

E Coli Project Paper Incredibly Rough Draft

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The dynamics of the Min-protein system help *Escherichia coli* bacteria regulate the process of cell division by identifying the center of the cell. We model the Min-protein system in bacteria that have been forced into unusual flattened shapes, as have recently been experimentally observed. We find that although the presence of Min oscillations is robust in a wide variety of cellular configurations, the location of the peaks is strongly affected by the cellular shape. In some cases no periodic oscillations are observed. In particular, we find that cellular shapes observed experimentally to present irregular oscillations do so in the theoretical model, consistently [or inconsistently?] with experiment. [**In agreement with previous theoretical and experimental results, we observe “rotating” behavior in certain shapes having three corners.**]

I. INTRODUCTION

It is vital that during the process of bacterial cell division a cell avoid minicelling, or splitting into daughter cells with lopsided volumes. During this process a long FtsZ polymer chain develops on the cell wall in the center region of the cell that dictates the plane of division[1] [2]. Previous experimental studies have shown that the MinC protein, known to inhibit the FtsZ polymer[3], exhibits a pole to pole oscillatory behavoir in conjunction with the MinD and MinE proteins, while the oscillating MinE tends to be more localized in the center of the cell [4] [5] [6] [7] [8]. The MinC protein will then have a higher time averaged concentration in the cell poles as opposed to the center region of the cell, aiding in prohibiting the FtsZ from developing in the wrong region.

A significant amount of work has been done to develop protein reaction and diffusion models that exhibit accurate macroscopic dynamics of the MinD protein system. Early models involved free proteins that affect eachothers' rates of diffusion and membrane attachment but do not combine into compound states[9]. In 2003 Huang [10] improved upon this work with a simple model based on MinD-MinE combination, ATPase hydrolysis, and MinD membrane attachment that when simulated exhibits accurate MinD oscillations in cylindrical cells[10]. In this model cytoplasmic MinD is more likely to attach to the membrane when MinD is already clustered there (following observed non-linear attachment of minD on the cell membrane), and is stationary once attached. A number of studies have used an approach similar in that they do not rely on the ability of MinD to move along the walls and cluster [11] [9] [12] [13] [14], while studies have been made as well of models which rely on MinD mobility and attraction on the cell membrane[15][16]. Variations of the Huang 2003 model that stochastically account for variations of molecular interaction [13] and as well monte-carlo simulations that implement stochastic version of Huang's mean filed reaction rates confirm the major results obtained by Huang's model and more successfully predict experimentally observed oscillations in round cell phenotypes.[12] [13] [17] Biochemical models of broader scope have also been used to study the MinD sys-

tem and show consistent results.[18]. However, in general the results of the stochastic and monte-carlo simulations are similar to those given by Huang's mean field results. They both show the characteristic oscillations in the pill shaped cells. We do not see any compelling reason to avoid using this simple and successful model.

Previous studies have been made of the Min system's association with the cell membrane [19][20]. Studies have shown as well that MinD binds preferentially to regions enriched with cardiolipin, an anionic phospholipid that collects on regions of high negative curvature. This mechanism has been incorporated into other models.[12, 21, 22] However, this mechanism of combined clustering, phospholipids and MinD has not been observed in real cells. [23]

It is vital for the mechanism by which the cell accurately divides to be robust and indeed it has been shown to be[24]. Studies have shown that the oscillations can effectively find poles in abberent cell shapes [25] [26]. Varma et al. have studied three pronged connected tube shapes, both experimentally and in simulation[27] and have shown that even in these oscillations develop in regular patterns. However, Mannik et al. have shown that there are limits to this robustness. Forcing cells into flattened, irregular cell shapes adversely effects the Min system's ability to maintain their regular oscillatory behavior [**this link doesn't work = citemannik2012robustness**] [28] [29]. We follow the experimental work of Mannik et al. and use Huang's mean field differential equation reaction model to explore the model's ability to find regular oscillatory behavoir in a series of abberent shapes.

A. What is the MinD system and why is it important?

B. How proteins move in cell

II. METHODS AND INITIAL CONDITIONS

The model for the behavior of the MinD and MinE proteins inside the cell implements the same set of 5 reaction-

diffusion equations studied in the work of Huang et al (equations 1, 2, 3, 4, and 5)[10]. A 3d grid is constructed in cartesian coordinates with a grid spacing of $.05 \mu\text{m}$. We define our cell shapes and solve the reaction-diffusion equations numerically to observe the time evolution of the MinD and MinE concentrations inside the cell.

We start our cells with the MinD and MinE concentrations that are reported as wild type concentrations by Huang et al . We as well use the same reaction diffusion constants and and reaction rates,

$$\begin{aligned} D_D = D_E &= 2.5 \mu\text{m}^2 / \text{sec}, \\ \sigma_D^{\text{ADP}} \rightarrow \text{ATP} &= 1/\text{sec}, \sigma_D = 0.025 \mu\text{m}/\text{sec}, \\ \sigma_{dD} &= 0.0015 \mu\text{m}^3/\text{sec}, \\ \sigma_{de} &= 0.7/\text{sec}, \sigma_E = 0.093 \mu\text{m}^3/\text{sec}. \end{aligned}$$

We study cell shapes that are similar to those studied by Huang and also new, flattened pancake-like shapes, inspired by the experimental work of Mannik [?]. Our pill shapes differ from Huangs in that they are cylinders with hemisphere endcaps instead of pure cylindridrical shapes. Our cylinderidrical radius is 0.50μ and the lengths of our cells (measured between the tips of the endcaps) are 5μ , 4μ , 3μ , and 2.5μ .

With our flattened cell shapes we mean to study cells that are parrallel to those lodged into crevices by Mannik so we give them height of 0.25μ [?]. Viewed from the top down the cells will have the shapes described below and viewed from the side they have at their edges a semicircular protrusion (one may imagine the edges of a pancake).

Our first group of (three) flattened cell shapes are triangular. One of them is equilateral, one has a 3-4-5 right angle shape, and the last is iscoscolese.

Our second group of (four) flattened cells are our own creations, designed to investigate different patterns of protein oscillation behavoir.

The cell shapes and the sections that we split them into in our data analysis are shown in Figure [We need a big figure with all of this!!].

Kubitschek[30] [31] has shown in multiple experiments that at the time of cell division cells have a volume that is within a range of roughly $1\mu\text{m}^3$ to $2\mu\text{m}^3$. We follow Huang's simulations[10] and Mannik's experiments [[this link doesn't work = citemannik2012robustness](#)] and model cells that are slightly larger than this range. Amoung our flattened cells the two dimensional length scales are tuned so that every total cell volume is very close to $3\mu^3$. [[should we just leave it to the reader to figure out for themselves what our pill shape volumes are?](#)]

To interpret the results, we generated several different plot views of the printed simulation data. These plots included a time averaged view of the protein densities in the cell; a plot tracking the location of protein concentrations that were global maxima in space and local maxima in time; and an animated view that showed the

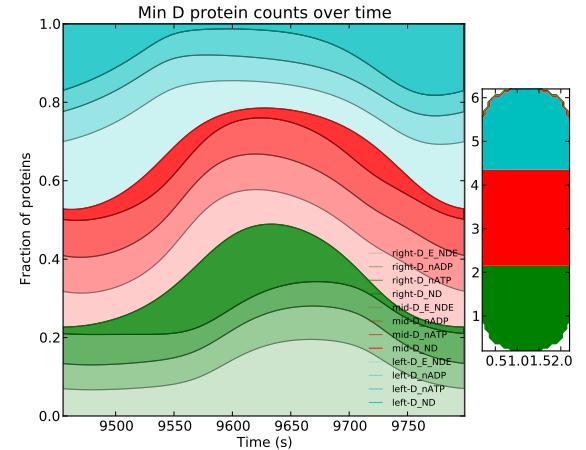


FIG. 1. Total protein fluctuation in the right, middle, and left parts of a $5\mu\text{m}$ by $1\mu\text{m}$ pill shaped cell (above) and a $3\mu\text{m}$ by $1\mu\text{m}$ pill shaped cell (below). The vertical axis shows stacked the total number of proteins that are of four different compound stages and in the right section of the cell (bottom four colors), in the middle of the cell (middle four colors) and in the left section of the cell (top four colors). The different forms of protein changes while the total number of MinD protein in the cell remains constant, shown by the fact that the very top line is horizontal.

actual dispersion of protein concentrations in the cell over time.

A. Mathematical Model

III. SPECIFIC RESULTS

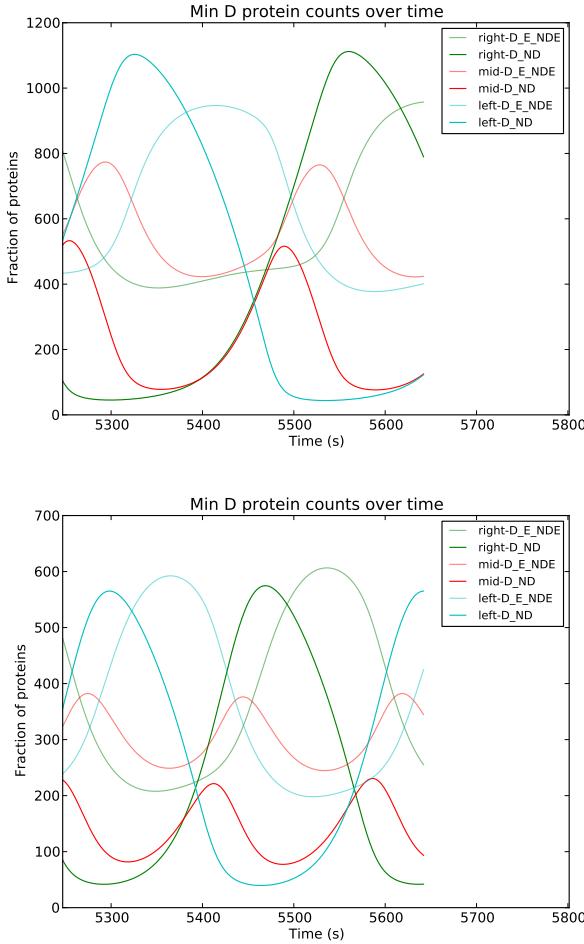
A. Pill Shape

Figure 1 shows total protein results for pill shaped cell of length 5μ and radius $.5\mu$. The plot shows both the transerence between different stages of protein compound and movement within the cell. Starting at the beginning of a period, we can see that first there is a spike on the left of MinD-ATP attached to the wall that is accompanied by a growing number of cytoplasmic MinD-ATP and MinD-ADP that are entering the left section, followed by a high peak in MinD-MinE-ATP. This peak gives way through the middle to peaks on the right side of the cell, the first being a small bump in cytoplasmic MinD-ATP that dissapears into a much larger spike in wall attached MinD-ATP, which is followed by a peak in wall attached MinD-MinE-ATP. The pattern during each period clearly shows the cycle of proteins rections, as well as the very regular nature of the pole to pole protein oscillations.

[our 5 length cells are labeled as 4 cells in the file labels, since 4 is the length of the cylinder. In the text I'll refer to the 5 length cells (file says 4) as 5 length cells. What huang's paper

Length(μ)	2.50	3.00	4.00	5.00
Period(sec)	sim	33	38	48

TABLE I. Period According to Pill Length

FIG. 2. Average protein density per unit area of wall for the wall attached stages of the protein cycle. Shown in the right, middle, and left parts of a $5\mu\text{m}$ by $1\mu\text{m}$ pill shaped cell (above) and a $3\mu\text{m}$ by $1\mu\text{m}$ pill shaped cell (below).

calls 4 length cells will be labeled by our files as 3 length cells, but will be refered in our text as 4 length cells]. We can see a clear difference in period between the 5μ , 4μ , and 2μ length cells. The oscillation periods for our 4μ show excellent agreement with the simulations of Huang et all [10]. This is to be expected since we begin our simulations with their reported wild type concentrations of MinD and MinE proteins. The periods for the different pill lengths are shown in the table below. We can see a direct correlation between size and period, as the proteins must diffuse further in a longer cell before they accumulate on the opposite wall.

Figure 2 shows the ave protein per unit area that is wall attached in the middle, right, and left sections of

the pill shaped cell. This is an important test of the central idea behind the utility of the Min protein system. The system is meant to place Min proteins on the walls in heavier concentrations in the caps of the cell and lower concentrations in the middle section, so that the FtZ polymer may build up along the center walls. We can see from the plot of the $3\mu\text{m}$ by $1\mu\text{m}$ cell that the MinD proteins reach concentration peaks in the caps that are roughly three times higher (for the MinD-ATP proteins) and roughly 1.5 times higher (for the MinD-MinE-ATP) than peaks in the center. Integrals of these plotted values show that the time averaged concentrations are likewise higher in the caps than in the center.
[am computing these integrals now in ave_plot.py but need to print out into maybe a bar chart]

B. Triangular Shapes

Figure 3 shows the equivalent motion and transference between compound states for the triangular states as Figure 1 does for the pill shaped cells. The equilateral triangle shows that in all three sections there is a spike in wall attached MinD-ATP directly followed by a spike in wall attached MinD-MinE-ATP. The pill had shown a similar pattern except that the mid section exhibited no such pronounced spike. Interestingly, the right and mid sections show spikes at similar times when compared to the left section spikes, which seem to oscillate in a manner shifted pi off from the other two. This behavior shows itself in the animations, which show a relaxation into a stable oscillation from a maxima in one corner to a double maxima in the other two corners, and back again. The left section heights are roughly twice that of the right section heights. The simulations are started with higher concentrations of proteins in primarily the right section, with some overlap into the middle section, so that perhaps this oscillation pattern seems reasonable. However, it's interesting to note that the oscillations, started with asymmetric density, do not naturally rotate around the corners of the triangle in a circular pattern.

It seems as well that there is a desired oscillation maxima for the particular shape and size of the cell, as can be seen by the relaxation, over the first four periods, to a regular maximal height of the right and left sections' total protein at the height of each spike.

[Note to make sure I'm interpreting the isosceles triangle data correctly: the triangle sides are 5,5,3, and the higher density starts in the corner that is opposite the short side. This corner is covered by the right section. I've verified this by looking at the protein_microscopy and drawing a little picture, which is on my desk...if you dare look for something on my desk!!!] The isosceles triangle starts its simulation with a higher concentration of protein in the section that is opposite the shorter side. It quickly relaxes into a stable oscillation pattern. This pattern shows that right corner section regularly has higher

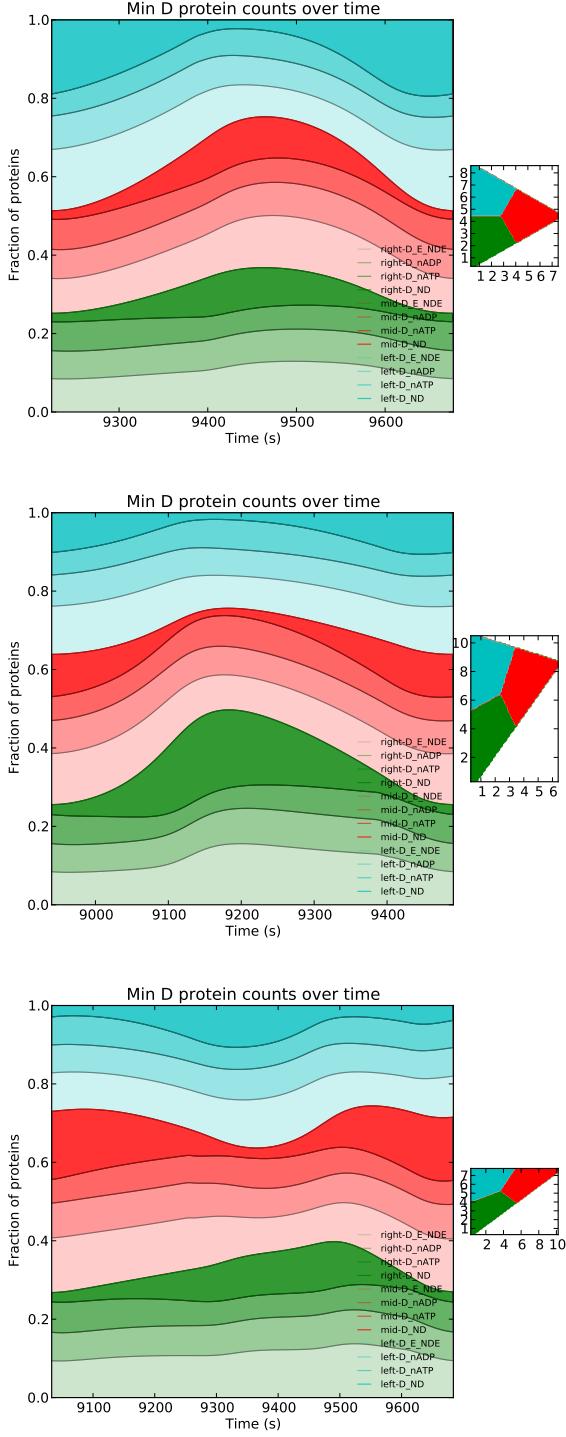


FIG. 3. Total protein fluctuation in the right, middle, and left parts of a equilateral (top), isosceles (middle), and 3-4-5 (bottom) triangle. The vertical axis shows stacked the total number of proteins that are of four different compound stages and in the different sections of the cell.

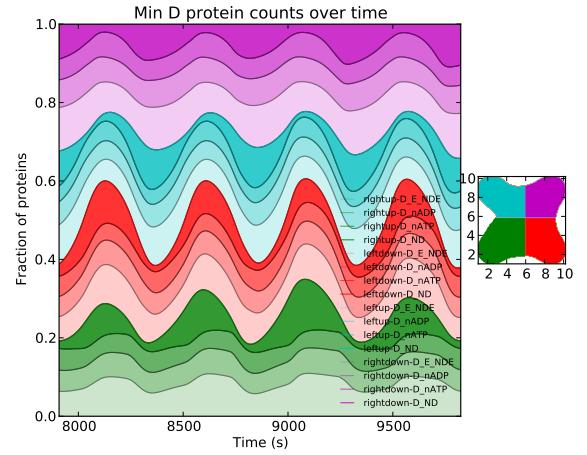


FIG. 4. Total protein fluctuation in the star cell shape. The vertical axis shows stacked the total number of proteins that are of four different compound stages and in the different sections of the cell.

spikes in total protein than the other sections, and that its spikes appear one pi removed from the spikes for the other two sections. This is similar behavior to that which is seen in the equilateral triangle, differing in that it converges more quickly towards this behavior and that it shows a greater exaggeration in the height of protein buildup in the right most section.

[Note on interpretation of 3-4-5 triangle: the higher density starts to the right of a vertical line that is parallel to the second longest side in the triangle (the longer of the two non-hypotenuse sides). This means the higher concentration covers the right angle and also the smaller angle in the triangle] The 3-4-5 triangle exhibits behavior that I need more data to properly observe. It does not converge as quickly as that for the isosceles or equilateral triangles.

C. Randst shape

[Cheat sheet for the shapes, need to put these into a big figure from the sections output: 96=star shape, 97=sideways sideways flying saucer pancake, 98=manniiks squished cell, 99=an A]

With arrow plots - have noticed that the wall attached proteins seem to climb along the walls with little arrows, so the maxima crawl along, in a way.

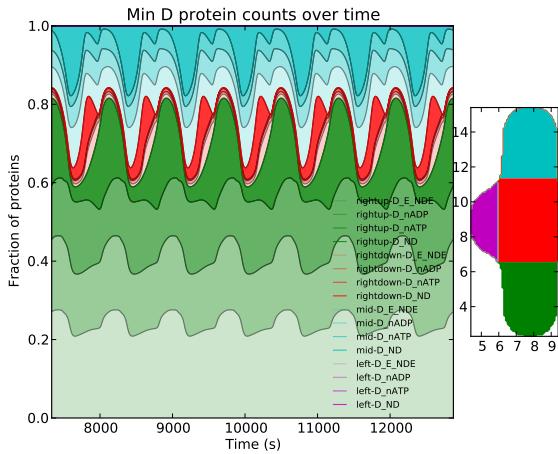


FIG. 5. Total protein fluctuation in the sideways flying saucer pancake cell shape. The vertical axis shows stacked the total number of proteins that are of four different compound stages and in the different sections of the cell.

1. Randst-96-Star

Looking at a few periods in detail. We find that there is a small increase in cytoplasmic MinE always directly before the beginning of climb in the membrane bound MinE peak. Also, there is at any one time a very large amount more of minE that is membrane bound than cytoplasmic in any one section. A difference of a factor of roughly 20 when membrane MinE is at its peak, and roughly 12 when membrane MinE is at its minimum. There seem to be two modes of oscillation, each exhibiting a density peak maxima that moves back and forth diagonally from one corner to the opposite, as if there were two overlapping, criss-crossed cells oscillating. In the box plot this is seen as roughly coinciding membrane minD peaks in the lower left and right corners, followed one pi later in peaks in the upper right and upper left corners. Interesting that this did not exhibit counterclockwise motion that Huang's 2008 paper might suggest. Should run sims that start in the wacky way that just started running with triangles.

We haven't simulated long enough yet, so no real pattern has shown itself. There's certainly oscillations, and it's almost as if there's competing modes - top to bottom, left to right, corner to corner, etc. Interesting.

[maybe?] The animations seem to relax into a pattern in which the oscillations go from the smallest corner, out to the other three, and then back to the smallest corner?
[There isn't enough data to see properly though, need to run for longer.]

2. Randst-97-

The sideways flying saucer pancake shape shows a truly interesting breakdown of an unstable equilibrium oscillation. For the first number of oscillations the maxima trade off right to left, from a high peak in the leftward compartment to vertically opposed peaks in the poles of the long axis of the cell. This is perhaps due to the fact that the starting density is highly concentrated to the right of a dividing line that runs parallel to the right vertical side. After a few oscillations the back and forth oscillations tip toward the vertical direction and the system loses its initial oscillation pattern. The total MinD animation shows that it falls quickly instead into a back and forth pattern between the two opposing endcaps, skipping right over the middle indentation. The oscillation at this point is reminiscent of the pills shape oscillation, showing the robust nature of the back and forth pattern.

Figure 5 shows between the top right and top left sections a pattern very close to the pattern seen in the pill shaped oscillations, with sharp spikes in wall attached MinD-ATP followed by lesser spikes in wall attached MinD-MinE-ATP, followed by the other end showing a similar behavior pi radius later. It is interesting to observe the middle section and the middle leftward indentation as well. It can be seen that the MinD-ATP shows moderate spikes in between the spikes on the top and the bottom, showing that the proteins are traveling through this section, but interestingly the left middle indentation shows this spike consistently later than the right middle section. As the protein travels back and forth through the cell, there is a certain amount of lag in the middle indentation. The system gets caught there for a little bit before moving on.

Figure 6 shows three arrow plots for this shape of cell. It's interesting that wall attached MinD never have global maxima in the middle flat region of the cell while both forms of MinE, the cytoplasmic MinE and the wall attached MinD-MinE-ATP, have a number of maxima in this region of the cell. It seems that the MinE never stray far from the middle of the cell - they approach the ends and react with the other proteins, sticking for a while on the walls. In order for a cytoplasmic MinE to reach the very end, it must pass a large amount of opportunity to react with MinD. **[I stopped writing this reasoning because I'm not sure it's valid. Interesting though. Look at this more.]**

3. Randst-98-C-Reminiscent of Mannik's cell

Our animations and total protein fluctuation plots (Figure 7 for our C shape (reminiscent of the flattened cell shown in Mannik's work) shows a very regular, stable, simple oscillation pattern. There are two clear ends of this cell that oppose one another, and the protein maximum travels to one end, where it reaches a peak, then travels through the cell to the other end, reaching another

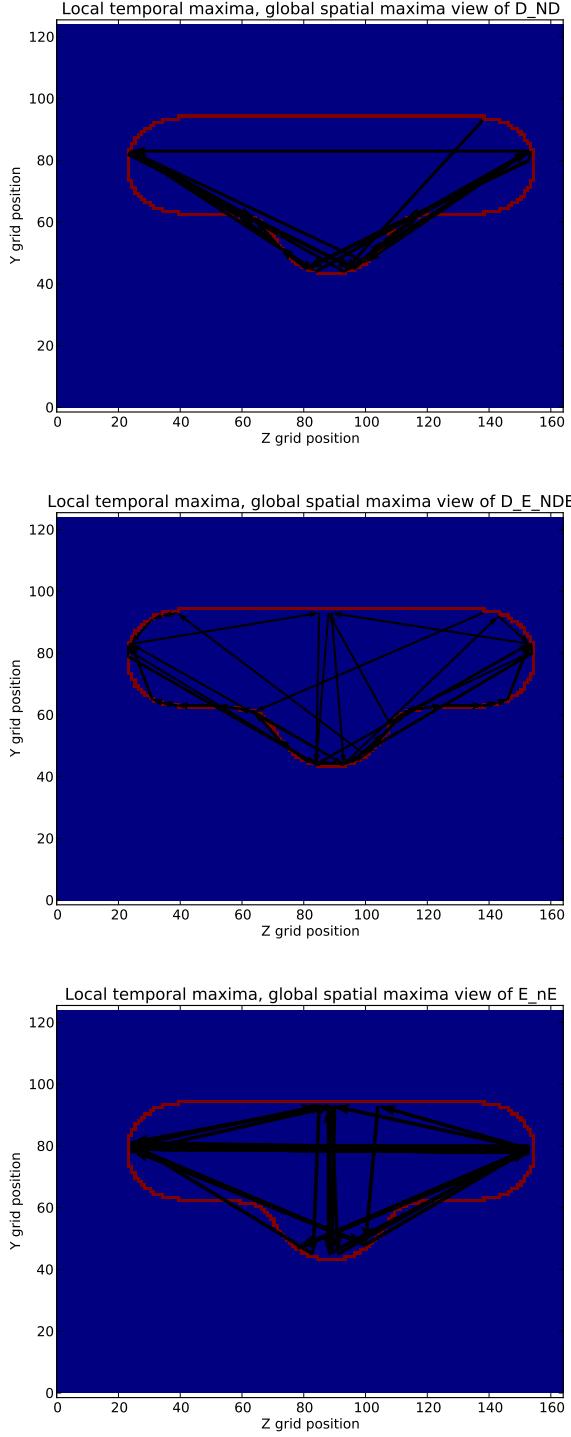


FIG. 6. Arrows that connect maxima that are global in space and local in time. The arrow heads touch spatial, time maxima and are adjacent to the tails of arrows that show the very next spatial, time maxima. The proteins shown are wall attached MinD-ATP (top), wall attached MinD-MinE-ATP (middle), and cytoplasmic MinE (bottom).

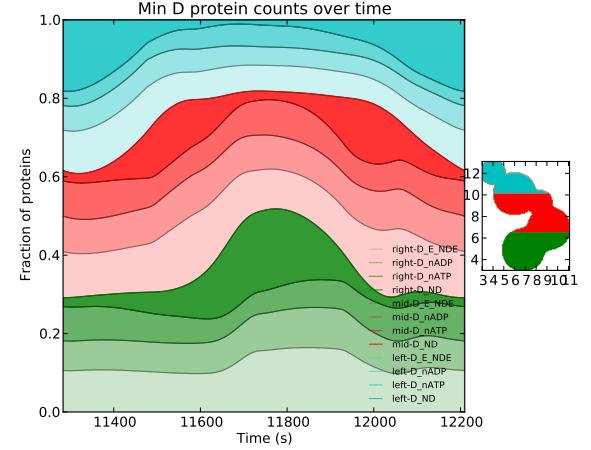


FIG. 7. Total protein fluctuation in the manniks squished cell shape. The vertical axis shows stacked the total number of proteins that are of four different compound stages and in the different sections of the cell.

peak, and then repeats the process. In a way it is similar to the simple pill shapes, in that there are two clear endcaps and a middle section (that is in this case bends and curves). The protein fluctuation plots show our familiar pattern of high spikes in wall attached MinD-ATP immediately followed by spikes in wall attached MinD-MinE-ATP, followed by a drop in both as the proteins move towards the other side.

The arrow plot shown here is typical of mannik's squished adnormal shape. It shows the protein maxima moving through the cell from one end to the other end, and back again. The effect of this movement can be seen in the time averaged plot directly below the arrow plot. The proteins spend a majority of time at the very ends of the cell.

4. Randst-99 our A shape

Our animations for this plot show a very interesting pattern. The maxima oscillates from the right corner to the top corner, then *back to the right corner* then to the top corner again, then to the left corner, then back to the top, then to the left, then to the top again, then back to the right side. It's fascinating that there must be two back and forth motions before proceeding to the other side [would this happen over and over or would there be a chaos, breakdown sort of thing at some point. Need longer sim]. The animations show that when there is a maxima in the center, if it is a maxima that is on the right side of the center upper cup then this maxima will be followed by a maxima in the lower left corner. This maxima in the lower left corner (immediately following the one on the right side of the upper cup) will reside for a bit longer than the left corner maxima preceding it. These longer residing maxima will

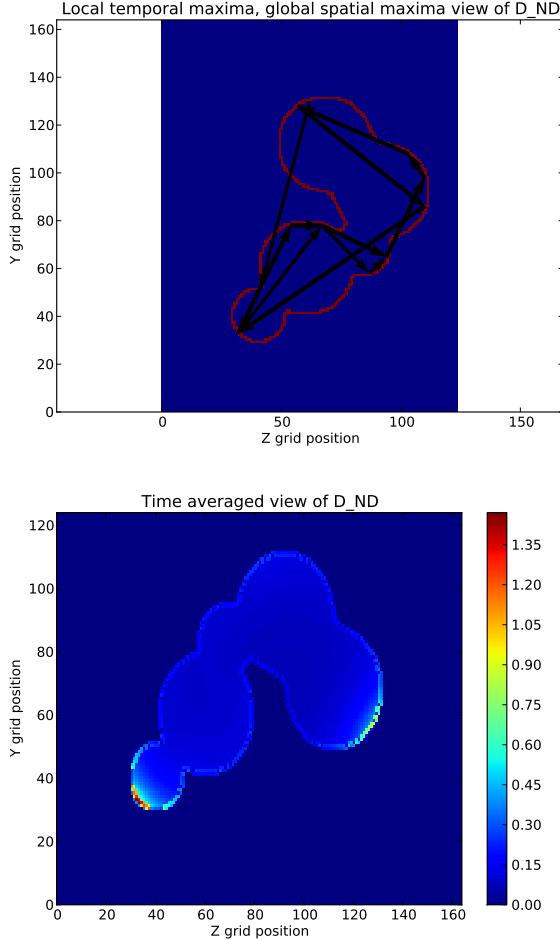


FIG. 8. (above) Arrows that connect maxima that are global in space and local in time. The arrow heads touch spatial, time maxima and are adjacent to the tails of arrows that show the very next spatial, time maxima. (below) An time averaged plot of the same proteins in the same cells shape. The protein that is shown is wall attached MinD-ATP.

then be followed by a switch, to the right corner, with a short less intensive maxima traveling through the center. The MinE shows the same pattern. So what does this say, keeping in mind our model of MinD diffusing and MinE comming in to eat it away? MinD starts in the lower left, diffuses up and maximizes on the right side of upper cup. Why right side of upper cup? Because there is more MinE on the left side than on the right side of that upper cup, because MinE has just spent time coagulating on left corner wall because there was more MinD there. The MinE attaches to the MinD on the membrane and begins to release it. Why at that point does the MinD go back to the left corner? When the MinE eats away at the wall bound MinD it starts at te edges and moves in. There's one frame where the MinE has maxima on either side of the MinD maxima on the right side cup. The center of the cup is a little further

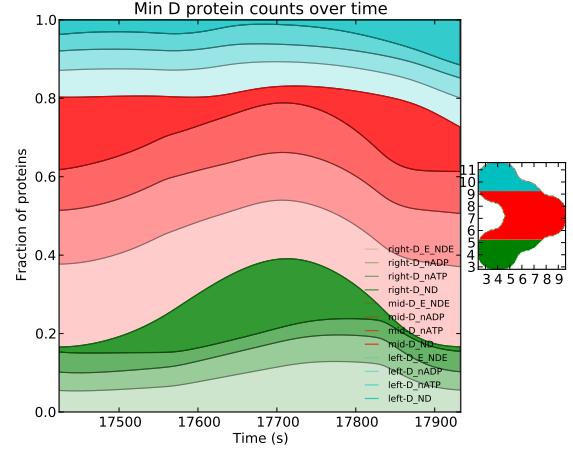


FIG. 9. Total protein fluctuation in the A shaped shape. The vertical axis shows stacked the total number of proteins that are of four different compound stages and in the different sections of the cell.

away so it attaches to these out ridges first and then moves in. After it eats at the corners and starts moving in why does the MinD go back to the left corner? Watching the cytoplasmic MinE, something very interesting: It as well maximizes twice in one corner, but in between the two maximizations it has a smaller maxima in the other corner. So when the MinD is getting eaten away from the top cup, the minE is moving over to maximize in the opposite corner. Why does it maximize there? there are more minD on the membrane to attach to on the left, so it can diffuse further down into the corner. Want to be able to construct a full frame by frame picture, with all the proteins layed out top to bottom, each frame onttop of its likewise frame. Actually, you can see this effect very slightly watching the 98 shape, between the center cap and the right corner.

Also - as to why the proteins like poles and corners. It's a diffusion model, so there's no momentum. When they are at an end or corner, they will go in all directions, and if there's a small hole way out, only a small percentage will go down it. As the others attach to the wall, there is a higher chance that diffusing particles will attach to that wall instead.

The total fluctuation plot shows this double maxima corner pattern, with two spikes on the right, then two spikes on the left, then two back on the right.

Figure 10 shows maxima that are global in space and local in time. It is interesting that the MinD-ATP, the stage that immediately proceeds attachment to the wall, show maxima in one base corner of the A shape and then immediate maxima in the other base corner, skipping any maxima steps in the middle (the top of the A), while the MinD-ADP, the stage that has just left the wall, shows no such behavoir. Every base corner maxima is followed by a top corner maxima, with very obvious symmetry.

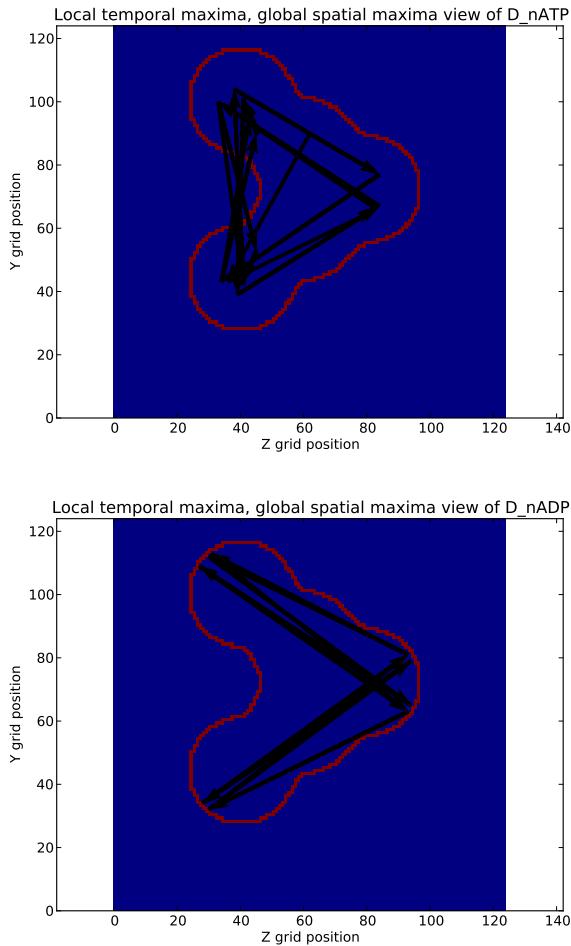


FIG. 10. Arrows that connect maxima that are global in space and local in time. The arrow heads touch spatial, time maxima and are adjacent to the tails of arrows that show the very next spatial, time maxima. The proteins shown are cytoplasmic MinD-ATP (above) and MinD-ADP (below).

IV. INTERPRETATION OF DATA

V. CONCLUSION

APPENDIX

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VI. BELOW ARE NOTES FOR THE WRITING

VII. INTRODUCTION

[I need a lot of references here!!!] need a lot of references here!!! (And the writing is just terrible) It is vital that during the process of bacterial cell division that the cell avoid minicelling, or splitting into daughter cells with lopsided volumes. During this process a long FtsZ polymer chain develops on the cell wall in the center region of the cell acting as a guide for the cell splitting process. in simulation that a system of Min protein interaction within the cell will lead to a natural oscillation of the MinC protein, which exhibits an aversion to the FtsZ polymer, from one cell end to the other that will leave the center free of any build up of MinC. This will allow for the PtsZ chain to develop in the center and not at the ends, where the two nucleoids are housed. The interaction takes place between MinE,MinD, and MinC, which naturally associates with MinD.

A significant amount of work has been done in simulating and studying the dynamics of the Min protein system and cells. A number of different models have been simulated and studied. They divide into two natural groups - stochastic (specific protein positions are tracked at all times) and deterministic (protein averages

are used along with rate constants to simulate interaction). They also split up into other groups - cooperative attachment (MinD on the cytoplasm is attracted to other MinD on cytoplasm, shown by non-linear attachment on walls experimentally). Huang's 2003 and Meinhart and De Boer 2001 are of this type. Huang's successfully predicts that breakdown of striped oscillation pattern occurs when exchange of ATP for ADP bound to cytosolic MinD is not too fast. There is as well aggregation current models, characterized by the proteins moving along the membranes in a way so that they're attracted to other proteins on the walls. We are simulating Huang's model, so a further test of the robustness of the oscillations with wild type values, and as well compare against experimental evidence given by Mannik. We use Huang's because of the success so far, and then also the other type (aggregation current models) depend on there being limited binding spots on the membrane for minD. This author also states that the deterministic models have largely verified stochastic model results.[11]

It has also been observed that the spatial characteristics of oscillation is not effected by broad ranges of temperature changes (although the frequency does vary) allowing for very robust cell division that is not deterred by environmental temperature fluctuations. [24]

We wish to subject Huang's seminal model to more harsh geometrical tests of robustness and compare results to the new experimental results of Mannik. **[this link doesn't work = citemannik2012robustness]**

Models also introduce the effect of cardiolipin, an anionic phospholipid that collects on regions of high negative curvature (the membrane of the cell poles) and attracts MinD, since it had been observed that minD binds preferentially in regions enriched with these phospholipids [12][21]. However, this mechanism of combined clustering, phospholipids and MinD has not been observed in real cells. [23]

A mathematical model that describes the building of the FtsZ polymer along the midsection cell membrane. Solved numerically using a finite volume method. [32]

Variations of the Huang 2003 model that attempt to more accurately account for the details of molecular interaction, focusing on the mechanism of MinD binding to other MinD on the cell membrane. These models confirm the major results obtained by Huang's model, while showing some interesting additional features. .[12][13]**[this last reference sounds like maybe the stochastic changes they made to the model cause the simulations to be more accurate in certain, odd cases. Should we have used this model?]**

More general biochemical models have also been used to study the MinD system and show consistent results.[18]

Stochastic models that are based on Huang's mean-field model but take chemical fluctuations into account in a discrete fashion show the same results as Huang's simulations in ordinary cell shapes, and better predict experimentally observed oscillations in round cell shapes.[13]

Purely theoretical models have been constructed that rely on min protein's mobility on the membranes and tendency to cluster together.[15][16] [read and write about these]

Huang has also made simulation studies of round cell shapes and shown that oscillations occur in these cell shapes, and that their behavior depends on the radius of the shape. They show that the Min proteins can select the long axis of the nearly round shapes based solely on the geometry of the cell.[17] [definately want to get this paper and read it]

Previous experimental studies have shown that MinC, known to inhibit the FtsZ polymer, exhibits an oscillatory, pole to pole behavoir in conjunction with MinD and MinE. [4]

The function of the Min system might not be restricted to proper cell division. There is evidence that it is instrumental in the disassembly of the cytokinetic ring directly after cell division, insuring that cell division takes place only once per cell cycle. [33]

Other simulations have been shown to exhibit oscillations in the cylinder shaped cell. While Huang's 2003 study involves stages of protein compounds (attached proteins), the differential equations governing protein mobility in this study are designed with free unattached proteins who interact with eachother. Huang does cite this paper so we can say that he followed and improved upon these simulations.[9]

The MinE has been observed in the center of the cell and its ability to counteract what's referred to as MinCD cell division inhibitor has been known. It's been found that it's not stationary but undergoes repetitive movement back and forth. [5]

Previous studies have been made of the Min system's association with the cell membrane [19][20]. Previous studies have shown that phospholipids on the cell wall attract MinD/MinE proteins and reduce ATPase activity. It's further been shown that the proteins interact preferably with anionic lipids that are localized at the poles of the cell.[22]

Experimental studies have been made of the process of septum formation in the middle of the pill shaped cell and its effect on MinD oscillations, leading to the conclusion that it is geometry of septum formation that allows for the creation of two MinD oscillation systems, one in each daughter cell. [26]

It is vital that the process of bacterial cell division result in a single nucleoid in each daughter cell. The cell must avoid minicelling, or splitting into daughter cells with lopsided volumes. One of the mechanisms the cell employs in order to avoid this is to allow a long FtsZ polymer chain to develop on the cell wall in the center of the cell. This chain acts as a guide for the cell splitting process. Huang et al have shown in simulation that a system of Min protein interaction within the cell will lead to a natural oscillation of the MinC protein, which exhibits an aversion to the FtsZ polymer, from one cell end to the other that will leave the center free of any build

up of MinC. This will allow for the FtsZ chain to develop in the center and not at the ends, where the two nucleoids are housed. The interaction takes place between MinE, MinD, and MinC, which naturally associates with MinD.

Mannik et all have shown that the formation of irregular cell shapes adversely effects the Min system's ability to maintain their regular oscillatory behavior (cramming into spaces).

A. What is the MinD system and why is it important?

-System of proteins in E.Coli and other cells. -Theorized to be instrumental in cell citokenisis. Reference experiments

B. How proteins move in cell

-Reference experimental showing proteins oscillating - Reference theory showing difEQ model shows oscillations -Reference Mannik showing into crevices. -Worthwhile studying effect of walls shape on the movement of cells (Sign post of what to expect from this paper)

VIII. METHODS AND INITIAL CONDITIONS

A. Mathematical Model

The model for the behavior of the MinD and MinE proteins inside the cell implemented the same set of 5 reaction-diffusion equations described in the paper by Huang et al (equations 1, 2, 3, 4, and 5). A 3d grid was constructed in cartesian coordinates with a grid spacing of .05 μm . From there, we were able to define a cell shape on the grid, and solve the reaction-diffusion equations numerically to observe the time evolution of the MinD and MinE concentrations inside the cell.

Our simulation used the same diffusion constants and reaction rates as Huang et al, which are

$$\begin{aligned} D_D &= D_E = 2.5 \mu\text{m}^2 / \text{sec}, \\ \sigma_D^{\text{ADP} \rightarrow \text{ATP}} &= 1/\text{sec}, \sigma_D = 0.025 \mu\text{m}/\text{sec}, \\ \sigma_{dD} &= 0.0015 \mu\text{m}^3/\text{sec}, \\ \sigma_{de} &= 0.7/\text{sec}, \sigma_E = 0.093 \mu\text{m}^3/\text{sec}. \end{aligned}$$

To test our computational model, we implemented a pill shaped cell, and tested using the same cell parameters as Huang et al, which were a radius of 0.5 μm in the middle and at the spherical endcaps, and two different cell lengths of 4 μm and 10 μm . We found the same type of oscillations as in their paper using these initial conditions, verifying that our model works as intended.

Below are snapshots of MinD and MinE concentrations at 5 second timestamps in the 4 μm cell:

[insert 5 second time stamps of 4 μm sim]

We then began to define other, non-traditional cell shapes for the purpose of modeling squished and perturbed E. coli cells, which were created experimentally in Mannick et al. To achieve this, we went with a cartesian lattice rather than the cylindrical lattice used in Huang et al's simulations, as it allows for more flexibility in defining the cell shape. Some of the cell shape models included a flattened pill (stadium shape), an ellipsoid, a spherical cell, and various randomly generated smooth shapes, such as those in the figures below.

[insert memf print of 2-3 cell shapes]

To interpret the results, we generated several different plot views of the printed simulation data. These plots included a time averaged view of the protein densities in the cell; a plot tracking the location of protein concentrations that were global maxima in space and local maxima in time; and an animated view that showed the actual dispersion of protein concentrations in the cell over time.

IX. SPECIFIC RESULTS

X. PILL SHAPE

[Be more exact about what exactly Nd is versus nATP, in terms of the unit and dimensions. The plotting may need to be changed] Figure fluctuations at a point in space adjacent to a polar wall. At each collection of peaks the proteins reach their zenith in an order that agrees with the qualitative picture described by Huang[10], except for nADP peak. The peaks start with a maxima of ATP-MinD accumulating at the walls **[Need a better way to refer to compounds]**. This peak is followed by a peak in Nde, or ATP-MinD-MinE, as the cell is converted on the wall from the former to the latter compound. As this compound splits apart and leaves the wall we see a peak in nE, or MinE in the cytoplasm. This is then followed by a broader peak in nATP, or the MinD-ATP compound, as minD-ADP is (changed but I forgot the name for it) into minD-ATP in the cytoplasm. The minD-ATP naturally diffuses away from the pole, which is shown by the broad nature of this peak. All of these peaks fit the qualitative picture except for the sharp MinD-ADP peak. One could expect that it is a sharp peak, meaning that the MinD-ADP proteins do not last in the cytoplasm for very long before they are converted (once again forgot the name) into MinD-ATP. The location of the peak in the time dimension is troubling, however, since that we would expect it to occur just after the peak in Nde, since the wall-bound MinD-

ATP-MinE proteins are the ones that split up and leave the wall, creating both the MinE and the MinD-ADP. One would expect to see the minD-ADP peak to coincide in time with the minE peak, but it instead coincides with the wall-bound MinD-ATP-MinE peak. (Although you can maybe sort of convince yourself otherwise, really looking at it).

The theory states that the aversion of the FtsZ polymer to the MinC protein is responsible for the FtsZ polymer setting up in the center of the cell as opposed to at the cellular poles. The MinC protein in turn naturally associates with the minD protein that is modelled in Huang's differential equations. **[look this up and write this again]** The theory is dependent on there being a significant difference in protein density between the center and polar regions. It is worthwhile examining this difference in detail. Figure fluctuation over time at a point adjacent to the polar cell wall and at a point adjacent to the cell wall in the center region of the cell. The different proteins show different relationships between their maxima and minima. The MinD-ADP show sharp spikes at the poles and much smaller oscillations at in the middle. The values at the poles are always greater than those in the middle, and the polar peaks exhibit densities that are roughly 2.5 times greater than the maxima in the center (**[this factor and the following factors will be put into a table of comparisons for each protein and for the different cell sizes]**). The MinD-ATP densities show a very different trend. For these it is the center density that is almost always greater than the polar density. The difference in density at the pole versus the middle is a factor of roughly .85 (once again we'll do this more officially in a table). The MinE is similar to the MinD-ADP comparison. It shows a difference in maxima by a factor of roughly 2. The MinC protein is associated with the minD protein, so perhaps the most important comparison is between the nfl-MinD (MinD protein in all its forms) at the ends and in the center of the pill. This shows sharp spikes at the pills and a maxima difference factor of roughly 1.25. **[I'd like to analyze further what this means for the FtsZ polymer. Would a difference like this really have a big effect? What sort of effect should it have, looking at experimental data? One thing is that Mannik (is it Mannik?) genetically deletes the MinC protein and they get 73 percent proper cell division. It really would be good to do a more extensive analysis here, referencing what other papers say about this.]**

It would be good here to add the time map plots and discuss the idea about the proteins spending most of their time in the center of the cell.

Figure ?? also shows that the middle density oscillates at a frequency that is twice that of the polar regions, which is to be expected considering the symmetry of the center compared to the that of the polar regions. Also, the difference between density maxima and minima each oscillation is smaller for the middle points than for the polar points. This is evident from simply watching the

simulation movies - the fluctuations are more extreme in the polar regions.

Add a table here that shows the period of oscillations versus the cell dimensions. Do the mathematical solution of the differential equations for an infinite long cylinder with different widths to see how fast the maxima move and compare (Dr. Roundy says that making the radial speed infinite would help).

Pill Normal section - Our goal with this project was to test whether or not the computational model developed by Huang et al was consistent with the newer experimental results (squishing E Coli) produced by Mannick et al. We generally see that:

- Protein concentrations bounce around to areas with high curvature. -- example: nflD randst 1 6 6 99 tri-polar zones with tri-polar concentrations -- nflE the same - There is some time delay between where different protein types appear.

The pills with larger cylindrical widths, 4.00 3.00 (the wider pill shapes) exhibit oscillations with maxima reached on either side (half period times) 30s, 70s, 110s, so first half oscillation occurred in 30s, the next two in 40s

The 4.00 2.00 pills exhibit a similar pattern, but max out at times 25s, 55s, 85s, 115s, so thirty seconds each half period. Seemed once again that the first oscillation had a higher max density, then settled down. I wonder if extremely increasing the starting condition density lopsidedness will still yield a settle down pattern with the same max densities? Is this dependent on shape/size?

The 4.00 0.50 pill seemed to show oscillations of 20 second half periods very consistently. Density maximum did not seem to lose intensity. Seemed to be the same each oscillation.

Also with this, when look at the periods and max density/min density ratio, consider the size dimensions of the pill shape and see if can see a mathematical relation.

The nflE 4.00 0.50 shows a very large difference in center highest density versus pole highest density (during a maxima). Is this perhaps the protein that's more important.

Looking at the extrema plots - one very interesting thing is the 4.00 2.00 ATP extrema, which shows the extrema only in the center of the cell. The other proteins for this shape show the extrema to be at the ends. Also, it seems the 4.00 3.00 cell shapes for these plots are missing. Very interesting to see if there is a certain protein that has its maxima in the center of the cell, while the others have maxima elsewhere. Tells a story that could maybe relate to the other shapes.

Also, try starting the cells with density only in the corner. Now the extrema go down the center of the cell, see if there would remain a lopsided nature of the oscillations if you started it like this instead.

The very long cells have very long periods

The time map plots seem inconsistent in that some show the highest densities on the poles, some in the centers, some on just one pole. For these want to run starting

from a time when the proteins are evenly spread out (so between two maxima) and stop at a similar pointe at the end.

The time map plots that I believe though sometimes show that the max density time-wise is actually in the middle of the cell! Make sure we have a good, longer view of this being true.

Important

Pill Short - Know how short is too short
Randst 99 -

XI. RANDST SHAPE

whats the difference between the extremes in density at different places (like the density max at the poles and at the rims in the center). Still need to do this.

There are sudden bursts in the nflE protein plots, at the poles. Their density maxima build very quickly then diffuse more slowly.

A. randst-96

The star shape (randst-96) Shows oscillations in nflD horizontally, two poles to two poles. There is sometimes a small amount of lag between the upper maxima moving horizontally and the lower. Half periods take roughly 25s. Dimensions are roughly 5.00 by 5.00? Check this. The nflE short bursts do develop lag between when the top and the bottom go off. Should coordinate this with the nflD lag.

Tried to see if there is a time correlation between nflE and nflD but the maxima seem to appear in the poles at roughly the same time.

Watched Nd and nE simulations next to each other at same time. Both have maxima that build up right at the walls (in the corners) and then subside. It's clear that the nE density bursts appear directly after the Nd bursts. The Nd bursts are subsiding as the nE bursts are rising up. The Nd reaches a time maxima just before the nE. This is true in each corner, so that when the maxima are lopsided top to bottom - when the top right corner maximizes first, say, the interplay between nE and Nd remains very clear in those corners where and when maxima occur.

Watched nATP and nADP together. nATP maxima seem to follow slightly after nADP maxima. This is not surprising since in rotation nATP follows after nADP. nATP maxima is more spread out - in-between pole maxima there is more spread out maxima in the center. This should show itself in the time map plotting. About this - the percentage difference seem to be the same between the non-max places and max places, roughly, its just that the nATP seems to have a max region that covers a wider area throughout the oscillation (or at least in between the maxima points in the oscillation).

Also watched the Nd next to the nATP. The Nd maximize more extreme in the corners up against the walls. The nATP density follows the Nd maxima, it looks like the nATP maxima follows the Nd. So if the Nd appears somewhere (in a corner) then the nATP will travel there to follow.

B. randst-97

Randst 97 nflE starts with small maxima, not much difference at all, then actually builds to high maxima at the poles.

C. randst-99

Randst 99 (sort of triangle) is roughly dimensions of 3.5 by 3.5? nflD oscillations appear to be half periods of 25s as well. Maxima appear at the poles and in the interim there are weak maxima in center pole.

D. randst-98

Funny randst shape 98 shows nflD oscillations of about 30s or so.

nADP and nATP side by side. nADP maximizes in the corners right before the nATP maxima follows. Makes sense looking at equations. Once again the nATP max-

ima is more spread out, where maxima in nADP appear more just at walls.

Nd and nATP side by side. Nd maximizes at walls and the nATP moves in to follow it

Nd and nE side by side. Nd maximizes in corners and then the nE also maximizes in the corners. The nE maxima appear in these sort of quick bursts in the corners, along with a slower broader movements away from that corner after the burst and into the next, where another sharp burst occurs. The Nd bursts occurs after the Nd as subsided in that corner, and really by the time the burst maximizes, the Nd is already on its way over and starting to build on the other side.

Randst nflD doesn't show much oscillation at all, but we start it so that the density is not max at the pole.

Randst 98 -

Randst 97 -

Randst 96 -

Triangle -

XII. INTERPRETATION OF DATA

- Discussion of conceptual reasons of why we see what we see
- Plots that are more interpretive (area-rating)
- Some sort of predictive claim?

XIII. ANIMATE?

XIV. CONCLUSION