Mini Review
Revisiting the Neighbor Exclusion Model and Its Applications

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ABSTRACT:

We review the neighbor exclusion model and some of its applications to analyze the binding data of DNA-ligand complexes. We revisit the closed form of the model developed by McGhee and von Hippel in 1974, showing that this classic model can be used to help studying the behavior of DNA contour and persistence lengths when interacting with intercalating ligands. We present methods to quantitatively analyze the variation of these two quantities, allowing one to determine important parameters of the interaction such as the intrinsic binding constant and the exclusion number of the ligand. © *2009 Wiley Periodicals, Inc. Biopolymers 93: 1–7, 2010.*

Keywords: neighbor exclusion model; DNA; persistence length; intercalation

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INTRODUCTION

DNA interactions with ligands such as drugs or proteins have been largely studied along the past years.[1–17] The study of these interactions is important to understand intracellular processes such as transcription, translation, and gene replication. In fact, these processes are controlled by several types of regulatory proteins that bind to specific DNA target sequences, playing the role to activate, or repress functions. In addition, an important biomedical application of this knowledge is directly related to the cancer chemotherapy treatment, in which the drugs inhibit DNA replication, annihilating cancer cells. In fact, a wide class of DNA binding ligands are chemotherapeutics, such as the anthracycline antibiotics. Even though they are nonspecific DNA binding molecules, these substances surprisingly have a great selectivity to cancer cells due to biochemical affinities. In this way, it is easy to notice the importance of characterizing DNA interactions with various types of ligands.

In this manuscript, we revisit a classic model, which attempts to explain DNA interactions with ligand molecules, the neighbor exclusion model. The closed form of this model was formulated in 1974 by McGhee and von Hippel, and even today it is largely used, specially to analyze experimental data. Therefore, a review is needed after 35 years of the original publication by the authors.

In addition, we discuss some important applications of the neighbor exclusion model. We show that the model can be used to study the variation of the contour length of DNA-ligand complexes. In fact, for intercalating ligands, we can determine the intrinsic binding constant of the interaction and the exclusion number of the ligand measuring the contour length as a function of total drug concentration in the sample. We also show that the model can be used to analyze the variation of the elasticity of DNA-ligand complexes. In this case, the neighbor exclusion model is combined with a new model called "entropic springs in series model."[18] This model was recently developed with the purpose to study the elasticity of the DNA-ligand complexes, providing an expression for the effective persistence length of these complexes, which depends on the concentration of ligand bound to DNA.

NEIGHBOR EXCLUSION MODEL

The neighbor exclusion model is used in many works to perform the analysis of the binding data of DNA-ligand interac-

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tions.[1,3,8,11,19] In this section, we revisit its classic closed form, which was developed by McGhee and von Hippel in 1974.[9]

Scatchard Model

Before the formulation of McGhee and von Hippel, a simpler model of interaction was first proposed by Scatchard in 1949.[20] This model works well for small ligands, which occupy only one binding site of a one-dimensional lattice (in our case, the bare DNA molecule). The interaction process can be summarized by the chemical reaction

$$C_{\mathrm{f}} + C_{\mathrm{fs}} \overset{k_1,k_2}{\longleftrightarrow} C_{\mathrm{b}}, \tag{1}$$

where C_{f}, C_{fs}, and C_{b} are the molar concentrations of free ligand molecules (in solution), free binding sites, and bound molecules, respectively. The parameters k_1 and k_2 are the association and dissociation rate constants, respectively. At chemical equilibrium, we can write

$$\frac{C_{\mathrm{b}}}{C_{\mathrm{f}} C_{\mathrm{fs}}} = \frac{k_1}{k_2} \equiv K_{\mathrm{i}}, \tag{2}$$

where K_{i} is the intrinsic binding constant.

The Scatchard model states that the number of free binding sites can be written as the total number of sites in the lattice C_{bp} (where the notation *bp* represents DNA base pairs, which are the sites of the lattice), less the total number of molecules bound to the lattice C_{b}. Equation (2) can then be written as

$$\frac{C_{\mathrm{b}}}{C_{\mathrm{f}}(C_{\mathrm{bp}} - C_{\mathrm{b}})} = K_{\mathrm{i}}, \tag{3}$$

which can be rewritten as

$$\frac{r}{C_{\mathrm{f}}} = K_i(1 - r), \tag{4}$$

where $r = C_{\mathrm{b}}/C_{\mathrm{bp}}$.

Equation (4) is known as Scatchard model. In this model, saturation occurs when $C_{\mathrm{b}} = C_{\mathrm{bp}}$, i.e., when all lattice sites are occupied. In this limit, $r \to 1$ and the model fails. Finally, observe that the intrinsic binding constant K_{i} can be determined from the curve slope in a plot of $C_{\mathrm{b}}/C_{\mathrm{f}}$ versus C_{b} [see Eq. (3)]. This type of plot is called a Scatchard plot, and their slopes and intercepts can be used to obtain estimations of the binding constant. When a ligand molecule occupies only one binding site this plot is linear, as predicted by Eqs. (3) and (4). Deviations from linearity indicate that the ligand occupies more than one lattice site, and Eq. (4) therefore fails for such ligands.

It is well known that many ligands of biological interest occupy more than one base pair in DNA molecule when interacting. The Scatchard model was then largely discussed by other authors who helped to develop some ideas behind the neighbor exclusion model.[21] Nevertheless, only in 1974 McGhee and von Hippel obtained the closed form of the model, which allowed a considerable advance to the study of more complex DNA ligands.

The Closed Form of the Neighbor Exclusion Model

To formulate their well-known expression of the neighbor exclusion model, McGhee and von Hippel have used the following procedure.

a. The bare DNA molecule is considered a one-dimensional lattice with numerous free binding sites. One can consider the lattice very long and therefore neglect end effects.
b. They first consider noncooperative phenomena, which is the experimental situation of many well-known ligands. This assumption implies that the intrinsic binding constant K_{i} does not depend on the number of previously bound ligand molecules.
c. In a lattice gap with g free binding sites situated between two bound molecules (see Figure 1), the number of linkable sites in the gap is $s = g - n + 1$ for $g \geq n$ or $s = 0$ for $g < n$, where n is the exclusion number, i.e., the number of linkable sites occupied by a ligand molecule. Figure 1 depicts the situation for $g = 5$ and $n = 3$, for which we have $s = 3$.
d. Observe that the volume of our system (i.e., the sample, in experimental situations) is constant, which implies that the number of molecules in solution is proportional to its molar concentration. Therefore, following McGhee and von Hippel, from now we treat the molar concentrations as the number of molecules in solution. The binding process can then be summarized by the chemical reaction

$$C_{\mathrm{f}} + \mathrm{S} \xrightarrow{K_{\mathrm{i}}} C_{\mathrm{b}}, \tag{5}$$

FIGURE 1 A gap with $g = 5$ linkable sites between two bound molecules. In this example we have $n = 3$ and $s = g - n + 1 = 3$.

where C_f is the number of free ligand molecules in solution, S is the average total number of linkable sites in the DNA molecule, and C_b is the number of bound ligand molecules.

At chemical equilibrium, we thus can write

$$K_i = \frac{C_b}{C_f S}. \tag{6}$$

The average number of linkable sites in a gap can be written as

$$s = \sum_{g=n}^{N} (g - n + 1) P_g, \tag{7}$$

where P_g is the probability of a particular gap to have exactly g sites.

Observe that, due to the lattice geometry, when there are C_b bound molecules in the lattice, there will be $C_b + 1$ gaps. Therefore,

$$S = (C_b + 1)s, \tag{8}$$

and using Eq. (6) and (7), we write

$$\frac{C_b}{C_f} = K_i (C_b + 1) \sum_{g=n}^{N} (g - n + 1) P_g. \tag{9}$$

The main problem is then to find the expression for the probability P_g. To perform this, we adopt the following notation:

- f is a free (linkable) lattice site.
- b_1, b_2,..., b_n are the first, second,..., and last bound sites by a single ligand. Figure 2 depicts this notation for $n = 3$. Observe that we call b_1 the left end of the ligand, while b_n is the right end of this same ligand.

Using this notation, we define the conditional probabilities:

- $P\{f|f\}$ is the probability to have a particular free site if the immediately former site is also free.
- $P\{f|b_1\}$ is the probability to have a bound site if the immediately former site is free.

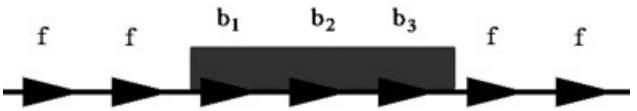

FIGURE 2 Notation used to calculate P_g. f is a free (linkable) lattice site and b_i is a bound site.

According to this notation, we must have $P\{b_1|b_2\} = P\{b_2|b_3\} = \cdots = P\{b_{n-1}|b_n\} = 1$ for a molecule with exclusion number n. In addition, we must have $P\{f|b_2\} = P\{f|b_3\} = \cdots = P\{f|b_n\} = 0$, since a free site can only be a left neighbor of another free site or of a first site (b_1) of a ligand molecule.

With this notation, we can write P_g as

$$P_g = P\{b_n|f\} \times [P\{f|f\}]^{g-1} \times P\{f|b_1\}. \tag{10}$$

The total number of bound sites in the lattice is $C_b n$, and the fraction of bound sites per total site number (base pair in the case of DNA) is

$$\frac{C_b n}{C_{bp}} = rn, \tag{11}$$

where $r = C_b/C_{bp}$ and C_{bp} is the total number of base pairs (lattice sites), as previously discussed.

Therefore, the fraction of free (linkable) sites is $1-nr$, which is also the probability that a lattice residue (chosen in a random way) has to be free (not bound).

Thus, the probability to choose a free site is $1-nr$. The former site, immediately left to this one, can be free (probability $1-nr$) or occupied (probability $nr/n = r$). Therefore, we can write

$$1 - nr = (1 - nr) P\{f|f\} + rP\{b_n|f\}. \tag{12}$$

Observe that, we must have $P\{f|f\} = P\{b_n|f\}$, as there is no preference for a site to be free or occupied. Therefore, using Eq. (12), we can immediately write

$$P\{f|f\} = P\{b_n|f\} = \frac{1 - nr}{1 - (n-1)r} \tag{13}$$

and

$$P\{f|b_1\} = P\{b_n|b_1\} = 1 - P\{f|f\} = \frac{r}{1 - (n-1)r}. \tag{14}$$

Substituting Eqs. (13) and 14 in Eq. (10), we finally get

$$P_g = \left[\frac{1 - nr}{1 - (n-1)r}\right]^g \left[\frac{r}{1 - (n-1)r}\right]. \tag{15}$$

With this expression for the probability P_g, we can easily calculate the sum of Eq. (7) letting $N \to \infty$ (with the assumption that the lattice is very long). The result is

$$s = \left(\frac{1 - nr}{r}\right) \left[\frac{1 - nr}{1 - (n-1)r}\right]^{n-1}. \tag{16}$$

Finally, we use Eq. (9) to get the McGhee and von Hippel expression. Since the lattice is very long, it is reasonable to

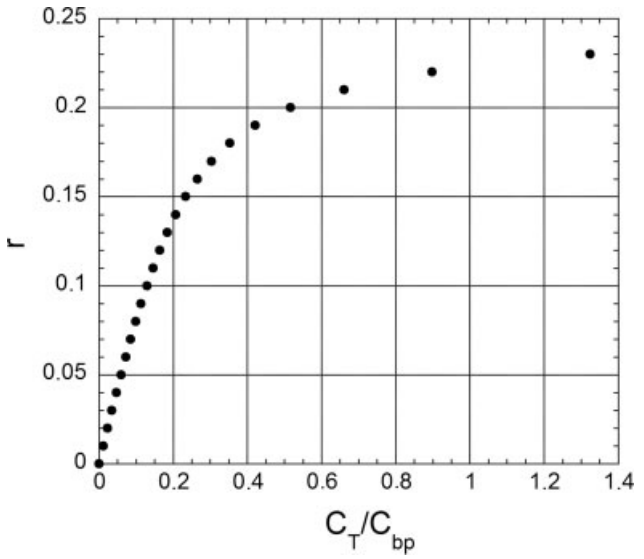

FIGURE 3 Graphical behavior of the neighbor exclusion model, Eq. (17). As the total drug concentration in the sample C_T increases, the parameter $r = C_b/C_{bp}$ increases until reaching a saturation value.

perform the approximation $C_b + 1 \approx C_b$ in Eq. (9), and therefore we get

$$\frac{r}{C_f} = K_i(1 - nr)\left[\frac{1 - nr}{1 - (n-1)r}\right]^{n-1}, \qquad (17)$$

which is the closed form of the neighbor exclusion model, obtained by McGhee and von Hippel in 1974.[9]

Observe that for $n = 1$, we get $r/C_f = K_i(1 - r)$, which is the Scatchard model, Eq. (4). This analysis proves that this model is really valid only for small ligands that occupy a single lattice site, as previously discussed.

Figure 3 shows the behavior of Eq. (17). We plot the parameter r ($y-$axis) as a function of total ligand concentration in the sample, $C_T = C_b + C_f$, normalized by the base pair concentration C_{bp} (x-axis). Observe that r initially increases approximately linearly with C_T/C_{bp}. This initial behavior occurs when C_b is still low and the Scatchard model is a reasonable approach. However, as C_T increases, the parameter $r = C_b/C_{bp}$ saturates, as expected.

APPLICATION: ANALYZING THE BINDING DATA

DNA molecules can interact with drugs, proteins, and other ligands in many different ways which, for simplicity, can be divided into two major groups: covalent and noncovalent interactions. Covalent bindings are usually irreversible and lead to complete inhibition of DNA processes. Noncovalent bindings are usually reversible and can be divided into three distinct classes: major groove binding, minor groove binding, and intercalation. Some ligands can also exhibit multimodal binding. As an example, we cite compounds from the ellipticine series, which can bind to DNA through intercalation, covalent binding or generating oxidizing species.[22] Another example is the drug actinomycin D (AMD), which can form at least five types of complexes: three types with double-stranded DNA and two types with single-stranded DNA.[23] The binding mode can also depend on the medium ionic strength or external conditions. As an example of external condition, which can modify the type of interaction, we cite ultraviolet-A illumination over DNA-psoralen complexes.[12,24] Later we present an overview of DNA-ligand binding modes.

DNA-Ligand Binding Modes

Covalent Binders. The covalent binding of drugs to DNA is usually irreversible and completely inhibits DNA processes. The metal-based complexes are examples of drugs, which interact with DNA by this type of binding. Cisplatin [*cis*-diamminedichloroplatinum(II)] and its analogues carboplatin and oxaliplatin are antitumor platinum-based complexes used in cancer chemotherapy. The covalent binding in this case is usually formed between the chloro groups of the drugs and the nitrogen of the DNA base pairs. The action of platinum complexes as anticancer drugs consist in damaging the DNA molecule with adducts that form various types of crosslinks. Nevertheless, their clinical usage is very limited due to several side effects.

Minor Groove Binders. This noncovalent binding is usually characterized by a combination of various types of interactions, such as electrostatic interaction, van der Waals and hydrogen bonds. This class of binding ligands presents an affinity to DNA minor groove floor and includes many compounds which are known by their antitumor and antibiotic functions. An example of minor groove binder is the anticancer compound distamycin A, which forms a reversible complex with DNA changing its elasticity and stabilizing the double-helix.[13]

Major Groove Binders. Major groove binding is also generally characterized by a combination of different types of interactions, specially the electrostatic one.[13] α-Helical [Ac-(Leu-Ala-Arg-Leu)$_3$-NH-linker] is a peptide, which interacts with DNA via major groove binding.[13] Another examples are

the intercalant and major groove binder YO and the bisintercalant and major groove binder YOYO.[25]

Intercalators. Intercalative binding is one of the most common interactions between DNA and ligands. It is characterized by the insertion of a flat aromatic molecule between two adjacent DNA base pairs. The complex is thought to be stabilized by the $\pi-\pi$ stacking interactions between the drug and the base pairs.[26] Intercalators also introduce strong structural perturbations in the DNA molecule. To accommodate the intercalated molecules, there is an increase in the DNA contour length, which is accompanied by an unwinding of the double-helix by a certain angle per intercalated molecule.[3,6,13]

Daunomycin and ethidium bromide (EtBr) are examples of drugs that intercalate the DNA molecule and can drastically modify its elasticity, depending on the drug concentration. Both drugs unwind the DNA double helix when intercalating.[6] Daunomycin is an anthracycline antibiotic used in the treatment of various cancers. It inhibits DNA replication and transcription, impeding cell duplication.[3] Ethidium bromide (EtBr) is commonly used as a nonradioactive marker for identifying and visualizing nucleic acid bands in electrophoresis and in other methods of nucleic acid separation.

The neighbor exclusion model, Eq. (17), can be used to help analyzing the binding data for all the discussed types of interactions. In fact, it can even be used to help determining the specific binding mechanism.[8,19] In the particular case of intercalative binding, the model is specially helpful because it allows one to determine the intrinsic binding constant K_i and the exclusion number n by measuring the contour length L of the DNA-ligand complexes as a function of total ligand concentration in the sample C_T. The contour length of the bare DNA molecule and of DNA-ligand complexes can be measured by performing DNA stretching experiments[11,12] or by analyzing the images of the complexes obtained by atomic force microscopy (AFM) or by scanning force microscopy (SFM),[4,27–29] for example.

The relative increase of the contour length, $\Theta = (L - L_0)/L_0$, can be related to $r = C_b/C_{bp}$ and to the total drug concentration C_T in the sample. In fact, observe that $(L - L_0)/L_0 = N_b\ \delta/N_{bp}\ \Delta$, where N_b is the number of bound drug molecules, N_{bp} is the number of DNA base pairs, $\Delta = 0.34$ nm is the mean distance between two consecutive base pairs in the bare DNA molecule at B-form and δ is the increase of this length due to an intercalated molecule. Using $\delta \approx \Delta$, which is valid for many ligands such as daumonycin, EtBr and psoralen,[6,13,15] we find that $\Theta = N_b/N_{bp} = C_b/C_{bp} = r$, using the concentrations in units of molar (M).

Observe that the neighbor exclusion model, Eq. (17), can be rewritten as[11]

$$C_T = C_{bp}\Theta + \frac{\Theta(1 - n\Theta + \Theta)^{n-1}}{K_i(1 - n\Theta)^n}, \qquad (18)$$

with $\Theta = r$.

This relation can used to fit experimental data of C_T versus Θ to determine the parameters K_i and n. This analysis was performed in Ref. 11 for DNA-EtBr complexes.

APPLICATION: ENTROPIC SPRINGS IN SERIES MODEL

The neighbor exclusion model can also be used to help studying the variation of DNA elasticity when interacting with ligands. To achieve this purpose, we introduce the entropic springs in series model, which can predict the behavior of DNA persistence length as a function of the parameter $\Theta = r$ when combined with the neighbor exclusion model. Therefore, we can use the combined model to study the effects of intercalating drugs on the DNA elasticity.

The idea behind this new model, which was recently presented in Ref. 18 is to determine the effective persistence length of a DNA molecule partially bound by a ligand. In fact, the partially bound DNA molecule can be considered as two entropic springs associated in series, as schematized in Figure 4. We associate a persistence length A_1 and a contour length L_1 to the portion free of drugs and the corresponding lengths A_2 and L_2 to the intercalated portion. The effective persistence length A_E of this association was calculated in Ref. 18. The result is

$$\frac{1}{A_E} = \frac{\left(1 + \frac{L_2}{L_1}\sqrt{\frac{A_1}{A_2}}\right)^2}{A_1\left(1 + \frac{L_2}{L_1}\right)^3} + \frac{\left(1 + \frac{L_1}{L_2}\sqrt{\frac{A_2}{A_1}}\right)^2}{A_2\left(1 + \frac{L_1}{L_2}\right)^3}. \qquad (19)$$

Observe that Eq. (19) gives the correct behavior of the persistence length at the corresponding limits. For $L_2 \to 0$,

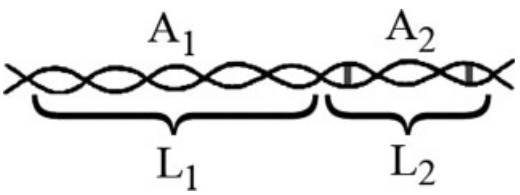

FIGURE 4 A partially intercalated DNA molecule. We associate a persistence length A_1 and a contour length L_1 to the portion free of drugs and the corresponding lengths A_2 and L_2 to the intercalated portion. Figure extracted from Rocha, M. S. Phys Biol 2009, 6; art no. 036013.[18]

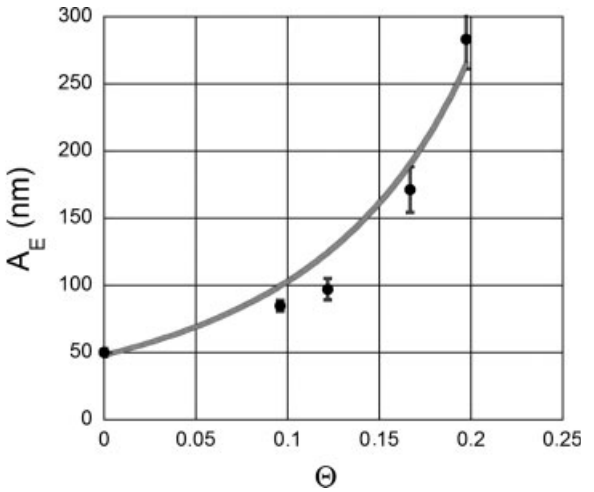

FIGURE 5 Effective persistence length A_E as a function of $\Theta = r$ for DNA-daunomycin complexes. *Circles*: measurements; *Solid line*: entropic springs in series model, Eq. (19), combined with the neighbor exclusion model. We use $n = 3.04$ and $K_i = 6.6 \times 10^4\ M^{-1}$ in this analysis.[5] Figure extracted from Rocha, M. S. Phys Biol 2009, 6; art no. 036013.[18]

we have $A_E = A_1$, which corresponds to a bare DNA molecule. For $L_1 \to 0$, $A_E = A_2$, which corresponds to a DNA saturated with drug molecules. Also, observe that if $A_1 = A_2$, we have $A_E = A_1 = A_2$ for any L_1 and L_2, which corresponds to a molecule uniform in elasticity. A more detailed discussion about this expression can be found in Ref. 18.

Equation (19) can be combined with the neighbor exclusion model, because L_1/L_2 depends on the parameter Θ. In Ref. 18, we have shown that

$$\frac{L_1}{L_2} = \frac{\delta}{(\delta + n\Delta)\Theta} - \frac{n\Delta}{\delta + n\Delta}, \tag{20}$$

where n is the exclusion number of the intercalating drug and the other variables were previously defined.

As mentioned, for many intercalating molecules we have $\delta \approx \Delta$[6,13] and the aforementioned relation can be simplified to

$$\frac{L_1}{L_2} \approx \frac{1 - n\Theta}{(1 + n)\Theta}. \tag{21}$$

If one measures the total drug concentration in the sample (which is trivial for experimentalists), the parameter $\Theta = r$ can then be determined solving numerically Eq. (18), for known values of n and K_i.

Equations (19) and (21) can be combined to plot the effective persistence length A_E as a function of $\Theta = r$. In Ref. 18, we have used this model to fit experimental data of DNA complexes formed with the drugs daunomycin and EtBr, finding an excellent agreement between the model and the experiments. Figure 5 reproduces this fit for DNA-daunomycin complexes. In this experimental data, we have measured the total drug concentration in the sample C_T, and $\Theta = r$ was determined for each value of C_T using Eq. (18), as explained earlier.

Other authors have used a similar method to study the variation of the persistence length of DNA complexes formed with ligands that interact by electrostatic binding.[8,19] They use a model proposed by Rouzina and Bloomfield for DNA bending by small cations,[30] which states that the effective persistence length of the DNA-ligand complex is given by

$$\frac{1}{A_E} = \frac{1}{A_1} + \frac{nr}{A_2}, \tag{22}$$

where all the parameters were previously defined.

Observe that this model is directly connected to the neighbor exclusion model by the parameter r, and therefore one can use the combined model to study the elasticity variation of these complexes as a function of the total concentration in the sample C_T.

CONCLUSION

We review the neighbor exclusion model and some of its applications to analyze the binding data of DNA-ligand complexes. We show that the model can be used to help studying the variation of both contour and persistence lengths of DNA molecules when interacting with a ligand.

It is worth to emphasize that studies concerning the size and elasticity of the DNA-ligand complexes are important to intracellular processes such transcription, translation, and gene replication, and also to biomedical applications, specially in cancer chemotherapy. Therefore, theoretical and experimental methods developed to characterize these interactions are extremely useful in both biology and medicine.

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