

Intracellular modelling of viral infections

Perdita Stevens

Laboratory for Foundations of Computer Science
School of Informatics
University of Edinburgh

with many thanks to

Alain Kohl, Laboratory for Clinical and Molecular Virology, University of
Edinburgh

Stephen Gilmore, LFCS

David Harel, Weizmann

Plan

1. Introduction
 - ▶ to virology
 - ▶ to stochastic and deterministic modelling
 - ▶ to modelling of viral infection
2. Some ongoing work:
 - ▶ One virology puzzle
 - ▶ Initial attempt to model
 - ▶ Some improvements
3. Discussion of challenges
4. Conclusion

Introduction

Virology in one slide

A virus is an obligate intracellular parasite. It has genetic material (RNA or DNA) inside a protein capsid, and sometimes an envelope.

Infection cycle (for purposes of this talk):

- ▶ a virion attaches to a receptor on a cell
- ▶ it moves into a cell, usually in an endosome
- ▶ it is uncoated, releasing the genetic material
- ▶ the genetic material is copied
- ▶ to make genetic material for new virions...
- ▶ ... and structural protein for the capsid etc.
- ▶ new virions are assembled
- ▶ and released either by budding or by cell lysis.

E.g., influenza

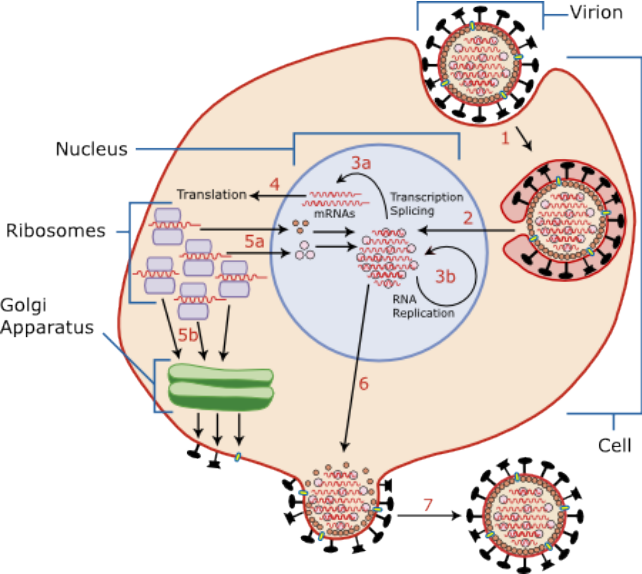


Image: YK Times at Wikimedia, GNU FDL

Why is modelling viral infection interesting?

Although the *qualitative* processes are often well understood, and also a lot of *quantitative* information is known, this is often from experiments in deliberately very simplified systems.

It's sometimes far from obvious how everything combines to produce an effect, and multi-factorial experiments can be much harder to do and interpret.

"I always tell my students, viruses don't always make sense" Alain Kohl

This is especially true once you add in the immune reaction to a viral infection – as always in biology, everything is unbelievably complicated...

State of the art

Many models of intracellular viral kinetics have been developed, some general and some incorporating knowledge of particular viruses.

- ▶ Coupled ODEs, e.g. Haseltine, Rawlings and Yin; Sidorenko and Reich; ...
- ▶ Stochastic, e.g. Zhdanov; Sidorenko et al.
- ▶ Also some specialised models of e.g. capsid formation
- ▶ Some models include aspects of immune reaction, typically interferon.

Particularly interesting to compare stochastic and deterministic approaches, as done by Srivastava et al...

Stochastic vs deterministic simulation

Suppose we write a chemical reaction



(with some information about its “rate”).

We can interpret the *same* information either

- ▶ **stochastically**, each simulation run having **discrete** quantities of A, B, C;
- ▶ **deterministically**, quantities of A, B, C being **continuous** variables.

Stochastic versus deterministic modelling of viruses

Srivastava, You, Summers and Yin (2002) wrote a very basic model, and implemented it both as a system of ODEs and as a system of reactions to be simulated stochastically using the Next Step method of the Gillespie algorithm.

(That is, quoting p312 of the paper:

1. Calculate the *potential* time at which each possible reaction will occur.
2. From the list of times calculated, allow *only* the fastest reaction to actually occur.
3. Update the number of the reactant and product molecules appropriately.

)

The two models made different predictions, especially at low MOIs: ODEs had an unstable steady state that was reachable by stochastic simulation, but ignored by deterministic simulation.

A simple model

this one is similar to that used by Krakauer and Komorova

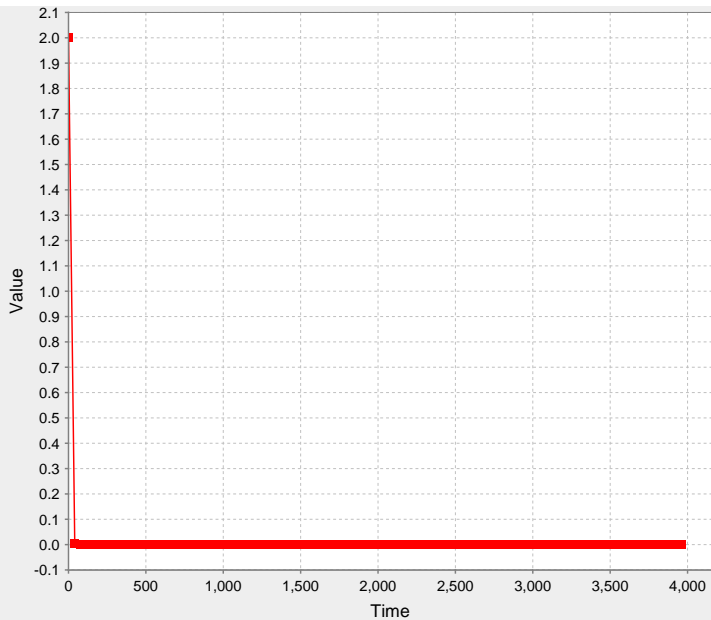
```
Genome + Polymerase -> RComplex
RComplex            -> Genome + Genome + Polymerase
Genome + Ribosome  -> TComplex
TComplex           -> Genome + Ribosome + Polymerase
TComplex           -> Genome + Ribosome + SProtein
Genome + SProtein  -> NewVirus
```

```
// decay/clearance processes
```

```
Genome          ->
Polymerase      ->
SProtein        ->
RComplex        ->
```

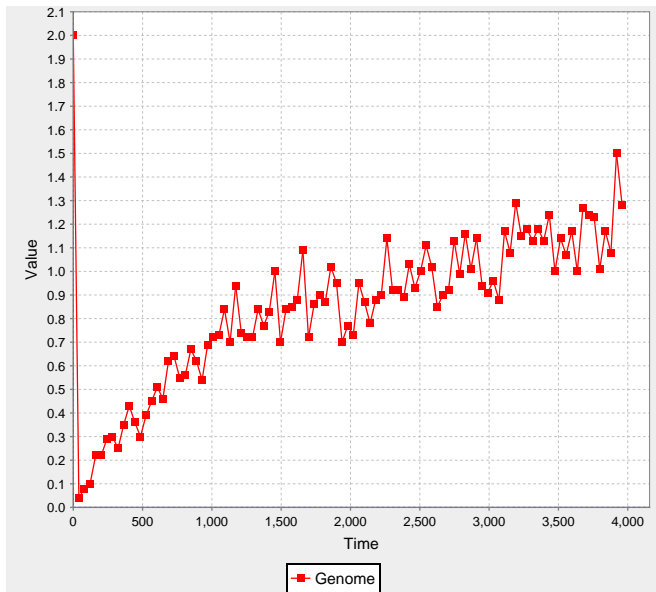
omitting rates, reaction names

Simulate using ODEs



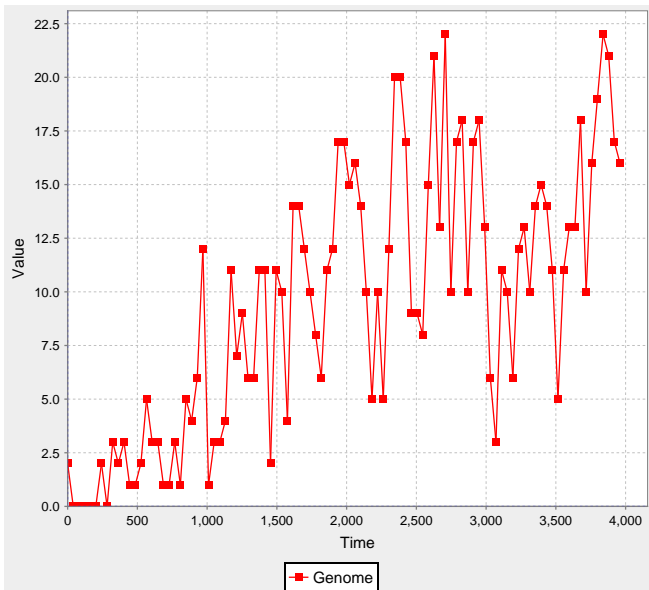
Genome

Simulate same model stochastically (average of 100 runs)



88/100 infections died out, but 12 were productive.

An individual simulation run: productive infection



Some ongoing work

*Preview of unfinished ongoing
work*



CDC, public domain

Arboviruses

Arthropod-borne viruses.

Examples: yellow fever, dengue, chikungunya, Semliki Forest virus, West Nile, Japanese encephalitis,...

Infect mosquito, causing *persistent infection without (much) cell death*

Mosquito bites small mammal, causing virus infection *with cell death*

More mosquitoes bite the mammal, perpetuating the cycle.

Humans become very ill, and thus are basically a dead-end or “accidental” host for the virus.

What's the difference? Why persistence in mosquitoes only?

Virus production in two cell cultures

SFV in mammalian cells:

- ▶ overwhelming infection: cells die
- ▶ almost all host transcription is shut off by virus, thus inactivating immune response

SFV in culture of *Aedes Albopictus* U4.4 cells:

- ▶ cells continue to divide, population increases as normal
- ▶ acute phase with high virus production (12-24hrs post infection)
- ▶ persistent phase, low but non-zero virus production (1-2% of cells productively infected)
- ▶ host transcription is significantly reduced, but not stopped completely

Why?

Obvious place to look at is the immune response: radically different in mosquitos compared to mammals. E.g.

- ▶ mosquitoes have limited immune memory and no adaptive immunity: innate immunity is all there is.
- ▶ important mechanism in mosquitoes is RNAi, which is not active (though still present) in mammals
- ▶ lots of details differ in the innate immune response.

But maybe this isn't the right approach: RNAi doesn't actually seem to be the difference (Kohl); the difference is apparent too soon for it to be very likely that mammalian adaptive immunity is relevant; no really convincing explanation so far.

What constitutes a convincing explanation?

It seems biologically natural to look for a particular *qualitative* difference between mammals and mosquitoes that explains the different dynamics of infection.

No shortage of candidates, given their diverse immune systems and cell behaviours.

But could it even be that minor *quantitative* differences suffice? E.g., just that the virus's parameters are a compromise between what's fit in mosquitoes and what's fit in mammals?

To investigate this, we need models.

Structural decisions

At this stage we didn't model, for example:

- ▶ entry or uncoating of the virus
- ▶ the behaviour of the non-structural protein: in fact, the initial translation produces polyprotein P1234 which is cleaved into P123 and nsP4, and later P123 becomes nsP1 ... nsP3
- ▶ degradation of most things
- ▶ the structural assembly of the new virions (properly)
- ▶ any compartmentalisation
- ▶ the cell's own behaviour, e.g. immune reactions.

Any or all of these decisions may have to be revisited – but which?

Numerical decisions

We have to choose

- ▶ rates at which different reactions occur (if reactants are at a given concentration) – reflecting both speed and affinity
- ▶ initial quantities

These are easier to deal with than structural decisions: e.g., easier to try different values.

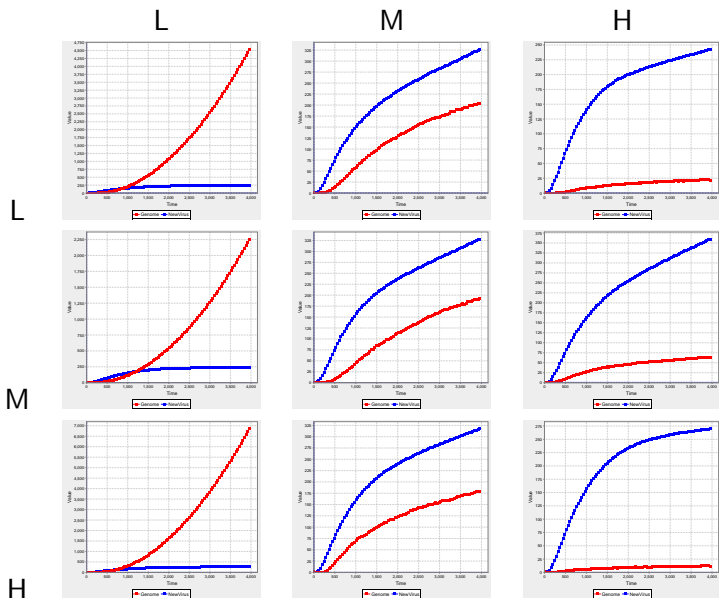
Some derived from experimental data; others guessed or deduced from analysis. (Alain Kohl's lab is beginning a series of experiments to confirm or deny initial guesstimates, for this modelling exercise.)

Disincentive to have structurally more complex model: need for more numbers!

Results from the simple model

Depending on parameters chosen, can get explosion or extinction of infection – but fail to model the initial surge followed by decline to persistence.

Encapsidation rate vs clearance rate of +ve RNA



Missing piece of story

Actually, it seems that no individual cell is persistently infected at all!

In the persistent phase, at any one time, only around 1% of cells are productively infected – but it isn't always the same 1%.

If we sop up virions *emitted* from cells using antibodies – without affecting virus inside cells – then the infection clears completely.

I.e., a cell is productively infected for a while, then the infection dies out in that cell (the cell does not die); meanwhile, other cells get infected.

Superinfection prevention

After a cell has been infected for about 24h, processes take place which prevent it from being reinfected by a similar virus.

This state persists – the cell is resistant to infection – for a few days.

So we think that at the single cell level, each infection dies out, most quickly, some slowly; before the virus dies out from the last cell, though, it can be reestablished in a newly susceptible cell or a descendant.

This would be interesting to explore with inter/intracellular modelling.

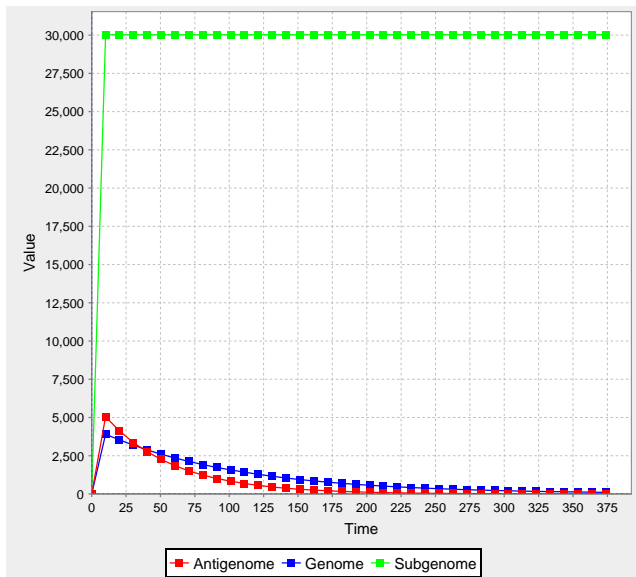
Next attempt

Experimental effect may be caused by cell “running out of steam” i.e. becoming short of some resource: energy, a substrate? We have little idea what.

Chose to model Steam, named to indicate uncertainty about what it is.

- ▶ initially set $\text{Steam} = 10000$;
- ▶ show Steam degraded and created at equal rates in cell
- ▶ show Steam consumed in each step of virus replication

With “steam”: average of 10 simulation runs



Not a bad match, considering.

Yet another wrinkle – or vital point?

8-10 days after infection of mosquito cells, there is a new surge of virus production – which turns out to be a “small plaque attenuated variant” (Davey and Dalgarno). This variant takes over: by day 16, wildtype is gone.

What's going on?

How does it relate to what happens in vivo?

Relation to formation of *defective interfering particles*? Or adaptation?

No real idea yet.

Discussion

Challenges, as seen by a newbie

We might split them into “boring engineering” and “true modelling” challenges – although of course the two are intertwined!

Modelling first:

1. Abstraction, because we don't **need to model** all details.
2. Abstraction, because we don't **understand** all details.
3. Confidence in the numbers we use – when is a good fit to experiment a sign that the model is right, and when is it just that we had enough parameters to fiddle?

Why do we need abstraction?

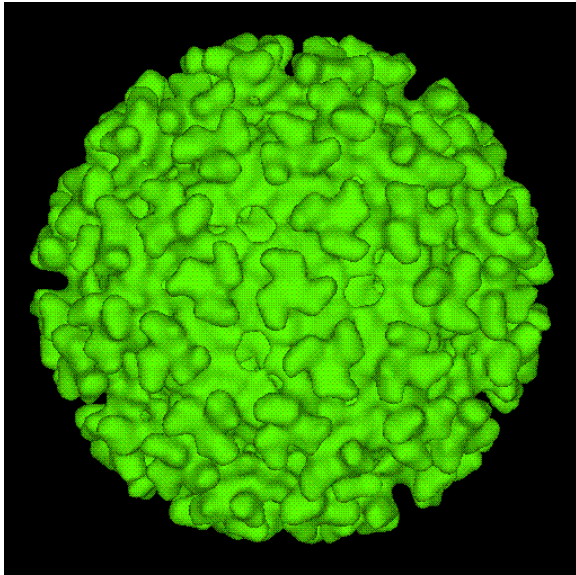


Image: CDC, public domain

Abstractions break (basic) modelling formalisms

Basic chemical model assumes at most 2 molecules combine.

Physically reasonable at the molecular level.

But when you model formation of new virion, you need something more like



where k may be large,

as shorthand for the whole process of capsid formation.

Even that abstracts away *a lot*: not all k protein molecules need to be there initially, there are several kinds, etc.

Engineering challenges

Biological computational modelling is in its infancy: heroic modellers achieve success against the odds.

How can the field mature?

1. Abstraction again! Want arbitrary flexibility to “zoom in” and “zoom out” from aspects of the system.
 - ▶ **Process algebra** is the popular approach,
 - ▶ but **model-driven development** may have as much to say about it... bidirectional transformations of biological models??
2. Usual challenges of (software) models: how to debug, maintain, diff, transform...
3. (Claim) A data-driven approach to intracellular modelling is infeasible (for reasonable values of “data”). But is there a “sweet spot” for a *virology workbench*?

Conclusions

- ▶ Virology is a particularly interesting area for systems biology
- ▶ with lots of potential for contributing to solving problems that are important in medicine and biology
- ▶ and perhaps for shedding light on modelling and model engineering issues that also arise elsewhere.
- ▶ The development of persistence of arbovirus infection in mosquitoes is one possible case study.

Questions, comments?