# Studying Viral Binding and Fusion Mechanisms with Host Cell Membranes

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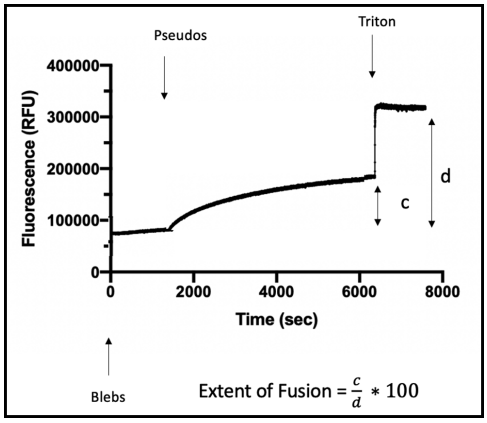
Primary CNF Tools Used: Malvern NS300 NanoSight

## Abstract:

**The current COVID-19 pandemic caused by the SARS-CoV-2 virus led to millions of deaths worldwide [1]. This virus is not the first of its kind; the SARS and MERS outbreaks in 2003 and 2012, respectively, were also caused by coronaviruses [1]. Additionally, the flu that we experience every year caused by the influenza virus continues to spread and we have yet to come up with a permanent solution for this viral infection [2]. Given the continuing threat these viruses pose to human health, our lab is focused on understanding the mechanisms of viral entry to determine antiviral targets. One such target is the viral fusion mechanism mediated by the spike protein in the case of coronaviruses and hemagglutinin (HA) in the case of influenza virus [1,2]. By fluorescently labelling these viruses or pseudoparticles, safer surrogates to live virus particles, we can study how they fuse with synthetic liposomes that contain receptors for the spike and HA proteins [3]. Additionally, the Daniel lab uses a chemical method to make blebs or vesicles that bud off cell membranes and can better mimic the cell membrane composition and be used for such fusion experiments [4]. These methods and experiments will provide a fundamental understanding of these viral entry events that in turn will enable us to be better prepared for future pandemics.**




## Summary of Research:

In order to test fusion between virus or viral like pseudoparticles with liposomes or cell membrane blebs, we use a method known as Ensemble Fluorescence Assay or bulk fusion assay. We label the membrane of either virus or host-cell mimic with a lipophilic dye that is self-quenched at high concentrations and then dequenches once fusion occurs. This helps us measure the extent of fusion when all the fluorescent dye is released on adding a detergent. However, in order to optimize the ratio between virus and membrane-mimic particles we rely heaving on dynamic light scattering and the NanoSight, which gives us information regarding the size, homogeneity and concentration of these particles. This concentration is critical in ensuring that we are able to get the best possible fluorescence signal and measure fusion events.

## References:

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[3] Millet JK, Tang T, Nathan L, Jaimes JA, Hsu HL, Daniel S, Whittaker GR. Production of Pseudotyped Particles to Study Highly Pathogenic Coronaviruses in a Biosafety Level 2 Setting. J Vis Exp. 2019 Mar 1;(145):10.3791/59010. doi: 10.3791/59010. PMID: 30882796; PMCID: PMC6677141.

[4] M.J. Richards, C.-Y. Hsia, R.R. Singh, H. Haider, J. Kumpf, T. Kawate, and S. Daniel. Membrane Protein Mobility and Orientation Preserved in Supported Bilayers Created Directly from Cell Plasma Membrane Blebs. Langmuir 2016 32 (12), 2963-2974. DOI: 10.1021/acs.langmuir.5b03415.