Technical Summary: Rational Engineering of In Vitro Transcription for RNA Synthesis

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The in vitro transcription (IVT) reaction is a necessary step for the manufacturing of RNA for mRNA vaccines and is typically performed as a liquid-phase batch reaction. In this thesis, I develop mathematical models of the thermodynamics and enzyme kinetics of this reaction to describe experimental input-output behavior, including reaction rates, undesired solid precipitation, and the formation of key impurities. These models are used for the design and optimization of reactor systems that go beyond the liquid-phase batch standard, including fed-batch reactions and heterogeneous flow reactor systems. These reactor designs are experimentally shown to improve the process economics and impurity profile of the in vitro transcription reaction. This work, performed as part of a larger industrial-academic collaboration for the manufacturing of RNA vaccines, was primarily motivated by questions which were broadly applicable to a wide set of industrial and academic practitioners of IVT. How can we decrease the cost to manufacture RNA vaccines and therapeutics? How can we decrease the formation of harmful byproducts? And how can we develop manufacturing processes faster, using fewer trial-and-error experiments?

The first half of this thesis develops computational models to describe the input-output relationships of IVT. For example, the second chapter of the thesis focuses on modeling the precipitation of the magnesium pyrophosphate byproduct during IVT. In this work, we developed a mechanistic model of IVT to include nucleation and growth of magnesium pyrophosphate crystals and subsequent agglomeration of crystals and DNA. The model explains previously unexplained trends in IVT data and quantitatively predicts the effect of adding the pyrophosphatase enzyme to the reaction system. The third chapter develops a quantitative understanding of the reaction kinetics of IVT. While the kinetics of the microscopic steps of this reaction (promoter binding, initiation, and elongation) were previously well-known, the rate law of overall RNA synthesis that emerged from this system was unclear. In this chapter, we showed that a model that incorporates both initiation and elongation steps is essential for describing trends in IVT kinetics in conditions relevant to RNA manufacturing. In contrast to previous reports, we found that the IVT reaction can be either initiation- or elongation-limited depending on solution conditions. This initiation-elongation model is also essential for describing the effect of salts, which disrupt polymerase-promoter binding, on transcription rates. Polymerase-polymerase interactions during elongation were incorporated into our modeling framework and found to have nonzero but unidentifiable effects on macroscopic transcription rates. Finally, we developed an extension of our modeling approach to quantitatively describe and experimentally evaluate RNA- and DNA-templated mechanisms for the formation of double-stranded RNA (dsRNA) impurities. We show experimental results that indicate that an RNA-templated mechanism is not appropriate for describing macroscopic dsRNA formation in the context of RNA manufacturing.

The latter half of this thesis sought to put these models to work for reaction engineering. In the fourth chapter we used our computational models to develop an optimized fed-batch

IVT process. Practitioners in the area of IVT seek to maximize the production of RNA and the incorporation of the 5-prime cap to the end of each RNA molecule while minimizing the use of expensive reagents. Fed-batch IVT is a promising technique for achieving these goals but is difficult to optimize by purely experimental means. In this chapter, we developed a mechanistic model for fed-batch IVT and used it to develop optimal fed-batch protocols which can produce over twice as much RNA as heuristic approaches. In addition, we observed and characterized for the first time the formation of magnesium phosphate crystals during the IVT reaction. We developed strategies informed by thermodynamic modeling to prevent this undesired crystallization during fed-batch IVT. Finally, we incorporated co-transcriptional capping into our model-based optimization approach and developed a strategy to maximize RNA formation while maintaining a high level of 5-prime cap incorporation.

The fifth and final chapter goes deeper into engineering applications: We developed models to describe the formation of truncated RNA, a critical manufacturing byproduct, and developed a heterogeneous flow reactor system that can help to decrease this byproduct formation. We found that the truncated RNA byproduct is produced by three discrete chemical pathways, which are dependent on reaction times, solution conditions, and catalyst concentrations. To mitigate this byproduct formation, we proposed a heterogeneous flow reactor in which the DNA template is immobilized on a solid porous matrix. With the aid of a process model describing the dynamics and steady-state reaction behavior of this system, we developed and evaluated an experimental reactor. We found that this reactor can decrease the formation of truncated RNA compared to an analogous batch reaction, and discussed challenges and opportunities for industrial adoption of this technology.