MAD CITY LABS INC.

Understanding Virus Mechanisms – One Particle At A Time

SINGLE-MOLECULE MICROSCOPY TECHNIQUES FACILITATE DIRECT STUDY OF MOLECULAR MECHANISMS, ENABLING LEAPS IN UNDERSTANDING SURROUNDING HOW VIRUSES ASSEMBLE, DISASSEMBLE, AND INTERACT WITH THEIR HOSTS.

Tijana Ivanovic, Ph.D., is an Assistant Professor of Biochemistry at Brandeis University (Waltham, MA) and Principal Investigator in the Ivanovic Lab, whose current research focuses on diverse virus pathogens and nonpathogenic model systems.

"We try to dissect cell entry mechanisms and describe the relationship between virus particle structure/organization and the early steps of infection, including virus particle sensitivity to immune system antibodies or manmade inhibitors," Ivanovic says of her 10-person team's research. "Once a virus is inside a cell, all hell breaks loose and too many complexities are introduced. Before the virus enters the cell, we have a convenient spot to prevent virus infection. You simply need to understand how the virus particle works and how it interfaces with the host."

Ivanovic followed her undergraduate degree by studying HIV cell entry as a lab technician at Aaron Diamond AIDS Research Center. She then studied virology in the lab of Max Nibert, Ph.D., M.D., at Harvard Medical School, examining reovirus[1] membrane penetration. Ivanovic remained at Harvard Medical School as a post-doctoral fellow under Stephen C. Harrison, Ph.D., at which point she started exploring more deeply biophysics and single-particle virus imaging.

"I'm integrating all of those various experiences [and model systems] now in my own lab. It's a very interdisciplinary group. We apply biological experiments, as well as cutting-edge imaging and analysis by physical approaches," Ivanovic says.

Tools and Techniques

Working on reovirus, the Ivanovic Lab team developed a way to study its assembly on single-molecule microscopes.

"We can watch a [nonenveloped] virus particle come together and we can watch it disassemble. And, for enveloped viruses, we dissect the mechanisms of membrane fusion," Ivanovic says.

Among the laboratory's tools used for this purpose are a pair of Mad City Labs RM21® microscopes that incorporate the Micromirror TIRF (Total Internal Reflection Fluorescence) technique and high-precision nanopositioning. These microscopes' ability to provide strong signal-to-noise ratios over multiple wavelengths (providing multicolor imaging) is vital to the study of these viral processes. Additionally, no other microscope comes equipped for sub-nanometer positioning, which is critical in single-molecule applications, where objects of interest often are separated by only a few nanometers.

"We build our instrumentation around [Mad City Labs'] platform and use the MadView™[2] to view split channels by color so we can send each channel to different parts of our CCD camera," Ivanovic explains. "For example, in one channel, we can get information on the particle size and, in another channel, we can watch the process of membrane fusion."

Using this instrumentation, Ivanovic Lab researchers dissect the mechanisms of membrane fusion for enveloped viruses, as well as membrane penetration for non-enveloped viruses. They can watch individual virus particles undergo different steps in the process of cell entry, observing intermediates that are otherwise "averaged out" in traditional experiments.

"This platform allows us to have multicolor imaging. We can not only dissect and see intermediates of a process, we can — at the same time, for example — quantify certain structural features for our virus particles or quantify how they're interacting with antibodies or inhibitors," Ivanovic explains. "We can look at a population of virus particles and identify it as pleomorphic – meaning particles may vary in shape and size. If you have a fluorophore attached to a virus particle in a way that scales with particle size, you can get information about particle size at a per-particle level, watching how size affects these processes."

Further, the Ivanovic Lab's instrumentation allows researchers to record movies of these processes over time, allowing for more dynamic study. The researchers can identify intermediates and group them based on features the system measures simultaneously: particle size, glycoprotein density, or how many inhibitors/antibodies they have bound to them.

In addition to observing virus' cell entry mechanisms, Ivanovic Labs researchers use their RM21® system to study virus mechanics.

[1] Reovirus is an animal virus, which is not enclosed in a lipid bi-layer.
[2] The MadView™ is an image splitter and RM21® accessory that allows up to four images to be collected.

"We look at how the virus assembles itself, again, using different colors we can observe simultaneously. For example, if you have a virus core particle labeled with one fluorescent dye and an outer capsid molecule labeled with a different fluorescent dye, we can see the secondary fluorescence when those two colors come together, meaning you can watch a virus particle assemble in real time," Ivanovic says (Figs. 1 & 2).

"We can also watch its disassembly, which typically accompanies cell entry and membrane penetration. Seeing multiple components simultaneously gives us a lot of flexibility to gather different kinds of information at each point in time."

Ultimately, by answering fundamental questions about the viral fusion mechanism and what available options a virus has to change, it is becoming possible to think a step ahead of the virus to devise novel treatment approaches.

Called To Battle: COVID-19

Ivanovic Lab researchers are evolving methods and developing model systems to study COVID-19 (SARS-CoV-2), a biosafety level (BSL) 3 pathogen, despite operating in a BSL-2 lab.

"One way is studying human coronavirus NL63, a BSL-2 coronavirus. We are developing a system that will allow us to swap NL63's

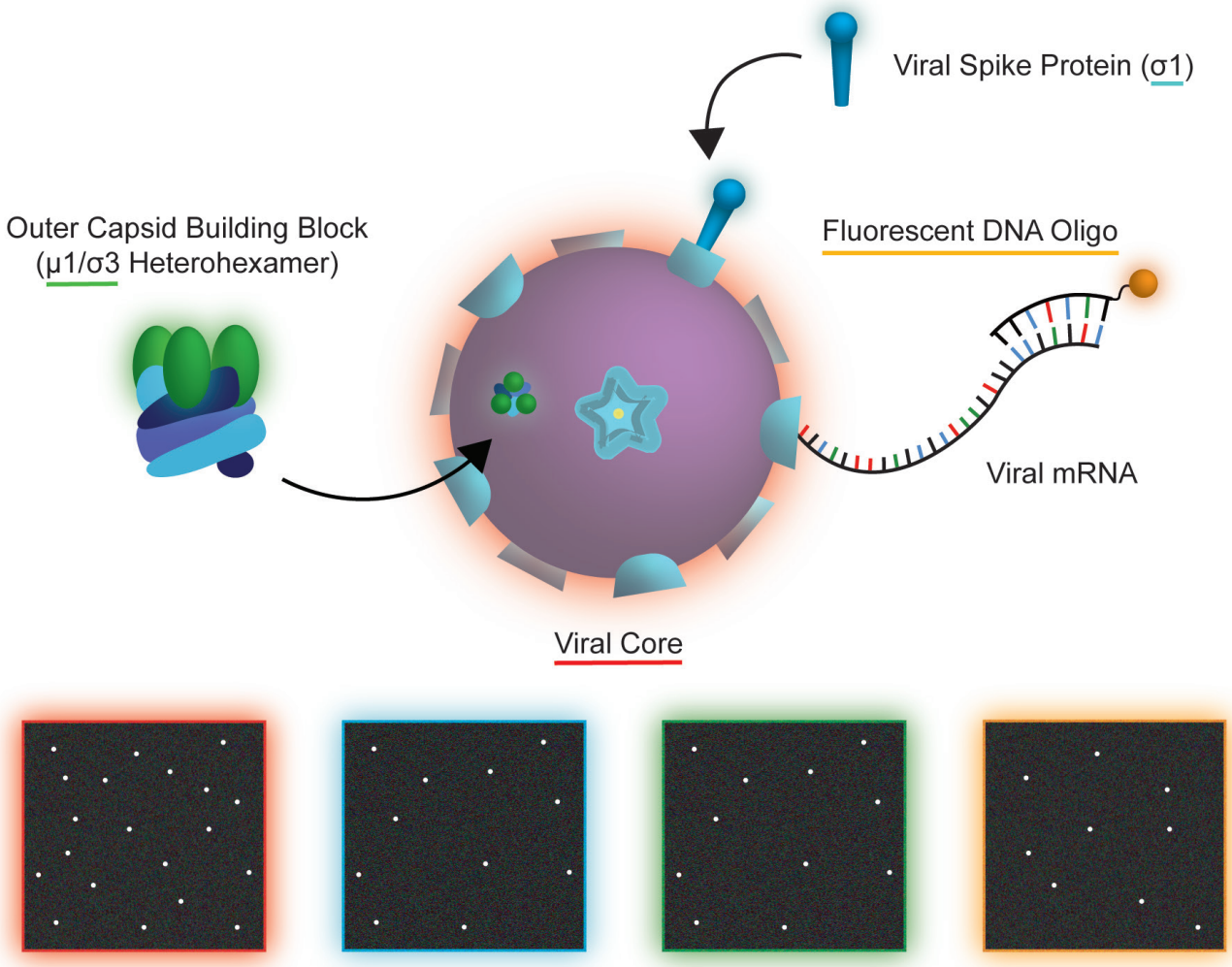

Fig1: *A cartoon depicting the principle of a 4-color version of this experiment. The virus components are labeled with different fluorescent dyes: viral core (red), viral spike protein (blue), outer capsid (green), viral mRNA (orange).*

[2] The MadView™ is an image splitter and RM21® accessory that allows up to four images to be collected.

cell entry protein with the cell entry protein of SARS-CoV-2, and then we can study it on this platform," Ivanovic explains. "We're also building virus-like particles. These are non-pathogenic versions of SARS-CoV-2, which means you can assemble the virus particle, but it will not have the genome inside, despite looking and functioning like an authentic virus particle on the outside."

Ivanovic continues, "It's critical to study these processes in the context of authentic structure, because there's a lot of contributions to the function of how the particle built itself."

For example: HIV, influenza, and coronaviruses are seemingly similar systems — they all use class I fusion proteins to mediate membrane fusion. However, HIV and coronaviruses assemble into compact virus particles, while influenza forms filamentous particle structure (with lengths ranging from ~50 nm to ~30 µm). HIV displays very few fusion proteins on its surfaces, while influenza and coronaviruses display hundreds of densely packed proteins on their surfaces. These structural features confer and/or reflect critical functions. The Ivanovic lab has recently shown that pleomorphic particles permit adaptation to any (including extreme) cell-entry pressure which has implications for the fusion mechanism.[3]

Building A Lab – And Rebuilding It

Mad City Labs helped set up the Ivanovic Lab's instrumentation and helped train lab members in its use. Mad City Labs also can assist system design based on user needs — a boon to researchers who lack experience in implementing such a system.

"Each time you're building a new system, you're doing something a little different, a little better, a little more convenient for your application as your experiments change and evolve," Ivanovic says. "First, you figure out what your experiment is — what must your system be capable of? A good strategy is to just talk to people who have built similar systems. Understanding setups others have devised, as well as their goals for those systems, can give you ideas you may not have thought of."

Ivanovic also suggests researchers take advantage of coming into a lab with an existing system by taking the time to learn its ins and outs, promoting creative approaches to solving scientific problems.

"[Researchers in the Ivanovic Lab] already use these systems and can participate in aligning, realigning, rebuilding, updating, etc.," Ivanovic says. "My goal is to have really motivated students and post-docs be able to take these systems apart and put them back together."

Conclusion

Single-molecule approaches enable direct study of molecular mechanisms by allowing the measurement of individual molecular "trajectories" within a population. They obviate the need

Viral Cores | µ1/σ3 Heterohexamers | Viral Cores and µ1/σ3 Heterohexamers

Fig 2: *Experiment data set demonstrating efficient particle (two-color) assembly on the microscope slide. All images courtesy of Ivanovic Lab, Brandeis University, Biochemistry Department*

[3] Phenotypic heterogeneity in particle size is a viral mechanism of persistence. Tian Li, Zhenyu Li, Erin E. Deans, Eva Mittler, Meisui Liu, Kartik Chandran, Tijana Ivanovic. bioRxiv 843177; doi: https://doi.org/10.1101/843177

for synchronization and indirect inferences inherent in ensemble-averaged approaches.

In the Ivanovic Lab, where researchers have studied viruses ranging from Ebola to COVID-19, Mad City Labs' instrumentation and assistance have enabled leaps in understanding surrounding how viruses assemble, disassemble, and interact with their hosts. Dr. Ivanovic can be reached at ivanovic@brandeis.edu.

Resources

- *Drier, Eric A. "Fluorescence Microscopy Technique Provides New Views Of Biological Processes" Photonics Online, Mad City Labs, 29 Nov. 2017, https://www.photonicsonline.com/doc/micromirror-tirf-microscopy-technique-and-applications-0001*
- *Con Foo, Jenice. "Is A DIY Microscope For You?" Photonics Online, Mad City Labs, 14 Jan. 2020, www.photonicsonline.com/doc/is-a-diy-microscope-for-you-0001*

About Mad City Labs

Mad City Labs designs and manufactures a complete product line of high-precision piezo nanopositioners, micropositioners, atomic force microscopes, and single molecule microscopes. We provide innovative instrument solutions from the micro- to pico-scale for leading industrial partners and academic researchers. Visit www.madcitylabs.com or email mclgen@madcitylabs.com for more information.

MCL®

MAD CITY LABS INC.