1. *Model Outline*

1.1 Introduction

Using our dynamic E.coli cell model, we were able to develop a set of 'host mindful' design parameters for our genetic circuit. The dynamic model for the cell was adapted from WEIßE. Y. et al. *Mechanistic Links between cellular trade-offs, gene expression, and growth.* PNAS, 2014.; explicitly analysing metabolism in light of the host cell constraints, therefore accounting for the burden the introduced circuit places on the cell. This is currently part of an emerging field within synthetic biology looking to improve design inline with the organism. The tradeoff this model gives between growth rate and cellulose output was then optimised using a multi objective genetic algorithm, as shown in Deb, Kalyanmoy. *Multi-Objective Optimization Using Evolutionary Algorithms.* This yields a map of parameters with different operation points, allowing for application orientated design. The model was created using MATLAB and simulated using the ODE15s package due to the fact that this is a stiff system.

1.2 Species

1.2.1 Species Definitions

gg = Glucose
ee = Energy
cc = Cyclic-di-GMP
gn = Cellulose
mT = Glucose Importer mRNA
cT = G-Importer Ribosome Complex
pT = Glucose Importer Protein
mE = Metabolism Reaction mRNA
cE = Metabolism Ribosome Complex
pE = Metabolism Reaction Protein
mH = Host Protein mRNA
cH = Host Protein Ribosome Complex
pH = Host Protein
mR = Ribosome mRNA
cR = Ribosome Ribosome Complex
pR = Ribosome
mM = Membrane Protein mRNA
cM = Membrane Protein Ribosome Complex
pM = Membrane Protein
pM^* = Activated Membrane Protein
mO = ompR mRNA
cO = ompR Ribosome Complex
pO = ompR Protein
pO^* = Activated ompR
mK = C-di-GMP Producer mRNA
cK = C-di-GMP Producer Ribosome Complex
pK = C-di-GMP Producer Protein
mP = C-di-GMP Decayer mRNA
cP = C-di-GMP Decayer Ribosome Complex
pP = C-di-GMP Decayer Protein

mI = tetR Protein mRNA
cI = tetR Protein Ribosome Complex
pI = tetR Protein
mS = Cellulose Machinery mRNA
cS = Cellulose Machinery Ribosome Complex
pS = Cellulose Machinery Protein
pS^* = Activated Cellulose Machinery

1.3 Parameters

1.3.1 Host Parameter Definitions

vT = Glucose Import Rate
kT = Michaelis Menton Constant for G-Importer Protein
vE = Rate of Catalysis for Metabolic Protein
kE = Michaelis Menton Constant for Metabolic Protein
wX = Default maximum transcription rate
wH = Host Protein maximum transcription rate
wR = Ribosome maximum transcription rate
oX = default transcription threshold energy for half maximal rate
oR = Ribosome transcription threshold energy for half maximal rate
dymX = Default decay rate
bX = Default RBS strength
uX = Default ribosome unbinding rate
nX = Default protein length
nR = Ribosome protein length
maxG = maximal elongation length
kG = Michaelis Menton constant for cellulose production
M0 = Cell mass
kH = Host Protein Hill function constant
hH = Host Protein Hill function constant

1.3.2 Circuit Parameter Definitions

wM = Membrane Protein transcription rate
wO = ompR Protein transcription rate
wK = C-di-GMP Producer transcription rate
wP = C-di-GMP Decayer transcription rate
wI = tetR protein transcription rate
wS = Cellulose machinery transcription rate
bM = Membrane protein RBS strength
bO = ompR protein RBS strength
bK = C-di-GMP producer protein RBS strength
bP = C-di-GMP decayer protein RBS strength
bI = tetR protein RBS strength

bS = Cellulose machinery RBS strength
kO = ompR Hill function constant
hO = ompR Hill function constant
kI = tetR Hill function constant
hI = tetR Hill function constant
vK = C-di-GMP producer enzymatic rate
kK = C-di-GMP producer Michaelis Menton constant
vP = C-di-GMP decayer enzymatic rate
kP = C-di-GMP decayer Michaelis Menton constant
sS = Cellulose enzymatic parameter
vS = Cellulose enzymatic parameter
kS = Cellulose enzymatic parameter
fs = cc to pS binding rate
rs = pS^*-cc unbinding rate
kM^* = Membrane protein activation reverse reaction constant
krO = $ompR^*$ degradation to ompR reaction constant

1.3.3 Translation Rate Parameters

$$translating - ribosomes = cT + cE + cH + cR + cM + cO + cK + cP + cI + cS$$

$$\gamma = \frac{maxG \times ee}{kG + ee}$$

$$\lambda = \frac{1}{M0} \times \gamma \times translating - ribosomes$$

1.4 Non Host Chemical Reactions

Note: This section neglects transcription.

1.4.1 Membrane Reporter Protein

$$mM \xrightarrow{\lambda + dymX} \emptyset$$

$$pR + mM \underset{uX}{\overset{bM}{\rightleftharpoons}} cM$$

$$cM \xrightarrow{\frac{\lambda}{nM}} pR + pM + mM$$

$$cM \xrightarrow{\lambda} \emptyset$$

$$pM \xrightarrow{\lambda} \emptyset$$

$$pM^* \xrightarrow{kM^*} pM$$

$$pM^* \xrightarrow{\lambda} \emptyset$$

1.4.2 ompR Protein

$$mO \xrightarrow{\lambda + dymX} \emptyset$$

$$pR + mO \underset{uX}{\overset{bO}{\rightleftharpoons}} cO$$

$$cO \xrightarrow{\frac{\lambda}{nO}} pR + pO + mO$$

$$cO \xrightarrow{\lambda} \emptyset$$

$$pO + pM^* \rightarrow pO^* + pM^*$$

$$pO \xrightarrow{\lambda} \emptyset$$

$$pO^* \xrightarrow{krO} pO$$

$$pO^* \xrightarrow{\lambda} \emptyset$$

1.4.3 C-di-GMP Producer

$$mK \xrightarrow{\lambda + dymX} \emptyset$$

$$pR + mK \underset{bK}{\overset{uK}{\rightleftharpoons}} cK$$

$$cK \xrightarrow{\frac{\gamma}{nK}} mK + pK + pR$$

$$cK \xrightarrow{\lambda} \emptyset$$

$$pK \xrightarrow{\lambda} \emptyset$$

1.4.4 C-di-GMP Decayer

$$mP \xrightarrow{\lambda + dymX} \emptyset$$

$$pR + mP \underset{bP}{\overset{uP}{\rightleftharpoons}} cP$$

$$cP \xrightarrow{\frac{\gamma}{nP}} mP + pP + pR$$

$$cP \xrightarrow{\lambda} \emptyset$$

$$pP \xrightarrow{\lambda} \emptyset$$

1.4.5 tetR gene Protein - yhjh gene inhibitor

$$mI \xrightarrow{\lambda + dymX} \emptyset$$

$$pR + mI \underset{bI}{\overset{uI}{\rightleftharpoons}} cI$$

$$cI \xrightarrow{\frac{\gamma}{nI}} mI + pI + pR$$

$$cI \xrightarrow{\lambda} \emptyset$$

$$pI \xrightarrow{\lambda} \emptyset$$

1.4.6 Cellulose Producing Proteins

$$mS \xrightarrow{\lambda + dymX} \emptyset$$

$$pR + mS \underset{uS}{\overset{bS}{\rightleftharpoons}} cS$$

$$cS \xrightarrow{\lambda} \emptyset$$

$$cS \xrightarrow{\frac{\lambda}{nS}} mS + pS + pR$$

$$pS \xrightarrow{\lambda} \emptyset$$

$$pS + cc \underset{rS}{\overset{fS}{\rightleftharpoons}} pS^*$$

$$pS^* \xrightarrow{\lambda} \emptyset$$

1.5 Non-Host Reaction Definitions

1.5.1 Transcription Rates

$$g2mM = \frac{wM \times ee}{oX + ee}$$

$$g2mO = \frac{wO \times ee}{oX + ee}$$

$$g2mK = \frac{wK \times ee}{oX + ee} \times \frac{(\frac{pO^*}{kO})^{hO}}{1 + (\frac{pO^*}{kO})^{hO}}$$

$$g2mP = \frac{wP \times ee}{oX + ee} \times \frac{1}{1 + (\frac{pI}{kI})^{hI}}$$

$$g2mI = \frac{wI \times ee}{oX + ee} \times \frac{(\frac{pO^*}{kO})^{hO}}{1 + (\frac{pO^*}{kO})^{hO}}$$

$$g2mS = \frac{wS \times ee}{oX + ee}$$

1.5.2 Translation Rates

$$m2pM = \frac{\gamma}{nM} \times cM$$

$$m2pO = \frac{\gamma}{nO} \times cO$$

$$m2pK = \frac{\gamma}{nK} \times cK$$

$$m2pP = \frac{\gamma}{nP} \times cP$$

$$m2pI = \frac{\gamma}{nI} \times cI$$

$$m2pS = \frac{\gamma}{nS} \times cS$$

1.5.3 Membrane Reporter Protein

$$\frac{dmM}{dt} = g2mM - (\lambda + dymX) \times mM + m2pM - bM \times pR \times mM + uX \times cM$$

$$\frac{dcM}{dt} = -\lambda \times cM - m2pM + bM \times pR \times mM - uX \times cM$$

$$\frac{dpM}{dt} = m2pM - \lambda \times pM + kM^* \times pM^*$$

$$\frac{dpM^*}{dt} = -\lambda \times pM^* - kM^* \times pM*$$

1.5.4 ompR Protein

$$\frac{dmO}{dt} = g2mO - (\lambda + dymX) \times mO + m2pO - bO \times pR \times mO + uX \times cO$$

$$\frac{dcO}{dt} = -\lambda \times cO - m2pO + bP \times pR \times mO - uX \times cO$$

$$\frac{dpO}{dt} = m2pO - \lambda \times pO - pO \times pM^* + krO \times pO^*$$

$$\frac{dpO^*}{dt} = pO \times pM^* - krO \times pO^* - pO^* \times \lambda$$

1.5.5 c-di-GMP Producer

$$\frac{dmK}{dt} = g2mK - (\lambda + dymX) \times mK + m2pK - bK \times pR \times mK + uX \times cK$$

$$\frac{dcK}{dt} = -\lambda \times cK - m2pK + bK \times pR \times mK - uX \times cK$$

$$\frac{dpK}{dt} = m2pK - \lambda \times pK$$

1.5.6 c-di-GMP Decay

$$\frac{dmP}{dt} = g2mP - (\lambda + dymX) \times mP + m2pP - bP \times pR \times mP + uX \times cP$$

$$\frac{dcP}{dt} = -\lambda \times cP - m2pP + bP \times pR \times mP - uX \times cP$$

$$\frac{dpP}{dt} = m2pP - \lambda \times pP$$

1.5.7 tetR Protein - yhjh inhibitor

$$\frac{dmI}{dt} = g2mI - (\lambda + dymX) \times mI + m2pI - bI \times pR \times mI + uX \times cI$$

$$\frac{dcI}{dt} = -\lambda \times cI - m2pI + bI \times pR \times mI - uX \times cI$$

$$\frac{dpI}{dt} = m2pI - \lambda \times pI$$

1.5.8 Cellulose Producing Proteins

$$\frac{dmS}{dt} = g2mS - (\lambda + dymX) \times mS + m2pS - bS \times pR \times mS + uX \times cS$$

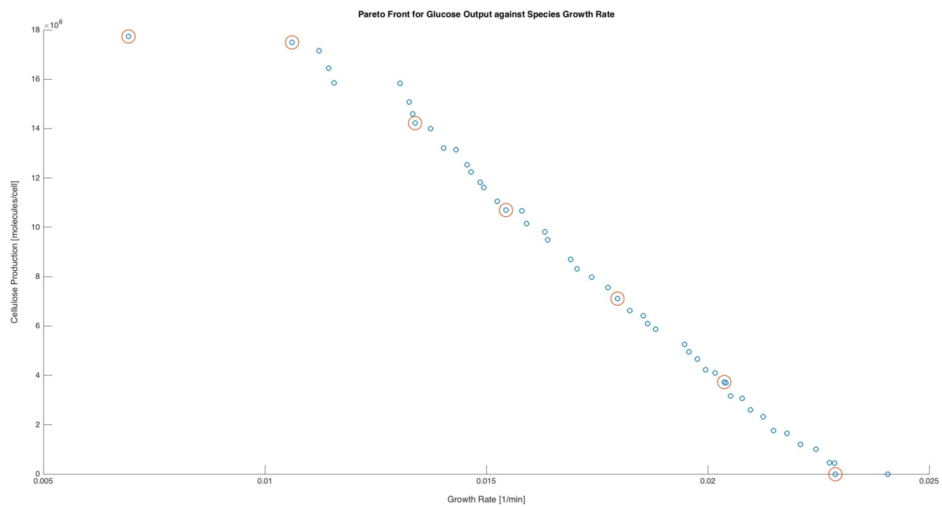
$$\frac{dcS}{dt} = -\lambda \times cS - m2pS + bS \times pR \times mS - uX \times cS$$

$$\frac{dpS}{dt} = m2pS - \lambda \times pS - fS \times cc \times pS + rS \times pS^*$$

$$\frac{dpS^*}{dt} = fS \times cc \times pS \times -rS \times pS^* - \lambda \times pS^*$$

2. *Multi Objective Optimisation Findings*

2.1 Pareto Front



Using the Genetic Algorithm to perform a multiobjective optimisation, we were able to produce a figure showing the cellulose output to growth rate trade off for different operational parameters. From this figure we selected seven individual operational points, as shown on the graph, that could be used to ensure the cell's performance suited its application.

2.2 Operation Points

This table shows the operation points for the cell and the corresponding parameter sets for transcription rate and ribosome binding strengths of the inserted gene circuit.

Table 2.1: Model Operation Parameters

Operating Point	wM	wO	wK	wP	wI	wS	bM	bO	bK	bP	bI	bS	λ	Cellulose Output
1	11.56	5.41	10.84	5.65	80.66	121.94	0.42	0.68	0.36	0.56	0.25	0.51	0.0069	1.77e+07
2	16.71	5.17	16.94	4.83	80.34	82.78	0.41	0.62	0.42	0.54	0.45	0.65	0.011	1.75e+07
3	4.91	3.08	62.78	1.44	179.45	32.39	0.45	0.92	0.78	0.25	0.91	0.93	0.013	1.42e+07
4	8.32	3.45	56.31	2.37	214.21	19.32	0.51	0.72	0.78	0.23	0.82	0.91	0.015	1.07e+07
5	4.49	2.73	29.14	1.98	124.71	14.19	0.14	0.78	0.59	0.42	0.89	0.79	0.017	7.11e+06
6	3.16	2.26	19.56	1.71	29.42	7.59	0.18	0.64	0.68	0.36	0.89	0.61	0.020	3.73e+06
7	2.76	1.00	2.69	1.00	1.00	47.40	0.00	0.00	0.65	0.45	1.00	0.015	0.023	1.65e-08

2.3

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