

Stochastic Modelling of Influenza Virus Fusion

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1 Introduction

Influenza virus binds to the receptors (R) of the host cell plasma membrane via its spike glycoprotein hemagglutinin (HA). After uptake of influenza virus into a host cell by the endocytic route the membrane of influenza virus fuses with the endosomal membrane of the host cell in order to release the viral genome. Membrane fusion and pore formation are also mediated HA.

Here we present stochastic simulations of HA-mediated fusion pore formation based on numerical solutions of a Master equation to predict the statistical distribution of various time-dependent stages in pore formation, namely the formation of the initial ion-permissive pore (IP) and of the subsequent lipid-permissive pore (LP), and to compare with experimental data [1]. The elementary steps of the fusion process are taken into account as the lateral diffusion of HA-trimers and receptors, the aggregation of HA-trimers to immobilized clusters, the reversible formation of HA-receptor contacts, and the irreversible conversion of HA-receptor contacts into stable links between HA and the target membrane.

2 Method and Results

Regions of the viral and the target membrane involved in a contact site are represented as continuous two-dimensional lattice constituted by small squared membrane units ('unit cells', Fig. 1) with an edge length of 6 nm which corresponds approximately to the spatial extension of HA trimers. Lateral diffusion of HA trimers and receptors is modeled by random transitions between adjacent cells of the simulation lattice [2].

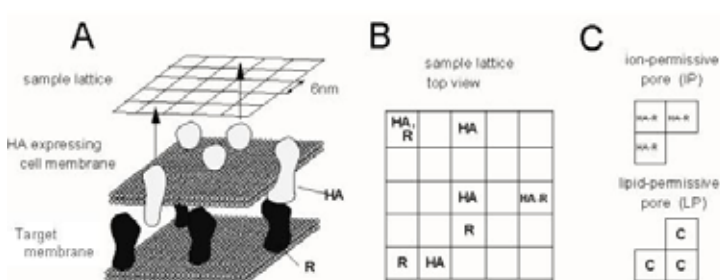


Figure 1. *The simulation lattice and model variables.* (A) The contact area between fusing cells. (B) Occupation state of the simulation lattice derived from the constellation in panel (A). Generally, a unit cell of the simulation lattice can be occupied by one of the shown variables.. (C) Definition of an ion-permissive pore (IP) and a lipid-permissive pore (LP). **HA**: Fusion activated hemagglutinin trimer; **HA-R**: HA-receptor contact; **HA***: Immobilized HA trimer captured in an HA cluster; **C**:

HA-receptor-membrane link; **IP**: Ion-permissive early fusion pore; **LP**: Lipid-permissive pore; Modified according to [2].

The cumulative distribution function $F_{\text{tot}}(t)$ for any fusion intermediate to occur within the time span t in the effective contact area between two fusing cells can be related to the corresponding distribution function $F_{\text{sample}}(t)$ for a simulation lattice by

$$F_{\text{tot}}(t) = 1 - [1 - F_{\text{sample}}(t)]^N \quad (1)$$

where N refers to the number of simulation lattices required to cover the effective contact area. The chain of events leading to the formation of a fusion pore between individual cells defines a Markov process which is governed by a Master equation. Let $p_{i,j}^X$ denote the probability to find a molecule of type X ($X = \text{HA}, \text{R}, \text{HA}^*, \text{HA-R}, \text{C}$) in the unit cell (i,j) at time t . The subscripts i and j ($i,j = 1, \dots, 50$) label the row and column of the simulation lattice. Generally, the time-evolution of the probability $p_{i,j}^X$ is governed by the Master equation

$$\frac{\partial p_{i,j}^X}{\partial t} = \sum_{i',j',X'} A(i,j,X \leftarrow i',j',X') p_{i',j'}^{X'} - \sum_{i',j',X'} A(i',j',X' \leftarrow i,j,X) p_{i,j}^X \quad (2)$$

The linear time-evolution operator $A(i,j,X \leftarrow i',j',X')$ gives the probability with which occupation of the unit cell (i,j) by the molecular species X is affected by the molecular species X' resident in the unit cell (i',j') .

From repeated simulations on a 50x50 simulation lattice we constructed the statistical distributions of the characteristic time span needed for the first occurrence of an IP pore and of a LP pore. The obtained theoretical distributions of IP and LP formation are both in reasonable concordance with the measurements [1] (Fig. 2)

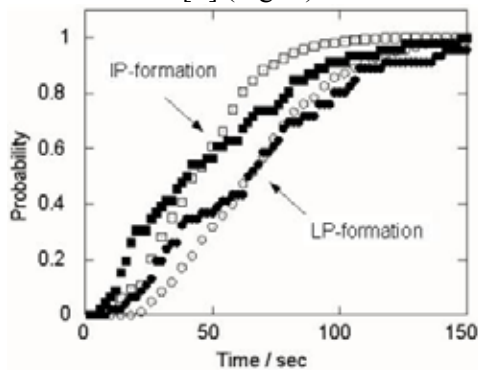


Figure 2 *Kinetics of single-cell fusion*. Cumulative distribution of delay times for IP-events and LP-events (first occurrence of a lipid-permissive pore). Closed symbols - experimental data of [1]. Open symbols - simulated distributions

Currently, we develop a user friendly program package which allows simulation of any of those lattice based systems including variable size of lattice, visualization of trajectories, statistics, etc (Kamm et al, in preparation).

3 Discussions

We have developed a mathematical model which explicitly takes into account the stochastic nature of the molecular events underlying the fusion process. A particular advantage of our approach is the possibility to model fusion signals measured in cell suspensions as a superposition of stochastic fusion signals arising from single-cell fusion events. This model can also be applied to fusion pore formation of other enveloped viruses is also applicable to other types of membrane-membrane interactions as, for example, cell-cell attachment [2].

References

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- [2] Schreiber, S., Ludwig, K., Herrmann, A., and Holzhütter, H.-G., Stochastic simulation of hemagglutinin-mediated fusion pore formation, *Biophysical J.* 81, 1360-1372, 2001.