:PAGEversion9R;
TTI System [compus] - fot686 /data/journal/bioc bioca.b bioc2612.k root Tue Aug 10 16:57:03 1999 full terminal narrow nolist laser paper newhyph port cols:1 /data/K2/bin/seta ul:3000 priority:10

ELSEVIER

Biophysical Chemistry 81 (1999) 7–21

Biophysical Chemistry

www.elsevier.nl/locate/bpc

Ligand binding on ladder lattices

Yong Kong*

Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, USA

Received 14 April 1999; received in revised form 2 June 1999; accepted 2 June 1999

Abstract

The ligand binding problems on two-dimensional ladders, which model many important binding phenomena in molecular biology, are studied in details. The model is represented by four parameters, the interactions between ligands when bound to adjacent sites on opposite legs of the ladder (τ), the interactions between bound ligands in the longitudinal direction of the ladder (σ), the number of binding sites that are covered by a bound ligand (m), and the intrinsic binding constant (K). The partition functions of ring ladders are approached with the transfer matrix method. A general relation is derived which connects the partition function of a linear ladder with that of a ring ladder. The results obtained apply to the general situation of multivalent binding, in which $m > 1$. Special attention is paid to the case where the ligand covers one site ($m = 1$). In this case explicit formulas are given for the partition functions of ring and linear ladders. Closed-form expressions are obtained for various properties of the system, including the degree of binding (θ), the midpoint in the binding isotherm ($1/\sqrt{\tau\sigma}$), the initial and end slopes of the Scatchard plots ($2\sigma + \tau - 4$ and $-\sigma^2\tau$, respectively). From these closed-form formulas, σ and τ may be extracted from experimental data. The model reveals certain features which do not exist in one-dimensional models. Using the general method discussed in [1], the recurrence relation is found for the partition functions. The analytical solution found for this model provides test cases to verify the numerical results for more complex two-dimensional models.

Keywords: Binding; Two-dimensional lattice model; Cooperativity; Scatchard plot; Protein–nucleic acid interactions; Ising model

* Current address: CuraGen Corporation, 555 Long Wharf Drive, New Haven, CT 06511, USA

0301-4622/99/$ - see front matter
PII: S0301-4622(99)00078-2

1. Introduction

Non-specific ligand binding, including protein–nucleic acid interactions, plays important structural and regulatory roles in various critical biological processes. These systems include histone–DNA complexes, protein binding to heparin, and protein binding to protein filaments (troponin–actin complexes, tropomyosin–actin complexes, etc.). The non-specific protein–nucleic acid interactions have received wide attention, not only because some of these interactions are directly involved in gene regulation, such as *E. coli lac* repressor, but also because the non-specific binding sites compete with those specific binding sites [2]. The understanding of non-specific interactions will enhance the understanding of protein–nucleic acid interactions in general and enrich our knowledge of specific binding. To understand these non-specific binding phenomena, quantitative measurements of the binding parameters involved are often required to distinguish competing models of the processes. Usually those binding problems are modeled by one-dimensional homogeneous lattices [3–8]. In such models, three thermodynamic parameters are used to describe the binding process: m, the number of binding sites covered by a bound ligand; σ, the unit-less cooperativity parameter, which specifies the nearest-neighbor interactions of bound ligands; and K, the intrinsic binding constant of the ligand for the transition from free state to bound state at each binding site, usually expressed in the unit of M^{-1} by mass-action equation if the free ligand and binding sites are expressed in molar concentrations. σ is the equilibrium constant of moving a bound ligand from an isolated site to a site which has one nearest neighbor occupied by another ligand. $\sigma > 1$ indicates that the interaction between the two bound ligands is favorable energetically, $\sigma < 1$ indicates negative cooperativity, and when $\sigma = 1$ there is no cooperativity. Although many systems have been investigated with this traditional model, the essential nature of the binding process under investigation may not be described adequately by the model due to its intrinsic simplicity. The model has been extended to include bidirectional cooperative binding [9], to include cooperativity longer than the nearest-neighbor interactions [4,10], and to treat the 'piggy-back' binding problem, in which the binding of a bigger ligand is modulated by the binding of a smaller ligand [11]. In this paper we extend the one-dimensional model to a two-dimensional ladder lattice model, and introduce another unit-less cooperativity parameter τ to specify the interactions between the bound ligands in different legs of the ladder (Fig. 1). Strictly speaking, the model is not truly 'two-dimensional' as the term is used in statistical mechanics literature, in which two-dimensional models usually mean that both dimensions have infinite sizes. The interaction along the other direction in the ladder model, however, gives the model a more realistic representation of many non-specific binding problems in molecular biology, such as the interactions between proteins and double-stranded nucleic acids. It should also be pointed out that in statistical mechanics, the behaviors of the models in the thermodynamic limit (i.e. when the number of sites in the model goes to infinity) are often more relevant for the investigation. In relation to the biological problems, on the other hand, the system under study is usually of finite size. For the infinite system, many techniques exist to simplify the model. For example, many properties of the system can be solely determined by the largest eigenvalue. Also, in such infinite systems, the properties of the system are not sensitive to the boundary condition, such as the often imposed periodical boundary condition where the first site and the last site are connected together. To study finite systems, we have to know the partition functions explicitly, or the knowledge of all eigenvalues. In addition, the effects of the boundary condition (end effects) may not be ignored [7]. In this paper, we will obtain the partition functions of finite systems by using the transfer matrix method for general m, and give explicit expressions of the partition functions for $m = 1$. A general relation is also obtained to calculate the partition functions for linear ladders from those of the ring ladders.

In Section 2, the binding on the ring ladders are approached with transfer matrix method. A general relation is derived in Section 3 which connects the partition function of a linear ladder

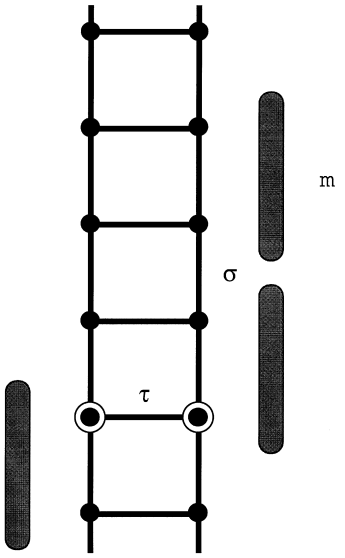

Fig. 1. The ladder lattice model. The ligands covers m sites on one leg when they bind to the ladder (here $m = 2$). The interactions between ligands bound on adjacent sites of the two legs are represented by τ, and the interactions between nearest-neighbor ligands bound on one leg are represented by σ. The circled sites are used to explain the construction of the transfer matrix. The circled site on the left is in the state of c_1, the one on the right is in the state of c_2. See text for details.

with that of a ring ladder. The results obtained in these two sections apply to the general situations where the ligands have an arbitrary size of m. The rest of the paper is devoted to the important special case of $m = 1$. Explicit formulas of partition functions and binding isotherm are derived for finite and infinite length ladders. Recurrence relations for the partition functions are developed using the general theory developed in [1]. Closed-form expressions of important properties of the binding system are given for the binding isotherm and Scatchard plots, from which relevant binding parameters can be extracted from experimental data.

2. Partition functions of ring ladders

The transfer matrix method [8] provides a convenient way to handle the partition functions of a small system if the boundary condition is imposed, i.e. the system is constructed to have the first and last sites connected. In this section we will use the transfer matrix method to obtain the partition functions of the ring ladders, and in the next section (Section 3) we will develop a formula to obtain the partition functions of linear ladders for different m values from those of ring ladders.

Transfer matrices for different m will be presented in this section. It turns out that they can be expressed in a rather compact form by using the transfer matrices for the one-dimensional lattices [9,12]. To simplify the notations, the intrinsic binding constant K and the ligand activity x are merged to define a scaled activity variable $\omega = Kx$. In the following ω is used throughout instead of K and x. Let's first consider the case of $m = 1$. In this case, for each site j and its adjacent site j' on the other leg of the ladder, there are four binding configurations: $\{c_0c_0\}$, $\{c_1c_0\}$, $\{c_0c_1\}$, and $\{c_1c_1\}$, where we use c_0 to denote an empty site and c_1 a bound site. The neighbor sites next to j and j' along the longitudinal direction of the ladder, sites $j + 1$ and $(j + 1)'$, also have these four binding configurations. These configurations determine the 4×4 transfer matrix for $m = 1$:

$$U_1 = \begin{bmatrix} 1 & 1 & 1 & 1 \\ \omega & \sigma\omega & \omega & \sigma\omega \\ \omega & \omega & \sigma\omega & \sigma\omega \\ \tau\omega^2 & \sigma\tau\omega^2 & \sigma\tau\omega^2 & \sigma^2\tau\omega^2 \end{bmatrix} \quad (1)$$

Here we assign each row for the configurations of sites j and j', in the order of $\{c_0c_0\}$, $\{c_1c_0\}$, $\{c_0c_1\}$, and $\{c_1c_1\}$, and assign each column for the configurations of sites $j + 1$ and $(j + 1)'$, in the same order. The ω and σ are assigned to site j or j', that is, when site j or j' is in ligated state, an ω is assigned; when j and $j + 1$, or j' and $j + 1'$, are in ligated state, a σ is assigned to the matrix element. When both sites j and j' are in ligated state, a τ is assigned. This assignment is not a unique assignment; alternative assignments are also possible [8].

For the general case, each site j on the ladder has $m + 1$ configurations, so does the adjacent site j' (see the case for $m = 2$ in Fig. 1, where the sites under consideration are circled). In addition to the empty configuration c_0, there are m-ligated configurations that depend on which part of the ligand the site has contact with. Imagine the

ligand moving upward along the ladder. When it first reaches the site under discussion (circled sites in Fig. 1), we assign c_1 to the configuration (the ligand on the left side of the ladder in Fig. 1). As it continues to move up one ladder site, we assign c_2 to the configuration (the ligand on the bottom right side of the ladder in Fig. 1). Hence for a pair of adjacent sites j and j', there are $(m+1)^2$ possible configurations. The pair of circled sites in Fig. 1 is in the configuration $\{c_1c_2\}$, while the pair of sites above them is in the configuration $\{c_0c_1\}$. These configurations determine a $(m+1)^2 \times (m+1)^2$ transfer matrix for a ligand of size m. For $m=2$, the transfer matrix is given by

$$U_2 = \begin{bmatrix} 1 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 1 \\ \omega^{1/2} & 0 & \sigma\omega^{1/2} & 0 & 0 & 0 & \omega^{1/2} & 0 & \sigma\omega^{1/2} \\ 0 & \omega^{1/2} & 0 & 0 & 0 & 0 & 0 & \omega^{1/2} & 0 \\ \omega^{1/2} & 0 & \omega^{1/2} & 0 & 0 & 0 & \sigma\omega^{1/2} & 0 & \sigma\omega^{1/2} \\ \tau\omega & 0 & \sigma\tau\omega & 0 & 0 & 0 & \sigma\tau\omega & 0 & \sigma^2\tau\omega \\ 0 & \tau\omega & 0 & 0 & 0 & 0 & 0 & \sigma\tau\omega & 0 \\ 0 & 0 & 0 & \omega^{1/2} & 0 & \omega^{1/2} & 0 & 0 & 0 \\ 0 & 0 & 0 & \tau\omega & 0 & \sigma\tau\omega & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \tau\omega & 0 & 0 & 0 & 0 \end{bmatrix}. \tag{2}$$

Here again we use rows for the sites j and j', and columns for the sites next to them, sites $j+1$ and $j+1'$. The order of configurations is: $\{c_0c_0\}$, $\{c_1c_0\}$, $\{c_2c_0\}$, $\{c_0c_1\}$, $\{c_1c_1\}$, $\{c_2c_1\}$, $\{c_0c_2\}$, $\{c_1c_2\}$, and $\{c_2c_2\}$. Since each ligand covers two sites, $\omega^{(1/2)}$ is assigned when one site is ligated. The zeros in the matrix arise from impossible situations or the fact that a particular site can only have one bound ligand. For example, $U_2(1,2)=0$ indicates that it is impossible for sites (j,j') in a configuration $\{c_0c_0\}$ while sites $(j+1,\ j+1')$ has a configuration $\{c_1c_0\}$; $U_2(2,2)=0$ indicates that site j cannot have two ligands bound at the same time. Also notice that except when either site j or site j' is in the empty configuration c_0, a factor τ will be assigned to the matrix elements.

It is evident from Eqs. (1) and (2) that in the general situation for arbitrary m, the transfer matrix can be written in a compact form as

$$U_m = T_m \cdot (W_m \otimes W_m) \tag{3}$$

where T_m is a $(m+1)^2 \times (m+1)^2$ diagonal matrix

$$T_m = \begin{bmatrix} I_{m+1} & & & & \\ & t_{m+1} & & & \\ & & t_{m+1} & & \\ & & & \ddots & \\ & & & & t_{m+1} \end{bmatrix}_{(m+1)^2 \times (m+1)^2} \tag{4}$$

in which I_{m+1} is a $(m+1)\times(m+1)$ identity matrix and t_{m+1} is defined as

$$t_{m+1} = \begin{bmatrix} 1 & & & & \\ & \tau & & & \\ & & \tau & & \\ & & & \ddots & \\ & & & & \tau \end{bmatrix}_{(m+1)\times(m+1)} \tag{5}$$

W_m in Eq. (3) is the transfer matrix for the binding of ligand with size m on a one-dimensional ring lattice [9,12]

$$W_m = \begin{bmatrix} 1 & 0 & 0 & \ldots & 0 & 1 \\ \omega^{1/m} & 0 & 0 & \ldots & 0 & \sigma\omega^{1/m} \\ 0 & \omega^{1/m} & 0 & \ldots & 0 & 0 \\ 0 & 0 & \omega^{1/m} & \ldots & 0 & 0 \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot & \cdot & \cdot \\ 0 & 0 & 0 & \ldots & \omega^{1/m} & 0 \end{bmatrix}_{(m+1)\times(m+1)} \tag{6}$$

The matrix direct product $A_{m\times n} \otimes B_{m'\times n'}$ used in Eq. (3) is defined as

$$A_{m\times n} \otimes B_{m'\times n'} = \begin{bmatrix} a_{11}B & a_{12}B & \ldots & a_{1n}B \\ a_{12}B & a_{22}B & \ldots & a_{2n}B \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ a_{m1}B & a_{m2}B & \ldots & a_{mn}B \end{bmatrix}_{mm'\times nn'} \tag{7}$$

When one goes from lower dimension to higher dimension, it is not uncommon to see the involvement of matrix direct product in the transfer matrix, such as in the study of the helix-coil transition coupled to dimerization of peptides [13]. The partition function ${}^{R}\Psi_{(n)}$ of a ring ladder with length of n is expressed as [8]

$$ {}^{R}\Psi_{(n)} = Tr(U_m^n). \tag{8} $$

When there is no cooperative interaction between the two legs of the ladder, $\tau = 1$. In this case T_m is an identity matrix, hence from Eq. (3) we have $U_m = W_m \otimes W_m$. Using the theorem of direct product $(A \otimes B)(C \otimes D) = (AC) \otimes (BD)$ and $Tr(A \otimes B) = TrA \times TrB$, it can be seen that the partition function of a ring ladder of length n is

$$ \begin{aligned} {}^{R}\Psi_{(n)} &= Tr(U_m^n) = Tr(W_m^n \otimes W_m^n) \\ &= (Tr(W_m^n))^2 = {}^{R}\Phi_{(n)}^2 \end{aligned} \tag{9} $$

where ${}^{R}\Phi_{(n)}$ is the partition function of a one-dimensional ring lattice with the ligand size of m. This result is expected from the physical nature of the problem: the two legs of the ladder is independent of each other when there is no co-operativity between the ligands bound on different legs. When τ is arbitrary, the partition function ${}^{R}\Psi_{(n)}$ is a complicated function of ${}^{R}\Phi$. From U_m we can easily get the partition functions for the ring ladder by using Eq. (8). Some of the partition functions for small m and n are listed in Appendix A.

3. Relation between linear ladder and ring ladder

In this section we will find a relation between the partition functions of linear ladders and ring ladders. This is the generalization of the equation we derived in an early paper for the multivalent binding in one-dimensional lattice [Eq. (2) in Di Cera and Kong [12]].

If we denote $X_{j(n)}$ as the possibility that the jth site in the ring ladder of length n is bound and $X_{j,j'(n)}$ as the possibility that both site j and site j' are bound (here j' is the adjacent site of j on the other leg of the ladder), we have the following relations [12,14]

$$ X_{j(n)} = \frac{\omega^{1,R}\Psi_{j(n)}}{{}^{R}\Psi_{(n)}} \tag{10} $$

$$ X_{j,j'(n)} = \frac{\omega^2\tau^{11,R}\Psi_{j,j'(n)}}{{}^{R}\Psi_{(n)}}. \tag{11} $$

Here we use the same nomenclature as in Di Cera and Kong [12]. ${}^{1,R}\Psi_{j(n)}$ is the contracted partition function for a ring ladder of length n (totally $2n$ binding sites) with site j fixed in ligated configuration. It describes all the possible configurations of the $2n-1$ sites other than site j of a ladder of length n with site j kept ligated. ${}^{11,R}\Psi_{j,j'(n)}$ is the contracted partition function for a ring ladder of length n with sites j and j' ligated, which describes all the configurations of the other $2n-2$ sites. Since the ring ladder is homogeneous, and the expected number of bound ligands is expressed as [8]

$$ L = \frac{\omega}{{}^{R}\Psi_{(n)}} \frac{\partial {}^{R}\Psi_{(n)}}{\partial \omega}, \tag{12} $$

we have

$$ X_{j(n)} = \frac{Lm}{2n} = \frac{m\omega}{2n\,{}^{R}\Psi_{(n)}} \frac{\partial {}^{R}\Psi_{(n)}}{\partial \omega}. \tag{13} $$

Similarly, using the same reasoning, we have

$$ X_{j,j'(n)} = \frac{\tau}{n\,{}^{R}\Psi_{(n)}} \frac{\partial {}^{R}\Psi_{(n)}}{\partial \tau}. \tag{14} $$

The site j in the above equations is quite arbitrary. The homogeneity of the ring ladder makes the equations applicable to every site in the ladder. Now we assign $j = n$. If we contract the contracted partition function ${}^{1,R}\Psi_{n(n)}$ again at site n', the site adjacent to site n on the other leg, we get

$$ {}^{1,R}\Psi_{n(n)} = {}^{10,R}\Psi_{n,n'(n)} + \tau\omega^{11,R}\Psi_{n,n'(n)}, \tag{15} $$

where ${}^{10,R}\Psi_{j,j'(n)}$ is the contracted partition function for a ring ladder of length n with site j

ligated and site j' unligated. Dividing Eq. (15) by ${}^{R}\Psi_{(n)}$ and using Eqs. (10) and (11), we have

$$X_{j(n)} = \frac{\omega^{10,R}\Psi_{n,n'(n)}}{{}^{R}\Psi_{(n)}} + X_{j,j'(n)}. \tag{16}$$

Rearranging Eq. (16) and substituting $X_{j(n)}$ and $X_{j,j'(n)}$ with Eq. (13) and Eq. (14), we obtain

$$\begin{aligned} {}^{10,R}\Psi_{n,n'(n)} &= \frac{{}^{R}\Psi_{(n)}}{\omega}(X_{j(n)} - X_{j,j'(n)}) \\ &= \frac{m}{2n}\frac{\partial {}^{R}\Psi_{(n)}}{\partial \omega} - \frac{\tau}{\omega n}\frac{\partial {}^{R}\Psi_{(n)}}{\partial \tau}. \end{aligned} \tag{17}$$

If we contract the partition function at sites n and n' at the same time, we have

$$\begin{aligned} {}^{R}\Psi_{(n)} = {}&{}^{00,R}\Psi_{n,n'(n)} + \omega^{10,R}\Psi_{n,n'(n)} + \omega^{01,R}\Psi_{n,n'(n)} \\ &+ \omega^2\tau^{11,R}\Psi_{n,n'(n)}. \end{aligned} \tag{18}$$

Since ${}^{10,R}\Psi_{n,n'(n)} = {}^{01,R}\Psi_{n,n'(n)}$ and ${}^{00,R}\Psi_{n,n'(n)}$, the contracted partition function for a ring ladder of length n with sites n and n' unligated, is equal to ${}^{L}\Psi_{(n-1)}$, the partition function for a linear ladder of length $n-1$, we get

$$\begin{aligned} {}^{R}\Psi_{(n)} = {}&{}^{L}\Psi_{(n-1)} + \frac{m\omega}{n}\frac{\partial {}^{R}\Psi_{(n)}}{\partial \omega} - \frac{2\tau}{n}\frac{\partial {}^{R}\Psi_{(n)}}{\partial \tau} \\ &+ \omega^2\tau^{11,R}\Psi_{n,n'(n)}. \end{aligned} \tag{19}$$

From Eqs. (11) and (14) we have ${}^{11,R}\Psi_{n,n'(n)} = \frac{1}{(n\omega^2)}\frac{\partial {}^{R}\Psi_{(n)}}{\partial \tau}$. Substituting this result into Eq. (19), and rearranging, we finally obtain

$${}^{L}\Psi_{(n-1)} = {}^{R}\Psi_{(n)} - \frac{m\omega}{n}\frac{\partial {}^{R}\Psi_{(n)}}{\partial \omega} + \frac{\tau}{n}\frac{\partial {}^{R}\Psi_{(n)}}{\partial \tau}. \tag{20}$$

This is an equation that expresses the partition function of a linear ladder of length $n-1$ ($2(n-1)$ binding sites) with that of a ring ladder with length n ($2n$ binding sites), as well as its derivatives with respect to σ and τ, which parameterize the nearest-neighbor interactions of the ligands bound along two different directions on the ladder. Using this equation, we can get the partition functions of the linear ladder from those of the ring ladders. Some of the partition functions of the linear ladders for small m and n are listed in Appendix B using the partition functions for ring ladders from Section 2, which are listed in Appendix A.

4. Finite ring and linear ladders when $m = 1$

The results obtained in previous sections apply to the general case of arbitrary m. In this section the important special case $m = 1$ will be discussed. When $m = 1$, we can find explicit expressions for the partition functions of ring and linear ladders of any length since the eigenvalues of the transfer matrix can be solved analytically.

4.1. Explicit expressions for finite ring and linear ladders when m = 1

The characteristic polynomial of transfer matrix U_1 [Eq. (1)] when $m = 1$ is given by

$$\begin{aligned} |\lambda I - U_1| = {}&(\lambda + \omega - \sigma\omega) \\ &\times(\lambda^3 - (\sigma^2\tau\omega^2 + \sigma\omega + \omega + 1)\lambda^2 \\ &+(\sigma - 1)\omega(\sigma^2\tau\omega^2 + \tau\sigma\omega + \tau\omega + 1)\lambda \\ &-\tau(\sigma - 1)^3\omega^3). \end{aligned} \tag{21}$$

One of the four eigenvalues of U_1 is $\lambda_0 = (\sigma - 1)\omega$, and the other three λ_i, $i = 1,2,3$ are the roots of the cubic equation

$$\lambda^3 + a\lambda^2 + b\lambda + c = 0 \tag{22}$$

where

$$a = -(\sigma^2\tau\omega^2 + \sigma\omega + \omega + 1) \tag{23a}$$

$$b = (\sigma - 1)\omega(\sigma^2\tau\omega^2 + \tau\sigma\omega + \tau\omega + 1) \tag{23b}$$

$$c = -\tau(\sigma - 1)^3\omega^3 \tag{23c}$$

In this case Eq. (22) has three real roots, and the solution can be expressed in trigonometric terms so as to avoid the complex cubic roots in

the Cardano's solution. The solutions of Eq. (22) are

$$\lambda_1 = -2\sqrt{\alpha}\cos\gamma - \frac{a}{3} \tag{24a}$$

$$\lambda_2 = 2\sqrt{\alpha}\cos\left(\gamma + \frac{\pi}{3}\right) - \frac{a}{3} \tag{24b}$$

$$\lambda_3 = 2\sqrt{\alpha}\cos\left(\gamma - \frac{\pi}{3}\right) - \frac{a}{3} \tag{24c}$$

where

$$\alpha = \frac{1}{3}\left(\frac{a^2}{3} - b\right) \tag{25a}$$

$$\beta = \frac{1}{2}\left(\frac{2a^3}{27} - \frac{ab}{3} + c\right) \tag{25b}$$

$$\gamma = \frac{1}{3}\cos^{-1}\frac{\beta}{\alpha^{3/2}} \tag{25c}$$

Since we have all the four eigenvalues, the partition function of the ring ladder of any length n can be evaluated by

$${}^{R}\Psi_{(n)} = \sum_{i=0}^{3} \lambda_i^n. \tag{26}$$

From Eq. (26) we can see that the partition functions of ring ladders obey a recurrence relation with an order of 4. For linear ladders, the partition functions are given by Eq. (20) with $m = 1$ as

$$\begin{aligned} {}^{L}\Psi_{(n)} &= {}^{R}\Psi_{(n+1)} - \frac{\omega}{n+1}\frac{\partial {}^{R}\Psi_{(n+1)}}{\partial\omega} \\ &\quad + \frac{\tau}{n+1}\frac{\partial {}^{R}\Psi_{(n+1)}}{\partial\tau} \\ &= \sum_{i=0}^{3}[\lambda_i - \omega(\lambda'_i)_\omega + \tau(\lambda'_i)_\tau]\lambda_i^n \\ &= \sum_{i=0}^{3} c_i\lambda_i^n, \end{aligned} \tag{27}$$

where $c_i = \lambda_i - \omega(\lambda'_i)_\omega + \tau(\lambda'_i)_\tau$ is independent of n.[1] Here $(\lambda'_i)\omega = \partial\lambda_i/\partial\omega$ and $(\lambda'_i)\tau = \partial\lambda_i/\partial\tau$. It is evident from $\lambda_0 = (\sigma - 1)\omega$ that $c_0 = 0$. Hence for the linear ladder we have

$${}^{L}\Psi_{(n)} = \sum_{i=1}^{3} c_i\lambda_i^n. \tag{31}$$

The same result for the linear ladder can also be obtained using the general method derived in [1]. The next subsection will discuss the use of this method.

4.2. Recurrence relation for linear ladder when $m = 1$

When $m = 1$ we can get a recurrence relation for the partition functions of finite linear ladders using the general methods discussed in [1]. In this case there are only two ligated configurations, one is the singly ligated configuration with one of the sites in a rung ligated, and the other doubly ligated configuration. The recurrence equation is given by

$$\begin{aligned} {}^{L}\Psi_{(n)} &= (\sigma^2\tau\omega^2 + \sigma\omega + \omega + 1)\,{}^{L}\Psi_{(n-1)} \\ &\quad - \omega(\sigma - 1)(\sigma^2\tau\omega^2 + \sigma\tau\omega + \tau\omega + 1) \\ &\quad \times {}^{L}\Psi_{(n-2)} + (\sigma - 1)^3\tau\omega^3\,{}^{L}\Psi_{(n-3)}. \end{aligned} \tag{32}$$

Clearly from this recurrence relation we can get the same explicit expression for the partition functions of linear ladder of any length n as Eq. (31). The method in [1] not only gives the recur-

[1] The other way to see that the coefficients c_I in Eq. (27) are independent of n, is to use [15].

$${}^{L}\psi_{(n)} = E_l U_m^{n+1} E_r \tag{28}$$

$$E_l = [1,0,\ldots,0] \tag{29a}$$

$$E_r = [1,0,\ldots,0]^t \tag{29b}$$

Since $Um = TAT^{-1}$, where

$$\Lambda = \begin{bmatrix} \lambda_0 & & & \\ & \lambda_1 & & \\ & & \lambda_2 & \\ & & & \lambda_3 \end{bmatrix}, \tag{30}$$

we have ${}^{L}\Psi_{(n)} = E_l T\Lambda^{n+1}T^{-1}E_r$. The coefficients c_i are determined by the elements of matrix T, which are not dependent on n.

rence equation, but also the contracted partition functions:

$$^{11,L}\Psi_{n(n)} = \frac{\sigma\,{}^{L}\Psi_{(n)} - \sigma(\sigma\omega+1)\,{}^{L}\Psi_{(n-1)} + \omega(\sigma-1)^2\,{}^{L}\Psi_{(n-2)}}{(\tau\sigma\omega+1)\omega} \tag{33}$$

$$^{10,L}\Psi_{n(n)} = \frac{{}^{L}\Psi_{(n)} + (\sigma^2\tau\omega^2-1)\,{}^{L}\Psi_{(n-1)} - \tau\omega^2(\sigma-1)^2\,{}^{L}\Psi_{(n-2)}}{2(\tau\sigma\omega+1)\omega} \tag{34}$$

From the recurrence relation [Eq. (32)] and using the partition functions listed in Appendix B for small n as the initial conditions, we can get the generating function for the partition functions of linear ladders as

$$G(z) = \sum_{n=0}^{\infty} {}^{L}\Psi_{(n)} z^n = \frac{1-\omega(\sigma-1)(\omega\tau\sigma+1+\omega\tau)z + \tau(\sigma-1)^3\omega^3 z^2}{1-(\sigma\omega+\sigma^2\tau\omega^2+\omega+1)z+\omega(\sigma-1)(\sigma^2\tau\omega^2+\omega\tau\sigma+1+\omega\tau)z^2-\tau(\sigma-1)^3\omega^3 z^3} \tag{35}$$

which can be expanded in series to generate ${}^{L}\Psi_{(n)}$.

4.3. Special cases regarding co-operativity for linear ladders when m = 1

In this subsection some special cases regarding the values of σ and τ will be discussed.

4.3.1. $\sigma = 1$

In this case there is no cooperativity in the longitudinal direction of the ladder. From Eq. (35) we can see that if $\sigma = 1$, the generating function reduces to

$$G(z) = \sum_{n=0}^{\infty} {}^{L}\Psi_{(n)} z^n = \frac{1}{1-(\tau\omega^2+2\omega+1)z}, \tag{36}$$

from which we can get

$${}^{L}\Psi_{(n)} = (\tau\omega^2+2\omega+1)^n = \sum_{i=0}^{2n}\sum_{j=0}^{i/2} \binom{n}{i-j}\binom{i-j}{j} 2^{i-2j}\tau^j\omega^i. \tag{37}$$

This equation can also be derived readily from Eq. (24), since two of the three eigenvalues will vanish, and the remaining one will be $\lambda_3 = \tau\omega^2 + 2\omega + 1$.

If we let $\tau = 1$ in Eq. (37), we obtain

$${}^{L}\Psi_{(n)} = (1+\omega)^{2n} \tag{38}$$

which is expected. From Eq. (37) we get the fraction of occupied sites (degree of binding) as

$$\theta(\omega,\sigma=1) = \frac{1}{2n}\frac{\partial \ln {}^{L}\Psi_{(n)}}{\partial \ln\omega} = \frac{\omega(\omega\tau+1)}{\tau\omega^2+2\omega+1} \tag{39}$$

which is independent of the length of the ladder. This is not surprising since there is no cooperativity in the longitudinal direction. No matter what τ is, the binding isotherm is the same for ladder of any size, including the infinite ladder. This can be easily checked from Eq. (47), discussed in Section 5.

4.3.2. $\tau = 1$

When $\tau = 1$, the partition function is the square of the partition function of the one-dimensional linear chain, as discussed in Section 2. In this case the problem reduces to the one-dimensional problem.

4.4. $\tau = 0$

When $\tau = 0$, the two adjacent sites on one rung of the ladder can not be occupied at the same time. In this case, $\lambda_1 = 0$, and the characteristic function Eq. (22) is reduced to a quadratic equation. The two non-vanishing roots are given by

$$\lambda_{2,3} = \frac{\sigma\omega + \omega + 1 \mp \sqrt{(\sigma\omega + \omega + 1)^2 - 4\omega(\sigma - 1)}}{2} \tag{40}$$

The partition function is given by ${}^{L}\Psi_{(n)} = c_2\lambda_2^n + c_3\lambda_3^n$, where $c_i = \lambda_i - \omega(\lambda'_i)_\omega$, $i = 2,3$. The leading term in ${}^{L}\Psi_{(n)}$ is $2(\tau + 1)^{n-1}\omega^n$, instead of $\tau^n\sigma^{2(n-1)}\omega^{2n}$ when $\tau \neq 0$. The limiting value for $\theta(\omega)$ when ω goes to infinity is always $1/2$. It should be noticed that there is certain similarity between the ladder model when $\tau = 0$ and the general one-dimensional model. Both are governed by a quadratic equation. For reference, the characteristic function of the general one-dimensional model is given by

$$\lambda^2 - (\sigma\omega + 1)\lambda + (\sigma - 1)\omega = 0. \tag{41}$$

4.4.1. $\sigma = 0$

When $\sigma = 0$ and $\tau \neq 0$, the coefficients of the characteristic function become

$$a = -(\omega + 1) \tag{42a}$$

$$b = -\omega(\tau\omega + 1) \tag{42b}$$

$$c = \tau\omega^3, \tag{42c}$$

hence the secular equation still has three non-vanishing roots. The leading term of the partition functions depends on whether n is even or odd: it is $\tau^k\omega^{2k}$ when $n = 2k - 1$, and $[(k + 1)\tau^k + 2\sum_{i=1}^{k} i\tau^{i-1}]\omega^{2k}$ when $n = 2k$. When $n = 2k - 1$, there is only one way in which the ladder can be bound with maximum $2k$ ligands, i.e. the rungs $1,3,\ldots, 2k - 1$ have two ligands bound, while rungs $2,4,\ldots, 2k - 2$ are empty. The situation when $n = 2k$ is more complicated, since there is more than one way to bind $2k$ ligands to $4k$ binding sites. The behavior of the binding isotherm when ω goes to infinity is given by

$$\lim_{\omega\to\infty} \theta(\omega,\sigma = 0) = \begin{cases} 1/2 & n = 2k, \quad k \text{ interger} \\ \dfrac{k}{2k-1} & n = 2k - 1 \end{cases} \tag{43}$$

In this case, the sites can never be saturated, as in the case when $\tau = 0$, except for $n = 1$.

5. Infinite linear ladder when $m = 1$

When the length of the linear ladder becomes large, the largest eigenvalue λ_3 dominates the expression of partition functions [Eq. (31)], so the binding isotherm becomes

$$\theta(\omega) = \lim_{N\to\infty} \frac{1}{2n} \frac{\partial \ln {}^{L}\Psi_{(n)}}{\partial \ln\omega} = \frac{\omega}{2\lambda_3} \frac{\partial\lambda_3}{\partial\omega} \tag{44}$$

The complicated derivative in Eq. (44) can be avoided in the following way. If we take derivative of Eq. (22) with respect to ω, we get

$$3\lambda^2\lambda' + (2a\lambda\lambda' + a'\lambda^2) + (b\lambda' + b'\lambda) + c' = 0 \tag{45}$$

where $\lambda' = \dfrac{\partial\lambda}{\partial\omega}$ and

$$a' = \frac{\partial a}{\partial a} = -\sigma - 2\sigma^2\tau\omega - 1 \tag{46a}$$

$$b' = \frac{\partial b}{\partial\omega} = (\sigma - 1)(3\sigma^2\tau\omega^2 + 2\omega\tau\sigma + 1 + 2\omega\tau) \tag{46b}$$

$$c' = \frac{\partial c}{\partial\omega} = -3\tau(\sigma - 1)^3\omega^2 \tag{46c}$$

From Eq. (45) we can solve λ'_3 and after substituting it into Eq. (44), we obtain

$$\theta(\omega) = \frac{\omega(a'\lambda_3^2 + b'\lambda_3 + c')}{2(a\lambda_3^2 + 2b\lambda_3 + 3c)} \tag{47}$$

in which no derivative of λ_3 will be needed.

It can be easily shown that as in the one-dimensional Ising model, no ferromagnetism exists in the ladder. In order for the ferromagnetism to exist, the second dimension also has to go to infinity.

$\theta(\omega)$ in Eq. (47) increases from zero when $\omega = 0$, to 1 as ω approaches infinity. The point when $\theta(\omega) = 1/2$ is where half the sites are occupied by ligands and half the sites are empty. If we put $\theta(\omega) = 1/2$ in Eq. (47), we can find

$$\omega|_{\theta=1/2} = \frac{1}{\sqrt{\tau}\,\sigma}. \quad (48)$$

When $\tau = 0$, the limiting value for θ is $1/2$ as ω approaches infinity. In this case, $\omega = 1/(\sigma + 1)$ when $\sigma = 1/4$.

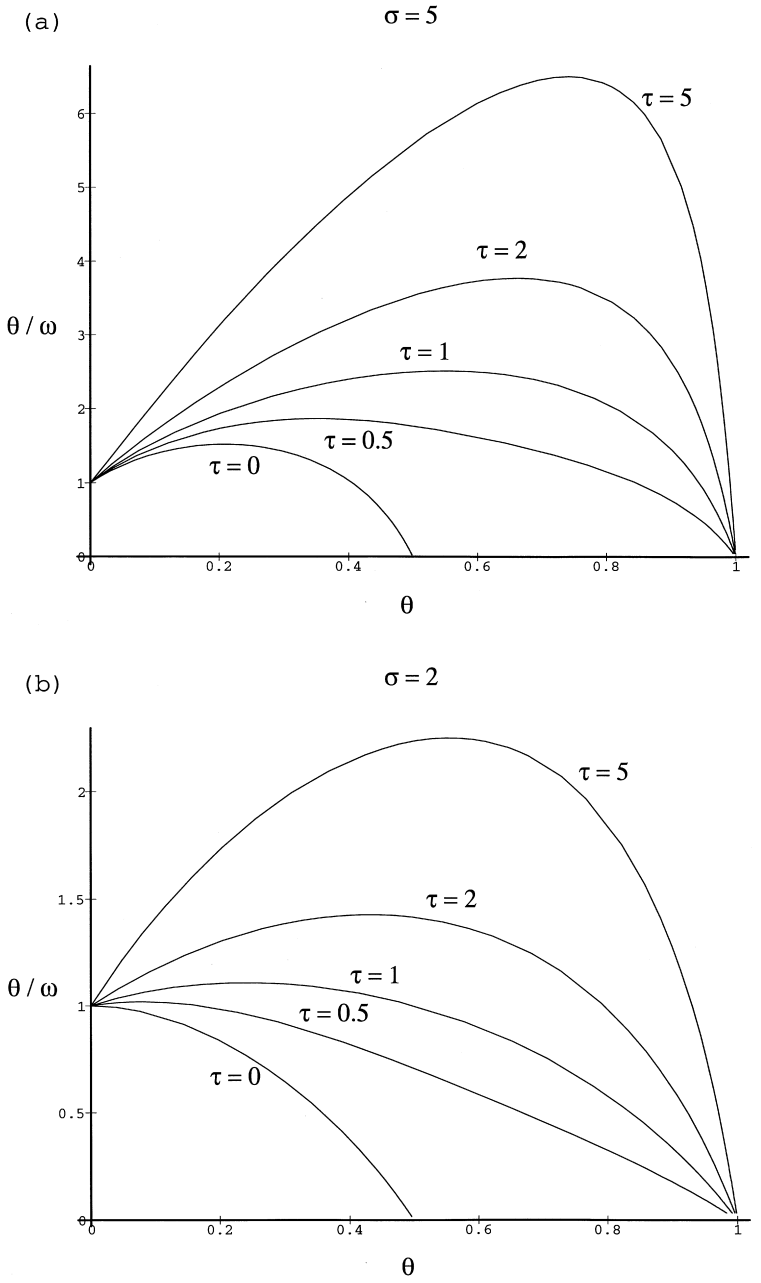

Fig. 2. Scatchard plots for infinite linear ladder when $m = 1$ with different σ and τ. In each figure τ takes values of 0, 0.5, 1, 2, and 5, a: $\sigma = 5$; b, $\sigma = 2$; c, $\sigma = 1$; d, $\sigma = 0.5$; and e, $\sigma = 0$.

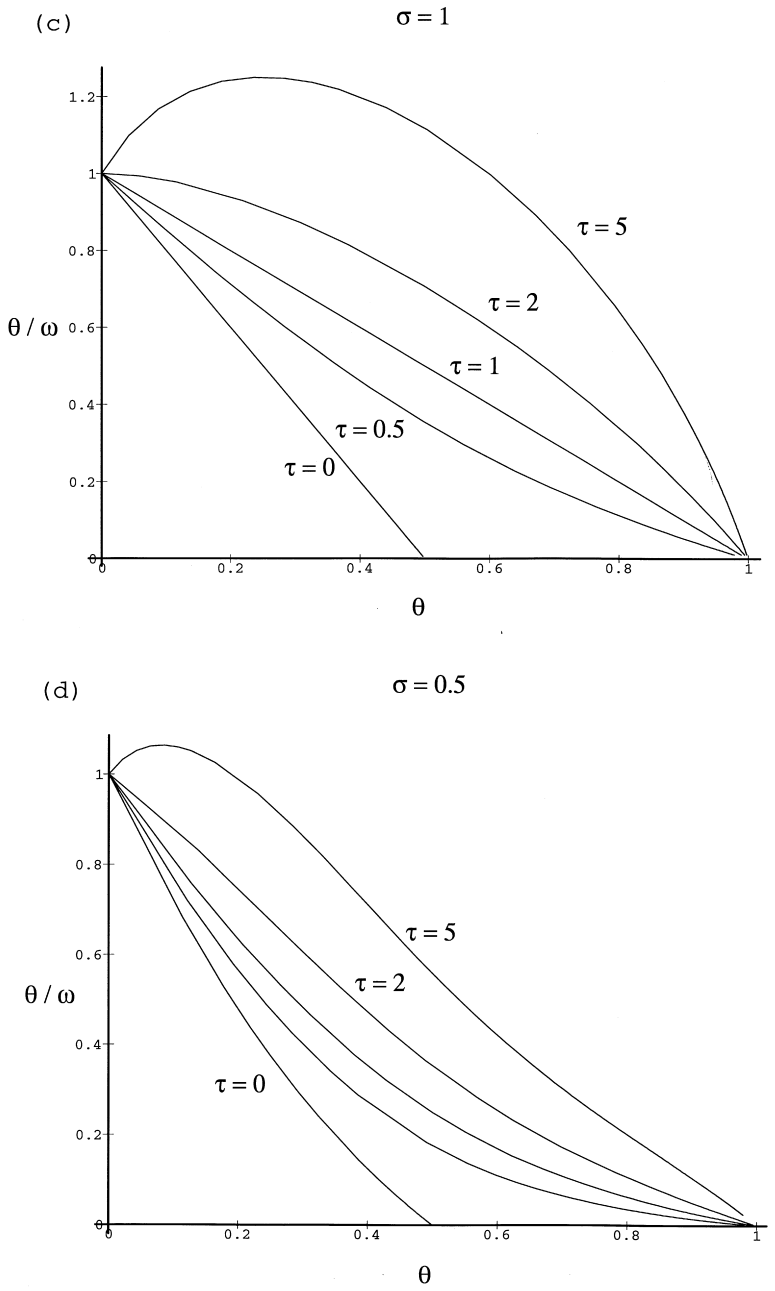

Fig. 2. *Continued.*

5.1. Scatchard plot

One of the most often used graphic representations of experimental data is the Scatchard plot, in which θ/ω is plotted against ω [5,6]. In simple case the Scatchard plot yields a straight line. For multivalent binding on one-dimensional lattice, a closed-form formula was developed [5] and its

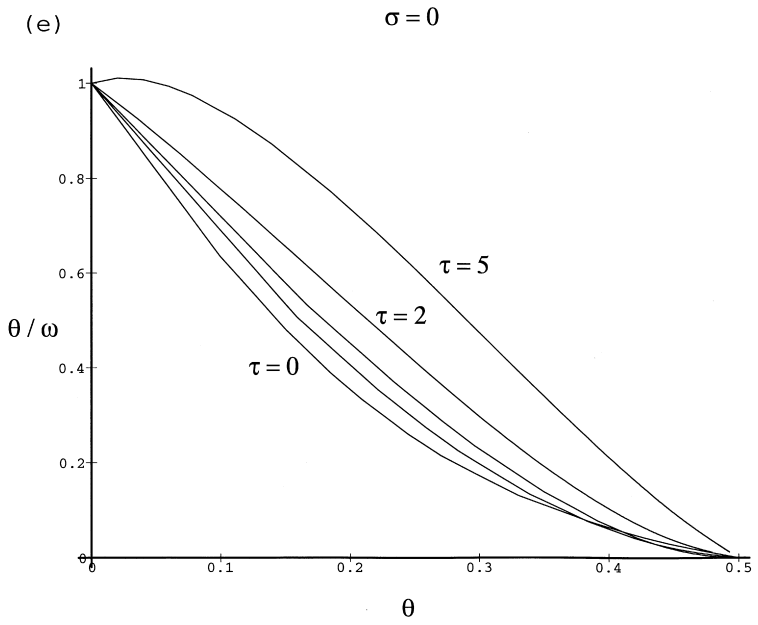

Fig. 2. *Continued.*

properties were characterized [5,6,9,12]. The Scatchard plots for infinite linear ladder when $m = 1$ with different σ and τ are shown in Fig. 2. From these plots we can see several features which do not exist in one-dimensional Scatchard plots. For one-dimensional lattices, a maximum in Scatchard plot implies that the system is positively cooperative with $\sigma > 3/2$ [5,6]. For the binding on a ladder, the cooperativities in two different directions compensate each other so that even when one cooperativity is negative, the other cooperativity still can generate a Scatchard plot with a maximum. Also we can see the inflection point in a Scatchard plot of two-dimensional ladders, which confirms the conclusion in Schwarz [6] that such inflection indicates complex binding mechanism involving at least two antagonistic cooperative interactions.

From Fig. 2 we can see that when $\sigma = 1$, the Scatchard plot is a straight line when $\tau = 1$ or $\tau = 0$. When $\tau = 1$, from Eq. (39) we can obtain $\theta/\omega = 1 - \theta$, which gives a Scatchard plot with a slope of -1. When $\tau = 0$, from Eq. (39) we obtain $\theta/\omega = 1 - 2\theta$, which gives a Scatchard plot with a slope of -2.

The beginning and end slopes of Scatchard plots for one-dimensional lattices are investigated in [5,6]. For the ladders, from Eq. (47) we have both θ and θ/ω as functions of ω, hence we can calculate the slope of the Scatchard plots for the ladders as

$$\frac{\partial}{\partial\theta}\left(\frac{\theta}{\omega}\right) = \frac{\frac{\partial}{\partial\omega}\left(\frac{\theta}{\omega}\right)}{\frac{\partial\theta}{\partial\omega}}. \tag{49}$$

From Eq. (49) we have the beginning slope as

$$S_0 = 2\sigma + \tau - 4 \tag{50}$$

and the ending slope as

$$S_\infty = -\sigma^2\tau. \tag{51}$$

Eq. (50) can also be obtained by the more intuitive method used in [5] for the one-dimensional lattices. When $\tau = 1$, Eq. (50) reduces to the one-dimensional result $2\sigma - 2m - 1$ with $m = 1$. From Eqs. (50) and (51) we can see that when $2\sigma + \tau > 4$, the Scatchard plots will have a maximum. In this case, the slope of Scatchard plots

changes from positive to negative, with a point in the middle where the slope is zero and the curve reaches the maximum. Eq. (51) needs special treatment when $\tau = 0$ or $\sigma = 0$. When $\tau = 0$, the ending slope is given by

$$S_\infty(\tau = 0) = -\frac{(\sigma + 1)^2}{2}, \tag{52}$$

and when $\sigma = 0$, the ending slope is

$$S_\infty(\sigma = 0) = \begin{cases} 2\sqrt{\tau}\,(1 - \sqrt{\tau})/(1 + \sqrt{\tau}), & \text{if } \tau \geq 1; \\ \dfrac{1}{2}(\tau - 1), & \text{if } \tau < 1. \end{cases} \tag{53}$$

From Eq. (53) we can see that $S_\infty(\sigma = 0)$ vanishes only when $\tau = 1$, and has finite negative values when $\tau \geq 1$ or $\tau < 1$. This is reflected in the crossing over of the Scatchard plots shown in Fig. 2. Eqs. (48), (50) and (51), as well as those for special cases, can be used to deconvolute the interaction parameters τ and σ from experimental binding data.

6. Discussion

In this paper we extend the traditional one-dimensional lattice model with three parameters (K, m and σ) to two-dimensional ladder models with four parameters (K, m, σ and τ). The model gives more detailed descriptions of the biological binding processes such as the interactions between proteins and double-stranded nucleic acids. The extra cooperativity parameter gives more flexibility to describe the ligand interactions in different directions. The binding isotherm reveals certain features, which cannot be modeled by the one-dimensional lattices. One prominent feature is that even when $\sigma = 0$ or $\tau = 0$, the Scatchard plots will show a maximum as long as $2\sigma + \tau > 4$ holds (for $m = 1$) (Fig. 2a,e). A maximum in Scatchard plots usually signifies positive cooperation in one-dimensional model [5,6]. In the ladder model, the maximum can show up when the interaction in one dimension is infinitely negative and that in the other direction is finitely positive. Preliminary data fitting experiments also show that while it is possible to find a reasonably good fit to binding curves of the ladder model by the binding curves from the one-dimensional model when the cooperativity in both directions are positive ($\sigma > 1$ and $\tau > 1$), it is impossible to find a one-dimensional binding curve to fit the binding curves from the ladder model when the cooperativity in one direction is positive and that in the other direction is negative (data not shown).

The parameters of the model can be extracted by full-range curve-fitting to experimental data using the explicit formulas provided, or can be estimated by using the closed-form formulas for the special properties on binding isotherms and Scatchard plots.

Acknowledgements

The author thanks Prof. E. Di Cera for his encouragement and help, and Prof. T. Lohman for discussion.

Appendix A. Partition functions for ring ladder

In this Appendix we list the partition functions of ring ladder for some small n, the length of the ring ladder, and small m, the size of the ligand. The interactions between ligands on one rung of the ladder are represented by τ, and those within one leg of the ladder are represented by σ. For $m = 1$, we have

$${}^R\Psi_{(1)} = \sigma^2\tau\omega^2 + 2\sigma\omega + 1 \tag{54a}$$

$${}^R\Psi_{(2)} = \sigma^4\tau^2\omega^4 + 4\sigma^2\tau\omega^3 + 2(\tau + \sigma^2 + 1)\omega^2 + 4\omega + 1 \tag{54b}$$

$${}^R\Psi_{(3)} = \sigma^6\tau^3\omega^6 + 6\sigma^4\tau^2\omega^5 + 3\tau\sigma^2(\tau + 2\sigma + 2)\omega^4 + 2\sigma(6\tau + \sigma^2 + 3)\omega^3 + 3(\tau + 2\sigma + 2)\omega^2 + 6\omega + 1 \tag{54c}$$

For $m = 2$, we have

$$^{R}\Psi_{(1)} = 1 \tag{55a}$$

$$^{R}\Psi_{(2)} = 4\sigma^2\tau^2\omega^2 + 4\sigma\omega + 1 \tag{55b}$$

$$^{R}\Psi_{(3)} = 3\tau(\tau + 2)\omega^2 + 6\omega + 1 \tag{55c}$$

$$^{R}\Psi_{(4)} = 4\sigma^4\tau^4\omega^4 + 16\sigma^2\tau^2\omega^3 + 4(\tau^2 + \sigma^2 + 2\tau + 1)\omega^2 + 8\omega + 1 \tag{55d}$$

$$^{R}\Psi_{(5)} = 5\sigma^2\tau^3(\tau + 4)\omega^4 + 10\sigma\tau(3\sigma + 2)\omega^3 + 5(\tau^2 + 2\tau + 2\sigma + 2)\omega^2 + 10\omega + 1 \tag{55e}$$

and for $m = 3$

$$^{R}\Psi_{(1)} = 1 \tag{56a}$$

$$^{R}\Psi_{(2)} = 1 \tag{56b}$$

$$^{R}\Psi_{(3)} = 9\tau^3\sigma^2\omega^2 + 6\sigma\omega + 1 \tag{56c}$$

$$^{R}\Psi_{(4)} = 4\tau^2(\tau + 3)\omega^2 + 8\omega + 1 \tag{56d}$$

$$^{R}\Psi_{(5)} = 5\tau(\tau^2 + 2\tau + 2)\omega^2 + 10\omega + 1 \tag{56e}$$

$$^{R}\Psi_{(6)} = 9\sigma^4\tau^6\omega^4 + 36\sigma^2\tau^3\omega^3 + 6(\tau^3 + 2\tau^2 + \sigma^2 + 2\tau + 1)\omega^2 + 12\omega + 1 \tag{56f}$$

$$^{R}\Psi_{(7)} = 7\tau^5\sigma^2(\tau + 6)\omega^4 + 14\tau^2\sigma(4\tau + 3)\omega^3 + 7(\tau^3 + 2\tau^2 + 2\tau + 2\sigma + 2)\omega^2 + 14\omega + 1 \tag{56g}$$

Appendix B. Partition functions for linear ladder

In this Appendix we list the partition functions of linear ladder for some small n, the length of the ring ladder, and small m, the size of the ligand. The interactions between ligands on one rung of the ladder are represented by τ, and those within one leg of the ladder are represented by σ. For $m = 1$, we have

$$^{L}\Psi_{(1)} = \tau\omega^2 + 2\omega + 1 \tag{57a}$$

$$^{L}\Psi_{(2)} = \sigma^2\tau^2\omega^4 + 4\sigma\tau\omega^3 + 2(\tau + \sigma + 1)\omega^2 + 4\omega + 1 \tag{57b}$$

$$^{L}\Psi_{(3)} = \tau^3\sigma^4\omega^6 + 2\tau^2\sigma^2(2\sigma + 1)\omega^5 + \tau(2\sigma^2\tau + 8\sigma^2 + \tau + 4\sigma)\omega^4 + 2(4\sigma\tau + \sigma^2 + 2\tau + 2\sigma + 1)\omega^3 + (3\tau + 4\sigma + 8)\omega^2 + 6\omega + 1 \tag{57c}$$

For $m = 2$,

$$^{L}\Psi_{(1)} = 1 \tag{58a}$$

$$^{L}\Psi_{(2)} = \tau^2\omega^2 + 2\omega + 1 \tag{58b}$$

$$^{L}\Psi_{(3)} = 2\tau(\tau + 1)\omega^2 + 4\omega + 1 \tag{58c}$$

$$^{L}\Psi_{(4)} = \sigma^2\tau^4\omega^4 + 6\sigma\tau^2\omega^3 + (3\tau^2 + 4\tau + 2\sigma + 2)\omega^2 + 6\omega + 1 \tag{58d}$$

$$^{L}\Psi_{(5)} = \tau^3[2(\tau + 1)\sigma^2 + 2\sigma + \tau]\omega^4 + 4\tau[(3\tau + 1)\sigma + \tau + 1]\omega^3 + (4\tau^2 + 6\tau + 4\sigma + 8)\omega^2 + 8\omega + 1 \tag{58e}$$

For $m = 3$,

$$^{L}\Psi_{(1)} = 1 \tag{59a}$$

$$^{L}\Psi_{(2)} = 1 \tag{59b}$$

$$^{L}\Psi_{(3)} = \tau^3\omega^2 + 2\omega + 1 \tag{59c}$$

$$^{L}\Psi_{(4)} = 2\tau^2(\tau + 1)\omega^2 + 4\omega + 1 \tag{59d}$$

$$^{L}\Psi_{(5)} = \tau(3\tau^2 + 4\tau + 2)\omega^2 + 6\omega + 1 \tag{59e}$$

$$^{L}\Psi_{(6)} = \tau^6\sigma^2\omega^4 + 8\tau^3\sigma\omega^3 + 2(2\tau^3 + 3\tau^2 + 2\tau + \sigma + 1)\omega^2 + 8\omega + 1 \tag{59f}$$

$$^{L}\Psi_{(7)} = \tau^5[2(\tau + 1)\sigma^2 + 4\sigma + \tau]\omega^4 + 2\tau^2[2(4\tau + 1)\sigma + 2\tau + 3]\omega^3 + (5\tau^3 + 8\tau^2 + 6\tau + 4\sigma + 8)\omega^2 + 10\omega + 1 \tag{59g}$$

References

[1] Y. Kong, J. Chem. Phys., in press.
[2] A. Revzin, The Biology of Nonspecific DNA-Protein Interactions, CRC Press, Boca Raton, 1990.
[3] D.M. Crothers, Biopolymers 6 (1968) 575.
[4] A.S. Zasedatelev, G.V. Gurskii, M.V. Volkenshtein, Mol. Biol. 5 (1971) 245.
[5] J.D. McGhee, P.H. von Hippel, J. Mol. Biol. 86 (1974) 469.
[6] G. Schwarz, Biophys. Chem. 6 (1977) 65.
[7] I.R. Epstein, Biophys. Chem. 8 (1978) 327.
[8] T.L. Hill, In Cooperativity Theory in Biochemistry, Springer-Verlag, , New York, 1985.
[9] T. Tsuchiya, A. Szabo, Biopolymers 21 (1982) 979.
[10] Y. Chen, Biophys. Chem. 27 (1987) 59.
[11] Y. Chen, A. Maxwell, H.V. Westerhoff, J. Mol. Biol. 190 (1986) 201.
[12] E. Di Cera, Y. Kong, Biophys. Chem. 61 (1996) 107.
[13] H. Qian, Biophys. J. 67 (1994) 349.
[14] E. Di Cera, Thermodynamic Theory of Site-Specific Binding Processes in Biological Macromolecules, Cambridge University, , Cambridge, UK, 1995.
[15] D. Poland, H.A. Scheraga, Theory of Helix-Coil Transitions in Biopolymers, Academic Press, , New York, 1970.