Allostery

Methods and Protocols

Edited by

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Dedication

I am convinced that all that is in the Universe revolves around my amazing wife; without her efforts, I could not do science. I have been in the fortunate position to have been trained by three mentors who are not only good people, but also believe in science at the highest caliber. Therefore, I would like to dedicate this book to these four individuals: Shellee Fenton and Drs. James B. Blair, Gregory D. Reinhart, and Gerald M. Carlson.
Preface

In the past 7 years, allostery has resurfaced as a major focus in understanding protein structure/function. Much of this rejuvenated interest has been driven by the ability of NMR to monitor protein dynamics and the potential of determining how these dynamics contribute to protein functions, including allostery (1–6). A second driving force for the recent interest is a growing appreciation that allosteric drugs offer safety advantages over conventional drugs (7–9). This renewed interest has resulted in several reviews on allostery (10–13).

At the onset of any discussion on allostery, it is beneficial to review the exact phenomenon included in the discussion. Shortly after the original use of “allosteric” (14), confusion over the definition of this term showed up in the literature. One source of confusion is whether “allostery” and “cooperative” should be treated as two synonyms to describe the same principle or if these words describe two different phenomena. To indicate similarities at the phenomenological level, it is now common to use “allostery” and “cooperative” interchangeably, with further definition as either “homotropic,” to indicate energetic coupling when the two ligands are identical, or “heterotropic,” to indicate energetic coupling when the two ligands are nonidentical. Even with these distinctions, the classification of homotropic and heterotropic as independent forms of regulation has been much debated. Alberto Sols articulated why these properties should be considered as related but independent properties by emphasizing that homotropic mechanisms require that the protein is an oligomer (15):

...because of confusion between two frequently linked but essentially independent concepts: (i) specifically regulatory sites and (ii) multiplicity of interacting equal sites in oligomeric proteins. ...

To compound the tendency to confusion, oligomerism is not only not necessarily linked to allosteric (heterotropic) effects but is not even the only basis for positive cooperativity (homotropic). ...

By contrast, a purely thermodynamic view led Harvey Fisher and coworkers to express the similarities in these two properties (16):

The term “cooperativity,” or, more precisely, “heterotropic cooperativity” has been used occasionally to describe systems such as that shown ... (in an allosteric energy cycle). ... in cases where the binding of one ligand either increases or decreases the affinity of a second, chemically distinguishable ligand. A majority of workers in the field, however, prefer to restrict the use of the term “cooperativity” to homotropic systems, and to refer to such effects in heterotropic systems as “positive and negative interactions.” ... however, such a formal distinction between homotropic and heterotropic systems (implying as it does that the two classes of systems require totally different mechanisms to achieve what is essentially the same result) is an unwarranted assumption and one which may prove to be misleading.

Given this long standing historical debate, we have found the most productive approach is to define the type of regulation that is being described. However, one distinction that should be noted is the additional challenges associated with the study of homotropic systems since the concentrations of the two ligands cannot be varied independently. In this book, the majority of the chapters focus on studies of heterotropic systems. However, given the historical association between heterotropic and homotropic effects, techniques specific to the study of homotropic systems are also represented.
A second level of confusion is whether “allostery” includes any reference to a change in protein conformation. The original definition given by Monod et al. in 1963 (14) had no reference to conformational changes. Shortly thereafter, Monod and coworkers offered a plausible model to explain allostery derived from assumed conformational changes (17). The 1965 reference has been used to suggest that the recent introduction of dynamics into the discussion of allostery offers a “new view” of allostery (3, 18, 19). Others have relied on the 1963 definition to emphasize that the original definition of allostery placed no constraints on the molecular source of allosteric regulation and that dynamics were always accounted for in the description of this phenomena (11, 12, 20, 21).

In the Fenton laboratory, we use the word “allostery” to refer to heterotropic coupling events, with no implication that the mechanism for this through-protein communication is restricted to a change in protein conformation. Therefore, allosteric regulation is defined functionally as how a macromolecule binds one ligand differently when a second ligand is or is not prebound to the macromolecule. Since the definition of allostery influences what is expected as the “molecular source of allostery” (22), the use of the same definition has been strongly encouraged throughout all chapters in this volume (12). However, unifying the use of terms across all structure/function studies is an unrealistic goal, and even in several chapters of this volume, the influence of historical deviations of our favored definition is apparent.

Despite the semantic debates regarding classification, the common feature of allosteric systems is ligand-induced, through-protein changes. Therefore, any technique that can be used to study protein structure/function questions can be applied to the study of allostery. As such, the primary value of this book is the logic that is necessary to study this phenomenon, a phenomenon that is well recognized through the history of the life sciences and very poorly understood at the molecular level.

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