# Stochastic modeling of septation in *Escherichia coli* using MCELL

A thesis submitted in partial fulfillment of the requirements for the award of the degree of

## Master of Technology


**Rahul**

School of Information Technology

Jawaharlal Nehru University

New Delhi - 110 067

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# DECLARATION

I hereby declare that the work carried out in this thesis is entirely original. It was carried out by me in the School of Information Technology, Jawaharlal Nehru University, New Delhi. I further declare that it has not formed the basis for the award of any degree, diploma, membership or similar title of any university or institution.


Rahul
School of Information Technology
Jawaharlal Nehru University
New Delhi

Indira Ghosh
Dean
School of Information Technology
Jawaharlal Nehru University
New Delhi, INDIA

Supratim Sengupta
Thesis Supervisor
School of Information Technology
Jawaharlal Nehru University
New Delhi, INDIA

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# Contents

# Chapter 1

# Introduction

Cell division is an important process in the life of every living organism. *E.coli* is the organism, which because of its simplicity has become model organism to study. *E.coli* cell division is very well regulated but the understanding of the process is still somewhat limited. The process has become area of intensive investigation through both experimental and mathematical modeling approaches. Many players are identified which leads to correct division of cells and will be discussed below.

## 1.1 Division Proteins

Hirota and co workers[6] identified many temperature sensitive proteins which in absence of cell division forms long filaments. Due to which it has been named as filamentous temperature sensitive(fts) proteins. Till date around 10 proteins have been identified which are known to assist in division process. They are FtsZ, A, K, Q, L, B, W, I, N and ZipA. All these proteins assemble at the site of division and forms a multi-protein complex called the divisome. The formation of septal ring is initiated by the assembly of FtsZ proteins at the site of division process[2]. The Z-ring, made only out of FtsZ polymers, is the first component of the septum that recruits other components of the multi-protein divisome complex to the mid-cell[5]. This multi-protein complex made out of other division proteins is responsible for

aiding the formation of the septum and the constriction of the FtsZ ring. The exact mechanism of septal closure is still a matter of research.

## 1.2 Spatial Regulation FtsZ Assembly

FsZ ring assembly initiates the septum formation eventually deciding where cell has to divide. The correct placement of FtsZ ring ensures that each daughter receives a copy of the newly replicated and segregated chromosome and other cellular machinery needed for the viability of daughter cells. The correct placement of FtsZ ring is regulated by two independent process Nucleoid Occlusion and Min Protein dynamics. These two process ensures that the septum formation at the middle of cell.

### 1.2.1 Nucleiod Occlusion

This is the phenomenon in which FtsZ ring is inhibited from forming over the nucleoids[31, 30]. In the early stages of cell cycle the nucleiod is present in the middle of cell, which makes it inaccessible for septum formation. Further after nucleoid segregation mid cell also becomes nucleus free which make it accessible for FtsZ ring formation. Thus, FtsZ ring assembly is spatially restricted to the nucleoid free regions of the cell, i.e. the cell poles and mid-cell following nucleoid segregation The mechanism of nucleiod occlusion is poorly understood and it is an area active investigation. Recently a SimA DNA binding protein in *E.Coli* has been identified as the one of the factors necessary for nucleoid occlusion[28].

### 1.2.2 Min proteins

Min proteins act independently of nucleiod occlusion and co-assist in the correct placement of FtsZ ring at midcell[10, 9]. It consists of MinC, MinD and MinE proteins whose spatio-temporal behavior ensures that only the middle of the the cell is viable for septum formation[3, 8].

**MinD** MinD is a peripheral membrane ATPase that acts as membrane assembly protein for both MinC and MinE. MinD exists in two forms namely MinD-ADP and MinD-ATP. The MinD-ATP has got higher affinity to associate itself to membrane compared to Min-ADP. The recent studies suggest that MinD can associate with the membrane even in the absence of other Min proteins whereas membrane association of MinE or MinC requires co expression of MinD. MinD is also known to be an activator of division inhibitory effect of MinC proteins. MinD shows sequence homology to par proteins that participate in plasmid and DNA partition system. Further more it's homologues are present in at least 17 bacterial species and so far in 8 of 12 bacterial genomes that have been sequenced completely.

**MinE** MinE is an 88 amino acid protein with two known functional domains. The N-terminal domain (amino acids 1-33) is required for it to interact with MinD and to counteract MinCD-mediated division inhibition. The C-terminal topological specificity domain (TSMinE, amino acids 34-88) is required to suppress the inhibitory activities of MinCD specifically at midcell. Additionally, this domain mediates homodimerization of the protein. The structure of the C-terminal domain as a dimer has been solved and reveals that the protein dimerizes in an anti-parallel fashion. The N-terminal domain is not shown in the structure but is predicted to be a nascent helix.

**MinC** MinC is a 231 amino acid protein comprised of two domains of roughly equal size. The N-terminal domain interacts with FtsZ and is required to inhibit FtsZ polymerization in vitro and Z-ring formation in vivo. MinC lacks site specificity and as result when it is expressed in absence of MinD and MinE, leads to inhibition of septation across the entire cell. Both MinD and MinE are required to provide site specificity to MinC. The mechanistic basis of how MinC inhibits FtsZ polymerization is still unknown.

### 1.2.3 Oscillation of Min proteins

The Min proteins show a remarkable oscillation pattern that ensures the assembly of the FtsZ ring at midcell only and prevent minicelling. To explain the oscillatory behavior of MinCDE it is necessary to understand quantitatively the assembly of MinCDE on the cell membrane at one pole, periodic release of Min proteins from membrane, its assembly at the other pole (following diffusion through the cytoplasm) instead of rebinding at the same pole.

Both MinD and MinE regulate the cellular distribution of MinC. In addition, MinD and MinE each influence the cellular distribution of the other. So the dynamics of MinC is regulated together by MinE and MinD. In cells expressing all three Min proteins MinC undergoes a rapid and dynamic localization cycle in which the protein oscillates from one cell pole to the other.

For the polar zone assembly of Min proteins two alternative hypothesis is proposed. The first hypothesis assumes self-assembly without any positional markers on the membrane for preferential MinD-ATP binding. The MinD-ATP molecules bind to the polar zones and start growing towards the middle of the cell. Moreover, once it associates with membrane it acts as a nucleation center for the assembly of other MinD-ATP. The idea that MinD-ATP can act as a self-associating system is compatible with the co-operative dynamics of the MinD-ATP observed in vitro[26, 8]. The alternative hypothesis says that the oscillations are not entirely due to spontaneous self-organization, but there are positional markers in either cell pole which distinguish the poles from the other portions of the membrane and hence guide oscillations.

The membrane bound MinD-ATP recruits MinC and MinE to cell membrane. MinE is known to trigger the dissociation of MinD-ATP from membrane, which is released back into the cytoplasm in MinD-ADP form together with the release of a phosphate. In the cytoplasm, MinD-ADP is converted to MinD-ATP via nucleotide exchange.

The membrane associated MinCDE proteins grows towards the center of the cell from the pole. As it reaches near the middle of cell membrane associated MinE assembles into a ring like structure at the leading edge of polar zone. This is called E ring from where disassembly of MinCD from membrane starts moving towards the polar regions. During disassembly process E ring remain associated with medial edge of the MinCD polar zone. As the MinE hydrolyzes the membrane-bound MinD off the mebrane and releases them back into the cytoplasm, a concentration gradient is created between the two poles. This concentration gradient drives the diffusion of cytoplasmic MinD to the opposite pole creating the new polar zone of MinD capped by the E-ring which lags behind the MinD-dynamics. The cycle is repeated leading to the pole-to-pole oscillation of Min proteins. This oscillatory behavior leads to an alternating peak of MinCD which shifts over time from one pole to another. When time-averaged over several oscillation periods, the resulting distribution of MinCD has a bimodal peak at both poles and a dip in the middle of the cell. As pointed out earlier, since MinC is a division inhibitor, such a bimodal distribution of MinC minimum allows for the formation of the FtsZ ring at mid cell only and not at the poles, thereby leading to viable daughter cells. Here it is further assumed that the length of time that the polar sites are free of the division inhibitor during each oscillatory cycle is too short to permit formation of a stable FtsZ ring. The period of oscillation is influenced by the cellular $\frac{MinD}{MinE}$ ratio and by growth conditions, which generally has periodicity in the range of 40s-120s. As a result oscillation occurs many times in each cell cycle. Also MinE always lags MinD dynamics. This phase lag is manifestation of large copy numbers of MinD compared to MinE inside the cell.

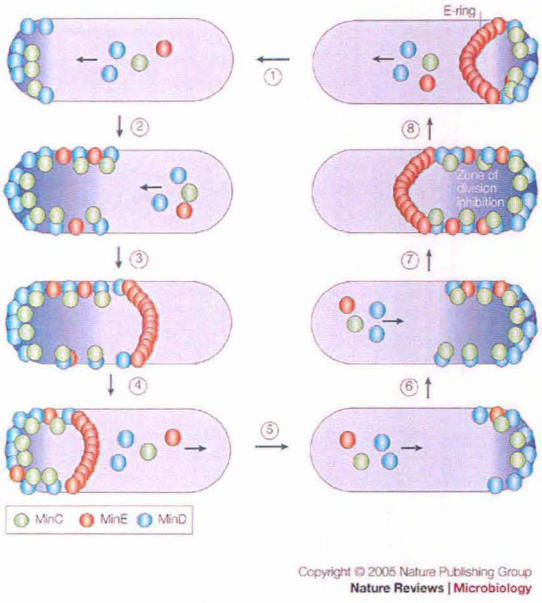



Figure 1.1: The oscillation cycle representation adapted from [17]. The figure 1-2 and 5-6 represents the assembley process of MinD and MinE at polar zones which grows towards the mid-cell. The MinE ring assembles at leading edge of the polar zone figure 3 and 7. Figure 4,5,8 and 1 shows the disassembly and re-assembly process of MinD and MinE at two poles.

## 1.3 Mathematical Models

Several mathematical models have been proposed to explain the oscillatory dynamics of Min proteins and they have been quite successful in capturing the in vivo phenomena[11, 14, 21, 13, 18, 7, 22, 19]. These mathematical models further suggest that these oscillations can generate itself in the absence of any specific polar nucleation site, based only on cytoplasmic diffusion, the relative association and dissociation properties of the proteins for the membrane and the rate of conversion of the released MinD-ADP to MinD-ATP. However, the explanation of Min oscillations in terms of the spontaneous self-organization of Min proteins, does not exclude the possible role of special polar binding sites in seeding the assembly of MinD-ATP complex at the poles.

## 1.4 Partioning of Min Proteins

While the above models looked into the oscillatory behavior of Min proteins in parent cells, there have been only a couple of attempts to study the oscillatory dynamics of Min proteins during constricting cells[23, 29]. The partitioning of Min proteins and regeneration of oscillation in daughter cells is important

to investigate because of the fast assembly rate of FtsZ ring[1]. Recent studies have reported that the new division site becomes available for FtsZ targeting almost at the same time of final contraction phase, in other words even before division of mother cell is complete[27]. It is further reported that the oscillation regenerate itself in almost all daughter cells. This is very important for the survival of daughter cells so that it can avoid mincelling[4]. Although there are few experimental attempts to look into Min oscillation during septation process and regeneration of oscillation in daughter cells but there are attempts to mathematically model the aforementioned process.

## 1.5 Deterministic Model of Septation

In order to investigate the partitioning of Min proteins and regeneration of oscillations in daughter cells, an important study was conducted using Huang et.al[11] model. The analysis is done in 3D using deterministic approach, which revealed many remarkable properties[23]. The study revealed that only 65 to 85 percent of the daughter cells (depending on parameter values) are able to regenerate Min oscillations. The Min oscillation models have reported that for the oscillation to occur the $\frac{MinD}{MinE}$ should have a threshold value otherwise oscillations will not happen. For the Huang et al[11] model, ratio of densities of $\frac{MinD}{MinE}$ has to be greater than 2.7 for the oscillations to regenerate in daughter cells. It was further observed that when the densities in parent cell was close to threshold, a large number of daughter cells do not oscillate but when the parent densities were far from the threshold, a smaller fraction of daughter cells did not oscillate. Also simultaneous equipartition of both MinD and MinE is never observed. This may be the effect of phase lag between the MinD and MinE oscillations. To study the effect of enhanced polar binding on polar membrane they also increased the polar binding rate of MinD-ATP with the constraint that oscillation in the parent cell is not affected. In addition to that densities of MinD and MinE were varied while all other parameters where kept constant. They observed that at the stability threshold of ratio of $\frac{MinD}{MinE}$ density there is some improvement in number of daughter cell going oscillations but away from this threshold no significant

improvement was observed. Variation of the other interaction parameters within plausible limits did not show any significant improvement in number of daughter cells undergoing oscillations either.

An important question that arises is: why does the 100 percent of daughter cells does not oscillate in the model, whereas in reality almost all daughter cell shows oscillation regeneration? The reason for this can be attributed to the phase lag between the oscillation pattern of MinD and MinE. Due to different phases of oscillations of MinD and MinE there is always some partitioning error in distribution of Min proteins in daughter cells. In order to regenerate oscillation in daughter cell the partitioning should happen in such a way that the ratio of $\frac{MinD}{MinE}$ is greater than the threshold value in both daughter cells. The oscillation is preserved if there is larger concentration of MinD and MinE near the middle of cell at the time of septal closure. Whereas if concentration is larger at the poles then only one daughter cells oscillates.

## 1.6 Stochastic Model

Although the above deterministic analysis gave important insights into the problem, the effect of stochastic variations cannot be ignored[24]. Biological systems are inherently noisy with perturbations from both external and internal factors[20]. While external factor includes environmental factors and any other similar external stimulus what people call external noise. On the other hand cellular process are subjected to fluctuations from many factors which comes from their inherent nature of existence and operation called intrinsic noise. The deterministic approach assumed continuous variation of Min proteins density in space and time whereas these proteins are present in low copy numbers inside the cell. This assumption is limited by the fact that randomness or fluctuations in a system are proportional to square root of number of particles or system size. This is the famous Schrodinger $\sqrt{n}$ law [24] which gives quantitative insight of stochasticity based on system size or particle number. The Min proteins are indeed found in finite copy numbers

roughly around 4400 MinD and 1000 MinE. Further these numbers are much less than that required to cover the cell membrane surface area. Assuming the dimension of cell to be 0.5 microns in radius and 4 microns in length, the total surface area of cell inner membrane comes out to be $4.71 \times (10)^{-12}$ microns including two end caps which is assumed to be hemisphere of radius 0.5 microns. Whereas considering MinD monomer to be 2.5nm in radius the total surface area it will be able to cover through linearly associating will be roughly around $.371 \times (10)^{-12}$[15, 16]. Which comes out to be 15 times lesser than total surface area available for association. Thus the stochastic effects are likely to play an important role in Min protein dynamics and partitioning after cell division.

## 1.7 Aim

Considering the inherent noisy nature of Min System our aim is to investigate whether stochasticity has any effect on the partitioning of Min proteins between daughter cells and consequent regeneration of oscillations in both daughter cells. We would like to explore the effect of stochasticity on the partitioning error.

We used the software package Mcell[12, 25] to perform stochastic analysis of Min protein partitioning after cell division. Mcell is capable of doing stochastic analysis in 3D and has been extensively validated. Like Kerr et al.[14], we adopted the model of Huang et al.[11] to model the MinD dynamics in Mcell. The model of Huang et al.[11] accurately captures the essential biochemical processes that are necessary for understanding the spontaneous generation of pole-to-pole Min oscillations. Furthermore it can be easily implemented into the Mcell environment to perform stochastic analysis.

## 1.8 Model

The Huang et.al[11] model uses following interactions with following parameters

$$MinDAdp \xrightarrow{k1} MinDAtp$$

$$MinDAtp + memberane \xrightarrow{k2} MinDmem$$

$$MinDAtp + MinDmem \xrightarrow{k3} MinDmem + MinDmem$$

$$MinDmem + MinE \xrightarrow{k4} MinDE$$

$$MinDE + MinDAtp \xrightarrow{k3} MinDE + MinDmem$$

$$MinDE \xrightarrow{k5} MinE + MinDAdp$$

| Rate Constants | k1 | k2 | k3 | k4 | k5 |
|---|---|---|---|---|---|
| Values | 1 | $3.8 \times 10^4$ | $9.0 \times 10^5$ | $5.6 \times 10^7$ | 0.7 |
| Units | $s^{-1}$ | $M^{-1} \times s^{-1}$ | $M^{-1} \times s^{-1}$ | $M^{-1} \times s^{-1}$ | $s^{-1}$ |

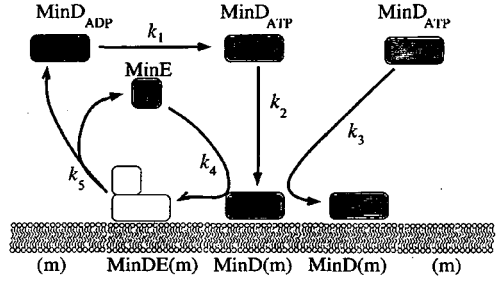



Figure 1.2: The interactions which are taken into account in modeling Min dynamics[11, 14]

# Chapter 2

# Material and Methods

Too realistically model the partitioning of the Min Proteins we need to take account the 3D structure of E.Coli cells. The simplest approach to modeling Min dynamics involves solving a set of continuous differential equations. This method has been successfully used to study many aspects of Min dynamics. Unfortunately, these models ignore the stochastic nature of protein dynamics in *E.coli* and often fall short of providing a satisfying, biologically accurate explanation of the phenomena being modeled. Biophysically realistic, stochastic 3D computer simulation is a powerful tool to explore Min partitioning. So we needed a 3D simulation environment were we can take into account the spatial features of Min Dynamics and implement the dynamics using Stochastic Algorithm to account for fluctuations. We chose the Mcell software package for this purpose.

## 2.1 Mcell

MCell is a highly optimized Monte Carlo simulation program that can be used to address complex biological problems (http://www.mcell.cnl.salk.edu)[12, 25]. The Mcell uses the Monte Carlo approach to perform realistic 3D simulation. This method provides approximate solution to variety of problems.

### 2.1.1 Monte Carlo Method for 3-D

For 3D reaction-diffusion problems, Monte Carlo methods replace sets of continuous differential equations with stochastic molecular events simulated directly within the volume of tissue. Individual molecules diffuse by undergoing a random walk. Movement trajectories can be reflected from arbitrary surfaces that can represent cell or organelle membranes, and thus a quantitative simulation of diffusion in complex 3D space is obtained. In addition, reaction transitions such as ligand binding, unbinding and protein conformational changes can be simulated probabilistically on a molecule by molecule basis. Thus, the Monte Carlo approach is very general and can be implemented with relative ease in complex structures. Furthermore, Monte Carlo simulations reproduce the stochastic variability and non-intuitive behavior of discontinuous, realistic 3D microenvironments that contain finite numbers of molecules.

MCell implements the Monte Carlo approach in very optimized way to give speed without sacrificing accuracy. In this method diffusion of individual ligand molecules is simulated using a random walk algorithm, and bulk solution rate constants are converted into Monte Carlo probabilities so that the diffusing ligands can undergo stochastic chemical interactions with individual binding sites such as receptor proteins, enzymes, transporters, etc. Thus, trial-to-trial variations can give important information about the system's stability, while the ensemble average of many trials yields the systems average behavior. Mcell was initially developed to extensively simulate activation of postsynaptic receptors at the neuromuscular junction.

To model Biological system using Mcell it is necessary to (i) specify the geometry of the sub cellular structures of the system, (ii) specify the diffusion constants and initial locations of diffusing molecules, (iii) define the stationary molecules on the surface of specified geometry (iv) specify the reaction mechanisms and kinetic rate constants governing the interaction of diffusing molecules with effector molecules, and (v) provide an appropriate time-step and number of iterations with which to simulate the spatial and temporal

evolution of the system. All these steps can be implemented in MCELL through the Model Descriptive Language (Mdl). This is a high level user interface and link between the steps of model design, simulation and output of results. How the simulation is performed, is controlled by the MDL language.

## 2.2 Introduction to the MCell Model Description Language (MDL)

MDL files consist of user comments, user-defined variables, keywords (capitalized), keyword statements, and keyword statement blocks that have subordinate statements enclosed in braces. User comments are begun with "/*" and end with "*/". Comments can be nested (comments within comments), and are ignored when the file is parsed. User variables are defined by equating an arbitrary name to a value. Names are typically given in lower case to distinguish them from upper case keywords. Types of variables include text, text expressions, numerical values, numerical expressions, and numerical arrays (elements may be values or expressions). Numerical expressions may include a variety of standard math functions.

**Keyword statements and blocks are used to:** Define values for simulation parameters. Two statements are required for every simulation, to define the timestep value and the number of time-step iterations. Many optional statements can be used to define other parameters, carry out runtime optimizations and define

**Logical Objects:** Logical objects have no physical location, and specify sets of input parameters for different types of ligand molecules, ligand release patterns, and chemical reaction mechanisms. Logical object definitions begin with a keyword that contains the word DEFINE.

**Physical Objects:** Physical objects have a location in space, and include various types of surfaces and ligand release sites. The user specifies an object name, and the first keyword describes the object. Physical objects are initially invoked as templates that can be modified in various ways, and

only exist in a simulation if instantiated.

**Design Metaobject Templates:** Physical object templates can be grouped into metaobjects, which in turn can be grouped into unlimited levels of higher order metaobjects.

**Instantiate Physical Objects and Metaobjects:** Creates actual simulation objects from templates.

**Output Data for Visualizations and Animations:** Here Commands for Visulization are specified.

**Output Reaction Data Statistics:** Here Reaction output Commands are specified.

**Output Other Data:** Uses syntax and formatting similar to the C programming language. Allows arbitrary file creation and write operations, printing of messages to the command line window, and conversion of numerical values to text variables.

### 2.2.1 General MDL File Organization

The left column shows an abbreviated version of an MDL file, as explained briefly in the right column. MDL keywords are capitalized, and italics indicate names, values, or expressions that would be supplied by the user. Subordinate statements within statement blocks have been omitted, and their positions are indicated by ellipsis marks. When the simulation is started, the file is read (parsed) from top to bottom. Some calculations for initialization are performed while parsing, so there is some order-dependence to the file layout. /* comment to describe purpose of Comments can appear anywhere to document the MDL file*/.

**variable-name-1** = text-expression User-defined variables can appear anywhere

**variable-name-2** = numerical-expression between statement blocks.

**variable-name-3** = numerical-array

**INCLUDE-FILE** = text-expression /* Other MDL files which is taking part in simulation */

**TIME-STEP** = numerical-expression /*Value given in seconds.*/

**ITERATIONS** = numerical-expression /*Total number of time-step iterations.*/

**EFFECTOR-GRID-DENSITY** = numerical-expression /* Optional keyword statements Global value for barycentric tiling.*/

**PARTITION-X** = [numerical array] /*Positions along x-axis to insert spatial partitions.*/

**PARTITION-Y** = [numerical array] /*Positions along y-axis to insert spatial partitions.*/

**PARTITION-Z** = [numerical array] /*Positions along z-axis to insert spatial partitions.*/

**CHECKPOINT-INFILE** = text-expression Name of checkpoint file to read during initialization.

**CHECKPOINT-OUTFILE** = Name of checkpoint file to write before stopping.

**CHECKPOINT-ITERATIONS** = Number of checkpoint iterations to run before numerical-expression stopping.

**DEFINE-LIGAND** . . . /*Defines Molecule */

**DEFINE-REACTION** . . . /*Define reactions and Rates*/

name-1 **BOX** ( . . . )/* Create 3D CUBE object*/

name2 **POLYGON-LIST** . . . /* Optional metaobject templates */

**INSTANTIATE** name-5 **OBJECT** . . .

/* Creates instance of how the defined objects have to simulated like ligand release time interval of release e.t.c */

**VIZDATA-OUTPUT** . . . /*Visualization output and Molecule position data*/

**REACTION-DATA-OUTPUT** . . . /*Reaction Output*/

### 2.2.2 Random number seed

This input parameter for Monte Carlo simulation whose value determines the values of random numbers used in the simulation.

### 2.2.3 Simulation time step

The numerical accuracy of MCell simulations depends primarily on the duration of the simulation time-step[25]. The time-step affects the average diffusion step length and the probability of reaction events. Validation of MCells Monte Carlo algorithms has shown that the average radial diffusion step-length should be no larger than about $\frac{1}{2}$ the radius of any diffusion barrier bottlenecks, and that simulation error of 1 or less can be achieved with probabilities of less than 0.2 for reaction events. In the present study a simulation time-step of $10^{-6}$ was used to satisfy these conditions. This value of time step is well verified and known to converge for most problems.

### 2.2.4 Spatial Partitioning

To speed up the simulation Mcell uses a unique technique of creating subspaces for simulating an entire 3D space. Spatial partitions creates transparent planes along x,y and z axis, which creates 3D subvolumes. Under optimal conditions each subvolume includes (wholly or partly) no more than a small number of mesh elements. The search for collisions between diffusing ligand molecules and mesh elements then can be restricted to individual subvolume, thus dramatically increasing execution speed. This partitioning is achieved by using appropriate key word in MDL language.

## 2.3 Implementation of Min Dynamics

To model the Min Dynamics we adopted the model of Huang et.al[11] which is so far most appropriate model to recover Min Oscillations. This is continuous 3D model for modeling Min oscillation. It is based on the most realistic biological phenomenology investigated so far. Furthermore it can be easily implemented into the Mcell environment to perform stochastic analysis.

### 2.3.1 Model Geometry

To create model 3D geometry of Cell we used Blender (www.blender.org), a computer aided design software. The blender is used because of three reasons. First it is open source. Second it has got a very good user group so learning is very easy because off large support base. Third, since the Mcell team supports blender, plugins are available which directly converts the 3 D geometry into Mdl format.

For our case we created 3 different kinds of geometry to evaluate the partitioning error.

1. In the first case we created a 20 vertices's cylinder having the following dimensions: 4 microns in length and .5 micron in length. This model geometry is used to validate the model and to check whether oscillations are generated prior to septation.

2. To model the case of gradual septation we created a cylinder, 5 microns in length and 0.5 micron in radius. To mimic septal growth, a mesh is created in the middle of the cell which grows in 10 steps from 0.5 microns to 0 microns. The radius is linearly spaced from 0.5 microns to 0 microns whereas area of the mesh increases quadratically. To achieve this 10 different configurations of the septating cell with gradually advancing levels of septum growth were created.

3. To model instantaneous septation the dimensions of cylinder are kept as above. Instead of creating 10 different configurations, only 2 are created. The first is a cylinder of length 5 microns length and radius 0.5 microns. The other is a cylinder of length 5 microns with an opaque (to diffusion of particles across the disk) circular disc in the middle of the cylinder, which completely isolates the two halves of the cylinder, thereby, mimicking two independent and disconnected daughter cells.

In Appendix A, we have provided a sample of Model geometry used to simulate the gradual septation process.

### 2.3.2 Implementation of Model in MDL

We created three different sets of simulation protocols to implement the problem.

1. **Oscillation Check**

   To verify that pole-to-pole oscillations are generated prior to septation, we first wrote an mdl file to implement Huang model[11] in the first geometry, which is described above. We set up the simulation for 20 minutes. The 4400 MinD and 1000 MinE are released from the 0.25 microns distance from the two poles of cylinder separately.

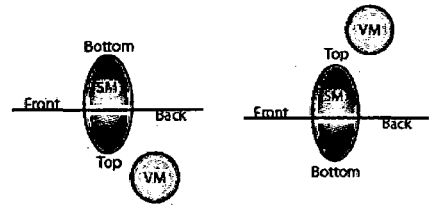
2. **Modeling of Gradual Septation**

   For this simulation we used the type of cellular geometry and configuration described in the second point of the above sub-section. The cylinder length was kept at 5 microns. To model gradual septation we needed to change the geometry of simulation during the entire period of simulation. To achieve this we used two important features of Mcell, namely check pointing and defining new geometry inside the already defined cell object. Check points are means to stop the simulation after executing it for a fixed number of iterations. After that, a new mdl file can be defined which will start from the point at which the simulation was suspended, together with new changes which might be new reactions, release of new molecules etc. This is implemented using keywords in mdl, namely checkpoint infile which specifies check point file from which simulation has to be started, checkpoint outfile which specifies check point file to be written at the end of executing defined number of iteration which can be used as initial condition for next simulation and checkpoint iterations this specifies number of iterations to execute before writing check point out file. Note if it is greater that Iterations then no outfile is written.

We wrote 10 Mdl files which has information about implementing the stochastic version of the Huang et al.[11] model and 10 Mdl files for

mimicking the model geometry during the gradual septation process. Each of those Mdl files are used to simulate Min dynamics during the process of gradual growth of the septum at midcell and were included at appropriate times in simulation. The entire septation process was assumed to occur in 10 steps each leading to an increase in the area of the growing septum from the circumference towards the central axis of the cylinder. The simulation is initiated with the geometry of cylinder with no septal region inside. Sufficient time was allowed for Min oscillations to be established in the cylindrical cell. We then check pointed to the time point up to which the oscillations establishes itself inside modeled cell from the observations we made from the oscillation check simulations. From this check point we simulate new Mdl file with new mesh which has an ingrowing septal ring at midcell. This new mesh contains a circular mesh extending from the circumference of the cylinder at a distance of 0.5 microns from the cylinder axis to 0.44 micron from the axis models the growing septal membrane. Then in the time span of 8-10 minutes 9 similar Mdl files are simulated to mimic the growth of the septum up to the point of complete septal closure through check pointing and new meshes which grows from outer radius of the cylinder towards the center of cylinder. After complete septal closure, which creates two independent daughter cells, the simulation is allowed to again run for 10 minutes to study whether oscillations are observed in both daughter cells. A point to noted here is that according to Mdl syntax we have to define physical objects for simulations, which includes geometry. Therefore, the geometry has to be defined as a physical object at the start of simulation. To introduce a new mesh through check point feature the geometry object name should remain shame throughout all Mdl files. In order to mimic septal growth the cylinder and septal region is treated as single object. We kept the same object name in new Mdl files in which these septal regions are defined. With these two considerations we were able to model the septation process using Mcell. The initial condition of molecule release is kept same as in the case of oscillation validation model.

After implementing septal growth another important thing that needed to be taken into consideration, were the reactions at the growing septum. Mcell gives the flexibility to restrict the particular reactions on the particular surfaces through the power full feature of surface class. A particular region in the model geometry can be specified to belong to a particular surface class and only those reactions will take place on that region which are defined with that particular surface class. Moreover, it is possible to specify the orientations of the surface molecules on surface region and restricting reactions to happen in specified orientation only. Utilizing these two powerful features, we defined new surface class for the septal region. We wrote the same set of interaction of Huang model[11] with new set of orientations with different surface class so as to differentiate between molecules distributed between two daughter cells and restrict the interaction accordingly. The two different orientation is shown in figure 2.1 which was used to differentiate between two sides of septum.




(a) The Top back orientation

(b) The Top Front orientation

Figure 2.1: Two different set of orientations to model reactions occurring on either side of the septum between two daughter cells. VM (Volume Molecules), SM (surface Molecule). Line represents the septal membrane

The Top back orientation is used to model reaction occurring in daughter cells geometry from 0 to 2.5 along z axis whereas the Top front orientation is used to model reaction occurring in daughter cells geometry from 0 to -2.5 along z axis

Third step was to time the septal growth. For this first simulation was allowed to run for 20 minutes so that stable oscillation is established. After that, at intervals of 1 minute, new septal rings are introduced in the middle of the cell to mimic the radial growth of the septum from the cell membrane towards the central axis of the cell. The septal closure was completed in 8-10 minutes so as to match with the experimentally observations. After complete septal closure when two daughter cells are physically separated the simulation is allowed to run for 20 minutes again to observe the oscillation regeneration in daughter cells.

3. **Instantaneous Septation**

   In this case all the condition are same as above, except that instead of separation happening gradually in 8-10 minutes, it happens instantaneously. After running for 20 minutes, the new cellular configuration with a circular disk at midcell (see description above) is introduced which completely separates the two daughter cells and it is again allowed to run for 20 minutes to check whether oscillations are regenerated in the daughter cells.



All the simulations were run on an AMD 3 Ghz Dual core processor, an HCL workstation, and a SUN opteron cluster which is part of the High Performance Computing Facilty(HPCF) of JNU. It took almost 5 days to finish oscillation validation model and approximately 10 days for a single run submitted to study protein partitioning after a cell division event using the septation (either gradual or instantaneous) model. We generated 20 realization of each model with 20 different seed values except for the case of instantaneous partitioning where 5 realizations were generated.

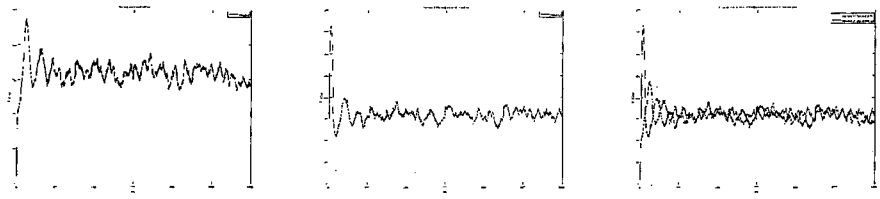
In Appendix B, we have provided a sample of the code (Mdl file) used to simulate the gradual septation process.

# Chapter 3

# Result

## 3.1 Validation of Model

A stochastic model of MinD and MinE oscillations was constructed following the deterministic set of reactions of Huang et.al [11] summarized in figure section 1.8. We initialized the simulation for 5400 proteins. Where we kept the ratio $\frac{MinD}{MinE} = 4$. So we simulated the model with 4400 MinD and 1000 MinE. In order to verify that the stochastic simulations could faithfully reproduce the oscillation we plotted the number of MinD on two end caps of cylinder versus time. For this we ran the stochastic simulation 16 times using different seed values. Then we averaged the data and plotted it against time. We found phase lag between peaks of MinD(m) which is accompanied by consistent noise.. This was expected for any system with finite number of discrete particles.



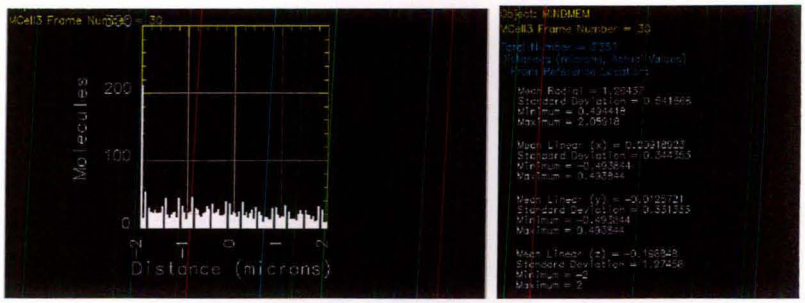
(a) MinD(m) on the end-cap at z=+2 micron

(b) MinD(m) on the end-cap at z=-2 micron

(c) MinD(m) on both the caps plotted together

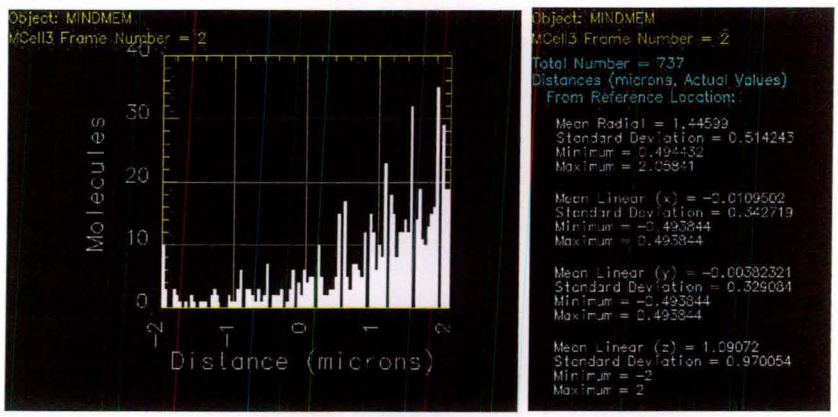
Figure 3.1: MinD(m) on two end caps

Then we ploted the distribution of MinD(m) along z axis at two different time points. We found peaks of MinD(m) on different end caps at different time points.




(a) Histogram plot of MinD(m) along z axis (b) Statistics of MinD(m) for this time point

Figure 3.2: Profile plot of MinD(m) where peak occurs at z=-2

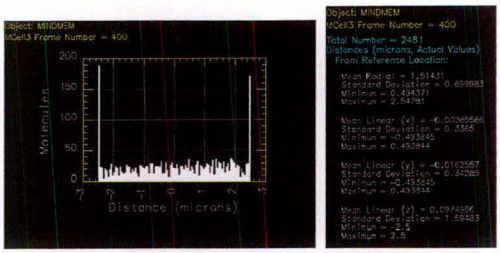



(a) Histogram plot of MinD(m) along z axis (b) Statistics of MinD(m) for this time point

Figure 3.3: Profile plot of MinD(m) where peak occurs at z=2

## 3.2 Gradual Septation

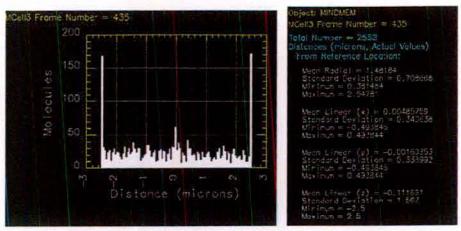
Once oscillations are observed in the simulations we started implementing septation process in model geometry by introducing septum in the middle of the cylinder. We kept the initial condition same as in model validation simulation. The first septum was introduced after 20 minutes of start of simulation. Total time of septal closure was timed to 8 minutes. After 8 minutes the complete isolation of two daughter cells is made. We observed following results in first simulation results.




(a) Histogram plot of MinD(m) along z axis

(b) Statistics of MinD(m) for this time point

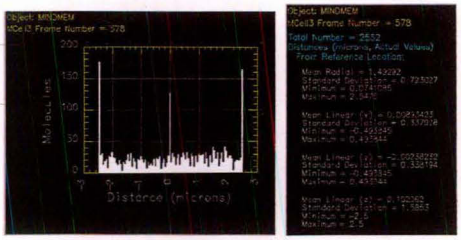
Figure 3.4: Profile plot of MinD(m) just before start of septation




(a) Histogram plot of MinD(m) along z axis

(b) Statistics of MinD(m) for this time point

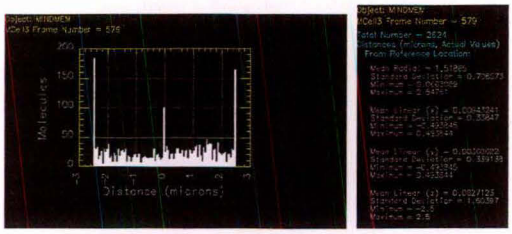
Figure 3.5: Profile plot of MinD(m) in the middle of septation




(a) Histogram plot of MinD(m) along z axis

(b) Statistics of MinD(m) for this time point

Figure 3.6: Profile plot of MinD(m) just before complete separation of the two daughter cells




(a) Histogram plot of MinD(m) along z axis

(b) Statistics of MinD(m) for this time point

Figure 3.7: Profile plot of MinD(m) in the just after complete separation of the two daughter cells

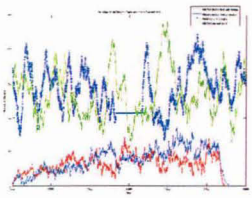


Figure 3.8: Plot of MinD(m) on the two endcaps of the cylinder and on the septum. The two different orientations of MinD(m) are plotted separately to differentiate between the two sides of the septum.

# Chapter 4

# Conclusion

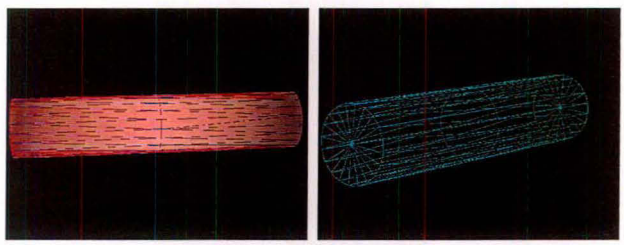
In this project we undertook a computer simulation study of the cell-division process in E.coli using a 3D stochastic model. Our aim was to study the effect of low protein copy numbers on the partitioning of Min proteins between daughter cells and the regeneration of oscillations in the two daughters after complete septation.

In the first run of gradual septation we observed that MinD(m) first increases on the septum which is expected because septum size increases with time. But after certain increase in septum size the MinD(m) fails to increase beyond certain threshold and shows variations around that threshold. Whereas the phase diffrence in MinD(m) on the ends of cylinder shows little variation once septum starts growing. Also there is little variation in the spatial distribution of MinD(m). This may be attributed to less diffusion space available at the center. More definite conclusion about the effect of stochasticity on partitioning can be made once enough samples of simulation is available. We are in the process of generating more data to compare the deterministic model and our stochastic 3D simulation.

More sensitive experimental observations of Min Oscillation during and after septation will aid in better understanding of the septation phenomena and help in realistically modeling the septation process to understand how Min oscillations are regenerated in the newly formed daughter cells.

# Appendix A

# Images of Model Cell Geometry



(a) Five microns cylinder (b) Wireframe image of cylinder

Figure A.1: Model cell geometry for model validation and septation Process

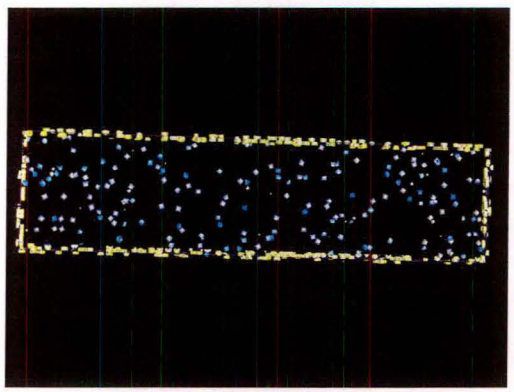
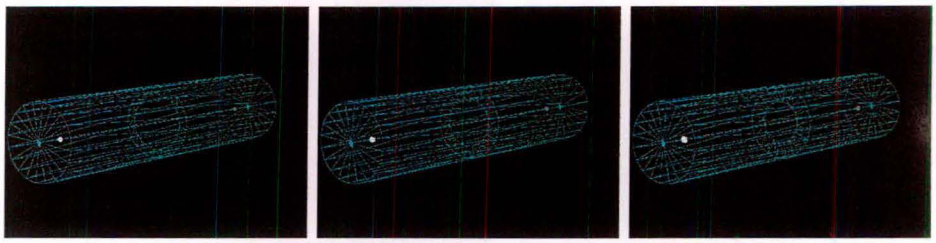


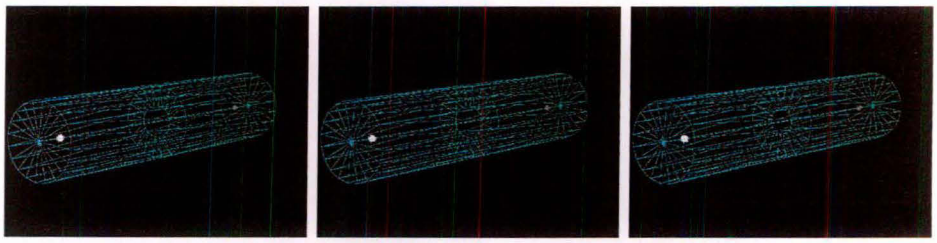
Figure A.2: Image of simulation running inside cylinder. Each color dots represents molecules. Yellow is surface molecule and blue is volume molecule



(a) Image of first septal disk

(b) Image of second septal disk

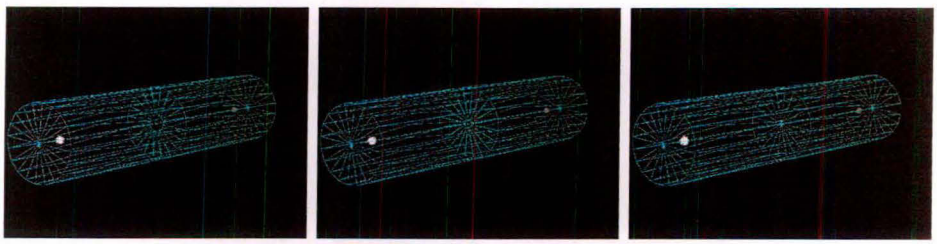
(c) Image of third septal disk



(d) Image of fourth septal disk

(e) Image of fifth septal disk

(f) Image of sixth septal disk



(g) Image of seventh septal disk

(h) Image of eighth septal disk

(i) Image of ninth septal disk. This marks the complete separation of the two daughter cells

Figure A.3: Images showing the progression of septation in ten stages.

# Appendix B

# Sample Mdl File

1. **Mdl Code for Model validation**

$ITERATIONS = 30e8$
$TIME\_STEP = 1e-6$
$CHECKPOINT\_INFILE = "chkpt\_1"$
$CHECKPOINT\_OUTFILE = "chkpt\_1"$
$CHECKPOINT\_ITERATIONS = 12e8$
$VACANCY\_SEARCH\_DISTANCE = .01$
$INTERACTION\_RADIUS = 0.003$
$PARTITION\_X = [0.125, 0, -.125]$
$PARTITION\_Y = [0.125, 0, -.125]$
$PARTITION\_Z = [[-2.2TO2.2STEP.025]]$
$DEFINE\_MOLECULES\{$
$MINDATP\{DIFFUSION\_CONSTANT\_3D = 2.5e-8\}$
$MINDMEM\{DIFFUSION\_CONSTANT\_2D = 0\}$
$MINE\{DIFFUSION\_CONSTANT\_3D = 2.5e-8\}$
$MINDE\{DIFFUSION\_CONSTANT\_2D = 0\}$
$MINDADP\{DIFFUSION\_CONSTANT\_3D = 2.5e-8\}$
$\}$
$DEFINE\_SURFACE\_CLASSES\{$
$surf\{REFLECTIVE = MINDATPTRANSPARENT = MINDMEM$
$REFLECTIVE = MINDADP\}$

$mem\{REFLECTIVE = MINDATPTRANSPARENT = MINDMEM$
$REFLECTIVE = MINDADP\}$
*/*mem surface class is for septum meshwhich will be used to model reaction on it*/}*
$INCLUDE\_FILE =$ "*Cylinder.mdl*"
$DEFINE\_REACTIONS\{$
$MINDADP \rightarrow MINDATP \quad [1] : rxn\_1$
$MINDATP, @surf' \rightarrow MINDMEM' \quad [3.8e4] : rxn\_2$
$MINDATP, +MINDMEM'@surf' \rightarrow MINDMEM'+MINDMEM' \quad [9e5] :$
$rxn\_coperative$
$MINE, +MINDMEM'@surf' \rightarrow MINDE' \quad [5.6e7] : rxn\_3$
$MINDATP, +MINDE'@surf' \rightarrow MINDMEM'+MINDE' \quad [9e5] :$
$rxn\_4$
$MINDE'@surf' \rightarrow MINDADP, +MINE, \quad [.7] : rxn\_5$
}
$INSTANTIATE \quad world \quad OBJECT\{$
$Cylinder \quad OBJECT \quad Cylinder\{\}$
$MINDADP\_release \quad SPHERICAL\_RELEASE_SITE\{$
$LOCATION = [0.0000, 0.000, 2.1]$
$MOLECULE = MINDADP$
$NUMBER\_TO\_RELEASE = 4400$
$SITE\_DIAMETER = 0.000$
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$LOCATION = [0.0000, 0.0000, -2.1]$
$MOLECULE = MINE$
$NUMBER\_TO\_RELEASE = 1000$
$SITE\_DIAMETER = 0.000$
}
}}
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$VIZ\_MOLECULE\_FORMAT = ASCII$
$FILENAME =$ "*septation*"

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$NAME\_LIST \quad \{ALL\_MESHES\}$
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$\}$
$MOLECULES\{$
$NAME\_LIST \quad \{ALL\_MOLECULES\}$
$ITERATION\_NUMBERS \quad \{ALL\_DATA@[[1TO30e8STEP3e6]]\}$
$\}$
$\}$
$INCLUDE\_FILE = "count\_output.mdl"$

2. **Mdl code which is used to model interactions from the start of septation and just before septal closure**

$ITERATIONS = 30e8$
$TIME\_STEP = 1e-6$
$CHECKPOINT\_INFILE = "chkpt\_1"$
$CHECKPOINT\_OUTFILE = "chkpt\_1"$
$CHECKPOINT\_ITERATIONS = 12e8$
$VACANCY\_SEARCH\_DISTANCE = .01$
$INTERACTION\_RADIUS = 0.003$
$PARTITION\_X = [0.125, 0, -.125]$
$PARTITION\_Y = [0.125, 0, -.125]$
$PARTITION\_Z = [[-2.2TO2.2STEP.025]]$
$DEFINE\_MOLECULES\{$
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$MINDMEM\{DIFFUSION\_CONSTANT\_2D = 0\}$
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$MINDE\{DIFFUSION\_CONSTANT\_2D = 0\}$
$MINDADP\{DIFFUSION\_CONSTANT\_3D = 2.5e-8\}$
$\}$
$DEFINE\_SURFACE\_CLASSES\{$
$surf\{REFLECTIVE = MINDATPTRANSPARENT = MINDMEM$

$REFLECTIVE = MINDADP\}$
$mem\{REFLECTIVE = MINDATPTRANSPARENT = MINDMEM$
$REFLECTIVE = MINDADP\}$
*/*mem surface class is for septum meshwhich will be used to model reaction on it*/\}*
$INCLUDE\_FILE = "Cylinder.mdl"$
$DEFINE\_REACTIONS\{$
$MINDADP \rightarrow MINDATP \quad [1] : rxn\_1$
$MINDATP, @surf' \rightarrow MINDMEM' \quad [3.8e4] : rxn\_2$
$MINDATP, +MINDMEM'@surf' \rightarrow MINDMEM'+MINDMEM' \quad [9e5] :$
$rxn\_coperative$
$MINE, +MINDMEM'@surf' \rightarrow MINDE' \quad [5.6e7] : rxn\_3$
$MINE; +MINDMEM; @mem; \rightarrow MINDE; \quad [5.6e7] : rxn\_8$
$MINDATP, +MINDE'@surf' \rightarrow MINDMEM'+MINDE' \quad [9e5] :$
$rxn\_4$
$MINDATP; +MINDE; @mem; \rightarrow MINDMEM; +MINDE; [ \quad 9e5] :$
$rxn\_9$
$MINDE'@surf' \rightarrow MINDADP, +MINE, \quad [.7] : rxn\_5$
$MINDE'@mem' \rightarrow MINDADP' + MINE' \quad [.7] : rxn\_1$
$MINDE, @mem' \rightarrow MINDADP, +MINE, \quad [.7] : rxn\_11$
$\}$
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$MOLECULE = MINDADP$
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$\}$
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$LOCATION = [0.0000, 0.0000, -2.1]$
$MOLECULE = MINE$
$NUMBER\_TO\_RELEASE = 1000$
$SITE\_DIAMETER = 0.000$

}
}}
$VIZ\_OUTPUT\{$
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}
$MOLECULES\{$
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$ITERATION\_NUMBERS \quad \{ALL\_DATA@[[1TO30e8STEP3e6]]\}$
}
}
$INCLUDE\_FILE = "count\_output.mdl"$

3. **Mdl code which is used to model interactions after septum closure**

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$CHECKPOINT\_OUTFILE = "chkpt\_1"$
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$INTERACTION\_RADIUS = 0.003$
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$PARTITION\_Y = [0.125, 0, -.125]$
$PARTITION\_Z = [[-2.2TO2.2STEP.025]]$
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$MINE\{DIFFUSION\_CONSTANT\_3D = 2.5e-8\}$
$MINDE\{DIFFUSION\_CONSTANT\_2D = 0\}$
$MINDADP\{DIFFUSION\_CONSTANT\_3D = 2.5e-8\}$
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$DEFINE\_SURFACE\_CLASSES\{$
$surf\{REFLECTIVE = MINDATPTRANSPARENT = MINDMEM$
$REFLECTIVE = MINDADP\}$
$mem\{REFLECTIVE = MINDATPTRANSPARENT = MINDMEM$
$REFLECTIVE = MINDADP\}$
*/*mem surface class is for septum meshwhich will be used to model reaction on it*/*
$INCLUDE\_FILE = "Cylinder.mdl"$
$DEFINE\_REACTIONS\{$
$MINDADP \rightarrow MINDATP \quad [1] : rxn_1$
$MINDATP, @surf' \rightarrow MINDMEM' \quad [3.8e4] : rxn\_2$
$MINDATP'@mem' \rightarrow MINDMEM' \quad [3.8e4] : rxn\_6$
$MINDATP, @mem' \rightarrow MINDMEM, \quad [3.8e4] : rxn\_6\_b$
$MINDATP, +MINDMEM'@surf' \rightarrow MINDMEM'+MINDMEM' \quad [9e5] :$
$rxn\_coperative$
$MINDATP'+MINDMEM'@mem' \rightarrow MINDMEM'+MINDMEM' \quad [9e5] :$
$rxn\_7$
$MINDATP, +MINDMEM, @mem' \rightarrow MINDMEM, +MINDMEM, \quad [9e5] :$
$rxn\_7\_b$
$MINE, +MINDMEM'@surf' \rightarrow MINDE' \quad [5.6e7] : rxn\_3$
$MINE' + MINDMEM'@mem' \rightarrow MINDE' \quad [5.6e7] : rxn\_8$
$MINE, +MINDMEM, @mem' \rightarrow MINDE, \quad [5.6e7] : rxn\_8\_b$
$MINDATP, +MINDE'@surf' \rightarrow MINDMEM'+MINDE' \quad [9e5] :$
$rxn\_4$
$MINDATP'+MINDE'@mem' \rightarrow MINDMEM'+MINDE' \quad [9e5] :$
$rxn\_9$
$MINDATP, +MINDE, @mem' \rightarrow MINDMEM, +MINDE, \quad [9e5] :$
$rxn\_9\_b$
$MINDE'@surf' \rightarrow MINDADP, +MINE, \quad [.7] : rxn\_5$
$MINDE'@mem' \rightarrow MINDADP' + MINE' \quad [.7] : rxn\_10$

$MINDE, @mem' \rightarrow MINDADP, +MINE, \quad [.7] : rxn\_11$

}

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}

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}

}}

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}

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$ITERATION\_NUMBERS \quad \{ALL\_DATA@[[1TO30e8STEP3e6]]\}$

}

}

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# Bibliography


[1] Lutkenhaus J Addinall S G, Cao C. Temperature shift experiments with an ftsz84(ts) strain reveals rapid dynamics of ftsz localization and indicate that the z ring is required throughout septation and cannot reoccupy division sites once constriction has initiated. *J. Bacteriol.*, 179:4277–4284, 1997.

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