The Effect of Membrane Receptor Clustering on Spatio-temporal Cell Signalling Dynamics

Bertrand R. Caré[1,3] and Hédi A. Soula[2,3]

[1] Université de Lyon,
Laboratoire d'InfoRmatique en Image et Systèmes d'information, CNRS UMR5205,
F-69621 VILLEURBANNE
bertrand.care@insa-lyon.fr
[2] Université de Lyon,
Cardiovasculaire, Métabolisme, Diabétologie et Nutrition, Inserm UMR1060,
F-69621 VILLEURBANNE
hedi.soula@insa-lyon.fr
[3] EPI BEAGLE INRIA

Abstract. Membrane receptors allow the cell to respond to changes in the composition of its external medium. The ligand-receptor interaction is the core of the signalling process and may be greatly influenced by the spatial configuration of receptors. As growing pieces of evidence suggest that receptors are not homogeneously spread on the cell surface, but tend to form clusters, we propose to investigate the implication of receptor clustering on ligand binding kinetics using a computational individual-based model. The model simulates the activation of receptors distributed in clusters or uniformly spread. The tracking of binding events allows the analysis of the effect of receptor clustering through the autocorrelation of the receptor activation signal and the empirical time distributions of binding events, which are still unreachable with in vitro or in vivo experiments. Results show that the apparent affinity of clustered receptors is decreased. Additionally, receptor occupation becomes spatially and temporally correlated, as clustering creates platforms of coherently activated receptors. Changes in the spatial characteristics of a signalling system at the microscopic scale globally affect its function in time and space.

Keywords: cell signalling, receptor, ligand, clustering, pathway, binding, kinetics, equilibrium, autocorrelation, individual-based model, computational biology.

1 Introduction

In cell signalling, most models describe the ligand as an external stimulus and the receptor as the binding target, based on the ground of chemical enzyme/substrate formalism [1, 2]. Such formulations are based on the law of mass-action, which evaluates local reaction rates from averaged chemical species densities over the medium volume. The law of mass-action is a mean-field approximation since it

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estimates local reaction rates on the basis of average values of the reactants densities over a large spatial domain. In addition, it amounts to assume that ligand-receptor interactions are independent with respect to time and space [3, 4].

In biology, these assumptions can be questioned, in particular when considering membrane receptors which are restricted to only 2 of the 3 spatial dimensions [5, 6]. On the specific case of membrane-restricted receptors (on spherical cells), the expression for reaction rate coefficients is a non-linear function of cell surface receptor density [7]. This pioneer study has been enriched by further works towards reversibility and rebinding, [8], receptor density [9], time dependency [10], and gradient sensing capabilities [11, 12].

Furthermore, the spatial organization of receptors *on the membrane itself* should also be taken into account. At first glance, since membrane receptors are bound to the cell membrane that allows for lateral degrees of freedom, one would expect a simple (and homogeneous) distribution of receptors on the membrane. Indeed, cell membrane is composed of a mixture of phospholipids in a fluid phase and as such, in the classical fluid-mosaic model of membrane [13], membranes components undergo isotropic random movement akin to Brownian motion [14, 15]. In this model, the resulting equilibrium distribution of components – and receptors among them – is therefore homogeneous. Recently, however, this picture has evolved considerably towards a non-homogeneous distribution of the usual components of cell membranes [16, 17, 18, 19, 20]. More and more evidence points towards the existence of micro-domains enriched in various lipids, such as cholesterol, as well as other proteins, such as receptors. In particular, receptor colocalization in lipid rafts and other membrane structures have been reported [21, 22, 23]. This specific localization and clustering may have a dramatic influence on signalling. This influence however remains unclear as literature reports contradictory effects of clustering/declustering on signalling (see e.g. [24, 25, 22]). The method used to disrupt the clusters of receptors may have significant side-effects on the cell signalling system.

On the modelling side, the impact of an inhomogeneous receptor density *on* the membrane itself has been studied only recently. Only few theoretical contributions have been reported in some specific cases : bacteria sensitivity[26] and chemotaxis [27], G-protein activation [28], simple model of trans-phosphorylation (implying two receptors only) [29]. In addition, several more detailed studies illustrate the possible effect of receptor clustering on receptor binding by inducing enhanced rebinding and ligand receptor switching [30, 31, 32, 33], or enhancing encounter probability of activated receptors with submembranar signalling proteins such as in GPCR signalling pathways [34]. Notably [32] proposes that clustering provides higher rebinding capabilities and therefore helps to obtain a better response – i.e. more binding events. However, another analysis [8] proposes that the forward rate constant is diminished when receptors are clustered, providing in that case less binding events. Both effects counteract themselves, and the final output remains to be studied.

In a previous paper, we investigated how receptor distribution may impact the primordial step of signalling that is ligand binding to receptor extracellular

domain [35]. We showed that in the case of a diffusion-limited reaction, receptor clustering impairs the sensitivity of the signalling system. While conserving the microscopic binding properties, the apparent affinity of a receptor for its ligand diminishes with clustering. We showed that this effect is based on spatial features and is diffusion-dependent. In the limit of high diffusion this impairment vanishes, whereas low diffusion amplifies it.

We present in this article a detailed study on how this effect takes place in terms of binding. Intuitively two effects are in action. Clustered receptors are "harder to find", as it diminishes their probability to be found by ligand molecules. In the other hand, when receptors are clustered, they are more likely to be found by a ligand that has been released by another nearby receptor. In other words, more rebinding events are expected in the clustered case. Obviously these two effects counter themselves and the outcome is not intuitively clear. Additionnaly, we show in this article several properties of the binding kinetics of receptors depending on their spatial configuration. Especially, we investigated not only how clustering affects the global amount of activation resulting from ligand stimulation, but also how the temporal dynamics of receptor activation changes with clustering, which translates a spatial correlation into a temporal one.

2 Models

As already mentioned, mathematical models of binding kinetics generally rely on the law of mass action. In the case of a correlated receptor spatial configuration, this hypothesis breaks down. In order to investigate this issue, we developped a simulation engine where the spatial characteristics of real signalling systems arises naturally by using an individual-based model. This simulation engine is defined and described in detail in another article [35] that we briefly describe here as well. The engine computes the equation of movement of punctual particles in a 2-dimension space with cylindric boundary conditions on the x-axis, and closed boundary on the y-axis, the membrane being at $y = 0$. This space is used to describe the extracellular medium. The membrane is the bottom line of the 2-dimension space. Receptors are positioned on the membrane and do not move during simulation, assuming that receptor diffusion is negligible compared to ligand diffusion. Ligand molecules are punctual particles which undergo a classical 2-dimension Brownian motion in the extracellular space. As mentioned above, motion is forbidden beneath the membrane or through the upper part of the simulation space. However, particles going through one lateral boundary appear across the other. Ligand molecules undergo Brownian motion in the overdamped regime via an explicit Euler scheme of step dt :

$$x(t + dt) = x(t) + \sqrt{Ddt}Z_1$$
$$y(t + dt) = y(t) + \sqrt{Ddt}Z_2$$

with D being the simulated ligand diffusion coefficient, and $Z_{1,2}$ are random values drawn from a normalized Gaussian variate. Binding can occur whenever

a ligand molecule is in the 'affinity zone' of a unoccupied receptor – a fixed square above the position of the receptor. If the receptor is free – not already bound to a ligand – binding can occur with a given probability p_1. Finally, an already bound ligand molecule can be released at the border of the affinity zone with a probability p_1 at each time step.

We studied two kinds of receptor spatial configurations in these simulations. The first is a reference – control – receptor distribution, in which they are uniformly spread on the 1-dimension membrane – referred hereafter as to the homogeneous distribution, or unclustered receptors case. The clustered case is obtained by positioning receptors next to each other – with adjacent but non-overlapping affinity zones – by groups of n. These clusters are then uniformly spaced. Most simulations will then compare several cluster sizes (various n) to the control. Note that the control case describes this reaction :

$$L + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \tag{1}$$

and [35] showed that we can relate reaction rates to the binding/unbinding probabilities via :

$$k_{-1} = p_{-1}$$
$$k_1 = \frac{p_1 S_r}{S_t}$$

with S_r being the area of the affinity zone and S_t the total area of the extracellular medium.

3 Results

Unless stated otherwise, the number of receptors for each simulation run was $N_r = 500$, the number of ligand molecules $N_l = 4.10^5$, $k_1 = k_{-1} = 10.0$, $dt = 10^{-3}$ giving $p_1 = p_{-1} = 10^{-2}$. The surface of each affinity zone was $S_r = 0.4$ and the total medium surface $S_t = 2.10^5$. The ligand diffusion coefficient was $D = 1.0$. The cluster size is noted n, $n = 1$ referring to the case of homogeneously spread receptors.

3.1 Transient Phase

Our previous results only dealt with receptor occupation at equilibrium, i.e. the average number of ligand-receptor complexes after the simulation reached a stationay state. The transient solution should yield the same result : clustering decreases the overall responses. As shown in Fig. 1 the fraction of occupied receptors through time was also cluster-dependent. The figures show a similar initial activation rise. Indeed, initially, ligand molecules were positioned uniformly, and since the global surface covered by receptor affinity zones was unchanged by clustering, the initial probability for a ligand to be in an unoccupied receptor

was equal no matter the cluster size. Quickly afterwards though, binding events began to decline steadily whenever receptor were clustered. This shows that the actual binding history for ligand molecules in the vicinity of receptor must be taken into account in order to understand this shift in complexation.

3.2 Binding Events Analysis

The occurence of specific events was tracked during simulation runs. The simulation yielded simultaneously the number of binding events and the number of ligand-receptor encounter events that took place at each time step. Binding events fell into two categories: the first binding events and the rebinding events. The former refers to ligand molecules binding to a receptor for the first time, from the ligand point of view. The latter refers to ligand molecules binding to a receptor for at least the second time, from the ligand point of view.

The relative contribution of binding events of each kind versus cluster size is reported on Fig. 2. In order to avoid any bias due to the decreasing in receptor occupation with clustering, the number of events were normalized on the total number of binding events recorded. As clustering increases, the contribution of first binding events dropped dramatically, while the amount of receptor activation due to rebinding increased. First binding events occured less often if receptors were clustered, but clustering was favorable to rebinding. This suggests that most of the receptor activation was performed by a small contingent of ligand that kept on rebinding.

By computing the ratio of the number of rebinding events to the number of first binding events versus cluster size (see Fig. 3), we obtained the average number of times a ligand molecule rebound to a receptor. As expected this ratio increased with cluster size. By having access to the index of each ligand molecule that generated a binding event, we also obtained the number of unique ligand molecules that had contributed to the total number of binding events. This gives an estimate of the average number of binding events generated by a single ligand molecule according to the cluster size – Fig. 3. Both curves have a similar trend : in the clustered case, receptor activation was induced through constant rebinding by the same set of ligands. Indeed, a high number of unique rebinding indicates a small contingent of ligand molecules involved in the signal. This put a strong emphasis on dependence on the binding history of ligands. On the other hand, in the unclustered case, most binding was performed by 'fresh' ligands newly coming from the medium, whereas rebinding was marginal.

3.3 Ligand Temporal Dynamics

The simulation also provided the time a ligand molecule had to wait between two consecutive binding events. Here, "consecutive" is defined in the ligand molecule referential. Consecutive binding events, that is, rebinding events, were sorted out in two classes : rebinding by a ligand molecule to the same receptor (self-rebinding) and rebinding by a ligand molecule to a different receptor (distinct rebinding). It was thus possible to investigate the qualitative effects of receptor

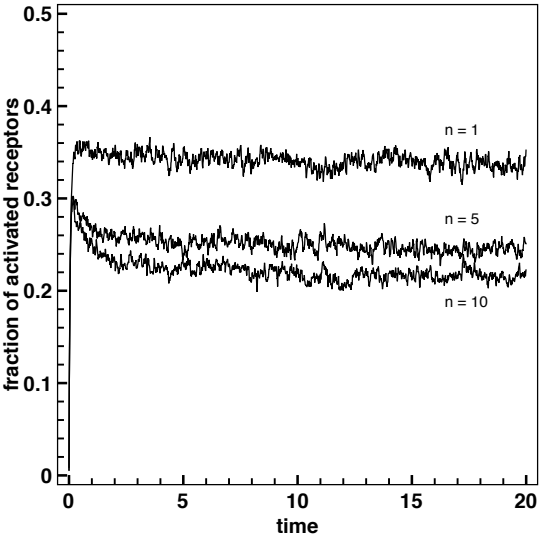

Fig. 1. Fraction of activated receptors versus time for various cluster sizes. The graph shows the signals of receptor activation for a single simulation run with the same parameters except the receptor clusters size. Clustering decreased the receptor activation at equilibrium.

clustering on the temporal dynamics of binding. Fig. 4 shows the mean time between rebinding events sorted in the two types mentioned above, plus the mean time of all rebinding times indifferently, for different cluster sizes. As expected, the time to rebind to another receptor decreased with clustering - since there were other receptors available in the vicinity when they were clustered. In the unclustered case, rebinding to another receptor was a marginal event, as suggested by its longer mean time (one order of magnitude above the others) and its quasi-inexistent influence on the overall rebinding time. Additionally, we noted that the self rebinding time also decreased with clustering, making the self-rebinding more frequent in the clustered case. This could be explained by the fact that, in the unclustered case, a bound receptor could be readily reoccupied by a new ligand molecule. We also had access to inter and intra-cluster rebinding times. Inter-cluster rebinding refers to rebinding of a ligand molecule to a receptor belonging to another cluster, unlike intra-cluster rebinding where rebinding occur to a receptor of the same cluster. Simply put, in the clustered case, there were no rebinding events (during simulation time) between clusters. All rebinding occured within the same cluster. As for the unclustered case, each receptor is a single cluster and we already mentioned that inter-cluster rebinding was extremely marginal.

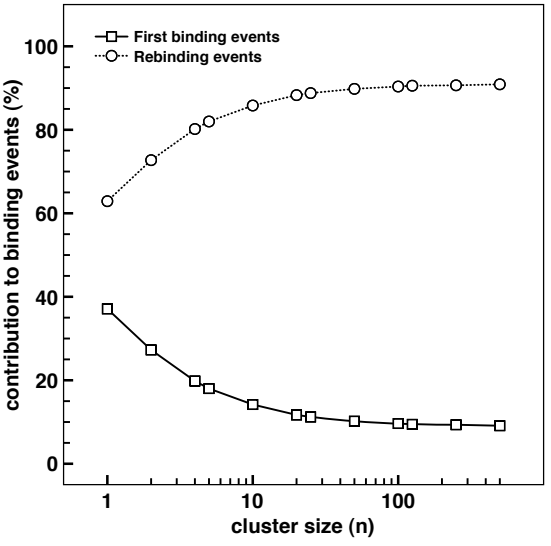

Fig. 2. Relative contribution of binding events from different types to the total binding. Binding events were splitted in two distinct types : the first binding type, i.e. when a ligand molecules bound to a receptor for the first time, and the rebinding type, i.e. when a ligand bound to a receptor and had already been bound in the past to any receptor.

3.4 Receptor Temporal Dynamics

From a receptor point of view, the change in the temporal dynamics of rebinding suggests that the spatial correlation of positions should induce a temporal correlation of activation. In order to investigate this coupling, the activation signal of each receptor was tracked for each time step in the form of a binary signal (0 : free, 1 : occupied by ligand). This signal was then averaged for 10 neighboring receptors. For all n, it simply means we sorted by groups of the 10 closest receptors. The autocorrelations of such signals were computed and are compared in Fig. 5 (dashed lines) with the autocorrelation of a spatially uncorrelated signal (solid line, $n = 1$). The autocorrelation is the correlation of the signal with itself shifted by a lag. Let $x(t)$ being a signal, we simply computed the following expression, the average being taken over the entire time course :

$$ac(lag) = \langle (x(t) - \bar{x})\,(x(t + lag) - \bar{x}) \rangle$$

The theoretical autocorrelation for binding events was expected to follow an exponential decay. Indeed, the curve for $n = 1$ presented a classical exponential decay. The correlation of the activation signal decreased with time. However, as clustering was introduced, the half-time of this decay increased. This means that the activation state of receptors correlated with their past state for a longer time

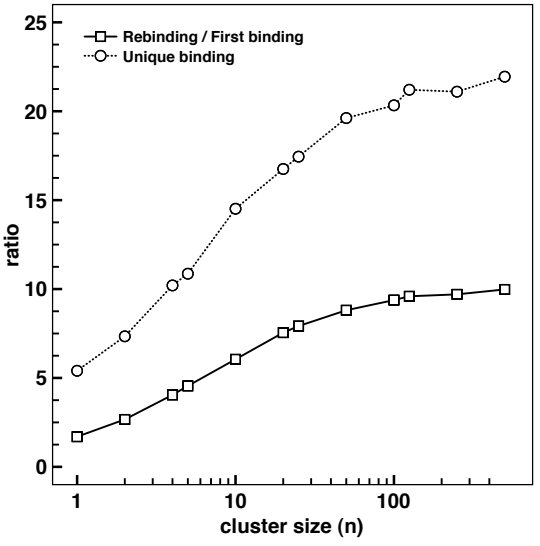

Fig. 3. Squares, solid line : ratio of rebinding events to first binding events (squares, solid line) versus cluster size. A ratio of 5 indicates that, in average, 5 out of 6 binding events occured through rebinding. Circles, dashed line : ratio of the number of individual ligands involved in binding events to the total number of binding events versus cluster size. In this case, a ratio of 5 means that, over the course of the simulation, a unique ligand molecule generated in average 5 binding events on its own (ignoring the ligand molecules that never bound to a receptor).

with clustering than in the unclustered case. The autocorrelation profiles suggest that the temporal correlation of the activation state of adjacent receptors was stronger with clustering.

Clustering introduced a spatial correlation on receptor activation, shown by an increase in rebinding events at the expense of first binding events. Globally, the fraction of activated receptors, at equal ligand stimulation, was decreased, as rebinding did not overcome the loss of encounter events between ligand molecules and receptor. The effect of clustering also appeard on the temporal dynamics of the receptor system, as the activation state of receptors correlated more with its past value. This illustrates how the spatial correlation of receptors translates into a temporal correlation of their binding with the ligand.

4 Discussion

A computational model was used to recreate ligand-receptor binding under specific spatial configurations similar to the ones observed experimentally. This kind of model allows for a detailed analysis of signalling systems, as each individual binding event can be tracked.

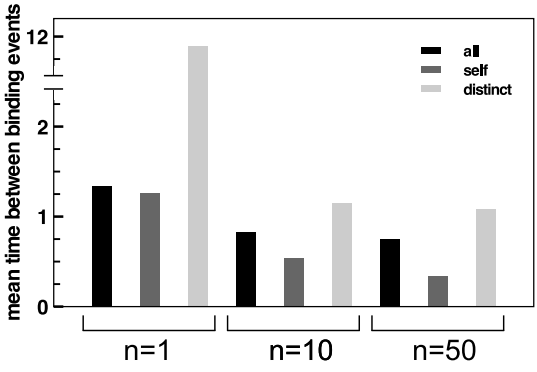

Fig. 4. The time spent by ligand molecules between two consecutive binding events was saved for each molecule during simulations. Theses durations were sorted out in two types : the times between rebinding to a distinct receptor (distinct rebinding) and the times between rebinding to the same receptor (self-rebinding). The mean time between consecutive binding events of such kinds (greyscales) are shown with respect to cluster size n, along with the mean time of consecutive binding when both types are pooled (black).

Receptor clustering seemingly induced a quantitative effect that decreased the global receptor activation by an external ligand. The behavior of the simulated signalling system could be examined in depth. When clustering was imposed to receptors, ligand binding occured more because of ligand molecules rebinding to receptors, at the expense of ligand molecules finding and binding for the first time to a receptor. Not only the crude number of such events was altered in favor of rebinding, the time spent between consecutive binding events also changed. The activation signal of receptors becomes space and time-dependent, showing how a different receptor spatial configuration introduced a shift in the temporal dynamics of the signal transmitted.

This suggests that the peculiar spatial distributions of receptors observed in nature might have a functional role in signalling. This role could possibly be not only quantitative, as the global receptor activation is reduced with clustering, but also qualitative. This study suggests that clustering introduces platforms of aggregated receptors whose activation becomes correlated in time and space, that is, the correlation of receptor position translates into a synchronization of receptor activation. This property is not available in the homogeneous receptor repartition scenario, where receptors are activated randomly in space and time. Making the activation of receptors time and space-dependent could be an advantage in terms of sensitivy, noise reduction and signal robustness. It could also improve signalling-associated cellular processes such as receptor trafficking, recycling, and interaction between parallel pathways. For instance, ligand-induced receptor internalization was observed in different pathways [36, 37], and could be partially relying on harmonization of receptor activation achieved by clustering : activated receptors can be internalized and recycled more efficiently if they

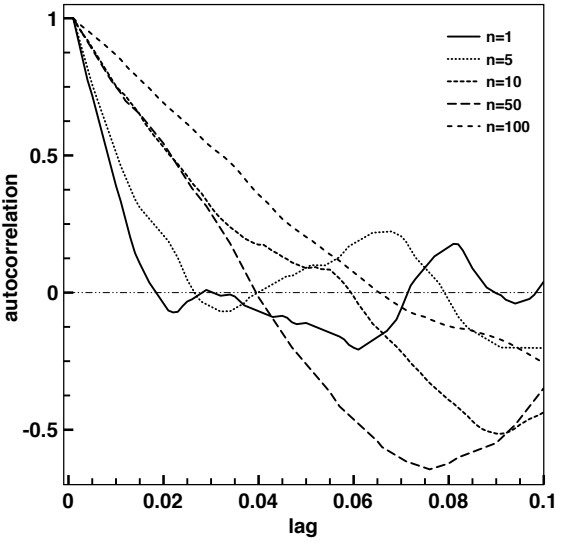

Fig. 5. Autocorrelation functions of the receptor activation signal for various cluster sizes. The binary occupation signal was computed for each receptor and each time step. The average signal of 10 neighboring receptors was used to perform an autocorrelation computation. Autocorrelation functions for clustered receptors show a longer exponential decay, suggesting that the spatial correlation between receptors translated into a temporal correlation.

are already grouped together, rather than spread ramdomly on the cell surface. The question remains to be investigated in studies integrating the spatial and temporal characteristics of such processes, using both modelling and biological experiments.

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