

Review

Cite this article: Montserrat-Canals M, Cordara G and Kregel U (2025). Allostery. *Quarterly Reviews of Biophysics*, 58, e5, 1–26 <https://doi.org/10.1017/S0033583524000209>

Received: 08 September 2024

Revised: 02 December 2024

Accepted: 03 December 2024

Keywords:

allostery concepts; aspartate transcarbamoylase; ATP synthase; biased signaling; chaperonin; GPCRs; hemoglobin; IDPs; inter-enzyme allostery; kinase; Lac repressor; methods to probe allostery; PAM and NAM; structural biology; structure-based drug discovery (SBDD)

Corresponding author:

Ute Kregel;

Email: ute.kregel@kjemi.uio.no

© The Author(s), 2025. Published by Cambridge University Press. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.

Allostery

Mateu Montserrat-Canals^{1,2} , Gabriele Cordara^{1,2}  and Ute Kregel^{1,2} 

¹Department of Chemistry, University of Oslo, Oslo, Norway and ²Hylleraas Centre for Quantum Molecular Sciences, University of Oslo, Oslo, Norway

Abstract

Allostery describes the ability of biological macromolecules to transmit signals spatially through the molecule from an *allosteric* site – a site that is distinct from *orthosteric* binding sites of primary, endogenous ligands – to the functional or active site. This review starts with a historical overview and a description of the classical example of allostery – hemoglobin – and other well-known examples (aspartate transcarbamoylase, Lac repressor, kinases, G-protein-coupled receptors, adenosine triphosphate synthase, and chaperonin). We then discuss fringe examples of allostery, including intrinsically disordered proteins and inter-enzyme allostery, and the influence of dynamics, entropy, and conformational ensembles and landscapes on allosteric mechanisms, to capture the essence of the field. Thereafter, we give an overview over central methods for investigating molecular mechanisms, covering experimental techniques as well as simulations and artificial intelligence (AI)-based methods. We conclude with a review of allostery-based drug discovery, with its challenges and opportunities: with the recent advent of AI-based methods, allosteric compounds are set to revolutionize drug discovery and medical treatments.

Table of contents

Introduction: allostery in context – historical overview	1
Classical example: hemoglobin	2
Other well-known examples	4
Aspartate transcarbamoylase	4
Lac repressor	5
Kinases	6
GPCRs, G-proteins and biased signaling	7
ATP synthase	9
Chaperonin	9
Interesting cases at the “extremes”	11
Monomeric allostery	11
Inter-enzyme allostery	11
Allostery of intrinsically disordered proteins	11
Dynamics, entropy, and ensemble nature of allostery	13
Methods to probe allostery	13
X-ray crystallography	13
Cryo-EM	15
NMR spectroscopy	15
HDX mass spectrometry	16
FRET	16
Other biophysical techniques	16
Deep mutational scanning, directed evolution, “resurrection” of evolutionary origins, and allosteric engineering	16
MD simulations, other computational methods, and AI	17
Allostery and drug discovery	18
Summary and outlook	19

Introduction: allostery in context – historical overview

Life costs energy. To preserve valuable resources, it is therefore essential that wasteful processes are limited to a minimum. Regulation is of the essence. Proteins are the workhorses of the cell. Their activity is tightly regulated by three main mechanisms: i) protein amount and lifetime, ii) direction to different compartments (e.g., organelles or membrane-less compartments in the cell), and iii) covalent modification or non-covalent binding of effectors; as so elegantly described in the textbook by Petsko and Ringe (2004). Regulation at the transcriptional level takes time, and protein degradation is

wasteful and requires recycling of components. The most efficient means is the direct regulation of protein activity by covalent modification or by ligand binding. The latter is the topic of this review.

Effector binding can either occur at the binding sites of primary, endogenous ligands (the so-called *orthosteric* sites) or at another location, *allos stereos* (Greek). If binding to a remote site nevertheless affects the biological outcome, one speaks of ‘allostery’. Ligand or substrate (= reactant) binding can be allosterically up- or down-regulated. This is often the case for enzymes catalyzing metabolic processes, prompting immediate response to elevated or depleted metabolite pools (Perutz, 1989), but also occurs in proteins without catalytic function.

The first protein structures determined were myoglobin and hemoglobin, by John C. Kendrew and Max Perutz in the late 1950s (Kendrew et al., 1958, 1960; Perutz et al., 1960). Hemoglobin was also the first protein ever reported to be crystallized (Schultz, 1836). It became the poster child for allosteric regulation (Monod et al., 1965; Koshland et al., 1966; Perutz, 1989; Changeux, 2013) and is featured in every biochemistry textbook. In contrast to myoglobin, hemoglobin adopts a multimeric structure: it is a tetramer composed of two α - and two β -subunits. Similar to catalytic substrates and orthosteric ligands, allosteric effectors often bind at subunit interfaces, along symmetry axes, where they trigger changes in subunit arrangement (Changeux and Edelstein, 2005). However, more recently, allostery has also been observed in monomeric proteins, including myoglobin (Frauenfelder et al., 2001; Ascenzi and Fasano, 2010; Larion et al., 2012), as well as in RNAs and ribozymes (Winkler and Dann III, 2006). In another twist, allostery has been observed to involve two different enzymes at once, referred to as ‘inter-enzyme allostery’ (Munack et al., 2016).

Already before the first protein structure was solved, there were indications of allosteric regulation in biological systems (Bohr, 1892; Bohr et al., 1904; Novick and Szilard, 1954; Umbarger, 1956; Yates and Pardee, 1956; Umbarger and Brown, 1958). The term ‘allosteric’ was, however, first coined by Monod and Jacob (1961) building on Changeux’s now classic experiments (Changeux, 1961). This work was further developed in 1963 (Monod et al., 1963) and culminated in the famous Monod–Wyman–Changeux (MWC) model of allostery in 1965 (Monod et al., 1965), characterized by the interconversion between tense (T) and relaxed (R) conformational states, exemplified for hemoglobin (Figure 1a). An alternative model (Koshland–Nemethy–Filmer (KNF); Figure 1b) (Koshland et al., 1966) described the observed data in terms of sequential changes of protein conformation. For a well-written historic account of the events we recommend the essay contributed by Morange (2012).

Although not initially described as such, the MWC model is commonly referred to as ‘conformational selection’ model, whereas the KNF model is also known as ‘induced-fit’ model. Both models originally focused on phenomenological changes, and not on the underlying stereochemical mechanism, which was first analyzed by Perutz in 1970 (Perutz, 1970; Perutz et al., 1998). More recently, the role of dynamics and thermodynamics in allosteric regulation have also been appreciated (Jardetzky, 1996; Kern and Zuiderweg, 2003; Popovych et al., 2006; Tsai et al., 2008; Boehr et al., 2009; Tzeng and Kalodimos, 2012). Allostery can even occur without any obvious change in conformation (Cooper and Dryden, 1984; Popovych et al., 2006; Tsai et al., 2008; Tzeng and Kalodimos, 2012; McLeish et al., 2013; Nussinov and Tsai, 2015). At the other end of the spectrum, very drastic conformational changes have been observed, for example, when intrinsically disordered proteins (IDPs) fold up upon binding to allosteric effectors (Ferreon et al., 2013). This can

be explained by the influence of allosteric effectors on the relative energies of an ensemble of conformational states, a model that has gained traction only recently (Ma et al., 1999; Lange et al., 2008; Boehr et al., 2009; Ferreon et al., 2013; Hilser, 2013; Motlagh et al., 2014; Wei et al., 2016), even though its principle was essentially already represented by the MWC model (Monod et al., 1965). Today, it is clear that both the conformational selection model and the induced-fit principles underlying the KNF model (Koshland, 1959; Koshland et al., 1966) are important features of allosteric regulation (Perutz, 1989; Silva et al., 2011; Motlagh et al., 2014; Tsai and Nussinov, 2014). It has even been postulated that *all dynamic proteins* may be allosteric (Gunasekaran et al., 2004) – and which proteins are not dynamic?

Classical example: hemoglobin

Hemoglobin is the major oxygen carrier in blood and responsible for transporting oxygen from the lungs to the rest of the body, where it is released to the tissues (Figure 2a). Oxygen uptake and release needs to be tightly regulated to preclude suffocation: in the lungs, oxygen uptake must be efficient, and in the tissues, oxygen must be promptly released. Oxygen binds to hemoglobin at four heme groups – one heme per subunit – to the iron ion at its center (Figure 2b) (Perutz et al., 1960, 1968). In contrast to monomeric myoglobin, which binds and stores oxygen in muscle, oxygen binding to hemoglobin is positively cooperative, characterized by a sigmoidal curve (Theorell, 1934; Morgan and Chichester, 1935) (Figure 2a): the more oxygen binds, the higher the protein’s affinity for it, until hemoglobin is loaded with four molecules of oxygen. This means that oxygen binding to one subunit affects oxygen binding to a distant site on another subunit. With this, hemoglobin is a perfect example of allostery and featured as the first of 24 examples in the publication by Monod et al. (1965), where they presented a relatively simple model of allostery that became known as ‘MWC’ or ‘symmetry model’ (Figure 1a). This model postulated that “allosteric effects are due to the displacement of an equilibrium between discrete states”, because ligands stabilize either one state or the other and “the conformation of each protomer is constrained by its association with the other protomers” (Monod et al., 1965). This model was challenged by KNF shortly afterward, who noted that “the fact that a good fit to hemoglobin data was obtained with rather simple models does not mean that these simple models are necessarily correct” and “a partial change in the A conformation might be caused by a change in an adjacent B structure, thus giving a conformation intermediate between A and B” (Koshland et al., 1966). The KNF model is therefore also known as ‘sequential model’ of allostery (Figure 1b).

Both models eventually turned out to be too simplistic (Colombo et al., 1992; Silva et al., 1992; Cui and Karplus, 2008; Fischer et al., 2011; Changeux, 2012). In a groundbreaking thermodynamic study in 1992, Parsegian et al. demonstrated the importance of solvation effects in allosteric regulation, and noted that the transition from deoxygenated T state to oxygenated R state in hemoglobin was accompanied by the binding of approximately 60 additional water molecules (Colombo et al., 1992), pointing to the effect of other factors than structure alone (reminiscent of the ‘hydrophobic effect’ underlying protein folding). In the same year, Arnone and coworkers reported a third quaternary structure of human hemoglobin (Silva et al., 1992).

Allosteric control of hemoglobin is in fact quite sophisticated. In addition to the *homotropic* effect of oxygen binding to different

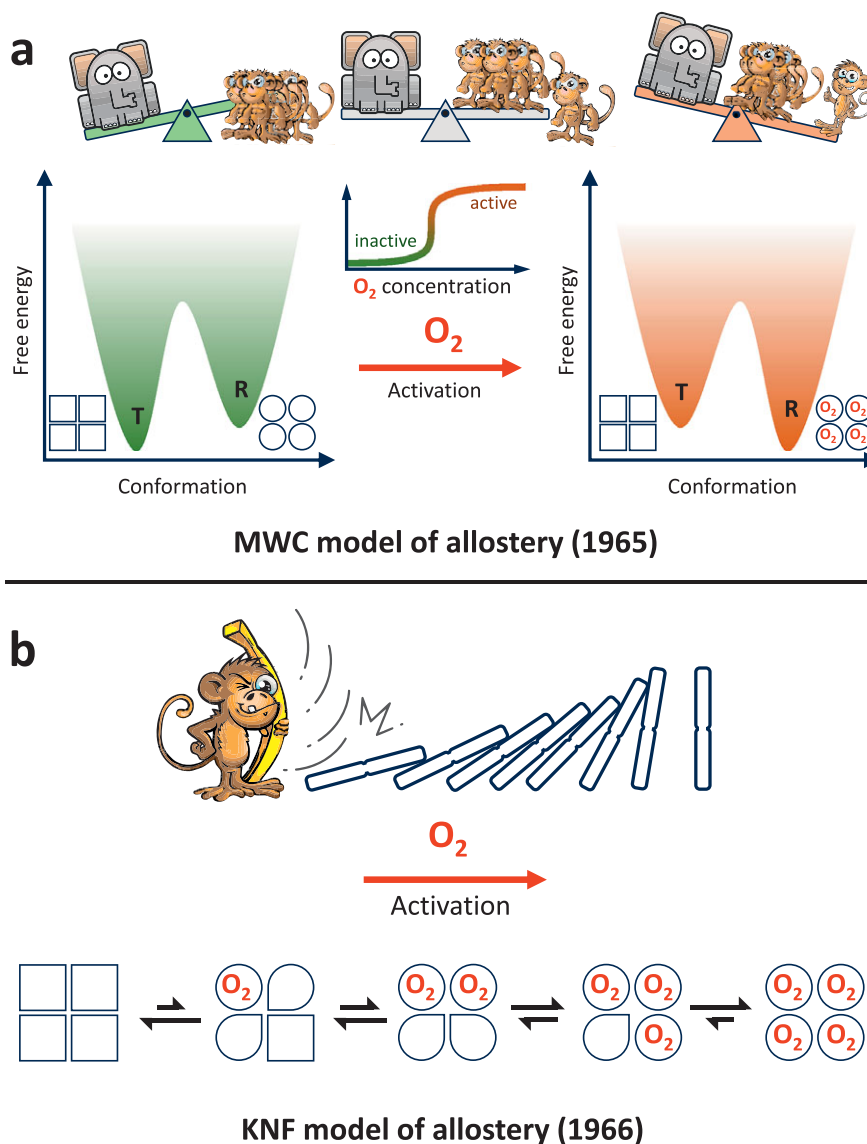


Figure 1. Classical allostery models for hemoglobin. (a) Monod–Wyman–Changeux model of allostery, put forward by Monod et al. (1965), here represented by a seesaw. This model is also known as ‘symmetry model’ (sometimes also as ‘concerted model’ or ‘conformational selection model’, although not initially described as such) and focuses on the conversion between two states: the inactive T (tense) state and the active R (relaxed) state poised for oxygen binding. Note the sigmoid curve for conversion between states. Panel adapted from Figure 1 in Tsai and Nussinov (2014). (b) Koshland–Nemethy–Filmer model of allostery, published by Koshland et al. (1966). This model is also known as ‘sequential model’ (or ‘domino model’), based on the assumption that conformational changes in one subunit likely affect the neighboring subunits, leading to a sequential change from T to R state. Panel adapted from Figure 1 in Kornev and Taylor (2015).

subunits, oxygen binding and release is also regulated by *heterotropic effects*, that is, by binding of other molecules or ions. In most jawed vertebrates, release of oxygen from hemoglobin to myoglobin in muscle is regulated by BPG (2,3-bisphosphoglycerate), a highly negatively charged small molecule, which binds to the heterotetramer interface at the center of hemoglobin by electrostatic interactions (Arnone, 1972) (Figure 2c). Binding of BPG causes subtle conformational changes that cause a realignment of the protein subunits, promoting oxygen release by interfering with oxygen binding to the heme groups. In other words, although oxygen and BPG bind to hemoglobin at different sites, binding is mutually exclusive.

BPG is not the only heterotropic allosteric regulator of hemoglobin. Also protons (i.e., low pH), carbon dioxide, and chloride interfere with oxygen binding by allosteric mechanisms involving salt bridges and hydrogen bonds (Perutz, 1970, 1989).

Inhibition of oxygen binding by protons is referred to as ‘Bohr effect’, after its discoverer Christian Bohr (the father of the famous Danish physicist Niels Bohr) (Bohr, 1892; Bohr et al., 1904). Inhibition by carbon dioxide also involves protons (in addition to N-terminal carbamate formation), which are released upon dissolving of the gas in the blood (with concomitant formation of bicarbonate – a process catalyzed by carbonic anhydrase).

Allosteric regulation of hemoglobin relies on its tetrameric structure. This makes it interesting to compare hemoglobin to its monomeric ancestor, which likely resembles myoglobin (displaying a hyperbolic oxygen binding curve suggesting non-cooperativity) (Theorell, 1934). Even though myoglobin has also been suggested to have allosteric features (Frauenfelder et al., 2001), its regulation is clearly less sophisticated compared to tetrameric hemoglobin (Antonini and Brunori, 1971; Baldwin and Chothia, 1979). By constructing a phylogenetic tree and subsequent experimental

characterization of “resurrected” family members, Pillai et al. were able to shed light on how complex regulation can arise from humble beginnings (Pillai et al., 2020; Berenbrink, 2020). Their rigorous study showed that strikingly few mutations are required to turn a simple monomeric hemoglobin precursor *via* a non-cooperative homodimer “missing link” into a tetrameric protein with regulatory features like hemoglobin.

Other well-known examples

Aspartate transcarbamoylase

Aspartate transcarbamoylase (ATCase) catalyzes a key step in pyrimidine biosynthesis, with cytidine triphosphate (CTP) as the final product. This enzyme featured a prominent role under the development of the MWC model (Monod et al., 1965) and is one of the best-known examples of allosteric regulation of enzymes. ATCase has a cage-like structure composed of six catalytic and six regulatory

subunits (Kantrowitz and Lipscomb, 1988) (Figure 3). In the T state, the cage is collapsed and the active sites are occluded. Upon binding of the substrates, the enzyme “relaxes” by a concerted transition into the R state (Macol et al., 2001). Bringing the two substrates L-aspartate and carbamoyl phosphate in close proximity is essentially all that is needed for the reaction to occur, which is a prime example for a ‘propinquity’-driven reaction.

ATCase is activated by feedforward control, provided by the end product of purine biosynthesis, adenosine triphosphate (ATP). Binding of CTP to the same regulatory sites inhibits the enzyme (Changeux et al., 1968), preventing a waste of resources (Yates and Pardee, 1956). Feedback inhibition and feedforward control are common mechanisms of metabolic allosteric regulation (Monod et al., 1963). The inhibitory effect of CTP has, however, been overestimated earlier and is rather weak on its own (Cockrell et al., 2013). *In vivo*, full feedback inhibition requires the combination of CTP, uridine triphosphate (UTP), and a divalent ion (and also feedforward activation is enhanced by a divalent ion) (Cockrell

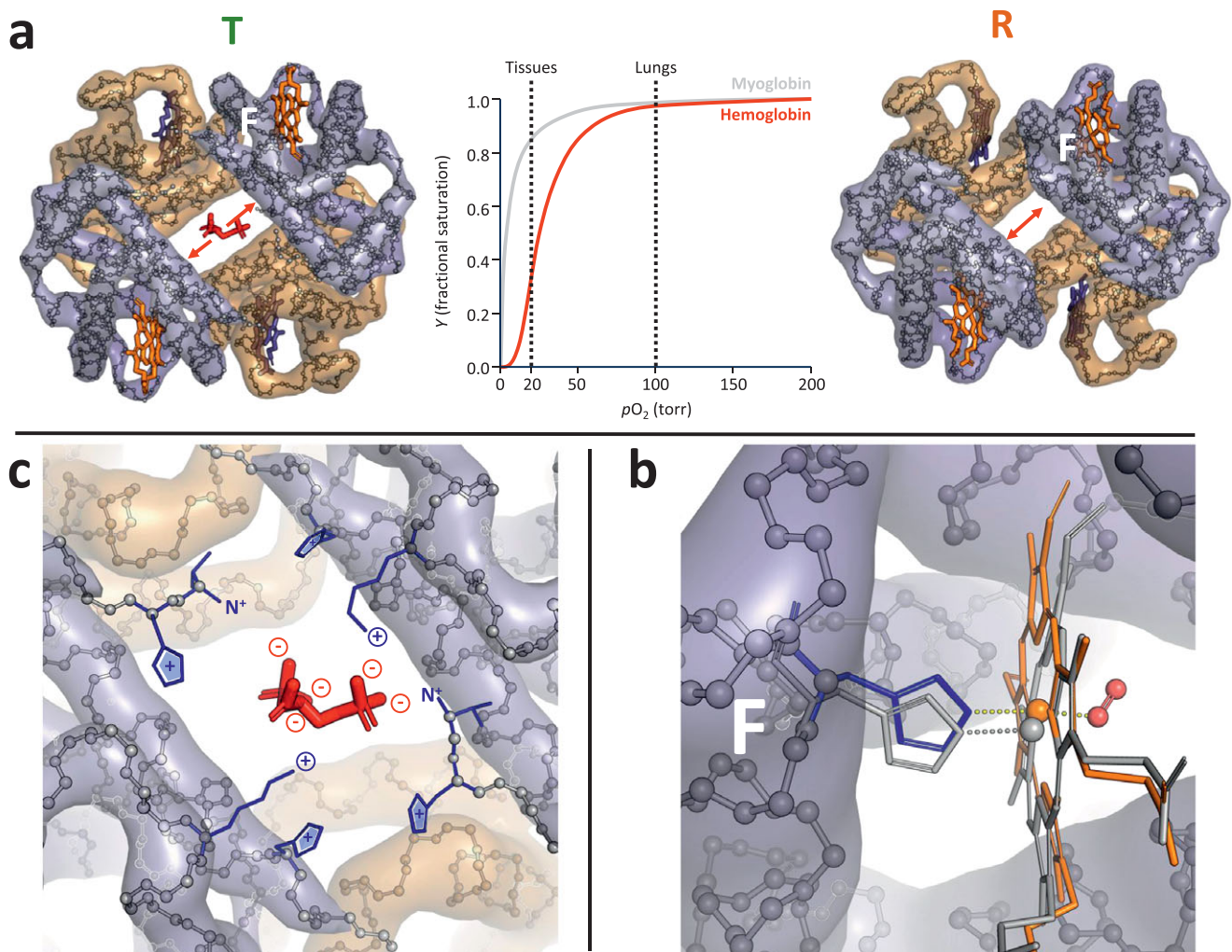


Figure 2. Allosteric transitions in hemoglobin. (a) Deoxy (T state, left; PDB ID: 5KSI) (Sun et al., 2017) and oxy forms of hemoglobin (R state, right; PDB ID: 3A0G (Etti et al., to be published)), depicted in Irving–Geis-like drawings. The hemoglobin α -subunits are colored in orange and the β -subunits in deep violet, with heme groups bound. In the middle, the sigmoid oxygen uptake curve of hemoglobin is shown, which ensures oxygen transport from the lungs to the tissues (adapted from Tymoczko et al., 2018, Figure 9.1). (b, lower right) Oxygen (red) binding to the heme group (gray/orange, with iron sphere at its center) converts the deoxy (gray) to the oxy (colored) form of hemoglobin. Note the change in conformation of the heme group and its effect on the proximal histidine (gray/blue sticks), which is part of helix F. This helix is close to the interface between α - and β -subunits, as shown in panel (a) above. (c, lower left) The negatively charged allosteric hemoglobin inhibitor 2,3-bisphosphoglycerate (BPG, red sticks) binds to the center of the hemoglobin $\alpha_2\beta_2$ -heterotetramer and interacts with positively charged groups in its vicinity. Binding of BPG stabilizes the tense (T) deoxy state of hemoglobin shown in panel (a) above.

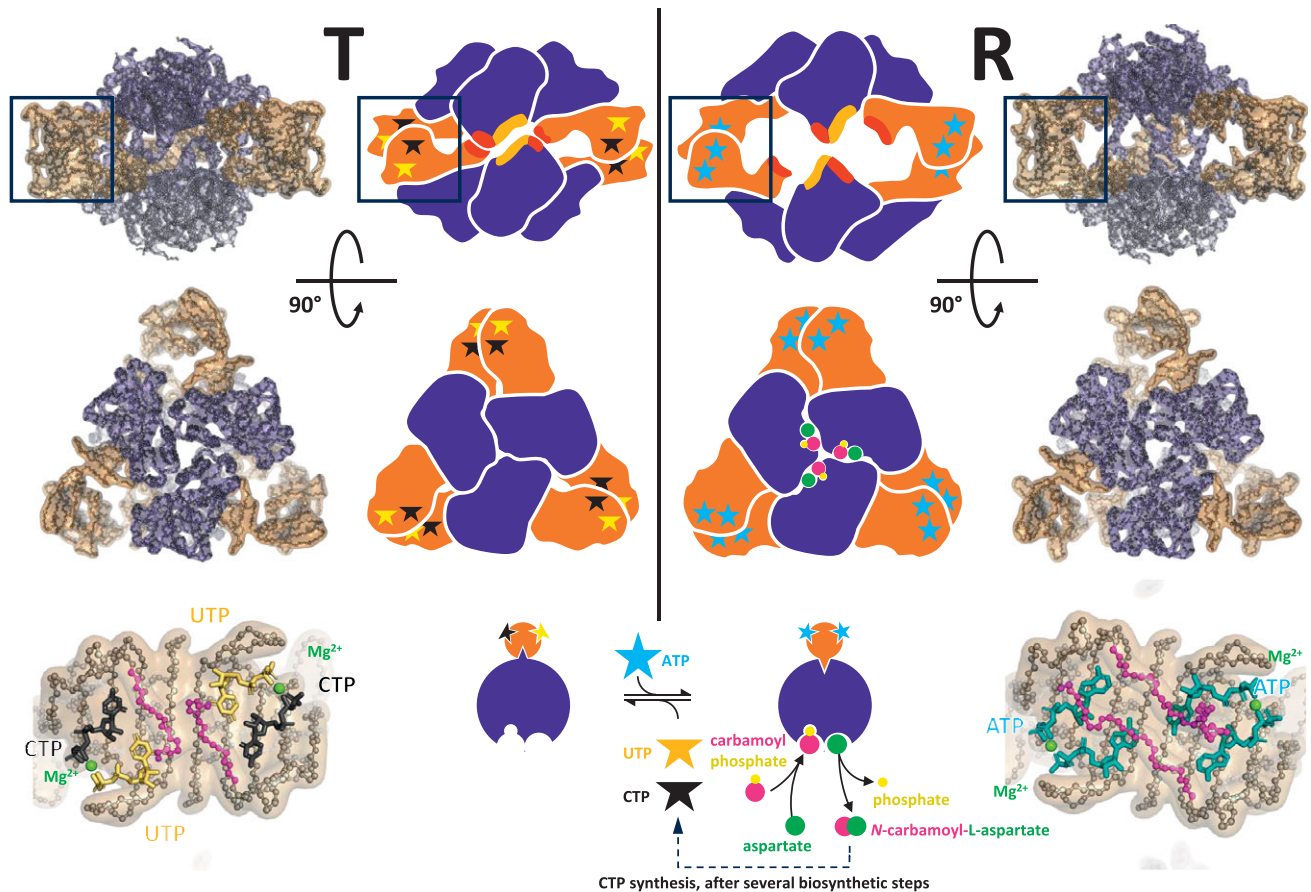


Figure 3. Allosteric transition in aspartate transcarbamoylase (ATCase). Left and right panels show the structure of the ATCase heterododecamer in its tense (PDB ID: 4KH1) and relaxed states (PDB ID: 4KH0), respectively (Cockrell et al., 2013). The upper and middle panels provide side and top views of the complex, with catalytic and regulatory subunits in different colors (deep violet and orange, respectively). Interfaces that are altered by the allosteric transition are highlighted in red and yellow. The schematic representations are based on Figures 3–10 from the textbook by Petsko and Ringe (2004). A black square contour marks the region of the regulatory subunit bound to the allosteric regulators (either the feedback inhibitors CTP + UTP, in black/orange, or the feedforward activator adenosine triphosphate (ATP), in blue), shown in detail in the bottom panel (based on the graphical abstract by Cockrell et al., 2013). Note the different conformations of the N-termini of the regulatory subunits (marked in magenta), which trigger opposite allosteric effects (inhibition or activation) through the same site. At bottom-center, the reaction catalyzed by ATCase and the feedback loop leading to its tense/relaxed transition are shown in schematic representation, with ATP as blue star and the smaller nucleotides CTP (black) and UTP (orange) represented by truncated stars.

et al., 2013). This new paradigm for ATCase allosteric regulation reconciles previous inconsistencies; for example, the requirement of divalent ions (Cockrell and Kantrowitz, 2012), the fact that ATP and CTP alone cannot induce the T to R transition (Howlett et al., 1977) and that UTP contributes to feedback inhibition, but binds to a unique regulatory site on ATCase (Peterson et al., 2013). The question how binding of inhibitors and activators to the same regulatory sites can cause so different effects, is elucidated by the unique – and alternatively exclusive – conformations of the N-terminal regions, stabilizing either the T or R state (Cockrell et al., 2013) (Figure 3).

Lac repressor

Bacterial genes are often organized in operons, which contain multiple genes under the control of a single promoter. Enzymes encoded by the *lac* operon enable cells to metabolize the disaccharide lactose when the bacteria’s preferred carbon source (glucose) is unavailable (Lewis, 2013). However, when glucose is plentiful or lactose is not present, transcription is shut down to avoid wasting resources. This is the function of the Lac repressor, a tetrameric protein, which prevents binding of

RNA polymerase to the promoter by binding to specific DNA sequences upstream of the operon genes. The Lac repressor has two domains: one for binding DNA and the other for tetramerization (Lewis et al., 1996) (Figure 4). Release of the repressor is triggered by allosteric regulation when an inducer (often derived from lactose, such as isopropyl β-D-1-thiogalactopyranoside (IPTG), and therefore signaling its presence) binds to the repressor at the distal, tetramerization site (Figure 4). Other metabolites activate repression. Despite the availability of crystal structures of the Lac repressor in complexes with DNA, inducer, and other allosteric effectors (Lewis et al., 1996; Daber et al., 2007), the precise allosteric model has only been elucidated recently, by high-resolution nuclear magnetic resonance (NMR) spectroscopy (Romanuka et al., 2023). Their data showed that the Lac repressor in the absence of ligands exists in a dynamic equilibrium between two conformational states, and the equilibrium is subtly shifted to the one or other side by DNA or inducers (Figure 4) – in full agreement with the MWC model.

Regulation of the *Escherichia coli lac* operon was famously analyzed and understood by Jacob and Monod (Jacob and Monod, 1961). Today, it is commonly exploited in biochemistry laboratories and the biotechnology industry for recombinant expression of genes.

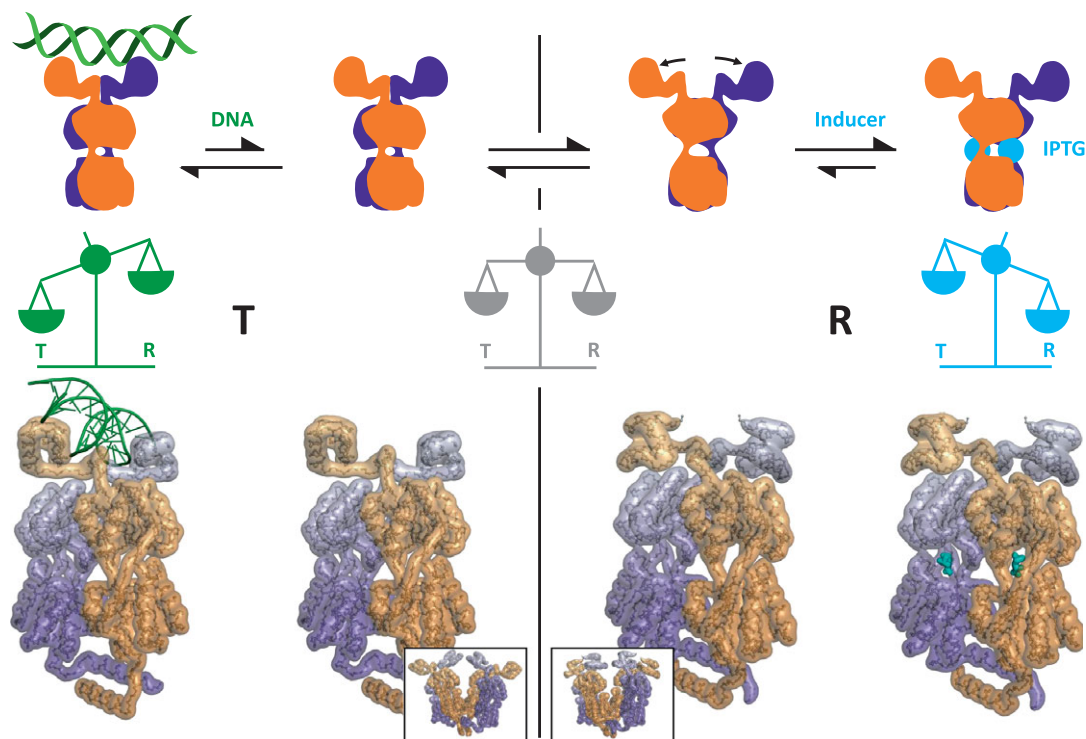


Figure 4. Allosteric transition of Lac repressor from *E. coli*. The scheme on top shows the equilibrium between tense (T, left) and relaxed states (R, right). The allosteric states are stabilized either by DNA binding to the promoter region of a gene (T), interfering with transcription, or by binding to an inducer (e.g., IPTG; R), permitting transcription, tipping the balance in the favor of one state or the other. The figure was adapted from Lewis (2013, Figure 3) and is based on experimental results by the Boelens lab, which showed strong evidence for the conformational selection model (Romanuka et al., 2023). The lower panel shows the structures of the *E. coli* Lac repressor in its tense (left; model based on PDB ID: 1EFA (Bell and Lewis, 2000) and PDB ID: 1LBG (Lewis et al., 1996) and relaxed states (right; PDB ID: 4RZS (Taylor et al., 2016)). The figure shows the homodimer, with the two protomers colored in orange and deep violet; in the biological unit, two homodimers associate with a homotetramer, shown in the insets at the bottom-center.

Kinases

Kinases are enzymes that catalyze the phosphorylation of molecules, either small or large. They serve important functions in metabolism and cell signaling. Their structure consists of two lobes or domains, separated by a catalytic cleft (Figure 5a). This makes them prime examples of the induced fit mechanism. While one domain serves to bind the substrate(s), the other acts like a lid, which clamps down on the substrate when it binds. Kinases can be subject to both covalent modification (predominantly by being phosphorylated themselves) and to non-covalent allosteric regulation, at their activation loop, which is positioned in the substrate-binding domain and serves as centerpiece of kinase allosteric regulation. Since allostery in protein kinases has been shown to involve dynamic interactions of clusters of amino acid (Masterson et al., 2010; Leroux and Biondi, 2020) rather than simple residue-to-residue information transfer between substrate recognition and catalysis, the allosteric mechanism of kinases has recently been likened to harmonics in a violin (Kornev and Taylor, 2015) (Figure 5a).

One of the best-known examples of regulated kinases is phosphofructokinase 1 (PFK-1), a tetrameric enzyme that catalyzes the third step of glycolysis, from fructose-6-phosphate to fructose-1,6-bisphosphate (Schirmer and Evans, 1990). This is one of the three irreversible steps of glycolysis ($\Delta G \ll 0$), which needs to be “bypassed” by another enzyme (a phosphatase) in the reverse pathway, gluconeogenesis. At the interface of several pathways, it is of critical importance that the activity of PFK-1 is tightly controlled. PFK-1 consumes ATP and is therefore dependent on the energy state of the cell. Metabolites that signal the energy charge

(ATP, ADP, AMP, as well as citrate and fructose-2,6-bisphosphate (F-2,6-BP), through hormone signaling) are therefore ideal allosteric regulators. As can be read up in every biochemistry textbook, metabolites signaling high energy charge (ATP and citrate) allosterically downregulate PFK-1 activity, whereas those associated with low energy (AMP, ADP, and F-2,6-BP) activate the enzyme’s activity by affecting the equilibrium between T and R states. The inactive T state binds ATP at the allosteric effector site, and the active R conformation has AMP or ADP bound to the same site. This conformation readily binds the enzyme substrate, fructose-6-phosphate. The structural basis for allosteric regulation of PFK-1 in bacteria is well described and serves as a textbook example of allostery (Schirmer and Evans, 1990). In contrast, no structural information existed for the eukaryotic enzyme until very recently. PFK-1 from human liver has now been shown to exhibit a slightly different allosteric mechanism than bacteria, with allosteric inhibition by ATP at multiple sites in the T state, and the C-terminus contributing through autoinhibition (Lynch et al., 2024). In addition to tetrameric PFK-1, higher-order PFK-1 filaments have been characterized by cryo-electron microscopy (cryo-EM) in the same study.

Another metabolically important enzyme is glucokinase, which phosphorylates glucose in the liver and pancreas. Also, this enzyme displays sigmoidal kinetics – despite exclusively functioning as a monomer (Cornish-Bowden and Cárdenas, 2004). NMR data suggest that the cooperative kinetic response at low glucose concentrations is caused by millisecond disorder–order transitions of the lid domain, which is bypassed at high glucose concentrations

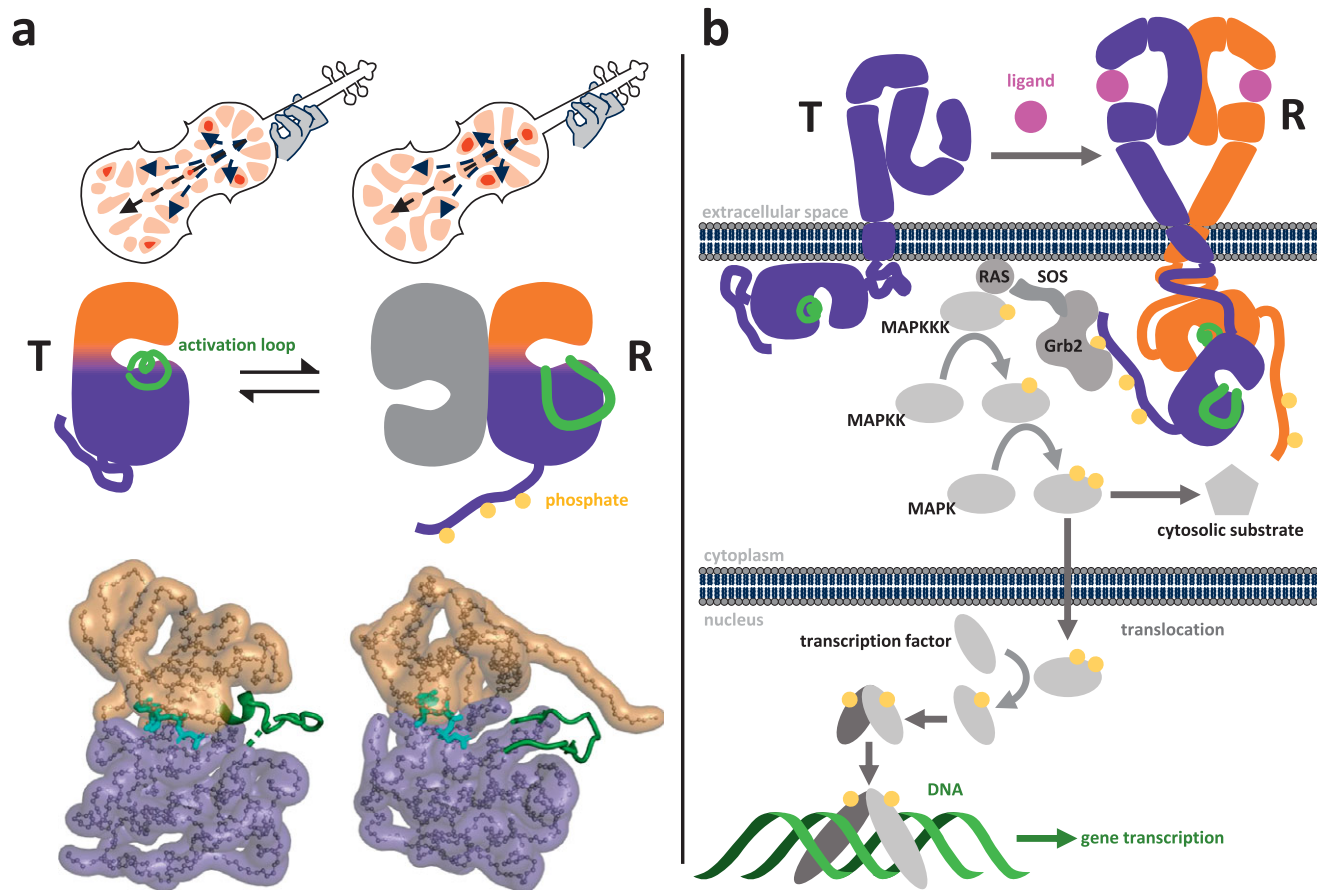


Figure 5. Allosteric transitions in protein kinases. (a) Transition between T and R states in protein kinases. The N-lobe is colored orange and the C-lobe deep violet, highlighting the stereotypical bilobal fold of protein kinases (here: EGFR kinase domain, with activation-induced dimerization indicated by second kinase, in gray). The activation loop, undergoing a conformational change during the transition, is highlighted in green. Allosteric transitions in protein kinases have been likened to the change in harmonics when playing a violin, now often referred to as ‘violin model’ (picture adapted from Kornev and Taylor, 2015, Figure 1). The bottom panel shows the structure of the EGFR kinase domain in tense/inactive (PDB ID: 2GS7) and relaxed/active state (PDB ID: 2GS6) (Zhang et al., 2006). (b) Ligand-induced EGFR dimerization leads to activation of one of its C-terminal tyrosine kinase domains, linked to the mitogen-activated protein kinase (MAPK) cascade. Ultimately, EGFR dimerization results in the activation of transcription factors.

(Larion et al., 2012), but this is not the only allosteric activation mechanism of this unique enzyme. Hyperinsulinemia-associated disease variants display a second activation mechanism, in which the ensemble structure is not perturbed (Whittington et al., 2015).

In addition to their function in metabolism, kinases are highly important transmitters of biological signals. Protein kinases are commonly organized in cascades, where one kinase phosphorylates the next (Figure 5b). This allows messages to be relayed extremely rapidly, due to signal amplification. Allosteric regulation of kinase cascades requires tight spatio-temporal control. This may be achieved and sped up in an interactive system of co-evolution based on co-localization, as suggested by Kuriyan and Eisenberg (2007). This hypothesis was bolstered by a study of serine kinase paralogs (and evolutionary related orthologs), one of which belongs to the MAP kinase cascade and is scaffold-dependent (i.e., co-localized with other kinases on the same scaffolding protein, which also contributes to allosteric regulation (Zalatan et al., 2012), whereas the other is scaffold-independent (Coyle et al., 2013)). Intriguingly, even the evolutionary precursors showed weak allostery, suggesting that latent allostery is intrinsic to the kinases and can be exploited by exposure to a novel regulator to evolve new modes of allosteric regulation (Coyle et al., 2013). A similar conclusion was reached by the Kern lab in a study involving ancestor sequence reconstruction (Pauling and Zuckerkandl, 1963)

and experimental resurrection of a Ser/Thr kinase (Aurora A kinase) (Hadzipasic et al., 2020). Also, this study strongly suggests that evolution of allosteric regulation is already encoded in the kinase, and did not *per se* require co-localization.

Initiation of protein kinase cascades is usually triggered by a Tyr kinase, as in the case of the epidermal growth factor receptor (EGFR) (Figure 5b). After ligand binding, the receptor dimerizes and the signal is transmitted through the membrane, modulated by lipids (Coskun et al., 2011; Maeda et al., 2022). This leads to auto-phosphorylation of tyrosine residues at the C-terminal tail on the other side of the membrane (Downward et al., 1984; Arkhipov et al., 2013; Kovacs et al., 2015). Autophosphorylation was long thought to be a symmetric process, like dimerization itself, but has more recently been shown to be asymmetric, with one tail domain acting as an activator and the other as a receiver (Zhang et al., 2006).

GPCRs, G-proteins and biased signaling

G-protein-coupled receptors (GPCRs) are the largest family of eukaryotic membrane receptors and the target of approximately one third of all small molecule drugs (Thal et al., 2018). GPCRs respond to a wealth of external signals, from hormones and other chemicals to light. Like tyrosine kinase receptors, GPCRs

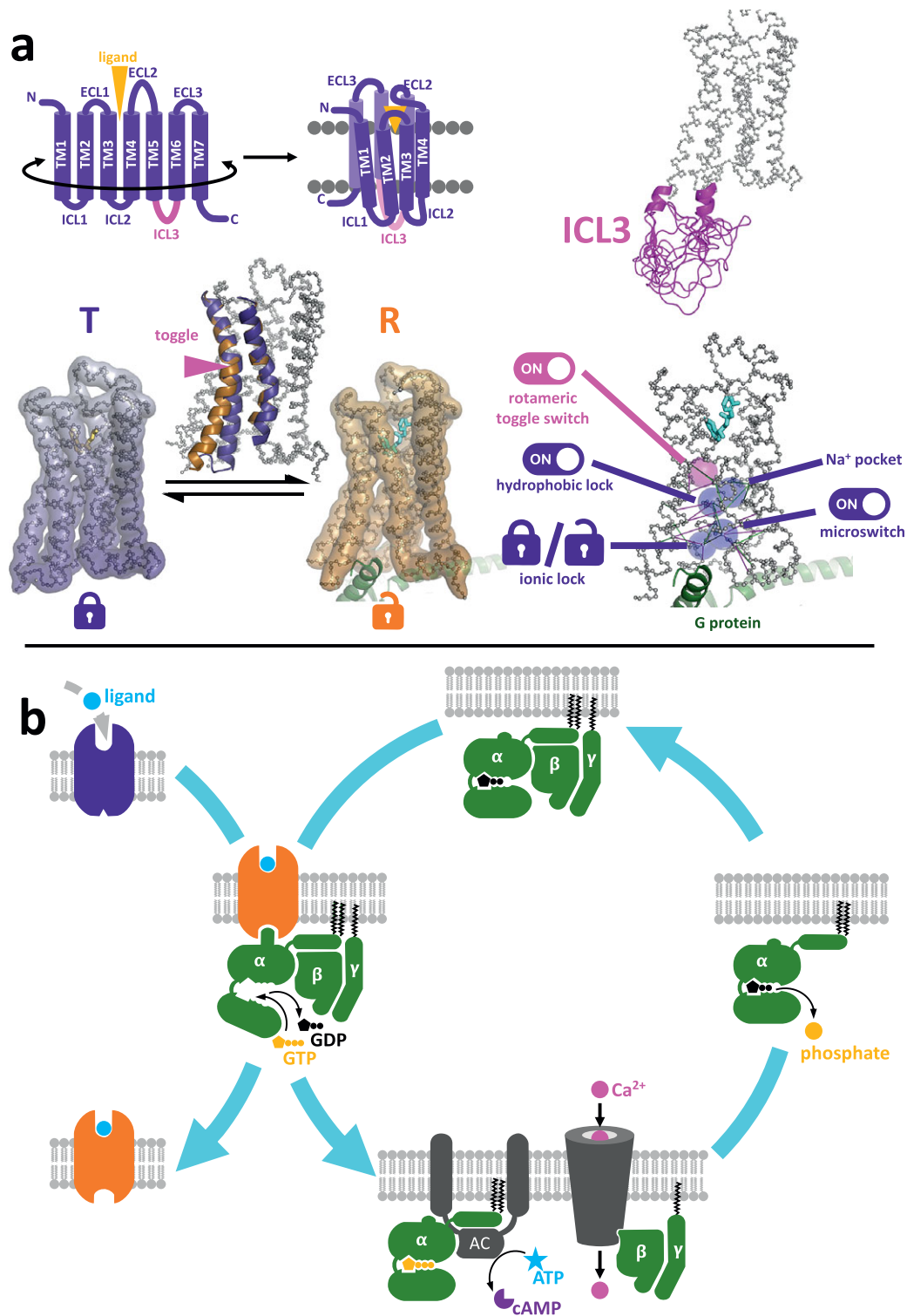


Figure 6. Allosteric transitions in G-protein-coupled receptors (GPCRs). (a) Top left: general topology of a GPCR, with transmembrane helices, extracellular (ECL) and intracellular (ICL) loops labeled. The position of the ligand-binding pocket is highlighted in yellow (illustration adapted from Schneider et al., 2018, Figure 2). Top right: NMR structure of β -2 adrenergic receptor (ADR2; PDB ID: 6KR8 (Imai et al., 2020)), with ICL3 loop sampling multiple conformations (magenta). Bottom left: ADR2 structure in its tense/inactive (PDB ID: 2RH1 (Cherezov et al., 2007)) and relaxed/active states (PDB ID: 3P0G (Rasmussen et al., 2011b)), colored in deep violet and orange, respectively. An inverse agonist (carazolol, yellow) is bound to the ligand pocket of the T state, whereas the agonist BI-167107 (cyan) is bound to the same pocket in the R state. The C-terminus of bovine $G_{\alpha s}$ is shown in green (structural superposition using the G_{α} coordinates from PDB ID: 3SN6 (Rasmussen et al., 2011a)). Bottom right: allosteric communication network underlying activation, as reconstructed by Zhou et al. (2019) and mapped on the backbone of human ADR2 in its active conformation (PDB ID: 3P0G (Rasmussen et al., 2011b)). Key functional signal relay hubs – including ‘toggle switch’ and ‘ionic lock’ – are labeled, a bound agonist molecule (BI-167107) is shown in cyan, and G_{α} in green (PDB ID: 3SN6 (Rasmussen et al., 2011a)). (b) G protein activation cycle. In their inactive GDP-bound form (top), G proteins exist as a trimeric complex attached to the cytoplasmic side of the cell membrane. Ligand-bound GPCRs, transitioning from T to R state, promote G protein activation by GDP–GTP nucleotide exchange in the G_{α} subunit (left). The activated trimeric complex dissociates, allowing its components to interact with cellular effectors (here: G_{α} with adenylate cyclase, AC, generating cAMP, and $G_{\beta\gamma}$ with an ion channel). Return to the inactive state occurs by GTP hydrolysis to GDP, promoted by RGS (Regulator of G-protein-signaling; not shown). Figure adapted from Rasmussen et al. (2011a, Figure 1) and Petsko and Ringe (2004, Figures 3–14).

allosterically relay signals through cell membranes (Figure 6a), and their activity is modulated by lipids (Oates and Watts, 2011; Bac-couch et al., 2022). The first GPCR-like structure was of bacteriorhodopsin (Henderson and Unwin, 1975), a protein responding to light (the first high-resolution structure of a GPCR was published by Palczewski et al. in 2000). This was also the very first membrane protein structure elucidated (by electron microscopy), and many years later led to the award of the Nobel prize in Chemistry to Richard Henderson (together with Dubochet and Frank) “for developing cryo–electron microscopy for the high-resolution structure determination of biomolecules in solution” (2017). As Editor in Chief of *Quarterly Reviews of Biophysics* (in which this review is published), Richard Henderson brought the journal to the respected status that it enjoys today.

The basic structural unit of GPCRs is a monomeric 7-transmembrane helix fold (Dixon et al., 1986) (Figure 6a). Upon agonist binding (or light capturing) on the extracellular side, a so-called ‘toggle’ helix kinks and conveys the signal through an allosteric network to the cytosolic side of the membrane, where an ‘ionic lock’ opens (Sprang, 2007). In addition, oligomerization has been shown to be important for the function of some GPCRs (Audet and Bouvier, 2012), giving rise to both positive and negative allosteric modulation (referred to as PAM and NAM, respectively). Importantly, GPCRs are often involved in more than one pathway, and signaling can be biased by different ligands, triggering either one signaling pathway or another (Liu et al., 2012; Hua et al., 2020; Suomivuori et al., 2020; Wingler et al., 2020).

As their name suggests, GPCRs are well-known to activate heterotrimeric G-proteins on the cytosolic side of the membrane. Like their smaller cousins (small G-proteins, with ras-p21 as their prototype) (Pai et al., 1989; Schlichting et al., 1990; Lu et al., 2016), heterotrimeric G-proteins also cycle between an active GTP-bound conformation and an inactive GDP form, subject to allosteric regulation (Audet and Bouvier, 2012; Zhou et al., 2019) (Figure 6b). When the signal arrives, the G-protein dissociates from the GPCR and prompts a response (actually, there are two responses: one conveyed through G_{α} and one through $G_{\beta\gamma}$). The signal is switched off when G_{α} gets deactivated by GTP hydrolysis, either through its intrinsic GTPase activity or by effector regulation, and the G-protein reassembles, ready to enter the next cycle. Instrumental for elucidating this mechanism were in particular two scientists, Lefkowitz and Kobilka (Dixon et al., 1986; Rasmussen et al., 2007, 2011a), who shared the Nobel prize in 2012.

In recent years, the molecular mechanism of GPCRs has been elaborated in more detail. Not only can GPCRs signal through different G-proteins; intriguingly, GPCRs can also use alternative signaling partners, like β -arrestins and others (Audet and Bouvier, 2012; Zhou et al., 2019). In a recent NMR analysis, by Kleist et al., the recruitment of either G-proteins or β -arrestins was shown to be guided by conformational selection at an allosteric hub (Kleist et al., 2022), and Förster resonance energy transfer (FRET) analysis revealed the importance of the third intracellular loop in biased GPCR signaling (Sadler et al., 2023) (Figure 6a). Moreover, different pockets in GPCRs were shown to be tuned by receptor activation and ligand recruitment (Kleist et al., 2022) (Figure 6a), suggesting that signaling through GPCRs is less straightforward than earlier anticipated, and may resemble the ‘violin model’ more than the traditional ‘domino model’.

ATP synthase

As its name suggests, ATP synthase is the molecular machine responsible for synthesizing the main cellular energy currency

ATP. In order to achieve this, it requires an energy supply itself (otherwise it functions in reverse, consuming ATP). Energy is provided in the form of protons that flow from one side of the membrane to the other, *with* the proton gradient, maintained by the electron-transport chain. In mitochondria, ATP synthase forms dimer ribbons, which likely act as proton traps (Strauss et al., 2008), enhancing the proton-motive force. The flow of protons through the membrane protein part of ATP synthase is directed through two half channels, driving the γ -subunit (Lau and Rubinstein, 2012), which rotates asymmetrically like a child playing hula hoop (Abrahams et al., 1994; Kato-Yamada et al., 1998; Rastogi and Girvin, 1999; Stock et al., 2000; Stewart et al., 2014) (Figure 7a). The ATP synthase heterohexameric $\alpha_3\beta_3$ ring represents the hoop (itself associated with one or several stalks) and cycles through alternating conformations during this process (O = open, L = loose, and T = tight), forcing ADP and phosphate to react and generate ATP (Figure 7b). This allosteric mechanism is referred to as ‘binding-change mechanism’ and functions through alternating sites cooperativity (Hackney et al., 1979; Boyer, 1993). The main credit for elucidating this intricate molecular mechanism goes to Boyer and Walker, who were awarded the Nobel prize in 1997 (Boyer, 1997; Walker, 1998). More recently, this asymmetric mechanism has been beautifully visualized by single-molecule FRET over the millisecond timescale, both for ATP synthesis and hydrolysis (Diez et al., 2004) (Figure 7c; for a description of the method, see section ‘FRET’). Nature itself has provided hundreds of exogenous allosteric regulators, which provide an exciting starting point for novel therapeutics (e.g., Cofas-Vargas et al., 2022).

Chaperonin

Cells are crowded with proteins and other molecules, making it challenging for proteins to fold. Chaperones and heat-shock proteins come to their rescue. They work like chambers or clamps and provide a suitable environment for protein folding. The best-characterized chaperone is the chaperonin GroEL/GroES from *E. coli*, which is an example of the chamber-like chaperones (Xu et al., 1997) present in all kingdoms (Figure 8). It is composed of two types of subunits, GroEL and GroES (where L stands for large and S for small), both of which form heptameric rings. The large subunits form two heptameric cages, which are arranged back to back and have an opening at either end. Most of the time, one of these openings is capped by a heptameric GroES lid. It is in this closed chamber (the so-called *cis* ring) that a polypeptide has a chance to fold; in fact, GroEL/GroES has been caught in the act of encapsulating a folding protein (Chen et al., 2008; Clare et al., 2009), providing a vivid image of ‘Anfinsen’s cage’ (Anfinsen, 1973; Ellis, 1994).

In this cage, the polypeptide enjoys a secluded environment and can fold undisturbed, without risking aggregation through exposed hydrophobic patches. The chaperonin also actively participates in protein folding (Lin and Rye, 2004; Horwich and Fenton, 2009; Hayer-Hartl et al., 2016): a large ATP-driven conformational change not only increases the size of the cavity, but additionally switches the properties of the cage walls from hydrophobic to hydrophilic. In this way, the polypeptide, which may get stuck to the cage wall, is pulled apart and given a new chance to fold. The folding trajectory itself is the same inside the chaperone as in solution (Horst et al., 2007).

The time in the cage is not random. It is timed by ATP hydrolysis, and coordinated between *cis* and *trans* rings in an interesting example of ‘nested’ allostery, with positive allostery (characterized by a sigmoid curve) within each ring, and negative allostery between

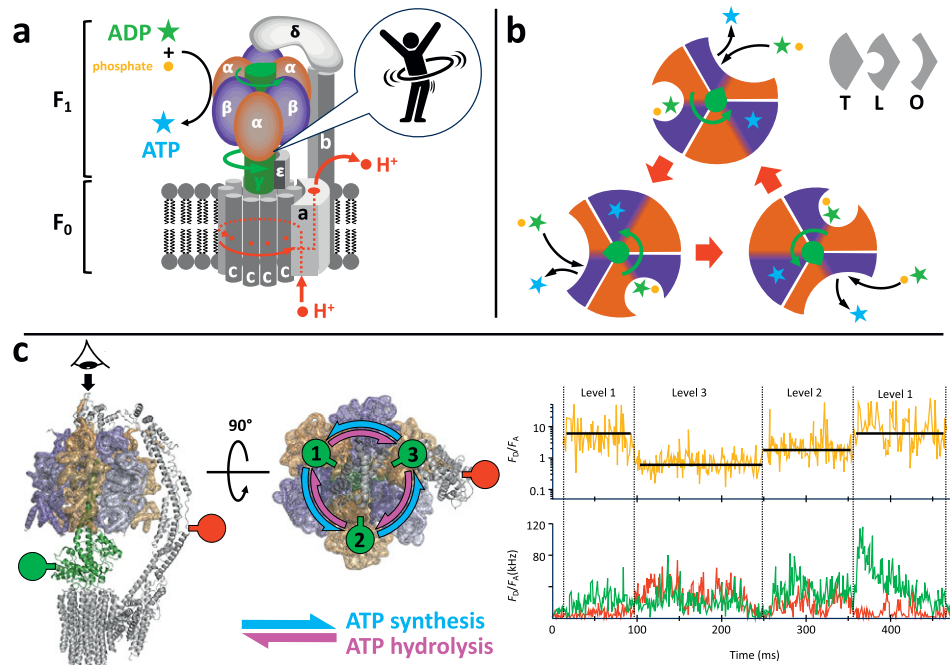


Figure 7. Allostery of ATP synthase. (a) Schematic representation of the mitochondrial ATP synthase, with subunits marked. The proton flow through the membrane subunits provides the mechanical force to rotate the γ subunit (green) in hula-hoop-like fashion, which promotes allosteric changes in the three $\alpha\beta$ units (colored orange, α , and deep violet, β), driving ATP synthesis. (b) For every rotation of γ , each $\alpha\beta$ unit cycles through open (O), loose (L), and tight (T) conformations, catalyzing the condensation of ADP and phosphate to ATP. This mechanism is referred to as ‘binding-change mechanism’ (Boyer, 1993). (c) Side and top views of ATP synthase structure (PDB ID: 8F39 (Sharma et al., 2024)). FRET fluorescent probes on the rotor (green) and stator (red) were used to visualize the rotary motion (Diez et al., 2004; right panel reproduced from Diez et al., 2004, Figure 2a with permission). As highlighted in the middle, the direction of rotation is opposite for ATP synthesis (counterclockwise, looking at ATP synthase from the top) and hydrolysis (clockwise).

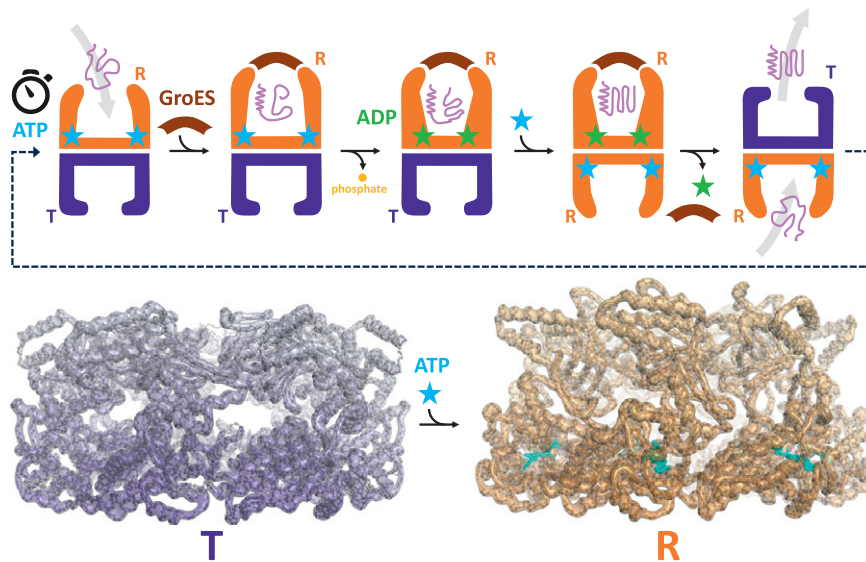


Figure 8. Allosteric transition in the GroEL–GroES chaperone. The scheme in the top panel shows the GroEL chaperonin double ring cycling between its tense (T, dark violet) and relaxed (R, orange) states, while folding a client protein (magenta). The process is driven by adenosine triphosphate (ATP) hydrolysis, serving as a timer for alternate cycling between *cis* ring (R state, where folding occurs) and *trans* ring (empty T state). The space enclosed by the GroEL ring in its R state and a GroES chaperonin cap (called ‘Anfinsen’s cage’ after Christian Anfinsen) serves as “safe space” for folding. The bottom panel shows the conformational transition of the GroEL ring from its tense (PDB ID: 4HEL (Meena and Saxena, 2016), deep violet) to its relaxed state (PDB ID: 8BL2 (Torino et al., 2023), orange) upon ATP binding (cyan).

cis and *trans* rings (Yifrach and Horowitz, 1995; Dyachenko et al., 2013; Saibil et al., 2013; Gruber and Horowitz, 2016). This antagonistic behavior between the two rings is driven by α -helix pistons at the inter-ring interface (Lorimer et al., 2018). Signaling networks and dynamic allosteric transitions in GroEL have been described in a review by Thirumalai and Hyeon (2018).

The overall molecular mechanism of the GroEL/GroES chaperonin was already reported in Horwich and Sigler’s insightful publication in 1997 (Xu et al., 1997). Another important structural paper was published in 2013, where the GroEL–ADP complex structure was resolved in the R state to 2.7 Å resolution (Fei et al., 2013). Unexpectedly, it revealed that the apical domains, which

bind the peptide substrate, do not obey the strict sevenfold symmetry; in fact, their arrangement is strikingly asymmetric. This flexibility may allow GroEL to bind to different substrates. Recently, it was found that the two rings transiently separate between cycles, and either reattach or assemble with a new partner (Yan et al., 2018). Ring separation was found to enable an efficient flux of folding polypeptides through the chaperonin system and convert the negative allosteric coupling of GroEL rings into a functionally efficient reaction cycle (Yan et al., 2018) – overcoming ‘Levinthal’s paradox’ (that most proteins fold quickly and reliably despite the astronomically high number of alternative conformations) (Levinthal, 1969).

Interesting cases at the “extremes”

Monomeric allostery

In their classic article introducing the MWC model of allostery, also known as symmetry model, Monod et al. 1965 stated as point 1 of the general properties of allosteric systems that “Most allosteric proteins are polymers, or rather oligomers, involving several identical units”. This opened for monomeric allostery from the very beginning. In this review, two cases have already been mentioned, regarding myoglobin (Frauenfelder et al., 2001) and glucokinase (Larion et al., 2012). Glucokinase is special among kinases, since its kinetics shows cooperative behavior for *homotropic* effects. However, many other kinases also function as monomers and are subject to *heterotropic* allosteric regulation. Myoglobin is generally known as a non-allosteric protein, functioning as an oxygen storage protein; however, it also interacts with a variety of small molecules, for example, NO and CO (Antonini and Brunori, 1971). There is evidence that myoglobin functions as a miniature chemical reactor, subject to allosteric regulation, and the protein is likely to have various other physiological functions than oxygen storage, including protection against oxidative damage (Galaris et al., 1989; Frauenfelder et al., 2001).

Another intriguing example of monomeric allostery is serum albumin (Ascenzi and Fasano, 2010), which is the most abundant protein in mammalian plasma and serves as a carrier and depot for steroids, fatty acids, and thyroid hormones. Serum albumin also binds heme and many other molecules, including drugs, which can bind to many different places in its structure (affecting pharmacokinetics). In fact, serum albumin has an extraordinary ligand binding capacity (Fasano et al., 2005). It also exhibits (pseudo-) enzymatic activity (Yang et al., 2007; Ascenzi and Gianni, 2013) and plays a key role in NO homeostasis and detoxification of reactive oxygen species (Fasano et al., 2008; Fanali et al., 2012), similar to myoglobin (Frauenfelder et al., 2001). Serum albumin is a monomeric protein with three domains that interact with each other, forming a heart-shaped structure (He and Carter, 1992). Heme binding and reactivity have been shown to be allosterically modulated by different serine albumin ligands, and *vice versa* (reviewed by Fasano et al., 2008; Ascenzi and Fasano, 2010). Transient heme binding to this non-classical heme protein can thus modulate the pharmacokinetics of administered drugs, for example, the blood-thinner warfarin, which can lead to severe or even fatal outcomes.

Given that latent allostery and allosteric potential has been observed in proteins without a strong allosteric profile (Coyle et al., 2013; Hadzipasic et al., 2020; Pillai et al., 2020), as evident from resurrection studies discussed in the sections ‘Classical example: hemoglobin’ and ‘Other well-known examples’, the

possibility has to be considered that also many other monomeric proteins are subject to allosteric regulation, if only weakly.

Inter-enzyme allostery

Allostery is common to many multimeric proteins and enzymes, and very important for the regulation of metabolic pathways, for example, in the form of feedback inhibition or feedforward activation, as originally proposed by Novick and Szilard (1954) and first observed by Umbarger (1956). Less well known is that allosteric regulation can even involve independent enzymes. Such a system has recently been discovered in the shikimate pathway (Blackmore et al., 2015; Munack et al., 2016), which links carbohydrate metabolism to the synthesis of important aromatic compounds in bacteria, fungi, and plants. The first enzyme of this pathway is 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase. This enzyme forms a complex with chorismate mutase (CM), a key branch point enzyme toward the synthesis of phenylalanine and tyrosine, and away from tryptophan (Sasso et al., 2009). CMs of some bacteria, for example, in *Mycobacterium tuberculosis*, have sub-optimal catalytic activity, but can be boosted to normal levels by their DAHP synthase partners (Sasso et al., 2009; Burschowsky et al., 2018). This involves the formation of a heterooctameric enzyme complex, where the DAHP synthase homotetramer is decorated by two CM dimers (Figure 9a). CM activity is subsequently switched off by enzyme release from the complex, preventing a waste of energy. Regulation of CM activity is even more intricate: Intriguingly, DAHP synthase was found to serve as a platform for feedback regulation of both enzymes (Blackmore et al., 2015; Munack et al., 2016; Burschowsky et al., 2018) – despite the fact that the allosteric regulators bind approximately 30 Å from the enzyme interface. Apart from very small realignments of subunits (<5°), no major structural changes were found, suggesting that this regulatory principle may be driven by dynamics (Munack et al., 2016). This is not the only time that this classic allosteric protein has surprised us (Sapienza et al., 2023).

Low-activity CMs also provided a golden opportunity to probe if their activity could be increased through directed evolution. Indeed, this was possible and the achieved k_{cat}/K_m even twice exceeded those of high-activity CMs (Fahrig-Kamaraukaitė et al., 2020). The fact that the evolutionary trajectories reached a plateau at the level of the best natural CMs furthermore exposed the natural limits of CM catalysis.

Allostery of intrinsically disordered proteins

It is estimated that more than 30% of all eukaryotic proteins are fully or to a large extent disordered (Ward et al., 2004; Uversky and Dunker, 2010). It may therefore seem surprising that such proteins can be functional and fold up upon ligand binding, but this is exactly what many IDPs do (Wright and Dyson, 1999; Chouard, 2011). None of the classical models of allostery, neither the symmetry model nor sequential adaptation, could explain such an effect, suggesting that other mechanisms must be at play. One extreme example of an IDP is the tumor suppressor p53, which is frequently implicated in human cancer and serves as an extraordinarily well-connected hub in multiple signaling networks (Oldfield et al., 2008). Another hub protein, adenovirus early region 1A (E1A) oncoprotein, was the target of the compelling allostery investigation by the Wright and Deniz labs (Ferreon et al., 2013; Hilser, 2013) (Figure 9b). Intriguingly, complexes with two different interaction partners, a transcriptional co-activator (CBP)

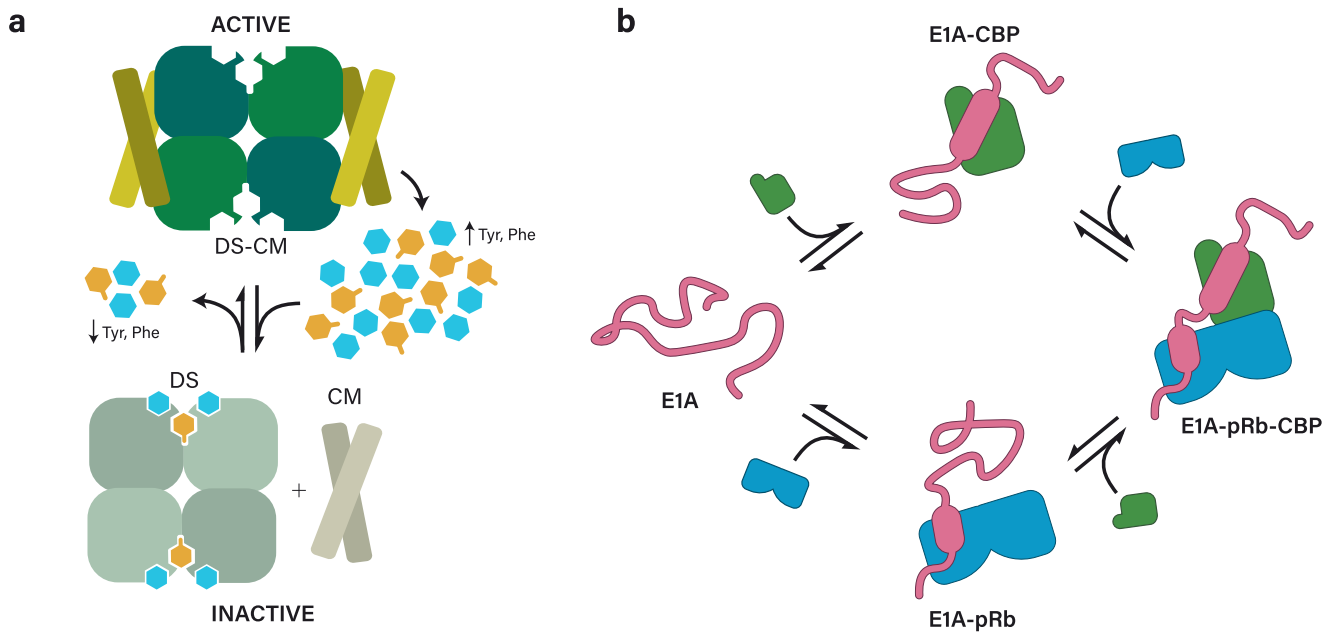


Figure 9. Fringe examples of allosteric regulation. (a) 'Inter-enzyme allosteric' regulation of chorismate mutase (CM) from *M. tuberculosis* (Munack et al., 2016). CM is only active when in complex with DAHP synthase. Upon binding of the allosteric feedback inhibitors Tyr and Phe to the partner enzyme, CM is released from the complex and rendered inactive. Intriguingly, the feedback inhibitors bind far from the CM-DAHP synthase interface, and induce hardly any conformational changes (Munack et al., 2016). (b) Adenovirus early region 1A (E1A) is an intrinsically disordered oncoprotein. Upon binding to the host regulators CREB binding protein (CBP) and retinoblastoma protein (pRb), E1A becomes structured and elicits responses through the host proteins (Ferreon et al., 2013).

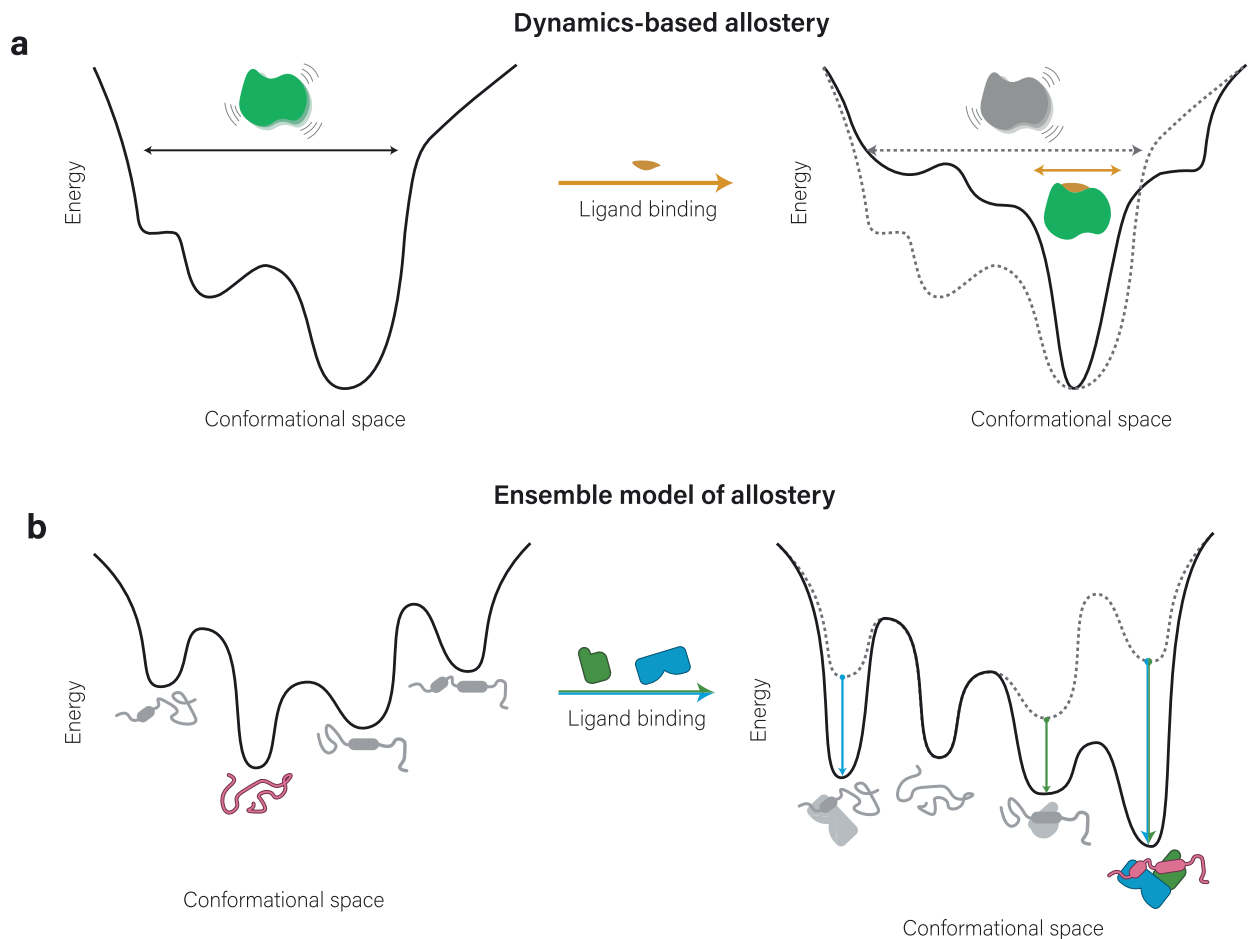


Figure 10. Modern models of allostery. (a) Allostery without conformational change can occur due to a difference in dynamics between apo and holoproteins. The most stable conformation does not change upon ligand binding, but the energetic landscape around it does. (b) The ensemble model of allostery understands protein conformations as a complex thermodynamic landscape that is altered upon ligand binding, illustrating the process in Figure 9b. Figure adapted from Hilser (2013).

and the retinoblastoma protein (pRb), led to the structuring of different segments of E1A, as determined by ensemble fluorescence spectroscopy and single-molecule FRET. Moreover, the binding of both proteins to E1A was positively cooperative, each increasing the probability that the other component binds. Remarkably, a truncated version of the E1A hub protein was found to switch cooperativity from positive to negative coupling (Ferreon et al., 2013).

The same transcriptional co-activator, CREB binding protein (CBP), was the target of another interesting investigation of IDP allosteric regulation, also by the Wright lab (Berlow et al., 2017; Chong and Forman-Kay, 2017): CBP is known to bind the α -subunit of hypoxia-inducible factor 1 (HIF-1), the transcriptional master regulator of cellular response to hypoxia (Dames et al., 2002; Freedman et al., 2002). Another effector of CBP is the protein CITED2, a negative feedback regulator of HIF-1, which binds to the same region on CBP as HIF-1 α , as revealed by NMR spectroscopy (Berlow et al., 2017). Both binding partners of CBP are intrinsically disordered when unbound. Counterintuitively, binding of CITED2 increases binding affinity for HIF-1 α in a concentration-dependent manner, despite binding to the same site. The authors could show that positive cooperativity involves a transient ternary complex of all three proteins. Apparently, CITED2 gets a foothold on CBP, on a partial overlapping sequence, and then squeezes HIF-1 α out of the binding site, thereby rapidly and efficiently attenuating the hypoxic response.

Dynamics, entropy, and ensemble nature of allostery

The fact that not all allosteric proteins exhibit changes of conformation, and that IDPs can also be subject to allosteric regulation, as described in several examples in the sections ‘Other well-known examples’ and ‘Interesting cases at the “extremes”’, challenges the classical models of allostery.

It has long been suspected that allostery can occur without conformational change (Cooper and Dryden, 1984) (Figure 10a). The first experimental evidence of such a system was provided by the Kalodimos lab in 2006, for the transcription factor CAP (catabolite activator protein) (Popovych et al., 2006). CAP is a homodimeric DNA-binding protein, which binds cAMP with negative cooperativity: binding of cAMP to one subunit decreases the probability of cAMP binding to the second subunit of CAP. This occurs without conformational change, as verified by NMR spectroscopy (Popovych et al., 2006). Instead, the dynamics of the system was modulated, with the first molecule of cAMP partially enhancing protein motions and the second molecule completely quenching the dynamics. By isothermal titration calorimetry (ITC), it was shown that the observed negative cooperativity was entirely driven by an unfavorable change in entropy, despite the enthalpic contribution favoring positive cooperativity (Popovych et al., 2006; Tzeng and Kalodimos, 2009, 2012).

A more recent example, from the Pai lab, on fluoroacetate dehydrogenase (FAD), shows how such allosteric communication may be achieved (Mehrabi et al., 2019). FAD is a homodimeric enzyme and exhibits half-of-the sites reactivity. Time-resolved serial crystallography revealed breathing motions in the enzyme, which correlated with catalytic snapshots of structural states, from substrate binding and the formation of a covalent intermediate, to placement of a hydrolytic water molecule and product release (Mehrabi et al., 2019). These conformational changes of the protein framework were correlated with the binding and release of surplus water molecules (Kim et al., 2017; Mehrabi et al., 2019), reminiscent of the case of hemoglobin (Colombo et al., 1992).

The allosteric regulation of IDPs is at the opposite end of the spectrum compared to allosteric systems not displaying a conformational change. However, also here, the conceptual challenge was to leave the traditional structure-centered view of allostery. The first discussion of allostery in terms of conformational transitions and ‘energy landscapes’ was in 1999 by the Nussinov lab (Tsai et al., 1999). An interesting early example was myoglobin (Frauenfelder et al., 2001). This paved the way to the ensemble model of allostery (Boehr et al., 2009; Hilser, 2013; Motlagh et al., 2014; Wei et al., 2016), which is now broadly accepted (additionally to structure-based factors, dynamics and kinetics) (Henzler-Wildman and Kern, 2007). The ensemble model describes allostery in terms of conformational selection (Ma et al., 1999; Boehr et al., 2009), that is, that ligand binding changes the thermodynamics of the system and with it the equilibrium (Figure 10b). This makes the requirement for conformational change, induced or not, obsolete. Thus, the classical allostery models can be understood as special cases of ensemble-based allostery.

It can be argued that the ensemble-based allostery model has an emphasis on thermodynamics and overlooks the role of kinetics. In contrast to thermodynamics, kinetics relates to the energy barriers between the states and to the energies and timescales required to overcome them. Not much is known about the kinetics in allosteric systems and it is possible that for some (if not many) processes, the rate-limiting steps are not governed by the absolute energies of special states, but by the ability of allosteric systems to reach them. This will be an interesting line of investigation for the future.

Methods to probe allostery

A plethora of methods are used to probe allostery. Many of these have been mentioned along with examples of allosteric regulation above. Below, we describe several of these methods, and their strengths and weaknesses, in somewhat more detail.

X-ray crystallography

The first protein structures were solved by X-ray crystallography (Kendrew, 1959): for the first time, protein structures could be directly visualized, including important cofactors and ligands. To date, 85% of all macromolecular structures have been solved by this method, which has the highest precision of all structural biology methods. As the name suggests, X-ray crystallography relies on the formation of protein crystals and their ability to diffract X-rays (Figure 11a), as a basis to generate electron density maps (good textbooks are, e.g., Blow, 2002; Rupp, 2009). This method yields the average structures of molecules constrained in a crystal lattice and is useful to the study of allostery when large conformational changes can be stabilized, for example, by the presence of an allosteric effector. Important examples are the initial allostery studies of hemoglobin (Baldwin and Chothia, 1979), described in the section ‘Classical example: hemoglobin’ of this review.

In most cases, X-ray crystallography provides only static snapshots of the structures in action. Recently, however, the traditional view of X-ray crystallography as a static enterprise has been shifting. Already in the 1980s and 1990s, Laue diffraction from protein crystals (using polychromatic X-rays) enabled time-resolved studies after triggering of conformational changes by a jump in pH or by flashing ‘caged’ compounds with laser light (Moffat et al., 1984). Higher-quality X-ray data sets were obtained by trapping reaction intermediates and collecting data with monochromatic techniques

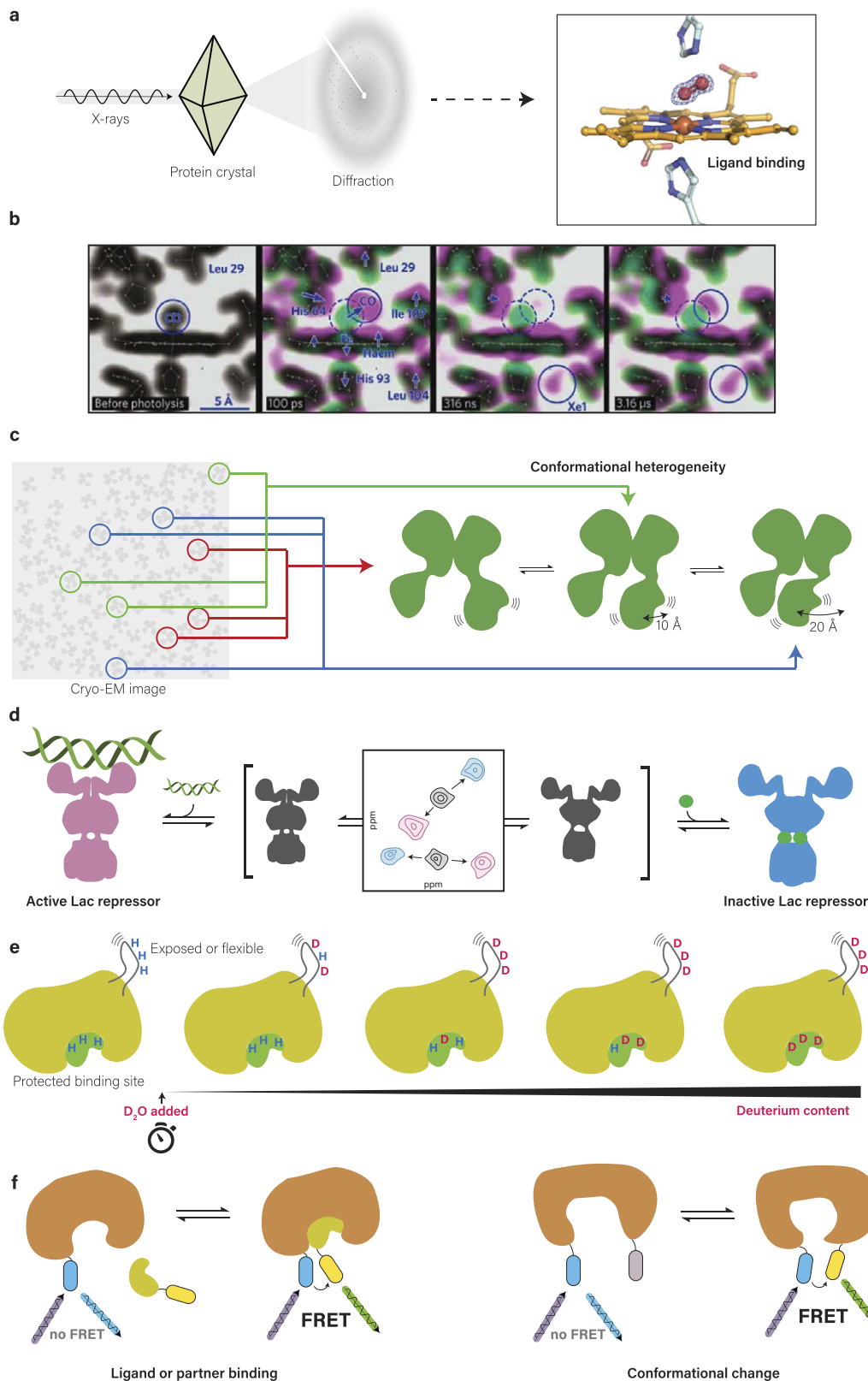


Figure 11. Experimental methods to study allostery. (a) X-ray diffraction relies on the ability of macromolecular crystals to diffract X-rays. After ‘phasing’ of the X-ray data (indicated by dashed arrow), the electron density is calculated, into which the model is built. Shown here: oxygen binding site of myoglobin (1 Å resolution; PDB ID: 1A6M (Vojtěchovský et al., 1999)). One of the main strengths of this method is its high precision. For allostery studies, crystallography is invaluable for the detailed study of ligands in protein pockets. (b) Time-resolved crystallography is a powerful tool to study dynamic processes occurring at timescales up to femtoseconds. Here: migration of carbon monoxide (CO) and subsequent structural relaxation of myoglobin (Figure adapted from Schotte et al., 2004). Recently, serial crystallography (using a series of micro- or nanocrystals) has evolved as powerful alternative for time-resolved studies. (c) Cryo-EM data processing and algorithms are increasingly efficient at separating conformational subpopulations in a sample and characterizing dynamic processes. A simplified example for the intrinsic motions described for the soluble angiotensin I-converting enzyme is shown (Lubbe et al., 2022). (d) NMR spectroscopy is particularly useful to study conformational selection mechanisms underlying allostery. Here: example of Lac repressor (see section ‘Lac repressor’), where the individual spectral peaks for the ligand-bound conformations can be observed and compared to the peaks in the absence of ligands (Romanuka et al., 2023). (e) Hydrogen-deuterium exchange mass spectrometry (HDX-MS) examines the exchange of labile hydrogens for deuterium over time and can thus reveal where in a protein a ligand binds. The exchange rate can be used as a proxy for dynamics since it is correlated with solvent exposure. (f) Förster resonance energy transfer (FRET) allows the study of binding events or conformational changes using two fluorescent probes: if the two sites are sufficiently close (but only then), they can transfer energy between them and transmit a read-out signal.

(Johnson, 1992; Hajdu and Andersson, 1993; Hajdu et al., 2000). In addition, information on dynamic processes and non-equilibrium states in proteins can be obtained from the characterization of alternative and low occupancy conformational states (Keedy, 2019) and from crystallographic *B*-factors (also referred to ‘temperature factor’ or ‘Debye–Waller factor’); however, characterizing dynamics and relating this information to specific functional outcomes is not trivial.

Since 2009, with the availability of the extremely intense beams produced by X-ray free-electron lasers (XFELs) and their femto-second pulses, the era of serial femtosecond crystallography (SFX) has begun (Neutze et al., 2000; Johansson et al., 2012; Schlichting and Miao, 2012), enabling molecular movies. In serial crystallography, a segmented flow of microdroplets containing microcrystals is exposed to the X-ray pulses. Currently, access to XFEL technology is still limited by the number of available X-ray sources. Promisingly, however, the developments and insights obtained from XFELs and serial crystallography have supported the parallel development of time-resolved X-ray diffraction studies in the much more available synchrotron X-ray sources. Although these limit the timescales that can be studied to nano- and microseconds, this is not a critical limit to most allostery studies, since most changes relevant for allostery such as binding and catalysis occur at such timescales (Henzler-Wildman and Kern, 2007).

One example of time-resolved serial crystallography has been discussed in the section ‘Dynamics, entropy, and ensemble nature of allostery’ (Mehrabi et al., 2019). Two other very exciting studies concern the pico-second time-resolved CO-migration analysis in myoglobin (Schotte et al., 2004) (Figure 11*b*) and time-resolved studies of a cooperative dimeric hemoglobin (Knapp et al., 2006). Another alternative to study allosteric mechanisms crystallographically is through multi-temperature crystallography (Keedy et al., 2018; Keedy, 2019). Most importantly, however, due to the high precision of X-ray crystal structures, this technique excels at identifying and characterizing the binding of allosteric ligands and fragments (reviewed in the section ‘Allostery and drug discovery’).

Cryo-EM

In many aspects, cryo-EM provides very similar information to X-ray crystallography, except that it additionally reveals information about charges. Traditionally, EM provided only low-resolution information; however, with the advent of more sensitive detectors (and other technological developments), this has changed (Kühlbrandt, 2014; Tan and Carragher, 2020). Today, the resolution of cryo-EM structures is often on par with X-ray crystallography, especially for large complexes. For this reason, and because this technique does not depend on crystals and requires only small amounts of sample, cryo-EM has quickly become one of the most important techniques for structural biology.

Cryo-EM relies on the freezing of macromolecules in vitreous ice, ideally imaging natively solvated molecules using a powerful electron microscope (reviewed by Nogales and Scheres, 2015; Nogales, 2018). Like X-ray crystallography, this technique visualizes averages of many individual, aligned particles; however, since cryo-EM is not limited by a crystal lattice, the whole conformational space is accessible. Information about dynamics can often be obtained directly, from the quality of the density (in EM based on Coulomb potentials) in different regions of a cryo-EM reconstruction. In addition, cryo-EM excels at separating conformational subpopulations of sufficient abundance in the sample (Figure 11*c*). New improvements in data processing algorithms, often involving

machine learning (ML) approaches (Punjani and Fleet, 2023; Jamali et al., 2024; Song et al., 2024), are pushing the limits on the intrinsic dynamics that can be observed in cryo-EM reconstructions. No two single protein particles have the exact same conformation, and the separation into smaller and smaller subpopulations yield cryo-EM models of increasing detail that reveal intrinsic, dynamic molecular processes. Since cryo-EM is a rapidly developing field, further advances are expected in the near future that will enable improved characterization of protein dynamics and the underlying biological processes, including allostery.

Time-resolved cryo-EM (Amann et al., 2023) provides another valuable tool to understand macromolecular dynamics for processes that can be triggered simultaneously for a whole population of molecules. Many different approaches have been tested for freezing cryo-EM samples at desired time points. These range from manual freezing, allowing the study of processes occurring within seconds or more, to automatically controlled thawing and refreezing within milliseconds. The field, however, still lacks standardized and commercially available solutions that make the different proof of concept methods available to the broader scientific community.

Systems benefiting most from cryo-EM methodology are large molecular complexes and membrane proteins, which are difficult to crystallize. It is therefore of no surprise that GPCRs (Zhang et al., 2023), but also ATCase, RTKs, ATP synthase, chaperonin, and many other proteins are highly amenable to this technique, as described in the section ‘Other well-known examples’. A beautiful example of time-resolved cryo-EM concerns the allosteric regulation of the human proteasome (Zhang et al., 2022).

NMR spectroscopy

NMR spectroscopy is a powerful tool to study biomolecular structures and dynamics, including transient conformations, and is thus a prime method to study allostery. A range of reviews have been dedicated to the topic (Tzeng and Kalodimos, 2011; Manley and Loria, 2012; Boulton and Melacini, 2016; Grutsch et al., 2016; Lisi and Loria, 2016; Gampp et al., 2024). NMR spectroscopy is most commonly used to characterize the structures of molecules in solution at physiological temperatures. It relies on the reorientation of atomic nuclei with a spin different from 0 (preferably with a spin of $\frac{1}{2}$) in a strong magnetic field, giving resonance frequencies that are dependent on the characteristic electronic environment of each nucleus. NMR-active nuclei include ^1H , ^{15}N , and ^{13}C . The main limitation of the technique is the size of amenable targets: since large molecules exhibit slower tumbling rates in solution, this leads to peak broadening and overlap, and, ultimately, the loss of signal. Development of procedures for site-specific isotope labeling (Tugarinov et al., 2006) as well as special pulse sequences (Pervushin et al., 1997; Xu and Matthews, 2013) have enabled the application of NMR spectroscopy to larger biomolecules; however, these are specialized techniques performed in expert labs.

The simplest NMR experiment to study allostery is chemical shift perturbation upon titration of an allosteric ligand, while more sophisticated structure determination can provide crucial information in 3D space. Importantly, structure determination with NMR spectroscopy yields an ensemble of states that can be described with high precision with the exact nuclear Overhauser enhancer method (Vögeli et al., 2012). Recent developments allow the establishment of unbiased structural correlations between different states (Ashkinadze et al., 2022a), providing an exhaustive description of protein dynamics. Recently, these techniques were used to

characterize the dynamic nature of allostery in a PDZ domain (Ashkinadze et al., 2022b).

In addition to 3D-structural information, NMR spectroscopy provides residue-specific information about the dynamics and thermodynamics. Particularly useful to the study of allostery are relaxation-dispersion experiments, which can characterize the exchange to lowly populated excited states occurring in the microsecond to millisecond timescale (Farber and Mittermaier, 2015). Other techniques allow the study of both the exchange at slower timescales and the faster dynamic processes occurring in the picosecond to nanosecond scale. For a thorough review of NMR methods to study allostery and dynamics at different timescales, we recommend Grutsch et al., 2016 or Lisi and Loria, 2016.

NMR spectroscopy is particularly well suited to decide if conformational selection is sufficient to explain allostery (Figure 11d) – as for the Lac repressor (Romanuka et al., 2023), ubiquitin (Lange et al., 2008), and the recruitment of β -arrestins by GPCRs (Kleist et al., 2022) – or if the mechanism is more complicated, as for protein kinases, the protagonists of the ‘violin model’ (Kornev and Taylor, 2015). NMR spectroscopy is also essential to the study of IDPs (see, e.g., Berlow et al., 2017) and for the experimental characterization of molecular dynamics (MD), as for the CAP protein (Popovych et al., 2006), which are two other important examples in this review (see sections ‘Other well-known examples’, ‘Interesting cases at the “extremes”’, and ‘Dynamics, entropy, and ensemble nature of allostery’).

HDX mass spectrometry

Hydrogen-deuterium exchange mass spectrometry (HDX-MS) is a powerful biophysical technique that is used to study ligand binding and other dynamic biomolecular processes (Masson et al., 2019). HDX-MS involves the dilution of a protein in a D_2O -based buffer, followed by monitoring of the exchange of labile hydrogen atoms (usually amide hydrogens) for deuterium. The exchange kinetics are directly dependent on solvent accessibility, influenced by dynamics and conformational changes upon perturbation, such as those caused by the binding of allosteric ligands (Figure 11e). The technique is limited to the study of processes occurring within seconds to hours. HDX-MS is often used in addition to other biophysical and structure determination techniques, as a complementary method. It works well for large biomolecular complexes and can also provide information about regions too dynamic to be studied with NMR spectroscopy (Peacock and Komives, 2021). More details and examples of its application can be found in recent reviews (Masson et al., 2019; Hodge et al., 2020).

Interestingly, other footprinting methods for probing solvent exposure can also be used. These include, for example, photochemical oxidation, which allows the study of much faster processes occurring in the microsecond time scale (Johnson et al., 2019).

FRET

FRET is a technique that depends on the communication of two fluorescent probes that are introduced either in two potential partner molecules or in distant places within a single molecule (Algar et al., 2019) (Figure 11f). If the two probes are sufficiently close, energy is transferred between them, and a signal can be read out. Since the efficiency of this energy transfer is inversely proportional to the sixth power of the distance between the chromophores, the method is very sensitive. Therefore, the technique can give very valuable information, despite its low resolution. Some examples from this review, where FRET was used, are the binding-change

mechanism of ATP synthase (Diez et al., 2004; Figure 7c) and biased signaling by GPCRs (Sadler et al., 2023) (both in the section ‘Other well-known examples’) as well as the study of the intrinsically disordered oncoprotein E1A (Ferreon et al., 2013) (section ‘Interesting cases at the “extremes”’). On a similar topic, a variation of the technique was used, bioluminescence resonance energy transfer (Galés et al., 2005), for in-depth pharmacological profiling of a large library of GPCR mutants (Heydenreich et al., 2023). This systematic study revealed an elaborate signaling network in GPCRs – much more extensive than previously anticipated – that represents a treasure trove for drug development.

Other biophysical techniques

Other structural biology techniques that can be useful to obtain information about biomolecular structure and interactions include small-angle scattering of X-rays (SAXS) (Brosey and Tainer, 2019) or neutrons (SANS) (Mahieu and Gabel, 2018). Both techniques provide low-resolution information of biomolecules in solution, with no upper limit for their size. As such, information from SAXS can, for example, be combined with NMR spectroscopy to characterize conformational ensembles (Venditti et al., 2016), and SANS allows contrast matching studies, where individual partners can be visualized in a biomolecular complex (Krueger, 2022).

Structural biology methods are, however, time- and resource-consuming techniques, and also simpler biophysical techniques can provide crucial information on ligand binding kinetics, thermodynamics, and stoichiometry. Different techniques exploit distinct properties of biomolecules and provide complementary information. These include ITC (Bastos et al., 2023), bilayer interferometry (Sultana and Lee, 2015), surface plasmon resonance (Nguyen et al., 2015), microscale thermophoresis (El Deeb et al., 2022), and differential scanning fluorimetry (Scott et al., 2016) as well as various spectroscopic techniques (electron paramagnetic resonance) (Galazzo and Bordignon, 2023), Raman (Kuhar et al., 2021), Fourier-transform and 2D infrared spectroscopy (Tumbic et al., 2021)), and other fluorescence-based techniques (Martino and Ferrone, 1989). Cooperativity between binding sites can be studied through dose/response curves and the calculation of Hill coefficients (where a Hill coefficient of 1 indicates independent binding, a value greater than 1 positive cooperativity, and a value below 1 negative cooperativity) (Hill, 1910; Prinz, 2010). This analysis, however, provides no information on the nature of the binding – allosteric or orthosteric – which requires additional, complementary studies.

For enzymes, a detailed kinetic analysis is invaluable for the characterization of the molecular mechanism.

Deep mutational scanning, directed evolution, “resurrection” of evolutionary origins, and allosteric engineering

Site-directed mutagenesis is a well-known tool to test hypotheses regarding protein catalysis and binding sites. For probing allosteric mechanisms, in particular, two mutagenesis-based methods are useful: deep mutational scanning (Fowler and Fields, 2014; Faure et al., 2022) and directed evolution (Zeymer and Hilvert, 2018; Yang et al., 2019). The latter method can also be used for protein design (Jäckel et al., 2008), with a beautiful example of allosterically switchable protein assemblies published recently by 2024 Nobel prize winner David Baker (Pillai et al., 2024).

In deep mutational scanning, the amino acid residues of a protein are substituted systematically one by one, usually by replacement with alanine (or glycine if the native residue is alanine).

Interpretation of the results is a complex task (Carlson and Fenton, 2016) and can be further complicated by compensatory effects and structural changes induced by the mutations. The large volume of data makes the application of computational approaches (Kalescky et al., 2015, 2016), particularly deep learning approaches, highly valuable (Verkhivker et al., 2023). Exciting recent examples are the mapping of allosteric landscapes of a GTPase switch (Mathy et al., 2023) and pharmacological profiling of a GPCR (Heydenreich et al., 2023), the latter described in the section ‘Allostery and drug discovery’.

Directed evolution mimics natural selection, where the selective pressure is applied by the researchers in various smart ways. This method was independently developed by the Arnold and Hilvert groups (Kast et al., 1996; Moore and Arnold, 1996), although Hilvert appears to have been forgotten for the 2018 Nobel prize. Interesting results have been obtained for tryptophan synthase (Buller et al., 2015, 2018), where directed evolution mimicked allosteric activation by stepwise tuning the conformational ensemble. In another example related to amino acid synthesis, directed evolution was applied by the Kast lab to the CM of *M. tuberculosis*, which requires activation by a partner enzyme (DAHPSynthase) for full activity (Sasso et al., 2009) (see section ‘Inter-enzyme allostery’). After four cycles of directed evolution, the catalytic efficiency of the enzyme was boosted 250-fold, to a stand-alone enzyme rivaling naturally top-performing CMs (Fahrig-Kamarauskaitė et al., 2020).

Instead of applying directed or systematic mutagenesis, allosteric mechanisms can also be mapped by comparing evolutionary-related proteins from genome sequencing data, and exploring their ancient origins, as pioneered by Pauling and Zuckerkandl (1963). Interesting examples of such studies concern the PDZ domain (Lockless and Ranganathan, 1999), protein kinases (Coyle et al., 2013; Hadzipasic et al., 2020), GPCRs (Süel et al., 2003), and hemoglobin (Süel et al., 2003; Pillai et al., 2020). Similarly, the origin of protein interactions and allostery in colocalization has been investigated, in an elegant study by Kuriyan and Eisenberg (2007). It is also possible to engineer allosteric regulation, as exemplified by metabolic engineering by the Parker group (Cross et al., 2013). A general strategy for engineering allosteric regulation was published by the Reynolds lab (Pincus et al., 2017).

MD simulations, other computational methods, and AI

Computational methods have become powerful and popular tools for the study of protein allostery (for recent reviews, see Schueler-Furman and Wodak, 2016; Greener and Sternberg, 2018; Ni et al., 2022a, 2022b; Colombo, 2023). Many different approaches exploiting different principles have been developed. These range from simple topology-based methods and normal mode analysis (NMA) to more complex MD simulations coupled to a plethora of algorithms and pipelines for the analysis of their results. Artificial intelligence (AI)-based methods and algorithms have also gained popularity in recent years and hold great promise for the near future (Xiao et al., 2023).

Topology-based models approximate protein structure as a network of nodes and edges. The nodes represent structural elements of the protein such as residues, while the edges are weighted based on the strength of interactions, allowing the study of perturbations and their propagation (Amor et al., 2016). Although simple, this approach has been validated on several important allosteric targets (Amor et al., 2016). For a review on emerging graphical representations of allostery, see Arantes et al., 2022. NMA-based approaches for studying allostery provide detailed characterization of protein

low-frequency normal modes at a reasonable computational cost. This is often achieved by using simple elastic or Gaussian network models. For example, NMA-based methods uncovered a novel allosteric site on matrix metalloproteinase-12, for which a highly specific allosteric inhibitor could be designed (Udi et al., 2013). NMA has also been successfully applied to map allosteric communication in large systems like the GroEL-GroES complex (Zheng et al., 2007).

MD simulations aim to fully characterize molecular trajectories over a time scale up to milliseconds. MD is computationally expensive, and different approaches have been developed to overcome the limitations of insufficient sampling through accelerated MD, replica-exchange MD, steered MD, or the use of coarse-grained models (Lazim et al., 2020). MD simulations are limited by the quality of the initial experimental models as well as the approximations intrinsic to the force fields used to govern interactions in the simulation, which are always an approximation of the underlying quantum mechanics principles (Lazim et al., 2020). Combining the results of MD with experimental data is a good way to mitigate these limitations. Both equilibrium and non-equilibrium processes can be studied with MD, and many different perturbations can be introduced in the system to study specific dynamic processes.

Crucially, a well-set-up MD simulation potentially reveals conformational couplings between distal regions, which are key to allosteric regulation. In practice, however, it can be difficult to filter these signals among the noisy dynamics inherent to MD. The analysis of correlated motions in the simulation is the most common analysis performed to extract information from MD trajectories, but many other strategies and variations can be used (Schueler-Furman and Wodak, 2016; Ni et al., 2022a), including AI-based methods (Tsuchiya et al., 2019; Do et al., 2022). A graph theory approach can be used to evaluate dynamic networks created by MD trajectory analysis. Clustering residues in communities with high intercorrelation, representing functional and structural regions of a protein, it is possible to evaluate how some areas couple and decouple, inferring the propagation of the allosteric signal (Arantes et al., 2022; Gheeraert et al., 2023). In addition, strategies for identifying allosteric binding sites have been developed based on the highly correlated motions occurring in the orthosteric and allosteric sites (Xu et al., 2018; Chen et al., 2019).

While a theory allowing accurate *de novo* prediction of allosteric sites is still out of reach, ML methods offer a viable alternative. Several research groups have developed computational frameworks aimed at predicting allosteric sites in proteins. Available tools have been reviewed by Nerin-Fonz and Cournin (2024) in a recent issue of *Current Opinion in Structural Biology*. Computational strategies to elucidate allosteric communication relevant for drug design were reviewed by Ni et al. (2022b). The most common type of ML methods is based on supervised classifiers, which are trained on a starting set of reference data. In this case, the classification algorithms are trained on databases collecting allosteric information (e.g., the Allosteric Database (ASD) (He et al., 2024); accessible online at <http://mdl.shsmu.edu.cn/ASD>). As the structural properties of a protein are encoded in its primary sequence, the recent advances in AI, including large language models (LLMs), have been successfully applied to proteins (e.g., AlphaFold (Jumper et al., 2021; Abramson et al., 2024; Lyu et al., 2024), RoseTTaFold (Baek et al., 2021; Krishna et al., 2024), and ESMFold (Lin et al., 2023)). These algorithms can successfully be exploited to generate information relevant to allostery (Fang et al., 2023; Jing et al., 2023; Vani et al., 2023).

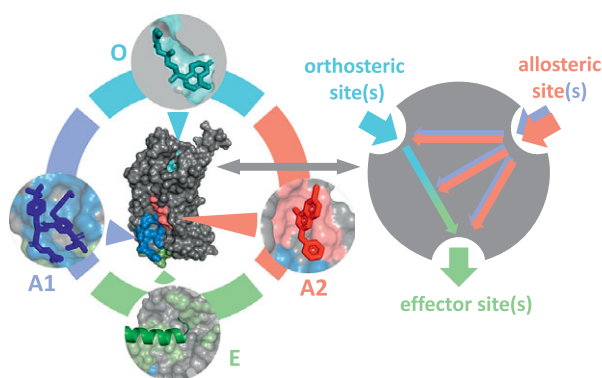


Figure 12. Allostery-based drug design. G-protein-coupled receptors are archetypal allosteric drug targets, as exemplified by ADRB2, shown on the left (and in Figure 6). The panel shows ADRB2 in its R state in surface representation (PDB ID: 3P0G (Rasmussen et al., 2011b)), with agonist BI-167107 (cyan) bound to its orthosteric site ('O') and G_s (green) bound to the effector site ('E', superimposed using PDB ID: 3SN6 (Rasmussen et al., 2011b)). The allosteric binding sites ('A1' and 'A2') for a positive (compound-6FA, blue; PDB ID: 6N48 (Liu et al., 2019)) and negative allosteric inhibitor (AS408, red; PDB ID: 6OBA (Liu et al., 2020)) were mapped onto the ADRB2 surface. The scheme on the right summarizes interactions among orthosteric, effector, and allosteric sites.

Allostery and drug discovery

Dysregulation of protein allostery leads to pathology (Nussinov and Tsai, 2013). While drug discovery traditionally targets orthosteric sites, the idea of employing allosteric effectors as drug molecules, either to rescue a malfunctioning protein (Foster et al., 1999; Liu and Nussinov, 2008) or to modulate/inhibit the activity of a selected target, is as old as the concept of allostery itself (DeDecker, 2000; Nussinov and Tsai, 2013; Tee and Berezovsky, 2024). Like traditional drug discovery, allosteric drug design strategies often depend on structure-based analysis methods (structure-based drug design, referred to as SBDD) (Śledź and Caflich, 2018), with allosteric effectors having the advantage of targeting evolutionarily less-conserved sites, leading to increased specificity and decreased side effects (Chatzigoulas and Cournia, 2021). This is especially relevant for drugs that affect several different cellular pathways, like GPCRs (Figure 12) (Smith and Milligan, 2010; Kenakin and Christopoulos, 2013; Wang et al., 2021; Filizola and Javitch, 2023; Heydenreich et al., 2023) – for this reason, GPCR-targeting drugs such as beta-blockers commonly have considerable side effects. Most importantly, however, the use of allosteric drugs expands the 'targetome', overcoming the "undruggability" of orthosteric sites. One such "undruggable" target is the ras oncogene (Weng et al., 2024), which is mutated in approximately 25% of human cancers (Karnoub and Weinberg, 2008). Recently, an allosteric pan-K-ras inhibitor has been developed that shows broad therapeutic potential (Kim et al., 2023) – in this context, it is interesting to note that both activating mutations and treatment may modulate signaling pathway and strength (Nussinov et al., 2022b). Another key advantage of allosteric drugs is that they can help overcome drug resistance (Ni et al., 2020).

Tailgating the increased understanding of allostery, drug discovery employing allosteric effectors and modulators is maturing into a promising research field (DeDecker, 2000; Nussinov and Tsai, 2013; Lu et al., 2014; Tan et al., 2022; Tee and Berezovsky, 2024). The ASD (<https://mdl.shsmu.edu.cn/ASD/m>) now counts 538 allosteric drugs, with status ranging from pre-clinical to approved (He et al., 2024) (July 2024); between 2018 and 2022, 24 out of 188 drugs approved by the Food and Drug Administration were allosteric

effectors (Nerín-Fonz and Cournia, 2024). Given that the first purposely designed allosteric drug was approved only in 2004, this is a rather impressive development.

Traditional drug development, targeting orthosteric sites, often starts with the screening of derivatives of the primary endogenous effector in search of an effective inhibitor (Bofill et al., 2019). Alternative starting points are provided by high-throughput screening or fragment-based screening, which may also yield allosteric effectors or modulators (Erlanson et al., 2016; Gong et al., 2017). However, given that allosteric ligands often bind to shallow, non-polar binding sites (Tan et al., 2022) and have low affinities, they pose considerable challenges to drug discovery (Chatzigoulas and Cournia, 2021). Several allosteric effectors fit the profile of 'beyond the Rule of Five' (Doak et al., 2014; Lipinski, 2016; Doak and Kihlberg, 2017) (bRo5) molecules, an extension of the Lipinski's 'rule of five' (Lipinski et al., 1997) (Ro5), a set of requirements on drug absorption and permeability. Relaxing the Ro5 criteria to include macromolecules and biologics, such as macrocycles and other larger compounds, dramatically expanded the toolbox available for allosteric drug discovery (Doak et al., 2014). Moreover, allosteric ligands often work best in concert with traditional drugs and depend on combinatorial optimization (Ni et al., 2020). Important examples of such combinatorial treatment strategies concern oxygen uptake by hemoglobin targeting cancer, cardiovascular diseases, and sickle cell disease (Duarte et al., 2010; Moody et al., 2024), the latter caused by pathogenic hemoglobin fibers.

The allosteric drug discovery workflow presents additional challenges compared to traditional drug development (Guarnera and Berezovsky, 2020; Fan et al., 2021; Pan and Mader, 2022), including the reverse perturbation analysis of allosteric signaling (Tee et al., 2018; de Vries et al., 2020). Additionally, the complementary effects of mutations (Guarnera and Berezovsky, 2020) need to be considered as well as the bias of compound libraries toward orthosteric sites, the high number of false negatives and the crosstalk among different allosteric sites in the same target (Jiao et al., 2012). The latter is both an obstacle and an opportunity, as the sites can be exploited through a combinatorial approach, designing medicines that combine the effect of two or more drugs on the same target (Kenakin and Christopoulos, 2013). This calls for the application of new approaches, including those involving AI (Wu et al., 2022; Cichońska et al., 2024; Lin et al., 2024; Tee and Berezovsky, 2024), as described in section 'MD simulations, other computational methods, and AI'. Another factor that needs to be considered is the evaluation of allosteric drugs: since these compounds often have a modulatory effect and bias signaling, one pharmacological parameter – efficacy – is insufficient to judge their potency, and concentration-response curves are more meaningful (Kenakin and Christopoulos, 2013). This will potentiate the needed effort but likely pay substantial dividends.

Several research groups have developed allosteric drug discovery pipelines, often combining experimental and computational methods, testing them on model systems with a recognized role in disease (Wagner et al., 2016). Recently, a full issue of the *Journal of Molecular Biology*, edited by Nussinov and Berezovsky, was entirely dedicated to allosteric drug design, covering topics from the identification of allosteric sites to the discovery or design of effectors targeting them (Berezovsky and Nussinov, 2022). For example, Tan et al. (2022) reported the use of an in-house-developed structure-based statistical mechanical model of allostery to derive an allosteric fingerprint for MAP kinase 7 and the GPCR rhodopsin and, based on their results, proposed a generic protocol for the design of

allosteric drugs. Another article in the collection describes innovative allosteric drugs, like molecular glues and proteolysis targeting chimera (PROTACs), which couple a target-binding module with a molecular warhead that prompts degradation (Nussinov et al., 2022a). *Current Opinion in Structural Biology* regularly publishes articles related to allostery, and a good overview of rationally designed allosteric modulators was recently published by Chatzigeorgoulas and Cournia (2021). Especially impressive examples are allosteric inhibitors against two important cancer targets: an inhibitor of the epidermal growth factor (EGFR) that is 1,000-fold more selective against an EGFR mutant that showed drug resistance (Jia et al., 2016) and an inhibitor that enabled dual targeting against BCR-ABL1 (Wylie et al., 2017).

Summary and outlook

There is increasing evidence for large-scale latent allostery in proteins, particularly from the study of protein homologs and ancestor resurrection studies, as discussed in this review for hemoglobin, protein kinases, and GPCRs (Süel et al., 2003; Pillai et al., 2020) (in sections ‘Introduction: allostery in context – historical overview’, ‘Classical example: hemoglobin’, and ‘Other well-known examples’). It is even possible that all (dynamic) proteins are allosteric (Gunasekaran et al., 2004). In combination with gene duplication and co-localization, either in organelles or pseudo-organelles, such as protein micro-compartments, membrane-less organelles, or the periplasm, allosteric regulation is subjected to selective pressures, allowing strong allosteric networks to evolve (Kuriyan and Eisenberg, 2007; Mathy and Kortemme, 2023). The reward for the cell and for life is a very efficient regulation system, which requires minimal input of energy to directly and rapidly respond to various environmental challenges.

Given that allostery is everywhere in biological systems, it is no surprise that allosteric drugs and modulators have enormous potential for the treatment of diseases – from cancer and other noncommunicable diseases to infectious diseases of bacterial and viral origin (section ‘Allostery and drug discovery’). The growing interest in allostery is also reflected by an enormous increase in publications on this topic, raising from approximately 10 publications annually before 2000 to >200 today. Due to the challenges in finding and characterizing allosteric binding sites and mechanisms, this field has long been lagging behind conventional drug discovery; however, with new technologies, including machine-based learning algorithms (section ‘Methods to probe allostery’), the future for allostery-based drug discovery looks bright. Already, the first mutant-selective drugs are entering the market, “undruggable” targets are being tackled, and allosteric modulators are countering drug resistance. The next frontier is the application of allosteric drugs in personalized medicine. Concerning mechanistic insights (sections ‘Interesting cases at the “extremes”’ and ‘Dynamics, entropy and ensemble nature of allostery’), we expect the next breakthroughs to be related to the kinetic characterization of allostery.

The genetic code provides the blueprint for the synthesis of proteins, and allostery the basis for protein communication and interaction. It is therefore fully deserved that allostery has been referred to as the “second secret of life” (Monod, 1971; Fenton, 2008). Approaching its 60th anniversary (Monod et al., 1965), allostery has not lost its appeal and is as relevant as ever.

Acknowledgements. We would like to thank Peter Kast, who is the main PI of the grant financing the salary of M.M.-C.

Author contribution. U.K. suggested the topic, conceptualized the review including the figures, and wrote most of the text. M.M.-C. and G.C. drafted the ‘Methods to probe allostery’ and the ‘Allostery and drug discovery’ sections, respectively. In addition, M.M.-C. and G.C. prepared the figures and drafted the figure legends. The manuscript was finalized by all the authors.

Financial support. The salary of M.M.-C. was funded by a grant from the Swiss National Science Foundation (Grant No. 310030M_219998).

Competing interest. The authors declare no competing interests.

Use of artificial intelligence tools. AI tools have not been used to generate text, images, or to analyze or extract insights. However, they are likely part of the literature search engines.

References

- Abrahams JP, Leslie AGW, Lutter R and Walker JE (1994) Structure at 2.8 Å resolution of F1-ATPase from bovine heart mitochondria. *Nature* **370**, 621–628.
- Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, Ronneberger O, Willmore L, Ballard AJ, Bambrick J, Bodenstein SW, Evans DA, Hung C-C, O’Neill M, Reiman D, Tunyasuvunakool K, Wu Z, Žemgulytė A, Arvaniti E, Beattie C, Bertolli O, Bridgland A, Cherepanov A, Congreve M, Cowen-Rivers AI, Cowie A, Figurnov M, Fuchs FB, Gladman H, Jain R, Khan YA, Low CMR, Perlin K, Potapenko A, Savy P, Singh S, Stecula A, Thillaisundaram A, Tong C, Yakneen S, Zhong ED, Zielinski M, Židek A, Bapst V, Kohli P, Jaderberg M, Hassabis D and Jumper JM (2024) Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **630**, 493–500.
- Algar WR, Hildebrandt N, Vogel SS and Medintz IL (2019) FRET as a biomolecular research tool — understanding its potential while avoiding pitfalls. *Nature Methods* **16**, 815–829.
- Amann SJ, Keihlsler D, Bodrug T, Brown NG and Haselbach D (2023) Frozen in time: analyzing molecular dynamics with time-resolved cryo-EM. *Structure* **31**, 4–19.
- Amor BRC, Schaub MT, Yaliraki SN and Barahona M (2016) Prediction of allosteric sites and mediating interactions through bond-to-bond propensities. *Nature Communications* **7**, 12477.
- Anfinsen CB (1973) Principles that govern the folding of protein chains. *Science* **181**, 223–230.
- Antonini E and Brunori M (1971) Hemoglobin and Myoglobin in Their Reactions with Ligands, Neuberger A and Tatum EL (eds.), *North-Holland Research Monographs*. Frontiers of Biology, Vol. 21. Amsterdam and London: North-Holland Publishing Company.
- Arantes PR, Patel AC and Palermo G (2022) Emerging methods and applications to decrypt allostery in proteins and nucleic acids. *Journal of Molecular Biology* **434**, 167518.
- Arkhipov A, Shan Y, Das R, Endres NF, Eastwood MP, Wemmer DE, Kuriyan J and Shaw DE (2013) Architecture and membrane interactions of the EGF receptor. *Cell* **152**, 557–569.
- Arnone A (1972) X-ray diffraction study of binding of 2,3-diphosphoglycerate to human deoxyhaemoglobin. *Nature* **237**, 146–149.
- Ascenzi P and Fasano M (2010) Biophysical chemistry allostery in a monomeric protein: The case of human serum albumin. *Biophysical Chemistry* **148**, 16–22.
- Ascenzi P and Gianni S (2013) Functional role of transient conformations: Rediscovering “chronosteric effects” thirty years later. *IUBMB Life* **65**, 836–844.
- Ashkinadze D, Klukowski P, Kadavath H, Güntert P and Riek R (2022a) PDBcor: An automated correlation extraction calculator for multi-state protein structures. *Structure* **30**, 646–652.
- Ashkinadze D, Kadavath H, Pokharna A, Chi CN, Friedmann M, Strotz D, Kumari P, Minges M, Cadalbert R, Königl S, Güntert P, Vögeli B and Riek R (2022b) Atomic resolution protein allostery from the multi-state structure of a PDZ domain. *Nature Communications* **13**, 6232.
- Audet M and Bouvier M (2012) Restructuring G-protein-coupled receptor activation. *Cell* **151**, 14–23.
- Baccouch R, Rascol E, Stoklosa K and Alves ID (2022) The role of the lipid environment in the activity of G protein coupled receptors. *Biophysical Chemistry* **285**, 106794.

- Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S, Lee GR, Wang J, Cong Q, Kinch LN, Schaeffer RD, Millán C, Park H, Adams C, Glassman CR, DeGiovanni A, Pereira JH, Rodrigues AV, van Dijk AA, Ebrecht AC, Opperman DJ, Sagmeister T, Buhlheller C, Pavkov-Keller T, Rathinaswamy MK, Dalwadi U, Yip CK, Burke JE, Garcia KC, Grishin NV, Adams PD, Read RJ and Baker D (2021) Accurate prediction of protein structures and interactions using a three-track neural network. *Science* **373**, 871–876.
- Baldwin J and Chothia C (1979) Haemoglobin: The structural changes related to ligand binding and its allosteric mechanism. *Journal of Molecular Biology* **129**, 175–220.
- Bastos M, Abian O, Johnson CM, Ferreira-da-Silva F, Vega S, Jimenez-Alesanco A, Ortega-Alarcon D and Velazquez-Campoy A (2023) Isothermal titration calorimetry. *Nature Reviews Methods Primers* **3**, 17.
- Bell CE and Lewis M (2000) A closer view of the conformation of the Lac repressor bound to operator. *Nature* **7**, 209–214.
- Berenbrink M (2020) Evolution of a molecular machine. *Nature* **581**, 388–389.
- Berezovsky IN and Nussinov R (2022) Multiscale allostery: Basic mechanisms and versatility in diagnostics and drug design. *Journal of Molecular Biology* **434**, 167751.
- Berlow RB, Dyson HJ and Wright PE (2017) Hypersensitive termination of the hypoxic response by a disordered protein switch. *Nature* **543**, 447–451.
- Blackmore NJ, Nazmi AR, Hutton RD, Webby MN, Baker EN, Jameson GB and Parker EJ (2015) Complex formation between two biosynthetic enzymes modifies the allosteric regulatory properties of both: AN EXAMPLE OF MOLECULAR SYMBIOSIS. *The Journal of Biological Chemistry* **290**, 18187–18198.
- Blow DM (2002) *Outline of Crystallography for Biologists*. Oxford: Oxford University Press.
- Boehr DD, Nussinov R and Wright PE (2009) The role of dynamic conformational ensembles in biomolecular recognition. *Nature Chemical Biology* **5**, 789–796.
- Boffill A, Jalencas X, Oprea TI and Mestres J (2019) The human endogenous metabolome as a pharmacology baseline for drug discovery. *Drug Discovery Today* **24**, 1806–1820.
- Bohr C (1892) Beiträge zur Lehre von den Kohlensäureverbindungen des Blutes. *Skandinavisches Archiv für Physiologie* **3**, 47–68.
- Bohr C, Hasselbalch K and Krogh A (1904) Ueber einen in biologischer Beziehung wichtigen Einfluss, den die Kohlensäurespannung des Blutes auf dessen Sauerstoffbindung übt. *Skandinavisches Archiv für Physiologie* **16**, 402–412.
- Boulton S and Melacini G (2016) Advances in NMR methods to map allosteric sites: From models to translation. *Chemical Reviews* **116**, 6267–6304.
- Boyer PD (1993) The binding change mechanism for ATP synthase – Some probabilities and possibilities. *Biochimica et Biophysica Acta* **1140**, 215–250.
- Boyer PD (1997) The ATP synthase—a splendid molecular machine. *Annual Review of Biochemistry* **66**, 717–749.
- Brosey CA and Tainer JA (2019) Evolving SAXS versatility: solution X-ray scattering for macromolecular architecture, functional landscapes, and integrative structural biology. *Current Opinion in Structural Biology* **58**, 197–213.
- Buller AR, Brinkmann-Chen S, Romney DK, Herger M, Murciano-Calles J and Arnold FH (2015) Directed evolution of the tryptophan synthase β -subunit for stand-alone function recapitulates allosteric activation. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 14599–14604.
- Buller AR, van Roye P, Cahn JKB, Scheele RA, Herger M and Arnold FH (2018) Directed evolution mimics allosteric activation by stepwise tuning of the conformational ensemble. *Journal of the American Chemical Society* **140**, 7256–7266.
- Burschowsky D, Thorbjørnsrud HV, Heim JB, Fahrig-Kamaraukaitė J, Würth-Roderer K, Kast P and Kregel U (2018) Inter-enzyme allosteric regulation of chorismate mutase in *Corynebacterium glutamicum*: Structural basis of feedback activation by Trp. *Biochemistry* **57**, 557–573.
- Carlson GM and Fenton AW (2016) What mutagenesis can and cannot reveal about allostery. *Biophysical Journal* **110**, 1912–1923.
- Changeux J-P (1961) The feedback control mechanism of biosynthetic L-threonine deaminase by L-isoleucine. *Cold Spring Harbor Symposia on Quantitative Biology* **26**, 313–318.
- Changeux J-P (2012) Allostery and the Monod–Wyman–Changeux model after 50 years. *Annual Review of Biophysics* **41**, 103–133.
- Changeux J-P (2013) 50 years of allosteric interactions: the twists and turns of the models. *Nature Reviews Molecular Cell Biology* **14**, 819–829.
- Changeux J-P and Edelstein SJ (2005) Allosteric mechanisms of signal transduction. *Science* **208**, 1424–1428.
- Changeux J-P, Gerhart JC and Schachman HK (1968) Allosteric interactions in aspartate transcarbamylase. I. Binding of specific ligands to the native enzyme and its isolated subunits. *Biochemistry* **7**, 531–538.
- Chatzigeorgoulas A and Cournia Z (2021) Rational design of allosteric modulators: Challenges and successes. *WIREs Computational Molecular Science* **11**, e1529.
- Chen D-H, Madan D, Weaver J, Lin Z, Schröder GF, Chiu W and Rye HS (2008) Visualizing GroEL/ES in the act of encapsulating a folding protein. *Cell* **153**, 1354–1365.
- Chen Z, Zhang X, Peng C, Wang J, Xu Z, Chen K, Shi J and Zhu W (2019) D3Pockets: A method and web server for systematic analysis of protein pocket dynamics. *Journal of Chemical Information and Modeling* **59**, 3353–3358.
- Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SGF, Thian FS, Kobilka TS, Choi H, Kuhn P, Weis WI, Kobilka BK and Stevens RC (2007) High-resolution crystal structure of an engineered human β_2 -adrenergic G protein-coupled receptor. *Science* **318**, 1258–1265.
- Chong A and Forman-Kay JD (2017) A hidden competitive advantage of disorder. *Nature*, **543**, 325–326.
- Chouard T (2011) Breaking the protein rules. *Nature* **471**, 151–153.
- Cichońska A, Ravikumar B and Rahman R (2024) AI for targeted polypharmacology: The next frontier in drug discovery. *Current Opinion in Structural Biology* **84**, 102771.
- Clare DK, Bakkes PJ, van Heerikhuizen H, van der Vies SM and Saibil HR (2009) Chaperonin complex with a newly folded protein encapsulated in the folding chamber. *Nature* **457**, 107–110.
- Cockrell GM and Kantrowitz ER (2012) Metal ion involvement in the allosteric mechanism of *Escherichia coli* aspartate transcarbamoylase. *Biochemistry* **51**, 7128–7137.
- Cockrell GM, Zheng Y, Guo W, Peterson AW, Truong JK and Kantrowitz ER (2013) New paradigm for allosteric regulation of *Escherichia coli* aspartate transcarbamoylase. *Biochemistry* **52**, 8036–8047.
- Cofas-Vargas LF, Mendoza-Espinosa P, Avila-Barrientos LP, Prada-Gracia D, Riveros-Rosas H and Garcia-Hernández E (2022) Exploring the druggability of the binding site of aurovertin, an exogenous allosteric inhibitor of F_0F_1 -ATP synthase. *Frontiers in Pharmacology* **13**, 1012008.
- Colombo G (2023) Computing allostery: From the understanding of biomolecular regulation and the discovery of cryptic sites to molecular design. *Current Opinion in Structural Biology* **83**, 102702.
- Colombo MF, Rau DC and Parsegian VA (1992) Protein solvation in allosteric regulation: A water effect on hemoglobin. *Science* **256**, 655–659.
- Cooper A and Dryden DTF (1984) Allostery without a conformational change. *European Biophysics Journal* **11**, 103–109.
- Cornish-Bowden A and Cárdenas ML (2004) Glucokinase: A monomeric enzyme with positive cooperativity. In Matschinsky FM and Magnuson MA (eds.), *Frontiers in Diabetes: Glucokinase and Glycemic Disease: From Basics to Novel Therapeutics*. Basel: S. Karger AG, pp. 125–134.
- Coskun Ü, Grzybek M, Drechsel D and Simons K (2011) Regulation of human EGF receptor by lipids. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 9044–9048.
- Coyle SM, Flores J and Lim WA (2013) Exploitation of latent allostery enables the evolution of new modes of MAP kinase regulation. *Cell* **154**, 875–887.
- Cross PJ, Allison TM, Dobson RCJ, Jameson GB and Parker EJ (2013) Engineering allosteric control to an unregulated enzyme by transfer of a regulatory domain. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 2111–2116.
- Cui Q and Karplus M (2008) Allostery and cooperativity revisited. *Protein Science* **17**, 1295–1307.
- Daber R, Stayrook S, Rosenberg A and Lewis M (2007) Structural analysis of lac repressor bound to allosteric effectors. *Journal of Molecular Biology* **370**, 609–619.
- Dames SA, Martinez-Yamout M, de Guzman RN, Dyson HJ and Wright PE (2002) Structural basis for Hif-1 α /CBP recognition in the cellular hypoxic

- response. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 5271–5276.
- DeDecker BS** (2000) Allosteric drugs: thinking outside the active-site box. *Chemistry and Biology* **7**, 103–107.
- Diez M, Zimmermann B, Börsch M, König M, Schweinberger E, Steigmiller S, Reuter R, Felekyan S, Kudryavtsev V, Seidel CAM and Gräber P** (2004) Proton-powered subunit rotation in single membrane-bound F_0F_1 -ATP synthase. *Nature Structural & Molecular Biology* **11**, R135–R141.
- Dixon RAF, Kobilka BK, Strader DJ, Benovic JL, Dohlman HG, Frielle T, Bolanowski MA, Bennett CD, Rands E, Diehl RE, Mumford RA, Slater EE, Sigal IS, Caron MG, Lefkowitz RJ and Strader CD** (1986) Cloning of the gene and cDNA for mammalian β -adrenergic receptor and homology with rhodopsin. *Nature* **321**, 75–79.
- Do HN, Wang J, Bhattarai A and Miao Y** (2022) GLOW: A workflow integrating Gaussian-accelerated molecular dynamics and deep learning for free energy profiling. *Journal of Chemical Theory and Computation* **18**, 1423–1436.
- Doak BC, Giordanetto F and Kihlberg J** (2014) Oral druggable space beyond the rule of 5: Insights from drugs and clinical candidates. *Chemistry and Biology* **21**, 1115–1142.
- Doak BC and Kihlberg J** (2017) Drug discovery beyond the rule of 5 – Opportunities and challenges. *Expert Opinion on Drug Discovery* **12**, 115–119.
- Downward J, Parker P and Waterfield MD** (1984) Autophosphorylation sites on the epidermal growth factor receptor. *Nature* **311**, 483–485.
- Duarte CD, Greferath R, Nicolau C and Lehn J-M** (2010) *myo*-inositol trispyrophosphate: A novel allosteric effector of hemoglobin with high permeation selectivity across the red blood cell plasma membrane. *ChemBioChem* **11**, 2543–2548.
- Dyachenko A, Gruber R, Shimon L, Horovitz A and Sharon M** (2013) Allosteric mechanisms can be distinguished using structural mass spectrometry. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 7235–7239.
- El Deeb S, Al-Harrasi A, Khan A, Al-Broumi M, Al-Thani G, Alomairi M, Elumalai P, Sayed RA and Ibrahim AE** (2022) Microscale thermophoresis as a powerful growing analytical technique for the investigation of biomolecular interaction and the determination of binding parameters. *Methods and Applications in Fluorescence* **10**, 042001.
- Ellis RJ** (1994) Opening and closing the Anfinsen cage. *Current Biology* **4**, 633–635.
- Erlanson DA, Fesik SW, Hubbard RE, Jahnke W and Jhoti H** (2016) Twenty years on: The impact of fragments on drug discovery. *Nature Reviews Drug Discovery* **15**, 605–619.
- Fahrig-Kamarauskaitė J, Würth-Roderer K, Thorbjørnsrud HV, Mailand S, Krengel U and Kast P** (2020) Evolving the naturally compromised chorismate mutase from *Mycobacterium tuberculosis* to top performance. *The Journal of Biological Chemistry* **295**, 17514–17534.
- Fan J, Liu Y, Kong R, Ni D, Yu Z, Lu S and Zhang J** (2021) Harnessing reversed allosteric communication: A novel strategy for allosteric drug discovery. *Journal of Medicinal Chemistry* **64**, 17728–17743.
- Fanali G, di Masi A, Trezza V, Marino M, Fasano M and Ascenzi P** (2012) Human serum albumin: From bench to bedside. *Molecular Aspects of Medicine* **33**, 209–290.
- Fang Y, Jiang Y, Wei L, Ma Q, Ren Z, Yuan Q and Wei D-Q** (2023) DeepProSite: Structure-aware protein binding site prediction using ESMFold and pretrained language model. *Bioinformatics* **39**, btad718.
- Farber PJ and Mittermaier A** (2015) Relaxation dispersion NMR spectroscopy for the study of protein allostery. *Biophysical Reviews* **7**, 191–200.
- Fasano M, Curry S, Terreno E, Galliano M, Fanali G, Narciso P, Notari S and Ascenzi P** (2005) The extraordinary ligand binding properties of human serum albumin. *IUBMB Life* **57**, 787–796.
- Fasano M, Fanali G, Fesce R and Ascenzi P** (2008) Human serum haemalbumin: An allosteric ‘chronosteric’ protein. In Bolognesi M, Prisc G and Verde C (eds.), *Protein Reviews: Dioxxygen Binding and Sensing Proteins*. Milano: Springer, pp. 121–131.
- Faure AJ, Domingo J, Schmiedel JM, Hidalgo-Carcedo C, Diss G and Lehner B** (2022) Mapping the energetic and allosteric landscapes of protein binding domains. *Nature* **604**, 175–183.
- Fei X, Yang D, LaRonde-LeBlanc N and Lorimer GH** (2013) Crystal structure of a GroEL-ADP complex in the relaxed allosteric state at 2.7 Å resolution. *Proceedings of the National Academy of Sciences of the United States of America* **110**, E2958–E2966.
- Fenton AW** (2008) Allostery: an illustrated definition for the ‘second secret of life’. *Trends in Biochemical Sciences* **33**, 420–425.
- Ferreon ACM, Ferreon JC, Wright PE and Deniz AA** (2013) Modulation of allostery by protein intrinsic disorder. *Nature* **498**, 390–394.
- Filizola BM and Javitch JA** (2023) Deciphering downstream receptor signaling. *Science* **382**, 1357–1358.
- Fischer S, Olsen KW, Nam K and Karplus M** (2011) Unsuspected pathway of the allosteric transition in hemoglobin. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 5608–5613.
- Foster BA, Coffey HA, Morin MJ and Rastinejad F** (1999) Pharmacological rescue of mutant p53 conformation and function. *Science* **286**, 2507–2510.
- Fowler DM and Fields S** (2014) Deep mutational scanning: A new style of protein science. *Nature Methods* **11**, 801–807.
- Frauenfelder H, McMahon BH, Austin RH, Chu K and Groves JT** (2001) The role of structure, energy landscape, dynamics and allostery in the enzymatic function of myoglobin. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 2370–2374.
- Freedman SJ, Sun Z-YJ, Poy F, Kung AL, Livingston DM, Wagner G and Eck MJ** (2002) Structural basis for recruitment of CBP/p300 by hypoxia-inducible factor-1 α . *Proceedings of the National Academy of Sciences of the United States of America* **99**, 5367–5372.
- Galaris D, Cadenas E and Hochstein P** (1989) Redox cycling of myoglobin and ascorbate: A potential protective mechanism against oxidative reperfusion injury in muscle. *Archives of Biochemistry and Biophysics* **273**, 497–504.
- Galazzo L and Bordignon E** (2023) Electron paramagnetic resonance spectroscopy in structural-dynamic studies of large protein complexes. *Progress in Nuclear Magnetic Resonance Spectroscopy* **134–135**, 1–19.
- Galés C, Rebois RV, Hogue M, Trieu P, Breit A, Hébert TE and Bouvier M** (2005) Real-time monitoring of receptor and G-protein interactions in living cells. *Nature Methods* **2**, 177–184.
- Gampp O, Kadavath H and Riek R** (2024) Structural biology NMR tools to detect protein allostery. *Current Opinion in Structural Biology* **86**, 102792.
- Gheeraert A, Lesieur C, Batista VS, Vuillon L and Rivalta I** (2023) Connected component analysis of dynamical perturbation contact networks. *The Journal of Physical Chemistry B* **127**, 7571–7580.
- Gong Z, Hu G, Li Q, Liu Z, Wang F, Zhang X, Xiong J, Li P, Xu Y, Ma R, Chen S and Li J** (2017) Compound libraries: Recent advances and their applications in drug discovery. *Current Drug Discovery Technologies* **14**, 216–228.
- Greener JG and Sternberg MJE** (2018) Structure-based prediction of protein allostery. *Current Opinion in Structural Biology* **50**, 1–8.
- Gruber R and Horovitz A** (2016) Allosteric mechanisms in chaperonin machines. *Chemical Reviews* **116**, 6588–6606.
- Grutsch S, Brüschweiler S and Tollinger M** (2016) NMR methods to study dynamic allostery. *PLoS Computational Biology* **12**, e1004620.
- Guarnera E and Berezovsky IN** (2020) Allosteric drugs and mutations: Chances, challenges, and necessity. *Current Opinion in Structural Biology* **62**, 149–157.
- Gunasekaran K, Ma B and Nussinov R** (2004) Is allostery an intrinsic property of all dynamic proteins? *PROTEINS: Structure, Function and Bioinformatics* **57**, 433–443.
- Hackney DD, Rosen G and Boyer PD** (1979) Subunit interaction during catalysis: Alternating site cooperativity in photophosphorylation shown by substrate modulation of [^{18}O]ATP species formation. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 3646–3650.
- Hadzipasic A, Wilson C, Nguyen V, Kern N, Kim C, Pitsawong W, Villali J, Zheng Y and Kern D** (2020) Ancient origins of allosteric activation in a Ser-Thr kinase. *Science* **367**, 912–917.
- Hajdu J and Andersson I** (1993) Fast crystallography and time-resolved structures. *Annual Review of Biophysics and Biomolecular Structure* **22**, 467–498.
- Hajdu J, Neutze R, Sjögren T, Edman K, Szöke A, Wilmouth RC and Wilmot CM** (2000) Analyzing protein functions in four dimensions. *Nature Structural Biology* **7**, 1006–1012.
- Hayer-Hartl M, Bracher A and Hartl FU** (2016) The GroEL–GroES chaperonin machine: A nano-cage for protein folding. *Trends in Biochemical Sciences* **41**, 62–76.
- He XM and Carter DC** (1992) Atomic structure and chemistry of human serum albumin. *Nature* **358**, 209–215.

- He J, Liu X, Zhu C, Zha J, Li Q, Zhao M, Wei J, Li M, Wu C, Wang J, Jiao Y, Ning S, Zhou J, Hong Y, Liu Y, He H, Chen F, Li Y, He X, Wu J, Lu S, Song K, Lu X and Zhang J (2024) ASD2023: Towards the integrating landscapes of allosteric knowledgebase. *Nucleic Acids Research* **52**, D376–D383.
- Henderson R and Unwin PNT (1975) Three-dimensional model of purple membrane obtained by electron microscopy. *Nature* **257**, 28–32.
- Henzler-Wildman K and Kern D (2007) Dynamic personalities of proteins. *Nature* **450**, 964–965.
- Heydenreich FM, Marti-Solano M, Sandhu M, Kobilka B, Bouvier M and Babu MM (2023) Molecular determinants of ligand efficacy and potency in GPCR signaling. *Science* **1378**, eadh1859.
- Hill AV (1910) The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. *The Journal of Physiology* **40**, I–VII.
- Hilser VJ (2013) Signalling from disordered proteins. *Nature* **498**, 308–310.
- Hodge EA, Benhaim MA and Lee KK (2020) Bridging protein structure, dynamics, and function using hydrogen/deuterium-exchange mass spectrometry. *Protein Science* **29**, 843–855.
- Horst R, Fenton WA, Englander SW, Wüthrich K and Horwich AL (2007) Folding trajectories of human dihydrofolate reductase inside the GroEL–GroES chaperonin cavity and free in solution. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 20788–20792.
- Horwich AL and Fenton WA (2009) Chaperonin-mediated protein folding: Using a central cavity to kinetically assist polypeptide chain folding. *Quarterly Reviews of Biophysics* **42**, 83–116.
- Howlett GJ, Blackburn MN, Compton JG and Schachman HK (1977) Allosteric regulation of aspartate transcarbamoylase. Analysis of the structural and functional behavior in terms of a two-state model. *Biochemistry* **16**, 5091–5099.
- Hua T, Li X, Wu L, Iliopoulos-Tsoutsouvas C, Wang Y, Wu M, Shen L, Brust CA, Nikas SP, Song F, Song X, Yuan S, Sun Q, Wu Y, Jiang S, Grim TW, Benchama O, Stahl EL, Zvonok N, Zhao S, Bohn LM, Makriyannis A and Liu Z-J (2020) Activation and signaling mechanism revealed by cannabinoid receptor-G_i complex structures. *Cell* **180**, 655–665.
- Imai S, Yokomizo T, Kofuku Y, Shiraiishi Y, Ueda T and Shimada I (2020) Structural equilibrium underlying ligand-dependent activation of β_2 -adrenoreceptor. *Nature Chemical Biology* **16**, 430–439.
- Jäckel C, Kast P and Hilvert D (2008) Protein design by directed evolution. *Annual Review of Biophysics* **37**, 153–173.
- Jacob F and Monod J (1961) Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular Biology* **3**, 318–356.
- Jamali K, Käll L, Zhang R, Brown A, Kimanius D and Scheres SHW (2024) Automated model building and protein identification in cryo-EM maps. *Nature* **628**, 450–457.
- Jardetzky O (1996) Protein dynamics and conformational transitions in allosteric proteins. *Progress in Biophysics and Molecular Biology* **65**, 171–219.
- Jia Y, Yun C-H, Park E, Ercan D, Manuia M, Juarez J, Xu C, Rhee K, Chen T, Zhang H, Palakurthi S, Jang J, Lelais G, DiDonato M, Bursulaya B, Michellys P-Y, Epple R, Marsilje TH, McNeill M, Lu W, Harris J, Bender S, Wong K-K, Jänne PA and Eck MJ (2016) Overcoming EGFR(T790M) and EGFR(C797S) resistance with mutant-selective allosteric inhibitors. *Nature* **534**, 129–132.
- Jiao W, Hutton RD, Cross PJ, Jameson GB and Parker EJ (2012) Dynamic cross-talk among remote binding sites: The molecular basis for unusual synergistic allostery. *Journal of Molecular Biology* **415**, 716–726.
- Jing B, Berger B and Jaakkola T (2023) AlphaFold meets flow matching for generating protein ensembles. [arXiv:2402.04845v2](https://arxiv.org/abs/2402.04845v2).
- Johansson LC, Arnlund D, White TA, Gergely K, DePonte DP, Weierstall U, Doak BR, Shoeman RL, Lomb L, Malmerberg E, Davidsson J, Nass K, Liang M, Andreasson J, Aquila A, Bajt S, Barthelmeß M, Barty A, Bogan MJ, Bostedt C, Bozek JD, Caleman C, Coffee R, Coppola N, Ekeberg T, Epp SW, Erk B, Fleckenstein H, Foucar L, Graafsma H, Gumprecht L, Hajdu J, Hampton CY, Hartmann R, Hartmann A, Hauser G, Hirsemann H, Holl P, Hunter MS, Kassemeyer S, Kimmel N, Kirian RA, Maia FR, Marchesini S, Martin AV, Reich C, Rolles D, Rudek B, Rudenko A, Schlichting I, Schulz J, Seibert MM, Sierra RG, Soltan H, Starodub D, Stellato F, Stern S, Strüder L, Timneanu N, Ullrich J, Wahlgren WY, Wang X, Weidenspointner G, Wunderer C, Fromme P, Chapman HN, Spence JC and Neutze R (2012) Lipidic phase membrane protein serial femtosecond crystallography. *Nature Methods* **9**, 263–266.
- Johnson LN (1992) Time-resolved protein crystallography. *Protein Science* **1**, 1237–1243.
- Johnson DT, di Stefano LH and Jones LM (2019) Fast photochemical oxidation of proteins (FPOP): A powerful mass spectrometry-based structural proteomics tool. *The Journal of Biological Chemistry* **294**, 11969–11979.
- Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Židek A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B, Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M, Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, Senior AW, Kavukcuoglu K, Kohli P and Hassabis D (2021) Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589.
- Kalescky R, Liu J and Tao P (2015) Identifying key residues for protein allostery through rigid residue scan. *The Journal of Physical Chemistry* **119**, 1689–1700.
- Kalescky R, Zhou H, Liu J and Tao P (2016) Rigid residue scan simulations systematically reveal residue entropic roles in protein allostery. *PLoS Computational Biology* **12**, e1004893.
- Kantrowitz ER and Lipscomb WN (1988) *Escherichia coli* aspartate transcarbamylase: The relation between structure and function. *Science* **241**, 669–674.
- Karnoub AE and Weinberg RA (2008) Ras oncogenes: split personalities. *Nature Reviews Molecular Cell Biology* **9**, 517–531.
- Kast P, Asif-Ullah M, Jiang N and Hilvert D (1996) Exploring the active site of chorismate mutase by combinatorial mutagenesis and selection: The importance of electrostatic catalysis. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 5043–5048.
- Kato-Yamada Y, Noji H, Yasuda R, Kinoshita Jr K and Yoshida M (1998) Direct observation of the rotation of ϵ subunit in F₁-ATPase. *The Journal of Biological Chemistry* **273**, 19375–19377.
- Keedy DA (2019) Journey to the center of the protein: Allostery from multi-temperature multiconformer X-ray crystallography. *Acta Crystallographica Section D: Biological Crystallography* **75**, 123–137.
- Keedy DA, Hill ZB, Biel JT, Kang E, Rettenmaier TJ, Brandañ-Neto J, Pearce NM, von Delft F, Wells JA and Fraser JS (2018) An expanded allosteric network in PTP1B by multitemperature crystallography, fragment screening, and covalent tethering. *eLife* **7**, e36307.
- Kenakin T and Christopoulos A (2013) Signalling bias in new drug discovery: Detection, quantification and therapeutic impact. *Nature Reviews Drug Discovery*, **12**, 205–216.
- Kendrew JC (1959) Three-dimensional structure of globular proteins. *Reviews of Modern Physics* **31**, 94–99.
- Kendrew JC, Bodo G, Dintzis HM, Parrish RG, Wyckoff H and Phillips DC (1958) A three-dimensional model of the myoglobin molecule obtained by X-ray analysis. *Nature* **181**, 662–666.
- Kendrew JC, Dickerson RE, Strandberg BE, Hart RG, Davies DR, Phillips DC and Shore VC (1960) Structure of myoglobin: A three-dimensional Fourier synthesis at 2 Å resolution. *Nature* **185**, 422–427.
- Kern D and Zuiderweg ERP (2003) The role of dynamics in allosteric regulation. *Current Opinion in Structural Biology* **13**, 748–757.
- Kim TH, Mehrabi P, Ren Z, Sljoka A, Ing C, Bezninov A, Ye L, Pomès R, Prosser RS and Pai EF (2017) The role of dimer asymmetry and protomer dynamics in enzyme catalysis. *Science* **355**, 262.
- Kim D, Herdeis L, Rudolph D, Zhao Y, Böttcher J, Vides A, Ayala-Santos CI, Pourfarjam Y, Cuevas-Navarro A, Xue JY, Mantoulidis A, Bröker J, Wunberg T, Schaaf O, Popow J, Wolkerstorfer B, Kropatsch KG, Qu R, de Stanchina E, Sang B, Li C, McConnell DB, Kraut N and Lito P (2023) Pan-KRAS inhibitor disables oncogenic signalling and tumour growth. *Nature* **619**, 160–166.
- Kleist AB, Jenjak S, Sente A, Laskowski LJ, Szpakowska M, Calkins MM, Anderson EI, McNally LM, Heukers R, Bobkov V, Peterson FC, Thomas MA, Chevigné A, Smit MJ, McCorvy JD, Babu MM and Volkman BF (2022) Conformational selection guides β -arrestin recruitment at a biased G protein-coupled receptor. *Science* **377**, 222–228.
- Knapp JE, Pahl R, Šrajcar V and Royer WE (2006) Allosteric action in real time: Time-resolved crystallographic studies of a cooperative dimeric hemoglobin. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 7649–7654.
- Kornev AP and Taylor SS (2015) Dynamics-driven allostery in protein kinases. *Trends in Biochemical Sciences* **40**, 628–647.

- Koshland Jr. DE (1959) Enzyme flexibility and enzyme action. *Journal of Cellular and Comparative Physiology* **54**, 245–258.
- Koshland Jr. DE, Némethy G and Filmer D (1966) Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry* **5**, 365–386.
- Kovacs E, Das R, Wang Q, Collier TS, Cantor A, Huang Y, Wong K, Mirza A, Barros T, Grob P, Jura N, Bose R and Kuriyan J (2015) Analysis of the role of the C-terminal tail in the regulation of the epidermal growth factor receptor. *Molecular and Cellular Biology* **35**, 3083–3102.
- Krishna R, Wang J, Ahern W, Sturmfels P, Venkatesh P, Kalvet I, Lee GR, Morey-Burrows FS, Anishchenko I, Humphreys IR, McHugh R, Vafeados D, Li X, Sutherland GA, Hitchcock A, Hunter CN, Kang A, Brackenbrough E, Bera AK, Baek M, DiMaio F and Baker D (2024) Generalized biomolecular modeling and design with RoseTTAFold All-Atom. *Science* **384**, ead12528.
- Krueger S (2022) Small-angle neutron scattering contrast variation studies of biological complexes: Challenges and triumphs. *Current Opinion in Structural Biology* **74**, 102375.
- Kuhar N, Sil S and Umopathy S (2021) Potential of Raman spectroscopic techniques to study proteins. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **258**, 119712.
- Kühlbrandt W (2014) The resolution revolution. *Science* **343**, 1443–1444.
- Kuriyan J and Eisenberg D (2007) The origin of protein interactions and allostery in colocalization. *Nature* **450**, 983–990.
- Lange OF, Lakomek N-A, Farès C, Schröder GF, Walter KFA, Becker S, Meiler J, Grubmüller H, Griesinger C and de Groot BL (2008) Recognition dynamics up to microseconds revealed from an RDC-derived ubiquitin ensemble in solution. *Science* **320**, 1471–1475.
- Larion M, Salinas RK, Bruschweiler-Li L, Miller BG and Brüschweiler R (2012) Order–disorder transitions govern kinetic cooperativity and allostery of monomeric human glucokinase. *PLoS Biology* **10**, e1001452.
- Lau WCY and Rubinstein JL (2012) Subnanometre-resolution structure of the intact *Thermus thermophilus* H⁺-driven ATP synthase. *Nature* **481**, 214–218.
- Lazim R, Suh D and Choi S (2020) Advances in molecular dynamics simulations and enhanced sampling methods for the study of protein systems. *International Journal of Molecular Sciences* **21**, 6339.
- Leroux AE and Biondi RM (2020) Renaissance of allostery to disrupt protein kinase interactions. *Trends in Biochemical Sciences* **45**, 27–41.
- Levinthal C (1969) How to fold graciously. *Mössbauer Spectroscopy in Biological Systems Proceedings* **67**, 22–24.
- Lewis M (2013) Allostery and the lac Operon. *Journal of Molecular Biology* **425**, 2309–2316.
- Lewis M, Chang G, Horton NC, Kercher MA, Pace HC, Schumacher MA, Brennan RG and Lu P (1996) Crystal structure of the lactose operon repressor and its complexes with DNA and inducer. *Science* **271**, 1247–1254.
- Lin Z, Akin H, Rao R, Hie B, Zhu Z, Lu W, Smetanin N, Verkuil R, Kabeli O, Shmueli Y, dos Santos Costa A, Fazel-Zarandi M, Sercu T, Candido S and Rives A (2023) Evolutionary-scale prediction of atomic-level protein structure with a language model. *Science* **379**, 1123–1130.
- Lin C-X, Guan Y and Li H-D (2024) Artificial intelligence approaches for molecular representation in drug response prediction. *Current Opinion in Structural Biology* **84**, 102747.
- Lin Z and Rye HS (2004) Expansion and compression of a protein folding intermediate by GroEL. *Molecular Cell* **16**, 23–34.
- Lipinski CA (2016) Rule of five in 2015 and beyond: Target and ligand structural limitations, ligand chemistry structure and drug discovery project decisions. *Advanced Drug Delivery Reviews* **101**, 34–41.
- Lipinski CA, Lombardo F, Dominy BW and Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* **46**, 3–26.
- Lisi GP and Loria JP (2016) Solution NMR spectroscopy for the study of enzyme allostery. *Chemical Reviews* **116**, 6323–6369.
- Liu J and Nussinov R (2008) Allosteric effects in the marginally stable von Hippel–Lindau tumor suppressor protein and allostery-based rescue mutant design. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 901–906.
- Liu JJ, Horst R, Katritch V, Stevens RC and Wüthrich K (2012) Biased signaling pathways in β_2 -adrenergic receptor characterized by ¹⁹F-NMR. *Science* **335**, 1106–1110.
- Liu X, Masoudi A, Kahsai AW, Huang L-Y, Pani B, Staus DP, Shim PJ, Hirata K, Simhal RK, Lefkowitz RJ and Kobilka B (2019) Mechanism of β_2 AR regulation by an intracellular positive allosteric modulator. *Science* **364**, 1283–1287.
- Liu X, Kaindl J, Korczynska M, Stöfel A, Dengler D, Stanek M, Hübner H, Clark MJ, Mahoney J, Matt RA, Xu X, Hirata K, Shoichet BK, Sunahara RK, Kobilka BK and Gmeiner P (2020) An allosteric modulator binds to a conformational hub in the β_2 adrenergic receptor. *Nature Chemical Biology* **16**, 749–755.
- Lockless SW and Ranganathan R (1999) Evolutionarily conserved pathways of energetic connectivity in protein families. *Science* **286**, 295–299.
- Lorimer GH, Fei X and Ye X (2018) The GroEL chaperonin: A protein machine with pistons driven by ATP binding and hydrolysis. *Philosophical Transactions B* **373**, 20170179.
- Lu S, Li S and Zhang J (2014) Harnessing allostery: A novel approach to drug discovery. *Medicinal Research Reviews* **34**, 1242–1285.
- Lu S, Jang H, Muratcioglu S, Gursoy A, Keskin O, Nussinov R and Zhang J (2016) Ras conformational ensembles, allostery, and signaling. *Chemical Reviews* **116**, 6607–6665.
- Lubbe L, Sewell BT, Woodward JD and Sturrock ED (2022) Cryo-EM reveals mechanisms of angiotensin I-converting enzyme allostery and dimerization. *EMBO Journal* **41**, e110550.
- Lynch EM, Hansen H, Salay L, Cooper M, Timr S, Kollman JM and Webb BA (2024) Structural basis for allosteric regulation of human phosphofructokinase-1. *Nature Communications* **15**, 7323.
- Lyu J, Kapolka N, Gumpfer R, Alon A, Wang L, Jain MK, Barros-Álvarez X, Sakamoto K, Kim Y, DiBerto J, Kim K, Glenn IS, Tummino TA, Huang S, Irwin JJ, Tarkhanova OO, Moroz Y, Skiniotis G, Kruse AC, Shoichet BK and Roth BL (2024) AlphaFold2 structures guide prospective ligand discovery. *Science* **384**, eadn6354.
- Ma B, Kumar S, Tsai C-J and Nussinov R (1999) Folding funnels and binding mechanisms. *Protein Engineering* **12**, 713–720.
- Macol CP, Tsuruta H, Stec B and Kantrowitz ER (2001) Direct structural evidence for a concerted allosteric transition in *Escherichia coli* aspartate transcarbamoylase. *Nature Structural & Molecular Biology* **8**, 423–426.
- Maeda R, Tamagaki-Asahina H, Sato T, Yanagawa M and Sako Y (2022) Threonine phosphorylation regulates the molecular assembly and signaling of EGFR in cooperation with membrane lipids. *Journal of Cell Science* **135**, jcs260355.
- Mahieu E and Gabel F (2018) Biological small-angle neutron scattering: recent results and development research papers. *Acta Crystallographica Section D: Structural Biology* **D74**, 715–726.
- Manley G and Loria JP (2012) NMR insights into protein allostery. *Archives of Biochemistry and Biophysics* **519**, 223–231.
- Martino AJ and Ferrone FA (1989) Rate of allosteric change in hemoglobin measured by modulated excitation using fluorescence detection. *Biophysical Journal* **56**, 781–794.
- Masson GR, Burke JE, Ahn NG, Anand GS, Borchers C, Brier S, Bou-Assaf GM, Engen JR, Englander SW, Faber J, Garlish R, Griffin PR, Gross ML, Guttman M, Hamuro Y, Heck AJR, Houde D, Jacob RE, Jørgensen TJD, Kaltashov IA, Klinman JP, Konermann L, Man P, Mayne L, Pascal BD, Reichmann D, Skehel M, Snijder J, Strutzenberg TS, Underbakke ES, Wagner C, Wales TE, Walters BT, Weis DD, Wilson DJ, Wintrode PL, Zhang Z, Zheng J, Schriemer DC and Rand KD (2019) Recommendations for performing, interpreting and reporting hydrogen deuterium exchange mass spectrometry (HDX-MS) experiments. *Nature Methods* **16**, 595–602.
- Masterson LR, Cheng C, Yu T, Tonelli M, Kornev A, Taylor SS and Veglia G (2010) Dynamics connect substrate recognition to catalysis in protein kinase A. *Nature Chemical Biology* **6**, 821–828.
- Mathy CJP and Kortemme T (2023) Emerging maps of allosteric regulation in cellular networks. *Current Opinion in Structural Biology* **80**, 102602.
- Mathy CJP, Mishra P, Flynn JM, Perica T, Mavor D, Bolon DNA and Kortemme T (2023) A complete allosteric map of a GTPase switch in its native cellular network. *Cell Systems* **14**, 237–246.

- McLeish TCB, Rodgers TL and Wilson MR (2013) Allostery without conformation change: Modelling protein dynamics at multiple scales. *Physical Biology* **10**, 1–9.
- Meena S and Saxena AK (2016) Crystal structure of *Escherichia coli* GroEL in substrate and ADP unloaded state. *Journal of Physical Chemistry & Biophysics* **6**, 1000222.
- Mehrabi P, Schulz EC, Dsouza R, Müller-Werkmeister HM, Tellkamp F, Miller RJD and Pai EF (2019) Time-resolved crystallography reveals allosteric communication aligned with molecular breathing. *Science* **365**, 1167–1170.
- Moffat K, Szebenyi D and Bilderback D (1984) X-ray Laue diffraction from protein crystals. *Science* **223**, 1423–1425.
- Monod J (1971) *CHANCE AND NECESSITY: An Essay on the Natural Philosophy of Modern Biology*. New York: Alfred A. Knopf.
- Monod J and Jacob F (1961) General conclusions: Teleonomic mechanisms in cellular metabolism, growth, and differentiation. *Cold Spring Harbor Symposium on Quantitative Biology* **26**, 389–401.
- Monod J, Changeux J-P and Jacob F (1963) Allosteric proteins and cellular control systems. *Journal of Molecular Biology* **6**, 306–329.
- Monod J, Wyman J and Changeux J-P (1965) On the nature of allosteric transitions: A plausible model. *Journal of Molecular Biology* **12**, 88–118.
- Moody AT, Narula J and Maurer TS (2024) Quantitative model-based assessment of multiple sickle cell disease therapeutic approaches alone and in combination. *Clinical Pharmacology & Therapeutics* **115**, 1114–1121.
- Moore JC and Arnold FH (1996) Directed evolution of a *para*-nitrobenzyl esterase aqueous-organic solvents. *Nature Biotechnology* **14**, 458–467.
- Morange M (2012) What history tells us XXVII. A new life for allostery. *Journal of Biosciences* **37**, 13–17.
- Morgan VE and Chichester DF (1935) Properties of the blood of the domestic fowl. *The Journal of Biological Chemistry* **110**, 285–298.
- Motlagh HN, Wrabl JO, Li J and Hilser VJ (2014) The ensemble nature of allostery. *Nature* **508**, 331–339.
- Munack S, Roderer K, Ökvist M, Kamaraukaitė J, Sasso S, van Eerde A, Kast P and Kregel U (2016) Remote control by inter-enzyme allostery: A novel paradigm for regulation of the shikimate pathway. *Journal of Molecular Biology* **428**, 1237–1255.
- Nerin-Fonz F and Cournia Z (2024) Machine learning approaches in predicting allosteric sites. *Current Opinion in Structural Biology* **85**, 102774.
- Neutze R, Wouts R, van der Spoel D and Weckert E (2000) Potential for biomolecular imaging with femtosecond X-ray pulses. *Nature* **406**, 752–757.
- Nguyen HH, Park J, Kang S and Kim M (2015) Surface plasmon resonance: A versatile technique for biosensor applications. *Sensors* **15**, 10481–10510.
- Ni D, Li Y, Qiu Y, Pu J, Lu S and Zhang J (2020) Combining allosteric and orthosteric drugs to overcome drug resistance. *Trends in Pharmacological Sciences* **41**, 336–348.
- Ni D, Chai Z, Wang Y, Li M, Yu Z, Liu Y, Lu S and Zhang J (2022a) Along the allostery stream: Recent advances in computational methods for allosteric drug discovery. *WIREs Computational Molecular Science* **12**, e1585.
- Ni D, Liu Y, Kong R, Yu Z, Lu S and Zhang J (2022b) Computational elucidation of allosteric communication in proteins for allosteric drug design. *Drug Discovery Today* **27**, 2226–2234.
- Nogales E (2018) Cryo-EM. *Current Biology* **28**, R1121–R1142.
- Nogales E and Scheres SHW (2015) Cryo-EM: A unique tool for the visualization of macromolecular complexity. *Molecular Cell* **58**, 677–689.
- Novick A and Szilard L (1954) II. Experiments with the chemostat on the rates of amino acid synthesis in bacteria. In Boell EJ (ed.), *Dynamics of Growth Processes*. Princeton, NJ: Princeton University Press, pp. 21–32.
- Nussinov R and Tsai C-J (2013) Allostery in disease and in drug discovery. *Cell* **153**, 293–305.
- Nussinov R and Tsai C-J (2015) Allostery without a conformational change? Revisiting the paradigm. *Current Opinion in Structural Biology* **30**, 17–24.
- Nussinov R, Tsai, C-J and Jang H (2022a) A new view of activating mutations in cancer. *Cancer Research* **82**, 4114–4123.
- Nussinov R, Zhang M, Maloney R, Liu Y, Tsai C-J and Jang H (2022b) Allostery: Allosteric cancer drivers and innovative allosteric drugs. *Journal of Molecular Biology* **434**, 167569.
- Oates J and Watts A (2011) Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. *Current Opinion in Structural Biology* **21**, 802–807.
- Oldfield CJ, Meng J, Yang JY, Yang MQ, Uversky VN and Dunker AK (2008) Flexible nets: Disorder and induced fit in the associations of p53 and 14-3-3 with their partners. *BMC Genomics* **9**, SI.
- Pai EF, Kabsch W, Kregel U, Holmes KC, John J and Wittinghofer A (1989) Structure of the guanine-nucleotide-binding domain of the Ha-ras oncogene product p21 in the triphosphate conformation. *Nature* **341**, 209–214.
- Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M and Miyano M (2000) Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* **289**, 739–745.
- Pan Y and Mader MM (2022) Principles of kinase allosteric inhibition and pocket validation. *Journal of Medicinal Chemistry* **65**, 5288–5299.
- Pauling L and Zuckerkandl E (1963) Chemical paleogenetics: Molecular “restoration studies” of extinct forms of life. *Acta Chemica Scandinavica* **17**, S9–S16.
- Peacock RB and Komives EA (2021) Hydrogen/deuterium exchange and nuclear magnetic resonance spectroscopy reveal dynamic allostery on multiple time scales in the serine protease thrombin. *Biochemistry* **60**, 3441–3448.
- Perutz MF (1970) Stereochemistry of cooperative effects in haemoglobin: Haem-haem interaction and the problem of allostery. *Nature* **228**, 726–734.
- Perutz MF (1989) Mechanisms of cooperativity and allosteric regulation in proteins. *Quarterly Reviews of Biophysics* **22**, 139–237.
- Perutz MF, Rossmann MG, Cullis AF, Muirhead H, Will G and North ACT (1960) Structure of hemoglobin: A three-dimensional Fourier synthesis at 5.5-Å resolution, obtained by X-ray analysis. *Nature* **185**, 416–422.
- Perutz MF, Muirhead H, Cox JM and Goaman LCG (1968) Three-dimensional Fourier synthesis of horse oxyhaemoglobin at 2.8 Å resolution: The atomic model. *Nature* **219**, 131–139.
- Perutz MF, Wilkinson AJ, Paoli M and Dodson GG (1998) The stereochemical mechanism of the cooperative effects in hemoglobin revisited. *Annual Review of Biophysics and Biomolecular Structure* **27**, 1–34.
- Pervushin K, Riek R, Wider G and Wüthrich K (1997) Attenuated T_2 relaxation by mutual cancellation of dipole-dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules in solution. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 12366–12371.
- Peterson AW, Cockrell GM and Kantrowitz ER (2013) A second allosteric site in *E. coli* aspartate transcarbamoylase. *Biochemistry* **51**, 4776–4778.
- Petsko GA and Ringe D (2004) *Protein Structure and Function*. London: New Science Press.
- Pillai AS, Chandler SA, Liu Y, Signore AV, Cortez-Romero CR, Benesch JLP, Laganowsky A, Storz JF, Hochberg GKA and Thornton JW (2020) Origin of complexity in haemoglobin evolution. *Nature* **581**, 480–485.
- Pillai A, Idris A, Philomin A, Weidle C, Skotheim R, Leung PJY, Broerman A, Demakis C, Borst AJ, Praetorius F and Baker D. (2024) De novo design of allosterically switchable protein assemblies. *Nature* **632**, 911–920.
- Pincus D, Resnekov O and Reynolds KA (2017) An evolution-based strategy for engineering allosteric regulation. *Physical Biology* **14**, 025002.
- Popovych N, Sun S, Ebright RH and Kalodimos CG (2006) Dynamically driven protein allostery. *Nature Structural & Molecular Biology* **13**, 831–838.
- Prinz H (2010) Hill coefficients, dose-response curves and allosteric mechanisms. *Journal of Chemical Biology* **3**, 37–44.
- Punjani A and Fleet DJ (2023) 3DFlex: Determining structure and motion of flexible proteins from cryo-EM. *Nature Methods* **20**, 860–870.
- Rasmussen SGF, Choi H-J, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VRP, Sanishvili R, Fischetti RF, Schertler GFX, Weis WI and Kobilka BK (2007) Crystal structure of the human β_2 adrenergic G-protein-coupled receptor. *Nature* **450**, 383–388.
- Rasmussen SGF, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, Thian FS, Chae PS, Pardon E, Calinski D, Mathiesen JM, Shah STA, Lyons JA, Caffrey M, Gellman SH, Steyaert J, Skiniotis G, Weis WI, Sunahara RK and Kobilka BK (2011a) Crystal structure of the β_2 adrenergic receptor-Gs protein complex. *Nature* **477**, 549–555.
- Rasmussen SGF, Choi H-J, Fung JJ, Pardon E, Casarosa P, Chae PS, DeVree BT, Rosenbaum DM, Thian FS, Kobilka TS, Schnapp A, Konetzi I, Sunahara RK, Gellman SH, Pautsch A, Steyaert J, Weis WI and Kobilka BK (2011b) Structure of a nanobody-stabilized active state of the β_2 adrenoceptor. *Nature* **469**, 175–180.

- Rastogi VK and Girvin ME** (1999) Structural changes linked to proton translocation by subunit *c* of the ATP synthase. *Nature* **402**, 263–268.
- Romanuka J, Folkers GE, Gnida M, Kovačić L, Wienk H, Kaptein R and Boelens R** (2023) Genetic switching by the Lac repressor is based on two-state Monod–Wyman–Changeux allostery. *Proceedings of the National Academy of Sciences of the United States of America* **120**, e2311240120.
- Rupp B** (2009) *Biomolecular Crystallography: Principles, Practice, and Application to Structural Biology*. New York: Garland Science.
- Sadler F, Ma N, Ritt M, Sharma Y, Vaidehi N and Sivaramakrishnan S** (2023) Autoregulation of GPCR signalling through the third intracellular loop. *Nature* **615**, 734–741.
- Saibil HR, Fenton WA, Clare DK and Horwich AL** (2013) Structure and allostery of the chaperonin GroEL. *Journal of Molecular Biology* **425**, 1476–1487.
- Sapienza PJ, Bonin JP, Jinasena HPD, Li K, Dieckhaus H, Popov KI, Aubé J and Lee AL** (2023) Mixed, nonclassical behavior in a classic allosteric protein. *Proceedings of the National Academy of Sciences of the United States of America* **120**, e2308338120.
- Sasso S, Ökvist M, Roderer K, Gamper M, Codoni G, Krengel U and Kast P** (2009) Structure and function of a complex between chorismate mutase and DAHP synthase: efficiency boost for the junior partner. *EMBO Journal*, **28**, 2128–2142.
- Schirmer T and Evans PR** (1990) Structural basis of the allosteric behaviour of phosphofructokinase. *Nature* **343**, 140–145.
- Schlichting I, Almoh SC, Rapp G, Wilson K, Petratros K, Lentfer A, Wittnghofer A, Kabsch W, Pai EF, Petsko GA and Goody RS** (1990) Time-resolved X-ray crystallographic study of the conformational change in Ha-Ras p21 protein on GTP hydrolysis. *Nature* **345**, 309–315.
- Schlichting I and Miao J** (2012) Emerging opportunities in structural biology with X-ray free-electron lasers. *Current Opinion in Structural Biology* **22**, 613–626.
- Schneider J, Korshunova K, Musiani F, Alfonso-prieto M, Giorgetti A and Carloni P** (2018) Predicting ligand binding poses for low-resolution membrane protein models: Perspectives from multiscale simulations. *Biochemical and Biophysical Research Communications* **498**, 366–374.
- Schotte F, Soman J, Olson JS, Wulff M and Anfinsen PA** (2004) Picosecond time-resolved X-ray crystallography: probing protein function in real time. *Journal of Structural Biology* **147**, 235–246.
- Schueler-Furman O and Wodak SJ** (2016) Computational approaches to investigating allostery. *Current Opinion in Structural Biology* **41**, 159–171.
- Schultz CH** (1836) Ref 132 as cited in: Hünefeld FL (1840) *Der Chemismus in der therischen Organisation*. Leipzig: FA Brockhaus.
- Scott DE, Spry C and Abell C** (2016) Differential scanning fluorimetry as part of a biophysical screening cascade. In Erlanson DA and Jahnke W (eds.), *Fragment-Based Drug Discovery: Lessons and Outlook* [Series: Methods and Principles of Medicinal Chemistry]. Weinheim: Wiley-VCH, pp. 139–172.
- Sharma S, Luo M, Patel H, Mueller DM and Liao M** (2024) Conformational ensemble of yeast ATP synthase at low pH reveals unique intermediates and plasticity in F_1 – F_o coupling. *Nature Structural & Molecular Biology* **31**, 657–666.
- Silva MM, Rogers PH and Arnone A** (1992) A third quaternary structure of human hemoglobin A at 1.7-Å resolution. *The Journal of Biological Chemistry* **267**, 17248–17256.
- Silva D-A, Bowman GR, Sosa-Peinado A and Huang X** (2011) A role for both conformational selection and induced fit in ligand binding by the LAO protein. *PLoS Computational Biology* **7**, e1002054.
- Śledź P and Caffisch A** (2018) Protein structure-based drug design: from docking to molecular dynamics. *Current Opinion in Structural Biology* **48**, 93–102.
- Smith NJ and Milligan G** (2010) Allostery at G protein-coupled receptor homo- and heteromers: Uncharted pharmacological landscapes. *Pharmacological Reviews* **62**, 701–725.
- Song X, Bao L, Feng C, Huang Q, Zhang F, Gao X and Han R** (2024) Accurate prediction of protein structural flexibility by deep learning integrating intricate atomic structures and cryo-EM density information. *Nature Communications* **15**, 5538.
- Sprang SR** (2007) A receptor unlocked. *Nature* **450**, 355–356.
- Stewart AG, Laming EM, Sobti M and Stock D** (2014) Rotary ATPases — dynamic molecular machines. *Current Opinion in Structural Biology* **25**, 40–48.
- Stock D, Gibbons C, Arechaga I, Leslie AGW and Walker JE** (2000) The rotary mechanism of ATP synthase. *Current Opinion in Structural Biology* **10**, 672–679.
- Strauss M, Hofhaus G, Schröder RR and Kühlbrandt W** (2008) Dimer ribbons of ATP synthase shape the inner mitochondrial membrane. *EMBO Journal* **27**, 1154–1160.
- Süel GM, Lockless SW, Wall MA and Ranganathan R** (2003) Evolutionarily conserved networks of residues mediate allosteric communication in proteins. *Nature Structural Biology* **10**, 59–69.
- Sultana A and Lee JE** (2015) Measuring protein–protein and protein–nucleic acid interactions by bilayer interferometry. *Current Protocols in Protein Science* **79**, 19.25.1–19.25.26.
- Sun K, D'Alessandro A, Ahmed MH, Zhang Y, Song A, Ko T-P, Nemkov T, Reisz JA, Wu H, Adebisi M, Peng Z, Gong J, Liu H, Huang A, Wen YE, Wen AQ, Berka V, Bogdanov MV, Abdulmalik O, Han L, Tsai A-I, Idowu M, Juneja HS, Kellems RE, Dowhan W, Hansen KC, Safo MK and Xia Y** (2017) Structural and functional insight of sphingosine 1-phosphate-mediated pathogenic metabolic reprogramming in sickle cell disease. *Scientific Reports* **7**, 15281.
- Suomivuori C-M, Latorraca NR, Wingler LM, Eismann S, King MC, Kleinhenz ALW, Skiba MA, Staus DP, Kruse AC, Lefkowitz RJ and Dror RO** (2020) Molecular mechanism of biased signaling in a prototypical G protein-coupled receptor. *Science* **367**, 881–887.
- Tan YZ and Carragher B** (2020) Seeing atoms: Single-particle cryo-EM breaks the atomic barrier. *Molecular Cell* **80**, 938–939.
- Tan ZW, Tee W-V and Berezovsky IN** (2022) Learning about allosteric drugs and ways to design them. *Journal of Molecular Biology* **434**, 167692.
- Taylor ND, Garruss AS, Moretti R, Chan S, Arbing MA, Cascio D, Rogers JK, Isaacs FJ, Kosuri S, Baker D, Fields S, Church GM and Raman S** (2016) Engineering an allosteric transcription factor to respond to new ligands. *Nature* **13**, 177–183.
- Tee W-V and Berezovsky IN** (2024) Allosteric drugs: New principles and design approaches. *Current Opinion in Structural Biology* **84**, 102758.
- Tee W-V, Guarnera E and Berezovsky IN** (2018) Reversing allosteric communication: From detecting allosteric sites to inducing and tuning targeted allosteric response. *PLoS Computational Biology* **14**, e1006228.
- Thal DM, Glukhova A, Sexton PM and Christopoulos A** (2018) Structural insights into G-protein-coupled receptor allostery. *Nature* **559**, 45–53.
- Theorell H** (1934) Kristallinisches Myoglobin. V. Mitteilung. Die Sauerstoffbindungskurve des Myoglobins. *Biochemische Zeitschrift* **268**, 73–82.
- Thirumalai D and Hyeon C** (2018) Signalling networks and dynamics of allosteric transitions in bacterial chaperonin GroEL: implications for iterative annealing of misfolded proteins. *Philosophical Transactions B* **373**, 20170182.
- Torino S, Dhurandhar M, Stroobants A, Claessens R and Efremov RG** (2023). *Nature Methods* **20**, 1400–1408.
- Tsai C-J, Ma B and Nussinov R** (1999) Folding and binding cascades: Shifts in energy landscapes. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 9970–9972.
- Tsai C-J, del Sol A and Nussinov R** (2008) Allostery: Absence of a change in shape does not imply that allostery is not at play. *Journal of Molecular Biology* **378**, 1–11.
- Tsai C-J and Nussinov R** (2014) A unified view of 'How allostery works'. *PLoS Computational Biology* **10**, e1003394.
- Tsuchiya Y, Taneishi K and Yonezawa Y** (2019) Autoencoder-based detection of dynamic allostery triggered by ligand binding based on molecular dynamics. *Journal of Chemical Information and Modeling* **59**, 4043–4051.
- Tugarinov V, Kanelis V and Kay LE** (2006) Isotope labeling strategies for the study of high-molecular-weight proteins by solution NMR spectroscopy. *Nature Protocols* **1**, 749–754.
- Tumbic GW, Hossan Y and Thielges MC** (2021) Protein dynamics by two-dimensional infrared spectroscopy. *Annual Review of Analytical Chemistry* **14**, 299–321.
- Tymoczko JL, Berg JM, Gatto GJ and Stryer L** (2018) *Biochemistry: A Short Course*. New York: Macmillan.
- Tzeng S-R and Kalodimos CG** (2009) Dynamic activation of an allosteric regulatory protein. *Nature* **462**, 368–372.
- Tzeng S-R and Kalodimos CG** (2011) Protein dynamics and allostery: an NMR view. *Current Opinion in Structural Biology* **21**, 62–67.
- Tzeng S-R and Kalodimos CG** (2012) Protein activity regulation by conformational entropy. *Nature* **448**, 236–240.

- Udi Y, Fragai M, Grossman M, Mitternacht S, Arad-Yellin R, Calderone V, Melikian M, Toccafondi M, Berezovsky IN, Luchinat C and Sagi I (2013) Unraveling hidden regulatory sites in structurally homologous metalloproteases. *Journal of Molecular Biology* **425**, 2330–2346.
- Umbarger HE (1956) Evidence for negative-feedback mechanism in the biosynthesis of isoleucine. *Science* **123**, 848.
- Umbarger HE and Brown B (1958) Isoleucine and valine metabolism in *Escherichia coli*. VII. A NEGATIVE FEEDBACK MECHANISM CONTROLLING ISOLEUCINE BIOSYNTHESIS. *The Journal of Biological Chemistry* **233**, 415–420.
- Uversky VN and Dunker AK (2010) Understanding protein non-folding. *Biochimica et Biophysica Acta – Proteins and Proteomics* **1804**, 1231–1264.
- Vani BP, Aranganathan A, Wang D and Tiwary P (2023) AlphaFold2-RAVE: From sequence to Boltzmann ranking. *Journal of Chemical Theory and Computation* **19**, 4351–4354.
- Venditti V, Egner TK and Clore GM (2016) Hybrid approaches to structural characterization of conformational ensembles of complex macromolecular systems combining NMR residual dipolar couplings and solution X-ray scattering. *Chemical Reviews* **116**, 6306–6322.
- Verkhivker G, Alshahrani M, Gupta G, Xiao S and Tao P (2023) From deep mutational mapping of allosteric protein landscapes to deep learning of allostery and hidden allosteric sites: Zooming in on “allosteric intersection” of biochemical and big data approaches. *International Journal of Molecular Sciences* **24**, 7747.
- Vögeli B, Kazemi S, Güntert P and Riek R (2012) Spatial elucidation of motion in proteins by ensemble-based structure calculation using exact NOEs. *Nature Structural & Molecular Biology* **19**, 1053–1058.
- Vojtěchovský J, Chu K, Berendzen J, Sweet RM and Schlichting I (1999) Crystal structures of myoglobin-ligand complexes at near-atomic resolution. *Biophysical Journal* **77**, 2153–2174.
- de Vries RMJM, Meijer FA, Doveston RG, Leijten-van de Gevel IA and Brunsveld L (2020) Cooperativity between the orthosteric and allosteric ligand binding sites of ROR γ t. *Proceedings of the National Academy of Sciences of the United States of America* **118**, e2021287118.
- Wagner JR, Lee CT, Durrant JD, Malmstrom RD, Feher VA and Amaro RE (2016) Emerging computational methods for the rational discovery of allosteric drugs. *Chemical Reviews* **116**, 6370–6390.
- Walker JE (1998) ATP synthesis by rotary catalysis (Nobel lecture). *Angewandte Chemie International Edition* **37**, 2308–2319.
- Wang Y, Yu Z, Xiao W, Lu S and Zhang J (2021) Allosteric binding sites at the receptor–lipid bilayer interface: novel targets for GPCR drug discovery. *Drug Discovery Today* **26**, 690–703.
- Ward JJ, Sodhi JS, McGuffin LJ, Buxton BF and Jones DT (2004) Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. *Journal of Molecular Biology* **337**, 635–645.
- Wei G, Xi W, Nussinov R and Ma B (2016) Protein ensembles: How does nature harness thermodynamic fluctuations for life? The diverse functional roles of conformational ensembles in the cell. *Chemical Reviews* **116**, 6516–6551.
- Weng C, Faure AJ, Escobedo A and Lehner B (2024) The energetic and allosteric landscape for KRAS inhibition. *Nature* **626**, 643–652.
- Whittington AC, Larion M, Bowler JM, Ramsey KM, Brüschweiler R and Miller BG (2015) Dual allosteric activation mechanisms in monomeric human glucokinase. *Proceedings of the National Academy of Sciences of the United States of America* **112**, 11553–11558.
- Wingler LM, Skiba MA, McMahon C, Staus DP, Kleinhenz ALW, Suominen C-M, Latorraca NR, Dror RO, Lefkowitz RJ and Kruse AC (2020) Angiotensin and biased analogs induce structurally distinct active conformations within a GPCR. *Science* **367**, 888–892.
- Winkler WC and Dann III CE (2006) RNA allostery glimpsed. *Nature Structural & Molecular Biology* **13**, 569–571.
- Wright PE and Dyson HJ (1999) Intrinsically unstructured proteins: Re-assessing the protein structure–function paradigm. *Journal of Molecular Biology* **293**, 321–331.
- Wu N, Yaliraki SN and Barahona M (2022) Prediction of protein allosteric signalling pathways and functional residues through paths of optimised propensity. *Journal of Molecular Biology* **434**, 167749.
- Wylie AA, Schoepfer J, Jahnke W, Cowan-Jacob SW, Loo A, Furet P, Marzinzik AL, Pelle X, Donovan J, Zhu W, Buonamici S, Hassan AQ, Lombardo F, Iyer V, Palmer M, Berellini G, Dodd S, Thohan S, Bitter H, Branford S, Ross DM, Hughes TP, Petruzzelli L, Vanasse KG, Warmuth M, Hofmann F, Keen NJ and Sellers WR (2017) The allosteric inhibitor ABL001 enables dual targeting of BCR–ABL1. *Nature* **543**, 733–737.
- Xiao S, Verkhivker GM and Tao P (2023) Machine learning and protein allostery. *Trends in Biochemical Sciences* **48**, 375–390.
- Xu Y and Matthews S (2013) TROSY NMR spectroscopy of large soluble proteins. In Heise H and Matthews S (eds.), *Modern NMR Methodology*. Topics in Current Chemistry, Vol. **335**. Berlin–Heidelberg: Springer, pp. 97–120.
- Xu Z, Horwich AL and Sigler PB (1997) The crystal structure of the asymmetric GroEL–GroES–(ADP) $_7$ chaperonin complex. *Nature* **388**, 741–750.
- Xu Y, Wang S, Hu Q, Gao S, Ma X, Zhang W, Shen Y, Chen F, Lai L and Pei J (2018) CavityPlus: A web server for protein cavity detection with pharmacophore modelling, allosteric site identification and covalent ligand binding ability prediction. *Nucleic Acids Research* **46**, W374–W379.
- Yan X, Shi Q, Bracher A, Miličić, G, Singh AK, Hartl FU and Hayer-Hartl M (2018) GroEL ring separation and exchange in the chaperonin reaction. *Cell* **172**, 605–617.
- Yang F, Bian C, Zhu L, Zhao G, Huang Z and Huang M (2007) Effect of human serum albumin on drug metabolism: Structural evidence of esterase activity of human serum albumin. *Journal of Structural Biology* **157**, 348–355.
- Yang KK, Wu Z and Arnold FH (2019) Machine-learning-guided directed evolution for protein engineering. *Nature Methods* **16**, 687–694.
- Yates RA and Pardee AB (1956) Control of pyrimidine biosynthesis in *Escherichia coli* by feedback mechanism. *The Journal of Biological Chemistry* **221**, 757–770.
- Yifrach O and Horowitz A (1995) Nested cooperativity in the ATPase activity of the oligomeric chaperonin GroEL. *Biochemistry* **34**, 5303–5308.
- Zalatan JG, Coyle SM, Rajan S, Sidhu SS and Lim WA (2012) Conformational control of the Ste5 scaffold protein insulates against MAP kinase misactivation. *Science* **337**, 1218–1222.
- Zeymer C and Hilvert D (2018) Directed evolution of protein catalysts. *Annual Review of Biochemistry* **87**, 131–157.
- Zhang X, Gureasko J, Shen K, Cole PA and Kuriyan J (2006) An allosteric mechanism for activation of the kinase domain of epidermal growth factor receptor. *Cell* **125**, 1137–1149.
- Zhang S, Zou S, Yin D, Zhao L, Finley D, Wu Z and Mao Y (2022) USP14-regulated allostery of the human proteasome by time-resolved cryo-EM. *Nature* **605**, 567–574.
- Zhang L, Mobbs JI, May LT, Glukhova A and Thal DM (2023) The impact of cryo-EM on determining allosteric modulator-bound structures of G protein-coupled receptors. *Current Opinion in Structural Biology* **79**, 102560.
- Zheng W, Brooks BR and Thirumalai D (2007) Allosteric transitions in the chaperonin GroEL are captured by a dominant normal mode that is most robust to sequence variations. *Biophysical Journal* **93**, 2289–2299.
- Zhou Q, Yang D, Wu M, Guo Y, Guo W, Zhong L, Cai X, Dai A, Jang W, Shakhnovich EI, Liu Z-J, Stevens RC, Lambert NA, Babu MM, Wang M-W and Zhao S (2019) Common activation mechanism of class A GPCRs. *eLife* **8**, e50279.