Analysis of Protein Binding to Receptor-Doped Lipid Monolayers by Monte Carlo Simulation

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ABSTRACT This paper presents a Monte Carlo simulation (MCS) method for estimating the parameters that characterize ligand-receptor binding directly from experimentally derived binding isotherms. Binding parameters are estimated by incorporating an MCS algorithm for ligand binding to a two-dimensional receptor array into a nonlinear regression program. The MCS method was tested by analyzing experimental isotherms of avidin binding to biotinylated lipid in Langmuir-Blodgett (LB) monolayers. The MCS-derived cooperativity coefficients and intrinsic association constants for avidin-biotin binding to LB films are correlated strongly ($R^2 > 0.93$) with the binding parameters determined from the same experimental data by a thermodynamic equilibrium binding model (Zhao et al. 1993. *Langmuir.* 9:3166–3173). This result shows MCS to be an accurate and potentially more versatile method for characterizing biomolecular interactions at surfaces.

INTRODUCTION

Monte Carlo simulation (MCS) is a useful numerical technique for studying systems when only their microscopic components can be precisely characterized (Hammersley and Handscomb, 1964; Binder, 1986). The reported applications of MCS to molecular binding have been limited, but they appear to fall into two categories. First, binding curves are generated from known parameters by MCS and compared with experimentally measured curves to test hypotheses or explain phenomena such as cooperativity and phase transitions (Crothers, 1968; Neville et al., 1972; Genest and Wahl, 1981; Hill, 1985). Most of these studies concern ligand binding to linear polymers such as DNA. Second, MCS is used to evaluate the uncertainties of binding parameters obtained either from graphical or nonlinear regression methods (Thakur et al., 1980; Saroff, 1989). This paper introduces a third application of MCS to molecular binding that determines the binding parameters directly from experimental data.

In the current paper an MCS method was developed to calculate isotherms of cooperative ligand binding to a two-dimensional receptor array from given parameters, where the MCS algorithm was incorporated into a nonlinear regression program for parameter estimation. This technique was tested by analyzing experimentally derived isotherms of avidin binding to biotin in lipid monolayers. Two important parameters estimated from this analysis were cooperativity coefficients and intrinsic association constants that characterize the level of avidin-avidin interaction and the affinity of avidin for biotin groups in lipid monolayers, respectively. The relative amount of avidin bound to the surface was also obtained. The MCS-derived cooperativity coefficients and intrinsic association constants correlated strongly ($R^2 > 0.93$) with the binding parameters obtained from the same experimental data by using a thermodynamic equilibrium binding model (Zhao et al., 1993). This result shows MCS to be an accurate and potentially more versatile method for characterizing biomolecular interactions at surfaces.

THEORY: PROBABILITY THAT A RECEPTOR IS BOUND

In order for MCS to simulate protein binding, the probability that a single receptor is bound by the protein needs to be determined. The free energy change accompanying the binding of protein A to an isolated receptor B is $\Delta G = \Delta G^0 - RT \ln a_A$, where ΔG^0 is the standard free energy change of this process, a_A is the activity of the protein molecule in solution, R is the universal gas constant, and T is the absolute temperature (Crothers, 1968). The activity of the protein is proportional to its concentration, i.e. $a_A = \gamma_A[A]$, where γ_A and $[A]$ are the activity coefficient and concentration of the protein, respectively (Tanford, 1961, p.188). If the receptor in question is not isolated (i.e., has close neighbors), the free energy change ΔG accompanying the binding is subject to perturbation from other adjacent bound protein molecules. If n neighbors of the receptor are bound by protein, the free energy change accompanying the binding is

$$\Delta G_n = \Delta G + \sum_{i=0}^{n} \delta G_i = \Delta G^0 - RT \ln a_A + \sum_{i=0}^{n} \delta G_i \qquad (1)$$

where $\delta G_0 = 0$ and δG_i ($i = 1, \ldots, n$) is the free energy perturbation induced by the ith bound neighbor.For a simple system consisting of a receptor at a surface and free protein molecules in solution, the receptor has two possible states: free or bound. Let G_f and G_b represent the energy levels of these two configurations, respectively. The energy difference

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between these two states is given by Eq. 1, i.e. $G_b - G_f = \Delta G_n$. The probability, P, that the receptor is bound by protein is given by Poland (1978)

$$P = \frac{\exp(-G_b/RT)}{\exp(-G_f/RT) + \exp(-G_b/RT)}$$

$$= \frac{\exp(-\Delta G_n/RT)}{1 + \exp(-\Delta G_n/RT)} = \frac{[A]K_0 \prod_{i=0}^{n} \eta_i}{1 + [A]K_0 \prod_{i=0}^{n} \eta_i} \quad (2)$$

where $K_0 = \gamma_A \exp(-\Delta G^o/RT)$ is the intrinsic association constant of the protein binding to receptor without interference from neighboring molecules (Tanford, 1961, p. 535) and $\eta_i = \exp(-\delta G_i/RT)$ is the cooperativity coefficient attributed to the perturbation from ith bound neighbor. $\eta_i > 1$, $\eta_i = 1$, and $\eta_i < 1$ indicate conditions for positive cooperativity, noncooperativity, and negative cooperativity, respectively. By assuming that the perturbation induced by each protein-bound neighbor is equivalent (i.e., $\delta G_i = \delta G$ or $\eta_i = \eta$ for $i = 1, \ldots, n$), Eq. 2 becomes

$$P = \frac{\eta^n K_0[A]}{1 + \eta^n K_0[A]} \quad (3)$$

MATERIALS AND METHODS

Avidin binding isotherms

An evanescent fiber-optic sensor system was used to obtain avidin binding isotherms. Details regarding the sensor fabrication and operation were given previously (Zhao and Reichert, 1992a, 1992b). Briefly, arachidic acid monolayers containing precise amounts of biotinylated phospholipid were deposited on the sensing tip of the fiber-optic sensor by the Langmuir-Blodgett (LB) technique. The biotinylated phospholipid used was a biotinylated lipid *N*-(6-(biotinoyl)-amino)hexanoyl)dipalmitoyl-L-α-phosphatidylethanolamine (B-x-DPPE, Molecular Probes, Eugene, OR). Fluorescein isothiocyanate (FITC)-labeled avidin (Sigma, St. Louis, MO) was dissolved to concentrations ranging from 0.05 to 10 μg/ml in 0.01 M phosphate-buffered saline (PBS, pH 7.5) with 0.5 M NaCl, and was introduced to the sensor surface through a flow cell. The procedures for binding isotherm experiment and signal collection was described elsewhere (Zhao et al., 1993). The binding isotherm experiments were carried out for various B-x-DPPE doping densities from 0.0 to 100 mol%. A molecular mass of 67,000 Daltons for avidin was used in calculation (Green, 1975).

Monte Carlo simulation

To model the protein binding by MCS, we assume that the distribution of protein receptors on the surface is a square lattice. Indirect support for this assumption comes from a crystallographic study of streptavidin bound to biotinylated lipid monolayers at air-water interface, showing that streptavidin molecules form such a structure (Darst et al., 1991). We also assume that a protein molecule interacts only with its nearest neighbors. For the square lattice distribution, each receptor has four nearest neighbors.

The state of the receptor array is represented by a N × N two-dimensional matrix S with elements S_{ij} ($i, j = 1, \ldots, N$), where (i, j) designates the receptor in ith row and jth column in the array. If receptor (i, j) is bound, $S_{ij} = 1$; if it is free, $S_{ij} = 0$. The probability that receptor (i, j) is bound by protein, P_{ij}, can be calculated from Eq. 3. The number of protein-bound nearest neighbors is determined by $n_{ij} = S_{i-1,j} + S_{ij+1} + S_{i+1,j} + S_{ij-1}$. Note that the receptors at edges of the array have only three neighbors, and those at the corners have only two. To minimize edge effects, we assume that the first row in the array is next to the last row and that the first column is next to the last column.

The MCS computation started with assigning all the elements of **S**, a 100x100 array, to zero. Eq. 3 was used to calculate the probability P_{ij} for each individual receptor. A random number, R, was obtained from a subroutine generating uniformly distributed random numbers between 0 and 1 (Zhao, 1992) and was compared with the calculated probability P_{ij}. If $R < P_{ij}$, the receptor was considered to be bound; otherwise, the receptor was free. Based on this calculation a new state array **S** was determined, and the number of bound receptors was counted. This new state array **S** was then used for next round of simulation. This process was repeated 50 times. Results from the first 10 rounds were discarded to eliminate the artifact of assigning all the elements of **S** to zero at the beginning. Results from the rest of 40 repetitions were averaged to give the fraction of bound receptors, denoted by x_1. To relate the calculated result to experimentally measurable quantity, we assumed that fluorescence intensity (F) received by the sensor system was proportional to the amount of surface-bound protein, i.e., $F = x_1 F_{max}$, where F_{max} was the fluorescence intensity when all the receptors were bound.

It has been shown that the number of bound receptors becomes stabilized, i.e., reaches equilibrium, in less than 10 rounds of simulation no matter how the elements of **S** are initially assigned, and that a few more repetitions thereafter are sufficient to give very reliable results due to the large number of receptors simulated (100x100) (Zhao, 1992). A random number generator discussed by Turner et al. (1985) was modified, tested, and used in this study.

Parameter estimation

The goal of performing the protein binding isotherm experiments is to obtain information on the affinity of the protein for its receptor and the cooperativity of the binding. This is usually done by fitting the experimental data to a model (i.e., an equation) and estimating the relevant parameters through regression. Unlike conventional models, MCS does not require explicit equations to describe the system. Nevertheless, parameter estimation can still be carried out through regression as long as the value predicted by the "model" for given parameters can be found (Zhao, 1992). Hence the procedure for estimating parameters from a MCS-based model is the same as that for the conventional nonlinear regression, except that the value predicted by model is obtained from MCS rather than from an equation. In our study a nonlinear regression program based on Levenberg-Marquardt method (Press et al., 1986) was adapted and supplemented with the MCS subroutine for parameter estimation. All the computation was carried out on a DEC Workstation 2100.

RESULTS AND DISCUSSION

Fig. 1 shows three binding isotherms produced via the MCS algorithm. The isotherms were calculated using intrinsic association constants and cooperativity coefficients that represent positive cooperativity ($\eta = 2.5$, $K_0 = 0.1$), noncooperativity ($\eta = 1$, $K_0 = 1$), and negative cooperativity ($\eta = 0.5$, $K_0 = 1$). $K_0 = 0.1$ was chosen in the positive cooperativity case to give a clearly sigmoid curve. Because $K_o = 0.1$ is small the fraction of bound receptors (x_1) in the region of low protein concentration in this case is smaller than those in the other two cases. However, the effect of positive cooperativity causes the fraction of bound receptors to increase more rapidly with protein concentration and soon to exceed those in the other two cases.

Fig. 2 contains representative, experimentally measured isotherms of avidin binding to B-x-DPPE of various densities in LB monolayers. The isotherms became noticeably sigmoid when the biotin surface density was 0.63 mol% or

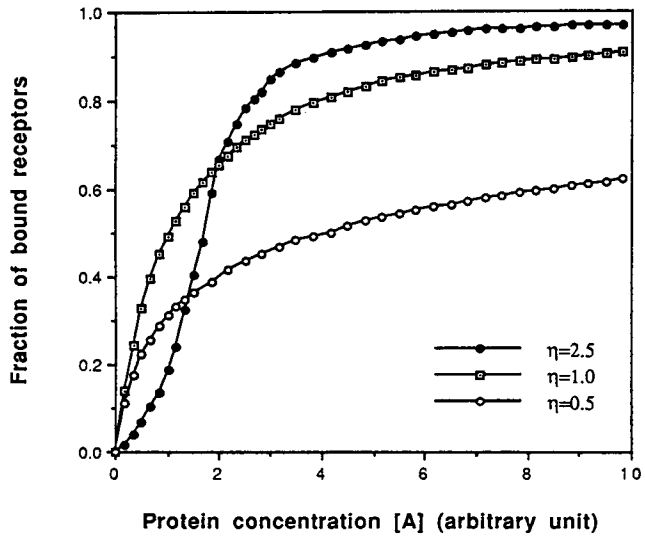

FIGURE 1 Three binding isotherms calculated from MCS, corresponding to positive cooperativity (η = 2.5, K_o=0.1), noncooperativity (η = 1, K_o=1), and negative cooperativity (η = 0.5, K_o=1).

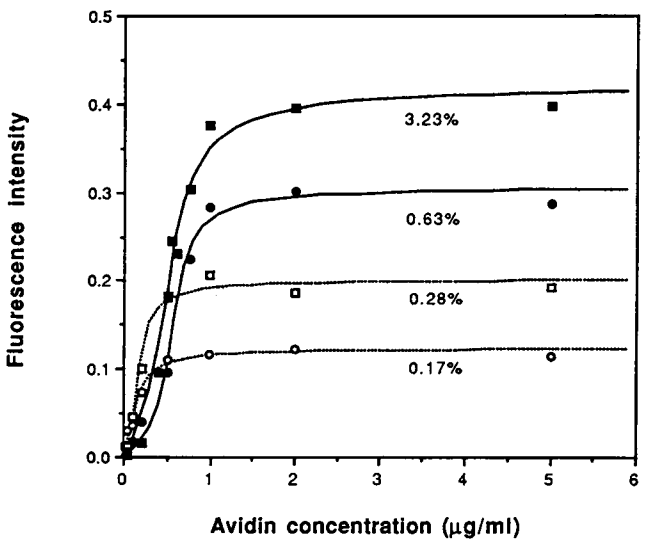

FIGURE 2 Representative isotherms of avidin binding to monolayers containing B-x-DPPE of various densities: 0.17 (○), 0.28 (□), 0.63 (●), and 3.23 (■) molar percent. The individual data points are experimentally measured fluorescence intensities. The solid and dotted lines are best-fit isotherms calculated from MCS.

greater, indicating the presence of positive cooperativity. The possible origin of the cooperativity present in these data has been discussed elsewhere (Zhao et al., 1993). The solid and dotted lines are the best fits of experimental data to the MCS-based model. The maximum fluorescence intensity (F_{max}), cooperativity coefficients (η), and intrinsic association constants (K_0) were estimated by nonlinear regression from the binding isotherms as described in Materials and Methods.

F_{max} values shown in Fig. 3 reflect the amount of avidin bound to B-x-DPPE in LB monolayers at equilibrium. Nonspecific avidin binding to the monolayer (0.0 mol% B-x-DPPE) was insignificant compared to the specific binding.

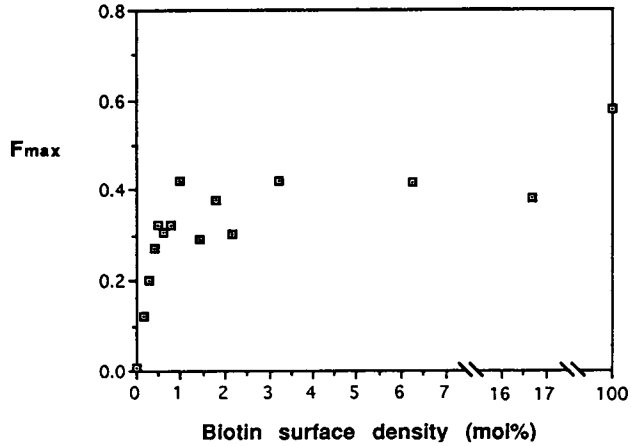

FIGURE 3 Maximum fluorescence intensity (F_{max}) corresponding to various B-x-DPPE surface densities. F_{max} is proportional to the amount of avidin bound to the LB monolayer.

The amount of avidin bound to the LB monolayer increased rapidly in the region of B-x-DPPE density <1.0 mol% and reached a plateau thereafter. One exception is the case of 100 mol% B-x-DPPE monolayer in which F_{max} was considerably greater than the plateau value. This pure phospholipid monolayer was much more fluid-like than those monolayers whose main composition was arachidic acid (Roberts, 1991), so that B-x-DPPE and bound protein molecules in this monolayer had greater mobility and possibly greater capacity to accommodate more avidin molecules.

Fig. 4 is a plot of cooperativity coefficients (η) for avidin binding at various surface densities of B-x-DPPE in LB monolayers. η increased rapidly with B-x-DPPE surface density in the low density region (<1.0 mol%) and appeared to level off at $\eta = 2.67 \pm 0.09$ thereafter. This was because increasing biotin surface density increased the adjacency of surface-bound avidin molecules and, therefore, increased the probability that avidin molecules interacted with one another. However, after a contiguous monolayer of avidin had

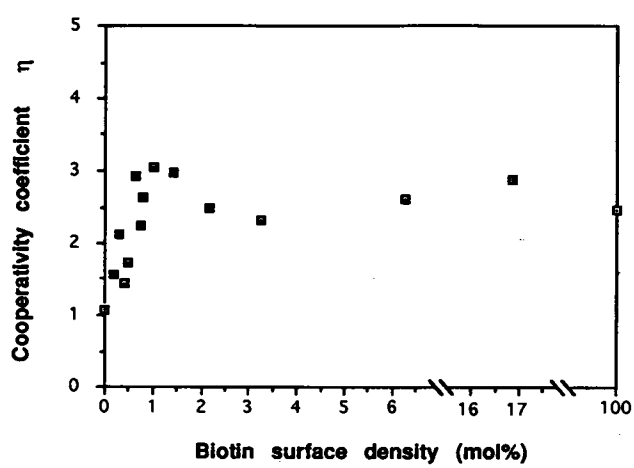

FIGURE 4 Cooperativity coefficients (η) for avidin binding to B-x-DPPE of various surface densities. η characterizes the level of avidin-avidin interaction in the binding.

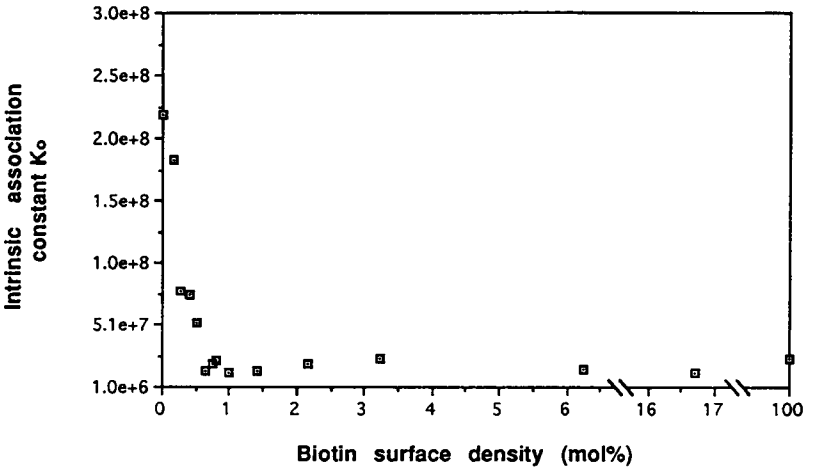

FIGURE 5 Intrinsic association constants (K_0) of avidin binding to B-x-DPPE of various surface densities. K_o characterizes the affinity of protein for its receptor in absence of protein-protein interactions among neighbors.

formed on the surface, further increases in B-x-DPPE surface density did not increase the adjacency of bound avidin, resulting in little change in the level of cooperativity. This is consistent with the fact that the amount of surface-bound avidin reached plateau at approximately 1.0 mol% biotin surface density (see Fig.3).

Fig. 5 is a plot of intrinsic association constant K_0 against B-x-DPPE surface density. K_0 decreased rapidly with B-x-DPPE surface density and then leveled off at $K_0 = 1.77 \pm 0.15$ ($\times 10^7$ M^{-1}) when B-x-DPPE density was 0.6–1.0 mol% and greater. This confirms a recent study showing that the kinetic association rate of avidin binding to B-x-DPPE in the LB film decreased rapidly with increased biotin surface density up to approximately 1.0% B-x-DPPE (Zhao and Reichert, 1992b). There it was concluded that steric hindrance from the crowding of neighboring B-x-DPPE receptors encumbered the binding of avidin to biotinylated lipid. It is surprising that K_0 for nonspecific avidin binding to LB monolayer (0.0 mol% B-x-DPPE) was greater than K_0 for any other specific binding. We believe that this is an artifact of data processing. Because the signal from nonspecifically bound avidin was only slightly above the background, the binding curve after background subtraction appeared to have reached its plateau at very low avidin concentration, resulting in a high K_0 value from data-fitting algorithm.

Recently, we used an equilibrium binding model (EBM) to characterize the isotherms of avidin binding to biotinylated lipid-doped LB films (Zhao et al, 1993). Therein we show that a less accessible biotinylated lipid, B-DPPE, exhibited noncooperative binding of avidin regardless of receptor density. However, avidin binding to the chain-extended biotin lipid B-x-DPPE showed increasing positive cooperativity with increasing receptor density up to approximately 1.0 mol% B-x-DPPE in the LB film. In the current paper we introduce a new method based upon MCS for estimating the binding parameters that characterize ligand-receptor binding. Here we only show the results for avidin binding to B-x-DPPE because the data for avidin binding to B-DPPE showed only noncooperative binding (Zhao, 1992).

The values of η, K_0, and F_{max} for avidin/B-x-DPPE binding determined by the MCS method described here, and by the EBM method described previously (Zhao et al., 1993), were compared by a linear regression test. This analysis resulted in the intercepts, slopes, and correlation coefficients (R^2) as listed in Table 1. In all cases, the intercepts are not different from zero at the 95% confidence level. The slopes for K_0 and F_{max} are not different from unity at the 95% confidence level. Therefore, the values of K_0 and F_{max} estimated by MCS and EBM were indistinguishable. Only in the case of η did the MCS-derived value systematically exceed the value estimated from EBM by 24 ± 9%. This was due to the higher sensitivity of EBM to cooperativity coefficient η. (In just two cases (0.0 and 0.4 mol%) did the value of η estimated by EBM exceed the value by MCS.)

TABLE 1 Correlation of binding parameters determined by EBM and MCS

	η	K_0	F_{max}
Intercept	−0.298 ± 0.188*	−0.386 ± 0.0397*	−0.006 ± 0.086*
Slope	1.244 ± 0.088	1.020 ± 0.049‡	1.004 ± 0.012‡
R^2	0.939	0.971	0.998

* intercept not different from zero at the 95% confidence level.
‡ slope not different than unity at the 95% confidence level.

It has been shown that for the same parameters EBM and MCS produce almost exactly the same binding isotherms, except for the case of highly positive cooperativity ($\eta > 2.0$) (Zhao, 1992). In the case of $\eta > 2.0$ the two models deviate from each other in the region where protein binding increases rapidly with bulk protein concentration. In this region the slope of binding curve calculated from EBM is greater than that from MCS, indicating a higher sensitivity of EBM to η. Therefore, to reach a certain level of binding a smaller η is required in EBM than in MCS. The EBM is formulated by assuming implicitly that the bound ligands are uniformly distributed and all the receptors behave in the same way as the "average receptor" (Zhao et al., 1993). In MCS a very large number of receptors are simulated to describe the overall behavior of the binding system. In the simulated receptor array there are aggregates or patches of bound receptors as well as free receptors. Within a patch of bound receptors a

positive cooperativity coefficient η is less effective in influencing the state of a receptor because all of its neighbors are bound, and it has a higher probability to remain bound. Within a patch of free receptors η has no effect on the probability that a receptor is bound or free ($n = 0$ in Eq. 3). This explains the difference in sensitivity to η in these two models. We believe that MCS gives a more accurate description of real ligand-receptor binding systems.

CONCLUSIONS

It has been demonstrated that MCS can be used to: (1) simulate isotherms of cooperative protein binding to a two-dimensional receptor array; and (2) numerically determine the relative amount of protein bound to the surface, the cooperativity coefficients, and intrinsic association constants from experimentally derived data. Results from this study are consistent with a previous analysis of avidin-biotin binding based on an equilibrium binding model (Zhao et al., 1993). Furthermore, the technique of incorporating MSC into a nonlinear regression algorithm is not limited to studying molecular binding, but also provides a general methodology to other types of modeling studies and data analyses.

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REFERENCES

Binder, K., editor. 1986. Monte Carlo Methods in Statistical Physics, 2nd ed., Springer-Verlag, Berlin. 376 pp.

Crothers, D. M. 1968. Calculation of binding isotherms for heterogeneous polymers. *Biopolymers* 6:575–584.

Darst, S. A., M. Ahlers, P. H. Meller, E. W. Kubalek, R. Blankenburg, H. O. Ribi,H. Ringsdorf, and R.D. Kornberg. 1991. Two-dimensional crystals of streptavidin on biotinylated lipid layers and their interactions with biotinylated macromolecules, *Biophys. J.* 59:387–396.

Genest, D., and P. Wahl. 1981. An interpretation of the binding of ethidium bromide to the core nucleosome based on Monte Carlo calculations, *Biochimie.* 63:561–564.

Green, N. M. 1975. Avidin. *Adv. Protein Chem.* 29:85–133.

Hammersley, J. M., and D.C. Handscomb. 1964. Monte Carlo Methods, Methuen & Co. Ltd, London.

Hill, T. L. 1985. Cooperativity Theory in Biochemistry: Steady-State and Equilibrium Systems. Chapter 10. Springer-Verlag, New York.

Neville, M. C., R. B. Kelman, and F. W. Briese. 1972. Monte Carlo simulation of co-operative adsorption of ligands onto polymers. *J. Theor. Biol.* 35:129–144.

Poland, D. 1978. Cooperative Equilibria in Physical Biochemistry. Clarendon Press, Oxford. 7 pp.

Press, W. H., B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling. 1986. Numerical Recipes. Chapter 14. Cambridge University Press, Cambridge.

Roberts, G., editor. 1991. Langmuir-Blodgett Films. Plenum Press, New York.

Saroff, H. A. 1989. Evaluation of uncertainties for parameters in binding studies: the sum-of-square profile and Monte Carlo estimation, *Anal. Biochem.* 176:161–169.

Tanford, C. 1961. Physical Chemistry of Macromolecules. John Wiley & Sons, New York.

Thakur, A., M. L. Jaffe, and D. Rodbard. 1980. Graphical analysis of ligand-binding systems: evaluation by Monte Carlo studies, *Anal. Biochem.* 107: 279–295.

Turner, J. E., H. A. Wright, and R.N. Hamm. 1985. A Monte Carlo primer for health physics. *Health Physics.* 48:717–733.

Zhao, S. 1992. Experimental and modeling study of avidin binding to biotin in lipid monolayers. Ph. D. dissertation, Department of Biomedical Engineering, Duke University, Durham, NC.

Zhao, S., and W. M. Reichert. 1992a. Langmuir-Blodgett affinity surfaces: targeted binding of avidin to biotin-doped Langmuir-Blodgett films at the tip of an optical fiber. *ACS Symposium Series 493: Macromolecular Assemblies in Polymeric Systems,* P. Stroeve, and A. C. Balazs, editors. 123:134.

Zhao, S., and W. M. Reichert. 1992b. The influence of biotin lipid surface density and accessibility on avidin binding to the tip of an optical fiber sensor. *Langmuir.* 8:2785–2791.

Zhao, S., D. S. Walker, and W. M. Reichert. 1993. Cooperativity in the binding of avidin to biotin-doped Langmuir-Blodgett films. *Langmuir.* 9:3166–3173.