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Minireview

# The structural basis of allosteric regulation in proteins

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ABSTRACT

**Allosteric regulation of protein function occurs when the regulatory trigger, such as the binding of a small-molecule effector or inhibitor, takes place some distance from the protein's, or protein complex's, active site. This distance can be a few Å, or tens of Å. Many proteins are regulated in this way and exhibit a variety of allosteric mechanisms. Here we review how analyses of experimentally determined models of protein 3D structures, using either X-ray crystallography or NMR spectroscopy, have revealed some of the mechanisms involved.**
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## 1. Introduction

Regulation of protein function is crucial to all organisms. Virtually all processes in the cell need to be carefully controlled and, if these controls fail, can lead to malfunction and disease. The control can be exerted at various different points in a protein's lifetime: from regulation of gene expression, through translation into protein, via control over its level of activity, to its final degradation [1]. Allosteric regulation is just one of many controls on activity, but a particularly interesting one. It entails a regulatory trigger, such as the binding of a small molecule to a protein or protein complex, which takes place at a site some distance from the active site. In some cases the two sites can be tens of Å apart, so it becomes interesting to ask how the signal is transmitted over such a distance.

Furthermore, allosteric triggers can increase as well as decrease the protein's activity. This differs from simple competitive inhibition wherein a small-molecule inhibitor, or indeed another protein molecule, binds to the active site and prevents the protein's natural substrate gaining access. It also differs in that, whereas a competitive inhibitor may need to be chemically similar to the substrate in order to compete against it, an allosteric regulator does not.

Much of the knowledge of allosteric mechanisms has come from analyses of protein 3D structures. There are over 100 cases of allosterically regulated proteins for which a structural model of either one, or both, of the active or inactive forms have been experimentally determined, using either X-ray crystallography or NMR spectroscopy. Comparison of the structures can suggest how the allosteric mechanism operates. Here we review some of the triggers and mechanisms that have been revealed by such structural studies.

## 2. Structural studies

In the 1960s, two alternative models were proposed for describing how allostery operates. Both apply to oligomeric protein assemblies of identical protein molecules, or subunits, and assume that each subunit is in either a relaxed state (R) or tense state (T). The R state is more receptive to ligand binding than the T state. In the Monod, Wyman and Changeux (MWC) model [2], also known as the concerted or symmetry model, the subunits in each protein assembly must either all be in the R state or all in the T state; that is, a conformational change in one subunit causes an equivalent change in all others. The ratio of the two types of assemblies is determined by thermal equilibrium. However, binding of a ligand to either state increases the equilibrium in favor of the R state, thus

*Abbreviations:* BoNT/A, botulinum neurotoxin type A; CAK, CDK-activating kinase; CDK, cyclin-dependent kinase; DAHP, 3-deoxy-D-*arbino*-heptulosonate-7-phosphate; DAHPS, DAHP synthase; DBD, DNA-binding domain; DHDPS, dihydrodipicolinate synthase; DHFR, dihydrofolate reductase; E4P, D-erythrose-4-phosphate; FlAsH, fluorescin arsenical hairpin binder; hPTP1E, human tyrosine phosphatase 1E; KNF, Koshland, Nemethy and Filmer model; MWC, Monod, Wyman and Changeux model; NBD, nucleotide binding domain; PDB, Protein Data Bank; PEP, phosphoenolpyruvate; PGDH, phosphoglycerate dehydrogenase; PTS, phosphotransferase system; RBD, regulatory binding domain; SBD, substrate binding domain

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making the ensemble more receptive to further ligand binding. This model seems to describe the mechanism that operates in many proteins including hemoglobin [3], membrane acceptors [4], and some enzymes [5]. In the second model, the Koshland, Nemethy and Filmer (KNF), or sequential, model [6], the subunits of each assembly do not all have to be in the same state. Binding of a ligand to one subunit occurs by induced fit, altering its conformation from the T to the R state. This change affects the structures of adjacent subunits, making them more receptive to ligand binding, without necessarily converting them to the R state as in MWC model.

Both of these models describe allosteric regulation of oligomeric assemblies. In this review we focus on examples where the allosteric and active sites are distinct sites on the same protein molecule. For the most part the examples come from 3D structural studies. Table 1 summarizes some of the different allosteric triggers that these studies have revealed and some of the mechanisms that have been proposed to describe how they operate. In the examples below, the structures are identified by their protein data bank (PDB) identifier which is a four-character code.

It should be noted that a 3D structure does not always provide the full story. There are cases where there is no, or very little, structural difference between the active and inactive forms of the protein. In these cases, the allosteric mechanism cannot be explained purely in terms of conformational change, and we discuss such cases towards the end of this review.

## 3. Allosteric regulation by small-molecule binding

The most common form of allosteric regulation is by the binding of small-molecule effectors or inhibitors. This is found in the negative feedback loops of many biosynthetic pathways where one of the products of the pathway inhibits further production of the product by closing down an enzyme involved in one of early steps of the pathway. Alternatively, a pathway can be activated by the presence of a specific molecule which switches on one of its crucial enzymes. The examples below are categorized by their allosteric mechanism; i.e. the effect that the binding of the allosteric molecule has on the protein's active site. The methods are schematically illustrated in Fig. 1.

### 3.1. Opening/closing of active site

The first examples are those where the conformational change in the protein is such that it opens up, or closes, the active site, either enabling it to carry out its function or shutting off its activity (Fig. 1a and d, respectively). The change can be quite dramatic, such as a hinge motion at the boundary of two domains, or more subtle, such as the rotation of a single side chain to control the entrance to the functional binding pocket.

**Table 1**
Triggers and mechanisms of allosteric regulation.

| Trigger | Mechanism | Effect |
|---|---|---|
| • Small-molecule binding<br>• Protein–protein binding<br>• Phosphorylation<br>• Modification of disulfide bonds | • Open/close active site<br>• Change active site conformation<br>• Change active site electrostatic properties<br>• Affect protein–protein complex formation<br>• Change protein flexibility<br>• "Population shift" in ensemble of conformers | Increase/decrease in protein's activity |

An example involving a hinge motion, albeit a subtle one, is provided by phosphoglycerate dehydrogenase (PGDH). This is a $NAD^+$-dependent enzyme that catalyses the first step in the biosynthesis of L-serine. It is allosterically inhibited by the binding of the serine end product. The protein consists of three domains: a regulatory binding domain (RBD), which is where the allosteric inhibitor binds, plus binding domains for substrate (SBD) and the NAD nucleotide (NBD). The protein's function is to oxidize 3-phosphoglycerate into 3-phosphohydropyruvate. Comparison of the *apo*-structure (PDB code 1psd) with the *holo*-structure, containing a bound serine in the allosteric site (1yba), showed that the serine binding causes a rigid-body hinge rotation of the RBD-SBD domains relative to the NBD domain through about 15°. As a result, in the complete tetrameric assembly, this rotation causes a closing off of the active site cleft and a change to the conformations of the catalytic residues. Together, these changes effectively shut down the enzyme [7].

The RBD belongs to a common fold called the ACT domain which is found in a number of other allosterically controlled proteins. An aspect that is common to most of these proteins is that they are involved in amino acid biosynthesis and the allosteric effector molecules are the end-product amino acids [8]. It is very likely that the ACT domain is an ancient one as most of these proteins are found in Bacteria and Archaea.

### 3.2. Changes to conformation of active site

Less dramatic than the opening and closing of an active site are minor modifications to its conformation which are nevertheless sufficient to affect the protein's ability to bind, or properly interact with, its substrates (Fig. 1b and e). An example here is provided by DAHP synthase which catalyzes the first step in the biosynthesis of the aromatic amino acids in microorganisms and plants. The catalytic reaction involves condensation of phosphoenolpyruvate (PEP) and D-erythrose-4-phosphate (E4P) to form 3-deoxy-D-*arbino*-heptulosonate-7-phosphate (DAHP). In *Escherichia coli,* DAHP synthase (DAHPS) exists in three different isomers, each inhibited by one of the three aromatic amino acid end products: Phe, Tyr or Trp. The 3D structures of the *apo*- and *holo*-forms of the Phe-regulated isozyme, DAHPS(Phe) are known: PDB entry 1kfl has the Phe bound, while PDB entries 1qr7 and 1gg1 have no Phe. Comparison of these structures showed that the binding of the inhibitor leaves most of the structure unchanged apart from four, short adjacent segments. The coordinated movement of these segments transmits the allosteric signal to the protein's active site which is deformed and dramatically reduces the interaction of DAHPS with both its substrates [9].

### 3.3. Changes to electrostatic properties of active site

In some cases the allosteric event seems to make very little change to the conformation of the protein's active site, or indeed, to the structure as a whole (more on this later). However, what little change does take place may be sufficient to alter the active site's electrostatic properties and hence have a significant impact on the protein's function. One example where this may be the case, is given by chorismate mutase (PDB codes 1csm and 2csm). This protein only exists in bacteria, fungi and plants and is involved in the biosynthesis of the amino acids tyrosine and phenylalanine. It catalyzes the conversion of dicarboxylic chorismate to prephenate, which is subsequently converted to one of these two amino acids. An alternative pathway for the dicarboxylic chorismate reactant leads to the synthesis of tryptophan. So, the chorismate mutase enzyme provides a branching point in the biosynthesis of all three aromatic amino acids and is controlled by the allosteric

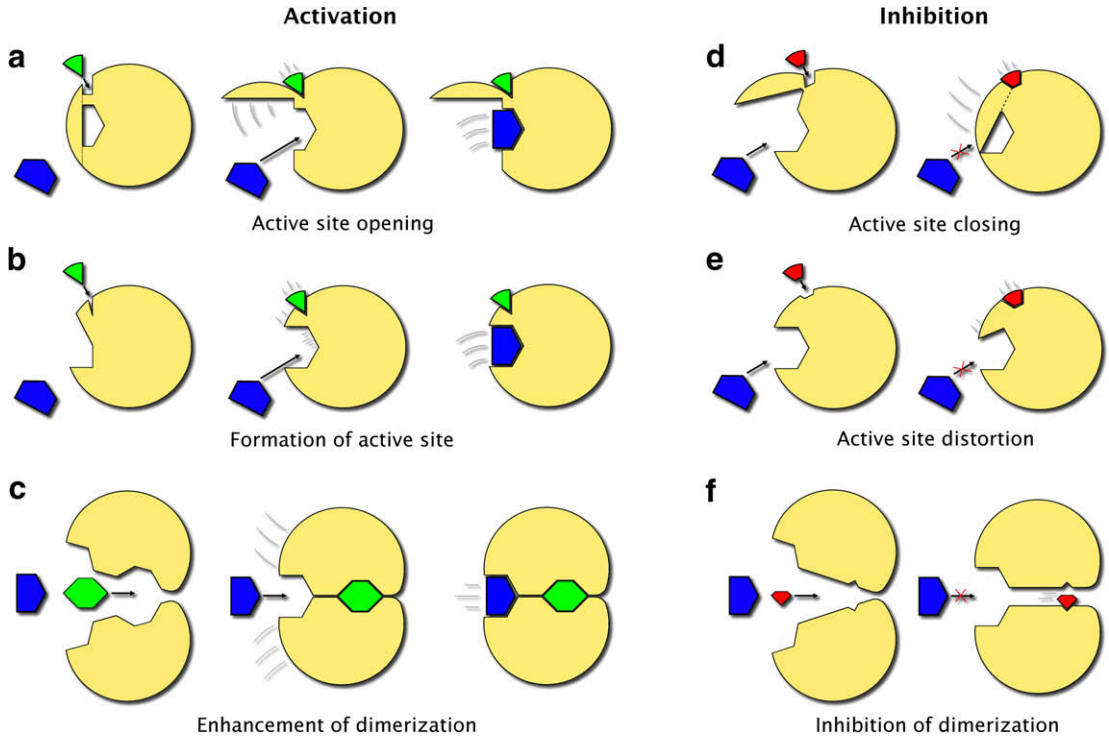


**Fig. 1.** Schematic diagram showing some of the allosteric mechanisms by which the binding of a ligand can alter the conformation of a distant active site. In all diagrams, the protein is colored cream, the substrate molecule is blue, and allosteric regulators are green for effectors and red for inhibitors. (a–c) Show activation triggers: (a) opening of the active site, (b) conformational change within the active site, and (c) formation of the active site by the dimerization of two protein molecules. (d–f) Show inhibitory effects: (d) closing of the active site, (e) conformational distortion of the active site, and (f) prevention of the dimerization necessary to create an active site.

binding of whichever end product is in abundance: activated by tryptophan, and inhibited by tyrosine.

The binding of either of these regulator molecules alters the relative orientation of the catalytic and allosteric domains; binding of the bulkier Trp pushes the domains further apart and affects the quaternary structure of the dimer. However, the effect on the conformation of the catalytic site is minimal. The only significant effect is on the conformation of the Glu23 side chain. In the active state, this residue is buried in the active site pocket but, on binding of the allosteric inhibitor and consequent structural changes, it swings into the binding site. The authors suggest that the appearance of this Glu in the active site causes a major change to its electrostatic properties which is sufficient to repel the substrate with its negative potential [10].

### 3.4. Allosteric control of complex formation

There are many cases where binding of a small-molecule ligand either enables or prevents the formation of a biologically functional multimer. The binding is allosteric as it may be far from the functional site and affects proteins that can only perform their function when in the correct multimeric assemblage (Fig. 1c and f).

An interesting example is ATP phosphoribosyltransferase. This catalyses the first step of the histidine biosynthetic pathway. Each protein chain consists of three domains: domains I and II contain the catalytic site in the cleft between them, while domain III is the regulatory domain. The functional quaternary structure is a dimer (PDB code 1nh7). The enzyme is allosterically inhibited by the end-product histidine. When histidine binds it causes a large twist of domain III relative to domains I and II which promotes the dimers to combine in groups of three, thus making hexameric assemblies of the protein. This grouping renders the protein inactive as the active sites become closed on formation of these complexes [11]. A similar mechanism operates in ribonuclease reductase [12].

More interesting still, is the case of AraC. Here the allosteric regulator switches the protein from one mode of operation to another by completely changing the way the protein dimerizes and dramatically affecting what it does (Fig. 2). AraC is a transcription regulator that, in *E. coli*, controls genes involved in the uptake and catabolism of the sugar arabinose. It has two domains: an arabinose-binding domain and a DNA-binding domain. So, when it dimerizes, the dimer has two arabinose- and two DNA-binding domains. The latter each bind to a different DNA site. With arabinose bound (PDB code 2arc), AraC dimerizes such that the two DNA sites to which the protein attaches itself are distantly separated (Fig. 2a and c), creating a 210-base pair loop in the DNA and repressing transcription of the promoters $p_{BAD}$ (the promoter for the arabinose operon) and $p_C$. If the effector molecule is absent (PDB code 2ara), the protein dimerizes along a completely different dimerization interface (Fig. 2b) and places the two DNA-binding domains close together. Now the dimer attaches itself to two adjacent DNA sites and promotes $p_{BAD}$ transcription instead of inhibiting it (Fig. 2d) [13].

## 4. Allosteric regulation by protein binding

Proteins can also act as allosteric effectors or inhibitors for other proteins. For example, the function of cyclin-dependent kinase 2 (CDK2) is controlled, as its name suggests, by the binding of the protein cyclin. CDK enzymes act as checkpoints in the eukaryotic cell cycle and function by activating downstream targets by phosphorylation. Two steps are required to switch these enzymes on. The first is the binding of the cyclin and the second is phosphorylation by a CDK-activating kinase (CAK). Structural studies of CDK2 in complex with cyclin A (PDB code 1fin) have revealed how the binding of the cyclin switches CDK2 on. Firstly, it displaces CDK2's activation segment and makes its substrate binding site accessible for binding ATP [14]. Secondly, it exposes

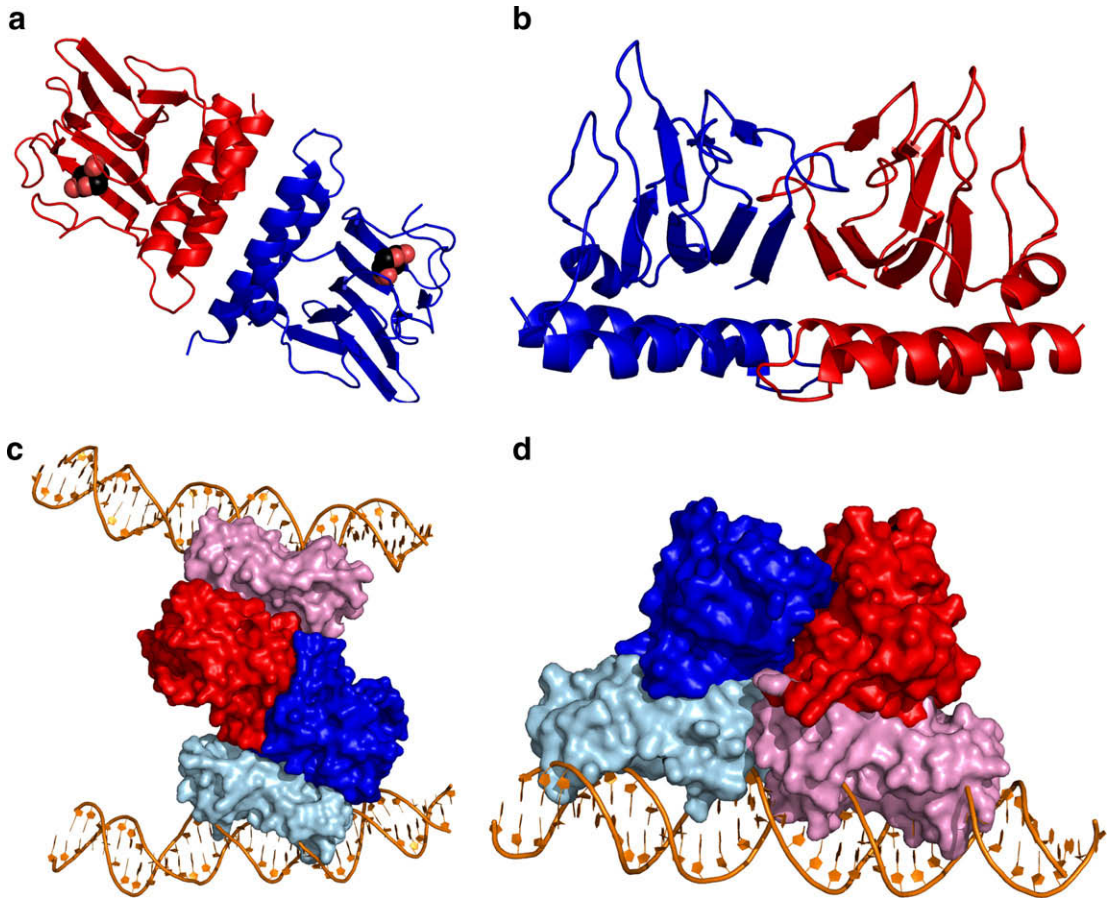


**Fig. 2.** Allosteric regulation by change of dimerization, as exemplified by the AraC protein from *Escherichia coli*. (a) Secondary structure representation of the dimer formed by the protein's arabinose-binding domains when arabinose (shown in spacefill representation) is bound (PDB code 2arc). The interface is formed by the α-helices. (b) When arabinose is absent, the domains bind along a completely different dimerization interface (PDB code 2ara), with the two molecules joining to form a beta barrel structure. (c) A schematic representation of the dimer in a) (red and blue surfaces) plus the DNA-binding domains (pink and light blue surfaces, respectively) modeled in at their expected locations at the C-terminus of each arabinose-binding domain. As a consequence of this mode of dimerization, the DNA-binding domains (DBDs) occur on either side of the dimer and so bind to very distantly separated sites on the DNA (shown in orange). (d) When, in the absence of arabinose, the arabinose-binding domains dimerize as in (b), the DBDs are placed side by side and attach to neighboring sites on the DNA. In (c) transcription of the regulated gene is repressed, and in (d) it is promoted.

the phosphorylation site (Thr160) which, when phosphorylated by CAK, forms the CDK2's peptide binding site and thus activates the enzyme [15].

A contrary example, of a protein acting as an allosteric inhibitor, is phosphocarrier protein $III^{Glc}$. This protein belongs to the bacterial phosphotransferase system (PTS) which acts as a sugar transport pathway. When there are ample PTS sugars available, $III^{Glc}$ becomes unphosphorylated and then inhibits enzymes involved in uptake and metabolism of non-PTS carbon sources. When PTS sugars become scarce, $III^{Glc}$ becomes phosphorylated and abandons its inhibitory function. One of the proteins it operates on is glycerol kinase (GK). This enzyme catalyses the reaction of glycerol and ATP to give glycerol-3-phosphate and ADP. Analysis of the GK/$III^{Glc}$ complex (PDB codes 1gla and 1glb) revealed that the phosphorylation site of $III^{Glc}$, His90, is buried in the interface between the two proteins. This suggested that, phosphorylation of His90 might prevent formation of the GK/$III^{Glc}$ complex, leaving the GK free to get on with its function [16].

## 5. Allosteric regulation by phosphorylation

Perhaps the most subtle allosteric trigger is the phosphorylation of a residue far from an active site. A good example is the CheY protein, a bacterial chemotaxis response regulator which transmits chemical stimuli to the bacterial flagella and is the end-point of a signal transduction cascade. CheY is activated by histidine kinase CheA which phosphorylates its Asp57 residue. When phosphorylated, CheY binds to the flagellar FliM protein, causing the flagella to turn clockwise and the bacteria to swim in a tumbling manner. Unphosphorylated CheY does not readily bind to FliM, causing the flagella to switch back to their default anti-clockwise rotation, resulting in smooth swimming. The residue responsible for triggering clockwise turning is Tyr106, which lies over 9.5 Å from the phosphorylated Asp57. In early structural studies of CheY, the allosteric mechanism was thought to operate via the highly conserved Thr87 residue which lies directly between Asp57 and Tyr106. This mechanism was named the "Y-T" coupling scheme [17] (PDB code 1djm). Subsequent structures seemed to point instead to a significant conformational change in the β4-α4 loop (Ala88 to Lys91) burying the Tyr106 in the active site (PDB codes 3chy and 1jbe) [18,19]. Molecular dynamics simulations of CheY tended to support the latter mechanism, with the role of the conserved Thr87 being thought to stabilize the active conformation of the β4-α4 loop [20].

A more dramatic consequence of single-residue phosphorylation is exemplified by mammalian glycogen phosphorylase in which the phosphorylation of Ser14, lying some 45 Å away from the catalytic site, causes a large-scale rotation through 10° of the two subunits of the dimer, thus opening up the catalytic site for business [21].

## 6. Allosteric disulfide bonds

The formation or breaking of disulfide bonds, distant from the protein's active site, can trigger a conformational change which affects the protein's active site [22]. Hence such disulfide bonds can be termed allosteric.

A fine example is that of botulinum neurotoxin type A (BoNT/A), shown in Fig. 3. This protein is synthesised in *Clostridium botulinum* as a single long chain consisting of a catalytic and a translocation domain. After translation, the two domains are proteolyzed and split into two separate chains. However, the domains remain connected by a single disulfide bond which keeps the translocation domain in place, blocking the active site of the catalytic domain and keeping it inactive (PDB code 3bta). When this disulfide is reduced, either in the acidic endosome or within the cytosol, the domains separate and the now-active catalytic domain is released into the cytoplasm [23].

A different use of regulatory disulfides is made by gelsolin, a protein involved in actin filament assembly and disassembly. It binds to actin, severing and capping the actin filaments before nucleating a new filament by binding actin monomers. One factor affecting its activity is the binding of $Ca^{2+}$ to its C-terminal domain. This activates the protein's functional N-terminal half. Another factor is a disulfide bond in the protein's second domain [24]. If the bond is absent, the rate at which $Ca^{2+}$ activates gelsolin is reduced (PDB code 1kcq). If the bond is present, $Ca^{2+}$-mediated activity is increased. The presence or absence of the bond is determined by the redox potential of the local environment; the bond tends to be present when the protein is in plasma, but not when it is in the cytoplasm [25].

A recent analysis of several proteins known to contain allosteric disulfides showed that the disulfides all had one thing in common: they tended to fall into the same conformational class, the –RHStaple bond [26]. This class has a high potential energy, meaning that the bonds are more easily cleaved than other types. This observation provides the possibility of predicting which disulfides might be allosteric.

## 7. Allosteric regulation by changes in protein flexibility

In some cases, structural studies have not been able to identify how the allosteric mechanism operates because there is no, or very little, conformational difference between the structures of the active and inactive forms. One such example is dihydrodipicolinate synthase (DHDPS) which catalyses the condensation of aspartate-β-semialdehyde and pyruvate to give 4-hydroxy-2,3,4,5-tetrahydro-2-dipicolinic acid. It is the first reaction in the biosynthesis of lysine in bacteria and plants. The activity of the enzyme is allosterically inhibited by lysine, the end product of the pathway. Structural studies of DHDPS from *E. coli* were unable to reveal how this inhibition operates. Neither tertiary nor the homo-tetrameric quaternary structures of the protein differed very much between the *apo*- and *holo*-conformations. The first study (PDB codes 1yxc and 1yxd) [27] suggested a decreased flexibility for an arginine side chain involved in substrate binding whereas the second study (PDB code 1dhp) found the opposite effect [28].

One explanation that has been advanced for cases such as this is that the allosteric effect is not always mediated by a conformational change. Or at least, not by one that can be detected by standard X-ray and NMR techniques which give a time-averaged snapshot of the 3D structure. In some cases, the allosteric signal may be relayed by an alteration to the dynamic behavior of the protein. After all, proteins are not static, solid entities but rather dynamic things, constantly vibrating and constantly in motion. Their low-frequency modes of vibration are determined by their shape and mass distribution so, any perturbation, such as the binding of an allosteric ligand, may affect distant sites via a change in the spectrum of vibrational frequencies [29–31].

One case where this effect has been experimentally tested is in PDZ domains. These are among the commonest of protein domains

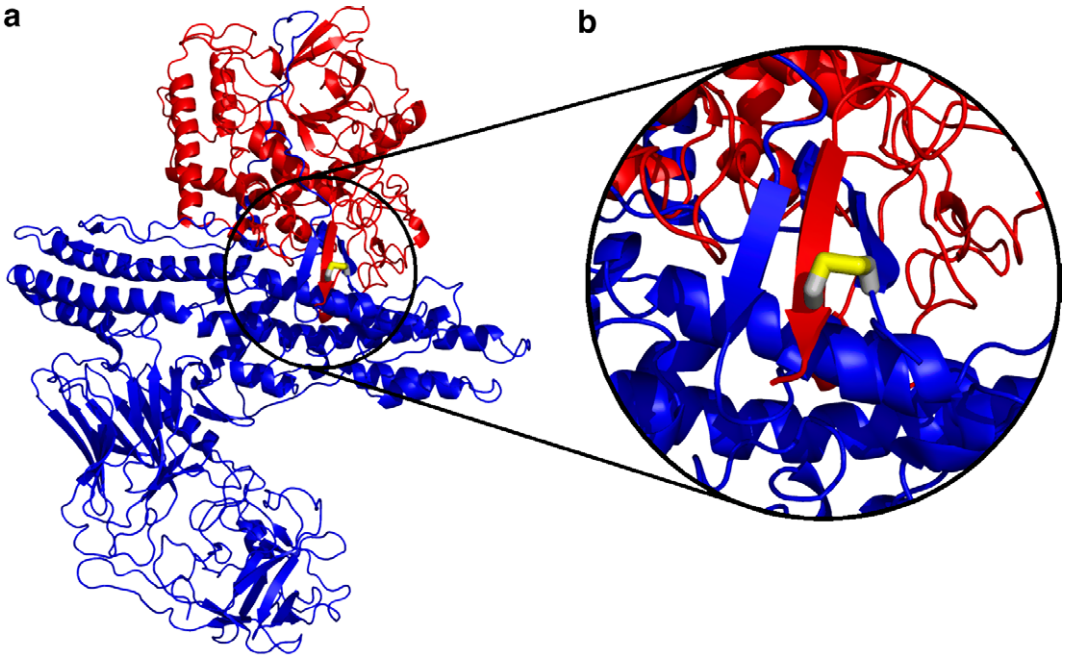


**Fig. 3.** An example of an allosteric disulfide bond. The diagram shows the secondary structure representation of botulinum neurotoxin type A (BoNT/A) from *Clostridium botulinum* (PDB code 3bta). The full-length chain of the protein is cleaved by proteolysis after translation, but the two parts (shown here in red and blue) are held together by a single disulfide bond (yellow). The active site of the catalytic domain (shown in red) is blocked by the other chain (blue) until the disulfide is reduced in the endosome or cytosol and the catalytic domain is released into the cytoplasm. (a) View of the whole structure and (b) a blow-up of the disulfide bond keeping the two chains tethered together.

and are found in many proteins. They act as protein–protein binding domains that recognize the C-terminal 4–6 residues of their target protein and are involved in protein targeting and protein complex assembly [32]. The signal, indicating successful recognition of the target, appears to travel a long distance through these domains via networks of physically linked "hot spot" residues, which can be identified by statistical analysis of sequence families [33]. How this signal is propagated was experimentally investigated using side chain ($^{2}$H-methyl) and backbone ($^{15}$N) NMR spin relaxation methods to probe the dynamics of the second PDZ domain from human tyrosine phosphatase 1E (hPTP1E) [34]. By comparing the differences in dynamics between the *apo*-form and a *holo*-form (containing a C-terminal peptide from the cognate-binding protein) significant differences were detected in the domain's ps–ns dynamics, thus providing a possible mechanism for propagating long-range signals within the protein.

A second example is provided by use of NMR and isothermal titration calorimetry to study the role of dynamics in the allosteric binding of cAMP to the catabolite activator protein (CAP) [35]. The authors were able to 'freeze' binding conformations at intermediate stages of cAMP binding and so show that, in this case at least, the allosteric effect could be explained purely by changes in protein dynamics.

## 8. Allosteric regulation by population shift

Another mechanism that has been posited both for explaining the difficult cases where there is no obvious conformational change, and as a general explanation of allosteric control, is that of "population shift". This has sometimes been termed the "new" view of protein allostery.

Proteins, because of their flexibility and internal motions and vibrations, exist in a complex statistical ensemble of conformers; many even contain intrinsically disordered domains [36] which are often associated with regulatory proteins [37]. Allosteric mechanisms may operate by causing a shift of the protein to a different ensemble of conformations and this change may not be observable in an experimentally determined 3D structure [38–40]. A similar explanation has been put forward for enzyme catalysis [41–43]. The CheY example described above, illustrating allosteric control by phosphorylation, has been used as a test case for molecular dynamics simulations to see whether the mechanism can be better explained by traditional ideas of conformational change, or more recent ideas of change in molecular populations [20]. The authors probed the protein's structural, energetic and dynamic properties as a function of its phosphorylation state and found that neither the "new" nor the "old" explanations of the allosteric mechanism were complete; a full explanation requires consideration of both the sequential local structural changes and the concerted collective motions of the ensemble.

## 9. Structural surveys

There are many allosteric proteins whose 3D structure has been determined and allosteric mechanism elucidated. Surprisingly, there have been few systematic analyses of all these many individual cases and no proper database where the details of the mechanisms are recorded. Extracting the information from the literature makes this an arduous task. Perhaps the first broad study was that performed by Daily and Gray [44]. They analyzed a data set of 51 pairs of known active and inactive allosteric protein structures in the PDB, including enzymes, signalling proteins and DNA-binding proteins. They compared each pair of structures to identify any local conformational differences between them. They found that the conformations of about 20% of the residues exhibited substantial changes, with most of the motion in weakly constrained regions of the structure, such as loops and surface residues. Allosteric binding resulted in motions in residues over distances that averaged 10–20 Å. Now, although the authors found that non-allosteric proteins also exhibited conformational adjustment on ligand binding, this was, on average, about half that of the allosteric proteins.

In a second study, the same group looked at the "coupled networks" of contacting residues and their rearrangement in the structures of 15 pairs of active and inactive allosteric proteins [45]. They were able to show that in 5 of these, the allosteric signal is communicated from the allosteric to the active site via changes in the network of residue-residue contacts between the two sites. In the remaining 10, the contact networks did not span the two sites, suggesting some other mechanism, such as large-scale rigid motions, was at play instead of, or in addition to, the contact networks.

Using the Daily and Gray data set of 51 structure pairs, the Nussinov group classified the allosteric mechanisms into three types according to the magnitude of the conformational change exhibited. The authors suggested the allosteric mechanisms in each types differed in the relative importance of entropic and enthalpic effects [46]. The classification can be found at http://protein3d.ncifcrf.gov/tsai/allostery which provides a useful resource for finding examples of allosteric effects of varying magnitudes.

## 10. Summary

Allosteric regulation of protein function is a fascinating and wide-ranging means of controlling protein function. It encompasses a variety of triggers and an assortment of mechanisms for relaying the signal from the allosteric to the active site. The signal may be one that activates the protein or inhibits it. Triggers include: binding of small molecules, binding of proteins, phosphorylation and modification of disulfide bonds. The mechanisms that these triggers activate can involve the opening or closing of the active site, modification of the conformation of the residues in the active site, changes to the rigidity or electrostatic properties of the active site, alteration of the dynamic properties of the protein as a whole or via a population shift in the protein's conformational ensemble. Indeed, allosteric effects may involve a combination of these mechanisms.

Understanding allosteric mechanisms is important in several fields. In drug discovery it may provide an alternative means of targeting a protein by small-molecule drugs. The allosteric binding site may provide a better target than the active site as, being under less evolutionary pressure, may be more species-specific [47,48]. One case where this has already been tried is in the structure-based design of a novel set of p38 map kinase inhibitors that target the allosteric site [49,50]. Moreover, protein–protein interactions are notoriously difficult to target with drugs; so, if the interaction is under allosteric control, targeting the allosteric site might prove a better bet.

Another area where knowledge of allostery could be useful is in protein design. Zhang and Bishop [51] describe how they managed to engineer a novel allosteric site into tyrosine phosphatase (PTP) using scanning insertional mutagenesis. The new site was regulated by a fluorescin arsenical hairpin binder (FlAsH) and, in one case, managed to reduce the protein's activity 12-fold. From examination of the X-ray structure, the authors deduced the most likely mechanism by which the inhibition was occurring was the prevention of closure of the PTP's activation loop.

Finally, the presence of an allosteric site in a newly solved protein structure might affect the determination of that protein's function. It is difficult enough to predict what ligand might bind in a binding site, so the presence of a second binding site must surely

complicate matters. Perhaps, as we learn more about allosteric systems it may become possible to develop algorithms to distinguish allosteric from active sites, and to design novel triggers to inhibit or activate proteins as required.

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