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Large conformational changes in proteins: signaling and other functions

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Guanine and adenine nucleotide triphosphatases, such as Ras proteins and protein kinases, undergo large conformational changes upon ligand binding in the course of their functions. New computer simulation methods have combined with experimental studies to deepen our understanding of these phenomena. In particular, a 'conformational selection' picture is emerging, where alterations in the relative populations of pre-existing conformations can best describe the conformational switching activity of these important proteins.

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Introduction

The first high-resolution myoglobin and hemoglobin crystal structures, helped to dispel the then prevalent view of proteins as rigid bodies (consistent with a simple lock and key mode of enzyme catalysis). Subsequently, Perutz proposed that myoglobin and hemoglobin must change conformation in order for O_2 to escape from the heme binding pocket. Indeed 'striking structural changes' were later noted when new crystal structures of oxyhemoglobin, carbonmonoxyhemoglobin and methemoglobin were compared to that of reduced hemoglobin. Fifty years thence, numerous experimental and theoretical studies have conclusively demonstrated that structural fluctuations of proteins are often intimately coupled to biochemical function. Indeed, regulated conformational changes control diverse cellular processes, such as signal transduction, membrane trafficking, vesicular transport, and polypeptide chain elongation. Key to the linkage of structural fluctuations and functional properties is the ability of alternate conformations to preferentially interact with different binding partners at different times or locations. This spatiotemporal modulation of function through dynamics allows eukaryotic cells to manage a large number of essential cellular processes with a limited number of distinct gene products. Perhaps the most prominent examples of proteins that undergo large-scale conformational changes to regulate cell processes are guanine and adenine nucleotide triphosphatases (NTPases). These ubiquitous enzymes function as conformational switches and regulators fueled by nucleotide binding and hydrolysis [1]. In this review we focus on two classes of such proteins: Ras-related G proteins and kinases. In the following sections we outline current techniques for studying the dynamics of such systems, with special emphasis on advanced simulation methods. We then describe the recent studies that highlight a shifting paradigm for how signaling enzymes operate, namely, via alterations in the relative populations of pre-existing conformations that are sampled through motions with a topologically preferred directionality (Figure 1).

Current techniques for probing conformational changes

Proteins exhibit a rich hierarchy of internal motions, from individual atomic displacements to collective large-scale motions, over a wide range of time scales [2,3]. The complex topography of the energy surface that underlies this dynamic behavior can give rise to multiple, significantly populated, conformational states that are separated by a distribution of energy barriers. Changes to the environment, such as temperature and pH, as well as binding events and enzymatic modifications can alter the extent of the accessible energy surface and thus both the equilibrium distribution of conformational states and their interconversion dynamics. Further, the internal motions and intrinsic dynamics of proteins have increasingly been recognized as essential for activities including ligand binding, enzymatic catalysis, and bimolecular recognition [4–7].

Crystallographic structures determined under different crystallization conditions or oligomerization states often yield valuable insights into conformational rearrangements. However, crystal structures represent only the average conformation for a particular condition. Important information on protein mobility has also been obtained from other experimental methods, such as nuclear magnetic resonance (NMR) spectroscopy, hydrogen-deuterium exchange, time-resolved X-ray crystallography, fluorescence spectroscopy, and inelastic neutron

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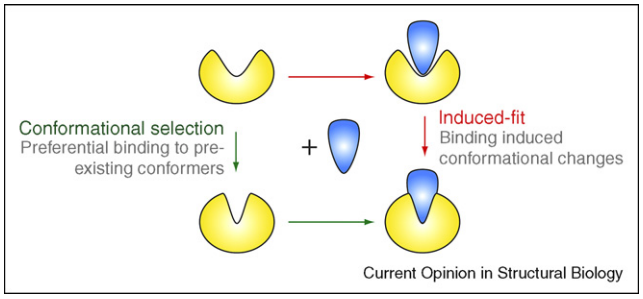
Figure 1



Conformational selection and induced-fit models. Conformational selection (green arrows) dictates that the unbound protein (left of figure) explores a range of conformations some of which are structurally similar to bound conformations. Interaction with binding partners (blue) leads to the preferential selection of favorable pre-existing conformations causing a corresponding shift in the population of microstates in the direction of bound conformations (right of figure). With the induced-fit model (red arrows) the bound-like conformations form only after interaction with a binding partner because of specific induced structural changes rather than selection from the already present unbound ensemble.

scattering [8,9]. For example, NMR measurements coupled with modeling and simulation have been instrumental in elucidating the dynamic properties of many small proteins (under 25 kDa) [10,11]. Computational approaches based on empirically derived potential energy functions, such as molecular dynamics (MD) and normal mode analysis (NMA), have enabled an understanding of the relationship between protein structure and dynamics at the atomic level [12]. For instance, MD simulation of the trypsin inhibitor protein provided the first insight into the rather 'fluid'-like nature of protein structures, when the 'rigid-body' view was still dominant [13]. Subsequent computational efforts, most prominently via MD, helped uncover many biological functions of protein flexibility (for recent reviews, see [12,14••,15–17]). Here we focus on some of the most recent developments that are expanding the scope of conventional MD.

Simulation approaches

A number of diverse computational methodologies have been developed for modeling protein dynamics with varying degrees of resolution. Among these, atomistic classical MD (cMD) and replica exchange MD (reMD) are the most widely used ones, while cutting-edge methods such as accelerated MD (aMD) are beginning to provide exciting results. cMD remains the method of choice for sampling nanosecond and Angstrom scale fluctuations [12,14••]. However, many biological processes involve motions that span microseconds to seconds and tens of nanometers or more. Such motions can in principle be sampled by cMD given sufficient computational resources. Indeed, microsecond length cMD simulations have been achieved for medium-sized proteins [16], though this required specialized hardware and software combinations.

A number of recently developed cMD adaptations employ elevated temperatures, an external force, or a biasing boost potential to facilitate the more rapid crossing of energy barriers separating distinct conformations. For example, the reMD method attempts to avoid kinetic traps by simulating frequently exchanging noninteracting replicas at different temperatures [18,19]. Another popular approach is the so-called steered MD (SMD), in which external forces are applied on the system of study to explore its mechanical responses (reviewed in [20,21]). Decreasing the depth of low-energy wells can also promote the sampling of high-energy conformations [22]. Along this line, the aMD method developed by Hamelberg *et al.* adds a bias potential (or boost energy) when the system's potential energy is below a certain threshold thus facilitating an accelerated rate of state-to-state evolution [23].

An alternative approach for probing large-scale motions involves coarse-grained (CG) models that typically utilize Cα atom or reaction center representations and simplified potential energy functions. Methods in this category include those that step atoms through time with MD or Brownian dynamics (BD) and those that employ collective motions estimated through normal mode approaches [24–26]. There are multiple flavors of the latter, including the elastic and anisotropic network models (ENM and ANM). Their fundamental assumption is that functionally significant fluctuations can be captured by a few low-frequency collective modes that behave much like an elastic material. Despite their simplifying assumptions these methods are often capable of capturing long time and length scale protein dynamics (see reviews [27•,28•,29,30]).

NTP-dependent cellular regulators

Proteins that hydrolyze guanosine or adenosine triphosphate (GTPases and ATPases) play a crucial role in many diverse cellular processes essential for growth and development. GTPase families, such as Ras, regulate processes including intracellular transport, sensory perception, and protein synthesis [1,31••]. ATPases including protein kinases control cell cycle regulation, cytoskeletal rearrangements, and motility [1,31••]. Remarkably, these structurally and functionally diverse families utilize the same underlying strategy: the addition or removal of inorganic phosphate to switch from active to inactive conformations.

Ras GTPases

Ras GTPases are ubiquitously expressed conformational switches that cycle between GTP bound on and GDP bound off states to mediate signaling pathways that control cell proliferation and differentiation [32••]. Recent

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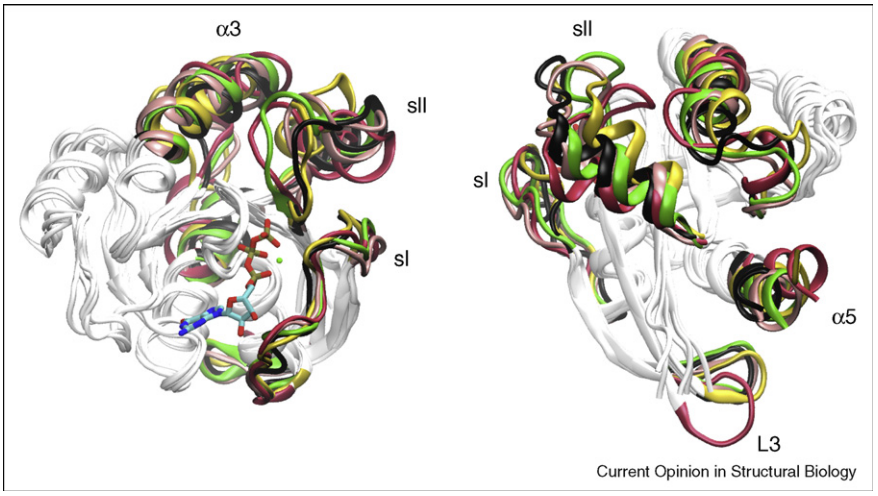
Figure 2



Major conformational clusters in a single aMD simulation of GTP bound H-ras include GDP, GTP, and intermediate like conformations. See [34] for further details.

analysis confirmed that the majority of H-ras crystal structures represent one of two major conformations, either GDP or GTP like [33]. Interestingly, a number of intermediate conformations for structures with oncogenic point mutations in the vicinity of the nucleotide-binding site were also noted [33]. cMD simulations initiated from one such intermediate, G12V with a docked GTP, resulted in a spontaneous transition toward the main GTP conformation [33,34••]. The activating transition involved a multiphase process in which L4-α2 (known as sII) reorganization was followed by that of L2 (known as sI, see Figure 2) [33]. The work also predicted flexibility at L3 and the C-terminus of α5 [33,34••]. This was recapitulated by subsequent aMD simulations [34••] on wild-type H-ras that revealed concerted motions and unique conformations for these regions during the step-wise transition of sI and sII [34••]. The combined application of cMD, aMD, and NMA [33,34••,35] indicated that conformational changes in Ras are best described by a population-shift mechanism rather than by the popular 'loaded-spring' [31••] or induced-fit on/off switch. First, nucleotide-free aMD simulations sampled the regions populated by the major GTP, GDP, and intermediate mutant conformers [34••]. Second, NMA qualitatively captured the differences between GDP and GTP conformations and had high overlap with the eigenvectors obtained from aMD trajectories. Together, these results suggest that nucleotide-dependent dynamics is facilitated by low-frequency global motions that are intrinsic to the structure. Other recent simulation [36] and ^{31}P NMR studies [37] have also highlighted the complex and global nature of the Ras dynamics. Furthermore, these results are consistent with earlier cMD simulations [35] and subsequent experiments [38••,39] of membrane-bound H-ras. For instance, cMD predicted that a pair of basic residues in helix α4 stabilizes the membrane orientation of GTP-H-ras while that of GDP-H-ras is stabilized by a different pair of basic residues at the flexible C-terminal region [35]. Experimentally, the ERK-phosphorylating ability of H-ras variants in which these residues were replaced by alanine was respectively weaker and significantly higher than the oncogenic G12V H-ras [38••,39]. This can be best understood in terms of a population-shift mechanism wherein mutations 'redistribute' the population of conformers centered around the two predicted conformations with distinct GDP and GTP membrane orientations [38••,39].

Protein kinases

Protein kinases are a large superfamily involved in the regulation of diverse cell signaling processes. They operate by transferring a phosphate group from ATP to specific serine, threonine, or tyrosine residues. The added phosphate alters the conformation and function of the target protein, with different kinases recognizing different target sequences. Structural studies have shown that major rearrangements occur during the inactive to active transition of kinases, including closure of the active-site cleft, packing of the activation loop, and rotation of the C-helix [40]. These rearrangements were found to involve set of conserved motifs that are anchored at the F-helix [41•]. A number of computational studies have probed the plasticity and allosteric nature of these rearrangements [42,43,44••,45,46]. In some kinases, structural changes during activation are linked to rearrangements of a conserved DFG motif that props open the active-site cleft between kinase domains [40]. Recently, Shaw and collaborators formulated a mechanism for DFG 'flipping'

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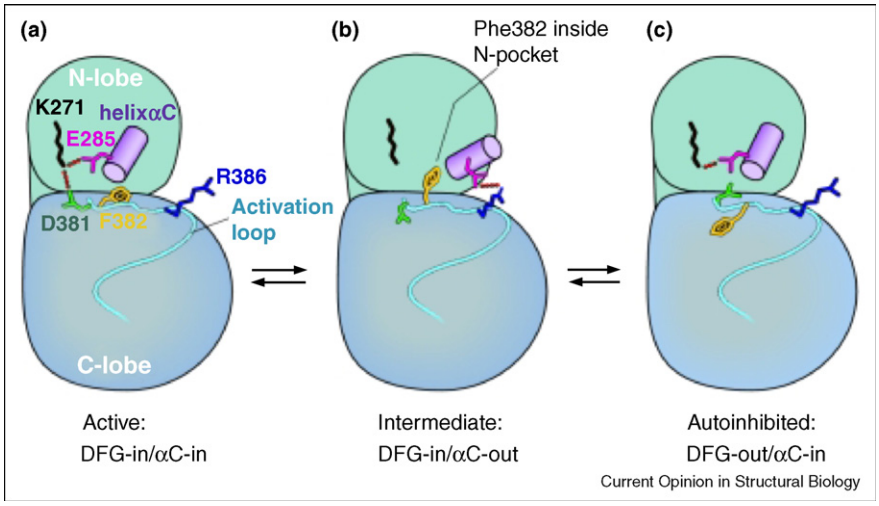
Figure 3



Key features of the proposed DFG flipping mechanism in Abl kinases include rearrangements of helix αC and the salt bridge partner of Glu-286. Schematics are based on the kinase domain of active **(a)** and auto-inhibited crystal structures **(c)**, along with a cMD-simulated intermediate **(b)**. See [47] for further details

based on microsecond cMD simulations together with crystallographic and kinetic experiments [47••]. The proposed mechanism involves a pH-dependent DFG rearrangement driven by electrostatic changes inherent to the catalytic cycle. This motion is correlated with a corresponding in–out displacement of the C-helix during activation (Figure 3). An intermediate structure that exhibits a DFG-in and C-helix-out configuration has also been identified [47••].

In another recent study Roux and Yang examined the catalytic domain of Src tyrosine kinase using a CG model [48••] and an atomistic 'string method with swarms-of-trajectories' [49]. By analyzing 'structural networks' among clusters of conformations from their CG simulations, the authors found two major ensembles of pathways for activation: one involving interactions of the C-helix, the activation loop, and the beta strands in the N-lobe, and another involving partial unfolding of the N-lobe [48••]. Free energy profiles from the 'string method' predicted a two-step activation pathway with opening of the activation loop preceding rotation of the C-helix [49]. A very similar pathway has been found in other kinases, such as in Lyn [50] and CDK5 [51]. A multiphase activation mechanism has also been proposed for Abl and EGFR kinases [52]. An intermediate structure that lies between the active and inactive structures of Src tyrosine kinase has also been identified [49]. Interestingly, both the two-step transition and the presence of an intermediate mirror the observations in Ras that were discussed in the previous section. Thus, conformational selection rather than induction might also operate in kinases.

Conclusions

Our current view of conformational transitions owes much to X-ray crystallography. Examination of different static structures of a protein or its complexes has led to a predominance of Koshland–Nemethy–Filmer like models that depict conformational interconversion as arising from induction by ligand binding or mutation [53]. As a consequence, signaling proteins such as Ras were believed to operate via a loaded-spring mechanism wherein the GTP gamma-phosphate forces rearrangement of distal sites [31••]. However, the simulation results discussed above, and other earlier studies [54], indicate that these proteins harbor an intrinsic susceptibility to sample multiple conformational states regardless of the bound nucleotide, that is, perturbations do not directly induce conformational change but rather shift or bias pre-existing conformations. It is increasingly apparent that the conformational plasticity of signaling proteins can be exploited for therapeutic benefit. For example, the cancer drug Gleevic/imatinib selectively binds and stabilizes an inactive form of Abl kinases [55,56]. Selectivity for Abl with respect to related kinases such as Src can be traced to distinct conformational preferences of the different families [57,58]. Clearly the knowledge of the complete conformational repertoire of signaling proteins such as Ras and kinases has applications to drug development and may facilitate the selective conformational targeting of distinct inactive conformations. The examples highlighted in this review demonstrate the power of modeling and simulation to decipher the dynamic features of signaling molecules and their increasing ability to provide

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mechanistic insight into large-scale conformational changes.

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