Counting defective interfering particles: Easy as 1, 2, 3, . . . ?

Laura Liao[1]

[1]Department of (Viro) Physics, Ryerson University
Supervisor: Prof. Catherine Beauchemin

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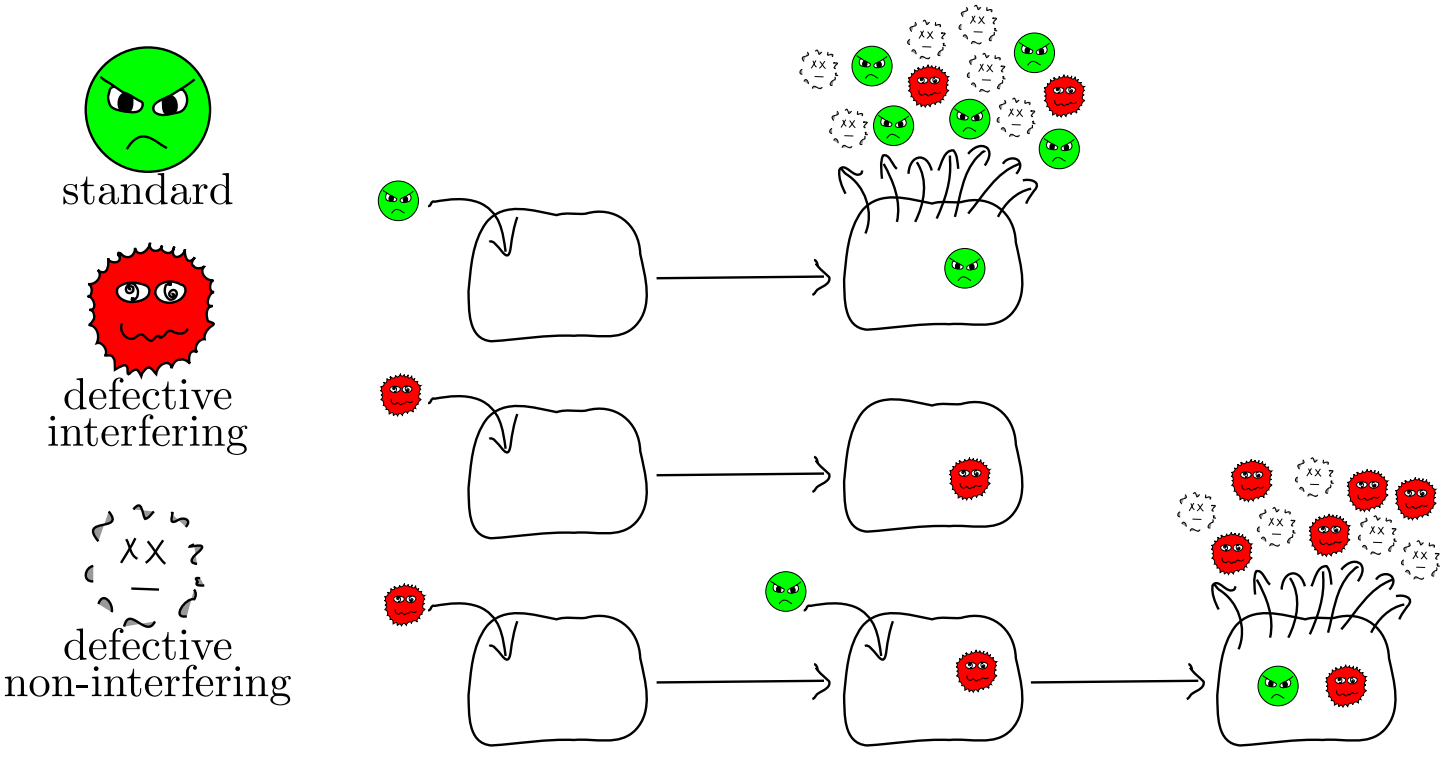
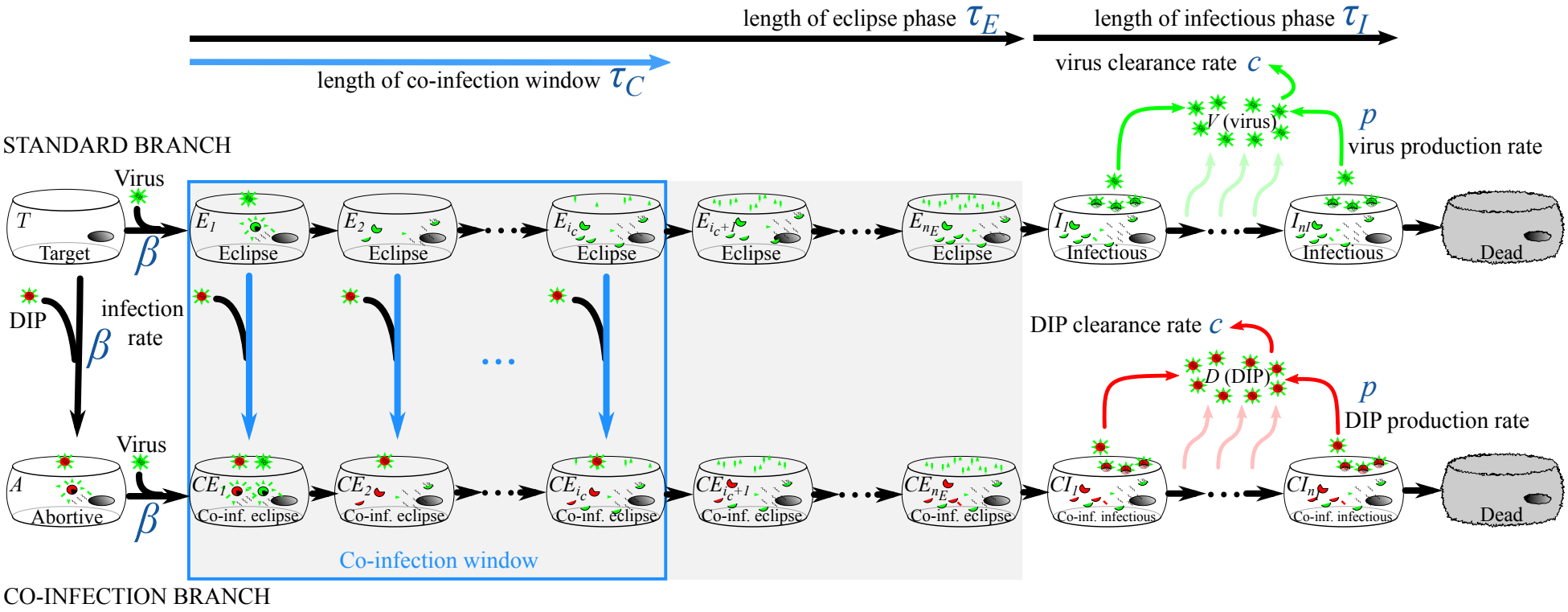
Defective interfering particles (DIPs)

Defective interfering particles (DIPs) are improperly formed virus.



DIPs impact the outcome of virus experiments.
Therefore, it is important to count them.

Influenza A viral replication cycle & model

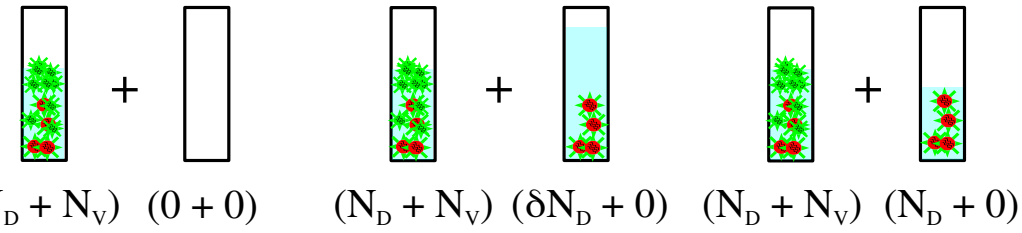


DIPs are indistinguishable from virus.

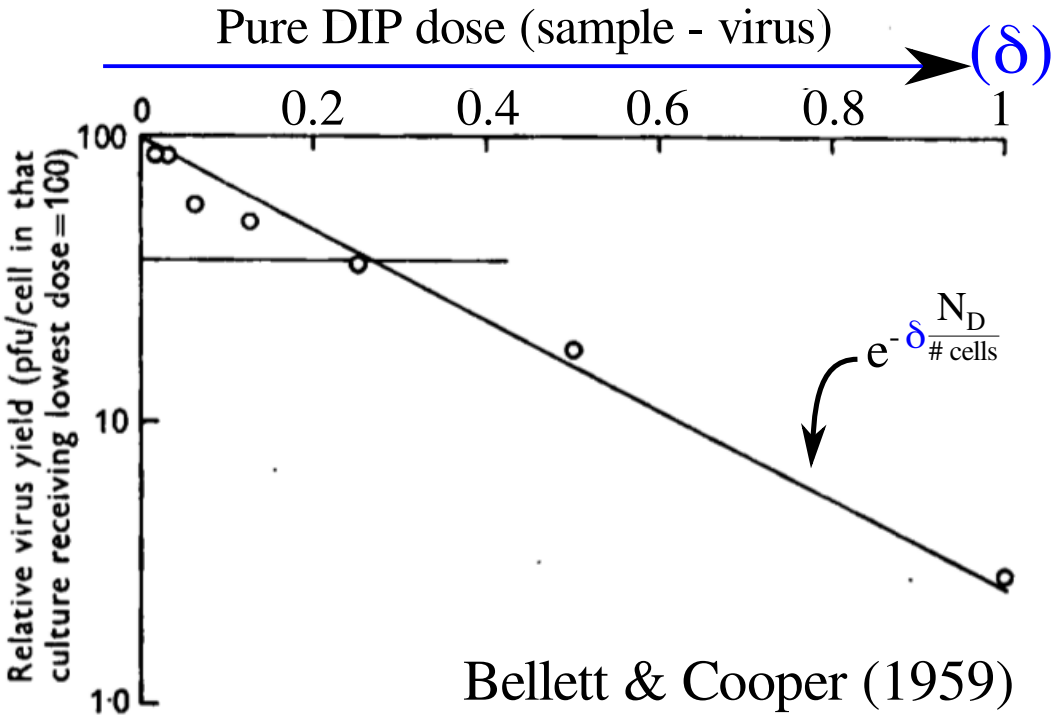
So, how have DIPs been counted?

Existing method to count DIPs

Infect with sample + diluted UV'd sample (pure DIP).



$(N_D + N_V)$ $(0 + 0)$ $(N_D + N_V)$ $(\delta N_D + 0)$ $(N_D + N_V)$ $(N_D + 0)$



Bellett & Cooper (1959)

$$f_{\text{cell receive one or more virus}} = 1\text{-}e^{-\frac{N_V}{\#\ cells}}$$

$$f_{\text{cell receive no DIP}} = e^{-\delta\frac{N_D}{\#\ cells} - \frac{N_D}{\#\ cells}}$$

$$f_{\substack{\text{cells receiving} \\ \text{one or more} \\ \text{virus but no DIP}}} = f_{\text{cells still producing virus}}$$

$$= (1\text{-}e^{-\frac{N_V}{\#\ cells}})e^{-\delta\frac{N_D}{\#\ cells} - \frac{N_D}{\#\ cells}}$$

So we have,

$$\text{relative virus yield} = \frac{f_{\text{cells receiving virus but no DIP}}}{f_{\text{cells receiving virus in absence of sample}}}$$

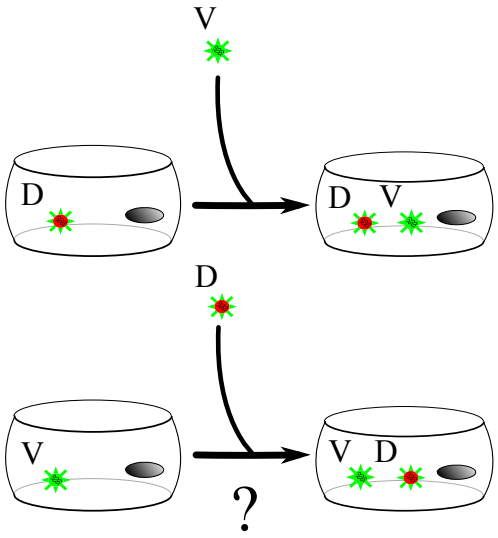
$$= \frac{(1\text{-}e^{-\frac{N_V}{\#\ cells}})e^{-\delta\frac{N_D}{\#\ cells} - \frac{N_D}{\#\ cells}}}{(1\text{-}e^{-\frac{N_V}{\#\ cells}})e^{-\frac{N_D}{\#\ cells}}}$$

$$= e^{-\delta\frac{N_D}{\#\ cells}}$$

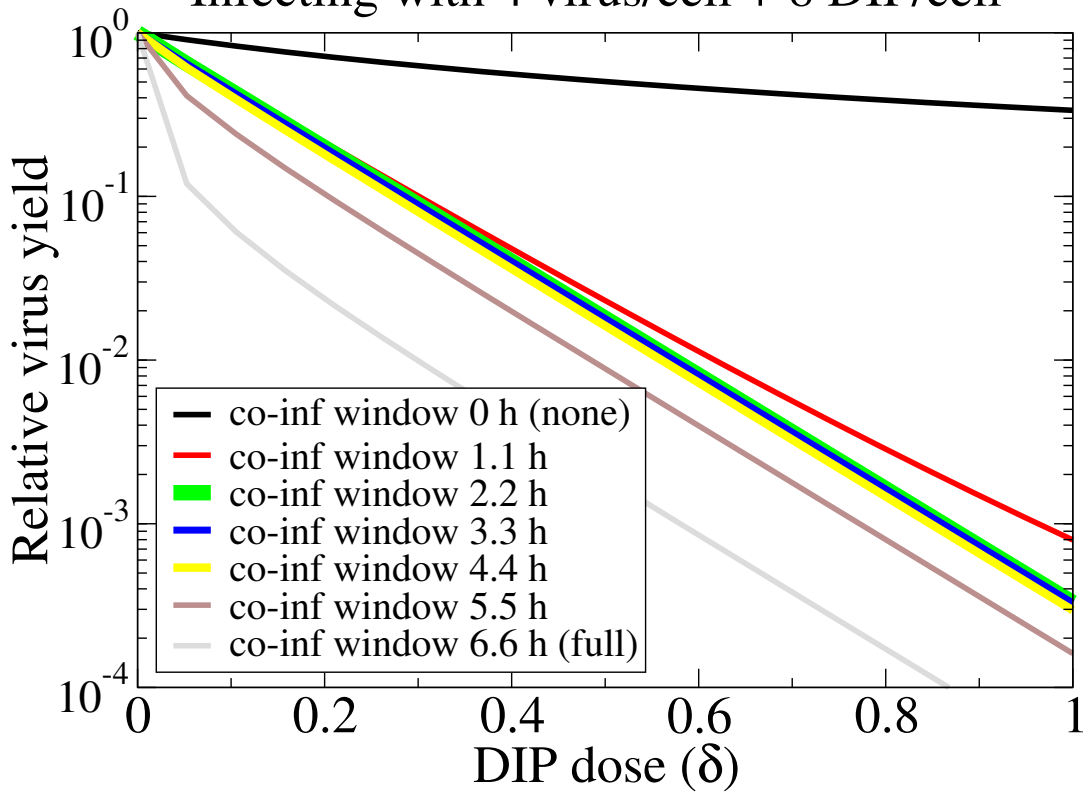
Bellett & Cooper (B&C) calculation uses Poisson distribution parameterized by $\frac{N_V = \#\ \text{virus}}{\#\ \text{cells}}$ or $\frac{N_D = \#\ \text{DIP}}{\#\ \text{cells}}$.

B&C does not account for order of infection events

B&C assay curves (predictions) impacted by co-inf window.



depends on co-infection window



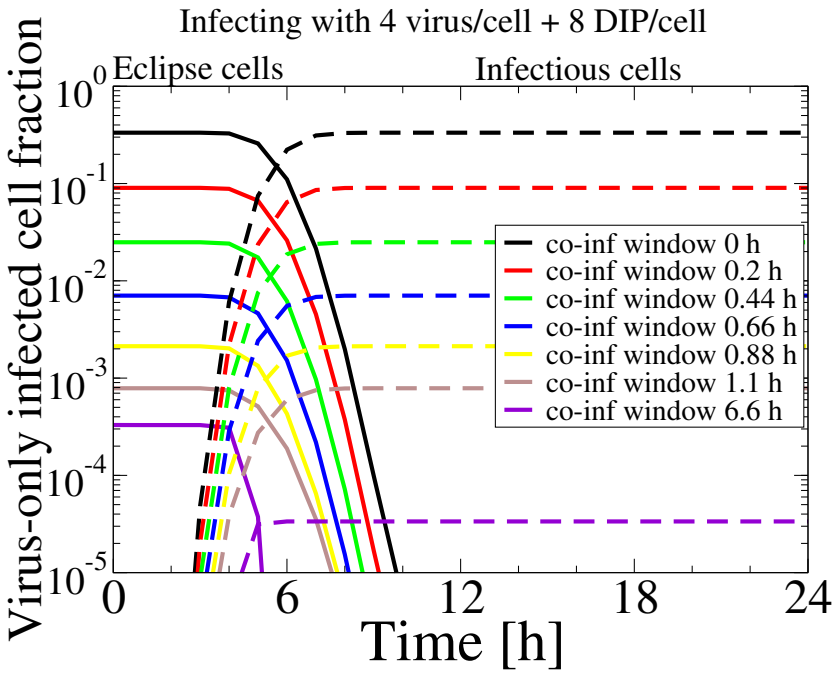
So order

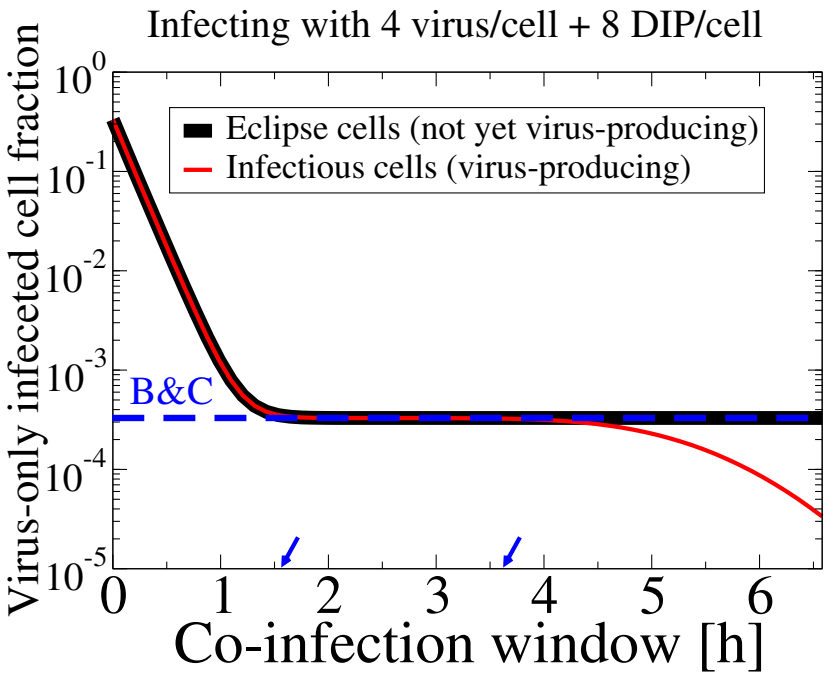
$D+V \neq V+D$

B&C valid for intermediate co-infection windows when order of events equally likely, *and* there are no newly produced DIPs.

B&C valid for intermediate co-infection windows

Compare frac virus producers for varying windows to B&C calc.





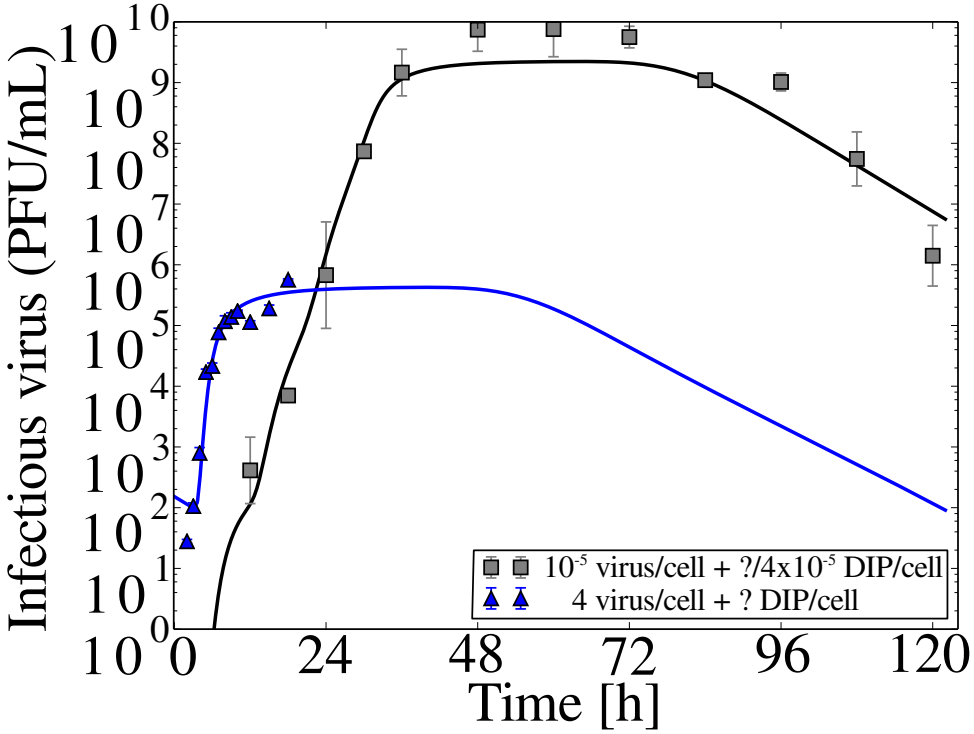
B&C valid for co-infection window between 1.5 h and 3.5 h.

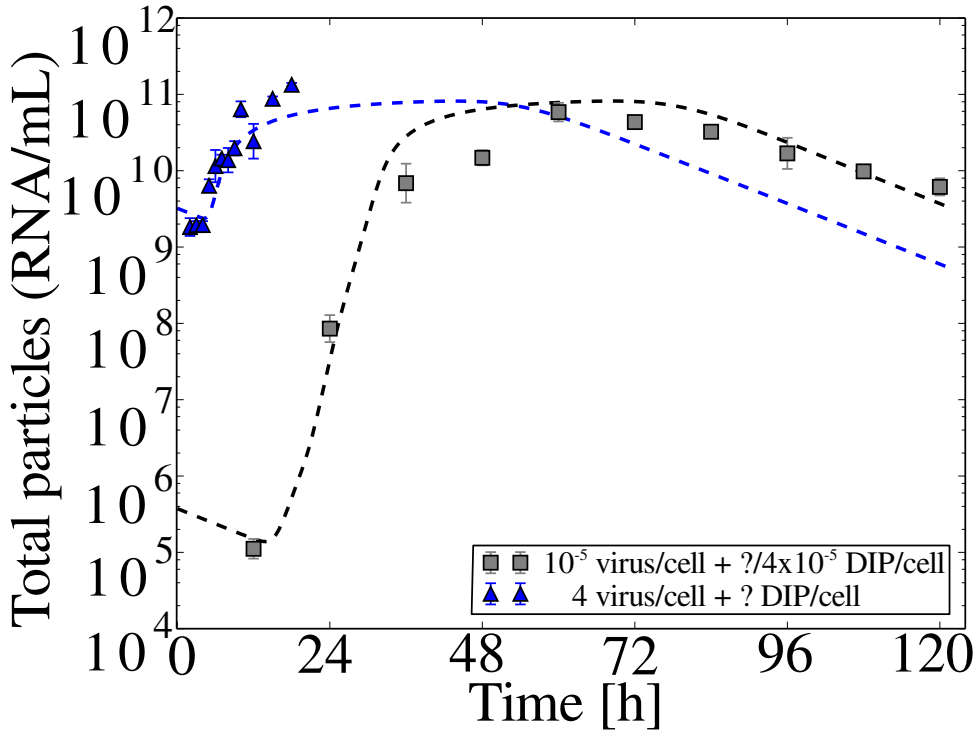
The biological co-infection window is between 1 h and 3 h.

So, B&C is suited for influenza A DIP counting.

Our method

Infect with sample (conc. and diluted). Equivalent total particles produced, but loss of infectious virus due to DIPs.

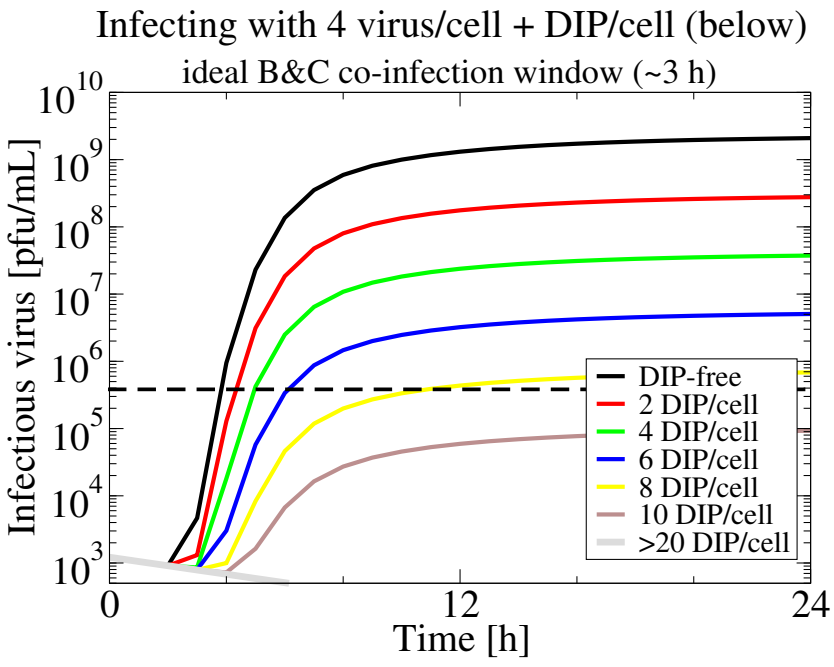


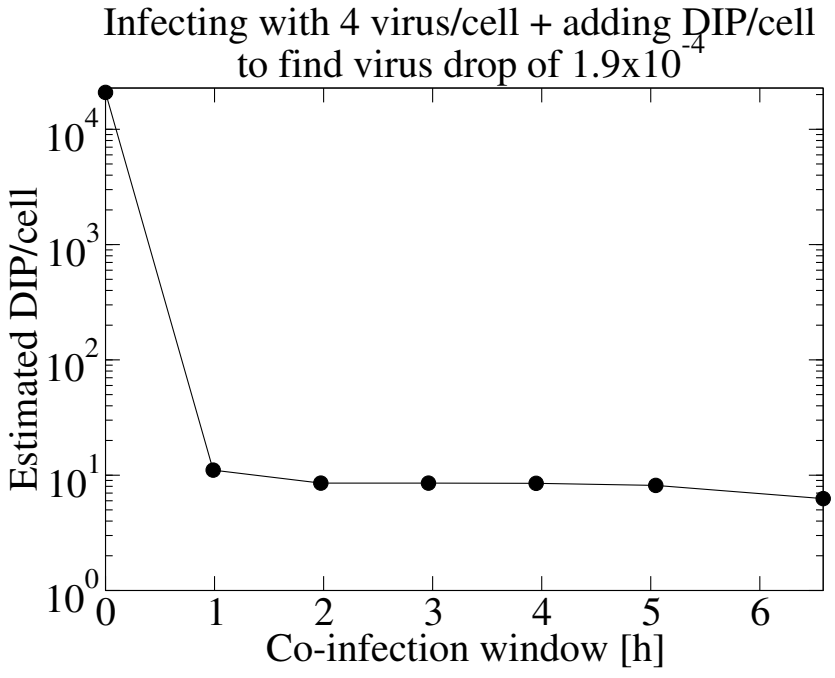


Our method proposes to use the drop in virus to estimate DIPs.

Adding DIP to achieve observed virus drop

Given a virus drop of 10^4, work backwards to find how much DIP was present in sample.



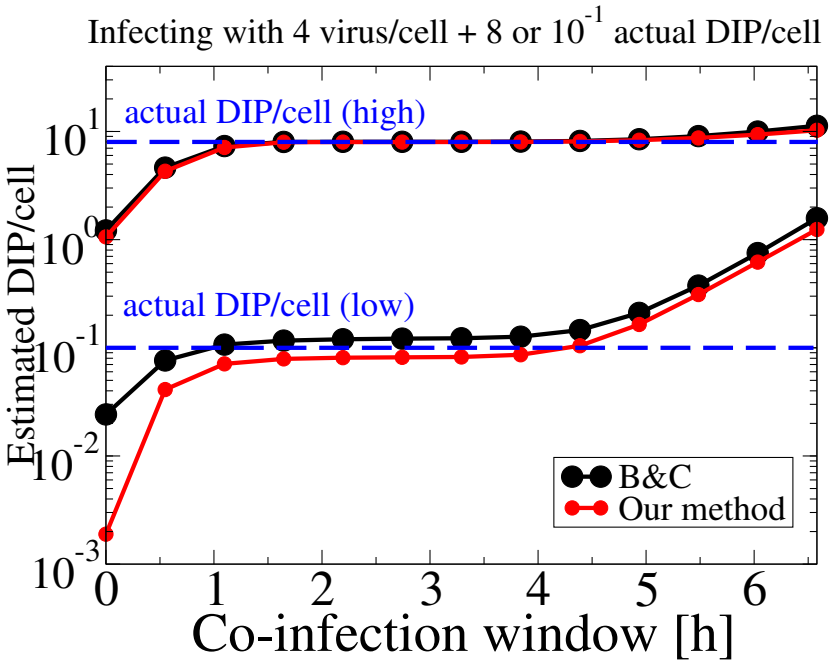


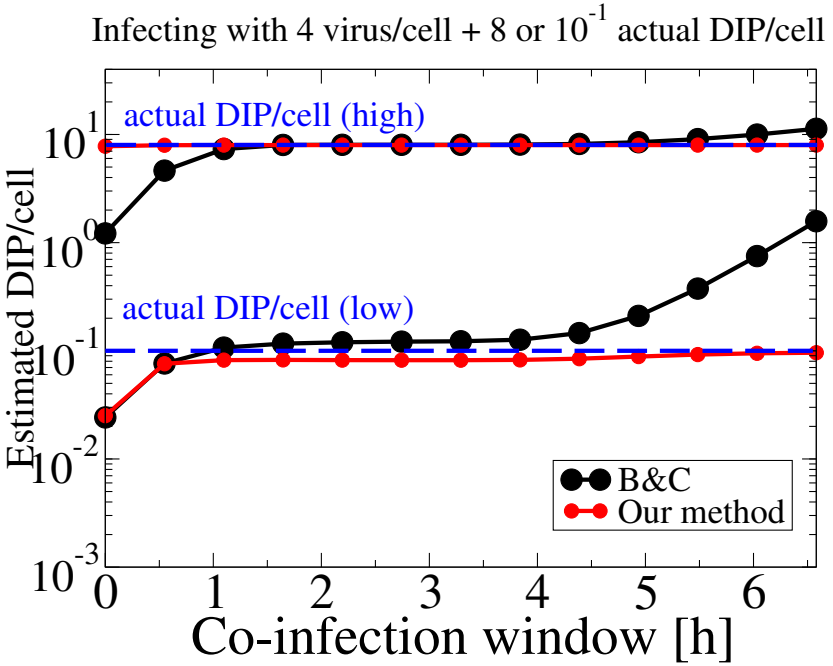
Our method estimates 8 DIP/cell that begin the infection, or 2 DIPs/virus present in the sample. Estimates consistent for all co-infection windows > 1 h.

Us vs. them

Infect with 4 virus/cell + 8 DIP/cell, varying co-inf window. Get virus drop as a fn of window. Fix window to 3 h, as in B&C (left); ask "How much DIP to add to achieve virus drop?".

- If co-inf window unknown, ours does just as well as B&C.
- If known, fix co-inf window to actual co-inf window (right). Ours performs better than B&C for long co-inf windows.





Conclusions on DIP counting

Have two methods (ours and B&C):

- if co-inf window not known, do as well as B&C.
- if co-inf window known, ours does better for long windows.
- ours does not use UV'd DIPs.

Validation — use both to count a sample:

disagree revise and test assumptions on DIP biology (exciting!).

agree evaluate how both methods fare with uncertainty in data.