Modeling a Bacterial Ecosystem Through Chemotaxis Simulation of a Single Cell

Nesrine Ouannes, NourEddine Djedi, Yves Duthen et Hervé Luga

Abstract We present in this paper an artificial life ecosystem in which the genes in the genome encode chemotaxis of bacteria that aim at: detecting resources (or sensing the environment), controlling the bacteria motion and producing a foraging behavior, and allowing bacteria to communicate together to obtain more sophisticated behaviors. The chemotaxis network of a cell is modulated by a hybrid approach that uses an algebraic model for the receptor clusters activity and an ordinary differential equation for the adaptation dynamics, and a metabolism model that is based on the transformation of matter from 'food'. The results show analysis of the motion obtained by some bacteria and their effects on the population behaviors generated by evolution. This evolution allows bacteria to have the ability to adapt themselves to better growth in the environment and to survive. As future work, we aim to improve the effect of the communication between bacteria to obtain bacteria that can emerge as new species, and to integrate the concept of colonies.

Keywords Artificial life · Bacterial chemotaxis · Single cell

1 Introduction

Simulation models in artificial life have focused metabolic, cellular systems and artificial chemistries. Artificial life research has also made progress in the study of adaptive behavior through computational models of artificial organisms. Remarkably simple chemical reactions can perform movements toward some attractants, and are therefore capable of modulating the behavior of artificial organisms.

We will also demonstrate whether a simple bacterial chemotaxis process of a cell can explain the evolution of more complicated behaviors such as bacterial population dynamics. One of the central questions of modern systems biology is the influence of microscopic parameters of a single cell on the behavior of a cell population. In terms of bacterial chemotaxis, this issue can be formulated as the influence of signaling network parameters on the spatiotemporal dynamics of bacteria that migrate towards chemical attractants and away from repellents. This chemotaxis is one of the simplest behaviors known, and it likely is one of the first behaviors to have existed in the history of life on earth.

In the bacterial chemotaxis process, when no attractant or repellant is present, or when the concentration of attractant or repellant is uniform, a bacterium such as *E.coli* tends to swim in a random walk, with periods of smooth swimming (or runs) interrupted by brief tumbles that changes the swimming direction. In response to attractant gradient, this random walk becomes biased and the bacteria tumble less frequently when encountering increasing concentrations of an attractant (i.e., they swim longer runs), and tumble more frequently when the attractant concentration is decreasing [1]. The motivation for studying such small organisms lies in the belief that elucidating the mechanisms controlling their behavior will help in understanding more complex biological pathways and organisms. Phosphorylation cascade in a chemotaxis network was first simulated by Bray et al [2], using a system of ODEs, and [3], a later version of their model, added adaptation. A major advance in chemotaxis modeling was achieved in [4]. Later, in [5], a theoretical analysis of a full ODE system with included phosphorylation cascade.

Here, we present a bacterial ecosystem by simulating bacteria chemotaxis network. The chemotactic Es-

cherichia coli bacterium model describes signal processing by mixed chemoreceptor clusters, which is a rapid-equilibrium (algebraic) model, adaptation through methylation simulated by ordinary differential equations (ODEs), and the running and tumbling of a cell with a flagella motor [6]. The metabolism of this bacterium is a set of chemical reactions that occur in the cell. These chemical reactions are designed digitally to perform different functions as *split*, *mutation* and *death*. the aim goal of this metabolic model is to demonstrate the importance of recycling the matter in an ecosystem environment.

2 Bacterial Chemotaxis

The chemotaxis process consists of three stages: chemoreception, signaling, and adaptation [7]. Methyl accepting chemotaxis proteins (MCPs) are located along the cell surface. These proteins act as chemoreceptors and bind with chemicals in the environment. If a nutrient attractant is detected outside of the cell, through MCP, the level of production of protein CheA decreases because the receptors state shifts to the off state. It has been shown that the activity of the receptor cluster depends on the local ligand concentration and the methylation level according to the MWC (Monod-Wyman-Changeux signal processing) model [8], [9]. CheA binds with phosphate in the cell (denoted CheA-P). And the phosphate group is transferred from the active CheA to the response regulator CheY. The concentration of CheY-P modulates the motor and its behavior makes the cell run or tumble.

2.1 MWC model

We applied the MWC model for a mixed receptor cluster [8], [9], where each receptor homodimer is described by a two-state model. The inactive state of a receptor has a higher affinity to the attractant than the active state. The entire complex exists with all of its receptor homodimers either active or inactive. The probability A that receptor cluster is active is dependent on ligand concentration and the methylation state of the receptors and calculated as:

$$A = 1/(1 + e^{F}) \tag{1}$$

Where $F = F_{on} - F_{off}$, and where $F^{on/off}$ is the free energy of the cluster to be on/off as a whole. Hence, the average activity per receptor in the cluster is A. The total free-energy difference in the mean-field approximation is $F = n_r f_r(m)$, which is just the sum of the individual free-energy differences between the receptor *on* and *off* states.

$$f_r(m) = f_r^{on} - f_r^{off} = \epsilon_r(m) + log(\frac{1 + [S]/K_r^{on}}{1 + [S]/K_r^{off}}) \tag{2}$$

where $[S]$ is the ligand concentration, $K_r^{on/off}$ is the dissociation constant for the ligand in the *on* and *off* state, respectively. The methylation state of the receptor enters via the "offset energy" $\epsilon_r(m)$.

2.2 Adaptation model

Adaptation is modeled according to the mean-field approximation of the assistance-neighborhood (AN) model [8], [10]. Adaptation in chemotaxis is mediated by two enzymes, methyltransferase CheR and methylesterase CheB. It is assuming that the demethylating enzyme CheB works only on active receptors and that the methylating enzyme CheR works only on inactive receptors within the AN. Each bound CheR adds methyl groups at a rate a(1-A), and each bound CheB removes methyl groups at a rate bA. It is assumed that both enzymes work at saturation ([CheR]= 0.16, [CheB]= 0.28) [11]:

$$dm/dt = a(1 - A)\ [CheR] - bA\ [CheB] \tag{3}$$

The average methylation level evolves in time as

$$m(t + \Delta t) = m(t) + kV\Delta t \tag{4}$$

The parameter k indicates the adaptation rate relative to the wild type adaptation rate V that is the rate of receptor methylation (see equation 3) [6].

2.3 Kinase activity

Both ligand binding and receptor methylation affect the activity of CheA. For example, the increase of an attractant inhibits CheA activity, but subsequently methylates a specific receptor. CheA kinase activity [6] is calculated as (varing into [0,1]):

$$CheA = CheA_{tot} AK_A/(AK_A + K_Y CheY_{tot}) \tag{5}$$

Where, A is the probability that receptor cluster is active, $CheY_{tot}$ is the total CheY concentration that is equal to 9.7 acoording to [11], K_A=5 and K_Y=100 are the rate constants acoording to [6].

2.4 CheY phosphorylation

The concentration of CheYp is obtained as a function of active CheA from the steady-state equation [12].

$$CheY = CheY_{tot}K_Y A/(K_Y CheA + K_Z CheZ + g_y) \quad (6)$$

Where, $CheY_{tot}$ is the total CheY concentration, and $CheZ$ is the total CheZ concentration, CheA is the active $[CheA\ ]$, and $k_y = 100\mu M^{-1}s^{-1}$, $k_Z = 30/\ [CheZ\ ]s^{-1}$, Y = 0.1 are the rate constants according to [12], [13], [14]. Receptor modification increases CheA activity and decreases sensitivity to attractants.

2.5 The CCW motor bias

The CCW motor bias depends on CheYp concentration in the following form [15].

$$mb = \frac{mb_0}{CheY(1 - mb_0) + mb_0} \quad (7)$$

Where, mb_0 (0.65) is the steady-state motor bias.

3 Bacterial Metabolism

The metabolism is responsible for essential cycles of growth, development and reproduction. Genes and movements of a bacterium affect the majority of these cycles. An organism's genome may contain instructions that encode the ability to metabolize one or more substrates present in the environment. Metabolism of a food either accelerates or decelerates a bacterium's replication rate by a factor that is positive or negative, signifying a nutrient or a toxin, respectively. In this model, every bacterium is represented by a genome from which it extract its basic properties describing how it moves, gains energy, expels toxins, and produces waste. These properties are updated in the genome at each time step, mutation is applied after each "split" operation.

Forrest and Jones' simulation [16] allows for simple material cycling through agent bodies. Materials are collected by the agents and stored for a time before being released back into the environment when the agent dies. From this point of view, we adopted this idea and the bacteria when they die, they will be transferred to a source of energy for the other bacteria.

Metabolism is calculated as the organism's total energy (energy obtained via the metabolizable food in addition to basal energy provided equally to all organisms) and subtracted to the 'cost of motion' generated from the tumble frequency produced by the bacteria network. This metabolic model, supports bacteria to stabilize their energy consumption in order to reach splitting threshold. After this each bacterium splits into two daughter cells. The food (i.e. food sources or waste of bacteria) is stored internally and used up as follows:

$$\Delta M_t = (M_0 + A(M_F + M_W) + M_T + mb_0 M_M + M_S)\Delta t \quad (8)$$

- ΔM_t is the total metabolic expenditure (by which the internal store or the energy gained from foods is depleted in each time step);
- M_0 is the base level metabolism (or the initial level at birth, which is equal to 25);
- A is the ability of bacteria to consume a food from the environment (according to the encoding genome described in the next section);
- M_F is the metabolic value stored from the food sources consumption that is +2 units;
- M_W is the metabolic value stored from the waste consumption (+1 unit);
- M_T is the metabolic cost of toxin consumption (-2);
- M_M is the metabolic cost of movement (-1 unit);
- mb_0 is the tumble frequency obtained from chemotaxis network of the bacteria;
- M_S is the metabolic cost of split operation ($M_t/2$).

4 Genetic representation

In the bacterial chemotaxis, there is a processing system of moderate complexity within the cell, triggered by its inputs and producing an output response. In *E.Coli* bacterium, this response corresponds to a change in the flagella rotation. The bacterial chemotaxis shows properties of receptor function, adaptation, memory and motor bias. To control these properties in order to simulate bacterial population behaviors, we use a genome that encodes the activities of each level in the chemotaxis network. In this genome, we have two different types of encoding as presented in figure 1. First, a binary encoding that describes the different capacities of a bacterium, that are: The capacities to detect a nutrient, and toxins with the same receptors. These capabilities serve as inputs for the network chemotaxis. The M and T values correspond to a small or large zone of nutrient detection. A consumption capacity of a bacteria, explaining how many a bacterium can consume from the food, this capacity is affecting the metabolism process (see equation 8). The gene AC represents the concentration of autoinducers (or the ability of detecting small diffusing autoinducers molecules), allowing bacteria to produce molecules and detect the molecules produced by other bacteria presented in their group, in order to communicate each other.

Second, a real encoding that encodes functions of the chemotaxis network of a bacterium that are: Cluster

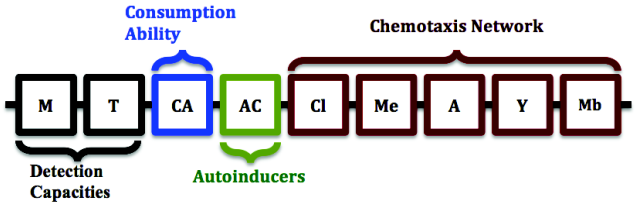

Fig. 1 The bacterium's genome.

activity (CI), kinase activity, methylation level (Me), CheY phosphorylation (Y) and motor bias (Mb), that are obtained from the equations (1) to (7).

Once a bacterium manages to accumulate enough energy to reach the division state, it divides immediately into two identical daughter cells, except that, the new bacterium copy will be mutated, in order to enable bacteria to evolve. This ensures that the parent's genetic material is preserved, while at the same time new genetic material is introduced in the population. A small probability p_m of mutation is proposed to be applied to the genome, by associating a noise to a selected gene. It must be emphasized at this point that, after the division process, the amount of energy of the parent's cell will be distributed equally to the two copies. This will guarantee that the parent cell continues to exist, and it can create many different offsprings during its lifetime and does not "die" after division.

5 Experimental Results

The objective of this work is to design an artificial ecosystem populated with bacteria. The set of experiments presented here are established in order to try to found a solution of the question of how to demonstrate if a simple bacterial chemotaxis process of a cell can explain the evolution of more complicated behaviors as bacterial population dynamics?

Simulated bacteria live and evolve in a 2-dimensional environment subdivided into discrete grid squares in which the bacteria exist as individual entities (i.e. the biotic element of the ecosystem). The developed model allows distinguishing three resources that are: (i) food (a source of energy) diffuses from multiple point of the environment and also (ii) from dead bacteria (or waste), and (iii) toxin resources. Although the environment has been discretized, bacteria are free to move in the continuous two-dimensional space by translating their location. Each bacterium has one cell, all have equal size, shape, chemotaxis network controlling their movements, and artificial genomes generated at each run. All other parameter setups used in the *Chemotaxis Network* are the same as presented in our previous work [17]. At the start of each run, the bacteria had random locations in the environment. We started each evolutionary run from 10 bacteria with randomly generated genomes. Dead bacteria are replaced by sources

All runs presented in figure 2 (left-hand) show a fast population increase in the first twenty simulation cycles (or generations). This increase leads to a population reproduction (or split), and then the population stays relatively constant for about 200 generations. From this level to the generation 300, the population decreases rapidly. This is a consequence of two facts. First a high number of bacteria die due to the depletion of food resources. Secondly, the speed of decrease is due to the bad MCP and toxin avoidance capacities. From generation 300, and every 300 cycles, the growth rate is often increased according to the capacities defined in the genome of each bacterium, which are also advanced. The number of species varies great during an experiment, which means that bacteria frequently split and die over time. It is important not simply relate this to food in the environment and to their own biomass, it is rather related the evolved capacities.

We test the changes in the values of the population's collective energy for all 30 runs, as presented in Figure 2 (right-hand) were we observe that the metabolizable resources are consumed, while the populations collective energy decreases in the beginning of the run (as new cells are created), at the division process's maximum speed, division process the biomass is exponentially decreased. Knowing that all sources are depleted. Within thirty generations, while many bacteria die because they did not have enough energy for movement, but fortunately not all the population, as, all simulation will stop in this case (but this has been tested before choosing the environment parameters). In iteration 300, when new nutrients resources are added to the environment, the bacteria consume nutrients, split, and when no more nutrient are present in the environment, their Energy decreases again but avidly than before. This means that the bacteria obtained after thousand of time steps are more stabilized and more effective in their use of energy. This effectiveness is due to the evolved capacities of detection (MCP capacities) of nutrients and mostly is due to the approved consumption ability.

The evolved capacities of bacteria effect the chemotaxis network response, for example: if a large zone is covered with a bacterium, it will conduct it to a long *run* movement (i.e. *Inactive State*). Also, if a good consumption ability is obtained, the bacteria metabolism will be better optimized. We also present data about the oscillations of the swimming of some bacteria. The figure 3 shows the path of (x,y) coordinates of some bacteria borrowed from the simulation, where each bacterium applies long *runs* and short *tumbles* in the pres-

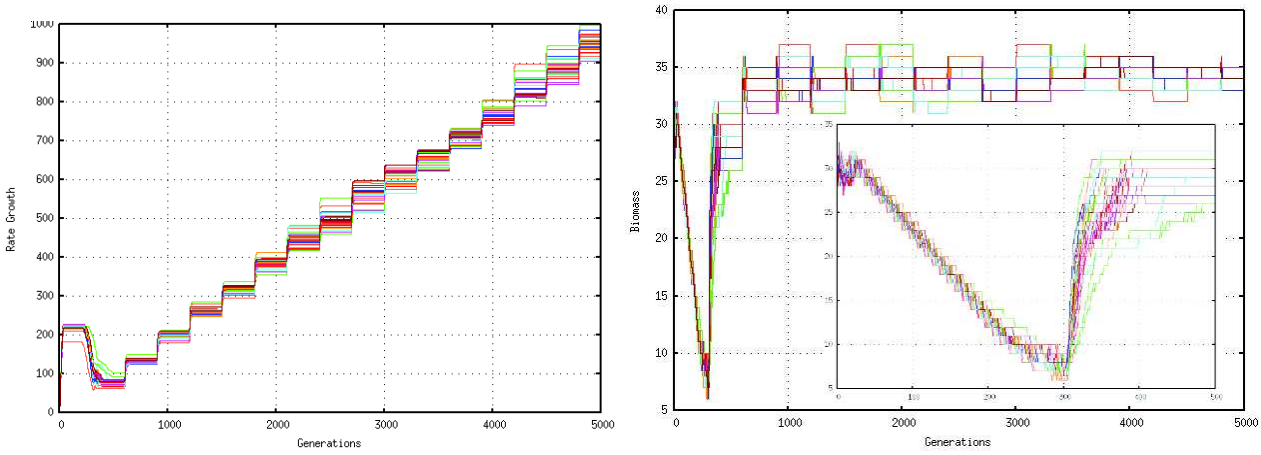

Fig. 2 Left-Hand. The Growth rate runs, which we have replicated 30 times with quantitatively the same results, representing the optimal values of the whole of the bacteria for 5000 steps. Right-Hand. The Energy of the evolved population of bacteria for 30 runs at 5000 generations. Inside the figure, a zoom in the same runs for 500 generations.

ence of nutrient sources (as response to the nutrient), and a *random walk* in the *Steady State*. *Run* is a period of long straight swimming, and *tumble* is when bacteria stop and abruptly change their orientations, which is seen in the figure as the angles formed between two *runs*. This path graph explained how bacteria moved from their initial positions toward a favorite zones, where two phases of evolution are remarked, in the first 300 cycles: bacteria are executing long *run* from a source to another, mostly in the first 20 cycles, and when all resources are depleted the oscillations became biased then a *random walk* is executed. The same thing is remarked in the second 300 cycles.

6 Discussion

The results show that a simple simulation model of single-celled creatures and biological mechanisms and simple chemical reactions allows us to model more complicated behaviors of a population of bacteria. We summarize that the growth rate (or bacteria number) continues to increase for several hundred epochs, as the resources are eventually present in the environment, and the population's collective lifespan is ameliorated because the evolved bacteria consume less energy with their optimal capacities and gather more sources. The behavior of the system is thus to favor emergence (or adaptation) of best capacities to detect food and avoid toxins, therefore to avoid death and to better reproduce and to survive longer.

When bacteria are moving, consuming and splitting, their chemotaxis network are optimized in order to control their evolution. The figure 4 explain how the chemoatxis network answer to changes happened in the environment and inside the bacterium cell. Four internal states are observed in this response; *Steady State*, *Fully Inactive*, *Adapted*, and *Fully Active State*. In each of these states the different protein's concentrations are observed and analyzed referred to [6].

In the *Steady State* the bacteria perform a *random walk*, and exploring the environment with the initial values: (the kinase CheA =0.0164, and methylation =1.92, the CheY =1.92. Finally the motor bias =0,65). All these values are used by the ordinary differential equations to calculate their changes over time. When bacteria detect food sources, they enter to a consumption state, where transmembrane receptors sense this changes of attractant and became inactive.The attractant binding inhibit the autophosphorylation activity of CheA. The CheY phosphorylated by the groups received from CheA (CheY-P), diffuses to the flagellar motors and changes of motor rotation, and causes a *run*. This increase of attractant concentration (realized by an attractant detection) shifts the equilibrium to *off* state of the receptors (i.e. *Fully Inactive State*), that results in an initial fast decreases of kinase activity (CheA) (to 0.002) and hence CheY level, and causes longer *runs* (i.e mb 0.75). The decrease of ChA activity is followed by a slow CheR dependent adaptation.

In the *Adapted State*, the probabilities of booth states of the receptors (*on*, *off*) are equal, and the booth CheR, and CheB enzymes are working for methylation and demethylation processes. In this state, methylation increases receptor ability to simulate CheA activity.

A removal of attractants shifts the system to the *on* state (or *Fully Active State*) that activates CheA autophosphorylation (0.047) and hence the downstream CheY phosphorylation. Methylation also decreases the activity of the receptor complex to attractant, thereby regulating the ligand binding to receptor complex.

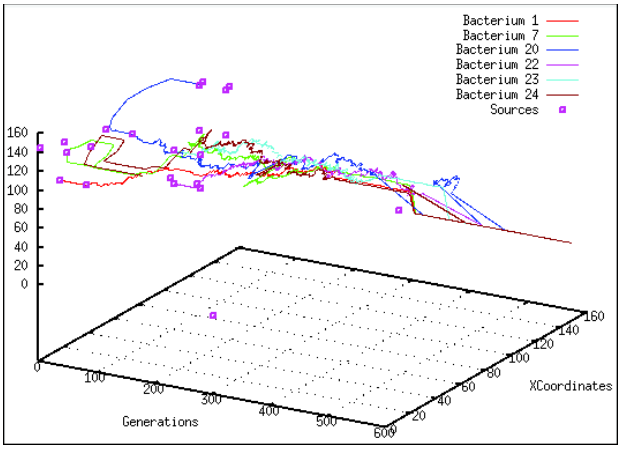

Fig. 3 Path realized by some bacteria in 2D space for the first 600 cycles. The squares present the sources of nutrients that are present until the generation 20 and again from 300.

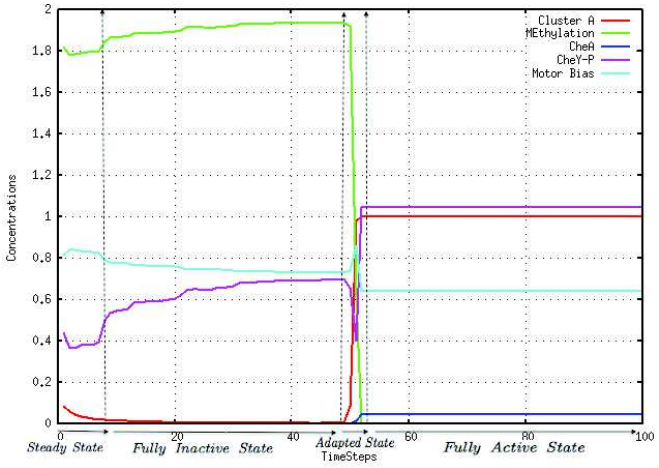

Fig. 4 Graph of variations in concentrations of proteins and enzymes used inside bacteria during the chemotaxis process.

7 Conclusion and Future work

Our model was been designed to simulate growth and behavior of bacterial ecosystem; it controls a group of bacteria cells at each time step. To analyze the obtained behaviors, we present data that characterizes bacteria positions in space, Energy, and, state in the cellular reproduction cycle. These results demonstrate that bacteria are still able to evolve through mutation. The constructed model of chemotactic *E.coli* employed a hybrid model for pathway simulation, with mixed algebraic, ODE, and stochastic components instead of a fully stochastic model with an evolutionary algorithm to evolve a population of bacteria.

In future work, we aim to improve the effect of the chemotaxis network to obtain more powerful bacteria that can emerge as new species which behaves differently from others, via the concept of colonies, and also to test this model on different environmental conditions and various changes.

References

1. Adler., J. (1975), Chemotaxis in bacteria. Annual, Review of Biochemistry, 44: 341-356.
2. Bray, D., Bourret, R. B., and Simon, M. I. (1993). Computer simulation of the phosphorylation cascade controlling bacterial chemotaxis. Molecular Biology of the Cell, 4(5):469.
3. Levin, M. D., Morton-Firth, C. J., Abouhamad, W. N., Bourret, R. B., and Bray, D. (1998). Origins of individual swimming behavior in bacteria. Biophysical journal, 74(1): 175-181.
4. Barkal, N. and Leibler, S. (1997). Robustness in simple biochemical networks. Nature, 387(6636):913-917.
5. Mello, B. A. and Tu, Y. (2003). Perfect and nearperfect adaptation in a model of bacterial chemotaxis. Biophysical journal, 84(5): 29-43.
6. Vladimirov, N., Lvdok, L., Lebiedz, D., and Sourjik, V. (2008). Dependence of bacterial chemotaxis on gradient shape and adaptation rate. PLoS computational biology, 4(12):e1000242.
7. Berg, H. C. (2000). Motile behavior of bacteria. Physics Today, 53(1):24-30.
8. Endres, R. G. and Wingreen, N. S. (2006). Precise adaptation in bacterial chemotaxis through assistance neighborhoods.
9. Keymer, J. E., Endres, R. G., Skoge, M., Meir, Y., and Wingreen, N. S. (2006), Chemosensing in escherichia coli: two regimes of two-state receptors. Proceedings of the National Academy of Sciences of the United States of America, 103(6):1786-1791.
10. Hansen, C. H., Endres, R. G., andWingreen, N. S. (2008), Chemotaxis in escherichia coli: a molecular model for robust precise adaptation, PLoS computational biology, 4(1):e1.
11. Li M, Hazelbauer GL (2004) Cellular stoichiometry of the components of the chemotaxis signaling complex. J Bacteriol 186: 3687-3694.
12. Kollmann, M., Lvdok, L., Bartholome, K., Timmer, J., and Sourjik, V, (2005), Design principles of a bacterial signalling network. Nature, 438(7067):504-507.
13. Stewart, R., Russell, C., Roth, A., and Dahlquist, F. (1988), Interaction of cheb with chemotaxis signal transduction components in Escherichia coli: modulation of the methylesterase activity and effects on cell swimming behavior, Cold Spring Harbor symposia on quantitative biology, 53: 27-40.
14. Sourjik, V. and Berg, H. C. (2002). Binding of the Escherichia coli response regulator chey to its target measured in vivo by fluorescence resonance energy transfer, Proceedings of the National Academy of Sciences, 99(20):12669-12674.
15. Cluzel, P., Surette, M., and Leibler, S. (2000), An ultrasensitive bacterial motor revealed by monitoring signaling proteins in single cells, Science, 287(5458):1652-1655.
16. Forrest, S. and T. Jones (1994). Modelling Adaptive Systems with Echo. In Complex Systems: Mechanisms of Adaptation. P 3-21.
17. Ouannes, N., Djedi, N., Luga, H., and Duthen, Y. (2014). Modeling a bacterial ecosystem through chemotaxis simulation of a single cell. pages 96-102, AROB , Beppu, Japan.