

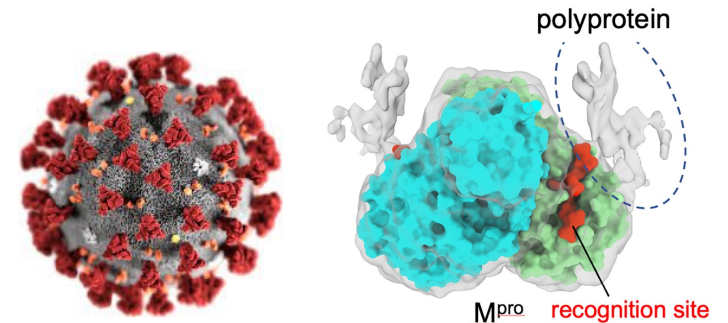
PENNSSTATE



# Cryo-EM Study of the SARS-CoV-2 Polyprotein Cleavage by the Main Protease M<sup>pro</sup>

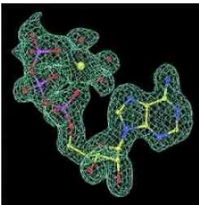
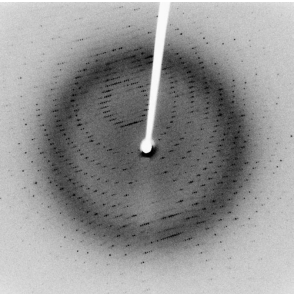
**Katsu Murakami**

Biochemistry and Molecular Biology  
Huck Center for RNA Molecular Biology  
Huck Center for Structural Biology  
Pennsylvania State University  
(<http://rnalab.org>)



Frederick National Laboratory Advisory Committee (FNLAC) meeting, 10/19/2023

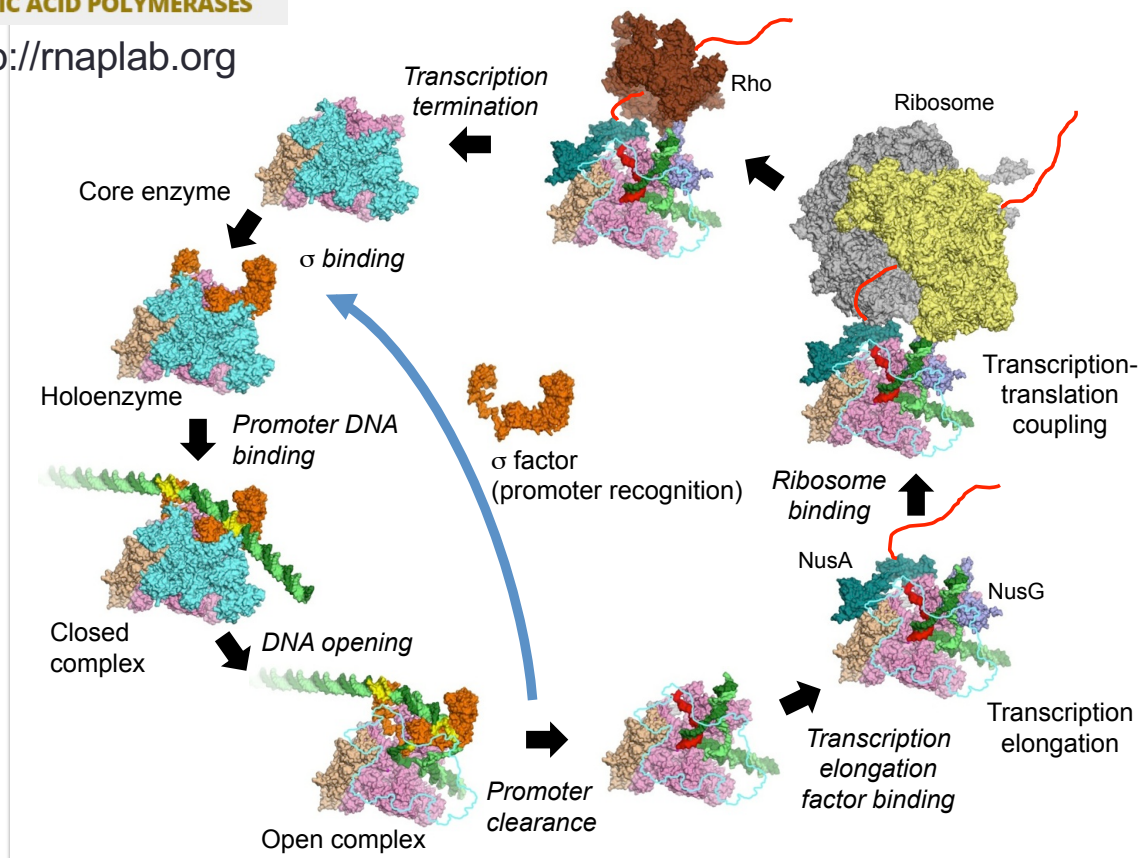
X-ray crystallography



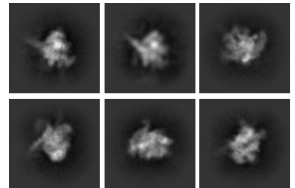
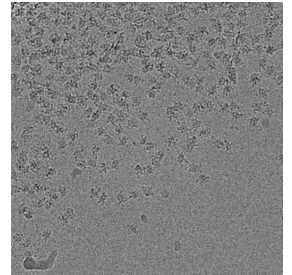
**MURAKAMI  
LABORATORY**  
STRUCTURAL BIOLOGY OF  
NUCLEIC ACID POLYMERASES

<http://rnalab.org>

## Transcription cycle



Cryo-EM

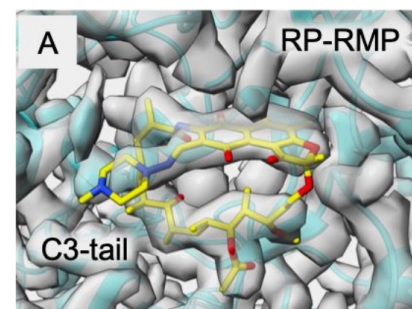


Qayyum, Shin and Murakami, *Encycl Biol Chem* 2020

# Optimization of Benzoxazinorifamycins to Improve *Mycobacterium tuberculosis* RNA Polymerase Inhibition and Treatment of Tuberculosis

Walajapet Rajeswaran, Shireen R. Ashkar, Pil H. Lee, Larisa Yeomans, Yeonoh Shin, Scott G. Franzblau, Katsuhiko S. Murakami, Hollis D. Showalter, and George A. Garcia\*

Cite This: *ACS Infect. Dis.* 2022, 8, 1422–1438 [Read Online](#)



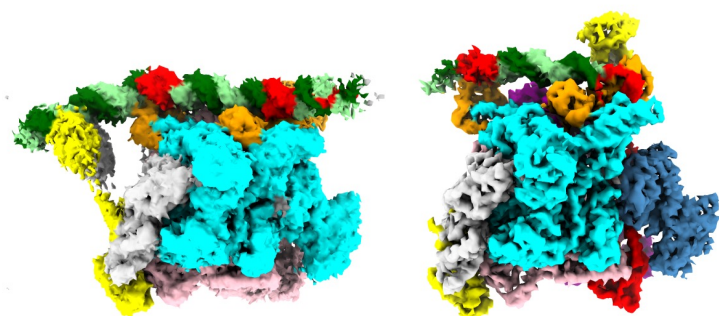
[Check for updates](#)

ARTICLE

<https://doi.org/10.1038/s41467-020-20776-y> **OPEN**

## Structural basis of ribosomal RNA transcription regulation

Yeonoh Shin <sup>1,3</sup>, M. Zuhaib Qayyum<sup>1,3</sup>, Danil Pupov <sup>2</sup>, Daria Esyunina<sup>2</sup>, Andrey Kulbachinskiy <sup>2</sup> & Katsuhiko S. Murakami <sup>1</sup>✉



**JBC RESEARCH ARTICLE**



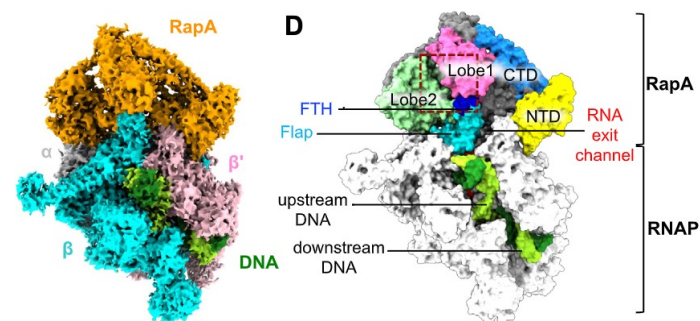
## Structural basis of RNA polymerase recycling by the Swi2/Snf2 family of ATPase RapA in *Escherichia coli*

Received for publication, May 5, 2021, and in revised form, November 2, 2021. Published, Papers in Press, November 12, 2021, <https://doi.org/10.1016/j.jbc.2021.101404>

M. Zuhaib Qayyum , Vadim Molodtsov, Andrew Renda, and Katsuhiko S. Murakami\* 

From the Department of Biochemistry and Molecular Biology, The Center for RNA Molecular Biology, The Center for Structural Biology, Pennsylvania State University, University Park, Pennsylvania, USA

Edited by Karin Musier-Forsyth





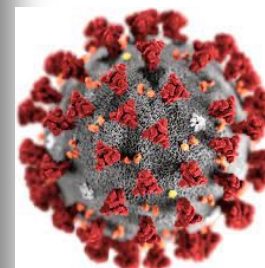


# SARS-CoV-2 polyprotein substrate regulates the stepwise M<sup>pro</sup> cleavage reaction

Received for publication, February 15, 2023, and in revised form, March 22, 2023. Published, Papers in Press, April 10, 2023, <https://doi.org/10.1016/j.jbc.2023.104697>

Manju Narwal<sup>1</sup>, Jean-Paul Armache<sup>1,2,\*</sup>, Thomas J. Edwards<sup>3</sup>, and Katsuhiko S. Murakami<sup>1,2,4,\*</sup>

From the <sup>1</sup>Department of Biochemistry and Molecular Biology, and <sup>2</sup>Center for Structural Biology, Huck Institute of the Life Sciences, Pennsylvania State University, University Park, Pennsylvania, USA; <sup>3</sup>National Cryo-EM Facility, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research Inc, Frederick, Maryland, USA; <sup>4</sup>Center for RNA Molecular Biology, Pennsylvania State University, University Park, Pennsylvania, USA



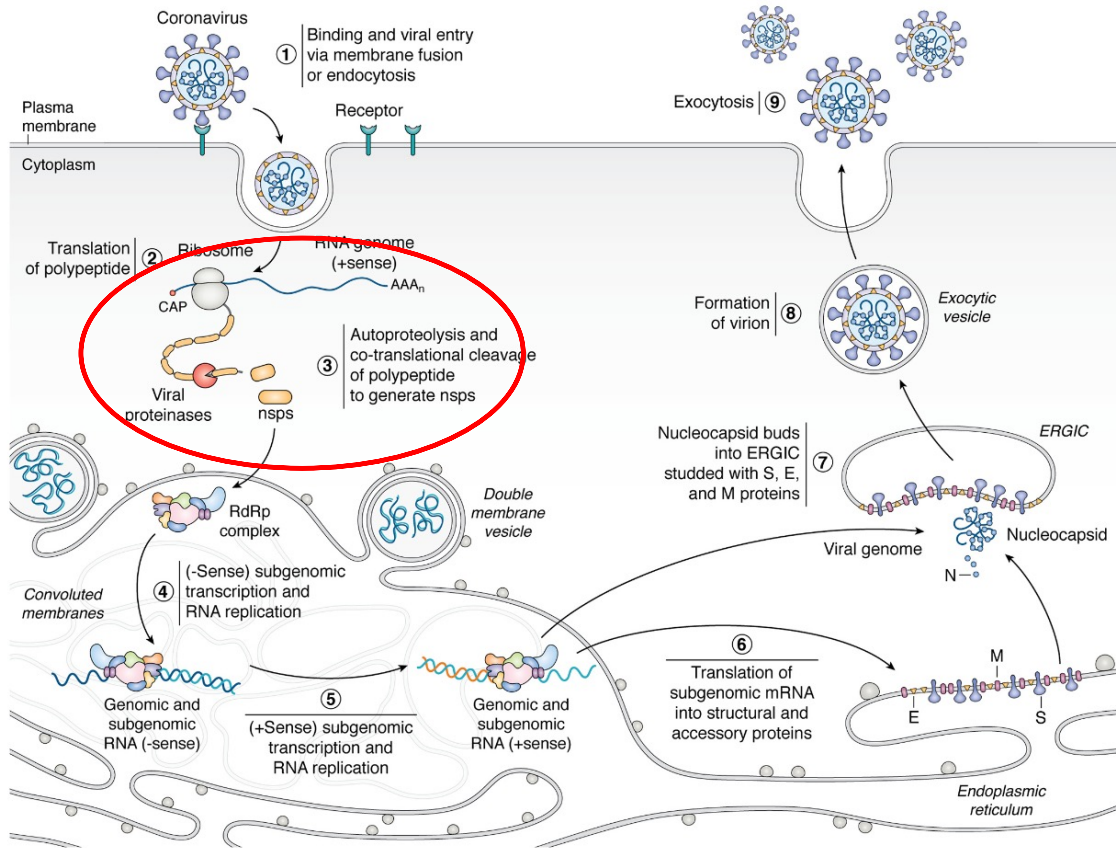
PENNSSTATE



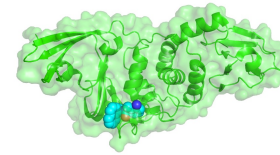
Frederick  
National Laboratory  
for Cancer Research



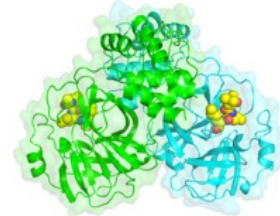
**JBC REVIEWS: The molecular virology of coronaviruses**



papain-like protease (PL<sup>pro</sup>)

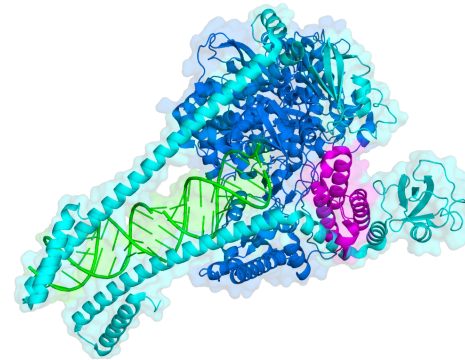


Main protease (M<sup>pro</sup>)



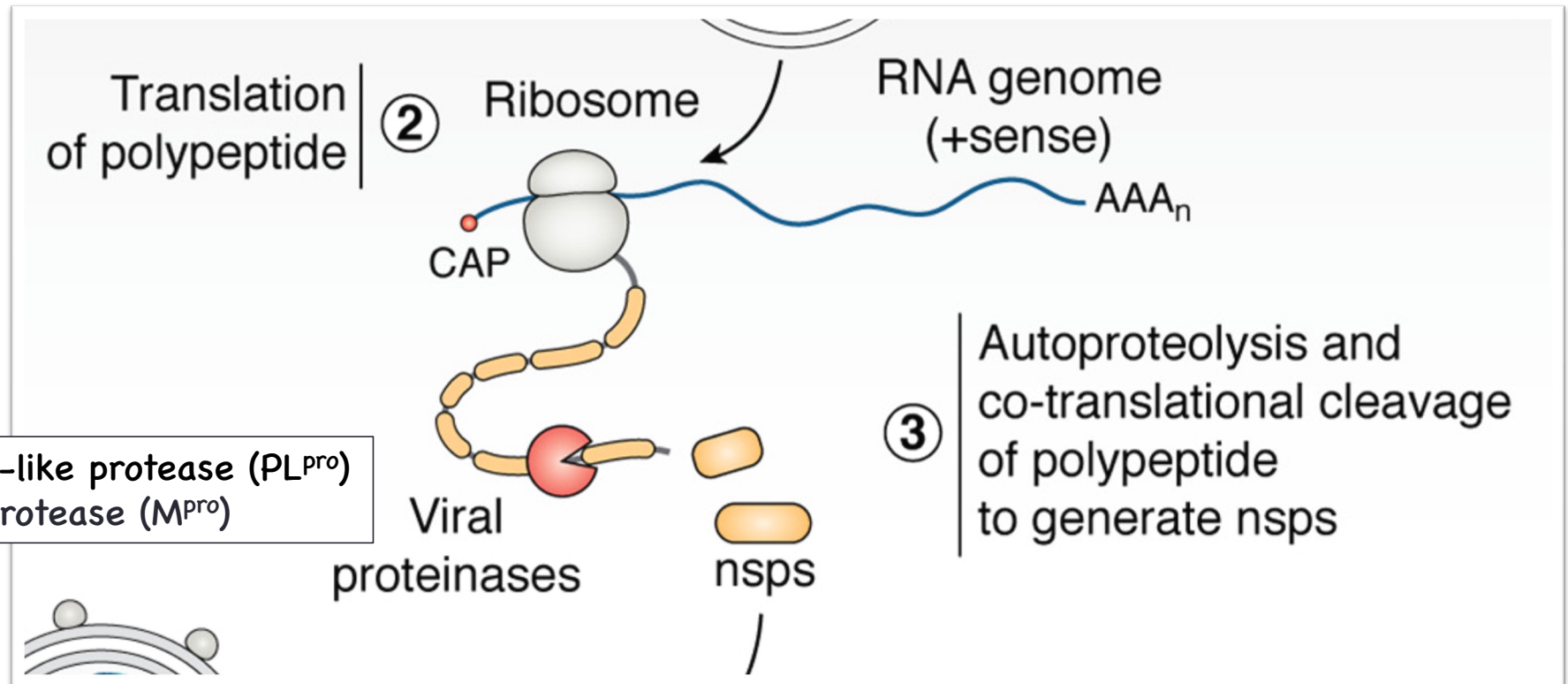
PAXLOVID

RNA-dependent RNA polymerase (RdRp)



REMDESIVIR  
MOLNUPIRAVIR

# Polyprotein processing by viral proteases



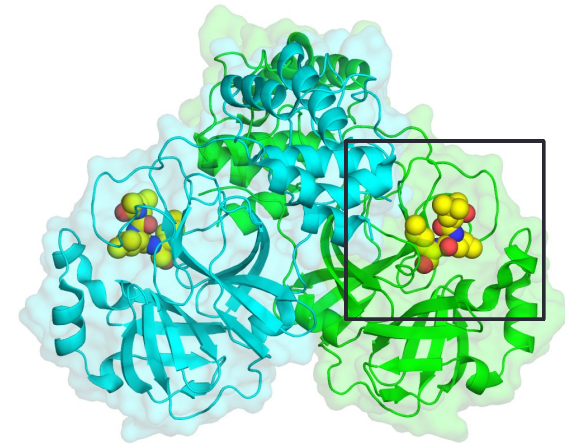
Hartenian, E. *et al.* The molecular virology of coronaviruses. *J Biol Chem* **295**, 12910–12934 (2020)

# SARS-CoV-2 Main protease M<sup>pro</sup>

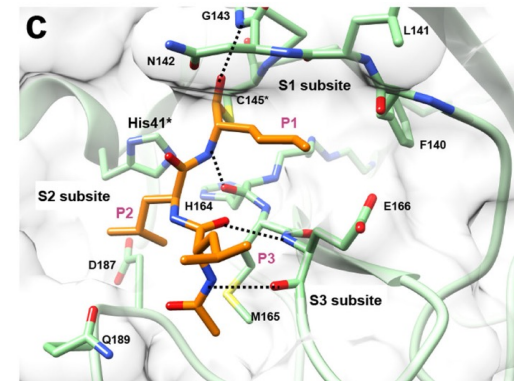
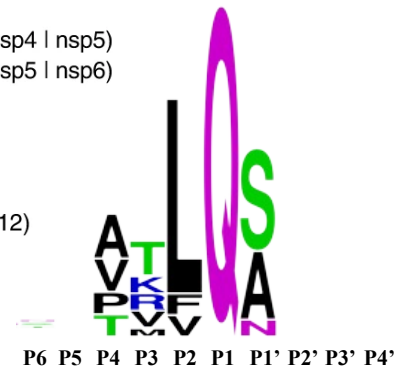
PDB: 7LBN

Narayanan *et al. Commun Biology* (2022)

- Chymotrypsin-like/3C-like protease (3CL<sup>pro</sup>)
- Homodimer
- *cis* and *trans* proteolytic activity
- Catalytic dyad: Cys145 and His41
- PAXLOVID: M<sup>pro</sup> inhibitor for COVID\_19 treatment
- Consensus Gln (Q) at substrate P1 position



	N-	P6	P5	P4	P3	P2	P1	∞	P1'	P2'	P3'	P4'	P5'	P6'	-C	
C4	T	S	A	V	L	Q		∞	S	G	F	R	K	M		(N-terminal auto-cleavage, nsp4   nsp5)
C5	S	G	V	T	F	Q			S	A	V	K	R	T		(C-terminal auto-cleavage, nsp5   nsp6)
C6	K	V	A	T	V	Q			S	K	M	S	D	V		(nsp6   nsp7)
C7	N	R	A	T	L	Q			A	I	A	S	E	F		(nsp7   nsp8)
C8	S	A	V	K	L	Q			N	N	E	L	S	P		(nsp8   nsp9)
C9	A	T	V	R	L	Q			A	G	N	A	T	E		(nsp9   nsp10)
C10	R	E	P	M	L	Q			S	A	D	A	Q	S		(nsp10   nsp11, nsp10   nsp12)
C12	P	H	T	V	L	Q			A	V	G	A	C	V		(nsp12   nsp13)
C13	N	V	A	T	L	Q			A	E	N	V	T	G		(nsp13   nsp14)
C14	T	F	T	R	L	Q			S	L	E	N	V	A		(nsp14   nsp15)
C15	F	Y	P	K	L	Q			S	S	Q	A	W	Q		(nsp15   nsp16)





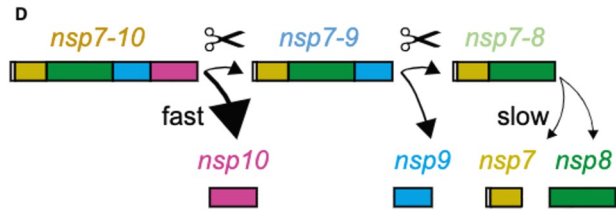
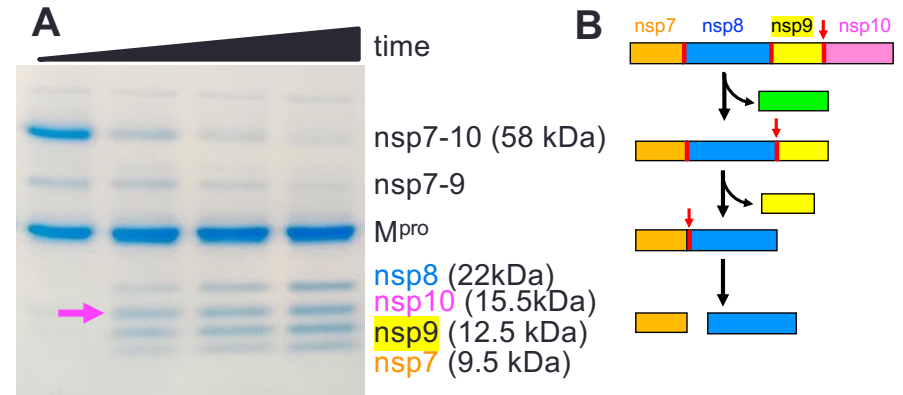
Research Article

# Processing of the SARS-CoV pp1a/ab nsp7–10 region

Boris Krichel<sup>1</sup>, Sven Falke<sup>2</sup>, Rolf Hilgenfeld<sup>3,4</sup>, Lars Redecke<sup>3,5</sup> and Charlotte Uetrecht<sup>1,6</sup>

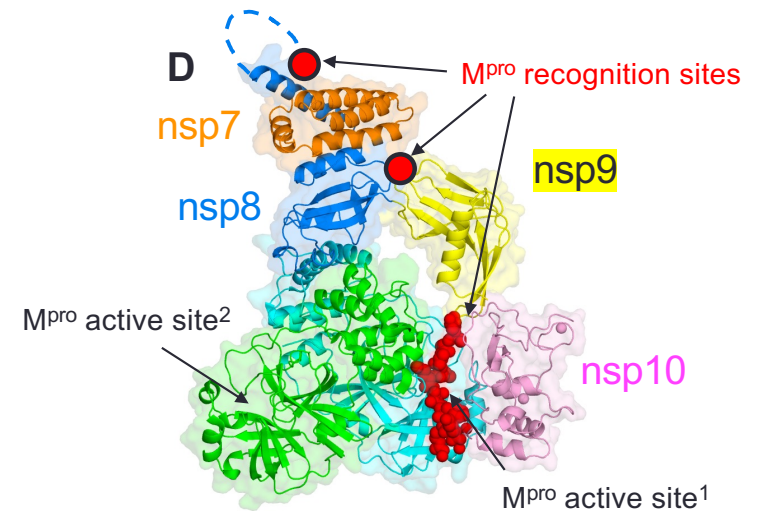
<sup>1</sup>Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Martinstraße 52, 20251 Hamburg, Germany; <sup>2</sup>University of Hamburg, Institut für Biochemie und Molekularbiologie, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany; <sup>3</sup>University of Lübeck, Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, Ratzeburger Allee 160, 23562 Lübeck, Germany; <sup>4</sup>German Center for Infection Research (DZIF), Hamburg-Lübeck-Borstel-Riems Site, University of Lübeck, Ratzeburger Allee 160, 23562 Lübeck, Germany; <sup>5</sup>Deutsches Elektronen Synchrotron (DESY), Notkestraße 85, 22607 Hamburg, Germany; <sup>6</sup>European XFEL GmbH, Holzkoppel 4, 22869 Schenefeld, Germany

Correspondence: Charlotte Uetrecht (charlotte.uetrecht@xfel.eu)



**Figure 3. SARS-CoV nsp7–10 processing monitored by native MS: signal over time of protease, substrate and products.**

SARS-CoV nsp7–10 processing monitored by native MS: signal over time of protease, substrate and products. (A) Substrate and enzyme. (B) Mass fractions over time show the order of nsp release. (C) Intermediate products. Error bars depict standard deviation ( $N = 3$ ). Time points (AVG  $\pm$  SD,  $N = 3$ ): 0.25  $\pm$  0.1 h, 1.2  $\pm$  0.1 h, 3.3  $\pm$  0.2 h, 5.3  $\pm$  0.3 h, 6.4  $\pm$  0.3 h and 20  $\pm$  0.5 h. (D) Schematic illustration of cleavage order and efficiency.



# No M<sup>pro</sup> structure studied by Cryo-EM

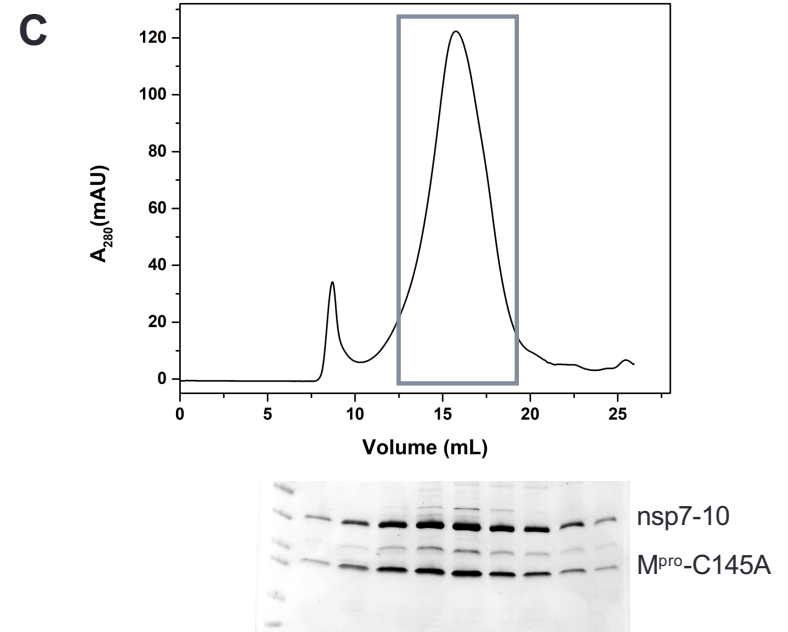
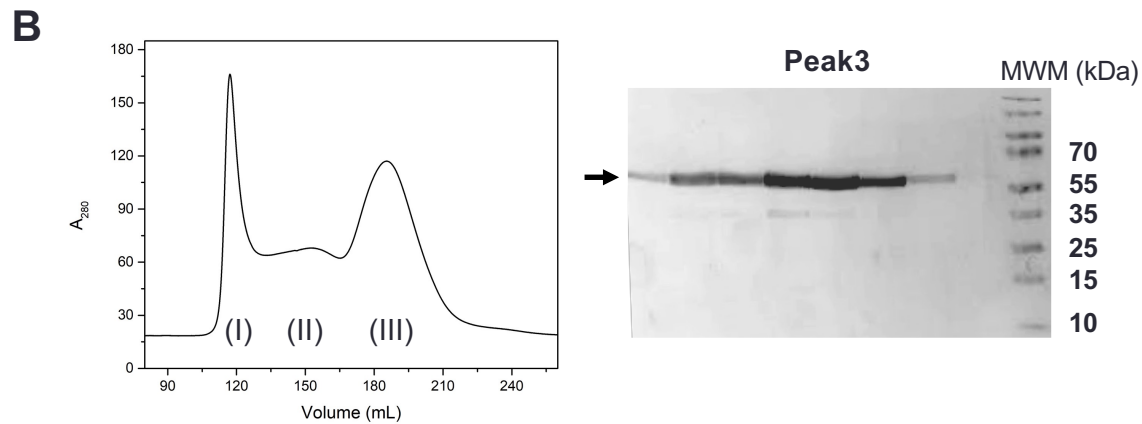
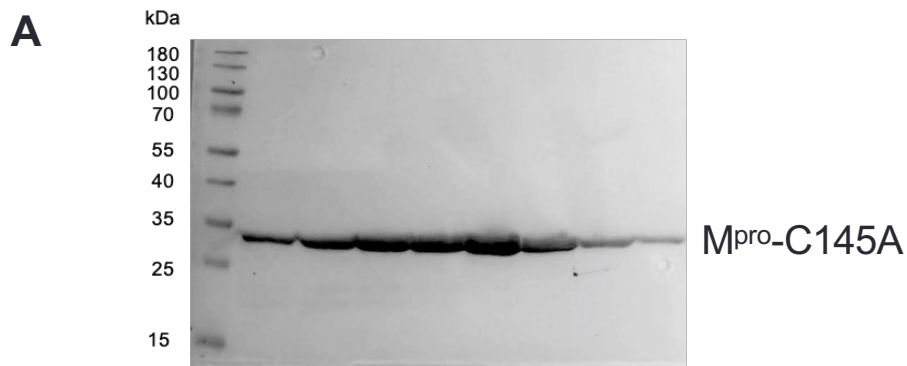
## COVID-19 / SARS-CoV-2

The first viral genome sequence: Jan 11, 2020

The first structure of M<sup>pro</sup>: Feb 05, 2020

The screenshot shows the RCSB PDB search results page. On the left, there are filter panels for 'Refinements', 'SCIENTIFIC NAME OF SOURCE ORGANISM', 'TAXONOMY', 'EXPERIMENTAL METHOD', 'POLYMER ENTITY TYPE', 'REFINEMENT RESOLUTION (Å)', and 'RELEASE DATE'. The 'EXPERIMENTAL METHOD' panel is circled in red, showing 'X-RAY DIFFRACTION (471)' and 'NEUTRON DIFFRACTION (4)'. The main search results area shows '1 to 25 of 471 Structures'. Two results are visible: 5R7Y and 5R7Z. Both are 'PanDDA analysis group deposition -- Crystal Structure of COVID-19 main protease in complex with Z45617795' and 'Crystal Structure of SARS-CoV-2 main protease in complex with Z1220452176' respectively. Both were released on 2020-03-11 and determined by X-RAY DIFFRACTION. Each result includes a 3D ribbon diagram of the protein structure and buttons for 'Download File' and 'View File'.

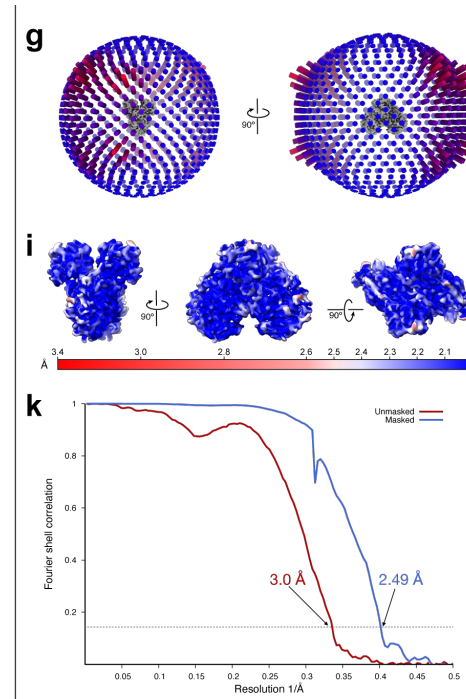
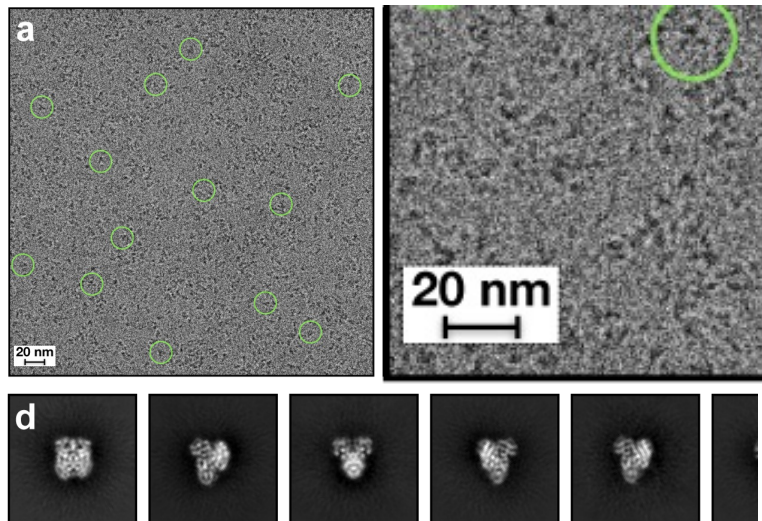
# Preparations of (A) $M^{\text{pro}}$ -C145A derivative (B) nsp7-10 polyprotein and (C) $M^{\text{pro}}$ /polyprotein complex



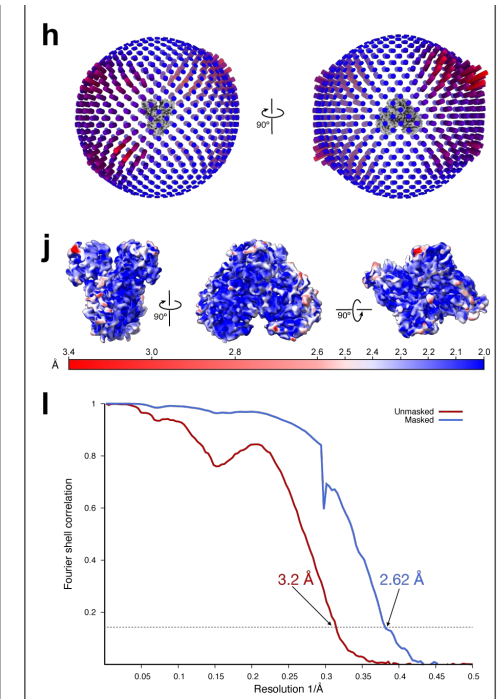
Narwal, Murakami *et al.*, *JBC* (2023)



# Cryo-EM structure of the polyprotein and M<sup>pro</sup> complex

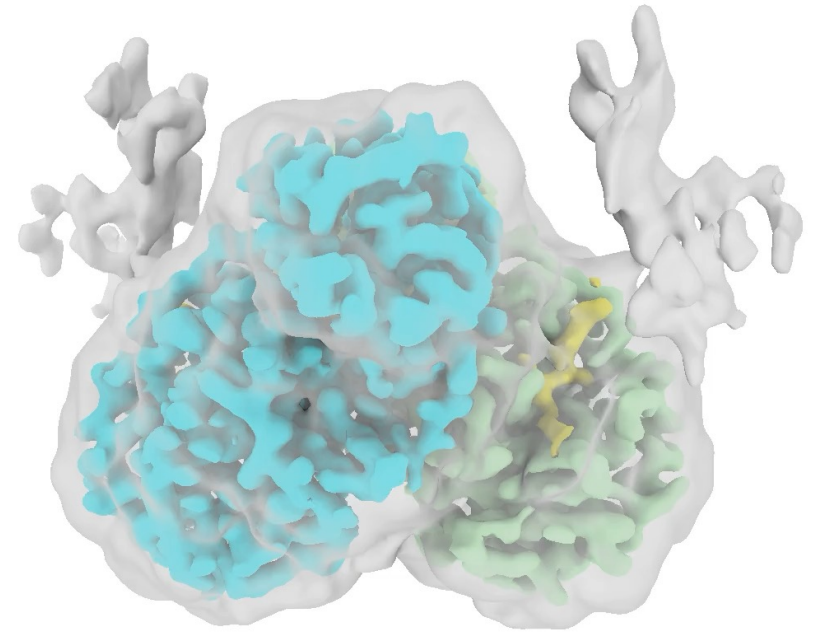
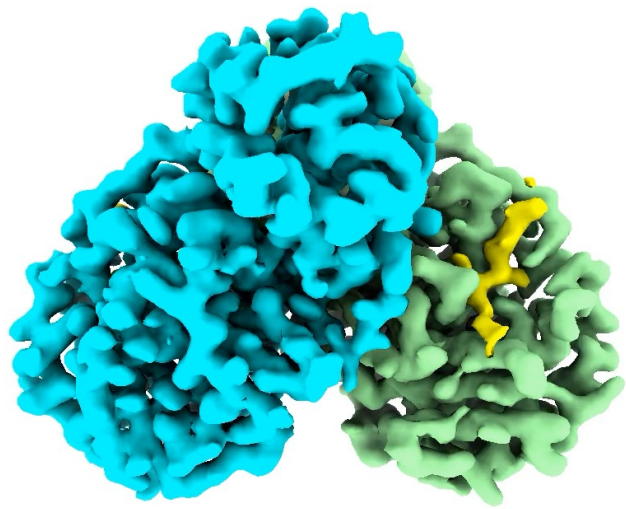


C2 symmetry

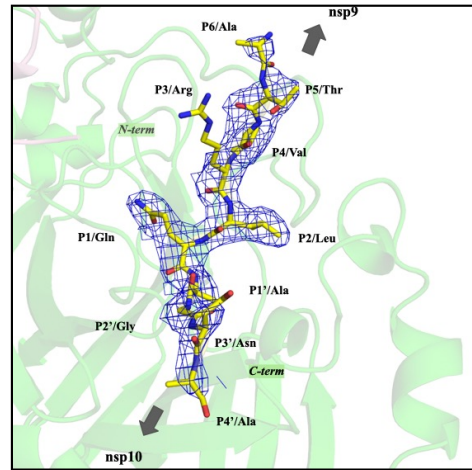
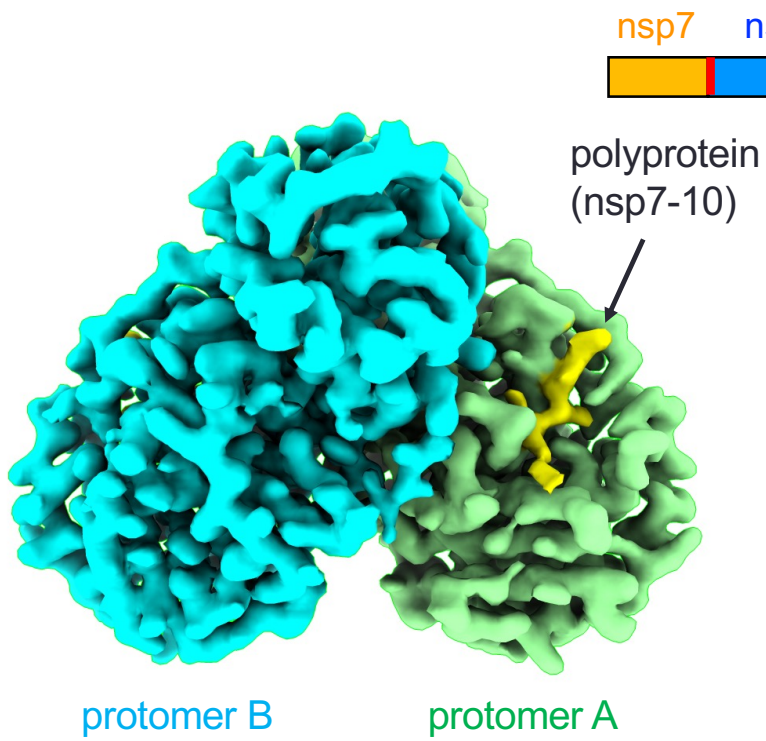


C1 symmetry

Narwal, Murakami *et al.*, *JBC* (2023)



Narwal, Murakami *et al.*, *JBC* (2023)



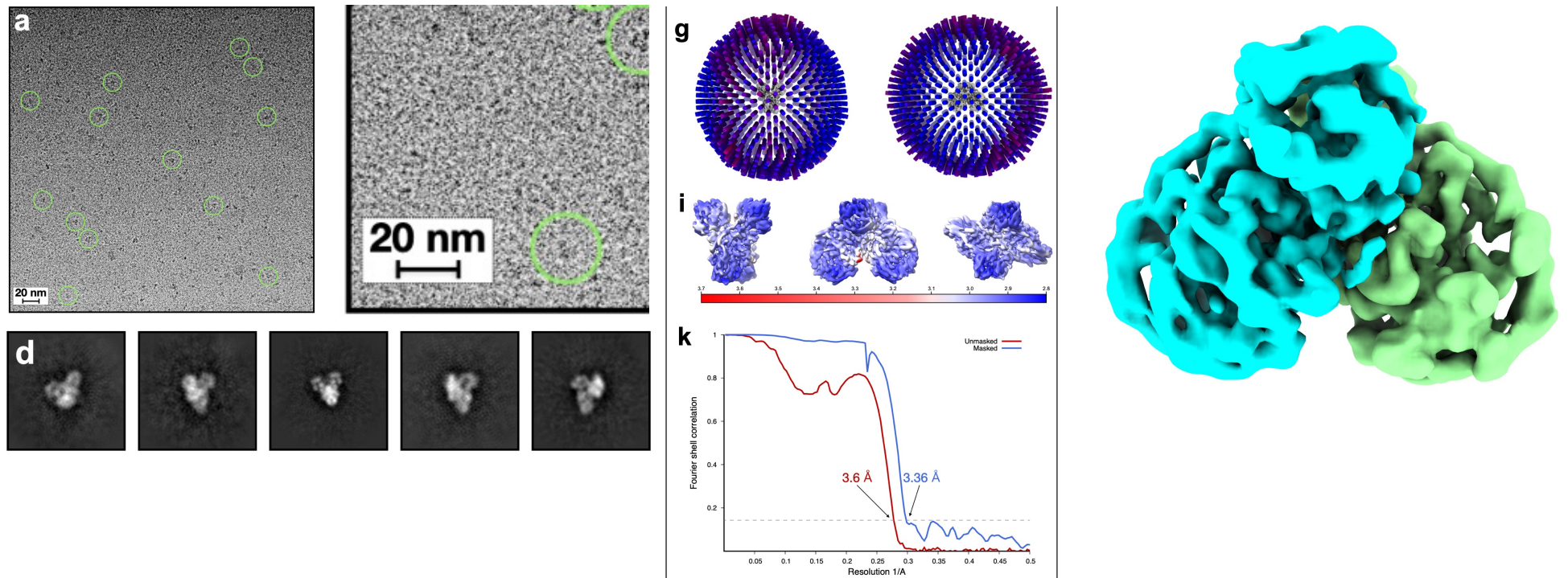
P6	P5	P4	P3	P2	P1	P1'	P2'	P3'	P4'
N	R	A	T	L	Q	A	I	A	S
nsp7				nsp8					
S	A	V	K	L	Q	N	N	E	L
nsp8				nsp9					
A	T	V	R	L	Q	A	G	N	A
nsp9				nsp10					

←

Narwal, Murakami *et al.*, *JBC* (2023)

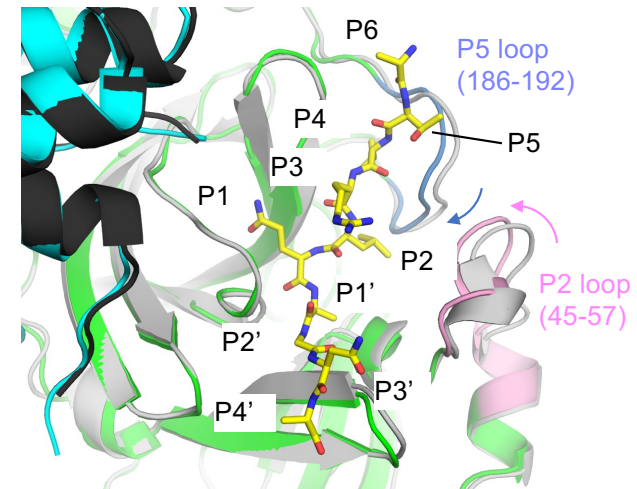
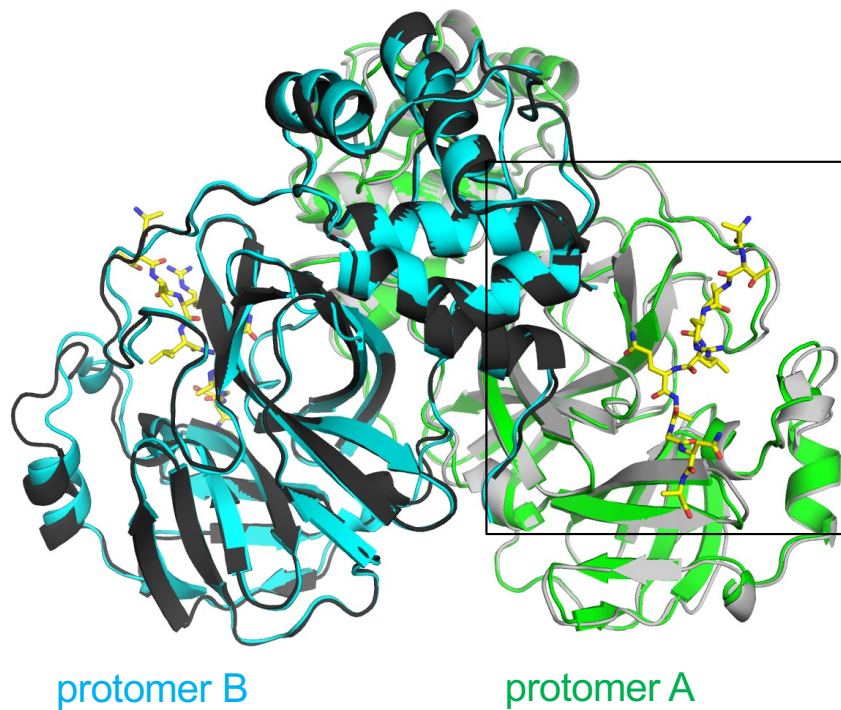


# Cryo-EM structure of the wild-type M<sup>pro</sup> (MW: 68 kDa)



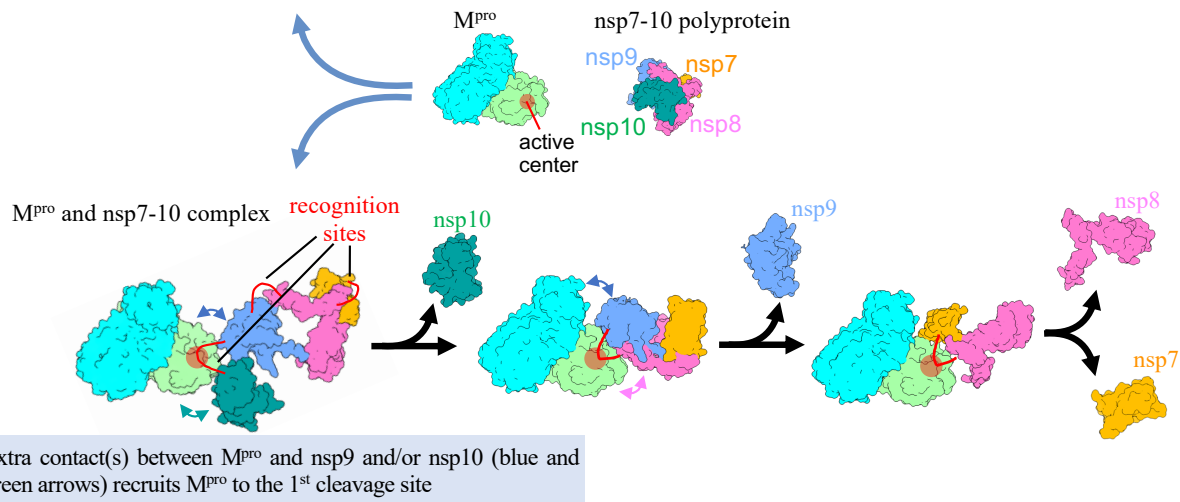
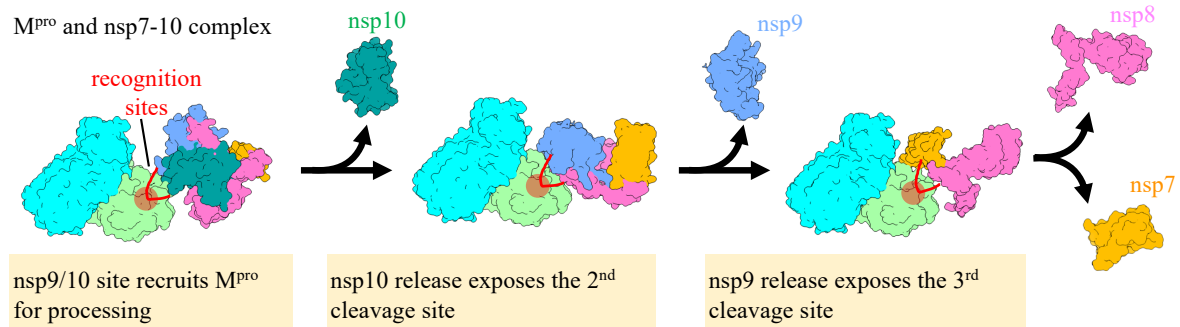
Narwal, Murakami *et al.*, *JBC* (2023)

# Comparison of the M<sup>pro</sup> structures in the apo-form and in the polyprotein (nsp7-10) complex



## A. Polyprotein driven model

Limited number of the recognition sites are exposed on surface of polyprotein for recruiting  $M^{pro}$ . Releasing protein exposes additional recognition sites to continue processing.



## B. $M^{pro}$ driven model

Recognition sites are already exposed on surface of polyprotein.  $M^{pro}$  selects a preferred cleavage site based on its affinity to  $M^{pro}$ .



## COVID-19 / SARS CoV 2





The first sequence: Jan 11, 2020

The first structure of M<sup>pro</sup>: Feb 05, 2020



## Experimental Method (total 807)

- X-ray Diffraction: 803
- Electron Microscopy: 4

Search Summary This query matches 807 Structures.

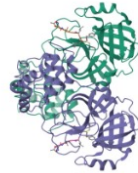
Refinements    -- Tabular Report  All  Selected


1 to 25 of 807 Structures Page 1 of 33 25 Sort by Release Date: Oldest to Newest



**6LU7**  

The crystal structure of COVID-19 main protease in complex with an inhibitor N3  
Liu, X., Zhang, B., Jin, Z., Yang, H., Rao, Z.  
(2020) Nature **582**: 289-293

**Released** 2020-02-05  
**Method** X-RAY DIFFRACTION 2.16 Å  
**Organisms** Severe acute respiratory syndrome coronavirus 2  
synthetic construct  
**Macromolecule** 3C-like proteinase (protein)  
N-[[5-METHYLISOXAZOL-3-YL]CARBONYL]JALANYL-L-VALYL-N-1-[(1R,2Z)-4-(BENZYL OXY)-4-OXO-1-[[[3R]-2-OXOPYRROLIDIN-3-YL]METHYL]BUT-2-ENYL]-L-LEUCINAMIDE (protein)









**6Y2E**  

Crystal structure of the free enzyme of the SARS-CoV-2 (2019-nCoV) main protease  
Zhang, L., Sun, X., Hilgenfeld, R.  
(2020) Science **368**: 409-412

**Released** 2020-03-04  
**Method** X-RAY DIFFRACTION 1.75 Å  
**Organisms** Severe acute respiratory syndrome coronavirus 2  
**Macromolecule** 3C-like proteinase (protein)

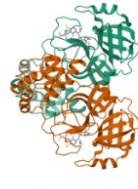





**6Y2F**  

Crystal structure (monoclinic form) of the complex resulting from the reaction between SARS-CoV-2 (2019-nCoV) main protease and tert-butyl 1-((S)-1-((S)-4-(benzylamino)-3,4-dioxo-1-((S)-2-oxopyrrolidin-3-yl)butan-2-yl)amino)-3-cyclopropyl-1-oxopropan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl)carbamate (alpha-ketoamide 13b)  
Zhang, L., Lin, D., Sun, X., Hilgenfeld, R.  
(2020) Science **368**: 409-412

**Released** 2020-03-04  
**Method** X-RAY DIFFRACTION 1.95 Å  
**Organisms** Severe acute respiratory syndrome coronavirus 2  
**Macromolecule** 3C-like proteinase (protein)  
**Unique Ligands** DMS, O6K





Structure Determination Methodology  
 experimental (807)

Scientific Name of Source Organism  
 Severe acute respiratory syndrome coronavirus 2 (807)  
 synthetic construct (24)  
 Homo sapiens (3)  
 Camelus bactrianus (2)  
 Escherichia coli (1)  
 Streptomyces exfoliatus (1)  
 Streptomyces roseus (1)

Taxonomy  
 Riboviria (807)  
 other sequences (24)  
 Eukaryota (5)  
 Bacteria (3)

Experimental Method  
 X-RAY DIFFRACTION (803)  
 ELECTRON MICROSCOPY (4)  
 NEUTRON DIFFRACTION (4)

Polymer Entity Type  
 Protein (807)

Refinement Resolution (Å)  
 1.0 - 1.5 (62)  
 1.5 - 2.0 (456)  
 2.0 - 2.5 (241)  
 2.5 - 3.0 (46)  
 3.0 - 3.5 (3)  
 3.5 - 4.0 (2)

Release Date  
 2020 - 2024 (807)

Enzyme Classification Name  
 Hydrolases (804)  
 Transferases (761)  
 Lyases (760)

Symmetry Type  
 C<sub>2</sub> (224)

# Acknowledgements

## Penn State (Murakami lab)

Manju Narwal

## Penn State

Jean-Paul Armache

## National Cryo-EM Facility (NCEF) at NIH

Thomas J. Edwards

## Huck Penn State Cryo-EM Facility

Sung Hyun (Joseph) Cho

Mike Carnegie

Carol Bator



R35 GM131860



National Cryo-EM Facility (NCEF) at NIH  
(HSSN261200800001)

A recruitment poster for Penn State. The top left features the Penn State Eberly College of Science logo. The top right identifies the Department of Biochemistry and Molecular Biology. A QR code is on the left. The main text reads: 'We are hiring! Faculty Positions in Cryo-EM Structural Biology at BMB, Penn State. Questions? Contact Katsu Murakami (kum14@psu.edu)'. The background shows a 3D molecular model. The bottom left has the Penn State Huck Institutes of the Life Sciences logo and the text 'Cryo-Electron Microscopy Facility' and 'Creating super high-definition 3D images of atoms and molecules'. The bottom right shows a photograph of a cryo-EM instrument.