Mechanistic Modeling of Viral Particle Production

by
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Technical Summary

Viral systems such as viral vectors and virus-like particles (VLPs) are essential components of modern biotechnology and medicine. Despite this, the commercialization of many of these biotherapeutics is limited by manufacturing processes that are incapable of meeting global demand. This thesis focuses on applying systems engineering techniques such as mechanistic modeling to improve the production of two difficult-to-manufacture therapeutic modalities: (1) recombinant adeno-associated virus (rAAV) for monogenic gene therapy and (2) homologous severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) VLPs for vaccination against SARS-CoV-2.

rAAV is a commonly used in vivo gene therapy vector because of its non-pathogenicity, long-term transgene expression, and broad tropism. However, rAAV vector production via transient transfection of mammalian cells is characterized by low yields and a low fraction of filled-to-total capsids (~1 – 30% of total capsids produced). This low percentage of full capsids leads to higher doses that may trigger immunogenic reactions in patients and necessitates costly downstream separations. We explored a multi-stage transfection strategy to understand effects of plasmid dosing on transient rAAV production. By differentially administering a set amount of plasmid DNA over multiple, time-separated doses we observed a modulation in Rep protein expression that allowed us to develop a mechanistic model accounting for the impact of Rep protein on rAAV capsid filling. Consistent with previous studies, the mechanistic model indicates that Rep proteins suppress both Rep and Cap protein synthesis while enhancing vDNA replication. This leads to a misalignment between capsid synthesis, vDNA replication, and encapsidation, the effects of which can be partially mitigated by reducing the intracellular concentration of Rep protein.

We used these mechanistic insights to develop an intensified process for rAAV production that combines continuous perfusion with high cell density re-transfection of human embryonic kidney (HEK) 293 cells. We demonstrate that performing multiple, time-separated doses at high cell density boosts both cell-specific and volumetric productivity and improves plasmid utilization when compared to a single bolus at standard operating conditions. Our results establish a new paradigm for continuously manufacturing rAAV via transient transfection that improves productivity and reduces manufacturing costs.

We next focused on using systems engineering approaches to improve the production of SARS-CoV-2 VLPs. VLPs are nanoparticles comprised of self-assembled viral or non-viral proteins that mimic the structure of the native virus without being infectious. Homologous VLPs constructed from the four SARS-CoV-2 structural proteins are promising vaccine candidates due in part to

their multi-antigenic structure: when a mutation occurs on one component of the virus, the vaccine remains effective due to its additional viral components. However, the deployment of these vaccines is currently constrained by the low productivity of stable producer cell lines.

We developed a mechanistic model that describes the recombinant production of SARS-CoV-2 VLPs within a cell. We then engineered and sampled multiple cell lines presenting varied stoichiometries of integrated genes encoding for the four structural proteins. Using an iterative workflow combining Bayesian parameter estimation and model-based design of experiments (MB-DOE), we consolidated kinetic information from the literature and converged on a useful model with minimal internal experimentation. We then used this model to demonstrate that that the underproduction of spike and nucleocapsid proteins limits the production of SARS-CoV-2 VLPs within our stable cell lines.

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