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Functional versus folding landscapes: the same yet different

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Protein functional landscapes are characterized by a modest number of states compared with the folding landscapes, allowing brute force sampling of these states for smaller proteins using computer simulations. On the other hand, because the functional landscape topographies are complicated, the native state dynamics are often difficult to interpret. Nevertheless, a number of experimental and computational techniques have recently emerged that are designed to reveal the essential features of the native landscape, such as the hierarchical organization of conformational substates. These studies also shed light on the mechanisms of protein function, for example, explaining how chemical energy is transduced in molecular motors. Overall, interpreting experimental results in the light of the functional landscape paradigm considerably enhances the understanding of complex biomolecular processes.

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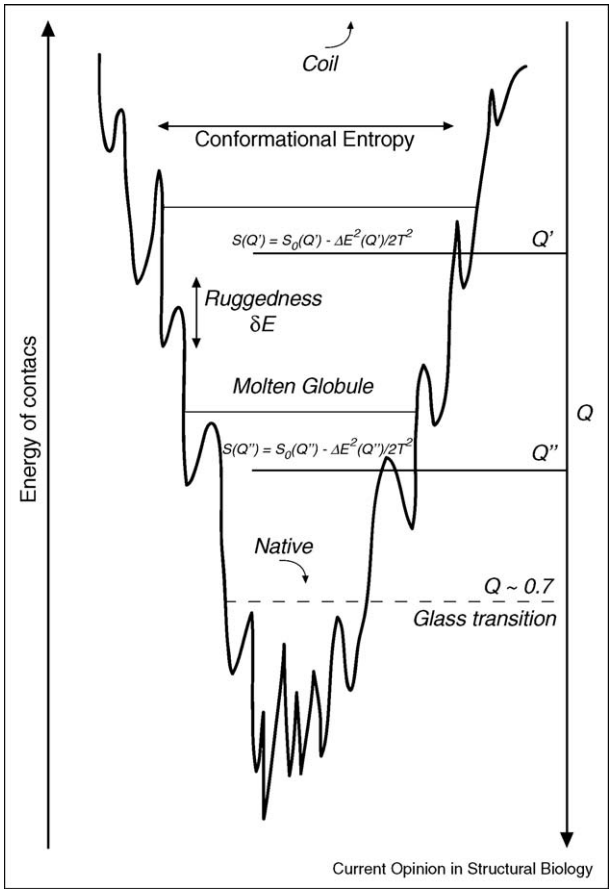
Introduction

Allosteric and functional transitions in proteins have been studied for many decades, starting from the successful kinetic models used to describe cooperative oxygen binding in hemoglobin [1]. However, as the microscopic understanding of proteins deepened, new questions kept arising. For example, despite a burgeoning understanding of the structure of the native state, the means by which a protein chain can fold into a unique 3D structure, predefined by its amino acid sequence, remained a mystery. This conundrum has been resolved with the emergence of the energy landscape theories, where it became apparent that protein landscapes are specialized compared with peptides with random sequences [2–9]. In particular, protein conformations which share many similar contacts with the native state are low in energy, introducing strong correlations in the energy landscape, which are absent in random heteropolymers. The energetic gradient toward the native state has been conveniently visualized in the form of a funnel [3], where conformational energy is plotted on the vertical axis and conformational entropy is indicated by the width of a funnel cross-section (see Figure 1). In addition to creating a funneled landscape, evolution also ensured that it is smooth, to avoid significant kinetic traps during the folding process. This is achieved by minimizing conflicts between the favorability of native contacts, which is known as the principle of minimal frustration [10]. These two landscape properties encourage a statistical view of the folding landscape, where specific details about various barriers and minima are of diminished importance, and are replaced by corresponding statistical distributions [11].

On the other hand, structural knowledge about the native state turned out to be incomplete, since it is not a single conformation at the bottom of the funnel, but a large ensemble of interconverting structures [12,13]. Ironically, the topography at the bottom of the funnel (the functional landscape) is statistically more complicated than the topography on the scale of the whole funnel (the folding landscape). When the protein chain moves from the middle part of the funnel, where it is highly dynamic (although compact), down to the bottom, it loses a significant amount of configurational entropy (see Figure 1). Subsequently, the chain dynamics are more strongly affected by the ruggedness of the landscape — specific barriers and minima — rather than being mainly driven by configurational entropy. Thus, the system starts to run out of states, signifying a transition to more glassy dynamics. For this reason, the statistical view, which is so helpful for describing the folding process, is less universal when describing functional motions (with some exceptions, such as the discovery of the hierarchical organization of native-like conformations [12]). For instance, a protein chain at the bottom of the funnel may reside in a particularly deep trap for a long time, waiting to overcome a large barrier, and this event may be more important in determining its functional dynamics than the distribution of other barriers and traps on the functional landscape. Therefore, it is most helpful to describe such a landscape with a detailed topographical map instead of statistical distributions of minima depths and barrier heights. Thankfully, the size (phase volume) of the bottom of the funnel is much smaller than in protein folding, and proteins in nature can fully explore it by brute force on biologically relevant timescales. Current computational resources are also approaching this limit; hence, functional landscapes of some smaller proteins have been sampled on microsecond timescales.

Figure 1



Protein folding landscape is schematically depicted as a funnel [3]. The vertical axis corresponds both to energy of contacts and also to Q, where the latter indicates similarity to the native state. In the upper region the protein chain is unfolded with a large conformational entropy. In the middle part of the funnel, the chain becomes compact, but retains diminished, yet still significant entropy. The lower part is a collection of similar low-energy conformations separated by barriers, known as the native state. The funnel can be stratified according to Q. At a fixed Q, configurational entropy is defined by the interplay between total number of states and ruggedness of the landscape for that particular Q stratum. At glass transition temperature T_g the configurational entropy almost vanishes. In the figure, $T_g(Q'') > T_g(Q')$. The funnel region around $Q \sim 0.7$, which corresponds to the onset of native-like conformations, is estimated to have a relatively high glass transition temperature [3].

Despite the number of conformations in the native state being significantly reduced relative to the unfolded protein, the atomistic dynamics are still highly multi-dimensional (hundreds or thousands of degrees of freedom) and must be projected to a few global or local coordinates to allow interpretation. This is critical for achieving broad conceptual insights, even if protein native dynamics can be adequately simulated with powerful computers. Otherwise, numerical simulations might fall into danger of simply becoming a surrogate for real experiments.

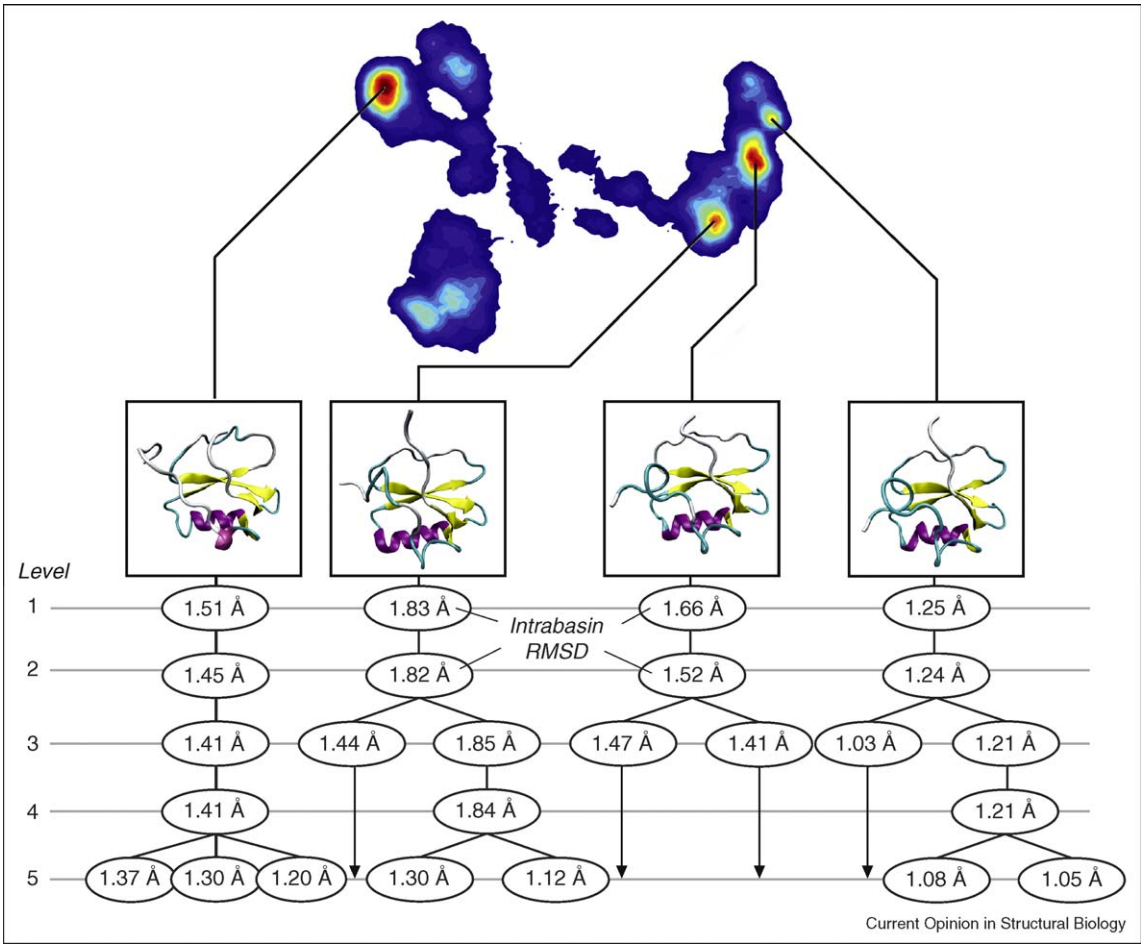
Choosing appropriate collective coordinates is a highly non-trivial task, however. For instance, Q, the fraction of native contacts, which is very useful when studying the folding landscape, because it provides a measure of correlation with the native state, is unlikely to be sufficient for describing the functional landscape of folded proteins. At the bottom of the funnel, one often is more interested in motion transverse to Q. However, excitations in a folded protein may include its partial unfolding [14], so Q still serves as one of many useful coordinates. In the discussion below we provide an overview of different collective coordinates that have been recently employed at high structural resolution or at long timescales to describe the native state dynamics. We also discuss techniques that try to avoid the use of global coordinates altogether. Finally, we overview some cases where native dynamics can be detected experimentally and fruitfully interpreted using the functional landscapes paradigm.

Trees and networks

A well-known method for dimensional reduction — principal component analysis (PCA) — can be applied to characterize protein dynamics [15–17,18••]. The main advantage of this approach is that it uses the actual protein dynamics itself to generate the appropriate collective coordinates for landscape description, relieving the concern of wondering if *a priori* chosen variables have captured all important motions. This technique has been frequently used to project the dynamics into first and second principal components, identifying conformational substates in the native state ensemble [15,16,19]. In a recent development, Materese *et al.* analyzed these basins using all relevant principal components, instead of just the first two, which allows one to follow basin splitting in higher dimensions [18••]. Hence, their approach directly reveals the hierarchical organization of protein native landscapes, in agreement with earlier experiments [20,21]. Thus, it has been shown that conformational substates in the native basin can be arranged into a tree, where the latter may potentially reflect organization of transition rates between the conformational substates [18••] (see Figure 2).

On a conceptually related note, a number of studies mapped protein folding landscapes by grouping the conformations visited during extensive Molecular Dynamics (MD) simulations to network nodes, building networks of states [22•,23–28]. There are a number of ways to group the visited conformations into network nodes, based on structure alone [22•], or on structure and features of the potential energy surface with the help of transition disconnectivity graphs [24,29]. At the next step, one estimates the transition rates between network nodes, subsequently, assigning the network edges. Finally, the resulting master equation is solved for various first passage time problems, to model long-timescale kinetic

Figure 2



Tree-like hierarchy of the functional landscape of eglin c is revealed by principal component analysis [18••]. For instance, a basin on a (PC1 and PC2) plot splits in two sub-basins on a (PC2 and PC3) plot. Five hierarchical tiers were identified. We thank Christopher K Materese for preparing the figure.

processes. Most of these studies relied on coarse-grained force-fields with implicit solvent because of the large phase volume of the folding landscape. Application of the network construction approach to functional landscapes of proteins is also fruitful [30], although with large proteins the existing efforts still encounter difficulties with sampling even in implicit solvent models and use phenomenological transition rates [24]. The main advantage of this class of methods is that they evade the problem of *a priori* choosing a complete set of collective coordinates. The kinetic network approach can also be combined with PCA and construction of free energy surfaces in hybrid techniques [22•].

Free energy surfaces

Free energy surfaces (FESs) may provide a powerful representation of the functional landscape [22•,31••,32••]. One way to take advantage of computed FESs is to run Brownian dynamics on top of them to investigate various first passage time problems [31••,32••]. For example, the first passage times of a transition between similar protein conformations can be used to characterize the nature of protein dynamics: whether it is diffusive like in a liquid or activated (glassy) like in a supercooled liquid. Wu *et al.* investigated this issue for the small protein, Trp-Cage, finding that at room temperature the protein dynamics is borderline between diffusive and activated [31••].

FESs may be computed using structure-based coordinates such as Q or root mean square deviation (RMSD). Since two such coordinates are required, slightly different native structures [31••] or different functional conformations [33••,34] have been used. Despite the correlation of Q with folding, these 2D surfaces resolve motions transverse to folding at high structural resolution in the native-like structural basin [31••]. A two-dimensional Q_1, Q_2 surface also accounts for unfolding excitations [14], as well as allosteric motions in which native contacts are broken in the *cracking* processes [33••,34–37]. Whether partial unfolding occurs is an important characteristic of a

functional transition that can be determined from a trajectory on a Q-based free energy surface [33••,34].

As discussed in the Introduction, characterization of functional landscapes requires many additional collective coordinates, which can be highly problem specific. For instance, to describe the motor protein kinesin function, the spatial motions of its parts are most important. Kinesin is a large multidomain protein, which moves along microtubules in discrete 8 nm long directed steps. It has two 'heads', the domains that interchange in binding to specific places on a microtubule. In this case, the spatial coordinates of tethered head center-of-mass (x, y, z) are convenient for computing a useful 3D FES. The surface, in turn, can be used to run Brownian dynamics simulations, to estimate various rates for kinesin steps and reveal the most favorable site for the tethered head to bind next on microtubule [33••]. In a different context, to describe the mechanisms behind the catalytic activity of enzymes or to compute the enzymatic rate constants, a chemical reaction coordinate is needed, such as the distance between the atoms which form new bonds or break old bonds [38]. It has been recently suggested, based on interpretation of various experiments, that allosteric motion and catalytic steps are tightly coupled in adenylate kinase, such that the protein motion facilitates the catalytic step [39–41]. In order to explore this issue, Pisliakov *et al.* used one conformational and one chemical coordinate to construct a 2D FES from higher resolution structural models [32••]. Subsequently, they ran Brownian dynamics simulations on this surface, finding no evidence for direct channeling of conformational excitations into the chemical transformation [32••]. This was explained by highly dissipative nature of protein conformational dynamics in explicit solvent, where coherent excitations of specific protein modes decay on timescales less than a nanosecond, while the chemical catalysis step usually occurs on the millisecond timescale [32••]. In yet another study, to understand causes of a particular allosteric mechanism, Okazaki *et al.* used a conformational coordinate together with binding coordinate (such as ligand binding energy) to obtain a 2D FES and plotted allosteric transition paths on it, suggesting that strong, long-ranged interactions lead to the induced-fit mechanism, while the pre-existing-equilibrium mechanism is favored by weak, short-ranged interactions [42].

Allostery

Allosteric transitions in proteins result in a global change in the native conformation upon local perturbation (ligand binding). They can be explored using functional landscape techniques. Allosteric landscapes have evolved to facilitate specific cooperative functional motions. Direct experimental observation of allosteric transition pathways is rarely possible, since energy landscapes are usually not probed directly, but instead only one or a couple of free energy minima are observed. A dimeric enzyme caspase-1 provides a nice example: in this protein, two cooperative binding sites are connected by a hydrogen bonding network which globally rearranges upon binding [43•]. Datta *et al.* used alanine mutation scanning to determine how residues in the hydrogen bonding network influence enzymatic activity. They identified two crucial residues which form a salt-bridge [43•]. It is expected that interpretation of allosteric transition ideas in molecules such as this using energy landscape should be productive. An example of studying the way specific contacts, including salt-bridges and water-mediated contacts sculpt the functional landscape is given by Materese *et al.* [18••].

Mapping of the allosteric functional landscapes in implicit solvent computer simulations can provide insight into important details, such as the adenylate kinase allosteric transition involving the pre-existing-equilibrium mechanism [44] or estimating the relative stability of two allosteric forms of C2 domain of coagulation factor V [45]. It is now even possible to use explicit solvent on a microsecond timescale, as Yang *et al.* have done for Src tyrosine kinase to build a connectivity map representing the energy landscape and provide simplified structural description of the concerted motions during the activation of the enzyme [46].

Techniques like paramagnetic relaxation enhancement measurements make it possible to observe transient dynamical events in protein native dynamics, providing a glance at the low-populated regions of the functional landscape [47,48]. A study by Thielges *et al.* exemplifies the benefit of increased usage of the energy landscape language by experimentalists: the authors connected multiple timescales detected by three-pulse photon echo peak shift (3PEPS) spectroscopy in antibody–fluorescein complexes with distribution of the barrier heights on the functional energy landscape [49]. Terahertz spectroscopy is yet another powerful technique for probing collective protein motions and their coupling to the hydration shell dynamics [50].

Coupling of folding and binding landscapes

Some intrinsically disordered proteins fold only upon ligand binding, representing a special type of allosteric transition [51]. A theory of coupling between binding and folding landscapes was developed by Papoian and Wolynes [52]. The resulting two-dimensional energy landscape was subsequently used by Wang and coworkers to study the detailed kinetics of binding and folding using path integral techniques [53]. Computer simulations using coarse-grained and atomistic models have also shed light on the kinetic mechanisms of the interplay between binding and folding [54,55]. One possible mechanism of coupling between binding and folding is localized frustration near the binding site that can be relieved upon binding [56]. In a very interesting recent development, a conformationally disordered enzyme was found to be

catalytically active [57]. Subsequent computer simulations indicated that the molten-globule-like phase partially folds upon substrate binding [58]. Since a meticulously defined three-dimensional structure has always been a cornerstone for explaining enzymatic catalysis, it was recently argued that proteins with weakly funneled energy landscapes challenge the long-held 'structure–function' paradigm [59]. However, there may be good reasons for a protein to evolve to be unfolded without a ligand. Unfolded chains occupy a larger volume, which may accelerate binding kinetics due to the increased cross-section for binding, according to the so-called fly-casting mechanism [33••,60]. Natively unfolded proteins are also degraded faster by the proteasome, providing an organism a finer temporal control over their functional activity [59]. Turjanski *et al.* [61] built *Q*-based free energy surfaces from computer simulations to elucidate coupling of a natively unstructured transcription factor folding to binding. They found that binding precedes folding, and the coupling of binding and folding is in accordance with NMR experimental data [62]. An explicit solvent all-atom simulations and subsequent representation of the energy landscape as 2D FESs gave insight into metal-coupled folding of a zinc-finger motif [63].

Conclusions

Current computational resources open new horizons in the detailed sampling of protein functional landscapes. Techniques developed to characterize the folding landscapes and frequently used with coarse-grained protein models can now be applied to investigate functional landscapes, even in the presence of explicit water. These studies provide detailed landscape maps, which, as explained in the Introduction, are more important for understanding protein function, than they are to describe folding, the latter being more amenable to the statistical treatment. In this review, we have discussed several recent works that have leveraged the energy landscape paradigm to explore protein dynamics and function, revealing its power in characterizing the organization and kinetics of the native state ensemble.

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- • of special interest
- •• of outstanding interest

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