Non-exponential delays in the influenza infection cycle: evidence from in vitro experiments

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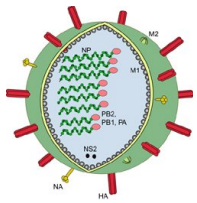
July 20, 2010

RYERSON UNIVERSITY

Spectrum of influenza research/investigation

microscopic and controlled ⟷ **macroscopic and natural**

virions



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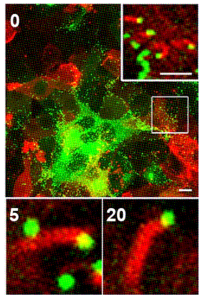

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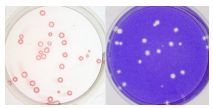

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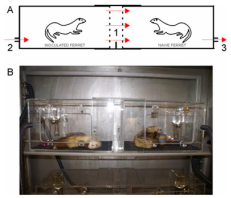

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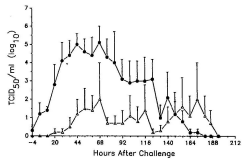

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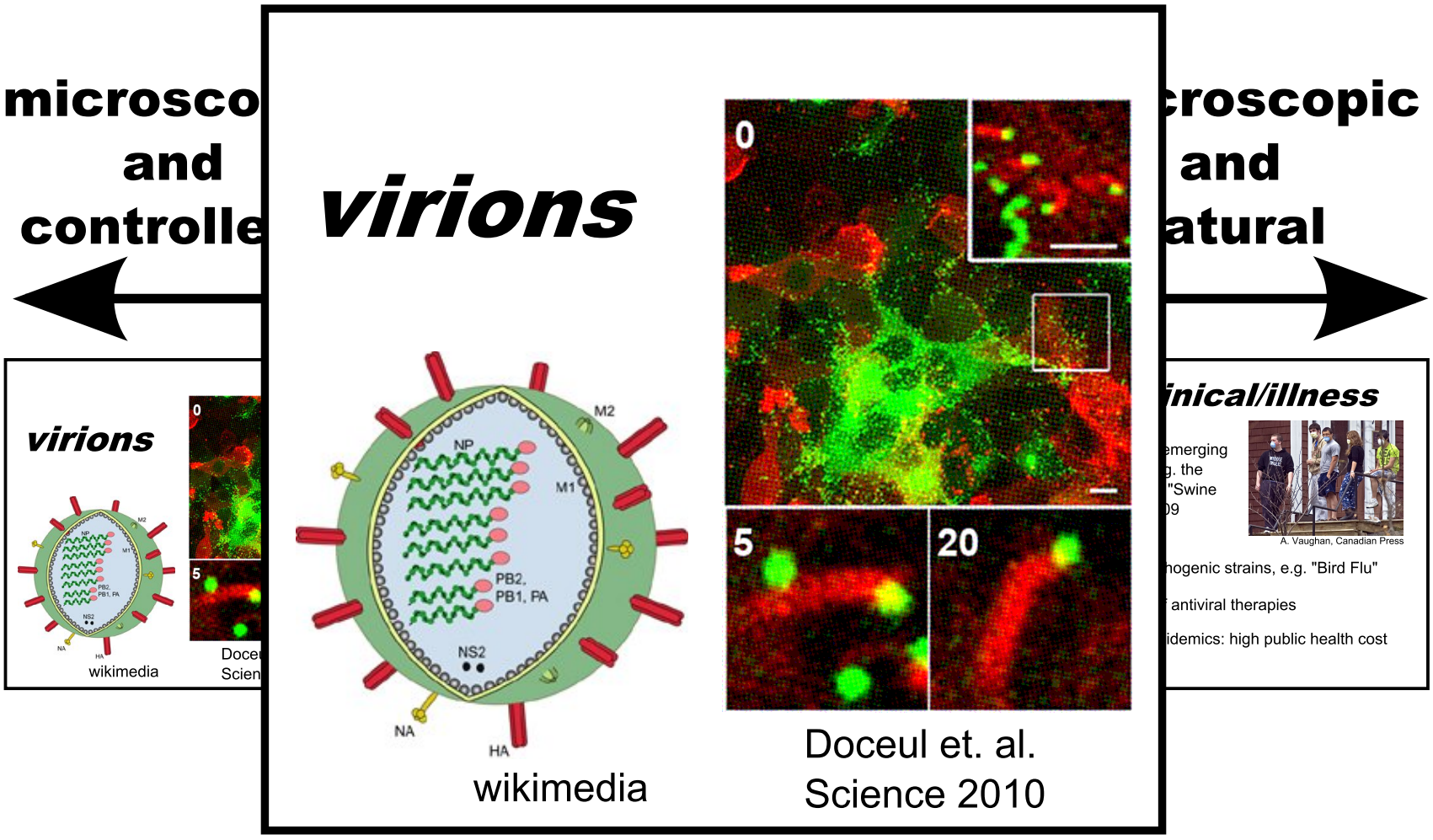

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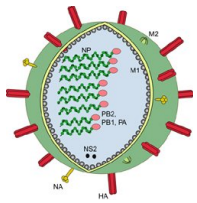

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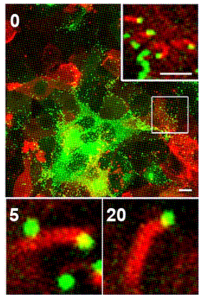

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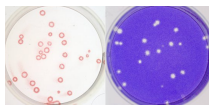

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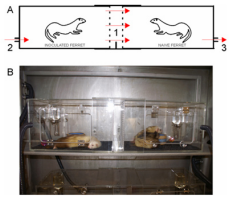

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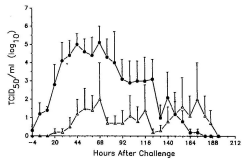

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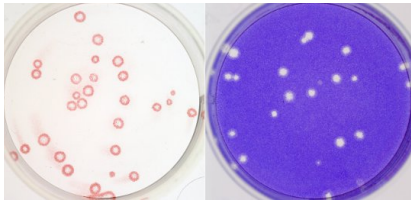

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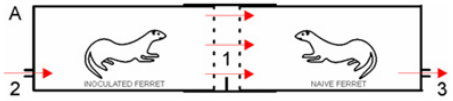

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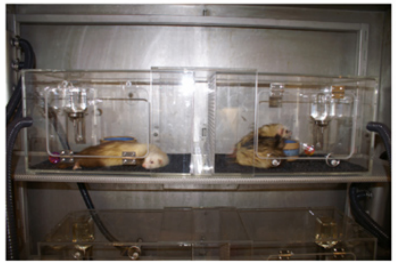

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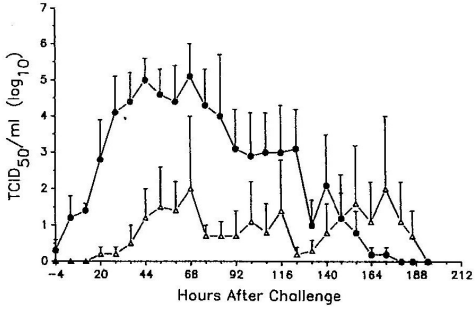

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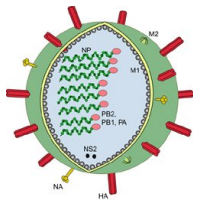

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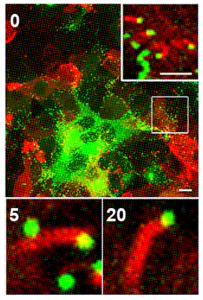

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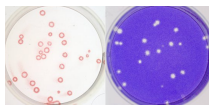

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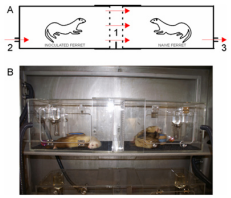

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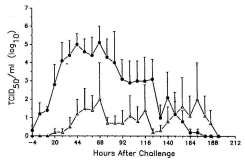

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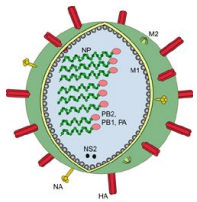

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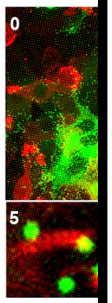

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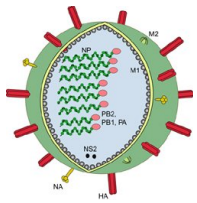

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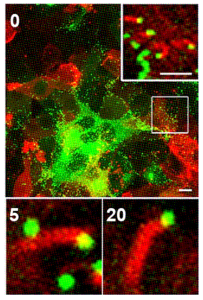

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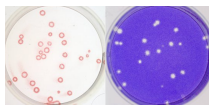

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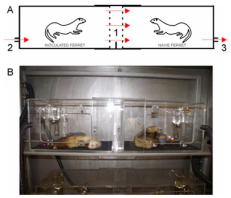

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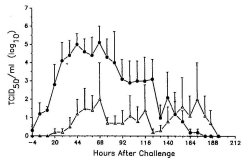

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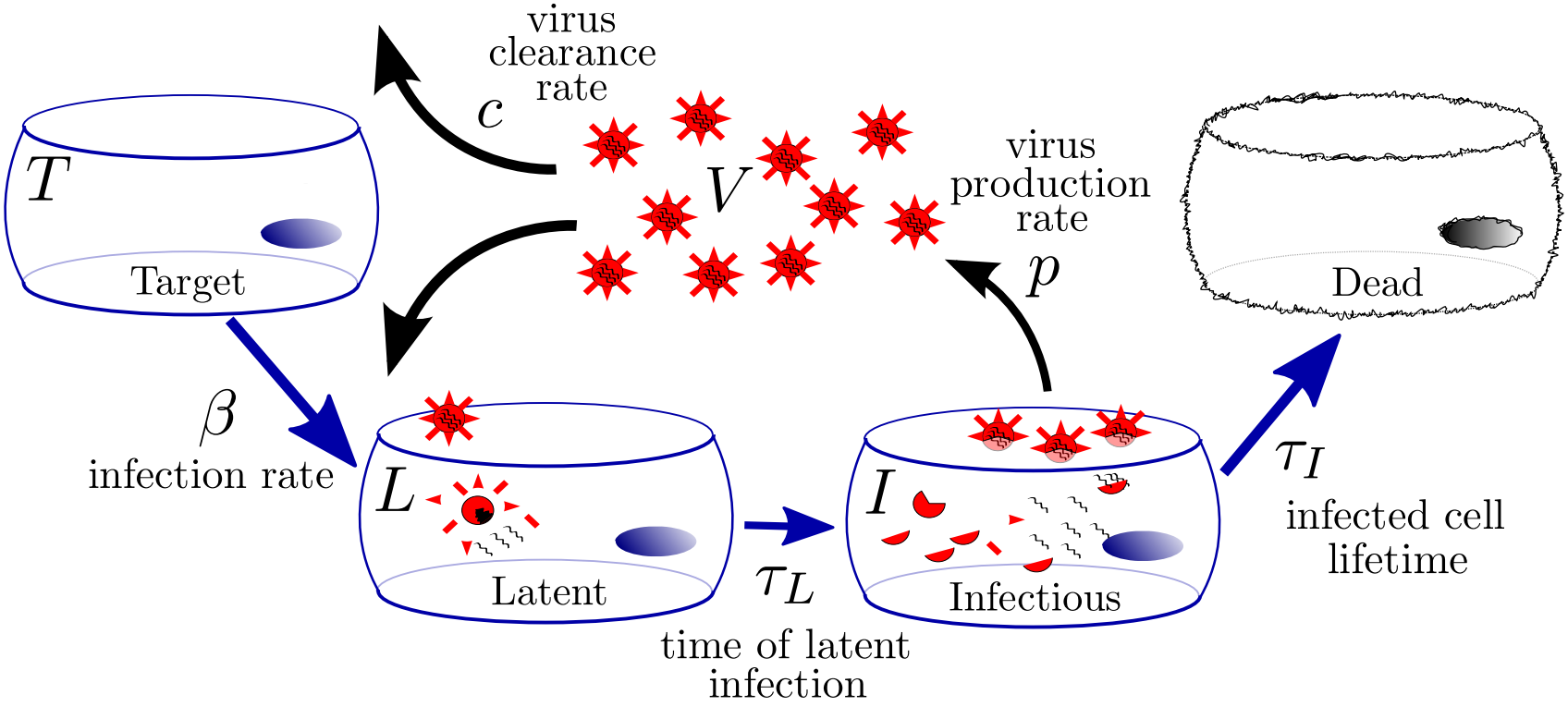
Basic viral infection model & Philosophy



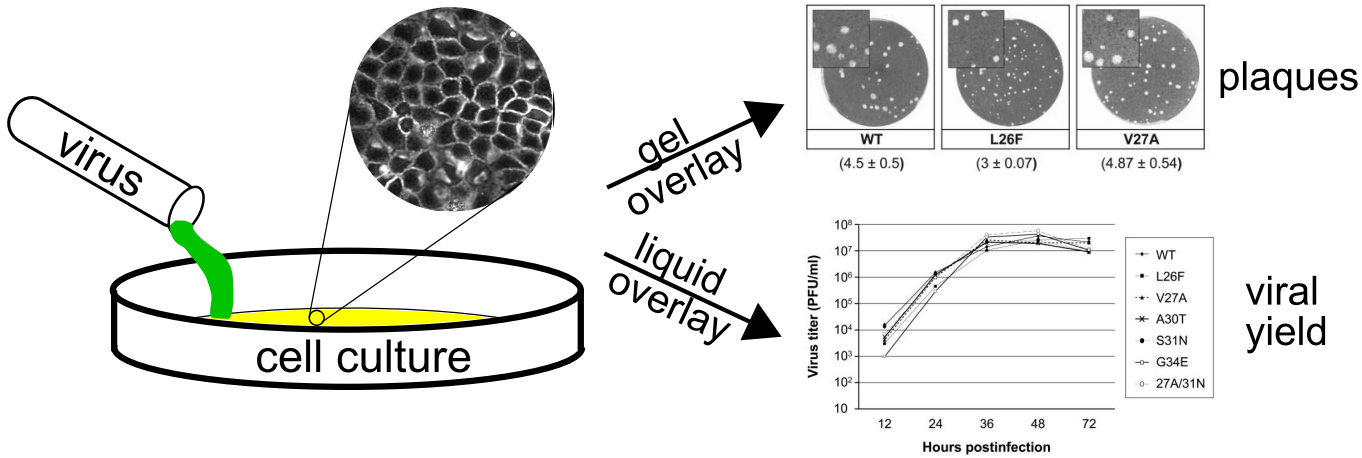
- Dependence on only a few key parameters of the virus-cell interaction.
- Knowledge of the parameter values, together with a dynamical model, yield complete information about infections of any scale.
- Basic problems:
  1. Can parameters be reliably extracted from noisy and limited data? (parameter identifiability).
  2. Is the model is correct? (reproduces data in *broad range* of scenarios)

A particular application: Drug-resistant viruses

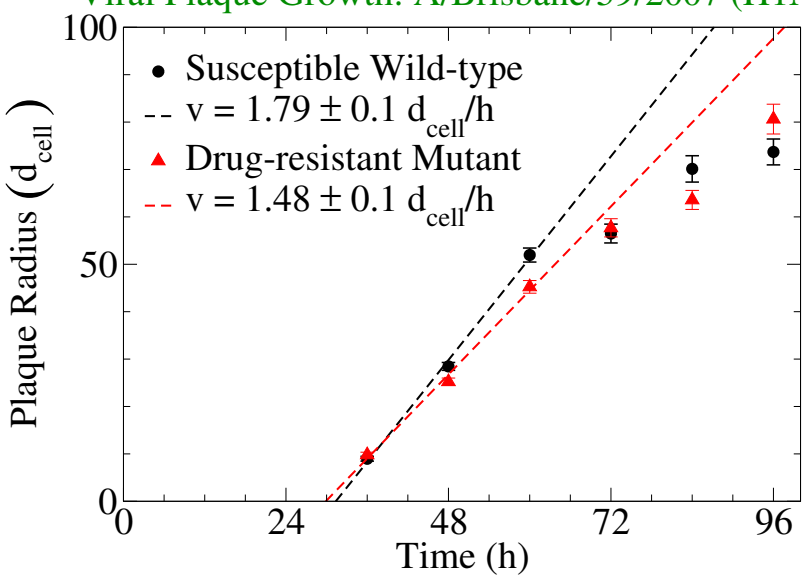
- In the last flu season (2008–2009), nearly 100% of seasonal virus strains were found resistant to Oseltamivir (Tamiflu).
- This was a surprise because it had been thought that the mutation conferring resistance (NA-H275Y) also reduced the viral *fitness*.
- We consider the laboratory replication capacity of a pair of viruses: one susceptible and one resistant to the drug.
- **Question**: What kinetic differences underlie the differences in strain genotype and fitness?

Experimental Overview

Two complementary views of virus replication:

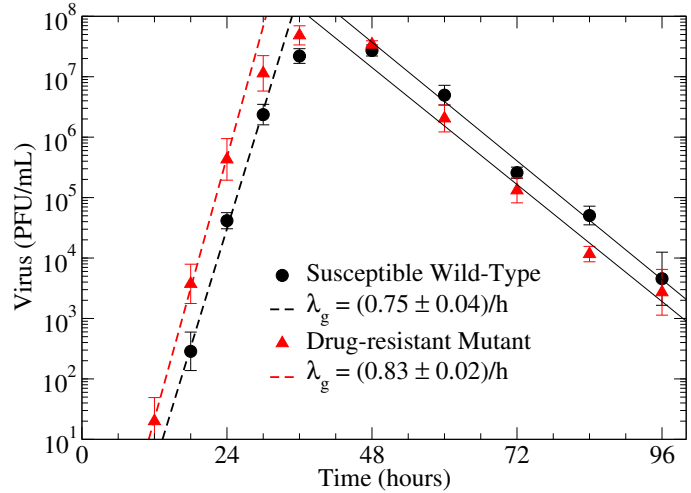


Viral Plaque Growth: A/Brisbane/59/2007 (H1N1)



→ Plaque Velocity, v_{plaque}

Viral Yield: A/Brisbane/59/2007 (H1N1)



→ Exp. Growth rate, λ_g

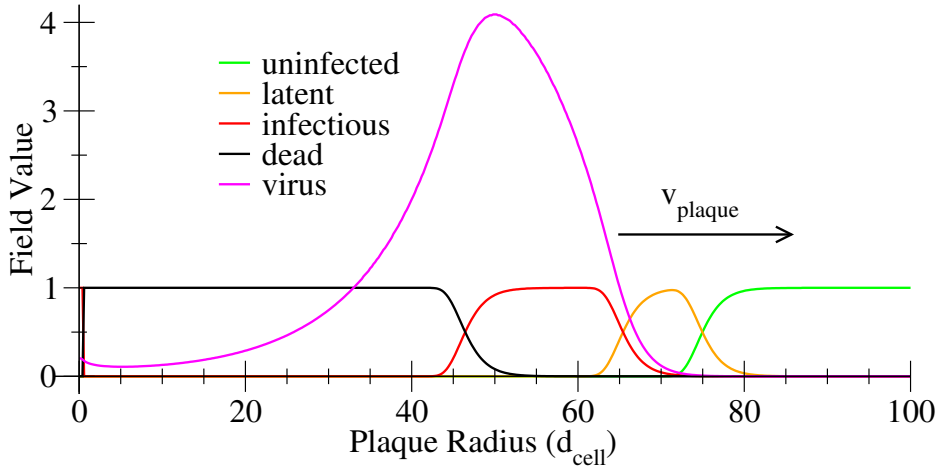
Models for Growth

Plaques: Reaction diffusion PDE with delay

$$\frac{\partial T}{\partial t} = -\beta T V$$

$$\frac{\partial I}{\partial t} = \beta T(t-\tau_L)V(t-\tau_L) - \beta T(t-\tau_L-\tau_I)V(t-\tau_L-\tau_I)$$

$$\frac{\partial V}{\partial t} = D\nabla^2 V + pI - cV = D\left(\frac{\partial^2 V}{\partial r^2} + \frac{1}{r}\frac{\partial V}{\partial r}\right) + pI - cV$$



Yield: Delay Differential Equations

$$\dot{T} = -\beta V T$$

$$\dot{I} = \beta V(t-\tau_L)\,T(t-\tau_L) - \beta V(t-\tau_L-\tau_I)\,T(t-\tau_L-\tau_I)$$

$$\dot{V} = pI/N_{\text{cells}} - cV$$

Assuming $T(t) = T_0$, $I(t) = I_0 e^{\lambda_g t}$, $V(t) = e^{\lambda_g t}$ for early times, growth rate is:

$$\lambda_g^2 + c\lambda_g = p\beta \exp(-\lambda_g \tau_L) .$$

Parameter reduction

- We have two experimental measures and 5 unknown parameters.
- The plaque velocity and the exponential growth rate depend on the rates of infection and production only through the quantity $p\beta$.

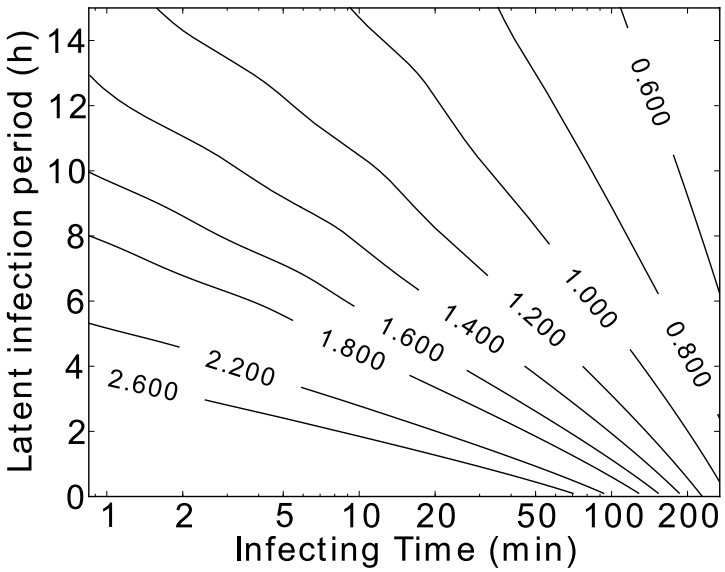
  Therefore we define a new characteristic time

  $$t_{\text{infecting}} \equiv \sqrt{\frac{2}{p\beta}}$$

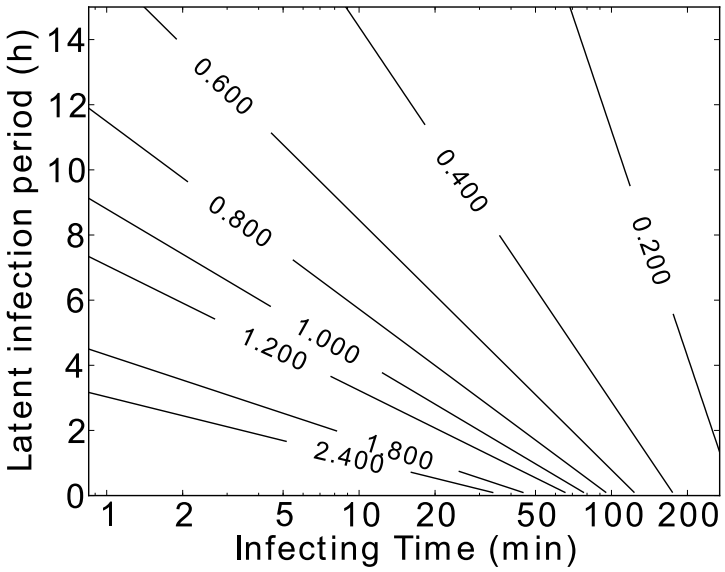
  which is the *time for one infectious cell to cause the latent infection of one more*, in a completely susceptible population.
- In model dynamics, the infectious lifespan plays only a very minor role, we fix it to $\tau_I = 12\ \text{h}$.
- Viral clearance c is extracted from the decay rate in viral yield experiments.
- Each experimental measure now depends only on:
  latent infection period and **infecting time**.

Model Results

Plaque Velocity (d_{cell}/h)

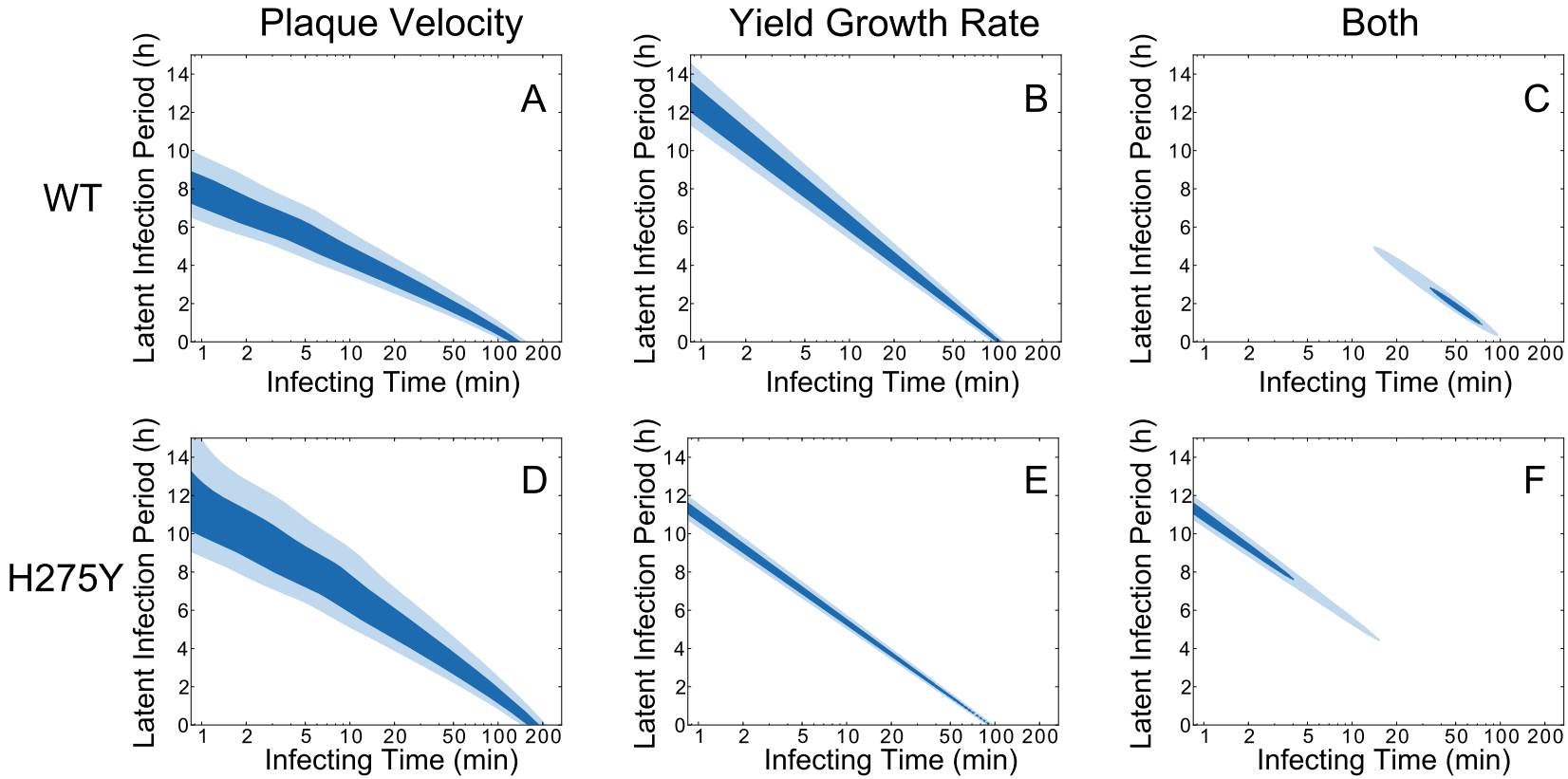


Exp. Growth Rate (h^{-1})



- Plaque velocity from simulation of delayed PDE model.
- Yield exponential growth rate from solution of transcendental equation (based on DDE).

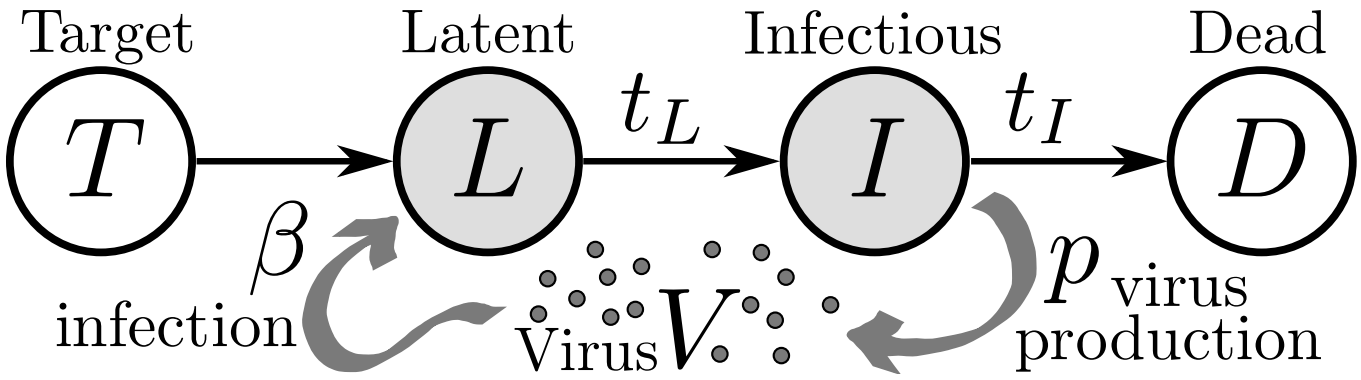
Application to A/Brisbane (H1N1) Susceptible-Resistant Strain Pair



- Oseltamivir-resistant mutant (NA-H275Y) has **longer latent infection period** but a **shorter infecting time** (higher infectivity).
- Consistent with recent result [Bloom et. al., Science 2010] showing enhanced surface neuraminidase expression (compared to earlier seasonal strains), but less for H275Y:
  less NA $\rightarrow$ more sticky (longer release but easier attachment)

BUT . . . solution to plaque+yield is not unique!

- Consider delays in transition out of infected states (grey)



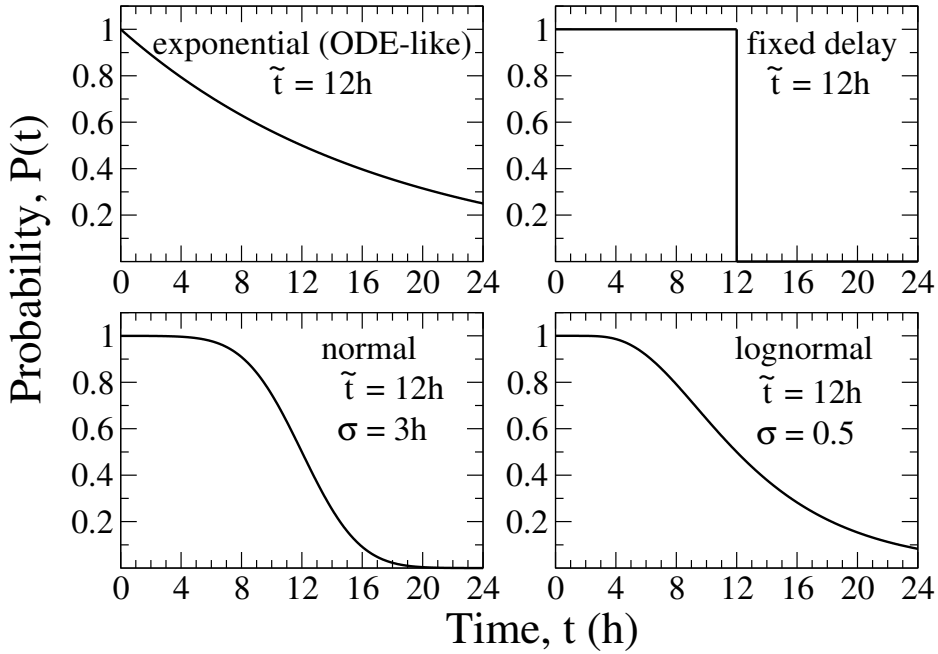
- We selected t_L and t_I from a Dirac-delta distribution (fixed delay).
- Models with alternatively distributed delays (e.g., exponential (PDE/ODE), normal, lognormal) can also reproduce plaque and viral yield dynamics.
- Basic result is robust, but specific parameter values change.
- In order to apply results to other systems, parameters must be correctly identified.
- Need:
  1. Model for generic delays
  2. Experimental system which allows for differentiation between models.

A model for generic delays

- Introduced originally for epidemiological models [Kermack & McKendrick 1927, Hethcote & Tudor 1980]
- Utilizes arbitrary pdf of state lifetime, $f(t)$, and

$$P(t) = 1 - \int_{-\infty}^{t} f(s)ds$$

the probability of remaining in that state for at least t.



$$\dot{T} = -\beta\, T\, V$$

$$L(t) = L(0)\, P_L(t) + \int_0^t \beta\, T(s)\, V(s)\, P_L(t-s)\, ds$$

$$I(t) = I(0)\, P_I(t) + \int_0^t P_I(t-s)\, L_0\, f_L(s)\, ds$$

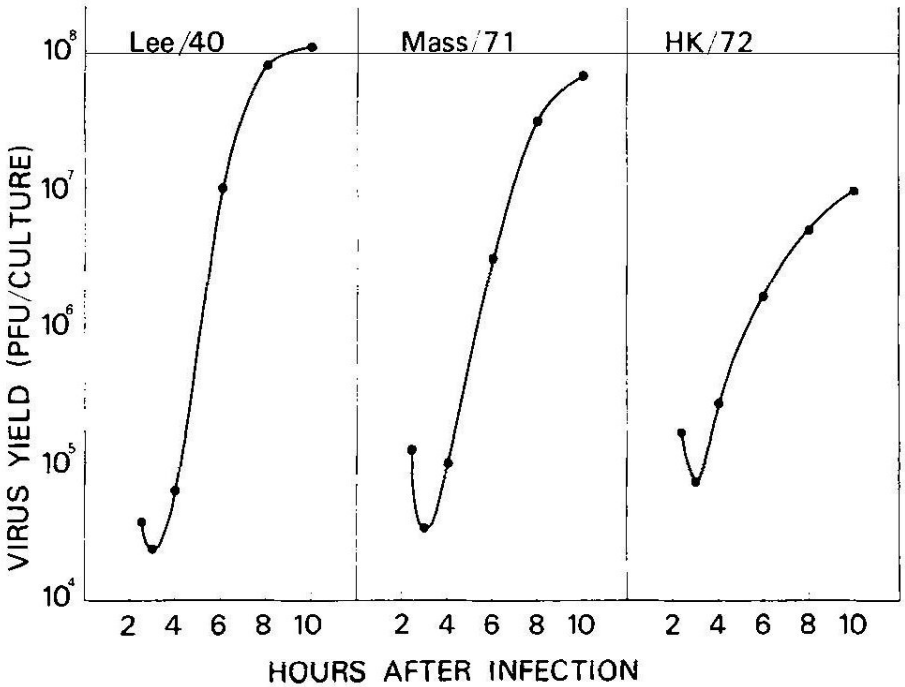
$$+ \int_{s_2=0}^{t} \int_{s_1=0}^{s_2} \beta\, T(s_1)\; V(s_1) \cdot f_L(s_2 - s_1) \cdot P_I(t - s_2)\; ds_1\, ds_2$$

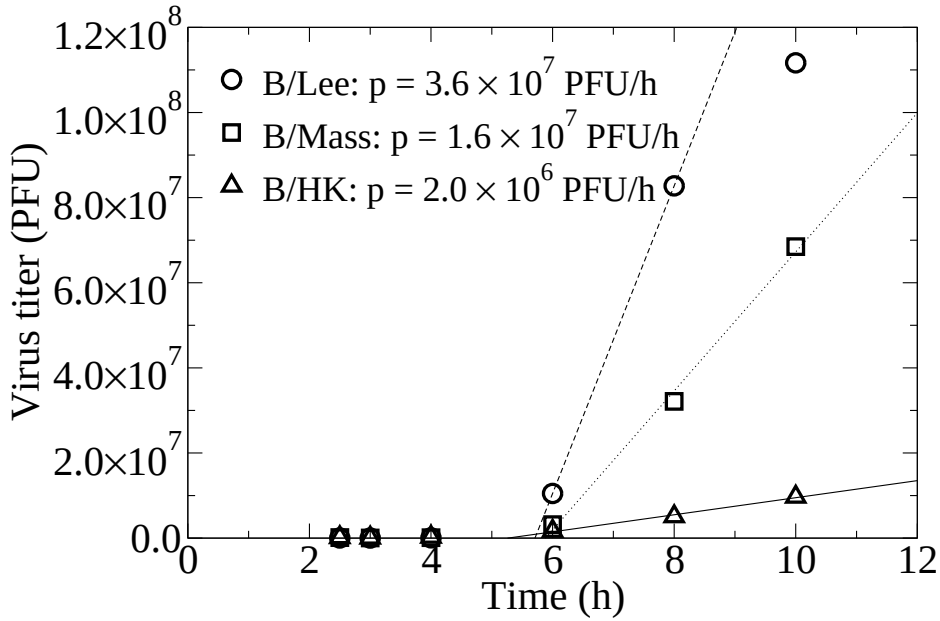
$$\dot{V} = p\frac{I}{N_{\text{cells}}} - cV$$

Single Cycle Growth (SCG) Viral Yield Experiments

- Infect cells with high virus concentration (MOI $>>$ 1)
  $\rightarrow$ all cells initially infected.

Tobita 1975

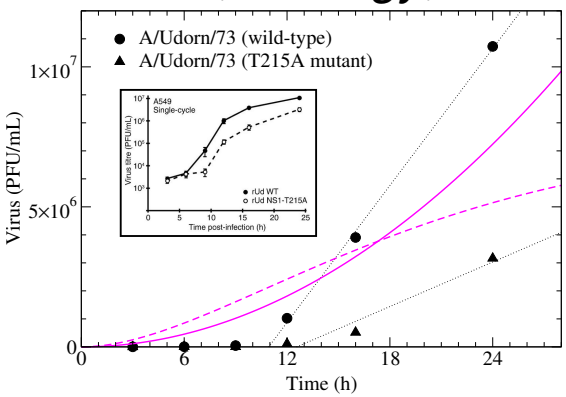


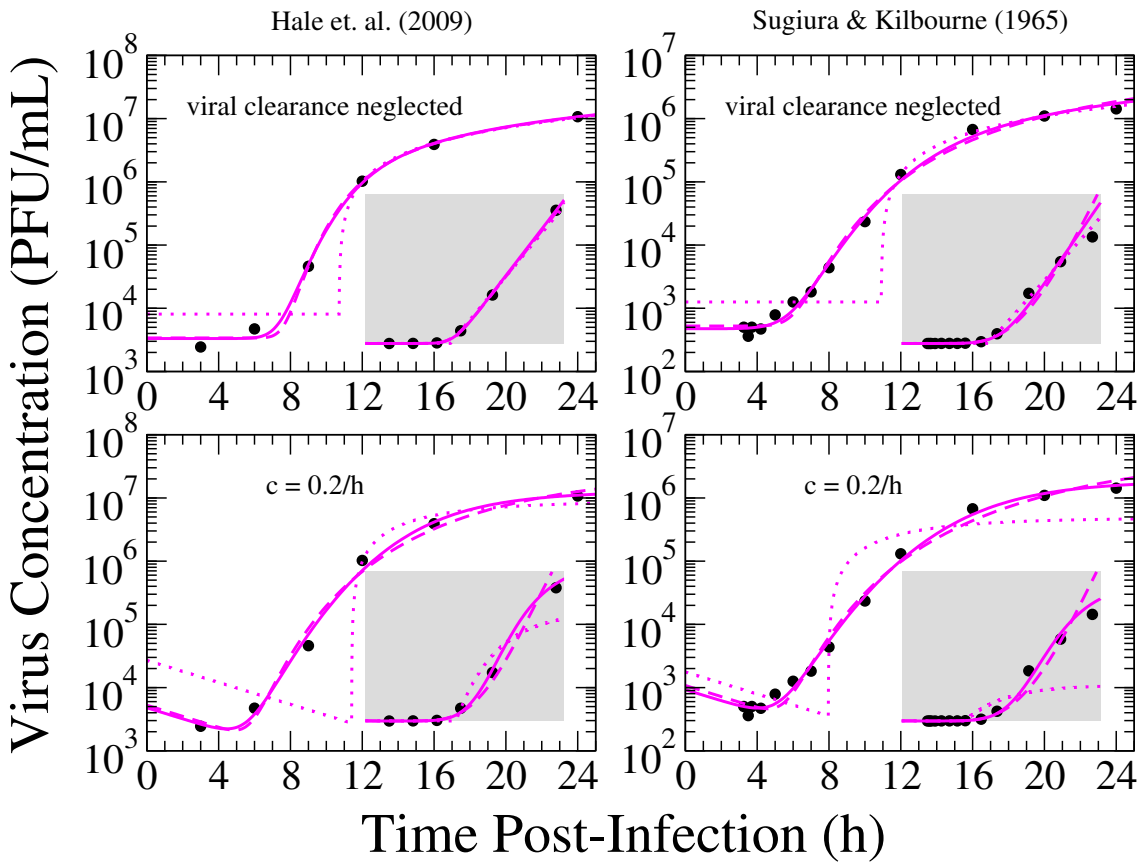


- Dynamics characterized by long delay *without viral production* and then linear growth.
- Reveals average production dynamics of a cell.

Fits to SCG with alternative delay models

Hale et al, *Virology*, 2009

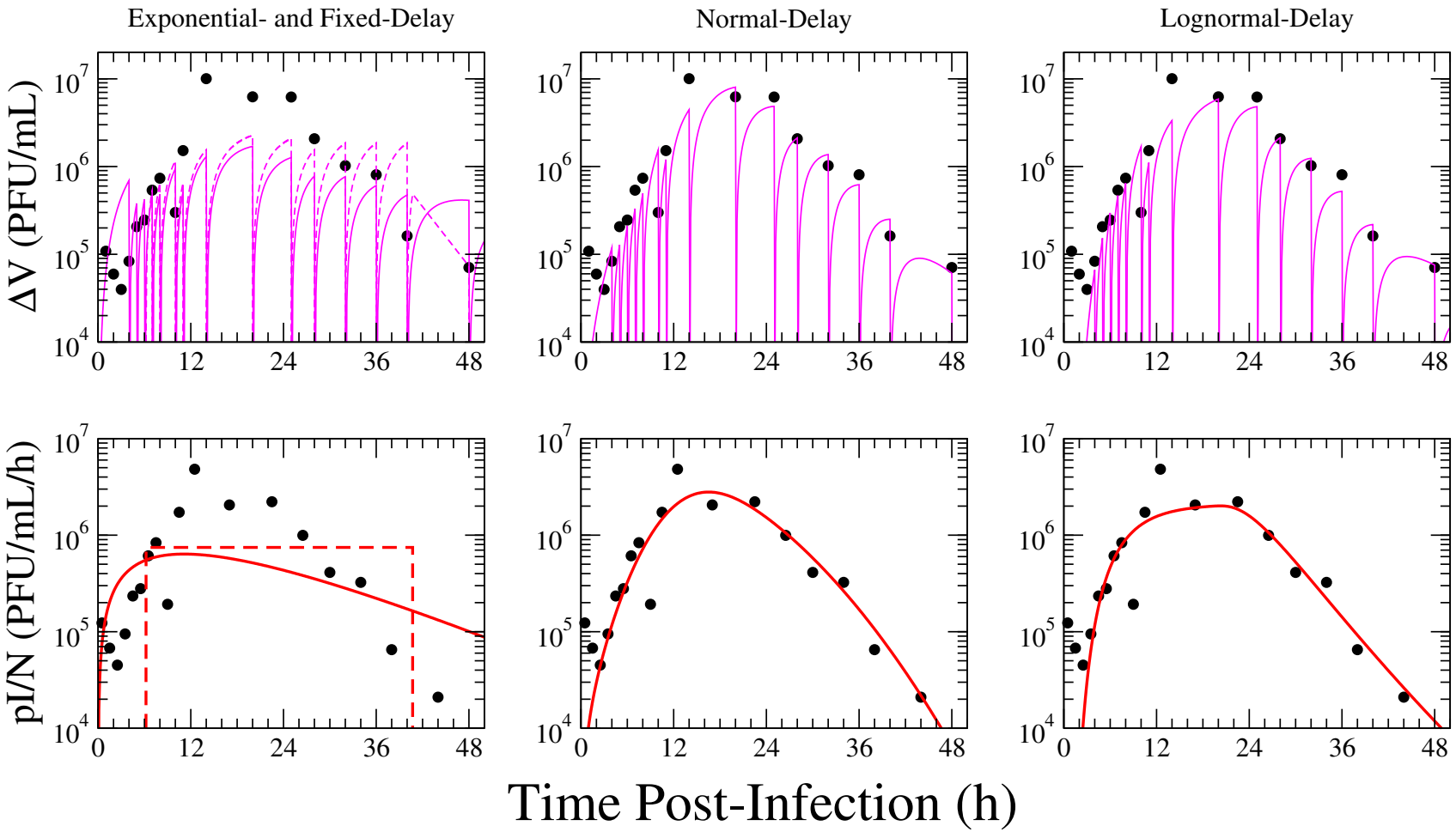




Exponential (left) and Dirac-delta (right, dotted) $\rightarrow$ poor fit
Normal (right, solid) and Lognormal (right, dashed) $\rightarrow$ good fit

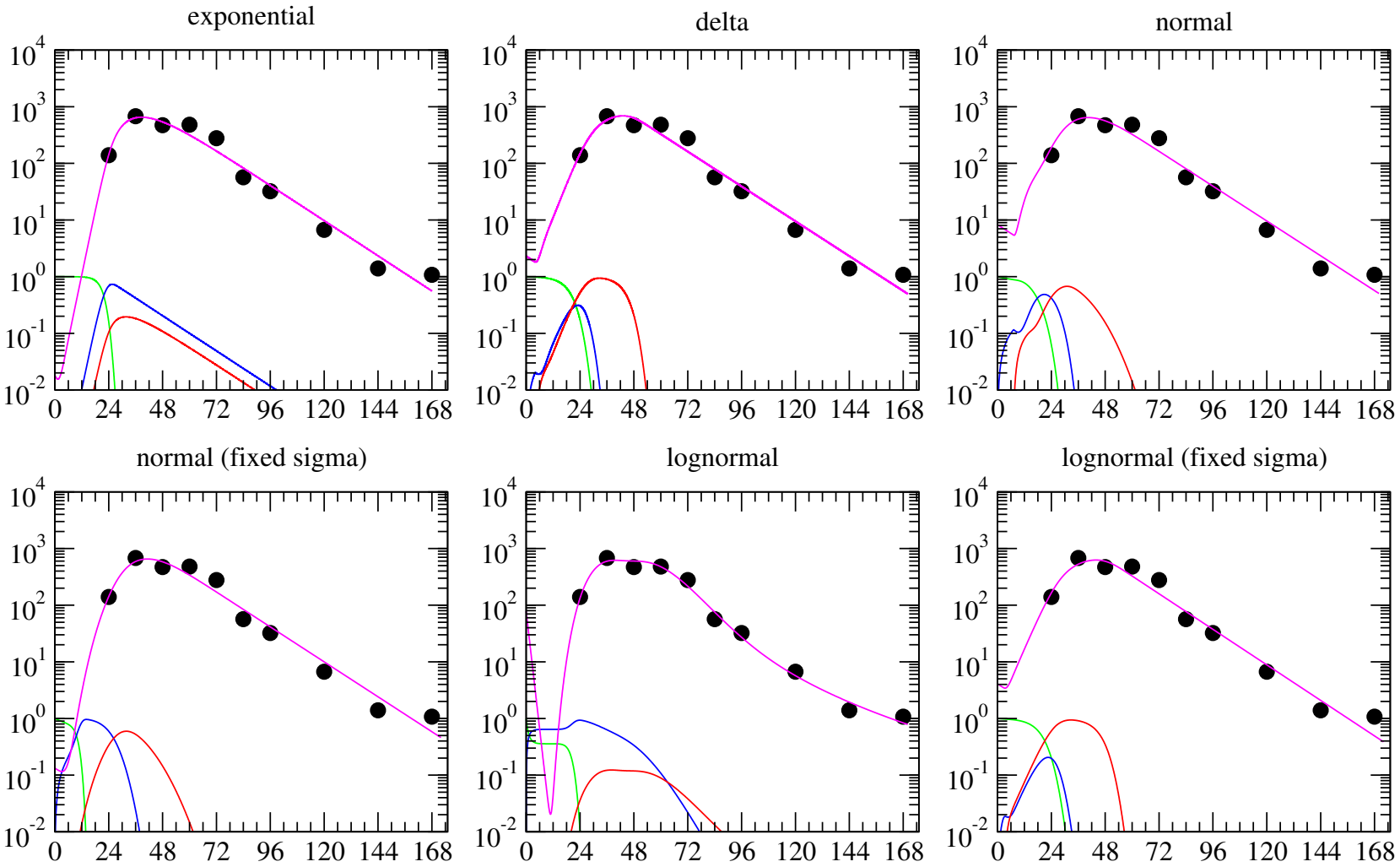
Single-Cycle Single-History Experiment

- Unique experiment in literature [Gaush & Smith 1968].
- Single well: virus is removed and medium is replaced at every time point.



Implications to Clinical Data

Virus dynamics alone are not enough to discriminate between delay models:



Human volunteer infected with A/Texas/36/91 (H1N1)

[From Barroso et al, *Antivir Ther*, 2005]

Summary

- Reliable extraction of some kinetic parameters, specific to a particular influenza strain, is possible using simple in vitro experiments.
- Many observable features of viral growth are reproducible by wide range of delay models.
- In single-cycle growth — where one can discriminate between models — exponential- and fixed-delay models are insufficient while normal- and lognormal-delays are reasonable.

Future Work and Further Info

- **Competition Experiments**: Test our results by allowing for virus competition $\rightarrow$ Use parameter estimates to predict results.
- **Influenza strain tool-kit**: Develop of a collection of laboratory experiments for the rapid parameterization of strains.
- **Model discrimination**: Work with experimentalists to refine the model discrimination procedure with single-cycle growth.

References (soon):

BP Holder and CAA Beauchemin. In-host influenza modeling and non-exponential delays, 2010.

BP Holder, P Simon, LE Liao, Y Abed, X Bouhy, CAA Beauchemin, G Boivin. Assessing fitness of an oseltamivir-resistant seasonal A/H1N1 influenza strain using a mathematical model, 2010.

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