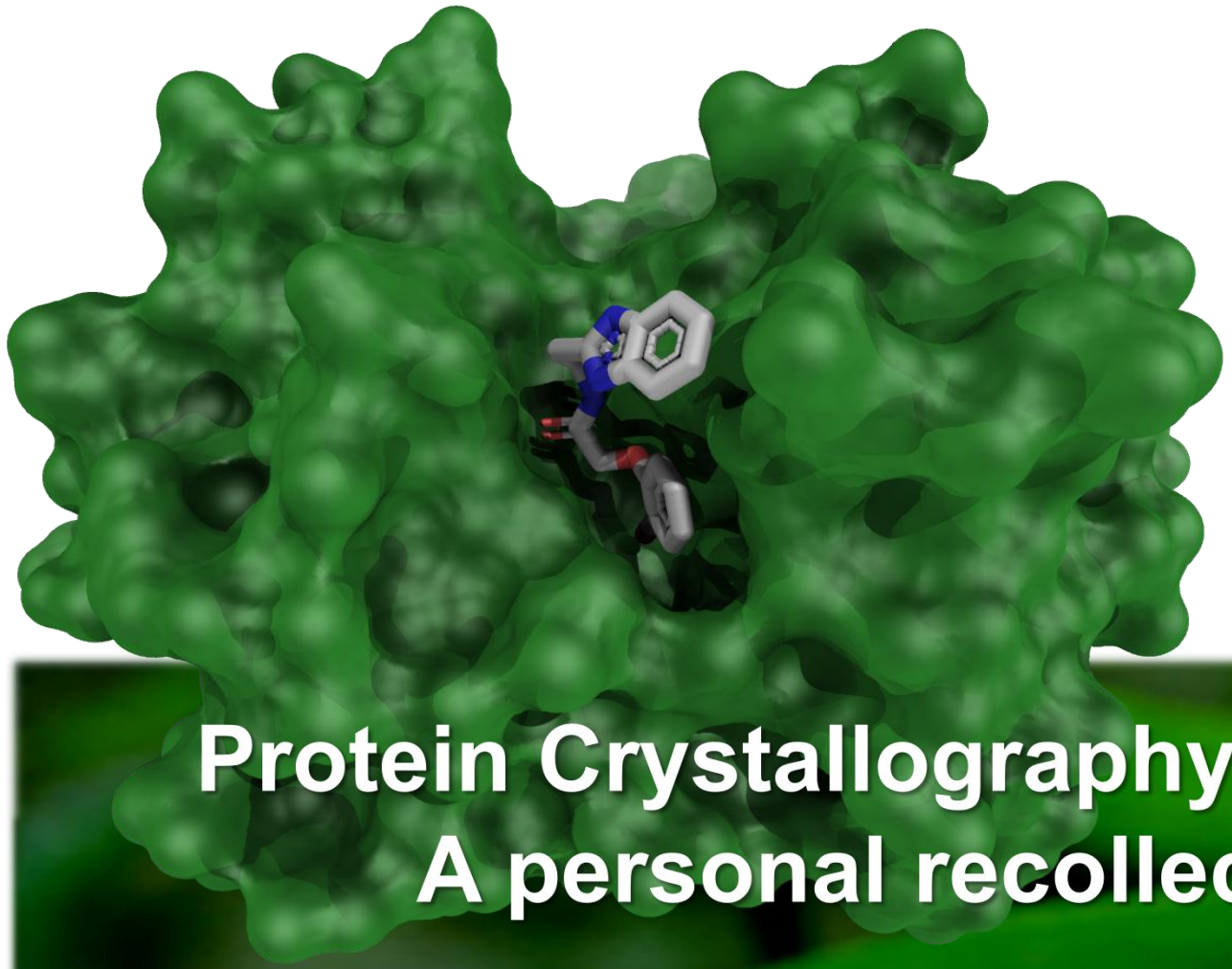


Protein Crystallography in Brazil: A personal recollection

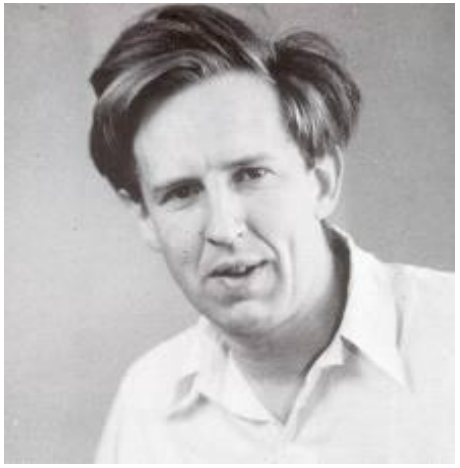
Glaucius Oliva
Instituto de Física de São Carlos – USP

Science & Development

CIBFar

- All human progress is directly related to the advancement of knowledge promoted by science.
- In the 21st Century, Information and Knowledge are the two main components of the wealth of nations
- All the great challenges of humanity, in health, food supply, energy, environment, sustainability, overcoming inequalities, employment, etc ... will only be answered by Science and Education.

THE SOCIAL FUNCTION OF SCIENCE



By

J. D. BERNAL, F.R.S.

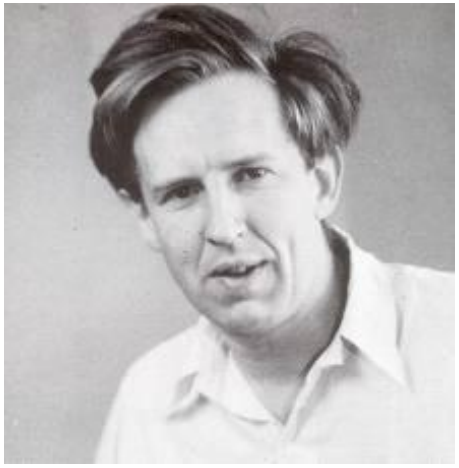
Birkbeck College, University of London

NEW YORK
THE MACMILLAN COMPANY
1939

There are here two sharply distinct points of view which might be called the ideal and the realist pictures of science. In the first picture science appears as concerned only with the discovery and contemplation of truth; its function, as distinct from that of mythical cosmologies, is to build up a world picture that fits the facts of experience. If it is also of practical utility, so much the better, as long as its true purpose is not lost. In the second picture utility predominates; truth appears as a means for useful action and can be tested only by such action.

The history of science shows that its growth follows, in the main, the general directions of economic development, and that the degree and scale in which science is pursued is roughly proportional to commercial and industrial activity. The leading industrial countries of the world are also the leading scientific countries.

THE SOCIAL FUNCTION OF SCIENCE



By

J. D. BERNAL, F.R.S.

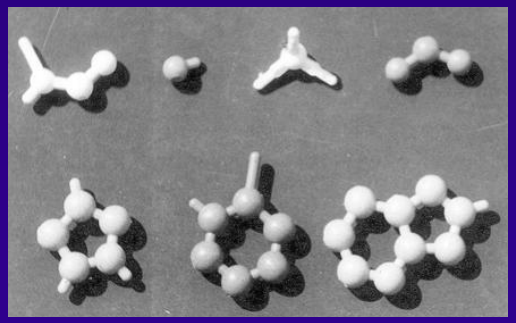
Birkbeck College, University of London

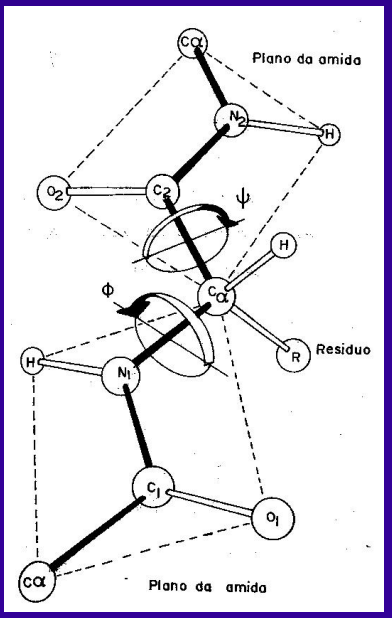
NEW YORK
THE MACMILLAN COMPANY
1939

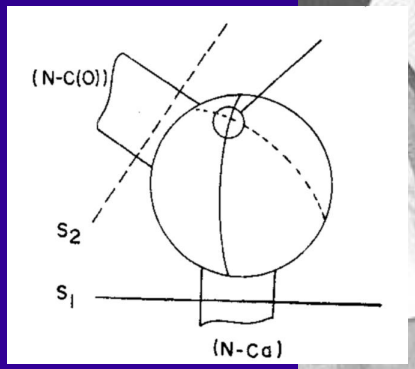
Science in Latin America has, until a comparatively recent date, suffered from the same disabilities as afflicted the mother country. In the colonial days, particularly at the beginning, something was done in natural history and mining, but the interest soon faded out and the revolution and civil wars which followed during most of the nineteenth century were not conducive to the development of science. In the present century, however, under the influence of the U.S.A. and of resurgent liberal thought, a renaissance in science from which much can be hoped is beginning, and already, particularly in Mexico and the Argentine, notable developments have been made in medicine, biology, and archaeology.

How it all began...

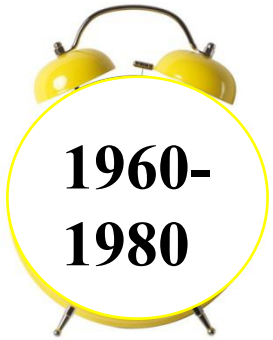
- The assembly of a molecular model of mioglobin, without the instructions (lost!!!) - 1979

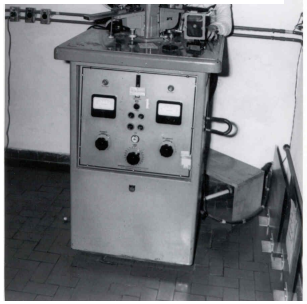






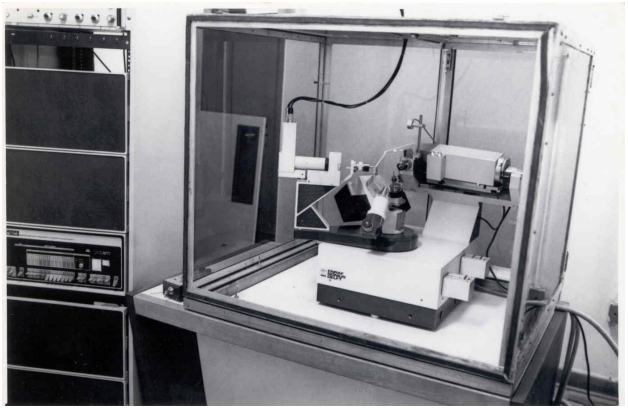
Before proteins





1958

first X-ray generator



1975

automated single crystal diffractometer

1976

school on direct methods

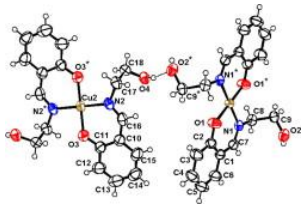
1977

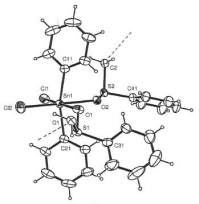
Eduardo Castellano visits

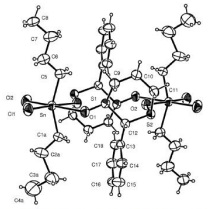
1971

founding of the Brazilian Association of Crystallography

Chemical crystallography in Brazil







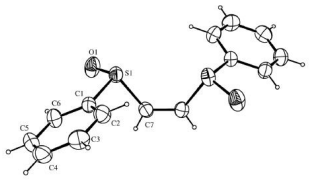
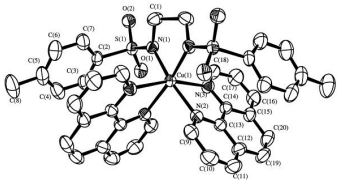


Figure 1. ORTEP plot of *rac*-,*cis*-cbpse, with the atom labeling scheme. Displacement ellipsoids are drawn at the 30% probability level.



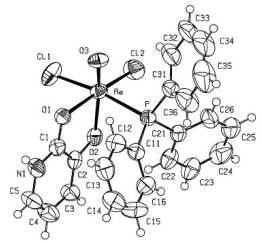
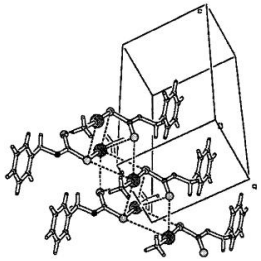
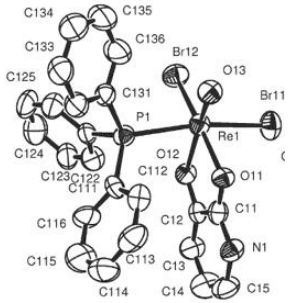
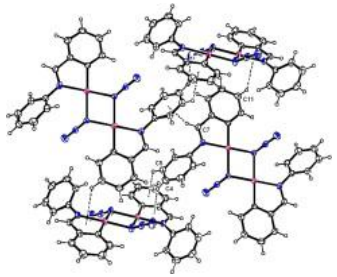


Figure 1. Ellipsoid representation[14] of **1**, with the thermal ellipsoids representing 50% probability.







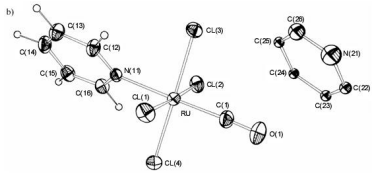
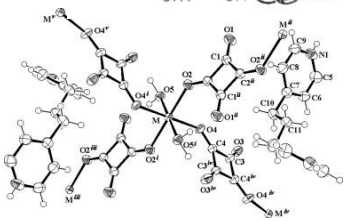
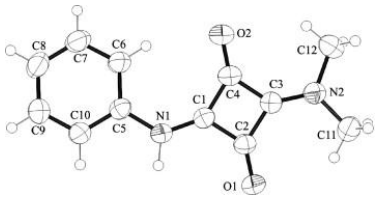
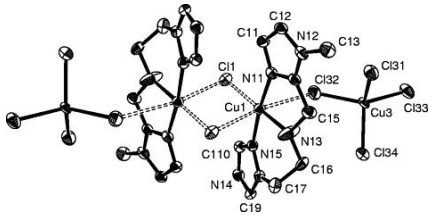


Figure 1. ORTEP drawings of pyH[*trans*-$RuCl_4(py)_2$] (a), and pyH[*trans*-$RuCl_4(CO)(py)$] (b).

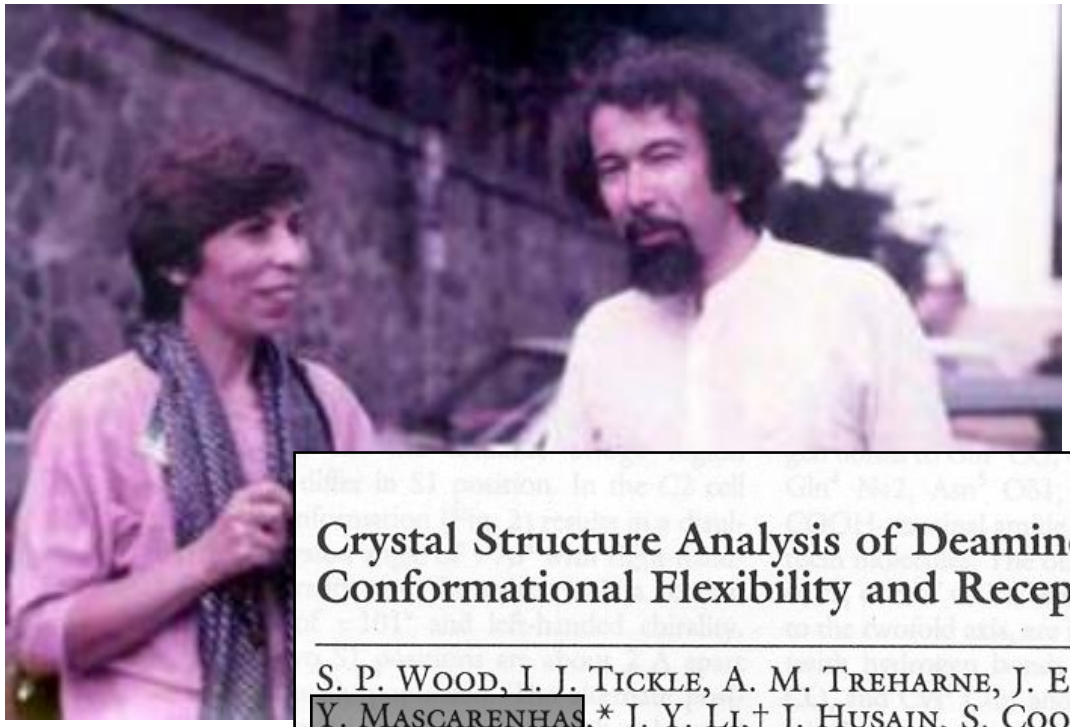






~3000 structures solved in Brazil

Yvonne does a sabbatical year with Tom Blundell in London



Crystal Structure Analysis of Deamino-Oxytocin: Conformational Flexibility and Receptor Binding

S. P. Wood, I. J. Tickle, A. M. Treharne, J. E. Pitts, Y. Mascarenhas,* J. Y. Li,† J. Husain, S. Cooper, T. L. Blundell, V. J. Hruby, A. Buku, A. J. Fischman, H. R. Wyssbrod

Two crystal structures of deamino-oxytocin have been determined at better than 1.1 Å resolution from isomorphous replacement and anomalous scattering x-ray measurements. In each of two crystal forms there are two closely related conformers with disulfide bridges of different chirality, which may be important in receptor recognition and activation.

Science (1986), v.232, p 633-636

Birkbeck
UNIVERSITY OF LONDON

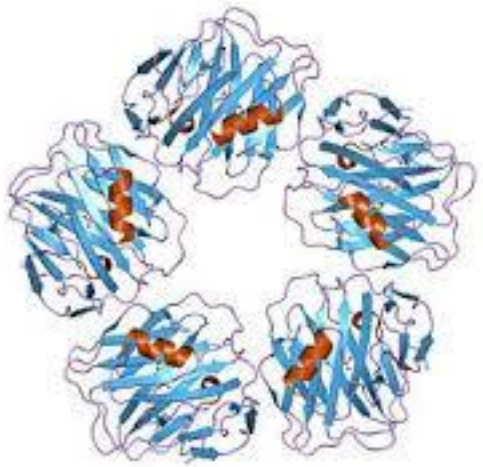
Room EB22 @ Birkbeck College, 1984

Structure of pentameric human serum amyloid P component

Jonas Emsley, Helen E. White, Bernard P. O'Hara*, Glaucius Oliva*, Narayanaswamy Srinivasan, Ian J. Tickle, Tom L. Blundell, Mark B. Pepys† & Steve P. Wood‡

Laboratory of Molecular Biology and ICRF Unit of Structural Molecular Biology, Department of Crystallography, Birkbeck College, London WC1E 7HX, UK
† Immunological Medicine Unit, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0NN, UK

The three-dimensional structure of pentameric human serum amyloid P component at high resolution, the first reported for a pentraxin, reveals that the tertiary fold is remarkably similar to that of the legume lectins. Carboxylate and phosphate compounds bind directly to two calcium ions; interactions with a carboxyethylidene ring are mediated by Asn 59 and Gln 148 ligands of the calcium ions. These X-ray results indicate the probable modes of binding of the biologically important ligands, DNA and amyloid fibrils.



Nature ***367****, 338 - 345 (27 January 1994)*

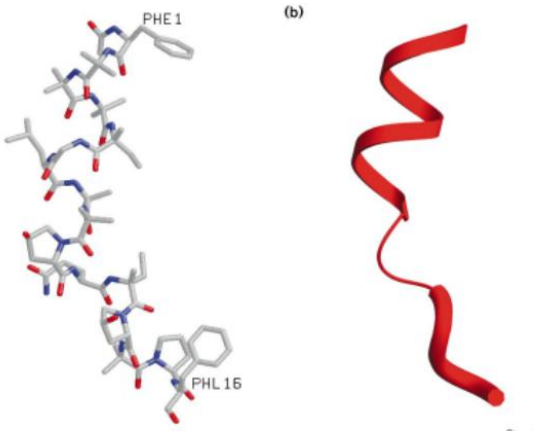
The structure and function of antiamoebin I, a proline-rich membrane-active polypeptide

CF Snook†, GA Woolley‡, G Oliva§, Vasantha Pattabhi#, SP Wood¶, TL Blundell¥ and BA Wallace*

Background: Antiamoebin is a member of the peptaibol family of polypeptides and has a unique antibiotic activity: it acts as an antiamoebic agent, but does not effectively haemolyze erythrocytes even though it does exhibit membrane-modifying activity.

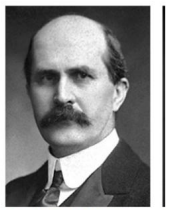
Address: Department of Crystallography, Birkbeck College, University of London, London, WC1E 7HX, UK.

Present addresses: †Department of Molecular

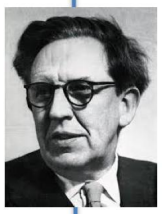


Structure ***6****, Issue 6, p783–792, 15 June 1998*

The Genealogy of Protein Crystallography in Brazil



W.H.BRAGG

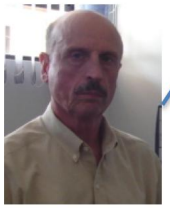


J.D. BERNAL

DOROTHY
HODGKIN

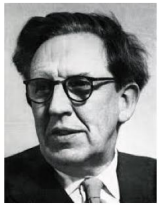
OXFORD/UK

T.L.BLUNDELL

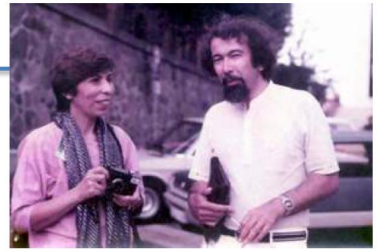


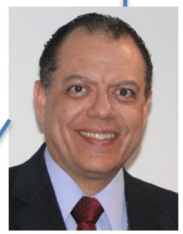
CASTELLANO

DEPARTMENT OF CRYSTALLOGRAPHY
BIRKBECK COLLEGE - LONDON/UK



1977-YVONNE





GLAUCIUS

RICHARD

OSMAR

PAULA

BERNAL LECTURE

Established in 1968, this annual lecture commemorates the life and work of JD Bernal (1901-71), with a particular focus on structural biology, X-ray crystallography and, especially, the social consequences of science.

2015

Sir Venki Ramakrishnan: 'The termination of translation in bacteria and eukaryotes (audio recording)'

2014

Dame Janet Thornton: 'The importance of genomics and bioinformatics for the future of medicine and agriculture (audio recording)'

2013

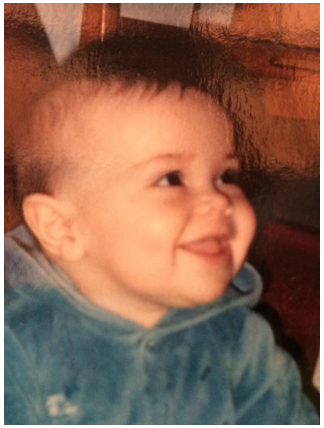
Professor Glaucius Oliva: 'From structural biology of neglected diseases to Brazilian science'

2012

Rt Hon David Willetts MP, Minister of State for Universities and Science: 'Bernal, science, risk and regulation' (cancelled owing to scheduling problems).

UK years...
PhD & kids

cellco
Biotecnologia ao seu alcance

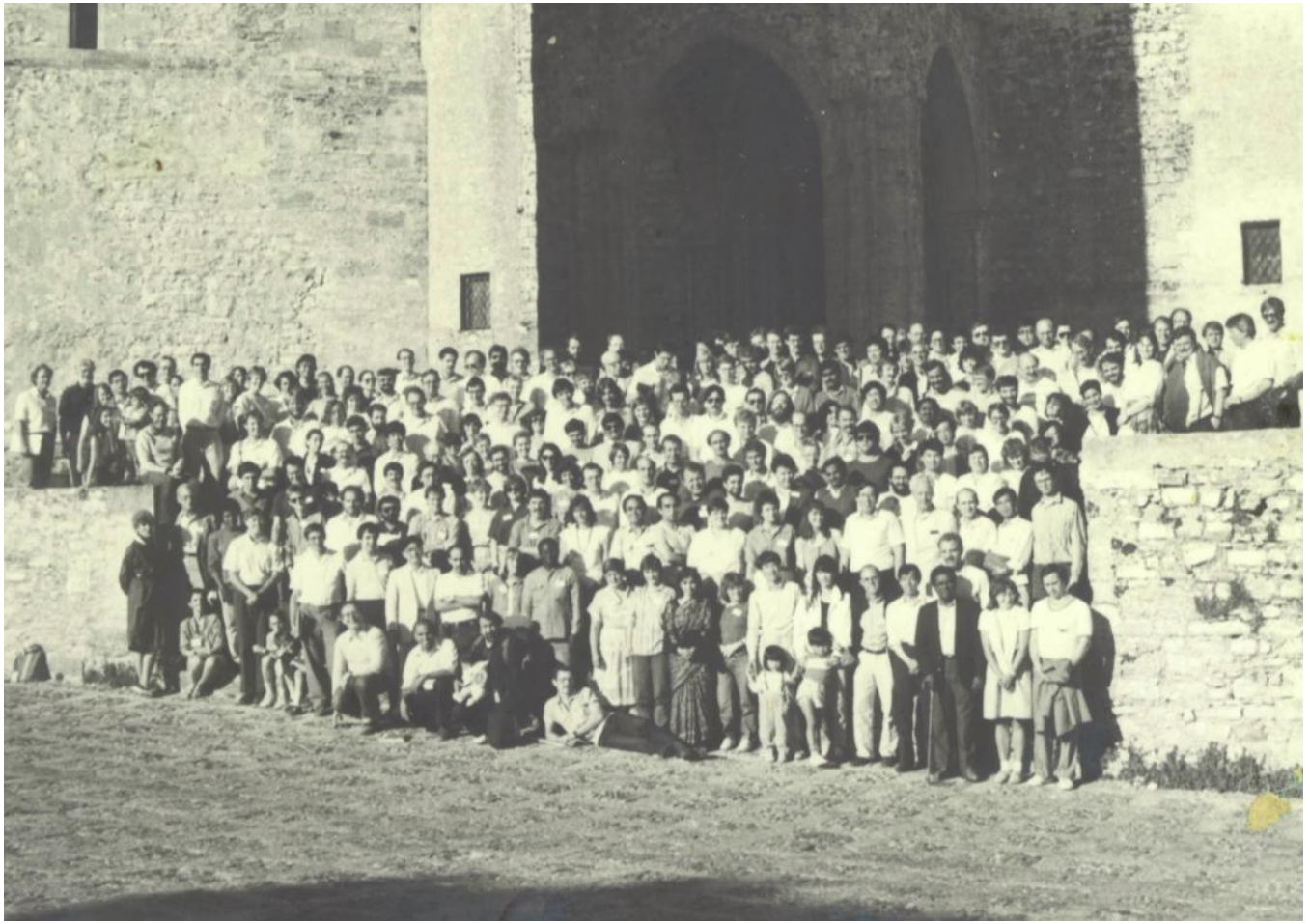


Ettore Majorana Foundation and Centre for Scientific Culture
President: Professor Antonino Zichichi

INTERNATIONAL SCHOOL of CRYSTALLOGRAPHY

Director: Sir Tom Blundell, FRS FMedSci

14th Course : Crystallography of Molecular Biology
ERICE, 29 May to 7 June 1988



Erice 1988

Erice 1988

Erice 1988

A singularity event @ Erice/1988...

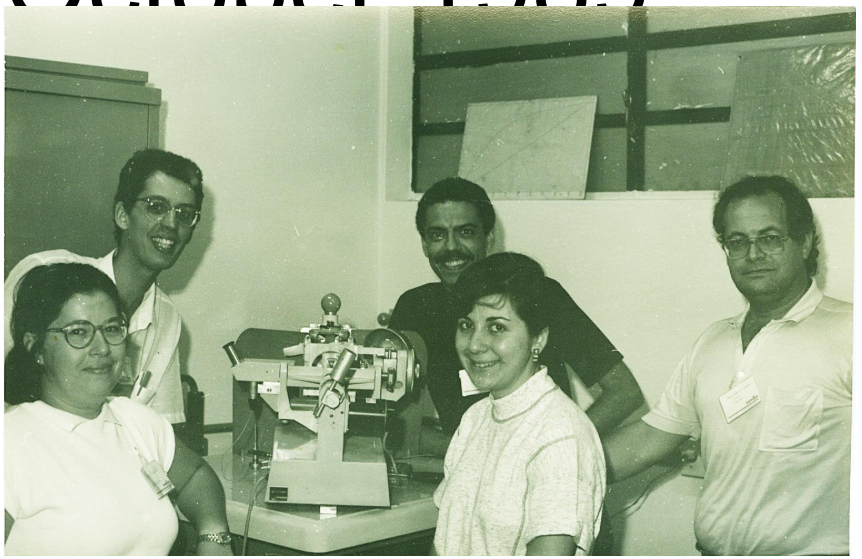
x N = Decision !!!

G.O: Richard, why not come to Brazil, and "own our own noses"

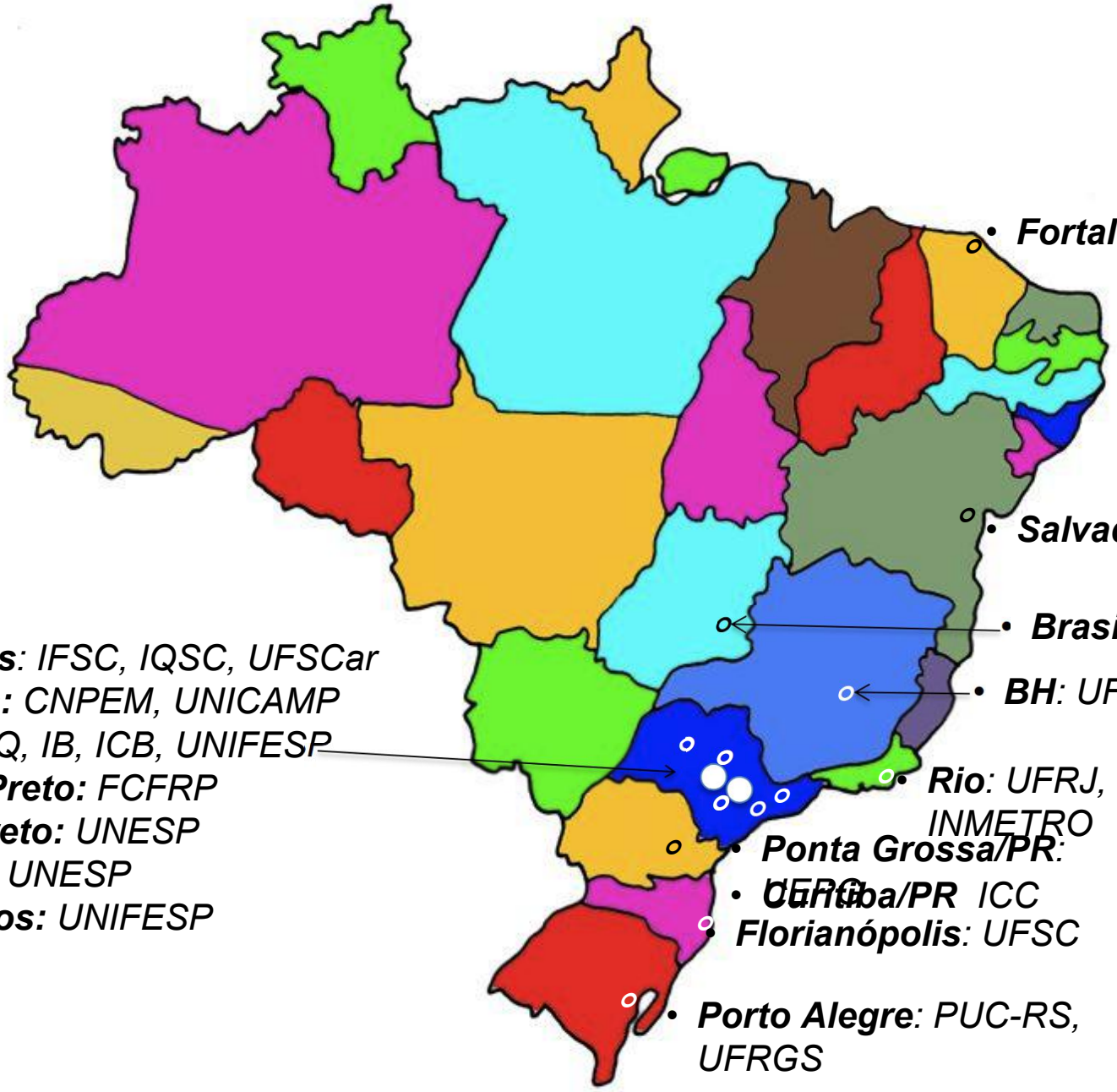
RCG: Ok Glaucius, let's do it.

PROTEIN CRYSTALLOGRAPHY IN BRAZIL - 1989

1st Course on Protein Crystallization
São Carlos, October 1989



PROTEIN CRYSTALLOGRAPHY IN BRAZIL - 2019



- ***São Carlos**: IFSC, IQSC, UFSCar*
- ***Campinas:** CNPEM, UNICAMP*
- ***S.Paulo:** IQ, IB, ICB, UNIFESP*
- ***Ribeirão Preto:** FCFRP*
- ***S.J.Rio Preto:** UNESP*
- ***Botucatu:** UNESP*
- ***S.J.Campos:** UNIFESP*

- ***Fortaleza**: UFC*
- ***Salvador**: UFBA*
- ***Brasilia**: UnB*
- ***BH**: UFMG*
- ***Rio**: UFRJ, INMETRO*
- ***Ponta Grossa/PR**: UEPG*
- ***Curitiba/PR** ICC*
- ***Florianópolis**: UFSC*
- ***Porto Alegre**: PUC-RS, UFRGS*

NODES IN ARGENTINA- BRASIL- PARAGUAY- URUGUAY

INSTITUTO LELOIR
FUNDACIÓN

I B R
CONICET
U N R

INQUIMAE
CONICET
U B A

CBME
Centro de
Biotecnologia
Molecular Estrutural

CNRMN

LNBio

USP

IFSC

INSTITUT PASTEUR
DE MONTEVIDEO

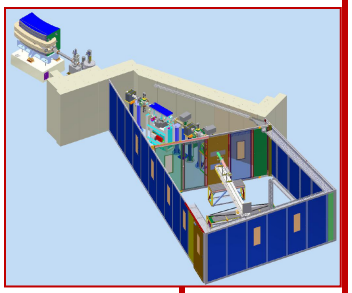
CENTER FOR FREE RADICAL AND BIOMEDICAL RESEARCH
-URUGUAY-

REPUBLICA DEL PARAGUAY
INSTITUTO DE INVESTIGACIONES EN CIENCIAS DE LA SALUD
UNIVERSIDAD NACIONAL DE ASUNCION

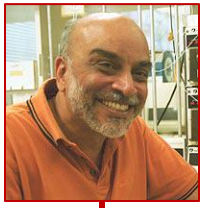
LNBIO

SMOLBNET

MX1



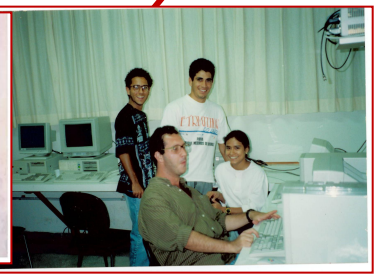
MX2



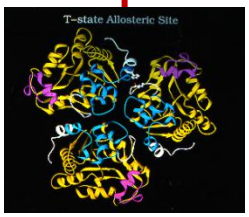
1988

2000

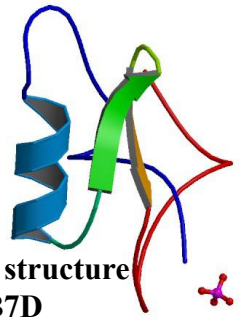
2018



FAPESP



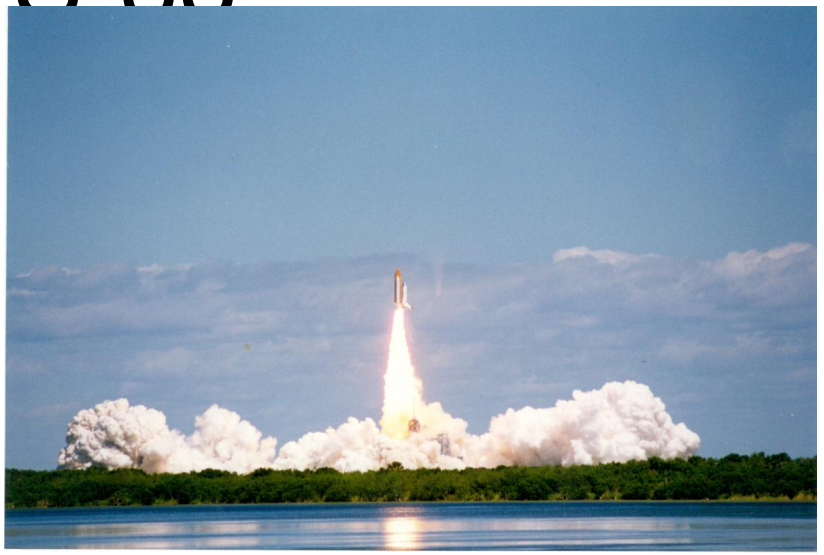
1st PX structure in Brazil 1DEA

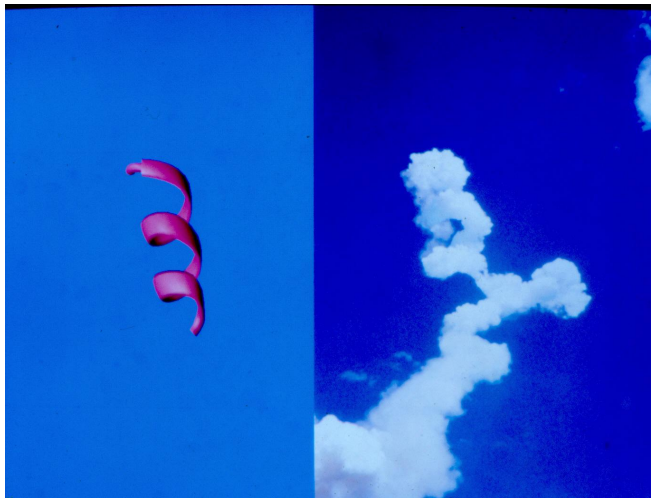


1st MX1 structure 1B7D

SIRIUS

CRYSTALLIZATION IN MICROGRAVITY
1997 – STS-83





Friends from DQ-UFSCar, great partners in the drug discovery endeavour in our group

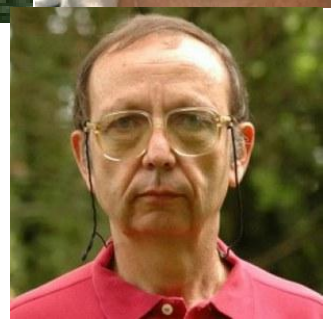






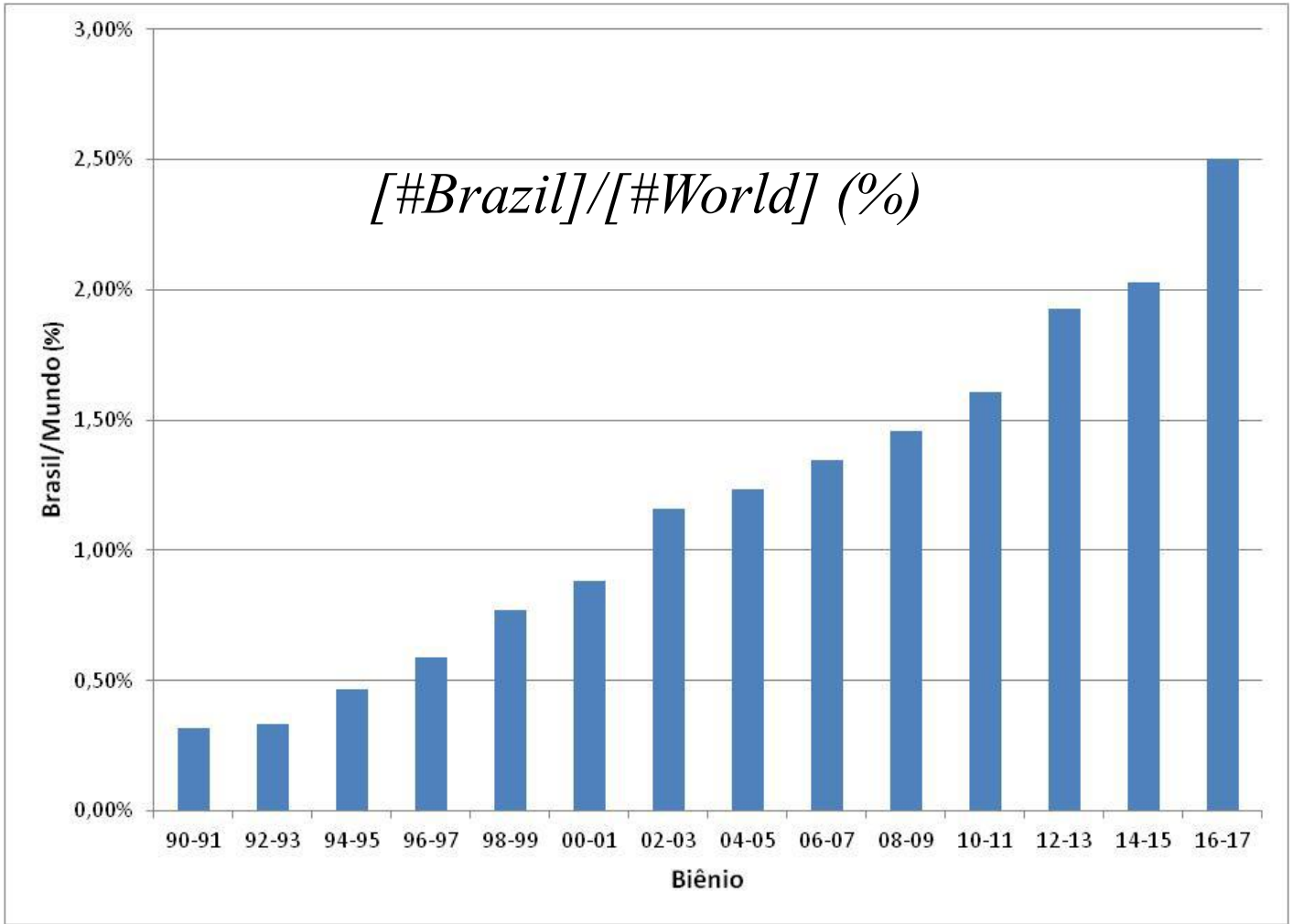






Publications in Structural Biology coming from Brazil

Query: *(X-ray or NMR or spectroscopy or fluorescence or cryst* or cryo-EM) and (enzyme or protein or carbohydrate or lipid or macromolecule)*



Nothing is possible without the people!

The pioneers

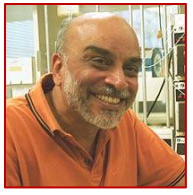

Help from abroad




The PX beginners... (the past?)







The present and the future....











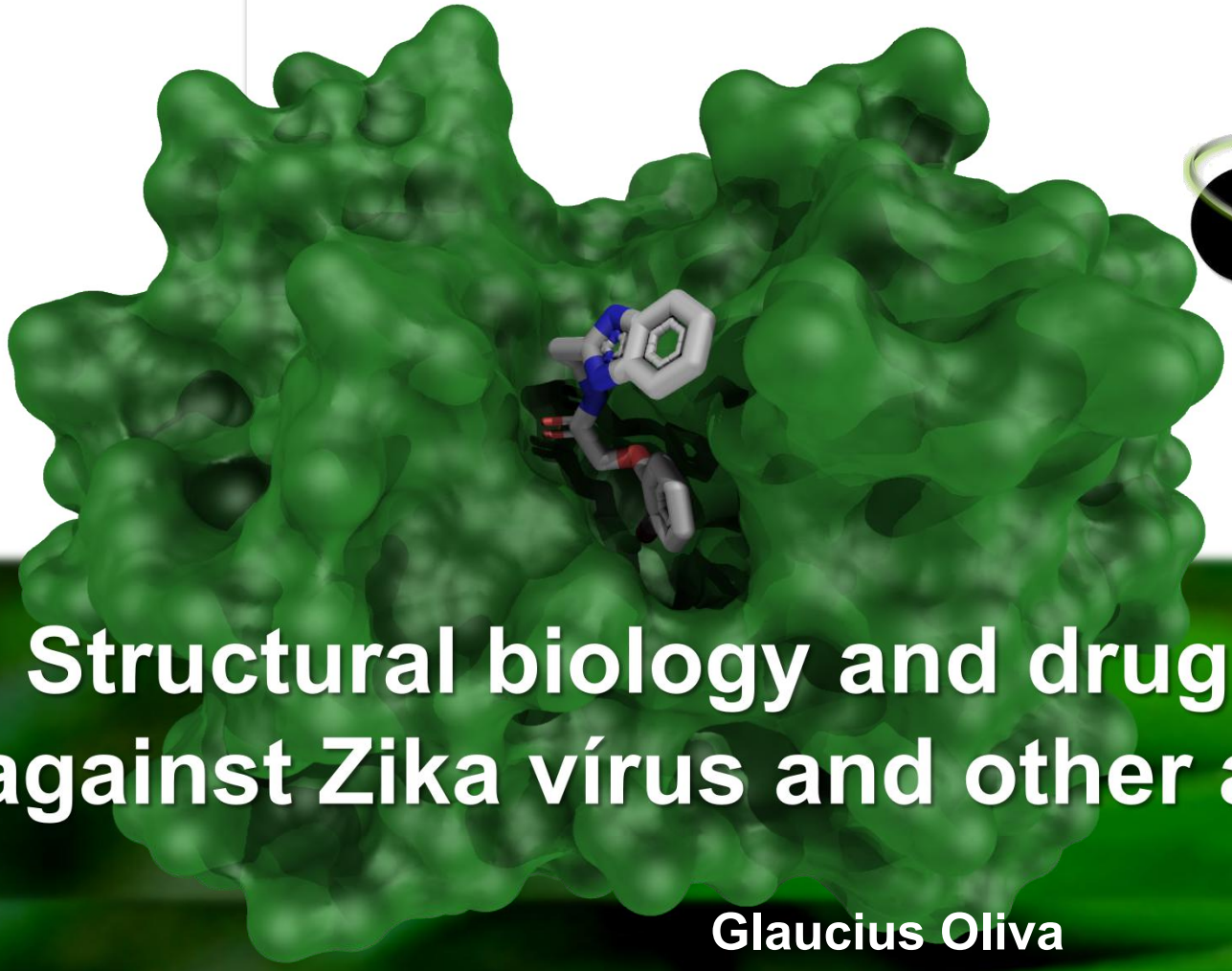









IFSC
LNLS
CBME
Centro de Biotecnologia Molecular Estrutural
USP
CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico
EESC • USP
1977
CIBFar
2019
hhmi
Howard Hughes Medical Institute
INBEQMeDI
SBBq
EMS



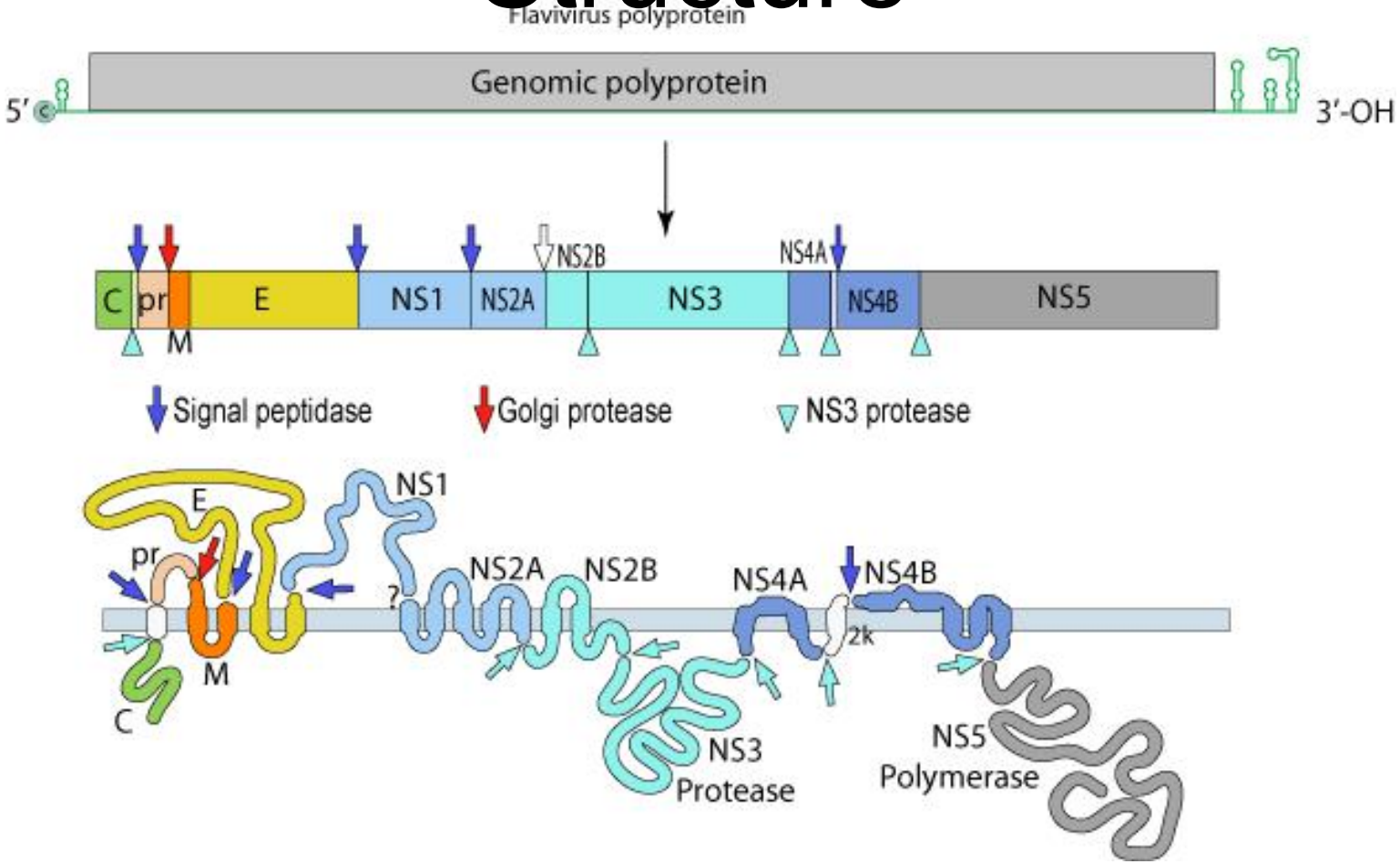
CIBFar

USP
Universidade de São Paulo

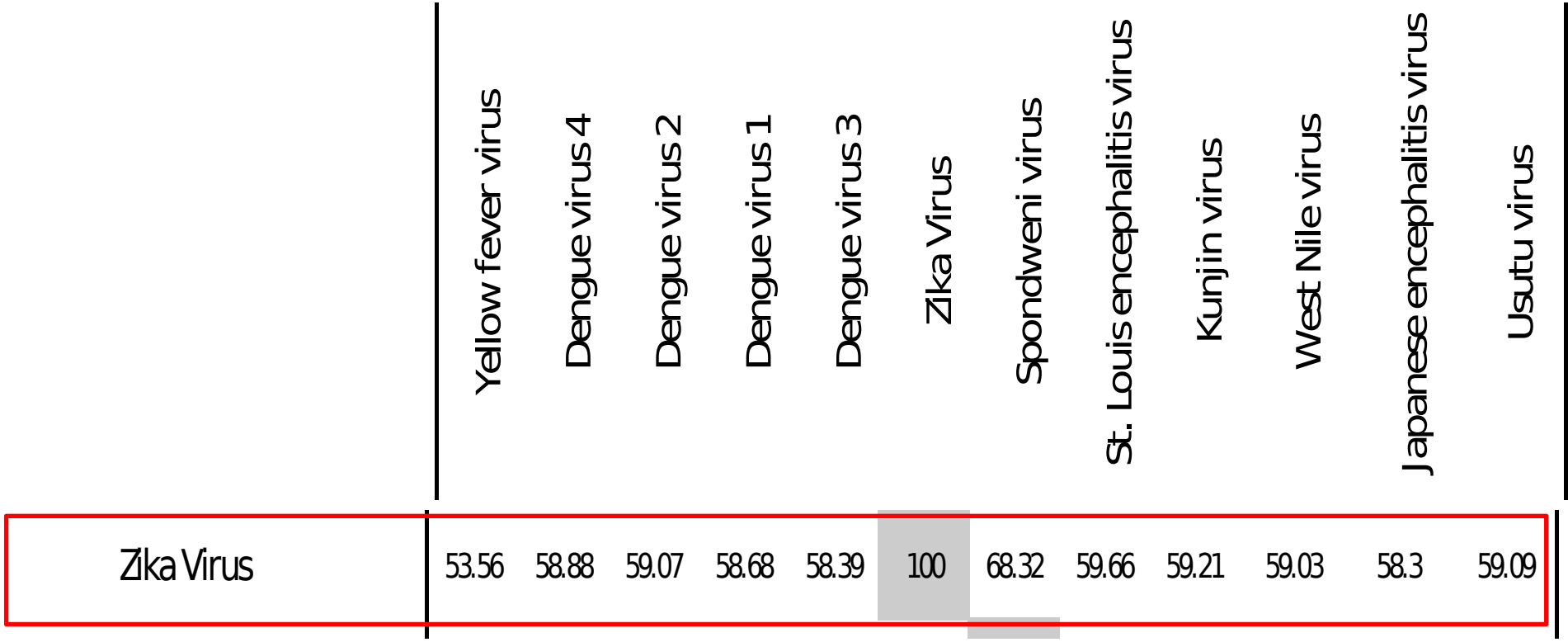
Structural biology and drug discovery against Zika vírus and other arboviroses

Glaucius Oliva
Instituto de Física de São Carlos / USP

Flavivirus: Genome and Structure



Matrix of identities
Complete genomes of several flaviviruses



	Yellow fever virus	Dengue virus 4	Dengue virus 2	Dengue virus 1	Dengue virus 3	Zika Virus	Spondweni virus	St. Louis encephalitis virus	Kunjin virus	West Nile virus	Japanese encephalitis virus	Usutu virus
Zika Virus	53.56	58.88	59.07	58.68	58.39	100	68.32	59.66	59.21	59.03	58.3	59.09

ClustalW 2.1

PUZZLE:

Relatively small molecular and structural differences, but hugely different pathological impacts and transmission

ZIKA VIRUS

Pathological impacts

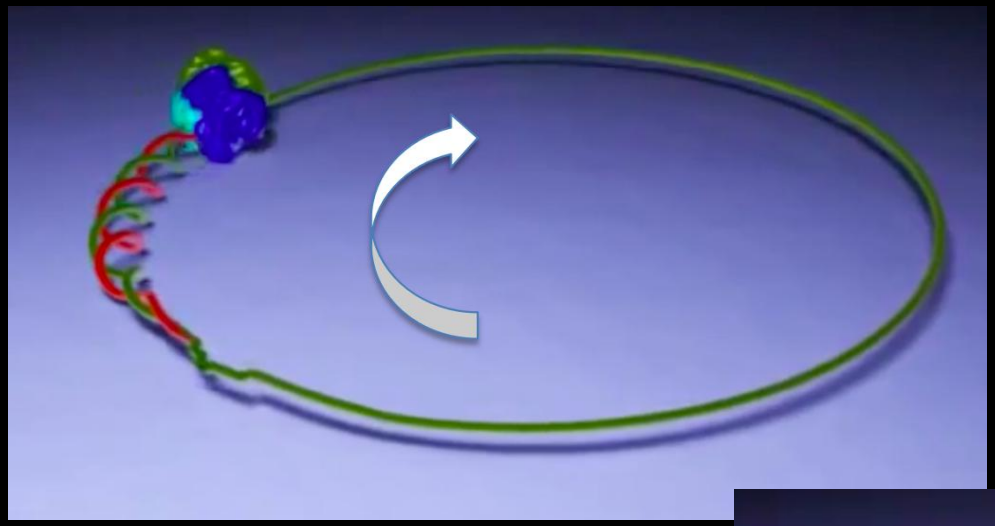
- Microcephaly
- Zika congenital syndrome
- Guillan-Barré Syndrome

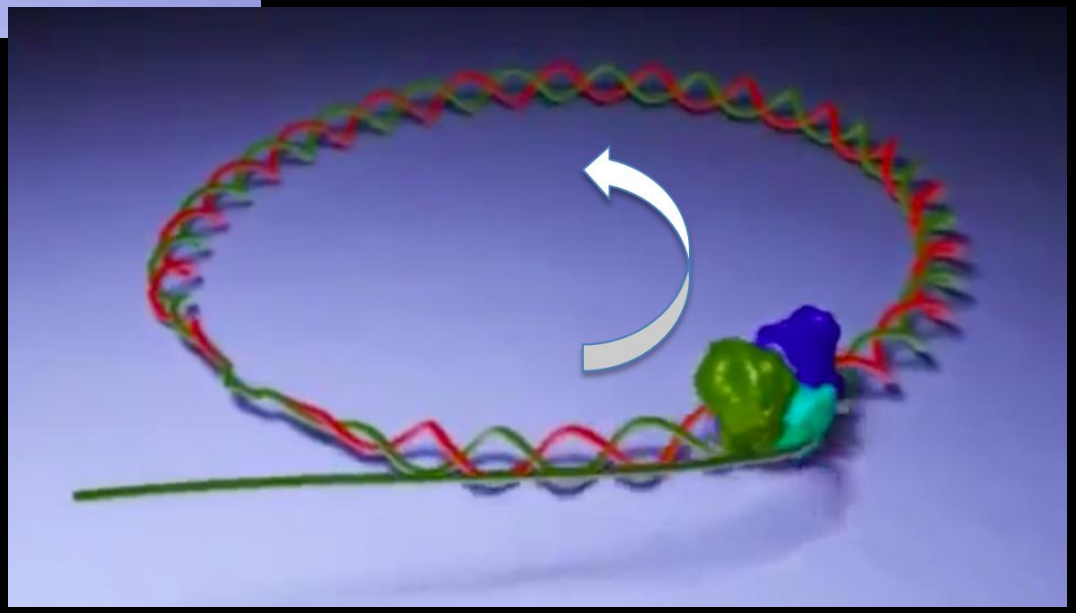
Transmission

	Dengue	Chikungunya	Zika
Vector	*Aedes aegypti*	*Aedes aegypti*	*Aedes aegypti*
Sexual	-	-	Yes
Pregnancy	-	-	Yes
Breast-feeding	-	-	Evidences
Blood transfusion & organ transp.	-	-	Yes

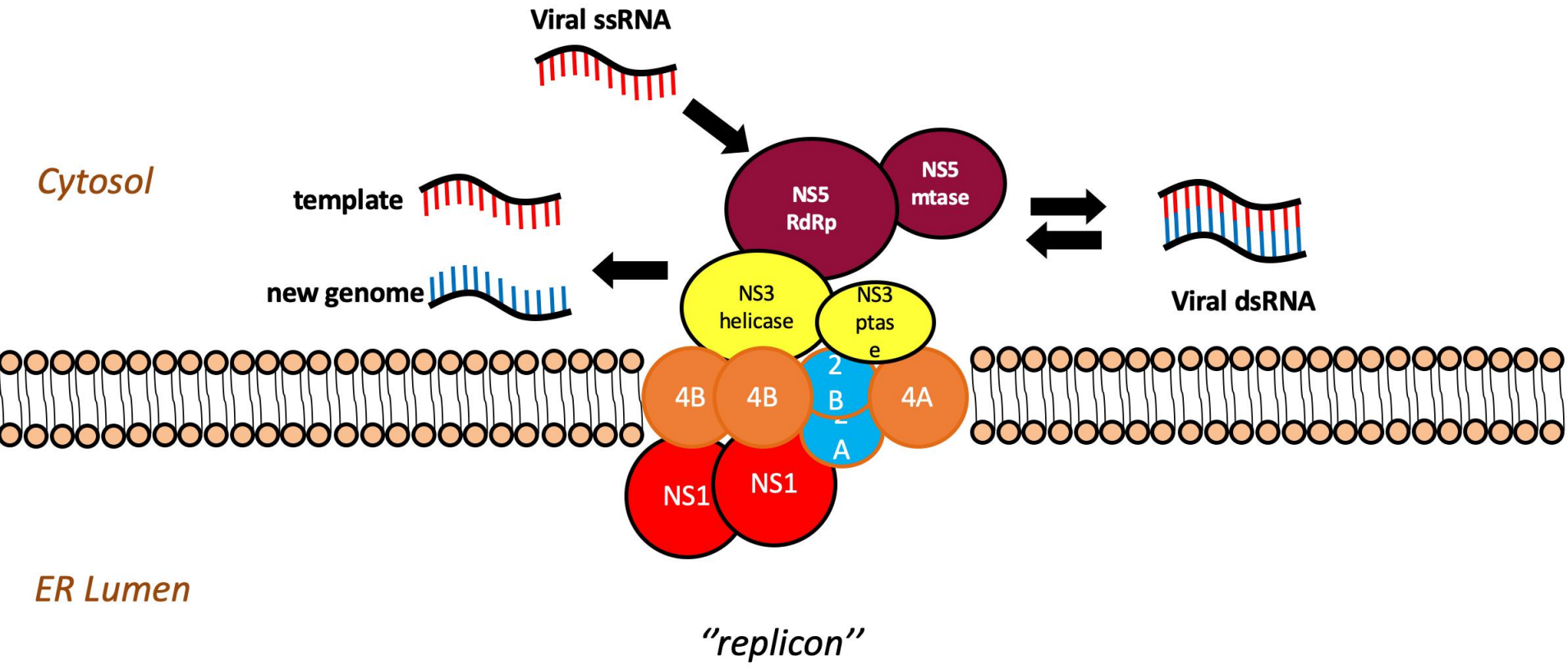
https://www.cdc.gov/

Targeting the Virus genome replication process





Non-structural proteins



"replicon"

nature COMMUNICATIONS

ARTICLE

Received 17 Oct 2016 | Accepted 30 Jan 2017 | Published 27 Mar 2017

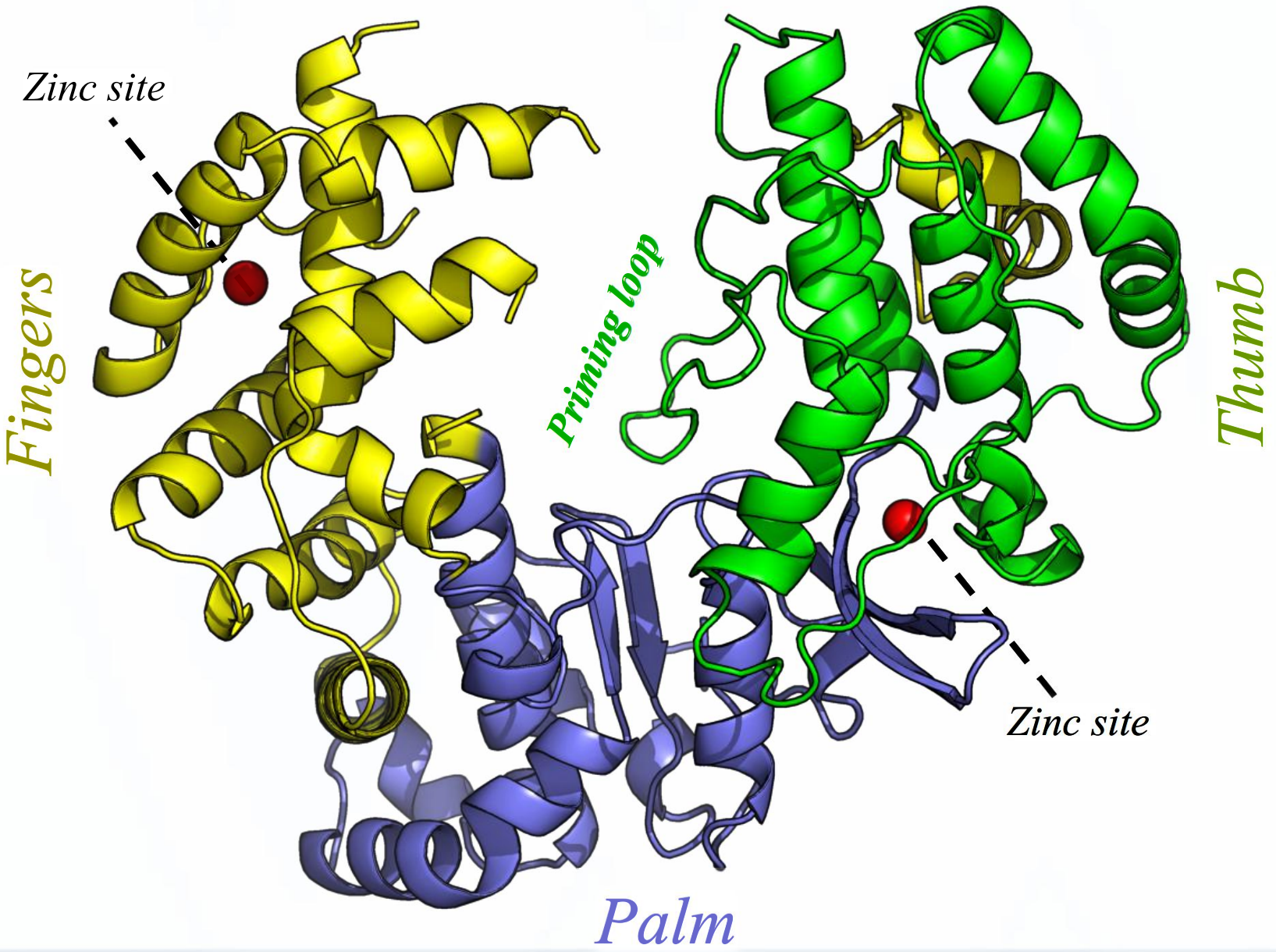
DOI: 10.1038/ncomms14764 OPEN

Crystal structure of Zika virus NS5 RNA-dependent RNA polymerase

Andre S. Godoy[1], Gustavo M.A. Lima[1], Ketllyn I.Z. Oliveira[1], Naiara U. Torres[1,2], Fernando V. Maluf[1,2], Rafael V.C. Guido[1] & Glaucius Oliva[1]

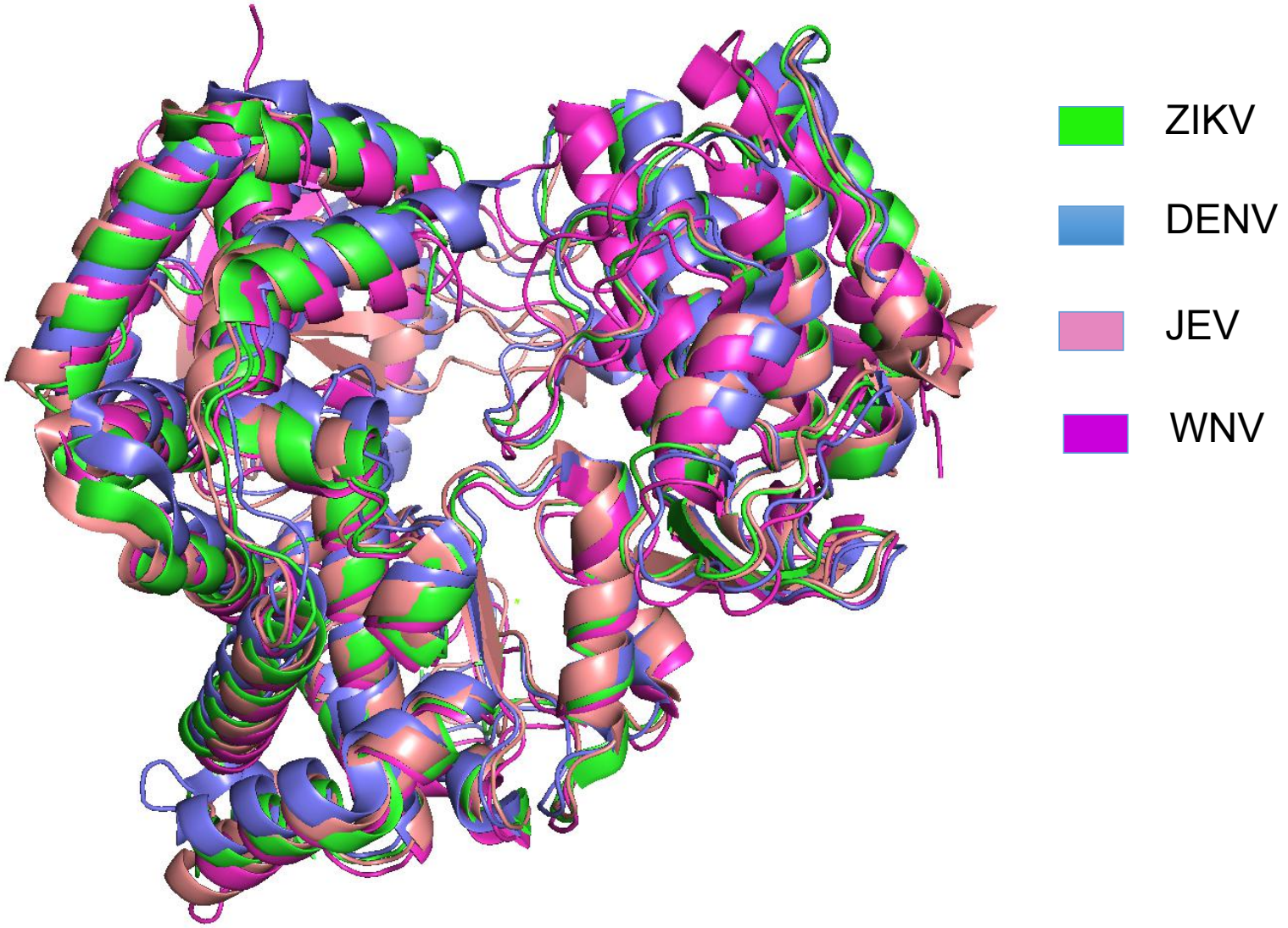
The current Zika virus (ZIKV) outbreak became a global health threat of complex epidemiology and devastating neurological impacts, therefore requiring urgent efforts towards the development of novel efficacious and safe antiviral drugs. Due to its central role in RNA viral replication, the non-structural protein 5 (NS5) RNA-dependent RNA-polymerase (RdRp) is a prime target for drug discovery. Here we describe the crystal structure of the recombinant ZIKV NS5 RdRp domain at 1.9 Å resolution as a platform for structure-based drug design strategy. The overall structure is similar to other flaviviral homologues. However, the priming loop target site, which is suitable for non-nucleoside polymerase inhibitor design, shows significant differences in comparison with the dengue virus structures, including a tighter pocket and a modified local charge distribution.

ZIKV NS5 RdRp



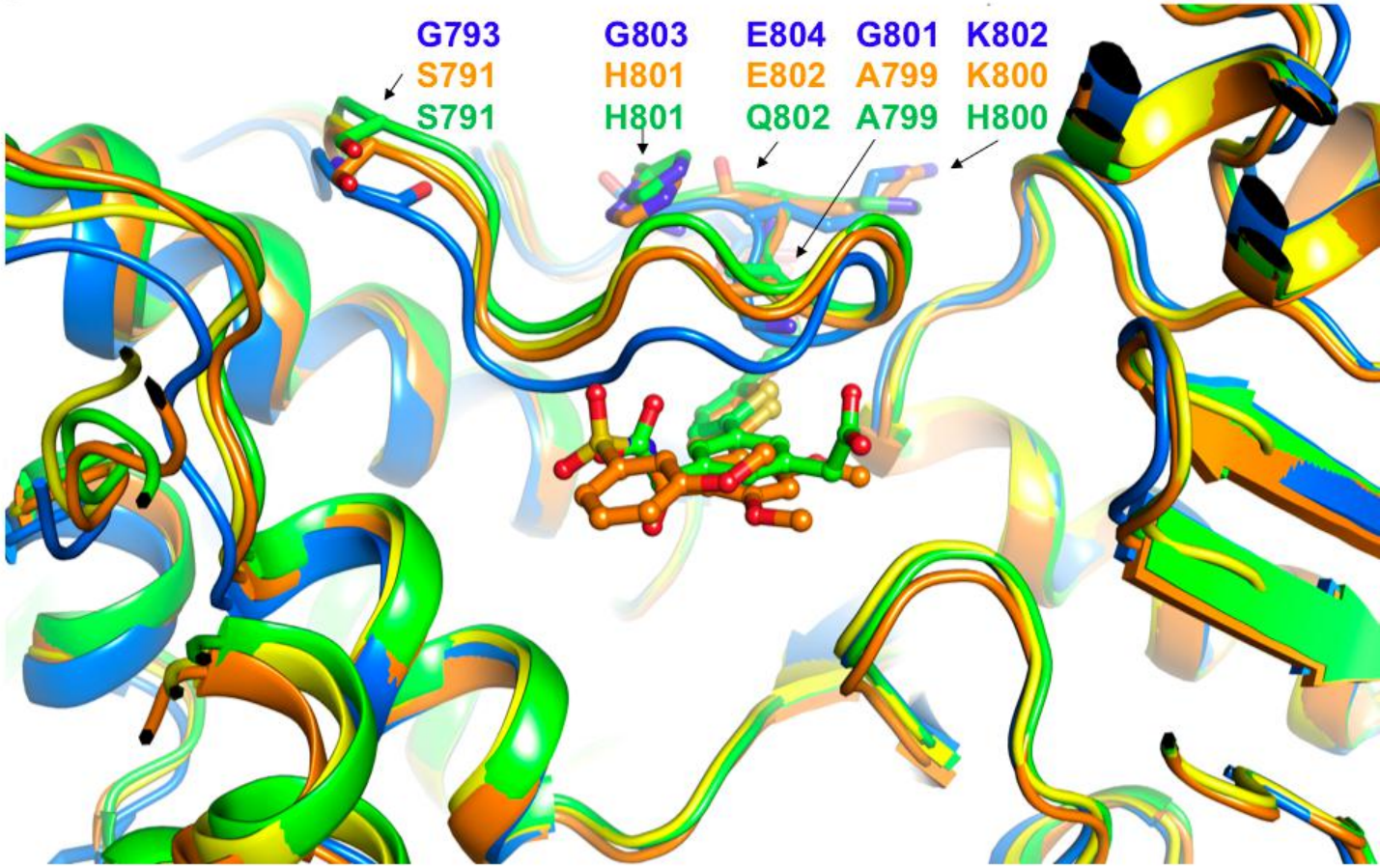
Godoy, AS. et al. Nat. Commun. v8, 2017

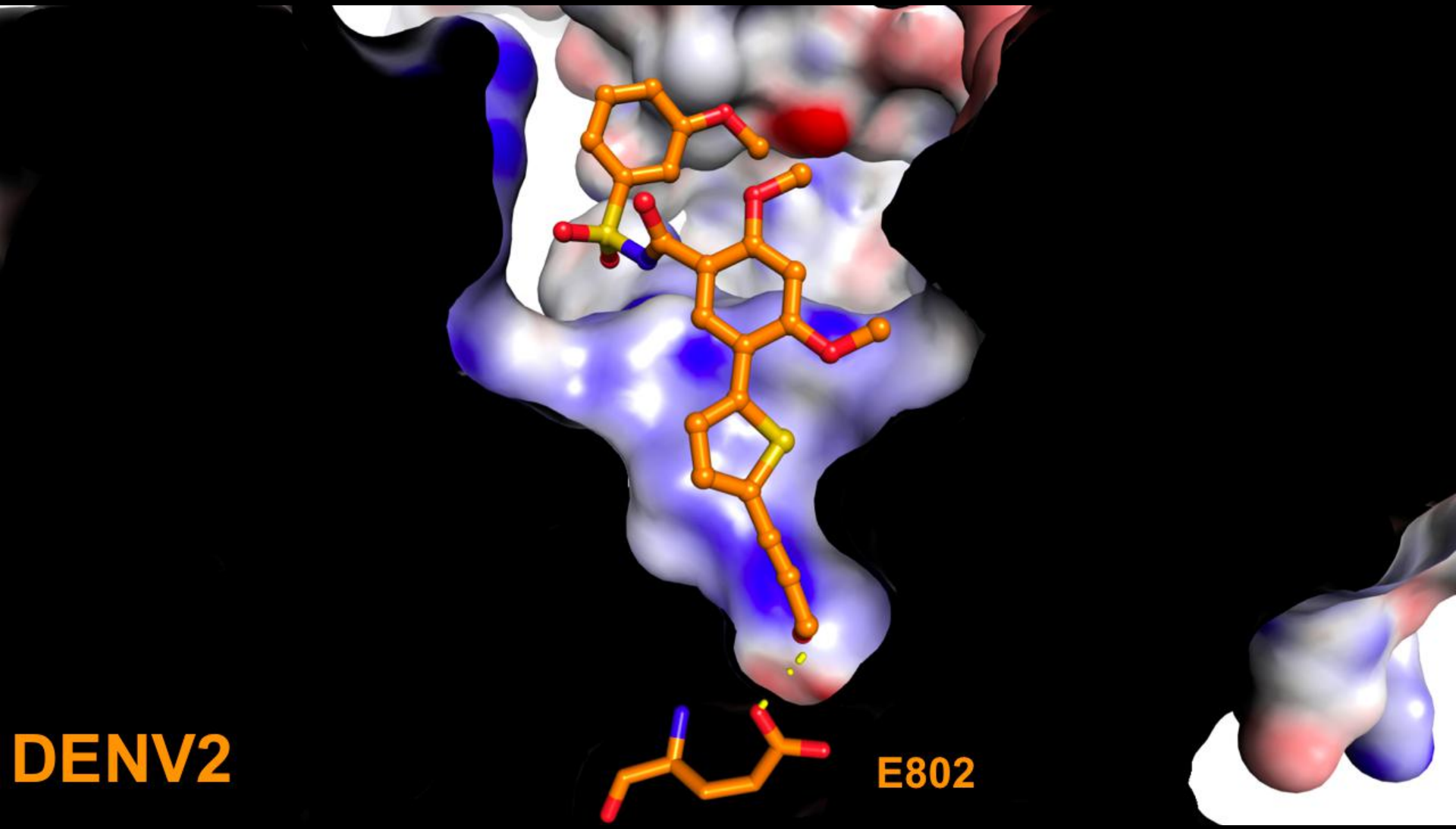
Structural Similarity amongst NS5 RdRps

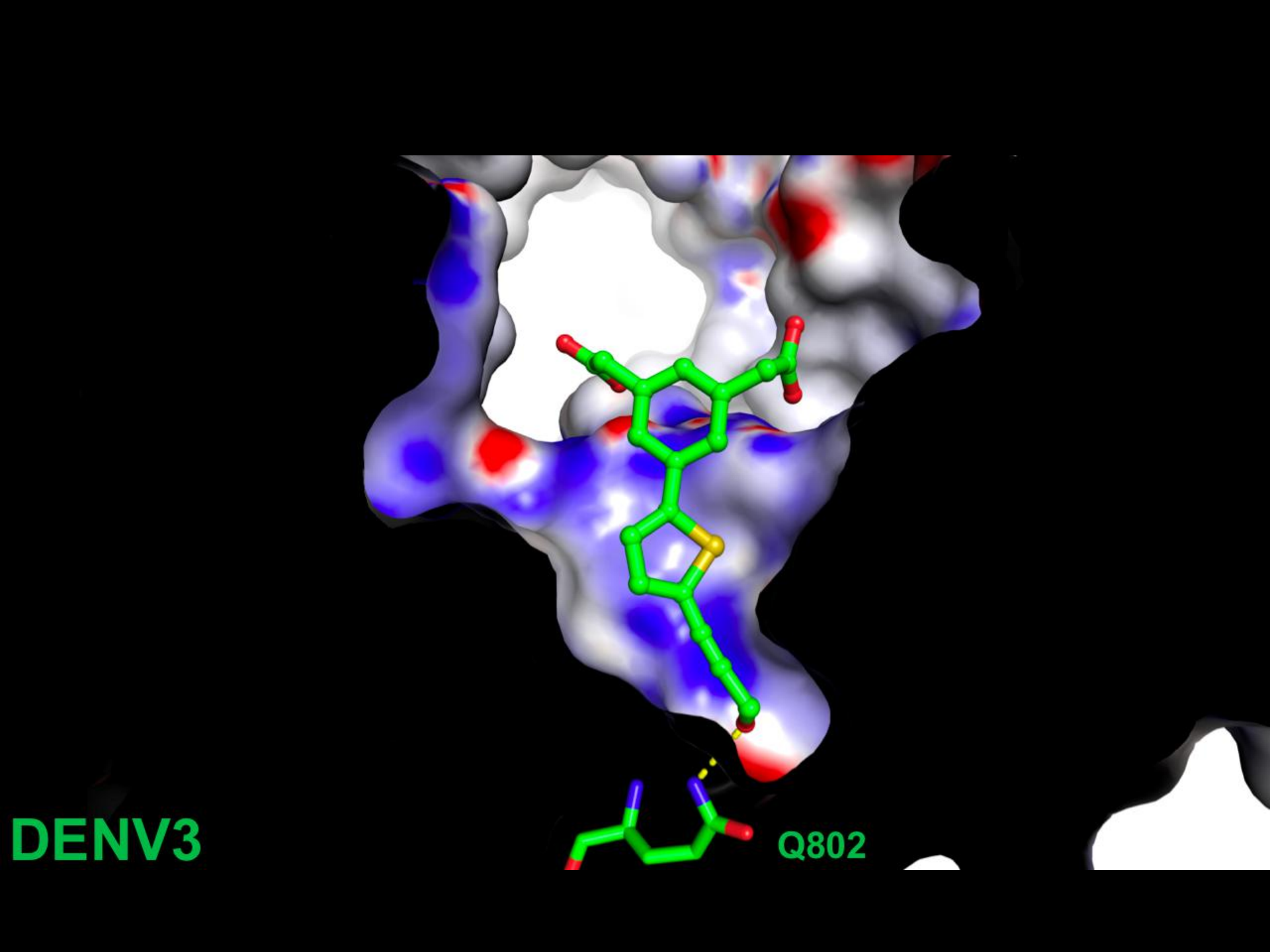


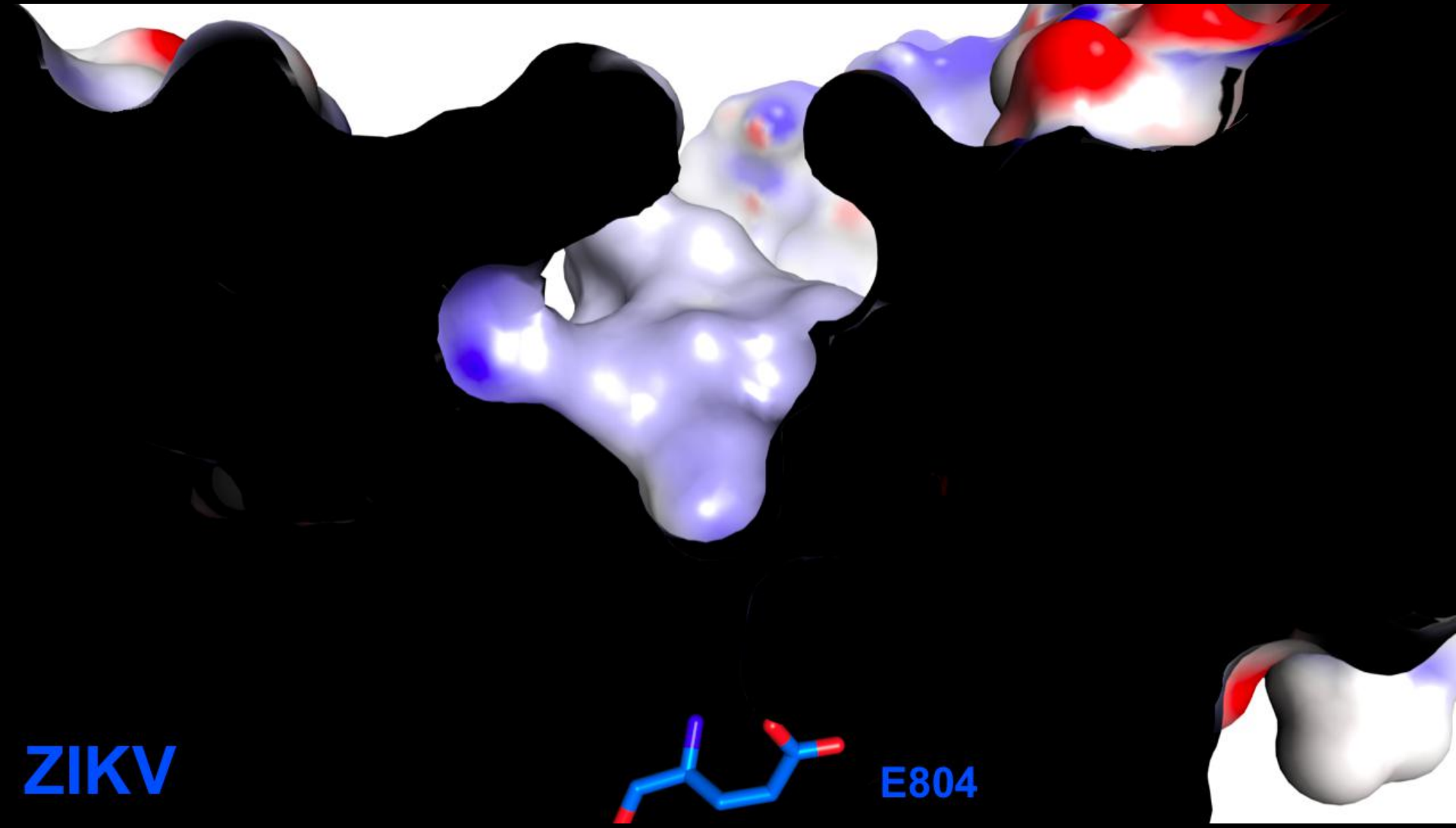
Priming loop

- ZIKV
- DENV2
- DENV3
- DENV3(apo)




DENV2
E802


DENV3
Q802


ZIKV
E804

NS5-RdRp
5U04

Virtual Screening

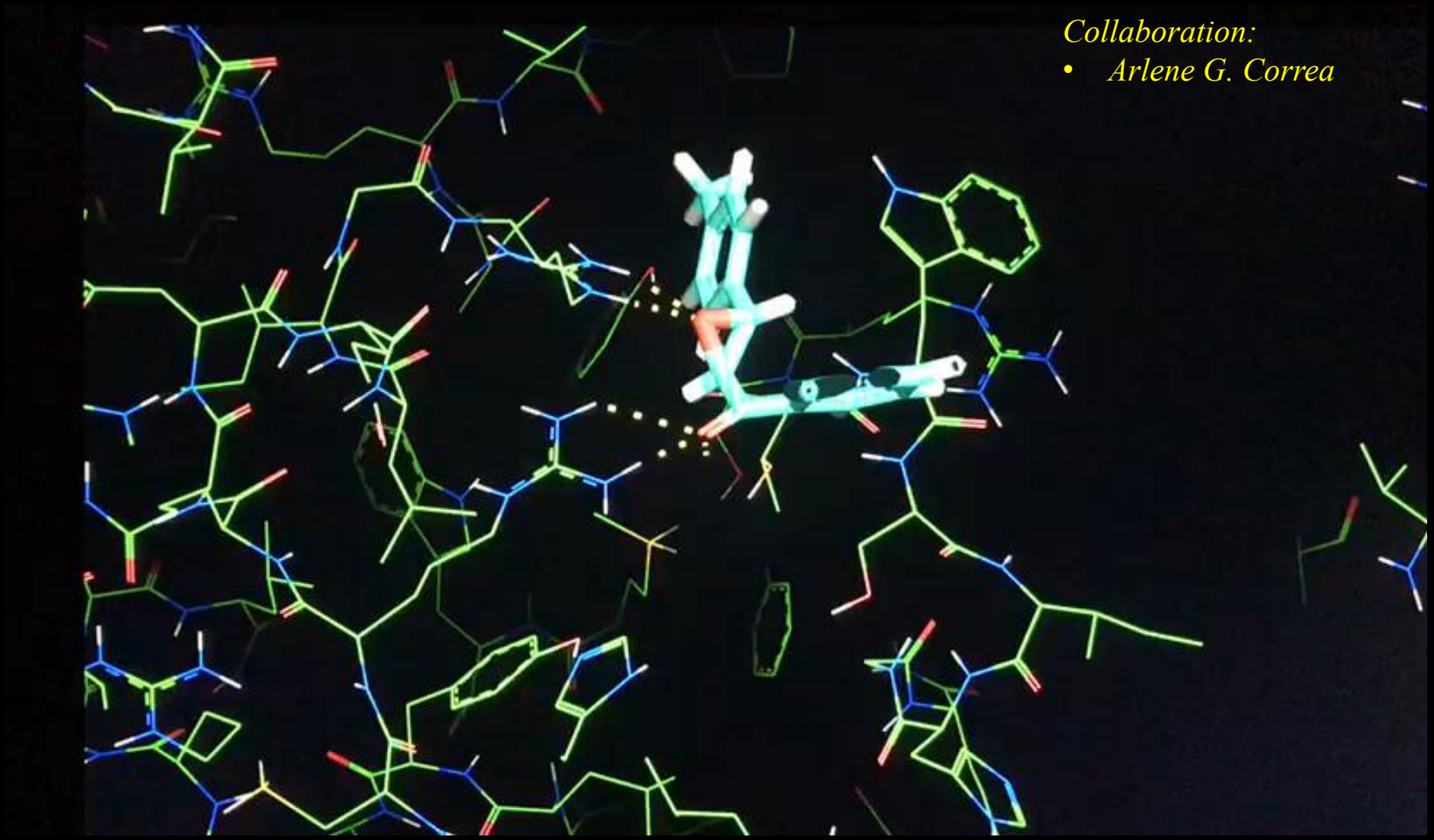
3.111 CIBFar compounds

CIBFar
CENTRO DE PESQUISA E INOVAÇÃO EM BIODIVERSIDADE E FÁRMACOS

Virtual Screening
- Rafael Guido
- Carolina H. Andrade
- Melina Mottin
- Bruna K. Sousa

Compound Libraries
- Arlene G. Correa
- Luiz C. Dias
- Fernando Coelho
- Carlos Roque
- NuBBE DB

Ex: VS against LSPN/UFSCar compound library

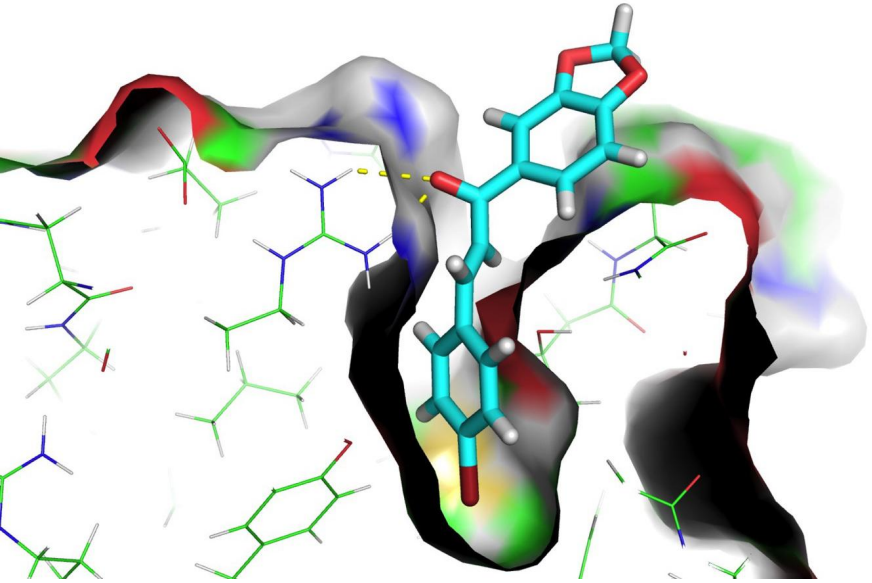


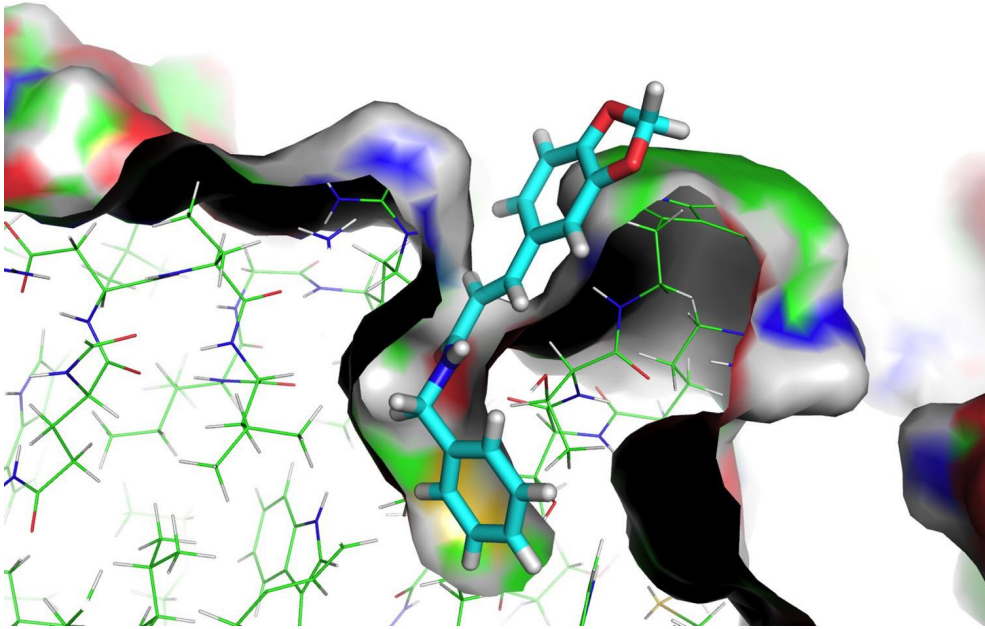
Collaboration:

- *Arlene G. Correa*

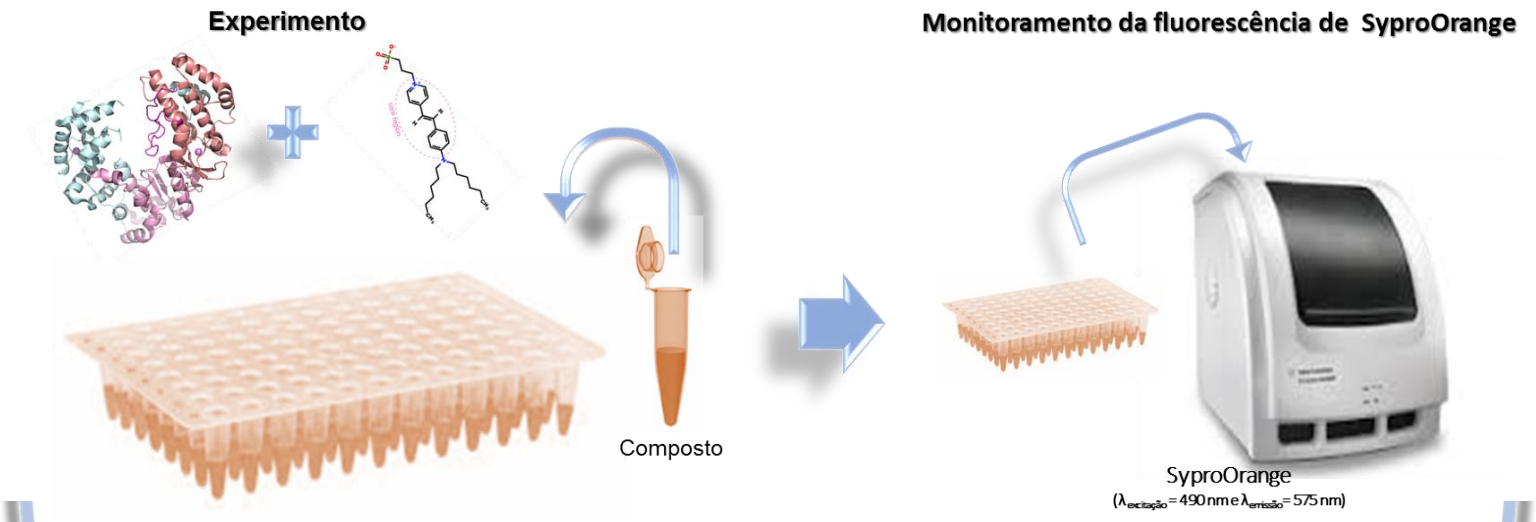
Top hits

CIBFar

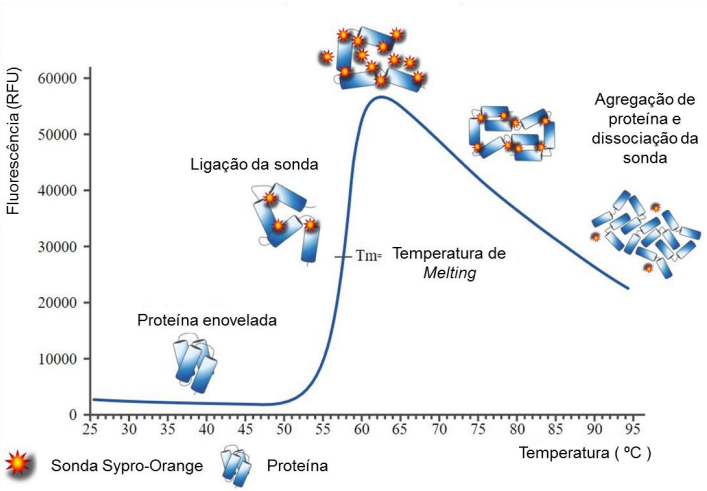




Fragments search using Differential Scanning Fluorimetry DSF)

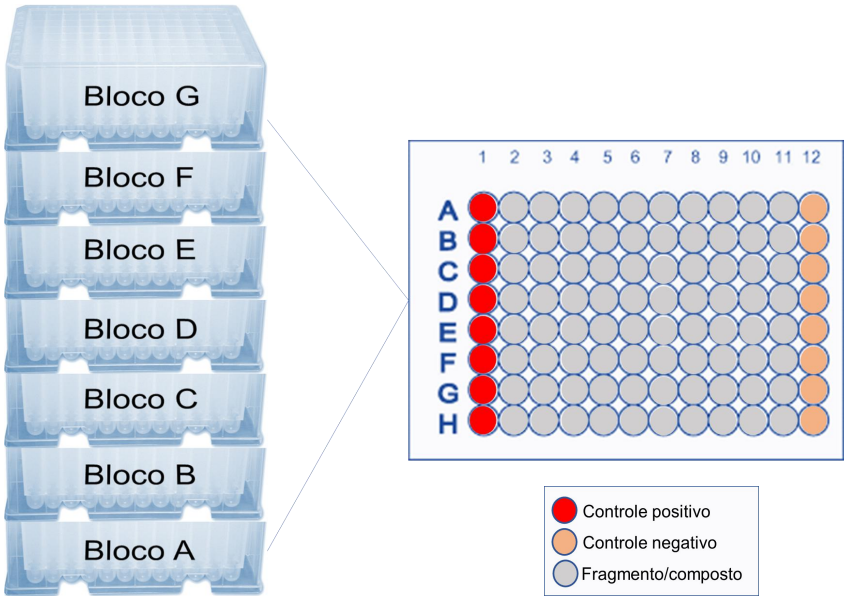


Resultado Esperado

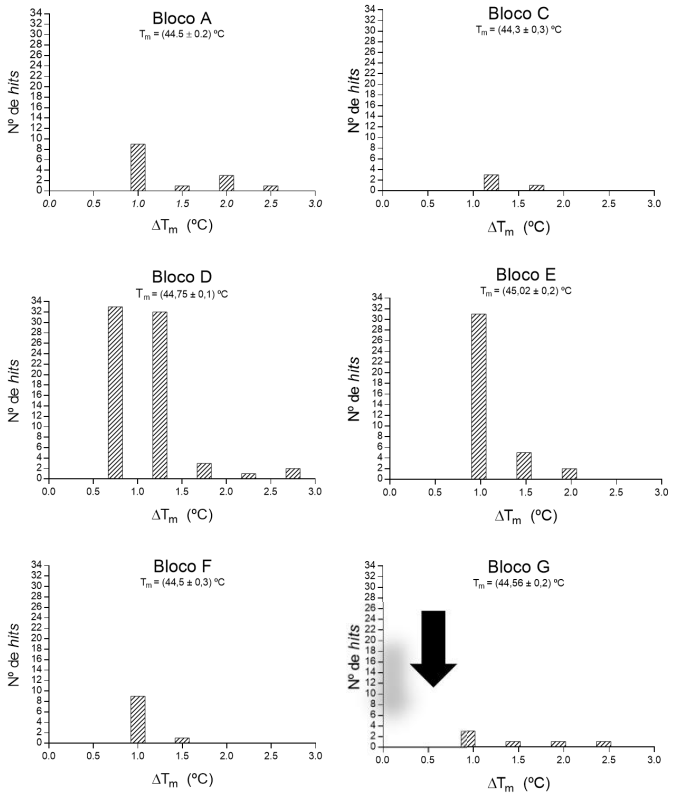


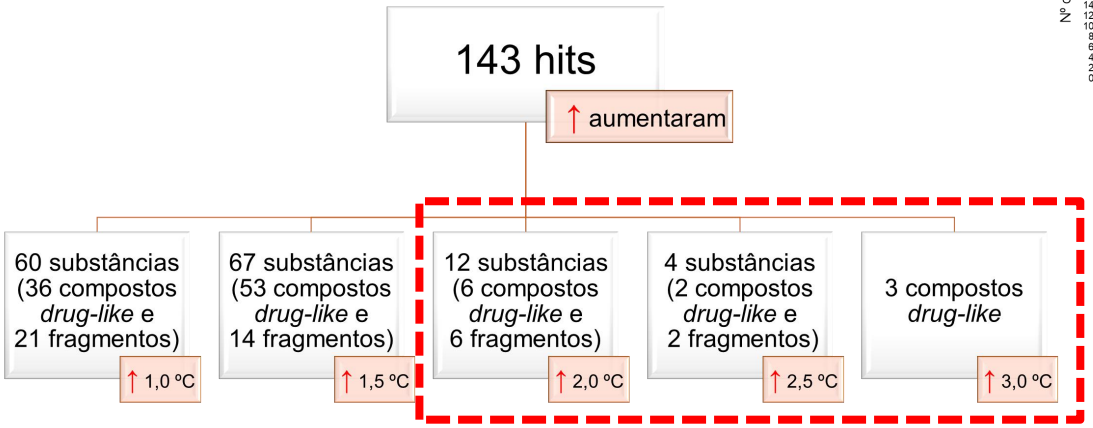
Compounds library from ICB/USP (Dr. M.Dias)

- ***514*** *compounds drug-like/fragments)*
- *organized in* ***7 blocks***



Número de compostos x Variação do Tm



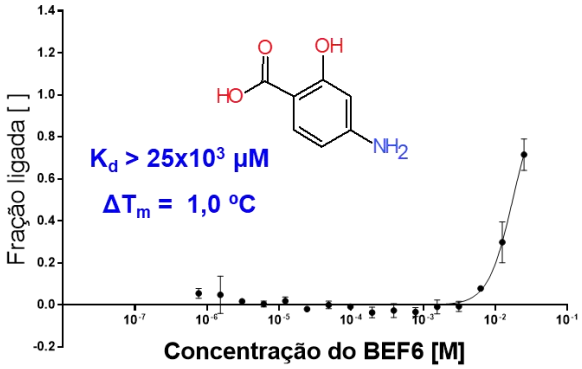


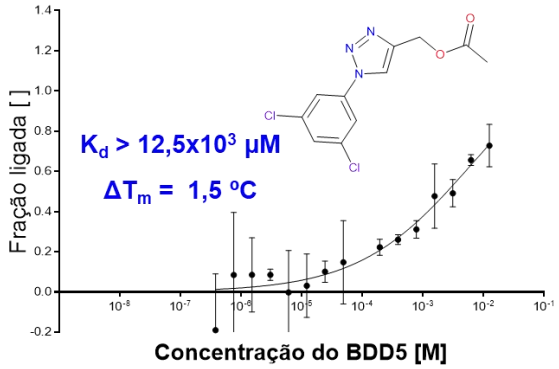
- *Measure affinity by MST*
- *Soaking & Co-crystallization experiments*

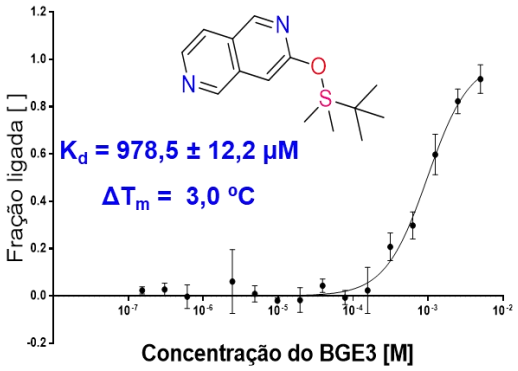
Measuring affinity by Microscale Thermophoresis (MST)

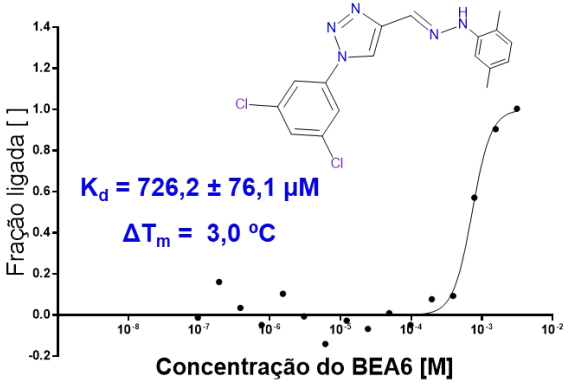
CIBFar

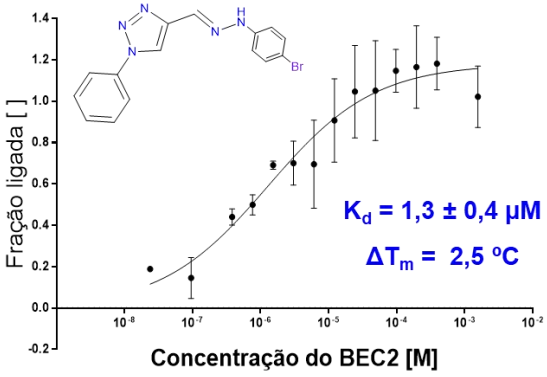
Métodos

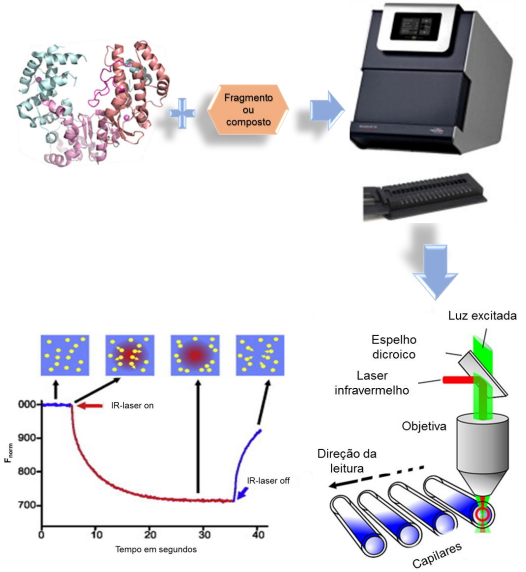






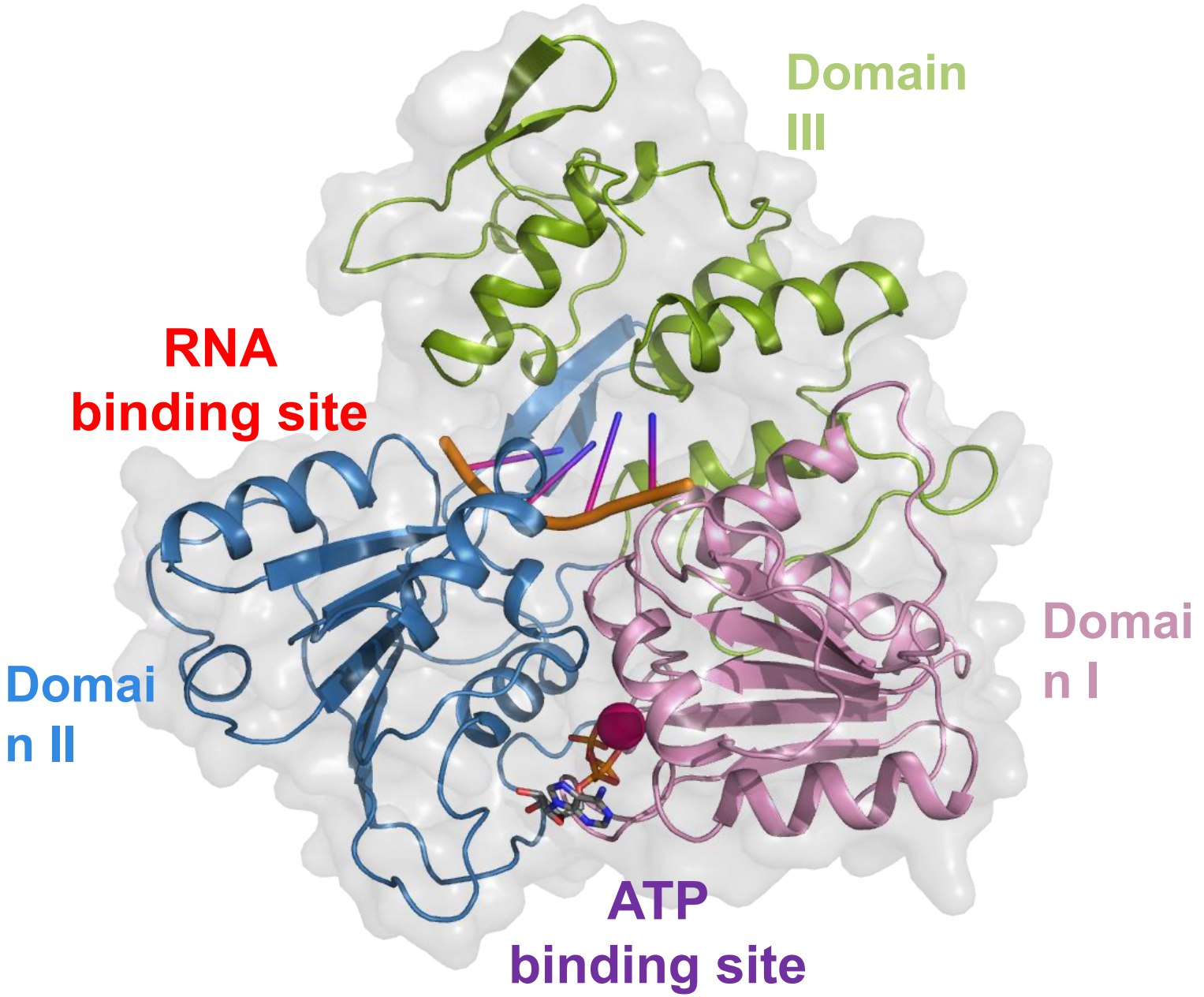






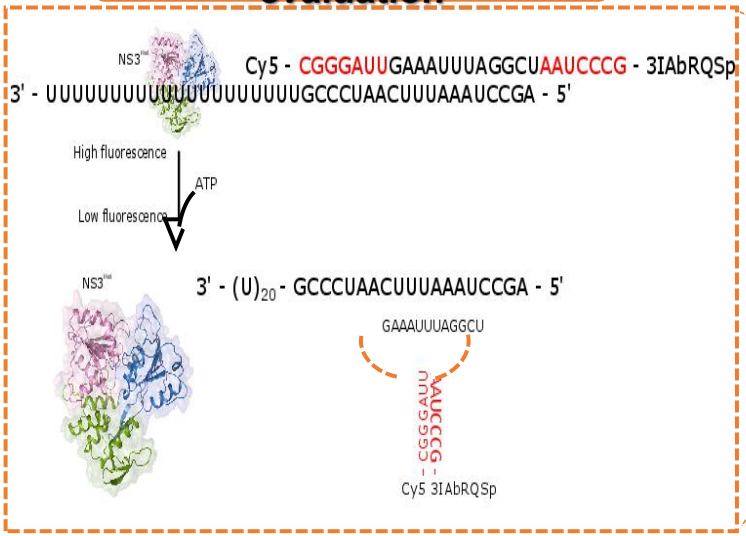
ZIKV_NS3^{Hel}: the Zika virus helicase

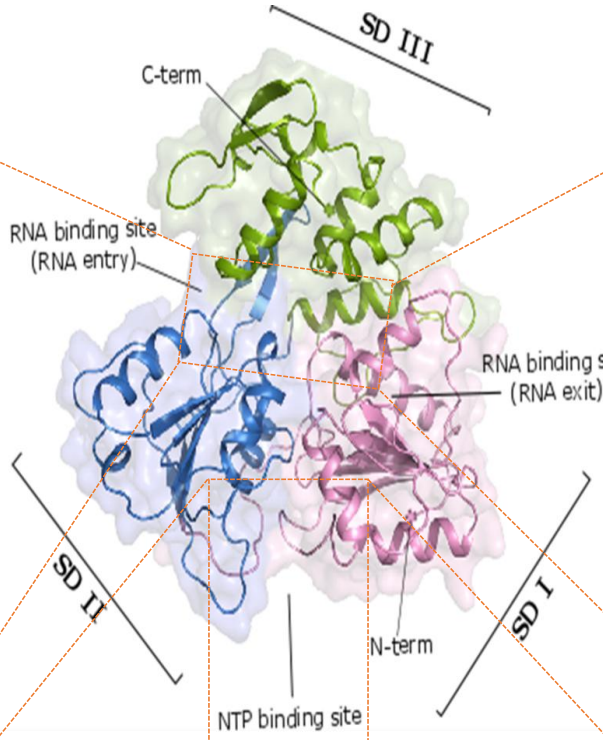
$NS3^{Hel}$-ZIKV: Structure



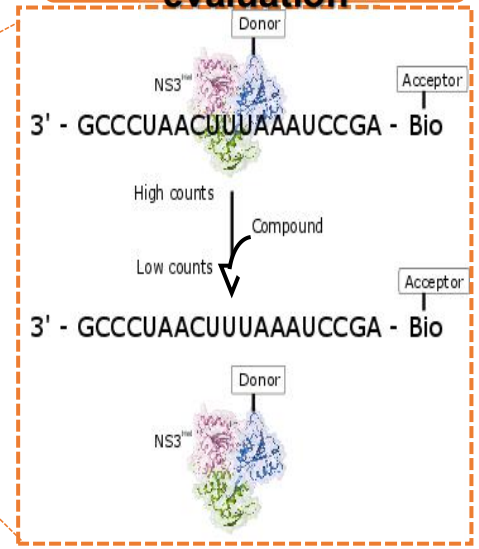
ZIKV_NS3^{Hel} Activity Evaluations

RNA unwinding evaluation

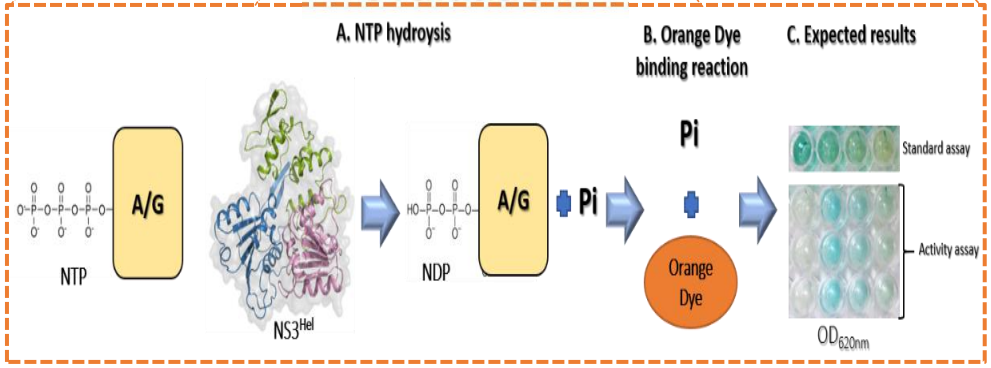




RNA binding evaluation

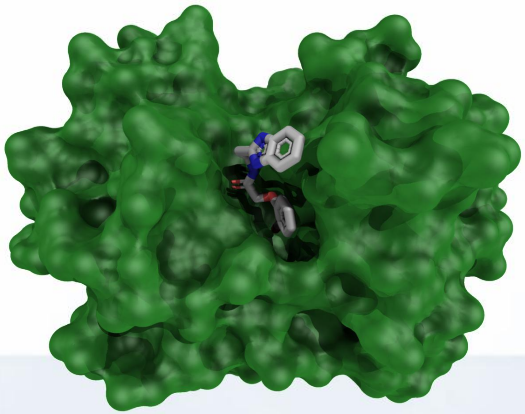


ATPase activity evaluation

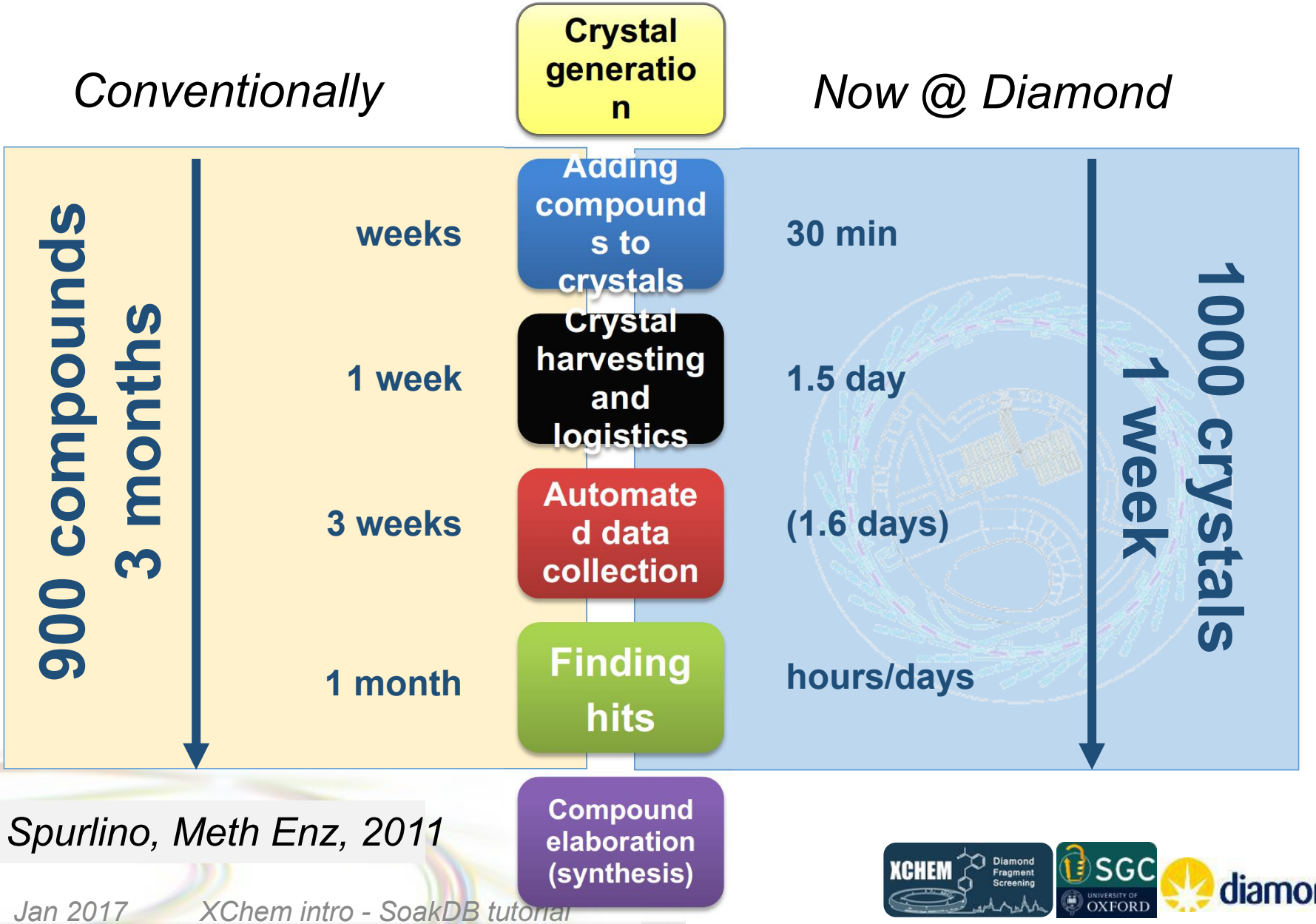


Fragment screening

Exploring NS3 Helicase domain



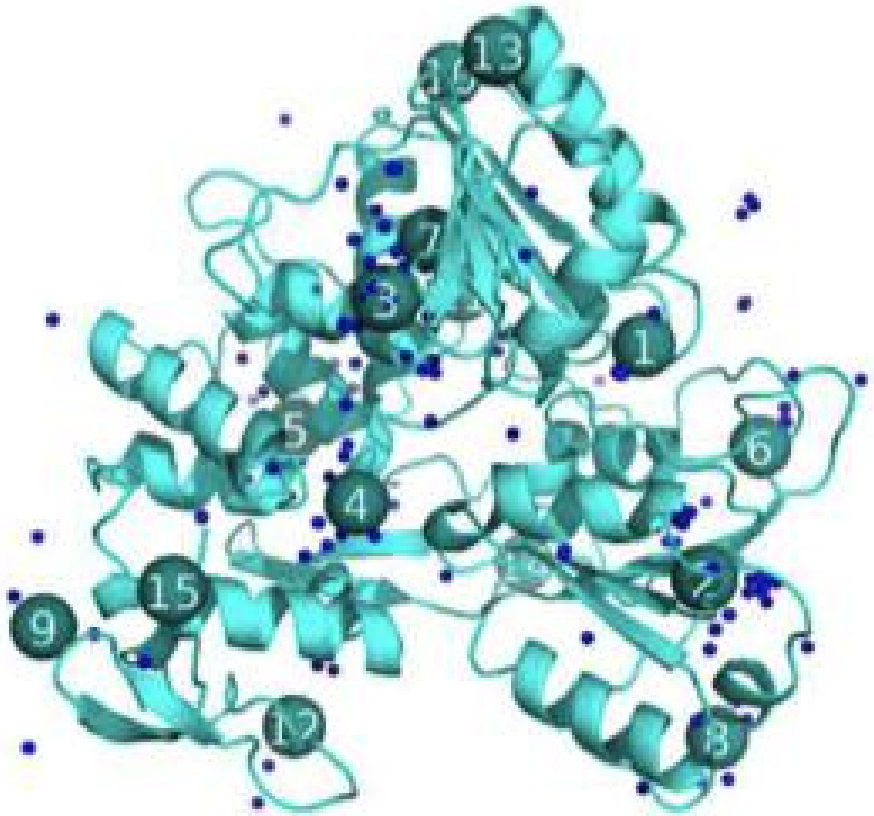
XChem: order of magnitude speedup

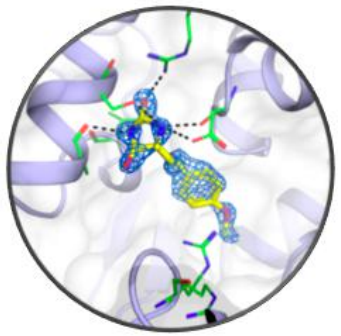


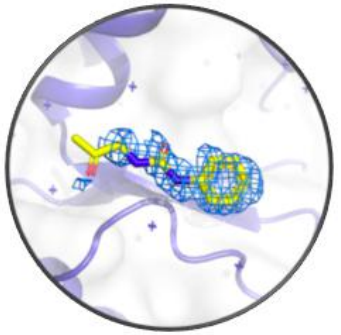
Spurlino, Meth Enz, 2011

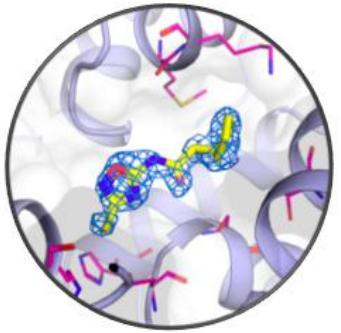
ZikV $NS3^{Hel}$ @ XChem Diamond

- *500 fragments tested (4 days)*
- *166 events (33%)*
- *about 16 sites (a lot in interfaces)*
- *44 reliable hits and on interesting sites (8%)*



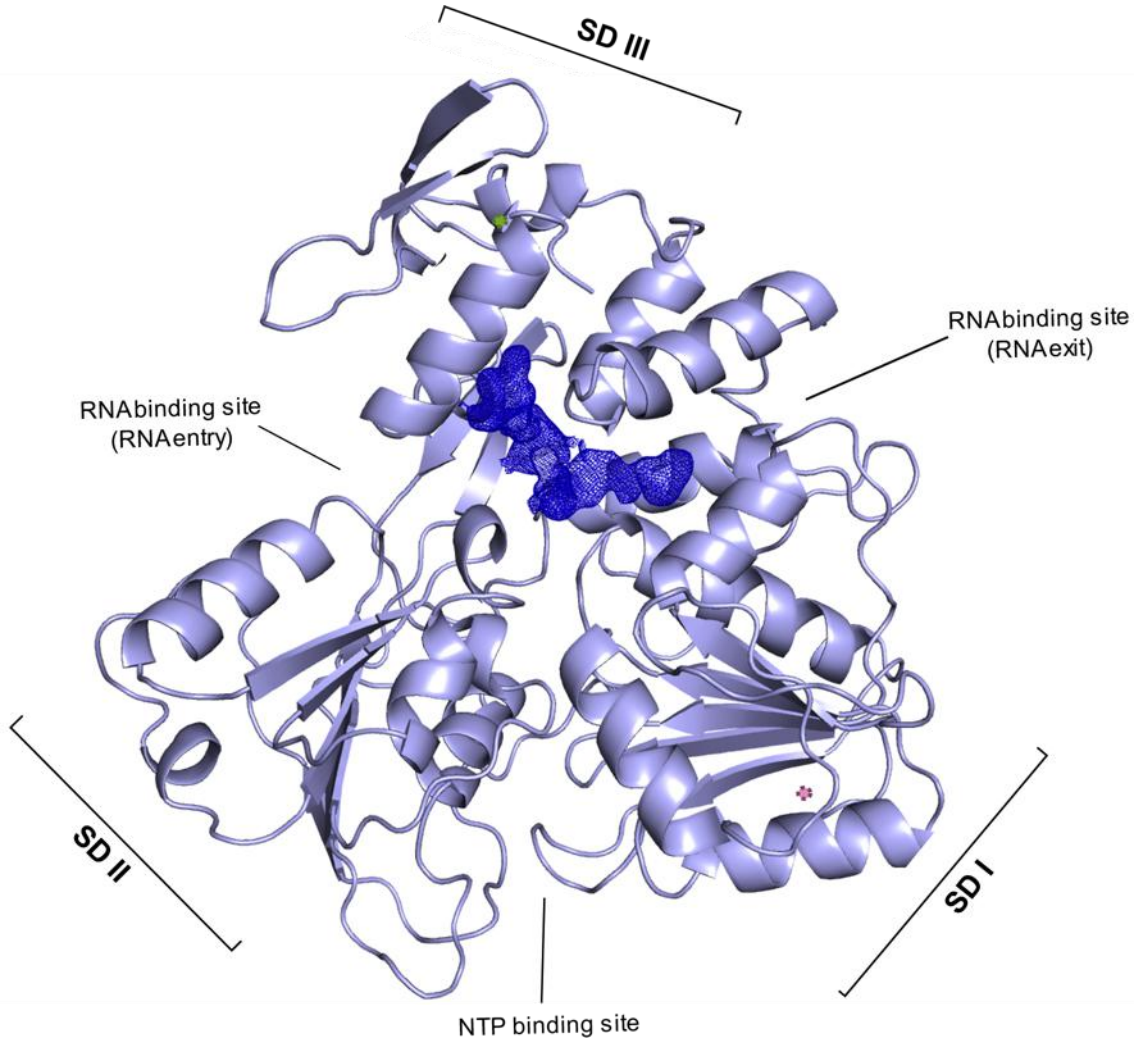


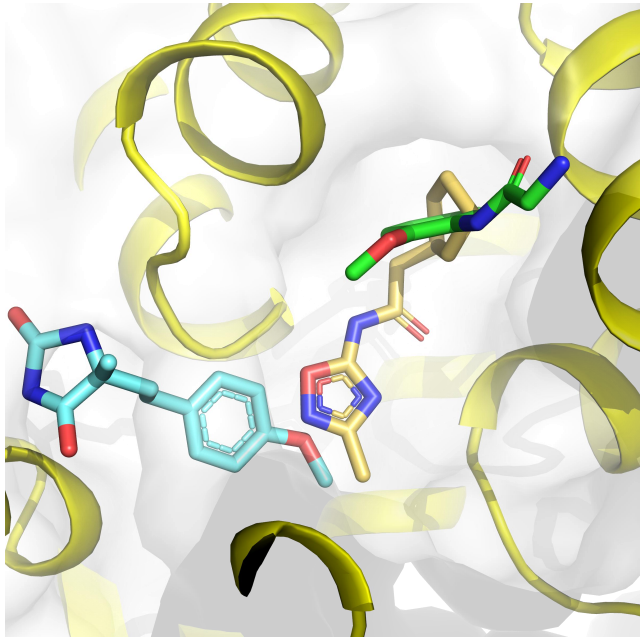




ZikV $NS3^{Hel}$ @ XChem Diamond

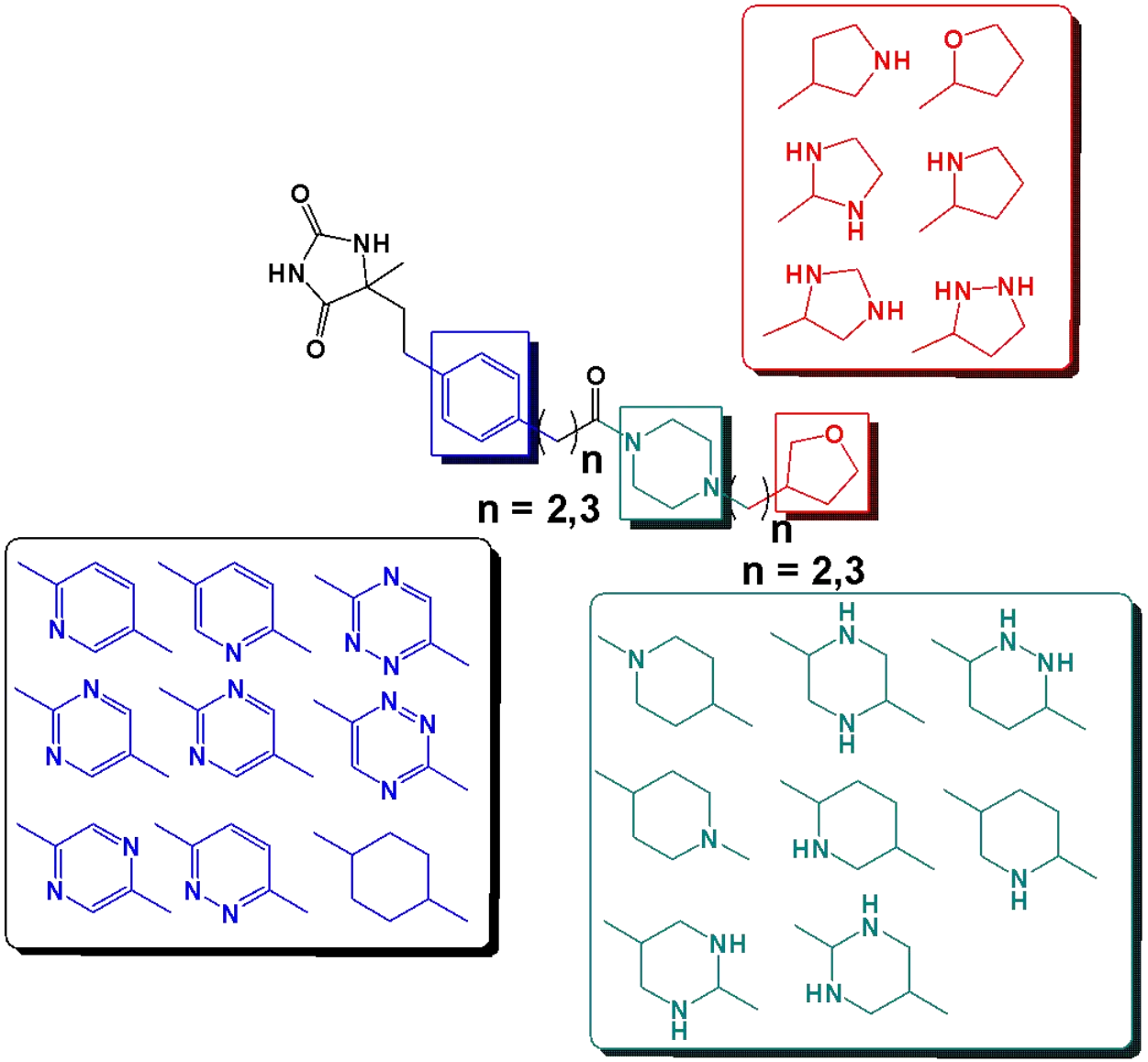
- *Two fragments close within binding distance at the RNA binding site*





Designed hydantoin derivatives

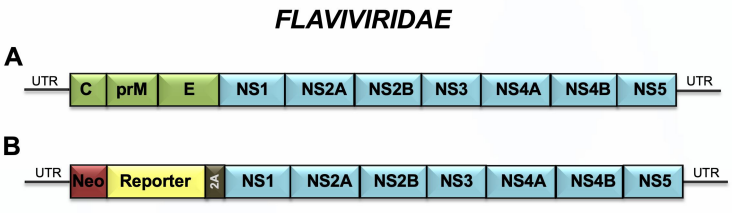
Synthesis by Prof. Arlene Correa (UFSCar)

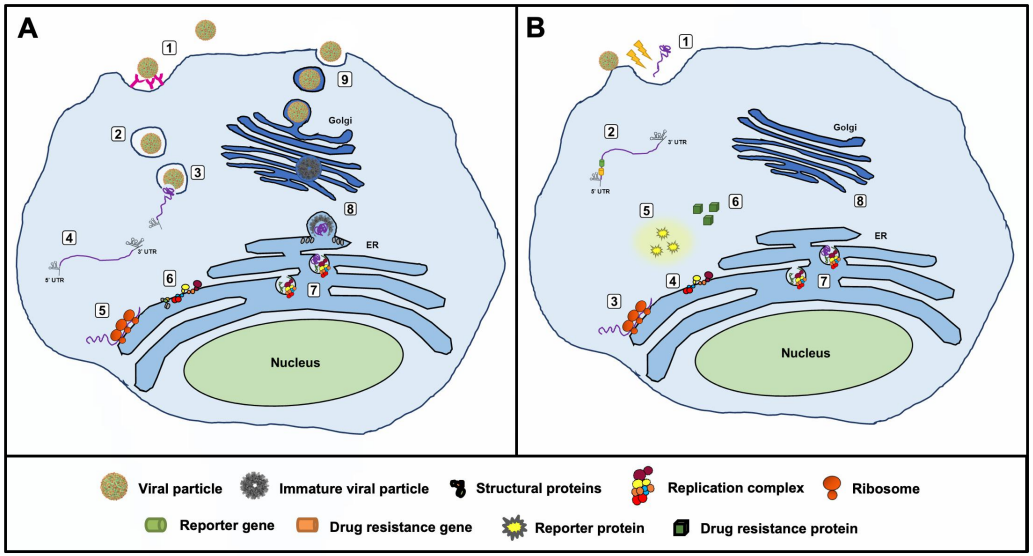


SIRIUS: the new 4th generation synchrotron facility recently inaugurated in Campinas/SP/Brazil

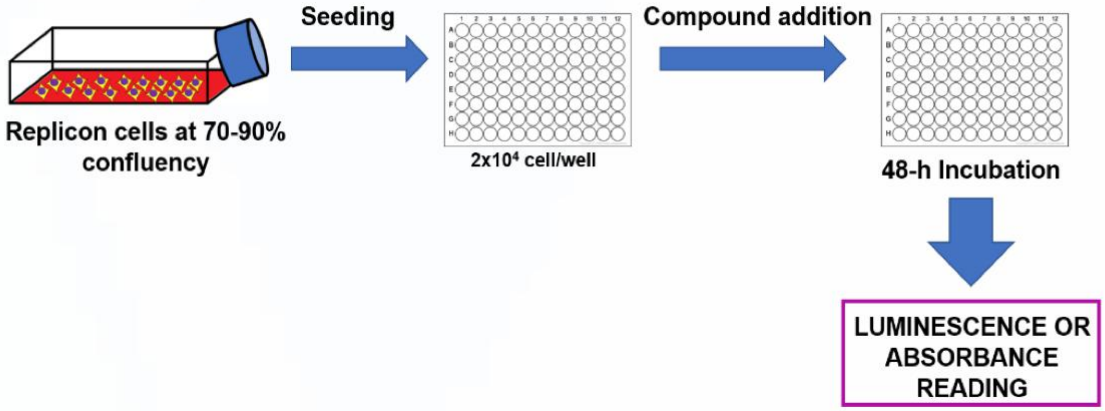
Development of stable cell lines expressing reporter containing viral replicons of ZIKV, YFV e CHKV, for antiviral compound screening

Collaboration with Profa. Laura Gil, Inst. Aggeu Magalhães, Fiocruz, PE)





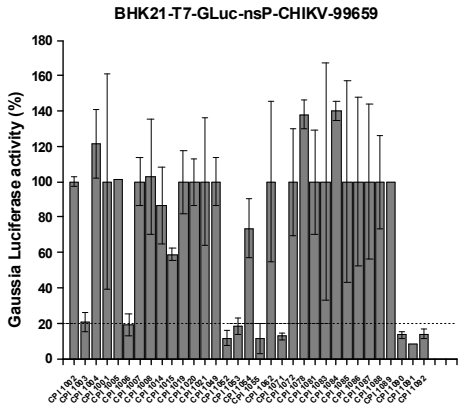
Antiviral screening – Luciferase activity and Cytotoxicity assay



Results

BHK-21-T7-Gluc-nsP-CHIKV-99650 antiviral screening

A



B

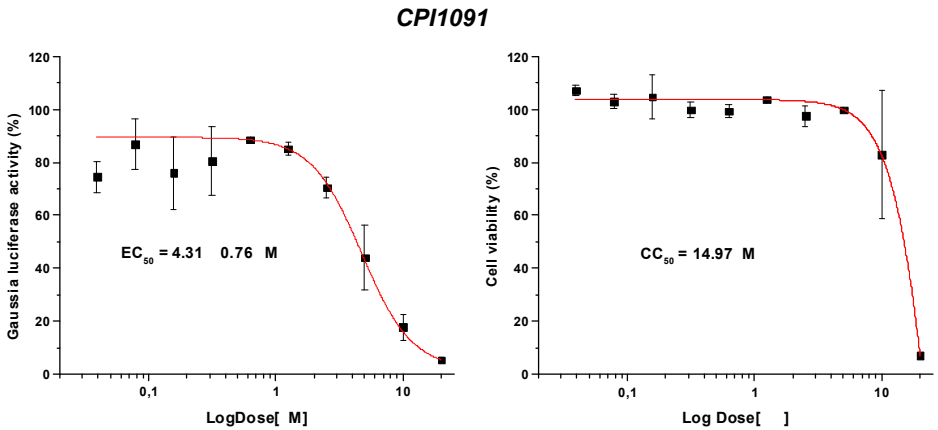


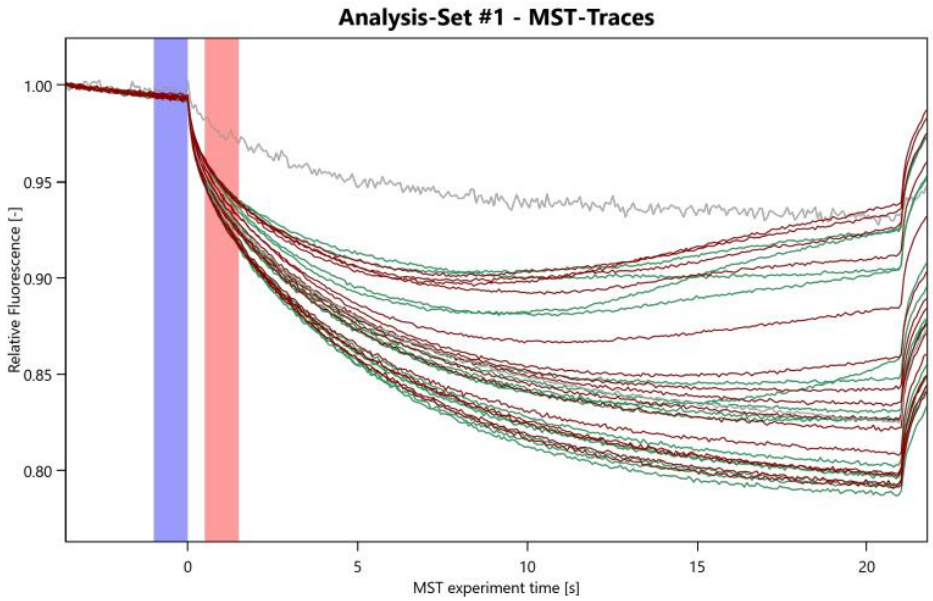
Figure 3. Antiviral assay using BHK-21 cell line expressing CHIKV reporter replicon:

(A) Replicon cell line was treated with compounds at 200 uM and those that inhibited the luciferase activity in ≥ 80% were evaluated in a dose-dependent manner (EC_{50}) and the cytotoxicity (CC_{50}) was determined by a MTT cell proliferation assay

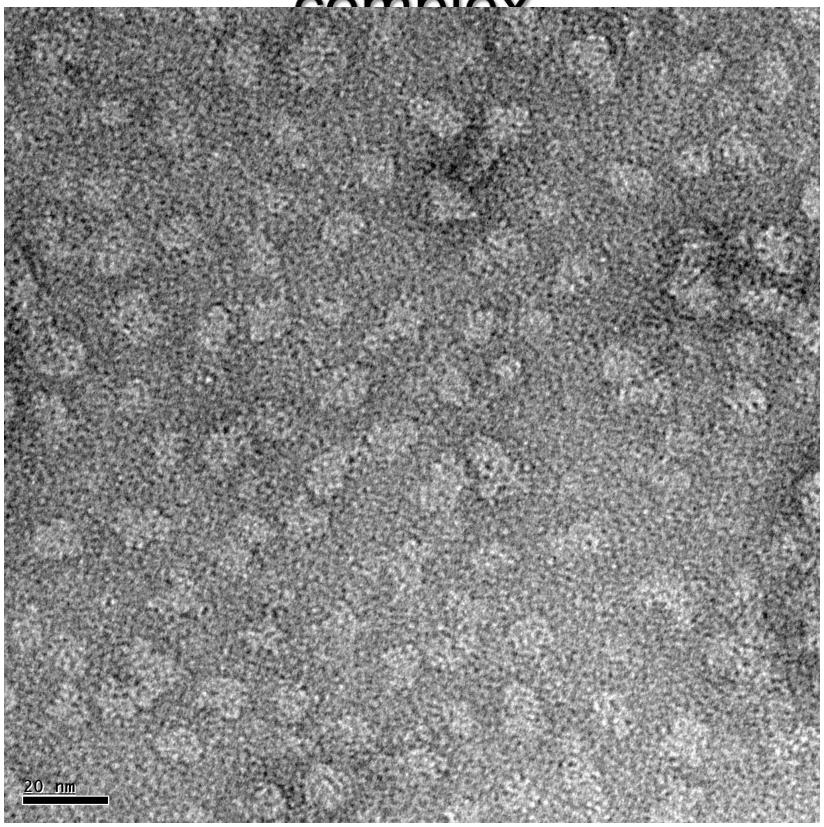
(B) EC_{50} and CC_{50} curves of compound CPI1091. Data represents mean and ± SD of three independent experiments performed in duplicates.

Towards the elucidation of the Replicon Organization and Structure

NS3Helicase-NS4B interaction seen by MST

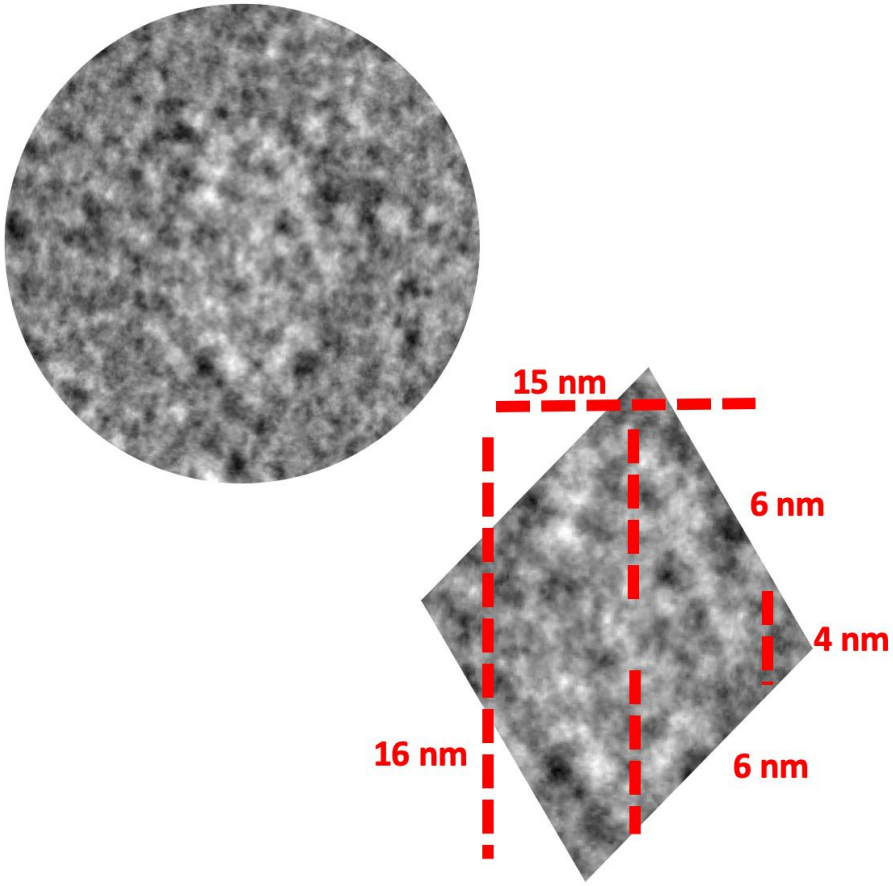


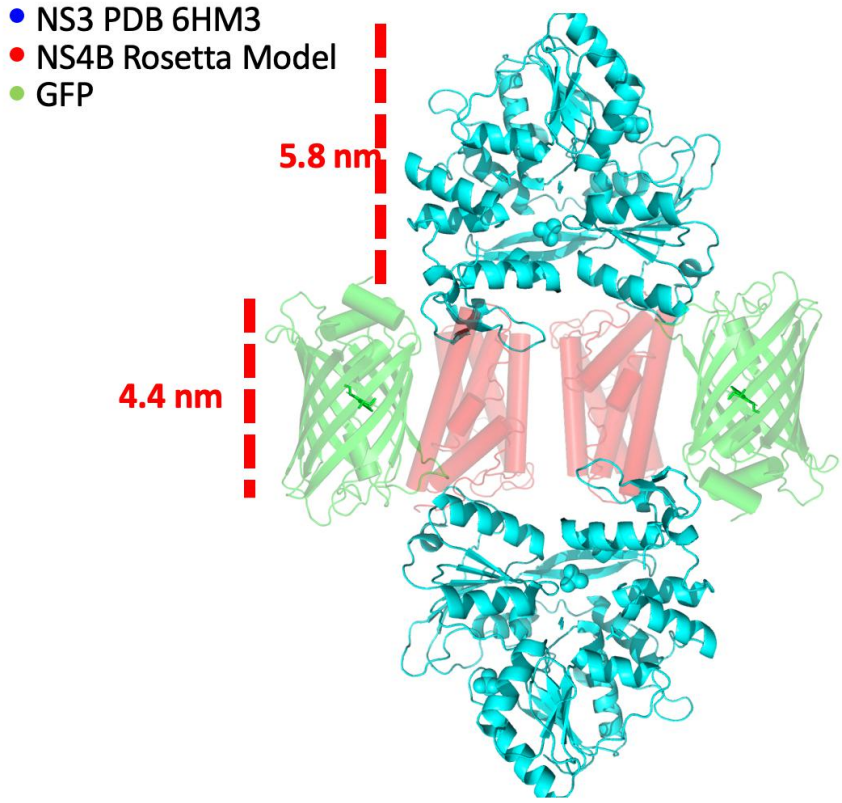
TEM Negative stain of NS3Helicase-NS4B complex



Towards the elucidation of the Replicon Organization and Structure

Particle measurements of NS3 Helicase NS4B complex and model comparation

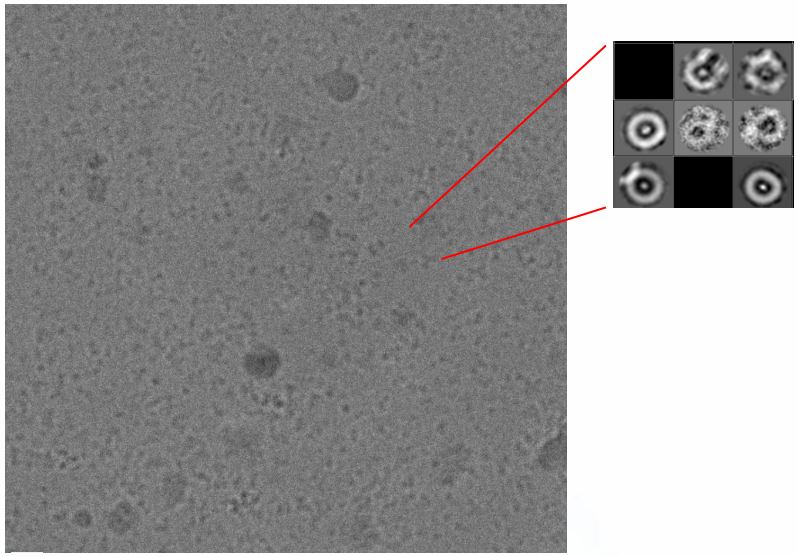




Towards the elucidation of the Replicon Organization

Titan Themis Cubed (FEI Company)

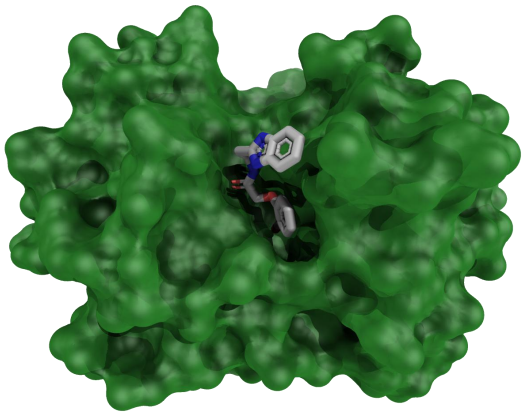
LNNano



Initial Cryo-EM Images

Structural elucidation

YFV NS3 protease domain



Resurgence of Yellow Fever in Brazil/2017

Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. *112*(6): 1-5, June 2017

Genome analysis of yellow fever virus of the ongoing outbreak in Brazil reveals polymorphisms

Myrna C Bonaldo[1]/+, Mariela Martínez Gómez[1], Alexandre AC dos Santos[1], Filipe Vieira Santos de Abreu[2,3], Anielly Ferreira-de-Brito[2], Rafaella Moraes de Miranda[2], Marcia Gonçalves de Castro[2], Ricardo Lourenço-de-Oliveira[2]

[1]Fundação Oswaldo Cruz-Fiocruz, Instituto Oswaldo Cruz, Laboratório de Biologia Molecular de Flavivírus, Rio de Janeiro, RJ, Brasil
[2]Fundação Oswaldo Cruz-Fiocruz, Instituto Oswaldo Cruz, Laboratório de Mosquitos Transmissores de Hematozoários, Rio de Janeiro, RJ, Brasil
[3]Instituto Federal do Norte de Minas Gerais, Montes Claros, MG, Brasil

The current yellow fever outbreak in Brazil is the most severe one in the country in recent times. It has rapidly spread to areas where YF virus (YFV) activity has not been observed for more than 70 years and vaccine coverage is almost null. Here, we sequenced the whole YFV genome of two naturally infected howler-monkeys (*Alouatta clamitans*) obtained from the Municipality of Domingos Martins, state of Espírito Santo, Brazil. These two ongoing-outbreak genome sequences are identical. They clustered in the 1E sub-clade (South America genotype I) along with the Brazilian and Venezuelan strains recently characterised from infections in humans and non-human primates that have been described in the last 20 years. However, we detected eight unique amino acid changes in the viral proteins, including the structural capsid protein (one change), and the components of the viral replicase complex, the NS3 (two changes) and NS5 (five changes) proteins, that could impact the capacity of viral infection in vertebrate and/or invertebrate hosts and spreading of the ongoing outbreak.

Resurgence of Yellow Fever in Brazil/2017

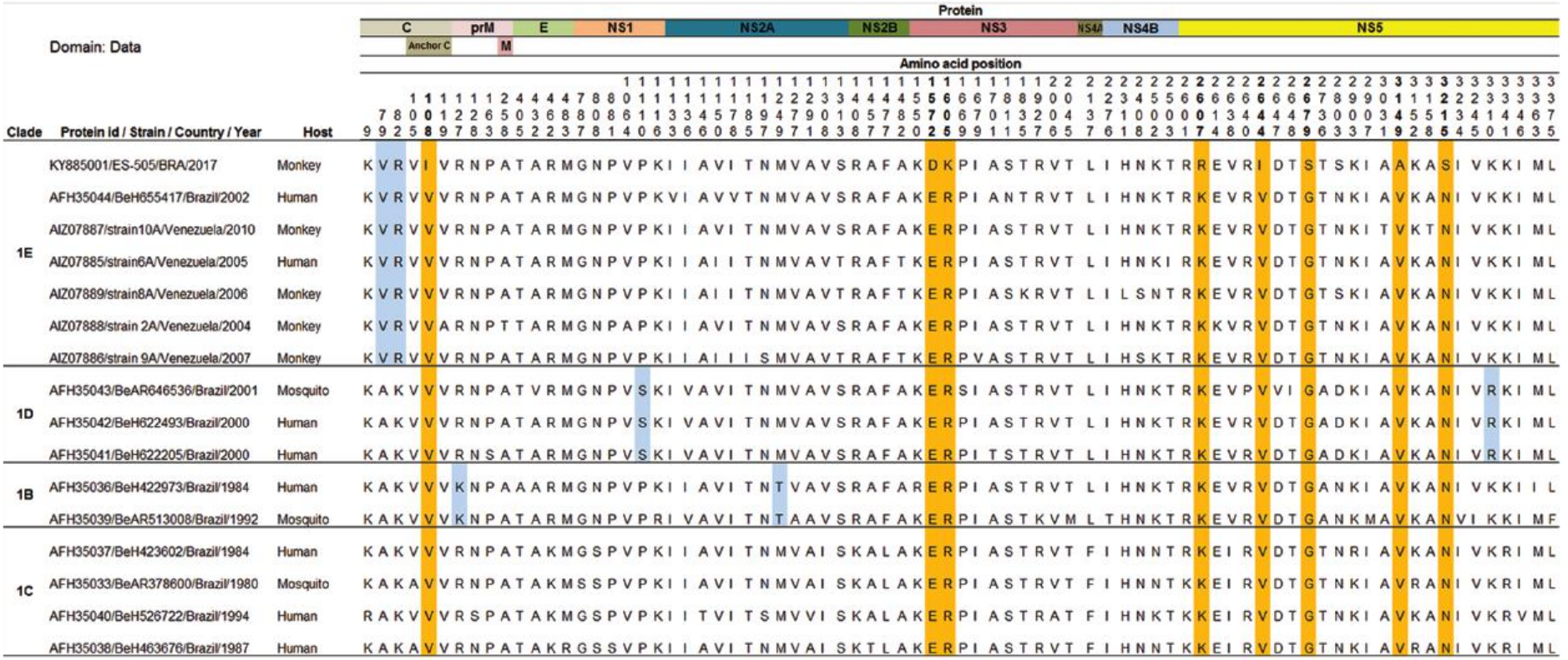
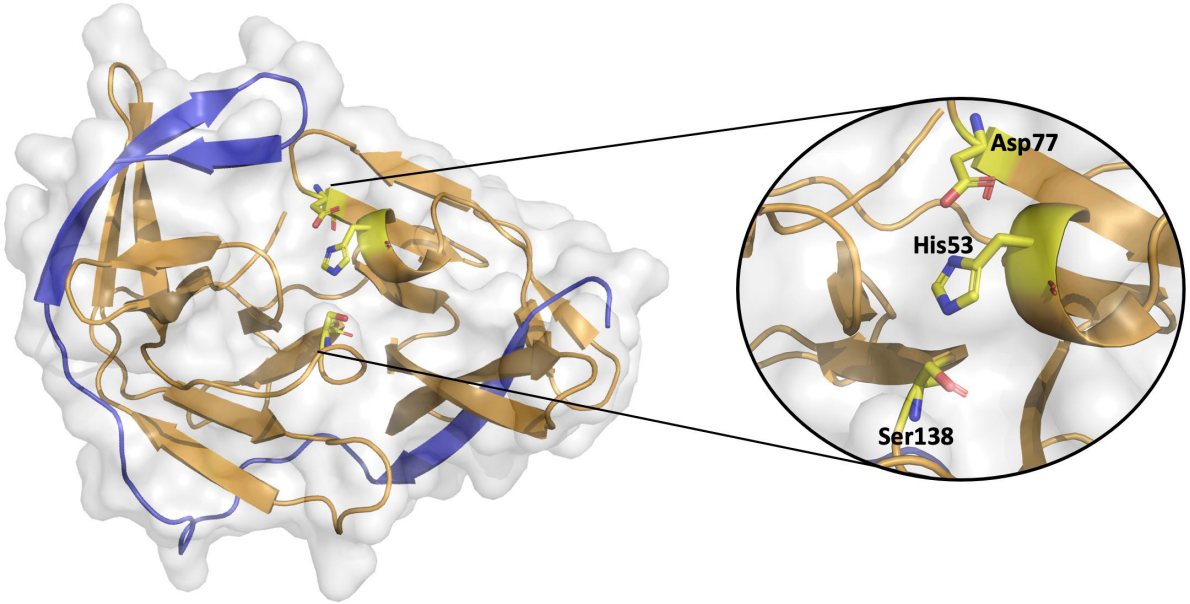
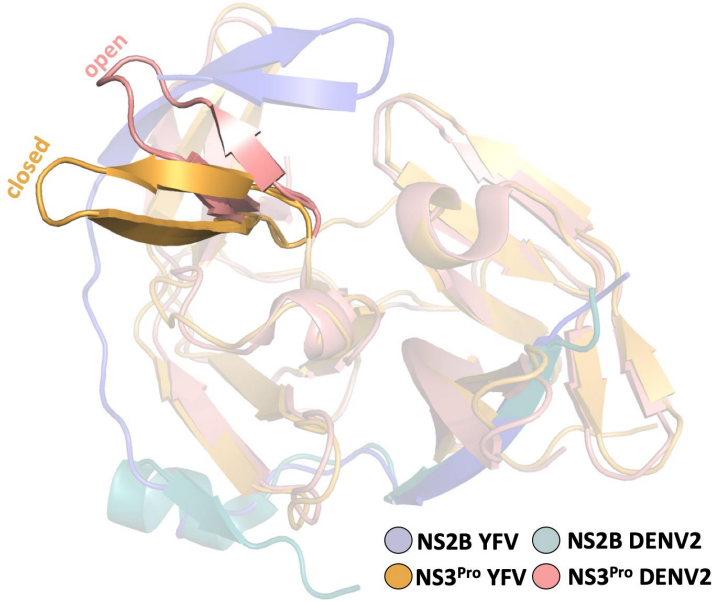


Fig. 3: amino acid (aa) differences revealed by the alignment of the precursor polyproteins of 16 Brazilian and Venezuelan yellow fever (YF) viruses detected since 1980. On the left of the alignment data, the identification of clades and yellow fever virus (YFV) sequences are supplied. On the top of the alignment, the YF viral proteins positions are indicated along with the aa positions of aa differences. The set of aa residues highlighted in blue indicate a related-clade pattern. The orange-highlighted aa indicate the position of the current YF sequences compared to the other YF sequences. For simplicity, only the ES-505/BRA/2017 strain sequence data were included in this figure.

Crystal structure of the NS2B-NS3 protease from the 2017 YFV Brazilian circulating strain

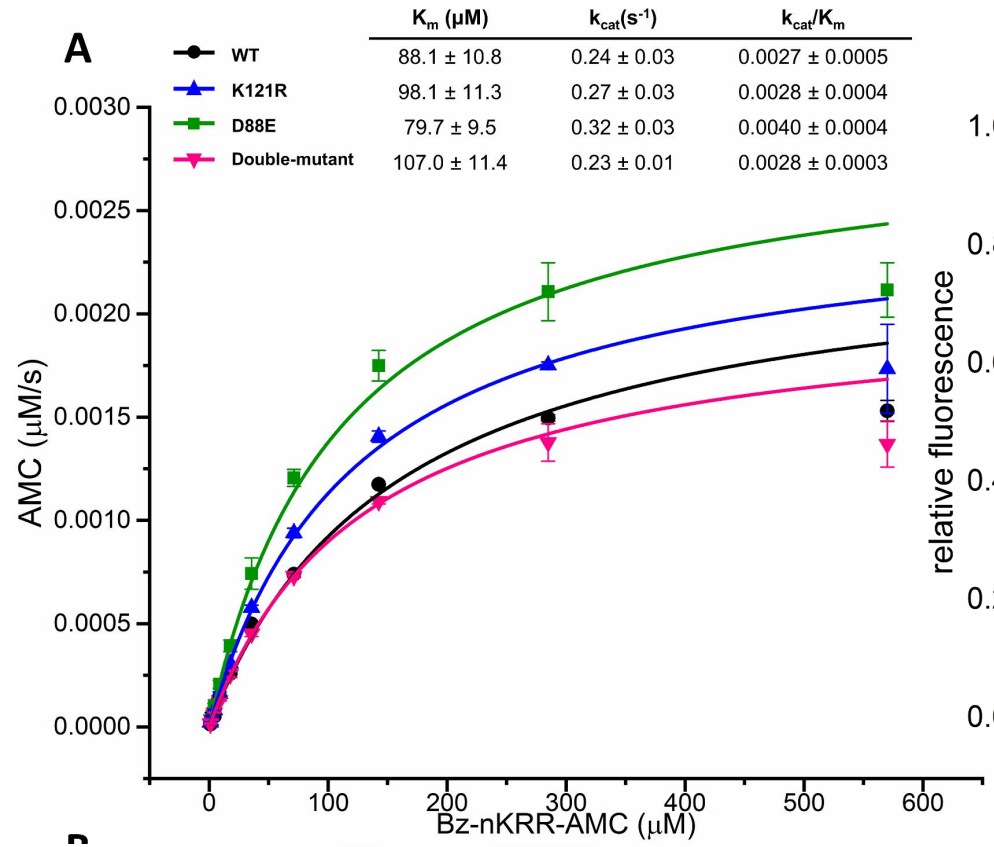
CIBFar

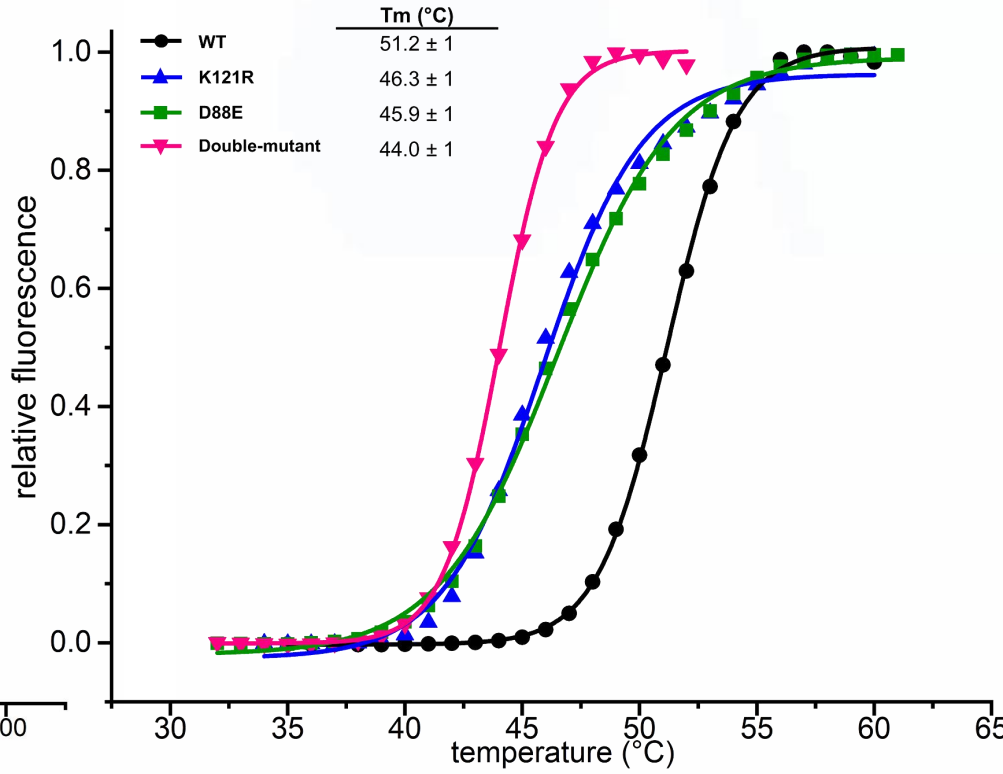




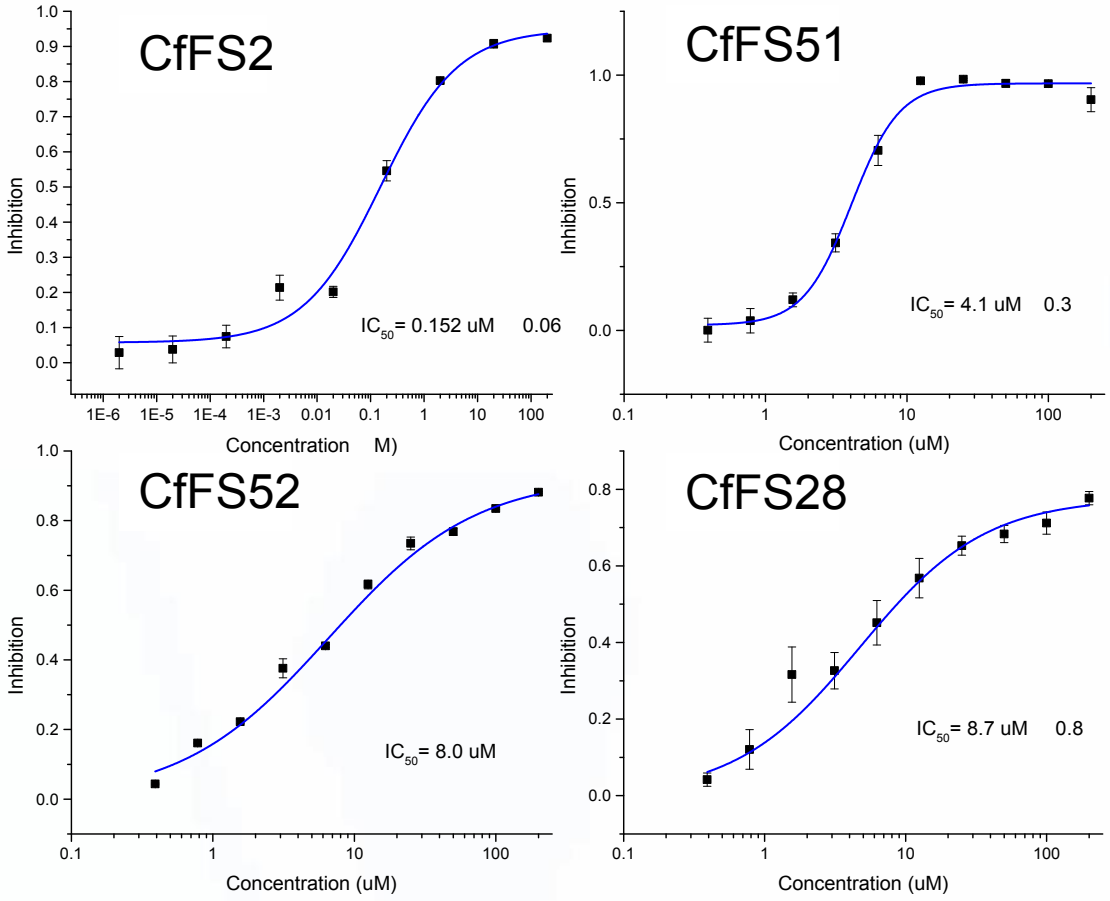
YFV NS2B-NS3 PROTEASE– TO BE PUBLISHED

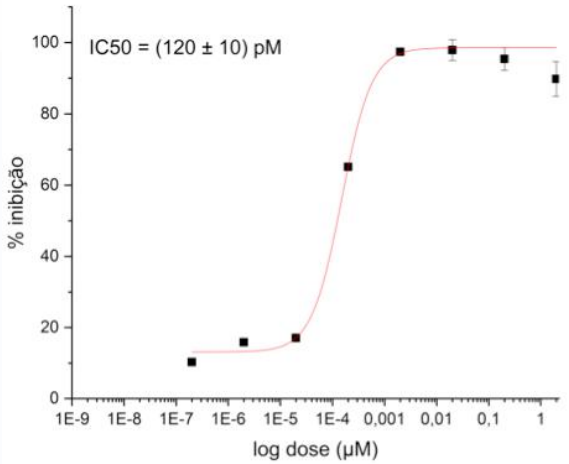
Polymorphism analysis

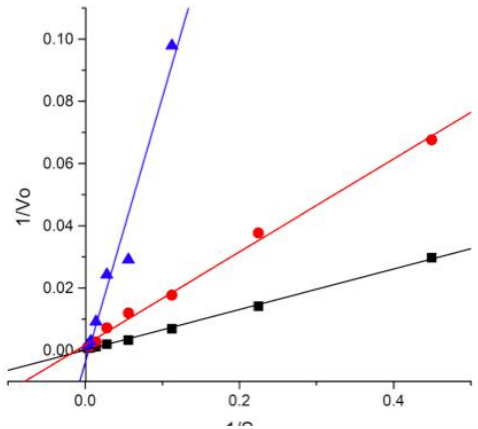




IS2B-NS3protease YFV





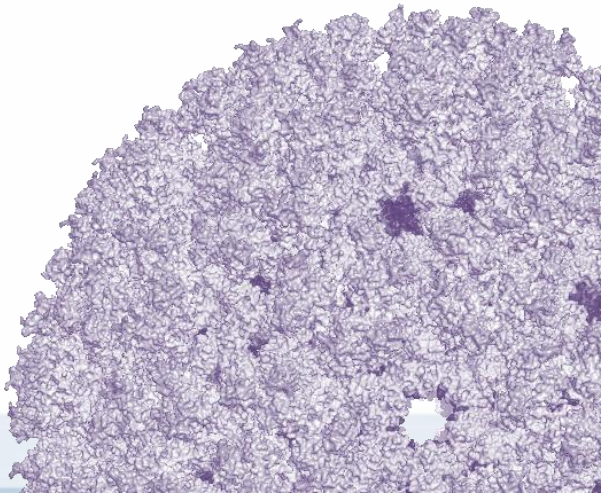


Enzyme kinetics of YFV protease versus synthetic peptide

Our first sub-nanomolar inhibitor !

- Zika Virus
- Yellow Fever Virus
- Chikungunya Virus
- Mayaro Virus

- Recombinant proteins
- Enzyme assays
- Biochemical assays
- Cell-based assays
- X-ray XTAL
- Cryo-EM



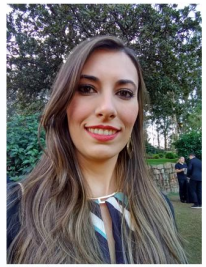
Acknowledgements

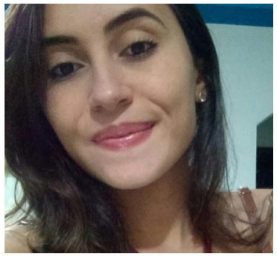
People @ IFSC/USP


Dr. Rafael V C Guido


André Godoy

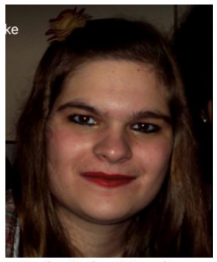

Nathalya Mesquita


Rafaela Fernandes


Renata Vieira


Victor Oliveira.


Ketllyn Oliveira


Gabriela Noske


Marjorie Freire

Collaborations

- Arlene G. Correa (DQ-UFSCar)
- Marcio Dias (ICB-USP)
- Carolina Andrade (UFG & Openzika Initiative)
- Melina Mottin (UFG & Openzika Initiative)
- Sean Ekins (Collaborations Pharmaceuticals, Inc & Openzika Initiative)
- Laura Gil (Instituto Aggeu Magalhães, FIOCRUZ-PE)
- Eduardo Cilli (IQ-UNESP-Ar)

Funding:

FAPESP

CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico

Acknowledgements

CIBFar

FAMILY

... and nothing would have been possible without the Institutional Support of the São Carlos Institute of Physics