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On the perturbation nature of allostery: sites, mutations, and signal modulation

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Regardless of the diversity of systems, allosteic signalling is found to be always caused by perturbations. This recurring trait of allostery serves as a foundation for developing different experimental efforts and theoretical models for the studies of allosteric mechanisms. Among computational approaches considered here particular emphasis is given to the structure-based statistical mechanical model of allostery (SBSMMA), which allows one to study the causality and energetics of allosteric communication. We argue that the reverse allosteric signaling on the basis of SBSMMA can be used for predicting latent allosteric sites and inducing a tunable allosteric response. Per-residue allosteric effects of mutations can also be explored and 'latent drivers' expanding the cancer mutational landscape can be predicted using SBSMMA. Most recent and important implementations of computational models in web-resources along with a brief outlook on future research directions are also discussed.

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Introduction

The progress from the first phenomenological models [1,2] to a consensus on the fundamental role of dynamics [3] in modulation of protein activity from remote sites, called allosteric regulation, took almost half a century [4,5]. It is even more exciting, therefore, to witness powerful expansion of the allostery studies, which nowadays span from exploring the nature of causality in allosteric signaling [5,6,7••,8••,9], to detecting allostery in diversity of biomolecules [10–13] and their complexes [14–17], finding new molecular processes with allosteric mechanisms [18,19], and to *in silico* and experimental design of allosteric structures [12,20] and materials [21,22]. In addition to different types of proteins [11,13,23,24] and molecular machines, such as chaperones [4,25,26], allosteric signaling and specifics of its causality are currently studied in four major receptor families [14], membrane proteins [16] and symporters [27,28], toxin-antitoxin operons [6], human glucocorticoid receptor [8••], and cardiac thin filaments [17], to name a few.

There are several causes of allosteric signaling, and, sometimes, they can act in combinations [25,29,30]. The most common case, binding of small ligands, works in many allosterically regulated systems from monomeric proteins to oligomers and protein complexes [31,32], motor proteins [33], molecular machines like chaperones [4,25,26], and receptors [14]. Specific lipid-protein interactions, such as binding of lipid to ammonia channel (AmtB) from *Escherichia coli*, can act as allosteric modulators for the binding to integral membrane proteins [16]. Post-translational modifications [4,25,29], glutathionylation and phosphorylation, work in allosteric regulation of caspase 3 function [9], and multiple phosphorylation sites in the N-terminal domain of the nuclear hormone receptor (NHR) affect transmission of allosteric signals in NHR [14]. Allosteric effect of mutations was shown to be an important component of cancerogenesis [34–36] and the origin of pathogenic changes in the cardiac thin filament (CTF, [17]). More than 1000 mutations allosterically act in both ligand- and voltage-gated ion channels (LGIC and VGIC), and hundreds of gain- or loss-of function mutations were identified in GPCRs [14]. Single-residue mutations were used for specific allosteric activation of Insulin-Degrading Enzyme (IDE) against the amyloid Aβ peptide [37,38]. An increasing evidence of the role of intrinsic disorder in allosteric coupling in proteins [39] currently includes different examples, such as the effect of frustration in intrinsically disordered regions (IDR) on the activity of Glucocorticoid Receptor (GR) [8••], allosteric modulation originated by the order-disorder organization in GPCRs [14], dynamically-driven attenuation of the substrate affinity and enzyme turnover in adenylate kinase [18], dimerization of the biotin repressor (Bir A) promoted by the allosterically initiated disorder-to-order transition in the binding interface [19].

Regardless of the size of molecules/systems and their allosteric effectors, the sizes of effectors and energetics of their actions are always negligible compared to those of the regulated objects, which reasonably suggests to define

their mode of action as a *perturbation*. For example, activating dimerization of RAF kinase is actually driven by the binding of only two residues, whose mutations prevents the activation of the protein [13]. A number of experimental techniques uses the perturbation nature of allostery, stimulating the development of corresponding theoretical models. Main focus of this review is to show how the understanding of the perturbation nature of allosteric signaling can guide the development of theoretical models and computational frameworks aimed at the efficient analysis of allosteric mechanisms. Recent advances, both experimental and computational, in the analysis of mechanisms, prediction of allosteric sites, and effects of signaling and communication will be discussed. We will also share our own experience in the developing of computational models for analyzing causality and energetics of allosteric signaling [4,7••,30,68••,73] which provide a quantitative description of the communication with known regulatory exosites, prediction of latent ones, and investigation of allosteric effects of mutations [36–38].

Experimental methods in the analysis of allosteric mechanisms

While X-ray analysis is sometimes successful in the investigation of different structural forms in the allosterically linked ensemble of conformations [40], the NMR spectroscopy is still the major experimental technique for exploring the dynamics of conformational transitions in allosteric mechanisms. The allosteric inhibition of DNA binding via the entropy redistribution caused by perturbations in the equilibrium picosecond-nanosecond motions is only one example, showing how NMR spectroscopy experiments (here, relaxation dispersion measurements) can characterize the perturbation nature of allosteric signaling [41]. Because of the methodological advancements in NMR, it became possible to study proteins and protein complexes of high molecular weight and to investigate micro- and millisecond processes and beyond. *Grutsch et al.* reviewed recent developments in relaxation dispersion and magnetization exchange experiments for the analysis of motions on slow timescales, spin relaxation measurements of functionally relevant pico-to-nanosecond dynamics, residual dipolar coupling and chemical shifts dynamically averaged in conformational ensembles [42]. Advances in NMR methods to map allosteric sites are also comprehensively discussed by *Boulton and Melachini* [43]. The variation in 1D saturation transfer difference (STD) NMR spectra of a molecular substrate in presence and absence of allosteric ligand was proposed to be used as an indicator of the effect of a potential allosteric drug [44].

A specific technique in the context of NMR spectroscopy, chemical shift perturbation (CSP), has been shown to efficiently detect protein structural changes caused by the effector binding, mutations, and other perturbations [45]. For instance, analysis of the single-effector bound state in the homodimeric enzyme thymidylate synthase using mixed ^{15}N-labeling CSP technique showed that long range intersubunit communication occurs only in the fully saturated protein state [46•]. A CSP technique was also used for the detection of hidden allosteric sites in the protein tyrosine phosphatase (PTP) via observation of the reciprocal signaling from the catalytic site with single-alanine mutations [47•]. Remarkably, a common exponential decay for the chemical shift changes caused by the ligand binding and mutations was observed in the analysis of perturbation-response distances in a large CSP protein dataset [48•]. It was found that 20–25 Å length of the signal propagation is a universal feature of all types of allosteric proteins.

Several other experimental methods are frequently used solely or in combination with computational approaches. Long-range allosteric communication [49] caused by the binding of lipid-like allosteric effector oleyl sulfate (OS) in soyebean lipooxugenase was detected by the hydrogen/deuterium exchange mass spectrometry (HDXMS). Isothermal calorimetry and elastic network modelling (ENM) showed how decrease of the cAMP binding affinity in CAP protein induces strong negative cooperativity by controlling the protein configurational fluctuations [50]. Isothermal Titration Calorimetry (ITC)-coupled with double-mutant cycle analysis and complemented by MD simulations concluded that binding of small molecular effector biotinoyl-5′-AMP activates the biotin repressor BirA by promoting its dimerization via modulation of the disorder-to-order transition at both allosteric and dimerization cites [19]. The combination of NMR and IR spectroscopy together with MD simulations showed that photoperturbation of the binding groove of PDZ domain can induce a conformational transition similar to that caused by the binding of allosteric ligand [51]. It was shown that combination of transient infrared spectroscopy (IR), NMR, and MD can fully characterize kinetics of the allosteric transition and potentially detect allosteric sites [52]. NMR relaxation techniques with single-site mutations (RASSMM) allowed the identification of residues in cyalophilin A (Cyp A) that are allosterically-coupled to active-site, facilitating the chemical-shift-restrained MD simulations used to explore and tune mechanisms of the allosteric coupling [53]. Role of mutations in perturbing allosteric regulation was shown in the analysis of 7000 cancer genomes, and statistical method AlloDriver was proposed for identification of proteins-enriched with somatic mutations altering native allosteric signaling [34]. 'Whole-protein alanine-scanning mutagenesis of allostery' performed on the human liver pyruvate kinase hL-PYK showed that a large percentage of the protein can contribute to the allosteric mechanism [54]. *Naganathan* recently reviewed a series of experimental and computational observations showing that mutational perturbations can consistently affect protein

stability and native conformational ensemble contributing to allosteric modulation of the function [55]. Using gene recombination method, Tyr-binding ACT domain in DAH7PS protein was interchanged with the non-homologous prephanate-binding CM domain, producing two protein chimeras with new functional allostery [56••].

Theoretical and computational studies of allostery

MD simulations are widely used in the studies of allostery [57,58]. Normal mode analysis and MD simulations performed on multidomain proteins suggest that allostery is a common mechanism of regulation in these proteins [59]. The analysis of the energetics of bound/unbound configurational ensembles in PDZ3 domain obtained from all-atom MD simulations shows that redistribution of electrostatic energy leads to significant changes in the protein local enthalpies in spite of negligible total changes, challenging thus the dominant view of entropy-driven dynamic allostery in PDZ domain [60]. Several structure/ensemble perturbation-based methods were used for exploring allostery in chaperonin GroEL [26], analyzing modulation of allosteric response in single chain-Fv (scFv) antibody [61•], and detecting the allosteric sites [62]. Ensemble- and rigidity theory-based perturbation approach was developed for the analysis of dynamic allostery, which identified experimentally observed key residues involved in the signal transmission [63]. Perturbation response scanning (PRS) combined with MD simulations described the role of hinges in transferring allosteric signal in human Pin1 [64•], and it helped to define allosterically relevant residues in Hsp 70 [65]. Based on the ideas of statistical coupling analysis, a new method - Rational Engineering of Allostery at Conserved Hotspots (REACH) - was recently proposed for the identification of allosteric hotspots and design of required allosteric responses in proteins [66]. Perturbation analysis of low frequency modes in elastic networks, combined with a feature-based screening of protein pockets are used in the AllositePro method for predicting allosteric sites [67].

Structure-based statistical mechanical model of allostery: from predicting allosteric sites to inducing and fine-tuning required allosteric response

In the context of the protein energy landscape paradigm the allosteric signaling is caused by the perturbation of a protein equilibrium state, which always results in a change of the free energy associated with the conformational space. Therefore, any allostery model should address two issues: first, the problem of causality, which links the perturbation to allosteric communication and, second, a quantification of the induced allosteric effects in terms of the thermodynamic changes in the system. In particular, ligand binding and mutations modulate protein functional activities by shifting the thermodynamic equilibria between corresponding configurational states, which is evaluated in terms of free energy changes. While these changes is a global property of the protein conformational space, concurrent allosteric communication involves signals that propagate from regulatory to functional sites and from mutated residues to functional regions, prompting a per-residue treatment of the energetics of this process.

The structure-based statistical mechanical model of allostery (SBSMMA) [7••] addresses problems of causality and energetics of allosteric communication, considering the source of allosteric signal as a perturbation. The method consists of three basic modules, which are reviewed below.

Causality and perturbations

Given a protein reference structure and its Cα harmonic description, two protein states, unperturbed (0) and perturbed (P), are defined. The energy function associated with the unperturbed state is

$$E^{(0)}(\boldsymbol{r}) = \frac{1}{2}\sum\nolimits_{i,j} k_{ij}\left(d_{ij} - d_{ij}^{0}\right)^{2} \tag{1}$$

where d_{ij} and d_{ij}^{0} are the interatomic distances between C a atoms in the generic $\boldsymbol{r}$ and reference $\boldsymbol{r}^{0}$ structures, respectively, and k_{ij} spring constants. As a result of either ligand(s) binding, mutation(s), or their combination, the protein energy in the perturbed state is:

$$E^{(P)}(\boldsymbol{r}) = E^{(0)}(\boldsymbol{r}) + \alpha_P V^{(P)}(\boldsymbol{r}) \tag{2}$$

where $V^{(P)}$ is an additional harmonic term (as in Eq. (1)) and α_P is a perturbation parameter ($\alpha_P \gg 1$ for stiffening perturbations and $\alpha_P \ll 1$ otherwise). Ligand binding perturbations are defined via the over stabilization of the interactions between all the pairs of residues in the binding site, which is assumed to mimic the presence of a ligand [7••,30] (Figure 1a). Two types of mutations, stabilizing (UP) and destabilizing (DOWN), are considered, allowing to investigate single-residue perturbations (Figure 1a) [30,38]

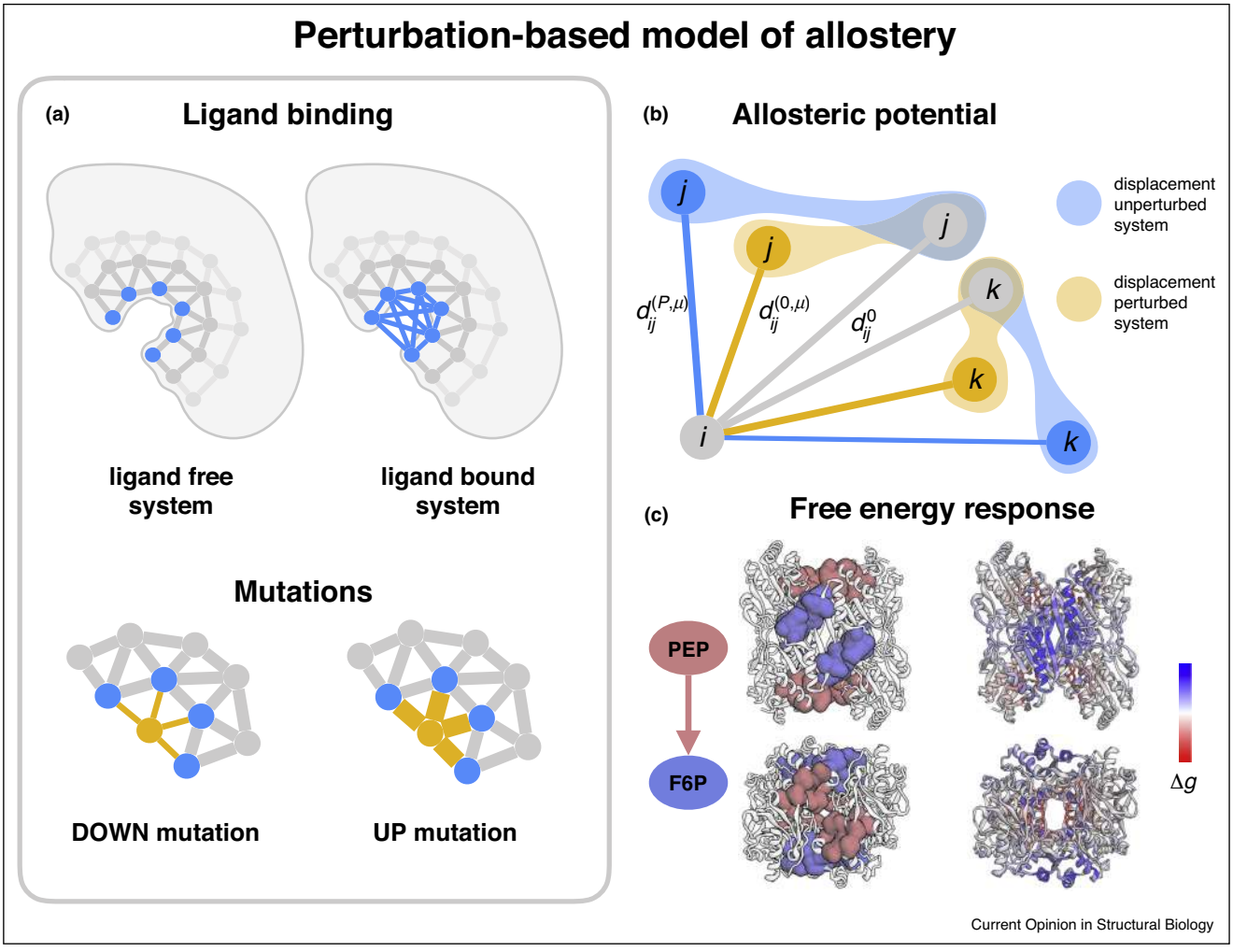
Microscopic allosteric potential

A microscopic allosteric potential measures energetics of the configurational ensemble at the single residue level, which can be altered by the perturbation. The potential quantifies the signal to a residue i provided by configurational changes in its neighborhood (Figure 1b) and evaluated in terms of the elastic work exerted on the residue:

$$U_i(\sigma) = \frac{1}{2}\sum_{\mu} \varepsilon_{\mu,i}\sigma_{\mu}^{2} \tag{3}$$

where $\varepsilon_{\mu,i} = \sum_j |\boldsymbol{e}_{\mu,i} - \boldsymbol{e}_{\mu,j}|^2$ are parameters defined via the normal modes obtained from the two protein

Figure 1



Structure-based statistical mechanical model of allostery (SBSMMA) evaluates free energy of allosteric signaling caused by the ligand binding and mutations as perturbations. **(a)** Ligand binding perturbations are defined by strengthening the interactions between the pairs of residues in a binding site. Mutational perturbations, stabilizing (UP) and destabilizing (DOWN), respectively, are defined by either increasing (stiffening) or decreasing (loosening) interactions with the residue's neighbors. **(b)** A microscopic allosteric potential measures the signaling delivered on a residue *i* as a result of the specific configurational changes taking place in the residue's neighborhood (Eq. (3)). C. Illustration of the free energy response in phosphofructokinase (PFK), as a result of the ligand binding perturbation in the inhibitor PEP site (all residues of the protein are colored according to the resulting per-residue allosteric free energy $\Delta g_i^{(P)}$, Eq. (4)).

states [7••] and $\sigma = (\sigma_1, \ldots, \sigma_\mu, \ldots)$ is a set of Gaussian distributed coefficients, which identify the displacement of the residue i as $\Delta \boldsymbol{r}_i(\sigma) = \sum_\mu \sigma_\mu \boldsymbol{e}_{\mu,i}$ (Figure 1b)

Free energy response

While the microscopic allosteric potential evaluates the allosteric signal delivered on a residue as a result of the elastic stress originated by the motion of its neighbors, the consideration of ensembles of configurations via integrating over all possible displacements of neighboring residues allows one to calculate the per-residue partition function as $z_i = \Pi_\mu \left(\pi 2 k_B T/\varepsilon_{\mu,i}\right)^{1/2}$ and, consequently, the free energy $g_i = -k_B T \ln z_i$ associated with the transmitted allosteric signals. Thus, considering two (unperturbed/perturbed) protein states, the allosteric signal delivered to a residue as a result of a perturbation (P) is calculated via the free energy difference:

$$\Delta g_i^{(P)} = \frac{1}{2} k_B T \sum_\mu \ln \frac{\varepsilon_{\mu,i}^{(P)}}{\varepsilon_{\mu,i}^{(0)}} \tag{4}$$

The free energy difference $\Delta g_i^{(P)}$ evaluates the total amount of work applied on a residue as a result of the perturbation: positive $\Delta g_i^{(P)} > 0$ suggests a potential for

Figure 2

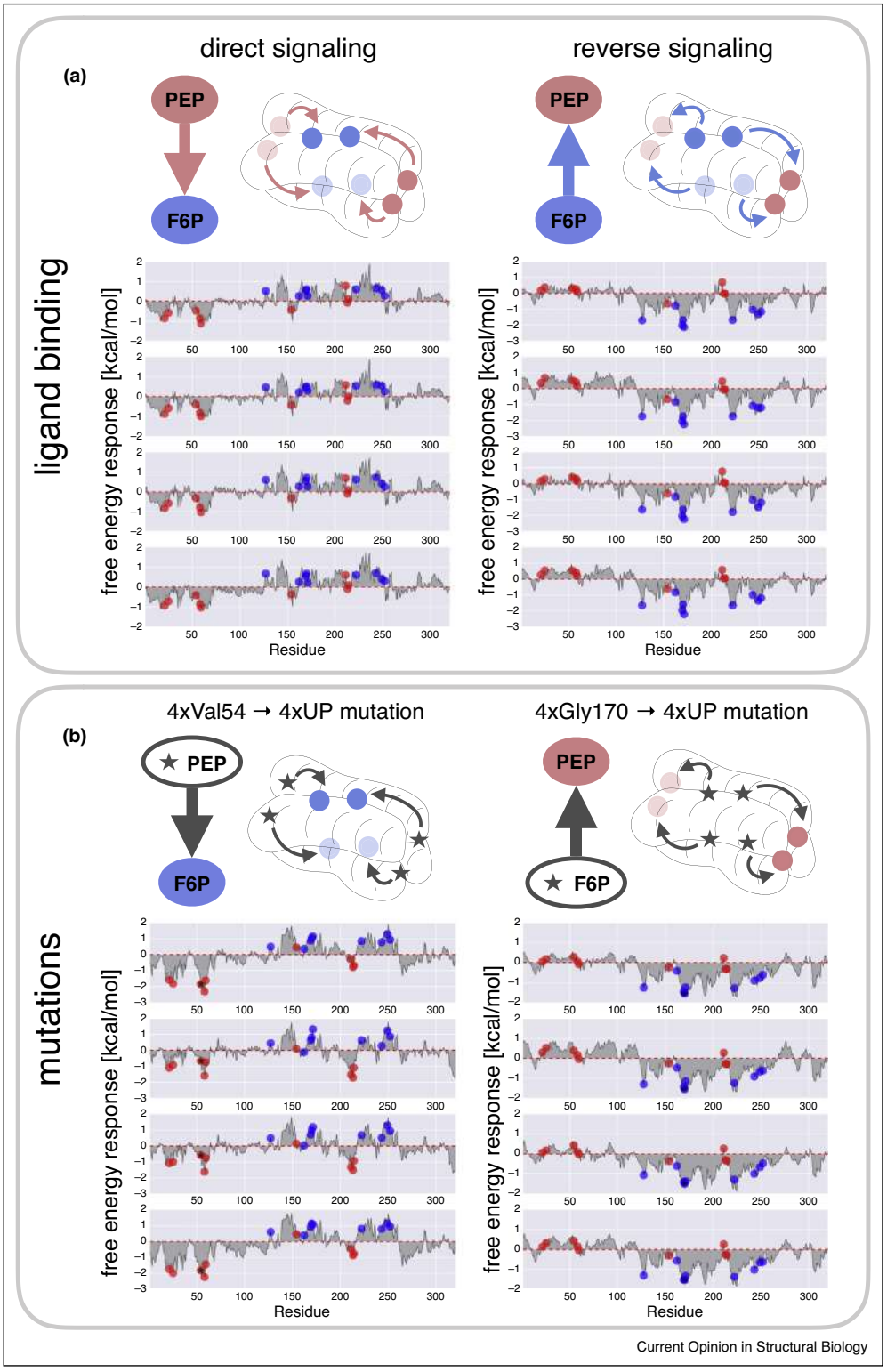


Illustration of the direct and reverse allosteric signaling and allosteric effects of mutations in PFK. **(a)** Direct signaling is shown for the signal sent from the bound inhibitor site PEP to the catalytic site F6P, reverse from the bound substrate site (F6P) to the inhibitor site (PEP).
The reversibility of the allosteric signal, effector vs. functional is clearly shown in the per-residue free energy response profiles in both direct and reverse cases of signaling (locations of residues belonging to PEP and F6P sites are shown by corresponding colors). **(b)**Two examples of the effects of stabilizing (UP) mutations in the PEP (Val54) and F6P (Gly170) sites of PFK (residues are 'mutated' in all fours subunits of the protein). In

conformational change of residue i, while preventing it in the case of negative $\Delta g_i^{(P)} < 0$. Since the unperturbed and perturbed protein states are both constructed on the same reference protein structure in the framework of SBSMMA, the free energy difference $\Delta g_i^{(P)}$ primarily accounts for the distortions of the equilibrium fluctuations induced by the perturbation, hence, not directly considering possible conformational changes (Figure 1c).

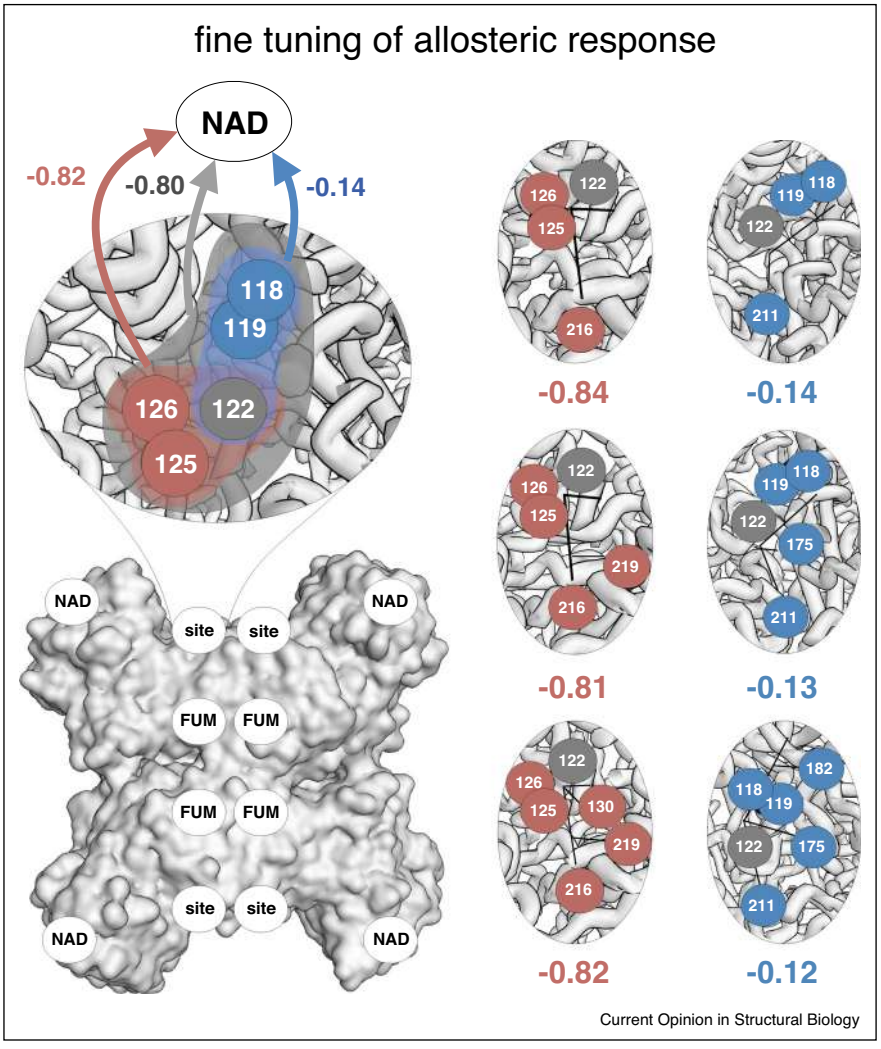
A variety of phenomenologies of allosteric communication can be observed from the application of the SBSMMA using ligand binding (Figure 2a, [7••,30]) and mutations (Figure 2b, [30,38]) as a source of perturbations. A possibility of reversing the perturbation was hypothesized earlier [4] on the basis of the observation of apparent symmetry of allosteric communication, according to which perturbation of functional sites can trigger a reverse signal (Figure 2a), aiding, thus, identification of allosteric sites. The reverse perturbation approach for prediction of allosteric sites was recently developed on the basis of SBSMMA [68••] and benchmarked on two sets of allosteric proteins, yielding the good predictive power [68••]. Per-residue approximation in the analysis of the energetics of reverse perturbation allows one to predict latent allosteric sites and to designate non-natural regulatory sites, which can provide a required allosteric response upon binding of corresponding ligands (Figure 3). This response can be fine-tuned by adjusting both allosteric sites and effectors. Another important implication of per-residue approximation in SBSMMA is a possibility to predict and explore so-called 'latent drivers' [36], that is not harmful passenger mutations that can drive cancer development and drug resistance upon some triggering events, such as coupling with other emerging mutations or start of therapy. Specifically, analysis of the combined allosteric effects of mutations with additional perturbations (other mutations, ligand binding etc.) may point to those that make an impact similar to the one of known cancerous 'drivers', expanding thus the cancer mutational landscape. The SBSMMA was implemented in the AlloSigMA web server [30], which is proven to be a useful guide for the exploration and engineering of allosteric regulation. For example, computational prediction of novel allosteric pockets in HIV-1 non-nucleoside reverse transcriptase [69], description of allosteric communication in HIV polyprotein Gag [70], and allosteric communication between the antibody constant and variable regions [71] were performed using the AlloSigMA [30] and earlier developed SPACER [72] servers. The SBSMMA was also used for building the AlloMAPS database [73] that provides a comprehensive information on the allosteric signaling in about 2000 proteins and protein chains.

Outreach and outlook on future direction

Since detail description of major allostery-related web-servers/databases was provided elsewhere [4,74,75], we review only the latest and most important web-resources. The CryptoSite web server (http://salilab.org/cryptosite, [76]) predicts cryptic binding sites on the basis of machine learning algorithm, which allowed to almost double the size of the potentially 'druggable' human proteome of disease-associated proteins without using experimental fragment-based ligand discovery or long MD simulations and fragment docking. The Allosteric Database (ASD v3.0, http://mdl.shsmu.edu.cn/ASD, [77]) is a comprehensive source of data on more than 70 000 allosteric modulators, allosteric molecules/functions, binding sites, pathways, and related diseases. The AlloFinder server (http://mdl.shsmu.edu.cn/ALF, [78]) is the interactive platform, which contains a pipeline for the allosteric site identification, screening and evaluation of allosteric modulators, complemented by the allosterome mapping of predicted allosteric sites and modulators in human proteome. The AlloSigMA server (http://allosigma.bii.a-star.edu.sg/home/, [30]) is an implementation of the SBSMMA [7••], which provides an interactive framework for estimating the allosteric free energy as a result of the ligand binding, mutations, and their combinations. Offering an aid for the detection of latent allosteric sites, 'latent driver' mutations [36], and evaluation of the allosteric effects of ligand binding and mutations, AlloSigMA can rationally guide the selection of allosteric sites/mutations, their modifications and combinations in order to achieve required and to prevent undesired signaling, facilitating, thus, experimental efforts on allosteric modulation of protein activity. The AlloMAPS database (http://allomaps.bii.a-star.edu.sg/home, [73]) contains data on allosteric signaling in 46 proteins with comprehensively annotated functional and allosteric sites, 1908 proteins and protein chains from PDBselect collection of chains with low (<25%) sequence identity, and 33 proteins with more than 50 characterized pathological SNPs in each molecule. The energetics of allosteric signaling between known functional and regulatory exosites, effects of SNPs, and allosteric modulation caused by the sites and mutations designated by the user can be explored using the AlloMAPS data. The Allosteric Signaling Maps (ASMs) of all proteins and protein chains obtained via exhaustive per-residue SBSMMA-based scanning contain a comprehensive information on the allosteric effects of stabilizing/destabilizing mutations

(Figure 2 Legend Continued) both cases mutations induce pronounced allosteric response: the free energy response in F6P on the mutations Val54 in PEP in all four chains is similar to the case of the direct signaling, whereas response in PEP on the mutations of all four Gly170 resembles the reverse signaling from F6P to PEP upon the ligand binding to the F6P site. In both, ligand binding and mutation cases, the free energy profiles resemble the outputs that can be obtained in the AlloSigMA server [30].

Figure 3



Inducing and fine-tuning of the allosteric response in the NAD-dependent malic enzyme (NADME) protein. The allosteric signal can be produced by the putative allosteric site in NADME, and its strength can be tuned by changing composition of the site. Combinations of blue-grey residues give the allosteric signal close to the one from natural allosteric (FUM) to functional (NAD) sites ($\Delta g^{(P)}_{NAD} = -0.14$ kcal/mol), whereas red-grey combinations provide much stronger signalling (see Ref. [68••] for details). Importantly, different site-ligand combinations can be used for obtaining similar signaling, suggesting a strategy of mutual design of the pairs of new allosteric sites and effectors.

and on the allosteric modulation range caused by every sequence position.

To conclude, as a general phenomenon allostery is detected in an increasing number of biological and non-biological systems, yielding a wide diversity of mechanisms. Among the most recent interesting twists are the role of dynamic allostery in driving the cold adaptation [18], biased agonism in receptors [14,15], and the emerging concept of allosteric materials [22]. Together with already classical yet unsolved problems of prediction of allosteric sites, agonism/antagonism, engineering and tuning of site-modulator pairs towards the design of allosteric drugs [79], new paradigms in allostery provide a lot of opportunities for the basic research and important biomedical applications.

Conflict of interest

No conflict of interests.

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- • of special interest
- •• of outstanding interest

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