Structural Biology and Drug Discovery

Colby Sandate

EPFL

08-05-2025

What is structural biology?

- Scientific field that produces information on the 3D structure of biological macromolecules (proteins, nucleic acids and their assemblies) using a variety of techniques such as:

  - X-ray crystallography
  - Nuclear magnetic resonance spectroscopy (NMR)
  - Cryo-electron microscopy (cryo-EM)
  - Small angle X-ray scattering (SAXS)
  - And others

The role of structural biology in biopharma

- 3D characterization of macromolecules provides critical insights into their function, dynamics, and interactions with potentially therapeutic compounds.
  - Provides targets for therapeutics development.

- Characterization of protein-ligand interactions (and often drug mechanism of action).
  - Resolving details of binding sites can lead to the development of compounds with improved selectivity and potency, minimizing off-target effects and improving efficacy.

The role of structural biology in biopharma

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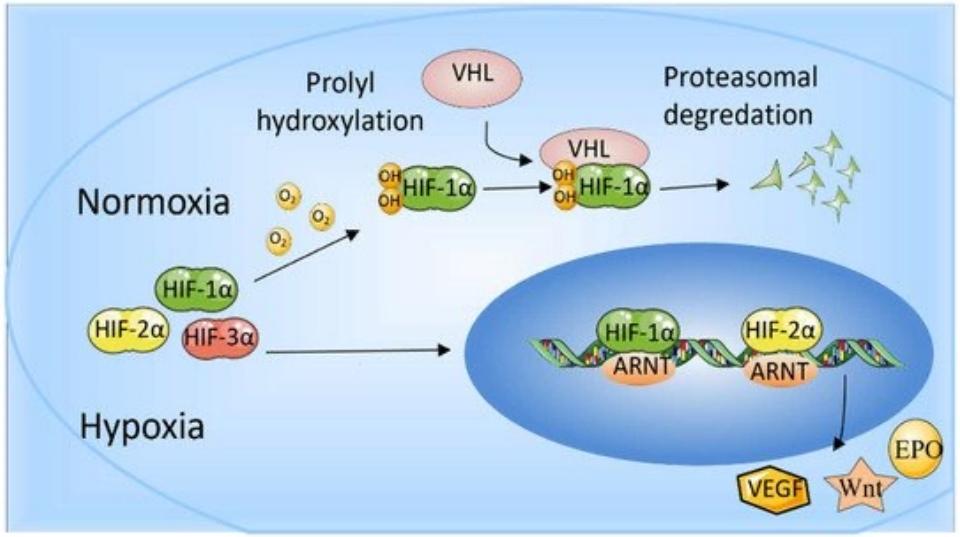
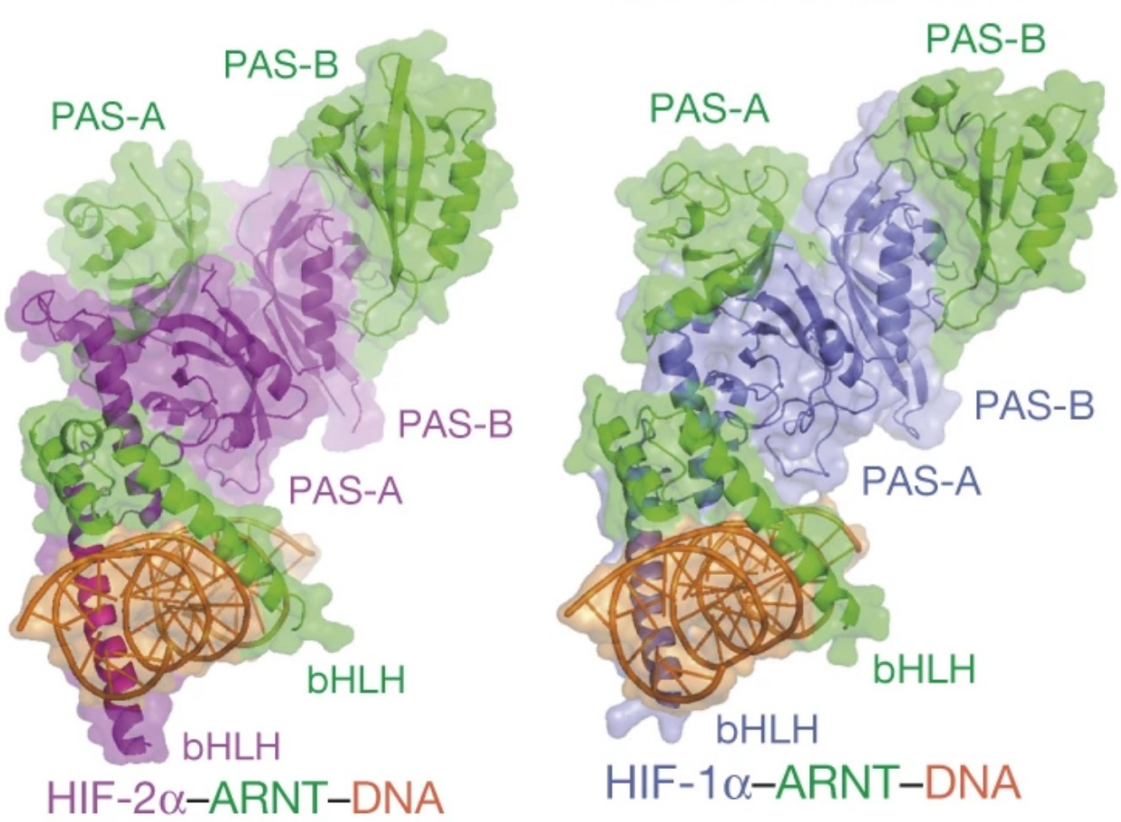
  - Resolving details of binding sites can lead to the development of compounds with improved selectivity and potency, minimizing off-target effects and improving efficacy.

Structural data provides critical information for biological processes – and opportunities for therapeutics

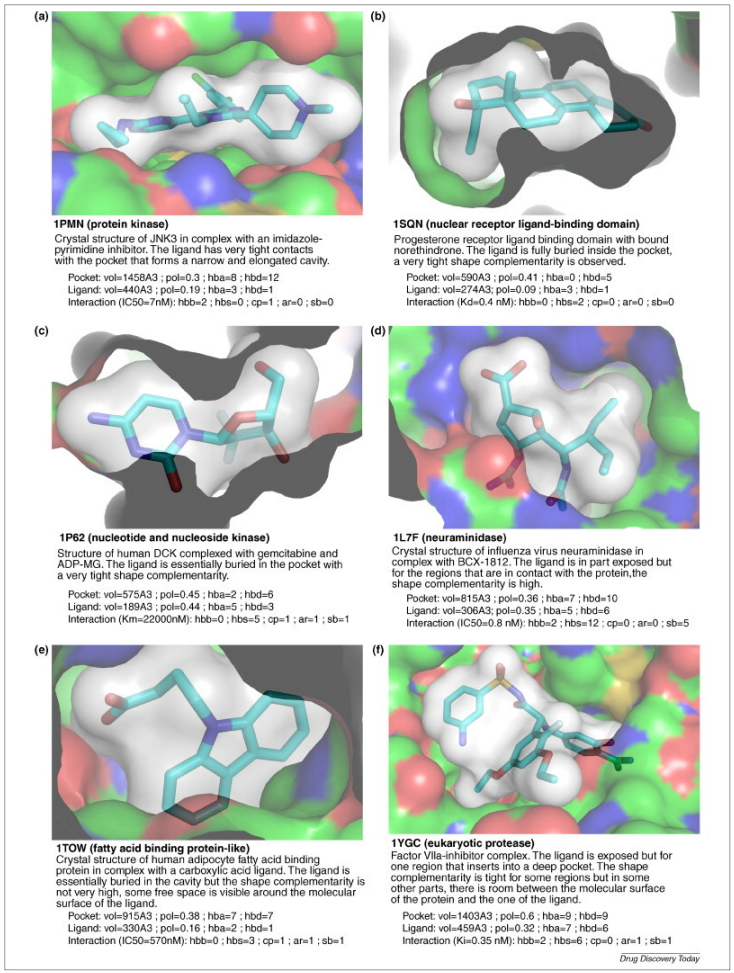


Qin Q, *et al.* (2022) *Int. J. Mol. Sci.*



We D, *et al.* (2015) *Nature*

Ligand binding pockets in proteins



Pérot S, *et al.* (2010) *Drug Discovery Today*

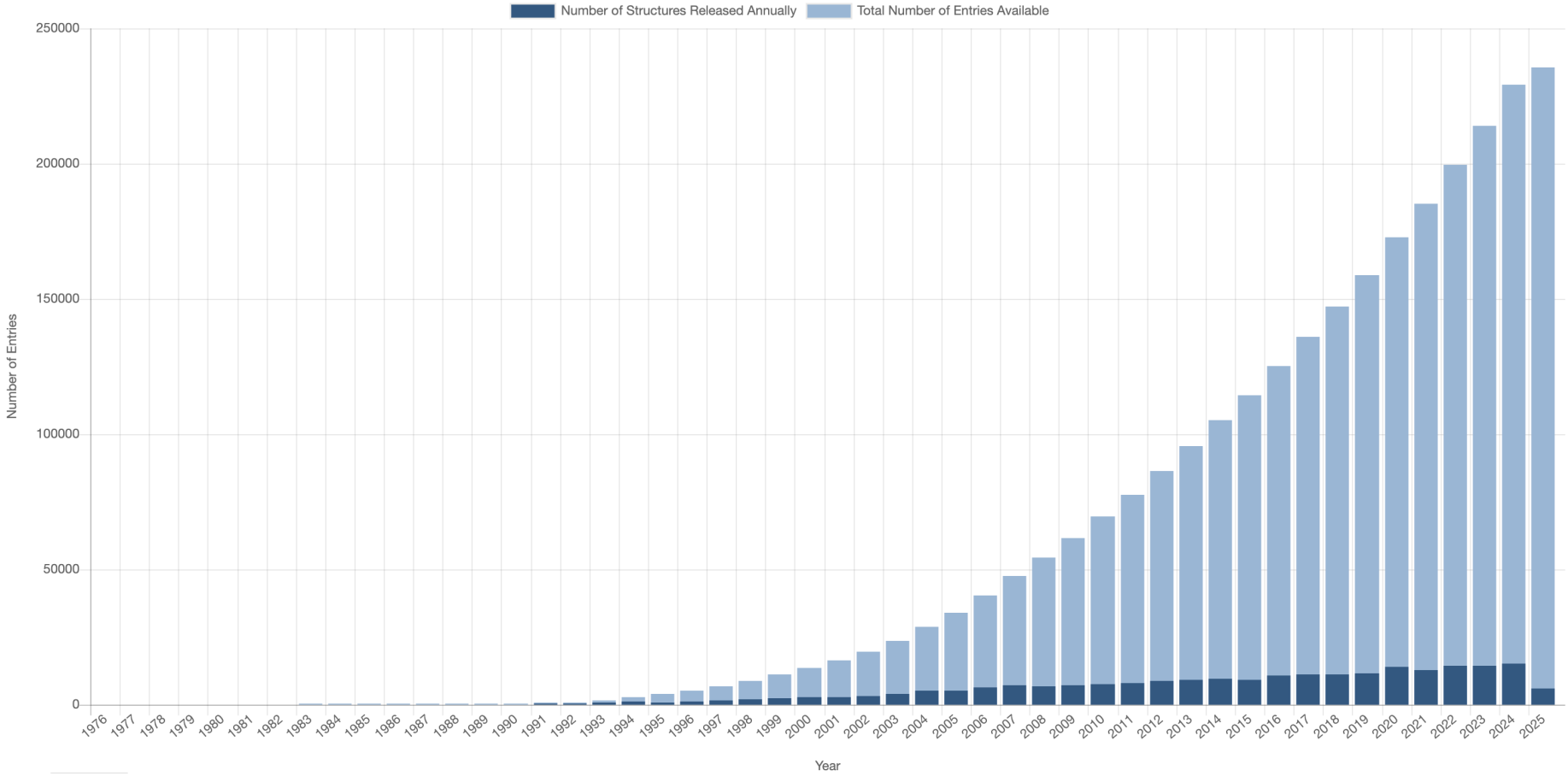
-Majority of drugs bind to cavities within proteins. Besides differing in shape & size, these cavities are generated by chiral residues which bring differing properties to the cavity's surface (charge, hydrophobicity, H-bond donors and acceptors).

-Identification of ligand binding pockets in proteins allows for structure-based drug design (SBDD).

-No consensus exists for definition or gold standard for binding pocket.

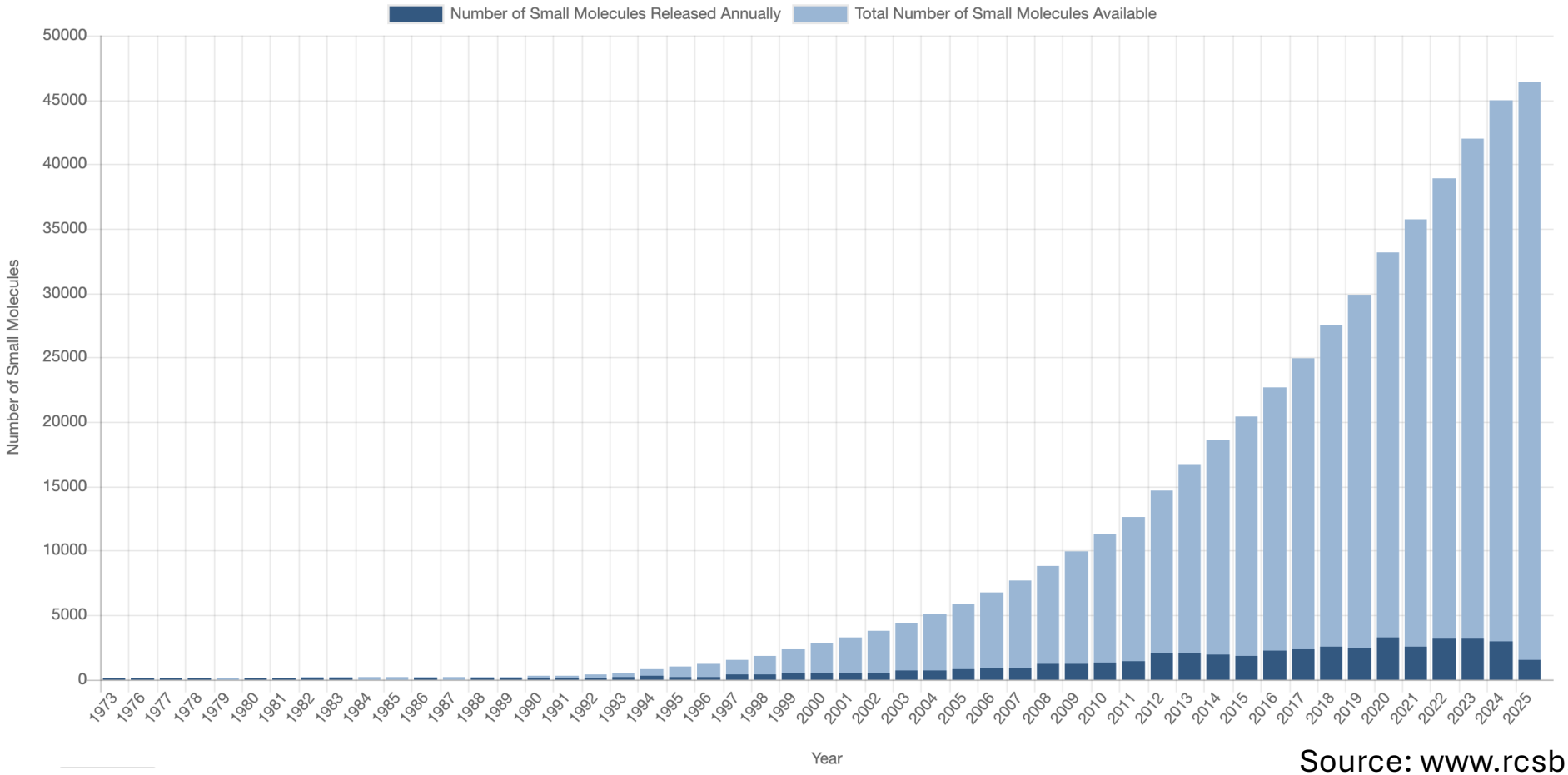
-Average volume of drug-binding cavity is ~930$Å^3$.

A golden age for structural biology



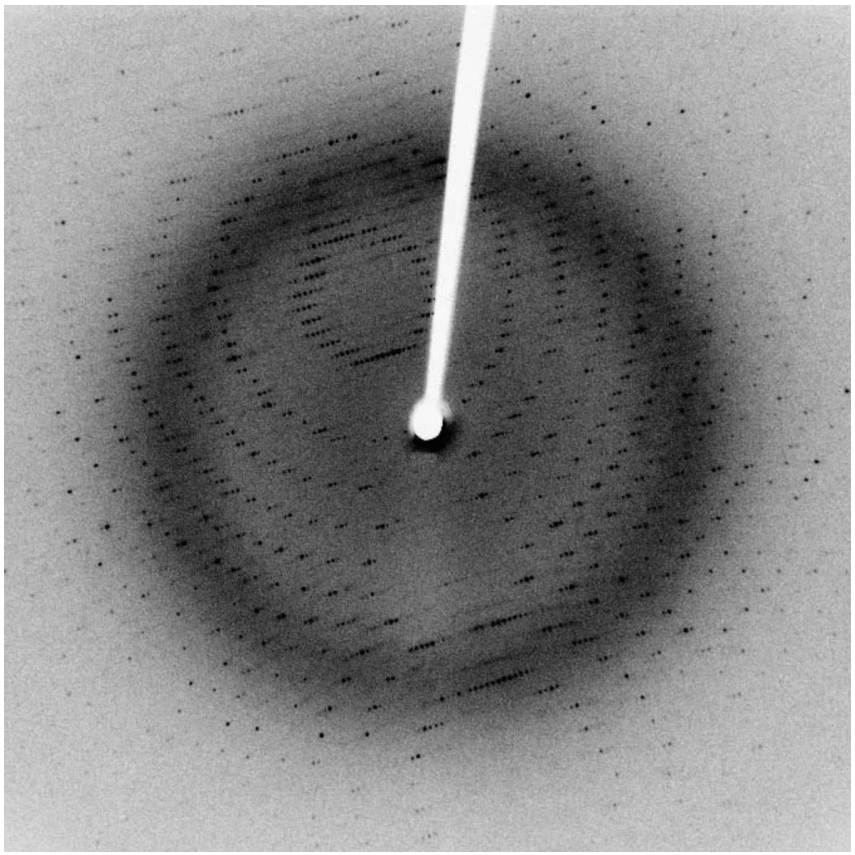
Source: www.rcsb.org

Growth of small molecules coordinates in the PDB



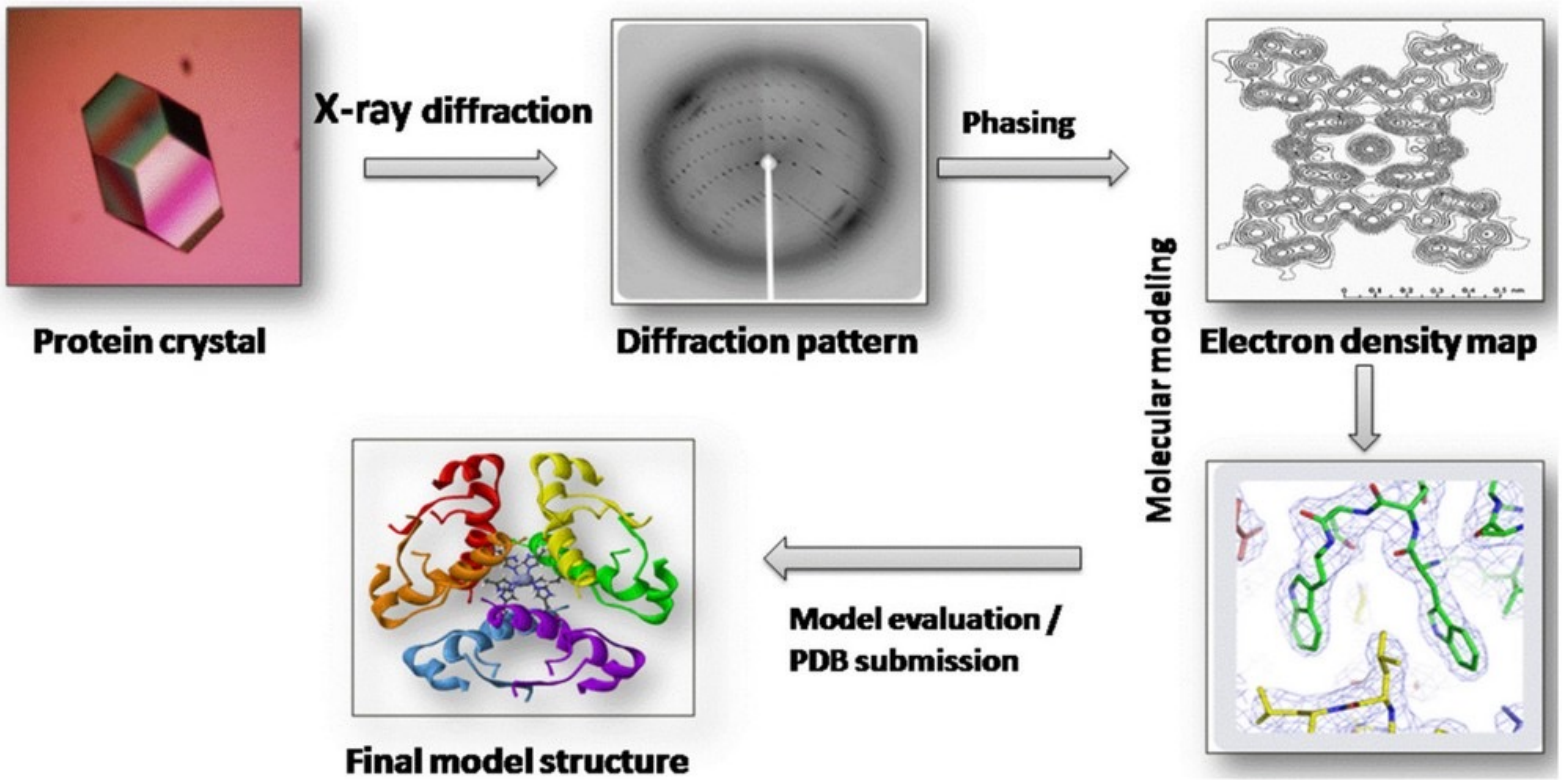
Source: www.rcsb.org

X-ray Crystallography



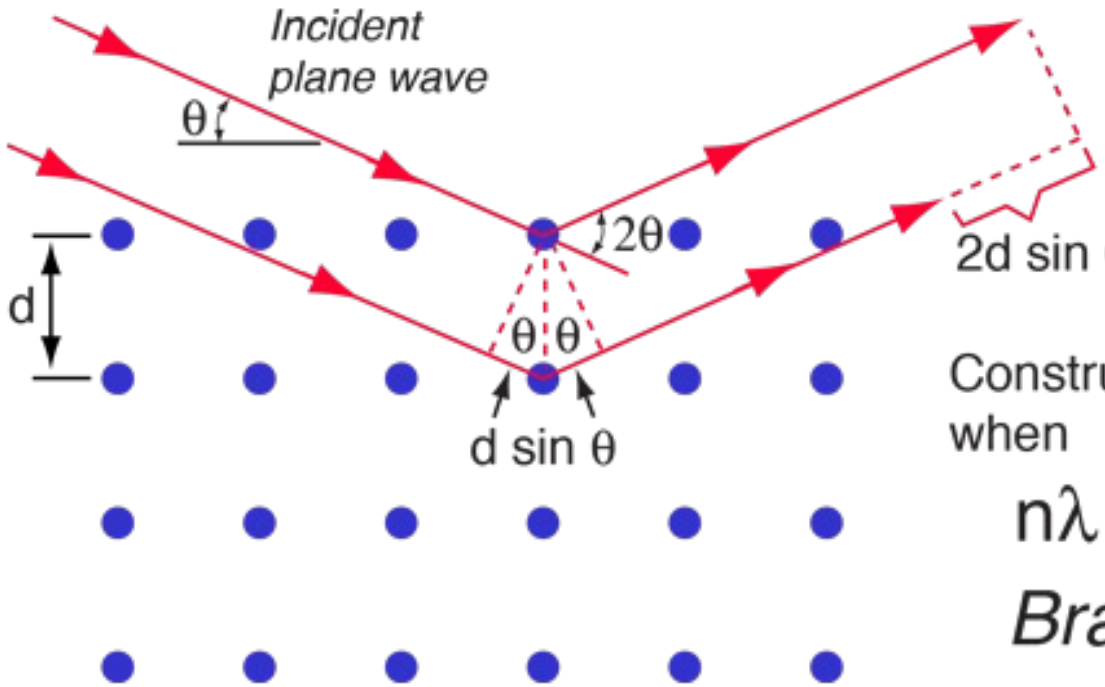
https://www.britannica.com/science/X-ray-diffraction

Simplified workflow



https://www.creative-biostructure.com/protein-crystallography-452.htm

Why are crystals necessary?

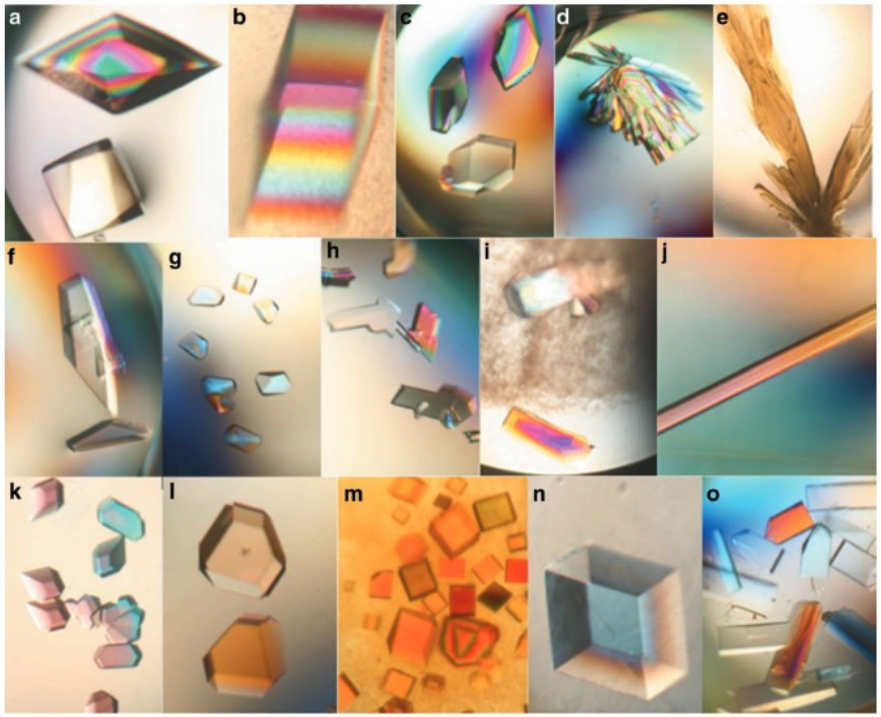


Constructive interference when

$$n\lambda = 2d \sin \theta$$

Bragg's Law

Protein crystallization



Wlodawer A, Dauter Z, Jaskolski M, (2017)
Springer Protocols: Protein Crystallography.

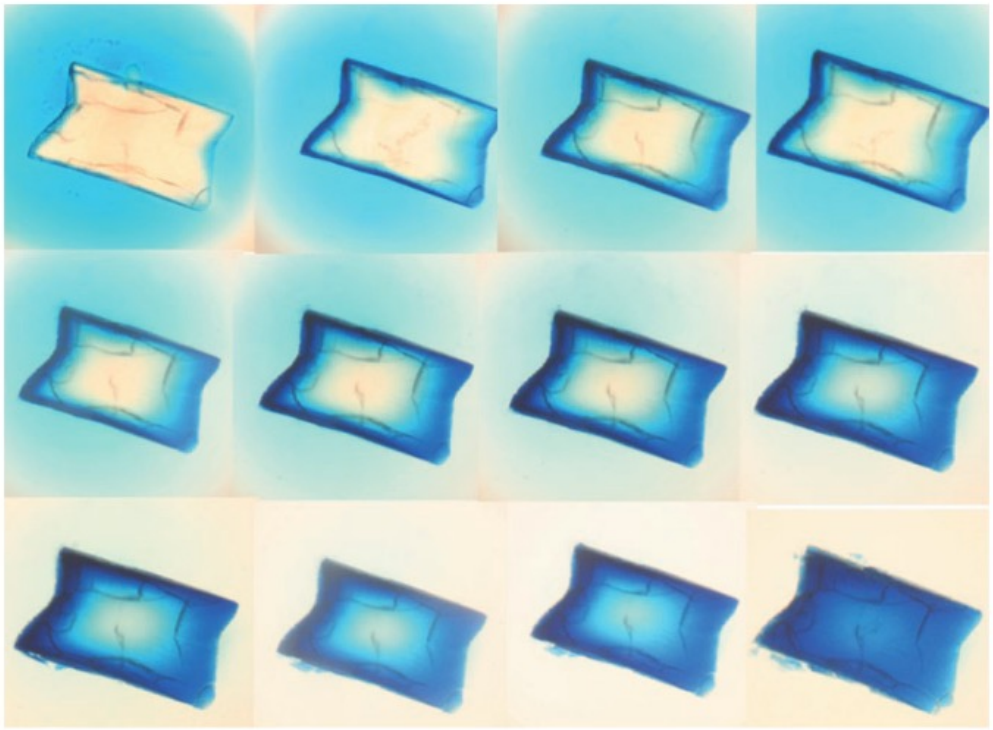
-Key bottleneck for X-ray crystallography.

-May require extensive condition optimization (buffers and additives, construct boundries and protein engineering).

-Flexibility, size, asymmetry and disorder may be refractory to crystallization.

-However optimized conditions allow for high throughput efforts.

Crystal soaking with small molecules



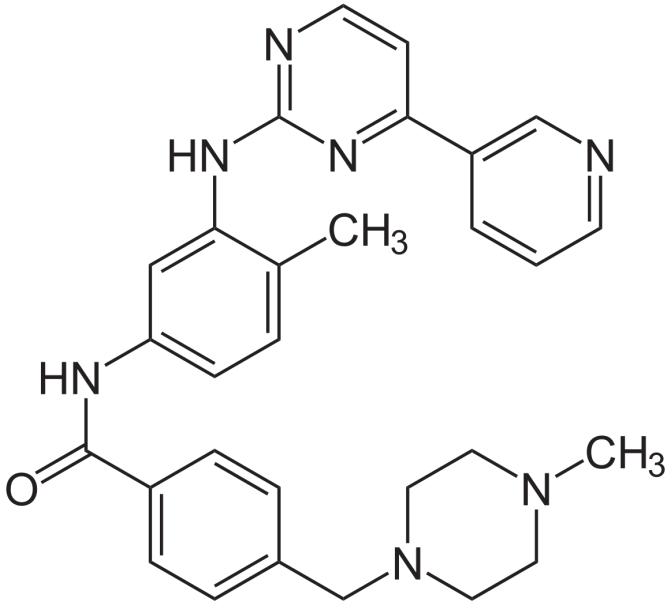
Wlodawer A, Dauter Z, Jaskolski M, (2017)
Springer Protocols: Protein Crystallography.

-Protein crystals contain many solvent-filled channels (~50% total volume).

-Small molecules diffuse through crystals and interact with proteins as if they were in solution.

-Can allow for high throughout work of determing protein-ligand complexes.

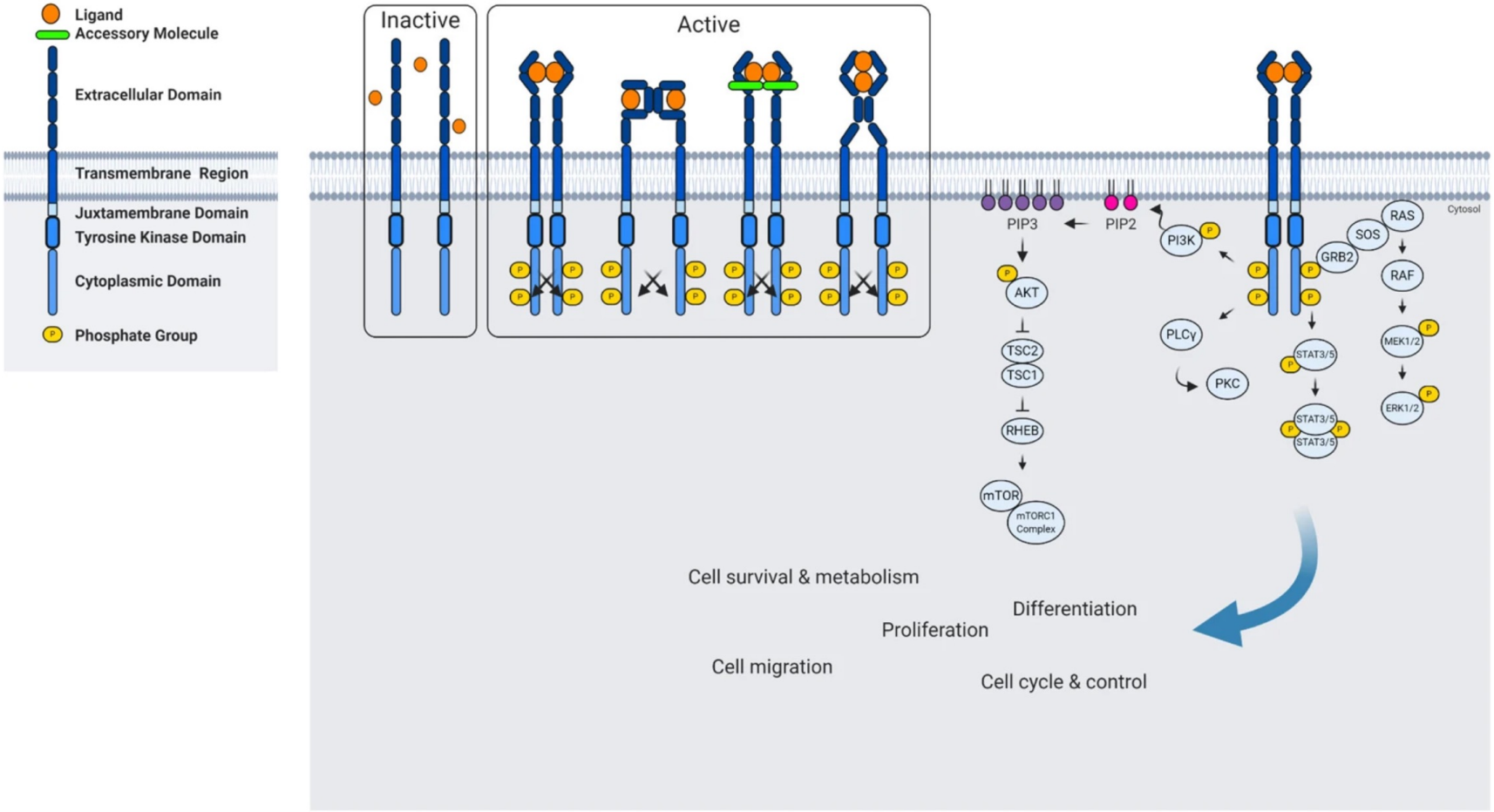
Case Study: Imatinib (Glivec)



https://rexmedical.co.nz/products/other/imatinib

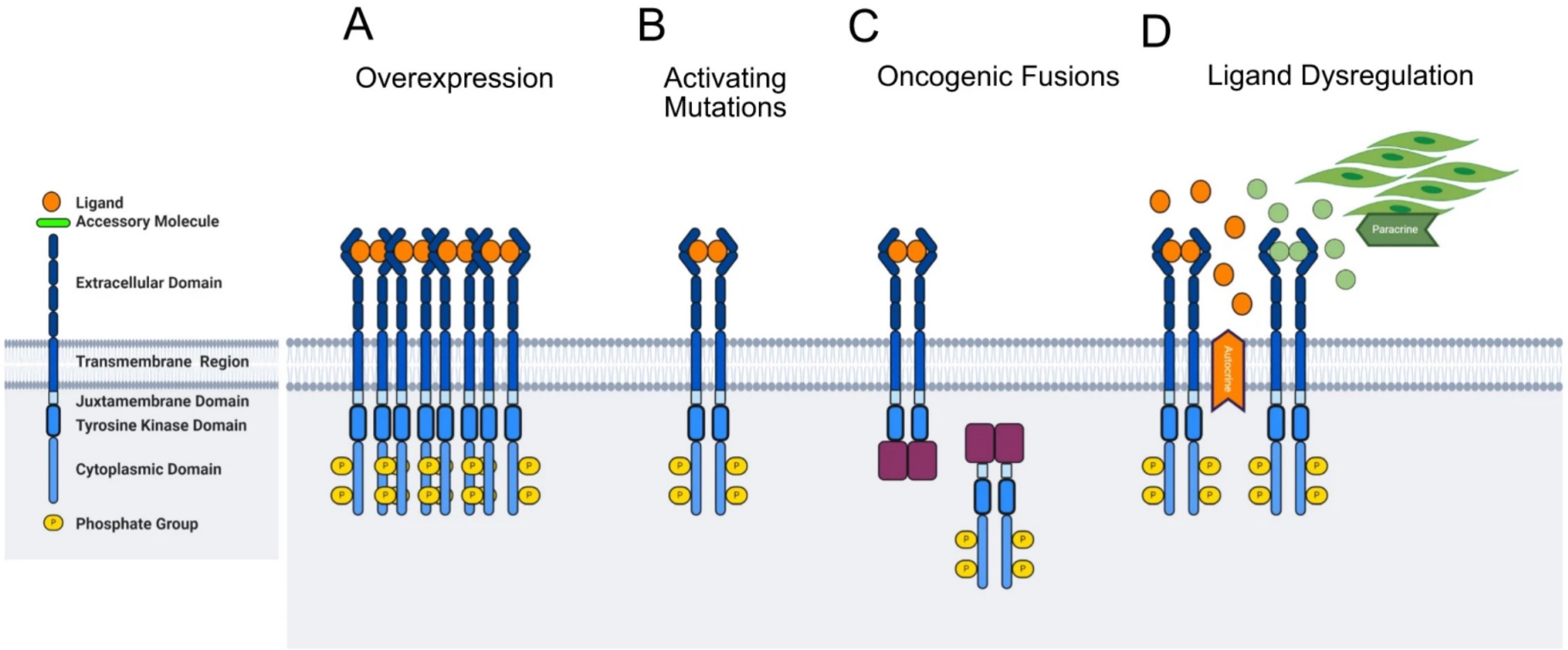
https://de.wikipedia.org/wiki/Imatinib

Receptor Tyrosine Kinases (RTK) overview



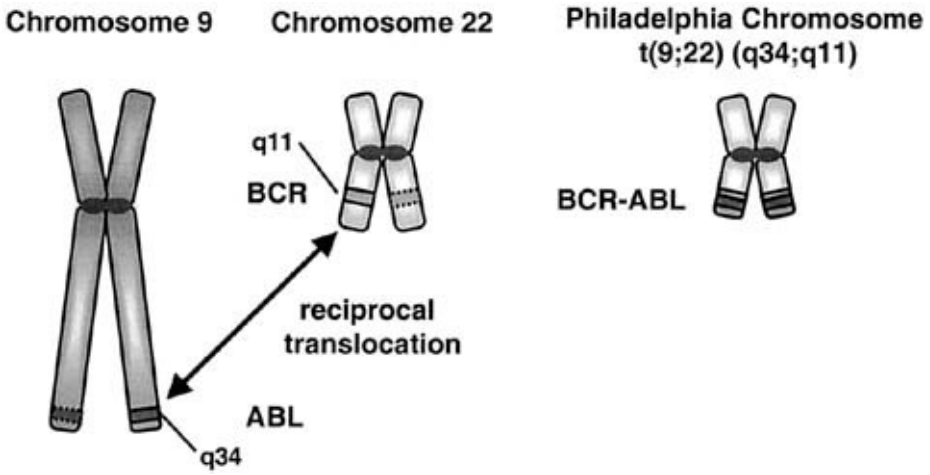
Saraon P, *et al*. (2021) *Oncogene*

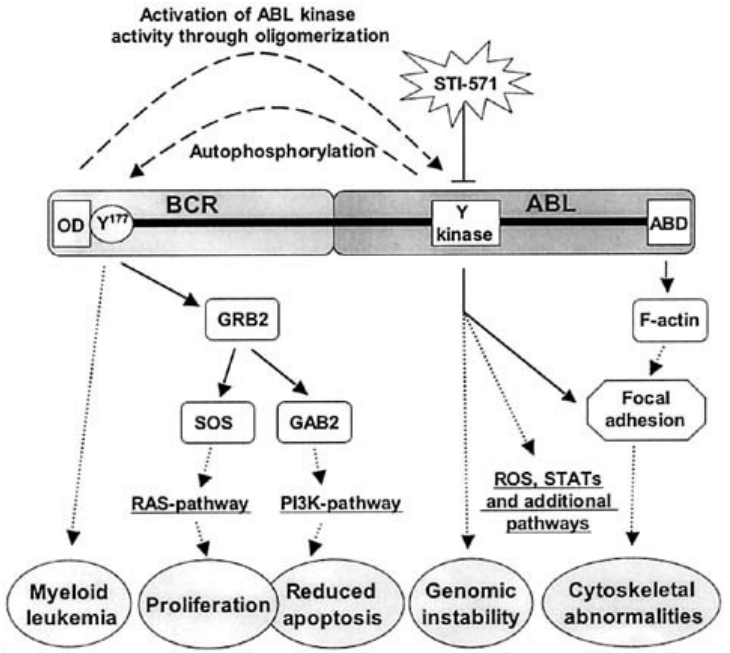
Mechanisms of RTK hyperactivation



Saraon P, *et al*. (2021) *Oncogene*

The BCR-ABL fusion protein is a result of chromosomal translocation, often in hematopoietic stem cells

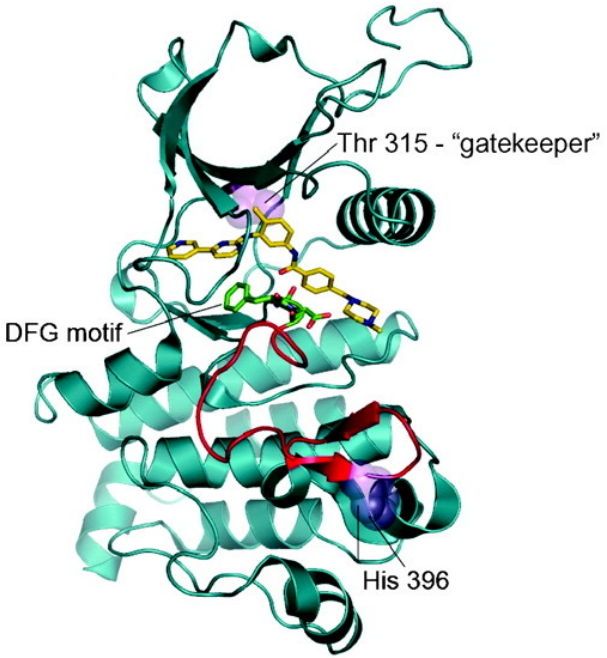




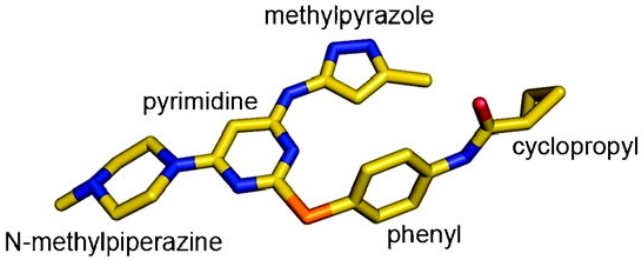
-Constitutively active kinase (does not need ligand or dimerization for activity).
-Globular and cytosolic (does not respond to receptor-based inhibitors).
-Upregulated intracellular signaling through phosphorylating substrates.

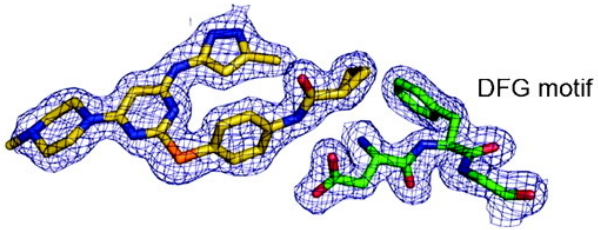
Sattlermc M, Griffin JD (2003) *Seminars in Hematology*

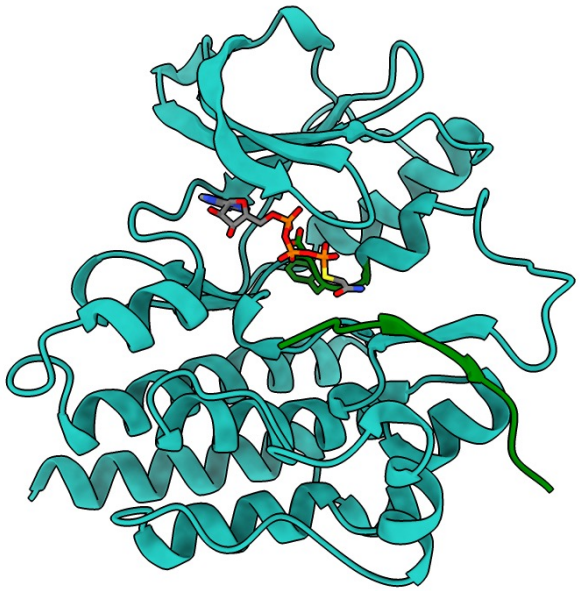
Imatinib binds the ATP binding pocket of ABL



Abl : Imatinib







Young MA, *et al.* (2006) *Cancer Res*

PDB: 2G1T

Large compound screens and structure-activity studies resulted in Imatinib

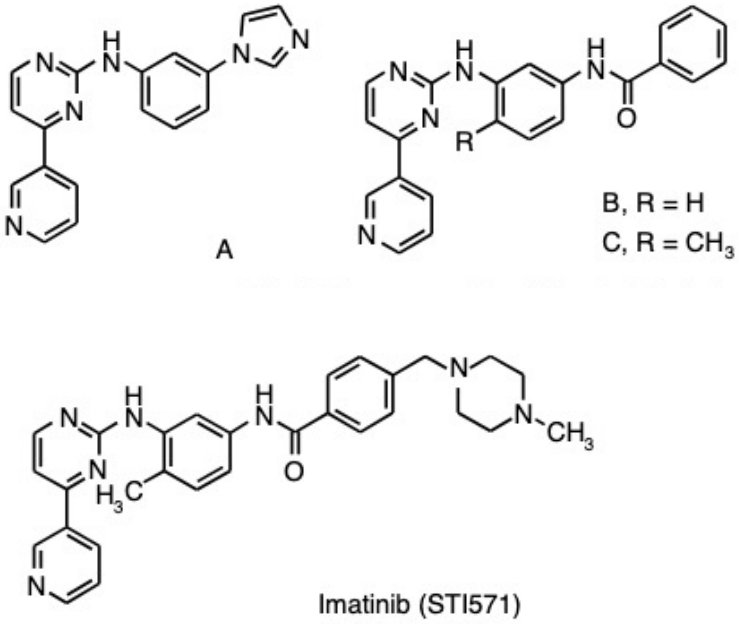


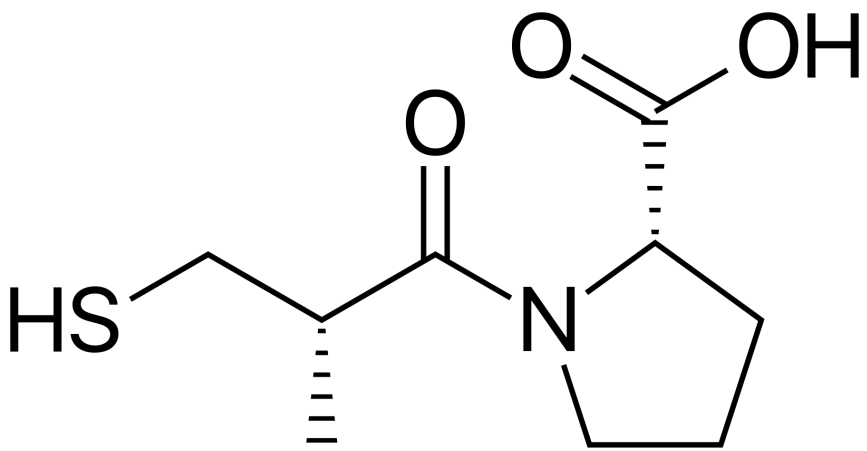
Table 1
Inhibitory profile against a panel of protein kinases

Kinase	Inhibitory concentration (IC_{50} nM)			
	A	B	C	Glivec
cAbl	3300 ± 1100	2800 ± 850	361 ± 48	188 ± 18
Kit	1100 ± 200	1100 ± 180	785 ± 140	413 ± 23
PDGFR-ß	390 ± 58	870 ± 110	400 ± 72	386 ± 111
VEGFR-2 (Human KDR)	1400 ± 210	1300 ± 310	10,000	10,000
EGFR (HER-1; Erb B)	>10,000	>10,000	>10,000	>10,000
FGFR-1	2500	>10,000	>10,000	>10,000
CMet	n.d.	>10,000	>10,000	>10,000
IGF-R	>10,000	>10,000	>10,000	>10,000
CDK1/cyclinB	92 ± 4	200 ± 37	>10,000	>10,000
CSrc	1700 ± 100	>10,000	>10,000	>10,000
PKC-α	1000	1200	72,000	>10,000

Data represent the mean ±SEM ($n \geq 3$) drug concentrations required to inhibit enzyme activity by 50% (IC_{50} value; nM) at ATP concentrations optimized for each kinase. Alternative nomenclature for the kinases is given in parenthesis. n.d. = not determined.

Manley PW *et al.* (2002) *European Journal of Cancer*

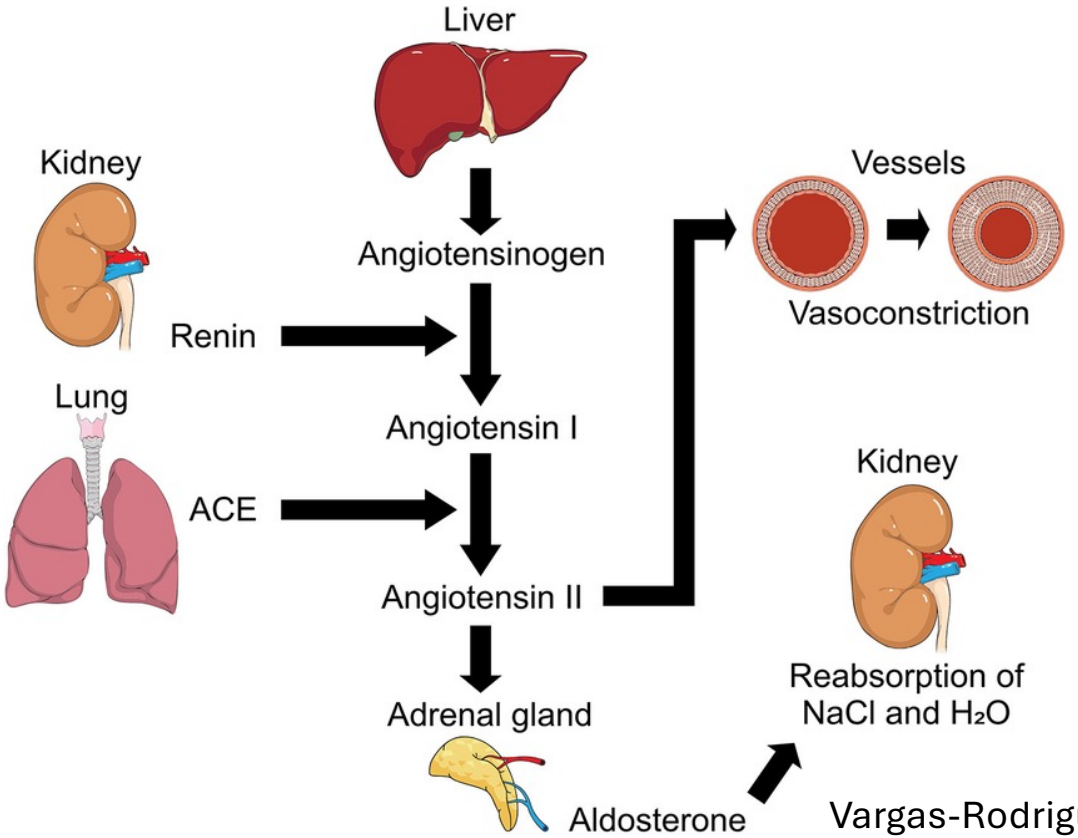
Case Study: Captopril - from snake venom to the clinic



https://www.denkpharma.com/products/pharmaceuticals/captopril

https://en.wikipedia.org/wiki/Captopril

The RAAS creates a proteolytic cascade that results in constriction of the vasculature



Vargas-Rodriguez JR, *et al*. (2022) *Frontiers in Medicine*

Peptides found within the venom of *Bothrops jararaca* inhibit the renin-angiotensin aldosterone system

https://en.wikipedia.org/wiki/Bothrops_jararaca

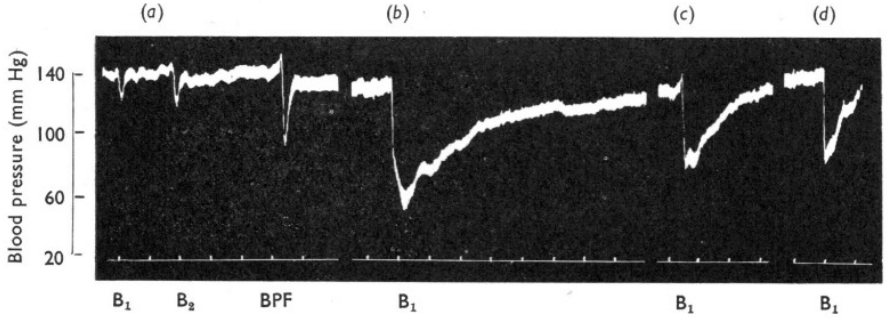
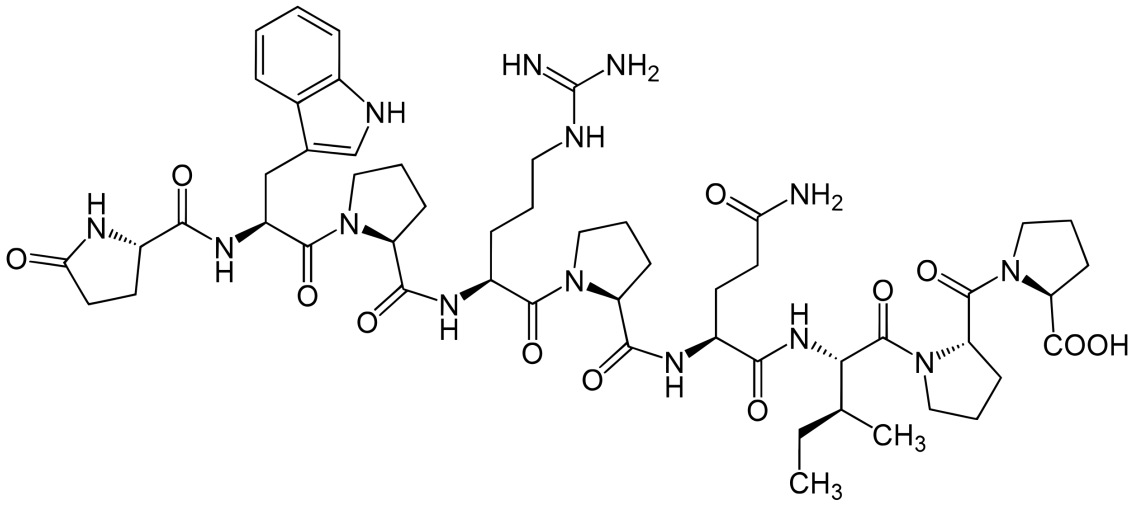
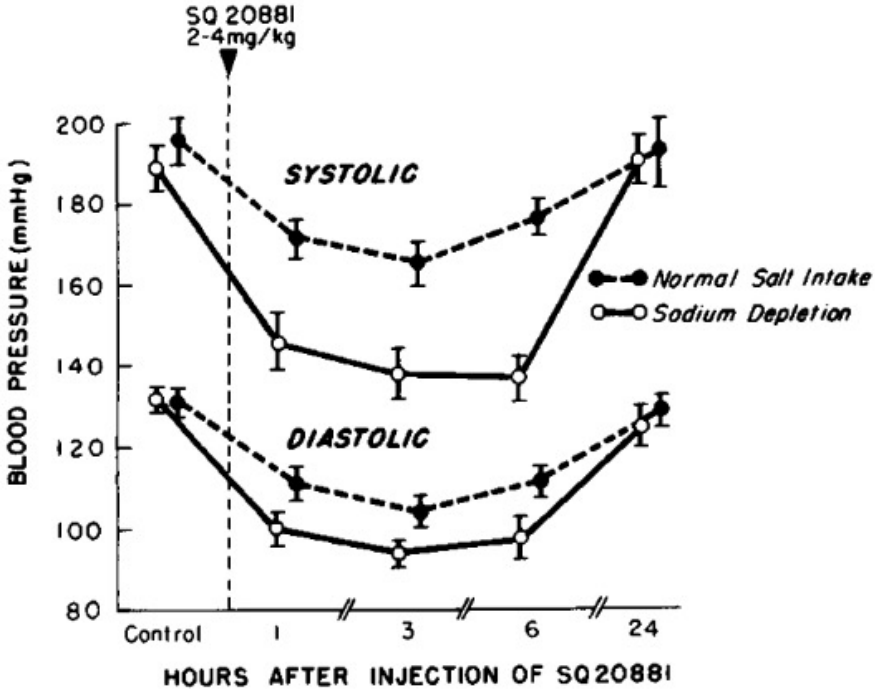


Fig. 4. The effect of bradykinin potentiating factor (BPF) upon the arterial hypotension produced in a cat by bradykinin. (*a*) Before; (*b*), (*c*) and (*d*) 3, 15 and 30 min after 2 mg/kg of BPF. B_1, 1 μg/kg and B_2, 2 μg/kg of synthetic bradykinin. Drugs were injected intravenously. Time marks, 1 min.

Ferreira SH (1965) *Brit. J. Pharmacol*

Teprotide, a synthetic ACE inhibitor





https://en.wikipedia.org/wiki/Teprotide#/media/File:Teprotide.svg

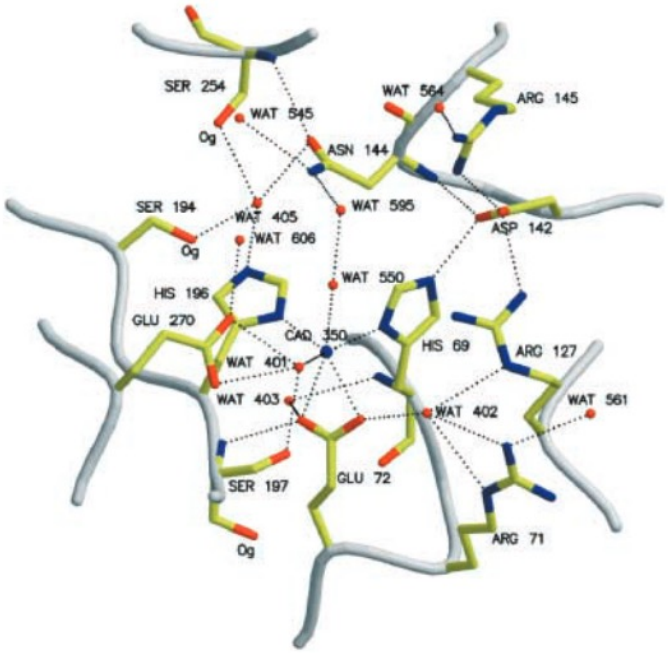
Gavras H, *et al*. (1974) *N. Engl. J. Med*.

The search for an orally available small molecule ACE inhibitor

- Teprotide has a short half-life due to proteolytic activity in animals.
- Commercial interest in peptide ACE inhibitors waned.
- Lead compound screens ended in failure.

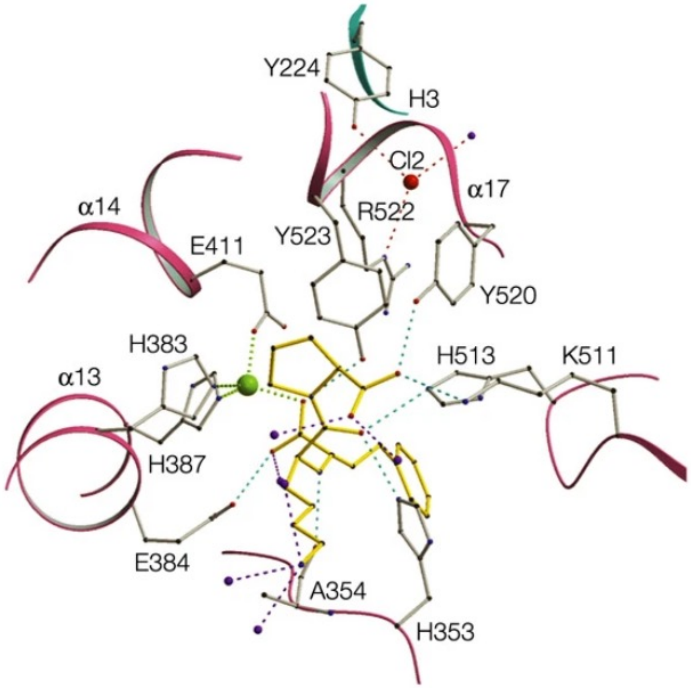
Faliure to find an small molecule ACE inhibitor lead to studies on carboxypeptidase A

ACE active site



Jensen AF, *et al.* (2002) *J Biol Inorg Chem*

Carboxypeptidase A active site



Acharya KR, *et al.* (2003) *Nature Reviews Drug Discovery*

L-Benzylsuccinate is an inhibitor of Carboxypeptidase A

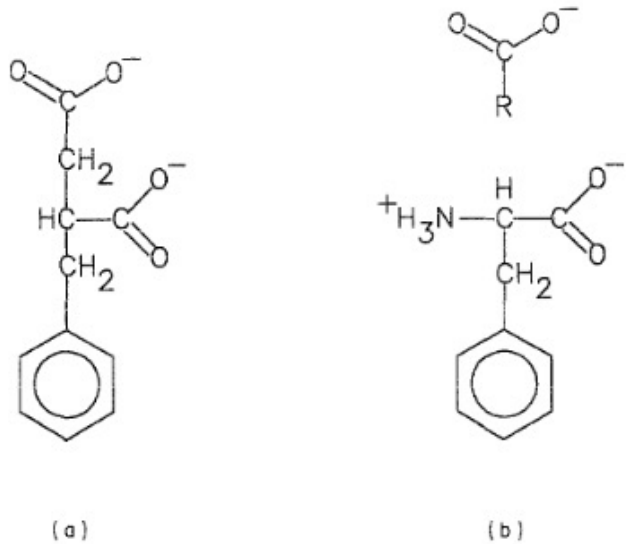
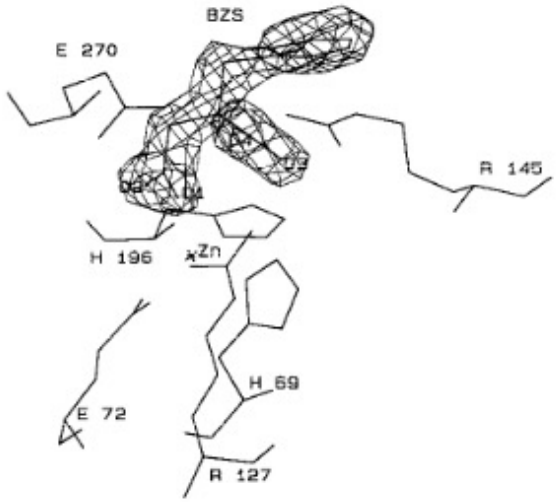
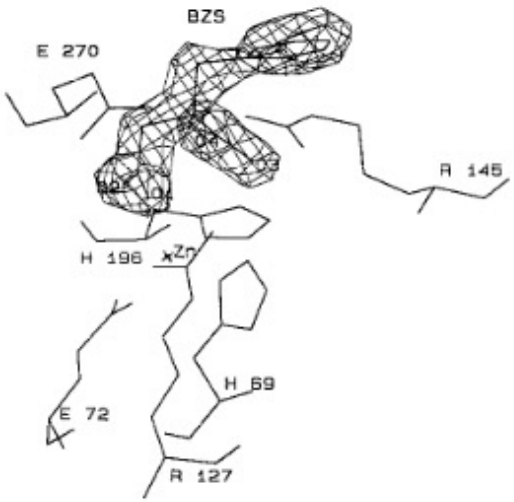


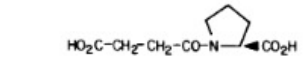
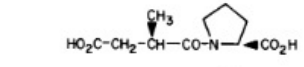
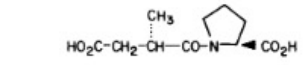
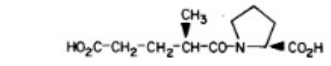
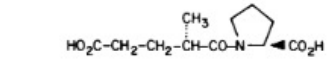
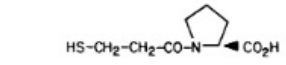
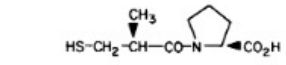
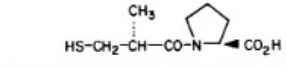
Figure 1. Scheme comparing (a) the benzylsuccinate molecule with (b) the products of peptide hydrolysis.





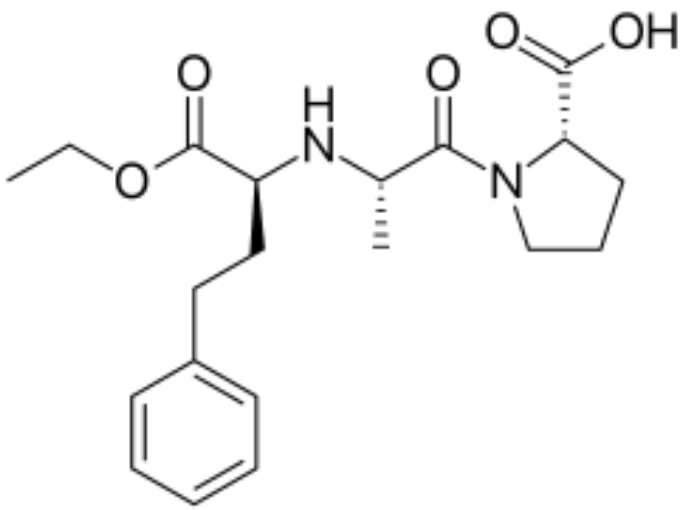
Mangani S, *et al.* (1991) *J. Mol. Biol.*

Captopril was inspired by L-benzylsuccinate and synthesized shortly after

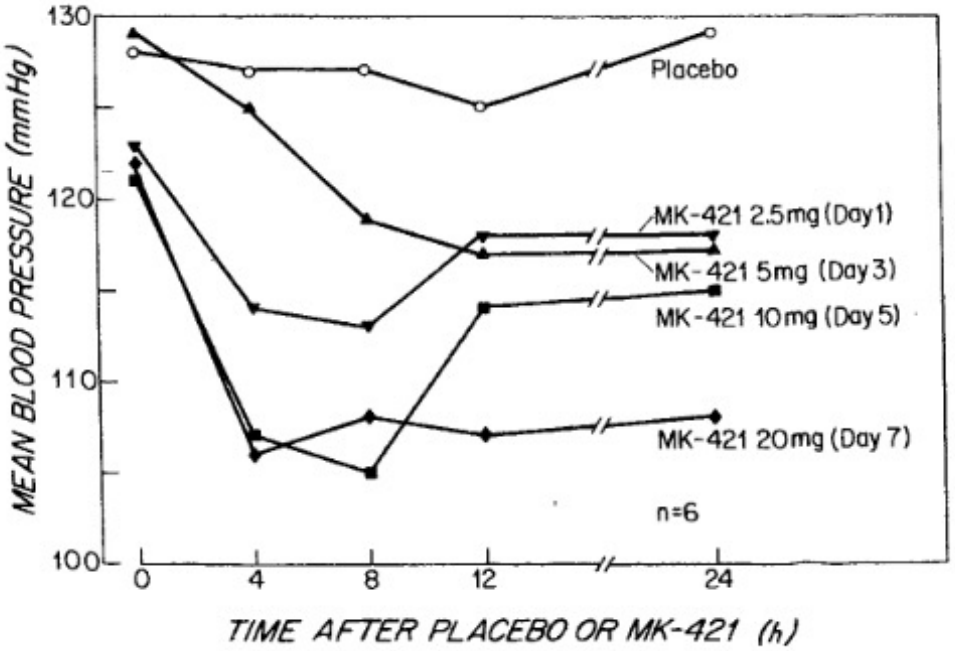
	Structure	Desig-nation	Activity (μg/ml)				
			Angiotensin-converting enzyme of rabbit lung (IC_{50})	Excised guinea pig ileum			
				AI (IC_{50})	AII (IC_{50})	Ach IC_{50}	BK (AC_{50})
1	<Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	(SQ20,881)	1.0	0.068	> 32	> 32	0.0017
2			135	94	>100	>100	8.0
3		(SQ13,297)	12	13	>100	>100	0.2
4			340	>100	>100	>100	15
5			1.0	4.6	>100	>100	1.0
6			230	>100	>100	>100	4.7
7		(SQ13,863)	0.04	0.06	>100	>100	0.005
8		(SQ14,225)	0.005	0.005	>100	>100	0.0007
9			0.50	1.7	>100	>100	3.1

Ondetti MA, *et al.* (1977) *Science*

Toxicity associated with high doses of Captopril leads to the development of Enalapril and others



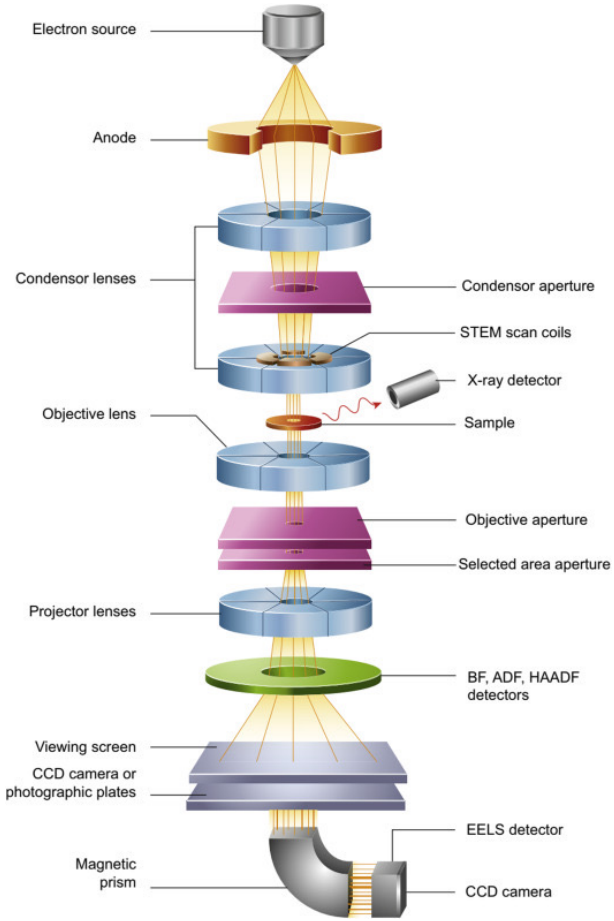
https://en.wikipedia.org/wiki/Enalapril



Gavras H, *et al.* (1981) *The Lancet*

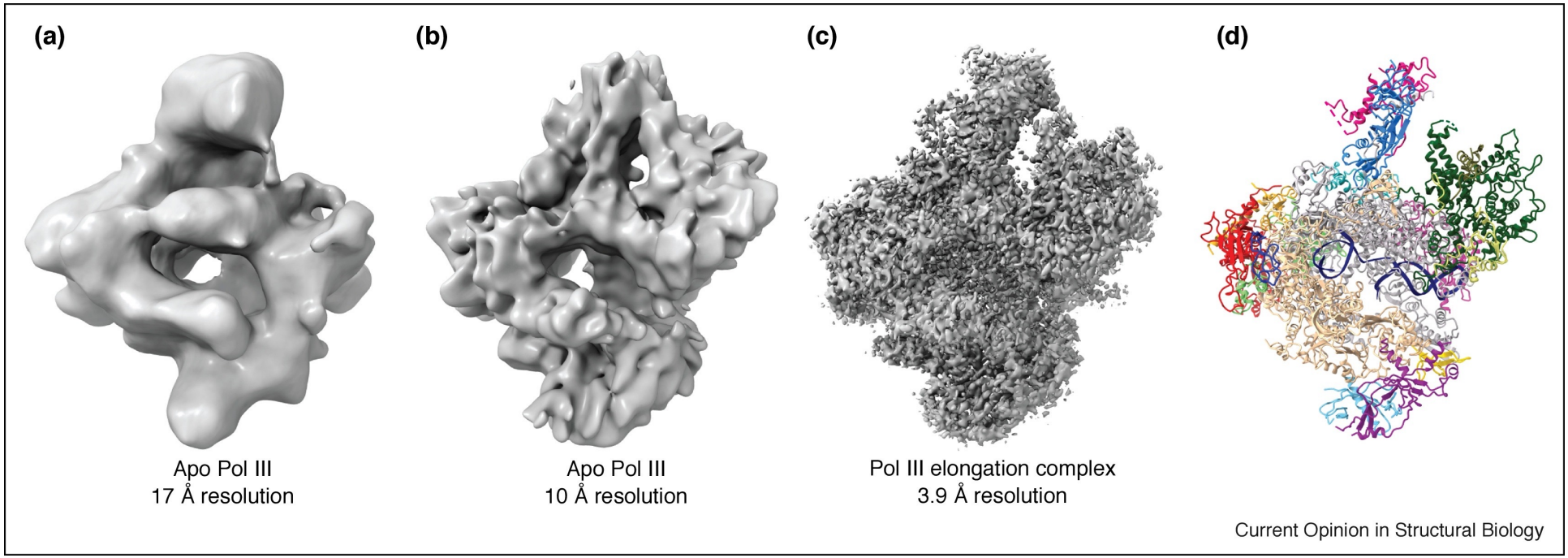
Cryo-EM Introduction

https://cfim.ku.dk/equipment/electron_microscopy/titankrios/

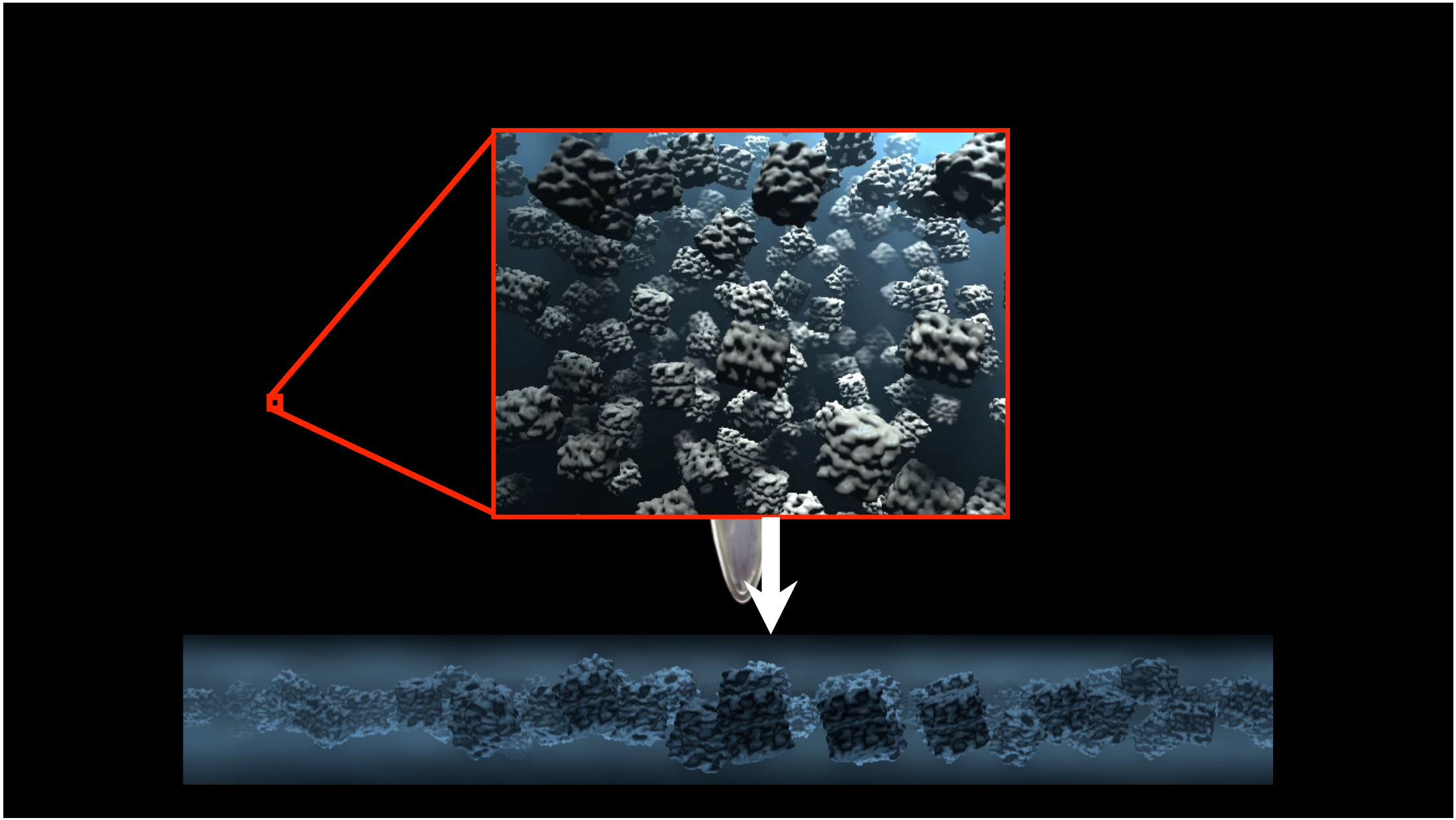


Inkson BJ, (2016) Materials Characterization Using Nondestructive Evaluation (NDE) Methods

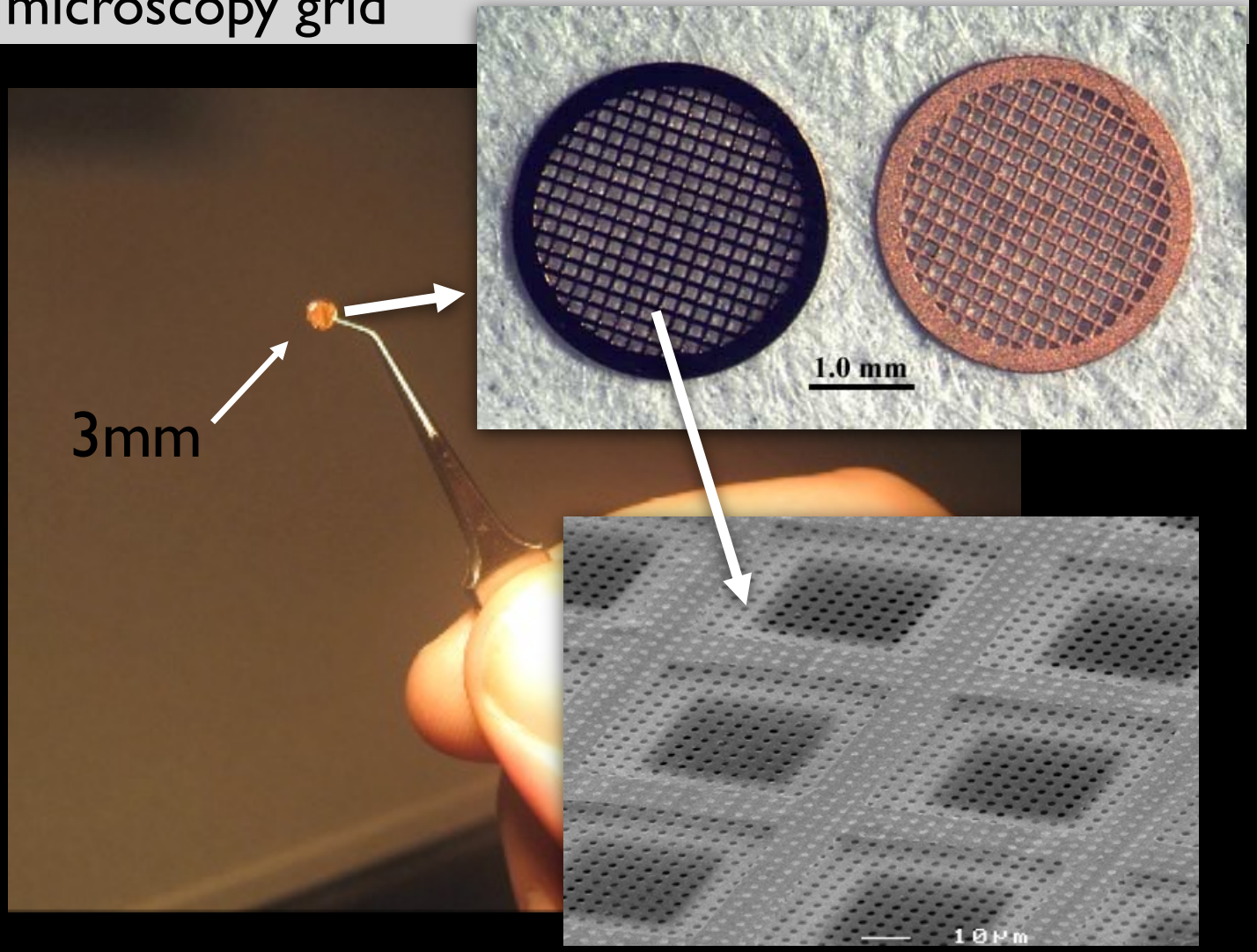
Resolution Revolution

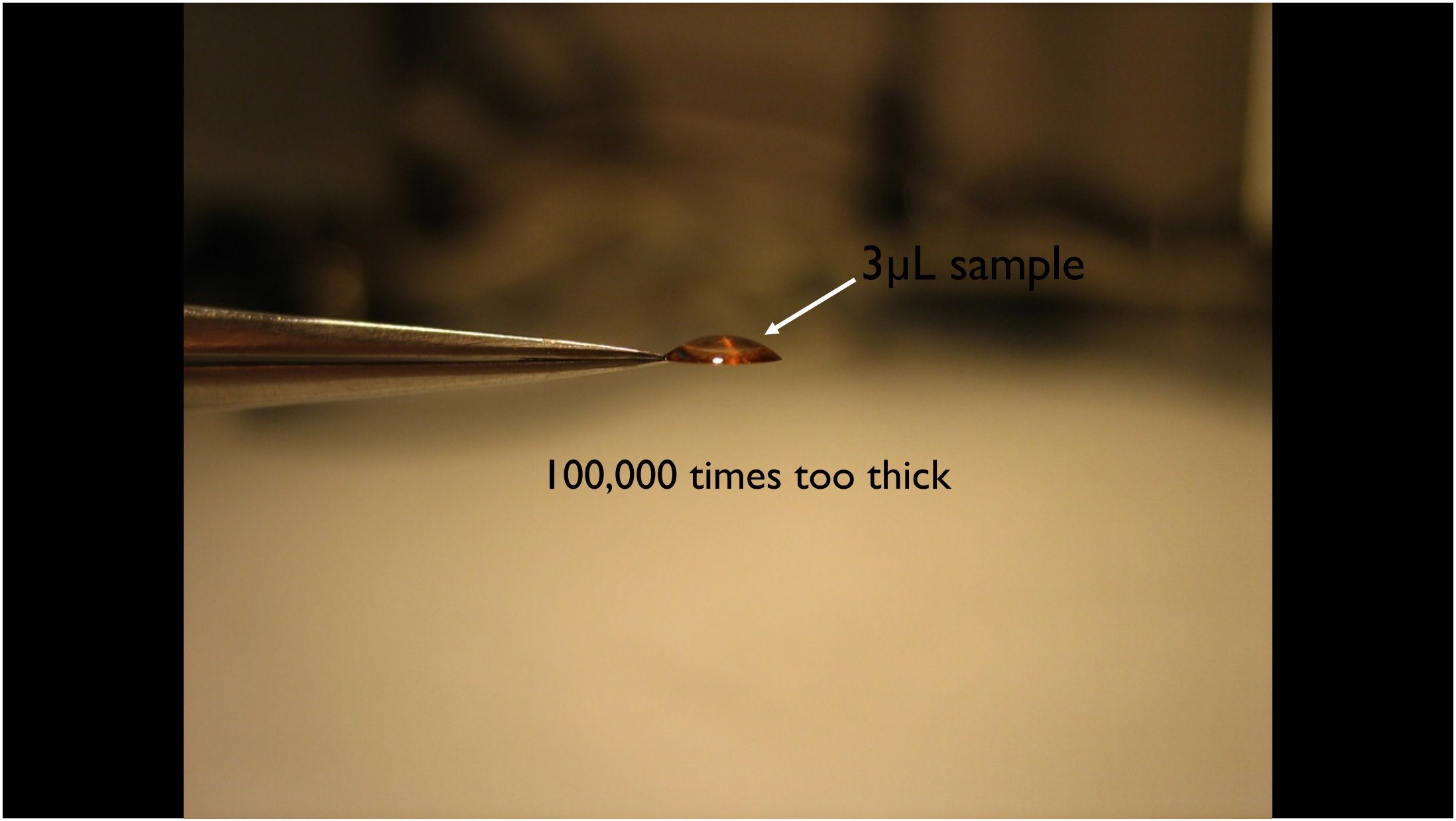


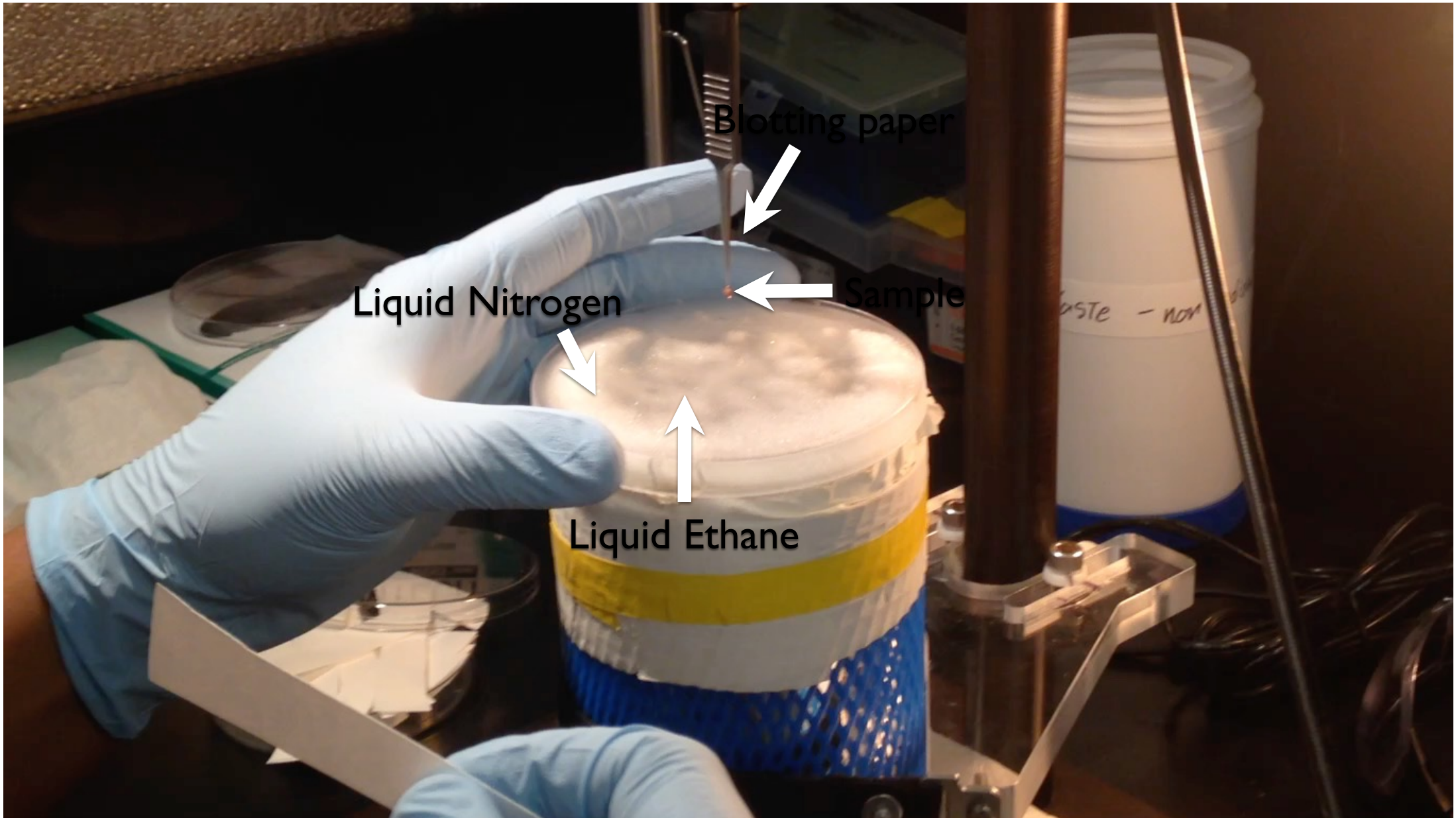
Hanske J, *et al.* (2018) *Curr Opin Struct Biol.*

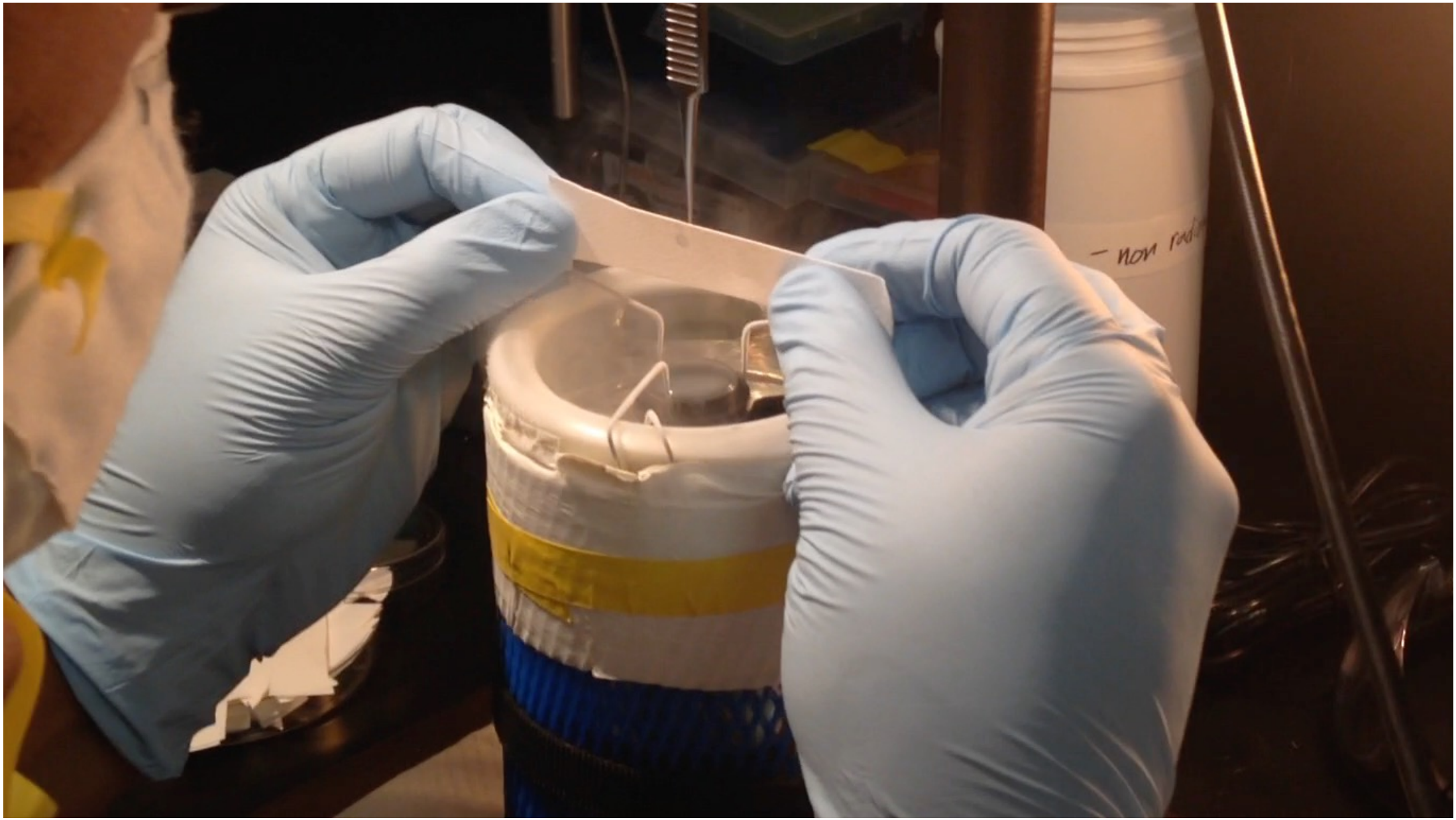


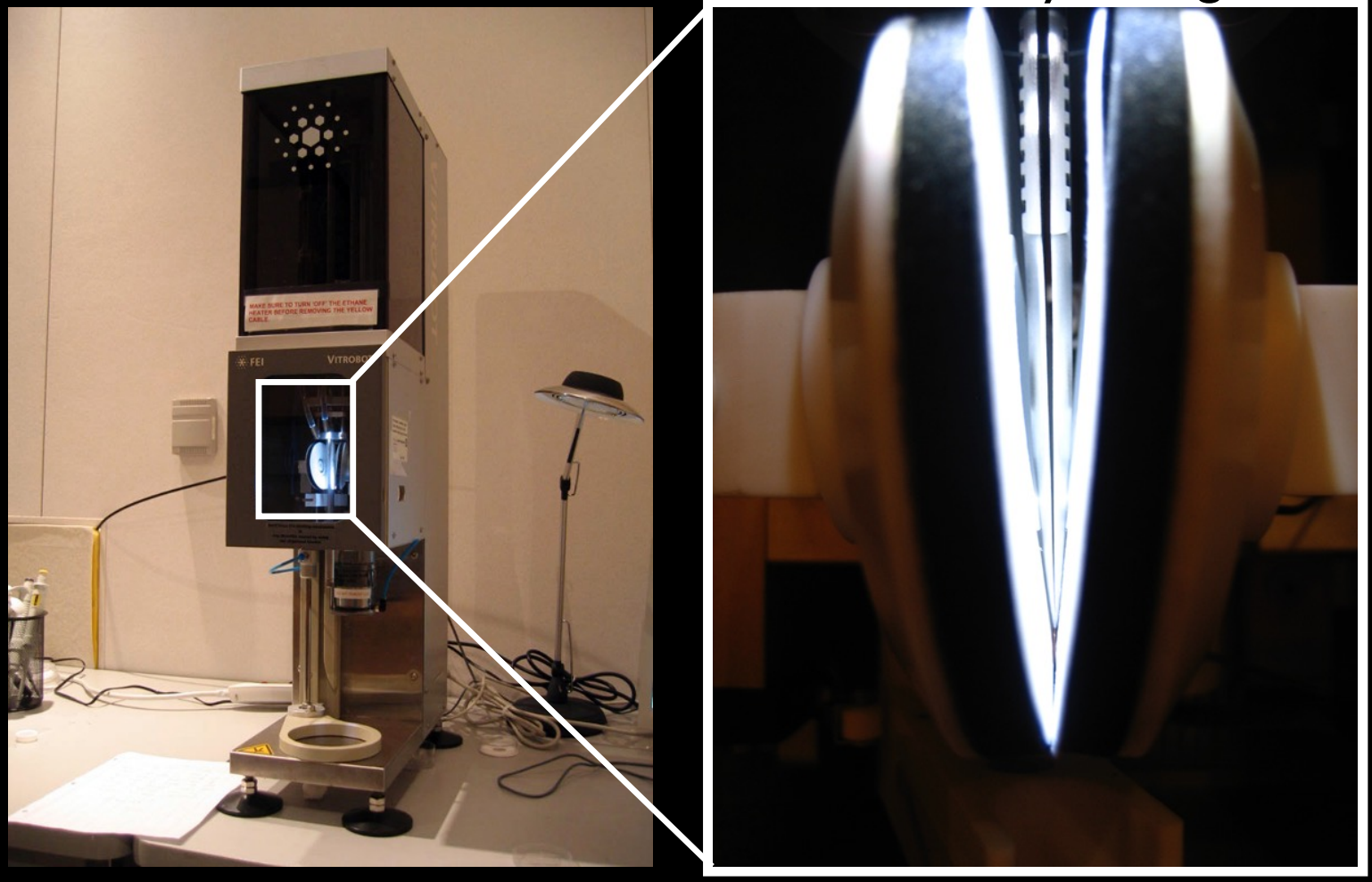
Electron microscopy grid

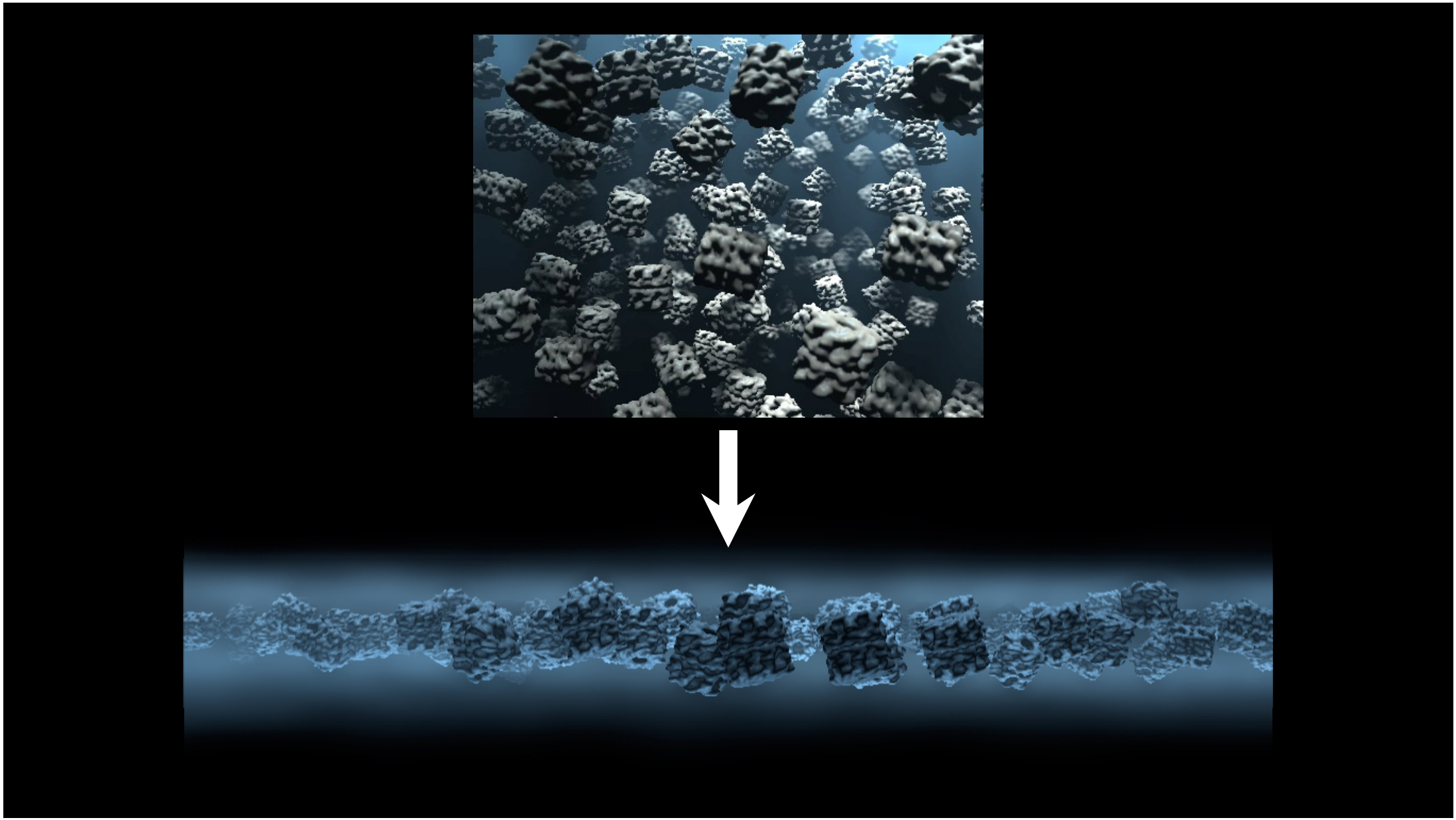


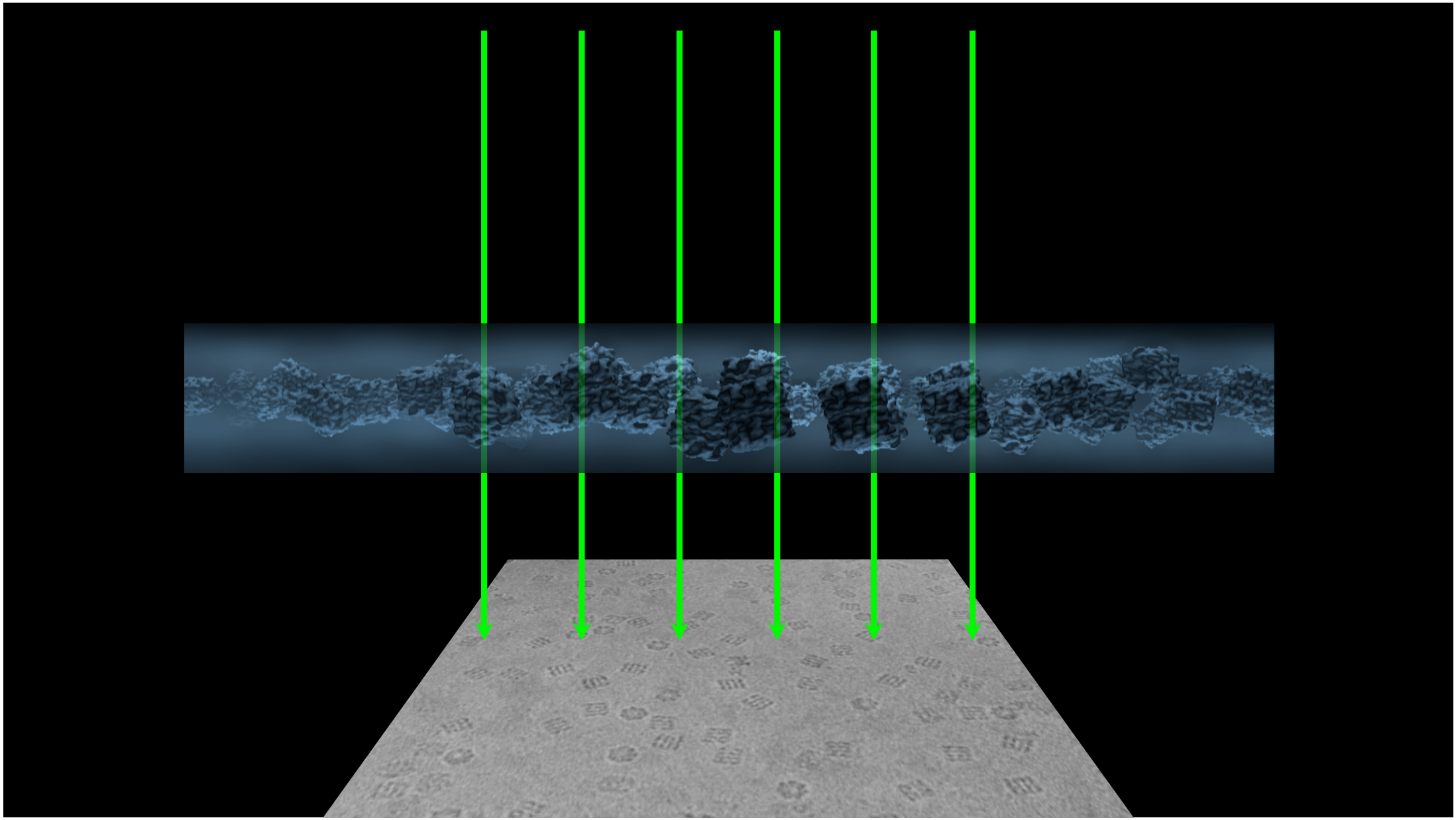

3µL sample
100,000 times too thick

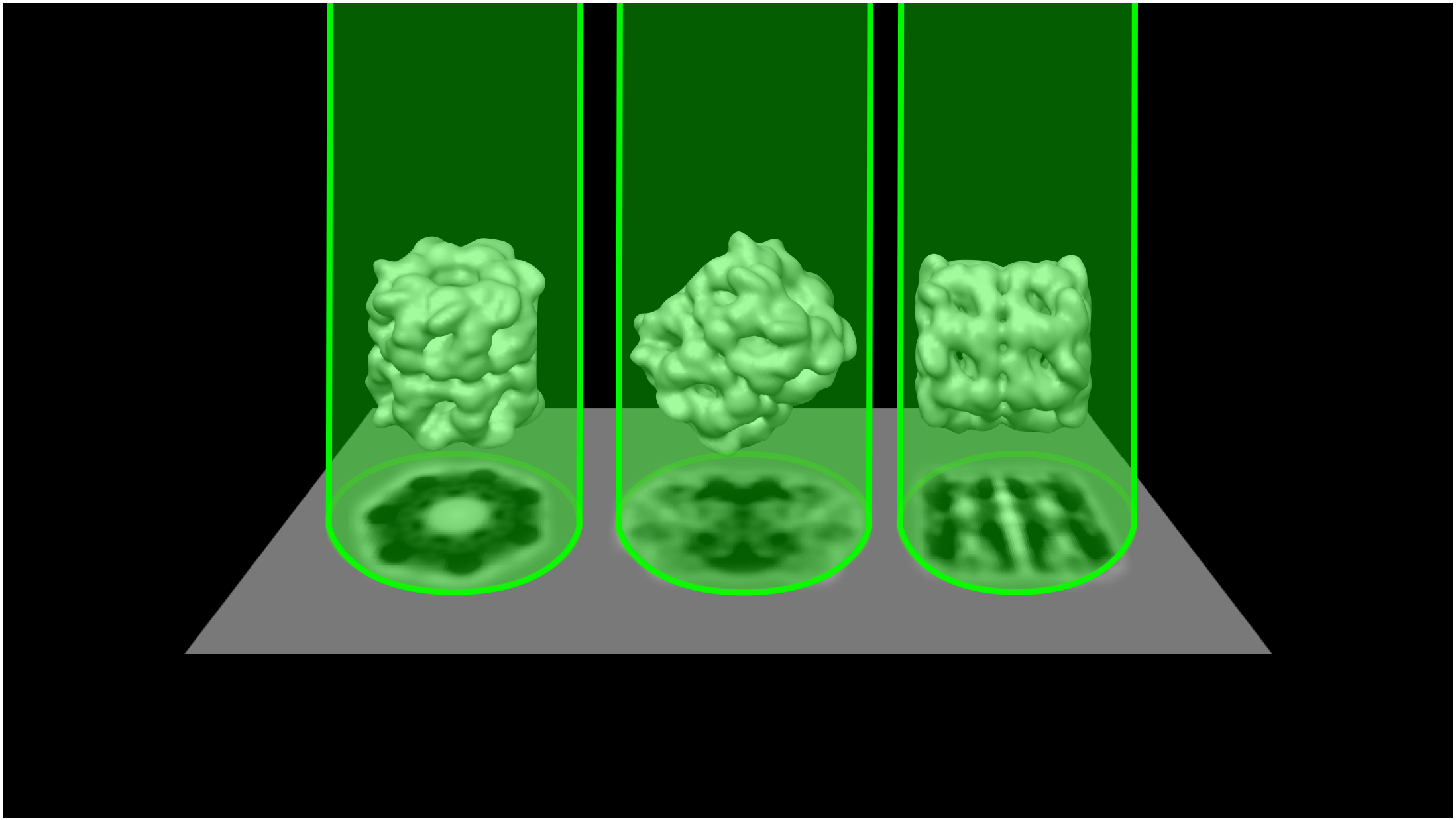

Blotting paper
Sample
Liquid Nitrogen
Liquid Ethane

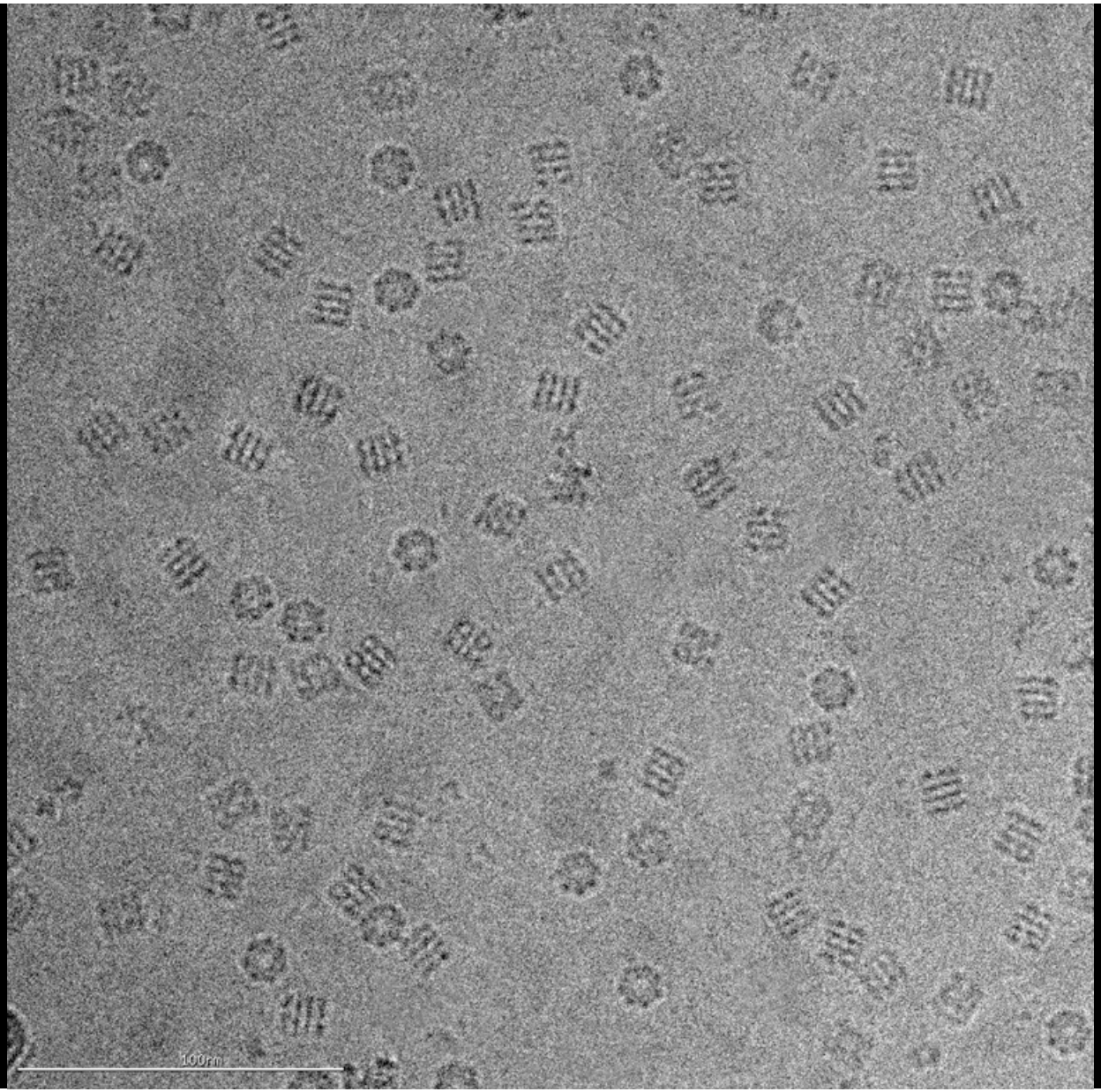


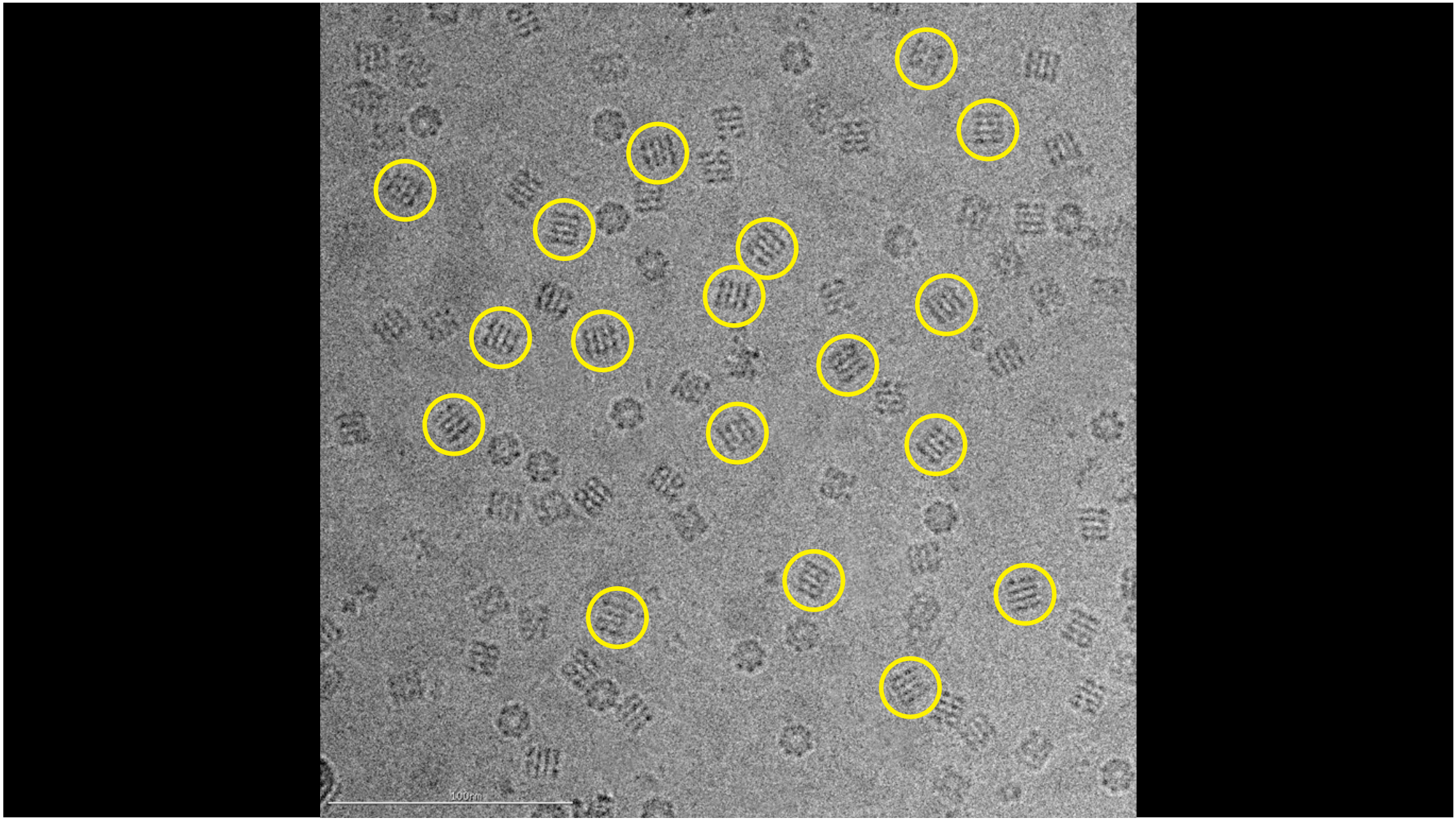

MAKE SURE TO TURN OFF THE ETHANE
HEATER BEFORE REMOVING THE YELLOW
CABLE
FEI

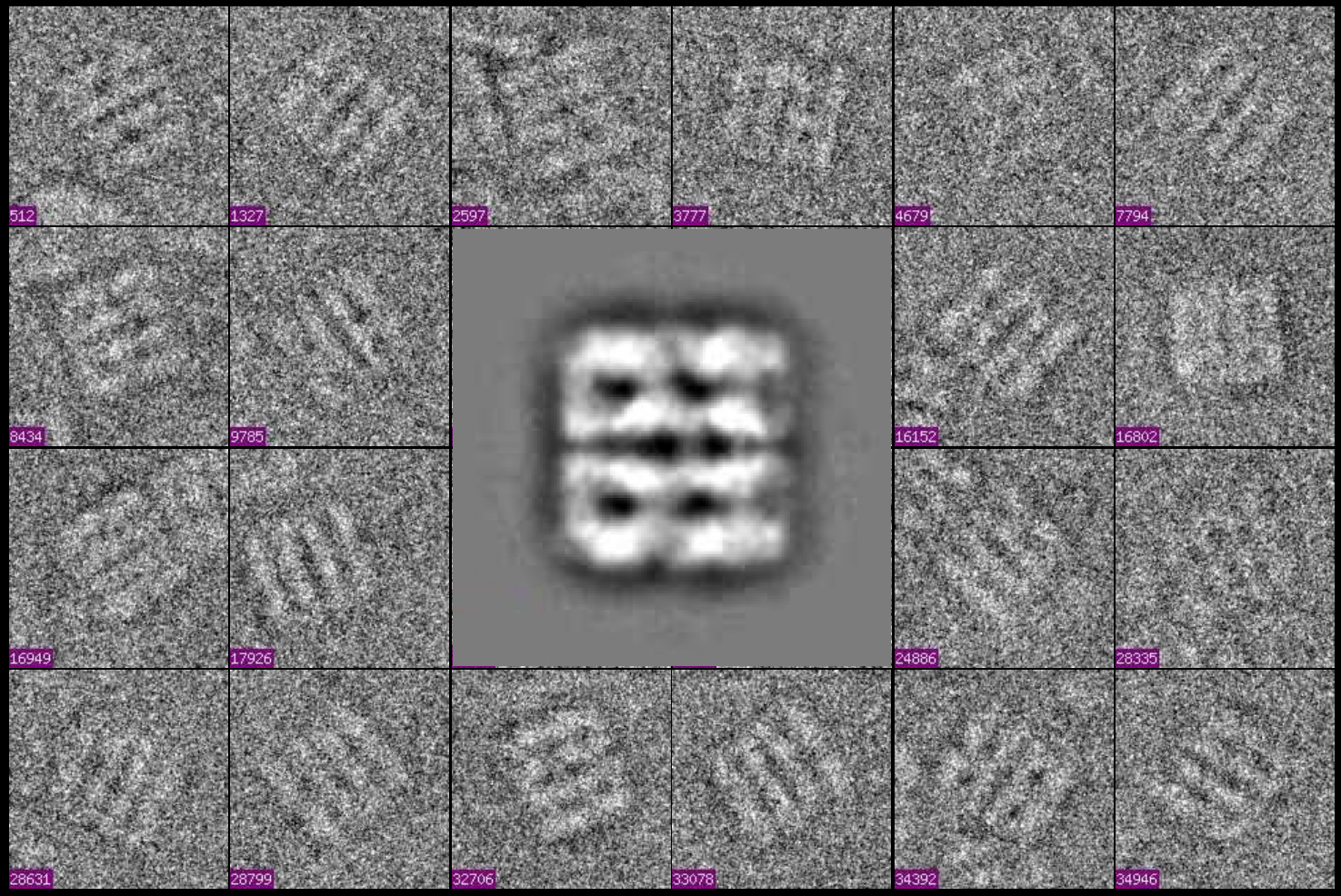







100nm


100nm


512
1327
2597
3777
4679
7794
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17926
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28631
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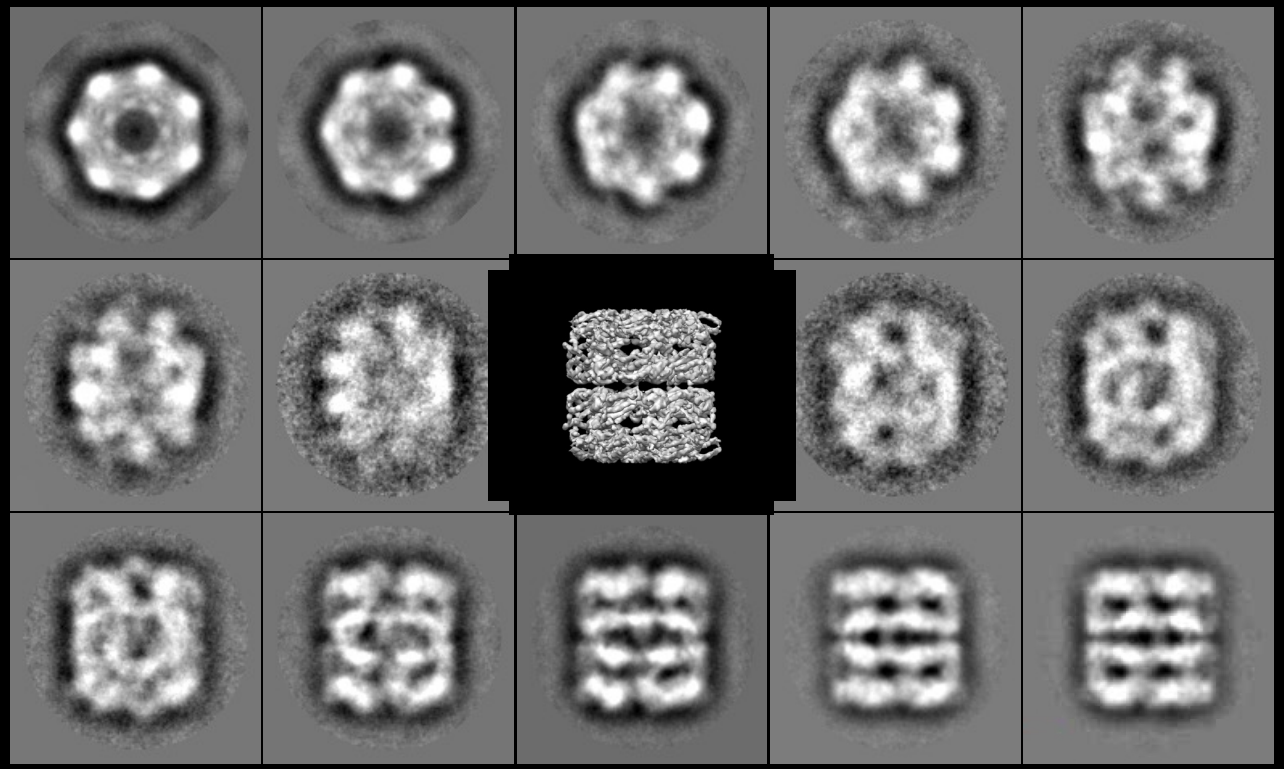
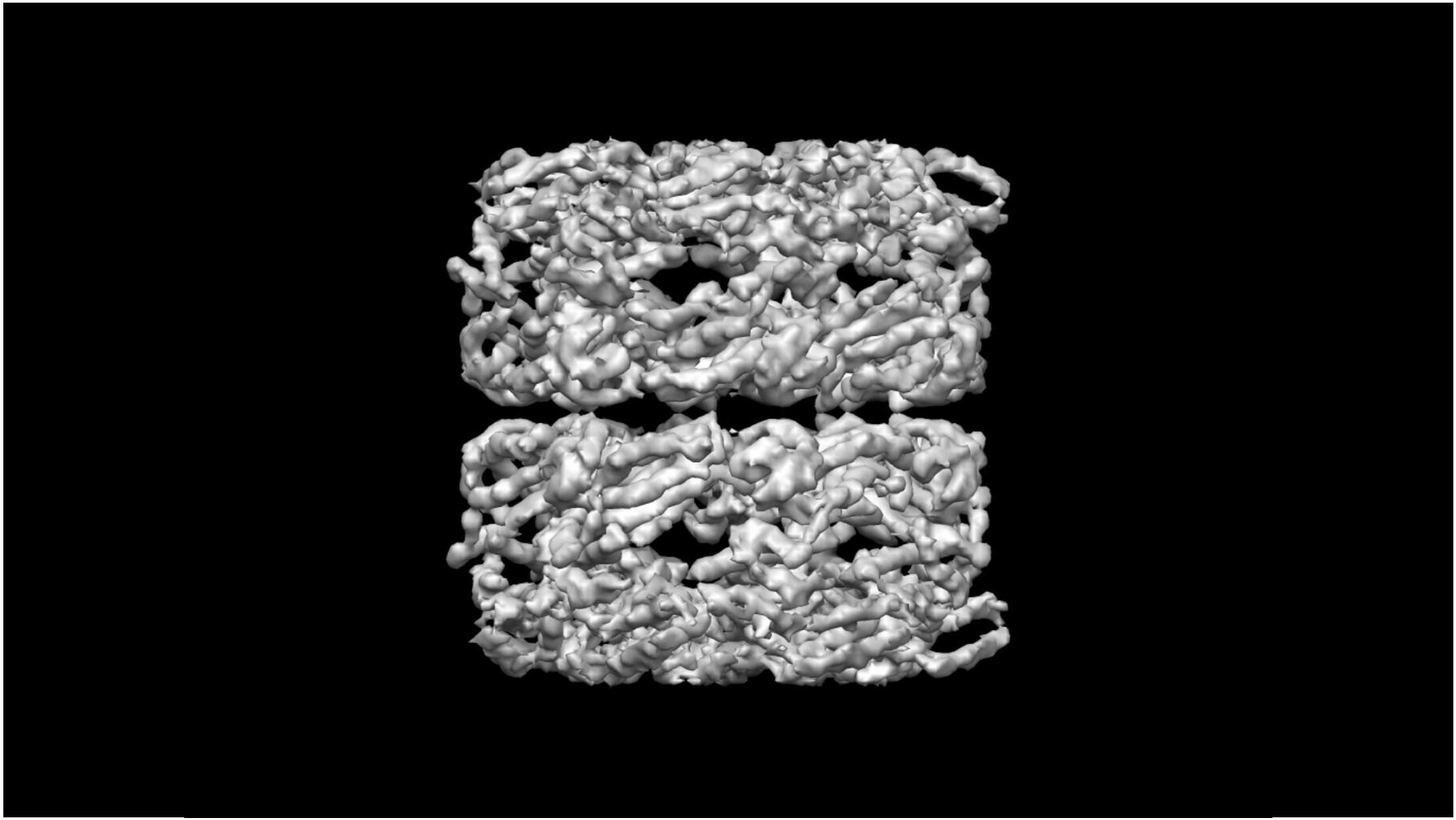
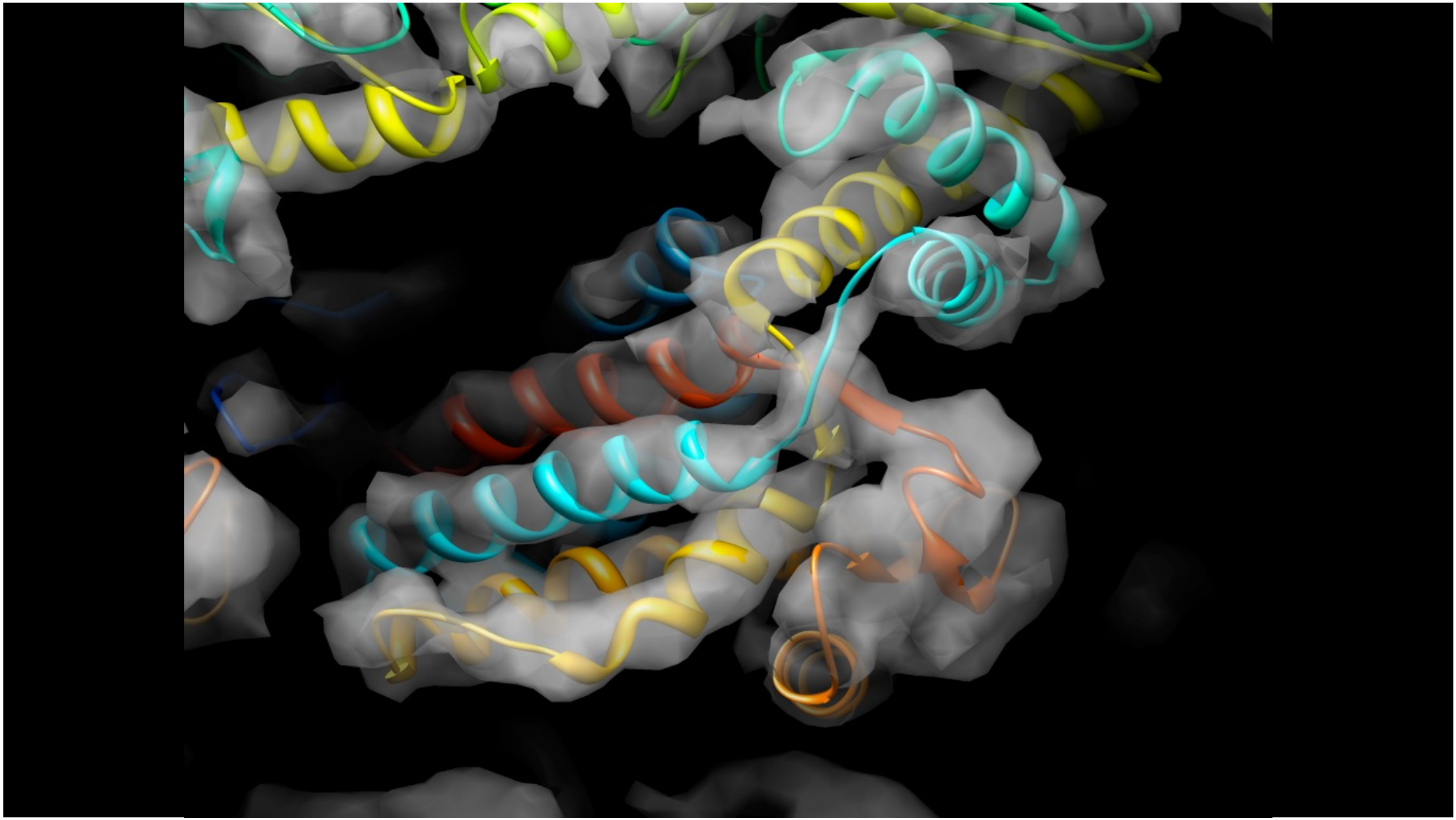
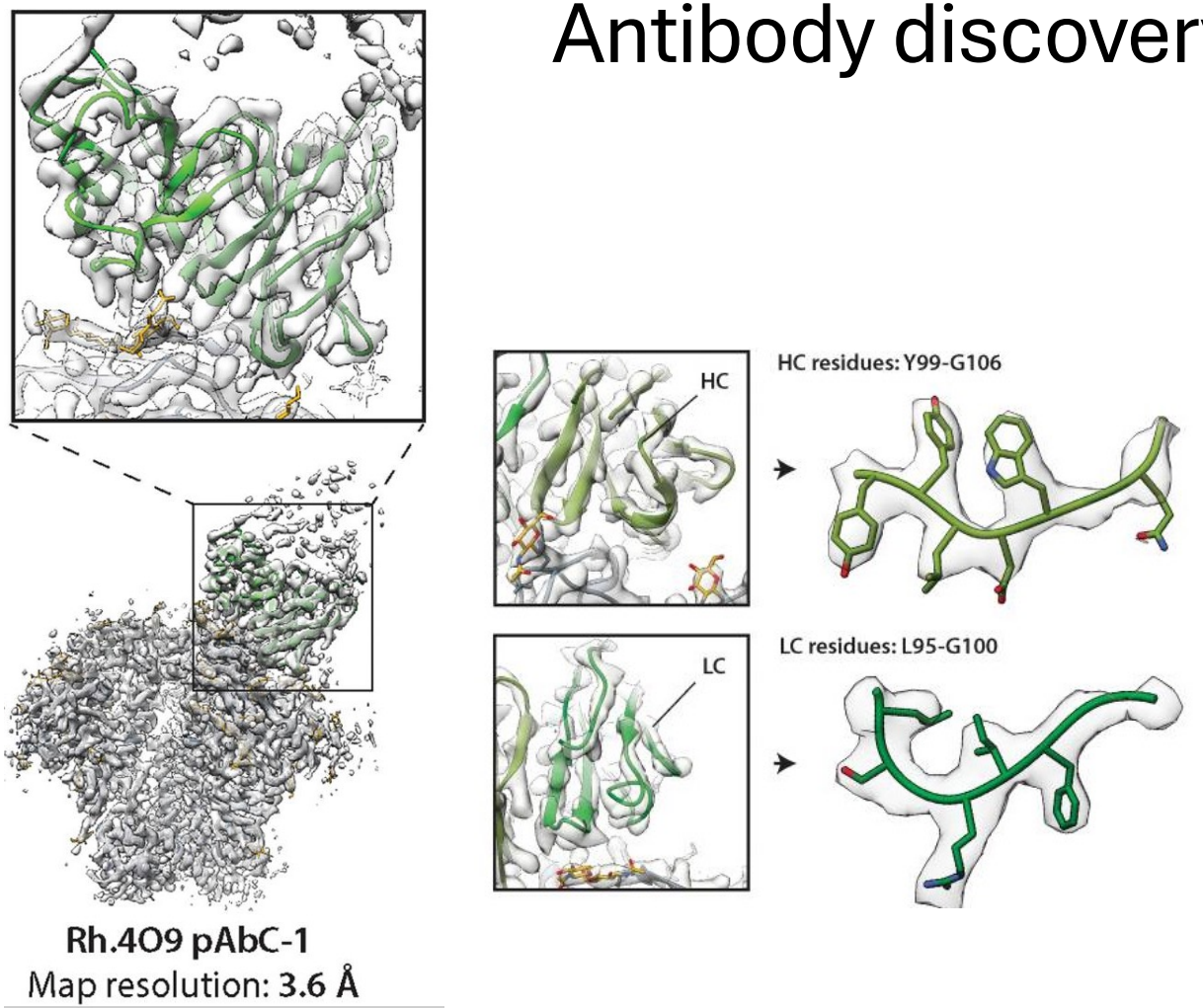
34946







Antibody discovery using cryo-EM



Antanasijevic A, Bowman CA *et al.* (2022) *Sci Adv.*

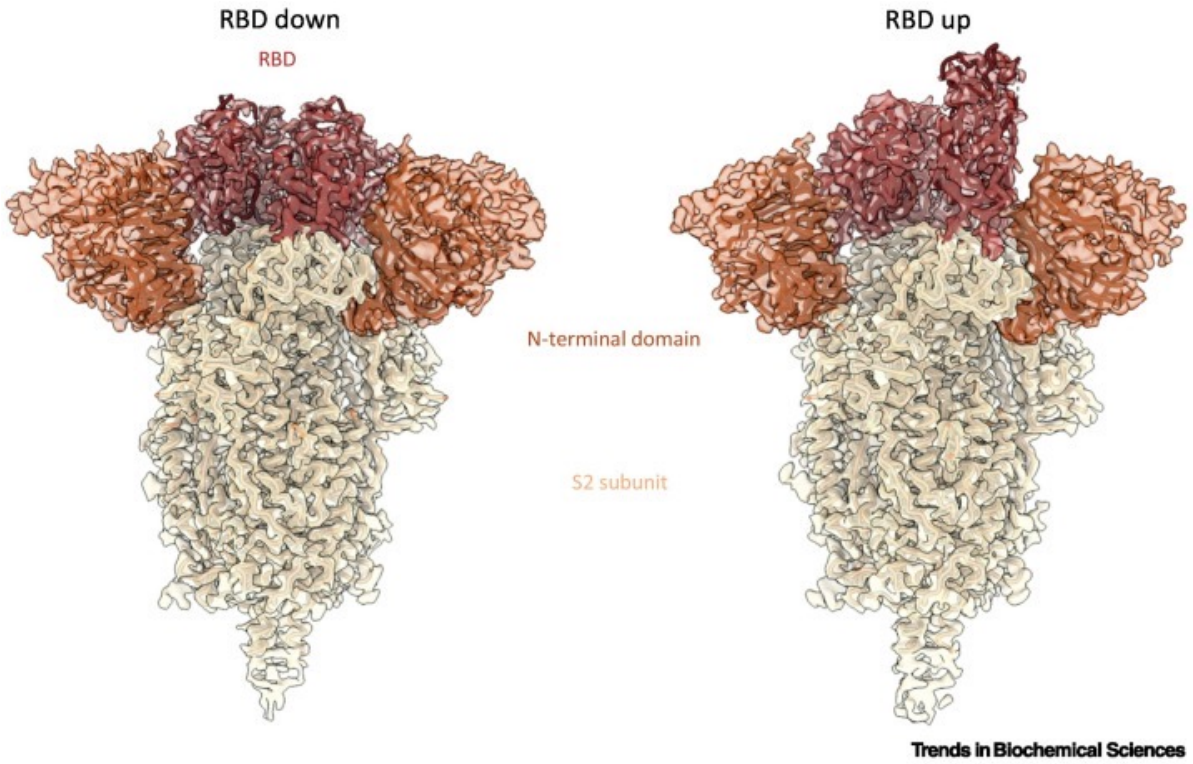
-Rhesus macaque were immunized with soluble HIV Env trimers.

-Trimers were purified by immunoaffinity chromatography and then subjected to single particle analysis.

-Fab-corresponding parts of bound antibodies were sequenced in a combined approach using the cryo-EM density and b cell receptor time of immunization sequencing databases.

-Hybrid structural and bioinformatics approach that identifies mAbs that bind to specific epitopes.

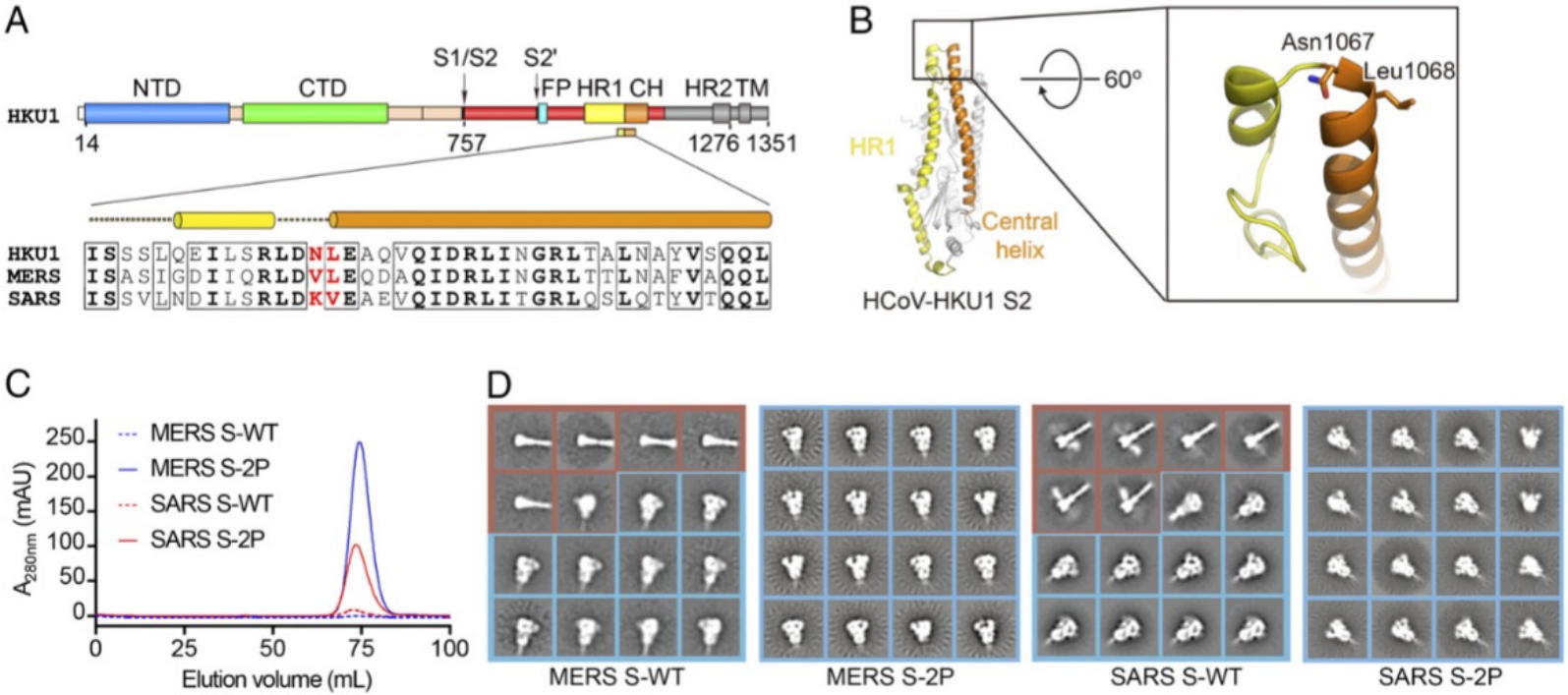
Cryo-EM and SARS-Cov-2



Rapid studies of the spike protein trimer revealed an active conformation, “RBD up”, which is ACE2 receptor accessible.

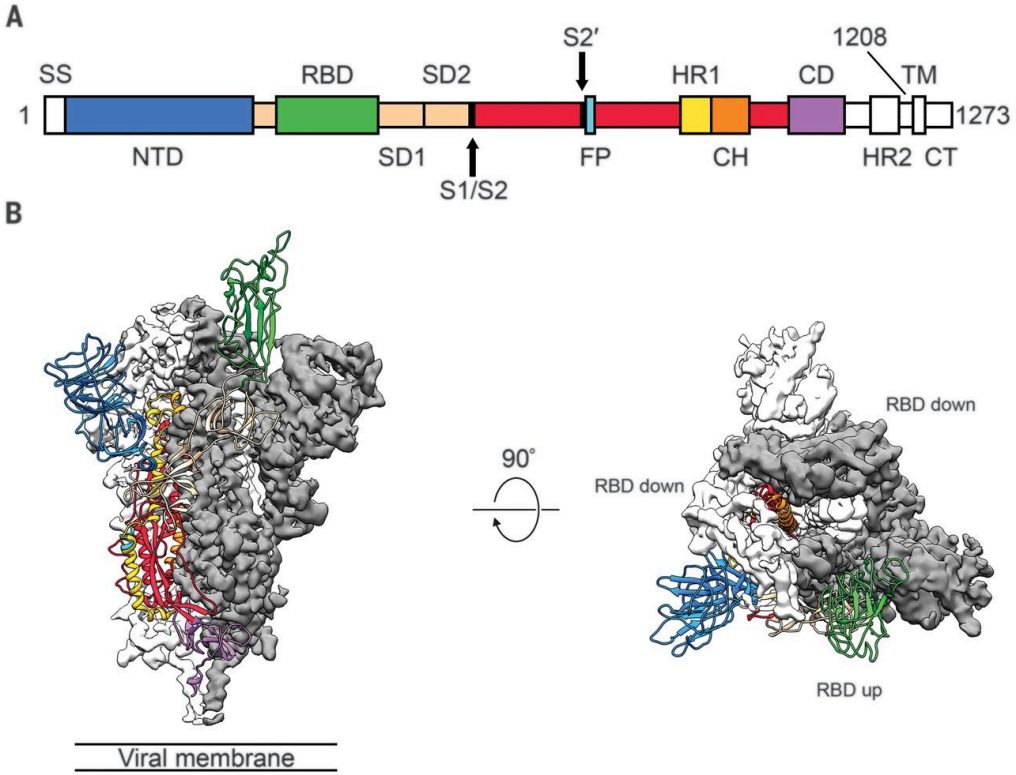
Rapp M, Shapiro L, Frank J (2021) *Trends Biochem Sci*.

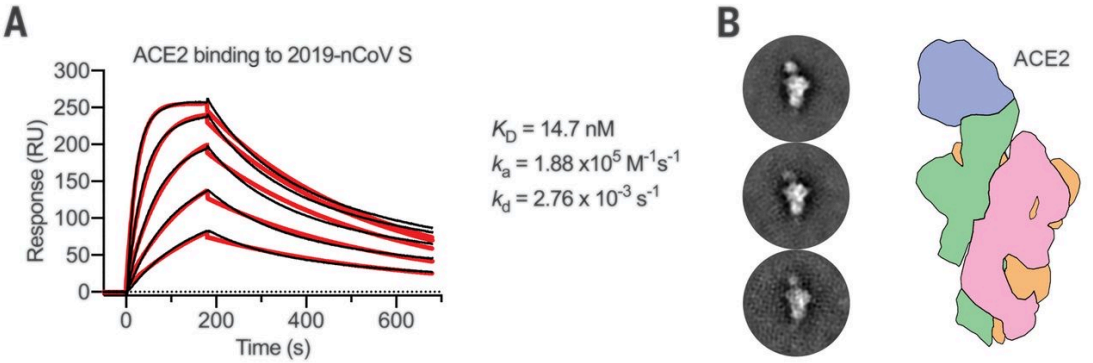
Previous structural data of MERS-CoV discovered two mutations in the spike protein that prevent a post-fusion conformation



Pallsen J et al. (2020) *PNAS*

The double proline mutation also stabilize the receptor-binding conformation of the SARS-CoV-2 Spike

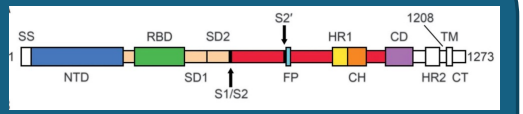




Wrapp D et al. (2020) *Science*

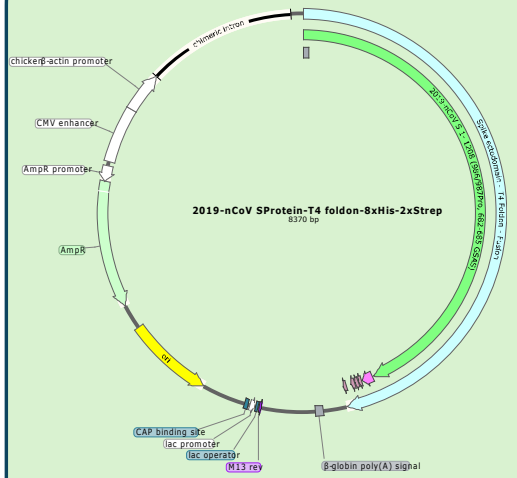
Construct was designed for cryo-EM experiments, but is also very well suitable for serological assays

Good thinking



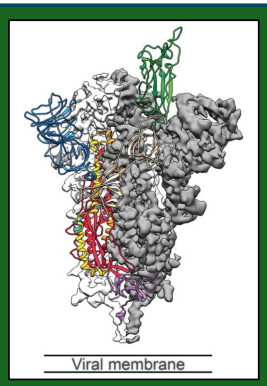
- Signal sequence removed
- Transmembrane domain removed
- Furin cleavage-site mutated
- Two stabilizing proline mutations added
- T4 trimerization domain added
- Purification tags added at C-term (twin-strep and His tags)

Vector Designed



Production in CHO cells
Purification by affinity tags

Great Results

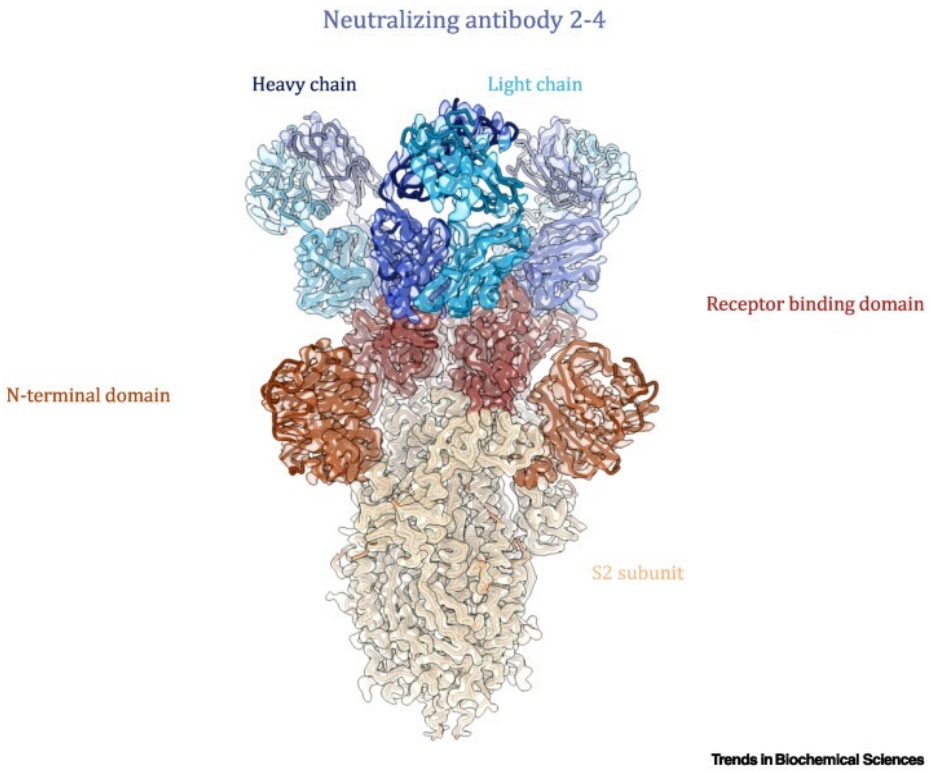


Structure of Spike by CryoEM

Serological tests (CHUV)

Wrapp D et al. (2020) *Science*

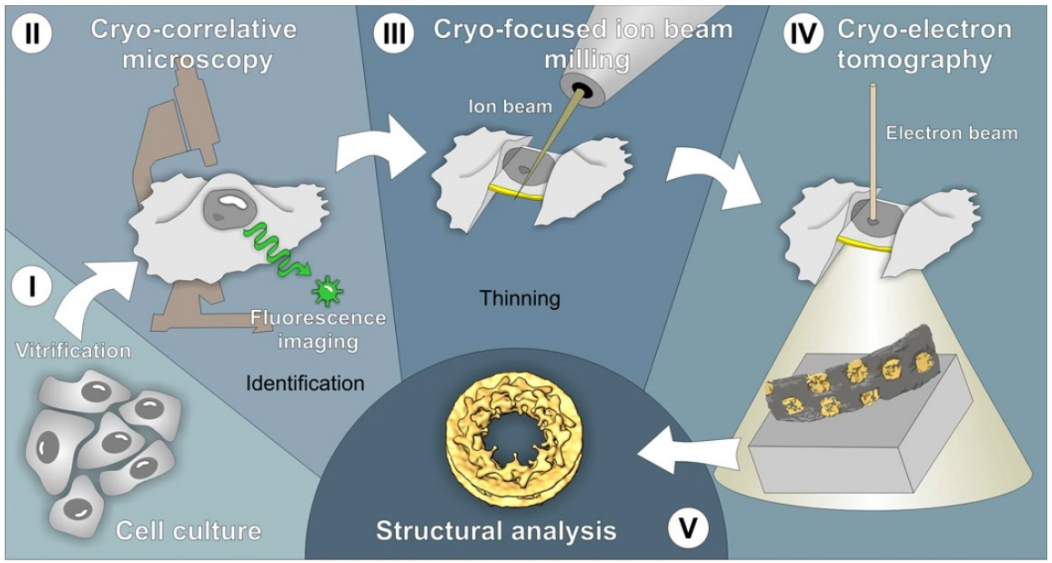
Identification of neutralizing antibodies derived from convalescent COVID-19 patients



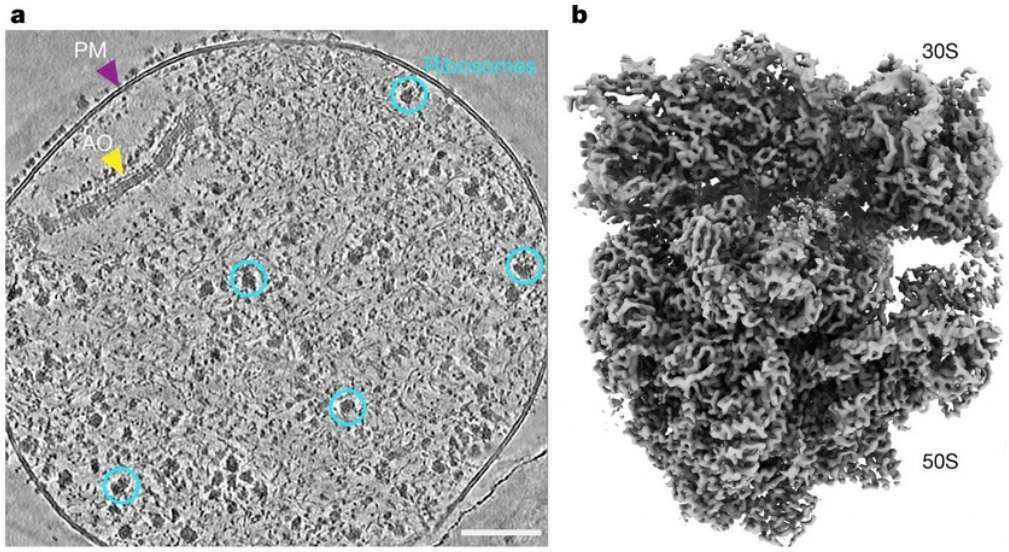
Neutralization occurs by sterically blocking ACE2 interaction or preventing binding-competent conformations.

Rapp M, Shapiro L, Frank J (2021) *Trends Biochem Sci*.

Cryo-electron tomography



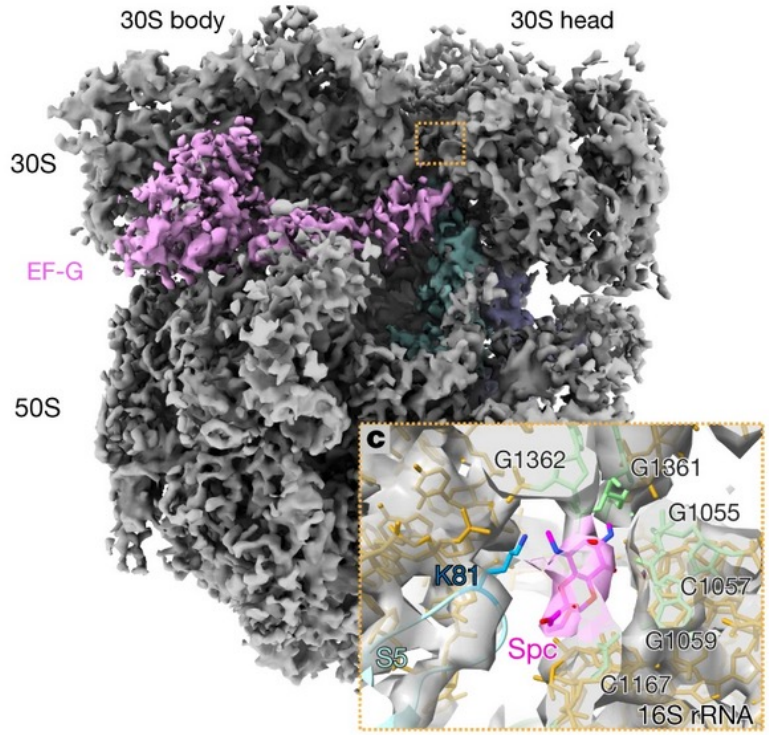
In situ workflow



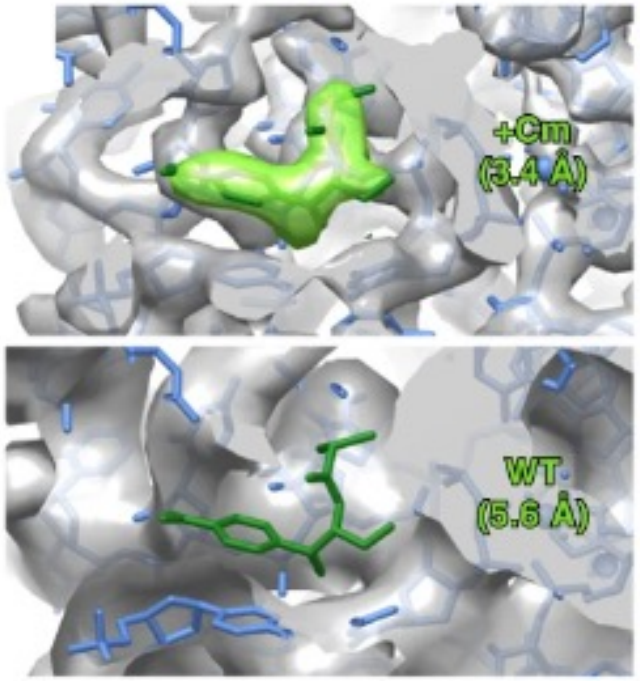
Single particle-like workflow resulting in high resolution reconstruction

Chen L, *et al*. (2024) *J Biochem*.

Antibiotic-bound ribosomes



Xue L, *et al.* (2022) *Nature*



Tegunov D, *et al.* (2021) *Nature Methods*

Cryo-ET Pros and Cons

- Pro

- Most native conditions compared to any structural technique.

- Full complexity of the cellular environment maintained (crowding, PPIs, ultrastructure..).

- Best when studying large and highly populated assemblies.

Cryo-ET Pros and Cons

- Cons

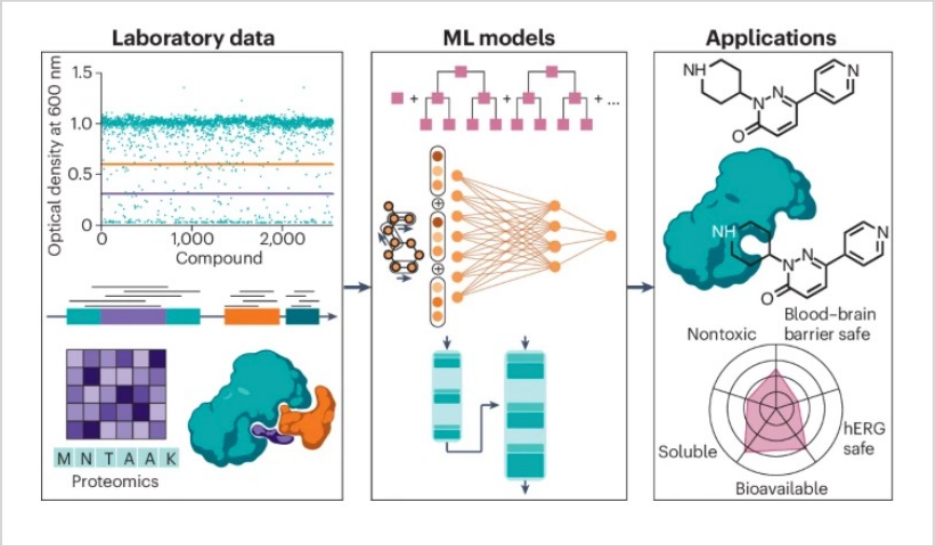
- Very low throughput, especially for in situ cryo-ET.

- Single particle workflow is currently limited to a few large and distiguishable complexes (ribosome, proteasome, nuclear pore complex…)

Machine learning in drug discovery

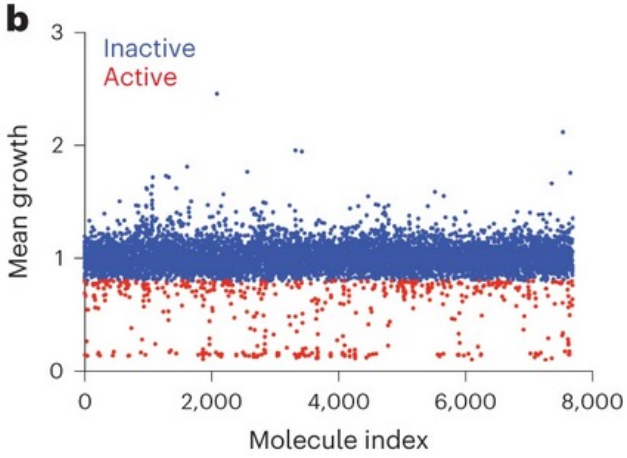
- Traditional approaches to drug discovery has a steep failure rate and incur significant costs.

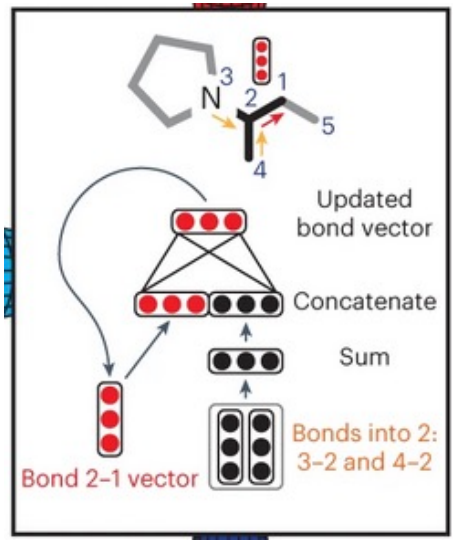
- Advances in AI-based techniques may accelerate drug discovery while reducing both failure rate and associated costs.

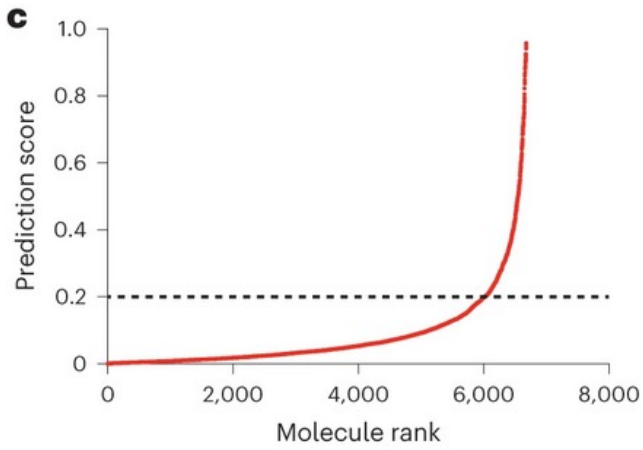


Catacutan DB, *et al.* (2024) *Nature Chemical Biology*

Identification of antibiotic abaucin through deep learning-guided discovery



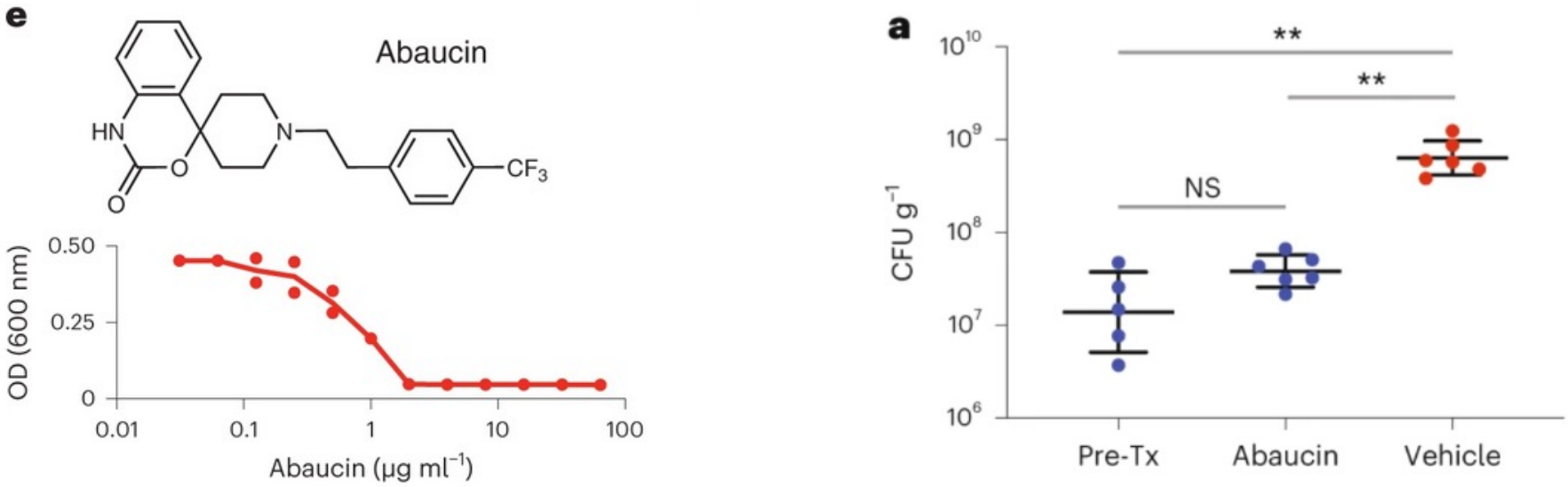




~7,500 molecules were screen for growth inhibition for *A. baumannii*, serving as a training dataset for ML which associated structural information with predicted antiobiotic activity.

Liu G, *et al.* (2023) *Nature Chemical Biology*

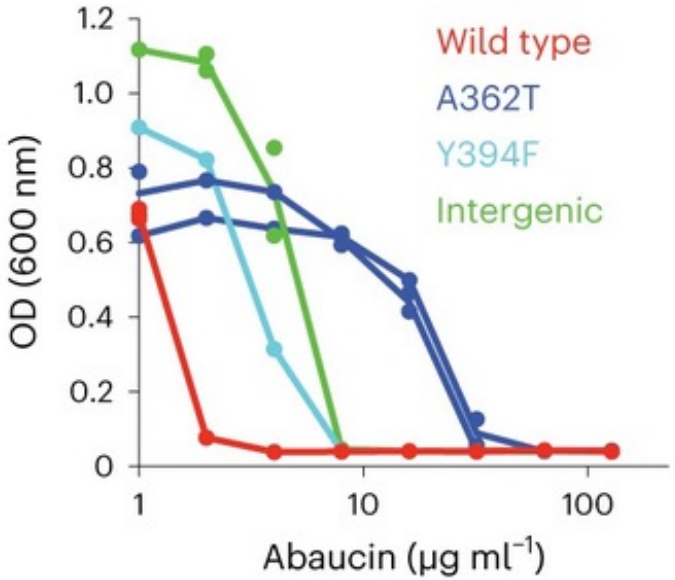
Identification of antibiotic abaucin through deep learning-guided discovery

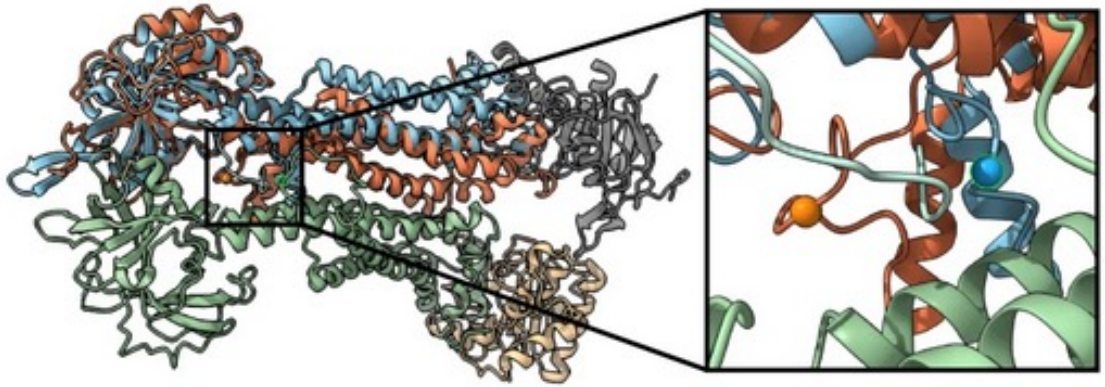


Abaucin shows selective potency against *A. baumannii*, and can suppress infection in a wound model.

Liu G, *et al.* (2023) *Nature Chemical Biology*

Abaucin-resistant clones displayed mutations in lipoprotein trafficking protein LolE





E. coli LolE structure compared to predicted structure of *A. baumannii* LolE

Liu G, *et al.* (2023) *Nature Chemical Biology*

Thank you!

Any Questions?