



Statistical thermodynamics of molecules with multiple adsorption states: Application to protein adsorption

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ABSTRACT

The statistical thermodynamics of molecules with multiple adsorption states has been studied using a lattice model. The thermodynamic functions, calculated for a system characterized by two adsorption states, were applied to describe the adsorption of antifreeze proteins onto an ice crystal. The theoretical formalism reproduces the classical Langmuir equation (one-state limit), leads to the exact statistical thermodynamics of molecules adsorbed in one dimension with n different adsorption states, and seems to be a promising way toward a more accurate description of the adsorption thermodynamics of structurally diverse proteins.

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1. Introduction

Protein adsorption plays an important role due to its possible applications in a variety of technological and biological processes [1–3]. The understanding of the fundamental factors that determine protein adsorption are of crucial importance in developments biosensors, biochip, medical device coatings, drug and the fabrication of novel materials [4–8]. Even more, the effective adsorption of bioparticles is important for many practical processes such as wastewater filtration, protein separation, enzymes immobilization, immunology assays and so forth [9–12].

The adsorption of proteins on surfaces is a complex process [13]. Due to the size and different shapes of these adsorbing particles, the interactions between the adsorbed proteins on the surface can be strongly influenced by the fact that the particles may undergo conformational changes upon adsorption [14–17].

Numerous studies about the adsorption of proteins have been reported in the literature. Of special interest is the case of antifreeze proteins (AFPs). AFPs are a structurally diverse group of proteins that allow certain organisms, such as fish, insects, and plants, to survive subzero temperatures [18–22]. AFPs bind to small ice crystals to inhibit growth and re-crystallization of ice. This inhibition process has been generally considered as one of the many cases of crystal-growth inhibition by impurity adsorption [20].

The interaction of AFPs with the ice crystal causes thermal hysteresis, which is a difference between freezing and melting temperatures. Despite the number of studies devoted to investigating the interactions between AFPs and the ice crystal, no direct experimental evidence has been found to demonstrate whether the

binding of AFPs to ice surfaces is reversible or irreversible. Thus, it has been proposed that the binding of AFPs to ice surfaces causes the growing ice fronts to advance in spaces between the AFP molecules leading to local surface curvatures. This physical change makes it energetically less favorable for water molecules to join the ice lattice, resulting in a local freezing-point depression. This mechanism, known as Kelvin effect [23–25], implies that AFPs adsorb to ice surfaces in an irreversible manner, since desorption of AFPs would allow supercooled water to join the ice lattice instantly. However, it has been difficult to reconcile irreversible adsorption with the observed concentration dependence of the thermal hysteresis. To understand this phenomenon, other theories have been developed based on reversible AFP adsorption [26–28].

Adsorption of AFPs is a very demanding problem both experimentally and theoretically. In fact, the inherent complexity of this system, mainly associated to the structural diversity of AFPs in the adsorbed state, still represents a major difficulty to the development of approximate solutions for the thermodynamic functions. To this respect, simple solvable models are useful as a basis to identify and characterize the most prominent features of this particular process, and to stimulate the development of more sophisticated models which are able to reproduce concrete experimental systems.

Recently, two interesting studies dealing with AFP adsorption have been reported [29,30]. In Ref. [29], the reversible adsorption of single- and two-domain AFPs onto an ice crystal was studied. On the basis of the well-known Langmuir model [31] and using kinetic arguments, the authors derived equations to describe the two adsorbed states of the protein: state I, with the protein adsorbed perpendicular to the surface on single sites; and state II, with the protein lying parallel to the surface and occupying 2 adjacent sites.

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In Ref. [30], a statistical mechanics model was proposed to study the thermal hysteresis activity of the type I AFP. In this framework, the AFP molecule was modeled as a chain, which occupies χ sites when adsorbed on the ice-crystal surface. In order to obtain the grand partition function of the adlayer, the authors calculated the total number of configurations of N AFP molecules on N_1 sites as $\Omega(N, N_1, \chi) = (N_1/\chi)! / \{N![(N_1/\chi) - N]!\}$. This factor is identical to the well-known Langmuir configurational factor for an effective lattice of N_1/χ “supersites” of size χ [32]. Consequently, the adsorption isotherm obtained by Li et al. corresponds to the standard Langmuir equation [see Eq. (4) in Ref. [30]].

The adsorption isotherm equations reported in Refs. [29,30] rely upon the same assumptions as the classical Langmuir model and no dependence is observed on adsorbate size and shape, lattice geometry and dimension space. However, the adsorption of the protein in the state II and the adsorption of a chain of length χ are clear examples of multisite adsorption process, and consequently, multisite statistics should be considered. In this sense, it has been shown in numerous studies [33–46] that the entropic effects of structured molecules are significant in the monolayer regime when compared with monatomic adsorption. Moreover, in contrast to the statistic for the simple particles, the structure of lattice space plays a fundamental role in determining the statistics of particles occupying more than one site on the lattice.

As a consequence of these limitations, there is a lack of research concerning the probable mechanism of protein adsorption and the configuration of the protein in the adsorbed state. In this context, the main purpose of the present Letter is to introduce a new theoretical scheme, which allows to include the role of adsorbate size and lattice structure on the main thermodynamic functions of molecules with multiple adsorption states. The new formalism (1) reproduces the classical Langmuir equation (one-state limit), (2) leads to the exact statistical thermodynamics of molecules adsorbed in one dimension with n different adsorption states, (3) provides a close approximation for two-dimensional systems and (4) can be applied to describe the adsorption of two-domain antifreeze proteins onto an ice crystal.

The letter is organized as follows: the new theory is presented in Section 2, along with the description of the modified Langmuir model developed in Ref. [29]. In order to test the accuracy and applicability of the proposed model, Section 3 is devoted to the analysis of two-domain type III AFPs adsorbed onto an ice crystal. The results of the present study are compared with previous published data and MC simulation results.

2. Theory

In Ref. [29], a modified Langmuir equation was presented to account for the two-step adsorption process of a two-domain protein onto an ice crystal. The two-domain protein was modeled as two identical units (or domains) connected by a flexible linker and two possible adsorption states: with one of the domains adsorbed to the ice surface allowing the second domain to freely diffuse only limited by the extent of the linker (state I, and with both domains adsorbed to the ice surface (state II, Fig. 1b). In Section 2.1, we will reproduce the calculations of Can and Holland [29].

On the other hand, the basis of a more general model, capable of describing the reversible adsorption of molecules with multiple adsorbed states, will be given in Section 2.2.

2.1. Modified Langmuir isotherm

The modified Langmuir isotherm was developed in Ref. [29] as a generalization of the classical Langmuir model, with the following additional assumptions: (i) the adsorption and desorption rate

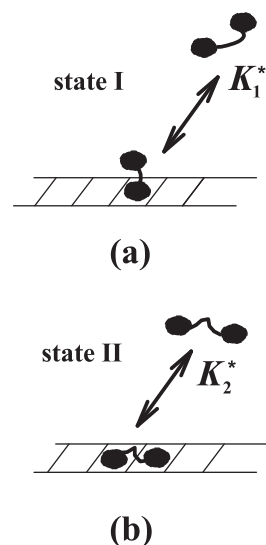


Figure 1. Schematic representation of the two different adsorption configurations for a two-domain protein onto the ice lattice: (a) state I, with one of the domains adsorbed to the ice surface allowing the second domain to freely diffuse only limited by the extent of the linker and (b) state II, with both domains adsorbed to the ice surface. The corresponding equilibrium binding constants are K_1^* and K_2^* .

constants are equivalent for each domain and (ii) the desorption rate constants are not affected by whether the other domain is bound or unbound. Under these conditions, the kinetic equation for state I is

$$\frac{d\theta_1}{dt} = k_{a1}C(1 - \theta) - k_d\theta_1 - k_{a2}\theta_1(1 - \theta) + k_d\theta_2, \quad (1)$$

where θ_1 (θ_2) represents the partial surface coverage corresponding to the state I (state II); $\theta = \theta_1 + \theta_2$ is the total surface coverage; C is the concentration of the protein in the solution; and k_{a1} [k_{a2}] is the adsorption rate constant from solution [state I] to state I [state II]. Since it is assumed that the desorption rate does not depend on the number of domains bound to the surface, desorption rate constants for the domains in each state were taken as k_d .

Similarly, a kinetic equation can be derived for state II:

$$\frac{d\theta_2}{dt} = 2k_{a2}\theta_1(1 - \theta) - 2k_d\theta_2, \quad (2)$$

where the factors of two arise because the surface coverage of a protein in state II is twice as large as a protein in state I. Interested readers are referred to Ref. [29] for a more complete description of Eqs. (1) and (2).

By equating to zero Eqs. (1) and (2) (equilibrium conditions), θ_1 and θ_2 can be obtained:

$$\theta_1 = \frac{-1 - K_1C + \sqrt{(1 + K_1C)^2 + 4K_1K_2C}}{2K_2}, \quad (3)$$

and

$$\theta_2 = \frac{-1 - K_2 - 2K_1C - K_1^2C^2 - 3K_1K_2C + (1 + K_2 + K_1C)\sqrt{(1 + K_1C)^2 + 4K_1K_2C}}{K_2 - K_1K_2C + K_2\sqrt{(1 + K_1C)^2 + 4K_1K_2C}}, \quad (4)$$

where $K_1 = k_{a1}/k_d$ [$K_2 = k_{a2}/k_d$] is the equilibrium binding constant between solution and state I [state I and state II]. Finally, the total surface coverage can be calculated from the sum of Eqs. (1) and (2),

$$\theta = \frac{1}{2K_1K_2C} + \frac{1}{2K_2} + 1 - \sqrt{\left(\frac{1}{2K_1K_2C} + \frac{1}{2K_2}\right)^2 + \frac{1}{K_1K_2C}} \quad (5)$$

2.2. Lattice model for adsorption of molecules with multiple adsorption states: statistical-mechanical derivation

Let us assume a lattice of M sites ($M \rightarrow |\infty$) with lattice constant a , connectivity c and periodic boundary conditions. Under this condition all lattice sites are equivalent, hence edge effects will not enter our derivation.

n molecules are adsorbed on the surface with the following considerations: (1) the molecules are constituted by n identical units connected by flexible linkers and can adsorb in n different adsorption states. (2) A molecule adsorbed in the i -state (or i -molecule) is assumed to be a molecule occupying i sites on the lattice ($i = 1, \dots, n$). (3) $N = \sum_{i=1}^n N_i$ (being N_i the number of molecules adsorbed in the i -state). In addition, double site occupancy is not allowed as to represent properties in the monolayer regime. Since different particles do not interact with each other, all configurations of $\{N_1, \dots, N_n\}$ particles on M sites are equally probable; henceforth, the canonical partition function $Q(M, \{N_1, \dots, N_n\}, T)$ for this system is

$$Q(M, \{N_1, \dots, N_n\}, T) = \Omega(M, \{N_1, \dots, N_n\}) \exp \left[-\frac{E(N_1, \dots, N_n)}{k_{\text{BT}}} \right], \quad (6)$$

where $\Omega(M, \{N_1, \dots, N_n\})$ is the number of ways to arrange N_1 molecules type 1, N_2 molecules type 2, ... and N_n molecules type n on M sites; $E(N_1, \dots, N_n)$ is the total adsorption energy between adparticles and lattice sites; T is the temperature and k_{B} is the Boltzmann constant. For simplicity, we have also assumed the internal and vibrational contributions to the partition factor to be a unitary factor in Eq. (6).

For $c = 2$ (one-dimensional lattice), $\Omega(M, \{N_1, \dots, N_n\})$ can be exactly calculated as the total number of permutations of the N_1 indistinguishable 1-molecules, N_2 indistinguishable 2-molecules, ... and N_n indistinguishable n -molecules out of n_e entities, being n_e

$$\begin{aligned} n_e &= \text{number of 1 - molecules} + \text{number of 2 - molecules} \\ &+ \dots + \text{number of } n \text{ - molecules} + \text{number of empty sites} \\ &= \sum_{i=1}^n N_i + M - \sum_{i=1}^n iN_i = M - \sum_{i=1}^n (i-1)N_i. \end{aligned} \quad (7)$$

Accordingly,

$$\Omega(M, \{N_1, \dots, N_n\}) = \frac{[M - \sum_{i=1}^n (i-1)N_i]!}{\prod_{i=1}^n N_i! [M - \sum_{i=1}^n iN_i]!}. \quad (8)$$

Additionally, $E(N_1, \dots, N_n)$ can be written as

$$E(N_1, \dots, N_n) = \sum_{i=1}^n \epsilon_i N_i, \quad (9)$$

where ϵ_i represents the adsorption energy of a molecule in the i -state.

In the canonical ensemble, the Helmholtz free energy $F(M, \{N_1, \dots, N_n\}, T)$ relates to $\Omega(M, \{N_1, \dots, N_n\})$ through

$$\begin{aligned} \beta F(M, \{N_1, \dots, N_n\}, T) &= -\ln Q(M, \{N_1, \dots, N_n\}, T) \\ &= -\ln \Omega(M, \{N_1, \dots, N_n\}) + \beta \sum_{i=1}^n \epsilon_i N_i, \end{aligned} \quad (10)$$

where $\beta = 1/k_{\text{BT}}$.

The chemical potential of the adsorbed species j , $\mu_{j,\text{ads}}$, can be calculated as [32]

$$\mu_{j,\text{ads}} = \left(\frac{\partial F}{\partial N_j} \right)_{N'_s, i \neq j} \quad (j = 1, \dots, n). \quad (11)$$

From Eqs. (8)–(11) it follows that

$$\begin{aligned} \beta \mu_{j,\text{ads}} &= (j-1) \ln \left[1 - \sum_{i=1}^n \left(\frac{i-1}{i} \right) \theta_i \right] \\ &+ \ln \frac{\theta_j}{j} - j \ln \left(1 - \sum_{i=1}^n \theta_i \right) + \beta \epsilon_j \quad (j = 1, \dots, n), \end{aligned} \quad (12)$$

where $\theta_i = iN_i/M$ represents the partial coverage of the species i . In addition, $\theta = \sum_{i=1}^n \theta_i$, being θ the total surface coverage.

At equilibrium, the chemical potential of the adsorbed and gas phase are equal,

$$\mu_{\text{ads}} = \mu_{\text{gas}}, \quad (13)$$

where μ_{gas} corresponds to the chemical potential of the molecule in an ideal gas, at temperature T and pressure P . Then,

$$\beta \mu_{\text{gas}} = \beta \mu^0 + \ln P, \quad (14)$$

where μ^0 is the standard chemical potential [32]. Now, equating Eq. (12) with Eq. (14), it results,

$$\begin{aligned} \ln(K_j^* P) &= (j-1) \ln \left[1 - \sum_{i=1}^n \left(\frac{i-1}{i} \right) \theta_i \right] \\ &+ \ln \frac{\theta_j}{j} - j \ln \left(1 - \sum_{i=1}^n \theta_i \right) \quad (j = 1, \dots, n), \end{aligned} \quad (15)$$

where $K_j^* = \exp[\beta(\mu^0 - \epsilon_j)]$ is the equilibrium binding constant between the gas phase and the molecule adsorbed in the j -state. Eq. (15) represents the partial adsorption isotherm corresponding to the j -molecule.

Hereafter, we address the calculation of approximated thermodynamical functions of molecules adsorbed on lattices with connectivity c higher than two (i.e., dimensions higher than one). In this case and for $i > 2$, the i -molecule can be adsorbed in two types of configurations: (i) as a linear array of monomers, which we call a “linear i -molecule” and (ii) as a chain of adjacent units with the following sequence. Once the first unit is in place, the second unit occupies one of the c nearest-neighbors of the first unit. The third and successive units occupy one of the $c-1$ nearest-neighbors of the preceding unit. This process continues until i units are placed without creating an overlap. We call this feature a “flexible i -molecule” [47].

In general, the number of states Ω for fixed M and N will be also a function of the lattice connectivity; henceforth $\Omega(M, \{N_1, \dots, N_n\}, c)$. In order to derive an explicit form for the $\Omega(M, \{N_1, \dots, N_n\}, c)$, we assume a connectivity ansatz used by different authors [34,38,47] to relate the configurational factor $\Omega(M, \{N_1, \dots, N_n\}, c)$ for any c with respect to the same quantity in one dimension ($c = 2$). Thus

$$\Omega(M, \{N_1, \dots, N_n\}, c) = \Omega(M, \{N_1, \dots, N_n\}, c = 2) \prod_{i=1}^n m_i(c, i)^{N_i} \quad (16)$$

where $\Omega(M, \{N_1, \dots, N_n\}, c = 2)$ can be obtained from Eq. (8) and $m_i(c, i)$ represents the number of available configurations (per lattice site) for an i -molecule at zero coverage. $m_i(c, i)$ is, in general, a function of the connectivity and the size/shape of the adsorbate. It is straight forward to demonstrate that,

$$m_i(c, i) = \begin{cases} c/2 & \text{for linear } i\text{-molecules} \\ [c(c-1)^{(i-2)}/2 - m'_i] & \text{for flexible } i\text{-molecules} \end{cases} \quad (17)$$

the term m'_i is subtracted in Eq. (17) since the first term overestimates $m_i(c, i)$ by including m'_i configurations providing overlaps in the i -molecule. In addition, $m_i(c, i) = 1$ for $i = 1$.

In this way, the partial adsorption isotherm corresponding to an adsorbed j -molecule/surface geometry results,

$$\ln[m_j(c,j)K_j^*P] = (j-1) \ln \left[1 - \sum_{i=1}^n \left(\frac{i-1}{i} \right) \theta_i \right] + \ln \frac{\theta_j}{j} - j \ln \left(1 - \sum_{i=1}^n \theta_i \right) \quad (j = 1, \dots, n). \quad (18)$$

3. Applications: adsorption of proteins to the ice lattice

Casen = 1: Classical Langmuir isotherm.

In the limit $n = 1$ ($j = 1$), $m_i(c, i) = 1$ and Eq. (18) adopts the form of the classical Langmuir isotherm:

$$\ln(KC) = \ln \theta - \ln(1 - \theta), \quad (19)$$

or

$$\theta = \frac{KC}{1 + KC}, \quad (20)$$

where the pressure P was replaced by the concentration of the protein in the solution C and, to simplify the notation, we write $K_1^* = K$ and $\theta_1 = \theta$.

Casen = 2: Adsorption isotherms for molecules with two adsorbed states.

For $n = 2$ ($j = 1, 2$), the scheme developed in Section 2.2 provides a theoretical framework to study the adsorption of molecules with two possible adsorption states as shown in Figure 1. Thus, from Eqs. (17) and (18), and replacing P by C , it results:

$$\ln(K_1^*C) = \ln \theta_1 - \ln(1 - \theta_1 - \theta_2), \quad (21)$$

and

$$\ln\left(\frac{C}{2}K_2^*C\right) = \ln \left[1 - \left(\frac{1}{2}\right)\theta_2 \right] + \ln \frac{\theta_2}{2} - 2 \ln(1 - \theta_1 - \theta_2). \quad (22)$$

By simple algebra, Eqs. (21) and (22) can be written in a more convenient form:

$$\theta_1 = \frac{K_1^*C}{1 + K_1^*C} \sqrt{1 - \frac{cK_2^*C}{cK_2^*C + \frac{1}{2}(1 + K_1^*C)^2}}, \quad (23)$$

$$\theta_2 = 1 - \sqrt{1 - \frac{cK_2^*C}{cK_2^*C + \frac{1}{2}(1 + K_1^*C)^2}}, \quad (24)$$

and

$$\theta = 1 - \frac{1}{1 + K_1^*C} \sqrt{1 - \frac{cK_2^*C}{cK_2^*C + \frac{1}{2}(1 + K_1^*C)^2}}. \quad (25)$$

Clearly, Eqs. (23)–(25) are different to the corresponding ones obtained by Can and Holland [29] [Eqs. (3)–(5)]. Note, for example, that (i) while K_2 (adimensional parameter) represents the equilibrium binding constant between state I and state II, K_2^* (parameter with units of C^{-1}) is the equilibrium constant between solution and state II and (ii) Eqs. (3)–(5) do not show dependence on adsorbate shape and lattice geometry, as expected for adsorption of molecules that occupy more than one site on the lattice. To rationalize these differences and to investigate the outcome and limitations of Eqs. (3)–(5) and Eqs. (23)–(25), theoretical results are compared below with Monte Carlo (MC) simulation data.

The adsorption process is simulated through a grand canonical ensemble method. Given a square lattice of M adsorption sites, the algorithm to carry out an elementary Monte Carlo Step (MCS) is the following:

- (1) Set the value of temperature T and pressure P .
- (2) An initial configuration of 1- and 2-particles adsorbed at random positions is generated.
- (3) One of the available states is chosen at random.

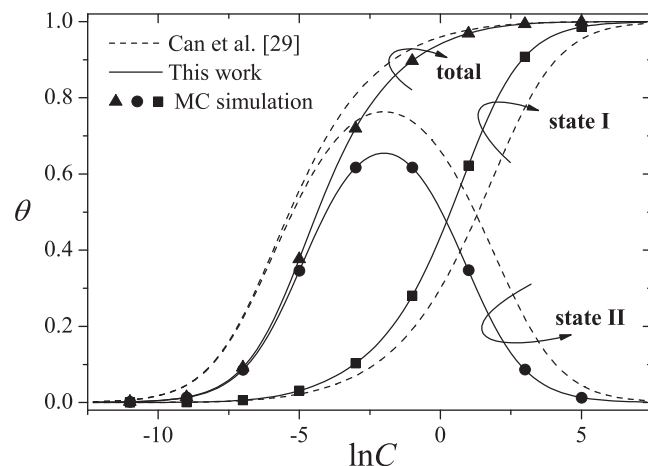


Figure 2. Total and partial adsorption isotherms (coverage vs $\ln C$) for a system of two-states molecules ($n = 2$) adsorbed on a one-dimensional lattice. Dashed lines represent results from Can and Holland [29] [Eqs. ((3)–(5))]; solid lines correspond to exact calculations from Eqs. ((23)–(25)); and symbols denote MC simulation data. Parameter values: $K_1^* = 7.39 \text{ mM}^{-1}$ ($\beta\epsilon_1 = -2$) and $K_2^* = 54.60 \text{ mM}^{-1}$ ($\beta\epsilon_2 = -4$).

(4) If the state selected in step (3) is the state I [II], then a site [pair of nearest-neighbor sites] is chosen at random and an attempt is made to change its occupancy state with probability given by the Metropolis scheme [48],

$$W_{\text{ads}} = \min\{1, P \exp(-\Delta E/k_B T)\} \quad (26)$$

and

$$W_{\text{des}} = \min\left\{1, \frac{1}{P} \exp(-\Delta E/k_B T)\right\}, \quad (27)$$

where W_{ads} is the adsorption probability, W_{des} is the desorption probability and $\Delta E = E_f - E_i$ is the difference between the energies of the final and initial states.

(5) Repeat from step (3) M times.

The approximation to thermodynamical equilibrium is monitored through the fluctuations in the number of adsorbed particles; this is usually reached in 10^6 MCS. After that, mean values of thermodynamic quantities, like total and partial isotherms are obtained as simple averages over m successive configurations:

$$\theta = \frac{\langle N \rangle}{M} \quad \theta_1 = \frac{\langle N_1 \rangle}{M} \quad \theta_2 = \frac{\langle N_2 \rangle}{M} \quad (28)$$

where the brackets mean the time average over the m MC simulation runs (in our calculations we have used $m = 10^6$).

The differences between K_2 and K_2^* , which derive from the different adsorption mechanisms considered in the calculations of Sections 2.1 and 2.2, do not allow a direct comparison between the model of Ref. [29] and the present theory. However, as will be seen from the analysis below, with the simple choice of K_1 equal to K_1^* , and K_2 equal to K_2^* (magnitude¹), both models provide qualitatively similar results.

A typical case corresponding to $n = 2$ is shown in Figure 2. In order to evidence clearly the multisite-occupancy effect associated to the molecules lying parallel to the surface, the adsorption energy assigned to the state II is set larger than the adsorption energy corresponding to state I: $\beta\epsilon_1 = -2$ ($K_1^* = 7.39 \text{ mM}^{-1}$) and $\beta\epsilon_2 = -4$

¹ Note that K_2 is an adimensional parameter, while K_2^* has units of C^{-1} .

($K_2^* = 54.60 \text{ mM}^{-1}$). The values of K_1 and K_2 were chosen according to the rule described in previous paragraph. Symbols represent MC simulation results, dashed lines denote data from Can and Holland [Eqs. ((3)–(5))] and solid lines denote data from the present theory [Eqs. ((23)–(25))]. For simplicity, a one-dimensional geometry has been considered for the comparison. Accordingly, the computational simulations have been developed for chains of $M = 10^4$ sites.

In the case of Eqs. ((23)–(25)), the behavior of the curves can be explained as follows. Due to the fact that the flat molecules have greater adsorption energy, they adsorb favorably at low concentrations. As the concentration is increased, the adsorption of molecules in state I starts becoming more energetically favorable, therefore this species displaces the flat molecules. Thus, the coverage corresponding to adsorbed molecules in state II has a maximum and later decreases asymptotically to zero. This behavior is known as adsorption preference reversal (APR) and has been observed in computational simulations, mean-field theory, and exact calculations for methane-ethane mixtures adsorption in silicalite [45,49,50]. MC simulations fully agree with the predictions from Eqs. ((23)–(25)).

With respect to the model derived by Can and Holland [29], partial and total isotherms qualitatively agree with simulation values and theoretical data from Eqs. ((23)–(25)). These results can be interpreted in the following way. At low concentrations, the molecules bind to the surface in a tilted orientation (state I) and change to a more connected state (state II). As the concentration is increased, the incoming molecules compete for the binding sites and state I dominates at high concentrations.

An identical situation is observed in Figure 3, where an experimental case, corresponding to the adsorption of single- and two-domain type III AFPs has been studied [29]. The corresponding equilibrium constants were taken from the calculations in Ref. [29]: $K_1 = 1.9 \text{ mM}^{-1}$ and $K_2 = 5$. In addition, K_1^* and K_2^* were chosen by following the same criteria used in the study of Figure 2. The resulting curves indicates that (1) Eqs. ((23)–(25)) provides the exact solution of the problem, and (2) the curves from Eqs. ((3)–(5)) show a good qualitative agreement with the simulation data.

Similar results have been obtained in two dimensions (so we do not show here). Even though the present theory does not provide the exact solution of the two-dimensional problem², it leads to a very good approximation of the simulation data.

In summary, the statistical thermodynamics of molecules with multiple adsorption states has been studied using a lattice model. In contrast to the existing development on multistate adsorption thermodynamics, where the arrangement of the adsorption sites in space is immaterial, the present theory includes the configuration of the molecule in the adsorbed state as a parameter of the model. In this way, adsorbate structure and lattice geometry play fundamental roles in determining the statistics of multistate adsorbed molecules.

The new theoretical framework (1) provides the first exact model of molecules adsorbed in one dimension with n different adsorption states; (2) includes, as a particular case ($n = 2$), the phenomenology of the model derived by Can and Holland [29]; (3) reproduces the classical Langmuir equation (one-state limit); (4) leads to a close approximation for two-dimensional systems and multiple adsorbed states and (5) seems to be a promising way toward a more accurate description of the adsorption thermodynamics of structurally diverse proteins.

² The problem in which a two-dimensional lattice contains isolated points (vacancies) as well as particles occupying k adjacent sites has not been solved in closed form and still represents a major challenge in surface science.

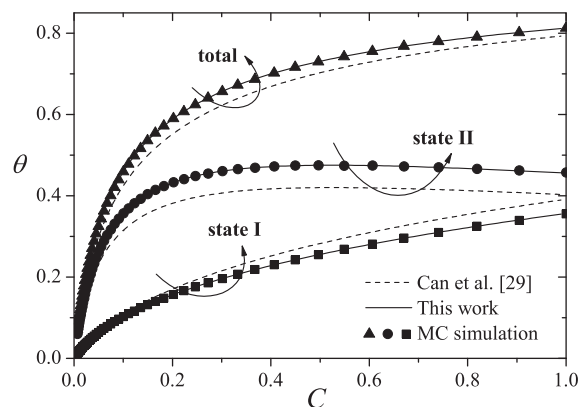


Figure 3. Same as Fig. 2, but for $K_1 = 1.9 \text{ mM}^{-1}$ and $K_2 = 5$. In this case, total and partial surface densities are plotted as a function of the concentration C .

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