Allosteric Interactions after 50 Years

The first article to use the term "allosteric" in its title was published by Monod, Changeux, and Jacob in 1963,[1] followed soon afterwards by another more mechanistically oriented text by Monod, Wyman, and Changeux, with heightened impact.[2] The four founding fathers of allostery are presented in Fig. 1 in photos from the initial period. This special issue is timed to coincide with a major colloquium entitled "Allosteric Interactions in Cell Signaling and Regulation" to be held May 14–17, 2013, at the Pasteur Institute to mark the 50th anniversary and provide an overview of the state of research on allostery. While the focus of this issue and the conference is the current spectrum of activities related to allostery in molecular and cellular biology, it is inevitable to glance backward to understand why allostery was such a seminal discovery. A partial answer is that a half-century ago, the concept of allostery captured important features of macromolecular dynamics that united four distinct streams of research: (1) feedback-inhibited enzymes, (2) bacterial genetic repressors, (3) hemoglobin, and (4) membrane receptors. The power of the concept and its generality led to continuously bourgeoning applications, samplings of which are presented in this special issue.

Concerning the first stream, enzymes displaying the phenomenon of feedback inhibition had been studied as a significant biological phenomenon beginning in the 1950s, but a deeper understanding required astute analytical experiments. The novel approach applied to threonine deaminase and the background for this research are eloquently captured in the Recollections by Jean-Pierre Changeux.[3] His pivotal exploitation of the properties of threonine deaminase, especially its sensitivity to heat and urea, clarified two essential points. First, he demonstrated that the sites for the substrate and the inhibitor could be separately inactivated by heating (see Fig. 2),[4] strongly suggesting at least partially independent structural elements and thereby providing the basis for *allosteric* sites—from the Greek *allos* ("other") and *stereos* ["solid (object)"]. Similar findings on aspartate transcarbamylase revealing loss of feedback inhibition by heating suggested a generalization of this fundamental feature.[5] Second—and critical for the rest of the allostery story—in the presence of 1.5 M urea (which inactivated the enzyme with a half-time of about 20 min),[6] substrates and activators accelerated inactivation by what we would now describe as favoring the R state,[2] whereas inhibitors slowed it down by favoring the T state, as

shown in Fig. 2 of Changeux's Recollection.[3] These observations were at the heart of the principle of quaternary constraint.[2] It may be generally assumed that in the course of evolution, natural selection led to enzyme efficiencies that were largely maximized. Therefore, in order to introduce regulatory features, the only room for maneuver would be *reductions* in efficiency. By binding together catalytically active subunits (protomers) into highly stable quaternary structures that stabilized conformations with diminished activity (the T state), allosteric regulation based on quaternary constraint could emerge. Stronger binding of substrates or activators by the R state could overcome the higher intrinsic stability of the T state, resulting in cooperative regulatory interactions. Subsequent structural determinations permitted visualization of the precise three-dimensional polypeptide conformations underlying these properties for the key enzymes studied in the initial studies,[7,8] as well as many others.[9]

In relation to the second stream, the sigmoidal saturation curves for feedback-inhibited enzymes hinted at a common ground with hemoglobin, as suggested publically by B. Davis in 1961 at the Cold Spring Harbor meeting,[3] although the parallel had already been applied to enzymes at least among scientists influenced by Jeffries Wyman.[10] Classical studies of sigmoidal oxygen binding by hemoglobin began at the start of the 20th century, and their quantification by A. V. Hill produced the equation still widely used as a measure of cooperativity.[11] An interesting functional connection to feedback-inhibited enzymes can be made from early hemoglobin experiments by Wyman on the effect of urea.[12] The notion of quaternary constraint was unknown at the time, but addition of urea led to an *increase* in the oxygen affinity of hemoglobin, consistent with the idea that its binding activity is "constrained" by subunit interactions in the deoxy form that are opposed (or relaxed) by urea. For hemoglobin, urea and the Bohr effect at high pH both exert their effect by destabilizing the T state.[13] This point is central for distinguishing between concerted[2] and sequential models of hemoglobin cooperativity.[14,15] The former *require* quaternary constraint, whereas the latter can yield equivalent functional properties with either quaternary constraint or quaternary enhancement.[16] Indeed, the original sequential model of Pauling,[14] as well as and its later incarnation by Koshland, Nemethy, and Filmer,[15] used *increased* interaction energy between liganded subunits to drive cooperativity.

Fig. 1. The founding fathers of allostery. Jacques Monod, upper left; Jean-Pierre Changeux, upper right; François Jacob, lower left; Jeffries Wyman, lower right.

Beyond its historical interest, there is still much to be learned about hemoglobin allostery as reported in this issue for the fascinating comparison between hemoglobins of humans and fish.[17]

For the third stream, the fact that the lac operon and the key role of the lac repressor were being studied along the same corridor in the Pasteur Institute where Jean-Pierre Changeux was working on threonine deaminase permitted two previously unrelated lines of research to be intimately connected. As presented in the two foundational publications,[1,2] genetic repressors and allosteric enzymes became two heads of the same coin. The many subsequent advances in the repressor field, particularly in the light of the atomic structures established by X-ray crystallography, are cogently described here in the review by Mitchell Lewis.[18] More generally, the flourishing superfamily of nuclear receptors follows the same themes, with variations.[19]

The fourth stream concerning membrane receptors was less evident in the early days of the allostery theory, but Jean-Pierre Changeux rapidly understood the potential and promptly directed his efforts in that direction, as he vividly recounts.[3] His determination soon met with success with the isolation of nicotinic ligand-gated ion channels and the field of allosteric
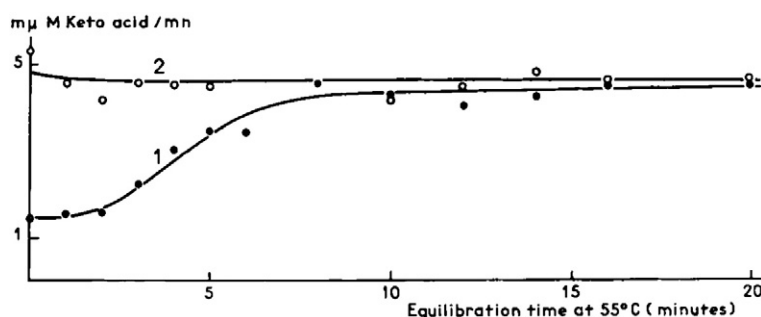
Fig. 2. Effect of temperature on threonine deaminase. Purified protein in the standard buffer was incubated at 55 °C for various lengths of time and then assayed in the presence of 0.01 M of the inhibitor isoleucine (curve 1) or without the inhibitor (curve 2). The inhibitory action initially present (curve 2) is progressively lost. Modified from Changeux.[4]

membrane proteins became a major focus of research, as represented by articles in this issue concerning both ligand-gated and G-protein-coupled receptors.[20,21] Moreover, an important early theoretical advance by Changeux and his colleagues[22] anticipated extended receptor membrane arrays, which proved to be of particular relevance to bacterial chemotaxis,[23,24] as reviewed in this issue.[25] Other work has characterized the flagellar motor as one of the most cooperative allosteric systems known to date.[26,27]

Part of the attraction of the allosteric concept was the relatively simple mathematical description that flowed from its basic assumptions. Nevertheless, alternative mathematical formulations were possible and one interesting approach was based on the notion of "equivalent monomers." This idea, formulated in 1965 by Francis Crick and Jeffries Wyman, permitted mathematical simplifications by postulating a hypothetical equilibrium between monomeric units as the basis of allostery. A number of novel equations and useful concepts (such as the "allosteric range") were also presented in their long-latent text now published in this issue for the first time.[28] For one particularly valuable equation relating the Hill coefficient and the number of binding sites, the authors presumed "that there is a simple derivation" but expressed frustration at having been "unable to discover it." The passage of time has revealed new mathematical insights that permit a simple derivation to be presented here.[29] In addition, the historical context and ramifications of the Crick–Wyman text are the subject of Henri Buc's illuminating account, including Monod's resistance to equivalent monomers, however hypothetical they might be, because they did not respect the principle of quaternary constraint.[30] Conceptual aspects of these distinctions are further detailed elsewhere in this issue.[20] Numerous pertinent equations for allostery have been published at regular intervals,[31–40] including some with novel implications for the MWC (Monod–Wyman–Changeux) model,

such as nesting,[41] binding-site non-equivalence,[42] or tertiary-level transitions.[43] A notable effort to provide a statistical mechanical treatment of the allosteric model is also provided in this issue.[44]

In addition to large-scale systems such as bacterial chemotaxis, other complex structures have come under scrutiny with respect to their allosteric features. A particularly impressive example is the allosteric regulation of 26S proteasome activities. Totaling 2.5 MDa, the complex achieves a network of finely regulated allosteric interactions involving the 20S core particle and the 19S regulatory particle.[45] Another large allosteric structure is described for the emerging picture of the chaperonin GroEL, with 14 identical protomers arranged in two 7-member rings. This complex performs a veritable allosteric choreography, with subunit domains twisting and turning to achieve substrate unfolding.[46] Applied to complex viruses, allosteric principles also provide insights into the unusually dynamic maturation of *Nudaurelia capensis* single-stranded RNA Omegavirus, involving a dramatic condensation of the 490-Å spherical procapsid to a 400-Å icosahedral-shaped capsid. The phenomenon involves specific auto-proteolysis and stabilization involving allosteric communication among the four quasi-equivalent subunits in the icosahedral asymmetric unit.[47] Finally, the massive molecular machinery of muscle contraction is also shown to use allosteric control, but with significant differences between cardiac and skeletal muscle. Swapping portions of the skeletal and cardiac forms of troponin T permits critical features of the allosteric regulation for cross-bridge recruitment and the tuning of calcium regulation in cardiac muscle to be identified.[48]

Along with applications of allosteric principles in so many diverse research areas, progress is also continuing in the founding field of allosteric enzymes. This subject remains very active and four communications in this issue provide examples of state-of-the-art developments with unnatural amino acids,[49]

with chemical shift covariance analysis to follow long-range allosteric interactions,[50] with photo-cross-linking and mass spectrometry to identify contacts between domains,[51] and with X-ray crystallography, mutagenesis, and isothermal titration calorimetry to characterize a network of three synergistic allosteric sites.[52]

The sampling of allostery presented in this issue covers various interesting facets, but cannot of course be exhaustive. Many classes of interactions were not included, including riboswitches, DNA-binding proteins, and hormone receptors, to mention just a few. These and several additional topics will be discussed in the meeting at the Pasteur Institute and in a second special issue on Allosteric Interactions to appear at a later date. Many of these subjects have also been covered in recent reviews.[53–58]

To close on a personal note, looking back to the early days of allostery, I realize how lucky I was to interact with the four founding fathers as a young scientist in the 1960s. I knew it was an exciting time to work on allosteric proteins but could not have imagined the wide impact the principles of allostery would have in the next 50 years, as illustrated by the selections in these two special issues. I wish to thank Peter Wright and Moshe Yaniv for arranging the special issues, Charalampos (Babis) Kalodimos for participating with me as Editor of this first issue, and Marina Ostankovitch and her colleagues at Elsevier, especially Meghan Jendrysik and Rowena Prasad, for their diligent efforts to make these issues possible. I also thank all the authors for their timely and enthusiastic participation.

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