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Implications of Decoupling the Intracellular and Extracellular Levels in Multi-level Simulations of Virus Infections

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Abstract

Virus infections are characterized by two distinct levels of detail: the intracellular level describing how viruses hijack the host machinery to replicate, and the extracellular level describing how populations of virus and host cells interact. Deterministic, population balance models for viral infections permit incorporation of both the intracellular and extracellular levels of information. Under appropriate assumptions, the interaction between the intracellular and extracellular levels decouples, permitting solution of first the intracellular level, and subsequently the extracellular level. This decoupling leads to (1) intracellular and extracellular models of viral infections that have been previously reported and (2) a significant reduction in the computational expense required to solve the model. However, the decoupling restricts the behaviors that can be modeled. Simulation of a previously reported multi-level model demonstrates this decomposition when the intracellular level of description consists of numerous reaction events. Additionally, this model demonstrates that viruses can persist even when the intracellular level of description cannot sustain a steady-state production of virus (i.e., has only a trivial equilibrium). We expect the combination of this modeling framework with experimental data to result in a quantitative, dynamic understanding of viral infections and cellular antiviral strategies, as well as how to best control both viral infections and cellular antiviral strategies.

Keywords

Mathematical Modeling; Population Balance; Systems Biology; Computational Biology; Virus Dynamics

1 Introduction

One of the simplest, yet most intriguing biological organisms is the virus. The virus contains enough genetic information to replicate itself given the machinery of a living host. So powerful is this strategy that viral infections are at once a threat to and a hope for human survival. According to the Joint United Nations Programme on HIV/AIDS (UN-AIDS) in 2002, 42 million people were living with human immunodeficiency virus (HIV), 5 million people were newly infected with HIV, and 3.1 million people died owing to acquired immune deficiency syndrome (AIDS) related illnesses. At the same time, viruses show

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promise in anti-tumor therapies as oncolytic agents (Bell et al., 2003), as delivery vehicles for gene therapy (Nathwani et al., 2004), and as therapeutic agents for drug-resistant bacteria (Levin and Bull, 2004). The common thread between these examples is that controlling the propagation of virus spread is essential, and doing so first requires understanding of how viruses propagate. Mathematical models offer one means of quantitatively understanding how viruses propagate, and how to best control this propagation. In particular, models can serve as a beneficial tool in proposing, identifying, and distinguishing between key biological and experimental phenomena contained in data.

Most mathematical models for viral infections have focused exclusively on events in either the intracellular or extracellular level. At the intracellular level, kinetic models have been applied to examine the dynamics of how viruses harness host cells to replicate more virus (Knijnenburg and Kreischer, 1983; Eigen et al., 1991; Endy et al., 1997; Arkin et al., 1998; Yin, 2004; il Lim et al., 2006), and how drugs targeting specific virus components affect this replication (Reddy and Yin, 1999; Endy and Yin, 2000; Kim and Yin, 2004). These models, however, consider only one infection cycle, whereas infections commonly consist of numerous infection cycles. At the extracellular level, researchers have considered how infection dynamics are affected by delays in infected-cell lysis (Levin et al., 1977; Weld et al., 2004) and age-dependent virus production (Nelson et al., 2004; Gilchrist et al., 2004) or how drug therapies affect the dynamics of populations of viruses (Perelson et al., 1996; Herz et al., 1996; Bonhoeffer et al., 1997; Nowak and May, 2000; Perelson, 2002; Wodarz and Nowak, 2002). These models, though, neglect the effect of intracellular events, such as the fact that drugs generally target specific intracellular viral or host components. The continuing elucidation of both intracellular and extracellular events in exquisite detail motivates the need for comprehensive models that include both levels of information. In particular, models fitted to *in vitro* data, which generally considers a single round of infection, could then be extrapolated to predict *in vivo* behavior, which usually constitutes multiple rounds of infection.

One way to couple both levels of information is to assume that intracellular kinetics occur on a much faster time scale than between cell transmission (Krakauer and Komarova, 2003). This assumption effectively requires the intracellular model to reach a steady state for viral infections to persist. In contrast, we recently proposed a more general description which incorporates both levels of information in a deterministic setting via cell population balances (Haseltine et al., 2005). This model provides a much more generic framework for combining the intracellular and extracellular levels of description without the separation of time scales assumption required by Krakauer and Komarova (2003). Just as combining within-host and between-host dynamics have elucidated features of the evolution of virulence (Gilchrist and Coombs, 2006) and the optimization of viral fitness (Gilchrist et al., 2004), we expect the dynamic linking of intracellular and extracellular levels to yield additional insights into understanding viral infections. Using multiple levels of dynamic description allows one to investigate the emergent properties obtained from interactions between levels, interactions that are neglected when these levels are decoupled.

In this paper, we propose limiting assumptions that effectively decouple the intracellular and extracellular levels. In this case, it is possible to first solve the equations governing the

intracellular description of the model, then use these results to solve the extracellular description of the model. We discuss how these assumptions lead to previously-reported models (intracellular models and extracellular age-structured models), and how the resulting model can be efficiently simulated. A previously-reported example illustrates the predictive power and limitations of this technique. In addition, this example demonstrates the nonintuitive result that viruses can persist even when the intracellular level of description cannot sustain a steady-state production of virus. Finally, we discuss the results and present conclusions.

2 Modeling Framework

We consider population balance models segregated by the age of the infected cell, τ . Such models can readily be extended to contain an arbitrary number of internal and/or external (i.e., spatial) segregations. As derived in Haseltine et al. (2005), the resulting segregated model is then

$$\frac{\partial f(t, \tau)}{\partial t} + \frac{\partial f(t, \tau)}{\partial \tau} = B - D \quad (1a)$$

$$\frac{\partial i_j}{\partial t} + \frac{\partial i_j}{\partial \tau} = R_j + E_j \quad j=1, \dots, n \quad (1b)$$

$$\frac{\partial e_k}{\partial t} = E_k + \int_0^{\tau_d} f(t, \tau) R_k(t, \tau) d\tau \quad k=1, \dots, m \quad (1c)$$

in which

- $f(t, \tau) d\tau$ is the concentration of infected cells,
- B is the birth rate of infected cells,
- D is the death rate of infected cells,
- i_j is the concentration of the j th intracellular viral component,
- e_k is the concentration of the k th extracellular species,
- R_j is the j th intracellular production rate,
- E_j is the j th extracellular production rate, and
- τ_d is the age of the oldest infected cell permitted by the model.

Equation (1) is a coupled set of integro-hyperbolic partial differential equations. In this framework, the intracellular state of an infected cell depends on both the time and the age of infection as demonstrated in equation (1b). Also, infected cells may interact with other extracellular species in an age-dependent manner; for example, virus may be produced and secreted at different rates in the infection cycle of a single cell. The integral term present in equation (1c) accounts for such age-dependent effects in the production rates of extracellular species.

We focus our attention on the intracellular reaction set, i.e., equation (1b). If the extracellular components do not affect the intracellular production rates, then the intracellular reactions

$$\frac{\partial i_j}{\partial t} + \frac{\partial i_j}{\partial \tau} = R_j + E_j \quad j=1, \dots, n \quad (2)$$

effectively decouple from the extracellular reactions and population balance

$$\frac{\partial f(t, \tau)}{\partial t} + \frac{\partial f(t, \tau)}{\partial \tau} = B - D \quad (3a)$$

$$\frac{\partial i_j}{\partial t} + \frac{\partial i_j}{\partial \tau} = R_j + E_j \quad j=1, \dots, n \quad (3b)$$

$$\frac{\partial e_k}{\partial t} = E_k + \int_0^{\tau_d} f(t, \tau) R_k(t, \tau) d\tau \quad k=1, \dots, m \quad (3c)$$

Next, consider redefining the relative cell age α as

$$\alpha = \frac{t - \tau}{\tau_d} \quad (4)$$

Assuming that the production rate expressions are independent of time, then rewriting the intracellular equations (2) in terms of this variable reduces the partial differential equations to ordinary differential equations

$$\frac{\partial i_j}{\partial \alpha} = R_j + E_j \quad j=1, \dots, n \quad (5)$$

Consequently, we may first solve the intracellular equations (5) to determine the production rate term $R_k(t, \tau) \triangleq R_k(\alpha)$, then use this term to solve the remaining equations (3). This decomposition results in significant reductions in both computational expense and the complexity of the resulting systems of equations. These claims are illustrated in the example.

The decoupled equations (5) represent the kinetic descriptions usually employed to account for viral infection at the intracellular level (see, for example, Knijnenburg and Kreischer (1983); Eigen et al. (1991); Endy et al. (1997)). The decoupled extracellular equations (3) demonstrate remarkable similarity to models designed to account for age-dependent lysis of phage-infected bacteria Levin et al. (1977) (which can be viewed as an age-discretized version of the the presented extracellular model) and the age-dependent infection of T cells by HIV (Nelson et al., 2004). These works, however, did not consider how intracellular kinetics mechanisms might give rise to extracellular virus production. Since our work incorporates the intracellular description, we are then in an excellent position to address which biological assumptions we must make to validate the decomposition giving rise to the extracellular description proposed by both of these works and equation (3). The decomposition clearly requires that the extracellular description have little or no interaction with the intracellular events. Physically, this requirement implies that each infected cell

exhibits precisely the same course of infection regardless of what time the cell was infected. The most restrictive assumption, then, is that each host cell is infected by identically the same virus (i.e., identical initial conditions for each infected cell), and that the infected cell may affect the extracellular environment but not vice versa. A less restrictive assumption would permit variation in the initial condition, but at the expense of requiring more substantial simulation of the intracellular equations (5). Accounting for more extensive interaction from the extracellular to intracellular descriptions, such as super-infection of infected cells, requires solving the full model, equation (1).

3 Example: Initial Infection for a Generic Viral Infection

In this section, we reconsider the initial infection example of Haseltine et al. (2005) to illustrate the efficiency of the proposed decomposition. This model considers intracellular species of genomic (gen) and template (tem) viral nucleic acids respectively, viral structural protein (str), and viral enzymes V_1 and V_2 . Intracellular reactions include:

Reaction rates are given by the a_j expressions. These events account for production of a viral template used to replicate the viral genome and mass-produce viral structural protein, and the assembly and secretion of viral progeny. We assume that host nucleotides and amino acids are available at constant concentrations.

Extracellularly, the model tracks uninfected host cells (unc), infected host cells (inf), and virus (vir) for the reactions

These events address the intuitive notions of cell growth, death, and infection by free virus. In this description, infected cells are segregated by the age of infection τ , and it is assumed that all infected cells die by the maximum age τ_d . The model equations for this system are then

$$\frac{\partial \eta(t, \tau)}{\partial t} + \frac{\partial \eta(t, \tau)}{\partial \tau} = k_6 e_{\text{unc}} e_{\text{vir}} \delta(\tau) - (k_8 + \delta(\tau - \tau_d)) \eta(t, \tau) \quad (8a)$$

$$\frac{\partial i_j(t, \tau)}{\partial t} + \frac{\partial i_j(t, \tau)}{\partial \tau} = R_j \quad j = \text{tem, gen, str, } V_1, V_2 \quad (8b)$$

$$\frac{de_{\text{unc}}}{dt} = k_{10} - k_9 e_{\text{unc}} - k_6 e_{\text{unc}} e_{\text{vir}} \quad (8c)$$

$$\frac{de_{\text{vir}}}{dt} = -k_7 e_{\text{vir}} - k_6 e_{\text{unc}} e_{\text{vir}} + \int_0^{\tau_d} \eta(t, \tau) R_{\text{vir}}(\tau) d\tau \quad (8d)$$

Initial conditions and rate constants used for this simulation are presented in the supporting information. These parameters are the same as those used by Haseltine et al. (2005).

For this example, the intracellular events decouple from the population balance (8a) in equation (8) owing to the fact that the governing equations for the intracellular species do not depend on time. Consequently, we can first solve for the intracellular age distribution, i.e.

$$\frac{\partial i_j(\alpha)}{\partial \alpha} = R_j \quad j = \text{tem, gen, str, } V_1, V_2 \quad (9)$$

and subsequently for the population-level dynamics

$$\frac{\partial \eta(t, \tau)}{\partial t} + \frac{\partial \eta(t, \tau)}{\partial \tau} = k_6 e_{\text{unc}} e_{\text{vir}} \delta(\tau) - (k_8 + \delta(\tau - \tau_d)) \eta(t, \tau) \quad (10a)$$

$$\frac{de_{\text{unc}}}{dt} = k_{10} - k_9 e_{\text{unc}} - k_6 e_{\text{unc}} e_{\text{vir}} \quad (10b)$$

$$\frac{de_{\text{vir}}}{dt} = -k_7 e_{\text{vir}} - k_6 e_{\text{unc}} e_{\text{vir}} + \int_0^{\tau_d} \eta(t, \alpha) R_{\text{vir}}(\alpha) d\alpha \quad (10c)$$

$$R_{\text{vir}}(\alpha) = k_5^i i_{\text{gen}}(\alpha) i_{\text{str}}(\alpha) \quad (10d)$$

To solve the coupled sets of integro-partial differential equations for the population-level dynamics, we use orthogonal collocation of Lagrange polynomials on finite elements (Villadsen and Michelsen, 1978) to discretize the age dimension. The discretized PDE yields a system of differential-algebraic equations, which we solve using the package DASKR, a variant of the predictor-corrector solver DASPK (Brown et al., 1994). For the decoupled system, we first solve for the intracellular reactions, equations (9), over the age range $[0, \tau_d]$ using one hundred evenly-incremented time points. The solution to these equations is shown in Figure. Virus production rates required by the cell population balance, equation (10), are calculated from this intracellular information by linearly interpolating between the time points. Further details on the model solution are presented in the supporting information.

Figure 2 plots the full and decoupled model solutions for all extracellular species. The decoupled solution provides results that are indistinguishable from the full solution, but at roughly one third the computation expense (15.6 CPU seconds versus 45.2 CPU seconds on a 1.6 GHz Intel Centrino processor). The majority of the decrease in computational expense is directly attributable to the reduced size of the state vector; the decoupled solution requires only discretization of the cell population balance, whereas the full solution also requires discretization of all intracellular species. Because the predictor-corrector method employed by DASKR requires the solution of a Newton iteration, an operation that scales cubically with the size of the state, we expect more dramatic decreases in computational expense for the decoupled solution as the number of intracellular species increases. Additionally, formulating the problem in this manner significantly reduces the potential for discretization problems owing to stiffness in the intracellular components.

Of additional interest to this model is the fact that the only steady state for the intracellular description given by equation (6) is the case of viral extinction; that is, no viral components are present in the cell. This assertion can be readily shown by setting the governing equations for the intracellular state to zero:

$$\frac{di_{\text{tem}}}{d\alpha} = 0 = a_1 \quad (11a)$$

$$\frac{di_{\text{gen}}}{d\alpha} = 0 = -a_1 + a_3 - a_5 \quad (11b)$$

$$\frac{di_{\text{str}}}{d\alpha} = 0 = a_2 - a_4 - a_5 \quad (11c)$$

Equation (11a) requires that $i_{\text{gen}} = 0$. Subsequently, it is straightforward to show that equations (11b) and (11c) require that $i_{\text{tem}} = 0$ and $i_{\text{str}} = 0$, respectively. Figure 1 illustrates this point: the str and tem species continue to increase over time, never settling to a steady state. The analysis of Krakauer and Komarova (2003) argues that the intracellular

description must attain a steady state for the virus to persist at the extracellular (population) level. However, Figure 2 (a) clearly demonstrates that the coupled intracellular and extracellular model is evolving towards an infected steady state (i.e., the viral infection is persisting); the existence of this steady state was previously computed in Haseltine et al. (2005). This persistence results from a careful balancing of intracellular viral production and extracellular viral transmission. This result points out a potential limitation in the analysis of Krakauer and Komarova (2003), and suggests that more in-depth study is required to fully understand the phenomenon of viral persistence.

4 Conclusions

We have proposed conditions for which multi-level viral infection models might give rise to traditional models describing either the extracellular or intracellular descriptions. In particular, one must assume that the extracellular description have little or no interaction with the intracellular events. Then the intracellular level decomposes from the extracellular level, leading to traditional descriptions of the intracellular and extracellular levels. In addition, this decomposition generates a more computationally tractable problem than the original formulation. An example illustrated the efficacy of this decomposition. The assumptions required for this decomposition restrict the ability of the model to compensate for instantaneous extracellular effects such as super-infection of infected cells and transfer of material from the extracellular to intracellular levels. However, we suspect that in many cases these effects may either be insignificant or indistinguishable from models making these assumptions given experimental data.

Additionally, we demonstrated the nonintuitive result that viral persistence can occur even when the intracellular level of description cannot sustain a steady-state production of virus. This persistence results from a careful balancing of intracellular viral production and extracellular viral transmission. Consequently, a comprehensive understanding of viral persistence requires analysis of models including both the intracellular and extracellular levels, as is the case in this paper.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Notation

α	relative cell age
a_j	j th reaction rate i_j concentration of the j th intracellular viral component species

B	birth rate of infected cells
D	death rate of infected cells
E_j	<i>j</i> th extracellular production rate
e_k	concentration of the <i>k</i> th extracellular species
f(t, τ)dτ	concentration of infected cells
k_j	rate constant for reaction <i>j</i>
k_jⁱ	rate constant for intracellular reaction <i>j</i>
R_j	<i>j</i> th intracellular production rate
t	time
τ	age of infection
τ_d	maximum age of infection

Subscripts

gen	genomic viral nucleic acid
str	viral structural protein
tem	template viral nucleic acid

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Figure 1.
Evolution of the intracellular infection as a function of the relative cell age, α .

Figure 2.

(a) Comparison of the full (lines) and decoupled (points) model solutions for the initial infection example. (b) Percent error for the decoupled model solution, assuming the full solution is exact.