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Computational approaches to mapping allosteric pathways

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Abstract

Allosteric signaling occurs when chemical and/or physical changes at an allosteric site alter the activity of a primary orthosteric site often many Angstroms distant. A number of recently developed computational techniques, including dynamical network analysis, novel topological and molecular dynamics methods, and hybrids of these methods are useful for elucidating allosteric signaling pathways at the atomistic level. No single method prevails as best to identify allosteric signal propagation path(s), rather each has particular strengths in characterizing signals that occur over a specific timescale range and magnitude of conformational fluctuation. With continued improvement of accuracy and predictive power, these computational techniques aim to become useful drug discovery tools that will allow researchers to identify allostery critical residues for subsequent pharmacological targeting.

Introduction

The recently broadened paradigm of allosteric regulation, which is inclusive of monomeric single-domain [1–3] as well as multimeric proteins [4], can be thought of as any mechanism by which an effector perturbation at one site propagates a signal to an orthosteric site and shifts the population of a preexisting conformational sub-state to become more predominant [5,6]. Effector perturbations can result from a wide range of biological and physical phenomena, including the binding of a small effector molecule, post-translational modifications, protein binding, temperature changes, and pH changes. Until recently, an atomistic understanding of allosteric propagation could only be inferred by projecting experimental results onto static structural models provided by x-ray crystallography. However, over the last decade advances in computational simulation have also provided qualitative and quantitative descriptions of allosteric phenomena that have been experimentally validated and are increasingly predictive of experimental observables. These computational techniques can be broadly divided into two categories: those which seek to predict the overall conformational impact of an allosteric event on the protein state (ie. apo

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vs. holo), and those which aim to elucidate specific atomic-level allosteric pathways through which effector perturbations are transmitted. As a number of excellent reviews focusing on the former methodologies already exist [7••,8], the focus of this opinion will be on the latter.

Mapping Networks by Topology Analyses

Many early computational approaches for elucidating allosteric pathways are based on protein topological analyses such as graph theory, statistical coupling analysis, and perturbation algorithms [9–11]. These approaches, which assume that allosteric networks can be defined solely through inter-residue non-bonding interactions, have generally been successful in identifying residues involved in functional and correlated motions.

A recent topology-based algorithm, CONTACT [12•], relies on a single crystal structure to identify residues that have side-chain contact heterogeneity. CONTACT defines “contact networks” by tracing paths of residues that can adopt alternate conformations. This method accurately predicts the identity of residues involved in DHFR allostery and residues whose mutation affects allostery, as previously characterized using NMR and biochemical experiments [13]. The remarkable agreement between topological methods like CONTACT and experimental mutagenesis studies is impressive given that the topological characterizations include only the steric inter-residue van der Waals interactions. This suggests that such interactions are a major component of allosteric signal propagation and is consistent with previous studies that utilize a measure of residue steric incompatibilities such as residue “frustration” [14,15].

Jenik et. al [16•] has formalized a previous residue frustration algorithm [14] as a webserver for elucidating specific pathways of allosteric transmission named the “protein frustratometer” (http://lfp.qb.fcen.uba.ar/embnet). In its most straightforward implementation, it systematically “mutates” each set of interacting residues *in silico* and predicts the energetic consequences of each mutation. Each interaction is thus associated with a spectrum of energies. If the energy of wild-type residues is favorable relative to the energies of this spectrum, the interaction is said to be minimally frustrated. If it is unfavorable, the interaction is highly frustrated. Typically, ~10–15% of residue contacts are judged “frustrated” by this metric [15]. By tracing out pathways of highly frustrated interactions, it is possible to predict sets of residues with alternative conformations that are potential pathways for propagating an allosteric signal.

Mapping Networks by Simulation Analyses

Simulation-based methods have long been used to study allosteric processes. Increasingly, molecular dynamics trajectories, whether conventional, accelerated, steered, coarse-grained, or umbrella sampled, have been used to generate conformational ensembles for subsequent allosteric network analysis (reviewed elsewhere [7••]). Multiple methods such as normal mode analysis [17–19], correlation matrices [20–22], community-network analysis [23], mutual information [24] and dynamical network analysis [25–27] have been applied to evaluate and identify pathways or regions of correlated residue motions presumably related to allostery. Recent advances in some of these methods are highlighted below.

ENM and Structural Perturbation Method

An elastic network model (ENM) represents a biomolecular structure as a coarse-grained network of nodes connected by a series of harmonic springs. ENMs are typically analyzed by normal mode analysis (NMA) [28]. In recent implementations electrostatic forces are incorporated into the model and selected spring-force constants can be modified to better account for water hydration effects at ionized interfacial surfaces [29•]. Once such a model has been constructed, techniques like the "structural perturbation method" can be used to identify potential allosteric pathways [30,31]. In brief, for each network node, the force constants of all connecting springs are modified, essentially mimicking a point mutation. The impact of this subtle modification on the overall motions of the system (as judged by normal-mode analysis) is assessed for each perturbed node. The nodes with the greatest impact are thought to be those that are most likely to participate in the allosteric mechanism. When applied to large protein systems such as GroEL and GroES, these methods have successfully identified correlated motions within and between the rings that link GroEL substrate binding site residues to the GroES ATPase activity [31].

Modified MD Simulations and Fourier Transform Analysis

Pump-probe molecular dynamics simulations apply an oscillating force to selected atoms or residues during the course of a molecular dynamics simulation [32]. Conformations are extracted from the simulation then a fluctuation power spectrum for distant atoms and/or atom groups is generated by applying a Fourier transform to their atomic Cartesian coordinates. This spectrum is then compared to similar spectra obtained when no oscillating force is applied, or when varied pump force magnitudes, periods and atom selections are used. In theory, atoms connected to the excited protein region by well defined pathways of transmitted momentum will be more affected by the generating oscillating force than other residues. These same residues are more likely to participate in allosteric pathways connecting the excited region of the protein to other biologically relevant domains (e.g., an orthosteric site).

MD and the Perturbation-Response Scanning Method

The perturbation-response scanning method [33,34] can also be used to gain insight into specific allosteric pathways. As a first step, multiple random forces are sequentially applied to each node to capture the linear response of the whole system. The resulting changes in the whole-system conformation are compared to allostery-induced structural changes observed experimentally (e.g., as captured by crystallographic or NMR structures resolved in the presence and absence of an allosteric effector). When applied to the bacterial ferric binding protein [33], this method suggested that residues distant from the primary active site are nevertheless associated with experimentally validated structural changes. These residues are most likely to belong to distant allosteric sites.

MD and Dynamical Network Analysis

Dynamical network analysis is a cross-correlation method that draws upon molecular dynamics simulations and is constructed by considering all pairwise interactions between protein residues [25–27]. Individual protein residues are each represented by a network node

and all node pairs are connected by edges with lengths that are inversely proportional to the correlation between their motions (i.e., the distance in network space between highly correlated residues is shorter). Pathways between “source” and “sink” nodes, typically associated with the allosteric and orthosteric sites, respectively, are represented by simple node lists the length of a given pathway in network space is calculated by summing the lengths of the edges between adjacent list entries. The number of paths between sink and source nodes increases combinatorially with the total number of interconnected system nodes. While efficient algorithms exist for finding the single optimal (shortest) path between two nodes, the identification of near optimal pathways quickly becomes computationally intractable as the number of nodes increases. Several techniques are therefore used to make subsequent path finding efforts more feasible.

A recently developed algorithm [35•] is capable of identifying nodes that cannot possibly participate in pathways that are shorter than a user defined cutoff length. These nodes can be effectively ignored, further simplifying the network and thus speeding up subsequent path-finding efforts. Following network simplification, a recursive algorithm is used to identify paths between source and sink nodes. Dynamical network analysis is greatly facilitated by publicly available programs like WISP (http://nbcr.ucsd.edu/wisp), Figure 1, among others.

Different methods sample different allosteric timescales, motions and pathway mechanisms

Comparisons of recent work utilizing these different methodologies demonstrate that there is no single method that best captures all the allosteric pathways for a given system. Presumably this is because of limitations in the conformational timescales and fluctuations sampled by each approach. These limitations become most apparent when NMR derived measures of protein dynamics are compared to those back calculated from computationally generated conformational ensembles.

Fuglestad and colleagues [36••] used a number of methods to characterize thrombin allosteric networks. They find that different methods correlate to fluctuations occurring on differing timescales. For example, an ensemble of conformations generated using accelerated molecular dynamics produced the highest degree of correlation between back-calculated residual dipolar couplings (RDCs) and NMR-derived RDCs for the thrombin-PPACK complex ($R^2 = 0.92$). In contrast, conventional molecular dynamics and a single crystal structure did not yield the same degree of correlation ($R^2 = 0.8$ and 0.72, respectively). RDCs are most sensitive to longer-timescale fluctuations, typically in the μs to ms regime. Comparison of NMR backbone order parameter values (S^2), a measure of ps to ns timescale fluctuations, to S^2 values back-calculated from the conventional molecular dynamics ensemble demonstrated the closest correlation with protein fluctuations on this timescale; results also seen in other work [37,38]. Interestingly, longer timescale motions are better correlated with the results of the frustratometer than are shorter-timescale events, suggesting topological methods are particularly well suited to characterizing conformational rearrangements that occur on these longer timescales.

Ever longer molecular dynamics simulations permit a direct comparison between simplified and atomistic techniques for studying allostery. In a recent study comparing soft modes derived from network models (such as ENM and ANM) to principal components derived from a millisecond-long Anton molecular dynamics simulation [39], the three lowest-frequency ANM modes captured the backbone conformations and motions observed in the first five principal components of the MD simulation [40••. In other words, the network method successfully identified the first three sub-states of the MD-generated energy landscape, but not the remaining five. The authors point out that the network method does not sample high energy, less populated sub-states, perhaps explaining why some were missed. Nevertheless, the network method did identify the most significant (*i.e.*, most highly populated) sub-states at considerably less computational expense to the Anton simulations.

Fenwick and colleagues [41•] report that using a molecular dynamics simulation method highly restrained by NMR derived RDC data, termed ERNST, is more sensitive to identifying pathways correlated through hydrogen bond networks than using unrestrained MD. Application of the ERNST method and subsequent correlation-coefficient matrix analysis based on residue backbone torsion angles map weak, long-range motions through the β-sheet hydrogen-bond network that propagates communication between distant residues and the UBD binding sites [41•,42•]. Such motion also allows for propagation of an allosteric signal through a pathway without breaking the secondary-structure hydrogen bond.

Finally, DuBay et al. [43•] sample very fast timescale motion that do not result in large conformational changes upon an allosteric event but rather identify allosteric pathways that utilize population shifts in side-chain entropies. Rather than using molecular dynamics to develop a conformational ensemble of structures, the authors use Monte Carlo sampling of side-chain torsions on a static protein backbone. They find long-range correlations, up to 60Å. The authors point out these long-range correlations allow for allosteric signal to propagate through a variety of ways; van der Waals, hydrogen bond, salt bridge or implicit solvent. This both expands the types of inter-residue interactions typically considered for signal transmission and incorporates new ways of thinking about population shifts without large conformational changes but through entropic, thermodynamic mechanisms [44,45].

A couple of recent publications have combined topology-based methods with one or more simulations in order to increase conformational sampling, in what can be considered hybrid methods, and therefore capture longer-timescale correlated motions [46–48]. For example, Weinkam and colleagues recently compared the structures of effector-bound and unbound crystal structures for three protein-effector systems [48]. They applied molecular dynamics to sample the conformations of the allosteric transition in order to identify residues affected by effector binding [48]. A similarity metric (QI_{diff}) thought to reflect residue involvement in the allosteric network was then calculated for each amino acid by comparing the inter-atomic contacts of each residue center of mass over the course of the trajectory to those of the apo and holo crystal structures. The residues predicted to participate in the allosteric signal were highly correlated with those that, when mutated, are known to disrupt allostery [48,49], thus validating the technique. Subsequent use of a machine-learning algorithm to predict allosteric-pathway residues based on QI_{diff}, as well as the inclusion of additional features into the model, led to even more robust predictions[49]. The Allosmod webserver,

which provides tools for developing energy landscapes and analyzing simulations, can be found at http://modbase.compbio.ucsf.edu/allosmod/.

A hybrid method combining multiple simulation methodologies have also been reported and applied to the study of adenylate kinase [47].

Future Directions and Conclusions

Computational methods for identifying specific allosteric pathways provide useful atomic-level information that complements existing experimental techniques. While impressive progress has been made, the methods developed thus far are still foundational and each shows some gap in being fully predictive of experimental observations. As with other areas of computational chemistry, methods for prediction of allosteric pathways will benefit from improved and/or polarizable force field development. These methods, hinted at by Martin et al. [29•], will improve on modeling pathway components dependent upon electrostatics and may reveal a larger role in hydrogen bonding interactions than previously appreciated. Also, despite the recognized importance of water in protein folding and molecular recognition [50], the observation that certain waters can have very long residence times in protein interiors [51], and recent experimental studies exploring water's role (reviewed in [45]), there is a surprising omission of water from covariance analysis or most of the computational allosteric pathway methods.

Nevertheless, as the accuracy and predictive power of computational methods improve, we expect them to become powerful tools for drug discovery. Current therapeutics focus principally on orthosteric sites, though the number targeting allosteric sites is on the rise [52,53]. Computational methods for identifying entire allosteric pathways, rather than just the endpoints of those pathways (e.g., allosteric and orthosteric binding pockets), may provide additional opportunities for the pharmacological modulation of pathogenic allosteric signaling [54•,55•].

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Highlights

- Advances in computational methods for allosteric pathways are reviewed.
- New methods are able to replicate many but not all experimental results.
- Each method accesses specific dynamic timescales or conformational amplitudes.
- Multiple webservers are available that automate allosteric pathway elucidation.
- Atomistic level allosteric pathway identification will be of use in drug discovery.

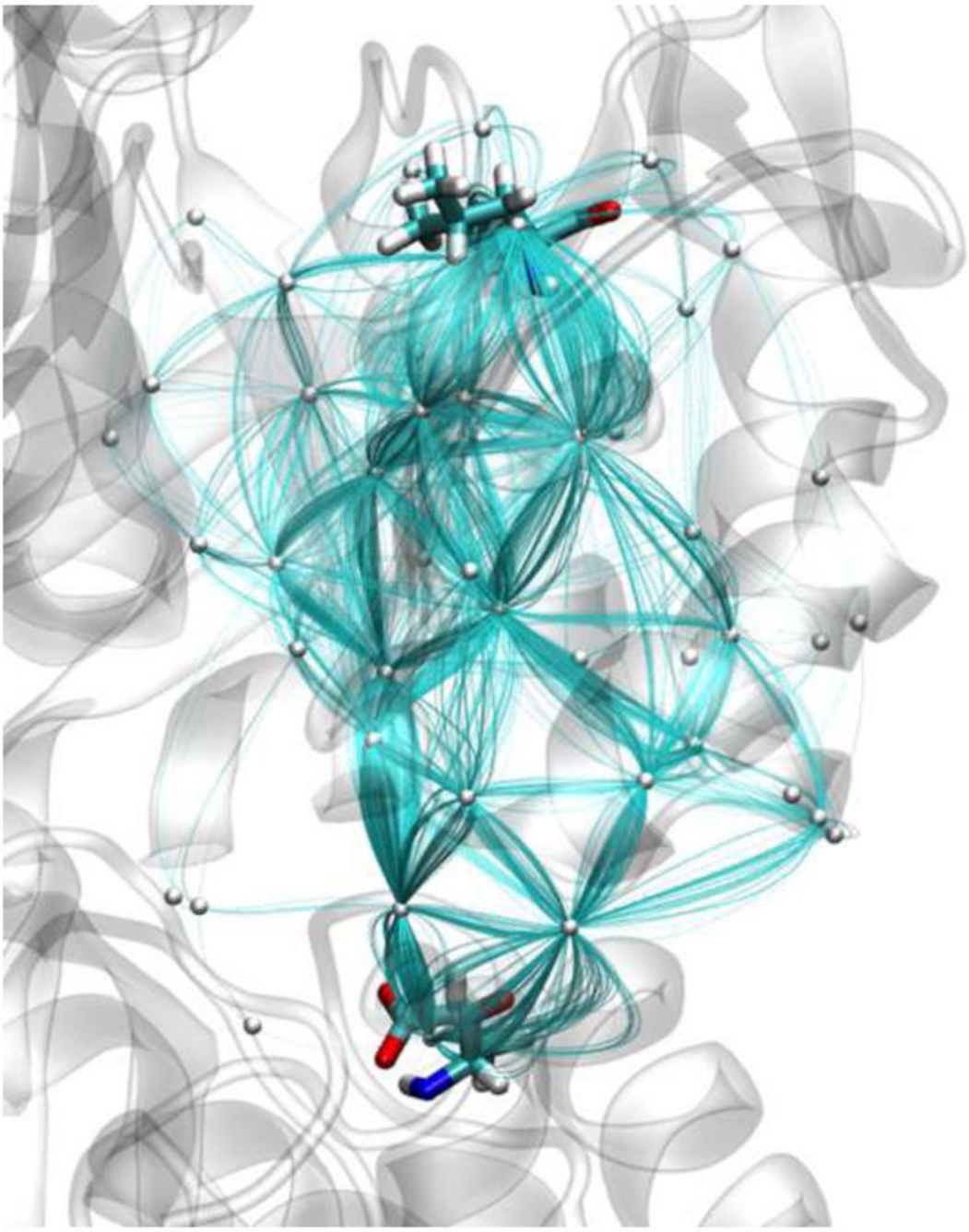

Figure 1.

A dynamical network analysis [35•] of the protein dimer HisH-HisF with bound effector molecule, PRFAR (effector not shown). The residues predicted to assist in propagating the allosteric signal between two residues of interest, Glu180:HisH (top) and Leu50:HisF (bottom), are depicted as white spheres along suboptimal signaling pathways, depicted as cyan lines. Expanding signaling pathways with lengths in network space near that of the

optimal pathway are believed to facilitate an allosteric signal and may provide insight into differences between allosterically active and inactive forms.