

Modern Methods in Protein Research

P

iology:



Aleš Hnízda

28 April 2021



Overview

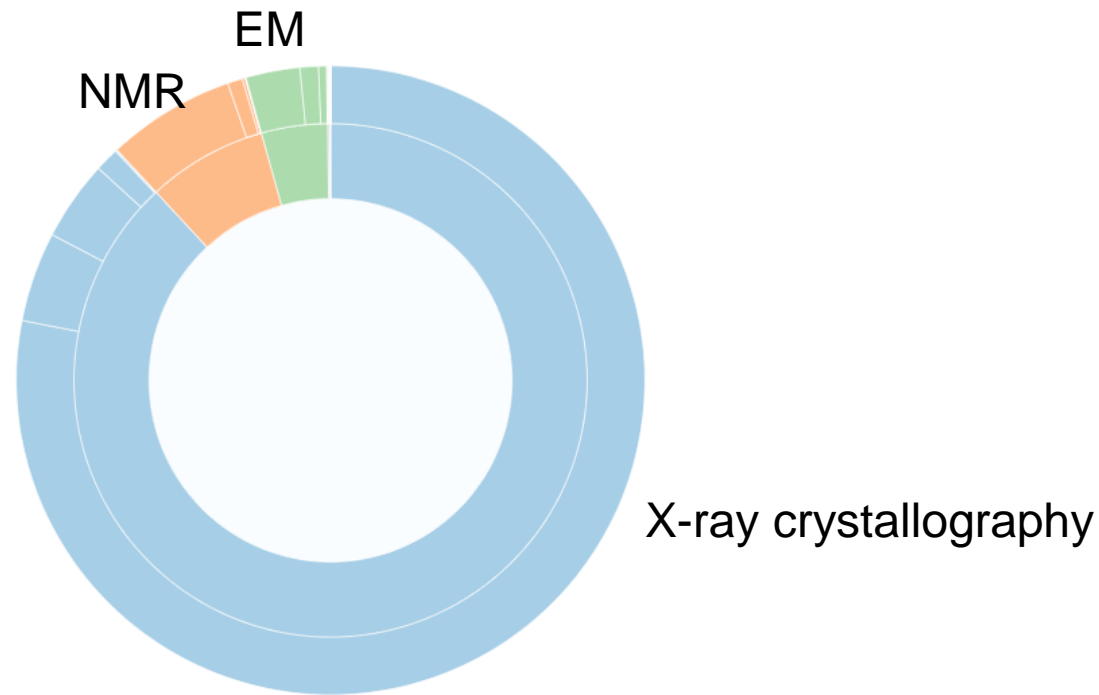
- Historical perspective
- Workflow
- Applications

Overview

- **Historical perspective**
- Workflow
- Applications

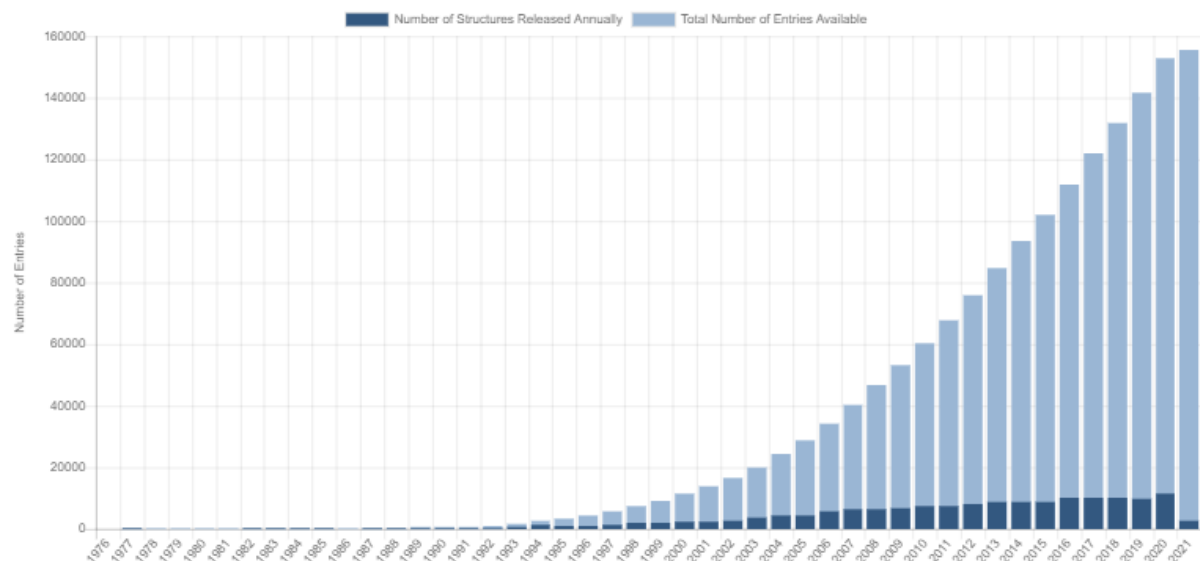
Techniques for getting structure

- NMR, X-ray crystallography, cryo-EM

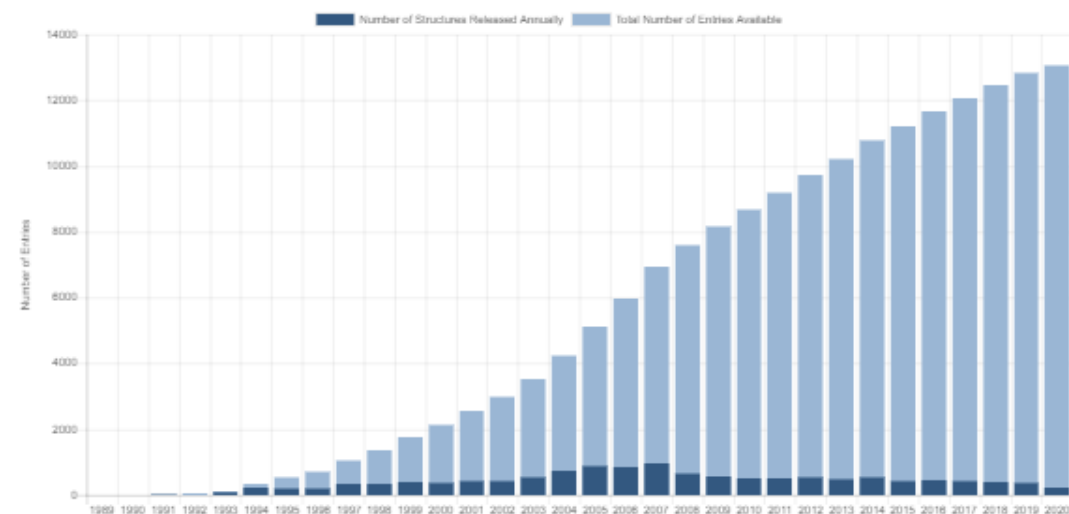


≈180,000 deposited structures in total

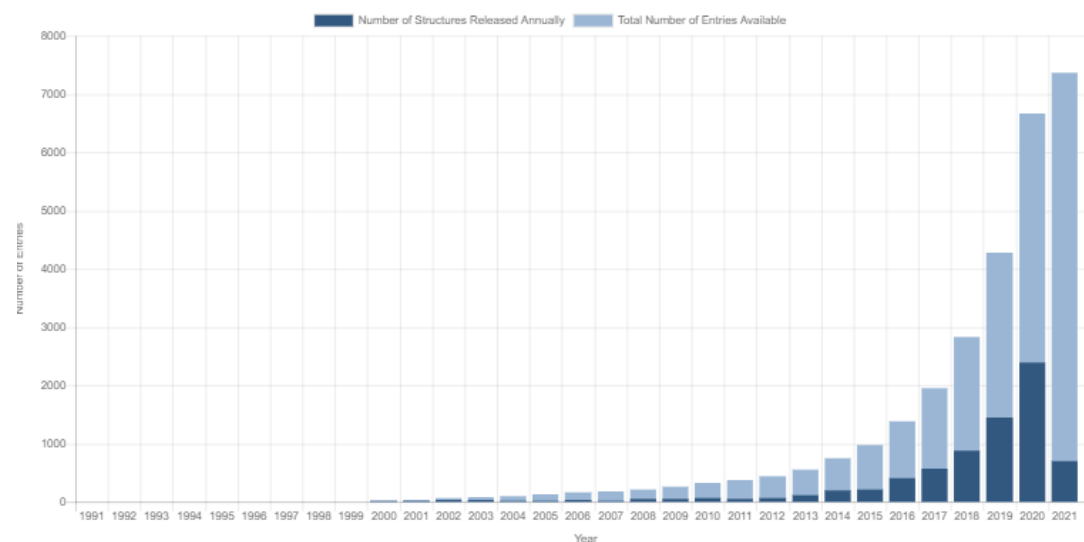
X-ray crystallography



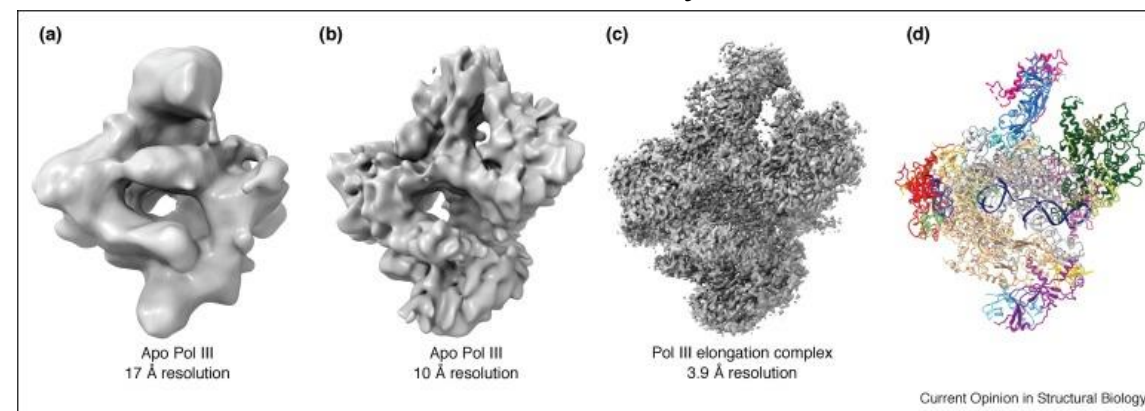
NMR



Cryo-EM



Resolution revolution in cryo-EM in 2015



Hanske et al. Curr.Opin. Str. Biol. 2018

The revolution will not be crystallized: a new method sweeps through structural biology

Move over X-ray crystallography. Cryo-electron microscopy is kicking up a storm by revealing the hidden machinery of the cell.

Ewen Callaway

09 September 2015

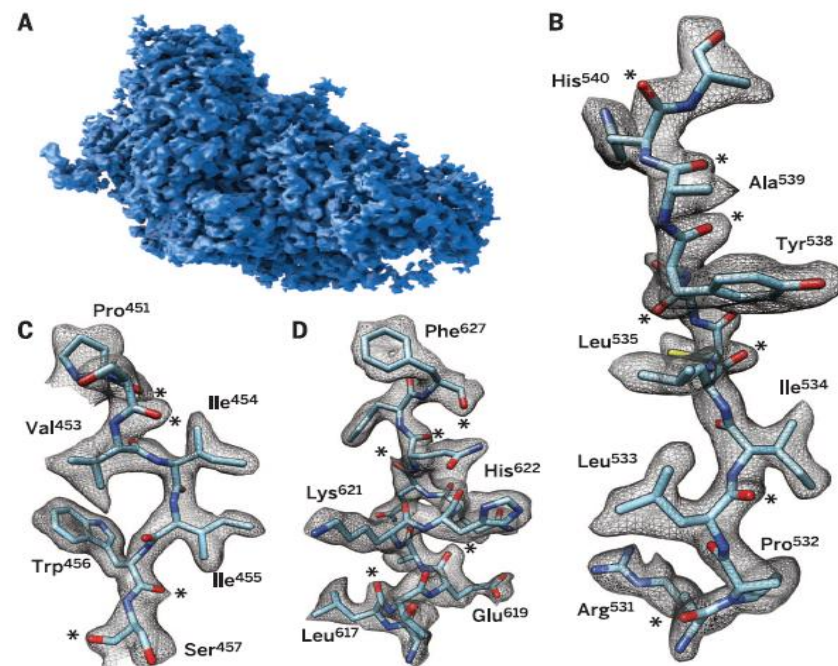
[PDF](#) [Rights & Permissions](#)

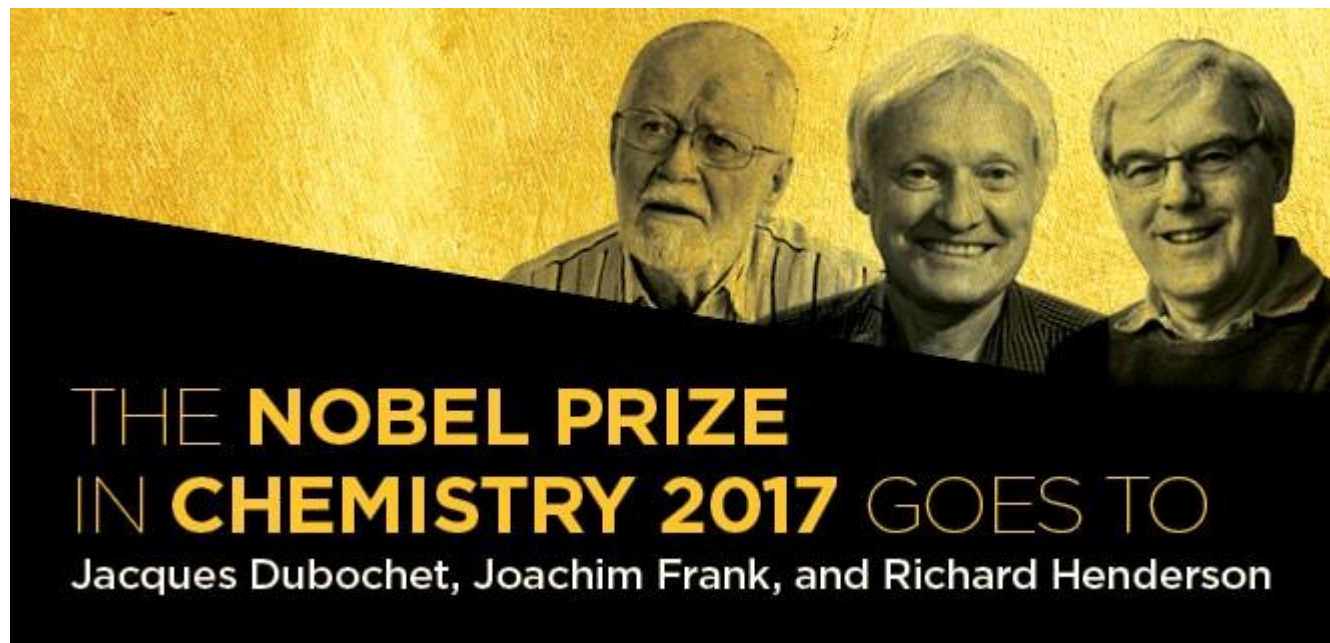


ELECTRON MICROSCOPY

2.2 Å resolution cryo-EM structure of β -galactosidase in complex with a cell-permeant inhibitor

Alberto Bartesaghi,^{1*} Alan Merk,^{1*} Soojay Banerjee,¹ Doreen Matthies,¹ Xiongwu Wu,² Jacqueline L. S. Milne,¹ Sriram Subramaniam^{1†}





Jacques Dubochet – sample preparation

Joachim Frank – creating 3-D model from 2-D projections

Richard Henderson – the first 3-D structure based on EM

Current state: Breaking limits in cryo-EM

Article

Single-particle cryo-EM at atomic resolution

<https://doi.org/10.1038/s41586-020-2829-0>

Received: 22 May 2020

Accepted: 27 August 2020

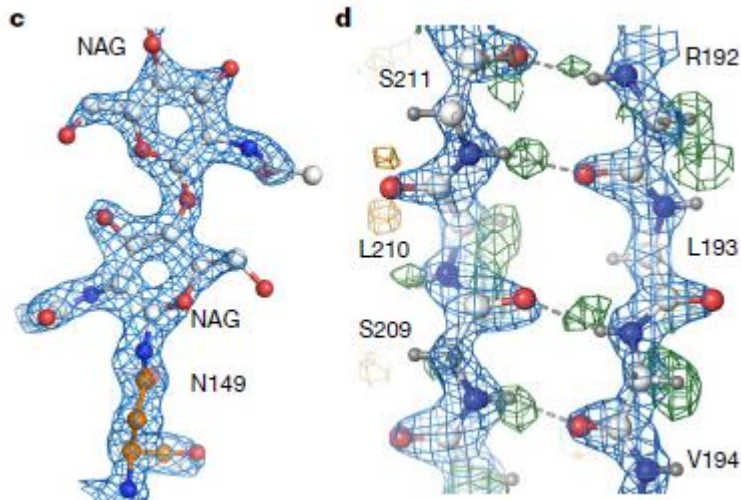
Published online: 21 October 2020

Takanori Nakane^{1,9}, Abhay Kotecha^{2,9}, Andrija Sente^{1,9}, Greg McMullan¹, Simonas Masiulis^{1,7}, Patricia M. G. E. Brown¹, Ioana T. Grigoras^{1,9}, Lina Malinauskaitė¹, Tomas Malinauskas³, Jonas Miehling¹, Tomasz Uchański^{1,5}, Lingbo Yu², Dimple Karia², Evgeniya V. Pechnikova², Erwin de Jong², Jeroen Keizer², Maarten Bischoff², Jamie McCormack², Peter Tiemeijer², Steven W. Hardwick⁶, Dimitri Y. Chirgadze⁶, Garib Murshudov¹, A. Radu Aricescu^{1,2} & Sjors H. W. Scheres^{1,2}✉

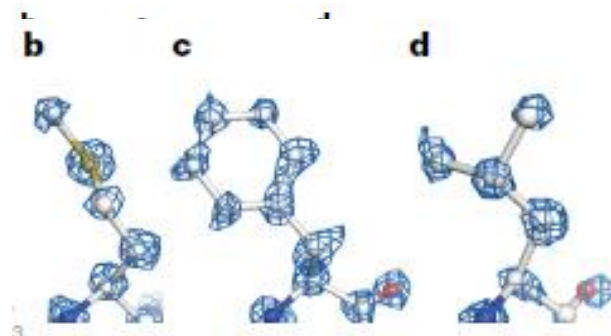
GABA receptor (1.7 Å)

N-acetylglucosamin

helices



Apo ferritin (1.22 Å)



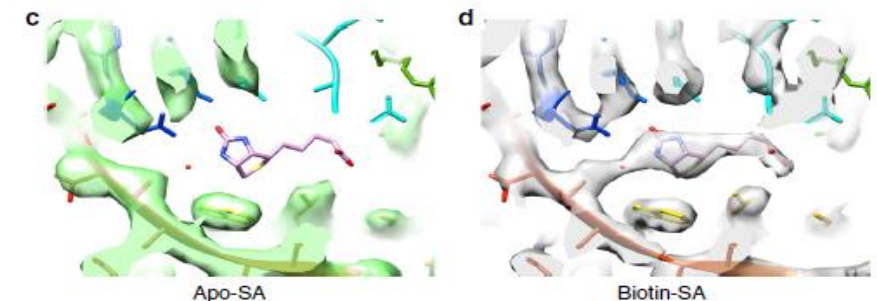
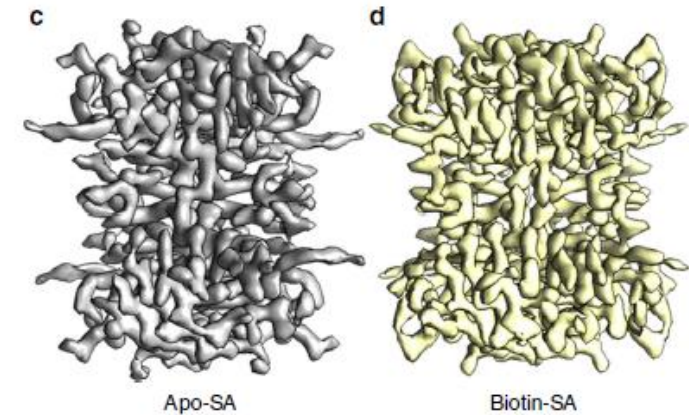
ARTICLE

<https://doi.org/10.1038/s41467-019-10368-w>

OPEN

Single particle cryo-EM reconstruction of 52 kDa streptavidin at 3.2 Angstrom resolution

Xiao Fan^{1,2,4}, Jia Wang^{1,4}, Xing Zhang¹, Zi Yang^{1,2}, Jin-Can Zhang³, Lingyun Zhao¹, Hai-Lin Peng³, Jianlin Lei^{1,2} & Hong-Wei Wang^{1,2}✉

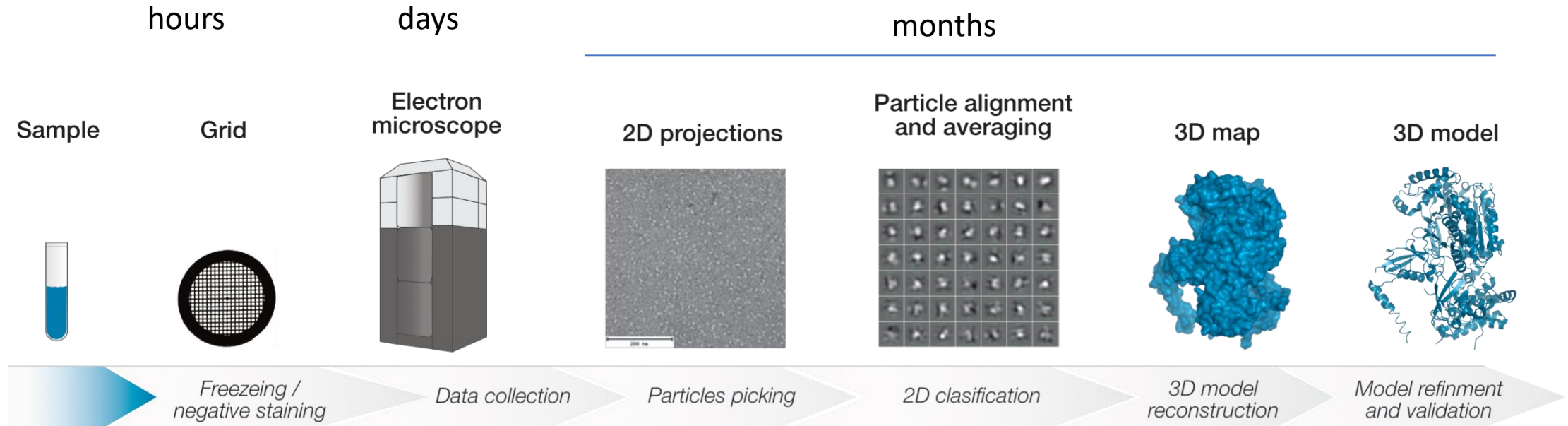


Overview

- Historical perspective
- **Workflow**
- Applications

Cryo-electron microscopy

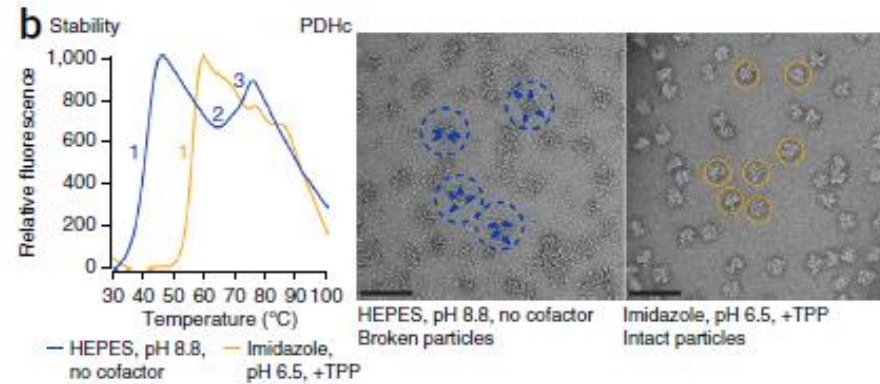
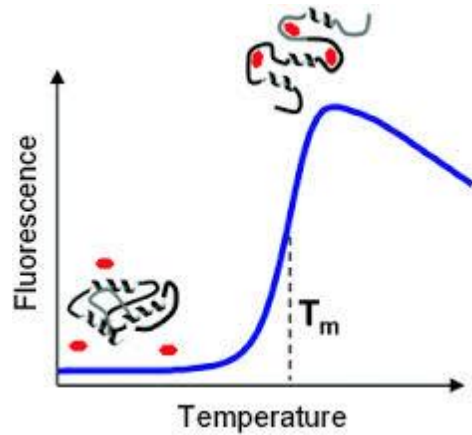
- Specifically single-particle analysis



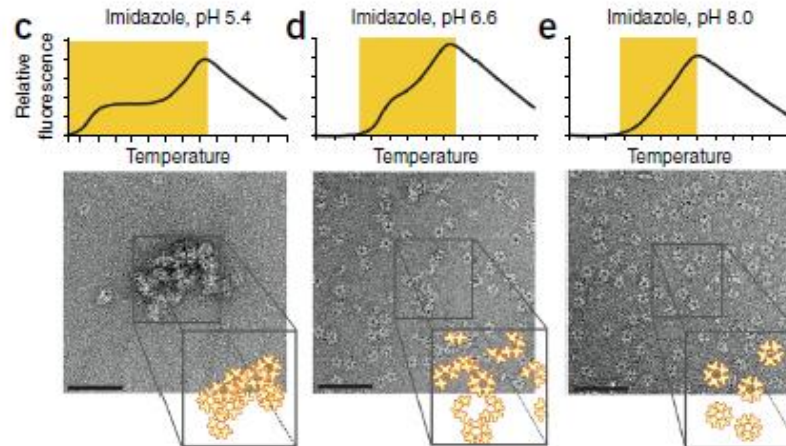
<https://leaderna.com/>

Choice of buffer for specimen: from Thermofluor to ProteoPlex

E.coli pyruvate dehydrogenase

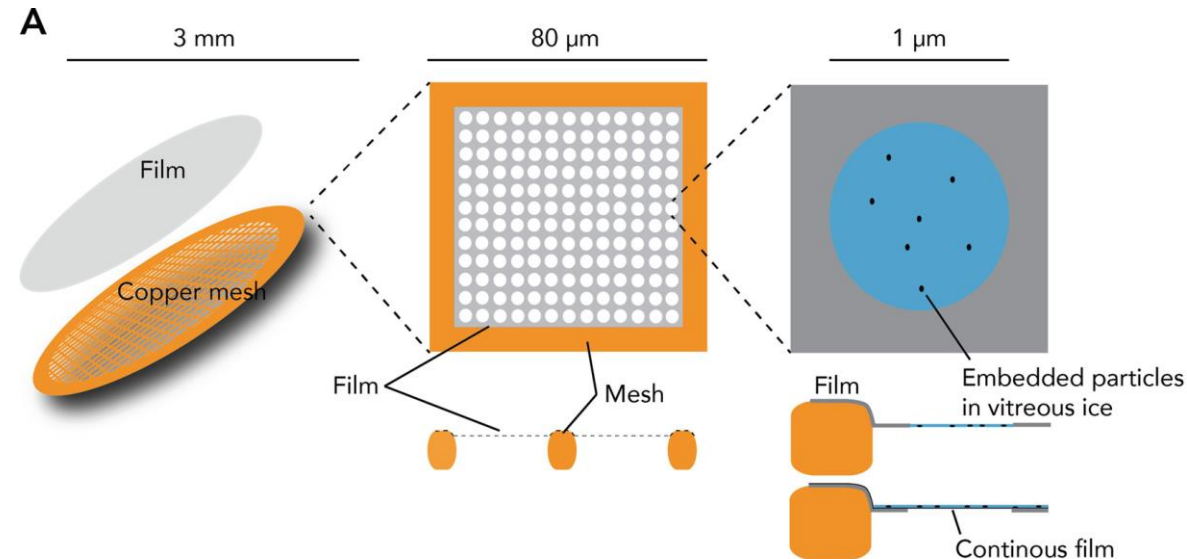


Bacterial tRNA synthetase Sela

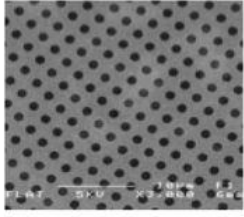
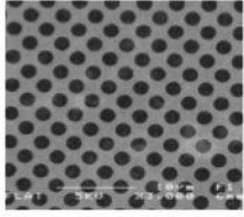
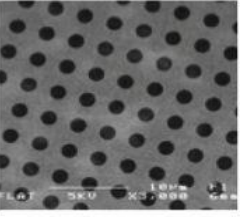


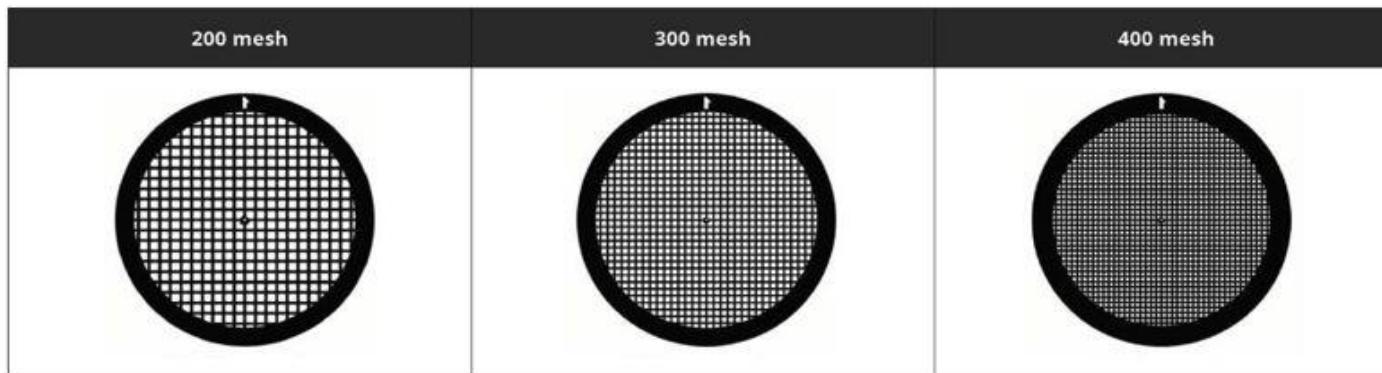
Sample preparation: grid making

- One sample – 3 μl of protein ($\approx 1 \text{ mg/ml}$)
- Application of sample on EM grid followed by vitrification using liquid ethane



Many different types of EM grids

Hole Pattern	1.2/1.3	2/1	2/2
Hole Size	1.2 μm	2 μm	2 μm
Hole Spacing	1.3 μm	1 μm	2 μm
			

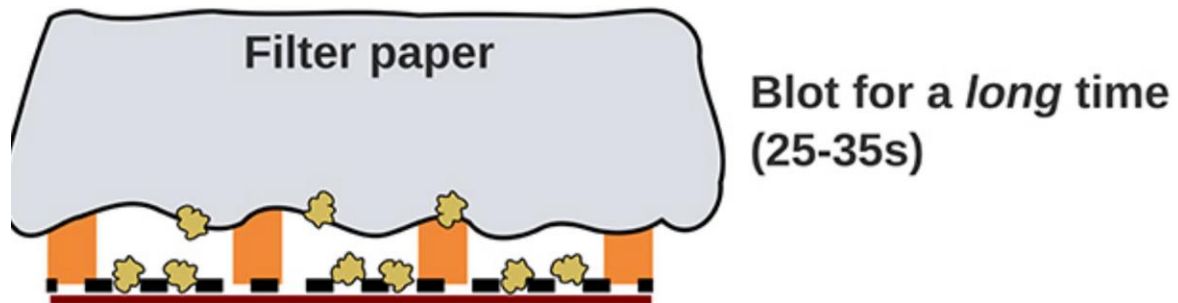
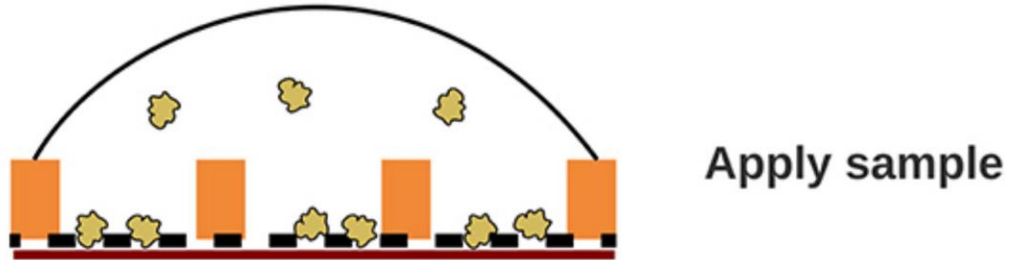


Glow discharge of EM grids: making them hydrophilic



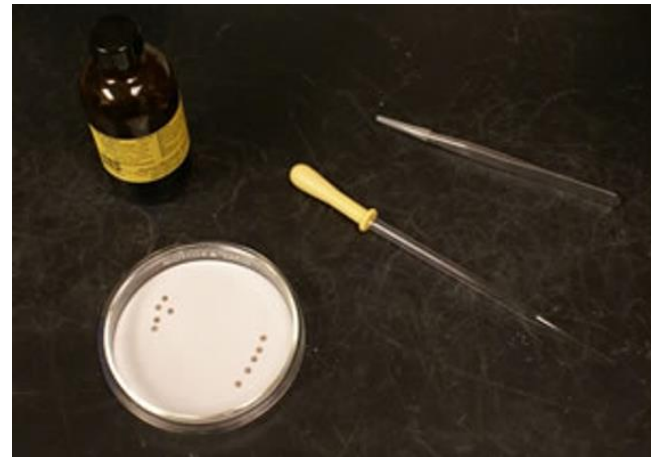
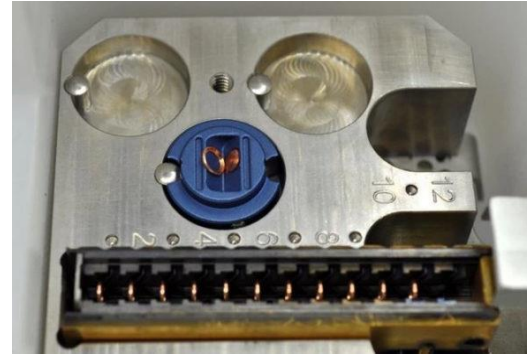
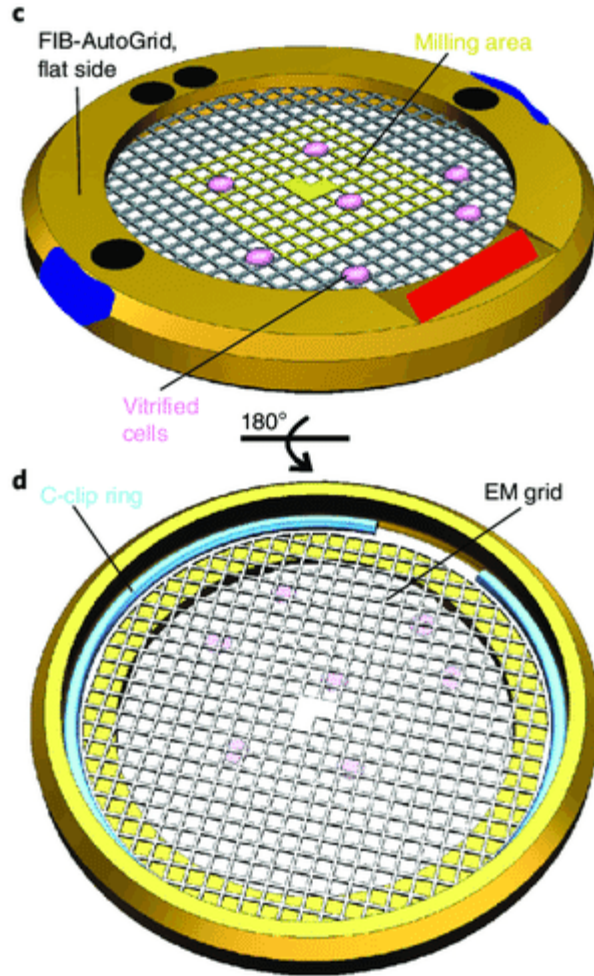
Vitrification using plunge freezing: Vitrobot





Major challenge: air-water interface

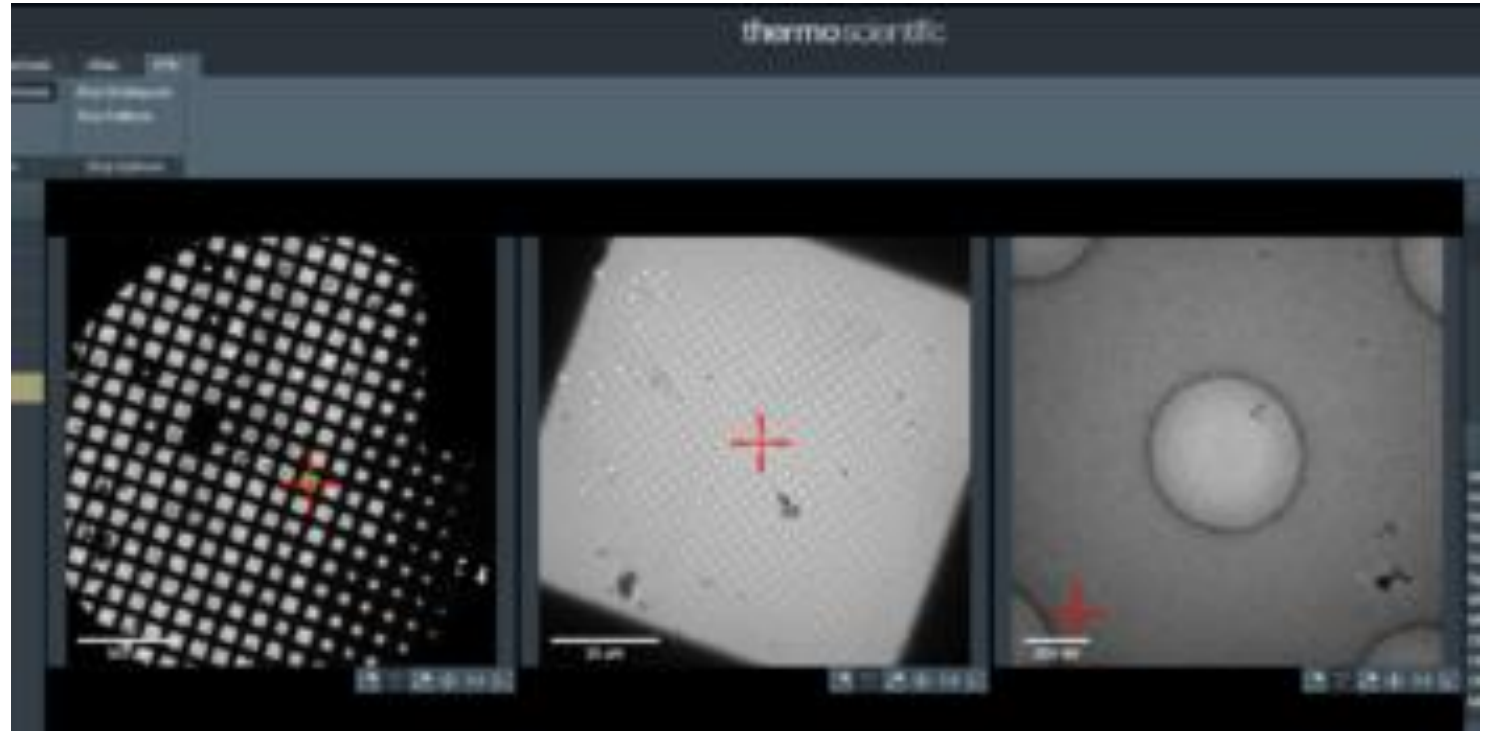
Grids are clipped and ready to go



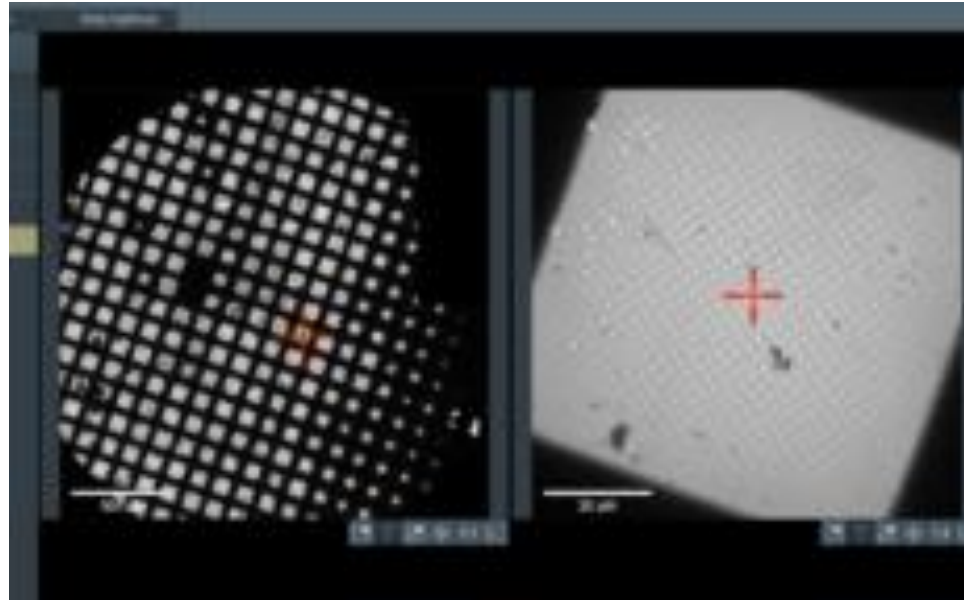
Screening of grids & data collection



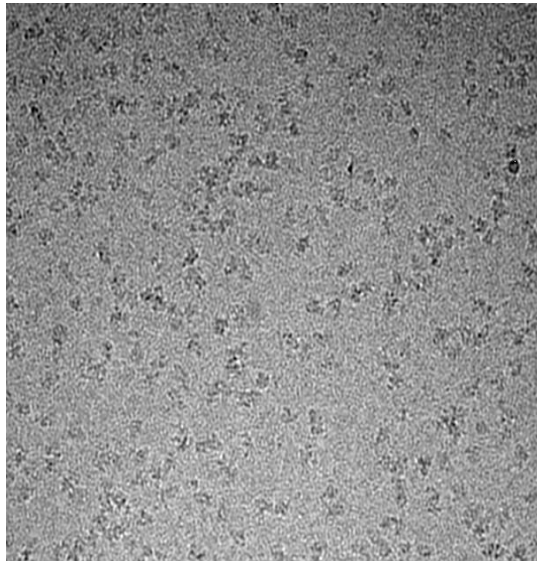
Talos Arctica Cryo-TEM



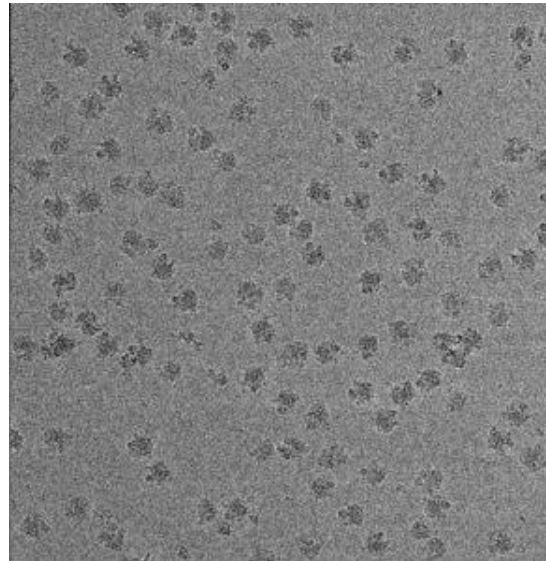
<https://blogs.urz.uni-halle.de/kastritislab/>



Ku70/80 (150 kDa)

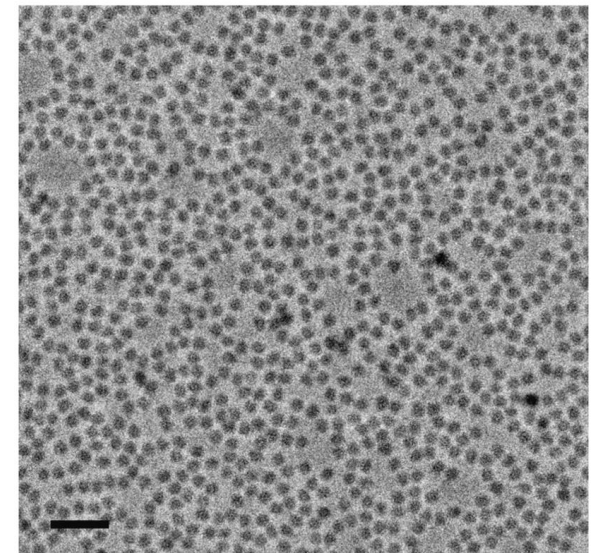


ribosome (3 MDa)



[/www.vanderbilt.edu](http://www.vanderbilt.edu)

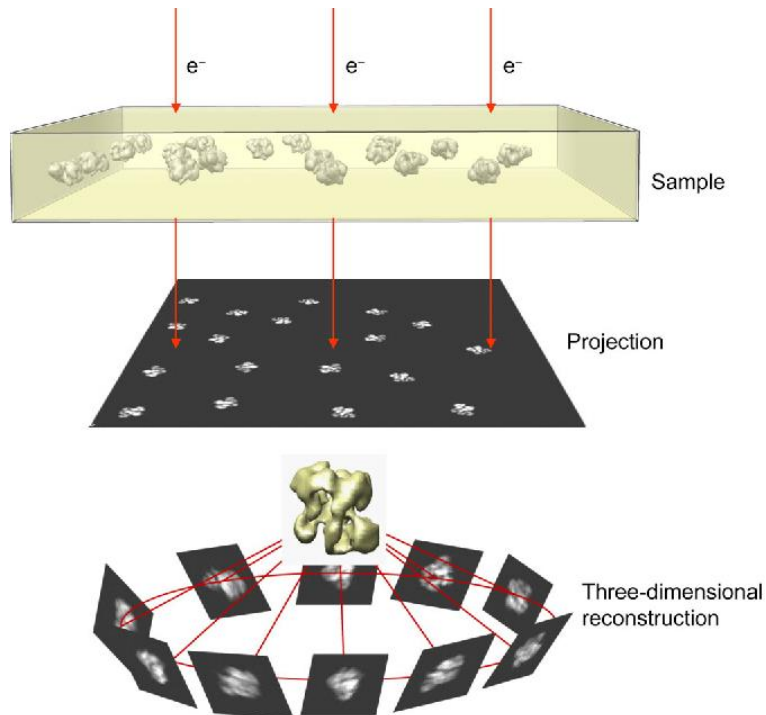
Streptavidin (52 kDa)



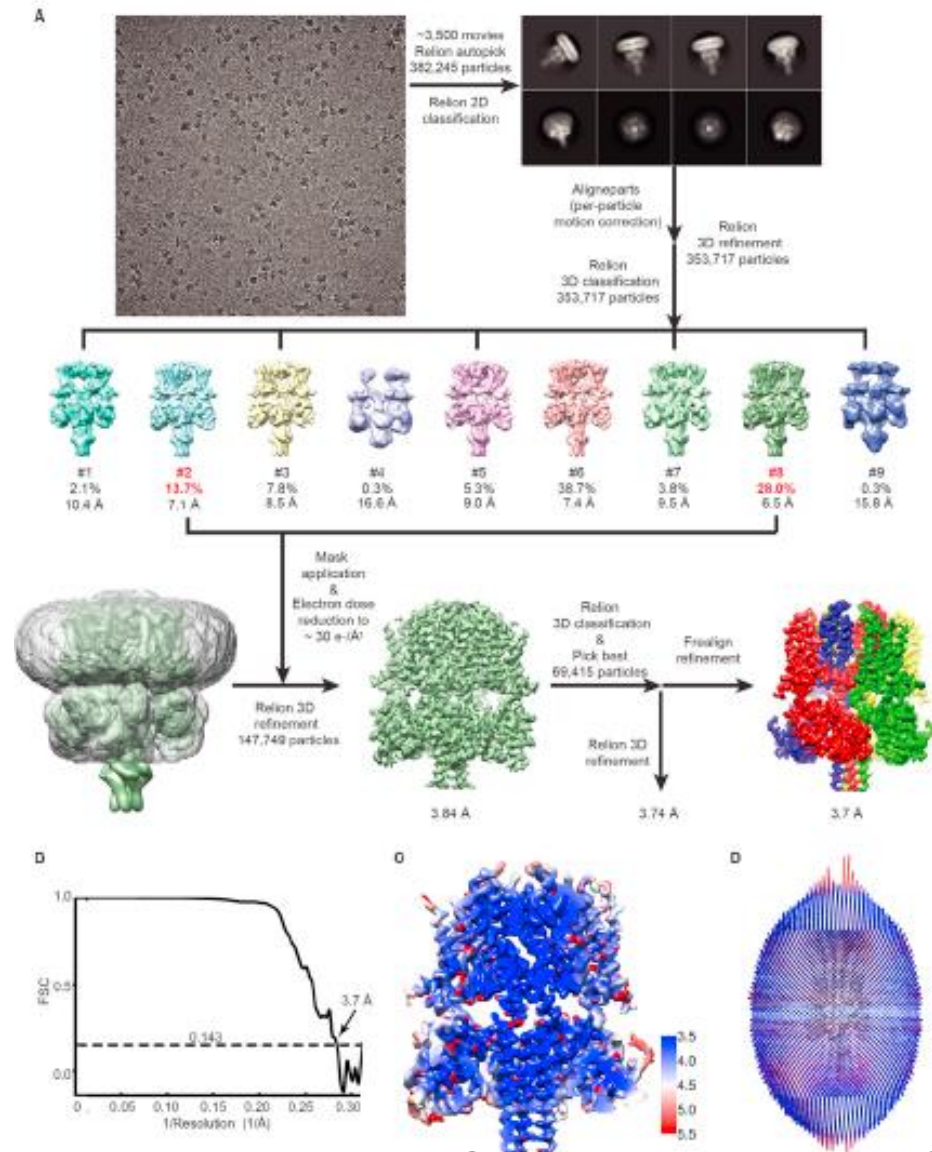
Fan X. et al. Nat Comm 2019

Data handling and processing: towards the structure

- Terabytes of data
- Computationally demanding



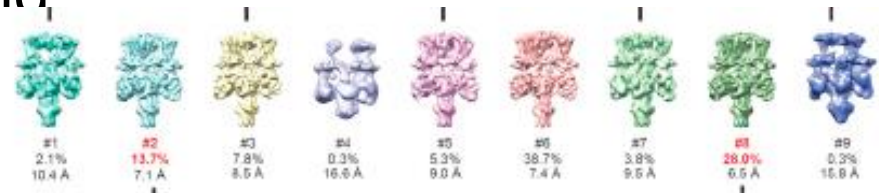
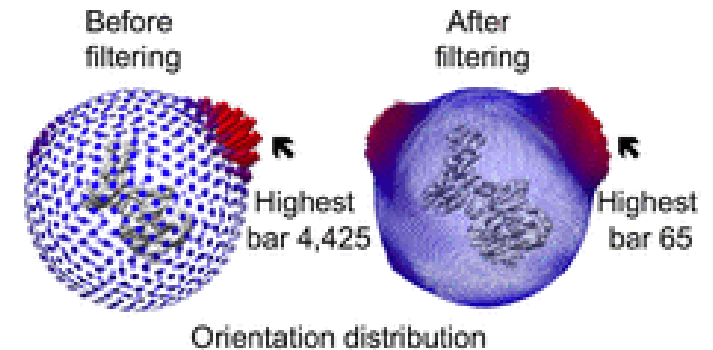
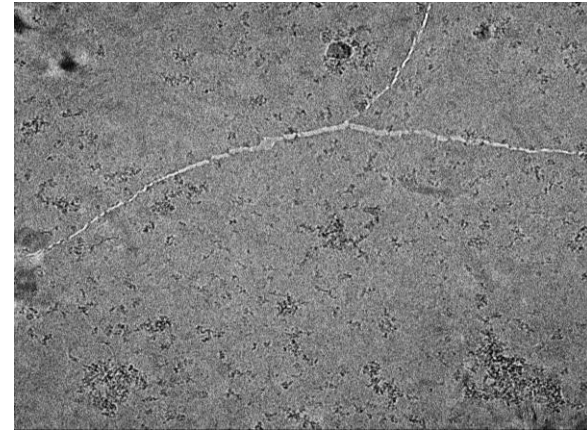
Complex of potassium channel KCNQ1 with calmodulin



Sun and MacKinnon, Cell, 2017

Limitations / critical points

- Sample preparation: water-air interface
- (choice of grids / detergents)
- Preferred orientation
- Relevance of 3-D classes
- Slow progress (low capacity of screening)
- Large datasets / computationally demanding



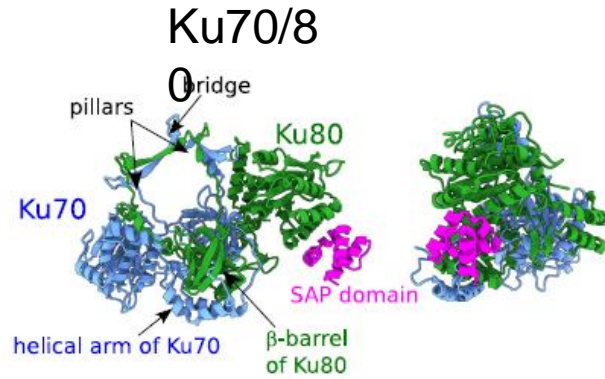
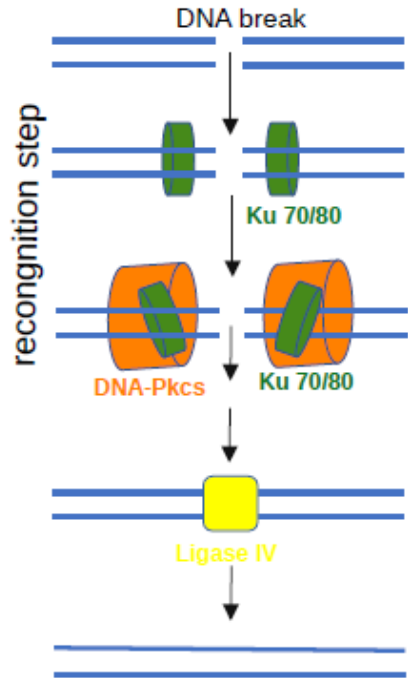
Overview

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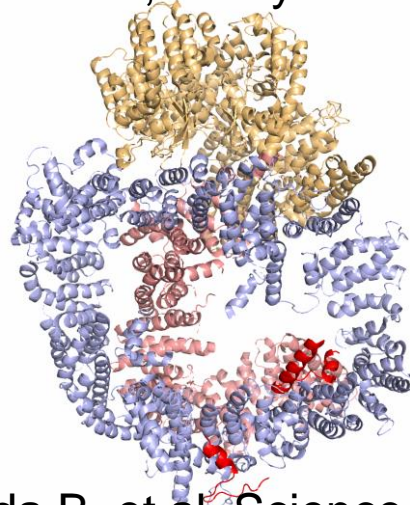
From X-ray crystallography to cryo-EM

DNA-dependent protein kinase: catalytic subunit + Ku7080

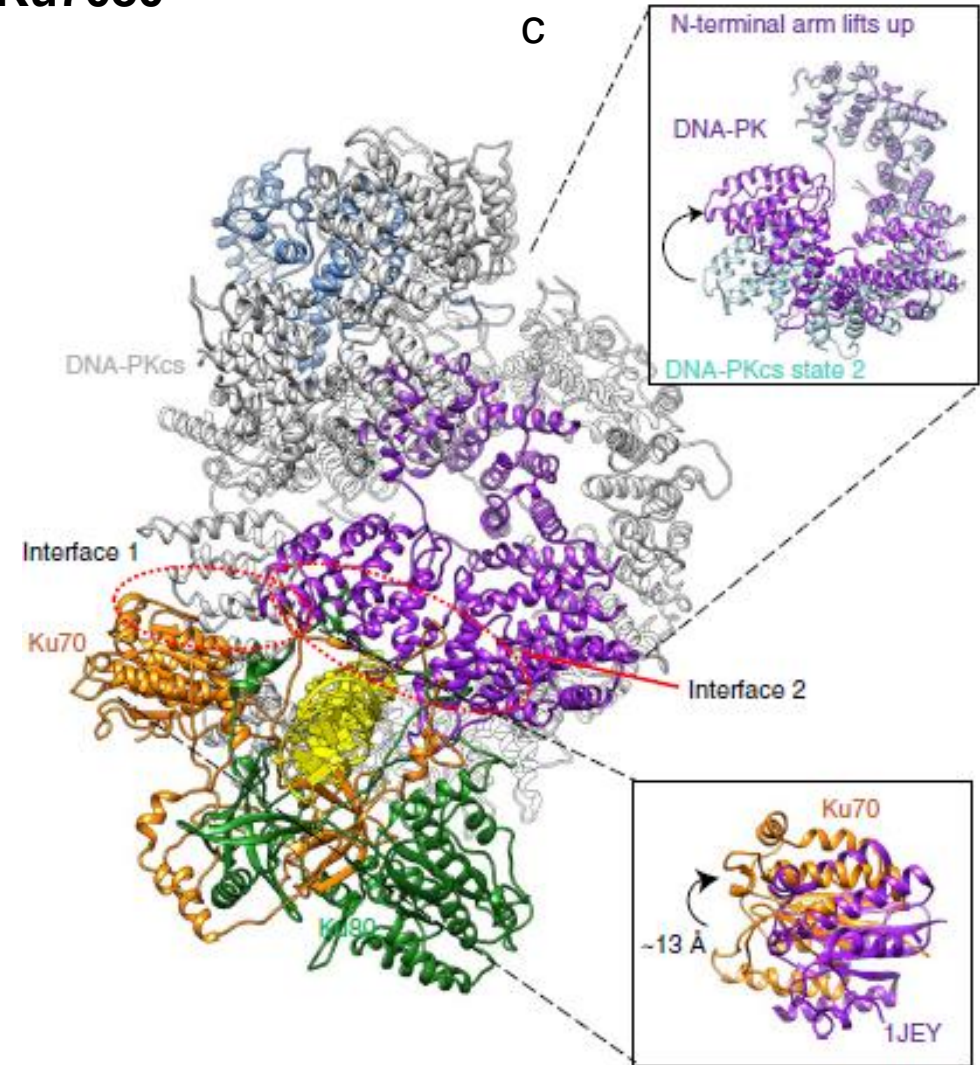
Non-homologous end joining



DNA-PK, catalytic subunit



Sibanda B. et al. Science

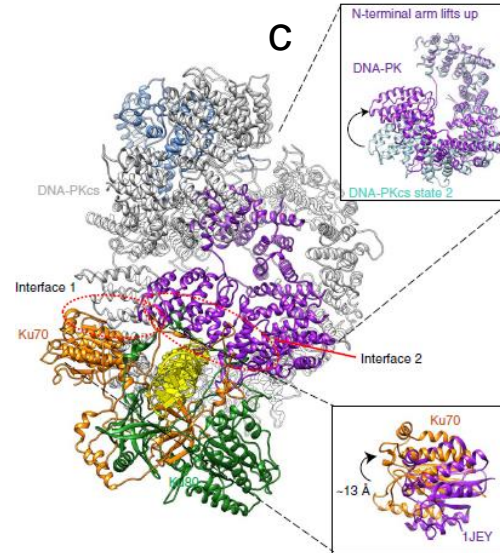


Chaplin A. et al. NSMB, 2020

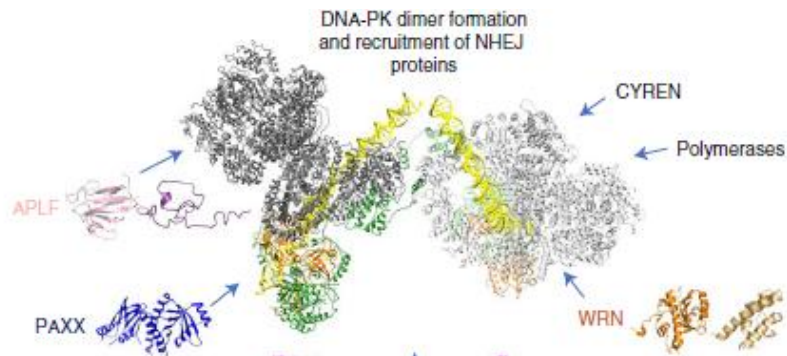
From X-ray crystallography to cryo-EM

Large multicomponent assemblies

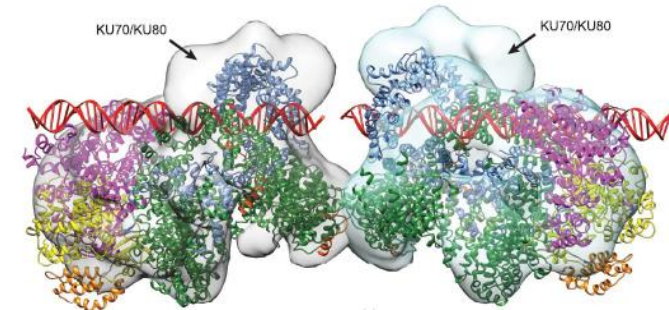
Major
class



Minor
class



Cryo-EM based model

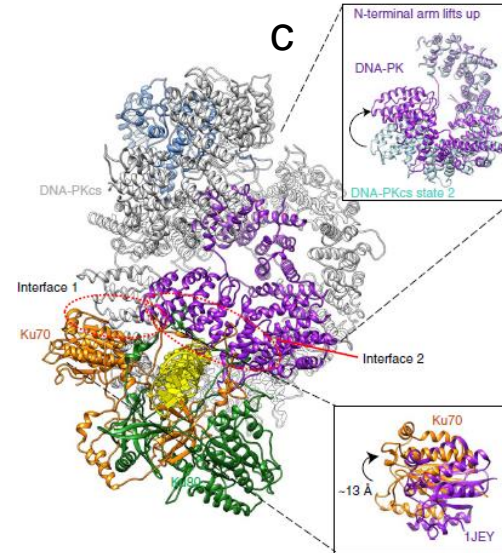


X-ray crystallography based model

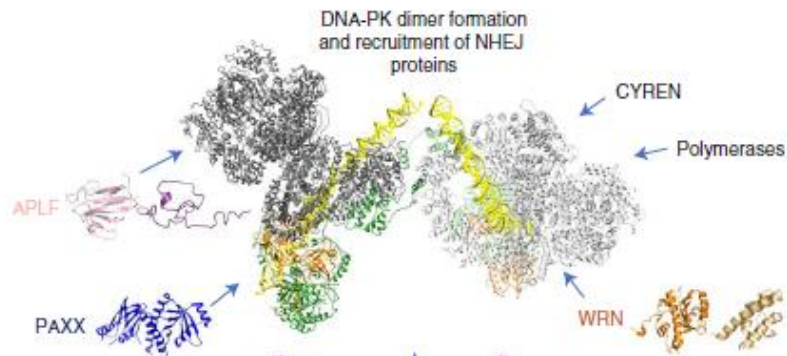
From X-ray crystallography to cryo-EM

Large multicomponent assemblies

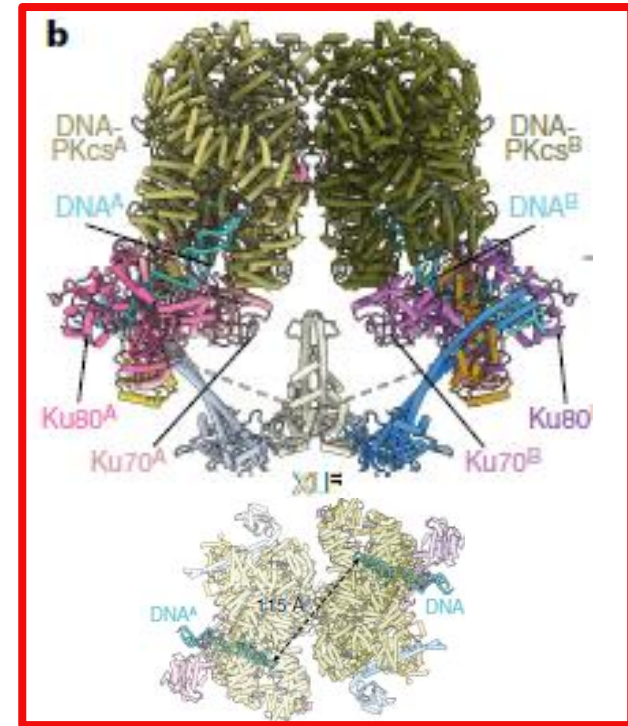
Major class



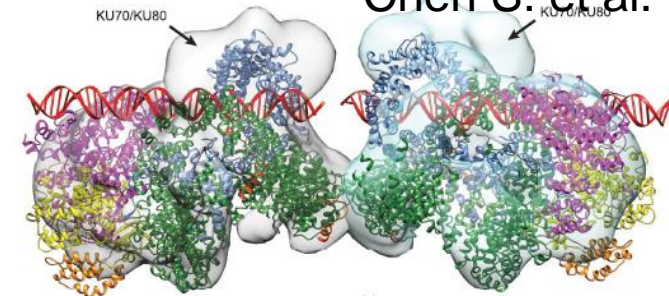
Minor class



Cryo-EM based model



Chen S. et al. Nature 2021



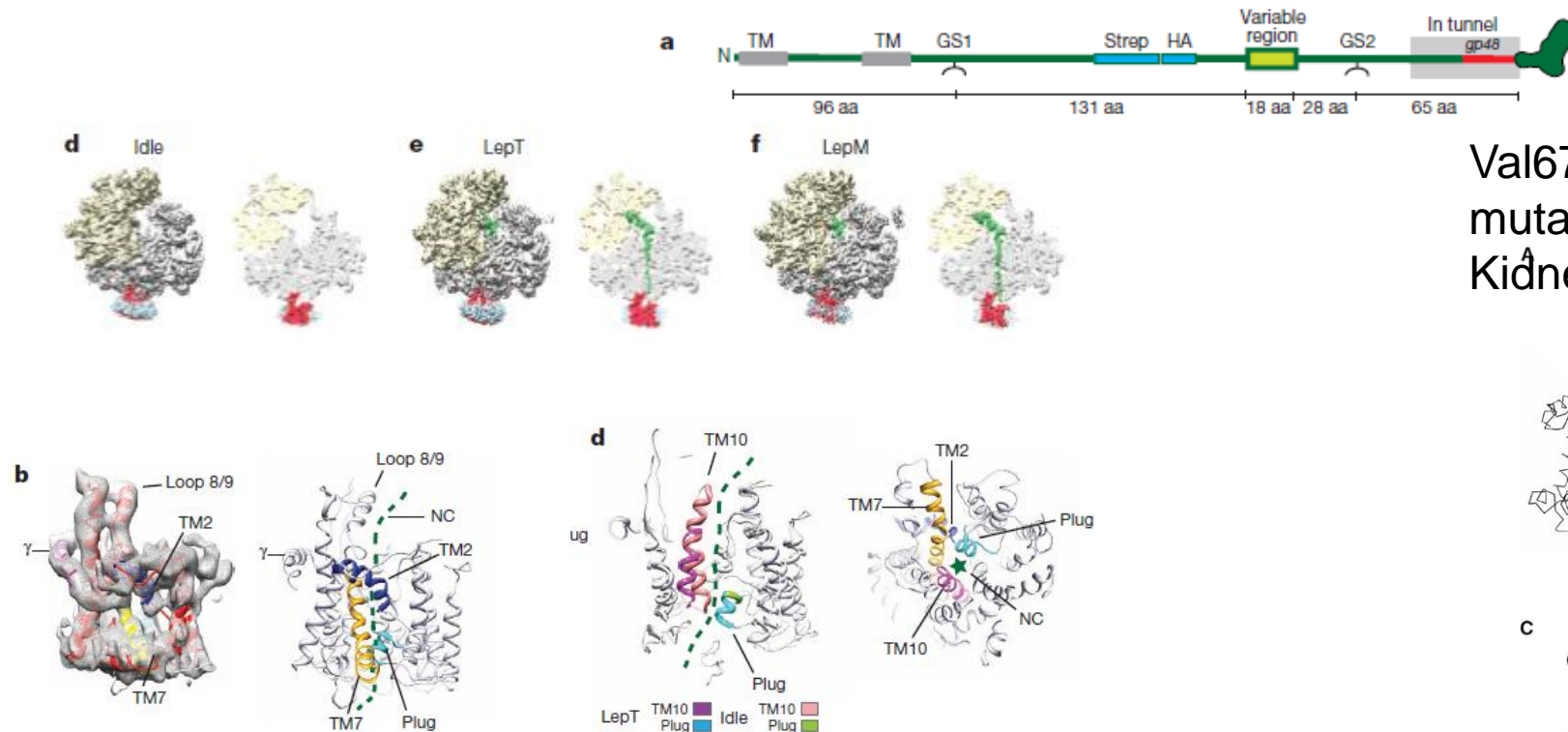
X-ray crystallography based model

From X-ray crystallography to cryo-EM

Cryo-EM can analyze biomolecules directly from biological samples

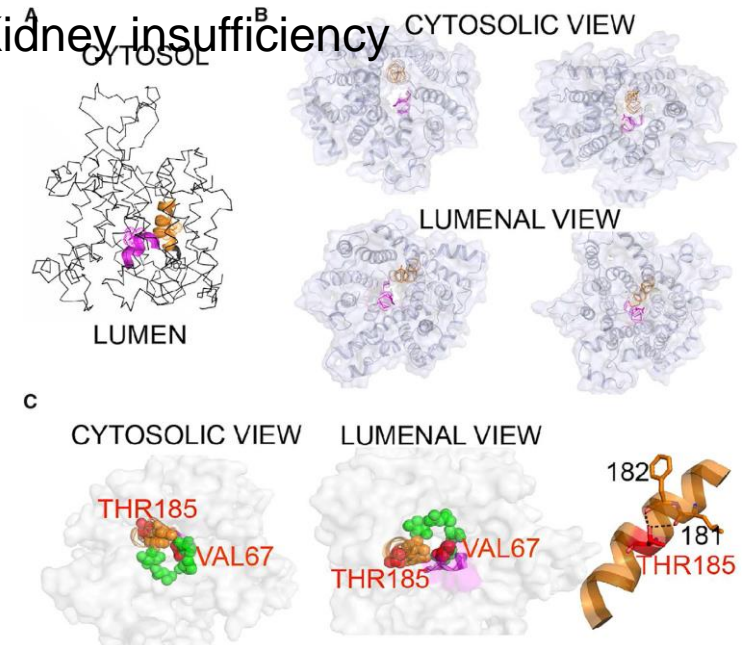
Translocation channel Sec61 bound to ribosome:

structural study of pull-downed complex directly from wheat-germ extract



Val67Gly and Thr185Ala mutations:

Kidney insufficiency



Gogala M. et al. Nature 2014

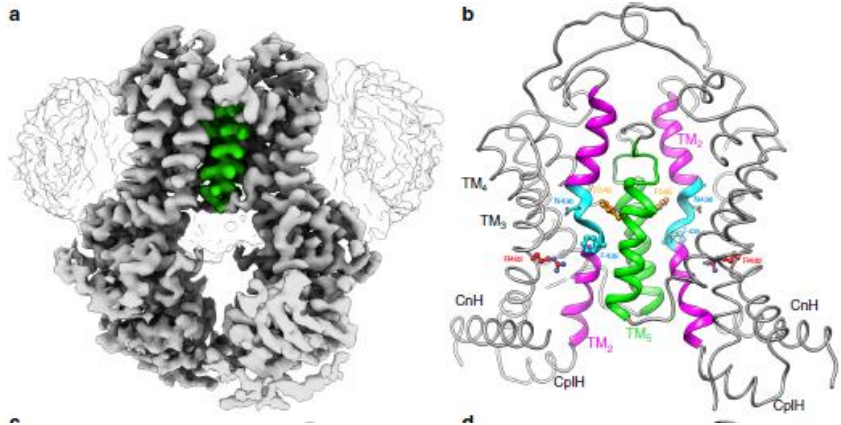
Bolar N. et al. AJHG
2016

From X-ray crystallography to cryo-EM

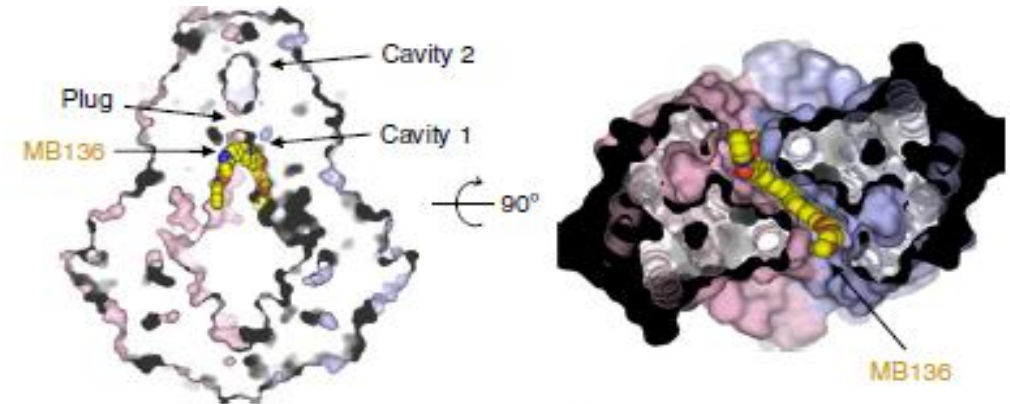
Progress in structural biology of **membrane proteins** and associated drug discovery

ABC transporter ABCG2 – export of chemotherapeutics

Apo form: closed state

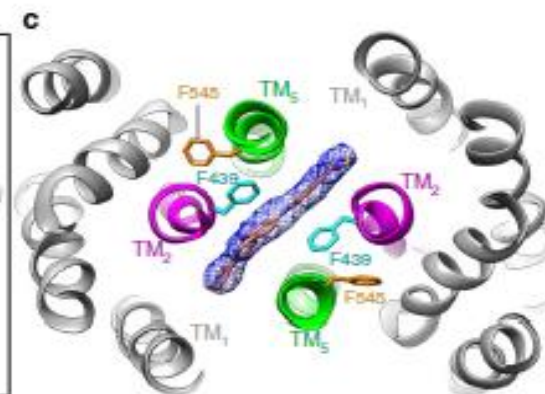
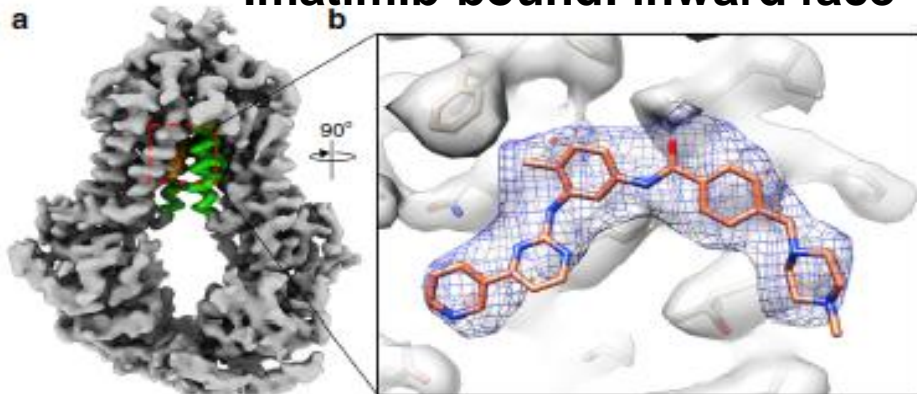


Inhibitor-bound: locked in inward face

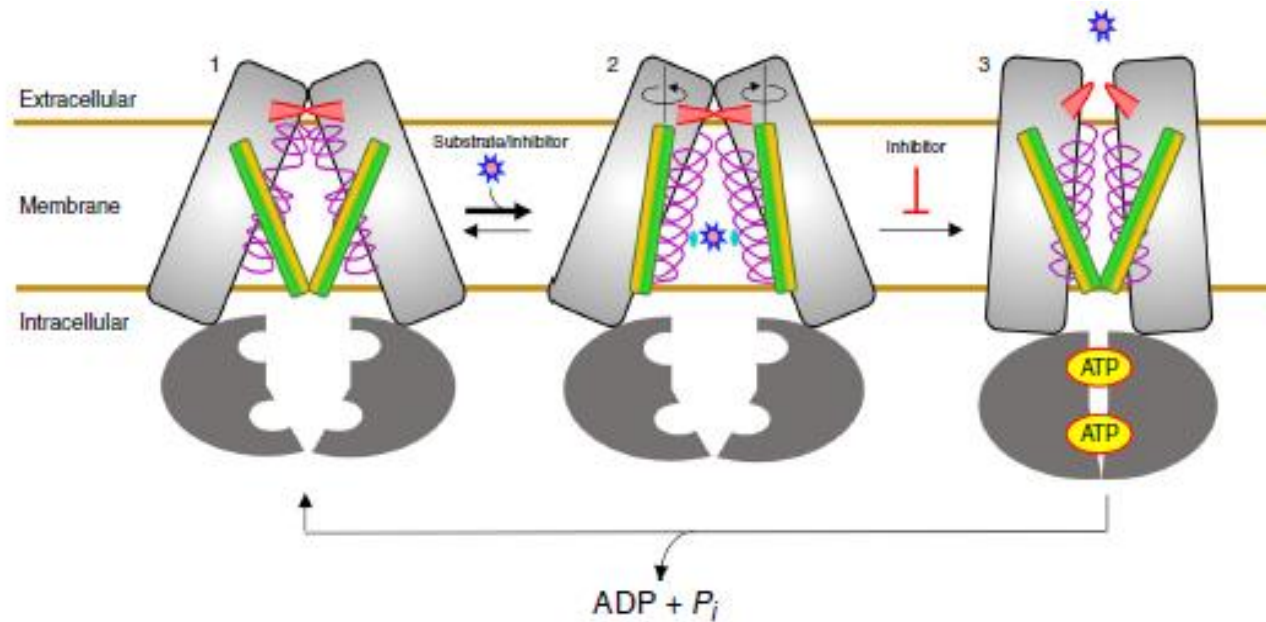


Jackson S. et al. NSMB 2018

Imatinib-bound: inward face

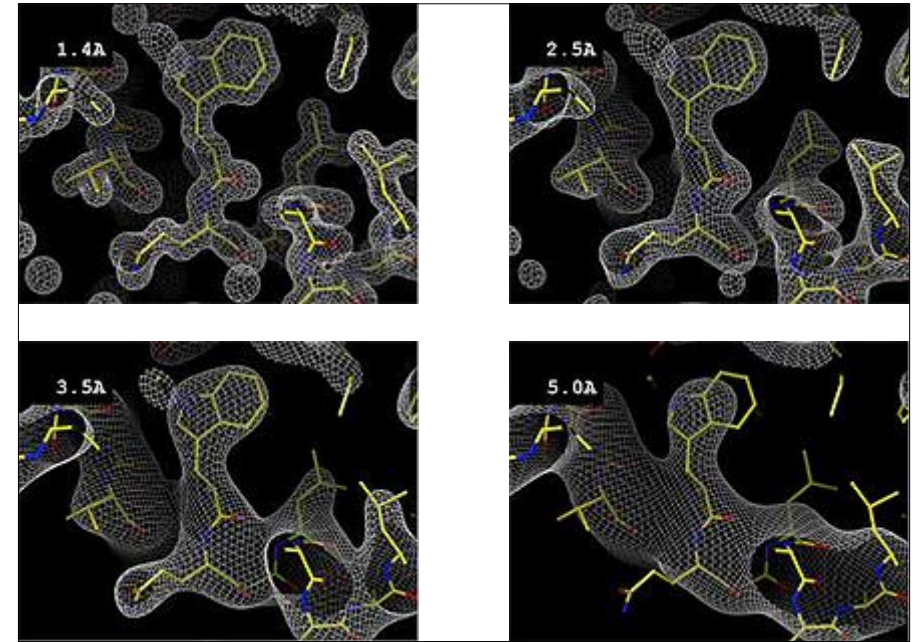
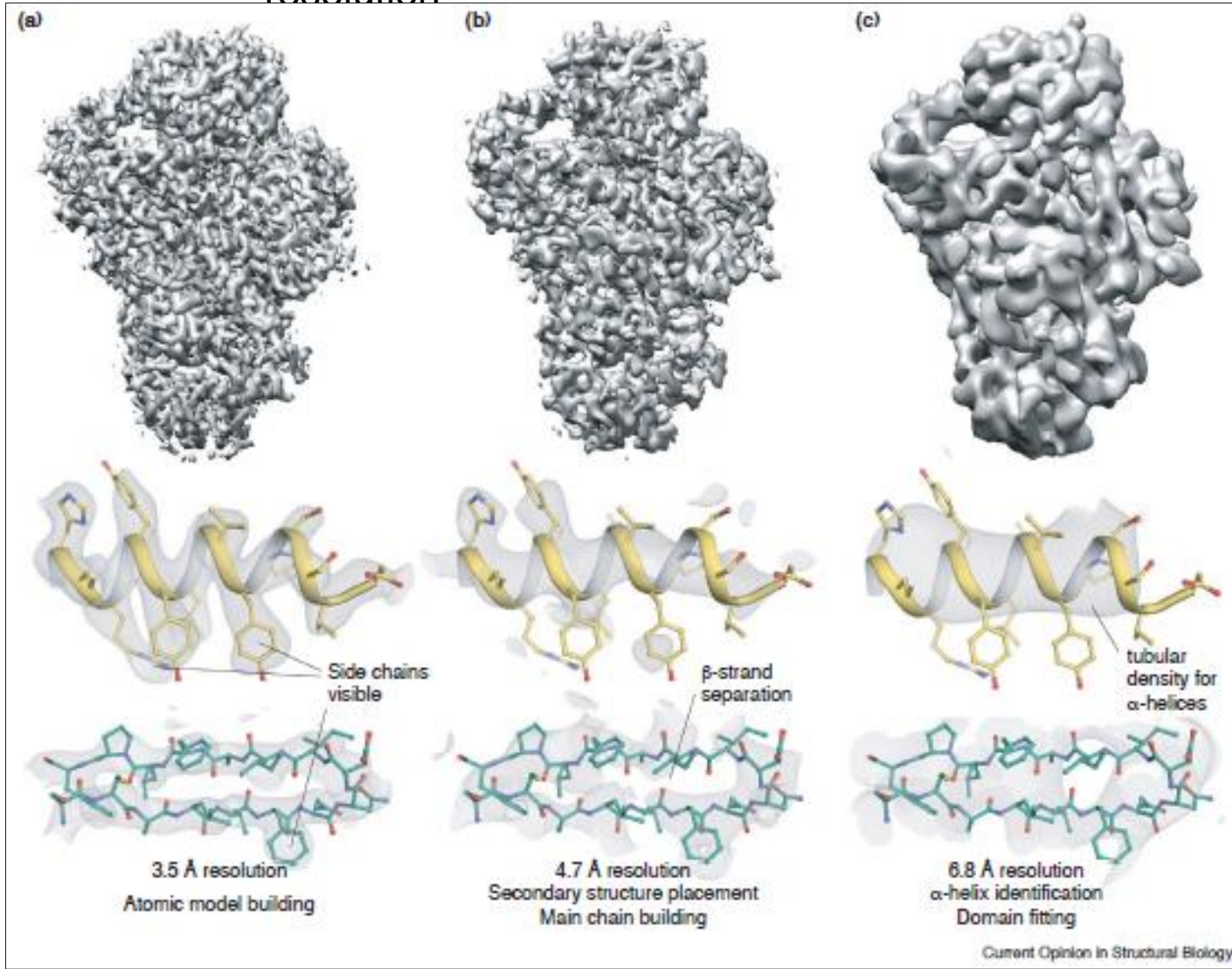
Orlando and Liao, Nat.Comm
2020

Cryo-EM based structures of ABCG2: mechanism for export of chemotherapeutics



From X-ray crystallography to cryo-EM

Cryo-EM often provides snapshots of large complexes with lower resolution



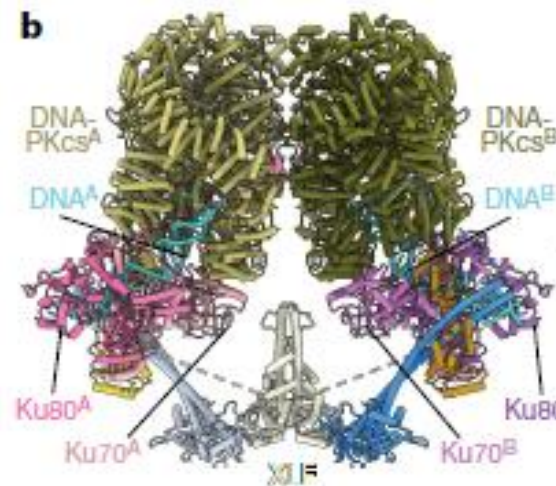
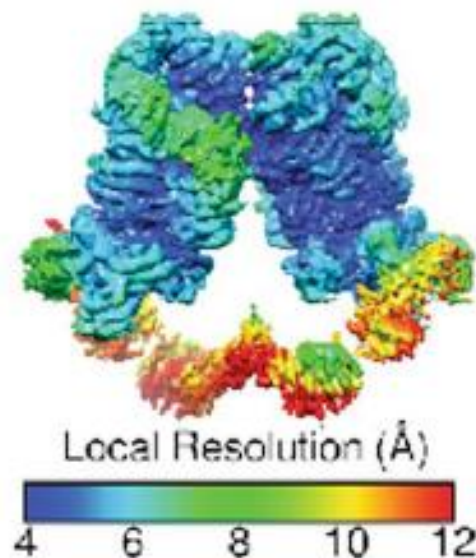
<https://proteopedia.org/>

Polyadenylation factor

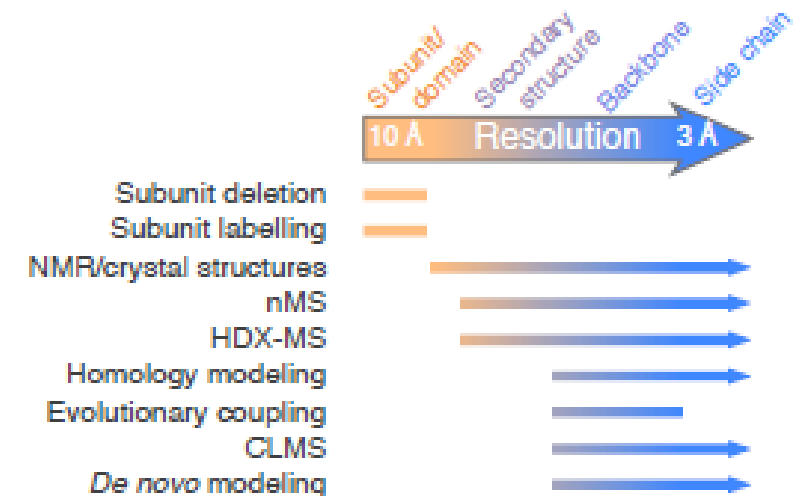
Casanal A. et al. Cur. Opin. Str.Biol. 2019

Cryo-EM based maps boost integrative structural biology

- Supramolecular complexes with lower resolution (lower than 4 Å)
- Flexible parts with lower local resolution or blurred map

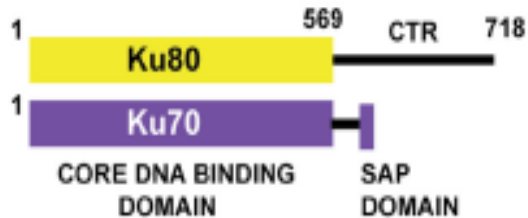
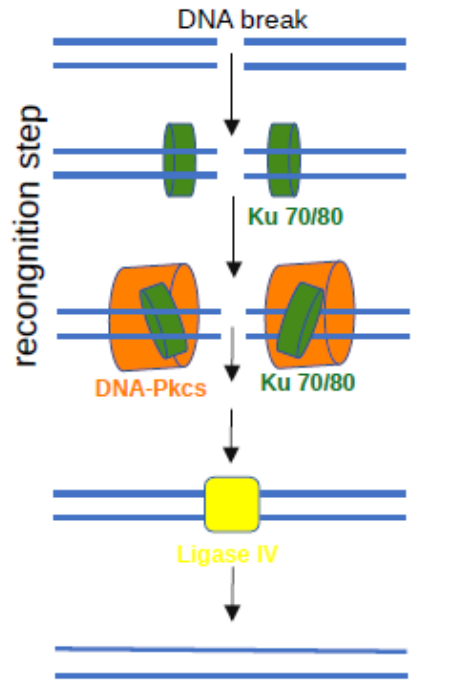


Need for validation using complementary technique



Cryo-EM can capture subpopulation of conformers missed in crystals

Non-homologous end joining



Ku70

Ku80

SAP domain

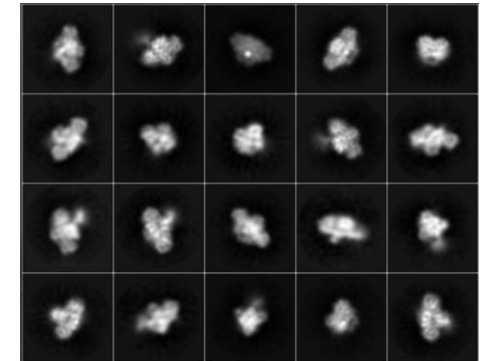
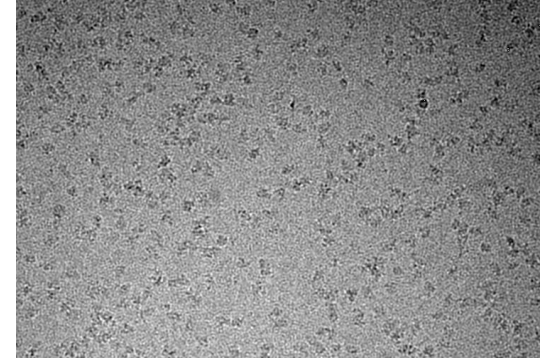
PDB ID 1JEQ

Ku70

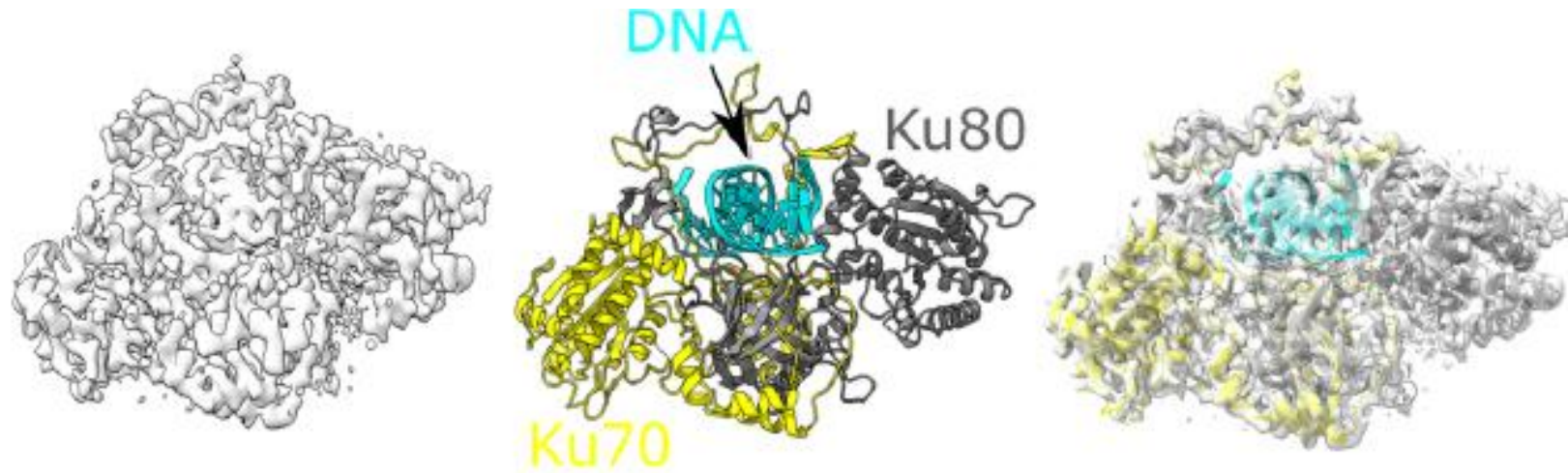
DNA

Ku80

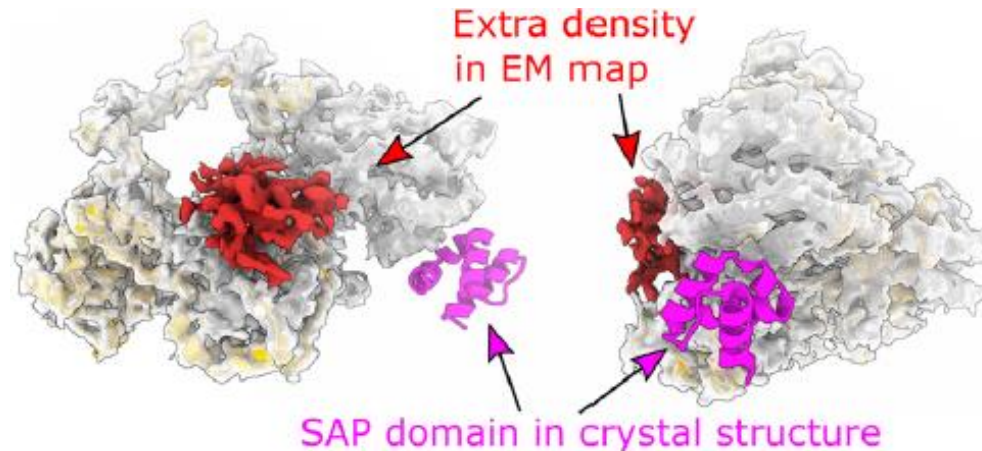
PDB ID 1JEY



Ku70/80 apo form: density for SAP domain found at DNA aperture

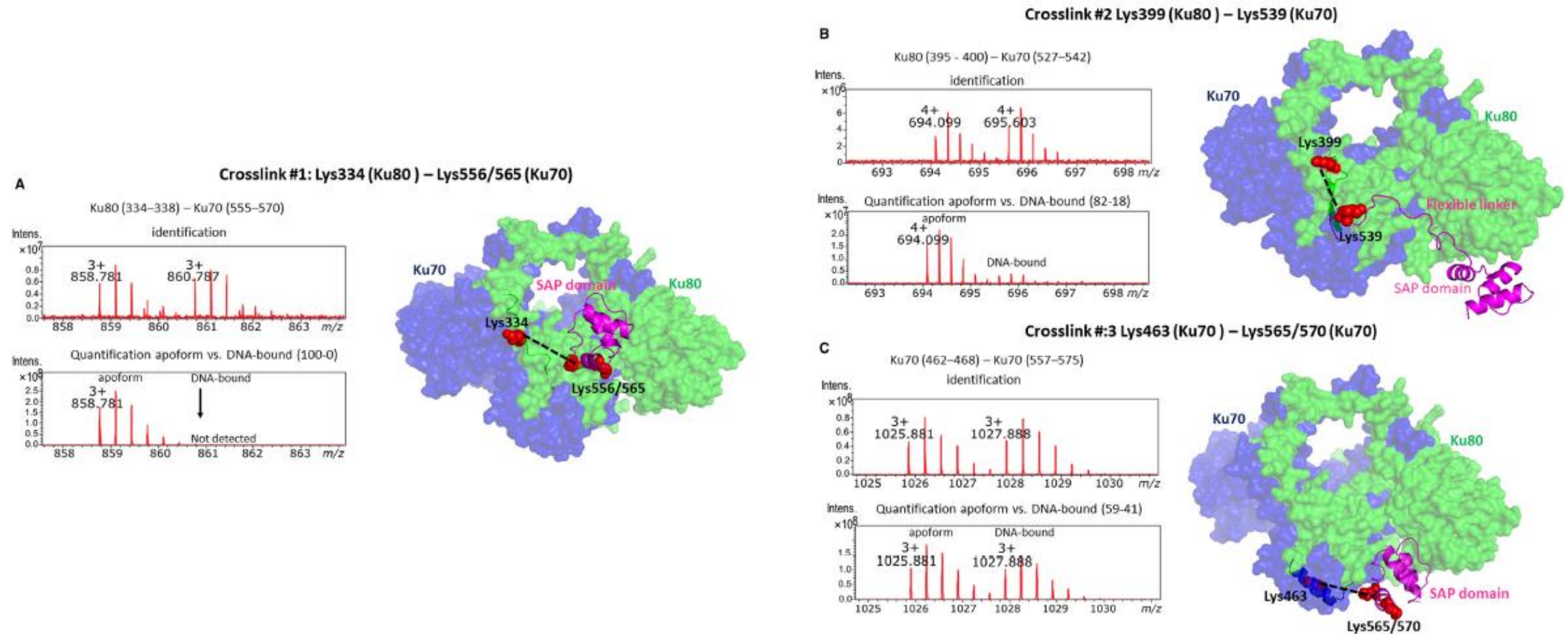


DNA- bound state:
No extra density

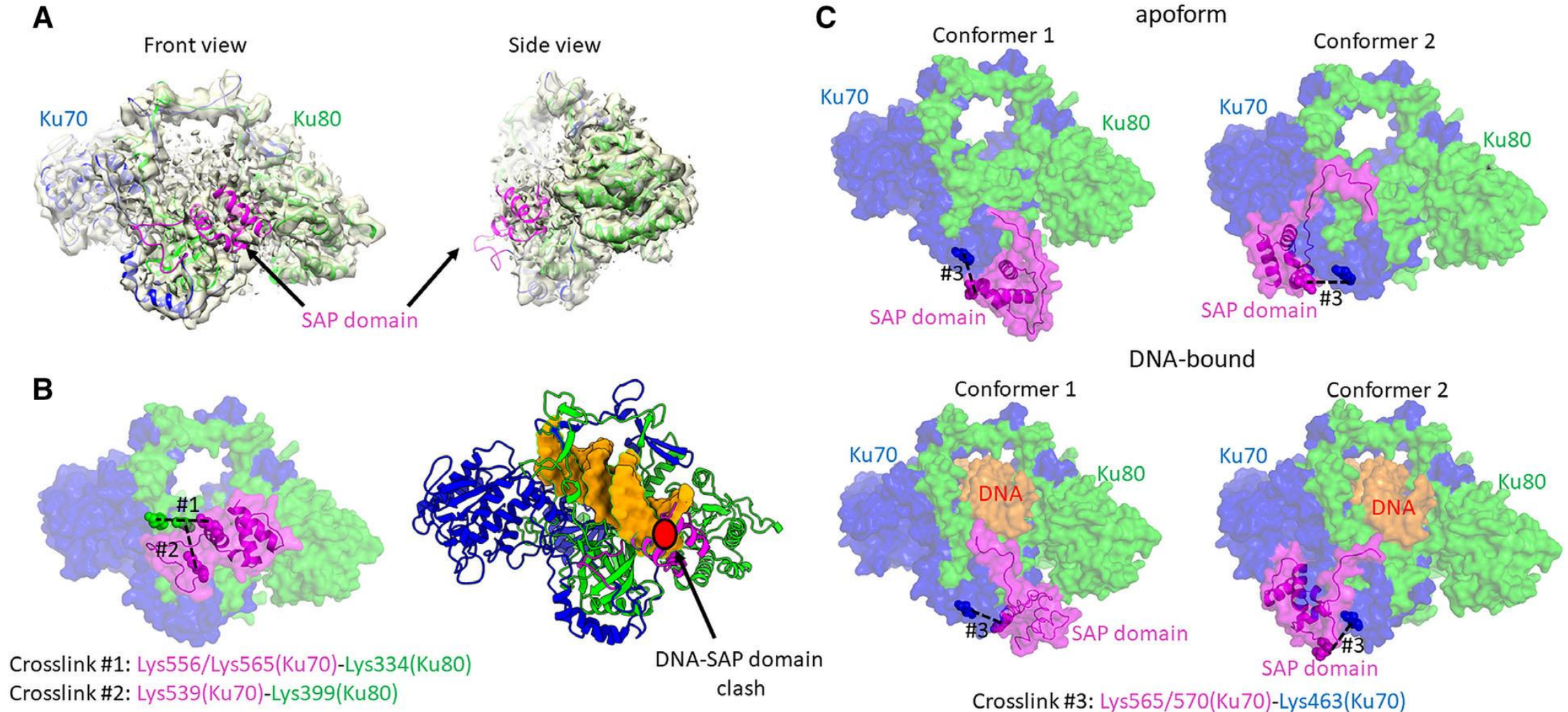


Apo state:
Extra density at DNA aperture

Protein cross-linking followed by mass spectrometry: Multiple positions of the SAP domain including DNA aperture



Molecular docking guided by cryo-EM and MS-based crosslinking: flexible movements of the SAP domain depending on DNA



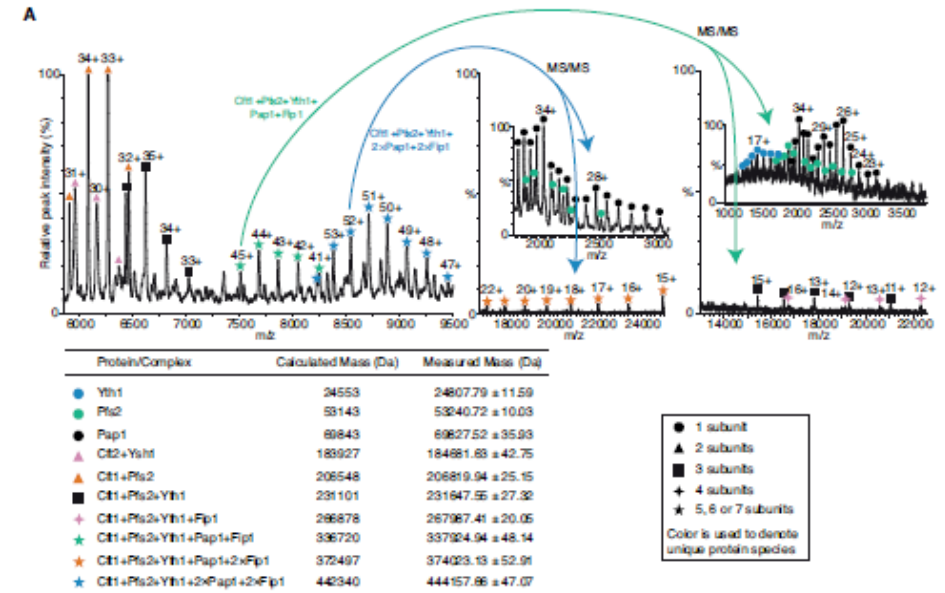
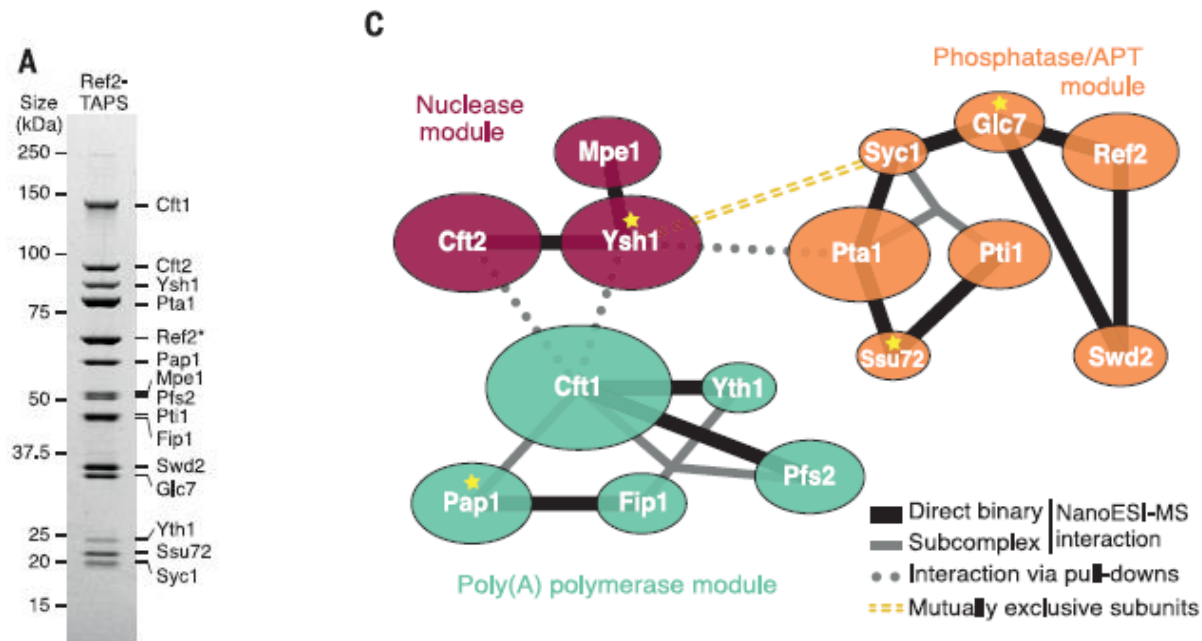
Case story of integrative structural biology for large complex

STRUCTURAL BIOLOGY

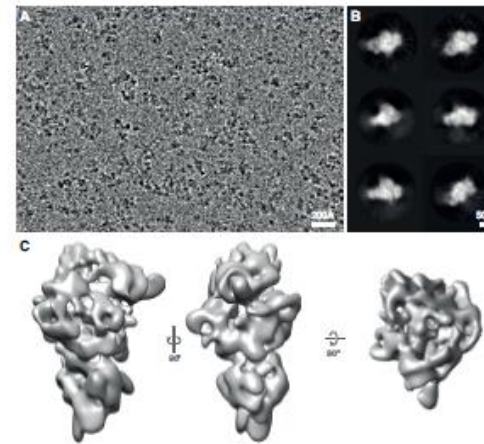
Architecture of eukaryotic mRNA 3'-end processing machinery

Ana Casañal,^{1*} Ananthanarayanan Kumar,^{1*} Chris H. Hill,¹ Ashley D. Easter,¹ Paul Emsley,¹ Gianluca Degliesposti,¹ Yuliya Gordiyenko,^{1,2} Balaji Santhanam,¹ Jana Wolf,¹ Katrin Wiederhold,¹ Gillian L. Dornan,¹ Mark Skehel,¹ Carol V. Robinson,² Lori A. Passmore^{1†}

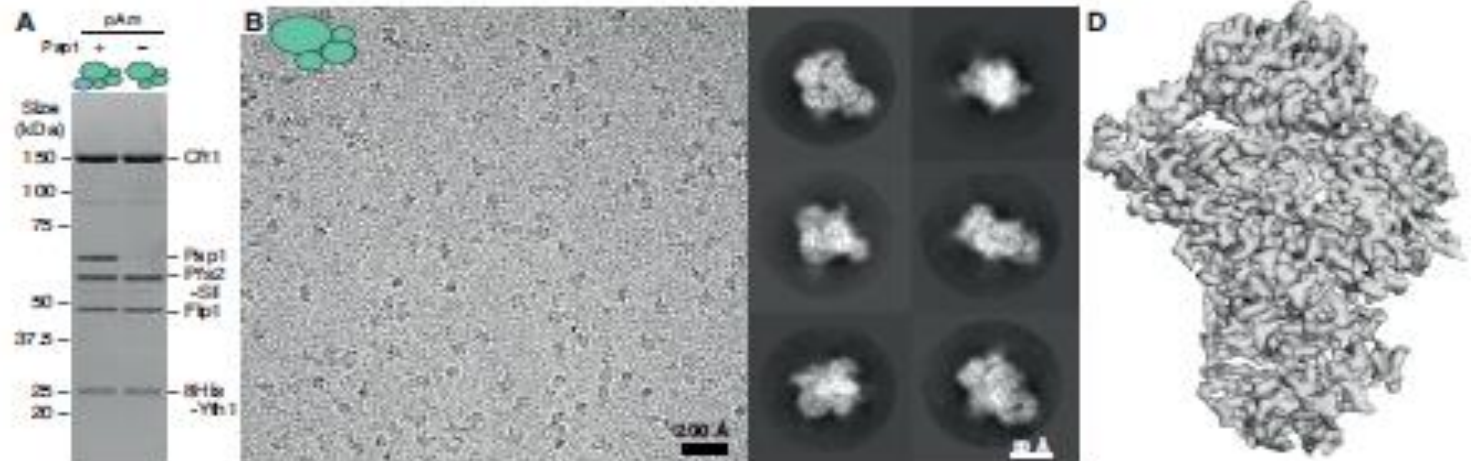
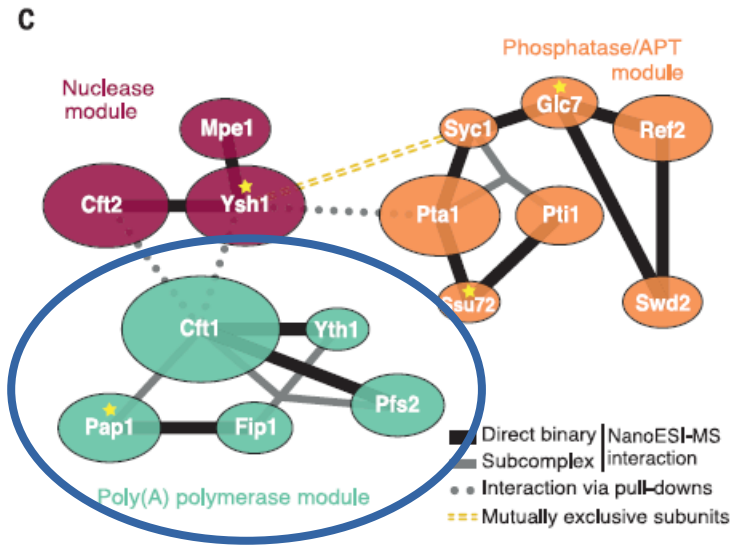
Native MS and pull-down for mapping interactions



CryoEM structure of polymerase module at 3.5 Å

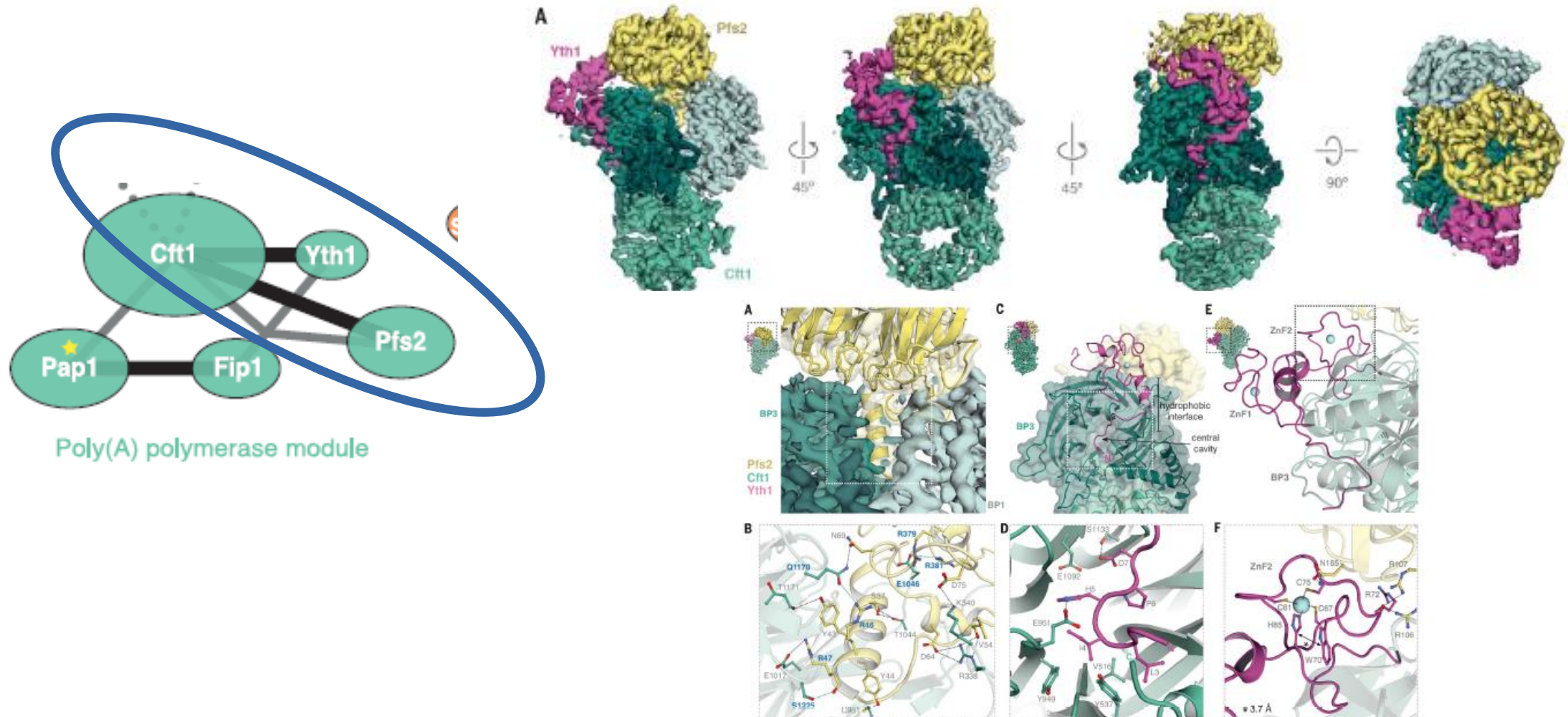


Entire complex at 12 Å
Pull down from yeasts

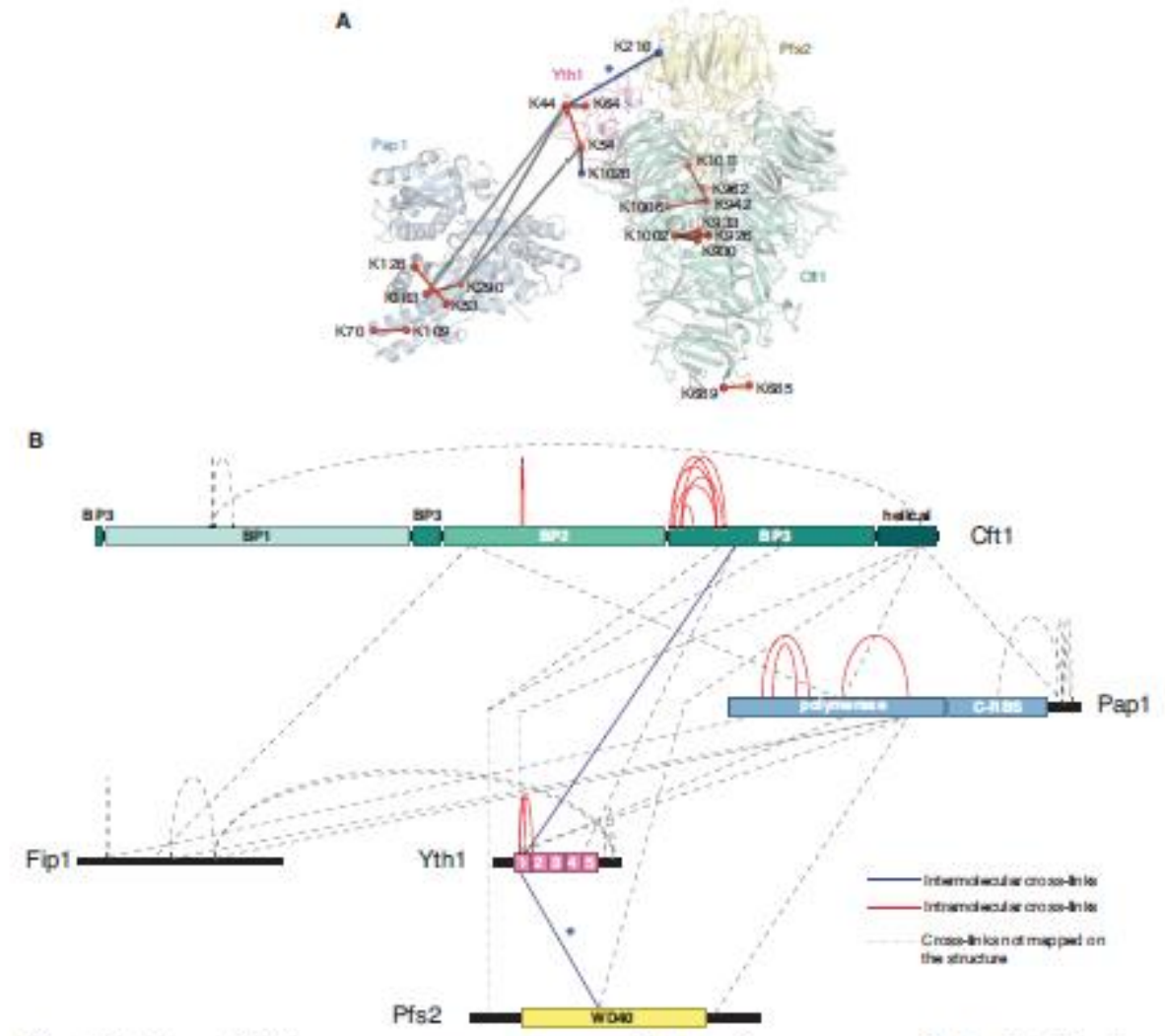
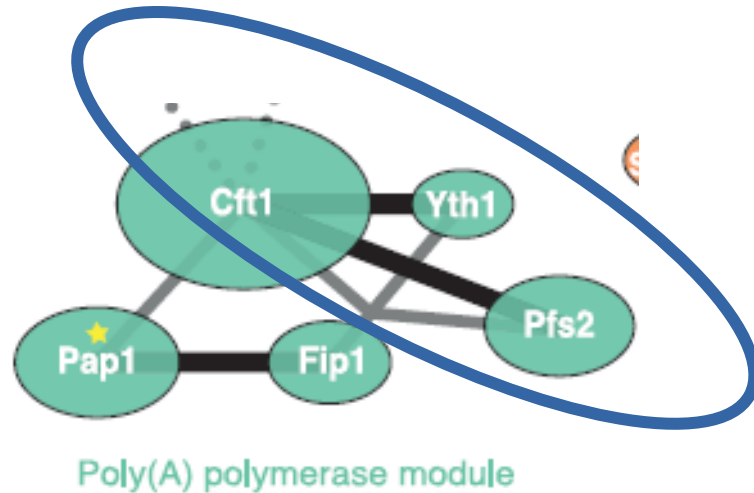


Overexpression in insect cells

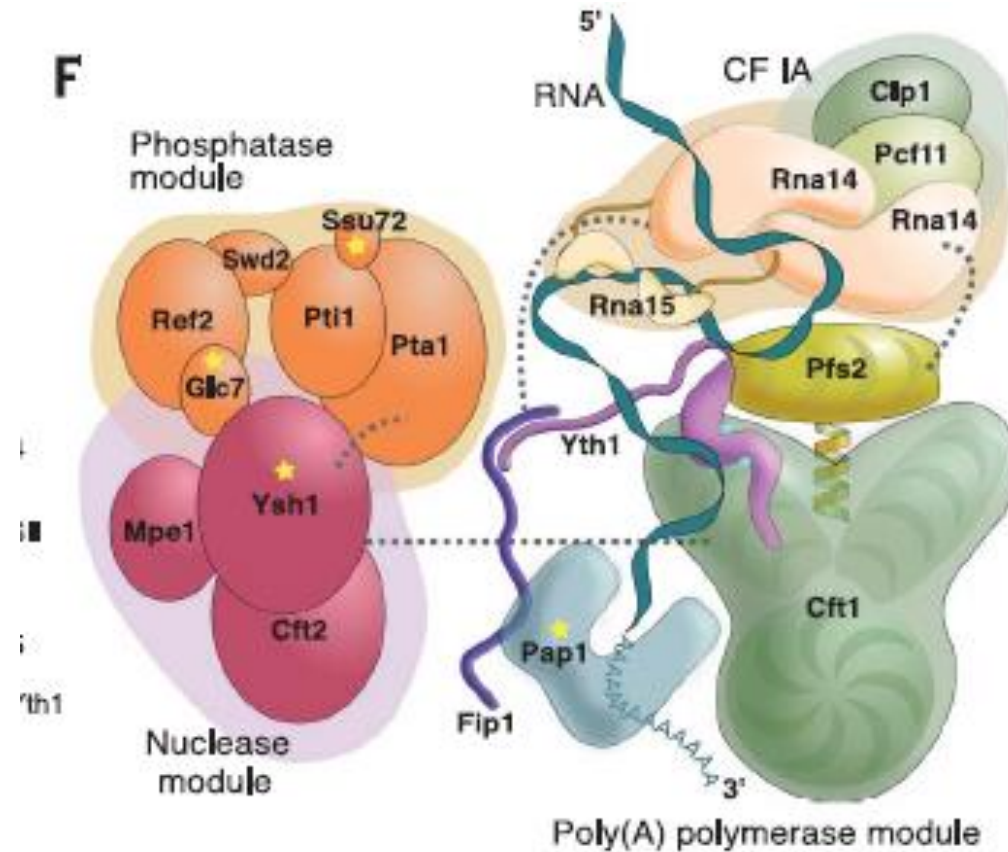
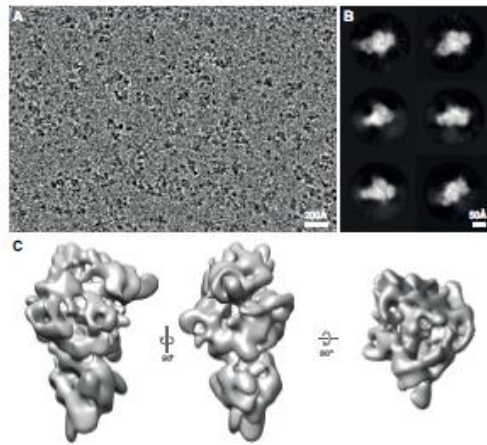
Cryo-EM defined the structured core of polymerase module



Mass spectrometry-analysed crosslinking supported and extended the model for polymerase module



First module solved, others will come ?



Cryo-EM – take home message

- relatively low consumption of sample (few μ l of protein)
- first-choice technique for multicomponent systems
- Slow progress (at least at the beginning)
- Demanding for computational resources

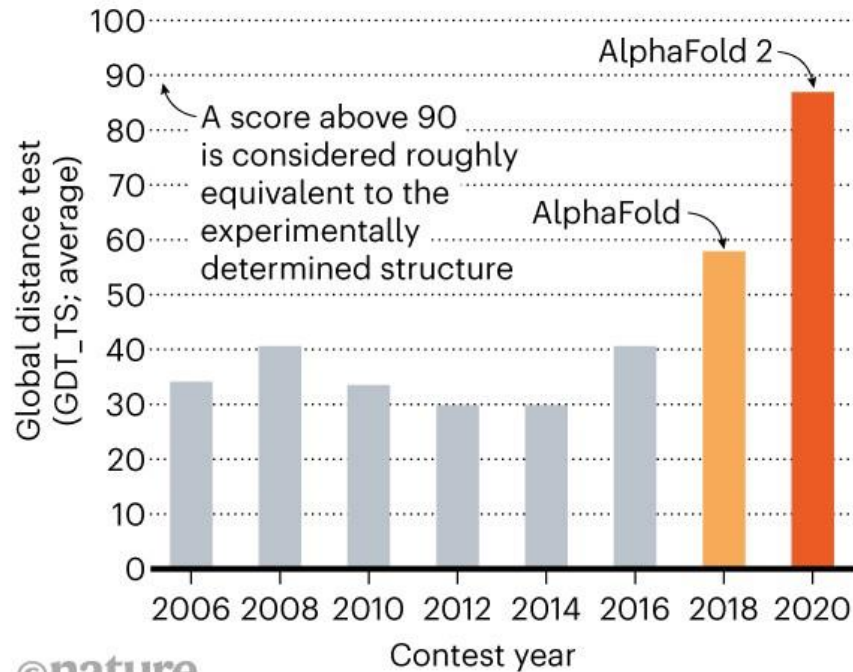
<https://em-learning.com/>

<https://www.ccpem.ac.uk/>

Future perspective: deep learning

STRUCTURE SOLVER

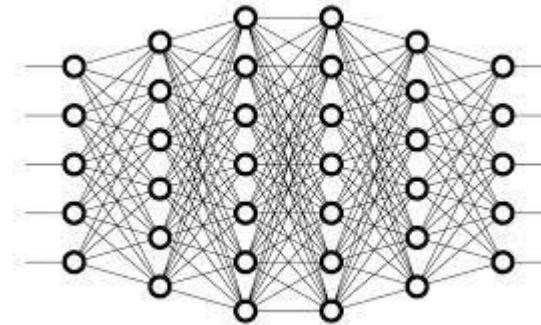
DeepMind's AlphaFold 2 algorithm significantly outperformed other teams at the CASP14 protein-folding contest — and its previous version's performance at the last CASP.



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AlphaFold2 – prediction software by DeepMind (Google)

Information released in November 2020



[https://www.nature.com/articles/d41586-020-03348-](https://www.nature.com/articles/d41586-020-03348-4)