Impact of receptor clustering on the membrane-based stage of a signalling pathway

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Abstract

Individual-based Monte Carlo simulations naturally introduce spatial-based constraints on simulated binding kinetics. As far as the membrane is concerned, these spatial constraints may have an important impact on the signalling cascade. Indeed, several works have shown that membrane receptors distribution is not uniform. Some membrane structures known as domains can contain several copies of a particular receptor. Additionally, the disruption of these structures widely affects the pathway. We propose here to simulate one particular pathway – the first stage of the membrane part of the insulin-dependent glucose uptake cascade. By using a simple mechanism of space-dependent diffusion, we are able to create dynamical receptor clusters. We show that adjusting the diffusion regime can modify drastically the resulting response.
Keywords: signalling, receptor clustering, kinetics, computational biology.

1 Introduction

Cells have the ability to respond to external stimuli by the means of membrane receptors. When activated, the receptor propagates the signal inside the cell by activating internal effectors [1]. Membrane receptors diffuse on the cell membrane which is in fluid-phase [2]. Under such conditions, one would expect a homogeneous receptor spatial distribution on the cell membrane. However, several studies show that receptors spatial distribution is far from uniform [3, 4, 5] for different receptor and cell types [6, 7], and that membrane receptors form clusters.

This spatial configuration of receptors must be taken into account in systems biology approaches. Indeed, all models assume mass-action kinetics, hereby implying a well-stirred medium and space-independent behavior of species. In that case, the spatial characteristics of the system of interest are ignored.

As receptor clustering was studied in different signalling systems, no clear consensus can be extracted regarding its functional impact for cell signalling [8, 9]. In a previous work, our results suggested that, at binding equilibrium, receptor clustering leads to a decrease in the apparent receptor affinity, and thus diminishes cell response at equal stimulation [10].

We propose to go further and study the impact of clustering in a later signalling stage that is restricted in the membrane. For this problem, insulin pathway presents some interesting characteristics that makes it a particulary suitable target. Firstly, insulin, the main hormone enabling the metabolic regulation of glucose, is able to bind to its cognate receptor (IR) which can then phosphorylate tyrosine residues of intracellular signal mediators [11]. The membrane-bound insulin receptor substrate 1 (IRS1) protein is the principal internal effector of insulin-induced cell response [12]. Secondly, insulin receptors are known to be localized in clusters on the membrane – inside structures known as caveolae [13]. When caveolae are disrupted, clusters unfold and IR redistribute themselves uniformly, and the cell response to an insulin stimulus seems significantly affected [14, 15].

In this work, we propose to investigate the effect of receptor clustering on the early internal stage of insulin signalling, that is, the IRS1 activation by IR. A Monte Carlo individual-based computational framework was developed in order to recreate the IR-IRS1 interaction under different IR and IRS1 spatial configurations.

In order to impose such spatial constraints, we chose a diffusion-based mechanism. By introducing a space-dependent diffusion, we are able to create dynamical clusters of either species. This space-dependent process known as non-homogeneous diffusion will be applied selectively to IRS1, allowing the simulation of insulin-induced cell response under experimentally relevant spatial configurations: an homogeneous distribution of IRS1 in the membrane or a colocalized with IR distribution of IRS1.

2 Model

The exact mechanism leading to receptors clustering remains unclear. Several models have accounted for a non-homogeneous spread of membrane molecules [16, 17]. In essence, most models include a specific static zone where the diffusion of species is constrained (see e.g [18]). One the simplest, yet non readily explored, is a non-homogeneous space-dependent diffusion as we will describe below.

2.1 Non-Homogeneous Diffusion

Throughout this paper, simulations will be perform on a lattice where particles (both IR and IRS1) will undergo a simple 2d random walk using toric boundary conditions. In addition, in order to be able to simulate various diffusion constants, we added a probability p to stay in place. A particle at position (x, y) at time t will have:

$$\begin{cases} x+1, y & \text{at} & t+1 & \text{with probability} & (1-p)/4 \\ x, y+1 & \text{at} & t+1 & \text{with probability} & (1-p)/4 \\ x-1, y & \text{at} & t+1 & \text{with probability} & (1-p)/4 \\ x, y-1 & \text{at} & t+1 & \text{with probability} & (1-p)/4 \\ x, y & \text{at} & t+1 & \text{with probability} & p \end{cases}$$

Basically, each particle has a probability $(1-p)/4$ to jump on an adjacent lattice cell at each time step. One can easily show that the resulting movement will be a classical diffusion process with $D = 1-p$. If we hypothesize that membrane diffusion is not constant, that is $p(x, y)$ is a non-constant function of the position, we will obtain a simple particle clustering process. Indeed, let us assume – in the 1d case for the sake of simplicity – a constant-by-part dependence of the diffusion coefficient $D(x) = D_1,\ \forall x \in [a, b]$ and $D(x) = D_2$ outside $[a, b]$ (where $[a, b]$ is a more viscous zone in the membrane – $D_1 < D_2$). Considering a single molecule, its probability $\pi(x, t)$ to be located at position x at time t is:

$$\begin{aligned} \pi(x, t+\delta t) = \quad & p(x)\pi(x, t) \\ + \quad & \pi(x-\delta x, t)\left(1-p(x-\delta x)\right)/2 \\ + \quad & \pi(x+\delta x, t)\left(1-p(x+\delta x)\right)/2 \end{aligned}$$

where $p(x)$ is our probability to stay in place at each time step and is defined, using the jump probability $q(x) = 2\delta t/(\delta x)^2 D(x)$ above, as $p(x) = 1-q(x)$. Noting $g(x, t) = (1-p(x))\pi(x, t)/2$ and developing $g(x \pm \delta x, t)$ in series of x, one obtains at order 2:

$$\begin{aligned} \pi(x, t+\delta t) \quad & = p(x)\pi(x, t) + 2g(x) + (\delta x)^2 \partial_{xx} g(x) \\ & = \pi(x, t) + (\delta x)^2 \partial_{xx} g(x, t) \end{aligned} \tag{1}$$

Dividing by δt, taking the limit $\delta t \to 0$, and setting $\delta t/(\delta x)^2 = 1$, one gets:

$$\partial_t \pi(x, t) = \partial_{xx}\left(D(x)\pi(x, t)\right) \tag{2}$$

where we used the expression of $p(x)$ above to define $D(x)$. Noting $u(x, \infty)$ the density of molecules at x at equilibrium, one expects from eq.(2) $D(x)u(x, \infty) = A$, where A is a constant. Now, using the constant-by-part function for $D(x)$ expressed above, this yields $u(x, \infty) = A/D_1\ \forall x \in [a, b]$ and $u(x, \infty) = A/D_2$ outside. The equilibrium concentration inside the $[a, b]$ patch equals the one outside the patch times the ratio D_2/D_1. Hence the larger the slowdown of the Brownian diffusion inside the patch, the larger the accumulation inside it at equilibrium.

This mechanism will serve as a simple mean to obtain dynamical clusters. We will therefore make the assumption that the stability of such diffusion gradients will be greater than the typical equilibrium time constants of all the reactions described below.

2.2 Spatial simulation of Insulin pathway

In order to test the impact of receptor clustering at the membrane level, we will consider the very first steps of the insulin signalling pathway.

$$IR \underset{a_{-1}}{\overset{a_1}{\rightleftharpoons}} IR^\star \tag{3}$$

$$IRS1 + IR^\star \xrightarrow{k_1} IR^\star + IRS1^\star \tag{4}$$

$$IRS1^\star \xrightarrow{m_1} IRS \tag{5}$$

where $IRS1$ is the non-phosphorylated form of IRS1 molecule and $IRS1^\star$ its phosphorylated form. The phosphorylation of IRS1 is induced by a phosphorylated insulin receptor $IR^\star$ whereas IR is its non-phosphorylated form.

In this simple model, receptor activation/deactivation is simulated using constant rates: a_1 and a_{-1} respectively. We do not explicitely model insulin ligand particles. Receptor activation is done every time step with probability a_1 when a receptor is not activated and a_{-1} in the other case (see Eq.3). Similarly, all phosphorylated IRS1 will have a probability m_1 to spontaneously dephosphorylate itself at each time step (Eq.5). The reaction itself in Eq.4 will occur with probability k_1 for each unphosphorylated IRS1 particle that resides on the same lattice cell as an activated IR.

Receptors will undergo a Brownian motion on the membrane using the space-dependent diffusion $D(x, y)$ as described in the section above. In all simulations, diffusion will be 1 everywhere except on the domains where diffusion will be lower D_s. These domains will

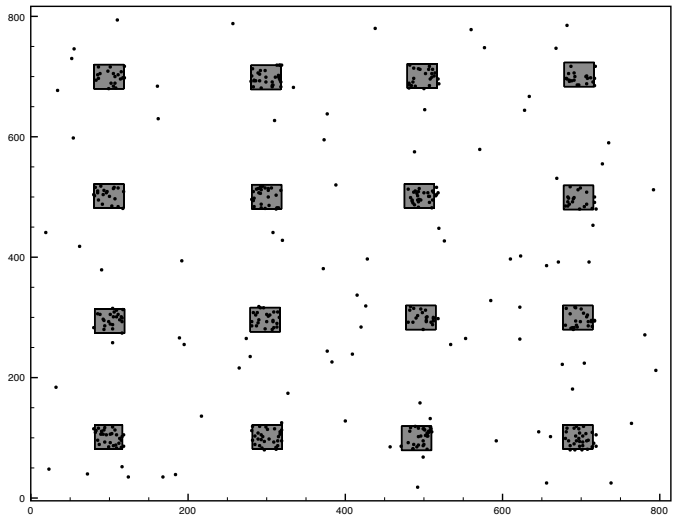

Figure 1: Receptor map at equilibrium. Black dots are receptors positions while grey squares are zones of slow diffusion $D_s < 1$. Parameters: $n = 2^4$, $D_s = 10^-2$ and total time is $T = 10^6$ steps.

be n squares (with $n = 1, 2^2, 2^4, 2^8$) positioned on an evenly spaced grid and whose sizes are such that the covered space is constant and equal to λS with S being the whole surface.

As such, a situation where $n = 1$ is a large slow patch which will accumulate all the particles and will describe an extremely clustered case for the receptors. Concerning the diffusion of IRS1 we will study two scenarios: one where IRS1 diffusion is not altered by the domain - $D_{irs} = 1$ everywhere on the lattice will be called $HD - IRS1$. In the second scenario, the IRS1 diffusion function will be equal to the IR diffusion one – $NHD - IRS1$. In the first scenario, the equilibrium distribution of IRS1 will cover homogeneously the whole membrane, whereas in the second scenario IR and IRS1 equilibrium distribution will coincide.

3 Results

Several situations were studied. At first, we can manipulate the equilibrium numbers of $IR^\star$ simulating various insulin stimulation. This yields dose-response functions of phosphorylated IRS1 versus stimulation. This is the obvious biological effect at this stage of the pathway. In all simulations the number of receptors (of any form) on the membrane will be $N_R = 500$ and the total IRS1 (of any form) will be $N_I = 10,000$. The initial distribution of both species is uniform on the membrane which is a 800×800 grid.

We tested several values of $D_s \in \{1, 10^{-1}, 10^{-2}, 10^{-3}\}$. Note that $D_s = 1$ stands as the control situation where all particles undergo the same Brownian motion. Simulations were performed on 10^6 time steps to ensure equilibrium.

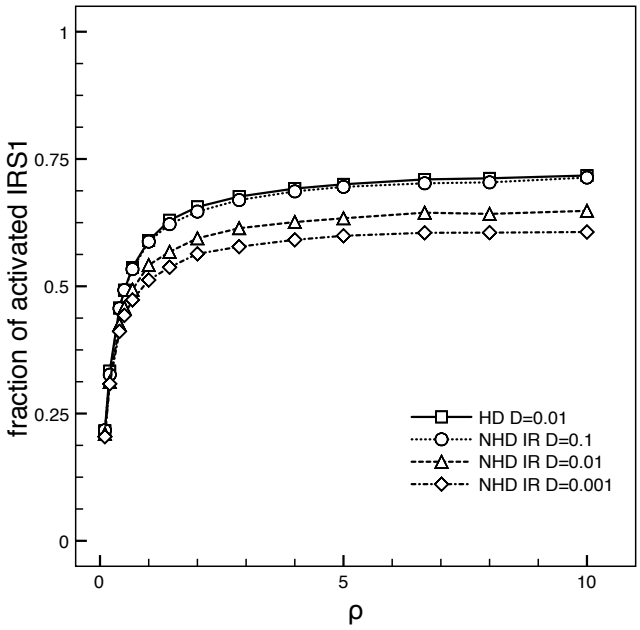

Figure 2: Dose-response of HD-IRS1: number of $IRS1^\star$ versus stimulation (ratio $\rho = a_1/a_{-1}$) for various diffusion coefficients in the slow zone $D_s = 1$ (□), $D_s = 10^{-1}$ (◯), $D_s = 10^{-2}$ (△) and $D_s = 10^{-3}$ (◇). All curves reach a plateau: a maximum amplification that decreases with the clustering, i.e. with lower D_s. As an indication of clustering, note that the equilibrium map of receptors in Fig. 1 is for $D_s = 10^{-2}$ here. Parameters: $n = 2^4$ and $T = 10^6$. Values are averaged over the last 10^4 time steps and over 3 different runs.

In order to assess the clustering effect of the non-homogeneous diffusion, a map of receptors at the equilibrium of a typical simulation $n = 2^4$ and $D_s = 10^{-2}$ is displayed on Figure 1. Note that this is a screenshot taken at a single time step and that all receptors keep on diffusing.

3.1 IRS1 diffuse homogeneously – HD-IRS1

In this section, IRS1 diffusion is not altered by the domains. We will suppose for simplicity's sake that its diffusion is the same for the phosphorylated form and is equal to the 'fastest' receptor diffusion ($D = 1$). In this case, we can have an insight of the result by noticing that unsphosphorylated IRS1

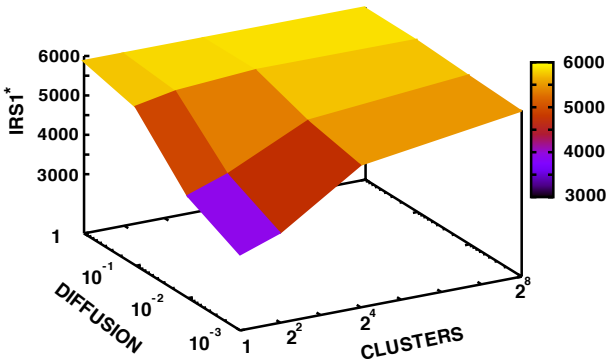

Figure 3: Maximal response for HD-IRS1 for all parameters: $n \in \{1, 2^2, 2^4, 2^8\}$ and $D_s \in \{1, 10^{-1}, 10^{-2}, 10^{-3}\}$. Note that the control cases are either $D = 1$ or $n = 0$ (not shown) and yield identical values ~ 5800. The maximal values were taken using the $\rho = a_1/a_{-1} = 10$ stimulation for the last 10^4 times step and for 3 runs.

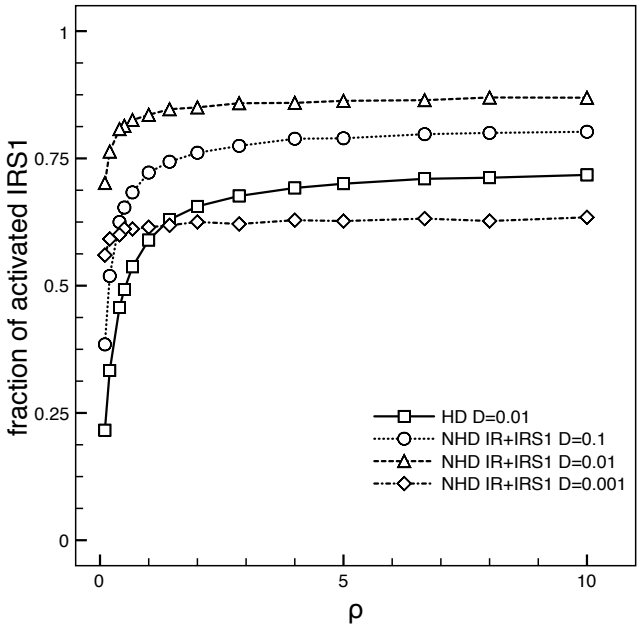

Figure 4: Dose-response of NHD-IRS1: number of $IRS1^\star$ versus stimulation (ratio $\rho = a_1/a_{-1}$) for various diffusion coefficients in the slow domain $D_s = 1$ ($\square$), $D_s = 10^{-1}$ ($\bigcirc$), $D_s = 10^{-2}$ ($\triangle$) and $D_s = 10^{-3}$ ($\diamond$). All curves reach a plateau: a maximum amplification that decreases with the clustering i.e. with lower D_s. As an indication of the clustering, note that the equilibrium map of receptors in Fig. 1 corresponds to $D_s = 10^{-2}$ here. Parameters: $n = 2^4$ and $T = 10^6$. Values are averaged over the last 10^4 time steps and over 3 different runs.

will have a harder time to find heavily clustered receptors. Moreover when a receptor is found and a IRS1 molecule is phosphorylated, the latter will have ample time to return to a receptor-free zone. We can expect this effect to be stronger with clustering: that is with n close to 1 and $D \ll 1$.

We display on Figure 2 the results of such simulations. The dose-response - the number of $IR^\star$ versus the ratio $\rho = a_1/a_{-1}$ – for three different diffusion $D_s \in \{10^{-1}, 10^{-2}, 10^{-3}\}$ for $n = 2^4$. Note that when $D = 10^{-2}$ the receptors clustering is as in Figure 1. As expected, there is a important decrease in the response – the number of phosphorylated IRS1 – versus the stimulation. By decreasing D_s we obtain less loose clusters and therefore less IRS1 activation. We can predict at this stage that this decrease will be sharper with bigger clusters, i.eI with $n = 1$ or $n = 2^2$.

Indeed, by compiling maximal responses for various diffusion values and profiles, one obtains the results on Figure 3. All maximal values are below the control case ($D_s = 1.0$) for all n. The worst case scenario is for the lowest diffusion ($D_s = 10^{-3}$) and the big square ($n = 1$) where $IRS1^\star$ maximal stimulation is almost 50% of the control.

In essence, we showed that deep clustering decreases the biological effect of insulin stimulation on the first phase of amplification. The main hypothesis here is that $IRS1$ are membrane bound and not colocalized with IR. We explore next the scenario where $IRS1$ is colocalized with the insulin receptors.

3.2 IRS1 diffuse non-homogeneously – NHD-IRS1

By submitting $IRS1$ to the same non-homogeneous diffusion mechanism as IR, we expect the opposite effect happening. Indeed, now both species will be colocalized in the same area and the reaction should come easier. However the picture is not as straightforward as it seems. Indeed as Figure 4 shows it, for $n = 2^4$. For $D = 10^{-1}$ and 10^{-2}, there are more $IRS1^\star$ compared to the control (squares). However for $D = 10^{-3}$, the reaction is severely downgraded.

Additionally, clustering also affects the results. Indeed and contrary to the previous scenario, the more there are clusters (higher n), the more there is an effect on the pathway. As displayed on Figure 5, the maxi-

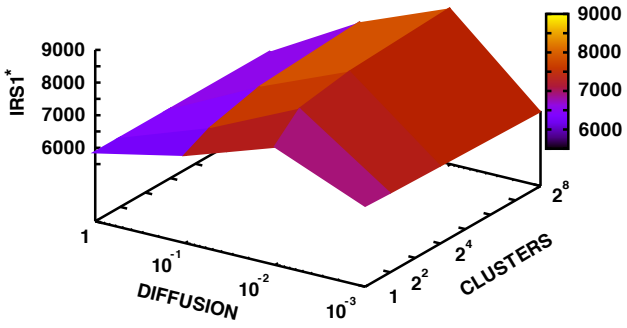

Figure 5: Maximal response as a function of diffusion and clustering in the case of NHD-IRS1: $n \in \{1, 2^2, 2^4, 2^8\}$ and $D_s \in \{1, 10^{-1}, 10^{-2}, 10^{-3}\}$. Note that the control case are either $D = 1$ or $n = 0$ (not shown) and yields identical values ~ 5800. The maximal values were taken using the $\rho = a_1/a_{-1} = 10$ stimulation for the last 10^4 times step and for 3 runs.

mal responses are higher for intermediate diffusion and small, sparse clustering (almost a 50% increase in the maximal response compared to the control). Note that as in the first scenario, controls were made for all diffusion coefficients without clustering and were identical.

4 Conclusion

As growing pieces of evidence suggest that membrane components are clustered into domains, functional properties of this clustering remain elusive. In the case of receptors, we previously showed using a simple individual-based model that ligand binding was hindered because of clustering [10]. In essence, ligand molecules spend more time in receptor-free zones than they would if receptors were spread homogeneously on the membrane.

By introducing a simple mechanism of non-homogeneous diffusion, we are able to simply create clusters of receptors while maintaining diffusion. In addition, this scheme allows us to create identical clusters for any other membrane species. In a signalling pathway such as the insulin one, the next step after receptor activation involves a diffusing membrane species: IRS1. Therefore we needed to consider two scenarios: either IRS1 is clustered with insulin receptor or not. Both cases are simply obtained by hindering the diffusion of IRS1 the same way as IR or not.

When IRS1 diffusion is not hindered and IRS1 position distribution is homogeneous – as this seems to be the case at least in human cells [15] – the effect of clustering is important: the phosphorylation of the insulin receptor substrate IRS1 is dramatically decreased with equal stimulation. This effect is stronger with high, dense clustering. Therefore we can conclude that the pathway is severely impaired by the clustering.

In the second scenario, the non-homogeneous diffusion apply to all species creating co-clustering between IRS1 and IR on the membrane. In that case, we showed that the pathway is upgraded and more phosphorylated IRS1 are available with small, sparse clustering. The effect of diffusion on the results is not monotonic and the effect is stronger with small clustering [18, 19].

Note that we ignored in our simulation an important mechanism pertaining to insulin receptors: transphosphorylation. Receptors can be activated by an already phosphorylated nearby receptor. This hereby can potentially increase the overall phosphorylated receptor pool and even more so in case of clustering. This feature should be added in a future version of the model.

This type of individual-based simulations allows to introduce spatial constraints naturally. We showed that these spatial constraints can drastically modify a simple pathway. Spatial and diffusion constraints will therefore be an important issue in the field of systems biology.

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