**Influenza Virus Structure and Pathways**

![Figure 1: A schematic of influenza virus infection in a host cell. The FluSim model and simulation closely mirror the pathways described above.](image)

**Influenza Virus Molecular Infection Model and Stochastic, Discrete-Event Simulation**

**Introduction**

Influenza virus is responsible for the greatest pandemic in human history causing 20 – 40 million deaths worldwide during the 1918 flu season. In 1997 fears of a future pandemic arose with the discovery of a new strain of H5N1 avian influenza. As we know the influenza virus is comprised of 8 segments coding for 11 known proteins. In figure 1 we see a graphical representation of the influenza virus life cycle within a single epithelial cell. Our model represents all 8 segments, 10 of the 11 proteins (PB1-F2 has been excluded) as well as intermediates such as cRNA and the vRNPs.

**Assumptions and Limitations**

- An excess of host cell components including nucleotides, amino acid molecules, ribosomes, others cellular molecules not directly modeled
- Stability of the host cell (no apoptosis or cell death)
- A lack of cross infection by multiple influenza virus to the same host cell.
- A negligible host immune response to influenza infection

**Model Design**

In an effort to better understand the dynamics of influenza infection, a new model of influenza virus infection at the molecular level has been developed. Comprising nineteen infection stages and six molecular classes (mRNA, cRNA, vRNA, vRNP, proteins and virions) the FluSim model includes all of the major discrete events in influenza infection beginning with virion binding, progressing through internalization, virion uncoating, vRNP nuclear import, mRNA transcription, cRNA replication, vRNA synthesis, viral protein translation, vRNP assembly, and ending with virion assembly and release. This intracellular infection model has been used as the framework for the implementation of a discrete event, stochastic simulation. The path of influenza virus infection and interplay between the various stages is shown in Figure 2.

**Simulation**

A discrete event, stochastic simulation method was chosen for this purpose because of its ability to mirror the true temporal and spatial nature of influenza infection, including the probabilistic treatment of the randomness apparent in biological systems at the molecular level. Simulation results are compared to experimental evidence (not shown) as well as an independent Gillespie simulation implementation for qualitative and quantitative validation of the results. The expected molecular counts match this validation at reasonable accuracy.

The simulation was implemented using the SimJava V2 simulation framework.

**Results and Future Work**

mRNA transcription shows mRNA counts are close to what is expected ("A" vs "B") but timing is off. The peak occurs experimentally at ~4.5 hpi. This is due to the reduced number of precursor mRNA in the system. (4,400 vs ~44,000 assuming MOI of 5). This was done to make the simulation run in a reasonable time. Figure "B" is produces using Dizzy software. cRNA synthesis rates ("C") approximately match Gillespie results ("D") while remaining approximately 10 fold lower then vRNA replication counts as experimentally observed. vRNA replication ("E") begins ~1 hpi and continues to climb for the remaining of the simulation as echoed in the Gillespie results ("F"). While PB2 vRNA replication shows an exponential like growth the remaining vRNAs show slower then expected replication. Taking an average of multiple runs of FluSim, while changing the initial random number seed, should give a better representation of the vRNA replication behavior.

**Proteins** ("G") and vRNP ("H") production offer, for the first time, a glimpse of what the system will do with the current rules in place.

While there is much work to be done but we have succeeded in creating the first molecular infection model of influenza virus infection.