

## MODELLING RANDOM ANTIBODY ADSORPTION AND IMMUNOASSAY ACTIVITY

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**ABSTRACT.** One of the primary considerations in immunoassay design is optimizing the concentration of capture antibody in order to achieve maximal antigen binding and, subsequently, improved sensitivity and limit of detection. Many immunoassay technologies involve immobilization of the antibody to solid surfaces. Antibodies are large molecules in which the position and accessibility of the antigen-binding site depend on their orientation and packing density.

In this paper we propose a simple mathematical model, based on the theory known as random sequential adsorption (RSA), in order to calculate how the concentration of correctly oriented antibodies (active site exposed for subsequent reactions) evolves during the deposition process. It has been suggested by experimental studies that high concentrations will decrease assay performance, due to molecule denaturation and obstruction of active binding sites. However, crowding of antibodies can also have the opposite effect by favouring upright orientations. A specific aim of our model is to predict which of these competing effects prevails under different experimental conditions and study the existence of an optimal coverage, which yields the maximum expected concentration of active particles (and hence the highest signal).

**1. Introduction.** Immunodiagnostics devices (or immunoassays) rely on the binding of antigens by antibodies and are used to detect biomarkers for a variety of diseases (such as cancer, HIV or cardiovascular disease) with high specificity and sensitivity in a range of media including blood or urine. Antibodies are glycoproteins produced by plasma cells whose primary function is to bind specifically to an antigen and elicit an immune response, thereby protecting the host from infection. Antibodies are large Y-shaped molecules composed of two regions: a fragment crystallizable (Fc) region at the base and a fragment antigen binding (Fab) region at the top (see Figure 1(a)). Each arm of the Fab region contains a hypervariable region

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at its tip, called a paratope, which is capable of binding strongly to one epitope on an antigen.

Many immunoassay technologies involve immobilization of the detection antibody to solid surfaces; this configuration also occurs on a large scale in physiological reactions *in vivo*. Widely used immobilization strategies include physical adsorption (based on electrostatic and van de Waals interactions) and covalent bonds, both of which result in a random particle distribution on the surface. It is, however, well known that such immobilization techniques have the unfortunate consequence of drastically reducing the ability of antibodies to efficiently bind antigen, through physical mechanisms such as molecule denaturation upon contact with the solid surface and crowding (or overlapping) of antibody fragments. (See, for example, [8], [14], [16].) Although better orientation of antibodies can be achieved through various molecular engineering strategies, most practical applications use the physical and chemical adsorption methods mentioned above. The passive adsorption of antibody particles onto the solid surface is achieved by incubation in a solution of known concentration and the resulting surface-adsorbed amount can be controlled experimentally, for example by varying parameters such as the antibody bulk concentration or incubation time, [9], [14], [16].

In this paper we consider the experimental configuration known as a direct binding assay, [12]. The sample containing the antigen is introduced over the sensor surface and the antigen then interacts with the immobilized antibody. This reaction generates an optical or electrochemical signal which is proportional to the reaction product; the dependence of this signal on the antigen or antibody concentration is then plotted as a calibration (or dose-response) curve for the assay. However, the model developed here can also be used for more general immunological platforms such as sandwich immunoassays, where the analyte to be measured is first captured to a surface by an immobilized antibody and then reacts with a second, labelled antibody in order to produce a detectable signal. The sandwich format has the advantage of increased sensitivity and robustness and forms the basis for many clinical and commercial detection tests.

The theory known as *random sequential adsorption* (RSA) has been successfully used over the past few decades to describe monolayer particle deposition, with wide applications in many physical and biological settings such as, for example, thin films of adsorbed colloidal particles, reactions on polymer chains, DNA sequencing, etc. [2], [7]. In the standard RSA model, rigid particles are placed at random, sequentially and irreversibly onto solid smooth surfaces in such a way that they do not overlap. If an incoming particle approaches an already covered part of the substrate, it is rejected. Eventually no more particles fit on the surface and the process stops in the so-called *jamming limit*. In one dimension, this process is commonly referred to as “the car parking problem” (or interval filling) and the jamming coverage, also known as the Rényi constant, has been calculated in [6] to be  $C_R \approx 0.74756$ . Many generalizations of this standard framework exist and include, for example, competitive RSA (where particles of two or more different sizes compete for adsorption, see [4] and references therein), cooperative sequential adsorption, [2], and RSA of overlapping particles, [7]. The original paper [6] calculates the jamming coverage using a recursive approach, however, recent papers dealing with applications to physical sciences have focussed on understanding the kinetics of RSA processes and have studied the time evolution of quantities such as gap distribution or total coverage during deposition, [4], [5].

Using the standard RSA theory for uniform size particles with no overlap, we now develop a simple procedure for evaluating the percentage of immobilized antibodies which are correctly oriented, with their binding site exposed, and therefore available for reaction with antigen. This percentage is expressed as a function of the total antibody coverage and used for evaluating the signal in a direct assay, which leads to a theoretical prediction of the calibration (or dose-response) curve. In many experimental situations an optimal antibody concentration has been identified beyond which the signal drops leading to a so-called *hook effect*. In such cases, a theoretical model is highly useful for optimizing assay design, as experimental studies are time consuming and antibodies are often patent protected and expensive. On the other hand, there are a number of situations when the hook effect does not occur. It has been suggested in recent work (see, for example, [8]) that, at high coverages, two competing effects are involved whereby particle crowding leads to overlaying and obstruction of binding sites (which is associated with signal decrease and a hook effect) but also favours improved (upright) antibody orientation which yields a signal increase. Representing particles by one-dimensional intervals is obviously a major simplification but we find that the model presented here reproduces many of the qualitative features of the adsorbed antibody activity (such as the competing effects described above) and gives us a good starting point for understanding such physical systems.

**2. Calculation of active percentage.** In this section we summarize the kinetic RSA calculation of the gap distribution and coverage as functions of time; these results are well-known in the literature so the derivation details are not included. A one-dimensional model for antibody activity is then introduced which estimates the active percentage using the gap distribution and predicts how this quantity changes with total surface coverage.

**2.1. Random sequential adsorption: The kinetic approach.** In the standard, one-dimensional RSA formulation, we start with a line segment of length  $L$ , assumed empty at  $t = 0$ . Unit length intervals are placed randomly and sequentially at a fixed rate onto the line, provided they do not overlap already deposited intervals. We introduce the function  $N(x, t)$  as the *gap length density function* at time  $t$  (so  $N(x, t)dx$  represents the mean number of gaps with length between  $x$  and  $x + dx$ ) and we let  $P(x, t) = N(x, t)/L$ . As  $L \rightarrow \infty$ , the evolution of  $P$  can be described by the following integro-differential equation, [5]

$$\frac{\partial P}{\partial t} = \begin{cases} -(x-1)P(x, t) + 2 \int_{x+1}^{\infty} P(y, t) dy, & \text{if } x \geq 1 \\ 2 \int_{x+1}^{\infty} P(y, t) dy, & \text{if } x < 1 \end{cases}$$

which essentially describes the rates at which gaps of length  $x$  can be created or destroyed. Using the initial conditions

$$P(x, 0) = 0, \quad \lim_{t \rightarrow 0} \int_0^{\infty} xP(x, t) dx = 1,$$

the solution to the above equations can be shown to be

$$P(x, t) = \begin{cases} t^2 F(t) e^{-(x-1)t}, & \text{if } x \geq 1 \\ 2 \int_0^t \tau F(\tau) e^{-x\tau} d\tau, & \text{if } x < 1 \end{cases} \quad (1)$$

where

$$F(t) = \exp \left[ -2 \int_0^t \frac{1 - e^{-u}}{u} du \right].$$

The total coverage is then given by

$$\theta(t) = 1 - \int_0^\infty xP(x, t) dx = \int_0^t F(\tau) d\tau,$$

which converges in the long term to the jamming limit (Rényi's constant)

$$\lim_{t \rightarrow \infty} \theta(t) = C_R = 0.74756\dots$$

Note that  $\theta$  is a non-dimensional quantity which represents the fraction of the line which is covered by intervals.

**2.2. Derivation of the active antibody coverage.** We introduce a simple model in which antibody molecules are represented by non-overlapping circles, where a certain fraction of the circumference denotes the active area (see Figure 1). In this representation, all antibodies are assumed to have the same dimension in all directions and the coverage of the substrate can be described using the one-dimensional RSA process described in the previous section (if we identify the molecule diameters with the filling intervals). An immobilized antibody is active if its binding

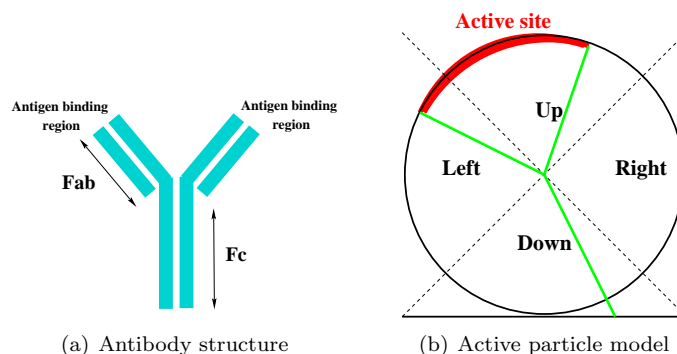


FIGURE 1. An antibody is active if it has the correct orientation.

site (Fab region) is correctly exposed and available to bind the incoming antigen. In the current model a particle is defined to be active if, either its binding site is pointing “up” (meaning its centre is contained within the relevant quadrant), or else pointing left or right and a gap of length at least  $\delta$  exists between the binding site and the neighbouring adsorbed molecule, where  $\delta$  depends on the size of the oncoming reactant molecules and it is assumed that  $\delta \leq 1$ . The antibody orientation is usually described in the immunoassay literature (see, for example, [13], [14]) by one of the following positions: “end on” (which would correspond to our “up” definition), “head on” (down), “flat on” and “side on” (both of which correspond, in our 1-dimensional model to a “side” orientation). The total number of active molecules at any given time,  $N_{\text{active}}$ , is then calculated by adding all “up” particles, all “left” particles with enough space on their left (which is obtained by multiplying the percentage of particles pointing left by the total number of gaps  $\geq \delta$ ) and all “right” particles with enough space on their right (obtained in the same way as

the left case). (The “down” particles are assumed inactive.) Moreover, if two adjacent particles are pointing towards the gap between them and this gap is not large enough to fit two antigens then only one of the immobilized particles is considered active. The active coverage is defined as  $\mathcal{B} = N_{\text{active}} \times \text{particle length}/L$  and can be calculated in terms of the gap density function (1) as follows,

$$\begin{aligned} \mathcal{B}(t) &= P_{\text{up}}\theta(t) + 2P_{\text{side}} \int_{\delta}^{\infty} P(x, t) dx - P_{\text{side}}^2 \int_{\delta}^{2\delta} P(x, t) dx \\ &= P_{\text{up}}\theta(t) + 2P_{\text{side}} \left( tF(t) + 2 \int_0^t (e^{-\delta\tau} - e^{-\tau})F(\tau) d\tau \right) \\ &\quad - P_{\text{side}}^2 \times \begin{cases} 2 \int_0^t (e^{-\delta\tau} - e^{-2\delta\tau}) F(\tau) d\tau, & \text{if } 2\delta < 1 \\ tF(t) (1 - e^{-(2\delta-1)t}) + 2 \int_0^t (e^{-\delta\tau} - e^{-\tau})F(\tau) d\tau, & \text{if } 2\delta \geq 1 \end{cases} \end{aligned} \quad (2)$$

where  $P_{\text{up}}$  is the percentage of particles in the “up” position and  $P_{\text{side}}$  is the percentage of particles with binding site facing either left or right (assuming these two positions are equally likely). We normalize the active and total coverages by defining

$$\bar{\mathcal{B}} = \frac{\mathcal{B}}{C_R}, \quad \bar{\theta} = \frac{\theta}{C_R},$$

so that  $0 \leq \bar{\mathcal{B}}(t), \bar{\theta}(t) \leq 1$ , for all  $t$ .

Experimental evidence suggests that antibodies are more likely to lie flat at low surface coverage while crowding may favour an upward orientation, [8], [9], [13]. Based on this observation, we propose a linear model which expresses the percentages of particles in different configurations as functions of coverage,

$$P_{\text{side}}(\bar{\theta}) = \left( \epsilon - \frac{1}{2} \right) \bar{\theta} + \frac{1}{2}; \quad P_{\text{up}}(\bar{\theta}) = \left( \frac{1}{2} - \epsilon \right) \bar{\theta}, \quad (3)$$

where  $0 < \epsilon < 1/2$  is a parameter which measures the gradient of this variation. Note that, as  $\bar{\theta} \rightarrow 1$  we have  $P_{\text{side}}(\bar{\theta}) \rightarrow \epsilon$  and  $P_{\text{up}}(\bar{\theta}) \rightarrow \frac{1}{2} - \epsilon$  so  $\epsilon$  also reflects the prevailing configuration as the coverage approaches the jamming limit. (For example, if  $0 < \epsilon < 1/4$ , antibody crowding will lead to more particles standing up.) It is difficult to assign precise values for  $\epsilon$  based on experimental information as, in general, the exact orientation of antibodies is unknown. This parameter could be linked, for example, to physical quantities such as the solution concentration of antibodies (it has been suggested, [8], that incubation with a higher concentration increases the adsorption rate and lowers the probability of flat-on or side-on particles due to the time constraint), surface properties or the method of immobilization. Note that  $2P_{\text{side}} + 2P_{\text{up}} = 1$  as we assumed that the “up” and “down” orientations are also equally likely.

The normalized concentration of active antibodies  $\bar{\mathcal{B}}$  is plotted in Figure 2 against the normalized total concentration  $\bar{\theta}$ , using the formulas (2) and (3). Recall that  $\bar{\theta} = 1$  corresponds to the jamming coverage (the maximum possible monolayer coverage achieved by random adsorption). The numerical integration is implemented in the C programming language while the data manipulation and graphics were performed using the IDL (Interactive Data Language) software. We consider the cases of large antigen particles ( $\delta = 1$ , so the antigen size is equal to that of the antibody) and small antigen particles ( $\delta = 0.1$ ), each of these relevant to various biosensing applications. Note that the behaviour of the active coverage function is qualitatively different in the two cases, primarily due to different lateral accessibility of large and

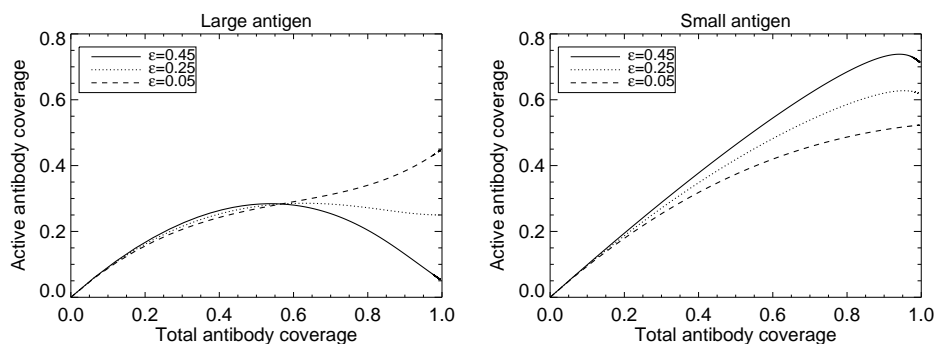
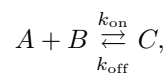


FIGURE 2. Active coverage,  $\bar{B}$ , as a function of total coverage,  $\bar{\theta}$ , for large ( $\delta = 1$ ) and small ( $\delta = 0.1$ ) antigen. The various curves in each diagram correspond to different orientation probabilities, as reflected by  $\epsilon$ .

small antigen particles. In particular, when dealing with large antigen sizes, the number of active antibodies is generally lower, the hook effect (when present) is more pronounced and appears at lower coverage values and the particle orientation near jamming (as reflected by  $\epsilon$ ) has a more dramatic influence on activity. These differences will be discussed in more detail in the context of the calibration curves presented in Figure 3 (Section 3).

**3. Modelling immunoassay response and kinetics.** The reaction between an antibody confined to a surface and antigen distributed in solution occurs in many physiological and industrial processes and has been studied extensively. The simplest and most commonly used model which describes the chemical binding kinetics between the two proteins is given by the bimolecular reaction,



where  $A$  is antigen,  $B$  is the bound antibody,  $C$  is the (bound) product, while  $k_{\text{on}}$  and  $k_{\text{off}}$  represent the association and dissociation rate constants, respectively. This can be cast as the following differential equation (see, for example, [1], [3], [8])

$$\frac{dC}{dt} = k_{\text{on}}A(t)(B_0 - C(t)) - k_{\text{off}}C(t),$$

where all the functions denote concentrations and  $B_0$  is the initial concentration of immobilized antibody. This equation (often derived from the Langmuir adsorption model) assumes, among other restrictions, homogeneous particle distribution and binding that is independent of mass transport (so the concentration of the antigen at the surface is equal to its bulk concentration). The equilibrium value of the product is given by

$$C^* = \frac{1}{2} \left[ A_0 + B_0 + k_a - \sqrt{(A_0 + B_0 + k_a)^2 - 4A_0B_0} \right], \quad (4)$$

where  $A_0$  is the initial antigen concentration and

$$k_a = \frac{k_{\text{off}}}{k_{\text{on}}}$$

is called the affinity (or equilibrium dissociation) constant.

Note that equation (4) expresses the signal as a function of  $B_0$ , which is the reacting (that is, active) concentration of antibody and is, essentially, unknown in experimental settings. To obtain a more useful model we now calculate the signal as a function of the total antibody present on the surface, by first converting concentrations into coverages. Thus, the initial active antibody concentration can be written as

$$B_0 = \frac{\mathcal{B}}{D} = \frac{\bar{\mathcal{B}}C_R}{D} \equiv \kappa\bar{\mathcal{B}},$$

where  $D$  is the diameter (or length, in our 1-dimensional model) of the antibody molecule, while  $\mathcal{B}$  is the initial active coverage, as calculated by (2). The constant  $\kappa = C_R/D$  represents the concentration of antibodies corresponding to the jamming coverage  $C_R$  and we let

$$\alpha = \frac{A_0D}{\theta} = \frac{A_0}{\kappa\bar{\theta}}$$

be the initial antigen to antibody concentration ratio. The signal (4) becomes

$$\mathcal{C} = \frac{1}{2} \left[ \alpha\bar{\theta} + \bar{\mathcal{B}} + K - \sqrt{(\alpha\bar{\theta} + \bar{\mathcal{B}} + K)^2 - 4\alpha\bar{\theta}\bar{\mathcal{B}}} \right], \quad (5)$$

where

$$\mathcal{C} = \frac{C^*}{\kappa}, \quad K = \frac{k_a}{\kappa}.$$

We take the non-dimensional quantity  $\mathcal{C}$  as a measure of the assay signal and plot (5) as a function of the total antibody coverage  $\bar{\theta}$ , since  $\bar{\mathcal{B}}$  has already been calculated in terms of  $\bar{\theta}$  in the previous section.

Figure 3 provides a qualitative comparison to experimental calibration curves. The assay signals are plotted for large and small antigen particles, two values of the  $\epsilon$  parameter and various antigen/antibody ratios ( $\alpha = 0.2 - 5$ ). Recall that  $\epsilon$ , introduced in (3), is a measure of the variation of particle orientation during the adsorption process. For example,  $\epsilon = 0.05$  corresponds to fast growth of the number of antibodies in the up/down orientation so that near the jammed state,  $\bar{\theta} \rightarrow 1$ , only 10% of them are lying flat. The corresponding graphs in Figure 3 show a steady increase in the signal, for both large and small antigens, which would seem to suggest that the optimal strategy in this case is to get as close as possible to a jammed monolayer configuration. By contrast, if  $\epsilon = 0.45$ , the side orientation predominates throughout the whole deposition process,  $0 \leq \bar{\theta} \leq 1$ , with 90% of antibodies found in this configuration at the jamming limit. Figure 3 shows that the behaviour of the signal now depends strongly on the antigen size. For large particles, the signal initially increases and then decreases, which indicates the existence of an optimal antibody coverage; moreover, this optimal value depends on  $\alpha$ , the antigen/antibody ratio (so that, for lower antigen concentrations, the signal growth lasts longer). This result is also observed experimentally in direct binding assays where adsorption of capture antibody at high concentration can result in a drop of antigen signal due to steric hindrance from overcrowding, [11], [14], [16]. For small antigen the hook effect is less pronounced and the optimal antibody coverage (at high antigen concentrations) occurs closer to the jamming limit; also, the signal values in this case are much higher than for large particles. These results are intuitively clear if we consider that (when the side orientation prevails), as the gaps get progressively smaller during deposition, it becomes increasingly more difficult for large antigens to bind and hence the signal drops.

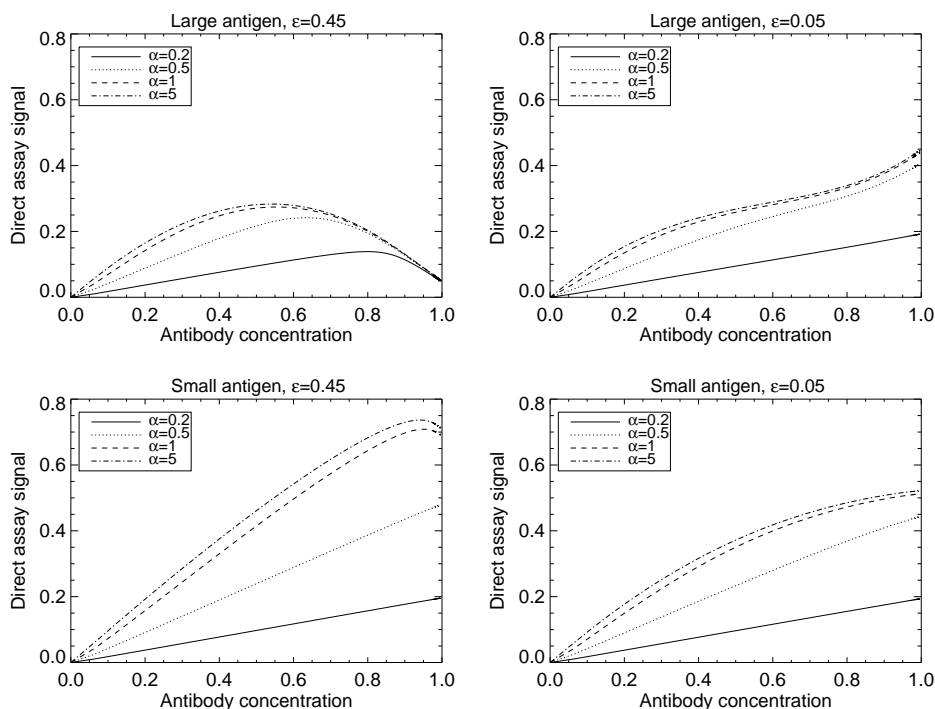


FIGURE 3. Assay signal as a function of total coverage,  $\bar{\theta}$ , for  $\delta = 1$  (large antigen) and  $\delta = 0.1$  (small antigen). The various curves in each diagram correspond to different antigen/antibody ratios,  $\alpha = 0.2 - 5$ .

**4. Conclusions and comparison with experimental data.** We have presented a new model for quantifying the activity of antibodies immobilized on a solid surface, based on the standard random sequential adsorption (RSA) theory. In spite of its simplicity, this model reproduces many qualitative features of immunoassays reported in the experimental literature. The results presented in Section 3 support the conclusion of [9] that the optimal performance of immunoassays is determined by the interplay between several factors such as immobilized antibody density, relative size of antigens and method of immobilization.

The main parameters used in this mathematical model are  $\epsilon$ , which quantifies antibody orientation, and  $\delta$ , which represents the ratio of antigen to antibody size. A direct comparison between our theoretical results and existing experimental data is only possible if enough information is provided regarding the size of the particles involved and their prevalent orientation. For example, the results of [14] and [16] report high antigen-binding rates at low surface coverage of antibody, which then decrease at higher antibody concentrations. A flat-on orientation of antibodies is determined by neutron reflection (NR) measurements and it is concluded that the signal drop at higher packing density is due to increased steric hindrance to antigen access. These conclusions qualitatively match our  $\epsilon = 0.45$  results, which also display the hook effect, especially so in the case of [14] where the antigen used is human chorionic gonadotrophin (hCG), which is characterized as a large molecule.



It was suggested in [8] that the antigen-binding activity at high coverage is determined by two competing effects: due to crowding, the antibodies may adopt a favourable outward orientation but, on the other hand, they may become shielded and less accessible. The increase of the signal at high antibody concentrations observed in the paper referenced above (where a decrease was not seen until after the monolayer limit was exceeded) was attributed by the authors to antibodies facing outward. Our results in Figure 3 also show that, if antibody crowding promotes improved particle orientation ( $\epsilon = 0.05$ ), the signal increases steadily which suggests that high surface concentrations are beneficial. Otherwise, if the prevailing effect is shielding of active sites, then the signal decreases (as seen in the case when  $\epsilon = 0.45$ ). The hook effect appears in our theoretical calibration curves for  $\epsilon = 0.45$ , is especially visible for large antigens and is associated with the existence of a well-defined optimal surface coverage, which should be relevant to assay design and functionality.

Many generalizations are possible for this model and include more complex antibody geometry (such as allowing different lengths for the side and upward orientations) and the possibility of partial overlap between adsorbed antibodies. Also, many papers (see, for example, [9], [15]) describe a phenomenon known as clustering (aggregation of antibodies due to attraction forces between neighbouring molecules) which would seem to suggest that, in some cases, adsorption is not uniform and would be more correctly modelled within a cooperative RSA framework. Regarding the antibody-antigen kinetics presented in Section 3, a more realistic approach would take into account the transport of the analyte to the surface (by convection and/or diffusion) and represent the kinetic rate constants as coverage-dependent functions (a fact which reflects the varying affinity of the immobilized antibodies towards antigen due to antibody denaturation at lower coverages, [8]). Such improvements will form the subject of further studies.

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## REFERENCES

- [1] D. A. Edwards, [Steric hindrance effects on surface reactions: Applications to BIAcore](#), *J. Math. Biol.*, **55** (2007), 517–539.
- [2] J. W. Evans, [Random and cooperative sequential adsorption](#), *Rev. Mod. Phys.*, **65** (1993), 1281.
- [3] V. Gubala, C. Crean, R. Nooney, S. Hearty, B. McDonnell, K. Heydon, R. O’Kennedy, B. D. MacCraith and D. E. Williams, [Kinetics of immunoassays with particles as labels: Effect of antibody coupling using dendrimers as linkers](#), *Analyst*, **136** (2011), 2533–2541.
- [4] M. K. Hassan, J. Schmidt, B. Blasius and J. Kurths, [Jamming coverage in competitive random sequential adsorption of a binary mixture](#), *Phys. Rev. E*, **65** (2002), 045103.
- [5] P. L. Krapivsky, [Kinetics of random sequential parking on a line](#), *J. Stat. Phys.*, **69** (1992), 135–150.
- [6] A. Rényi, On a one-dimensional problem concerning random space-filling (In Hungarian), *Publ. Math. Inst. Hung. Acad. Sci.*, **3** (1958), 109–127.
- [7] J. C. Roach, V. Thorsson and A. F. Siegel, [Parking strategies for genome sequencing](#), *Genome Research*, **10** (2000), 1020–1030.
- [8] B. Saha, T. H. Evers and M. W. J. Prins, How antibody surface coverage on nanoparticles determines the activity and kinetics of antigen capturing for biosensing, *Anal. Chem.*, **86** (2014), 8158–8166.

- [9] W. Schramm and S. Paek, [Antibody-antigen complex formation with immobilized immunoglobulins](#), *Anal. Biochem.*, **205** (1992), 47–56.
- [10] J. Talbot, G. Tarjus, P. R. Van Tassel and P. Viot, [From car parking to protein adsorption: An overview of sequential adsorption processes](#), *Colloids Surf. A*, **165** (2000), 287–324.
- [11] M. L. M. Vareiro, J. Liu, W. Knoll, K. Zak, D. Williams and A. T. A. Jenkins, [Surface plasmon fluorescence measurements of human chorionic gonadotrophin: role of antibody orientation in obtaining enhanced sensitivity and limit of detection](#), *Anal. Chem.*, **77** (2005), 2426–2431.
- [12] D. Wild (editor), *The Immunoassay Handbook*, 3<sup>rd</sup> edition, Elsevier, 2005.
- [13] M. E. Wiseman and C. W. Frank, [Antibody adsorption and orientation on hydrophobic surfaces](#), *Langmuir*, **28** (2012), 1765–1774.
- [14] H. Xu, J. R. Lu and D. E. Williams, [Effect of surface packing density of interfacially adsorbed monoclonal antibody on the binding of hormonal antigen human chorionic gonadotrophin](#), *J. Phys. Chem. B*, **110** (2006), 1907–1914.
- [15] H. Xu, X. Zhao, C. Grant, J. R. Lu, D. E. Williams and J. Penfold, [Orientation of a monoclonal antibody adsorbed at the solid/solution interface: A combined study using atomic force microscopy and neutron reflectivity](#), *Langmuir*, **22** (2006), 6313–6320.
- [16] X. Zhao, F. Pan, B. Cowsill, J. R. Lu, L. Garcia-Gancedo, A. J. Flewitt, G. M. Ashley and J. Luo, [Interfacial immobilization of monoclonal antibody and detection of human prostate-specific antigen](#), *Langmuir*, **27** (2011), 7654–7662.

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