National Center for CryoEM Access and Training

CryoEM

August 2020

The mission of NCCAT is twofold: to provide nationwide access to advanced cryoEM technical capabilities, and to assist users in the development of cryoEM skills needed for independent research. NCCAT provides access to state-of-the-art equipment required to solve structures to the highest possible resolution using cryoEM methods. Supported by the **NIH Common Fund** Transformative High Resolution Cryo-Electron Microscopy program (U24 GM-129539).

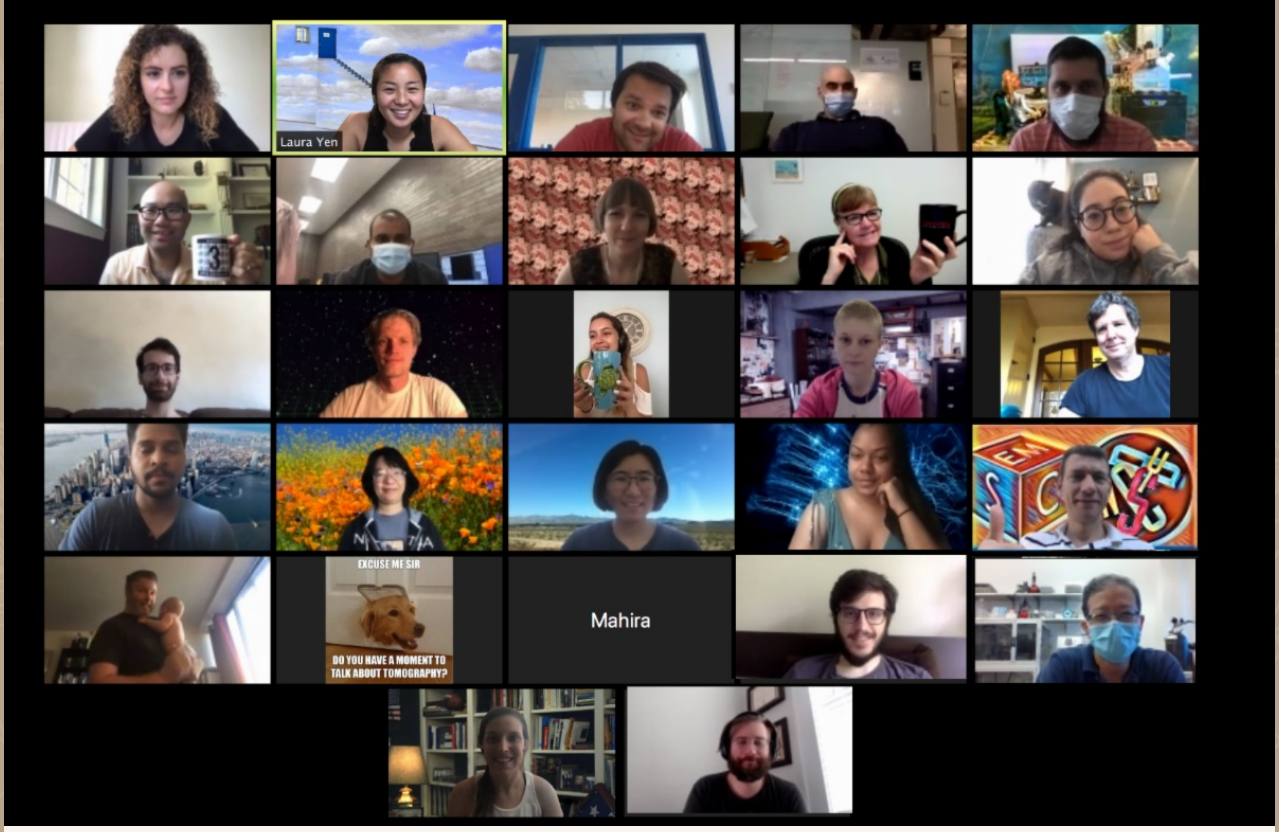
Phased Reopening of NCCAT

NCCAT continued operating during the New York City pandemic lockdown with minimal on-site staff and everyone else working remotely.

With New York City currently in phase IV of reopening we now have staff on site every day and all three installed Krios instruments (which we call Krios #4, #5, and #6) are available for all NCCAT projects, with one microscope continuing to be dedicated to COVID-19 related research.

Users are currently allowed on site only by appointment to drop off samples. At this time we will not be inviting embedded trainees to the facility. We will continue to evaluate on-site access for users as New York City continues to open up.

You can visit the NCCAT website for more information our**COVID-19 operations** including some **Frequently Asked Questions**.



Week 20 of remote working! Despite great temptation, nobody tried to cut their own bangs

Highlighting a SARS-CoV-2 Project

By Cathleen Castello

The COVID-19 pandemic has turned the entire world upside down. Statewide lockdowns, cancelled events, working and schooling from home are just a few things the planet has had to deal with for the past five months and counting. New York emerged early as the epicenter of the pandemic in the United States which left many of us to figure out how to adapt. Fortunately for all of us at NCCAT we were able to transition to remote work with minimal interruption. Additionally we invited researchers with COVID-19 projects to apply for a special rapid access category which underwent an expedited review. One of the first projects we received was from Dr. Jeff Bonnano in Dr. Steve Almo's group at the Albert Einstein College of Medicine.

The Almo lab mainly focuses on using structural and mechanistic principles to develop new therapeutic strategies to treat illnesses like cancer, autoimmune and infectious disease. Their work to determine the structure of cell surface proteins that regulate immunity using x-ray crystallography has lead to the founding of the immunotherapy company **Cue Biopharma**. Moreover, their unique capability to study oxygen-sensitive proteins in an aerobic environment has resulted in the discovery of new therapeutic opportunities, including naturally occurring antiviral drugs.

When the pandemic hit, their research focus, technology, and passion for structure was easily transitioned into work on SARS-CoV-2. In fact, their project to characterize the structure SARS-CoV-2 Spike protein was the perfect opportunity to introduce the group to cryoEM, since all of their previous targets were too small and better suited for x-ray crystallography. Quickly, members of his lab (Natalie Herrera, Nick Morano, and Scott Garforth) began a collaborative effort with Einstein colleague Jon Lai to develop enhanced expression systems for two commonly utilized constructs of the Spike protein. The samples were then transferred to the team at NCCAT who assisted in grid preparation, initial screening, data collection and processing, and structure characterization. Additionally the NCCAT scientists were able to provide basic training, instruction, and guidance in the cryoEM pipeline, as well as data processing and reconstructions while working remotely. In fact, Jeff Bonnano, already an experienced

structural biologist and x-ray crystallographer, is now self sufficient with the data processing/structural determination processes of cryoEM.

With full assistance of the NCCAT staff, and collaborative efforts at Einstein, they were able to produce a 3.2 Å resolution structure of the SARS-CoV-2 Spike protein in less than 48 hours. Dr. Almo credits this as “a remarkable, testament to the phenomenal infrastructure that NCCAT has assembled” as well as the competence and commitment of his team members. The results of this work have been submitted for publication and a preprint of the manuscript is available now on **biorxiv**. Dr. Almo’s team is now using this structure as the foundation to determine the structures of the SARS-CoV-2 Spike protein bound to Fab fragments derived from broadly neutralizing antibodies cloned from the B cells of convalescent patients at Montefiore Medical Center (with Jon Lai and Kartik Chandran). These studies will define the mechanisms responsible for the clinical activity of these naturally occurring antibodies and may afford new therapeutic opportunities. Last week Dr. Anthony Fauci, director of NIAID, referenced the cryoEM structure of the Spike protein in his opening statement to the House of Representatives, emphasizing the importance of these studies.

These days it can feel like there is not much good news in the world. Dr. Almo has assured me that he does not believe this is a sign of the end of days. His lab, NCCAT, and countless other workplaces have been able to adapt to these socially distanced times. Not just adapt, but persevere and accomplish great things. The challenges of the pandemic resulted in even greater synergy and collaborations between the basic science investigators, the clinicians at Einstein and Montefiore Medical Center, and the broader scientific community. If there is any good to be found in this current crisis it’s the amazing penchant of humanity to work together in times a great adversity.

*Much of this story was excerpted from an informal interview with Dr. Steve Almo, which can be read in its entirety on the **NCCAT website.**

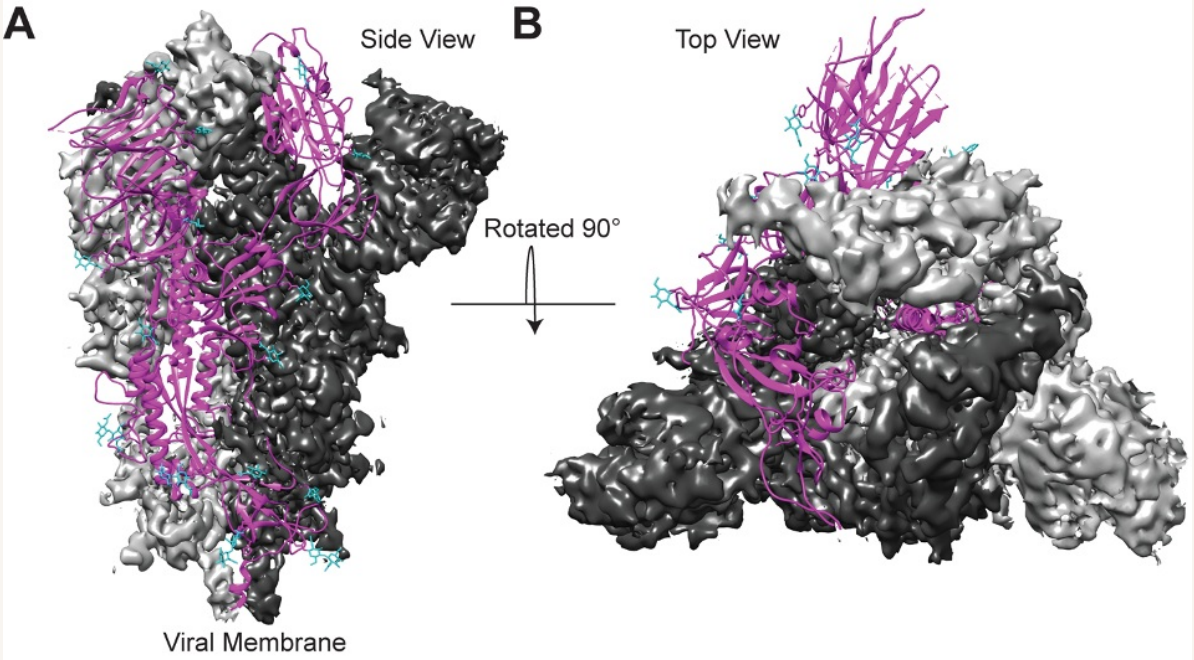


Figure 6 of Herrera *et. al.* "Cryo-EM structure of OptSpike1-CHO in the closed state"

COVID-19 Publications

In April we began accepting projects related to COVID-19 and have so far collected data for 10 of these projects, several papers have already been submitted for publication, and two have been accepted. You can read these papers now at *bioRxiv*, *Nature*, and *Nature Structural & Molecular Biology*.

- **Acharya P, *et. al*.** **A glycan cluster on the SARS-CoV-2 spike ectodomain is recognized by Fab-dimerized glycan-reactive antibodies**. *bioRxiv.* 2020.

- Chen J, *et al*. Structural basis for helicase-polymerase coupling in the SARS-CoV-2 replication-transcription complex. *bioRxiv.* 2020.
- Henderson R, *et. al.* Controlling the SARS-CoV-2 Spike Glycoprotein Conformation. *Nat Struct Mol Bio.* 2020.
- Herrera NG, *et.al.* Characterization of the SARS-CoV-2 S Protein: Biophysical, Biochemical, Structural, and Antigenic Analysis. *bioRxiv.* 2020.
- Liu L, *et al.* Potent neutralizing antibodies directed to multiple epitopes on SARS-CoV-2 spike. *Nature.* 2020.
- Zhou T, *et. al*. Structure-Based Design with Tag-Based Purification and In-Process Biotinylation Enable Streamlined Development of SARS-CoV-2 Spike Molecular Probes. *bioRxiv.* 2020.

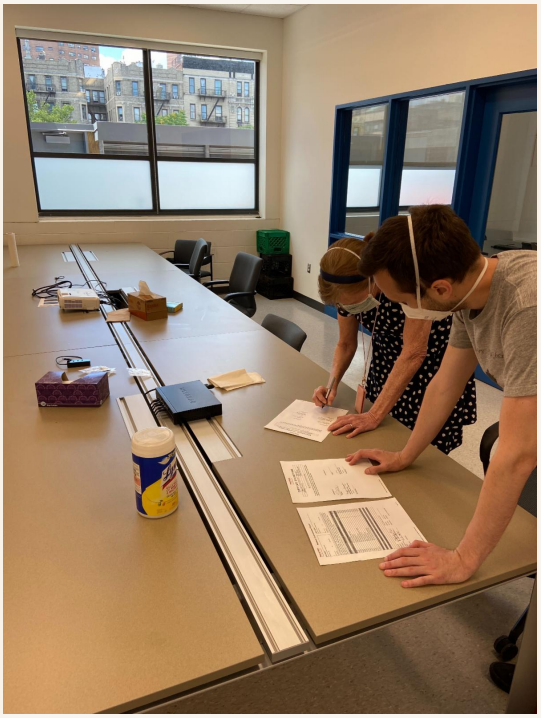
NCCAT is still accepting COVID-19 projects under the **Rapid Access Proposal COVID-19 research (RAP-c)** program. These proposals receive an expedited review. Further details can be at our at our website. Additional inquiries can be emailed to **nccatuseroffice@nysbc.org**.

Krios 5 Sign-Off

On June 26, 2020 NCCAT finally welcomed Krios 5 a.k.a "Nathaniel" to the family.

Nathaniel has been a bit of a problem child for the ThermoFisher engineers and NCCAT staff, but it's been said that great things take time.

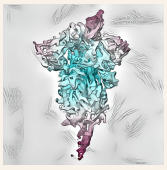
He is now running regular NCCAT projects after successfully completing his benchmark testing and reaching sub 2 Å resolution on our standard apoferritin test sample.



Submit Your Proposal to NCCAT







NCCAT GUP1 PROPOSAL SUBMISSION

The **GUP1 access program** supports single

NCCAT GUP2 PROPOSAL SUBMISSION

The **GUP2 access program** supports use

NCCAT RAP1 PROPOSAL SUBMISSION

The **RAP access program** allows users with

NCCAT BAG PROPOSAL SUBMISSION

The **BAG access program** allows

NCCAT RAPc PROPOSAL SUBMISSION

The **RAPc access program** is a special

particle cryoEM data collection on one of our existing Titan Krios instruments using a Gatan K2 direct-electron detector.	of Chameleon (the commercialized version of Spotiton) and an exploratory screening microscope session.	GUP proposals that have an active URC/UAC score to request additional time on their project without submitting an entirely new proposal.	researchers greater flexibility in instrumentation access by combining General User Proposal (GUP) access proposals	access category for researchers working on COVID-19 related projects. These applications are accepted on a rolling basis and will receive an expedited review.
Submit Now!	**Submit Now!**	**Submit Now!**	**Submit Now!**	**Submit Now!**

VISIT OUR WEBSITE

FOLLOW NCCAT

NCCAT is supported by the NIH Common Fund Transformative High Resolution Cryo-Electron Microscopy program (U24 GM-129539).