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When a virus binds to a cell, the cell engulfs it into an endosome that becomes acidic over time due to proton pumps (Fig. 3). At a low pH, HA undergoes conformational changes that promote membrane fusion and allow viral RNA to enter the host cell for infection (Fig. 4).

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**Introduction**

The influenza pandemic of 1918 is responsible for an estimated 50 million deaths. Despite nearly a century long research on the Influenza virus, much is unknown about its fusion kinetics and factors that make it pandemic and virulent. The virus contains a lipid bilayer at the outer surface, which contains membrane proteins called hemagglutinin (HA). HA governs the binding and fusion behaviors of virus to the cell (Fig. 1).

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**Objectives**

- To develop a virus fusion assay that utilizes confined micro-volumes and supported lipid bilayers (SLB).
- To test the viability of TBHQ as a fusion inhibitor.
- Characterize the fusion kinetics of X31 influenza virus.

**Experimental Methods**

**Single-particle fusion assay**

Fluorescently-labeled viruses are attached to an artificially-made bilayer supported on a glass surface (supported lipid bilayer). Fusion is detected when the viral membrane fluorophores dequench upon lipid mixing with the bilayer (Fig 6). Rapid acidification is achieved by photolysing o-nitrobenzaldehyde with a UV laser, which releases protons (Fig 7).

**Results & Data Analysis**

More than one HA is believed to act together in order for a fusion event to occur, due to the high energy barrier hindering the penetration and bending of a bilayer. The probability that N number of HA will act at the same time at a rate k to achieve fusion can be described by a gamma distribution. Fusion data are fitted to this distribution function, as shown below in Fig. 10.

**Discussion**

- Compared to literature, at pH 3, the N is between 1 to 2 and k is between 0.08/s to 0.11/s. Our data matches this.
- Little or no effect of hydroquinone on fusion kinetics for those viruses that did fuse. However, more data is needed to see if TBHQ affects the “fraction” of viruses that fuse, which may be independent of fusion kinetics.
- The effectiveness of TBHQ on inhibiting fusion may depend on other factors, such as temperature, TBHQ concentration, and incubation time.

**References**


**Acknowledgements**

Funded by G-K-12 NSF and BME department at Cornell