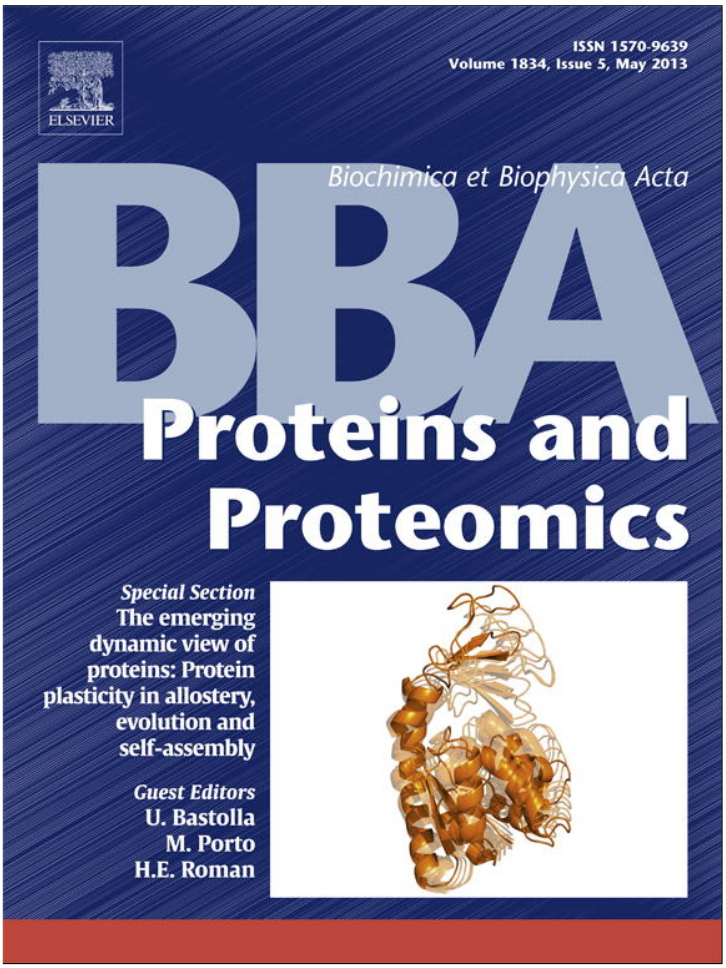

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Review

Thermodynamics of allostery paves a way to allosteric drugs ☆

Igor N. Berezovsky *

Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel

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ABSTRACT

We overview here our recent work on the thermodynamic view of allosteric regulation and communication. Starting from the geometry-based prediction of regulatory binding sites in a static structure, we move on to exploring a connection between ligand binding and the intrinsic dynamics of the protein molecule. We describe here two recently introduced measures, binding leverage and leverage coupling, which allow one to analyze the molecular basis of allosteric regulation. We discuss the advantages of these measures and show that they work universally in proteins of different sizes, oligomeric states, and functions. We also point the problems that have to be solved before completing an atomic level description of allostery, and briefly discuss ideas for computational design of allosteric drugs. This article is part of a Special Issue entitled: The emerging dynamic view of proteins: Protein plasticity in allostery, evolution and self-assembly.

1. Introduction

Allostery is the regulation of protein function via sites not directly adjacent to the site of altered activity [1,2]. The very concept of allostery emerged in 1935, when Linus Pauling proposed a model of positive cooperativity in the binding of oxygen to hemoglobin [3]. The phenomenological MWC (Monod–Wyman–Changeux) [4] and KNF (Koshland–Némethy–Filmer) [5] models developed in 1960s were devised to explain this cooperativity. Briefly, the MWC model is based on the following postulates: (i) oligomers are made of identical monomers, (ii) ligand binding leads to a change in the quaternary structure with preservation of the oligomer's symmetry, (iii) different quaternary structures are accessible in both liganded and unliganded forms, and (iv) the allosteric effect is a result of a change in the equilibrium between conformers upon ligand binding. The major assumptions of the KNF model are: (i) ligand binding causes tertiary conformational changes, and (ii) sequential transition between the states is based on the induced-fit mechanism. Negative cooperativity was not described by the original MWC model, and it was an important distinction between the MWC and KNF models [1]. Eventually, the extended MWC model that includes negative cooperativity [6] was introduced. Numerous studies have since been performed of the MWC and KNF models at different levels of coarse-graining, such as analysis of local perturbations [7,8], using normal modes [9] for quantifying energetic and entropic contributions to allostery [10–12] and associated conformational changes [13,14], and implementing network description of allosteric signaling [15–19]. At some point, original restrictions of the MWC and KNF models on considering only oligomeric structures were dropped, and allostery is currently analyzed in proteins with a wide range of sizes, shapes [20], and functions [15,21–25]. One of the questions that remain under intense discussion [1,26,27] is the apparent dichotomy between MWC and KNF models and their counterparts in the energy landscape-based "new view" of allostery [27–30] — conformational selection and induced fit. The difference is in ligand binding preceding conformational change or not, which is primarily a matter of kinetics. Thermodynamically, however, these models are not mutually exclusive, because in both cases there is a shift in the population of different functional states upon effector binding [21,22,31,32]. In 1975, Frauenfelder with colleagues devised a concept of the energy landscape with multiple conformational states existing in thermal equilibrium, which can be changed by both solvent and ligands [33–37]. Considering "conformational mobility as a common route between allosteric regulation and catalysis" or, in other words, relying on the role of intrinsic dynamics in the protein function and its regulation [38–40], one can explore how the relevant conformational states determine allosterically connected functional and regulatory sites [26,41]. We discuss here the thermodynamic view of allostery, where the effect of regulation is determined by the relative stability of free and ligand-bound conformations [21,22], whereas an analysis of transition pathways and other aspects of kinetics are avoided. We overview our recent works on the static predictor of allosteric sites — local closeness [23], and the newly introduced concepts, binding leverage [22] and leverage coupling [21], that provide quantifiable measures for characterizing the molecular basis of allosteric regulation and communication. We briefly address technical issues that have to be resolved, describe immediate projects of keen

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* Tel.: +972 8 9342794; fax: +972 8 9344118.
E-mail address: Igor.Berezovsky@weizmann.ac.il.

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interest, and discuss several ideas for the computational design of allosteric drugs [42].

2. From geometry of effector binding sites to thermodynamics of allosteric communication

Functional motions and allosteric mechanisms of proteins utilize the inherent dynamics of the protein and depend on the balance between its different conformational states. Therefore, it is important to: (i) predict and characterize substrate- and effector-binding sites; (ii) study the coupling between ligand binding and functional dynamics, and the role of effector binding in the switching between active and inactive states in the protein's conformational ensemble; and (iii) analyze the molecular basis of communication between the allosteric and catalytic sites.

2.1. A static geometry-based predictor for allosteric sites

We have started from the task to find a predictor for the native and latent allosteric sites based on a single structure and without any knowledge of the structure's dynamics, interactions in binding sites, and sequence conservation. In other words, we wanted to have a parameter-free, fast, and efficient approach for scanning numerous structures currently available in structural proteomics. The predictor should detect potential allosteric sites that are not characterized by specific chemical groups selected in evolution and manifested in sequence conservation. We devised a local centrality measure, dubbed local closeness [23], which can be used as a predictor of ligand binding sites involved into regulation of the protein function. First, the residue interaction graph (RIG) where each residue in the protein is a node has to be built. Second, residues of the binding sites are determined by ranking exposed residues by the local closeness in the RIG. The local closeness of degree m for a node is defined as

$$C_m = \sum_{k=1}^{m} \frac{n_k}{k^2}. \quad (1)$$

The n_k is the number of nodes whose shortest distance from a given node is exactly k. Local closeness serves thus as a measure of the residue connectivity for neighbors at a maximal distance m in the graph. The number of neighbors, n_k, is proportional to the area of a shell in an isotropic system and scales approximately as k^2. Therefore, the contribution of a potentially large number of residues at large distances is weighted by k^2 to make it comparable with the contribution from the fewer number of residues at closer distances. We found that $m=4$ gives the best performance of predictions. This value of m effectively means that only residues closer than 30–40 Å are included in the calculation, which roughly corresponds to the length scale of single domains. Fig. 1c illustrates for an idealized 2D network a straightforward geometrical rationale for why local closeness can predict ligand binding sites. The two large circles in the figure represent the nodes covered in the calculation of C_4, and their shade the factor $1/k^2$. Surface nodes in cavities (red circle) will have higher local closeness than at a flat or ridged surface patch (yellow circle) because more nodes are included in the calculation. The local closeness has obvious advantages over other static predictors of binding sites. First, the local closeness clearly outperforms the global closeness measure [43], which is defined as the inverse of the average shortest distance to all other residues in the RIG. The global closeness essentially indicates closeness of the geometrical center of the protein to the given residues, providing good predictive ability for globular domains. It fails, however, in non-globular structures and assemblies, such as for example the linear tetramer of tryptophan synthase (PDB ID: 1bks, Fig. 1a,b). In this case the global closeness is simply high at the waist of the protein, while the correspondence to surface geometry is completely lost (Fig. 1a). The invention of the local closeness resolved this issue. Since local closeness is weighted by the power two of the distance from the residue, it correctly predicts ligand binding sites in non-globular geometries where global closeness clearly degenerates [23]. Second, local closeness performs better than the most advanced local network measure weighted based on the amino acid identity [44]. The latter helps in identifying residues that take an active part in catalysis, but lowers the chance of finding effector binding sites, which do not have typical catalytic chemistry. Local closeness predicts catalytic sites almost as well as residue-type weighted measure developed in [44]. However, local closeness has a clear geometrical meaning and is free of any fitting parameters, in contrast to the score in [44]. Using a set of allosteric proteins (see Table 1 in [23]), we showed that local closeness performs better in finding non-catalytic effectors sites. To conclude, introduction of local closeness fulfills a need for the generic structure-based and sequence-independent measure that allows one to predict allosteric sites. The obvious advantages of this measure are: clear and intuitive geometrical interpretation, independence from any information about sequence or amino acid composition, absence of

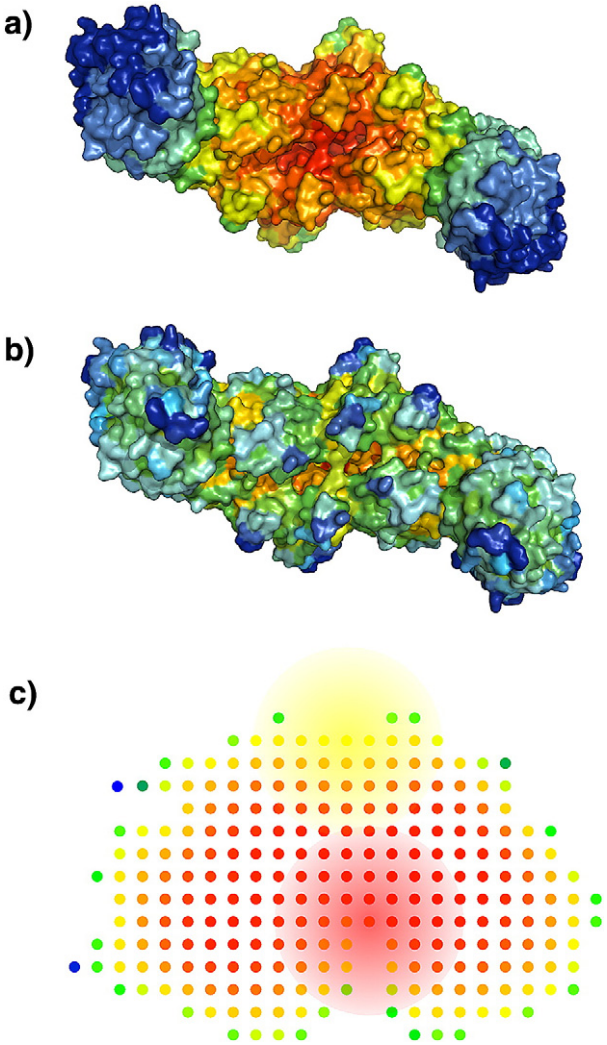

Fig. 1. Local closeness provides correct predictor for functional and allosteric sites in single- and multidomain proteins. Global (a) and local (b) closeness for tryptophan synthase (PDB ID: 1bks). To calculate the network measures, a residue interaction graph (RIG) where each residue is a node that is generated. Residues are defined to be in contact if any of their atoms are within a distance of 5 Å. Panel (c) illustrates how local closeness can be interpreted geometrically for an idealized RIG (as described in the main text).
Reproduced with permission from [23].

any fitting parameters, and equally good performance for both monomers and oligomers [23].

2.2. Molecular basis for allosteric regulation

Turning to protein dynamics, we have conceived a generic quantity, binding leverage, which measures ability of a binding site to couple to intrinsic motions of the protein [22]. Fig. 2 illustrates how the binding of a ligand can affect the local deformation of the binding site and, as a consequence, to modulate conformational changes of the whole protein. The curved arrow describes idealized function-related movement in a particular conformational degree of freedom, and small arrows show the direction of motion for specific C_α atoms in the same degree of freedom. Ligand X attracts surrounding atoms and prevents the opening its binding pocket, and ligand Z sterically blocks its binding site from the closing. Binding in either of these locations will affect conformational changes in the other one. Ligands X and Z, can be an allosterically coupled pair effector–substrate, whereas ligand Y is an example of binding which does not interact with the depicted motion, and hence cannot affect these function-related conformational changes. Binding leverage quantifies the cost of deforming the binding site when a ligand is present in the site. In the following analyses binding sites are defined by docking dummy ligands to the surface of a protein through Monte Carlo simulations, to find sets of residues that are located next to each other on the surface. First, springs of length d_{ij} (dashed lines in Fig. 2) are placed between each pair of C_α atoms i and j, whose connecting line passes within 3.5 Å of any ligand atom. Second, the change in potential energy of the spring due to structural change is calculated as:

$$\Delta U = \frac{k}{2}\sum_{ij}\Delta d_{ij}^2, \tag{2}$$

where the indices i and j run over all relevant residue pairs and k is an arbitrary spring constant. If there is a pair of active/inactive conformations, a vector describing the difference between two aligned structures can be used in calculations. In the case of a single known structure, the conformational changes can be simulated using coarse-grained potentials, for example by calculating the normal modes of an elastic network model [8]. If ΔU_k represents this change for a normal mode k, the binding leverage L_A for a set of modes A is then calculated as

$$L_A = \sum_{k\in A}\Delta U_k. \tag{3}$$

The binding leverage of a site both depends on the range of motion at the site and how tightly bound the ligand is, i.e. how many pairs of residues connect with the ligand. A ligand that binds to a site with high binding leverage has a potential to lock one or more collective degrees of freedom, thus shifting the balance between the states that are sampled along those coordinates. Binding leverage thus is an indication of the strength of coupling between ligand binding and functional dynamics. Detection and analysis of sites with high binding leverage allow one to predict both catalytic and allosteric sites as well as latent binding sites which can be potential drug targets [22].

2.3. Allosteric communication

We have also addressed the question of how allosteric communication takes place between the sites. We introduced the concept of leverage coupling, which provides a quantitative characteristic of allosteric communication. The concept is based on the following assumption: sites that have high binding leverage for the same motion are more likely to be allosterically coupled than sites that only have high binding leverage for motion along independent degrees of freedom. Fig. 3 contains an example of a toy protein, which illustrates the role of independent degrees of freedom in allosteric communication. There are four binding sites in the protein: W, X, Y, and Z. Two conformational degrees of freedom, shown by red and green arrows, are used in the illustration. Both degrees of freedom cause opening of the site X, red closes site Y and green closes Z. Pairs of site X–Y and X–Z have high binding leverage under the red and green degrees of freedom, respectively. As a result, X–Y and X–Z are allosterically coupled, whereas the other pairs are only weakly coupled. It is easy to imagine that site X could be a catalytic site, Z an activator site and Y an inhibitor site. Alternatively, if the protein was an oligomer, X, Y, and Z sites can be identical sites with positive

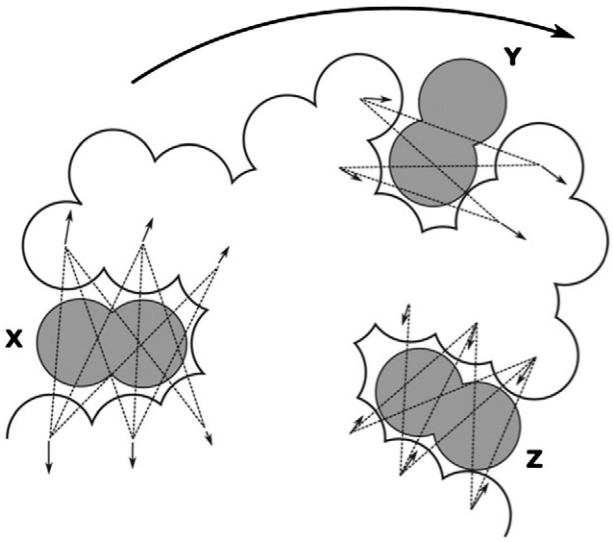

Fig. 2. Illustration of binding leverage for one degree of freedom. The outline represents the protein surface, and the gray dumbbell ligands. The curved arrow shows the general direction of motion in one degree of freedom and the small arrows the direction of motion for specific C_α atoms in the same degree of freedom. Dashed lines indicate pairs of atoms whose interconnecting line crosses the ligand.
Reproduced with permission from [22].

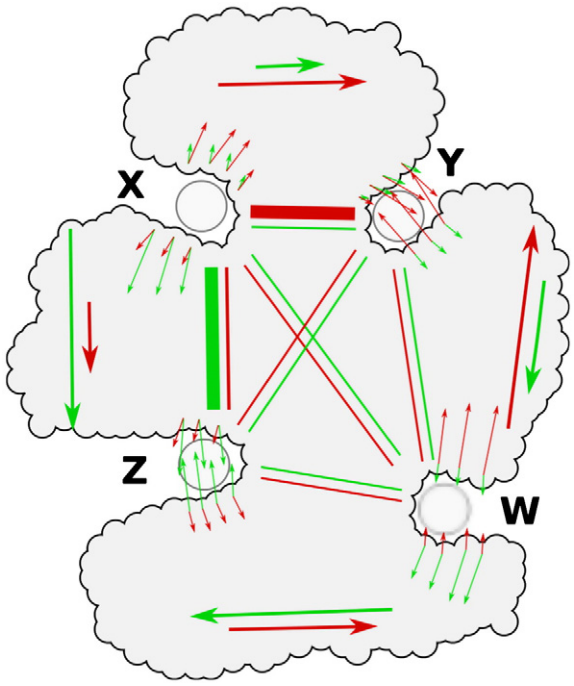

Fig. 3. Illustration of the concept of sites communicating through leverage coupling.
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or negative binding cooperativity. To quantify the strength of communication between two sites P and Q (each defined by a set of residues), we introduce the leverage coupling, D_{pq}, defined as a dot product of the binding leverages, λ_p and λ_q, of these sites:

$$D_{PQ} = \lambda_P \cdot \lambda_Q. \quad (4)$$

The vector $\lambda_p = (\lambda^*_{p1}, \ldots, \lambda^*_{pn})$ characterizes binding leverage of the site P. Vector elements $\lambda^*_{P\mu}$ are binding leverages of sites P caused by degrees of freedom μ:

$$\lambda^*_{P\mu} = \frac{\sum_{i \in P} \lambda_{i\mu}}{||P||}, \quad (5)$$

where the norm of P is the number of residues in the site. Fig. 3 illustrates that leverage couplings D_{XY} and D_{XZ} are large because of red and green degrees of freedom, respectively. Further, the matrix $C_{PQ} = D^2_{PQ}/D_{PP}D_{QQ}$ measures the normalized leverage coupling and has the range $0 \le C_{PQ} \le 1$. The normalized leverage coupling C_{PQ} is necessary for the analysis of big molecular machines like chaperones [21], where the conformational change at the binding sites is small compared to the large-scale functional motions. In this case the task is to compare the values between different sites and to find the most correlated pairs of sites for a given protein. The measure C_{PQ} is thus used (instead of D_{PQ}) to analyze how binding sites are correlated with different modes of functional motion. Overall, leverage coupling allows one to investigate allosteric communication in enzymes regulated by ligand binding and phosphorylation in proteins with different sizes and degree of oligomerization [21].

2.4. Contemporary approaches to the analysis of protein flexibility and dynamics, and future tasks

The role of the fluctuations and structural dynamics [39,40,45] in allosteric signaling [15,17,46] is currently analyzed by many different methods, such as MD simulations [15,28], normal mode analysis [8,13], and network approaches [18,47]. The most advanced recently developed methods are: Rosetta-based modeling [30] and simulations with structure-based dual basin Gô models [48,49]. Among the few remaining caveats of these methods is the absence of a quantifiable description of the coupling between the effector binding and protein dynamics. Another challenge is elucidating the mechanisms underlying allosteric communication between the effector and active site [30]. On the technical side, most of the above approaches require knowledge of both bound and unbound forms of the protein. The concept of binding leverage discussed above allows one to quantify the strength of connection between ligand binding and functional dynamics, even when only the structure of one functional state is known [22]. Leverage coupling detects communicating regulatory and functional sites and describes mechanism of communication by motion along coherent structural degrees of freedom [21]. Both measures can be calculated using only one structure, in a bound or unbound form. One of the remaining limitations in the binding leverage and leverage coupling measures is related to the usage of the normal modes-based energy functions. Indeed, normal modes correctly capture the thermodynamic nature of allosteric regulation [15–18]. However, as it is sharply stated by Levitt and colleagues in their seminal work on normal modes "the faults and virtues of the two methods are complementary in that molecular dynamics provide an approximate numerical solution to the exact equations of motion, whereas normal-mode dynamics provide an exact analytical solution to the approximate equations of motion" [9]. As a result, it was not possible to predict allosteric site in the catabolite activator protein (CAP, [22]). The plausible explanation for the case of the transcription factor CAP is that it is an example of "purely entropic" allostery without conformational change [50], which does not involve the collective motion that normal modes describe. Noteworthy, the local closeness, being a static geometry-based measure, correctly predicts CAP's binding site because it captures high residue interconnectivity in the deep surface pocket. Success of the prediction with local closeness hints to the role of local fluctuations. The latter are not directly connected to the collective motion, but they propagate through the protein and cause a global rigidification of the structure–conformational changes that cannot be correctly captured by normal modes. Coarse-grained simulations also do not provide a correct picture of allosteric regulation by metal binding, e.g. in thrombin [21], where a proper consideration of electrostatics appears to be necessary. Overall, development of a reliable energy function that discriminates between the different types of interactions, detailed consideration of global and local fluctuation, and improvement of conformational sampling are necessary future steps towards a complete description of allostery [51–56]. Elaborate energy functions, along with sufficient conformational sampling, are also necessary for the analysis of allosteric regulation in functional (super) families of structural homologs with different substrate specificity and in orthologs from different species [21]. Comparative analysis of homologs/orthologs is indispensable, because it can help in establishing the rules of fine tuning that provide specificity of allosteric regulation in individual proteins. This knowledge is crucial for exploring ways of design of allosteric drugs discussed below.

3. Towards design of allosteric drugs

Back in 1987, Paul Sigler with colleagues showed in the classic structural work on Trp repressor that binding of tryptophan can change the state of the transcriptor factor, making it unable to bind DNA [57]. The main advantage of allosteric drugs is in that they bind outside the catalytic site. As a result the allosteric effectors should not possess specific and conserved structures, contrary to the substrate ligands. Despite simplicity and non-specificity, allosteric effectors can provide highly specific regulation of the function via binding to the sites that communicate selectively with corresponding functional sites. Fig. 4a illustrates how effectors E1 and E2 can selectively regulate biochemical transformations of substrates S1 and S2, respectively. Fig. 4b shows an example where the allosteric effector E1 can activate/inhibit the ATP binding. At the same time, regulation of the ATP binding in this protein would not interfere with functions of other ATP-dependent proteins, where ATP binding is not regulated or regulated by other than allosteric mechanisms.

What important features should allosteric drugs possess? First, evolutionary selection presumably works against all ligand binding besides the binding of the specific substrates. In other words, most of the caves/cliffs on the protein surface, besides the catalytic ones, should be shielded from interaction with other small molecules present in the cell. What could be a generic and non-specific mechanism of protecting the protein surface? Water plays an important role in protein folding, stability, and function [58–60], and it is common knowledge that soluble proteins are surrounded by several layers of structured water [61–64]. One can imagine, therefore, that the shell of structured water is an efficient universal mechanism of shielding the protein surface from undesired interactions with potential ligands. In this case presence of polar group(s) that would provide penetration through the shell of the structured water will be required for allosteric drugs. Further, the strength of binding and non-specificity of interactions are also necessary. Therefore, ligands that contain groups interacting via big in number and non-specific interactions, such as van der Waals [65,66], could be the first candidates in the search for allosteric effectors.

To conclude, almost 50 years ago Monod and colleagues said [67]: "despite latent dangers …, the allosteric regulation is of such value that natural selection must have used it to the limit". Nowadays, we achieved a stage of a design of allosteric drugs [68–71], which is a rather unexplored area with a great potential for experimental and theoretical endeavors [72].

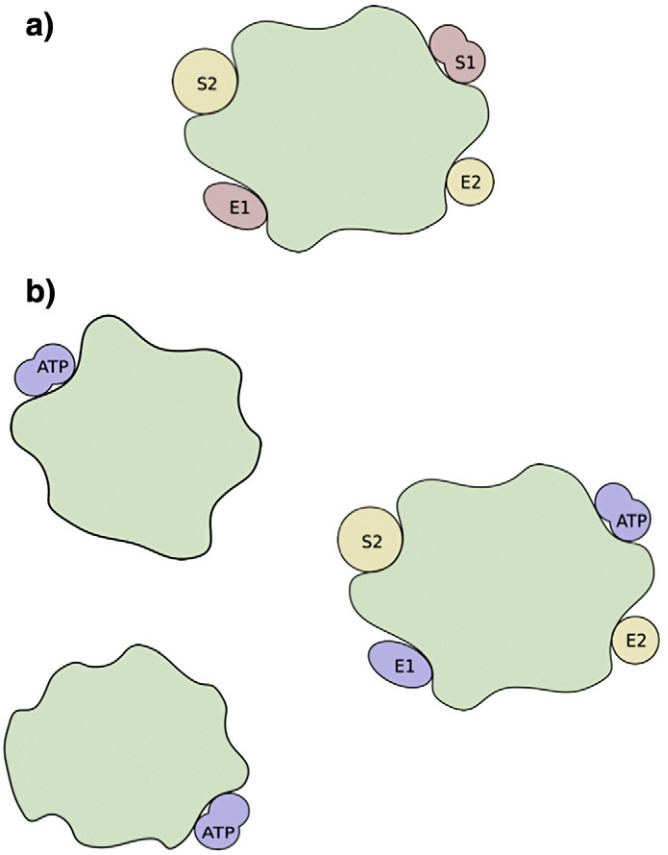

Fig. 4. Illustration of specificity of regulation provided by allosteric drugs. (a) Example of effectors E1 and E2 that selectively regulate biochemical transformations of substrates S1 and S2, respectively. (b) Example of specific activator/inhibitor of the ATP-binding site.

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