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REVIEW

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Mechanistic modeling of viral particle production

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Abstract

Viral systems such as wild-type viruses, viral vectors, and virus-like particles are essential components of modern biotechnology and medicine. Despite their importance, the commercial-scale production of viral systems remains highly inefficient for multiple reasons. Computational strategies are a promising avenue for improving process development, optimization, and control, but require a mathematical description of the system. This article reviews mechanistic modeling strategies for the production of viral particles, both at the cellular and bioreactor scales. In many cases, techniques and models from adjacent fields such as epidemiology and wild-type viral infection kinetics can be adapted to construct a suitable process model. These process models can then be employed for various purposes such as in-silico testing of novel process operating strategies and/or advanced process control.

KEYWORDS

dynamics, mathematical modeling, upstream biomanufacturing, viruses, virus-like particles

1 | INTRODUCTION

Viral systems such as wild-type viruses, viral vectors (e.g., adeno-associated viruses [AAVs] and retroviruses [Warnock et al., 2011]) and virus-like particles (VLPs) have become essential components of modern biotechnology and medicine. These systems are used in the production of vaccines (e.g., inactivated or attenuated whole viral vaccines [Plotkin, 2014], viral vector vaccines [Ura et al., 2014], and VLP-based vaccines [Mohsen et al., 2017]), vaccine adjuvants and antiviral therapies (Frensing, 2015), gene therapies (Warnock et al., 2011), recombinant protein production (Shah et al., 2013; Wurm & Bernard, 1999), and drug delivery (Ma et al., 2012; Wen & Steinmetz, 2016).

The successful development and commercialization of many of these biotherapeutics require large-scale and high-yield production of these viral systems. Scaling up and optimization of viral system manufacturing processes are currently active areas of research. Some of the challenges are generally applicable to most viral systems, for example, inefficient downstream separation and purification, (Singh & Heldt, 2022), while others are system specific, for example, low yields in recombinant AAV (rAAV) production due to inefficient filling of capsids with the desired genetic material (Nguyen et al., 2021). These challenges have motivated significant research activity throughout the manufacturing and development pipeline, for example, engineering and optimizing cell lines (Genzel, 2015; Kiesslich & Kamen, 2020), engineering the viral system itself to facilitate production (Jordan et al., 2013; C. Li & Samulski, 2020), bioreactor process development and intensification (Gallo-Ramírez et al., 2015; Kiesslich & Kamen, 2020), and improving the performance of downstream separation processes (Singh & Heldt, 2022; Wolf & Reichl, 2011).

One of the comparatively nascent developments in the viral systems production literature, which motivates this review, is the development and application of mathematical modeling approaches to understanding the manufacturing process both at the cellular and reactor scales. The use of modeling strategies in biotechnology is well-established (a cursory search of the literature will reveal a plethora of studies and reviews on the topic, [e.g., Bailey, 1998; Lee et al., 2005]) and has significantly contributed to the development of the field. Incorporating modeling into the process research and development workflow can contribute several benefits such as providing deeper process insights into the dynamics and multivariable interactions present in the system (Nguyen et al., 2021; Udugama et al., 2021), enabling the deployment of advanced process control and monitoring strategies (Hong et al., 2018; Udugama et al., 2021), enhance process optimization methodologies (Narayanan et al., 2020), and guide and accelerate the development of novel manufacturing strategies (Gernaey et al., 2010; Nguyen et al., 2021).

Fortunately, the development of process models for viral systems manufacturing is significantly aided by a comprehensive pre-existing literature in adjacent fields such as wild-type viral infection, epidemiology, and chemical/biochemical reactor engineering. At the cellular scale, detailed studies on the viral life cycle and infection kinetics provide the basis for mapping the intracellular process steps and can serve as initial kinetic estimators. At the reactor scale, the interactions between viruses and cells can be coarse-grained and approximated by functional forms that are analogous to the compartment-modeling frameworks used to study infection at the scale of an individual human or a population. These human-scale (e.g., Gastine et al., 2021; Hadjichrysanthou et al., 2016; Hill et al., 2018) and population-scale models (e.g., Annas et al., 2020; Carcione et al., 2020) are commonly used tools to understand disease progression and the impact of specific interventions like antiviral therapies or vaccination programs. Multiscale approaches that combine cellular- and reactor-scale modeling are also commonly employed in the literature for similar applications.

This review aims to consolidate existing literature on the dynamical modeling of upstream viral systems manufacturing and outline a robust approach for constructing novel models. By breaking down the required model constituents and processes at both the cellular and reactor scales, we show how a model can be formulated using information and strategies from the various aforementioned fields.

2 | CELLULAR-SCALE MODELS

2.1 | Model constituents

Cellular-scale models of viral processes describe the steps that map the viral production processes within an individual cell. The specific steps vary, but in general capture uptake, unpacking, transport, replication, transcription, translation, budding, and release processes occurring within the cell. Figure 1 provides an overview of the steps often included in cellular-scale reaction-transport models of viral systems.

When establishing a cellular-scale model, the delineation of the model steps depends broadly on four characteristics:

1. Virus Type: The type of wild-type virus, viral vector, or VLP.
2. Cell Type: The type of cells being used to generate the virus, e.g., mammalian cells such as HEK293 or insect cells such as Sf9. The cell type also impacts the kinetic parameter values used in the model.
3. Genetic Expression Approach: The method of expressing the gene (s) of interest, e.g., transient transfection, stable expression, or wild-type infection.
4. Data Availability: The utility of the model depends on the type and quality of the available experimental data.

Unlike wild-type infection models, which seek to understand the timing and dynamics of wild-type viral infection, recombinant models depict the cellular production of viral vectors or VLPs for therapeutic or experimental use. Although recombinant viral system modeling is a less mature area of development, models have been created for viral vectors (Dinh et al., 2005; Nguyen et al., 2021), attenuated viruses for vaccines (Laske et al., 2019), and VLPs (Hu & Bentley, 2000; Roldão et al., 2007). Figure 2 shows predictions from an exemplar cellular-scale recombinant model. This work—which describes a transiently transected rAAV system—is a demonstration of a cellular-scale modeling workflow: after the reaction-transport network is determined and mathematically formulated, the model is fit to experimental data, validated, and used to make predictions. In this case, the model was used to make operational recommendations to increase the proportion of filled rAAV capsids. For a thorough overview of wild-type infection models, refer to Ref. Yin and Redovich (2018).

The focus of this section of the review is the mathematical approaches that enable cellular-scale viral modeling. These mathematical strategies are often application agnostic, and can be readily applied to either wild-type or recombinant systems.

2.2 | Mathematical approaches

Cellular-scale models can be broadly categorized as either deterministic or stochastic. Deterministic models neglect the inherent randomness and noise of the biological system and provide precise predictions. While the insights gleaned from deterministic models are often useful, biological systems are inherently random, and stochastic elements can be included to describe variations observed in experiments and to capture a more complete understanding of the system dynamics (Bressloff, 2014; Tsimring, 2014). Srivastava et al. (2002) is a helpful primer on the differences of applying deterministic and stochastic modeling approaches to viral systems.

2.2.1 | Deterministic modeling

Deterministic models of viral systems often employ a system of ordinary differential equations (ODEs) or differential-algebraic equations (DAEs) to describe the cellular-scale mass action kinetics. This approach assumes species homogeneity within the cell. The steps outlined in Figure 1 provide an overview of the steps often included in ODE- or DAE-based reaction-transport models of recombinant viral systems. Three methods of gene delivery and production are highlighted: (a) gene delivery through plasmid transfection, (b) gene delivery through recombinant viral infection and receptor-mediated

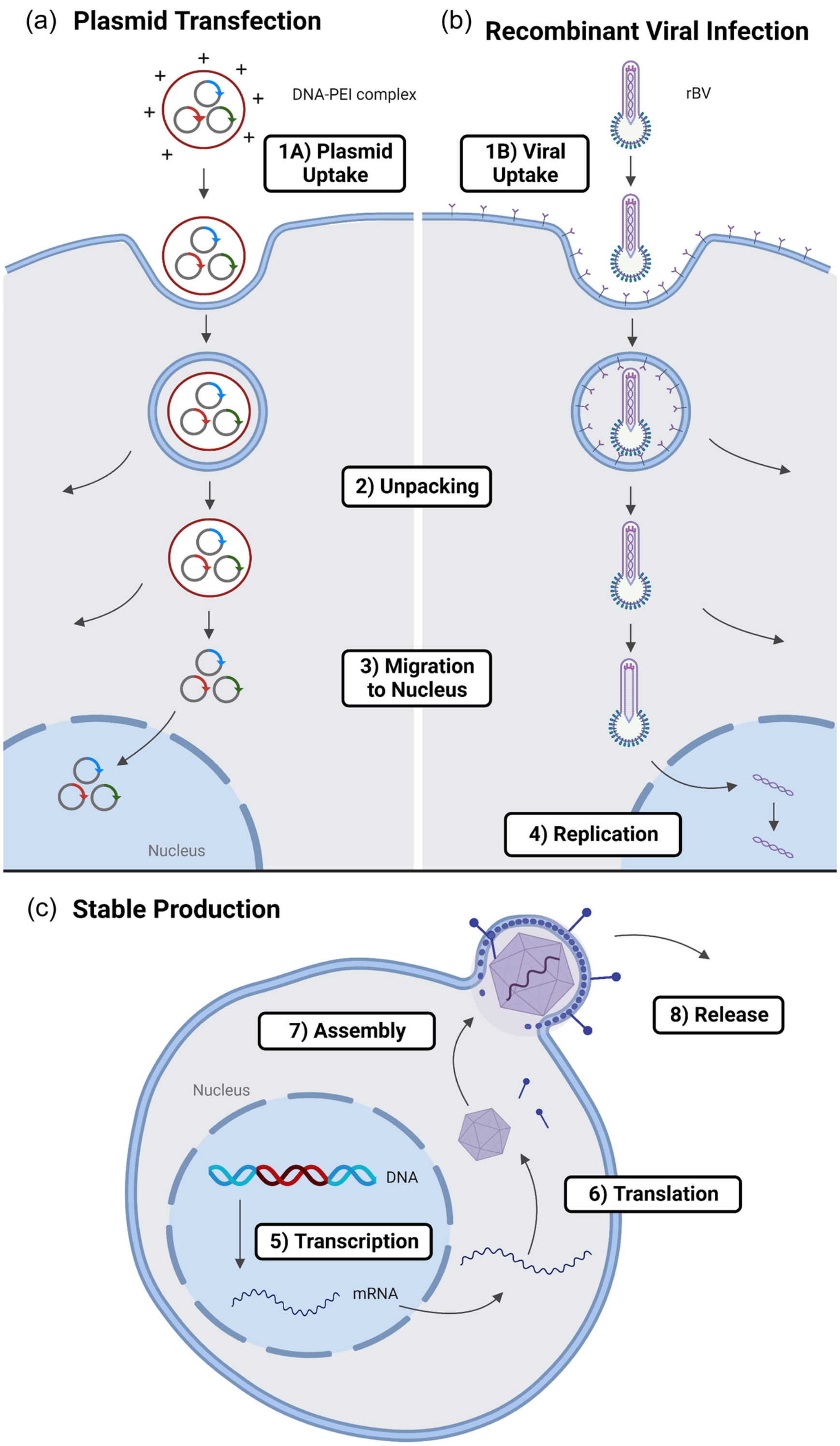

FIGURE 1 Typical steps included in cellular-scale models of recombinant viral systems. Transient gene delivery to a host cell via (a) plasmid transfection and (b) recombinant viral infection. First, the plasmids or virus delivering the genes are taken up by the cell (uptake), where they escape the endosomal vesicles (unpacking), migrate to the nucleus, and replicate. (c) The production of a viral vector via a stable cell line, an alternative to transient gene delivery, is used to depict the transcription of viral genes, translation of viral proteins, assembly of the recombinant viral system, and its release from the cell. The transient systems shown in (a) and (b) may also incorporate these steps. Created with BioRender.

endocytosis, and (c) a stable producing cell line. Note that not all steps are relevant to all viral systems.

There is no consensus on the mathematical approaches used to describe many of the cellular-scale steps depicted in Figure 1. Some mathematical approaches are summarized in Tables 1 and 2. Many of the differences in approach are driven by differences in virus or application, but differences are also caused by a lack of understanding of the precise mechanisms occurring at the process step, which is often rooted in experimental limitations that make it difficult to extract the granular kinetic details of the complex biological interactions occurring in each step. Because of this, the steps included in cellular-scale models often describe lumped phenomena.

As an example, consider the assembly step. Viral assembly is a complex process with many substeps: the capsid proteins assemble, the nucleic acid becomes encapsulated within the capsid, and, in some cases, the virus obtains a membrane coat. Modeling these interactions is an

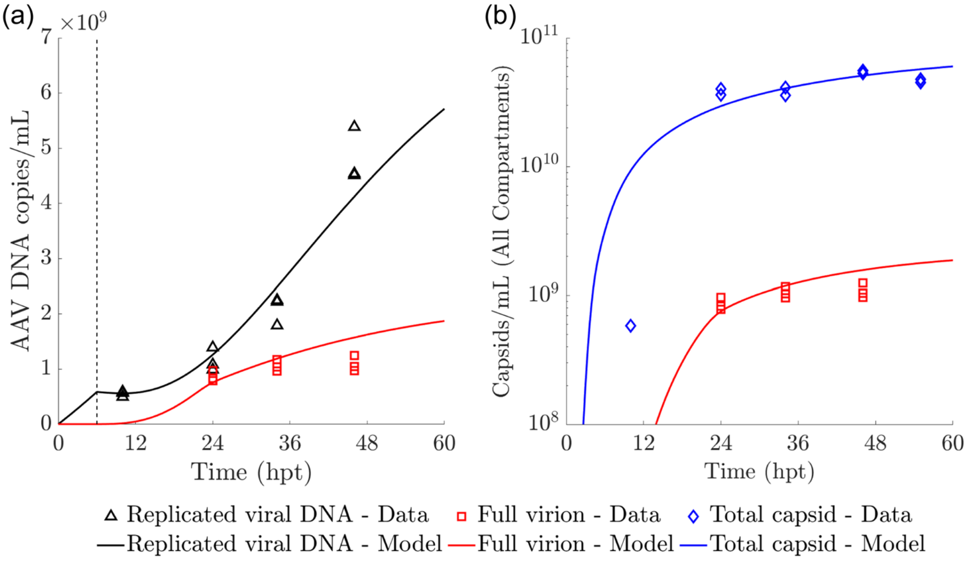

FIGURE 2 Dynamical cellular-scale model outputs for a recombinant adeno-associated virus (AAV) production process. In this study, Nguyen et al. (2021) created a mechanistic model that describes the generation of recombinant AAV (rAAVs) via transient transfection. The model was fit to experimental results and used to elucidate the mechanisms driving the low proportion of full capsids produced by the system (b). These model predictions were then used to generate hypotheses about ways to increase the proportion of full capsids, such as dosing the plasmids at different time points. These plots were regenerated using software available at GitHub (Nguyen, 2021).

active area of research, and detailed mechanistic and physics-based models exist that can provide a thorough description for many viral and VLP systems (Hagan, 2014; Perlmutter & Hagan, 2015). However, as shown in Table 1, the assembly rate of the viral particles and VLPs is often approximated in cellular-scale dynamical models using Michaelis–Menten kinetics (Grebennikov et al., 2021; Heldt et al., 2012; Zitzmann et al., 2020), limiting-substrate kinetics (Aunins et al., 2018; Nguyen et al., 2021; Shcherbatova et al., 2020), or thermodynamic (Hu & Bentley, 2000) approaches. When building an integrated cellular-scale model, a balance between detail and computability needs to be struck. If the approximation accurately predicts the rate and stoichiometry of the step, the model conclusions should be sufficient for making population-scale productivity and product quality assessments.

Even when a step's high-level mathematical structure is the same, the equation details can differ. For example, multiple groups have modeled the transcriptional regulation of gene expression by tracking the number of ribosomes available for translating the mRNA into protein (Aunins et al., 2018; Binder et al., 2013; Lim et al., 2006; Zitzmann et al., 2020). The models of Binder et al. (2013) and Zitzmann et al. (2020) include ODEs for the translation complexes that form when an mRNA binds to a ribosome. Each translation complex represents a polyribosome, and a cap is placed on the number of ribosomes available inside the cell. Aunins et al. (2018) also uses ODEs to describe the formation of translation complexes, but include an additional ODE that tracks the number of available ribosomes. Lim et al. (2006) instead uses an algebraic approach in which ribosomes are assigned to the various mRNAs based on their length.

2.2.2 | Stochastic modeling

The propagation of stochastic effects often leads to cell-to-cell heterogeneity in viral systems. Including stochastic elements in cellular-scale models can capture this heterogeneity, providing a more robust assessment of the range of possible outcomes. For viral systems, these stochastic effects become increasingly relevant at low multiplicity of infection (MOI) (Heldt et al., 2015; Srivastava et al., 2002). However, even at high MOI, a range of cell-to-cell productivity spanning multiple orders of magnitude is often observed (Heldt et al., 2015; Hensel et al., 2009). Wild-type viral stochastic modeling is an active area of development, and models have been built for human immunodeficiency virus (Sazonov et al., 2021), influenza (Heldt et al., 2015), vesicular stomatitis virus (VSV) (Hensel et al., 2009), poliovirus (Schulte et al., 2015), and others.

That said, these stochastic approaches have not been widely applied to recombinant systems. For linear systems and systems operating near the thermodynamic limit at higher numbers of substrates, deterministic and stochastic approaches should give similar results (Hahl & Kremling, 2016). For some recombinant systems, such as monoclonal stable cell lines with multiple genomic copies, these assumptions likely hold, and deterministic approaches are sufficient. However, for recombinant systems operating with a lower or variable number of reactants, such as attenuated virus production processes, these assumptions may not be valid and stochastic elements can be considered.

TABLE 1 Mathematical approaches used in cellular-scale viral models

Mathematical approach	Equation structure	Step	References
Power law	$v_o = k\prod_i^N [S_i]^{\alpha_i}$	Plasmid Uptake (1A)	Nguyen et al. (2021)
		Viral Uptake (1B)	Lim et al. (2006), Zitzmann et al. (2020), Grebennikov et al. (2021)
		Unpacking (2)	Heldt et al. (2012)
		Migration to Nucleus (3)	Dee and Shuler (1997), Heldt et al. (2012)
		Replication (4)	Roldão et al. (2007), Aunins et al. (2018)
		Transcription (5)	Roldão et al. (2007)
		Translation (6)	Heldt et al. (2012), Grebennikov et al. (2021)
		Assembly (7)	Aunins et al. (2018), Shcherbatova et al. (2020), Nguyen et al. (2021)
		Release (8)	Grebennikov et al. (2021), Nguyen et al. (2021)
Power law with limiting or regulating protein	$\frac{d[C_i]}{dt} = k_b[P_i][S_i]\frac{d[S_{i+1}]}{dt} = k[C_i]$	Viral Uptake (1B)	Dee and Shuler (1997), Nunes-Correia et al. (1999), Roldão et al. (2007), Heldt et al. (2012)
		Replication (4)	Lim et al. (2006), Heldt et al. (2012), Zitzmann et al. (2020)
		Transcription (5)	Lim et al. (2006), Zitzmann et al. (2020)
		Translation (6)	Lim et al. (2006), Binder et al. (2013), Aunins et al. (2018), Zitzmann et al. (2020)
Power law with time delay	$v_o = k[S_i](t - \delta_t)$	Migration to Nucleus (3)	Roldão et al. (2007)
		Translation (6)	Hu and Bentley (2000)
Michaelis–Menten	$v_o = \frac{v_{max}[S_i]}{[S_i] + K_M}$	Transcription (5)	Grebennikov et al. (2021)
		Translation (6)	Roldão et al. (2007)
		Assembly (7)	Heldt et al. (2012), Zitzmann et al. (2020), Grebennikov et al. (2021)
Thermodynamics	$[n] = \prod_{i=2}^n K_i[1]^n$	Assembly (7)	Hu and Bentley (2000)

Note: Many of the process steps summarized in Figure 1 have been described using multiple mathematical approaches.

3 | REACTOR-SCALE MODELS

3.1 | Model constituents

A mechanistic model of a viral bioreactor at bioreactor length and time scales needs to consider four aspects,

1. Bioreactor Configuration: The reactor design and operating conditions are vital in writing the governing conservation and balance equations describing the bioreactor.
2. Substrates and metabolites: A description of how key substrates and metabolites are transported into and out of the bioreactor along with their production and/or consumption by cells.
3. Viral Kinetics: A suitable model describing the kinetics of the viral infection and viral particle production process is needed to specify the kinetics terms in the model.
4. Biomass: A description of how the biomass evolves within the system.

While this review focuses primarily on reactor-scale viral kinetics and biomass descriptions, the other reactor model constituents will also be briefly considered.

3.2 | Bioreactor configuration

A variety of different bioreactor configurations for viral particle production have been explored in the literature (Gallo-Ramírez et al., 2015; Grein et al., 2017; Gutiérrez-Granados et al., 2018). The bioreactor configuration can have a significant impact on various aspects of the process such as its dynamics, control, and optimization (Gallo-Ramírez et al., 2015; Yamuna Rani & Ramachandra Rao, 1999). For example, undesirable oscillatory behavior in viral titers can be eliminated by employing a tubular plug-flow reactor (PFR) instead of a continuously stirred tank reactor (CSTR) as accumulation of large concentrations of defective interfering particles (DIPs) can be avoided in PFRs (Tapia et al., 2019). While computational fluid

TABLE 2 Variables used in Table 1 to describe cellular-scale modeling mathematical approaches

Variable	Description
C_i	Protein complex *i*
k	Rate constant
K_i	Association constant
K_m	Concentration of substrate needed to achieve half the maximal reaction rate
n	Number of subunits in VLP
N	Number of reacting species
P_i	Binding protein *i*
S_i	Reactant *i*
v_{max}	Maximum achievable reaction rate
v_o	Rate of reaction
α_i	Order of reactant *i*
δ_t	Time delay

dynamics (CFD) models can be constructed for bioreactor configurations to explore and identify an optimal design for a given process and viral system, commercial availability and practical limitations such as avoiding excessive shear stress and the need to get enough oxygen to the cells limit the choice of bioreactor configurations. Also, experimental validation of CFD models for some commercial bioreactor configurations are not available, so the predictive accuracy of these models for those configurations is unknown (KarimiAlavijeh et al., 2022; Löffelholz et al., 2013; Nadal-Rey et al., 2022; Scully et al., 2020).

Given a choice of a bioreactor configuration, the corresponding macroscopic model equations can be derived by considering mass, species, and energy balances across the bioreactor, drawing upon concepts from an extensive chemical engineering literature (e.g., see Fogler, 2016; Froment et al., 2010; Levenspiel, 1999 and citations therein). The model equations for CSTRs and batch bioreactors tend to be comparatively more straightforward (often expressible as ODEs and/or DAEs) than other bioreactor configurations (typically partial differential equations [PDEs] with terms containing spatial derivatives). The commonly employed assumption of perfect mixing in CSTR/batch reactors eliminate the need for the model to capture spatial variations in key variables. In subsequent sections, only CSTR/batch reactor models will be considered to simplify the presentation. The development of models for other bioreactor configurations is much more computationally expensive and in many cases remain an area of research (X.-R. Li et al., 2019; Teng et al., 2021).

3.3 | Substrates and metabolites

The transport and consumption/production of key substrates (e.g., glucose, glutamate, and oxygen) and inhibitory metabolites (e.g., lactate and ammonium) can be captured by including suitable transport equations based on the bioreactor configuration (e.g., see Brotherton & Chau, 1996; Craven et al., 2013, 2014; Hong & Braatz, 2021). The rate expressions should include substrate- and metabolite-dependent effects for those processes in which such effects are significant (Pörtner & Schäfer, 1996). For example, viral production in Sf9 insect cell cultures can be limited by nutrient depletion, rather the accumulation of inhibitory metabolites, so that supplementing glucose and other key substrates can improve viral production (Fernandes et al., 2013). The addition of other supplements such as pyruvate or anti-oxidants can further increase viral production by manipulating the cellular metabolic pathways (Carinhas et al., 2010; Rodrigues et al., 2013). Metabolic shifts arising from infection and/or bioreactor operation can also take place and impact productivity (see Petiot et al., 2015 and citations therein). In some cases, the bioreactor model can be simplified by omitting the model equations related to substrates and metabolites when these variables are kept constant as part of the reactor's regulatory control policy and/or when the concentrations of specific substrates/metabolites of interest do not impact the process (Genzel et al., 2004; Möhler et al., 2005).

3.4 | Viral kinetics and biomass

For many viral systems, especially systems of infectious viral particles, the relationship between the virus particles and the biomass is closely coupled and are often considered together. Before formulating the model, it is helpful to consider a flow diagram to understand how the system dynamics impact the various components. As a first case, consider a simplified batch bioreactor with three species of interest: target cells (T), infected cells (I), and virus particles (V). The following assumptions are employed: Target cells grow exponentially with a rate constant μ, target cells are infected by attachment of free viral particles with a rate constant k_1, target cells die with a rate constant k_2, infected cells undergo apoptosis with a rate constant k_3, virus particles are released upon apoptosis of infected cells with a proportionality constant k_4, and free virus particles degrade with a rate constant k_5. It is often helpful to construct a flowchart describing the processes of the system.

Figure 3 corresponds to one of the simplest models for viral particle production in a batch bioreactor,

$$\begin{aligned} \frac{dT}{dt} &= \mu T - k_1 TV - k_2 T, \\ \frac{dI}{dt} &= k_1 TV - k_3 I, \\ \frac{dV}{dt} &= -k_1 TV + k_4 I - k_5 V. \end{aligned} \tag{1}$$

This TIV model (and its variants, e.g., with additional terms to account for flow into and out of a bioreactor in a continuous CSTR or modifying various terms within (1) to more accurately reflect actual processes) has been used to model the production of viral particles of various viruses, e.g., Influenza (Frensing et al., 2013; Möhler et al., 2005; Schulze-Horsel et al., 2009), polio (Jiang et al., 2019;

Ursache et al., 2015), and dengue (Abbate et al., 2019, 2016). As previously mentioned in Section 1, it is helpful to note the parallels between the TIV model and both patient viral infection dynamical models (e.g., see Hernandez-Vargas & Velasco-Hernandez, 2020; Perelson & Ribeiro, 2013; Saenz et al., 2010 and citations therein) and compartment models in epidemiology (see Brauer et al., 2008 and citations therein). These related fields have a rich literature which can serve as a source for formulating models and extensions. For recombinant systems where the viral particle does not infect cells in the bioreactor, the reactor-scale model would be much more closely aligned with well-established bioreactor models for recombinant protein production (e.g., see S. Liu, 2020; McDuffie, 1991).

Multiple avenues for model extension are available. In some cases, it might be necessary to account for additional viral or cell species. For example, some viruses are known to produce DIPs during replication which can impact system dynamics (Frensing et al., 2013; Shirogane et al., 2021). Frensing et al. (2013) extended the TIV model by including three additional equations to track DIPs, cells infected with DIPs, and cells coinfected with DIPs and standard virus particles (STVs), and the proposed model was successfully able to qualitatively match experimental bioreactor results (see Figure 4 for exemplar simulations). To integrate deeper insights of the cell population into the model, the equations for one or more species of interest can be reformulated as a population balance model (PBM) with suitable intrinsic variables capturing the dimensions in which the population varies (Inguva & Braatz, 2022; Inguva et al., 2022; Villadsen et al., 2011). Table 3 summarizes exemplar intrinsic variables considered in cell PBMs with a focus on viral particle production.

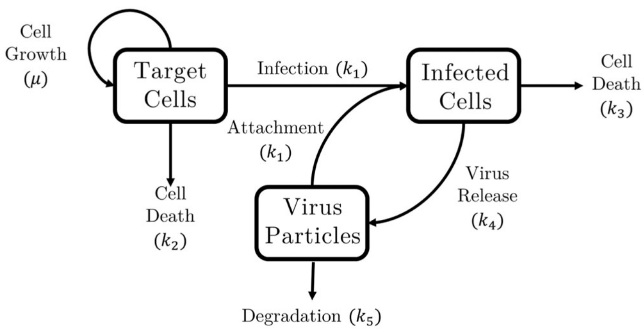

FIGURE 3 Flowchart for a simplified TIV system adapted from Möhler et al. (2005); see Müller (2015) for additional examples of flow charts for more complex systems.

4 | MULTISCALE MODELS

Viral systems are inherently multiscale; infection and recombinant viral production are the result of a dynamic relationship between different time and length scales. Coupling the cellular- and reactor-scale dynamics can improve model accuracy and predictability for viral systems. This coupling often comes with increased model complexity and computational requirements, which can be mitigated via strategic application of simplifying assumptions and/or referral to the literature on the modeling and simulation of multiscale systems (Ingram et al., 2004; Raimondeau & Vlachos, 2002).

The literature is sparse regarding multiscale modeling of recombinant viral systems. However, as with cellular- and reactor-scale models, analogous multiscale wild-type viral models can be leveraged for recombinant multiscale modeling (Garira, 2017). These wild-type models are typically motivated by mapping the spread and treatment of a virus within a population, tissue, or cellular system, but many of the high-level mathematical approaches and considerations

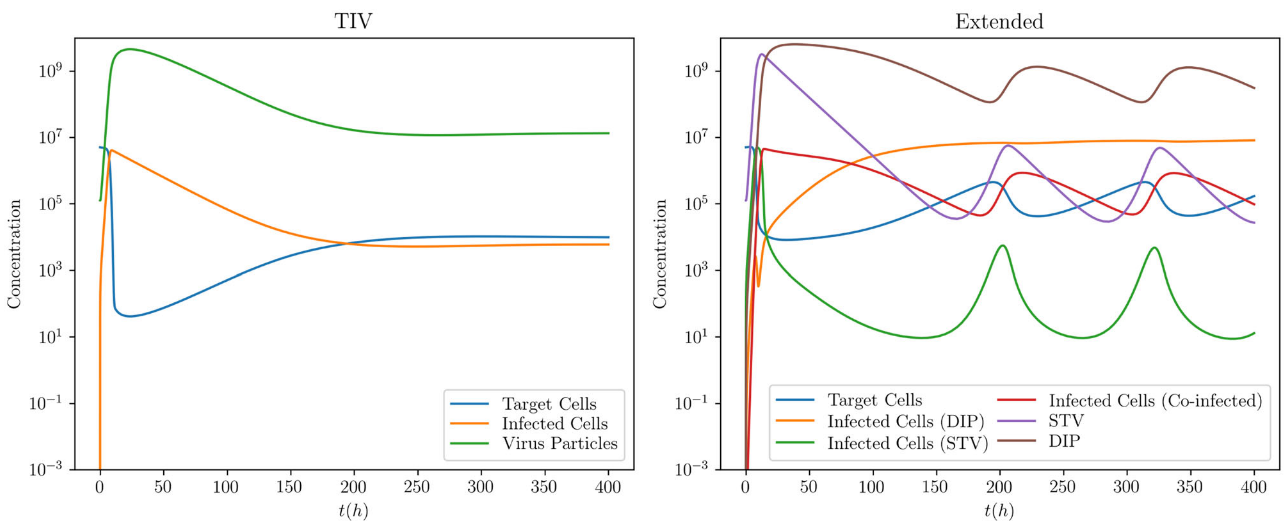

FIGURE 4 Exemplar reactor-scale simulations performed using the TIV (left) and extended model (right) in a continuously stirred tank reactor (CSTR) from Frensing et al. (2013). Frensing et al. (2013) observed oscillatory behavior during experimental runs which was explained by the presence of defective interfering particles (DIPs). This effect was captured in the extended model which introduced additional species to track DIPs and cells either infected with DIPs or coinfected cells.

TABLE 3 Exemplar intrinsic variables considered in PBMs for biotechnology

Intrinsic variable	Description	Reference
Cell age/postinfection cell age	Enables age-based effects, e.g., cell death and reproduction to be captured	Schmidt and Sawodny (2017), Haseltine et al. (2008), Haseltine et al. (2005), Kurtz et al. (1998)
Cell mass/volume/size	These properties can be measured and used to capture similar effects as cell age	Quedeville et al. (2018), Abia et al. (2009), Mantzaris et al. (1999)
Intracellular DNA/RNA content	Provide deeper insights into the cell state	Fischer et al. (2019), Dürr et al. (2017), Fadda et al. (2012), Y.-H. Liu et al. (2007)
Fluorescence	Provides information on the extent/progress of infection in the cell population. Data can be obtained from flow cytometry	Müller et al. (2013), Dürr et al. (2012), Müller et al. (2008)

Note: Where possible, references to the literature considering viral systems are provided.
Abbreviation: PBM, population balance model.

governing these systems are directly relevant to recombinant application (Garira, 2017). Additionally, there are relevant multiscale publications on nonviral recombinant cellular systems (e.g., monoclonal antibodies), which are summarized by Kyriakopoulos et al. (2018). Note that multiscale models in which cellular-scale dynamics are paired with reactor-scale dynamics are often referred to as "structured" models in the literature (Bailey & Ollis, 1986).

One approach to incorporating multiscale dynamics is to couple the cellular-scale production of virus or VLP with the infectivity state of the cell population. Information is often transferred unidirectionally from the reactor-scale to the cellular-scale, such that the intracellular dynamics are affected by the infection state of the cell. Haseltine et al. (2005) demonstrated this approach for a general viral infection system using a series of integro-PDEs. The group used simplifying assumptions to decouple the system (Haseltine et al., 2008), an approach that was also successfully employed to model Influenza A infection and antiviral efficacy (Heldt et al., 2013). Dürr et al. (2017) extended this Influenza A infection model by using approximate moment methods to solve a population balance system, which was used to predict productivity effects due to heterogenous gene overexpression and expedited the screening of suitable cell line candidates (Duvigneau et al., 2020). Hu and Bentley (2000) instead used a stochastic method to predict the infection pattern of a baculovirus insect system producing VLPs. Protein synthesis and VLP formation within individual cells was then tied to the time since infection and the number of infecting baculoviruses.

An alternative way to incorporate multiscale dynamics is to couple extracellular process variables to cellular-scale dynamics, which can prove useful for recombinant systems that do not contain live infection dynamics. For example, Ho et al. (2006) linked antibody production to bioreactor glucose concentration, building on the model built by (Bibila & Flickinger, 1991). Also, Jedrzejewski et al. (2014) used Monod kinetics to link extracellular metabolites to the glycosylation model, building on the model published by (Jimenez del Val et al., 2011).

These approaches linking cellular-scale dynamics to process variables have not been widely extended to segregated cell population models, where, for example, the infection status of individual cells is tracked. This simplification enables the model equations to remain a system of ODEs, greatly simplifying the solution approach. Future applications can extend these approaches to include the cellular-scale effects from other industry-relevant process variables such as dissolved oxygen and pH.

5 | PARAMETER ESTIMATION

Estimating the model parameters of a viral system is not trivial. Many states cannot be measured using existing measurement techniques and, when measurement techniques are available, the outputs are often noisy or measured on a relative scale. Because of this, care should be taken to understand a model's structural and practical identifiability when performing parameter estimation. Structural identifiability is achieved if a unique set of parameters exists for a given model output and can be assessed before model fitting (Wieland et al., 2021). Practical identifiability instead evaluates the ability of the available experimental measurements to define finite confidence intervals for the fit parameters.

A variety of parameter estimation techniques have been employed in the biological modeling literature to fit model parameters to experimental data. Methods include linear and nonlinear least squares (Mendes & Kell, 1998), heuristic search algorithms (Calvez & Hutzler, 1996; Srinivas & Patnaik, 1994), and Kalman filtering (Lillacci & Khammash, 2010), among others. A common parameter estimation approach is maximum likelihood (ML) estimation, which seeks to find the parameters that make the fit data most likely. Assuming zero-mean, normally distributed and additive noise, the ML estimator can be written as a function of the vector of measurements $\boldsymbol{Y}$ and model outputs $f(\boldsymbol{X}, \boldsymbol{\theta})$ (Beck and Arnold (1977) by

$$\min_{\theta} \, (\boldsymbol{Y} - f(\boldsymbol{X}, \boldsymbol{\theta}))^{\mathrm{T}} \boldsymbol{V}_{\epsilon}^{-1} (\boldsymbol{Y} - f(\boldsymbol{X}, \boldsymbol{\theta})), \tag{2}$$

where $\boldsymbol{X}$ is the vector of state variables, $\boldsymbol{\theta}$ are the model parameters, and $\boldsymbol{V}_{\epsilon}$ is the measurement error covariance matrix.

Identifiability concerns often dictate that select parameters be held constant during fitting. Uncertainty analysis methods can be used to select the most effective fitting parameters; fitting sensitive parameters that drive model uncertainty can improve the predictive power of the model (Blower & Dowlatabadi, 1994; Nagy & Braatz, 2007). Meanwhile, the parameters that are held constant during model fitting should be sourced from prior knowledge, such as literature or other models. For example, Nguyen et al. (2021) initially fit parameters related to plasmid delivery when constructing their cellular-scale model of rAAV production. These model-derived plasmid delivery parameters were then held constant along with four parameters leveraged from literature when fitting the rAAV production model, the outputs of which are shown in Figure 2. Predetermined parameters affect the determination of the fit parameters ($\boldsymbol{\theta}$). Because of this, the group substituted a matrix of overall experimental data covariance for the measurement error covariance matrix in (2) that accounts for both the predetermined parameter variance and the measurement noise.

For models of viral systems in which the available data are limited and noisy, Bayesian techniques strike a balance between complexity and usefulness (Beck & Arnold, 1977). Since Bayesian estimation techniques allow inference of the entire probability distributions of the estimated parameters, they are also able to quantify the uncertainty in the parameter estimates. Bayes theorem describes the *a posteriori* distribution $P(\boldsymbol{\theta}|\mathbf{Y})$ as a function of the experimental data $\mathbf{Y}$ and the a priori distribution $P(\boldsymbol{\theta})$ of the model parameters,

$$P(\boldsymbol{\theta}|\mathbf{Y}) = \frac{P(\mathbf{Y}|\boldsymbol{\theta})P(\boldsymbol{\theta})}{P(\mathbf{Y})}. \quad (3)$$

Assuming zero-mean normally distributed noise and normally distributed priors with means $\boldsymbol{\mu}$ and variances $\mathbf{V}_{\mu}$, the maximum *a posteriori* estimator is Beck and Arnold (1977)

$$\min_{\theta} \ (\mathbf{Y} - f(\mathbf{X}, \boldsymbol{\theta}))^{\mathrm{T}} \mathbf{V}_{\epsilon}^{-1} (\mathbf{Y} - f(\mathbf{X}, \boldsymbol{\theta})) + (\boldsymbol{\theta} - \boldsymbol{\mu})^{\mathrm{T}} \ \mathbf{V}_{\mu}^{-1} (\boldsymbol{\theta} - \boldsymbol{\mu}). \quad (4)$$

While ML estimation is straightforward to implement and finds the parameter values that make the fit data most likely, it does not generalize beyond the observed data. The MAP estimator extends the ML estimator by incorporating prior knowledge about the fitting parameters. This prior knowledge can be leveraged from literature results or previous experimentation.

6 | CONCLUSIONS

Demand is surging for products manufactured in viral systems. Nevertheless, the yields of even the most state-of-the-art recombinant viral systems often fall short of market needs. Improvements to recombinant viral processes are urgently needed to meet this demand and ensure the consistent manufacture of high-quality viral products. Dynamical modeling is one way to realize high-impact process gains; understanding the cellular- and reactor-scale dynamics can increase specific productivity, improve control of critical quality attributes (CQAs), and decrease the amount of time required for process development by encouraging targeted experimentation. Modeling can also inform other methods of upstream process enhancement such as media development and process intensification, leading to even greater gains.

Many of the modeling methodologies summarized in this review were first applied to wild-type viral systems. Extending these approaches to recombinant systems is not trivial, and often requires experimentation for model validation. That said, advances in measurement technologies and synthetic biology will continue to improve the informativeness of experiments, enabling even more comprehensive models. Additionally, as more products made in viral systems mature and scale into manufacturing systems, dynamical models can be created with manufacturing-specific applications.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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