Population Balance Modeling of Conjugation in *Enterococcus faecalis*

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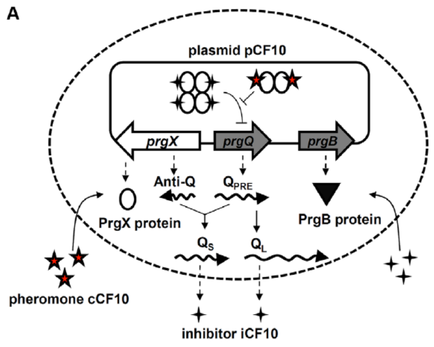
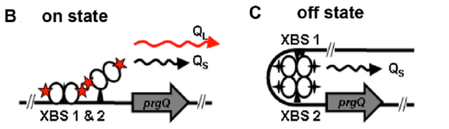
Motivation and Introduction to the pCF10 System

Conjugation is one process by which bacteria can exchange genetic information. In the bacteria *Enterococcus faecalis,* successful conjugation leads the spread of antibiotic resistance in certain populations

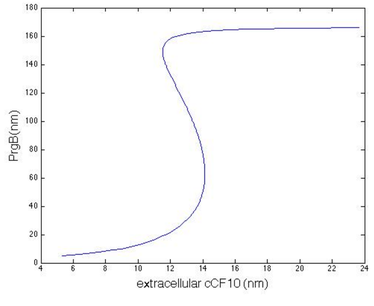


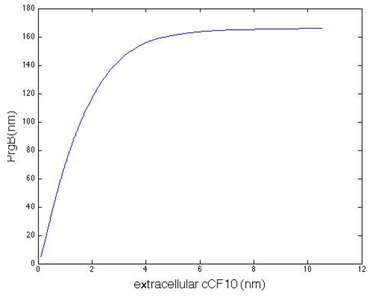


- In the bacteria *Enterococcus faecalis,* the *prgX-prgQ* gene pair on plasmid pCF10 regulates the process of conjugation[1]
- Donor cells produce inhibitor iCF10
- Recipient cells produce pheromone cCF10
- Inhibitor iCF10 and pheromone cCF10 compete for binding to PrgX protein
- Figure C depicts the *prgQ* gene in a *repressed* state. iCF10-bound PrgX tetramers form a DNA loop, leading to a decrease in *prgQ* gene expression.
- Figure B depicts the *prgQ* gene in a *derepressed* state. cCF10-bound PrgX dimers allow for successful expression of the *prgQ* gene.
- Anti-Q + Qpre = Qs = no conjugation
- Ql RNA leads to successful expression of PrgB protein, the concentration of which indicates the extent of the onset of conjugation

Bistability

- The pCF10 regulatory network has two stable states owing to both RNA polymerase collisions about the *prgQ-prgX* gene pair and RNA-interactions between Anti-Q and Qpre RNA[2]
- The upper level of PrgB protein indicates the "on" level of conjugation in donor cells
- The lower level of PrgB protein indicates the "off" level of conjugation in donor cells
- Bistability can be excluded from the system by setting parameter values referring to the degradation rate of Qs and Ql RNA equal to one another





Why a Population-based Approach is Needed

The *pCF10* system traditionally has been studied from the point of view of an "average" cell. All cells are considered identical in the sense that all intracellular and extracellular species are modeled with a set of deterministic ordinary differential equations. More accurate are stochastic single cell models that have been introduced that seek to describe the inherent fluctuations that exist for such a small number of signaling molecules. However, any single cell approach suffers from the fact that individual cells indeed act independently and that extracellular concentrations change as a result of the interactions between these many unique cells in a single population.

Theory

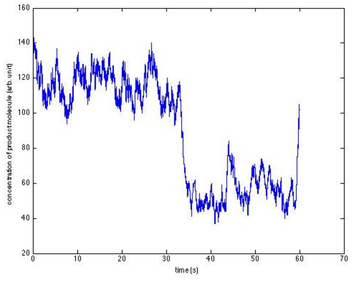
- As stated earlier, a set of ODEs can be written describing the change in concentration of various species such as intracellular inhibitor, extracellular pheromone, etc.
- Here, we attempt to describe the same system through a population balance model.
- The population balance model distinguishes between a set of internal coordinates, a vector X containing various quantities related to the cell such as intracellular concentrations, and external coordinates, the position vector r of the cell. Cells with identical internal *and* external coordinates are viewed as indistinguishable.
- Additionally, a vector Y is used to describe extracellular quantities influencing intracellular processes.
- A population balance equation incorporating stochastic behavior can be derived for the *pCF10* system, shown below, as laid out in detail in Ramkrishna[3]. In essence, it is a number balance on cells of state X at time t with plasmid copy number k=5

$$\frac{\partial f_5(\mathbf{x},t)}{\partial t} = -\nabla_{\mathbf{x}}\cdot\dot{\mathbf{X}}_5(\mathbf{x}|\mathbf{Y})f_5(\mathbf{x},t) + \frac{1}{2}\nabla_{\mathbf{x}}\nabla_{\mathbf{x}} : \mathbf{B}_5(\mathbf{x}|\mathbf{Y})\mathbf{B}_5(\mathbf{x}|\mathbf{Y})^T f_5(\mathbf{x},t) + \mu f_5(\mathbf{x},t)$$

- The leftmost term represents the rate at which cells of state x and plasmid copy number equal to 5 accumulate at time t.
- The term to the right of this represents the net flux of cells by "convective" transport in internal coordinate space
- The next term to the right accounts for random fluctuations (i.e. Brownian motion)
- This equation coupled with a balance equation for the vector Y as well as appropriate initial and boundary conditions allow for a solution for the expected number density f_k

Methodology

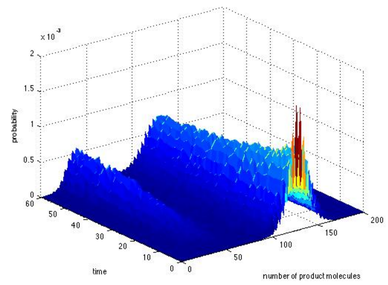
- The bistability curve found to the left was created by solving a set of deterministic ODEs for various intracellular quantities as derived from mass-action kinetics. As the concentration of PrgB was iterated through an array of values, MATLAB's solver function was used to solve for each quantity from a system of equations derived from setting the time derivative of each species concentration equal to 0 (i.e. solving for steady state values)
- The time evolution of the probability density function for the single cell, incorporating stochastic behavior, was created by running a number of stochastic simulations through the use of the exact Gillespie stochastic simulation algorithm and compiling each of the sample paths. An example of a single path is shown below.
- The population balance equation formulated for the plasmid pCF10 system was numerically solved simultaneously with an equation for the extracellular variables by treating the variables as continuous entities. This leads to the formation of a stochastic differential equation that may be solved numerically by the Euler-Maruyama method (essentially a more general version of the well known Euler method for solving ODEs)
- Initial values for use in the above Euler-Maruyama numerical method for both the off-state and on-state were found from the aforementioned bistability curve. These two sets of values were found by locating the two distinct levels of PrgB protein for which the extracellular concentration of cCF10 = 13.5

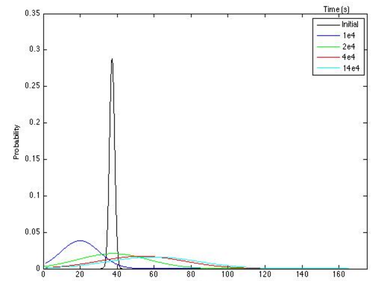


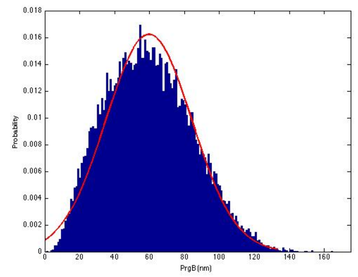
- It should be noted that the equation shown in *Theory* can be turned into a Fokker-Planck type equation with a suitable change of variables and that the resulting PDE could in theory be solved

Bistability versus Bimodal Distribution

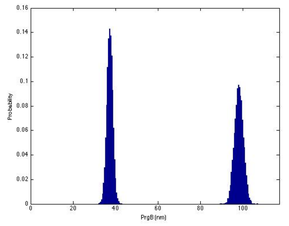
- Shown to the right is a figure of the time evolution of the probability density function for a toy reaction system that exhibits bistability. Created using the single cell approach, the figure shows a unimodal distribution developing into a bimodal distribution.
- In contrast to this is the result produced from the population balance approach, shown below:
- Importantly, the population balance approach does NOT show the emergence of bimodality
- Identical results to the above two graphs were found for a system initially in the off state.



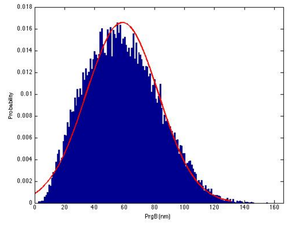




- To exclude the possibility that enough time wasn't allowed for the formation of a bimodal distribution, another figure (shown below) was generated showing what the population balance model predicts for a population that is initially half in an on-state and half in an off-state.



Initial bimodal distribution at 100 seconds forms a unimodal distribution by 14e4 seconds



Conclusions and Future Work

- It is apparent that the population balance approach leads to results distinct from those of the single cell approach. The latter fails to differentiate between bistability and bimodality
- The population balance approach shows how a unimodal distribution may result even in the presence of bistability due to the effects cells have on their environment and their neighbors
- Current work is focused on developing more efficient algorithms for solving these stochastic systems near exactly. A parallel approach is being developed to simulate many pathwise solutions concurrently to decrease the CPU time needed to render statistics about the entire population. Additionally, this approach is making use of the recently developed tau-leap method that essentially leaps over periods of time where no significant reactions occur in the stochastic simulation

References

(1) Shu, C.-C., Chatterjee, A., Dunny, G., Hu, W.-S., & Ramkrishna, D. (2011). Bistability versus bimodal distributions in gene regulatory processes from population balance. *PLoS Computational Biology*, *7*(8), e1002140. doi:10.1371/journal.pcbi.1002140
(2) Chatterjee, A., Johnson, C. M., Shu, C.-C., Kaznessis, Y. N., Ramkrishna, D., Dunny, G. M., & Hu, W.-S. (2011). Convergent transcription confers a bistable switch in Enterococcus faecalis conjugation. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(23), 9721–6. doi:10.1073/pnas.1101569108
(3) Ramkrishna, D. (2000). *Population Balances*. Elsevier.