Allostery turns 50: Is the vintage yet attractive?

Maurizio Brunori*

Department of Biochemical Sciences, Sapienza University of Rome, Rome, Italy

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The Monod–Wyman–Changeux (MWC) model for allosteric proteins,¹ an elegant and powerful theoretical work published in 1965 in the *Journal of Molecular Biology*, was a remarkable conceptual evolution in the field of biological regulation insofar as it introduced a conformational selective mechanism to allow enzymes to cope with the ever-changing physiological demands of the cell. However the expression “allosteric inhibition” had already been coined by Jacques Monod and Francois Jacob years before, in 1961, in the written comments to the experiments on L-threonine deaminase carried out at the *Institut Pasteur* by Jean-Pierre Changeux as a PhD student of Monod. In this volume of *Protein Science*, Changeux *lui meme* writes the story that led to suggest the nonoverlapping mechanism of control coupled to the binding of signaling molecules at sites removed from the active site of enzymes.²

The MWC model has been inspiring and remarkably successful; it is reported/illustrated in every Biochemistry textbook and was cited thousands of times, possibly a record for a theoretical paper in protein science. The rigorous and elegant thermodynamic expression was the result of conceptually original thoughts and a growing collaboration between Jacques Monod and Jeffries Wyman (at the time working in the Biochemistry Institute and the

Regina Elena at the University of Rome), as detailed by Buc³ in an excellent historical paper based on original documents from the Archives of the *Institut Pasteur*. Scientists at *Pasteur* were primarily interested in the regulation of enzymatic activity; however, their interest in hemoglobin as a prototype of an allosteric protein is already spelled out in a seminal paper by Monod *et al.*⁴ entitled “Allosteric Proteins and Cellular Control Systems.” The sigmoidal oxygen binding curve typical of hemoglobin and the Bohr effect discovered at the beginning of the 20th century had been quantitatively characterized and extensively analyzed. The 3D structure of hemoglobin, just solved by Perutz *et al.*,⁵ showed the hemes to be separated by a large distance (approx 30 Å) implying that co-operativity must be mediated by the protein; soon afterward, the structure of deoxygenated hemoglobin revealed the tertiary and quaternary conformational changes associated to oxygenation. Gibson⁶ had discovered in 1959, a quickly reacting form of hemoglobin using flash photolysis to study ligand recombination; the enhanced rate proved later to be characteristic of R-state hemoglobin. In 1951, Wyman and Allen⁷ had proposed (rather prophetically) that to explain the Bohr effect, a ligand-linked conformational change of the protein was necessary: “the reason why certain acid groups are affected by oxygenation is simply the alteration of their position and environment which results from the change of configuration of the hemoglobin molecule as a whole accompanying oxygenation,”

*Correspondence to: Maurizio Brunori, Department of Biochemical Sciences, Sapienza University of Rome, Rome, Italy. E-mail: maurizio.brunori@uniroma1.it.

Figure 1. Energy level diagram indicating the 10 states (T_0 to T_4 and R_0 to R_4) and the fundamental equilibrium constants of the MWC model, namely: K_T and K_R , the oxygen dissociation constants of the two allosteric states T (tense) and R (relaxed); and L_0 , the population ratio of the two states in the fully deoxygenated tetrameric hemoglobin. Drawings depict the two different quaternary states; the assumption implicit is the fully concerted quaternary transition which (in the specific case of a symmetric binding curve) occurs at the level T_2 – R_2 (switch-over point). Typical values of the parameters for human hemoglobin at neutral pH and 20 °C are approximately: $L_0 = [T_0]/[R_0] = 10^5$, and $c = K_R/K_T = 0.01$.

and “if we are prepared to accept hemoglobin as an enzyme, its behavior might give us a hint as to the kind of process to be looked for in enzymes more generally.” In the early 1960s, Antonini and coworkers (see Ref. ⁸) had shown that oxygen binding to deoxyhemoglobin was associated with a conformational change as detected by differential binding of bromthymol blue and differential accessibility to proteolytic attack by carboxypeptidases. Wyman⁹ quickly appreciated the impact of the message embedded in the new word coined by Monod and Jacob; he presented these novel results obtained in Rome in a paper entitled “Allosteric Effects in Hemoglobin.”

The MWC paper was finally submitted to the *Journal of Molecular Biology* in December 1964. It went through a rather demanding and peculiar refereeing process, as Wyman¹⁰ recalls: “I vividly remember a meeting Jacques and I had with several of our friends and colleagues at the MRC Laboratory in Cambridge to discuss the manuscript and its suitability for publication in the *Journal of Molecular Biology*. As I recall it, these included Max Perutz, John Kendrew, Francis Crick and Sydney Brenner, a rather formidable group of critics. The attack mainly centered on the symmetry ideas, and Jacques bore

the brunt of it. But afterward, in the calmer atmosphere at tea, we all agreed that the paper, symmetry and all, should be published as it stood, a decision which in retrospect no one I thought could possibly question.” After publication of the MWC model, the hemoglobin field took off and for the next 20 years or so, hot debates concerning its applicability to hemoglobin and the limits of consistency to the canonical formulation were perceived at meetings and seen in the press. The essentials of the two-state model are depicted in Figure 1, showing the energy level diagram for the quaternary states of hemoglobin in equilibrium at every degree of saturation with the ligand.

Perutz was captivated by the MWC theory, but by comparing the 3D structures of oxyhemoglobin and deoxyhemoglobin, he realized that some modification was necessary. A stereochemical mechanism for cooperativity and a detailed interpretation of the Bohr effect, involving specific interactions between residues at the interfaces and at the C-terminals, was published in 1970.¹¹ Perutz’s mechanism has been for years the target of many scientists round the world, who published data by advanced biophysical techniques to falsify or support the proposal. Background knowledge on hemoglobins was by-and-large summarized in the book I wrote with Antonini¹² published almost exactly 40 years ago. The scientific debate about the applicability of the MWC model to hemoglobin continued and to some extent it is still going on. Many reviews and provocative papers have been published over the years (see for example Refs. 13–18 and references therein). In a nutshell, it is clear that some of the initial assumptions of the canonical allosteric model had to be dropped along the way. An oligomeric structure is not a *conditio sine qua non*, since we know that also monomers display allosteric behavior; symmetry conservation and the invariance of the two binding constants are not tenable; a quaternary change is not always required; and negative cooperativity (when clearly distinguished from chain heterogeneity) demands description by the alternative “induced fit” approach championed by Koshland.¹⁹

For years, only a limited number of complex proteins and allosteric enzymes, starting with aspartate trans-carbamoylase,^{20,21} were extensively studied; particularly intriguing to me has been the successful application of the allosteric model to membrane receptors^{22,23} and to the chaperonins.²⁴ But over the last 10 years, it was appreciated that allostery is widespread among functioning proteins and indeed the applicability of the model was extended to many other proteins, including monomeric ones (see Ref. ¹⁷ and references therein).

The combination of sophisticated experimental methods and theoretical approaches based on molecular dynamic simulations has made it possible to

unveil the role of tertiary and quaternary conformational changes in functional regulation, a problem that intrigued Changeux from the very beginning.² The progress made for different systems ranging from oligomeric to monomeric macromolecules has been impressive. In particular, one should recognize the role of dynamics in mediating allosteric interactions even in simple systems, and the expansion of the repertoire of functionally distinct modules involved in molecular recognition.^{25–27} The population shift of conformational variants represents an interesting extension of the basic concept of allostery applied to several interesting monomers crucial to protein–protein recognition events that are, at the same time, selective and promiscuous; in these cases, it may be advisable not to use the term “cooperative” which was intended to indicate homotropic interactions among sites binding the same ligand. Likewise, it may be misleading to let people believe that population shift of conformational variants implies a “new view” of allostery; indeed, this was the founding idea of the MWC model, the two allosteric states being both populated even in the absence of the ligand/substrate (see Fig. 1), a concept basic to natural selection and biological evolution. It may be proper to close by reporting a quotation from Buc³ when he refers to Monod’s philosophy in his approach to writing the MWC paper: “...But part of the seduction of the paper rests also on its speculative character. Proteins are considered there not only as transducers working close of to their theoretical limit of efficiency, but also as historical objects that evolution has optimized. In its two facets, radiant and convincing, or speculative and questionable, the article bears Monod’s label, an amateur in physical-chemistry, a visionary insofar as molecular Darwinism is concerned.”

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