In-situ single particle cryo-EM

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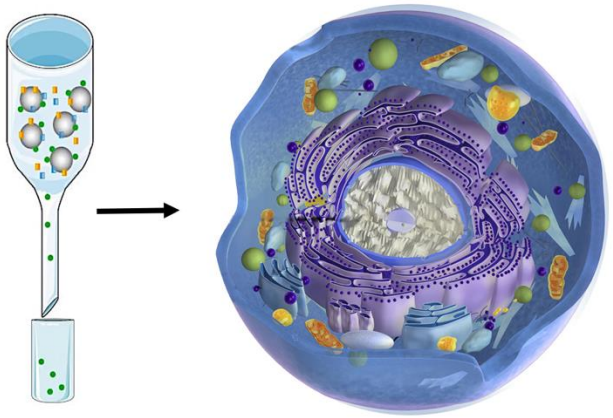
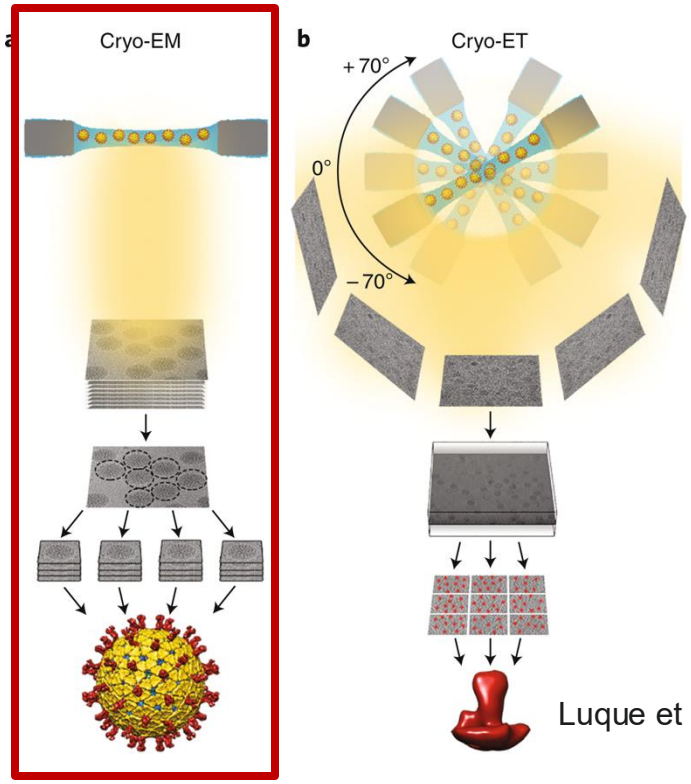
The cryo-EM Resolution Revolution

Method of the Year 2015:

THE REVOLUTION WILL NOT BE CRYSTALLIZED

Next Frontier: High-Resolution Visualization *in situ* *(in native environments)*





Luque et al, 2020

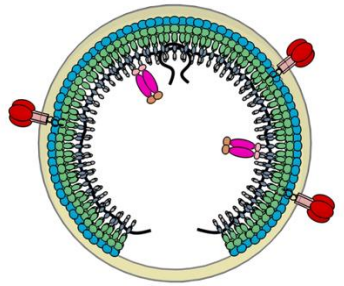
Challenges of *in situ* Samples:

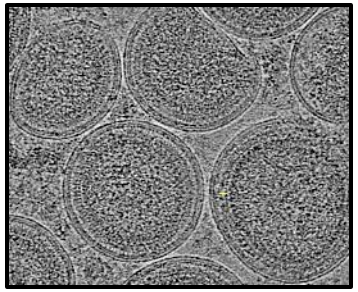
- Large, thick samples need specifical preparation
- Very high background with all the other molecules/cellular contents (both a challenge and a blessing), size limit
- Intrinsic heterogeneity/dynamics of the samples (both a challenge and a blessing)
- Abundance challenge

***in situ* single particle cryo-EM**

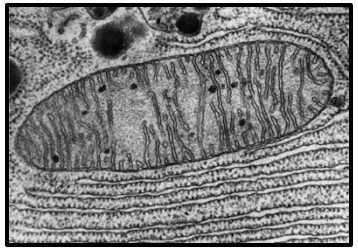
Examples and outline:

- Viruses

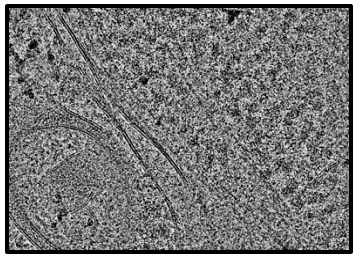




- Purified cellular organelles

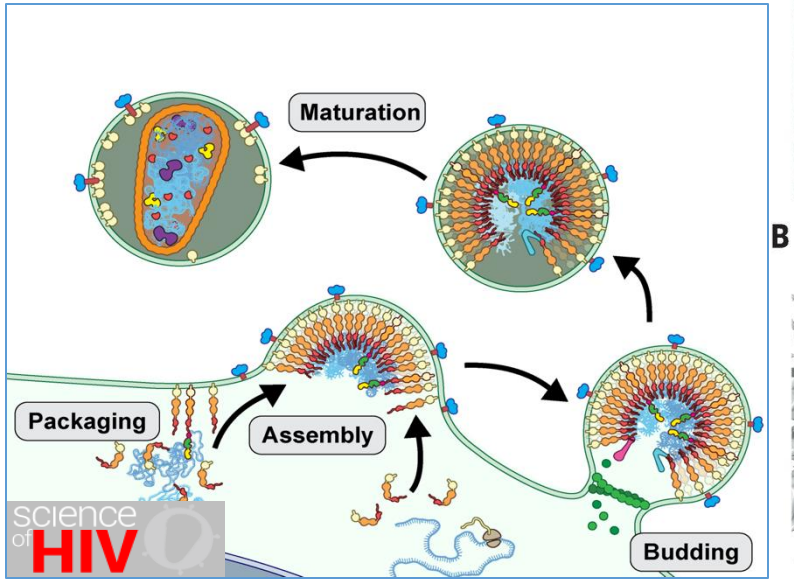


- Thin-sliced cells



Virus Example: HIV-1

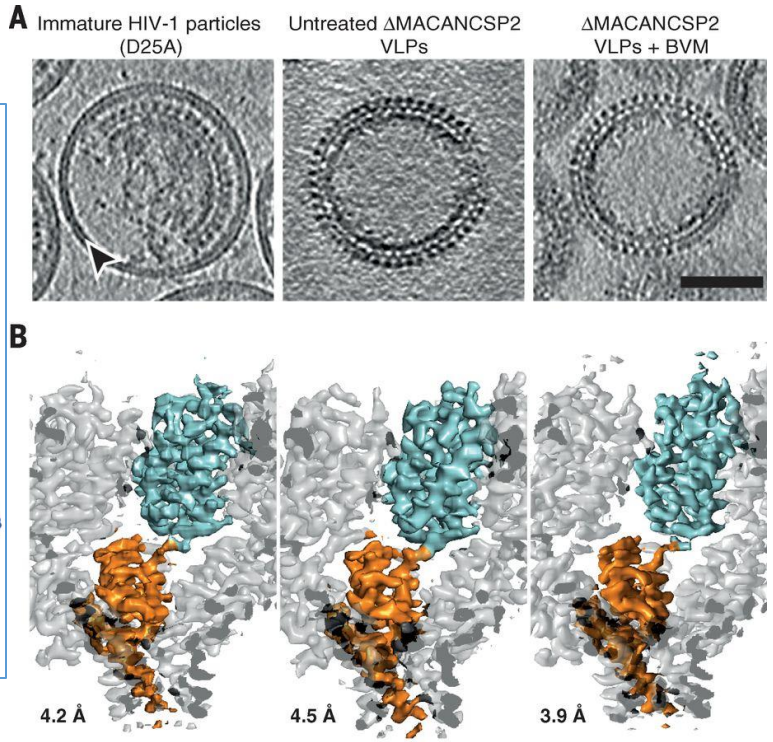
Heterogeneous HIV capsids are traditionally major challenges, only solvable in specific cases by cryo-electron tomography (cryo-ET) techniques



An atomic model of HIV-1 capsid-SP1 reveals structures regulating assembly and maturation

FLORIAN K. M. SCHUR, MARTIN OBR, WIM J. H. HAGEN, WILLIAM WAN, ARJEN J. JAKOBI, JOANNA M. KIRKPATRICK, CARSTEN SACHSE, HANS-GEORG KRÄUSSLICH, AND JOHN A. G. BRIGGS Authors Info & Affiliations

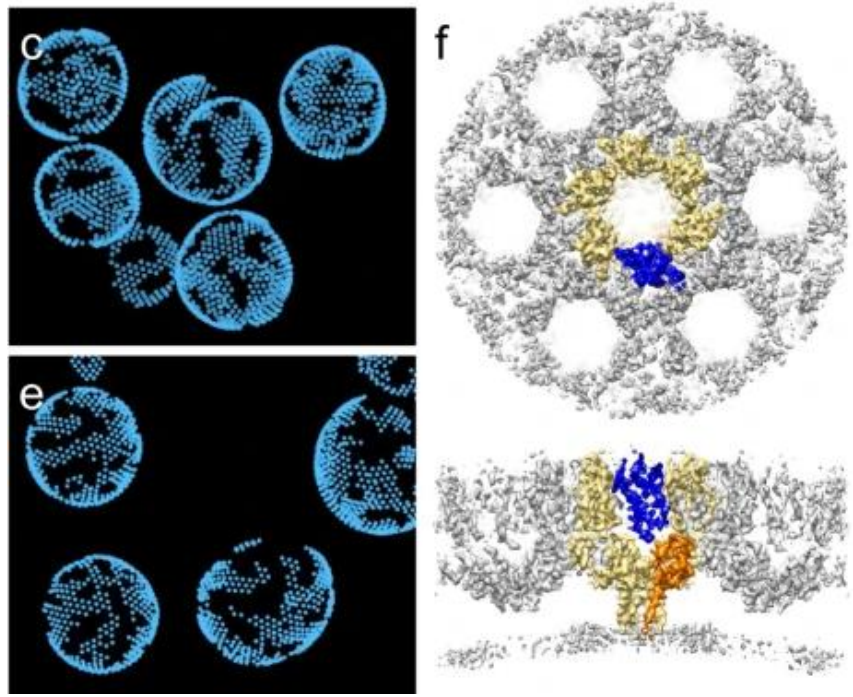
SCIENCE · 14 Jul 2016 · Vol 353, Issue 6298 · pp. 506-508



CryoET structures of immature HIV Gag reveal six-helix bundle

Luiza Mendonça, Dapeng Sun, Jiying Ning, Jiwei Liu, Abhay Kotecha, Mateusz Olek, Thomas Frosio, Xiaofeng Fu, Benjamin A. Himes, Alex B. Kleinpeter, Eric O. Freed, Jing Zhou, Christopher Aiken & Peijun Zhang

Communications Biology **4**, Article number: 481 (2021) | Cite this article



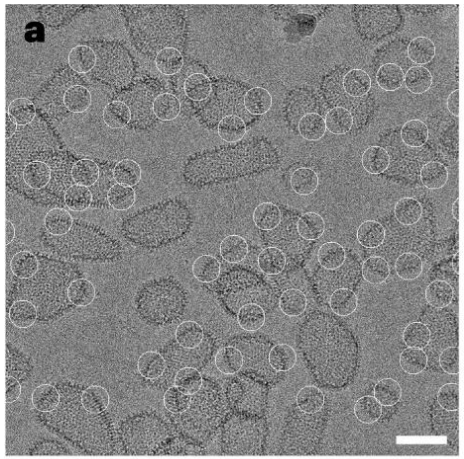
Single Particle cryo-EM of Mature HIV-1 Particles

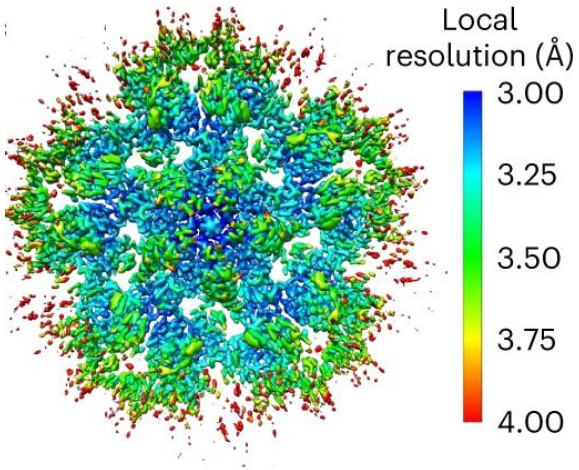
Article | Open access | Published: 09 February 2023

A molecular switch modulates assembly and host factor binding of the HIV-1 capsid

Randall T. Schirra, Nayara F. B. dos Santos, Kaneil K. Zadrozny, Iga Kucharska, Barbie K. Ganser-Pornillos & Owen Pornillos

Nature Structural & Molecular Biology **30**, 383–390 (2023) | Cite this article



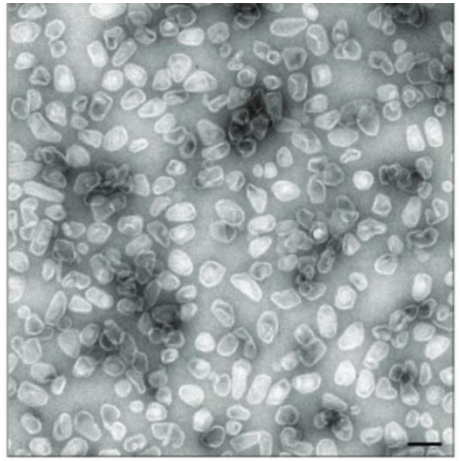


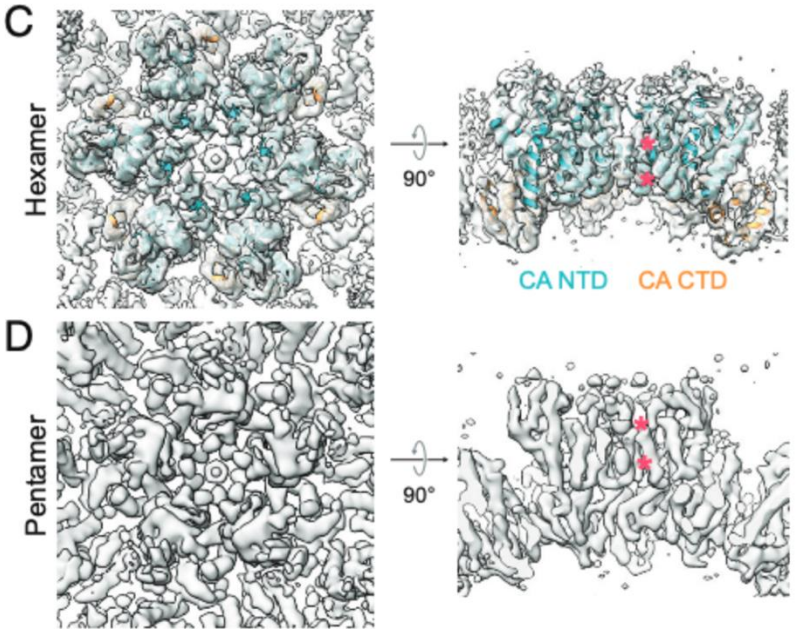
PNAS RESEARCH ARTICLE | BIOCHEMISTRY OPEN ACCESS

Structural insights into HIV-1 polyanion-dependent capsid lattice formation revealed by single particle cryo-EM

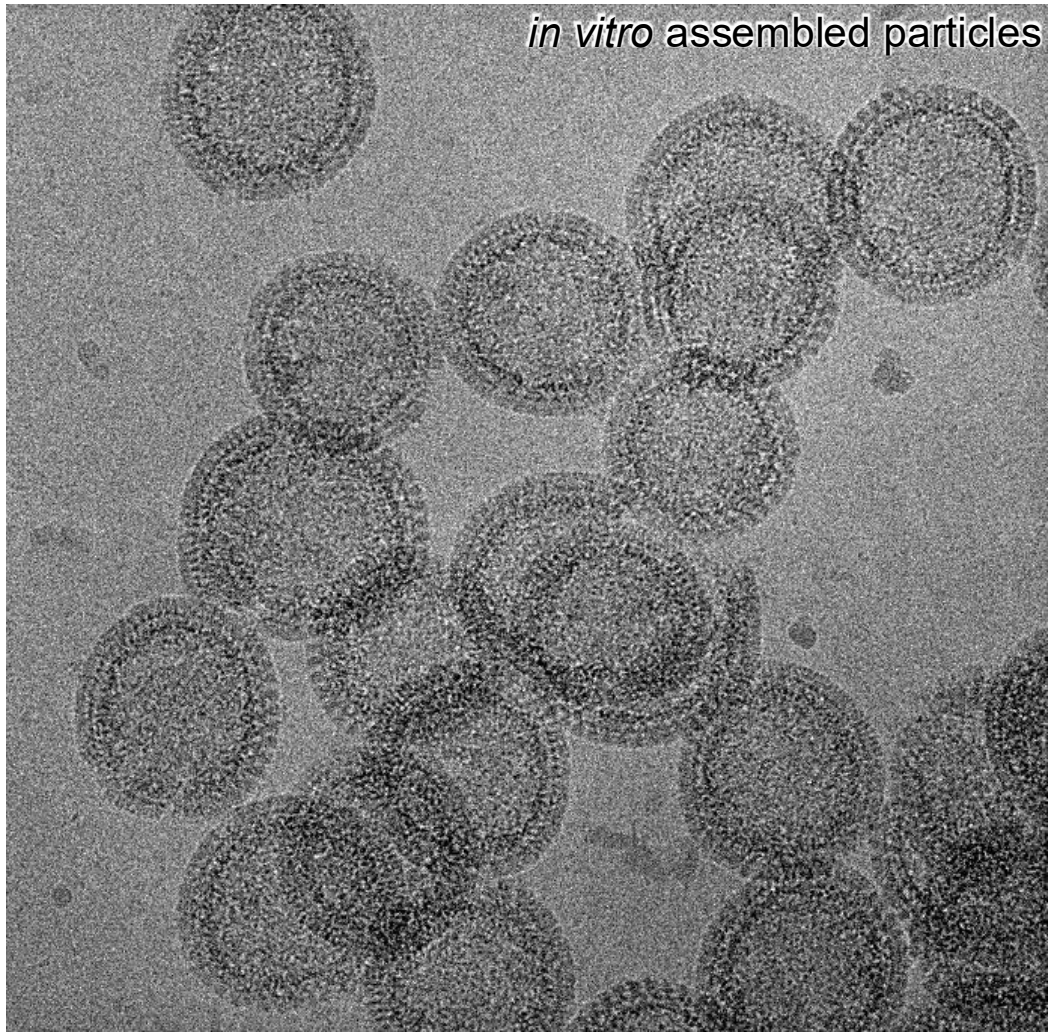
Carolyn M. Highland[a,b], Aaron Tan[c,1], Clifton L. Ricaña[a], John A. G. Briggs[c,d], and Robert A. Dick[a,2]

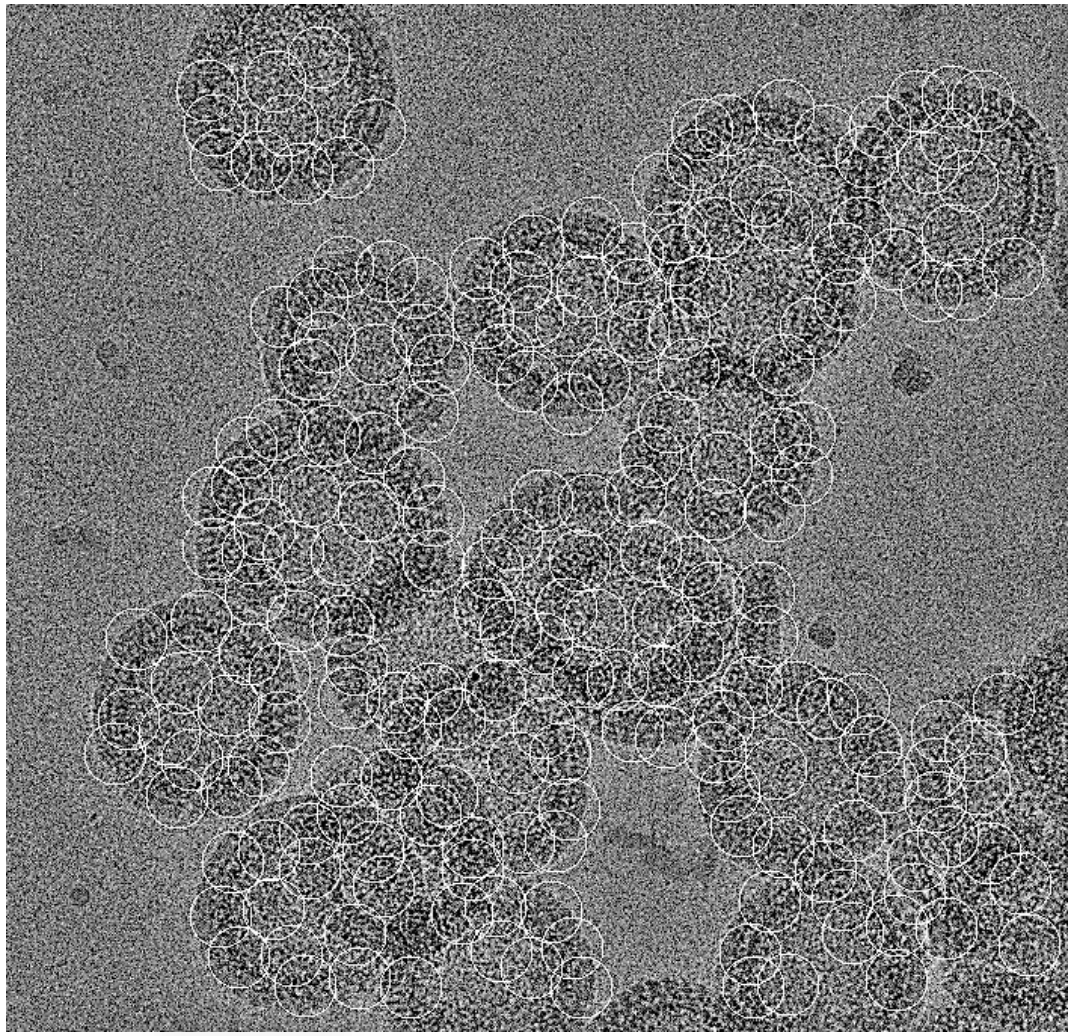
Edited by James Hurley, University of California, Berkeley, CA; received December 2, 2022; accepted March 12, 2023





Single Particle cryo-EM of HIV-1 Immature Particles





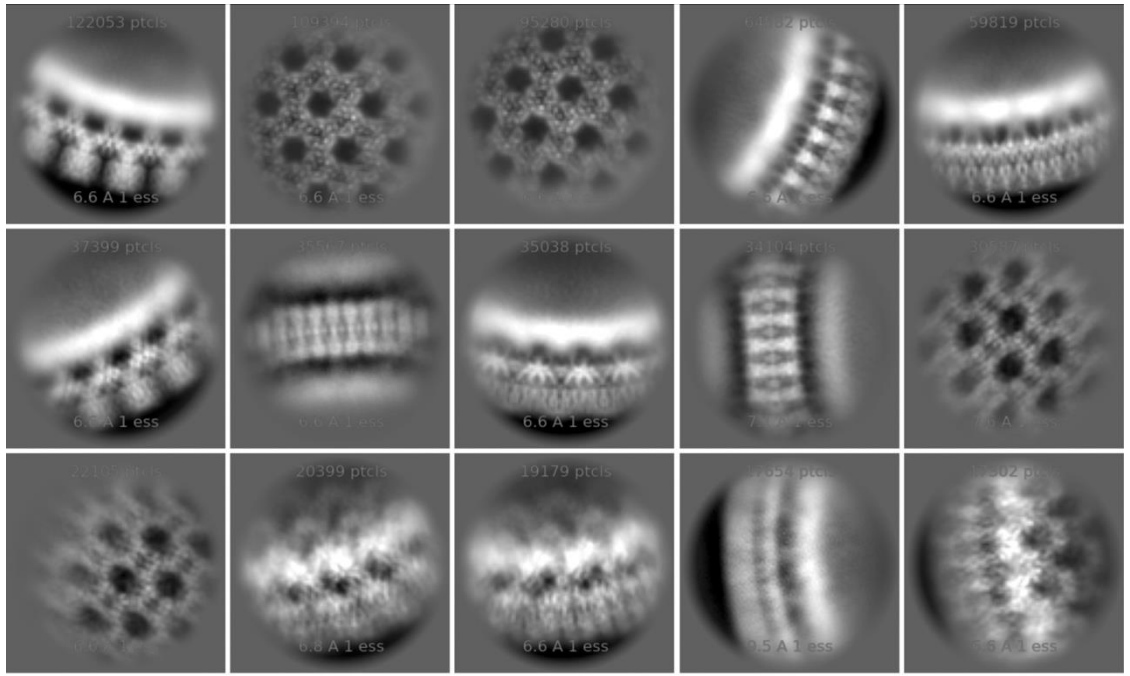
Chunxiang (Charlie) Wu

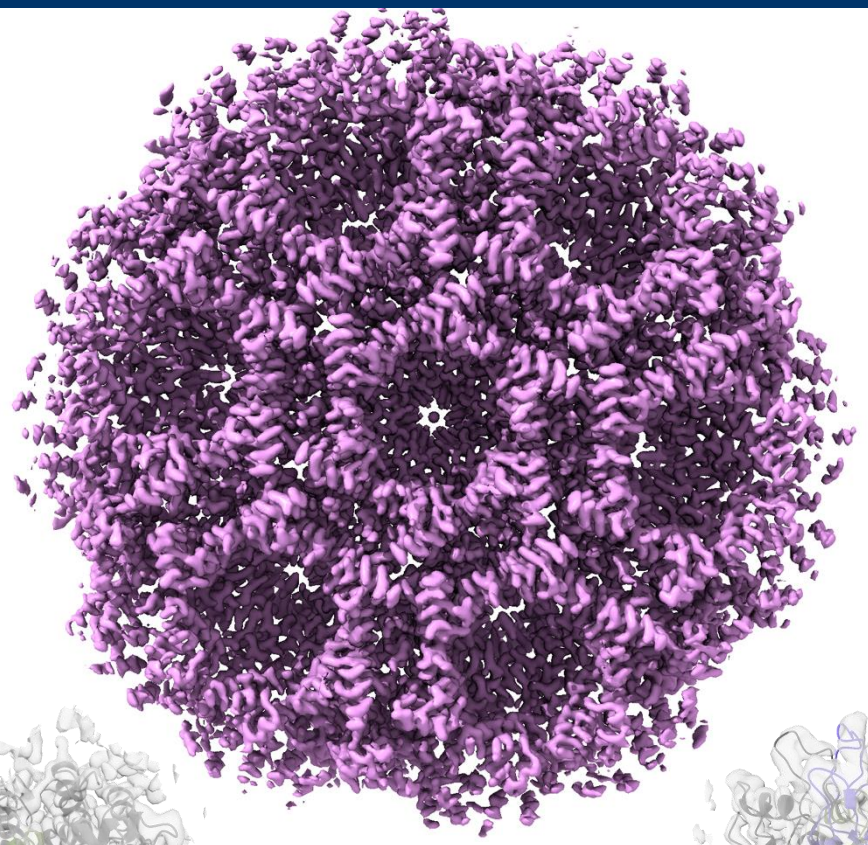
Megan Meuser

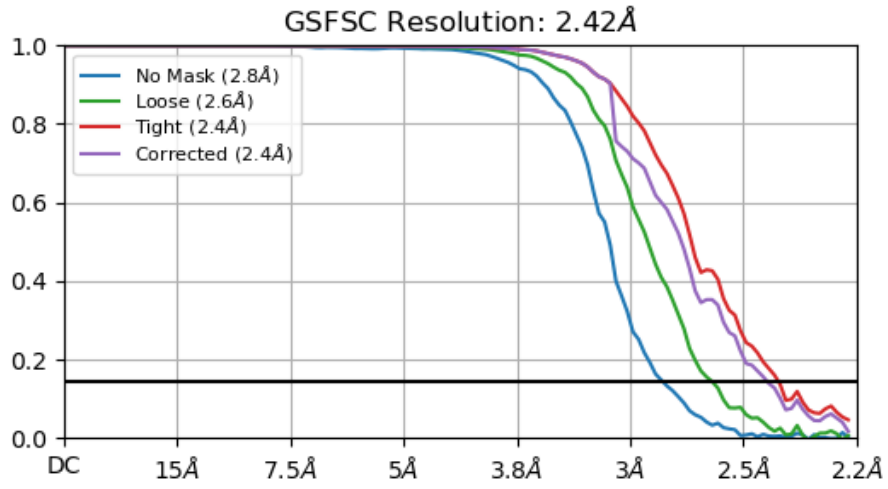
Rachel Yang

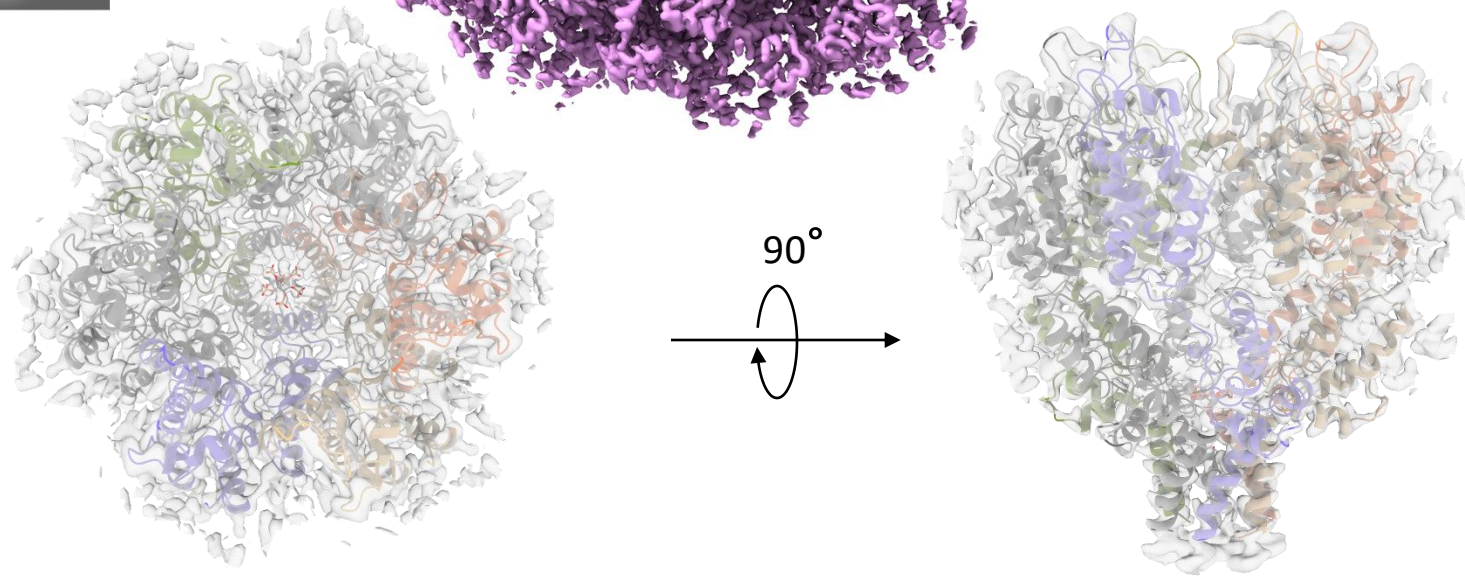
Swapnil Devakar

Single Particle cryo-EM of HIV-1 Immature Particles

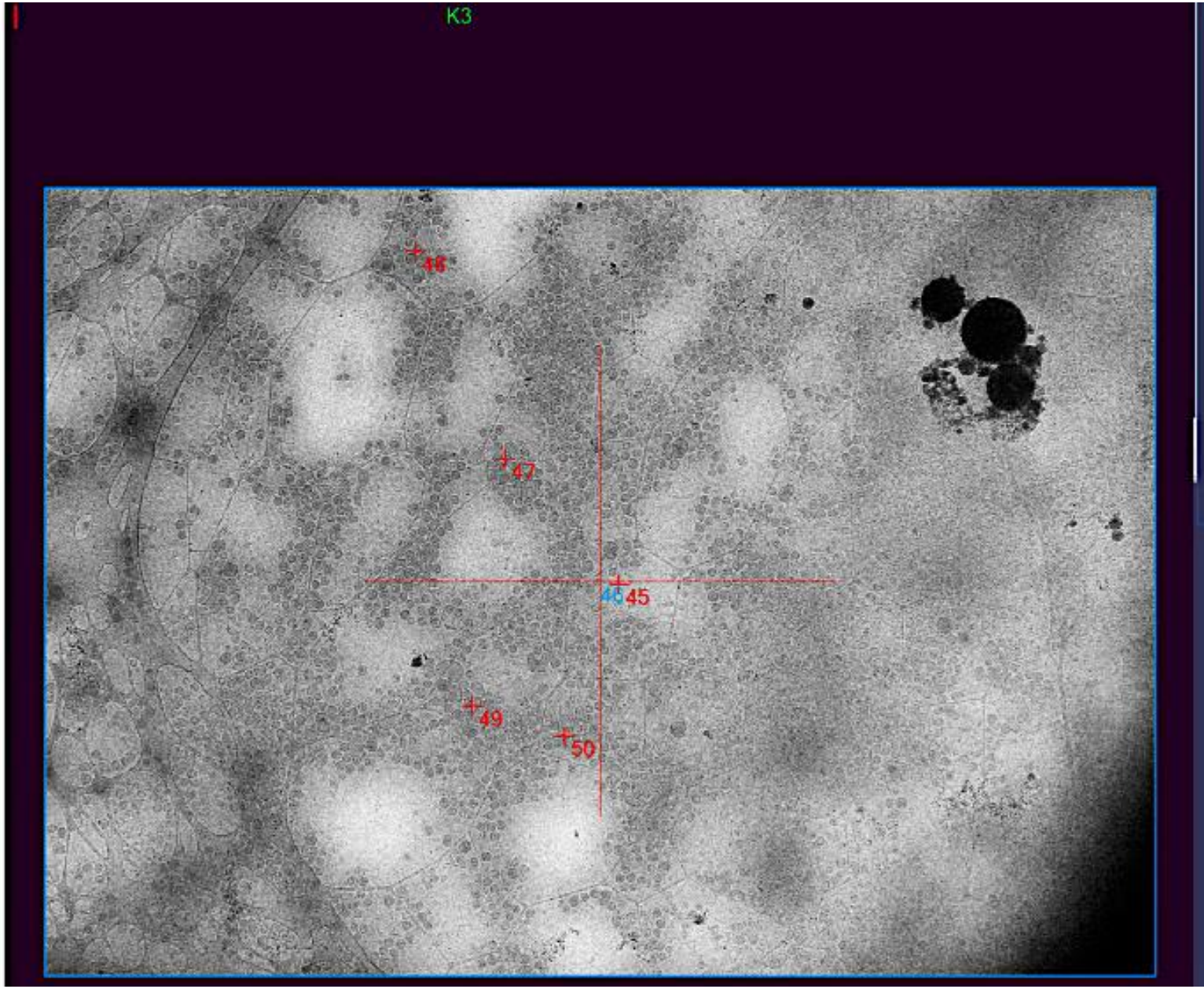


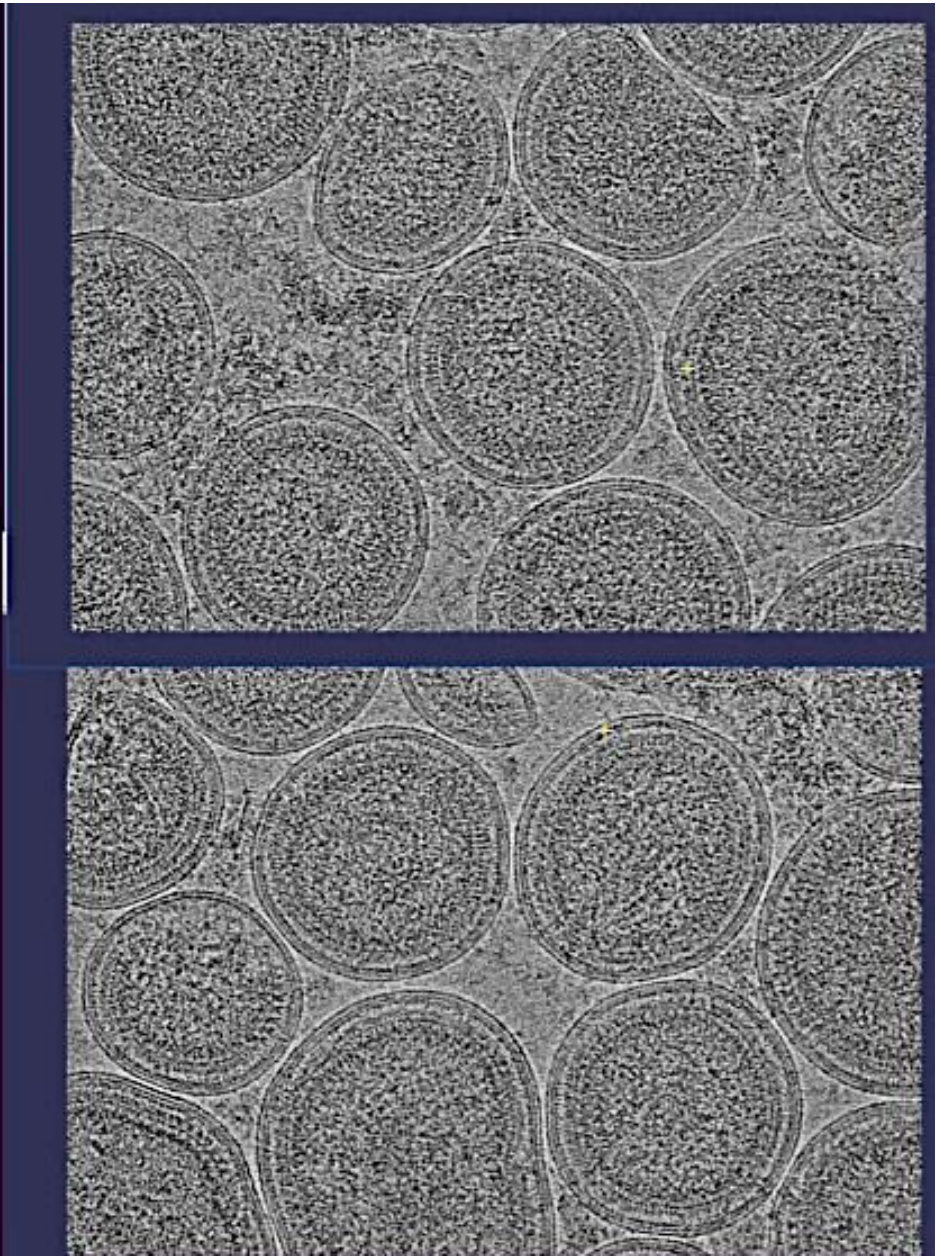




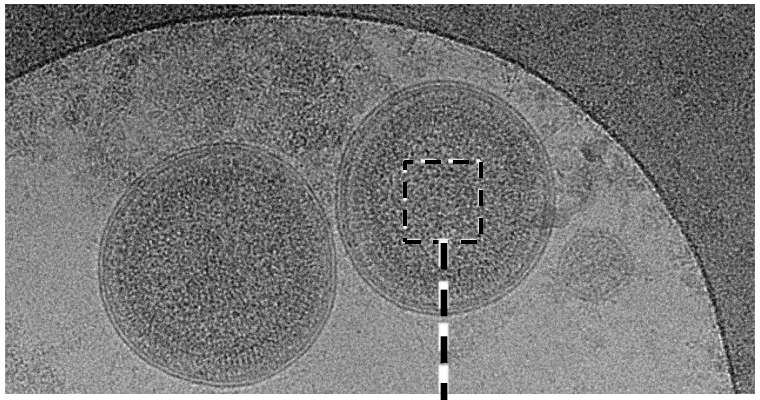


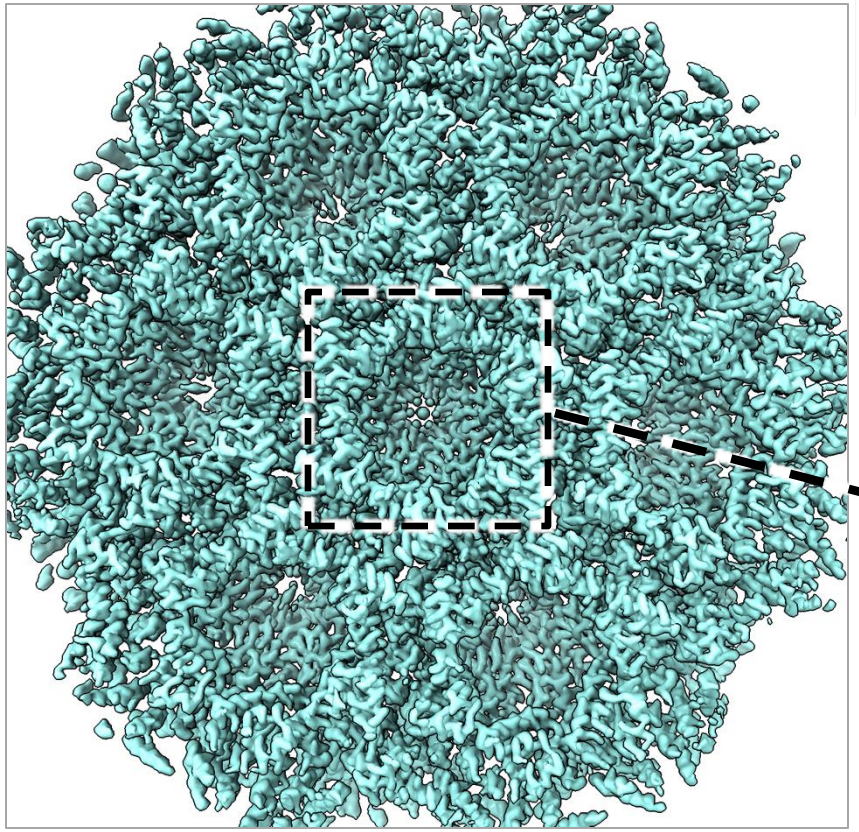
Single Particle cryo-EM of Immature HIV-1 Virus Like Particles (VLPs)



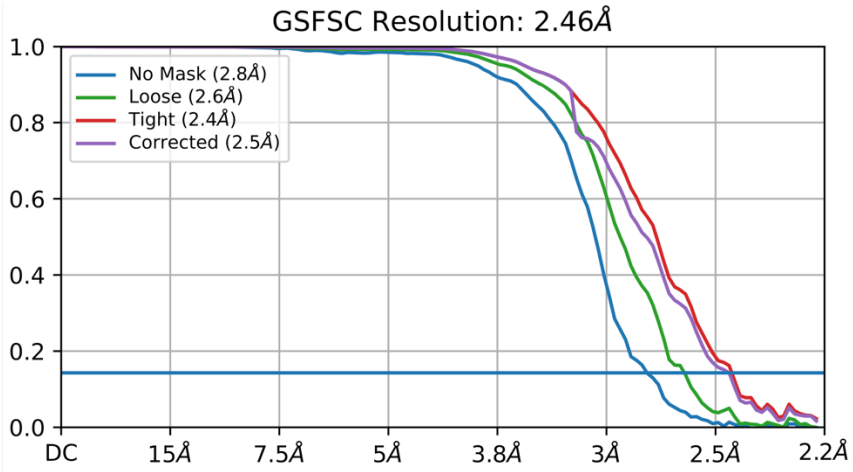


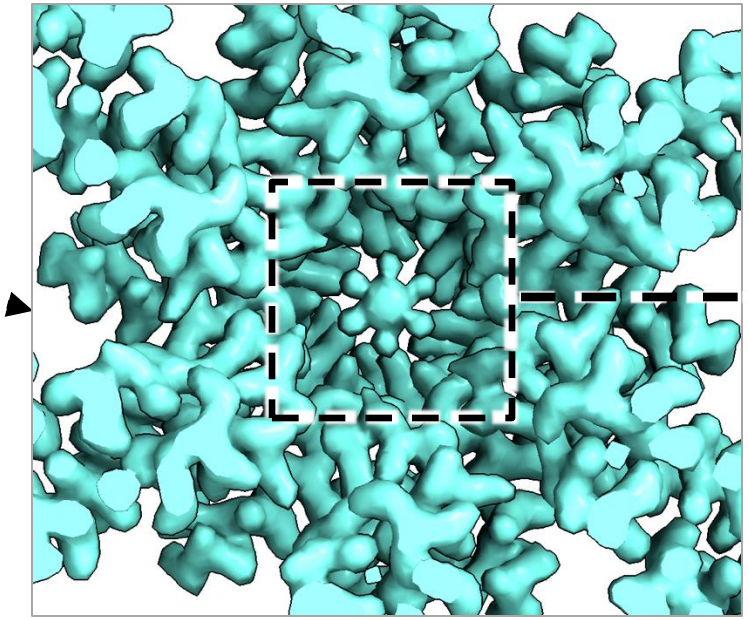
Cryo-EM studies of Immature Immature HIV-1 Virial Particles

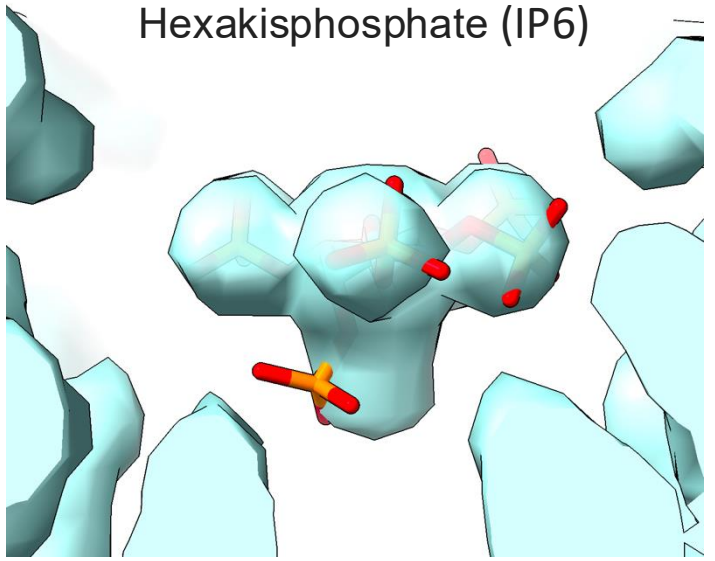


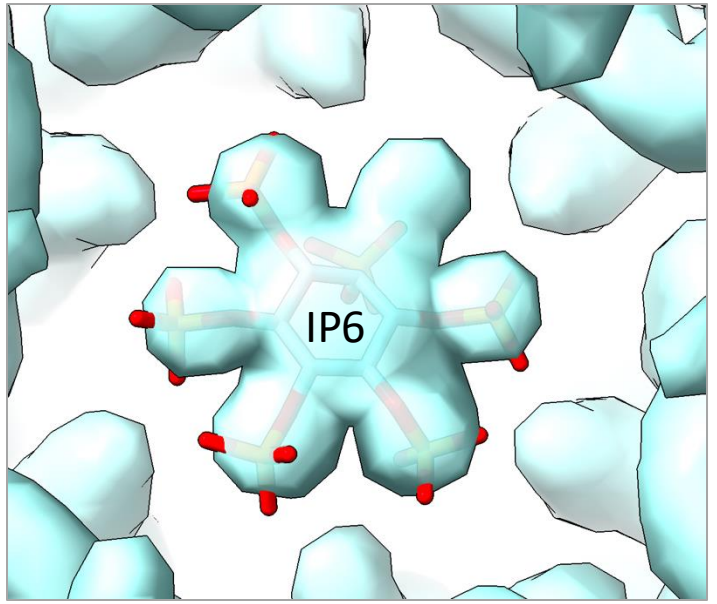


HIV-1 VLP



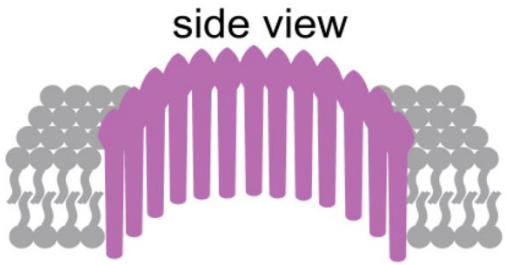




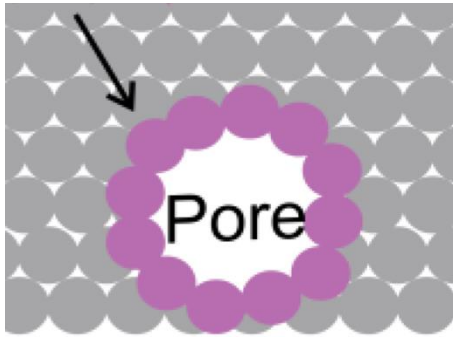


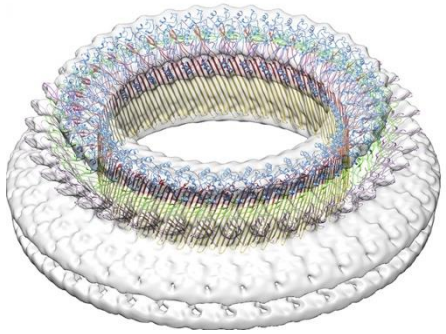
Poking Holes on Virus Membrane to Help Drug Influx

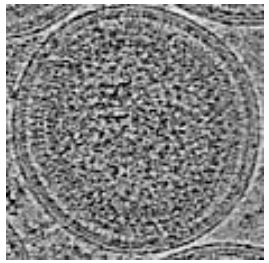
Bacterial toxin perforingolysin O (PFO)

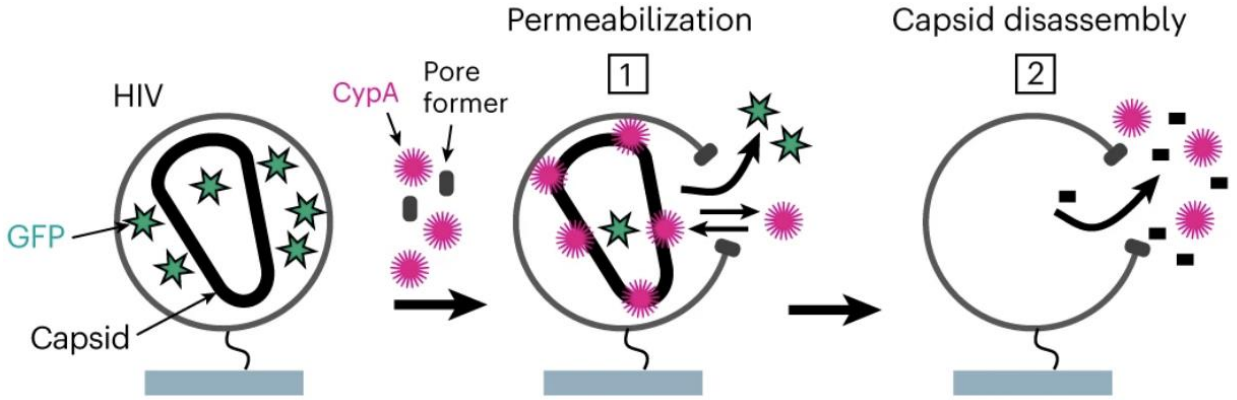


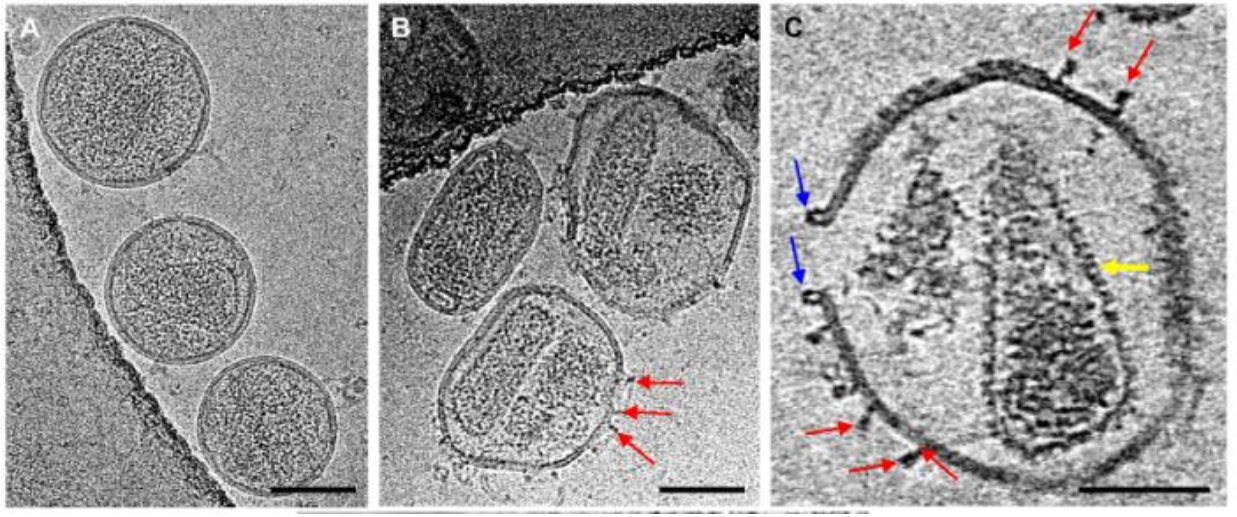
Top view









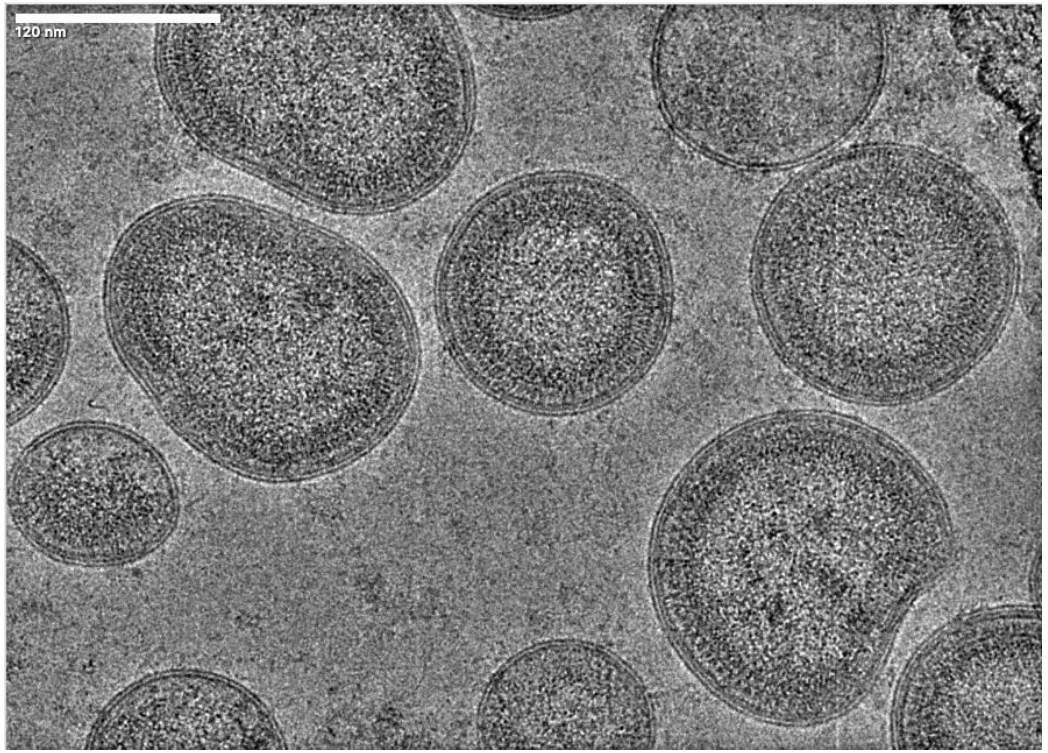


Can we apply PFO treatment to immature *in situ* VLPs?

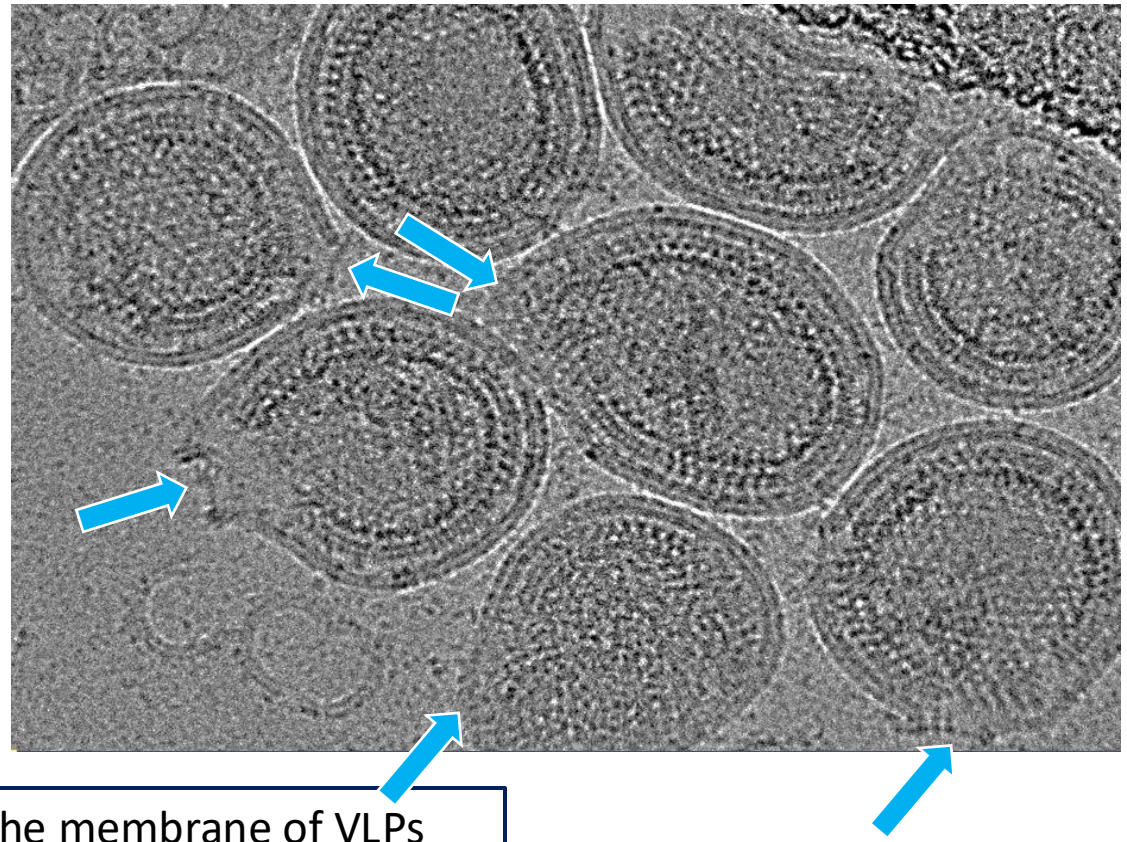
Chantal L Márquez, et al. (2018) eLife 7:e34772
Tao Ni *et al.*, *.Sci. Adv.***7**,eabj5715(2021).
Renner, N. *et al. Nat Struct Mol Biol* **30**, 370–382 (2023).

Poking Holes on Virus Membrane to Help Drug/Inhibitor Influx

- PFO: Intact membrane

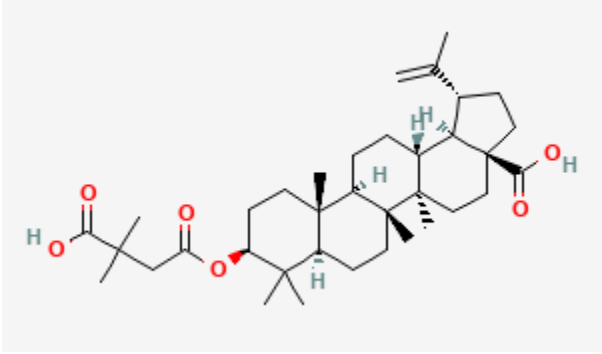


+PFO: Broken membrane

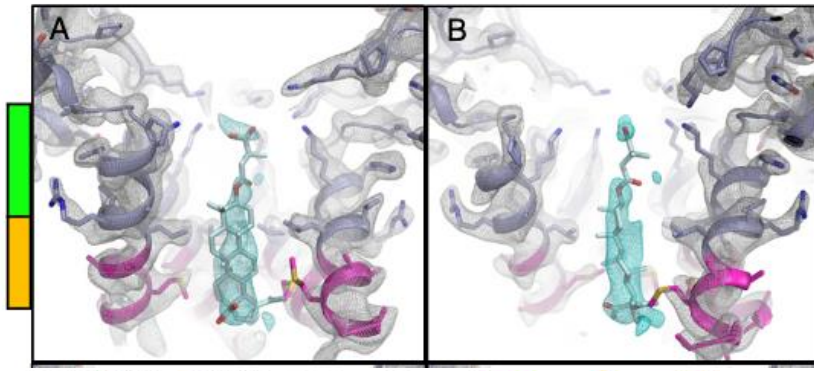


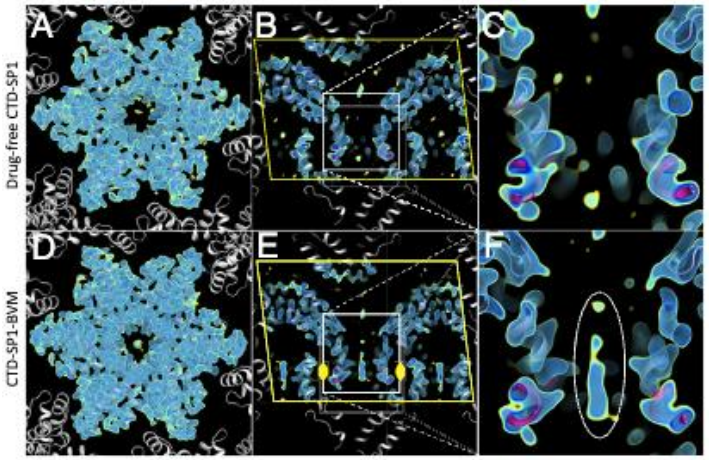
- PFO forms holes in the membrane of VLPs
- PFO treatment does not alter the Gag lattice
- PFO treatment allows for “soaking in” of binders

Bevirimat (BVM)

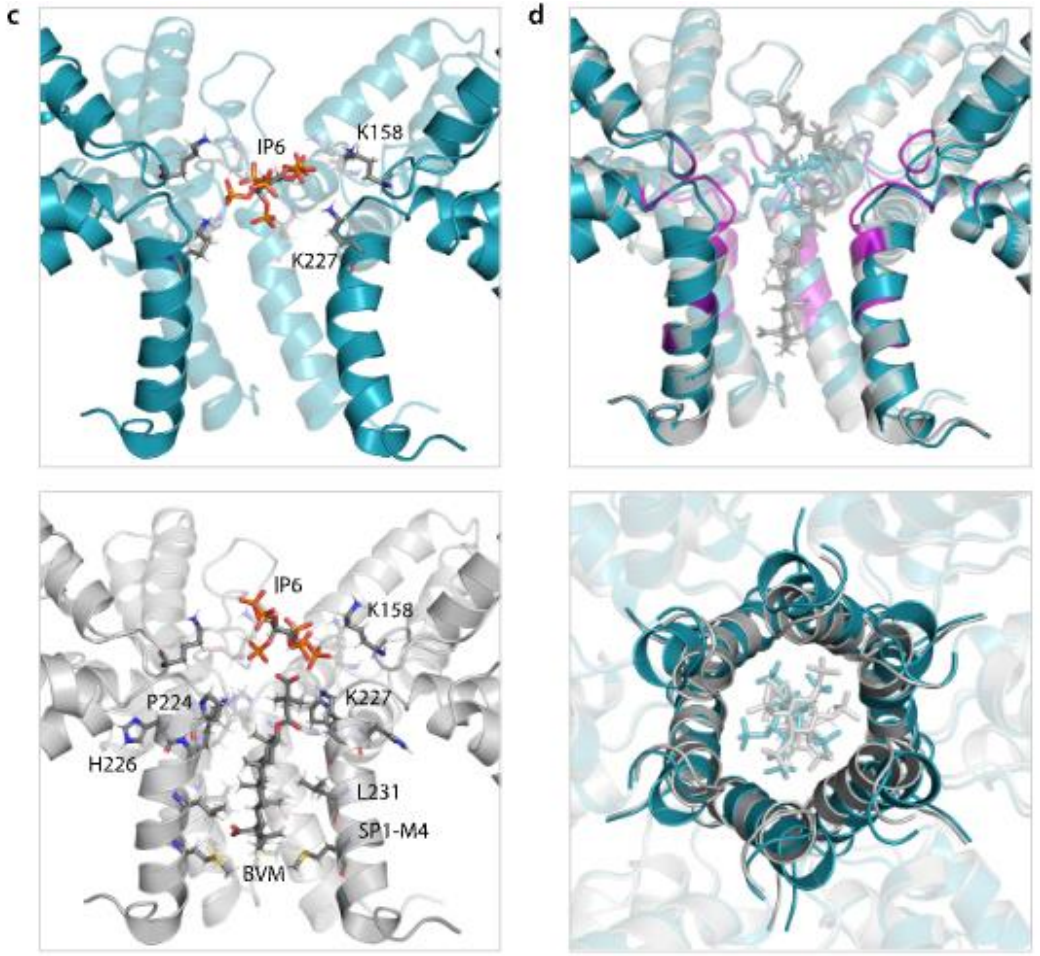


microED structure of BVM
with CA_{CTD}-sp1 hexamer (2018)

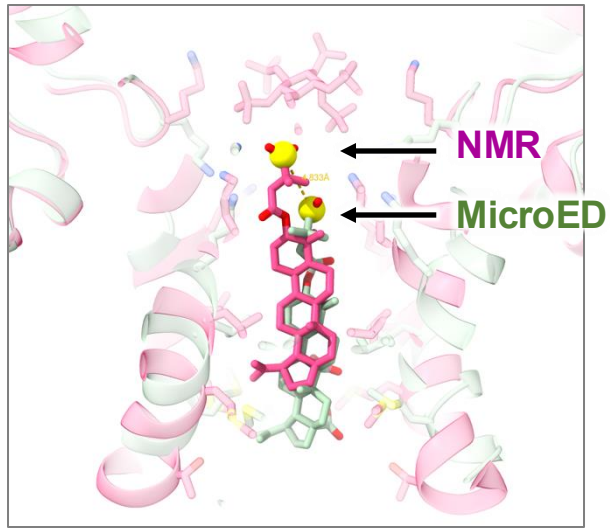




MAS NMR structure of BVM
With CA_{CTD}-sp1 hexamer (2023)

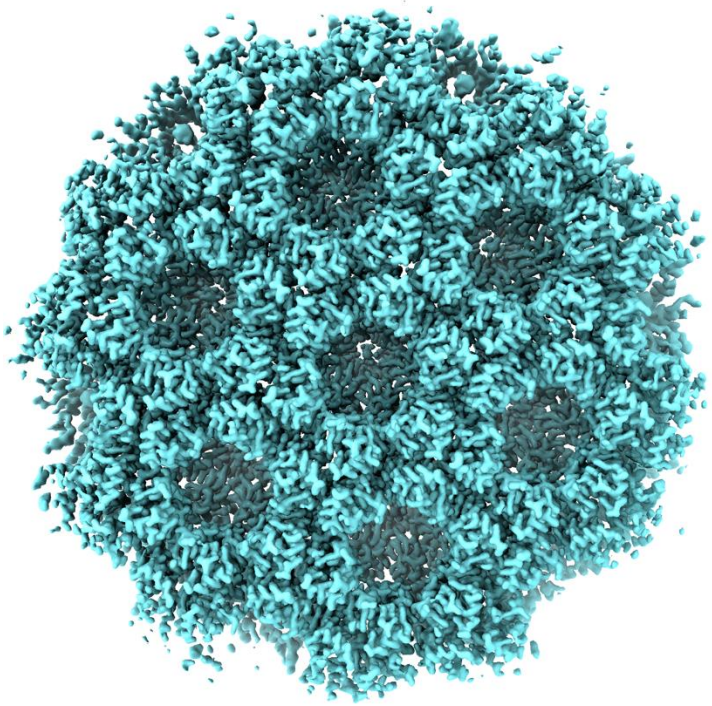


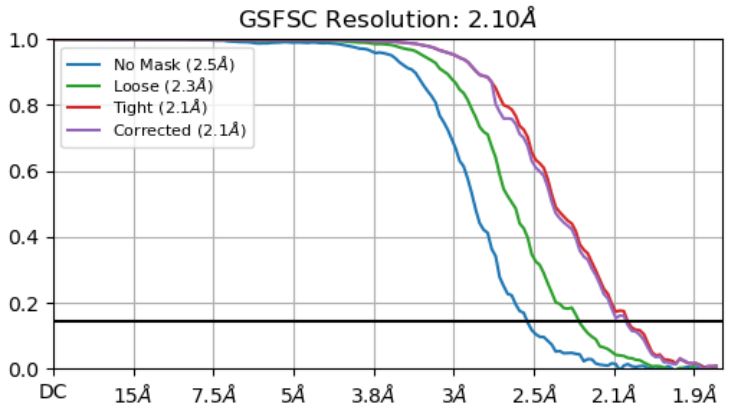
MicroED:NMR



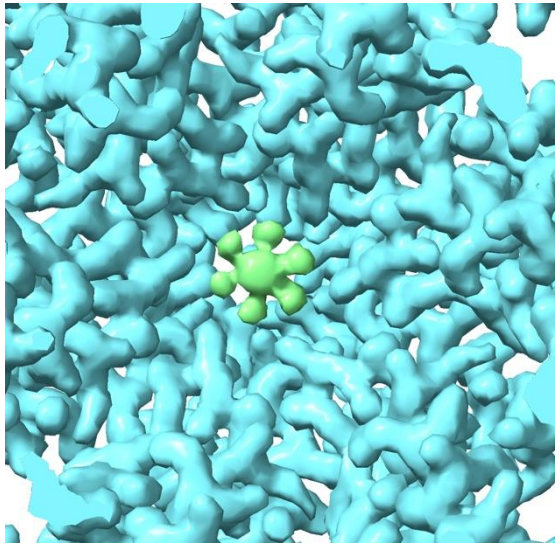
Purdy MD, Yeager M et al. MicroED structures of HIV-1 Gag CTD-SP1 reveal binding interactions with the maturation inhibitor bevirimat. Proc Natl Acad Sci U S A. 2018 Dec 26;115(52).
Sarkar S, Polenova T et al. Structural basis of HIV-1 maturation inhibitor binding and activity. Nat Commun. 2023 Mar 4;14(1)

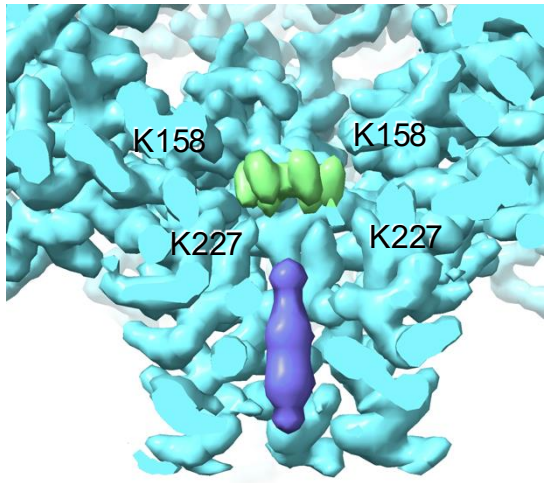
BVM Binding to PFO-treated Immature HIV-1 VLPs



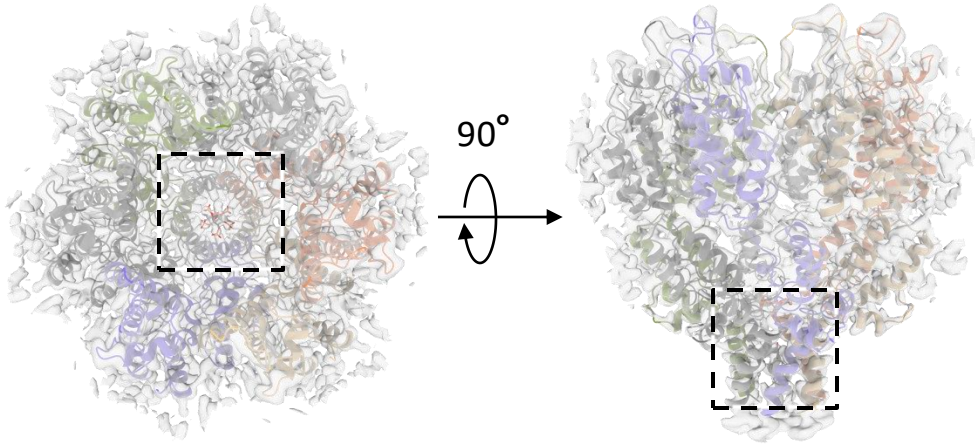


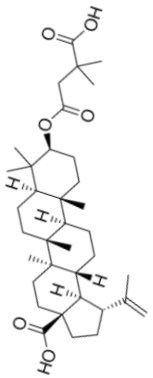
IP6

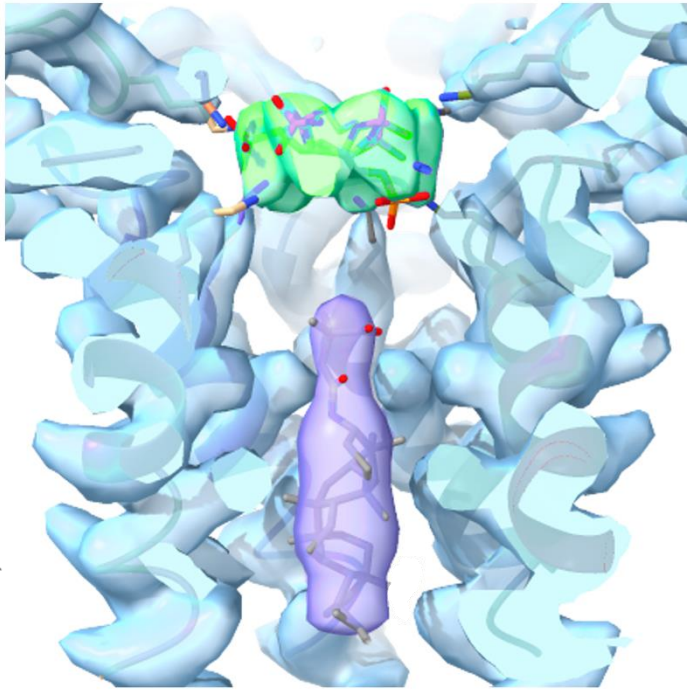




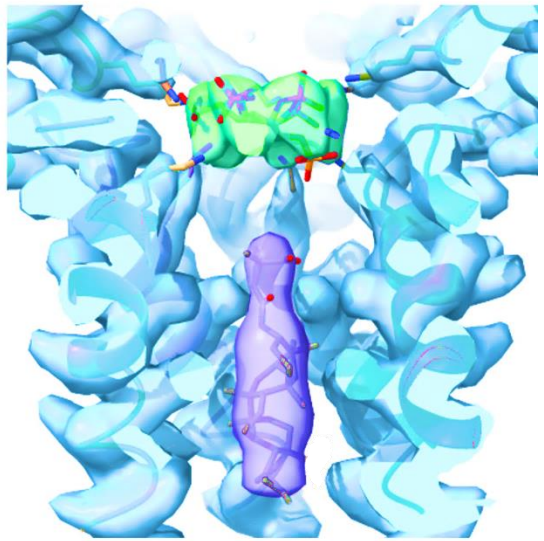
Bevirimat



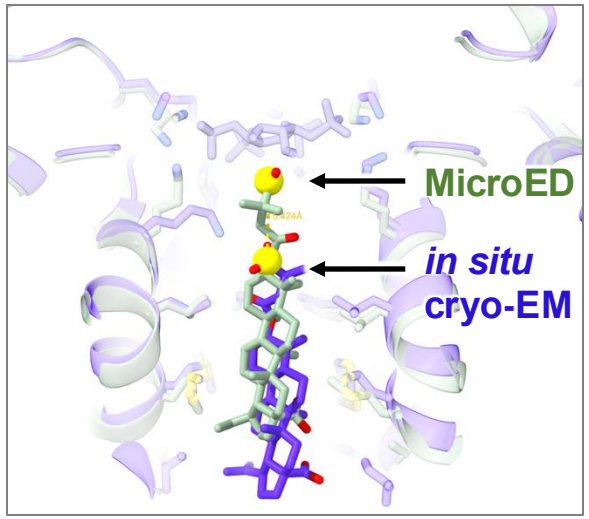




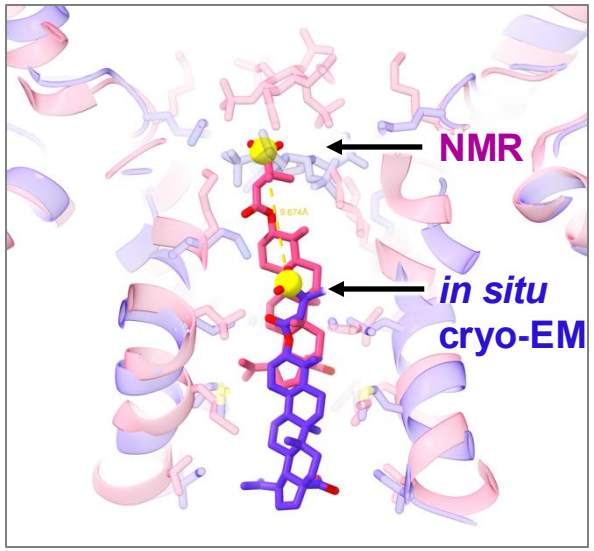
Comparisons with Previous BVM Structures

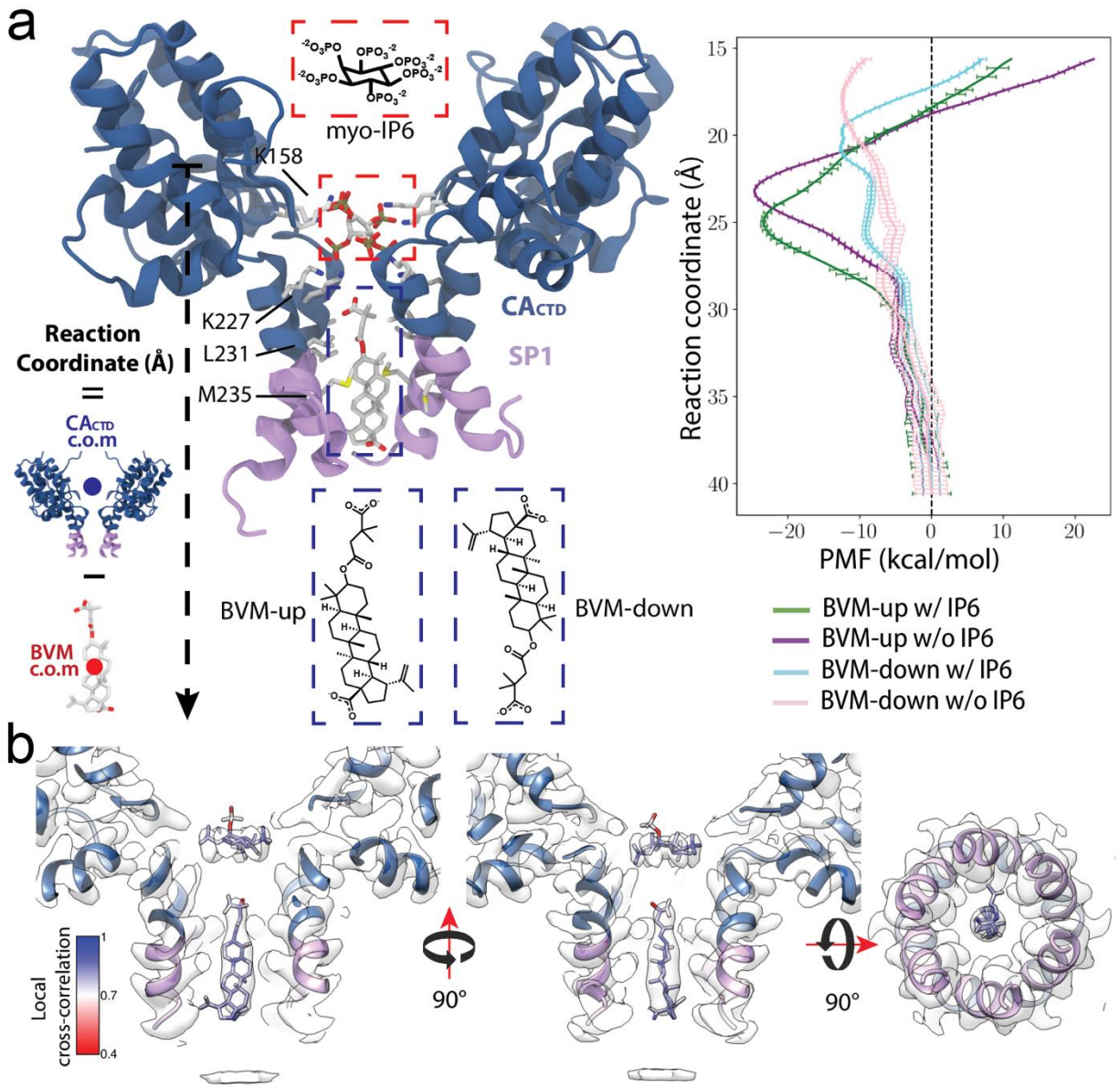


cryo-EM : MicroED



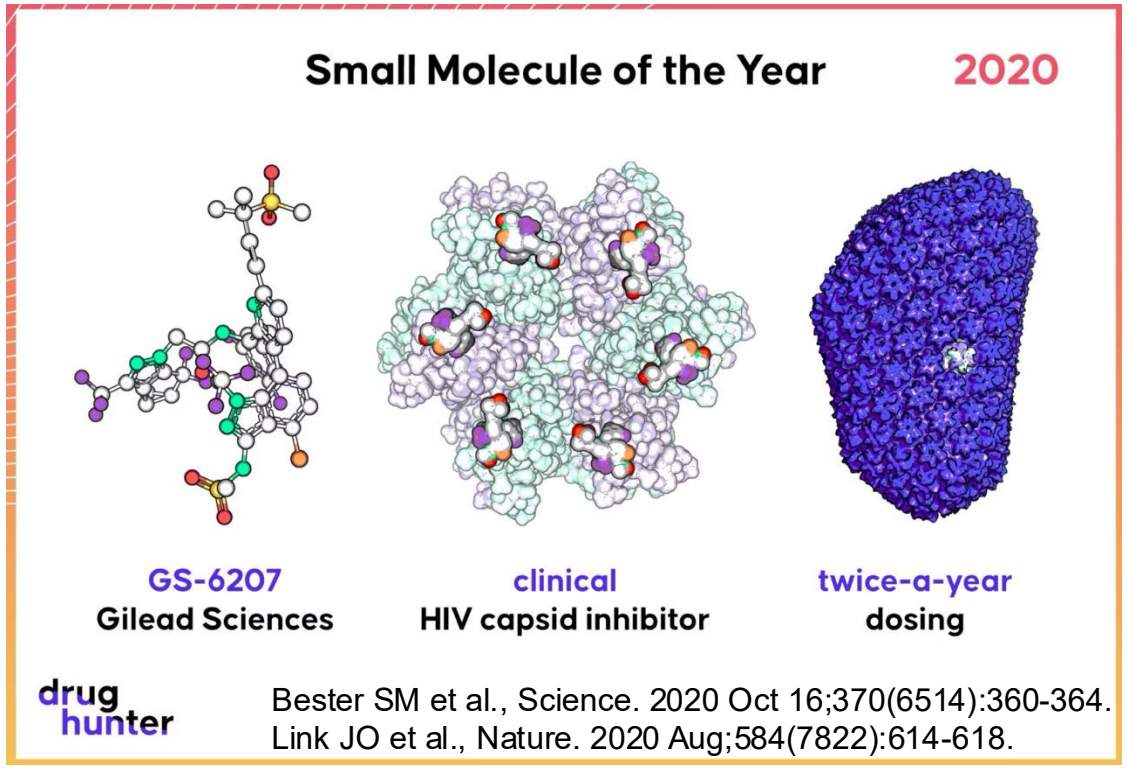
cryo-EM : NMR



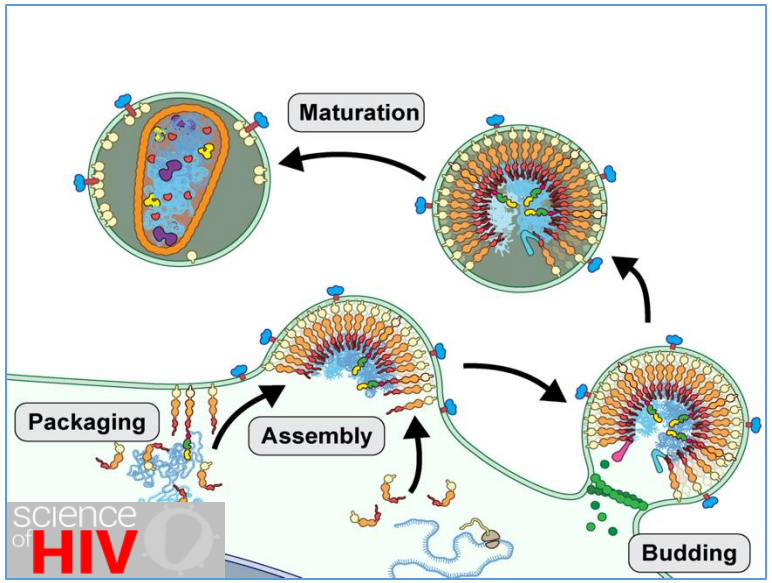


Full-atom molecular dynamic simulation (Juan Perilla, U Delaware)

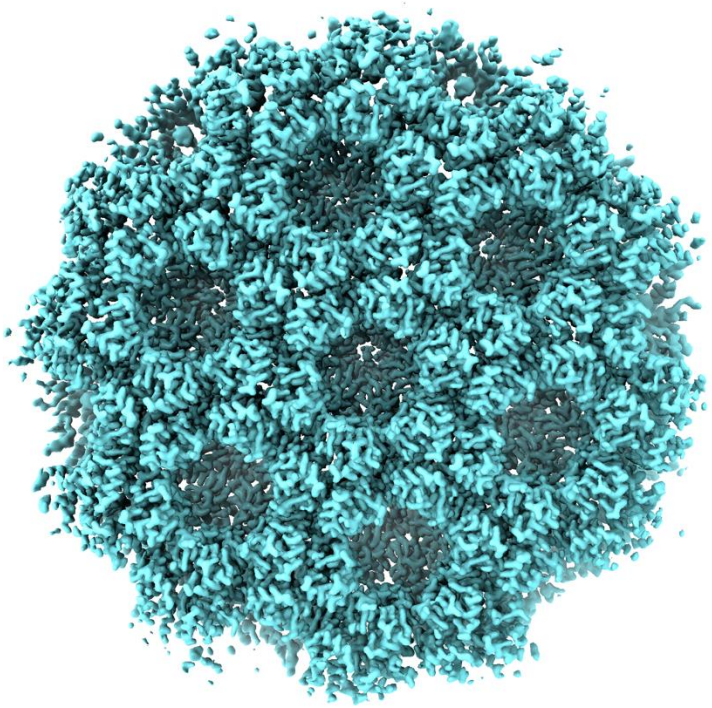
Lenacapavir (LEN)

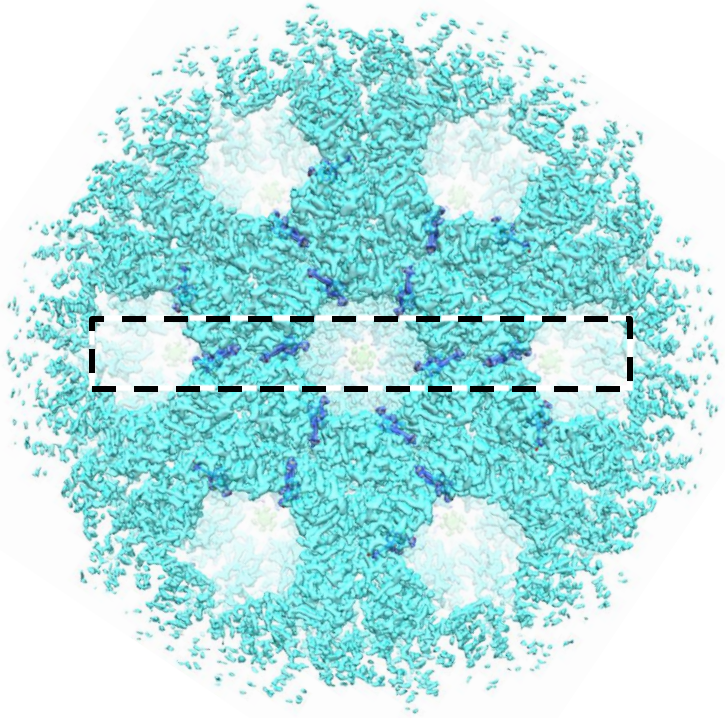


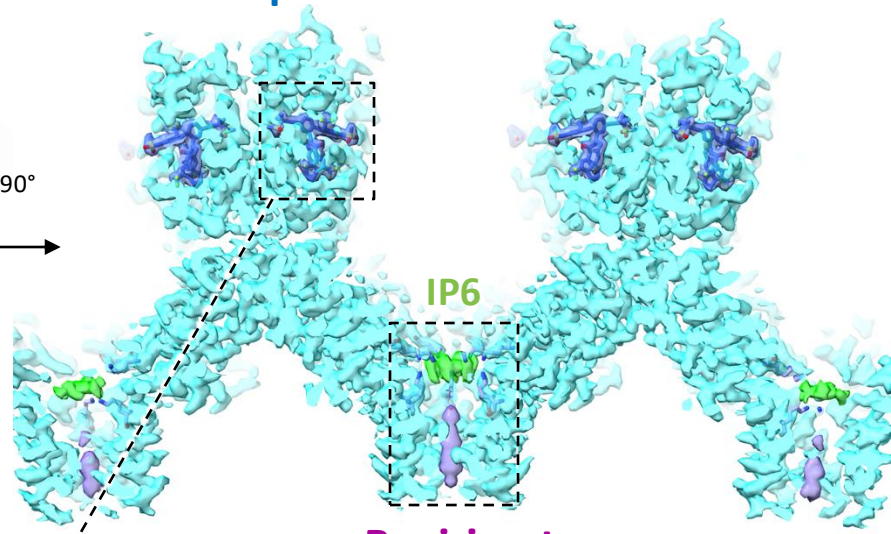
Bester SM et al., Science. 2020 Oct 16;370(6514):360-364.
Link JO et al., Nature. 2020 Aug;584(7822):614-618.

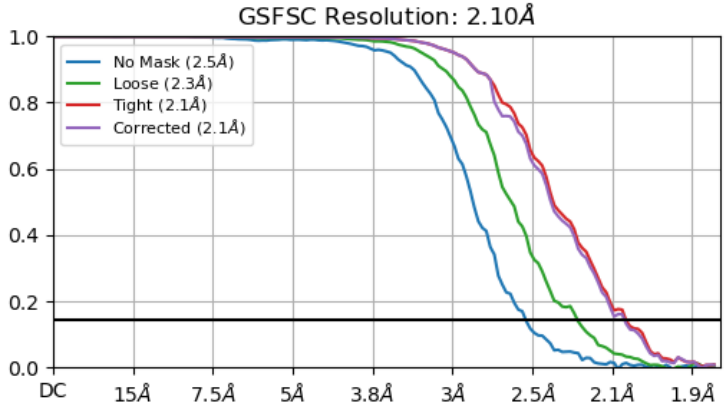


LEN Binding to PFO-treated Immature HIV-1 VLPs

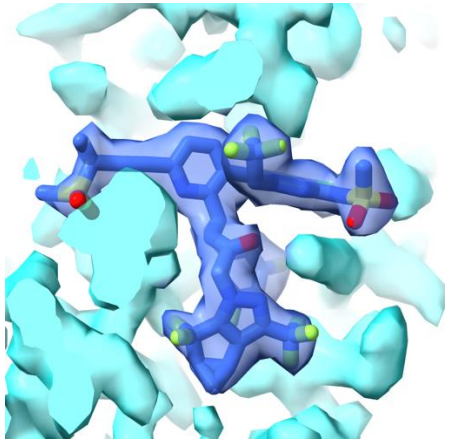


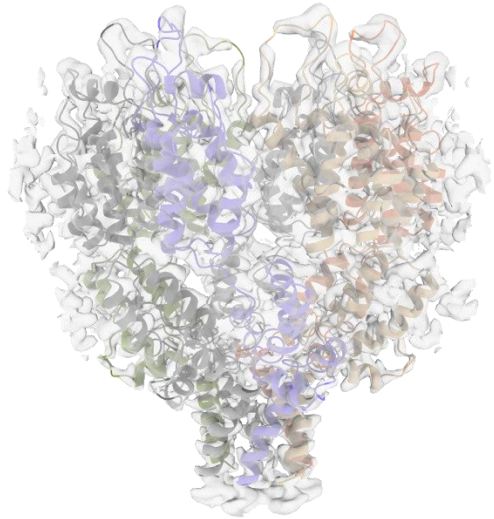






Lenacapavir

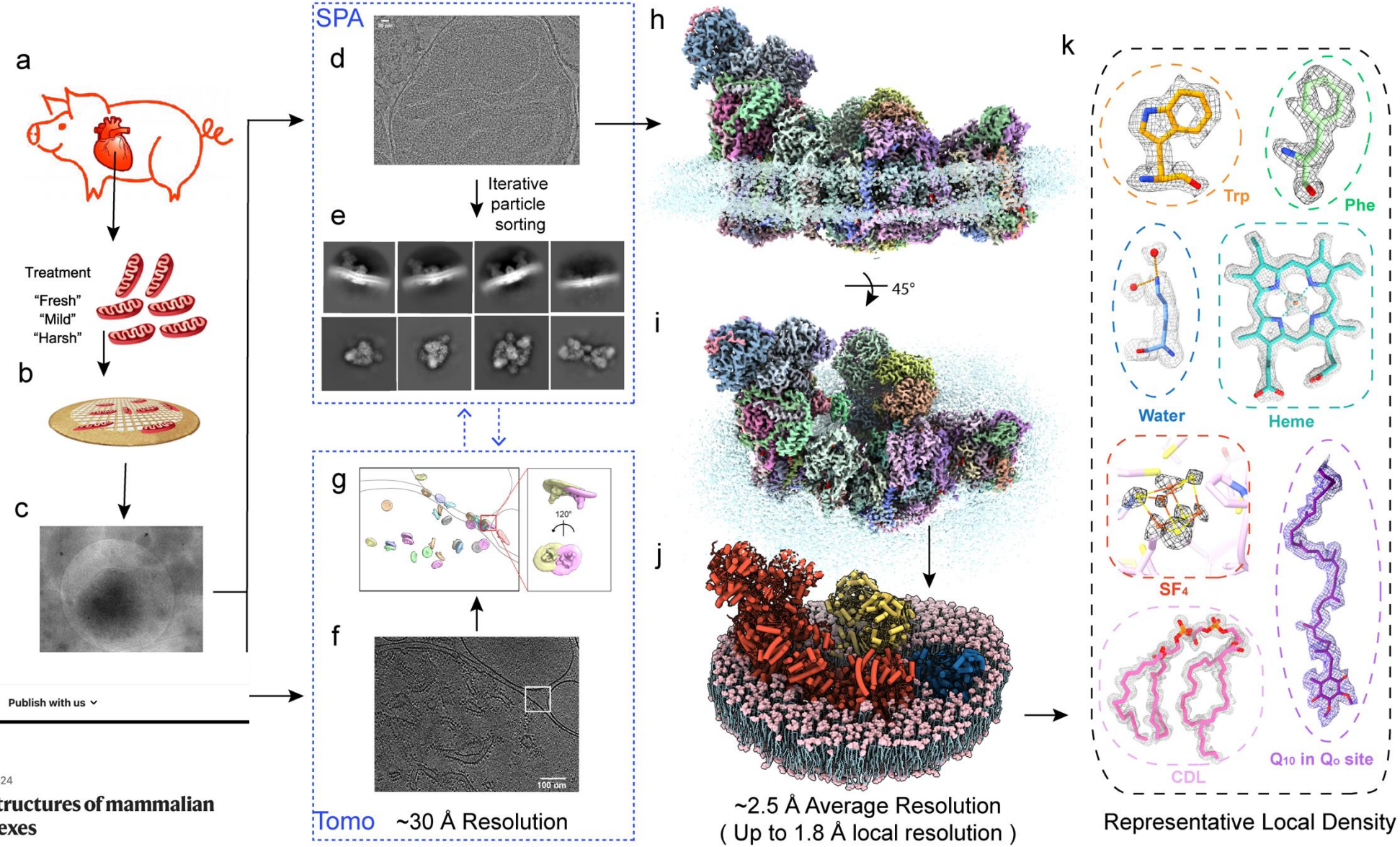




***in situ* structural studies in the native virus environment provide great opportunities for virology research and drug development**

Part 2—in situ cryo-EM of Purified Organelles

High-resolution *in situ* Structures of Mitochondrial Respiratory Supercomplexes in Reaction within Purified Mitochondria



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High-resolution in situ structures of mammalian respiratory supercomplexes

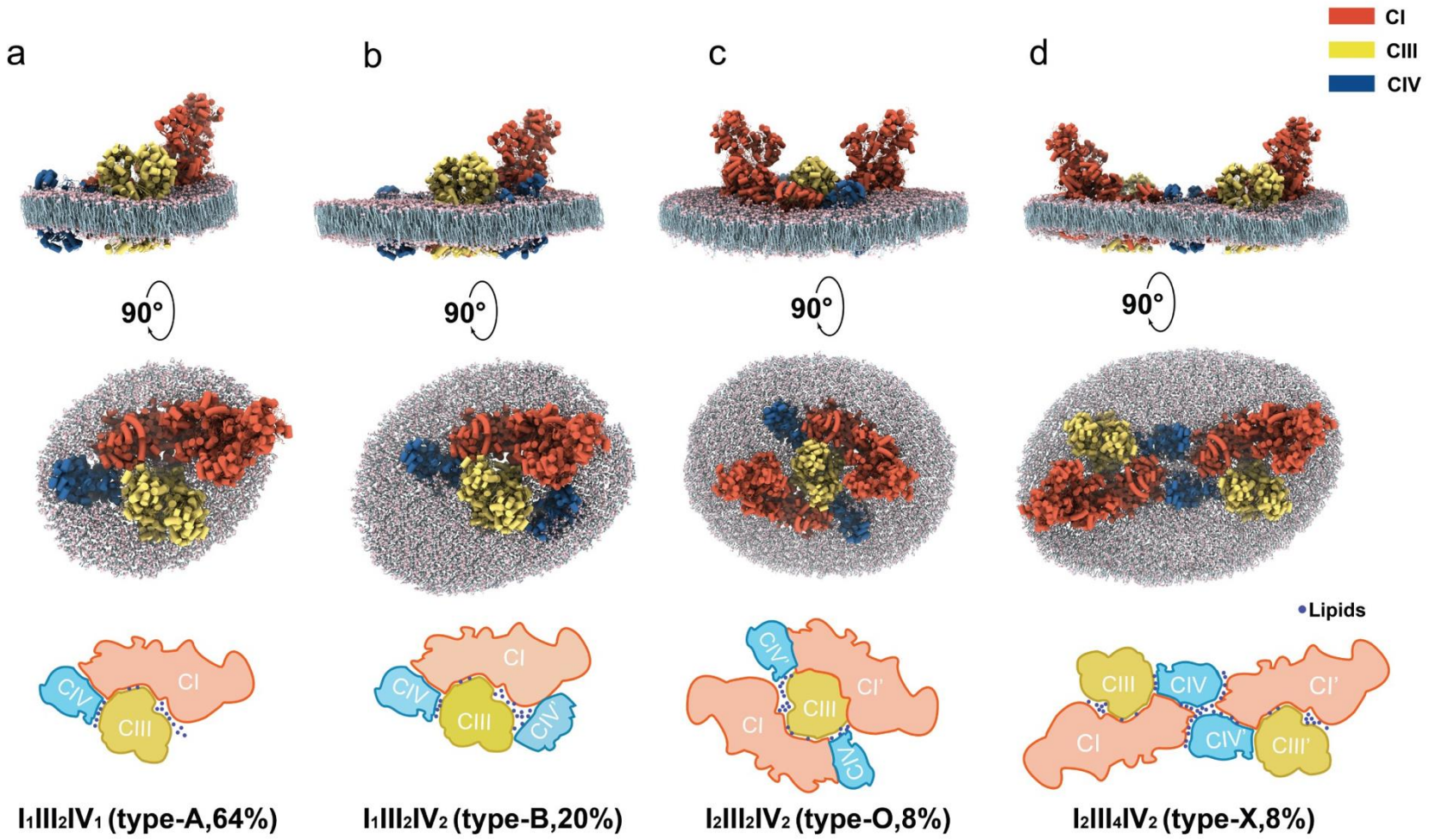
Wan Zheng, Pengxin Chai, Jiapeng Zhu & Kai Zhang

Nature (2024) | Cite this article

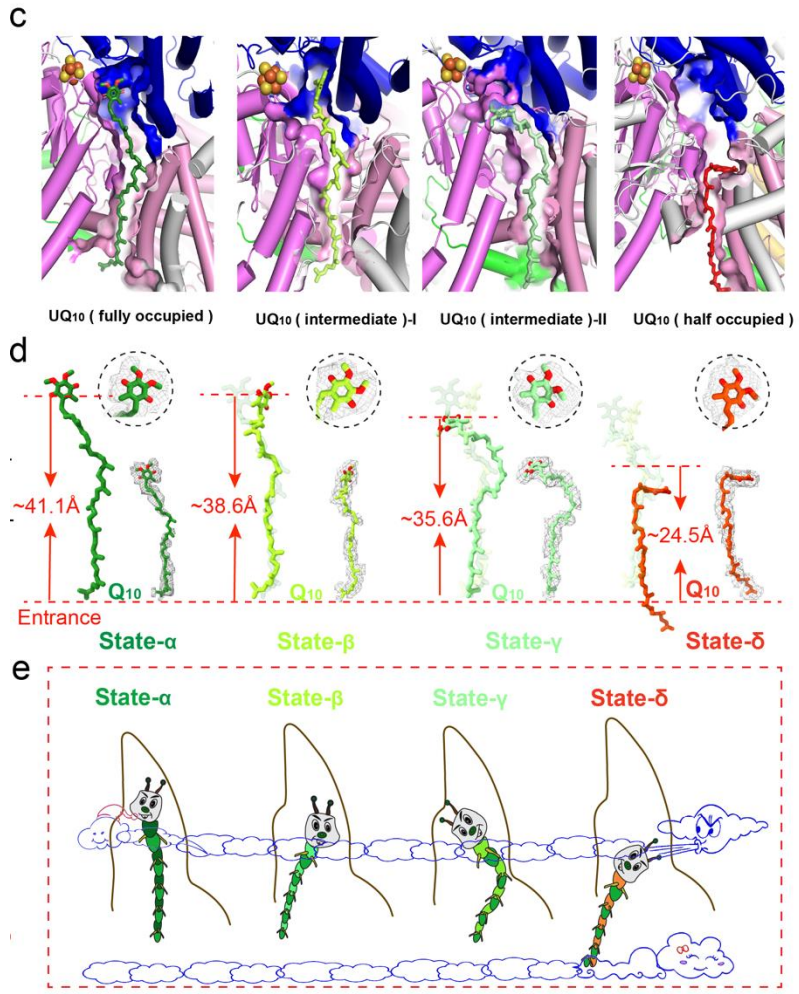
Kai Zhang, Yale

Bridging Biochemistry to the Cellular Environment

Discovery of various 'weakly-bound' forms of supercomplexes

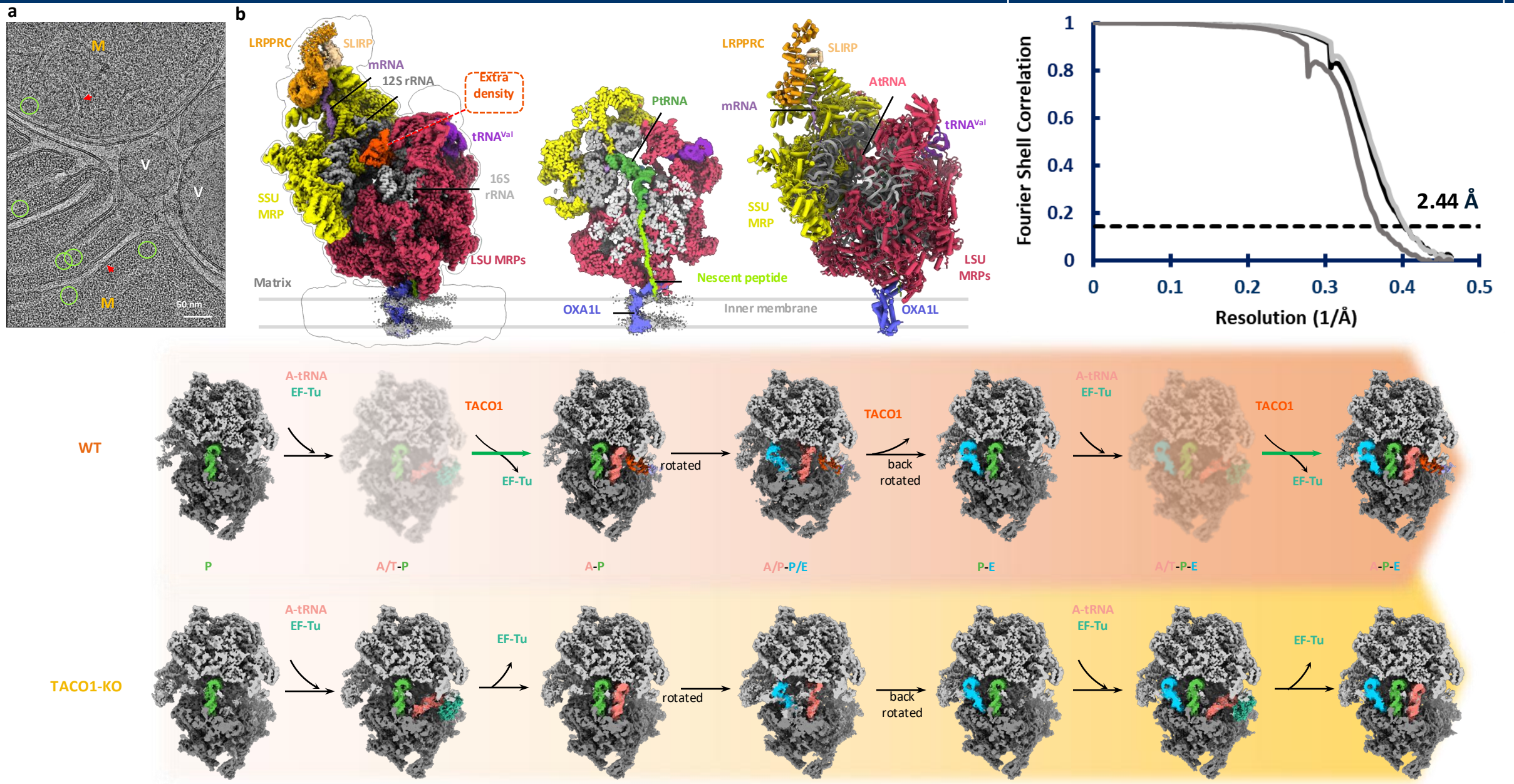


Visualizing the dynamic process of Q/QH2 turnover in Complex I



Kai Zhang, Yale

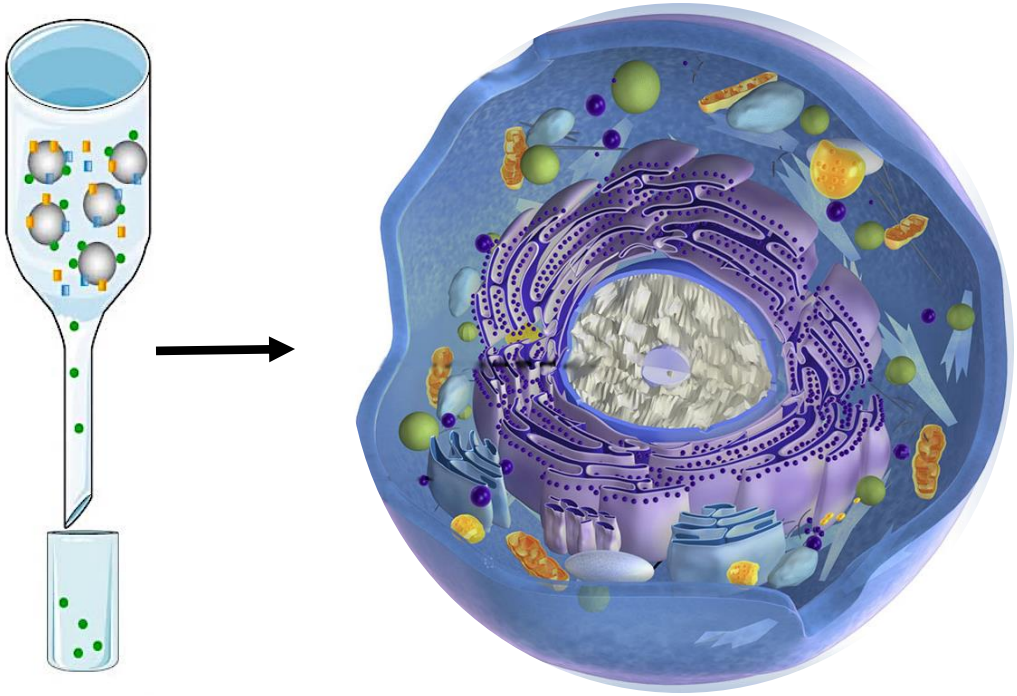
Identification of New Regulators of Mitochondria Ribosome



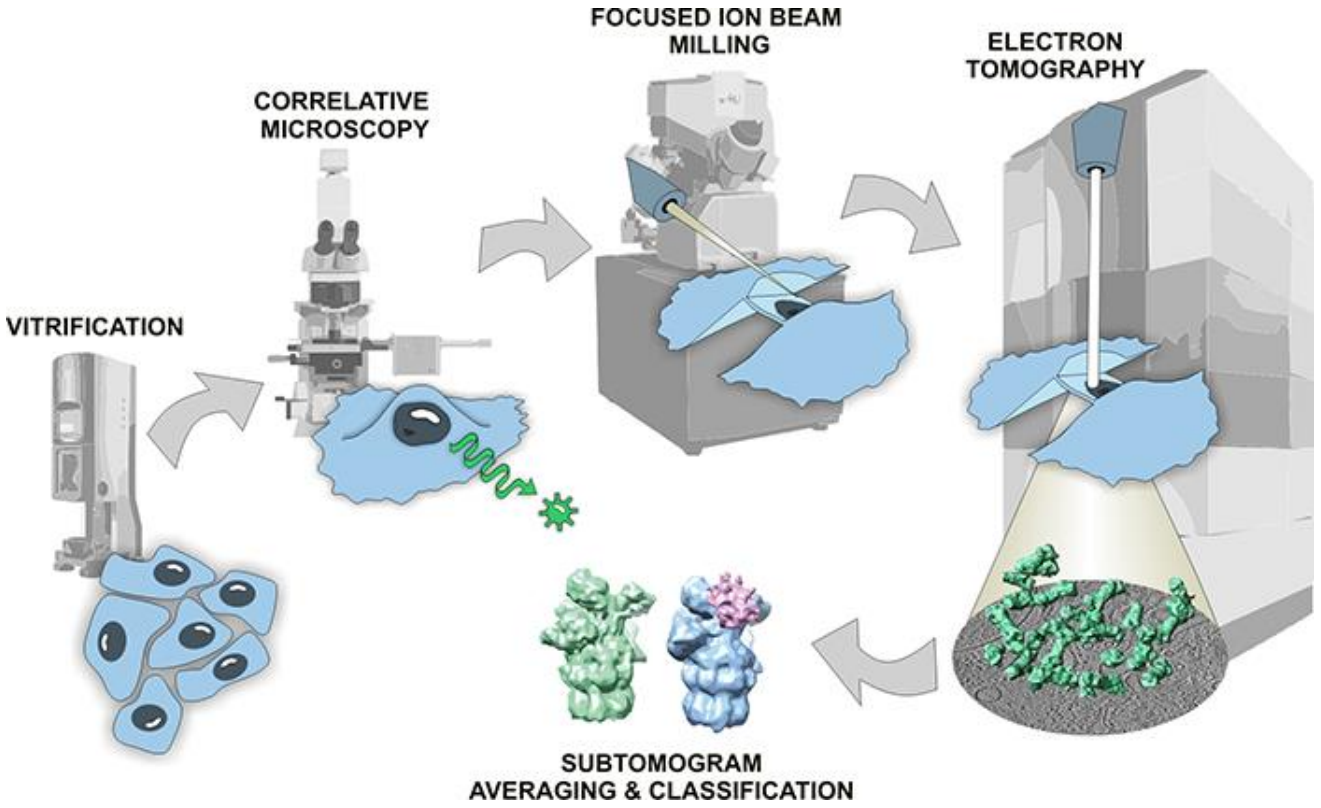
***in situ* structural studies with purified organelles provide great opportunities for fundamental biological research**

High-Resolution Visualization in Cells

From test tubes to cells



Cryo-FIB and cryo-ET are recently combined to resolve high-resolution structures in cells



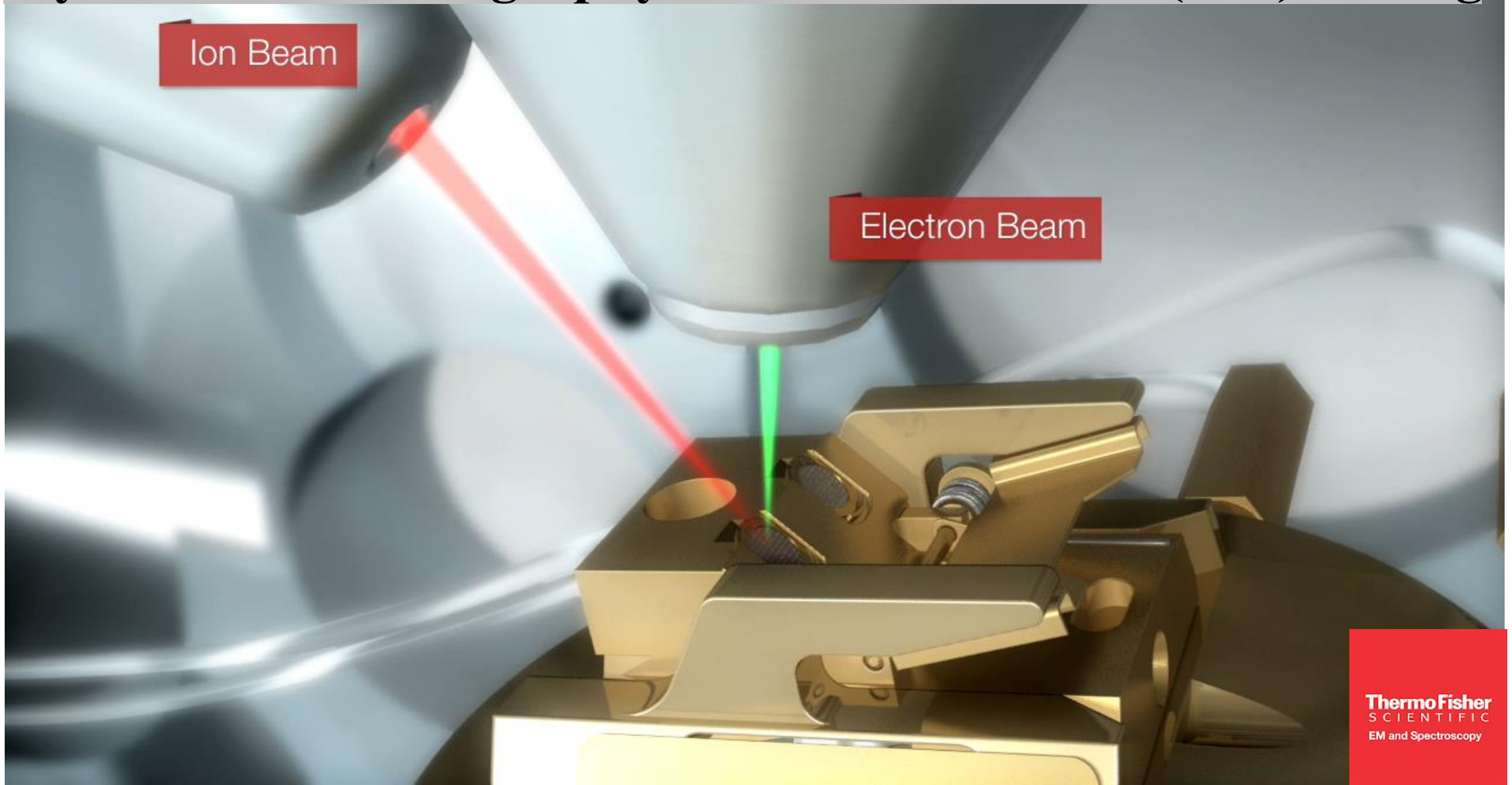
Rigort et al, 2018

Prepare Cell Samples for *in situ* cryo-EM Studies

ThermoFisher

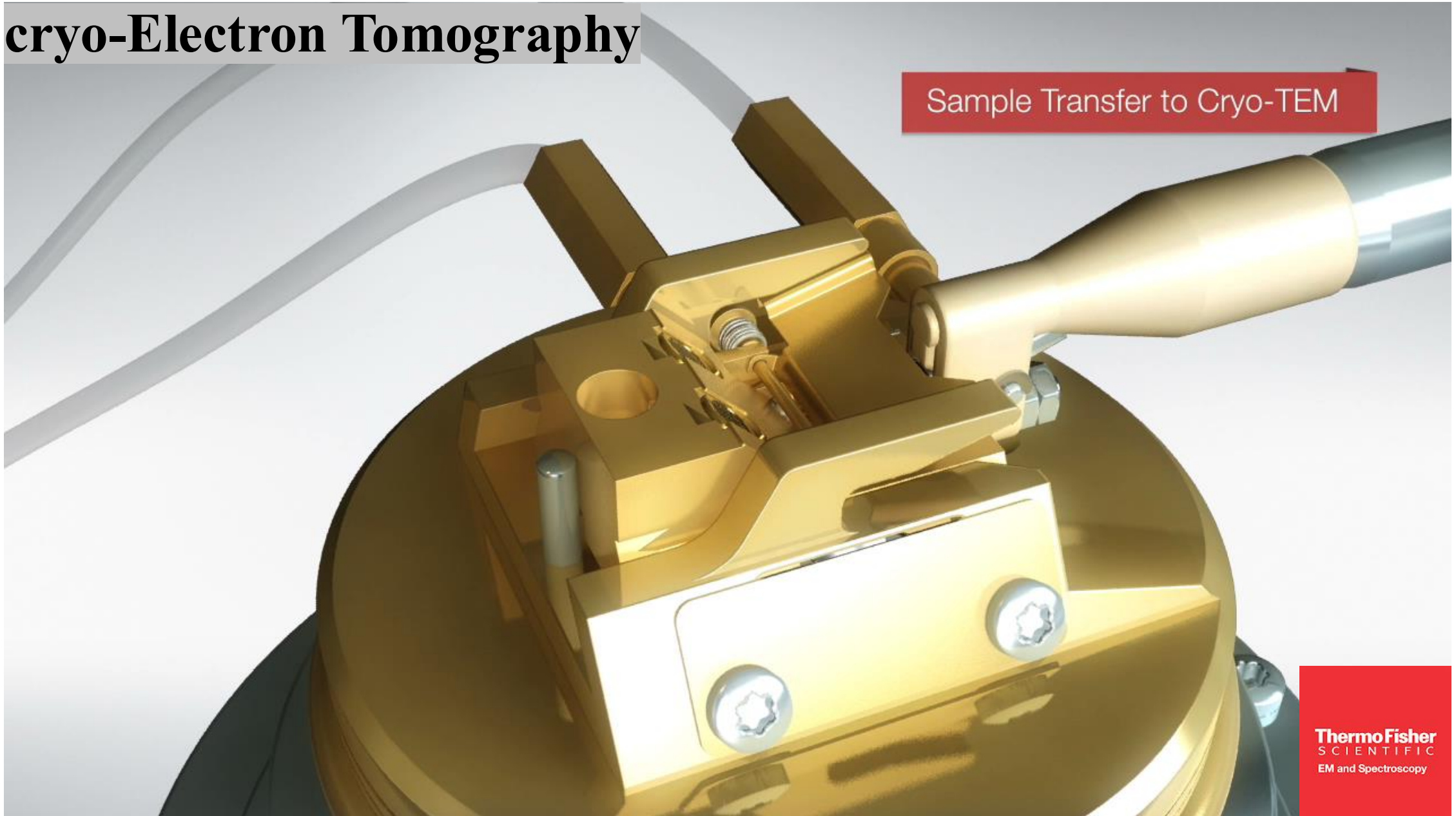
Cryo-ET of Viruses in Cells

cryo-Electron Tomography: Focused Ion Beam (FIB) milling

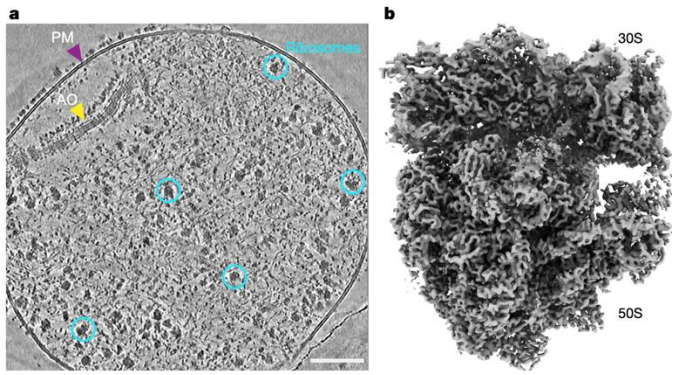


Cryo-ET of Viruses in Cells

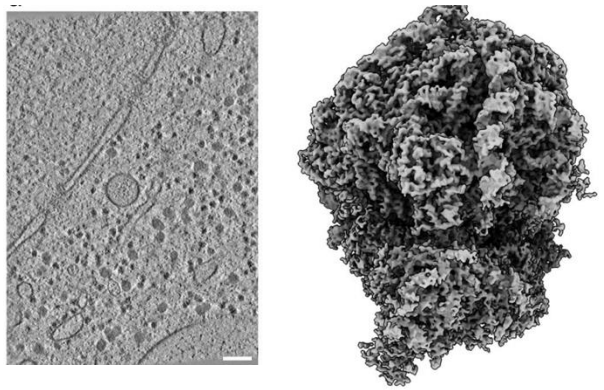
cryo-Electron Tomography



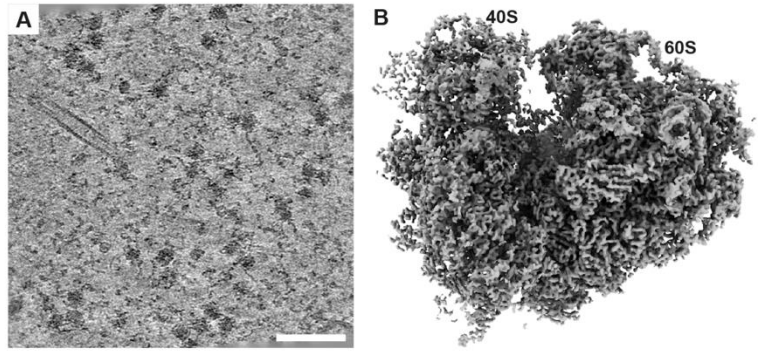
High-Resolution Ribosome Structures in Cells



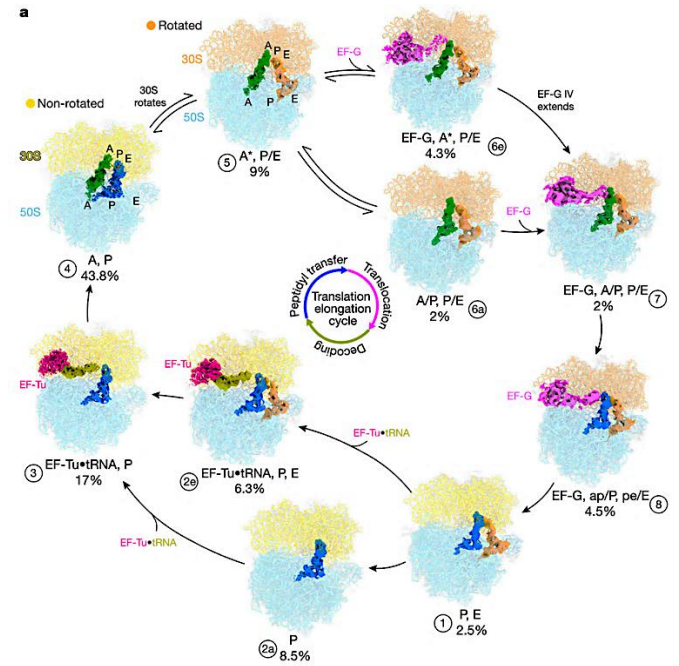
70S (3.5 Å) in *M. pneumoniae*



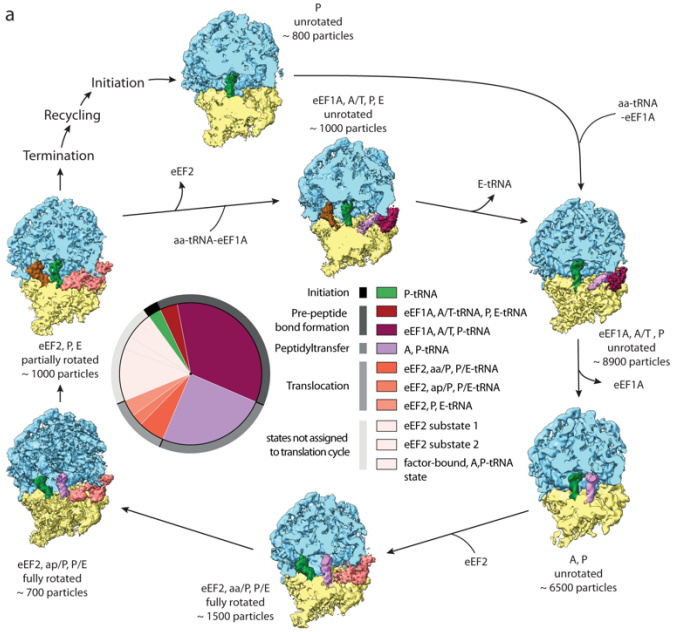
80S (3.8 Å) in *D. discoideum*



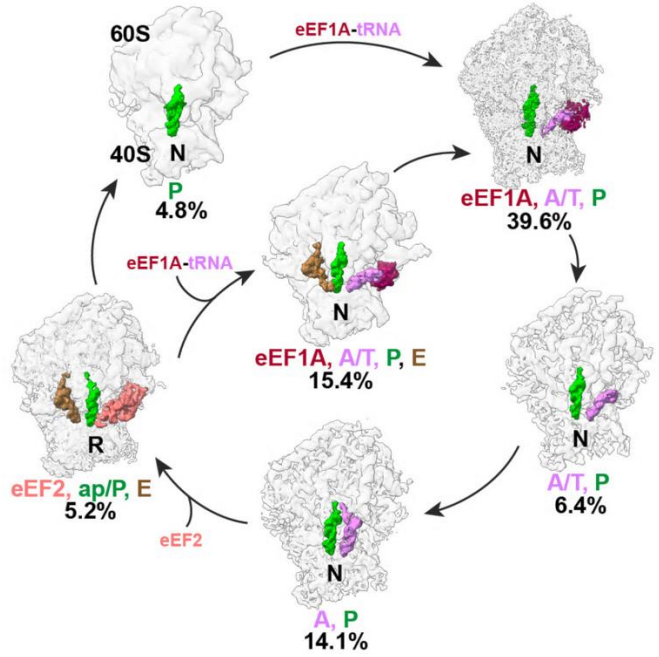
80S (3.2 Å) in Human 293T Cells



Xing et al., Nature, 2022

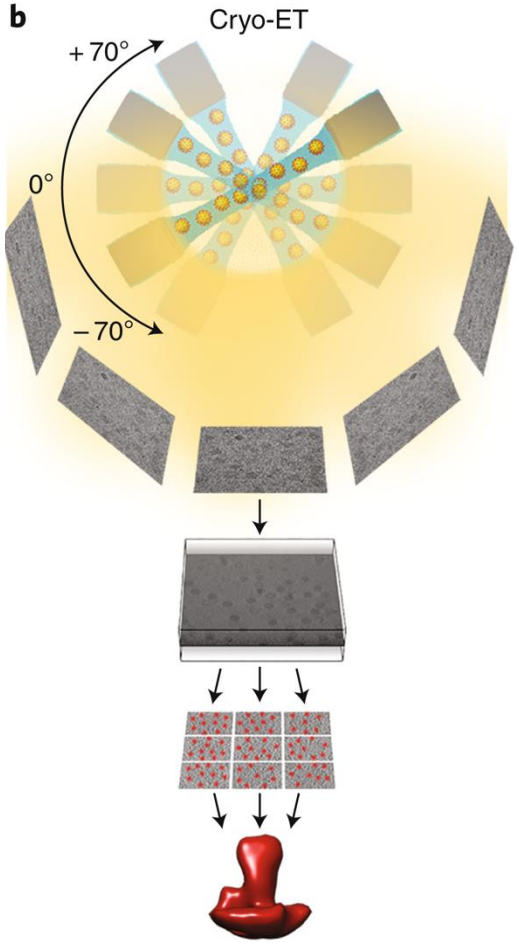


Hoffmann et al., Nat. Commun, 2022



Xue et al., Science, 2023

cryo-ET vs. single Particle cryo-EM for *in situ* Studies

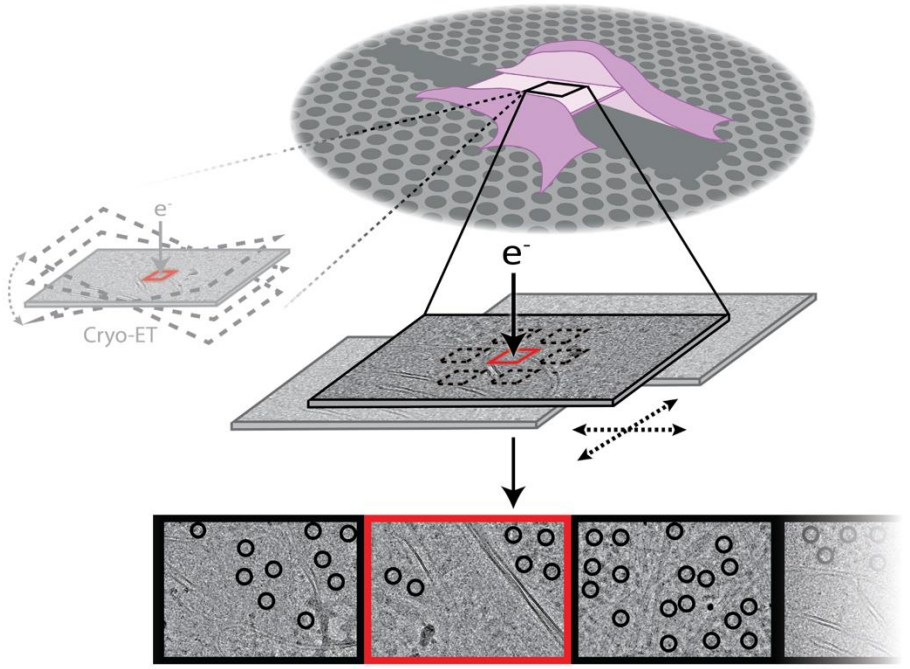


Luque et al, 2020

Cryo-ET is extremely powerful for *in situ* work, but….

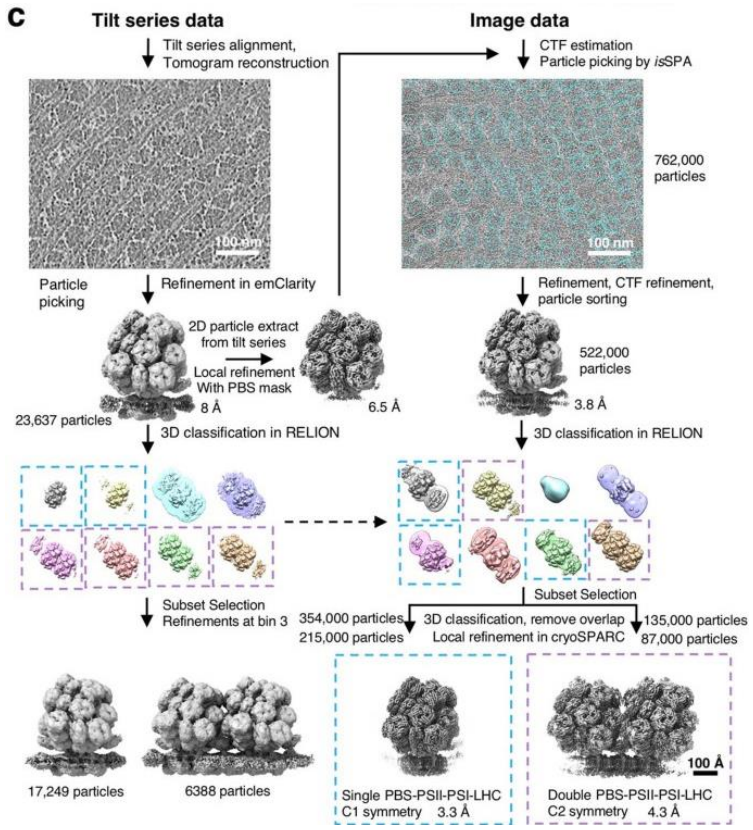
- High tilt affects signal-to-noise ratio and data quality
- Time/resource consuming for data collection and processing
- Experience dependent, not user-friendly

Can we combine cryo-FIB with single particle cryo-EM to study the in-situ structure at high resolution?



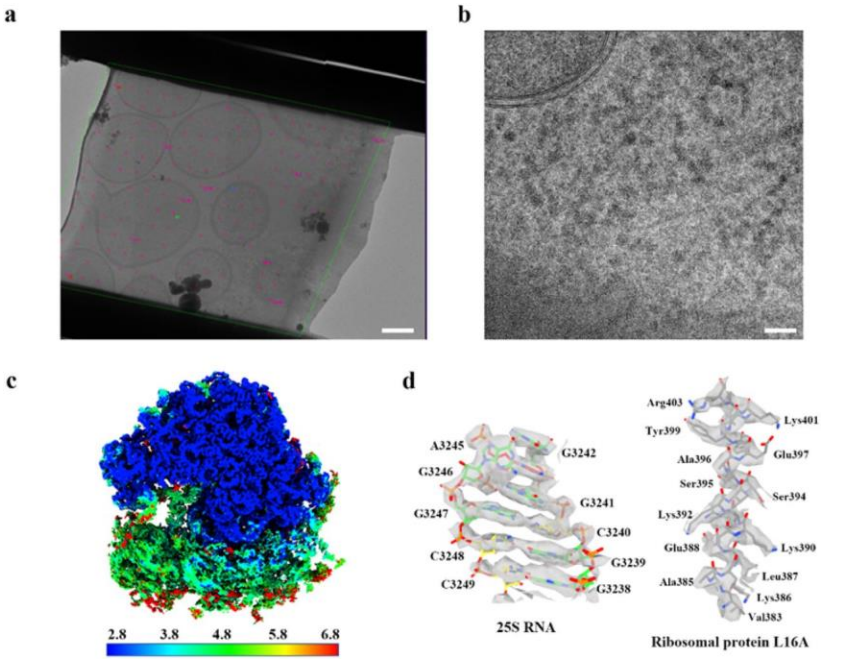
in situ Single Particle cryo-EM Studies

Phycobilisome–PSII–PSI–LHC megacomplex from red algae

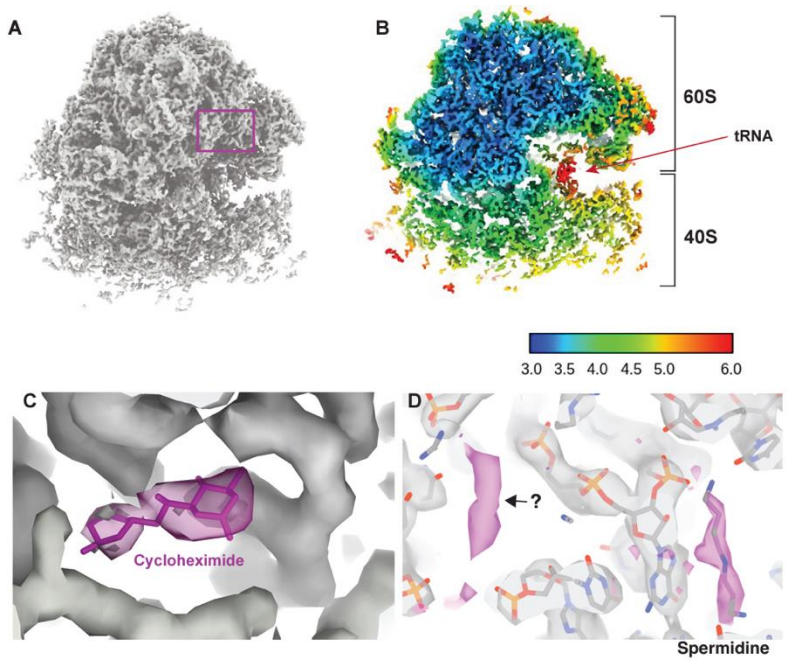


3.3 Å, You et al., Nature, 2023

Yeast 80S ribosomes



2.90 Å (60S), Cheng et al., BioRxiv, 2023



3.15 Å, Lucas et al., BioRxiv, 2023

isSPA/GisSPA

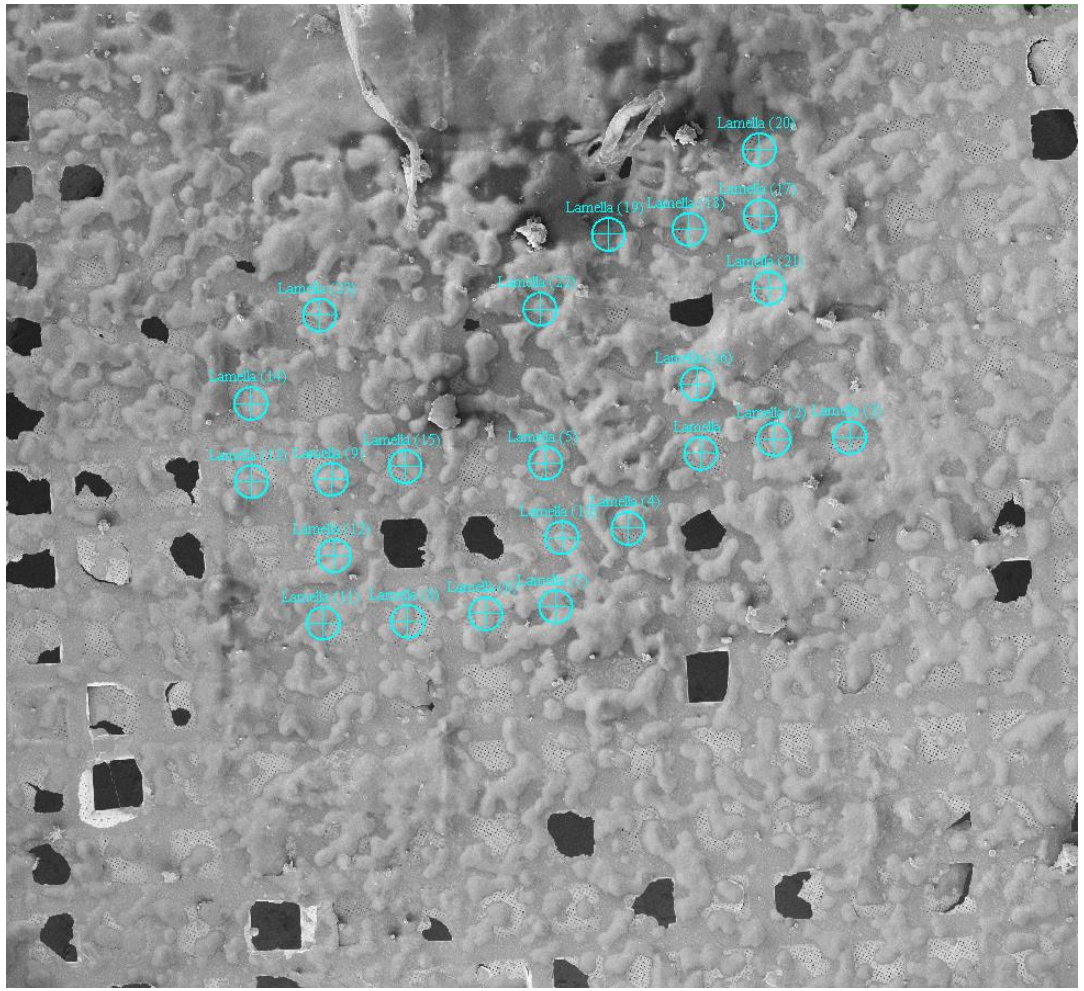
J Cheng, B Li, L Si, X Zhang. *The Innovation,* 2021
J Cheng, T Liu, X You, F Zhang, SF Sui, X Wan, X Zhang . *Nat Common,* 2023

2DTM

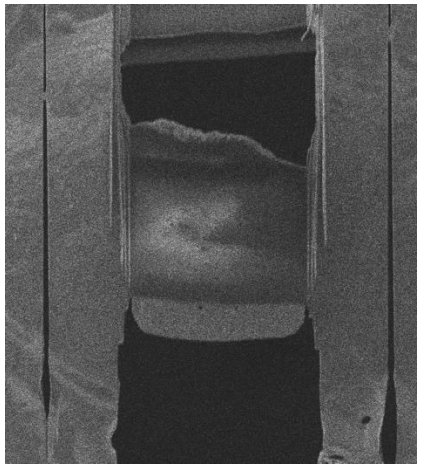
JP Rickgauer, N Grigorieff , W Denk. eLife, 2017
Elferich J, Schiroli G, Scadden DT, Grigorieff N. eLife, 2022
Lucas BA, Zhang K, Loerch S, Grigorieff N. eLife, 2022

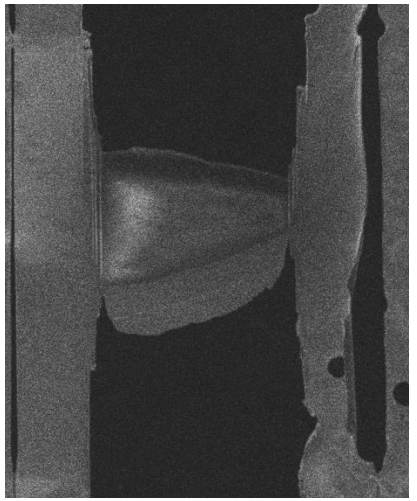
FIB-milling of Human 293A Cells

Frozen cells on EM grid



Typical lamellae





Wei Zheng

Wangbiao Guo

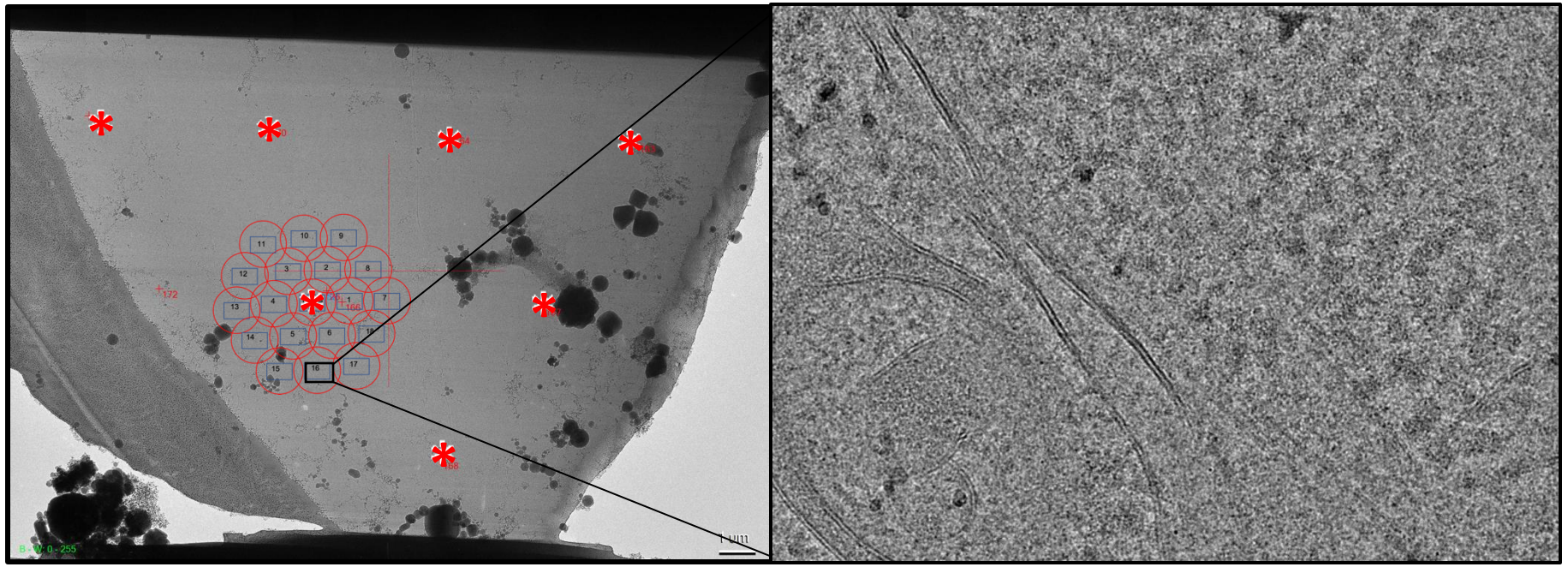
Jun Liu

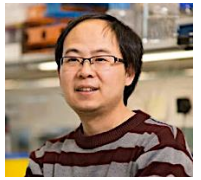
Jianfeng Lin

Lamellae (100nm-200nm thick) used for final data collections:
Native cells: 195 lamellae from 12 days of milling
Inhibitor-treated cells: 110 lamellae from 6 days of milling
(Training/experimenting to get there: months)

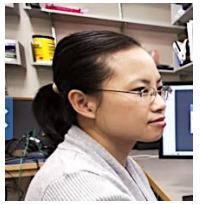
Single Particle cryo-EM data Collection on the Lamellae

High efficiency data collection





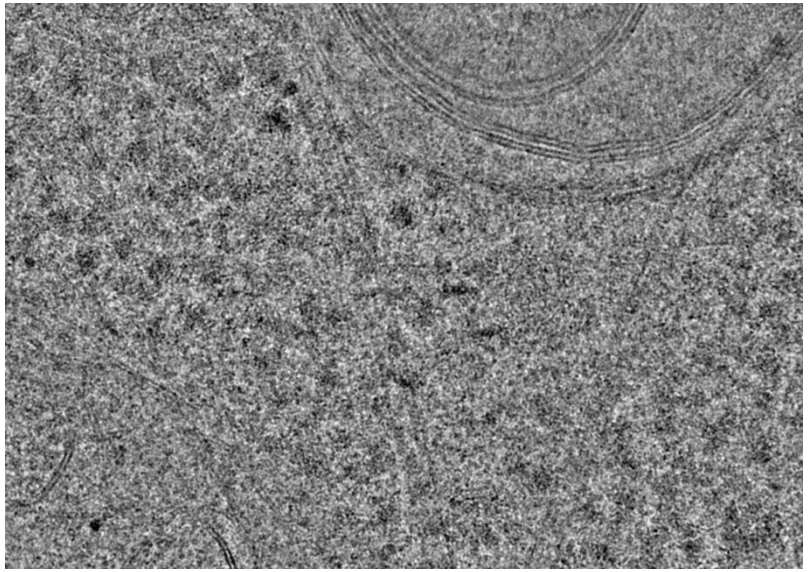
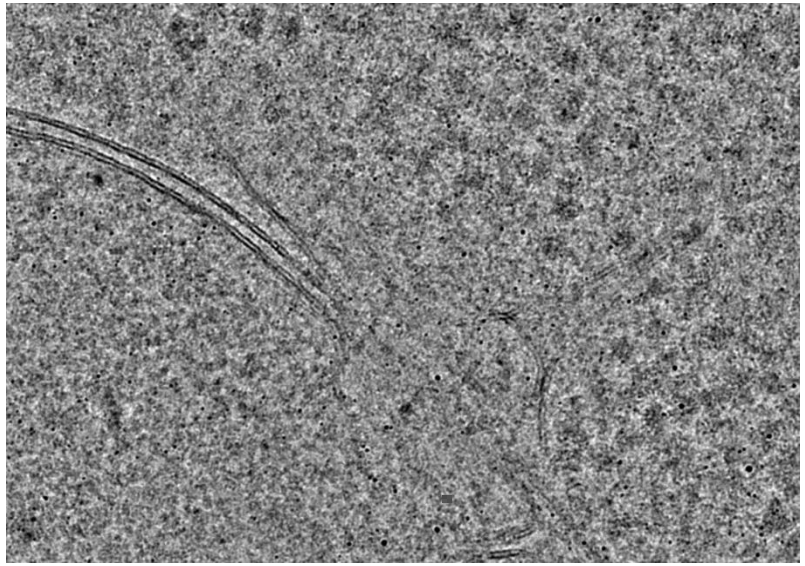
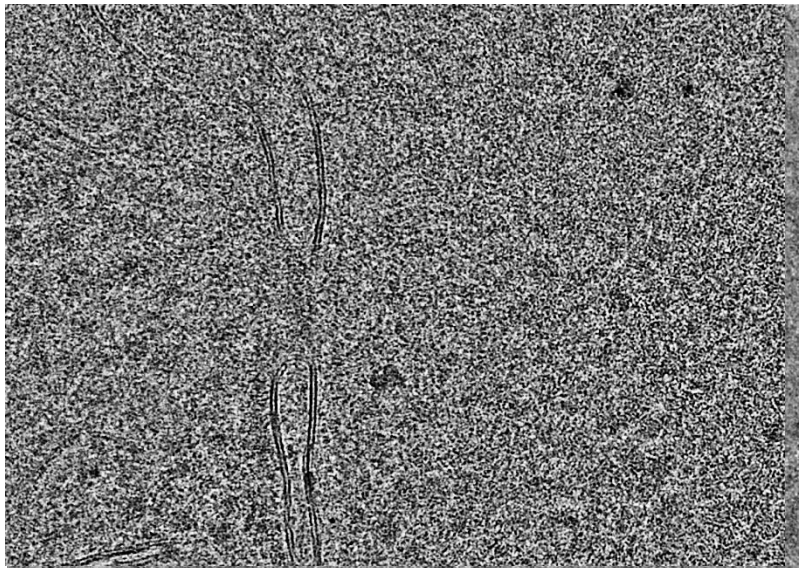
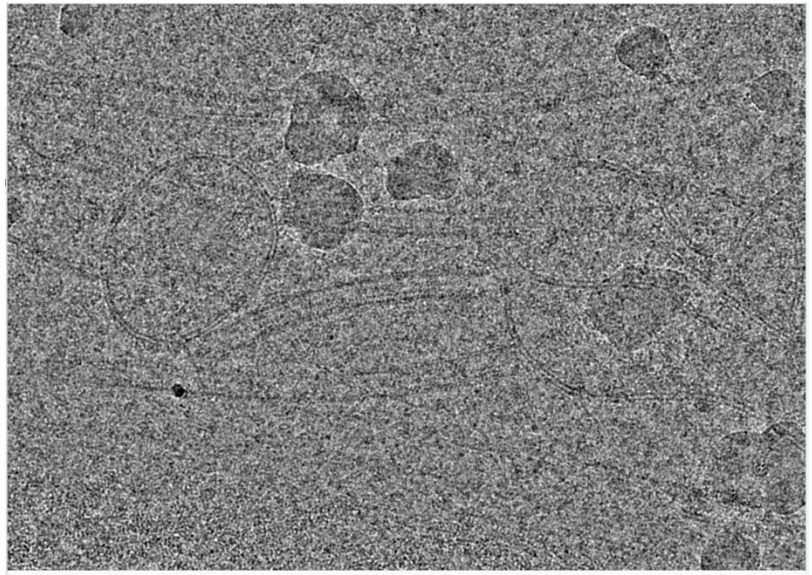
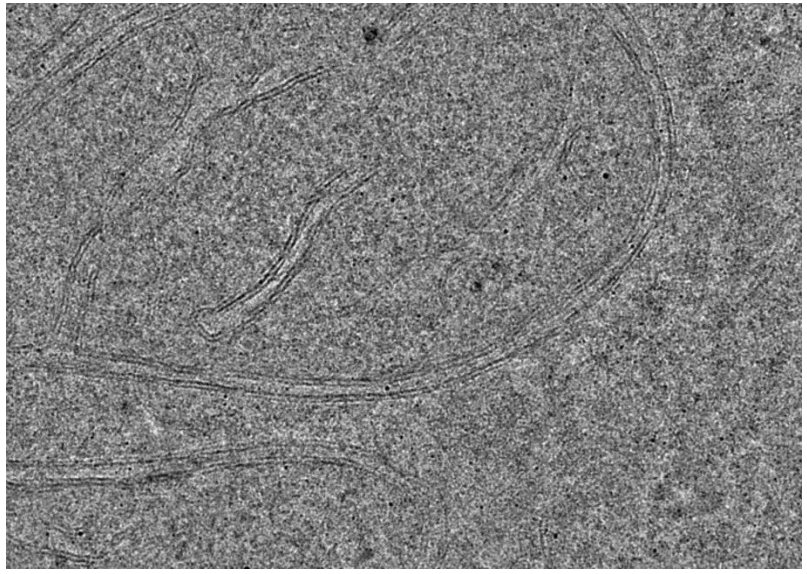
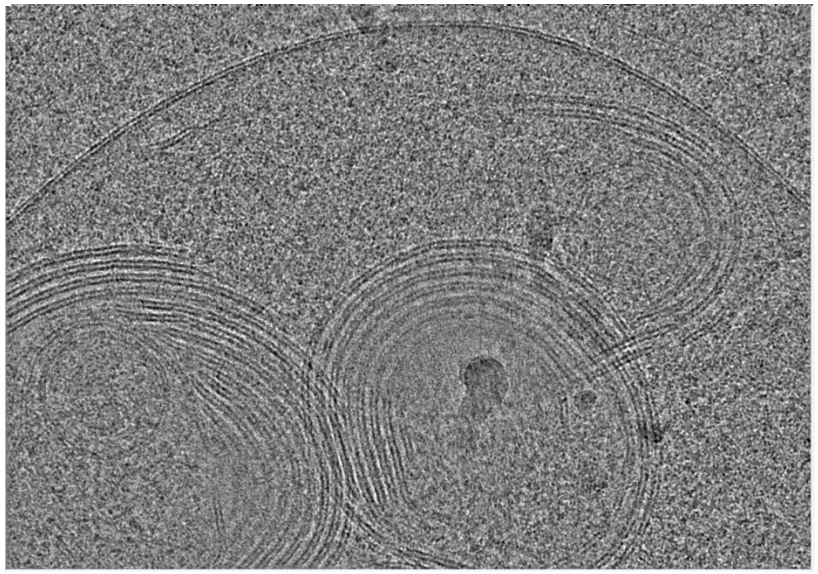
Kai Zhang



Shenping Wu

Native cells: 15,274 micrographs (3.5 days)
Inhibitor treated cells: 6,479 micrographs (1.5 days)

Features in Cells

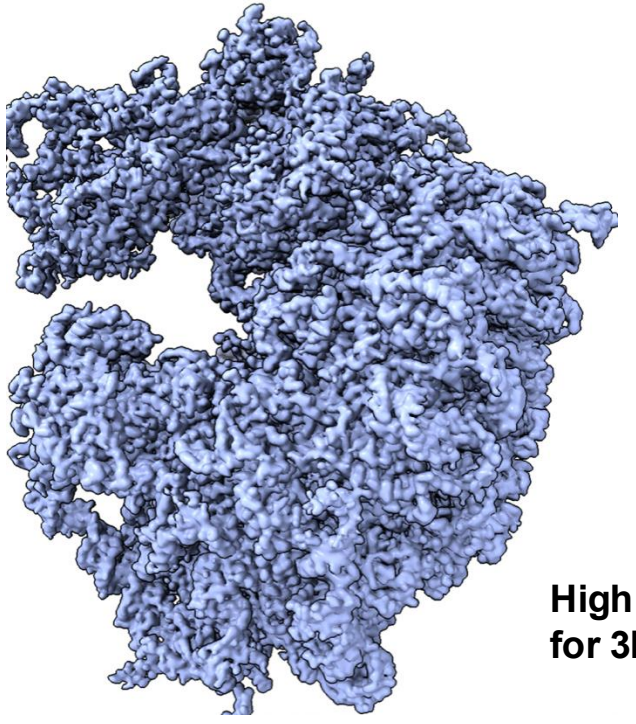







High-resolution *in situ* Single Particle Cryo-EM Structures of Ribosomes

Yong Xiong 3/10/23
cell lamella data 80s
To: Wu, Shenping & 7 more Details

It worked! A prelim reconstruction from partial data and incomplete analysis (an initial class of ~30K particles, within a day after collecting data :). It is now at ~3.6 Å resolution and the map looks good. We have about a million particles to mine for different conformation classes and the final resolution will also be quite a bit better….

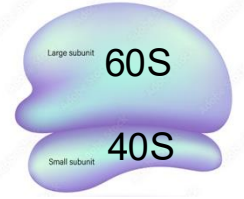
Best,
Yong



High Efficiency for 3D reconstruction

Penxin Chai

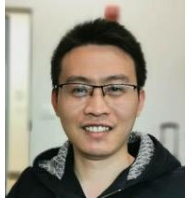
GisSPA template matching using 60S refence (EMD-15113)



80S

Wei Zheng

Yuekang Zhang



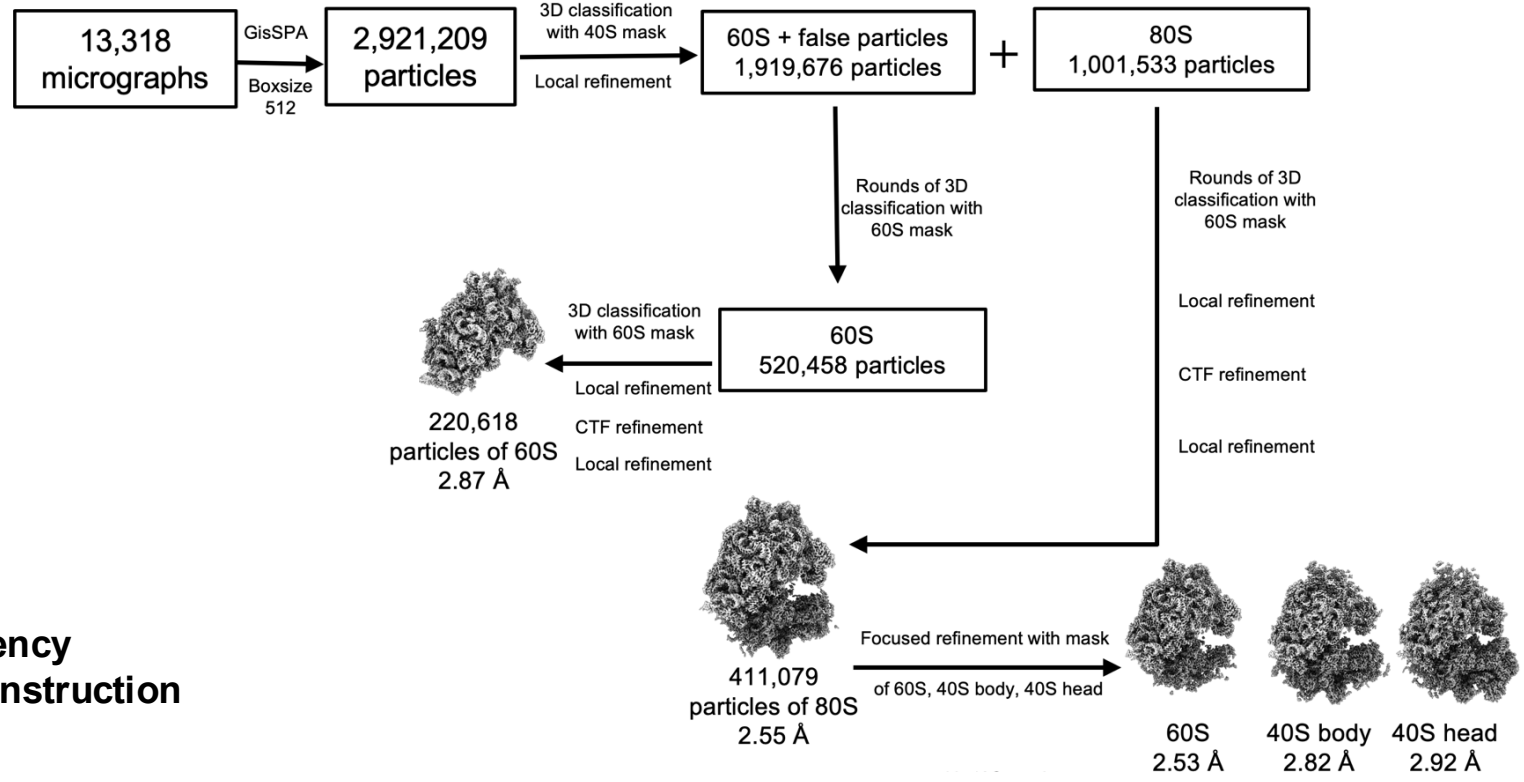
Shuhui Wang

Liz Bailey

Swapnil Devarkar

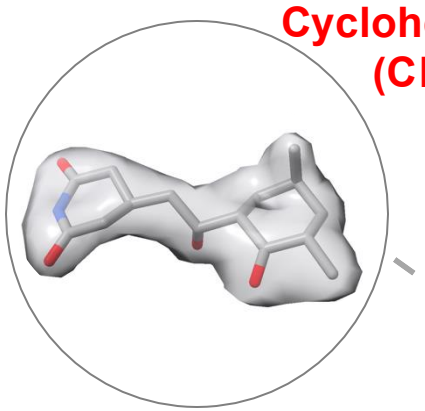
Ivan Lomakin

Jimin Wang

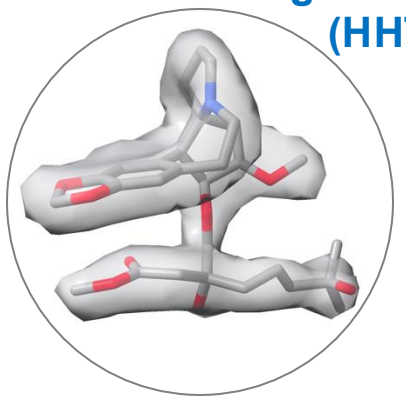


Atomic Level *in situ* Single Particle Cryo-EM Structures of Ribosomes

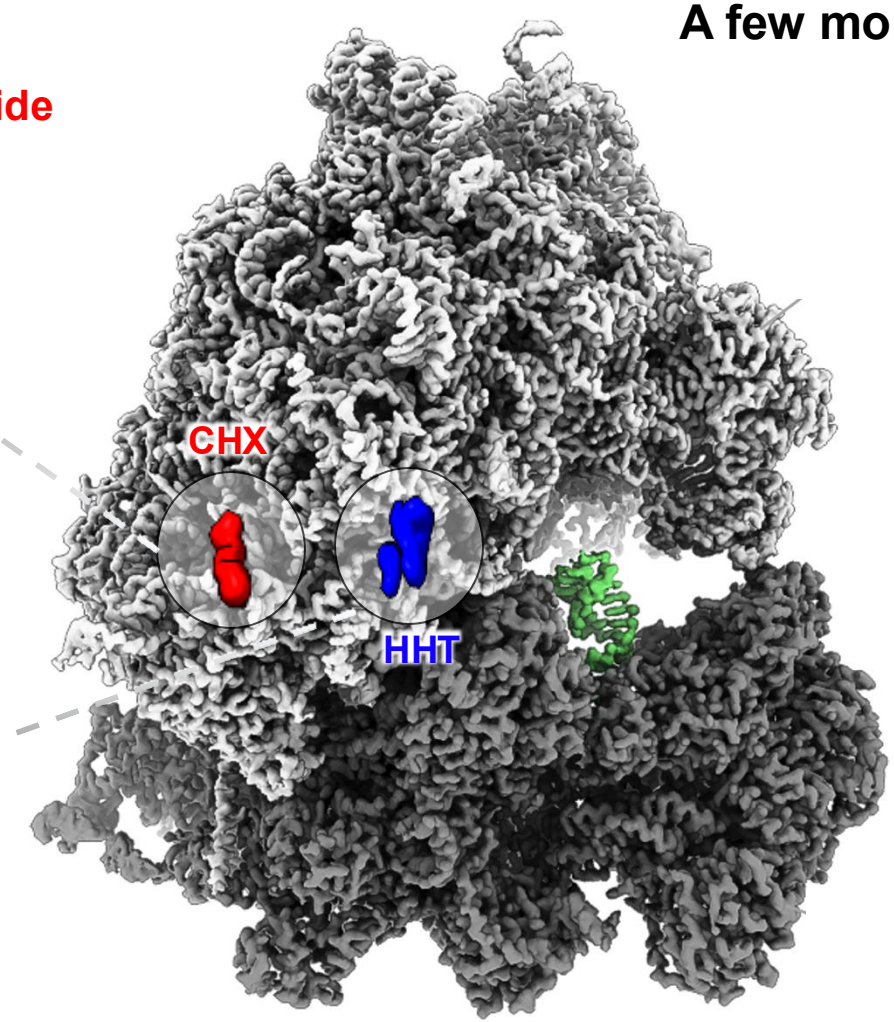
High Resolution



Cycloheximide
(CHX)



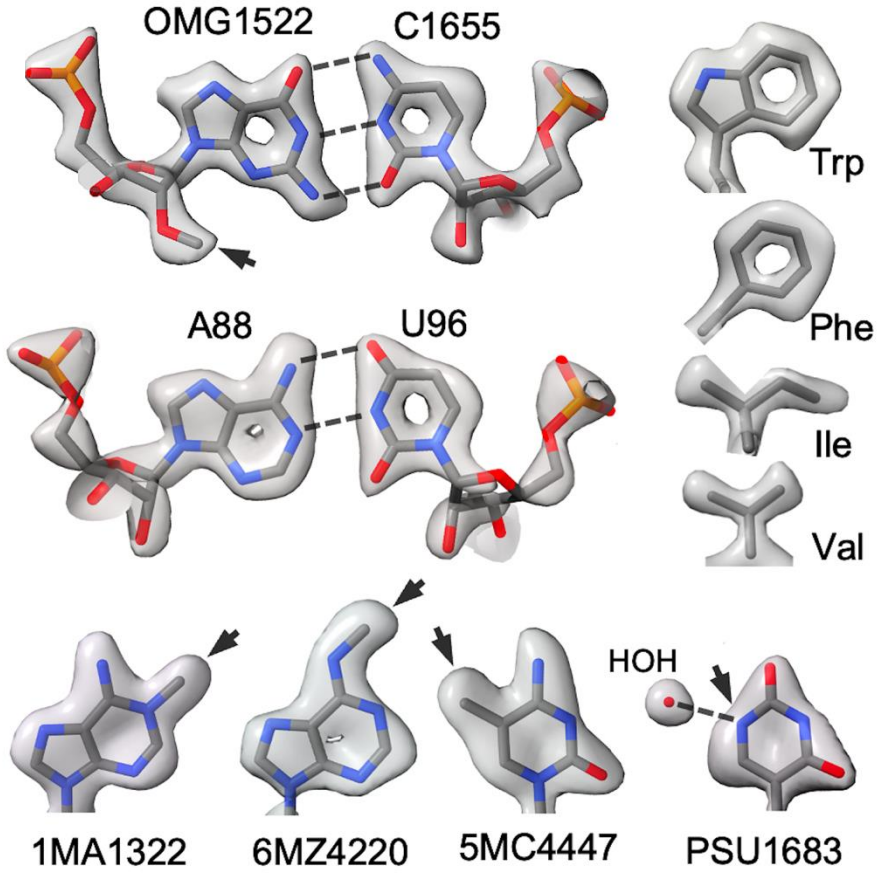
Homoharringtonine
(HHT)



Allowing for high-resolution
drug/inhibitor studies
in the native cell environment

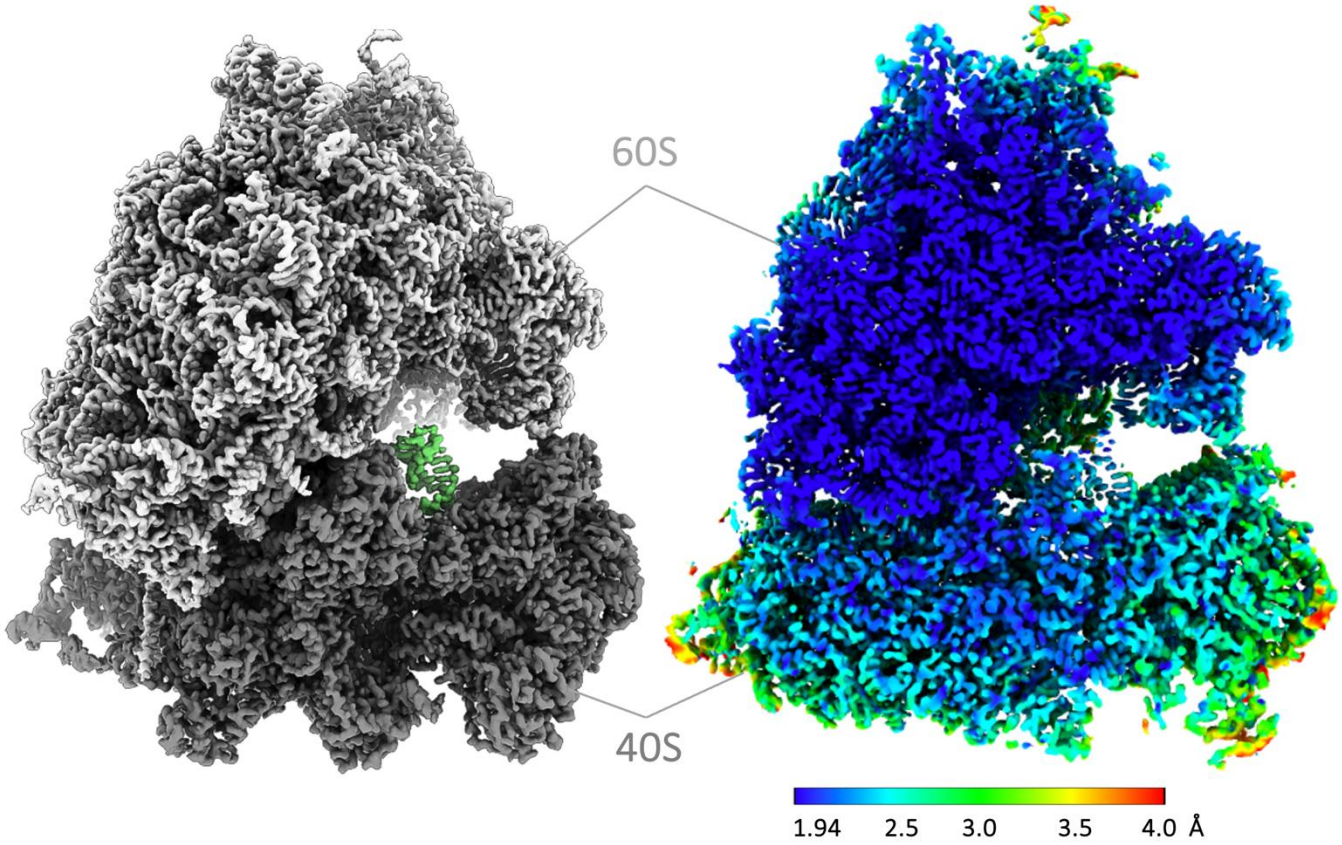
2.16 Å resolution

A few more months

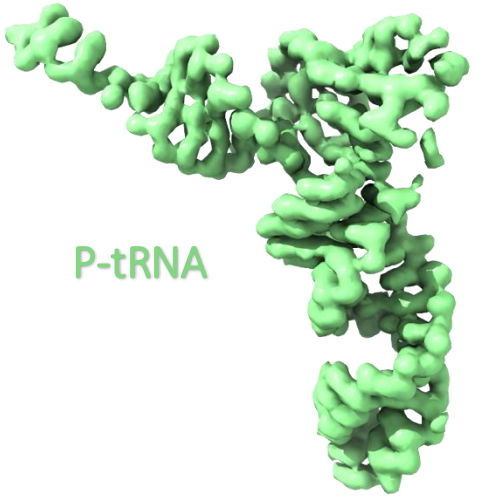


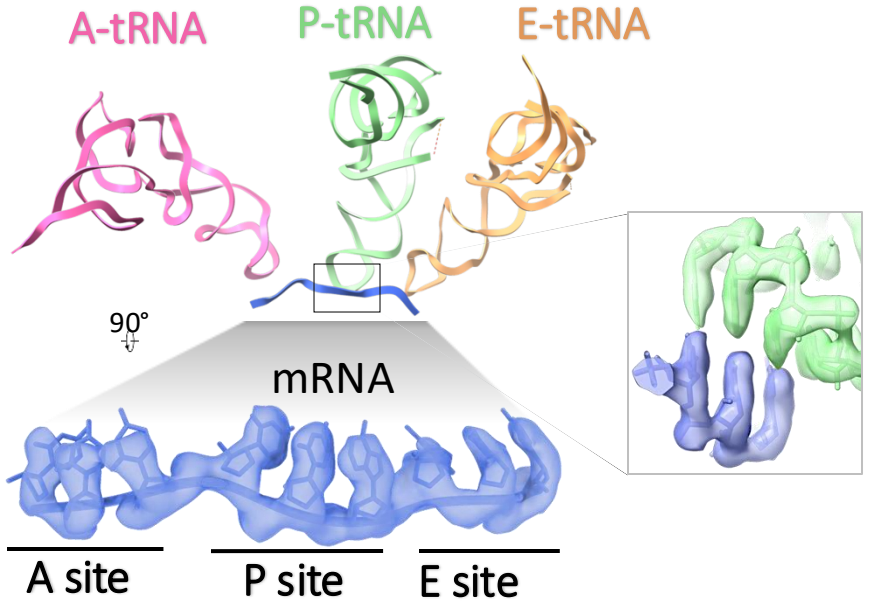
Allowing for atomic model building
in the native cell environment

High-Resolution *in situ* cryo-EM Maps

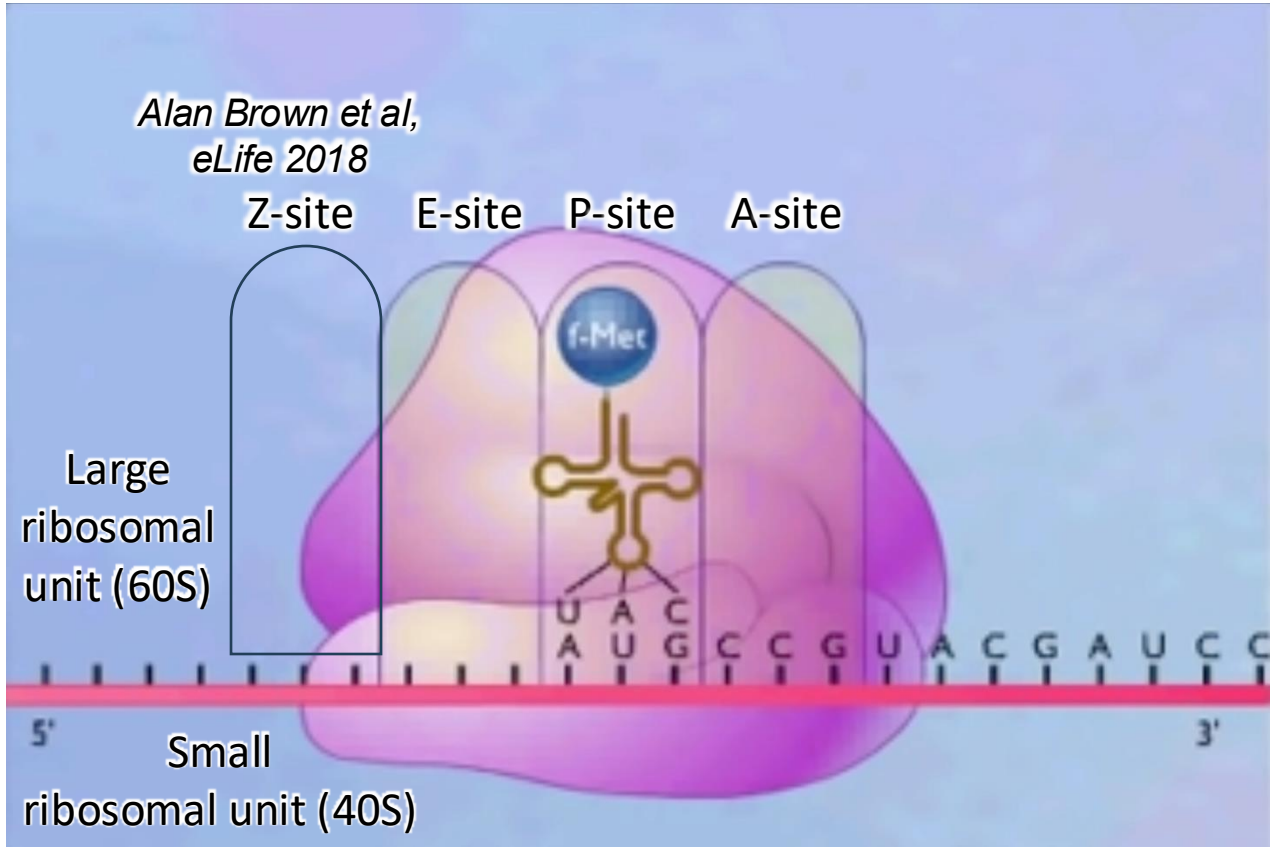


tRNA and translating mRNA





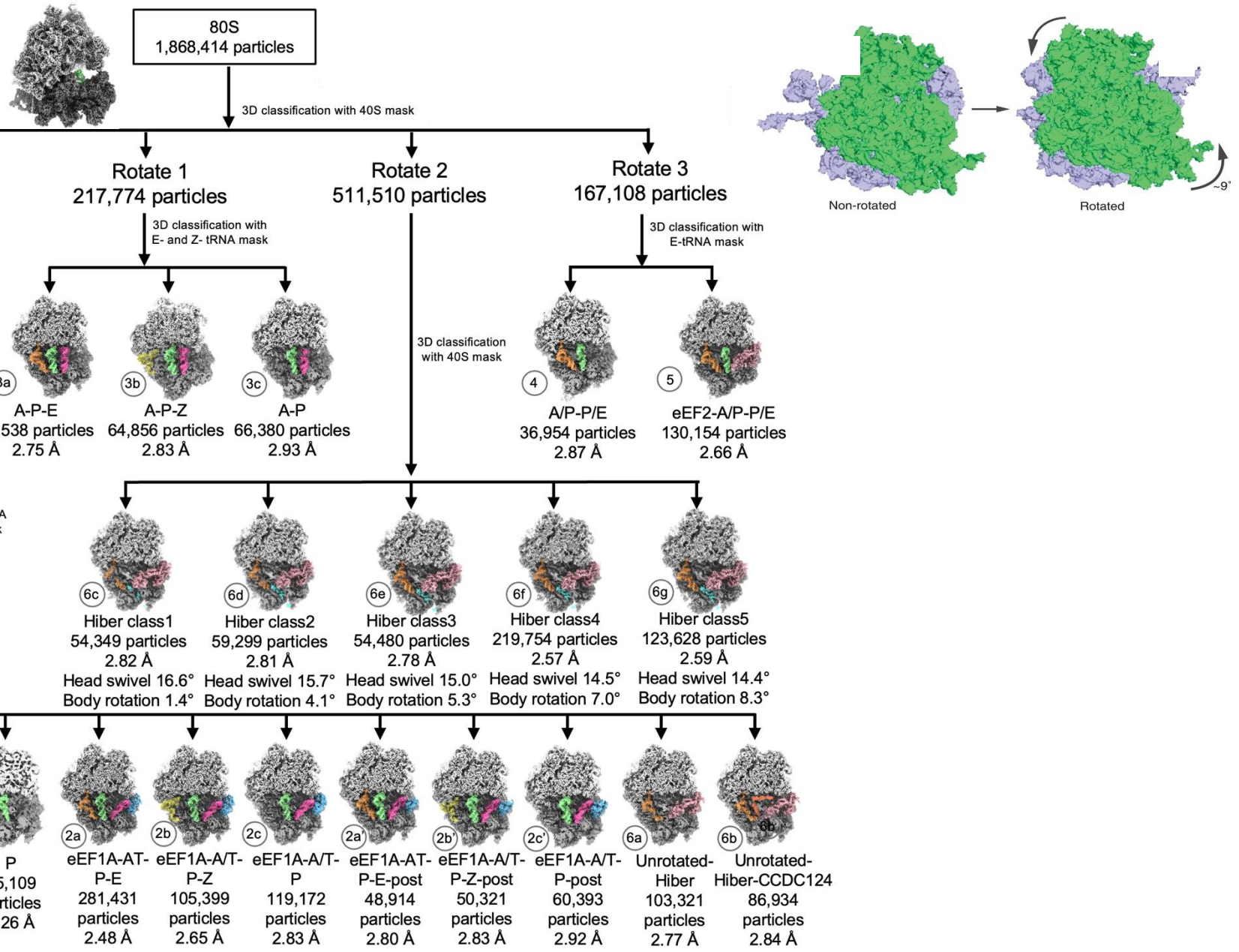
Protein Translation Cycle is Highly Dynamic and Complex



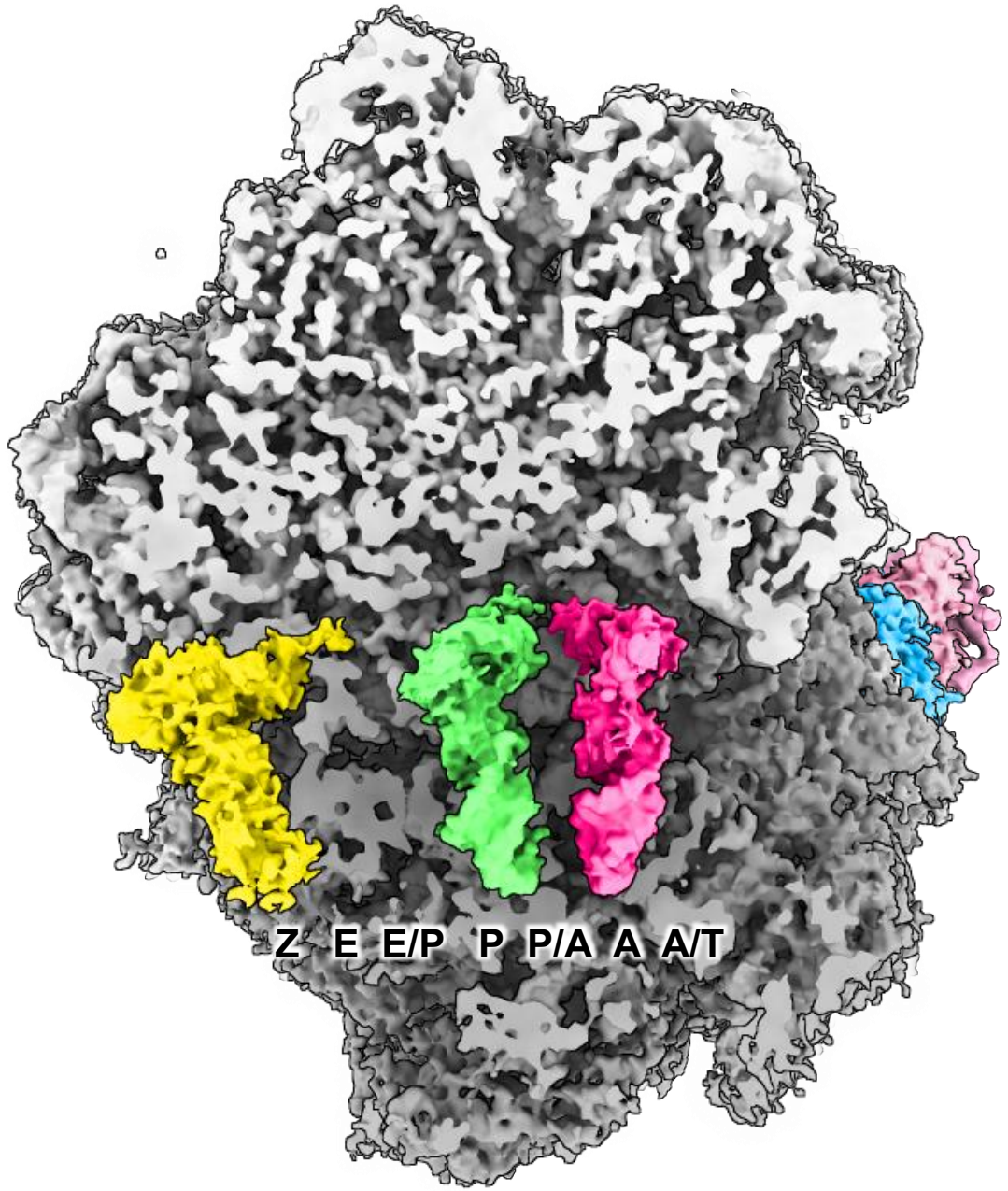
https://www.youtube.com/watch?v=Ikq9AcBcohA

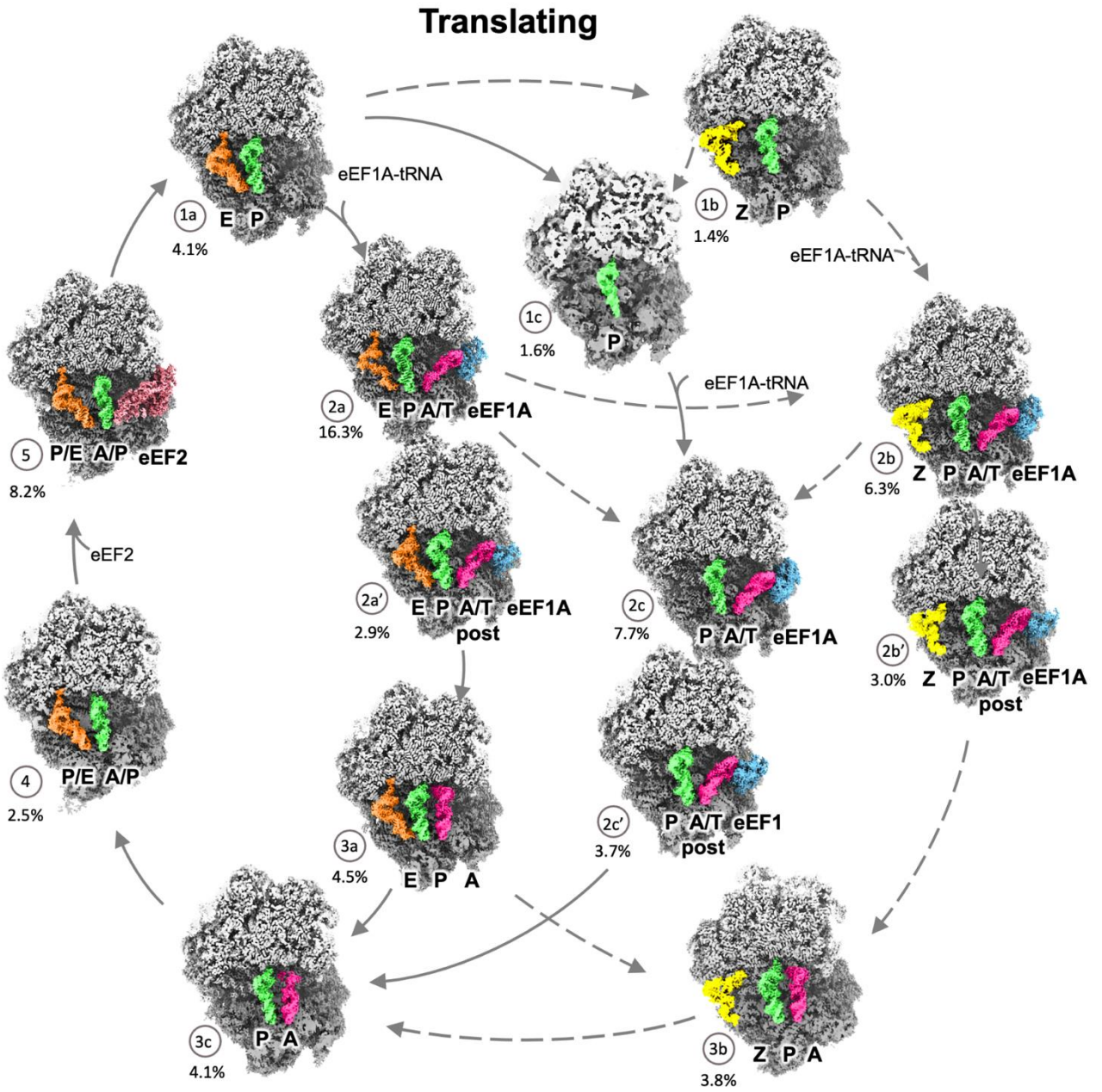
Everything, everywhere, all at once….

Purification *in silico*—3D Classification of Different Ribosome States

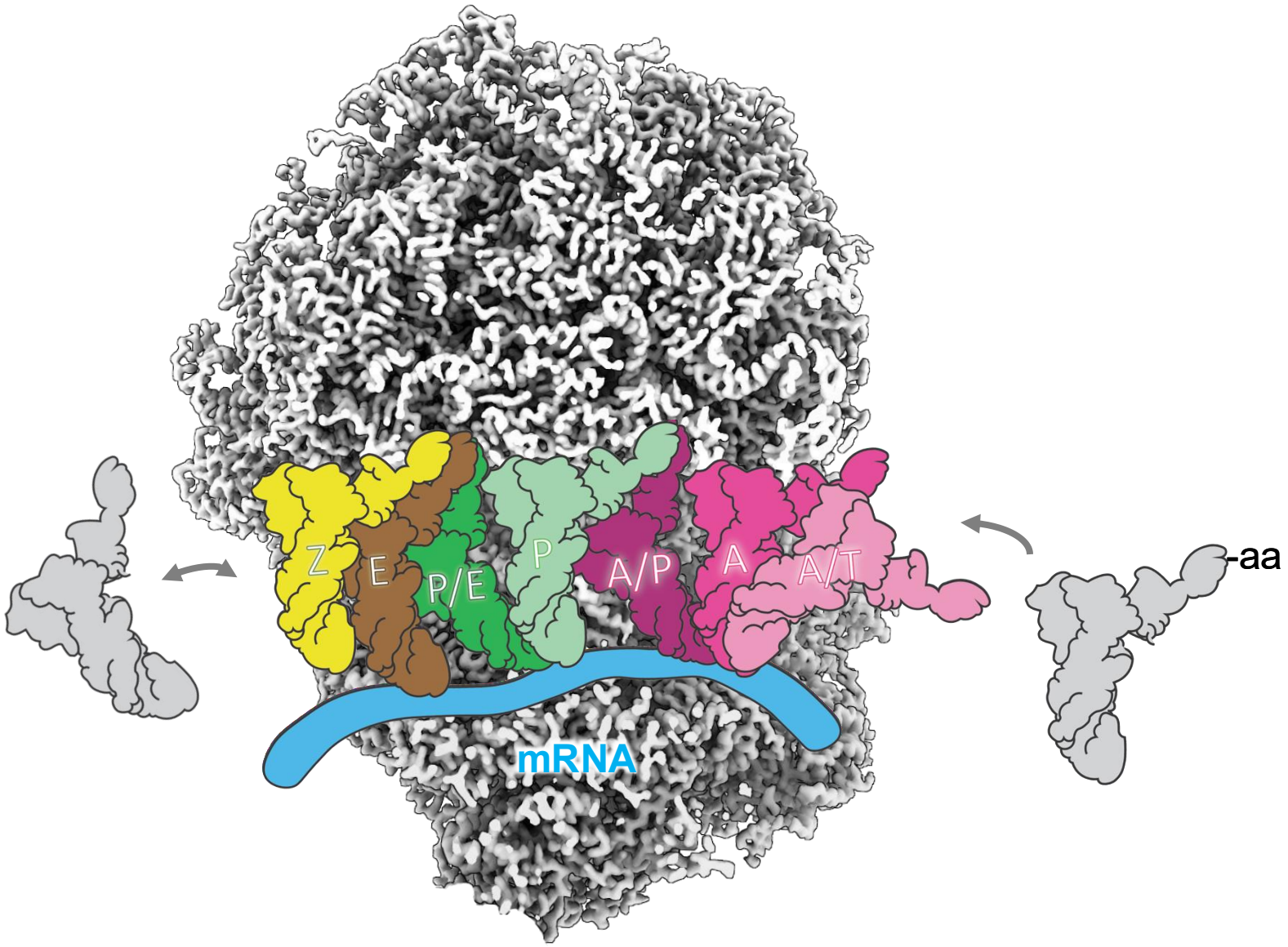


The Most Extensive Translational Cycle States Observed in Cells

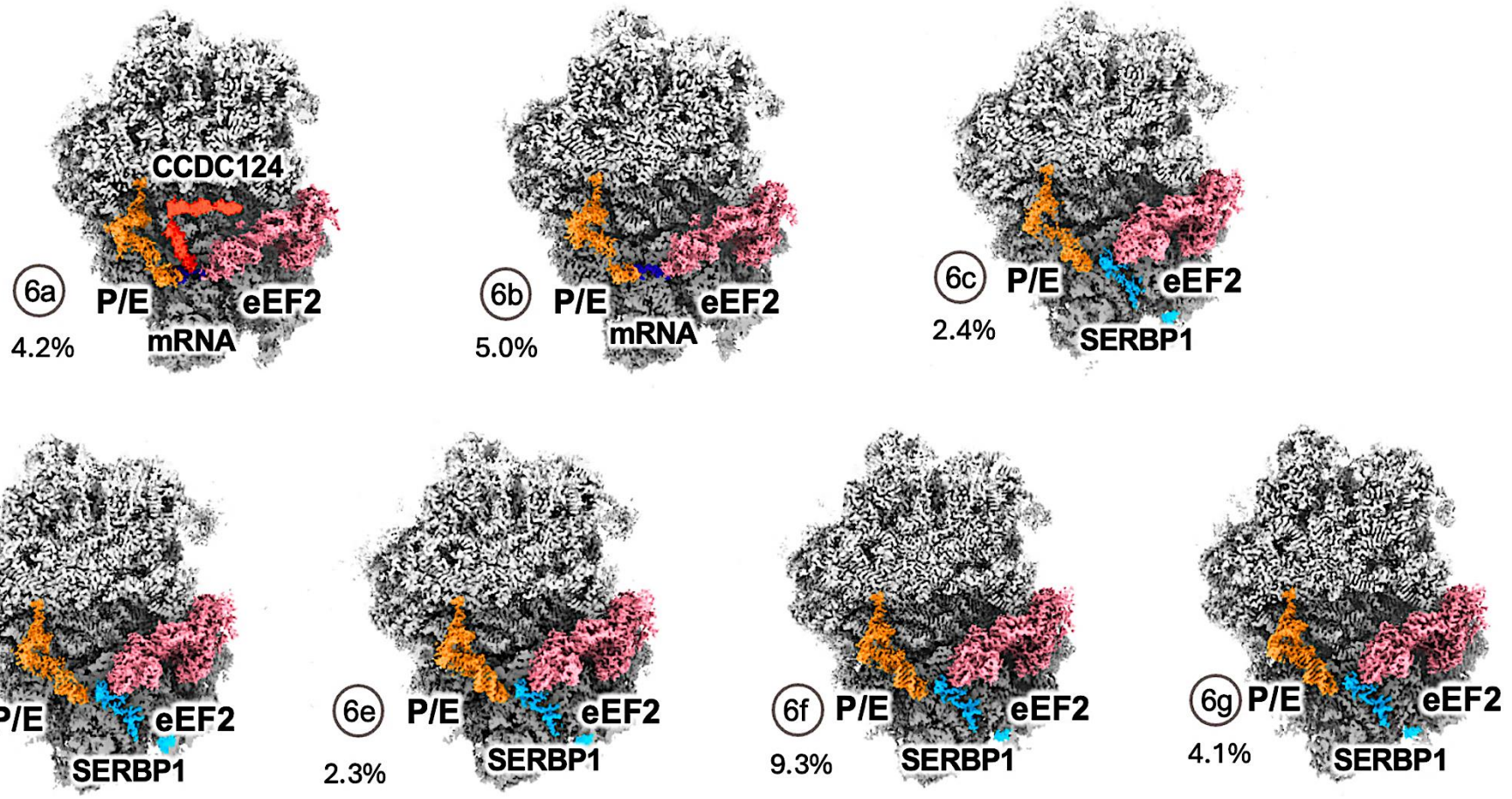




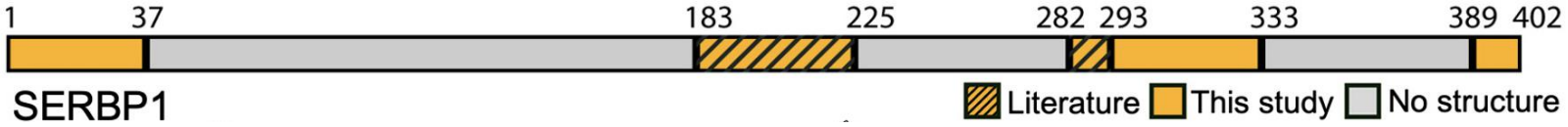
tRNA Trajectory during Translation

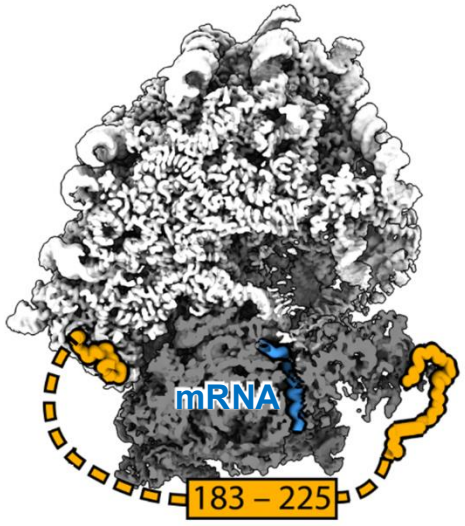


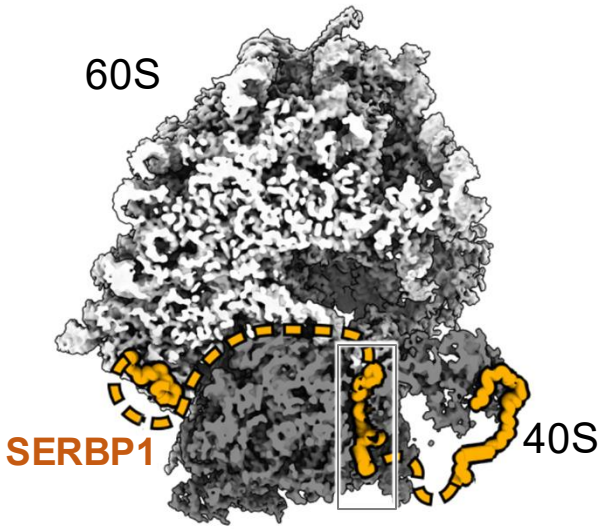
Extensive Non-Translating States

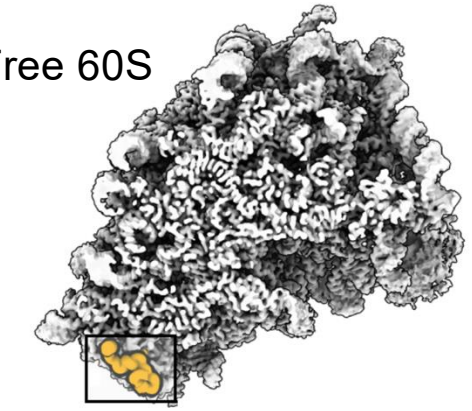


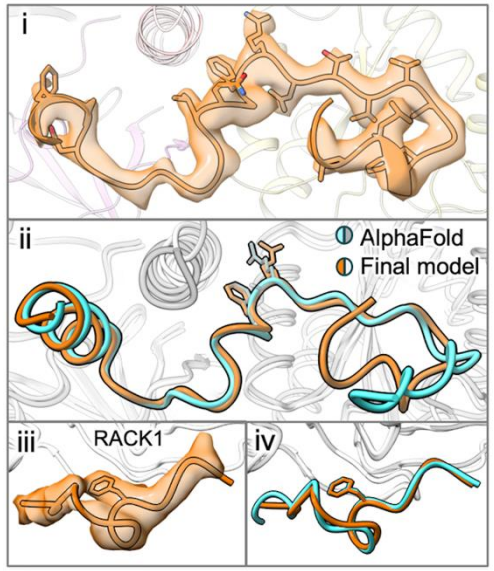
New Biological Observations on SERBP1

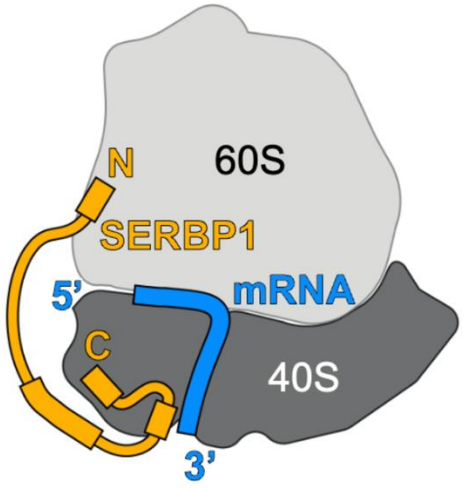




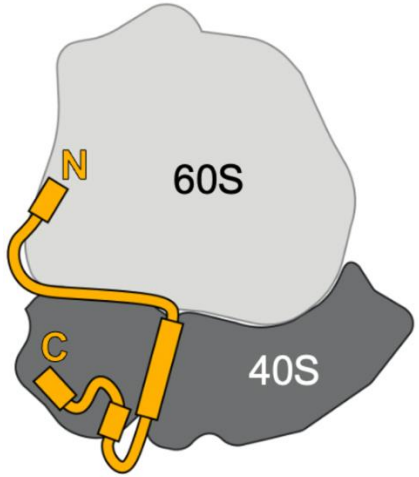




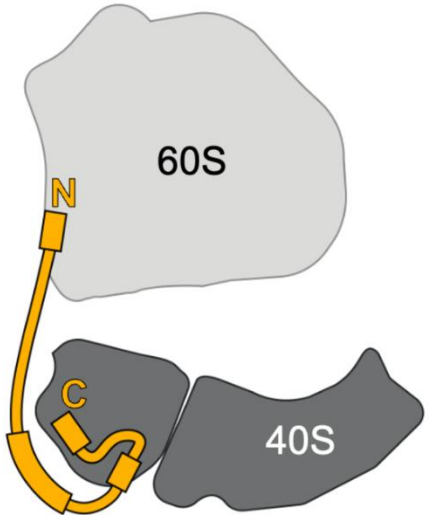




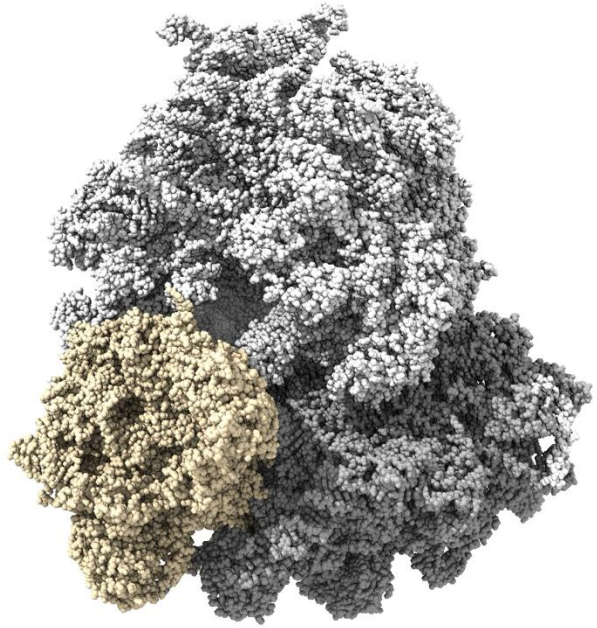
Translating State



Non-translating State



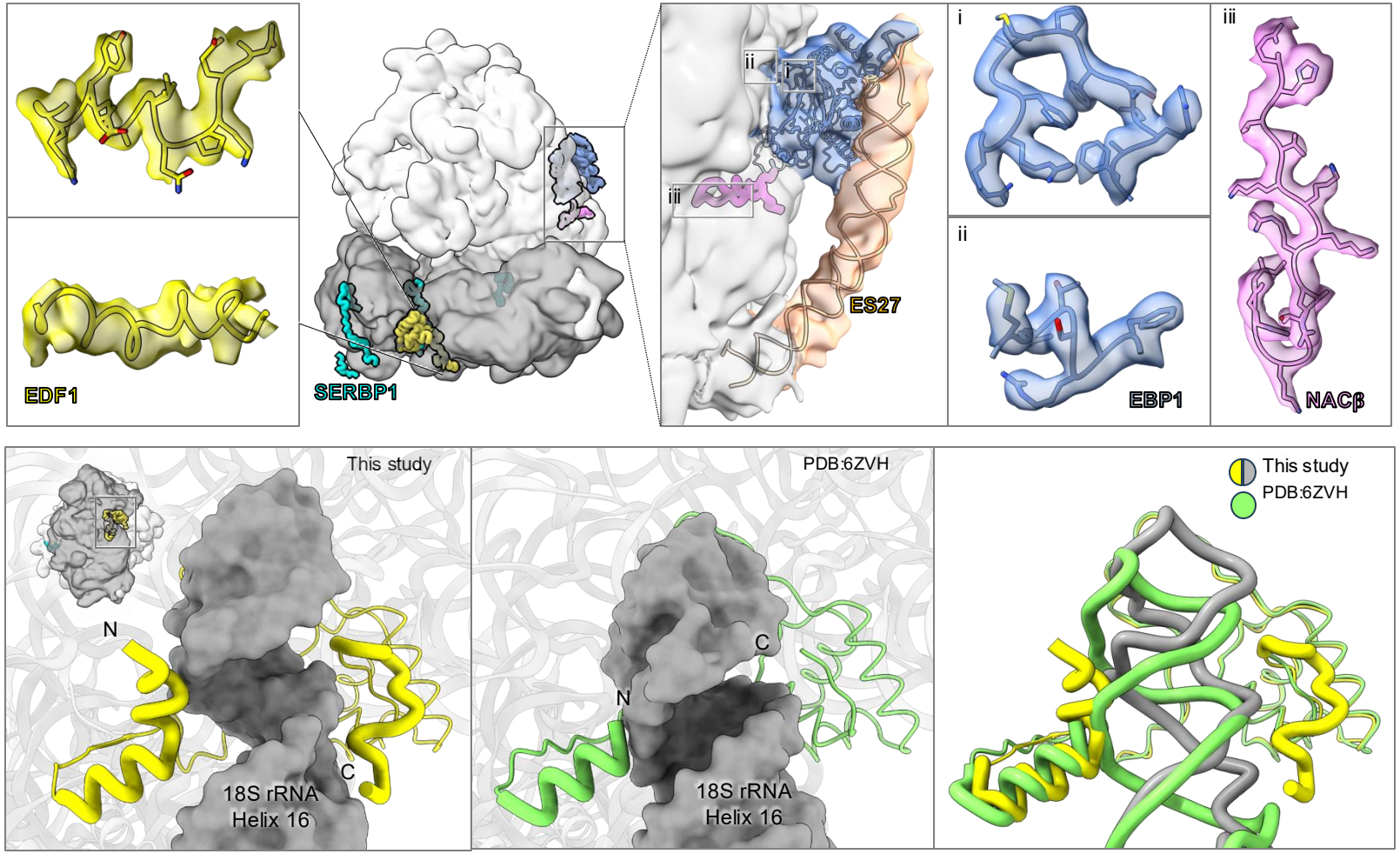
“Free” 60S and 40S



New Biological Insights

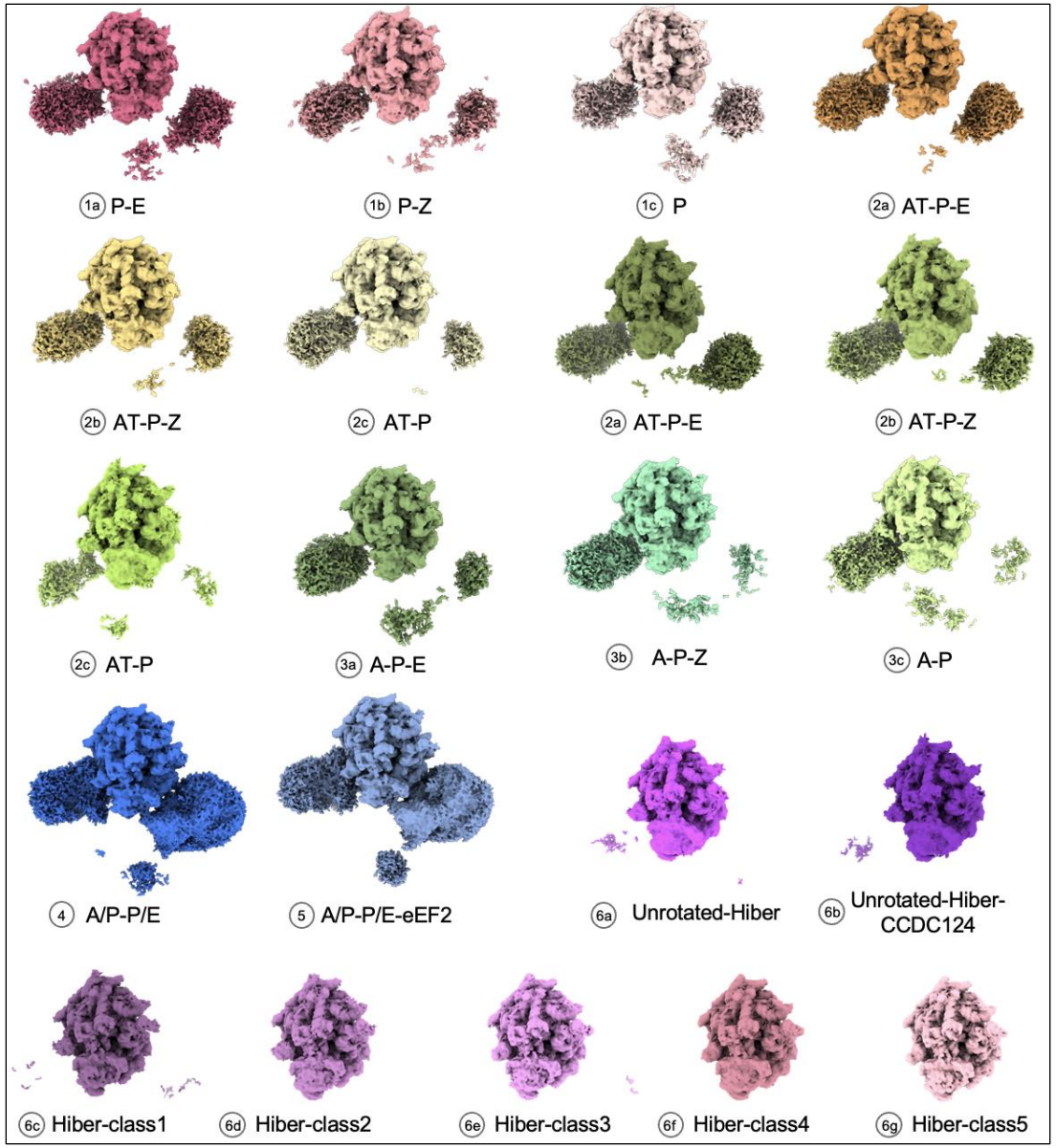
New interaction mode of the regulatory factor EDF1

NACβ is involved in nascent peptide processing/trafficking
EBP1 caps the nascent peptide exit

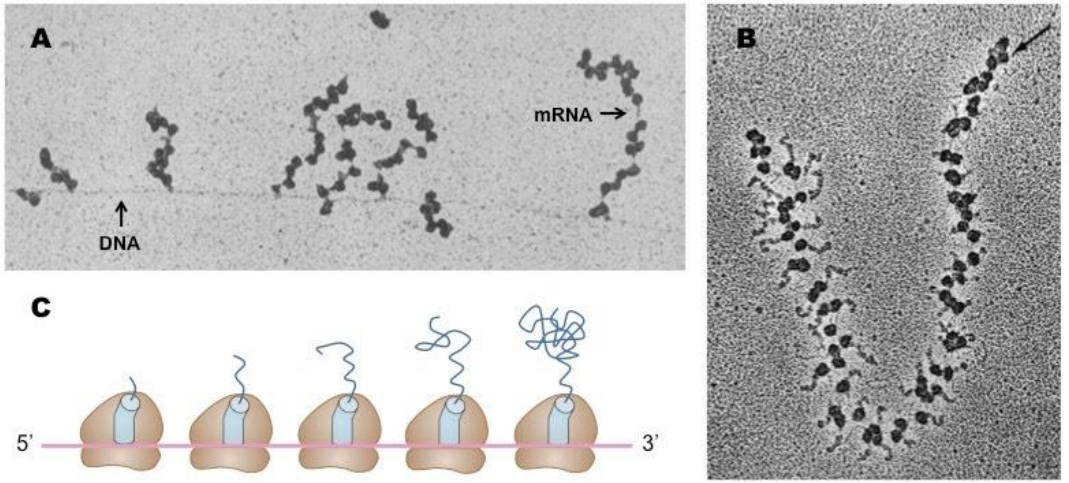


Communications between Neighboring 80Ss in the Polysome

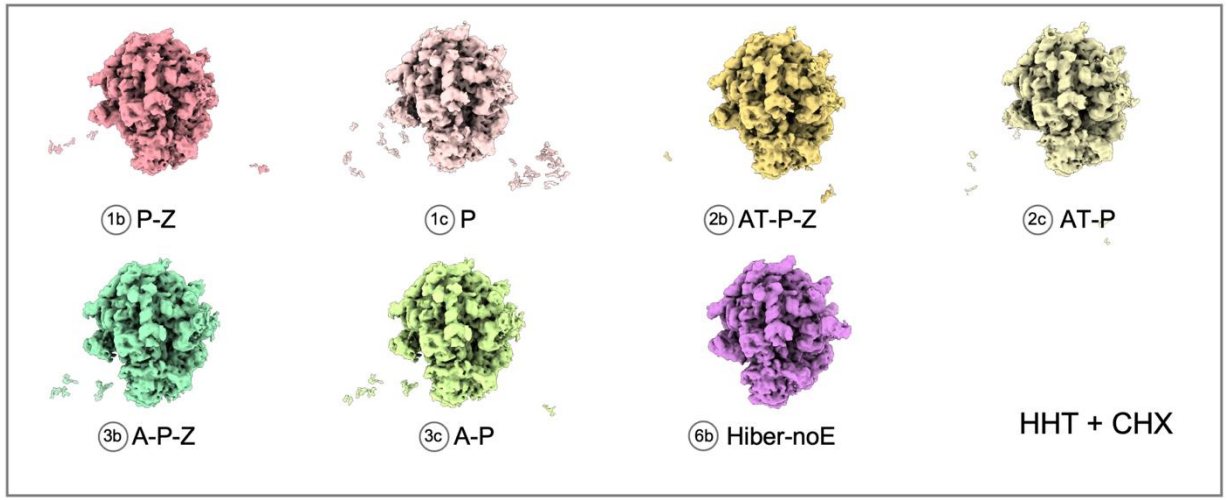
Native Cells



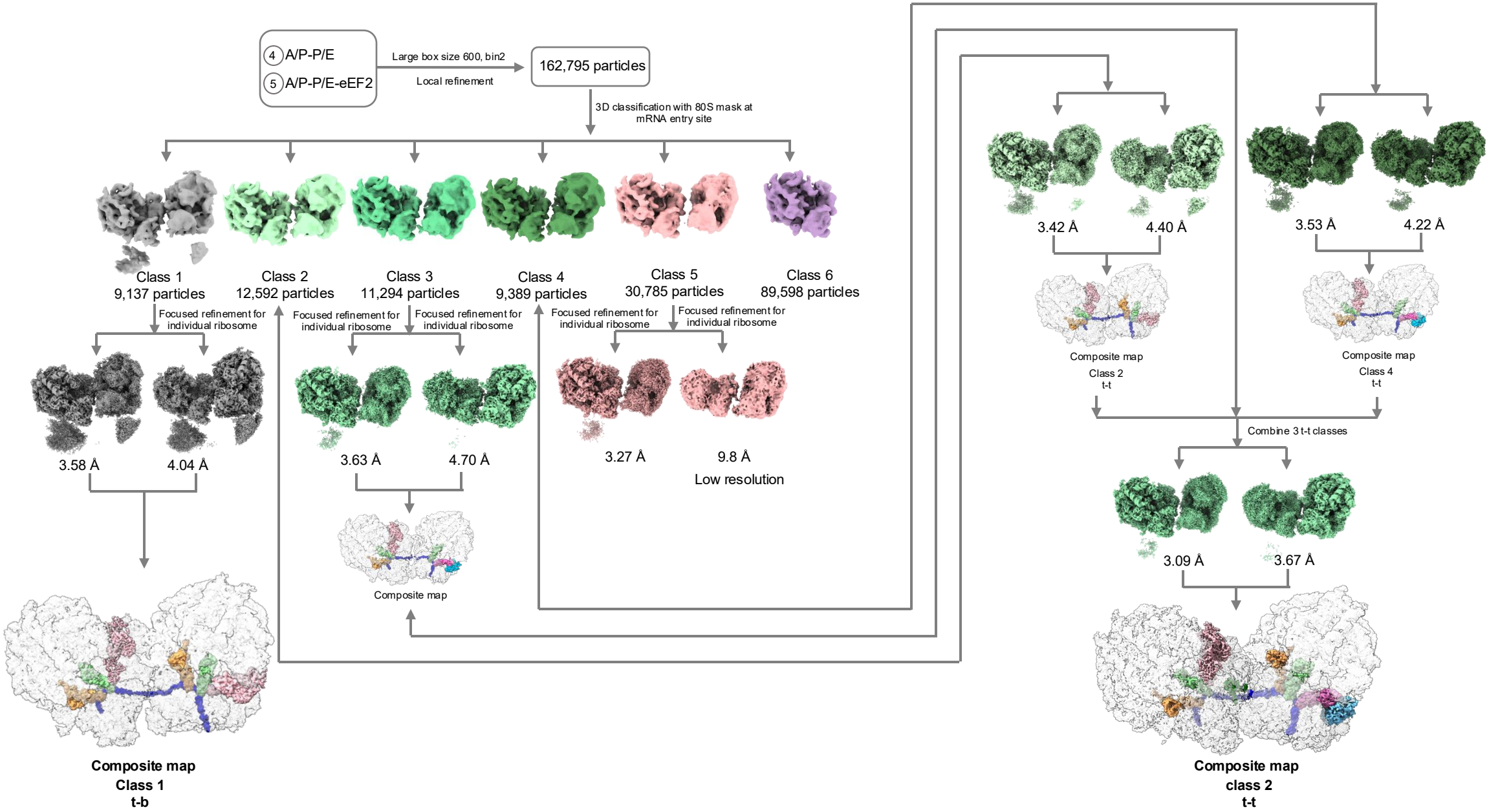
Polysome—Many copies of ribosomes translate the same mRNA simultaneously



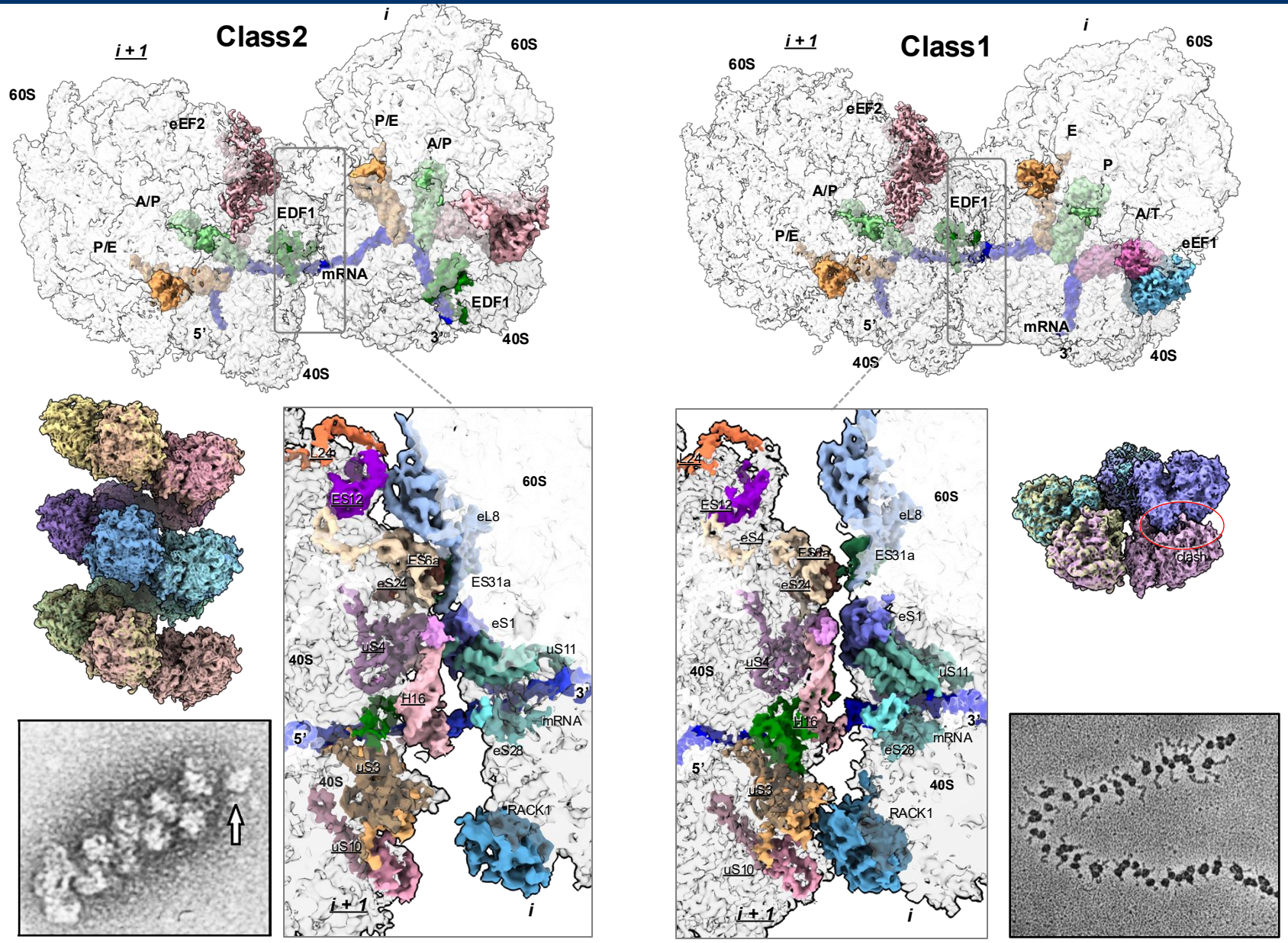
Treated with HHT + CHX



Communications between Neighboring 80Ss in the Polysome

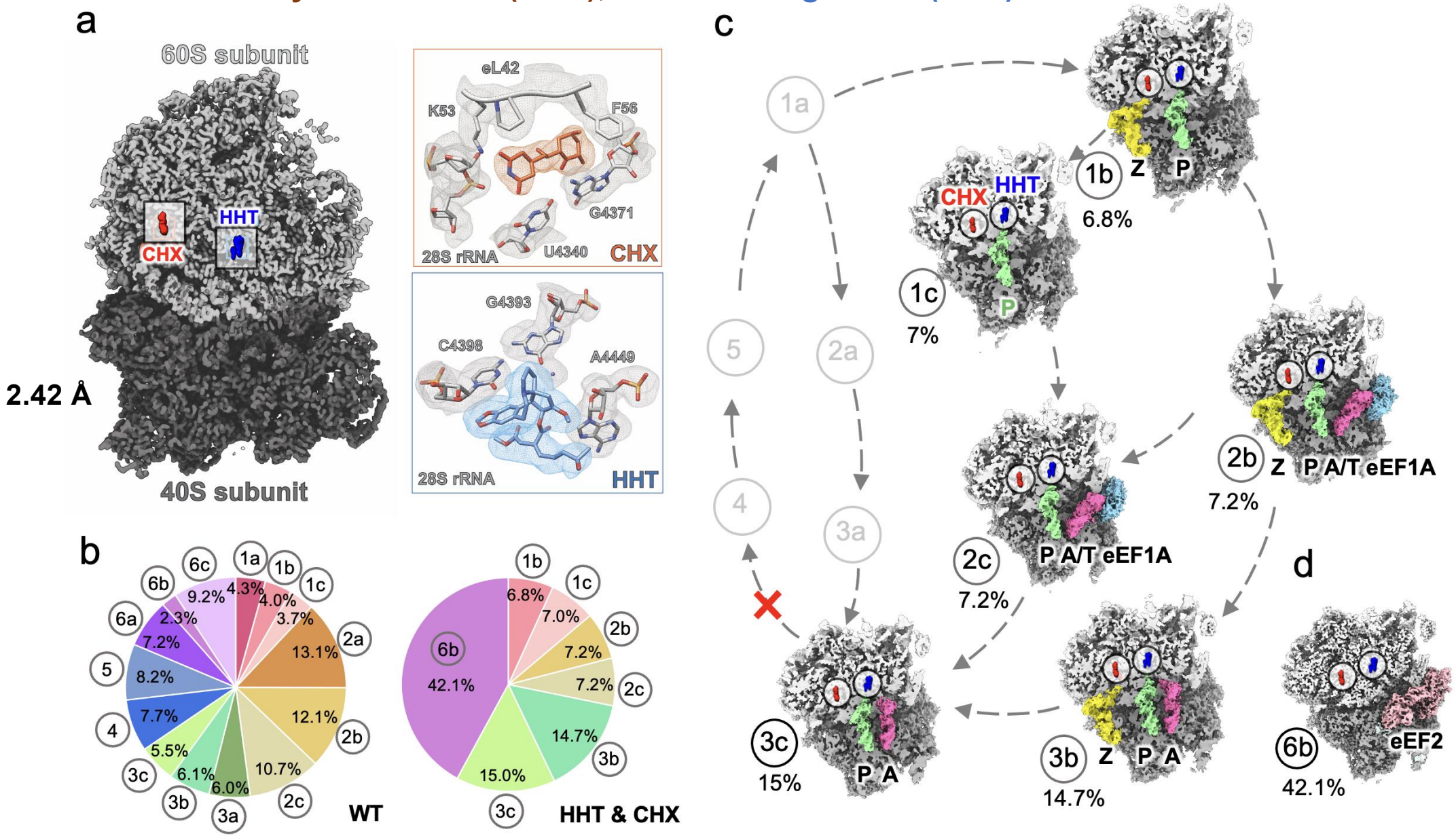


Coordination between Neighboring 80Ss in the Polysome



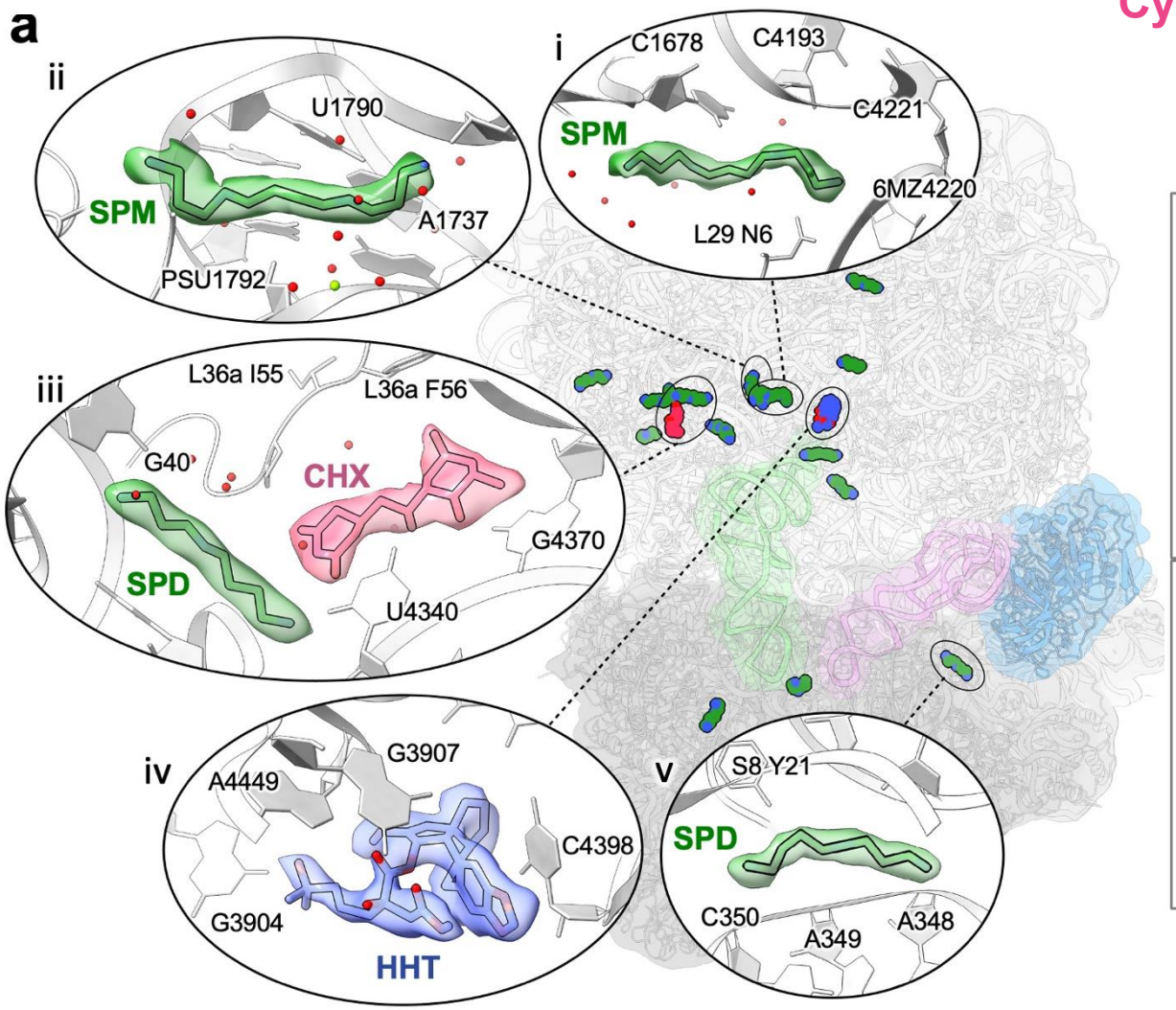
Inhibitors and Anti-Cancer Drugs Alter Translation Landscape

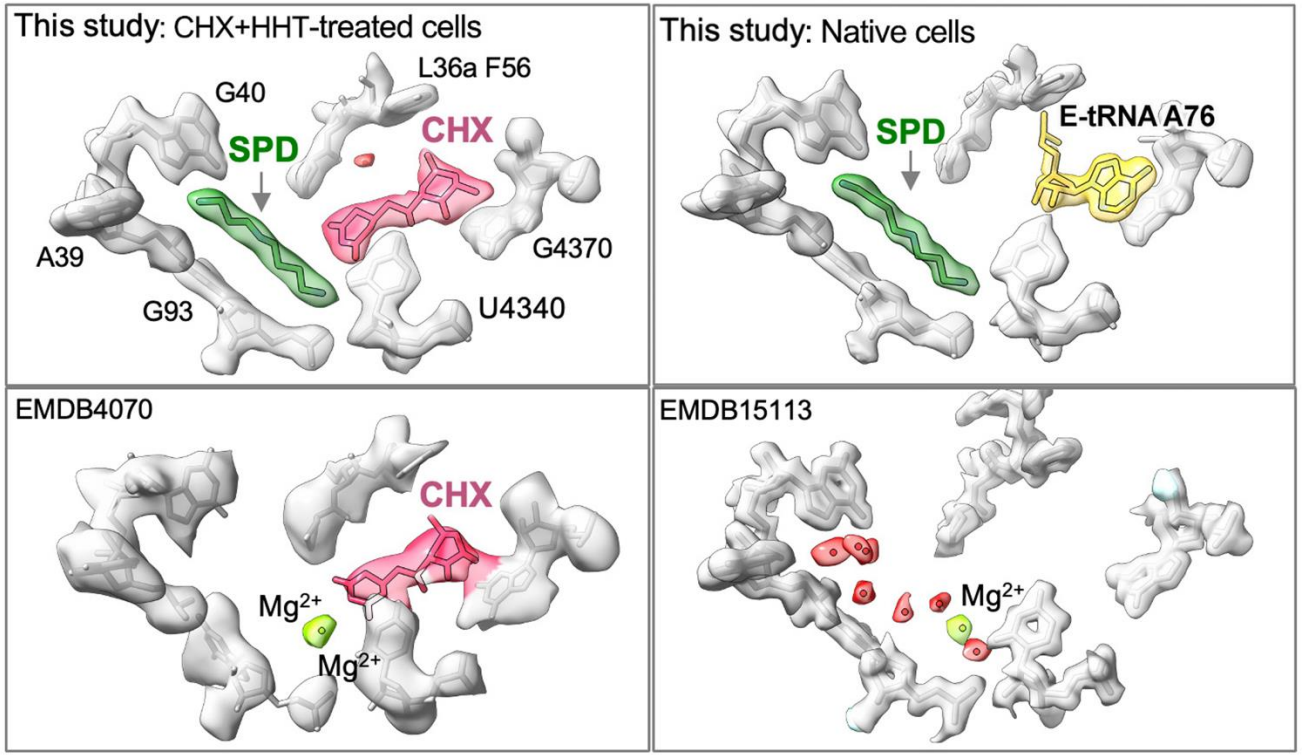
Cycloheximide (CHX), Homoharringtonine (HHT)



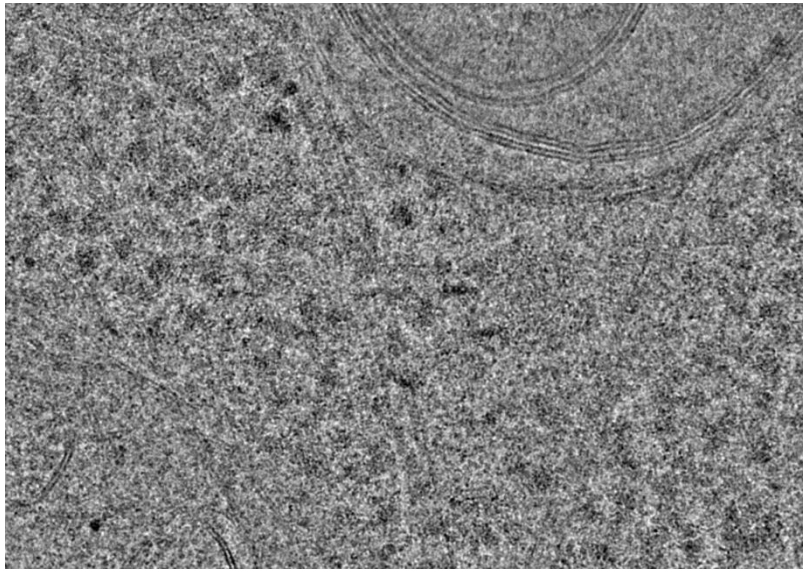
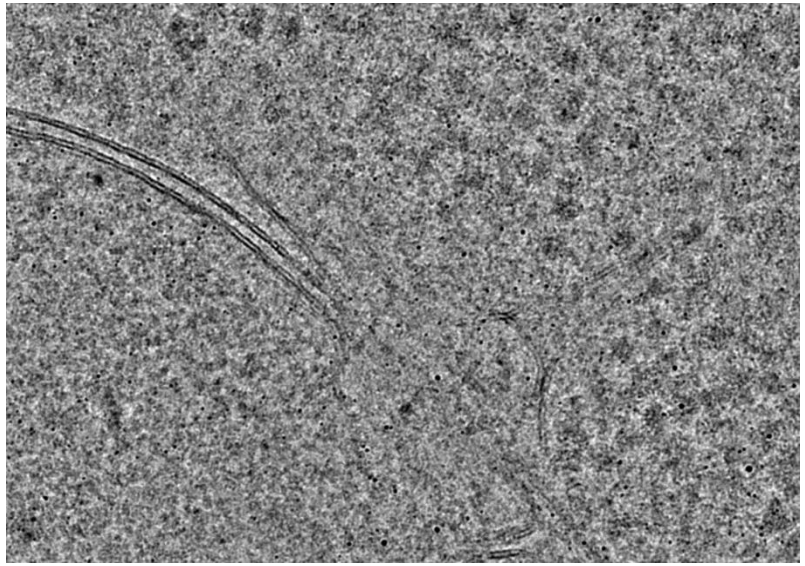
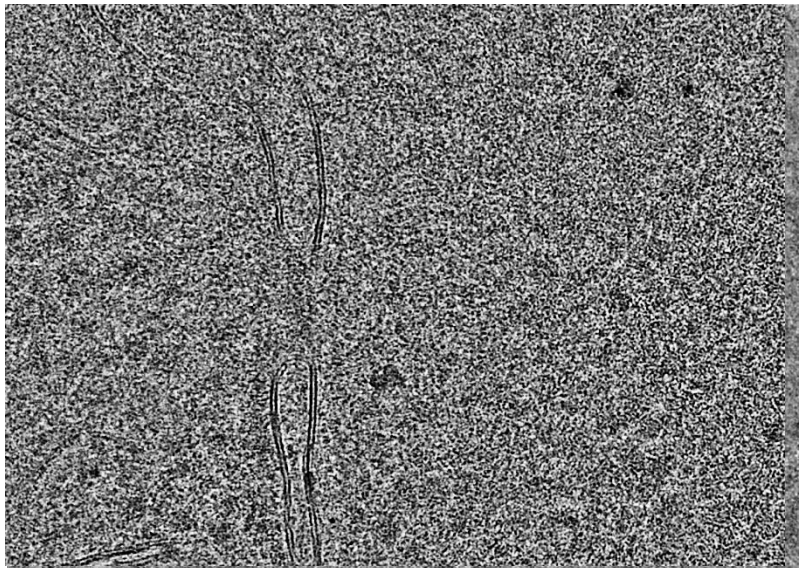
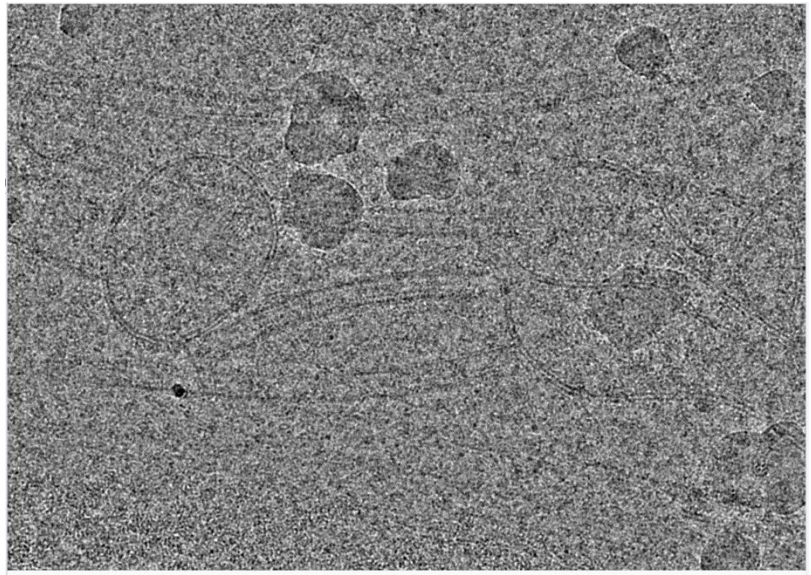
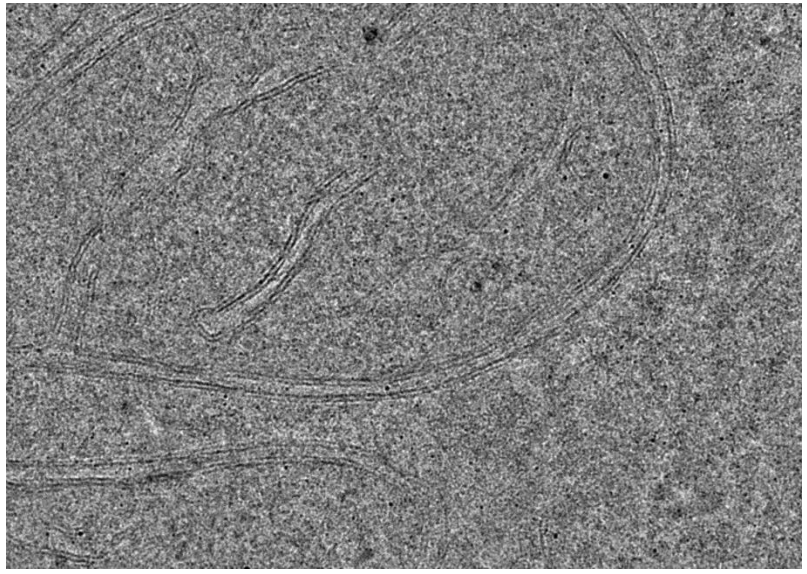
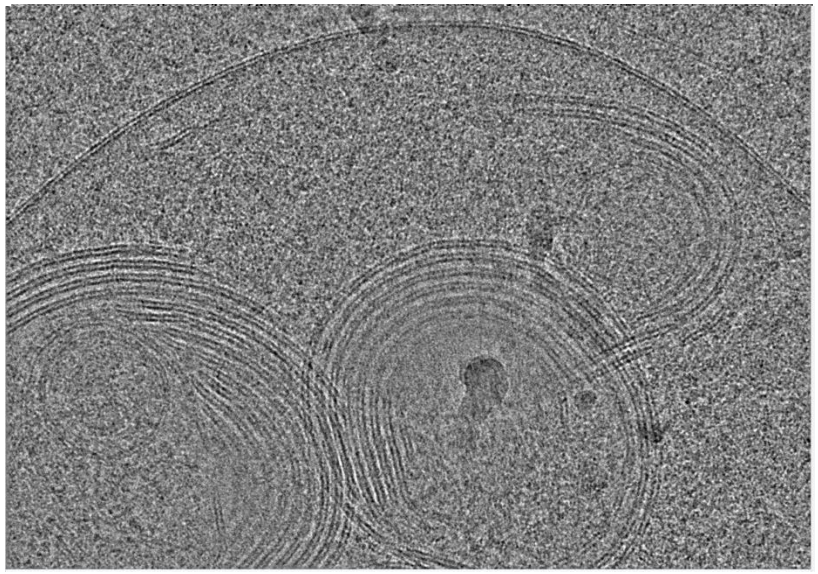
Drugs and Important Small Molecule Cofactors *in situ*

Spermine (SPM), Spermidine (SPD)
Cycloheximide (CHX), Homoharringtonine (HHT)





The Future in Cells

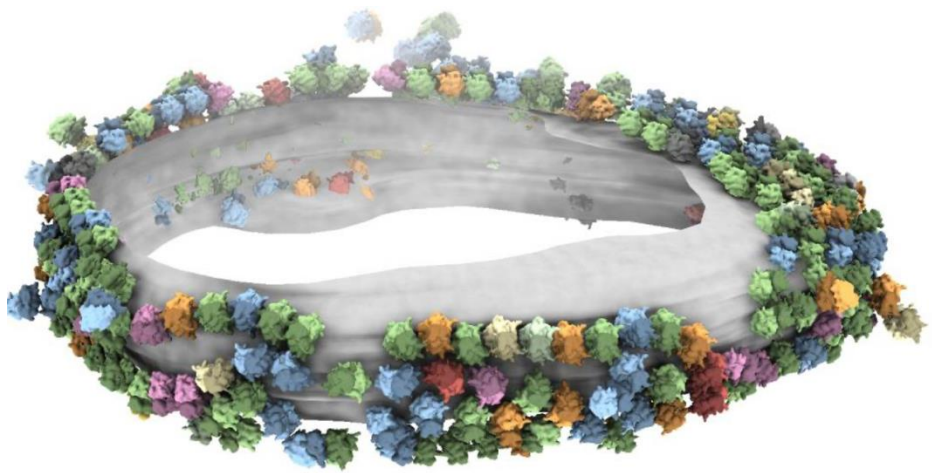







Summary

Single particle cryo-EM holds great potential
for high-resolution *in situ* structural biology

Much more to be optimized and much more can be done….

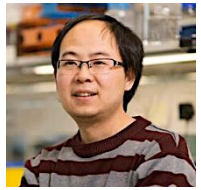
A good complement to cryo-ET

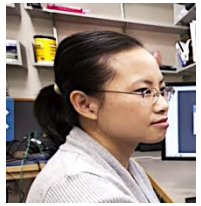


Gemmer et al, Nature 2023

- Better milling with higher throughput and less damage
- Can we identify smaller molecules?
  thinner samples, better algorithms, machine learning…
- Better classification and data science tools—network in cells
- Low abundance?
  molecular biology, CLEM, large (and forever) datasets
- Combination with cryo-ET for low abundance cases and for cellular mapping (Z-height)

Acknowledgment





Funding:
HIV : NIH grants
In situ translation work: Yale startup