



Published in final edited form as:

Curr Protein Pept Sci. 2009 April ; 10(2): 128–132.

Allosteric Transitions in Biological Nanomachines are Described by Robust Normal Modes of Elastic Networks

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Abstract

Allostery forms the basis of intra-molecular communications in various enzymes, however the underlying conformational changes are largely elusive. Recently, we have proposed to employ an elastic model based normal mode analysis to investigate the allosteric transitions in several molecular nanomachines (including myosin II, DNA polymerase and chaperonin GroEL). After combining with bioinformatics analysis of the evolutionary sequence variations, we have been able to identify the highly conserved and robust modes of collective motions that are capable of transmitting molecular signals over long distances.

Keywords

Allostery; chaperonin; elastic network model; myosin; normal mode analysis; polymerase

INTRODUCTION

A common theme in a diverse range of biological nanomachines --- from motor proteins to various enzymes, is that they undergo large-scale domain movements in response to the binding or release of ligands or other partner molecules. These highly collective movements naturally couple the structural changes at distant sites to facilitate the transmission of signals over long distances. Indeed, the hallmark of allostery is the transmission of signals through a network of residues in response to local perturbations. Therefore, an ultimate understanding of the microscopic basis of allostery in large enzymatic systems hinges on the detailed revelation of the underlying conformational changes and the structural elements involved. This challenging task is made even more difficult by the lack of effective computing techniques to simulate and analyze these long-time-scale biomolecular motions. Standard computational techniques such as all-atom molecular simulations are usually limited to nanosecond time scale, while biomolecular movements span a wide range of time scales up to seconds or minutes.

To obtain insights into the ‘slow’ biomolecular motions, the normal mode analysis (NMA) of an elastic network model (ENM) representation of large biomolecular complexes has

been proposed [1, 2] In ENM, the alpha carbon atoms of neighboring amino acid residues are connected by springs with a uniform force constant. Despite such a drastic simplification, numerous studies have shown that many crystallographically observed large-scale conformational changes in proteins are dominated by one or a few lowest ENM modes [3–6]. The success of this low-resolution approach has been attributed to the robustness of the lowest modes in describing domain movements which are essentially determined by molecular shapes and are insensitive to detailed interactions [6].

Recently, we have sought to combine the structure-based ENM modeling with a sequence-based bioinformatics analysis to quantify the conservation of biomolecular motions as described by ENM modes. Our goal is to investigate the relationship between the global dynamics at the macromolecular level and the amino acid variations at the microscopic level. By analogy with the celebrated finding by Anfinsen that a protein's native structure is determined by its amino acid sequence [7], we propose that a protein's conformational dynamics is subject to modulations by evolutionary variations in its amino acid sequence, and the sequence variations are constrained by the conservation of functional dynamics. Thus, we expect that 'neutral' mutations that do not affect the functional dynamics adversely are tolerated while the 'hot spot' residues critical to the functional dynamics must be strongly conserved [8].

To test the above proposal and explore how evolutionary sequence variations shape the functional dynamics, we have introduced a novel method to compute the robustness of an ENM mode to the variations in ENM force constant. Such variations naturally arise from differences in the sequences of homologous proteins. Since an ENM is built by adding a spring for every inter-residue contact, the variation of residue types forming those contacts may change the force constant of the corresponding springs [8]. Such perturbations to ENM force constants would in turn change the ENM modes. Therefore, the functional constraint dictates that the ENM modes that describe the functionally relevant movements must be robust to the sequence variations.

We have implemented the above ideas using a structural perturbation method (SPM) [9, 10]. We have established, by probing the response of ENM modes to perturbations of residue-contact interactions, that certain low-frequency ENM modes are robust to sequence variations across a given protein family. For these robust modes, the extent to which the residue contacts are conserved in a protein family correlates well with their dynamical importance. We have validated these ideas for three biological nanomachines, namely, DNA polymerase, myosin II and Chaperonin GroEL. In these three cases we have found that the functionally relevant low-frequency modes are also the most robust to sequence variations. Thus, it appears that the robustness of the ENM modes is an indicator of their functional relevance [9, 10].

The SPM method can be used to identify the network of hot spot residues that cooperatively orchestrate the large-scale domain movements. By assessing the degree of response to residue-specific perturbations the importance of a given residue position in facilitating allosteric transitions can be gauged. We will demonstrate the applications of the SPM in the above three biological nanomachines. For further details see [9, 10].

DNA POLYMERASE

DNA polymerases play central roles in DNA replication and repair. Despite substantial variations in amino acid sequences, the global structural features of DNA polymerases are described using the hand metaphor [11]. In its reaction cycle, the first step involves the binding of the duplex DNA to the unliganded polymerases which triggers the closing of the thumb domain around the DNA. Subsequent binding of dNTP to the binary complex results

in the rotation of the fingers from the open conformation to the closed one. Waksman and coworkers [12] solved the structures of two ternary complexes of the large fragment of *Thermus Aquaticus* (TAQ) DNA polymerase I bound to a DNA duplex and dNTP. The transition from the open form (PDB: 2KTQ) to the closed form (PDB: 3KTQ) results in the closing of the finger-palm crevice.

The NMA of the open form structure yielded a number of low-frequency ENM modes that describe the observed open/closed conformational changes [9]. The open/closed transition is dominated by mode #4 (overlap=0.50) that describes the fingers domain bending toward the active site. We also found several subdominant modes (#5, #7, #10, with overlap smaller than for #4 but higher than average), which supplement mode #4 in describing the fine details of the observed structural changes. The robustness analysis revealed that mode #4 is the most robust mode while the other subdominant modes (#7, #10) are also highly robust. Thus there is a significant correlation between the functional relevance and the robustness of an ENM mode. This finding is in accord with a number of experiments that have probed the fidelity of DNA polymerases to specific mutations [9]. The strong conservation and robustness of the dominant and a few subdominant modes may be a requirement for the high-fidelity replication function of DNA polymerase I.

For the TAQ DNA polymerase I we have identified a sparse network of residues that is involved in the open/closed transition. Among these hot-spot residues, I614 [13] and F667 [14] were shown to be critical for the fidelity of DNA replication. 13 mutations of residues R660, A661, A662, T664, N666, V669, L670 were found to cause decreased fidelity [15]. In addition, R659, K663, F667, and Y671 were shown to be immutable or absolutely conserved. Kermekchiev *et al.* [16] reported 4 cold-sensitive mutations (E626K, W706R, I707L, E708D) and one deleterious mutation (Q690R). These residues are clustered on the outside surface of the enzyme's finger domain, which is far from the active site, but at the hinge of the fingers domain.

MYOSIN II

Myosin II belongs to a super-family of motor proteins that generate movements along actin filaments powered by ATP hydrolysis. The enzyme is capable of sensing and responding to the binding of actin and the presence or absence of a γ -phosphate and transmitting this information along a pathway of increasingly larger conformational changes that ultimately results in a force-generating event [17]. The available crystal structures and cryo-EM results of myosins capture their conformations in four structural states --- transition state, near-rigor state, detached state [18] and rigor-like state [19–21].

We have studied the conformational changes from the transition state (PDB: 1VOM) to the near-rigor state (PDB: 1MMA) [9]. This transition (1VOM \rightarrow 1MMA) is dominated by mode #1 (overlap=0.558) that describes a swinging motion of the converter relative to the rest of the structure. We also found mode #2 as a subdominant mode (overlap= 0.388). The robustness analysis revealed that both mode #1 and #2 are highly robust. The hot spot residues of mode #1 and #2 are mostly distributed over the relay helix, SH1 helix and the converter. These hinge residues may be critical in mediating signals that ultimately lead to force generation.

Many of the hot spot residues have been shown to be functionally important by mutational studies. Some of them are highlighted as follows. Five hypertrophic cardiomyopathy mutations are mapped to residues E492, F506, R695, A699, F745 in *Dictyostelium* myosin. The mutant A699R [22] exhibits the lowest level of force with preserved actin-activated MgATPase activity. The E706K mutation causes a human myopathy in human skeletal myosin IIa which is mapped to E683 in *Dictyostelium* myosin [23]. The motor functions of

the mutants I499A and F692A are highly compromised [24]. The mutants Y494K, W501L, G740D produce a cold-sensitive phenotype *in vivo* [25]. The mutant G680V myosin exhibits a substantially enhanced affinity for several nucleotides, decreased ATPase activity, and over-occupancy or creation of a novel strongly actin-binding state [26]. The mutants F481C/N483K and H484Q are functionally defective [27]. *Dictyostelium* cells transformed with F487A or F506G [28] myosin were found to be unable to undergo processes that require myosin II function.

GroEL CHAPERONIN

Molecular chaperones play an essential role in helping proteins that have low spontaneous yield reach their native states by mediating their productive folding. Among this class of nanomachines, the most extensively studied is the *E. Coli* chaperonin GroEL, which has two heptameric rings stacked back-to-back (for review see 29). During the reaction cycle GroEL goes through a number of allosteric states that are triggered by ATP binding and interactions with the co-chaperonin GroES [29, 30]. The substrate protein (SP), which also affects the allosteric transitions, is most efficiently recognized when GroEL is in the T state, and ATP-binding shifts the equilibrium to the R state. Binding of GroES and subsequent ATP hydrolysis result in the formation of the R" state. The T → R → R" allosteric transitions result in the formation of the *cis*-ring. When ATP or SP binds to the opposite or *trans* ring the ligands (the inorganic phosphate, ADP and GroES) are ejected from the *cis* ring. It has been appreciated that the dramatic allostery observed in GroEL is intimately related to its function [31]. Indeed, in the course of the allosteric transitions the polarity of the inner lining of the cavity changes from hydrophobic in the T state to hydrophilic in the R" state. The SP-GroEL interaction is attractive when GroEL is in the T state and becomes less so as the T → R → R" transitions occur. The change in polarity of the inner cavity is required for the annealing function of the GroEL machinery [30].

In an earlier work [9] we analyzed the conformational change of a single GroEL subunit from the ADP+GroES-bound state to the apo state (PDB: 1AON_A → 1GRL) using the ENM modes of the first structure. This conformational change is dominated by mode #1 (overlap=0.806) that describes multiple hinge motions of the apical (A), intermediate (I) and equatorial domains (E). The robustness analysis indicated that mode #1 is also the most robust to sequence variations. There are several other modes among the lowest 10 which are highly robust.

For mode #1, the hot-spot residues are clustered around domain I and the A-I and E-I interfaces where many functionally important and highly conserved residues are distributed. Our analysis correctly identified many hot spot residues validated as being functionally relevant. Mutant Y360E has reduced ATPase activity while D361K lacks the ability to bind to the GroES [32]. More recently, using genetic experiments, Klein and coworkers [33] identified two mutants (GroEL44 mutant: E191G) and (GroEL673 mutant: G173D and G337D). They found intragenic suppressor mutations at 13 residues (G173S, V174F, V174I, T176N, V189E, V190I, V190L, Q194P, Q194H, Q194H, R322G, T331S, T331S, T331A, T331N, D359Y, D359N, D359G, Q366R, K371N, G375C, V378G, V378A) that restore growth at elevated temperatures to the mutant *E. Coli*. GroEL44 bacteria. 12 of these 13 residues were also identified using our method as being allosterically relevant. Murai *et al.* cross-linked the E and A domains through a disulfide in the D83C/K327C double mutant (with additional mutations C138S, C458S, C519S) [34]. This mutant can still bind ATP, but it fails to process further reaction steps such as ATP hydrolysis, binding of GroES, dissociation of substrate protein from GroEL, and facilitating protein folding.

More recently, we have studied the conformational changes in the entire double-ring GroEL machine [10]. We started from a GroEL-GroES structure (PDB code: 1AON) which describes the R" T state: the *cis*-ring is bound with 7 ADPs and GroES (R" state) and the *trans*-ring is unliganded (T state). We focused on the R" T→TR" transition which involves a series of large-scale conformational changes that eventually invert the bullet configuration (*trans* to *cis*, and *cis* to *trans*) as GroEL completes one-half of its working cycle. During this transition, the *cis*-ring releases the SP and ADP, while the *trans*-ring binds ATP and encapsulates SP.

We solved the normal modes for the ENM built from the double-ring structure 1AON. By comparing each normal mode with the observed R" T→TR" conformational changes, we found a single dominant mode #18 (with overlap=0.68). Several subdominant modes (#3, #8, #17, #38–39) also have lower but relatively significant overlaps. We then used the robustness to sequence variations as a criterion to justify the importance of mode #18. The robustness is determined by two scores: $f_{\delta E}$ ($f_{\delta V}$) is the fractional variation of the eigenvalue (eigenvector amplitude) of each mode in response to random perturbations to the ENM force constant caused by sequence variations [9]. The lower these scores are, the higher is the robustness. We found mode #18 has the highest robustness (or lowest value in $f_{\delta E}$), and modes #19, #20 rank second and third respectively. The results for $f_{\delta V}$ pinpoint four most robust modes (#3, #8, #17, #18) as nearly degenerate global minima, and several other highly robust modes (say #39) as local minima. All of these modes correspond to either the dominant mode (#18) or sub-dominant modes (#3, #8, #17, #38–39). Because the motions from one allosteric state to another take place along the eigenvectors, $f_{\delta V}$ appears to be a more effective indicator of functional relevance than $f_{\delta E}$.

A detailed analysis of the dominant mode #18 has offered key insights into the complex dynamics governing the R" T → TR" transition. This mode accounts qualitatively for the puzzling allosteric couplings within and between the two rings. A dynamic symmetry (asymmetry) within (between) the *cis* and the *trans* rings is evident from this mode which tidily explains the positive cooperativity within one ring and the negative inter-ring cooperativity. The structural displacements of mode #18 for all seven *trans*-ring and *cis*-ring subunits are found to be nearly symmetric (obeying seven fold rotational symmetry). Therefore, this mode indeed describes a highly concerted set of motions in both the *cis* and the *trans* rings. However, there are substantial differences between the two rings. In the *trans* ring, mode #18 captures the observed T→R" changes in the E and I domains, but in the A domain mode #18 predicts significantly reduced motions than are observed. This is consistent with the two-stage T → R" transition where the I domain moves into the R"-state configuration (clamping downward to close the nucleotide binding site) before the A domain fully opens (twisting in the clockwise direction and swinging upward) for GroES binding. In the *cis* ring, however, mode #18 predicts larger-amplitude motions in the A and E domains of the *cis* ring than in the *trans*-ring. The substantial differences in motions between the two rings imply that the inter-ring interface may be structurally distorted due to the unequal motions in E domains between the *trans* and *cis* rings, which may then facilitate the transmission of allosteric signals from the *cis* ring to the *trans* ring.

The response of the mode #18 to structural perturbations (*in silico* mimic of mutations), allows us to construct the allosteric ‘wiring diagram’ which gives a network of hot spot residues that are involved in the allosteric transitions. Many hot spot residue positions are shared between the single subunit mode #1 [9] and the double-ring mode #18. Additional hot spot residues were found in mode #18 due to inter-subunit couplings, which are mostly located at inter-subunit interfaces (some are intra-ring, others are between rings). These residues were predicted to be involved in the signal transmissions between different subunits

of the two rings. Several of the identified residues have been found to be functionally important by experiments or in computational studies [10]:

In the *cis* ring, residues Y203, F204, T210 form contact with I305, G306, Q351 of the adjacent subunit. Residues V263 and V264 also form contact with I305 and G306 of the neighboring subunit. In the *trans*-ring, residues G269 and I270 form contact with residue E257 of the neighboring subunit. Experiments have shown that mutants Y203E and F204E both do not show GroES or SP bindings [32]. Residue T210 is at the hinge of the loop (199–204) between strands 6 and 7 that binds SP in the T state. Residues V263 and V264 bind to SP in the T state, and form part of the GroEL-GroES interface in the R" state [35]. Mutant V263S shows no GroES/SP binding, and V264S shows reduced ATPase activity and no GroES/SP bindings [32]. Residue R268 is also a potential SP-binding site [36]. Mutants L309K and L314K show reduced SP folding [32]. Residues I301-K311 were found to be indirectly involved in peptide binding [37]. Danziger and coworkers found that E257 is a sensor involved in coupling SP binding to stimulation of ATP hydrolysis [38]. Stan *et al.* have emphasized the role of E257 in the release of SP using all atom simulations [39]. R58 and M267 were previously found as part of the wiring diagram in a bioinformatic study by Kass and Horovitz [40]. Residue Y506 forms contact with residues A384 and T385 of neighboring subunit. Mutants A503V and A507T were found to give active single-ring GroEL [41].

In the *trans* ring, residue F281 forms contact with residues T181, A383-E386 of a neighboring subunit. Experimentally, the F281D mutant has decreased ATPase activity and decreased SP folding [32]. Mutant A383E has no ATPase activity, no GroES binding and no SP refolding [32]. A salt bridge forms between R197 and E386 in the T state and a new E386-K80 salt bridge may be formed in the ATP-bound state [42]. Mutants T385I, E388K, M389I were found to give active single-ring GroEL [41].

CONCLUSIONS

The ability to transmit signals over long distances so that specific function can be carried out is the hallmark of allostery. Mutational and genetic studies on a number of biological nanomachines have revealed some of the residues that are important to allosteric functions. Given the importance of the ligand-induced conformational changes for function it is logical to hypothesize that the network of residues through which allostery is transmitted must be encoded in the structure which is conserved to a much greater extent than the sequence. Furthermore, the ENM modes that describe these conformational changes should be robust to sequence variations. In our recent work reviewed here, we have shown a significant connection between functionally relevant motions that describe allosteric transitions in three distinct biological nanomachines and their robustness to changes in the sequence.

We have also identified a network of hot spot residues in DNA polymerase, myosin motor and chaperonin GroEL that are responsible for allostery. Many of the key residues predicted have also been found to be relevant in a number of experimental studies. Remarkably, not only do we find that residues in this network are strongly conserved but also that the network is sparsely connected. Similar observation for other systems has been made by using a sequence-based method [43, 44]. It is likely that efficient transmission of allosteric signals is achieved through a small network of distantly connected residues and hence may be an evolutionary requirement for all protein families.

The combination of structural and bioinformatics methods reviewed here is general enough that it can be used to examine allostery in other biological systems as well. We propose that it is important to assess if the dynamics encoded in a mode is robust enough to evolutionary

sequence variations because it is the structural dynamics, rather than the sequence, that is more strongly conserved for functional reasons.

Acknowledgments

This research was supported in part by a grant from the National Institutes of Health (1R01GM067851-01) to DT and by the Intramural Research Program of the NIH, NHLBI to WZ and BRB. WZ is also indebted to the support from the intramural funding of University at Buffalo.

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