# Density Dependence: A Nonlinear Analysis of HIV Syncytium in Drug-Treated Transfection

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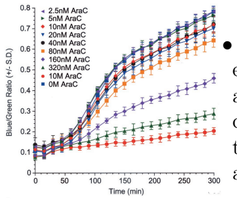
## Background

- HIV Type-1 is a virus that affects the immune system of millions around the globe.
- HIV Type-1 allows for infected cells to fuse with healthy cells, forming multi-nucleated cells called syncytia.
- HIV infected cells express viral glycoproteins that allow for binding to other cells expressing CD4 receptor, which then leads to syncytia formation.
- From assays based on syncytia spread, we can quantify characteristics like fusion times and syncytia formation rate.
- By looking at the inhibition of these glycoproteins via a chemical agent, we can see how much of a dependence syncytia formation has on viral glycoproteins.

## Cell Fusion Assay

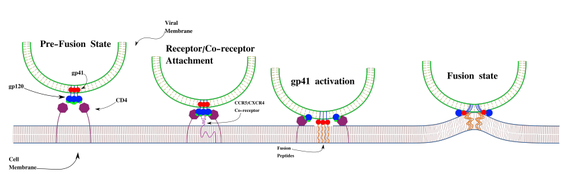



(Taken from Lineberger et al. (2002) J Virol.)

- Cell fusion assays use a donor cells expressing the viral surface protein and acceptor cells expressing the cell receptor to observe fusion, here the viral glycoproteins are gp120 and gp41.
- We used data from cell fusion assays performed by Lineberger et al. (2002) J Virol. that examined fusion for 2 HIV variants that bind in slightly different ways. This was done with different drug-dosages as seen in the data.

## Cell-cell fusion

- Glycoprotein expression in transfected cells will form clusters, which allows for cell-cell fusion with acceptor cells expressing receptor. The glycoprotein structure consists of a surface protein (gp120) and a transmembrane protein (gp41).
- Cytosine Arabinoside (AraC) was hypothesized to be used to limit the transcription and synthesis of gp120/gp41. This was done due to it's effect on the precursor protein, gp160, which then is synthesized into gp120/gp41.



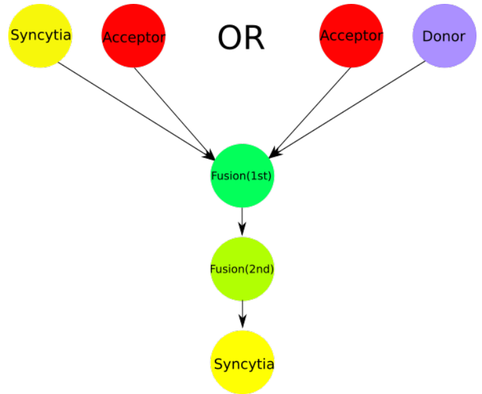

## Methods

- We fit the mathematical model to data using minimization of the sum of squared residuals. SSR is the following:

$$SSR = \sum_{i=1}^{n} (y_i - f(t_i))^2$$

- The syncytia formation rate, initial number of donor cells, and fusion times were estimated.
- Bootstrapping was used to estimate the posterior distributions of the free parameters.
- All code was written using python.

## Cell Fusion Assay Model

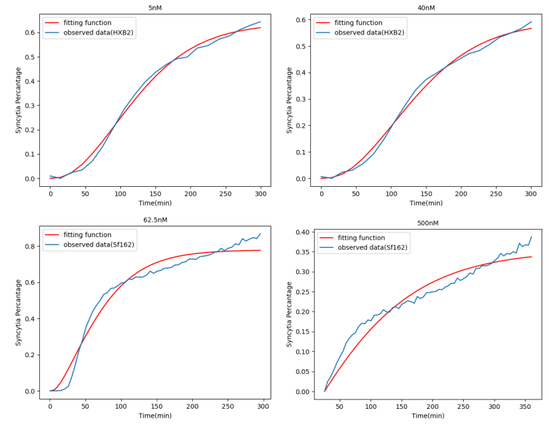



- We used a mathematical model to fit data from the cell fusion assay, with Erlang time distributed fusion states.

$$\begin{aligned}
\frac{\mathrm{d}D}{\mathrm{d}t} &= -\gamma DA \\
\frac{\mathrm{d}A}{\mathrm{d}t} &= -\gamma DA - \gamma SA \\
\frac{\mathrm{d}F_1}{\mathrm{d}t} &= 2\gamma DA + \gamma SA - kF_1 \\
\frac{\mathrm{d}F_2}{\mathrm{d}t} &= kF_1 - kF_2 \\
\frac{\mathrm{d}S}{\mathrm{d}t} &= kF_2.
\end{aligned}$$

- $D$ are the donor cells, $A$ are the acceptor cells, $F_1$ are the first fusion state, $F_2$ are the second fusion state, and $S$ are cells that have fused into syncytia.
- The syncytia formation rate is represented by $\gamma$, and fusion time is represented by $1/k$. These are two of the three parameters we fit for.

## Density Independent fits




The fits are not as great as they possibly could be, particularly the Sf-162 strain of HIV has bad SSR. The difference between model and data was enough to consider additions to the model.

## Density Dependence

- The difference in model and data was thought to have been related to spatial effects in viral spread, as donor cells run out and more syncytia form, the less of a chance there is for fusion to occur.

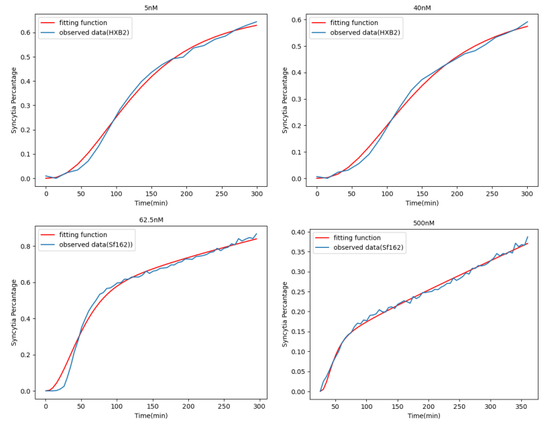
$$\gamma_d = \frac{\gamma}{1 - \alpha S}$$

- We implemented density-dependent syncytia formation, dependent on $\alpha$ and the amount of syncytia already present. If $\alpha$ is large, then as syncytia grows the formation rate decreases.
- To include $\gamma_d$ this in the model, we simply change the $\gamma$ in the ODE system to $\gamma_d$.

$$\begin{aligned}
\frac{dD}{dt} &= -\gamma_d DA \\
\frac{dA}{dt} &= -\gamma_d DA - \gamma_d SA \\
\frac{dF_1}{dt} &= 2\gamma_d DA + \gamma_d SA - kF_1 \\
\frac{dF_2}{dt} &= kF_1 - kF_2 \\
\frac{dS}{dt} &= kF_2
\end{aligned}$$

- This adds a new parameter to fit to, $\alpha$, which gives a measure of the amount of dependence of syncytia formation rate on density of syncytia.

## Density Dependent Fits




- These fits are improved from the original model fits, especially in the Sf-162 strains. This is also seen in a reduction in SSR, which improved by almost three times in some cases. (Example: 62.5nM AraC Sf162 independent: $1.221 \times 10^{-1}$ and 62.5nM AraC Sf162: $4.572 \times 10^{-2}$).)
- This leads us to assume there is density-dependence involved in our drug-treated system. This dependence rises as we increase the dosage of AraC, meaning that there is an even greater increase due to less expression of glycoproteins.

## Conclusions

- The formation of syncytia is affected by transcription inhibitor AraC. The inhibition of these glycoproteins leads to a significant change in syncytia formation rate.
- The analysis seems to show a density dependence in syncytia formation rate of HIV, especially when drug dosages are very high. This has not been investigated before, and could lead to better modeling of HIV.

## Future Directions

- Measure syncytia formation rate and fusion duration for different syncytia-forming viruses, like respiratory syncytial virus (RSV).
- Use an agent-based model, which includes explicit spatial dependence, to investigate syncytia formation.
- Perform imaging and experiments on syncytia to help validate models.
- Investigate temperature and pH dependence of parameters.