Glucose distribution over *S. aureus* cytoplasm

ChemE 422

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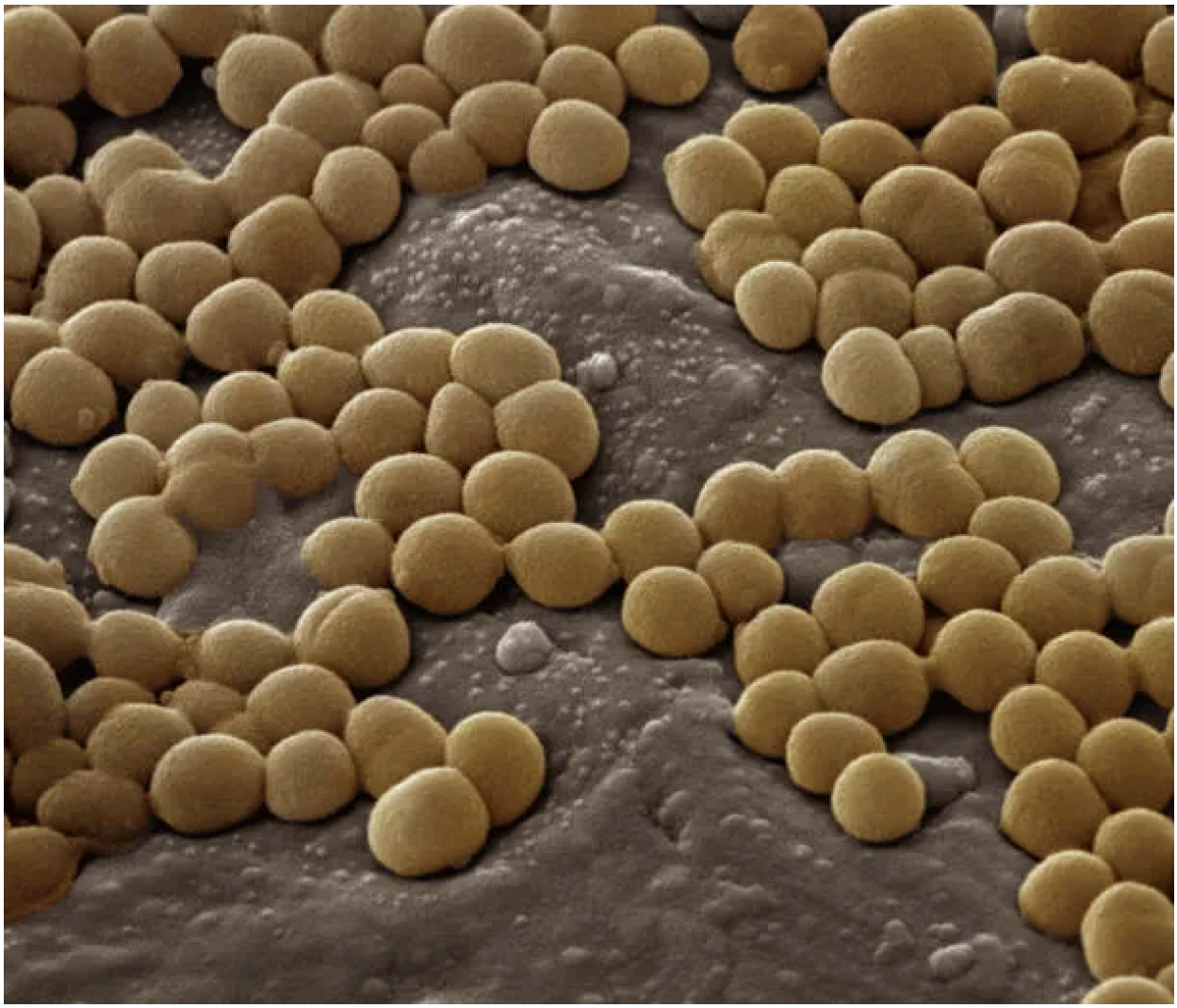

Abstract

The glucose concentration across a cellular cytoplasmic environment was investigated to rigorously benchmark the predictions from metabolic models, which are the basis of my research. I explored several different solution methods: 1) non-dimensionalized linear kinetics, which employed a Bessel solution, and 2) dimensional non-linear (Michaelis-Menten) kinetics, which was solved numerically. Both experimentally-based and synthetic parameters were explored to create a model that generated a reasonable concentration distribution. The models all necessitated either a very large reaction term (k or v_{max} for linear or Michaelis-Menten, respectively) or an incredibly small diffusion term (D), which I suspect is the result of these models omitting regulatory processes and feedback systems that prevent limitless passive diffusion *in situ*.

Approach and results

All of the models generated in this study extend from the steady-state microbalance $\frac{DC}{Dt} = 0 = D\nabla^2 C + \sum_{rxn}^{RXN} (C_{rxn})$ and the associated Neumann and Dirichlet BCs: $\frac{dC}{dr}|_{r=0} = 0$ & $C_{r=R} = C_{\infty}$, respectively. The full derivations of each method are communicated in the Appendix, however, notably for the non-dimensionalized linear kinetics solution, the Damkohler number appears and concisely describes the contribution of glucose consumption within the cell to the diffusion of glucose into the cell. Reasonable concentration distributions were achieved from $Da > 20$.

Discussion

The FBA simulation predicted $99\ \frac{mmol}{hr}$ of glucose consumption. While this value technically has units of per time, fluxes from metabolic models are generally considered to represent ultimate behavior of the cell since individual bacteria operate on timescales faster than hours. Reproducing this value required very large Da values. **Figure 1** illustrates the case for linear glucose metabolism ($Da > 20$), which corresponds to either large reaction parameters in **Table 1** ($k > 4E7\ \frac{1}{hr}$) or tiny diffusion coefficients.

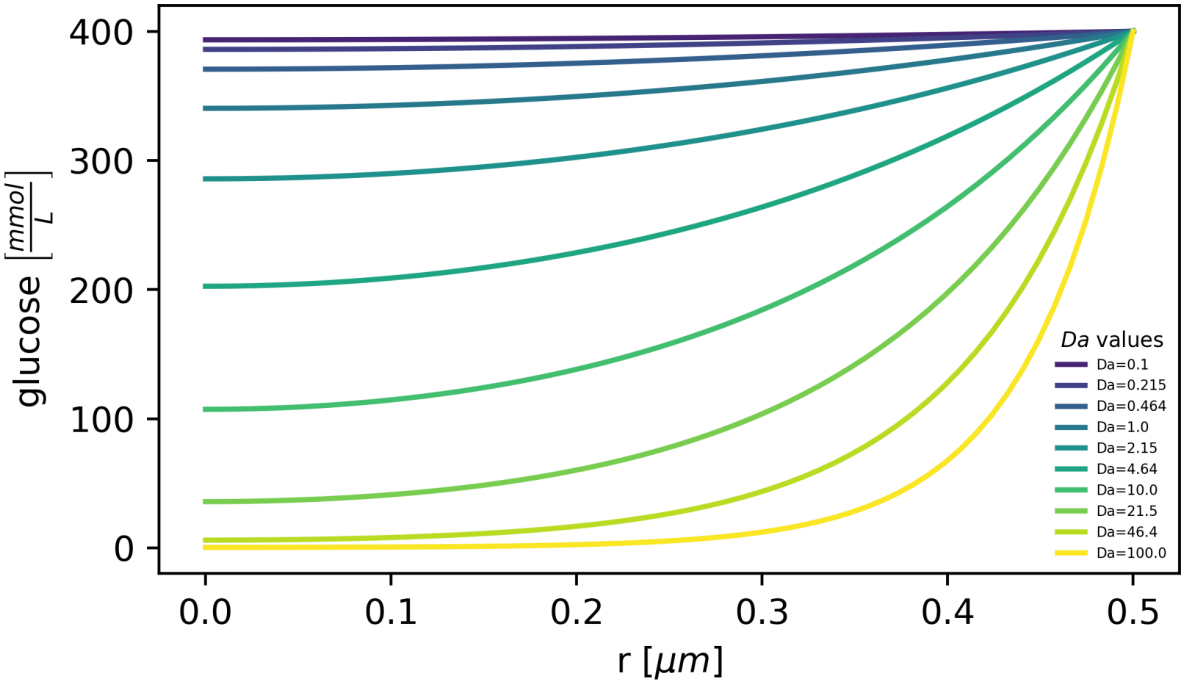

Figure 1: A comparison of the analytical, non-dimensional, solution method at various *Da* numbers that each represent different rate constants.

Table 1: The average concentrations of each *Da* trial, corresponding with the plots from **Figure 1**. A *Da* around 25, translating to a reaction rate of ~1E7 given a Diffusion constant of $3E-5\ \frac{cm^2}{hr}$ and a spherical radius of $5E-5\ cm$, best matches the FBA simulations predictions.

Da Number	Reaction rate ($\frac{1}{hr}$)	Average Concentration (mM)
0.1	2.40E5	395
0.215	5.16E5	390
0.464	1.11E6	380
1.0	2.40E6	359
2.15	5.16E6	322
4.64	1.11E7	262
10.0	2.40E7	188
21.5	5.16E7	119

46.4	1.11E8	72
100.0	2.40E8	45

The case for non-linear Michaelis-Menten kinetics is complicated by the inability to concisely non-dimensionalize the system and by the use of experimental v_{max} & Km parameters predicting complete diffusion of the media into the cell, illustrated in **Figure 2**. A range of diffusion constants and MM parameters were therefore explored to find the combinations that created a similar profile as the linear kinetics model and a similar total consumption to the FBA model.

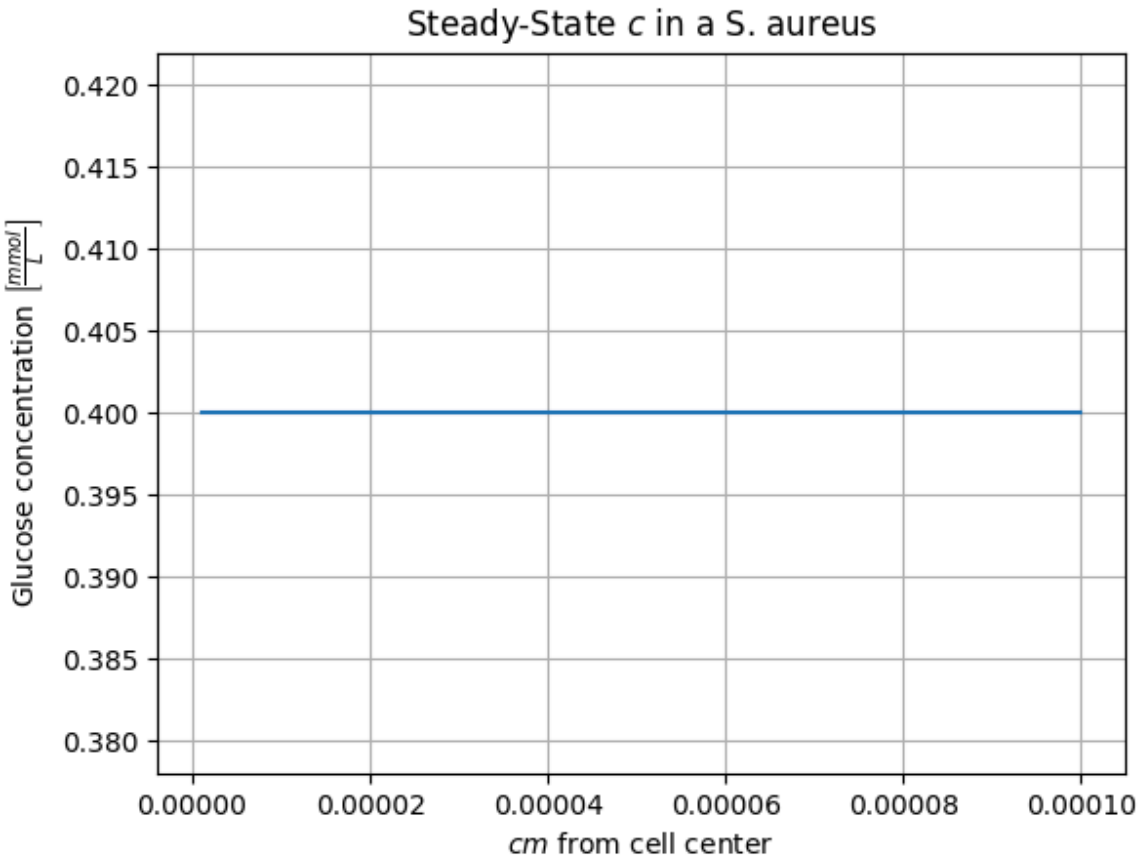

Figure 2: The concentration distribution using the experimentally derived parameters from **Table A2**. The near negligence of glucose consumption and the overwhelming effect of diffusion across the cellular cytoplasm motivated altering the model design to have a constant diffusion term while using the experimental kinetic values to possibly capture regulatory effects that are not in this abiotic model.

Table 2: Parameter values for solving the dimensional system with non-linear kinetics.

Parameter	Value	Source
D	$1.8E5\ \frac{\mu m^2}{hr}$	Mika et al., 2010. https://doi.org/10.1111/j.1365-2958.2010.07201.x
v_{max}	$4\ \frac{mmol}{mg*hr}$	Approximated from Siebers et al. 1998, 10.1128/JB.180.8.2137-2143.1998
K_M	$0.3\ mM$	Approximated from Siebers et al. 1998, 10.1128/JB.180.8.2137-2143.1998

Improvement

The results presented here make it apparent that more complex diffusion and kinetic expressions are needed to replicate biological processes such as substrate transport and utilization. This would further solidify the inability to non-dimensionalize the problem, but the problem could be nevertheless solved numerically.

Another future direction would to be simulate the dynamic system and evaluate at what time the substrate consumption matches the FBA prediction, since it is possible that the per hour calibration for the model was conducted on another model organism such as *E. coli* and is therefore not completely accurate itself.

A final point of an improvement would be to find experimental data of total glucose uptake for *S. aureus* in a given timeframe, against which the model could be predicted. This is not a straightforward data set to find because bacterial cultures divide continuously, so determining the total glucose consumption per cell would require aggregate consumption of glucose by a culture and simultaneously determining the cell count. This data, in addition to fluxomics data that evaluated glucose concentration as a function of cellular radius, would be immensely useful in benchmarking these predictions and assessing their relative strengths and weaknesses.

Appendix

All of the calculations and code construction occurred in this Python Jupyter Notebook.

Validation: Flux Balance Analysis

The FBA validation simulation was developed from the iYS854 *S. aureus* metabolic model (http://bigg.ucsd.edu/models/iYS854). This model consists of 1335 metabolites and 1455 reactions that extend from 866 metabolic genes. The maximum uptake fluxes for media nutrients in [mM/hr] were defined as follows

{'EX_h2o_e': 10.0,
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 'EX_k_e': 10.0,
 'EX_ca2_e': 10.0,
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where the highlighted line for glucose consumption was augmented to represent the 400 mM concentration of the media of this system. The compound and reaction abbreviations are detailed in the BiGG database. The metabolic model was then optimized to maximize biomass production given these uptake possibilities and the innate metabolic structure of *S. aureus* in essentially a steady-state mass balance problem. The resultant solution of fluxes for $1E3$ reactions was parsed to find the glucose consumption of 99.1 $\frac{mmol}{hr}$.

Problem statements and solutions

Governing equation and BCs

The governing equation

$$\frac{Dc_{glu}}{Dt} = D\nabla^2 c_{glu} + R_{glu}$$

is a species microscopic balance on the substrate glucose, involving the passive diffusion of glucose into the cell $D\nabla^2 c_{glu}$ and all of the reactions that consume glucose R_{glu}. The comparison with FBA supported that a steady-state solution could be acquired, which conveniently simplifies the problem into an ODE:

$$0 = D\frac{1}{r^2}\left(\frac{d}{dr}\left(r^2\frac{dc}{dr}\right)\right) + R_{glu}.$$

Two Bound Conditions in *r* are needed to solve the $2^{nd} - order$ ODE, which are a Neumann boundary $\frac{dc}{dr}_{r=0} = 0$ and a Dirichlet boundary $c(r = R) = C_{\inf}$.

Two kinetic models of glucose consumption were considered: linear and non-linear Michaelis-Menten. The linear relationship $k * c$ created a relatively easy problem to solve analytically but is likely not representative of the biology captured in the metabolic models against which these solutions are validated. The Michaelis-Menten relationship $\frac{v_{max}{}^{*}c}{K_m + c}$ is designed to capture enzymatic reactions, but requires defining 2 parameters: v_{max} as the maximum reaction rate for a given amount of enzyme and K_m as the concentration at which the reaction rate is $\frac{v_{max}}{2}$. The rate limiting enzyme for glucose metabolism is by all accounts Phosphofructokinase-1 (PFK-1), so this is the enzyme that was used for the Michaelis-Menten relationship of glucose consumption.

Derived quantities

Two solution methods were pursued, which each yielded different expressions and solutions that will each be discussed. The first method built on the non-dimensional, first-order kinetics, solution from Lecture 5. The second method applied a finite approach to estimate the relationship with non-linear Michaelis-Menten kinetics.

Method 1: Non-dimensional, $1^{st} - order$ kinetics

The first method non-dimensionalized the system wth $\zeta = \frac{r}{R}$ and $\theta = \frac{c}{c_{inf}}$ to create a new governing equation

$$0 = \frac{1}{\zeta^2}\left(\frac{d}{d\zeta}\left(\zeta^2\frac{d\theta}{d\zeta}\right)\right) + Da * \theta$$

that importantly includes the *Da* number. This equation with the new BCs $\frac{d\theta}{d\zeta}_{\zeta=0} = 0$ and $\theta(\zeta = 1) = 1$ is solved with the modified spherical Bessel's equation

$$\theta = A\frac{sinh(\sqrt{Da}*\zeta)}{\sqrt{Da}*\zeta} + B\frac{cosh(\sqrt{Da}*\zeta)}{\sqrt{Da}*\zeta}.$$

Applying the Neumann BC results in $\frac{lim}{\zeta\to 0}\left[\frac{d}{d\zeta}\left(\frac{cosh(\sqrt{Da}*\zeta)}{\sqrt{Da}*\zeta}\right)\right] \to \inf$, which necessitates that $B = 0$. Applying the Dirichlet BC results in $\frac{lim}{\zeta\to 1}\left[A\frac{sinh(\sqrt{Da})}{\sqrt{Da}}\right] = 1$ and therefore $A = \frac{\sqrt{Da}}{sinh(\sqrt{Da})}$.

The final expression is then

$$\theta = \frac{\sqrt{Da}*sinh(\sqrt{Da}*\zeta)}{\sqrt{Da}*\zeta*sinh(\sqrt{Da})} = \frac{sinh(\sqrt{Da}*\zeta)}{\zeta*sinh(\sqrt{Da})}.$$

This solution to the PDE was modeled over a range of radii $[0, 1]$ μm and a range of Da values $[0.1, 100]$, which are captured in **Figure 1**, to explore how concentration varies as a function of rate constant, where convention is assumed to be constant.

Method 2: Non-linear, Michaelis-Menten, kinetics

The non-linear, Michaelis-Menten, approach complicated the governing equation

$$0 = D\frac{1}{r^2}\left(\frac{d}{dr}\left(r^2\frac{dc}{dr}\right)\right) + \frac{{v_{max}}^*c}{K_m+c}$$

and was solved numerically via a Finite Method. Solving the system with the parameter values in **Table 2** led to the concentration gradient in **Figure 2**. Exploring a logspace of both v_{max} & K_M led to plots in **Figure A1** that exhibited the more expected concentration distribution and approximately the total glucose consumption that was predicted from FBA. The most realistic concentration distributions were achieved with $v_{max} \in [5E6, 10E6]$ $\frac{mmol}{cm^3*min}$. The concentration profile was found to be mostly independent of K_M, which makes sense considering that v_{max} is directly proportional to the reaction rate while realistic values for K_M are drowned by the summed c_{glu}.

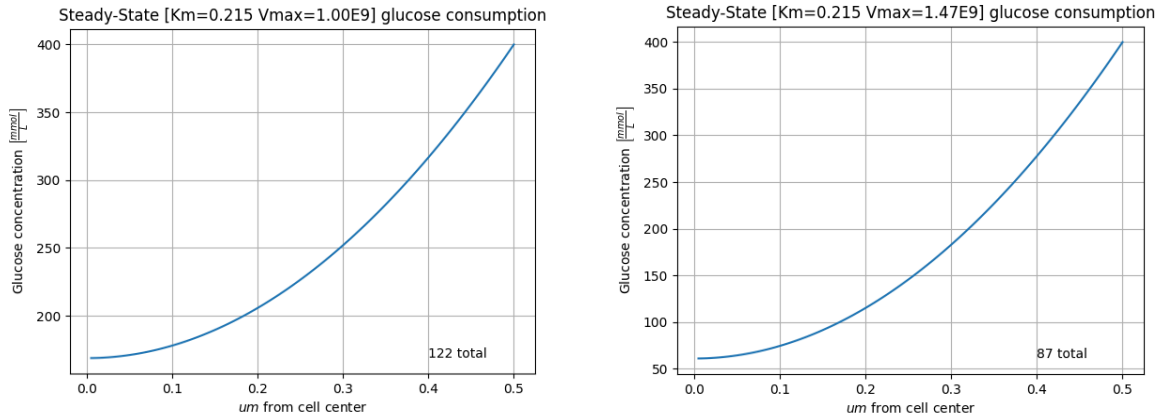

Figure A1: The substrate distribution when $v_{max} \in [1E9,\ 1.5E9]$ at a close to experimental $K_M = 0.2$ approximates the FBA simulation results in terms of total glucose consumed. The distribution of glucose is also biologically sensible, as the innermost of the cell is somewhere between $\frac{1}{3}$ and $\frac{1}{6}$ of the media concentration.

An opposite adjustment was done, where a range of values were explored $D \in [1E-20,\ 1]$ with the experimentally defined kinetic parameters to find the diffusion a sensible concentration distribution over the cellular cytoplasm. The optimal diffusion coefficient was $D \sim -13$ in **Figure A2**. Biological assumptions employed to convert experimental values into the units of this system include: $\frac{5E-16\ g_{PFK}}{1\ cell}$; $\frac{3E-13\ g_{dry-weight}}{1\ cell}$; $\frac{1\ g_{protein}}{2\ g_{dry-weight}}$; & $\frac{3.3\ g_{PFK}}{1000\ g_{protein}}$ (sourced from ChatGPT DeepSearch).

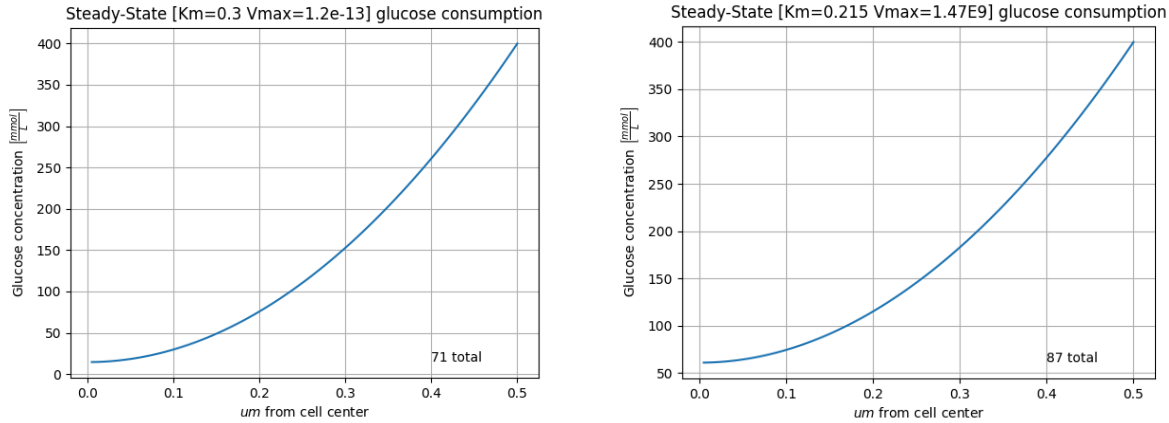

Figure A2: The glucose concentration distribution for different combinations of experimental and fitted parameters. The left panel contains the experimentally-based

$v_{max} = 1.2E-13 \frac{mmol}{cell*hr}$ and $K_M = 0.3\,mM$ values and solves for a diffusion constant of $D = 1.29E-17 \frac{cm^2}{hr}$. The right panel fixes the experimentally-based $K_M = 0.3\,mM$ and $D = 1.8E5 \frac{\mu m^2}{hr}$ and solves for $v_{max} = 1.5E9 \frac{mmol}{cm^3*hr}$. The average concentration in these plots is $\approx 80 \frac{mmol}{L}$, which translates to $\approx 40E-10\,mmol$ of glucose consumed by the cell.