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Simulations of allosteric transitions

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Allosteric transitions are one of the subtler mechanisms used by nature to fine tune protein activity. Effector binding to a specific site on the protein surface induces significant activity change, and initiates a conformational transition that frequently includes domain motions and is very large. From a theoretical and biophysical perspective two problems are particularly intriguing. The first is the way in which a launching signal, which is spatially confined and includes only a few interacting atoms, is propagated to a large-scale conformational transition we frequently see in allosteric transitions. Hence, there is the question of how a small perturbation is magnified to yield motions of thousands of atoms. The second puzzle is of focus, coherence, and efficiency. The impact of the binding of the effector is sometimes extended over tens of angstroms. How the signal is transmitted and kept significant over such large distances in the 'noisy' molecular environment is another major direction of investigation. In the present review we examined different theoretical and computational attempts to solve the questions.

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Living systems are using controlled and subtle modifications of protein structures to alter activity, to respond to environmental changes, and to better manipulate cellular processes. Allosteric transitions are examples of such events and are the focus of the present manuscript. We emphasize theory and methodologies used to study these transitions rather than specific applications and outcomes. These transitions are induced by binding of effectors (typically small molecules), which are followed by adjustments of the structure and function of the proteins.

Shifts in protein conformations during allosteric transitions can be spatially large but are rarely complex. By 'rarely complex' we mean that typical allosteric motions usually include quaternary shifts (as proposed by Monod *et al.* in 1965 [1]), or more local displacements as a few side chain transitions or rearrangements of solvation shells [2,3]. These motions are described qualitatively by a small number of coarse variables and are therefore not complex. Only rarely more elaborate events such as partial folding and unfolding are found in allosteric transitions. For example, the conversion of a helix to an extended chain during an allosteric transition is an event that we do not find frequently [4].

If the 'rarely complex' hypothesis is correct in the general case, it suggests that the binding of effector tilts the free energy surface toward the alternate conformation in a small number of coarse-grained variables, supporting quick and efficient search to end products. Extreme cases will have one coarse variable to follow (the reaction coordinate). The reaction path defines a tube in which most reactive trajectories pass. Identifying these few coarse variables, and illustrating their capacity to quantitatively explain functional data, charts a clear path to the solution of the allosteric problem. Functional data may include kinetic and thermodynamic measurements of the transitional events and variations of the last upon point mutations.

What is an ideal simulation of an allosteric transition (at least in the author's view)? An ideal simulation will make it possible to first, follow sequence of events and understand the process at a microscopic and coarse-grained levels; second, follow biochemical variations of the process and explain subtle changes in function at residue and atomic resolution; and third, have ample opportunities to compare computational data to experiments. The last point is particularly important since a significant role of simulations is to provide a unified picture. The simulation should bring together multiple experimental probes of different components of the process to create a single coherent model.

All the approaches discussed in this article are models based on physics. We critically relate these technologies to the ideal simulation mentioned above. For better focus, we do not discuss approaches that use evolutionary data, though interesting information on critical residues can be extracted from multiple sequence alignments of allosteric proteins (e.g. [5]).

The following computational approaches of allosteric transitions are considered: first, elastic networks [6–10]; second, Go models [11,12,13•] and other simplified

approaches [14•,15]; third, atomic reaction coordinates [16,17•,18–21]; forth, (relatively) short time atomically detailed simulations to examine local events of allostery [22,23•,24]; and fifth, atomically detailed simulations of the whole transition using theories of long-time dynamics [17•,18].

An approach that has become popular in the last few years because of its simplicity is the Elastic Network Model (ENM) [9,14,19,25–27,28•]. It represents 'continuum of protein matter' by a selected set of point masses that are frequently placed at the coordinates of the alpha carbons. However, there are many choices, and an alternative follows predetermined electronic density [29]. Each of the points is connected by springs to neighbors determined by proximity. The actual chemical connectivity and diversity of interaction strengths between sites are disregarded in most cases. The lowest frequency modes of the ENM provide soft directions of motion that were conjectured to shape allosteric transitions and to imply a reaction coordinate [10,25,30,31]. Analysis of network structure also helped identify critical residues by solving a master equation and follow signal propagation in the network [32]. Another intriguing view is the perturbation and stability analyses [10]. It is argued that modes that are biologically important are insensitive to perturbation (mutation). The analysis of the myosin–actin system was particularly intriguing [14•].

A few drawbacks come to mind. An ENM has one harmonic minimum while a conformational transition requires at least two mechanically stable states. The plastic network model is an interpolation between two ENMs [33] making it possible to define a reaction coordinate. In conjunction with functional optimization [19] it sometimes provides a sensible reaction path [28•]. Another drawback is the limitation to large-scale motions. If the transition is localized, or have localized events (e.g. the allosteric transition in Scapharca hemoglobin is dominated by one side chain transition [3]), ENM cannot capture it. Of course, the effector binding is a local transition. Finally, testing ENM-generated hypotheses is limited. Coarse-grained ENM structures are compared to trapped experimental structures assumed to be along the reaction pathway [15,33].

More detailed coarse-grained approaches are provided by the Self Organizing Polymer (SOP) [14•] and the Go model [11,12,13•]. Coarse-grained models are used at the atomic or residue levels and the solvent is modeled implicitly. The Go model also adds a potential bias toward the native conformation. Both approaches retain correct chemical bonding and sequence details and therefore able to better connect between the global transition and local binding, like in the analysis of kinase [13•]. These coarse-grained theories provide mechanistic and thermodynamic properties. It is more difficult to use them for the study of kinetics. While useful estimation of kinetics has been made and interesting kinetic hierarchy was pointed out [13•], some clever theoretical and modeling choices are made. The lack of explicit solvation requires a friction kernel or an approximation to it that are difficult to obtain from first principles. Native contacts are set to make specific contribution to the energy, and the impact on kinetics is not obvious.

A technique that brings in atomic energy and microscopic details at moderate cost is the calculation of a reaction coordinate. Most algorithms for computing reaction pathways in large systems are global in nature (the whole path is considered). Examples are the LUP [34], the related algorithms NEB [35] and the zero-temperature string [36], and other functionals [37]. In this formulation equations for the whole curve are derived and solved simultaneously. For example, a minimum of the following action $S = \int_{\text{reactant}}^{\text{product}} \sqrt{\nabla U^t \nabla U} dl$ provides the Steepest Descent Path (SDP) [37] (U is the potential energy and dl is a length element along the path). Recent examples of application of the functional approach for allosteric transitions are in [17•,18]. Yet another technique (conjugate peak refinement [16]) was used to study the recovery stroke in myosin and an intriguing mutation for myosin II recovery was proposed, a prediction which was verified experimentally [38,39].

However, some challenges remain. First, a reaction coordinate does not provide kinetics and thermodynamics. Second, searches for SDP are usually limited to the neighborhood of the initial guess of the path. Therefore the significance of a computed path is not known since alternative pathways are rarely explored. One solution to the problem is to estimate reaction coordinates that are temperature dependent. Targeted Molecular Dynamics (TMD) [40] is a finite temperature molecular dynamic trajectory that is biased toward the products using an additional constraint and was applied to allosteric problems [41]. It suggested a different mechanism of the myosin recovery stroke compared to Minimum Energy Path (MEP) [16] that coupled global motions to ATP binding and therefore expanded the search for alternative pathways. However, the computed TMD path is bias dependent and the connection to function (a similar restriction is present in MEP) is unclear. A promising direction is the use of minimum free energy pathways [42] that enable estimation of local thermodynamics.

The advantage of the reaction path approach is that full atomic picture is retained. The disadvantages are views confined to the neighborhood of a path (a tube around the one-dimensional coarse variable) and the necessity to conduct additional calculation to determine thermodynamics and kinetics. Specifically for allosteric transitions, empirical observations suggest a relatively small number of coarse-grained variables, supporting the idea of

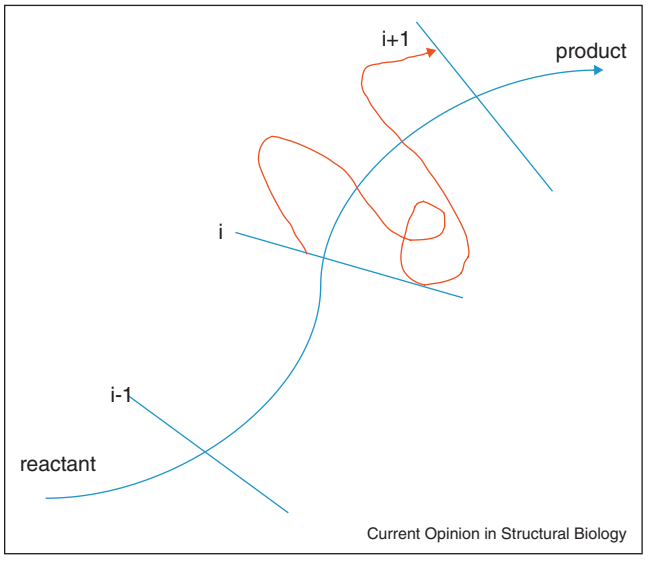
reaction tube. On the other hand, lessons from protein folding [43] suggest that for complex systems multiple pathways are more efficient route to product.

Molecular Dynamics (MD) is a promising candidate for 'ideal' simulation technology. The functional form and parameters of the energy function are obviously not perfect; however, they are independent of the allosteric problem at hand, which is clearly an advantage. The significant drawback of MD is that typical times of MD simulations are too short to study kinetics and thermodynamics of allostery. A few trajectories were pushed with clever partial ligation states to a rapid, computationally induced, allosteric transition [44] in Scapharca hemoglobin. However, this example is an exception and a few (or one) trajectories are not sufficient for kinetic or thermodynamic calculations. Recent investigations focused on local events such as the escape of P_i from the binding site in myosin [23•].

Considerable progress was made in the last few years in developing techniques to study long-time dynamics at atomic details. A focus was on rare events in which the system passes rapidly (but infrequently) over a significant (free) energy barrier. Of interest are TIS (Transition Interface Sampling) [45], PPTIS (Partial Path Transition Interface Sampling) [46], Forward Flux [47] and Milestoning [48]. All these methods build on the availability of a reaction coordinate. Transition events are recorded on interfaces along the reaction coordinate, collecting information from short trajectory fragments (see illustration in Figure 1). The generation of the short trajectories and their processing differ from method to method. The TIS and the Forward Flux are exact techniques; however, the focus on short (rare) trajectories makes them inappropriate for allosteric transitions dominated by diffusive dynamics and individually long trajectories. Both PPTIS and Milestoning are aimed at diffusive processes and are quite similar in their assumptions of phase space decorrelation between interfaces or Milestones. Applications of PPTIS were for an assumed reaction coordinate (e.g. a particular bond rotation). It is not a trivial matter to guess a reasonable reaction coordinate for allosteric transitions (e.g. the myosin recovery stroke). The use of a computed reaction coordinate by functional methods [37] is therefore vital and enables the application of Milestoning to two allosteric systems (Scapharca hemoglobin [18] and myosin [17•]). These applications compared favorably with experimental data yielding additional insight into mechanisms. For example it was suggested that the entropy of switch II, computed from the atomically detailed simulations, is a drive to force release in myosin.

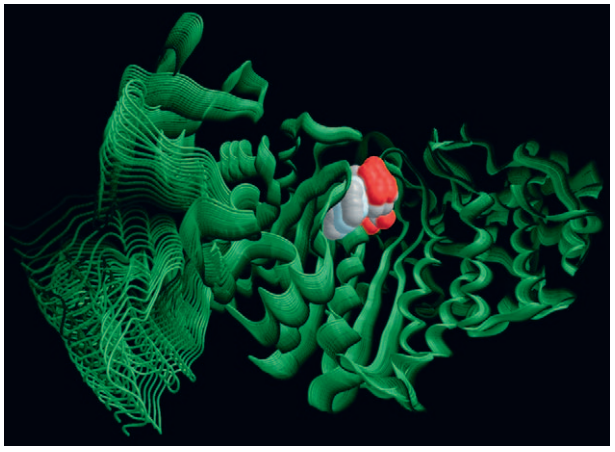
In Milestoning [49•,50] we start with a set of structures distributed along a one-dimensional reaction coordinate. Hypersurfaces orthogonal to the reaction coordinate define Milestones. First we sample configurations at equilibrium constrained to each of the interfaces. Second we release the constraints and run unconstrained trajectories until they 'touch' for the first time another milestone and terminate. We bin the terminating trajectories and estimate the kernel $K_{s,s'}(t)$ which is the probability density that a trajectory starting at Milestone s will terminate at Milestone s' after time t. Since the Milestones are close to each other the trajectories terminate quickly. The short trajectories are independent and run using massive (and trivial) parallelism allowing for efficient estimate of the kernel. The kernel includes only local transition information, but is sufficient to determine the equilibrium and kinetic of the system using a statistical mechanic theory [48,50,51]. For example the mean first passage time $\langle\tau\rangle$ is determined by $\langle\tau\rangle = \mathbf{1}^t \int_0^\infty \tau \cdot \mathbf{K}(\tau) f\tau \cdot \left[\mathbf{I} - \int_0^\infty \mathbf{K}(\tau) d\tau\right]^{-1} \mathbf{P}(0)$ where $\mathbf{K}(\tau)$ is the matrix with elements $K_{s,s'}(\tau)$ and $\mathbf{P}(0)$ the vector of initial conditions. $\mathbf{I}$ is the identity matrix and $\mathbf{1}$ is a vector with all elements 1. The time scale for allosteric transition in myosin II was ~0.1–1 ms [17•] which is not at significant variation from the experiment (~1 ms). In Figure 2 we illustrate the transition by overlapping structures of myosin along the reaction path of the recovery stroke. Hence Milestoning makes it possible to use a computed reaction coordinate and to build a bridge to thermodynamics and kinetics. At the least it can be used to test kinetics and thermodynamics of a

Figure 1



A schematic view of Milestones along a reaction coordinate. The curved line is the reaction coordinate and the 'planes' (lines in two dimensions) are the Milestones. Trajectories initiated at a Milestone (the red trajectory is initiated from Milestone '*i*') are integrated until they hit for the first time another Milestone (Milestone '*i* + 1' in the example below). The statistics of terminating trajectories is used to estimate the transition kernel and the overall kinetics and thermodynamics (see text for more details).

Figure 2



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Sample structures from a reaction coordinate calculation for the myosin recovery stroke. There are 241 structures along the reaction coordinate for which only 15 are shown in the figure. The reaction coordinate is computed with the functional approach and is used to initiate a Milestoning calculation. The ATP is shown using a space filling model and the arm is on the left. See text for more details. The figure was prepared with the graphic module of the MOIL program [57] http://clsb.ices.utexas.edu/prebuilt/.

proposed mechanism (reaction path) against experiment and a proposed path.

The most significant limitation of the approaches mentioned above is that they generate trajectories in the neighborhood of a reaction coordinate. Techniques that avoid the use of a reaction coordinate are known, for example, Markov State Models [52•–55•], Markovian Milestoning [56], and Directional Milestoning [49•]. The Markov State Model is an analysis and an extension of initial MD trajectories. The trajectories provide the scaffold for more detailed exploration of phase space and enhanced sampling between metastable states by short trajectories in the spirit of the technologies mentioned earlier. However, MD trajectories between allosteric states are extremely hard to sample directly. This is the promise of computed reaction coordinates in providing the initial scaffold. At present only Milestoning combines the use of a computed reaction coordinate with the evaluation of a transition operator based on short trajectories. In conjunctions with methods like Directional Milestoning [49•], the search space can start with a single reaction coordinate and expand to higher dimension if the short trajectories drift from the central tube. This combination is likely to get us closer to an ideal simulation of allostery.

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