Series
DOI 10.1007/s12038-012-9186-6

What history tells us XXVII. A new life for allostery

MICHEL MORANGE
Centre Cavaillès, CIRPHLES USR 3308, Ecole normale supérieure, 29 rue d'Ulm, 75230 Paris Cedex 05, France

(Fax, 33-144-323941; Email, morange@biologie.ens.fr)

1. Introduction

The number of articles published on allostery has rapidly increased in recent years. Since the 1960s, the word 'allostery' has always had different meanings. On the one hand, allostery simply describes the conformational changes of proteins (and other macromolecules) following the binding of ligands. On the other hand, allostery may explicitly refer to the theory proposed by Jacques Monod, Jeffries Wyman and Jean-Pierre Changeux in 1965 – also known as the MWC model, or model of the concerted transition (Monod *et al.* 1965). In this model, the allosteric change in conformation of proteins is not directly induced by the interaction of the proteins with their effectors, but results from the displacement of equilibrium between conformational states.

It is the latter vision of allostery that is flourishing at present, but with three significant extensions: the number of structural states is no longer limited to two but to an ensemble; allostery is not characteristic just of oligomeric proteins comprising different subunits but also concerns monomeric proteins and other macromolecules such as RNA and DNA; and the catalytic process also requires the dynamic reequilibration of an ensemble of pre-existing states. In the new unifying vision, 'allosteric regulation and catalysis emerge via a common route' (Goodey and Benkovic 2008). I will successively remind the conditions in which the adjective 'allosteric' emerged 50 years ago, and why Monod hypothesized in his model the existence of different states pre-existing in equilibrium. Then I will discuss the different reasons for the recent renewed interest in allostery. And, finally, I will underline the importance, both theoretical and practical, of this new vision, but sharply contrast it with apparently related views on the importance of plasticity in the behaviour of biological systems (West-Eberhard 2003), and on the extension of Darwinism to the molecular level (Kupiec 2009).

2. The origin of the allosteric model

The roots of allostery were in the discovery by H Edwin Umbarger of the end-product inhibition of the first enzymes of metabolic pathways (Umbarger 1956). Arthur Pardee and Jean-Pierre Changeux simultaneously confirmed these early observations, and discovered the possibility of desensitizing proteins to the action of their regulators without altering their catalytic activities (Changeux 2003). More than the difference in chemical structures between the substrates and regulators, desensitization was the main argument suggesting the existence of different sites on proteins. The word 'allosteric' was introduced by Monod in the conclusion of the Cold Spring Harbor Symposium of 1961 to designate simultaneously the differences in the structure of molecules and the consequent necessary existence of different sites for substrates and allosteric regulators (Monod and Jacob 1961). To explain how an inhibitor binding to another site could nevertheless affect the catalytic reaction, Monod used the 'induced-fit' model proposed earlier by Daniel Koshland (1958): binding of the regulatory ligand at the allosteric site induced a conformational change of the protein that modified the active site.

Apparently nothing was different in the 1963 review written by Monod, Changeux and François Jacob on 'allosteric proteins and cellular control systems', except for an extension of the early observations to new categories of proteins, hormone receptors, the newly described repressors, and an emphasis on the oligomeric structure of the allosteric proteins, haemoglobin being the most studied and best known among them (Monod *et al.* 1963).

Keywords. Allostery; energy landscape; molecular dynamics; NMR

Published online: 30 January 2012

The path from these early observations to the 1965 allosteric model has been amply explored by historians (Creager and Gaudillière 1996; Buc 2006). Whereas the 1963 article was a summary of all the observations made in the field, the 1965 model was presented in a dogmatic way. It was also in this 1965 article that appeared for the first time the idea that two different conformational states exist in the absence of any ligand.

The rigid 1965 model was a reaction against the critique of the 1963 article that allostery was a decadent theory susceptible to explain every biological observation on regulation (Changeux 2003). Monod was eager to find strong physical principles behind what he considered as 'the second secret of life'. He favoured the existence in protein structure of a 'principle of symmetry': the symmetry of protein structures is conserved during conformational changes. Symmetry is what physicists call an invariant. The pre-existence of the different conformational states was a characteristic unique to the allosteric model. The existence of an equilibrium between different conformational states could be looked for by using the fast kinetic methods developed by Manfred Eigen. The positive or negative result of this search allowed to choose between the model of Monod, and the extension, made by Daniel Koshland, of the induced-fit model to regulatory, multi-subunit proteins (Koshland *et al.* 1966). But the pre-existence of conformational states was a collateral effect of the principle of symmetry: the best and simplest way to justify the conservation of symmetry was to imagine that the symmetric structures of the proteins existed beforehand. In addition, this hypothesis generated simple equations, and drastically reduced the number of parameters necessary to describe the systems under study. 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 (Yudkin 1938).

Jeffries Wyman did not fully agree with the emphasis on the principle of symmetry (Wyman 2003). Nevertheless, the latter became the most visible mark of the system. In 2005, 40 years after its introduction, at a time when the new dynamic vision of protein structure and function was already emerging and promised that allostery would be 'born again' (see later), Changeux continued to support the allosteric model by searching in the recent scientific literature for all the observations in favour of the existence of a structural symmetry in oligomeric proteins (Changeux and Edelstein 2005).

Everyone who has taught the induced-fit model and the MWC model has realized how different these two models are. The induced-fit model is understood much more rapidly: it looks more 'natural', probably because it describes a causal succession of events, from the binding of the regulatory ligand to the modification of the active site (or vice versa). The MWC model is counterintuitive: the change in protein conformation is only an indirect consequence of the binding of the ligands. For the same reasons, the Lamarckian model of evolution, by a direct – transmissible through generations – modification of the organisms by the environment, is easier for lay people to grasp than the Darwinian model in which the environment only indirectly modifies some of the characteristics of organisms, by favouring the reproduction of those who bear these characteristics. The Lamarckian and induced-fit models are instructive, whereas the Darwinian and MWC models are selective, a resemblance which did not displease Monod.

3. The multiple paths to the present renewal

The allosteric behaviour of proteins has gained an increasing place in the descriptions of molecular and cell biologists, particularly in the description of cell signalling pathways. The transmission of signals can result from covalent modifications of proteins by, for instance, protein kinases. But in most cases the transmission of signals results from the allosteric conformational changes of the protein components of these pathways, the consequence of the binding of small second messengers or, more often, of interactions with other proteins. The model that was consciously or even more frequently unconsciously favoured was the induced-fit model: molecules in these signalling pathways interacted with their protein targets, and changed their conformation to induce their interaction with other downstream components, or to help them to acquire new functions.

Nevertheless, the importance of these studies for the recent change was the fact that more and more single-chain monomeric proteins were shown to have allosteric properties (for a recent example, see Yang *et al.* 2010). Similarly, RNAs and ribozymes, which only exceptionally form oligomers, were also shown to be able to have allosteric properties (Winkler and Dann 2006).

Two scientific advances of a very different nature were important for the revival of the allosteric theory, and to generate a dynamic view of proteins, in which they are considered as a moving ensemble of conformational states in equilibrium. The first was the progressive establishment, at the end of the 1990s, of a new conception of protein folding. After Cyrus Levinthal had shown that the rapid process of protein folding could not be explained by a random search for the most stable thermodynamic state, the hypothesis that there was a folding pathway was adopted. But experimental observations did not fit such a simplistic model: there were multiple parallel pathways for folding. The new vision of protein folding progressively emerged as the displacement in an energy landscape of multiple partially folded conformations. This landscape is represented as an imperfect, irregular funnel. Although initially the existence of a unique native state was not questioned, this singularity

became an abnormality (Zhuravlev and Papolan 2010). The notion of an energy landscape was applied to the native state and its conformational changes. The native state itself may be a disordered state (Uversky 2002; Chouard 2011).

However, the major impetus towards the new dynamic vision was the possibility offered recently by two techniques, NMR and molecular dynamics. NMR is not new, but recent developments as well as its combination with other techniques – such as hydrogen–deuterium exchange – allowed NMR to demonstrate the existence of an ensemble of conformational states, and to have access to the dynamics of interconversion between these different states (Palmer and Massi 2006). Molecular dynamics is no longer limited to the local, most rapid conformational changes (Shaw *et al.* 2010), but also provides information on the slowest, global conformational changes, those which are essential to an understanding of both catalysis and allosteric regulation. Combined, these two technological developments gave access not only to an enumeration of the different states but to a precise structural description of them. Additional information came from the use of fluorescent probes (FRET), X-ray scattering in solution, and the tedious work of mutagenesis of the different residues involved in these conformational changes (Henzler-Wildman and Kern 2007).

The impact was greater on the description of catalysis, the preferred domain for explanations by induced-fit mechanisms. The need to consider catalysis in a new way was also a distant consequence of the efforts made to engineer new proteins and enzymes. Initial successes were followed by repeated failures to confer on these newly designed enzymes a catalytic efficiency comparable to that of 'natural' enzymes. The case of abzymes is emblematic of these difficulties (Hilvert 2000), despite the discovery that abzymes exist naturally, and their potential use in medicine. The difficulty of mimicking induced-fit processes with these artificial enzymes was part of the explanation, but the problem appeared more general: what cannot be reproduced is the dynamics of protein behaviour.

A radically new vision emerged (Boehr *et al.* 2006; Benkovic *et al.* 2008). Each catalytic step corresponds to an ensemble of thermodynamic and structural states. The transition from one catalytic step to the next one corresponds more to a re-equilibration between these different states than to the formation of new states: what was a minor species at one step becomes a major one at the next. All happens as if the ensemble of conformational states anticipated the progression of the reaction.

The same statistical description could easily be applied to allosteric transitions (del Sol *et al.* 2009). The most interesting thing is that allosteric regulation and enzymatic catalysis no longer appear as distinct phenomena but as the manifestations of the same intrinsic dynamic characteristic of proteins. The fact that the time scales of events linked with catalysis and allosteric transitions are often different is no longer considered as the sign of a difference in nature between the two phenomena. The recent extension of this dynamic view to DNA (Nikolova *et al.* 2011) is the last step in this process of unification of macromolecular functional properties: modifications in DNA structure are not induced by the binding of proteins, but different conformations of DNA pre-exist and they are differentially stabilized by the binding of proteins.

The precise description of this unified vision of catalysis and allostery would require much more time and space, but this is beyond the scope of this article. Is it something new? Specialists in molecular dynamics argue that they had this dynamic vision of protein structure and function from the beginning, and that only the technological developments that allowed the community of biologists to acknowledge its importance are new (Cui and Karplus 2008). In this case, the novelty can also be considered as the displacement of equilibrium: the dynamic vision of protein structure is now shared by the majority of researchers working on proteins, instead of being supported by a small group of theoreticians working on computers to model the behaviour of proteins. This dynamic vision is clearly not a simple extension of the 1965 model. The latter was limited to oligomeric proteins and to the existence of only two different conformational states; no relations between the allosteric transition and the modifications in protein structure that take place during catalysis were imagined. In the new vision, allosteric behaviour can even result from a change in the dynamics of the system, without any conformational change (Tzeng and Kalodimos 2009). Nevertheless, the new vision is based on the same selective conception as the allosteric model: ligands do not induce the formation of new conformational states, but select one state among the ensemble of pre-existing ones. When students have fully understood the MWC model, they feel comfortable with the new conception of protein structure and function. This does not mean that the new model totally excludes the possibility of the existence of induced-fit phenomena (Silva *et al.* 2011).

Retrospectively, the vain efforts of Monod to demonstrate that β-galactosidase, the enzyme on which he had spent so much time, was allosteric seem less absurd (Ullmann 2003, p 201).

4. The place of the new vision in the contemporary landscape of biological models

This new vision of macromolecular behaviour opens up original avenues of research. One consists in characterizing the network of amino acid residues that are responsible for this dynamic behaviour. The best strategy to identify the residues forming these networks is to look for their conservation in evolution, an approach made possible by the recent accumulation of sequences. It is interesting to relate the present use of evolutionary information to access the dynamic function of proteins to the efforts made by Monod and his colleagues in

the 1965 paper to justify the allosteric model by evolutionary considerations (Monod *et al.* 1965).

This unified view of catalysis and allostery also offers new opportunities to modify the activity of proteins and enzymes. When the description of the allosteric network has been completed, it becomes possible to look for new hidden allosteric sites on the surface of the proteins, and to design molecules that might bind these cryptic allosteric sites (Gunasekaran *et al*. 2004), or to engineer chimeric proteins in which the allosteric networks are artificially linked (Lee *et al.* 2008).

This new vision of catalysis and allostery seems to be in harmony with the numerous contemporary discourses outlining the role of plasticity both in development and evolution (West-Eberhard 2003). Similarly, the increasing place of 'noise' (Eldar and Elowitz 2010) – stochastic variations in genetic circuits – has the same roots in molecular dynamics as the catalytic and allosteric changes in macromolecular structures. The recent efforts to extend Darwinism to the molecular and cellular levels bear an obvious similarity to the capacity of substrates and effectors to select one of the different pre-existing conformations of a macromolecule (Kupiec 2009). It was not pure chance that an article on the transition between the different states of ES cells (Kamiya *et al.* 2011) immediately followed an article showing the allosteric behaviour of DNA (Nikolova *et al.* 2011) in the same issue of *Nature*.

But are these resemblances significant? Are those sharing the new vision of catalysis and allostery supporting the same fight against determinism as those arguing for the importance of plasticity and random variations in development?

I think that the answer is clearly negative, and that this conclusion can help us to go beyond the vagueness of many contemporary discourses, and the ambiguities of the metaphysical message they deliver. The theory of allostery is never mentioned in these books and in articles dealing with plasticity and stochasticity (Oyama *et al.* 2001; West-Eberhard 2003; Kupiec 2009). The reason for this absence is very clear: the new, dynamic vision of protein structure and function, of catalysis and allostery, does not oppose a deterministic view of these processes. This molecular form of plasticity has been exploited by natural selection to design extraordinarily efficient macromolecular agents. Opposing determinism on the one hand and stochastic variations on the other has no general value. Maybe this is the best lesson that the recent extension of the theory of allostery can afford us!

Acknowledgements

I am indebted to David Marsh for his critical reading of the manuscript, and to Professor Michèle Reboud for my renewed interest in enzymology.

References

Benkovic SJ, Hammes GG and Hammes-Schiffer S 2008 Free-energy landscape of enzyme catalysis. *Biochemistry* **47** 3317–3321

Boehr DD, McElheny D, Dyson, HJ and Wright PE 2006 The dynamic energy landscape of dihydrofolate reductase catalysis. *Science* **313** 1638–1642

Buc H 2006 Interactions between Jacques Monod and Jeffries Wyman (or the burdens of co-authorship); in *Allosteric proteins – 40 years with Monod-Wyman-Changeux – Rendiconti di scienze fisiche e naturali* Serie IX 17 (2) (Roma: Bardi)

Changeux J-P 2003 A Ph.D. with Jacques Monod: Prehistory of allosteric proteins; in *Origins of molecular biology: a tribute to Jacques Monod* (ed) A Ullmann (Washington: ASM Press) pp 229–242

Changeux J-P and Edelstein SJ 2005 Allosteric mechanisms of signal transduction. S*cience* **308** 1424–1428

Chouard T 2011 Breaking the protein rules. *Nature* **471** 151–153

Creager ANH and Gaudillière J-P 1996 Meanings in search of experiments or *vice-versa*: The invention of *allosteric regulation* in Paris and Berkeley (1959-1967). *Hist. Stud. Phys. Biol. Sci.* **27** 1–89

Cui O and Karplus M 2008 Allostery and cooperativity revisited. *Protein Sci.* **17** 1295–1307

del Sol A, Tsai CJ, Ma B and Nussinov R 2009 The origin of allosteric functional modulation: multiple pre-existing pathways. *Structure* **17** 1042–1050

Eldar A and Elowitz MB 2010 Functional roles for noise in genetic circuits. *Nature* **467** 167–173

Goodey NM and Benkovic SJ 2008 Allosteric regulation and catalysis emerge via a common route. *Nat. Chem. Biol.* **4** 474–482

Gunasekaran K, Ma B and Nussinov R 2004 Is allostery an intrinsic property of all dynamic proteins? *Proteins* **57** 433–443

Henzler-Wildman K and Kern D 2007 Dynamic personalities of proteins. *Nature* **450** 964–972

Hilvert D 2000 Critical analysis of antibody catalysis. *Annu. Rev. Biochem.* **69** 751–793

Kamiya D, Banno S, Sasai N, Ohgushi M , Inomata H, *et al.* 2011 Intrinsic transition of embryonic stem-cell differentiation into neural progenitors. *Nature* **470** 503–509

Koshland DE Jr. 1958 Application of a theory of enzyme specificity to protein synthesis. *Proc. Natl. Acad. Sci. USA* **44** 98–104

Koshland DE, Nemethy G and Filmer D 1966 Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry* **5** 365–385

Kupiec JJ 2009 *The origin of individuals* (Singapore: World Scientific Publishing Company)

Lee J, Natarajan M, Nashine V C, Socolich M, Vo T, *et al.* 2008 Surface sites for engineering allosteric control in proteins. *Science* **322** 438–442

Monod J and Jacob F 1961 General conclusions: Teleonomic mechanisms in cellular metabolism, growth, and differentiation. *Cold Spring Harbor Symp. Quant. Biol.* **26** 389–401

Monod J, Changeux J-P and Jacob F 1963 Allosteric proteins and cellular control systems. *J. Mol. Biol.* **6** 306–329

Monod J, Wyman J and Changeux J-P 1965 On the nature of allosteric transition: A plausible model. *J. Mol. Biol.* **12** 88–118

Nikolova EN, Kim E, Wise AA, O'Brien PJ, Andricioael I and Al-Hashimi HM 2011 Transient Hoogsteen base pairs in canonical duplex DNA. *Nature* **470** 498–502

Oyama S, Griffiths P E and Gray R D (eds) 2001 *Cycles of contingency: Developmental systems and evolution* (Cambridge: The MIT Press)

Palmer AG III and Massi F 2006 Characterization of the dynamics of biomacromolecules using rotating-frame spin relaxation NMR spectroscopy. *Chem. Rev.* **106** 1700–1719

Shaw DE, Maragakis P, Lindorff-Larsen K, Plana S, Dror RO, *et al.* 2010 Atomic-level characterization of the structural dynamics of proteins. *Science* **330** 341–346

Silva DA, 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 Comput. Biol.* **7** e1002054

Tzeng S-R and Kalodimos CG 2009 Dynamic activation of an allosteric regulatory protein. *Nature* **462** 368–372

Ullmann A 2003 Being around; in *Origins of molecular biology: a tribute to Jacques Monod* (ed) A Ullmann (Washington: ASM Press) pp 199–205

Umbarger HE 1956 Evidence for a negative-feedback mechanism in the biosynthesis of isoleucine. *Science* **123** 848

Uversky VN 2002 Natively unfolded proteins: A point where biology waits for physics. *Protein Sci.* **11** 739–756

West-Eberhard MJ 2003 *Developmental plasticity and evolution* (Oxford: Oxford University Press)

Winkler WC and Dann III CE 2006 RNA allostery glimpsed. *Nat.e Struct. Mol. Boil.* **13** 569–571

Wyman J 2003 Recollections of Jacques Monod; in *Origins of molecular biology: a tribute to Jacques Monod* (ed) A Ullmann (Washington: ASM Press) pp 263–267

Yang S, Blachowicz L, Makowski L and Roux B 2010 Multidomain assembled states of Hck tyrosine kinase in solution. *Proc. Natl. Acad. Sci. USA* **107** 15757–15762

Yudkin J 1938 Enzyme variation in micro-organisms. *Biol. Rev.* **13** 93–106

Zhuravlev PI and Papolan GA 2010 Protein functional landscapes, dynamics, allostery: a tortuous path towards a universal theoretical framework. *Q. Rev. Biophys.* **43** 295–332