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Adaptability of protein structures to enable functional interactions and evolutionary implications

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Several studies in recent years have drawn attention to the ability of proteins to adapt to intermolecular interactions by conformational changes along structure-encoded collective modes of motions. These so-called soft modes, primarily driven by entropic effects, facilitate, if not enable, functional interactions. They represent excursions on the conformational space along principal low-ascent directions/paths away from the original free energy minimum, and they are accessible to the protein even before protein–protein/ligand interactions. An emerging concept from these studies is the evolution of structures or modular domains to favor such modes of motion that will be recruited or integrated for enabling functional interactions. Structural dynamics, including the allosteric switches in conformation that are often stabilized upon formation of complexes and multimeric assemblies, emerge as key properties that are evolutionarily maintained to accomplish biological activities, consistent with the paradigm sequence → structure → dynamics → function where 'dynamics' bridges structure and function.

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Introduction

With the accumulation of structural and dynamic data and the rapid advances in the visualization of the spatio-temporal dynamics of protein–protein interactions [1] as well as the conformational dynamics of proteins in living cells [2], and with the availability of efficient models and methods for analyzing structural dynamics and allostery [3,4••,5], there is increasing support for the significance of structure-encoded dynamics as a major determinant of protein–protein and protein–ligand interaction mechanisms.

Structure-encoded dynamics, also called intrinsic dynamics, represents the conformational motions, or the spectrum of modes, uniquely defined by the 3-dimensional structure. The most favorable modes, also called 'soft modes' are usually distinguished by their cooperativity, hence their involvement in allosteric switches or global changes in structure [3,4••,6]. The functional significance and robustness of these modes of motions suggest new design and engineering principles, such as the need to enjoy suitable conformational flexibility, or substrate adaptability, rather than a high stability exclusively. Conformational flexibility appears to be essential to optimizing protein–substrate interactions [7,8], enabling allosteric responses [9] or mediating multispecificity [10–12]. In line with these concepts, the intrinsic dynamics of proteins is emerging as a factor closely related to the evolutionary selection of structures [13,14••,15•].

We present here recent studies, in support of the significance of structural dynamics in determining binding geometry, assembly and/or oligomerization mechanisms and facilitating allostery. We also highlight recent work on the relationship between the evolutionary selection of structures and their intrinsic dynamics.

The functional motions of proteins are not random: they are robustly favored by the structure

Proteins engage in many complex interactions in the cell. These are usually accomplished by changes in their structure, varying over a broad range, from highly localized movements at the level of single-residues, to cooperative rearrangements of multiple domains or subunits. While conformational changes have been broadly described as 'wigglings and jigglings', this description falls short of reflecting the cooperative nature of many functional interactions. In particular molecular machines require precise integration of functional movements (often driven by ATP binding). Increasing evidence supports the propensities of many complexes and assemblies to undergo non-random changes in their structures. These changes are usually predictable by simple models such as elastic network models (ENMs) which take account of the cooperative nature of biomolecular dynamics [16].

A few principal modes of motion, also called soft modes, mediate intermolecular interactions

The old concept of a single 'native' structure has long given way to that of an 'ensemble of substates in the native state' which usually share the same fold. The protein essentially samples a multitude of conformers, which are transiently stabilized during its biological activity. These conformers are accessible through local changes in structure (e.g. loop motions or side chain rotations) or global rearrangements (domain/subunit movements). Yet, these are all 'native' substates for a given protein, the relative probabilities of which change under different conditions, or at different stages of the biological processes (e.g. allosteric cycle) in which they take part, or in the presence or absence of their natural substrates — a phenomenon usually referred to as 'conformational shift'. Such shifts between pre-existing states may also occur due to mutations. There is increasing attention on the opportunities (and limitations) of modulating conformational shifts for controlling binding affinities and/or biomolecular functions [17].

An important observation is that these different conformers are along a few 'principal modes of motion' intrinsically accessible to the fold that they share [3,4••,18–21]. One of the early studies demonstrating that experimentally observed structural variations simply represent reconfigurations of different sizes along one or two principal modes encoded by the structure is that of de Groot, Gresinger and coworkers [22] for ubiquitin. This highly versatile protein adopts a variety of conformations while binding its substrates, and these are simply those sampled along one or two principal directions of motions accessible to the unbound ubiquitin, also seen by NMR residual dipolar coupling. A more recent example is the single-molecule Förster resonance energy transfer analysis of phosphoglycerate kinase (PGK) dynamics by Fitter and coworkers [23•]. In that study, Fitter and coworkers elegantly showed that first, the experimentally detected functional (hinge-bending) motions of the enzyme are encoded by the fold, as predicted by ENMs, and second, those motions are already performed in the ligand-free state of PGK domains, before substrate-binding.

Soft modes define pre-existing pathways of reconfiguration selected for modulating binding, assembly or multimerization

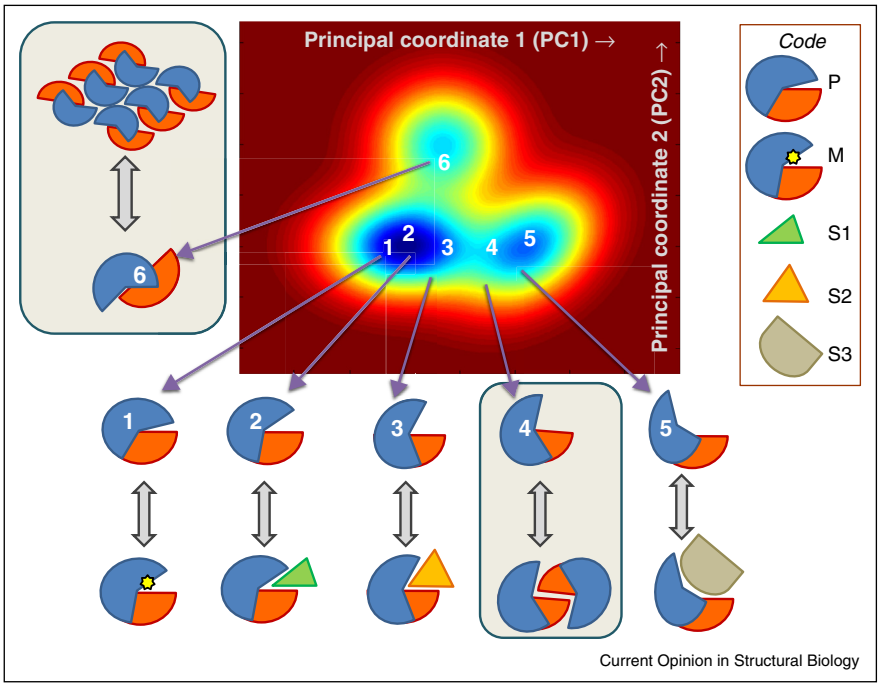
For a better visualization of the conformational space and accessible conformers, let us consider the free energy surface in Figure 1. The surface depicts the most favorable region, or the global minimum, of a much broader energy landscape. In principle, the protein (P) would sit at the lowest energy well, for example, position '**2**' on the landscape. But because of the different crystallization conditions and the inherent conformational flexibility of P, the structures resolved for P (as well as the models determined by NMR) would be distributed in the vicinity of this well. The conformers designated as '**1**'–'**6**' display such alternative structures, or *substates* of the native state. The important observation, from experiments and computations, is that these substates are not randomly distributed in space, but more or less aligned along a few principal directions of reconfiguration and these directions are nothing else than the soft/principal modes of motions accessible to P.

The soft modes may therefore be viewed as pre-existing paths or valleys on the conformational energy landscape, away from the lowest energy minimum [24]. Some are steeper; others are easy or soft. In the same way as these regions will be the first to be flooded when there is a rise in the water level, these modes are the first to be 'recruited' in response to a perturbation (substrate binding, mutation, etc.). In other words, the conformational changes undergone by the protein upon formation of multimers or complexes with different substrates (S1–S3), or in the presence of mutations (M), schematically illustrated in the last row (Figure 1) are simply those conformers already accessible to the (unbound) protein via deformations along its softest modes/paths.

In summary, the emerging picture is the following. The alternative structures resolved for a given protein — for example, ligand-bound/unbound, active/inactive, open/closed, outward-facing/inward-facing, or at different stages along an allosteric cycle — usually represent substates accessible via soft modes [18,21,25,26] predictable with the help of physics-based approaches such as ENMs and normal mode analysis [3,4••]. Excursions on the conformational energy landscape thus define the type of substates accessible to the protein to adapt to its interactions. Allosteric effects often arise by triggering or altering these pre-existing modes upon ligand binding.

We present here a few recent studies supporting these concepts: Dima and coworkers showed that the conformational diversity attained via excursions on the conformational landscape underlie the mechanosensing functionality of a muscle anchoring complex observed in atomic force microscopy (AFM) [27]; Gur *et al.* showed that the reconfiguration of adenylate kinase between its open and closed forms upon ligand binding takes place along such valleys of the conformational space [28]. Further, the conformational pathways described by a single mode starting from the open state were shown to successfully predict the closed state for a set of proteins that undergo large hinge-bending motions [29]. Shi and coworkers showed that the changes induced by Na^+ binding on the intramolecular interaction network correlate well with the principal mode of motion intrinsically accessible to the dopamine D2-like receptor [30]. Similarly, an evolutionary conserved interaction network was shown to connect Na^+ binding to global conformational

Figure 1



Schematic representation of conformers accessible to a given protein under physiological conditions, and pre-disposition to bind different substrates or favor different multimerization states. The energy landscape in the middle represents the vicinity of the native state or global energy minimum for a hypothetical protein P. It represents the projection of this region onto the subspace spanned by two principal coordinates, PC1 and PC2. Numbers 1–6 on the surface depict the location of various structures, or substates that might be resolved for the same protein. They would be distinguished by the type and extent of rearrangements between the two subunits (colored *red* and *blue*) of the protein. There is a series of conformers along PC1, differing in the extent of 'opening' of the cleft between the two subunits, from the most compact (labeled '**1**'; leftmost) to the most exposed ('**5**', *rightmost*), predisposed to bind different substrates (S1–S3). Structure '**6**' shows a different inter-subunit packing arrangement, for example, a twisting motion, defined by PC2. Some of the structures are pre-disposed to form multimers (e.g. '**4**' favors dimer formation, and '**6**' favors hexamer formation). The mutant M structure resembles an already accessible structure ('**2**'). Thus, the protein may accommodate different substrate binding (multispecificity) or adapt to different oligomerization states by reconfigurations along two principal coordinates. PC1 and PC2 are generally along the two softest modes intrinsically accessible to P, predictable ENM analysis. The soft modes thus represent pre-existing directions of structural change, probabilistically accessible under equilibrium conditions, and can be selected for mediating ligand binding, mutations or oligomerization.

changes crucial to neurotransmitter transport [31]. The transition of aspartate transporter between inward-facing and outward-facing states is essentially accommodated by a single mode predicted by the ENM in the presence of membrane environment [32]. Modulation of soft modes underlies allosteric regulation in CRP/FNR family of transcription factors [33–35]. Residues acting as hinges in the softest mode of ASC protein allosterically modulate the binding surface to promote the formation of ASC speck assembly [36•]; CO_2 binding stabilizes the open form of connexin26 by interfering with the softest mode accessible to the protein (which otherwise drives the opening/closing of the hemichannel) [37]. ENM modes that enable the contraction/dilation of the extracellular vestibule in a series of GPCR family members correlate with the formation of the cavity for G-protein binding on the intracellular side [38•]. Also, the global modes of motion provide mechanistic insights into how the function of voltage-gated potassium channel Kv7.1 is regulated by the binding of its auxiliary subunit KCNE [39]. Finally, the binding of the transactivation domain of MLL protein and c-Myb to CREB KIX domain is enabled by reconfiguration along soft modes [40].

Binding is mediated by not only local interactions but global dynamics that alter surface properties and regulate allosteric responses

Several observations point to the interplay between stability and dynamics in shaping protein's binding landscape. The significance of shape complementary and local physiochemical characteristics is well-established. But binding is not necessarily a local phenomenon. It also entails global changes in conformation that may allosterically alter surface properties at distal regions. Examples are pre-existing structural fluctuations that expose binding epitopes or drive the formation of a cavity for substrate binding [41•], stabilization of the selected conformational

shift that enables binding and controls the binding affinity [17], or the modulation of the global dynamics upon complex formation [33].

We anticipate that design studies that involve substrate binding will increasingly require a thorough understanding of the nature of the global modes accessible to the unbound protein, in addition to the usual examination of its structure and surface properties. On a local scale, protein–protein interfaces show dynamic patterns with distinct thermal fluctuations [42]; on a global scale, allosteric changes in structure may explain some of the entropic gain in binding [43]; a distant mutation that induces a large conformational change may disrupt the dimerization of an enzyme [44]; or, the functional effect of *N*-glycosylation is not through changes in protein structure but decreases in protein dynamics, and an increase in protein stability modulates the oligomerization and aggregation states of the glycosylated protein [45]. Likewise, a nucleotide-mimetic was shown to modulate the oligomerization state of the oncoprotein reptin by altering its global conformation and protein-binding activity [46•]. Finally, an approach based on the maximization of information entropy change associated with the global modes between bound and unbound structures significantly helps in distinguishing the native protein complex structures from the designed complex structures [47].

Triggering or altering of pre-existing dynamics is a means of modulating biological activity

Proteins present key sites that have the capacity to trigger or alter global modes of motion. Hinge regions are such sensitive sites [3,29]. Hinge-bending and large twisting/untwisting motions are common mechanisms of allosteric regulation, as shown in numerous ENM applications. Enhanced hinge flexibility facilitates kinase activation [48]; conversely, substrate coevolution in HIV-1 protease restore the hinge axis deformed by drug resistant mutations, highlighting the functional importance of hinge motions [49]. Allosteric hot spots constitute another group of target sites that modulate protein–protein interactions [50]. Notably, an allosteric inhibitor recently discovered for neuropeptidases presumably disrupted activity by preventing a hinge-like motion associated with substrate binding and catalysis [51•]. In some cases, key sites may be on the surface, for example, some residues serve as sensors, and others as effectors for efficiently sensing and rapidly communicating perturbations. A recent study invited attention to such residues on the ATPase domain of Hsp70, which mediate interdomain allostery [52•]. In the case of the PyrR family of pyrimidine operon attenuators, key mutations all distant from the interface and outside ligand-binding pockets were identified to control the oligomeric state; these mutations introduced structural changes comparable to the conformational shift observed between the unbound and nucleotide-bound conformations of the protein [14••]. Distant dynamic couplings between variable (V_H) and constant (C_H2) domains and the hinge region (C_H1–C_H2 interface) were also observed within an IgG1 monoclonal antibody during its reversible self-association [53]. Allostery through DNA is also an important modulator of DNA functions; the coalescence of protein-induced DNA bubbles was suggested to regulate DNA's flexibility and the assembly of the transcription machinery [54]. Binding of an antibiotic 60 Å away from the DD-transpeptidase active site has been shown to allosterically stimulate the opening of the active site, predisposing the penicillin binding protein 2a to inactivation [55].

Evolution of sequences and structures to enable intrinsic dynamics in favor of functional interactions

Emphasis in classical studies has been on the requirement to conserve biochemical (e.g. catalytic) activity and overall stability, and on the evolution (or conservation) of amino acids to ensure them. However, with the emergence of structural dynamics as a major determinant of mechanisms of interaction, it is clear that the conservation of the conformational mechanics (not only chemistry) is another equally important evolutionary requirement. ENM-based normal mode analyses have helped elucidate the shared dynamics of homologous proteins starting from the original work of Echave and coworkers [56] and Ortiz and coworkers [57]. An important observation has been the correlation between the structural core change among family members (for a series of protein families) and the soft modes intrinsically favored by the shared architecture of family members [57]. A more recent study helped elucidate the conserved ENM modes crucial to the switching function of Ras GTPase family, as well as the modes specific to particular family members [58].

Systematic study of sequence conservation patterns and conformational mobilities demonstrate that regions that enjoy higher conformational mobility are also sequentially variable, and vice versa [59,60]. Furthermore, not only conserved residues, but also co-evolving pairs of residues are of interest toward gaining a better understanding of the functional interactions (intramolecular or intermolecular) that are presumably maintained by compensating mutations. These analyses clearly demonstrate that co-evolving pairs of residues relate to 3-dimensional contacts [59,61–65]. Furthermore, coevolving pairs of residues often populate conformational flexible regions such as substrate-recognition sites [52•,59,66••], suggestive of the need to modulate specificity by sequence coevolution. Finally, proteome-wide analysis of conformational dynamics indicates that the interface sites enriched in disease-associated non-synonymous single nucleotide variants play a crucial role in functional dynamics [67]. It remains to be seen if such studies can assist in the identification of allosterically coupled sites and in the

design of allosteric inhibitors. Efforts to push forward these efforts combined with druggability considerations [68] may open new avenues for identifying potential sites for allosteric regulation.

An interesting observation is that pathways of assembly are also under evolutionary pressure [13,69]. In a recent review, Marsh and Teichmann emphasized how local protein flexibility and disorder, as well as large-scale motions and quaternary structure assembly correlate with evolutionary changes in protein sequence and structure [70]. Electrospray mass spectroscopy experiments show assembly intermediates that are in accord with those observed evolutionarily [13]. Teichmann and coworkers also showed that the conformational changes allosterically induced by selected mutations (called *allosteric mutations*) are similar to those stabilized upon ligand binding or by intersubunit geometry changes occurring upon oligomerization [14••]. This observation again highlights the intrinsic preferences of the original structure to undergo changes along well-defined (soft) modes of deformation, as illustrated in Figure 1. The relationship between intrinsic dynamics and evolution appears to be twofold: evolution selectively maintains the structures that lend themselves to functional intrinsic dynamics, and evolution employs the intrinsic dynamics of the protein to promote allosteric switches or oligomerization mechanisms.

Conclusion

A wide range of events/processes implicated in a given protein's interactions, function and evolution appear to proceed via similar mechanisms: allosteric response of the protein to specific substrate binding, its structural changes triggered or stabilized by ligand-binding or drug-binding, its evolutionarily selected modes of assembly or oligomerization. Our current understanding is that these are 'similar' because they represent all excursions along a few dominant directions on the energy landscape: the soft modes of motions uniquely defined by the protein architecture. Methods that exploit the intrinsic dynamics of proteins are probably to open the way to new strategies that for design, discovery and therapeutics.

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