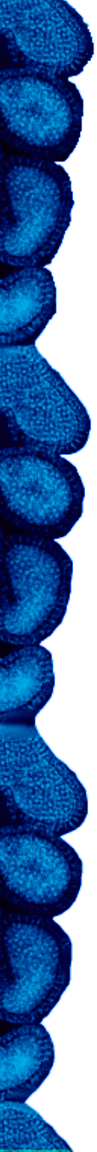

RYERSON UNIVERSITY

Modelling the spatial spread of hepatitis C virus infection in vitro

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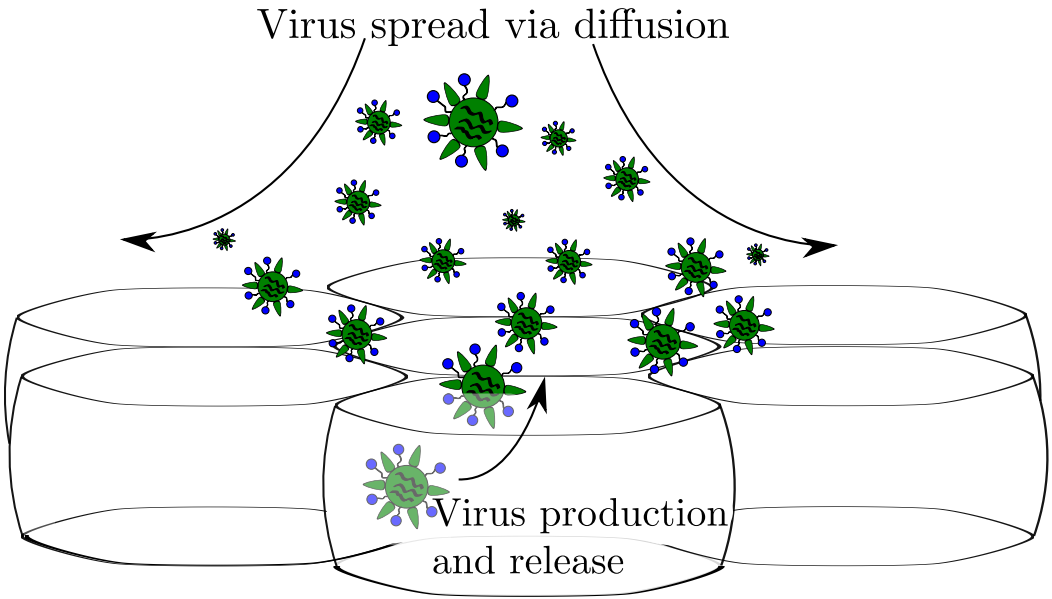
Collaborator: *Prof. Jordan Feld, MD*

Hepatitis C virus

- Hepatitis C is a liver disease caused by the hepatitis C virus (HCV).
- Nearly 200 million people worldwide are infected with the disease.
- Approximately 75% to 85% of people infected will become chronically infected and could develop liver cancer.
- To date, there are no models spatially describing the spread of HCV at the tissue/cell culture level in vitro.

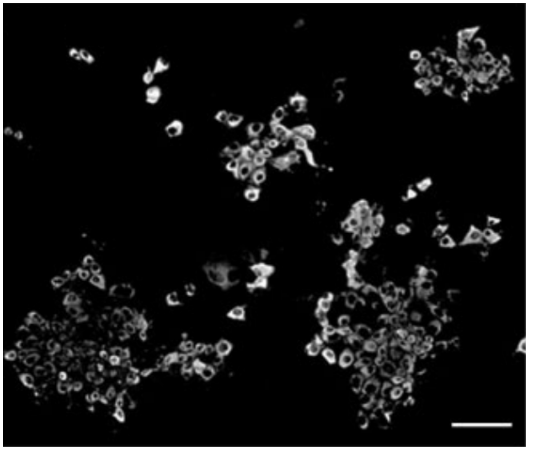
How does virus infection proceed in vitro?

- A virus inoculum is initially deposited into the medium covering a monolayer of cells.
- Cells infected by that virus begin producing their own virus progeny which is also released into the medium.
- The newly produced virus diffuses through the medium, outwards from its origin, and goes on to infect other cells.

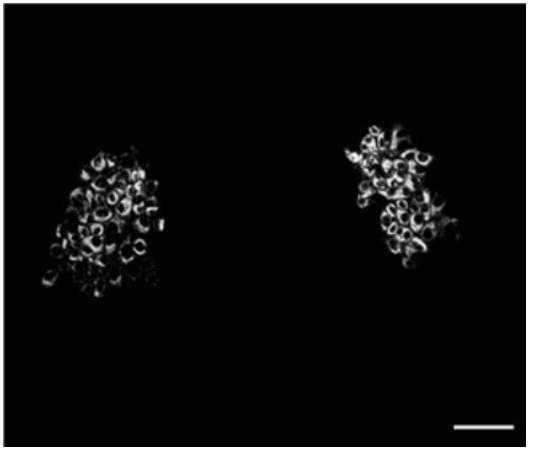


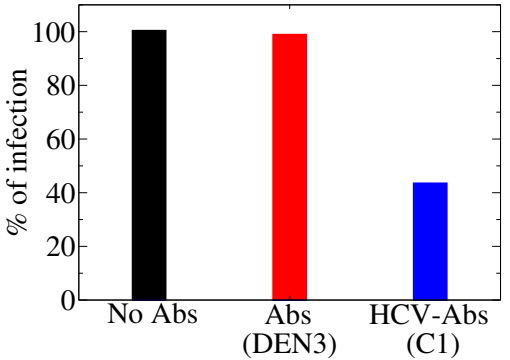
Spatial distribution of HCV in vitro

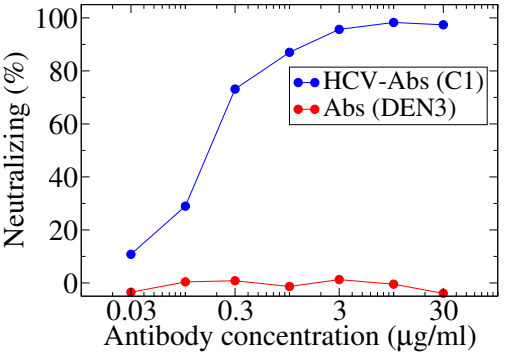
Liquid medium (high diffusion)



Thick medium (low diffusion)



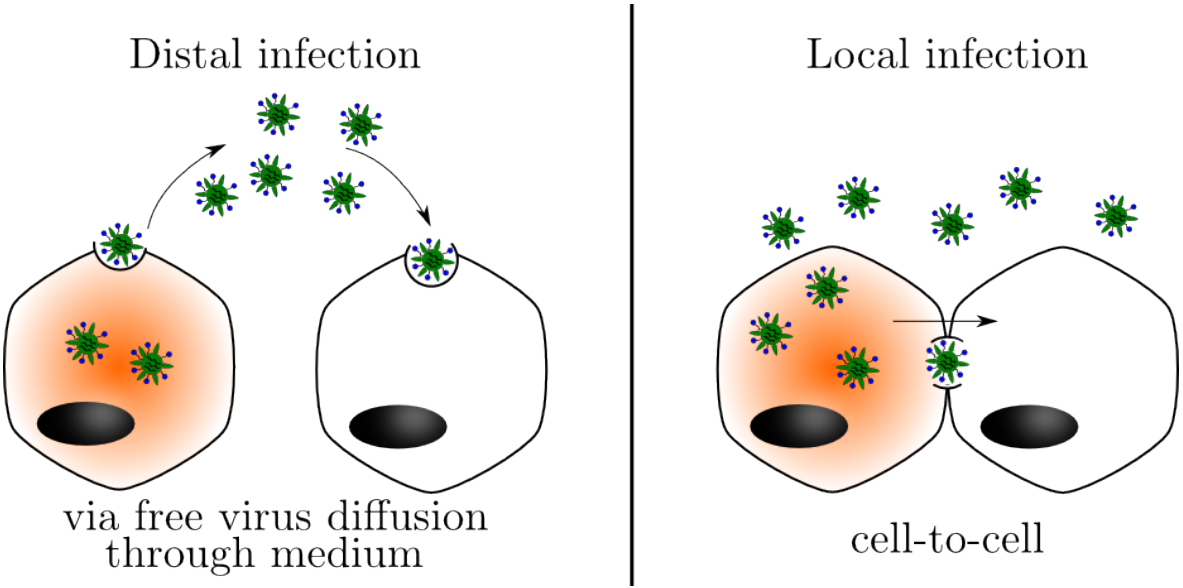




[From: Timpe et al., Hepatology, 47():17–24, 2008. DOI:10.1002/hep.21959]

The two modes of infection

It would appear there are two processes leading to cell infection:



The **distal** (**free virus, fv**) process would be responsible for the wide distribution of satellite foci away from infected cells.

The **local** (**cell-to-cell, c2c**) process would be responsible for the dense infection of cells within each foci, close to infected cells.

So my goal is to...

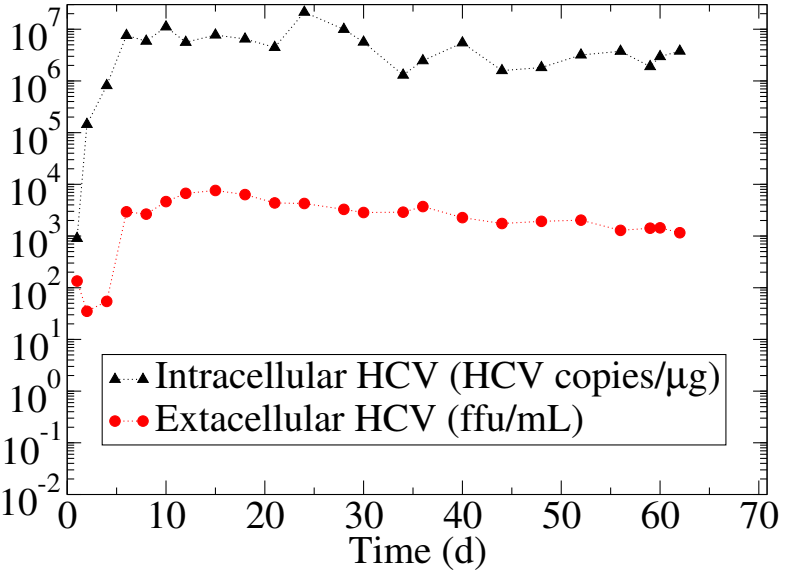
- *build a computer/mathematical model which implements the two modes of infection for HCV; and*
- *confront the model to experimental data to evaluate the relative contribution of each mode (% due to cell-to-cell infection?).*

with a long term goal to...

- *use the model and in vitro system as a platform to quantify the course of HCV infections in vitro and characterize the effect of strains, mutations, antivirals, etc.*

Our Model

The time course of HCV infection in vitro



[From: Sainz and Chisari, J. Virol., 80(20), 2006. DOI:10.1128/JVI.01059-06]

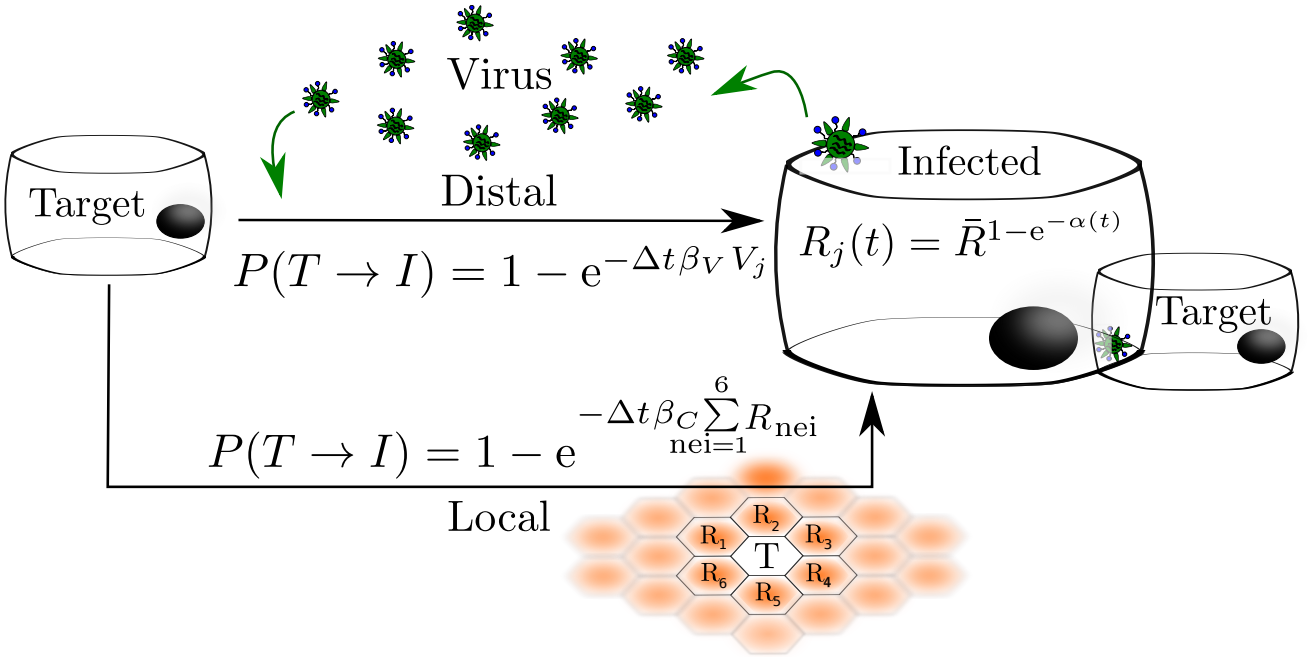
intracellular virus is a count of positive HCV RNA strands contained within the cells of the culture [# of HCV copies/μg of total cell RNA]

extracellular virus is the concentration of **infectious** virus (focus forming units or ffu) in the medium [ffu/mL]

So our model will need to track both intracellular virus, and extracellular infectious virus...

Proposed model: A 2D hexagonal grid of cells

Our two-scale spatial model tracks intracellular viral replication at the **single-cell** scale, and **multi-cell** spread of the infection through the monolayer.



R_j — intracellular HCV copies within cell j.
V_j — extracellular infectious HCV concentration above cell j.

The extracellular HCV model

The amount of virus at each site over time is affected by

Diffusion on the hexagonal grid at rate D

$$V_j(t+\Delta t) = \left(1 - \frac{4D\Delta t}{(\Delta x)^2}\right) V_j(t) + \frac{2D\Delta t}{3(\Delta x)^2} \sum_{N_{\text{nei}}} V_{N_{\text{nei}}}(t)$$

Virus production by infected cells containing R_j HCV copies

$$V_j(t+\Delta t) = V_j(t) + \Delta t\, p_V(t)$$

Virus clearance due to loss of infectivity at rate c (we use $c = 0.056\ \text{h}^{-1}$)

$$V_j(t+\Delta t) = V_j(t) \text{e}^{-c\Delta t}$$

[estimates for c range between $0.06\ \text{h}^{-1}$–$0.3\ \text{h}^{-1}$]

Confronting the model to experimental data

Our complete model has 8 parameters, **5 of which are not set by the data**:

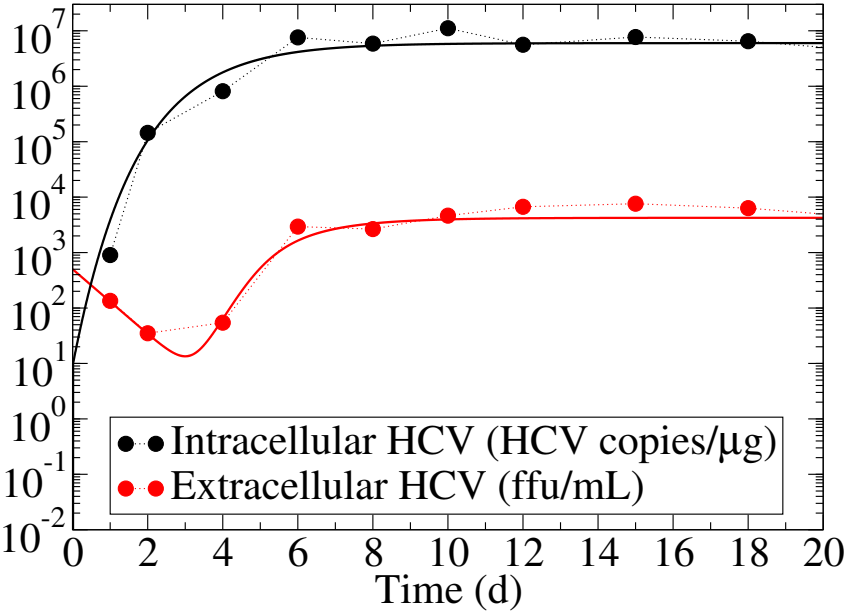
cell-to-cell infection rate: β_C

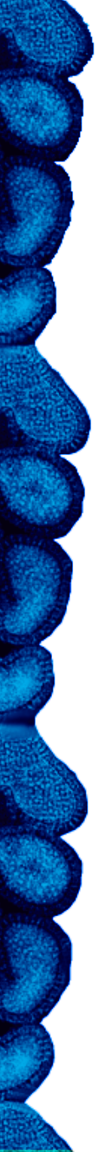
infection rate by free virus: β_V

$\bar{R}, \alpha$ — intracellular HCV copies

$\bar{V}, \rho, f_{\text{mid}}, c$ — extracellular infectious HCV

Our model is generally able to reproduce the shape of the data quite well. This is not surprising given the large number of parameters in the model.

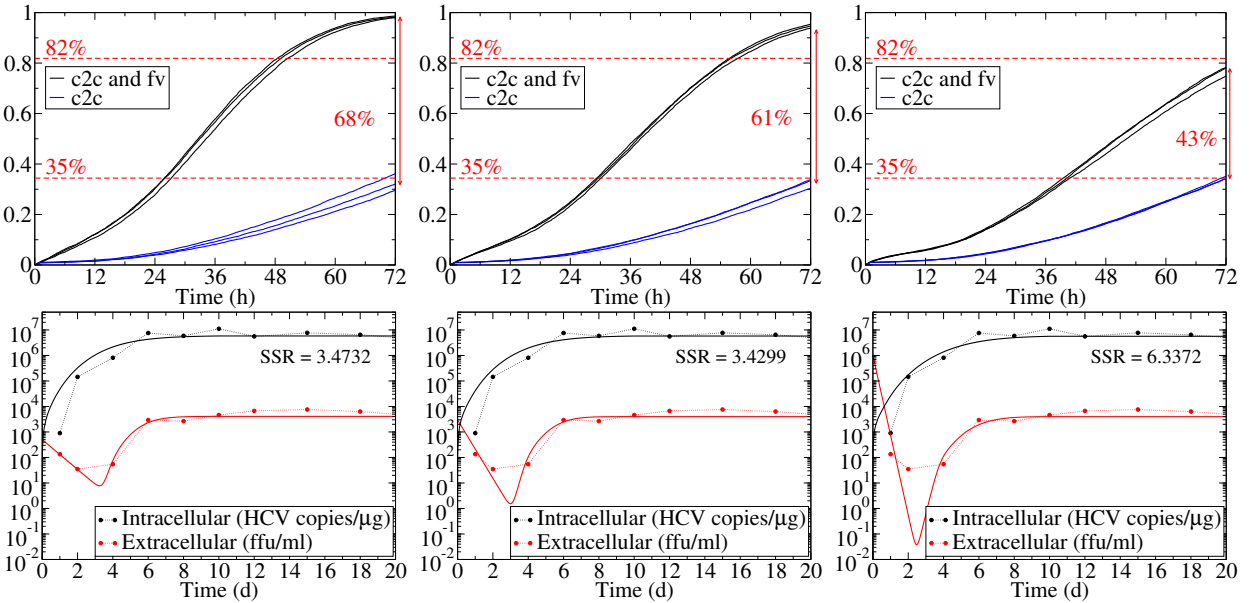




Cell-to-cell (c2c)
vs.
free virus (fv)
infection in vitro

Reproducing % of cells infected

We want to reproduce the correct percentage of cells infected while maintaining a good SSR for R and V. [$\rho = 30$]



c	V_0	β_V	α	β_C	f_{mid}	SSR	c2c	fv
0.056	500	2.0e-5	0.025	5.8e-3	0.6	3.47	86%	14%
0.11	3000	3.5e-6	0.025	5.8e-3	0.6	3.43	91%	9%
0.3	1.0e6	1.1e-8	0.025	5.8e-3	0.6	6.64	96%	4%

Future work

1. Double-check everything to ensure the 90% c2c + 10% fv is truly constrained by the data and seek additional data for further confirmation.
2. Once the ratio c2c:fv is firmly established, show how the efficacy of an antiviral acting on either or both transmission routes is modulated by this ratio.
3. Apply the model to answer specific, topical HCV questions. E.g., evaluate antiviral efficacy using the model, evaluate how the c2c:fv ratio in vivo might differ from in vitro.

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http://phymbie.physics.ryerson.ca

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And my supervisor's funding which covers my salary!

Questions?

What does that look like?

The rate of diffusion of virus through the medium is given by

$$D = \frac{k_B T}{6\pi\eta\, r_V},$$

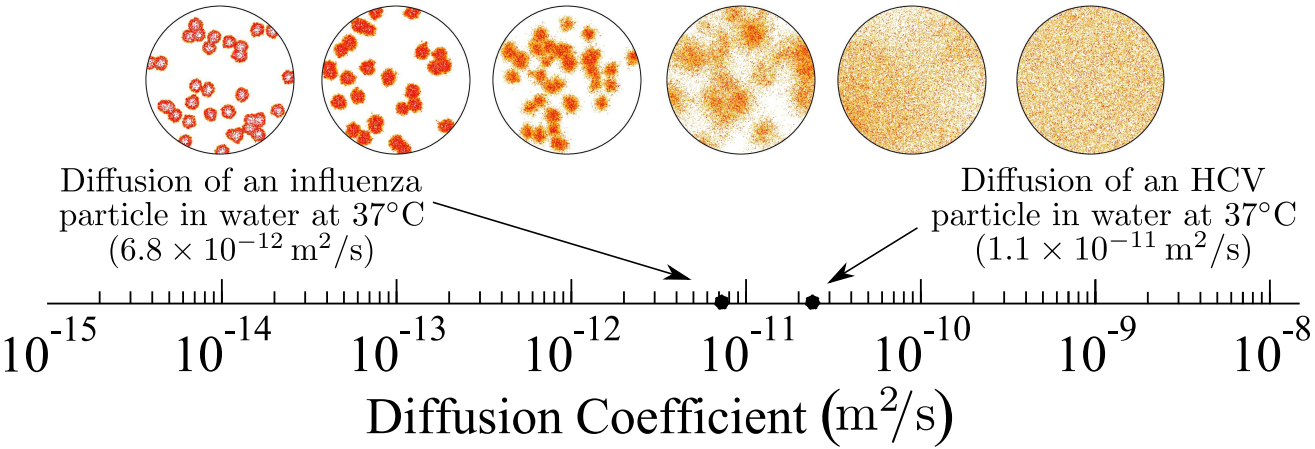
where

k_B the Boltzmann constant (1.38×10^{-23} J/K);

T temperature of the medium ($37°\mathrm{C} = 310.15$ K);

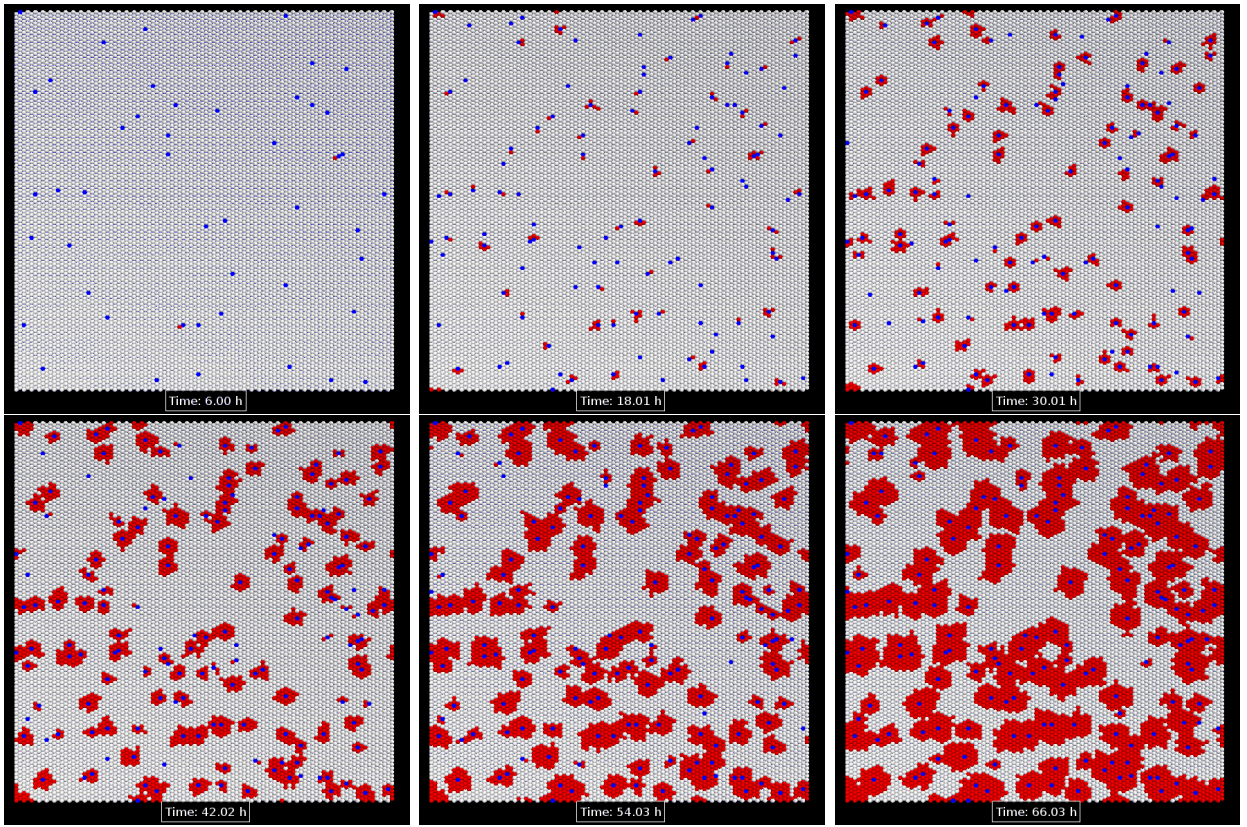
η viscosity of the medium (water, 6.7×10^{-4} J · s/m^3); and

r_V radius of virus particles (m).



So if you infect cells with flu or HCV in a liquid medium, infection should distribute mostly evenly...

Visualising the spatial HCV infection



Uninfected, Infected by virus (distal), Infected cell-to-cell (local)