Bull Math Biol
DOI 10.1007/s11538-011-9677-x

ORIGINAL ARTICLE

Periodic Multidrug Therapy in a Within-Host Virus Model

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Received: 21 October 2010 / Accepted: 11 July 2011

Abstract Floquet theory and perturbation techniques are used to analyze a classical within-host virus model with periodic drug treatment. Both single and multidrug treatment strategies are investigated. Specifically, the effects of both RT-inhibitors and P-inhibitors on the stability of the infection-free steady state are studied. It is found that when both classes of drugs have periodic drug efficacy functions, then shifting the phase of these functions can critically affect the stability of the infection-free steady state. A numerical study is conducted to illustrate the theoretical results and provide additional insights.

Keywords Within-host virus model · Periodic drug treatment · Structured treatment interruption · HIV · Optimization

1 Introduction

Mathematical modeling of within-host viral infections has played an important role in the understanding of viruses over the past couple decades. These models usually involve a system of nonlinear ODEs comprised of at least three state variables corresponding to concentrations of healthy cells, infected cells, and free virus particles. Antiviral medications used to treat these infections can be incorporated into mathematical models and insights may be gained into the overall effect that the drugs have on the dynamics of the system. The administration of a drug is typically periodic on a day to day time scale (for example, in HIV treatment the RT-inhibitor *tenofovir DF* is usually taken once every 24 hours (Fung et al. 2002)). The drug efficacy function

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Published online: 06 August 2011

is periodic of the same period; within a cycle it is usually characterized by a quick rise to a maximum soon after drug intake, followed by a slower decay (Dixit and Perelson 2004; Rong et al. 2007). Inclusion of periodic treatment can be modeled by a nonlinear time periodic ODE system, which in general is difficult to analyze.

In this paper, we consider a classical within-host virus model (Perelson et al. 1993; Perelson and Nelson 1999; Nowak and May 2000) which encompasses several important infections such as HIV (Richman 2004), hepatitis B (Ganem and Prince 2004; Locarnini and Lai 2003) and C (Gilchrist et al. 2004), influenza (Earn et al. 2002), and even the malaria parasite *P. falciparum* (Molineaux and Dietz 2000). We will apply perturbation techniques and the Floquet theory to explore the consequences of periodic variations of the efficacy of antiviral treatment. Our results should be interpreted with caution as many factors which may be important to the dynamics of viruses are not included in the model. For example, drug resistance, immune response, and latent reservoirs of virus all may have important consequences for the dynamics of the HIV virus and are often included in some form in other models. See, for example, Ball et al. (2007), De Leenheer and Pilyugin (2008) for inclusion of drug resistance, Silliciano et al. (2003), Rong and Perelson (2009) for a discussion of latent reservoirs and viral persistence, and Adams et al. (2004) for a model with immune response. It also should be noted that current medications cannot completely eradicate HIV virus. The fact that viral eradication is theoretically possible shows that all the relevant dynamics of the HIV virus are not captured in the standard model or that current drugs are not potent enough. Viral mutation to drug resistant strains (Bonhoeffer and Nowak 1997), the presence of latent reservoirs of HIV (Silliciano et al. 2003; Rong and Perelson 2009), and residual viral replication during treatment (Rong and Perelson 2009) have all been proposed to explain the treatment failure.

The standard within-host virus model considered here is a system of three nonlinear ODEs. In the case of HIV, its states include the concentrations of healthy CD4+ T cells (the targets of the virus), infected T cells, and free virus particles. Upon infection of a healthy T cell, the viral RNA is converted into DNA using the enzyme called reverse transcriptase. This step, which is error-prone and leads to mutations, can be blocked by reverse transcriptase (RT) inhibitors. Once the viral copy has been made, the double stranded viral DNA is integrated into cell's nucleus as a provirus. Subsequently, viral proteins are produced according to the genetic information encoded in the provirus. These proteins are constructed, mature, and new viruses bud off from the infected cell's surface which go on to infect other healthy T cells. During the maturation stage, the protease enzyme cleaves long protein chains, and the protease (P) inhibitors are the drugs that target this step.

In the case of HIV, using a "drug cocktail" consisting of both RT-inhibitors and P-inhibitors is common treatment protocol, which is often referred to as HAART (highly active antiretroviral therapy). As stated above, these drugs work at different stages in the viral replication process and their corresponding efficacy functions are periodic in time. We will investigate the effect that phase shifts of these drug efficacy functions have on the dynamics of the system. Shifting the phase of a drug efficacy function corresponds to changing the daily drug administration time (assuming the drug is taken once each day; the assumption of a periodic efficacy function, of course, means that the patient takes the drug at the same time each day). If just one drug is

used in a treatment, shifting the phase of the drug efficacy function does not change the system dynamics. If, however, both an RT-inhibitor and P-inhibitor are used, then these phase shifts can completely change the dynamics. With this in mind, we will search for optimal phase shifts of the RT-inhibitor and P-inhibitor drug efficacy functions (optimal in the sense of maximizing overall treatment effectiveness). Again, results should be viewed with caution as we do not use the actual pharmacokinetic models of the drug efficacies. Refer to Dixit and Perelson (2004) and Rong et al. (2007) for detailed pharmacokinetic models.

Another avenue of research in HIV treatment is the use of Structured Treatment Interruptions (STIs). These regimens provide patients with relatively long breaks from taking medications, sometimes called drug holidays. Antiviral drugs can have numerous side effects, so STIs can reduce the treatment burden on patients in an organized manner, which in turn increases patient adherence to drug regimens. Also, theoretically, STIs may help to combat drug resistance in patients. Finally, antiviral medications are often expensive or in limited supply, therefore, STIs can help to alleviate this problem. There is much research on the optimal control of STIs (Adams et al. 2004; Rosenberg et al. 2006). It will be seen that our results, as viewed in the context of STIs, are significant but again need to be viewed with much caution as the deficiencies of the model also become more dramatic with the larger time period under which treatments vary in STIs. There are a multitude of possible treatment regimens and doctors create STI regimens with what can be described best as an "educated guess" of what would be most effective (Rosenberg et al. 2006), so the search for optimal STIs certainly calls for more research.

This paper is organized as follows. First, we introduce periodic perturbations of a constant drug efficacy. We do this for general periodic functions and then look at the specific case where the perturbation function is sinusoidal, where we discover that the phase shifts mentioned earlier have a significant influence on the dynamics of the system. To illustrate this result, we provide simulations using current estimates for HIV parameters. Because of some shortcomings of the standard model with respect to generating a persistent low level virus concentration, we simulate two modifications of the model and obtain the same conclusion that phase shifts of the drug efficacy functions can significantly affect treatment outcome. We then shift back to the original model and state a simple result about optimizing the phase shift of arbitrary periodic drug efficacy functions in a combination therapy. Finally, we consider drug efficacies that are piecewise constant periodic functions and numerically investigate the effect that varying phase shifts has on the long term dynamics of the system.

2 The Model

As a starting point, we consider the classical within-host viral model (Perelson et al. 1993; Perelson and Nelson 1999; Nowak and May 2000)

$$\begin{aligned} \dot{T} &= f(T) - kVT, \\ \dot{T}^* &= kVT - \beta T^*, \\ \dot{V} &= N\beta T^* - \gamma V, \end{aligned} \tag{1}$$

where T, T^*, and V denote the concentrations of healthy and infected cells, and virus particles, respectively. All parameters are assumed to be positive. The parameters β and γ are the decay rates of infected cells and virus particles, respectively. The infection is represented by a mass action term kVT, and N is the average number of virus particles budding off an infected cell during its lifetime. The homeostatically regulated growth rate of the uninfected cell population is given by the smooth function $f(T) : \mathbb{R}_+ \to \mathbb{R}$, which is assumed to satisfy the following:

$$\exists T_0 > 0 : f(T)(T - T_0) < 0 \quad \text{for all } T \neq T_0 \text{ and } f'(T_0) < 0.$$

The class of admissible functions $f(T)$ is chosen to be quite large. It contains the two most popular choices for $f(T)$, namely, $a - bT$ for some positive a and b (Nowak and May 2000), and $s + rT(1 - T/T_{\max})$ for some positive s, r, and $T_{\max}$ (Perelson and Nelson 1999).

Since continuity of f implies that $f(T_0) = 0$, it follows that $E_0 = (T_0, 0, 0)$ is an equilibrium of (1), and will be referred to as the infection-free equilibrium. A second, positive equilibrium (corresponding to an infection) exists if the following quantities are positive:

$$\overline{T} = \frac{\gamma}{kN}, \qquad \overline{T}^* = \frac{f(\overline{T})}{\beta}, \qquad \overline{V} = \frac{f(\overline{T})}{k\overline{T}}.$$

This is the case iff $f(\frac{\gamma}{kN}) > 0$, or equivalently $\overline{T} = \frac{\gamma}{kN} < T_0$. In terms of the basic reproductive number $R_0 := \frac{T_0}{\gamma} kN$, the positive equilibrium exists whenever $R_0 > 1$.

Theorem 1 (De Leenheer and Smith 2003) *If $R_0 > 1$, then E_0 is unstable and the infection persists (there exists $\delta > 0$ such that $T(t), T^*(t), V(t) > \delta$ for all sufficiently large t). If $R_0 < 1$, then E_0 is globally asymptotically stable (GAS).*

If, in addition, the function $f(T)$ satisfies the "sector condition": $(f(T) - f(\overline{T}))(T - \overline{T}) \leq 0$ for all $T > 0$, then the positive equilibrium is GAS when $R_0 > 1$ (De Leenheer and Smith 2003; De Leenheer and Pilyugin 2008).

Suppose now that $R_0 > 1$, so that a treatment is needed to clear the infection. To study this scenario, we modify the model by including two types of drugs, i.e., the RT-inhibitors and P-inhibitors:

$$\begin{aligned} \dot{T} &= f(T) - k\big(1 - \eta_{\mathrm{RT}}(t)\big)VT, \\ \dot{T}^* &= k\big(1 - \eta_{\mathrm{RT}}(t)\big)VT - \beta T^*, \\ \dot{V} &= N\big(1 - \eta_P(t)\big)\beta T^* - \gamma V, \end{aligned} \tag{2}$$

where $\eta_{\mathrm{RT}}(t), \eta_P(t) : \mathbb{R} \to [0, 1]$ are the drug efficacy functions of the RT-inhibitor and P-inhibitor (respectively). Suppose also $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t)$ are periodic and share a common period τ.

Since we consider the drug treatment as a periodic forcing, we will treat the infection-free equilibrium E_0 as a τ-periodic solution of (2). The stability of E_0 can

be deduced from the linearized system

$$\dot{x} = B(t)x, \tag{3}$$

where

$$B(t) = \begin{pmatrix} f'(T_0) & 0 & -k(1-\eta_{\mathrm{RT}}(t))T_0 \\ 0 & -\beta & k(1-\eta_{\mathrm{RT}}(t))T_0 \\ 0 & N(1-\eta_P(t))\beta & -\gamma \end{pmatrix}.$$

We want to investigate the Floquet multipliers of $B(t)$ since they determine the local stability properties of E_0 for system (2). Since $B(t)$ is block triangular, its Floquet multipliers are $\lambda_1 = e^{f'(T_0)\tau}$ and λ_2 and λ_3 that are also the Floquet multipliers of the τ-periodic system

$$\begin{pmatrix} \dot{x_2} \\ \dot{x_3} \end{pmatrix} = \begin{pmatrix} -\beta & k(1-\eta_{\mathrm{RT}}(t))T_0 \\ N(1-\eta_P(t))\beta & -\gamma \end{pmatrix} \begin{pmatrix} x_2 \\ x_3 \end{pmatrix}. \tag{4}$$

The Floquet multipliers λ_2 and λ_3 are the eigenvalues of $X(\tau)$ where $X(t)$ is the principal fundamental solution to system (4). $\lambda_1 = e^{f'(T_0)\tau} < 1$ since $f'(T_0) < 0$, so we focus our attention on λ_2 and λ_3.

Since the matrix in (4) is quasipositive, $X(t)$ has nonnegative entries for all $t \geq 0$. Therefore, by the Perron–Frobenius theorem the spectral radius of $X(\tau)$ is an eigenvalue (i.e., the eigenvalue with maximal modulus is positive). We denote the spectral radius by $\rho(X(\tau))$ and let λ_2 be the dominant Floquet multiplier of (4) (i.e. $\lambda_2 = \rho(X(\tau))$).

The following theorem is due to d'Onofrio.

Theorem 2 (d'Onofrio 2005) *Let the Floquet multipliers of system* (4) *be contained inside the open unit disk in the complex plane. Then E_0 is GAS for system* (2)*, hence the infection is cleared.*

If $\rho(X(\tau)) > 1$, then E_0 is unstable. If $\rho(X(\tau)) < 1$, then by Theorem 2, E_0 is GAS. We note that the spectral radius $\rho(X(\tau))$ is really a measure of treatment effectiveness when $\rho(X(\tau)) < 1$. The smaller it is, the faster the convergence to E_0 will be.

3 Perturbation Technique

Because this system is difficult to analyze for general periodic functions, we will first look at small periodic perturbations from constant drug efficacies.

Assuming the drug efficacies are constant over time ($\eta_{\mathrm{RT}}(t) = e_1 \in [0, 1]$ and $\eta_P(t) = e_2 \in [0, 1]$), we can consider the total drug efficacy as an ordered pair $(e_1, e_2) \in [0, 1] \times [0, 1]$. The stability of E_0 is determined by whether or not the modified basic reproduction number $\tilde{R}_0(e_1, e_2)$ is less than 1, where

$$\tilde{R}_0(e_1, e_2) = \frac{T_0}{\gamma} k(1-e_1)N(1-e_2) = R_0(1-e_1)(1-e_2). \tag{5}$$

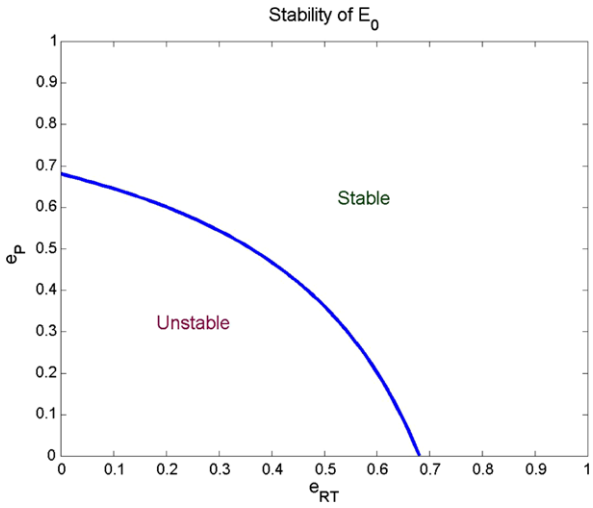


Fig. 1 The stability diagram for constant efficacy treatments. This curve represents the threshold efficacies (i.e., where $(1-e_{\mathrm{RT}})(1-e_P)=\frac{1}{R_0}$). Outside the curve E_0 is stable, inside the curve E_0 is unstable. In this figure, $1-\frac{1}{R_0}\approx 0.681$, as in Rong et al. (2007)

The infection-free equilibrium E_0 is GAS when

$$(1-e_1)(1-e_2)<\frac{1}{R_0},$$

and is unstable, (also the infection persists), when the reversed strict inequality holds. The stability threshold curve $(1-e_1)(1-e_2)=\frac{1}{R_0}$ has intercepts $(e^*,0)$ and $(0,e^*)$ where $e^*=1-\frac{1}{R_0}$ and is shown in Fig. 1.

The goal now is to investigate how periodically perturbing constant drug efficacies affects the system. Fix $e_{\mathrm{RT}}\in[0,1]$ and $e_P\in[0,1]$. Let

$$\eta_{\mathrm{RT}}(t,\epsilon)=e_{\mathrm{RT}}-\epsilon\cdot\varphi_{\mathrm{RT}}(t),\qquad \eta_P(t,\epsilon)=e_P-\epsilon\cdot\varphi_P(t), \tag{6}$$

where $\epsilon>0$ is small and $\varphi_{\mathrm{RT}}(t),\varphi_P(t):\mathbb{R}\to\mathbb{R}$ are τ-periodic analytic functions.

In Fig. 2, we depict a sinusoidal perturbation from a constant efficacy.

Proposition 3 (Noncritical case) *Consider system* (2) *with* $\eta_{\mathrm{RT}}(t,\epsilon)$, $\eta_P(t,\epsilon)$ *as in* (6). *Suppose that* $(1-e_{\mathrm{RT}})(1-e_P)\neq\frac{1}{R_0}$.

(i) *If* $(1-e_{\mathrm{RT}})(1-e_P)>\frac{1}{R_0}$, *then for all sufficiently small* ϵ, E_0 *is unstable.*

(ii) *If* $(1-e_{\mathrm{RT}})(1-e_P)<\frac{1}{R_0}$, *then for all sufficiently small* ϵ, E_0 *is GAS.*

The proof of local stability is based on the continuity of Floquet multipliers with respect to small perturbations. Then global stability of E_0 in case (ii) follows from d'Onofrio's result. We consider this the noncritical case because small perturbations do not alter the stability of E_0.

The more interesting case is the critical one where (e_{RT},e_P) is on the threshold curve, i.e.,

$$(1-e_{\mathrm{RT}})(1-e_P)=\frac{1}{R_0}.$$

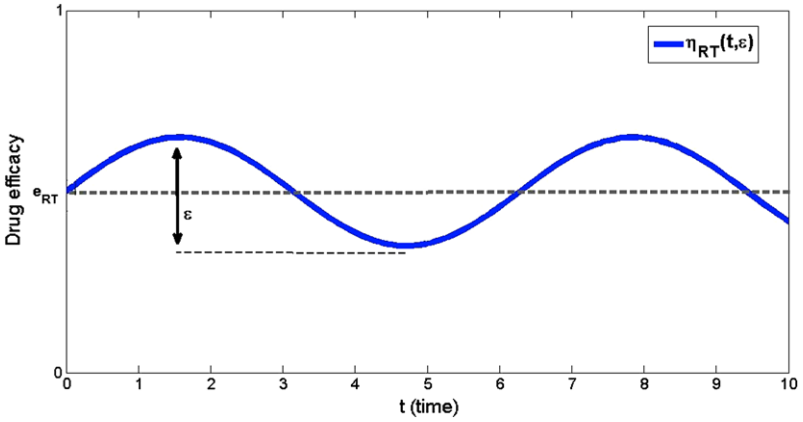


Fig. 2 This shows a small amplitude sinusoidal perturbation (here, we let $\varphi_{\mathrm{RT}}(t) = -\sin t$ in (6))

For the rest of this section, we concentrate exclusively on the critical case. Now for each $\epsilon > 0$, we substitute (6) into (4) and obtain the τ-periodic system

$$\begin{pmatrix} \dot{x_2} \\ \dot{x_3} \end{pmatrix} = \begin{pmatrix} -\beta & k(1-(e_{\mathrm{RT}} - \epsilon \cdot \varphi_{\mathrm{RT}}(t)))T_0 \\ N(1-(e_P - \epsilon \cdot \varphi_P(t)))\beta & -\gamma \end{pmatrix} \begin{pmatrix} x_2 \\ x_3 \end{pmatrix}.$$

Observing that

$$\begin{aligned} k\big(1-\eta(t,\epsilon)\big)T_0 &= kT_0\big(1-e_{\mathrm{RT}}+\epsilon\cdot\varphi(t)\big) \\ &= \frac{kT_0}{R_0(1-e_P)} + kT_0\epsilon\cdot\varphi(t) \\ &= \frac{\gamma}{N(1-e_P)} + kT_0\epsilon\cdot\varphi_{\mathrm{RT}}(t), \end{aligned}$$

the above system can be written as

$$\begin{aligned} \begin{pmatrix} \dot{x_2} \\ \dot{x_3} \end{pmatrix} &= \begin{pmatrix} -\beta & \frac{\gamma}{N(1-e_P)} \\ N(1-e_P)\beta & -\gamma \end{pmatrix} + \epsilon \cdot \begin{pmatrix} 0 & kT_0\varphi_{\mathrm{RT}}(t) \\ N\beta\varphi_P(t) & 0 \end{pmatrix} \begin{pmatrix} x_2 \\ x_3 \end{pmatrix} \\ &= \begin{pmatrix} -\beta & \frac{\gamma}{N(1-e_P)} \\ N(1-e_P)\beta & -\gamma \end{pmatrix} \\ &\quad + \epsilon \cdot \left(kT_0\varphi_{\mathrm{RT}}(t) \begin{pmatrix} 0 & 1 \\ 0 & 0 \end{pmatrix} + N\beta\varphi_P(t) \begin{pmatrix} 0 & 0 \\ 1 & 0 \end{pmatrix} \right) \begin{pmatrix} x_2 \\ x_3 \end{pmatrix}. \end{aligned} \tag{7}$$

For each $\epsilon > 0$, the Floquet multipliers of (7) determine the stability of E_0 for system (2) (with the functions of (6) as the efficacy functions in (2)). We let $Y(t,\epsilon)$ denote the principal fundamental solution to (7). We also let

$$B_0 = \begin{pmatrix} -\beta & \frac{\gamma}{N(1-e_P)} \\ N(1-e_P)\beta & -\gamma \end{pmatrix}, \qquad B_{\mathrm{RT}} = \begin{pmatrix} 0 & kT_0 \\ 0 & 0 \end{pmatrix}, \quad \text{and} \quad B_P = \begin{pmatrix} 0 & 0 \\ N\beta & 0 \end{pmatrix}.$$

Then $Y(t,\epsilon)$ is a principal fundamental solution to

$$\dot{x} = \big(B_0 + \epsilon \cdot \big(\varphi_{\mathrm{RT}}(t) B_{\mathrm{RT}} + \varphi_P(t) B_P\big)\big)x,$$

and the Floquet multipliers of (7) are the eigenvalues of $Y(\tau,\epsilon)$.

The eigenvalues of B_0 are $-(\beta+\gamma)$ and 0. Hence, diagonalizing B_0, we obtain

$$B_0 = Q \begin{pmatrix} 0 & 0 \\ 0 & -(\beta+\gamma) \end{pmatrix} Q^{-1},$$

where

$$Q = \begin{pmatrix} 1 & 1 \\ \frac{N(1-e_P)\beta}{\gamma} & -N(1-e_P) \end{pmatrix} \quad \text{and} \quad Q^{-1} = \frac{1}{1+\beta/\gamma} \begin{pmatrix} 1 & \frac{1}{N(1-e_P)} \\ \beta/\gamma & \frac{-1}{N(1-e_P)} \end{pmatrix}.$$

For computational convenience, we introduce the auxiliary quantities $a := \beta/\gamma$, $b := (\beta+\gamma)$ and $M := N(1-e_p)$. We also define

$$\begin{aligned} A_0 &:= Q^{-1} B_0 Q = \begin{pmatrix} 0 & 0 \\ 0 & -b \end{pmatrix}, \\ A_{\mathrm{RT}} &:= Q^{-1} B_{\mathrm{RT}} Q = \frac{kT_0}{1+a} \begin{pmatrix} Ma & -M \\ Ma^2 & -Ma \end{pmatrix}, \\ A_P &:= Q^{-1} B_P Q = \frac{\beta}{(1+a)(1-e_P)} \begin{pmatrix} 1 & 1 \\ -1 & -1 \end{pmatrix}, \\ X(t,\epsilon) &:= Q^{-1} Y(t,\epsilon) Q. \end{aligned}$$

Upon introducing $A_1(t) := \varphi_{\mathrm{RT}}(t) A_{\mathrm{RT}} + \varphi_P(t) A_P$, $X(t,\epsilon)$ becomes the principal fundamental solution to

$$\dot{x} = \big(A_0 + \epsilon \cdot A_1(t)\big)x. \tag{8}$$

The eigenvalues of $X(\tau,\epsilon)$ coincide with those of $Y(\tau,\epsilon)$ since the matrices are similar. Thus, the spectral radius of $X(\tau,\epsilon)$ determines the stability properties of E_0.

The proofs of the following propositions rely upon expanding $X(t,\epsilon)$ as a Taylor series and using the Implicit Function theorem to expand the dominant eigenvalue of $X(t,\epsilon)$ as an analytic function of ϵ (for small ϵ), then calculating derivatives of this function at $\epsilon = 0$ in order to see where the perturbation takes the dominant eigenvalue. The proofs are deferred to the Appendix.

Throughout the remainder of this paper, we will denote the time average of a τ-periodic function φ as $\overline{\varphi} := \frac{1}{\tau}\int_0^\tau \varphi(t)\,dt$ and define the following quantity Δ_1 as

$$\Delta_1 := \frac{\overline{\varphi_{\mathrm{RT}}} kT_0 N(1-e_P)\beta}{\gamma} + \frac{\overline{\varphi_P}\beta}{(1-e_P)}. \tag{9}$$

Proposition 4 *Let $\epsilon > 0$ be sufficiently small and $\eta_{\mathrm{RT}}(t,\epsilon)$, $\eta_P(t,\epsilon)$ be defined as in (6) (with $(1-e_{\mathrm{RT}})(1-e_P) = \frac{1}{R_0}$). If $\Delta_1 < 0$, then $\rho(X(\tau,\epsilon)) < 1$. Hence, the infection-free equilibrium E_0 for system (2) is GAS. If $\Delta_1 > 0$, then $\rho(X(\tau,\epsilon)) > 1$ and hence E_0 is unstable.*

If $\overline{\varphi_P} = 0$ and $\overline{\varphi_{\mathrm{RT}}} \neq 0$, then stability is determined by $\mathrm{sgn}(\overline{\varphi_{\mathrm{RT}}})$. This is certainly expected since $\overline{\varphi_{\mathrm{RT}}} < 0$ implies $\overline{\eta_{\mathrm{RT}}} > e_{\mathrm{RT}}$, and $\overline{\varphi_{\mathrm{RT}}} > 0$ implies $\overline{\eta_{\mathrm{RT}}} < e_{\mathrm{RT}}$. An analogous statement can be made for when $\overline{\varphi_{\mathrm{RT}}} = 0$ and $\overline{\varphi_P} \neq 0$.

In what follows, we investigate a more subtle question, namely, what happens when both $\overline{\varphi_{\mathrm{RT}}} = 0$ and $\overline{\varphi_P} = 0$ (which implies $\Delta_1 = 0$).

We introduce

$$\begin{aligned} C &:= \int_0^\tau e^{-A_0 t} A_1(t) e^{A_0 t}\, dt, \\ E &:= \int_0^\tau e^{-A_0 t} A_1(t) e^{A_0 t} \left(\int_0^t e^{-A_0 s} A_1(s) e^{A_0 s}\, ds \right) dt, \end{aligned} \tag{10}$$

and

$$\Delta_2 := \left(E_{11} + \frac{e^{-b\tau}}{1 - e^{-b\tau}} C_{12} C_{21} \right). \tag{11}$$

Proposition 5 *Suppose that $\epsilon > 0$ is sufficiently small and $\eta_{\mathrm{RT}}(t, \epsilon)$, $\eta_P(t, \epsilon)$ are as defined in (6). Also, suppose that $\overline{\varphi_{\mathrm{RT}}} = 0$ and $\overline{\varphi_P} = 0$. If $\Delta_2 < 0$, then $\rho(X(\tau, \epsilon)) < 1$. Hence, the infection-free equilibrium E_0 for system (2) is GAS. If $\Delta_2 > 0$, then $\rho(X(\tau, \epsilon)) > 1$, and hence E_0 is unstable.*

This proposition implies that when $\overline{\varphi_{\mathrm{RT}}} = 0$ and $\overline{\varphi_P} = 0$, stability of E_0 is determined exclusively by the sign of Δ_2.

3.1 Perturbation by Sinusoidal Functions

In this section, we consider a specific example where $\varphi_{\mathrm{RT}}(t)$ and $\varphi_P(t)$ are sinusoidal functions.

Proposition 6 *Let $\epsilon > 0$ be sufficiently small and $\varphi_{\mathrm{RT}}(t) = \alpha_1 \sin t$, $\varphi_P(t) = \alpha_2 \sin t$ in (6) where $\alpha_1, \alpha_2 \in \mathbb{R}$. Let $\Lambda := -R_0(\frac{\alpha_1}{\alpha_2}(1 - e_P)^2 + \frac{\alpha_2}{\alpha_1}(1 - e_{\mathrm{RT}})^2) + (\frac{\gamma}{\beta} + \frac{\beta}{\gamma})$. Then $\Delta_2 = K\Lambda$ where $K > 0$. Hence, the infection-free equilibrium E_0 for system (2) is GAS when $\Lambda < 0$. E_0 is unstable when $\Lambda > 0$.*

Some remarks are in order after this proposition. First, if either $\alpha_1 = 0$ or $\alpha_2 = 0$ (but not both zero), then $\Lambda < 0$. Hence, if we periodically perturb the RT-inhibitor about a critical efficacy while holding the P-inhibitor at a critical efficacy or vice-versa, then E_0 is GAS.

Second, suppose α_1 and α_2 have the same sign. This corresponds to the case where $\varphi_{\mathrm{RT}}(t)$ and $\varphi_P(t)$ are completely in phase with each other. In this case, for certain choices of parameters, Λ is positive.

We now analyze when $\Lambda > 0$ in this case, denote the above expression for Λ as

$$\Lambda = \beta^2 \alpha_1 \alpha_2 \left[-g(e_{\mathrm{RT}}, e_P) + \left(\frac{\gamma}{\beta} + \frac{\beta}{\gamma} \right) \right],$$

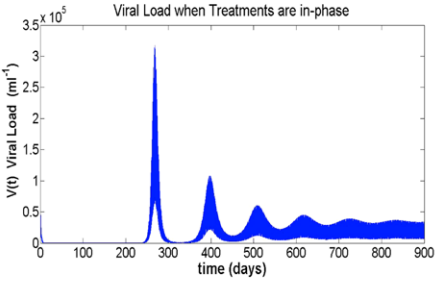
where $g(e_{\mathrm{RT}}, e_P) = -R_0(\frac{\alpha_1}{\alpha_2}(1-e_P)^2 + \frac{\alpha_2}{\alpha_1}(1-e_{\mathrm{RT}})^2)$ and $(e_{\mathrm{RT}}, e_P) \in [0, e^*] \times [0, e^*]$ (since $(1-e_{\mathrm{RT}})(1-e_P) = \frac{1}{R_0}$). Assume, without loss of generality, that $|\alpha_2| \geq |\alpha_1|$. Then the maximum of $g(e_{\mathrm{RT}}, e_P)$ occurs when $e_{\mathrm{RT}} = 0$, $e_P = e^*$, where we have $g(0, e^*) = \frac{\alpha_1}{\alpha_2 R_0} + \frac{\alpha_2 R_0}{\alpha_1}$. Hence, if $\frac{\gamma}{\beta} > \frac{\alpha_2 R_0}{\alpha_1}$ or $\frac{\gamma}{\beta} < \frac{\alpha_1}{\alpha_2 R_0}$, then $\Lambda > 0$ for all possible $(e_{\mathrm{RT}}, e_P) \in [0, e^*] \times [0, e^*]$. Next, assume $e_{\mathrm{RT}} = e_P$ $(= 1 - \sqrt{\frac{1}{R_0}})$. Then $\Lambda > 0$, if $\frac{\gamma}{\beta} > \frac{\alpha_2}{\alpha_1}$ or $\frac{\gamma}{\beta} < \frac{\alpha_1}{\alpha_2}$. Therefore, if $\alpha_1 = \alpha_2$ and $e_{\mathrm{RT}} = e_P$, then $\Lambda \geq 0$ no matter what virus/host parameters are chosen. Hence, when α_1 and α_2 have the same sign, E_0 can become unstable. In other words, small "in-phase perturbations" can destabilize the infection-free equilibrium.

Finally, we consider the last case: α_1 and α_2 have opposite signs (i.e., $\varphi_{\mathrm{RT}}(t)$ and $\varphi_P(t)$ are completely out of phase sinusoidal functions). Then Λ is always negative, and the infection-free equilibrium E_0 is GAS. Therefore, these small "out-of-phase perturbations" always stabilize the infection-free equilibrium.

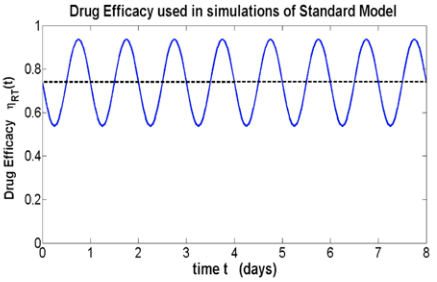
In Fig. 3, there are some simulations illustrating this phenomenon using estimates of HIV parameters from Rong and Perelson (2009). In the simulations, we see that the in-phase perturbations do not clear the infection. Also, notice that there are two periods of oscillations that occur. There are high frequency oscillations (period = 1 day) due to the periodic forcing from the sinusoidal drug efficacies. The low frequency damped oscillations are due to the fact that the positive steady state of system (1) is a locally asymptotically stable spiral point. In addition, we see that the out-of-phase sinusoidal forcing clears the infection.

A criticism of system (2) (assuming constant drug efficacies) is that it cannot robustly generate a low level viral steady state often observed in patients undergoing therapy (Rong and Perelson 2009). As the drug efficacies approach the threshold level, the magnitude of the viral steady state is very sensitive to small changes in efficacy. In addition, as the viral steady state decreases in magnitude, the viral load will spend a rapidly increasing length of time very close to zero. These features of the model still exist with periodic drug efficacies, although at least for small amplitude periodic perturbations, the viral steady state becomes a periodic solution. The standard model with treatment, system (2), does not seem to capture some relevant dynamics which contribute to low level viral persistence. An excellent review of modeling HIV persistence is provided by Rong and Perelson (2009). We briefly discuss and display simulations for two slightly modified versions of (2) that partially attempt to address the aforementioned criticism.

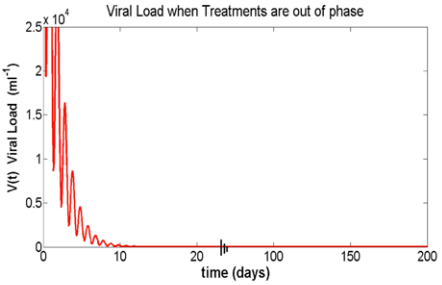
First, we consider a model which changes the per capita decay rate of T^* from the constant, β, to a function, $\delta(T^*)$ (Callaway and Perelson 2002). A simple form for $\delta(T^*)$, which was shown to robustly generate low level viremia, is $\delta(T^*) = (T^*)^\omega$ where $\omega > 0$. This model does not display such sensitivity of viral steady state with respect to changes in drug efficacies when (e_{RT}, e_P) are located close to the threshold curve. Also, the model was shown to fit data fairly well (Holte et al. 2006). The existence and validity of a biological mechanism behind this particular form of density dependent per capita decay rate for infected cells is questionable, however. In Fig. 4, we show simulations of this density dependent model with both in-phase and out-of-phase sinusoidal drug efficacy functions. It is seen that the out-of-phase treatments control the virus to levels just below 50 copies/ml. The in-phase treatments initially



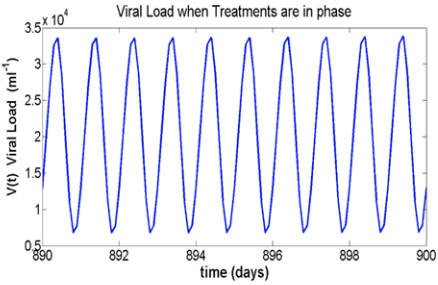
(a) In-phase treatment



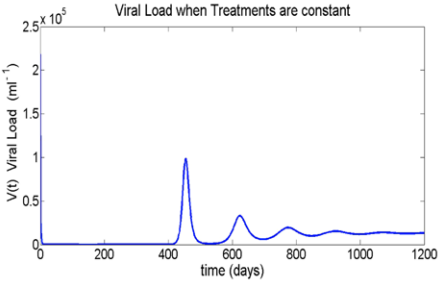
(b) Drug Efficacy used in simulations



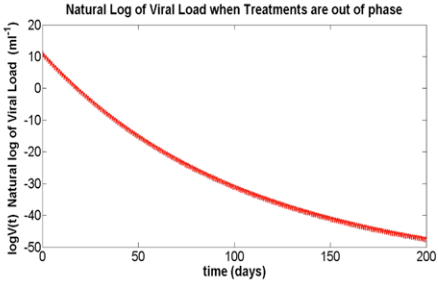
(c) Out-of-phase treatments



(d) Steady-state periodic orbit of viral load with in-phase treatments



(e) Constant Efficacy



(f) Out-of-phase treatment clears virus

Fig. 3 Viral Load vs. time for model (2) with $\eta_{\mathrm{RT}}(t) = (e - 0.03) - 0.2\sin(2\pi t)$ and $\eta_{\mathrm{RT}}(t) = (e-0.03) - 0.2\sin(2\pi t + \psi)$, where $e = 1 - \sqrt{\frac{1}{R_0}} = 0.7673$. $\eta_{\mathrm{RT}}(t)$ is depicted in (**b**). Note that the drug efficacies are perturbed about the value 0.7373 and have amplitude 0.2. The phase shift $\psi = 0$, corresponding to the in-phase treatments, was used in (**a**), (**b**), and (**d**). $\psi = 0.5$, corresponding to the out-of-phase treatments, was used in (**c**) and (**f**). In (**e**), the drug efficacies used, $\eta_{\mathrm{RT}}(t) = \eta_P(t) = e - 0.03$, are constant. The values of the parameters used in these simulations are taken from Rong and Perelson (2009). They are as follows: $f(T) = a - bT$ with $a = 10^4$ ml^{-1} day^{-1} and $b = 0.01$ day^{-1} (which implies that $T_0 = 10^6$ ml^{-1}), $k = 8 \times 10^{-7}$ ml day^{-1}, $\beta = 0.7$ day^{-1}, $N = 300$, $\gamma = 13$ day^{-1}. The initial conditions are taken to be the positive steady state of (1). The in-phase treatment initially brings the viral load close to zero, but then there is large amplitude viral oscillations, (**a**) before converging to a periodic solution with fairly high viral load, (**d**). The out-of-phase treatments cause the viral load to decay to zero, (**c**), (**f**). The viral load, under the assumption of a constant drug efficacy of magnitude 0.7373 ($= e - 0.03$), is shown in (**e**)

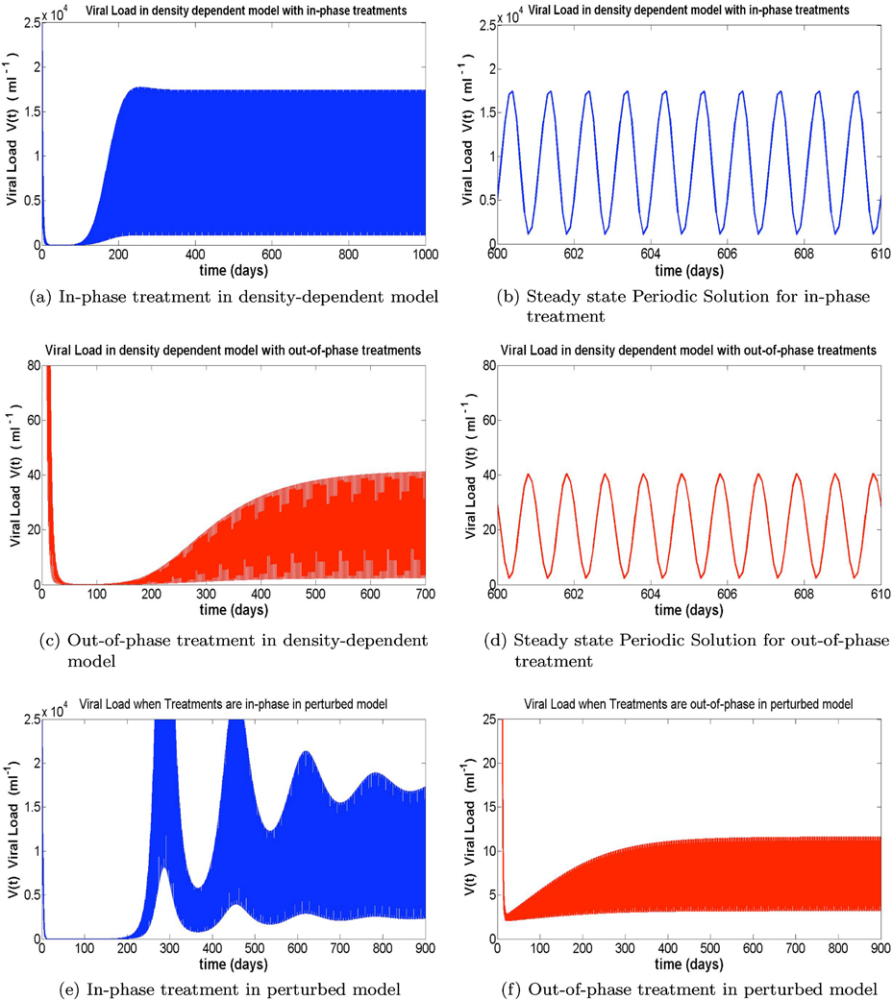
drive the virus to low levels, but then the virus rebounds to levels in excess of 10,000 copies/ml. At these viral levels, the in-phase treatments would certainly be a failure, where as the out-of-phase treatments would be successful in supressing the virus to levels below detection by standard assays. The contrast in results when varying the phase difference (while keeping the drug strengths constant) is certainly striking.

A rather naive way of keeping the virus level away from zero, is to simply perturb model (2) by a constant. Explicitly, we just add a constant, $A > 0$, to the $\dot{V}$ equation. The motivation behind this can be a very slow replicating drug sanctuary which leaks virus particles at a rate A day^{-1} and also produces virus at that same constant rate. Hence, the drug sanctuary remains fixed in size. For sufficiently small values of A, the qualitative behavior of the family of solutions will not change; however, $V(t)$ will always be strongly persistent no matter what the drug efficacy, i.e., $\exists\, \epsilon > 0$ (dependent on A) such that $V(t) > \epsilon\ \forall t > 0$. A simulation of this model with our periodic drug efficacies is shown in Fig. 4.

These results illustrate the importance of understanding the pharmacokinetics of the drugs in a combination therapy. Drugs and their dosing regimens are often designed to limit the variability of drug efficacy over the course of treatment. Interestingly, we have shown that when inserting periodic drug efficacies into a dynamical model of the virus, the phase difference between the RT-inhibitor, and P-inhibitor efficacy functions can greatly influence the treatment outcome. This can be viewed as an affirmation of the goal of minimizing variability in drug efficacy over time. Alternatively, especially given toxicity of medications, time-varying efficacy functions can be viewed as something to exploit if the phase difference between the RT- and P-inhibitor can be controlled. There are many factors which affect how the drug efficacy functions; $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t)$, might look in reality and how much drug design can effectively "shape" these drug efficacy functions. The complexities of this issue constitute a significant portion of pharmaceutical research. In a way, our results add an extra factor of complexity into the equation.

As we vary the phase difference of the drug efficacy functions in these models, a wide range of viral rebound levels are realized. In clinical studies of viral rebound during HAART, patients have displayed variable results. For example, in a study of viral level in patients 1 year after the initiation of HAART, 71% achieved viral levels below 500 copies/ml, 10% had viral levels at 500–5000 copies/ml, and 19% had a viral rebound above 5000% copies/ml (Abgrall et al. 2003). The reason for differing viral rebounds in patients is not always known. As patients certainly might have different dosing schedules, levels of adherence, and different pharmacokinetic/pharmacodynamic parameters, our results offer a possible explanation for some of the variance in results.

We conclude that shifting the phase of $\eta_P(t, \epsilon)$ in (6), which in this case amounts to changing the phase difference between $\eta_{\mathrm{RT}}(t, \epsilon)$ and $\eta_P(t, \epsilon)$, can affect the stability of E_0. How much this phase difference affects the treatment outcome depends on the parameters. For certain choices of the parameters, phase difference has a dramatic effect on the stability of E_0. Our results indicate that the timing between periodic dosages of RT-inhibitors and P-inhibitors can affect treatment effectiveness. There might be an "optimal dosing schedule" for treatment involving both RT-inhibitors and P-inhibitors. Thus, given the above observations, we further investigate varying phase shifts of the two drug efficacy functions.



(a) In-phase treatment in density-dependent model

(b) Steady state Periodic Solution for in-phase treatment

(c) Out-of-phase treatment in density-dependent model

(d) Steady state Periodic Solution for out-of-phase treatment

(e) In-phase treatment in perturbed model

(f) Out-of-phase treatment in perturbed model

Fig. 4 Simulations for the two modified models. (**a**), (**b**), (**c**), and (**d**) are based on the standard model, system (2), with density dependent decay rate for infected cells, i.e., $\dot{T}^* = k(1-\eta_{\mathrm{RT}}(t))VT - \delta(T^*)^{\omega+1}$. The drug efficacy functions used are: $\eta_{\mathrm{RT}}(t) = 0.79 - 0.2\sin(2\pi t)$ and $\eta_P(t) = 0.80 - 0.2\sin(2\pi t + \psi)$ where $\psi = 0$ in (**a**), (**b**), and $\psi = 0.5$ in (**c**), (**d**). All the remaining parameters are the same as used in the simulations in Fig. 3, except there are two new parameters, ω and δ. We choose $\omega = 0.4$ and $\delta = 0.274$ day^{-1}, as was used in Rong and Perelson (2009). δ is chosen so that the pretreatment viral steady state is the same as with the standard model. (**e**), (**f**) are based on the perturbed standard model, i.e., system (2) with $\dot{T}^* = k(1-\eta_{\mathrm{RT}}(t))VT - \beta T^* + A$. Here, we choose $A = 1$ day^{-1} and $\eta_{\mathrm{RT}}(t) = 0.78 - 0.2\sin(2\pi t)$ and $\eta_P(t) = 0.80 - 0.2\sin(2\pi t + \psi)$ where $\psi = 0$ in (**e**) and $\psi = 0.5$ in (**f**)

4 Optimizing Phase Shifts for General Periodic Functions

Let $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t)$ be given τ-periodic drug efficacy functions on $\mathbb{R}$. For $\psi_1, \psi_2 \in \mathbb{R}$, we consider the (linearized) phase shifted system:

$$\dot{x} = B(t, \psi_1, \psi_2)x, \tag{12}$$

where

$$B(t,\psi_1,\psi_2)=\begin{pmatrix} f'(T_0) & 0 & -k(1-\eta_{\mathrm{RT}}(t-\psi_1))T_0 \\ 0 & -\beta & k(1-\eta_{\mathrm{RT}}(t-\psi_1))T_0 \\ 0 & N(1-\eta_P(t-\psi_2))\beta & -\gamma \end{pmatrix}.$$

Proposition 7 *Let $\lambda_2(\psi_1,\psi_2)$ be the dominant Floquet multiplier of* (12)*. Then, $\lambda_2(\psi_1,\psi_2)=\lambda_2(0,(\psi_2-\psi_1)$ modulo $\tau)$.*

Denote $\lambda_2(0,\psi)$ as $\lambda_2(\psi)$. Observe the following:

1. The map $\psi\longmapsto\lambda_2(\psi)$ is a τ-periodic function on $\mathbb{R}$.
2. When optimizing phase shifts of $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t)$, we only need to consider phase shifts, ψ, where $\psi\in[0,\tau)$ and ψ shifts $\eta_P(t)$ to $\eta_P(t-\psi)$ (η_{RT} is not shifted). Hence, the *timing between* administered dosages of RT-inhibitors and P-inhibitors is the variable which affects the system dynamics.

We define the optimal phase shift $\psi^*\in[0,\tau)$ as the minimizer of $\lambda_2(\psi)$. For $\psi\in[0,\tau)$ let $X(t,\psi)$ be a principal fundamental solution to system (4) with $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t-\psi)$ as the drug efficacy functions in the system. The following theorem may be useful when numerically optimizing phase shifts.

Theorem 8 *The optimal phase shift ψ^* is the minimizer of* $\operatorname{tr}X(\tau,\psi)$.

The proofs of these results can be found in the Appendix.

5 A Numerical Study with Bang-Bang Efficacies

We now numerically explore the effect that phase shifts have on the dyanamics of system (2), when the drug efficacies are of the bang-bang type. Throughout this section, the dominant Floquet multiplier (of the system: $\dot{x}=B(t,0,\psi)x$) is denoted as λ_2. We consider drug efficacy functions of the bang-bang type and sums of these functions which are just piecewise constant functions. In the following examples, $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t)$ are assumed to be of the same type of periodic functions. Hence, we refer to the phase shift, $\psi\in[0,\tau)$, as the phase difference between $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t-\psi)$. First, we define $\eta_{\mathrm{RT}}(t),\eta_P(t):\mathbb{R}\to[0,1]$ as periodic functions with period $\tau=1$ such that

$$\eta_{\mathrm{RT}}(t)=\begin{cases} e_{\mathrm{RT}} & \text{if } t\in[0,\frac{1}{2}), \\ 0 & \text{if } t\in[\frac{1}{2},1) \end{cases} \quad\text{and}\quad \eta_P(t)=\begin{cases} e_P & \text{if } t\in[0,\frac{1}{2}), \\ 0 & \text{if } t\in[\frac{1}{2},1). \end{cases}$$

Hence, if the phase difference $\psi\in[0,\frac{1}{2}]$, then on $[0,1)$:

$$\eta_P(t-\psi)=\begin{cases} e_P & \text{if } t\in[\psi,\frac{1}{2}+\psi), \\ 0 & \text{if } t\in[0,\psi)\cup[\frac{1}{2}+\psi,1]. \end{cases}$$

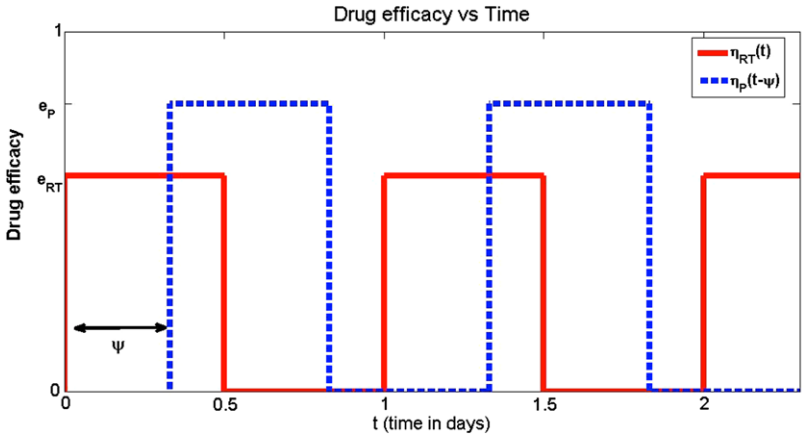


Fig. 5 (Color online) An example of the bang-bang functions with a phase difference ψ. Here, $\eta_{\mathrm{RT}}(t)$ is the *red curve* and $\eta_P(t-\psi)$ is the *dashed blue lines*

If $\psi \in (\frac{1}{2}, 1)$, then on $[0, 1)$:

$$\eta_P(t-\psi) = \begin{cases} e_P & \text{if } t \in [\psi, 1) \cup [0, \psi - \frac{1}{2}), \\ 0 & \text{if } t \in [\psi - \frac{1}{2}, \psi). \end{cases}$$

Here, e_{RT} and e_P are fixed in $[0, 1]$. Hence, the efficacy of the RT-inhibitor and P-inhibitor are e_{RT} and e_P (respectively) for 12 hours in a day and 0 for the other 12 hours. The graph of this function is shown in Fig. 5. This bang-bang control is certainly not perfect for modeling the real drug efficacy functions, but it allows us to explicitly calculate Floquet multipliers and thus gives us an idea of how varying the phase difference can affect treatment effectiveness. De Leenheer (2009) has shown that the Floquet multipliers of this system are $e^{f'(T_0)}$ and the eigenvalues of:

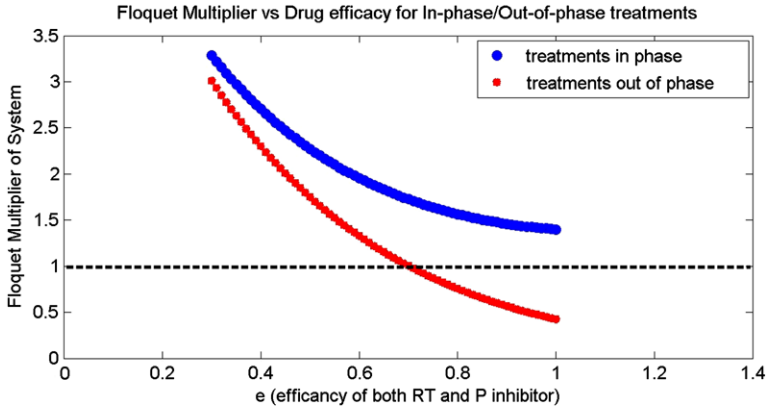
$$\begin{aligned} \Lambda(e_{\mathrm{RT}}, e_P, \psi) = {} & \exp\big[(0.5-\psi)E(0,0)\big]\exp\big[\psi E(0, e_P)\big] \\ & \times \exp\big[(0.5-\psi)E(e_{\mathrm{RT}}, e_P)\big]\exp\big[\psi E(e_{\mathrm{RT}}, 0)\big] \end{aligned}$$

when $\psi \in [0, \frac{1}{2}]$, and

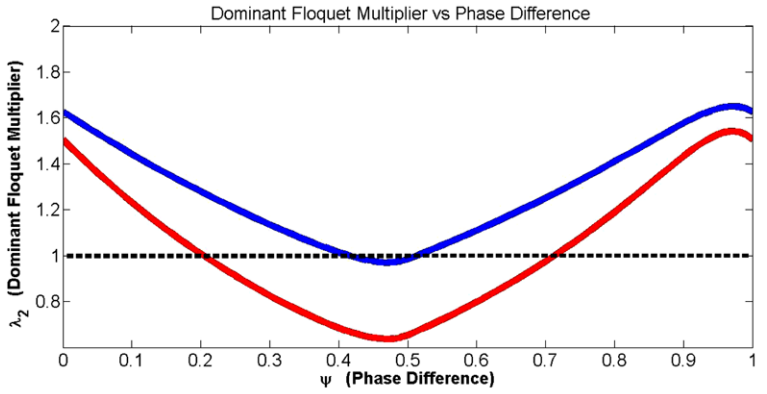
$$\begin{aligned} \Lambda(e_{\mathrm{RT}}, e_P, \psi) = {} & \exp\big[(\psi-0.5)E(0,e_P)\big]\exp\big[\psi E(0, 0)\big] \\ & \times \exp\big[(\psi-0.5)E(e_{\mathrm{RT}}, 0)\big]\exp\big[\psi E(e_{\mathrm{RT}}, e_P)\big] \end{aligned}$$

when $\psi \in (\frac{1}{2}, 1)$; where

$$E(e_{\mathrm{RT}}, e_P) = \begin{pmatrix} -\beta & k(1-e_1)T_0 \\ N(1-e_2)\beta & -\gamma \end{pmatrix}.$$



(a) Floquet Multiplier vs. efficacy for In-phase and out-of-phase treatments



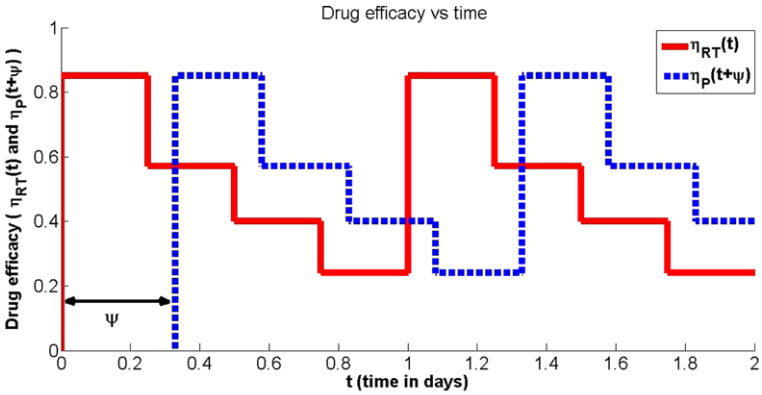
(b) Floquet Multiplier vs. phase difference

Fig. 6 (Color online) In (**a**), we assume the drug efficacy functions to be of the type depicted in Fig. 5 with $e_{\mathrm{RT}} = e_P = e \in [0.3, 1]$. The *blue line* graphs λ_2 as a function of efficacy e when $\psi = 0$. The *red line* depicts λ_2 as a function of efficacy e when $\psi = 0.5$. The *dashed gray line* is $\lambda_2 = 1$. We see the in-phase treatments do not clear the infection while the out of phase treatments do clear the infection. In (**b**), λ_2 is plotted as a function of the phase difference, ψ, when the drug efficacy functions, $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t - \psi)$, are assumed to be of the type displayed in Fig. 5. The *red curve* (the curve which is closer to 0) represents the case when $e_{\mathrm{RT}} = e_P = 0.85$ and the *blue curve* represents the case when $e_{\mathrm{RT}} = 0.9, e_P = 0.5$ or $e_{\mathrm{RT}} = 0.5, e_P = 0.9$ (both give the same graph)

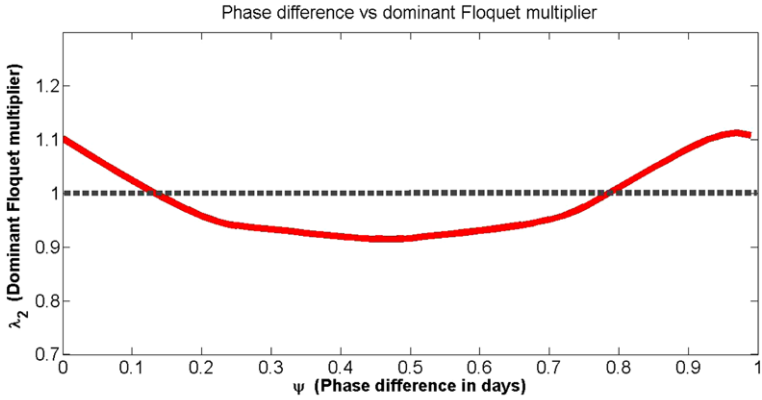
We show some numerical calculations of these eigenvalues. We use the parameters given by Rong et al. (2007), and they are as follows: $f(T) = a - bT$ with $a = 10^4$ ml^{-1} day^{-1} and $b = 0.01$ day^{-1} (which implies that $T_0 = 10^6$ ml^{-1}), $k = 2.4 \times 10^{-8}$ ml day^{-1}, $\beta = 1$ day^{-1}, $N = 3000$, $\gamma = 23$ day^{-1}. The difference in parameter choice from the prior simulations does not change qualitative results.

We first assume that $e_{\mathrm{RT}} = e_P = e$ where $e \in [0, 1]$.

We evaluate the dominant Floquet multiplier, λ_2, as a function of drug efficacy, e, for the cases $\psi = 0$ (completely in-phase) and $\psi = \frac{1}{2}$ (completely out-of-phase). The results are shown in Fig. 6.



(a) Piecewise constant drug efficacy



(b) Floquet Multiplier vs. phase difference

Fig. 7 (Color online) In (**a**), the drug efficacy functions $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t-\psi)$ are assumed to have a the shape above, which is a closer approximation to real drug efficacies. In (**b**), the dominant Floquet multiplier, λ_2, is shown as a function of phase difference, ψ, (the *red curve*) when $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t-\psi)$ are as in (**a**). The *dashed line* is just the function $\lambda_2 = 1$. It is seen that phase difference still can affect whether or not the infection-free equilibrium E_0 is stable

Remember E_0 is GAS if $\lambda_2 < 1$ and unstable if $\lambda_2 > 1$. Now we fix e_{RT} and e_P and calculate λ_2 as a function of the phase difference ψ as shown in Fig. 5.

Notice, in Fig. 6, λ_2 is a periodic function of ψ, which has a minimum and a maximum. The minimum, which occurs just before $\psi = \frac{1}{2}$, corresponds to the optimal phase difference. The phase difference is the determining factor in whether or not the infection is cleared in these examples.

We now better approximate an actual drug efficacy function with a piecewise constant function, whose graph is shown in Fig. 7, and evaluate the dominant Floquet multiplier as a function of the phase difference ψ as shown in Fig. 7. In this case, the phase difference still determines whether or not $\lambda_2 < 1$ (i.e., whether or not the infection clears).

5.1 Structured Treatment Interruptions

It is interesting to consider the consequences of increasing the period τ. During a STI regimen, a patient will take a drug for a certain period of time (the "on-days") and then take a "break" from that medication for another period of time (the "off-days"). This cycle may repeat. The bang-bang drug efficacy functions used here can be fairly close to the real drug efficacy functions for STIs. Thus, one can consider the drug efficiency function as being nearly constant, e_{RT} (or e_P), for the on-days and roughly 0 for the off-days. The period and duration of the drug efficacy function for an STI depends on the nature of the program. Duration here is meant as an open interval $I \in \mathbb{R}$ such that the drug efficacy function $\eta(t)$ supported in I, is periodic with period τ. In general, an STI might have a drug efficacy function $\eta_1(t)$ with period τ_1 on an interval of time (t_1, t_2), then change the drug efficacy function to $\eta_2(t)$ with period τ_2 on (t_2, t_3) and so on. A dynamic STI would choose $\eta_2(t)$ based on information reflecting the infection status at t_2 (Rosenberg et al. 2006).

We consider the bang-bang functions described above with the period τ increased to 60 days. Explicitly $\eta_{\mathrm{RT}}(t), \eta_P(t) : \mathbb{R} \to [0, 1]$ are periodic functions with period $\tau = 60$ such that

$$\eta_{\mathrm{RT}}(t) = \begin{cases} e_{\mathrm{RT}} & \text{if } t \in [0, 30), \\ 0 & \text{if } t \in [30, 60) \end{cases} \quad \text{and} \quad \eta_P(t) = \begin{cases} e_P & \text{if } t \in [0, 30), \\ 0 & \text{if } t \in [30, 60). \end{cases}$$

If the phase difference $\psi \in [0, 30]$, then

$$\eta_P(t - \psi) = \begin{cases} e_P & \text{if } t \in [\psi, 30 + \psi), \\ 0 & \text{if } t \in [0, \psi) \cup [30 + \psi, 60]. \end{cases}$$

If $\psi \in (30, 60)$, then

$$\eta_P(t - \psi) = \begin{cases} e_P & \text{if } t \in [\psi, 60) \cup [0, \psi - 30), \\ 0 & \text{if } t \in [\psi - 30, \psi). \end{cases}$$

Then the Floquet multipliers are the eigenvalues of

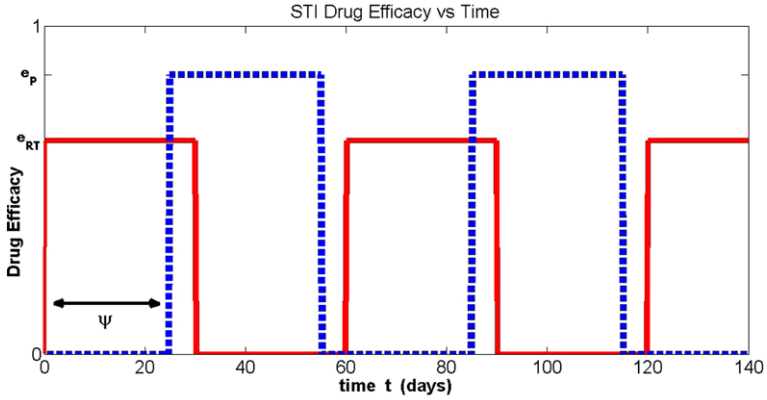
$$\begin{aligned} \Lambda(e_{\mathrm{RT}}, e_P, \psi) = {} & \exp\big[(30 - \psi)E(0, 0)\big] \exp\big[\psi E(0, e_P)\big] \\ & \times \exp\big[(30 - \psi)E(e_{\mathrm{RT}}, e_P)\big] \exp\big[\psi E(e_{\mathrm{RT}}, 0)\big], \end{aligned}$$

when $\psi \in [0, 30]$, and

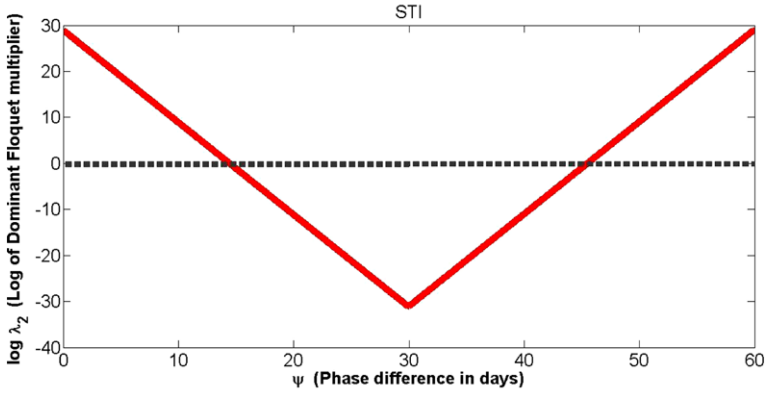
$$\begin{aligned} \Lambda(e_{\mathrm{RT}}, e_P, \psi) = {} & \exp\big[(\psi - 30)E(0, e_P)\big] \exp\big[\psi E(0, 0)\big] \\ & \times \exp\big[(\psi - 30)E(e_{\mathrm{RT}}, 0)\big] \exp\big[\psi E(e_{\mathrm{RT}}, e_P)\big], \end{aligned}$$

when $\psi \in (30, 60)$; where

$$E(e_{\mathrm{RT}}, e_P) = \begin{pmatrix} -\beta & k(1 - e_1)T_0 \\ N(1 - e_2)\beta & -\gamma \end{pmatrix}.$$



(a) STI drug efficacy function



(b) Floquet Multiplier vs. phase difference

Fig. 8 (Color online) In (**a**), an example showing the STI drug efficacy functions. $\eta_{\mathrm{RT}}(t)$ is the *red curve* and $\eta_P(t-\psi)$ is the *blue dashed lines*. In (**b**), the natural logarithm of the dominant Floquet multiplier, $\log\lambda_2$, is shown as a function of phase difference, ψ, when $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t-\psi)$ are of the type depicted in (**a**). In these calculations, $e_{\mathrm{RT}} = e_P = 0.85$. Notice the large range of values we obtain for λ_2 ($\min\lambda_2 \approx e^{-30}$ and $\max\lambda_2 \approx e^{30}$). The *dashed line*, $\log\lambda_2 = 0$, represents the threshold for clearing the infection

These are examples of static STIs where the drug efficacy functions, $\eta_{\mathrm{RT}}(t)$ and $\eta_P(t-\psi)$, have the on-days in a consecutive block of days. The period is $\tau = 60$ days and the on-days for the RT-inhibitor are 30 consecutive days (the off-days are similarly a 30 day block), likewise for the P-inhibitor. The functions are depicted in Fig. 8.

The graph of $\log\lambda_2$ as a function of ψ for $e_{\mathrm{RT}} = e_P = 0.85$ is shown in Fig. 8. These numerical calculations show there is an enormous variability in the results one obtains when varying the phase difference between bang-bang RT- and P-inhibitor functions on the larger time scale setting of STIs. Certainly, there is a much larger set of possible regimens which include treatment schedules that have multiple blocks of on-days and off-days within a period. Numerical optimal control methods have been used to address optimization over a larger set of possible STIs (Adams et al. 2004).

If we do choose a regimen where, within a period, the on-days are in a consecutive block and off-days are in a consecutive block, our results show that the on-days of the RT-inhibitor should for the most part coincide with the off-days of the P-inhibitor and vice-versa (the optimal phase difference $\psi^* \approx 30$ days in the example shown in Fig. 8b). As mentioned in the Introduction, one should view these results on STIs with a healthy dose of caution as the increase in the period of the drug treatment will also act to increase the already existing error inherent in the model.

6 Conclusions and Discussion

The effect which the periodic variations of drug efficacy functions have upon the dynamics of within-host virus models is difficult to analyze in general. In this paper, we analyzed, with considerable rigor and detail, how periodic forcing of threshold drug efficacies affected the infection-free equilibrium. This shed some light on the dynamical interplay between periodically administered RT-inhibitor and P-inhibitor treatment in a within-host virus model. Combination therapy has become a standard procedure in the treatment of HIV. Hence, the notion that the timing between periodic dosages of the two classes of drugs may affect the treatment outcome could be of value to researchers.

In our numerical study, we considered two different time scales for the treatment of a viral infection. We briefly considered the larger time scale of STI regimens. In most cases of HIV, for one reason or another, lifelong use of antivirals is not feasible (Rosenberg et al. 2006). This is why STIs have garnered much attention and research efforts. For the model used in this paper, we showed the timing of RT-inhibitor on-days relative to P-inhibitor on-days can have a strong effect on the treatment outcome. Even though the amount of drug used in each regimen was the same, simply varying this phase difference could give wildly different results. Our results, while dramatic, have their limitations. First, the model is too simplistic and probably does not capture all relevant dynamics, especially on a larger time scale. Second, we only numerically analyzed a small subset of possible regimens. Nevertheless, using the Floquet theory to explore STIs is novel and furthers the ideas presented in De Leenheer (2009). To analyze a larger number of regimens, optimal control might be the preferred method. However these methods also can have drawbacks, for example, the optimization of the STI regimen depends upon the choice of a cost functional.

The other treatment time scale considered was the day to day time scale. Varying the phase shift ψ in $\eta_P(t-\psi)$ on this time scale can be interpreted as changing the timing between daily dosages of RT-inhibitors and P-inhibitors. For example, if a patient takes both an RT-inhibitor and P-inhibitor every 24 hours, then the time in between dosages of the two drugs each day would be the phase shift ψ. Now to optimize treatment on this scale, with no consideration of negative effects of medication, phase shift is clearly the only variable to consider (assuming the patient has to follow a periodic schedule), since drug efficacies can be chosen to be as large as currently feasible. When side effects or costs of medication are considered, optimization of phase shift may be even more important. The toxicity of HIV medications is a serious obstacle in treating some patients. Potentially, drugs can be designed so that their

corresponding efficacy functions reduce the toxicity induced in patients by shortening drug exposure time, but through optimizing the phase difference between the RT- and P-inhibitors, treatment effectiveness can still be achieved. Phase shift contrasts from other treatment parameters in that it does not depend on the efficacies of the medications. So, an optimal phase shift for a given set of drugs, if found, possibly can give better treatment results for "free." Certainly, it is not known whether this phase shift affects viral dynamics in an actual setting and we do not claim it will. More realistic models and drug efficacy functions would have to be used in order to accurately gauge how much phase shifts actually affect the dynamics of a within-host virus. However, this paper does illustrate how the inherent periodicity of drug efficacies can be an important factor in the dynamics of our model and suggests that future research may need to incorporate time varying drug efficacies into models of within-host viruses with treatment.

Acknowledgements We thank the anonymous reviewers for their comments and suggestions. E. Pilyugin supported in part by NSF grant DMS-0818050.

Appendix

Proof of Proposition 4 The right-hand side of (8) is analytic, therefore, the principal fundamental solution $X(t,\epsilon)$ is analytic in ϵ. So, we can expand $X(t,\epsilon)$ as a Taylor series with respect to ϵ

$$X(t,\epsilon)=\sum_{n=0}^{\infty}\epsilon^n X_n(t)=X_0(t)+\epsilon X_1(t)+\epsilon^2 X_2(t)+\cdots.$$

Then we have

$$\begin{aligned}&\big(\dot X_0(t)+\epsilon\dot X_1(t)+\epsilon^2\dot X_2(t)+\cdots\big)\\&\quad=\dot X(t,\epsilon)\\&\quad=(A_0+\epsilon\cdot A_1(t)X(t,\epsilon)\\&\quad=A_0X_0(t)+\epsilon\big(A_0X_1(t)+A_1(t)X_0(t)\big)+\epsilon^2\big(A_0X_2(t)+A_1(t)X_1(t)\big)+\cdots.\end{aligned}$$

Since $X(t,\epsilon)$ is a principal fundamental solution, $X(0,\epsilon)=I$ for all ϵ.

Letting $\epsilon\to 0$, we see that $X_0(0)=I$, so that $X_i(0)=0$ for all $i\geq 1$.

Hence, we obtain the following system of differential equations:

$$\dot X_0(t)=A_0X_0(t), \qquad X_0(0)=I, \tag{13}$$

$$\dot X_1(t)=A_0X_1(t)+A_1(t)X_0(t), \qquad X_1(0)=0, \tag{14}$$

$$\dot X_2(t)=A_0X_2(t)+A_1(t)X_1(t), \qquad X_2(0)=0. \tag{15}$$

We note that

$$e^{A_0 t} = \begin{pmatrix} 1 & 0 \\ 0 & e^{-bt} \end{pmatrix}, \qquad e^{-A_0 t} = \begin{pmatrix} 1 & 0 \\ 0 & e^{bt} \end{pmatrix}.$$

The solutions to (13), (14), and (15) at $t = \tau$ (respectively) are:

$$X_0(\tau) = e^{A_0 \tau},$$
$$X_1(\tau) = e^{A_0 \tau} \int_0^\tau e^{-A_0 t} A_1(t) e^{A_0 t}\, dt,$$
$$X_2(\tau) = e^{A_0 \tau} \int_0^\tau e^{-A_0 t} A_1(t) e^{A_0 t} \left(\int_0^t e^{-A_0 s} A_1(s) e^{A_0 s}\, ds \right) dt.$$

To investigate the eigenvalues of $X(\tau, \epsilon)$, we consider the characteristic polynomial of $X(\tau, \epsilon)$

$$F(\lambda, \epsilon) = \lambda^2 - \operatorname{tr} X(\tau, \epsilon)\lambda + \det X(\tau, \epsilon). \tag{16}$$

We let $\lambda_2(\epsilon)$ and $\lambda_3(\epsilon)$ be the eigenvalues of $X(\tau, \epsilon)$. In agreement with previous notation, $\lambda_2(\epsilon)$ is the largest eigenvalue, which we know is $\rho(X(\tau, \epsilon))$. At $\epsilon = 0$ the eigenvalues are $\lambda_2 = 1$ and $\lambda_3 = e^{-b\tau}$ since $X(\tau, 0) = e^{A_0 \tau}$. The eigenvalues of $X(\tau, \epsilon)$, $\lambda_2(\epsilon)$, and $\lambda_3(\epsilon)$, are analytic functions of ϵ in some neighborhood of $\epsilon = 0$ since all of the hypotheses in the Implicit Function theorem are met:

$$\frac{\partial F}{\partial \lambda}(1, 0) \neq 0, \quad \text{and} \quad \frac{\partial F}{\partial \lambda}\left(e^{-b\tau}, 0\right) \neq 0,$$
$$\frac{\partial F}{\partial \lambda}(1, 0) = 2 - \operatorname{tr} X(\tau, 0) = 2 - \operatorname{tr}\left(e^{A_0 \tau}\right) = 2 - \left(1 + e^{-b\tau}\right) = 1 - e^{-b\tau} > 0,$$

and

$$\frac{\partial F}{\partial \lambda}\left(e^{-b\tau}, 0\right) = e^{-b\tau} - \operatorname{tr} X(\tau, 0) = e^{-b\tau} - 1 < 0.$$

So, there exists $\epsilon_0 > 0$ such that $\lambda_2(\epsilon)$ and $\lambda_3(\epsilon)$ are analytic functions in $B(0, \epsilon_0)$, where $B(0, \epsilon_0)$ is the ball centered around 0 with radius ϵ_0. Therefore,

$$\lambda_2(\epsilon) = 1 + \lambda_2'(0)\epsilon + \frac{1}{2}\lambda_2''(0)\epsilon^2 + O\left(\epsilon^3\right),$$
$$\lambda_3(\epsilon) = e^{-(\beta+\gamma)\tau} + O(\epsilon),$$

where

$$\lambda_2'(0) := \frac{d\lambda}{d\epsilon}(1, 0) \quad \text{and} \quad \lambda_2''(0) := \frac{d^2\lambda}{d\epsilon^2}(1, 0).$$

For small enough ϵ, $\lambda_3(\epsilon)$ stays inside the unit disk. But it is not clear whether $\lambda_2(\epsilon)$ goes inside or outside the unit disk for small ϵ. The sign of $\lambda_2'(0)$ determines whether $\lambda_2(\epsilon) < 1$ or $\lambda_2(\epsilon) > 1$ for sufficiently small $\epsilon > 0$. If $\lambda_2'(0) < 0$, then $\lambda_2(\epsilon) < 1$,

and hence $\rho(X(\tau,\epsilon)) < 1$. If $\lambda_2'(0) > 0$, then $\lambda_2(\epsilon) > 1$, and hence $\rho(X(\tau,\epsilon)) < 1$. If $\lambda_2'(0) = 0$, then we need to look at higher order derivatives. Hence, we begin by computing $\lambda_2'(0)$.

$$\lambda_2'(0) = \left.\frac{-\partial F/\partial\epsilon}{\partial F/\partial\lambda}\right|_{(1,0)}, \qquad \lambda_2'(0) = -\frac{[\frac{d}{d\epsilon}(\det X(\tau,\epsilon) - \operatorname{tr} X(\tau,\epsilon))]_{\epsilon=0}}{1-e^{-b\tau}}.$$

Now

$$\begin{aligned}&\left[\frac{d}{d\epsilon}\big(\det X(\tau,\epsilon) - \operatorname{tr} X(\tau,\epsilon)\big)\right]_{\epsilon=0}\\ &\quad= \det\big(e^{A_0\tau}\big)\frac{d}{d\epsilon}\det\big(I+\epsilon C+\epsilon^2 E+O\big(\epsilon^3\big)\big)\Big|_{\epsilon=0} - \operatorname{tr} X_1(\tau),\end{aligned}$$

where

$$\begin{aligned}C &= \int_0^\tau e^{-A_0t}A_1(t)e^{A_0t}\,dt,\\ E &= \int_0^\tau e^{-A_0t}A_1(t)e^{A_0t}\left(\int_0^t e^{-A_0s}A_1(s)e^{A_0s}\,ds\right)dt,\\ \operatorname{tr} X_1(\tau) &= \operatorname{tr}\big(e^{A_0\tau}C\big) = \operatorname{tr}\left(\begin{pmatrix}1 & 0\\ 0 & e^{-b\tau}\end{pmatrix}C\right) = C_{11} + e^{-b\tau}C_{22},\\ &\det\big(e^{A_0\tau}\big)\frac{d}{d\epsilon}\det\big(I+\epsilon C+\epsilon^2E+O\big(\epsilon^3\big)\big)\Big|_{\epsilon=0}\\ &\quad= e^{-b\tau}\frac{d}{d\epsilon}\det\left(\begin{pmatrix}1+\epsilon C_{11}+O(\epsilon^2) & \epsilon C_{12}+O(\epsilon^2)\\ \epsilon C_{21}+O(\epsilon^2) & 1+\epsilon C_{22}+O(\epsilon^2)\end{pmatrix}\right)\Big|_{\epsilon=0}\\ &\quad= e^{-b\tau}(C_{11}+C_{22}).\end{aligned}$$

Combining the above results, we obtain

$$\lambda_2'(0) = \frac{C_{11}+e^{-b\tau}C_{22}-e^{-b\tau}(C_{11}+C_{22})}{1-e^{-b\tau}} = \frac{C_{11}(1-e^{-b\tau})}{1-e^{-b\tau}} = C_{11}. \tag{17}$$

In what follows, we will need the following quantity:

$$\begin{aligned}e^{-A_0t}A_1(t)e^{A_0t} &= \varphi_{\mathrm{RT}}(t)e^{-A_0t}A_{\mathrm{RT}}e^{A_0t} + \varphi_P(t)e^{-A_0t}A_Pe^{A_0t}\\ &= \varphi_{\mathrm{RT}}(t)\begin{pmatrix}1 & 0\\ 0 & e^{bt}\end{pmatrix}A_{\mathrm{RT}}\begin{pmatrix}1 & 0\\ 0 & e^{-bt}\end{pmatrix}\\ &\quad+\varphi_P(t)\begin{pmatrix}1 & 0\\ 0 & e^{bt}\end{pmatrix}A_P\begin{pmatrix}1 & 0\\ 0 & e^{-bt}\end{pmatrix}\\ &= \varphi_{\mathrm{RT}}(t)\frac{MkT_0}{1+a}\begin{pmatrix}a & -e^{-bt}\\ a^2e^{bt} & -a\end{pmatrix}\end{aligned}$$

$$+\varphi_P(t)\frac{\beta}{(1+a)(1-e_P)}\begin{pmatrix}1 & e^{-bt}\\ -e^{bt} & -1\end{pmatrix}. \tag{18}$$

Hence, the matrix C is given by

$$C=\frac{1}{1+a}\int_0^\tau \varphi_{\mathrm{RT}}(t)N(1-e_P)kT_0\begin{pmatrix}a & -e^{-bt}\\ a^2e^{bt} & -a\end{pmatrix}$$
$$+\varphi_P(t)\frac{\beta}{1-e_P}\begin{pmatrix}1 & e^{-bt}\\ -e^{bt} & -1\end{pmatrix}dt.$$

From which it follows that

$$\lambda_2'(0)=\frac{1}{1+a}\left(\overline{\varphi_{\mathrm{RT}}}kT_0N(1-e_P)a+\frac{\overline{\varphi_P}\beta}{1-e_P}\right),$$

which proves Proposition 4. □

Proof of Proposition 5 Let $\overline{\varphi_{\mathrm{RT}}}=0$ and $\overline{\varphi_P}=0$. We evaluate the second derivative of $\lambda_2(\epsilon)$ with respect to ϵ:

$$\lambda_2''(0):=\frac{d^2\lambda}{d\epsilon^2}(1,0)=\frac{\left[\frac{d^2}{d\epsilon^2}(\operatorname{tr}X(\tau,\epsilon)-\det X(\tau,\epsilon))\right]_{\epsilon=0}}{1-e^{-b\tau}},$$

where

$$\left[\frac{d^2}{d\epsilon^2}\big(\operatorname{tr}X(\tau,\epsilon)-\det X(\tau,\epsilon)\big)\right]_{\epsilon=0}$$
$$=2\operatorname{tr}X_2(\tau)-\det\big(e^{A_0\tau}\big)\frac{d}{d\epsilon}\det\big(I+\epsilon C+\epsilon^2E+O\big(\epsilon^3\big)\big)\Big|_{\epsilon=0}$$
$$=2\operatorname{tr}\big(e^{A_0\tau}E\big)-\det\big(e^{A_0\tau}\big)\frac{d^2}{d\epsilon^2}\det\big(I+\epsilon C+\epsilon^2E+O\big(\epsilon^3\big)\big)\Big|_{\epsilon=0}$$
$$=2\big(E_{11}+e^{-b\tau}E_{22}\big)-e^{-b\tau}(2E_{11}+2E_{22}-2C_{12}C_{21})$$
$$=2\big(E_{11}\big(1-e^{-b\tau}\big)+e^{-b\tau}C_{12}C_{21}\big).$$

Therefore,

$$\lambda_2''(0)=2\left(E_{11}+\frac{e^{-b\tau}}{1-e^{-b\tau}}C_{12}C_{21}\right), \tag{19}$$

which concludes the proof. □

Proof of Proposition 6 We prove this theorem in 3 steps. First, let us assume that $\varphi_{\mathrm{RT}}(t)=\sin t$ and $\varphi_P=0$ in (6). Then

$$E=\int_0^{2\pi}\frac{kT_0\sin t}{1+a}\begin{pmatrix}Ma & -Me^{-bt}\\ * & *\end{pmatrix}\int_0^t\frac{kT_0\sin s}{1+a}\begin{pmatrix}Ma & *\\ Ma^2e^{bs} & *\end{pmatrix}ds\,dt$$

$$= \frac{(kT_0)^2}{(1+a)^2}\int_0^{2\pi} \sin t \begin{pmatrix} Ma & -Me^{-bt} \\ * & * \end{pmatrix} \begin{pmatrix} Ma(1-\cos t) & * \\ Ma^2\int_0^t e^{bs}\sin s\,ds & * \end{pmatrix} dt,$$

where asterisks denote nonessential entries. We find that

$$\begin{aligned} E_{11} &= \frac{-(kT_0Ma)^2}{(1+a)^2}\int_0^{2\pi}\left(\sin t(\cos t - 1)\right. \\ &\quad \left. + (\sin t)e^{-bt}\left(\frac{be^{bt}\sin t - e^{bt}\cos t + 1}{b^2+1}\right)\right)dt \\ &= \frac{-(kT_0Ma)^2}{(1+a)^2(b^2+1)}\int_0^{2\pi} b\sin^2 t + e^{-bt}\sin t\,dt \\ &= \frac{-(kT_0Ma)^2}{(1+a)^2(b^2+1)^2}\big(\pi b\big(b^2+1\big) + 1 - e^{-2\pi b}\big), \\ C_{12}C_{21} &= \frac{-(kT_0Ma)^2}{(1+a^2)}\left(\int_0^{2\pi} e^{-bt}\sin t\,dt\right)\left(\int_0^{2\pi} e^{bt}\sin t\,dt\right) \\ &= \frac{-(kT_0Ma)^2}{(1+a)^2(b^2+1)^2}\big(1-e^{-2\pi b}\big)\big(1-e^{2\pi b}\big). \end{aligned}$$

So,

$$\frac{e^{-b\tau}}{1-e^{-b\tau}}C_{12}C_{21} = \frac{-(kT_0Ma)^2}{(1+a)^2(b^2+1)^2}\big(e^{-2\pi b}-1\big).$$

Therefore, by Proposition 5,

$$\lambda_2''(0) = \frac{-(kT_0Ma)^2}{(1+a)^2(b^2+1)^2}\big(\pi b\big(b^2+1\big)\big). \tag{20}$$

Next, suppose that $\varphi_{\mathrm{RT}} = 0$ and $\varphi_P(t) = \sin t$ in (6). It follows that

$$\begin{aligned} &\frac{e^{-b2\pi}}{1-e^{-b2\pi}}C_{12}C_{21} \\ &\quad = \left(\frac{e^{-b2\pi}}{1-e^{-b2\pi}}\right)\frac{-\beta}{(1-e_P)^2(1+a^2)}\left(\int_0^{2\pi} e^{-bt}\sin t\,dt\right)\left(\int_0^{2\pi} e^{bt}\sin t\,dt\right) \\ &\quad = \left(\frac{e^{-b2\pi}}{1-e^{-b2\pi}}\right)\frac{-\beta}{(1-e_P)^2(1+a)^2(b^2+1)^2}\big(1-e^{-2\pi b}\big)\big(1-e^{2\pi b}\big) \\ &\quad = \frac{-\beta}{((1-e_P)(1+a))^2(b^2+1)^2}\big(e^{-2\pi b}-1\big), \\ E &= \int_0^{2\pi}\beta\frac{\sin t}{(1+a)(1-e_P)}\begin{pmatrix} 1 & e^{-bt} \\ * & * \end{pmatrix}\int_0^t \frac{\beta\sin s}{(1+a)(1-e_P)}\begin{pmatrix} Na & * \\ -e^{bs} & * \end{pmatrix} ds\,dt. \end{aligned}$$

From the computation involved in evaluating (20), it can be seen that

$$E_{11} = \frac{-\beta^2}{((1-e_P)(1+a))^2(b^2+1)^2}\big(\pi b\big(b^2+1\big)+1-e^{-2\pi b}\big),$$

and by Proposition 5 we obtain

$$\lambda_2''(0) = \frac{-\beta^2}{(1-e_P)^2(1+a)^2(b^2+1)^2}\big(\pi b\big(b^2+1\big)\big). \tag{21}$$

Finally, we let $\varphi_{\mathrm{RT}} = \alpha_1 \sin t$, $\varphi_P = \alpha_2 \sin t$ in (6) where $\alpha_1, \alpha_2 \in \mathbb{R}$. Then

$$\begin{aligned}
C &= \int_0^{2\pi} \big((\alpha_1 \sin t)e^{-A_0 t} A_{\mathrm{RT}} e^{A_0 t} + (\alpha_2 \sin t)e^{-A_0 t} A_P e^{A_0 t}\big)\, dt \\
&= \frac{1}{1+a}\int_0^{2\pi} \Big(\alpha_1 k T_0 \begin{pmatrix} * & -Me^{-bt}\sin t \\ Ma^2 e^{bt}\sin t & * \end{pmatrix} \\
&\quad + \frac{\alpha_2 \beta}{1-e_P}\begin{pmatrix} * & e^{-bt}\sin t \\ -e^{bt}\sin t & * \end{pmatrix}\Big)\, dt, \\
E &= \frac{1}{(1+a)^2}\int_0^{2\pi} \left\{\left[k T_0 \alpha_1 \sin t \begin{pmatrix} Ma & -Me^{-bt} \\ * & * \end{pmatrix} + \frac{\beta\alpha_2 \sin t}{1-e_P}\begin{pmatrix} 1 & e^{-bt} \\ * & * \end{pmatrix}\right]\right. \\
&\quad \times \left[k T_0 \alpha_1 \begin{pmatrix} Ma(1-\cos t) & * \\ Ma^2 \int_0^t e^{bs}\sin s\, ds & * \end{pmatrix}\right. \\
&\quad \left.\left. + \frac{\beta\alpha_2}{1-e_P}\begin{pmatrix} (1-\cos t) & * \\ \int_0^t e^{bs}\sin s\, ds & * \end{pmatrix}\right]\right\} dt.
\end{aligned}$$

Using (20), (21), and Proposition 5, it can be seen that

$$\begin{aligned}
\lambda_2''(0) &= \frac{\pi b}{(1+a)^2(b^2+1)}\left[-(\alpha_1 k T_0 Ma)^2 - \frac{(\alpha_2\beta)^2}{(1-e_P)^2} + \alpha_1\alpha_2 k T_0 N\beta\big(1+a^2\big)\right] \\
&= \frac{\pi b}{(1+a)^2(b^2+1)}\beta^2\alpha_1\alpha_2\left[-R_0\left(\frac{\alpha_1}{\alpha_2}(1-e_P)^2 + \frac{\alpha_2}{\alpha_1}(1-e_{\mathrm{RT}})^2\right)\right. \\
&\quad \left. + \left(\frac{\gamma}{\beta} + \frac{\beta}{\gamma}\right)\right],
\end{aligned}$$

which concludes the proof. □

Proof of Proposition 7 Let $\Gamma(t)$ be a PFS (principal fundamental solution) to (12). Let $\Phi(t)$ be a PFS to: $\dot{x} = B(t+\psi_1, \psi_1, \psi_2)x = B(t, 0, (\psi_2 - \psi_1)\text{modulo } \tau)x$. Then $\widetilde{\Phi}(t) := \Phi(t-\psi_1)$ is a FS to (12) with $\widetilde{\Phi}(\psi_1) = I$. Using Floquet's theorem, we obtain: $\Phi(\tau) = \widetilde{\Phi}(\tau+\psi_1) = \Gamma(\tau+\psi_1)\widetilde{\Phi}(0) = \Gamma(\psi_1)\Gamma(\tau)\widetilde{\Phi}(0) = \widetilde{\Phi}(\psi_1)\widetilde{\Phi}^{-1}(0)\Gamma(\tau)\widetilde{\Phi}(0) = \widetilde{\Phi}^{-1}(0)\Gamma(\tau)\widetilde{\Phi}(0)$. Hence, $\lambda_2(\psi_1, \psi_2) = \lambda_2(0, (\psi_2 - \psi_1)\text{modulo } \tau)$. □

Proof of Theorem 8 Let $X(t, \psi)$ be the principal fundamental solution of

$$\dot{x} = \begin{pmatrix} -\beta & k(1-\eta_{\mathrm{RT}}(t))T_0 \\ N(1-\eta_P(t-\psi))\beta & -\gamma \end{pmatrix} x.$$

The optimal phase difference ψ^* where $0 \le \psi < \tau$ is the ψ for which the spectral radius of $X(\tau, \psi)$, λ_2, is minimized. The characteristic equation of this matrix is

$$F(\lambda, \psi) = \lambda^2 - \operatorname{tr} X(\tau, \psi)\lambda + \det X(\tau, \psi) = 0.$$

We note that

$$\frac{\partial F}{\partial \lambda}(\lambda_2, \psi) = 2\lambda_2 - \operatorname{tr} X(\tau, \psi) > 0, \tag{22}$$

since λ_2 is the spectral radius of $X(\tau, \psi)$. Therefore, we can implicitly differentiate with respect to ψ at ψ^*:

$$\lambda_2\lambda_2' - \left(\lambda_2 \frac{d}{d\psi} \operatorname{tr} X(\tau, \psi) + \operatorname{tr} X(\tau, \psi)\lambda_2'\right) + \frac{d}{d\psi} \det X(\tau, \psi) = 0.$$

By applying Liouville's theorem, we find that $\frac{d}{d\psi} \det X(\tau, \psi) = 0$. Also, we are differentiating at ψ^*, so $\lambda_2' = 0$.

Hence, $\frac{d}{d\psi} \operatorname{tr} X(\tau, \psi) = 0$. Now we show that $\frac{d^2}{d\psi^2} \operatorname{tr} X(\tau, \psi) > 0$. Taking the second implicit derivative of λ_2 with respect to ψ at ψ^* and substituting $\lambda_2' = 0$ and $\frac{d}{d\psi} \det X(\tau, \psi) = 0$, we obtain

$$\lambda_2''\big(2\lambda_2 - \operatorname{tr} X(\tau, \psi)\big) - \frac{d^2}{d\psi^2} \operatorname{tr} X(\tau, \psi)\lambda_2 = 0.$$

Since $\lambda_2'' > 0$ and by (22), we see $\frac{d^2}{d\psi^2} \operatorname{tr} X(\tau, \psi) > 0$.

So, ψ^* is where $\operatorname{tr} X(\tau, \psi)$ is minimized. □

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