HAJIM SCHOOL OF ENGINEERING & APPLIED SCIENCES
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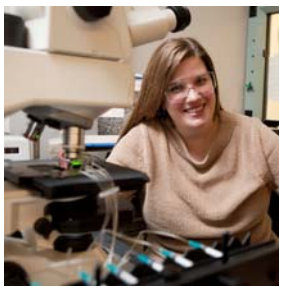

A Single Particle Approach for the Study of Viral Entry Kinetics

Wednesday, February 13, 2013
2:15 p.m.
101 Goergen Hall

Influenza is a membrane-enveloped virus, which infects a host cell through the endocytotic pathway. An essential viral coat protein, hemagglutinin, is responsible for both the attachment of the virus to the host cell (binding) and the fusion of the viral membrane with the host membrane. The virus binds to a glycolipid containing sialic acid groups present on the surface of many host cells, which initiates its uptake into an endosome. Once inside the endosome, the viral membrane must fuse the endosomal membrane in order to delivery its genetic material to the cytosol for replication. In nature, viral fusion to the endosomal membrane is initiated by a conformational change in hemagglutinin, triggered by acidification of the endosome.

Different strains and serotypes of virus can have markedly different binding and fusion characteristics and characterizing this behavior is important for basic biological studies of virus entry, identifying new anti-viral drugs that target viral entry processes, and creating new diagnostic tools for differentiating virus types. The development of such platforms requires an appropriate mimic for the host cell surface that presents the receptors for viral interaction and a strategy to acidify the system to mimic the drop in pH inside the endosomal compartment to initiate membrane fusion. We developed an in vitro method for assaying binding and fusion of a single virion particle using an individual virion imaging technique and stochastic analysis of data. In this work, we mimic the host membrane chemistry in a supported bilayer coating the walls of a microfluidic device. The physicochemical properties of the bilayer can be controlled to present different receptors and surface features to modify virus interaction. In this work, we monitor residence times of viruses bound to the membrane using total internal reflection microscopy, hemifusion (the merging of the two outermost lipid leaflets) using fluorescence dequenching, and the formation of fusion pores by release times of fluorescently-labeled viral contents. The increased sensitivity gained by monitoring individual events can facilitate comparison of binding and fusion kinetics between different influenza strains to better characterize and diagnose mutants and identify host membrane properties that enhance viral entry processes and infection.