Cooperativity of Allosteric Receptors

Stuart J. Edelstein and Nicolas Le Novère

The Babraham Institute, Cambridge CB22 3AT, UK

Correspondence to Stuart J. Edelstein: stuart.edelstein@unige.ch

http://dx.doi.org/10.1016/j.jmb.2013.03.011

Edited by P. Wright

Abstract

Cooperativity of ligand binding to allosteric receptors can be quantified using the Hill coefficient (nH) to measure the sigmoidal character of the binding curve. However, for measurements of the transition between conformational states, nH values can be misleading due to ambiguity of the reference state. For cooperative ligand binding, the reference state is a hyperbolic curve for a monomer with a single binding site characterized by nH=1. Therefore, binding curves with nH>1 provide a direct measure of cooperativity. For the dependence of the conformational state on ligand concentration, curves with nH<1 are observed, but in virtually all cases, the equivalent allosteric monomer has a value of nH=1. The ratio of the two nH values defines the effective cooperativity and always corresponds to nH=N (the number of protomers in the oligomer) for concerted transitions as specified by the Monod–Wyman–Changeux model. Dose–response curves for homopentameric α7 nicotinic receptors illustrate this relationship for both wild-type and mutant forms. For functional allosteric monomers such as G-protein-coupled receptors, normalization stretches the dose–response curve along the y-axis, thereby masking the “allosteric range” and increasing the apparent cooperativity to a limit for monomers of nH=1. The concepts of equivalent monomer and allosteric range were originally proposed in 1965 by Crick and Wyman in a manuscript circulated among the proponents of allostery, but only now published for the first time in this special issue.

Introduction

Understanding the molecular basis of cooperative interactions has been an active research subject since observations in the early 20th century described the sigmoidal curve for oxygen binding by hemoglobin, designated “heme–heme interactions.” A simple explanation for heme–heme interactions of hemoglobin was proposed by A.V. Hill in 1910 based on the hypothesis that an integral number, n, of oxygen molecules bind simultaneously to hemoglobin. For example, when the fractional oxygen binding, \(Y\), is presented in the form of a “Hill plot,” that is, \(\log[Y/(1-Y)]\) versus \(\log[O_2]\), the sigmoidal curve is linearized and the slope at \(Y=0.5\) provides an index of cooperativity known as the Hill coefficient, nH, with a value for hemoglobin typically of nH≈3. The corresponding curve for myoglobin with a single heme for binding oxygen is hyperbolic and in a Hill plot yields a slope of nH=1, which sets the lower limit of positive cooperativity. Heme–heme interactions are important physiologically, because hemoglobin is nearly saturated by oxygen at the partial oxygen pressure in the capillaries of the lung, while a substantial fraction of the bound oxygen is released at the partial oxygen pressure in the capillaries near active muscle. In contrast, were hemoglobin replaced by myoglobin in vertebrate red blood cells, saturation in the lungs would be complete, but only a small fraction of the bound oxygen would be released.

The pioneering work of Adair in 1925 established that hemoglobin contains four oxygen-binding heme sites. Therefore, the Hill coefficient does not describe the mechanism of oxygen binding, since the value of nH≈3 would imply three rather than four hemes, but the Hill coefficient is nevertheless a useful index of cooperativity on a scale from 1 to N (the number of binding sites and hence the theoretical upper limit to nH). Adair also determined values for the four phenomenological oxygen binding constants that describe the sigmoidal curve but did not propose a mechanistic model to explain the increase in affinity as oxygen binding proceeds.© 2013 Elsevier Ltd. Open access under CC BY-NC-ND license.
The first molecular mechanism for cooperative tetrarsers was proposed by Pauling in 1935 based on interactions between the four sites that are progressively stabilized as oxygen is bound. His equations provided the basis for the sequential Koshland–Nemethy–Filmer model, which postulated a ligand-induced conformation change upon oxygen binding to each subunit.

A turning point in the research on hemoglobin occurred when Perutz and Kendrew provided the first crystal structures of hemoglobin and myoglobin, revealing the close resemblance of each of the four hemoglobin subunits (two α chains and two β chains) to single-chain myoglobin. Moreover, pursuing the observation of different crystal forms in the presence and absence of oxygen by Haurowitz in 1938, Muirhead and Perutz established the structures of the two distinct forms for liganded hemoglobin and deoxyhemoglobin.

Beginning in the 1950s, cooperative interactions were also observed in studies of the kinetics of feed-back inhibited enzymes, as well as for dose–response phenomena of receptor–ligand interactions. The notion of “cooperative interactions” based on the Hill coefficient was applied to enzymes and receptors, although for certain physiological processes, the related terms “ultrasensitivity” and “supralinearity” have also been employed.

Introducing the concept of “allosteric interactions” provided an important step in the understanding of the nature of cooperative interactions and their modulation by effectors binding at allosteric sites that are structurally distinct from the active site. A particularly fruitful model for cooperative interactions to develop further this concept was proposed by Monod, Wyman, and Changeux. The Monod–Wyman–Changeux (MWC) model postulates a spontaneous, concerted equilibrium between two symmetric, oligomeric conformational states, the high-affinity R state and the low-affinity T state. The distribution between the two states is set by their intrinsic interconversion equilibrium in the absence of ligand specified by L, the allosteric constant, where $L = [T]/[R]$. As applied to hemoglobin, the model provided a simple explanation for cooperative oxygen binding based entirely on only the two conformational states of Perutz. Extensions of the MWC model also explained the paradoxical result that mutant hemoglobins with either very high or very low affinities display very low cooperativity. The high-affinity mutants are hyper-stabilized in the R state, whereas the low-affinity mutants are hyper-stabilized in the T state, with cooperativity greatly diminished in both cases.

A salient feature of the MWC model concerns differences in $T$, the binding function, compared to $R$, the state function, defined as the fraction of molecules in the R state. In contrast to the Koshland–Nemethy–Filmer model, the two functions do not necessarily superimpose and this distinction was an important element in support of the MWC model, for example, in early studies on the enzyme aspartate transcarbamylase. In this case, the binding of the substrate analog succinate under the conditions examined is cooperative with a value for $T$ of $n_0 \sim 1.5$. Under the same conditions, measurement of the conformational transition, $R$, by changes in the sedimentation coefficient or PMB reactivity produced a sigmoidal curve significantly to the left of $T$. More recent work on the bacterial flagellar motor has revealed even more striking differences between in $T$ and $R$.

Equilibrium curves for $T$ as a function of ligand concentration are measured by monitoring a conformational probe or functional assay [i.e., ion flow for a ligand-gated channel or downstream response for a G-protein-coupled receptor (GPCR)]. Although the analysis of cooperativity based on the Hill coefficient is often applied to receptors, the applications to dose–response phenomena are complicated by end points that are not at 0 and 1. In contrast to ligand binding measurements with values of $T$ strictly from 0 and 1, the $R$ values for conformational transitions as formulated by the MWC model fall into the range between $R_{\text{min}} > 0$ in the absence of ligand and $R_{\text{max}} < 1$ under conditions of ligand saturation. The concept $Q$, the “allosteric range,” where $Q = R_{\text{max}} - R_{\text{min}}$, was initially introduced in a 1965 manuscript by Crick and Wyman, which is published for the first time in this special edition. Applications of $Q$ were further refined by Rubin and Changeux. Since absolute values of $R_{\text{min}}$ and $R_{\text{max}}$ are not readily determined experimentally, data are commonly normalized to a scale of 0–1, but the resulting stretching in the vertical direction enhances the apparent cooperativity.

While normalization can clearly distort the measurement of cooperativity, interpretations of the true cooperativity of allosteric transitions also require comparison to a monomeric reference state. This point was emphasized in the manuscript by Crick and Wyman by postulating an “equivalent monomer” that can undergo transitions between two states, while emphasizing “that the equivalent monomer is a mathematical fiction and is not the actual monomer to which the oligomer may dissociate.” The “equivalent monomer” is thus distinct from a “functional monomer” that may exist in solution, as in the case of lamprey hemoglobin, which is largely dissociated in the oxygenated state. For mammalian hemoglobins, similar considerations apply, but at the level between tetramers and dimers, since $\alpha_2 \beta_2$ hemoglobin in both the T state and the R state dissociates to high-affinity, non-cooperative $\alpha \beta$ dimers. Functional monomers also provide the basis of allosteric lattice models since the energy of interaction between monomers differs for the T and R states.
Indeed, the concept of quaternary constraint is essential in the MWC formulation for which each protomer is constrained by quaternary bonds in T, in comparison to weaker quaternary bonds in the "relaxed" R state. In contrast, with respect to Crick–Wyman equivalent monomers, the interaction energies within the oligomeric states are strictly identical, as in the applications we have recently described.26 As we elaborate further here, hypothetical conformational transitions for equivalent monomers serve as reference states to measure the cooperativity of oligomeric allosteric proteins.

Considerations of allosteric monomers are further complicated by adding non-symmetric intermediate states to the MWC model in which individual subunits may occupy a tertiary conformation distinct from the quaternary conformation.30–33 Therefore, problems of nomenclature and definition must be addressed to avoid confusions arising from attributing distinct allosteric roles to monomer units under widely different circumstances. Once they are properly characterized, considering hypothetical equivalent monomers aids in revealing the precise degree of cooperativity for conformational transitions of oligomeric allosteric proteins. More directly, equivalent monomers can provide insights for stable functional monomers, such as monomeric GPCRs, in relation to dose–response data, as well as conformational selection of other monomeric proteins as examined recently.34–36 However, it is important to distinguish the concepts of hypothetical equivalent monomers versus physically real functional monomers that may play a significant role for allosteric proteins in certain cases. For this reason, we introduce specific nomenclature for the two cases as described in the following section. The contrasting implications for equivalent and functional monomers are also considered in Discussion, including presentation of distinct energy diagrams.

**Conformational Equilibria of Equivalent Monomers versus Functional Monomers**

While a single-chain protein such as myoglobin provides an unambiguous non-cooperative reference state for comparison with hemoglobin, interpretations are less straightforward for conformational changes measured by $\mathcal{R}$ or for dose–response behavior in general. In contrast to the use of the Hill coefficient to assess $Y$, $\mathcal{R}$ changes in the conformational state as described by the MWC model with interconversion between two symmetrical states are always fully concerted. Hence, the true cooperativity of $\mathcal{R}$ is always given by $N$, the number of subunits.38 For measurements under experimental conditions, the apparent cooperativity can only be evaluated with respect to conformational transitions of equivalent monomeric reference states. Efforts to relate these states to the oligomeric T and R states have taken various forms, including designating the monomer states and their interconversion by lowercase letters, that is, replacing R, T, and L by r, t, and l, respectively.24,33 While logical, this nomenclature can lead to confusions in spoken language. Moreover, it is important to distinguish between hypothetical equivalent monomers ("a mathematical fiction," as noted above) and physically plausible functional monomers. For this reason, we propose a distinct nomenclature for the two cases and adopt $\lambda$ for the allosteric transition of equivalent monomers, as we previously applied, to mark a clear distinction with $L$.29 By analogy, we designate furthermore the equivalent monomers corresponding to the T and R states by the Greek letters tau and rho, $\tau$ and $\rho$, respectively, producing $\lambda = [\tau]/[\rho]$. Concerning functional monomers, we propose representation of the conformational change at the monomer level in terms of two monomeric states by $T^*$ and $R^*$ (read as "T-star" and "R-star") corresponding to the oligomeric states T and R, while emphasizing that this nomenclature is distinct from an earlier usage we made for these terms.29 The equilibrium between $T^*$ and $R^*$ is defined by a monomeric allosteric constant, $L^*$, where $L^* = [T^*]/[R^*]$. The functional monomer is assumed to possess a single ligand binding site; as a result, single-polypeptide proteins with multiple sites, such as calmodulin, would not be included, and as allosteric proteins, they must be treated as heterotetramers.37 For both the equivalent monomeric states, $\tau$ and $\rho$, as well as the functional monomeric states $T^*$ and $R^*$, we assume that they share the same respective affinities for each ligand considered, as do T and R. Finally, we propose retaining the terms t and r solely to designate the tertiary conformation with a quaternary state.33

Based on linkage principles, the relationship of the allosteric constant, $L$, corresponding to T and R oligomeric states with $N$ identical subunits with respect to equivalent monomers is given by $L = \lambda^N$.24,29 However, the equation $L = \lambda^N$ requires identical energies of interaction between protomers in the T and R states, with no particular requirements for their magnitudes (other than the implicit requirement that the T and R states are stable oligomers). This formulation of identical stabilities for T and R, while useful for certain applications (see below), departs from the underlying principle of "quaternary constraint," which implies stronger protomer interactions within the T state compared to the R state, as pointed out by Monod in an unpublished response to the manuscript of Crick and Wyman.38 Hence, a more physically realistic expression for a functional monomer would take the form $L = \left(\frac{K_T}{K_R}\right)^N \left(\frac{K_T}{K_R}\right)^N$, where $K_{N,1}$ and $K_{N,1}$ are the oligomer-to-monomer dissociation equilibrium constants for the R and T.
states, respectively, and \( N \) is the number of monomers (also referred to as protomers) in the oligomer. Fundamentally, the energy difference between the \( T \) and \( R \) states implied by \( L > 1 \) can arise in two different ways. The hypothetical equivalent monomer of Crick and Wyman represents the protomer within the oligomer, with all the transition energy of the oligomer coming from the transition energy of the monomers. In contrast, Monod’s functional monomer represents the free protomer, with the transition energy of the oligomer arising both from the transition energy of the monomers and from stronger inter-subunit interaction in the \( T \) state (see Discussion).

With these concepts in mind, we can return to the evaluation of cooperativity of \( R \). The degree of cooperativity can be estimated directly by the derivative of \( R \) curve, and for this purpose, we have assigned the Greek letter \( \nu \) (nu), where \( \nu = dR/dX \) and \([X]\) is the concentration of ligand.\(^{29}\) The more familiar Hill coefficient can also be used as an empirical measure but will yield reliable results only when judiciously applied to take into account the allosteric range and the relationship to equivalent monomers in the light of the following considerations.

Concerning the allosteric range, the principal difficulty for use of the Hill coefficient with \( R \) measurements is the determination of end points. For ligand binding, measurements of \( Y \) performed by direct experimental methods fix the end points of binding data, since values are readily established at \( Y = 0 \) in the absence of ligand and \( Y = 1 \) at saturating concentrations of ligand. For measurements of \( R \), the reading at zero ligand concentration for whatever signal is being monitored reflects a mixture of \( T \) and \( R \) states, with \( R_{\text{min}} = 1/(1 + L) \). Thus, for values of \( L < 100 \), a significant fraction of molecules will be present in the \( R \) state even in the absence of ligand. Similarly at saturating concentrations of ligand, a significant fraction of molecules may be present in the \( R \) state, since \( R < 1 \). The exact value is given by

\[
R_{\text{max}} = 1/(1 + Lc^\alpha),
\]

where \( c \) is the ratio of ligand dissociation constants of the \( R \) and \( T \) states \( (c = K_R/K_T) \) and \( N \) is the number of ligand binding sites.\(^{24,25,39}\)

Variations in \( R_{\text{min}} \) and \( R_{\text{max}} \) are illustrated for a series of curves with increasing values of the allosteric parameter \( c \) (Fig. 1a). For each curve, the corresponding value of \( \lambda \) is fixed by \( \lambda = 1/\sqrt{c} \). Under these conditions, all of the curves are symmetric about their midpoint at \( R = 0.5 \). Since the end points for \( R \) measurements are fixed by \( R_{\text{min}} > 0 \) and \( R_{\text{max}} < 1 \), the value of the Hill coefficient for any experimentally determined data set will depend on the precise estimation of the end points. This feature of cooperativity is directly related to the allosteric range, \( Q=R_{\text{max}}-R_{\text{min}} \).\(^{24,25}\) For allosteric monomers as described in Fig. 1a, the relationship between cooperativity and allosteric range is simple and \( n_H \), as shown in Fig. 1a (inset). Moreover, for any \( R \) (or dose–response) curve for monomers normalized to the range 0–1, the corresponding value of the Hill coefficient will necessarily be \( n_H = 1 \), as shown in Fig. 1b for the data from Fig. 1a after normalization. Allosteric modulators can influence \( Q \) by altering \( \lambda \) (or \( L \) for oligomers), but the changes can only be ascertained clearly for data that are not normalized.
Reevaluating Cooperativity for α7 Nicotinic Receptors based on Equivalent Monomers

The importance of the equivalent monomers concept can be illustrated by considering published data for nicotinic receptors, particularly α7 homopentamers. Mutations introduced in the gene of α7 corresponding to residues lining the ion channel have been shown to exhibit dramatic gain-of-function phenotypes. For example, V251T displays a dose–response curve shifted to the left (EC₅₀ = 0.002 mM versus 0.1 mM for wild type), with higher cooperativity (nₜ = 2.1 versus 1.3 for wild type). Analysis in terms of the MWC model revealed that these gain-of-function properties could be explained by a substantial reduction in the value of the allosteric constant L. The initial analysis was based on normalized curves as presented in Fig. 2a, with the values of nₜ noted above. However, when the same data are presented without normalization and curves for the corresponding equivalent monomers are added in Fig. 2b, major differences are observed. Cooperativity is unaltered for V251T by normalization because the allosteric range without normalization is close to 1. In contrast, for the wild-type curves, cooperativity without normalization is only nₜ = 0.7, much lower than the normalized value of 1.3. Examination of the cooperativity of the equivalent monomers at the same values of α at which the nₜ of the pentamers were determined gives values of nₜ = 0.42 (V251T) and 0.14 (wild type). Clearly, in both cases, the ratio of pentamer/monomer values is exactly 5.0, in accord with the argument presented above that the intrinsic cooperativity of conformational transitions for the two states of allosteric oligomers must be equal to the number of subunits.

Discussion

By revisiting concepts formulated at the early stages of allostery, their impact on recent observations can be examined. This is particularly fruitful for the exchange initiated in 1965 by Crick and Wyman in reaction to a footnote on page 115 of the MWC article in which the properties of a tetramer and a monomer were compared using the same L value for both. The Crick–Wyman text “A Footnote on Allostery” was originally intended for publication, but as exchanges progressed and Monod drafted “A Third-power Footnote to Allosteric Transitions” for which he planned to associate Changeux and Wyman as co-authors, the impetus to publish waned and the texts never advanced beyond the “unpublished” status. Up to now, the texts and the lively exchanges in related letters were only available in archives of the pro-agonists’ papers at the Wellcome Library, Harvard University, and the Pasteur Institute, but the original manuscript of Crick and Wyman was judged to be of sufficient interest for historical and practical reasons to publish it now in this volume, nearly a half-century after it was drafted. In the associated letters between

Fig. 2. Properties of α7 nicotinic receptors wild-type and V251T mutant. (a) Dose–response (R) curves with normalization. (b) Dose–response (R) curves without normalization. The concentration of acetylcholine is indexed by the pair of dashed vertical lines, with values of nₜ = 0.7 and 0.014 for wild-type pentamers and equivalent monomers, respectively, and nₜ = 0.7 and 0.014 for wild-type pentamers and equivalent monomers, respectively. The value of nₜ for the wild-type equivalent monomers is calculated at the value of α corresponding to the midpoint of the R curve for wild-type pentamers to permit exact comparison. At the midpoint of the R curve for wild-type monomers (★), nₜ = 0.47. Parameter values are c = 0.1 for all curves; V251T: λ = 1.82 (L = λ⁵ = 20); wild type: λ = 15.2 (L = λ⁶ = 8 × 10⁶).
Monod and Crick, the humor, camaraderie, and obvious pleasure of friendly disputation are fully in evidence as they debate conflicting intentions to "Mother Nature." Some aspects of the exchange have been discussed previously in the context of a wider examination of the interactions between Monod and Wyman, and they are further developed in the article in this volume by H. Buc.\(^{45}\)

Since the intentions of Mother Nature remain moot points, the importance for posterity lies in the concepts of equivalent monomer and allosteric range first suggested by Crick and Wyman. With respect to the former, the principle contribution of Monod was to emphasize that the relationship between monomer and oligomer summarized by the equation \(L = \lambda^N\) requires identical inter-monomer binding energies for the T and R states. In other words, \(\lambda\) is fixed by \(\lambda = \sqrt[3]{L}\). For this reason, we also defined a distinct relationship, as discussed above, that would apply to a functional monomer, with a monomer allosteric constant given by \(L^*\) involving in addition distinct interaction energies between protomers of the R and T states. As emphasized in the MWC article, the physical basis of the allosteric model is the tighter interactions between protomers in the T state than in the R state, constituting the concept of "quaternary constraint."\(^{19}\) In this case, \(L = (L)^N (K_{N,1}^R / K_{N,1}^T)\), as defined earlier in this text. In contrast to \(\lambda\), which depends exclusively on \(L\) and \(N\), the relationship between \(\lambda\) and \(L^*\) is then given by \(\lambda = (L^*)^N / (K_{N,1}^R / K_{N,1}^T)\). In other terms, the energy of conformational transition for the equivalent monomer is equal to the sum of the energy of the conformational transition for the functional monomer plus the difference in the binding energies of the monomers in the oligomer.

A graphical representation of the distinctions between equivalent monomers and functional monomers is presented in Fig. 3 in terms of free-energy differences for the two cases. In the case of equivalent monomers, the energy of stabilization of the unliganded oligomeric T state compared to the R state is precisely \(N\) times the energy of stabilization of the monomeric unliganded t state compared to the p state, but the energy difference between monomers and oligomers is never defined, other than to assume it is large enough to stabilize the oligomers at physiological concentrations. In the case of functional monomers, for the hypothetical parameter values selected, the unliganded oligomeric T state is more stable than the R state, but in keeping with the concepts of "quaternary stability" and the "relaxed" condition of the R state, at the monomeric level, R* is more stable than T*.

The concepts of allosteric range and equivalent monomer are particularly relevant for allosteric receptors, since their functional properties are generally quantified by dose–response measurements directly related to \(R\), in comparison to enzymes or binding proteins such as hemoglobin more commonly characterized with respect to \(Y\). For estimations of the degree of cooperativity for dose–response data, the concept of allosteric range is indispensable in order to recognize the consequences of normalization of the experimental results, which increases the estimated value of the Hill coefficient \(n_H\) by vertical stretching of the data. This effect was illustrated for data obtained for \(\alpha_7\) nicotinic receptors (Fig. 2), for which normalization increased the apparent value of \(n_H\) for wild-type receptors from 0.7 to 1.3. It is therefore important to recognize the distinctions arising from normalization. These effects can be contrasted to stretching

---

**Fig. 3.** Energy diagrams for oligomer–monomer interactions for equivalent monomers in the left panel and functional monomers in the right panel. For the T state, on the left, the dissociation constant for the T oligomers to \(\tau\) monomers, indicated by \((a)\), is identical with the constant for R state dissociation to \(\rho\) monomers, that is, \((a) = (b)\), as required by the relationship \(L = \lambda^N\). In contrast, on the right panel, the dissociation constant for the T state, indicated by \((a')\), corresponds to stronger interactions, that is, \((a') > (b')\). In this case, \(L > 1\), but \(L' < 1\). Energies are calculated for the various parameters indicated using standard thermodynamic values.\(^{46}\) The T state is in blue and the R state is in green.
along the horizontal axis due to ligand depletion, which can reduce the apparent cooperativity.29

Considerations of multiple effectors binding to an allosteric protein can also lead to the opposite effect of contraction of the horizontal axis due to accretion of sequestered ligands.47

While many of the concepts presented here are of theoretical interest, applications to monomeric forms of GPCRs may be of more practical concern, particularly with respect to models of functional monomers rather than equivalent monomers. Although the range of GPCR is vast, many common properties appear across the spectrum of diversity.48 Special features of GPCRs must be taken into account such as multiple conformational states with distinct functions and ligands with distinct signaling profiles.49–51 In addition, various forms of homo- and hetero-oligomerization are involved and can alter the dynamic range because oligomerization increases the L value.52–54 The concept of dynamic range to interpret data without normalization is reflected by the importance of inverse agonists and related effects produced by modulators that act at allosteric sites.54–56 Similar reasoning leads to the understanding that ligand molecules with apparent properties of competitive agonists in systems with high L values can be revealed to be partial agonists at lower L value.43 Constitutively active GPCRs confirm the presence of preexisting allosteric equilibria between conformations, providing insights into both normal and pathological receptors.56–58 GPCRs are therefore a rich source for extensions of the allosteric principles, particularly in the light of recent advances in structural studies.59

Future developments of allostery in the domain of GPCRs are likely to bring additional insights.

Acknowledgement

We thank Benedetta Frida Baldi for helpful comments.

Received 12 February 2013; Accepted 1 March 2013

Available online 21 March 2013

Keywords:
pentameric ligand-gated channels; GPCRs; Hill coefficients; equivalent monomers; allosteric range

Abbreviations used:
GPCR, G-protein-coupled receptor; MWC, Monod–Wyman–Changeux

References


