Notch-Ligand kinetics (using *ChemicalReactions* toolkit)

Introduction

In our ENABLE, Notch-Ligand system collects intercellular signal for further processing, which is one of the most significant parts for a transmembrane logic gate. What's more, according to recent research [link here to Notch optimization], noise-signal-ratio (SNR) depend on the structure of Notch, which indicates related biological processes affect the component efficiency of Notch-Ligand system.

In previous research, some quantitative descriptions of Notch-Ligand system are published for explanation and exploration of system design based on this system. For example, cis-inhibition was modeled via chemical kinetics, which makes the mechanism for Notch-induced pattern formation more precise. However, stochastic model for Notch-Ligand system simulation haven't been reported yet.

Here we present a mathematical modeling for Notch-Ligand kinetics using Stochastic Petri nets, taking random intercellular processes into consideration. We find that SNR of our system is not only dependent on the affinity of Notch-Ligand pairs, but also share of Secretase Complex. Our modeling lifts the molecular-level chemical reaction up to cell colony-level, which offers clues for oriented optimization and precise application of Notch-Ligand system. Last but not least, our OOP (object-oriented programing) makes it easy to transplant Notch-Signal kinetics into further application [link here for software].

Method

Not like chemical reactions happening in tubes, Notch-Ligand interaction occurs in a 2D manner. That is to say, chemical reactions between Notch and Ligand takes place on the membrane of two neighboring cells. Though two cells may exchange their components on the membrane by touching to each other, the chemical constitution of those cells remains relatively independent. However, when Ligand proteins binding to the extracellular domain of Notch protein, proteolytic cleavage and release of the intracellular domain are induced.

Proteolytic cleavage of Notch needs few key steps, including S2-cleaveage by metalloprotease ADAM10, S3-cleavage by γ-secretase complex or γ-secretase and S4-cleavage [Ref. 1]. Here we simplify the cleavage of Notch-Ligand complex, and suppose that the cleavage is a one-step reaction with the smallest rate constant of all cleavages mentioned above. This simplification is coming down to the *rate-limiting step* in physics chemistry. The simplified equations are as follows.

$$N + L \rightleftharpoons NL$$

$$NL + S \rightleftharpoons NLS \rightarrow icd$$

Here N refers to Notch, L refers to Ligand, NL Notch-Ligand complex, S protease, NLS Notch-Ligand-protease complex, and icd means the intracellular domain of Notch.

Then it comes to our mathematic tools, *Petri net*. The history and precise definition of Petri net can be referenced in detail [Ref.2]. In short, this method views each chemical or reaction intermediate as nodes in a network, element reactions as edges, and weight and direction of each edge for stoichiometric number and reaction direction. Especially, we use $\{P, T, Pre, Post, M\}$ to describe a Petri net precisely: $P = \{p1, \dots, pu\}$ is the space of chemicals, $T = \{t1, \dots, tv\}$ is the spaces of all transitions (element reactions), Pre is a $\mathrm{v} * \mathrm{u}$ integer matrix containing the weights from chemicals to transitions (the (i, j)th element of this matrix is the weight of the arc going from chemical j to transition i, and $Post$ is a $\mathrm{v} * \mathrm{u}$ integer matrix containing the weights from transitions to chemicals (the (i, j)th element of this matrix is the weight of the arc going from transition i to chemical j). M is a u-dimensional integer vector that represents the current state of the system (i.e. the number of molecules).

For system with only a kind of Notch and a kind of Ligand, we have

$$P = \{N_1, L_1, NL_1, NLS_1, S_1, icd_1, N_2, L_2, NL_2, NLS_2, S_2, icd_2\}$$

Here the subscripts refer to the index of a cell in a cell-pair, and the index is designated arbitrarily. Also, we have

$$T = \{gN_1, gL_1, gN_1L_2, dN_1L_2, gN_1L_2S, dN_1L_2S, gicd, gS, dicd\}$$

Here the lower capital g and d refer to the generation and degradation of following chemicals.

Similarly, we can write out the matrix $Post$ and Pre (results not shown here). We can set W as a u-dimensional zero vector for initialization. Then we need to designate when and how this system chooses to finish a certain element reaction.

For this purpose, we consider that the occurrence of an element reaction is a heterogeneous

Poisson process, and certain reaction selection via sampling. That's, the possibility that an element reaction R_i happening in the time interval $(\mathrm{t}, \mathrm{dt}]$ is given by $h_i(x, c_i)dt$. With additivity assumption, we can get the possibility an arbitrary reaction happening in the time interval $(\mathrm{t}, \mathrm{dt}]$ is

$$h_0 = \sum_i h_i(x, c_i)dt$$

The form of $h_i(x, c_i)$ is given by mass-action stochastic kinetics. For a given system, $h_i(x, c_i) = c_i * C(n_{i1}, n_{i2}, \dots, n_{in})$, where c_i is the rate constant for reaction i, n_{in} refers to the molecule number of reactant k for reaction i, and function $C()$ means combinatorial number of all n_{in}. In practice, $C()$ can be replaced by $\prod()$ with some modifications for c_i.

Similarly, we can derive the expression for system with i kinds of Notch and j kind of Ligand (results not shown here).

Results

Notch-Signal component may be equivalent to constitutive expression for stationary system.

For certain parameters, Notch-Signal component may be equivalent to constitutive expression for stationary system [Fig. 1A]**.** It's a good property for using Notch-Ligand system in colony design, which can use the simplified version for higher-layer system simulation and shorten the simulation time without side effect of poor prediction.

Cleavage rate affects system response in a linear way. Notch-Ligand specificity may not affect system response.

For certain parameters, generation of Notch-Ligand-protease complex may be the rate-limiting step [Fig.1A]**.** For example, decreased rate constant of Notch-Ligand-protease complex generation reduce the icd generating rate in a linear manner [Fig. 1B with rate constant of gN_ALS decreased to one tenth of that of Fig. 1A]. Also, changing Notch-Ligand binding affinity may not significantly change icd generating rate, which strongly corroborated this view [Fig. 1C with rate constant of gN_AL_B decreased to one tenth of that of Fig. 1A; and Fig. 1D with rate constant of gN_AL_B decreased to compared to Fig.1A, where heter/homo refers to the rate constant ratio of gN_AL_B/gN_AL_A].

SNR can be tuned via Notch-Ligand binding affinity in a power-law manner.

For certain parameters, signal-noise-ratio can be tuned via Notch-Ligand binding affinity in a power-law manner [Fig. 1E with rate constant of gN_AL_B varied compared to Fig.1A]. This offers clue for Notch optimization. For more details, please refer to our experiment design [link to Notch optimization].

Amplification is required for Notch-Ligand system.

For certain parameters, signal of a certain pair of Notch-Ligand coupling may be diluted by occurrence of other reactions [Fig. 1F with types of overall Notch/Ligand varied compared to Fig.1A].

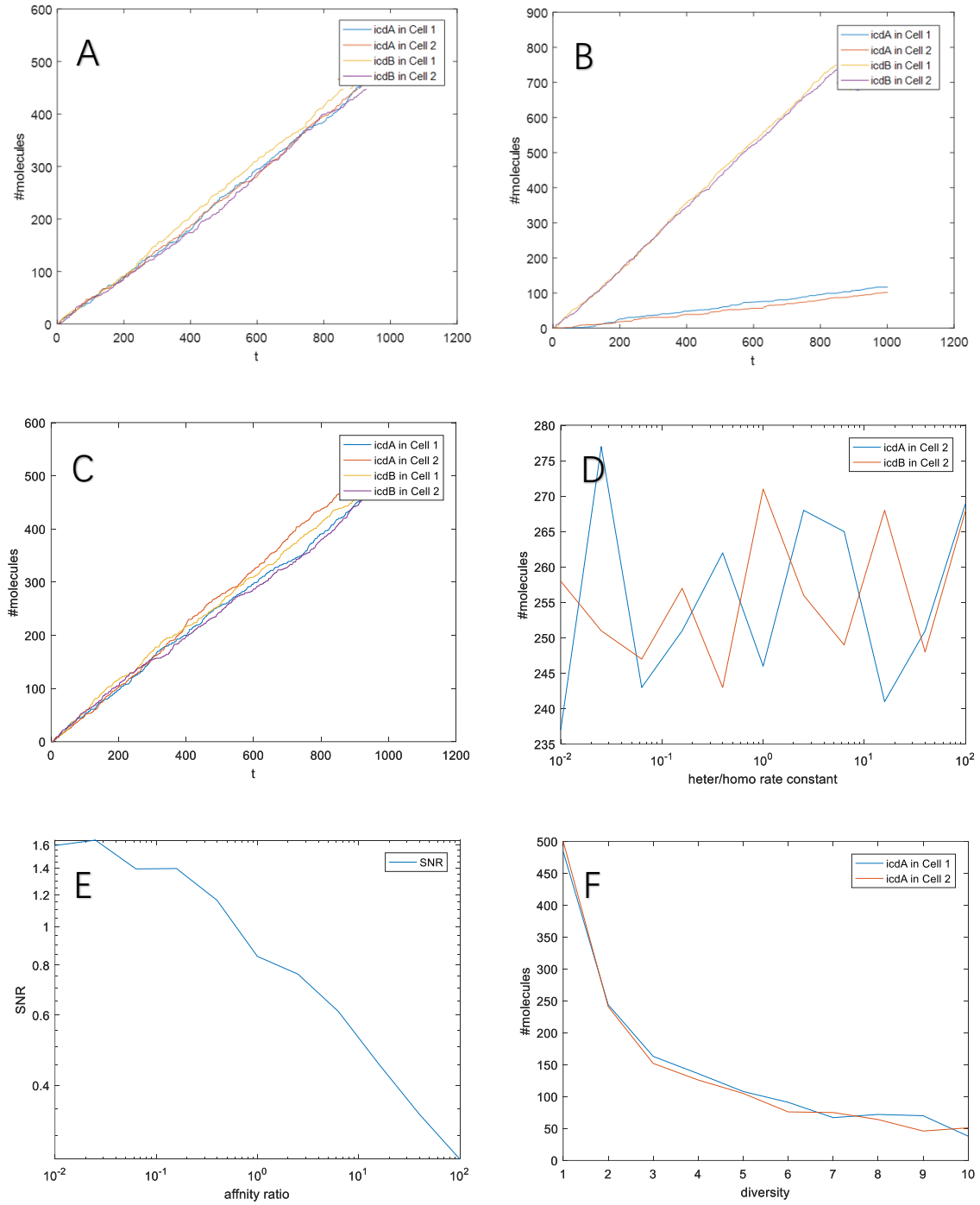

Fig. 1A | Simulation of a 2 Notch-2 Ligand system. The graph's horizontal axis shows the time range, and #molecules shows the number of 2 kinds of intracellular domain (icdA and icdB) in 2 neighboring cells. Both Cell1 and Cell2 are armed with all four kinds of Notch/Ligand A/B.

Fig. 1B | Weak binding affinity for cleavage leads to low response. The graph's horizontal axis shows the time range, and #molecules shows the number of 2 kinds of intracellular domain (icdA and icdB) in 2 neighboring cells. The affinity of protease to NotchB-LigandB complex is weakened, leading to poor NotchB response to Ligand signal.

Fig. 1C | High Notch-Ligand specificity may not affect system response. The graph's horizontal axis shows the time range, and #molecules shows the number of 2 kinds of

intracellular domain (icdA and icdB) in 2 neighboring cells. The specificity of Notch-Ligand binding is enhanced, making no significant difference of system response with the cleavage being the rate-limit reaction in this system.

Fig. 1D | Variant Notch-Ligand specificity may not affect system response. The graph's horizontal axis shows the ratio of rate constant of $gN_iL_j(i \neq j)$ and that of $gN_iL_j(i = j)$, and #molecules shows the number of 2 kinds of intracellular domain (icdA and icdB) in Cell2. The specificity of Notch-Ligand binding is tuned, making no significant difference of system response with the cleavage being the rate-limit reaction in this system. This strongly corresponds to the fact that the cleavage reaction is the rate-limit reaction in this system.

Fig. 1E | SNR can be tuned by Notch-Ligand specificity. The graph's horizontal axis shows the ratio of rate constant of $gN_iL_j(i \neq j)$ and that of $gN_iL_j(i = j)$, and the SNR are defined as the ratio of the number of 2 kinds of intracellular domain (icdA and icdB) in Cell2, with Cell1 only have LigandA. The specificity of Notch-Ligand binding is tuned, changing SNR in a power-law manner.

Fig. 1F | Amplification is needed for multi-Notch/Signal system. The graph's horizontal axis shows the diversity of the Notch-Ligand system, and the diversity are defined as the species number of Notch/Ligand. With the diversity increasing, the number of a certain type of Notch intracellular domain molecules reduces.

Discussion

Our modeling lifts the molecular-level chemical reaction up to cell colony-level, which offers clues for oriented optimization and precise application of Notch-Ligand system. For certain parameters, some properties are simulated, which fits experimental results well [link to experiment].

Also, this stochastic Notch-Ligand kinetics can be simplified as a single chemical constant for some certain conditions. Though this may me greatly reduce the workload of transplant our model into a higher-scale application (e.g. to model a cell colony made up of cells armed with Notch and Ligand), our OOP (object-oriented programing) modeling style makes it easy to transplant Notch-Signal kinetics for macroscale perspective [link here for software].

Reference

[1] Tetering, G. V., & Vooijs, M. (2011). Proteolytic cleavage of notch: “hit and run”. *Current Molecular Medicine*, 11(4), -.

[2] Wilkinson, D. J. (2006). *Stochastic modelling for systems biology*. Chapman and Hall/CRC.