Allostery and the Monod-Wyman-Changeux Model After 50 Years

Jean-Pierre Changeux

Collège de France & Institut Pasteur, URA CNRS 2182, Paris Cedex 15 75724, France; email: changeux@noos.fr

Annu. Rev. Biophys. 2012. 41:103–33

First published online as a Review in Advance on January 6, 2012

The *Annual Review of Biophysics* is online at biophys.annualreviews.org

This article's doi: 10.1146/annurev-biophys-050511-102222

1936-122X/12/0609-00103$20.00

Keywords

allosteric proteins, signal transduction, conformational selection versus induced fit, drug design, receptor diseases

Abstract

The Monod-Wyman-Changeux (MWC) model was conceived in 1965 to account for the signal transduction and cooperative properties of bacterial regulatory enzymes and hemoglobin. It was soon extended to pharmacological receptors for neurotransmitters and other macromolecular entities involved in intracellular and intercellular communications. Five decades later, the two main hypotheses of the model are reexamined on the basis of a variety of regulatory proteins with known X-ray structures: (*a*) Regulatory proteins possess an oligomeric structure with symmetry properties, and (*b*) the allosteric interactions between topographically distinct sites are mediated by a conformational transition established between a few preestablished states with conservation of symmetry and ligand-directed conformational selection. Several well-documented examples are adequately represented by the MWC model, yet a few possible exceptions are noted. New questions are raised concerning the dynamics of the allosteric transitions and more complex supramolecular ensembles.

Contents

INTRODUCTION

The word allosteric was coined to qualify the mechanism of feedback inhibition exerted on bacterial regulatory enzymes by their regulatory ligands (19, 28, 111). In contrast to the classical mechanism of inhibition by mutual exclusion due to steric hindrance, the allosteric mechanism for feedback inhibition takes place between non-overlapping (19), stereochemically distinct sites for substrates and regulatory ligands. Such indirect interactions mediated by discrete, reversible alterations of the molecular structure of the protein were named allosteric interactions (110, 111). The original terminology remains widely used. Its conceptual and biological implications have been presented (28).

The underlying mechanism suggested to account for the conformational change (110) initially relied on the induced-fit theory proposed by Koshland (97) for the specificity of enzyme action. The fit occurs "only *after* a change in shape of the enzyme molecule had been induced by the substrate" (97; italics mine). By analogy, binding of the regulatory ligand would induce the protein to adopt the adequate conformation (110). The results obtained with L-threonine deaminase (19–24), in particular the effects of regulatory ligands on the cooperativity of substrate binding and their

concomitant loss upon desensitization, as also noted for aspartate transcarbamylase (63, 64), soon led to a paradigmatic shift from instruction to selection (30). The new concept proposed that, instead of being induced by the ligand, the conformational transition is established as a preformed equilibrium between a few discrete states—independent of ligand structure and occupancy—differentially stabilized by the ligands. This conformational selection mechanism became one of the main hypotheses of the Monod-Wyman-Changeux (MWC) model, conceived in 1965, with the aim of bringing a "general interpretation of allosteric effects in terms of *certain features* of protein structure," in particular their organization into symmetrical oligomers (112; italics mine). This review compares what these features were in 1965 and how they stand in 2012 on the basis of the new structural, kinetic, and molecular dynamics simulation data that have become available since then.

MWC: Monod-Wyman-Changeux model 1965

HYPOTHESES OF THE MONOD-WYMAN-CHANGEUX MODEL

At the time the MWC model was elaborated, little was known about the detailed structure of regulatory proteins, except for the hemoglobin X-ray structure established by Perutz et al. (124, 126). In this context, the MWC theory included general reflections and hypotheses about the quaternary organization and symmetry properties of regulatory proteins as the basis for a conformational mechanism mediating signal transduction.

The original hypotheses of the model focus on structure and conformational transition.

- Structure: "[*A*]*llosteric proteins are oligomers* resulting from the assembly of protomers associated in such a way that the molecule possesses *at least one axis of symmetry;* . . . the conformation of each protomer is *constrained* by its association with the other protomers" (112; italics mine). In other words, the oligomeric structure creates a potentially cooperative assembly of subunits
- Conformational transition: "[T]wo (at least two) states are reversibly accessible to allosteric oligomers; these states differ by the distribution and/or energy of interprotomer bonds, and therefore also by the conformational constraints imposed upon the protomers; as a result, the affinity of one (or several) of the stereospecific sites toward the corresponding ligand is altered when a transition occurs from one to the other state; when the protein goes from one state to another state, its *molecular symmetry* (including the symmetry of the conformational constraints imposed upon each protomer) is *conserved*" (112; italics mine). The cooperative ligand binding thus follows from the cooperative interactions between subunits. The notion that the ligands selectively stabilize the state(s) to which they preferentially bind and thereby mediate signal transduction via selection of conformational states arises directly from the formal description of the MWC model (112) (**Figure 1*a***).

In the absence of ligand, the two states, R_0 and T_0, are assumed to spontaneously establish an equilibrium characterized by an intrinsic equilibrium constant, $L_0 = T_0/R_0$, called the allosteric constant. The ligand differentially binds to each state with microscopic dissociation constants, K_R and K_T, that "are the same for all homologous sites in each of the two states" (112) independent of their ligand occupancy. Thus, the model distinguishes a "function of state," $\bar{R}$, which describes the conformational equilibrium, and a "binding function," $\bar{Y}$, which distinctly evolves as a function of ligand concentration. The signal transduction mechanism then results exclusively from the displacement, or conformational shift, of the spontaneous equilibrium between the *R* and *T* states. The general equation, which includes the nonexclusive binding of substrate and allosteric modulator to the two states, was then established (133).

The MWC model was subsequently reformulated and applied to larger—unlimited—assemblies of proteins, such as membrane proteins, by Changeux et al. (35). This leads to a general

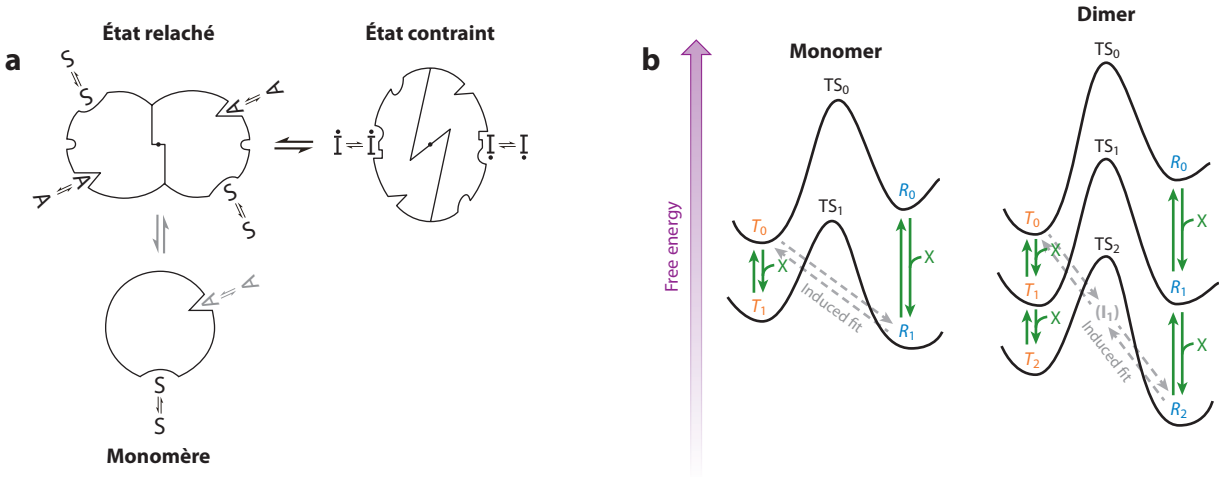

Figure 1

The Monod-Wyman-Changeux (MWC) model. (*a*) The two-state concerted MWC 1965 model (112) drawn from the original diagram by Changeux (22, 24) (translations: *État relaché*, relaxed state; *État contraint*, constrained state; *Monomère*, monomer). Adapted from References 22 and 23 with permission. (*b*) Recent thermodynamic representation by Changeux & Edelstein (30) of the MWC 1965 model for a dimer (*right*) and of the Changeux et al. 1967 model (35) for a hypothetical monomer (*left*). *T* and *R* states are presented on a vertical free energy scale and include the transition state (TS) kinetic barriers for their interconversion, estimated according to linear free energy principles for the dimer (see 51 and references therein). The pathway for the Koshland-Némethy-Filmer induced-fit mechanism (98) is represented by the gray dashed arrows and includes an intermediate (I) state for the dimer. Adapted from Reference 30 with permission.

thermodynamic formulation based on the conformational transition of single units (or protomers) modulated (or not) by the interaction with other protomers.

A single protomer equilibrium is established between a minimum of two states with different affinity for the ligand *f*:

$$r_0 \leftrightarrow s_0$$

$$r_1 \leftrightarrow r_0 + f \quad \text{and} \quad s_1 \leftrightarrow s_0 + f$$

with the isomerization constant: $(s_0)/(r_0) = l'$.

In a system of interacting protomers, for instance within a membrane lattice, the free energy ΔF of the transition $(s \leftrightarrow r)$ is proposed to depend on the fraction of protomers that are already in the *r* state and expressed as $\Delta F = (\varepsilon - \eta(r))$.

The isomerization constant $l' = (s)/(r)$ is then simply

$$l' = \exp[\beta \Delta F] = l\Lambda^{(r)}.$$

Depending on the value of Λ and thus on the free energy of the interaction between protomers (other formulations can be made), the model predicts the existence of various classes of responses to specific regulatory signals exhibited by biological systems: from a graded response of a single-receptor protomer or oligomeric receptor (MWC model) to an all-or-none phase transition response in large and periodic protein assemblies (**Figure 1*b***).

In more general terms, the Changeux et al. model developed in 1967 (35) lays the groundwork for a general thermodynamic mechanism of conformational selection (30). The model was subsequently documented in the case of globular proteins and pharmacological receptors, with a

distinct focus on either single protomer transitions in G-protein-coupled receptors (GPCRs) (16, 47, 92) or an energy landscape theory of protein structure and dynamics (13, 47, 72).

Another generalization of the MWC model was proposed by Wyman (160, 161). The generalization is based on the concept that the responses of a macromolecular system are linked to a diversity of chemical variables and, by extension, to large systems such as hemocyanins, incorporating a hierarchy of conformational equilibria and their "nesting" at each structural level. Soon after the MWC model was published, Koshland, Némethy, and Filmer (98) suggested a sequential, induced-fit mechanism (the KNF model), which posits multiple conformational states, including stable "intermediate" (or mixed states), with tertiary changes adapted to fit the ligand structure induced by its occupancy. This model involves "a progressive change" of conformation and assumes "that a subunit in conformation *b* is present only when *s* is bound to it" (98). The appropriate equations for the MWC and KNF models were derived, and a general scheme covering both models is discussed by Eigen (53). As in the Changeux et al. (35) models, Eigen states that the induced-fit KNF model formally appears as a limiting case in which the unbound active R_0 state of MWC is simply omitted (13, 47, 72, 92). Accordingly, the experimental predictions differ strikingly between the two models. In the past 50 years, these predictions have been experimentally tested in a large variety of systems. For the sake of clarity, I have selected only a limited number of examples focused on proteins whose full X-ray structure is known.

GPCR: G-protein-coupled receptor

KNF: Koshland-Némethy-Filmer model 1966

OLIGOMERIC STRUCTURE AND SYMMETRY PROPERTIES

The MWC model states that "allosteric proteins are oligomers possessing at least one axis of symmetry" (112). The modes of association of the subunits in an oligomer fall into two classes. In isologous associations, the domain of bonding between subunits involves two identical binding sets, confers a twofold axis of rotational symmetry, and gives rise to closed and even-numbered oligomers. In heterologous associations, the domain of bonding is made up of two different binding sets with no element of symmetry and may give rise not only to polydisperse, possibly large, helical polymers but also to closed symmetrical structures such as trimers, tetramers, and pentamers provided that the angles defined by the domains of bonding are adequate. The isologous mode was at that time privileged because of its simplicity from an evolutionary point of view. Yet the heterologous mode is also frequently encountered in regulatory proteins. Although this distinction has somewhat fallen out of use (69, 86), it is still helpful for interpreting the diversity of known allosteric protein crystallographic structures, because a large fraction of the well-characterized signal-transducing proteins are oligomers.

Regulatory Enzymes

As anticipated, isologous dimers or tetramers are common among regulatory enzymes. For example, glycogen phosphorylase is an isologous dimer with a dyad axis of symmetry (141). Phosphofructokinase is an isologous tetramer with four identical protomers occupying the corners of a squashed tetrahedron and three mutually perpendicular axes of dyad symmetry (134). Biosynthetic L-threonine deaminase, which had an important place in the early studies on allosteric proteins (19, 28), is an isologous tetramer with C_{222} symmetry (60). The "honorary" allosteric enzyme, hemoglobin X-ray structure was recognized by Perutz et al. (124, 126) as a tetramer $(\alpha\beta)_2$ with pseudotetrahedral symmetry, a true dyad axis relating the chains $\alpha 1$ to $\alpha 2$ and $\beta 1$ to $\beta 2$, and right-angle pseudodyad axes relating $\alpha 1$ to $\beta 1$ and $\alpha 2$ to $\beta 2$ (123). Protein kinase A holoenzyme contains two catalytic (C) subunits and one regulatory (R) subunit dimer activated cooperatively

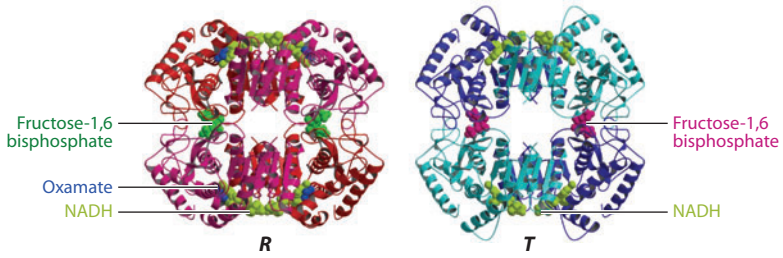

Figure 2

Allosteric transition captured by X-ray crystallography illustrating the conservation of symmetry with L-lactate dehydrogenase from *Bifidobacterium longum* (83). The ribbon structures illustrate the location of the catalytic and regulatory binding sites at the boundary between subunits and the conservation of symmetry in the *R* and *T* end states. Adapted from Reference 83 with permission.

by cAMP. Additional, critical cooperativity is associated with the organization of the enzyme as a symmetrical tetramer, which in reality is a dimer of dimers (14).

Escherichia coli aspartate transcarbamylase (ATCase), an allosteric protein much studied in previous decades (62, 90), is composed of distinct catalytic and regulatory subunits. The catalytic subunits form two heterologously associated trimers, which in turn form equilateral triangles, and the regulatory subunits make three isologous dimers. An axis of threefold symmetry passes through the center of the molecule and three axes of twofold symmetry are perpendicular to the triad (90). The molecule of ATCase thus combines isologous and heterologous associations.

These few examples, among many other well-characterized regulatory enzymes, illustrate the validity of a basic structural hypothesis of the MWC. Allosteric proteins are symmetrical oligomers (**Figure 2**). Yet, as discussed below, a few exceptions may exist.

Membrane Proteins

A critical level of supramacromolecular organization is the cell membrane and its component proteins mediating intercellular communication and signal propagation. The suggestion was made in the 1960s that the concept of allosteric interaction might extend to membrane proteins and specifically those that mediate synaptic transmission (24, 25, 35).

Symmetry restrictions were noted as a consequence of the integration of the proteins into the membrane (26, 34). First, twofold rotation axes are either normal to or coplanar with the membrane; second, rotation axes of order higher than two are necessarily normal to the lattice. Interestingly, axes of symmetry perpendicular to the membrane confer a transverse polarity to the membrane protein such that its outside face differs from the cytoplasmic one; on the other hand, a protein with a coplanar rotation axis exposes the same face to both membrane sides. As we shall see, transverse polarity most commonly occurs in allosteric membrane proteins but exceptions may exist. Two principal categories of allosteric membrane proteins are the ligand-gated ion channels and the GPCRs.

Ligand-gated ion channels. This family of allosteric membrane proteins includes an important group of receptors for neurotransmitters that incorporate an ion channel.

nAChR: nicotinic acetylcholine receptor

Nicotinic acetylcholine receptor family of pentameric receptors. The nicotinic acetylcholine receptor (nAChR) is the first identified neurotransmitter receptor, ion channel, and ligand-gated ion

channel (27, 29, 32). It is an integral membrane protein comprising five identical or homologous subunits symmetrically arranged around a central ionic channel located in the C_5 axis of symmetry (146, 151). Each subunit consists of a large amino-terminal extracellular domain carrying the ACh binding site and a transmembrane domain comprising four segments (TM1–TM4), with the TM2 segment lining the ion channel (**Figure 2**). In agreement with the physiological data, the molecule possesses a transverse polarity. This organization is conserved in a superfamily of ligand-gated ion channels referred to as pentameric receptors, which includes, in addition to nAChRs, 5-HT_3, $GABA_A$, $GABA_C$, and glycine receptors, some glutamate, histamine, and 5-HT-activated anionic receptors.

The discovery that bacterial orthologs of nAChRs (148) behave as ligand-gated ion channels (12) led to high-resolution crystal structures of *Gloeobacter violaceus* and *Erwinia chrysanthemi* receptors (11, 76, 77) (**Figure 3**). Their structures disclose a fivefold axis of symmetry perpendicular to the membrane and a core structure that is strikingly conserved in the superfamily of pentameric receptors from prokaryotic to eukaryotic. Recently, indeed, the crystal structure of the homopentameric *Caenorhabditis elegans* glutamate-gated chloride channel (GluCl) revealed the same overall

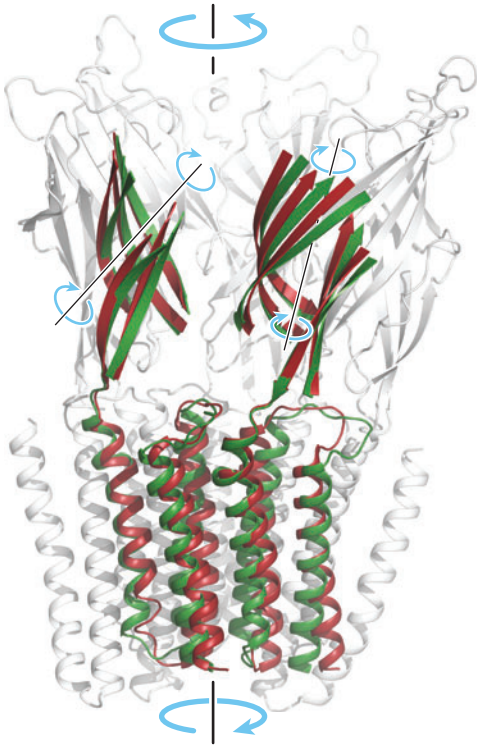

Figure 3

Allosteric transition captured by X-ray crystallography illustrating the conservation of symmetry with prokaryotic pentameric ligand-gated ion channels in open (*green*, from *Gloeobacter*) and closed (*red*, from *Erwinia*) channel conformations (11). Common core superimposition illustrates that the *Gloeobacter* subunits display a quaternary twist compared with *Erwinia* subunits, with counterclockwise (versus clockwise) rotation in the upper (versus lower) part of the pentamer (when viewed from the extracellular compartment) with conservation of symmetry. It further shows a significant reorganization of the tertiary structure of the subunits. Adapted from Reference 11 with permission.

architecture at the atomic level (74). In agreement with the MWC model, pentameric receptors are symmetrical oligomers.

Glutamate receptor family of tetrameric receptors. Excitatory neurotransmission in the central nervous system is mediated mainly by cationic ionotropic glutamate receptors (150). The family includes AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) (GluA1–GluA4), kainate (GluK1–GluK5), and NMDA (*N*-methyl D-aspartate) (GluN1, $GluN2_A$–$GluN2_D$, $GluN3_A$–$GluN3_B$) receptors. Whereas NMDA receptors are obligatory heterotetramers, AMPA and kainate subunits form functional homotetramers, although native receptors are usually heterotetramers. Each subunit possesses a large extracellular amino-terminal domain that participates in subtype-specific receptor assembly, trafficking, and modulation; a ligand-binding domain; and a transmembrane domain that includes the ion channel. The crystal structure of the AMPA-sensitive, homotetrameric rat GluA2 receptor (140) displays an overall axis of twofold symmetry, with the extracellular domains organized as pairs of local dimers, and concomitantly a fourfold symmetry of the ion channel. Each extracellular domain possesses its own local intradimer axis of twofold symmetry. This is a rare, though not unique, situation in which the protomer is not an asymmetrical unit but possesses its own symmetry in addition to the overall symmetry of the molecule. A twofold-to-fourfold-symmetry mismatch takes place with an abrupt transition located at the extracellular boundary of the membrane bilayer, with possible implications for the channel-gating process (see Reference 65).

ATP P2X receptors of trimeric receptors. P2X receptors are cation-selective ion channels gated by extracellular ATP and are implicated in diverse physiological processes, from the nervous system to the immune system. The crystal structure for the $P2X_4$ receptor (91) shows an unusual trimeric organization: a chalice-like shape, with the large extracellular domain protruding 70 Å above the membrane plane with three vestibules, and a smaller transmembrane stem extending 28 Å through the membrane. The three subunits are related by a C_3 axis of symmetry perpendicular to the plane of the membrane, with strong similarities to the acid-sensing ion channel structure (68).

Ion channels. The large population of ion channels that regulate membrane potential is only briefly mentioned, although they can be modulated allosterically by electric fields and various pharmacological agents, including ions or toxins. The X-ray structure of the KcsA potassium channel from *Streptomyces lividans* reveals four identical protein subunits in a symmetric (C_4) complex with a central ion-conducting pore (48). The crystal structure of a voltage-gated Na^+ channel from *Arcobacter butzleri* captured in a closed-pore conformation consists of four homologous repeat domains (I–IV) each comprising six transmembrane segments with four activated voltage sensors and a C_4 axis of pseudosymmetry (121).

Conclusion. All the ligand-gated ion channels and other ion channels with known X-ray structures are oligomers with symmetry axes perpendicular to the membrane plane and with transmembrane polarity. Hence, they all belong to the heterologous mode of association between subunits. This arrangement favors the accommodation of different types of homologous subunits within the same oligomer. In this respect, the nAChR is exemplary, with 17 genes known to encode nAChR subunits in vertebrates and with many possible combinations, although not all have been found (29, 70). Rules of inclusion/exclusion exist based on complementary interfaces between subunits. Complementarity occurs between principal faces carried by the α2–α4, α6–α9 subunits and complementary faces carried by the β2, β4, α7–α10 subunits (29, 70). The diversity ranges

from straightforward homo-oligomer such as $\alpha 7_5$ to combinations such as $\alpha 4_2\beta 2_3$, $\alpha 1_2\beta\gamma\delta$, $(\alpha 4\beta 2)_2\alpha 5$, and $\alpha 4\alpha 6\beta 2\beta 3\beta 4$, among others. Similar situations occur with other ligand-gated ion channels, particularly the $GABA_A$ receptors. The heterologous mode of association, which has a much broader structural diversity of nAChR oligomers than the isologous mode does, permits marked physiological and tissue targeting differences.

G-protein-coupled receptors. The three-dimensional structures of several GPCRs in their monomeric state, such as rhodopsin (115, 119), β2-adrenergic (37) and β1-adrenergic (157) receptors, adenosine A_{2A} (84) receptors, CXCR4 chemokine receptors (159), D_3 dopamine receptors (38), and histamine receptors (136), have been solved. Regulatory ligand binding is systematically located within a cavity or pocket at approximately 35 Å from the G-protein-binding domain in the cytoplasmic face, and much farther for class C receptors such as mGlu with a large, bilobed N-terminal domain.

The relationship between the formation of homodimers or heterodimers of GPCRs and signal transduction is still a debated issue and may vary among the class A, B, and C GPCRs (96, 128). Rhodopsin and the β2-adrenergic receptor, which belong to class A GPCRs, signal efficiently through G proteins when reconstituted into lipid nanodiscs containing only a single receptor molecule. Thus, class A GPCRs, at variance with the MWC model, may function without oligomerization.

Numerous studies, however, have revealed more complex activation mechanisms due to the ability of diverse GPCRs to form dimers or even larger oligomeric complexes. For the class A serotonin receptor $5HT_4$, activation of one protomer in a dimer is sufficient for G protein activation, but coupling efficiency increases by 100% when both protomers are activated (122). For the class C GPCRs mGlu and $GABA_B$, dimerization is mandatory for receptor activation: mGlu receptors form homodimers stabilized by an intersubunit disulfide bridge, and $GABA_B$ receptors are obligatory heterodimers ($GABA_{B1}$ + $GABA_{B2}$) (41, 128). Furthermore, time-resolved FRET experiments show that $GABA_B$ heterodimers form stable tetramers (that are present in the brain) with a decrease of G-protein-coupling efficiency (41). Still, no X-ray structure of a multimeric GPCR is available.

Dual topology proteins. According to von Heijne (154), dual topology refers to proteins that are undecided in terms of their overall orientation in the membrane and can insert in two opposite orientations with an approximate 1:1 stoichiometry. This feature would correspond to proteins possessing a coplanar twofold axis of symmetry and no transverse polarity (26, 34). Their existence is still debated. The X-ray structure of EmrE, a multidrug transporter from *E. coli*, reveals that the first three helices form a three-helix bundle packed against the equivalent helices of another EmrE molecule, forming a dimer with a dyad axis of symmetry coplanar to the membrane. Selenomethionine markers confirm an antiparallel orientation for the monomers, supporting a dual topology model (36). Other examples of a dual topology protein have been reported (154), yet this disposition is rare among membrane proteins.

Other examples of allosteric membrane receptors. Among the broad diversity of membrane receptors three examples have been selected for their physiological importance and/or singular transmembrane organization.

Receptor tyrosine kinase. Receptor tyrosine kinases (RTKs) are the high-affinity cell surface receptors for many polypeptide growth factors, cytokines, and hormones, but they also have a critical role in the development and progression of many types of cancer. The crystal structure of the extracellular ligand-binding domain of several RTKs (i.e., insulin, EGFR, and FGFR), but

not of the full-length receptor, is known. Yet the active form is a homodimer either covalently linked (insulin RTK) or stabilized by the specific ligands (2, 6).

PPARγ: peroxisome proliferator-activated receptor γ

RXRα: retinoid X receptor α

Toll receptor. The Toll-like receptor family comprises allosteric membrane receptors that recognize pathogen-associated molecular signals and initiate inflammatory responses. Toll-like 3 receptor extracellular domain recognizes double-stranded RNA, a viral replication intermediate, and recruits the adaptor protein TRIF (TIR-domain-containing adapter-inducing interferon-β) to its cytoplasmic Toll interleukin-1 receptor domain, thereby initiating a signaling cascade that results in the secretion of type I interferons and other inflammatory cytokines. The crystal structure of a complex between two mouse extracellular domains and double-stranded RNA (106) reveals a symmetrical isologous horseshoe dimer that binds a double-stranded RNA string at two sites located at opposite ends of the horseshoe, and an intermolecular contact between the two terminal domains coordinates and stabilizes the dimer. This juxtaposition mediated by the RNA stick could mediate transmembrane signaling by stabilizing the dimer of the cytoplasmic Toll interleukin-1 receptor domains. Toll receptors are authentic allosteric membrane receptors.

Nuclear Receptors

The *lac* repressor is a well-studied bacterial allosteric protein that, together with the specific DNA element of its operator, forms a genetic switch of the *lac* operon (**Figure 4**). The X-ray structure of the *E. coli lac* repressor (103) reveals a roughly V-shaped homotetramer that is a dimer of isologous dimers with a pseudosymmetric interface. The repressor is essentially a tethered dimer. Each repressor subunit consists of four domains: (*a*) an N-terminal domain, or headpiece, with a helix-turn-helix motif that interacts with the operator; (*b*) a hinge region, or linker, devoid of canonical secondary structure, that connects the DNA-binding domain to the core of the repressor and becomes ordered in the presence of DNA; (*c*) a sugar-binding domain, or repressor core, that is composed of two subdomains that are topologically similar but without amino acid sequence similarity; and (*d*) a C-terminal helix. Each monomer binds one inducer molecule with equal affinity, but isologous dimer formation is required for DNA binding. In the absence of DNA, the repressor molecule is partially disordered. When the dimer binds to the operator it becomes a fully symmetrical molecule with a dyad axis of symmetry from the N-terminal domain to the C-terminal helix and a change in properties of the ligand-binding domain.

In eukaryotes nuclear hormone receptors control numerous physiological processes through the regulation of gene expression. The recent elucidation of the X-ray structure of the PPARγ–RXRα (peroxisome proliferator-activated receptor–retinoid X receptor) heterodimer bound to DNA (18) reveals an overall architecture strikingly similar to that of the *lac* repressor, particularly the similar distinct ligand- and DNA-binding domains. Despite manifest sequence differences between PPARγ and RXRα, the relative arrangements of the two receptors, their domain-domain interactions, and the receptor interfaces with DNA are similar, indicating a pseudosymmetrical organization around an axis perpendicular to the DNA element. Synchrotron radiation X-ray scattering, small-angle neutron scattering, and steady-state and time-resolved fluorescence spectroscopy studies of several heterodimers in solution including PPAR-RXR reveal a common architecture on DNA direct repeat elements, pointing to the important role played by the hinge domains in establishing and maintaining, such as in *lac* repressor, the integrity of the structures in the process of signal transduction (131).

Supramolecular Allosteric Ensembles

Ensembles of allosteric units interacting in assemblies larger than the standard oligomers can also occur. Chaperonins are the complex enzymatic machines found in bacteria as well as chloroplasts

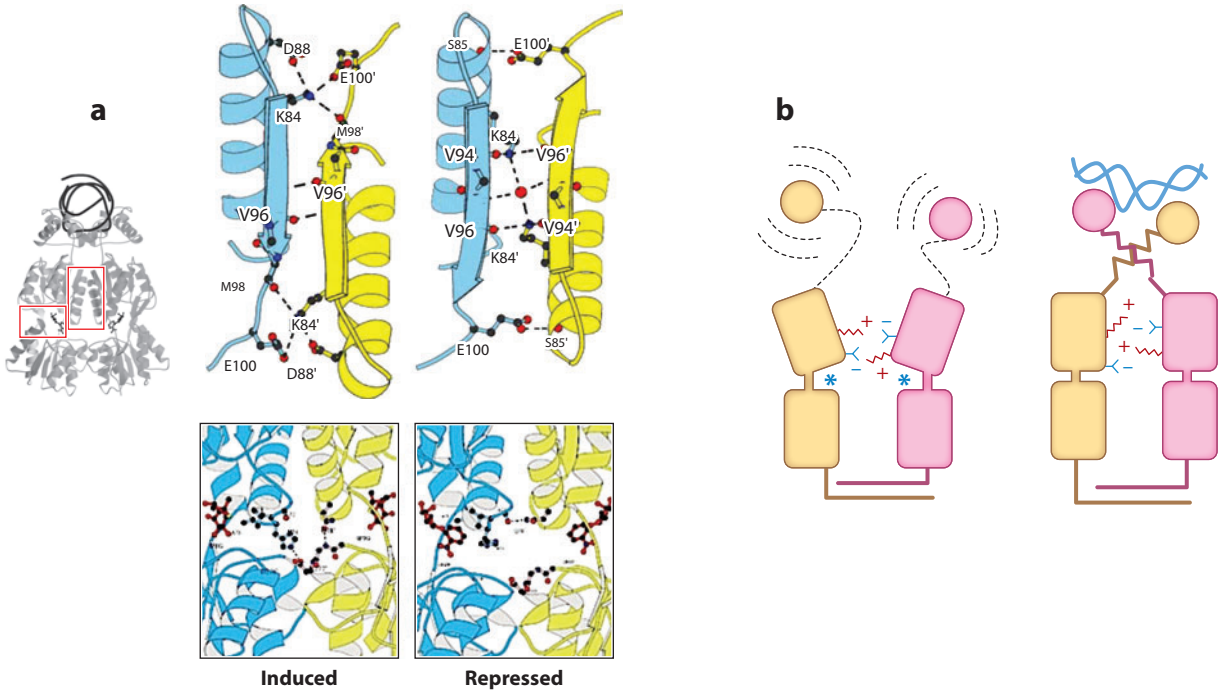

Figure 4

Allosteric transition captured by X-ray crystallography illustrating the conservation of symmetry with *Escherichia coli lac* repressor (103). (*a*) Comparison of the induced and repressed structures at the level of the intersubunit boundary (*top*) and at the level of the ligand-binding domain (*bottom*). Adapted from Reference 103 with permission. (*b*) Schematic illustrating the organization of the hinge domain and enhanced symmetry of the *E. coli lac* repressor protein upon DNA operator binding accompanied by changes of inducer-binding properties. Drawn from Reference 103 with permission.

and mitochondria that catalyze the native folding of proteins. They bind unfolded proteins via a hydrophobic lining of an open ring and then mediate ATP-triggered release followed by folding to the native state in an encapsulated cavity. The *E. coli* GroEL edifice is a double-ring 14-mer with one sevenfold symmetry axis and seven twofold axes that are all perpendicular to the sevenfold axis (79). GroEL binds and hydrolyzes ATP according to a nested allosteric MWC model of cooperativity (160, 161) that would include negative cooperativity (78).

Another example is that of the chemotactic receptors in bacteria. They show a sharp chemotactic signaling response (49) that has been attributed to a two-dimensional lattice of chemotactic receptors. In this arrangement, the cytoplasmic ends of the chemotactic receptor dimers are inserted into an hexagonal array of the autophosphorylating kinase CheA and the small transducing protein CheW (138). The number of chemotactic receptors in a single *E. coli* cell ranges from ~1,500 to ~4,500, creating a patch 0.2–0.6 μm in diameter. The close contact between CheA monomers allows this signal to pass to three surrounding units, resulting in a spread of activity across the receptor network and a sharp cooperative response (35).

Proteases as Monomeric Allosteric Proteins?

Other cases with allosteric interactions mediated by a single independent monomer, such as proteases, may sensu stricto contradict the oligomeric hypothesis of the MWC model. Trypsin-like

proteases are responsible for diverse physiological functions ranging from digestion and coagulation to immunologic reactions. NMR studies and rapid kinetic measurements reveal two distinct conformations of the active site: one fully accessible to substrate (E) and the other occluded by the collapse of a specific segment (E*) (67). The allosteric E*–E equilibrium provides a reversible mechanism for activity and regulation in addition to the irreversible conversion of zymogen to protease.

Ligands that may regulate protease activity through an allosteric mechanism include the calcium activation of calpain (94), as well as the controversial regulation of thrombin activity by Na^+. When bound to Na^+, thrombin adopts a fast conformation, which cleaves all procoagulant substrates more rapidly, and when free of Na^+, thrombin reverts to a slow state, which preferentially activates the protein C anticoagulant pathway, thus modulating the hemostatic balance (82). Additional structural studies are needed to firmly establish the allosteric character of these regulations.

Conclusions

In agreement with the MWC model, a large majority of signal-transducing proteins possess a symmetrical oligomeric structure. Nevertheless, possible exceptions have been noted. For instance, a few GPCRs may function without oligomerization, and some proteases are allosterically regulated as monomers.

A survey for the occurrence of biological protein assemblies deposited in the Protein Data Banks reveals interesting features (**Table 1**). In agreement with MWC preference for dyad axes of symmetry, dimeric proteins that compose the cyclic group of crystallographic point symmetry group are by far the most abundant oligomeric species. Oligomers with dihedral symmetry and an even number of protomers (such as 4-mers, 6-mers, 8-mers, and 12-mers) are comparatively more common than those with odd symmetry (3-mers, 5-mers, 7-mers). Homo-oligomers also predominate among protein assemblies with even symmetry, but surprisingly the opposite seems to be the case for oligomers with odd symmetry, in particular 3-mers and 5-mers. This might be relevant to the fact that, as mentioned above, the heterologous mode of association between subunits permits odd oligomers to accommodate different types of homologous subunits within the same molecule.

Table 1 Natural occurrence of oligomeric proteins[a]

	Homo-oligomers	Hetero-oligomers
Dimer	25,796	9,693
Trimer	3,166	3,804
Tetramer	6,784	4,050
Pentamer	394	497
Hexamer	2,054	1,234
Heptamer	119	108

[a]These data were provided by Nicolas LeNovère using information at the Protein Data Bank Europe (PDBe). More than approximately 72,802 proteins, including 10,413 monomers, have been examined. Note that this is a preliminary screening, a first-pass analysis, of the entire PDBe and has not been filtered to remove redundancies. The oligomers do not always correspond to multimeric quaternary structures but also contain functional complexes. The qualitative finding has been confirmed by Pedro Alzari with the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank USA after elimination of redundancies.

BINDING SITES FOR ORTHOSTERIC AND ALLOSTERIC LIGANDS

The original paper (112) on the MWC model extensively discussed protein oligomerization but did not specify the exact localization of the ligand-binding sites in the structure of allosteric oligomers, except that the biologically active site and the regulatory sites were topographically distinct. Since then, biochemical and structural studies on many allosteric proteins have revealed novel, unexpected features about the topology of binding sites for ligands involved in signal transduction (orthosteric) as well as in the modulation of signal transduction (allosteric). The most striking fact, fully supportive of the MWC model, is that a significant fraction of the sites are located at subunit interfaces.

Sites at Subunit Boundaries

Intersubunit boundaries are strategic areas of the protein able to "sense" but also control the quaternary transitions of an allosteric oligomer.

Regulatory enzymes. In the crystal structures of a variety of regulatory enzymes, substrate and regulatory binding sites have been identified and localized at subunit boundaries. For instance, in the case of the glycogen phosphorylase isologous dimer (141), each of the two subunits consists of two distinct domains. The two activating AMP sites, the two inhibiting glucose-6-phosphate-binding sites, and a small part of the catalytic site are located at the subunit boundary between N-terminal domains, which also includes the two phosphorylation sites at Ser-14. All these binding sites are located 15 to 60 Å from each other. In the *E. coli* phosphofructokinase isologous tetramer, the four catalytic sites for fructose-6-phosphate and the four allosteric sites for the activators ADP or GDP span the subunit boundaries (134). Another example is the *Bifidobacterium longum* L-lactate dehydrogenase, an isologous tetramer in which the four subunits are related by three molecular twofold axes: P, Q, and R. The molecule has subunit contacts only in the subunit interfaces along the P-axis and Q-axis, but not the R-axis. The tetramer has four active sites but only two sites for the allosteric activator fructose 1,6-bisphosphate at the P-axis interface. The active NADH binding site lies at the Q-axis subunit interface, in which His-195 is essential for enzyme catalysis and substrate binding (**Figure 2**).

Hemoglobin carries two classes of sites (125). In the first class, each of the four hemes is enveloped in a deep globin pocket. The second class of sites accommodates the potent allosteric modulator, 2,3-diphosphoglycerate (DPG) (10), which binds as a single molecule in the axis of symmetry of the $\alpha 2\beta 2$ tetramer, entering a multisubunit boundary cleft flanked by the N termini and helix H of the β-chains in the *T* structure (123). Hemoglobin also binds antisickling compounds such as clofibric acid and bezafibrate within the central cavity bound at sites approximately 20 Å away from the binding site of DPG but which have additive effects on the binding of DPG. Sites located at the boundary between subunits in the axial cavity constitute a novel category of sites important for drug design (59), and they are associated exclusively with the oligomeric organization of the protein. There are many additional examples of sites located at subunit boundaries in regulatory enzymes.

Ligand-gated ion channels. Early biochemical evidence with nAChR showed that the ACh-binding sites span the interfaces between subunits (118, 146). This arrangement was confirmed by the X-ray structure of the acetylcholine-binding proteins (AChBPs) from snails; AChBPs are soluble pentameric homologues of the nAChR extracellular domain (139), with five identical binding sites for ACh (or nicotine) located at the boundary between subunits. The binding site includes loops A, B, and C from the principal component and loops D, E, and F from the complementary

component, with conserved aromatic amino acids residues (146). This structural motif holds true for the whole family of pentameric receptors, particularly for prokaryotic pentameric receptors (11, 76, 77). For example, *Erwinia* receptor shows an activating response to a variety of compounds, including amino-butanol, cysteamine, and putrescine, and, remarkably, also to high concentrations of the neurotransmitter GABA (but not β-alanine and glycine). The X-ray structure shows that the *Erwinia* receptor ligand-binding pocket lies as expected at subunit interfaces of the extracellular domain and is framed by aromatic side chains with conserved residues at the homologous positions of loops B, C, and D found in all members of the family, with the relationship most similar to GABA and glycine receptors (163). The three-dimensional structure of the homopentameric *C. elegans* GluCl (74) further confirms the interfacial neurotransmitter site. The "sausage-shaped electron density" (74) assigned to glutamate maps to all five classical agonist-binding sites in the extracellular domain between subunits. The architecture of the site shares the standard loop's pocket, but with loop C adopting a closed conformation consistent with AChBP structures bound by agonists and the activated state of the receptor. In conclusion, prokaryotic receptors, *C. elegans* GluCl receptors, AChBPs, and most likely nAChRs show a remarkably well-conserved organization of the neurotransmitter-binding site at subunit boundaries.

Yet in the family of pentameric ligand-gated ion channels, the number and fine structure of binding sites per pentamer may vary depending on the composition of subunits, which, because of their heterologous associations, can be quite diverse in different organs and different species (102). For instance, in the case of the nAChR, the number of sites binding ACh and nicotinic agents depends on the composition of α-subunits, extending from two (as in the muscle 2α-1β-1γ-1δ nAChR) to five (as in the neuronal α7-homopentamer) (29). Nonequivalent binding sites formed by different subunits with different affinities for agonists and antagonists may coexist within a given oligomer, as is the case for the α-δ and α-γ subunit interfaces for *Torpedo* nAChR (108) or the α4-β2 and α4-α4 interfaces for human brain $(\alpha4\beta2)_2$ α4 receptors (109). Such diversity exists also for other pentameric receptors, notably for GABA receptors. Of special interest are the benzodiazepine ligands that are nonselective anxiolytics acting as potent positive allosteric modulators of $GABA_A$ receptors. Biochemical, pharmacological, and modeling evidence has shown that benzodiazepine ligands bind to intersubunit sites homologous to the GABA site but that they do not bind GABA. They are present on $GABA_A$ receptors that contain α1, α2, or α3 subtypes (3).

Ligands referred to as channel blockers were initially found to bind to sites located in the axis of symmetry of nAChR within the transmembrane channel lined by the α-helical TM2 (66, 146). X-ray crystallography established the same organization in *Gloeobacter* receptor (11, 77), and the sites for tetrabutylammonium and tetraethylammonium and the local anesthetic lidocaine were identified at different levels of TM2 (75). Smaller divalent transition metal ions such as Cd^{2+} and Zn^{2+} bind to the narrow intracellular entry of TM2. Similarly, in *C. elegans*, GluCl electron density associated with the channel blocker picrotoxin is apparent within the TM2 axial pore near the cytosolic side of TM2, on the fivefold axis of molecular symmetry (74).

Homologous dispositions for neurotransmitter and channel-blocker-binding sites at subunit boundaries are also found with ATP P2X trimeric receptors (91). The occurrence of binding sites at subunit interfaces thus appears to be a common feature of ligand-gated ion channels.

Sites Within Intrasubunit Domain Interfaces

Ligand-binding sites may lie not at the interface but within subunits at the boundary between domains. For *E. coli lac* repressor (104), the inducer and anti-inducer ligand-binding pocket is located at the interface of two N- and C-terminal subdomains of the core at ~40 Å from the C-terminal helix-turn-helix motif and the operator binding site. The structure of the core domain is essentially unchanged upon binding of the inducer and anti-inducer ligand (**Figure 4**).

The heterodimeric PPARγ-RXRα receptor in its active conformation (18) shows a general organization of individual subunit and domain interactions similar to that of the *lac* repressor, yet with asymmetrical ligand-binding pockets that are similar to previously defined structures with isolated ligand-binding domains and a more compact core. The thiazolidinediones, which include the drug rosiglitazone, an effective insulin sensitizer, bind to PPAR-γ, and 9-*cis*-retinoic acid binds to RXR-α. As a group, rosiglitazone and the antagonists GW9662 and BVT.13 give rise to a Y-shaped pocket in the intact PPAR-γ that is distinct from the positive allosteric modulator LXXLL peptide site and is distantly located from this site as from all other protein–protein interaction sites. As with the *lac* repressor, superposition of crystal structures obtained with the different PPAR-γ ligands shows that the overall conformation, including all the domain-domain, receptor-DNA, and receptor-coactivator interactions, is not significantly altered, although signal transduction takes place upon ligand binding (see next section).

Allosteric Modulatory Sites in Transmembrane Domains

In the case of membrane receptors, ligand-gated ion channels, and GPCRs, new categories of sites have been recently discovered within the transmembrane domain. These sites bind pharmacological agents structurally unrelated to the physiological ligands that interact with the common (orthosteric) ligand-binding site. These pharmacological agents, referred to as allosteric modulators, positively or negatively regulate receptor activity.

For instance, in the case of pentameric receptors, ivermectin behaves as a positive allosteric modulator of α7 nAChR, and its action is altered by mutations within the transmembrane domain TM2 (100). Furthermore, affinity labeling with general anesthetics (that are known to negatively modulate excitatory nAChRs and positively modulate inhibitory GABA receptors) identified both inter- and intrasubunit sites in the transmembrane domain (58). General anesthetics also negatively modulate prokaryotic *Gloeobacter* receptor. X-ray analysis of *Gloeobacter* receptor has recently identified a common general anesthetic binding site for propofol and desflurane within the upper part of the transmembrane domain of each protomer inside a cavity delimited by TM1, TM3, and TM2 within each subunit (117). The general anesthetics cavity is accessible from the lipid bilayer, and its entrance is obstructed by a lipid alkyl chain that clashes with propofol binding. Lipids might thus be the endogenous ligands of this membrane allosteric site (117). Molecular simulation studies of ethanol binding and equilibrium exchange for the homomeric α1 glycine receptor (GlyRα1), modeled on the structure of *Gloeobacter* receptor, confirm the location of ethanol-binding sites both between and within the GlyR subunits transmembrane domains (80, 114).

Ivermectin, which also positively modulates GluCl (74), binds at subunit interfaces between the TM3 and TM1 α-helices, making important contacts with the TM2 and the TM2-TM3 loop. The ivermectin-binding site in GluCl crystals appears to be homologous to many important modulators of pentameric receptors such as alcohol, anticonvulsants, anesthetics, and diuretics acting on the $GABA_A$ receptor, as well as ivermectin acting on α7 nAChR (100).

The first explicit mention of allosteric mechanisms in ligands interacting with GPCRs can be traced to an early demonstration with muscarinic ACh receptors showing that a ternary complex may form with more than one type of ligand (40, 93, 144). As a result of the enormous diversity of the GPCR superfamily, the structure of the orthosteric binding sites displays multiple modes (93). To date, no GPCR crystal structure in complex with an allosteric modulator has been characterized. However, mutagenesis experiments have pointed to key residues, particularly in the class C GPCRs, within the entire transmembrane-spanning region (104)—a "cornucopia" of allosteric sites (93).

Conclusions

Most well-characterized signal-transducing proteins possess binding sites for orthosteric and allosteric ligands at subunit interfaces. This disposition is particularly appropriate to sense the conformational transitions that, as postulated by MWC, affect the quaternary organization of the protein oligomer and thus subunit interfaces.

THE ALLOSTERIC TRANSITION: CONFORMATIONAL SELECTION WITH CONSERVATION OF SYMMETRY OR INDUCED FIT?

A central hypothesis of the MWC theory was that two (or more) states are reversibly accessible to allosteric oligomers in the absence of ligand, and when the protein undergoes transitions from one state to another state, its molecular symmetry is conserved. A variety of biophysical methods, including X-ray crystallography, NMR, time-resolved X-ray diffraction, and subnanosecond spectroscopic techniques, have been used together with molecular dynamics simulations to test this hypothesis.

X-Ray Structure of Resting and Active States

X-ray crystallography has been widely used to follow the conformational transitions of proteins.

Hemoglobin. Comparisons of the oxy-(*R*) and deoxy-(*T*) hemoglobin X-ray structures (60, 124, 126) revealed a quaternary $R \rightarrow T$ transition consisting of a rotation of the dimers $\alpha 1\beta 1$ relative to $\alpha 2\beta 2$ by 12° to 15°, and a translation of one dimer relative to the other by 0.8 Å, both movements accompanied by the conservation of the molecular symmetry of the protein. The iron atoms relative to the porphyrin plane change from out-of-plane in *T* deoxyhemoglobin to in-plane in *R* oxyhemoglobin. Moreover, the tertiary structure is altered, accompanied by changes of the steric constraints at the subunits' contact interfaces. In the *T* structure, the two β-subunits form a site that binds the allosteric modulator 2,3 diphospho-glycerate, which becomes too narrow to accommodate it in the *R* structure. Perutz et al. (127) and Edelstein and colleagues (50, 95) interpreted the hemoglobin data in terms of a simplified MWC model according to an $R \rightarrow T$ equilibrium where *T* predominates at low oxygen pressure and *R* (with higher oxygen affinity) predominates at high oxygen pressure. The variations of the hemoglobin oxygen-binding equilibrium curve with pH, ionic strength, and allosteric effectors could be described by the model but not by the Bohr effect, which, according to Perutz, requires a sequential rupture of several hydrogen bonds in the *T* structure. Locally induced changes would then follow oxygen binding, with negative cooperativity in support of the KNF model. Yet heterogeneity between the two types of chains readily accounts for such behavior, reconciling the data with the MWC scheme (9).

Regulatory enzymes. The X-ray structure of several regulatory enzymes in both *T* and *R* conformations has been resolved. The allosteric transition of dimeric phosphorylase b consists of each of the two protomers rotating by 5° about axes pointing in opposite directions normal to the molecular dyad axis of symmetry. Communication between catalytic sites of the dimer is provided by a change in packing geometry of two helices linking each site with the subunit interface (7). Similarly, the transition of tetrameric phosphofructokinase from *T* to *R* involves one rigid pair turn relative to the other by 7° about the *p*-axis (134). In both cases the rotations affect both the substrate and regulatory ligand-binding sites located at subunit interfaces. The end state of all these transitions obeys the same rule of symmetry conservation.

Another much studied protein is *E. coli* hexameric aspartate transcarbamylase. A comparison of X-ray structures of the low-activity *T* state and high-activity *R* state (in the presence of substrates

or substrate analogs) of aspartate transcarbamylase (105, 156) revealed that during the $T \rightarrow R$ transition, the two catalytic trimers increase their separation along the threefold axis by ~11 Å and rotate ~5° around the same axis, and the regulatory dimers rotate ~15° around their respective twofold axes with symmetry preserved (105).

Monitoring the 11 Å expansion during the $T \rightarrow R$ transition by small-angle X-ray scattering in solution (73, 158) indicated that the distance between the two catalytic trimers in the *R* conformation was 2.8 Å larger than in the *T* state. The data further revealed in solution a spontaneous and reversible equilibrium between the *R* and *T* states in the absence of ligand, critical evidence in favor of the MWC mechanism (56). The use of highly deuterated, $^{1}H,^{13}C$-methyl-labeled ATCase in concert with methyl-transverse relaxation optimized spectroscopy (TROSY) NMR (152) allowed the shift from the *T* state to the *R* state by substrate analogs or ATP to be quantitatively followed, along with tracking the complete disappearance of the *R* conformer with CTP and measuring directly the equilibrium constant (L_0) between *R* and *T* (152). Such changes for both homotropic and heterotropic effects are quantitatively accounted for by the MWC model. They confirm the results of early tests of the MWC model with ATCase based on comparisons between the equilibrium binding of the substrate analog succinate and the corresponding changes in the enzyme conformation, without the superimposition of ligand binding and conformational change expected from the KNF model (31, 33).

Macol et al. (107) constructed a version of aspartate transcarbamylase in which only one of the six catalytic protomers is able to bind a substrate analog while the others were inactivated by a single amino acid substitution in the active site. The combination of X-ray crystallography and small-angle X-ray scattering in solution provided structural evidence that the transition of only one catalytic monomer is sufficient to cause the transition of the entire enzyme into the *R* state, lending strong support to the MWC concerted model (107). Also consistent with a fully concerted transition is the observation by Iwata and colleagues (83) of *T* state and *R* state cocrystals of bacterial L-lactate dehydrogenase obtained at a concentration of oxamate (analogue of the substrate pyruvate) that pulls the allosteric equilibrium 50% to the *R* state. The crystals revealed equal quantities of the two symmetrical states, without any significant mixed or hybrid states, as would have been predicted by the KNF model (**Figure 2**).

These examples of regulatory enzymes validate the conservation of symmetry. They also illustrate how the structures of the catalytic and regulatory sites distributed at subunit interfaces are modified in the course of the quaternary transition, thus mediating homotropic and heterotropic ligand interactions.

Pentameric receptors and ion channels. A comparison of common core structure of bacterial *Erwinia* and *Gloeobacter* receptors in closed versus open conformations by X-ray crystallography (11, 77) reveals a global quaternary twist around the fivefold axis of rotational symmetry with additional tertiary deformations (**Figure 3**). These deformations involve a substantial rearrangement of the subunit interfaces and a downward motion of the β1–β2 loop, which is apparently coupled to a tilt of the TM2 and TM3 segments and generates a wide opening in the upper part of the pore (from 2 to 12 Å in diameter). The recently determined structure of *C. elegans* GluCl (74) crystallized in the presence of the positive allosteric modulator ivermectin and/or the endogenous agonist L-glutamate reveals an open conformation that superimposes closely, at the atomic level, with the previously identified prokaryotic *Gloeobacter* receptor structure (11), further indicating a highly conserved mechanism of channel gating from prokaryotes to eukaryotes. Prior to the availability of the structural data, in silico normal-mode analysis, initially applied to a model of α7 nAChR (147), predicted the counterclockwise motion in the upper part of the nAChR pentamer accompanied by a structural reorganization of the ACh-binding site, a bending of the subunits,

and the pore opening. The model accounts for at least 29% of the structural transition between *Erwinia* versus *Gloeobacter* receptor structures described above (11).

KcsA channels are gated as the cytoplasmic pH becomes increasingly acidic. Among the many convergent studies on the gating mechanism, a particularly elegant one uses a single KcsA channel with an attached gold nanocrystal irradiated by white X-rays. Motions of the diffraction spot from the nanocrystal were tracked in real time. These motions corresponded to concerted rotational movements around the fourfold axis of symmetry of the channel, twisting of the channel and bending of the gating helices, resulting in the open state (137). Also, the selectivity filter of the KcsA channel undergoes a variety of gating events, from flicker transitions (at the microsecond timescale) to C-type inactivation (millisecond to second timescale), for which the structures have been identified (45, 44). The KcsA channels thus undergo a set of conformational transitions that correlate with different states of channel opening. The quaternary twist mechanism that preserves molecular symmetry appears to be a highly conserved mechanism of pore opening in the broad ensemble of pentameric receptors and ion channels.

GPCRs: common X-ray structure of resting versus active states. GPCRs may at first glance appear to be in disagreement with the MWC model for allosteric proteins because class A GPCRs apparently function without oligomerization. Rhodopsin is a member of this class and has been important for understanding the activation transition of GPCRs in general (39). The rhodopsin structure is stabilized by multiple hydrogen-bonding networks within the TM7 core that involve structurally bound waters and conserved motifs forming an "ionic lock" that constrains TM3 and TM6. Receptor activation is driven by ultrafast isomerization of retinal, followed by a cascade of steps leading to conformational activation within milliseconds. The transition from inactive to active states is accompanied by a global and concerted rearrangement of the helix bundle sufficient to break the ionic lock that appears to be a prerequisite for an active receptor state and shifts the cytoplasmic end of TM6 (and to a lesser extent TM5) away from the bundle core (TM1–TM4 and TM7). This shift occurs by a rotation of TM6 that leaves the shape of the helix intact. On the cytoplasmic side, the conformational change is amplified by the characteristic bend caused by a conserved proline (142).

Recent X-ray structures of β_2-adrenergic and adenosine A_{2A} GPCRs in antagonist-resting versus agonist-bound states show a strikingly similar global conformational change, suggesting a common activation mechanism in GPCRs independent of the specific structure of the ligand (39, 132, 162). Non-rhodopsin GPCR structures show, in addition to a highly variable organization of the ligand-binding domain, that the extracellular ends of the TM segments and conformations of the connecting loops are more diverse than previously thought. Yet common features emerge from this diversity. For instance, residues in the binding pocket that interact with the ligand are, as in the case of nAChRs, more contracted or compact in the active conformation (helices III, V, and VII) that is stabilized by the agonist, whatever its nature (excluding class C GPCRs with a large extracellular domain). On the cytoplasmic end of this structure, there is an opening out of the helices on the cytoplasmic surface, particularly helices V and VI, creating a cavity to which the C terminus of G-α protein binds.

Thus, strikingly similar global conformational changes mediate a common activation mechanism in GPCRs, possibly due to their common evolutive origin. Moreover, crystallization of ligand-free opsin (120) and constitutively active rhodopsin that retains retinal (142) reveals a common active GPCR structure. These observations rule out a ligand-induced conformational change for the activation process, and are consistent with the single protomer conformational selection mechanism of Changeux et al. (35) (13, 47, 72, 92). The conformational changes of GPCRs oligomers have not yet been explored.

***lac* repressor.** The X-ray structures of the *lac* repressor in the induced and the repressed states do not reveal any change of tertiary conformation in the individual N-terminal and C-terminal subdomains (103), but the orientations of the subdomains change through a small hinge motion of the N-terminal subdomain relative to the C-terminal subdomain (**Figure 4**). When the repressor binds to its operator DNA, the two N-terminal subdomains rotate while preserving the twofold axis of the dimer. The conformational change links the effector site, through the dimer interfaces to the hinge helices, and the DNA-binding domains. Binding of the inducer stabilizes a subtle structural change in the N-terminal subdomain, which is sufficient to destabilize the repressor-operator complex and reduce the repressor's affinity for the operator by several orders of magnitude, thus triggering the genetic switch. The overall data are quantitatively fitted by the MWC model (104).

Dynamics of the Conformational Transition and the Intermediate States

The MWC model essentially describes the $T \rightarrow R$ transition for signal transduction under thermodynamic equilibrium. Assessing the conformational dynamics of oligomeric proteins in solution offers new insights into the mechanics of the transition.

Hemoglobin. Time-resolved wide-angle X-ray scattering has achieved an important analysis of the allosteric transition of hemoglobin. The method revealed the main structural change that takes place in the 2-μs range, after photolysis of CO-hemoglobin (17). An early transition signal fully develops at 300 ns corresponding to a tertiary relaxation followed by the main quaternary relaxation likely involving the αβ dimers' relative rotation and translation occurring in a concerted way at about 2 μs. The authors assigned this main structural change to the $R \rightarrow T$ quaternary transition of the MWC model and insisted that the partially ligated states present under their conditions do not modify the pattern, thus excluding a KNF mechanism. It should also be mentioned that an additional, albeit smaller, structural change occurs in a later 20-μs step.

In silico calculations based on the method of conjugate peak refinement are consistent with such a two-step quaternary transition (57). In the $R \rightarrow T$ direction, the first step would consist of an early large quaternary change characterized by a 3° rotation of each α-subunit relative to the $\beta_1\beta_2$ dimer, and the second step would consist of a late and smaller quaternary change characterized by a 6° rotation of the $\alpha_1\beta_1$ and $\alpha_2\beta_2$ dimers. Overall, the current kinetic measurements demonstrate that the cooperative $R \rightarrow T$ quaternary transition does not take place in an all-or-none fashion within the oligomeric tetramer but includes intermediate quaternary conformations (30).

Pentameric ligand-gated ion channels. An exceptional advantage of the electrophysiological recordings of ligand-gated ion channels is that they reach time ranges similar to those of wide-angle X-ray scattering with hemoglobin, giving hope for an improved resolution of the intrinsic conformational change that mediates signal transduction. In patch-clamp recordings of nAChR at the neuromuscular junction, the rise times are within a microsecond and the duration of the steady current is of the millisecond timescale. The durations of the full openings vary with the nature and binding affinity of the agonist but not with the maximum unitary current or intrinsic conductance, indicating, in agreement with the MWC model, invariance of the amplitude for the gating response irrespective of the structure of the ligand (51, 99, 113).

Yet additional events have been noted in the course of the gating transition. In mutant muscle AChR, the ultimate closed-to-open transition is preceded by two primed closed states (113). Further, with homomeric α2 glycine receptors, even at the lowest glycine concentration (20 μM), openings occurred in long (>300-ms) groups, with shut-time intervals within groups of openings

dominated by short shuttings of 5–10 μs interpreted as a "flip" mechanism (99). Some refinements of details may be required when linear free energy relationships are considered (51).

In an attempt to understand the dynamics of the structural changes involved in the gating mechanism and to eventually capture such primed or flip states, 1-μs molecular dynamics simulation were carried out with *Gloeobacter* receptor following its closure by a rapid jump in pH (116). Channel closure entails two major events initiated by large fluctuations in the pore at the top of the M2 helix, followed by global tertiary relaxation and a progressively increasing quaternary twist of the whole molecule. The two-step transition of the first subunit begins within the first 50 ns and is followed in 450 ns by its immediate neighbor in the pentamer, which proceeds with a similar scenario (116). The data, which are consistent with a global twist motion (147), may also reveal the possible occurrence of intermediate conformations in the course of the allosteric transition whose structure and functional significance are still unknown.

Conclusion

Early critics of the MWC model frequently mentioned the unnecessary character of the conservation of symmetry hypothesis. However, X-ray crystallography of several regulatory enzymes, ligand-gated ion channels, and nuclear receptors unambiguously shows that, in agreement with the MWC model, the same molecular symmetry is found in the resting and active end states, although exceptions have been noted. Moreover, the future analysis of the dynamics of their transition may disclose unexpected scenarios.

CONSTITUTIVE MUTATIONS AND RECEPTOR DISEASES

An important consequence of the MWC model is that the spontaneous $R \rightarrow T$ equilibrium can be shifted by mutations in addition to ligands. This is true for the hemoglobins Chesapeake and Kansas, whose mutations symmetrically alter the α1-β2 interface and modify the isomerization constant L in opposite directions (50, 95). This finding was further documented with many regulatory enzymes. Another consequence is that if the conformational equilibrium is significantly shifted in favor of the active state, a basal or spontaneous activity would be observed in the absence of ligand, thus ruling out the ligand induced character of the activation process.

In the case of ligand-gated ion channels, in particular the nAChR, spontaneous channel openings blocked by the antagonist α-bungarotoxin are indeed recorded in the absence of acetylcholine (85). Moreover, mutations were discovered, initially in the channel domain of α7-nAChR (130) but also scattered throughout the receptor structure (54, 143), that either promote a constitutive spontaneous channel opening or conversely stabilize the resting conformation (30, 52). The data are simply interpreted in terms of the MWC model by assuming that the mutation alters the unliganded equilibrium between discrete channel states (open and closed) without changing the energy for ligand binding (4, 52). Mutations in L can also convert competitive antagonists to agonists (30, 52, 130). Thus, the data bring solid evidence in favor of the conformational selection scheme for pentameric receptors. Some of the same mutations in nAChRs are responsible for human diseases such as autosomal dominant nocturnal frontal lobe epilepsy and congenital myasthenia (145).

Similarly, mutations in GPCRs cause more than 30 different human diseases. Among these mutations, about a 100 are constitutive mutations that are themselves responsible for more than 10 diseases (135). More than 40% of all wild-type GPCRs tested exhibit a significant basal activity in the absence of ligand (135). The α_{1B}-adrenergic receptor was the first GPCR in which point mutations were shown to trigger receptor activation in the absence of agonist (43), and the generality of this

finding was extended to the β2- and α2-adrenergic receptors, which are coupled to G_s-mediated stimulation and G_i-mediated inhibition of adenylyl cyclase, respectively (101). The constitutive effects of these mutations, together with the basal activity and its shift toward the resting conformation by negative allosteric modulators (or inverse agonists), were interpreted by Lefkowitz et al. (101) in terms of an adapted version of the MWC scheme. The spontaneous equilibrium between R and R^* and the relevant value of L would allow a significant level of R^* to be present in the absence of agonist. The occurrence of such disease-causing constitutive mutations that result in active receptor molecules in the absence of agonist rules out the induced-fit mechanism of receptor activation.

Last, similar constitutive mutations with eukaryotic nuclear receptors that may also be responsible for human diseases have been reported (1). In the particular case of *E. coli lac* repressor, the equilibrium between the high operator affinity/low inducer affinity conformation and the low operator affinity/high inducer affinity conformation can be altered by substituting particular amino acids at the dimer interface in the repressor (103, 104). In conclusion, mutational analyses of the diverse classes of allosteric proteins examined in this review not only support the MWC model but initiate its application to the understanding and pharmacology of certain human diseases that can be considered receptor diseases.

QUESTIONS ABOUT THE MONOD-WYMAN-CHANGEUX MODEL

The examples presented in this review illustrate that unexpectedly, after 50 years, the MWC model successfully applies to an increasing number of regulatory proteins, from bacterial enzymes to brain receptors. Nevertheless, it shows intrinsic limitations due to the necessarily simplifications inherent to model building. In a paper entitled "Allostery and Cooperativity Revisited," Cui & Karplus (46) agree that "the investigators decided not to sacrifice elegance for completeness" but point to a number of assumptions of the MWC model that, they assert, need to be revisited (though, in my opinion, not necessarily revised).

Oligomeric Structure: Allostery in Monomers?

Many signal transduction systems, including regulatory enzymes, ligand-gated ion channels, ion channels, and nuclear receptors, possess a symmetrical oligomeric structure. Some systems belong to the cyclic group, such as C_2 dimers, or to the dihedral group, such as D_2 tetramers. Such organization provides interfaces that may possibly lead to more stability and more allosteric control (69). On the other hand, integral membrane-bound ligand-gated ion channels and ion channels do show a heterologous mode of association, a disposition that allows the association of homologous but not identical subunits, thus creating a much broader structural diversity of oligomers.

In agreement with Cui & Karplus, notable exceptions may exist, the most debated one being the GPCRs (96, 128). Rhodopsin and the β2-adrenergic receptor may possibly mediate signal transduction as a single receptor unit made up of the compact assembly of transmembrane helices that undergo a basic concerted transition, involving tilts of TM5 and TM6, together with TM7 and TM3, common to all GPCRs (39, 162) (see above). Would a single monomer operate as a sort of "mini-oligomer" of transmembrane α-helices? In addition, many studies have emphasized the ability of diverse GPCRs to form dimers or even larger oligomeric complexes (41).

Other exceptions are proteases, some of which may exist under two distinct conformations of the active site (67) with an equilibrium modulated by ligands, in particular Na^+ or Ca^{2+} ions. The mechanism of this regulation is not known (82). Further, the monomeric enzyme glucokinase displays a sigmoidal saturation curve for its substrate as a consequence of a global conformational

change that according to the authors would obey a "mnemonical mechanism" rather than the concerted MWC model (89).

Finally, mention should be made of protomers that form supramolecular and cooperative assemblies, such as chemotaxis receptors, bacterial flagellar proteins, and contractile proteins, among others, that are relevant to the mechanism proposed by Changeux et al. (35). There are thus established exceptions to the oligomeric rule.

Conservation of Symmetry: Not Always Satisfied?

Many examples of allosteric oligomers have been reported, of which only a few have been presented in this review, that unambiguously demonstrate a $R \rightarrow T$ transition for which initial and end states captured by X-ray crystallography show conserved symmetry. Yet the development of novel technologies to follow the dynamics of conformational change, together with advanced molecular dynamics simulations, may reveal conformations that are transiently asymmetric. These have been reported in microsecond molecular dynamics simulations with pentameric *Gloeobacter* receptor (116) and with tetrameric K^+ channels (71, 87). Their physiological significance is not fully understood and needs further investigation.

Two-State or Multiple States: Induced Fit Versus Conformational Selection?

At a fine-structure level, ligand-dependent movements in the angstrom range associated with substrate binding have been interpreted in terms of an induced fit mechanism. A related but distinct case is the reordering of the C-loop of AChBP homolog or nAChR following nicotinic ligand binding that primarily maps the size of the bound ligand (149). For ionotropic glutamate receptors, changes in the degree of closure of the bilobed agonist-binding site quantitatively control the open probability of the discrete subconductance states of the ion channel (88).

An additional case related to substrate specificity is the phenomenon described for the allosteric enzyme ribonucleotide reductases, whose active site specificity is modulated by the binding of different regulatory ligands (129). Changes in signaling specificity with ligand binding are also observed for GPCRs, notably for a given β-adrenoceptor for which different agonists can elicit coupling to different G proteins, a process referred to as "ligand-directed signaling" (55). At the present level of resolution, the mechanism for such modulation may equally involve selective stabilization by ligands of preexisting conformations or an adequate induced-fit mechanism. As for a wide variety of receptor systems, including muscular and neuronal nAChRs (29, 58, 146), as well as K^+ channels (44, 45), desensitization processes that require more than two states have been identified within a broad range of timescales.

Last, negative cooperativity, which is only rarely observed, was not treated by the simple MWC model with equivalent subunits, but as mentioned by Cui & Karplus, it can be accounted for by an extended MWC model (50, 95). In any case, at variance with the simplest, and deliberately minimal, two-state formulation of the MWC model, a diversity of additional discrete conformations may have to be postulated to fit the data but within the conformational selection scheme.

Do Oligomers Behave as Rigid Units?

Finally, Cui & Karplus (46) state that "the implied assumption that *the oligomers behave as rigid units* preventing the transition from the mathematical MWC model to an atomic level description" (italics mine). This might possibly be the case if one were to reduce the MWC model to its mathematical formulation. However, this is certainly not in the spirit of the structural transitions

suggested by the MWC model, which proposed that within the oligomer "the conformation of each protomer is *constrained* by its association with the other protomers" and also that the "two states . . . reversibly accessible to allosteric oligomers . . . differ by the distribution and/or energy of interprotomer bonds, and therefore also by the conformational constraints imposed upon the protomers" (112). The protomers were viewed from the start as rigid but flexible units with tertiary changes accompanying the global quaternary transition, which structural (17), biophysical (153), and molecular dynamics (57, 81) studies have demonstrated to be true for hemoglobin, pentameric ligand-gated ion channels, and possibly a few other systems.

CONCLUSIONS: ALLOSTERY, THE MONOD-WYMAN-CHANGEUX MODEL, AND THE FUTURE

After exactly 50 years, the notion that allosteric interactions between topographically distinct protein sites may be mediated by a conformational change has reached the status of an indisputable reality (19, 28, 111). All the examples presented in this review on signal-transducing proteins (or receptors) illustrate the validity of the mechanism that complements, on chemical grounds, the classical mechanism of direct interaction between ligands by steric hindrance at the level of a common binding site. Many more examples can be found.

The MWC model, which was published in 1965, four years later deals with an additional property commonly encountered with signal transduction proteins: the occurrence of cooperative interactions between multiple ligand-binding sites. The model relies on two main principles: the occurrence of an oligomeric structure, which is indeed the case for most signal-transducing proteins, and a pre-existing conformational equilibrium, differentially stabilized and shifted by the ligands. As presented in this review, several well-established examples can be fitted better by this scheme than by the KNF induced-fit mechanism. However, this does not exclude exceptions.

An important outcome of the MWC model is that it has had major influences on rational drug design and on our understanding of human pathologies. As a result of symmetry properties, hetero-oligomers may occur more frequently in heterologous oligomers. The resulting diversity is particularly extraordinary with regard to ligand-gated ion channels, creating new opportunities for drug design but also substantial challenges for the targeting of drugs to particular circuits in the brain.

Further, the allosteric transition paradigm adds a new dimension to drug conception. In the past, both agonist and antagonist drugs were designed to fit a rigid site in a single conformation (15). By contrast, the allosteric scheme implies that agonists and antagonists, as well as positive and negative allosteric modulators, select and stabilize structurally different conformations that may be modeled for the active, resting, and eventually desensitized states, exploiting the considerable amount of data rapidly emerging from structural and molecular dynamics studies (146). Moreover, both structural and computational evidence reveals intermediate conformations in the dynamics of the allosteric transition (57, 81, 116) that need to be further explored from structural and physiological points of view.

The MWC paradigm also states that diverse conformations of nAChR may spontaneously exist in the absence of ligand. From a drug design perspective, this suggests the possibility that antagonists may not only inhibit the effect of agonists, but also exhibit an intrinsic activity in blocking background activity (inverse agonists). It also led to the discovery of novel allosteric modulators that regulate the activity of ligand-gated ion channels, as well as GPCRs, and are located within the transmembrane domain (58, 74, 117). It also predicts that by altering the unliganded equilibrium between discrete conformational states mutations may cause constitutive receptor activation with important pathological consequences in what may be referred to as receptor diseases.

Turning now to a physicist's point of view, Garcia et al. (61) mention the "unreasonable effectiveness" of the MWC model (cited 5,957 times as of November 15, 2011) and argue that MWC models "serve in the same capacity in biology that the Ising model introduced to describe the magnetic properties of materials does in physics. Both the MWC model and the Ising model make the extremely useful simplifications that, first, the individual elements within a complex system can exist only in a countable number of discrete states (rather than in a continuum), and that an individual element can sometimes change its state" (35). They further argue that the "MWC framework can be even more usefully applied to an unreasonably broad range of biological problems."

Indeed the MWC formalism has already been extended to supramolecular assemblies of proteins that show ordered structures. An interesting example is the 14-mer chaperonins (79). These complex machines bind and hydrolyze ATP, following a nested allosteric MWC model of cooperativity that, according to the authors, includes negative cooperativity (78). Other examples are the chemotactic receptors in bacteria, in which sharp signaling response (49) has been assigned to a conformational change spreading across the receptor two-dimensional lattice (35, 49, 61). Similarly, the bacterial flagellar switch that controls the direction of flagellar rotation during chemotaxis has a highly cooperative response, which is in quantitative agreement with the general model of conformational spread (5, 35, 49). The notion might possibly be extended along these lines to the collective bending of multiple myosin heads (all in the same direction) that combine to move the actin filament relative to the myosin filament, resulting in muscle contraction. Also, Garcia et al. (61) have suggested the application of the MWC model to eukaryotic transcriptional regulation, from nucleosomes to enhancers, as long as the system can exist in two overall states, such as DNA conformational states (accessible and inaccessible), and the affinity of the relevant ligands for their target sites depends on which of the two overall conformational states the system is in. This opens the analysis to more complex molecular assemblies than those discussed in this review, as they are composed of sets of different proteins that form reproducible but poorly symmetrical supramolecular edifices such as, for instance, the polymerase transcription factor complexes. This might be the case for the viral polymerase and allosteric inhibitors that block polymerizing activity at the level of a far distant site (155). A particularly interesting example is the anaphase-promoting complex engaged in cell division, which is composed of proteins encoded by 13 different genes, resulting in a 1.4-MDa protein assembly in eukaryotes (7). The proteasome or even the ribosome may belong to that category. Their conformational transitions are largely unknown. But are the changes local, or does the whole complex undergo global conformational changes between a minimum of two active and resting conformations (35, 61)? These studies should bridge the gap between the molecular biology of globular proteins and cellular organelle structure and physiology. In short, after 50 years the MWC theory still has an impressive future.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The author thanks the Woods Hole Marine Biological Laboratory, where this review was written, and Leonard Warren, Jean-Jacques Slotine, Stuart Edelstein, Marc Delarue, Felix Rey, and Pedro Alzari for constructive remarks and suggestions on the manuscript. Nicolas Le Novère is thanked for help with a first-pass analysis of the Protein Data Bank Europe, and Pedro Alzari is thanked

for a closer examination of the not redundant homo-oligomers versus hetero-oligomers in the Research Collaboratory for Structural Bioinformatics (RCSB).

LITERATURE CITED

1. Achermann JC, Jameson JL. 2003. Human disorders caused by nuclear receptor gene mutations. *Pure Appl. Chem.* 75:1785–96
2. Arighi E, Borrello MG, Sariola H. 2005. RET tyrosine kinase signaling in development and cancer. *Cytokine Growth Factor Rev.* 16:441–67
3. Atack JR. 2010. $GABA_A$ receptor $\alpha2/\alpha3$ subtype-selective modulators as potential non-sedating anxiolytics. *Curr. Top. Behav. Neurosci.* 2:331–60
4. Auerbach A. 2012. Thinking in cycles: MWC is a good model for acetylcholine receptor-channels. *J. Physiol.* 590:93–98
5. Bai F, Branch RW, Nicolau DV Jr, Pilizota T, Steel BC, et al. 2010. Conformational spread as a mechanism for cooperativity in the bacterial flagellar switch. *Science* 327:685–89
6. Baloh RH, Enomoto H, Johnson EM Jr, Milbrandt J. 2000. The GDNF family ligands and receptors—implications for neural development. *Curr. Opin. Neurobiol.* 10:103–10
7. Barford D. 2011. Structure, function and mechanism of the anaphase promoting complex (APC/C). *Q. Rev. Biophys.* 44:153–90
8. Barford D, Johnson LN. 1989. The allosteric transition of glycogen phosphorylase. *Nature* 340:609–16
9. Bellelli A, Brunori M. 2011. Hemoglobin allostery: variations on the theme. *Biochim. Biophys. Acta* 1807:1262–72
10. Benesh RE, Benesh R, Yu CI. 1969. The oxygenation of hemoglobin in the presence of 2,3-diphosphoglycerate. Effect of temperature, pH, ionic strength, and hemoglobin concentration. *Biochemistry* 8:2567–71
11. Bocquet N, Nury H, Baaden M, Le Poupon C, Changeux JP, et al. 2009. X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. *Nature* 457:111–14
12. Bocquet N, Prado de Carvalho L, Cartaud J, Neyton J, Le Poupon C, et al. 2007. A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family. *Nature* 445:116–19
13. Boehr DD, Nussinov R, Wright PE. 2009. The role of dynamic conformational ensembles in biomolecular recognition. *Nat. Chem. Biol.* 5:789–96
14. Boettcher AJ, Wu J, Kim C, Yang J, Bruystens J, et al. 2011. Realizing the allosteric potential of the tetrameric protein kinase A RIα holoenzyme. *Structure* 19:265–76
15. Bovet D. 1959. Isosterism and competitive phenomena in drugs: a study of structure-activity relationships in agents acting upon autonomic effector cells. *Science* 129:1255–64
16. Burgen AS. 1981. Conformational changes and drug action. *Fed. Proc.* 40:2723–28
17. Cammarata M, Levantino M, Wulff M, Cupane A. 2010. Unveiling the timescale of the RT transition in human hemoglobin. *J. Mol. Biol.* 400:951–62
18. Chandra V, Huang P, Hamuro Y, Raghuram S, Wang Y, et al. 2008. Structure of the intact PPAR-γ-RXR-α nuclear receptor complex on DNA. *Nature* 456:350–56
19. Changeux JP. 1961. The feedback control mechanisms of biosynthetic L-threonine deaminase by L-isoleucine. *Cold Spring Harb. Symp. Quant. Biol.* 26:313–18
20. Changeux JP. 1962. Effect of L-threonine and L-isoleucine analogs on L-threonine desaminase. *J. Mol. Biol.* 4:220–25
21. Changeux JP. 1963. Allosteric interactions on biosynthetic L-threonine deaminase from *E. coli* K12. *Cold Spring Harb Symp. Quant. Biol.* 28 497–504
22. Changeux JP. 1964. Allosteric interactions interpreted in terms of quaternary structure. *Brookhaven Symp. Biol.* 17:232–49
23. Changeux JP. 1964. *Sur les propriétés allostériques de la L-thréonine déaminase d'Escherichia coli K12*. PhD thesis. Fac. Sci. Univ. Paris. 102 pp.
24. Changeux JP. 1965. On the allosteric properties of biosynthesized L-threonine deaminase. VI. General discussion. *Bull. Soc. Chim. Biol.* 47:281–300

25. Changeux JP. 1966. Responses of acetylcholinesterase from *Torpedo marmorata* to salts and curarizing drugs. *Mol. Pharmacol.* 2:369–92
26. Changeux JP. 1969. Symmetry and cooperative properties of biological membranes. In *Symmetry and Function of Biological Systems at the Macromolecular Level*, ed. A Engstrom, B Strandberg, pp. 235–56. Nobel Symp. 11. Stockholm: Almqvist & Wiksell
27. Changeux JP. 2010. Allosteric receptors: from electric organ to cognition. *Annu. Rev. Pharmacol. Toxicol.* 50:1–38
28. Changeux JP. 2011. 50th anniversary of the word "allosteric". *Protein Sci.* 20:1119–24
29. Changeux JP, Edelstein S. 2005. *Nicotinic Acetylcholine Receptors: From Molecular Biology to Cognition*. New York: Odile Jacob. 284 pp.
30. Changeux JP, Edelstein S. 2011. Conformational selection or induced fit? 50 years of debate resolved. *F1000 Biol. Rep.* 3:19
31. Changeux JP, Gerhart JC, Schachman HK. 1968. Allosteric interactions in aspartate transcarbamylase. I. Binding of specific ligands to the native enzyme and its isolated subunits. *Biochemistry* 7:531–38
32. Changeux JP, Kasai M, Lee CY. 1970. Use of a snake venom toxin to characterize the cholinergic receptor protein. *Proc. Natl. Acad. Sci. USA* 67:1241–47
33. Changeux JP, Rubin MM. 1968. Allosteric interactions in aspartate transcarbamylase. III. Interpretations of experimental data in terms of the model of Monod, Wyman, and Changeux. *Biochemistry* 7:553–61
34. Changeux JP, Thiéry J. 1968. On the excitability and cooperativity of biological membranes. In *Regulatory Functions of Biological Membranes*, ed. J Järnefelt, pp. 116–38. BBA Libr. 11. Amsterdam: Elsevier
35. Changeux JP, Thiéry J, Tung Y, Kittel C. 1967. On the cooperativity of biological membranes. *Proc. Natl. Acad. Sci. USA* 57:335–41
36. Chen YJ, Pornillos O, Lieu S, Ma C, Chen AP, Chang G. 2007. X-ray structure of EmrE supports dual topology model. *Proc. Natl. Acad. Sci. USA* 104:18999–9004
37. Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, et al. 2007. High-resolution crystal structure of an engineered human β2-adrenergic G protein-coupled receptor. *Science* 318:1258–65
38. Chien E, Liu W, Zhao Q, Katritch V, Han GW, et al. 2010. Structure of the human dopamine D_3 receptor in complex with a D_2/D_3 selective antagonist. *Science* 33:1091–95
39. Choe HW, Park JH, Kim YJ, Ernst OP. 2011. Transmembrane signaling by GPCRs: insight from rhodopsin and opsin structures. *Neuropharmacology* 60:52–57
40. Clark AL, Mitchelson F. 1976. The inhibitory effect of gallamine on muscarinic receptors. *Br. J. Pharmacol.* 58:323–31
41. Comps-Agrar L, Kniazeff J, Norskov-Lauritsen L, Maurel D, Gassmann M, et al. 2011. The oligomeric state sets GABA(B) receptor signalling efficacy. *EMBO J.* 30:2336–49
42. Conn PJ, Christopoulos A, Lindsley CW. 2009. Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. *Nat. Rev. Drug Discov.* 8:41–54
43. Cotecchia S, Exum S, Caron MG, Lefkowitz RJ. 1990. Regions of the alpha 1-adrenergic receptor involved in coupling to phosphatidylinositol hydrolysis and enhanced sensitivity of biological function. *Proc. Natl. Acad. Sci. USA* 87:2896–900
44. Cuello LG, Jogini V, Cortes DM, Pan AC, Gagnon DG, et al. 2010. Structural basis for the coupling between activation and inactivation gates in K^+ channels. *Nature* 466:272–75
45. Cuello LG, Jogini V, Cortes DM, Perozo E. 2010. Structural mechanism of C-type inactivation in K^+ channels. *Nature* 466:203–8
46. Cui Q, Karplus M. 2008. Allostery and cooperativity revisited. *Protein Sci.* 17:1295–307
47. Deupi X, Kobilka BK. 2010. Energy landscapes as a tool to integrate GPCR structure, dynamics, and function. *Physiology* 25:293–303
48. Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, et al. 1998. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science* 280:69–77
49. Duke TA, Le Novère N, Bray D. 2001. Conformational spread in a ring of proteins: a stochastic approach to allostery. *J. Mol. Biol.* 308:541–53
50. Edelstein SJ. 1971. Extensions of the allosteric model for hemoglobin. *Nature* 230:224–27

51. Edelstein SJ, Changeux JP. 2010. Relationships between structural dynamics and functional kinetics in oligomeric membrane receptors. *Biophys. J.* 98:2045–52
52. Edelstein SJ, Schaad O, Changeux JP. 1997. Myasthenic nicotinic receptor mutant interpreted in terms of the allosteric model. *C. R. Acad. Sci. III* 320:953–61
53. Eigen M. 1968. New looks and outlooks on physical enzymology. *Q. Rev. Biophys.* 1:3–33
54. Engel AG, Ohno K, Sine SM. 1999. Congenital myasthenic syndromes: recent advances. *Arch. Neurol.* 56:163–67
55. Evans BA, Sato M, Sarwar M, Hutchinson DS, Summers RJ. 2010. Ligand-directed signalling at β-adrenoceptors. *Br. J. Pharmacol.* 159:1022–38
56. Fetler L, Kantrowitz ER, Vachette P. 2007. Direct observation in solution of a preexisting structural equilibrium for a mutant of the allosteric aspartate transcarbamoylase. *Proc. Natl. Acad. Sci. USA* 104:495–500
57. Fischer S, Olsen KW, Nam K, Karplus M. 2011. Unsuspected pathway of the allosteric transition in hemoglobin. *Proc. Natl. Acad. Sci. USA* 108:5608–13
58. Forman SA, Miller KW. 2011. Anesthetic sites and allosteric mechanisms of action on Cys-loop ligand-gated ion channels. *Can. J. Anaesth.* 58:191–205
59. Fylaktakidou KC, Duarte CD, Jogireddy R, Koumbis AE, Nicolau C, Lehn JM. 2011. Polyphosphates and pyrophosphates of pentopyranoses and pentofuranoses as allosteric effectors of human hemoglobin: synthesis, molecular recognition, and oxygen release. *Chem. Med. Chem.* 6:1495–508
60. Gallagher DT, Gilliland GL, Xiao G, Zondlo J, Fisher KE, et al. 1998. Structure and control of pyridoxal phosphate dependent allosteric threonine deaminase. *Structure* 6:465–75
61. Garcia H, Kondev J, Orme N, Theriot JA, Phillips R. 2011. Thermodynamics of biological processes. *Methods Enzymol.* 492:27–59
62. Gerhart JC, Pardee AB. 1962. The enzymology of control by feedback inhibition. *J. Biol. Chem.* 237: 891–96
63. Gerhart J, Pardee A. 1964. Aspartate transcarbamylase, an enzyme designed for feedback inhibition. *Fed. Proc.* 23:727–35
64. Gerhart J, Schachman H. 1965. Distinct subunits for the regulation and catalytic activity of aspartate transcarbamylase. *Biochemistry* 4:1054–62
65. Gielen M, Le Goff A, Stroebel D, Johnson JW, Neyton J, Paoletti P. 2008. Structural rearrangements of NR1/NR2A NMDA receptors during allosteric inhibition. *Neuron* 57:80–93
66. Giraudat J, Dennis M, Heidmann T, Chang JY, Changeux JP. 1986. Structure of the high-affinity site for noncompetitive blockers of the acetylcholine receptor: serine-262 of the delta subunit is labeled by [3H]chlorpromazine. *Proc. Natl. Acad. Sci. USA* 83:2719–23
67. Gohara DW, Di Cera E. 2011. Allostery in trypsin-like proteases suggests new therapeutic strategies. *Trends Biotechnol.* 29:577–85
68. Gonzales EB, Kawate T, Gouaux E. 2009. Pore architecture and ion sites in acid-sensing ion channels and P2X receptors. *Nature* 460:599–604
69. Goodsell DS, Olson AJ. 2000. Structural symmetry and protein function. *Annu. Rev. Biophys. Biomol. Struct.* 29:105–53
70. Gotti C, Moretti M, Gaimarri A, Zanardi A, Clementi F, Zoli M. 2007. Heterogeneity and complexity of native brain nicotinic receptors. *Biochem. Pharmacol.* 74:1102–11
71. Haliloglu T, Ben-Tal N. 2008. Cooperative transition between open and closed conformations in potassium channels. *PLoS Comput. Biol.* 4:e1000164
72. Hammes GG, Chang YC, Oas TG. 2009. Conformational selection or induced fit: a flux description of reaction mechanism. *Proc. Natl. Acad. Sci. USA* 106:13737–41
73. Herve G, Moody MF, Tauc P, Vachette P, Jones PT. 1985. Quaternary structure changes in aspartate transcarbamoylase studied by X-ray solution scattering: signal transmission following effector binding. *J. Mol. Biol.* 185:189–99
74. Hibbs RE, Gouaux E. 2011. Principles of activation and permeation in an anion-selective Cys-loop receptor. *Nature* 474:54–60
75. Hilf R, Bertozzi C, Zimmermann I, Reiter A, Trauner D, Dutzler R. 2010. Structural basis of open channel block in a prokaryotic pentameric ligand-gated ion channel. *Nat. Struct. Mol. Biol.* 17:1330–36

76. Hilf RJ, Dutzler R. 2008. X-ray structure of a prokaryotic pentameric ligand-gated ion channel. *Nature* 452:375–79
77. Hilf RJ, Dutzler R. 2009. Structure of a potentially open state of a proton-activated pentameric ligand-gated ion channel. *Nature* 457:115–18
78. Horovitz A, Willison KR. 2005. Allosteric regulation of chaperonins. *Curr. Opin. Struct. Biol.* 15:646–51
79. Horwich AL, Fenton WA, Chapman E, Farr GW. 2007. Two families of chaperonin: physiology and mechanism. *Annu. Rev. Cell Dev. Biol.* 23:115–45
80. Howard RJ, Murail S, Ondricek KE, Corringer PJ, Lindahl E, et al. 2011. Structural basis for alcohol modulation of a pentameric ligand-gated ion channel. *Proc. Natl. Acad. Sci. USA* 108:12149–54
81. Hub JS, Kubitzki MB, de Groot BL. 2010. Spontaneous quaternary and tertiary T-R transitions of human hemoglobin in molecular dynamics simulation. *PLoS Comput. Biol.* 6:e1000774
82. Huntington JA. 2011. Thrombin plasticity. *Biochim. Biophys. Acta* doi:10.1016/j.bbapap.2011.07.005
83. Iwata S, Kamata K, Yoshida S, Minowa T, Ohta T. 1994. T and R states in the crystals of bacterial L-lactate dehydrogenase reveal the mechanism of allosteric control. *Nat. Struct. Biol.* 1:176–85
84. Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EY, et al. 2008. The 2.6 angstrom crystal structure of a human A2A adenosine receptor bound to an antagonist. *Science* 322:1211–17
85. Jackson MB. 1984. Spontaneous openings of the acetylcholine receptor channel. *Proc. Natl. Acad. Sci. USA* 81:3901–4
86. Janin J, Bahadur RP, Chakrabarti P. 2008. Protein-protein interaction and quaternary structure. *Q. Rev. Biophys.* 41:133–80
87. Jensen MØ, Borhani DW, Lindorff-Larsen K, Maragakis P, Jogini V, et al. 2010. Principles of conduction and hydrophobic gating in K^+ channels. *Proc. Natl. Acad. Sci. USA* 107:5833–38
88. Jin R, Banke TG, Mayer ML, Traynelis SF, Gouaux E. 2003. Structural basis for partial agonist action at ionotropic glutamate receptors. *Nat. Neurosci.* 6:803–10
89. Kamata K, Mitsuya M, Nishimura T, Eiki J, Nagata Y. 2004. Structural basis for allosteric regulation of the monomeric allosteric enzyme human glucokinase. *Structure* 12:429–38
90. Kantrowitz ER, Lipscomb WN. 1988. *Escherichia coli* aspartate transcarbamylase: the relation between structure and function. *Science* 241:669–74
91. Kawate T, Michel JC, Birdsong WT, Gouaux E. 2009. Crystal structure of the ATP-gated P2X4 ion channel in the closed state. *Nature* 460:592–98
92. Kenakin T. 2007. Allosteric theory: taking therapeutic advantage of the malleable nature of GPCRs. *Curr. Neuropharmacol.* 5:149–56
93. Keov P, Sexton PM, Christopoulos A. 2011. Allosteric modulation of G protein-coupled receptors: a pharmacological perspective. *Neuropharmacology* 60:24–35
94. Khorchid A, Ikura M. 2002. How calpain is activated by calcium. *Nat. Struct. Biol.* 9:239–41
95. Kister J, Poyart C, Edelstein SJ. 1987. An expanded two-state allosteric model for interactions of human hemoglobin A with nonsaturating concentrations of 2,3-diphosphoglycerate. *J. Biol. Chem.* 262:12085–91
96. Kniazeff J, Prezeau L, Rondard P, Pin JP, Goudet C. 2011. Dimers and beyond: the functional puzzles of class C GPCRs. *Pharmacol. Ther.* 130:9–25
97. Koshland DE Jr. 1959. Enzyme flexibility and enzyme action. *J. Cell Comp. Physiol.* 54:245–58
98. Koshland DE Jr, Némethy G, Filmer D. 1966. Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry* 5:365–85
99. Krashia P, Lape R, Lodesani F, Colquhoun D, Sivilotti LG. 2011. The long activations of $\alpha 2$ glycine channels can be described by a mechanism with reaction intermediates ("flip"). *J. Gen. Physiol.* 137:197–216
100. Krause RM, Buisson B, Bertrand S, Corringer PJ, Galzi JL, et al. 1998. Ivermectin: a positive allosteric effector of the $\alpha 7$ neuronal nicotinic acetylcholine receptor. *Mol. Pharmacol.* 53:283–94
101. Lefkowitz RJ, Cotecchia S, Samama P, Costa T. 1993. Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. *Trends Pharmacol. Sci.* 14:303–7
102. Le Novère N, Corringer PJ, Changeux JP. 2002. The diversity of subunit composition in nAChRs: evolutionary origins, physiologic and pharmacologic consequences. *J. Neurobiol.* 53:447–56
103. Lewis M. 2005. The *lac* repressor. *C. R. Biol.* 328:521–48

104. Lewis M. 2011. A tale of two repressors. *J. Mol. Biol.* 409:14–27
105. Lipscomb WN. 1994. Aspartate transcarbamylase from *Escherichia coli*: activity and regulation. *Adv. Enzymol. Relat. Areas Mol. Biol.* 68:67–151
106. Liu L, Botos I, Wang Y, Leonard JN, Shiloach J, et al. 2008. Structural basis of Toll-like receptor 3 signaling with double-stranded RNA. *Science* 320:379–81
107. Macol CP, Tsuruta H, Stec B, Kantrowitz ER. 2001. Direct structural evidence for a concerted allosteric transition in *Escherichia coli* aspartate transcarbamoylase. *Nat. Struct. Biol.* 8:423–26
108. Martinez KL, Corringer PJ, Edelstein SJ, Changeux JP, Mérola F. 2000. Structural differences in the two agonist binding sites of the *Torpedo* nicotinic acetylcholine receptor revealed by time-resolved fluorescence spectroscopy. *Biochemistry* 39:6979–90
109. Mazzaferro S, Benallegue N, Carbone A, Gasparri F, Vijayan R, et al. 2011. Additional acetylcholine (ACh) binding site at α4/α4 interface of (α4{β}2)2α4 nicotinic receptor influences agonist sensitivity. *J. Biol. Chem.* 286:31043–54
110. Monod J, Changeux JP, Jacob F. 1963. Allosteric proteins and cellular control systems. *J. Mol. Biol.* 6:306–29
111. Monod J, Jacob F. 1961. Teleonomic mechanisms in cellular metabolism, growth, and differentiation. *Cold Spring Harb Symp. Quant. Biol.* 26:389–401
112. Monod J, Wyman J, Changeux JP. 1965. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* 12:88–118
113. Mukhtasimova N, Lee WY, Wang HL, Sine SM. 2009. Detection and trapping of intermediate states priming nicotinic receptor channel opening. *Nature* 459:451–54
114. Murail S, Wallner B, Trudell JR, Bertaccini E, Lindahl E. 2011. Microsecond simulations indicate that ethanol binds between subunits and could stabilize an open-state model of a glycine receptor. *Biophys. J.* 100:1642–50
115. Murakami M, Kouyama T. 2008. Crystal structure of squid rhodopsin. *Nature* 453:363–67
116. Nury H, Poitevin F, Van Renterghem C, Changeux JP, Corringer PJ, et al. 2010. One microsecond molecular dynamics simulation of channel gating in a nicotinic receptor homologue. *Proc. Natl. Acad. Sci. USA* 107:6275–80
117. Nury H, Van Renterghem C, Weng Y, Tran A, Baaden M, et al. 2011. X-ray structures of general anaesthetics bound to a pentameric ligand-gated ion channel. *Nature* 469:428–31
118. Oswald RE, Changeux JP. 1982. Crosslinking of α-bungarotoxin to the acetylcholine receptor from *Torpedo marmorata* by ultraviolet light irradiation. *FEBS Lett.* 139:225–29
119. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, et al. 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* 289:739–45
120. Park JH, Scheerer P, Hofmann KP, Choe HW, Ernst OP. 2008. Crystal structure of the ligand-free G-protein-coupled receptor opsin. *Nature* 454:183–88
121. Payandeh J, Scheuer T, Zheng N, Catterall WA. 2011. The crystal structure of a voltage-gated sodium channel. *Nature* 475:353–58
122. Pellissier LP, Barthet G, Gaven F, Cassier E, Trinquet E, et al. 2011. G protein activation by serotonin type 4 receptor dimers: evidence that turning on two protomers is more efficient. *J. Biol. Chem.* 286:9985–97
123. Perutz MF. 1989. Mechanisms of cooperativity and allosteric regulation in proteins. *Q. Rev. Biophys.* 22:139–237
124. Perutz MF, Bolton W, Diamond R, Muirhead H, Watson HC. 1964. Structure of haemoglobin. An X-ray examination of reduced horse haemoglobin. *Nature* 203:687–90
125. Perutz MF, Fermi G, Abraham DJ, Poyart C, Bursaux CE. 1986. Hemoglobin as a receptor of drugs and peptides: X-ray studies of the stereochemistry of binding. *J. Am. Chem. Soc.* 108:1064–78
126. Perutz MF, Rossmann MG, Cullis AF, Muirhead H, Will G, North AC. 1960. Structure of haemoglobin: a three-dimensional Fourier synthesis at 5.5-A. resolution, obtained by X-ray analysis. *Nature* 185:416–22
127. Perutz MF, Wilkinson AJ, Paoli M, Dodson GG. 1998. The stereochemical mechanism of the cooperative effects in hemoglobin revisited. *Annu. Rev. Biophys. Biomol. Struct.* 27:1–34
128. Pin JP, Comps-Agrar L, Maurel D, Monnier C, Rives ML, et al. 2009. G-protein-coupled receptor oligomers: two or more for what? Lessons from mGlu and $GABA_B$ receptors. *J. Physiol.* 587:5337–44

129. Reichard P. 2010. Ribonucleotide reductases: substrate specificity by allostery. *Biochem. Biophys. Res. Commun.* 396:19–23
130. Revah F, Bertrand D, Galzi JL, Devillers-Thiéry A, Mulle C, et al.1991. Mutations in the channel domain alter desensitization of a neuronal nicotinic receptor. *Nature* 353:846–49
131. Rochel N, Ciesielski F, Godet J, Moman E, Roessle M, et al. 2011. Common architecture of nuclear receptor heterodimers on DNA direct repeat elements with different spacings. *Nat. Struct. Mol. Biol.* 18:564–70
132. Rosenbaum DM, Zhang C, Lyons JA, Holl R, Aragao D, et al. 2011. Structure and function of an irreversible agonist-β_2 adrenoceptor complex. *Nature* 469:236–40
133. Rubin M, Changeux JP. 1966. On the nature of allosteric transitions: implications of non-exclusive ligand binding. *J. Mol. Biol.* 21:265–74
134. Schirmer T, Evans PR. 1990. Structural basis of the allosteric behaviour of phosphofructokinase. *Nature* 343:140–45
135. Seifert R, Wenzel-Seifert K. 2002. Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn Schmiedebergs Arch. Pharmacol.* 366:381–416
136. Shimamura T, Shiroishi M, Weyand S, Tsujimoto H, Winter G, et al. 2011. Structure of the human histamine H1 receptor complex with doxepin. *Nature* 475:65–70
137. Shimizu H, Iwamoto M, Konno T, Nihei A, Sasaki YC, Oiki S. 2008. Global twisting motion of single molecular KcsA potassium channel upon gating. *Cell* 132:67–78
138. Shimizu TS, Le Novère N, Levin MD, Beavil AJ, Sutton BJ, Bray D. 2000. Molecular model of a lattice of signalling proteins involved in bacterial chemotaxis. *Nat. Cell Biol.* 2:792–96
139. Smit AB, Celie PH, Kasheverov IE, Mordvintsev DY, van Nierop P, et al. 2006. Acetylcholine-binding proteins: functional and structural homologs of nicotinic acetylcholine receptors. *J. Mol. Neurosci.* 30:9–10
140. Sobolevsky AI, Rosconi MP, Gouaux E. 2009. X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. *Nature* 462:745–56
141. Sprang SR, Acharya KR, Goldsmith EJ, Stuart DI, Varvill K, et al. 1988. Structural changes in glycogen phosphorylase induced by phosphorylation. *Nature* 336:215–21
142. Standfuss J, Edwards PC, D'Antona A, Fransen M, Xie G, et al. 2011. The structural basis of agonist-induced activation in constitutively active rhodopsin. *Nature* 471:656–60
143. Steinlein OK. 2010. Animal models for autosomal dominant frontal lobe epilepsy: on the origin of seizures. *Expert Rev. Neurother.* 10:1859–67
144. Stockton JM, Birdsall NJ, Burgen AS, Hulme EC. 1983. Modification of the binding properties of muscarinic receptors by gallamine. *Mol. Pharmacol.* 23:551–57
145. Taly A, Corringer PJ, Grutter T, Prado de Carvalho L, Karplus M, Changeux JP. 2006. Implications of the quaternary twist allosteric model for the physiology and pathology of nicotinic acetylcholine receptors. *Proc. Natl. Acad. Sci. USA* 103:16965–70
146. Taly A, Corringer PJ, Guedin D, Lestage P, Changeux JP. 2009. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. *Nat. Rev. Drug Discov.* 8:733–50
147. Taly A, Delarue M, Grutter T, Nilges M, Le Novère N, et al. 2005. Normal mode analysis suggests a quaternary twist model for the nicotinic receptor gating mechanism. *Biophys. J.* 88:3954–65
148. Tasneem A, Iyer LM, Jakobsson E, Aravind L. 2005. Identification of the prokaryotic ligand-gated ion channels and their implications for the mechanisms and origins of animal Cys-loop ion channels. *Genome Biol.* 6:R4
149. Taylor P, Talley TT, Radic Z, Hansen SB, Hibbs RE, Shi J. 2007. Structure-guided drug design: conferring selectivity among neuronal nicotinic receptor and acetylcholine-binding protein subtypes. *Biochem. Pharmacol.* 74:1164–71
150. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, et al. 2010. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol. Rev.* 62:405–96
151. Unwin N. 2005. Refined structure of the nicotinic acetylcholine receptor at 4 Å resolution. *J. Mol. Biol.* 346:967–89

152. Velyvis A, Yang YR, Schachman HK, Kay LE. 2007. A solution NMR study showing that active site ligands and nucleotides directly perturb the allosteric equilibrium in aspartate transcarbamoylase. *Proc. Natl. Acad. Sci. USA* 104:8815–20
153. Viappiani C, Bettati S, Bruno S, Ronda L, Abbruzzetti S, et al. 2004. New insights into allosteric mechanisms from trapping unstable protein conformations in silica gels. *Proc. Natl. Acad. Sci. USA* 101:14414–19
154. von Heijne G. 2006. Membrane-protein topology. *Nat. Rev. Mol. Cell Biol.* 7:909–18
155. Voss JE, Vaney MC, Duquerroy S, Vonrhein C, Girard-Blanc C, et al. 2010. Glycoprotein organization of Chikungunya virus particles revealed by X-ray crystallography. *Nature* 468:709–12
156. Wang J, Stieglitz KA, Cardia JP, Kantrowitz ER. 2005. Structural basis for ordered substrate binding and cooperativity in aspartate transcarbamoylase. *Proc. Natl. Acad. Sci. USA* 102:8881–86
157. Warne T, Serrano-Vega MJ, Baker JG, Moukhametzianov R, Edwards PC, et al. 2008. Structure of a β1-adrenergic G-protein-coupled receptor. *Nature* 454:486–91
158. West JM, Xia J, Tsuruta H, Guo W, O'Day EM, Kantrowitz ER. 2008. Time evolution of the quaternary structure of *Escherichia coli* aspartate transcarbamoylase upon reaction with the natural substrates and a slow, tight binding inhibitor. *J. Mol. Biol.* 384:206–18
159. Wu B, Chien EY, Mol CD, Fenalti G, Liu W, et al. 2010. Structures of the CXCR4 chemokine GPCR with small-molecule and cyclic peptide antagonists. *Science* 330:1066–71
160. Wyman J. 1967. Allosteric linkage. *J. Am. Chem. Soc.* 89:2202–18
161. Wyman J. 1984. Linkage graphs: a study in the thermodynamics of macromolecules. *Q. Rev. Biophys.* 17:453–88
162. Xu F, Wu H, Katritch V, Han GW, Jacobson KA, et al. 2011. Structure of an agonist bound human A_{2A} adenosine receptor. *Science* 332:322–27
163. Zimmermann I, Dutzler R. 2011. Ligand activation of the prokaryotic pentameric ligand-gated ion channel ELIC. *PLoS Biol.* 9:e1001101