Brookhaven National Laboratory

U.S. DEPARTMENT OF ENERGY

Laboratory for BioMolecular Structure (LBMS)

Liguo Wang

The 3rd LBMS annual cryo-EM course

June 20th, 2023

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Laboratory *for* BioMolecular Structure

LBMS *is a center for life science imaging that offers access to state-of-the-art cryo-electron microscopes and laboratory equipment for studies on the building blocks of all living organisms and their behavior.*

Mission: to support and enhance BER mission research through the **development, operation and continued improvement** of a state-of-the-art electron microscopy facility optimized for solving BER-related challenges.

Focus: **complex interactions** specifying the **function of entire biological systems**
— from molecules to organelles, cells and multicellular organisms

LBMS leadership

Science Advisory Committee

Name	Affiliation
Huilin Li (Chair)	Van Andel Institute
Daniela Nicastro	UT Southwestern Medical Center
Alexis Rohou	Genentech Inc.
John Shanklin	Brookhaven National Laboratory
Fred Sigworth	Yale University
Sharon Wolf	Weizmann Institute of Science, Israel
~~Elizabeth~~ Wright	U. of Wisconsin-Madison
Chen Xu	U. of Massachusetts Medical School

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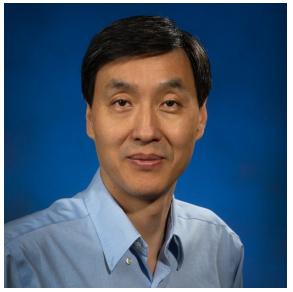
LBMS team

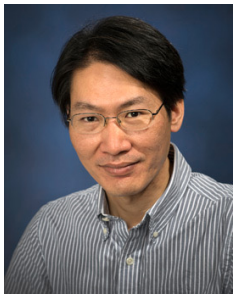
Sean McSweeney
Director of LBMS



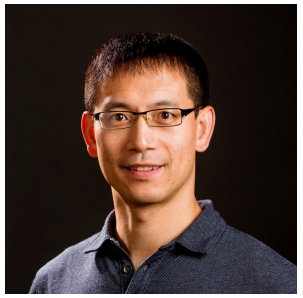
Liguo Wang
Scientific Operations Director

Guobin Hu
Scientist

Jake Kaminsky
Scientific Associate



Qun Liu
PI, Biology Dept.
BNL



Yong Xiong
Professor
Yale

Dongyan Tan
Assistant Professor
SBU

Jun Liu
Professor
Yale

LBMS open for general research

- Proposal lifetime is 2 years
- 2 proposal cycles per year:
  - January – June (proposal deadline September 15)
  - July – December (proposal deadline March 15)
- General User – most common form of user access for routinely-supported experiments
- Block Allocation Groups (BAGs) – groups of researchers that want to combine their short microscope time requests into a single proposal to permit greater flexibility in beam time scheduling
- Rapid Access (6 month) – rapid access to instrument time for “hot topics” or for straightforward experiments with a fast turnaround time
- Proprietary – full cost-recovery instrument time
- BER outreach activity (no proposal required)

- Guaranteed: 200 days/year
- Users GU/BAG: 52.5%
- Users Rapid: 7.5%
- Outreach: 15.0%
- Development: 10.0%
- Collaboration: 7.5%
- Proprietary: 7.5%

https://www.bnl.gov/cryo-em/userguide/

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Access to Electron Microscopes (EMs) at LBMS

Screening EMs
Talos 120C & Jeol 2100

Proposal process
- Proposal submission
- Feasibility review
- Peer review

Biochemical screening
- Mono disperse sample
- High enough concentration
- Enough quantity

NS screening
- Iterative negative staining (NS) grid preparation
- Image using screening EMs
- Assess sample quality (e.g. homogeneity)

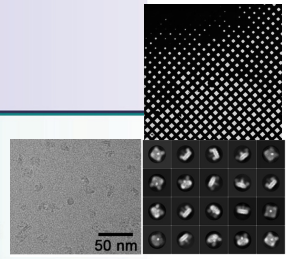
Cryo-EM screening
- Iterative cryo-sample preparation
- Image using screening EMS
- Assess sample quality: Image (e.g. ice thickness, particle density) 2D classification (e.g. no preferred orientation)

Users will be trained in person and must be onsite.

High-resolution EM
Krios with K3 & GIF

Proposal process
- Proposal submission
- Feasibility review
- Peer review

Screened cryo-sample
- Present preliminary results e.g. raw image, 2D class averages
- Send only prescreened cryo-samples



High resolution data collection
- Select optimal imaging conditions
- Setup automated data collection
- Collect data

Image processing
- Transfer data back to home institution
- Reconstruct 3D EM density map
- Build atomic models
- Validate structure and publish

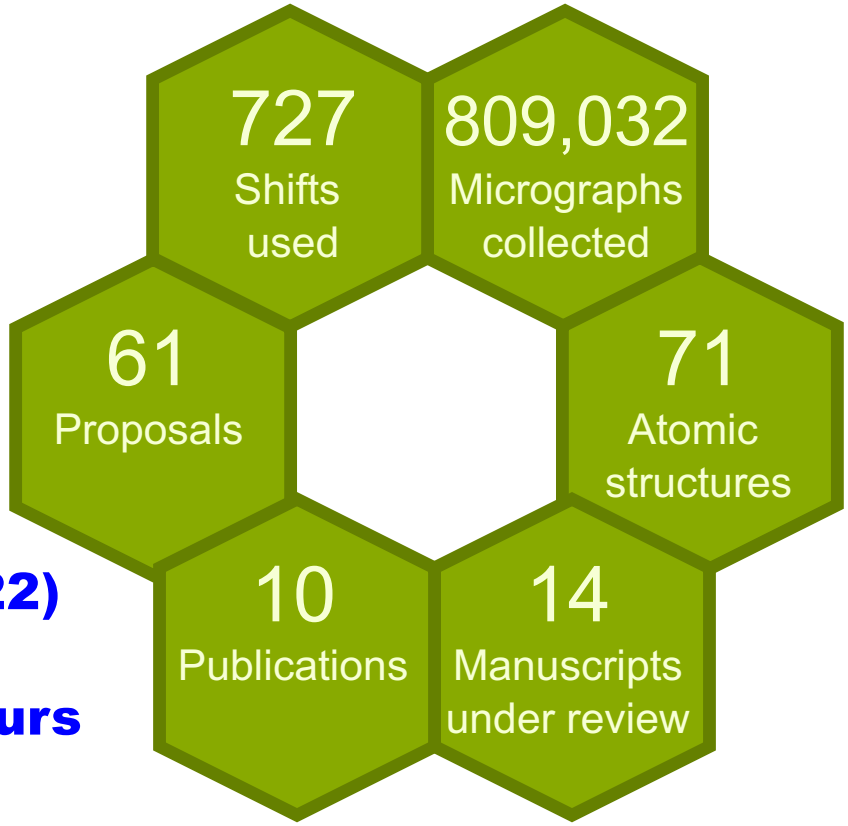
Users will be trained virtually.
Users' presence is required (Zoom meeting).

https://www.bnl.gov/cryo-em/userguide/

LBMS statistics: Krios is 5X over subscribed

Planned usage per year

- Guaranteed: 600 shifts/year
- Users GU/BAG: 315 shifts
- Users Rapid: 45 shifts
- DOE Outreach: 90 shifts
- Development: 60 shifts
- Collaboration: 45 shifts
- Proprietary: 45 shifts



Actual usage (1/1-12/31/2022)

121% of the guaranteed hours

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LBMS: 3-tiers training

1) Annual 4-day cryo-EM course to the public.
2) Quarterly cryo-EM workshops for current and potential LBMS users.
3) On-demand 5-day in-person training on screening EMs for LBMS users and on-demand remote training on the high-end EM.

https://www.bnl.gov/cryoemcourse/

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Second LBMS annual cryo-EM course
(June 14-17, 2022)

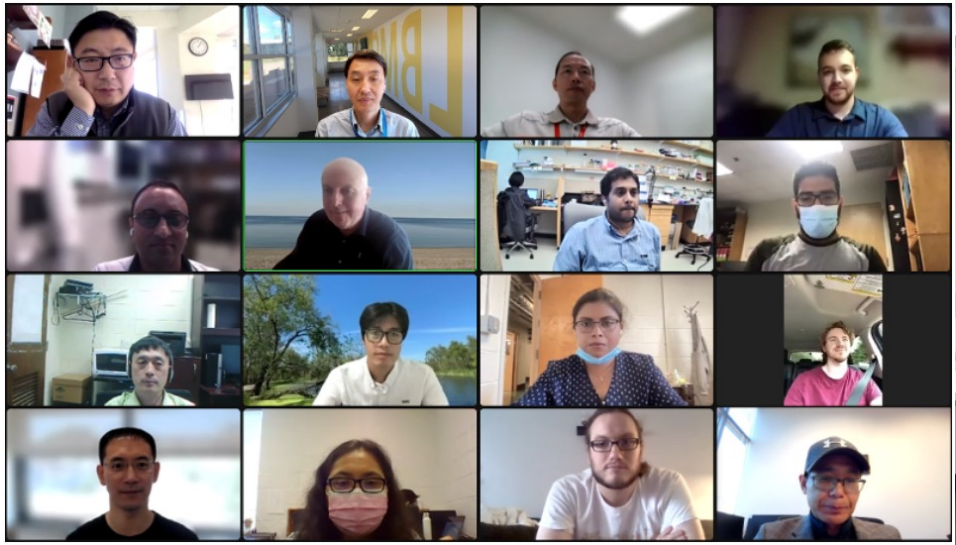
Trend in CY 2022:

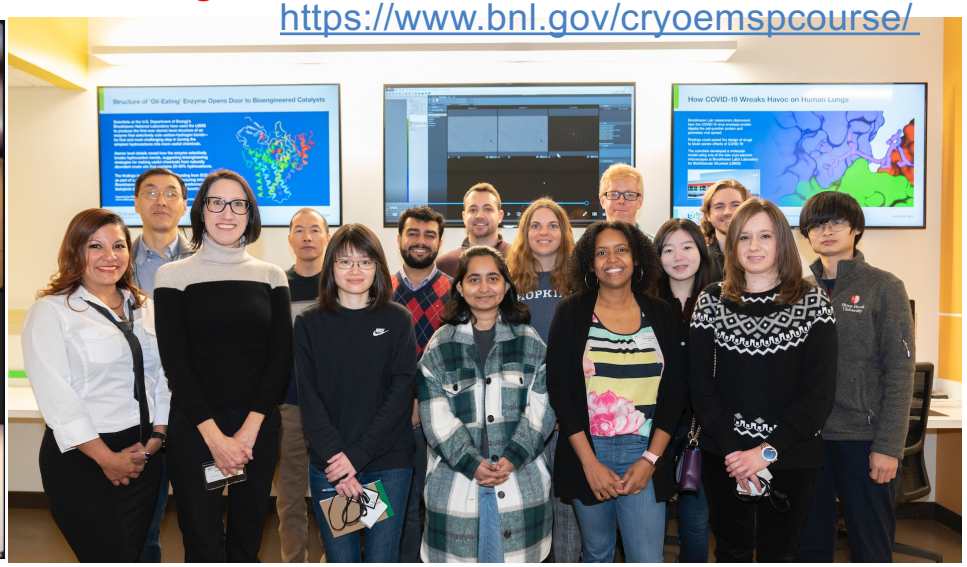
- More people interested in cryo-ET: 25% of attendees
- More industrial attendees including Pfizer and Moderna

Quarterly cryo-EM workshop

- Sample preparation: February 4, 2022
- Automated data collection: May 6, 2022
- Cryo-EM SPA data processing: August 5, 2022
- Cryo-ET data analysis: November 4, 2022
- 2023-1 sample preparation and screening (**in-person**): February 3, 2023
- 2023-2 workshop (NSLSII, CFN and LBMS user meeting): April 26, 2023
- 2023-3 cryo-EM SPA data processing (Virtual): August 4, 2023

https://www.bnl.gov/cryoemspcourse/



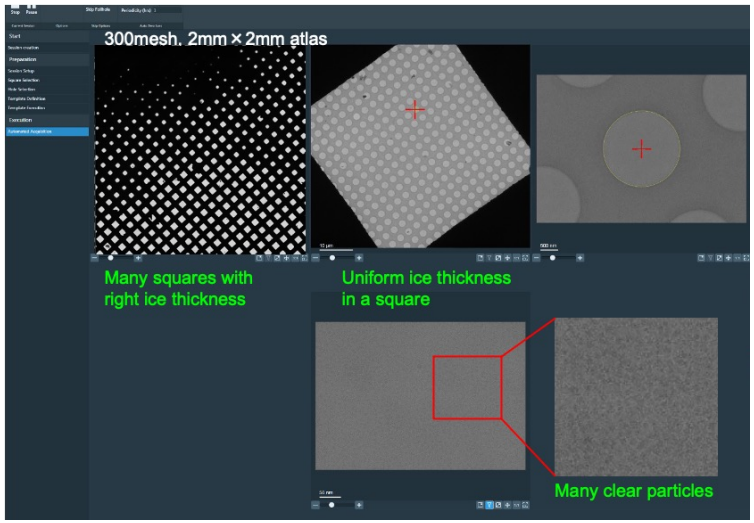


Average rating: 4.5/5.0, Likely to recommend: 100%

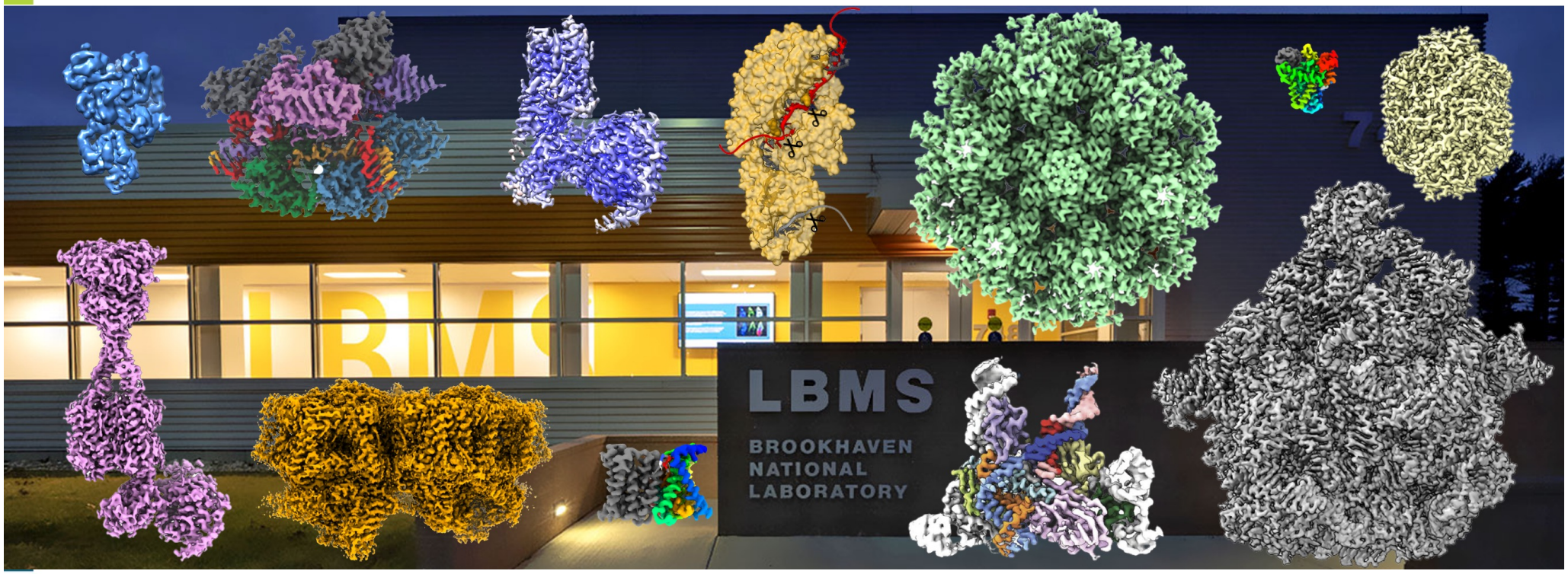
Training: in-person and remote

- Negative staining sample preparation: Half day for up to three people
- Talos operation with room temperature holder/sample: One day for each person including sample loading/unloading, EM alignment, EPU for lacey/continuous carbon grid
- Cryo-sample preparation with Vitrobot: One day for up to three people, and one more day to practice (preferred to have their own samples).
- Talos operation with Cryo-sample/holder (require completion of training with room temperature holder/sample): A two-day session for each person including sample loading/unloading, EM alignment, EPU for holey carbon grids
- **Krios data collection**: remote

Name	Proposal	Institution
Haijiao Liu	307854	SBU
Enju Lima	307995	SBU
Chi-Lin Tasi	310009	University of Texas MD Anderso
Martín Dodes Traian	310182	Scripps at Florida
Brittany Wheatley	310182	Scripps at Florida
Jyothi Chandras Sistla	307795	SBU
Martien	307854	BNL
Lingshuang Wu	307795	SBU
Kreitler, Dale	307854	BNL
Shujuan Gao	307795	SBU
Blanford, Jantana	307854	BNL
Sajina Bhandari	307854	BNL
Khiem Nguyen	308683	UConn



Structures determined from data collected at LBMS in 2022



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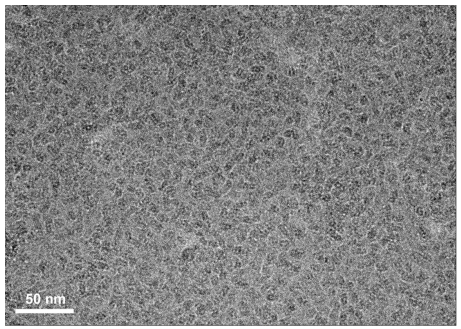
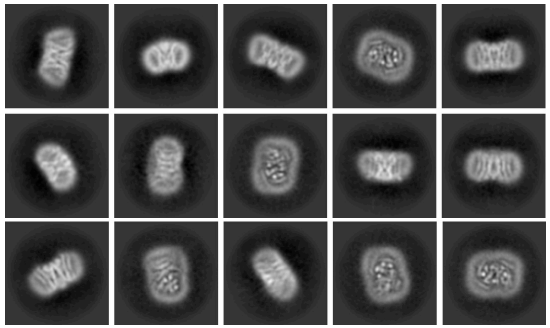
- Micrographs collected: 809,032
- Atomic structures determined: 71
- Smallest structure: 48 kDa (2.7 Å)

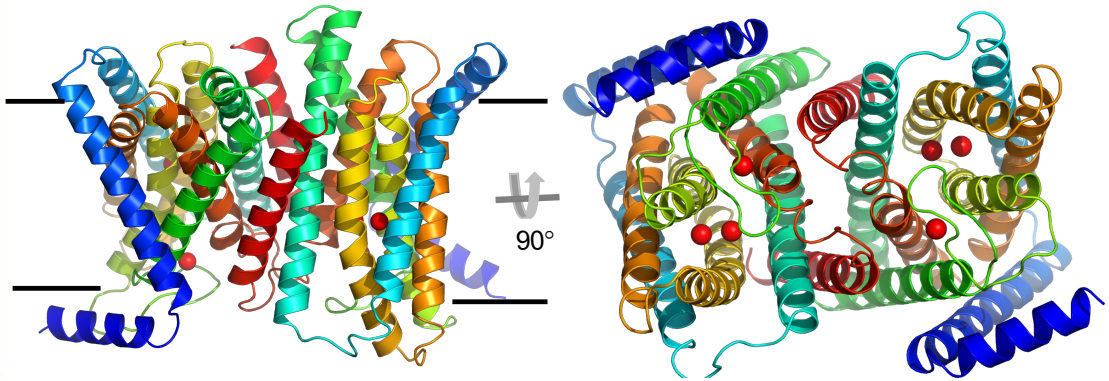
Cryo-EM structure of zinc uptake across membranes



- A representative cryo-EM micrograph



- Averaged 2D classes show features of a dimeric transporter structure



- Cryo-EM structure of a zinc transporter dimer. Captured metals (cadmium) are shown as red spheres.

Motivation To provide structural basis for engineering the zinc uptake process for promoting growth of bioenergy crops on zinc-deficient marginal land.

Approach The Liu group at BNL QPSI used the LBMS cryo-EM to determine a zinc uptake transporter structure to understand the zinc uptake process from the environment.

Results

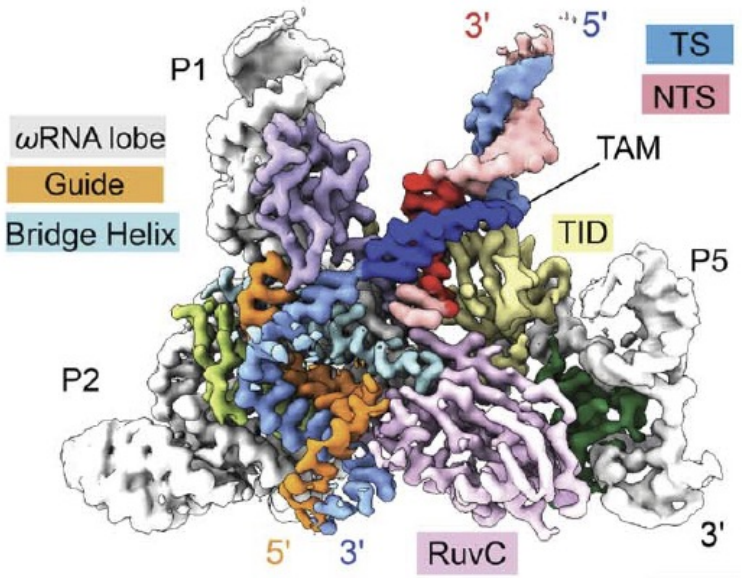
- The cryo-EM structure was determined at 3.1 Å resolution which reveals an inward-facing, occlude dimer structure of its own kind.
- The structure suggests **a novel intracellular zinc sensing and autoregulation mechanism** on zinc uptake.

Impact The structure will provide insights into the design of improved bioenergy crops growing on zinc-deficient marginal land.

Funded by BER

Pang, C., et al, Nature Communications 14, 3404, (2023).

Structural Insight for Advancing Gene-Editing Technology



Cryo-EM reconstruction at 2.78 Å of the IscB-ωRNA/target DNA complex. TS=target strand DNA; NTS=nontarget strand DNA.

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Scientific Achievement

Scientists showed that the evolution of the IscB enzyme to the popular gene-editing Cas9 enzyme involved dwarfing the associated ωRNA and the introduction of protein domain replacements.

Significance and Impact

Structure-guided insight into miniaturizing the Cas9 enzyme is important to developing the next generation of CRISPR-Cas9 genome editors.

Research Details

- **Cryo-electron microscopy at the LBMS was used to determine the high-resolution structure of** IscB-ωRNA bound to a double-stranded DNA.
- The structure explained target-adjacent motif recognition, R-loop formation, and **DNA cleavage mechanisms, providing a detailed comparison between IscB and Cas**9.

Ke laboratory, Cornell University

G. Schuler, C. Hu, A. Ke. Structural basis for RNA-guided DNA cleavage by IscB-ωRNA and mechanistic comparison with Cas9. *Science*, **376**, 1476-1481 (2022).

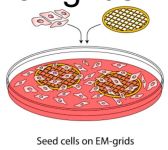
Cryo-Electron Tomography (cryo-ET)

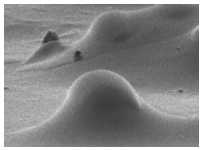
Plunge freezer

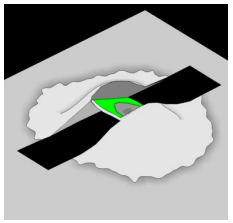
Cryo-FIB Installed in a month!

Cells grown on grids

Up to 10 µm thick

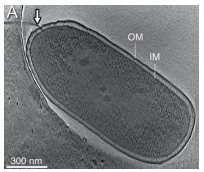






Cells deposited on grids

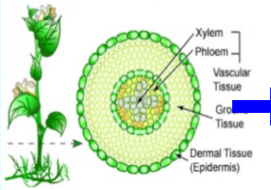
Small or peripheral



Thinner than 0.5 µm

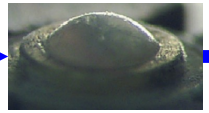
Cryo-ET

Tissues

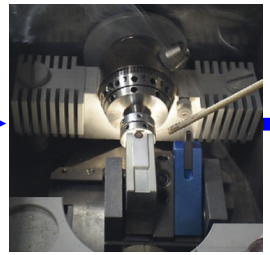


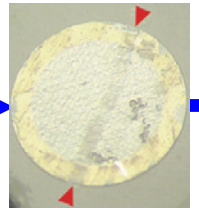
High pressure freezer

Being acquired!



Cryo-microtome





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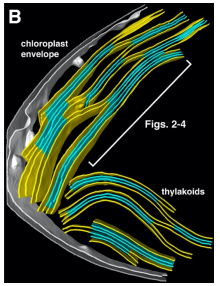
Up to 200 µm thick

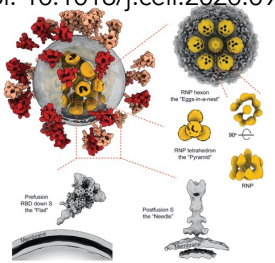
Chloroplast cup

doi: 10.7554/eLife.04889



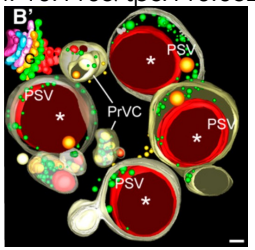
SARS-CoV-2

doi: 10.1016/j.cell.2020.09.018



Endosperm Cells

doi: 10.1105/tpc.110.082156



Important websites and contact information

- LBMS website: https://www.bnl.gov/cryo-em/
- Online calendar: https://lbmscalendar.bnl.gov
- Registration & training: https://www.bnl.gov/cryo-em/userguide/next-steps.php
- PASS for proposal management: https://pass.bnl.gov
- Forms: http://www.bnl.gov/cryo-em/forms.php.
- LBMS mailing list: lbms-em-l@lists.bnl.gov
- Quarterly cryo-EM workshop: https://www.bnl.gov/cryoemspcourse/

- Nancye Wright, Proposal Coordinator: 631-3445132, wright@bnl.gov
- Guobin Hu, EM Scientist: 631-3447915, ghu@bnl.gov
- Jake Kaminsky, Scientific Associate: 631-3448980, jkaminsky@bnl.gov
- Liguo Wang, Director of Scientific Operations: 631-3447011, lwang1@bnl.gov

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Thank you!

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