Cryo-electron microscopy (cryo-EM) has rapidly emerged as a transformative technology in structural biology, enabling the detailed visualization of biomolecular structures at near-atomic resolution. However, the successful adoption and integration of cryo-EM into research programs often require significant initial investment, both in terms of financial resources and technical expertise. Pilot funding plays a critical role in this context, serving as a catalyst for cryo-EM projects that might otherwise face insurmountable barriers. Pilot funds provide researchers with the opportunity to explore innovative hypotheses, optimize experimental conditions, and generate preliminary data essential for securing larger, more sustained funding. This initial support is particularly vital in cryo-EM, where the complexity and cost of equipment, sample preparation, and data analysis can be prohibitive. Through targeted pilot programs, researchers can establish proof of concept, demonstrating the feasibility and potential impact of their projects. The success of cryo-EM pilot projects is often measured by their outcomes, notably the number and quality of publications and the deposition of high-resolution structures in public databases. These successes not only validate the initial investment but also contribute to the broader scientific community by providing new insights into molecular mechanisms and potential therapeutic targets. Moreover, the data generated through pilot-funded projects can significantly enhance the likelihood of obtaining subsequent funding from major grant agencies, thereby ensuring the continued advancement of cryo-EM research. Therefore, pilot funds are indispensable in fostering the growth and success of cryo-EM projects. By enabling researchers to overcome initial hurdles and achieve significant milestones, these funds play a pivotal role in advancing the field of structural biology and expanding our understanding of complex biological systems.

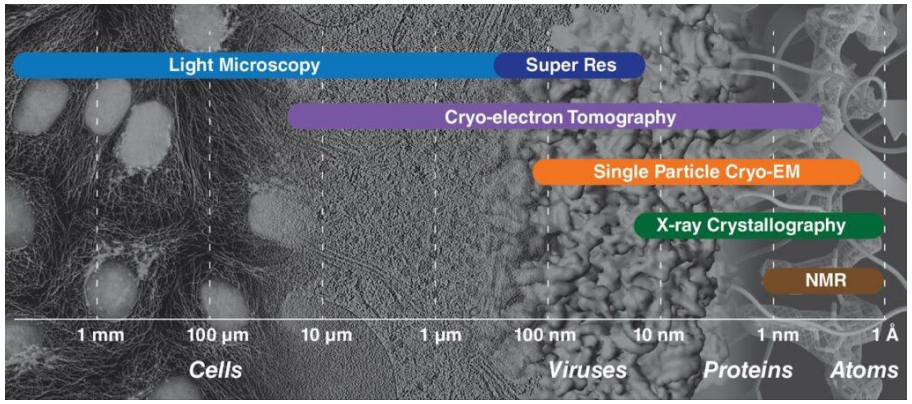
Driving Cryo-EM Success: Leveraging Pilot Programs to Grow Your Userbase

Vitor Hugo Balasco Serrão

Director of the Biomolecular Cryo-Electron Microscopy Facility
Department of Chemistry and Biochemistry
University California - Santa Cruz

WACD 2024 – San Diego

What is (cryo)-EM?



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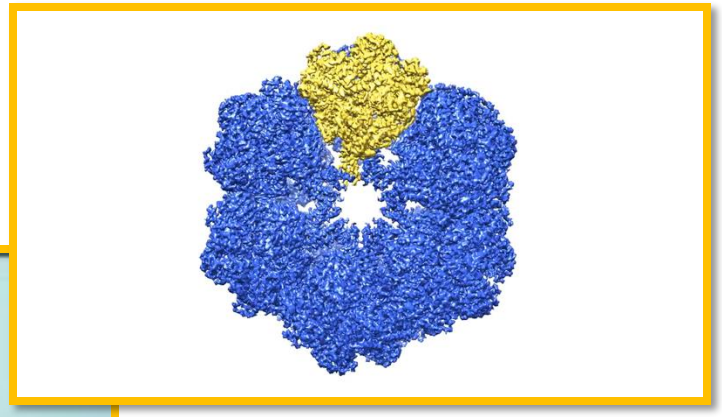

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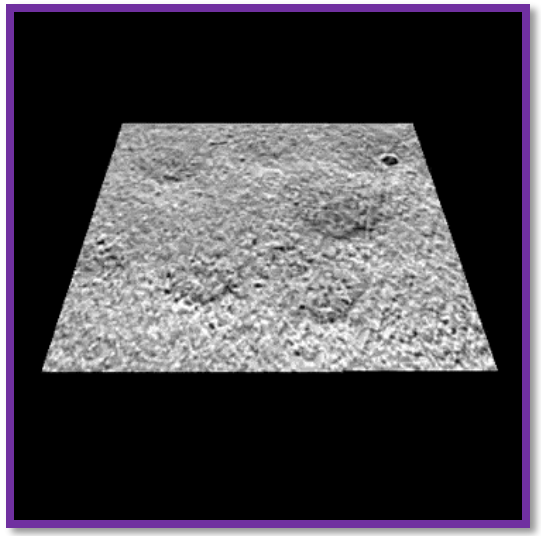

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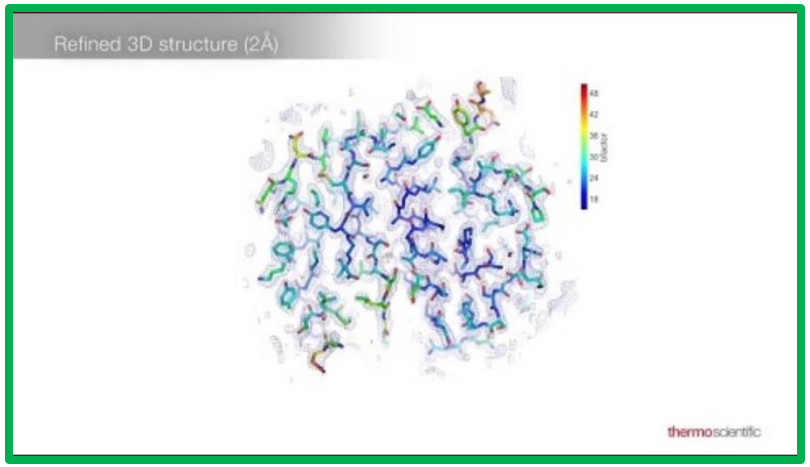

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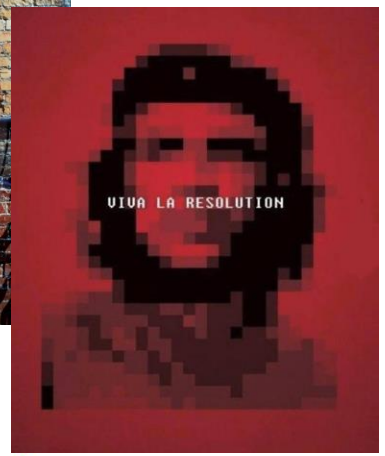
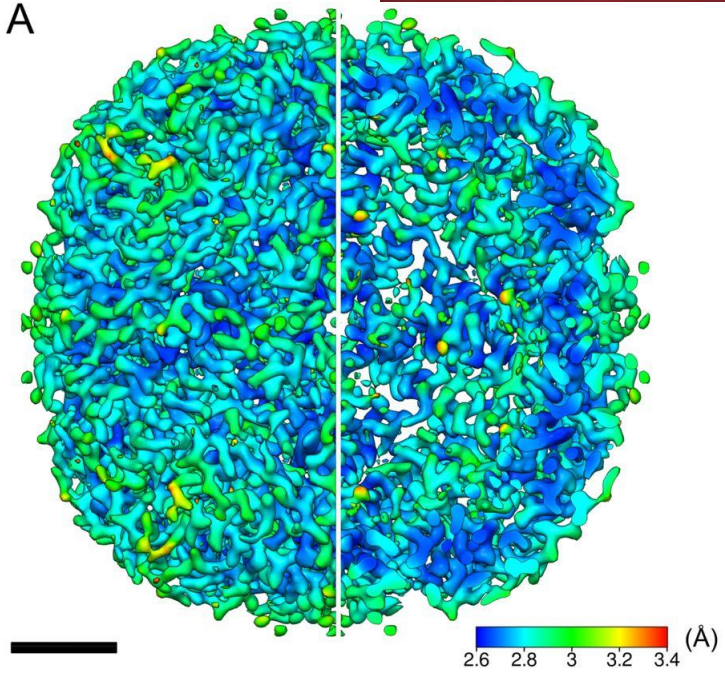
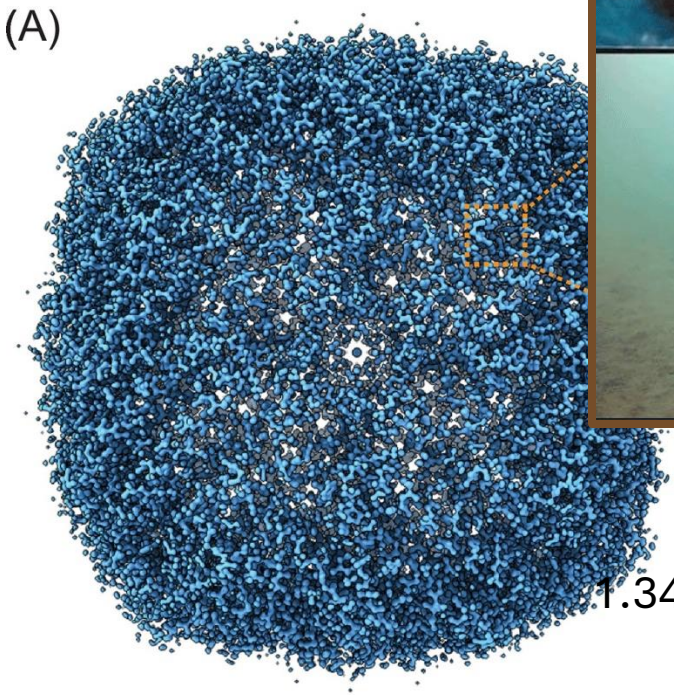
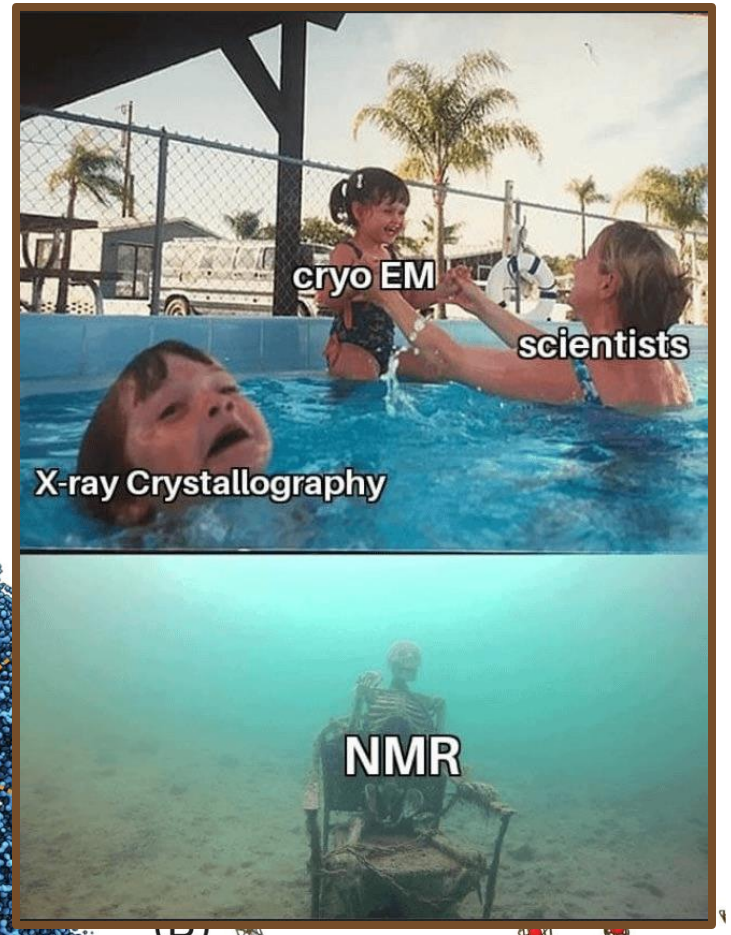
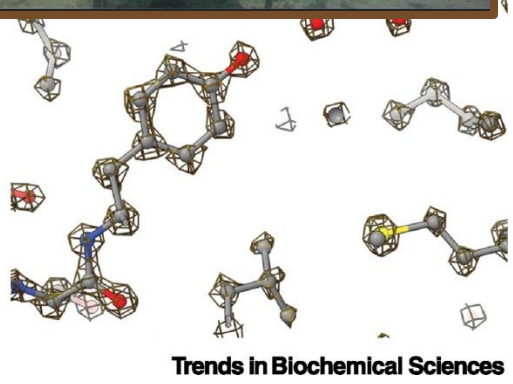

VIVA LA RESOLUTION


A
B
2.6 2.8 3 3.2 3.4 (Å)
C
HIS65 HIS60 ARG43 MET37 PHE132 LYS146 TYR32 TRP93 TYR34


(A)
1.34 Å


cryo EM
scientists
X-ray Crystallography
NMR


Trends in Biochemical Sciences

Once Upon a Time

NIH
National Institutes of Health

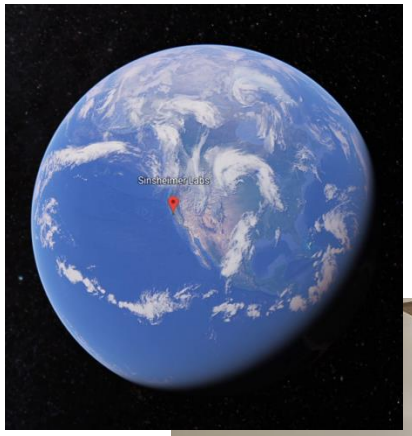
+

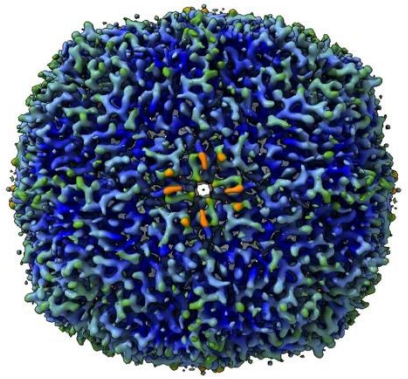
Office of Research
UC SANTA CRUZ

S10 High-End Instrumentation Grant
TFS Glacios 200 kV

R01 Supplements
Gatan K2 Direct Detector
4x workstations
250 TB Storage

Renovations & Infrastructure
50% Staff's salary
$30,000 to create user's base – Pilot Program





TFS Falcon4i (*Agouron Institute*)
2.6 Å / 585 images (1h)

TFS Glacios 200 kV

- Gatan K2 Summit
- Recently installed TFS Falcon4i
  - MicroED capabilities
  - HTP-Single-particle cryoEM

Live-data processing

- CS-Live
- TFS Tomo-Live
- Auto-indexing/integrating diffraction

Sample preparation

- Manual plunger
- Leica GP2
- TFS Vitrobot Mark IV

Data management

- 2.5 PB storage
- 13 x remotely access workstations
- 32 x GPUs (RTX3090 / RTX4090)

Data acquisition	SPA		cryoET & STA	microED	Model building & refinement
EPU	cryoSPARC v4.5.1	cryoCARE	Dynamo	AutoMicroED	Model-Angelo
SerialEM	Relion 4.0	cryoDRGN	IMOD	XDS	AlphaFold Multimer / ColabFold
Tomo5	Relion 5.0	cryoID	IsoNet	DIALS	Coot 0.9
EPU-D	cisTEM	Topaz	Napari	MicroED tools	Phenix 1.19.2
	EMAN 2.91	crYOLO			Chimera
		Spisonet			ChimeraX

UC SANTA CRUZ CRYO-EM

User's base

21 research groups at UCSC

11 external academic labs
(3x University of São Paulo; 4x University of Toronto; 1x Queen's University, 1x Brandeis University / Scripps, 1x Standford, 1x University of Madrid)

10 companies

Active projects

- 30+ UCSC
- 10 – non-UCSC

Projects at national / high-end facilities

- PNCC – 16
- SLAC – 1
- UCSF – 4
- Janelia/HHMI – 2
- Berkeley - 2

https://www.ucsccryoem.org/
Twitter > 600 followers
Instagram > 260 followers

Pilot Program (2021-2022)

Total amount - $30,000

Maximize number of users

- Small packages for sample preparation/screening ($1,500)
- If successful, a second package for data collection ($1,500)
- Max of 10 projects
- Internal committee (4 PIs + Facility's Director) – evaluation of the one-page proposal

UC SANTA CRUZ Cryo-EM

CALL FOR PROPOSALS

My First Freezing: cryo-EM Project

Cryo-electron microscopy (cryo-EM) is revolutionizing structural investigations of biological and material samples, and the new UCSC Biomolecular cryo-EM facility is here for you to exploit this exciting new technology. Cryo-EM can contribute to biomedical research focusing on structural biology, enzymology, microbiology, cell biology, and ligand discovery, to name a few applications. Material sciences and soft materials characterization can also benefit from the atomic resolution information accessible by cryo-EM and is compatible with our instrumentation.

With the establishment of our new facility, the UCSC Office of Research has made funding available to kick-off new projects and collect preliminary data for future extramural grant applications. We are pleased to announce a call for applications for this funding entitled "*My First Freezing: cryo-EM Project*". Awardees will receive a **$1500 "recharge voucher"** for the UCSC cryo-EM facility, which can be used for sample preparation and EM supplies that will allow preliminary data acquisition and analysis. Based on project progress and availability of funds, a second voucher may also be requested.

Who can apply?
UC-affiliated researchers that aim to visualize biological and/or material samples in nano-to-micrometer size scale. Sample purification and previous characterization may not be required depending on the goals.

What should you expect?
Please contact the facility manager (Vitor Serrão - vbalasco@ucsc.edu) to discuss your project ideas and feasibility. This discussion will clarify what is typically needed to get going- types and amounts of samples that are ready (or near ready) to be put on the microscope – and what preliminary data can be obtained with the initial budget and how it will impact the future grant applications and projects achievability.

Eligibility Conditions
- UC-affiliated researchers (PIs/postdocs/grad-students).
- New facility users and projects that are not already included as major users and/or have funded opportunities for cryo-EM projects will be considered during the selection.
- The proposal should present an appropriate and suitable target and methodology.
- The researchers should provide enough evidence of a well-behaved sample of interest.

Submission
Submit a one-page PDF file containing a brief overview of your project (including goals and/or preliminary data that might support project feasibility) and how cryo-EM will contribute to its success and new extramural funding to cryoem@ucsc.edu. Applications will be evaluated by a technical advisory working committee on a rolling basis, with the first round of results announced on February 28th 2022.

Total of 3 applicants!

My First freezing – 1st round

Imaging Semiconducting Coacervates – Ayzner's Lab

4 students/trainees using (cryo)-TEM
NSF proposal submitted and after accepted
1 Publication

$9,797

Cryo EM of UPF1::ribosome complexes – Arribere's Lab

3 students using SPA-cryoEM -> (Enisha Sehgal – PhD candidate at UW Baker's Lab AI/SPA-cryoEM)
NIH R35 renewal
PNCC proposal (160263) accepted – 4 datasets collected at high-resolution
1 Publication (cover page)

$10,511

Using cryo-EM to study conformational changes of the Gin4 kinase – Kellogg's Lab

1 PhD thesis/training (Francisco Mendez – Postdoc at UCSD doing cryoEM)

$2,831

~$23,000

Pilot Program (2022-2023)

Total amount ~$7,000 left

Maximize number of users

- Small packages for sample preparation/screening ($1,500)
- If successful, a second package for data collection ($1,500)
- Max of ~3 projects
- Internal committee (4 PIs + Facility's Director) – evaluation of the one-page proposal

UC SANTA CRUZ CRYO-EM

CALL FOR PROPOSALS

My First Freezing: cryo-EM Project

Cryo-electron microscopy (cryo-EM) is revolutionizing structural investigations of biological and material samples. The UCSC Biomolecular cryo-EM facility is here to help you exploit this exciting new technology. Cryo-EM can contribute to biomedical research focused on structural biology, enzymology, microbiology, cell biology, ligand discovery, etc. Material sciences and soft materials characterization can also benefit from the atomic resolution information accessible by cryo-EM with our instrumentation.

The UCSC Office of Research made funding available to kick off new projects and collect preliminary data for future extramural grant applications. We are pleased to announce a second round of "*My First Freezing: cryo-EM Project*". Awardees will receive a **$1500 Recharge Voucher** for the UCSC cryo-EM facility. The voucher can be used for EM sample preparation and preliminary data acquisition and analysis. Based on the project's progress and the availability of funds, a second voucher may also be requested. Our initial awardees were able to successfully obtain preliminary data to apply for grants, start high-resolution data collection at National Facilities, and publish results.

Who should apply? UC-affiliated researchers aiming to visualize biological and/or materials samples on a nano-to-micrometer size scale. Be aware that highly purified samples and previous characterization *may not be necessary* depending on the goals of the project. Please contact the facility manager (Vitor Serrão - vbalasco@ucsc.edu) to discuss your project ideas and feasibility. This discussion will clarify what is typically needed to get started- types and amounts of samples that are ready (or near ready) to be put on the microscope; what preliminary data can be obtained with the initial budget; and potential impact on project achievability and future grant applications.

How to apply?
Submit a one-page PDF file containing a brief overview of your project (including goals and/or preliminary data that might support project feasibility) and explaining how cryo-EM will contribute to its success and new extramural funding to cryoem@ucsc.edu. Applications will be accepted on a rolling basis.

Evaluation Criteria
- UC-affiliated researchers (PIs/postdocs/grad-students).
- The proposal should present an appropriate and suitable target and methodology.
- The researchers should provide enough evidence of a well-behaved sample of interest.

Applications will be evaluated by the Cryo-EM working committee, with the first round of results announced on February 10th 2023.

Total of 4 applicants!

My First freezing – 2nd round

General properties and hypothesis for VHPO from Enhygromyxa salina (esVHPO) for cryo-EM studies – Mckinnie's Lab $2,999

1 student using (cryo)-TEM
PNCC proposal (160337) accepted – 4 datasets collected at high-resolution
2 datasets collected at UCSF
1 Publication (submitted)

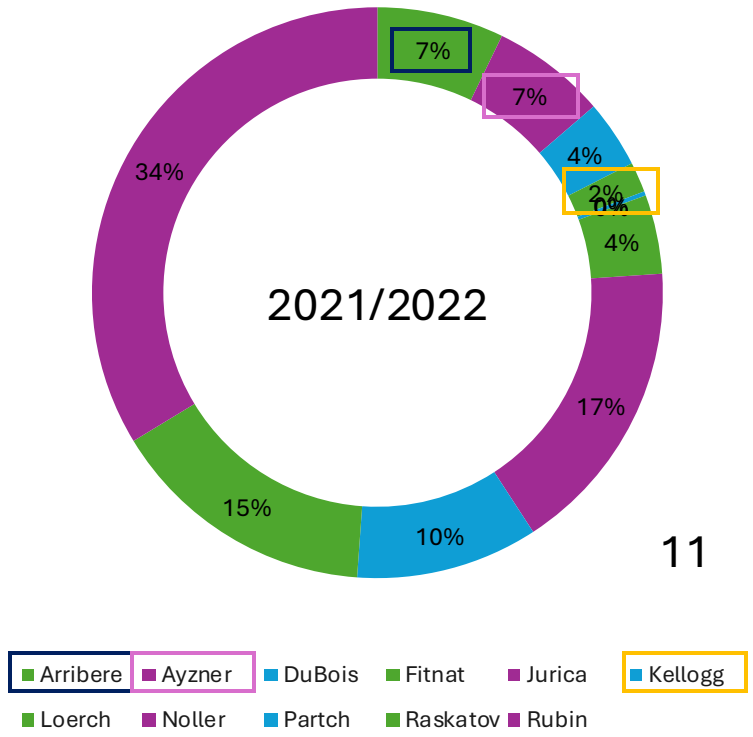
Cyclic Peptide – RNA Complex Visualization – Lokey's Lab $2,937

1 PhD thesis/training (Kevin Yang – Working on Biotech/Biopharma interacting with cryoEM)

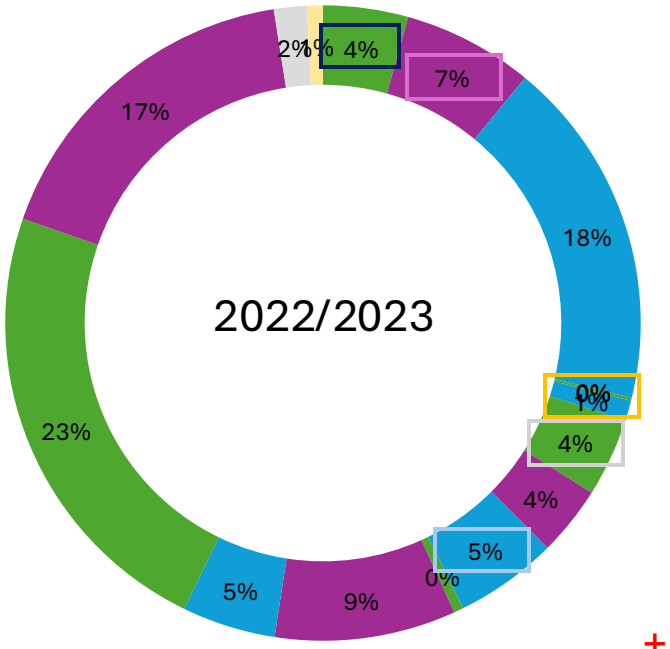
Cryo-EM studies of the antimicrobial activity of functional nanocomposites – Chen's Lab $1,482

1 student using (cryo)-TEM
1 cryoET (tilt series) dataset collected at BNL – cryoEM Facility

~$7,500

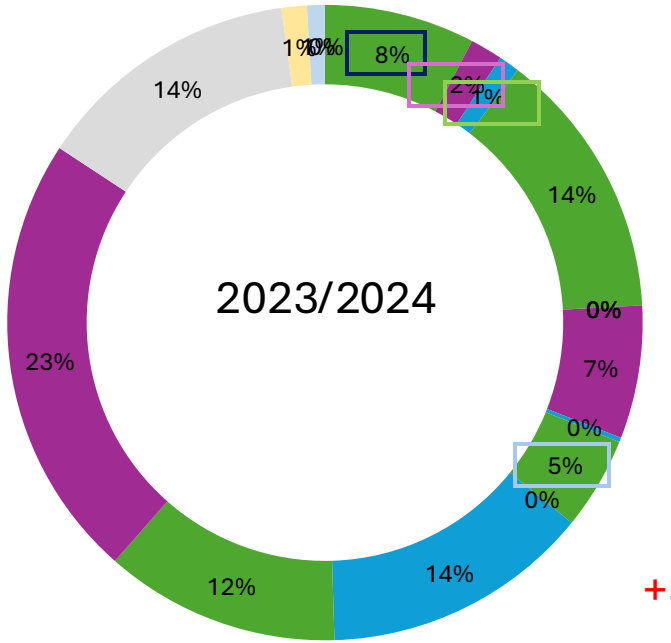


11



+5

Arribere
Ayzner
DuBois
Fitnat
Jurica
Kellogg
Loerch
Lokey
McKinnie
Millhauser
Noller
Partch
Raskatov
Rubin
Shanks
Zhang



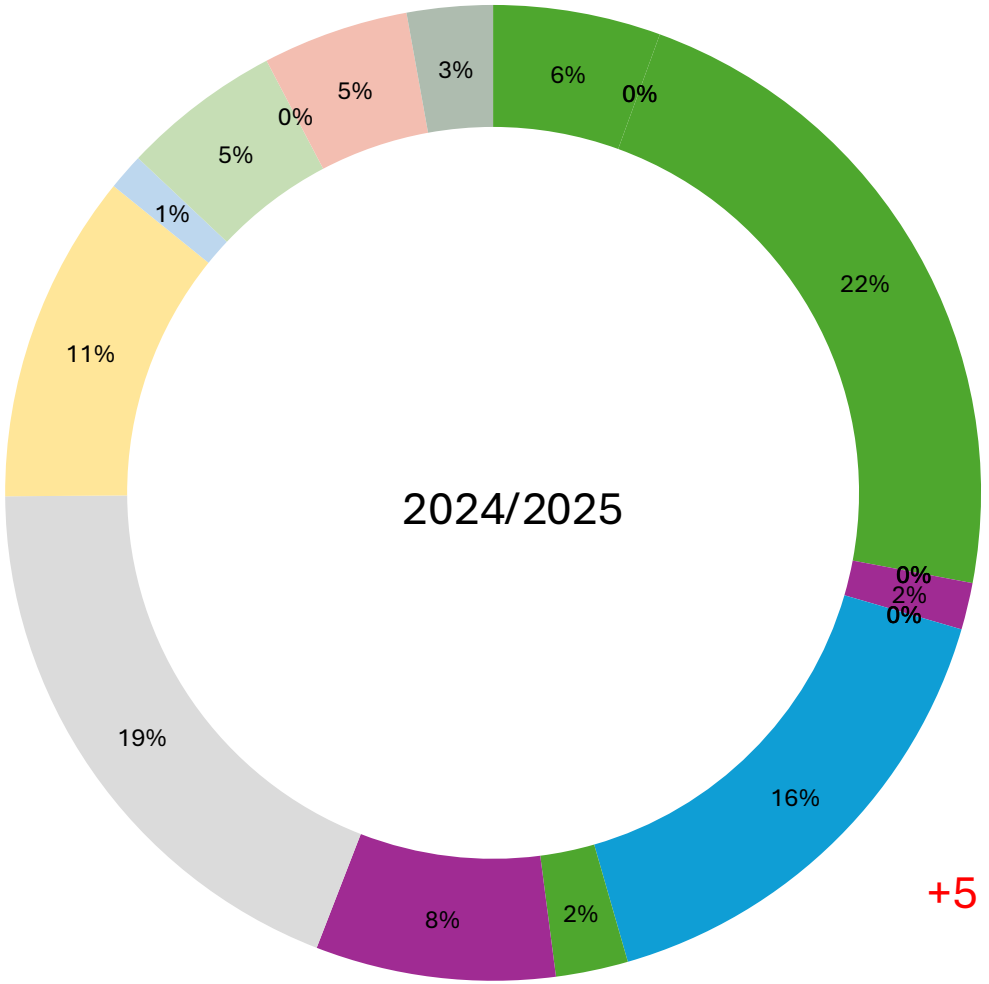
+3

Arribere
Ayzner
Chen
DuBois
Fitnat
Jurica
Kellogg
Loerch
Lokey
McKinnie
Millhauser
Noller
Partch
Raskatov
Rubin
Shanks
Zehr
Zhang

Growing user base

21/22	22/23	23/24	24/25
16%	21%	16%	8%

User	Rank
Arribere	#6
Ayzner	#8
Chen	#15
Kellogg	#14
Lokey	#11
McKinnie	#9



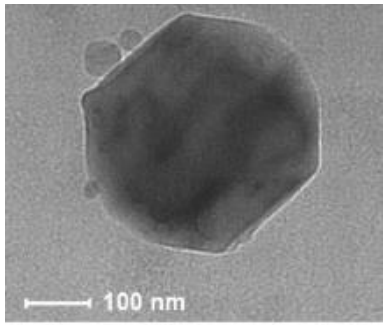
+5

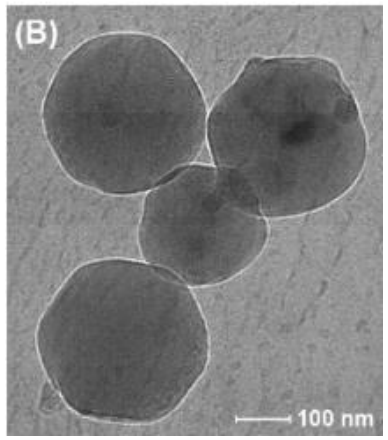
Publications

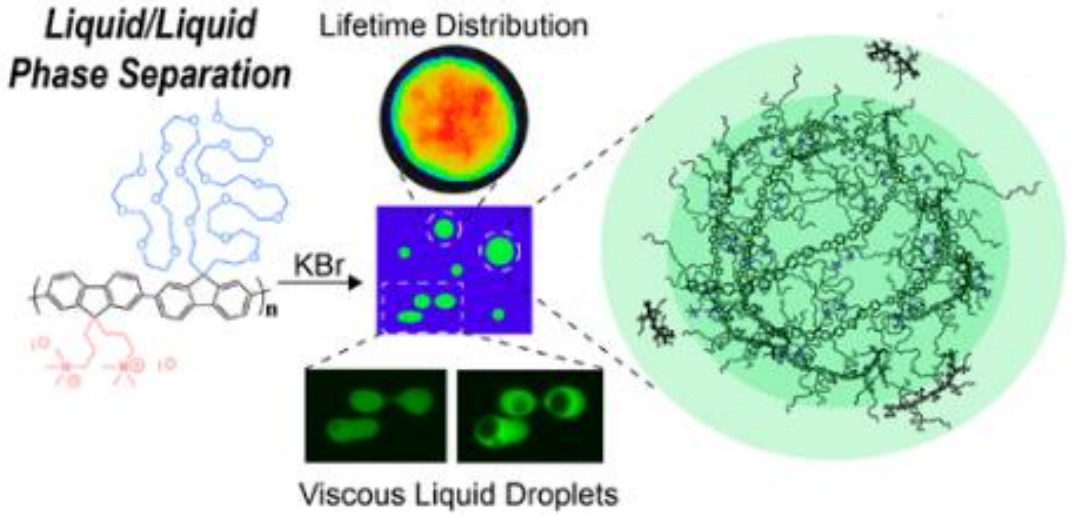
Excitonically Coupled Simple Coacervates via Liquid/Liquid Phase Separation.

Anna R. Johnston, Gregory M. Pitch, Eris D. Minckler, **Ivette G. Mora**, Vitor H. Balasco Serrão, Eric A. Dailing, and Alexander L. Ayzner

The Journal of Physical Chemistry Letters 2023





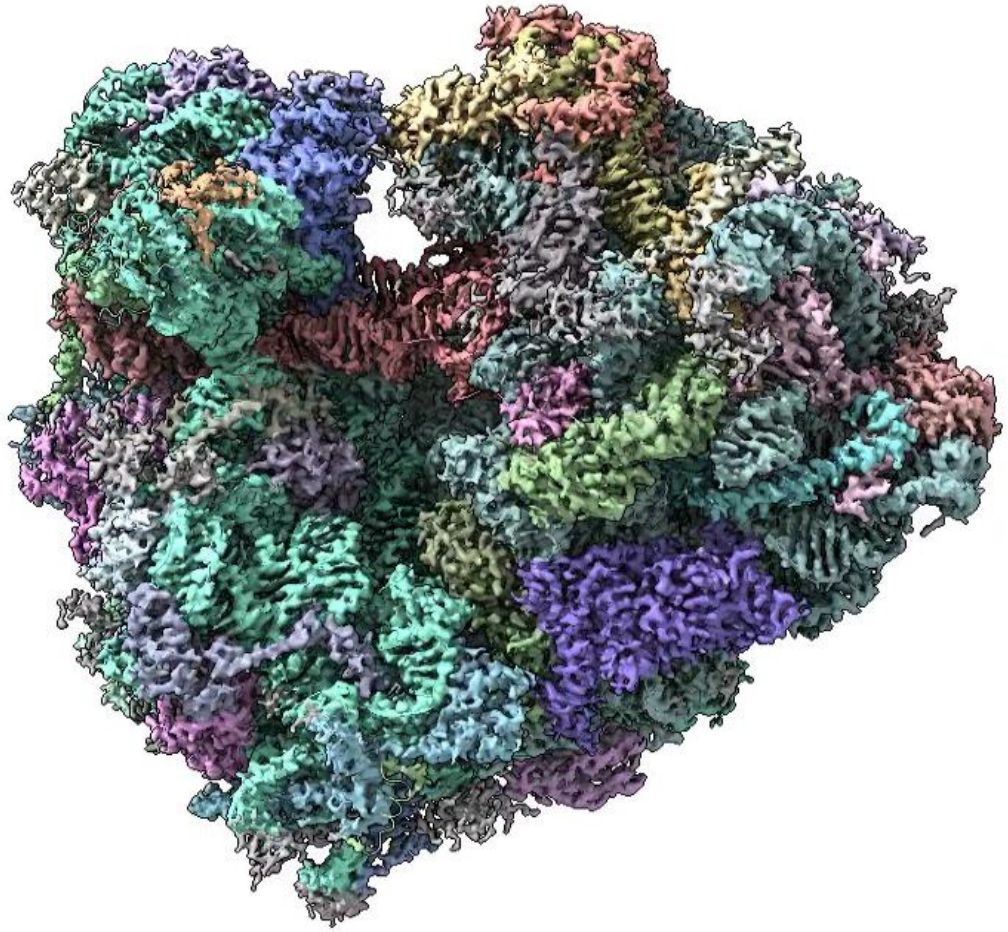


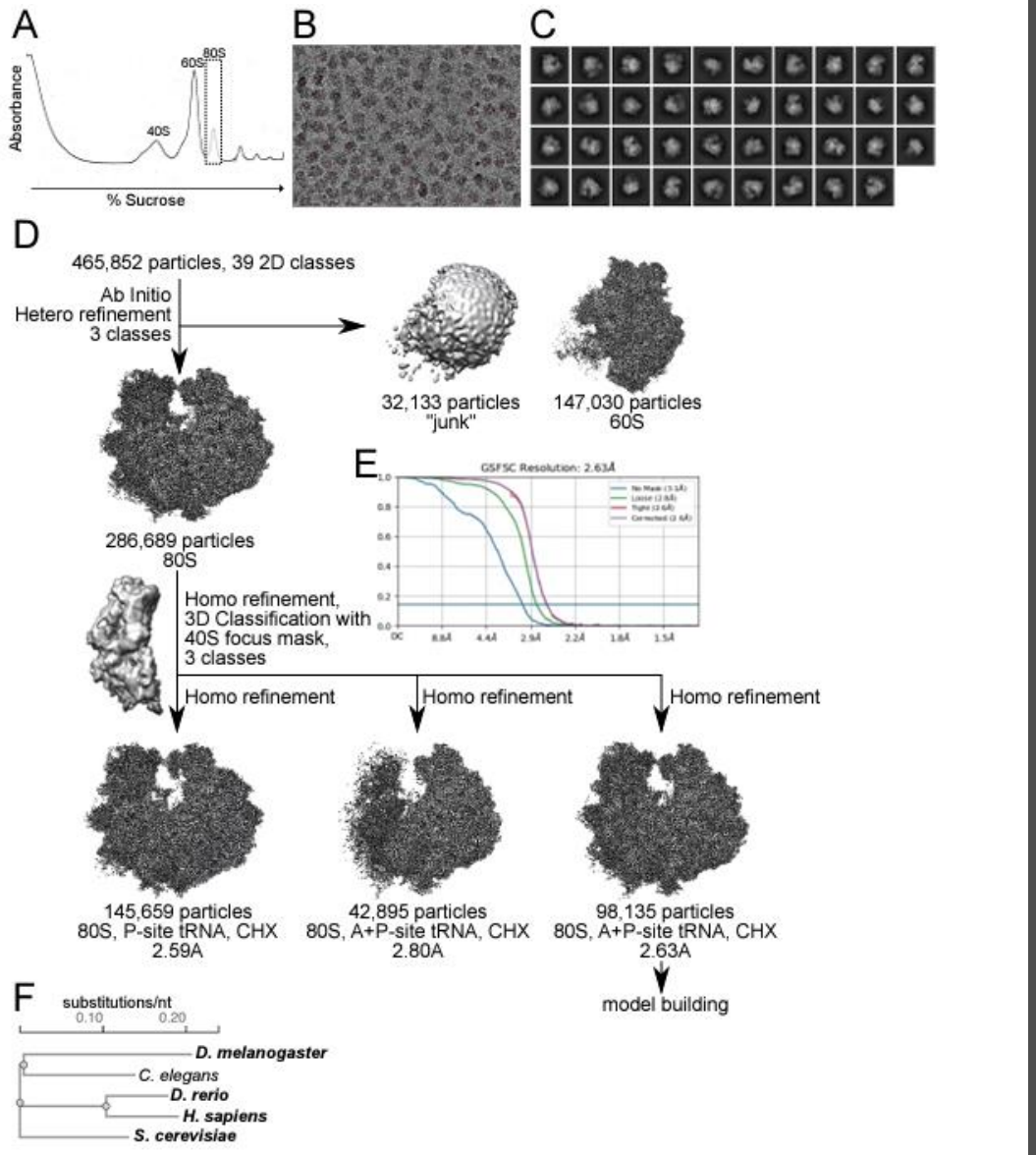
Publications

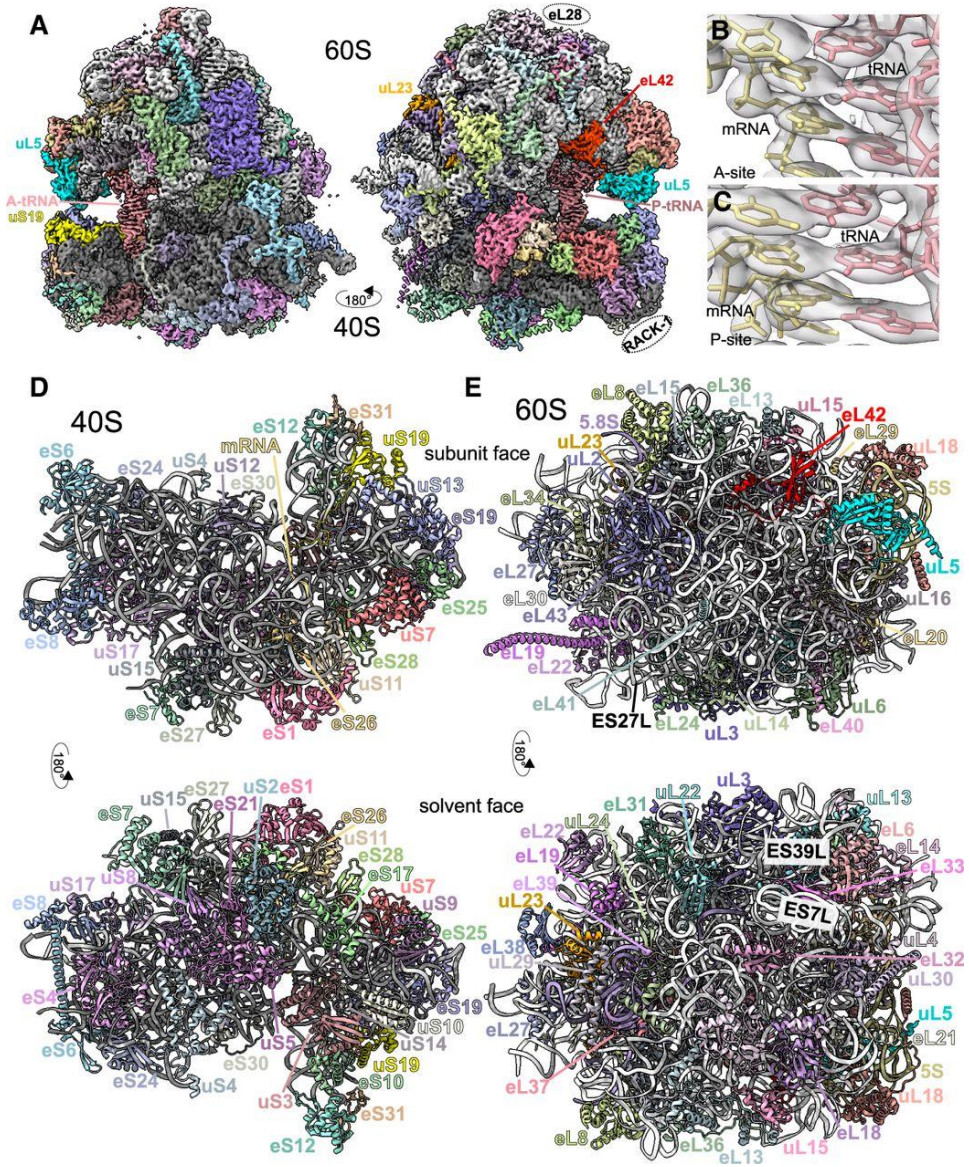
High-Resolution Reconstruction of a *C. elegans* Ribosome Sheds Light on Evolutionary Dynamics and Tissue Specificity

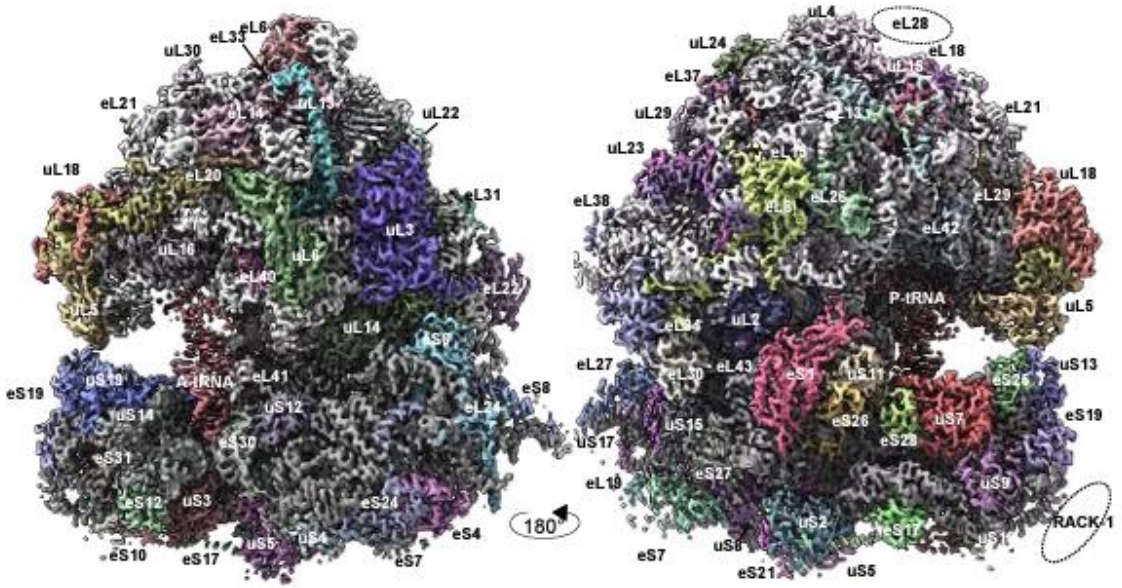
Enisha Sehgal, Chloe Wohlenberg, Evan M Soukup, Marcus J Viscardi, Vitor Hugo Balasco Serrão and Joshua A Arribere

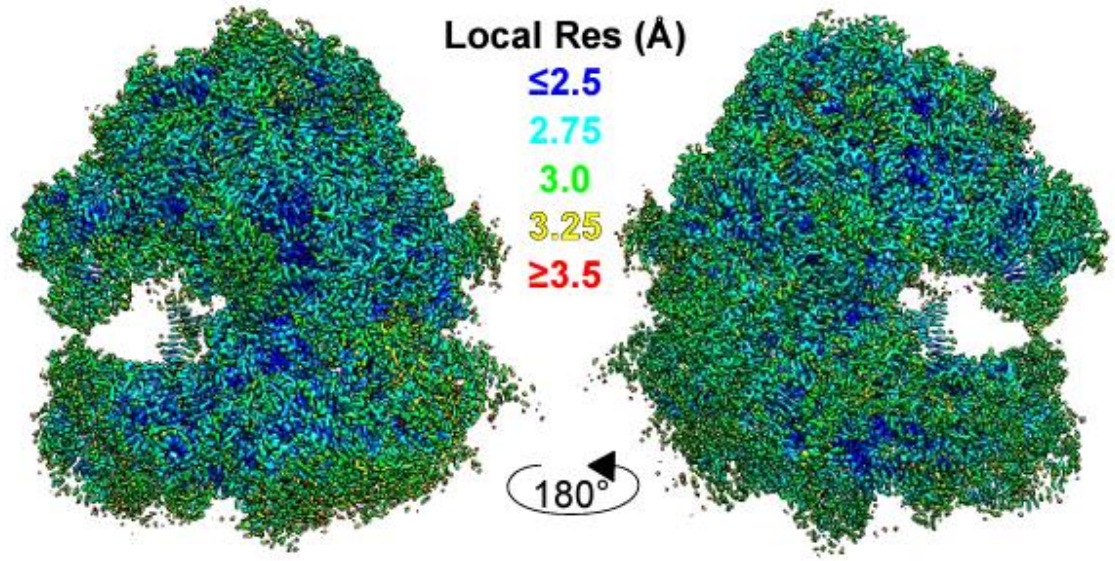
RNA - 2024

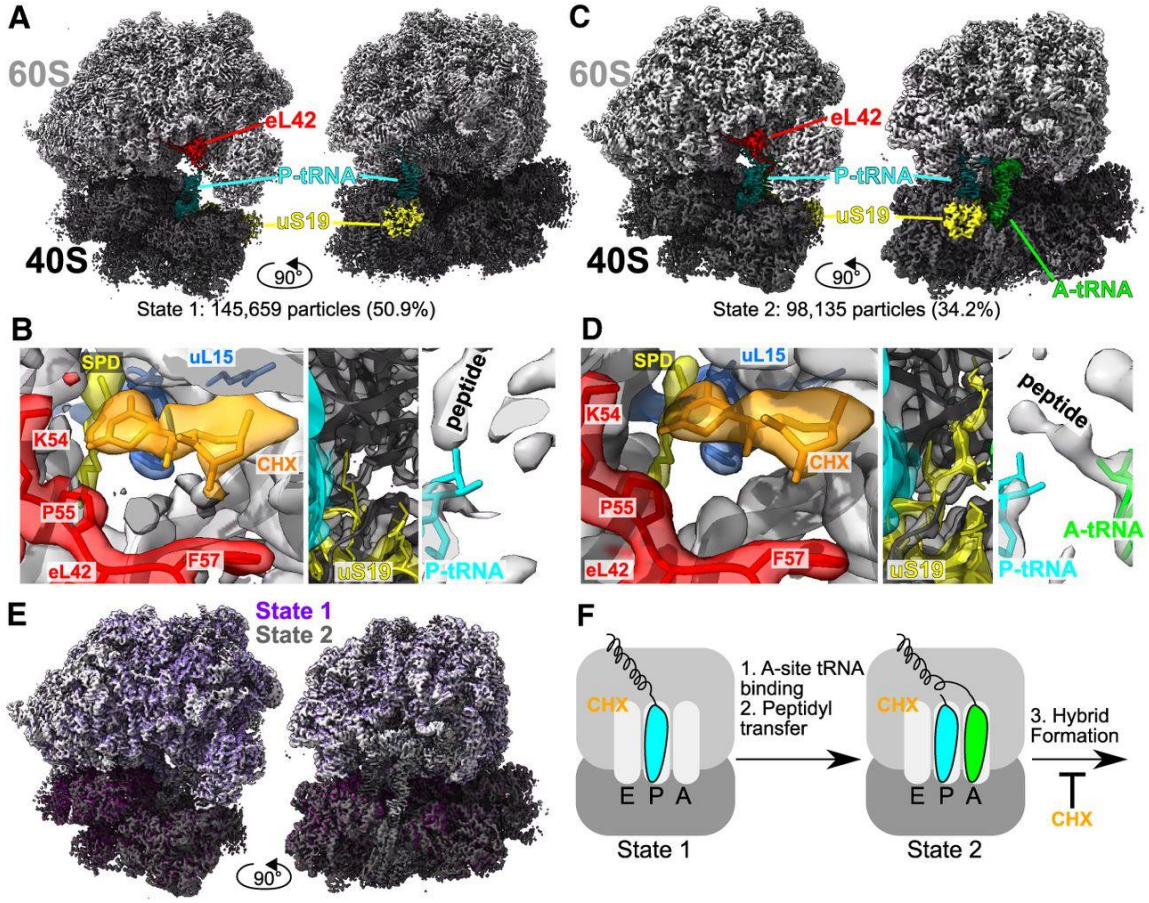



A
Absorbance
40S
60S
80S
% Sucrose
B
C
D
465,852 particles, 39 2D classes
Ab Initio
Hetero refinement
3 classes
32,133 particles
"junk"
147,030 particles
60S
286,689 particles
80S
Homo refinement,
3D Classification with
40S focus mask,
3 classes
Homo refinement
Homo refinement
Homo refinement
145,659 particles
80S, P-site tRNA, CHX
2.59A
42,895 particles
80S, A+P-site tRNA, CHX
2.80A
98,135 particles
80S, A+P-site tRNA, CHX
2.63A
model building
E
GSFSC Resolution: 2.63Å
F
substitutions/nt
0.10
0.20
D. melanogaster
C. elegans
D. rerio
H. sapiens
S. cerevisiae

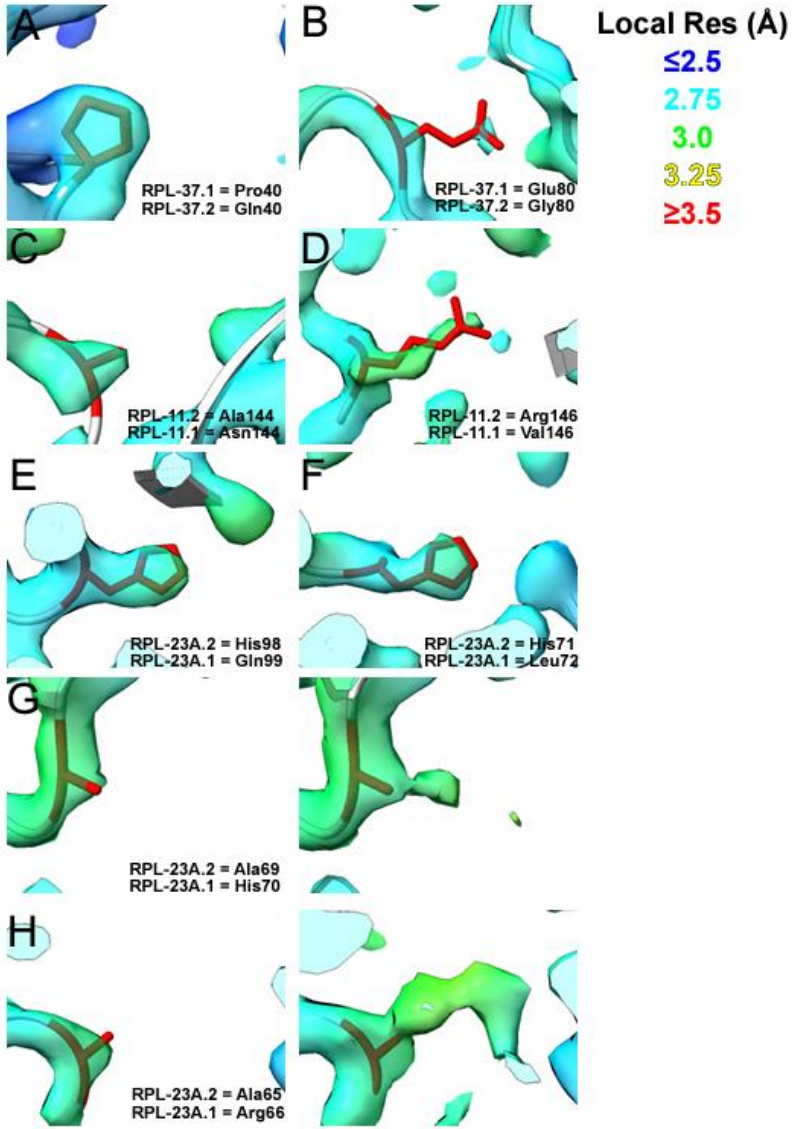

A
60S
40S
eL28
uL23
eL42
uL5
P-tRNA
A-tRNA
uS19
RACK-1
180°
B
tRNA
mRNA
A-site
C
tRNA
mRNA
P-site
D
40S
subunit face
mRNA
E
60S
5.8S
ES27L
solvent face
ES39L
ES7L

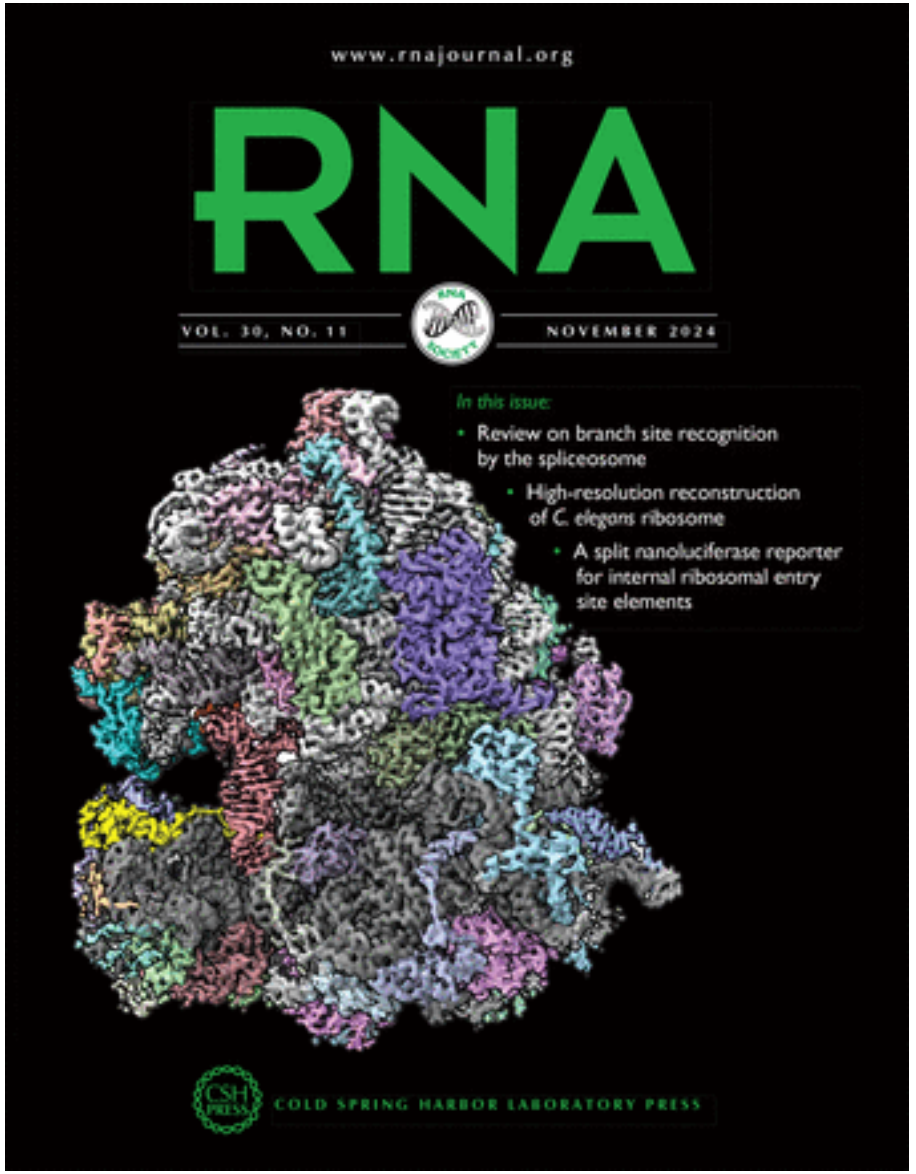






"Here we report the first high-resolution single-particle cryo-electron microscopy (cryo-EM) map of an endogenous *C. elegans* ribosome at an overall resolution ($FSC_{0.143}$) of 2.63 Å. **The map represents the highest resolution map reported to date from whole animals, and it will serve as a launching point for future translational studies in this organism.**"


A
RPL-37.1 = Pro40
RPL-37.2 = Gln40
B
RPL-37.1 = Glu80
RPL-37.2 = Gly80
C
RPL-11.2 = Ala144
RPL-11.1 = Asn144
D
RPL-11.2 = Arg146
RPL-11.1 = Val146
E
RPL-23A.2 = His98
RPL-23A.1 = Gln99
F
RPL-23A.2 = His71
RPL-23A.1 = Leu72
G
RPL-23A.2 = Ala69
RPL-23A.1 = His70
H
RPL-23A.2 = Ala65
RPL-23A.1 = Arg66
Local Res (Å)
≤2.5
2.75
3.0
3.25
≥3.5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VOL. 30, NO. 11
NOVEMBER 2024
In this issue:
• Review on branch site recognition by the spliceosome
• High-resolution reconstruction of C. elegans ribosome
• A split nanoluciferase reporter for internal ribosomal entry site elements
COLD SPRING HARBOR LABORATORY PRESS

PI/Group Melissa Jurica

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NIH High-End Instrumentation program - S10OD02509

UC SANTA CRUZ CRYO-EM