**Title.** A Systems Biology Approach for Investigating Host Limitation of Viral Replication

**Key words.** host-viral interactions, virology, systems biology, T7 phage, *E. coli*

**Introduction**. Understanding how viruses interact with their hosts is key to controlling unwanted viral infections in humans, agricultural crops, and industrial processes. Viruses are parasites completely dependent on their cellular hosts to produce the proteins, DNA/RNA, and lipids necessary for their replication. What aspects of host metabolism limit the rates of viral synthesis and assembly? It is well known that the rate of production of many bacteriophages increases with the rate of bacterial growth [1], possibly due to increased host biosynthetic metabolic capacity and concomitant removal of host metabolic constraints on viral growth [2]. Using a theoretical approach, Yu *et al*. found the rate of T7 replication in is sensitive to the number of ribosomes per infected cell but insensitive to the counts other macromolecular components such as RNA polymerase [3]. Based on these findings Yu *et al* suggested that the rate-limiting step of phage growth is the synthesis of phage proteins. However, it is unclear how these results translate to a systems wide picture of host limitation of viral replication.

**Hypothesis**. I propose to use a systems biology approach to test the hypothesis that cellular production of viral protein and DNA limits the rate of T7 intracellular replication in *E. coli*.

Specifically, I propose to: (1) Develop an integrated model of *E. coli* metabolism and T7 replication. I will analyze the sensitivity of the rate of T7 production to the rates of transcription, translation, and replication. I will employ linear optimization to model metabolism, growth correlations to model *E. coli*’s macromolecular machinery, and ordinary differential equations (ODEs) to model T7 replication. (2) Experimentally test if *E. coli* production of T7proteins or DNA limits viral replication using small molecule transcription, translation, and replication inhibitors and amino acid and deoxynucleotide genetic auxotrophs. I will determine the sensitivity of T7 production to each of these perturbations and use differences between my experimental measurements and my model’s predictions to drive biological discovery.

**Specific Aim 1: Computational sensitivity analysis of host requirements for T7 production.**

I have spent the past six months working with my advisor, Prof. Markus Covert, and fellow graduate student, Elsa Birch, building an integrated biochemical model of *E. coli* metabolism and T7 replication. Specifically, we are integrating two previously published models to create a two-part multi-scale hybrid model: (1) a model of T7 replication based on ordinary differential equations that describe the transcription and translation of 55 T7 genes [2, 3], and (2) a model of *E. coli* metabolism based on flux balance analysis (FBA) – a linear optimization-based method that predicts the steady-state rate of each metabolic reaction given metabolite, enzymatic, and regulatory constraints [4]. The model captures the dynamics of all of *E. coli*'s metabolic pathways as well as the detailed dynamics of T7 replication.

The model employs the following key assumptions: (1) Separation of timescales – changes in *E. coli* intracellular metabolite concentrations and the accumulation of T7 components occur at distinct timescales, and thus each can be modeled assuming the other process is time-independent. (2) Metabolite accumulation – I will relax the conventional FBA constraints which prevent metabolite accumulation to allow the production of small molecules required for T7 production.

Upon model completion I will perform a sensitivity analysis of T7 intracellular production rate to ribosome, RNA polymerase, and T7 DNA polymerase copy numbers, and to the fluxes of amino acid and deoxynucleotide synthesis reactions. I will vary the value of each parameter individually and then use the hybrid model to predict the T7 production rate. This sensitivity analysis I will enable me to determine which aspect of protein or DNA synthesis has the greatest effect on viral production, and therefore greatest anti-viral target potential.

*Possible Outcomes and Further Studies*: I predict that I will not only verify You *et al*.’s findings, but also obtain a systems view of the sensitivity of T7 production to ribosome copy number. I will also identify the components of small molecule metabolism which affect T7 production. Furthermore, our integrated model will enable investigation of other aspects of host-viral interaction including transcriptional regulation and protein-protein interactions.

**Specific Aim 2: Experimental sensitivity analysis of host requirements for T7 production.**

During my third year I will experimentally determine the sensitivity of T7 production to the rate cellular metabolism. I will use small molecule antibiotics to inhibit transcription, translation, and replication. I will use publicly available LD50 data to determine drug concentrations that will inhibit with efficiencies varying 25% - 100%. Specifically, I will use tetracycline and chloramphenicol to inhibit the 30S & 50S ribosomal subunits, rifamycin to inhibit mRNA polymerase, mupirocin to inhibit isoleucyl-tRNA synthetase, and ciprofloxacin to inhibit DNA polymerase. Additionally, I will obtain auxotrophs for amino acids and deoxynucleotides from the *E. coli* Kieo collection [5]. I will determine the sensitivity of T7 production to metabolismby (1) varying the amount of limiting amino acid or deoxynucleotide in the infection media an order of magnitude below the concentration that produces wild type growth, (2) adding antibiotics, (3) inoculating with T7 phage, and (4) quantitating the rate of T7 production using a standard plaque assay time course. The relative change in T7 production rate compared to wild type will indicate how sensitive the T7 production rate is to the perturbation of protein or DNA synthesis. This analysis will determine the metabolic pathways which most strongly limit viral production.

*Possible Outcomes and Further Studies*: I expect these experiments will positively confirm approximately 75% of the model predicted sensitivities of T7 production. I expect the remaining 25% will highlight the knowledge gaps of our computational model and provide opportunities to improve our understanding.

**Conclusion**. Using the combined computational and experimental approach outlined here, I expect to construct a quantitative, and biologically accurate model of host-viral interaction within 3 years. In the future I plan to use my model of host-viral interactions to inform treatment of viral infections in medicine, agriculture, and industry by 1) identifying host drug targets for anti-viral medication, and 2) engineering novel mechanisms for host resistance or immunity to viral infection.

**Statement of originality**. I hereby declare this submission to be my own work.

**References**

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