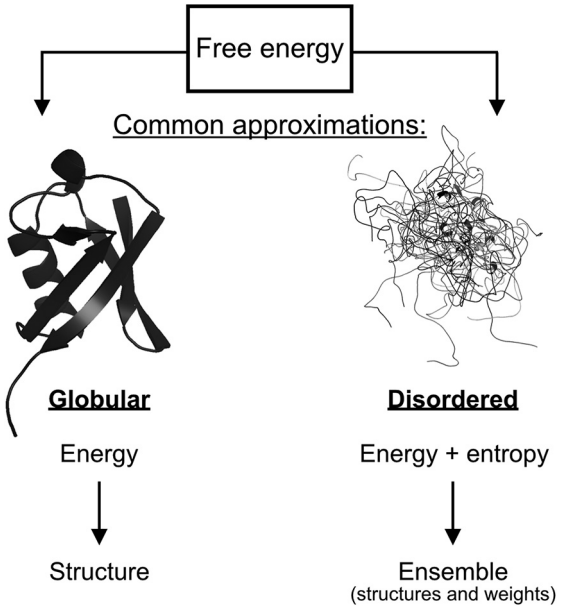

Fig. 1. The more populated states of a protein correspond to the minima of its free energy landscape. For folded proteins, the native state is often represented by a single structure, where the conformational entropy is neglected. This is an approximation, and it is increasingly recognized that entropy plays an important role for folded proteins, for example through conformational fluctuations and functional motions [10–13]. For disordered proteins, native states must be represented as structural ensembles, as their conformational entropy is always sizeable.

toward a single predominant state. As a result of the large loss of conformational entropy, such an event may have a free energy cost that must be paid through other interactions, for example, release of water molecules or favorable enthalpic contributions. At the other extreme, it has been recently suggested that a small molecule may bind a disordered protein according to the "entropic expansion" model [3]. In this model, the presence of the small molecule enables the disordered protein to more readily explore (i.e., with higher probability) structural conformations, or in other words, induces a "disorder-to-more-disorder" transition, thus favorably contributing to the free energy of binding. This is also a shift in population that might lead to invisibles states becoming detectable. Midway between these two extrema, there is the case in which the conformational entropy of the protein remains approximately constant, which we refer to as "isentropic shift." The cases in which the entropy of the bound state remains sizeable are of "disorder-to-disorder" transitions, also known as "fuzzy" binding [21]. We emphasize that in all cases, when binding occurs, the bound ensemble experiences a shift, however small, in the populations of the observable states.

As examples of small-molecule binding to disordered proteins are emerging, we can use the conceptual framework of the structural ensemble modulation mechanism to interpret recently reported experimental observations. For instance, a detailed study of the D2 domain of $p27^{Kip1}$ (p27-D2) was recently carried out using nuclear magnetic resonance relaxation dispersion and small-angle X-ray scattering experiments, in which a minimal model of five distinct states was introduced to describe the p27-D2 structural ensemble [6]. The changes in this minimal ensemble were described upon the binding of the small molecule SJ572403, and a population shift without entropic expansion was suggested. In the language presented here, population shift and entropic expansion are not mutually exclusive, as by population shift we indicate the general process by which a small molecule gives rise to a modulation of a structural ensemble (Fig. 2). In this sense, a binding event is always associated with a population shift. We also note that, while the evidence reported in the case of p27-D2 does not directly suggest an entropic expansion, it is still possible that some states, which may be invisible to the specific experimental techniques used, could have become more populated upon binding, or that the changes in the populations across the five observed states ultimately resulted in an increase in entropy. Addressing these challenging issues will require more investigations. More generally, to establish whether or not an entropic expansion mechanism is present, the change in the overall conformational entropy in the presence and absence of a small molecule should be accurately calculated. This task is daunting but could in principle be carried out using molecular dynamics simulations, possibly incorporating experimental measurements as structural restraints [11,19,22].

Another study reported an example of small-molecule binding in the isentropic scenario [7]. In that case, the use of advanced simulation techniques [22] enabled the characterization of the binding of the small molecule 10058-F4 to a disordered peptide binding region from the protein c-Myc. The observed changes in the bound ensemble were largely within the statistical accuracy of the calculations, yet the small molecule displayed signs of sequence specificity while retaining a diffuse binding mechanism.

Given the relatively small but increasing number of other examples of small molecules binding to disordered proteins, including Aβ [5,23,24], α-synuclein [25], tau [26], c-Myc [1], p27kip1 [27], PTP1B, [28], osteopontin [29], and unfolded β2-microglobulin [30], we expect that our understanding of the structural ensemble modulation mechanism will systematically improve in the near future.

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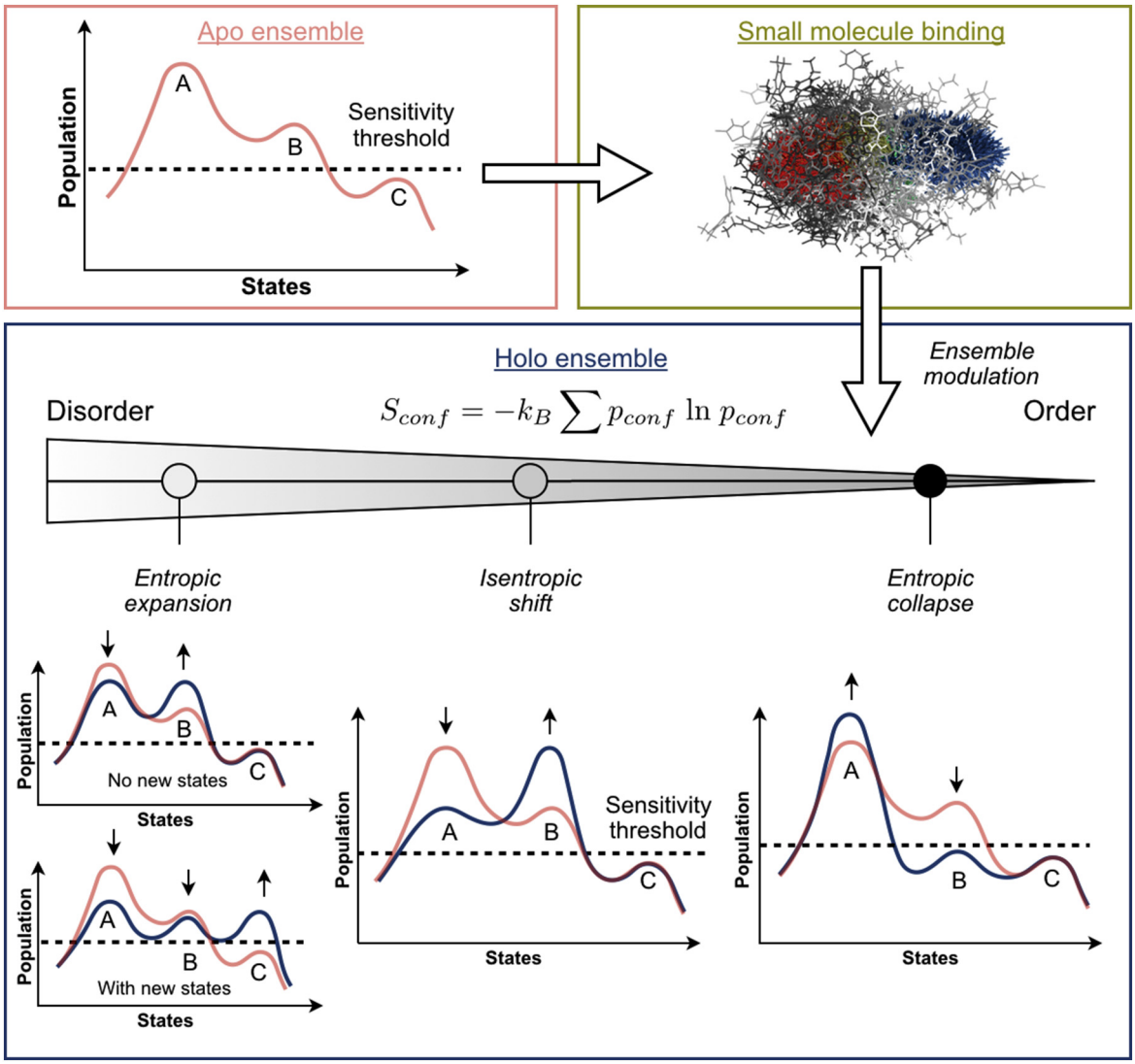

Fig. 2. Schematic representation of the structural ensemble modulation mechanisms of small-molecule binding to disordered proteins. The figure illustrates the case of a disordered protein that populates three main states in its free (apo) state, although only two of them are above the threshold of detectability (dashed line in the left upper panel). In this figure, we use populations rather than free energies to emphasize the similarity between the population shift and ensemble modulation descriptions. Upon binding a small molecule, one of the states (state A in the lower right panel) can become more populated, thereby reducing the overall entropy ("entropic collapse"). It is also possible that the populations are redistributed among the three states (lower central panel) without an overall change of the entropy ("isentropic shift"). Finally, the number of observable states can increase (lower left panel), with an overall increase in entropy ("entropic expansion").

Questions for the community

As the number of small molecules being identified to interact with disordered proteins is increasing, key questions still remain to be addressed, including the following:

1) Is the ensemble modulation mechanism the unifying model to describe the binding of small molecules and disordered proteins?
2) Can one identify examples of small molecules that bind disordered proteins by entropic collapse, isentropic shift, and entropic expansion? Can such small molecules be specific?
3) Can one characterize the effects of such small molecules on the behavior of their disordered protein targets, thereby defining their potential in drug discovery?

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Additional questions, of a more technical nature, include the following:

4) Which level of resolution in the structures and in the accuracy of their corresponding statistical weights is required to meaningfully compare structural ensembles? Which techniques should be used to achieve such resolution? Are the statistical weights necessary?
5) What is the role of the transition rates between conformational states in the ensemble modulation model?
6) Can docking to many conformations within the unbound ensemble, as commonly done for folded proteins, be effective to identify a small-molecule binder to a disordered protein?
7) Which forces are typically driving the binding process in the case of entropic collapse, isentropic shift, and entropic expansion? What is the role of water molecules in modulating the structural ensembles in these modes of binding? Can one optimize the binding specificity using these forces?

We anticipate that to answer to these questions it will be important to develop high-resolution, quantitative structural ensemble characterization techniques through combination of experimental and computational methods [14,15,17,19,22,31]. This field is in its infancy, but we believe that it is already possible to define a common language based on the robust conceptual framework provided by statistical mechanics. This language is very convenient to describe ensembles of conformations together with their statistical weights, as well as their changes upon external perturbations, including the binding of small molecules.

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