A Footnote on Allostery

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Abstract

A manuscript on allostery signed by Francis Crick and Jeffries Wyman was sent by Crick to Jacques Monod in 1965. Monod transmitted a copy of the manuscript, upon which he had written several comments, to Jean-Pierre Changeux, then a post-doctoral fellow at the University of California Berkeley in the laboratory of Howard Schachman. Changeux provided a copy to Stuart Edelstein, a graduate student in the same laboratory. The manuscript was never submitted for publication, but Edelstein retained his copy since that time and has edited it for publication in the special issue on allostery. The text emphasized the interpretation of the properties of an allosteric oligomer by characterizing its equivalent monomer. The text also developed the concept of the allosteric range and included a simple equation for calculation of the Hill coefficient from the parameters of the Monod-Wyman-Changeux model.

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Editor's Preface

The text by Francis Crick (1916–2004) and Jeffries Wyman (1901–1995) published posthumously here for the first time was written in 1965, principally in reaction to a footnote on page 115 of the article by Monod, Wyman, and Changeux that appeared in the same year in the Journal of Molecular Biology.1 The manuscript circulated among a few scientists interested in allostery who were associated with Jacques Monod's group at the Pasteur Institute, but it was never submitted for publication. Although prompted by the footnote in question, the text also introduces a number of original insights that clearly illustrate extensive reflections on allosteric theory, especially the concepts of “allosteric range” and “equivalent monomer”, which precede the direct discussion of the footnote in the final section of the text. Other mathematical subtleties of allostery that undoubtedly required close examination and extensive reworking of the relevant equations are presented. This effort reveals considerable acumen on the part of Crick in the area of allostery, a subject with which his name is not generally associated.

The original manuscript was skillfully typed except for the more complicated equations, which were handwritten. The text did not contain an abstract, and the headings as they appeared in the original version are maintained here. The original formatting is also respected, except for some minor modifications for consistency and the addition of sequential numbers to the individual equations to facilitate their discussion. Images of two of the pages are presented in Fig. 1 in order to provide a glimpse of the original manuscript, the first with an equation added by hand and the second including the final sentences of the text with a remark added by Jacques Monod as it appears on the photocopy of the entire manuscript in my possession since 1965. To check for any errors of transcription or interpretation, I have validated all equations numerically.

The text presents authorship by F. H. C. Crick and Jeffries Wyman, but the version reproduced here appears to be largely the work of Crick since Wyman in a letter to Crick dated March 30, 1966, suggests that portions of the material were not previously seen by Wyman, who commented “…your very neat and useful n − n' theorem has no very simple proof that I can find, though I have written out one that is not too long and is probably essentially the same as yours".

Wyman’s laudatory remarks, coming from someone with a long and distinguished career in the study of thermodynamics, cooperativity, and linkage relations, confirm Crick’s prowess in a domain in which he had little or no earlier experience. His surprising
and astute \( n - n' \) mathematical relationship for computing the difference between the number of binding sites and the Hill coefficient is Eq. (16) in the edited version presented here. I have generated a graphical description of the underlying principles of the \( n - n' \) theorem presented in Fig. 2 of this preface to facilitate comprehension of its unfamiliar properties. The text states that Eq. (16) can be shown "by rather tedious algebra... One naturally suspects that there is a simple derivation of it, but we have been able to discover it." A compact derivation by Poitevin and Edelstein is published in this special issue.²

Monod drafted "A Third Power Footnote about Allosteric Transitions" to comment on the Crick-Wyman text, which he sent to Wyman with a letter dated October 6, 1965 (the text and the associated correspondence with Crick and Wyman are in the Monod Archives of the Pasteur Institute). Monod’s text is relatively brief and its essential points are discussed in the article in this special issue by Edelstein and Le Novère,³ which analyses in detail the implications of the "allosteric range" for evaluating the cooperativity of allosteric receptors, along with distinctions between Crick and Wyman’s hypothetical equivalent monomers with identical interaction energies for the T and R states versus Monod’s physically realistic functional monomers characterized by stronger interaction energies in the T state. The concept of the allosteric range was previously incorporated (with significant embellishments), under the citation “Crick & Wyman, manuscript in preparation”, in the 1966 article by Rubin and Changeux.⁴

I thank Nicolas Le Novère for his encouragement to pursue publication of this text, his valuable comments on this preface, and his help in obtaining the necessary authorizations kindly provided by Christopher Hilton of the Wellcome Library and Anne Cabot Wyman.

Stuart J. Edelstein
Paris, 12 January 2013

References for Preface

Fig. 1. Images of the original Crick–Wyman manuscript. Upper panel: section “On the Nature of the Saturation Function”. Lower panel: final sentences, with the annotation made by Monod on this copy, underlining “the formation of co-operative oligomers may” and adding by hand below the text: shift from “mathematical abstraction” to physical reality.

Fig. 2. Graphical representation of the equation for \( n - n' \). The equation is illustrated for \( \bar{Y} \) as a function of \( \alpha \) in the lower panel for curves corresponding to trimers (in red) and tetramers (in blue), that is, \( n = 3 \) and \( n = 4 \), respectively. The continuous values of the Hill coefficient, \( n' \), for each \( \bar{Y} \) curve are presented in the middle panel in red (trimers) and blue (tetramers). The top panel presents the corresponding values of \( n - n' \) for \( n = 4 \) as the black continuous curve and the open circles are the solution computed from the relationship on the right side of Eq. (16) from the text by Crick and Wyman:

\[
 n - n' = (n - 1) \left( \frac{\bar{Y}^{n-1}}{\bar{Y}^n} \right)
\]

In this case, \( 4 - n' = 3 \left( \frac{\bar{Y}}{\bar{Y}^4} \right) \). The curves were computed with \( L = 100 \) and \( c = 0.1 \), but the equation for \( n - n' \) holds for any values of \( L > 1 \) and \( 0 < c < 1 \). However, for parameter values producing higher levels of cooperativity, the peaks of the middle panel may exceed the value of 2.0 (up to the limit of \( n \)) and the minimum of the curve in the upper panel may descent below the value of 2.0 (down to the limit of 0).
Introduction

Recently, Monod, Wyman, and Changeux (1965) published a very interesting paper entitled “On the Nature of Allosteric Transitions: A Plausible Model.” Their theory assumed that there are no direct interactions between different sites in an allosteric protein, either between sites for the same ligand or between sites for different ligands. Instead, the protein is considered to exist in just two possible conformations, which they call R and T. Each ligand has a different affinity for these two states, and for this reason, indirect interactions can occur between the binding of the various ligands. In particular, they develop the theory for symmetrical oligomers, and by assuming that a mixed oligomer (in which some of the protomers are in the R configuration and some are in the T configuration) cannot occur, they easily show that their model can give strong co-operative effects.

Toward the end of their paper, they point out that their theory could be applied equally well to an allosteric monomer (n = 1 in their terminology). However, they argue that there are advantageous amplifying properties associated with the molecular symmetry of oligomers and that “the molecular symmetry of allosteric proteins is used to amplify and effectively translate a low-energy signal”. This argument is supported by a footnote (p. 115) giving a simple numerical example that purports to show that a tetramer can be as much as 1000 times as sensitive to a ligand as a monomer.

This footnote has prompted us to look more closely into the mathematical properties of their model. We first introduce the concept of the equivalent monomer and give an easy derivation of the general equation for the binding or any number of different non-competitive ligands. We then discuss some of the properties of the saturation curve for their model in the case of a single ligand. In particular, we consider when it is “symmetrical” and the value of its “slope”. Next we consider the physical restrictions on the various parameters involved and show how much these co-operative or allosteric effects reduce the overall sensitivity of the protein to the substrate. The tables give some numerical examples for certain special values of the constants.

Finally, we consider the argument in the footnote mentioned above, demonstrate that it is misleading, and give an example to illustrate that, under certain conditions, their claim for the “amplification” effect of an oligomer over a monomer is well justified.

The Model Described

We shall consider a model identical in its essential features to that of Monod, Wyman, and Changeux (1965). For a fuller description of this model, the reader is referred to their paper, p. 90. In addition, we shall limit ourselves to what they called (p. 95) “K systems” and will not consider “V systems” in which the two states of the protein have the same binding affinities but differ in their catalytic activities.

The protein is an oligomer consisting of n identical protomers, arranged symmetrically. There are distinct sites for the different ligands F_1, F_2, F_3, ..., F_m. There is no direct interaction between any of these sites, whether they are on the same protomer or they are on different protomers. The protein exists in two and only two distinct configurations, the R state and the T state. All mixed states, in which some of the protomers are in the R state and some are in the T state, are considered to be so unfavorable energetically that we can ignore them. In general, the affinity of a ligand for the T state is different from its affinity for the R state. We define:

$$F_m = \text{concentration (activity) of the } m^{th} \text{ ligand.}$$
$$K_m, K_m' = \text{microscopic dissociation constants for the binding of the } m^{th} \text{ ligand to the R and T states, respectively.}$$
$$\alpha_m = F_m/K_m,$$
$$c_m = K_m/K_m'. \text{ Thus, } \alpha_m c_m = F_m/K_m'.$$
$$R_0, T_0 = \text{concentration (activities) of the R and T states, respectively, with no ligands attached.}$$
$$L = \text{equilibrium constant between the R and T states such that } T_0 = LR_0.$$

The Equivalent Monomer

It is convenient at this point to introduce “the mathematically equivalent monomer” that, for convenience, we shall abbreviate to “the equivalent monomer”. This is a hypothetical monomer that can exist in two states, the r state and the t state closely analogous to the R and T states of the oligomer. It has the following properties:

(1) The microscopic binding constants of all the various ligands have the same values for the equivalent monomer as they have for each protomer in the oligomer.

(2) The free-energy difference between the R and T states is, for the oligomer, n times the corresponding value in the monomer (n is the number of equivalent monomers making up the oligomer).

The crucial assumption is the second one. As stated above, Monod, Wyman, and Changeux used the symbol L for the equilibrium constant between the two states, defining it as

$$T_0 = LR_0 \quad (1)$$

for the oligomer. We shall use, in addition, the terminology

$$t_0 = c_0 \quad (2)$$
where the small letters apply to the equivalent monomer, and we shall retain the capital letters for the oligomer. Thus, the second assumption is equivalent to

$$L = \ell^n \quad (3)$$

Our actual oligomer, in its mathematical properties, can be considered to be constructed by assembling together \(n\) equivalent monomers in such a way that their \(r\) states are completely coupled together and that their \(t\) states are also completely coupled but that this coupling does not affect the free-energy difference per protomer between the two states nor does it affect the microscopic binding constants of any of the ligands.

It is of course important to realize that the equivalent monomer is a mathematical fiction and is not the actual monomer to which the oligomer may dissociate.

The usefulness of the concept of the equivalent monomer springs from the theorem

$$\frac{\sum T}{\sum R} = \left(\frac{\sum \ell}{\sum r}\right)^n \quad (4)$$

In fact, this theorem, which is true for any given concentrations whatsoever of the various possible ligands, applies even if there are direct interactions between different sites on the same equivalent monomer (or protomer) provided there is no direct interaction between any two sites on different protomers.

The theorem is almost self-evident. Imagine that, in the chosen solution of the ligands, the protein molecule is artificially constrained to be in the \(R\) state. Then the amount of the binding of the various ligands to the equivalent monomer (restrained in the \(r\) state) will be exactly the same as to a protomer of the oligomer. In the same way, if the protein is artificially held in the \(T\) state, the amount of the various ligands bound (though different from the amounts bound to the \(R\) state) will once again be the same for the equivalent monomer (restrained in the \(t\) state) as for a protomer of the oligomer. If the equivalent monomer is not constrained, it will spend part of its time in the \(r\) state and part in the \(t\) state. This can be represented by an equilibrium constant between different sites on the same equivalent monomer (or protomer) provided there is no direct interaction between any two sites on different protomers.

The equivalent monomer enables us to derive very easily the general formula for the binding of the \(m\) different ligands. It is easy to show for the equivalent monomer that \(p\) is given by

$$p = \ell(1 + c_1a_1)(1 + c_2a_2)\ldots(1 + c_ma_m) \left/ \left(1 + a_1\right)\left(1 + a_2\right)\ldots\left(1 + a_m\right)\right. \quad (9)$$

Since for the corresponding oligomer

$$\frac{\sum T}{\sum R} = p^n \quad (10)$$

we easily derive the saturation function \(Y_m\) for the ligand \(F_m\) as

$$Y_m = \frac{\left(\frac{a_m}{1 + a_m}\right) + p^n\left(\frac{c_m}{1 + c_m}\right)}{1 + p^n} \quad (11)$$

This useful and general formula can also be derived by considering the binding potential.

The Nature of the Saturation Function

We shall consider first the behavior with a single ligand. For simplicity, we drop the subscripts and write

$$Y = \frac{\left(\frac{a}{1 + a}\right) + p^n\left(\frac{c}{1 + c}\right)}{1 + p^n} \quad (12)$$

and

$$p = \ell(1 + a) \left/ \left(1 + a\right)\right. \quad (13)$$
It is convenient to introduce several new symbols. Following Wyman, we define \( r' \) (which he called \( n \)) by the equation

\[
n' = \frac{d \ln \left( \frac{\bar{Y}}{1-\bar{Y}} \right)}{d \ln \alpha} = \frac{\alpha}{\bar{Y}(1-\bar{Y})} \left( \frac{d \bar{Y}}{d\alpha} \right)
\]  

(14)

Thus, \( n' \) is the slope of \( \bar{Y} \) against \( \alpha \), each plotted on a log scale (a Hill plot). Wyman showed that \( n' \) is unity when there are no co-operative effects and that the slope is usually greatest when \( \alpha \) is close to 1. The midpoint occurs when \( \bar{Y} = 1/2 \). This depends upon \( n \).

We shall assume that \( L \) is an integer. We shall assume that \( c \leq 1 \). This merely amounts to saying which we are calling the R state and which we are calling the T state. \( L \) can take any positive value, but as allosteric effects are usually greatest when \( L \) is large, we shall mainly consider values of \( L \) larger than unity. We first dispose of certain special cases that are of little interest.

1. When \( L = 0 \), the protein is always in the R state.
2. When \( L = \infty \), the protein is always in the T state.
3. When \( c = 1 \), the ratio of the R state to the T state does not change as \( \alpha \) varies (because the ligand has equal affinity for the two states).

In all these cases, the saturation function \( \bar{Y} \) is a simple hyperbola, and \( n' = 1 \). In what follows, we shall not consider these special cases, although we shall consider what happens when \( c = 0 \).

When \( \alpha = 0, \bar{Y} = 0 \); when \( \alpha = \infty, \bar{Y} = 1 \). At all other values of \( \alpha \) (\( c \neq 1; L \neq 0, L \neq \infty \)), the saturation function has the following properties that are easily proved. All other variables being kept constant,

1. A decrease in \( L \) always increases \( \bar{Y} \).
2. An increase in \( c \) always increases \( \bar{Y} \).
3. An increase in \( n \) (\( L \) being constant, not \( i \)) always increases \( \bar{Y} \).

If, on the other hand, \( \ell \) (not \( L \)), \( c \), and \( \alpha \) are kept constant, an increase in \( n \) increases or decreases \( \bar{Y} \) depending on whether \( p \) is less than or greater than unity. If \( p = 1 \), a change of \( n \) makes no difference to \( \bar{Y} \).

The Symmetry of the Binding Curve

We ask under what conditions is the curve of \( \bar{Y} \) against \( \log \alpha \) symmetrical about the midpoint \( \bar{Y} = 1/2 \). This depends upon \( n \).

1. For \( n = 1 \), it is always symmetrical. The midpoint occurs when \( \alpha = \frac{1+L}{1-Lc} \).
2. For \( n = 2 \), it is always symmetrical. The midpoint occurs when \( \alpha = \left( \frac{1+L}{1-Lc} \right)^{1/2} \).
3. For \( n = 3 \) or greater, it is only symmetrical if \( \ell^2 c = 1 \). The midpoint occurs when \( \alpha = \ell = \frac{1}{\sqrt{c}} \).

It is of interest to ask what value of \( p \) has under these various conditions. When \( n \) is greater than 2, the condition for symmetry, \( \ell^2 c = 1 \), makes \( p = 1 \) at the symmetrical midpoint \( \alpha = \ell \). For the case \( n = 1 \) or \( n = 2 \), the curve is always symmetrical, but the midpoint in general does not occur when \( p = 1 \). In the special cases in which \( \ell^2 c = 1 \), however, \( p \) is usually unity at the symmetrical midpoint, when \( \alpha = \ell \), whatever the value of \( n \).

In physical terms, the condition \( \ell^2 c = 1 \) implies that the value of \( p \) when \( \alpha = 0 \) (i.e., \( p = \ell \)) is the reciprocal of the value of \( p \) when \( \alpha = \infty \) (i.e., \( p = \ell c \)). That is, the balance in favor of the T state when \( \bar{Y} \) is zero is exactly equal to the balance in favor of the R state when \( \bar{Y} \) goes to unity. It is thus not surprising that the Hill plot is always symmetrical under these conditions. Conversely, if \( \ell c > 1 \) and \( \ell > 1 \), the T state is favored over the whole range, and if \( \ell c < 1 \) and \( \ell < 1 \), the R state predominates everywhere. In fact, as a rough rule, one can say that whether the T or the R state is the more common depends upon whether \( \ell^2 c \) is greater or less than unity.

In the special case \( c = 0 \), the value of \( p \) is unity when \( \alpha = \ell - 1 \).

The Case of \( n \to \infty \)

One gets some insight into the behavior of the saturation function for higher values of \( n \) by considering the unrealistic case of \( n \) being very large. In these circumstances, \( p^n \) approximates to a step function, the step going from \( p^n = \ell^n \) down to \( p^n = (\ell c)^n \) at the point where \( \alpha = \left( \frac{1-\ell}{1-\ell c} \right) \), since this makes \( p = 1 \), assuming that \( \ell \) and \( c \) are such that \( \alpha \) can attain this value. If \( \ell > 1 \) and \( \ell c < 1 \), then \( p^n \) jumps down from infinity to zero at this point if \( n \) is infinite. Thus, the saturation function will start off, at low \( \alpha \), following the hyperbola \( \bar{Y} = \frac{c_0}{1+\alpha} \), and at the point \( \alpha = \left( \frac{1-\ell}{1-\ell c} \right) \), and will jump up to the hyperbola \( \bar{Y} = \frac{c_0}{1+\alpha} \) as the entire protein swings suddenly from the T state to the R state.

It is easy to see that the binding curve will be symmetrical (plotted against log \( \alpha \)) if the value of \( \bar{Y} \)
just before the jump is equal to the value of \(1 - \bar{\gamma}\) just after the jump. That is, if the jump occurs when \(\alpha\) is given by
\[
\frac{c\alpha}{1 + c\alpha} = 1 - \frac{\alpha}{1 + \alpha}
\]
This equation is satisfied if \(\alpha = \frac{1}{1+c}\). Since the jump occurs when \(\alpha = \frac{1}{1+c}\), these two conditions imply that \(\ell^2 c = 1\), as might have been expected from our previous approach.

**The Maximum Value of \(n'\)**

The parameter \(n'\) is in effect a convenient measure of the slope of the binding curve. It is thus useful to ask how it varies with \(\alpha\). When \(\alpha = 0\) or \(\alpha = \infty\), \(n'\) takes the value 1.0. In between, it rises smoothly to a maximum except when \(n = 1\), when naturally \(n'\) is always unity. The maximum is usually near the point: \(\bar{\gamma} = 1/2\), but need not occur exactly at that value unless the curve of \(\bar{\gamma}\) against \(\log\alpha\) is symmetrical.

By rather tedious algebra, it can be shown that, for an oligomer made of \(n\) protomers, \(n'\) is given by the formula
\[
n' = \frac{(n-1)\left(1 \frac{n-1}{\bar{\gamma}_n} \right)}{\bar{\gamma}_n}
\]
and is thus the saturation function for the oligomer and where \(\bar{\gamma}_{n-1}\) is obtained by substituting \((n-1)\) for \(n\) in this equation. This formula is true for all relevant values of \(n\), \(L\), \(c\), and \(\alpha\). One naturally suspects that there is a simple derivation of it, but we have been unable to discover it. It is easily shown that \(\bar{\gamma}_{n-1}\) is never greater than \(\bar{\gamma}_n\).

We now consider how the maximum value of \(n'\) (as \(\alpha\) varies), which we have called \(n'_{\text{max}}\), varies with \(n\), \(c\), and \(\ell\). We have not obtained the general formula for \(n'_{\text{max}}\) as it appears to be very cumbersome but have instead studied certain special cases. We consider first that \(n\) is given.

(1) For a given value of \(\ell\), but differing values of \(c\), \(n'_{\text{max}}\) is always a maximum when \(c = 0\). For these conditions (i.e., \(c = 0\)), \(n'_{\text{max}}\) is given by
\[
n'_{\text{max}} = n\left(1 - \frac{\alpha_{\text{max}}}{1 + \alpha_{\text{max}}}\right)
\]
where \(\alpha_{\text{max}}\) (the value of \(\alpha\) that makes \(n'\) a maximum) is that value of \(\alpha\) that satisfies the equation
\[
(1 + \alpha)^{n-1}[\alpha(n-1)-1] = L
\]
(2) For a given value of \(c\) (\(c \neq 0\)) but differing values of \(\ell\), \(n'_{\text{max}}\) is a maximum when \(\ell^2 c = 1\) and occurs when \(\alpha_{\text{max}} = \ell = \frac{1}{\sqrt{c}}\). This is the condition that makes the binding curve symmetrical. For this special case, \(n'_{\text{max}}\) is given by the formula
\[
n'_{\text{max}} = \frac{4(n-1)}{(1 + \ell)^2} = \frac{4\sqrt{c}(n-1)}{(1 + \sqrt{c})^2}
\]
Equivalent formulas are
\[
n'_{\text{max}} = \frac{2(n-1)(1 + \ell^2 c)}{(1 + \ell)(1 + c)}
\]
As \(c \to 0\) (and thus \(\ell \to \infty\)), \(n' \to n\).

It is worth pointing out that, when \(\ell^2 c \neq 1\), the value of \(n'\) at the special value of \(\alpha\) which makes \(p = 1\) is given by
\[
n' = \frac{4(n-1)}{(1 + \ell)^2} = \frac{4\sqrt{c}(n-1)}{(1 + \sqrt{c})^2}
\]

**The Allosteric Range**

It is useful to have a parameter to denote how much an allosteric protein changes as the concentration of a ligand goes from zero to infinity. We thus introduce the concept of the "allosteric range", which is the modulus of the difference between the values \(\sum Q_T\) at these two extremes. Thus, if the protein is entirely in the T state when \(\alpha = 0\) and entirely in the R state when \(\alpha = \infty\), then \(Q\), the allosteric range, will be unity. For the case of a single ligand, \(Q\) is given by
\[
Q = \frac{L}{1 + L} - \frac{Lc^p}{1 + Lc^p} = \frac{L(1-c^p)}{(1 + L)(1 + c^p)}
\]
It is easy to see, for a given \(L\), that \(Q\) increases when \(c^p\) decreases. Thus, for a given \(n\), \(Q\) is a maximum when \(c = 0\). For a given \(c\), \(Q\) increases as \(n\) increases (\(c < 1\)).

When \(n\) and \(c\) are both given, we can easily show that \(Q = 1\) is a maximum when \(L^2 c^p = 1\). That is, when \(\ell^2 c = 1\). When this is so, the inaccessible range (1 - \(Q\)) is \(\frac{1}{L^2}\). This is tabulated in Table 2 for various values of \(\alpha_{\text{bis}}\) and \(n\).
inaccessible range is in two parts, one where \( \sum T = \sum R \) is near zero and other one when it is near unity. For the symmetrical case, \( c^2 = 1 \), these two inaccessible regions are equal. However, when \( c = 0 \), the inaccessible region occurs only when \( \sum T = 0 \) is near zero, since when \( \alpha = \infty \), all the protein is then in the R state.

When there is more than one ligand involved, we can carry over the same formalism by using an “effective value” of \( L \). This is the value of \( \rho^n \) when the particular ligand we are considering has zero concentration. Thus, for example, we define

\[
Q_2 = \frac{L_2(1-c_2^0)}{(1+L_2)(1+L_2c_2^0)}
\]

where

\[
\left( L_2 \right)^\frac{1}{n} = \frac{(1+c_1\alpha_1)(1+c_2\alpha_2)\ldots(1+c_n\alpha_n)}{(1+\alpha_1)(1+\alpha_2)\ldots(1+\alpha_n)}
\]

that is, when \( c_2 \) is equal to zero. \( Q_2 \) is thus the allosteric range produced by the ligand \( F_2 \) for given concentrations of all the other ligands. Naturally, its value depends on these other concentrations.

**Physical Limits on the Constants of the Protein**

We must now consider what physical restrictions there will be on the various parameters. Naturally, at this stage that our understanding of protein structure being somewhat primitive, we can only provide suggestive arguments. It will help to consider first the order of magnitude of the energies involved. To do this, we may take hemoglobin as a handy example. Monod, Wyman, and Changeux have fitted the binding curve of hemoglobin (for which \( n = 4 \)) with the parameters \( L = 9054 \) and \( c = 0.014 \). The value of the free energy corresponding to a ratio of 0.014 is \( RT \ln 0.014 \), which is approximately 2.5 kcal. In the same way, the value of \( L \) corresponds to about 5.5 kcal. We thus see that the energies involved are relatively small.

Whatever the ligand (assuming it to be a small molecule), it seems certain that there will be an upper limit to its affinity for the active site of the protein; in other words, that there will be a lower limit to \( K_R \). Moreover, it is reasonable to assume that, in many cases, the protein will have evolved; thus, this lower limit of \( K_R \) is closely approached. Naturally, there will be circumstances in which the physiology of the cell may require a lower affinity, but in most cases, one would suspect that the highest possible affinity would be to the advantage of the organism. We may thus consider \( K_R \) as given. This has the effect of fixing the scale of \( \alpha \) so that we can consider the range of \( \alpha \) as also given. In any case when we come to compare an oligomer with a monomer, the comparison is only meaningful (“fair”) if we assume that, for the purposes of comparison, \( K_R \) is the same for both.

The value of \( K_T \), on the other hand, which is higher than \( K_R \) (remember that \( c = K_R \) and \( c < 1.0 \)), depends upon the affinity for the substrate in the T state of the protein. This affinity could be very much less, since the conformation of the active site in the T state might have little attraction for the substrate. Exactly what upper limit one should put upon \( \frac{1}{L} \) at this stage is unclear, but a value of \( c \) as low as 0.001 might not be unreasonable, assuming that it was an advantage to have it. This is so close to zero that, to a first approximation, we may assume for many purposes that there are no serious restrictions on \( c \).

The value of \( L \) presents greater difficulties. It is a striking fact that, so far, no unmodified allosteric protein has been found, which dissociates into subunits under physiological conditions and concentrations. There may well be a requirement that, in most cases, this should not occur to an appreciable extent. However, protein subunits are large molecules and their affinity for each other could easily be very high, since the area of contact between them will typically be greater than that between a small ligand and the protein. If we make the tentative assumption that, in the monomeric state, the protein occurs largely, if not entirely, in the R configuration, then (since \( L \) is usually large) we see that the R state will disaggregate very much more easily than the T state (in all cases without ligand). Since there is an upper limit to the affinity for aggregation and since we want the R aggregate to be fairly stable, we see that \( L \) cannot be as large as we please. In other words, we cannot make the R state too unfavorable or it would dissociate.

It is difficult to decide how far to trust this argument, but it certainly suggests that there may be an upper limit to \( L \). However, this may be so high that, in practice, the value of \( L \) is likely to be decided by other considerations.

As far as we can see, there are no obvious joint restrictions on \( L \) and \( c \) together, that is, to say one can imagine a protein with \( L \) big and \( \frac{1}{L} \) small, with \( L \) small and \( \frac{1}{L} \) big, or with both of them big. No doubt certain special kinds of conformational change may tie them together, but we cannot see any general restriction.

In summary, then we see that, in considering the design of an allosteric protein

1. The range of \( \alpha \) can usually be taken as given.
2. There are likely to be upper limits on both \( L \) and \( \frac{1}{L} \), but in practice, they may be rather large.
The Design of an Allosteric Protein

We are now in a position to lay down the broad design requirements for a good allosteric protein. In the first place, over the saturation range, the protein must be swung by the ligand from being predominantly in the T state to being mainly in the R state. This implies, as we have seen, that both i and (1/2) should be somewhat greater than unity.

If a rather sharp swing is required, then we need a co-operative effect between several protomers and we shall expect n to be as big as convenient. Presumably, the upper limit to n is set by the difficulty of designing an oligomer with high n having strong interactions between all its protomers.

Assuming then that n is greater than unity and is fixed, we have seen that, for a given i to get the steepest slope on the Hill plot (i.e., to make n as large as possible), we must have i^2c = 1. Actually, the maximum is fairly flat so that i^2c ≈ 1 would be satisfactory. To make n approach n', we make ℓ → ∞ and c → 0. The effect of this, however, is to reduce the overall affinity of the protein for the ligand.

This is best seen by computing some special cases. We first consider the special case be i^2c = 1 and consider how α_{02}, the value of α when Y = 1/2, is related to n'. Now we have already seen that (when i^2c = 1) α_{02} is equal to i. Thus, the higher we make i, the further we shift the binding curve to the right. The expression for n' for the special case i^2c = 1 reduces to

\[ n' = \frac{4i(n-1)}{(1+i)^2} = \frac{4(n-1)\alpha_{02}}{(1+\alpha_{02})^2} \]  \hspace{1cm} (26)

Another special case that is easy to compute is when c = 0. These relationships are displayed in Table 1. The relation between α_{02} and n'_{max}

<table>
<thead>
<tr>
<th>α_{02}</th>
<th>n'_{max} for a dimer</th>
<th>n'_{max} for a tetramer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1^a</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>1.11</td>
<td>1.33</td>
</tr>
<tr>
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<td>1.44</td>
<td>2.33</td>
</tr>
<tr>
<td>10</td>
<td>1.67</td>
<td>3.11</td>
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<tr>
<td>10</td>
<td>1.82</td>
<td>3.52</td>
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<td>1.82</td>
<td>3.46</td>
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<tr>
<td>1.91</td>
<td>3.75</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>1.92</td>
<td>3.77</td>
</tr>
<tr>
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<td>1.90</td>
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</tr>
<tr>
<td>1.60</td>
<td>4.00</td>
<td></td>
</tr>
</tbody>
</table>

Underlined values are for the special condition i^2c = 1. Boldfaced values are for the special case c = 0.

\[ \sum T = 1000 \]  \hspace{1cm} (27)

Taking α = 9, for example, we would have, for a tetramer, \[ \sum T = 0.1 \]. In order to reach the same value for the T/R ratio with a monomeric system, the concentration of F would have to be more than one thousand times larger.

Table 2. The relation between α_{02} and (1 - Q)

<table>
<thead>
<tr>
<th>α_{02}</th>
<th>Monomer ( n = 1 )</th>
<th>Dimer ( n = 2 )</th>
<th>Tetramer ( n = 4 )</th>
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</thead>
<tbody>
<tr>
<td>1^a</td>
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<tr>
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<td>0.0025</td>
<td>0.000</td>
</tr>
<tr>
<td>+∞</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Underlined values are for the special case i^2c = 1.

Boldfaced values are for the special case c = 0.

*a No allosteric effect.

Table 1, where numerical values are given for a dimer (N = 2) and a tetramer (n = 4). It can be seen that the only way to make n' approach n is to make α_{02} large.

Then, the price of allosterism is that the ligand is only bound well at higher concentrations than would be needed if there were no co-operative effects or allosteric interactions. Notice that this argument applies even more strongly to a monomer. Even though n' is then always unity, if i^2c = 1, then α_{02} = i; to get a big allosteric range, we need i to be large, and thus, α_{02} becomes large, as shown in Table 2.

An allosteric protein, therefore, will often be a compromise between the requirements for a large allosteric range and for a high effective affinity for the ligand. The higher the value of n, the easier it is to meet these conflicting demands, as can be easily seen from the numbers in Tables 1 and 2.

The Tetramer–Monomer Comparison

The footnote on p. 115 of the paper of Monad, Wyman, and Changeux (1965)^1 reads

"Consider for example an allosteric system with an intrinsic equilibrium constant (L = T0/R0) of 1000. Assume, that the R state has affinity 1/K'F for a ligand F, and set F/K'F = α. In the presence of the ligand, the ratio of the two states will be

\[ \frac{\sum T}{\sum R} = \frac{1000}{1 + \alpha} \]  \hspace{1cm} (27)"
It is easy to see why this footnote is misleading. The authors have tacitly assumed that the value of $L$ is in some way fixed and have chosen the same value for the monomer as for the tetramer. As we have argued earlier, there is no justification for this, although it is certainly reasonable to assume that $K_R$ is unaltered and that the comparison should be made at the same value of $\alpha$. If we assume, as they do, that $L = 1000$ for the tetramer but select for illustration the value $\sqrt[4]{1000} = 5.6$ for the monomer, then as we have already shown for such cases (since this monomer is mathematically the same as the "equivalent monomer") when $p = 1$, which now occurs when $\alpha = 4.6$, both tetramer and monomer bind exactly the same amount of any ligand. When $\alpha = 9$ (the value they consider), $p = 0.1$ for the tetramer and 0.56 for our monomer. To reduce this latter to 0.1, we require $\alpha = 55$. This is a 6-fold increase in the concentration of F, not more than a thousand times, as they claim.

Therefore, we see that, for the special conditions $p = 1$ that will often occur near half-saturation, it need make no difference at all whether we have a monomer or an oligomer. However, the strong cooperative effects in an oligomer will naturally make a tremendous difference to the shape of the saturation curve on either side of this special point, a feature clearly brought out by the authors in their discussion. It is this effect that makes an oligomer so much more responsive to a ligand. Consider a simple example. Let

\[ n = 3 \text{(a trimer)} \]
\[ \ell = 10 \]
\[ \text{and thus } L = 1000 \]
\[ \text{Take } c = 0 \text{ for simplicity} \]

and consider the effect of an inhibitor whose concentration (using their terminology) is proportional to $\beta$. When no inhibitor is present, we find that $\alpha$ (proportional to the substrate concentration) must be equal to 9 for $\Sigma T = \Sigma R$. At this value, the sites are 45% saturated. What value of $\beta$ will inhibit this to 1%? The necessary formula [from their Eq. (4)] is

\[ \Upsilon_s = \frac{\alpha}{1 + \alpha} \left[ \frac{1}{\ell^n \left(1 + \beta\right)^n + 1} \right] \]  

Putting in the values $\ell = 10$ and $\alpha = 9$, this reduces to

\[ \Upsilon_s = \frac{9}{10} \left[ \frac{1}{(1 + \beta)^n + 1} \right] \]  

We see that, for $n = 1$, we need $\beta = 88$, but for $n = 3$, we need only to have $\beta = 3.5$ to make $\Upsilon = 0.01$. Thus, to inhibit the monomer to 1% activity requires, in this case, about 25 times the concentration of inhibitor needed to do the same thing to the trimer.

In short, the formation of cooperative oligomers may make a very large difference to the behavior of the protein at the extremes of the saturation curve but a much smaller one near the middle.

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allostery; equivalent monomer; allosteric range

**References**