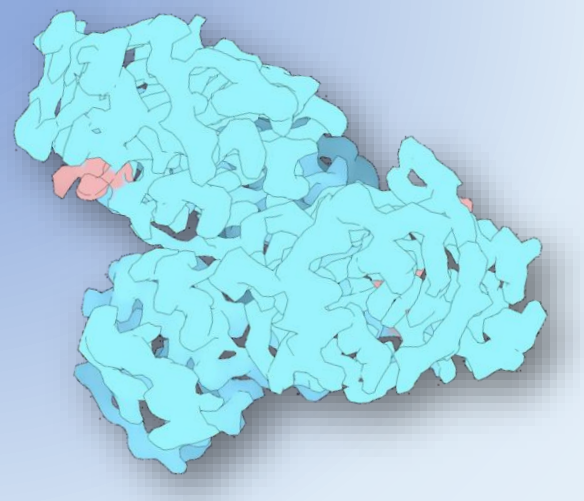
Structural insights on the SARS-CoV-2 Main Protease maturation process and inhibition

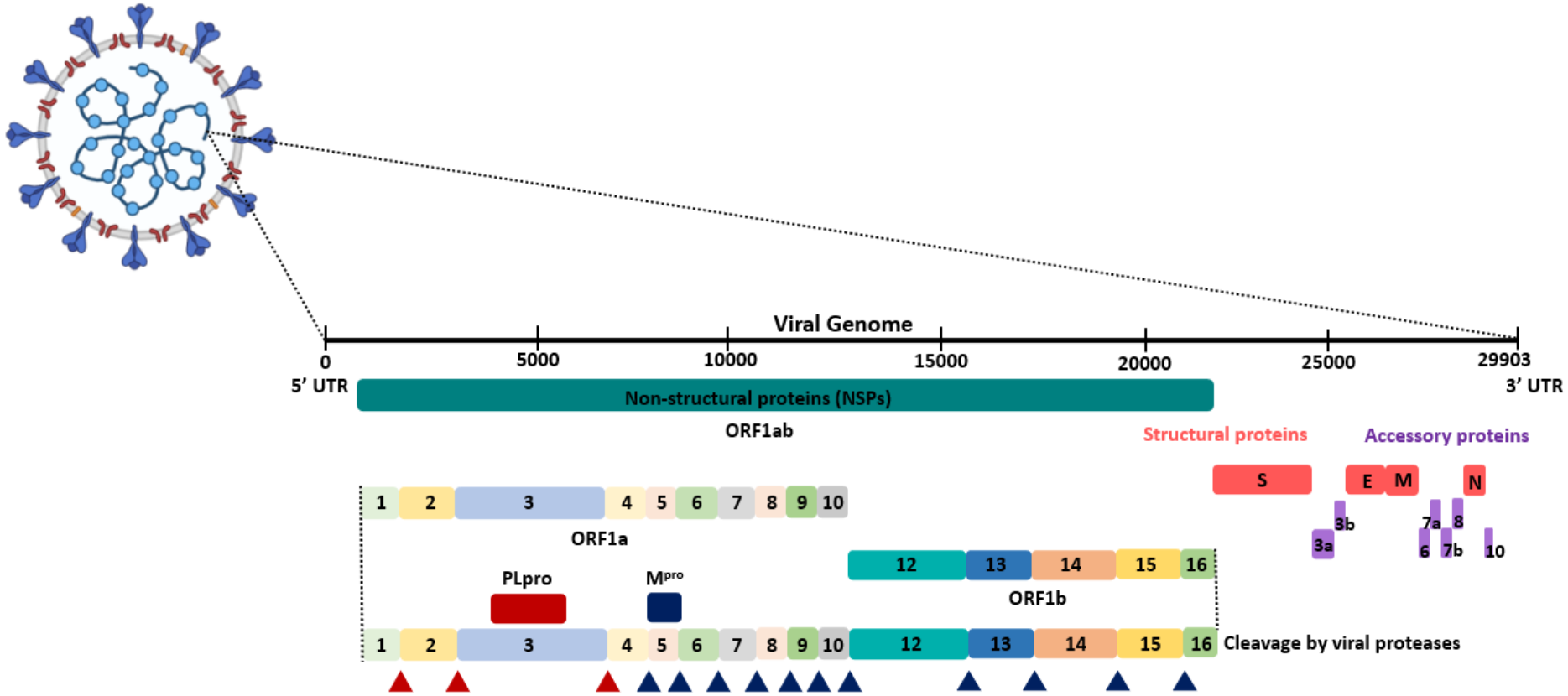


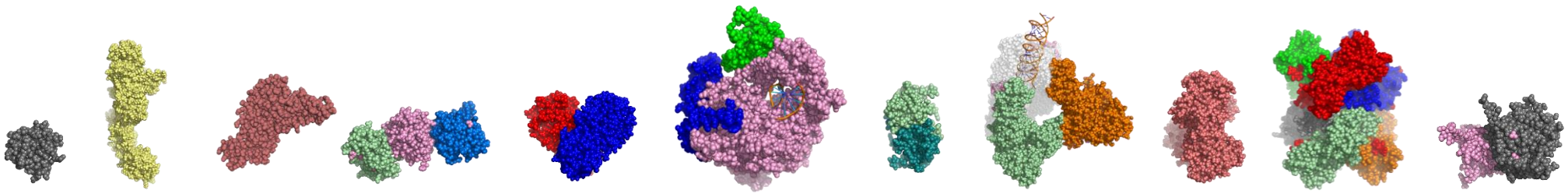
Andre Schutzer Godoy, PhD
X-ray crystallography, cryo-EM, fragment screening, virology

University of Sao Paulo - Brazil
AI-driven Structure-enabled Antiviral Platform
NIH AviDD U19 Center

andregodoy@ifsc.usp.br, andre.schgodoy@gmail.com

SARS-CoV-2 Genome Organization





7K3N *NSP1*

7MSW *NSP2*

6XA9 *NSP3 PLpro*

6YWM *NSP3 macrodomain*

6XMK *NSP5 Mpro*

6XQB *NSP12-NSP7-NSP8 complex*

6W9Q *NSP9*

6XEZ *NSP13*

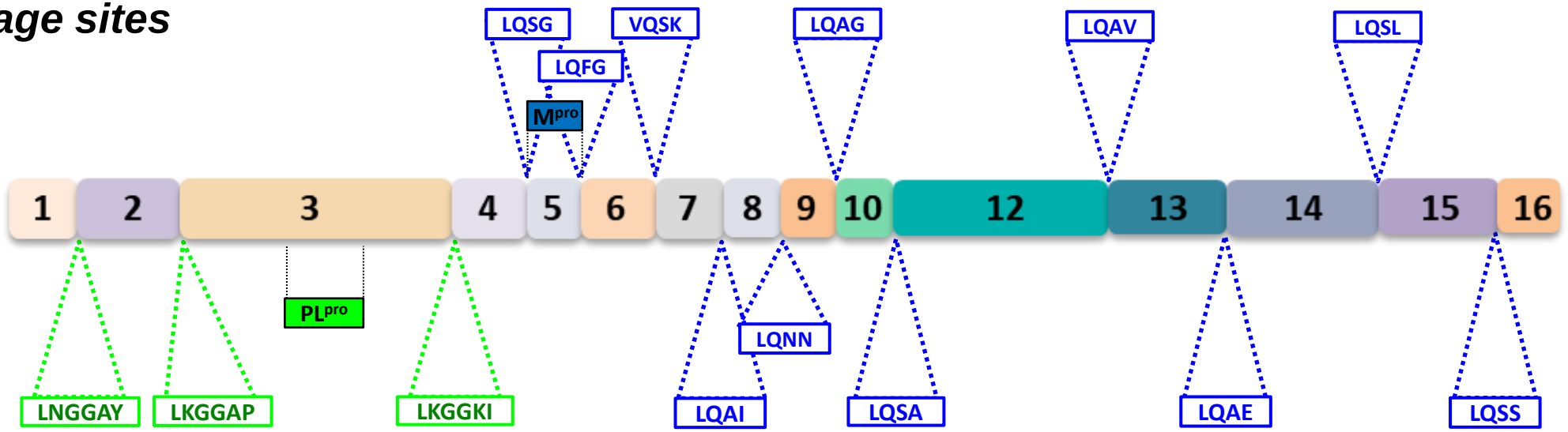
7R2V *NSP14*

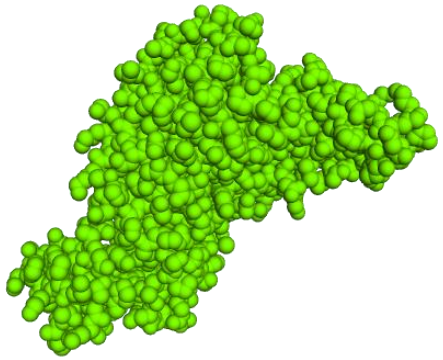
6X1B *NSP15 NendoU*

6WQ3 *NSP16-NSP10*

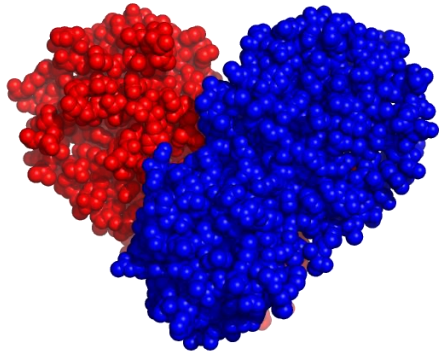
SARS-CoV-2 proteases

Cleavage sites





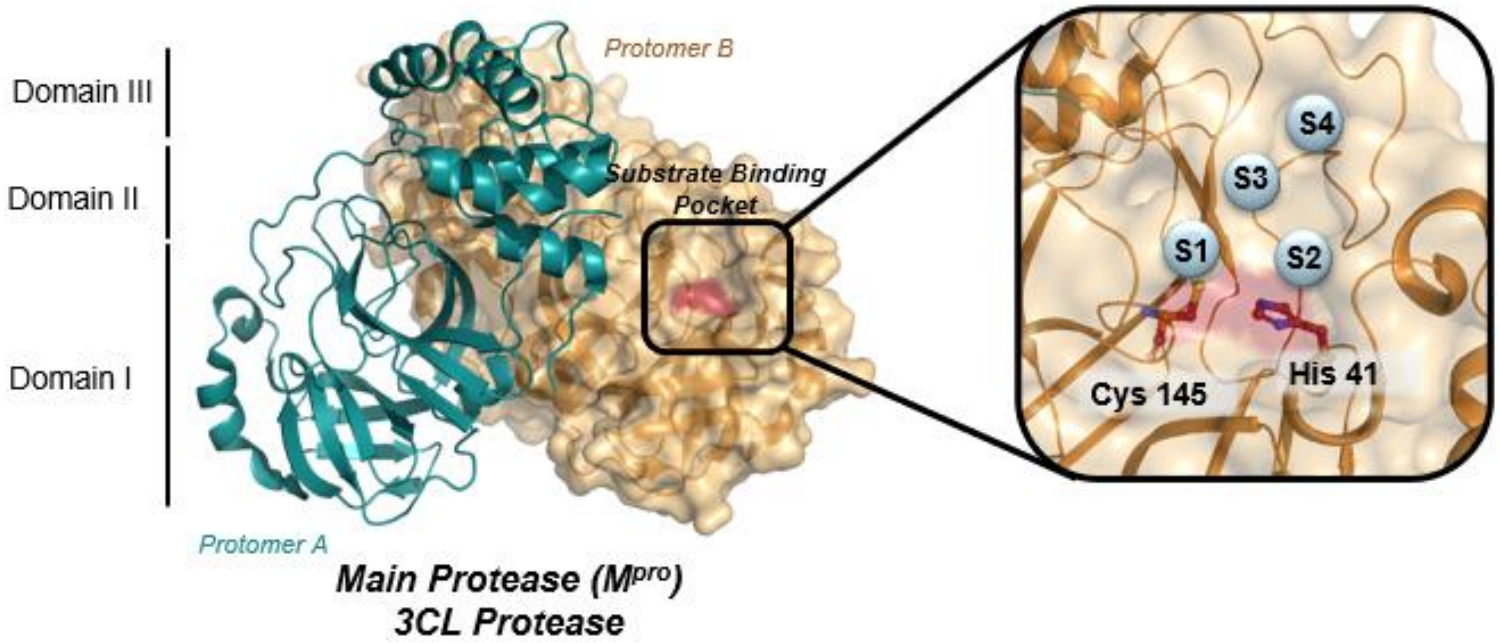
Papain-like protease



Main protease
3CL protease

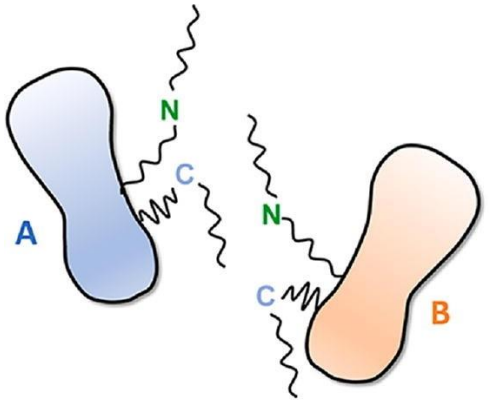
SARS-CoV-2 Main Protease (M^{pro})

- Cysteine protease with dimeric structure
- Responsible for the cleavage of 11 sites of the polyprotein, including its own N and C-terminal
- Over 600 structures deposited on PDB (March 2023)
- Key target for antiviral development
- Lack of information about its self-maturation process



SARS-CoV-2 Main Protease Maturation

- If the protein is active only as a dimer, how copies of immature M^{pro}, still as part of the entire viral poliprotein, get together to conduct its self-cleavage ?
- The N and C-terminus processing occurs within a dimer (*cis*-cleavage) or between two distinct dimers (*trans*-cleavage)
- What are there conformational changes involved ?
- Can NEW CAVITIES be identified in the immature M^{pro} , that could be explored for early inhibition of the protease activity ?

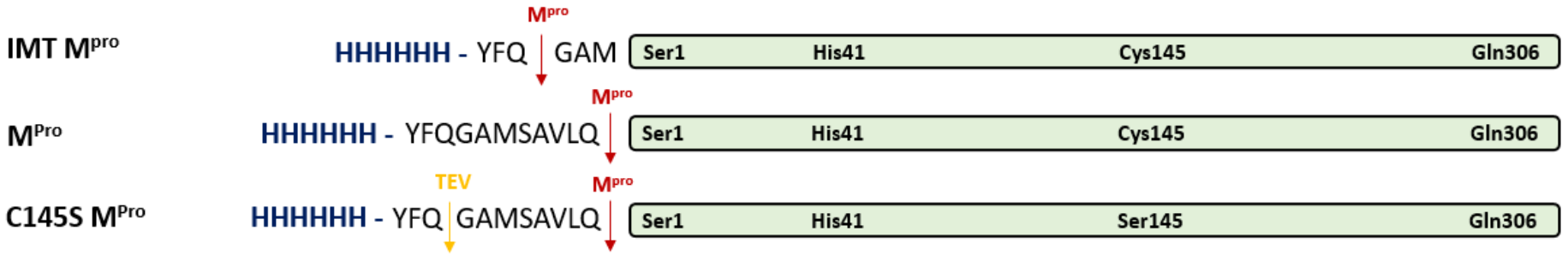


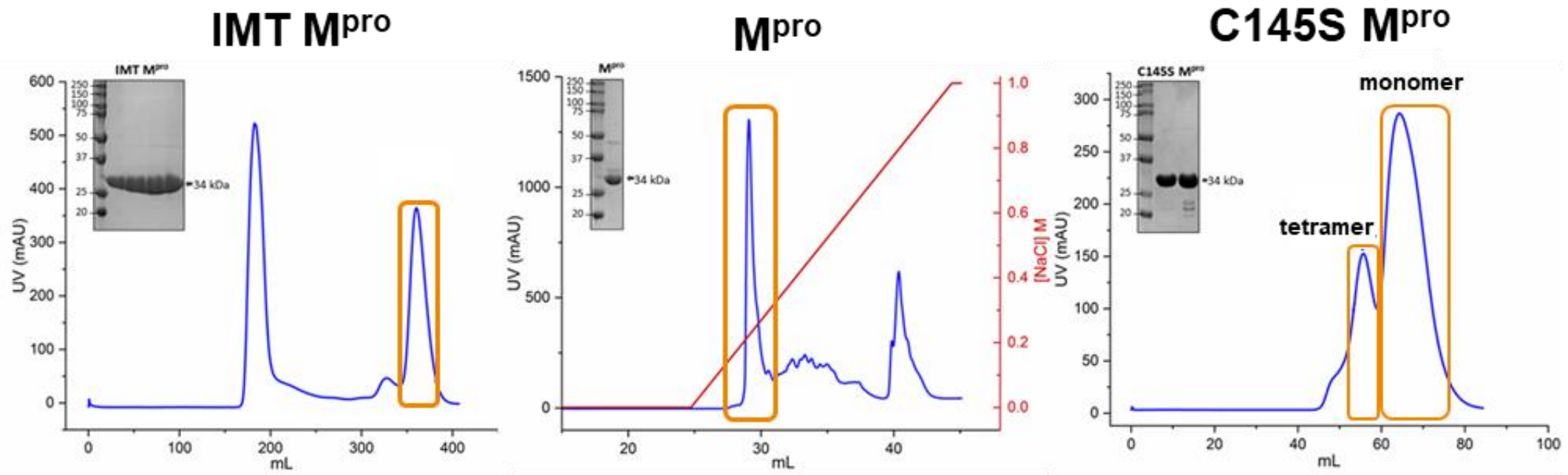
Immature M^{pro} protomers

j.jmb.2021.167118

SARS-CoV-2 M^{pro} constructs

- Immature form
- Mature or native
- C145S mutant



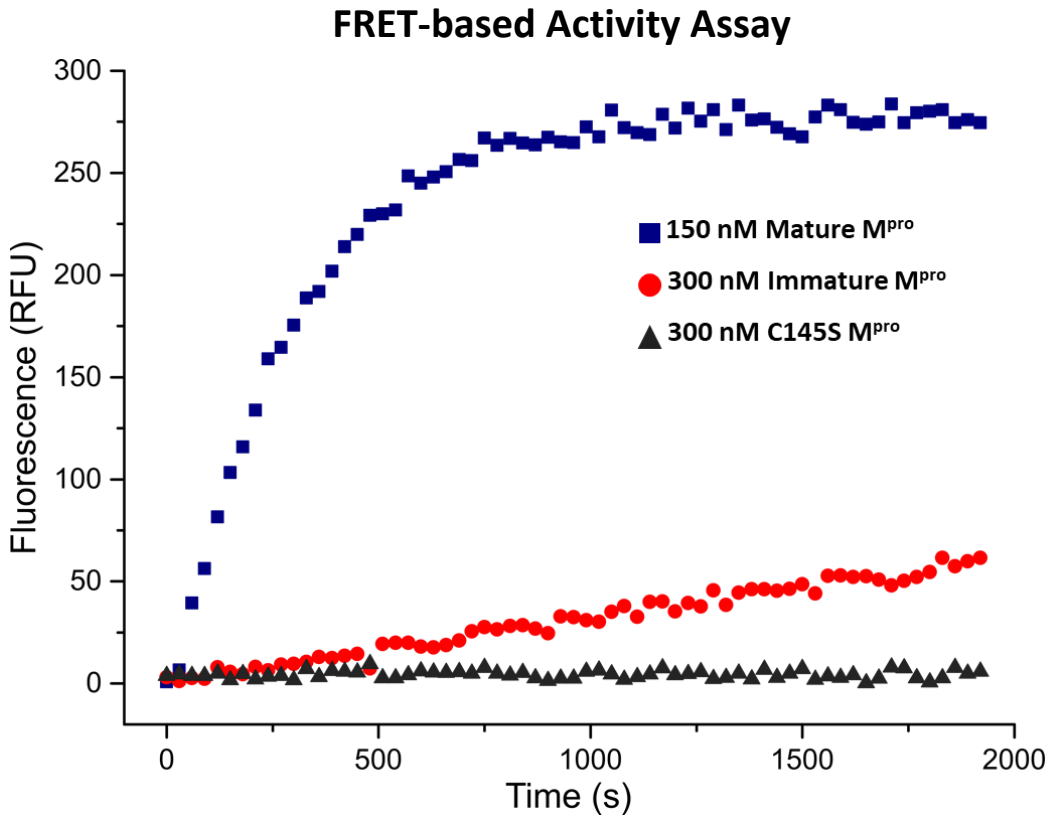


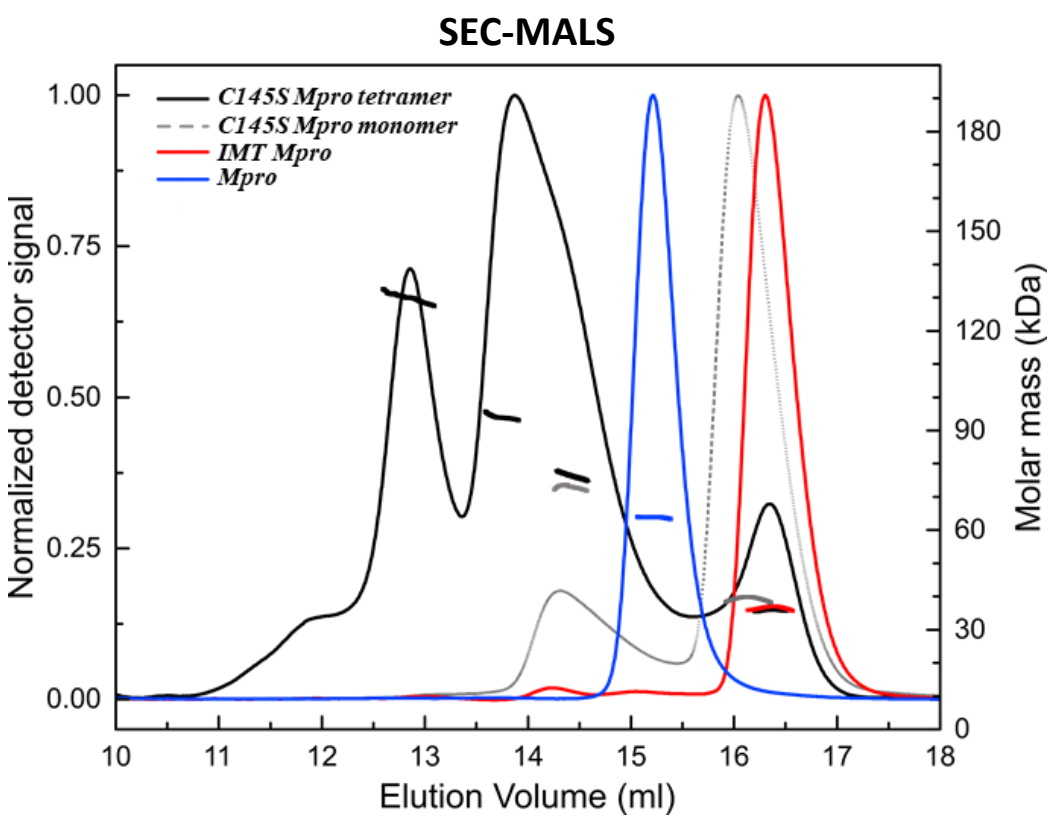
NOSKE, G.D. et al, JMB, 2021

SARS-CoV-2 Main Protease

In-solution characterization:

- IMT-Mpro has reduced activity and is monomeric in solution
- C145S Mpro is inactive and a mixture of oligomeric states ranging from monomers to tetramers



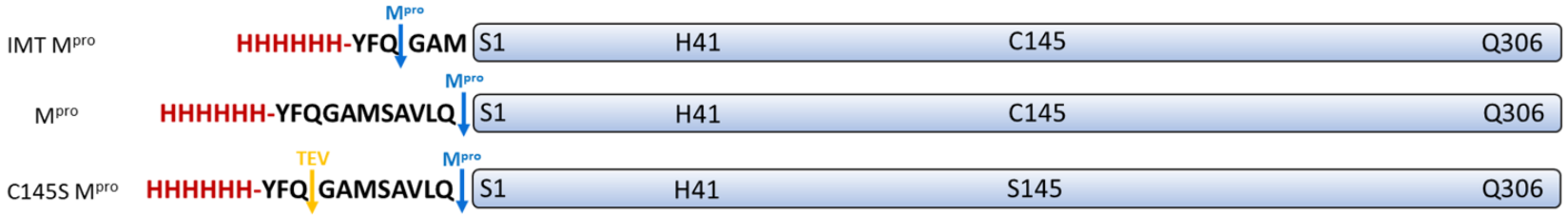


NOSKE, G.D. et al, JMB, 2021

SARS-CoV-2 Main Protease

SARS-CoV-2 Main-Protease Constructs:

- ***Immature form***
- ***Mature or native***
- ***C145S mutant***



IMT M^{pro}

M^{pro}

C145S M^{pro}

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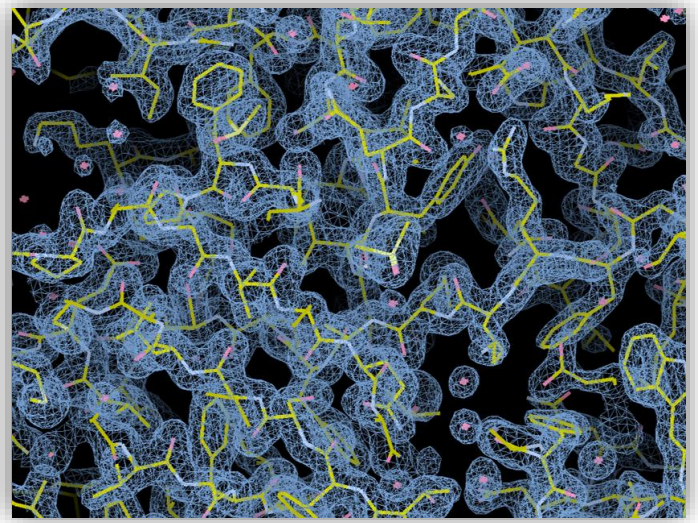
1st SIRIUS External users experiment

SIRIUS, Campinas – SP - September 2020



Agência FAPESP NOTÍCIAS AGENDA VÍDEOS ASSINE

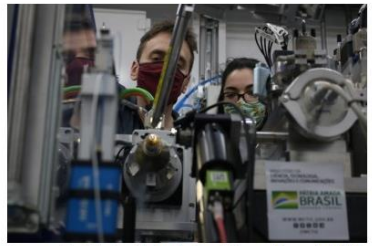
Primeiro experimento realizado no Sirius busca desenvolver fármaco para COVID-19

20 de outubro de 2020

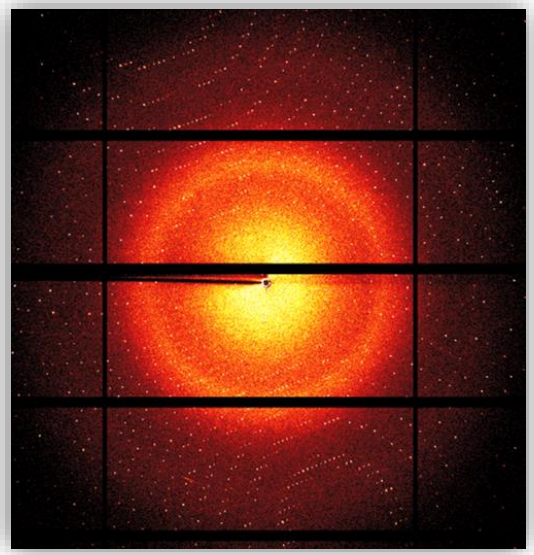
EN ES

Maria Fernanda Ziegler* | Agência FAPESP – Por meio de um potente feixe de luz síncrotron foi possível determinar, em três dias, a estrutura de mais de 200 cristais de duas proteínas do novo coronavírus (SARS-CoV-2).

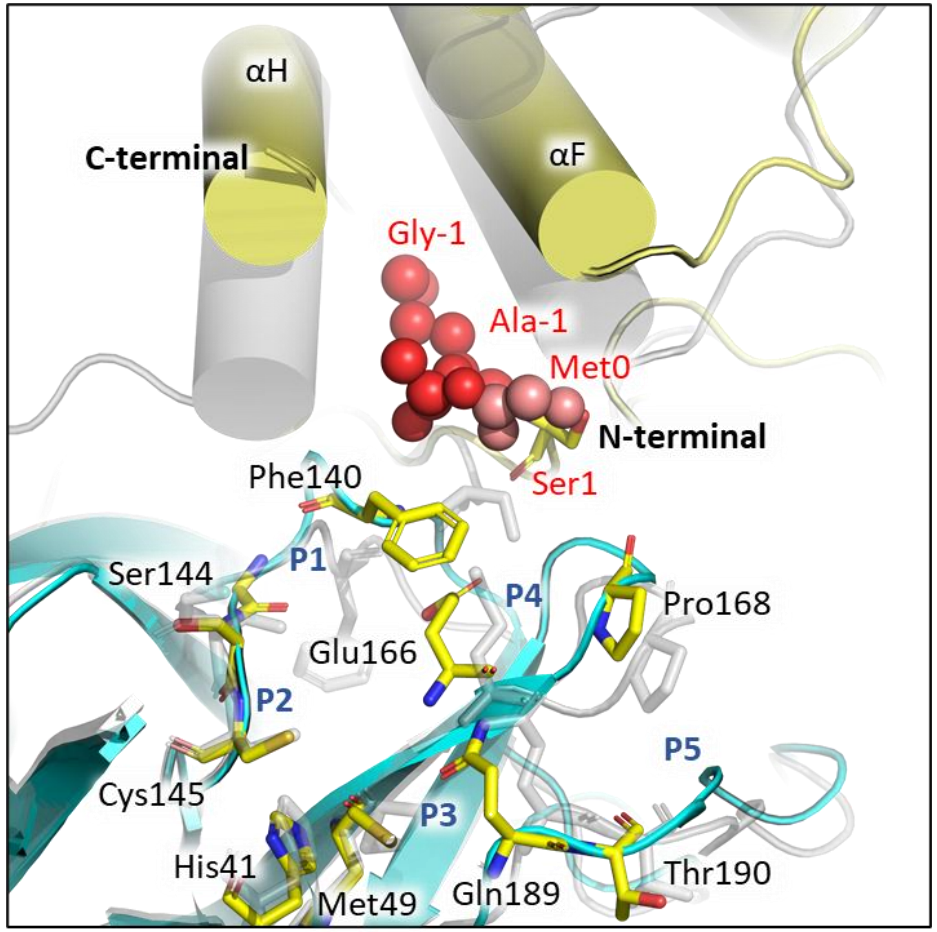
A investigação realizada por pesquisadores do Instituto de Física de São Carlos da Universidade de São de Paulo (IF-USP) tem importância não só pela temática – essencial para o desenvolvimento de um possível fármaco contra a COVID-19 –, mas também pelo seu caráter de ineditismo.

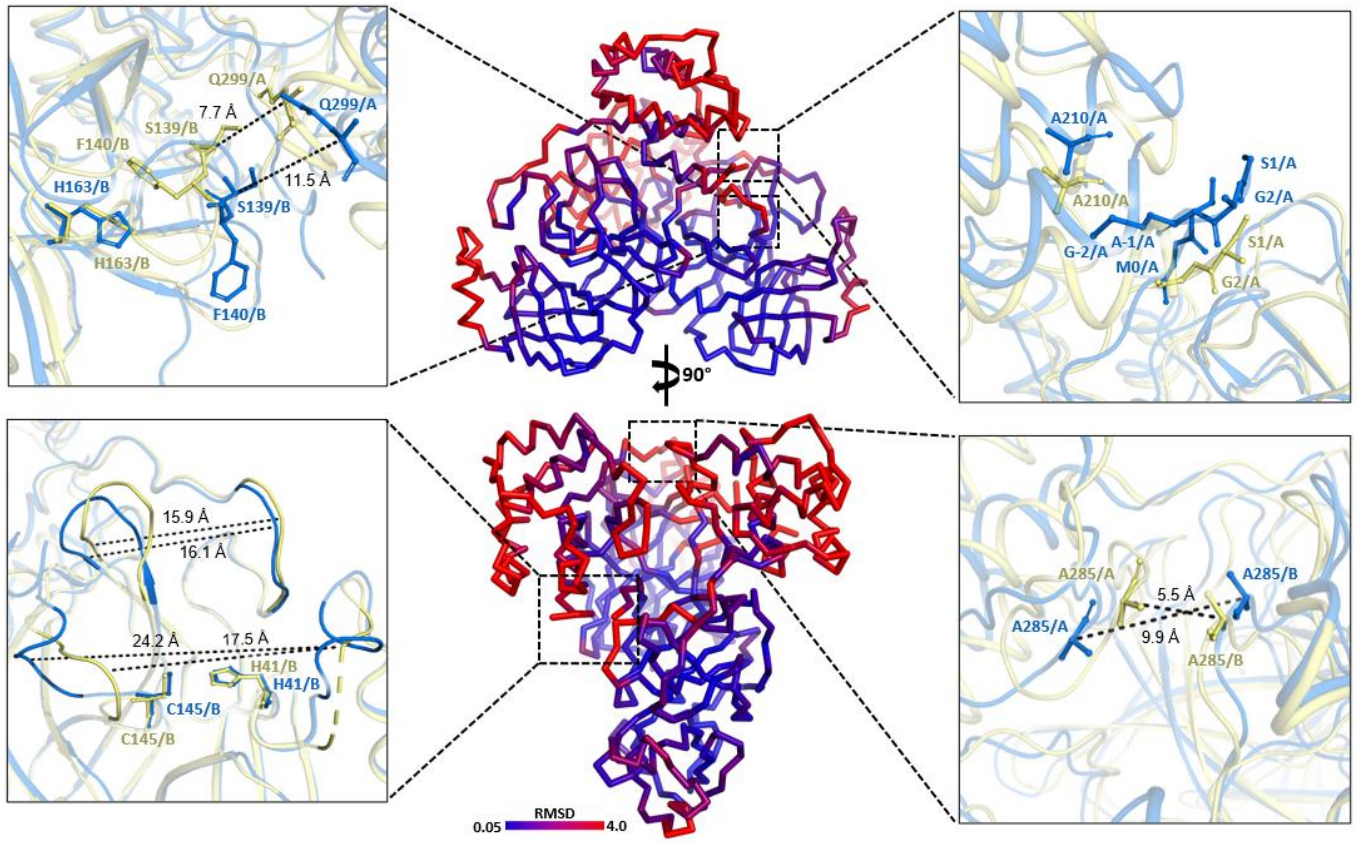


Em sua estreia, linha de cristalografia de proteínas analisou mais de 200 cristais de duas proteínas do novo coronavírus, expostos a pequenas moléculas que são partes de fármacos conhecidos. Expectativa é que, ao identificar essas estruturas, seja possível detectar substâncias que se encaixem perfeitamente nas proteínas, bloqueando sua ação no vírus (*André Godoy e Aline Nakamura posicionam cristal de proteína de SARS-CoV-2 para análise no Sirius; foto: CNPEM/divulgação*)



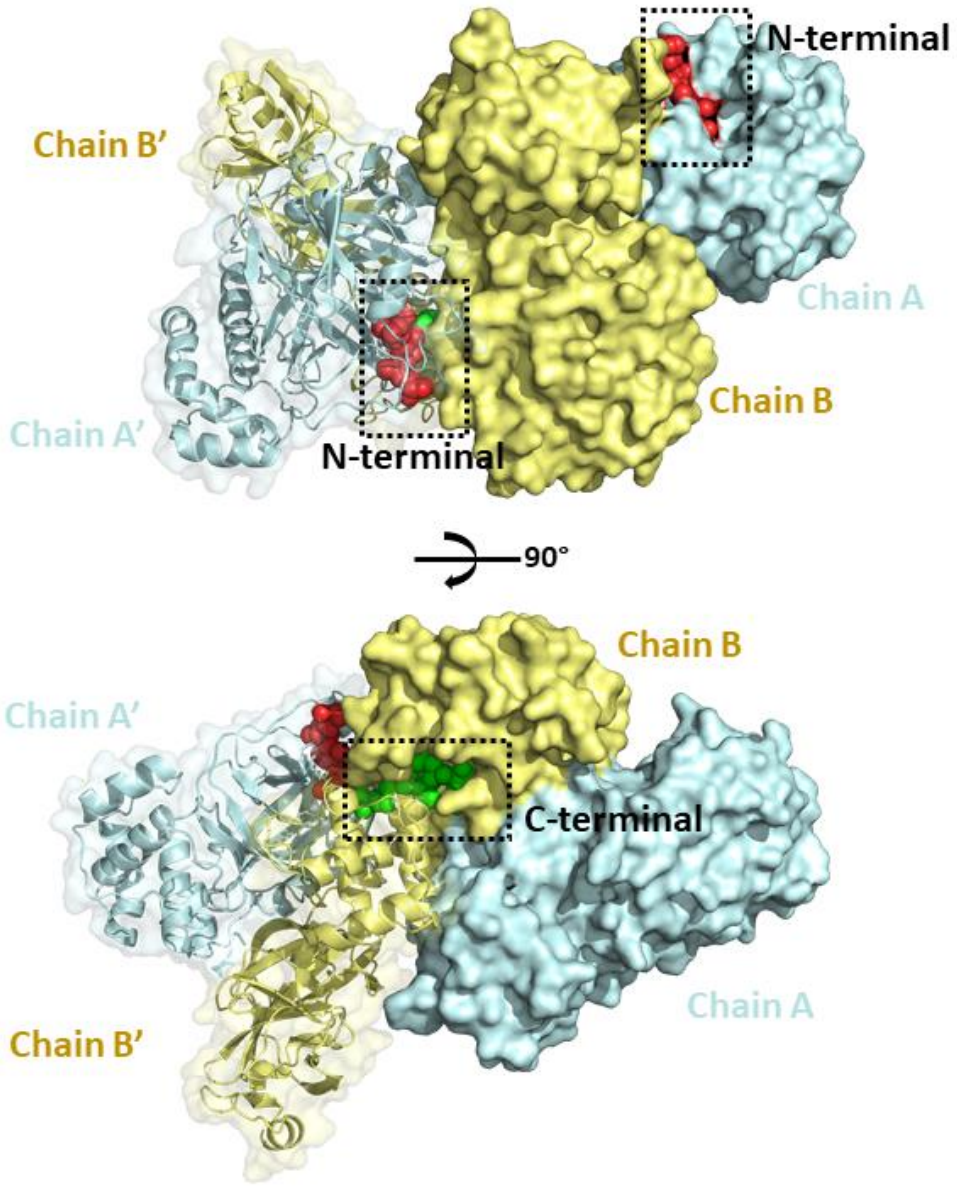
Crystal structure of IMT-M^{pro}

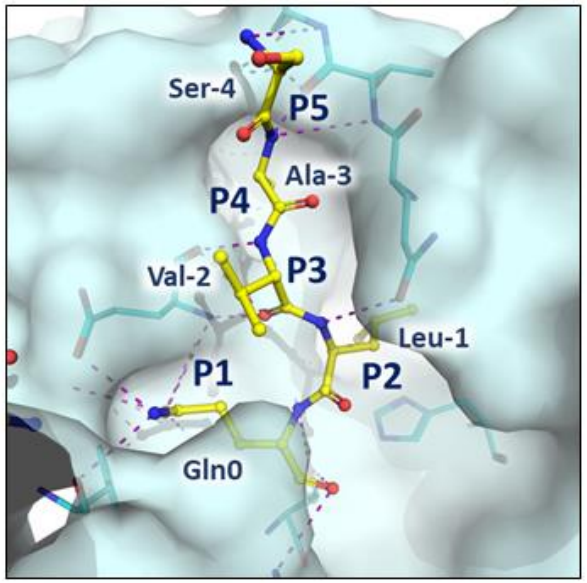


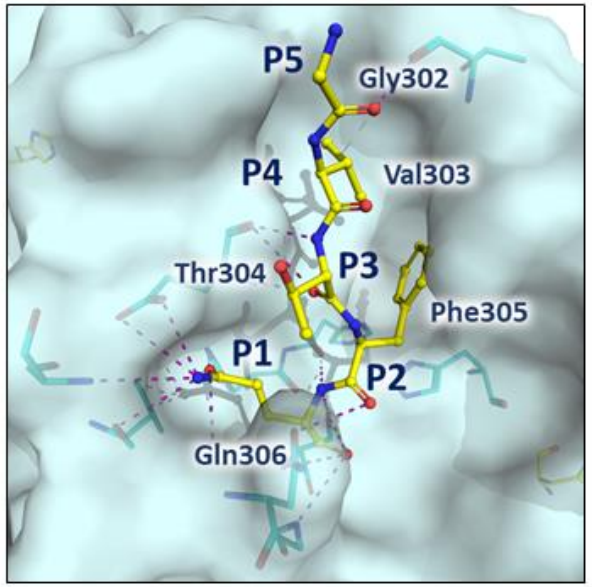


NOSKE, G.D. et al, JMB, 2021

Crystal structure of C145S M^{pro}

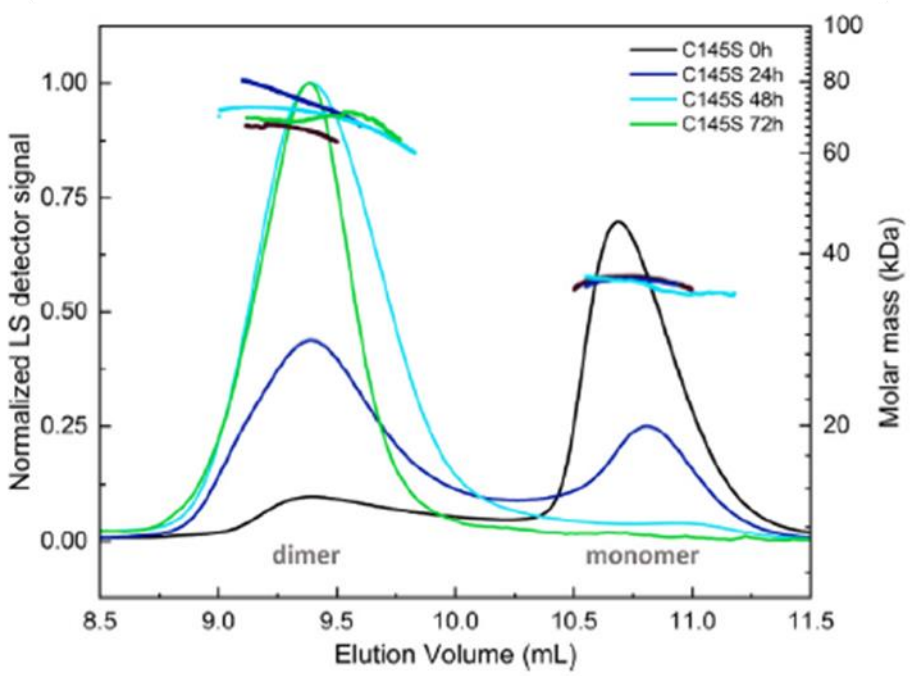


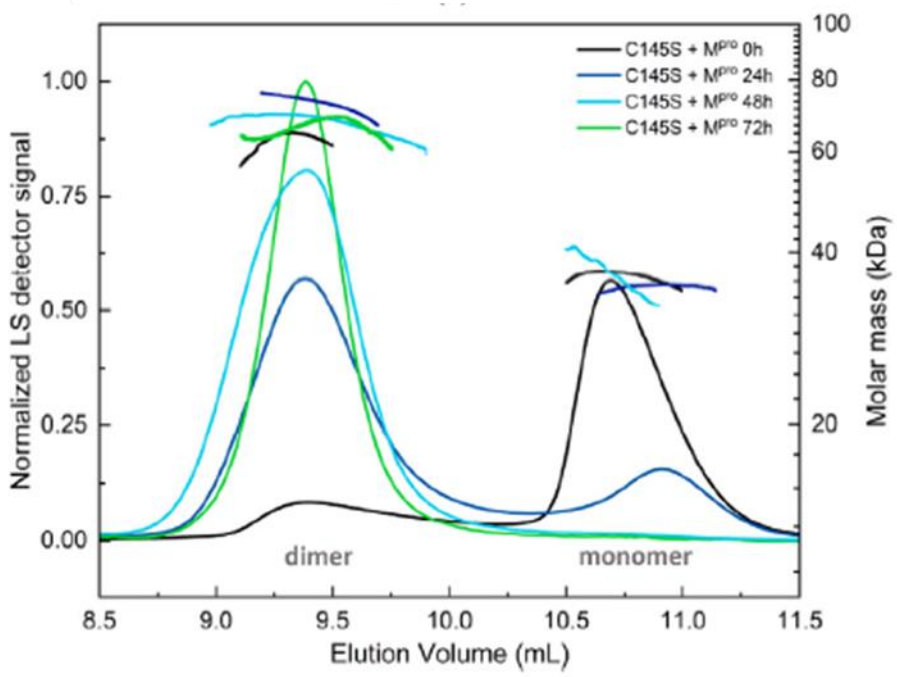


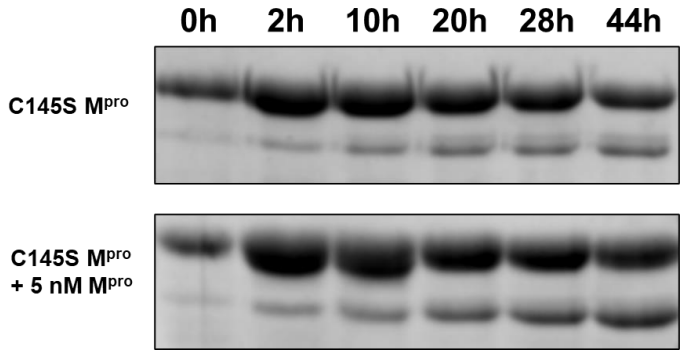


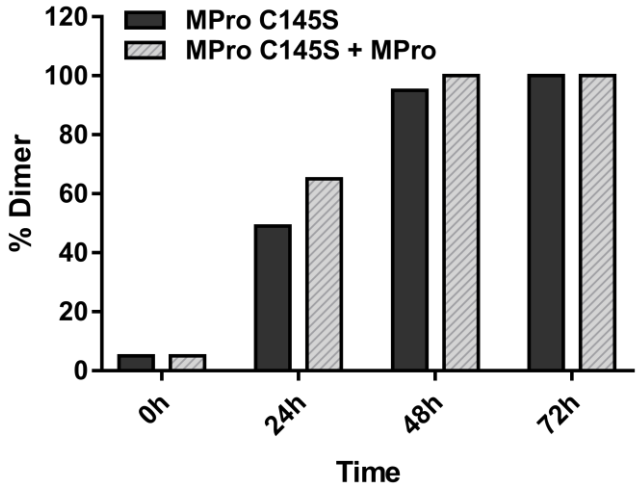
Adapted from NOSKE, G.D. et al, JMB, 2021

C145S M^{pro} self-cleavage analysis



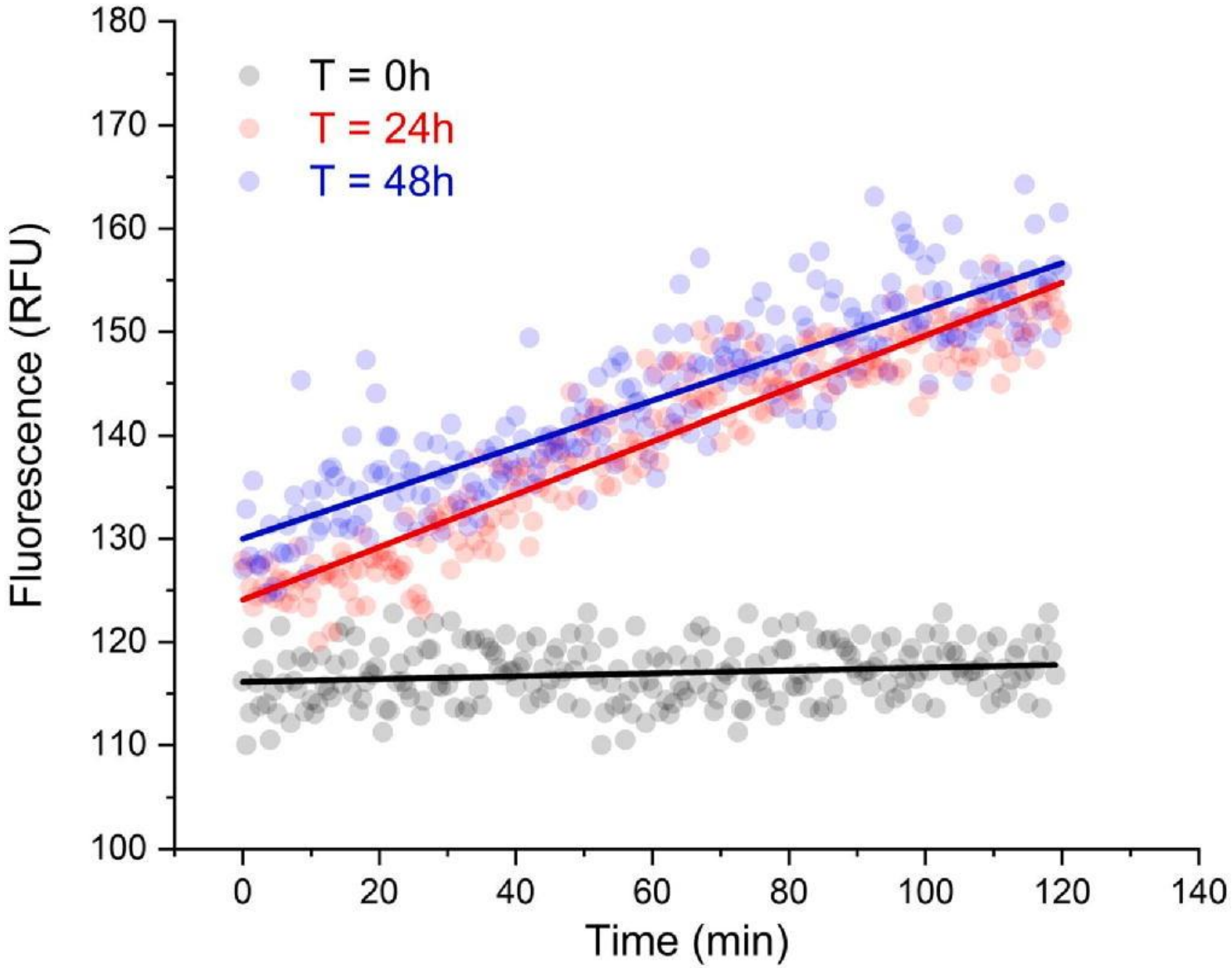




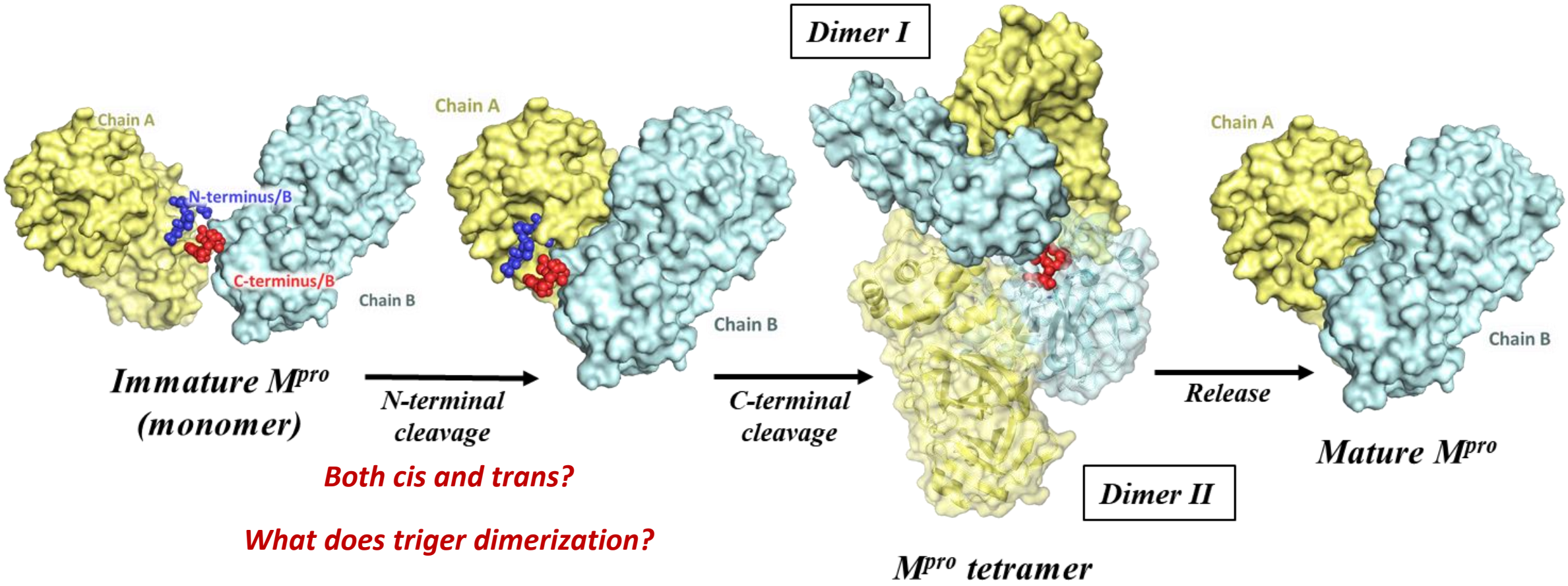


Adapted from NOSKE, G.D. et al, JMB, 2021

C145S M^{pro} self-cleavage analysis



Model for M^{pro} maturation process



Both cis and trans?

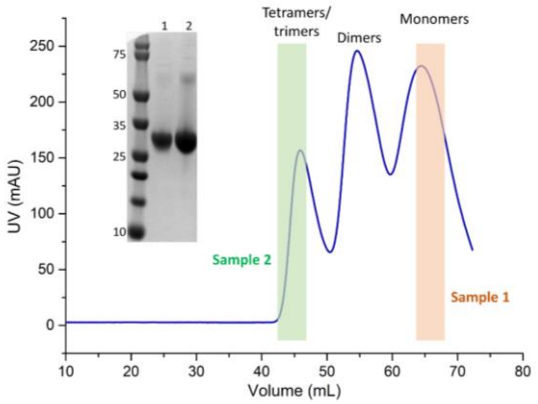
What does triger dimerization?

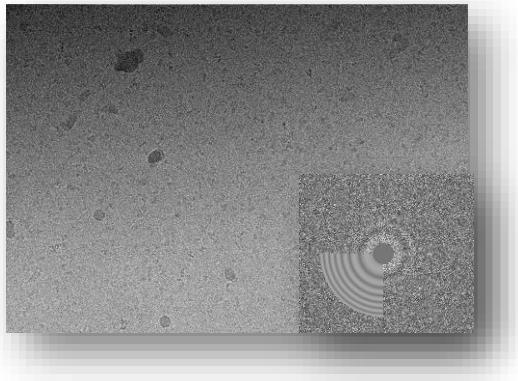
Adapted from NOSKE, G.D. et al, JMB, 2021

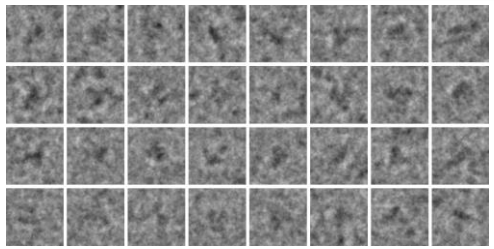
A Crystallographic Snapshot of SARS-CoV-2 Main Protease Maturation Process

j.jmb.2021.167118

Cryo-EM structure of C145S M^{pro}





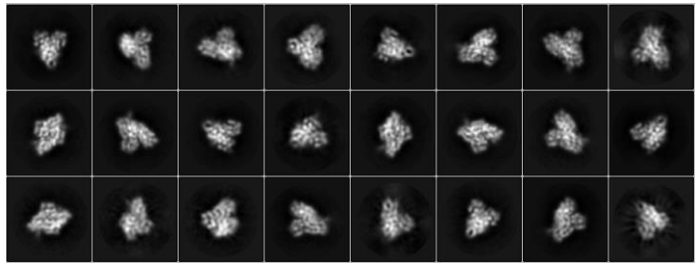


Import 13,770 Movies in Relion v3.1

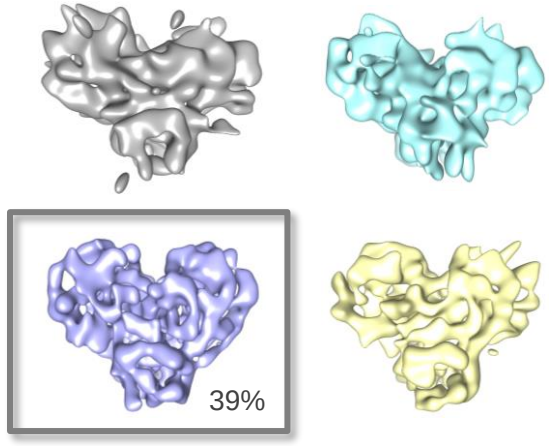
MotionCor2, CTFFIND v4.1

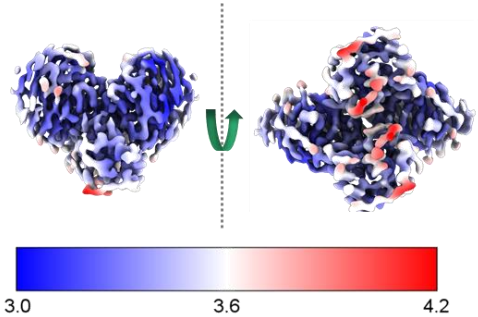
Particle picking crYOLO (1,956,496 particles)

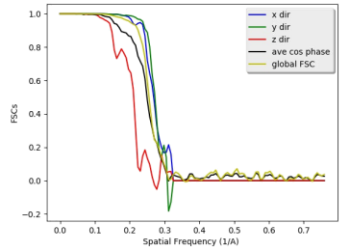
2D Classification (3 rounds)



Initial model and 3D Classification (569,725 particles)







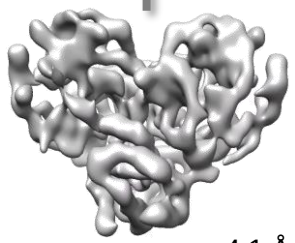
Local refinement (C2 symmetry)
3.5 Å

Non-uniform refinement (C2 symmetry)
~3.6 Å

Homogeneous refinement (C2 symmetry)
~3.8 Å

imported in cryoSPARC v3.2.0

CTF Refine
Bayesian polishing



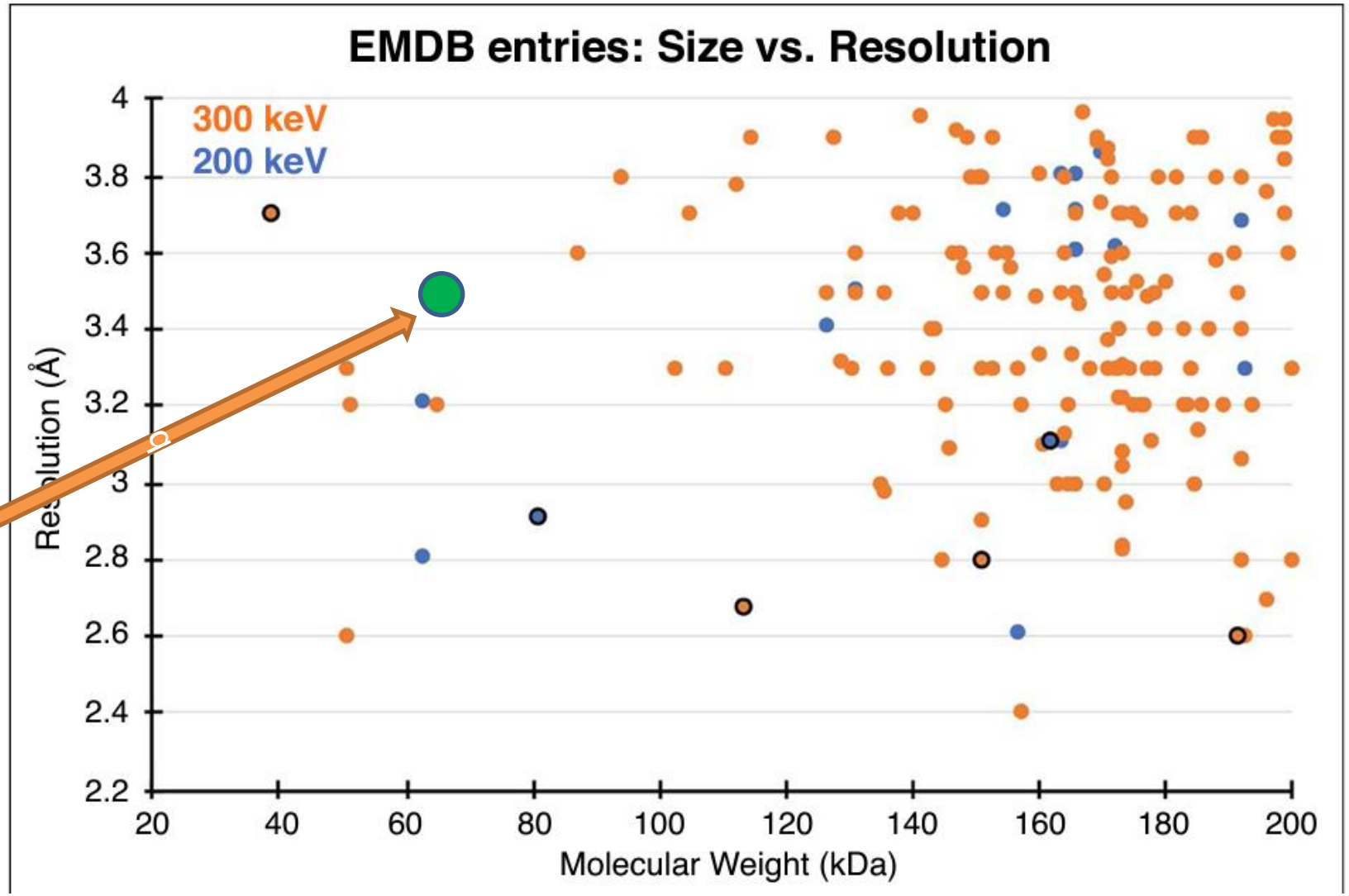
~4.1 Å

Refine3D (227,534 particles)

Adapted from NOSKE, G.D. et al, Nat. Commun, 2023

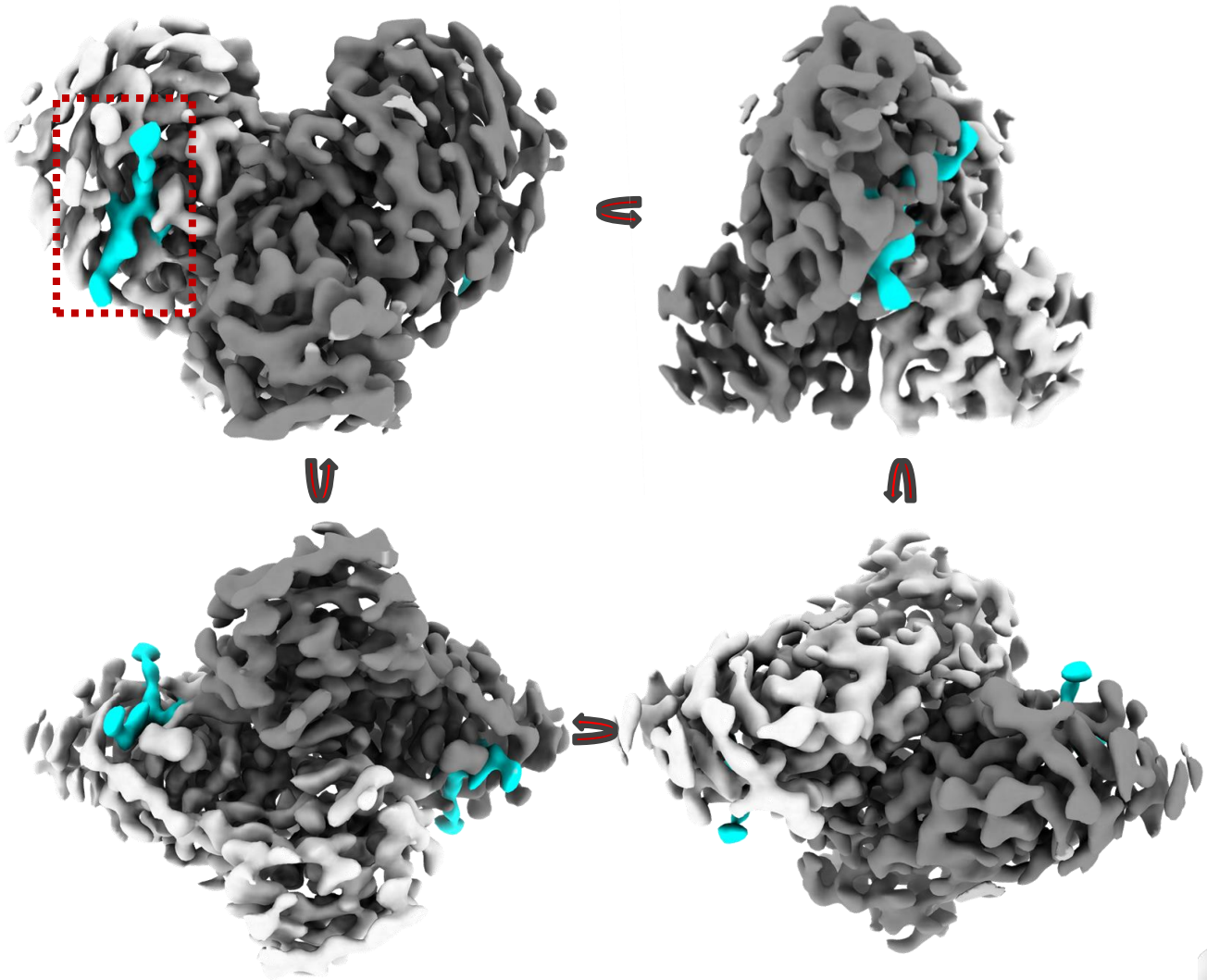
Cryo-EM of M^{pro} - Only 68 kDa at 3.5 A !!

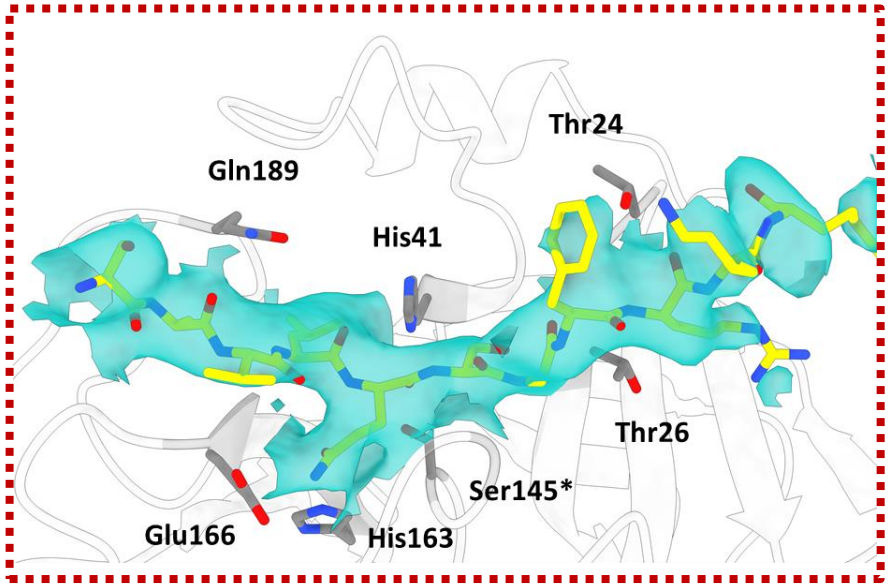
SARS-CoV-2 main protease@CIBFAR

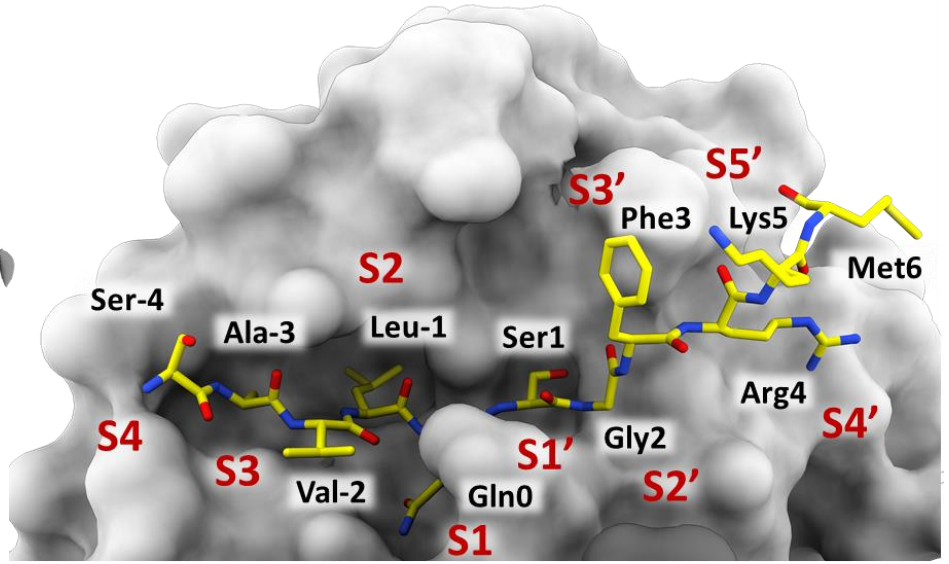


Current Opinion in Structural Biology 2020, 64:9–16

Cryo-EM structure of C145S M^{pro}

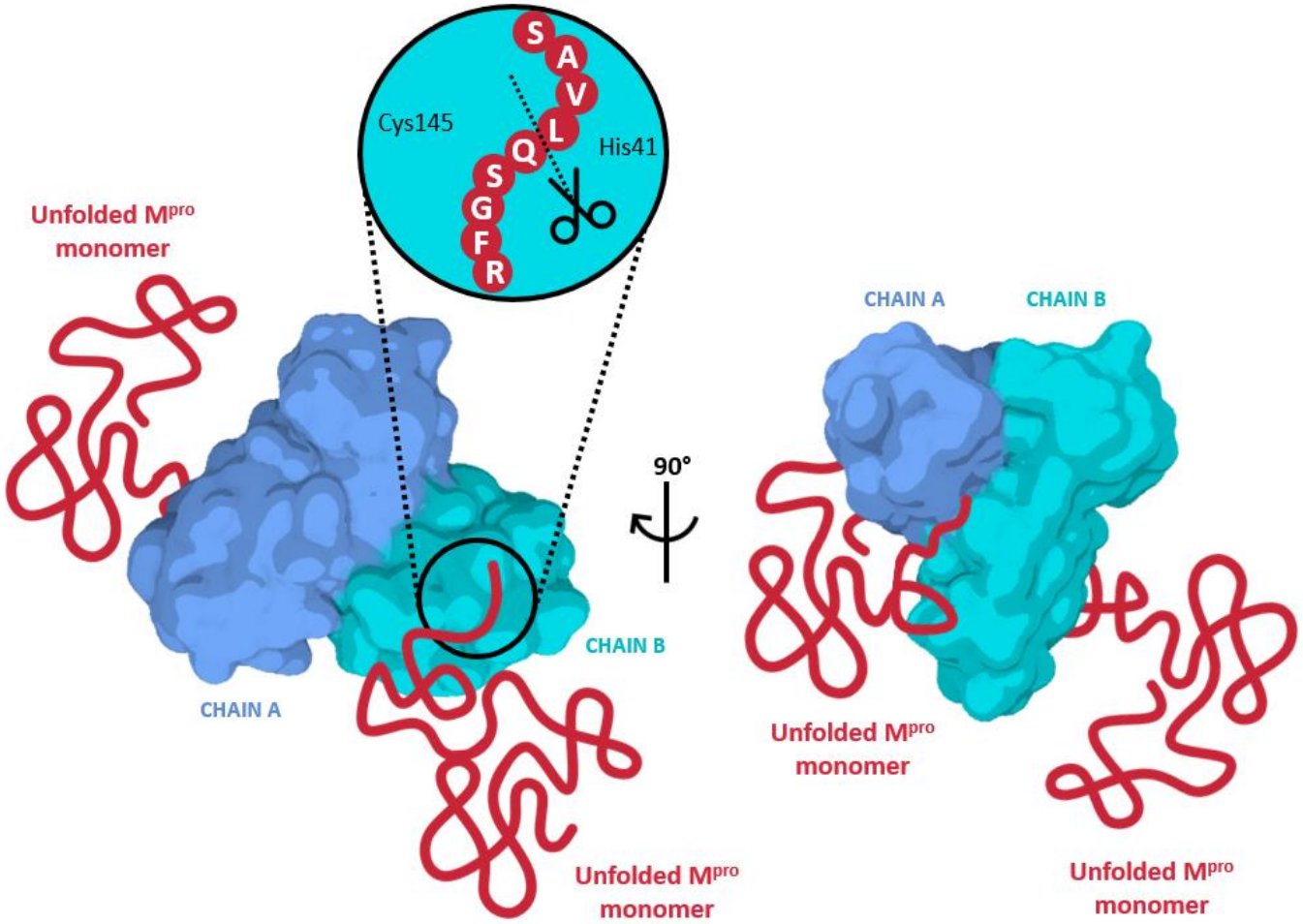


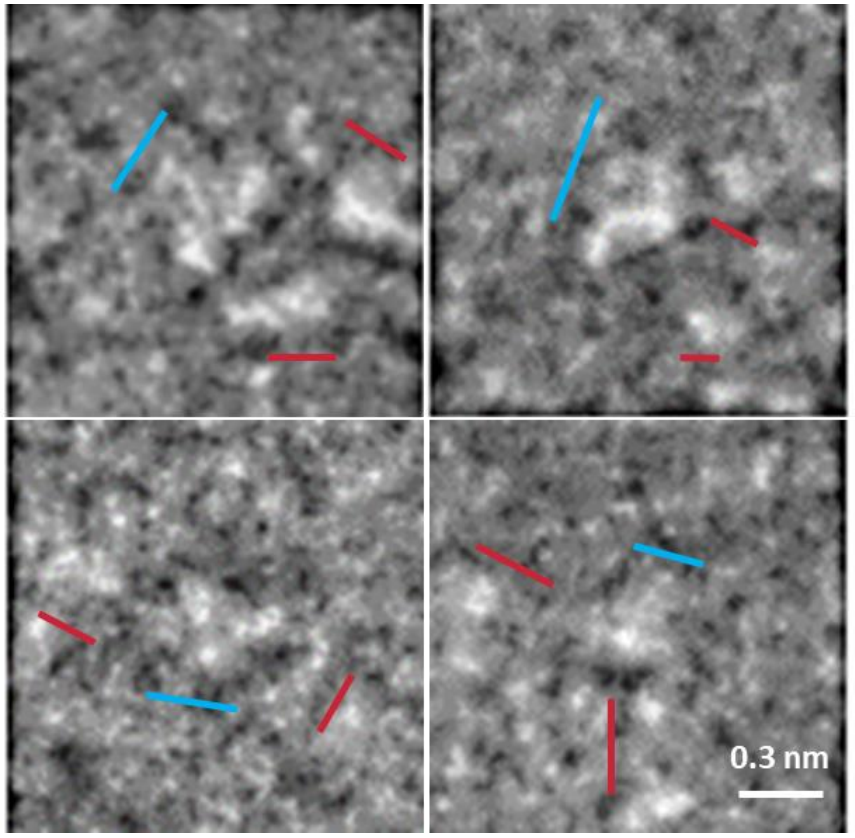




Adapted from NOSKE, G.D. et al, Nat. Commun, 2023

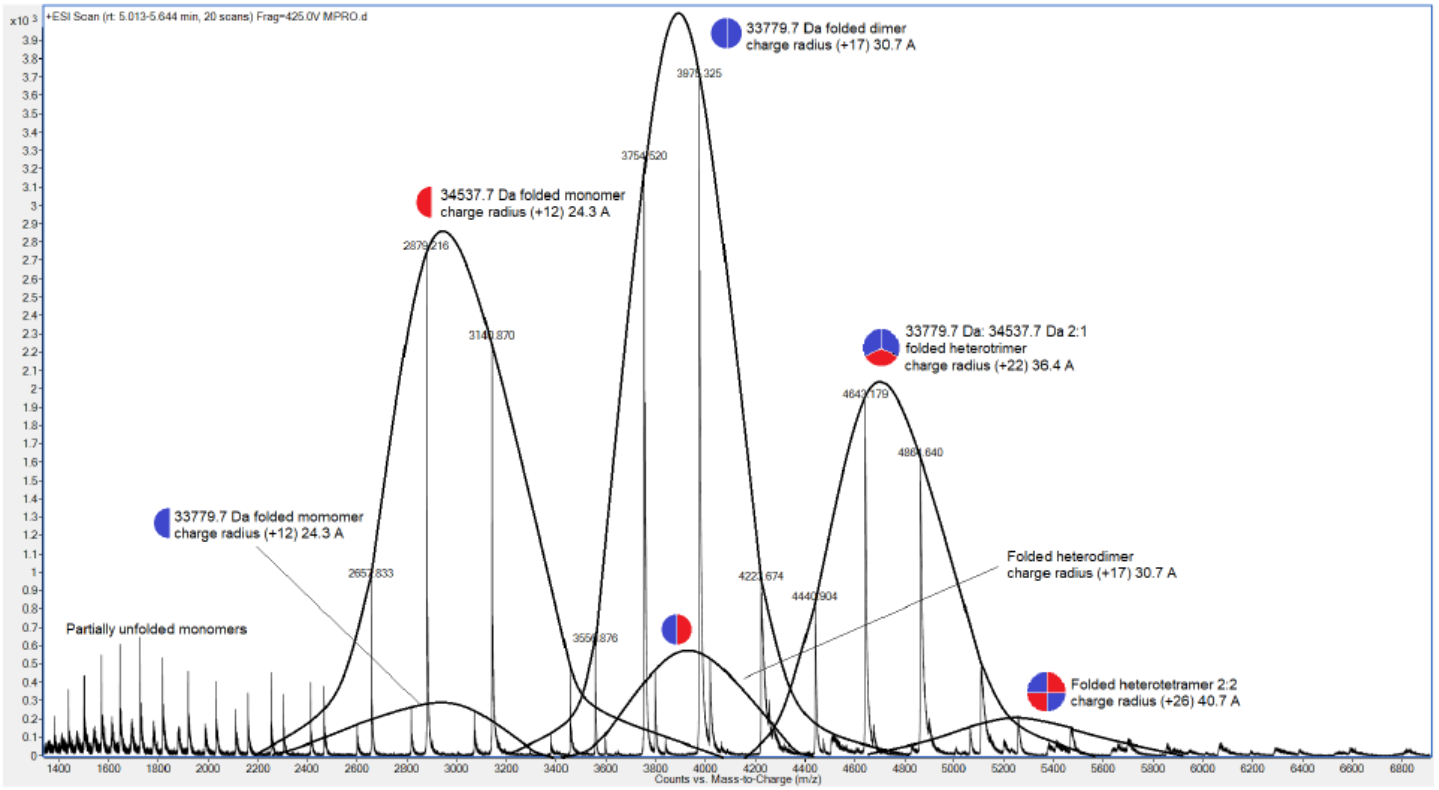
Cryo-EM structure of C145S M^{pro}

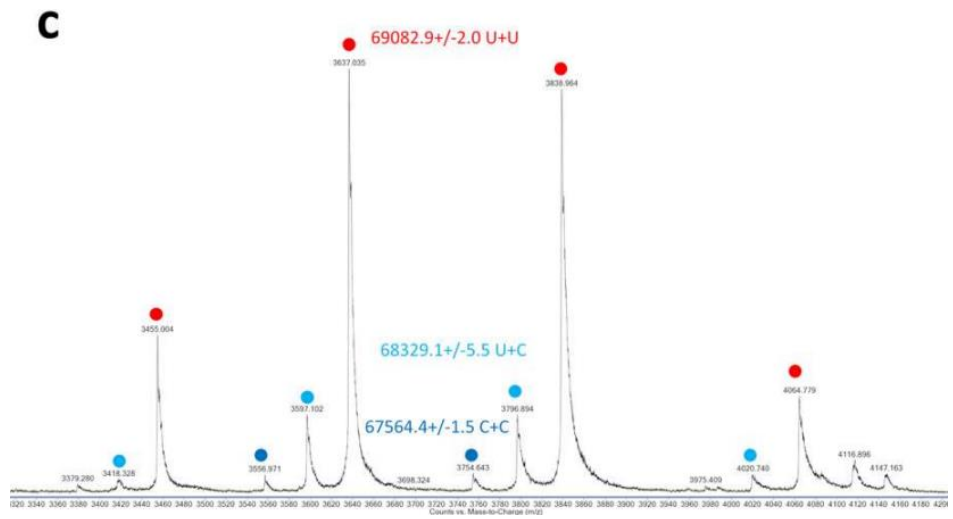


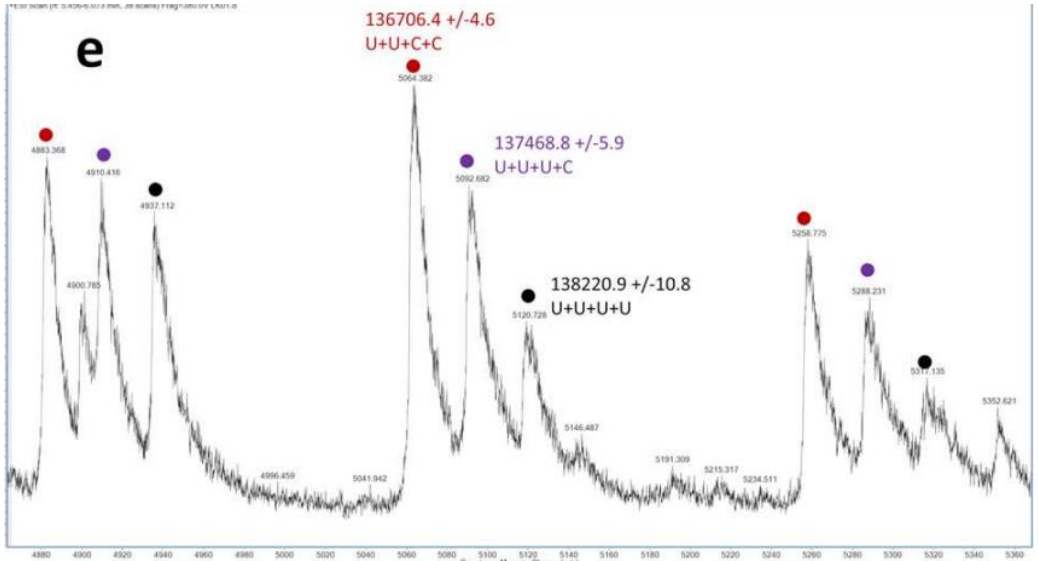


Adapted from NOSKE, G.D. et al, Nat. Commun, 2023

Native MS - C145S M^{pro}

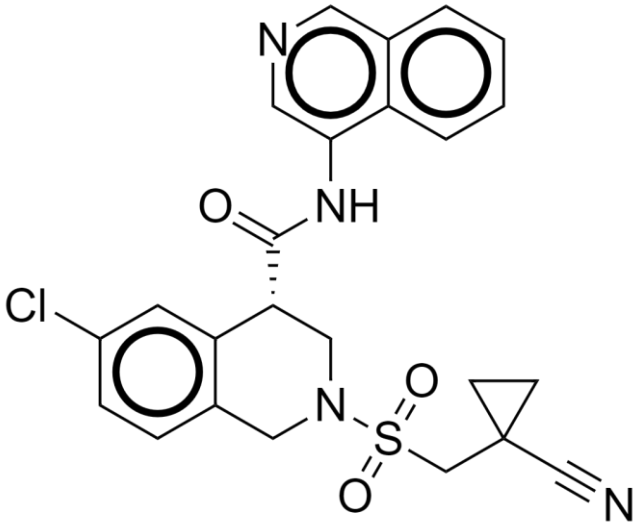




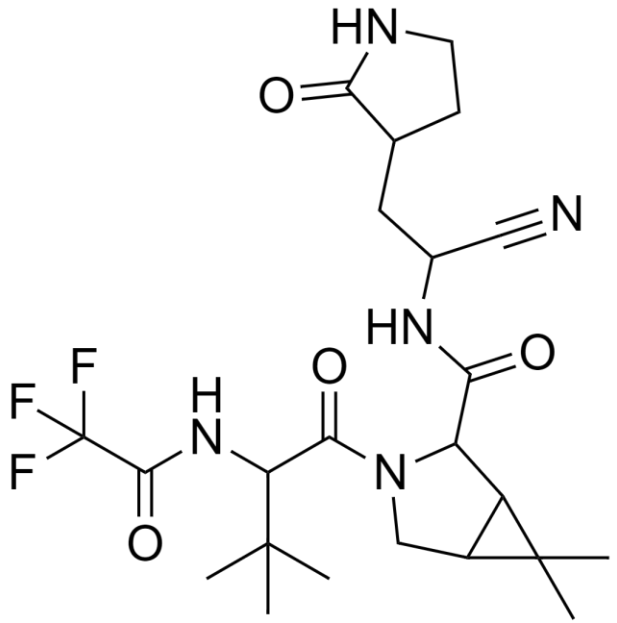


NOSKE, G.D. et al, Nat. Commun, 2023

Inhibitor effects in oligomerization



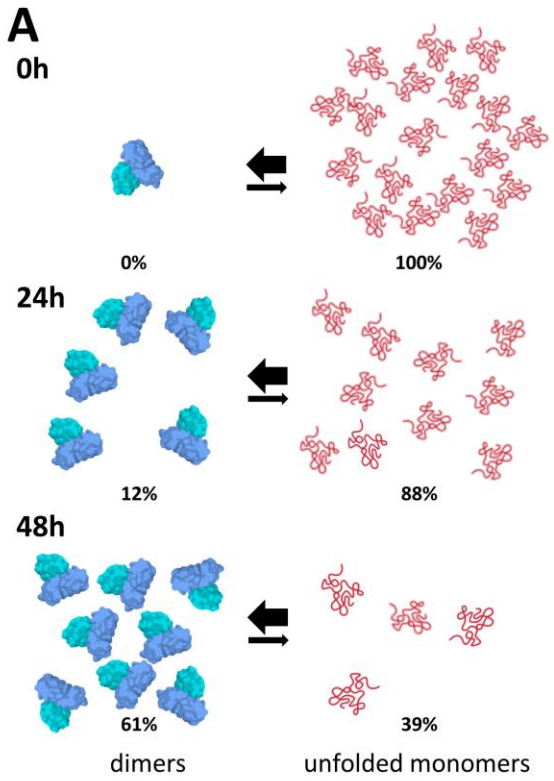
MAT-POS-e194df51-1
Developed by The Moonshot Consortium
pIC50 7.5
Non-covalent binding mode



Nirmatrelvir
Developed by Pfizer
pIC50 7.7
Covalent binding mode

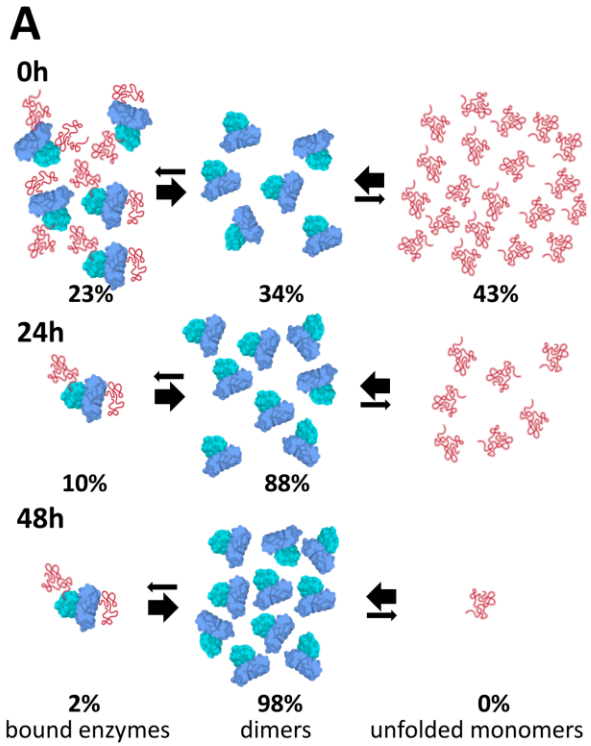
Native MS - C145S M^{pro}

C145S M^{pro} monomers

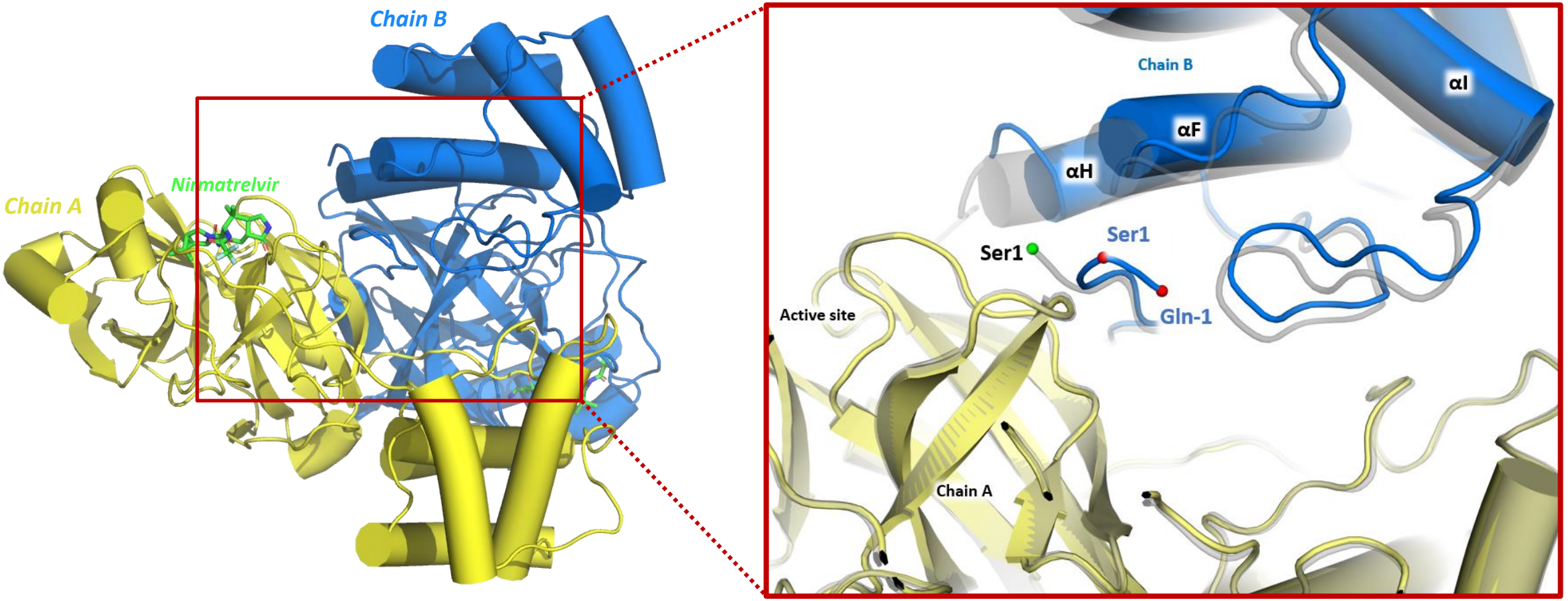


Native MS - C145S M^{pro}

C145S M^{pro} tetramers

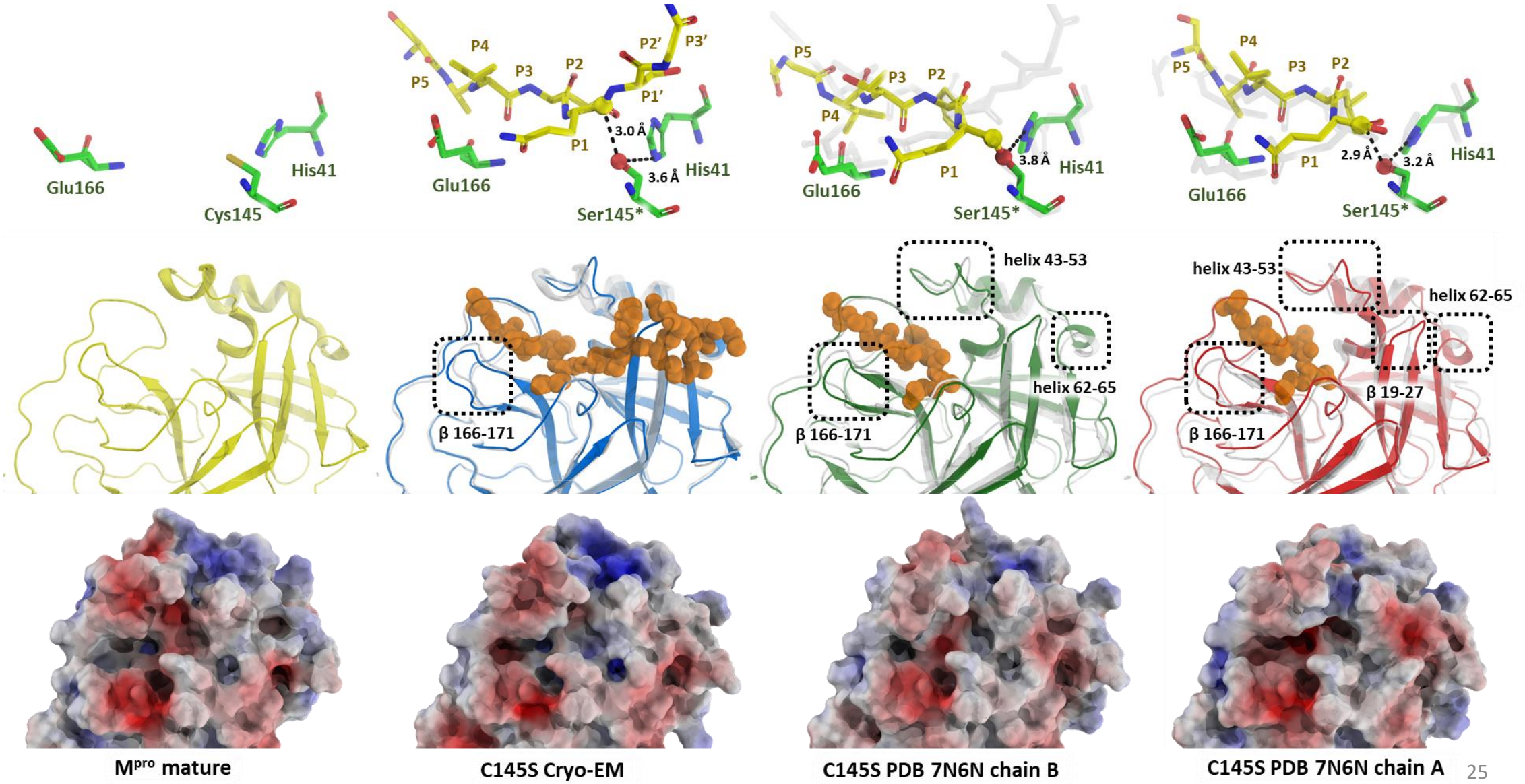


Crystal Structure of M^{pro} in complex with nirmatrelvir



Adapted from NOSKE, G.D. et al, Nat. Commun, 2023

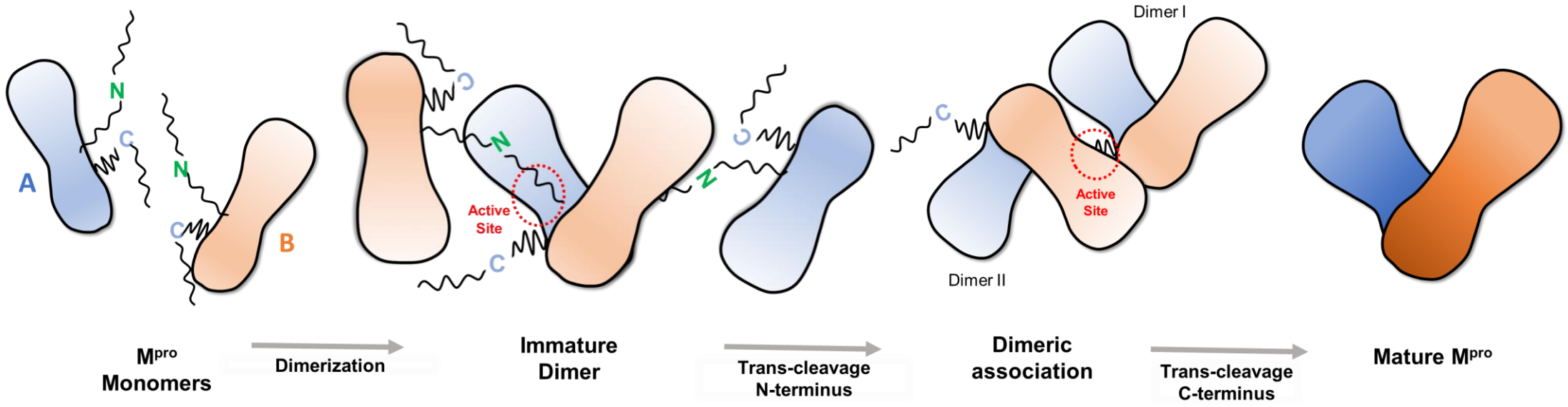
Overview of M^{pro} cleavage process



NOSKE, G.D. et al, Nat. Commun, 2023

Conclusions and perspectives

- N-terminal cleavage is **NOT** critical for dimerization
- Dimerization is induced by covalent linkage
- Multiple oligomeric states can co-exist and act both cis and trans during maturation
- Structural information can guide the development of a new generation of M^{pro} inhibitors targeting intermediate steps of the maturation process



Adapted from NOSKE, G.D. et al, JMB, 2021

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An in-solution snapshot of SARS-COV-2 main protease maturation process and inhibition

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Check for updates

Gabriela Dias Noske[1,6], Yun Song[2,6], Rafaela Sachetto Fernandes[1], Rod Chalk[3], Haitem Elmassoudi[3], Lizbé Koekemoer[3], C. David Owen[2], Tarick J. El-Baba[4,5], Carol V. Robinson[4,5], The COVID Moonshot Consortium*, Glaucius Oliva[1] & Andre Schutzer Godoy[1]

The main protease from SARS-CoV-2 (M^{pro}) is responsible for cleavage of the viral polyprotein. M^{pro} self-processing is called maturation, and it is crucial for enzyme dimerization and activity. Here we use C145S M^{pro} to study the structure and dynamics of N-terminal cleavage in solution. Native mass spectroscopy analysis shows that mixed oligomeric states are composed of cleaved and uncleaved particles, indicating that N-terminal processing is not critical for dimerization. A 3.5 Å cryo-EM structure provides details of M^{pro} N-terminal cleavage outside the constrains of crystal environment. We show that different classes of inhibitors shift the balance between oligomeric states. While non-covalent inhibitor MAT-POS-e194df51-1 prevents dimerization, the covalent inhibitor nirmatrelvir induces the conversion of monomers into dimers, even with intact N-termini. Our data indicates that the M^{pro} dimerization is triggered by induced fit due to covalent linkage during substrate processing rather than the N-terminal processing.