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A general framework improving teaching ligand binding to a macromolecule ☆

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ABSTRACT

The interaction of a ligand with a macromolecule has been modeled following different theories. The tenants of the induced fit model consider that upon ligand binding, the protein–ligand complex undergoes a conformational change. In contrast, the allosteric model assumes that only one among different coexisting conformers of a given protein is suitable to bind the ligand optimally. In the present paper, we propose a general framework to model the binding of ligands to a macromolecule. Such framework built on the binding polynomial allows opening new ways to teach in a unified manner ligand binding, enzymology and receptor binding in pharmacology. Moreover, we have developed simple software that allows building the binding polynomial from the schematic description of the biological system under study. Taking calmodulin as a canonical example, we show here that the proposed tool allows the easy retrieval of previously experimental and computational reports. This article is part of a Special Issue entitled: Calcium Signaling in Health and Disease. Guest Editors: Geert Bultynck, Jacques Haiech, Claus W. Heizmann, Joachim Krebs, and Marc Moreau.

1. Introduction

The interaction of a ligand with a protein may induce at least three sequential steps, namely the binding of the ligand to the protein, the induction of a conformational change and finally the biological effect. The binding step is followed by technical means measuring direct ligand binding such as free and ligand bound separation, equilibrium and flux dialysis, surface plasmon resonance and in some specific case, supramolecular mass spectrometry [1–4]. The conformational step is monitored by biophysical techniques such as fluorescence spectroscopy, NMR, microcalorimetry [5–7] and finally, the biological outcome may be an enzymatic activity, a change in cellular morphology or the generation of a second messenger transient signal [8].

Description of such a biological system has been started more than a century ago, starting with the key and lock model of Emil Fischer (1890), followed by the model of the activity of an enzyme able to bind a substrate by Henry (1908) and Michaelis and Mentens (1912). Since then, the subject of ligand binding to oligomeric proteins has been covered by numerous reviews and text books, for a historical personal point of view see [9].

Describing the behavior of a putative biological model needs the extraction of macroscopic parameters from dose–response curve. These include the so called Adair–Klotz constants and the maximum and minimum signals [10–12]. These macroscopic parameters may be linked to the molecular description of the model. The experimental and theoretical reasoning to obtain these parameters start with the choice of the experimental readouts (i.e. bound ligand, conformational changes, enzymatic activity, cellular signal or changes in signaling) and obtention of the dose–response curves. Fitting of the latter is achieved to obtain Adair–Klotz phenomenological parameters. These are then deconvoluted to get microscopic parameters linked to the proposed molecular model. This molecular description allows predicting the behavior of the system upon specific experimental conditions and therefore designing new experiments in order to address the relevance of the model. For these reasons, we will make the link with the proposed framework and phenomenological parameters in the different sections. Our first aim in this paper is to demonstrate that for a given Adair–Klotz or Fletcher model that in any case fits to experimental binding data, it is always possible to derive either an induced fit model or an allosteric model. Our second aim is to pinpoint the fact that this formalism may be used to help students to link several models that they approach in different training programs, namely multiple ligand binding to monomeric or polymeric proteins addressed in

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biophysics, competitive and non-competitive models in enzymology and receptor binding in pharmacology.

Here, we begin with introducing the general framework, for the modeling of one type of ligand on several sites on the macromolecule. We will extend this approach to address the case of multiple ligands. A relationship between model and observable signal is also given in this first section. Thereafter, links between the proposed generic models, the standard induced fit model, allosteric model and mathematical models (eg. Hill's equation [13]) are discussed. The last section concerns the development of a MATLAB toolbox designed to build easily the binding polynomial from the description of the biological system under study. As we have analyzed the calmodulin system since 1981, we use it to show the potentiality of such an approach to simulate previous models.

2. Framework description

2.1. Binding of one ligand to a macromolecule

In this first section, let us consider the interaction of a macromolecule P with n binding sites for a given ligand L. This system can be studied in different ways. Firstly, one may consider a macroscopic approach in which focus is put on the number of bound ligand (Fig. 1A). The ratio between $n+1$ different configurations (P, PL, PL_2 … PL_n) can be modeled according to Adair–Klotz K_i constants:

$$K_i = \frac{[PL_i]}{[L]\cdot[PL_{i-1}]} \tag{1}$$

and a macroscopic binding polynomial $P(x)$:

$$P(x) = 1 + \sum_{i=1}^{n}\left(\prod_{j=1}^{i} K_j\right)\cdot x^i \tag{2}$$

where $x = [L]$.

An alternative to this macroscopic approach is to consider the occupancy of each binding site independently (Fig. 1B). As a consequence, we distinguish between a PL molecule with the ligand on the j-th site a PL molecule with the ligand on the k-th site. A given configuration is now $PL^1_{i_1}\ldots L^n_{i_n}$ written with, for each L, the site number as exponent and a binary i_k index which is equal to 1 when the site is occupied and 0 elsewhere. The equilibrium is now computed through 2^n microscopic $\beta_{i_1,\ldots,i_n}$ constants defined as following:

$$\beta_{i_1,i_2,\ldots,i_n} = \frac{\left[PL^1_{i_1}\ldots L^n_{i_n}\right]}{[P]\,[L]^{i_1}\ldots[L]^{i_n}}. \tag{3}$$

From these microscopic constants, two sets of parameters are defined: the individual affinity constant for each site ($k_j = \beta_{0,\ldots,1,\ldots,0}$ where the only 1 is on the j-th position) and the coupling factors between sites deduced from β and k_j parameters according to the following expression:

$$c_{i_1,i_2,\ldots,i_n} = \frac{\beta_{i_1,i_2,\ldots,i_n}}{\prod_{j=1}^{n} k_j^{\,i_j}}. \tag{4}$$

With this new set of parameters, the binding polynomial $P(x)$ can be rewritten:

$$P(x) = 1 + \sum_{j=1}^{n} x^j \sum_{\Sigma_q i_q = j} c_{i_1,\ldots,i_n}\cdot\prod_{m=1}^{n} k_m^{i_m}. \tag{5}$$

By identification, a relationship between Adair–Klotz constants and microscopic parameters can be obtained:

$$K_j = \sum_{\Sigma_q i_q = j} c_{i_1,\ldots,i_n}\cdot\prod_{m=1}^{n} k_m^{i_m}\cdot\prod_{m=1}^{j-1}\frac{1}{K_m}. \tag{6}$$

It must be noticed that there is a link between each complex represented in the scheme of Fig. 1 and each term of the binding polynomial. For the macroscopic scheme, the complex P is associated with the binding polynomial term of degree zero, the complex PL with the term of degree one, and so on. The same observation holds for the microscopic scheme.

Although such equations have already been described in numerous textbooks and reviews [14,15], the possibility to derive the binding polynomial from the description of the biological system has not been underlined. Moreover, using such a framework when teaching undergraduate students (since 1990 for one of us) leads to an improvement in their capability to generate any biological model starting from a physical description of any enzyme or receptor binding model.

2.2. General framework for the binding of different ligands

Let us now consider a macromolecule P with different ligands that may bind on different sites. The proposed framework to study such a system is to consider that each ligand has a unique binding site. This framework encompasses all the considered cases:

- P has actually r sites and each of them are associated to a given ligand L_r.
- P has actually some sites that are associated to the same ligand L, each binding site is distinguished according to the site number (as for the case described in Section 2.1).
- P has a given site on which different classes of ligands can bind; we consider as many virtual binding sites as necessary for the given actual binding site and fix the coupling factors between each virtual site to 0. By this way, as soon as a ligand binds on the site, the binding affinity of other virtual sites is 0.

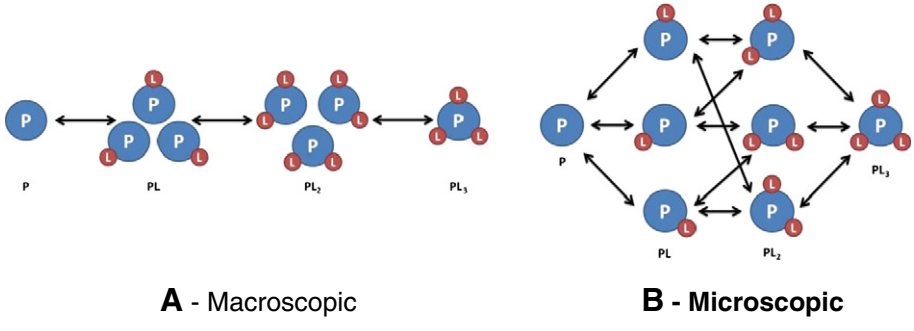

Fig. 1. Comparison of macroscopic and microscopic approaches. The macromolecule P has three sites for ligand L. The different entities with none, one, two or three ligands are represented.