RUTGERS

NCCAT Tomography Short Course 2021

Introduction to Cryo-electron Tomography

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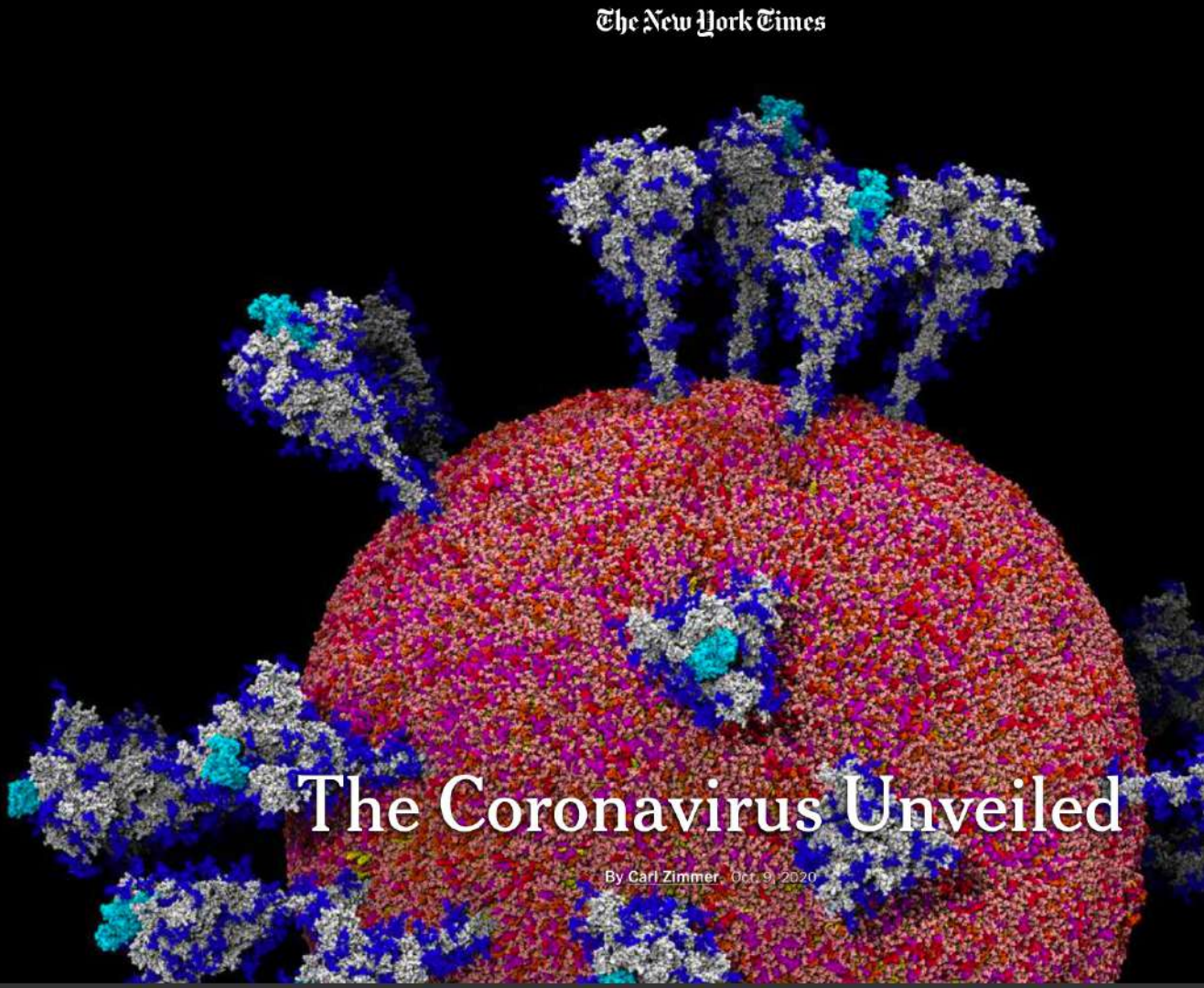
Department of Cell Biology and Neuroscience

Institute for Quantitative Biomedicine

Rutgers University

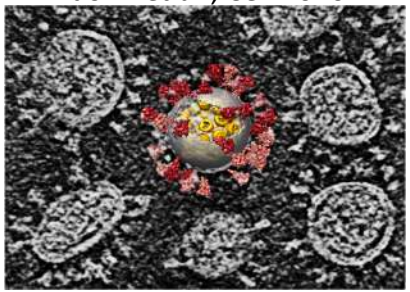
April 12, 2021

Resolving Structures to Drive Scientific Discoveries during the Pandemic



How does the virus look like?

Yao H. *et al.*, Cell 2020



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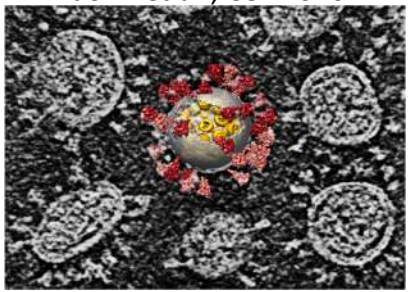
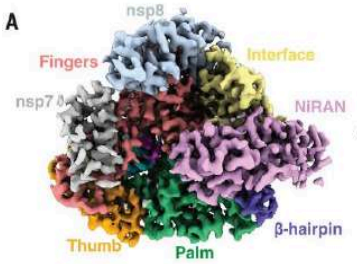
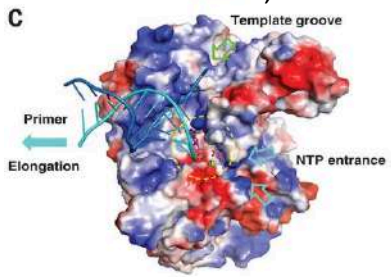
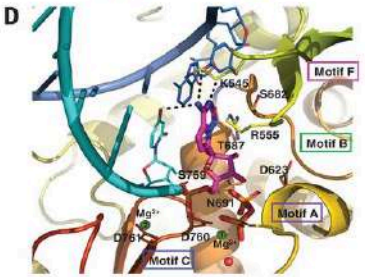


How does Remdesivir interact with SARS-CoV-2?

Yin W. *et al.*, Science 2020







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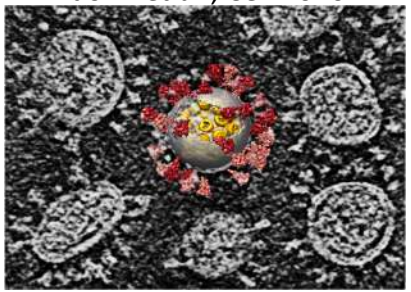

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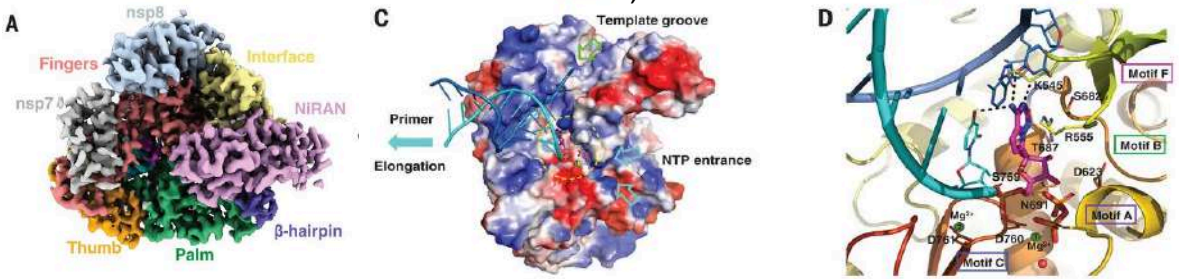
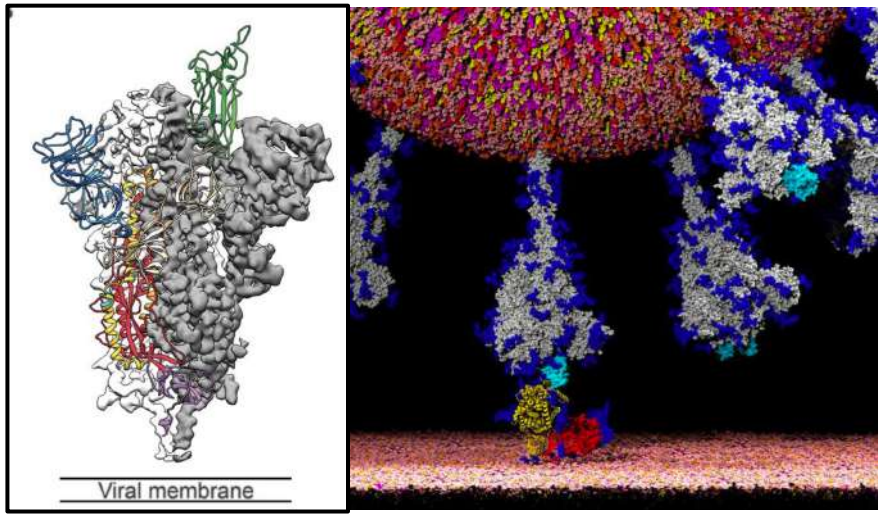


How does SARS-CoV-2 enter human cells?

Wrapp D. *et al*., Science 2020; Simulation by Amaro lab, UCSD



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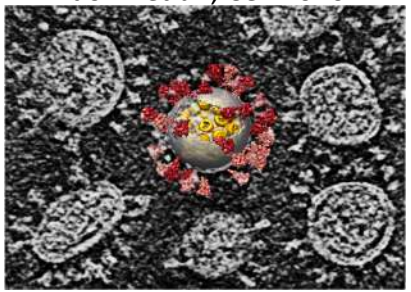

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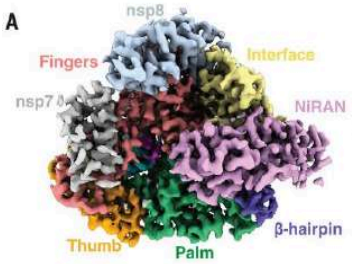

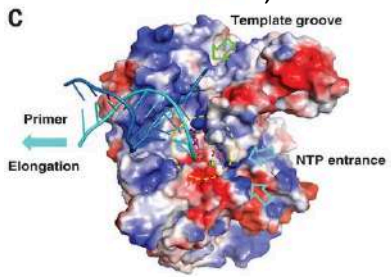

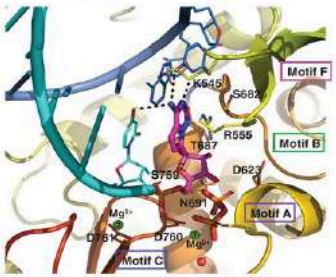

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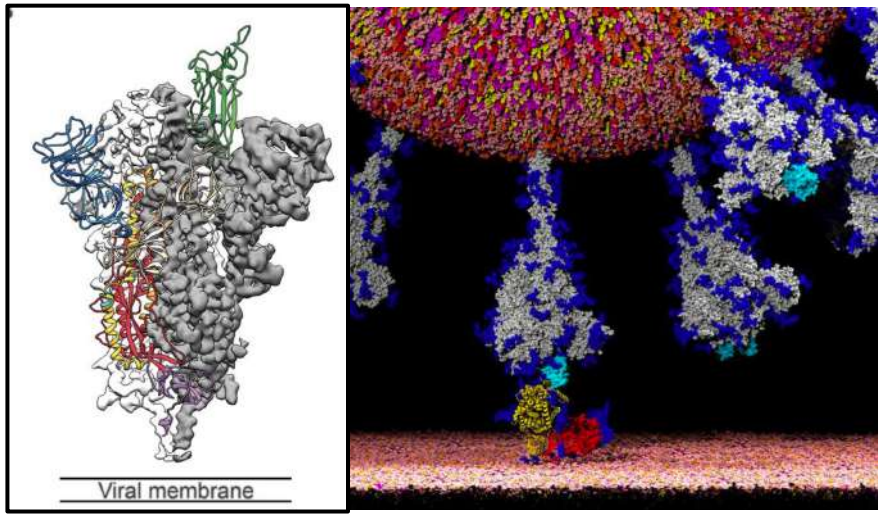
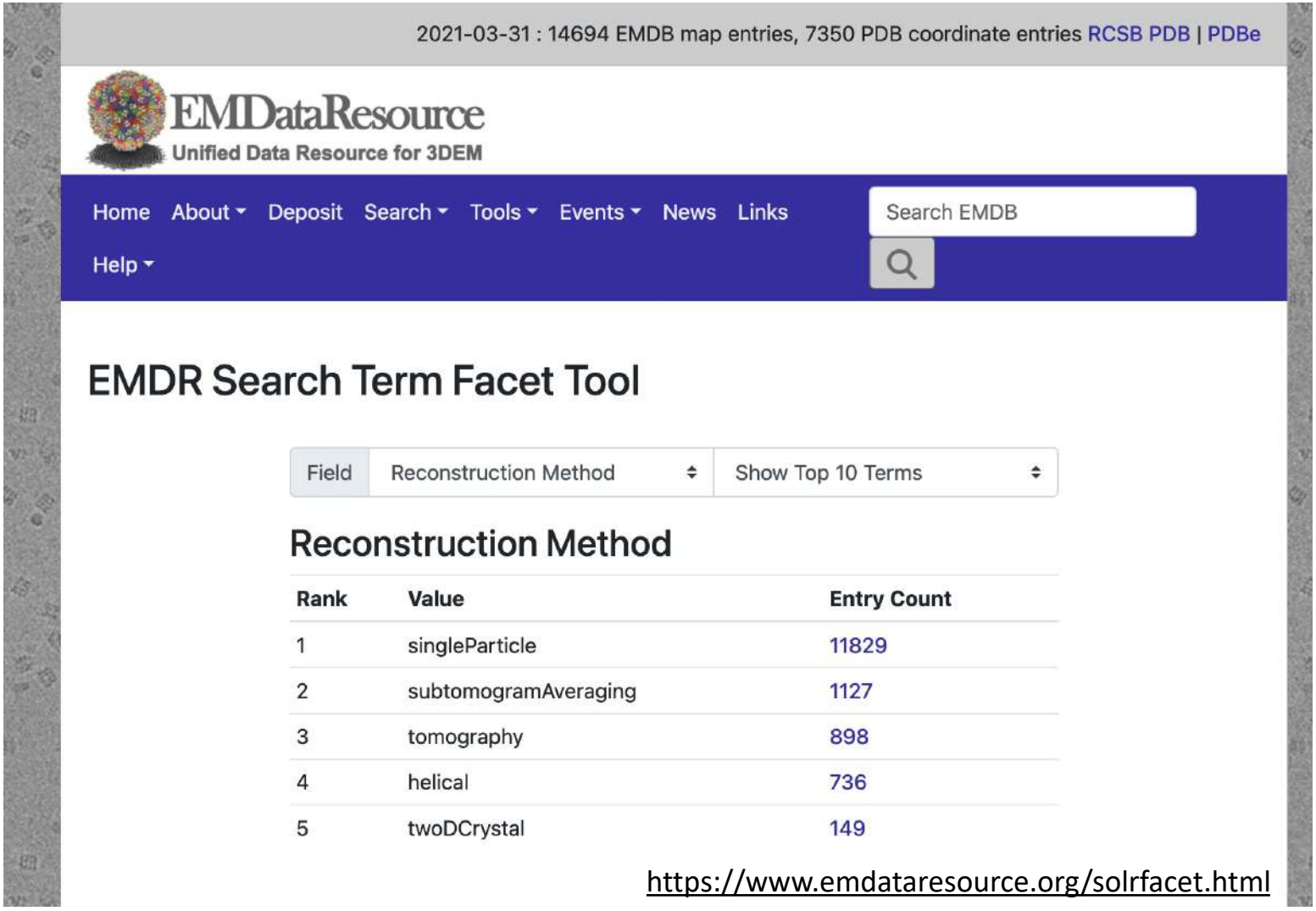


How does SARS-CoV-2 assemble inside cells?

Klein S. *et al*., Nature Communications 2020


2021-03-31 : 14694 EMDB map entries, 7350 PDB coordinate entries RCSB PDB | PDBe
EMDataResource
Unified Data Resource for 3DEM
Home
About
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Search
Tools
Events
News
Links
Help
Search EMDB
EMDR Search Term Facet Tool
Field
Reconstruction Method
Show Top 10 Terms
Reconstruction Method
Rank
Value
Entry Count
1
singleParticle
11829
2
subtomogramAveraging
1127
3
tomography
898
4
helical
736
5
twoDCrystal
149
https://www.emdataresource.org/solrfacet.html

Cryo-EM Single Particle Analysis

The Nobel Prize in Chemistry 2017

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Prize share: 1/3

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Richard Henderson
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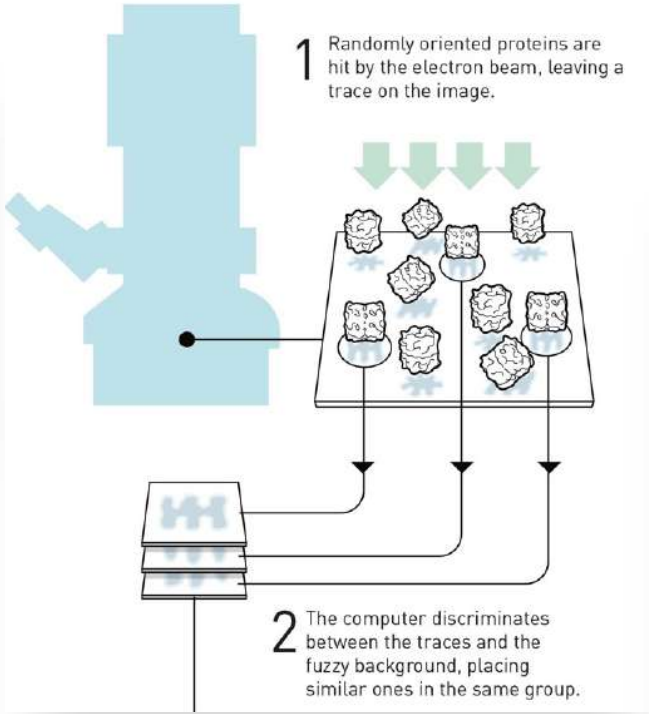
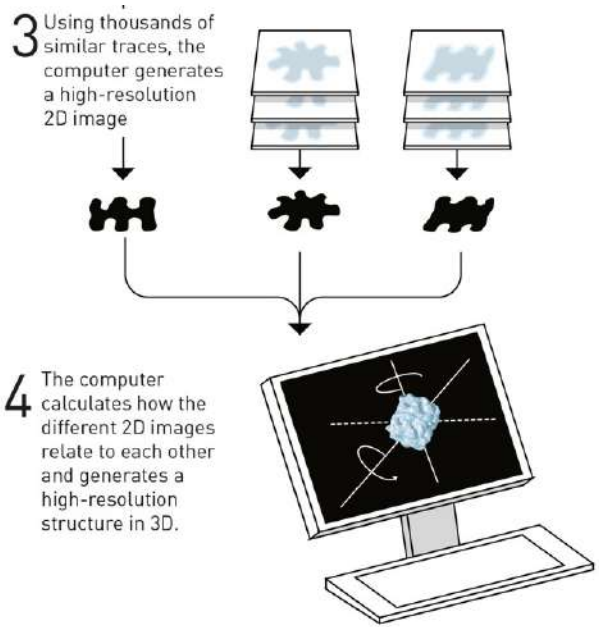
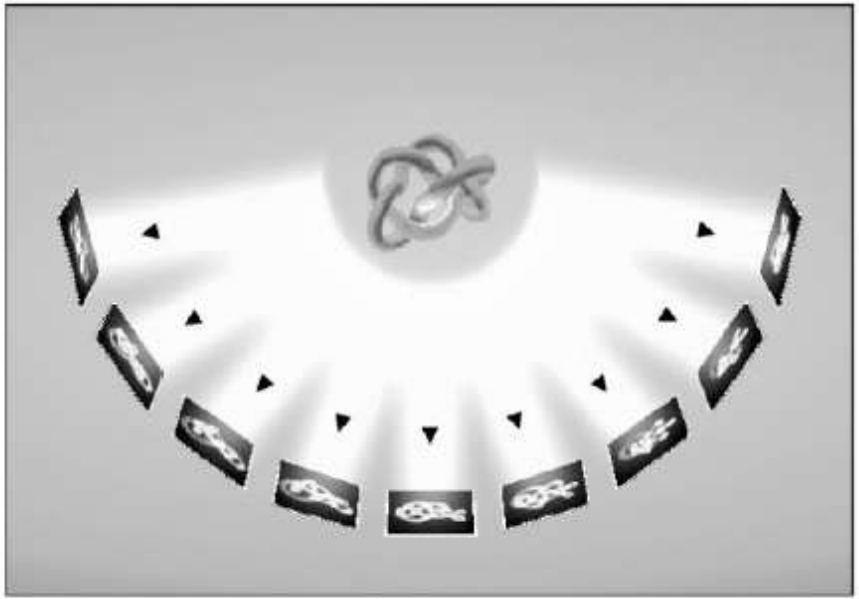




Cryo-electron Tomography

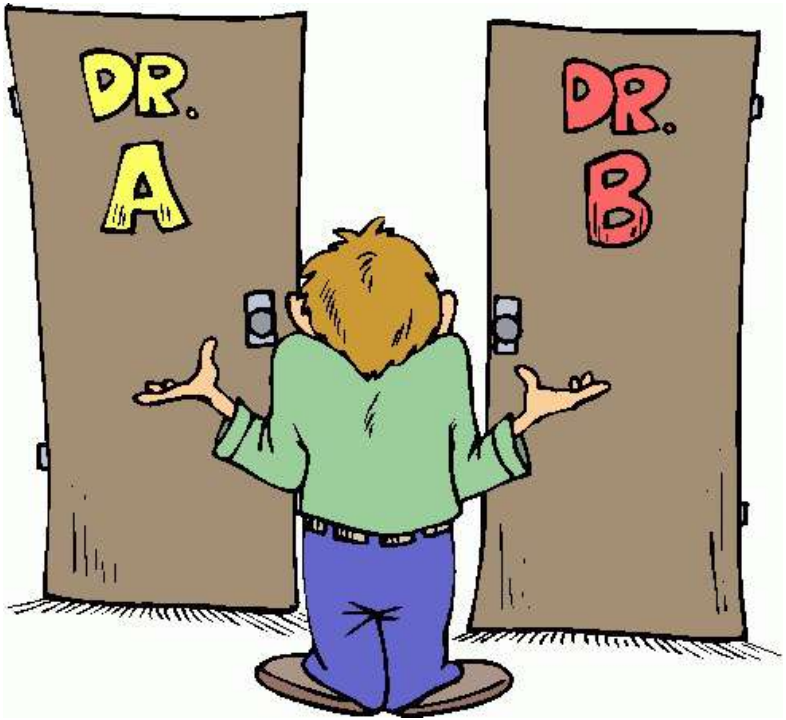
On a TEM: 3D structures → 2D images



On a computer: 2D images → 3D structures

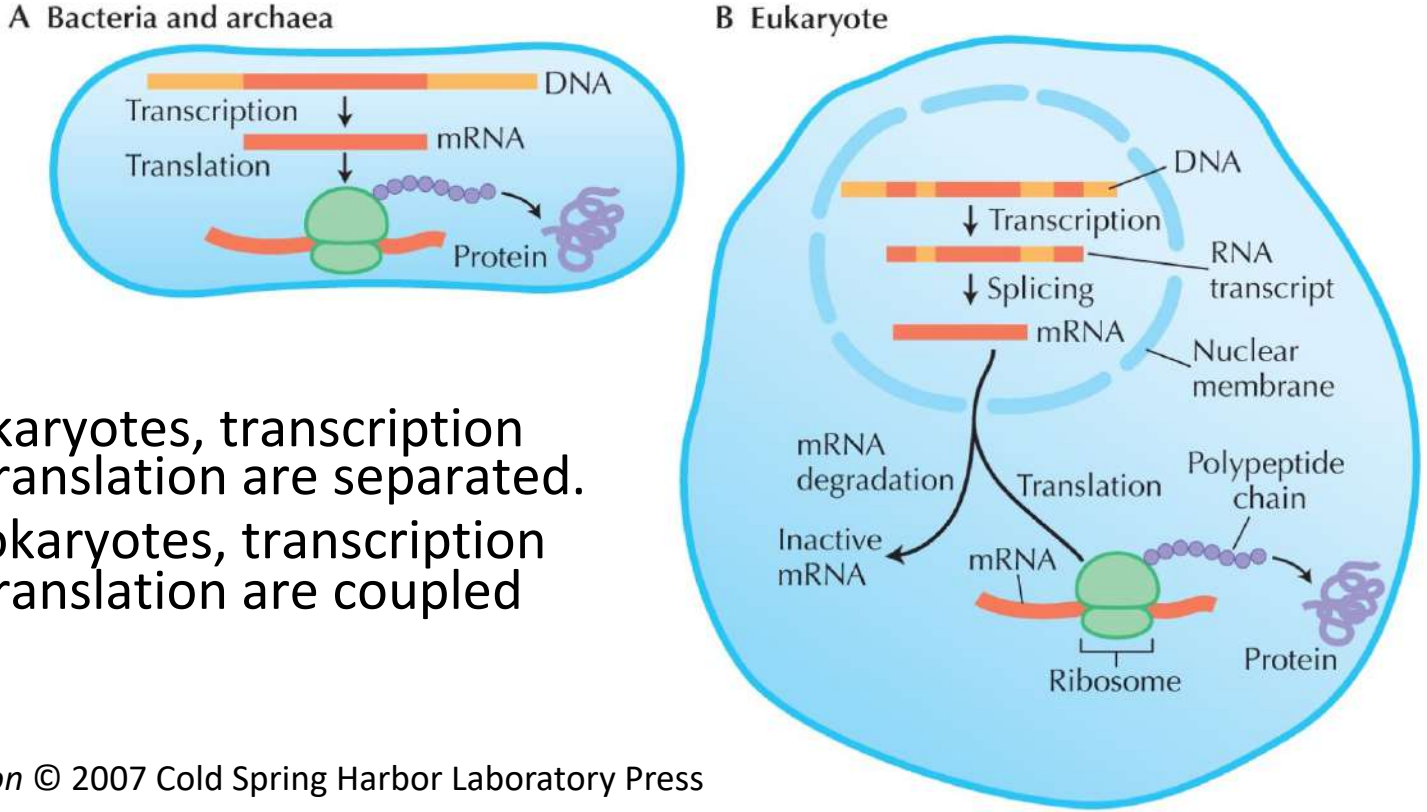
Baumeister W. 1999 Trends in Cell Bio.

Single Particle *or* Tomography



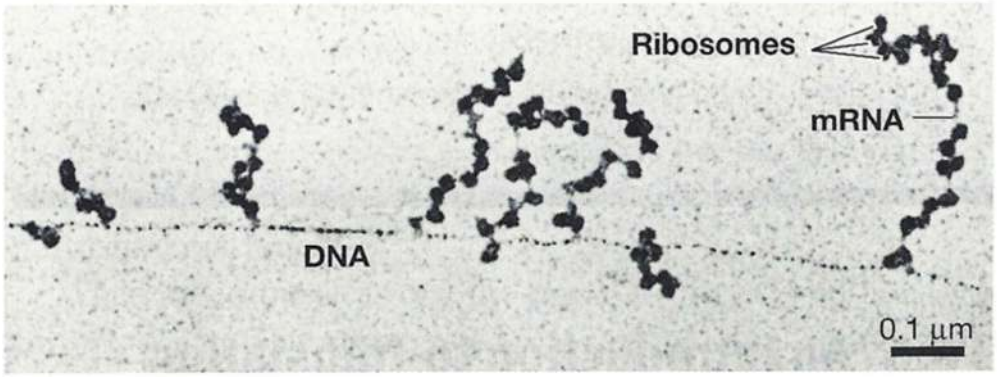
https://clipart-library.com

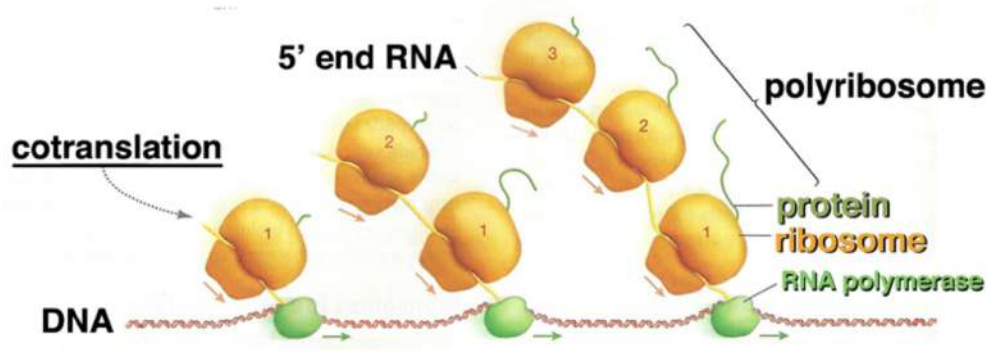
Transcription-translation Coupling



- In eukaryotes, transcription and translation are separated.
- In prokaryotes, transcription and translation are coupled

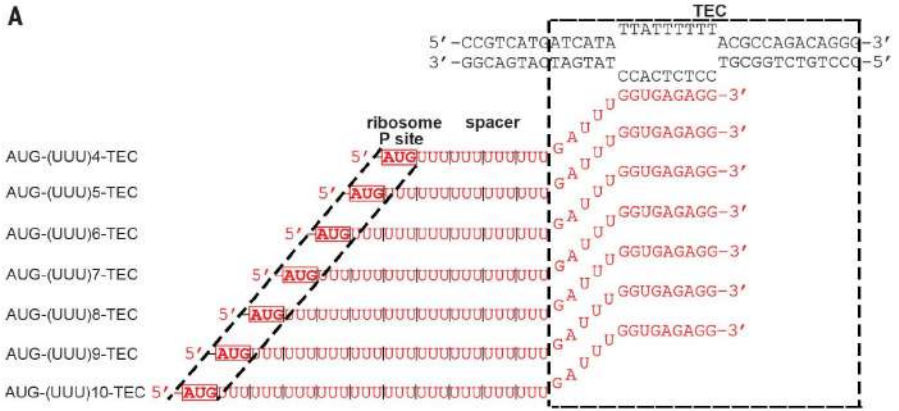
Transcription-translation Coupling





Structures of Transcription-translation Coupling Complexes Resolved by Single Particle Analysis

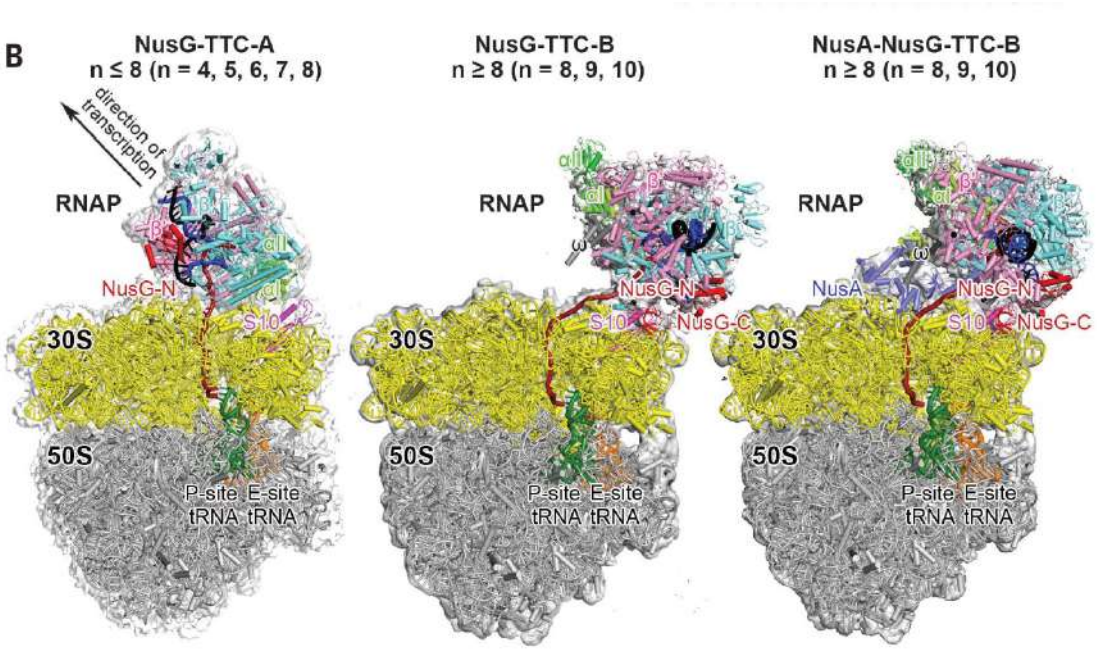
- Sample preparation:
  - Synthetic nucleic acid scaffolds
    - DNA and mRNA determinants for formation of transcription elongation complex (TEC)
    - An mRNA AUG codon: formation of translation complex
    - An mRNA spacer between TEC and AUG
  - RNAP
  - Ribosome and $tRNA^{fMet}$
  - NusG and NusA: coordination of TEC and robosome



Wang C. *et al*., Science 2020

Structures of Transcription-translation Coupling Complexes Resolved by Single Particle Analysis

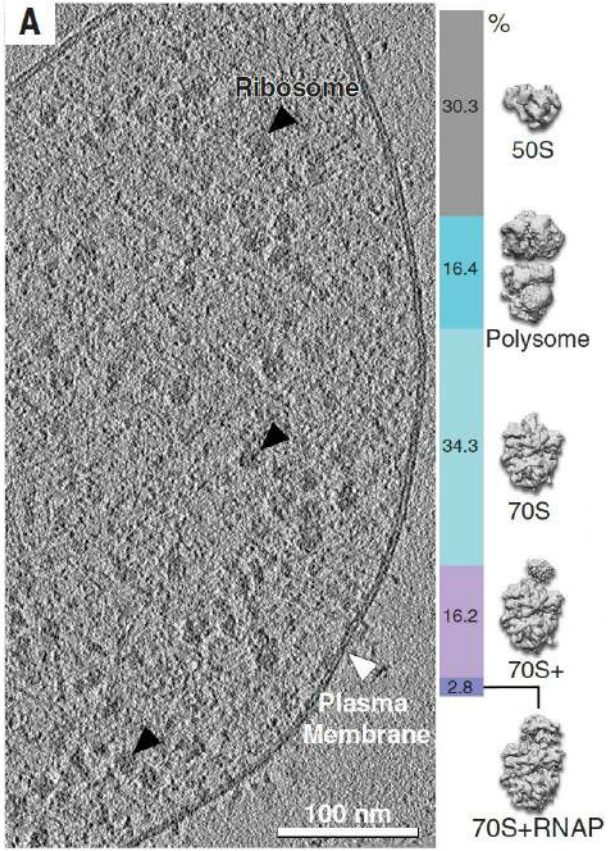
- Structures
  - TTC-A vs TTC-B
  - Unambiguous rigid-body docking of RNAP and Ribosome
  - mRNA spacer at RNAP – ribosome interface
  - Manual fitting of NusA and NusG at RNAP-ribosome interface → only TTC-B is physiologically relevant



Wang C. *et al*., Science 2020

In-cell Architecture of an Actively Transcribing-translating Expressome

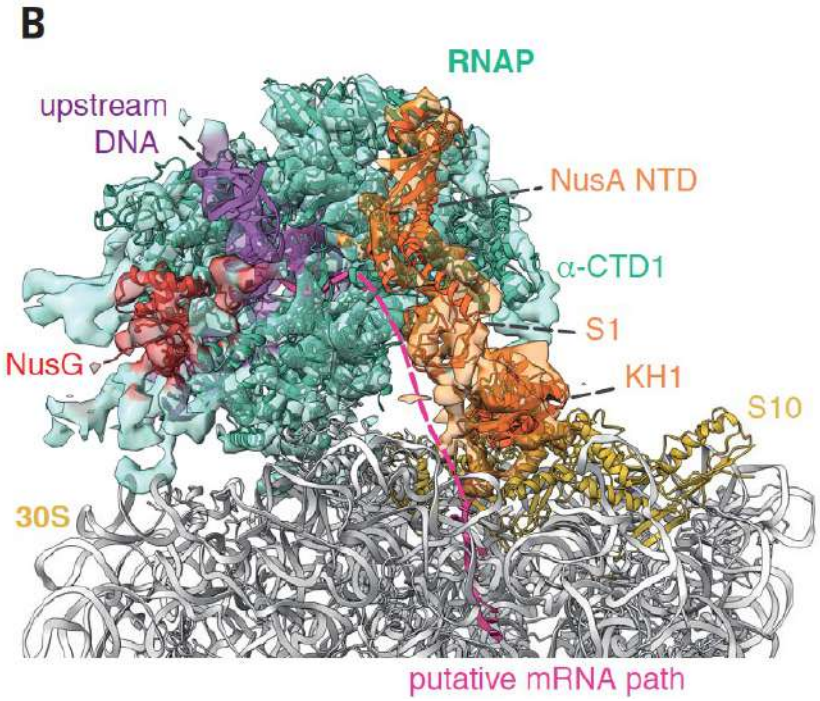
- Sample preparation
  - Cell system: *Mycoplasma pneumoniae*
  - Cryo-ET of whole cell
- Extract ribosome subtomogram
- Classification and refinement



O'Reilly, F. J. *et al.*, Science 2020

In-cell Architecture of an Actively Transcribing-translating Expressome

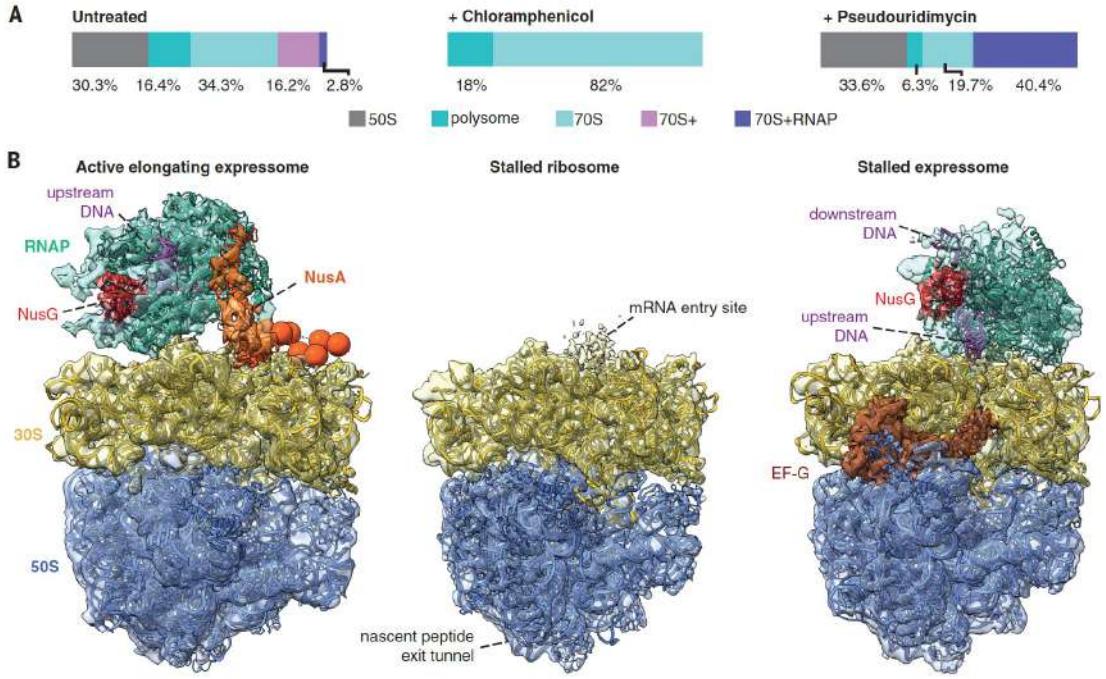
- Structure of transcription-translation coupling complex
  - In-cell cross-linking mass spectrometry data guide density assignment and model fitting
  - Integrative model to understand RNAP-ribosome interface and to resolved binding sites for NusG and NusA
  - mRNA path not resolved



O'Reilly, F. J. *et al.*, Science 2020

In-cell Architecture of an Actively Transcribing-translating Expressome

- Functional studies using translation and transcription inhibitors
  - Changed the percentage of expressome
  - Changed expressome architecture



O'Reilly, F. J. *et al.*, Science 2020

Let's Summarize: Single Particle *vs* Tomography

- Sample preparation
  - Purification & *in vitro* reconstituted system *vs* cells
- Imaging & data processing

Single Particle

Table S1. Cryo-EM structures: NusG-TTC-A, NusG-TTC-B, and NusA-NusG-TTC-B (n = 4, 5, 6, 7, 8, 9, or 10; with CHAPSO)

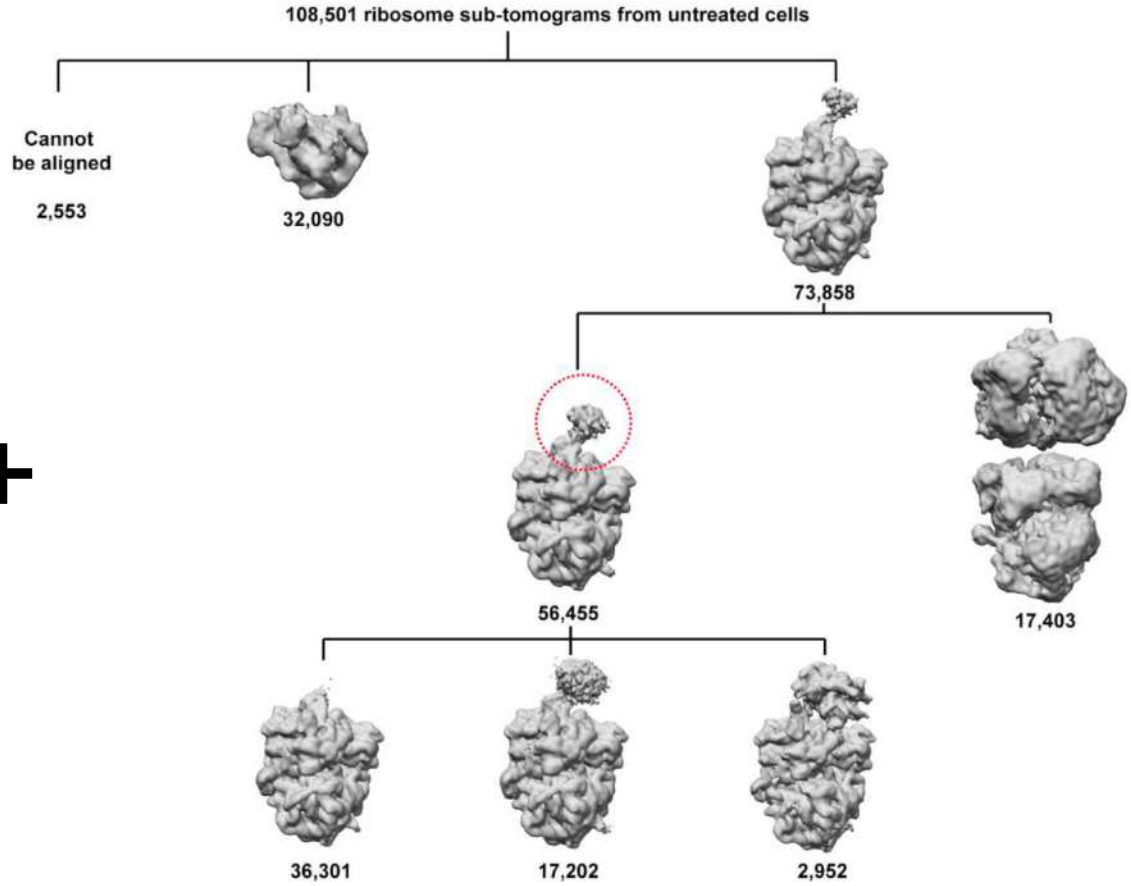
mRNA spacer	TTC class	TTC subclass	cryo-EM facility	particles	resolution	EMDB code	PDB code
4	NusG-TTC-A	TTC-A	NCCAT	139,302	3.7 Å	21386	6VU3
5	NusG-TTC-A	TTC-A	Rutgers	27,378	3.7 Å	21468	6VYQ
6	NusG-TTC-A	TTC-A	Rutgers	24,582	3.8 Å	21469	6VYR
7	NusG-TTC-A	TTC-A	Rutgers	29,704	3.7 Å	21470	6VYS
8	NusG-TTC-A	TTC-A	Rutgers	1,957	6.3 Å	22193	6XIJ
5	TTC-A	TTC-A	Rutgers	27,650	4.1 Å	21494	6VZJ
8	TTC-A	TTC-A	Rutgers	10,379	3.9 Å		
8	NusG-TTC-B	TTC-B	Rutgers	435	12.6 Å	22192	6XII
9	NusG-TTC-B	TTC-B	Rutgers	6,121	4.7 Å	22142	6XDR
10	NusG-TTC-B	TTC-B	Rutgers	4.617	5.0 Å	22181	6XGF
8	NusA-NusG-TTC-B	TTC-B1	NCCAT	38,958	3.2 Å	22082	6X6T
8	NusA-NusG-TTC-B	TTC-B2	NCCAT	45,451	3.5 Å	22084	6X7F
8	NusA-NusG-TTC-B	TTC-B3	NCCAT	61,683	3.1 Å	22087	6X7K
9	NusA-NusG-TTC-B	TTC-B1	Rutgers	2,558	5.9 Å		
9	NusA-NusG-TTC-B	TTC-B2	Rutgers	21,740	4.2 Å		
9	NusA-NusG-TTC-B	TTC-B3	Rutgers	11,509	4.8 Å	22107	6X9Q
10	NusA-NusG-TTC-B	TTC-B1	Rutgers	4,236	4.9 Å		
10	NusA-NusG-TTC-B	TTC-B3	Rutgers	19,968	3.7 Å	22141	6XDQ
8	NusA-TTC-X	TTC-X	Rutgers	759	9.3 Å		

Wang C. *et al*., Science 2020

Cryo-ET

- Cross-linking mass spectrometry
- Functional studies

+



O'Reilly, F. J. *et al*., Science 2020

Let's Summarize: Single Particle *vs* Tomography

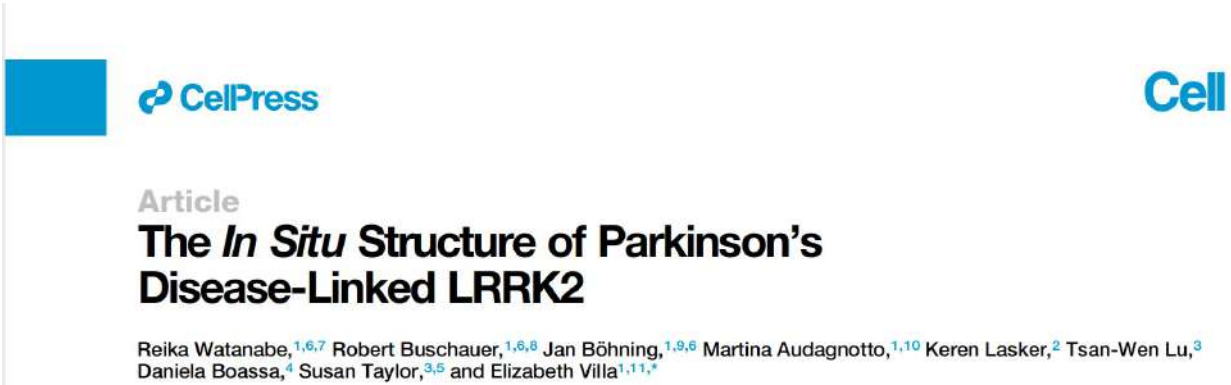
- Sample preparation
  - Purification & in vitro reconstituted system *vs* cells
- Imaging & data processing
- Resolution & interpretation
  - Single particle: **atomic** resolution maps to allow unambiguous fitting and direct modeling of individual protein/RNA components
  - Cryo-ET: **subnanometer** resolution subtomogram averages combined with integrative modeling to reveal complex architecture in cellular & functional settings

Why Tomography?

- Sample has a unique structure or is heterogenous

- Sample in a complex environment

Applying Cryo-ET to Reveal Protein Structure *in situ* – The Workflow



CellPress

Cell

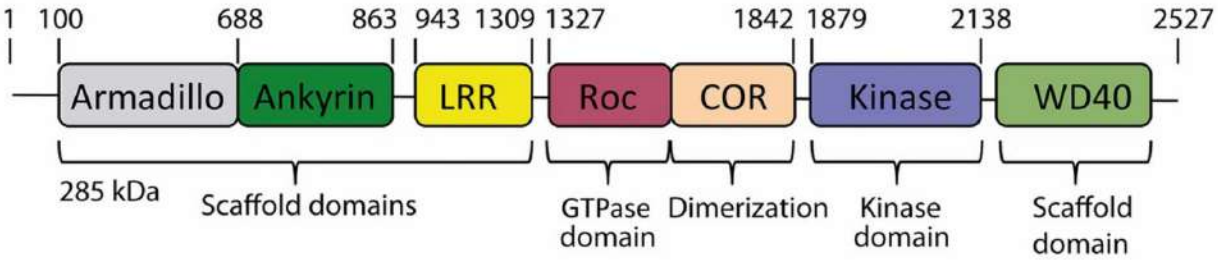
Article

The *In Situ* Structure of Parkinson's Disease-Linked LRRK2

Reika Watanabe,[1,6,7] Robert Buschauer,[1,6,8] Jan Böhning,[1,9,6] Martina Audagnotto,[1,10] Keren Lasker,[2] Tsan-Wen Lu,[3] Daniela Boassa,[4] Susan Taylor,[3,5] and Elizabeth Villa[1,11,*]

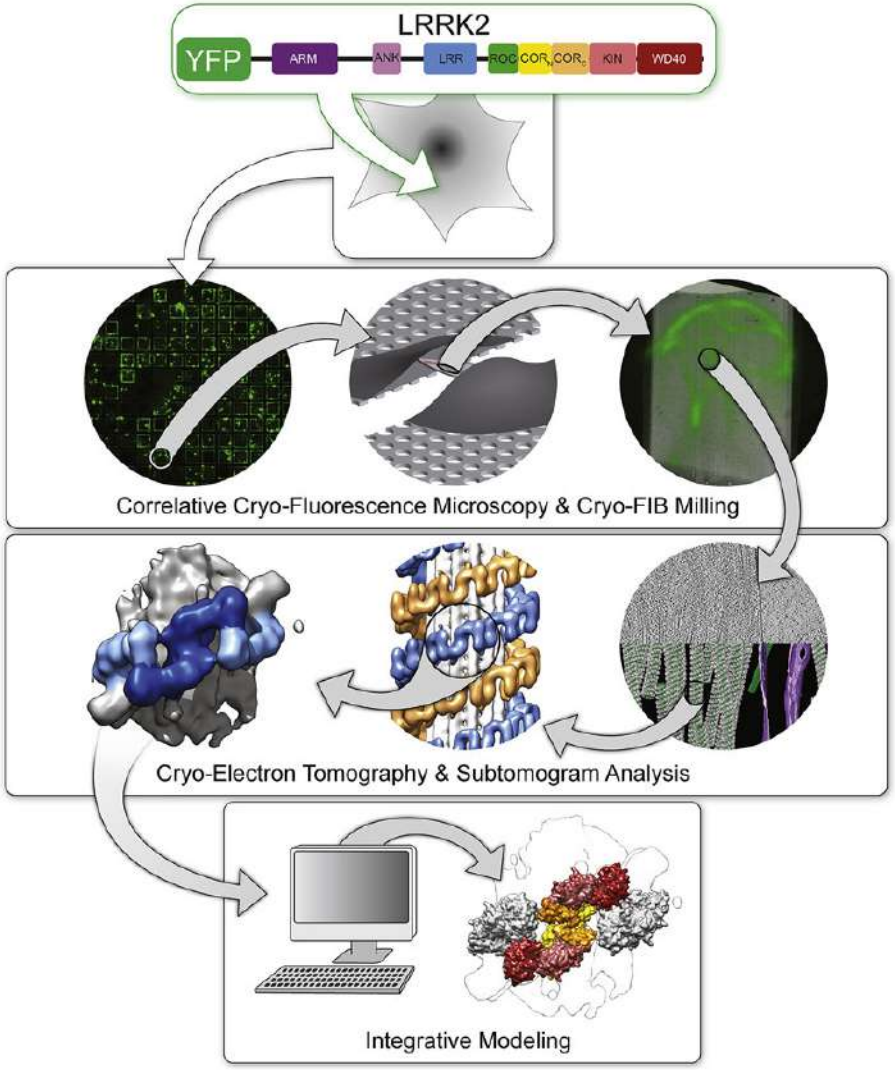
Structure of LRRK2

- LRRK2: (Leucine-rich repeat kinase 2) the most mutated gene in familial Parkinson's disease
- Functions in neurite outgrowth, membrane trafficking, autophagy
- Mutations or pharmacological inhibition of kinase activity recruit LRRK2 to microtubules
- Multi-domain protein; structure of the full-length protein is not available.



Guaitoli, G. *et al.*, PNAS 2016

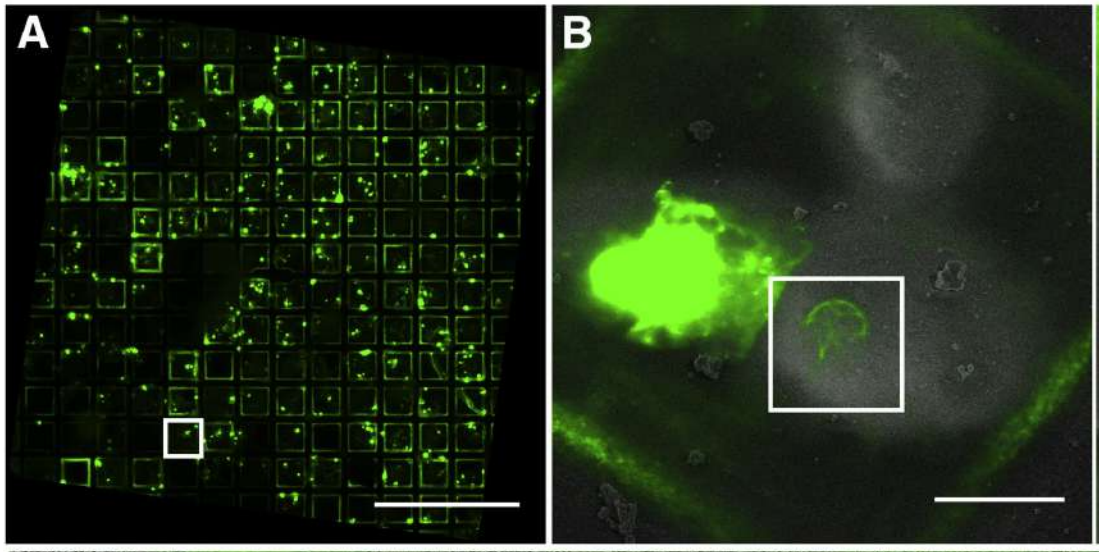
Workflow



Watanabe, R. *et al*., Cell 2020

Step 1: Design and Prepare Cells to Allow Detection of Targets in the Crowded Environment

- Correlative Light and Electron Microscopy (CLEM)
- Increasing abundance for easy detection



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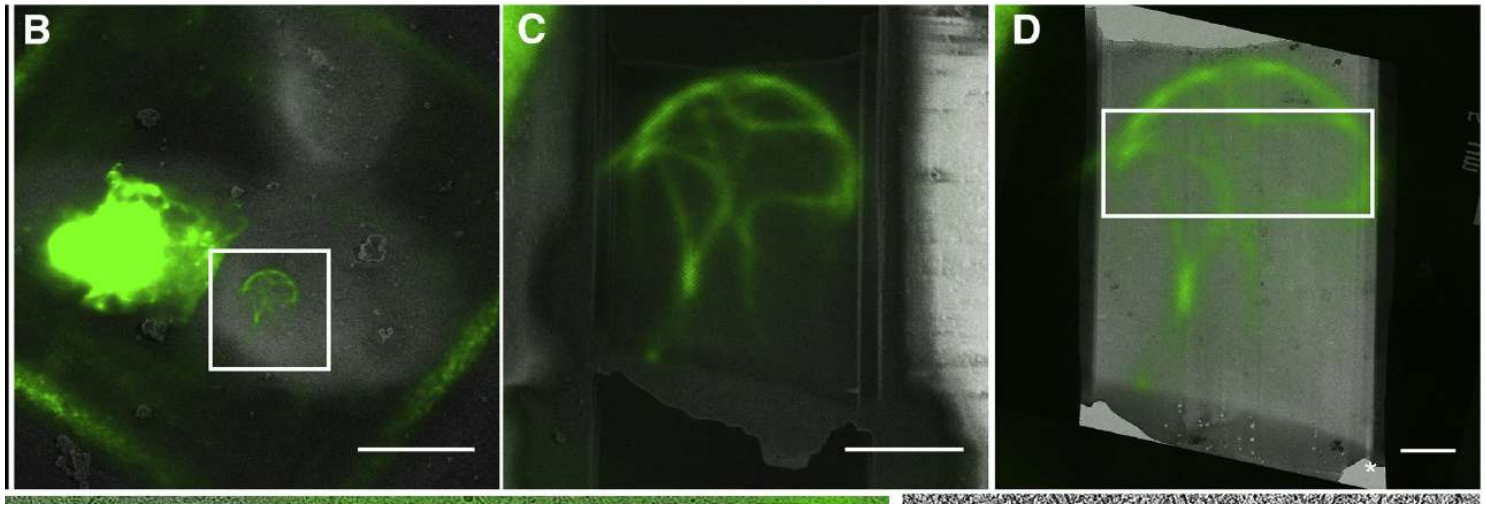
Step 2: Focused Ion Beam Milling to Generate Thin Cell Lamella for Cryo-ET

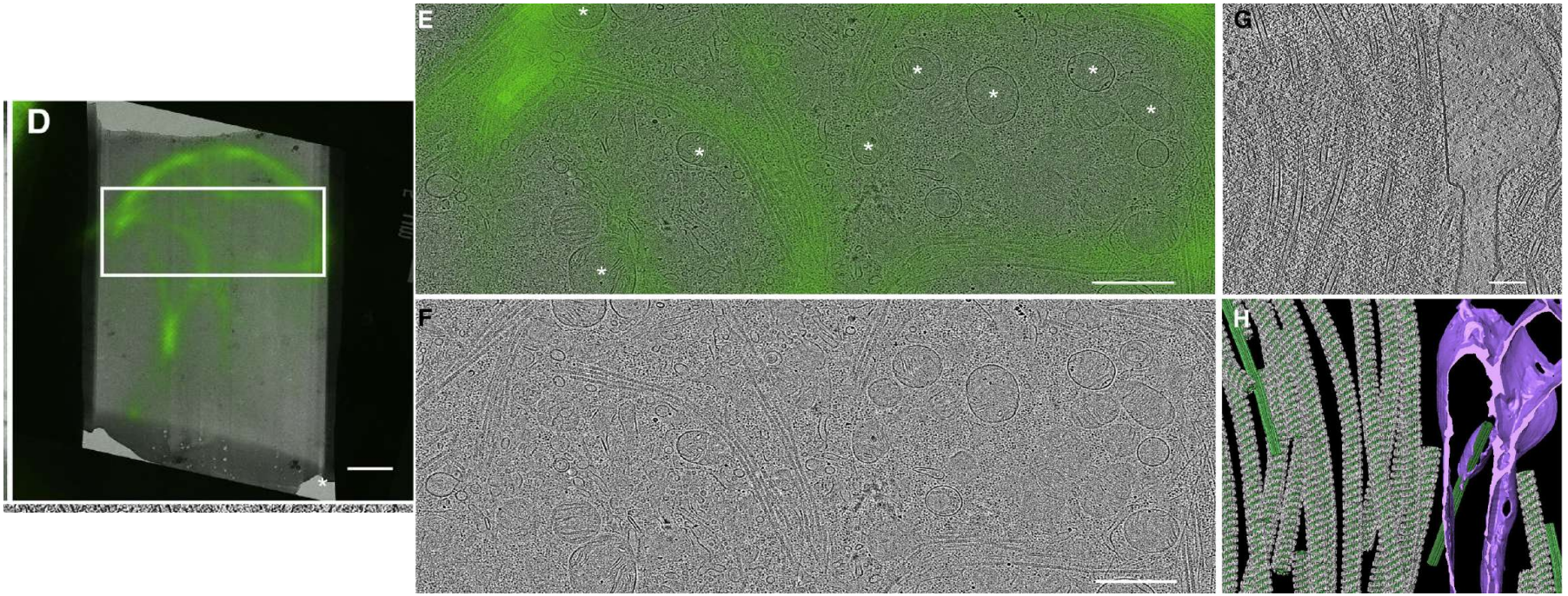
- Cells on grids: 1 - 5 µm
- Lamella: 100– 150 nm



Watanabe, R. *et al*., Cell 2020

Step 3: Cryo-ET Imaging and Tomogram Reconstruction

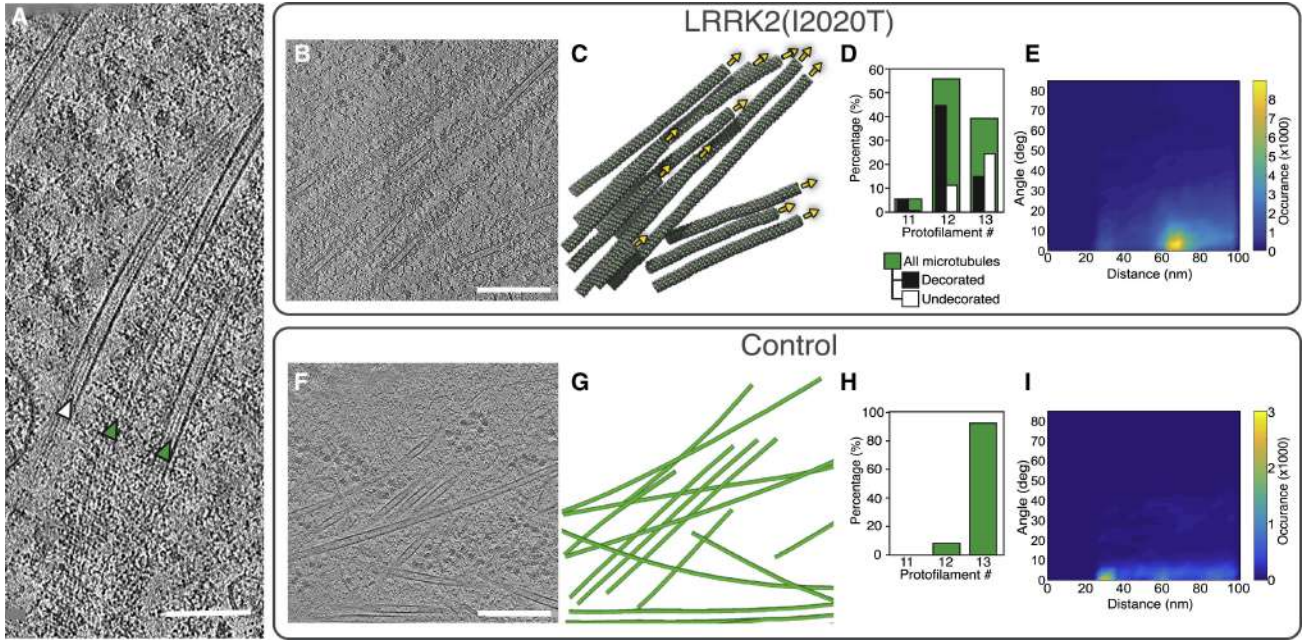
- Use CLEM to guide tilt series data collection



Watanabe, R. *et al*., Cell 2020

Step 4: *In situ* Structure Analysis

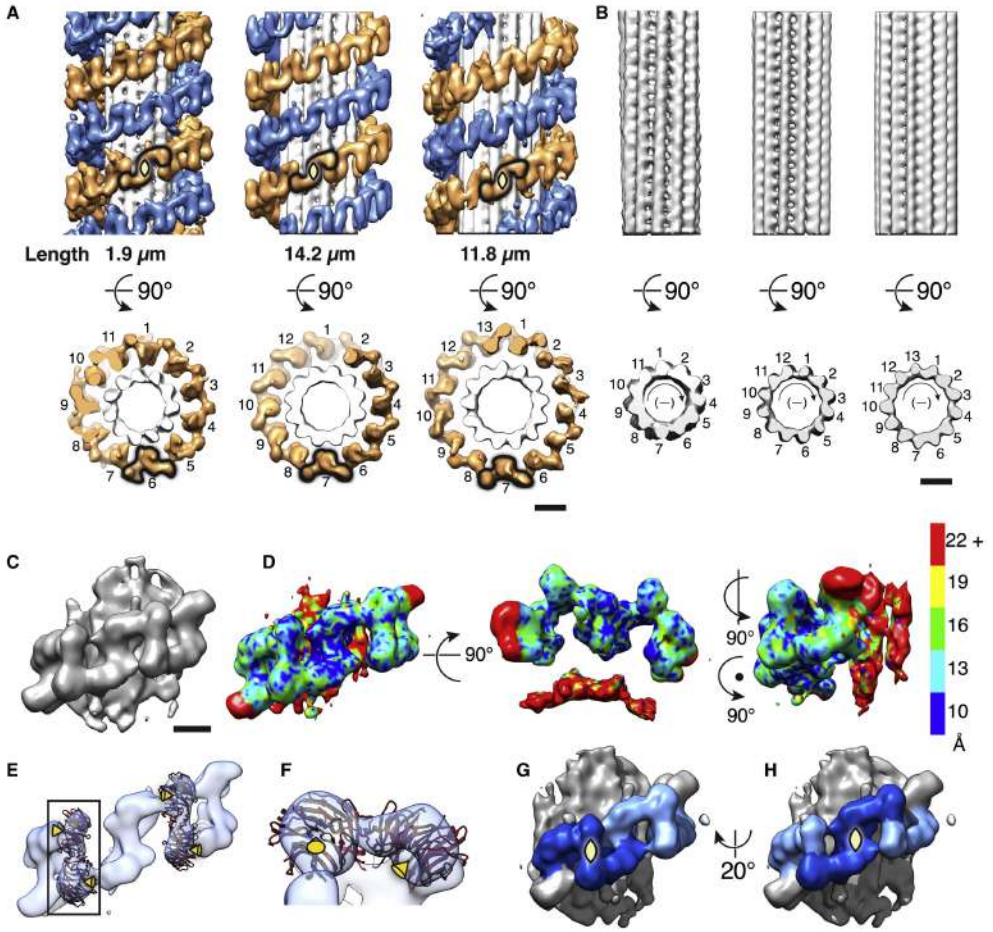
- Distribution and dynamics in cells



Watanabe, R. *et al*., Cell 2020

Step 5: Subtomogram Analysis

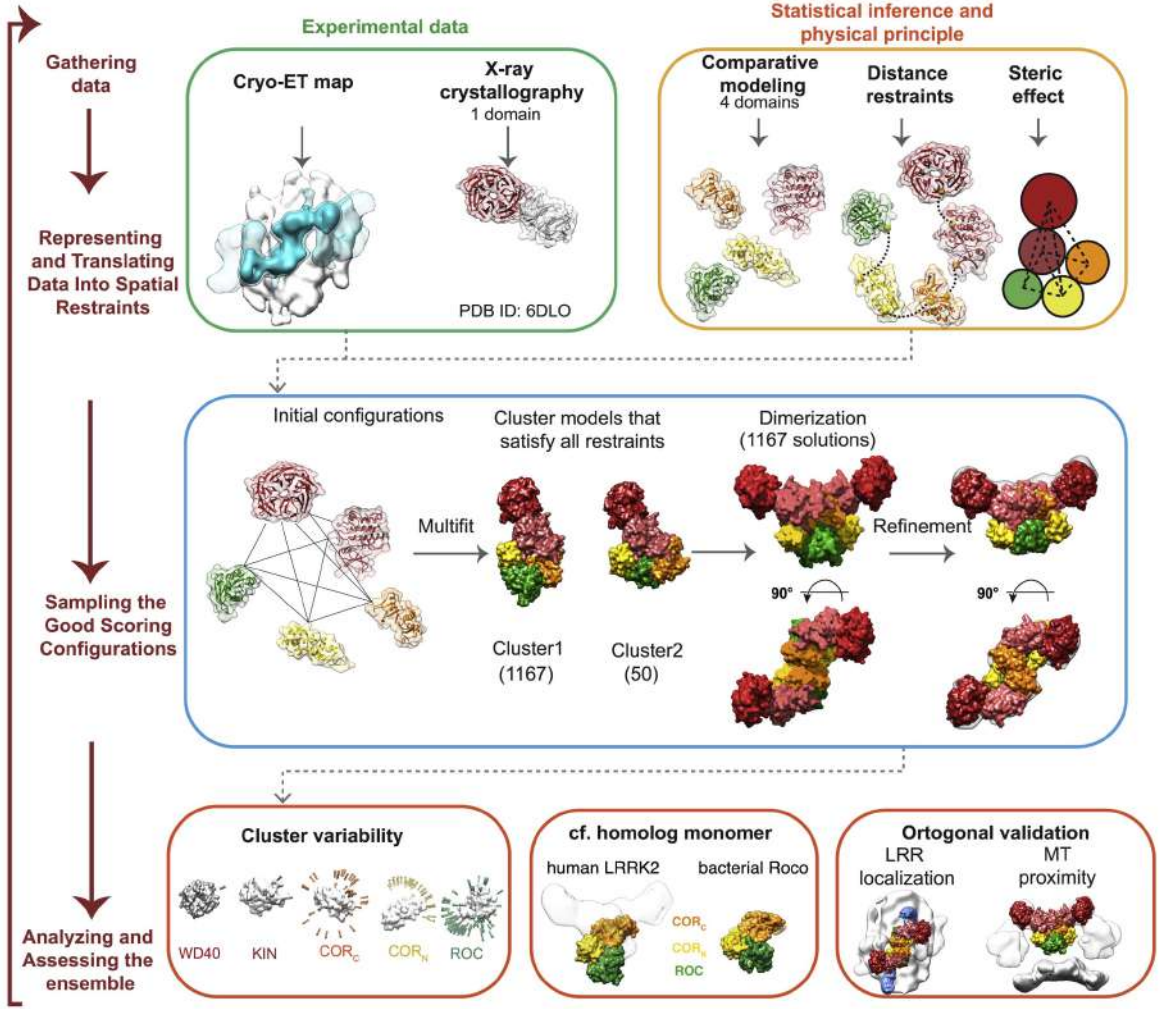
- Extraction
- Classification
- Averaging
- Model fitting



Watanabe, R. *et al*., Cell 2020

Step 6: Integrative Modeling

- Details in domain organization can be deduced from nanometer resolution maps



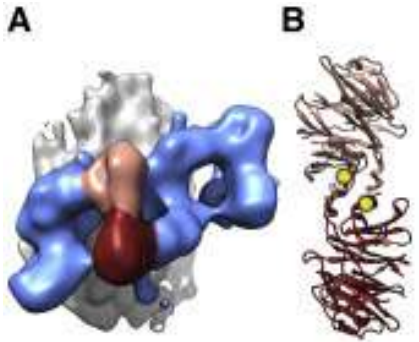
Watanabe, R. *et al*., Cell 2020

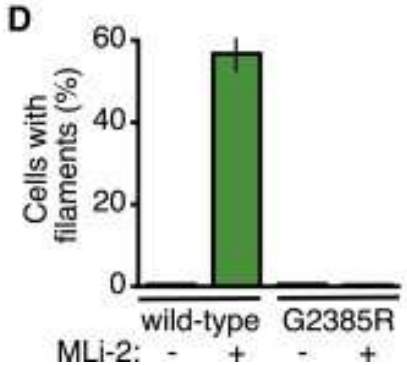
Step 7: Functional Analysis

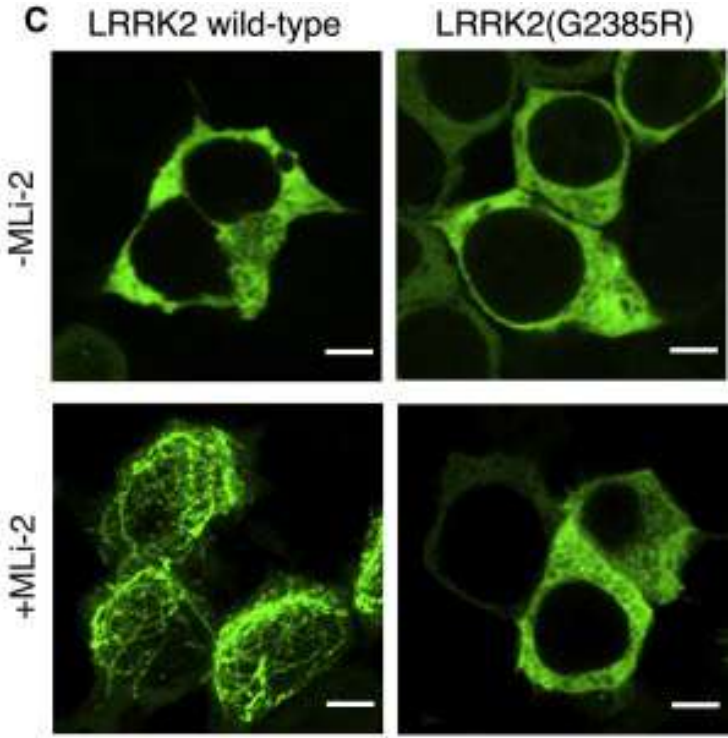
Disturbing structure

↕

Variations of functions







Watanabe, R. *et al*., Cell 2020

Summary

- What is cryo-ET
- Single particle vs cryo-ET
- Cryo-ET workflow
  - Sample/cell preparation
  - CLEM to identify targets in crowded cellular environments
  - FIB milling to prepare thin lamella for cryo-ET imaging
  - Subtomogram analysis
  - Integrative modeling to reveal details in domain organization

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