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Allosteric sites: remote control in regulation of protein activity

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The presence of multiple allosteric sites in proteins motivates development of allosteric drugs — modulators of protein activity with potentially higher specificity and less toxicity than traditional orthosteric compounds. A quest for allosteric control of any protein starts from the identification and characterization of allosteric sites. Protein dynamics is the basis for allosteric communication. Binding of effector molecules to allosteric sites modulates structural dynamics, thus affecting activity of remote functional sites. We review here theoretical concepts and experimental approaches for exploring allosteric sites, their role in allosteric regulation, and ways to assess their druggability. Key steps of the design procedure aimed at obtaining allosteric drugs with required agonistic/antagonistic effect are proposed, and their computational and experimental elements are discussed.

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Introduction

Allosoteric sites are the ''other objects'', from Greek allos (ἄλλοζ, other) and stereos (στερεὸζ, object), which regulate activity of proteins by remotely affecting their active sites. Molecular mechanisms of allosteric communication are rooted in the dynamic nature of proteins [1–3] — a common trait of all types of molecules from small single-domain structures to complex oligomeric enzymes [4••], receptors (e.g. GPCR [5,6]), huge molecular machines such as chaperones [4••,7] and AAA+ proteases [8]. The physics of protein structure and dynamics is, therefore, a cornerstone in the study of causality and energetics of allosteric signaling [9,10,11•]. The biological implications of allosteric effects were envisioned by Monod, Changeux, and Jacob in their pioneering work 'Allosteric proteins and cellular control systems' [12]. On the basis of the assumption that 'the allosteric effector does not need to bear any chemical or metabolic relation of any sort with the substrate', the authors hypothesized that 'the absence of any inherent obligatory chemical analogy or reactivity between substrate and allosteric effector appears to be a fact of extreme biological importance' [12]. It took about half a century before the significance of allosteric regulation was fully recognized, and it became apparent that potential allosteric drugs are free from major drawbacks of traditional orthosteric compounds. In particular, non-competitive action [13–15] via modulation of protein structural dynamics [4••,16••,17] is among the major advantages of prospected allosteric drugs. GPCRs and protein kinases are two groups that make up to about half of the current drug targets. Allosteric inhibitors of kinases [18,19] do not affect non-specifically conserved ATP binding sites, avoiding off-target inhibition and preventing drug toxicity. In the case of GPCRs [5,6], allosteric modulators do not cause receptor desensitization typical of treatment with orthosteric activators. Additionally, allosteric effectors provide functional selectivity allowing the regulation of different subsets of downstream signaling pathways via binding of specific effector molecules to distinct locations of the same receptor. Detection of communication between the allosteric and functional sites, as well as design of effector molecules with required agonist/antagonist activities are the key steps in obtaining allosteric drugs [15,20•]. Here, we review major theoretical concepts, computational models, and state-of-the-art experimental approaches currently used in the study of allosteric regulation. We also briefly discuss future tasks and sketch a generic approach for design of allosteric effectors with required agonist/antagonist characteristics.

From phenomenological models to atomic level description of allostery

The view of allostery has undergone significant transformation since it was first introduced in phenomenological models with conformational selection (Monod–Wyman–Changeux model [21], MWC) and induced fit (Koshland–Nemethy–Filmer [22], KNF) scenarios. The energy landscape concept [1,2,23] supported by vast experimental data [24–26] unraveled the omnipresence of allosteric control [3] in all types of proteins [24]. On the basis of accumulated NMR data, the original model of protein as a fluctuation around the averaged structure [27] was further elaborated into a more general conformational equilibrium view with configurational states coexisting in a structural

ensemble [10]. Using statistical physics arguments Cooper and Dryden showed that the allosteric free energy due to ligand binding can be chiefly entropic, and the minor changes in fluctuations (one per cent RMS per atom) may result in a large free energy change (of the order of kT per molecule). NMR studies substantiated this view [26,28], unraveling, for example, major role of entropy in regulation of CAP transcriptional activity [29,30••], in relaxation dynamics of calmodulin [31••,32], and in regulation of proteins with intrinsically disordered regions [11•,33].

A recent comprehensive phenomenological model of allostery accounts for the switching between agonist and antagonist effects via a population shift in the protein's conformational ensemble [34•], opening an opportunity for design of protein switches [35•]. At atomic level resolution, simple harmonic models implemented in the context of normal mode analysis (NMA) [36–38] provide a proper description of the protein dynamics modulated by allosteric effectors. A coarse grained elastic network model (ENM) approach was used for predicting the allosteric effects due to backbone fluctuations in CRP/FNR family transcription factors, CAP and GlxR, followed by the experimental verification via X-ray and calorimetric analysis [39••]. Ultimately, modeling and experimental analysis of allosteric regulation should properly take into account the balance between the background contribution from the structural enthalpy and the conformational entropy modulated as a consequence of the ligand binding. In order to obtain the required agonist/antagonist mode of the activity modulation [40••], atomic level description of the ligand–protein interactions is a prerequisite.

Experimental identification and characterization of allosteric sites

The starting point in a search for allosteric control of any protein is an identification of the protein's binding exosites [6,13–15,20•,41,42,43•]. Existence of multiple latent allosteric sites has been recognized on many occasions in different proteins, albeit a systematic approach to their detection and characterization is yet to be developed. A wealth of the proteomic data combined with libraries of potential ligands has made high-throughput [44] and fragment-based screening [45] integral parts of pharmaceutical research, detecting target binding sites and providing primary compounds in drug discovery projects. Site-directed approaches, such as alanine scanning [46], disulfide trapping [40••,47], hydrogen/deuterium exchange mass spectrometry [48,49••] (HDXMS), fluorescent [50] and photoaffinity [51,52] labeling are complementary to the high-throughput techniques. Given a protein of interest, the latter methods allow one to find allosteric sites and to investigate their function-related conformational dynamics. HDXMS and disulfide trapping are particularly suitable for exploring the allosteric sites and the modes of their regulation. Specifically, it has been shown that upon binding to the same regulatory site different disulfide-containing fragments can occupy individual subsites and, thereby, generate more potent activating or inhibiting effects [40••,47]. Therefore, disulfide trapping can be an efficient and systematic protocol in hypothesis-driven ligand discovery, addressing the question of agonism and antagonism in the function regulation [40••]. Analysis of protein–ligand interactions and allosteric signaling in the cAMP/protein kinase A [48] shows that time-dependent HDXMS monitoring can be instrumental in exploring conformational transitions related to function and its allosteric regulation [49••]. It is worth noting that standard biochemical experiments and work with biased ligand libraries are typically expensive and time consuming. Given the strong demand for investigation and screening of a rapidly growing number of allosteric drug targets [14•,15], computational *in silico* methods have become indispensable part of current research in allostery [13,41,43•,53].

Computational approaches for prediction of allosteric sites, and web-based databases and servers for allostery

Sequence-based/structure-based approaches developed for detecting allosteric sites are reviewed elsewhere [41]. In brief, sequence-based approaches utilize conservation of the protein sequences, which reflects similarity of the corresponding 3D structures. For example, statistical coupling analysis [54] of multiple sequence alignments was recently shown to be helpful in detecting the communication between allosteric and functional sites. Structure-based models rely upon analysis of known binding pockets and ligands [41]. A major drawback of these approaches is the static nature of the objects under consideration complemented with a bias towards traits typical for binding sites in the training set. Allosteric modulation of protein activity is non-competitive, which is manifested in a low evolutionary pressure on allosteric sites and in their distinct pharmacophoric characteristics compared to those of orthosteric pockets. For example, while allosteric sites can be very shallow, structure-based approaches are focused around deep surface pockets typical of 'traditional' drug targets. The problem of shallow binding pockets is addressed in DARC (Docking Approach using Ray-Casting) by matching topographies of the surface pockets observed within the protein and obtained by viewing potential ligands from the same vantage point [55].

The major future challenge is a detection and druggability assessment of the latent allosteric sites. It can be resolved only by exploring the dynamics of the structure, because latent sites are active in certain structures of the conformational ensemble, determined by the structural dynamics, binding of other ligands, and mutations [39••,56•,57••]. A key characteristic of any allosteric site is its ability to couple to the intrinsic dynamics of the