

Ligand-receptor binding kinetics in surface plasmon resonance cells: A Monte Carlo analysis

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Abstract. Surface plasmon resonance (SPR) chips are widely used to measure association and dissociation rates for the binding kinetics between two species of chemicals, e.g., cell receptors and ligands. It is commonly assumed that ligands are spatially well mixed in the SPR region, and hence a mean-field rate equation description is appropriate. This approximation however ignores the spatial fluctuations as well as temporal correlations induced by multiple local rebinding events, which become prominent for slow diffusion rates and high binding affinities. We report detailed Monte Carlo simulations of ligand binding kinetics in an SPR cell subject to laminar flow. We extract the binding and dissociation rates by means of the techniques frequently employed in experimental analysis that are motivated by the mean-field approximation. We find major discrepancies in a wide parameter regime between the thus extracted rates and the known input simulation values. These results underscore the crucial quantitative importance of spatio-temporal correlations in binary reaction kinetics in SPR cell geometries, and demonstrate the failure of a mean-field analysis of SPR cells in the regime of high Damköhler number $Da > 0.1$, where the spatio-temporal correlations due to diffusive transport and ligand-receptor rebinding events dominate the dynamics of SPR systems.

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1. Introduction

The accurate measurement of the reaction rates between different species of chemicals is a crucial component in the process of understanding and manipulating the biochemical processes which perpetuate or extinguish life [1, 2].

A common method of measuring these rates is via surface plasmon resonance (SPR) [3, 4]. SPR allows the binding dynamics between two species of chemicals to be measured in real time, and is performed by binding one of the two chemical species to a substrate (the receptor species), and then measuring the change in index of refraction as the other chemical species (the ligand species) flows over the substrate and the two chemicals interact [5, 6, 7]. See Fig. 1 for a schematic of the experimental setup.

Ideally, the data from this experiment allows for the easy extraction of the binding and unbinding rates. However, in SPR cells the rates of transport to the reaction surface can be quite slow relative to the reaction rates, i.e., it may take much longer to diffusively transport down to the receptor surface than it does to bind to that surface, so the well-mixed assumption of first-order reaction kinetics may not necessarily be valid. The rate of transport to the reaction surface combines with the intrinsic reaction rates to create the effective reaction rates that are measured in an SPR assay. In order to determine the intrinsic reaction rates the influence of the transport rate must be properly accounted for [8].

Most of the attempted approaches to the problem of decoupling the transport and reaction rates model the system with a deterministic process, where the dependence on the parameters of the SPR system is governed by a set of coupled differential partial rate equations [9, 10, 11]. Simulations for SPR systems are often derived from numerical solutions to these PDEs, but these solutions often fail to capture the spatial and temporal correlations between the ligands and the receptors as they interact, and ignore statistical fluctuations [12].

Monte Carlo simulations are a computational tool developed to numerically solve the basic master equation for stochastic processes, and faithfully encode account for the presence of fluctuations and correlations in the modeled system. Monte Carlo methods have found widespread application in the modeling of physical, chemical, and biological systems. Since we cannot provide a comprehensive overview of Monte Carlo techniques in this brief paper, we refer the reader to Ref. [13] as a recent review of stochastic modeling for biological systems.

In this paper, we present results from Monte Carlo simulations of SPR cells for a broad range of binding and unbinding rates that allow for the observation of how the presence of correlations and fluctuations influence SPR data. Reaction rates derived from the standard mean-field model of the reaction kinetics[‡] will be compared with known intrinsic reaction rates used in the simulations in order to determine the degree

[‡] The mean-field model of reaction kinetics is physics nomenclature for the well-mixed assumption of the law of mass action (i.e., physical and temporal correlations are ignored). The term ‘mean-field’ will be used to refer to this model throughout this paper, but the two terms are equivalent [14, 15].

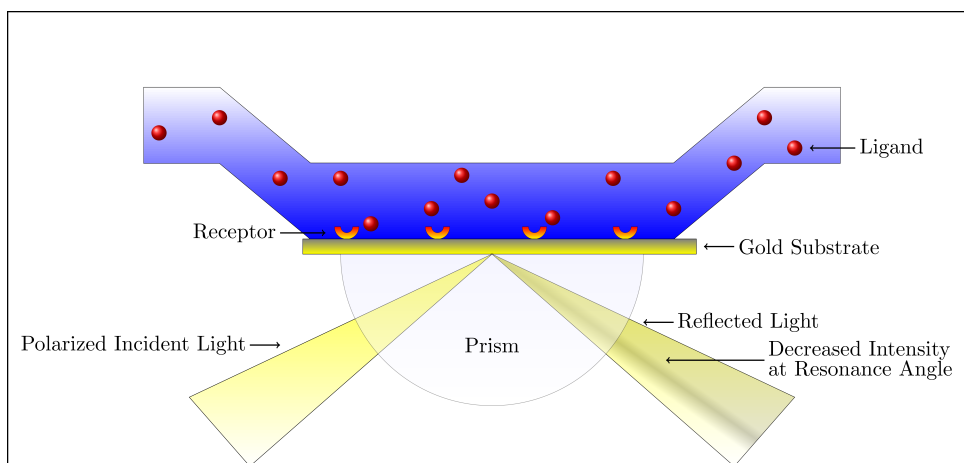


Figure 1. Surface plasmon resonance cell (schematic). A gold substrate is embedded at the bottom of a flow cell. Receptors (red-orange half circles) are distributed evenly across the substrate. Ligands (red spheres) are dissolved in a non-reactive solvent, and allowed to flow across the receptor surface with a constant concentration and flow rate. Ligands will be transported down to the receptor surface where they bind and unbind to the receptors according to their dynamics. Incident p-polarized light is shown through a prism onto the receptor surface. The angle at which resonance between the incident beam and the standing waves of electrons (plasmons) in the gold substrate can be measured by recording the angle at which the reflected light has a decreased intensity [3, 9, 17]. The extracted data of this resonance angle as a function of time can be rescaled to indicate the bound ligand density (i.e. the number of bound ligands normalized by the concentration of ligands in the flow cell) as a function of time [18].

to which spatio-temporal correlations and fluctuations are important to the dynamics of the system.

2. Surface plasmon resonance

2.1. The structure of the surface plasmon resonance cell

The structure of the surface plasmon resonance cell is discussed in more detail in literature [9, 16], but the following section will attempt to give a brief overview.

A surface plasmon resonance cell (schematically detailed in Fig. 1) is constructed by embedding a gold substrate into the bottom of a flow cell with linear dimensions on the order of millimeters. Two chemical species are chosen with the goal of determining the binding dynamics between them. One of these species is designated the receptors, and the other the ligands. The receptors are typically distributed randomly along the gold substrate and fixed in place, creating the receptor surface. A non-reactive solvent has a predetermined concentration of ligands dissolved into it, and this solution is allowed to flow over the receptor surface at a constant flow velocity.

The ligands in the solution are transported diffusely down to the receptor surface, where they bind and unbind to the receptors according to their respective dynamics.

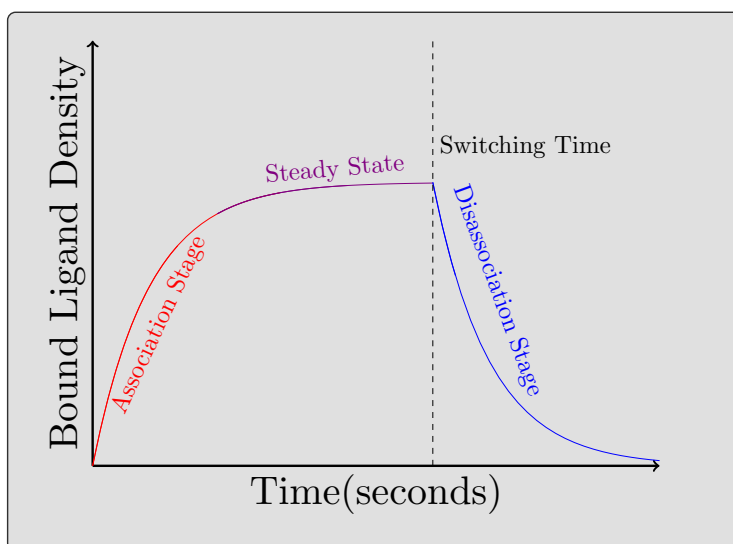


Figure 2. Stages of an SPR experiment. The red line indicates the association state of the SPR experiment, where a solution of ligands flows over the receptor surface with a constant concentration and fixed flow velocity. The bound concentration reaches a steady state (depicted in purple), at which point the concentration of incoming ligands is cut off, letting the bound ligands decay off the receptors in the dissociation stage, represented by the blue line.

The binding and unbinding of the ligands to the receptors cause the resonance energy of the surface plasmon waves in the gold substrate to change [3, 9, 17]. This change in the energy of the waves can be measured by shining a p-polarized beam of light onto the substrate through a prism. The prism allows the momentum of the incident beam to be varied, and when the momentum of the incident beam and the surface plasmons of the gold substrate are the same, the beam and plasmons couple and create a surface plasmon polariton in the gold substrate. This coupling results in a decrease in energy of the reflected beam of light, and the momentum at which this occurs can be measured by recording the angle where the resonance between the incident beam and the surface plasmon appears. The change in resonance angle as a function of time can then be rescaled into a plot of bound ligand-receptor pairs as a function of time [18].

2.2. Stages of the surface plasmon resonance experiment

The experimental process of surface plasmon resonance is typically performed in two stages. First, the solution of ligands is allowed to flow over the receptor surface with a constant concentration of ligands and fixed flow velocity. The system is allowed to evolve in this state until a steady-state concentration of bound ligands is observed. This stage of the experiment is referred to as the association stage. Subsequently, the concentration of incoming ligands is cut off, and the number of bound ligand-receptor pairs is allowed to decay away, as the ligands gradually unbind. This stage of the experiment is known as the dissociation stage. The concentration of bound ligands is measured throughout

both stages, and data similar to the kind depicted in Fig. 2 is generated. This paper aims to replicate both stages via Monte Carlo simulations, in order to determine the role that the spatio-temporal correlations induced by diffusion-limited association and repeated ligand rebinding processes play in the dynamics of the SPR cell.

3. SPR cell model

3.1. Cell geometry

We model the SPR cell as a rectangular lattice, with lattice spacing of 10nm. The lattice is constructed with maximum dimensions of L_x, L_y, L_z on the x, y , and z axes, which correspond to the laboratory dimensions of the SPR chip. Periodic boundary conditions are imposed on the z axis, and a reflective boundary condition imposed along the $y = L_y$ top of the y axis. Ligands are introduced at the $x = 0$ surface, and perform a random walk to adjacent lattice sites until they encounter the $x = L_x$ surface, at which point they are removed from the lattice.

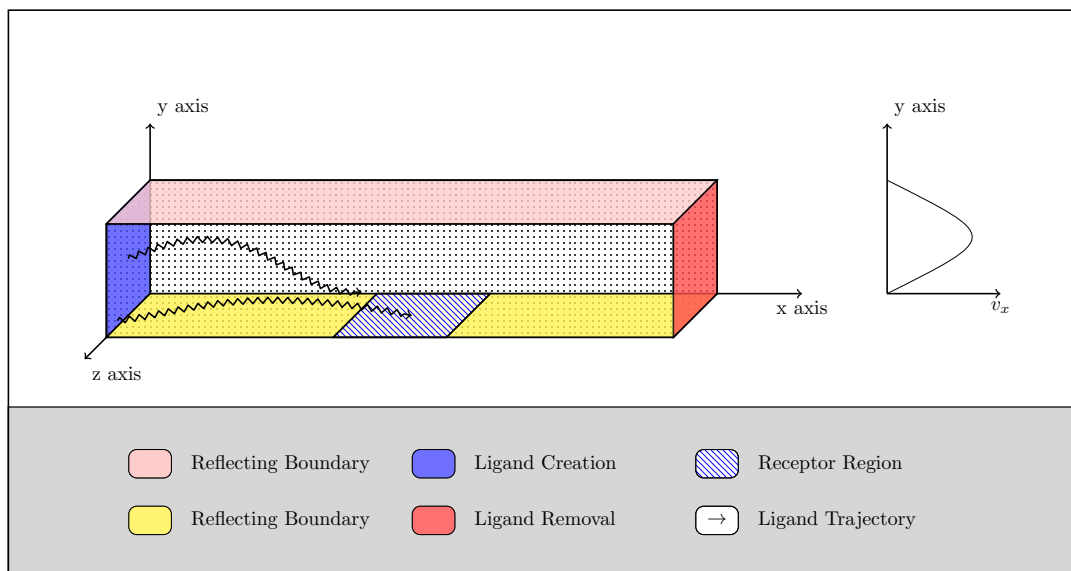


Figure 3. The discretized model of the SPR cell. Ligands are introduced at the ligand creation region depicted in solid blue, and perform a random walk through the lattice. This random walk is biased to create a parabolic flow profile (shown in the plot to the right of the schematic) that would be expected in the regime of laminar flow typical of SPR cells. The top and bottom planes of the lattice (depicted by the solid pink and solid yellow planes) form reflecting boundaries for the ligands. Receptors are evenly distributed in the receptor region (the dashed blue plane), and any ligand directly adjacent above a receptor has a chance to bind to it according to the association rate k_+ . Ligands perform their random walk through the lattice sites until they encounter the ligand removal region (depicted by the solid red plane), where they are removed from the simulation. In the association stage of the simulation, a ligand is immediately introduced at the ligand creation region to keep the concentration of ligands in the SPR cell constant, while in the dissociation stage of the simulation, the ligands are removed and not reintroduced, to allow the concentration of bound ligands to decay.

A subsection of the $y = 0$ surface is selected to model the receptor surface, from $x = x_0$ to $x = x_1$. Receptors are distributed evenly over this subsection with density R_0 , and the receptors are modeled such that if a ligand is directly adjacent above the receptor, the ligand can bind to the receptor with a probability \widetilde{k}_+ . Once the ligand is attached to a receptor it can no longer move, but can unbind from the receptor with probability \widetilde{k}_- . Ligands are assumed to be small enough that they do not interact in the lattice, and a receptor that is bound to a ligand cannot bind to another ligand until the first ligand unbinds.

A summary of the laboratory parameters of the SPR chip is given in Table 1, and a schematic representation of the simulation cell shown in Fig. 3.

Table 1. The laboratory parameters of a surface plasmon resonance chip.

Parameter	Description	Value	
L_x	Lattice size along x axis	4.80	mm
L_y	Lattice size along y axis	0.0500	mm
v	Mean flow velocity	1.33	mm/s
D	Diffusion coefficient	30.0	$\mu m^2/s$
R_0	Receptor concentration	5000	μm^{-2}
C_0	Ligand concentration	100	nM
k_+	Association rate	—	$M^{-1}s^{-1}$
k_-	Dissociation rate	—	s^{-1}
x_0	Start of SPR scanning region	2.9	mm
x_1	End of SPR scanning region	4.3	mm

While SPR regions are three-dimensional, the dynamics themselves are captured sufficiently in a two-dimensional representation if enough simulations are performed. Thus, only the x and y dimensions of the SPR chip are of concern for the model. The laboratory parameters are then discretized using the lattice constants detailed in Table 2, which give the SPR model parameters listed in Table 3.

Table 2. The lattice constants used to discretize the SPR model.

Parameter	Description	Value	
λ	Lattice size constant	10	nm
δt	Time step	1.51×10^{-6}	s

3.2. Ligand movement

Surface plasmon resonance cells are small, on the order of millimeters. This results in SPR cells having very small Reynolds numbers [19]. This in turn means that SPR cells reside in the regime of almost ideal laminar flow, so the movement of ligands in our simulation is biased to reflect this laminar transport.

Table 3. The discretized parameters of the surface plasmon resonance model.

Parameter	Relation to lab param.	Value
\widetilde{L}_x	L_x/λ	4.80×10^5
\widetilde{L}_y	L_y/λ	5×10^3
\widetilde{v}	$v \cdot (\delta t/\lambda)$	200.8
\widetilde{D}	$D \cdot (\delta t/\lambda^2)$	0.453
\widetilde{R}_0	$R_0 \cdot \lambda^2$	0.5
\widetilde{C}_0	$C_0 \cdot N_A \cdot \lambda^3$	6.022×10^{-5}
\widetilde{k}_+	$k_+ \cdot \delta t/(N_A \cdot \lambda^3)$	—
\widetilde{k}_-	$k_- \cdot \delta t$	—
\widetilde{x}_0	x_0/λ	2.90×10^5
\widetilde{x}_1	x_1/λ	4.30×10^5

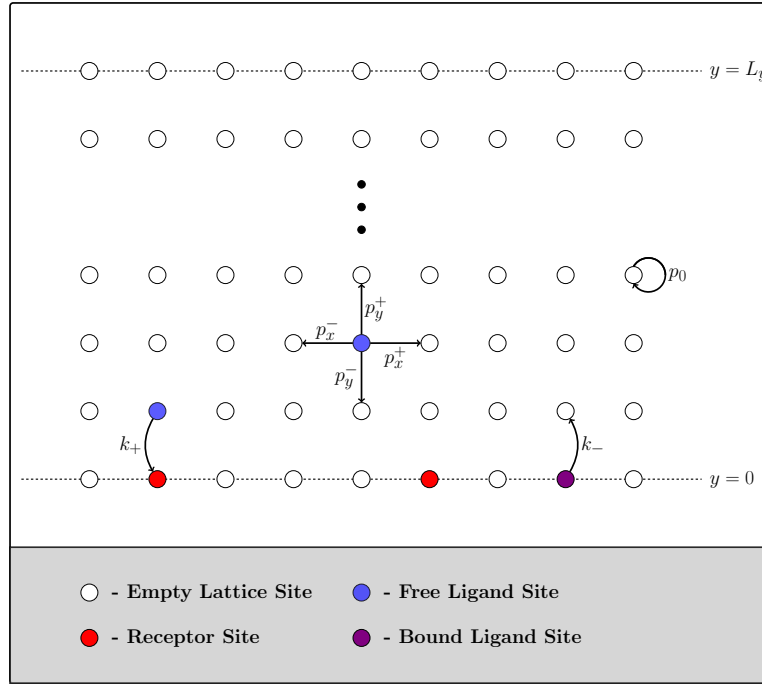


Figure 4. The two-dimensional dynamics of the SPR model. This plot shows all the possible actions that a ligand might take as it moves through the lattice sites. A ligand has probabilities p_μ^\pm of moving forward (+) or backwards (-) in the $\mu \in \{x, y, z\}$ direction, and a probability p_0 of stay put. Additionally, if a ligand is above a receptor it has probability \widetilde{k}_+ of binding to the receptor (independent of the probabilities of movement; for the purposes of the simulation the ligands are stepped in a direction determined by the movement probabilities, and then check if they could bind to a receptor). If a ligand is bound, it can no longer move, but has a probability \widetilde{k}_- to unbind. Once unbound, the ligand continues the random walk through the lattice.

The movement of the ligands through the lattice is modeled via a biased random walk, where the probabilities of moving parallel to the flow velocity are adjusted to create a parabolic flow profile as is expected in the case of laminar flow. The first

moment of the ligand position is taken from the flow velocity in that direction,

$$p_\mu^+ - p_\mu^- = \tilde{v}_\mu, \quad (1)$$

The second cumulant of the ligand position is taken from diffusion in the fluid,

$$(p_\mu^+ + p_\mu^-) - (p_\mu^+ - p_\mu^-)^2 = \tilde{D}_\mu = \tilde{D}/3. \quad (2)$$

The probabilities of ligand movement can be extracted from these conditions along with a normalization condition:

$$p_0 + \sum_{\mu} p_\mu^\pm = 1. \quad (3)$$

Here p_0 is the probability of staying still, p_μ^\pm respectively denote the probability of moving in the positive or negative μ direction; v_μ and D_μ are the flow velocity and diffusion constant in the μ direction, where μ can be either x , y , or z . Diffusion in the system is isotropic while the following bias velocities are chosen to model laminar flow:

$$\tilde{v}_y = \tilde{v}_z = 0, \quad (4)$$

$$\tilde{v}_x = \frac{6\tilde{v}_y(\tilde{L}_y - y)}{\tilde{L}_y^2}. \quad (5)$$

The probabilities of movement perpendicular to the flow velocity are unchanged. The parameters with a ' \sim ' superscript are dimensionless simulation parameters related to the physical parameters of the SPR chip via Table 3. The dimensional mean flow velocity v is related to the pressure gradient ΔP across the system as well as the viscosity η [20] via

$$v = -\frac{L_y^2 \Delta P}{12\eta L_x}. \quad (6)$$

As the ligands propagate through the lattice and encounter receptors in the receptor surface on the lattice floor, some percentage of the ligand population will bind to the receptors. This percentage is measured every time step for both the association and dissociation stages of the simulation. An example of these results is shown in Figure 6. A brief summary of the algorithm used for the Monte Carlo simulations is given in Appendix C.

3.3. Analysis

The system described in Table 3 was then simulated, with the parameters scaled by a factor of $\alpha = 0.025$ as described in Appendix B. Nine different association rates and two different dissociation rates were selected from the range of known values (detailed in Fig. 5, with values ranging from $10^3 M^{-1} s^{-1}$ to $10^7 M^{-1} s^{-1}$ and $10^{-2} s^{-1}$ to $10^{-3} s^{-1}$ respectively).

All possible pairs of these association and dissociation rates were then simulated giving eighteen different simulations. In order to obtain statistically significant results, each of these eighteen simulations was performed five hundred times (each time the

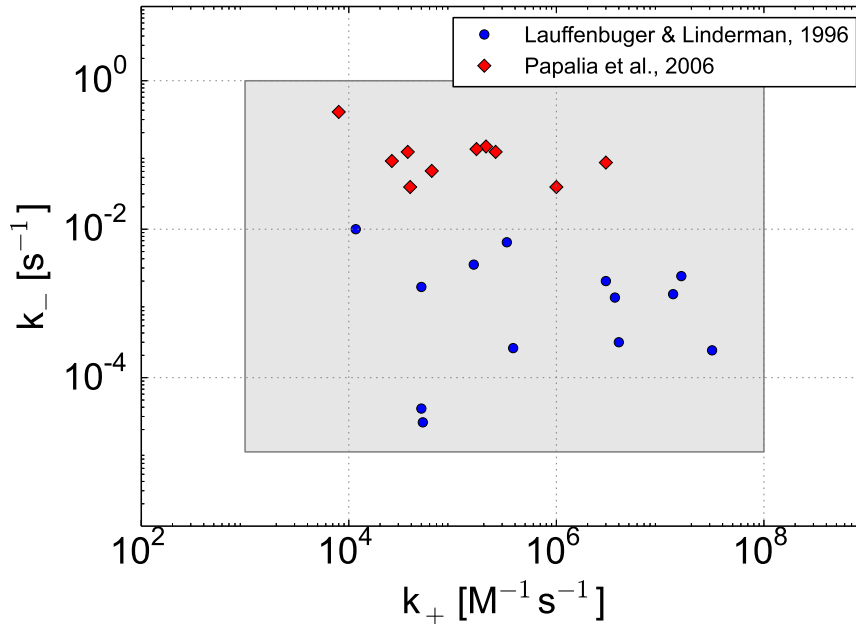


Figure 5. The range of experimentally determined reaction rates between pairs of different chemical species. The dissociation rate k_- of the chemical pair is plotted against the association rate k_+ on a log-log plot in order to give a representation of the range of values that these rates can take. The blue circles represent pairs recorded by Papalia et al. [21], while the red diamonds represent pairs recorded by Lauffenburger and Linderman [22]. The shaded region represents the regime of typical association and dissociation rates.

simulation is independent of all others), with new random initial conditions for each realization of the simulation. The number of realizations of each simulation was chosen to be five hundred in order to shrink the associated error while still being computationally feasible. Figure 6 shows example results of an averaged set of five hundred runs of an association-dissociation rate pair simulation. The example simulation data in Fig. 6 displays fits for both the association stage (red circles), and the dissociation stage (blue triangles). The mean field prediction of the dissociation phase is represented by the (green) dashed line with square markers. The error bars are not included because they are the same size as the (gray) data points. The inset in Figure 6 highlights the non-exponential behavior of the dissociation phase, by showing a logarithmic plot of the dissociation stage of Fig. 6. The (blue) line with triangular markers is the non-exponential fit of the (gray) data points, and the (green) dashed line with square markers is the mean-field prediction. Again, error bars are excluded because they are the same size as the (gray) data points. This plot of a high association rate is chosen to showcase the non-exponential behavior of the dissociation stage at high Da . This behavior does not coincide with the prediction of the mean-field analysis, and will be discussed in Section 4.

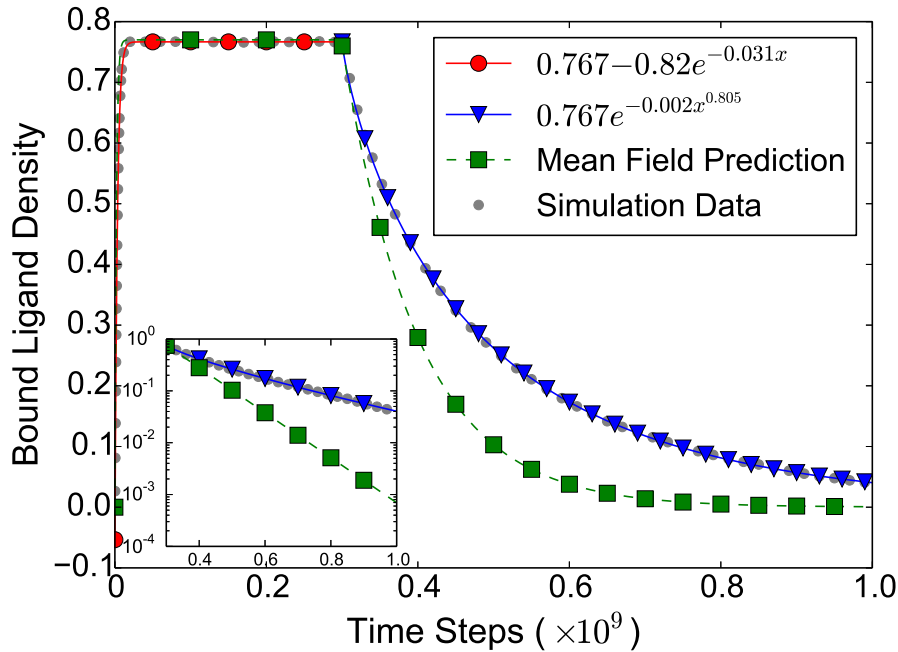


Figure 6. An example of simulation data for an association rate of $10^6 M^{-1}s^{-1}$ and a dissociation rate of $10^{-3}s^{-1}$. Error bars are the same size as the data points, and are thus excluded. The simulation results for the density of bound ligands is represented by the (gray) dots. A subset of the simulation data points is shown to ensure that the data points do not overlap and are easily visible. The fit of the association stage of the simulation is represented by the (red) line with circular markers, and the fit of the dissociation stage is represented by the (blue) line with triangular markers. For comparison, the mean-field prediction for a dissociation rate of $10^{-3}s^{-1}$ is shown by the (green) squares. The inset is a logarithmic (base ten) plot of the dissociation data of the main panel, again plotting the bound ligand density versus simulation time steps. The (blue) line marked with triangles is the stretched exponential fit of the data, represented by the (gray) dots, and the mean field prediction is represent by the (green) squares. This particular rate pair was selected because it demonstrates the non-exponential behavior of the dissociation phase at high Da . This is easily seen in the form of the fit for the dissociation phase, which is a stretched exponential (i.e., $p(t) \propto e^{-\alpha t^\beta}$ for $\alpha, \beta \in \mathbb{R}$) rather than simple exponential (i.e., $p(t) \propto e^{-\alpha t}$ for $\alpha \in \mathbb{R}$). This contradicts the predictions of the mean-field analysis, and will be discussed in more detail in Sec. 4.

3.4. Mean-field approximation

The mean-field rate equation for the SPR system is given by the first-order differential equation for the bound ligand concentration p §,

$$\dot{p} = C_0 k_+ (\gamma - p) - k_- p, \quad (7)$$

Where C_0 , k_+ , and k_- are described in Table 1 and $n_l = C_0(x_1 - x_0)L_y L_z$ and $n_r = R_0(x_1 - x_0)L_z$ are the number of ligands and receptors in the SPR scanning

§ In this case p is defined as the number of bound ligand-receptor pairs normalized by the number of ligands in the volume of the SPR cell bounded by the receptor surface. This number of ligands has a value of: $n_l = C_0(x_1 - x_0)L_y L_z$.

region, respectively. The factor γ is the ratio of the number of ligands in the volume of the SPR cell bounded by the receptor surface, to the number of receptors on the receptor surface: $\gamma = n_l/n_r$.

The mean-field association and dissociation rates were extracted via several parameters (summarized in Table 4) that are easily extracted from the numerical data. These values are often employed in the analysis of sensogram|| data [23, 24]. The mean-field model, eq. (7), provides predictions for these parameters which are summarized in eqs. (8)-(12) below. Specifically, the parameters listed in Table 4 are: the time derivative $f_0 = \dot{p}(0)$ of the bound ligand concentration at the initial time¶; f_∞ , which is the change in the time derivative \dot{p} with respect to the bound ligand concentration p at the switching time between the association and dissociation stages; the change r_0 in $\ln(p)$ with respect to time at the switching time; the change r_∞ in $\ln(p)$ with respect to time as time goes to infinity; and the saturation concentration p^* of bound ligands as they reach a steady state in the association phase:

$$f_0 = \gamma k_+ C_0, \quad (8)$$

$$f_\infty = k_+ C_0 + k_-, \quad (9)$$

$$p^* = \frac{\gamma k_+ C_0}{k_+ C_0 + k_-}, \quad (10)$$

$$r_0 = k_-, \quad (11)$$

$$r_\infty = k_-. \quad (12)$$

Table 4. The sensogram metrics.

Parameter	Definition
f_0	$\dot{p}(0)$
f_∞	$-\lim_{p \rightarrow p^*} (\frac{\partial^2}{\partial p \partial t} p)$
r_0	$-\frac{\partial}{\partial t} \ln p(t) _{t=t_{\text{switch}}}$
r_∞	$-\frac{\partial}{\partial t} \ln p(t) _{t=t_\infty}$
p^*	$p(t_{\text{switch}})$

To measure the association and dissociation rates, f_0 , f_∞ , and r_0 were used. These parameters were chosen because they are easily extracted from the numerical data, and provide simple relations to the association and dissociation rates. The numerical values of each of the three parameters was taken from the simulation data for each of the rate pairs, and the association rates and dissociation rates were solved for twice, namely via

$$k_+ = \frac{f_0}{\gamma C_0}, \quad (13)$$

|| A sensogram is a plot of SPR data vs. time. Figure 6 is an example sensogram, generated via simulations.

¶ Because the concentration of ligands in the flow cell is not constant at the beginning of the simulation, the time used to calculate this was not $t = 0$, but instead the time when the concentration began to behave like an exponential.

or

$$k_+ = \frac{f_\infty - r_0}{C_0}. \quad (14)$$

In each case the dissociation rate of the system is

$$k_- = r_0. \quad (15)$$

The two different association rates k_+ are paired with the one dissociation rate k_- , and compared with the actual input simulation values of these rates.

4. Results

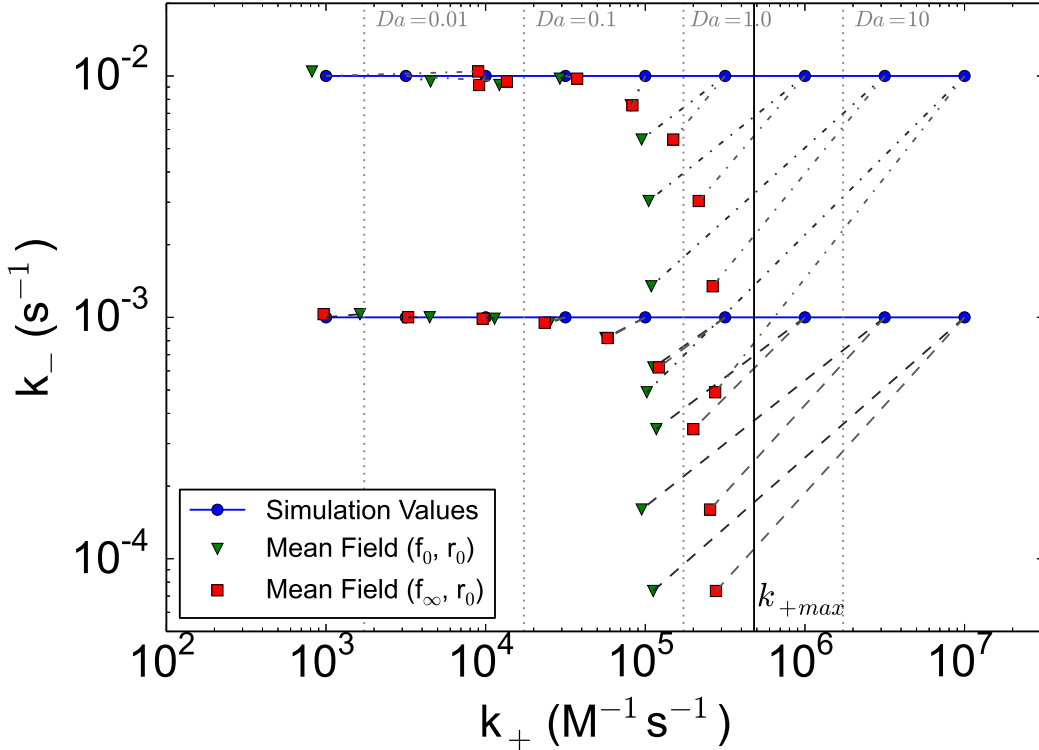


Figure 7. The comparison of extracted and simulation association and dissociation rates. The plot shows the dissociation rates k_- plotted against the association rates k_+ on a log-log scale for the eighteen different simulated pairs of association and dissociation rates. The intrinsic simulation rates are denoted by the (blue) circles, the rates extracted using the f_0 and r_0 sensogram metrics, eqs. (13) and (15), are denoted by the (green) triangles, and the rates extracted by f_∞ and r_0 sensogram metrics, eqs. (14) and (15), are indicated by the (red) squares. The (gray) dashed lines connect the mean-field rates with the corresponding simulations from which they were extracted from. The dotted lines denote different values of constant $Da = k_+ R_0 (L_x L_y / 6vD^2)^{1/3}$. The solid (black) line labeled k_{+max} represents a theoretical maximum that can be extracted from the mean-field theory for this particular system. Note that the highest value of k_+ that can be accurately predicted is much lower, and occurs around $Da \sim 0.1$.

The comparison of the simulation rates and the rates extracted from the data by applying the mean-field analysis can be seen in Fig. 7. The true simulation rates are denoted by the (blue) circles, the rates extracted using f_0 and r_0 , eqs. (13) and (15), are denoted by the (green) triangles, and the rates extracted by f_∞ and r_0 , eqs. (14) and (15), are indicated by the (red) squares. The (gray) dashed lines connect the mean-field rates with the corresponding simulations that they were extracted from. The dotted lines denote different values of constant $Da = k_+ R_0 (L_x L_y / 6vD^2)^{1/3}$. The solid (black) line labeled k_{+max} marks a theoretical maximum that the mean-field theory can predict, which will be discussed below. These results were replicated with various values of the lattice spacing constant λ and time step Δt in order to ensure these results are independent of the discretization of the system. The values used in this paper were chosen because they accurately model the average receptor size and binding timescale of a SPR cell.

It is immediately apparent from Fig. 7 that the extracted mean-field rates diverge rapidly from the simulation values as Da increases, though it is interesting to note that the mean-field measurements of k_+ using f_0 and r_0 are better than those using f_∞ and r_0 for $Da < 0.1$ and high k_- , while the the predictions of f_∞ and r_0 are slightly more accurate for $Da > 0.1$ than those of f_0 and r_0 . The better predictive abilities of (f_0, r_0) at low Da and high k_- are due to the high sensitivity of the association rate k_+ to the sensogram metric f_∞ at low Da and high k_- .

4.1. Sensitivity

Sensitivity in this context means the ratio of relative change in the extracted rate to the relative change in the sensogram metrics. To clarify, if $y = f(x)$, then the sensitivity S_y , of y to x is defined by the relation $dy/y = S_y dx/x$. Thus $S_y(x) = (x/f(x))df/dx$. The sensitivity of k_+ to f_0 and f_∞ is given by the equations

$$S_{k_+}(f_0) = 1, \quad (16)$$

$$S_{k_+}(f_\infty) = \frac{f_\infty}{f_\infty - r_0} = \frac{C_0 k_+ + k_-}{C_0 k_+} = 1 + K. \quad (17)$$

For extraction of rate constants, the ideal value for sensitivity is 1; sensitivities $\ll 1$ would indicate that the rate constants are independent of the sensogram metrics, while sensitivities $\gg 1$ indicate that small errors in the measurement of sensogram metrics will be amplified into large errors in the interpreted rate constants. The sensitivities are plotted in Fig. 8 for the range of k_+ values used in the simulations, as well as both values of k_- . The (green) dashed line is the sensitivity of k_+ to f_∞ with a constant $k_- = 0.01s^{-1}$, the (red) dashed-dotted line is the sensitivity of k_+ to f_∞ with a constant $k_- = 0.001s^{-1}$, and the solid (blue) line is the sensitivity of k_+ to f_0 for all values of k_- . As can be seen, in the regime where k_+ is relatively low and therefore $Da < 1$, k_+ is less sensitive to changes in the the sensogram metric f_0 than f_∞ . The results extracted from the (f_0, r_0) interpretation therefore predict the rates more accurately in this regime. Additionally, k_+ is approximately an order of magnitude less sensitive to

f_∞ for the smaller k_- at low Da , and so the the predictions of the (f_∞, r_0) metric at $k_- = 0.001s^{-1}$ are more accurate than those of the same interpretation at $k_- = 0.01s^{-1}$ for low Da .

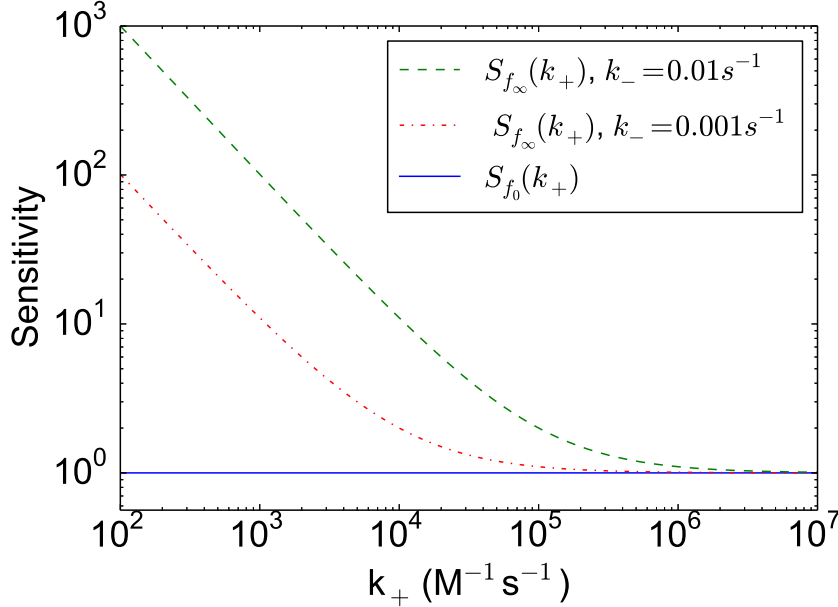


Figure 8. A log-log plot of the sensitivity of the attachment rate to the sensogram metrics f_0 and f_∞ , eqs. (8) and (9), as a function of k_+ . The (green) dashed line is the sensitivity of k_+ to f_∞ for $k_- = 0.01s^{-1}$, the (red) dashed-dotted line is the sensitivity of k_+ to f_∞ for $k_- = 0.001s^{-1}$, and the (blue) solid line is the sensitivity of f_0 to k_+ for all values of k_- . The concentration of ligands C_0 was taken to be 100nM.

4.2. The diffusion-limited regime

In the regime of high Da , f_∞ becomes the more accurate of the the metrics. This (as noted in Ref. [23]) is because f_∞ is less affected by the transport of ligands, since it is extracted from later parts in the experiment, where most of the ligands in the system are near the binding surface. There is still a qualitative increase in the error of the sensogram metrics' predictions as Da increases. One cause of this deviation is the effect of diffusive transport on the ligands. As k_+ increases, the average time for a ligand to bind to a receptor begins to be dominated by the time it takes for a ligand to be transported to the receptor surface [23]; however, at low association rates, $C_0k_+ < D/(L_y/2)^2$, the time delay an average ligand will experience before binding will be due to the association rate. As the association rate increases into the regime of $C_0k_+ > D/(L_y/2)^2$, the time delay will not be due to the association rate, but instead will be dominated by the much longer time it takes to be diffusely transported to the receptor.

The mean-field approximation can only interpret the time spent before binding as being due to the association rate, and so the time scale it takes to diffusely transport

ligands to the receptor surface gives a theoretical maximum on the association rate that the mean-field theory can predict,

$$k_{+max} \approx \frac{1}{C_0} \frac{D}{(L_y/2)^2}. \quad (18)$$

This value is marked with a (black) solid line in Fig. 7. In this figure the asymptotic approach of the (f_∞, r_0) prediction comes close to this value as Da increases, while the prediction of (f_0, r_0) approaches an asymptote at a lower value because it is more sensitive to the diffusive transport in the system.

4.3. Ligand-receptor rebinding events

The remaining effect to mention is that of ligand rebinding, which is assumed not to happen in the mean-field dissociation phase of the SPR experiment. However, the ligands may still perform random walks back to the receptor surface after they have unbound. As the association rate increases, the likelihood of a ligand rebinding to a receptor increases. This causes ligands to on average stay on the receptor surface longer. The mean-field interpretation of this is a lowered dissociation rate, which is why the extracted dissociation rate decreases as the simulation association rate increases.

Additionally, it was predicted by Gopalakrishnan et al. [8] that ligand dissociation from a surface with uniform receptor density R_0 into a semi-infinite domain in the absence of advective transport results in non-exponential late time dissociation of the form $p(t) \propto e^{ct} \text{erfc}(ct)$ where c is a parameter that depends on the density of receptors and the dissociation rate, and $\text{erfc}(z) = 2/\sqrt{\pi} \int_z^\infty e^{-x^2} dx$. As seen in Fig. 6, the dynamics of the dissociation phase are indeed non-exponential for high Da , but are stretched exponentials (i.e. $p(t) \propto e^{-\alpha t^\beta}$ for $\alpha, \beta \in \mathbb{R}$) instead of error functions. This difference from the predictions of Ref. [8] is likely due to the presence of advective transport in the SPR cell. For low Da , the behavior of the late-time dissociation corresponds to exponential kinetics, as the effects of the temporal correlations of ligand-receptor rebinding and diffusion are negligible compared to the time it takes for association. This exponential behavior at low Da corresponds to the agreement between the simulation rates and the mean-field predictions at low Da , as seen in Fig. 7.

5. Conclusion

These Monte Carlo simulations of ligand-receptor binding kinetics in SPR cells provide a testing ground for different analysis techniques. They were used in this paper to determine the regime in which a mean-field analysis of SPR is applicable. The system in Table 1 was modeled using these methods, and the dynamics of many ligand-receptor species with differing association and dissociation rates were simulated. The sensogram metrics defined in Table 4 were employed to relate the mean-field approximation of the system to parameters easily extracted from the simulation data.

The predictions of the sensogram metric were close to the actual simulation values for $Da < 0.1$, but after that point the association rate begins to get large enough that diffusive transport begins to dominate the time scale on which ligands interact with receptors, and the probability of ligand rebinding events becomes very high. By ignoring these two temporal correlations, the mean-field predictions begin to drastically differ from the simulation parameters, and within a factor ten increase in the association rate, the error between the mean-field predictions and the simulation parameters increased by a factor of one hundred. Thus, these simulations show that a mean-field analysis of surface plasmon resonance is only valid for small values of $Da < 0.1$, due to the importance of the diffusive and ligand-rebinding temporal correlations. Further work could be done on looking at the effects of the ligand-rebinding correlations on different receptor topologies. In biological systems, such as cells, receptors are not evenly distributed like those on the bottom of the SPR flow cell, but appear in clusters on the cell surface. This clustering could increase the likelihood that a ligand rebinding event occurs, allowing ligands to remain on the cell surface longer than would strictly be predicted from their binding rates, c.f. Ref. [14]. This would further distance the dynamics of these biological systems from mean-field predictions.

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Appendix A. Reaction-diffusion-advection PDE

This appendix is added to present a model of the SPR system described by Table 1, and to show that this can be reduced to a system of three dimensionless parameters Da , D_D , K , and a time scale τ .

The simplification of the advection-diffusion PDE follows from a derivation performed by Ref. [9]. We start with the PDE for ligand concentration in a flow cell with a receptor surface on the $y = 0$ plane,

$$C_t = D(C_{xx} + C_{yy}) - \left(\frac{6v}{L_y^2}\right)y(L_y - y)C_x, \quad (\text{A.1})$$

where subscripts on C denote differentiation with respect to the subscript. Eq. (A.1) can be recast in terms of the scaled variables $\hat{x} = x/L_x$, $\hat{y} = y/L_y$, $\hat{z} = z/L_z$ and $\hat{t} = 6vt/L_x$,

$$C_{\hat{t}} = Pe^{-1}(\varepsilon^2 C_{\hat{x}\hat{x}} + C_{\hat{y}\hat{y}}) - \hat{y}(1 - \hat{y})C_{\hat{x}}, \quad (\text{A.2})$$

where $\varepsilon = L_y/L_x$ is a dimensionless parameter, and Pe denotes the P eclét number

$$Pe = \frac{6vL_y^2}{DL_x}, \quad (\text{A.3})$$

which represents the ratio of the advective transport rate to the diffusive transport rate. The surface density of bound receptors ($R(\hat{x}, \hat{t})$) evolves according to the reaction rate equation

$$R_{\hat{t}}(\hat{x}, \hat{t}) = k_+C(\hat{x}, 0, \hat{t})(R_0 - R) - k_-R, \quad (\text{A.4})$$

and the boundary condition for the receptor surface is given by

$$C_{\hat{y}}(\hat{x}, 0, \hat{t}) = \frac{Pe}{L_y} R_{\hat{t}}(\hat{x}, \hat{t}). \quad (\text{A.5})$$

SPR systems typically have a Peclét number on the order of 100.

Now we can show that for systems with large Peclét numbers, close to the receptor surface (A.2) simplifies and Pe becomes irrelevant. First we redefine the \hat{y} and \hat{t} variables to a more useful form:

$$\eta = Pe^\alpha \hat{y}, \quad \tau = Pe^\beta \hat{t}, \quad (\text{A.6})$$

where α and β are quantities that will be determined later. Using these substitutions, eq. (A.2) becomes

$$C_\tau = Pe^{-(\alpha+\beta)} (\varepsilon^2 C_{\hat{x}\hat{x}} + Pe^{2\alpha} C_{\eta\eta}) - (Pe^{-(\alpha+\beta)} \eta + Pe^{-(2\alpha+\beta)} \eta^2) C_{\hat{x}}. \quad (\text{A.7})$$

If we require the Péclet coefficients on $C_{\eta\eta}$ and $\eta C_{\hat{x}}$ to be unity, the exponents α and β must be $\alpha = 1/3$ and $\beta = -1/3$. Eq. (A.7) then reduces to

$$C_\tau = Pe^{-2/3} \varepsilon^2 C_{\hat{x}\hat{x}} + C_{\eta\eta} - (\eta - Pe^{-1/3} \eta^2) C_{\hat{x}}. \quad (\text{A.8})$$

Because η is a rescaling of \hat{y} , the only part of eq. (A.8) that determines the binding dynamics is the region where $\eta \rightarrow 0$. In this limit (A.8) simplifies to

$$C_\tau = Pe^{-2/3} \varepsilon^2 C_{\hat{x}\hat{x}} + C_{\eta\eta} - \eta C_{\hat{x}}. \quad (\text{A.9})$$

Then, in the regime where $Pe^{-2/3} \varepsilon^2$ is small, the ligand concentration is governed by the reduced equation

$$C_\tau = C_{\eta\eta} - \eta C_{\hat{x}}. \quad (\text{A.10})$$

Finally, the ligand and receptor concentrations can be rendered dimensionless by the transformation

$$\begin{aligned} c(\hat{x}, \eta, \tau) &= C(\hat{x}, \eta, \tau)/C_0, \\ r(\hat{x}, \eta, \tau) &= R(\hat{x}, \eta, \tau)/R_0. \end{aligned} \quad (\text{A.11})$$

Under this transformation, the boundary conditions on the receptor surface given by eqs. (A.5) and (A.4) become

$$\begin{aligned} c_\eta(\hat{x}, 0, \tau) &= D_D^{-1} r_\tau(\hat{x}, \tau), \\ r_\tau(\hat{x}, \tau) &= Da D_D \{c(\hat{x}, 0, \tau)(1 - r) - Kr\}, \end{aligned} \quad (\text{A.12})$$

where Da , D_D , K , and τ are defined in eqs. (B.1)–(B.4).

Appendix B. Scaling Method for Simulation Parameters

Taking the laboratory parameters from Table 1 and converting them into simulation parameters as listed in Table 3 yields values too large to simulate in a reasonable amount of time. Therefore, it is necessary to find a method of scaling that can shrink this dynamical system down to an equivalent simulation cell.

There are four parameters that characterize the system [9]. These are derived in Appendix A, and are summarized below. These are τ , the time scale of the diffusive reactive system:

$$\tau = \left(\frac{6v}{L_x}\right)^{2/3} \left(\frac{D}{L_y^2}\right)^{1/3} t. \quad (\text{B.1})$$

The Damköhler number Da is the ratio of the rate of ligand binding action at the receptor surface to the rate of transport to that surface:

$$Da = k_+ R_0 \left(\frac{L_x L_y}{6v D^2}\right)^{1/3}. \quad (\text{B.2})$$

D_D is the ratio at which ligands diffuse across the vertical axis of the lattice, to the rate of transport to the receptors:

$$D_D = \frac{C_0}{R_0} \left(\frac{L_x L_y D}{6v}\right)^{1/3}. \quad (\text{B.3})$$

Finally, K represents the equilibrium dissociation constant for the reaction, normalized by the ligand concentration:

$$K = \frac{k_-}{C_0 k_+}. \quad (\text{B.4})$$

Any method of scaling that preserves the dynamics of the system must keep these values unchanged. We may hence scale each of the physical parameters in these four values by a scale parameter α specified such that the values Da , D_D , and K remain fixed:

$$\begin{aligned} L_x &\rightarrow \alpha^{\gamma_x} L_x, & k_+ &\rightarrow \alpha^{\gamma_+} k_+, & v &\rightarrow \alpha^{\gamma_v} v, \\ L_y &\rightarrow \alpha^{\gamma_y} L_y, & k_- &\rightarrow \alpha^{\gamma_-} k_-, & D &\rightarrow \alpha^{\gamma_D} D, \\ C &\rightarrow \alpha^{\gamma_C} C, & R &\rightarrow \alpha^{\gamma_R} R, & t &\rightarrow \alpha^{\gamma_t} t, \end{aligned}$$

where the constant α is a positive real number. We choose the exponents such that

$$\begin{aligned} 0 &= \gamma_t + \frac{1}{3}(\gamma_D + 2\gamma_v - 2\gamma_x - 2\gamma_y), \\ 0 &= \gamma_+ + \gamma_R + \frac{1}{3}(\gamma_x + \gamma_y - \gamma_v - 2\gamma_D), \\ 0 &= \gamma_C - \gamma_R + \frac{1}{3}(\gamma_x + \gamma_y + \gamma_D - \gamma_v), \\ 0 &= \gamma_- - \gamma_C - \gamma_+. \end{aligned} \quad (\text{B.5})$$

The above requirements ensure that none of the four parameters are affected by this scaling. At this point any exponents that satisfy the above requirements can be chosen. For simplicity's sake, the exponents of v , D , and R were chosen to be zero. γ_y and γ_x were chosen to be 1 and 2 respectively. This yields the following definitions

$$\begin{aligned} \gamma_x &= 2, & \gamma_t &= 2, & \gamma_v &= 0, \\ \gamma_y &= 1, & \gamma_+ &= -1, & \gamma_D &= 0, \\ \gamma_C &= -1, & \gamma_- &= -2, & \gamma_R &= 0. \end{aligned} \quad (\text{B.6})$$

Fig. B1 shows the results of simulations of the system described in Table 3 with association rate $k_+ = 10^6 M^{-1} s^{-1}$ and dissociation rate $k_- = 10^{-2} s^{-1}$ scaled with various

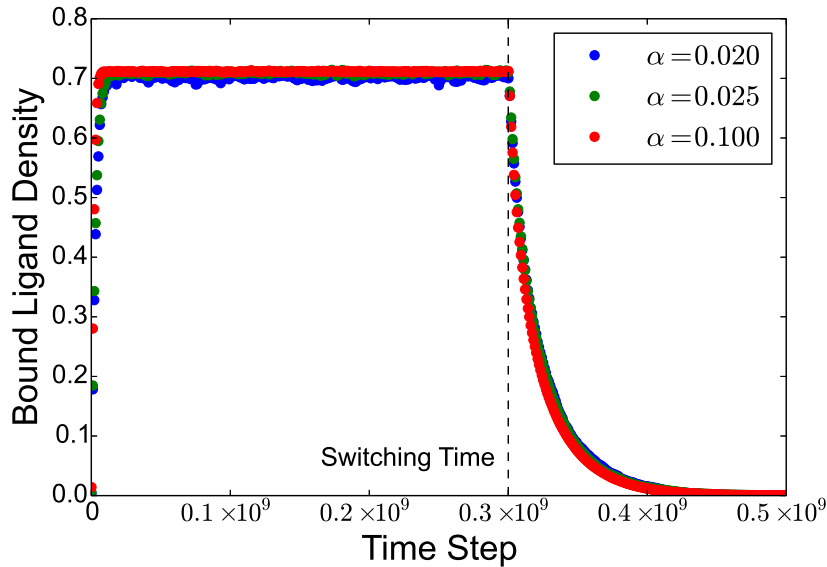


Figure B1. An example of scaling for various values of α . The density of bound ligands is plotted against the unscaled Monte Carlo time step for three realizations of the system described in Table 3 with association rate $k_+ = 10^6 M^{-1} s^{-1}$ and dissociation rate $k_- = 10^{-2} s^{-1}$. The simulations were performed using three different values of the scaling parameter α . Note that changing the values of α by a factor of 5 implies a rescaling of the system length in the x direction and of the overall time scales by a factor of 25. The results of these simulations were unscaled by multiplying by the reciprocal of the scaling factors when needed, and plotted versus the unscaled time steps. The unscaled concentration of ligands is 100nM for each simulation. This concentration is held constant for the duration of the association phase, which lasts until the 0.3×10^9 time step. At this point, marked by the (black) dashed line and labeled as the ‘switching time’, the concentration of incoming ligands is set to zero, to initiate the dissociation phase. The three data sets are represented by the (blue, red, and green) dots, and as expected, each of the three sets of data coincide.

scaling constants α . The range of values of α shown here is actually representative of a whole order of magnitude of values after α has been raised to the appropriate exponents. Note that the coincidence of the differently scaled simulation results confirms the assertion that the results of scaled simulations of the system described in Table 3 will accurately represent the dynamics of the unscaled system.

Appendix C. Algorithm for Monte Carlo Simulation

A summary of the algorithm used for the Monte Carlo simulation is as follows.

- 1) Select a random ligand and generate a random number r uniformly distributed between zero and one.
- 2) If the ligand is not bound to a receptor:
 - a) If $r < p_0$ the ligand remains at the same location.

- b) If instead $r < p_0 + p_x^+$ the ligand is stepped in the positive x direction.
 - i) If the ligand encounters the end of the SPR cell ($x = \widetilde{L}_x$), remove the ligand.
 - ii) If the simulation is in the association phase, introduce a new ligand at the $x = 0$ plane to maintain ligand concentration.
- c) If instead $r < p_0 + p_x^+ + p_x^-$ the ligand is stepped in the negative x direction.
 - i) If the ligand encounters the beginning of the SPR cell ($x = 0$), do not move the ligand.
- d) If instead $r < p_0 + p_x^+ + p_x^- + p_y^+$, step the ligand in the positive y direction. Otherwise if $r < p_0 + p_x^+ + p_x^- + p_y^+ + p_y^-$, step the ligand in the negative y direction.
 - i) If the ligand encounters either the top or bottom planes of the SPR cell (i.e. $y = 0$ or $y = \widetilde{L}_y$), reflect the ligand back one lattice spacing into the lattice to ensure reflective boundary conditions.
- e) If instead $r < p_0 + p_x^+ + p_x^- + p_y^+ + p_z^+$, step the ligand in the positive z direction. Otherwise if $r < p_0 + p_x^+ + p_x^- + p_y^+ + p_y^- + p_z^+ + p_z^-$, step the ligand in the negative z direction.
 - i) If the ligand moves past either of the z axis boundaries of the SPR cell (i.e. $z = 0$ or $z = \widetilde{L}_z$), place the ligand on the opposite boundary to create periodic boundary conditions.
- f) After the ligand is stepped, if it is one lattice site above an empty receptor, generate a random number q evenly distributed between zero and one.
 - i) If $q < \widetilde{k}_+$, bind ligand and receptor, and set ligand position to receptor position.
- 3) If the ligand is bound to a receptor, check if $r < \widetilde{k}_-$. If it is, unbind the ligand.
- 4) Repeat the above process n times every time step, where $n = \widetilde{C}_0 \cdot (\widetilde{L}_x \cdot \widetilde{L}_y \cdot \widetilde{L}_z)$ is the number of ligands in the SPR cell.
- 5) Count the number of bound ligand receptor pairs and divide by the number of ligands in the volume of the SPR cell bounded on the bottom by the receptor surface during the association phase to retrieve the bound ligand density. Record this every time step.
- 6) After a steady-state concentration of bound ligand-receptor pairs has been reached, change from the association stage to the dissociation stage.

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