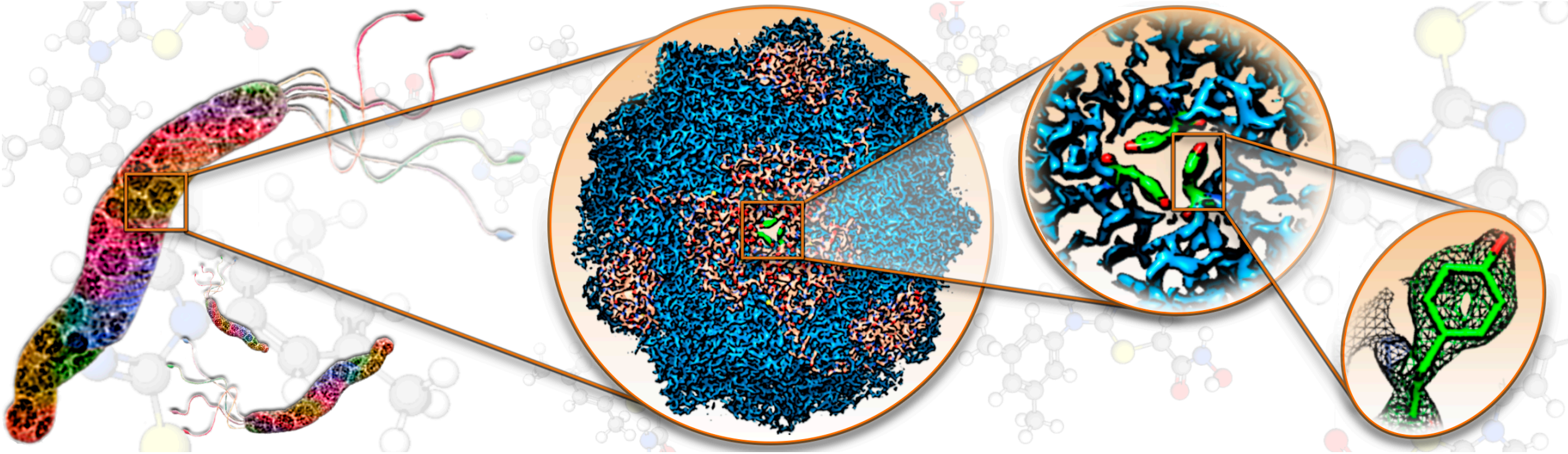
NCMM
Centre for Molecular Medicine Norway
Nordic EMBL partnership for Molecular Medicine

Breaking cryo-EM resolution limits enabling structure based drug discovery targeting Helicobacter pylori, a WHO class I carcinogen

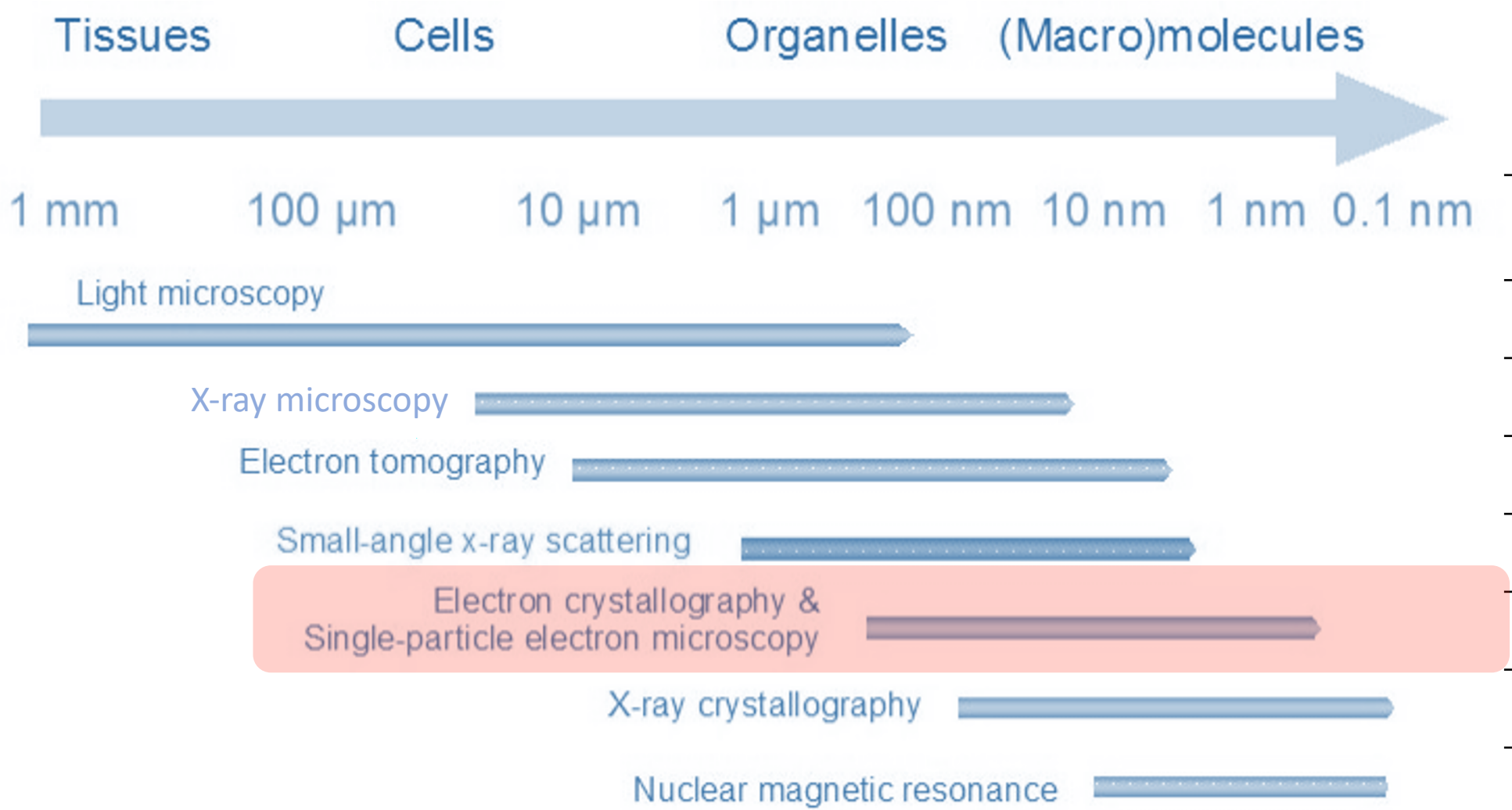
NORDIC **EMBL**
PARTNERSHIP FOR
MOLECULAR MEDICINE



Cunha E. S.* *et al. (Nature Communications, 2021)*

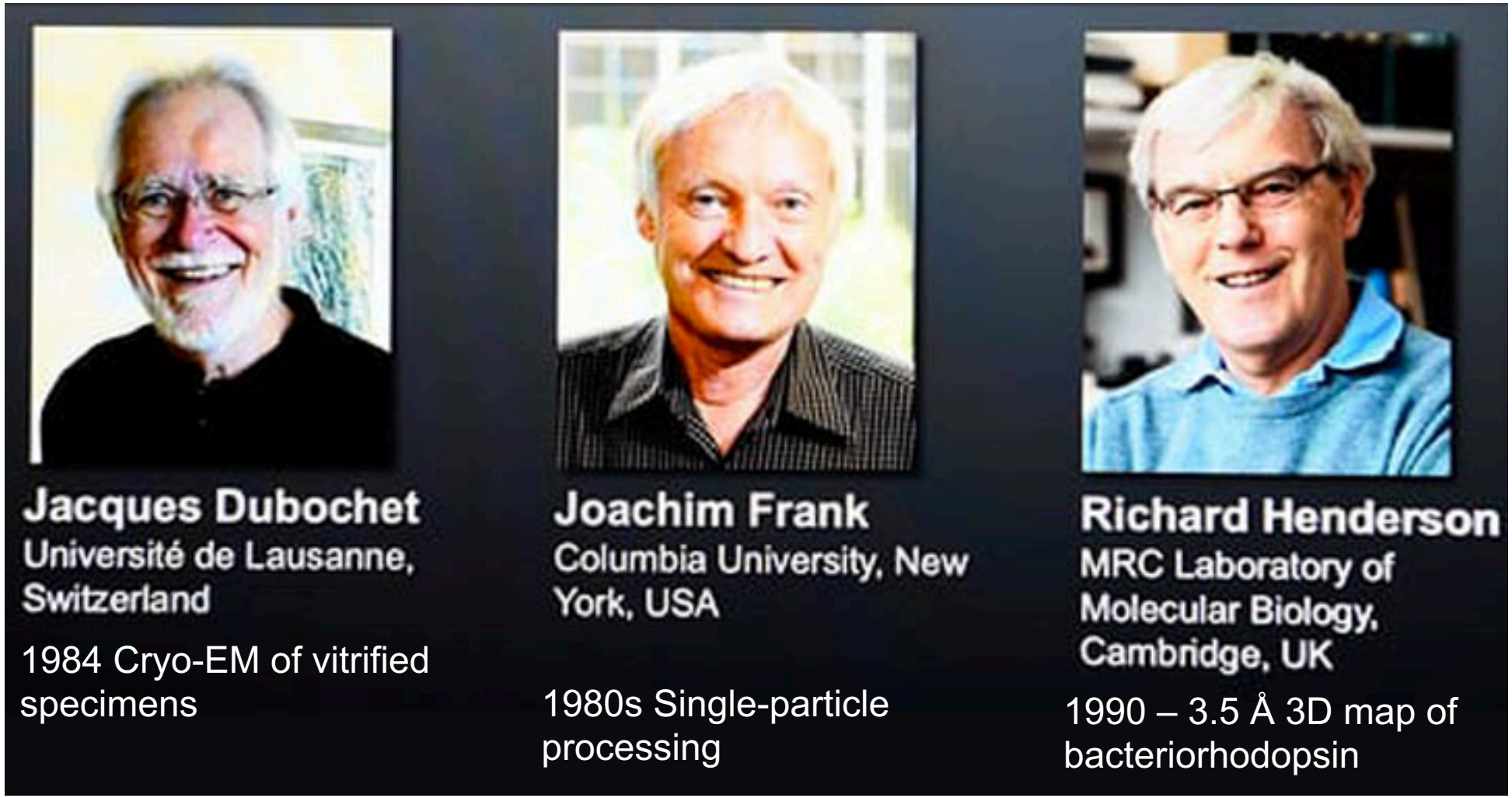
Eva S. Cunha
Researcher @ Hartmut ‘Hudel’ Luecke group
Structural Biology and Drug Discovery Group

What can we see with Cryo-EM?

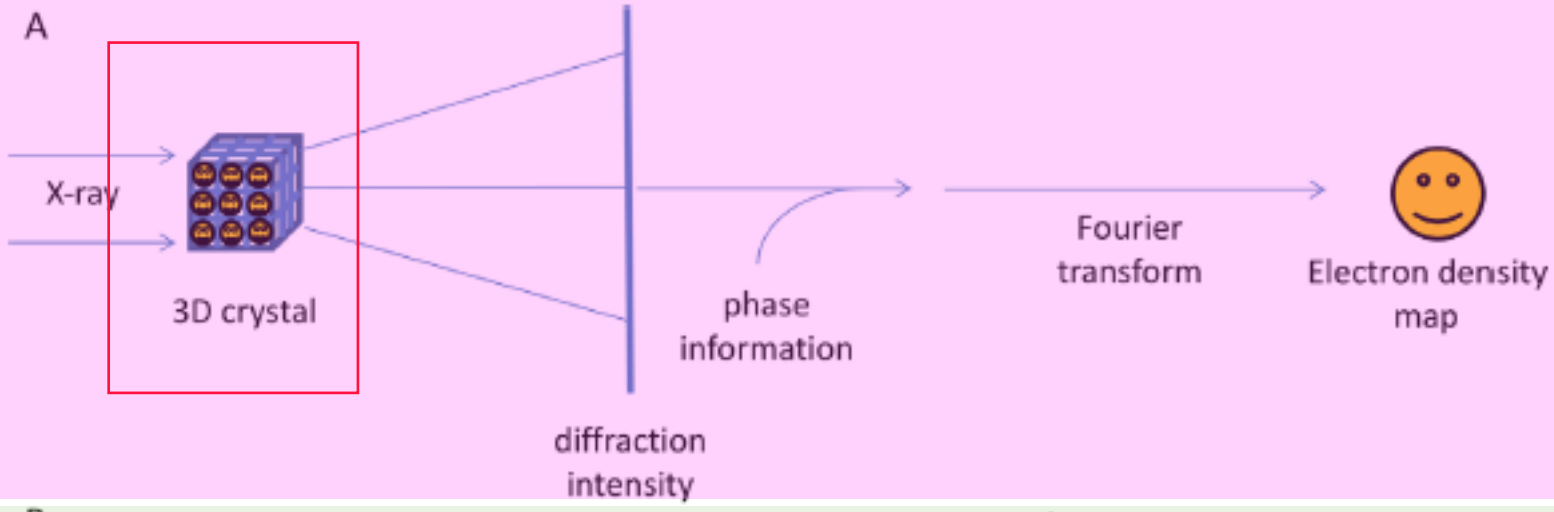


Approximate Max Res/ nm
200
30
2
2
0.2
0.1
0.1

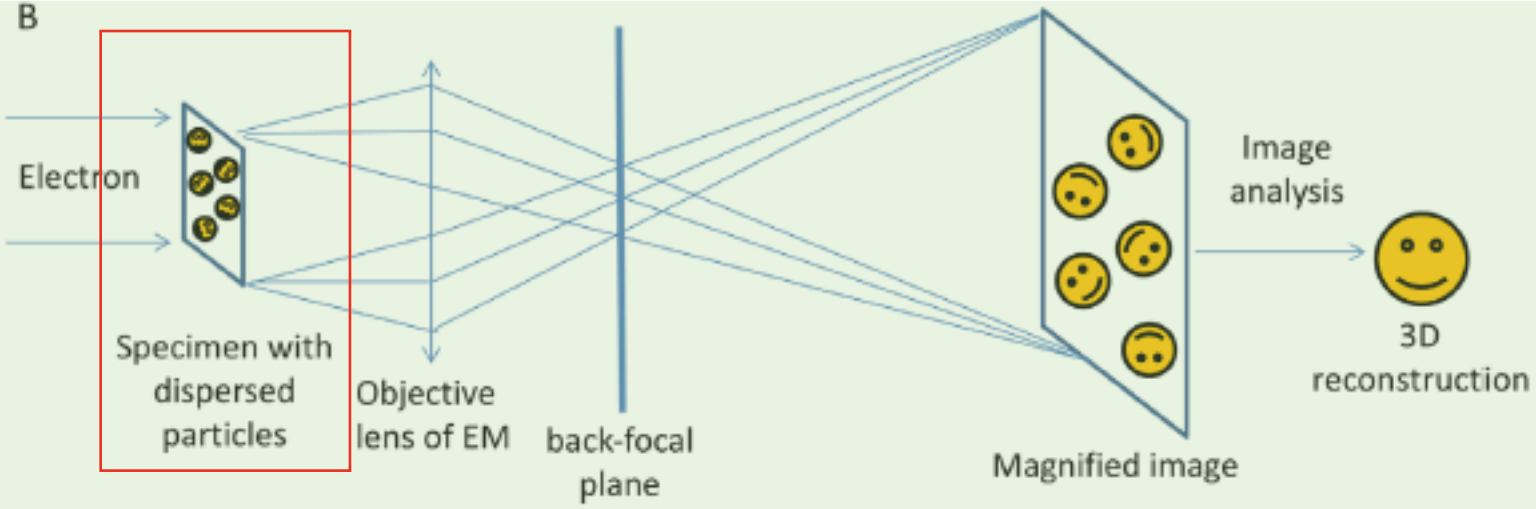
Nobel prize in chemistry in 2017



X-ray crystallography vs Cryo-EM



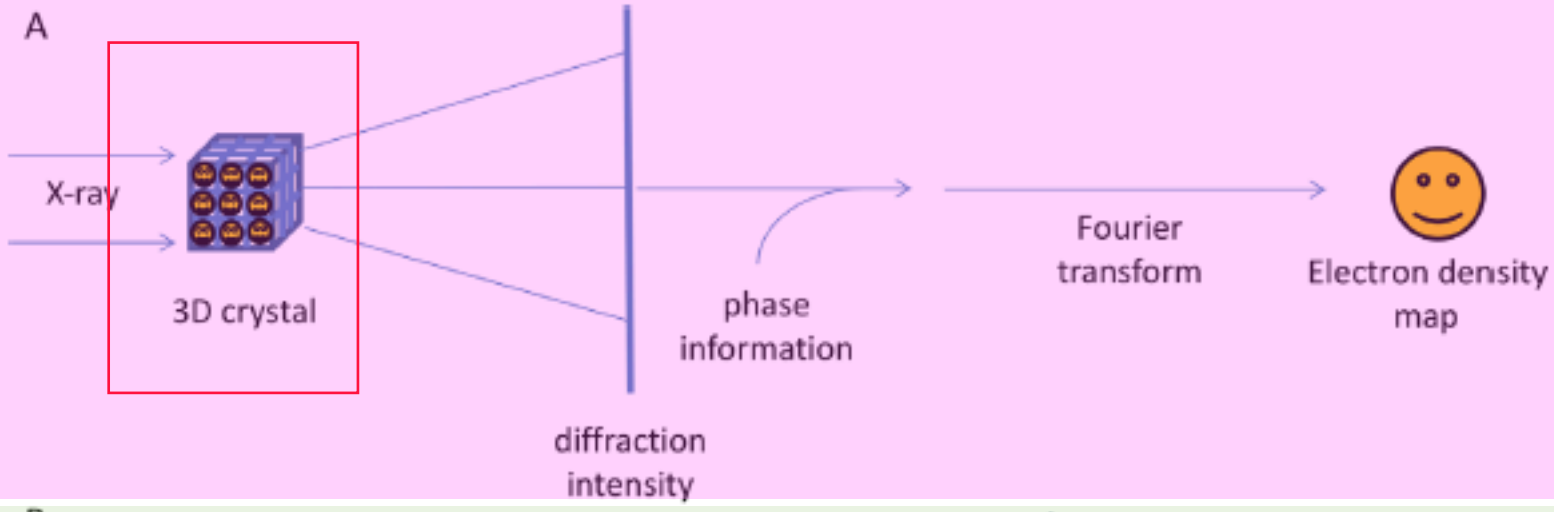
- Larger amount of sample (mg)



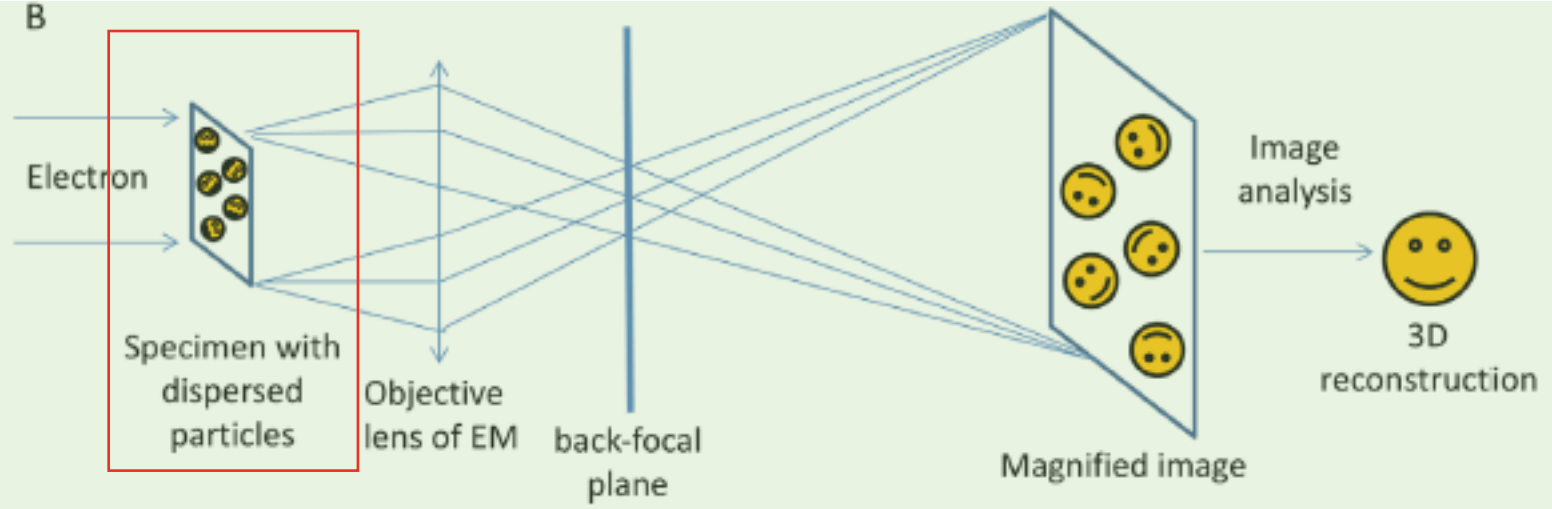
- Lower amount of sample (ug)

Wang, 2016

X-ray crystallography vs Cryo-EM



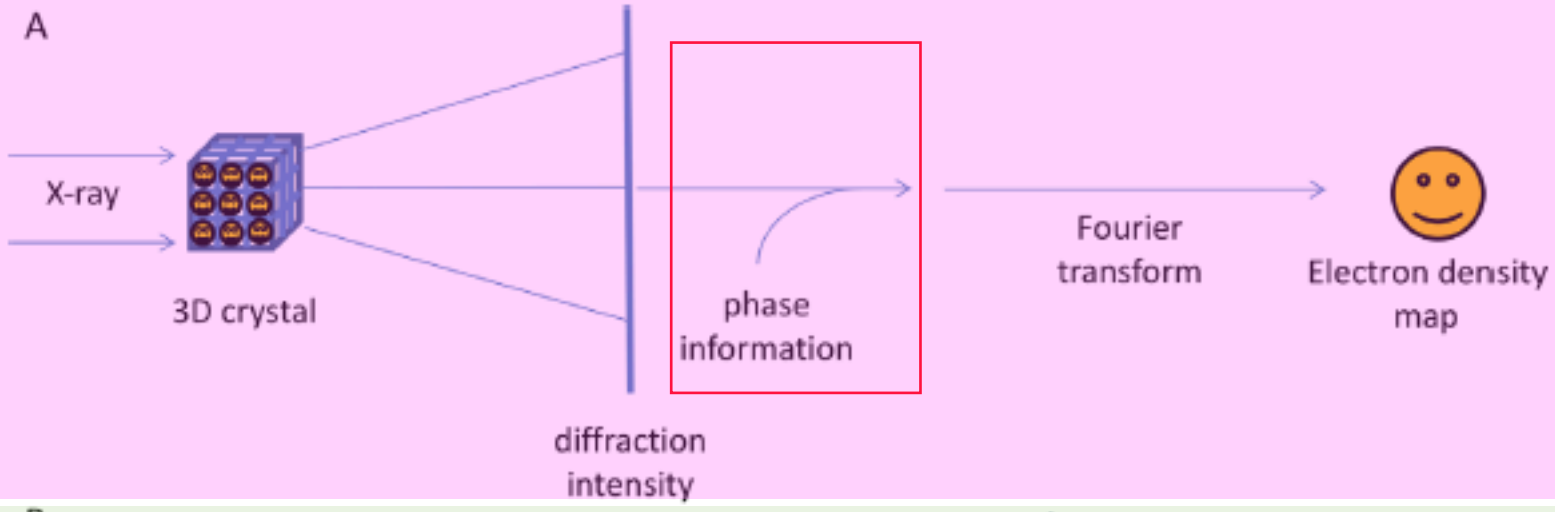
- Larger amount of sample (mg)
- Needs well ordered 3D crystals



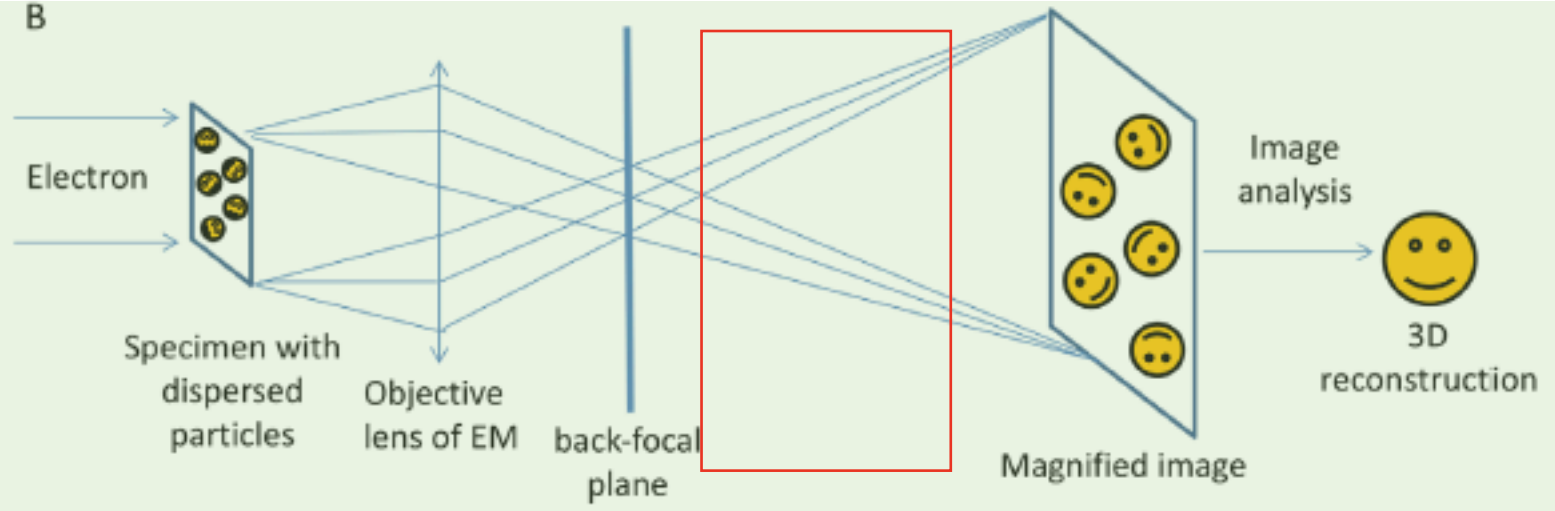
- Lower amount of sample (ug)
- Needs a optimized cryo-grid
- Structure in more “native-like” conditions

Wang, 2016

X-ray crystallography vs Cryo-EM



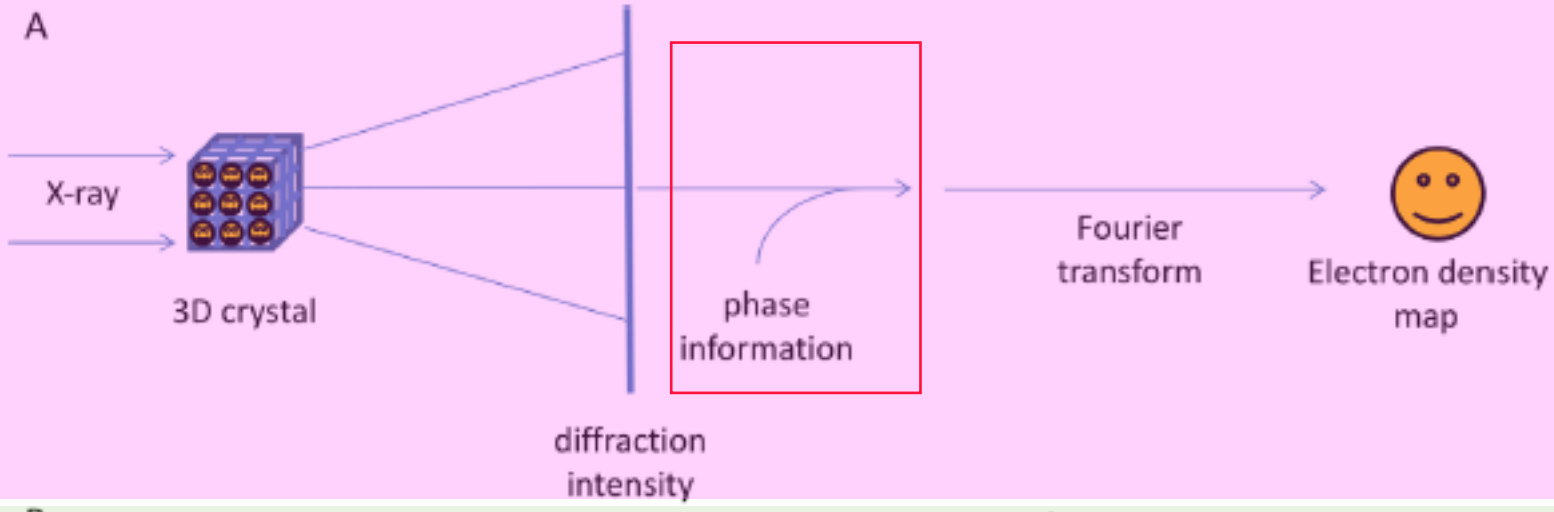
- Larger amount of sample (mg)
- Needs well ordered 3D crystals
- Needs phases
- Can achieve higher resolution (1-3Å)



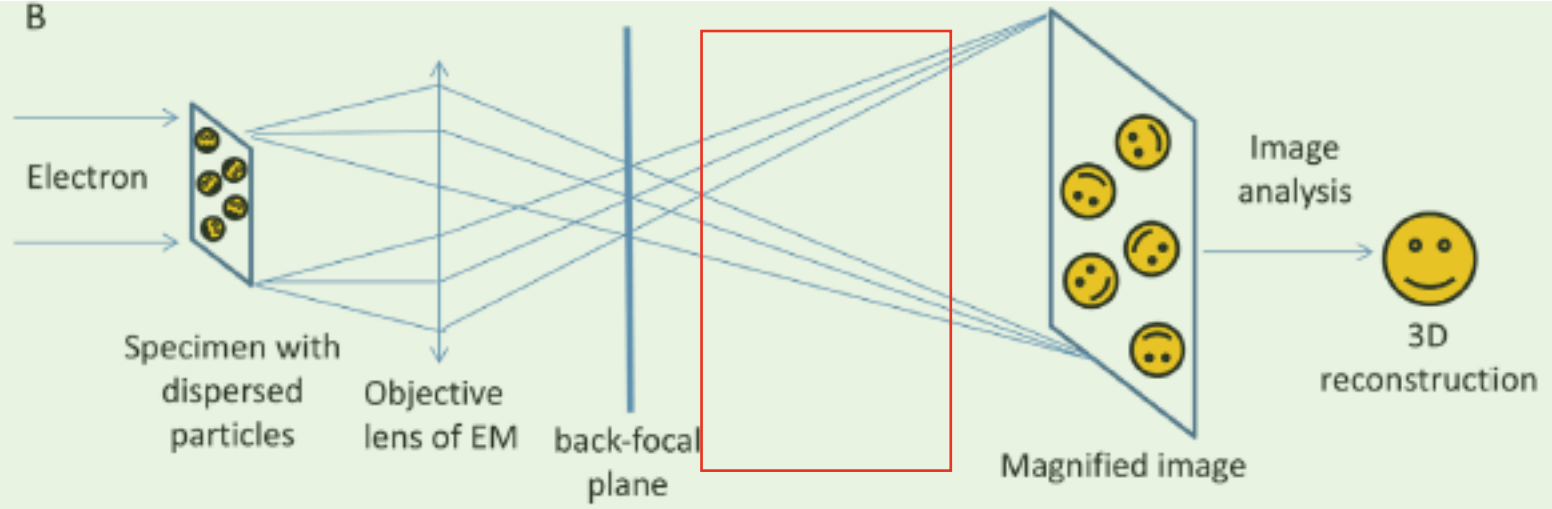
- Lower amount of sample (ug)
- Needs a optimized cryo-grid
- Structure in more “native-like” conditions
- Does not need phases
- Typical lower resolution (3-5Å)

Wang, 2016

X-ray crystallography vs Cryo-EM



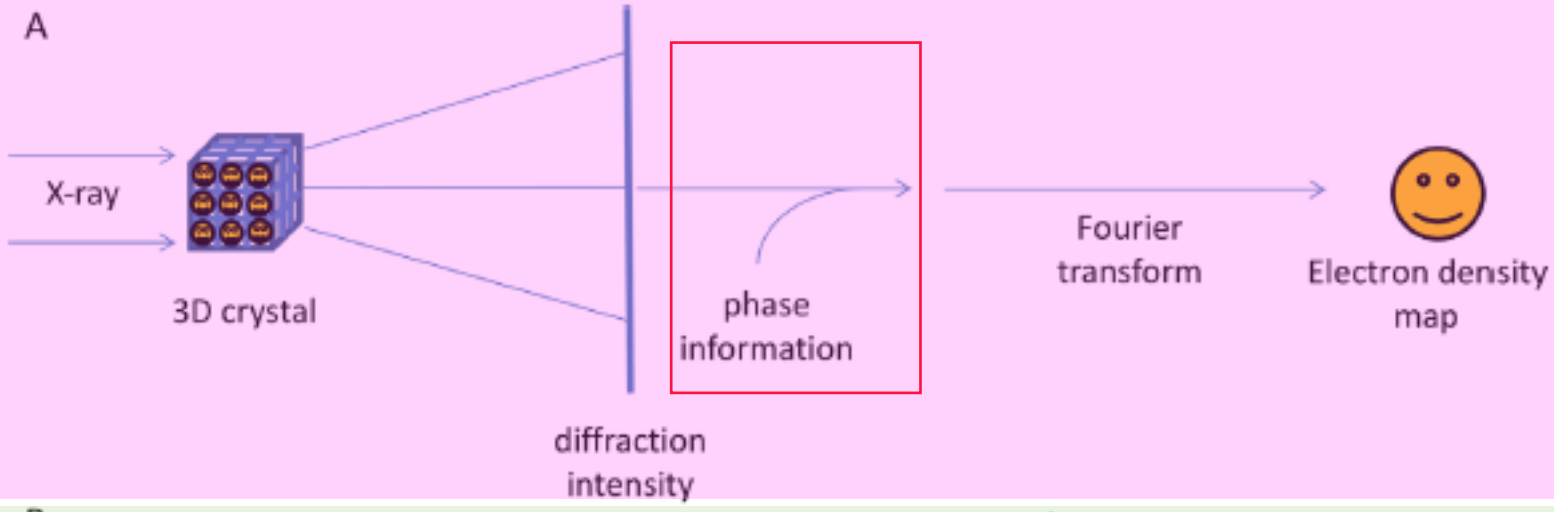
- Larger amount of sample (mg)
- Needs well ordered 3D crystals
- Needs phases
- Can achieve higher resolution (1-3Å)
- Typically one conformation in a crystal



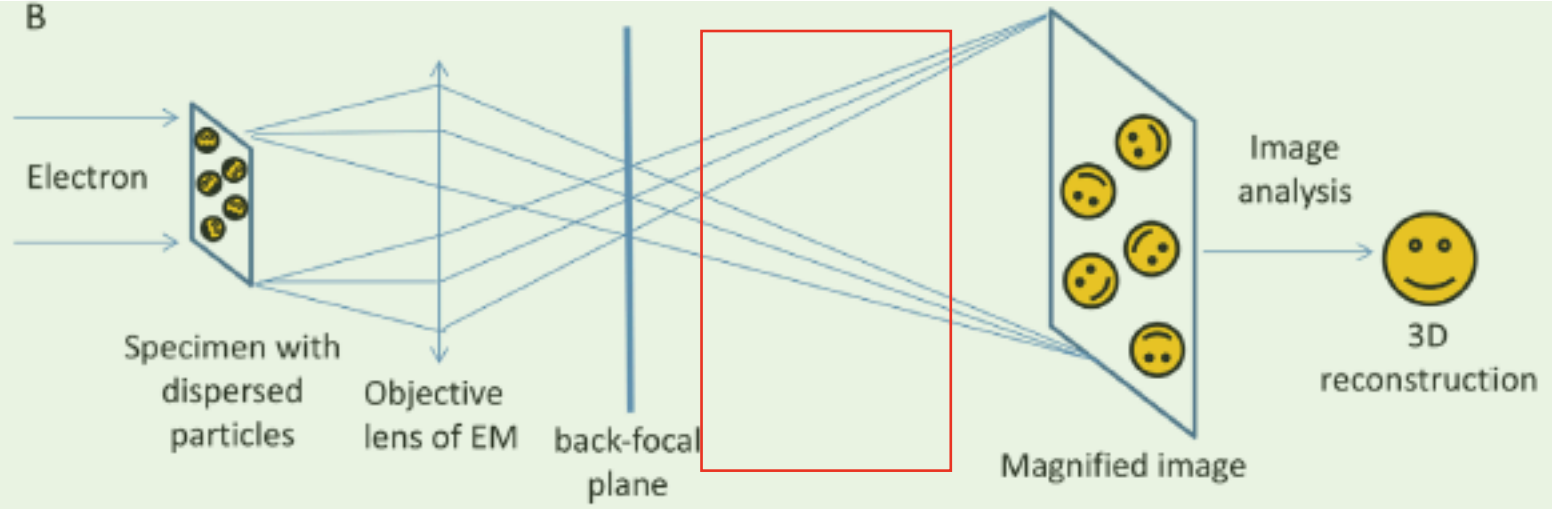
- Lower amount of sample (ug)
- Needs a optimized cryo-grid
- Structure in more “native-like” conditions
- Does not need phases
- Typical lower resolution (3-5Å)
- Allows conformational heterogeneity in one dataset

Wang, 2016

X-ray crystallography vs Cryo-EM



- Larger amount of sample (mg)
- Needs well ordered 3D crystals
- Needs phases
- Can achieve higher resolution (1-3Å)
- Typically one conformation in a crystal
- Data collection in minutes



- Lower amount of sample (ug)
- Needs a optimized cryo-grid
- Structure in more “native-like” conditions
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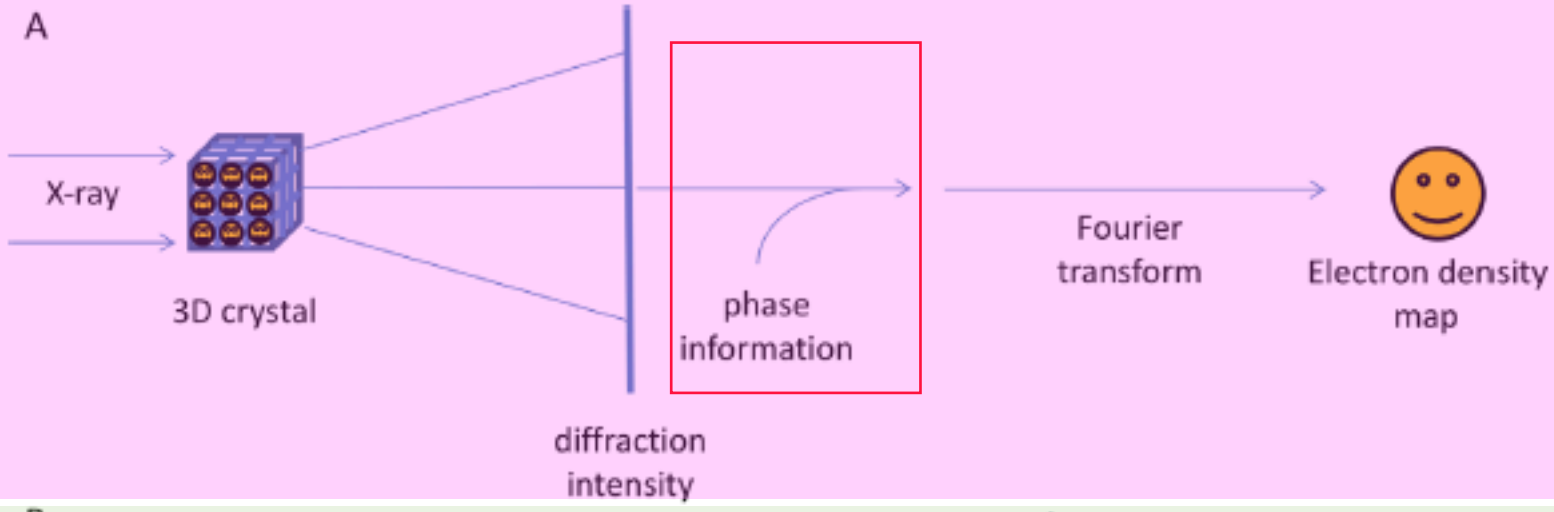
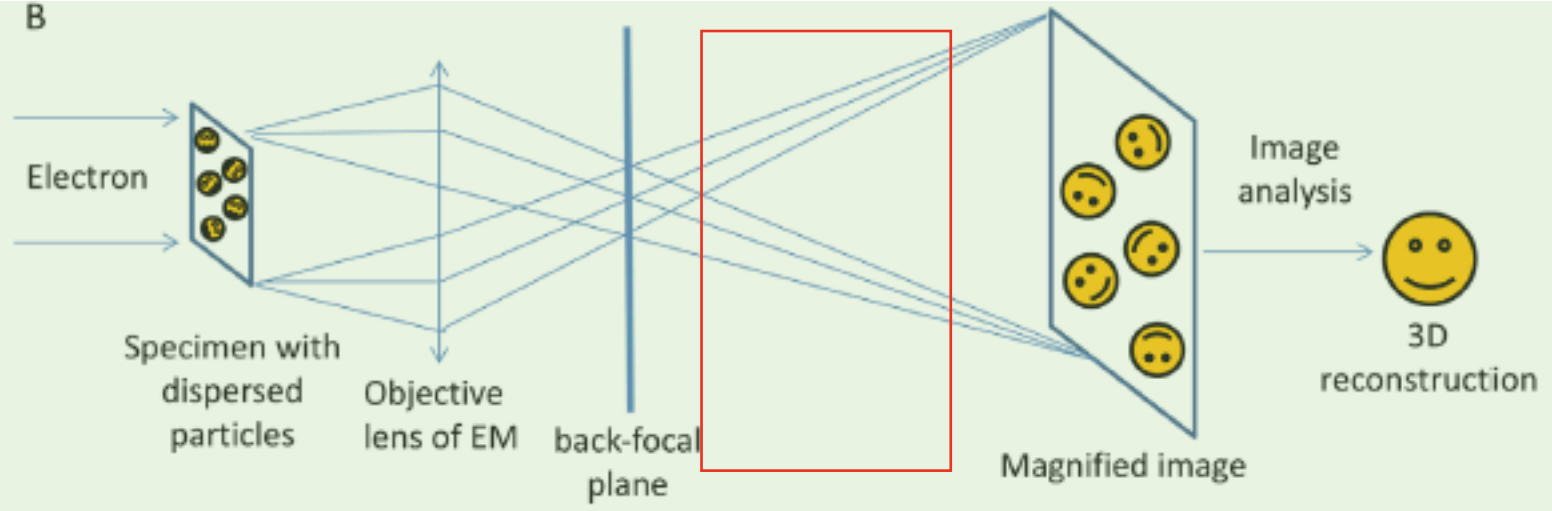
- Data collection in days

Wang, 2016

X-ray crystallography vs Cryo-EM



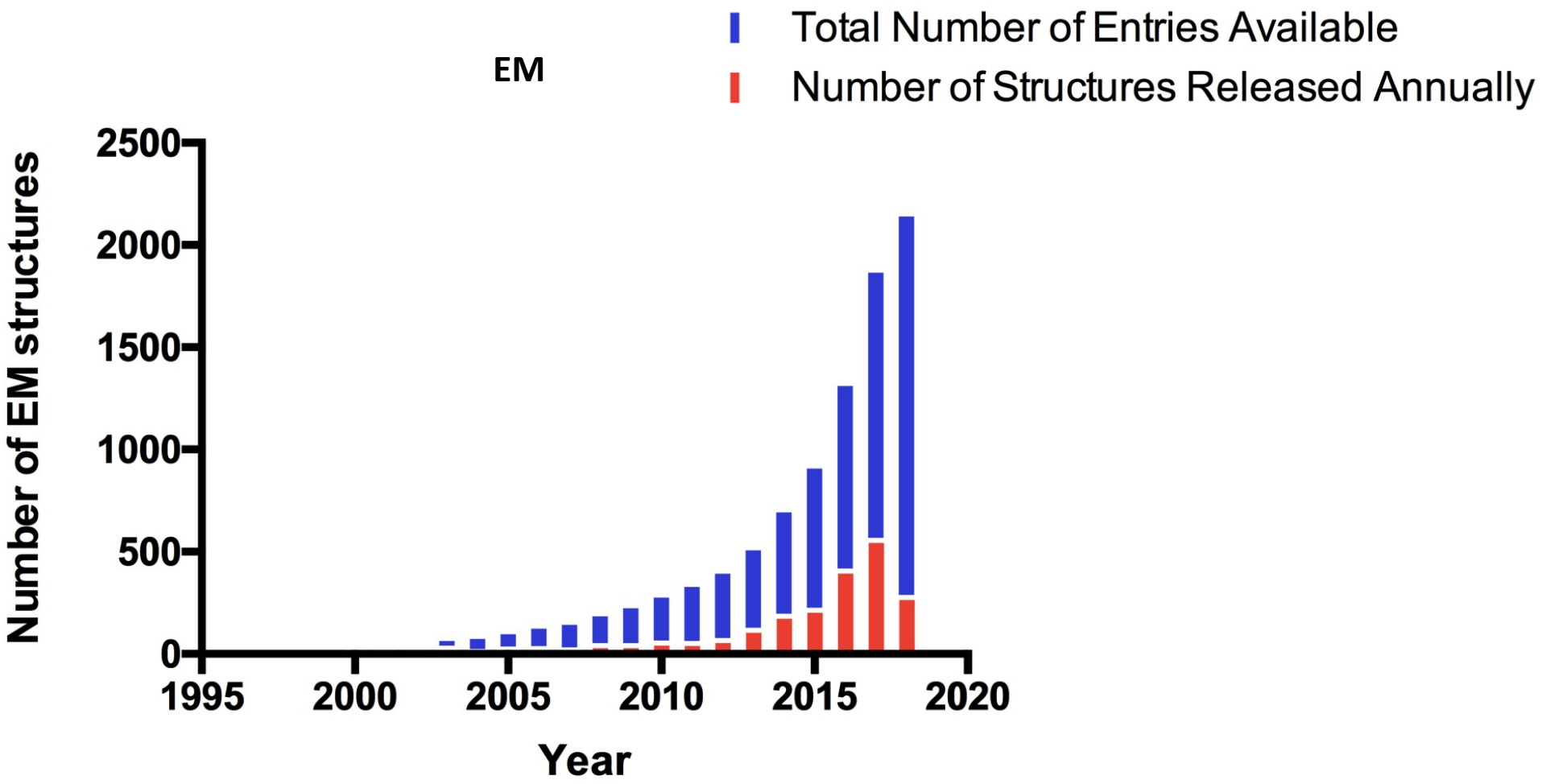
- Larger amount of sample (mg)
- Needs well ordered 3D crystals
- Needs phases
- Can achieve higher resolution (1-3Å)
- Typically one conformation in a crystal
- Data collection in minutes
- Needs GBs of data storage



- Lower amount of sample (ug)
- Needs a optimized cryo-grid
- Structure in more “native-like” conditions
- Does not need phases
- Typical lower resolution (3-5Å)
- Allows conformational heterogeneity in one dataset
- Data collection in days
- Needs TBs of data storage

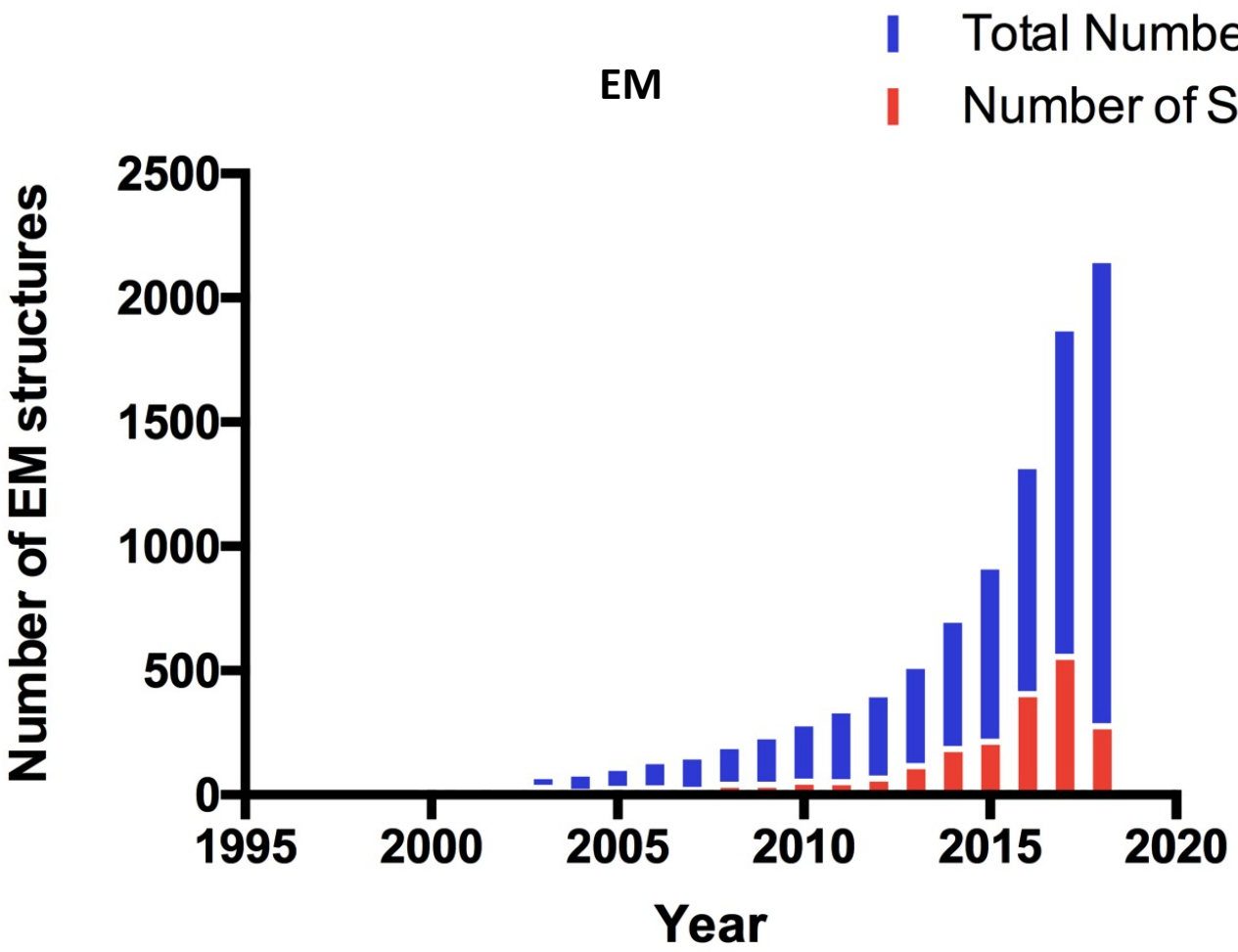
Wang, 2016

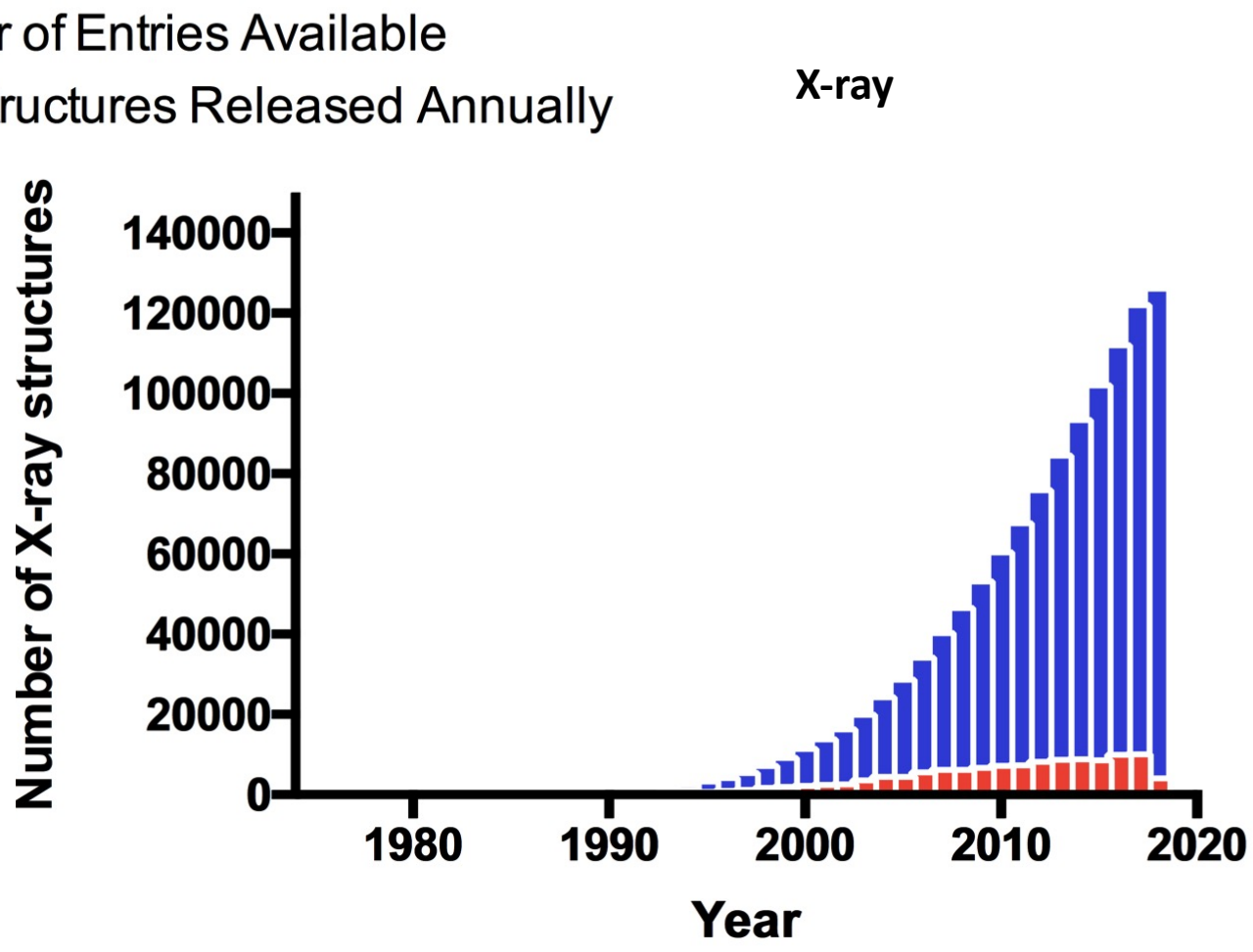
Cryo-EM vs X-ray crystallography



https://www.ebi.ac.uk/pdbe/emdb/

Cryo-EM vs X-ray crystallography





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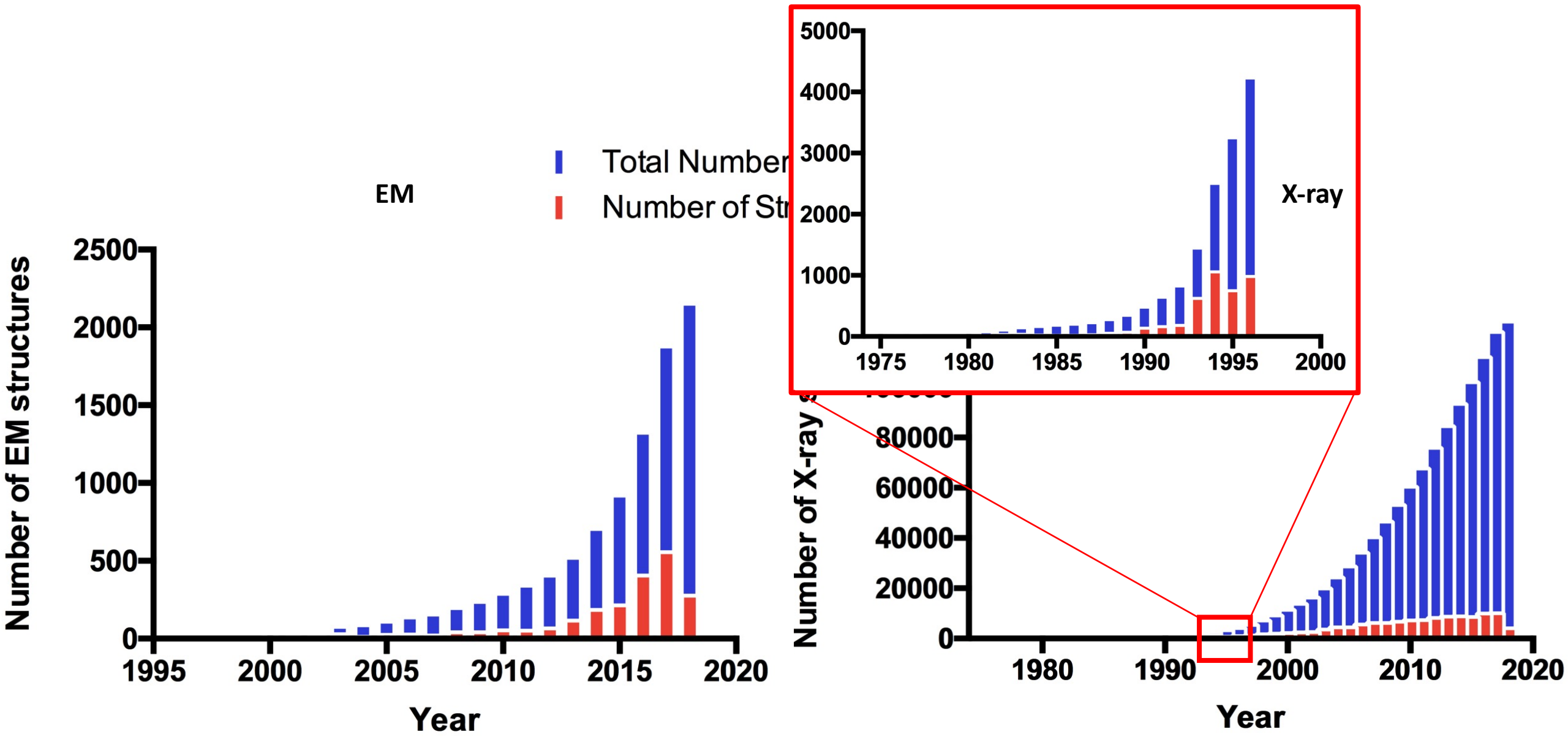
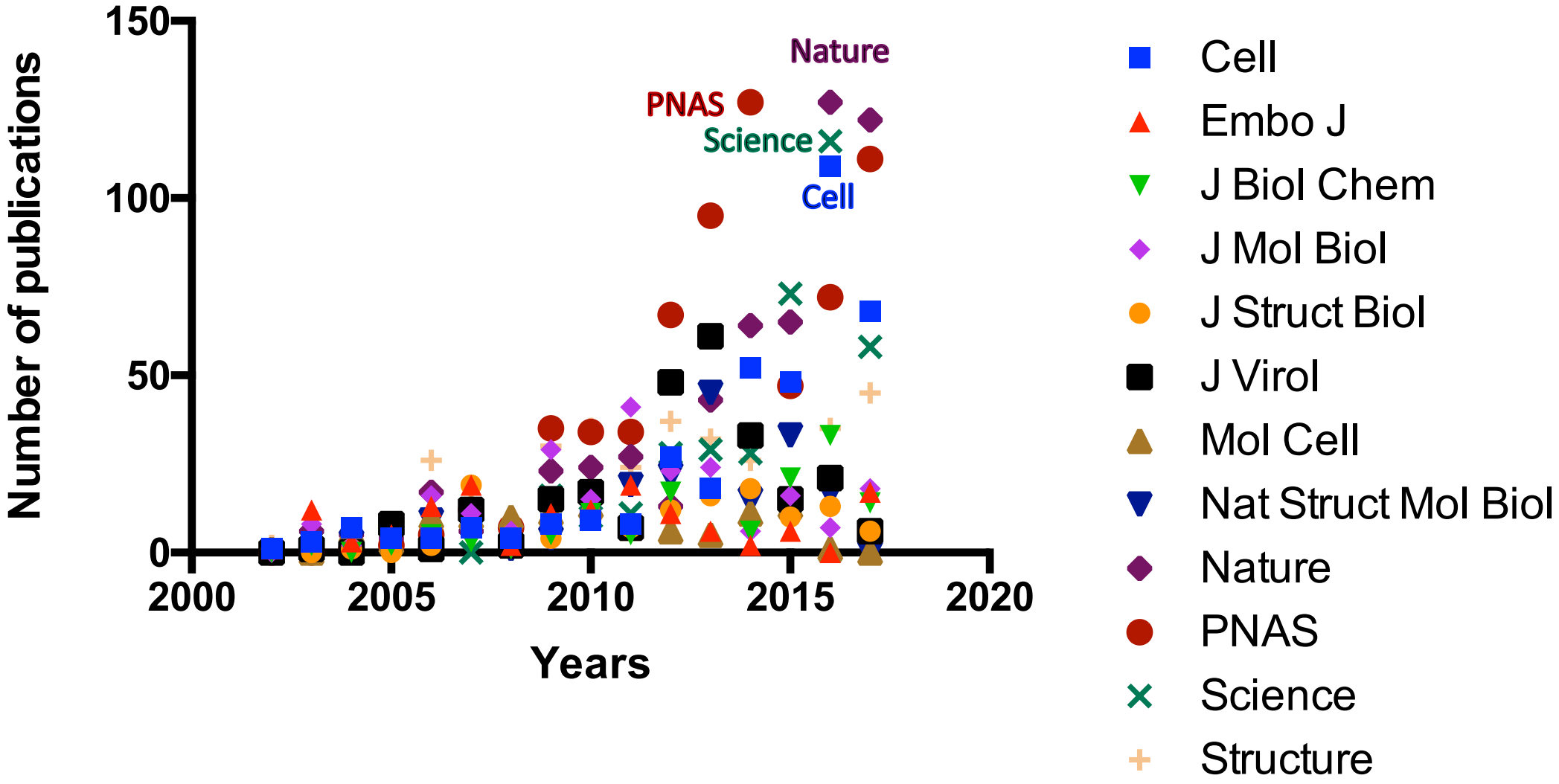
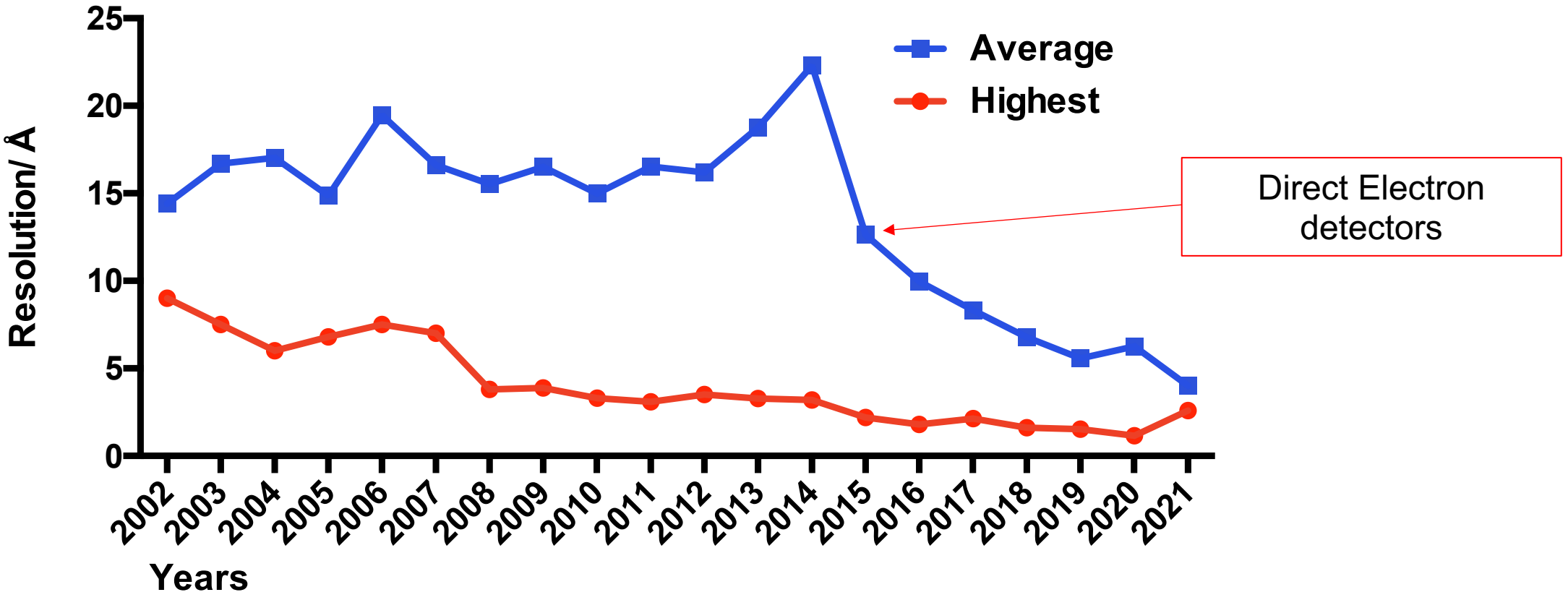


https://www.ebi.ac.uk/pdbe/emdb/

Cryo-EM resolution revolution



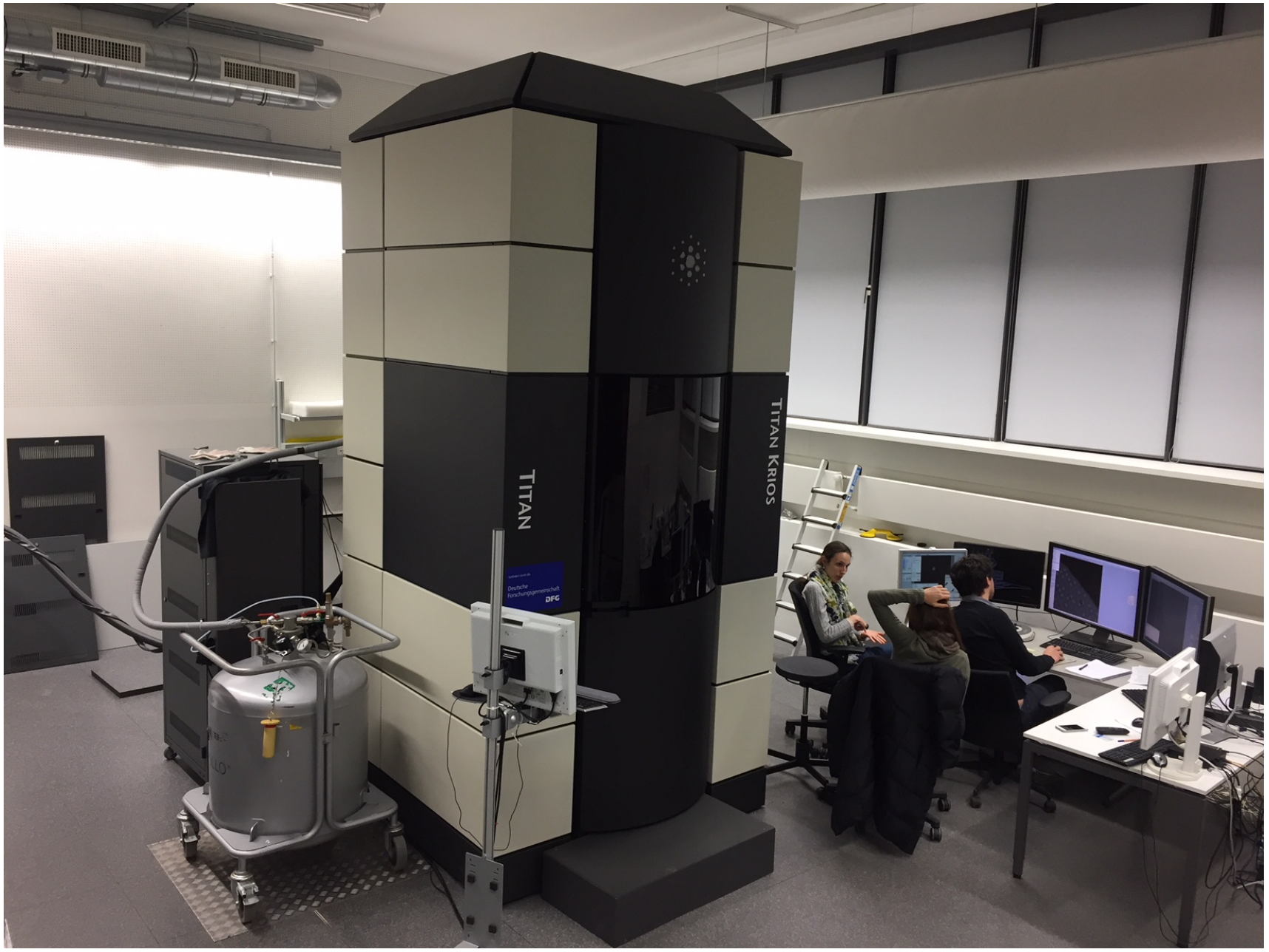
Cryo-EM resolution revolution



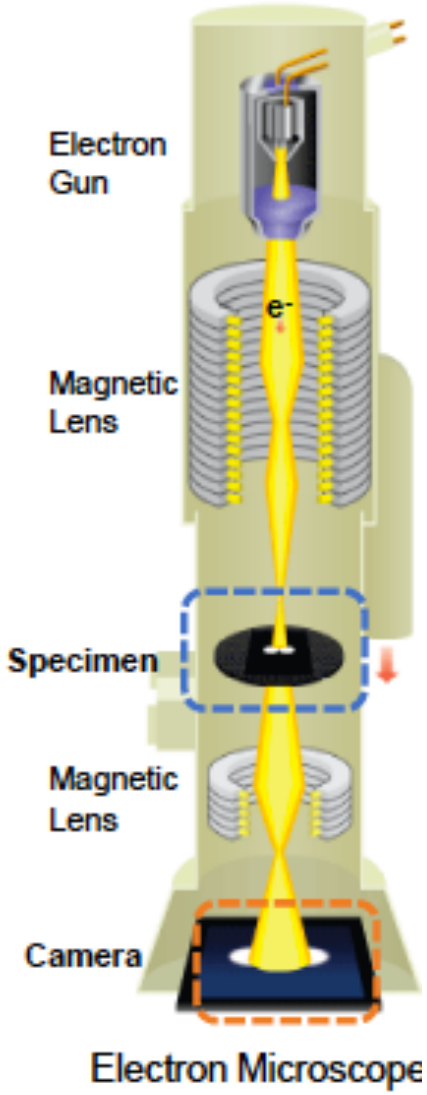
https://www.ebi.ac.uk/pdbe/emdb/

A Titan Krios requires a shielded room about 6 meters high

5 Million Euros



Cryo-EM microscope composition and types

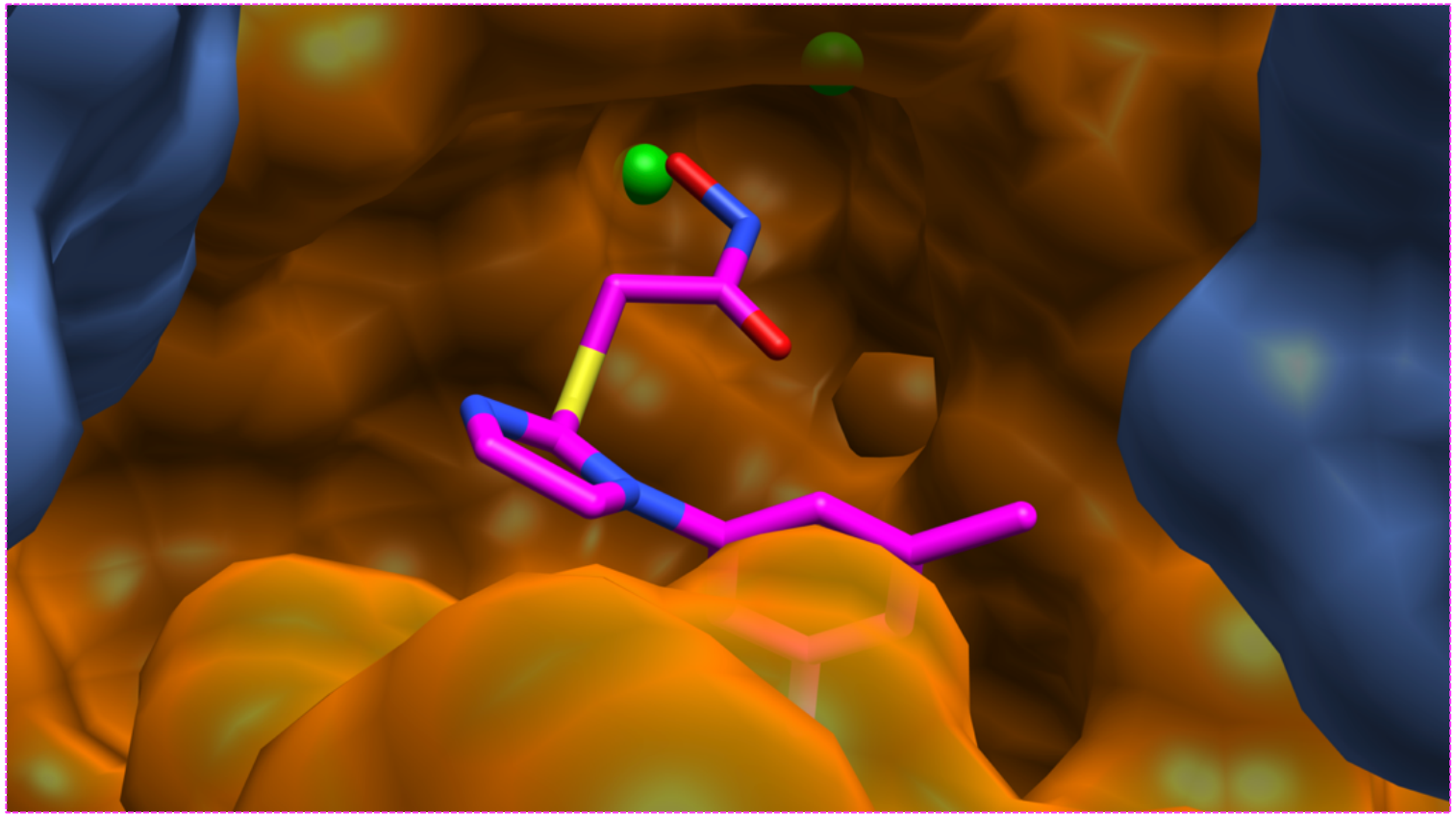


Microscope class	Typical examples	~ marginal cost/day (in 2016 Euros, including detectors)
Entry level	FEI T12, JEOL 1400	250
Mid-range	FEI F20/Talos, JEOL 2100F	600
Upper-mid-range	FEI F30/Polara, JEOL 3200FS	1000
High-end	FEI Titan Krios	3000

https://www.med.uio.no/ncmm/english/news-and-events/news/2018/the-case-for-cryo-em-in-norway.html

Passmore, 2016

What is important in a cryo-EM laboratory?



Cunha E. S. *et al.** *(Nature Communications, 2021)*

Cryo-EM state-of-the-art installation for high-resolution structure determination

< 30 % ambient humidity

Stable temperature

Hood for liquid ethane

Clean liquid nitrogen

Cold FEG

Gatan 20 eV/Selectris 10 eV energy filter

K3 or Falcon 4 direct electron detector

128 Cores

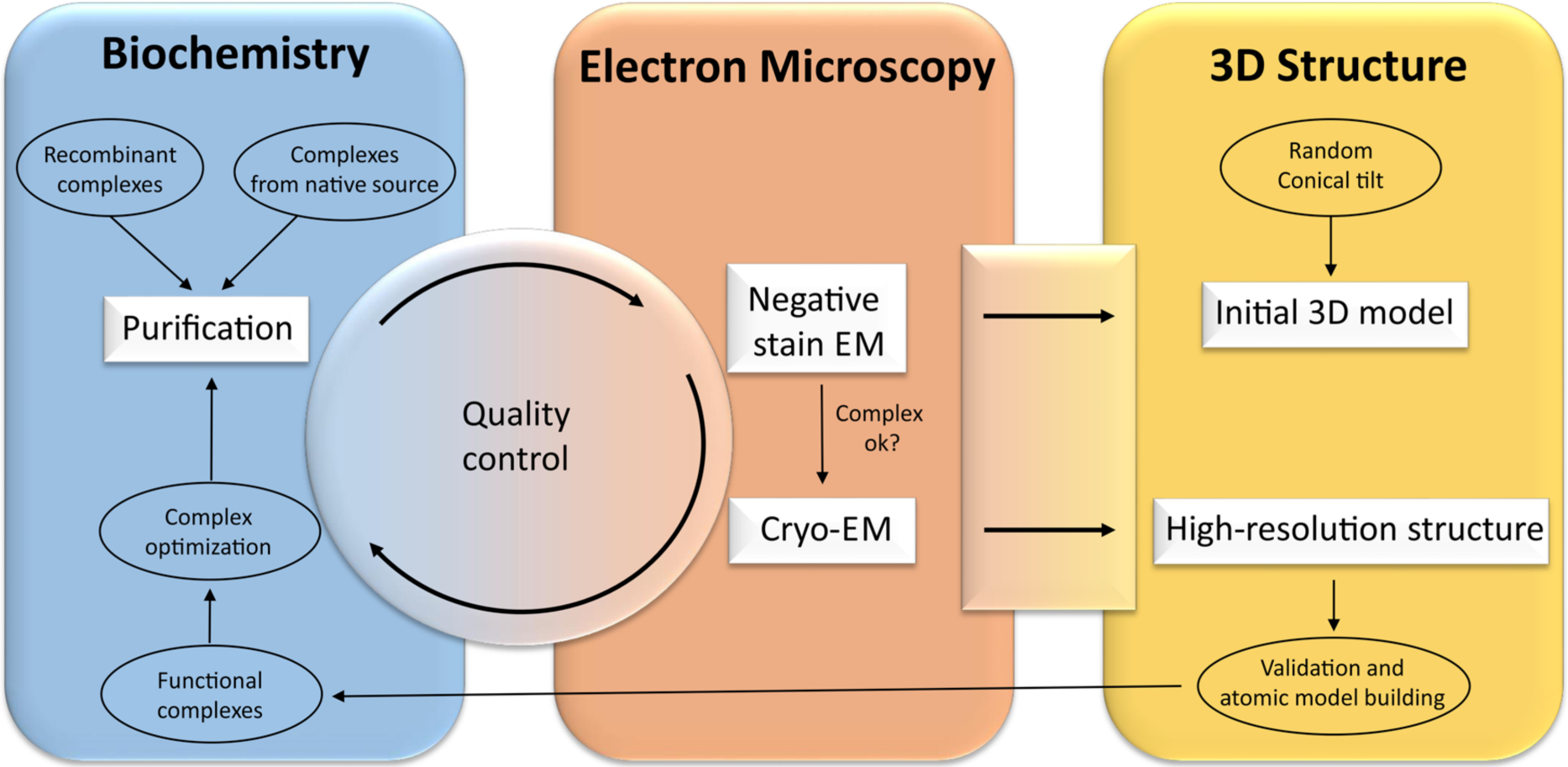
Large memory (1 TB)

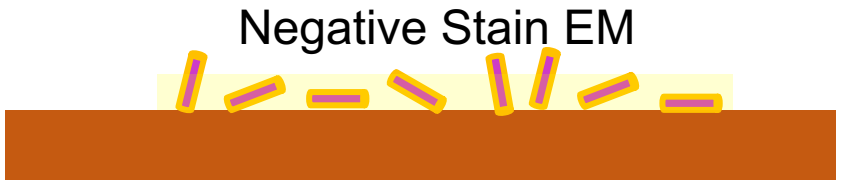
GPUs (40 GB each)

100s TB of storage

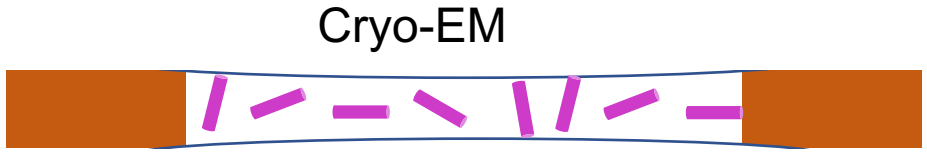
High-throughput data transfer (Infiniband)

Cryo-EM sample preparation pipeline





~15-20 Å
Lower concentration of sample



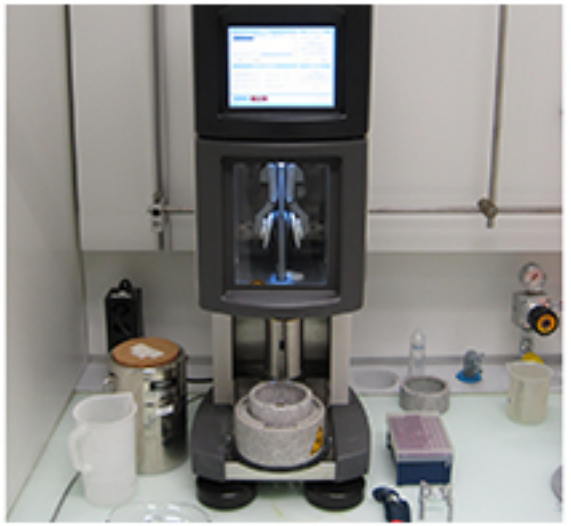
~2-5 Å
Higher concentration of sample

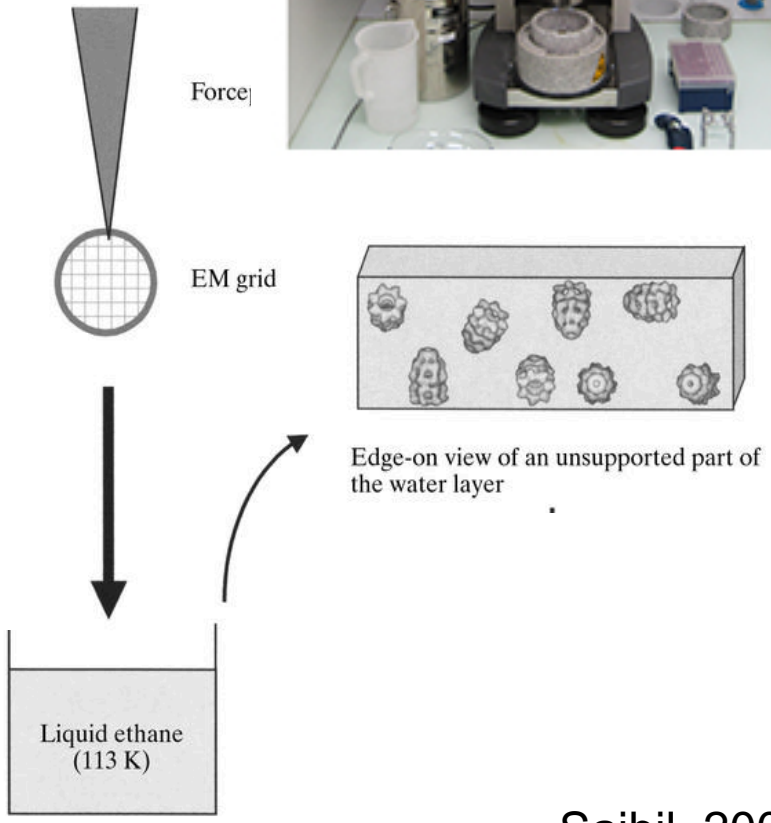
Cryo-samples are frozen in liquid ethane to get vitrified ice

1. Apply sample to grid

2. Blot away excess buffer (controlled force/ time/ humidity/ temperature)

3. Plunge grid into liquid ethane. For water to vitrify, the temperature has to drop faster than 10^5-10^6 K/s (Dubochet et al 1988)
   - Liquid nitrogen boils on contact - poor cooling capacity
   - Water is a poor thermal conductor (thin sample is mandatory < 3 µm)
   - Plunge at > 1 m/s





Saibil, 2000

Cryo-samples are frozen in liquid ethane to get vitrified ice

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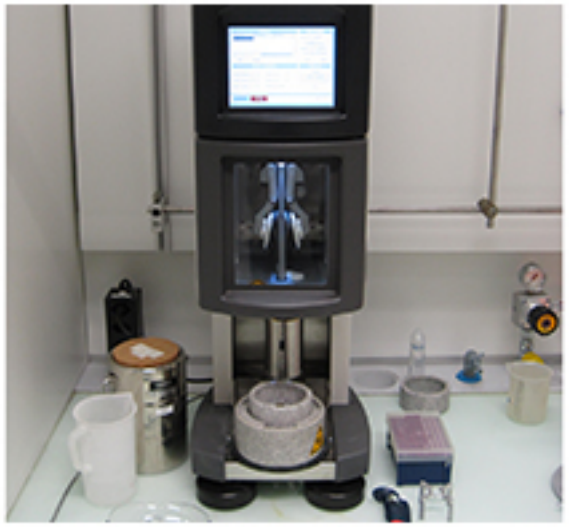
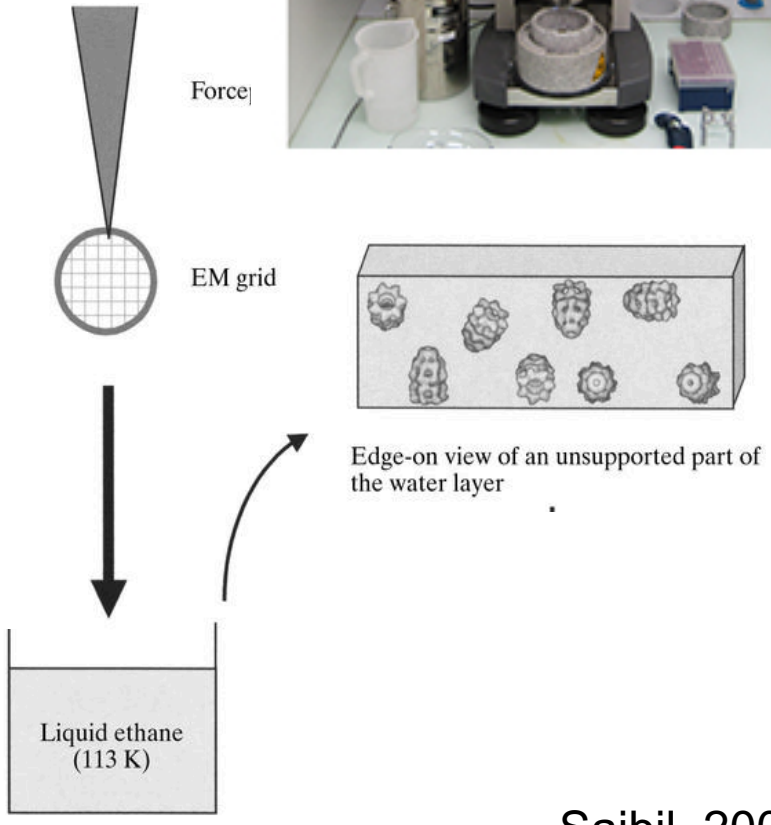
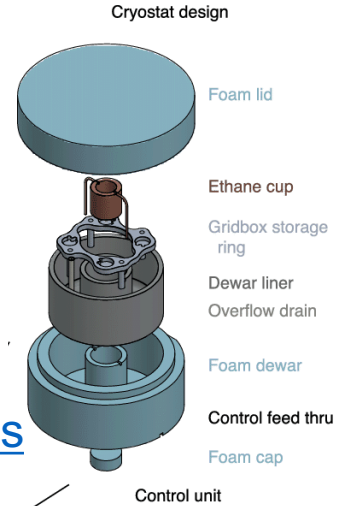




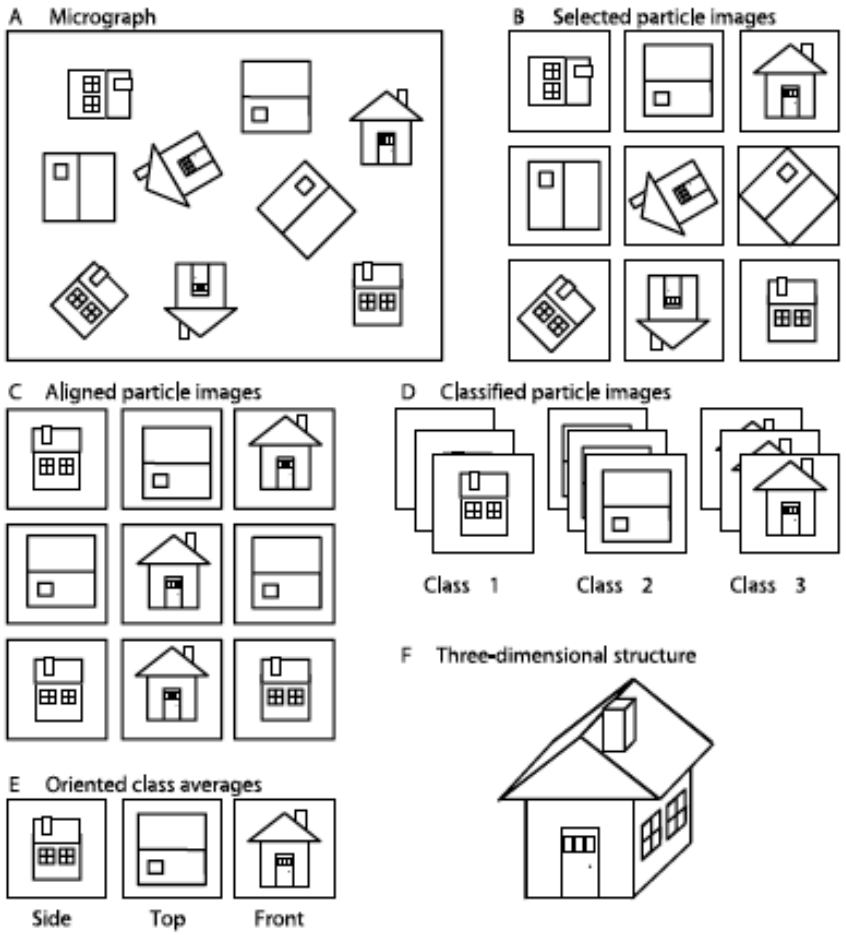


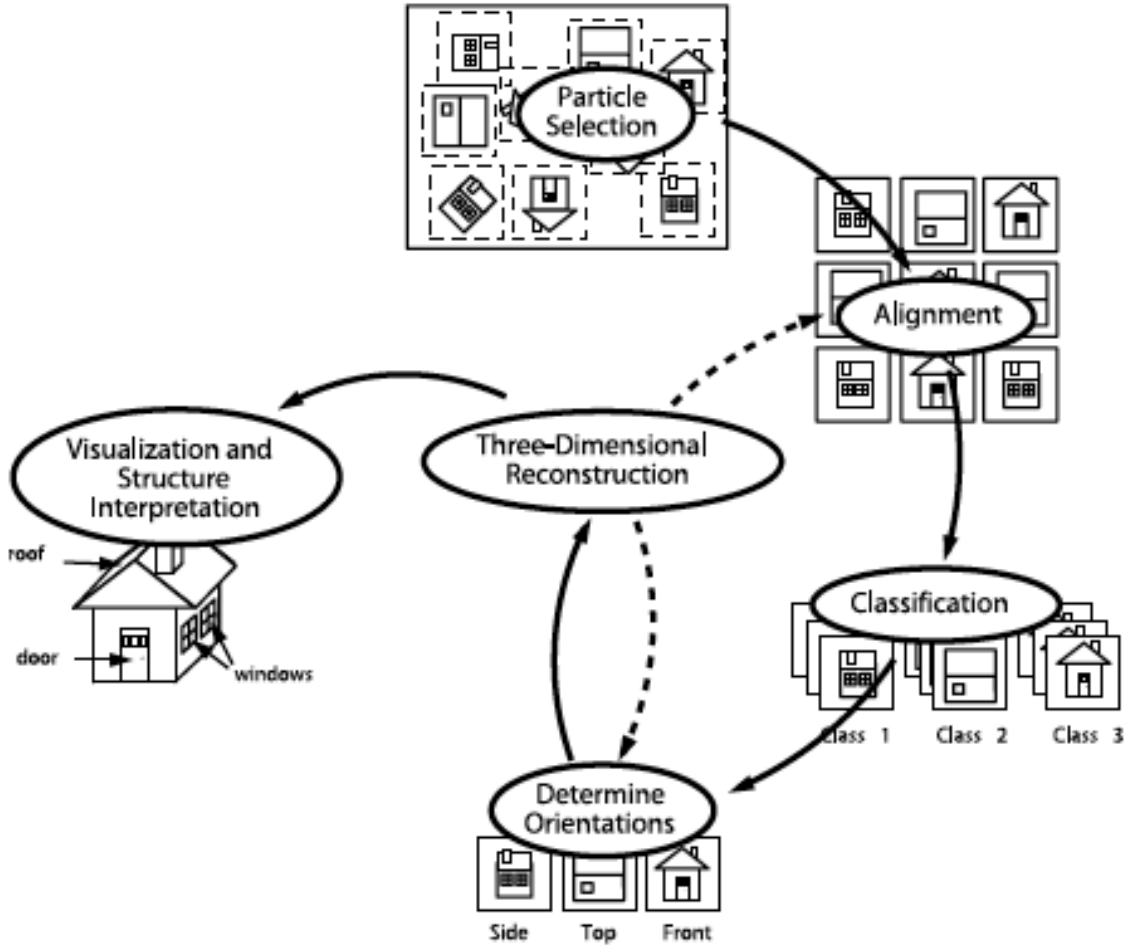
https://www.youtube.com/watch?v=M0LHiAwKKes

Saibil, 2000

Processing pipeline for cryo-EM images

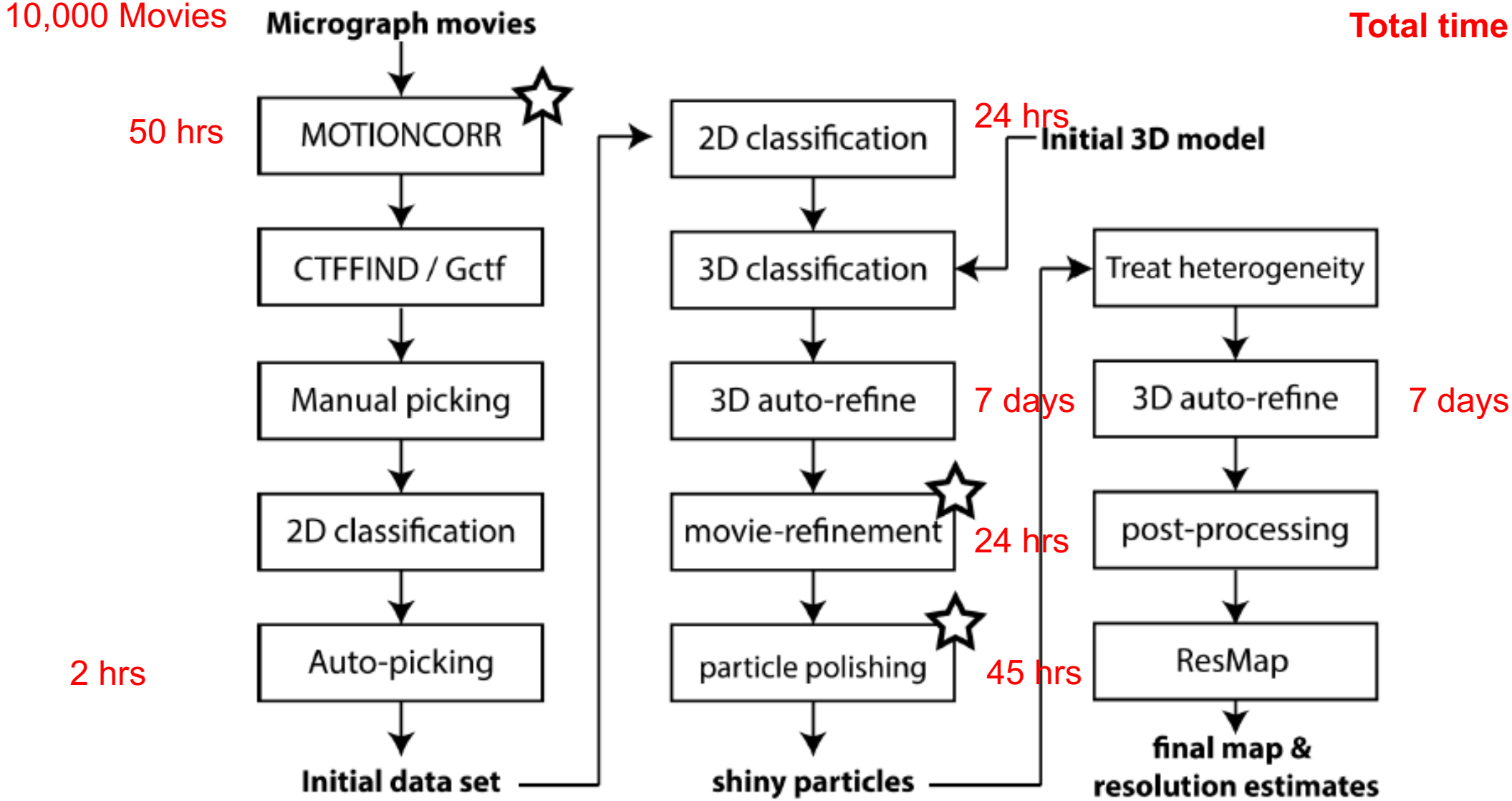
Single particle 3D reconstruction is based on averaging. We need many images of the same molecule in random orientations, however every individual image is very noisy with unknown orientation.





Thuman-Commike, 2001

Cryo-EM processing workflow for high-resolution structure determination



Scheres, 2016

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Breaking cryo-EM resolution limits enabling structure based drug discovery targeting Helicobacter pylori, a WHO class I carcinogen

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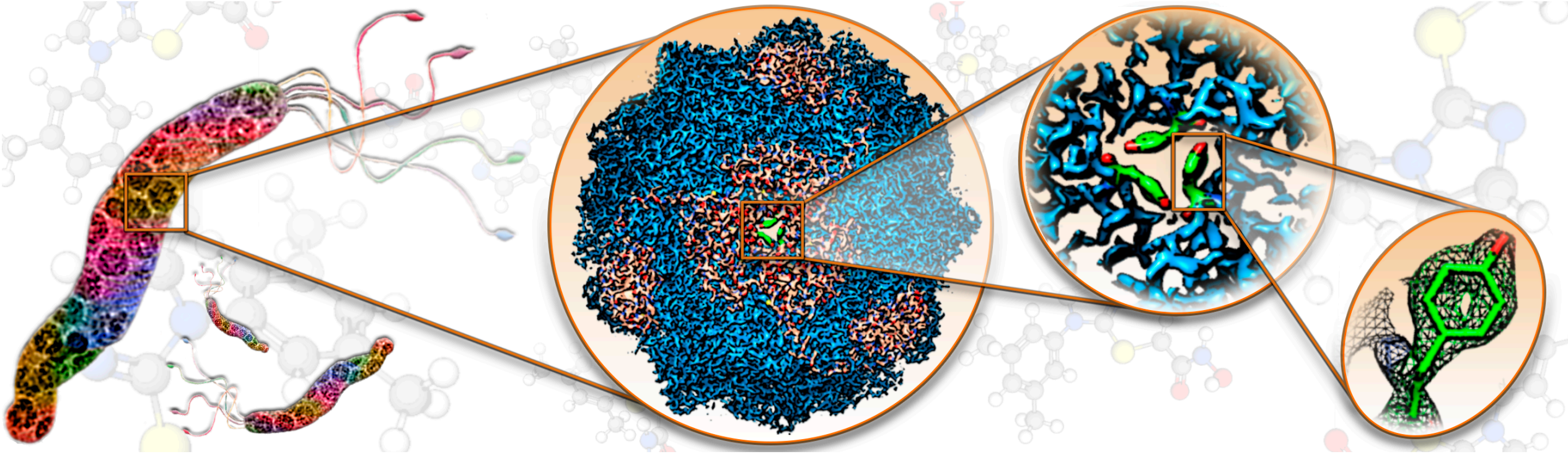

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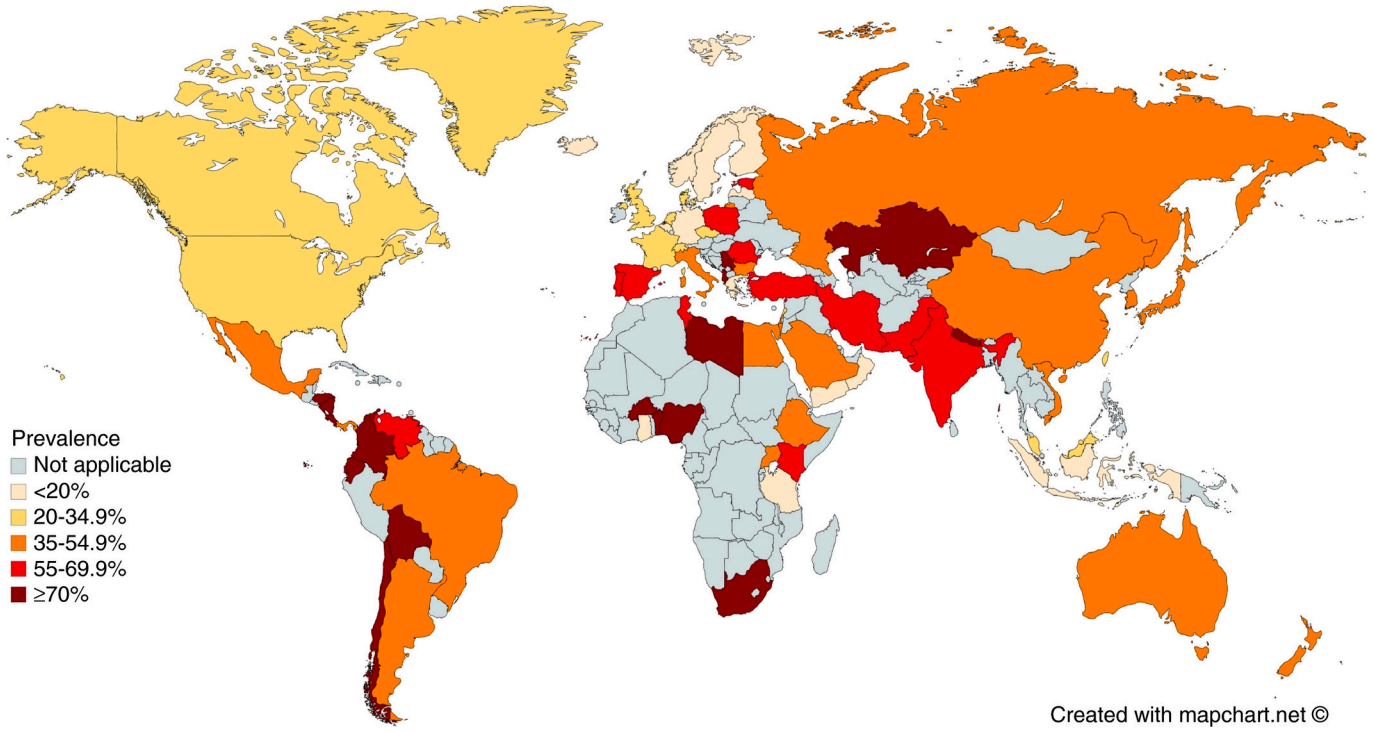
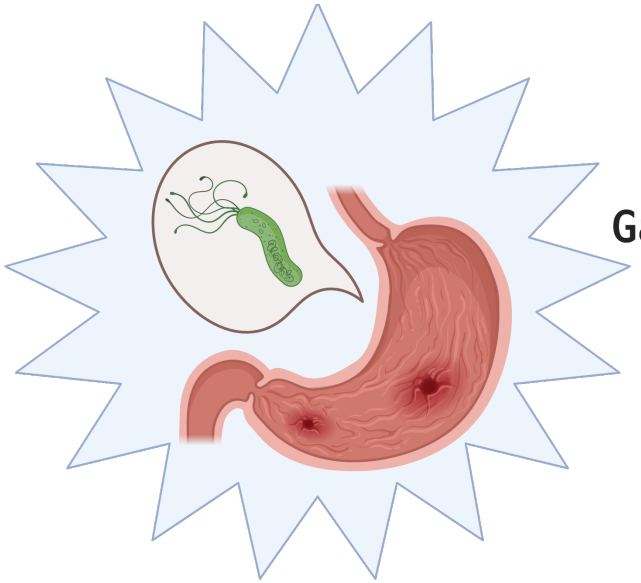
Eva S. Cunha
Researcher @ Hartmut ‘Hudel’ Luecke group
Structural Biology and Drug Discovery Group

Helicobacter pylori, a WHO Class 1 carcinogen



Zamani, M, *et al.,* Aliment Pharmacol Ther. 2018

Ulceration
10-20%

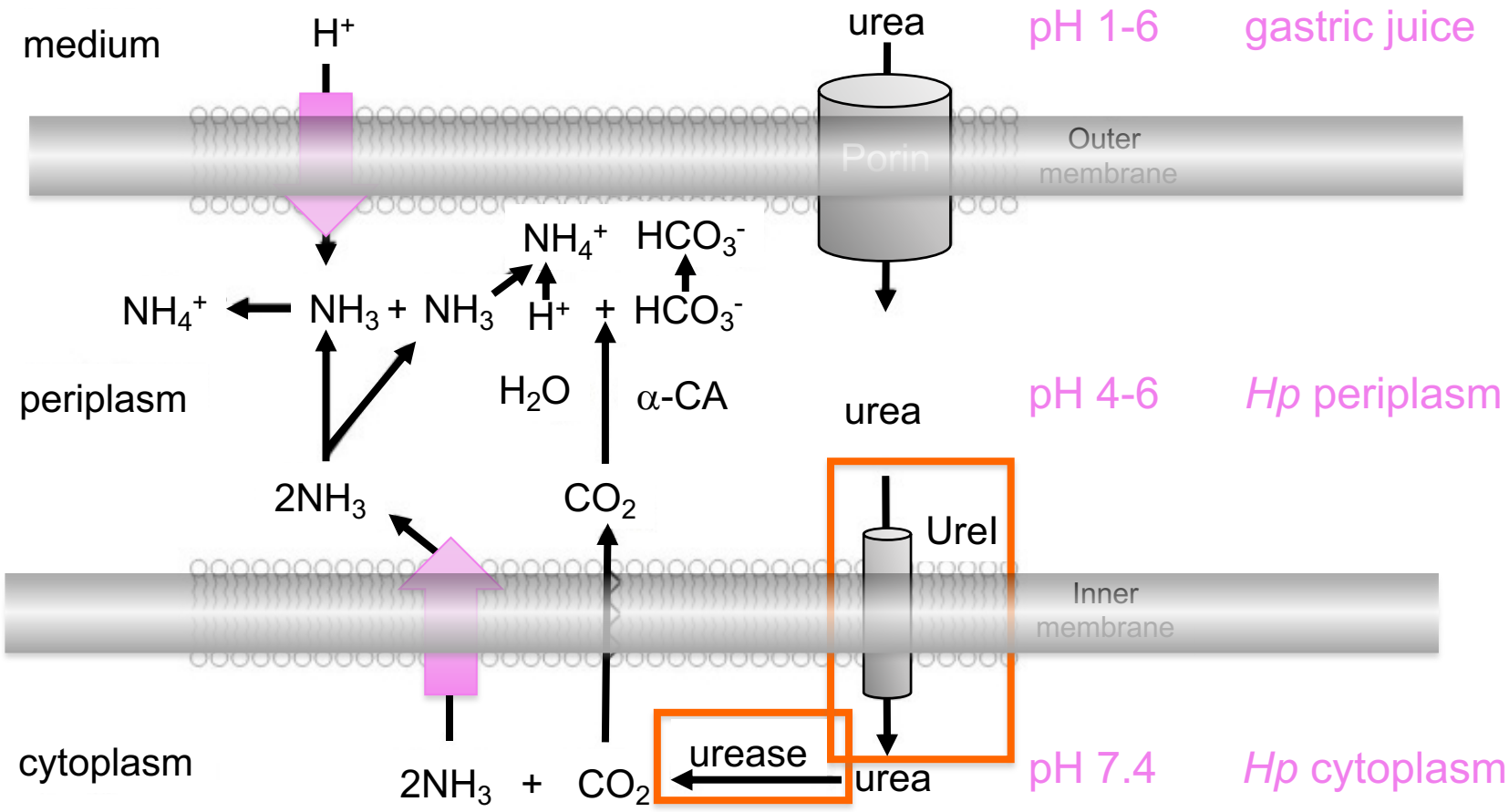


Gastritis
Majority

Cancer
1-2%

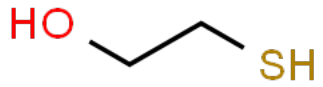
- Over 50% of the world population are chronically infected
- Resistance to antibiotic treatment rising rapidly and has already reached 30% eradication failure

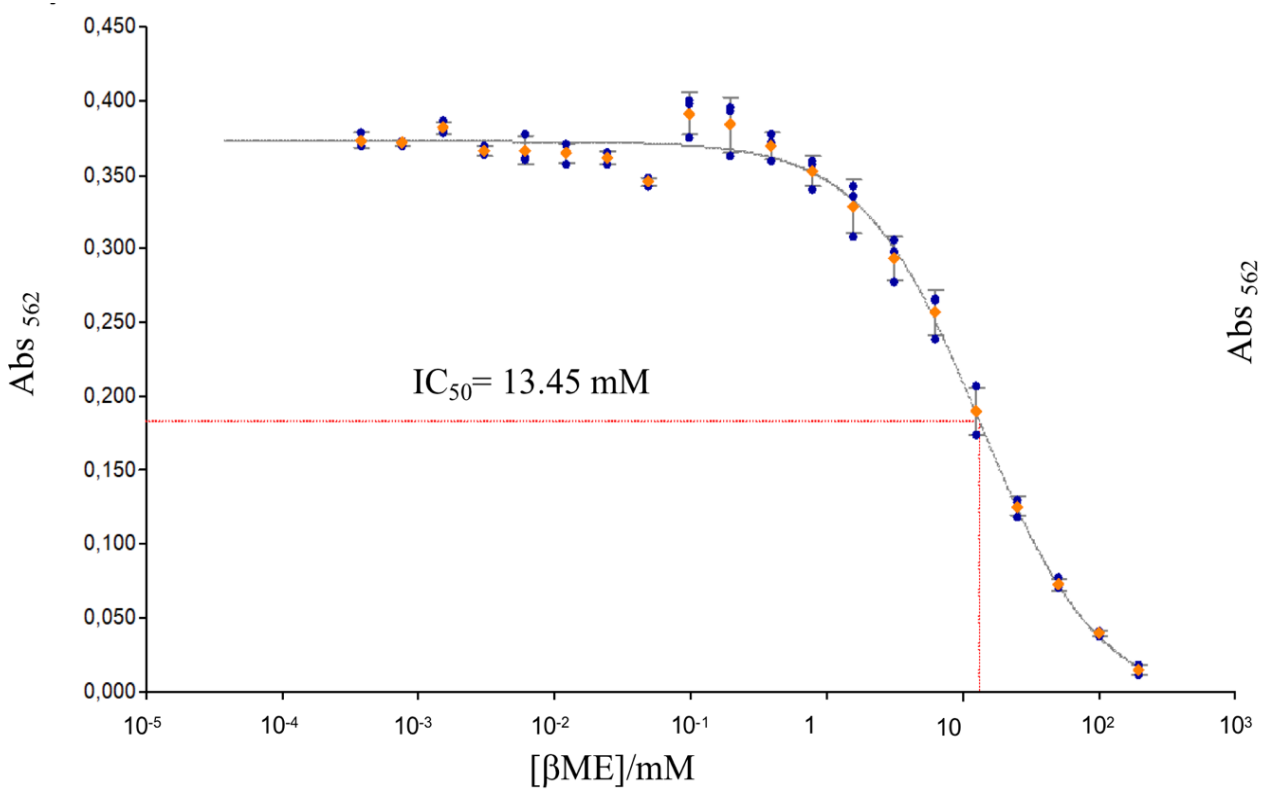
Survival at acidic pH requires cytoplasmic urease



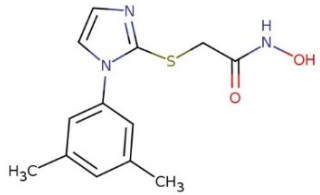
Inhibitor identified through high-throughput screening is an hydroxamic acid

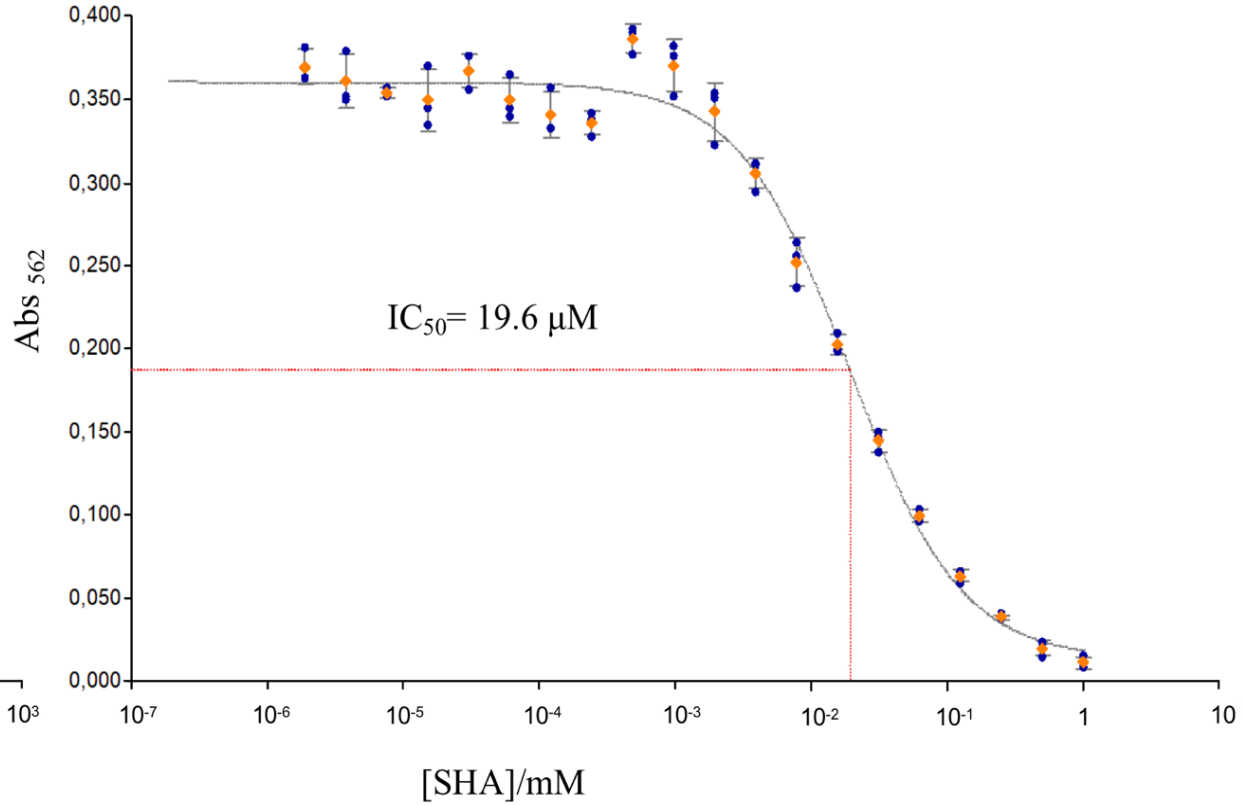
BME





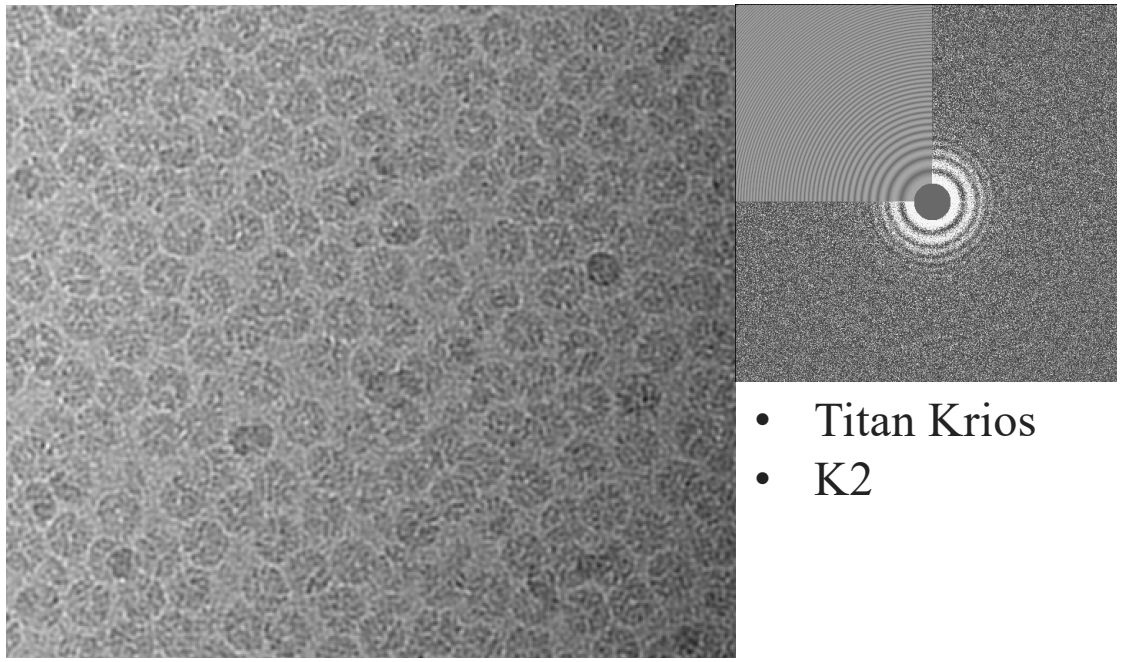
SHA



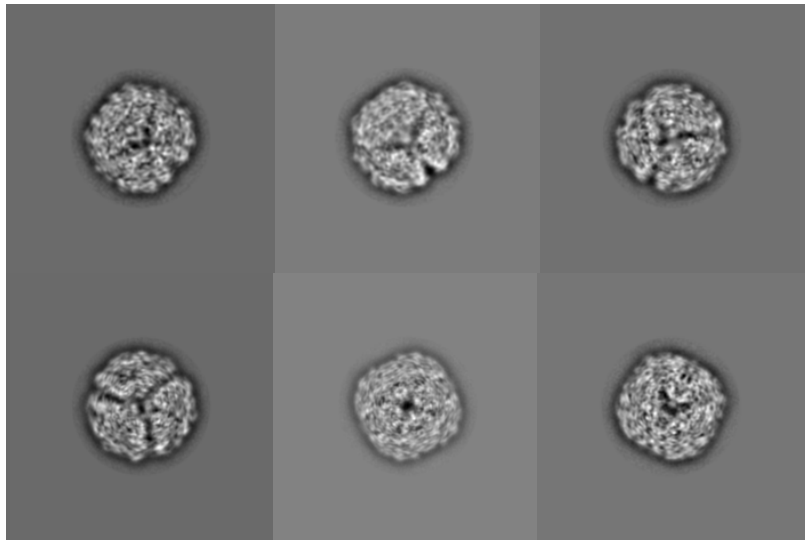


Cunha E. S.* *et al. (Nature Communications, 2021)*

U-BME and U-SHA structures show a dodecameric arrangement of the 1.1 MDa urease

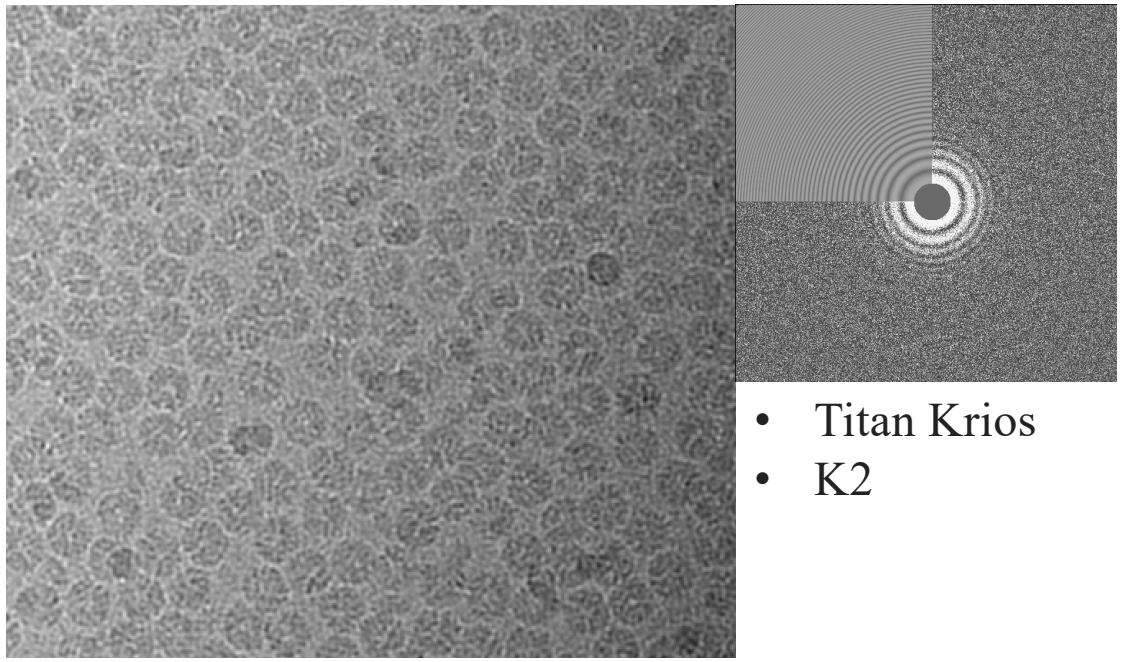


- Titan Krios
- K2

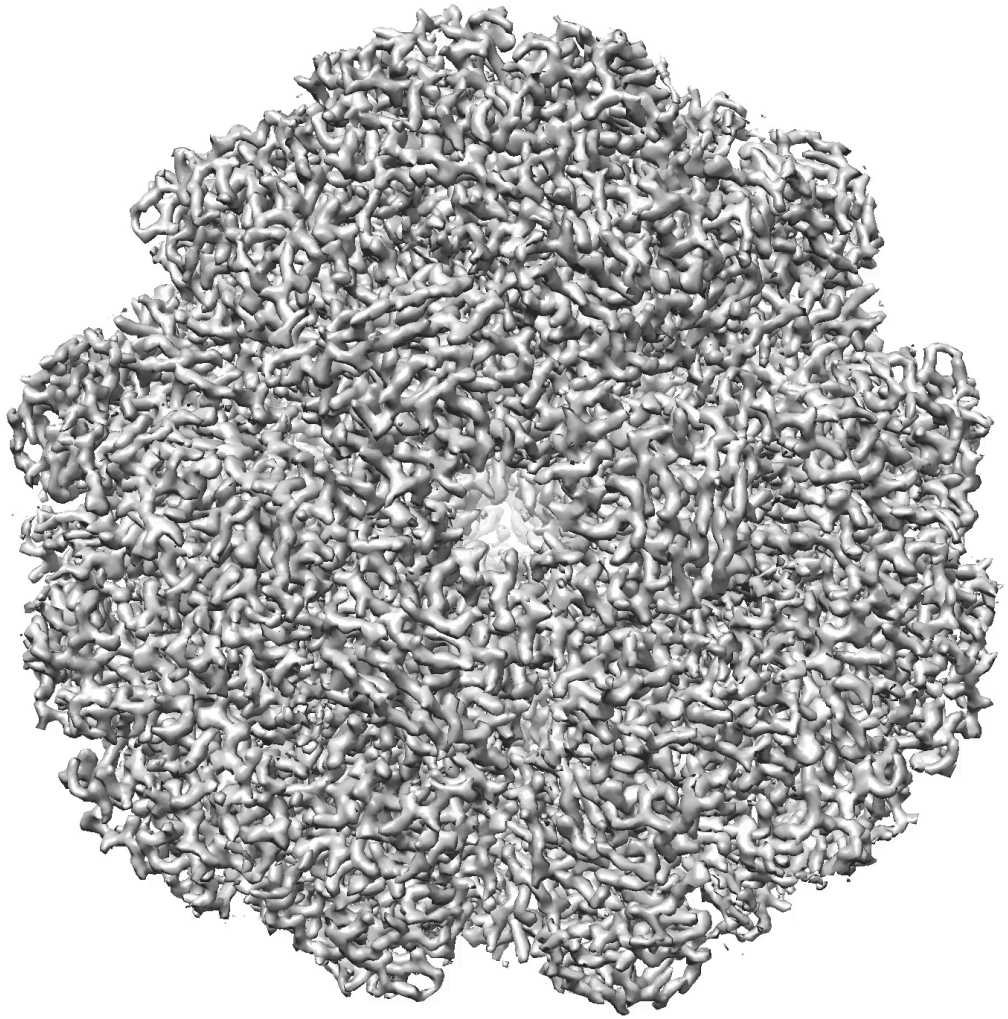


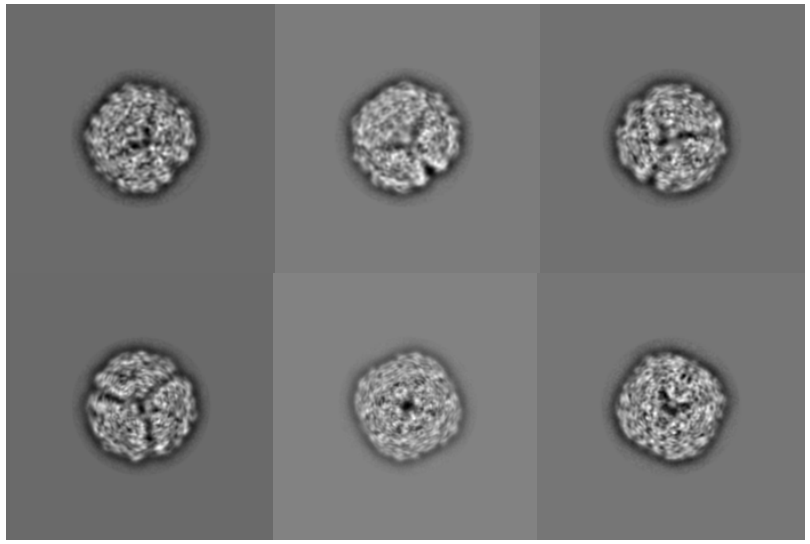
- 175,895 particles for U-BME, resolution 2.5 Å
- 187,461 particles for U-SHA, resolution 2.0 Å

U-BME and U-SHA structures show a dodecameric arrangement of the 1.1 MDa urease



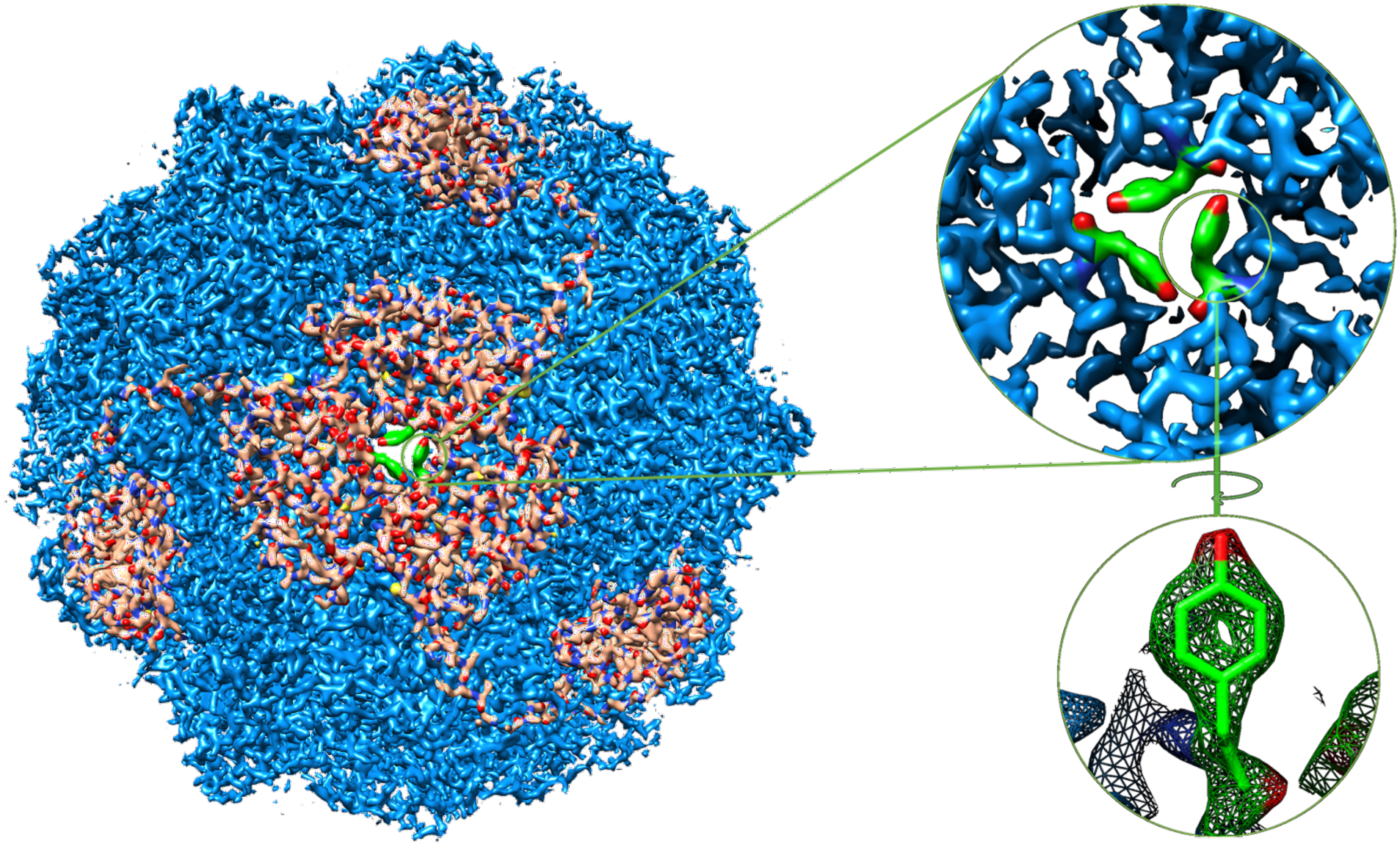
- Titan Krios
- K2





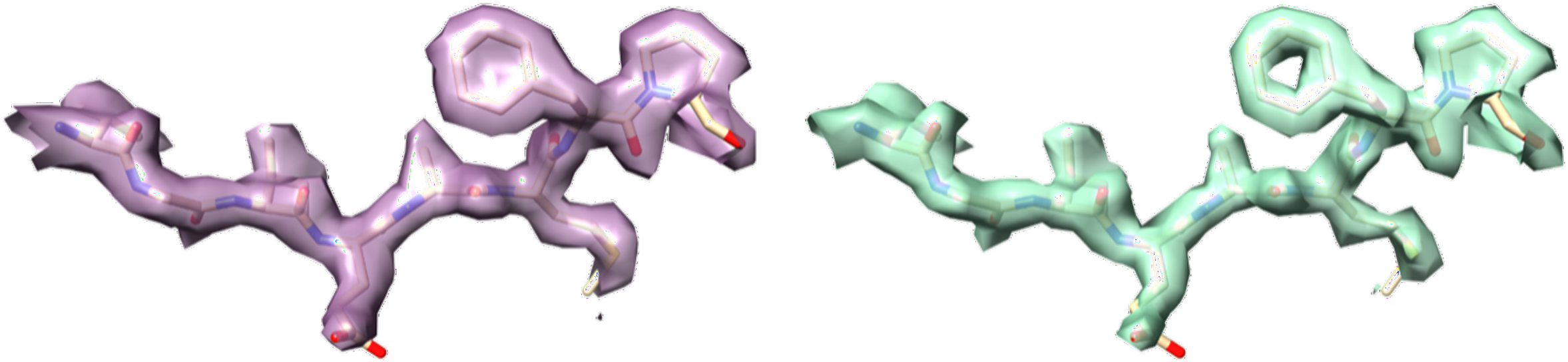
- 175,895 particles for U-BME, resolution 2.5 Å
- 187,461 particles for U-SHA, resolution 2.0 Å

Tetrahedral arrangement of *H. pylori* urease composed of two subunits



Cunha E. S.* *et al. (Nature Communications, 2021)*

Likelihood-based density modification improves map quality and nominal resolution

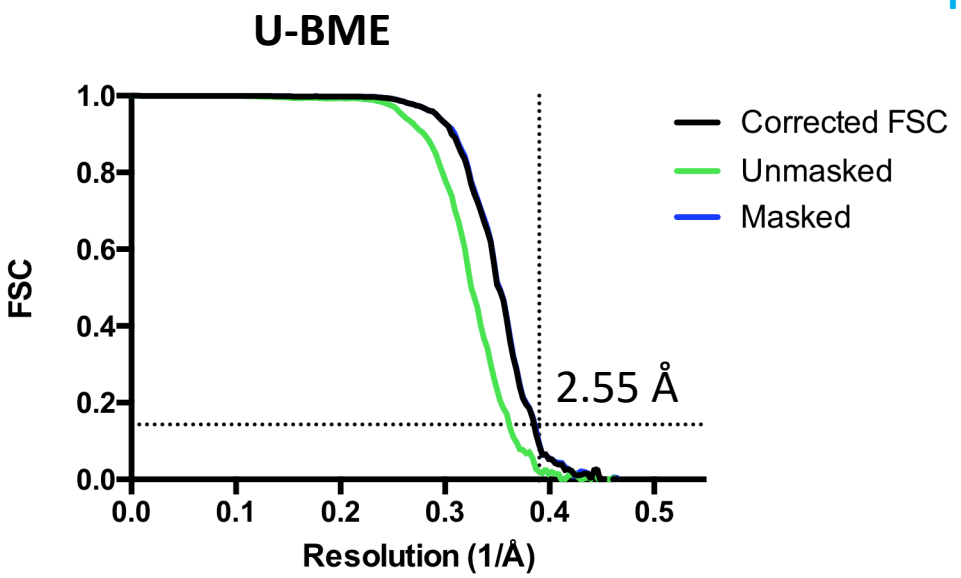


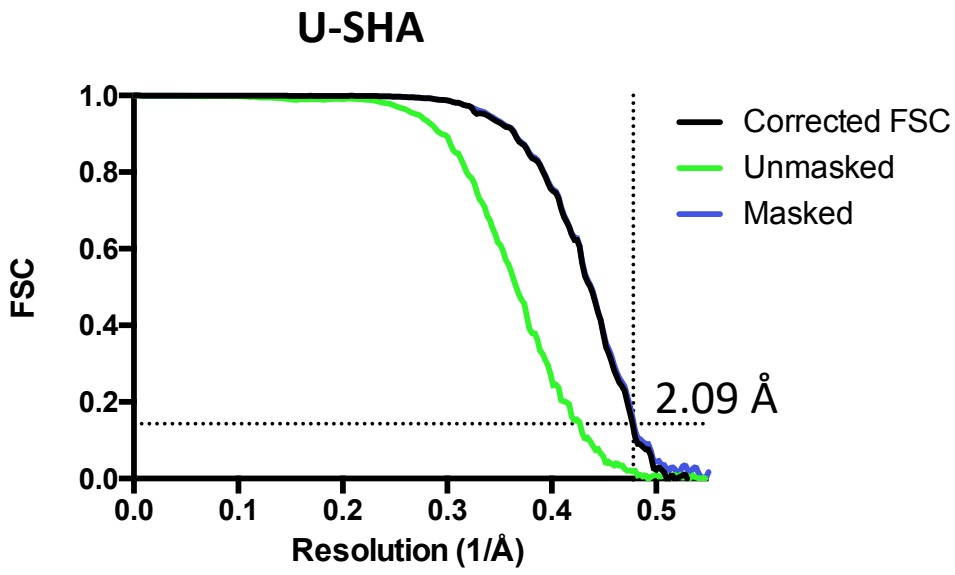
Cunha E. S.* *et al. (Nature Communications, 2021)*

Terwilliger, T.C., Ludtke, S.J., Read, R.J. *et al*. *Nat Methods* **17,** 923–927 (2020)

Likelihood-based density modification improves map quality and nominal resolution

Map to map





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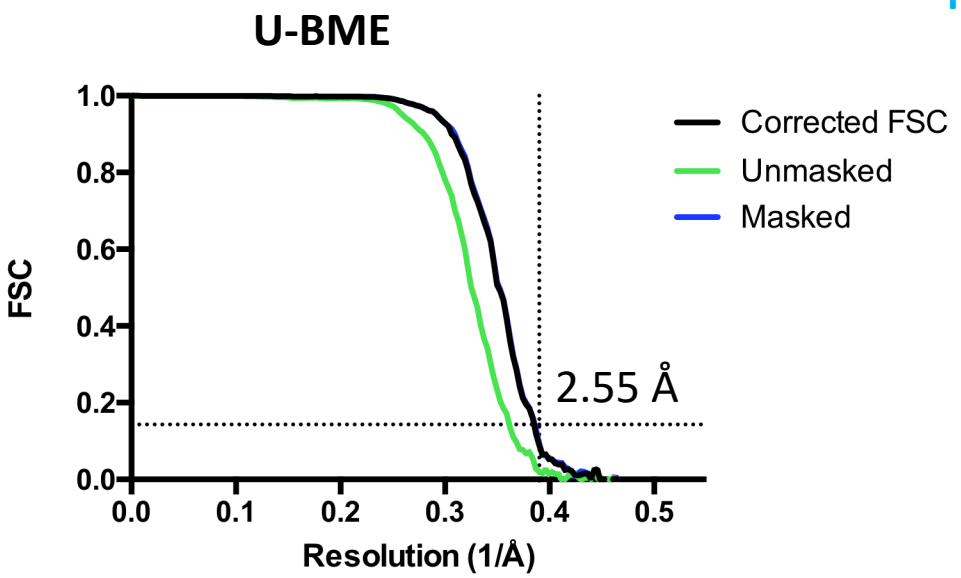
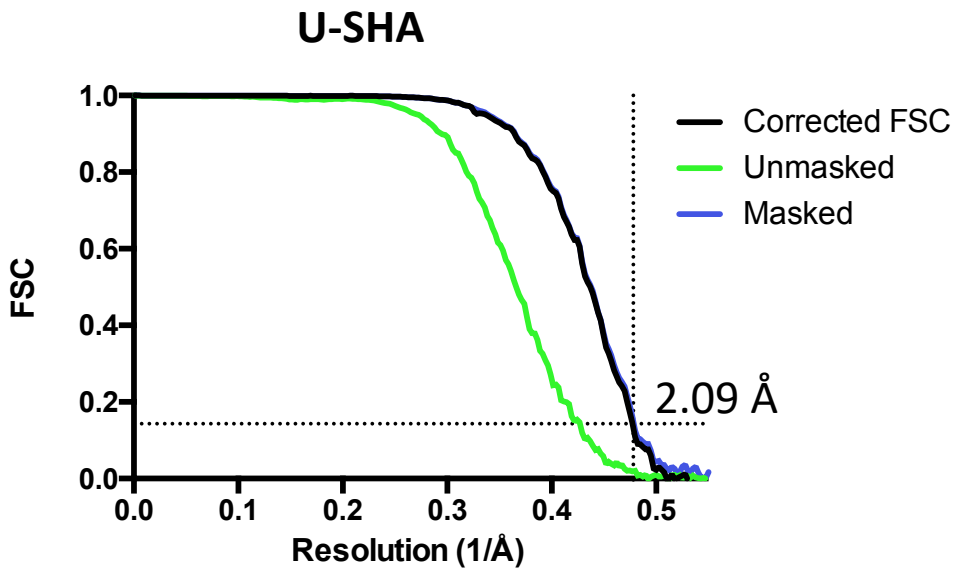
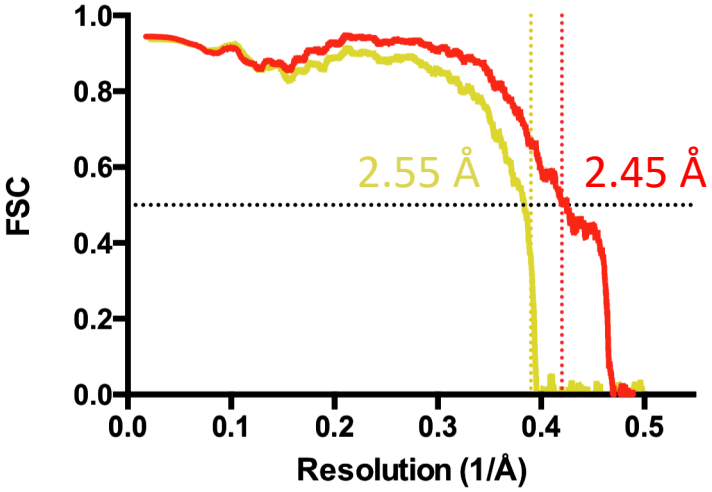
Map to map

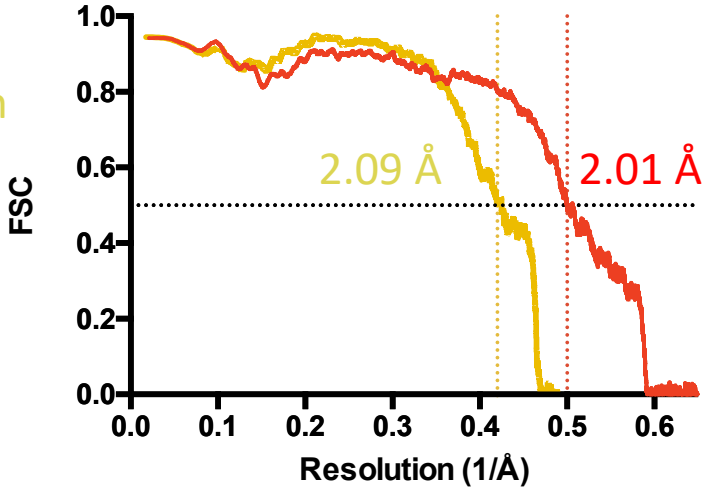




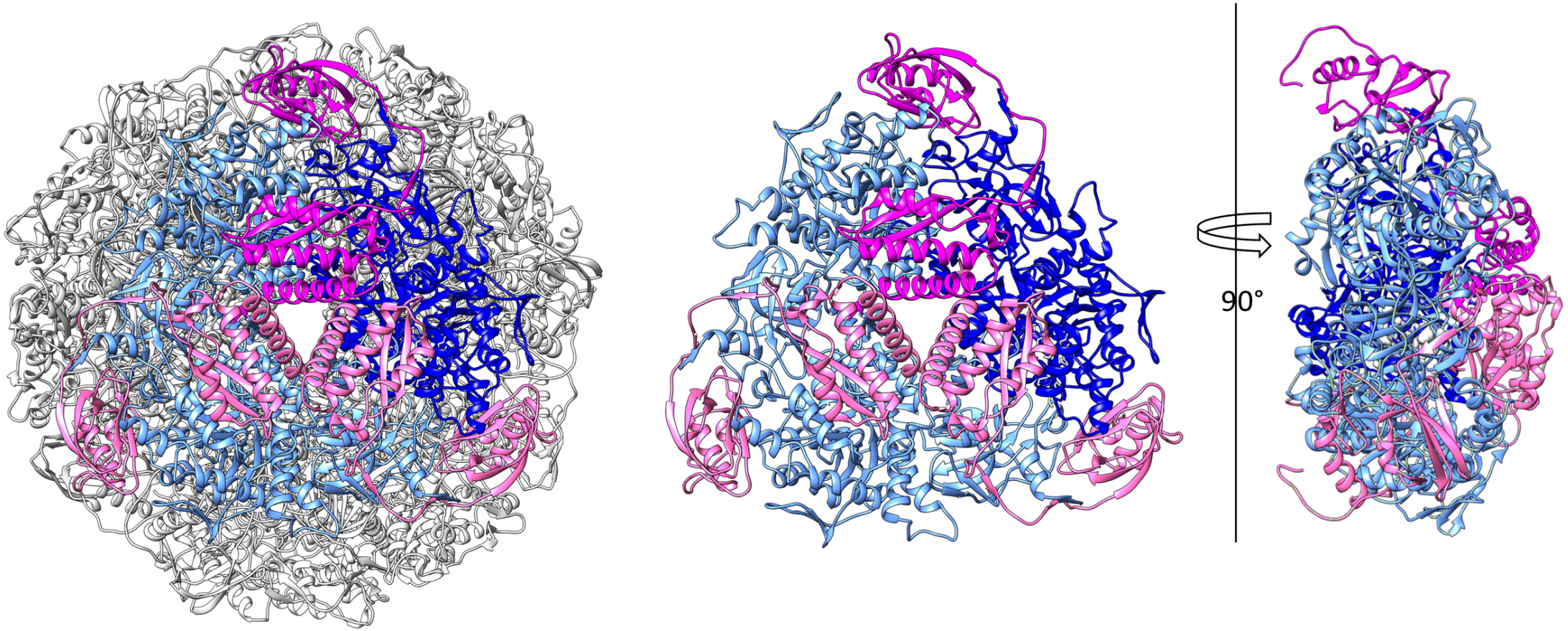
Map to model



Before density modification
After density modification

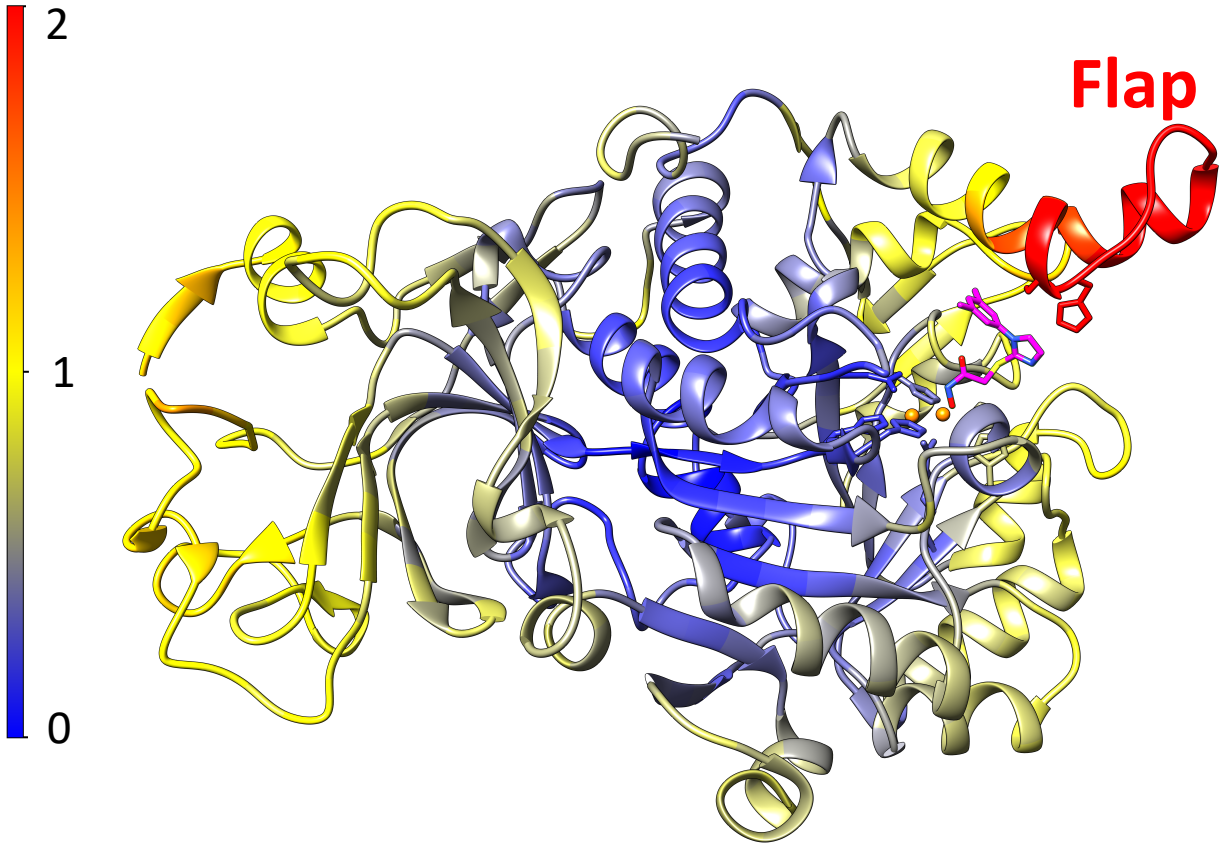


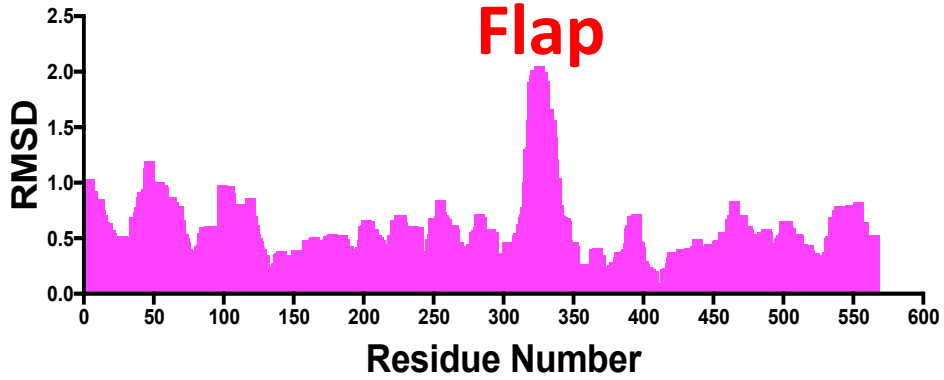
Tetrahedral arrangement of *H. pylori* urease composed of two subunits



Cunha E. S.* *et al. (Nature Communications, 2021)*

RMSD analysis between BME and SHA bound urease shows highest variation at the flap region covering the active site





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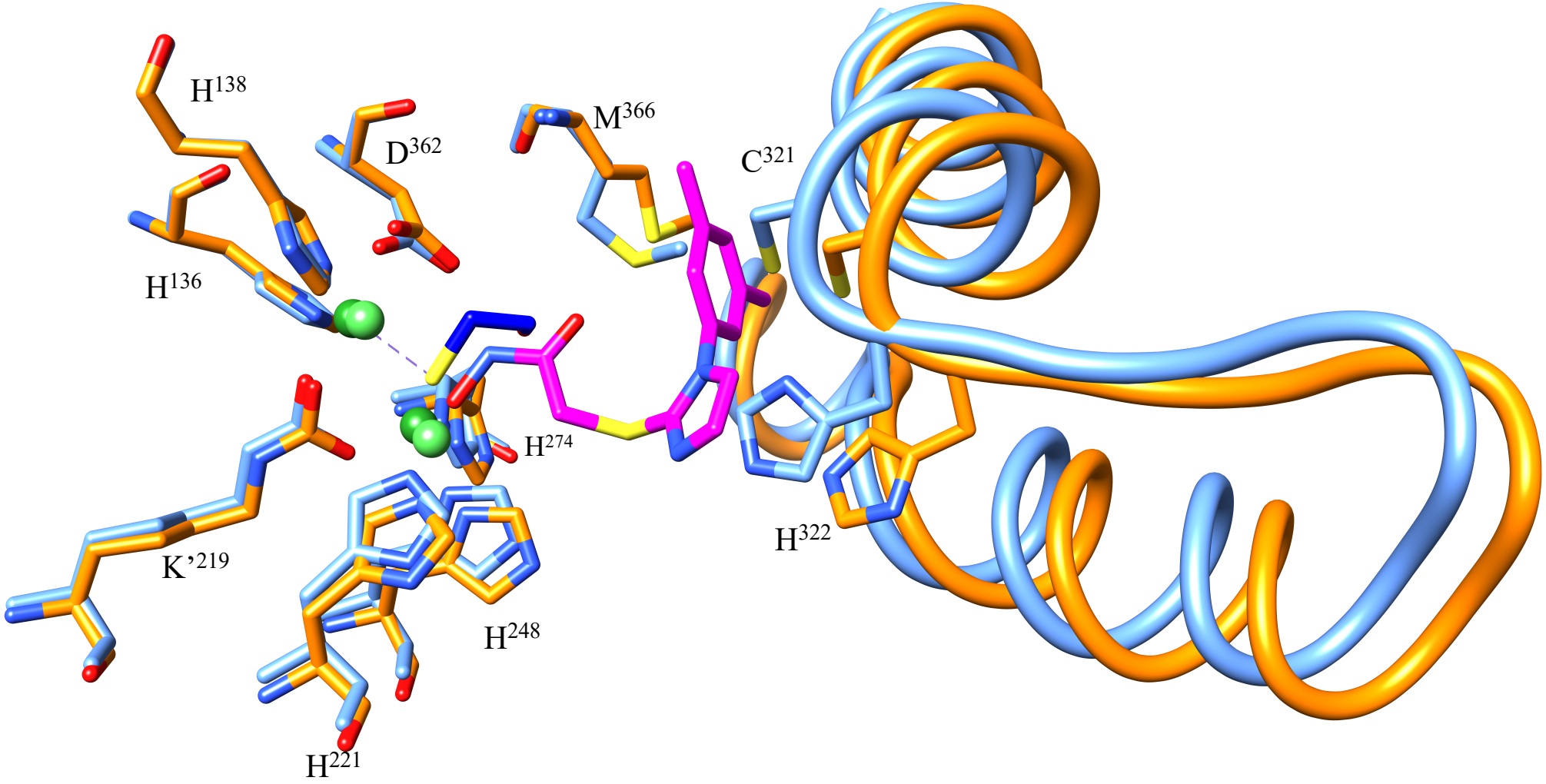
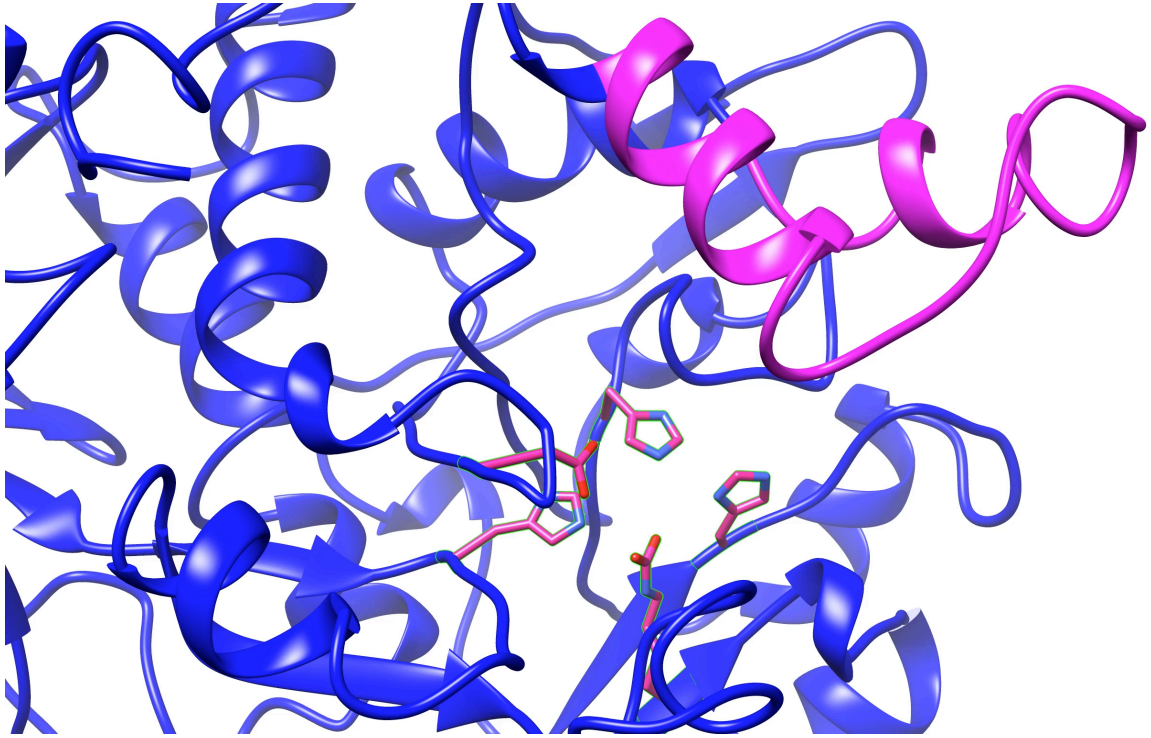
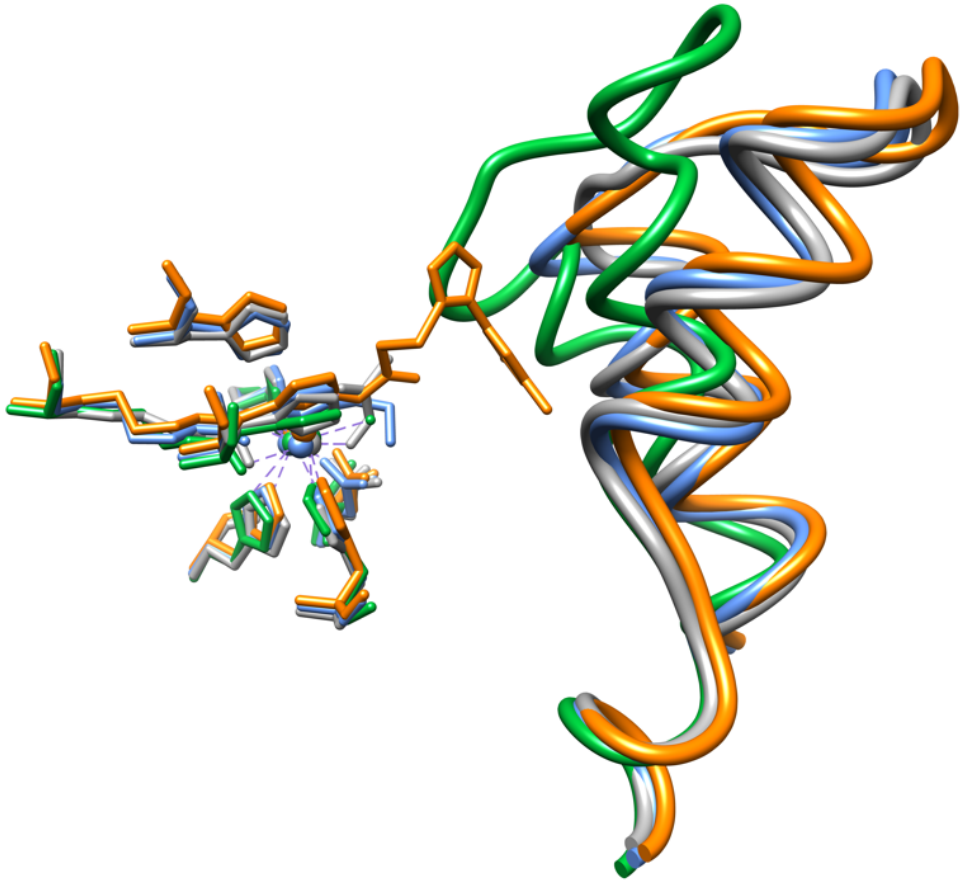


Active site with Ni^{2+} **SHA/BME** **Flap**

Cunha E. S.* *et al. (Nature Communications, 2021)*

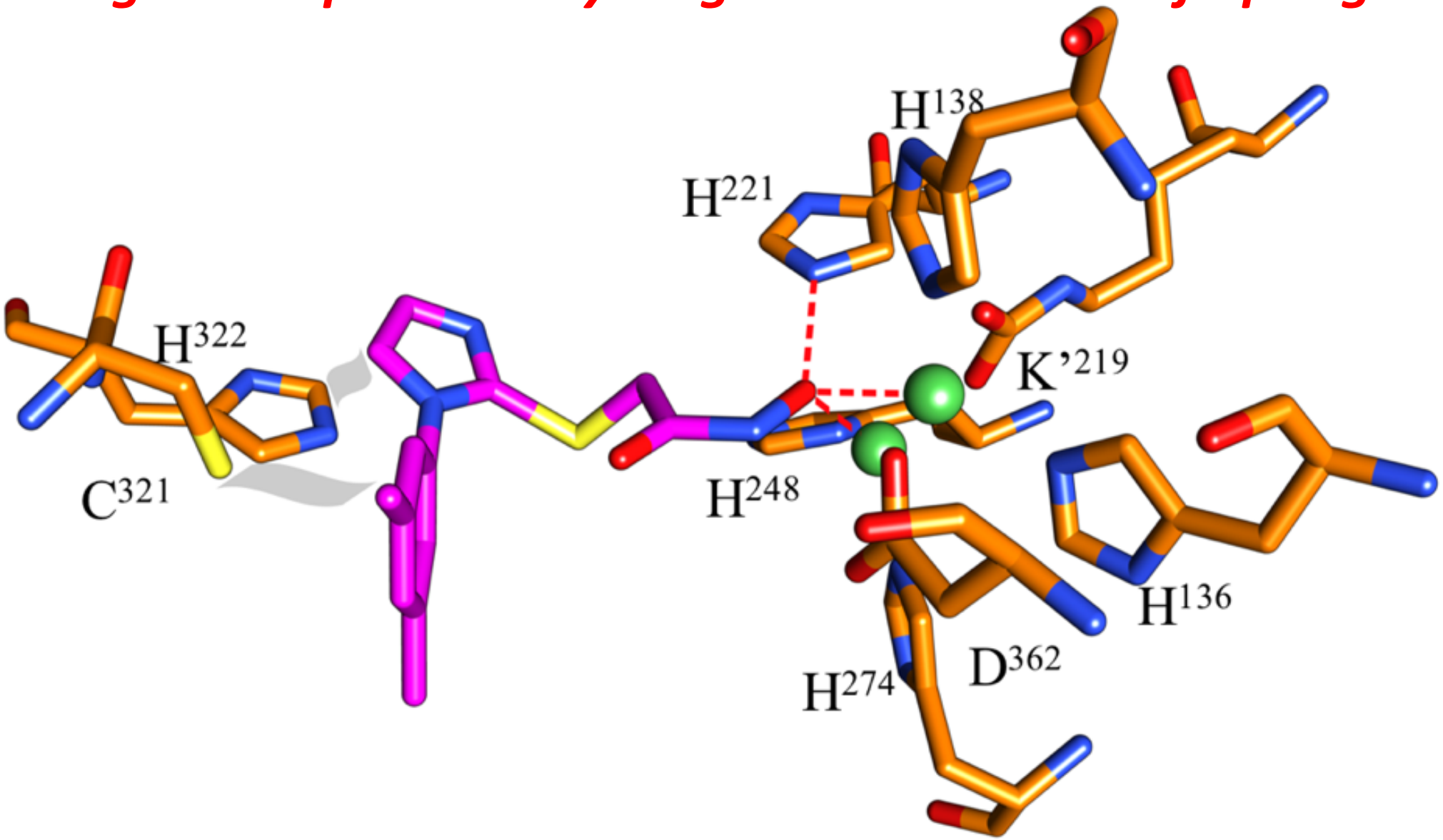
U-SHA provides the most open flap region structural snapshot to date





Structure	Clash score	Rama outliers [%]	Rama allowed [%]	Rama favored [%]
Crystal PDB 3.0 Å NAT	51.00	7.11	16.71	76.18
Crystal PDB 3.0 Å AHA	38.00	2.86	13.21	83.92
Crystal, rerefined 3.0 Å NAT	23.69	3.90	14.25	82.12
Crystal, rerefined 3.0 Å AHA	18.09	1.60	9.25	89.50
Cryo-EM U-SHA, 2.0 Å	0.60	0.00	4.15	95.85
Cryo-EM U-BME, 2.5 Å	4.57	0.00	4.55	94.45

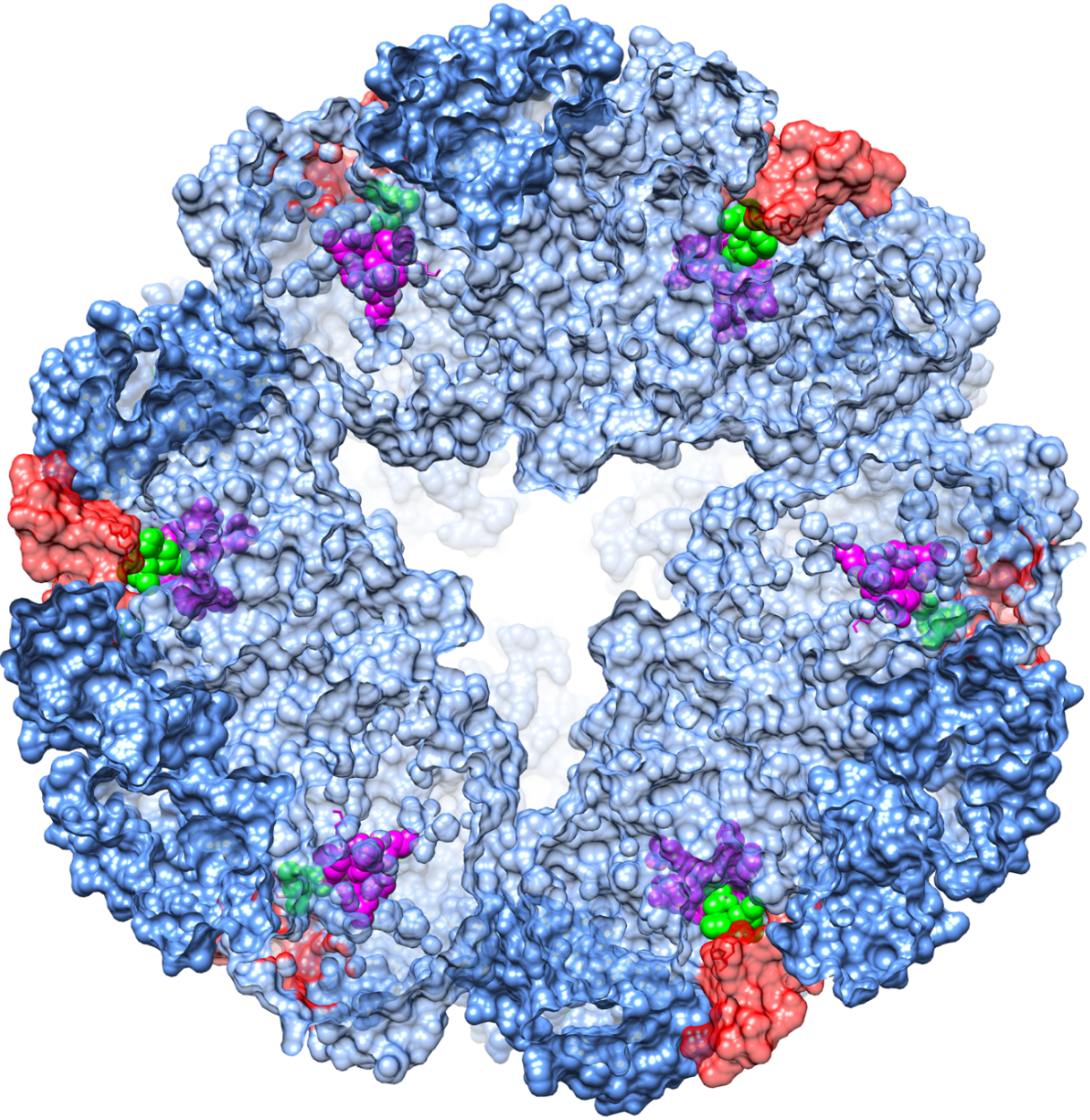
Drug development may target residues in the flap region



Flap **Inhibitor (SHA)** **Active site with Ni^{2+}**

Cunha E. S.* *et al. (Nature Communications, 2021)*

Single particle Cryo-EM can be used for drug discovery targeting *H. pylori* urease



- U-SHA adds a structural snapshot with the most open flap region observed experimentally to date
- Increasing the interactions between SHA and the flap region might lead to compounds with lower IC_{50} values.
- The distance from the bi-nickel center to the outer surface of the dodecamer is about 30 Å and presumably requires movement of the flexible flap region for access.

Cunha E. S.* *et al. (Nature Communications, 2021)*

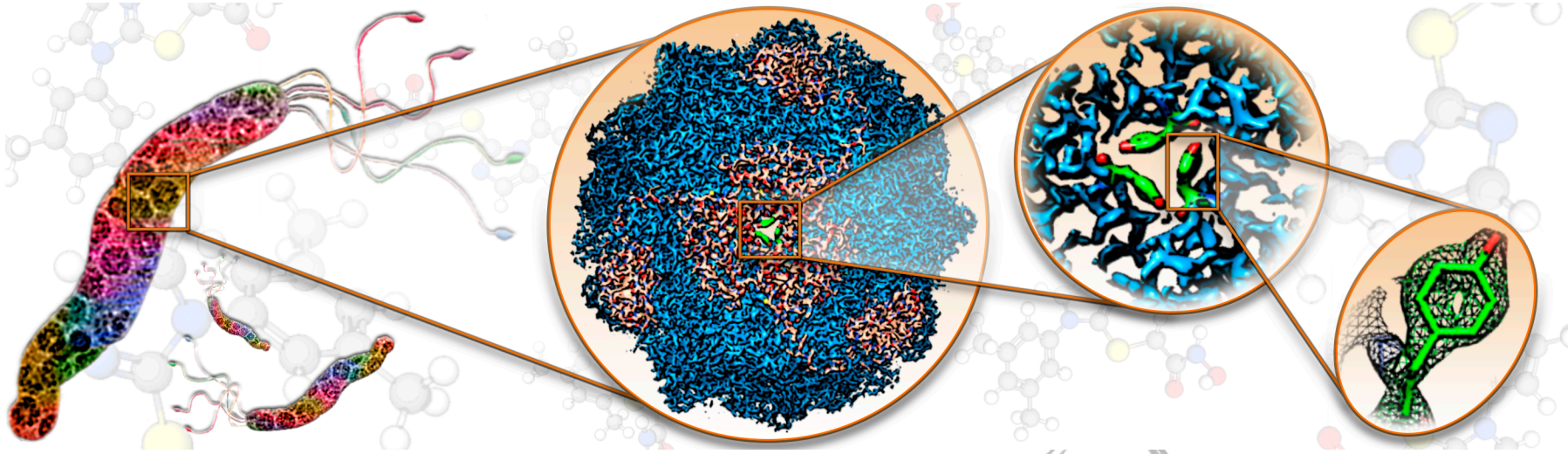
Thank you for your attention!

Dr. Hartmut Lücke (Hudel)
Dr. Marta Sanz Gaitero

Members of the Hudel lab

Collaborators!
Xiaorui Chen – University of California, Irvine
Deryck Mills – Max Planck Institute for Biophysics, Frankfurt

Cryo-EM facilities:
Umeå
Dr. Linda Sandblad
Dr. Michael Hall
Aarhus
Dr. Thomas Boesen
Dr. Andreas Bøggild



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MARIE CURIE ACTIONS