TIMELINE

50 years of allosteric interactions: the twists and turns of the models

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Abstract | The concept of indirect or 'allosteric' interaction between topographically distinct sites, and the subsequent 1965 Monod-Wyman-Changeux (MWC) model for the conformational change mediating them, arose around 50 years ago. Many classic regulatory proteins (including haemoglobin, Asp transcarbamylase and nicotinic acetylcholine receptor) follow the central paradigm of the MWC model, which has been expanded and challenged as a result of novel technologies. Importantly, the concept of allosteric interaction has aided our understanding of human diseases and drug design.

Allosteric interactions between proteins and their regulatory ligands were initially defined, and are still defined today, as indirect interactions that are established between topographically distinct sites and mediated by a discrete reversible alteration in the molecular structure of the protein. The concept of allosteric interaction was first proposed in 1961 to account for the feedback inhibition mediating the activity of particular enzymes in metabolic pathways in which the inhibitor is not a steric analogue of the substrate[1–4]. This concept was expanded in 1963 (REF. 5) to explain the properties of regulatory proteins in general, including haemoglobin. In 1965, the attention focused on the observation that the substrates and regulatory ligands often interact with more than one binding site at the same time, and thus that the regulatory proteins function as 'molecular switches'. This led to the idea that ligand–protein interactions selectively stabilize a global cooperative conformational change of the protein quaternary structure, which is the foundation of the Monod–Wyman–Changeux (MWC) model[6].

In this Timeline article, I briefly discuss the origin of the concept of allosteric interaction, which arose 50 years ago, the early evidence supporting it and how the definition of this concept has developed over time (FIG. 1 (TIMELINE)). Then, I describe some of the key breakthroughs that have occurred in the field over the past 50 years and present some of the still debated issues. I end with a summary of recent progress and thoughts on the key outstanding questions that need to be addressed at the atomic level.

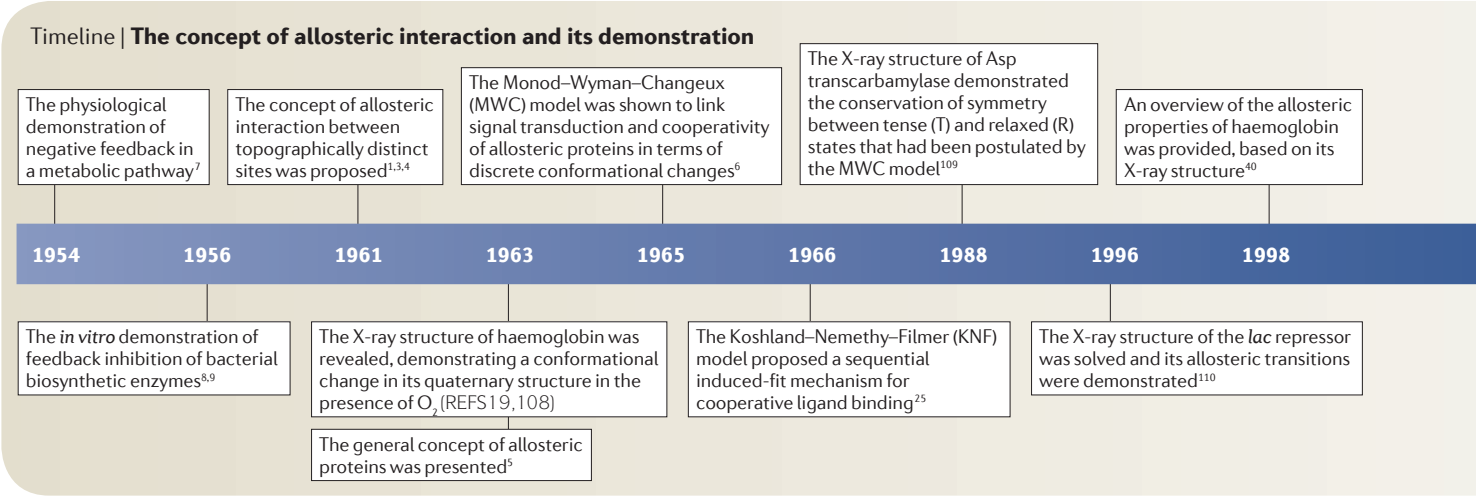
Models of allosteric interactions

In this section, the origin and development of the concept of allosteric interaction are presented, together with the evidence supporting it and the debate it initiated.

Feedback inhibition in metabolic pathways. In 1954, Novick and Szilard[7] noticed that the synthesis of indole-3-glycerol phosphate, the precursor of the amino acid Trp, was rapidly inhibited by the addition of Trp to *Escherichia coli* growing in chemostats. Thus, they proposed that an enzyme early in the pathway was inhibited by negative feedback from the end product of this pathway. In 1956, using cell-free extracts, Umbarger discovered that L-Thr deaminase, the first enzyme in the L-Ile biosynthetic pathway, was inhibited by L-Ile[8]. Troublesomely, he also noted that his measurements of substrate production, and of the inhibitor binding to the substrate, did not follow the expected Michaelis–Menten hyperbolic kinetics but produced sigmoidal curves. In parallel, Yates and Pardee observed that the first enzyme of the pyrimidine biosynthetic pathway, Asp transcarbamylase (ATCase), was inhibited by feedback from the pyrimidine CTP[9]. A new class of enzymes, referred to as 'feedback inhibited enzymes', emerged. The discovery of these enzymes was the 'springboard' for the delineation and generalization of the concept of allosteric interaction.

Defining allosteric sites and interactions. Following the discovery of feedback inhibited enzymes, the question became: which mechanism accounts for the apparent 'competitive' inhibition between a substrate and its 'feedback inhibitor'? Observations made independently and simultaneously by two graduate students, myself using L-Thr deaminase in the laboratory of Monod[1,4,10,11] and Gerhart using ATCase in the laboratory of Pardee[2,3,12], provided novel insight. Both noticed that the sensitivity of the enzyme to the feedback inhibitor decreased progressively with time. Heat treatment of L-Thr deaminase eliminated its sensitivity to L-Ile without significantly decreasing its enzymatic activity[1]. Treatment of ATCase with a sulphhydryl group reagent decreased its sensitivity to CTP, although the enzyme retained full catalytic activity[2,3]. In both cases, Michaelis–Menten hyperbolic kinetics replaced sigmoidal substrate saturation kinetics of the enzyme that were seen in the absence of treatment. The term 'desensitization' was coined to refer to this phenomenon, which was soon confirmed as a general property of regulatory enzymes.

To account for the data on L-Thr deaminase, I proposed two models[1] (FIG. 2a). In the first, I put forward that the protein-binding sites for the substrate and regulatory inhibitor are 'partially overlapping', so that the interaction is a type of classic competition by steric hindrance. In the second model, I suggested that the two sites do not overlap and that the substrate and regulatory ligand interact with topographically distinct sites on the protein. The second model, which was referred to as 'no-overlapping', was favoured on the basis that loss of feedback inhibition is accompanied by a normalization of the substrate kinetics[1]. Around the same time, Gerhart and Pardee concluded that ATCase "has additional groups



for binding its feedback inhibitor, CTP" (REF. 3). The word 'allosteric', which originates from the Greek words for 'different' (αλλο–) and 'solid' with reference to three-dimensionality (στερεο–), was first used in Monod's and Jacob's written conclusions of the 1961 Cold Spring Harbor Symposium on Quantitative Biology, where my data were first presented[4]. It reflects the idea that there is a difference in stereospecificity between the binding sites for the substrate and feedback inhibitor of a protein and was used to qualify and generalize the 'no-overlapping' mechanism[1] and its mediation by a conformational change in the protein. This article marked the birth of the term allosteric. In the symposium conclusions it was further stated that the effect "need not be restricted to 'end-product inhibition', which may turn out to constitute only one class of allosteric effects" (REF. 4).

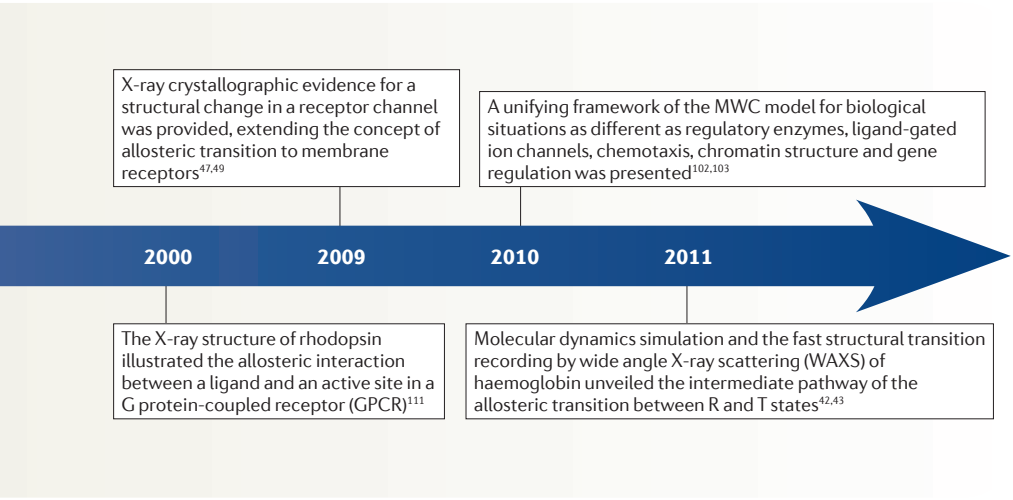
In 1963, a paper by Monod, Changeux and Jacob further developed the concept of allosteric interaction by excluding that there is any direct interaction between the substrate and regulatory inhibitor of an enzyme (the Monod–Changeux–Jacob (MCJ) models[5] are shown in FIG. 2b). Such direct interactions were eliminated by data showing that ligands such as L-Leu[10] and ATP[3] can act as both activators in their own right and as antagonists of feedback inhibitors of Thr deaminase and ATCase, respectively. This demonstrated the formation of ternary complexes, ruling out the steric hindrance mechanism[1,5].

The MCJ model presented in the paper also illustrates the concept of allosteric transition, the conformational change that mediates allosteric interactions, with data collected from experiments using Glu dehydrogenase[13–16], acetyl-CoA carboxylase[17] and muscle phosphorylase *b*[18]. The data suggested that the conformational change frequently, but not always, involves the dissociation and association of subunits within the protein. The paper also presented X-ray data for haemoglobin[19], which showed that the four haem groups of this molecule are far apart (a distance of 25–36 Å). This excluded the possibility that they directly interact with each other in their cooperative binding of oxygen molecules. The then unpublished observation that the distance between certain SH residues in the protein may shift by ~19% upon oxygenation provided direct evidence of a conformational alteration of haemoglobin when it is bound by its ligand. It was stated that "in hemoglobin there is complete evidence that the regulatory effect, ie the cooperative binding of oxygen, is related to a reversible, discrete conformational alteration of the protein" (REF. 5). Last, in the paper by Monod, Changeux and Jacob, it was proposed that the conformational change linking the topographically distinct binding sites in allosteric proteins was caused by an 'induced-fit' sequential mechanism, which was initially proposed by Koshland for enzyme activation[20,21], and that according to this mechanism the ligand 'instructs' rather than 'selects' the conformational change.

The MWC model

From instruction to selection. The attempt to further understand, in terms of protein structure, the conformational change mediating the allosteric interactions between distinct sites on proteins led to a striking paradigm shift. The MWC model[6] (FIG. 2c) was designed to establish a causal link between the structural organization of the known regulatory proteins, including the cooperative binding of substrate and regulatory ligands (activators and inhibitors), the interrelationships between cooperative ligand binding and the binding of regulatory ligands, and last, the uncoupling of all these interactions through desensitization. An essential assumption was that the cooperativity observed between the multiple binding sites for substrate and regulatory ligand (activators and inhibitors in FIG. 2c) relies on a particular structural design of the protein. "Allosteric proteins are oligomers the protomers of which are associated in such a way … that the molecule possesses at least one axis of symmetry" (REF. 6). Furthermore, "the conformation of each protomer is constrained by its association with the other protomers" (REF. 6). The idea that the allosteric transition involves a change in the aggregation state of the protein was abandoned, and the concept that more discrete, yet cooperative, conformational changes occurred was favoured.

Studies on the effect of urea on L-Thr deaminase suggested that ligands strengthened or loosened the assembly of subunits in the protein without causing their dissociation[22]. It was thus proposed that regulatory oligomers spontaneously exist in 'R' (for relaxed) and 'T' (for tense) states in thermodynamic equilibrium, in the absence of ligand, the states differing "by the distribution and/or energy of inter-protomer bonds" (REF. 6). The addition of ligands (substrate, activator or inhibitor) would shift the equilibrium, stabilizing the oligomer conformation for which they have the highest affinity; substrate and activator would stabilize the R state, and the inhibitor would stabilize the T state. The model assumes that the conformational transition occurs for all



subunits of an oligomer or for none; that is, it is 'concerted' and thus conserves oligomer symmetry. The equations describing this model distinguish a 'function of state', R, representing the conformational equilibrium, and a 'binding function', Y, which differentially evolve as a function of ligand concentration. Several of these statements have since been reformulated and discussed in terms of 'conformational selection' or 'shape or population shift' (see REFS 23,24). The MWC model created a theoretical landmark in biochemical thinking by suggesting that a selectionist model of protein regulation, rather than the traditional induced-fit scheme, accounts for the conformational change of allosteric proteins in response to ligands. Yet, in 1966, Koshland, Nemethy and Filmer proposed a sequential induced-fit mechanism of allosteric transition (termed the KNF model[25]) (FIG. 2d), which involves a progressive change of conformation assuming "that *a* subunit in conformation *b* is present *only* when *s* is bound to it" (REF. 25) and which excludes any conformational change of the protein in the absence of ligand.

Later, the MWC model was applied to larger, unlimited protein assemblies, such as those possibly occurring between membrane proteins[26]. This led to the idea that the conformational transition of single protomers (see also FIG. 2e) is modulated by their interaction with other protomers. This model predicts that, depending on the value of the free energy of the interaction between protomers, different responses to specific regulatory signals can occur. These range from the graded response of an oligomeric receptor (for a small number of protomers, as proposed by the MWC model) to an all-or-none phase transition response in large protein assemblies[26,27].

The first attempt to determine whether the MWC model or the KNF model was adequate involved the analysis of the cooperative binding of NAD to glyceraldehyde-3-phosphate dehydrogenase by temperature-jump measurements[28]. The data were consistent with an all-or-none interconversion between two conformations of the enzyme, as postulated by the MWC model. In another attempt to discriminate between these models, the conformation and binding functions of ATCase were assessed. At variance with the KNF model, the equilibrium dialysis binding data for ATCase differed from the conformational change data measured at variable ligand concentrations and composition. Under these conditions, the binding and conformation data were adequately explained by generalized MWC equations (REFS 29–31; see also REF. 32). In summary, it is generally accepted that allosteric interactions between topographically distinct sites do occur and are mediated by a conformational transition of the protein. The cooperative interactions, which, in many instances, take place between active and regulatory sites (see below), are better accounted for by the conformational selection MWC mechanism rather than by the induced-fit KNF mechanism, although the debate still continues.

The MWC model in context

In the early 1960s, only the crystallographic structures of myoglobin and haemoglobin had been solved. Since then, 92,350 structures, including proteins in complex with nucleic acids, have been deposited in the Protein Data Bank (PDB)[33,34]. Abundant examples from the PDB validate the concept that, in regulatory proteins, the regulatory and active sites are topographically distinct. The average topological distance between sites is ~35 Å, but reaches ~60 Å between the acetylcholine-binding sites and the ion channel within the nicotinic acetylcholine receptor (AChR) and between the CTP-binding site and catalytic sites within ATCase[35]. As the interaction between these sites takes place 'at-a-distance', it is, by definition, allosteric (FIG. 3).

An interesting feature of allosteric proteins not mentioned by the MWC model is that a significant fraction of the sites for both orthosteric and allosteric ligands lie at subunit interfaces (reviewed in REF. 35) (FIG. 3). These areas of the protein adequately sense and control the quaternary transitions of an allosteric oligomer.

The symmetrical oligomeric structure postulated by the MWC model is a common, but not universal, property in proteins[33–36]. Among the 88,000 protein structures deposited in the PDB (excluding nucleic acid complexes), oligomers (44,735) are approximately as abundant as monomers (43,160). Another important feature not mentioned in the MWC model is the occurrence of hetero-oligomerization, which generates a broad combinatorial diversity of oligomers that exhibit different physiological, pharmacological or stability properties of critical biological importance[37,38].

The following examples of classic regulatory proteins illustrate the central paradigm of the MWC model; that is, that R and T symmetrical end-states spontaneously occur under equilibrium conditions, in the absence of a regulatory signal, and they expand on the model as a result of new kinetics and molecular dynamics data.

Haemoglobin. From X-ray crystallographic studies of haemoglobin, it was initially proposed that an equilibrium exists between a T and an R state (which have low and high affinity for oxygen, respectively) in line with a simplified MWC model[39]. However, Perutz believed that the Bohr effect (whereby a decrease in pH causes a decrease in the oxygen-binding affinity of haemoglobin), would require the sequential rupture of hydrogen bonds in the T state, which would support the KNF model[40]. However, studies with fish haemoglobin suggested that heterogeneity between the α- and β-types of chain in the T structure might reconcile data with the MWC model[41]. Molecular dynamics simulations[42] revealed that the motion of haemoglobin-α and haemoglobin-β subunits in the T (deoxy) to R (tetraoxy) transition involves two sequential quaternary rotations, which, in

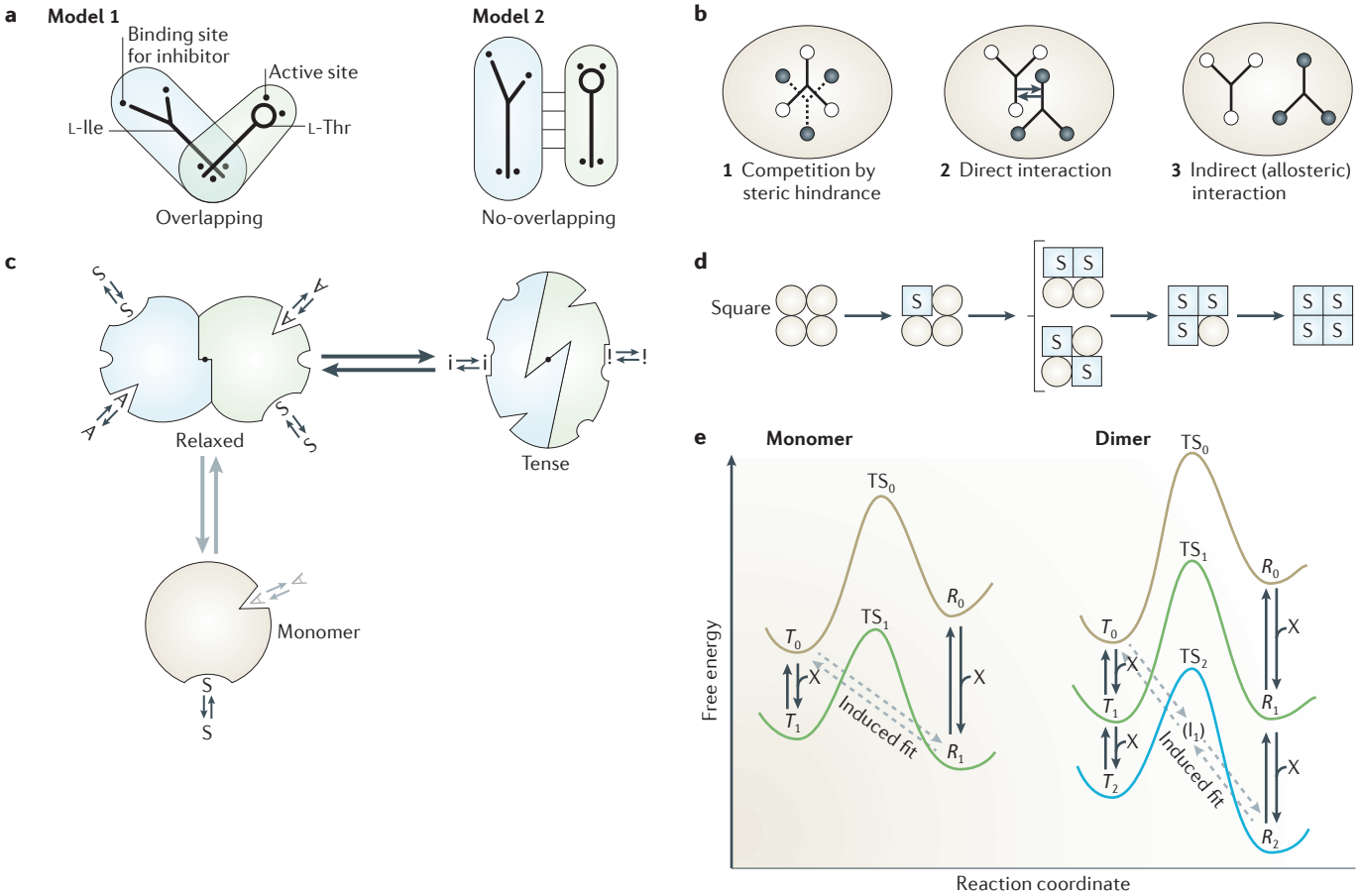


Figure 2 | **Models of allosteric interactions.** **a** | Models proposed by myself in 1961 for the feedback inhibition of L-Thr deaminase by L-Ile[1]. Model 1, named 'overlapping', describes the classic model of competitive inhibition by mutual exclusion through steric hindrance of a common site. In this model the substrate and inhibitor compete for the same site on the protein. In model 2, named 'no-overlapping', the binding-sites for the substrate and inhibitor are topographically distinct and the interaction between them is indirect or 'allosteric'. **b** | Shown are the models proposed by Monod, Changeux and Jacob in 1963 (REF. 5) for the interaction between a substrate and an inhibitor binding to different groups on the surface of the enzyme. In addition to the two models proposed in 1961 (left panel and right panel), a third possibility of a 'direct interaction' between the substrate and inhibitor as a result of them binding to topologically close sites on the target protein was considered. **c** | Schematic representation of the two-state concerted model of Monod, Wyman and Changeux (MWC) in 1965 (REF. 6), that was originally displayed in my Ph.D. thesis in 1964 (REF. 104). The principal conformational transition between two discrete states of a dimer, R (relaxed) and T (tense), occurs in the absence of ligand and preserves the symmetry of the quaternary structure of the protein. It involves alterations of the tertiary structure of the protein (quaternary constraint) and the binding interface between subunits. The three classes of molecules that bind to the enzyme and differentially stabilize the R and T states are shown: A (activator), i (inhibitor), and S (substrate). A hypothetical monomeric state, to which A is possibly bound, is also shown and indicated by the grey arrow. **d** | Sequential representation of the Koshland–Nemethy–Filmer model proposed in 1966 (REF. 25). At variance with the MWC model, the conformational transition (between the round and square subunits) does not take place in the absence of ligand but is 'induced' by the ligand, S. All the unbound subunits are round and all the ligand-bound subunits are square. There is no unbound square subunit. Once initiated at the level of one subunit, the conformational change propagates within the oligomer, with different mixed states occurring during the transition. **e** | Energy landscape representation of the model of conformational selection for an hypothetical monomeric (left) and dimeric (right) allosteric protein proposed by Changeux and Edelstein in 2011 (REF. 105). All the individual valleys correspond to the functionally relevant substrates, which pre-exist to ligand binding. T and R states are presented on a vertical free energy scale and include the transition state (TS). Kinetic barriers for their interconversion are estimated according to linear free energy principles for the dimer. Subscript numbers correspond to the number of ligand molecules (0, 1, 2) bound. The hypothetical pathway for the induced-fit mechanism is presented by the arrows in grey and includes an intermediate (I) state for the dimer. X represents a ligand; the grey line shows the ligand-free, the green line the mono-liganded and the blue line the bi-liganded state. The models in part **a** are reproduced, with permission, from REF. 1 © (1961) Cold Spring Harbor Laboratory Press. The models in part **b** are reproduced, with permission, from REF. 5 © (1963) Elsevier. The model in part **c** is reproduced, with permission, from REF. 104 © (1965) Elsevier. The model in part **d** is reproduced, with permission, from REF. 25 © (1966) ACS Publications. The model in part **e** is reproduced, with permission, from REF. 105 © (2011) Faculty of 1000 Ltd.

agreement with the MWC model, ultimately conserve symmetry. The quaternary transitions include an early large change in quaternary structure (which is characterized by a 3° rotation of each α-subunit relative to the β1β2 dimer), with a lower energy barrier, and a smaller late quaternary change (characterized by a 6° rotation of the α1β1 and the α2β2 dimers) with a higher energy barrier[42]. The results are consistent with the recent wide-angle X-ray scattering measurements (WAXS measurements) of a fast global (2 μs) quaternary transition and a slow (20 μs) transition component that includes tertiary changes[43]. The ongoing research (see REF. 44)

thus reveals new and largely unexplored features of the transition dynamics between the T and R end-states of the MWC mechanism.

The ATCase hexamer. ATCase comprises two catalytic subunits of three identical polypeptide chains and three regulatory subunits that are composed of two identical chains. X-ray studies of this hexamer identified structurally distinct and symmetrical T (low activity) and R (high activity) states in the presence of substrates or substrate analogues (FIG. 4a). Nuclear magnetic resonance (NMR) analysis further revealed a spontaneous equilibrium between discrete T and R states in solution, allowing the equilibrium constant (L_0) between R and T forms of the enzyme, and the shift from T to R by substrate or ATP binding, to be measured quantitatively. X-ray data further show that, when ATCase undergoes the T to R transition, it expands by 10.6 Å, and the upper catalytic trimer rotates 12° relative to the lower trimer along the threefold axis. All the data adequately fit a simplified MWC model[45].

Nicotinic AChR. The X-ray structures of two bacterial homologues[46] of nicotinic AChR, one stabilized in a closed conformation and the other in an open symmetrical conformation, were compared at the atomic level[47–50]. The data revealed that a quaternary twist accounts for at least 29% of the closed to open transition, and it contributes to the substantial tertiary rearrangement of the subunit interfaces that results in a tilt of the transmembrane 2 (TM2) and TM3 segments to widen the opening in the upper part of the pore from 2 Å to 12 Å in diameter[47,49,50] (FIG. 4b).These data are consistent with a former normal mode computational analysis of a model for α7 nAChR[51] and with recent atomistic molecular dynamics simulations[52] of both eukaryotic and bacterial homologues. The data are globally explained by the MWC model for the end-states for the activation transition[52,53]. Yet, the dynamics of the conformational reorganization, which occurs in a few microseconds, involves progressive motions and intermediate conformations between the symmetrical end-states. For example, rate-equilibrium free energy analysis of muscle nicotinic AChR reveals that the extracellular domain moves before that of the transmembrane domain (TMD) in the course of channel opening[53]. In addition, single-channel electrophysiology experiments suggest the occurrence of a hypothetical 'flip' or intermediate conformation, possibly including fast and/or slow desensitized states. Interestingly, a mixed

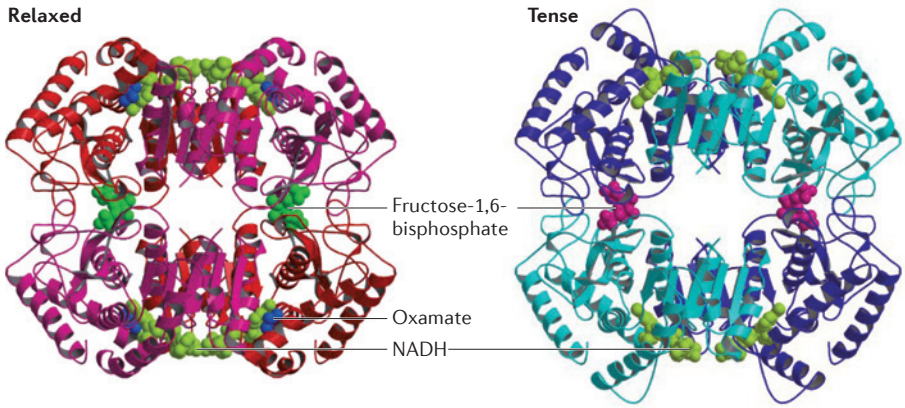


Figure 3 | **An example of a typical allosteric protein.** The X-ray structure of L-lactate dehydrogenase from *Bifidobacterium longum* in its R (relaxed) and T (tense) allosteric states. This illustrates the oligomeric structure of the protein and highlights that the catalytic site (which binds fructose-1,6-bisphosphate) and the regulatory site (which binds NADH) are topographically distinct and located at different subunit interfaces. The conformational transition between symmetrical R and T states (which coexist in the same crystal) preserves the symmetry of the protein. The tertiary folding of the subunits is shown in shades of red for the R state and in shades of blue for the T state. Fructose-1,6-bisphosphate is shown in green in the R state and in pink in the T state so that it stands out. Oxamate is a structural analogue of the substrate lactate that preferentially binds to the R state. The images are reproduced, with permission, from REF. 106 © (1994) Macmillan Publishers Limited.

'locally closed' but symmetrical structure has been identified[54] in which an open conformation of the extracellular domain is associated with a closed pore[50]. Also, more than the two states proposed in the MWC model are needed to account for the different steps of pharmacological desensitization. In other words, the original MWC model offers a general frame that needs to be further refined and expanded to account for the abundant data available[52,53].

New insights into conformational changes

The MWC model shows intrinsic limitations that have been introduced, in particular, by its founding postulates inspired by bacterial regulatory enzymes and haemoglobin. In the past decades, the development of novel technologies (see REFS 24,43,52) has increased our understanding of protein structure and conformational changes, providing new insights and, in specific cases, challenging features of the original MWC model.

Do allosteric monomers exist? Although the MWC model suggests that allosteric proteins are oligomeric, some monomeric proteins, including proteases[35], thrombin[55] and phospholipases[56], are thought to mediate signal transduction and therefore undergo an allosteric transition[24,35]. G protein-coupled receptors (GPCRs), which contain seven TMDs, may belong to this category. Reconstitution of single molecules of β_2 adrenergic receptor (β2AR) in lipid nanodiscs demonstrated that a monomer can activate a G protein *in vitro*. Structural studies were performed using β2AR and adenosine A2A receptor 'monomers' in which their unbound or antagonist- and agonist-bound states were compared. The transition between these states involved conformational rearrangements that are similar to those initially observed during the activation of the GPCR rhodopsin; that is, a global and concerted rearrangement of the helix bundle that shifts the cytoplasmic end of TM6 (and to a lesser extent TM5) away from the bundle core (which comprises TM1–TM4 and TM7), offering an additional interacting surface for the G protein (discussed in REF. 57). Moreover, NMR analysis of ^{19}F-labelled β2AR in complexes with various ligands, revealed that the cytoplasmic ends of helices TM6 and TM7 adopt two major conformational states. Changes in the NMR signals demonstrate that agonist binding primarily shifts the equilibrium towards the G protein-specific active state of TM6. By contrast, β-arrestin-biased ligands predominantly affect the conformational states of TM7 (REF. 58). These data support the notion that, in agreement with the conformational selection mechanism postulated by the MWC model, GPCRs may undergo spontaneous transitions between discrete conformational states, but, at variance with the MWC model, at the level of a single monomer. Would GPCR monomers then possibly be viewed as 'mini-oligomers' of seven transmembrane helices?

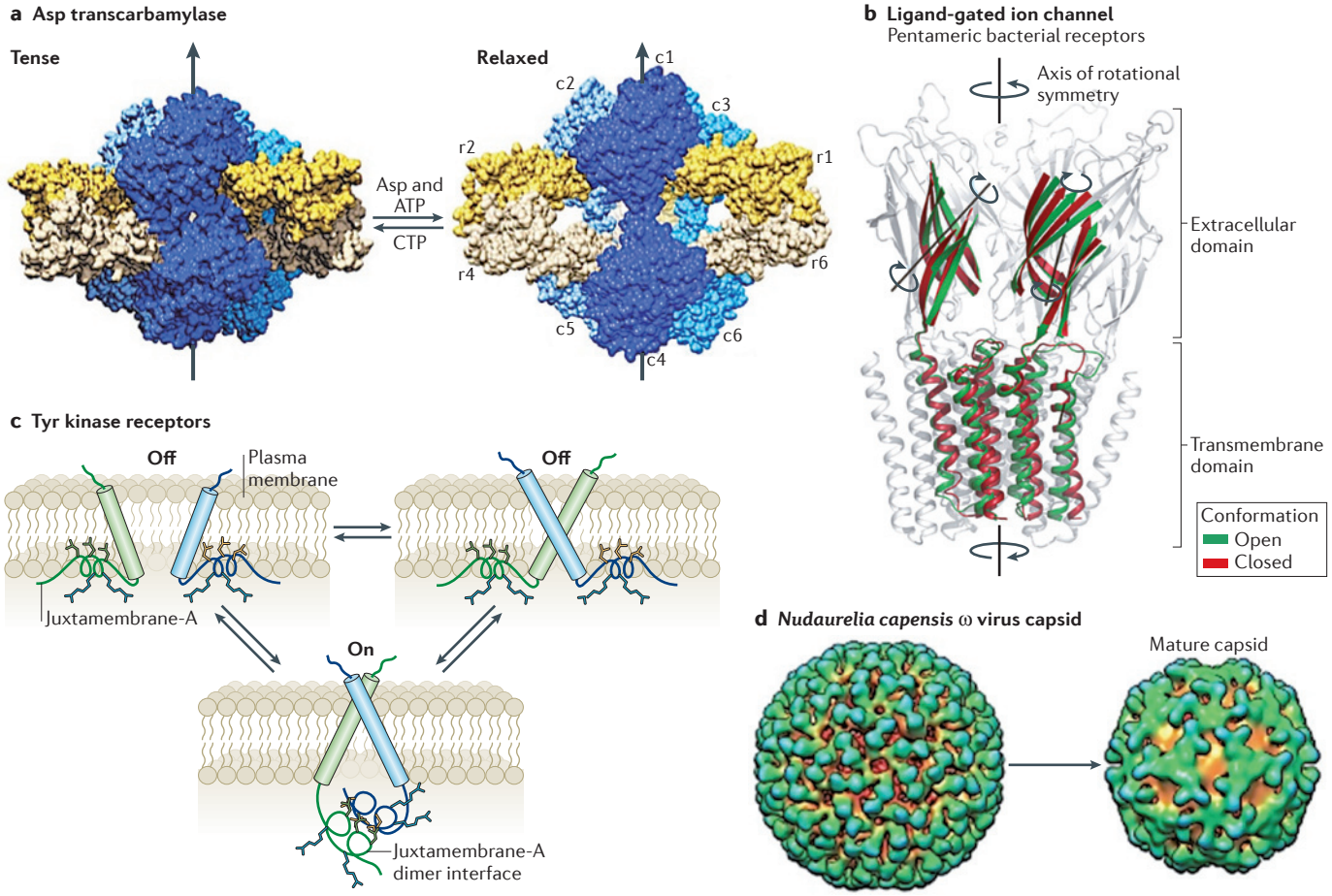


Figure 4 | **Diversity of allosteric mechanisms.** **a** | X-ray structure of Asp transcarbamylase (ATCase) in the tense (T) and relaxed (R) states. In agreement with the Monod–Wyman–Changeux (MWC) model, the symmetry between these two states is conserved. The catalytic subunits are in shades of blue and the regulatory subunits in yellow and tan. The axes of symmetry along the catalytic trimer (c) and regulatory dimer (r) are shown. The molecule expands 11 Å along the threefold axis during the allosteric transition. During the T to R transition, the regulatory dimers rotate ~6^0 around their respective twofold axes, and the catalytic trimers rotate ~7.5^0 around the threefold axis[109]. **b** | Superimposed X-ray structure of two bacterial pentameric ligand-gated ion channels (these are bacterial homologues of the nicotinic acetylcholine receptor (AChR)). One is crystallized at pH 4.6 in an open conformation (*Gloeobacter violaceus;* shown in green) and the other in a closed conformation (*Erwinia chrysanthemi*; shown in red). The conformational transition involves a global quaternary twist between closed and open states, which is accompanied by changes in the tertiary structure with conservation of symmetry along the fivefold rotational axis. The extracellular domain carrying the orthosteric ligands (top) and the transmembrane domain with the ion channel in the axis of symmetry (bottom) are shown. **c** | Molecular dynamics model of the transmembrane domain of Tyr kinase receptors transitioning from inactive monomers (top left) to an inactive dimer (top right) and active dimer (bottom). Also depicted is the 'off' to 'on' signal transduction transition, which involves a change in the relative position of the transmembrane domains and reorganization of the cytoplasmic kinase domain (top right and bottom). **d** | The *Nudaurelia capensis* ω virus capsid undergoes a global change of shape when the basic icosahedral units comprising the capsid undergo an allosteric transition. This transition involves proteolysis and results in capsid maturation. The images in part **a** are reproduced, with permission, from REF. 45 © (2012) ACS Publishing, and REF. 107 © (2012) Elsevier. The image in part **b** is reproduced, with permission, from REF. 47 © (2009) Macmillan Publishers Limited. The image in part **c** is adapted from REF. 67 © (2013) Cell Press. The images in part **d** are reproduced, with permission, from REF. 83 © (2013) Elsevier.

However, it should be noted that rhodopsin has been detected as dimers and as larger oligomers, both in crystals and in solution[59]. The first β2AR structure was also solved as a dimer, and dimers were found in the crystal structure of the CXC chemokine receptor 4 (CXCR4) and μ-opioid GPCRs, although these dimers had variable dimerization interfaces[57]. Some class C GPCRs, such as the $GABA_B$ (γ-aminobutyric acid B) and glutamate receptors, form obligatory heterodimers with an asymmetric mode of activation whereby only one protomer within a dimer activates the G protein. Furthermore, fluorescence resonance energy transfer (FRET) studies indicate that β2AR reconstituted in a model lipid bilayer can form tetramers, which would represent an inactive form of the receptor[60]. Thus, the signal transduction mediated *in vivo* by some GPCRs that are functionally active *in vitro* as monomers might possibly be modulated by higher-order aggregation transitions[57,60,61].

Unstructured proteins and allostery. The occurrence of intrinsically disordered protein segments noted in several systems illustrates the difficulty of establishing unequivocal relationships between the primary structure and the three-dimensional organization of proteins[62–64]. Evidently, in the presence of these segments, protein symmetry may not be rigorously established. Yet, as outlined below, they may contribute to the modulation and diversification of allosteric transitions.

The *lac* repressor is a DNA-binding protein in *E. coli* that represses the transcription of genes encoding proteins involved in lactose metabolism[65]. It was proposed to be an allosteric protein[5] that spontaneously binds to DNA but is released to allow transcription in the presence of a lactose analogue (inducer). Its minimal structure is a homodimer of a subunit type that consists of an amino-terminal DNA-binding domain (which interacts with the DNA sequence of the *lac* operator), a disordered hinge region (also termed linker), a ligand-binding domain and a carboxy-terminal helix[65]. Crystallographic data show that the *lac* repressor is partially disordered in the absence of DNA. However, when it is bound to DNA, the repressor dimer becomes a fully symmetrical molecule, with a dyad axis of symmetry from the N-terminal domain to the C-terminal helix. Binding of the inducer to the *lac* repressor stabilizes a subtle hinge motion in its N-terminal subdomain relative to the C-terminal subdomain. This reduces the affinity of the repressor for the operator by several orders of magnitude, thus triggering its release from DNA and ultimately transcription. It has been suggested that the data collected in the presence or absence of an inducer can be quantitatively accounted for by the MWC model[65].

Tyr kinase receptors are high-affinity cell surface receptors that are activated by polypeptide growth factors, cytokines and hormones. Long timescale molecular dynamics simulations of membrane-embedded epidermal growth factor receptor (EGFR) reveal that its N lobe dimerization interface is intrinsically disordered in the monomer and becomes ordered and activatable only upon dimerization (FIG. 4c). In ligand-bound dimers, the extracellular domains assume conformations that favour the dimerization of each single transmembrane helix near the N terminus, and thus the formation of asymmetric (active) kinase dimers[66,67] (FIG. 4c). These simulations need experimental confirmation but are nevertheless authentic breakthroughs in the understanding of this exceptionally important class of receptors. They suggest that Tyr kinase receptors follow a MWC mechanism during activation, even though the cytoplasmic kinase dimer is asymmetric, and thus uncharacteristic of the MWC model, in the active state.

Glucokinase (GCK) shows particularly striking behaviour, as it displays a sigmoidal kinetic response to increasing blood glucose levels, even though it is a monomer that harbours only one glucose-binding site. NMR studies revealed large-scale, glucose-mediated disorder to order transitions that would account for the observed apparent cooperative effects[68]. A mechanism such as this, based on an unstructured protein organization, would be unrelated to the MWC model.

Last, it has been noted that disordered segments in protein kinase A (PKA) contribute to the diversity of the oligomers by driving the assembly of distinct tetramers that have different allosteric properties but also binding partners, resulting in distinct cellular locations[69]. Under these conditions, the flexibility generated by the disordered segments would contribute not only to the diversification of allosteric oligomers mediating distinct MWC processes but also to their different distribution in the cell and thus to their role in metabolism.

Glossary

Congenital myasthenic syndrome
An inherited neuromuscular disorder caused by defects at the neuromuscular junction, for instance, by mutations at the level of the postsynaptic receptors. This syndrome is distinct from autoimmune myasthenia gravis.

Induced-fit scheme
(also interpreted as a 'Lamarckian' mechanism). When a ligand binds to a protein site, it instructs a conformational change in the protein that is complementary to its structure and that does not exist in its absence.

Intrinsically disordered protein
Often referred to as a naturally unfolded protein or disordered protein. These proteins are characterized by a lack of a stable and well-defined three-dimensional tertiary structure when the protein exists as an isolated polypeptide chain (a subunit) under physiological conditions.

Ising model
A mathematical model of ferromagnetism in statistical mechanics, named after the physicist Ernst Ising. This model represents magnetic dipole moments of atomic spins arranged in a lattice, allowing each spin to interact with its neighbours and yielding sharp phase transitions, as a simplified model of a strongly cooperative change of state.

Michaelis–Menten hyperbolic kinetics
The simplest and best-known models of enzyme kinetics. It involves an enzyme (E) binding to a substrate (S) to form a complex (ES), which is converted into a product (P) and E. The relation between enzyme velocity, as a function of substrate concentration, follows an hyperbolic law.

Molecular dynamics simulations
A computational method to calculate the time-dependent behaviour of a molecular system. These provide detailed information on the fluctuations and conformational changes of proteins and nucleic acids.

Normal mode computational analysis
A computational method that can reveal the overall change in the conformation of large proteins, without the need to calculate the specific molecular mechanism, such as the motion of specific bonds. For instance, for proteins, each amino acid is represented as a bead and all pairs of beads are connected by springs.

Orthosteric sites
The principal binding sites carried by proteins, for instance, the neurotransmitter-binding sites of receptors in the brain.

Population shift
A formulation, in terms of energy landscape, for proteins of the conformational selection mechanism of the Monod–Wyman–Changeux (MWC) model.

Protomers
The repeated units of a symmetrical oligomeric protein.

Quaternary structure
The arrangement of multiple folded protein units in a multisubunit oligomeric complex.

Rate-equilibrium free energy analysis
An analysis that provides information on transition-state structures. It revealed, for instance, the temporal sequence in which the different regions of an ion channel protein move during a closed–open conformational transition.

Selectionist model of protein regulation
(also interpreted as a 'Darwinian' mechanism). The ligand selectively stabilizes pre-existing conformations when it binds to the state for which it exhibits preferential affinity. That is, the variability of the conformations takes place before selection occurs.

Sigmoidal substrate saturation kinetics
A classic deviation from Michaelis–Menten hyperbolic kinetics, revealing the cooperative interaction between several identical binding sites, as is the case when oxygen binds haemoglobin.

Single-channel electrophysiology
This patch clamp technique affords high resolution of the detailed properties of single-ion channel currents from many cell types.

Startle disease
(also known as hyperekplexia or exaggerated surprise). A neurological disorder classically characterized by pronounced startle responses to tactile or acoustic stimuli and hypertonia, and caused by genetic mutations in a number of different genes (such as the gene encoding Gly receptor).

Temperature-jump measurements
A technique for measuring rapid chemical kinetics, in which the solution is rapidly heated, for example, by the output of a pulsed laser.

The free energy landscape paradigm of protein folding
A statistical description of a potential surface of a protein. It assumes that folding occurs through organizing an ensemble of structures rather than through only a few uniquely defined structural intermediates.

Wide-angle X-ray scattering measurements
(WAXS measurements). An X-ray-diffraction technique that is used to determine the crystalline structure of proteins.

Allostery without conformational change? An interesting condition has been revealed by the catabolite activator protein (CAP) from *E. coli*, the allosteric interactions of which are claimed to be mediated by "changes in protein intrinsic motions in the absence of structural change" (REF. 70). CAP is an activator of transcription initiation, the DNA-binding domain of which is modulated by cyclic AMP (cAMP) binding to it at a distance. This allosteric interaction does not seem to involve structural changes, but NMR analysis revealed that the intrinsic motion of residues in the unliganded subunit of CAP that cannot be predicted by the inspection of static structures[70] are strongly affected by cAMP binding. This would provide a means of propagating the allosteric signal to the distal site[71]. Last, structure analysis of the cAMP-mediated CAP switch from the inactive to the active state provides one of the clearest examples of population shift (REF. 72). Additional examples of 'allostery without conformational change' have been considered (see REF. 73).

Supramolecular assemblies. The concept of allosteric interaction was initially extended to 'supramolecular' assemblies of proteins organized in two-dimensional lattices, as mentioned above[26]. Since then, *E.coli* chemotaxis receptors that regulate flagellar rotation have been shown to exist in a thermal equilibrium between two or more conformations that establish cooperative interactions between 'nearest neighbours' within a regular two-dimensional hexagonal lattice. The data can be accommodated by the model that myself and colleagues proposed in 1967 and by some of its statistical derivatives[74] which, at variance with the original MWC model, yield very sharp, almost all-or-none, response curves to the chemoattractant accompanied by the spread of the conformational change throughout the membrane lattice[74].

More limited in size, the chaperonin complex GroEL comprises 14 identical protomers, arranged in two seven-member rings, that twist and turn as a result of a single set of discrete allosteric transitions[75,76]. Also, the inositol 3-phosphate (IP3) receptor forms a 'balloon' with numerous holes and pockets that interact with multiple molecular effectors, thus providing a 'platform' for a diversity of cell signalling events[77]. Other examples of large assemblies are the 2.5 megadalton proteasome complex[78], the cyclosome[79], the anaphase-promoting complex[80] and the molecular machinery that regulates striated muscle contraction[81,82] and even virus capsid maturation[83] (FIG. 4d). All of these execute their function through networks of at-a-distance transitions (in a cascade) that are far more complex than can be predicted by a simple MWC mechanism.

In conclusion, despite its simplicity and sometimes its limitations, the MWC model has opened many new avenues of active research on protein conformational changes. The recent research discussed in this section further helps to develop and adapt the MWC model to situations that were largely unanticipated at the time it was conceived.

Allostery, disease and drug design

The concept of allosteric interaction had important practical consequences for the understanding of human diseases and the design of pharmacological agents.

Constitutive mutations and 'receptor diseases'. An important prediction of the MWC model is that the shift of the T to R equilibrium of allosteric proteins by mutations, causing loss or gain of function, might be associated with disease states. This was initially found to be true for haemoglobins[39] and several regulatory enzymes. As a result of mutation, the T to R equilibrium becomes considerably shifted in favour of the active state (R) and the enzyme shows spontaneous (or basal) activity in the absence of ligand. In the case of ligand-gated ion channels, a wealth of electrophysiological studies, including single-channel recordings, first with α7 nicotinic AChR[84] and then with more than 1000 mutants of muscle nicotinic AChR[53], showed that full channel openings may take place spontaneously and with high frequency in the absence of agonist. This definitively rules out the induced-fit mechanism proposed by the KNF model. Many of the mutations in nicotinic AChR cause congenital myasthenic syndrome (muscle nicotinic AChR) or autosomal dominant nocturnal frontal lobe epilepsy (neuronal nicotinic AChR). These mutations are preferentially located either at the interface between subunits or within a given subunit at the interface between rigid domains of the receptor protein[85]. Mutations have been found in similar locations in the 5-HT_3, $GABA_A$ and Gly receptors and are responsible for several diseases, including startle disease. The disease phenotype of these receptor mutations can in most instances be explained by the very high level of steady receptor activation resulting from the shift of the MWC equilibrium in favour of the active R state[35,53].

About 100 constitutive mutations in GPCRs have been identified and found to be responsible for more than ten diseases. These mutations frequently increase the basal activity of the receptor; this can be restored to the 'resting activity' by the use of negative allosteric modulators (also known as inverse agonists)[57,86]. Mutations in Tyr kinase receptors that increase their activity in the absence of ligand have also been reported, for decades, to be oncogenic as they allow receptor proteins to dimerize and undergo autophosphorylation. In addition, constitutive mutations of nuclear receptors cause many human diseases[35]. In general, these disease data are simply accounted for by adapted versions of the MWC model. Interestingly, the AlloSteric Data Base mentions a total of 571 allosteric-related diseases.

Allosteric modulation and drug design. Until recently, drugs were almost exclusively designed to either mimic or competitively inhibit ligands binding to the principal sites (orthosteric sites) of their targets. A major breakthrough was the discovery that various molecules acting at allosteric sites that are topographically distinct from the orthosteric sites modulate signal transducing proteins, selectively potentiating or inhibiting their physiological activity without directly affecting the ongoing signalling processes. In the case of ligand-gated ion channels, several categories of allosteric modulatory sites have been identified[87], and these are distributed throughout the protein[88].

First, in the extracellular domain, the binding sites for Ca^{2+} or Zn^{2+} are located at the subunit interface below the neurotransmitter-binding pocket. In heteropentameric receptors like neuronal nicotinic AChRs, defined subunit interfaces bind orthosteric ligands such as ACh and others do not. This is also the case for $GABA_A$ receptors for which the sites located at 'non-agonist' interfaces were suggested, a while ago[89], and biochemically demonstrated to be the target of the benzodiazepines (the most commonly prescribed psychopharmaceutical drug), which behave as potent allosteric modulators of $GABA_A$ receptors[89,90].

Second, the TMD is the target of various allosteric modulators, including general anaesthetics, ethanol and other alcohols, neurosteroids, lipids and cholesterol, as well as several synthetic compounds[91–93]. These target three distinct loci: an intrasubunit cavity; an intersubunit cavity; and a lipid bilayer interface. Lipids, free fatty acids and steroids may behave as the endogenous allosteric modulators of these ligand-gated ion channels.

Third, other modulatory sites, such as phosphorylation sites[94], are present in the cytoplasmic domain of ligand-gated ion channels[87,88] and may have important roles in the clustering, stabilization and modulation of receptor functions (such as desensitization).

Moreover, many allosteric modulatory sites have been identified in GPCRs[95], although so far the only GPCR the crystal structure of which has been solved in complex with an allosteric modulator is in complex with Na^+ ions[96]. On a final note, the AlloSteric Database contains 22,004 allosteric modulator entries and 16,842 allosteric druggable entries, which highlights the potential for targeting allosteric modulation with drugs.

Conclusion

The original concept of allosteric interactions as indirect, at-a-distance interactions that are established between topographically distinct sites and mediated by a discrete conformational change of the protein[1–5] has 'held true' throughout the years for various biological systems. The MWC model[6] further attempted to specify the property that such 'cybernetic sensors' frequently behave as all-or-none molecular switches through a cooperative organization of the protein into a symmetrical oligomer. Since the models were first published, the MCJ paper[5] has been cited 1730 times, and the MWC paper[6] 6317 times (a number which is still increasing at a rate of about 150 per year), as determined using Google scholar. The MWC model proposes a conformational mechanism and a mathematical formulation, the simplicity (some even say 'beauty') of which made it easy to use and widely apply. Retrospectively, it seems to be a fundamental step in the recognition of the central importance of protein folding and conformational reorganizations in protein function. However, its simplicity introduces necessary restrictions on the validity of some of its detailed applications. As a result, three principal issues are still under active debate.

First, the MWC model was, by design, limited to a minimum number of conformational states (two) under a thermodynamic equilibrium. It is now established that, in several systems, more than two states have to be taken into consideration. Moreover, since then, the detailed description of the kinetics of allosteric transitions and of their nested intermediate conformations has become a lively field of research. Second, the symmetry of allosteric oligomers, a feature that was initially proposed to specify the cooperativity of the structural transition mediating cooperative binding, is also debated. Many classic allosteric proteins are unambiguously symmetrical oligomers, the conformational end-states of which preserve the symmetry of the molecule. Yet, several well-documented cases have been described in which signal transduction is mediated by non-oligomeric structures, such as monomers (for example, GPCRs) or even supramolecular assemblies (for example, a ribosome, a myosin molecule or a riboswitch), that do not exhibit evident symmetrical properties throughout the diverse conformational transitions that they undergo.

Third, studies on the physics of allosteric communications between far distant sites in proteins are ongoing, using their own concepts and methods. One may mention, for instance, the free energy landscape paradigm of protein folding[23,97–99] (FIG. 2e), which encompasses the native conformation as well as any non-native conformations sampled during folding or catalysis. From a functional standpoint, there are two key points: first, all the individual valleys, which are the functionally relevant substrates, pre-exist; and second, the landscape is dynamic, and the relative populations of the substrates will change following, for instance, mutations or binding of a drug at an allosteric site (FIG. 2e). To optimize the newly formed interactions, atoms within the protein move and reorient. This creates strain energy (frustration), which forces the next layer of atoms to also move, which in turn affects the next layer, and so on, yielding perturbations that travel across the structure as a 'wave'. Thus, allostery works via a population shift. Amazingly, this conclusion is, at this stage, fully consistent with the conformational selection mechanism postulated by the MWC model (FIG. 2e).

In parallel, several theoretical approaches have attempted to provide a 'unifying framework for allosteric interactions'. First, the relative contribution of the conformational transition of 'equivalent monomers' in cooperation within an oligomer were re-evaluated[100,101]. Second, using the MWC model, six 'sensing characteristics', used to specify the complex conformational change taking place during the allosteric transition, have in fact been shown to be strongly interdependent, (that is, specifying one characteristic strongly constrains the others)[102]. Last, a general mathematical implementation of the MWC model has been proposed, using statistical mechanics, which links biological situations that otherwise do not seem to be connected, including regulatory enzymes, ligand-gated ion channels, chemotaxis, chromatin structure and gene regulation[103]. This illustrates the 'design' constraints faced by these molecules which, according to the authors, are "beginning to assume similar proportions in biology as those of the Ising model in physics" (REF. 103).

As for any scientific theory, the MWC model did not aim to give an exhaustive description of biological reality. However, perhaps one of its most positive values is that it has been, and still is, a strong incentive for empirical research on proteins and now on supramolecular protein assemblies, carried out using the broad diversity of biophysical and molecular dynamic methods that are presently available. In short, 50 years on, the MWC model still provides a useful basis for future research.

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1. Changeux, J. P. The feedback control mechanisms of biosynthetic-threonine deaminase by L-isoleucine. *Cold. Spring Harb. Symp. Quant. Biol.* **26**, 313–318 (1961).
2. Gerhart, J. C. & Pardee, A. B. Separation of feedback inhibition from activity of aspartate transcarbamylase (ATCase). *Fed. Proc.* **20**, 224 (1961).
3. Gerhart, J. C. & Pardee, A. B. The enzymology of control by feedback inhibition. *J. Biol. Chem.* **237**, 891–896 (1962).
4. Monod, J. & Jacob, F. General conclusions: teleonomic mechanisms in cellular metabolism, growth, and differentiation. *Cold Spring Harb. Symp. Quant. Biol.* **26**, 389–401 (1961).
5. Monod J., Changeux J. P. & Jacob, F. Allosteric proteins and cellular control systems. *J. Mol. Biol.* **6**, 306–329 (1963).
6. Monod, J., Wyman, J. & Changeux, J. P. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* **12**, 88–118 (1965).
7. Novick, A. & Szilard, L. in *Dynamics of Growth Processes*. (ed. Boell, E. J.) 21–32 (Princeton University Press, 1954).
8. Umbarger, H. E. Evidence for a negative feedback mechanism in the biosynthesis of isoleucine. *Science* **123**, 848 (1956).
9. Yates, R. A. & Pardee, A. B. Control of pyrimidine biosynthesis in *Escherichia coli* by a feedback mechanism. *J. Biol. Chem.* **221**, 757–770 (1956).
10. Changeux, J. P. Effect of L-threonine and L-isoleucine analogs on L-threonine deaminase. *J. Mol. Biol.* **4**, 220–225 (1962).
11. Changeux, J. P. The origins of allostery: from personal memories to material for the future. *J. Mol. Biol.* **425**, 1396–1406 (2013).
12. Gerhart, J. From feedback inhibition to allostery: the enduring example of aspartate transcarbamoylase. *FEBS J.* http://dx.doi.org/ doi: 10.1111/febs.12483 (2013).
13. Frieden, C. Glutamic dehydrogenase. I. The effect of coenzyme on the sedimentation velocity and kinetic behavior. *J. Biol. Chem.* **234**, 809–814 (1959).
14. Frieden, C. Coenzyme binding, observed by fluorescence enhancement, apparently unrelated to the enzymic activity of glutamic dehydrogenase. *Biochim. Biophys. Acta* **47**, 428–430 (1961).
15. Yielding, K. L. & Tomkins, G. M. Structural alterations in crystalline glutamic dehydrogenase induced by steroid hormones. *Proc. Natl Acad. Sci. USA* **46**, 1483–1488 (1960).

16. Yielding, K. L. & Tomkins, G. M. Inhibition of glutamic dehydrogenase by *o*-phenanthroline and its analogs. *Biochim. Biophys. Acta* **62**, 327–331 (1962).
17. Vagelos, P. R., Alberts, A. W. & Martin, D. B. Activation of acetyl-CoA carboxylase and associated alteration of sedimentation characteristics of the enzyme. *Biochem. Biophys. Res. Commun.* **8**, 4–8 (1962).
18. Krebs, E. G. & Fischer, E. H. Molecular properties and transformations of glycogen phosphorylase in animal tissues. *Adv. Enzymol. Relat. Subj. Biochem.* **24**, 263–290 (1962).
19. Perutz, M. F. *et al.* Structure of hæmoglobin: a three-dimensional Fourier synthesis at 5.5-Å˚ resolution obtained by X-ray analysis. *Nature* **185**, 416–442 (1960).
20. Koshland, D. E. Jr. Enzyme flexibility and enzyme action. *J. Cell Comp. Physiol.* **54**, 245–258 (1959).
21. Koshland, D. E. Jr. The role of flexibility in enzyme action. *Cold Spring Harb. Symp. Quant. Biol.* **28**, 473–482 (1963).
22. Changeux, J. P. Allosteric interactions on biosynthetic L-theonine deaminase from *E. coli* K12. *Cold Spring Harb. Symp. Quant. Biol.* **28**, 97–504 (1963).
23. Boehr, D. D., Nussinov, R. & Wright, P. E. The role of dynamic conformational ensembles in biomolecular recognition. *Nature Chem. Biol.* **5**, 789–796 (2009).
24. Cui, Q. & Karplus, M. Allostery and cooperativity revisited. *Protein Sci.* **17**, 1295–1307 (2008).
25. Koshland, D. E. Jr, Némethy, G. & Filmer, D. Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry.* **5**, 365–385 (1966).
26. Changeux, J. P., Thiéry, J., Tung, Y. & Kittel, C. On the cooperativity of biological membranes. *Proc. Natl Acad. Sci. USA* **57**, 335–341 (1967).
27. Shimizu, T. S., Le Novere, N., Levin, M. D., Beavil, A. J., Sutton, B. J., Bray, D. Molecular model of a lattice of signalling proteins involved in bacterial chemotaxis. *Nature Cell Biol.* **2**, 792–96 (2000).
28. Kirschner, K., Eigen, M., Bittman, R. & Voigt, B. The binding of nicotinamide–adenine dinucleotide to yeast d-glyceraldehyde-3-phosphate dehydrogenase: temperature-jump relaxation studies on the mechanism of an allosteric enzyme. *Proc. Natl Acad. Sci. USA* **56**, 1661–1667 (1966).
29. Changeux, J. P., Gerhart, J. C. & Schachman, H. K. Allosteric interactions in aspartate transcarbamylase. I. Binding of specific ligands to the native enzyme and its isolated subunits. *Biochemistry* **7**, 531–538 (1968).
30. Gerhart, J. C. & Schachman, H. K. Allosteric interactions in aspartate transcarbamylase. II. Evidence for different conformational states of the protein in the presence and absence of specific ligands. *Biochemistry* **7**, 538–552 (1968).
31. Changeux, J. P. & Rubin, M. M. Allosteric interactions in aspartate transcarbamylase. III. Interpretations of experimental data in terms of the model of Monod, Wyman, and Changeux. *Biochemistry* **7**, 553–561 (1968).
32. Blangy, D., Buc, H. & Monod, J. Kinetics of the allosteric interactions of phosphofructokinase from *Escherichia coli*. *J. Mol. Biol.* **31**, 13–35 (1968).
33. Berman, H. M. *et al.*Trendspotting in the Protein Data Bank. *FEBS Lett.* **587**, 1036–1045 (2013).
34. Rose, P. W. *et al.* Protein Data Bank: new resources for research and education. *Nucleic Acids Res.* **41,** D475–D482 (2013).
35. Changeux, J. P. Allostery and the Monod–Wyman–Changeux model after 50 years. *Annu. Rev. Biophys.* **41**, 103–133 (2012).
36. Levy, E. D. & Teichmann, S. Structural, evolutionary, and assembly principles of protein oligomerization. *Prog. Mol. Biol. Transl. Sci.* **117**, 25–51 (2013).
37. Kerszberg, M. & Changeux, J. P. A model for reading morphogenetic gradients: autocatalysis and competition at the gene level. *Proc. Natl Acad. Sci. USA* **91**, 5823–5827 (1994).
38. Tsigelny, I. F., Kouznetsova, V. L., Baitaluk, M. & Changeux, J. P. A hierarchical coherent-gene-group model for brain development. *Genes Brain Behav.* **12**, 147–165 (2013).
39. Edelstein, S. J. Extensions of the allosteric model for haemoglobin. *Nature* **230**, 224–227 (1971).
40. Perutz, M. F., Wilkinson, A. J., Paoli, M. & Dodson, G. G. The stereochemical mechanism of the cooperative effects in hemoglobin revisited. *Annu. Rev. Biophys. Biomol. Struct.* **27**, 1–34 (1998).
41. Levantino, M. *et al.* The Monod–Wyman–Changeux allosteric model accounts for the quaternary transition dynamics in wild type and a recombinant mutant human hemoglobin. *Proc. Natl Acad. Sci. USA* **109**, 14894–14899 (2012).
42. Fischer, S., Olsen, K. W., Nam, K. & Karplus, M. Unsuspected pathway of the allosteric transition in hemoglobin. *Proc. Natl Acad. Sci. USA* **108**, 5608–5613 (2011).
43. Cammarata, M., Levantino, M., Wulff, M. & Cupane, A. Unveiling the timescale of the R–T transition in human hemoglobin. *J. Mol. Biol.* **400**, 951–962 (2010).
44. Tekpinar, M. & Zheng, W. Coarse-grained and all-atom modeling of structural states and transitions in hemoglobin. *Proteins* **81**, 240–252 (2013).
45. Lipscomb, W. N. & Kantrowitz, E. R. Structure and mechanisms of *Escherichia coli* aspartate transcarbamoylase. *Acc. Chem. Res.* **45**, 444–453. (2012).
46. Bocquet, N. *et al.* A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family, *Nature* **445**, 116–119 (2007).
47. Bocquet, N. *et al.* X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. *Nature* **457**, 111–114 (2009).
48. Hilf, R. & Dutzler, R. X-ray structure of a prokaryotic pentameric ligand-gated ion channel. *Nature* **452**, 375–379 (2008).
49. Hilf, R. J.& Dutzler, R. Structure of a potentially open state of a proton-activated pentameric ligand-gated ion channel. *Nature* **457**, 115–118 (2009).
50. Corringer, P. J. *et al.* Structure and pharmacology of pentameric receptor channels: from bacteria to brain. *Structure.* **20**, 941–956 (2012).
51. Taly, A. *et al.* Normal mode analysis suggests a quaternary twist model for the nicotinic receptor gating mechanism. *Biophys. J.* **88**, 3954–3965 (2005).
52. Calimet, N., Simoes, M., Changeux, J. P., Karplus, M., Taly, A. & Cecchini, M. A gating mechanism of pentameric ligand-gated ion channels. *Proc. Natl Acad. Sci. USA* http://dx.doi.org/110.1073/pnas.1313785110 (2013).
53. Auerbach, A. The energy and work of a ligand-gated ion channel. *J. Mol. Biol.* **425**, 1461–1475 (2013).
54. Prevost, M. S. *et al.* A locally closed conformation of a bacterial pentameric proton-gated ion channel. *Nature Struct. Mol. Biol.* **19**, 642–649 (2012).
55. Pozzi, N., Vogt, A. D., Gohara, D. W. & Di Cera, E. Conformational selection in trypsin-like proteases. *Curr. Opin. Struct. Biol.* **22**, 421–431 (2012).
56. Mosior, M., Six, D. A. & Dennis, E. A. Group IV cytosolic phospholipase A2 binds with high affinity and specificity to phosphatidylinositol 4,5-bisphosphate resulting in dramatic increases in activity. *J. Biol. Chem.* **273**, 2184–2191 (1998).
57. Bouvier, M. Unraveling the structural basis of GPCR activation and inactivation. *Nature Struct. Mol. Biol.* 2013 **20**, 539–541 (2013).
58. Liu, J. J., Horst, R., Katritch, V., Stevens, R. C. & Wüthrich, K. Biased signaling pathways in β2-adrenergic receptor characterized by 19F-NMR. *Science* **335**, 1106–1110 (2012).
59. Fotiadis, D. *et al.* Structure of the rhodopsin dimer: a working model for G-protein-coupled receptors. *Curr. Opin. Struct. Biol.***16**, 252–259 (2006).
60. Fung, J. J. *et al.* Ligand-regulated oligomerization of β_2-adrenoceptors in a model lipid bilayer. *EMBO J.* **28**, 3315–3328 (2009).
61. Audet, M. & Bouvier, M. Restructuring G-protein-coupled receptor activation. *Cell* **151**, 14–23 (2012).
62. Daily, M. D. & Gray, J. J. Allosteric communication occurs via networks of tertiary and quaternary motions in proteins. *PLoS Comput. Biol.* **5**, e1000293 (2009).
63. Ferreiro, D. U., Hegler, J. A., Komives, E. A. & Wolynes, P. G. On the role of frustration in the energy landscapes of allosteric proteins. *Proc. Natl Acad. Sci. USA* **108**, 3499–3503 (2011).
64. Ferreon, A. C., Ferreon, J. C., Wright, P. E. & Deniz, A. A. Modulation of allostery by protein intrinsic disorder. *Nature.* **498**, 390–394 (2013).
65. Lewis, M. Allostery and the lac operon. *J. Mol. Biol.* **425**, 2309–2316 (2013).
66. Arkhipov, A. *et al.* Architecture and membrane interactions of the EGF receptor. *Cell* **152**, 557–569 (2013).
67. Endres, N. F. *et al.* Conformational coupling across the plasma membrane in activation of the EGF receptor. *Cell* **152**, 543–556 (2013).
68. Larion, M., Salinas, R. K., Bruschweiler-Li, L., Miller, B. G. & Brüschweiler, R. Order-disorder transitions govern kinetic cooperativity and allostery of monomeric human glucokinase. *PLoS Biol.* **10**, e1001452 (2012).
69. Zhang, P. *et al.* Structure and allostery of the PKA RIIβ tetrameric holoenzyme. *Science.* **335**, 712–716 (2012).
70. Kalodimos, C. G. Protein function and allostery: a dynamic relationship. *Ann. N.Y. Acad. Sci.* **1260**, 81–86 (2012).
71. Tzeng, S. R. & Kalodimos, C. G. Protein activity regulation by conformational entropy. *Nature.* **488**, 236–240 (2012).
72. Popovych, N., Tzeng, S. R., Tonelli, M., Ebright, R. H. & Kalodimos, C. G. Structural basis for cAMP-mediated allosteric control of the catabolite activator protein. *Proc. Natl Acad. Sci. USA* **106**, 6927–6932 (2009).
73. McLeish, T. C., Rodgers, T. L. & Wilson, M. R. Allostery without conformation change: modelling protein dynamics at multiple scales. *Phys. Biol.* **10**, 056004 (2013).
74. Bray, D. The propagation of allosteric states in large multiprotein complexes. *J. Mol. Biol.* **425**, 1410–1414 (2013).
75. Saibil, H. R., Fenton, W. A., Clare, D. K. & Horwich, A. L. Structure and allostery of the chaperonin GroEL. *J. Mol. Biol.* **425**,1476–1487 (2012).
76. Dyachenko, A., Gruber, R., Shimon, L., Horovitz, A. & Sharon, M. Allosteric mechanisms can be distinguished using structural mass spectrometry. *Proc. Natl Acad. Sci. USA* **110**, 7235–7239 (2013).
77. Mikoshiba, K. The discovery and structural investigation of the IP_3 receptor and the associated IRBIT protein. *Adv. Exp. Med. Biol.* **740**, 281–304 (2012).
78. Sledź, P., Förster, F. & Baumeister, W. Allosteric effects in the regulation of 26S proteasome activities. *J. Mol. Biol.* **425**, 1415–1423 (2013).
79. Sudakin, V. *et al.* The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis. *Mol. Biol. Cell.* **6**, 185–197 (1995).
80. Barford, D. Structural insights into anaphase-promoting complex function and mechanism. *Phil. Trans. R. Soc. B* **366**, 3605–3624 (2011).
81. Oliveira, D. M., Nakaie, C. R., Sousa, A. D., Farah, C. S., & Reinach, F. C. Mapping the domain of troponin T responsible for the activation of actomyosin ATPase activity. Identification of residues involved in binding to actin. *J. Biol Chem.* **275**, 27513–27519 (2000).
82. Gollapudi, S. K., Mamidi, R., Mallampalli, S. L. & Chandra, M. The N-terminal extension of cardiac troponin T stabilizes the blocked state of cardiac thin filament. *Biophys. J.* **103**, 940–948 (2012).
83. Domitrovic, T. *et al.* Virus assembly and maturation: auto-regulation through allosteric molecular switches. *J. Mol. Biol.* **425**, 1488–1496 (2013).
84. Revah, F. *et al.* Mutations in the channel domain alter desensitization of a neuronal nicotinic receptor. *Nature.* **353**, 846–849 (1991).
85. Taly, A. *et al.* Implications of the quaternary twist allosteric model for the physiology and pathology of nicotinic acetylcholine receptors. *Proc. Natl Acad. Sci. USA* **103**, 16965–16970 (2006).
86. Cotecchia, S., Ostrowski, J., Kjelsberg, M. A., Caron, M. G. & Lefkowitz, R. J. Discrete amino acid sequences of the α1-adrenergic receptor determine the selectivity of coupling to phosphatidylinositol hydrolysis. *J. Biol. Chem.* **267**, 1633–1639 (1992).
87. Taly, A., Corringer, P. J., Guedin, D., Lestage, P. & Changeux, J. P. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. *Nature Rev. Drug Discov.* **8**, 733–750 (2009).
88. Changeux, J. P. The concept of allosteric modulation: an overview. *Drug Discov. Today Technol.* **10**, e2203–e228 (2013).
89. Galzi, J. L. & Changeux, J. P. Neurotransmitter-gated ion channels as unconventional allosteric proteins. *Curr. Opin. Struct. Biol.* **4**, 554–565 (1994).
90. Sawyer, G. W., Chiara, D. C., Olsen, R. W. & Cohen, J. B. Identification of the bovine γ-aminobutyric acid type A receptor α-subunit residues photolabeled by the imidazobenzodiazepine [^{3}H]Ro15-4513 *J. Biol. Chem.* **277**, 50036–50045 (2002).
91. Li, G. D., Chiara, D. C., Cohen, J. B. & Olsen, R. W. Numerous classes of general anesthetics inhibit etomidate binding to γ-aminobutyric acid type A (GABAA) receptors. *J. Biol. Chem.* **285**, 8615–8620 (2010).

92. Nury, H. *et al.* X-ray structures of general anaesthetics bound to a pentameric ligand-gated ion channel. *Nature.* **469**, 428–431 (2011).
93. Hibbs, R. E. & Gouaux, E. Principles of activation and permeation in an anion-selective Cys-loop receptor. *Nature.* **474**, 54–60 (2011).
94. Teichberg, V. I., Sobel, A. & Changeux, J. P. *In vitro* phosphorylation of the acetylcholine receptor. *Nature.* **267**, 540–542 (1977).
95. Canals, M., Lane, J. R., Wen, A., Scammells, P. J., Sexton, P. M. & Christopoulos, A. A. Monod–Wyman–Changeux mechanism can explain G protein-coupled receptor (GPCR) allosteric modulation. *J Biol Chem.* 287, 650–659 (2012).
96. Liu, W. *et al.* Structural basis for allosteric regulation of GPCRs by sodium ions. *Science.* **337**, 232–236 (2012).
97. Frauenfelder, H., Sligar, S. G. & Wolynes, P. G. The energy landscapes and motions of proteins. *Science.* **254**, 1598–1603 (1991).
98. Nussinov, R. & Tsai, C. J. Allostery in disease and in drug discovery. *Cell.* **153**, 293–305 (2013).
99. Kar, G., Keskin, O., Gursoy, A. & Nussinov, R. Allostery and population shift in drug discovery. *Curr. Opin. Pharmacol.* **10**, 715–722 (2010).
100. Crick, F. H. & Wyman, J. A footnote on allostery. *J. Mol. Biol.* **425**, 1500–1508 (2013).
101. Edelstein, S. J. & Le Novère, N. Cooperativity of allosteric receptors. *J. Mol. Biol.* **425**, 1424–1432 (2013).
102. Martins, B. M. & Swain, P. S. Trade-offs and constraints in allosteric sensing. *PLoS Comput. Biol.***7**, e1002261 (2011).
103. Marzen, S., Garcia, H. G. & Phillips, R. Statistical mechanics of Monod–Wyman–Changeux (MWC) models. *J. Mol. Biol.* **425**, 1433–1460 (2013).
104. Changeux, J. P. On the allosteric properties of biosynthetic L-threonine deaminase. VI. General discussion. *Bull. Soc. Chim. Biol.* **47**, 281–300 (1965).
105. Changeux, J. P. & Edelstein, S. Conformational selection or induced fit? 50 years of debate resolved. *F1000 Biol. Rep.* **3**,19 (2011).
106. Iwata, S., Kamata, K., Yoshida, S., Minowa, T. & Ohta, T. T and R states in the crystals of bacterial L-lactate dehydrogenase reveal the mechanism for allosteric control. *Nature Struct. Biol.* **1**, 176–185 (1994).
107. Kantrowitz, E. R. Allostery and cooperativity in *Escherichia coli* aspartate transcarbamoylase. *Arch. Biochem. Biophys.* **519**, 81–90 (2012).
108. Perutz, M. F. X-ray analysis of hemoglobin. *Science* **140**, 863–869 (1963).
109. Kantrowitz, E. R. & Lipscomb, W. N. *Escherichia coli* aspartate transcarbamylase: the relation between structure and function. *Science* **241**, 669–674 (1988).
110. Lewis, M. *et al.* Crystal structure of the lactose operon repressor and its complexes with DNA and inducer. *Science* **271**, 1247–1254 (1996).
111. Palczewski, K. *et al.* Crystal structure of rhodopsin: a G protein-coupled receptor. *Science.* **289**, 739–745 (2000).

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Competing interests statement
The author declares no competing interests.

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