

Targeting Flavivirus Non-structural Proteins For Drug Discovery

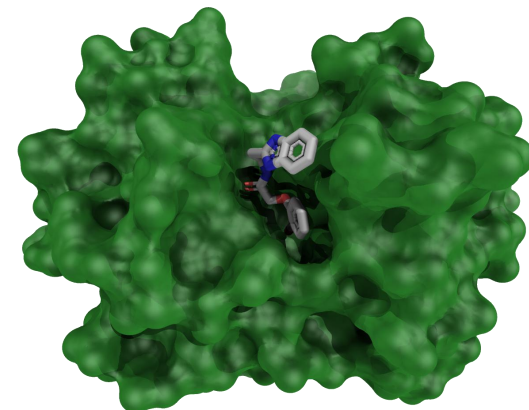
Workshop em Celebração
"60 anos do Prof. Glaucius Oliva"
"30 anos de Cristalografia de Proteínas"
no IFSC/USP

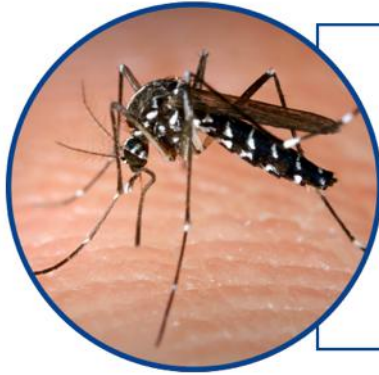
A large, stylized number "60" in gold, with a portrait of Prof. Glaucius Oliva integrated into the zero. Below the number is a yellow ribbon graphic.

A row of logos for various institutions, including USP, IFSC, and CIBFar.

Save the Date
31/10/2019
Auditório "Prof. Sérgio Mascarenhas", no IFSC/USP - São Carlos-SP

Andre S. Godoy





ZIKA VÍRUS

2015-16

Ministério confirma que novo vírus 'primo' da dengue foi encontrado por pesquisadores

Circulação da Febre Zika no País, no entanto, só será confirmada após testes feitos em laboratórios de referência

Fabiana Cambricoll, O Estado de S.Paulo
05 Maio 2015 | 19h05

[SIGA O ESTADÃO](#)

Surto de microcefalia em bebês faz País decretar emergência sanitária nacional

Foram notificados 141 casos no Nordeste; uma das suspeitas da equipe que investiga é a de ligação com o zika vírus

Lígia Formenti, O Estado de S. Paulo
11 Novembro 2015 | 15h00

[SIGA O ESTADÃO](#)

Le Monde

Dimanche 7 Lundi 8 Février 2016 - 7h 21:30 - N° 2201 - 2,40 € - France métropolitaine - www.lemonde.fr - Fondateur: Hubert Bourde-Méry - Directeur: Brune Pignatelli

Zika : l'épidémie rouvre le débat sur l'avortement en Amérique latine

POLITIQUE
NICOLAS SARKOZY
VEUT RENOUVER
AVEC LE PS/PSI F

HEALTH

The New York Times

Zika Virus, a Mosquito-Borne Infection, May Threaten Brazil's Newborns

Global Health

By DONALD G. McNEIL Jr. DEC. 28, 2015



Zika cases



January 2016

- Confirmed
- Reported

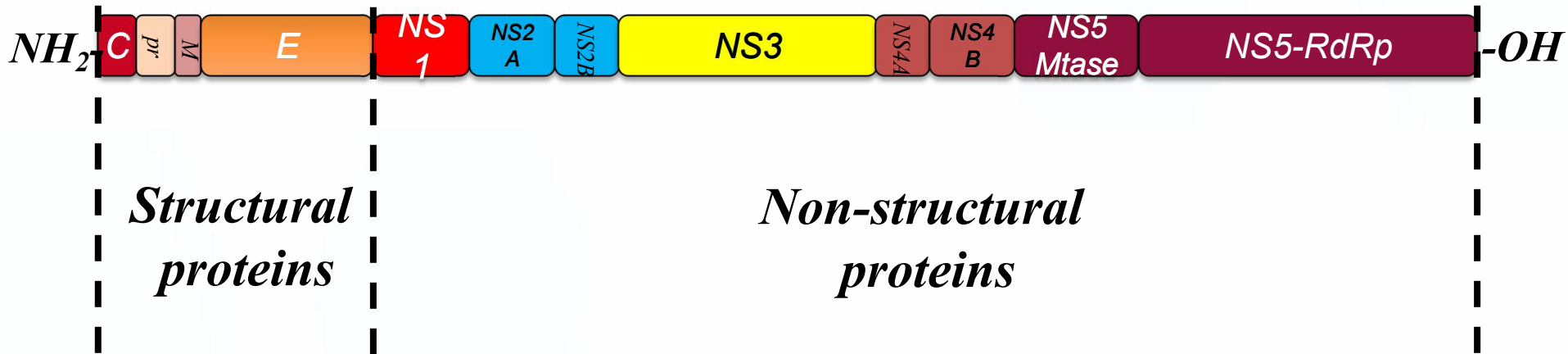
Zika cases



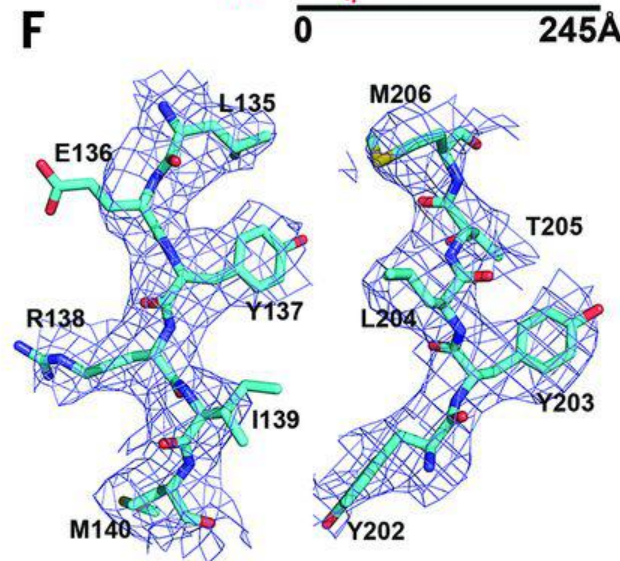
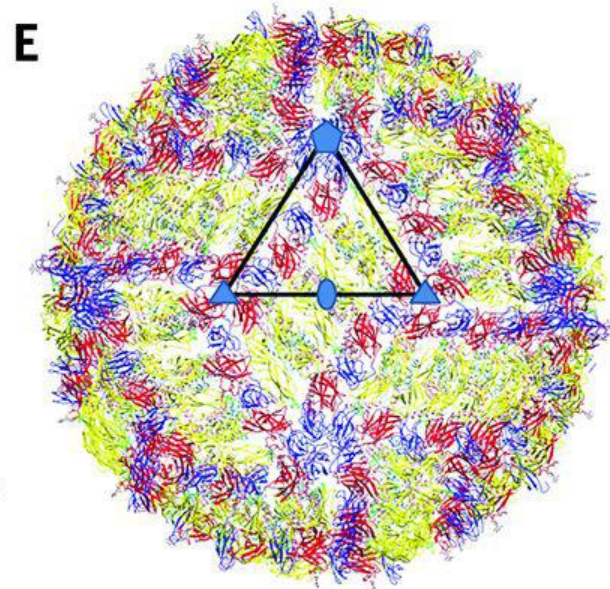
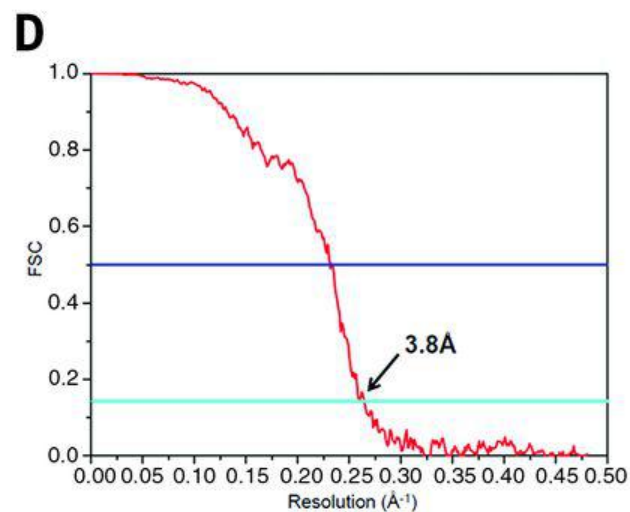
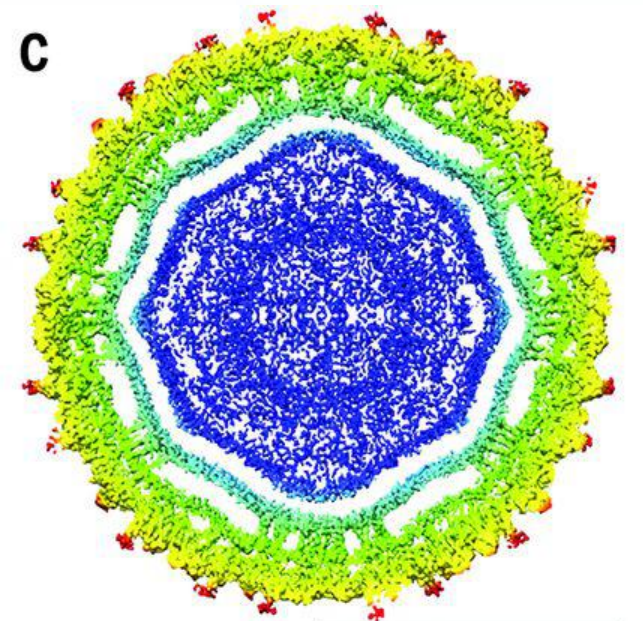
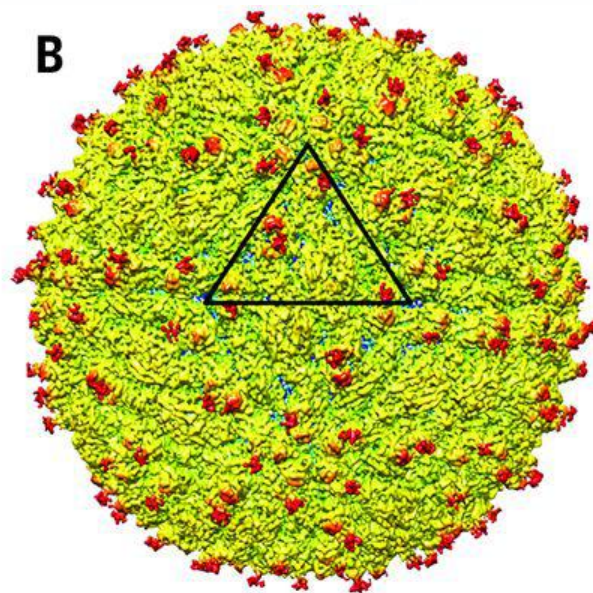
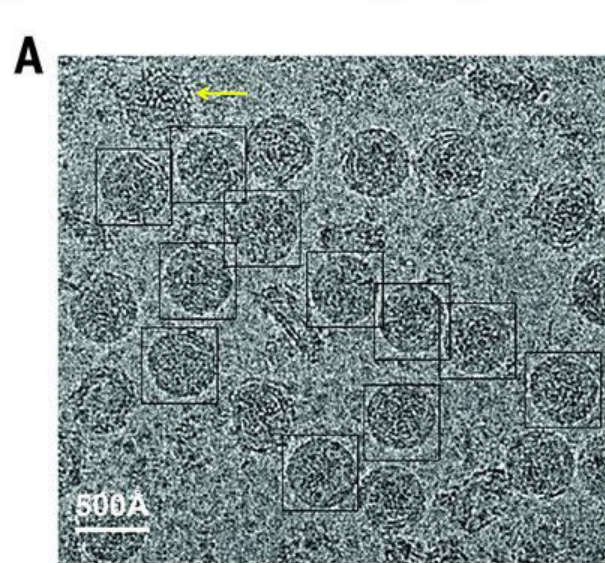
March 2016

- Confirmed
- Reported

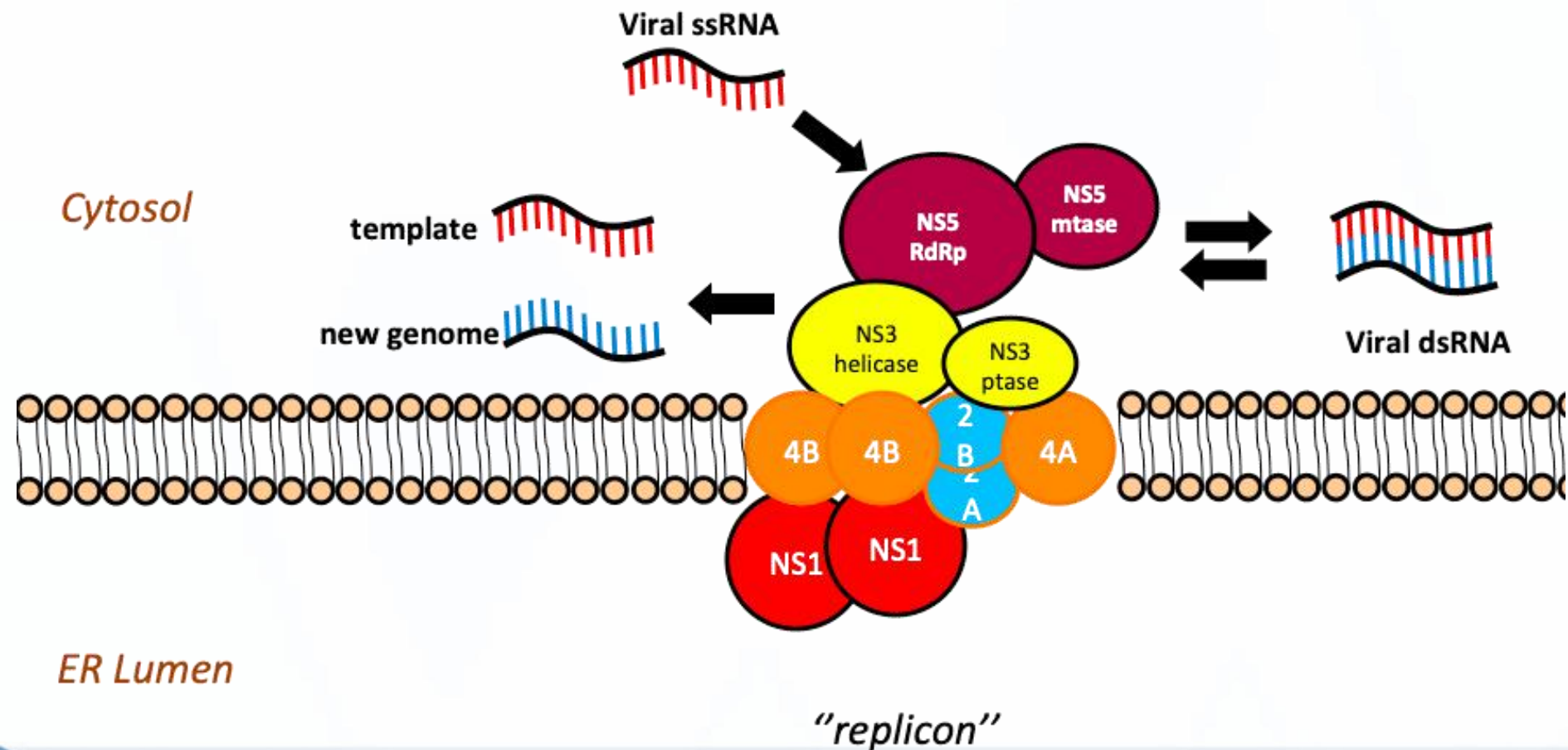
Targeting Flavivirus Non-structural Proteins



ZIKV Structural Proteins

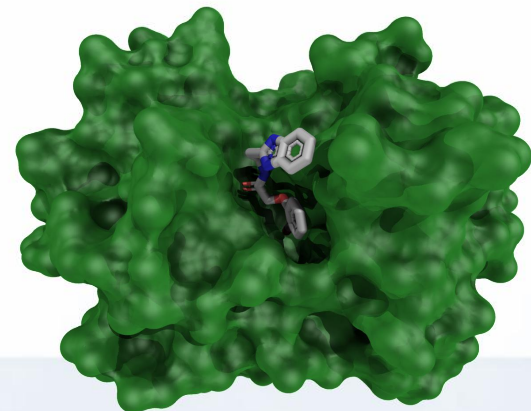


Targeting Flavivirus Non-structural Proteins



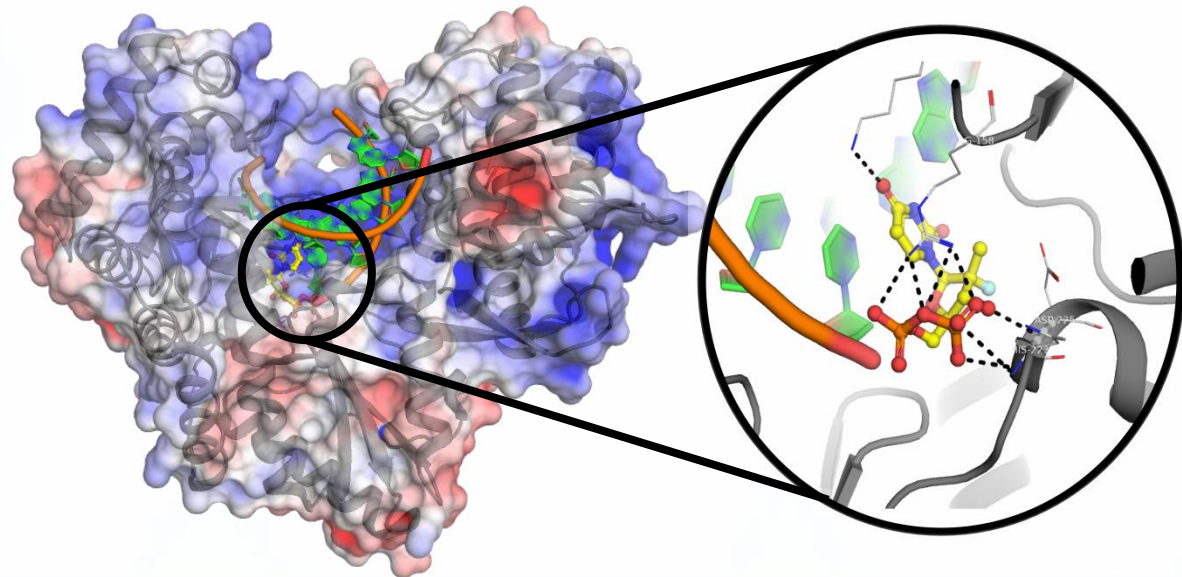
Structural Elucidation

ZIKV NS5 RNA-dependent RNA
polymerase

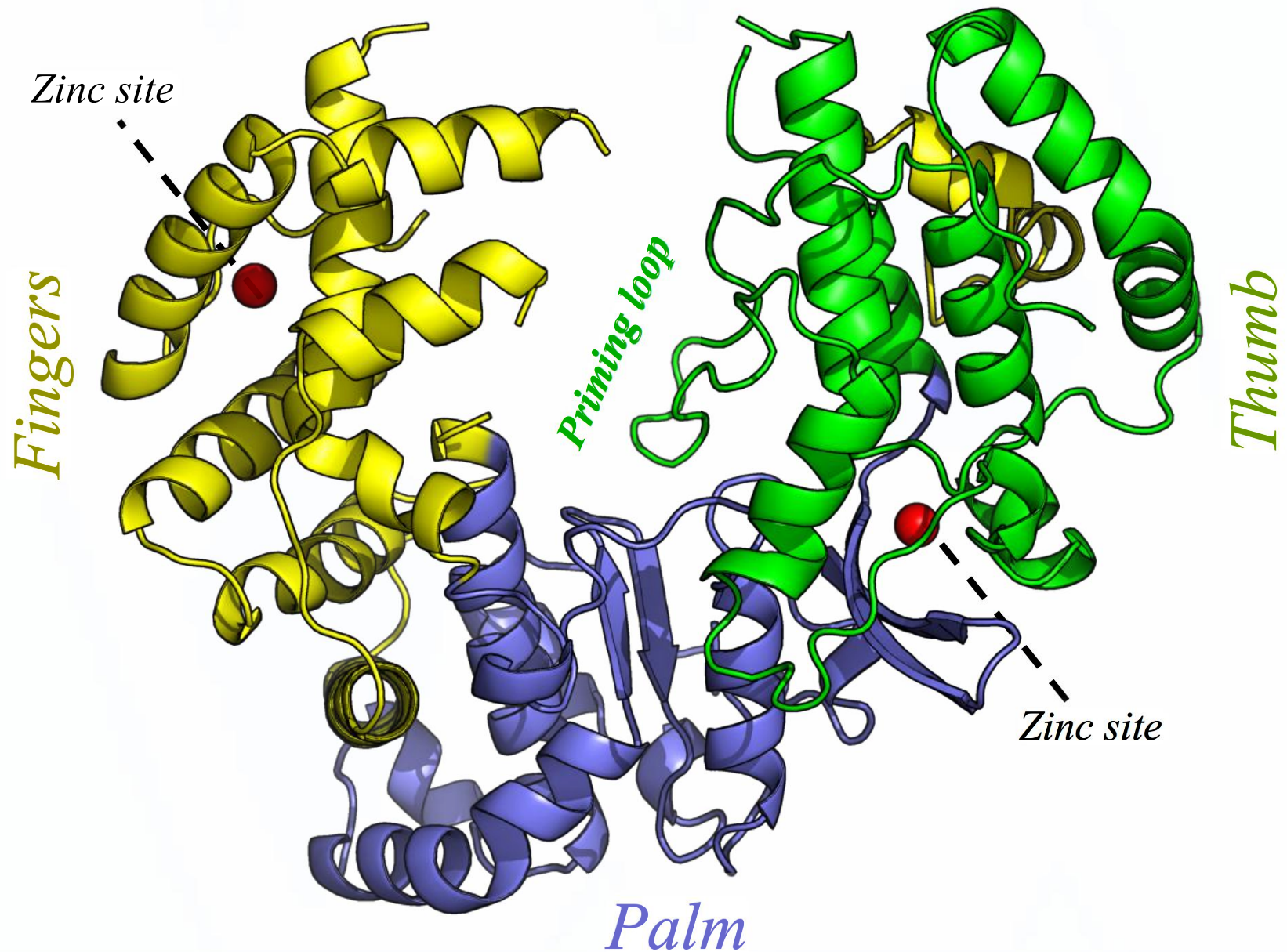


ZIKV NS5 RdRp

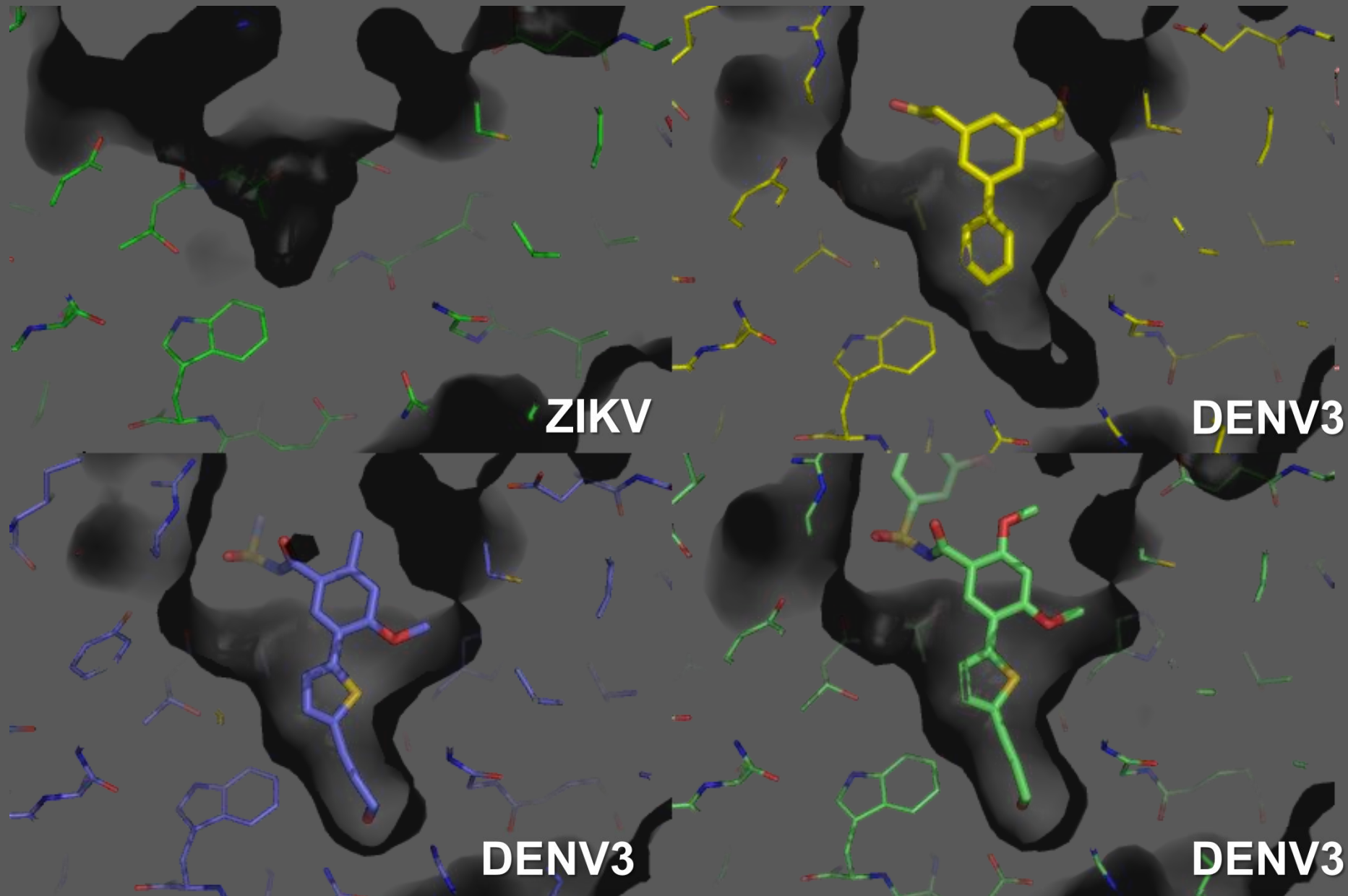
- *RNA-dependent RNA polymerase (RdRp) is an enzyme that catalyzes the replication of RNA*
- *Initiation is primer-independent (de novo)*
- *Essential for virus replication – exciting target !*



ZIKV NS5 RdRp

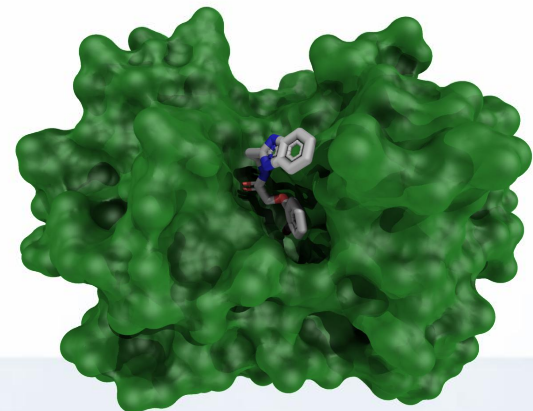


ZIKV NS5 RdRp Priming loop



Fragment screening

Exploring NS3 Helicase domain



Fragment-based drug design (FBDD)

- *Each heavy atom (HA) increases required Chemical Space (CS) by 8 fold*
- *10^3 molecules of MW 190 Da explore similar CS of 10^{18} molecules of MW 440 Da*



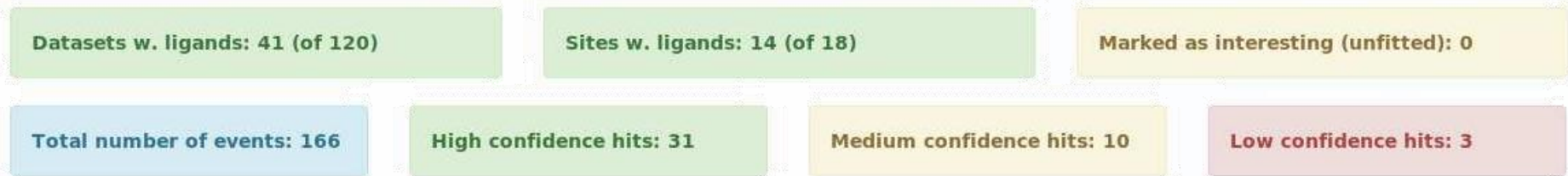
PANDDA Inspect Summary

Summary of Inspection of Datasets

Fitting Progress

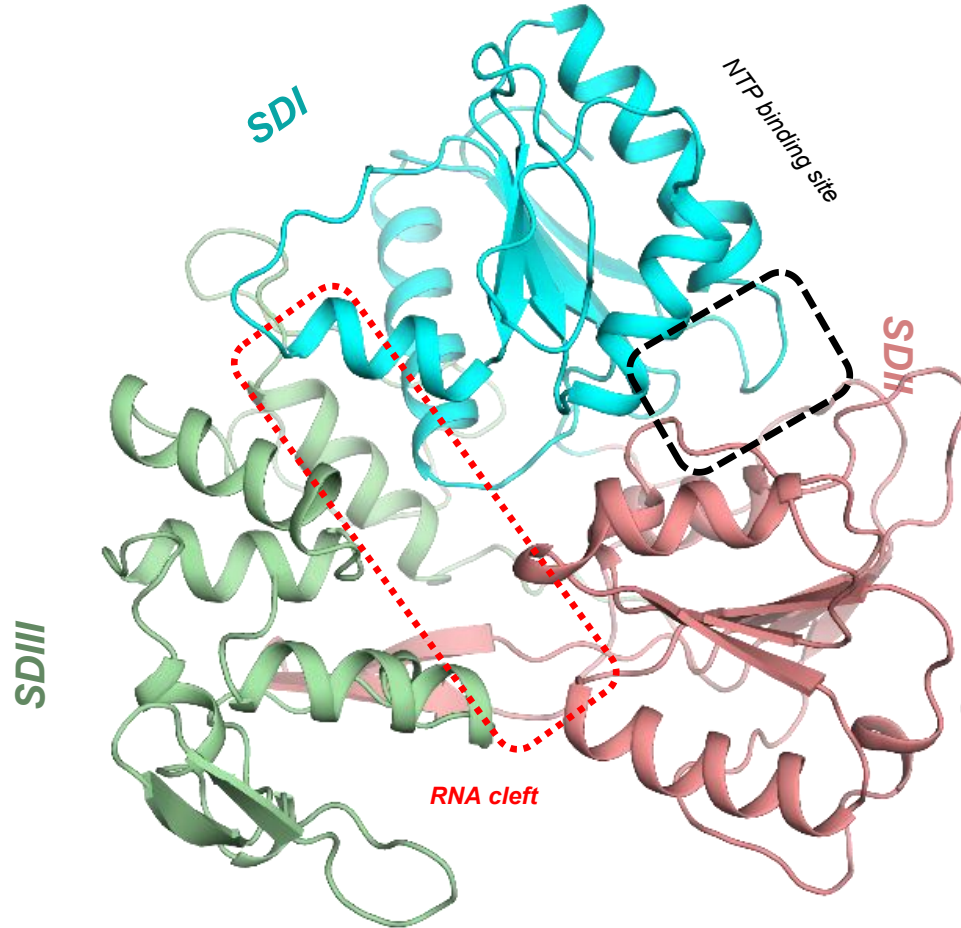


Identified Ligands by Site

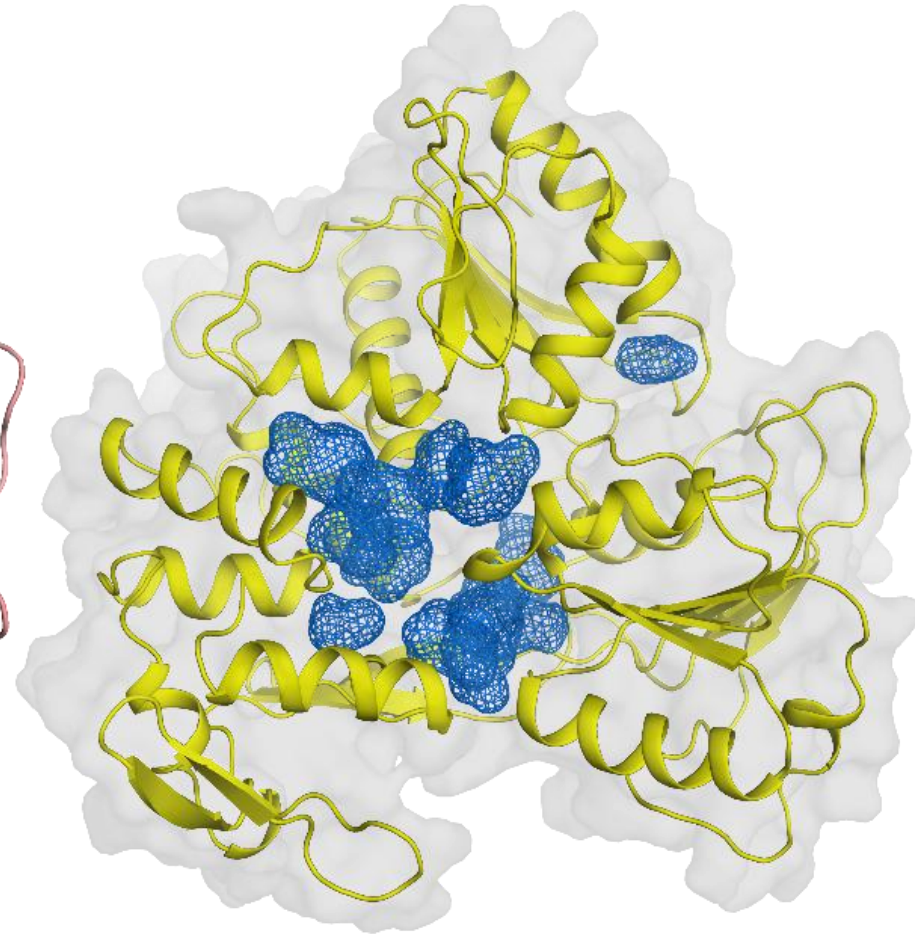


- *500 fragments tested*
- *About 450 datasets collected and processed (From 1.3 – 3.0 Å)*
- *166 events (33%)*
- *about 16 sites (a lot in interfaces)*
- *44 reliable and on interesting sites (8%)*

- **Several hits at the RNA binding site, previously unexplored**

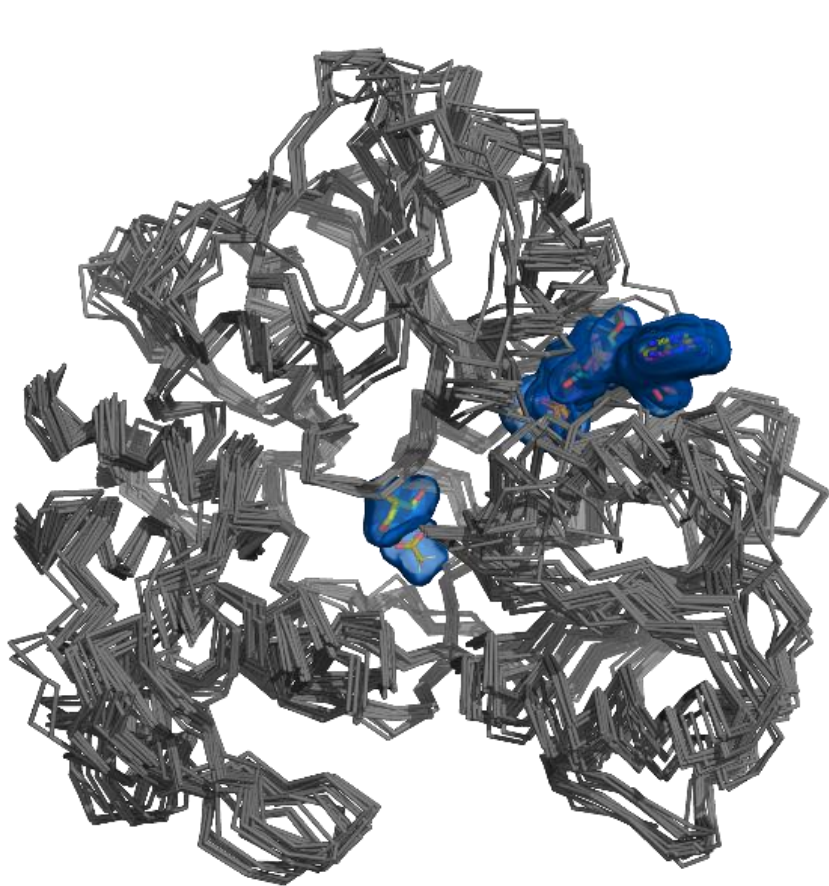


ZIKV NS3-Hel structure

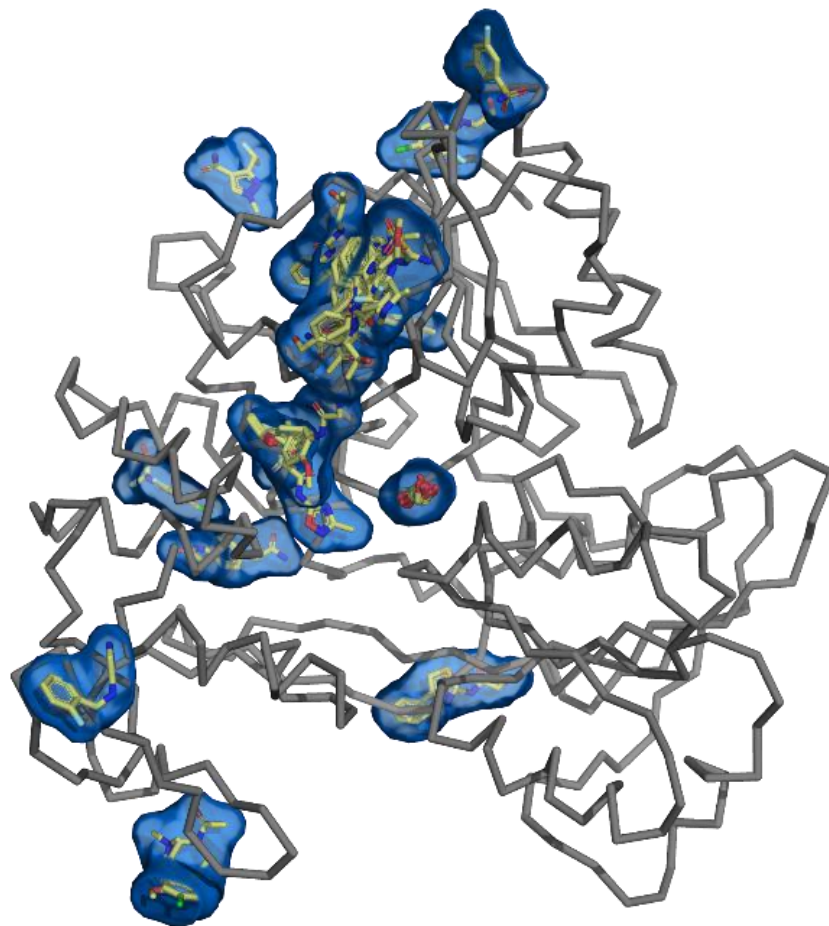


Theoretical predicted sites

- *Several hits at the RNA binding site, previously unexplored*



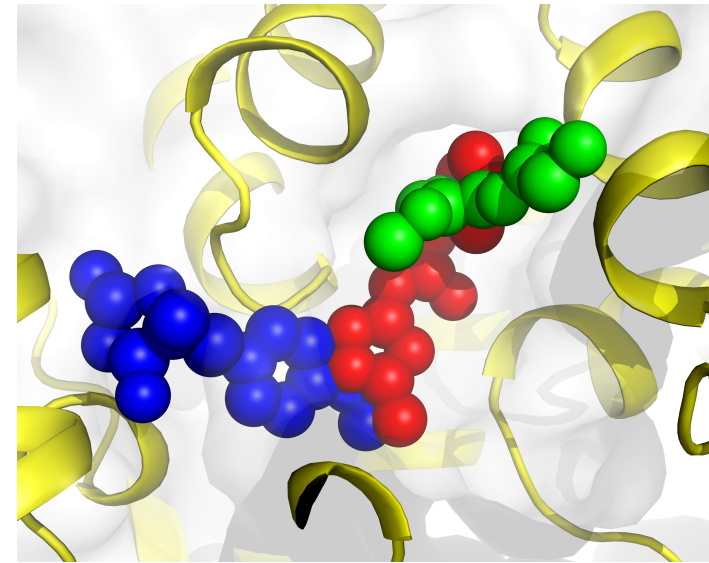
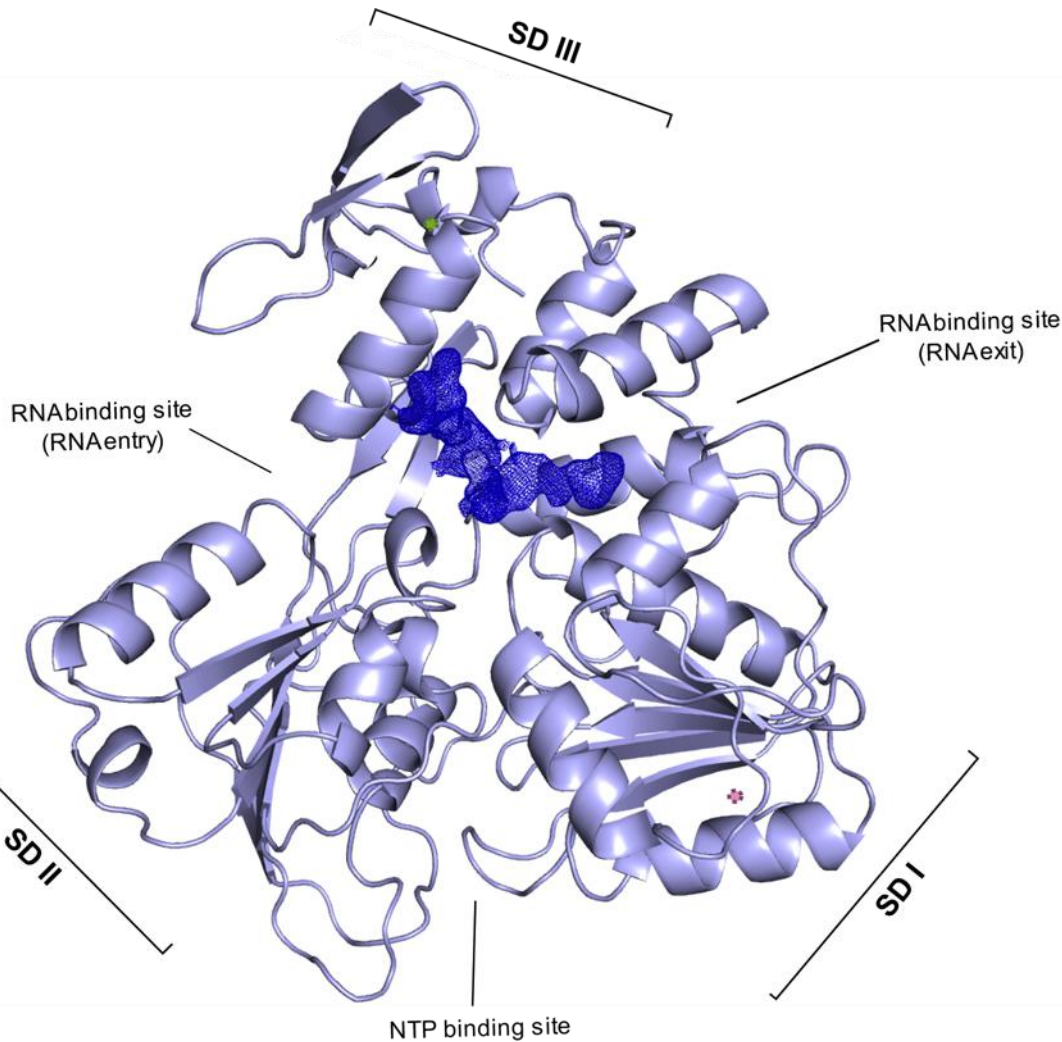
*protein ligand complexes for all
flaviviral NS3-Hel in the PDB*



*Results from the X-Chem
experiment*

Diamond

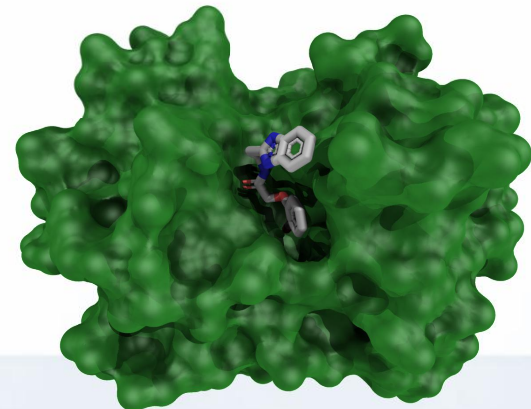
- *Three fragments close within binding distance at the RNA binding site*



under development...

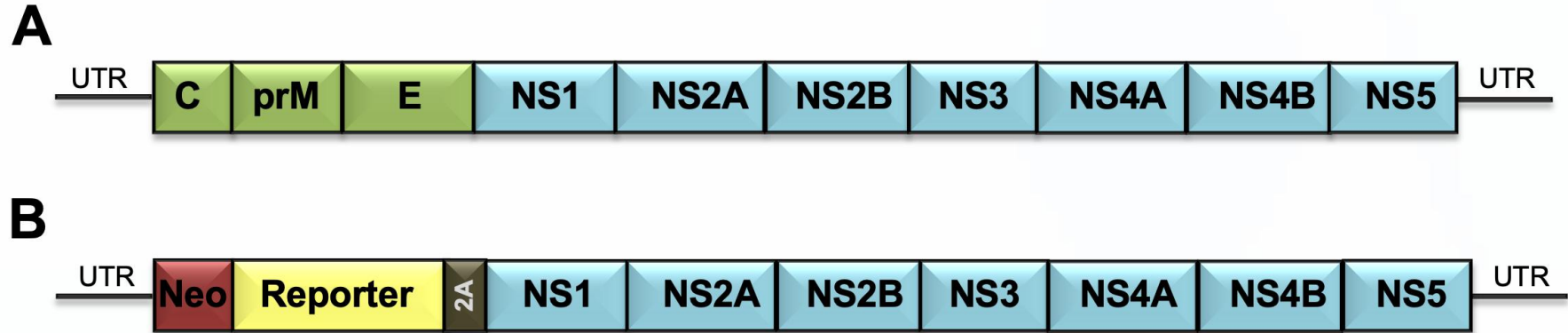
Replicons

Cell based assays using
non-infectant clones

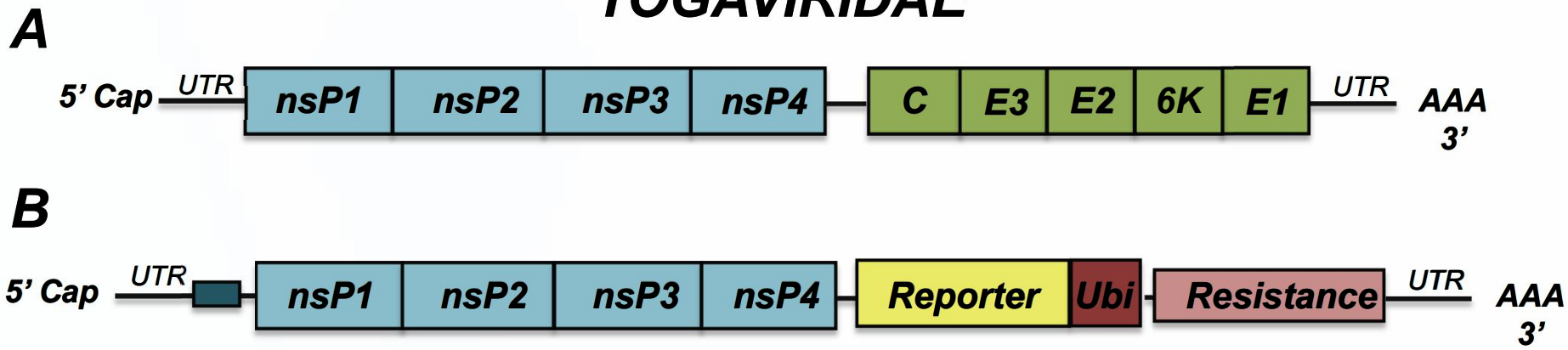


Cell lines expressing reporter containing viral replicons

FLAVIVIRIDAE

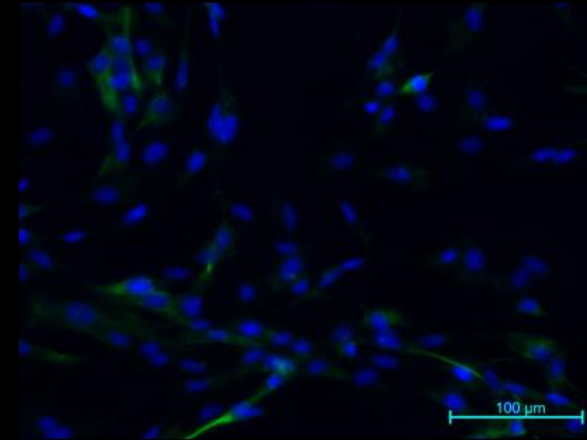
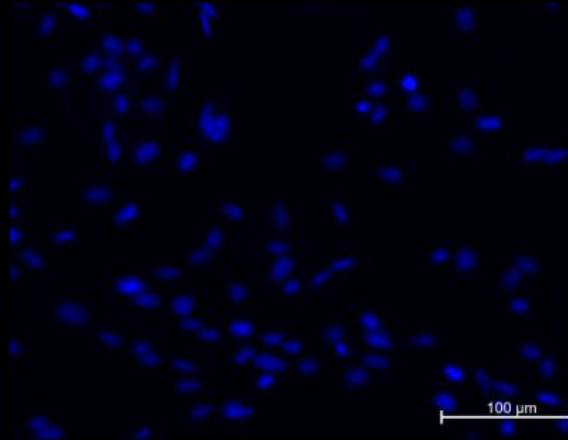
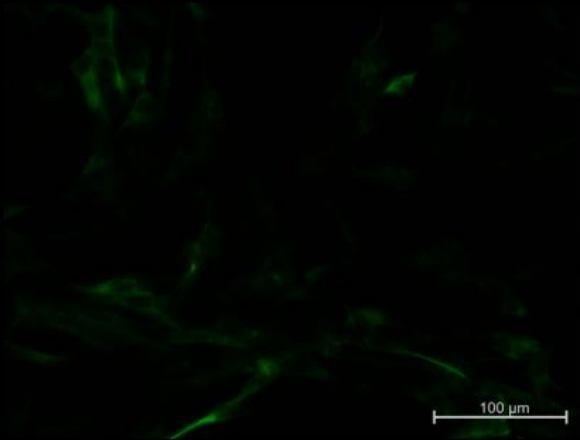


TOGAVIRIDAE

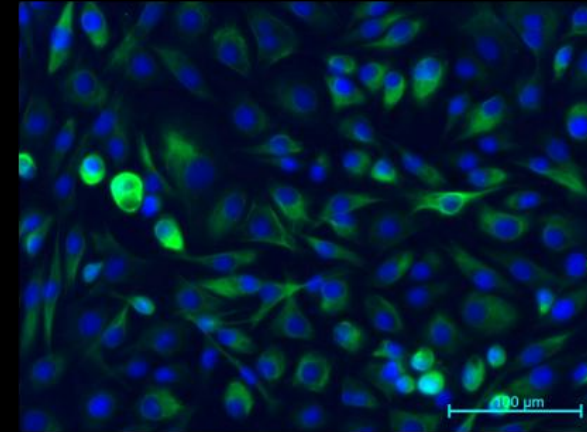
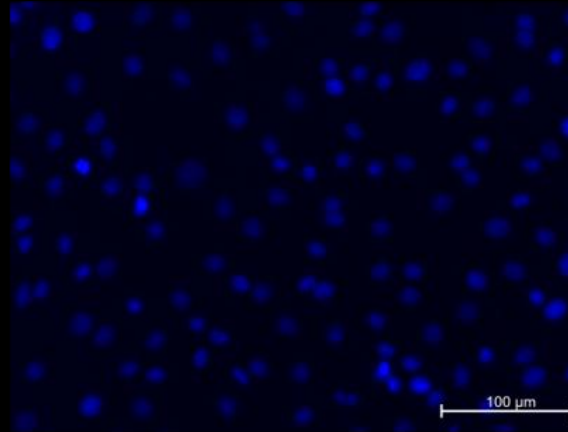
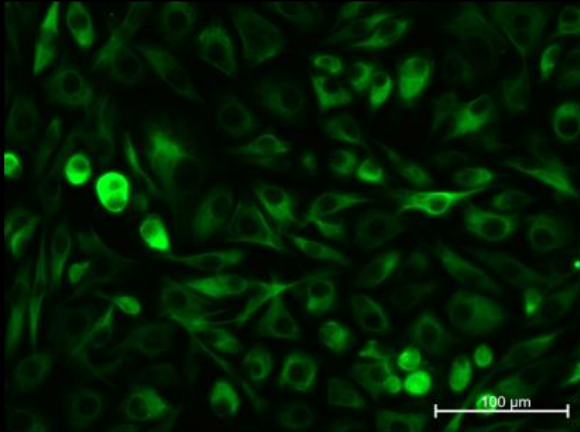


Flaviviral Replicons

BHK-21



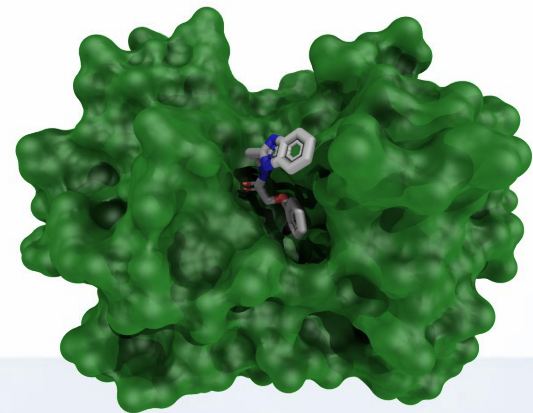
BHK-21-RepYFV7D-LUC



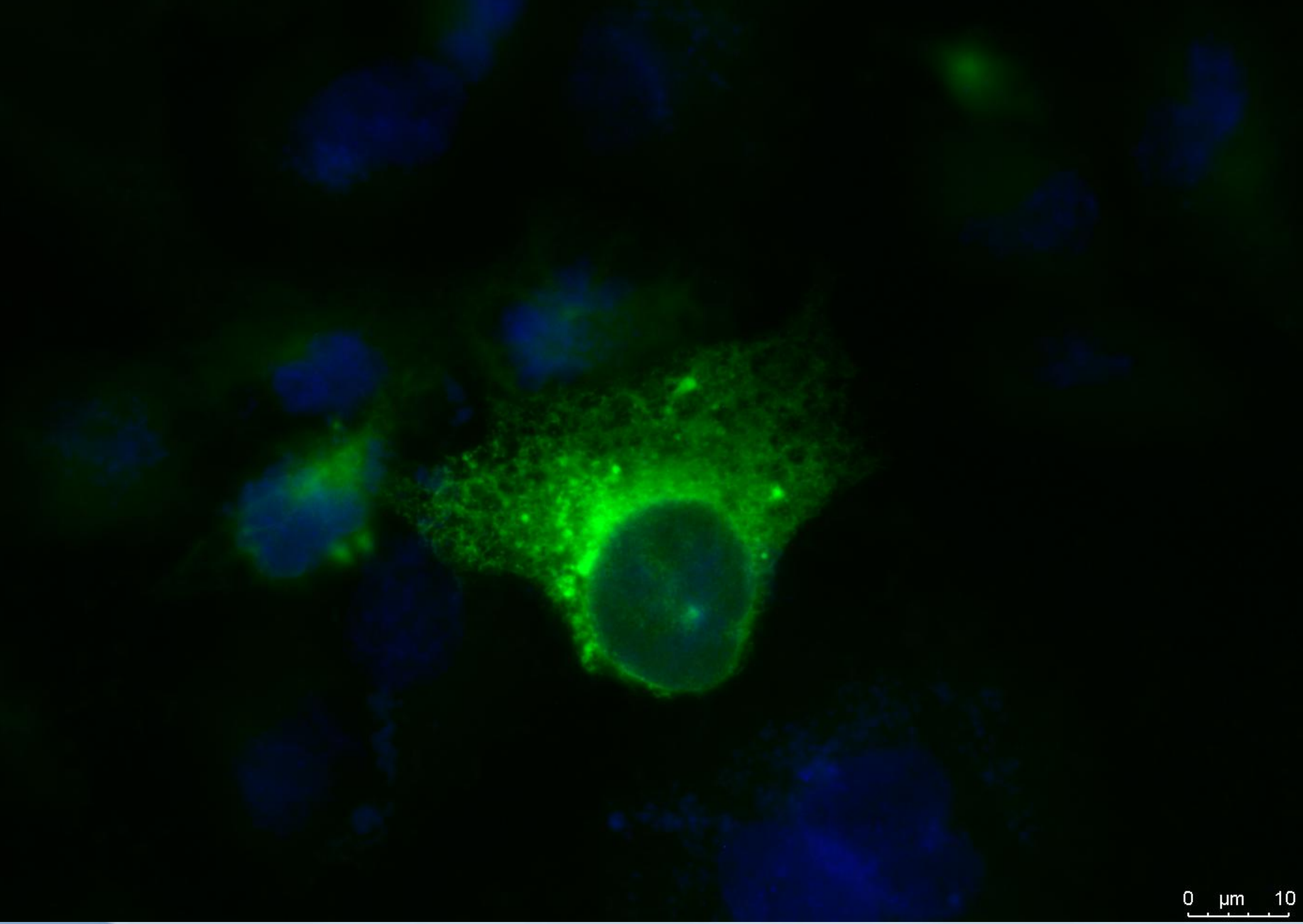
Antiviral assay using BHK-21 cell line expressing YFV reporter replicon. Indirect immunofluorescence assay (IFA). NS1 protein expression was evidenced using a monoclonal primary antibody (mAbF2) antibody and FITC-conjugated secondary antibody.

Viral assembly

Replication complex

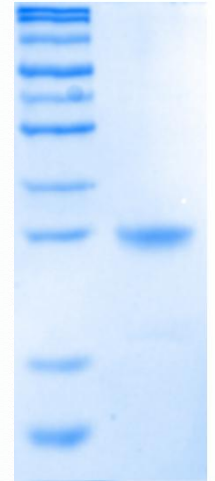
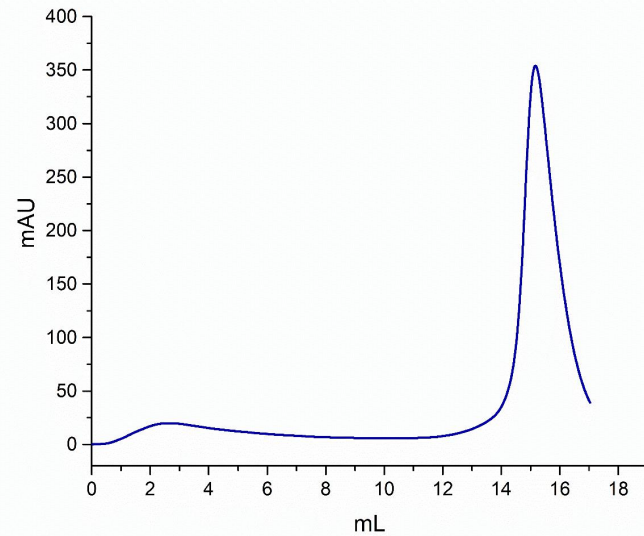
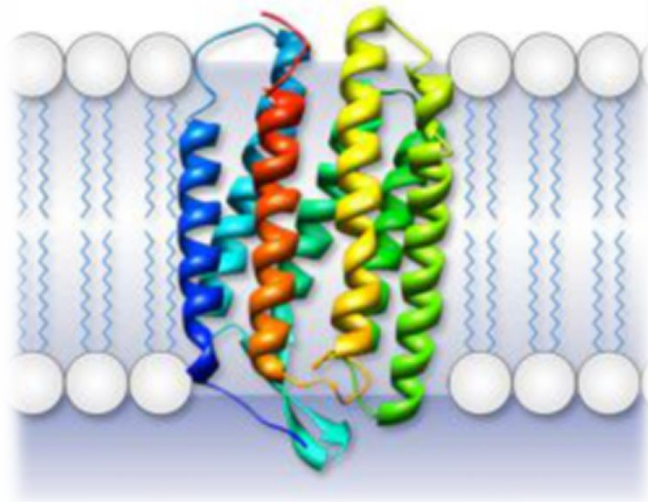


Transmembrane viral complex

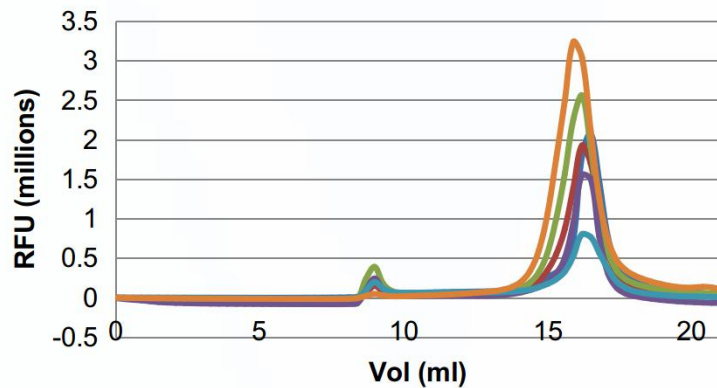


0 μm 10

Transmembrane viral complex

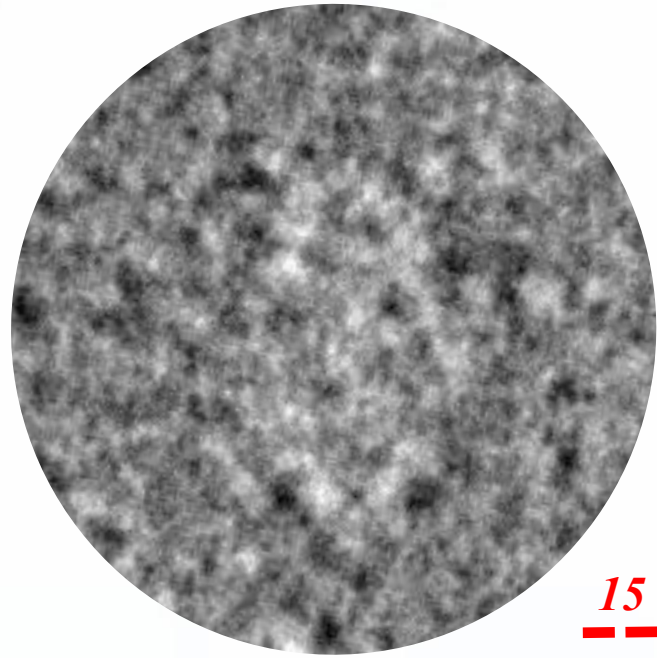


FSEC - Detergents

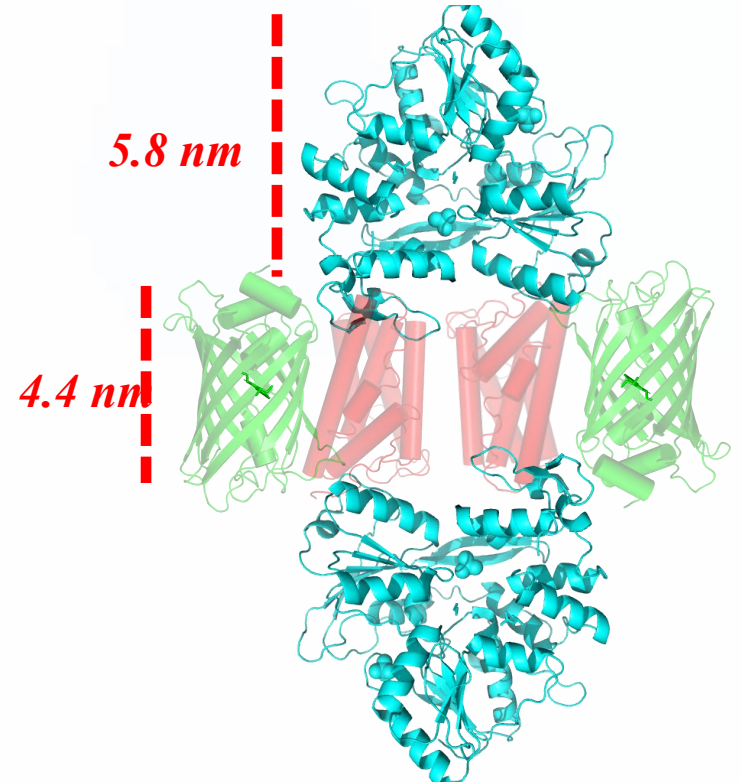
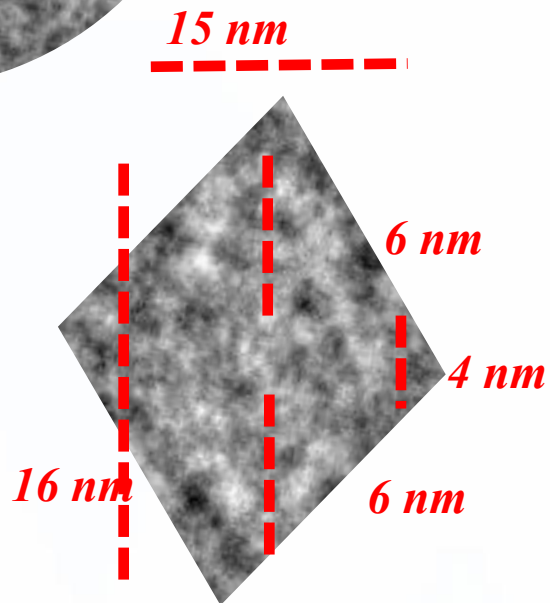


Ms. Edwin Quesnay
Profa. Andre Ambrosio

Transmembrane viral complex



- *NS3 PDB 6HM3*
- *NS4B Rosetta Model*
- *GFP*



Resurgence of Yellow Fever in Brazil/2017

MINAS GERAIS 

Sete mortes por febre amarela são confirmadas em Minas Gerais

Ministro da Saúde, Ricardo Barros, vai participar de videoconferência com autoridades do estado e do Espírito Santo para discutir casos da doença.

Por G1 MG — Belo Horizonte
18/01/2017 09h55 - Atualizado há 2 anos



Casos de febre amarela põem cidades do litoral paulista em alerta no feriado

Quem ainda não se vacinou deve, ao menos, passar repelente e evitar áreas de mata



William Cardoso

SÃO PAULO | AGORA O registro de mortes por [febre amarela em cidades do litoral](#) paulista põe em alerta quem pretende passar o feriado de 7 de

SCIENTIFIC REPORTS

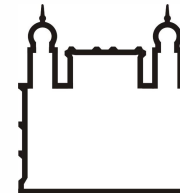
OPEN Phylodynamics of Yellow Fever Virus in the Americas: new insights into the origin of the 2017 Brazilian outbreak

Received: 4 May 2017
Accepted: 30 June 2017
Published online: 07 August 2017

Daiana Mir¹, Edson Delatorre¹, Myrna Bonaldo², Ricardo Lourenço-de-Oliveira³, Ana Carolina Vicente⁴ & Gonzalo Bello¹

Yellow fever virus (YFV) strains circulating in the Americas belong to two distinct genotypes (I and II) that have diversified into several concurrent enzootic lineages. Since 1999, YFV genotype I has spread outside endemic regions and its recent (2017) reemergence in non-endemic Southeastern Brazilian states fuels one of the largest epizootic of jungle Yellow Fever registered in the country. To better understand this phenomenon, we reconstructed the phylodynamics of YFV American genotypes using sequences from nine countries sampled along 60 years, including strains from Brazilian 2017 outbreak. Our analyses reveals that YFV genotypes I and II follow roughly similar evolutionary and demographic dynamics until the early 1990s, when a dramatic change in the diversification process of the genotype I occurred associated with the emergence and dissemination of a new lineage (here called modern). Trinidad and Tobago was the most likely source of the YFV modern-lineage that spread to Brazil and Venezuela around the late 1980s, where it replaced all lineages previously circulating. The modern-lineage caused all major YFV outbreaks detected in non-endemic South American regions since 2000, including the 2017 Brazilian outbreak, and its dissemination was coupled to the accumulation of several amino acid substitutions particularly within non-structural viral proteins.

CIBFar



Ministério da Saúde

FIOCRUZ

Fundação Oswaldo Cruz

Gabriela Noske

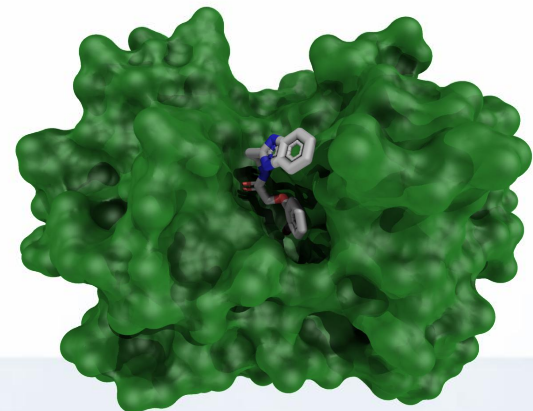
Victor Oliveira

Ms. Nathalya Furtado

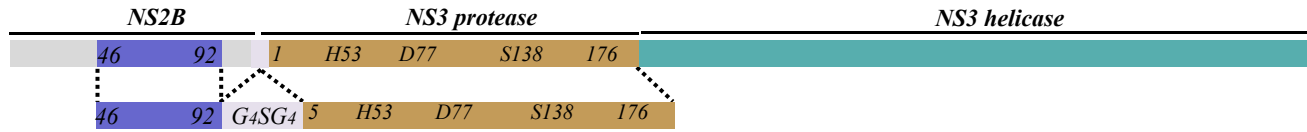
Dr. Myrna Bonaldo

Structural elucidation

YFV NS3 protease domain

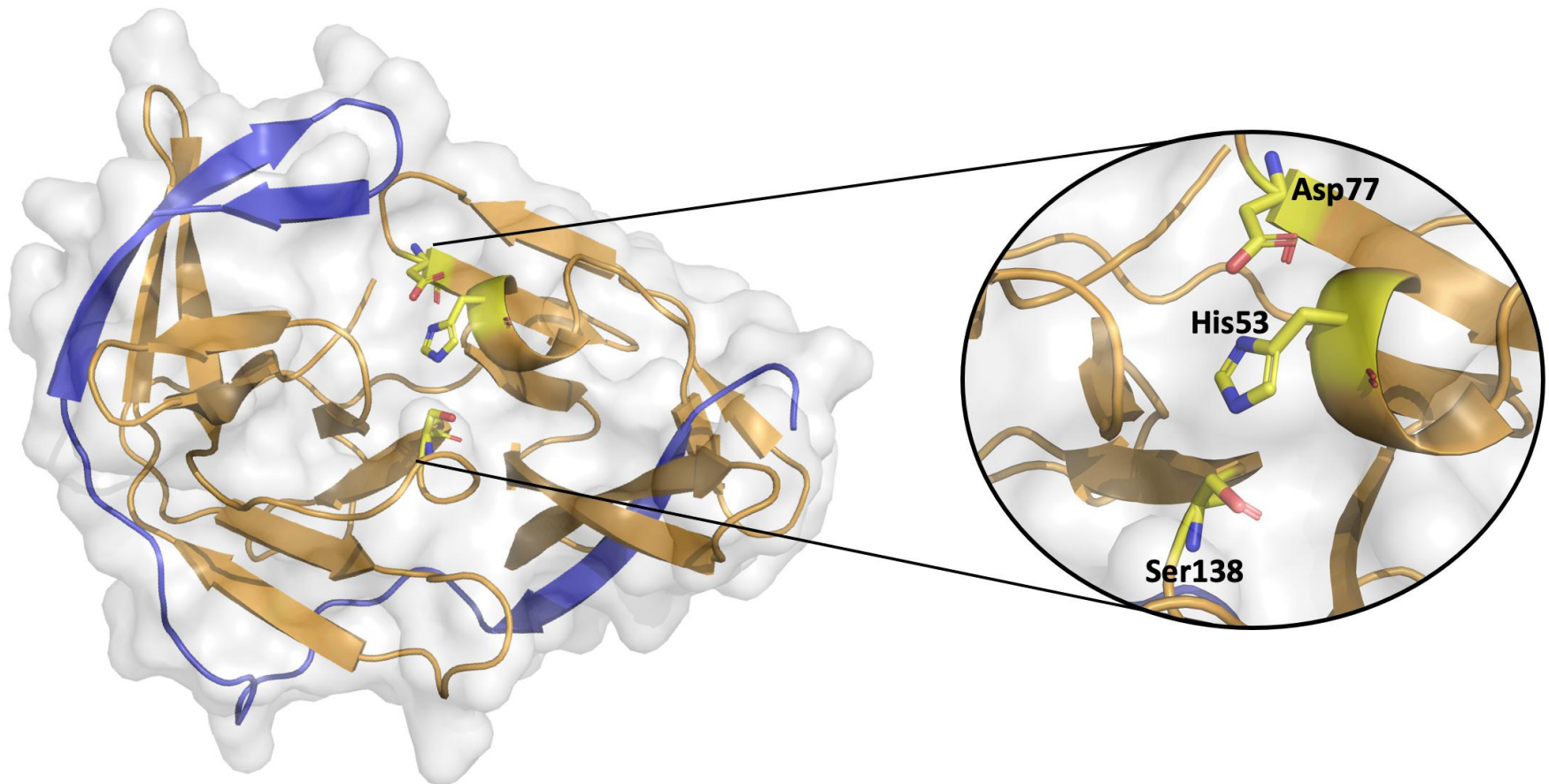


YFV NS2B-NS3 protease— to be published

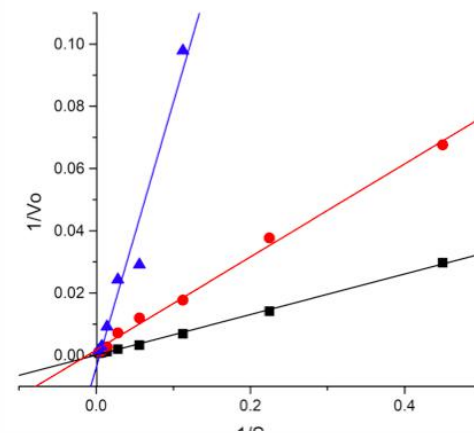
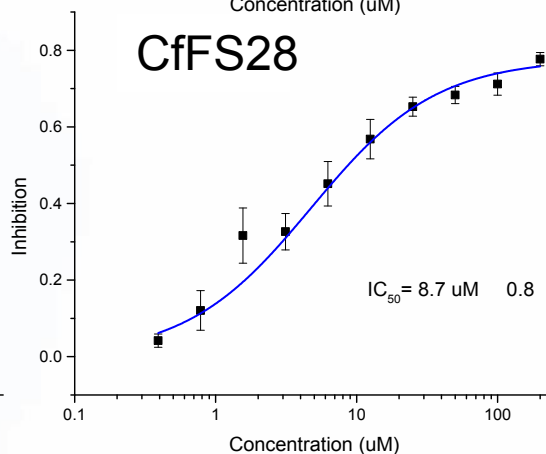
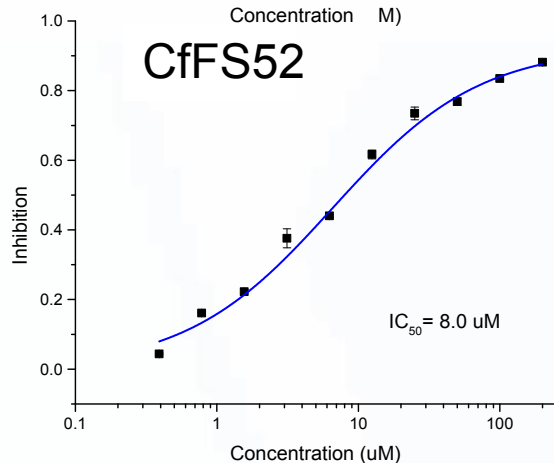
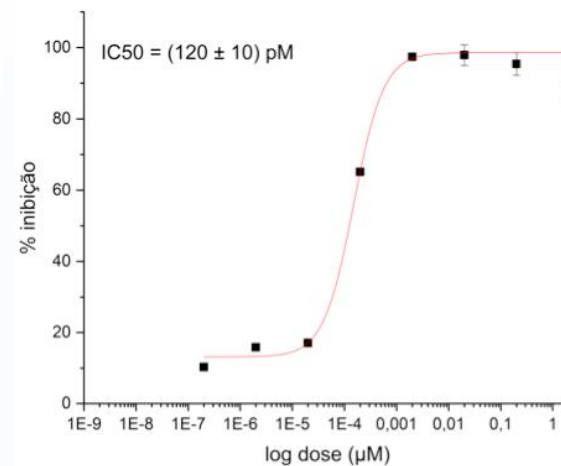
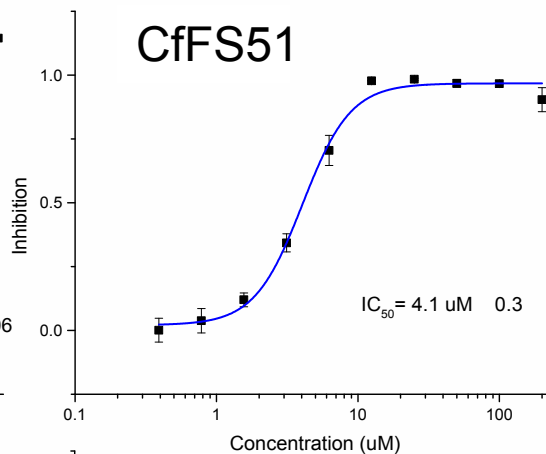
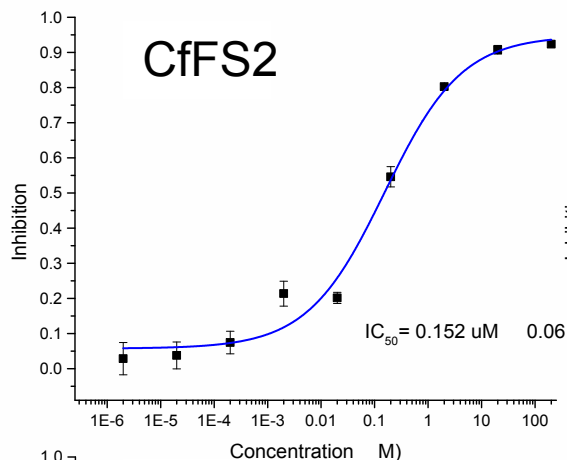


● NS2B

● NS3 protease



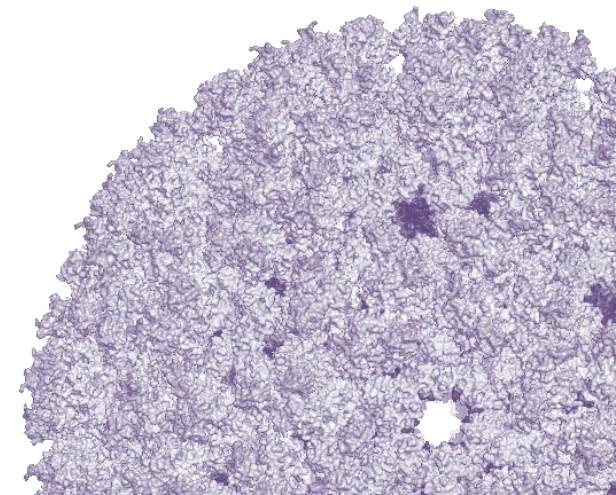
S2B-NS3protease YFV



Enzyme kinetics of YFV protease versus synthetic peptide

compounds series examples (about 1,000 tested so far)

- Zika Virus
 - Yellow Fever Virus
 - Chikungunya Virus
 - Mayaro Virus
- Recombinant proteins
 - Enzyme assays
 - Biochemical assays
 - Cell-based assays
 - X-ray XTAL
 - Cryo-EM



Acknowledgements



People @ IFSC/USP



Dr. Rafael V C Guido



André Godoy



Nathalya Mesquita



Rafaela Fernandes



Renata Vieira



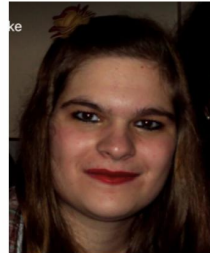
Bruna Macedo



Victor Oliveira.



Ketilyn Oliveira



Gabriela Noske



Marjorie Freire



Gustavo Lima



Andre Ambrósio



Congratulations and thank you !

