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REVIEW

Is Allostery an Intrinsic Property of *All* Dynamic Proteins?

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ABSTRACT **Allostery involves coupling of conformational changes between two widely separated binding sites. The common view holds that allosteric proteins are symmetric oligomers, with each subunit existing in "at least" two conformational states with a different affinity for ligands. Recent observations such as the allosteric behavior of myoglobin, a classical example of a nonallosteric protein, call into question the existing allosteric dogma. Here we argue that all (nonfibrous) proteins are potentially allosteric. Allostery is a consequence of re-distributions of protein conformational ensembles. In a nonallosteric protein, the binding site shape may not show a concerted second-site change and enzyme kinetics may not reflect an allosteric transition. Nevertheless, appropriate ligands, point mutations, or external conditions may facilitate a population shift, leading a presumably nonallosteric protein to behave allosterically. In principle, practically any potential drug binding to the protein surface can alter the conformational redistribution. The question is its effectiveness in the redistribution of the ensemble, affecting the protein binding sites and its function. Here, we review experimental observations validating this view of protein allostery. Proteins 2004;57:433–443.**

Key words: allostery; conformational ensembles; allosteric transition; drug discovery; population redistribution; energy landscape; function

INTRODUCTION

Allostery, or a "different shape," is the coupling of conformational changes between two widely separated sites. Allosteric proteins have two identical (homotropic) or different (heterotropic) ligands. The binding of one ligand increases (or decreases) the affinity of the protein toward the second (Fig. 1). The two binding sites may be on the same polypeptide chain though in different domains, or in different subunits. Allostery is crucial to living cells. It has long been shown to control metabolism either through positive feedback regulation or negative inhibition.[1–4] In 1965, Monod et al. analyzed 24 allosteric enzyme systems and proposed a "plausible model on the nature of allosteric transition" (the "concerted" or "MWC" model).[5] The proposition was inspired by the observation of two conformational states of deoxy- and oxyhemoglobin. The MWC model suggested that allosteric proteins are symmetric oligomers with identical protomers. Each protomer exists in "at least" two conformational states (tense, T; relaxed, R) with different affinities for ligands.[5] The basic assumption of the model is that the protein interconverts between two conformations, R and T, in a concerted manner. Subunits in the oligomers cannot exist in a hybrid form such as TR. Koshland et al. challenged the MWC model and proposed their sequential hypothesis (the "KNF" or "sequential" model).[6] In the sequential model, subunits change conformation, one at a time. Thus, a hybrid form such as TR can exist in the sequential model. Here, the binding of a ligand will change the conformation of a protomer without affecting the neighboring subunits. Eigen combined the MWC and KNF extreme models leading to a general model.[7] However, decades of research have lead to the conclusion that the exact mechanisms by which allostery is achieved may span a broad range, and can be extremely different from each other, although a few prototypes do exists.[8,9]

It has been broadly accepted that since the binding of a ligand to one site can affect the other through a propagated change in the protein shape, this strategy is used by nature to regulate protein activity.[10] It is remarkable how subtle and effective the communication between the two

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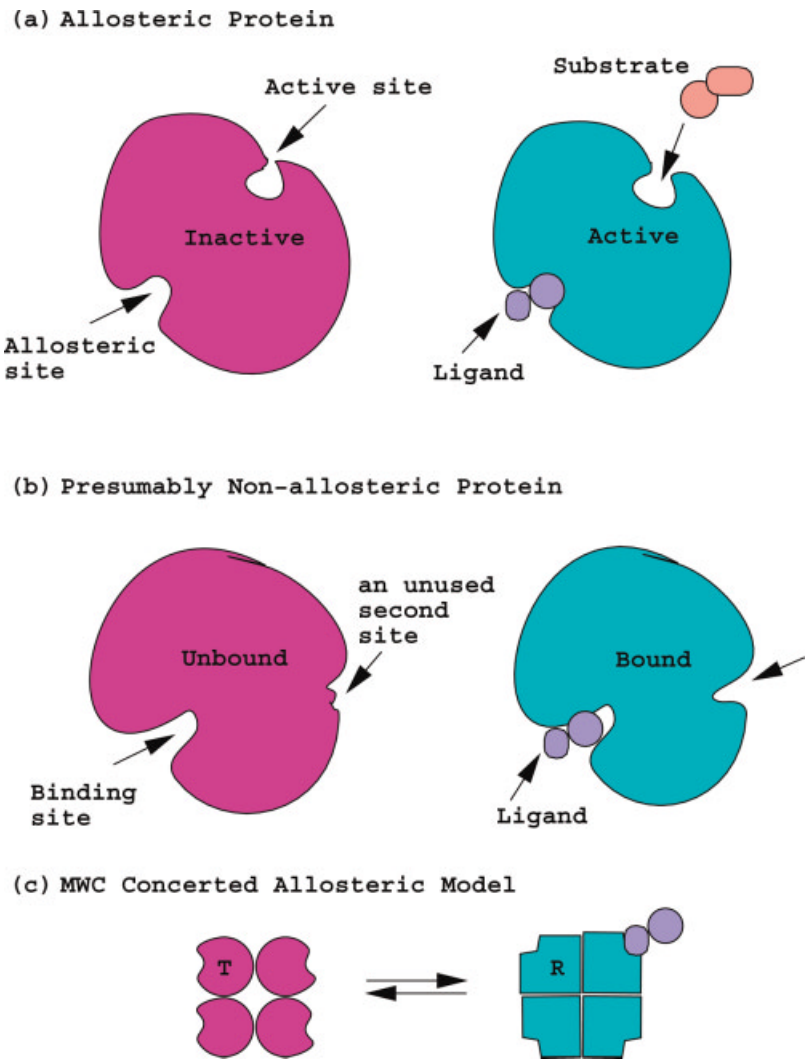

Fig. 1. In this figure, we present a unified concept of allostery encompassing known allosteric and presumably nonallosteric proteins. **a:** According to the classical model, an allosteric protein contains two or more topologically distinct binding sites that are interconnected functionally. Binding of a ligand at one site alters the properties of the other leading to a higher affinity for the second ligand or substrate. **b:** According to the proposition made in this work, a similar situation occurs in presumably nonallosteric proteins: The binding of a ligand or a point mutation may lead to a conformational change at a remote (second) site. However, this (second) site is not known to have been used by a ligand/effector before. Consequently, the change at that site was not considered to be similar to the so-called classical allosteric second-site-linked change. Nevertheless, if a suitable binder is present, it may now bind to the favorable site shape. Such a mechanism appears useful in drug design. The drug may bind at a site different from that of the natural ligand. The binding leads to a conformational change at the "native" binding site, which now is unfavorable for binding. **c:** A schematic representation of the concerted hypothesis of allostery, put forward by Monod et al. (MWC).[5] The model assumes that an allosteric enzyme consists of a number of subunits that can exist in two different conformations, active (R) and inactive (T). Further, the subunits within an enzyme must all have the same conformation.

binding sites can be. In the aspartate receptor, a mere 1.0Å conformational change 100Å away is sufficient to lead to an enormous amplification in the response.[10,11] The mechanism of such structural change propagation remains largely elusive.

Weber was the first to propose that the process of ligand binding merely shifts the population of the conformational states in the dynamic ensemble.[12] This has been substantiated by recent experiments that have revealed that conformational states in the pre-existing equilibrium can influence function.[13–15] Population shift or re-distribution of protein conformational states is a powerful concept for rationalizing binding mechanisms and allosteric regulation.[16–28] All protein structures obey the same physical principles. All proteins exist as a population of conformational states, with the probable exception of fibrous proteins. Hence, if indeed a population shift is the underlying mechanism of allosteric regulation,[26–28] are all proteins potentially allosteric? Recent extensive experimental studies of allosteric proteins led us to re-examine the nature of allostery in an attempt to

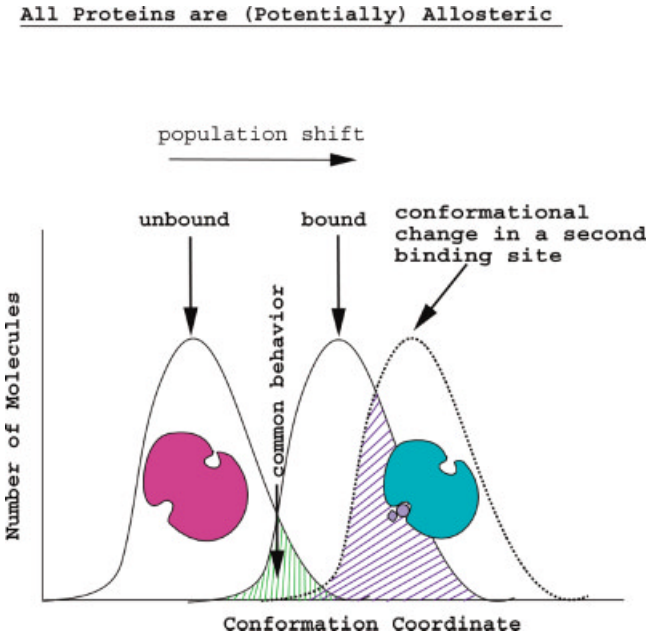

Fig. 2. The "new view" of protein allostery: all proteins are potentially allosteric when thought in terms of population redistribution. Ligand binding leads to a conformational redistribution potentially effecting a conformational change in a second binding site.

answer this question. Below, we review the classical allosteric model. We proceed to describe and generalize allostery in terms of population shift. In this regard, we point to the dependence of protein function on conformational flexibility. We conclude that *allostery is likely to be an intrinsic property of all proteins*. This concept is schematically illustrated in Figure 2. Finally, we highlight the broad implications to drug discovery.

Classical Allosteric and Non-Allosteric Proteins

Thirty-five years ago, when the MWC model was proposed,[5] there were 24 allosteric enzymes (a classical allosteric protein example is shown in Fig. 3). Although no such recent compilation of allosteric proteins exists, a literature search reveals hundreds of allosterically regulated proteins, which include important drug targets (Table I lists some examples). The database of macromolecular motions lists as many allosteric as nonallosteric examples (7%).[29] Recent observations of allostery in single domain proteins have extended the common view that allosteric regulation is always associated with multidomain proteins. Further, proteins assumed to be nonallosteric are increasingly observed to display allosteric transitions. Recent experiments suggest that allostery can be introduced into presumably nonallosteric proteins, either by interactions with a stronger binder, chemical modifications, or engineering a few point mutations.[30–33] Ikeda et al.[31] have shown that pyruvate kinase M1, a nonallosteric isozyme, can be converted into an allosteric enzyme by replacing a single amino acid at the subunit interface. Phosphofructokinase provides yet another example of a nonallosteric to allosteric conversion by site-directed mutagenesis.[34] Myoglobin was a traditional example of a nonallosteric protein. Yet, Frauenfelder et al.[35] have shown that the existence of substates with different catalytic properties enables controlling the reactions of myoglobin with diatomic molecules such as O_2 and H_2O_2. This example further indicates that allostery does not require multimeric proteins. That allostery can involve monomeric proteins was also observed in the human p38 MAP kinase, where a new allosteric binding site was reported.[36] The NMR study by Volkman et al. on NtrC provides another excellent example for allostery in a single-domain signaling protein.[13] Further, allostery is not conserved between species and allosteric and nonallosteric proteins have similar evolutionary origins.[37] For example, L-lactate dehydrogenase (LDH) from *Lactobacillus pentosus* is a nonallosteric enzyme that is highly homologous to allosteric LDHs from bacteria.[38] Hemoglobin, the classical model of allostery, is only a moderately cooperative oxygen carrier if

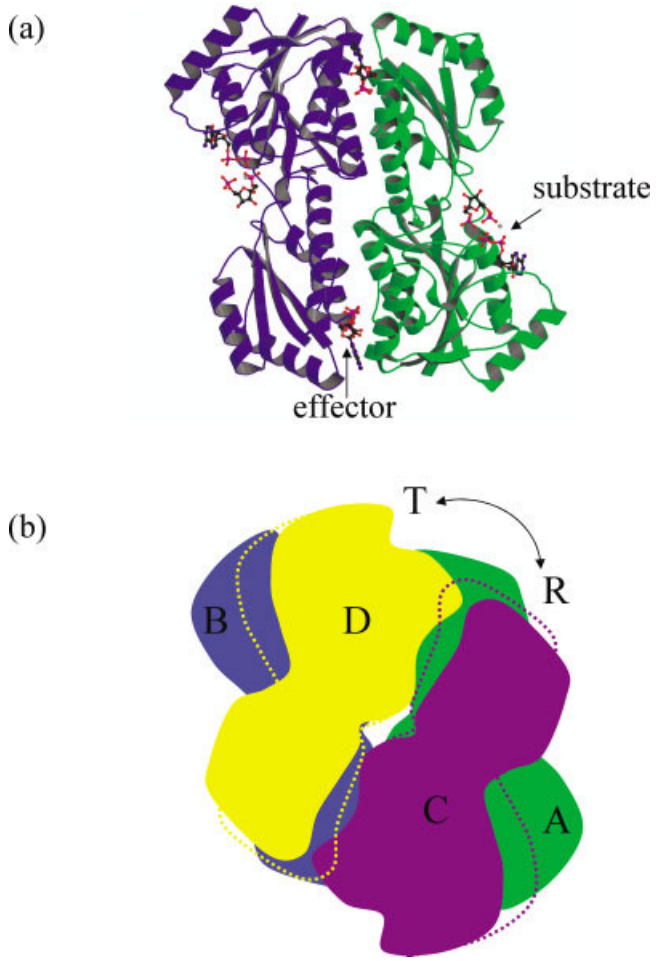

Fig. 3. **a:** Ribbon representation of crystal structure of one dimer of phosphofructokinase (PFK), an allosteric enzyme. The substrate and effector molecules are shown in ball-and-stick representation. The effector site of one monomer is linked to the substrate binding site of the other monomer through a loop. **b:** Schematic representation of functional form (tetramer) of PFK. The interface area between the two dimers, AB and CD, is much smaller compared to that between either A and B or C and D. The CD dimer undergoes about a 7° rotation with respect to AB dimer upon allosteric activation.

TABLE I. Examples of Allosteric Proteins That Include Important Drug Targets†

Proteins	Allosteric modulator(s)	Remarks	Refs.
P38 MAP kinase	BIRB 796	Blocking p38 MAP kinase is an effective strategy for the treatments of many inflammatory diseases	36
Muscle glycogen phosphorylase	Glucose, glucose 6-phosphate, ATP and purines	GP catalyzes glycogenesis, which is a target for type 2 diabetes	93, 94
Thrombin	Sodium (Na)	Thrombin plays a major role in hemostasis by regulating the procoagulant, anticoagulant, and fibrinolytic pathways	95
Lac repressor	Isopropyl-β-D-thiogalactoside (IPTG)	Involved in gene regulation (synthesis of lac mRNA) and helps to understand protein–DNA interactions	96
Aspartate transcarbmoylase	CTP, UTP, ATP	Involved in pyrimidine biosynthesis and catalyzes the carbamoylation of the amino group of aspartate	97
HIV-1 reverse transcriptase	Nevirapine, delavirdine, and efavirenz	Converts viral RNA to DNA, and is a key target for the development of anti-HIV drugs	98

†Examples of ligand-gated ion channels and G-protein-coupled receptors, which are not included here, are given in the review by Christopoulos.[16]

heterotropic effectors are absent but has a higher affinity and cooperativity for oxygen in their presence.[39] Nicotinic acetylcholine receptor is yet another example.[40] The two-state models cannot fully account for these recent experimental observations.

What then differentiates between allosteric and nonallosteric proteins? Or, are all proteins potentially allosteric and evolution made use of it to its own advantage to regulate and optimize cellular pathways? If so, can drugs be engineered to utilize this property to regulate protein activity? Some approaches to accommodate protein flexibility in computational drug design have already been outlined.[22,41] Below, we address these questions invoking the concept of population shift or re-distribution.

BINDING SITE FLEXIBILITY AND POPULATION SHIFT

The prevalent view now accepts that proteins are not rigid as it appears when looking at crystal or averaged NMR structures, using graphics workstations.[18,42] Hydrogen/Deuterium (H/D) exchange clearly indicates that native proteins exist as statistical ensembles[43–45] distinguished by locally unfolded region(s) in the binding sites or elsewhere. The pioneering work of Elber and Karplus demonstrated that the potential energy surface of myoglobin is characterized by a large number of thermally accessible minima around the native structure.[46] These observations suggest that the Gibbs energy of stabilization is not equally distributed in the structure. Since local unfolding occurs in the functional state, its significance is beyond protein folding per se.

Alber and his colleagues[47,48] showed the importance of flexibility in the activation of the allosteric aspartate transcarbamoylase. The structure of the active nonallosteric catalytic subunit resembles the low activity T state. However, remarkably, the difference is that the active subunit is flexible. Consistently, mutations that activate the holo-enzyme (in the absence of ligands) yield multiple conformations, with different structures. Kurbanov et al.[47] propose that the regulation involves a transition from the relatively rigid inactive state to a more flexible, active ensemble and that the increased flexibility facilitates the conformational transitions during enzyme turnover. Barbar et al.[49] have shown that upon dissociation of the dynein light chain from the LC8 dimer, there is an increase in flexibility at sites remote from the dimer interface. Their hydrogen exchange and heteronuclear NMR relaxation data indicate that residues in two surface helices rigid in the dimer are highly flexible in the monomer, probably playing a role in dynein assembly.

Analyzing interactions between biological molecules cannot be reduced to a static description of molecular structures. The binding partner should be considered, as well as the time component of the interaction.[50] Experiments have suggested that alterations that affect the relative populations may lead to varying protein functions.[51–55] Hence, in principle a potential allosteric regulation is already built into the protein.

There is experimental and theoretical support that binding at one site can effectively shift the population, showing conformational changes at some other sites. NMR conformers generated from NOE constraints can provide clues for backbone flexibility in solution. Often flexible regions do not yield strong NOEs. Figures 4 and 5 show a comparison of NMR conformers of the apo and holo structures of the biotinyl domain (E77 to E156) from acetyl co-enzyme A carboxylase[56] and trp repressor[57] (H16 to S102), respectively. Even regions far away from the ligand binding site show differences in the flexibility between the two states. Molecular dynamics simulations on proteins have illustrated the effect of distant mutations on the functional loops. For example, simulations on β1,4 galtactosyltransferase I (β4Gal-T1) reveal that mutating residues (in particular, glycines) in a small loop leads to a large effect on the long funtional loop[58] (Fig. 6). β4Gal-T1 undergoes a large conformational change upon binding UDP-gal.[59] The long functional loop (I345 to H365) moves by as much as 20Å between the apo and holo conformations.

Using NtrC, a single domain signaling protein, Volkman et al. showed that the inactive (unphosphorylated) protein samples the active state even in the absence of the ligand, or a covalent modification.[13] Phosphorylation does not induce a new structure; rather, it shifts a pre-existing equilibrium.[20,21] In the case of calmodulin, it was shown that the unbound form exists in a predominantly closed conformation, with a smaller population of more open conformations,[14] thus providing yet another example for the pre-existing equilibrium view. The spirit of the population shift theory relies on the static and the dynamic: one being the existence of key functional conformations (static) and the other is the interchanges of the populations. The latter appears crucial: it focuses on the integration of the entire protein energy landscape and the dynamic interconversion of the functional conformations. It is the change in the dynamic interconversion of the functional conformations that relates to allosteric regulation.

ALLOSTERY IN LIGHT OF MOLECULAR ENSEMBLES AND POPULATION RE-DISTRIBUTION

There is increasing realization that proteins should be treated as a dynamic ensemble of conformational states. Ligand binding re-distributes the molecular ensemble leading to altered conformations at some other locations.[20,21,26–28] In the classical allosteric protein view, the two binding sites are targeted by the inducer (or inhibitor) and the substrate. To begin with, there are only relatively few conformers in which the substrate binding site "fits" the substrate. These conformers bind the substrate even in the absence of the inducer. Since there is a considerably larger population in which the inducer binding site "fits" the inducer compared to the substrate case, the inducer will bind altering the substrate binding site shape. Thus, the population is now re-distributed, leading to a larger proportion that has a binding site fitting the substrate.

In the case of nonallosteric proteins, the first scenario holds, similar to the allosteric proteins in the absence of an inducer: conformers whose binding sites fit the substrate will bind to it. Redistribution of the remaining populations propagates binding. Thus, if we were able to induce such a population shift, we could create allosteric proteins from nonallosteric proteins. Since shifts are a function of conditions,[20,21,60] it may take place in the test tube or in vivo. Similar to oligomers, a monomer is also a dynamic protein ensemble. In principle, a monomer too can show a nonallosteric to allosteric transition. Therefore, allosteric transitions should not be restricted to multimers.

EXAMPLES OF ALLOSTERY

Hemoglobin

Hemoglobin is a classical example of an allosteric protein. This oxygen carrier consists of four heme-containing subunits, paired as two dimers. It exists in the deoxy (T) and oxy (R, high affinity to oxygen) states. The binding of oxygen is cooperative: Binding at one site changes the conformation of the distal site leading to a higher affinity to the second oxygen molecule. Hemoglobin has traditionally been viewed as fitting well into the MWC model, but recent experiments question the adequacy of the MWC model in explaining the hemoglobin behavior. Yonetani et al.[39] produced oxygen-binding curves under different conditions, and found that the oxygen affinity and the cooperativity effect of hemoglobin are modulated principally by tertiary structural changes induced by its interaction with allosteric effectors (such as DPG, GZF, and IHP). In their absence, hemoglobin is only a moderately cooperative oxygen carrier with a limited functional diversity. The authors proposed a "global" allosteric model in which the difference in the free energies between the T and R states becomes zero under appropriate conditions. This example raises the possibility that both (T and R) states are equally accessible. In contrast, the MWC model prefers one state over the other at any given time.

Cooperative and Competitive Allosteric Regulation

Allostery may involve cooperative or competitive binding. Hexokinase, a classical example of cooperative allostery,[61] catalyzes the transfer of a phosphate group from ATP to glucose, to yield glucose-6-phosphate. The term "cooperativity" is used to describe binding data that do not have hyperbolic dependence upon substrate concentration. On the other hand, in negative linkage, there is competitive binding: The two ligands bind to different protein conformations. The binding of the end product of a pathway lowers the protein affinity to its substrate. Aspartate transcarbamoylase, which catalyzes the reaction carbamoylphosphate + aspartate → N-carbamoylaspartate, has been a particularly well studied example.[62–64] One of the final products, CTP, functions as an inhibitor in over-production. In both cases, the principle is the same. In the first cooperative case, the population shifts toward a substrate binding-favorable state. In the second competitive inhibition case, the shift is toward a substrate-unfavorable state. Either way, binding leads to a change in the energy landscape.

Large Oligomers

Aspartate transcarbamoylase (ATC) and GroEL provide examples of large allosteric oligomers. ATC consists of six catalytic and six regulatory subunits, with the catalytic subunits being arranged in two trimers, each as an equilateral triangle, joined by three regulatory dimers. The movement is a concerted allosteric transition between the active and inactive states. The GroEL chaperonin is a large multi-subunit assembly mediating ATP-dependent protein folding. It consists of two stacked rings, each with 7 radially-arranged subunits, with three domains in each.[65,66] ATP binds cooperatively to a ring promoting subsequent binding of GroES.[67,68] The intermediate domain swings down toward the equatorial domain and the central channel. The apical domain swivels up and rotates. The movement couples the binding of the GroES to that of ATP. Molecular dynamics simulations of a single subunit suggest that the ATP binding-induced early perturbation triggers the larger domain movements.[54] It was long proposed that in a symmetric arrangement the binding of

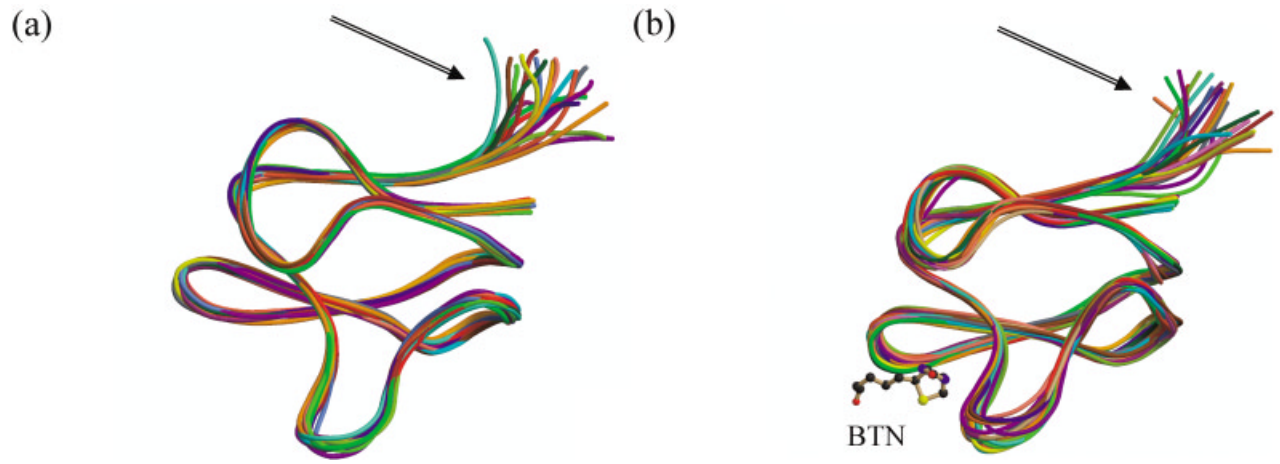

Figure 4.

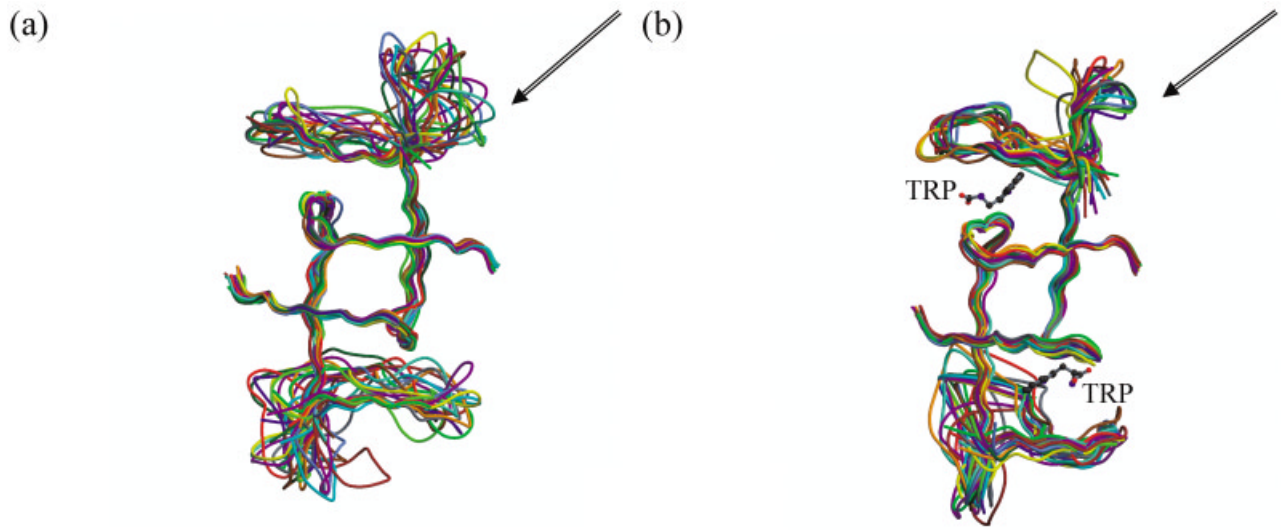

Figure 5.

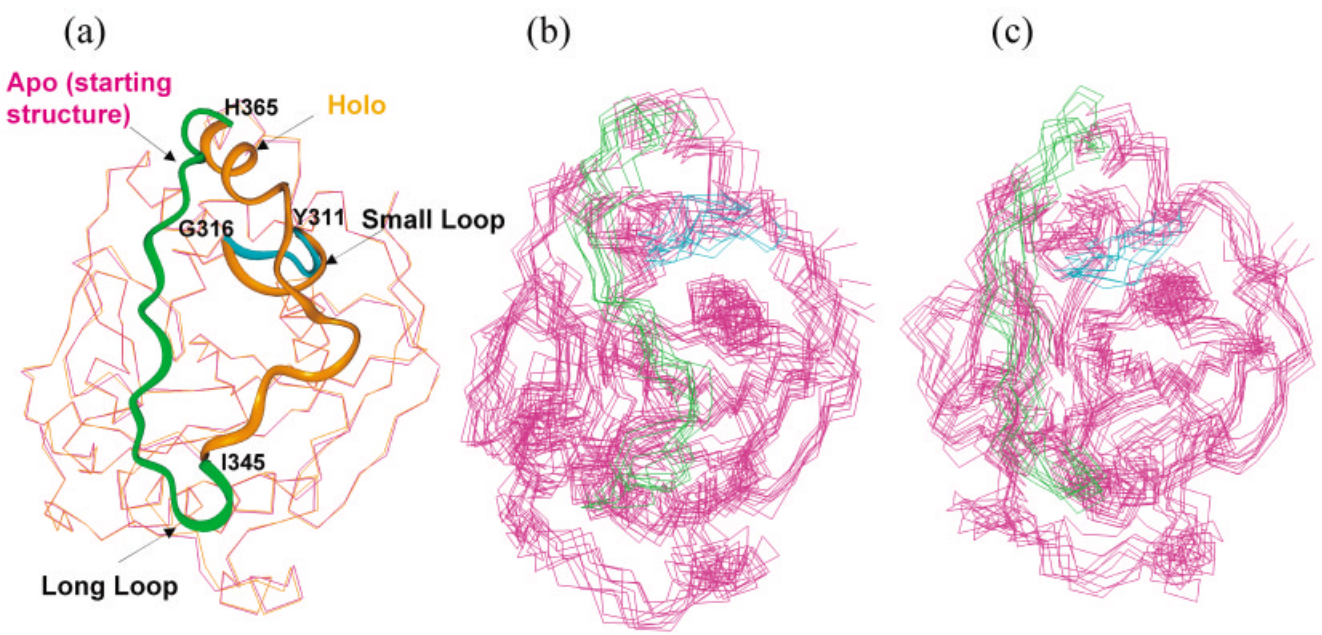

Figure 6.

one ligand molecule to one subunit can trigger a conformational change transmitted to neighboring subunits. Symmetrical assemblies are favorable, since they undergo a cooperative allosteric transition, leading to a cellular switch in response to an even small change in the ligand concentration.[1,69–71] Indeed, most allosterically-regulated enzymes consist of symmetrical assemblies of identical subunits.

GENERALIZED ALLOSTERY CONCEPT AND DRUG DESIGN

If under appropriate conditions all proteins are allosteric, then it might be possible to design drugs to regulate any proteins of interest. In the case of cell surface receptors, allosteric ligands interact with binding sites that are spatially distinct from the classical agonist site and modulate receptor activity through conformational changes. Extensive studies on G-protein-coupled receptors (GPCRs) and ligand-gated ion channels (LGICs) have indeed shown that allostery can be very useful in drug discovery.[16,72–74] Christopoulos[16] notes that there are at least three advantages to using allosteric modulators for cell-surface receptors: (1) they are saturable and therefore there is a ceiling to the effects of a drug; (2) allosteric ligands have the ability to selectively tune responses in specific tissues; and (3) allosteric drugs have the potential for greater receptor subtype selectivity. These attributes may be expected to similarly apply to allosteric modulators in general.

A nice example for the last two advantages can be seen in the P38 MAP kinase, selectively blocking this enzyme. Table I provides a few additional examples of allosteric proteins where selectivity could potentially play an important role in the treatment of various diseases. Several diseases of autoimmunity, such as rheumatoid arthritis, diabetes, and inflammatory bowel disease, have been associated with higher levels of proinflammatory cytokines. p38 MAP kinase plays an important role in the signal transduction cascade leading to the production of proinflammatory cytokines. Therefore, inhibiting the p38 MAP kinase is an effective strategy for the treatments of many inflammatory diseases.[36] Pargellis and co-workers determined the crystal structure of human p38 MAP kinase in complex with a diaryl urea class of inhibitor.[36] The structure revealed a new allosteric binding pocket that is distinct from the ATP binding site (see Fig. 7). While the diaryl urea compounds inhibit p38 MAP kinase by altering the ATP binding site conformation, most other kinase inhibitors use the ATP-binding pocket and compete with ATP binding. Based on the diaryl urea compounds, the authors have further attempted to chemically synthesize a compound that would bind the protein better. The new compound, termed BIRP 796, has a 12,000-fold increase in the binding affinity. The binding of these compounds creates a large conformational change in the conserved Asp-Phe-Gly motif. This motif assumes a conformation with the Phe residue buried in a hydrophobic pocket in all the known Ser/Thr kinase structures. However, in the diaryl urea compounds bound conformation, the Phe

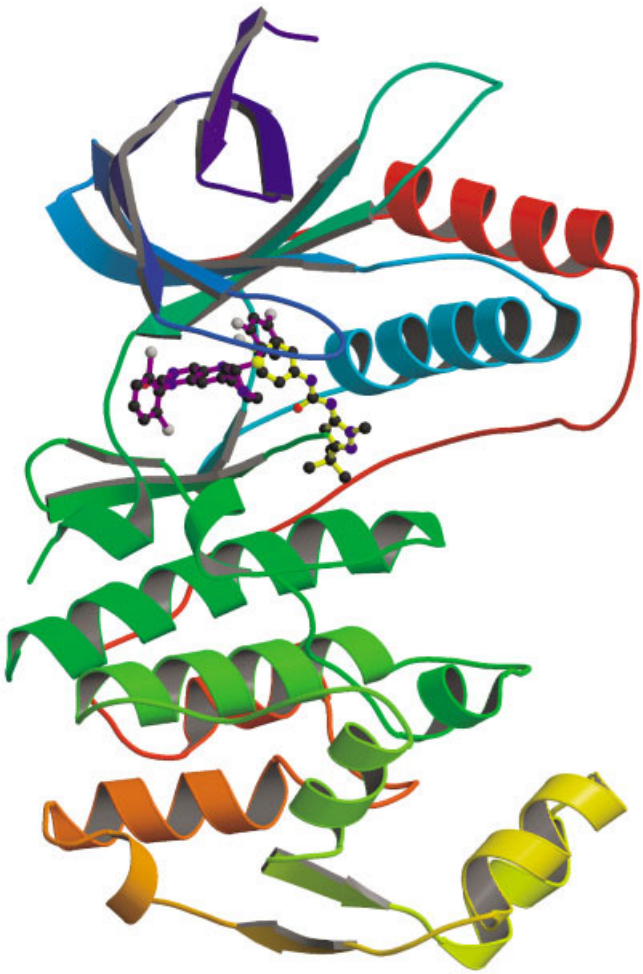

Fig. 7. Ribbon representation of p38 MAP kinase. This enzyme provides an example where structural studies and structure-based ligand design could lead to the identification of novel allosteric binding site. Compound 1 of diaryl urea class of inhibitors (shown with bonds colored in yellow) binds a region that is distinct from the ATP-binding site where most other P38 MAP kinase inhibitors bind (such an inhibitor is shown with bonds colored in purple).[36] The structure was drawn using the coordinates deposited as 1KV1 and 1OUY in PDB.

Fig. 4. Superposition of 23 NMR conformers of (**a**) apo- and (**b**) holo-biotinyl domain (Glu77-Glu156) from acetyl coenzyme A carboxylase of *E. coli* (PDB codes: 3BDO and 2BDO, respectively). Although the N-terminus is far away from the ligand (BTN, biotin) binding site (indicated by arrow), there is a difference in flexibility in solution. Only C^{α} traces are shown for clarity.

Fig. 5. Superposition of 15 NMR conformers of (**a**) apo- and (b) holo- trp repressor, as deposited in the PDB (1WRT and 1WRS, respectively; H16-S107). The regions that show a difference in flexibility away from the ligand (TRP, tryptophan) binding site are indicated by arrow. Only C^{α} positions are shown for clarity.

Fig. 6. **a:** Superposition of apo and holo crystal structures of β1,4 galtactosyltransferase I. The small and long loops are shown in a ribbon model, while the rest of the structure is shown in C^{α} trace. The conformers obtained between 2.5 and 3.0 nsec during explicit water simulations starting with apo conformation are also shown: (**b**) wild type sequence, (**c**) residues in the small loop (Y311-G316) are mutated to alanine, which affects the movement of the long loop (I345-H365). This example illustrates mutations away from the functional loop that could affect loop flexibility and movement.

side chain moves by about 10Å to a DFG-out conformation. The authors propose that the conformational variability of the DFG motif may be a general phenomenon that can be utilized in the design process.[36]

How to make use of "hidden" allosteric sites to design new drugs for the classical nonallosteric proteins? Nature has devised fascinating ways from which we can possibly learn how to take advantage of allostery. Analyses of collections of mutations of several enzymes have shown that regardless of the locations of the mutations, the changes are largely expressed at the same sites.[23] Rose et al.[75] have shown why HIV protease mutations in drug-resistant strains are often spatially removed from the binding sites: These mutations lead to rigid body rotation of five domains, changing the binding site size and epitope. Further, mutations at the interdomain interfaces that favor the unliganded form increase the off-rate of the inhibitor. This allows the substrate greater access suggesting a potential mechanism of resistance to competitive inhibitors. Interestingly, it was further found[76] that in the HIV-1 protease, "compensatory mutations" that occur far away from the binding sites can increase the protease activity, which is decreased by active site mutations. Phosphofructokinase (PFK) provides an interesting example of a nonallosteric to allosteric conversion by site-directed mutagenesis. PKF from *Dictyostelium discoideum* (DdPFK) differs from other eukaryotic PFKs in that it has nonallosteric kinetics. Santamaria and co-workers found that deletions at the C-tail region of DdPFK convert it to an allosteric enzyme.[34]

Computational drug design can follow nature's ingenuity in designing allosteric drugs. The key issue is *how* to find drugs that bind to the allosteric sites. There are two problems here: (1) to find the allosteric binding site and (2) to design a ligand that binds the site. These problems may well be interrelated; a potential allosteric binding site may depend on the ligands. As described below, several existing approaches may be adapted towards allosteric drug design.

Analysis Based on Static Protein Structure and Sequences

Protein crystal and NMR structures provide information about structural dynamics. Luque and Freire,[77] Freire,[78] and Pan et al.[28] have performed a structure-based thermodynamic stability analysis using the COREX algorithm, which detects smaller scale motions.[79] COREX has discovered that binding sites have a dual character,[77] characterized by the presence of regions of low and high stability. Larger (hinge bending) motions can be investigated by normal mode analysis or molecular dynamics simulations. Large motions predicted from the Gaussian Network Model (GNM) analysis can be correlated with protein function. It has been nicely shown that inhibitor binding alters the directions of domain motions in HIV-1 reverse transcriptase.[80] In the case of the HIV protease, the GNM indicated that cooperative fluctuations also change between the unliganded and the substrate-bound HIV-1 protease.[81] These types of structure-based analyses may be useful in identifying potential allosteric binding sites and the effects of the ligand binding on the active sites.

Lockless and Ranganathan[82] and Suel et al.[83] devised a sequence-based method to quantitatively map global networks of amino acid interactions in a protein. Their analysis of three structurally and functionally distinct allosteric protein families reveals a subset of residues that form physically connected networks that link distant functional sites in the tertiary and quarternary structure. These residues are evolutionarily conserved (or co-evolve) in the respective families. This finding suggests that the existence of networks of interactions in nonallosteric proteins may provide clues to possible sites where, should a compound bind, the primary ligand binding site conformation may be altered. This suggestion is supported by studies on other proteins. For example, the aspartate receptor study has led Yu and Koshland[10] to argue that the conformational change and kinetics must relate to evolution. They proposed that evolution selects for an initial conformation that attracts a ligand or that can rapidly convert to a conformation that attracts a ligand. They speculate that the original conformational change was not the one we see now in the aspartate receptor, but was improved by evolution to optimize propagation, thermodynamics, and kinetics.

Dynamic Analysis Based on Molecular Dynamics Simulations

Molecular dynamics simulations of target proteins have not yet been applied to drug design directly. Protein fragments may move cooperatively, suggesting that the conformational distribution is not random. The time scale of such movements may range from the fast (nsec), to medium (10 to 100 nsec), to the slow. Ligand binding will change the motion and the conformational distribution. This approach is computationally expensive. However, direct observations from simulations can provide insight into molecular mechanisms for drug design.

Piana et al.[84,85] have carried out simulations to probe drug resistance by compensatory mutations. Their findings have established that in HIV-1 drug-resistant variants, compensatory mutations that are usually located far from the binding site can affect the enzymatic activity through molecular mechanisms related to differences in the conformational flexibility. In the β4Gal-T1, analysis of the covariance of the spatial displacement of residues reveals that loops correlated in their motions have highly conserved residues involved in the loop-loop interactions.[86,87] Recently, a novel application of time-resolved X-ray crysallography on myoglobin revealed that correlated side-chain motions play a role in protein function.[88] Thus, analysis of correlated motions between side chains and loops can provide clues to possible allosteric sites in classically nonallosteric proteins.

CONCLUSIONS: ALLOSTERY, SIGNALING AND POPULATIONS

Structural perturbation at any site leads to a redistribution of the population. Thus, evolution has no need to

invent a change of shape for allostery; it can take advantage of it. One source of structural perturbation is the binding of inhibitors (or inducers). Other sources include mutations, binding to sister molecules, changes in pH, ionic strength, temperature and covalent modification such as phosphorylation and acetylation. Redistributed conformations are not a manifestation unique to allostery. Rather, they are physical attributes of proteins.

Allostery derives from populations. Thus, there is no well-defined path, nor a distinct series of steps molecules follow. Rather than every single molecule undergoing a series of steps to reach the conformational change observed in the snapshot of a shape of a site that is far away, *what we observe is the outcome of the ensemble*. The perturbations at one site do not yield a homogeneous distribution. Since some portions of the molecule are less stable than others, these parts will manifest larger variability. When thought of in these terms, allosteric activation should not produce an alternate rigid binding site shape, which fits the ligand (substrate). Rather, the perturbation upon inducer (or inhibitor) binding leads to a redistribution of the ensemble, which would be largely reflected in a priori less stable binding sites. Nevertheless, we should remember that the "active" conformer is also present in the presumably "inactive" ensemble, albeit at a lower concentration. Upon binding, there is an equilibrium shift in its direction, further driving the binding reaction.

Hence, to conclude, depending on their function, proteins may be dynamic or stiff and fibrous. Here we argue that all dynamic proteins (simply called proteins above) are potentially allosteric. Allostery derives from a redistribution of the conformational ensemble. Even though the behavior of enzymes (or other proteins) may show nonallosteric kinetics, they are likely to be allosteric for proper ligands or if modified by a few mutations. Ligands or mutations facilitate the shift of populations.

The effect of drugs on protein conformations has been strikingly illustrated by Fersht and his colleagues.[89–92] Drugs such as CDB3 rescue the conformation of unstable mutants of p53. They maintain the mutant in its folded state and allow it sufficient time to bind its sequence-specific target DNA or the p53 binding proteins that will stabilize it.[91] An effective allosteric drug will lead to a considerable change in the active site size and chemical properties, effectively altering its specificity. While currently there are some allosteric drugs, these have been designed to known allosteric sites in proteins known to be allosteric. For these proteins, the designed drugs have been shown to be highly effective, providing several advantages over traditional drugs, such as greater selectivity and saturability of their effect.[16] Applying this approach to *all* proteins is considerably more difficult to realize, since the allosteric sites are unknown. Nevertheless, such a route may vastly broaden the horizon of potential drugs and drug discovery.

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