BARRY M. GOLDWATER SCHOLARSHIP
AND EXCELLENCE IN EDUCATION PROGRAM

Nominee's Essay

Discuss a significant issue or problem in your field of study that is of particular interest to you.

Your essay must include a description of the issue or problem, discuss an idea for research that could have significant impact on the issue or problem, describe an aspect of the research in which you would be involved, and explain the relevance of the issue or problem to you as a mathematician, scientist, or engineer. The content and style of your essay will be important to the success of your scholarship application. Assume that your reader is knowledgeable in mathematics, science, and engineering; and will have the expertise to read, review, and understand the complexities of your field of specialty.

If your essay involves research in which you are or were involved, please indicate if you are or were the sole researcher or if you collaborated with another individual.

Include a bibliography, references, or illustrations, when appropriate, as part of the essay. Font size may not be smaller than 12 characters per inch, or 11 point. Your essay must be typed and confined to two pages (one side only). **Be sure to include your signature at the end of your essay.**

Title: Sindbis virus infection visualized through single-particle fluorescence

Sindbis virus infects both mammalian and insect cells. Although not pathogenic in humans, Sindbis is a model for many mosquito-borne viruses that do cause human disease, such as West Nile virus. Understanding fundamental aspects of the infection process of Sindbis virus will ultimately lead to progress in the treatment and prevention of human diseases caused by other related viruses. It will also help to further our general understanding of viral infection, an understanding that is essential for improved development of anti-viral therapies.

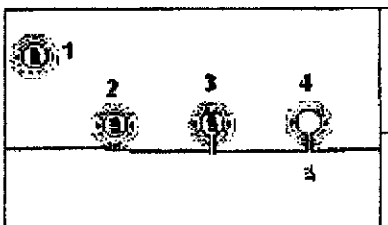

Figure 1 Protein pore infection model (*1*) Sindbis virion; (*2*) binding to cell membrane; (*3*) creation of protein pore; (*4*) release of RNA.

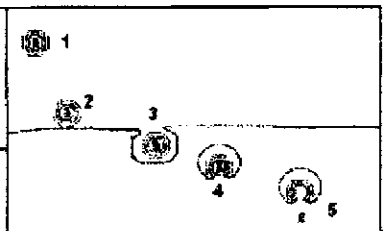

Figure 2 Endocytotic pathway infection model (*1*) Sindbis virion; (*2*) binding to cell membrane; (*3*) endocytosis; (*4*) membrane fusion; (*5*) release of RNA.

After viruses bind to the surface of a cell, there are two general mechanisms they use to deliver genetic material into the cell: 1) direct penetration of the external cell membrane by either a protein pore or membrane fusion; or 2) internalization via endocytosis (see figures 1 and 2). More detailed knowledge of these two pathways will be applicable to many other viruses in addition to Sindbis. While working for [redacted] in the Physics Department at [redacted] and with [redacted], professor of Biochemistry, and [redacted] a graduate student in Biochemistry, I have used single particle fluorescence microscopy to observe individual Sindbis viruses as they infect cells in order to distinguish between these two infection models. The power of this technique is that it allows us to visualize infection in real-time. Thus far, we have incorporated fluorescent labels in the viral coat proteins and the lipid envelope, and we have used this labeled virus to infect live baby hamster kidney (BHK) cells. This technique has enabled us to measure a range of viral mobilities, including free diffusion in solution, slower diffusion inside cells, sticking to spots on the cell surface, and directed motion characteristic of motor protein transport (see figure 3). We have also observed some evidence of virus-cell membrane fusion (see figure 4).

There are several ways in which I am interested in developing this research to elucidate the infection mechanism for Sindbis virus. First, we have begun to label Sindbis with multiple types of fluorescent labels simultaneously. This has several advantages. When we can see fluorescence from multiple labels, we have additional verification that we are looking at viral particles. In addition, the lipid envelope label is quenched, so in the event of membrane fusion its quenching will decrease, resulting in increased

fluorescence. However, changes in intensity can also occur when particles move vertically in and out of the focus field or if one particle enters on top of another. Since the intensity of the protein label will not change with membrane fusion, using both labels simultaneously allows us to distinguish between change in fluorescence intensity due to movement of particles and due to membrane fusion.

One of our early results is that most of the diffusivities we have measured for Sindbis virus indicate much less mobility than is measured in similar experiments with viruses that exhibit endocytosis. For example, Lakadamyali, et al., have studied influenza (1), which is well known to infect via endocytosis. The minimum diffusivity constant they measure is 0.001 $\mu m^2/s$, while, with some exceptions, the particles we have observed have diffusivity constants of at least an order of magnitude slower than that. These data suggest that we are seeing viral particles stuck to the cell surface rather than undergoing endocytosis, supporting the protein pore model. To confirm this, we will employ fluorescent probes designed specifically for observing endocytosis. We will then be able to directly compare the motion of these probes in the same cellular system as Sindbis virus.

Figure 3 Types of observed motion (*1*) *Constrained Motion.* DIC image of BHK cell with overlay of the time trace lasting about 600 s. This virus appears to be constrained, and has a diffusivity constant of 0.0003 $\mu m^2/s$. (*2*) *High mobility.* DIC image of BHK cell with overlay of time trace lasting about 550 s. This virus is much more mobile than most, with a diffusivity constant of 0.015 $\mu m^2/s$. (*3*) *Directed Motion.* DIC image of BHK cell with overlay of time trace lasting about 600 s. Each image is approximately 24 by 24 μm.

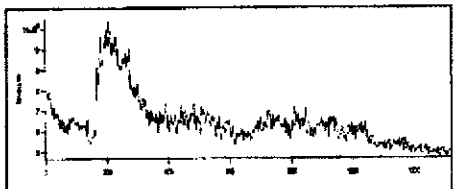

Figure 4 Self quenched membrane label The sudden increase in fluorescent intensity at about 200 s may indicate membrane fusion. The following decrease in intensity is consistent with photobleaching.

In addition to comparing the motion of Sindbis virus in cells to that of other endocytosed substances, we will track the RNA of Sindbis virus with a label such as SYTO (Molecular Probes). SYTO is a membrane permeable, intercalating nucleic acid probe, whose fluorescent quantum yield increases dramatically when bound to RNA. Labeling the RNA will act as another test of the protein pore model, which predicts that the genetic material is injected at the surface of the cell. By labeling the RNA in addition to our existing label of the protein shell, we will be able to monitor the motion of the RNA in relation to the protein shell, as well as the motion of the RNA once inside the cell. If the protein shell remains on the cell surface while the viral RNA moves away from it, this would further support the protein pore model. Observation of endocytotic probes and labeling the viral RNA should help us to further analyze both possible infection mechanisms.

This work has other possible applications beyond the understanding of the infection of this specific virus and the development of viral treatments for similar viruses. For example, it may further develop knowledge of membrane fusion, a possible step in the infection pathway that is also involved in numerous biological processes, including neurotransmitter release, gamete formation in sexual reproduction, and, of course, other viral infections. In addition, the comparison of infection mechanisms among different viruses may improve understanding of viral evolution. Finally, this technique of using fluorescent probes to study viral infection in real-time is new and is still being refined. Our work in developing this technique will allow others to apply it to the study of additional viruses.

1. Lakadamyali, M., Rust, M. J., Babcock, H. P. & Zhuang, X. W. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 9280–9285.

Nominee's Signature [redacted] Date [redacted]