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Intrinsic dynamics is evolutionarily optimized to enable allosteric behavior

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Abstract

Allosteric behavior is central to the function of many proteins, enabling molecular machinery, metabolism, signaling and regulation. Recent years have shown that the intrinsic dynamics of allosteric proteins defined by their 3-dimensional architecture or by the topology of inter-residue contacts favors cooperative motions that bear close similarity to structural changes they undergo during their allosteric actions. These conformational motions are usually driven by energetically favorable or soft modes at the low frequency end of the mode spectrum, and they are evolutionarily conserved among orthologs. These observations brought into light evolutionary adaptation mechanisms that help maintain, optimize or regulate allosteric behavior as the evolution from bacterial to higher organisms introduces sequential heterogeneities and structural complexities.

Keywords

intrinsic dynamics; allosteric response; evolutionary adaptation; chaperonins; normal modes; elastic network models

Introduction

Allosteric regulation of protein function takes place in diverse biological processes, such as metabolism, signal transduction and molecular machinery. Two classical models of allostery have found broad utility in interpreting the behavior of allosteric proteins: the concerted (all-or-none) model and the sequential model, with respective underlying mechanisms of (i) selection from pre-existing states and (ii) induced fit, stabilized or elicited upon ligand

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binding [1]. However, recent experimental and computational data on the subject have exposed the need to go beyond simple phenomenological models and acquire a physics-based or mechanistic understanding of allostery as well as its evolutionary fine-tuning. As such, protein flexibility and structure-encoded collective dynamics have come forth in forming the link between folded structures and allosteric regulation. Furthermore, the need to understand the allosteric events in the light of the conformational space accessible to the protein under equilibrium conditions became clear [1–4]. While the thermodynamics-based concept of an ensemble of pre-existing states is plausible, the ease of undergoing allosteric switches/shifts triggered by local binding events calls attention to the role of conformational dynamics, or pre-existing paths that ought to be easily accessible on the conformational landscape, near the original equilibrium state [5].

The correlation between the allosteric mechanisms of conformational changes and the ‘soft’ modes of motions intrinsically accessible to folded structures, uniquely defined by the inter-residue contact topology, is now borne out by numerous studies [6,7]. Here ‘soft’ refers to minimal increase in energy for a given excursion away from the equilibrium state. Furthermore, given the functional significance of allosteric events, emerging topics of interest are the relationship of allosteric dynamics to evolution, the ability of proteins to evolve so as to favor/retain allosteric functions [7,8] (*arrow **1*** in Fig 1); or the possibility of designing allosteric proteins by learning from evolution [9,10].

In this review, we focus on the relationship between biomolecular action, evolution and dynamics (Fig 1). Actions involve both chemical (e.g. catalytic, orthosteric) and physical (e.g., binding, allosteric) events. We focus here on the physical changes that underlie allosteric responses, how they are uniquely encoded by the structure, and how the structure (and sequences) that allow for these allosteric switches/shifts are uniquely selected by evolution.

Allosteric responses are triggered when stimuli exploit pre-existing dynamics

Conformational coupling between spatially distant functional sites, e.g. allosteric and orthosteric sites, is a key requirement for regulating allosteric function [11]. This way a first binding event (e.g. ATP binding) induces, or facilitates, a second (e.g. substrate binding) often taking place at a remote location on the structure. While classical examples of allostery have focused on ligand binding (e.g. oxygen binding to one of the subunits of hemoglobin that prompts cooperative binding to other subunits; or ATP-binding in ATP-regulated molecular machines), recent studies show that allosteric responses can be stimulated by a variety of mechanisms in addition to binding of an ion, small molecule, another protein, or DNA [12,13] (*arrow **4*** in Fig 1), such as chemical change or posttranslational modification (PTM)[14,15] (*arrow **5***), complex formation or assembly [16] (*arrow **6***), or mutations or amino acid substitutions [17–19] (*arrow **7***). In fact, all proteins, including monomers and complexes/assemblies, have been pointed out to be prone to allosteric modulation[20].

While the ability to exhibit allosteric responses has been observed for a broad range of proteins, including membrane proteins (transporters, receptors) in recent years, not all

binding/complexation events result in allosteric responses. Allosteric responses are robustly dependent on the overall architecture [1], or topology of contacts often described by network models. The site, and even the direction, of application of the perturbation or alteration matters. The point is that the allosteric response would not be induced if the protein were not already *predisposed* to undergo that specific conformational change; and only those stimuli that exploit those pre-existing propensities do effectively induce cooperative, allosteric responses, hence the concept of selecting from an existing pool of collective modes (at the center of 'dynamics' box in Fig 1). Essentially, the first binding event acts on a site, and in a direction, that is highly *sensitive* to the specific perturbation, or should stimulate an energetically favorable movement in order to elicit an allosteric response [21,22]. In the energy landscape description of the conformational space, that direction is the first to be flooded/occupied upon a rise in energy. Perturbations due to energy input, such as that released by ATP hydrolysis, also fall in this category, as demonstrated in a study of GroEL [23], that the system is driven by the ATP hydrolysis-induced directional force to move along existing transition pathways on the free energy landscape.

Using these concepts, new methods have been developed for assessing the allosteric potential of individual residues or for predicting allosteric communication pathways [24–33]. A recent review [34] provides an overview of structure- or topology-based approaches for predicting allosteric mechanisms, including elastic network model (ENM)-based analyses, molecular dynamics (MD) simulations, and graph theoretical methods.

Allosteric changes in structure conform to evolutionarily conserved soft modes

The above described site- and direction-specific sensitivity to perturbations is an intrinsic property of the structure. Each structure encodes a spectrum of normal modes under equilibrium conditions. Among them the softest modes, also called global modes, are manifested in the largest changes in conformation (for a given energy increase), and they are often distinguished by their high collectivity, i.e. the motion is distributed over a large portion of the structure. Many studies, including those in recent years [35–38], demonstrated that these 'cooperative' modes are 'used' when the protein undergoes allosteric changes in its structure, i.e. the different types of perturbations (*arrows **4–7*** in Fig 1) elicit allosteric responses only if/when they selectively operate on those sites that stimulate the soft modes that lend themselves to allosteric behavior (*arrow **2*** in Fig 1). Likewise, alterations in these soft modes due to mutations or aberrant interactions may lead to dysfunction.

How can we determine these soft modes? A broadly used approach is to adopt an ENM for modeling the topology of inter-residue contacts. ENMs provide an analytical solution for the spectrum of normal modes uniquely accessible to each protein/complex, which is then decomposed to extract the soft modes. User-friendly interfaces have been developed to facilitate these computations; among them, *DynOmics* [39] also takes account of the environment such as the lipid bilayer for membrane proteins. More advanced methods combine ENM-based methods and MD simulations to determine the conformational subspace spanned by soft modes [40–42]. ClustENM [41] is such an unbiased hybrid

method. Application to a broad range of proteins, from calmodulin to the ribosome, and to DNA-protein and protein-protein docking [43], as well as trigger-factor-ribosome complexation [44], provided evidence for the role of soft modes in sampling populated conformers, including those visited during allosteric machinery, binding and assembly.

A number of studies have shown that soft modes are conserved within protein families and "fine-tuned for specific function" [7] (*arrow **3*** in Fig 1). A recent systematic analysis of 116 CATH superfamilies showed that each superfamily is characterized by a *signature dynamics*, defined by 1–4 softest modes shared among all members [45]. Note that these modes include those that underlie, or facilitate, allosteric couplings. Detailed analysis for selected families further showed that the differentiation within families are mainly imparted by a second group of prominent modes, immediately following these highly conserved modes, termed low-to-intermediate frequency modes (LTIF) [45].

Interestingly, a few modes at the other end of the spectrum, the highest frequency modes, also exhibit a certain level of conservation among family members [45]. These usually refer to sites severely constrained, due to stability (e.g. folding nuclei) or chemical activity (orthosteric) requirements. A recent ENM analysis [11] highlighted the dynamic coupling between allosteric and orthosteric events. The coupling between these respective events is consistent with their concurrent evolutionary conservation.

Modularity and evolutionary adaptation mechanisms between orthologs facilitate allosteric responses

Many allosteric proteins contain conserved domains or subunits that serve as modular units for regulating their allosteric behavior. Examples of such domains in multidomain proteins are the ATPase domains, the Src module of cytoplasmic tyrosine kinases [46], the PDZ domains [47], the cAMP-binding domains of PKA [3], or the ATP-cones that regulate ribonuclease reductases [48]. Likewise, the individual subunits of allosteric complexes often possess intrinsic abilities to undergo conformational switches in accord with the allosteric transitions of the complex. The intrinsic abilities, or modular allostery, of these subunits are thus used, with suitable evolutionary adaptations originating from various effects (e.g. sequence changes, intermolecular interactions, PTMs), for enabling the more complex machinery of biological assemblies [49]. A recent example is the microseconds dynamics of an allosteric switch domain (M domain) in the AAA+ disaggregation machine, a hexameric chaperone that rescues aggregated proteins [50]. FRET experiments showed a broad population of conformers compatible with the active and inactive states of this domain, with interchanges much faster than the time scale of either ATP hydrolysis or disaggregation activity, thus prompting the AAA+ to its allosteric machinery.

A closer look shows the role of these modular units in enabling allostery, and the level of conservation, or differentiation, by evolutionary adaptation mechanisms. Figure 2 compares the behavior of bacterial (GroEL) and mammalian (CCT) chaperonins, both functioning as allosteric machines. The individual subunits of the former readily favor the transition between R (ATP-bound) and T (-unbound) forms, as can be seen in **panel B**: a high overlap (correlation cosine > 0.80) is observed between the softest mode predicted by the ENM (k =

1) and the experimentally observed structural change between these two states [51]. This means that the individual GroEL subunits function as allosteric modules that predispose the heptameric ring to cooperatively undergo all-or-none transitions. In contrast, CCT exhibits a more complex behavior, consistent with the heterogeneity of its octameric rings, as shown in **panel E**. Certain subunits undergo slower transitions than others, presumably resulting in a sequential transition. In parallel, the overall transition of the CCT hetero-16-mer (**panel F**) is not as efficient as that of the GroEL 14-mer (**panel C**). Overall, this example demonstrates that (i) the individual subunits of GroEL operate as allosteric modules that enable an all-or-none transition of the rings in support of the GroEL allosteric behavior, and (ii) the heterogeneity of the CCT results in a distribution of soft modes resulting in a slower, sequential mechanism of allostery, consistent with the result from experiments [52–54] and an Arrhenius analysis [55].

A *dynamics-based regulation* is therefore distinguished as the evolutionary adaptation mechanism that underlie the distinctive allosteric behavior of CCT compared to that of GroEL. A comparison of eukaryotic (Hsp70s, HspA1 and Hsc70) and bacterial (DnaK) chaperones also reveals the modulation of allosteric interactions by ‘evolutionary diversification’, so as to fine-tune Hsp70 function [56]. The significance of such dynamics-based fine-tuning of allosteric activity by evolution has been pointed by Hilser and coworkers in the context of the thermal adaptation of enzymes [57], and by Sjöberg and coworkers who noted the evolutionary flexibility of ATP-cones [48]. Likewise, Ozkan and coworkers invited attention to the evolving allosteric dynamics and resilience between ancient and extant thioredoxins [58], or to the evolution of β-lactamases to allosterically modulate resistance to antibiotics [59].

How does small molecule binding affect pre-existing states or dynamics?

The allosteric response triggered by small molecule binding can be viewed in the broadest sense as a change in the conformational energy landscape of the protein. The change in the energy landscape can be realized in multiple ways: the locations of existing minima do not change, but their population (or the corresponding depth or width/curvature) does; or their locations may also exhibit some shifts. Both effects were seen for example in the energy landscape generated for dopamine transporter in the presence and absence of its substrate, dopamine [60]. A change in the depth of the energy minimum could originate from an enthalpic effect such as electrostatic attractions with the ligand; whereas a change in the width or curvature of the energy minimum without altering the depth would be entropic. ENM modes are purely entropic. Their relevance to allosteric dynamics suggests that entropy is a major factor driving allosteric changes, or on the topology of inter-residue contacts. Yet, recent studies also invite attention to the importance of enthalpic effects that may also contribute to allostery [47,61].

Figure 3 provides a view of the change induced in the frequency of the protein’s pre-existing soft modes upon binding an allosteric modulator. Shifts in the ENM mode frequency dispersion were computed by populating the local density in the vicinity of each residue, in analogy to an analysis on triosephosphate isomerase [62]. The frequency shift in the soft modes is illustrated for each residue of the holo-enzyme, glutamate racemase (Figure 3A).

The residues interacting with the allosteric sites stand out in terms of their higher z-scores, as can be seen in the color-coded diagram in Fig 3B. Computations performed for a dataset of 315 proteins using RESPEC [63], a residue-specific ENM, showed that ligand-binding generally tends to shift the frequencies toward higher values, i.e., producing a constraining effect [64] on soft modes (Figure 3C).

Dynamics-based regulation of allostery as sequence and structure evolve

The above examples illustrate the significance of intrinsic dynamics in defining and evolutionarily conserving the allosteric function of proteins with suitable fine-tuning to mediate their sequence and structure diversification. Comparison of GroEL and CCT in Fig 2 showed how the distinctive allosteric dynamics of the bacterial and mammalian chaperonins can be traced to the intrinsic dynamics of their modular subunits, which in turn are encoded by their evolutionarily diverging sequences and structures. Fig 3 demonstrated how ligand binding regulates dynamics by changing the curvature of the energy landscape along the soft modes. On the other hand, mutations introduced through directed evolution, which increase the catalytic activity of tryptophan synthase, were reported to act allosterically through shifting the conformational ensemble [65]. Allosteric mutations located away from the subunit interface can modify the conformational dynamics, similar to allosteric ligands, and thereby induce changes in the oligomeric state of homologous proteins during evolution [66]. Thus, dynamics-based conservation or regulation of allosteric behavior emerges as an effective mechanism for maintaining or modulating function, as the protein sequence and structure evolve.

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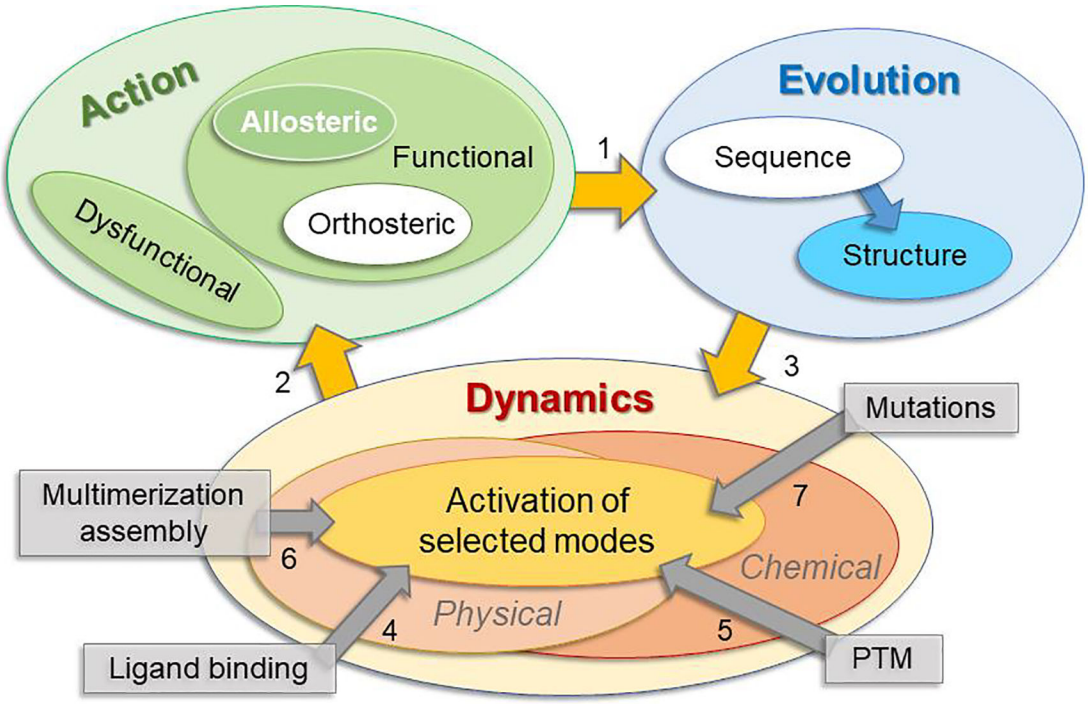

Figure 1.

Schematic representation of relationships between mechanisms of action, evolution and dynamics. *Green round box* represents the mechanisms of action. If an action is functional it may have allosteric and/or orthosteric effects. If it is dysfunctional, it will not be selected against by the evolutionary pressure (*Blue round box*). The protein sequences and structures filtered by evolution will then perform their functions defined by intrinsic dynamics, during which various perturbations act through either physical or chemical changes (*orange round box*). These changes may alter the favorability of selected modes through which the protein perform actions. *Gray square boxes* represent four main stimuli that trigger allosteric responses.

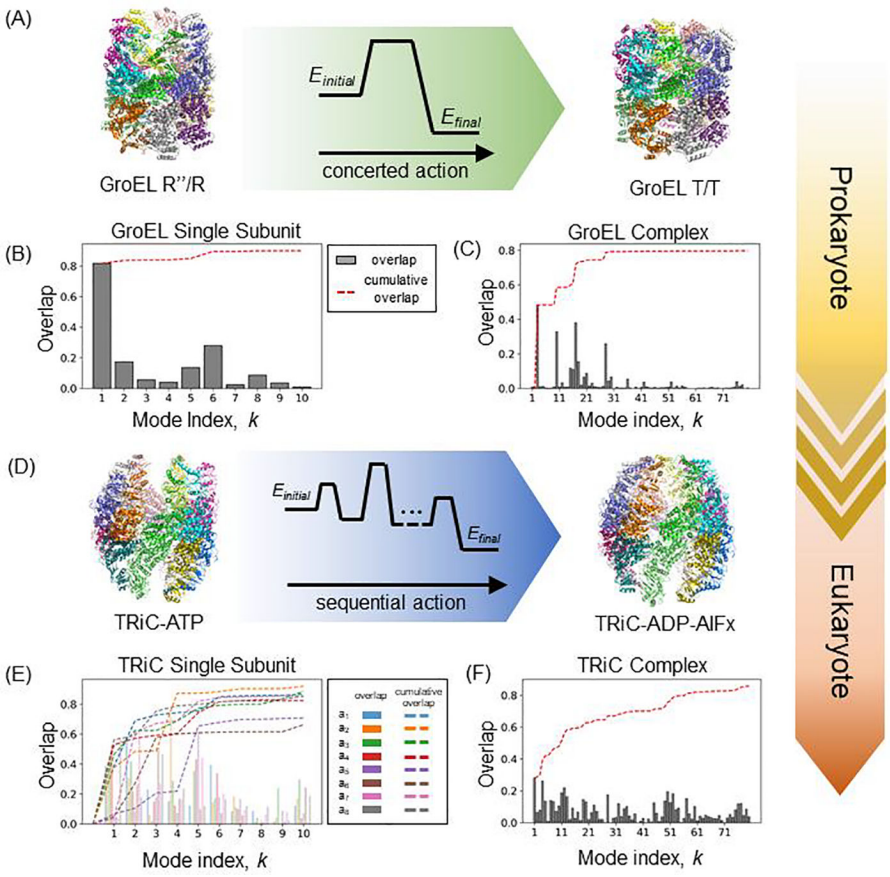

Figure 2. Differential modes of action of prokaryotic and eukaryotic chaperonins.

(A and D) The transition of GroEL from *cis*-ring GroES-bound (R") state (PDB id: 1gru) to apo (T) state (PDB id: 1gr5) follows a concerted mode of action, which surmounts a single energy barrier. Whereas the transition of TRiC/CCT from ATP-bound, open state (PDB id: 4a0v) to an ADP-P-bound, closed state (PDB id: 4a0w) follows a sequential mode of action, which involves several possibly smaller energy barriers. Co-chaperonin GroES is not included for the purpose of direct comparison between the two states of GroEL. (B) Overlaps between the ten softest modes predicted by ENM to be accessible to a single subunit of GroEL in the R form, and the experimentally observed deformation undergone by the subunit during its transition to the T form. The *bars* display the correlation cosines for each mode, and the *red curve* displays the *cumulative overlap* (summed over consecutive modes starting from mode $k = 1$. Panel (C) displays the behavior of softest 80 modes accessible to the entire complex. The cumulative contribution of ~ 30 soft modes is sufficient to reach a correlation cosine of ~ 0.80 with the experimentally observed transition between the two endpoints shown in panel (A). (D-F) Counterparts of the respective panels (A) -(C) shown for TRiC/CCT (mammalian chaperonin). CCT rings are each hetero-octameric, hence eight overlap curves and *bar plots* corresponding to each of the (sequentially different) subunits are displayed in (E). Overall the passage between the two end points necessitates a larger ensemble of modes of motions, compared to the bacterial chaperonin, suggesting a more controlled/restrained allosteric machinery evolutionarily endowed by sequence/structure divergence.

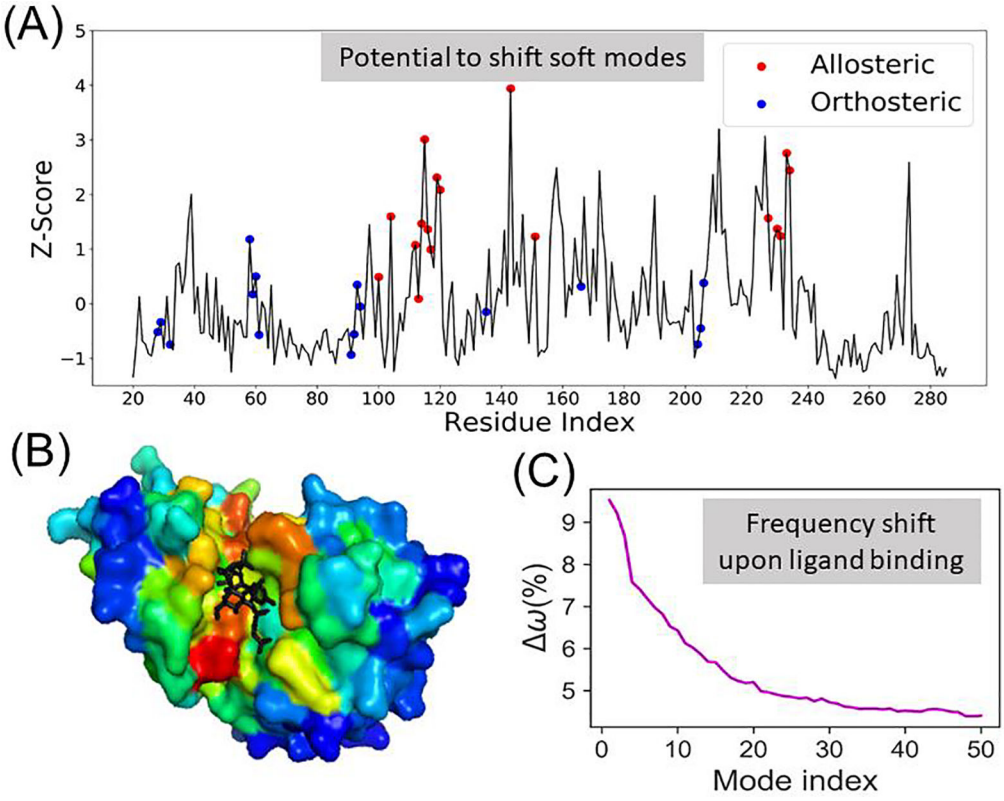

Fig 3. Potential of residues to induce a shift (increase) in the frequency of soft modes if targeted by a ligand.

Panel (A) illustrates the results obtained by scanning all residues in glutamate racemase (PDB id: 2JFN). The ordinate, z-score, gives a measure of the extent of frequency shifts in the global modes (averaged over ten softest modes), with peaks referring to those sites that would induce the highest shift, if targeted. Experimentally known binding/coordination sites for allosteric ligands (residues within 4.5 Å from allosteric ligands) are indicated by *red circles*, and those for orthosteric ligands, by *blue circles*. (B) Glutamate racemase in complex with an allosteric activator (UMA, shown in *black sticks*) and the orthosteric ligand (L-Glu, not seen from this perspective). The enzyme is color-coded by z-scores, *red* and *blue*, representing highest and lowest values, respectively. The allosteric ligand binding cavity is distinguished by its high potential to induce a frequency shift. (C) Results for a dataset of 315 protein complexes resolved in the presence of orthosteric and/or allosteric ligands. The curve displays the percent shift in the frequency of each of the 50 softest modes, computed by RESPEC, between the complex and the holo forms of the protein, averaged over all members of the dataset. The frequencies shift toward higher values, indicating a stiffening in the soft modes.