A mathematical model for dynamics of CD40 clustering

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Received: 11 April 2013 / Revised: 28 June 2013 / Accepted: 5 July 2013 / Published online: 17 July 2013
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Abstract Ligand bound-receptors in a signalosome complex trigger signals to determine cellular functions. Upon ligand binding, the ligand–receptor complexes form clusters on cell membrane. Guided by the previous experimental reports on the cluster formation of CD40, a trans membrane receptor for CD40-ligand, we built a minimal model of the receptor cluster formation. In this model, we studied co-operative and non-co-operative clustering of a maximum of four CD40 molecules assuming a positive mediator of clustering such as cholesterol to be present in both cases. We observed that co-operative interactions between CD40 molecules resulted in more of the largest CD40 clusters than that observed with the non-co-operatively interacting CD40 molecules. We performed global sensitivity analysis on the model parameters and the analyses suggested that cholesterol influenced only the initial stage of the co-operatively clustering CD40 molecules but it affects both the initial and the final stages in case of the non-co-operatively clustering CD40 molecules. Robustness analyses revealed that in both co-operative and non-co-operative interactions, the higher order clusters beyond a critical size are more robust with respect to alterations in the environmental parameters including the cholesterol. Thus, the role of co-operative and

non-co-operative interactions in environment-influenced receptor clustering is reported for the first time.

Keywords CD40 · Clustering dynamics · Kinetic model for receptor clustering · Cholesterol in receptor clustering · Co-operativity in receptor clustering and robustness

Introduction

Cellular responsiveness to an extracellular stimulus is determined by the signals triggered by the ligand-bound receptors on cell membrane (Stanford and Horton 2001). Once a ligand binds to its receptor, it induces lateral movement of the ligand–receptor complex on a cell membrane (Cherry et al. 1998). This leads to the cluster formation (Caré and Soula 2011). In case of the trans-membrane receptor CD40, it was experimentally observed that environmental factors such as cholesterol present in the cell membrane plays a pivotal role in positively mediating the cluster formation process (Rub et al. 2009). Also study shows that receptor clustering is a dynamic process (Rub et al. 2009).

Because neither the mechanism of each receptor–ligand complex interacting in the lipids on cell membrane nor the nature of interaction between two or more such receptor–ligand complex of the same type is known, we decided to model the interaction between such ligand–receptor complexes. As a model receptor, we used CD40, a trans membrane receptor that plays crucial roles in immune responses (Banchereau et al. 1994). It is possible that a CD40-CD40-ligand complex can interact with another such complex co-operatively or non-co-operatively. In case of co-operative interactions, the stability of the growing cluster of ligand–receptor complex would be expected to

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be more than that observed with the non-co-operative interactions. On the other hand, because the clustering is dependent on the interaction strength between the ligand–receptor complex through membrane lipids and integral membrane proteins, the cholesterol content in the membrane can be another factor in enhancing the clustering of receptors, which was indeed indicated from the experiments as well (Rothberg et al. 1990; Rub et al. 2009). Therefore, in our model, we examined the effect of environmental factors including cholesterol on CD40 clustering when (a) Receptors cluster in non-cooperative way and (b) Receptors cluster in a co-operative way. Our observations lead to a novel mathematical model for receptor clustering where co-operative interactions between receptors lead to more robust and environmentally sensitive phenomenon.

Methodology

CD40 receptors signal from two distinct membrane micro-domain: one deficit in cholesterol and other rich in cholesterol. It was observed that the receptors cluster in the cholesterol rich micro-domain (Raffaella et al. 2006), although not exactly in the context of CD40. Besides, the dynamic characteristics of the *clustered* or *clustering* receptors are not understood. Besides Cholesterol, co-operative effects between receptors could play a role in receptor clustering.

Here we have built a computational model of ligand receptor dynamics and rigorously studied the evolution of clustering process where maximum size of clustered receptors was kept to four. The small cluster size makes it possible to understand the role of various parameters involved in the process of cluster formation. For the current four receptor model, we assume that the receptors are embedded in a cholesterol rich medium close to each other (but not clustered without/before ligand binding) such that the role of lateral diffusion can be ignored for the sake of simplicity. We tested how the interactions between such closely spaced receptors impacts clustering when they interact with each other cooperatively or non-cooperatively.

Below are shown the model chemical reactions used for designing the case study

where R_0 represents the total number of receptors (CD40) in a particular cell out of which R is the number of receptors available for the binding to the applied ligand. L is the ligand. $[LR]$ is the ligand bound receptor which could exist in two different forms $[LR1]$ and $[LR2]$ where $[LR1]$ is the fraction of the receptor that remains in the cholesterol deficit micro-domain of the membrane and $[LR2]$ represents the fraction that is relocated in the cholesterol rich micro-domain of the membrane. $[LR1_a]$ and $[LR2_a]$ are the respective active fractions of the receptor.

The system is simulated as a deterministic system. The reason being it is reported that there are roughly around 5,000–10,000 CD40 receptors per cell (Karmann et al. 1995). Experimental studies from our lab also show that the ligand concentration for which CD40 clustering occurs is very high (6–8 $\mu\text{g/ml}$). So we have assumed the interactions to be deterministic in nature.

Next, a dynamic evolution process is introduced in the model where the $[LR2_a]$ is allowed to bind with another $[LR2_a]$ to form a dimer. The dimers are independently allowed to form tetramers in one step. Alternatively, a two step mechanism is also introduced where dimers combining with isolated receptors form trimers and the resultant trimers when independently combines with an isolated receptor results in a tetramer. Reactions (7)–(10) represent the systems evolution with all the intermediate stages. Degradation of individual receptor types and their intermediate forms is represented through reactions (11)–(17).

The simulations are carried out using the software COPASI (Hoops et al. 2006). Such explicit details of the on and off rates of the ligand receptor binding are not known experimentally for CD40 receptor. However, we choose values which are similar in order of magnitude to other

biochemical studies (Kholodenko et al. 1999; Chen et al. 2009; Yamada et al. 2003; Vilar et al. 2006).

In this case, we do not have any experimental work which reports the size distribution or even average size of clusters of ligand-bound receptors. Of course, time evolution of their sizes is difficult to obtain experimentally but can be well captured by the differential equation based simulations. In this work, we have modeled the ligand receptor interactions and tried to determine indicators which can distinguish between cooperative and non-cooperative cluster formation process.

Model equations

Dynamics of ligand binding stage

$$\frac{d([L] \cdot V_c)}{dt} = -V_c \cdot (k1_{(2)} \cdot [L] \cdot [R] - k2_{(2)} \cdot [LR]) - V_c \cdot (k1_{(9)} \cdot [L])$$

$$\frac{d([LR] \cdot V_c)}{dt} = V_c \cdot (k1_{(2)} \cdot [L] \cdot [R] - k2_{(2)} \cdot [LR]) - V_c \cdot (k1_{(3)} \cdot [L] \cdot [R] - k2_{(3)} \cdot [LR1]) - V_c \cdot (k1_{(4)} \cdot [L] \cdot [R] - k2_{(4)} \cdot [LR2])$$

$$\frac{d([R] \cdot V_c)}{dt} = V_c \cdot (k1_{(1)} \cdot [R_0] - k2_{(1)} \cdot [R]) - V_c \cdot (k1_{(2)} \cdot [L] \cdot [R] - k2_{(2)} \cdot [LR])$$

Dynamics of receptors separated as the non-clustered fraction

$$\frac{d([LR1] \cdot V_c)}{dt} = V_c \cdot (k1_{(3)} \cdot [LR] - k2_{(3)} \cdot [LR1]) - V_c \cdot (k1_{(5)} \cdot [LR1]) + V_c \cdot (k1_{(6)} \cdot [LR1_a] - k2_{(6)} \cdot [LR1])$$

$$\frac{d([LR2] \cdot V_c)}{dt} = V_c \cdot (k1_{(4)} \cdot [LR] - k2_{(4)} \cdot [LR2]) - V_c \cdot (k1_{(8)} \cdot [LR2_a]) - k2_{(9)} \cdot [LR2] - V_c \cdot k1_{(7)} \cdot [LR2]$$

Receptor separated as the clustered fraction

The process of cluster formation is studied considering two possible mechanisms of cluster formation. The first condition is clustering solely as a function of environmental contribution (such as high viscosity mediated resistant in lateral movement of the receptors leading to their gathering) which is model 1 (M1). The second condition is condition in model 1 + an additional cooperative effect imparted by the receptors on each other during the process of clustering, which we describe as model 2 (M2).

Dynamics of clustered fraction in M1

$$\begin{aligned} \frac{d([LR2_a] \cdot V_c)}{dt} = & -2 \cdot V_c \cdot \left(\frac{\left(\frac{vf_{(11)} \cdot [LR2_a]}{kms_{(11)}} - \frac{vr_{(11)} \cdot [LR2_a_2]}{kmp_{(11)}} \right) A_chol_{(11)}}{k11a + A_chol_{(11)} + \left(\frac{[LR2_a]}{kms_{(11)}} + \frac{[LR2_a_2]}{kmp_{(11)}} \right) (k11b + A_chol_{(11)})} \right) \\ & - V_c \cdot \left(\frac{\left(\frac{vf_{(13)} \cdot ([LR2_2] + [LR2_a])}{kms_{(13)}} - \frac{vr_{(13)} \cdot [LR2_a_3]}{kmp_{(13)}} \right) A_chol_{(13)}}{k13a + A_chol_{(13)} + \left(\frac{[LR2_a_2] + [LR2_a]}{kms_{(13)}} + \frac{[LR2_a_3]}{kmp_{(13)}} \right) (k13b + A_chol_{(13)})} \right) \\ & - V_c \cdot \left(\frac{\left(\frac{vf_{(14)} \cdot ([LR2_a_3] + [LR2_a])}{kms_{(14)}} - \frac{vr_{(14)} \cdot [LR2_a_4]}{kmp_{(14)}} \right) A_chol_{(14)}}{k14a + A_chol_{(14)} + \left(\frac{[LR2_a_3] + [LR2_a]}{kms_{(14)}} + \frac{[LR2_a_4]}{kmp_{(14)}} \right) (k14a + A_chol_{(14)})} \right) \\ & - V_c \cdot k1_{(15)} \cdot [LR2_a] + V_c \cdot k1_{(7)} \cdot [LR2] - V_c \cdot (k1_{(8)} \cdot [LR2_a] - k2_{(8)} \cdot [LR2]) \end{aligned}$$

$$\begin{aligned} \frac{d([LR2_a_2] \cdot V_c)}{dt} = & -2 \cdot V_c \cdot \left(\frac{\left(\frac{vf_{(12)} \cdot [LR2_a_2]}{kms_{(12)}} - \frac{vr_{(12)} \cdot [LR2_a_4]}{kmp_{(12)}} \right) A_chol_{(12)}}{k12a_{(12)} + A_chol_{(12)} + \left(\frac{[LR2_a_2]}{kms_{(12)}} + \frac{[LR2_a_4]}{kmp_{(12)}} \right) (k12b_{(12)} + A_chol_{(12)})} \right) \\ & - V_c \cdot \left(\frac{\left(\frac{vf_{(13)} \cdot ([LR2_a_2] + [LR2_a])}{kms_{(13)}} - \frac{vr_{(13)} \cdot [LR2_a_3]}{kmp_{(13)}} \right) A_chol_{(13)}}{k13a + A_chol_{(13)} + \left(\frac{[LR2_a_2] + [LR2_a]}{kms_{(13)}} + \frac{[LR2_a_3]}{kmp_{(13)}} \right) (k13b + A_chol_{(13)})} \right) \\ & + V_c \cdot \left(\frac{\left(\frac{vf_{(11)} \cdot [LR2_a]}{kms_{(11)}} - \frac{vr_{(11)} \cdot [LR2_a_2]}{kmp_{(11)}} \right) A_chol_{(11)}}{k11a + A_chol_{(11)} + \left(\frac{[LR2_a]}{kms_{(11)}} + \frac{[LR2_a_2]}{kmp_{(11)}} \right) (k11b + A_chol_{(11)})} \right) \\ & - V_c \cdot k1_{(16)} \cdot [LR2_a_2] \end{aligned}$$

$$\begin{aligned} \frac{d([LR2_a_4] \cdot V_c)}{dt} = & V_c \cdot \left(\frac{\left(\frac{vf_{(12)} \cdot [LR2_a_2]}{kms_{(12)}} - \frac{vr_{(12)} \cdot [LR2_a_4]}{kmp_{(12)}} \right) A_chol_{(12)}}{k12a + A_chol_{(13)} + \left(\frac{[LR2_a_2]}{kms_{(12)}} + \frac{[LR2_a_4]}{kmp_{(12)}} \right) (k12b + A_chol_{(12)})} \right) \\ & - V_c \cdot \left(\frac{\left(\frac{vf_{(14)} \cdot ([LR2_a_3] + [LR2_a])}{kms_{(14)}} - \frac{vr_{(14)} \cdot [LR2_a_4]}{kmp_{(14)}} \right) A_chol_{(14)}}{k14a + A_chol_{(14)} + \left(\frac{[LR2_a_3] + [LR2_a]}{kms_{(14)}} + \frac{[LR2_a_4]}{kmp_{(14)}} \right) (k14b + A_chol_{(14)})} \right) \\ & - V_c \cdot k1_{(18)} \cdot [LR2_a_4] \end{aligned}$$

$$\begin{aligned} \frac{d([LR2_a_3] \cdot V_c)}{dt} = & V_c \cdot \left(\frac{\left(\frac{vf_{(13)} \cdot ([LR2_a_2] + [LR2_a])}{kms_{(13)}} - \frac{vr_{(13)} \cdot [LR2_a_3]}{kmp_{(13)}} \right) A_chol_{(13)}}{k13a + A_chol_{(13)} + \left(\frac{[LR2_a_2] + [LR2_a]}{kms_{(13)}} + \frac{[LR2_a_3]}{kmp_{(13)}} \right) (k13b + A_chol_{(13)})} \right) \\ & - V_c \cdot \left(\frac{\left(\frac{vf_{(14)} \cdot ([LR2_a_3] + [LR2_a])}{kms_{(14)}} - \frac{vr_{(14)} \cdot [LR2_a_4]}{kmp_{(14)}} \right) A_chol_{(14)}}{k14a + A_chol_{(14)} + \left(\frac{[LR2_a_3] + [LR2_a]}{kms_{(14)}} + \frac{[LR2_a_4]}{kmp_{(14)}} \right) (k14b + A_chol_{(14)})} \right) \\ & - V_c \cdot k1_{(17)} \cdot [LR2_a_3] \end{aligned}$$

Dynamics of clustered fraction of M2

$$\begin{aligned} \frac{d([LR2_a] \cdot V_c)}{dt} = & -2 \cdot V_c \cdot \left(\frac{\left(\frac{vf_{(11)} \cdot [LR2_a]}{kms_{(11)}} - \frac{vr_{(11)} \cdot [LR2_a_2]}{kmp_{(11)}} \right) \left(1 + \frac{b_{(11)} A_chol_{(11)}}{a_{(11)} kd_{(11)}} \right)}{1 + \frac{A_chol_{(11)}}{kd_{(11)}} + \left(\frac{[LR2_a]}{kms_{(11)}} + \frac{[LR2_a_2]}{kmp_{(11)}} \right) \left(1 + \frac{A_chol_{(11)}}{a_{(11)} kd_{(11)}} \right)} \right) \\ & - V_c \cdot \left(\frac{\left(\frac{vf_{(13)} \cdot ([LR2_a_2] + [LR2_a])}{kms_{(13)}} - \frac{vr_{(13)} \cdot [LR2_a_3]}{kmp_{(13)}} \right) \left(1 + \frac{b_{(13)} A_chol_{(13)}}{a_{(13)} kd_{(13)}} \right)}{\left(1 + \frac{A_chol_{(13)}}{kd_{(13)}} + \left(\frac{[LR2_a_2] + [LR2_a]}{kms_{(13)}} + \frac{[LR2_a_3]}{kmp_{(13)}} \right) \left(1 + \frac{A_chol_{(13)}}{a_{(13)} kd_{(13)}} \right) \right)} \right) \\ & - V_c \cdot \left(\frac{\left(\frac{vf_{(14)} \cdot ([LR2_a_3] + [LR2_a])}{kms_{(14)}} - \frac{vr_{(14)} \cdot [LR2_a_4]}{kmp_{(14)}} \right) \left(1 + \frac{A_chol_{(14)}}{a_{(14)} kd_{(14)}} \right)}{\left(1 + \frac{A_chol_{(14)}}{kd_{(14)}} + \left(\frac{[LR2_a_3] + [LR2_a]}{kms_{(14)}} + \frac{[LR2_a_4]}{kmp_{(14)}} \right) \left(1 + \frac{A_chol_{(14)}}{a_{(14)} kd_{(14)}} \right) \right)} \right) \\ & - V_c \cdot k1_{(15)} \cdot [LR2_a] + V_c k1_{(7)} \cdot [LR2] - V_c \cdot (k1_{(8)} \cdot [LR2_a] - k2_{(8)} \cdot [LR2]) \end{aligned}$$

$$\begin{aligned} \frac{d([LR2_a_2] \cdot V_c)}{dt} = & V_c \cdot \left(\frac{\left(\frac{vf_{(11)} \cdot [LR2_a]}{kms_{11(11)}} - \frac{vr_{(11)} \cdot [LR2_a_2]}{kmp_{11(11)}} \right)}{1 + \frac{A_chol_{(11)}}{kd_{(11)}} + \left(\frac{[LR2_a]}{kms_{(11)}} + \frac{[LR2_a_2]}{kmp_{(11)}} \right) \left(1 + \frac{A_chol_{(11)}}{a_{(11)} \cdot kd_{(11)}} \right)} \right) \\ & - 2 \cdot V_c \cdot \left(\frac{\left(\frac{vf_{(12)} \cdot [LR2_a_2]}{kms_{(12)}} - \frac{vr_{(12)} \cdot [LR2_a_4]}{kmp_{(12)}} \right)}{1 + \frac{A_chol_{(12)}}{kd_{(12)}} + \left(\frac{[LR2_a_2]}{kms_{(12)}} + \frac{[LR2_a_4]}{kmp_{(12)}} \right) \left(1 + \frac{A_chol_{(12)}}{a_{(12)} \cdot kd_{(12)}} \right)} \right) \\ & - V_c \cdot \left(\frac{\left(\frac{vf_{(13)} \cdot ([LR2_a_2] + [LR2_a])}{kms_{(13)}} - \frac{vr_{(13)} \cdot [LR2_a_3]}{kmp_{(13)}} \right) \left(1 + \frac{b_{(13)} \cdot A_chol_{(13)}}{a_{(13)} \cdot kd_{(13)}} \right)}{1 + \frac{A_chol_{(13)}}{kd_{(13)}} + \left(\frac{[LR2_a_2] + [LR2_a]}{kms_{(13)}} + \frac{[LR2_a_3]}{kmp_{(13)}} \right) \left(1 + \frac{A_chol_{(13)}}{a_{(13)} \cdot kd_{(13)}} \right)} \right) \\ & - V_c \cdot k1_{(16)} \cdot [LR2_a_2] \end{aligned}$$

$$\begin{aligned} \frac{d([LR2_a_4] \cdot V_c)}{dt} = & V_c \cdot \left(\frac{\left(\frac{vf_{(12)} \cdot [LR2_a_2]}{kms_{(12)}} - \frac{vr_{(12)} \cdot [LR2_a_4]}{kmp_{(12)}} \right) \left(1 + \frac{b_{(12)} \cdot A_chol_{(12)}}{a_{(12)} \cdot kd_{(12)}} \right)}{1 + \frac{A_chol_{(12)}}{kd_{(12)}} + \left(\frac{[LR2_a_2]}{kms_{(12)}} + \frac{[LR2_a_4]}{kmp_{(12)}} \right) \left(1 + \frac{A_chol_{(12)}}{a_{(12)} \cdot kd_{(12)}} \right)} \right) \\ & + V_c \cdot \left(\frac{\left(\frac{vf_{(14)} \cdot ([LR2_a_3] + [LR2_a])}{kms_{(14)}} - \frac{vr_{(14)} \cdot [LR2_a_4]}{kmp_{(14)}} \right) \left(1 + \frac{b_{(14)} \cdot A_chol_{(14)}}{a_{(14)} \cdot kd_{(14)}} \right)}{1 + \frac{A_chol_{(14)}}{kd_{(14)}} + \left(\frac{[LR2_a_3] + [LR2_a]}{kms_{(14)}} + \frac{[LR2_a_4]}{kmp_{(14)}} \right) \left(1 + \frac{A_chol_{(14)}}{a_{(14)} \cdot kd_{(14)}} \right)} \right) \\ & - V_c \cdot k1_{(18)} \cdot [LR2_a_4] \end{aligned}$$

$$\begin{aligned} \frac{d([LR2_a_3] \cdot V_c)}{dt} = & V_c \cdot \left(\frac{\left(\frac{vf_{(13)} \cdot ([LR2_a_2] + [LR2_a])}{kms_{(13)}} - \frac{vr_{(13)} \cdot [LR2_a_4]}{kmp_{(13)}} \right) \left(1 + \frac{b_{(13)} \cdot A_chol_{(13)}}{a_{(13)} \cdot kd_{(13)}} \right)}{1 + \frac{A_chol_{(13)}}{kd_{(13)}} + \left(\frac{[LR2_a_2] + [LR2_a]}{kms_{(13)}} + \frac{[LR2_a_4]}{kmp_{(13)}} \right) \left(1 + \frac{A_chol_{(13)}}{a_{(13)} \cdot kd_{(13)}} \right)} \right) \\ & - V_c \cdot \left(\frac{\left(\frac{vf_{(14)} \cdot ([LR2_a_3] + [LR2_a])}{kms_{(14)}} - \frac{vr_{(14)} \cdot [LR2_a_4]}{kmp_{(14)}} \right) \left(1 + \frac{b_{(14)} \cdot A_chol_{(14)}}{a_{(14)} \cdot kd_{(14)}} \right)}{1 + \frac{A_chol_{(14)}}{kd_{(14)}} + \left(\frac{[LR2_a_3] + [LR2_a]}{kms_{(14)}} + \frac{[LR2_a_4]}{kmp_{(14)}} \right) \left(1 + \frac{A_chol_{(14)}}{a_{(14)} \cdot kd_{(14)}} \right)} \right) \\ & - V_c \cdot k1_{(17)} \cdot [LR2_a_3] \end{aligned}$$

The differential equations capture the time evolution of the system as clustered and non-clustered fraction. In the equations V_c is the volume of the reaction compartment which is assumed as 1 ml for the current case study. In the model M1, A_chol is the contribution from the cholesterol influencing the clustering process positively. In model M2, in addition to A_chol , b and a are the parameters capturing the cooperative effect of the ligand bound receptors when they combine. As mentioned above, a simulator Complex Pathway Simulator (COP-ASI) is used for simulating above equations. We assume 5,000–10,000 receptors (R0) (Karmann et al. 1995) and 500–5,000 ligands (L) in simulation of various conditions. All other quantities are assumed to be zero in beginning. The reaction parameters are as below.

$$\begin{aligned} k1_{(1)} &= k2_{(1)} = k1_{(2)} = k2_{(2)} = k2_{(3)} = k1_{(4)} = k2_{(4)} \\ &= k1_{(6)} = k2_{(6)} = k1_{(8)} = k2_{(8)} = 0.1/s, \\ k1_{(3)} &= 1/s, k1_{(5)} = 0.01/s, k1_{(7)} = 0.001/s, k1_{(9)} \\ &= k1_{(10)} = k1_{(15)} = k1_{(16)} = k1_{(17)} = k1_{(18)} = 0.0001/s, \\ vf_{(11)} &= vf_{(12)} = vf_{(13)} = vf_{(14)} = 1, kms_{(11)} = kms_{(12)} \\ &= kms_{(13)} = kms_{(14)} = kmp_{(11)} = kmp_{(12)} = kmp_{(13)} \\ &= kmp_{(14)} = 50/\text{ml}, vr_{(11)} = vr_{(12)} = vr_{(13)} = vr_{(14)} \\ &= 0.1/(\text{ml s}), a_{(11)} = a_{(12)} = a_{(13)} = a_{(14)} = 10, b_{(11)} \\ &= 500, b_{(12)} = b_{(13)} = b_{(14)} = 10, A_chol_{(11)} = 10, \\ A_chol_{(12)} &= A_chol_{(13)} = A_chol_{(14)} = 5, kd_{(11)} \\ &= kd_{(12)} = kd_{(13)} = kd_{(14)} = 500, k11a = k12a \\ &= k13a = k14a = k11b = k12b = k13b = k14b = 100. \end{aligned}$$

Sensitivity analysis of the system

Local sensitivity analysis

Mathematically, the sensitivity coefficients are the first order derivatives of model outputs with respect to the model parameters; $S_{ij} = \frac{\partial O_i}{\partial p_j}$, where O_i is the i th model output and p_j is the j th model parameter. This calculation yields unnormalized sensitivity of O_i w. r. t perturbation in parameter p_j . We have used the sensitivity analysis toolbox SBML_SAT which calculates these sensitivity coefficients with the centered difference assumption which is expressed as:

$$s_{ij} = \frac{O_i(p_j + \delta p_j) - O_i(p_j - \delta p_j)}{2\delta p_j}$$

Upon normalization the sensitivity coefficient S_{ij} is given as [43]:

$$s_{ij}^{normalized} = \frac{S_{ij}}{O_i/P_j}$$

The perturbation coefficient δp_j in the above equations is considered as $\delta p_j = 0.001 * p_j$ for any of the model parameter p_j . Variation of δp_j in the range $0.0001p_j - 0.01p_j$ didn't alter the results of local sensitivity analysis (data not shown). During the sensitivity analysis, each parameter is subjected to perturbations individually keeping all the other parameters constant. Their respective effect in the outputs (the clustered or un-clustered receptor fractions) was measured and the sensitivity coefficients were calculated simultaneously.

Global multi-parametric sensitivity analysis

Multi-parametric sensitivity is a tool that could be used to quantitatively characterize the relative importance of the model variable in determining the fate of the system outputs (Choi et al. 2000). The goal here is to subject uncertainty in the model variable by randomly selecting parameter values from a probability distribution rather than using fixed values.

First the set of model parameters subjected to uncertainty were sampled using Latin Hypercube sampling method (McKay et al. 1979) which divides the given range of parameter value in n equidistant points. We have chosen the parameter range to be $0.01p_j - 100p_j$, where p_j is the j th model parameter subject to change. For all the simulations number of sampling points was chosen to be 5,000. Hence, for example in the model M1 when the parameters were subjected to perturbations, the sampling points were chosen from hypercube of the dimension equal to 48 (number of parameters) dimensions and each dimension

has 5,000 equidistant sampling points in the range $0.01p_j - 100p_j$. Difference of a model output in normal and perturbed state is calculated using an objective function and the results are categorized as acceptable or unacceptable based on a given threshold. Finally, based on the results of the objective functions sensitivity of model output were calculated using Kolmogorov–Smirnov statistics.

We have investigated the dynamics sensitivity, i.e. the above sensitivity analysis was performed during the evolution of the system where we tested the sensitivity at regular predefined intervals. Here for a simulation of 10,000 s (arbitrary), 5,000 equidistant sampling points were considered where sensitivity of the system was evaluated.

Integrated response of the output sensitivity

The integrated response of the model output (O_i) is the total amount of the O_i during the time course of simulation when the sensitivity analysis was performed. If O_i is plotted with respect to time, it corresponds to the area under the curve. The integrated response for state variable O_i is given by

$$I_i = \int_{t=t_0}^{t_{end}} [O_i] \cdot dt$$

This expression is evaluated approximately by the discrete summation using trapezoid rule:

$$I_i = \int_{t=t_0}^{t_{end}} [O_i] \cdot dt = \frac{\Delta t}{2} (O_i(t_0) + 2O_i(t_1) + \dots + 2O_i(t_{n-1}) + O_i(t_n))$$

Results and discussion

Receptors spanning the cell membrane are CD40 receptors signal from two distinct membrane micro-domain: one deficient in cholesterol and other rich in cholesterol. It was observed that the receptors cluster in the cholesterol rich micro-domain, although the dynamics of the clustered or clustering receptors are not understood. We investigated the process of cluster formation in presence and in absence of cooperative effect between the ligands. Hence we built two computational models which exposed the difference between two systems of receptors: one without inter-receptor cooperative effect (M1) and the other with cooperative effect (M2) among the receptors. In both the models environmental factors (cholesterol) were considered to be equal.

Figure 1 shows the dynamics of the system with un-clustered fraction (LR1) and the clustered fraction (LR2) of the receptor. Figure 1a shows the activation of LR1 where

LR1 quickly reaches its peak in synchrony with LR. Later due to degradation, which is a slow process, the total LR1a decreases continuously until it is completely eliminated (Fig. 1a). Figure 1b, c shows the various forms in which LR2 could exist during its evolution to form a tetramer. Figure 1b represents the evolution of the system for M1 and Fig. 1c represents the same for M2. It could be noticed that the system where cluster formation is function of both environmental contribution and inter-receptor cooperative effect, the number of receptors with largest cluster size comprises the major fraction of ligand bound receptor (Fig. 1c). Also when the cluster formation is coupled to the environmental contribution and cooperative effect, the intermediate steps (such as dimer formation) are significantly suppressed as compared to the condition when only environmental factors trigger cluster formation (Compare Fig. 1b, c). Biologically, it suggests that cooperative effect between the receptors themselves (together with environmental factors) would result in large size and small numbered clusters. However, if cooperative effect is not present among the receptors, clusters of all sizes could be observed in the system.

A clustering system with receptor cooperatively exposed to spectrum of ligand doses

The figures expose interesting fact about the dynamics of the system undergoing clustering. Firstly, with the increase in signal dose, the unclustered fraction decreases and clustered fraction increases (Fig. 2a) this is also an experimentally observed fact (Rub et al. 2009). In addition, the simulations also suggest that for each value (concentration) of unclustered receptor fraction (LR1_a, X axis of Fig. 2b), there can exist

two values of the clustered fraction with highest receptor number (LR2_a_4, Y axis of Fig. 2b). As the signal dose increases, the maximum differences in its values that LR2_a_4 can assume for a given value of LR1_a also increase.

Global dynamic sensitivity analysis of the clustered and unclustered fraction

The un-clustered receptor fraction shows a uniform response to the variation of parameters where each line in the figures corresponds to the response to the variation of a particular parameter during the time-evolution (Fig. 3a, b). It could be clearly noticed that the clustered fraction shows similar sensitivity profile as compared to its un-clustered counterpart till the time point 600, but undergoes a phase transition immediately and shows a remarkably different (almost steady) sensitivity profile in the later time points (Fig. 4b). We suspect an underlying switching mechanism for such behavior, possibly due to the cooperative effect between the receptors, but it needs deeper mathematical investigations to understand the causality behind such emergent behavior.

Sensitivity of clustering process on the environmental factors

Evaluation of the contribution of environmental effect parameter (A_{chol}) on clustering in both M1 and M2 through global sensitivity analysis

Value of A_{chol} (shown as $A_{re_11, 12, 13, 14}$ in Fig. 4) is varied in the range $0.01 * p_j - 100 * p_j$ of its current value and the sensitivity of integrated response was calculated.

Fig. 1 Dynamics of evolution of the clustered and un-clustered fraction of the receptor. The unclustered fraction exhibit similar profile of activation as the [LR] early complex (a), whereas the clustered fraction in both M1 (b) and M2 (c) exhibit prolonged activation. In the figure LR = ligand receptor bound complex, LR1 = unclustered receptor fraction, LR1_a = Active receptor fraction taking part in the cluster formation processes, LR2 = inactive dimer, LR2_a_2 = active dimer fraction, LR2_a_3 = active trimer fraction, LR2_a_4 = active tetramer fraction

Fig. 2 Ligand dependent activation of un-clustered and clustered fraction of the receptor and their phase plot. Data shown for M2 where *arrow* near the “Ligand” shows the direction of ligand dose increment (**a**, **b**). LR1_a and LR2_a_4 are the un-clustered and clustered fraction of the receptors respectively

Results provide interesting information regarding the sensitivity of cluster formation to perturbation in the environmental condition as implemented and tested through M1 and M2. It could be noted that in M1, where clustering is solely a function of A_chol the largest size of the cluster (LR2_a_4) is most sensitive to change in the A_chol value of last reaction but is extremely robust against change in the A_chol value of all the intermediate processes (Fig. 4a). For the identical set of analysis model M2 is sensitive to the A_chol value of the first step only but all the subsequent reactions are robust to the change in their A_chol value (Fig. 4b). The model thus suggest, if a biological system with inter-receptor cooperatively is targeted to be perturbed (inhibited or activated), first step of cluster formation would be maximally effective for the treatment.

Robustness analysis

Robustness of the model output was calculated using the simulation software SBML-SAT. Following the previous studies, here the output robustness is calculated against the total parameter variation (TPV) where TPV represents the set of parameters that are subjected to random variations.

TPV is given as,

$$TPV = \sum_{n=1}^L \log \left(\frac{k_n}{k_{n0}} \right),$$

k_n = randomly generated perturbed model parameter value and k_{n0} is the corresponding model parameter value in the unperturbed system. L is the total number of parameters subjected to variation.

Robustness quantifies the change in the output characteristics of a model induced by the TPV and represented as

$$R_{f,TPV}^M = - \frac{\sum_{p=1}^N \left(\frac{f_p}{f_0} \right)}{N}$$

where $R_{f,TPV}^M$ is the robustness coefficient of the output f for a model “M” where f_0 and f_p are the output under the unperturbed and perturbed conditions respectively and N is total number of parameters varied.

Thus in the model, for the ligand receptor complexes with maximum bound receptor size of four (largest cluster size), robustness coefficient was measured as:

$$R_{LR2_a_4,TPV}^M = - \frac{\sum_{p=1}^N \left(\frac{LR2_a_4_p}{LR2_a_4_0} \right)}{N},$$

Robustness by its definition is a negative quantity implying that value of robustness coefficient closer to zero corresponds to more robust systems.

The models had parameters and concentration values in the biologically observed range. For robustness calculations, the perturbed parameters were sampled in the range of 0.1–10 times their reference values (reference values are the values used for the simulations in the unperturbed

Fig. 3 Multi-parametric dynamic sensitivity analysis of the evolution of un-clustered and clustered receptor fraction. The lines, 48 in each plot, correspond to the respective model parameters, where **a** shows the systems evolution as un-clustered fraction and **b** shows the systems evolution as a clustered fraction

Fig. 4 Multi-parametric sensitivity of integrated response is plotted as a function of value of A_{chol} in reactions 11, 12, 13 and 14 for both models

system) and the sample parameter sets with their sampling ranges are given in additional table 3. The parameters were sampled using Latin Hypercube sampling (LHS) where

2,000 equidistant samples from the minimum to the maximum (0.1–10 times the reference value) were drawn, for each parameter subjected to the perturbations. The

Fig. 5 Different quantities are plotted for various realizations of parameter variations in M1

Fig. 6 Different quantities are plotted for various realizations of parameter variations in M2

perturbations were applied globally, where a set of values of all the perturbed parameters were randomly chosen from the sample space for one simulation and for the next simulation another random set of parameters were picked from the sample space. The robustness coefficient was calculated

as an averaged quantity, obtained from 5,000 simulations for each individual case study.

The results show (Figs. 5, 6) that with increase in cluster size the robustness of the individual clusters decreases till the cluster size of 3 receptors, beyond which the robustness

increases again which is shown for cluster size of four. As we increase the model size (not shown here) the robustness of higher order clusters decreases further. Hence the model predicts that for identical perturbation conditions, the higher order clusters will be more stable than the lower order clusters, once a certain threshold of cluster size is crossed. For a system with maximum cluster size of four (and six which is not shown here) the threshold size is cluster with three receptors. Figures 5 and 6 show that the threshold is independent of whether the simulated system is assumed as a co-operative or non-cooperative receptor system. Such predictions are subjected to experimental validation once we obtain our dynamic data of cluster formation from the live cell imaging. Though difficult, experimentalists should attempt to perturb the receptor expression rate (model equivalent is variation of receptor number) and ligand doses (model equivalent is ligand number that were varied). The wild type case should be compared with the perturbed case and the number of clusters in both the conditions could be computed.

Conclusions

Based on the previous experimental guidelines a phenomenological model representing the characteristics of CD40 receptor has been built. The model mimics the CD40's ability to relocate in the cholesterol less and cholesterol deficit membrane micro-domains. As there is no prior information available on the dynamics of the receptors clusters formation, we implemented and tested two probable conditions that could trigger the formation (and adhere) of the clusters. In condition 1 which is implemented in M1 cluster formation is studied only as functions of contribution from the surrounding (cholesterol) and in M2 cluster formation was studied as a function of both surroundings contribution and inter-receptor cooperative effect. Both models were subjected to sensitivity analysis. Analysis shows that clustering could enhance the time for which a ligand bound receptor could be activated as compared to its un-clustered counterpart.

We believe that establishing experimental technologies which allow us to know more about dynamics of cluster formation will be extremely useful for understanding such basic dynamics as it will help extending such minimal model with explicit details of ligand receptor interaction to the spatial dimensions as well. The experiments should capture cluster formation as function of signal dose and time of exposure to the dose. From the global sensitivity analysis, it seems, if we observe few predominant big clusters, the current model (M2) would suggest inter-receptor co-operativity coupled to contribution from

cholesterol contributes to the clustering of CD40 receptors. The model built is of small size and in the real system the number of receptors can in principle be orders of magnitude larger than the ones considered for the study. But in the conceptual level, the working principles of the larger system wouldn't be very different from the smaller one: if the system has inter-receptor co-operativity we would mostly observe either unclustered fraction of the receptors or large size receptor clusters.

Another feature that distinguishes the cooperative and non-cooperative cluster formation is the dynamic sensitivity analysis where a distinct switch like sensitivity profile was found to emerge in the system M2 indicating that the system's parameters will be responsive to perturbation only till a certain time during the evolution of the system after which all the parameters show strong robustness to the identical perturbation. Although characterizing the exact underlying mechanism behind such switch like transition in the sensitivity profile would require further intense mathematical analysis the results expose a time dependent switch like sensitivity profile which can only be observed in cooperative cluster formation process. We also found that a system with both inter-receptor cooperative effect and a positive environmental influence could be more robust to perturbations and the critical perturbation points in such system could be the first step of cluster formation.

Such model drive hypothesis is open to experimental validation. For example, as we know that cholesterol plays a major role in CD40 clustering experiments can be designed to perturb the total concentration of cholesterol in the cell membrane (as done by Rub et al. 2009) for the single cell level and observe the differences in 1] Cholesterol inhibited 2] Cholesterol overexpressed and 3] unperturbed condition. The studies can be done in different doses of CD40 ligand and for each perturbation condition the cluster formation can be compared. As our sensitivity analysis shows that non-cooperative and cooperative receptor interaction mediated clusters can evolve distinctly one can hypothesize that cholesterol depletion/inhibition will reduce the number of largest cluster size (Fig. 4a, LR2_a_4) in the non-cooperative system but intermediate clusters can be observed. On the other hand if the cluster formation process is cooperative cholesterol depletion will dramatically reduce the clusters of any size as the first step of cluster formation will be affected by such depletion (Fig. 4a, LR2_a_4). Thus our minimal model can be used for generating such experimentally testable hypothesis that can distinguish between a cooperative and non-cooperative clustering process.

Acknowledgments All authors thank Department of Biotechnology for financial assistance.

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