

# Understanding allosteric and cooperative interactions in enzymes

Athel Cornish-Bowden

Unité de Bioénergétique et Ingénierie des Protéines, Institut de Microbiologie de la Méditerranée, Centre National de la Recherche Scientifique and Aix-Marseille Université, France

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## Correspondence

A. Cornish-Bowden, Unité de Bioénergétique et Ingénierie des Protéines, Institut de Microbiologie de la Méditerranée, Centre National de la Recherche Scientifique, Aix-Marseille Université, 31 chemin Joseph-Aiguier, 13402 Marseille Cedex 20, France  
Fax: +33 491 16 40 97  
Tel: +33 491 16 41 38  
E-mail: acornish@imm.cnrs.fr

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The paper that introduced biochemists to the idea of allosteric feedback inhibition [Monod J, Changeux J-P & Jacob F (1963) *J Mol Biol* **6**, 306–329] is now 50 years old, and the two papers on models for enzyme cooperativity that followed it [Monod J, Wyman J & Changeux J-P (1965) *J Mol Biol* **12**, 88–118; Koshland DE, Némethy G & Filmer D (1966) *Biochemistry* **5**, 365–385] are almost as old. All of these papers continue to be heavily cited today – more in the 21st century than they were in the last two decades of the 20th. This is because they continue to be central for understanding enzyme regulation, and increasingly important in the age of systems biology.

## Introduction

The paper that popularized the idea of allosteric feedback inhibition is now 50 years old [1], although the concept [2] and the word ‘allosteric’ had appeared a little earlier, in the latter case only in the printed record [3], not spoken at the symposium itself. Its importance is that it explained some puzzling observations that had been accumulating. General ideas of enzyme inhibition had been known for many years, starting with Henri’s observations on invertase [4,5] and developed by Michaelis and his co-workers [6–8]. However, the older observations seemed to require the inhibitor to be structurally similar to one or more of the reactants, so that it could directly interact with the active site of the enzyme. The discovery of feedback inhibition [9,10] showed that inhibitors could have physiological roles independent of any structural

similarity with the molecules involved in the catalysed reaction. For example, phosphoribosyl-ATP pyrophosphorylase catalyses the first committed step in the biosynthesis of histidine, and is inhibited by histidine, which is structurally quite different from the substrates (phosphoribosyl-ATP and water) and products (phosphoribosyl-AMP and pyrophosphate) of the reaction. Moreover, mild treatment of the enzyme with  $\text{Hg}^{2+}$  ions destroys the sensitivity of the catalytic activity to histidine but affects neither the uninhibited activity nor the binding of histidine [11]. Many regulatory enzymes also display allosteric *activation*, and a single effector often acts in opposite directions on opposing reactions: for example, in many organisms AMP is an allosteric inhibitor of fructose bisphosphatase and an allosteric activator of phosphofructokinase.

Observations of this kind were difficult to explain in terms of classical ideas of enzyme catalysis. The first satisfactory explanation came from the concept of an *allosteric site* (ἄλλος στερεός: other solid), whereby a regulatory molecule could bind to an enzyme at a specific site clearly distinct from the catalytic site. The existence of such sites implied that changes at one site could affect behaviour at another that was not necessarily adjacent, and that in turn was most easily explained in terms of protein flexibility. If a protein molecule were conformationally rigid, an assumption that might not have been explicitly made in earlier discussions of protein structure but was usually implied, then it was not obvious how information could be passed from one site to another unless they were very close to one another. Thus to explain the cooperative binding of oxygen to haemoglobin, Pauling [12] argued that the binding sites needed to be close enough to one another to interact electronically: 'in hemoglobin four hemes form a conjugated system'.

Pauling's interpretation proved to be untenable when the X-ray crystal structure [13] showed the oxygen-binding sites to be too far apart, the authors saying that 'the four haem groups are actually wide apart (40 Å) excluding any possibility of direct interaction' [14]. As early as 1951 Wyman and Allen [15] had suggested that conformational changes could account for long-range effects in haemoglobin. This idea was taken up in the allosteric model of cooperativity [14], to be discussed shortly; conformational mobility as an explanation of various enzyme properties received a major stimulus from the theory of induced fit [16,17]. Koshland originally proposed this to explain puzzling aspects of enzyme specificity – for example, why is water a very poor substrate for yeast hexokinase, despite being more reactive than glucose and easily able to reach the active site? At that time he was not primarily interested in enzyme regulation, and in his later account of the origins of the theory [18] he did not mention it:

One of the great lessons of my life derived from the publication of the induced-fit theory. I was supposed to give a talk at a well-known symposium on my oxygen 18 (O<sup>18</sup>) work, but decided to speak instead about new research on the specificity of proteins. As I was preparing the talk I was going through the classical explanation for the manner in which substrates are excluded from active sites and decided the 'key-lock' or 'template' theory of Emil Fischer [19] was too simple. The theory provided no explanation for the low enzymatic reactivity of water...

Although we did many experiments that in my opinion could only be explained by the induced-fit theory, gaining acceptance for the theory was still an uphill fight. One referee wrote, 'The Fischer Key-Lock theory has lasted 100 years and will not be overturned by speculation from an embryonic scientist'. [Reference added]

Induced fit had the broader implication that protein flexibility could have a physiological function, however, so it was more than just a side effect of the forces that maintain protein structure. Its importance went beyond specificity alone, therefore, as was already recognized in the original account of allosteric interactions [3].

It was understood very soon that these ideas were not restricted to soluble regulatory enzymes and that they could also be applied to membrane proteins, and specifically those that mediate synaptic transmission [20–22]. In the subsequent half-century this has become a major field of research on its own, central to the development of neurochemistry. The systems studied include, for example, nicotinic acetylcholine receptors [23], glutamate receptors [24], G-protein-coupled receptors [25,26] and many others.

Conformational regulation then became a central feature of the *allosteric model* of cooperativity. The two essential features that distinguish it from other models of cooperativity are first that an equilibrium exists between two conformational states, usually known as the R ('relaxed') and T ('tense') states, in the absence of any ligand, and second that symmetry is maintained, so that all of the subunits in an oligomeric protein change between the R and T states in a concerted manner. Different ligands bind preferentially to one of the two states, and thus perturb the equilibrium between them. Some authors [27–29] have preferred to call it the *symmetry model* or *concerted model*, but here I shall follow the term used by the original authors [14].

The *sequential model*, the principal alternative, appeared a little later [27] and did not require conformational symmetry but did require a strict application of induced fit, with one conformation existing only when ligand is bound and another existing only when ligand is not bound. Many authors [29–32] have noted that both models can be regarded as special cases of more general models in which neither conformational symmetry nor strict induced fit is maintained. Although these generalizations are clearly correct, they have not proved to be very useful, and most experimentalists have preferred to interpret their data in terms of one of the original models, nowadays almost always the allosteric model. To this day they constitute

the foundation on which nearly all attempts to understand cooperative interactions in enzymes are built. Nonetheless, Cui and Karplus [32] point out that

Both the MWC [Monod–Wyman–Changeux model] and the Pauling-KNF [Pauling/Koshland–Némethy–Filmer] models are phenomenological, and so do not answer the fundamental question of how the binding of a ligand or its modification yield the observed allosteric effect at an atomic level of detail. Present day applications of computational methods to biomolecular systems, combined with structural, thermodynamic, and kinetic studies, make possible an approach to that question, so as to provide a deeper understanding of the requirements for allostery.

Any such deeper understanding will surely involve models in which induced fit or conformational symmetry are not assumed but follow automatically from the mechanism. Recently, Dyachenko and co-workers [32a] have described how mass spectrometry can be used to determine the amounts of all the different species that occur as a ligand binds to a protein, and have thus made an important step in the direction foreseen by Cui and Karplus. [This sentence and reference 32a added after original online publication].

New scientific ideas rarely spring from nowhere, and the term ‘Pauling-KNF model’ used in the quotation (with similar terms used by other authors) is a reminder that the sequential model [27] depended on algebra similar to Pauling’s [12], though it was based on a fundamentally different view of the mechanism in which binding sites interact at a distance through conformational changes. In a recent discussion of the history of allostery the philosopher Michel Morange [33] pointed out that

The allosteric model was not so different from the model proposed by John Yudkin as early as 1938 to explain the phenomenon of enzymatic adaptation, a model favoured by Monod when he initiated the study of this phenomenon: a protein precursor is able to adopt, in the presence of different ligands, multiple conformations with different enzymatic activities [34]. [*Reference number edited*]

Two other explanations of cooperativity are sometimes needed. It may happen that an enzyme changes its state of aggregation when a ligand binds. For example, glutamate dehydrogenase from cattle liver undergoes a change from oligomer to monomer when ligands such as GTP bind to it [35], and this can generate cooperativity in a manner analogous to that for the allosteric model. Tomkins and Yielding had

already argued that this could be a general explanation of cooperativity [36], and this possibility was later analysed by two groups [37,38]. Any enzyme for which such an effect is proposed should also show a dependence of cooperativity on the enzyme concentration: this was observed for glutamate dehydrogenase but not for others such as threonine deaminase [39], so it could not be a universal explanation of cooperativity. In practice, therefore, it is evident when such a model needs to be considered, as it will be known whether different states of oligomerization exist, as for example  $\text{NAD}^+$ -specific isocitrate dehydrogenase from yeast, which was recently reviewed [40].

All of these explanations of cooperativity attribute it to interactions between subunits in oligomeric enzymes. They cannot therefore explain cooperativity in monomeric enzymes, but as no clear examples of this were known until some years afterwards that was not considered to be a problem, and two mechanisms that would allow cooperative kinetics (but not cooperative binding at equilibrium) [41,42] were not initially thought to be necessary for explaining any real cases. (Rabin’s stated objective [42], in fact, was not so much to analyse a particular example as to point out that kinetic cooperativity could arise without the need to consider quaternary structure.) However, rat liver hexokinase D (‘glucokinase’) proved to display cooperativity with respect to its substrate glucose [43–45], as recently reviewed [46,47], so the various models that had been proposed to explain cooperativity in monomeric enzymes needed to be examined [48]. Allosteric interactions as such, of course, do not require subunit interactions and can therefore regulate the activity of monomeric enzymes, such as trypsin-like proteinases [49] and calpain [50].

Not all mechanisms for regulating enzyme activity fall into any of the classes so far mentioned, and the most important one involving neither allosteric nor cooperative interactions is regulation by covalent modification, typically phosphorylation and dephosphorylation. In a recent review of this subject, starting from the discovery of the phosphorylation of glycogen phosphorylase [51], Fischer [52] wrote about the atmosphere at the end of the 1960s that

The question that seemed of concern to most everyone was whether the regulation of a particular system followed the ‘allosteric’ model of Monod [14] or the ‘induced-fit’ model of Koshland [27]. And it was perfectly clear to me, for instance, that Jacques [Monod], who was a very close friend, never believed one minute that covalent regulation by protein phosphorylation could play any fundamental role in enzyme regulation. [*Reference numbers edited*]

Such scepticism was probably justified at the time, but it is now known from many examples that covalent-modification mechanisms are fundamental in metabolic regulation. Action of an allosteric modifier on an enzyme that catalyses a reaction of a covalent-modification system allows a very high degree of sensitivity: classical regulatory enzymes almost never have Hill coefficients greater than 4, but systems of interconvertible enzymes can have the equivalent of much higher Hill coefficients, up to 30 with tight constraints on the possible rate constants and much higher if wider ranges of rate constants are allowed [53–55]. The price to be paid for this increased sensitivity, however, is that they need to consume ATP in order to function, because the interconversion reactions need to be irreversible in both directions, and hence they must have different co-substrates. It is not necessary, of course, for one single model to account for the whole of any regulatory effect: for example, phenylalanine hydroxylase is regulated both by phosphorylation and by allosteric effects of phenylalanine and tetrahydrobiopterin [56,57].

Returning to the two principal models of cooperativity, it is clear that the allosteric model is now the preferred model for most biochemists, and most of the papers today that mention the sequential model mention the allosteric model as well, as illustrated in Fig. 1, and they often include a statement similar to the following [58]:

The kinetics and regulation of GS [glycogen synthase] appear to be adequately described by an MWC model in which phosphorylation acts like a

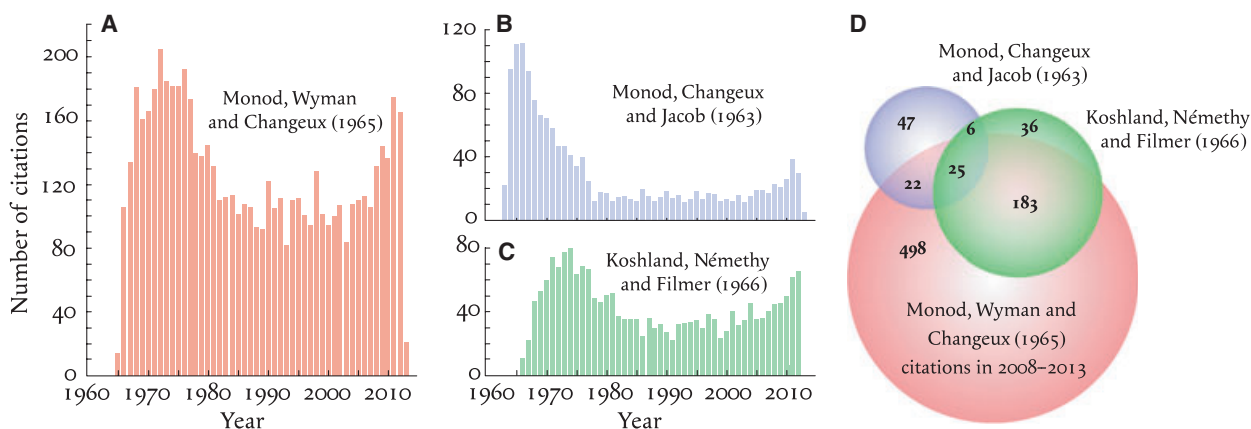
classic allosteric modifier. However, the sequential or Koshland–Némethy–Filmer model [27] is expected to provide an equally adequate, although more complicated, description. [Reference number edited]

Notice that the paper refers to the ‘MWC model’ but does not give a reference: presumably the point has been reached where the model is so well known that a reference is superfluous. There are, in fact, rather few papers that use the sequential model as the principal basis for analysing experimental data. Some mention it in passing [59], or refer to ‘a classical KNF paradigm’ without giving any details [60], or mention it as an explanation of negative cooperativity [61], but papers that include a thorough analysis of experimental data in terms of the sequential model have become virtually non-existent.

## Refinements

### Heterotropic interactions

The two classical models of cooperativity can deal almost equally well with interactions between equivalent ligands: substrate with substrate, inhibitor with inhibitor or activator with activator. Interactions of this kind are called *homotropic effects*. For positive cooperativity they predict binding curves that are so similar that it is very difficult to distinguish experimentally between them. The sequential model can also account for negative homotropic effects, or *negative cooperativity*, whereas the allosteric model cannot.



**Fig. 1.** Citations to classic papers. (A) Monod, Wyman and Changeux [14]; (B) Monod, Changeux and Jacob [1]; (C) Koshland, Némethy and Filmer [27]; (D) citations to the same papers during 5 years up to March 2013. Notice that of papers that cite Koshland, Némethy and Filmer [27] only 14% (36/250) cite neither of the others. Even this low percentage may be misleading, however, as the allosteric model is now so well known that authors may feel it unnecessary to give a reference, and at least one [58] of the 36 refers to the ‘MWC model’ without a citation. The figure was drawn from data in Web of Science, checked in March 2013, so the data for 2013 are very far from complete, and those for 2012 are probably incomplete also. The classic paper of Michaelis and Menten [6] exhibits an even more dramatic increase in citations during the 21st century [62].

However, the appearance of negative cooperativity can also arise for less interesting reasons, for example if the enzyme preparation is impure, or if it is pure but has subunits that are not identical; the latter explanation applies, for example, to the binding of oxygen to haemoglobin IV from trout, which is cooperative at pH 7.7 but negatively cooperative at pH 6.1 [63].

The difference is much greater for *heterotropic effects*, or effects of binding of one kind of ligand, such as an inhibitor, on the binding of another, such as the substrate. Heterotropic effects are of great physiological importance, and most examples of cooperativity are examples of cooperative binding of inhibitors or activators; cooperative binding of substrates is rather rare except when it is induced as a side effect of allosteric inhibition or activation [64]. The allosteric model can handle heterotropic effects in such a natural and simple way that they were included in the original description of the model [14]: an allosteric inhibitor binds to the opposite conformation from the one that favours binding of substrate, and an allosteric activator binds to the same conformation as the one that favours binding of substrate: it is just a matter of displacing an equilibrium, with the ligand ‘selecting’ a particular state from those available. In a study of phosphofructokinase from *Escherichia coli*, therefore, no special assumptions needed to be added to the basic model to account quantitatively for the reciprocal effects of binding the substrates fructose 6-phosphate and ATP, the inhibitor ADP and the activator phosphoenolpyruvate [65].

By contrast, the sequential model requires so many different cases to be considered that it becomes very complicated [66–68], with far more possibilities to be checked than one can hope to distinguish experimentally. This does not mean, of course, that the sequential model is incorrect, but it does mean that there have been very few attempts to apply it to real experimental examples of heterotropic effects, as discrimination between the different possibilities would require an unrealistically large amount of data.

### Reversibility and disequilibrium

The classical models for handling subunit interactions ignore two points that are normally regarded as important for discussing enzyme-catalysed reactions: that they are reversible and that the binding of substrate in the steady state is not the same as binding at equilibrium [69]. Reversibility is less of a problem than it may appear, however, because tightly regulated reactions, such as that catalysed by phosphofructokinase, normally have large equilibrium constants

favouring the physiological direction of reaction, and if necessary they are reversed by different reactions: for example, conversion of fructose 1,6-bisphosphate to fructose 6-phosphate is catalysed by fructose bisphosphatase, also a tightly regulated enzyme, not by phosphofructokinase. Reversibility complicates the already complicated equations for the classical models to the point that it becomes impractical to apply them, but is more easily incorporated in the Hill equation, originally proposed [70] as an empirical equation to describe the binding of oxygen to haemoglobin and now very useful for quantifying the degree of cooperativity. In its usual irreversible form it is

$$v = \frac{Va^h}{a_{0.5}^h + a^h} \quad (1)$$

in which  $v$  is the rate at substrate concentration  $a$ ,  $V$  is the limiting rate at saturation,  $a_{0.5}$  is the concentration at which  $v = 0.5 V$  and  $h$  is the Hill coefficient, which varies from 1 for non-cooperative enzymes to larger values (not usually exceeding 4) for cooperative enzymes. It can be generalized to the following reversible equation [64]:

$$v = \frac{\frac{Va}{a_{0.5}} \cdot \left(1 - \frac{p/a}{K}\right) \cdot \left(\frac{a}{a_{0.5}} + \frac{p}{p_{0.5}}\right)^{h-1}}{1 + \left(\frac{a}{a_{0.5}} + \frac{p}{p_{0.5}}\right)^h} \quad (2)$$

in which  $p$  is the product concentration,  $p_{0.5}$  is the value of  $p$  at half saturation and  $K$  is the equilibrium constant, i.e. the value of  $p/a$  at equilibrium. Although this equation is certainly more complicated than Eqn (1), in part because of the need to separate the factor expressing the degree of disequilibrium from the other terms, it is much simpler than the full reversible equations for the classical models would be [71]. In practice it gives a reasonable fit to experimental data, and it can handle both homotropic and heterotropic effects without great complication [64].

Since Briggs and Haldane [69] showed that Michaelis–Menten kinetics could be explained as readily in terms of non-equilibrium steady-state binding of substrates as by the equilibrium process assumed originally, it has been generally recognized that it is neither necessary nor desirable to assume that substrates bind at equilibrium. However, except in the case of models for cooperativity in monomeric enzymes (or, more exactly, enzymes with only one binding site on each molecule), for which cooperativity of substrate binding at equilibrium is impossible [48], it is assumed that cooperative kinetics simply mimic

what would be observed in equilibrium binding. Although this is in theory unsatisfactory, it is virtually unavoidable if analysis is not to be made hopelessly complicated. Although there have been attempts to handle subunit interactions without assuming equilibrium binding [72], these have not been widely applied, but an example is provided by the RNA packaging motor in bacteriophages of the *Cystoviridae* family [73], which shows cooperativity of ATP binding in kinetic experiments but not in equilibrium measurements.

### Three-dimensional structures of enzymes

At the time when the models for allosteric interactions and cooperativity were developed, extremely few three-dimensional structures of proteins were known, just myoglobin [74] and haemoglobin [13]. Neither of these is an enzyme, although haemoglobin was often called an 'honorary enzyme' in recognition of its great contribution to understanding interactions between subunits of oligomeric enzymes. Although structures appeared a little later for lysozyme [75],  $\alpha$ -chymotrypsin [76] and ribonuclease [77], these are relatively simple enzymes without regulatory properties, and their structures shed no light on the regulatory properties of oligomeric enzymes. Other structures were initially slow to appear, so understanding of allosteric interactions and cooperativity was heavily dependent on kinetics and chemical modification.

In these early years there was no investigation of protein structures by magnetic resonance, but nuclear magnetic resonance started to be used a little later for enzymes such as phosphorylase [78,79], as well as for peptides such as oxytocin [80] and other small proteins such as cytochrome  $c_3$  [81]. In the last case the paramagnetic iron atom allowed the nuclear data to be supplemented with measurements of electron paramagnetic resonance, which has become an essential tool for studying enzymes containing transition-metal ions in their catalytic sites. It can also be applied to proteins that do not contain paramagnetic centres by labelling with nitroxides. This is most easily done with cysteine residues [82], but these have the disadvantage that they are relatively rare in proteins and often have functional roles; however, other choices are now possible, such as tyrosine residues [83]. Labelling of iodoacetamide haemoglobin with a paramagnetic derivative of iodoacetamide was in fact used many years ago in one of the first attempts to distinguish experimentally between the models of cooperativity: Ogawa and McConnell [84] used this approach to estimate the change in conformation that accompanies oxygenation;

they found a strict proportionality, as expected for the sequential model, which requires the conformational change to be induced by ligand binding and not to occur otherwise, whereas the allosteric model predicts that the conformational change should initially run ahead of the degree of oxygenation.

Thanks to the great advances in X-ray crystallography and magnetic resonance in the subsequent decades, many three-dimensional structures are now known and constitute an essential part of the study of allosteric and cooperative mechanisms. In general these support the idea of conformational symmetry.

### Enzyme regulation in the age of systems biology

At the time when allosteric and cooperative regulation of enzymes first started to be analysed, attention was focused almost entirely on the individual enzymes that were strictly regulated, which were treated as if they were solely responsible for the flux through a whole pathway. In reality, however, flux control is shared (unequally) among all the enzymes in the system [85,86], and with the development of systems biology in the past decade this has become widely recognized. This does not mean, of course, that the classical mechanisms of regulation have no physiological role, but it does mean that their roles are different from what was thought. In the absence of any feedback inhibition the flux through a pathway would, other things being equal, be determined mainly by the activities of the enzymes at the beginning of the pathway, but that is not usually what is needed: feedback inhibition, in fact, allows flux control to be transferred to the pathways that require the end-product; in other words it allows control by demand rather than by supply [87,88]. The reality may be more complicated, however, as other things will often not be equal [88]. Computer simulation of some model pathways, such as a simple branched pathway with two different end-products [89], showed, in fact, that simple product inhibition, with no feedback inhibition of the first step of the entire pathway, or the 'first committed steps', i.e. the first steps after the branch-point, was quite sufficient to regulate production of end-products according to demand. The price to be paid for this flux regulation is much too high to be tolerated by a living organism, however, because the variation in flux will be accompanied by enormous changes in metabolite concentration. Cooperative feedback inhibition must be seen, therefore, not as a mechanism for regulating fluxes but as a mechanism for maintaining homeostasis when fluxes respond to changes in demand, and to

allow information about metabolite concentrations at the end of a metabolic pathway to be transmitted to upstream enzymes [90].

### Negative cooperativity

In normal (positive) cooperativity binding of a molecule of ligand increases the capacity to bind others, but sometimes one observes the opposite, known as *negative cooperativity*. This was first reported under that name for glyceraldehyde 3-phosphate dehydrogenase from rabbit muscle, in which each molecule of  $\text{NAD}^+$  that binds to the tetrameric protein decreases the capacity of further molecules to bind [91]; a somewhat earlier example, for glutamate dehydrogenase, had been described as ‘antagonistic homotropic interactions’ [92]. At that time the principal and almost the only interest of negative cooperativity was that the sequential model could explain it and the allosteric model could not, unless some of the original restrictions were relaxed [93–95]. Later it was found that various enzymes, such as cytidine triphosphate synthetase [96], could readily be brought close to half-saturation but not to full saturation: in such enzymes a tetramer of apparently identical subunits would behave like a dimer. This behaviour came to be known as *half-of-the-sites reactivity*, but its function remained obscure. The physiological value of increasing the sensitivity of a response to a signal seemed obvious, but what could be the value of decreasing it? Levitzki [97] suggested that low sensitivity at high ligand concentrations might be a price that needed to be paid for obtaining high sensitivity at low concentrations, but it was unclear if this explanation was tenable [98], and the physiological role of negative cooperativity has remained mysterious.

A possible rationale may be found by focusing, as suggested by Atkinson [99], on the sensitivity of concentrations to rates rather than the opposite. As kinetic experiments are usually designed so that the experimenter chooses concentrations of substrate and effectors and the enzyme responds with the appropriate rates, this reversal of dependent and independent variables may seem strange. In the physiological context, however, an enzyme operating in the middle of a metabolic pathway has little influence on the rate of the reaction it catalyses and must adjust the concentrations of substrate and product to the rate at which the substrate arrives: then Atkinson’s point of view makes more sense. It follows that if a particular metabolite concentration acts as an inhibitor or activator of a different pathway then negative cooperativity in the pathway for which it is a substrate will increase its effectiveness as a signal [100].

## Current applications to experimental data

Nearly half a century after they were first proposed, the classical models of cooperativity [14,27], together with the theory of allosteric interactions [1,3], remain the basis for nearly all attempts to analyse the mechanistic basis of regulation, not only for enzymes such as aspartate transcarbamoylase, but also for similar but different systems, such as ion channels. Examples of these two cases are now illustrated, and others may be found elsewhere [101].

### Aspartate transcarbamoylase

Aspartate transcarbamoylase, which regulates the biosynthesis of pyrimidine nucleotides, has long been the textbook example of an enzyme analysed in terms of allosteric feedback inhibition (by cytidine triphosphate) and the allosteric model of cooperativity [102–104], and remains so today [105]. Its three-dimensional structure, first determined in 1972 at 5.8 Å resolution [106], is now known with high precision, both for the holoenzyme with various ligands bound, such as *N*-phosphonacetyl-L-aspartate (PALA) [107], and for the catalytic subunit [108]. This structural information, combined with numerous studies of the kinetic and catalytic mechanism, have allowed the nature of the two states of the enzyme required by the allosteric model, and the transition between them, to be understood in considerable detail. Kantrowitz’s review [105] describes the history of these investigations and the present state of knowledge, which continues to be in accordance with the two-state allosteric model suggested half a century ago.

### Allosteric coupling in ligand-gated channels

Although enzyme mechanisms remain the principal focus of interest in allosteric interactions and models of cooperativity, they are also widely evoked in other domains, and in a recent paper Colquhoun and Lape [109] discuss their application to ion channels. They begin by noting that the word *allosteric* is used very loosely, and sometimes means little more than ‘we have got an antagonist and we are not sure what it does, but it appears not to be competitive’, but they point out that the original meaning given by Monod and Jacob [3] was much more precise: ‘From the point of view of mechanisms, the most remarkable feature of the [inhibition of the synthesis of a tryptophan precursor by tryptophan] is that the inhibitor is not a steric analogue of the substrate. We therefore

propose to designate this mechanism as “allosteric inhibition”.’

As an ion channel can be either ‘open’ or ‘shut’, and as any ligand may favour one or the other state, it may be an ideal system to interpret in terms of the allosteric model [14]. In practice, although it has been applied with some success to some channels, such as the nicotinic receptor [110], Colquhoun and Lape consider that it provides less than perfect results because it does not allow for the number of distinct shut states that can exist.

## Concluding remarks

It has become clear that the allosteric model [14] has essentially overwhelmed the sequential model [27] in the eyes of nearly all biochemists. When the latter is mentioned at all it is usually as an afterthought, as in the example quoted earlier [58]. It is not just a popularity contest, however, but a matter of which model has proved the more capable of accounting for new experimental data. I have said rather little about such new data, preferring to concentrate on the historical aspects, which are in the process of being forgotten (in the last two or three years I have attended lectures, in major international congresses, by scientists who appear to think that the lock-and-key model [19] remains the basic theory of enzyme specificity, or that only now are magnetic resonance studies revealing the existence of protein flexibility). However, the classical models of cooperativity continue to show great vitality and to be the focus of many new experiments, such as studies of chaperonins [111], clathrins [112] and many others, as is already evident from Fig. 1.

At first sight one might think that there is no relationship between the arguments over these two models and the arguments over the nature of evolution, whether by natural selection or by a ‘Lamarckian’ process (the quotation marks are to take account of the fact that the adjective does not accurately represent Lamarck’s thinking [113] and that in his later years Darwin was no less ‘Lamarckian’ than Lamarck). There are parallels, however, because the sequential model, and in particular induced fit, assumes a Lamarckian process in which the ligand ‘instructs’ a protein to adopt a particular conformation, whereas the allosteric model assumes a process in which the ligand ‘selects’ one out of two or more pre-existing conformations [114]. As Morange [33] has noted, the parallels between models of evolution and models of cooperativity ‘did not displease Monod’.

I must end on a sad note. François Jacob died on 19 April 2013, while this paper was being written. Of the other principal originators of the ideas discussed, Jacques Monod died in 1976, George Némethy in 1994, Jeffries Wyman in 1995 and Daniel Koshland in 2007.

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