**Towards a whole-cell modeling approach for synthetic biology**

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Towards a whole-cell modeling approach for synthetic biology

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Despite rapid advances over the last decade, synthetic biology lacks the predictive tools needed to enable rational design. Unlike established engineering disciplines, the engineering of synthetic gene circuits still relies heavily on experimental trial-and-error, a time-consuming and inefficient process that slows down the design cycle. This reliance on experimental tuning is because current modeling approaches are unable to make reliable predictions about the in vivo behavior of synthetic circuits. A major reason for this lack of predictability is that current models view circuits in isolation, ignoring the vast number of complex cellular processes that impinge on the dynamics of the synthetic circuit and vice versa. To address this problem, we present a modeling approach for the design of synthetic circuits in the context of cellular networks. Using the recently published whole-cell model of Mycoplasma genitalium, we examined the effect of adding genes into the host genome. We also investigated how codon usage correlates with gene expression and find agreement with existing experimental results. Finally, we successfully implemented a synthetic Goodwin oscillator in the whole-cell model. We provide an updated software framework for the whole-cell model that lays the foundation for the integration of whole-cell models with synthetic gene circuit models. This software framework is made freely available to the community to enable future extensions. We envision that this approach will be critical to transforming the field of synthetic biology into a rational and predictive engineering discipline.

Synthetic biology aims to engineer synthetic genetic circuits to endow cells and organisms with the ability to address new applications, including the production of drugs and industrial products, the design of new therapeutics for human diseases, and the study of basic biological processes. Despite significant advances in the field over the last decade, the synthetic biology design cycle has been hampered by the lack of predictive models. In contrast, more established engineering disciplines, such as civil and electrical engineering can rely on rational design by using models that can capture the behavior of real-world systems with a high degree of accuracy. This is currently not the case for synthetic biology, as models are generally only able to make qualitative predictions about system behavior. As a result, producing a functional engineered biological system usually requires extensive manual tuning by trial-and-error, a time-consuming process that significantly slows the design process. The lack of predictive power in current models is partly because these models account for only the synthetic circuits themselves, but not other ongoing processes in the cells in which they reside. However, the complex dynamics of synthetic circuits may affect the host cell and vice versa, leading to divergence between current models and experimental results. Recently, a whole-cell model of a small and simple bacterium was developed and shown to capture numerous aspects of this organism’s behavior. Here, we adapt this model to enable the easy incorporation of synthetic circuits and investigate its use for design in synthetic biology. We show that synthetic circuits can be integrated into the model and demonstrate the effects that synthetic genes can have on the host cell. We anticipate that this whole-cell modeling approach for synthetic gene circuits will enable more predictive and rational design for the field of synthetic biology.

I. INTRODUCTION

Synthetic biology promises to revolutionize many areas of technology, including bio-manufacturing, therapeutics and diagnostics.1,2 To meet this promise, synthetic biology must become a rigorous engineering discipline based on rational and predictive design.3–6 Modeling efforts within synthetic biology have so far focused on synthetic components and their interactions, where more detailed models have tried to refine these descriptions.7 For example, Tigges et al.8 considered how opposing RNA polymerases interact, while Stricker et al.9 incorporated DNA looping into their

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model of transcriptional regulation. The increasing use of stochastic models as a means of describing synthetic gene circuits further intends to improve these network-level descriptions.9–12

Interactions between the host cell and synthetic circuits have been recognized since the observation that oscillations in the repressilator stopped upon entry of the cell into stationary phase.10 More recently, interactions between the host cell and synthetic circuits have been used to uncover novel aspects of host physiology13 and population-level altruism14,15 and to guide design. These interactions are also an important consideration when porting circuits between organisms.16 However, modeling could be further used to accelerate synthetic biological design if it can account for the critical role of the cellular host in a comprehensive and systematic way, the state (e.g., mass, volume, energy status, metabolite concentrations) of which governs the parameters of the synthetic circuit. Consequently, biological circuit design has primarily relied on ad hoc engineering, requiring extensive experimental tuning, with post hoc modeling rather than predictive models and systematic design rules.

Many synthetic circuits have been modeled in isolation or only in the context of simplified host parameters due to the lack of comprehensive host models. This is a highly simplified approach for designing synthetic gene circuits that are expected to function inside of living cells. However, this situation is beginning to change. Recently, Karr et al.17 developed the first “whole-cell” model of Mycoplasma genitalium, which attempts to describe this organism in its entirety. M. genitalium is an ideal candidate for a whole-cell model because it has one of the smallest known genomes, containing only 525 genes.17,18 The model comprises 28 sub-models of distinct cellular processes including transcription, DNA repair, metabolism, and cell division. Each sub-model is implemented using the most appropriate mathematical representation. For example, metabolism is modeled using flux-balance analysis,19 whereas RNA degradation and protein degradation are modeled as Poisson processes.20 The model independently executes the sub-models for 1 s of real time and integrates the inputs and outputs of the sub-models at longer time-scales. In total, the whole cell model comprises 1423 chemical reactions and processes (e.g., formation of protein complexes) and approximately 1800 parameters. Each simulation starts at the beginning of the cell cycle and continues until the in silico cell “divides.” The whole-cell model captures the intracellular dynamics of M. genitalium remarkably well; it predicts previously observed gene essentiality with 79% accuracy and qualitatively agrees with measured metabolite concentrations, reaction fluxes, and RNA expression levels.17 Here, we adapted the whole-cell model of M. genitalium to enable the straightforward inclusion of artificial synthetic gene constructs into the in silico simulation. This updated software framework is made freely available to the community.

Towards a whole-cell modeling approach for synthetic biology, we investigate a critically important question: How do synthetic gene circuits affect the host cell? This question is relevant not only to the synthetic biology community but to the wider biological community because expression of non-native genes and genetic engineering are ubiquitously used throughout basic biological research, medicine, and biological engineering.

We used the M. genitalium whole-cell model to investigate the interactions between synthetic gene circuits and their hosts. First, we added lacI genes to the whole-cell model to study the effects of synthetic genes on the host cell and found a clear relationship between the number of genes added and the length of the cell cycle. We found that a substantial portion of this effect was due to the increased time required to replicate the DNA, and that another probable factor was the effect on the production of mRNAs from the native genome. We then used the whole-cell model to examine the impact of codon usage on gene expression. Codon optimization is a simple and commonly used approach to increase protein expression. We chose to investigate codon usage to test the predictive capacity of whole-cell models. Consistent with experimental findings,20 the model predicts little difference in the protein expression levels of codon-optimized and non-optimized genes but captures growth differences in the host cell. Finally, we implemented a Goodwin oscillator in the whole-cell model. We found that the oscillator displayed the characteristic noisy and irregular dynamics of a Goodwin oscillator.

II. THE EFFECTS OF SYNTHETIC GENES ON THE HOST CELL

We sought to investigate how synthetic circuits might affect the dynamics and behavior of the host cells in which they reside. Furthermore, we wanted to see how these effects vary with circuit size. As a proxy for complex gene circuits, we added varying numbers of lacI genes, with identical promoters (promoters having the exact same DNA sequence), in tandem. Terminator sequences (sequences that halt the process of transcription and demarcate the “end” of the transcriptional unit) were not included into the genome (see Appendix for details). We used lacI as our model gene, as it is extensively studied21 and a commonly used gene in the implementation of synthetic gene networks.9,10,22,23 The most compelling effect was on the length of the cell cycle. Figure 1(a) (solid line) shows the relationship between the number of lacI genes added (which correlates approximately linearly with the relative fraction of LacI protein, see supplementary information24 Figure 1) and the length of the cell cycle. The length of the cell cycle increases quickly with the addition of the first few genes and continues to increase up to 100 added genes. We saw similar results for the genes araC, tetR, and gfp (also well-understood genes which are commonly used in synthetic biology10,25–27) (see supplementary information24 Figure 2) and similar relationships (both quantitatively and qualitatively) between the number of genes added and the relative fraction of the protein (see supplementary information24 Figure 1). To ease comparison between results for lacI and araC, tetR, and gfp, we artificially set all proteins to form tetramers (a complex of four proteins) in all the studies reported here.

We then determined the contribution that the replication of additional DNA made to the increased cell cycle time.
This was done by preventing the lacI genes from being transcribed in the model, thus eliminating the production of LacI protein. Figure 1(a) (dashed line) shows the cell cycle time when the lacI genes were non-expressed. The relationship between the number of non-expressed lacI genes and the cell cycle length is similar to that of expressed lacI—the cell cycle length generally increases with the addition of non-functional lacI. However, the magnitude of the increase is not as great as with expressed lacI genes.

We also examined the effect on the extra expressed lacI genes on the number of mRNAs produced from the native genome. We found that as the number of expressed lacI genes increased, the mean (per time point, averaged over the length of the cell cycle) number of mRNAs produced from the native genome decreased (Figure 1(b)). We saw a similar relationship for araC, tetR, and gfp (see supplementary information24 Figure 3).

III. THE EFFECTS OF CODON USAGE ON SYNTHETIC GENE EXPRESSION

Codon optimization is a common method adopted in attempts to increase gene expression for the purposes of large-scale protein purification. From the perspective of synthetic biology, codon optimization is a potential way of tuning the dynamics of a synthetic gene network as well as the burden it imposes on the cell. We used the whole-cell model to investigate how codon optimization of four commonly used genes in synthetic biology, lacI, araC, tetR, and gfp, affects their expression. We used the JCat online codon
optimization tool\textsuperscript{28} to re-encode the gene sequences for expression in \textit{M. genitalium} (see supplementary information\textsuperscript{24} for sequences). Briefly, JCat calculates the Codon Adaption Index (CAI) of the sequence for a particular organism; higher numbers are considered more optimal. Our re-encoded \textit{lacI}, \textit{araC}, \textit{tetR}, and \textit{gfp} genes had CAI’s of 0.99, 0.96, 0.98, and 0.99, compared to 0.15, 0.18, 0.41, and 0.57 for the unoptimized sequences, respectively. Figure 2 shows the expression profiles of the optimized and unoptimized genes. In all cases, the protein expression profiles increase at approximately the same rate. This is in agreement with experimental observations in \textit{E. coli}, where the CAI has no correlation with gene expression level.\textsuperscript{20,29} However, experimental data have confirmed that the growth rate of \textit{E. coli} correlates with the CAI; the higher the CAI, the faster the growth.\textsuperscript{20} We examined the effect of the codon-optimized versions of our genes on the length of the cell cycle (Figure 3). For \textit{lacI} and \textit{araC} (Figures 3(a) and 3(b), respectively), which had differences in the CAI’s of the original and optimized genes of 0.84 and 0.78, respectively, we found agreement with the experimental observations. Optimized \textit{lacI} and \textit{araC} had a lesser effect on the growth of the cell than their unoptimized counterparts (mean difference across all data points was 0.22 and 0.29 h, respectively). Conversely, for \textit{tetR} and \textit{gfp} (Figures 3(b) and 3(c), respectively), which had lower differences in their CAI’s (0.57 and 0.42, respectively), the difference between the growth rates was approximately zero (mean difference across all data points was −0.06 and −0.01 h, respectively).

We performed a statistical analysis of the data using 1-tailed paired t-tests to test the hypothesis that the mean cell cycle length with the unoptimized gene is greater than the mean cell cycle length with the optimized gene. In the case of \textit{lacI} and \textit{araC}, we found the cell cycle length with the unoptimized gene to be significantly greater than the cell cycle length with the optimized gene (p-values of 0.046 and 0.015, respectively). We found no significant difference in the cases of \textit{tetR} and \textit{gfp} (p-values of 0.8 and 0.53, respectively). Table I summarizes the results of the t-tests.

### IV. GOODWIN OSCILLATOR DYNAMICS IN THE WHOLE-CELL MODEL

We implemented a Goodwin oscillator\textsuperscript{9,30} in the context of the \textit{M. genitalium} whole-cell model. This was done to demonstrate that a simple synthetic gene circuit could be made to function in the whole-cell model. A Goodwin oscillator is a simple genetic oscillator comprising a single gene that represses its own expression. The Goodwin oscillator was conceived 50 years ago\textsuperscript{30} but only recently implemented as a gene network in \textit{E. coli}.\textsuperscript{9} We used the \textit{lacI} sequence that is not optimized for \textit{M. genitalium} to construct an \textit{in silico} Goodwin oscillator within the whole-cell model (Figure 4).

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<td>\textit{gfp}</td>
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Figure 5(a) shows the dynamics of the oscillator for two representative in silico cell simulations (see supplementary information24 Figure 4 for additional in silico cell simulations). To ease comparisons between our simulations and experimental observations from E. coli9 (Figure 5(c)), Figure 5(b) shows the simulations from Figure 5(a) with a low-pass filter applied. The simulations agree qualitatively with experimental observations in E. coli;9 oscillations are irregular and noisy.

V. DISCUSSION

In this study, we used a whole-cell model of M. genitalium to investigate several fundamental questions in synthetic biology. Specifically, we presented a number of simple examples to demonstrate the potential uses of whole-cell models in synthetic biology and to study the effects of artificial gene circuits on their hosts.

We first investigated how the addition of genes into the genome affected the dynamics of the cell. This is a proxy for understanding how synthetic circuits, formed from externally added genes, may influence host cell behavior. We found a clear positive relationship between the number of genes added and the length of the cell cycle which was consistent for all four genes examined. Externally added genes commonly affect the growth rate of the host20,32 although the magnitude of the effect varies between organisms.33 The relationship between the number of lacI genes added and the length of the cell cycle was approximately linear. As the fraction of the total cell protein comprised of LacI was approximately linear with the number of lacI genes added, there is an approximately linear relationship between the fraction of total cell protein comprising LacI and the cell cycle length. This linear relationship is in agreement with results seen with the overexpression of genes in E. coli, where it has been observed that growth rate corresponds approximately linearly with the fraction of both β-galactosidase or an inactive form of the elongation factor TU.34

By performing the same analysis using non-expressed lacI genes, we delineated the contribution to the additional cell cycle time that was caused by the increased time required to replicate the DNA containing the lacI genes. Additional DNA replication time was found to account for approximately half of the increased cell cycle time. We then examined the effect of the additional genes on the level of mRNAs produced from the native genome. It might be expected that the additional genes sequester cellular proteins and resources, slowing down cellular processes and therefore growth. Reduced mRNA levels, due to reduced transcription, would be a clear indication of this sequestration. We found that for all genes, there was indeed a clear effect, with the additional genes causing the number of mRNAs produced from the native genome to decrease. This behavior indicates that one of the effects of adding an increasing number of expressed genes is to reduce the overall cellular resources that can be dedicated to transcription of the native genes. Other likely contributions to the observed growth effects could be additional genes diverting limited nucleic acids and amino acids from other DNA, RNA, and proteins in the cell. This would lead to the lower production of metabolic enzymes, RNA polymerase, and ribosomes, resulting in a
lower growth rate.\textsuperscript{35–38} It should be noted that in our studies, the expression of added genes was always on and thus, these results do not capture the fact that in many complex synthetic circuits, only a subset of the genes will be expressed at any given time.

We then examined the effect of codon usage on the expression profile of four genes commonly used in synthetic biology: \textit{lacI}, \textit{araC}, \textit{tetR}, and \textit{gfp}. In agreement with experimental results from \textit{E. coli} (on codon usage in \textit{gfp}),\textsuperscript{20} we observed no difference in the rates of expression between unoptimized and optimized versions of the genes, despite substantial differences in their CAI’s. In further agreement with the findings of Kudla \textit{et al.},\textsuperscript{20} we found that codon optimization could have a positive effect on the growth rate. We only observed this difference for \textit{lacI} and \textit{araC}, where there was the greatest difference in the CAI values between the unoptimized and optimized genes. We observed no difference in the growth rate of unoptimized and optimized \textit{tetR} or \textit{gfp}, which had lower differences in CAI values. A gene with a higher CAI will use codons that are translated by aminoacylated-tRNAs that are more abundant within the cell. In the model, translation is affected by the availability of aminoacylated-tRNA. By using an added gene with a higher CAI, the expression of the added gene is less likely to use the less abundant aminoacylated-tRNA species, and these are therefore less likely to becoming limiting for the translation of genes in the native genome. As the expression of the genes in the native genome is directly related to the growth of the cell, an added gene with a higher CAI will have less effect on growth. This proposed mechanism is a theory, and further analysis is required to understand the relationship between the changes in the level of optimization and the corresponding growth effects. However, the relationship may be non-linear, with smaller differences in optimization causing negligible growth effects. If the observed optimization effects can be verified experimentally in \textit{M. genitalium}, these findings would further demonstrate the predictive power of the whole-cell model. Finally, we described the successful implementation of a Goodwin oscillator within the whole-cell model and found that its behavior qualitatively matched the \textit{in vivo} dynamics observed in \textit{E. coli}.\textsuperscript{9}

Integrating synthetic gene circuits within accurate whole-cell models has the potential to enable more rapid and reliable design of biological systems with less trial and error. We believe that whole-cell models will ultimately aid synthetic biologists in understanding and describing why synthetic circuits do not behave \textit{in vivo} as expected from the predictions of isolated computational models.\textsuperscript{10,39} This, in turn, should lead to models that can more accurately predict and thus enable the rational design of synthetic circuits. We intend this work to be taken as a preliminary investigation into the use of whole-cell models within synthetic biology. A caveat to the work presented here is that without complementary \textit{in vivo} data, we cannot directly test the \textit{in silico} predictions made with the whole-cell model. Nonetheless, given the previous accuracy of this whole-cell model in Karr \textit{et al.} (e.g., its qualitative agreement with measured metabolite concentrations, reaction fluxes, and RNA expression levels)\textsuperscript{17} and the consistency of our findings with existing experimental data, we hope that this effort may launch new studies to compare experimental results with model predictions using this approach. Such efforts will enable the further optimization of whole-cell synthetic circuit models for more rapid and accurate design.

\section*{VI. FUTURE OUTLOOK}

There are many intriguing questions that a whole-cell approach to modeling synthetic gene circuits could be used to address. Ideally, any future investigations would be closely accompanied with experimental work, with the aim of comparing model predictions with experimental results and improving the accuracy of this approach. \textit{M. genitalium} is not an ideal model organism; it is a small organism with a long doubling time, has few known genetic tools, and no known defined medium. However, \textit{Mycoplasma} have been successfully engineered in prior work—for instance, with functioning GFP and TetR proteins\textsuperscript{30,41} and on a much larger genome-wide scale in the recent work of Gibson \textit{et al.}.\textsuperscript{32}

This study has focused on one side of the relationship between a synthetic circuit and the host—the effect of the synthetic circuit on the host. We have not examined the other side of the relationship—the effect of host processes on the behavior of the synthetic circuit. Ultimately, the effects between the host and the synthetic circuit are intertwined and feed back on each other, and whole-cell models have the potential to uncover details of these likely complex relationships.

The whole-cell model necessarily makes numerous assumptions and simplifications. A current limitation of the whole-cell model for use in synthetic biology is the absence of non-specific binding of transcription factors to DNA, although the non-specific binding of other proteins, such as RNA polymerase, is captured in the model. Though a transcription factor may have a single specific binding site, it is thought that transcription factors that are not bound specifically are likely to be bound non-specifically, elsewhere in the genome.\textsuperscript{43,44} The remainder of the genome therefore acts as a sink for transcription factors and consequently has important implications for the dynamics of gene circuits.\textsuperscript{43,44} For synthetic biology, a useful extension to the whole-cell model would therefore be the ability to define non-specific binding for added transcription factors.

The use of whole-cell models for synthetic biology is in its infancy, and thus advantages to using such models for biological engineering will need to be demonstrated with experimental data. Nonetheless, whole-cell models are likely to form the backbone of future modeling approaches in synthetic biology and will help transform the field into a more predictive engineering discipline. Within the workflow of the synthetic biology design and construction process, we envisage whole-cell models will serve as advanced biophysical models that will exist in-between higher-level graphical design tools such as Clotho\textsuperscript{45} and GenoCAD\textsuperscript{46} and the physical implementation of the circuit in the cell. The software advancements made here allow for synthetic circuits to be easily integrated into the whole-cell model, allowing users to programmatically add and remove model components including genes,
transcription units, protein monomers, complexes, reactions, and regulatory interactions (see Appendix). Further improvements of the model may incorporate transcriptional, 47,48 RNA-based, 49,50 and recombinase-based circuitry.31 We hope this will encourage the application of the whole-cell modeling approach to synthetic biology. Beyond synthetic biology, we anticipate whole-cell models will be useful for interpreting experiments and guiding biological discovery.

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APPENDIX: IMPLEMENTATION OF SYNTHETIC CIRCUIT WHOLE-CELL MODELS

1. Whole-cell models

The Mycoplasma whole-cell model was implemented as described previously.17 The knowledge base Application Programming Interface (API) was improved to enable users to programmatically add and remove model components in a straightforward fashion, including genes, transcription units, protein monomers, complexes, reactions, and regulatory interactions. In addition, we created new a class called SimulationRunner to encapsulate all of the model components and parameter modifications required for specific simulations and to separate these perturbations from the submodel classes. Researchers can use the revised code to easily simulate perturbations including gene additions and deletions.

2. Synthetic circuit whole-cell models

The lacI knock-in model was implemented by adding a single protein-coding gene to the MATLAB M. genitalium whole-cell model.12 First, we appended the E. coli K-12 W3110 lacI (b0345) DNA sequence with start and stop codons modified for M. genitalium as well as 100 immediate upstream random nucleotides (which includes the promoter) to the end of the M. genitalium chromosome at position 580077 (see supplementary information24 for sequences). Second, we defined a new promoter with 6 nucleotides (nt) −35 and −10 boxes at the −35 and −10 positions relative to lacI. The lacI promoter RNA polymerase binding probability was set equal to the 0.001 mean RNA polymerase mRNA promoter binding probability, and the lacI mRNA half-life was set equal to the 4.53 min mean mRNA half-life. The half-lives of both the LacI monomers and tetrameric complexes were set to 20 h (the default half-life for proteins in the whole-cell model). The same procedure and parameters were used for the genes araC, tetR and gfp.

The codon-optimized lacI knock-in model was implemented similarly using the codon-optimized lacI sequence calculated by JCat28 (see supplementary information24 for sequence). The tandem repeat lacI knock-in model was also implemented similarly by repeatedly appending the E. coli lacI gene and immediate upstream region to the end of the M. genitalium chromosome 20, 50, and 100 times. The same procedure was used for the genes araC, tetR and gfp.

The Goodwin oscillator model was implemented by first adding lacI to the whole-cell model as described above. Next, we implemented LacI auto-repression by adding a 10 nt LacI tetramer DNA-binding site at the −40 position relative to the lacI gene and setting the fold-change effect of bound LacI to the lacI RNA polymerase recruitment rate to 10%. Finally, because the whole-cell model only simulates a single ~9 h cell cycle, oscillations were not observed when using a 20 h half-life. We, therefore, decreased the half-lives of the LacI protein monomers and complexes from 20 h to 24 min. A short half-life may be obtainable using ssrA tags, commonly employed to increase protein degradation rates in oscillators implemented in E. coli9,10 and which are also present in Mycoplasma.52

The interested reader is referred to Data S1, Sec. 1.4 of Karr et al.17 for further discussion of the whole-cell model initial conditions.

3. Numerical simulations and data analysis

All simulations and data analysis were performed using MATLAB 2012b. Each whole-cell simulation typically took between 20–30 h, and 2 h, respectively. Statistical analysis was carried out using MATLAB 2012b.

4. Availability

All of the simulation code and numerical results are freely available open-source at SimTK (http://simtk.org/home/wholecell).


1B. Xia et al., “Developer’s and user’s guide to Clotho v2.0 A software platform for the creation of synthetic biological systems,” Methods Mol. Biol. 498, 97–135 (2011).