

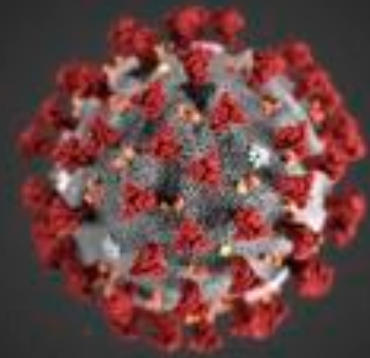
# Going Viral: Stealthy but deadly virus with a serious sweet tooth

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Melissa Maginnis, Ph.D.  
Molecular and Biomedical Sciences



# COVID-19 Outbreak



SARS-CoV2  
2019-nCoV



**COVID 19** STOP THE SPREAD OF GERMS

Help prevent the spread of respiratory diseases like COVID-19.

- Avoid close contact with people who are sick.
- Cover your cough or sneeze with a tissue, then throw the tissue in the trash.
- Avoid touching your eyes, nose, and mouth.
- Clean and disinfect frequently touched objects and surfaces.
- Stay home when you are sick, except to get medical care.
- Wash your hands often with soap and water for at least 20 seconds.

For more information, [www.cdc.gov/COVID19](http://www.cdc.gov/COVID19)

# Emerging and Re-emerging Viruses

Swine Flu



Ebola virus



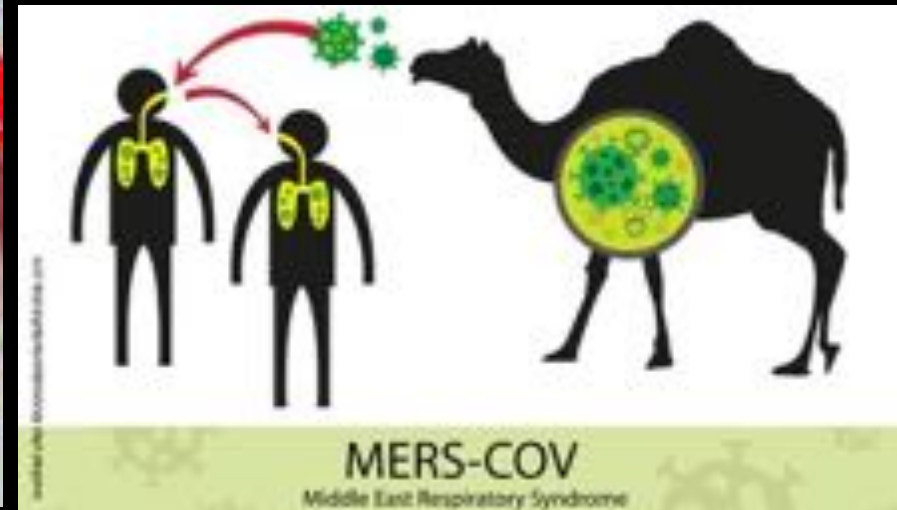
Zika virus



SARS Coronavirus

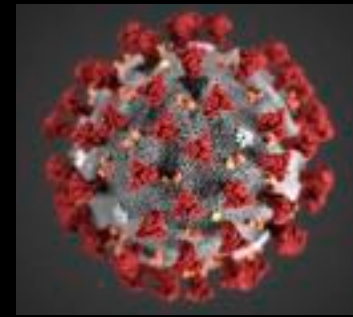


MERS Coronavirus





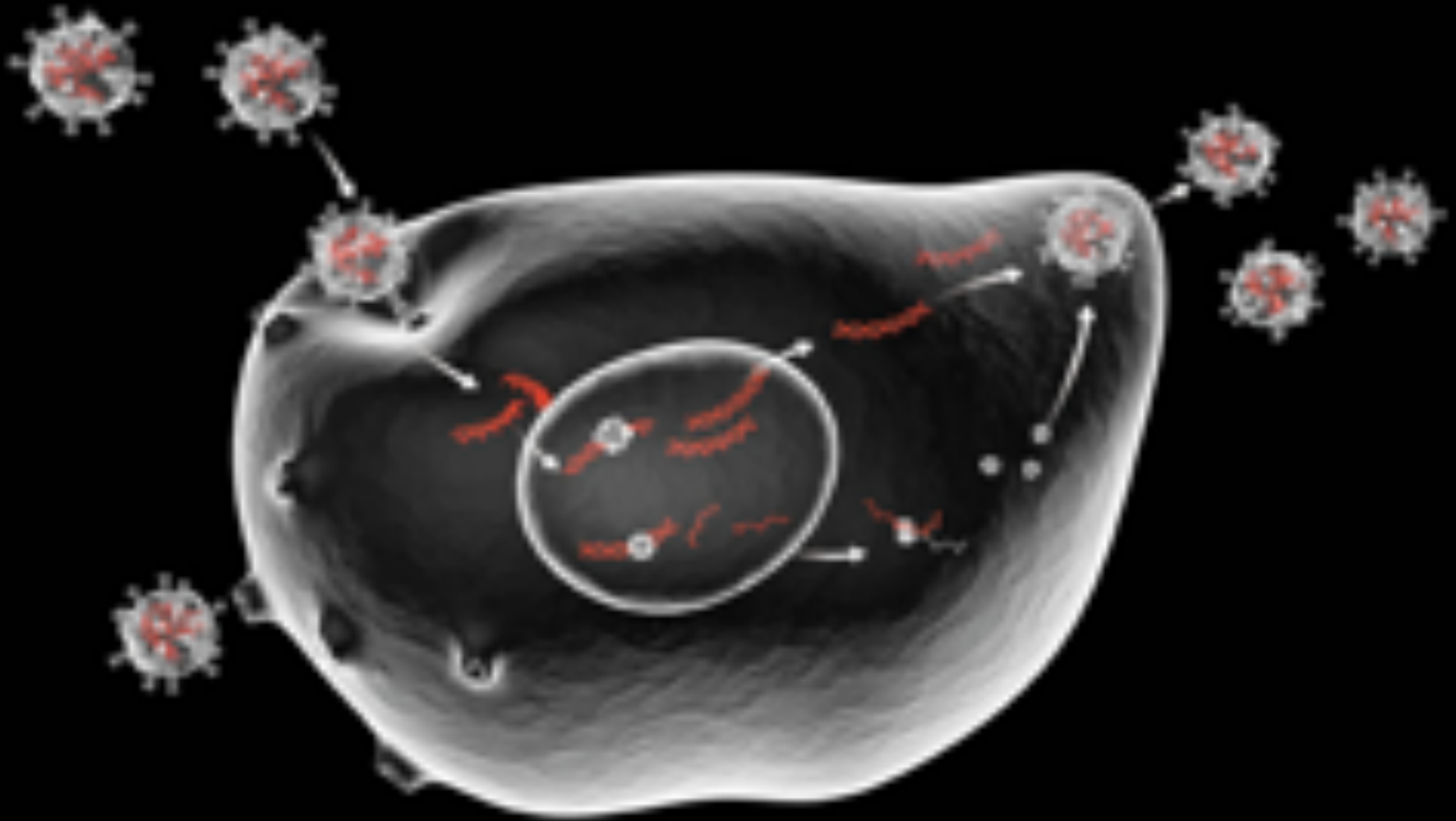
# COVID-19 Pandemic



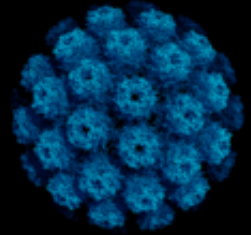
- Declared a **pandemic** by WHO yesterday
- Virus emerged from a bat reservoir
- Cases as of March 12, 2020: **127,863**
- Countries/regions affected: **116**
- 80,932 cases in China, 70% recovered
- United States: **1,323**
- Death toll: **4,718**
- **Fatality rate: 0.6-5% (Global average = 3.4%)**
- Stable on surfaces for **3-9** days
- Prevention and control: hygiene and social distancing



# Virus-Host Cell Interactions



# JC Polyomavirus Infection



Initial infection occurs via peroral transmission



**Healthy**

Subsequent infection in the kidney

**Shed in urine**

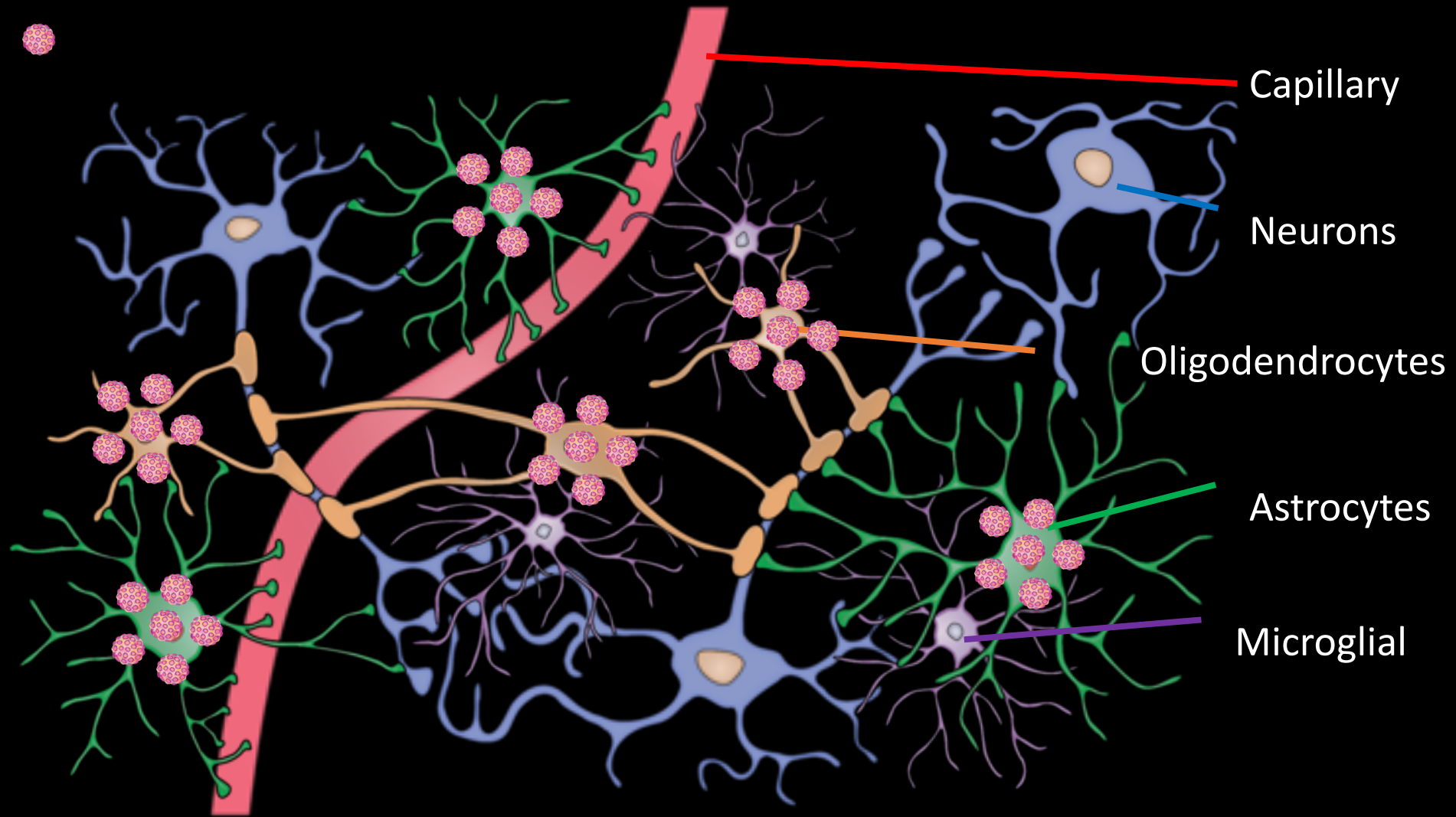
Immunosuppression



**Immune compromised**

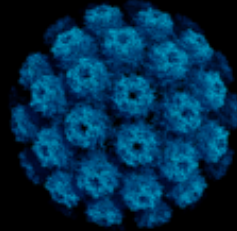
JCPyV spreads to the brain causing PML

# JCPyV Infection in the Brain

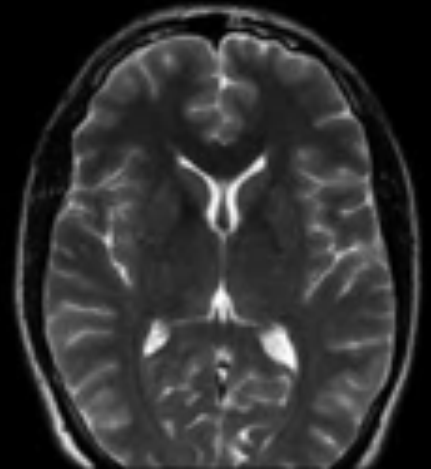
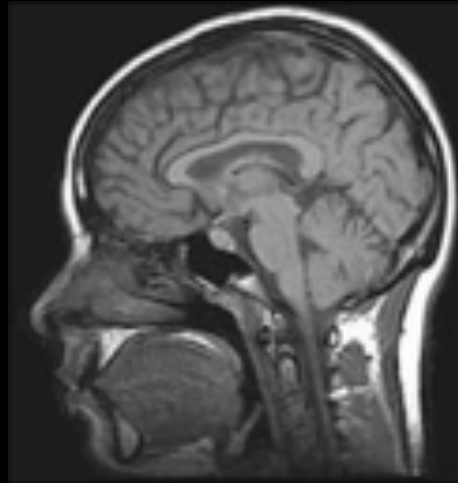




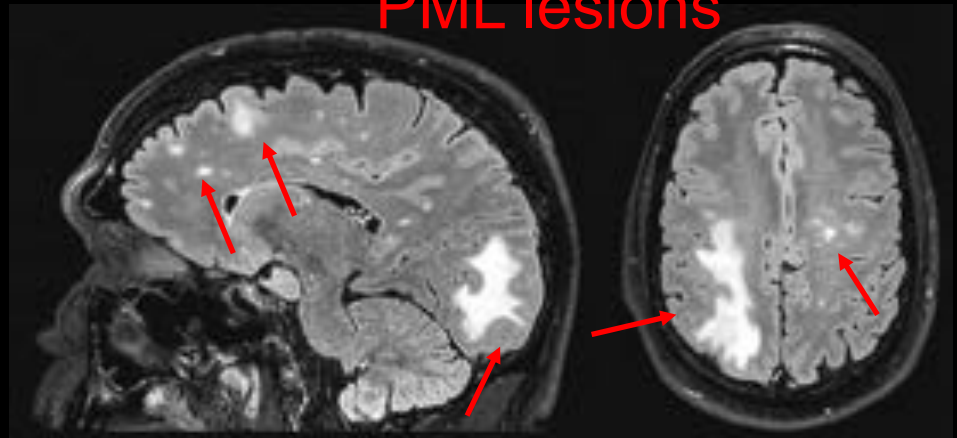
# JCPyV Causes PML



Healthy  
brain



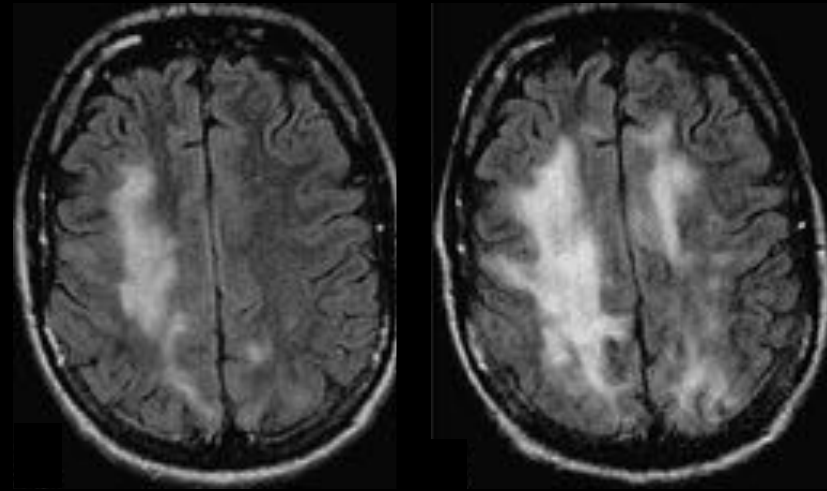
PML  
brain



# PML

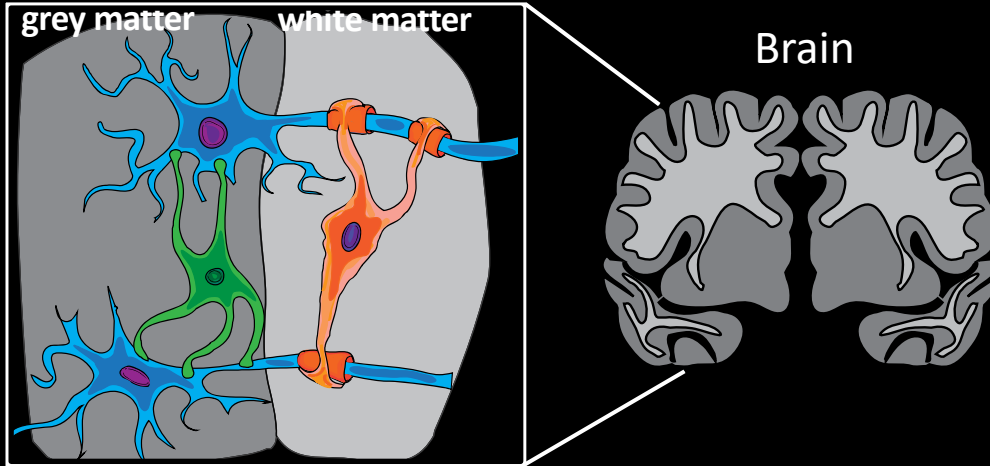
- Symptoms: partial paralysis and cognitive impairments
- 5% of HIV-1 positive patients
- 1:500 patients receiving immunomodulatory therapies
- Debilitating and fatal
- Limited treatment options

PML progression over 4 months

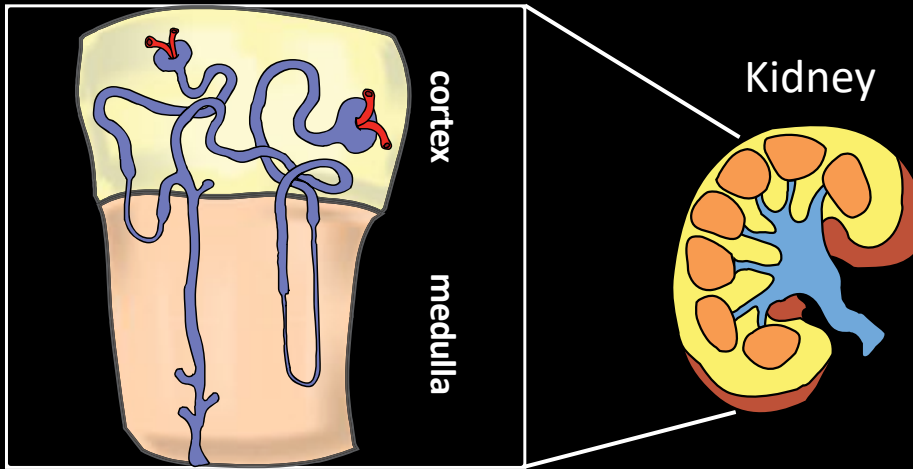
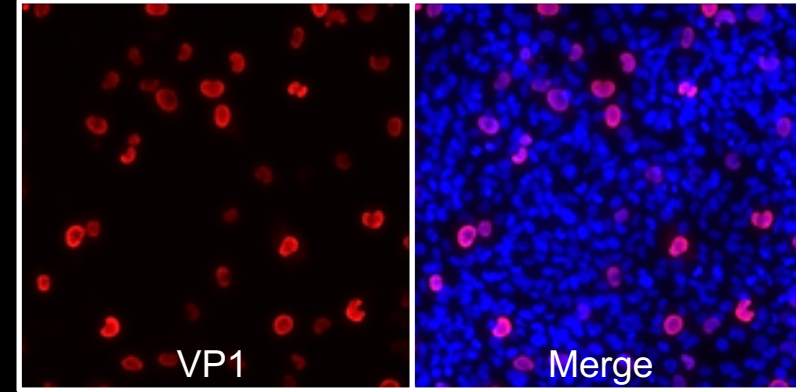


Bag et al. AJNR. 2010.

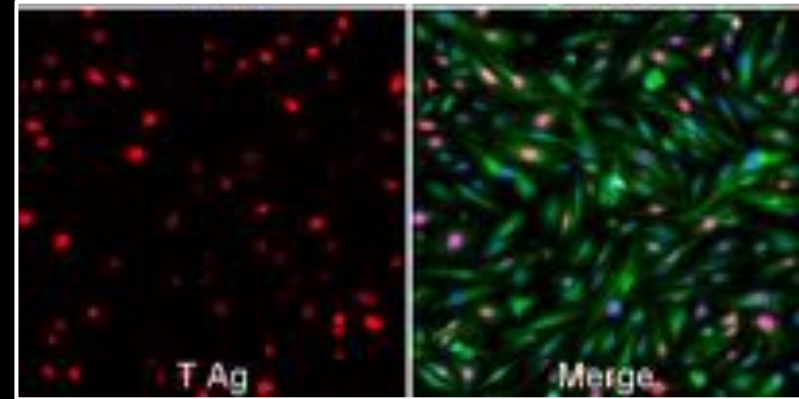
# Laboratory Cell Culture Models for JCPyV Infection



Human astrocytes and oligodendrocytes

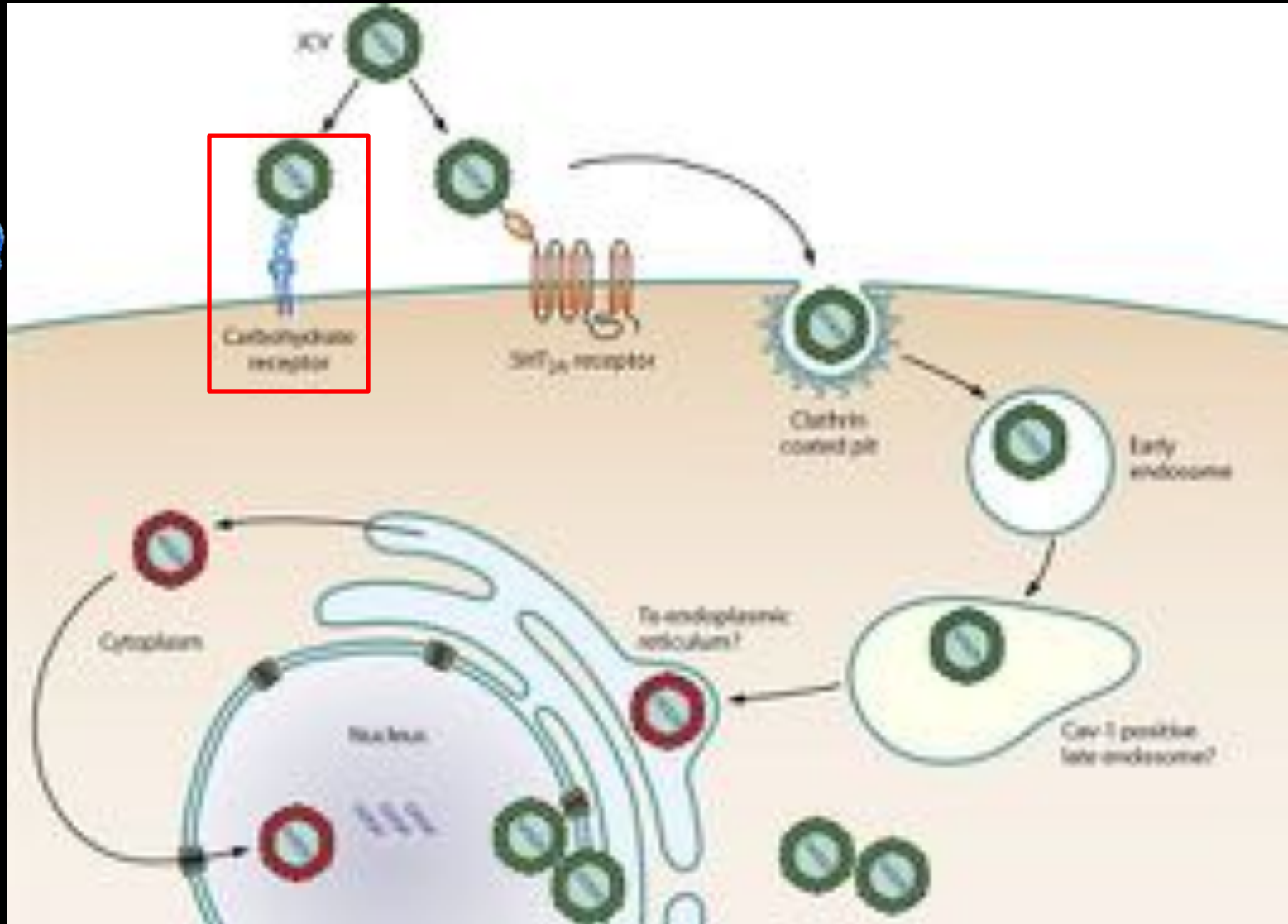
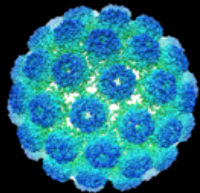


Kidney cells

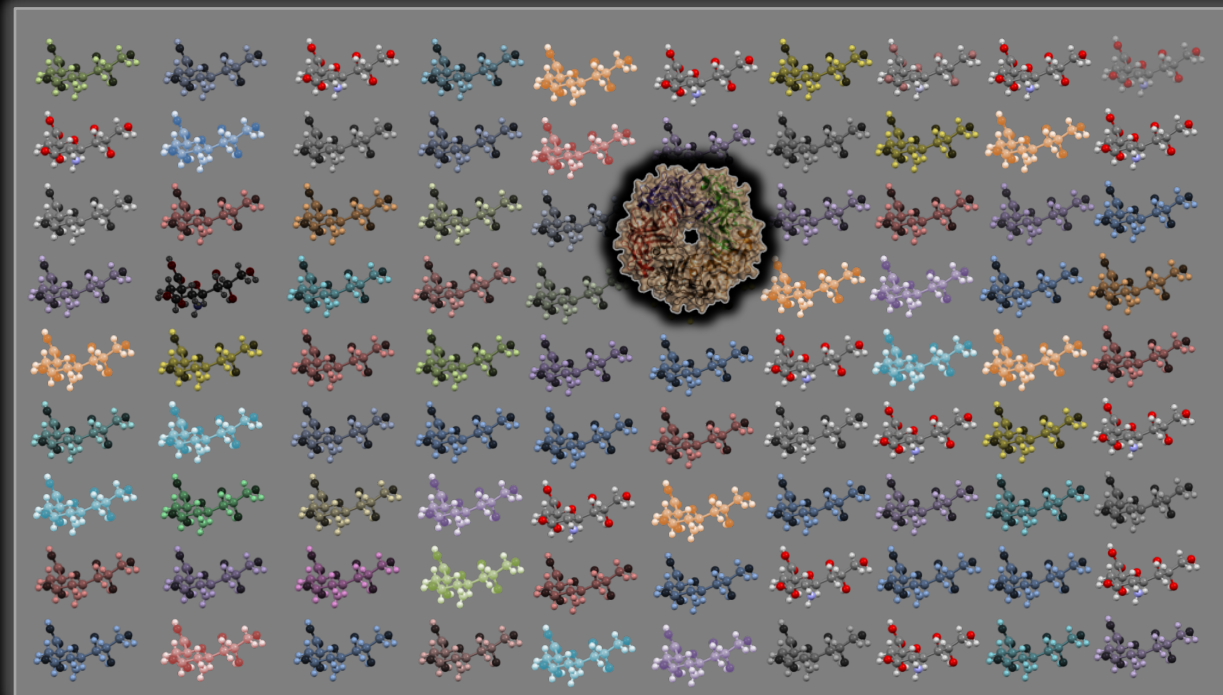
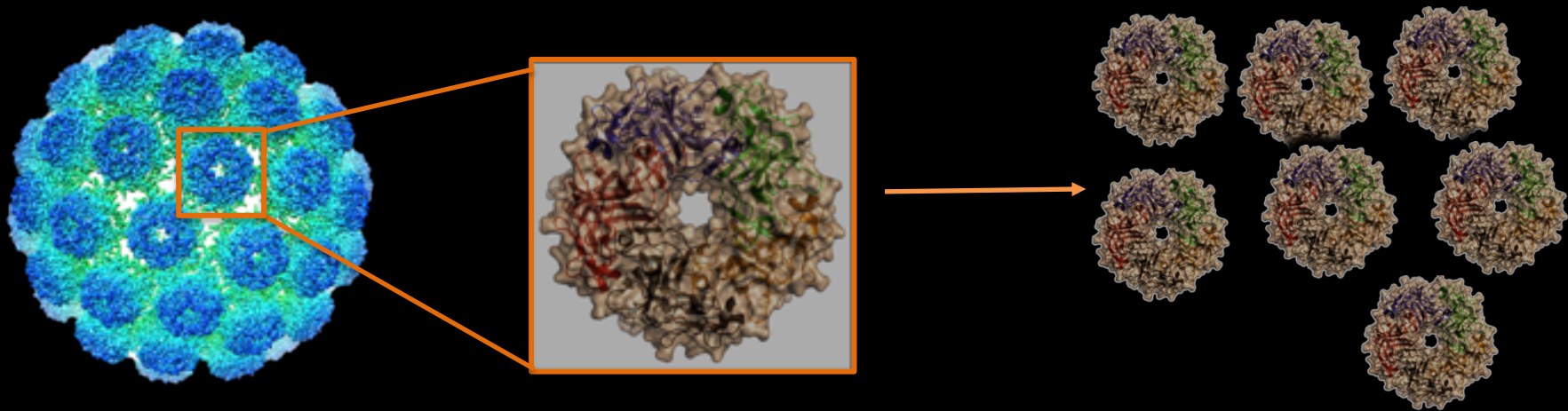




# How does JC PyV select target cells?



# Identification of a JCPyV Receptor



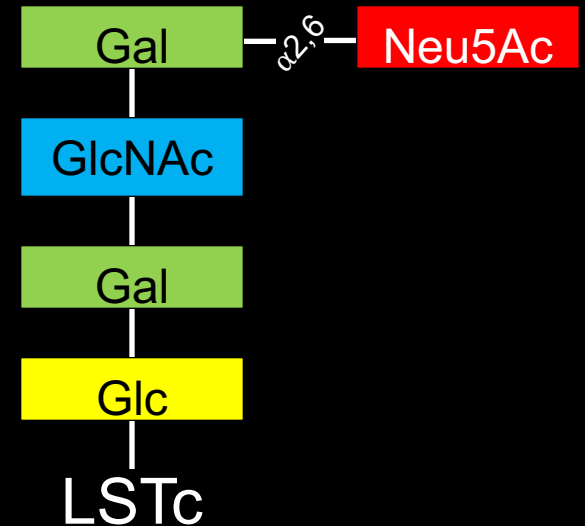
Angelina Palma

Feizi Lab, Imperial College of London

Glycan Array Screen

# LSTc

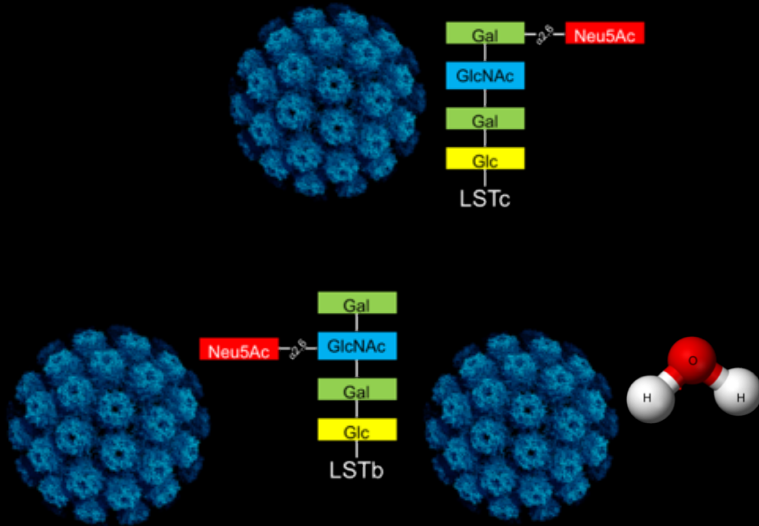
- $\alpha$ 2,6-linked-lactoseries tetrasaccharide c
- Expressed on a glycoprotein or glycolipid
- Present on many glycan structures on human cells
- Receptor fragment for influenza A strains





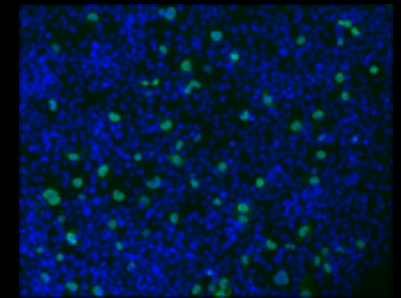
# Methods to Study JCPyV-LSTc Interactions

Incubate JCPyV with LSTc or controls

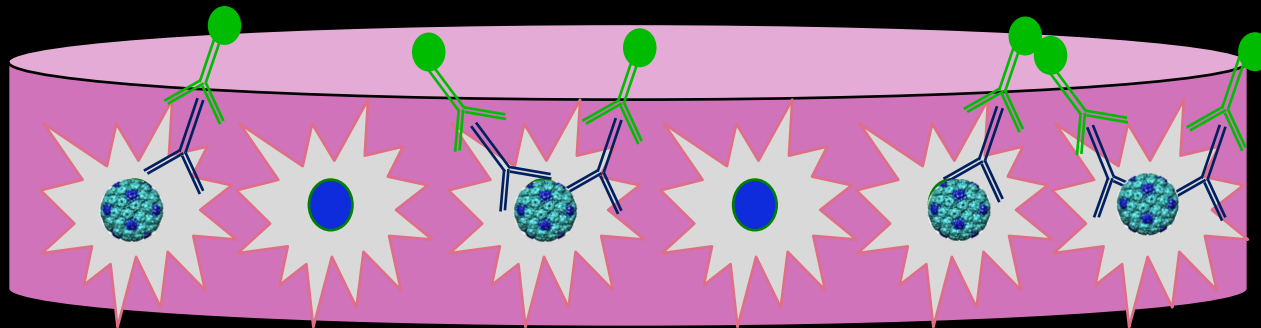


Add complex to cells

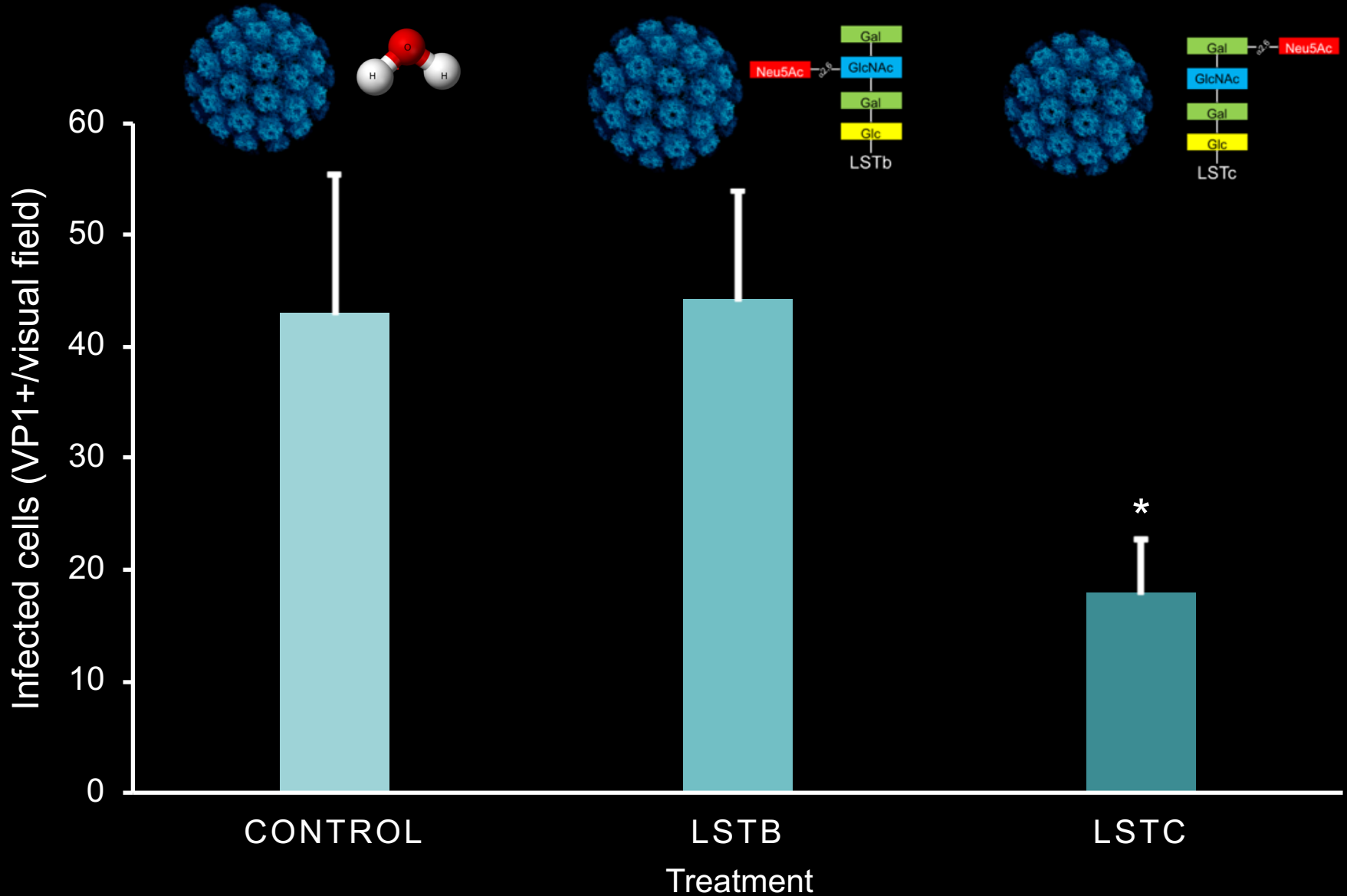
Measure viral infectivity



Colleen Mayberry

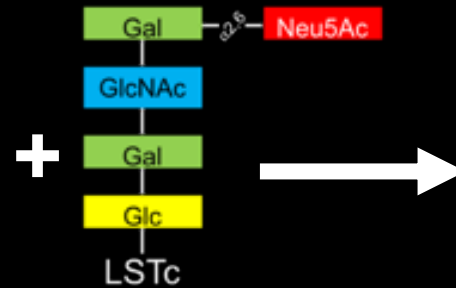
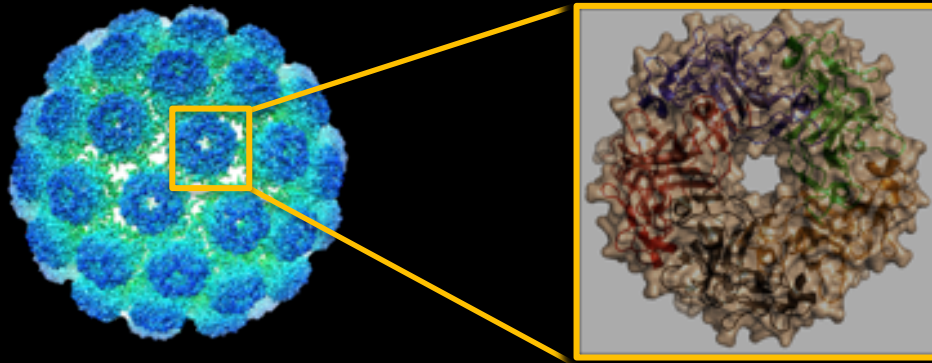


# LSTc Inhibits JCPyV Infection

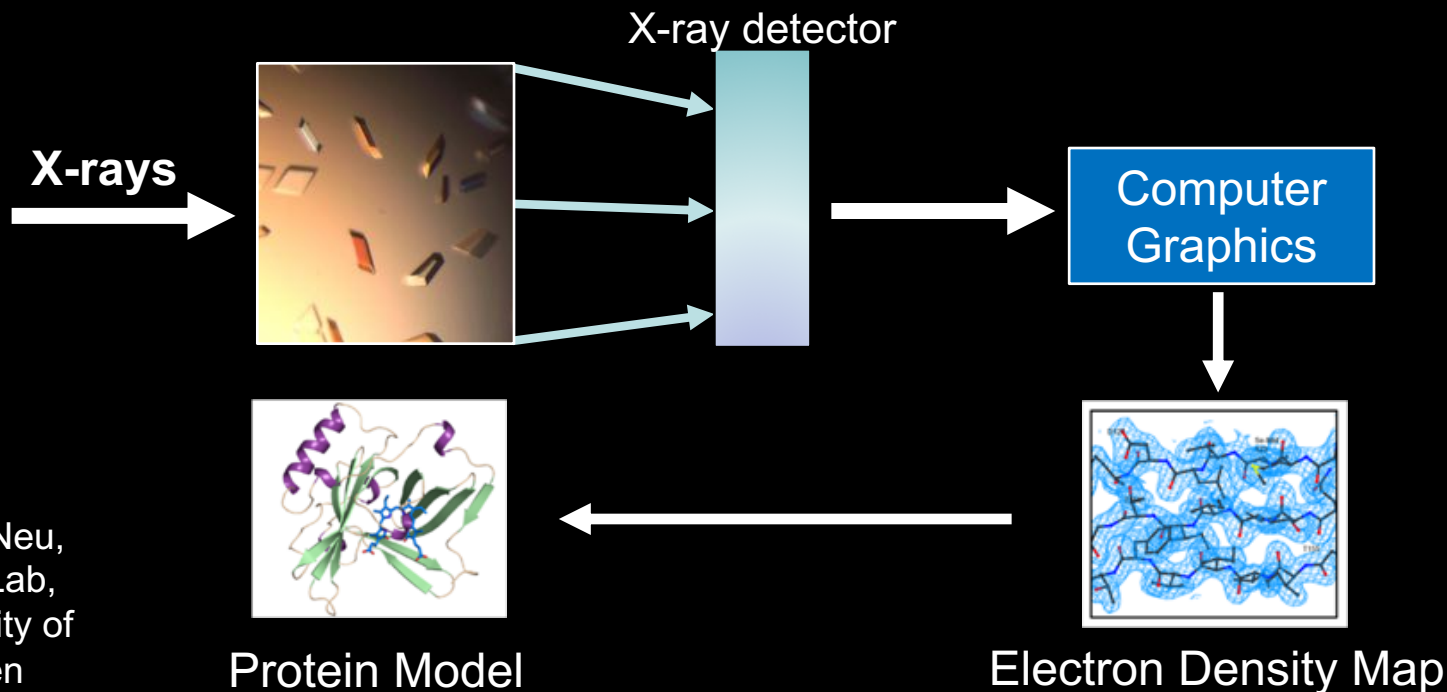
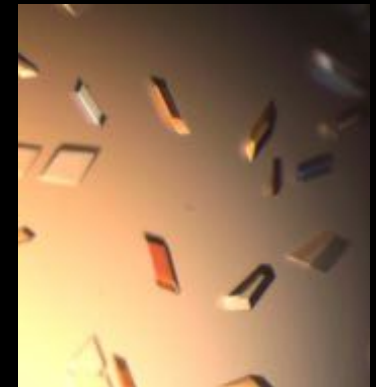


# Structural Studies: JCPyV-LSTc Interactions

## Purification of VP1 pentamers

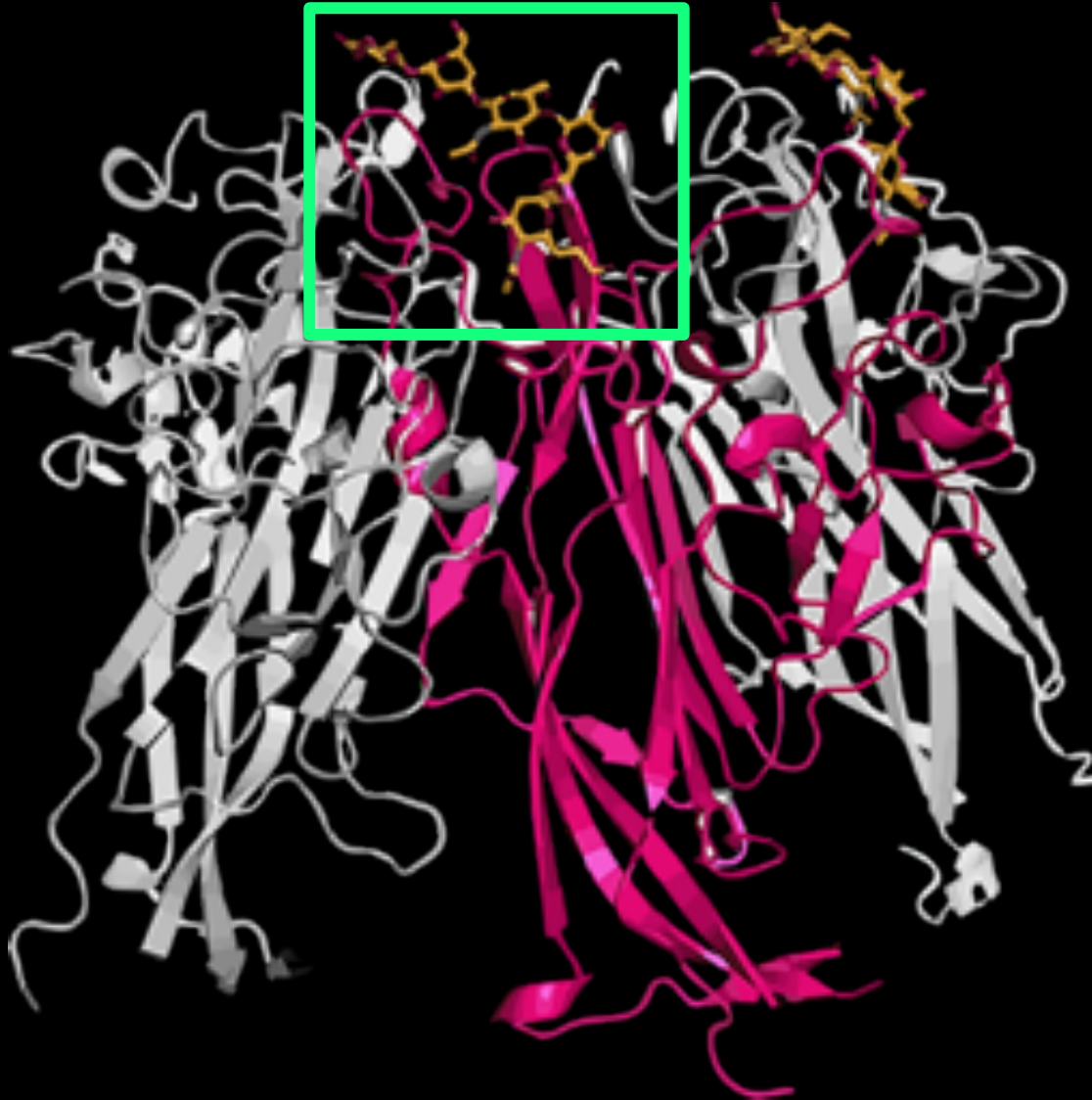


## Crystals





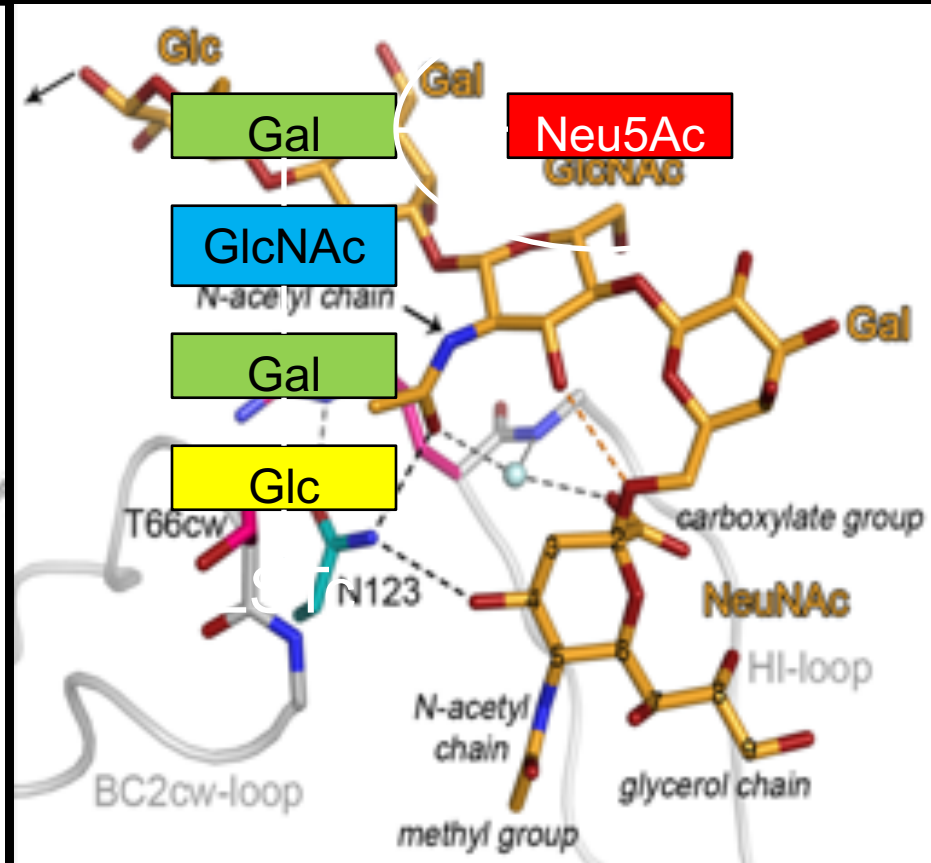
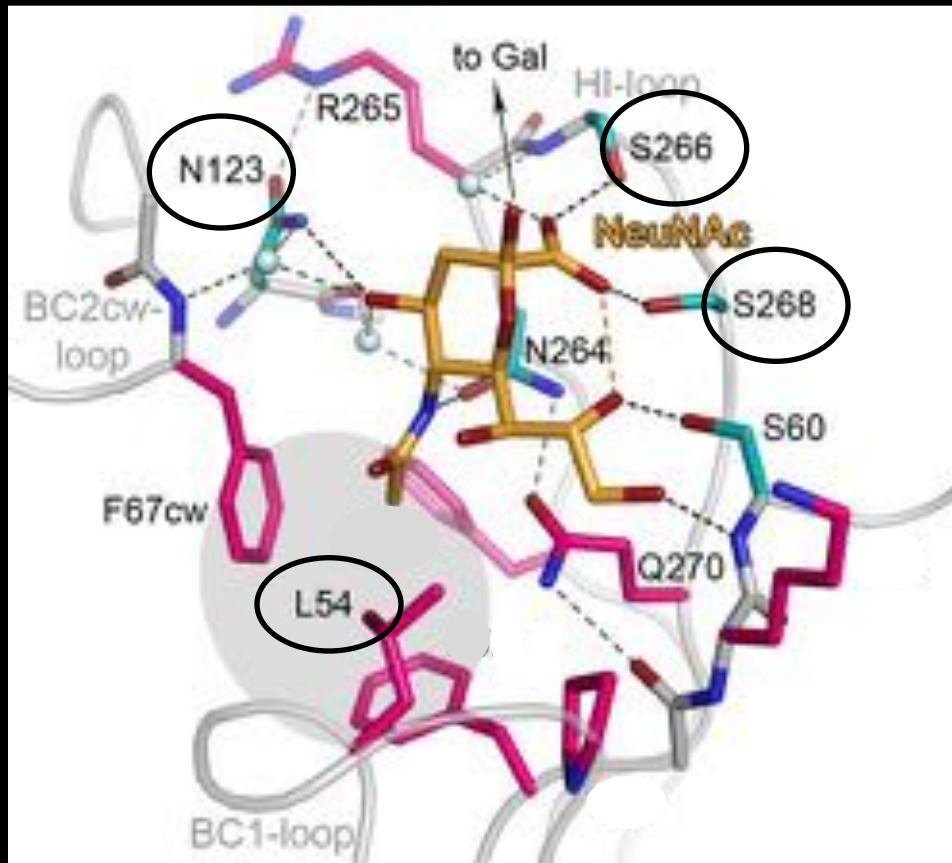
# JCPyV VP1 in Complex with LSTc



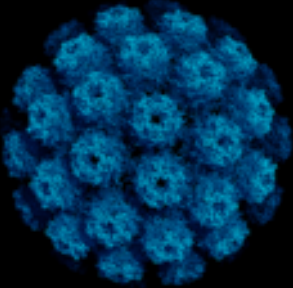
# Specific Contacts Between JCPyV VP1 and LSTc

## Key H-bonds with NeuNAc

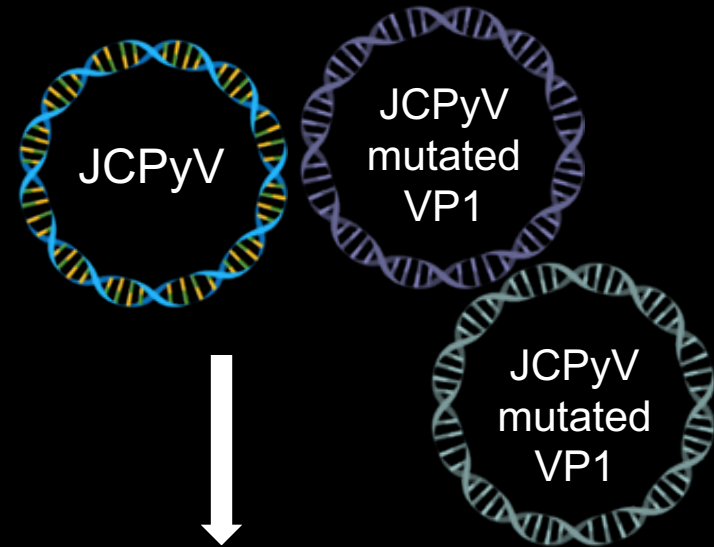
## LSTc L-shaped conformation



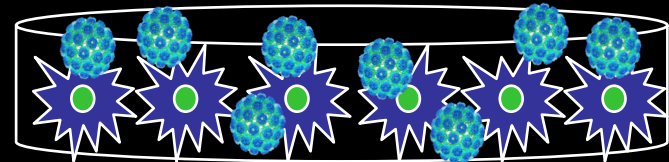
# Engineering Viruses with Mutated Genomes



Mutate viral genome in infectious clone

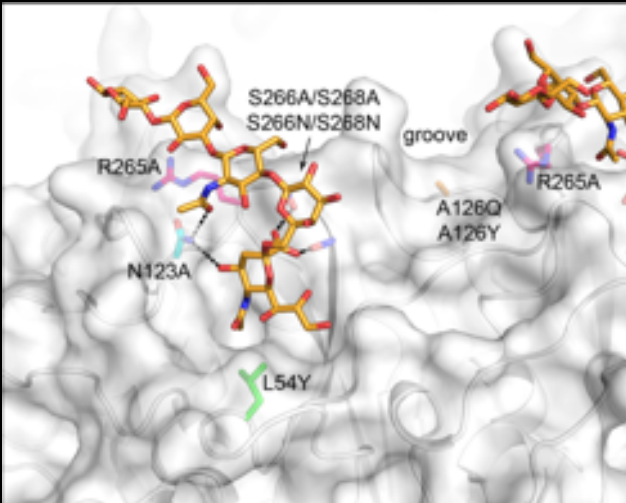


Transfect cells with infectious clones

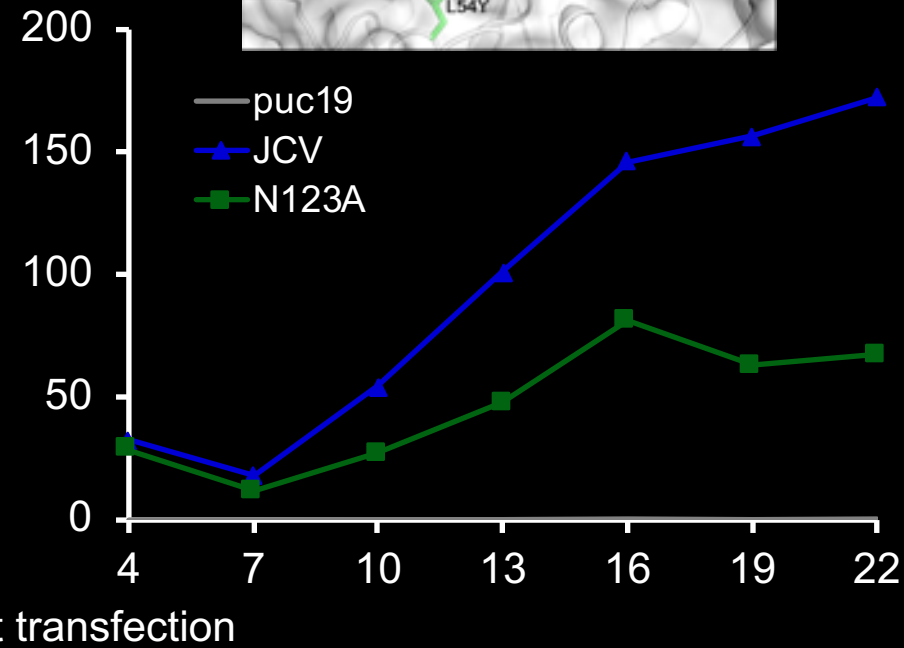
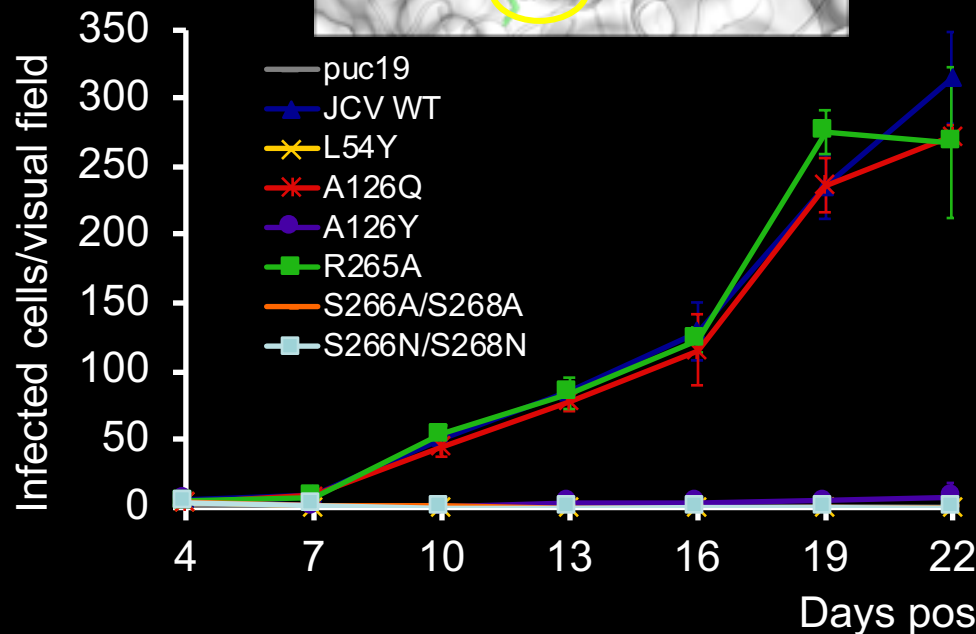
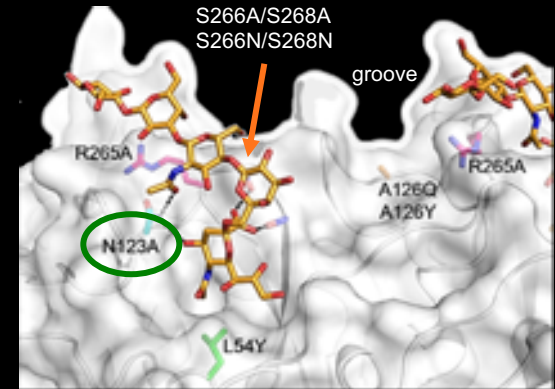
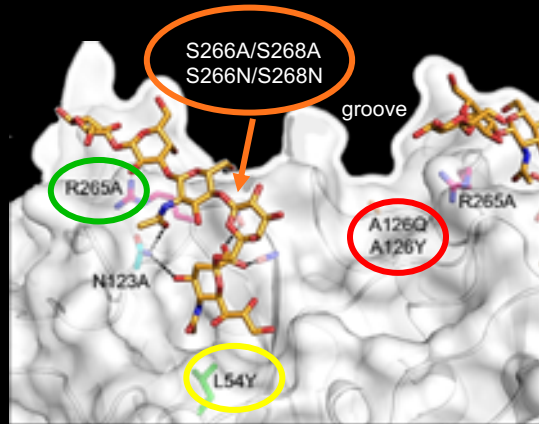


Infectious virions produced?

Measure viral spread

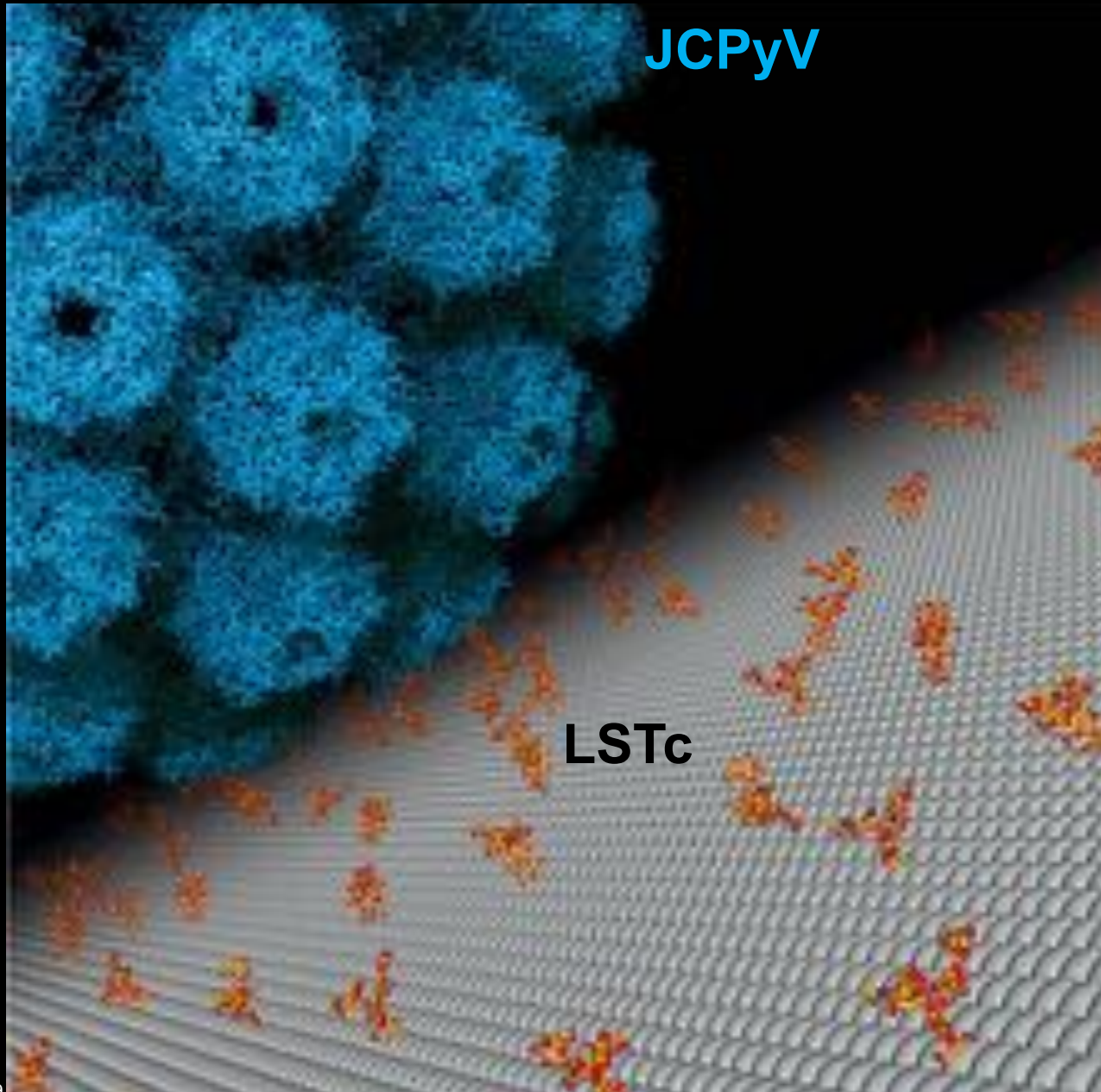


# Mutation of JCPyV Sialic Acid-Binding Sites Abolishes Viral Growth

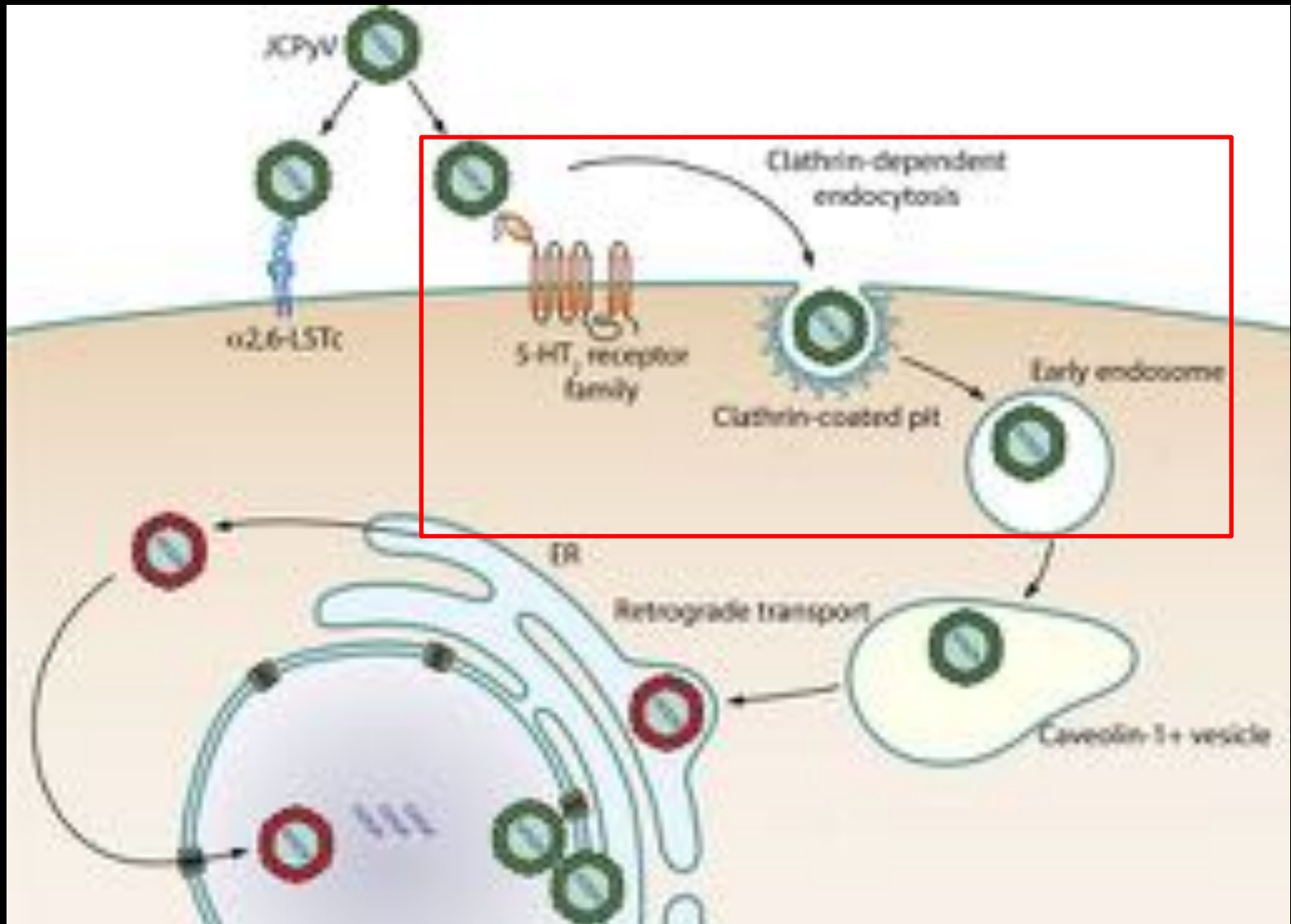




# JCPyV has a Sweet Tooth for LSTc

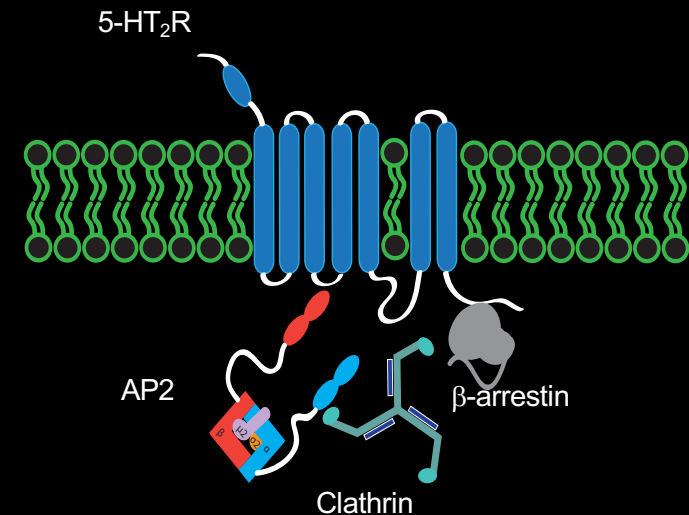


# How does JCPyV enter target cells?



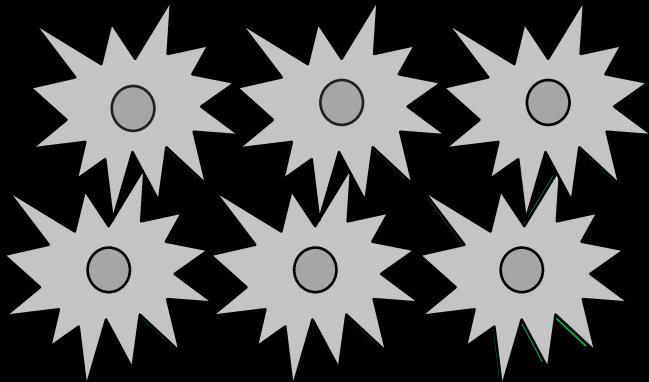
# Serotonin (5-HT) Receptors

- G-protein coupled receptors
- Mediates endocytosis of neurotransmitter 5-HT
- Functions in mood, sleep, and behavior
- Activates signaling events
- 15 known 5-HTRs
- 5-HT<sub>2</sub>Rs (2A, 2B, 2C) are necessary for JCPyV infection

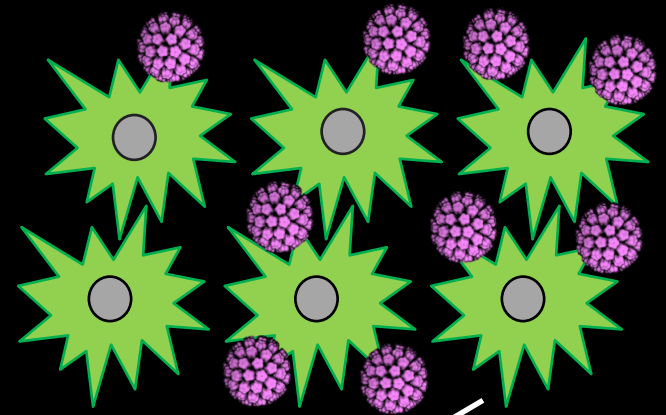


# Analysis of JCPyV Interactions with 5-HT<sub>2</sub>Rs by Super Resolution FPALM

Express photoactivatable 5-HT<sub>2</sub>Rs in HEK cells



Infect cells with labeled JCPyV



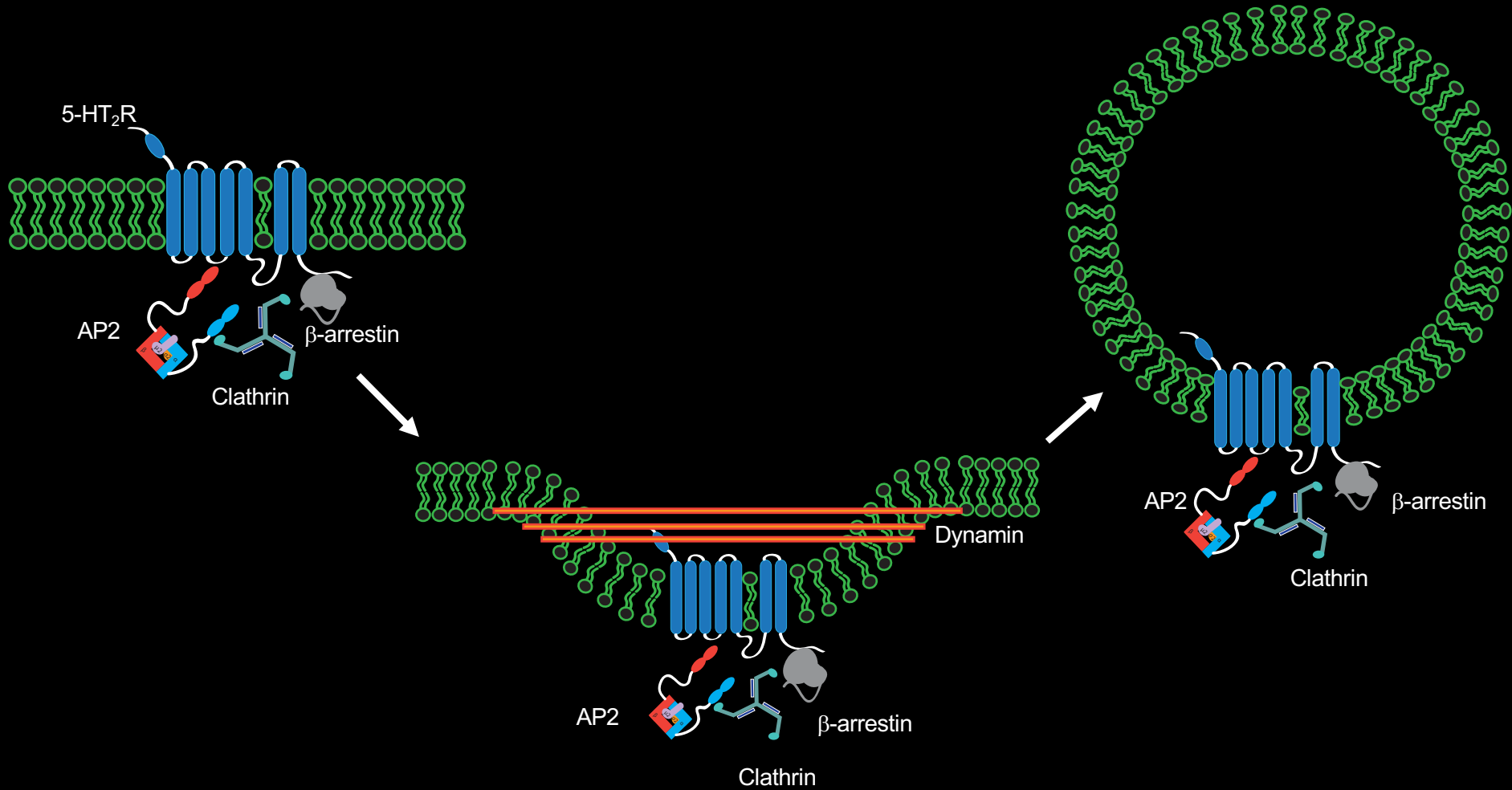
Analyze interactions by FPALM



Kashif Mehmood, Jeanne DuShane

Matthew Parent, Sam Hess

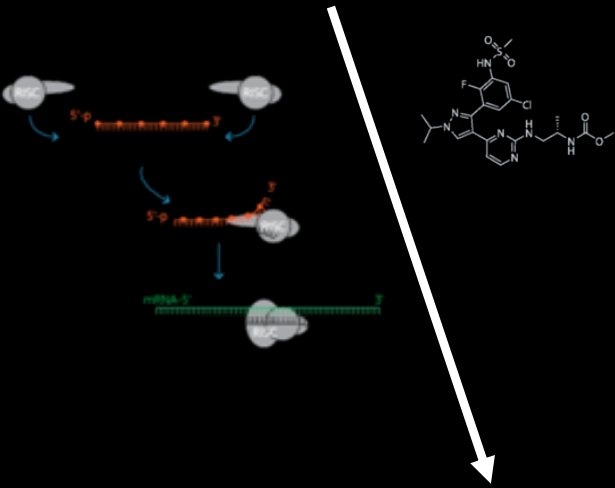
# Serotonin Receptor Endocytosis



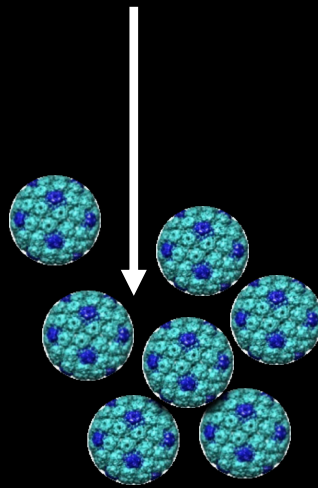


# Methods to Study Virus-Host Cell Interactions

Inhibit cellular proteins with siRNAs or chemicals



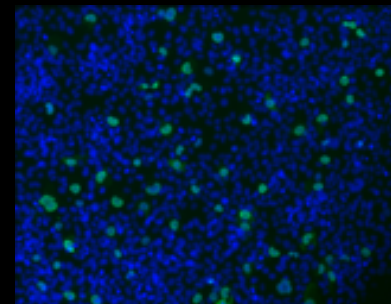
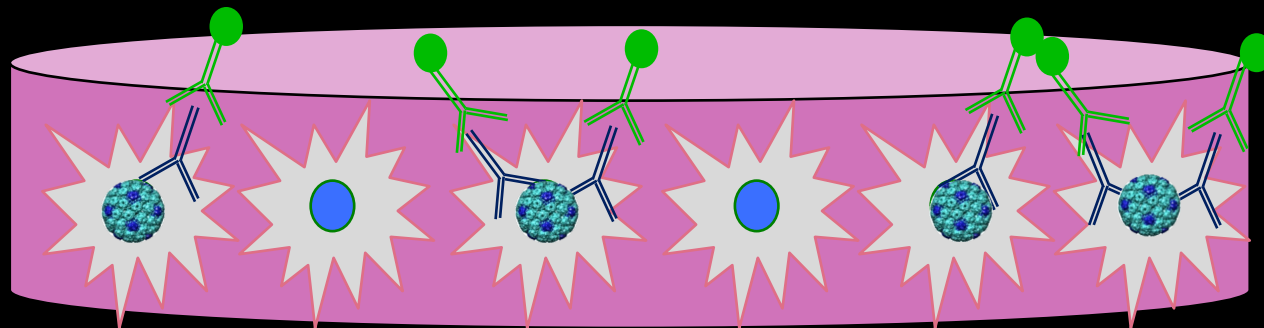
Infect with JCPyV



Stain with Abs to detect JCPyV

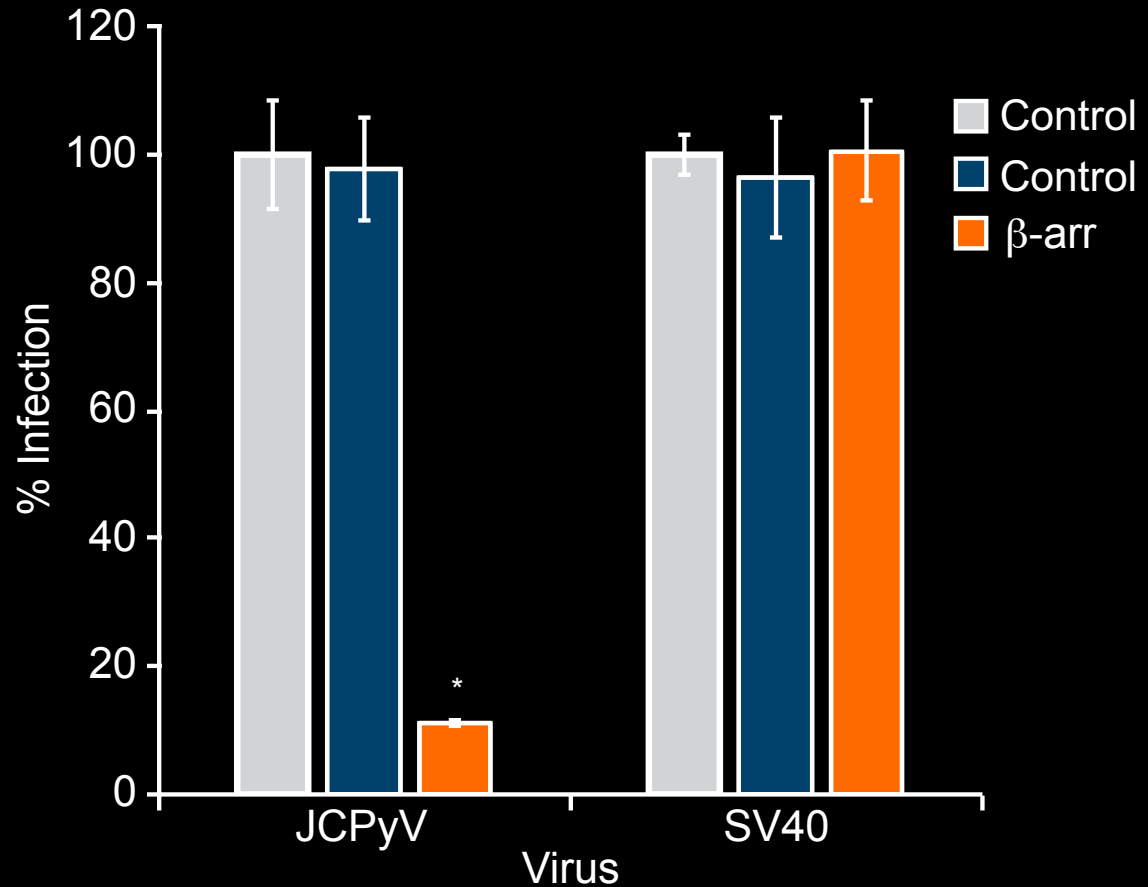
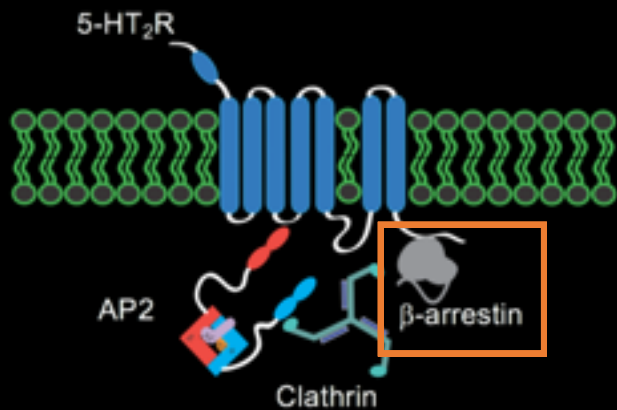


Quantify infection using microscopy



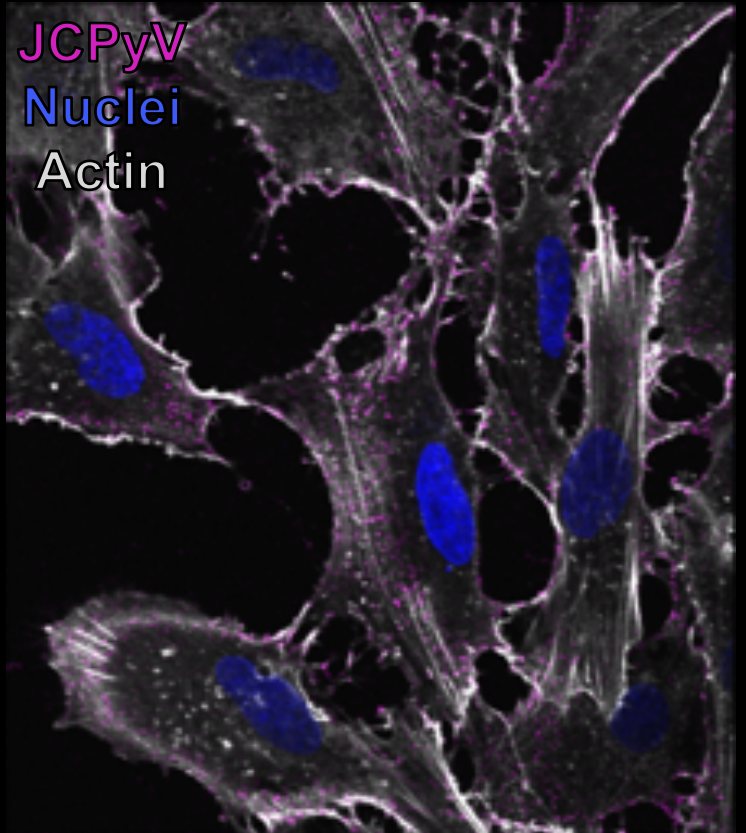
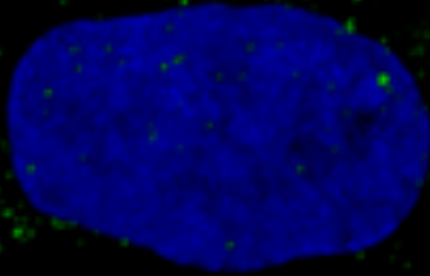
Colleen Mayberry

# Serotonin Scaffold Protein $\beta$ -arrestin is Required for JCPyV Infection



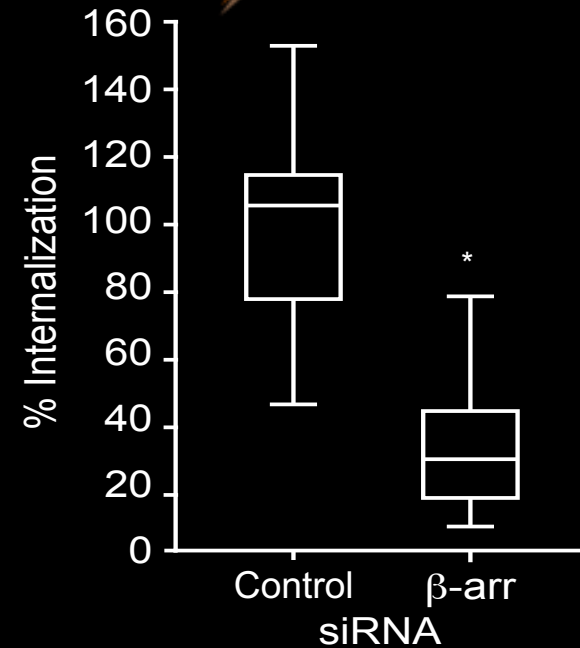
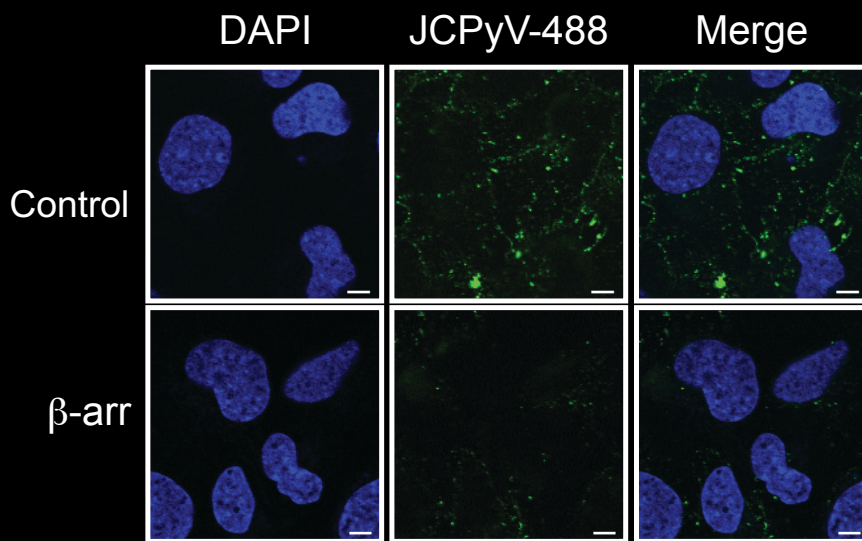
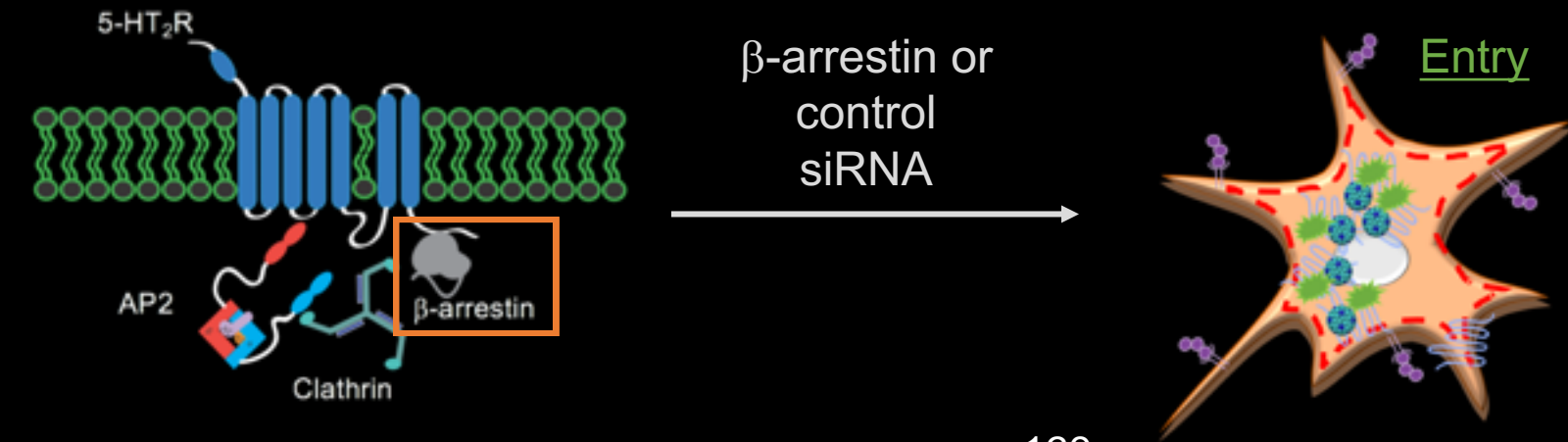
# Confocal Microscopy to Study Viral Entry

JCPyV  
Nuclei

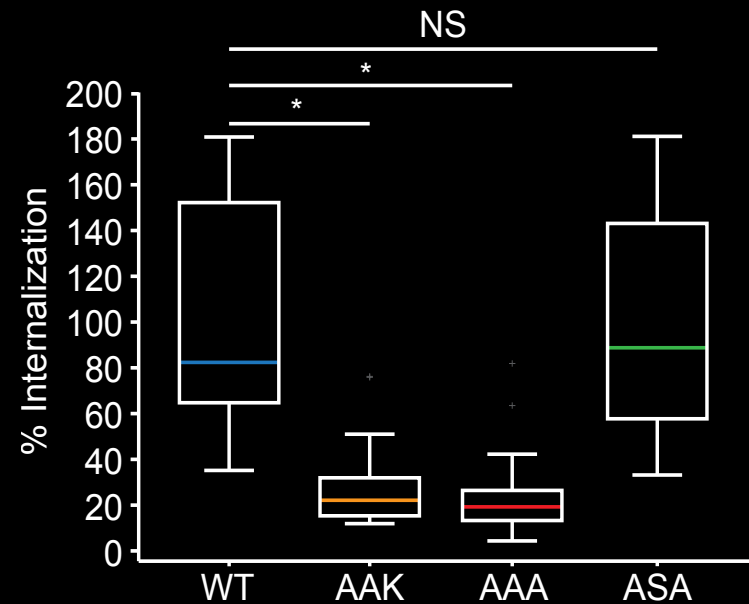
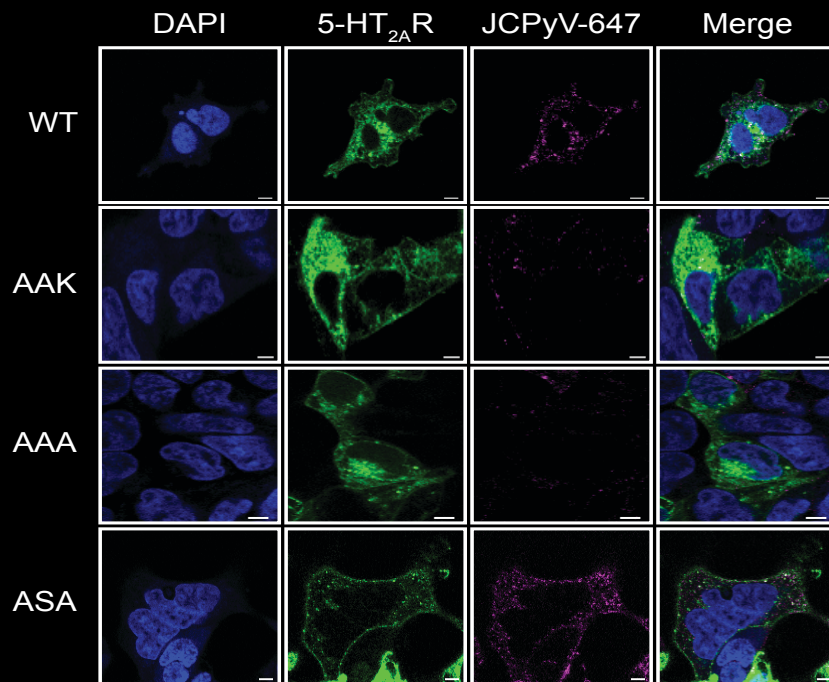
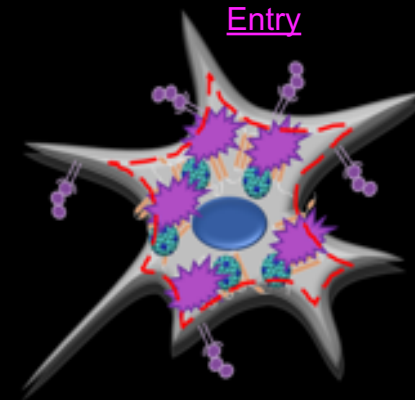
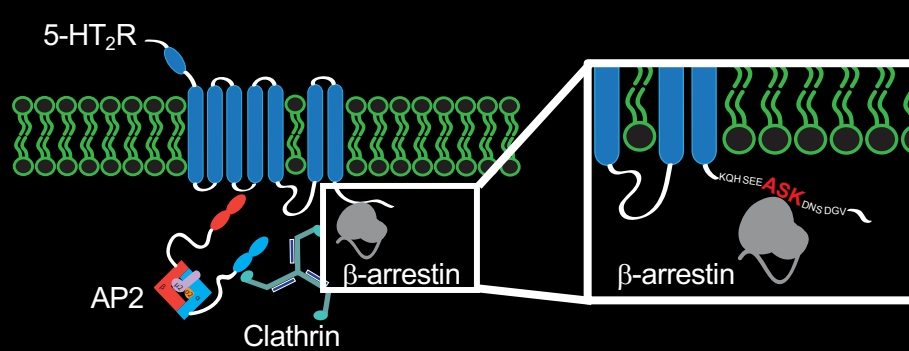


Colleen Mayberry

# Serotonin Scaffold Protein $\beta$ -arrestin is Required for JCPyV Entry



# Disruption of $\beta$ -arrestin Binding Motif in 5-HT<sub>2A</sub>R Reduces JCPyV Entry

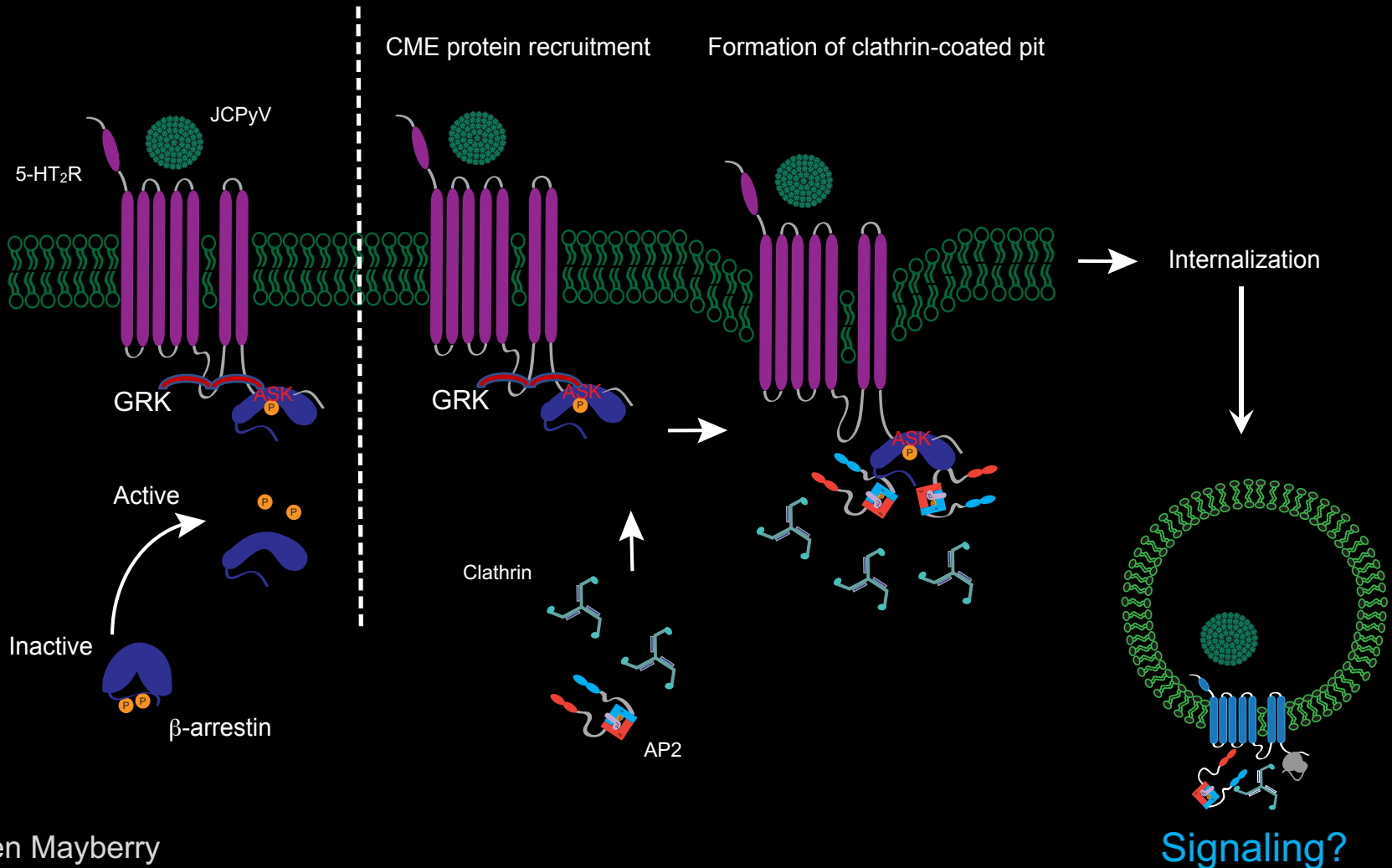




# Model of JCPyV Internalization

$\beta$ -arrestin binding to 5-HT<sub>2</sub>R

Initiation of clathrin-mediated endocytosis

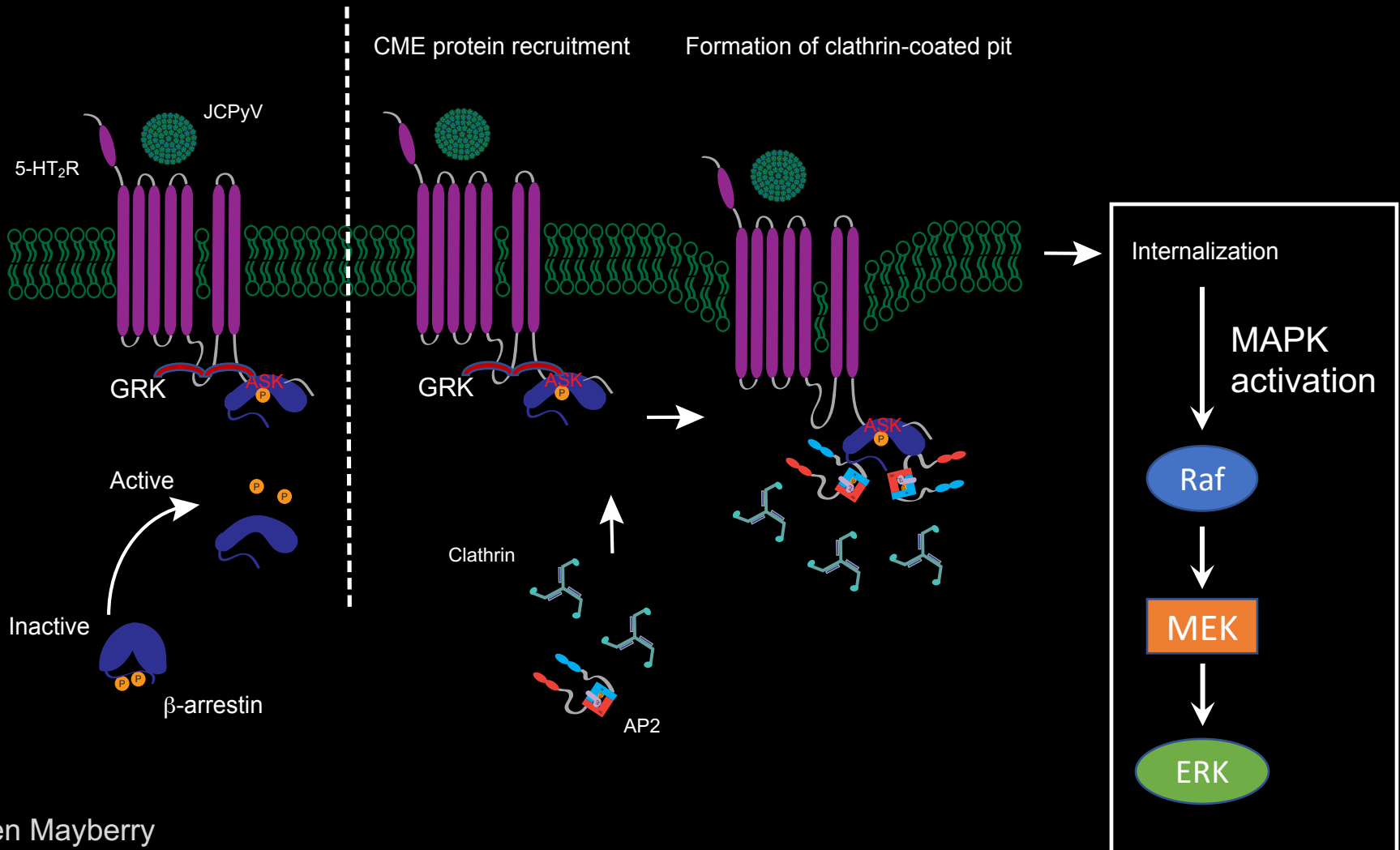




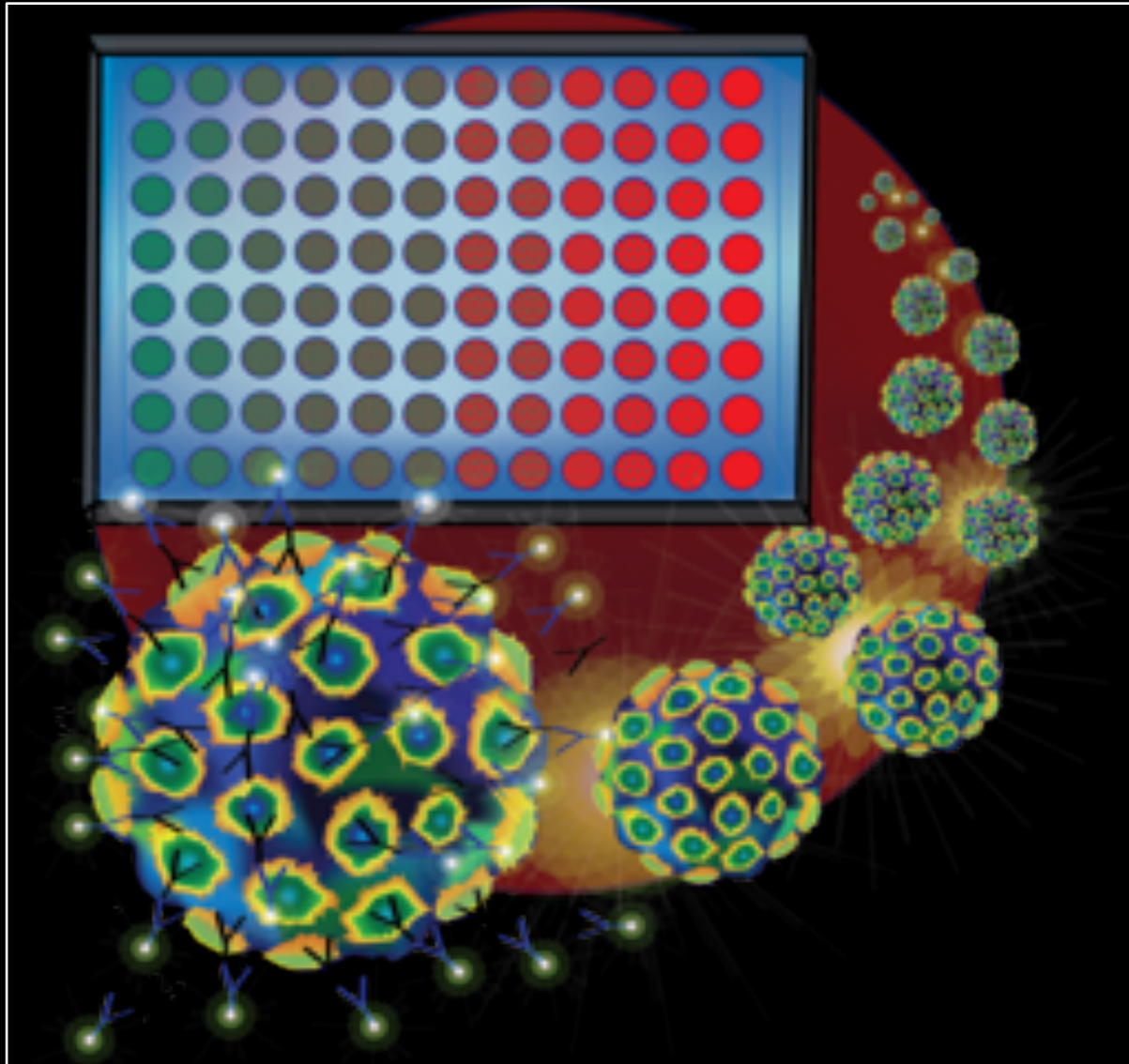
# Model of JCPyV Internalization and Signaling

$\beta$ -arrestin binding to 5-HT<sub>2</sub>R

Initiation of clathrin-mediated endocytosis



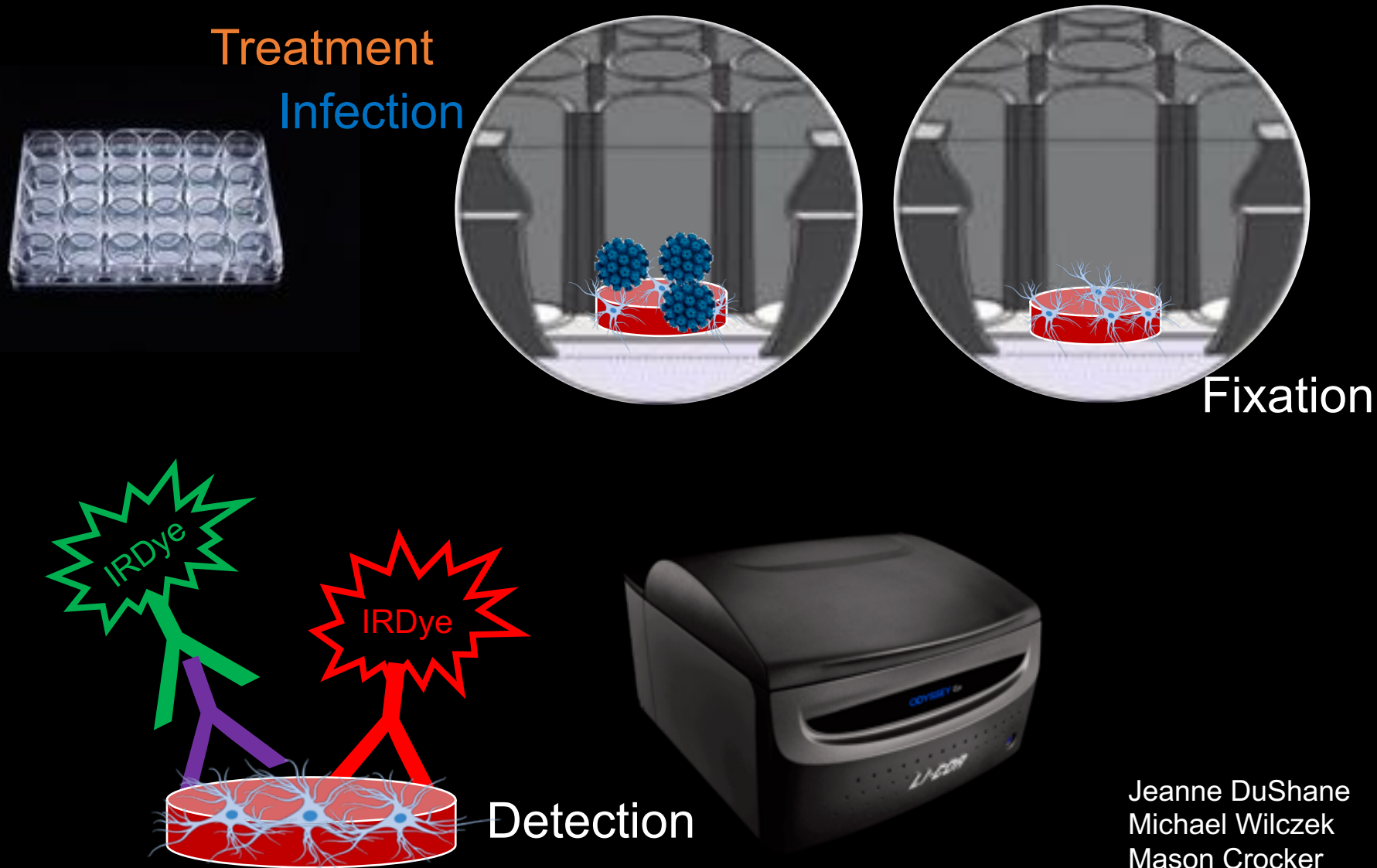
# In-Cell Western Assay to Detect Changes in Viral and Cellular Proteins during Infection



Michael Wilczek

DuShane et al. *Frontiers in Microbiology*. 2019.

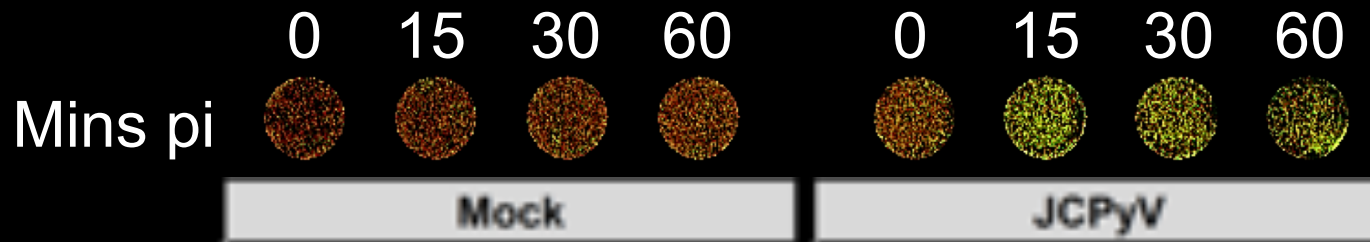
# In-Cell Western Assay to Detect Changes in Viral and Cellular Proteins during Infection



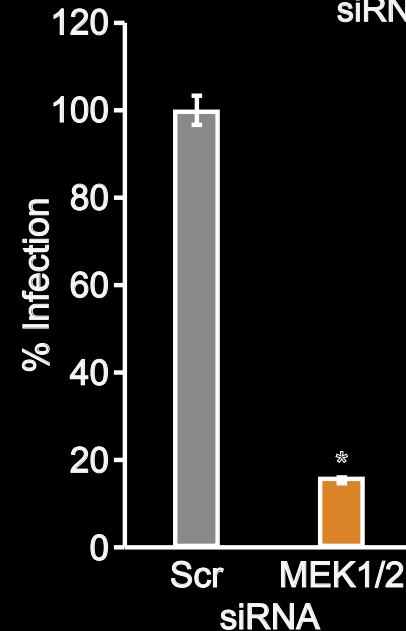
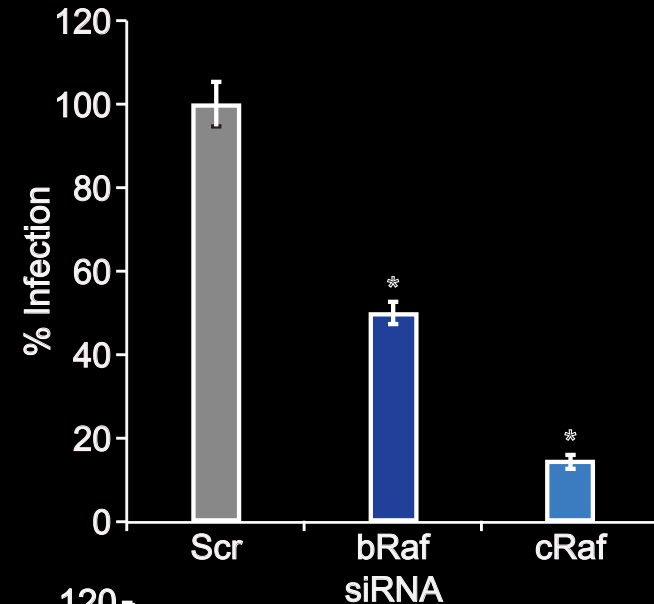
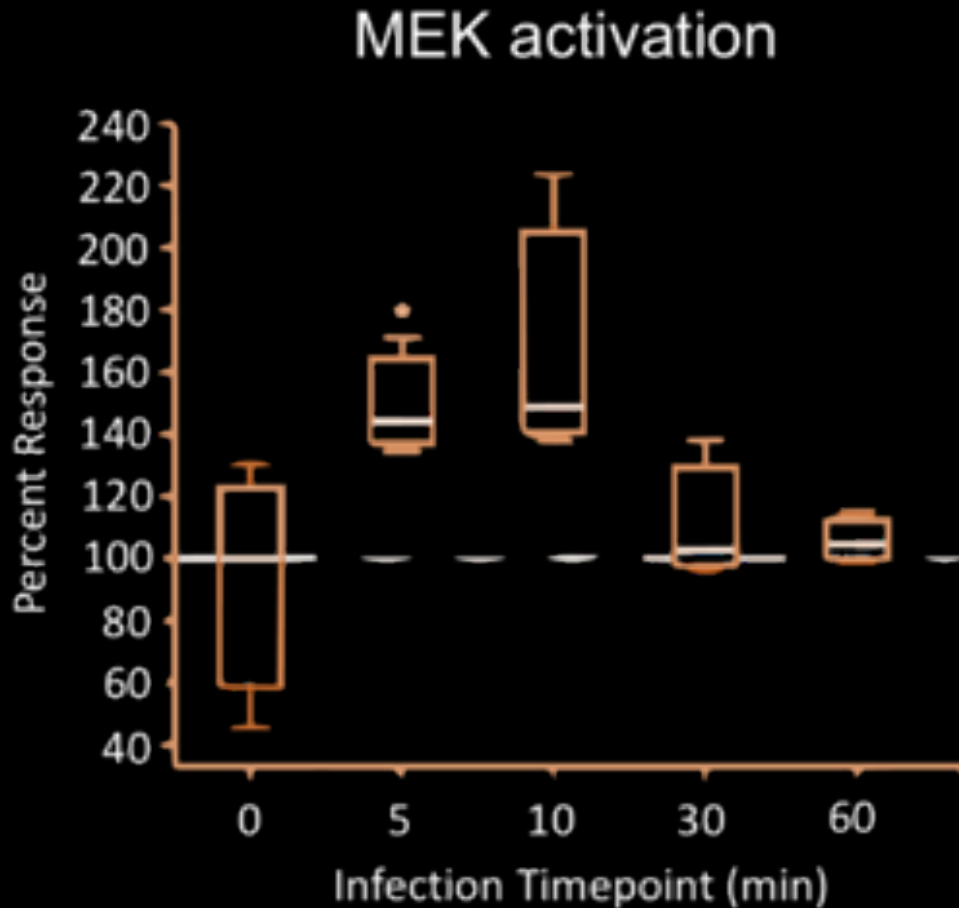
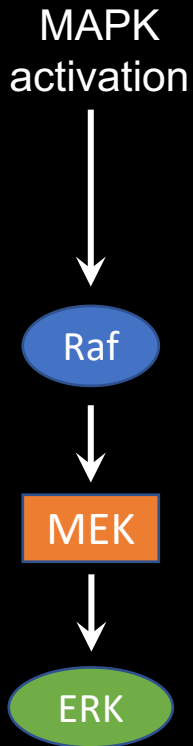
Jeanne DuShane  
Michael Wilczek  
Mason Crocker



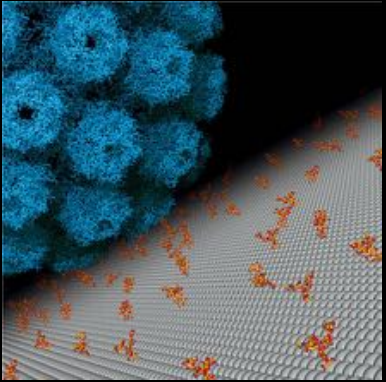
# MAPK ERK is Activated during JCPyV Infection



# JCPyV Infection Requires Raf and MEK Activity



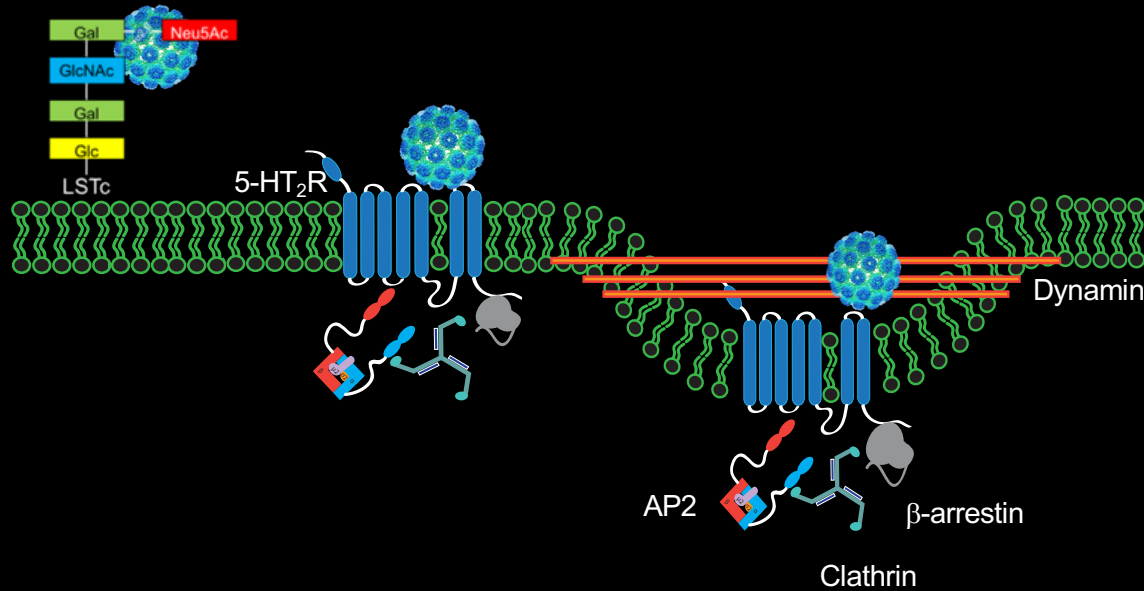
# Recap



Binding to  
LSTc

Cell Entry via 5-HT<sub>2</sub>R<sub>s</sub>

JCPyV-induced Cell  
Signaling



**MAPK  
activation**

Raf

MEK

ERK

# Acknowledgements

## Maginnis Lab

Francesca Armstrong  
Avery Bond  
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Francois Levasseur  
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Sarah Nichols  
Stephen Rezack  
Michael Wilczek

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Jeanne DuShane (Excerpta Medica)  
Ashley Soucy (GSBSE, UMaine)

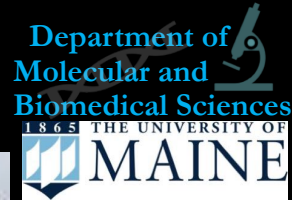
## Funding Sources

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NIH-NIAID: R15AI144686  
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NIH Incentive Grant  
Maine Space Grant Consortium, CUGR, Radke



## Collaborators

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Feizi Lab, Imperial College of London  
Stehle Lab, University of Tübingen  
Sam Hess, Matthew Parent, UMaine  
Ben King, UMaine  
Andrew Smerekanicz, Emera Astronomy Center  
Shawn Laatch, Emera Astronomy Center



# Thank you!



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 @MelissaMaginnis



