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Thermodynamic *versus* Kinetic Discrimination of Cooperativity of Enzymatic Ligand Binding

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Abstract

In this work, we have introduced thermodynamic measure to characterize the nature of cooperativity in terms of the variation of standard free energy change with the fraction of ligand-bound sub-units of a protein in equilibrium, treating the protein-ligand attachment as a stochastic process. The fraction of ligand-bound sub-units of cooperative ligand-binding processes are calculated by the formulation of stochastic master equation for both the KNF and MWC Allosteric cooperative model. The proposed criteria of this cooperative measurement is valid for all ligand concentrations unlike the traditional kinetic measurement of Hill coefficient at half-saturation point. A Kullback-Leibler distance is also introduced which indicates how much average standard free energy is involved if a non-cooperative system changes to a cooperative one, giving a quantitative synergistic measure of cooperativity as a function of ligand concentration which utilizes the full distribution function beyond the mean and variance. For the validation of our theory to provide a systematic approach to cooperativity, we have considered the experimental result of the cooperative binding of aspartate to the dimeric receptor of Salmonella typhimurium.

Keywords: Cooperativity of ligand binding; Free energy; Kullback-Leibler distance; Hill coefficient

Introduction

Cooperative binding of ligands to multimeric protein is a very important biological process [1-6]. It plays a crucial role in the physiological regulation of protein [7] and enzyme function [8]. Many proteins found in nature are multimeric consisting of two or more subunits usually linked to each other by non-covalent interactions [9]. Depending on the binding affinity of the ligands to the multimeric proteins, the cooperative binding kinetics can be generally classified as positive, negative and non-cooperative [6,10-12]. The phrase 'binding affinity' is a thermodynamic term which refers how strong the binding is and it is expressed in terms of the standard free energy change, ΔG^0 [13]. Usually ΔG^0 is determined in terms of the experimentally measurable equilibrium binding constant, Keq using the relation $\Delta G^0 = -RT \ln Keq$ where R and T are the universal gas constant and absolute temperature, respectively [1-16]. This relation can assist to determine the nature of cooperativity, particularly if one considers the binding of ligands to the protein in a stepwise manner [1-5,10]. For example, if the value of K_{eq} increases in successive binding steps, ΔG^0 becomes more negative and the protein shows positive cooperativity [13- 17]. The case is reverse for negative cooperative process. If the value of K_{eq} remains the same for all the binding steps, no cooperativity will be observed [13-17].

Indeed, the thermodynamic characterization of cooperativity in terms of ΔG^0 is a well accepted approach in biophysics [13,14-17]. But, it is hard to the unavailability of all the successive equilibrium binding constant values [18,19]. To avoid the problem, experimentalists usually prefer to characterize the nature of cooperativity of a protein in terms of the Hill coefficient [20,21] which is defined in terms of the fraction of occupied sites, θ that can be easily estimated from the kinetic experiments [20,21]. However, this method does not yield any details of the stepwise binding mechanism generating the resultant cooperative behavior. Recently, Bordbar et al. proposed a theory to characterize cooperativity in terms of ΔG^0 and the Hill coefficient [18]. The theory has been constructed on the basis of the consideration that only one intermediate step is experimentally separable among all the intermediate binding steps [18]. But sometimes it is possible to separate more than one intermediate-binding steps in the ligand-binding assay. Later we have studied entropy production of cooperative binding of substrate molecules on the active sites of a single oligomeric enzyme in a chemiostatic condition to characterize spatial and temporal binding in enzyme catalysis [22-24] So construction of an unified theory is essential which provides the thermodynamic as well as kinetic description of cooperativity.

Keeping the above developments in mind, here we have provided a thermodynamic characterization of cooperative binding of ligands to a single multimeric protein using the chemical master equation. We have considered two seminal models of allosteric cooperativity, i.e., Koshland-Nemethy-Filmer (KNF) and Monod-Wyman-Changeux (MWC) models to describe the cooperative ligand-binding process in equilibrium. We have calculated ΔG^0 for the overall ligand-binding process in both the models. We have introduced a thermodynamic variable by which the nature of cooperativity as well as some thermodynamic information about such cooperative process can be obtained. To examine the acceptability of our proposed thermodynamic variable, we have applied it on the cooperative Aspartate binding to the dimeric receptor of Salmonella typhimurium and judged the results against the experimental conclusions [25]. It is observed that the description of the nature of cooperativity tally very

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well with that of the Hill coefficient estimated from the experiment [25]. We have also discussed on another estimate of cooperativity using the relative entropy or the Kullback-Leibler distance [26] in terms of the standard free energy change. This measure is well known in the information theory literature and has been used in various contexts, a particularly relevant case being the measure of non-exponentiality of waiting-time distributions applied on protein folding statistics [27].

In what follows we have discussed on the cooperative binding and Hill coefficient from the chemical master equation in section II. Then we have introduced a measure of cooperativity in terms of the Gibbs free energy in section III. We have numerically explored the theory on step-wise Aspartate receptor binding in section IV. In section V, we have studied the cooperativity entropy in terms of Kullback-Leibler distance. The paper is concluded in section VI.

Description of Cooperative Binding using Chemical Master Equation and Hill Coefficient

In the protein-ligand binding kinetics, ligands usually bind to the subunits of the protein in a stepwise manner with different affinities. This was first proposed by Adair to explain the cooperativity phenomenon observed in the oxygen-binding to hemoglobin at equilibrium [2]. By extending the concept of Adair, later Koshland, Nemethy and Filmer and Monod, Wyman and Changeux proposed two different models (KNF and MWC models, implement for protein-ligand binding having a large number of steps due to respectively) to describe the allosteric cooperativity. In this section, we have provided a stochastic description of the ligand-binding kinetics for an oligomeric enzyme for KNF and MWC models.

The KNF model

Here we have discussed the cooperative ligand-binding kinetics for a single multimeric protein by considering the KNF model through master equation approach. The binding scheme is depicted in (Figure 1) for a multimeric protein consisting of n_T number of identical subunits. The dynamics of the ligand binding mechanism has been explained by counting the number of sub-units of the protein molecule occupied by ligands at a particular instant of time. As the reactions occur randomly so the number of occupied sites becomes a fluctuating quantity [38-40]. Here $k_1'^{(n-1)}$ and $k_{-1}^{(n)}$ are the association and dissociation rate constants in the nth reaction step, respectively. We have taken the pseudo first-order rate constant] with the constant ligand concentration $k_1^{(n-1)} = k_1'^{(n-1)}$ [L]. If 'n' number of occupied sites are present at time t, then at time $t + dt$, the number may increase or decrease by one unit due to an association or a dissociation reaction. So the master equation for this cooperative binding mechanism can be written as

$$\frac{\partial P^{KNF}(n,t)}{\partial t} = k_1^{(n-1)}(n_T - n + 1)P_{KNF}(n-1,t) + k_{-1}^{(n+1)}(n+1)P^{KNF}(n+1,t) - k_1^{(n)}(n_T - n)P^{KNF}(n,t) - k_{-1}^{(n)} n P^{KNF}(n,t) \quad (1)$$

Here, $P^{KNF}(n,t)$ is the probability of having n number of occupied sites at time t with $k_1^{(-1)} = k_{-1}^{(0)} = k_1^{(n_T)} = k_{-1}^{(n_T+1)} = 0$ to match the boundary terms. Solving the master equation (Equation 1) at the steady state, we obtain the probability distribution, $P^{KNF}(n)$ as well as the fractional occupancy, θ^{KNF} as

$$P^{KNF}(n) = \frac{I_n}{\sum_{n=0}^{n_T} I_n}, \quad (2)$$

and

$$\theta^{KNF} = \langle n \rangle^{KNF} / n_T = \frac{\sum_n n I_n}{n_T \sum_{n=0}^{n_T} I_n}, \quad (3)$$

where $(n)^{KNF}$ is the average number of occupied sites at steady state. Here $I_n = \binom{n_T}{n} \prod_{j=0}^{n-1} Z^{(j)}$ and the stepwise equilibrium constants are defined as $Z^{(j)} = K^{(j)}$ [L] with $K^{(j)} = k_1'^{(j)} / k_{-1}^{(j+1)} (j = 0,1,....,(n_T - 1))$. For describing the cooperative ligand-binding process, here we have considered $K^{(j)} = f^j K^{(0)}$. So In becomes, $I_n = \binom{n_T}{n} f^{n(n-1)/2} (Z^{(0)})^n$. The parameter f induces the cooperativity in ligand binding in this scheme. For $f > 1$, the successive equilibrium constants continue to increase giving rise to positive cooperativity, whereas for $f < 1$, they go on decreasing resulting in negative cooperativity. For $f = 1$, the system becomes non-cooperative with the probability distribution

$$P_{(nc)}^{KNF}(n) = \binom{n_T}{n} \frac{[Z]^n}{[1+Z]^{n_T}}, \quad (4)$$

where $Z^{(j)} = Z^{(0)} = Z \forall j$ and $Z = k_1 / k_{-1}$. By inserting the value of Z, the above equation can be written as a binomial distribution [38] given by

$$P_{(nc)}^{KNF}(n) = \binom{n_T}{n} \left(\frac{k_1}{k_1 + k_{-1}}\right)^n \left(\frac{k_{-1}}{k_1 + k_{-1}}\right)^{(n_T - n)} \quad (5)$$

In this case, the average number of occupied sites at steady state can be written as

$$\langle n \rangle_{(nc)}^{KNF} = n_T \left(\frac{Z}{1+Z}\right) = n_T \left(\frac{k_1}{k_1 + k_{-1}}\right) \quad (6)$$

The fractional occupancy, $\theta_{nc}^{KNF} = \frac{\langle n \rangle_{(nc)}^{KNF}}{n_T}$ is independent of the number of sub-units of the protein molecule.

The MWC scheme

In this section, we have described the cooperativity of the allosteric protein-ligand binding kinetics [3] by considering the MWC scheme. In this scheme, the sub-units of an oligomeric protein remains in two conformations, R and T, which are in equilibrium. However, they differ according to their affinities for the ligand with the R conformation being preferred. The protein exists largely in the T conformation in the absence of ligand. However, as the R conformation has greater affinity for the ligand, the conformational equilibrium is gradually shifted to the R state in the presence of ligand. The ligand binding kinetics can be described in terms of the number of bound subunits with the protein being in R or T conformation. For a single oligomeric enzyme, this quantity is a fluctuating one. In (Figure 2), we have schematically described the MWC model for ligand-binding kinetics where the protein has n_T number of sub-units. Here k_s^+[L] and k_s^- are the forward and the backward transition rates between the dynamical states of the system and [L] is the constant ligand concentration. The corresponding master equations for the two conformations, R and T can be written as

$$\frac{\partial P_{(R)}(n,t)}{\partial t} = k_R^+[L](n_T - n + 1)P_R(n-1,t) + k_R^-(n+1)P_R(n+1,t) - k_R^+[L](n_T - n)P_R(n,t) - k_R^- n P_R(n,t) - v_R(n)P_R(n,t) + v_T(n)P_T(n,t) \quad (7)$$

and

$$\frac{\partial P_T(n,t)}{\partial t} = k_T^+[L](n_T - n + 1)P_T(n-1,t) + k_T^-(n+1)P_T(n+1,t) - k_T^+[L](n_T - n)P_T(n,t) - k_T^- n P_T(n,t) - v_T(n)P_T(n,t) + v_R(n)P_R(n,t) \quad (8)$$

Here $P_s(n,t)$ (s =R,T) is the probability of the system to remain in the state, n at time t. For ion channel, n denotes the state with n number sub-units in active state (for ligand-binding, n represents the state with n number of bound sub-units). $v_R(n)$ and $v_T(n)$ are the transition rates from conformation R to T and vice versa in the n^{th} state, respectively. The steady state solution of the master equation can be written as

where

$$P^{MWC}(n) = P_R(n) + P_T(n) \quad (9)$$

$$P_R(n) = \binom{n_T}{n} \frac{K_R^n [L]^n}{z([L])} \quad (10)$$

and

$$P_T(n) = \binom{n_T}{n} \frac{Dr^n K_R^n [L]^n}{z([L])} \quad (11)$$

Here $z([L]) = (1+K_R[L])^{n_T} + D(1+K_T[L])^{n_T}$. The equilibrium constants are defined as

$$K_R([L]) = k_R^+([L])/k_R^-([L]), K_T([L]) = k_T^+([L])/k_T^-([L]), r = K_T([L])/K_R([L])$$

and $D = v_R(0)/v_T(0)$. Here D is independent of the concentration of ligand. By using the solution of the master equations, the average fractional occupation, θ^{MWC} can be written as

$$\theta^{MWC} = \langle n \rangle^{MWC} / n_T = \frac{\sum_{n=0}^{n_T} n\left(P_R(n) + P_T(n)\right)}{n_T}$$

$$= K_R[L] \frac{(1+K_R[L])^{n_T-1} + Dr(1+rK_R[L])^{n_T-1}}{z([L])}, \quad (12)$$

where $(n)^{MWC}$ is the average number of occupied sub-units of the protein at steady state when the ligands bind to the protein according to the MWC scheme.

Estimation of hill coefficient

Traditionally cooperativity is characterized by the Hill coefficient [34,35,41]. For positive and negative cooperative cases, the Hill coefficient becomes greater than or less than one, respectively. The non-cooperative case has unit Hill coefficient. Experimentally it is obtained by determining the fractional saturation, θ (= $(n)/n_T$) at various ligand concentrations [L], constructing the Hill plot [34] ($ln(\theta/1-\theta)$ *vs* $ln[L]$) and then finding the slope at the halfsaturation point, θ = 0-.5 or at a point where the slope deviates maximum from unity. On the other hand, Hill coefficient is theoretically defined as the ratio of the variances of the binding number of the cooperative and non cooperative cases at the half-saturation point [31-34,35].

Here we briefly mention the features of the Hill plot for both the KNF and MWC model binding schemes. The Hill slope, H can be generally written as [35]

$$H = \frac{[L](d\theta/d[L]}{\theta(1-\theta)}. \quad (13)$$

Now the fractional saturation in our case can be written as see (Equation 3)

$$\theta = \frac{\sum_n nB_n[L]^n}{n_T \sum_n B_n[L]^n}, \quad (14)$$

where $B_n = \binom{n_T}{n} \prod_{j=0}^{n-1} K^{(j)}$ with $B_0 = 1$. Then one gets

$$H = \frac{\langle n^2 \rangle - \langle n \rangle^2}{n_T \theta(1-\theta)} = \sigma_{coop}^2 / \sigma_{bino}^2, \quad (15)$$

where σ_{coop}^2 and σ_{bino}^2 are the variances of the binding numbers of the cooperative and non-cooperative cases, respectively. The Hill coefficient, n_H is given at the half-saturation point as

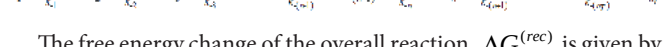
$$n_H = 4\sigma_{coop}^2 / n_T \quad (16)$$

Thermodynamic Description of the Cooperative Ligand Binding Process

In this section, we have calculated the free energy change in the cooperative ligand-binding process and introduced a thermodynamic variable defined in terms of the rate of change of ΔG^0 with θ. This quantity can act as an indicator of cooperativity in equilibrium. We have also calculated the cooperativity free energy in terms of the Kullback-Leibler distance which is widely used in information theory.

Calculation of the free energy change due to binding of ligands to amultimeric protein

Here we have estimated the free energy change due to the binding of ligands to a multimeric protein in equilibrium. Let us first consider a linear chemical reaction network with n_T number of species like



The free energy change of the overall reaction, $\Delta G_{tot}^{(rec)}$ is given by

$$\Delta G_{tot}^{(rec)} = \sum_{i=1}^{n_T-1} \Delta G_{X_i, X_{i+1}}^0 + \sum_{i=1}^{n_T-1} RT \ln \frac{[X_{i+1}]}{[X_i]} \quad (17)$$

Here $\Delta G_{X_i, X_{i+1}}^0$ is the standard free energy change due to the transformation of species X_i to X_{i+1} and is defined as $\Delta G_{X_i, X_{i+1}}^0 = -RT \ln K_i^{eq}$ with $K_i^{eq} = k_i / k_{-i}$. $[X_i]$ is the concentration of the species X_i.

Using (Equation 17), we can determine the free energy change of a proteinligand binding kinetics where n_T number of sub-units of a single protein get gradually occupied by the ligands. In this case, the concentration terms of (Equation 17) should be replaced by the corresponding probabilities of the relevant binding states and R would be changed to the Boltzmann constant, kB. The reaction scheme of this linear chemical reaction is similar with the scheme of the KNF model of allosteric cooperativity (Figure 1). Therefore, the overall free energy change for the KNF scheme, $\Delta G_{tot}^{(KNF)}$ can be calculated as

$$\Delta G_{tot}^{(KNF)} = \sum_{n=0}^{n_T} \Delta G_{(n-1),n}^{(KNF)} = \sum_{n=0}^{n_T} \Delta G_{(n-1),n}^{0(KNF)} + \sum_{n=0}^{n_T} k_B T \ln \frac{P(n)}{P(n-1)} = \Delta G_{tot}^{0(KNF)} + k_B T \ln \frac{P(n_T)}{P(0)} \quad (18)$$

Here P(n-1) and P(n) are the steady state probabilities of the conformations, (n-1) and n, respectively. $\Delta G_{(n-1),n}^{0(KNF)}$ is the standard free energy change due to changing the $(n-1)^{th}$ protein's conformation to n^{th} conformation. $\Delta G_{tot}^{0(KNF)}$ is the standard free energy change for the overall process. P(0) and $P(n_T)$ are the steady state probabilities of the protein's conformations when it remains fully vacant and fully occupied by the ligands, respectively. At equilibrium $\Delta G_{tot}^{0(KNF)}$ becomes zero. So (Equation 18) becomes

$$\Delta G_{tot}^{0(KNF)} = -k_B T \ln \frac{P(n_T)}{P(0)} \quad (19)$$

Now substituting the values of $P(n_T)$ and P(0) from (Equation 2) into (Equation 19), we obtain

$$-\frac{\Delta G_{tot}^{0(KNF)}}{k_B T} = -\Delta \bar{G} = n_T \ln Z^{(0)} + (n_T - 1) \ln f \quad (20)$$

For notational simplicity, here we have considered. From $\frac{\Delta G_{tot}^{0(KNF)}}{k_B T} = \Delta \bar{G}$ (Equation 20), it is observed that $\Delta \bar{G}$ depends only

on the equilibrium ligand-binding constant of the first step, $Z^{(0)}$, the cooperativity inducing parameter, f and the total number of sub-units, n_T . Using this relation, one can easily determine $\Delta\overline{G}$ for a non-cooperative ligand-binding process by measuring only $Z^{(0)}$ as f becomes unity for this process. However, for positive and negative cooperative cases, when $f \neq 1$ then it becomes essential to measure the individual equilibrium binding constants experimentally to determine f.

To compare the standard free energy change for positive and negative cooperative processes, we write the above equation (Equation 20) as

$$-\Delta G^{-}_{\pm c} = n_T \ln Z^{(0)} + (n_T - 1)\ln f^{\pm} \quad (21)$$

The notations +c and −c indicate positive and negative cooperativity, respectively. Similarly, f^{+} and f^{-} are the cooperativity inducing parameter for positive and negative cooperative processes, respectively. For simplicity,

here we have considered $f^{+} = f = 1/f^{-}$. Then we obtain

$$\left[-\Delta\overline{G}_{+c}\right] - \left[-\Delta\overline{G}_{-c}\right] = (n_T - 1)\ln\left(\frac{f^{+}}{f^{-}}\right) = (n_T - 1)\ln\left(f^{2}\right) = 2(n_T - 1)\ln f \quad (22)$$

As $f \geq 1$, so we can say that standard free energy change of a positive cooperative binding process will be always greater than that of negative cooperative process.

Free energy as an indicator of cooperativity

For a better understanding about the cooperative binding process from the thermodynamic point of view, here we have calculated the change of the conformational free energy, $\Delta\overline{G}$ with respect to the fraction of binding sites, θ. Taking the derivative of the expression of $\Delta\overline{G}$ in (Equation 20)with respect to θ, we obtain

$$-\frac{d\Delta\overline{G}}{d\theta} = S = \frac{n_T}{[L]}\frac{d[L]}{d\theta} \quad (23)$$

Now for a certain increment of θ, the required change in ligand concentration is smaller for positive cooperativity compared to that of the negative cooperativity. Therefore, the value of $\frac{d[L]}{d\theta}$ in the right hand side of (Equation 23) is expected to be less for positive cooperative cases than that of the negative one. As a result, at a certain ligand concentration, the rate of change of $\Delta\overline{G}$ with θ becomes smaller for positive cooperative cases than negative cooperativity. In thermodynamics, the Gibbs free energy is related to the non-mechanical work performed by the system. So we can say that work done due to a certain increment of θ is smaller for positive cooperative binding than that of the negative cooperative binding indicating more efficient binding in positive cooperativity. Now putting $\frac{d[L]}{d\theta}$ from (Equation 13) into (Equation 23), we obtain

$$S = \left[\frac{n_T}{H(1-\theta)\theta}\right] \quad (24)$$

In (Equation 24), the Hill slope H, fraction of occupied site, θ and total number of sub-units, n_T all are experimentally measurable quantities. Hence, by using this expression, one can easily calculate the standard free energy change with θ. Usually experimentalists measure the Hill coefficient, n_H which is the Hill slope at θ = 0.5 where (Equation 24) becomes

$$s\big|_{\theta=0.5} = \left[\frac{4n_T}{n_H}\right] \quad (25)$$

For non-cooperative cases, the Hill coefficient n_H becomes unity and the corresponding value of S will be $4n_T$. For positive cooperative cases, the value of n_H is greater than unity and for the negative cooperative cases its value becomes less than unity. Hence, at θ = 0.5, the value of S will be greater than $4n_T$ for negative cooperative cases and for positive cooperativity, S should be less than $4n_T$. Therefore, the quantity, S can characterize the nature of cooperativity in terms of the thermodynamic quantity, ΔG^0. It is also important to note that by using the Hill coefficient one can identify the nature as well as the degree of cooperativity. However, no information about the efficiency of the ligand binding process can be directly obtained form it. From the thermodynamic approach, one can also get some information regarding this. Using the expression of n_H from (Equation 16) and putting this into (Equation 24), we obtain

$$s\big|_{\theta=0.5} = \left(\frac{n_T}{\sigma_{coop}}\right)^2 \quad (26)$$

By determining the distribution of a cooperative ligand-binding process, one can easily calculate the variance of the binding number and hence, **S** at θ = 0.5.

Cooperativity entropy and free energy: Kullback-Leibler formalism

In defining the cooperativity index in our earlier work, we had used the probability associated with the fully bound state of the protein and not the binding probability distribution. Now in this work it is useful to compare the entire probability distributions corresponding to the cooperative and the non-cooperative cases that should, in principle, give further understanding of the phenomenon. A natural choice comes from the field of information theory called the relative entropy or the Kullback-Leibler (KL) distance [26]. The KL distance between two probability distributions $u(n)$ and $v(n)$ is defined as

$$D(u\|v) = \sum_n u(n)\ln\left(\frac{u(n)}{v(n)}\right) \quad (27)$$

where we take $u(n)$ to be the reference distribution [36,26]. $D(u\|v) \geq 0$ always but it is not a metric [36]. Here we give an information entropic estimate of the amount of cooperativity in the ligand binding in terms of the KL distance where obviously the non-cooperative system with binomial distribution is the reference. Hence we can measure the amount of cooperativity quantitatively in terms of the relative entropy between the binomial distribution, $P^{(bino)}(n)$ and the distribution of the cooperative system as

$$D(P^{(bino)}\|P^{ss}_{coop}) = \sum_n P^{(bino)}(n)\ln\frac{P^{(bino)}(n)}{P^{ss}_{coop}(n)} = -S^{(bino)}_{sys} - \sum_n P^{(bino)}(n)\ln(P^{ss}_{coop}(n)) \quad (28)$$

Here $S^{(bino)}_{sys}$ is the system Shannon entropy or information entropy [36] associated with the binomial distribution and the cross term in the above equation actually gives the contribution of cooperativity. With $P^{ss}_{coop}(n) = P^{(bino)}(n)\forall n$, the KL distance becomes zero for a non-cooperative system. So here we call the relative entropy as the cooperativity entropy, S_{coop} because a non-zero value of S_{coop} indicates the system to be cooperative. As it can not be negative, it is unable to detect the nature of cooperativity. Despite that, if one knows the nature of the cooperativity by some other means then the variation of the cooperativity entropy with ligand concentration can give valuable insight on the evolution of such cooperative behavior.

The cooperativity free energy can be interpreted in terms of the

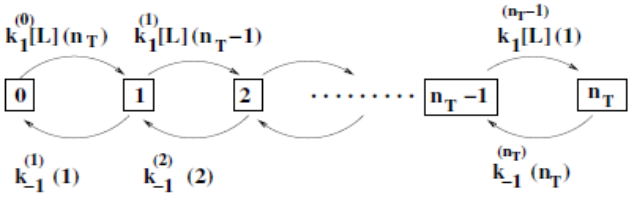


Figure 1: Schematic diagram of the KNF model and are the association and dissociation reaction rates of the dynamical states of the system expressed in terms of the number of occupied sites by the ligands and [L] being the constant ligand concentration.

Kullback-Leibler distance using the following way. From (Equation 18), it can be written as, $G^{0(conf)}(n) = -k_B T \ln P^{(eq)}(n)$. So the standard free energy of the nth ligand-binding state for cooperative and non-cooperative binding cases can be expressed as

$$G_c^{0(conf)}(n) = -k_B T \ln P_c^{eq}(n)$$

and

$$G_{nc}^{0(conf)}(n) = -k_B T \ln P_{nc}^{(eq)}(n) \quad (29)$$

respectively. The difference between $G_c^{0(conf)}(n)$ and $G_{nc}^{0(conf)}(n)$ gives the standard free energy change due to cooperative ligand-binding processes for the nth binding step and it can be estimated as

$$G_c^{0(conf)}(n) - G_{nc}^{0(conf)}(n) = \Delta G_{coop}^{0(conf)}(n) = k_B T \ln \frac{P_{nc}^{(eq)}(n)}{P_c^{(eq)}(n)} \quad (30)$$

Now multiplying both side by $P_{nc}^{(eq)}(n)$ and summing over all n, we obtain

$$\sum_{n=0}^{n_T} P_{nc}^{(eq)}(n)\Delta G^0(n)_{coop} = \left\langle \Delta G_{coop}^0 \right\rangle = k_B T D(P_{nc}^{(eq)}(n) \| P_c^{(eq)}(n)) \quad (31)$$

where $D(P_{nc}^{(eq)}(n) \| P_c^{(eq)}(n)) = \sum_{n=0}^{n_T} P_{nc}^{(eq)}(n) \ln \frac{P_{nc}^{(eq)}(n)}{P_c^{(eq)}(n)}$ is the relative entropy or the distance from the non-cooperative probability distribution, $P_{nc}^{(eq)}$ (reference distribution), to a cooperative distribution, $P_c^{(eq)}$ (target distribution). From (Equartion 31), it is observed that this KL distance measures the ensemble average standard free energy change due to cooperative ligand-binding processes, $\left\langle \Delta G_{coop}^0 \right\rangle$ [36,26] in equilibrium.

Cooperative Aspartate Binding to the Dimeric Receptor of Salmonella Typhimurium

Understanding of structural basis for cooperative binding mechanisms with detailed information of interactions responsible for a protein to being allosteric, comprises a large volume of recent literature. In the spirit of the experimental report of Kolodziej et al., we have theoretically investigated the origin of cooperative binding of the aspartate to the dimeric receptor of Salmonella typhimurium. The aspartate receptor is a dimeric one that binds aspartate with negative cooperativity. Serine68 residue located within the aspartate binding pocket and at the subunit interface, acts as an allosteric switch in this receptor. From their experiment, it is observed that mutations at this position (68) by several amino acid residues show different types of cooperative behavior. For example, amino acids threonine, leucine, asparagine and isoleucine show positive cooperativity whereas cysteine, valine show negative cooperativity. No cooperativity is observed for amino acids aspartate and alanine.

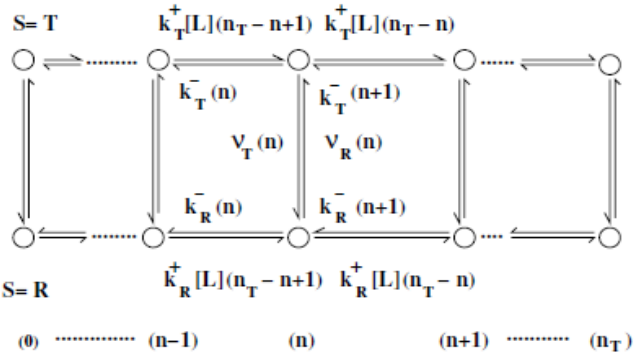


Figure 2: Schematic diagram of the MWC model. $k_s^+[L]$ and k_s^-(s =R, T) are designated as the forward and backward transition rates of the dynamical states of the system and [*L*] being the constant ligand concentration. $v_R(n)$ and $v_T(n)$ are the transition rates from conformation R to conformation T and vice versa, respectively, in the n^{th} state (for detailed description see main text).

Table 1: The stepwise Aspartate binding constants, K_1' and K_2' ($in\ \mu M^{-1}$) for different amino acid residues at position 68 of Aspartate receptor taken from the experimental study of Kolodziej *et al* [25]. The cooperativity index, C is determined at the ligand concentration where θ= 0.5 and also at the limiting condition (very high ligand concentration). C characterizes the cooperative behavior successfully as can be seen by comparing it with n_H.

Amino acid	$K^{(0)}(=K_1')\ K^{(1)}(=K_2')\ n_H$			n_H	
			(expt.)	(theo.)	(half-saturation)
serine	0.7	0.2	0.7	0.696	11.483
cysteine	0.5	0.2	0.8	0.775	10.325
valine	1.0	0.8	0.9	0.944	8.472
aspartate	0.1	0.1	1.0	1.0	7.999
alanine	0.6	0.6	1.0	1.0	8.000
threonine	0.4	0.9	1.2	1.199	6.667
leucine	0.3	1.1	1.2	1.314	6.089
asparagine	0.1	0.5	1.3	1.382	5.789
isoleucine	0.4	2.8	1.4	1.451	5.512

Here our main aim is to find out a thermodynamic quantity by which we can characterize the nature as well as degree of cooperativity like the Hill coefficient for better understanding about the cooperative ligand-binding processes. As a first step of investigation, we have plotted $\Delta\overline{G}$ as a function of [L] for the amino acids showing negative, non- and positive cooperative behavior in (Figure 3a,3b,3c) , respectively. It is observed that in all the cases, $\Delta\overline{G}$ becomes more negative with increasing [L] indicating that the ligandbinding process becomes more efficient with increasing [L] at equilibrium. From (Figure 3a), we observe that the magnitudes of $\Delta\overline{G}$ at a particular ligand concentration for different amino acids does not follow the trend shown by the corresponding Hill-coefficients, n_H see (Table 1). According to n_H value, amino acid serine shows highest negative cooperativity but in terms of $\Delta\overline{G}$, valine shows the highest value. Similar behavior is also observed for the amino acids showing positive cooperativity. Therefore, we can say that the standard free energy change of a cooperative process does not depend on the degree of cooperativity. From (Equation 20), it is observed that the value of $\Delta\overline{G}$ depends on the first equilibrium binding-constant, $Z^{(0)}$,

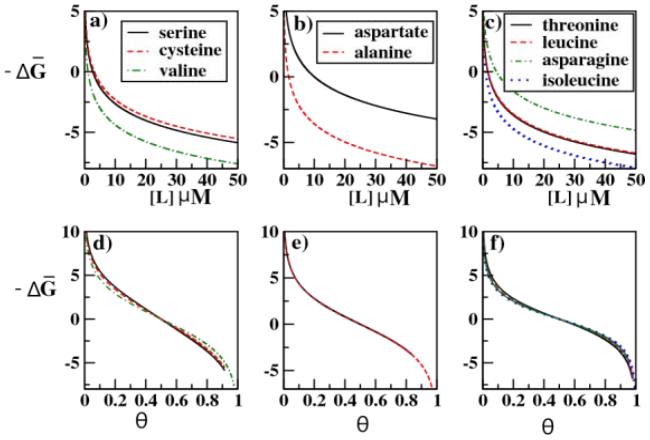


Figure 3: The variation of free energy change of cooperative processes, K_1' is plotted with [L] for amino acid residues showing (a) negative, (c) positive and (b) non-cooperative cases, respectively.

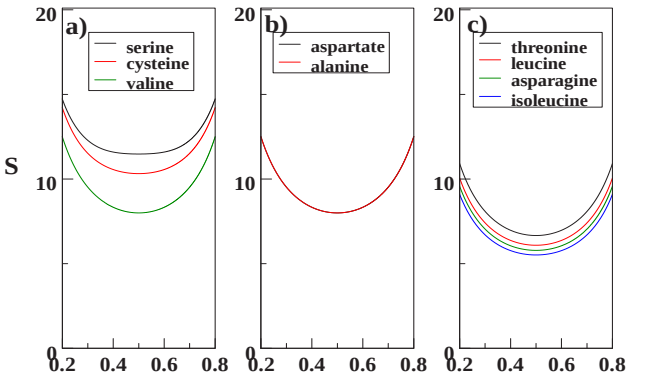


Figure 4: Plot of **S** versus $\theta$for the amino acids showing (a) negative cooperativity (b) no cooperativity and (c) positive cooperativity, respectively.

number of sub-units, n_T and the cooperativity inducing parameter, *f*. In the right side of (Equation 20) there are two terms; the first term $n_T lnZ^{(0)}$ and the second term $(n_T - 1)\ln f$. For the positive and negative cooperative cases, $\Delta\bar{G}$ is strictly governed by the first term for the step wise equilibrium constants considered here from the experimental work of Kolodziej et al.. However, if the first term becomes same due to having same initial equilibrium binding constant, then the second term governs the $\Delta\bar{G}$. For example, the amino acids threonine and isoleucine showing positive cooperativity, have same $K^{(0)}$ as well as $Z^{(0)}$ values. As isoleucine has higher f value so the magnitude of $\Delta\bar{G}$ is grater than that of threonine for this case. Therefore, the variation of $\Delta\bar{G}$ with [L] does not have a direct connection to the degree of cooperativity. Next, We have plotted the variation of standard free energy change, $\Delta\bar{G}$ with the fraction of occupied sites, θ for getting some answer about the question, 'How the nature of cooperativity can be characterized in terms of the thermodynamic quantity'. In (Figure 3d,3e,3f) we have plotted $\Delta\bar{G}$ as a function of θ for the amino acids showing the negative, non- and positive cases, respectively. For all the three cases, the nature of the plots are similar. The value of $\Delta\bar{G}$ is positive when θ < 0.5. It passes through zero at θ = 0.5 and becomes negative when θ > 0.5. Therefore, these plots can not characterize the nature as well as do not provide any information about the degree of

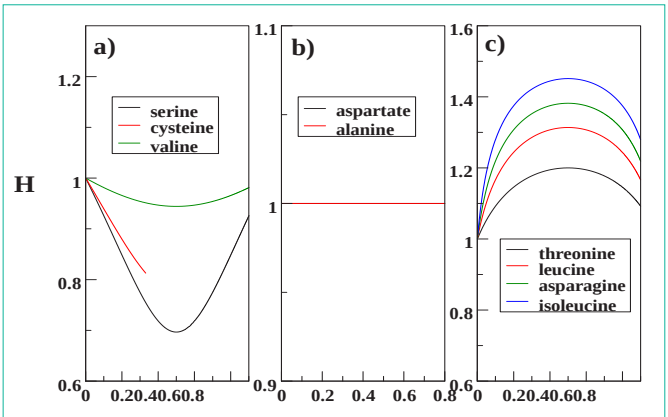


Figure 5: Plot of Hill slope, H with the variation of θ. (a) negative cooperativity (b) no cooperativity and (c) positive cooperativity.

cooperativity. Next, we have plotted S as a function of θ in (Figure 4a,4c) for the amino acids responsible for showing negative and positive cooperativity, respectively. For the non-cooperative case, the same plot is drawn in (Figure 4b). From these figures it is observed that all the curves passes through a minimum at θ = 0.5. In (Figure 4a), we observed that the magnitude of S at θ = 0.5 for the amino acids showing negative cooperativity follow the similar trend as exhibited by the corresponding Hill coefficients. Similar trend is also observed for the amino acids showing positive cooperativity which is depicted in (Figure 4c). However, for the non-cooperative case, the magnitude of S becomes equal for the amino acids, aspartate and alanine. This can be easily understood from (Equation 25). For non-cooperative cases, the value of n_H is unity and as here $n_T = 2$, the value of S should be 8 at θ = 0.5 for all the amino acids showing no cooperativity. S value greater than 8 indicates the negative cooperativity whereas for positive cooperativity, the value of S becomes less than 8 which is also observed from (Figure 4).

To compare n_H and S, we have also plotted the Hill slope, H as a function of θ for negative and positive cooperative cases which is depicted in (Figure 5a,5c), respectively. H is less than one for negative cooperativity, greater than one for positive cooperativity and remains equal to one for the noncooperative case, shown in (Figure 5b). Therefore, we can say that the quantity, S is an alternative thermodynamic measure to characterize the nature as well the degree of cooperativity like the Hill slope. The only difference is in the reference frame, i.e., the magnitude of H and S for the non-cooperative case. For more clarity, we have illustrated the utility of the S in detecting cooperativity by comparing it with the Hill coefficients determined in the work of Kolodziej et al. [25]. The theoretical values of Hill coefficients calculated for different cases tally very well with the experimental data [25]. It is evident from (Table 1). that S detects the presence and absence of cooperativity successfully. Also the extent or degree of positive or negative cooperative behavior is equally well characterized by S. This can be seen by comparing the values of n_H and S for the cases of serine and cysteine showing negative cooperativity as well as for threonine and isoleucine showing positive cooperativity. Therefore, the thermodynamic parameter, S can characterize the nature as well as degree of cooperativity very well. We have plotted Scoop with [L] in (Figure 6) for the cases studied by Kolodziej et al [25] as mentioned already. We have compared the

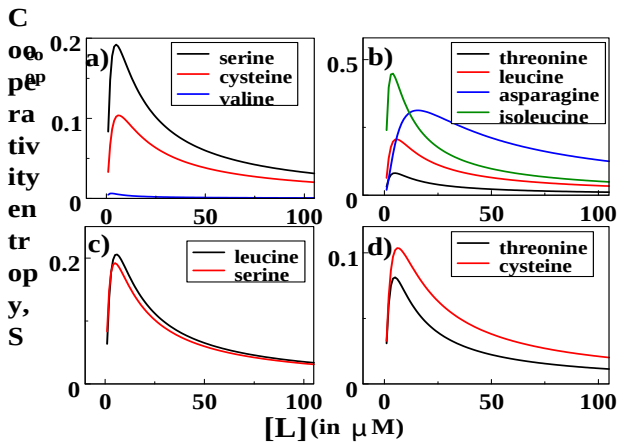


Figure 6: The variation of cooperativity entropy, S_{coop} with [L] for amino acid residues showing (a) negative cooperativity and (b) positive cooperativity. In (c) and (d) we compare the cases of the amino acid residue pairs leucine-serine and threonine-cysteine showing positive and negative cooperativity, respectively.

negative cooperative cases in (Figure 6a) and the positive cooperative cases in (Figure 6b). It is clear from the plots that the maximum value of S_{coop} is larger, greater the degree of cooperativity. After reaching the maxima, in all the cases S_{coop} approaches zero asymptotically with increasing [L] as then the protein goes to saturation and the difference between the cooperative and the non-cooperative binding diminishes. One can see this effect drastically in the case of the valine residue with very small negative cooperativity where the S_{coop} curve lies always very close to zero see (Figure 6a). On the other hand, for the strongest positive cooperativity with isoleucine residue, peak value of the S_{coop} curve is highest and it approaches saturation faster compared to the asparagine residue case which has lower values of Hill coefficient as well as the index C see (Figure 6b). Hence S_{coop} can be used to compare different cooperative systems. The approach of S_{coop} to saturation as a function of [L] is guided by the ratio of the stepwise equilibrium constants as well as by their actual values. Next we extend this comparison to the cases of pairs of amino acid residues, one with positive and the other with negative cooperativity with a comparable factor of rise or fall of the second equilibrium constant compared to the first one. We compared the cases of the leucine-serine pair in (Figure 6c) and that of the threonine-cysteine pair in (Figure 6d). For the leucine residue case, the second equilibrium constant increases by a factor of 3.66 whereas for the serine residue case it decreases by a factor of 3.5.

Conclusion

A systematic approach to cooperativity based on the kinetic mechanism of ligand binding of a multimeric proetinis developed by using chemical master equation description. As a next step the basic models of cooperativity are utilized to provide an information theory approach to address both the issues of thermodynamics and kinetics of cooperativity and the possible discrimination in their characterization for the examples of real situation. The Probability distribution of cooperative ligand-binding processes are formulated for both the KNF and MWC Allosteric cooperative models. We have introduced a thermodynamic measure to characterize the nature of cooperativity in terms of the change of standard free energy involved with the fraction of ligand-bound sub-units due to binding of ligands on a protein in equilibrium. The proposed criteria of cooperativity in terms of $\overline{G}_\theta$ is shown to be similar to that of the Hill coefficient which is defined kinetically and it is valid at any constant ligand concentration at equilibrium. We have thoroughly analyzed the connection of $\overline{G}_\theta$ to the Hill coefficient using some relevant experimental data on cooperativity and demonstrated that $\overline{G}_\theta$ successfully characterizes the cooperative behavior. A Kullback-Leibler distance is also introduced which indicates that how much average standard free energy will be involved if a non-cooperative system changes to cooperative one and it is applied for the aforesaid aspartate binding case, giving a quantitative measure of cooperativity as a function of ligand concentration. The proposed information theory approach gives an understanding of the mechanistic development of cooperativity over the traditional approach of Hill coefficient at half-saturation point. The probabilistic approach uses the full distribution function beyond the mean and variance. To address the subtle issue of cooperativity, the full distribution function is more meaningful for small value of the number of subunits as the distribution is intrinsically defined in finite dimension. The competition of binding of more than one kind of substrate may develop qualitatively new types of cooperativity which can open up many challenges in bio-engineering of proteins.

References

1. Hill AV. A new mathematical treatment of changes of ionic concentration in muscle and nerve under the action of electric currents, with a theory as to their mode of excitation. J Physiology. 1910; 40: 4-7.
2. Adair GS.The Hemoglobin System: VI. The Oxygen Dissociation Curve of Hemoglobin. Biol J Chem. 1925; 63: 529-545.
3. Monod J, Wyman J, Changeux JP. On the Nature of Allosteric Transitions: A Plausible Model. J Mol Biol 1965; 12: 88-118.
4. Koshland DE Jr., Nemethy G, Filmer D.Comparison of Experimental Binding Data and Theoretical Models in Proteins Containing Subunits. Biochemistry. 1966; 5: 365-385.
5. Weber G, Anderson SR.Biochemistry. 1965; 4: 1942-1947.
6. Hill TL. Cooperativity Theory in Biochemistry. Springer. 1985.
7. Banerjee K, Das B, Gangopadhyay G. On the estimation of cooperativity in ion channel kinetics: activation free energy and kinetic mechanism of Shaker K+ channel. J Chem Phys. 2013;138: 165102.
8. Fersht A, Structure and Mechanism in Protein Science: a guide to enzyme catalysis and protein folding. Freeman WH. New York, 1999.
9. Voet D,Voet JG. Biochemistry. 2010.
10. Hammes GG, Wu CW. Kinetics of allosteric enzymes. Annu Rev Biophys Bioeng. 1974;3: 1-33.
11. Goldbeter A, On the role of enzyme cooperativity in metabolic oscillations: analysis of the hill coefficient in a model for glycolytic periodicities. Biophysical Chemistry. 1976; 6: 95-99.
12. Ricard J, Cornish-Bowden A. Co-operative and allosteric enzymes: 20 years on. Eur J Biochem. 1987; 166: 255-272.
13. Cera ED. Thermodynamic Theory of site-specific binding process in biologcal macromolecule, Cambridge university press. 1995.
14. Cera ED. Site-Specific Thermodynamics: Understanding Cooperativity in Molecular Recognition. Chem. Rev. 1998; 98: 1563-1592.
15. E. Di Cera. Thermodynamic basis of site-specific cooperativity. Biopolymers. 1994; 34: 1001-1005.
16. E. Di Cera. Chen Biophys J. 1993; 65: 164-170.

17. J. R. Williamson, Cooperativity in macromolecular assembly. Nature Chem Biol. 2008; 4: 458-465.

18. Bordbar AK, Saboury AA, Housaindokht MR, Moosavi-Movahedi AA, Statistical Effects of the Binding of Ionic Surfactant to Protein. Colloid J Interface Sci. 1997; 192: 415-419.

19. Bordbar K, Mousavi SHA.Dazhampanah H. Analysis of oxygen binding by hemoglobin on the basis of mean intrinsic thermodynamic quantities.Acta Biochim Pol. 2006; 53: 536-568.

20. Bindslev N. Drug-acceptor interactions: modeling theoretical tools to test and evaluate experimental equilibrium effects. Co-Action Publishing. 2008.

21. Palmer T, Bonner P. Enzymes: Biochemistry, Biotechnology and Clinical Chemistry. 2007.

22. Banerjee K, Das B, Gangopadhyay G.Entropic estimate of cooperative binding of substrate on a single oligomeric enzyme: an index of cooperativity. JChem Phys. 2012; 136: 154502-154515.

23. Banerjee K, Math J. Chem. 2014; 52: 741-753.

24. Pal K, Das B,Banerjee K, Gangopadhyay G. Nonequilibrium thermodynamics and a fluctuation theorem for individual reaction steps in a chemical reaction network. J Phys Conf Ser. 2015; 638: 012002.

25. Kolodziej F, Tan T, Koshland DE.Producing Positive, Negative, and No Cooperativity by Mutations at a Single Residue Located at the Subunit Interface in the Aspartate Receptor of Salmonella typhimurium. Biochemistry. 1996; 35:14782-14792.

26. Kullback S, Leibler RA, Ann Math Stat. On Information and Sufficiency. 1951; 22: 79-86.

27. Chekmarev SF. Phys Rev E. 2008; 78: 066113-066120.

28. Conway A, Koshlandv DE. Negative cooperativity in enzyme action. The binding of diphosphopyridine nucleotide to glyceraldehyde 3-phosphate dehydrogenase. Biochemistry. 1968; 7: 4011-4022.

29. Levitzki A, Koshland DE. Negative cooperativity in regulatory enzymes. Proc. Natl. Acad. Sci. USA. 1969; 62: 1121-1128.

30. Levitzki A. Quantitative Aspects of Allosteric Mechanisms. SpringerVerlag. 1978.

31. Wyman JJ. Linked Functions and Reciprocal Effects in Hemoglobin: A Second Look. Adv Protein Chem. 1964; 19: 223-286.

32. Schellman JA. Macromolecular binding. Biopolymers. 1975; 14: 999-1018.

33. Cera ED, Gill SJ, Wyman.Canonical formulation of linkage thermodynamics. J Proc Natl Acad Sci. 1988; 85: 449-452.

34. Dahlquist FW.The meaning of Scatchard and Hill plots. Methods Enzymol. 1978; 48: 270-299.

35. Abeliovich H.An empirical extremum principle for the hill coefficient in ligand-protein interactions showing negative cooperativity. Biophys J. 2005; 89: 76-79.

36. Cover TM, Thomas JA. Elements of Information Theory, second ed. Wiley. 2006.

37. Seifert U.Entropy Production along a Stochastic Trajectory and an Integral Fluctuation Theorem. Phys. Rev Lett. 2005; 95: 040602-040606.

38. Das B, Gangopadhyay G.Master equation approach to single oligomeric enzyme catalysis: mechanically controlled further catalysis. J Chem Phys. 2010; 132: 135102-135111.

39. Ge H.Sensitivity amplification in the phosphorylation-dephosphorylation cycle: Nonequilibrium steady states, chemical master equation, and temporal cooperativity. Qian M J Chem Phys. 2008; 129: 015104-015120.

40. Qian H. Cooperativity and Specificity in Enzyme Kinetics: A Single-Molecule Time-Based Perspective. Biophys J. 2008; 95: 10-17.

41. Weiss JN.The Hill equation revisited: uses and misuses. FASEB J. 1997; 11: 835-841.

42. Tochtrop GP, Richter K, Tang C, Toner JJ, Covey DF, Cistola DP. Energetics by NMR: site-specific binding in a positively cooperative system. Proc Natl Acad Sci. USA. 2002; 99: 1847-1852.

43. Freire E, Murphy KP, Sanchez-Ruiz JM, Galisteo ML, Privalov PL. The molecular basis of cooperativity in protein folding. Thermodynamic dissection of interdomain interactions in phosphoglycerate kinase. Biochemistry. 1992; 31: 250-256.

44. Rouzina I, Bloomfield VA.Force-induced melting of the DNA double helix 1. Thermodynamic analysis.. Biophys J. 2001; 80: 882-893.

45. Horovitz A. The relation between cooperativity in ligand binding and intramolecular cooperativity in allosteric proteins. Proc R Soc. 1995; 259: 85.