NanoImaging Services

CryoEM & Drug Discovery

Lessons learned, challenges and opportunities

Giovanna Scapin PhD

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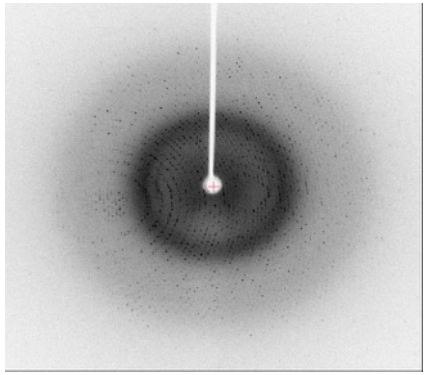

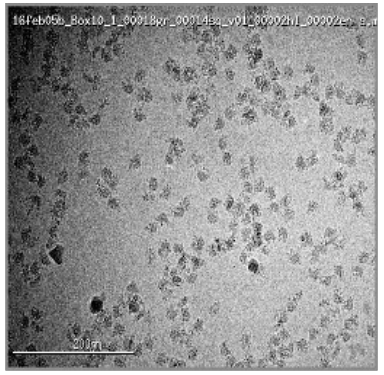

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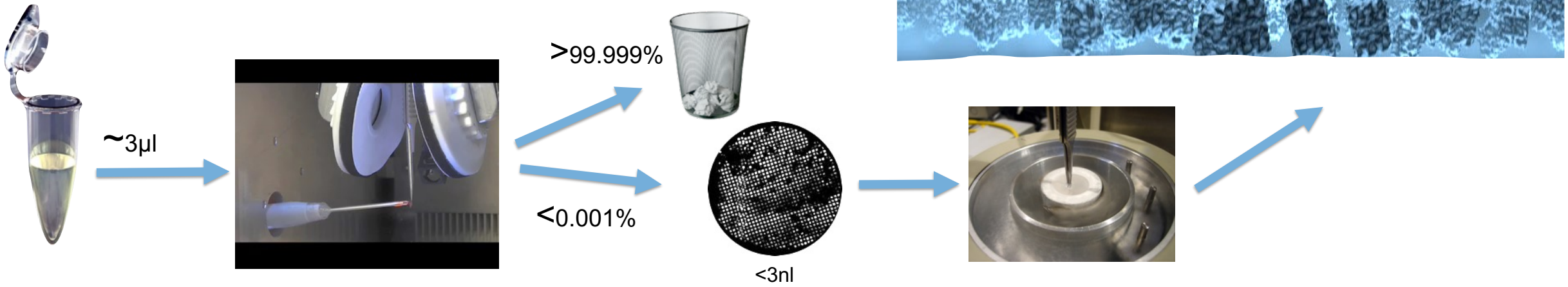
CryoEM: the process

SAMPLE PREPARATION



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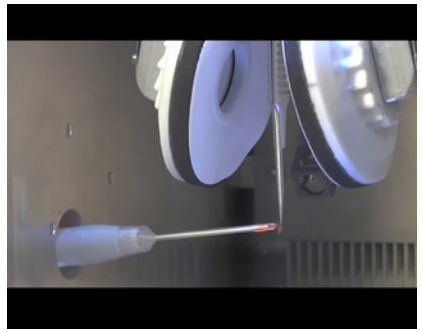

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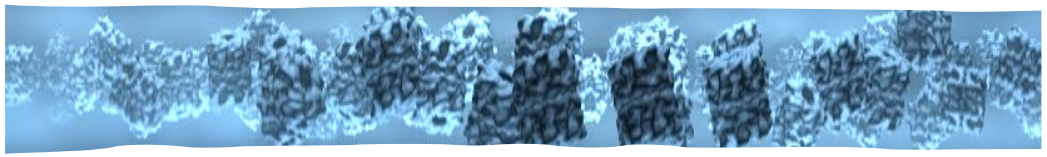
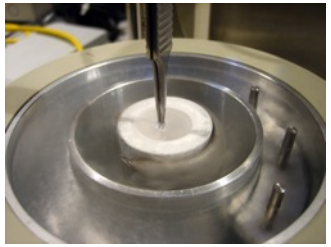
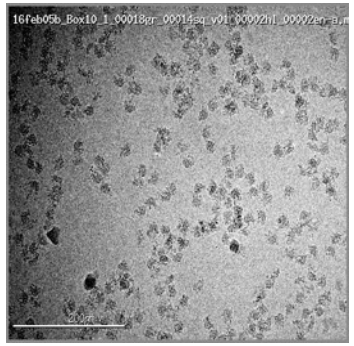
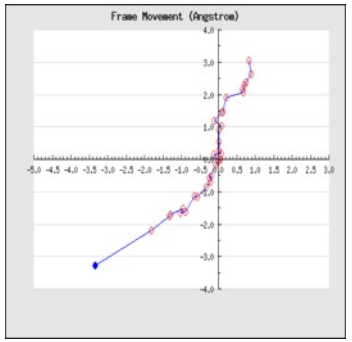
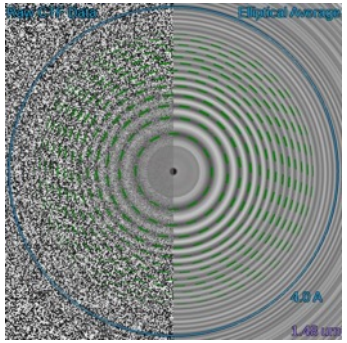


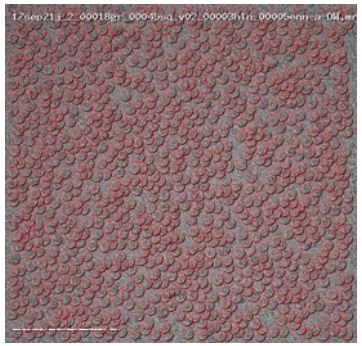


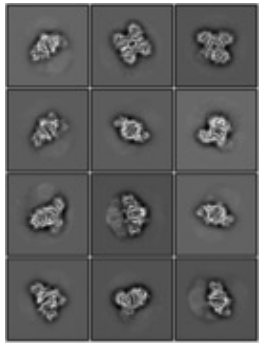
DATA COLLECTION and PROCESSING

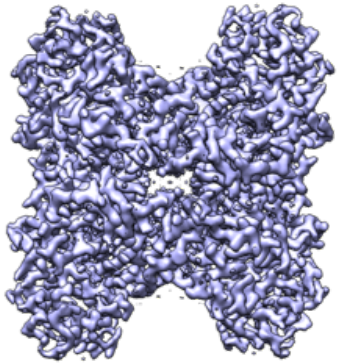












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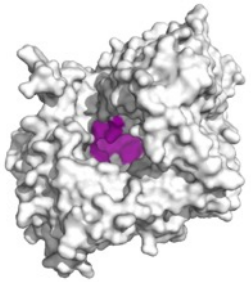
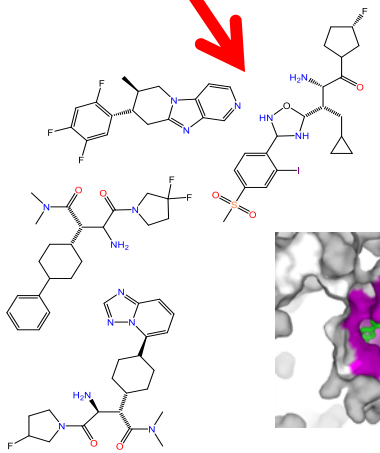
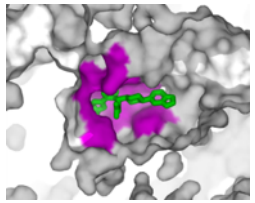
Structural Biology in Drug Development

Target ID → Hit ID → Lead ID/OPT → Pre-Clinical → Phase I, II, III



Biological insight, substrate binding, mechanism





Protein-ligand structures of early chemical matter to focus chemistry

Find out what the chemical structure really is, MOA

Iterative structure-guided evolution to:
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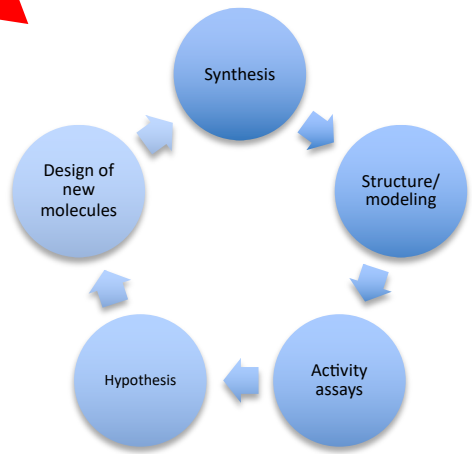

Scapin,G., Potter,C.S. & Carragher, B. (2018) Cell Chem Biol 25 (11), 1318-1325

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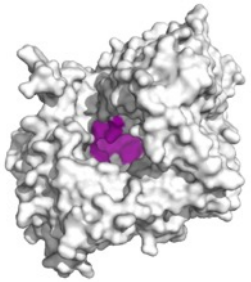
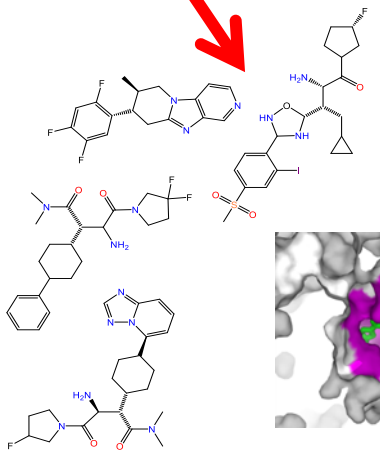
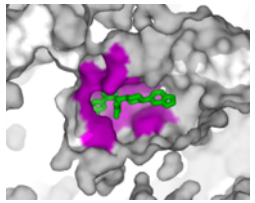


Biological insight, substrate binding, mechanism

1-2 structures, timing and resolution not limiting





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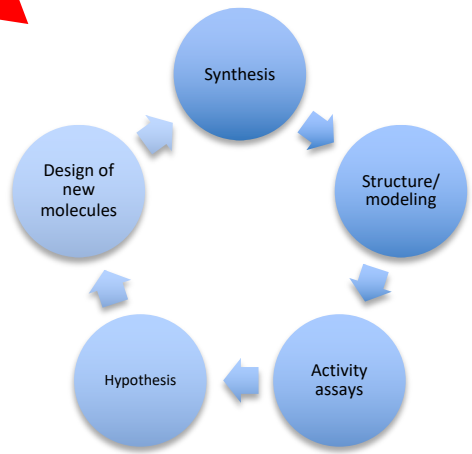

Many structures. Timing, throughput and resolution are important

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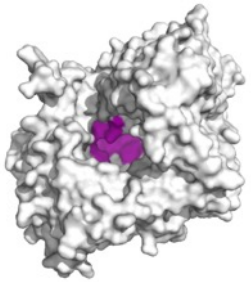

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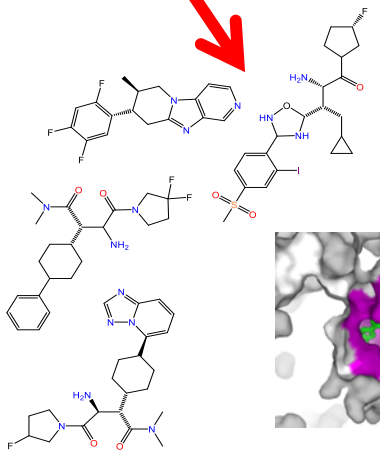

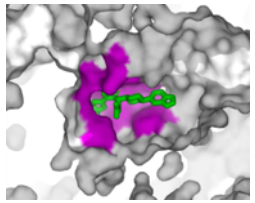

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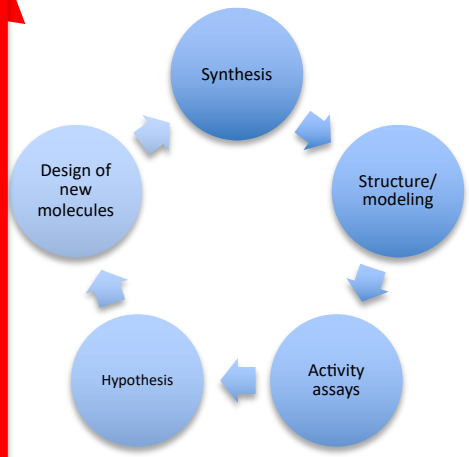

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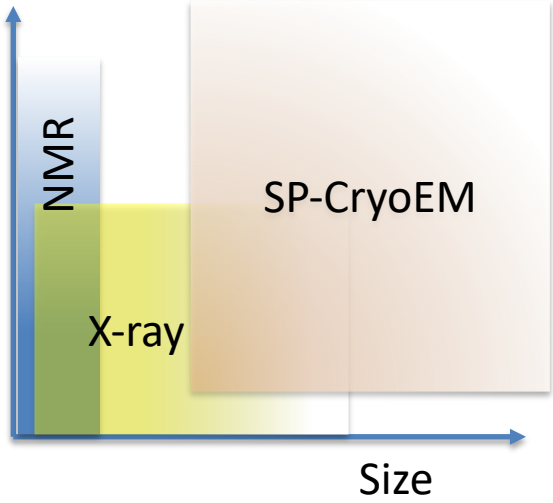

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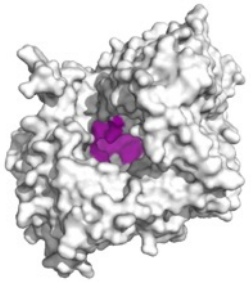

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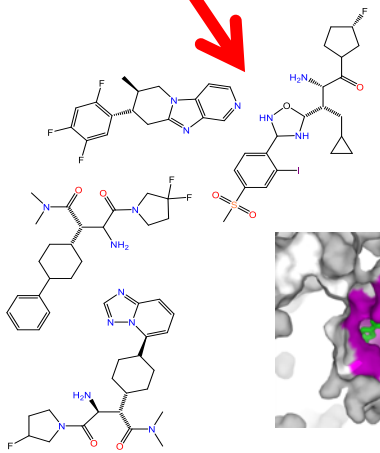

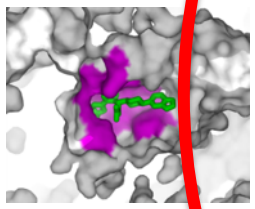

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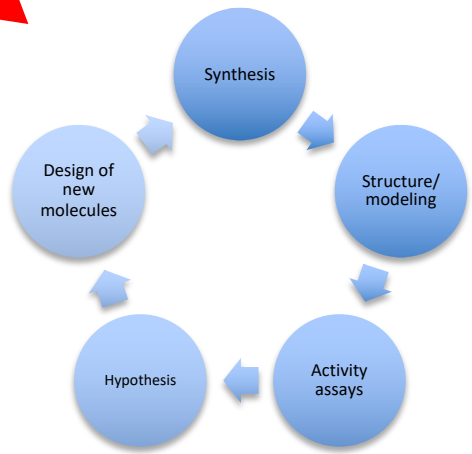

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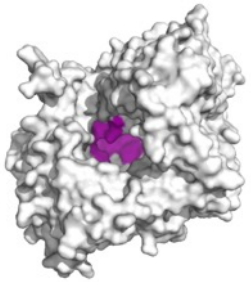

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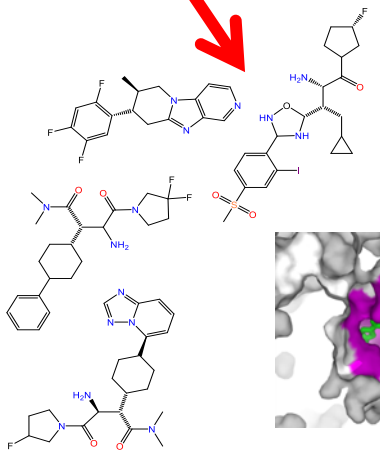

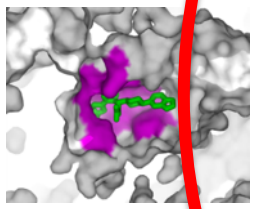

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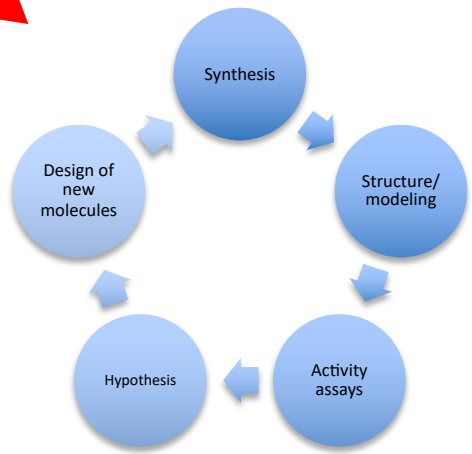

Many structures. Timing, throughput and resolution are important

Resolution

Timing

Throughput

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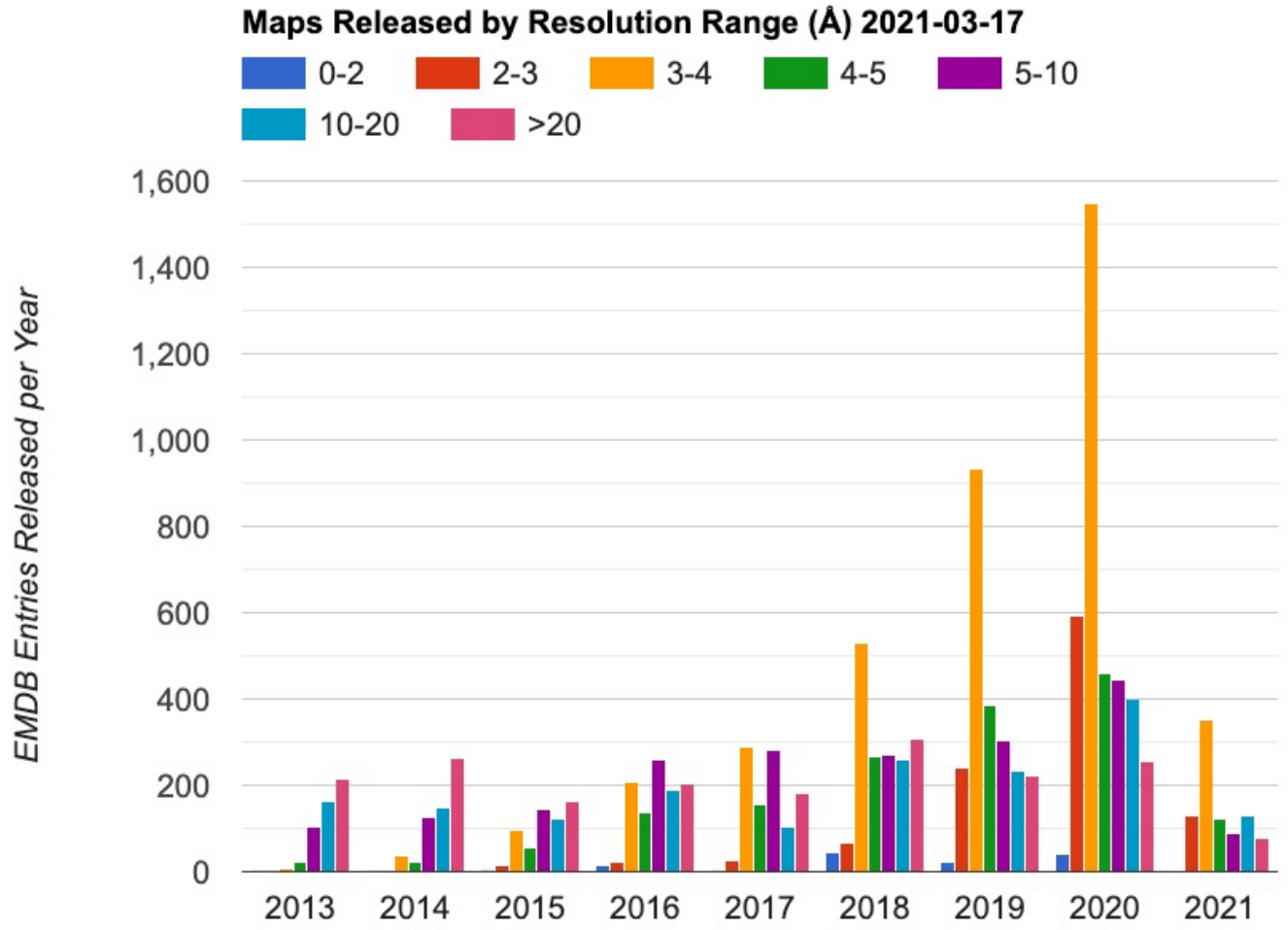
Resolution

Atom positions
Hydrogen bonds
Ligand conformation
Water molecules……



http://www.emdataresource.org/statistics.html

Resolution

Atom positions
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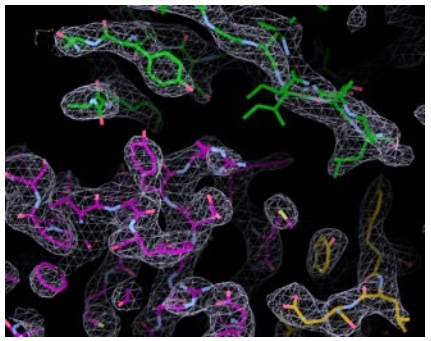
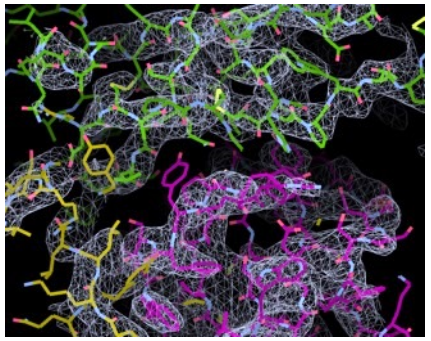
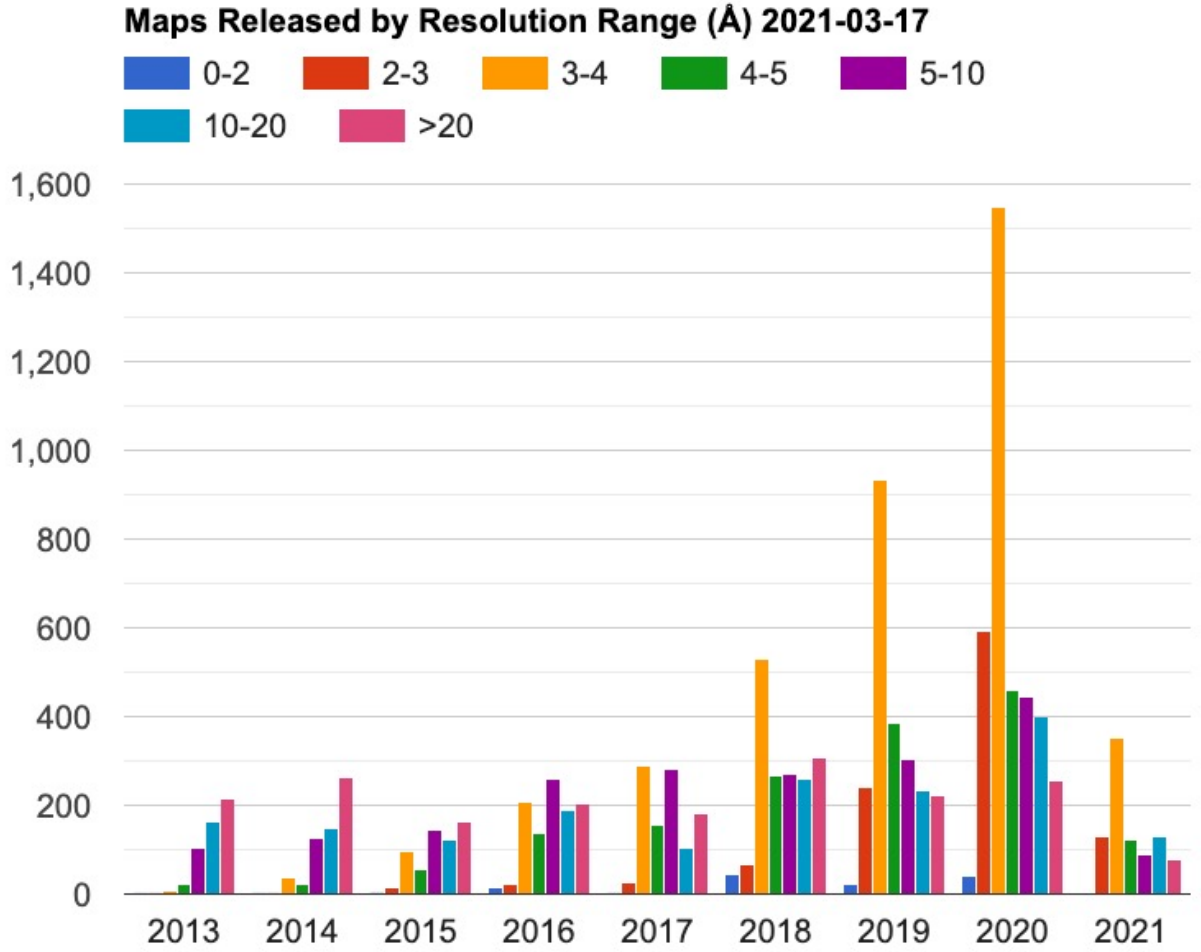
3 Å



3.5 Å





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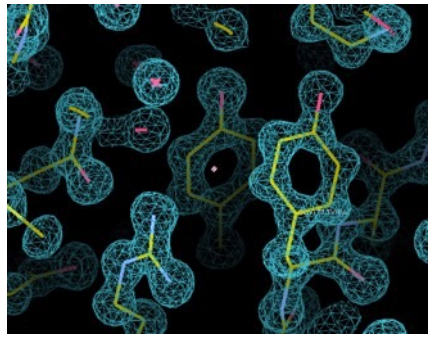
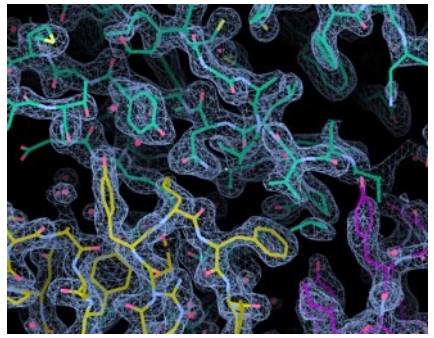
Resolution

1 Å



2 Å



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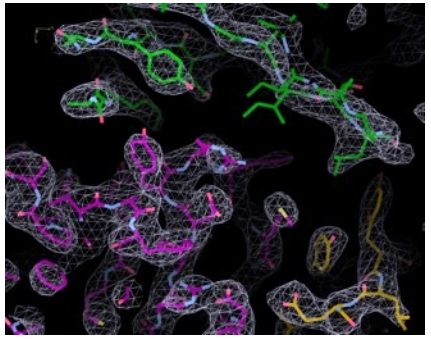

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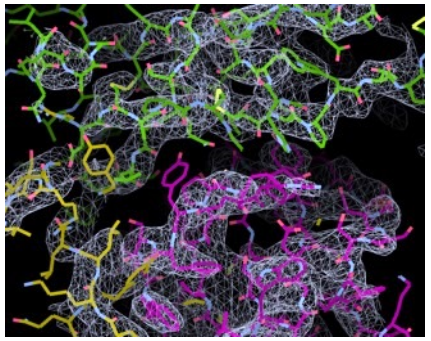

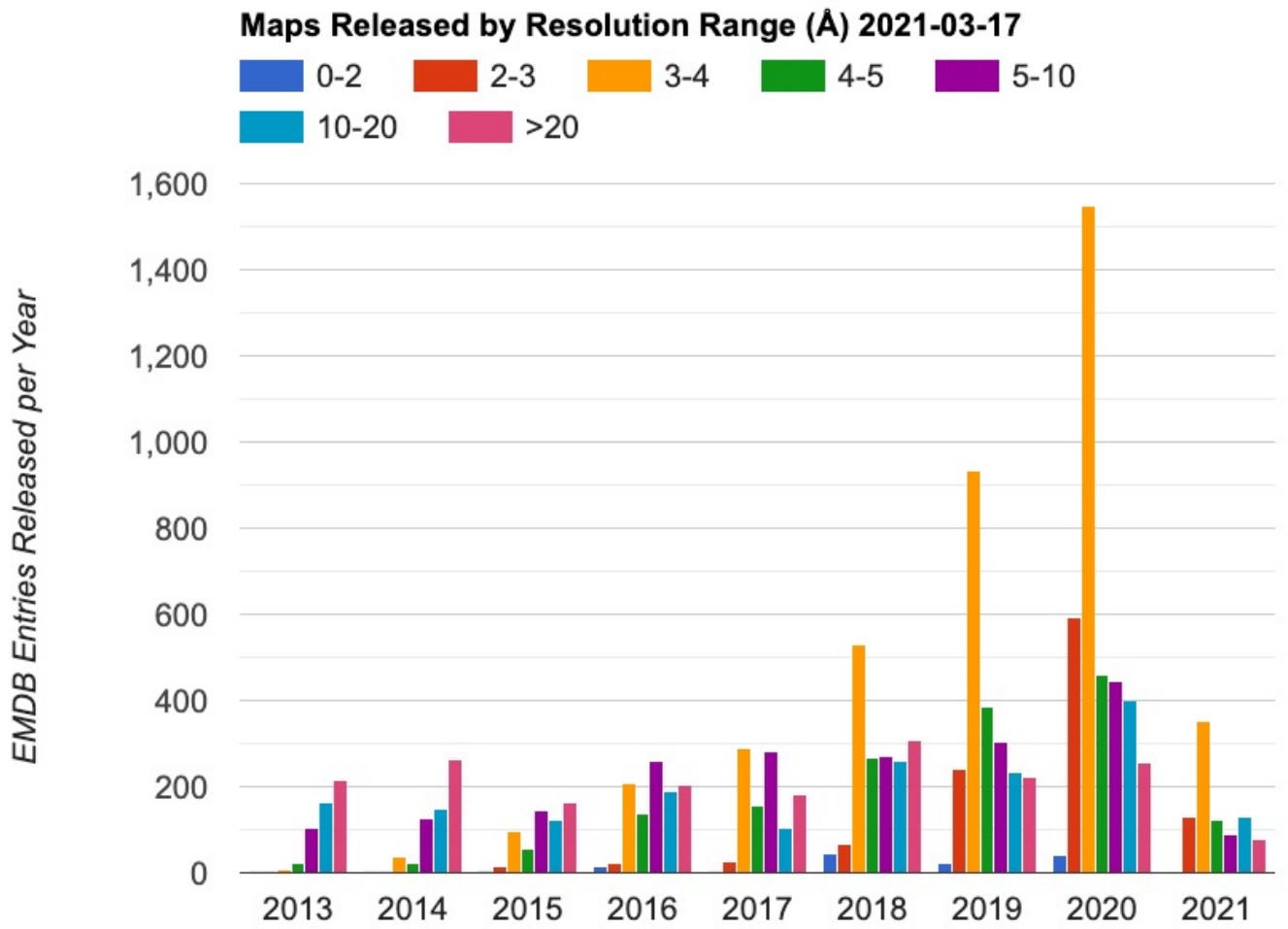

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Resolution

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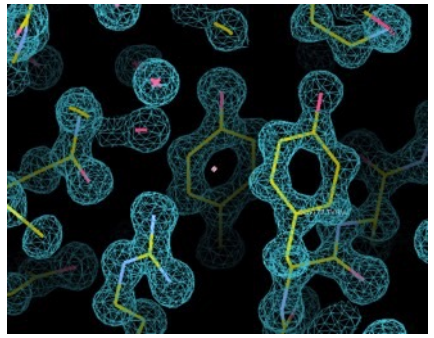

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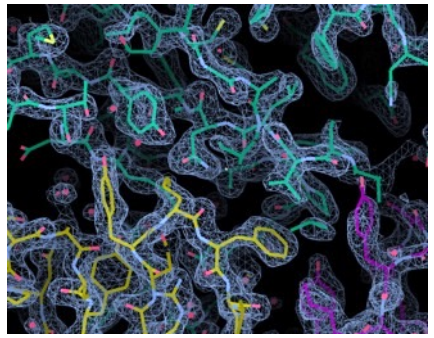

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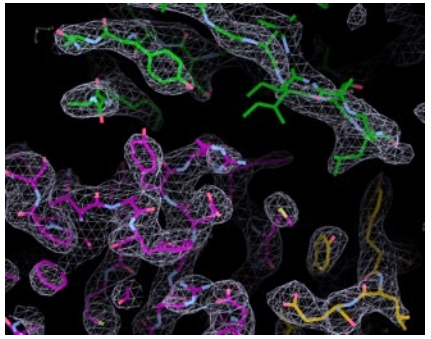

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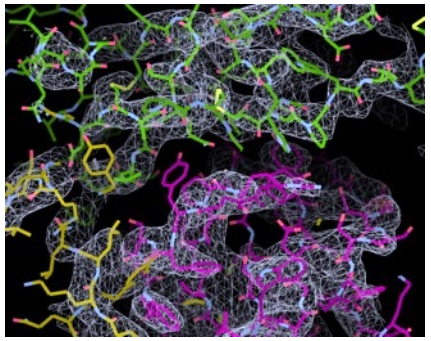

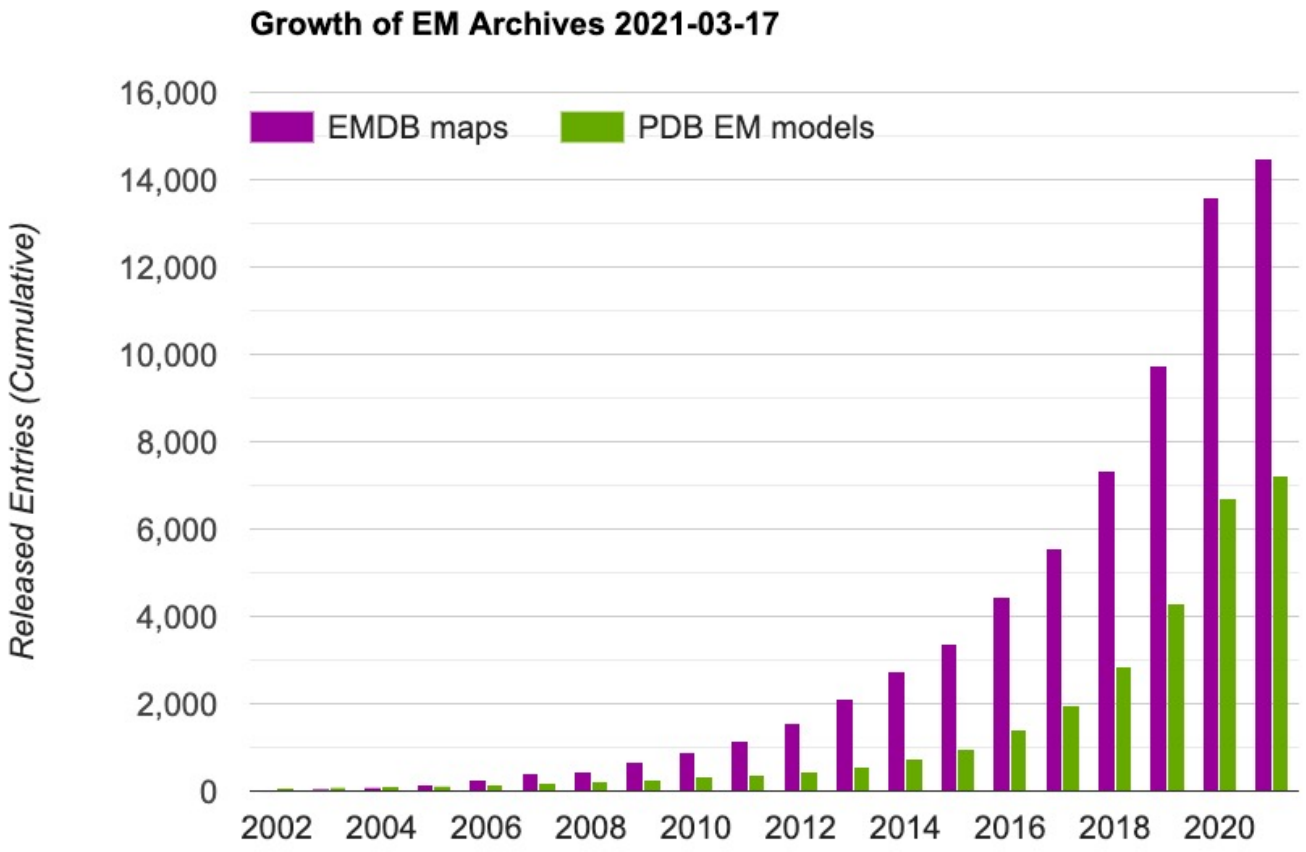

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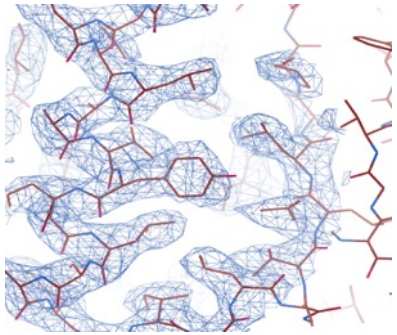
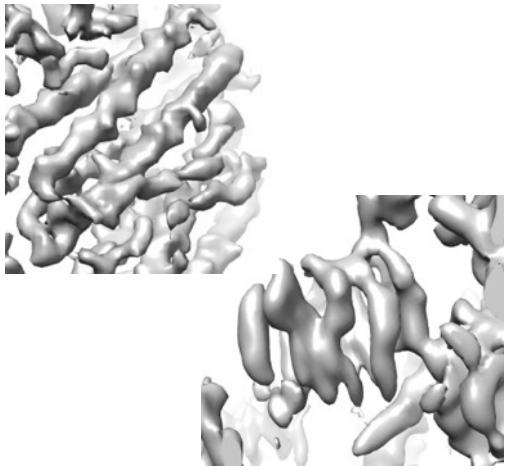
Model building & Validation

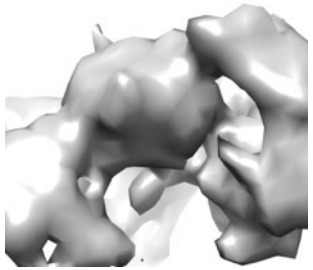


A map is a map, what we need for drug design is a structure

Maps at or better than 3.5 Å → “Standard” MR, model building/rebuilding, real space refinement can be used; ligands are visible

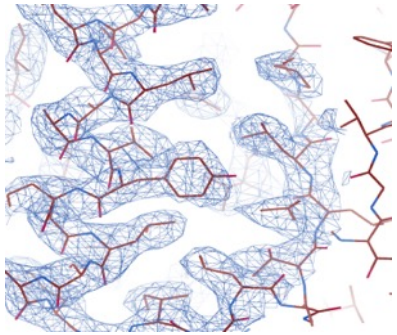


Maps between ~5 and 3.5 Å → “Standard” MR still possible, but model building/rebuilding and refinement need to be “guided”. Ligands are a guessing game



Maps worse than 5 Å → “Standard” MR likely to fail, model building/rebuilding (when even possible) and refinement need to be “guided”. Ligands? What ligands?

Model building & Validation



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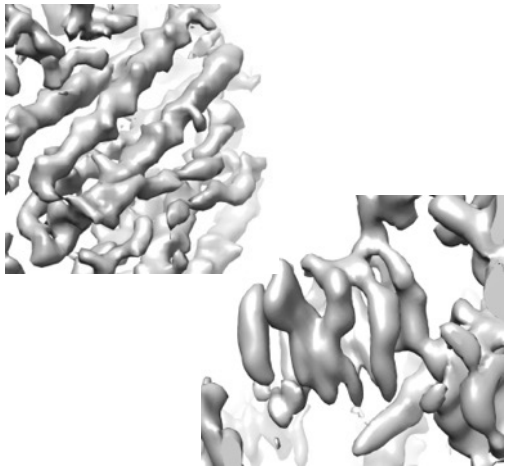
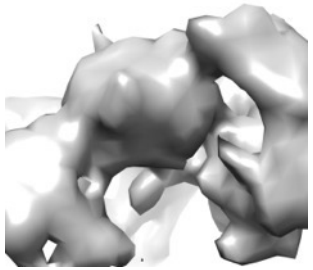
- Chimera, Coot, Phenix-Refine, Rosetta…
- CM&I Engagement and MD driven fitting



Map lding/rebuilding and

A good model is essential for good modeling – VALIDATION! (PDB)

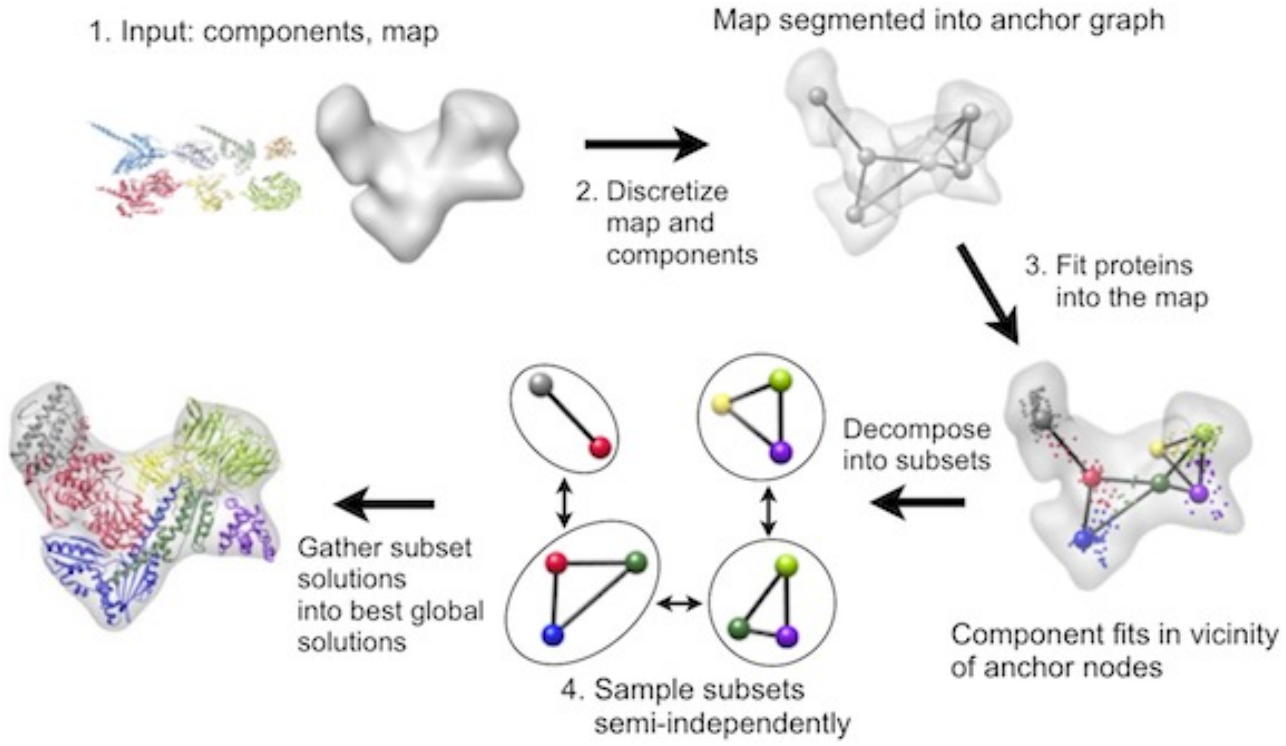
→ Map-to-structure pipeline



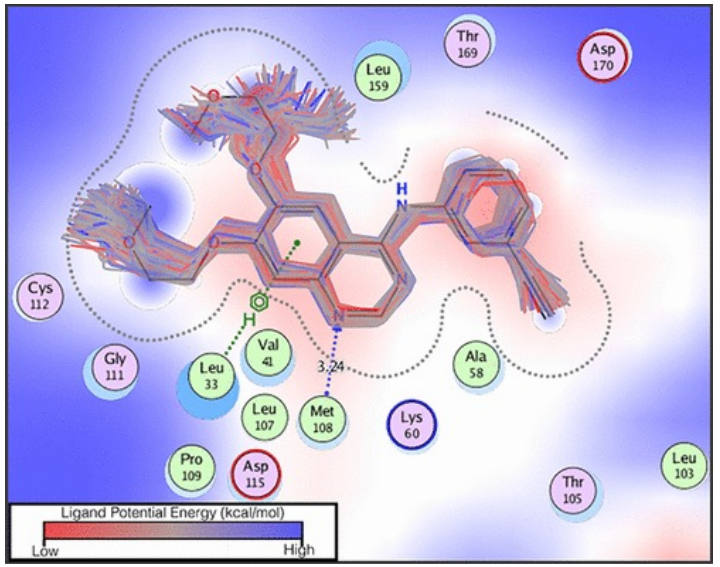
Maps worse than 5 Å → "Standard" MR likely to fail, model building/rebuilding (when even possible) and refinement need to be "guided". Ligands? What ligands?

NANOIMAGING SERVICES

Even low-res maps are better than no maps



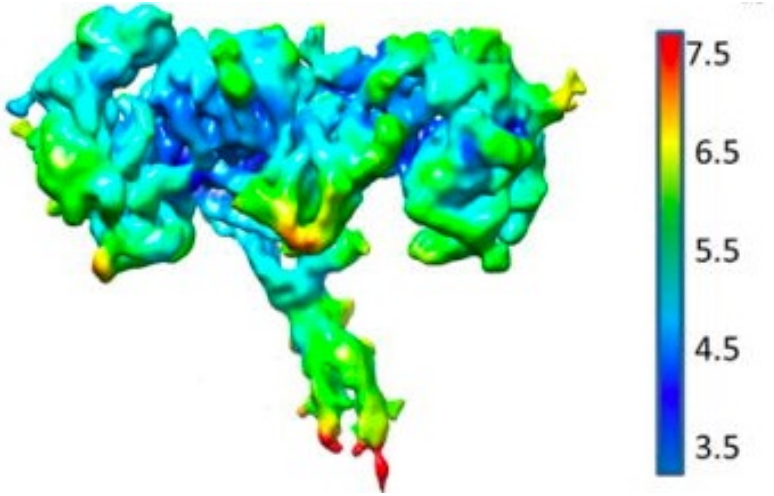
https://integrativemodeling.org/1.0/tutorial/multifit.html



Flexible Fitting of Small Molecules into Electron Microscopy Maps Using Molecular Dynamics Simulations with Neural Network Potentials

J. Chem. Inf. Model. 2020, 60, 5, 2591–2604

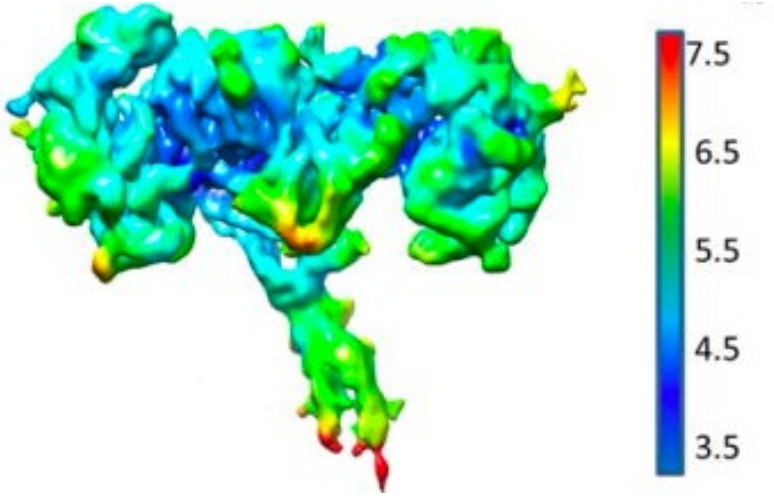
Local resolution



Scapin, G et al., *Nature* 2018 **, 556,** pg 122-125

Nominal resolution 4.7 Ang

Local resolution



Scapin, G et al., *Nature* 2018 **, 556,** pg 122-125

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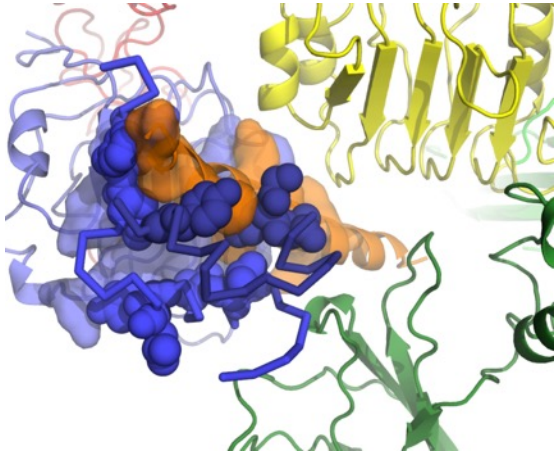
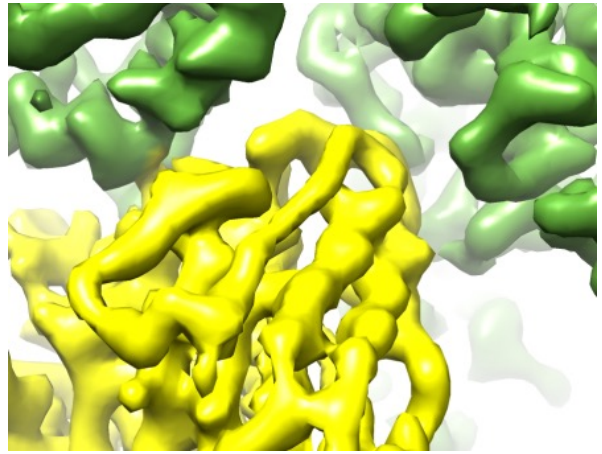
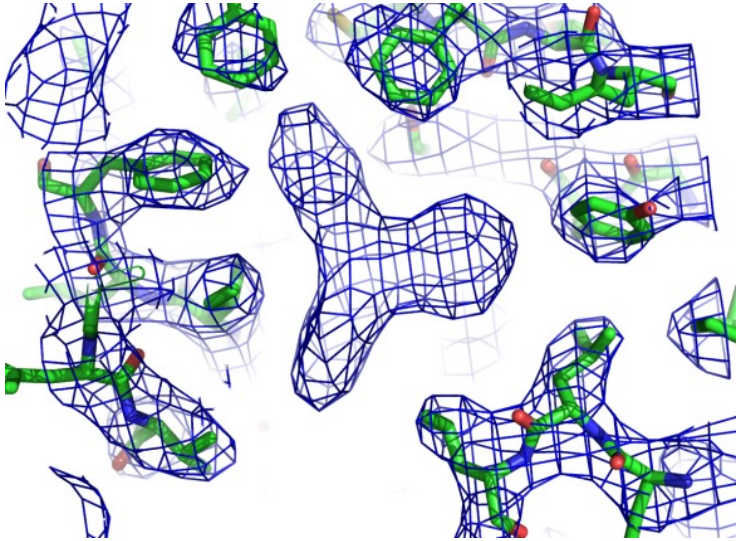




Conformational changes
Ligand binding
Epitope mapping



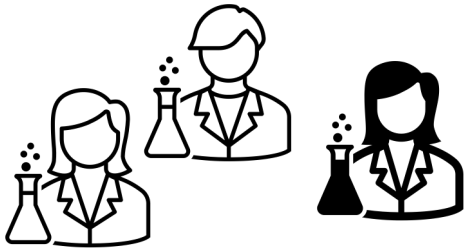
NanoImaging Services

Bottleneck # 1: cost and accessibility









STRUCTURA BIOTECHNOLOGY

RELION

Phenix

Estimated cost: $8-10M up front, $2.5-3.0M/year running costs (Facilities costs, salaries, equipment service contracts, IT storage, software licensing and fees)

NanoImaging Services

Academic centers, Consortia & CROs

PACIFIC NORTHWEST Cryo-EM Center

Cambridge Pharmaceutical Cryo-EM Consortium

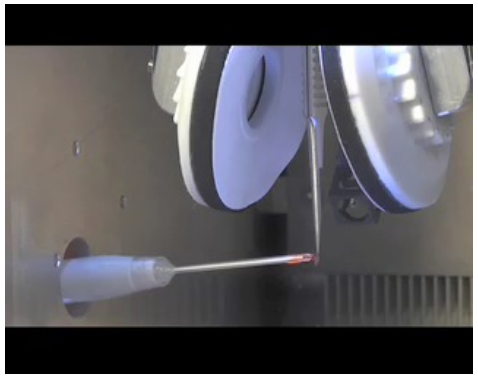
NCCAT National Center for CryoEM Access and Training

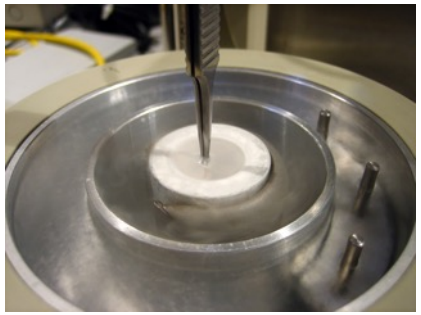
S²C² | Stanford-SLAC Cryo-EM Center

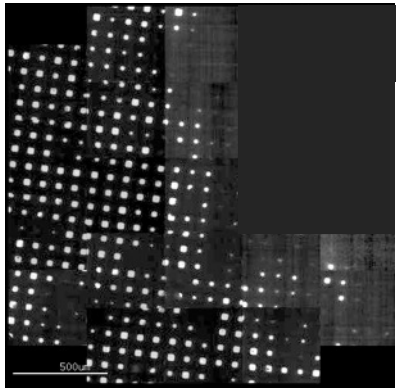
ThermoFisher SCIENTIFIC

NanoImaging Services

Bottleneck #2: vitrification

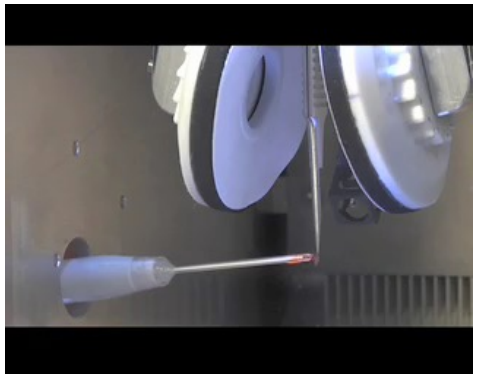


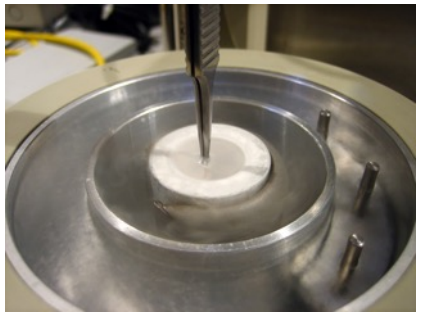


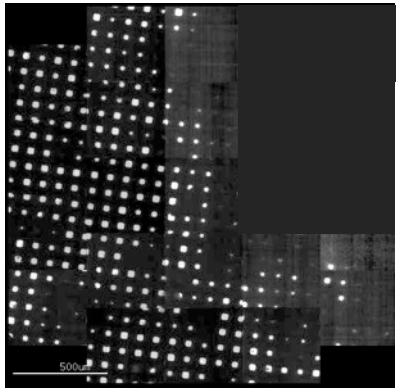


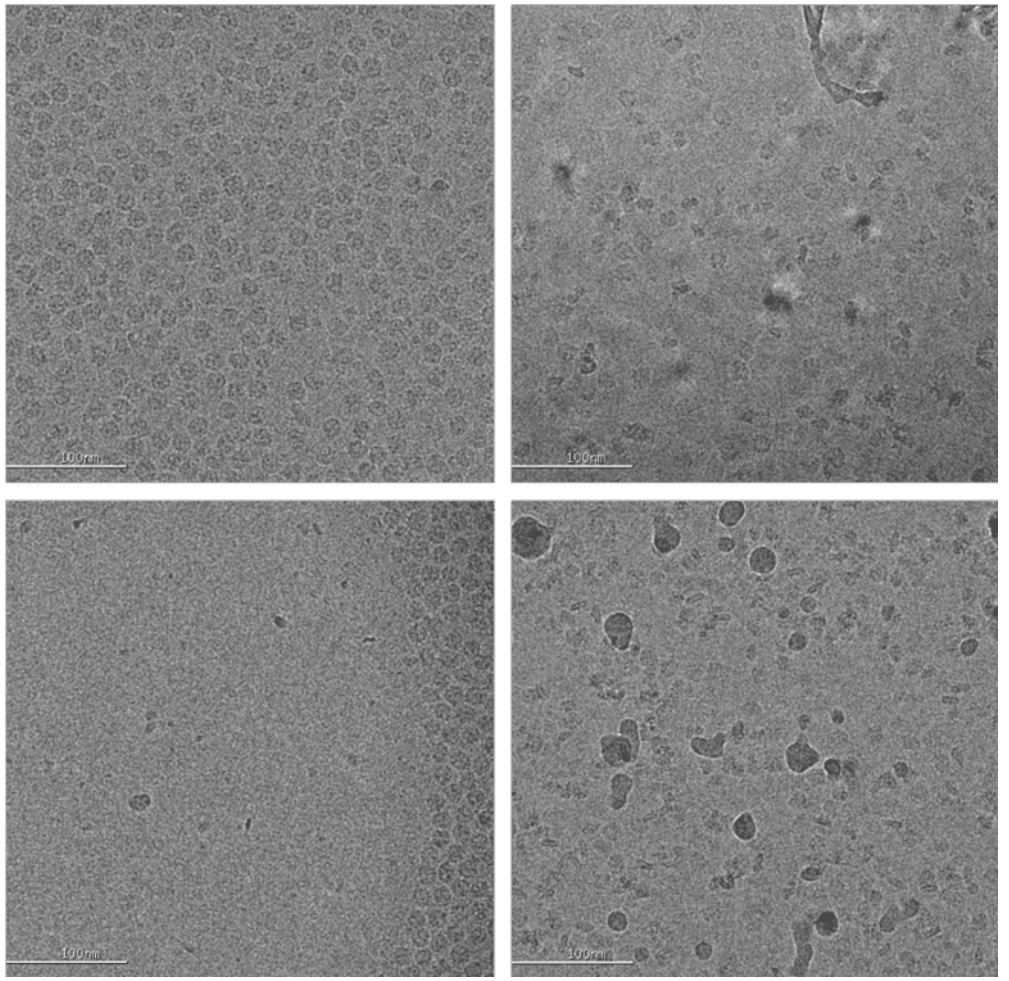
NanoImaging Services

Bottleneck #2: vitrification



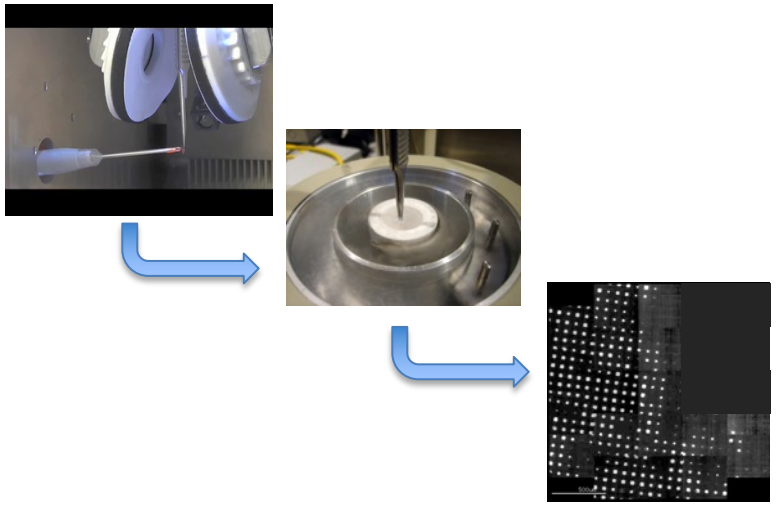


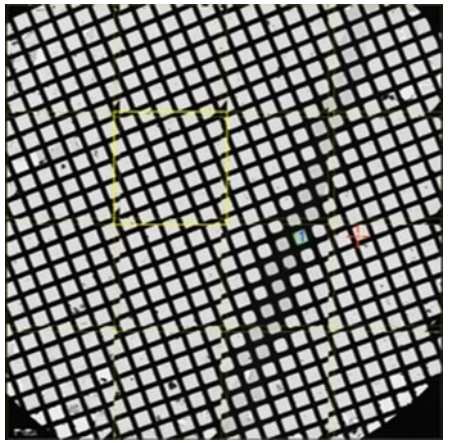


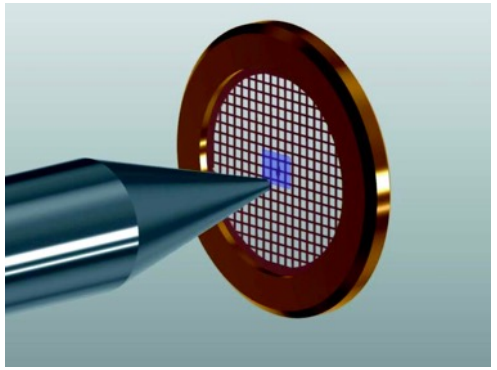


NanoImaging Services

New approaches to vitrification







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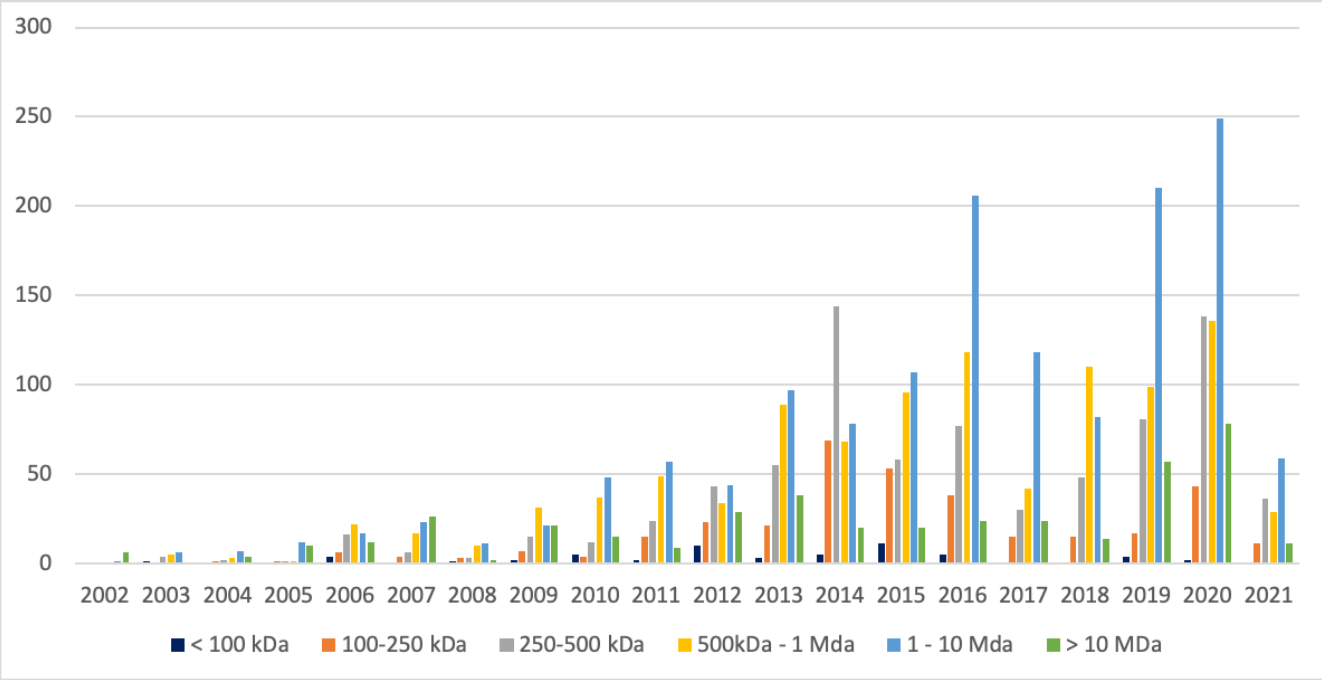
Bottleneck # 3: sample

Size

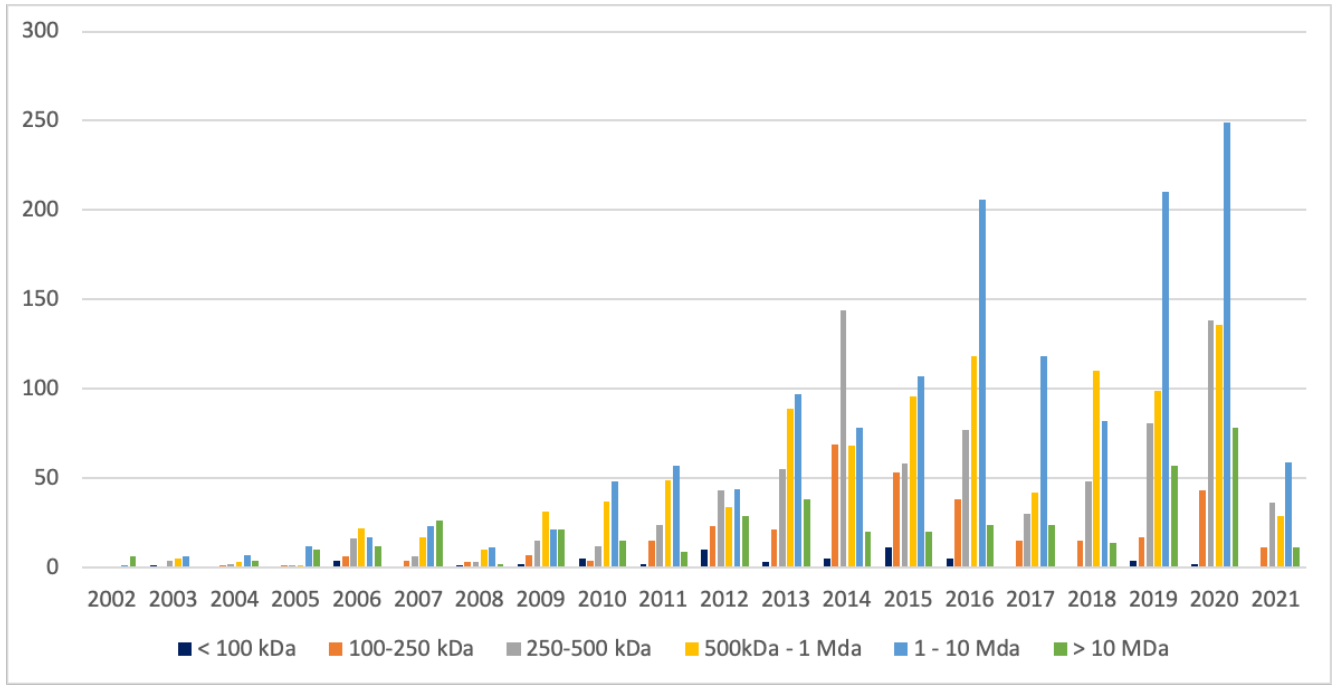
Molecular weight trends for SP maps



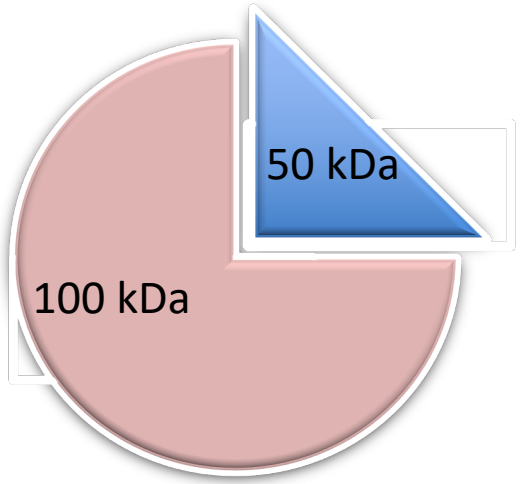
Bottleneck # 3: sample

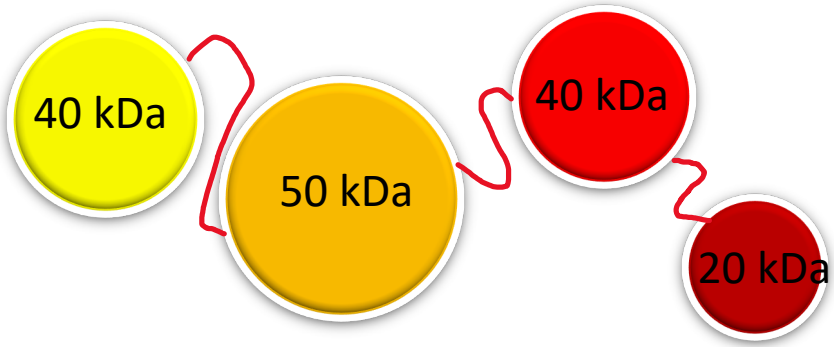
Size

Molecular weight trends for SP maps



Absolute mass vs. ordered mass





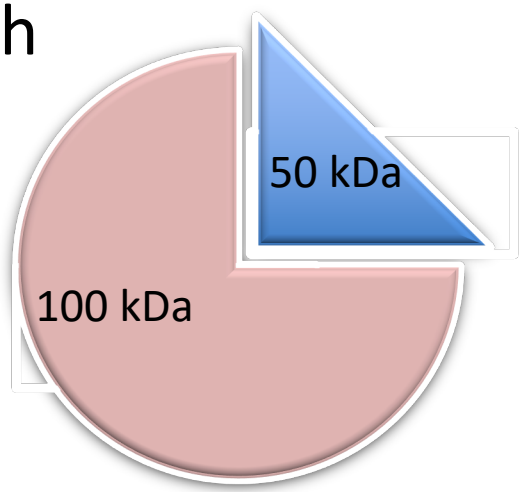
Increasing the size…

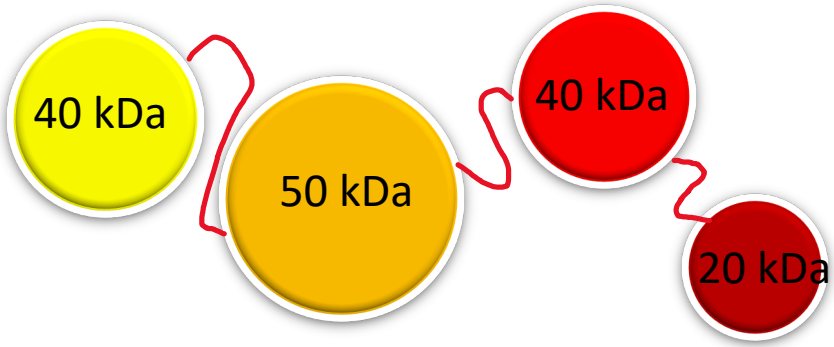
Size

- Increase size of ordered mass by complexing with
  - FAB
  - Biological partners
  - Large substrates
  - Stabilizing binders

- Ordered matter

Absolute mass vs. ordered mass





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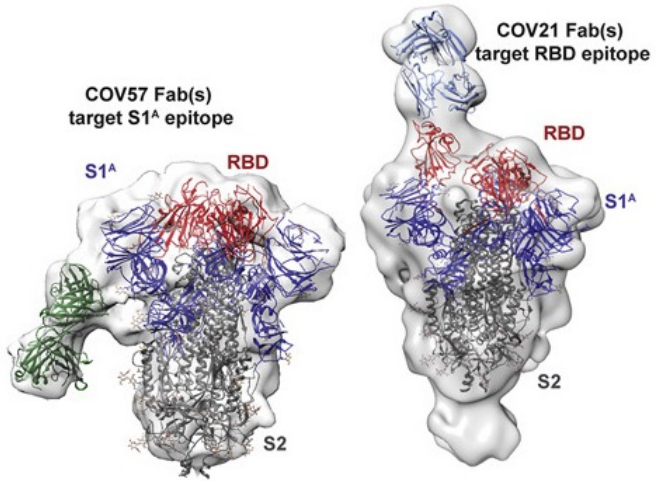
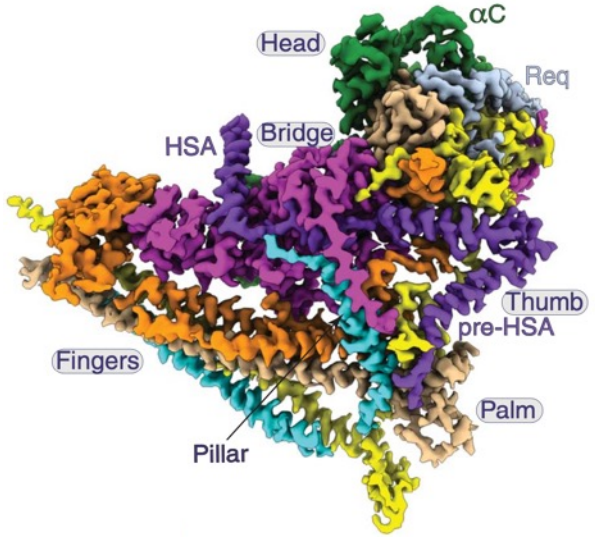
Bottleneck # 3: sample

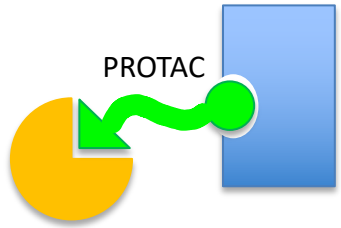
Complexes

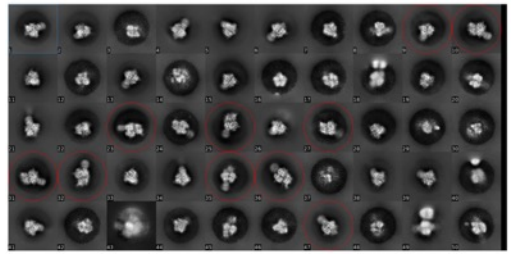


Barnes, et al. Cell 182 (4) 2020, 828-842 (2020)



He et al., Science 367, 875–881 (2020)





Bussiere et al., www.biorxiv.org/content/10.1101/737510v1 (2020)

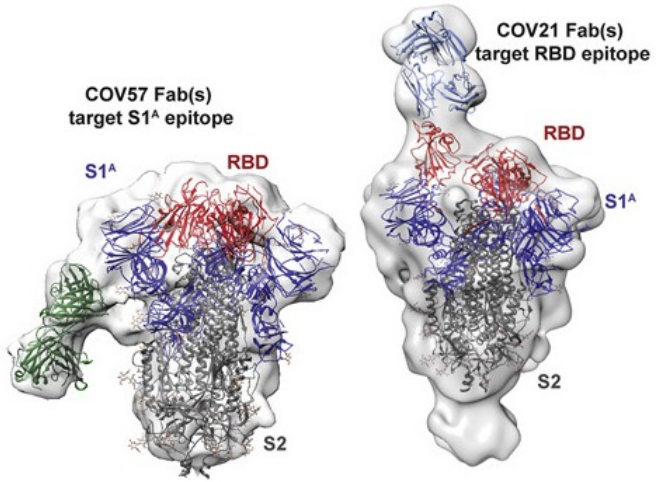
Average sample concentration in

Negative Staining	0.05 – 0.2 nM
Vitrified grids	3 – 50 nM

NANOIMAGING SERVICES

Bottleneck # 3: sample

Complexes



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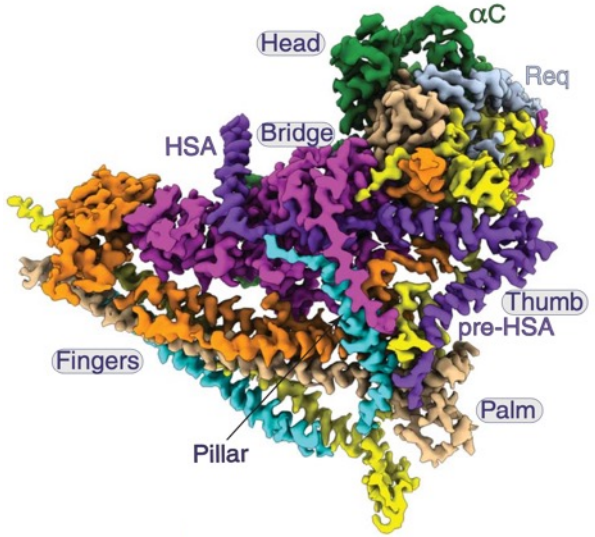
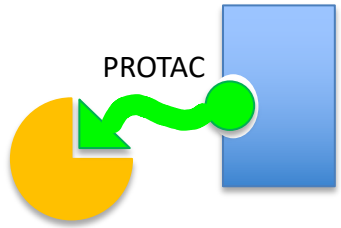
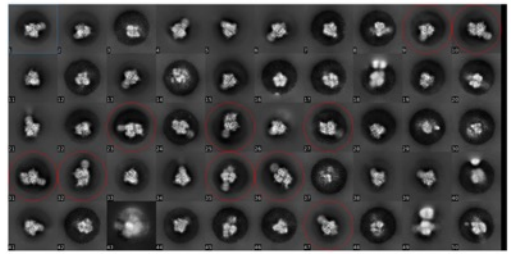


He et al., Science 367, 875–881 (2020)





Bussiere et al., www.biorxiv.org/content/10.1101/737510v1 (2020)

Average sample concentration in

Negative Staining	0.05 – 0.2 nM
Vitrified grids	3 – 50 nM

Upper nM to low μM Kd may result in **complex dissociation** under cryoEM condition

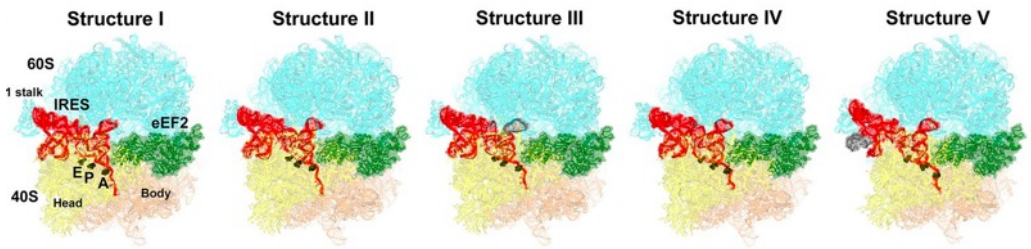
Possible solutions:

Higher protein concentration
Increase the ratio of one component
Cross-linking
Beware of Negative Staining results

Bottleneck # 3: sample

Flexibility

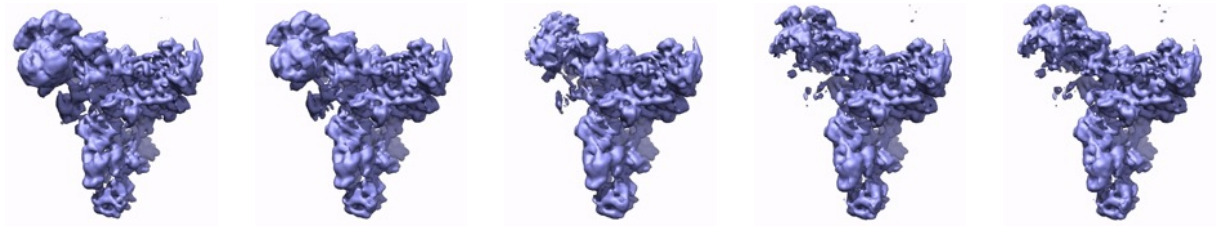
Discrete



Abeyrathne, et al.
https://elifesciences.org/articles/14874 (2020)

Can be resolved by multiple rounds of classification (2D or 3D)
Focused refinement, local refinement or multibody refinement can be used to optimize the variable areas

Continuous



https://guide.cryosparc.com/processing-data/tutorials-and-case-studies/tutorial-3d-variability-analysis-part-one

Punjani, et al. bioRxiv. https://doi.org/10.1101/2020.04.08.032466, (2020)

3D Variability Analysis (3DVA) is a computational method to resolve continuous and discrete heterogeneity from single particle cryo-EM data of protein molecules.

Acknowledgements

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Christina Cheng

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All all the crew at NYSBC/SEMC

National Resource for Automated Molecular Microscopy
http://nramm.nysbc.org

Questions?