COVID-19 Research Update

Andrzej Joachimiak

Argonne National Laboratory

Structural Biology Center, X-ray Science Division, Advanced Photon Source

The University of Chicago

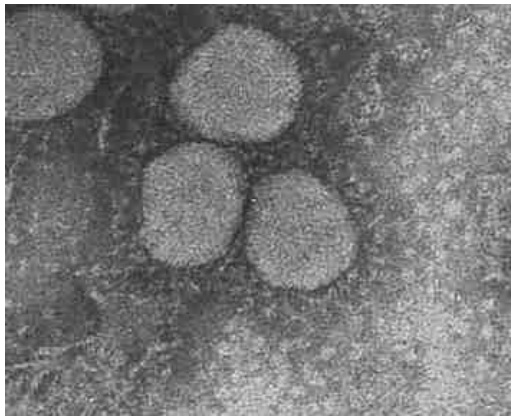
Center for Structural Genomics of Infectious Diseases

October 23, 2020

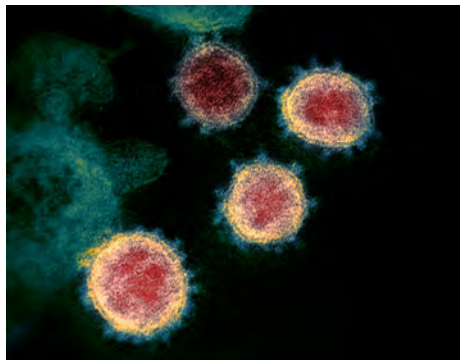
Funding is provided by: the National Institute of Allergy and Infectious Diseases of NIH under Contract No. HHSN272201700060C, the DOE Office of Science through the National Virtual Biotechnology Laboratory, a consortium of DOE national laboratories focused on response to COVID-19 (the Coronavirus CARES Act) and the use of Structural Biology Center beamlines at the Advanced Photon Source is supported by the U.S. DOE Office of Science and operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357.

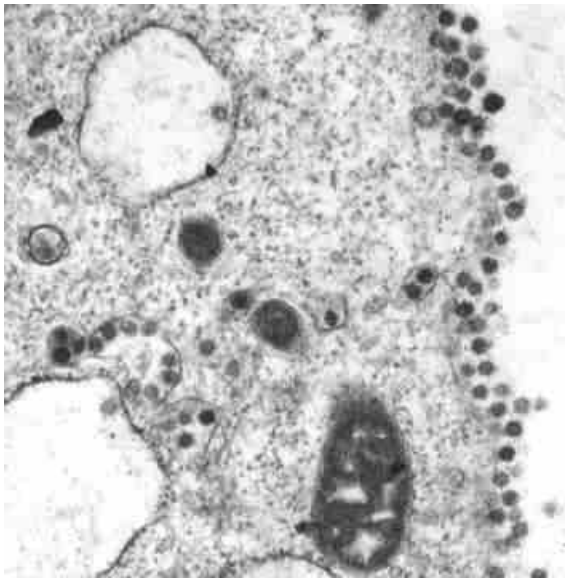
COVID-19 Pandemic : Images of SARS-CoV-2

- Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is an etiologic agent responsible for the current outbreak of Coronavirus Disease 2019 (COVID-19) - at present there is no effective vaccine or proven drug to prevent infections and stop virus proliferation.
  - SARS-CoV-2 isolated in FRhK-4 cells
  - Thin section electron micrograph and negative stained virus particles



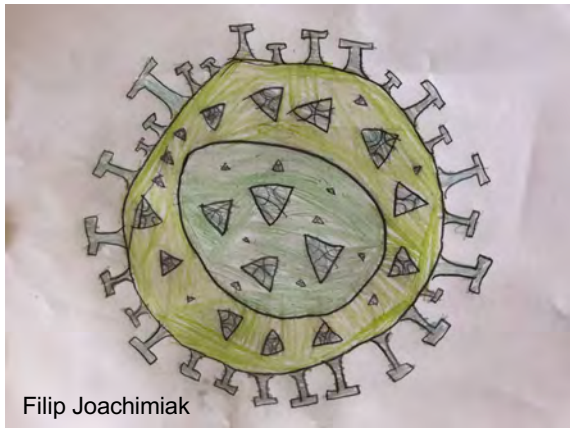
  - Department of Microbiology, The University of Hong Kong and the Government Virus Unit, Department of Health, Hong Kong SAR China



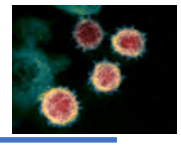


Infected: >41.5M
Death: >1.14 M

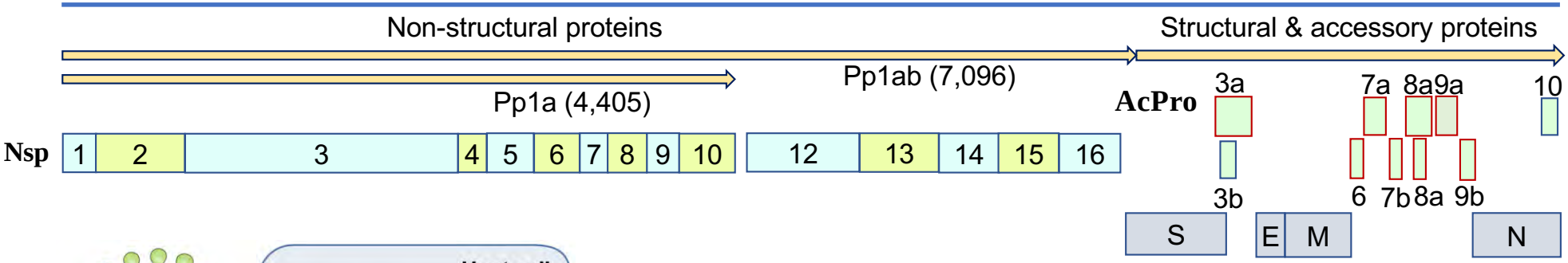
SARS-CoV-2 isolated from first US patient (NIAID)

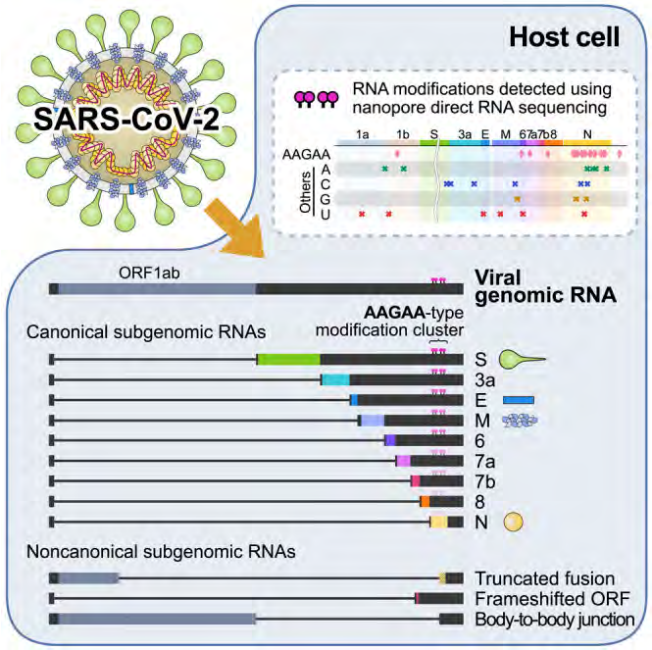


SARS-CoV-2 Genome



(+) RNA (29,903)





Kim *et al. Cell*, 2020

- SARS-CoV-2 is spherical, enveloped, non-segmented, (+) sense RNA betacoronavirus with a large ~30 kbs ssRNA genome.
- The RNA genome is coding for 29 proteins.
- The 4 structural, 15 non-structural and 9-10 accessory proteins are translated from subgenomic RNAs.
- Polyprotein processing is essential for the release and maturation of the 15 Nsps and assembly into cytoplasmic, ER membrane-bound multicomponent replicase-transcriptase complex.
- This complex is responsible for directing the replication, transcription and maturation of the viral genome and subgenomic mRNAs.
- Although this virus is similar to human and animal SARS- and MERS-CoVs, the detailed information about SARS-CoV-2 proteins structures and functions is urgently needed to rapidly develop effective therapeutics.

NIH: NIAID

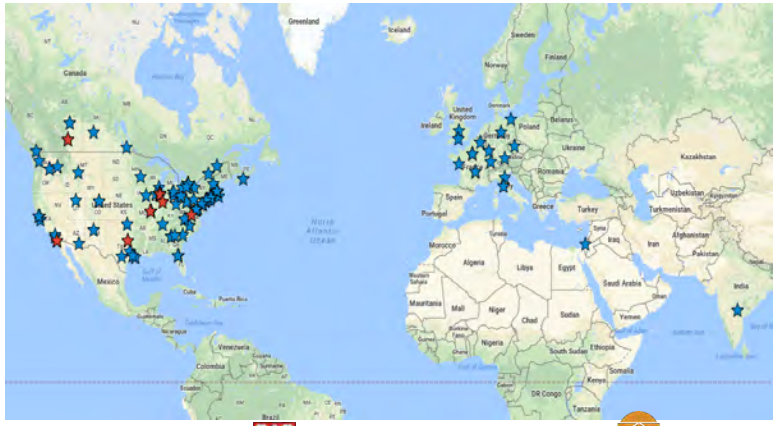
Center for Structural Genomics of Infectious Diseases

CSGID

We are a consortium of laboratories using state-of-the-art structural biology methods to determine the 3-D structures of proteins from pathogens in the NIAID Category A-C priority lists and organisms causing emerging and re-emerging infectious diseases. We do this as a free service to the scientific community! Members of the scientific community are encouraged to submit their targets of interest to CSGID by using our online form: Submit/check your proposal now!
For more detailed information about our mission, please refer to the **CSGID brochure (pdf)** and the **Overview.**

Who we are? | Community Services | Scoreboard | Structures | Publications

Targets:	12005
Targets requested by the community:	5391
Community Interest targets:	157
Targets with structures:	864
Structures:	1330 (X-ray: 1318, NMR: 7, Cryo-EM: 5)
Structures complexed with ligands:	560
Solved proteins requested by community:	365 (567 structures)
Solved Community Interest targets:	53 (122 structures)
Publications:	201 (131 structural, 70 methodological/other)
Citations:	4990 (2376 for structural, 2614 for methodological/other)



Northwestern University Feinberg School of Medicine · The University of Chicago · Argonne National Laboratory · Washington University in St. Louis · UT Southwestern Medical Center · UC Riverside · University of Calgary · Purdue University · University of Virginia

DOE broad capabilities for addressing COVID-19 crisis

- Light and neutron sources
- Nanoscience centers
- Computational resources
- People with deep expertise relevant to:
  - Testing
  - Antiviral drug discovery
  - Vaccine discovery
  - Supply chain bottlenecks
  - Modeling and understanding disease spread
  - Molecular and structural biology

About

DOE User Facilities

NVBL Structure

NVBL Coordination Team

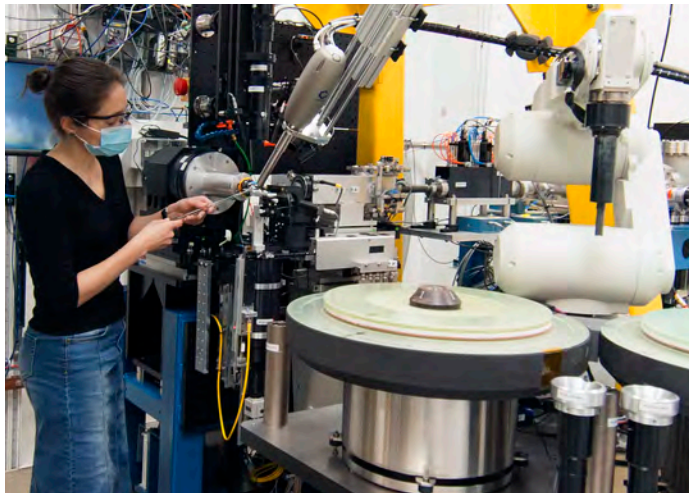
National Virtual Biotechnology Laboratory (NVBL)

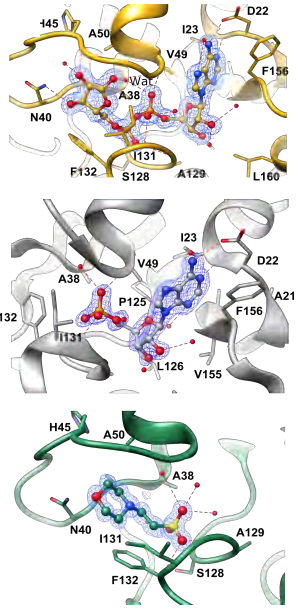
https://science.osti.gov/nvbl

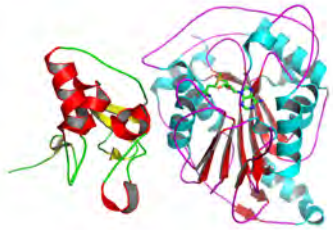
- Consortium of 17 DOE National Laboratories
- Takes advantage of DOE user facilities
- Initial activities include:
  - Epidemiological and logistical support
  - Addressing supply chain bottlenecks by harnessing advanced manufacturing
  - Medical therapeutics: computational drug discovery and structural biology
  - Innovations in testing capabilities
  - New project in understanding fate and transport of virus in the environment

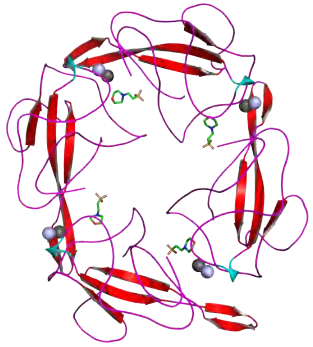
NVBL COVID-19 Task 5 Team

- **Structure-Based Protein Design for Diagnostics**
  - Argonne National Laboratory
  - Lawrence Berkeley National Laboratory
  - Los Alamos National Laboratory
  - Oak Ridge National Laboratory
- **Structural Biology and Structure-based Drug Discovery**
  - University of Chicago
  - Northwestern University
  - Argonne National Laboratory
  - Auburn University
  - University of Iowa
  - Diamond Light Source
  - University of Texas SWMC
  - NIH/NIAMS



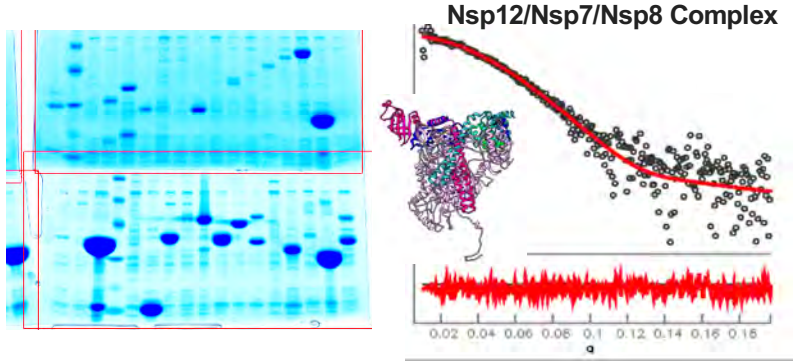


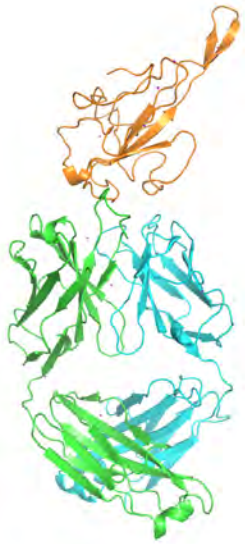




NVBL Structure-Based Protein Design for Diagnostics

- An integrated approach for utilizing emerging structural data has been applied to develop novel targets and demonstrate high-affinity reagents for non-nucleic acid-based detection systems.
  - The ANL team provided proteins for affinity reagent development for COVID-19 diagnostics.
  - The LBNL team performed HTP SAXS experiments at the ALS.
  - The ORNL team contributed to neutron protein crystallography studies and structure-based development of diagnostics.
  - LANL used protein design tools, available structures and HTP screening to develop affinity reagents for diagnostic tests.





Advanced Photon Source Beamlines and APCF (APS Sector 84) Rapid COVID-19 Response

- The APS has stepped up as a world-leading source of information about SARS-CoV-2.
- More than 275 scientists from around the country have used 20 APS beamlines (remotely and mail-in) for nearly 7,700 hours, studying the virus with potential drugs, antibodies, and human proteins, and materials for better and less expensive N95 masks.
- 94 detailed structures for viral proteins, both alone and in complexes with potential therapeutic molecules, were determined using data collected at the APS.
- Argonne researchers have determined 40 of the structures identified using the APS.

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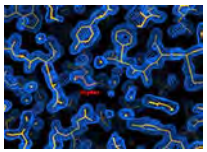
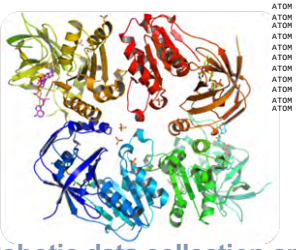
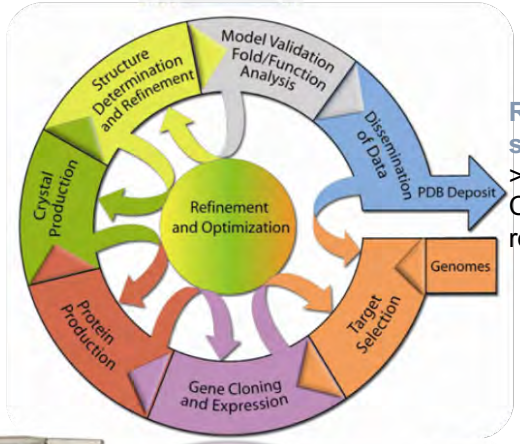
Argonne HTP Structural Biology Pipeline Applied to COVID-19

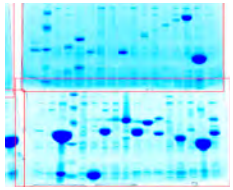




HTP crystallization
12 million drops to date, Mosquito, Robohotels

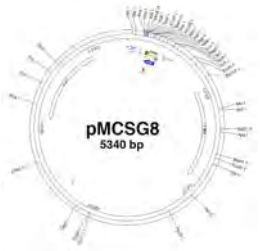


Robotic data collection and semi-automated structure determination
>3,300 PDB deposits to date (MCSG, SBC and CSGID) (4/week) SBC-Collect, HKL-3000, robotics



Mg-scale protein purification
>20,000 proteins to date (max: 300/week)
AktaXpress systems

Cloning and small-scale protein expression
>65,000 clones to date (max: 4000/week)
>50 ug-scale protein production (max: 4000/week) 250 genomes; >90 vectors; *E. coli* and eukaryotic systems



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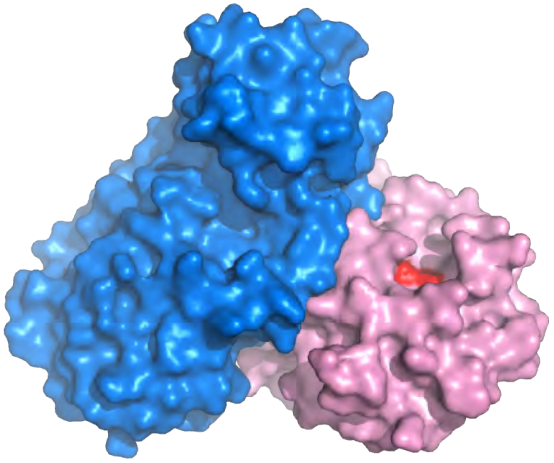
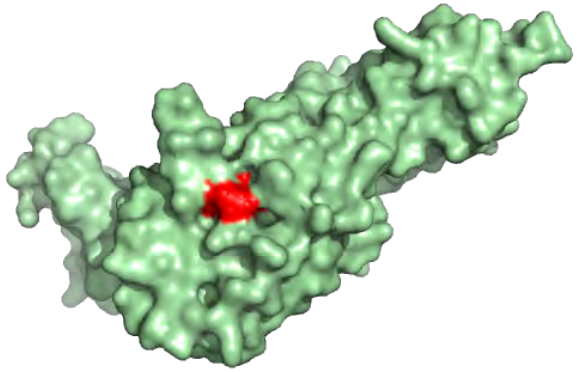
SARS CoV-2 Drug Targets

- Proteases are important SARS-CoV-2 enzymes potentially targetable with antivirals: papain-like protease (PLpro) and main protease (Mpro).
- Proteases are especially attractive targets because they play an essential role in several viral replication processes, including cleavage and maturation of viral polyproteins, assembly of the replicase-transcriptase complex, and disruption of host viral response machinery to facilitate viral proliferation and replication.
- PLpro and Mpro are conserved across different coronaviruses and promising inhibitors have already been discovered for their SARS-CoV-2 variant.]



Structure of SAR-CoV-2 Mpro dimer

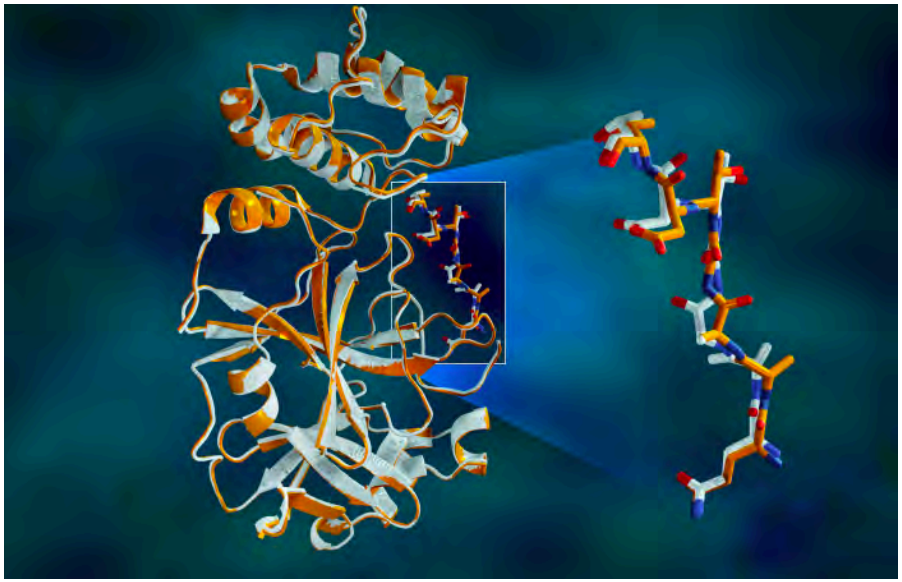


Structure of SAR-CoV-2 PLpro monomer

Structural Plasticity of SARS-CoV-2 3CL M^{pro} Active Site Cavity Revealed by Room Temperature X-ray Crystallography

- A team of researchers at the DOE's Oak Ridge and Argonne National Laboratories has performed the first room-temperature X-ray measurements on the SARS-CoV-2 main protease — the enzyme that enables the virus to reproduce.

- The model will be used to advance supercomputing simulations aimed at finding drug inhibitors to block the virus's replication mechanism. Research results are publicly available (PDB id: 6WQF) and have been published in the journal Nature Communications.



Overlapping X-ray data of the SARS-CoV-2 main protease shows structural differences between the protein at room temperature (orange) and the cryogenically frozen structure (white). Credit: Jill Hemman/ORNL, U.S. Dept. of Energy

17 structures determined by ANL and ORNL teams

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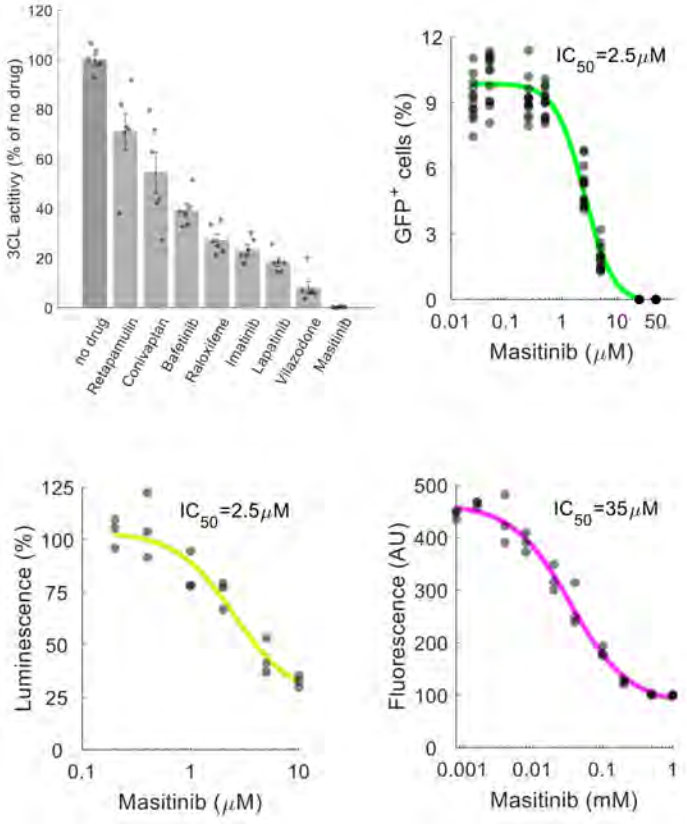
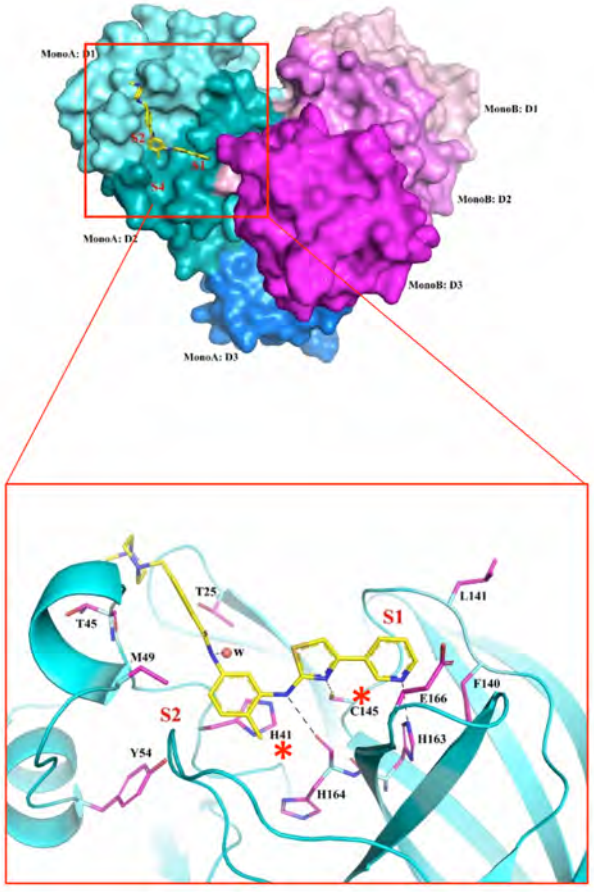
Argonne NATIONAL LABORATORY

Structural plasticity of SARS-CoV-2 3CL Mpro active site cavity revealed by room temperature X-ray crystallography. Kneller DW, Phillips G, O'Neill HM, Jedrzejczak R, Stols L, Langan P, Joachimiak A, Coates L, Kovalevsky A. Nature Communications volume 11, Article number: 3202 (2020)

Structure of SARS-CoV-2 Mpro with Masitinib





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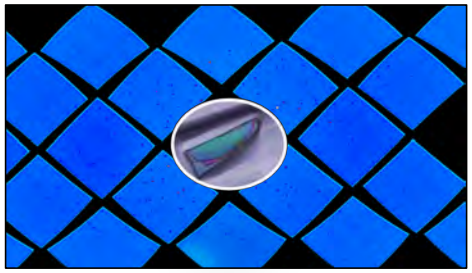
Drayman *et al.* (2020) submitted, posted on bioRxiv server : https://doi.org/10.1101/2020.08.31.274639

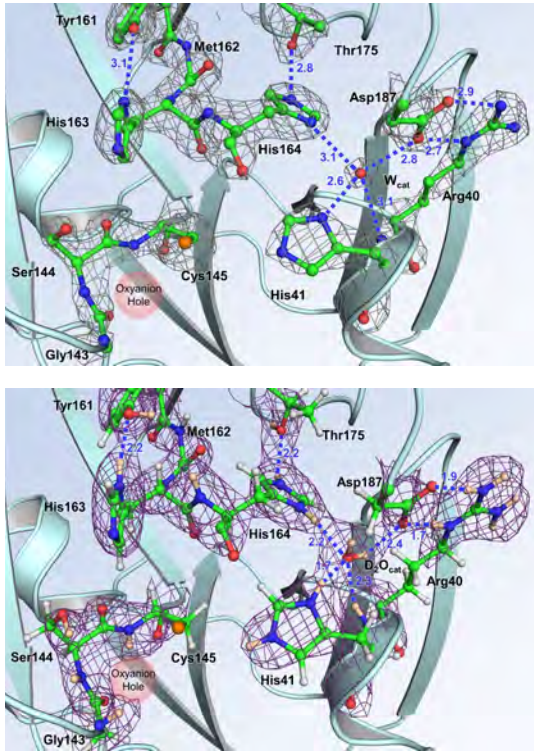
Room temperature X-ray and neutron crystallography of SARS-CoV-2 M^{pro} for the design of specific inhibitors

- Main Protease (3CL M^{pro}) is a major target for structure-guided drug design against SARS-CoV-2.
- The neutron structure shows that the catalytic site natively adopts a zwitterionic reactive state
  - His41 is doubly protonated and positively charged, whereas Cys145 is negatively charged in the thiolate state
- Combined X-ray and neutron studies play a critical role for *in silico* docking, clinical drug repurposing, and rational drug design efforts

- Scientific contributions
  - 5 papers published
  - 9 deposited X-ray structures reveal malleability of the active site, rare oxidization state, and binding mode clinical protease inhibitors



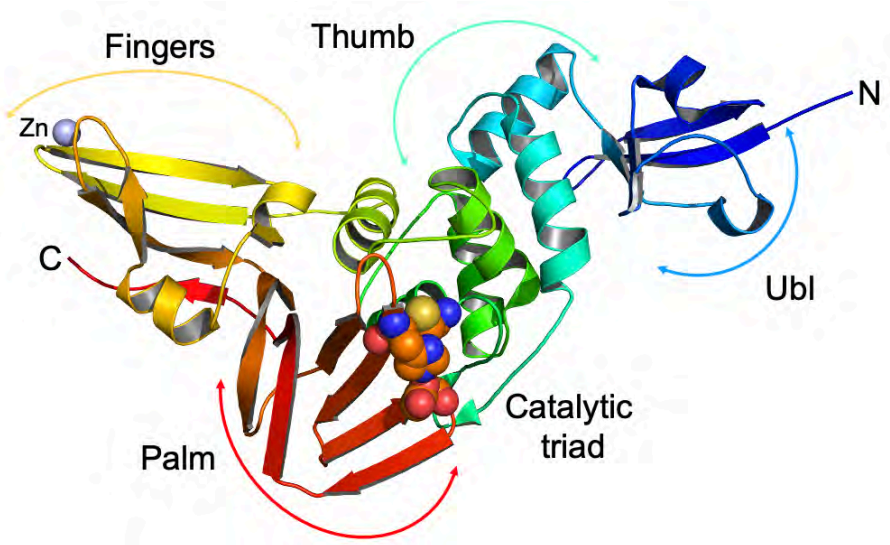
Catalytic site of SARS-CoV-2 3CL^{Mpro}



Top: Electron density map (no hydrogen atoms visible)
Bottom: Nuclear density map allowing visualization of protonation states and hydrogen bonding interactions

Structure of SARS-CoV-2 Nsp3 PLpro

- Cysteine PLpro protease is highly conserved and found in all coronaviruses, often in two copies
- PLpro exhibits multiple functions, in addition to processing polyproteins and recognizing LXGG sequence motif it has deubiquitinating activity and deISG15ylating (interferon-induced gene 15) activities
- Inhibitors block the peptidase activity of PLpro *in vitro* and some can block SARS-CoV-2 replication in cell culture assays.
- Our collection of structures provides fundamental molecular and mechanistic insight to PLpro and illustrates details for inhibitors recognition and interactions.



9 structures determined at ANL

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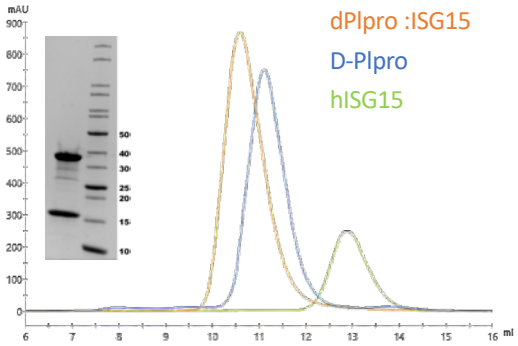
Osipiuk et al. submitted to *Nature Communications*
Posted on bioRxiv server https://doi.org/10.1101/2020.08.06.240192

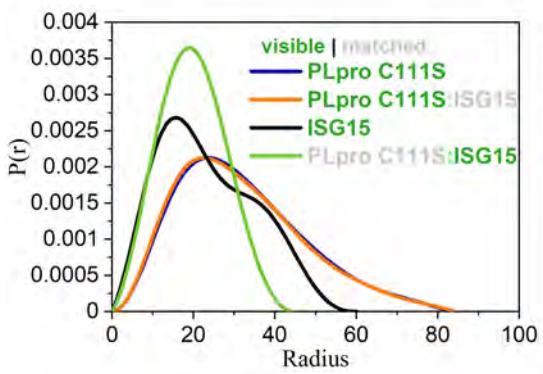
SANS provides insights into the deISGylating activity of PLpro and can be used to guide anti-viral drug design

- The PLpro plays important roles in viral polyprotein processing and deubiquitination and deISGylation of host proteins to counteract innate immunity
- The PLPro complex with human interferon-stimulated gene 15 (ISG15) is as an important structural target
- SANS with contrast matching showed an unexpected conformational rearrangement of ISG15 that was not observed in previously reported crystal structures of the complex

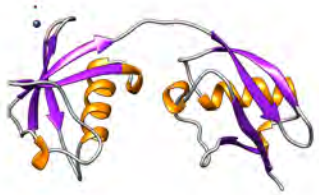
Size exclusion chromatography



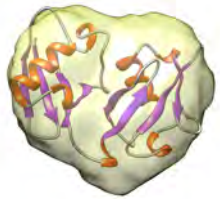
Small-angle neutron scattering



Free ISG15



PLproC111S:ISG15 (100D)



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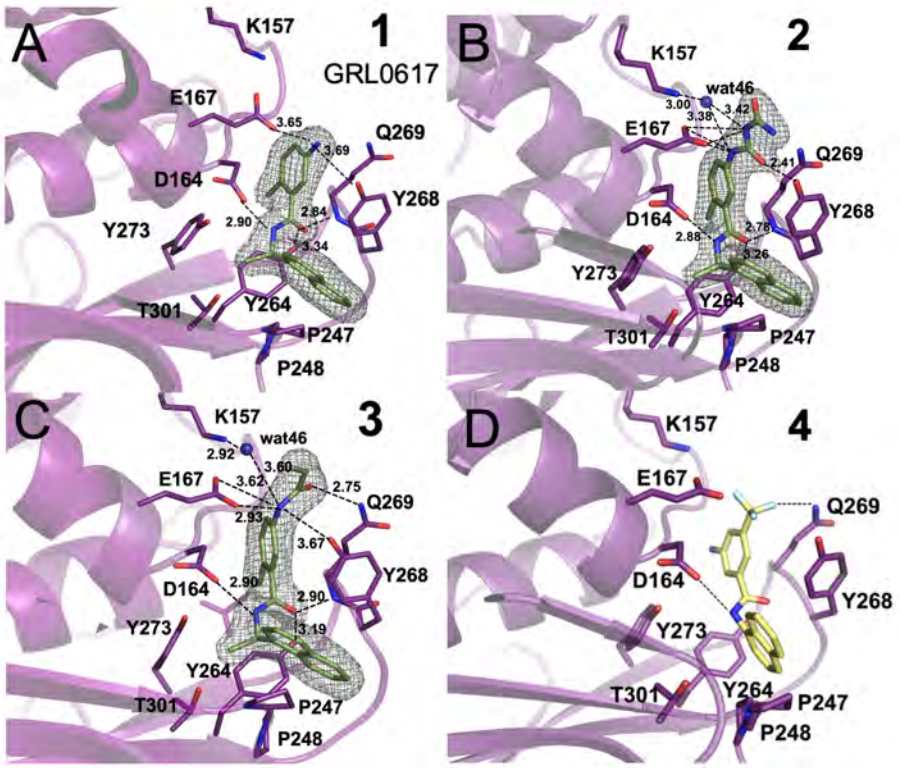
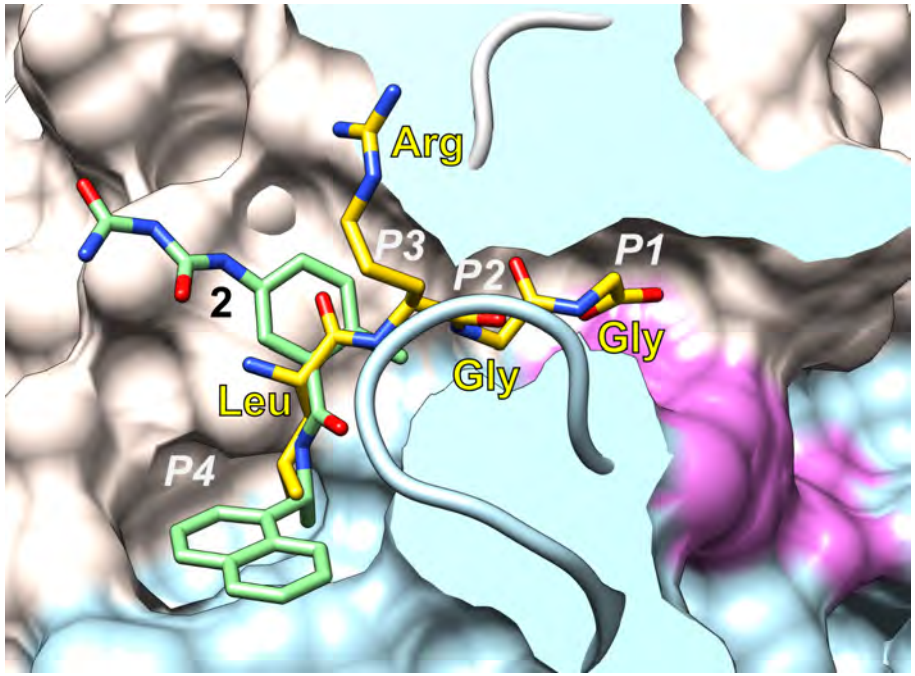
Structures of PLpro in Complex with Inhibitors Shows Details of Interactions





Inhibitor blocks access to PLpro active site

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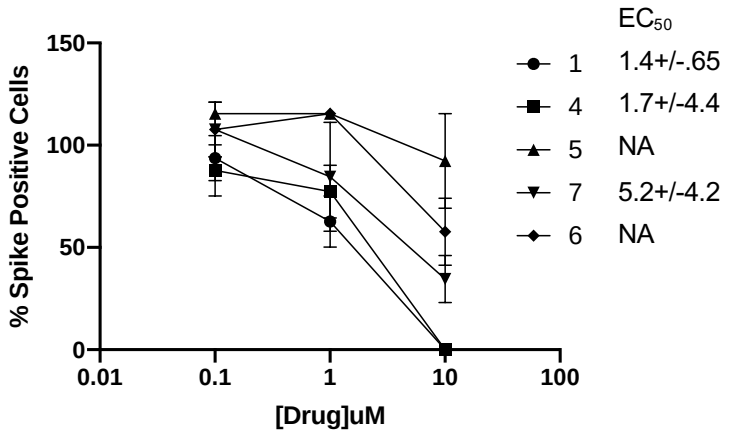
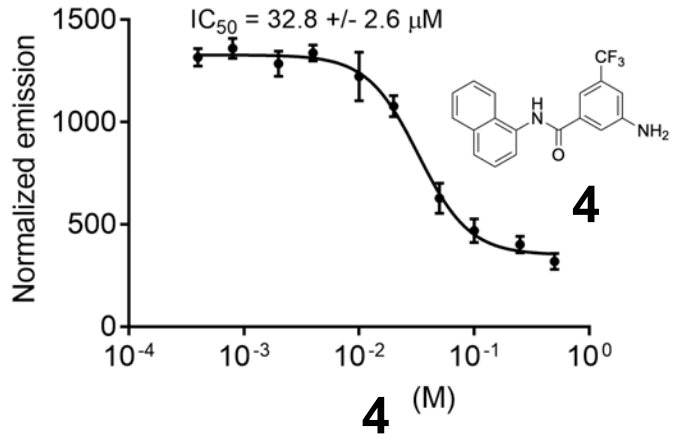
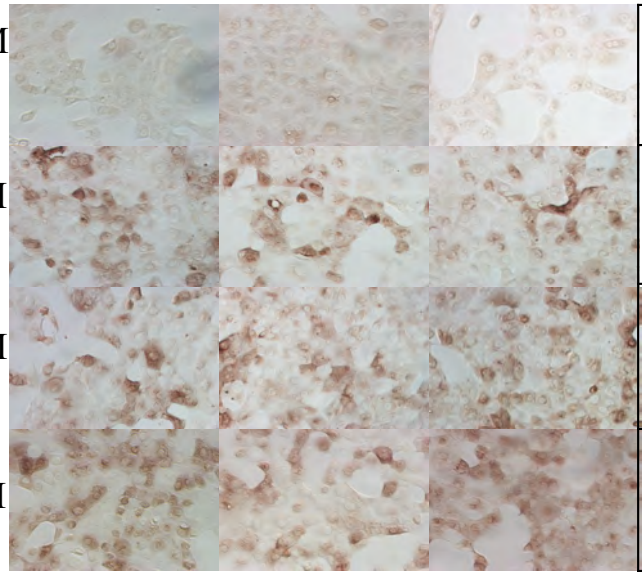
Compounds Targeting PLpro Inhibit SARS-CoV-2 Replication





4

% Positive cells in view (**4**)



0%	0%	0%
70%	65%	50%
60%	80%	70%
80%	70%	90%

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Assembly, Mechanism and Inhibition of SARS-CoV-2 RNA Transcription Complex (Nsp7/8/12)

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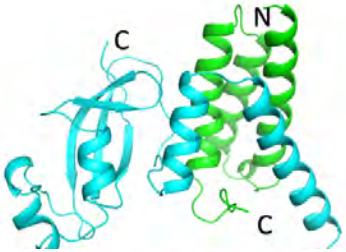
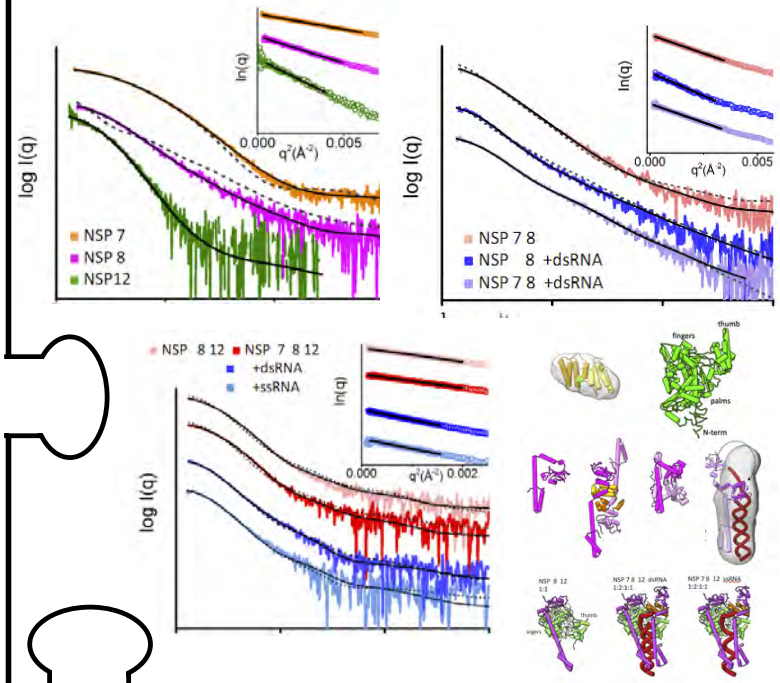
Crystallography & Protein Expression for Atomic Detail



Nsp7/8 Crystal Structures

BERKELEY LAB

Solution Scattering SAXS for High-Throughput Structures Constrained by Crystallography



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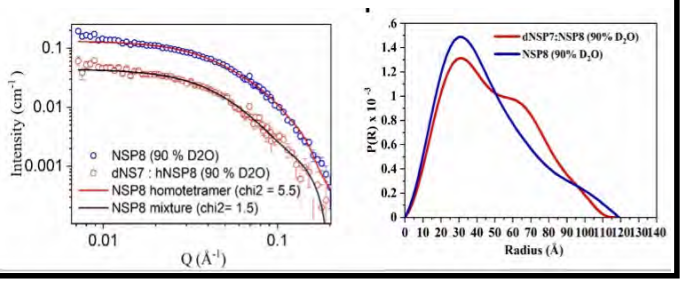
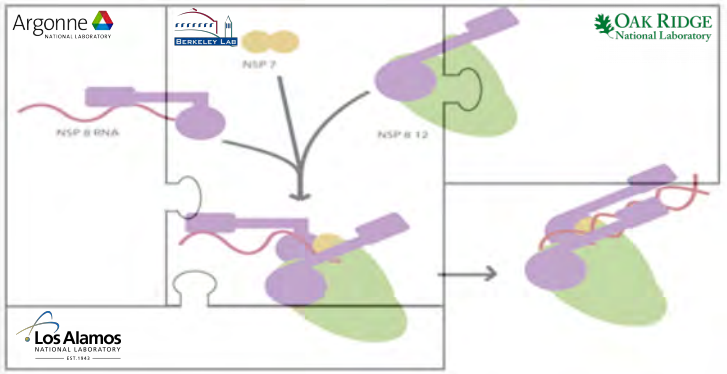
Contrast Matching SANS to Visualize Conformational Changes Induced During Complex Formation



Los Alamos NATIONAL LABORATORY EST. 1943

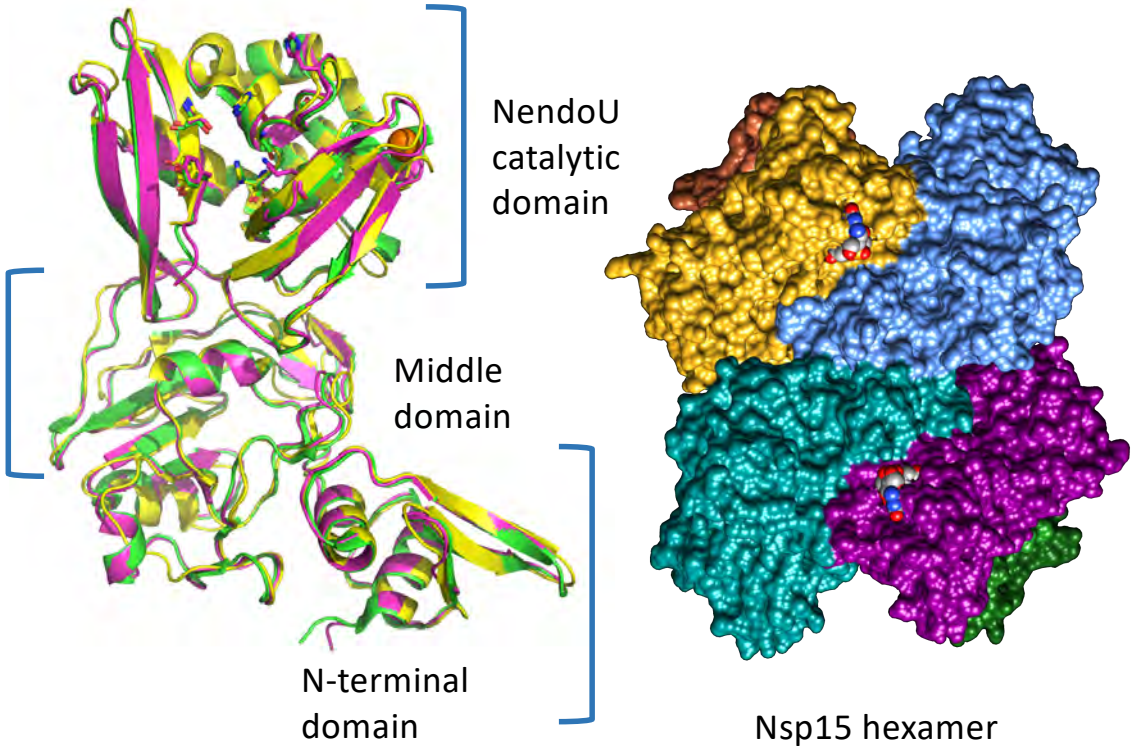
Computational Design of Complex Disrupting Molecules



Integrated Data for Mechanism and Leads on Inhibition

Nsp15 Endoribonuclease NendoU from SARS-CoV-2

- Nsp15 is a uridylate-specific endoribonuclease.
- NendoU activity interfere with the innate immune response and is essential in coronavirus biology.
- Nsp15 was shown to degrade viral RNA the polyuridine extensions on (-) sense strand of RNA.
- Nsp15 is highly conserved in coronaviruses.
- Several structures have been determined with ligands including complex with FDA approved drug Tipiracil, GpU product, 3'UMP Uridine Vanadate and 5'UMP.



SARS-CoV-2 vs SARS-CoV-1 vs MERS-CoV

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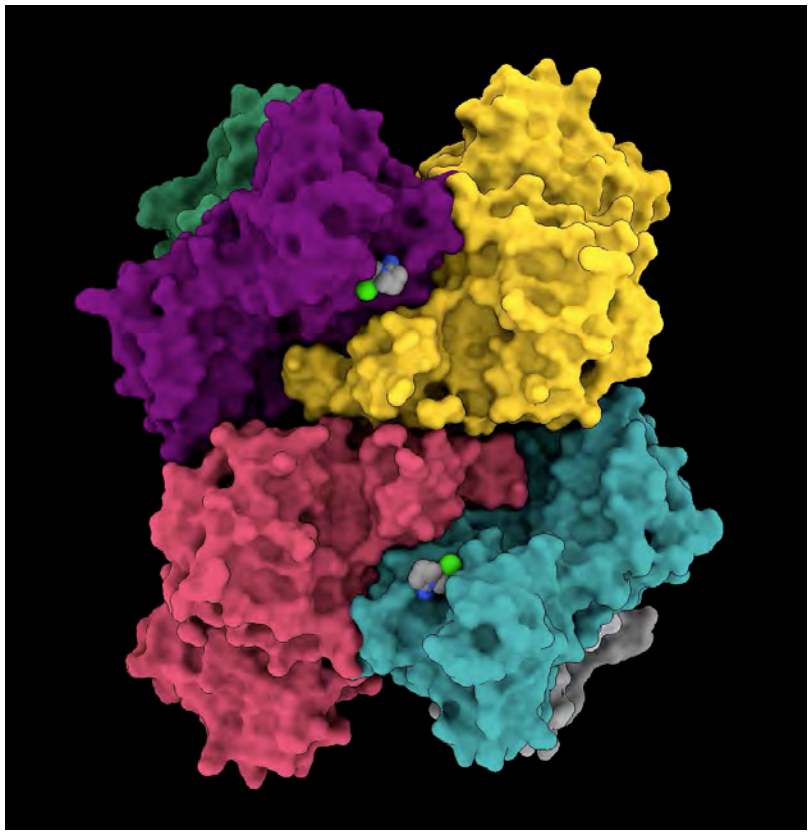
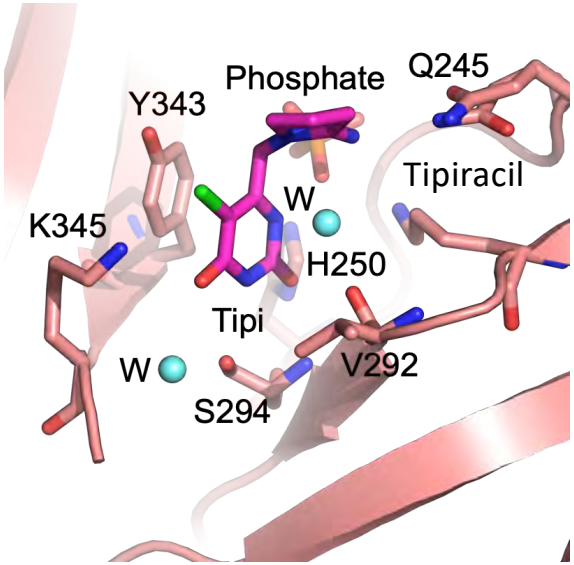
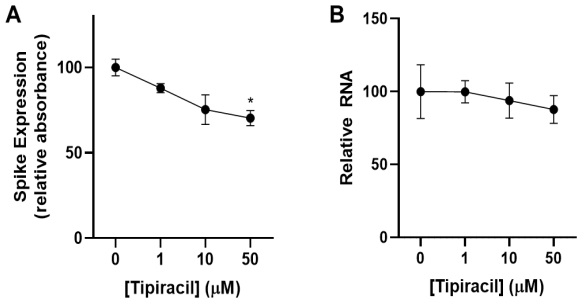
Nsp15 Endoribonuclease Complex with Tipiracil





Tipiracil



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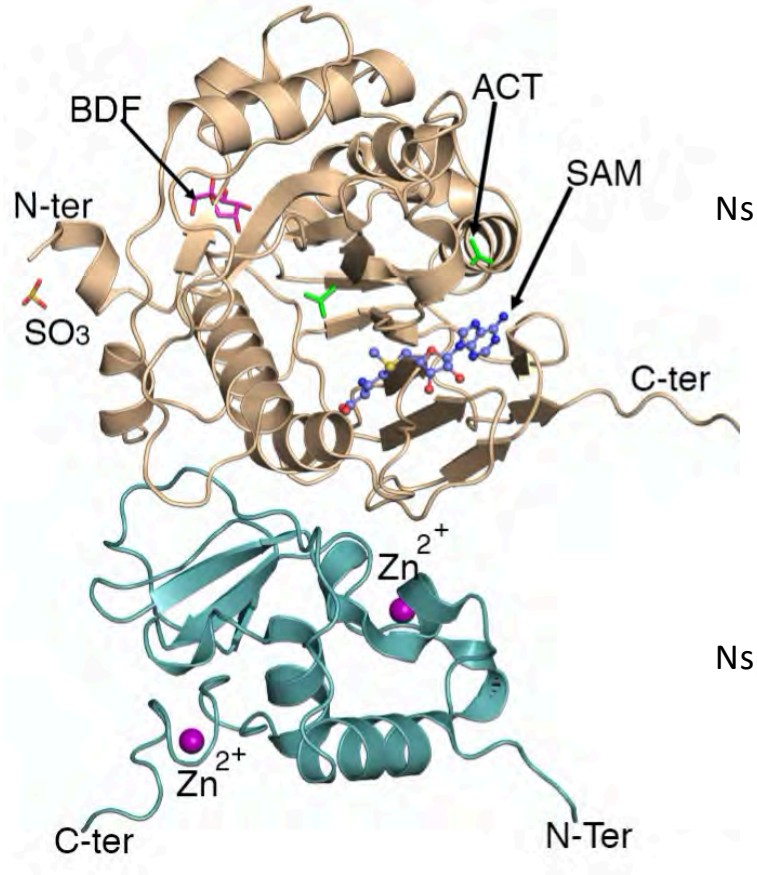
Nsp10/Nsp16 2'-O-methyltransferase from SARS-CoV-2

- Nsp10 and Nsp16 is responsible for the capping of mRNA at the 5′ terminus which is critical for virus replication and fidelity.
- The methylation of the mRNA Cap is essential for efficient translation of viral transcript in a eukaryotic host.
- The presence of Cap-1 makes viral RNAs mimic the host transcripts and prevents their degradation.
- The active site of Nsp16 MTase is conserved in the coronavirus family; they utilize a K-D-K-E catalytic tetrad which is essential for enzymatic activity.



Nsp16

Nsp10

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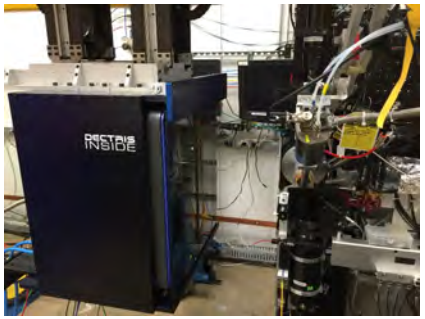
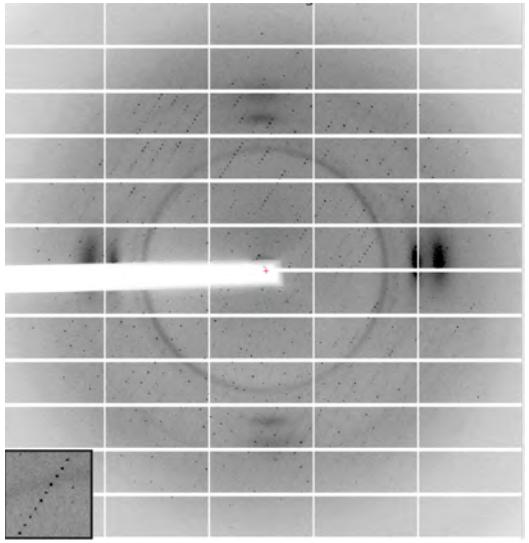
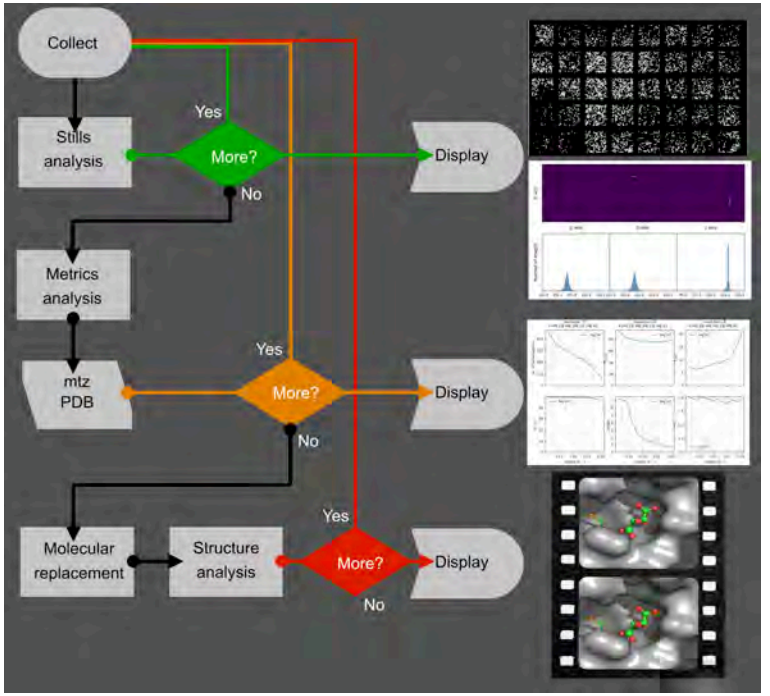
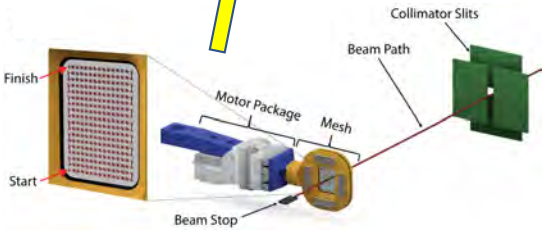
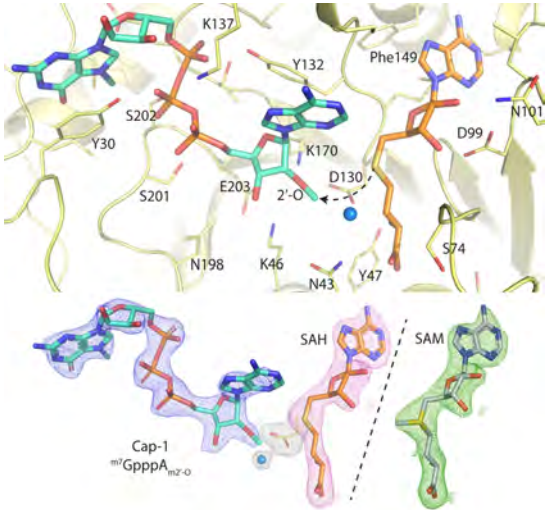
Serial Crystallography of SARS-CoV-2 Nsp10/Nsp16 Complex at 19ID

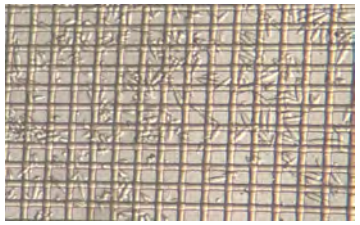












Capturing methyl transfer reaction in the crystal

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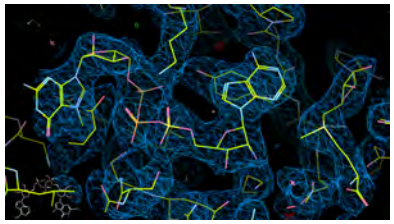
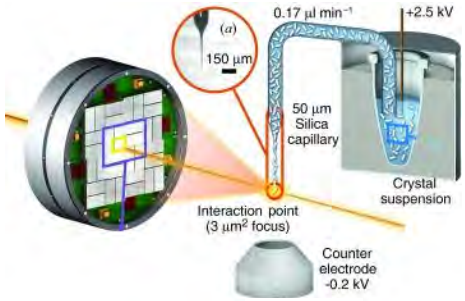
NVBL Partnerships in COVID-19 Research – Nsp10/Nsp16 and Mpro

Experiments at LCLS-MFX (SLAC) examine radiation sensitive Nsp10/16 structures and short-lived intermediates to provide new opportunities for the rational discovery of small molecule inhibitors.

- Damage free structures of Cap-1, the final stage in viral RNA maturation will verify serial synchrotron results.
- Visualization of acutely radiation sensitive catalytic metals (Mn or Mg) not observed in synchrotron data
- Capturing the rapid intermediate steps involved in docking of Cap-0 within Nsp10/16.



The SAM and m7GpppA ligands in the XFEL structure

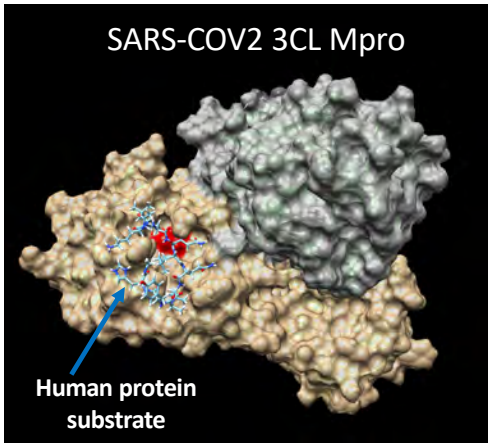


The electro-spinning injector delivers tiny crystals into fs-scale X-ray pulses

First experiments September/October, data analysis in progress. Fixed-target time-resolved measurements planned for 2021

A novel drug design concept against SARS-CoV-2 proteases

- 3CL Mpro interacts with human proteins affecting immune responses provoking side effects
- PLpro resembles human proteases (De-Ubiquitome)



Model of SARS-COV-2 3CL Mpro with human protein substrates

- ❖ Extensive modeling and MD simulations
- ❖ Co-crystallization efforts ongoing
- ❖ SANS (ORNL), SAXS(SLAC-SSRL) planned

Novel inhibitor design concept for SARS-COV2 protease specificity, avoiding human-virus protein interactions

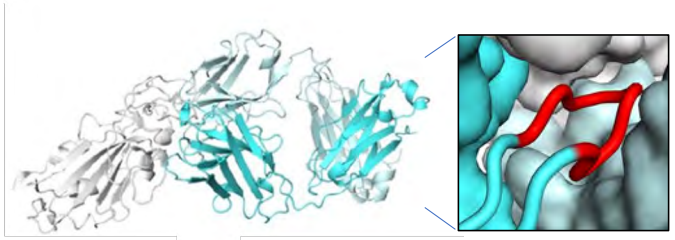
A Rapid Response at SLAC to Combat COVID-19

Fast Tracked Research Leads to Three Drugs in Clinical Trials + Two Entering Clinical Trials

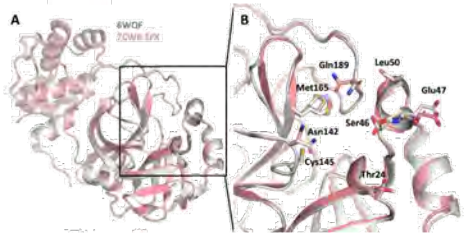
Synchrotron & CryoEM research started in March, LCLS in August

so far

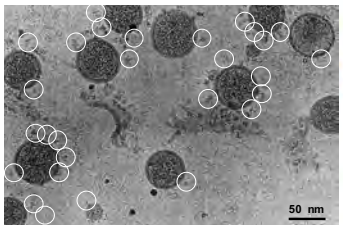
Proposals awarded time: **36** (5 international)
Fragments/inhibitors screened: **868**
PDB deposits: **18**

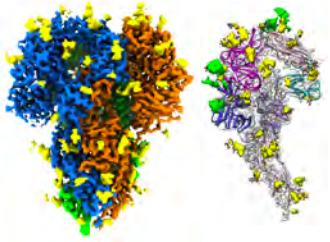


SSRL BL12-1: Human antibody (cyan) bound to the spike protein binding domain (grey). Close-up of the antibody and binding domain interface (Yuan, Science 2020)



LCLS-MFX: SARS-CoV-2 main protease structures at near-physiological temp to guide drug repurposing (Durdagi, bioRxiv/2020)





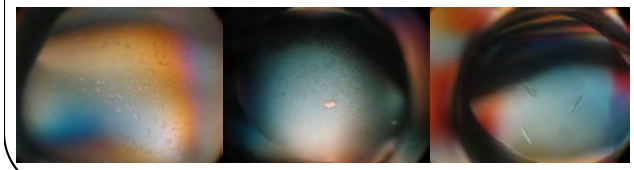
Cryo-EM: Structure of spike proteins and glycans of human coronavirus NL63 directly from virus particles (Zhang, bioRxiv/2020/245696)

Groups from across the US and abroad used SSRL, LCLS and CryoEM facilities at SLAC for COVID-19 related research

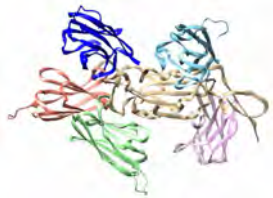
Diagnostic and Blocking Antibodies and Nanobodies Against Spike and Nucleocapsid

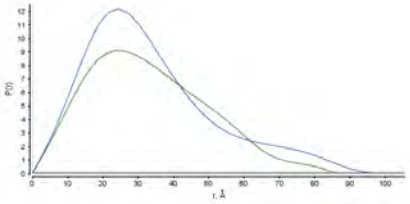
Nanobodies Against Spike (RBD)

Nanobody Production:
8/week – DNA to Crystal Trials



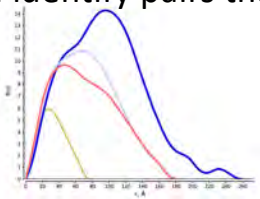
Using SAXS to test simultaneous binding by multiple nanobodies and conformational change to RBD



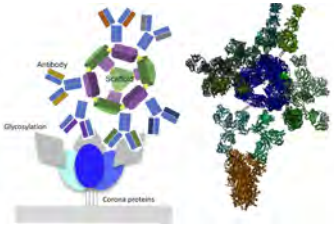


Antibodies Against Spike (RBD)

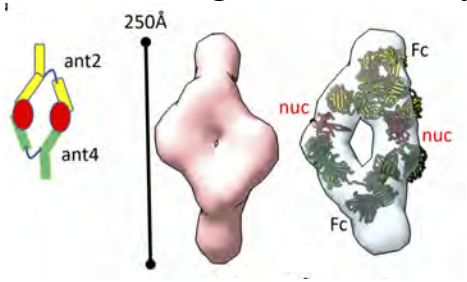
Using SAXS to identify variation in modes of binding and identify pairs that can bind simultaneously



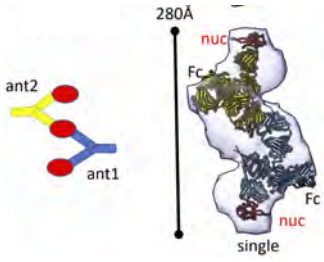
Using designed proteins to display multiple antibodies for increased avidity



Antibodies Against Nucleocapsid



Using SAXS to distinguish modes of binding by pairs with and without detergents

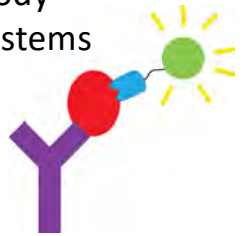


Goals & Applications

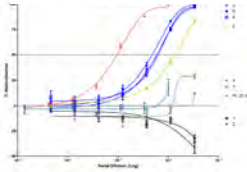
Enhanced Blocking

Antigen Detection

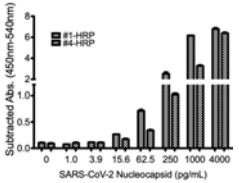
Hybrid Antibody Nanobody Systems



Neutralization Assays



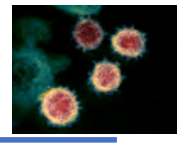
ELISA



Have systems with 100% blocking and pg/mL detection

SARS-CoV-2 Genome and CSGID (57) /UoC/ANL (40) Structures

(+) RNA (29903)



Non-structural proteins

Structural & accessory proteins

Pp1ab (7096)

Pp1a (4405)

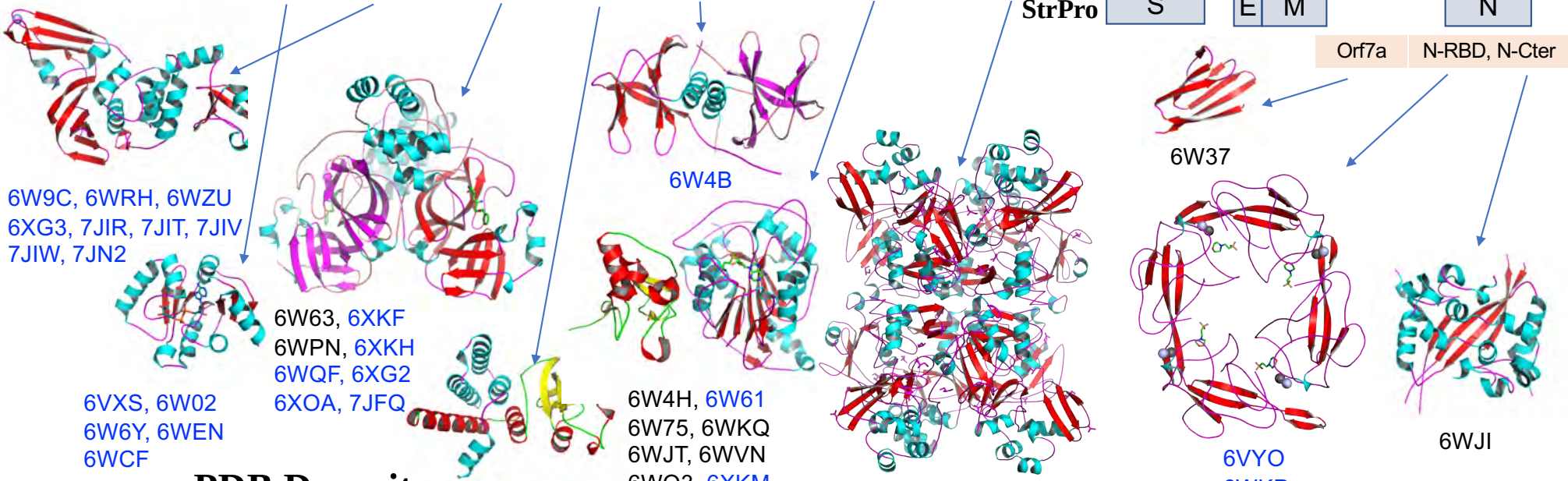
Nsp 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 12 | 13 | 14 | 15 | 16

AcPro 3a | 3b | 7a | 6 | 8a | 7b | 8a | 9b | 9a | 10

StrPro S | E | M | N

Structures

ADRP | PLpro | Mpro | Nsp7/8 | Nsp9 | Nsp10/16 | Nsp15 | Orf7a | N-RBD, N-Cter



6W9C, 6WRH, 6WZU
6XG3, 7JIR, 7JIT, 7JIV
7JIW, 7JN2

6W63, 6XKF
6WPN, 6XKH
6WQF, 6XG2
6XOA, 7JFQ

6W4B

6VXS, 6W02
6W6Y, 6WEN
6WCF

6W4H, 6W61
6W75, 6WKQ
6WJT, 6WVN
6WQ3, 6XKM
7JHE, 7JIB
7JPE

6W37

6VYO
6WKP

6WJI

6WIQ, 6WQD
6WTC, 6XIP

6W01, 6VWW, 6WLC
6WXC, 6X1B, 6X41
7K1O, 7K1L

PDB Deposits

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Acknowledgements

ANL/UoC/CSGID

C. Chang
G. Babnigg
M. Endres
R. Jedrzejczak
Yc. Kim
N. Maltseva
K. Michalska
J. Osipiuk
L. Stols
K. Tan
C. Tesar
M. Wilamowski

SBC, ANL/APS/XSD

D. Sherrell
A. Lavens
K. Lazarski

Univ. of Chicago
A. Baranczak
B. Dickinson
G. Dong
N. Drayman
T. Kossiakoff
G. Randall
S. Snyder
J. Solway
S. Tay
P. Wilson

BioCARS/UoC
V. Srayer

Auburn Univ.
J. Wower

CSGID
D. Borek, UT, SWMC
D. Fremont, WashU
A. Godzik, UCR
R. Kuhn, PU
Z. Otwinowski, UT, SWMC
W. Minor, UVA
A. Mesecar, PU
K. Satcher, NU
A. Savchenko, UC

SLAC/LCLS/SSRL
S. Boutet
A. Cohen
M. Hunter
A. Lyubimov
R. Sierra
S. Wakatsuki
J. Wierman
The construction of the MFX instrument at LCLS was BER funded and operates as a partnership between LCLS and SSRL-SMB

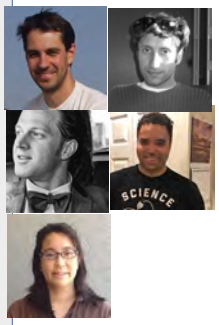
ORNL
H. O'Neil
L. Coates
A. Kovalevsky
J. Parks

LANL
R. Jha

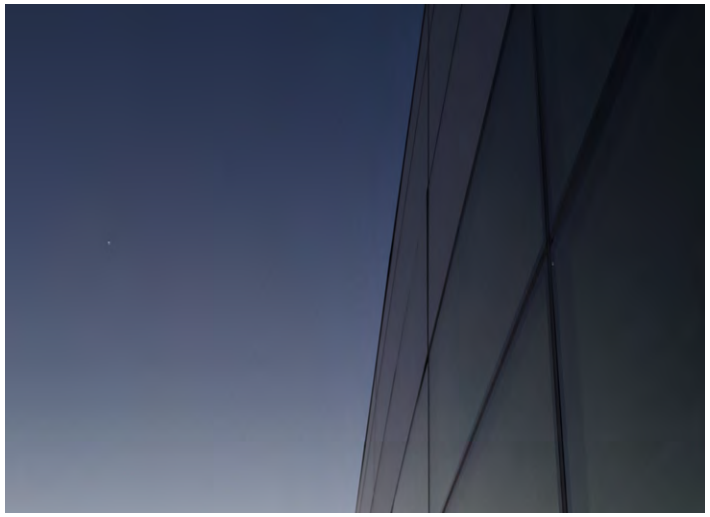
ALS/LBNL/SIBYLS
P. Adams
G. Hura
M. Hammel
C. Hodge
D. Rosenburg
S. Tsutakawa

Oxford/Diamond
F. von Delft
M. Walsh

ANL/CELS
R. Stevens
A. Ramanathan



Funding is provided by: the National Institute of Allergy and Infectious Diseases of NIH under Contract No. HHSN272201700060C, the DOE Office of Science through the National Virtual Biotechnology Laboratory, a consortium of DOE national laboratories focused on response to COVID-19 (the Coronavirus CARES Act) and the use of Structural Biology Center beamlines at the Advanced Photon Source is supported by the U.S. DOE Office of Science and operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357.



Thank you