Statistical Mechanical theory of binding-induced stresses and consequences for virucidal activity of nanoparticles

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I derive a theoretical model, based on the statistical mechanical theory of multivalent interactions [1–4], to calculate the adsorption of multivalent nanoparticles from a bulk solution to a set of cells. A comparison with previous theory from Xu and Shaw as implemented by Lennart Lindfors (AZ) is also made. The main result is that assuming a specific binding geometry should be done with care (and possibly not done at all), as this arbitrary choice, which can be relieved making a complete treatment as presented here, has a strong influence on the results obtained.

I. CALCULATING THE ADSORBED FRACTION OF NANOPARTICLES

The scenario I aim to describe is the following. A group of N_C cells, each bearing an average surface density of receptors σ_R on their surface, is immersed in a solution containing nanoparticles coated with a surface density of ligands σ_L. This solution could be, for example, the blood plasma, where nanoparticles are injected to deliver a certain drug. As a side-note, I am building this theory to use as much as possible experimentally controllable quantities. Thus, I am building it as a function of the surface density of ligands/receptors rather than assuming any specific number of potentially interacting binders (a binder is either a ligand or a receptor). Connections to the theory presented in both [5] and thus our previous discussion will be made clear in the next section.

The *effective* bond strength is characterised by the number $\chi = \exp(-\beta\Delta G_{\text{bond}})$, where $\Delta G_{\text{bond}})$ is the bond energy and $\beta = 1/k_BT$ is the inverse thermal energy, where k_B is Boltzman's constant and T the absolute temperature of the system. Note that, as thoroughly described by Varilly *et al* [1], the bond energy is a function of the relative position of ligands and receptors, as well as dependent on the specific properties of the linker used to graft the ligands on the nanoparticles and, in the most general case, can be different for each single ligand-receptor pair.

We seek a formula that can give us f the fraction of particles, relative to their initial number in solution, which will be bound to a cell. To do that, we first consider the "reaction"

$$NP + M \rightarrow NP - M \tag{1}$$

where M is a binding site on the cell and NP a nanoparticle. In practice, we define a binding site as a region on the cell surface that can only be occupied by a single nanoparticle. If we assume the cell has a surface area A_{cell}, we will have that $M \approx \frac{A_{\text{cell}}}{A_{\text{NP}}} N_{\text{cell}}$, where $A_{\text{NP}} = \pi R_{\text{NP}}^2$ is the effective area of a spherical nanoparticle, R_{NP} being its radius, and A_{cell} the exposed area of a cell. We can use standard chemical equilibrium together with mass conservation equations to derive the following equilibrium equation for the chemical reaction described in Eq. 1:

$$K_{\text{bind}} = \frac{[NP-M]_e}{[NP]_e[M]_e} = \frac{[NP-M]_e}{([NP]_0 - [NP-M]_e)([M]_0 - [M]_e)} \tag{2}$$

where $[X]_e(0)$ is the concentration of species X, either at equilibrium (e) or its initial value (0). Equation 5 can be solved for $[NP-M]_e$ since it is a simple 2nd order equation, its solution is:

$$[NP-M]_e = \frac{([NP]_0 + [M]_0)K_{\text{bind}} + 1 - \sqrt{([([NP]_0 + [M]_0)K_{\text{bind}} + 1]^2 - 4[NP]_0[M]_0K_{\text{bind}}}}{2K_{\text{bind}}} \tag{3}$$

to obtain all the equilibrium concentrations as a function of the equilibrium constant and the initial concentration of the species in solution. These quantities can then be used to find the fraction of bound nanoparticles, which is simply given by:

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$$f = 1 - \frac{[NP]_e}{[NP]_0} = 1 - \frac{[NP]_0 - [NP - M]_e}{[NP]_0} = \frac{[NP - M]_e}{[NP]_0} \tag{4}$$

$$= \frac{([NP]_0 + [M]_0)K_{\text{bind}} + 1 - \sqrt{([([NP]_0 + [M]_0)K_{\text{bind}} + 1]^2 - 4[NP]_0[M]_0K_{\text{bind}}^2}}{2K_{\text{bind}}[NP]_0} \tag{5}$$

We now have an equation that allows us to calculate the fraction of particles adsorbed to a cell, given the initial bulk concentration of particles, the concentration of cells (since the initial concentration of binding sites is $[M]_0 = \frac{A_{\text{cell}}}{A_{\text{NP}}}[N_{\text{cell}}]$), and the *effective* binding constant of a nanoparticle to a binding site. It should be noted that this equation (unlike the previous one implemented in the initial model from AZ) has the correct limit, and the maximum value of f for $K_{\text{bind}} \to \infty$ is $\min(1, \frac{[M]_0}{[NP]_0})$, as expected in the case where the total number of adsorption sites is not enough to adsorb potentially all nanoparticles in the bulk. The rest of these notes focus on calculating this quantity for our system of cells and nanoparticles as a function of their design parameters.

II. CALCULATING K_{bind} BASED ON THE SYSTEM'S MOLECULAR PROPERTIES

The effective binding constant is formally given by the following formula:

$$K_{\text{bind}} = \int_{v_{\text{bind}}} \exp\left(-\beta A(\vec{r})\right) d\vec{r} \tag{6}$$

where $A(\vec{r})$ is the interaction free-energy between the nanoparticle and the surface of the cell, and the integral is over the "binding volume" v_{bind}. This is the region of space where the nanoparticle's centre of mass should reside when the nanoparticle is bound to a given binding site. In calculating this number, it is important to consider the fact that the nanoparticle should not be able to overlap neighbouring sites. In other words, this region is the region of space within which the nanoparticle can fluctuate while still bound to a specific site. In practice, assuming a homogeneous density of receptors (and thus that the free energy is only a function of the distance between the nanoparticle and the surface), we have that:

$$K_{\text{bind}} \approx A_{\text{L}} \int_0^{z_{max}} \exp\left(-\beta A(z)\right) d\vec{z} \tag{7}$$

where $A_{\text{L}} \approx R_{ee}^2 = Na^2$ is the approximate area spanned by a ligand (described as a Gaussian chain associated to a polymer with N monomers of length a), $z_{\max} \approx Na$ the maximum distance at which a bond can be formed and $A(z)$ the free-energy as a function of the distance from the surface. Note that in general $A(z)$ is measured in such a way that it represents the difference in free energy between a nanoparticle in the bulk of the solution vs a bound particle, and thus $A(z) \to 0$ when $z \to z_{\max}$.

A. The Derjaguin approximation

In order to calculate the effect of nanoparticle curvature on the free-energy $A(z)$, we will use the Derjaguin approximation. This approximation states that:

$$F(h) = 2\pi R_{\text{eff}} W(h) \tag{8}$$

where $F(h)$ is the *force* between two particles with surface-to-surface distance h from each other, and $W(z)$ their interaction energy *per unit area*, calculated for two infinite parallel planes. The effective radius appearing in Eq.10 is given as a function of the radii of the two particles R_1 and R_2 as:

$$R_{\text{eff}}^{-1} = R_1^{-1} + R_2^{-1} \tag{9}$$

which for a nanoparticle interacting with a cell, considering that $R_{\text{cell}} \gg R_{\text{NP}}$) approximates to:

$$R_{\text{eff}} \approx R_{\text{NP}}. \tag{10}$$

The Derjaguin approximation works better the shorter is the interaction range between the two particles compared to the nanoparticles size, that is, when $z_{\max}/R_{\text{NP}} \ll 1$. In our case, $z_{\max}$ is dictated by the effective radius of the polymer tethering the ligand $R_P \approx \sqrt{N} a_{\text{mono}}$, which for a typical LNP is much smaller than the nanoparticle radius. Using Eq. 10, we can calculate the interaction free energy at a distance z as:

$$A(z) = \int_z^\infty F(h)dh = \int_z^\infty 2\pi R_{\text{NP}} W(h)dh \tag{11}$$

All is left to be done now to calculate the binding constant, and thus the fraction of bound nanoparticles, is an expression for $W(h)$, which we will describe below.

1. *Calculating $W(h)$*

Calculating $W(h)$ - and thus later the integral defining K_{bind} - requires calculating different terms:

$$W(h) = W_{\text{bond}}(h) + W_{\text{steric}}(h) + W_{\text{ns}}(h), \tag{12}$$

The first term in Eq.12 is the attractive interaction W_{bond} due to the formation of ligand-receptor bonds. For a system of binders (we call a binder both a ligand or a receptor), the free-energy of interaction is given by [2]:

$$\beta A_{\text{bond}} = \sum_i \ln p_i + \frac{1}{2} \sum_i \sum_j p_{ij} \tag{13}$$

where $\beta = 1/k_B T$ is the thermal energy and p_i, can be interpreted as the probability that binder i is *not* bound and p_{ij} is the probability that binders i, j bind to each other [6] . These probabilities are given by the solution of the following system of coupled equations:

$$p_i + \sum_j p_{ij} = 1 \tag{14}$$

$$p_{ij} = p_i p_j \chi_{ij} \tag{15}$$

where χ_{ij} is the effective bond strength between binder i and binder j, related to the bond energy by $\chi_{ij} = \exp(-\beta \Delta G_{ij})$. A few things to notice. By using Eq.14 and 18 we can simplify Eq. 13 so that it is purely written as a function of the p_is, to:

$$\begin{aligned}
\beta A_{\text{bond}} &= \sum_i \ln p_i + \sum_i \sum_{j<i} p_{ij} \\
&= \sum_i \ln p_i + \frac{1}{2} \sum_i \sum_j p_{ij} \\
&= \sum_i \ln p_i + \frac{1}{2} \sum_i (1 - p_i) \\
&= \sum_i \ln p_i + \frac{1}{2} \sum_i (1 - p_i) \\
&= \sum_i \ln p_i + \frac{1}{2} (1 - p_i)
\end{aligned} \tag{16}$$

The second point to notice is a bit more important. As we will discuss more in detail later, the bond strength for a ligand-receptor pair (ij), which we call $\chi_{ij}(\vec{r_{ij}})$, is a function of the exact position of the binders relative to each other, $\vec{r_{ij}} = \{x_{ij}, y_{ij}, z_{ij}\}$. In practice, this means that in a system no two ligand-receptor pairs are the same. In the most general case then, Eqs.14 and 18 represent a *coupled system of equations*, one for each binder in the system, that must be solved simultaneously, with knowledge of the exact position of all binders in the system. Without a detailed molecular model where we have the precise position of each ligand and receptor, this is not possible. However, we can simplify the problem and obtain an approximate solution by averaging the bond energy over all the possible positions of the binders. Even this average can be done in different ways. Here, because we will need it later, we will consider that the binders reside on two infinite parallel surfaces facing each other at a distance h, and the spatial average is taken over all possible grafting positions of the ligands/receptors on these two surfaces. In this case, we have:

$$
\begin{aligned}
&p_i + \sum_{j=1}^{N} p_{ij} = 1 \rightarrow \\
&p_i + \sum_{j=1}^{N} p_i p_j \chi_{ij} = 1 \rightarrow \\
&p_\alpha + \sum_{\beta=1}^{N_{\text{types}}} N_\beta \langle \chi_{\alpha\beta} \rangle p_\alpha p_\beta = 1 \qquad (17) \\
&p_\alpha + \sum_{\beta=1}^{N_{\text{types}}} \sigma_\beta K_{\alpha\beta} p_\alpha p_\beta = 1 \qquad (18)
\end{aligned}
$$

where the sum over all distinct, discrete partners j has been substituted with the average interaction with the N_β partners found in the region A_{bind} spanned by the binder α, and σ_β is the surface density of the binder of type β. Because we have lost spatial lateral resolution, in this new expression discrete binders i and j are substituted by an average binder of a given type (highlighted by the switch to Greek letters α and β). In this view, $K_{\alpha\beta}$ is the average bond strength multiplied by the binding area, and only depends on the surface-to-surface separation h:

$$
K_{\alpha\beta}(h) = A_{\text{bind}} \langle \chi_{\alpha\beta} \rangle = \int_{A_{\text{bind}}} \chi_{\vec{r}}(\vec{r}) d^2 r \qquad (19)
$$

where $\vec{r} = \{x, y, z\}$ is the vector connecting the grafting points of two binders and the integral is over the lateral position (x, y) and $z \equiv h$. To calculate this integral, we describe the receptor epitope and the ligand as two point particles, and consider that the ligand is tethered to the nanoparticle by a polymer that behaves as a gaussian chain of average end-to-end distance $R_{ee} \approx Na^2$, where a is the length of a monomer and N the number of monomers in the polymer [7]). Considering the case of two flat, parallel opposing surfaces for the nanoparticle and the cell, we obtain the (almost)-exact analytical expression [1]:

$$
K_{\alpha\beta}(h) = K^0_{\alpha\beta} \exp\left(-\frac{3h^2}{4Na^2}\right) \sqrt{\frac{12}{\pi N a^2}} \frac{\text{erf}\left(\sqrt{-\frac{3h^2}{4Na^2}}\right)}{\text{erf}\left(\sqrt{-\frac{3h^2}{2Na^2}}\right)} \qquad (20)
$$

where K^0 is the binding constant of free ligands and receptors measured in solution, and erf is the error function, defined by:

$$
\text{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x dt e^{-t^2} \qquad (21)
$$

We can finally further specialise these expressions to a system with a single ligand(L)-receptor(R) pair type, that is $\alpha \equiv L$, $\beta \equiv R$, obtainingfor the probabilities, starting from Eq. 18:

$$
\begin{aligned}
&p_L + p_L p_R \sigma_R K_{LR} = 1 \\
&p_R + p_R p_L \sigma_L K_{LR} = 1
\end{aligned} \qquad (22)
$$

in which case we have an analytical solution:

$$p_L = \frac{(\sigma_L - \sigma_R)K_{LR} - 1 + \sqrt{4\sigma_L K_{LR} + (1 + (\sigma_R - \sigma_L)K_{LR})^2}}{2\sigma_L K_{LR}}$$
$$p_R = \frac{(\sigma_R - \sigma_L)K_{LR} - 1 + \sqrt{4\sigma_R K_{LR} + (1 + (\sigma_L - \sigma_R)K_{LR})^2}}{2\sigma_R K_{LR}}. \tag{23}$$

The last thing to notice here is that in the Derjaguin approximation, we need the free-energy per unit area, which we obtain from Eq. 16 by considering that in a region of area S there are $\sigma_L S$ ligands and $\sigma_R S$ receptors (all indistinguishable in this averaged description), giving:

$$W_{\text{bond}}(h) = \frac{\beta A_{\text{bond}}}{S} = \frac{\sum_i \ln p_i + \frac{1}{2}\sum_i \sum_j p_{ij}}{S} \tag{24}$$
$$= \frac{\sum_i \ln p_i + \frac{1}{2}(1 - p_i)}{S} \tag{25}$$
$$= \frac{N_L\left[\ln p_L + \frac{1}{2}(1 - p_L)\right] + N_R\left[\ln p_R + \frac{1}{2}(1 - p_R)\right]}{S} \tag{26}$$
$$= \sigma_L\left[\ln p_L + \frac{1}{2}(1 - p_L)\right] + \sigma_R\left[\ln p_R + \frac{1}{2}(1 - p_R)\right]. \tag{27}$$

For a system of ligands and receptors, besides the bond energy there is also generally a second steric interaction. This second interaction is due to the polymer tethering the ligand to the nanoparticle surface, which cannot penetrate the cell surface. This contribution is proportional to the number of ligands and is generally given by:

$$A_{\text{steric}} = A_{\text{rep}}(z) = -N_L k_{\text{B}} T \ \ln\left(\frac{Q(z)}{Q_(\infty)}\right), \tag{28}$$

where Q is the partition function of the tethering polymer. Even in this case, if we consider the case of two flat surfaces facing each other at a distance z, and describe the polymer as a gaussian chain, Eq. 28 can be calculated almost exactly as [1]:

$$W_{\text{steric}}(h) = -k_{\text{B}} T \ \sigma_L \ln \text{erf}(\frac{h}{\sqrt{\frac{2}{3}Na^2}}), \tag{29}$$

Combining the previous expressions, as implemented in the Python code, we can calculate the steric and bond energy contribution, and use to calculate the interaction free-energy for the entire particle as a function of distance to the cell. Using Eq.5, the fraction of bound particles can the be found.

2. Approximating the integral

The interaction free energy will generally display a minimum around the equilibrium binding distance, let us call this minimum z_{bind}. Because we are integrating the exponential of a function with a minimum, the integral will be dominated by contributions around this minimum and other details (e.g., z_{max}) will not matter. Because of this behaviour, we can further approximate the integral using a standard procedure called a saddle point approximation [8], resulting in the following formula:

$$K_{\text{bind}} = A_{\text{L}} \int_0^{z_{max}} \exp\left(-\beta A(z)\right) d\vec{z} \tag{30}$$
$$\approx \exp(-\beta A(z_{\text{bind}}))\sqrt{\frac{2\pi}{\beta A''(z_{\text{bind}})}} \tag{31}$$

where $A''(z') \equiv \frac{d^2A}{dz^2}|_{z=z'}$. The minimum of $A(z)$ can be found observing that:

$$A'(z) = \frac{d}{dz}\int_z^\infty F(h)dh \tag{32}$$

$$= \frac{d}{dz}\left(\mathcal{F}(\infty) - \mathcal{F}(z)\right) \tag{33}$$

$$= F(z) = -2\pi R_{\rm NP} W(z), \tag{34}$$

where we used the fact that $\mathcal{F}$ is the integral function of F, so the minimum $A'(z) = 0 \leftrightarrow W(z) = 0$. For the same reason, we have:

$$A''(z) = -2\pi R_{\rm NP}\frac{d}{dz}W(z) \tag{35}$$

In general, because the steric repulsion is very sharp once $z < Na^2$ while the bond energy monotonically decreases with z, we have that $z_{\rm min} \approx Na^2$, but this should be taken as an initial guess for numerical refinement rather than a decent approximation. In practice, the saddle point approximation might be useful for numerical efficiency more than anything else. In fact, even when the resulting expressions for $A(\rm min), z_{\rm min}$ and $A''(\rm min)$ are analytical, they can be extremely complex, and not particularly useful.

Analysis of different models

3. *Intro: The effective bond energy ΔG_{ij}*

In order to understand and compare with other models, I will come back here to the definition of the bond energy, following the treatment presented in [1]. The single bond energy between a ligand and a receptor can be expressed as the sum of two terms:

$$\Delta G_{ij} = \Delta G^0_{ij} + \Delta G^{\rm cnf}(\vec{r}_i, \vec{r}_j) \tag{36}$$

The term ΔG^0_{ij} depends on the free energy of binding for *free* ligands and receptors in solution, related to the experimentally measurable equilibrium dissociation constant K_D for that specific pair (i,j) by $\Delta G^0_{ij} = k_B T \ln\left(K_D^{ij}/\rho^\circ\right)$. The second term, $\Delta G^{\rm cnf}(\vec{r}_i, \vec{r}_j)$, depends on the properties of the polymer used to graft ligands to the nanoparticle, and can be shown to be equal to [1]:

$$\Delta G^{\rm cnf}(\vec{r}_i, \vec{r}_j) = k_B T \ln\left(\frac{1}{\rho^\circ}\frac{Q_{ij}}{Q_i Q_j}\right) \equiv k_B T \ln\left(\frac{1}{\rho^\circ V_{\rm cnf}}\right) \tag{37}$$

where Q_{ij} is the partition function of the polymer tether when ligand i and receptor j for a bond and Q_i, Q_j are the partition functions for the free (but grafted) state of i and j, respectively. For dimensionality reasons, and given their definition, the expression has the dimensionality of a density, hence its inverse represents some form of effective volume and thus we wrote $\frac{Q_{ij}}{Q_i Q_j} \equiv V_{\rm cnf}^{-1}$. In practice, if we assume that both ligands and receptors behave as gas particles free to roam within a volume $V_{\rm eff}$, and upon formation of a bond they are simply co-localised but still free to move as a pair in the same region, given their definition $Q_{\rm ij} = Q_i = Q_j = V_{\rm eff}$ and thus $V_{\rm cnf} = V_{\rm eff}$ in this case. In general, however, the expression for $\Delta G^{\rm cnf}$ are not that simple and in fact the precise form of this latter term is specific to the polymer properties, since it measures the configurational cost to stretch the tether so that the ligand can bind to its receptor. For PEG, we will use in all plots a description assuming an ideal (gaussian) chain. The expressions used were previously derived using the formulas in [1]. It should be noted that the polymer brush around a nanoparticle is usually quite dense but the exact regime (mushroom vs brush vs fluid) matters, especially if one wants to obtain accurate results. Here I will just notice that in a typical nanoparticle with both a short polymer (PEG2K) coating with ligands attached on longer polymers (PEG3.4K), different parts of the polymer will be potentially in different regimes, depending on the fraction of chains of each type.

Connection to other approximate treatments (Xu and Shaw's paper)

The connection between the previous formulas and those found in Xu and Shaw's paper [5] is explained here, which should also clarify the assumptions made and their validity (or not). In practice, it turns out that there is a cancellation of error that makes the model "better" than equivalent calculations with the right physics but wrong assumptions about the binding geometry - see the discussion later.

Ref.
[5] reports the following equations:

$$K_{\rm diss} = K_0 \exp\left(N_R \ln \frac{(N_R - \bar{n})}{N_R} + N_L \ln \frac{(N_L - \bar{n})}{N_L} + \bar{n} \right) \tag{38}$$

where $K_{\rm diss} \equiv K_{\rm bind}^{-1}$ is the effective dissociation constant between the nanoparticle (or virus) and the cell, K_0 the contribution from *non-specific forces*, and $\bar{n}$ the equilibrium number of bonds between ligands and receptors, given by the solution of the following equation:

$$\frac{(N_R - \bar{n})(N_L - \bar{n})}{\bar{n}} = K_D V_{\rm eff} \tag{39}$$

where K_D again is the dissociation constant for the single ligand-receptor bond and $V_{\rm eff}$ the volume of the binding zone, that is, the region between the nanoparticle in proximity to the cell surface, where ligands and receptors are close enough to form bonds. The expression in the exponential can be identified with the binding free energy:

$$\exp\left(N_R \ln \frac{(N_R - \bar{n})}{N_R} + N_L \ln \frac{(N_L - \bar{n})}{N_L} + \bar{n} \right) = \exp(\beta A_{\rm bond}) \tag{40}$$

because this is equivalent to our previous expression, Eq.16. That this is the case can be readily seen by noting that if $\bar{n}$ is the average number of bonds formed, then:

$$\frac{(N_R - \bar{n})}{N_R} = p_R; \qquad \frac{(N_L - \bar{n})}{N_L} = p_L \qquad p_{LR} = \frac{\bar{n}}{N_L N_R} \tag{41}$$

are the probability that a receptor is *not* bound (p_R), that a ligand is *not* bound (p_L), and that one of the $N_L N_R$ potential ligand-receptor pairs in the system is formed (p_{LR}). With this connection, and using $K_D V_{\rm eff} \equiv \chi^{-1}$ we see that Eq. **??** can be re-written as Eq. 39:

$$\frac{(N_R - \bar{n})(N_L - \bar{n})}{\bar{n}} = K_D V_{\rm eff} \tag{42}$$

$$\frac{(N_R - \bar{n})(N_L - \bar{n})}{\bar{n} N_R N_L} = \frac{K_D V_{\rm eff}}{N_L N_R} \tag{43}$$

$$\frac{p_L p_R}{K_D V_{\rm eff}} = p_{LR} \tag{44}$$

$$p_L p_R \langle \chi_{LR} \rangle = p_{LR} \tag{45}$$

which is exactly Eq.18. Finally, with these definitions for p_L, p_R we obtain:

$$\begin{aligned} N_R \ln \frac{(N_R - \bar{n})}{N_R} + N_L \ln \frac{(N_L - \bar{n})}{N_L} + \bar{n} &= N_R \ln \frac{(N_R - \bar{n})}{N_R} + N_L \ln \frac{(N_L - \bar{n})}{N_L} + \frac{1}{2}\bar{n} + \frac{1}{2}\bar{n} = \\ &= N_R \ln p_R + N_L \ln p_L + N_L N_R p_L p_R \langle \chi_{LR} \rangle \\ &= N_R \ln p_R + N_L \ln p_L + \frac{1}{2} N_L N_R p_L p_R \langle \chi_{LR} \rangle + + \frac{1}{2} N_L N_R p_L p_R \langle \chi_{LR} \rangle \\ &= N_R \ln p_R + \frac{1}{2} N_R (1 - p_R) + N_L \ln p_L + \frac{1}{2} N_L (1 - p_L) \\ &= N_R \left[\ln p_R + \frac{1}{2}(1 - p_R) \right] + N_L \left[\ln p_L + \frac{1}{2}(1 - p_L) \right] \end{aligned} \tag{46}$$

where in the next to last line we exploited the fact that from the definition of p_L, p_R we also have $\bar{n} = N_R(1-p_R) = N_L(1-p_L)$. Note that, as we wanted to prove, Eq. 46 is the same as 26.

Connecting the two theories is helpful because it shows the kind of approximation made by Xu and Shaw in their model for the nanoparticle(virus) / cell interaction. In practice, to derive their equations from the more general theory we had to assume:

1. There is a single possible ligand-receptor pair type.
2. The configurational penalty for forming a bond is equivalent to that of an ideal gas of ligands and receptors confined to a region V_{eff}. In other words, there is no dependence at all on the type of polymer used to tether the ligand to the nanoparticle.
3. To calculate V_{eff} , one needs to assume that the particle is effectively bound at a specific distance from the surface, and that bonds are confined in a region dictated by this distance and the maximum length considered for potential bonds. The exact choice for these two quantities greatly influences the final result. Later in Fig.1 , we will show th effect of different choices.

   To connect A_{bond} to the effective binding constant between the nanoparticle and the cell (K_{bind}), we need to make another assumption on the non-specific forces present. Xu & Shaw leave it as a free-parameter in their theory, K_0 (which has dimensions of a number density or, equivalently, the inverse of a volume). However, we can calculate this number from the exact statistical mechanical definition of the binding constant (see Eq. 7). To do this, we make three more assumptions:
4. Non-specific interactions are negligible compared to the steric and ligand-receptor bonding contributions, in other words, *in the absence of bonds* the average density in the bulk of the solution or near the cell surface is the same.
5. The bond energy remains constant and equal to its value calculated at a binding distance $z_{\text{bind}} \approx R_{ee,L}$, and
6. The nanoparticle can wiggle laterally within an area $R^2_{ee,L}$ while still remaining bound. In other words, Eqs. 4–6 are equivalent to saying that the particle is bound whenever its centre of mass resides within a volume $V_{\text{bind}} \approx R^3_{ee,L}$ and within a distance $R_{ee,L}$ from the surface of the cell. With these approximations, we get $K_0 \approx R^{-3}_{ee,L}$, where $R_{ee,L}$ is the average end to end distance of the polymer tethering our ligands.

We now discuss the effect of different assumptions, see Fig.1 for reference, where the results of different models/assumptions are reported.

Of all the previous assumptions, Assumption 1 is valid whenever ligand-receptor binding is specific, in which case ligands will only interact appreciably with their cognate receptors but not spuriously with others. Assumptions 4-6 can also be released if one had any experiment measuring the binding for nanoparticles without any ligand, in which case K_0 can be recovered exactly including all contributions. However, for nanoparticles coated with PEG in water, we expect such interactions to be neglible, and our approximation to hold quite nicely.
Assumptions 2 and 3 are instead both delicate and quite strong in terms of their effects, as can be evaluated by comparison with the "exact" theory, where these assumptions are *not* made. "Guessing" the right binding geometry / binding distance, or even assuming there is a fixed one, is complicated. On the one side, **the binding distance is not a constant dependent only on the nanoparticle**, because it depends on the interplay between bonding and steric contributions, and thus on the receptor density. That this is the case can be evinced by comparing the free energy density as a function of the distance for two systems that only vary by the receptor density and nothing more, see Figure 2. On the other side, as can be seen from the saddle-point treatment of the "exact" solution, it is not only the binding distance but also the *curvature* of the effective potential around the binding distance that strongly influences the effective dissociation constant of the nanoparticle. Notably, this curvature depends on the polymer properties. Moreover, it is also clear in these equations that the curvature of the nanoparticle itself plays a role, not correctly accounted for in the other treatments.
An additional problem with Assumption 2 is that, at least in principle, one cannot disregard the loss of configurational freedom (stretching) of the polymer tether required for a ligand to reach a receptor and, again, this configurational term depends on the polymer properties of the tether, which themselves also depend on the "state" of the polymer. For example, whether it can be described by an ideal chain, or as a self-avoiding walk in the mushroom (Flory's theory) or brush regime (Alexander-DeGennes). It turns out that in practice this approximation as a somewhat smaller effect compared to Assumption 3, at least not for the type of polymers modelled here. We can see that this in Figure 1 by comparing the two "exact" models using two different polymers (ideal vs self-avoiding walk/Flory),vs two

fixed-geometry models where the binding geometry is the same but an exact description of the bond energy is taken vs one where all bonds are assumed to have the same average length. In both cases, the effect is smaller compared to assuming a different binding distance and a different treatment of curvature effects. To see the effect of these approximations, one can compare the two models based on Xu & Shaw equations when one assumes that the binding distance is approximately that of an ideal ligand with the same model but where the ligand is considered in the brush state (what I called "Lennart's model"). Or, equivalently, the "exact" vs "mine" treatment, where the same polymer description is used but in the second case a fixed geometry is assumed and nanoparticle curvature is only used to determine the approximate number of interacting ligands, rather than the proper treatment using the full distance dependence of the interaction energy $A(z)$ (and its dependence on nanoparticle curvature/radius) to calculate the full integral determing K_{bind}, i.e. Eq. 7 .

III. SUMMARY

I summarise the main points / messages here:

1. The effective dissociation constant (and thus adsorption) estimated with a model that naively assumes only the binding distance is important to calculate the nanoparticle/cell effective dissociation constant is strongly dependent on the choice of the latter. Full/'Exact' modelling (Eq. **??** evaluated through proper integration using Derjaguin's approach) shows that both the binding distance and the curvature of the interaction potential (which also depend on the curvature of the nanoparticle) are both important and should thus be properly accounted for.

2. The correct binding geometry (binding distance, curvature) depends on the interplay of binding vs steric properties, which in turn depend on the specific model assumed for the polymer. Using a Flory or ideal polymer model gives comparable results, but they both differ strongly from assuming a naive fixed-geometry.

3. Xu and Shaw's model does not consider directly any effect of the polymer that tethers the ligands, and should probably be used only in cases where the ligands and the receptors are not grafted but mobile.

4. Lennart's initial calculation over-estimates the effective dissociation constant quite a lot. Using a gaussian model to determine the geometry seems to correct this problem, at least partially, but this is due to a cancellation of error which will be strongly system depedent and should probably not be used.

5. In the end, doing the full, correct treatment is numerically easy and tractable (calculations take less than a second), so probably better use the full model.

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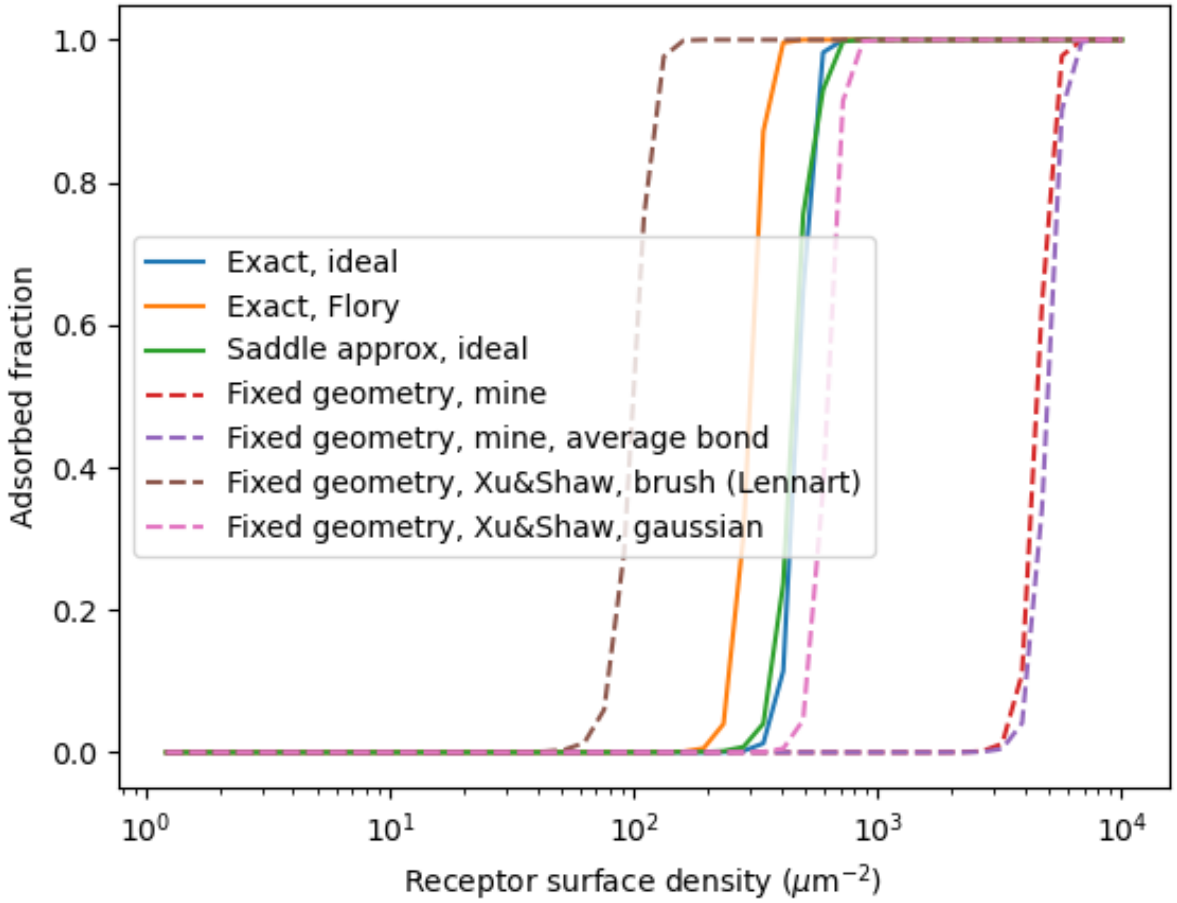
[5] Huafeng Xu and David E Shaw. A simple model of multivalent adhesion and its application to influenza infection. *Biophysical journal*, 110(1):218–233, 2016.

[6] A binder is either a ligand or a receptor, but the distinction between them is arbitrary. Purely for clarity, here we use the term ligand for a binder on a nanoparticle and receptor for a binder on a surface, but the theory is agnostic to this definition and, as long as i and j can form bonds between each other, the distinction between who is who does not matter

[7] In theory, a is the Kuhn length but for PEG, which is very flexible, to a good approximations, these are the same

[8] This formula can be simply derived by doing a Taylor expansion of the function around the minimum, truncating to second order and calculating the resulting analytical Gaussian integral



[h!]

Figure 1: Calculated fraction of adsorbed nanoparticles for different models. "Exact" is based on the Derjaguin approach and does not assume any binding distance (but different polymer models, "ideal/gaussian" vs "self-avoiding-walk/Flory"), and K_{bind} is calculated by doing the full integral in Eq. 7, while "Saddle" uses a saddle point approximation to calculate the relevant integrals. "Fixed geometry" (dashed lines) are calculations assuming a specific nanoparticle-cell surface distance upon binding. In "mine", the exact form of the bond energy is taken into account to solve Eqs.14,18 assuming the ligand behaves as a gaussian chain that can sample potential binding partners with a probability that varies continuously depending on the exact bond energy. In "average", a gaussian chain behaviour is still assumed but all ligand-receptor pairs are assumed to have the same average bond length. In "Xu & Shaw", the model proposed in Ref. [5] is used and equations 38,39 are solved. As we have shown, this is equivalent to solving the correct equations but using a rough ideal gas approximation to calculate the bond strength χ_{ij} (ligands and receptors are treated as an ideal gas of particles confined in the binding region V_{eff}). We use this model but choose 2 different binding distances, based on assuming the ligands behave as an ideal polymer vs a polymer in the brush state ("Lennart's" model)

. Due to a cancellation of errors, Xu and Shaw's model assuming a binding distance based on a gaussian polymer is close to the correct theoretical treatment, at least for the bond strength of 1nM and specific nanoparticle size used here. Parameters used $K_D = 1$nM, the other various parameters can be found in the *.py* files implementing the code and where chosen to represent a typical concentration of T cells in the spleen.

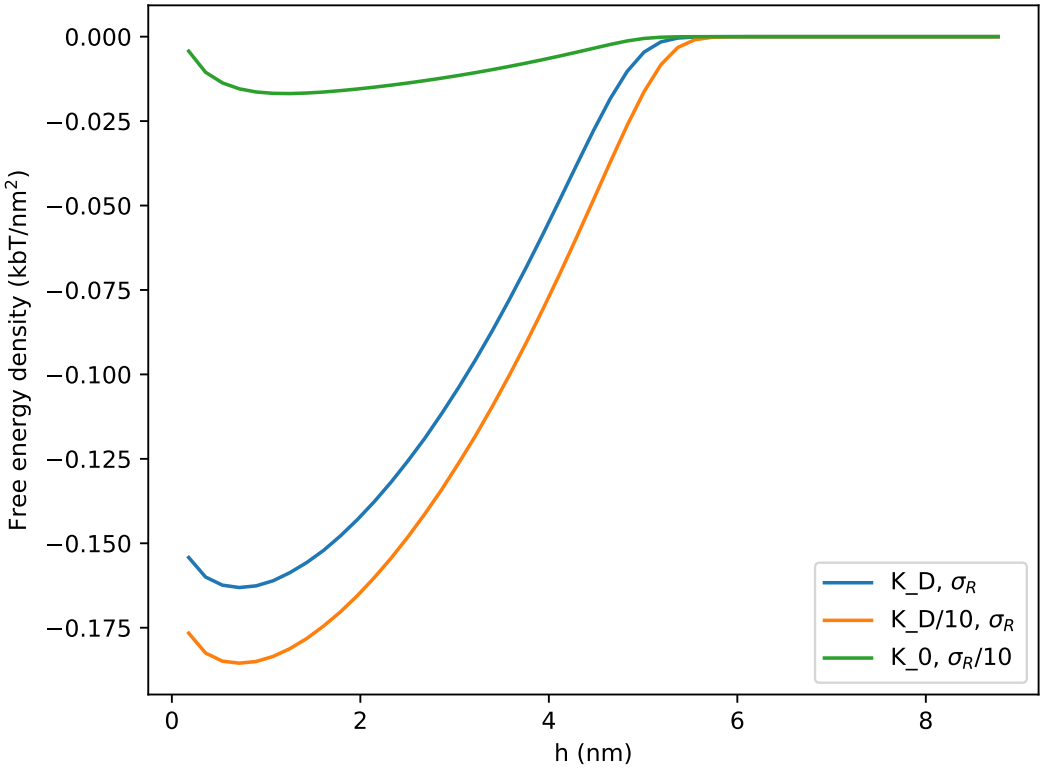


Figure 2: Free energy density as a function of distance for nanoparticle/cell systems differing by either the bond strength (as measured by the equilibrium binding constant for a single bond, K_0, or the average receptor density σ_R. Notably, the distance at which the free-energy density is minimum is different in all three cases, meaning that the binding geometry is not a constant. Because the latter has a large effect on the calculated value of nanoparticle adsorption, it would be better to use a model, like we do here, where such a distance is not guessed, but calculated by the theory from first principles.