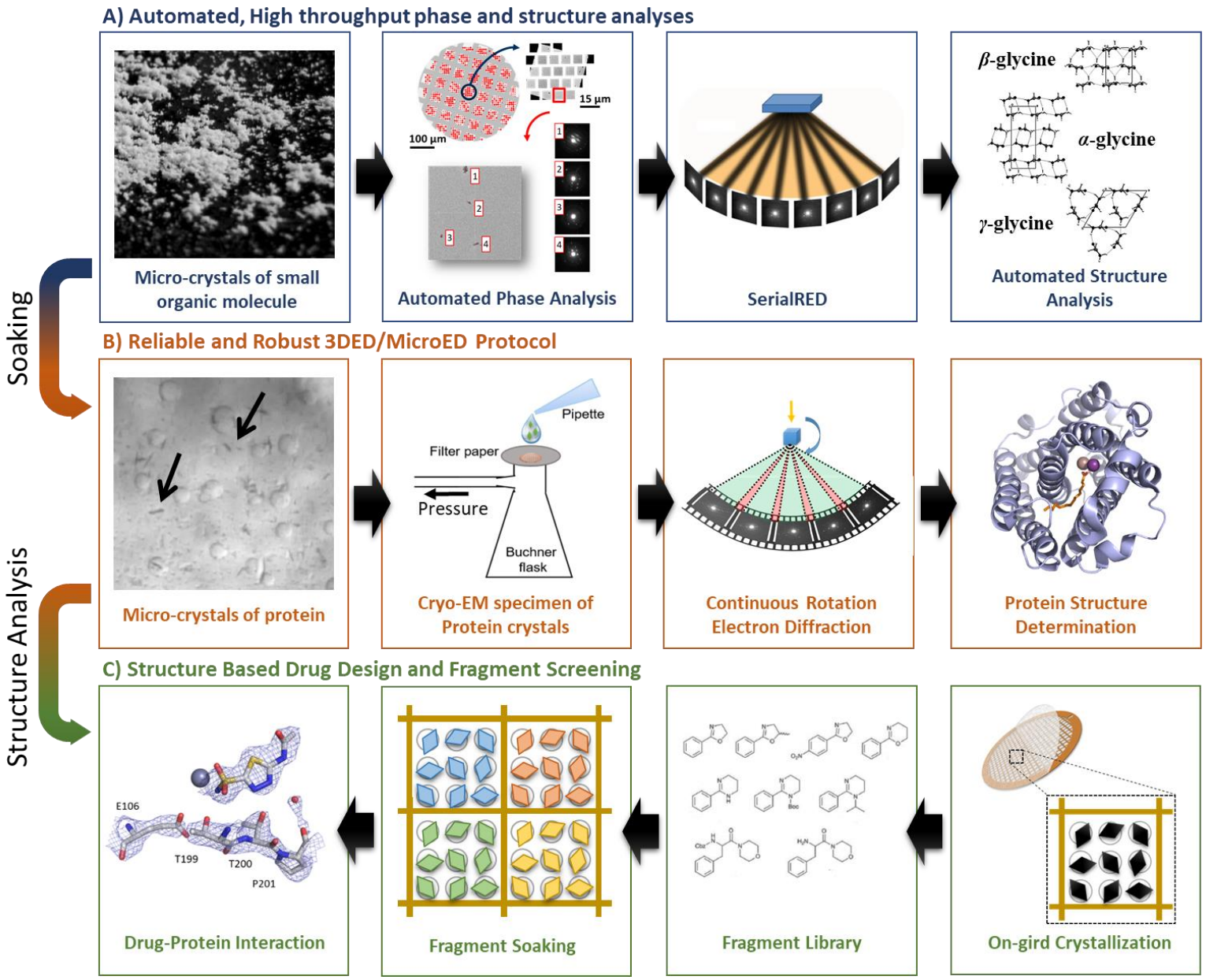
H2020-MSCA ITN
Grant n. 956099

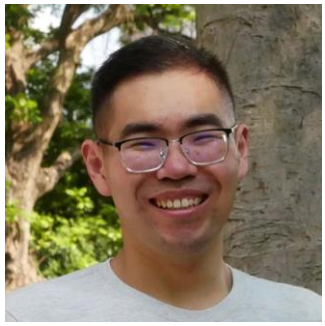
Stockholm University

ESR 10 – Structure-based Drug Discovery by 3D ED

Lei Wang and Hongyi Xu – Stockholm University

Outline





Lei Wang

- **Polymorphism**
- **Crystal engineering**
- Phase analysis (ESR 11)

FZU: Dynamical refinement on organics
Supervisor: **P. Brázda**

- **Structure determination of drug targets**

UBA: 3D ED ab-initio phasing on proteins
Supervisor: **J-P. Abrahams**
eBIC: Single particle cryo-EM
Supervisor: **P. Zhang**
Thermo Fisher: State of art of cryo-TEM

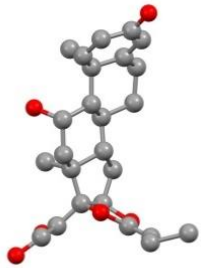
- **Protein-Ligand interaction**

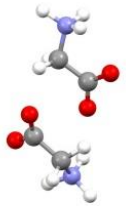
AstraZeneca: Protein ligand interaction
Supervisor: **H. Käck**

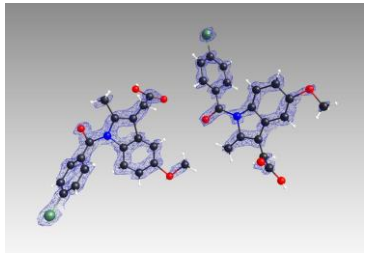
Alignment with Our Research at SU

Molly Lightowler

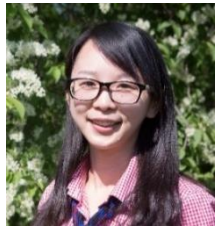
Drug Molecules







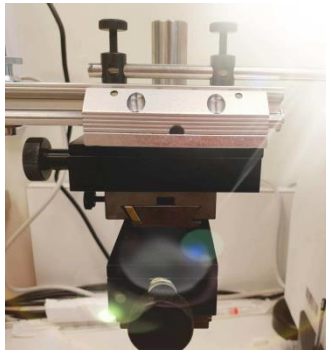
ChemRxiv **2021**
Submitted



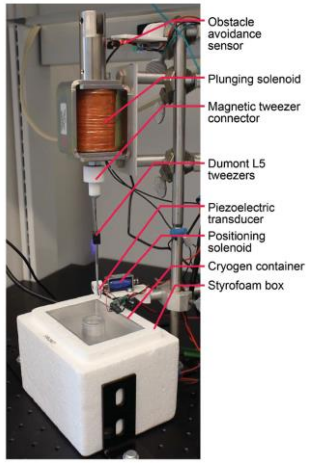
Jingjing Zhao

Jiaoyan Xu

cryo-EM specimen preparation

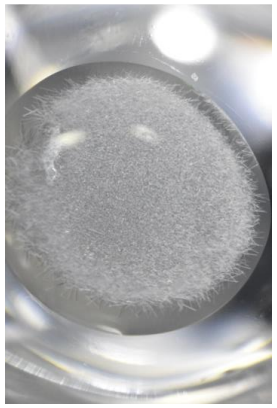


Nat. Commun. **2021**

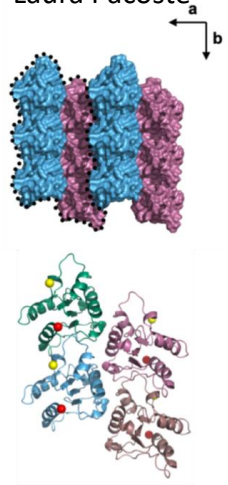


Acta D. **2019**

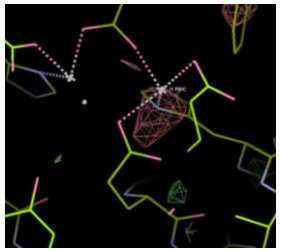
Dr. Gerhard Hofer



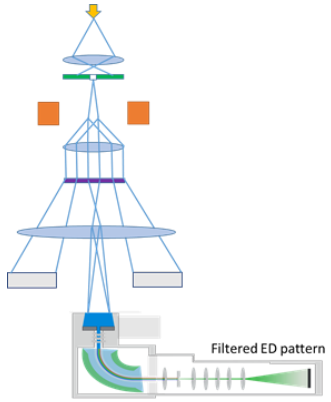
Laura Pacoste



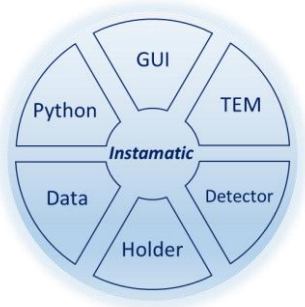
Nat. Commun. **2021**



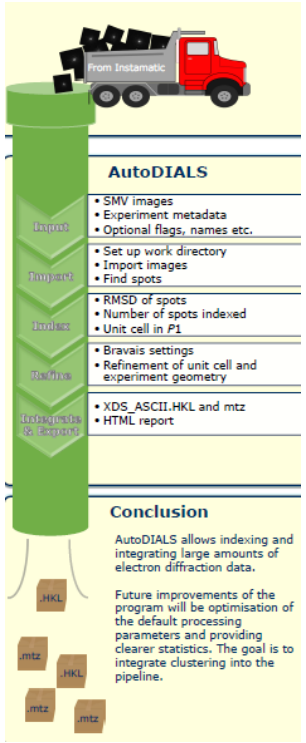
Dr. Taimin Yang



aRxiv **2021**



Viktor Bengtsson



Dr. Mathieu Coincon

MicroED
@

SciLifeLab

SCIPION

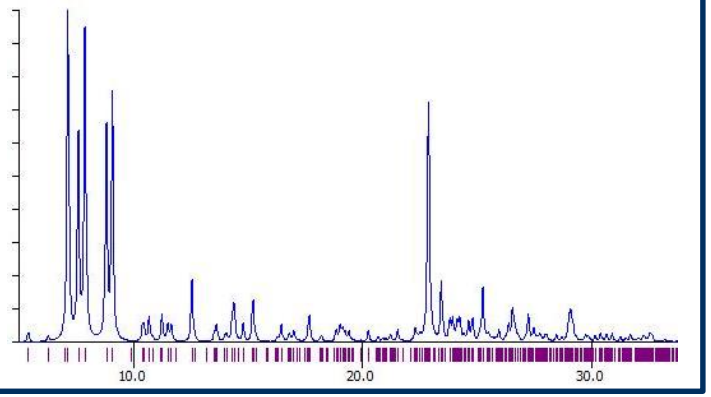
DIALS

Small Molecules

Small Organic Molecules – Sample Prep.

Typical Sample

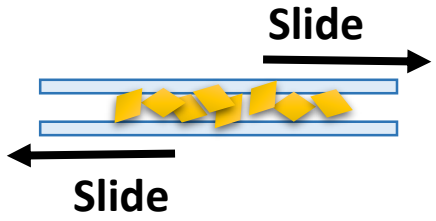
Screening with PXRD



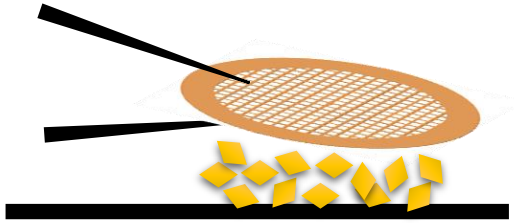
Specimen Preparation

Crush

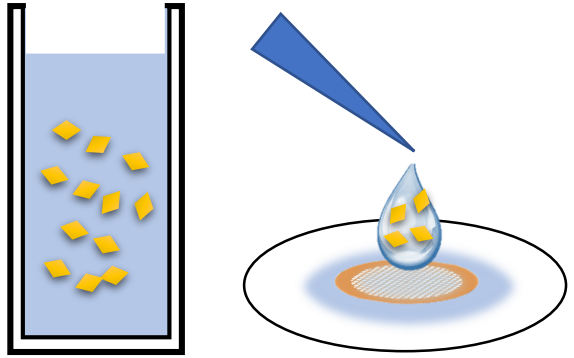
or



Specimen Transfer to TEM grid

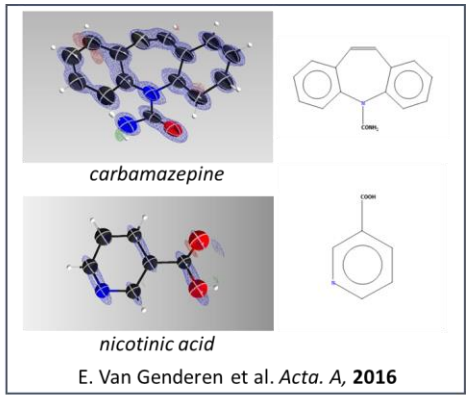


or

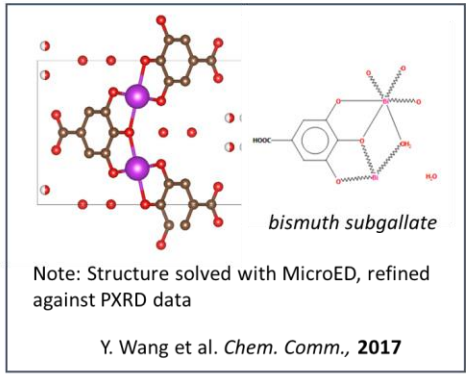


Small Organic Molecules – Examples

- Routine structure determination
- Reliable structure models
- Need more attention to structural details
  - ADPs
  - Data completeness
  - Hydrogens
- Typical turn around: 1-2 days

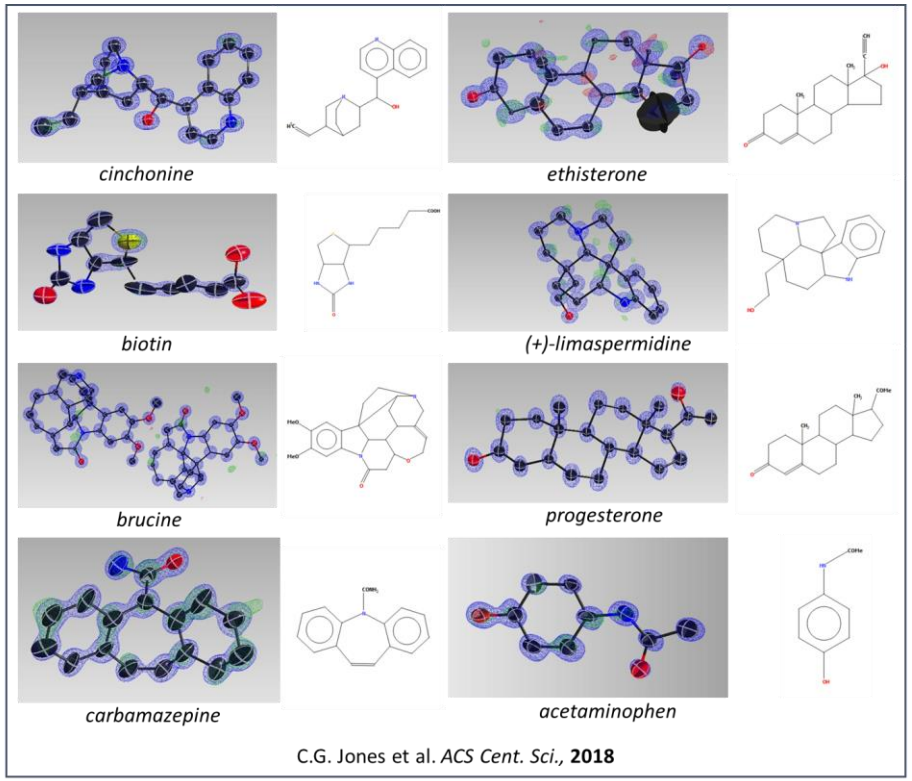


E. Van Genderen et al. *Acta. A,* **2016**

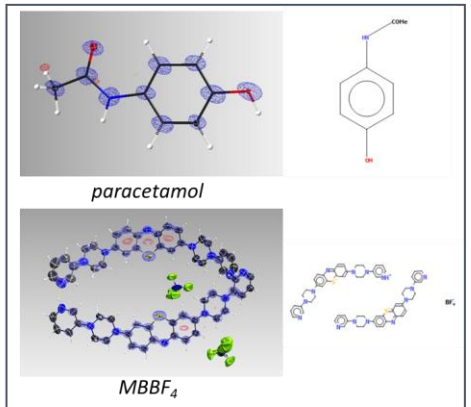


Note: Structure solved with MicroED, refined against PXRD data

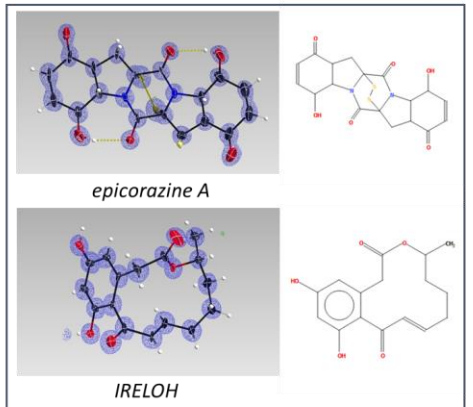
Y. Wang et al. *Chem. Comm.*, **2017**



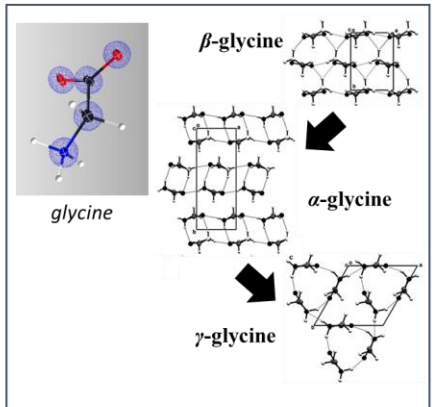
C.G. Jones et al. *ACS Cent. Sci.*, **2018**



T. Grüne et al. *Angew. Chem.*, **2018**



M.T.B Clabbers et al. *Acta. A.*, **2019**



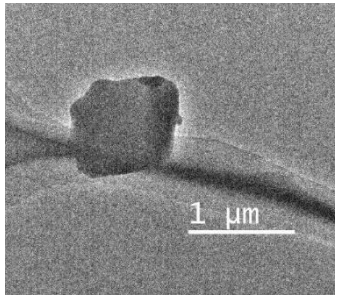
E.T. Broadhurst et al. *IUCrJ,* **2020**

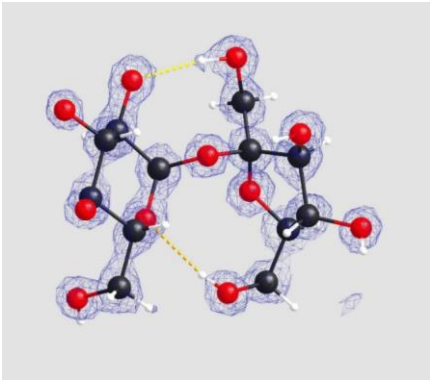
Clabbers and Xu, *Drug Discovery Today: Technology* **2020**

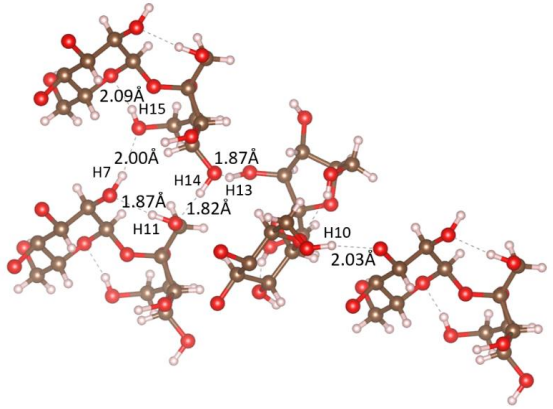
Small Organic Molecules – Examples

- Sample source: Sugar cube for coffee and tea
- Data collection parameters:
  - Rotation speed: 1.13 °/s
  - Exposure: 0.3 s/frame
  - **Total range: ~ 60° per dataset** (190 frames)
  - Electron dose rate: 0.08 $e^-/Å^2/s$
  - **Total electron dose: ~ 5 $e^-/Å^2$**
- Data collected at both room temperature and 100K
- In average, 15 datasets per 4 hour session

Refinement			
	Sucrose	Sucrose Single Dataset	Sucrose @ 100K
a	10.86	10.86	10.91
b	8.71	8.71	8.78
c	7.76	7.76	7.85
β [°]	102.9	102.9	103.4
Resolution [Å]	0.77	0.77	0.77
No. of total refined data	3203	1778	2093
No. of parameters	242	94 (isotropic)	102 (isotropic)
No. of restraints	5	1	1
R_1 Fo > 4σ (Fo)	0.1438	0.2890	0.2097
R_1 all	0.1666	0.3183	0.2197
R_{int}	0.5544	0.0640	0.5205
GooF	1.017	0.974	1.203
wR_2	0.3965	0.6649	0.4530

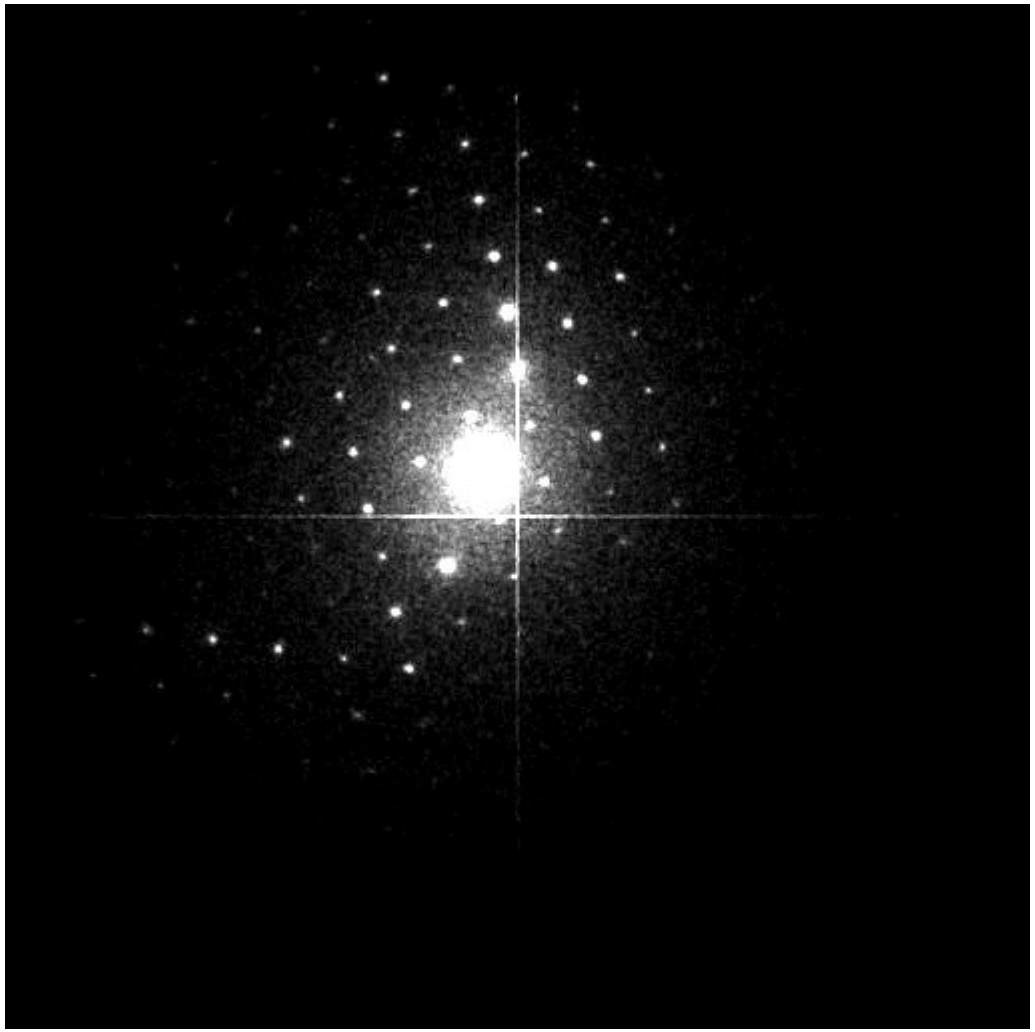




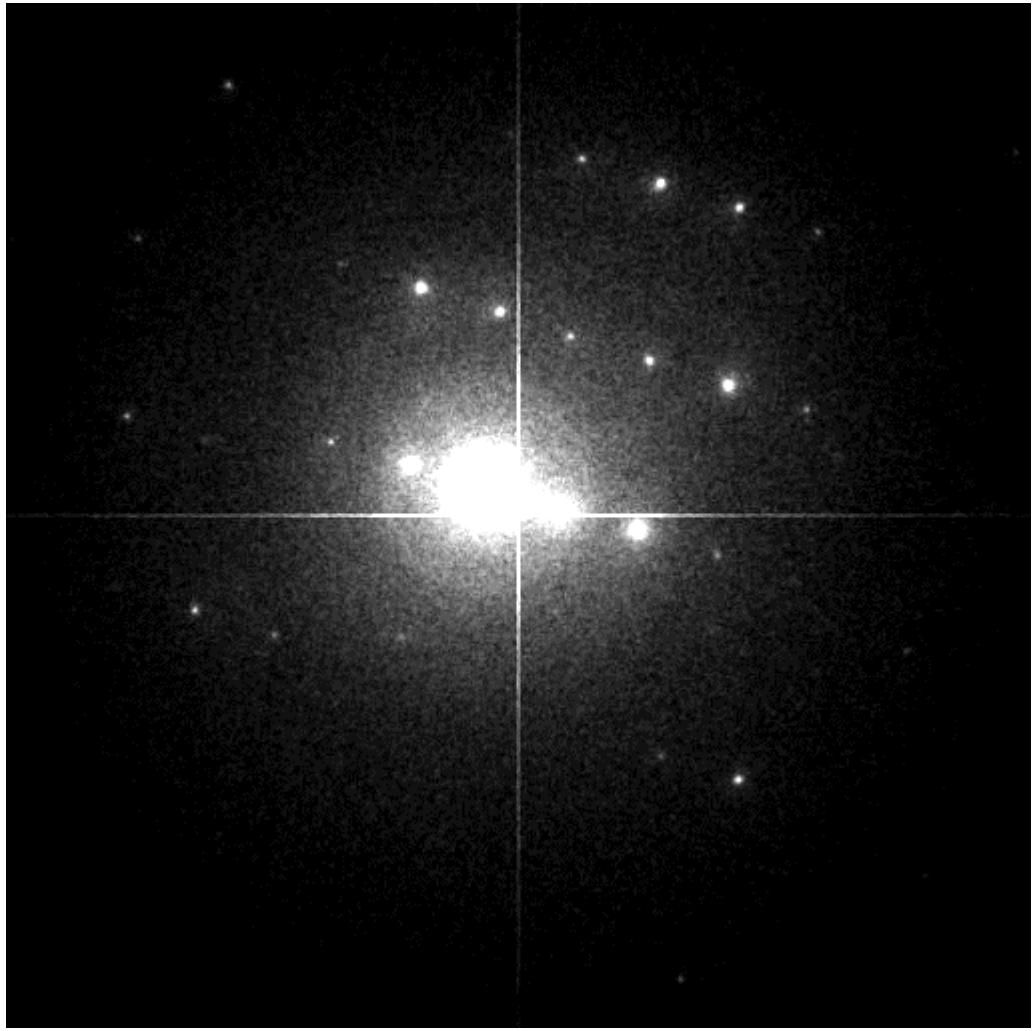


Yang et. al., *Symmetry* **2021**

Small Organic Molecules – Examples



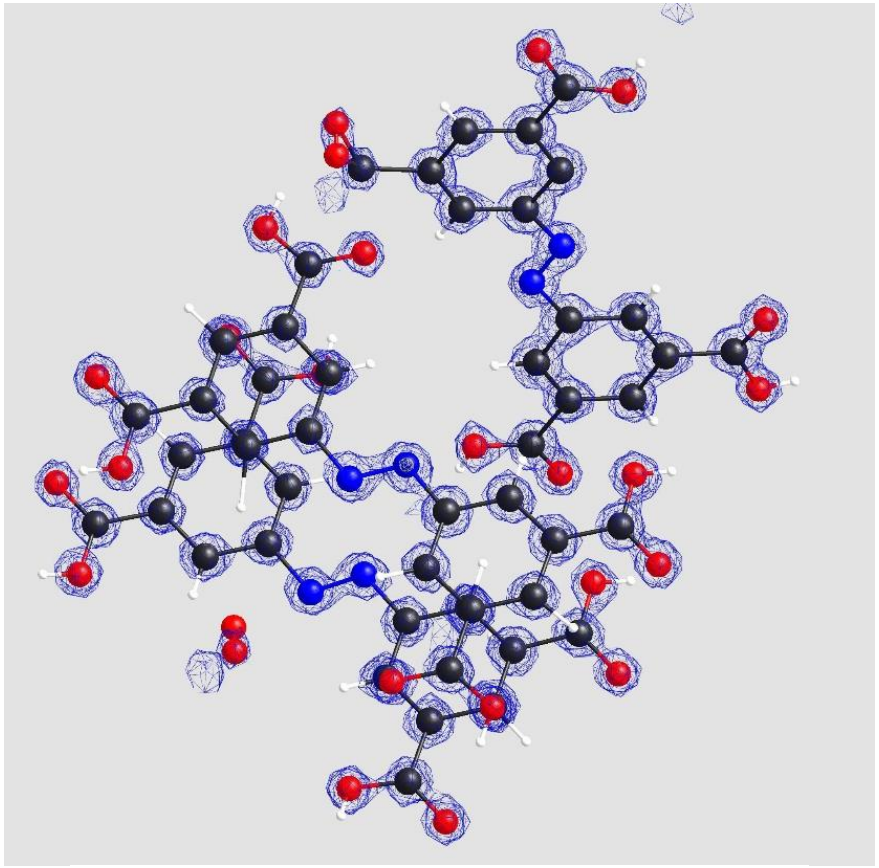
Sucrose Data Collection at **Room Temperature**
4 x playback speed

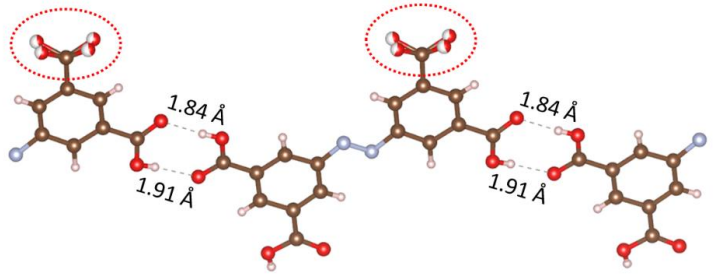


Sucrose Data Collection at **100 K**
4 x playback speed

Yang et. al., *Symmetry* **2021**

Small Organic Molecules – Examples





Yang et. al., *Symmetry* **2021**

	H_4ABTC	H_4ABTC @ 100K
	Data Processing	
Number of crystals	5	9
a	13.47	13.52
b	14.63	14.63
c	14.65	14.70
α [°]	98.55	99.40
β [°]	105.90	106.06
γ [°]	112.92	113.99
Resolution [Å]	0.80	0.90
I/σ	4.77 (1.99)	2.63 (1.35)
$CC_{1/2}$ [%]	97.1* (88.4*)	90.8* (73.0*)
Redundancy	4.89	7.08
R_{meas}	0.209 (0.595)	0.395 (0.648)
No. of reflections	48822	48984
No. of unique reflections	8936	12999
Space group	*P*-1	*P*-1
Completeness	0.897 (0.814)	0.935 (0.752)
	Refinement	
Resolution [Å]	0.83	0.90
No. of total refined data	8143	6740
No. of parameters	767	704
No. of restraints	0	0
R_1 Fo > 4σ (Fo)	0.1842	0.2199
R_1 all	0.2102	0.2648
R_{int}	0.1843	0.3353
GooF	1.144	1.862
wR_2	0.4531	0.4773

78 unique atoms

Ave. bond Lengths:

C-C: 1.52(2) Å

C-C in benzene: 1.43(2) Å

C-O: 1.31(3) Å

C=O: 1.27(3) Å

C-N: 1.45(2) Å

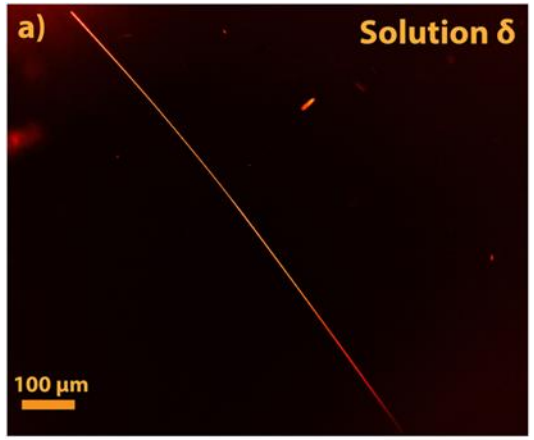
N=N: 1.27(2) Å

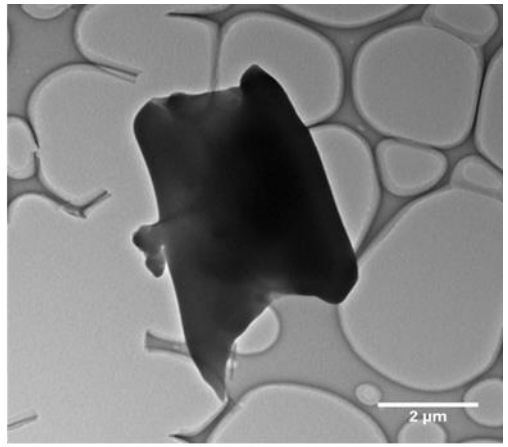
Indomethacin

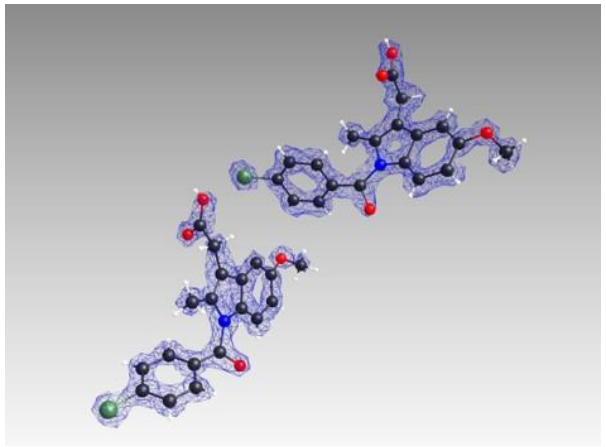
- **δ**-Indomethacin
- Misunderstood for 47 years

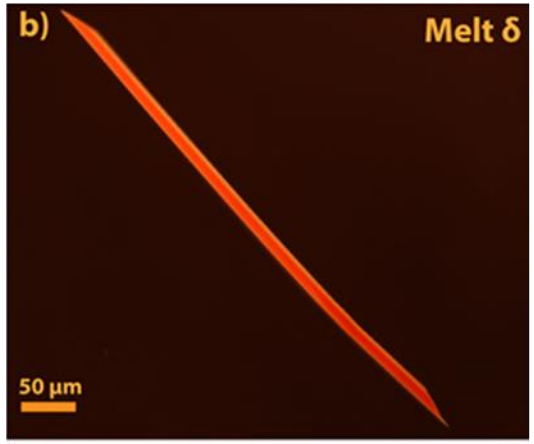
Sun Yat-sen University, China
Associate Prof. Ming Lu
Ms. Shuting Li

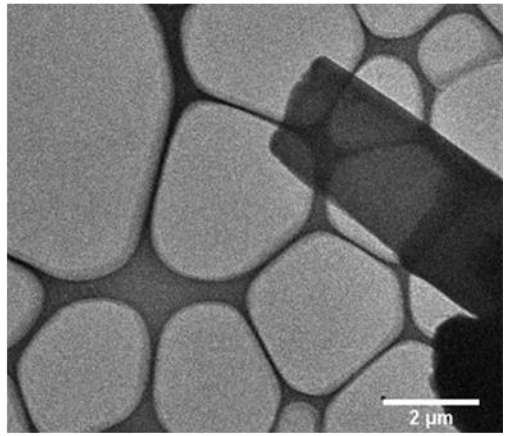
Molly Lightowler

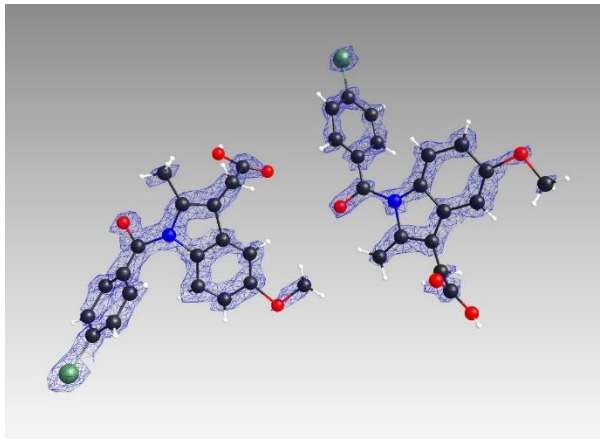


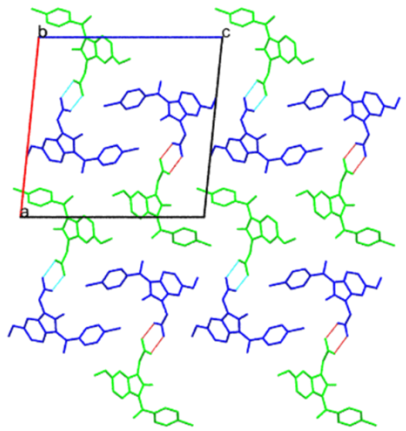


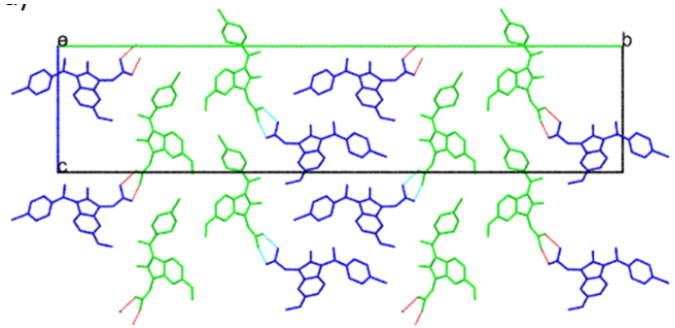












New Phase:
θ-Indomethacin
b-axis: 58.98 Å

M. Lightowler et al. *Angewandte Chemie* **2021**

Indomethacin and Other Examples

Structure determination, thermal stability and dissolution rate of δ-indomethacin

Iryna Andrusenko [a], Victoria Hamilton [b], Arianna E. Lanza [a], Charlie L. Hall [b], Enrico Mugnaioli [a], Jason Potticary [b], Asma Buanz [c], Simon Gaisford [c], Anna M. Piras [d], Ylenia Zambito [d] ✉, Simon R. Hall [b] ✉, Mauro Gemmi [a] ✉

Solution-**δ**-Indomethacin
Independently solved in Mauro Gemmi's group

2594
doi:10.1017/S1431927621009223

Microsc. Microanal. 27 (Suppl 1), 2021

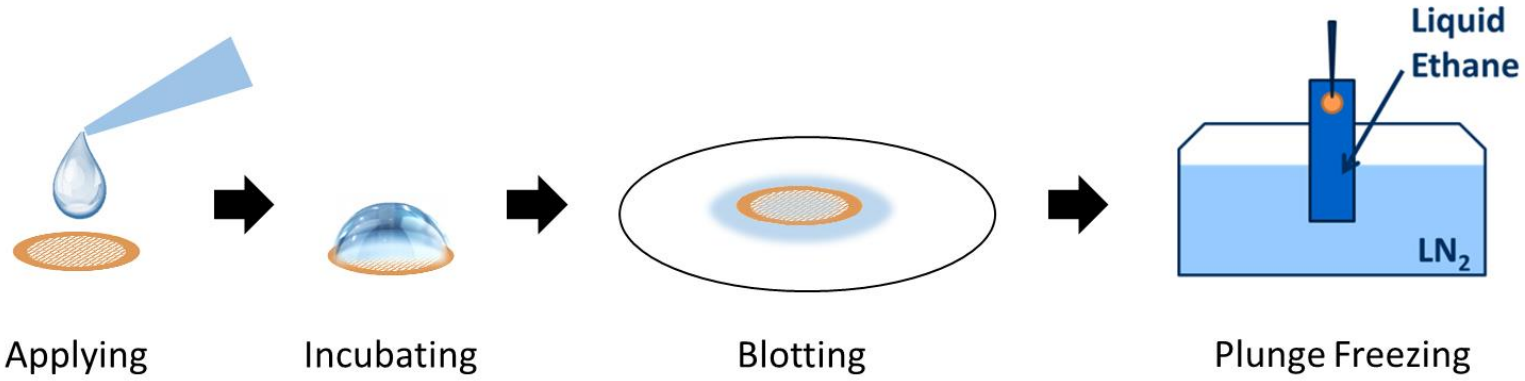
Small Molecule Microcrystal Electron Diffraction (MicroED) for the Pharmaceutical Industry – Results from Examining Over Fifty Samples

Jessica Bruhn[1], Giovanna Scapin[2], Anchi Cheng[1], Thejusvi Ganesh[1], Sargis Dallakyan[1], Brandon Read[1], Travis Nieusma[3], Kyle Lucier[3], Megan Mayer[3], Nicole Chiang[3], Nicole Poweleit[3], Philip McGilvray[3], Timothy Wilson[3], Michael Mashore[3], Camille Hennessy[3], Sean Thomson[3], Clinton Potter[3] and Bridget Carragher[4]

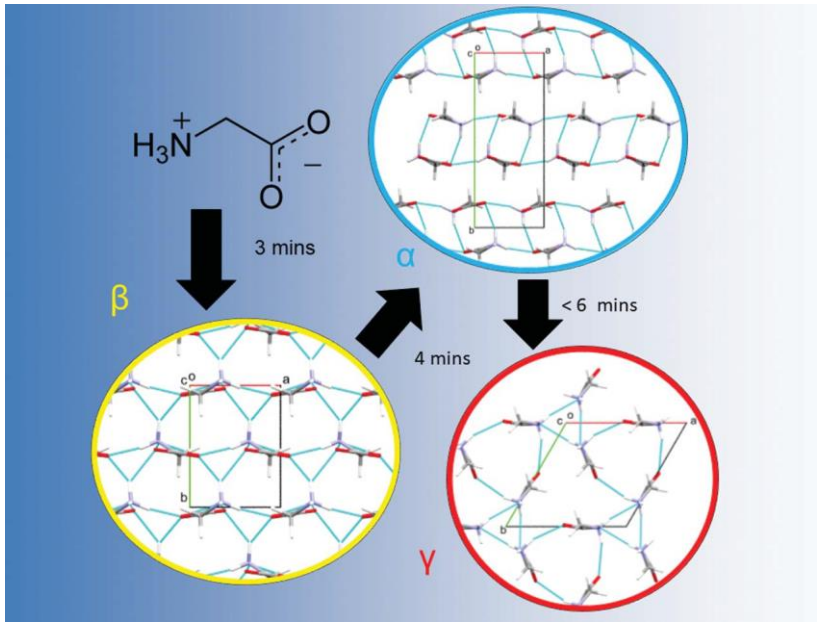
Putting MicroED to the Test: An Account of the Evaluation of 30 Diverse Pharmaceutical Compounds

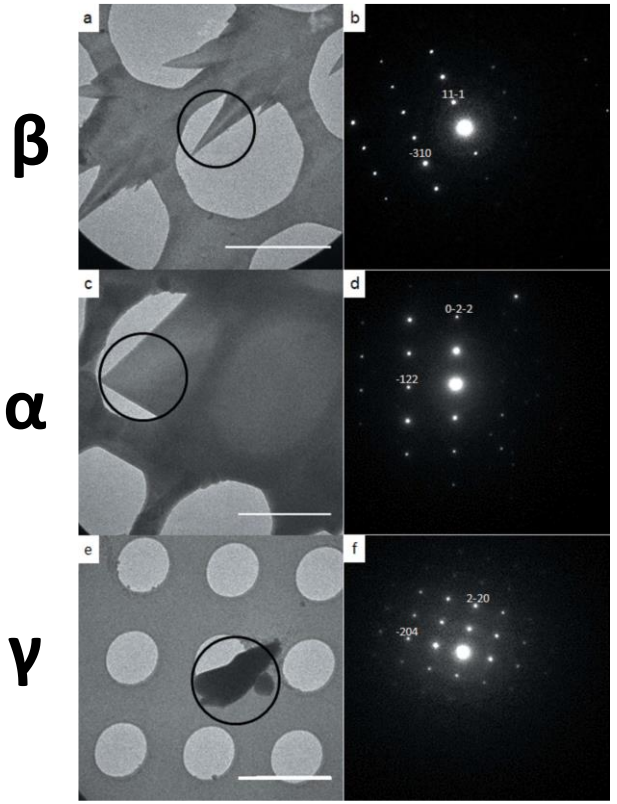
Jessica E. Burch[1]*, Austin G. Smith[2†], Seb Caille[2], Shawn D. Walker[2†], Ryan Wurz[3], Victor J. Cee[3†], Jose Rodriguez[1], D. Gostovic[4], Kyle Quasdorf[2]*, Hosea M. Nelson[1,5]*

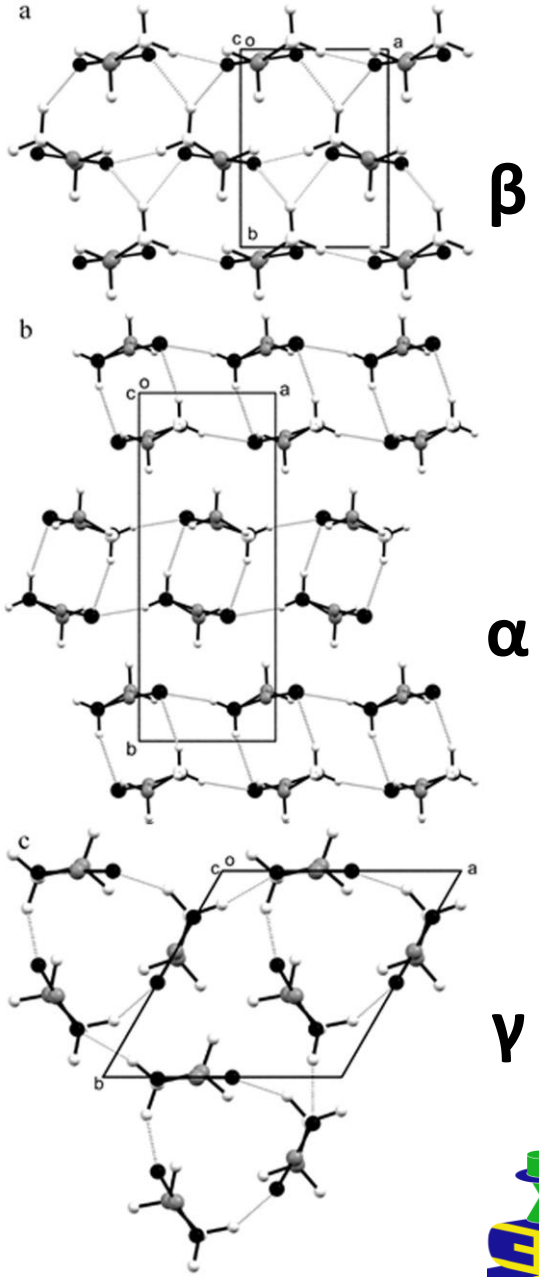
On-grid Crystallizaton – Glycine



- The time of incubation is varied, Time resolution: second
- stability order β < α < γ





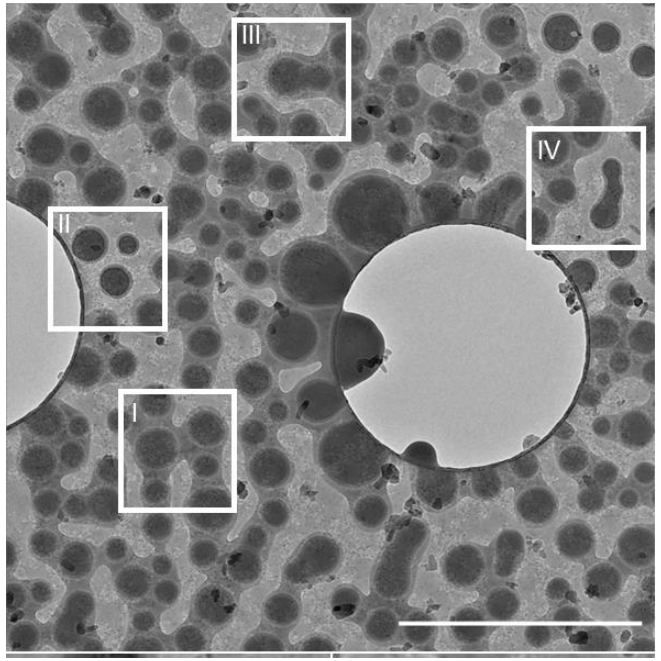


Broadhurst et al. *IUCrJ* **2019** **University of Edinburgh, UK** **Mr. Edward Broadhurst, Prof. Simon Parsons, Dr. Fabio Nudelman**

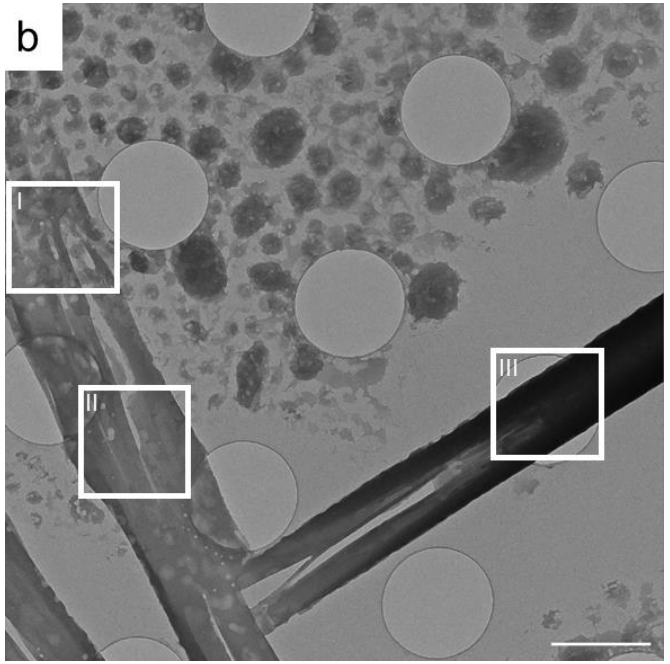
On-grid Crystallizaton – Carbamazepine

Carbamazepine dihydrate:
A dibenzazepine that acts as a sodium channel blocker

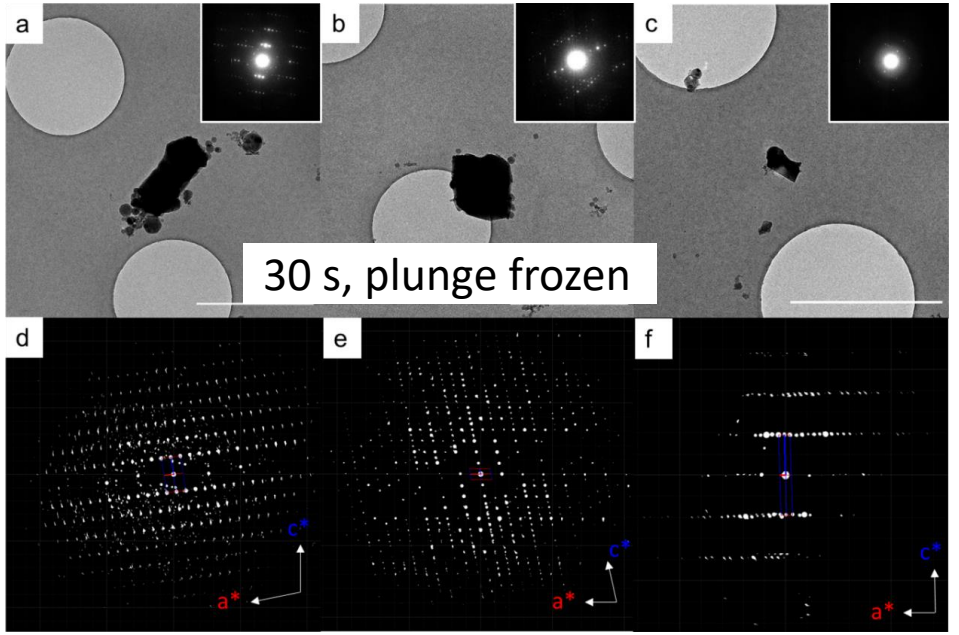
20 s, plunge frozen



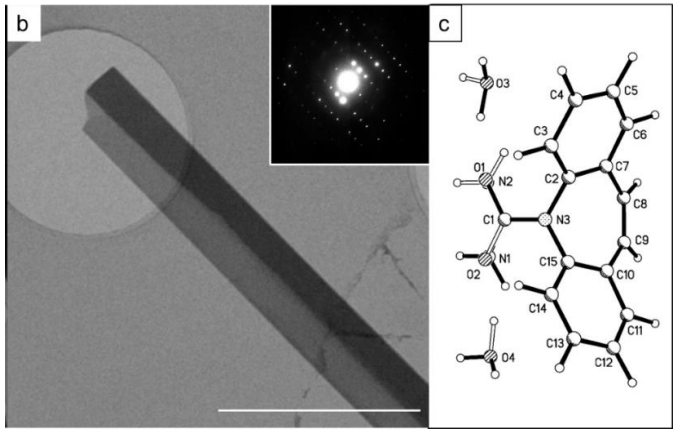
20 s, Room Temperature
Crystallization time ~ 1-2 min



Relative Stability: CBZDH, III > I > V > IV > II



form III **form IV** **form II**

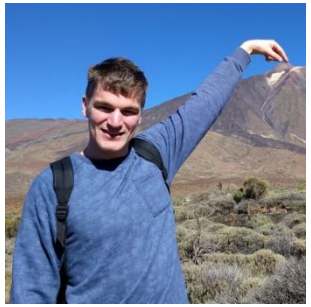


180 seconds

carbamazepine dihydrate

University of Edinburgh, UK

Mr. Edward Broadhurst
Prof. Simon Parsons
Dr. Fabio Nudelman



Manuscript Submitted

Macromolecules

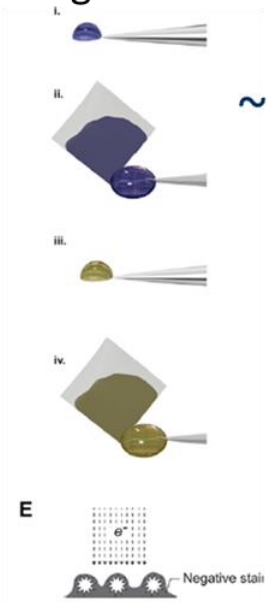
Macromolecules – Screening

High Throughput Crystallization Facility

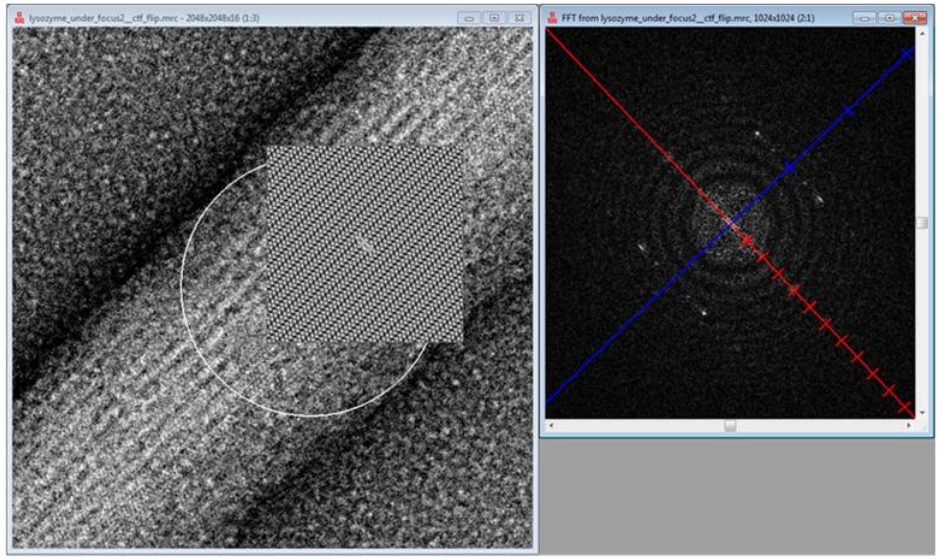
Karolinska Institutet

Automated screening (based on imaging)

Negative Staining



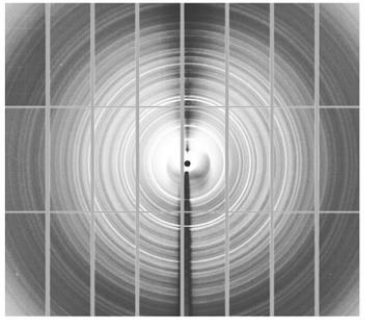
~ 25Å

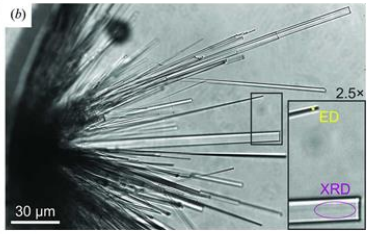


Arnold et. al., *Proteomics* **2018**

Hovmoller et. al., *Ultramicroscopy* **1992**

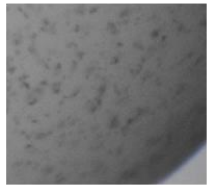
Powder XRD (on a single crystal X-ray diffractometer)

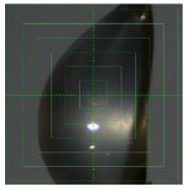




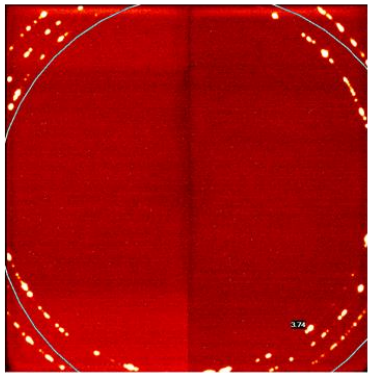
XRD1 beamline @ ELETTRA
Needle shaped lysozyme

Lanza et al. *IUCrJ* **2019**

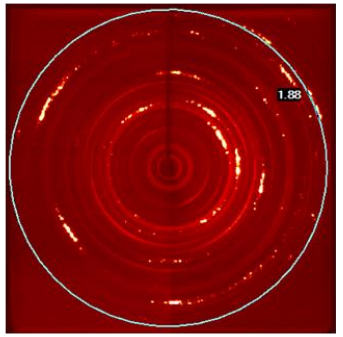




- Concentration
- Cryo/room temp
- Cryo-protectant

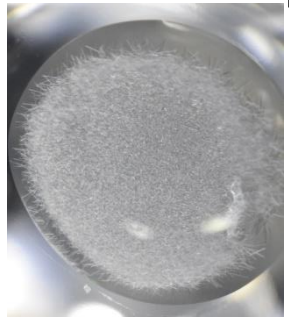


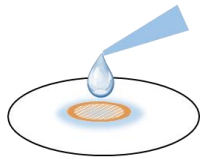
ZSM-5 in water

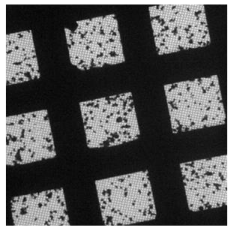


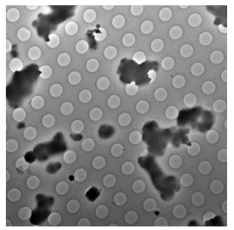
Jiaoyan Xu

Macromolecules – Specimen Preparation

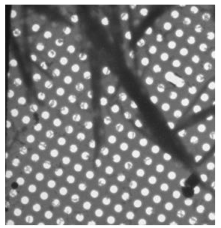




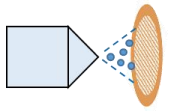


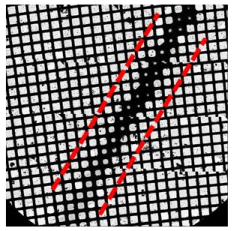


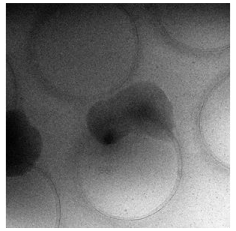
One-sided blotting or pressure-assisted blotting



Double-sided blotting

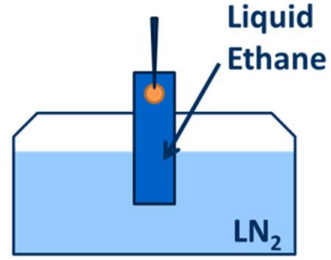




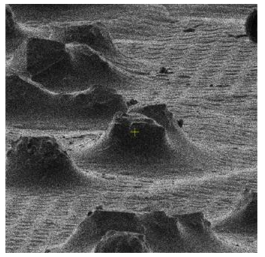


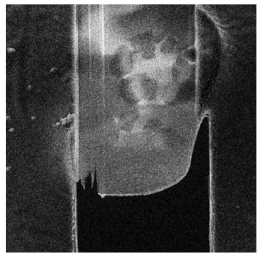
Ink-jet dispenser

cryo-EM specimen preparation



Plunge freezing





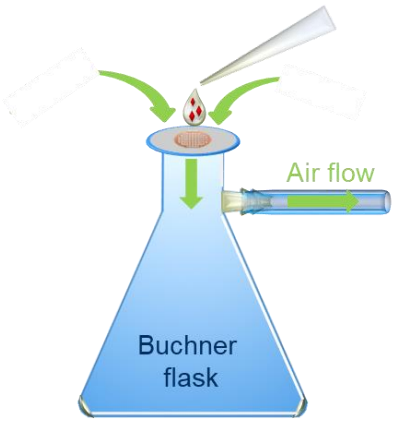
Cryo-FIB Milling

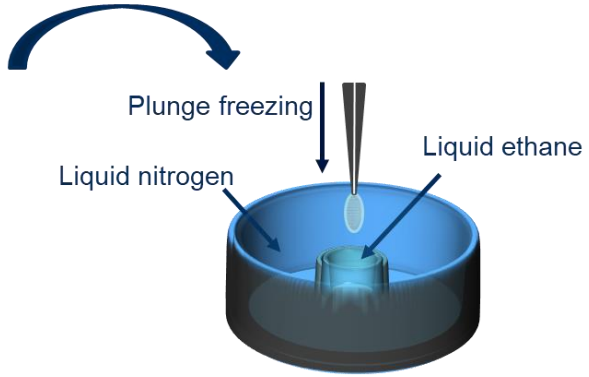
Electron Diffraction Data Collection

Data collection

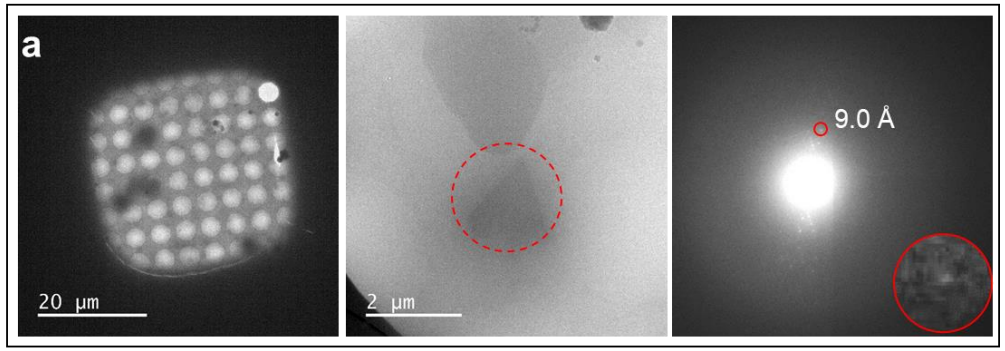
Clabbers and Xu, *Acta D* **2020**

Macromolecules – Preassis

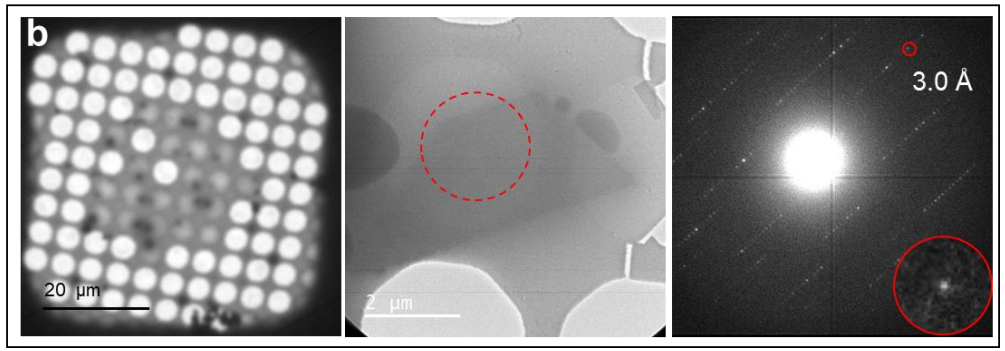




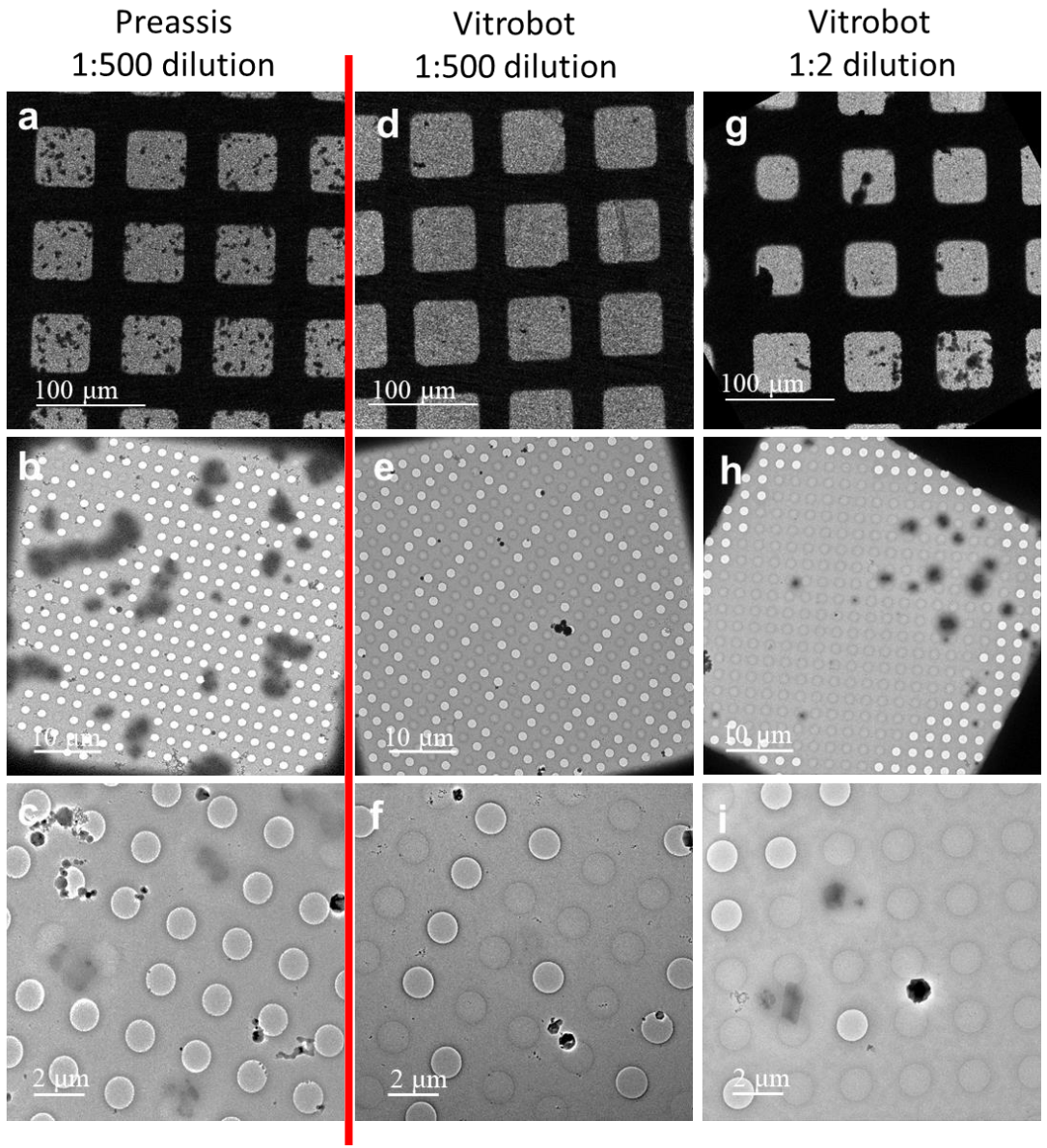
Vitrobot



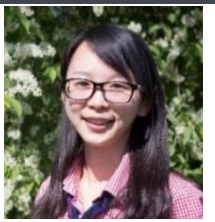
Preassis



44% PEG 400, Viscous Mother Liquor

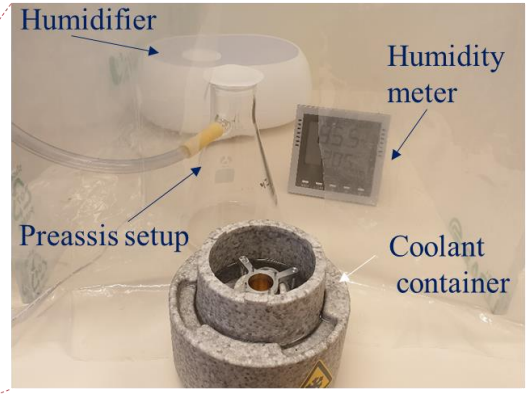


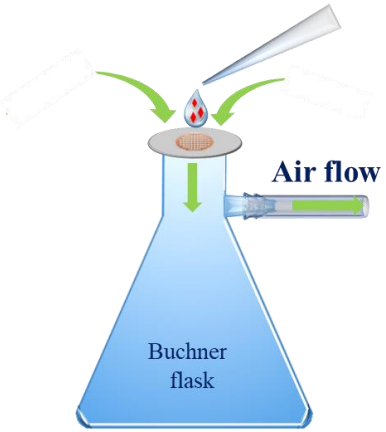
Zhao et al. *Nat. Commun.* **2021**

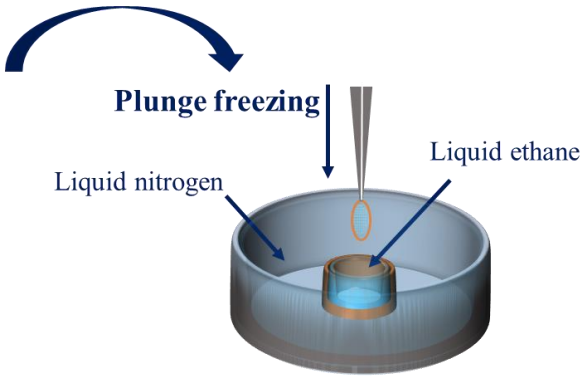


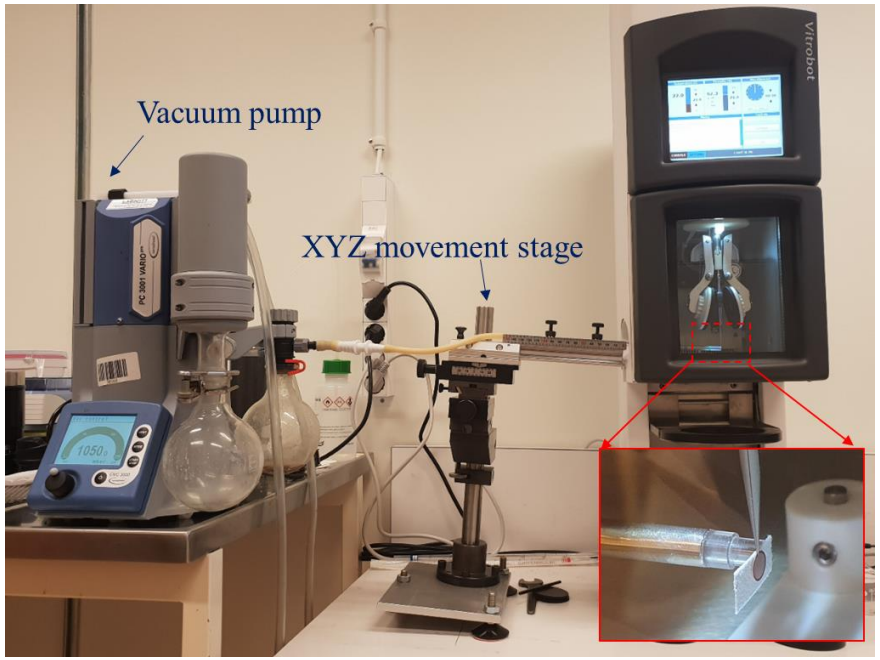
Jingjing Zhao

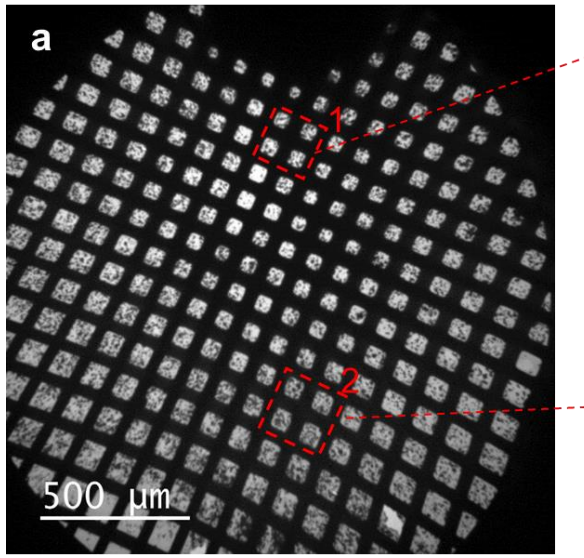
Macromolecules – Preassis

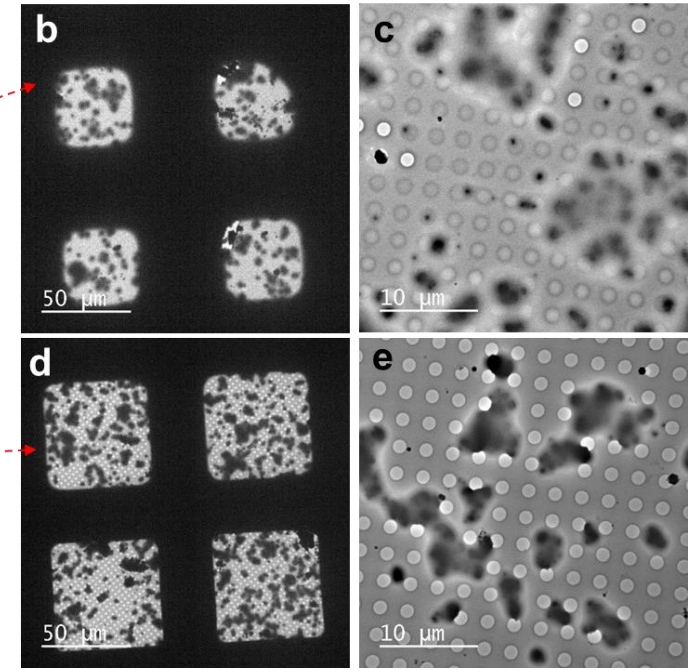


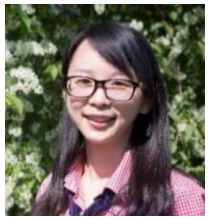










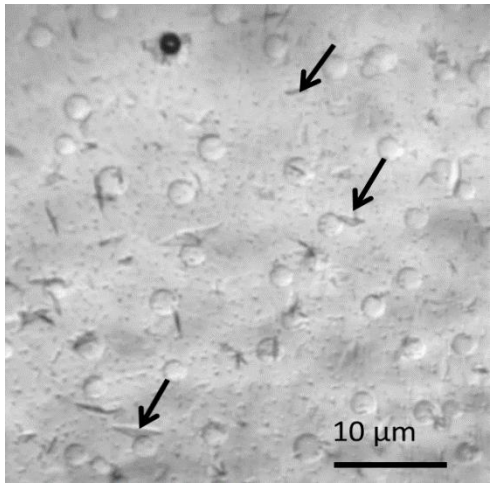


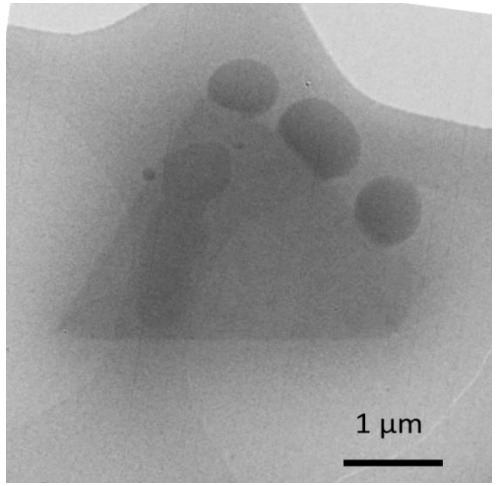
Jingjing Zhao

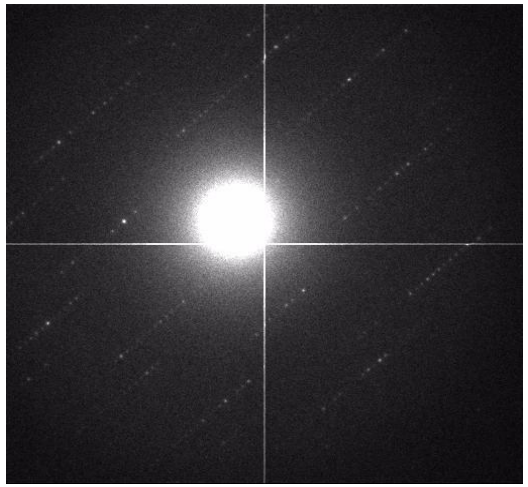
Zhao et al. *Nat. Commun.* **2021**

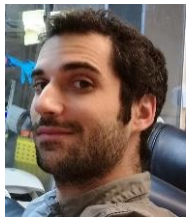
Macromolecules – *Sa*R2lox

- *Sulfolobus acidocaldarius* R2-like ligand-binding oxidase
- Phased from a homologue of 35% sequence identity
- First unknown protein structure solved by 3D microcrystal ED





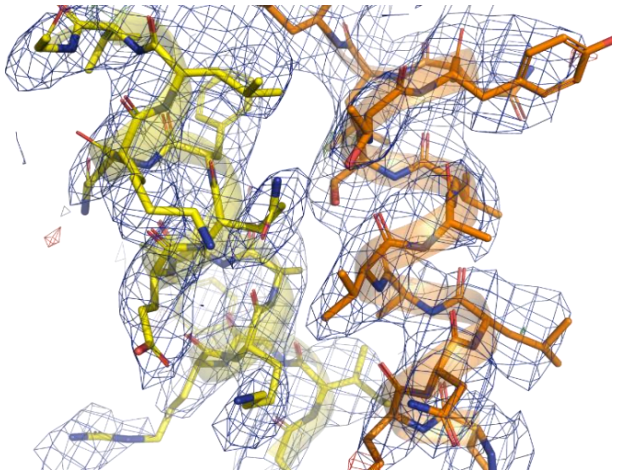


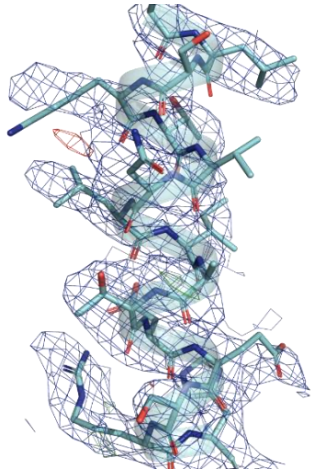


Hugo Lebrette

Max Clabbers

Electrostatic scattering potential maps 2Fo-Fc (1.0σ)





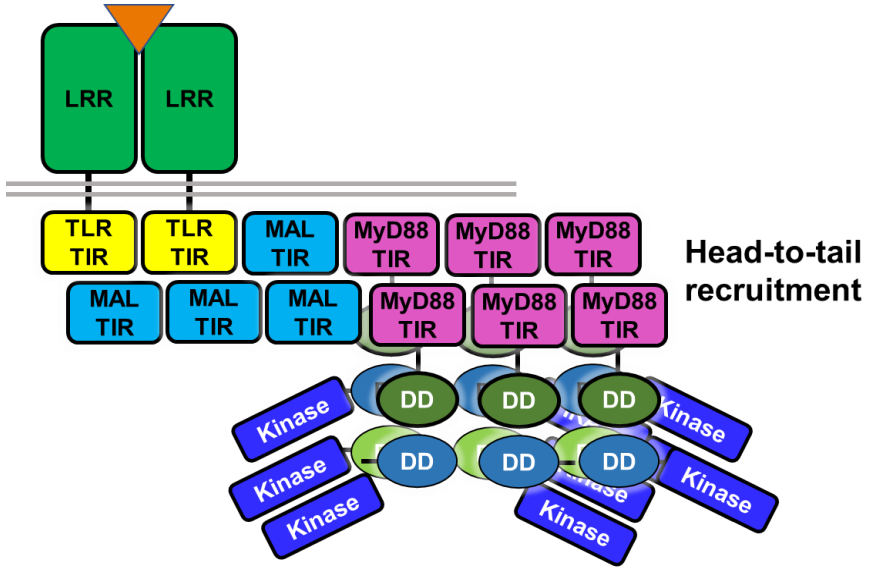
Resolution range	29.00-3.00 (3.08-3.00)
Space group	$P2_12_12$
a, b, c (Å) α, β, γ (°)	63.31, 108.93, 48.17 90.00, 90.00, 90.00
Multiplicity	32.4 (8.5)
Completeness (%)	62.8 (52.9)
Mean I/σI	6.12 (0.75)
R_{meas}	0.561 (3.995)
$CC_{1/2}$	0.981 (0.597)
Reflections used in refinement	4423 (343)
Reflections used for R_{free} *	233 (15)
R_{work}	0.3179 (0.4299)
R_{free} *	0.3347 (0.4623)
macromolecules	2241
ligands	2
Protein residues	274
RMSD(bonds) **	0.002
RMSD(angles) **	0.41
Ramachandran favoured (%)	96.67
Ramachandran allowed (%)	3.33
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.82
Clashscore	10.26
Average B-factor	48.37

Xu et al. *Science Advances* **2019**

Macromolecules – MyD88

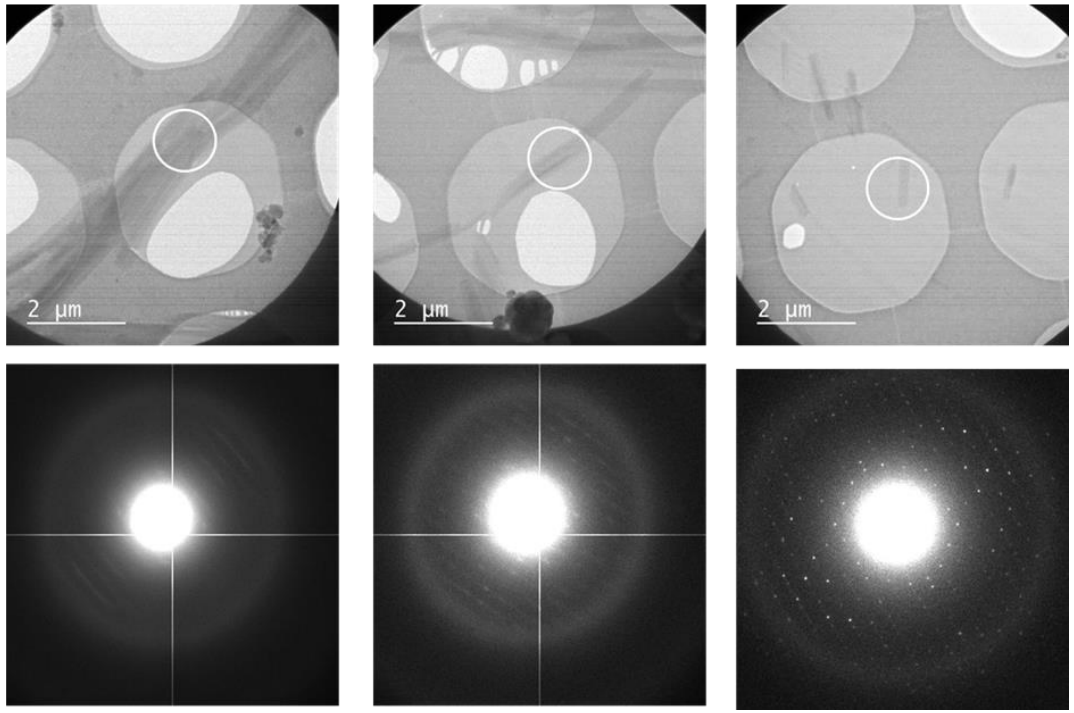
Max Clabbers

TLR signaling



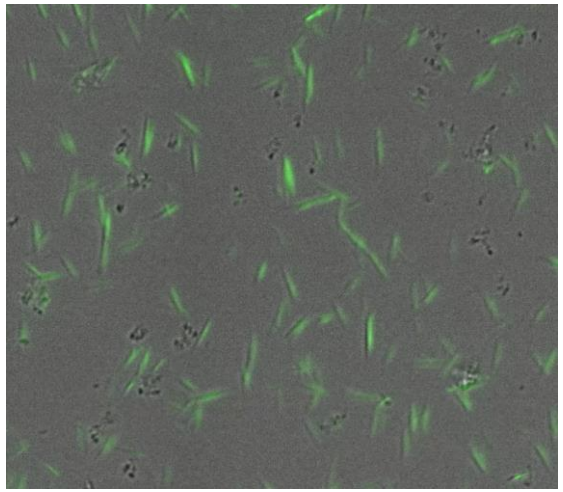
Head-to-tail recruitment

3DED/MicroED



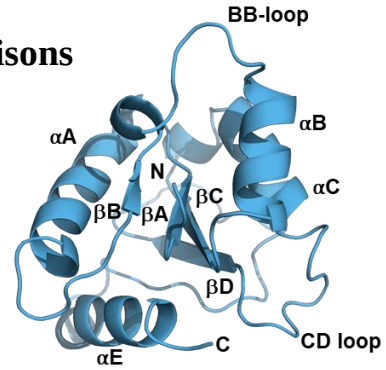
Clabbers et al. *Nat. Commun.* **2021**

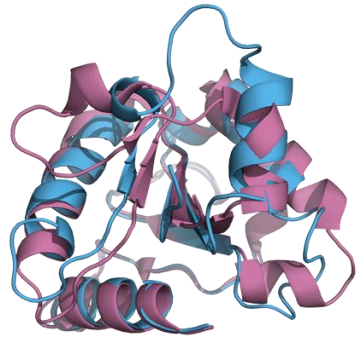
Crystal Growth



Structure Comparisons

MicroED: Dimer





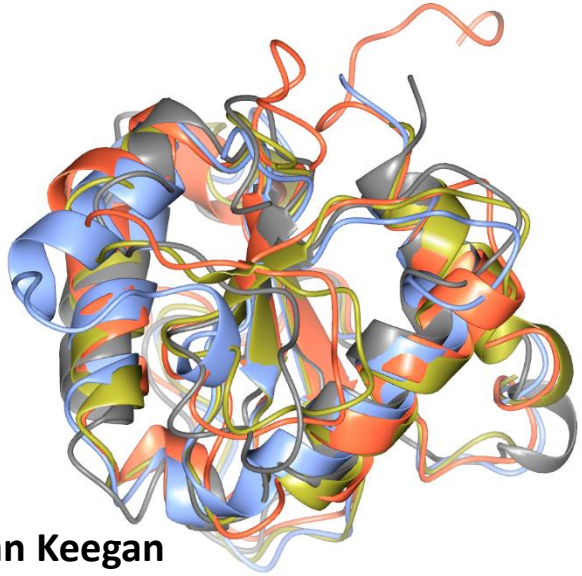
X-ray: Monomer

MyD88^{TIR} MicroED structure

MyD88^{TIR} X-ray structure

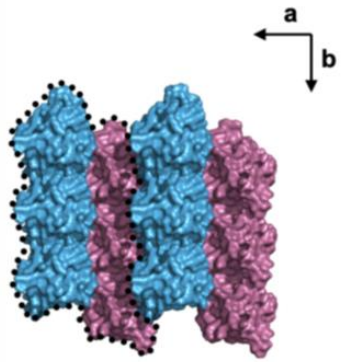
Macromolecules – MyD88

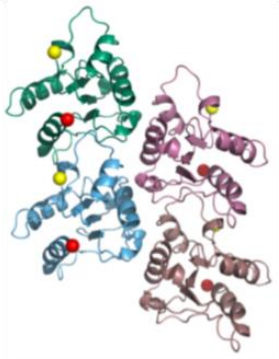
Target (7BEQ)
99% 4EO7 (chain A)
30% 2JS7 (chain A)
2Z5V (chain A)

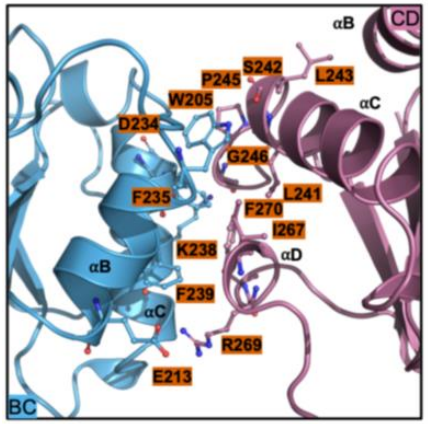


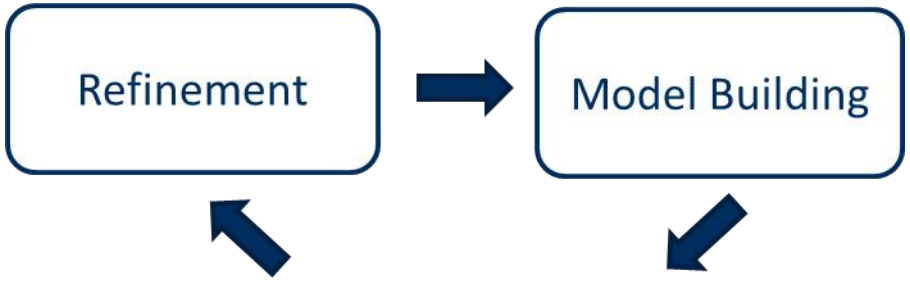
Molecular Replacement

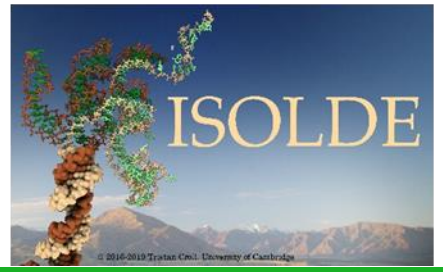
Image Courtesy: **Ronan Keegan**











Alphafold 2 Predictions

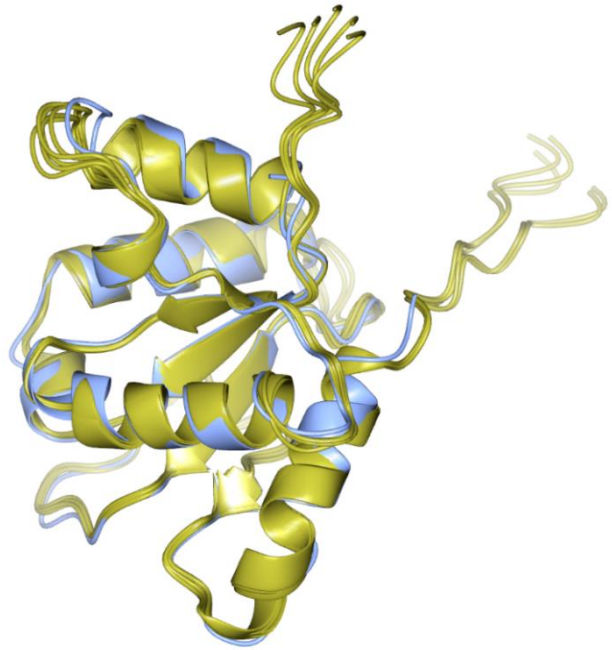
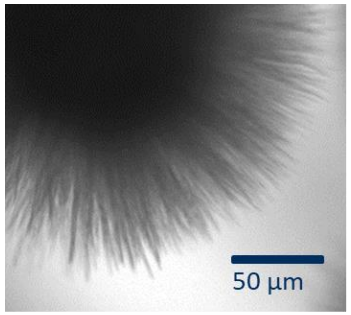
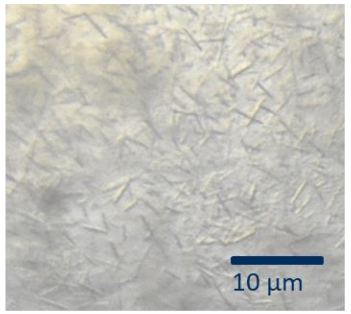


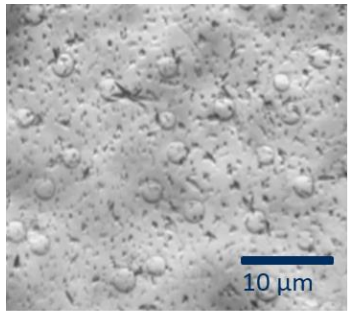
Image Courtesy: **Ronan Keegan**

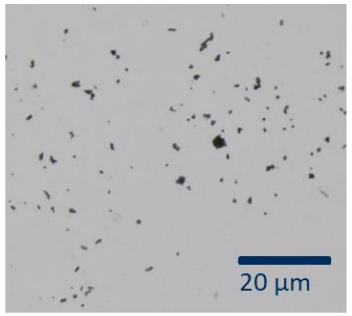
Macromolecules – Protein Crystal Gallery

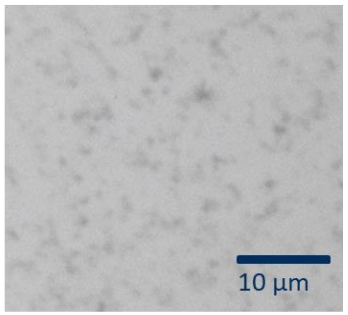
Courtesy:
Mathieu Coinçon

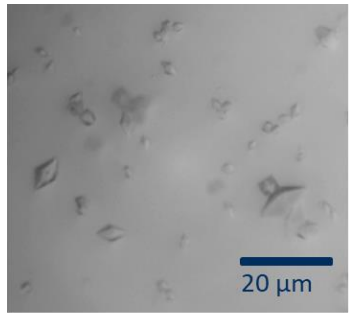


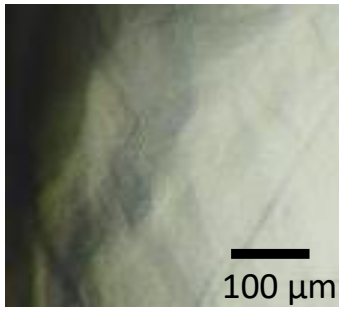


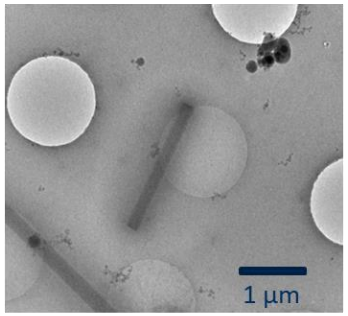


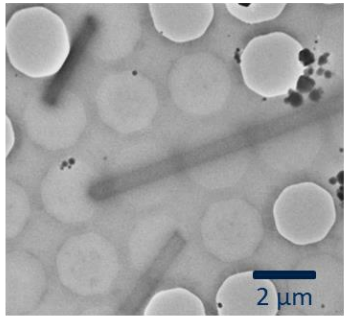


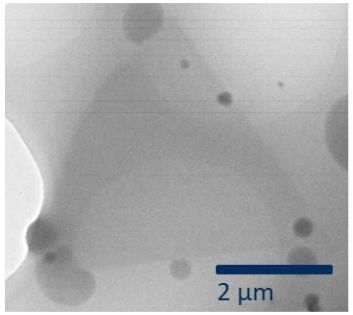


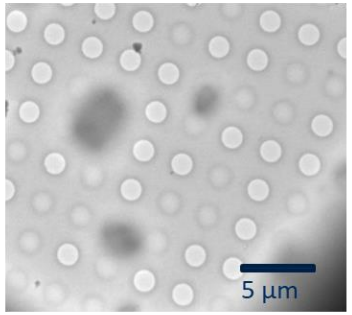


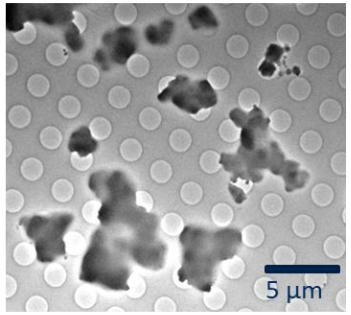


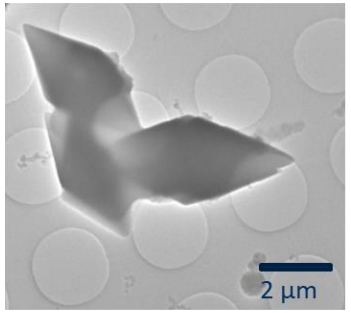


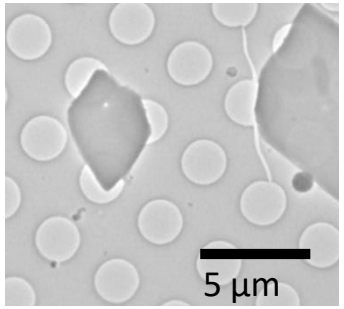










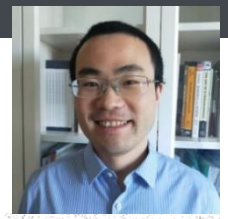


needle-like | plate-like | fragmented | diamond-like | Stacked plates

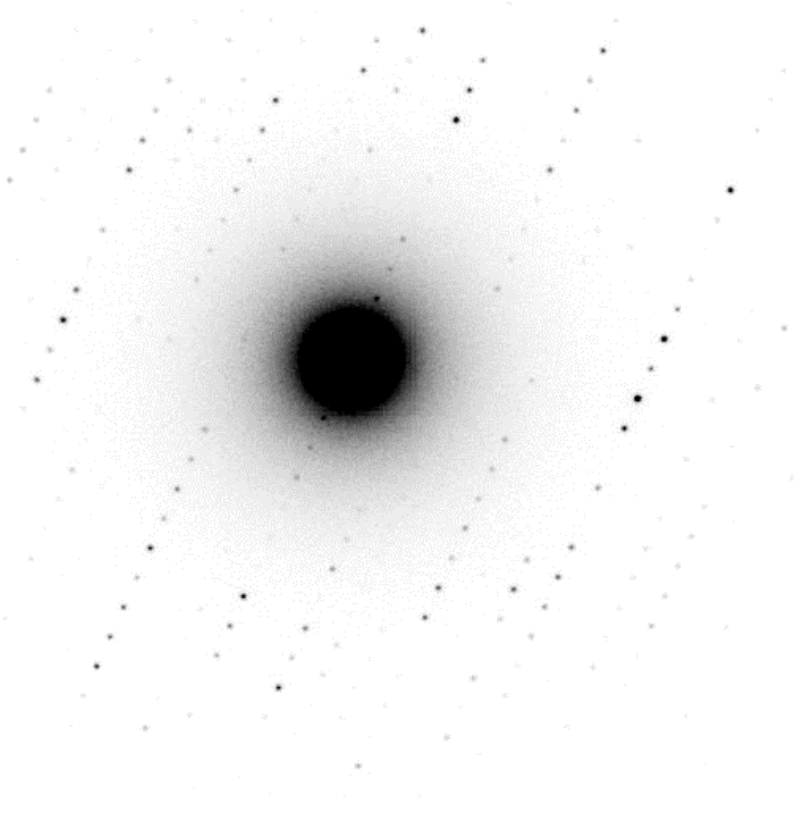
Clabbers and Xu, *Acta D* **2020**

Instrumentation

Taimin Yang

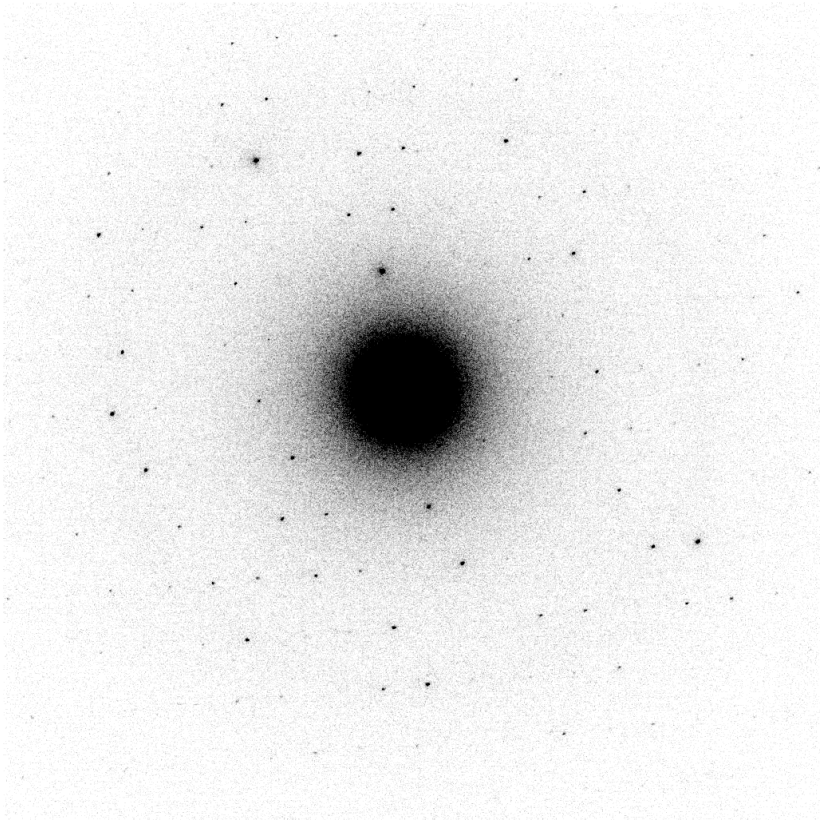


Electron microscopy centre, MMK, SU



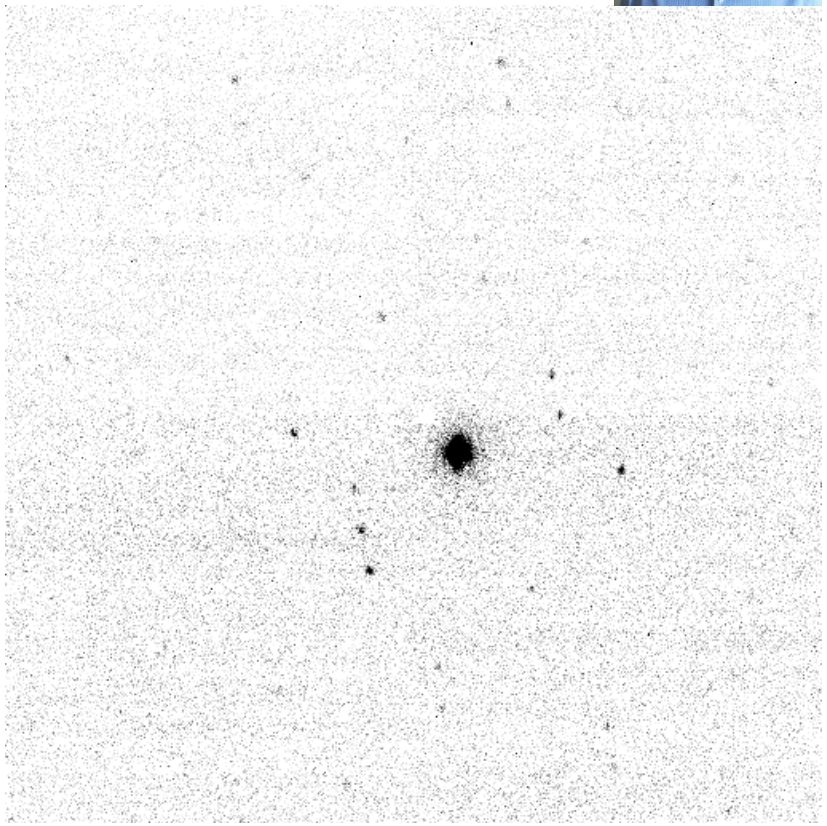
JEOL 2100 (200kV) LaB_6 + **Timepix**
Human **C**arbonic **A**nhydrase **II**

2.3 Å



Themis Z (300kV) FEG + **Oneview**
Lysozyme

2.2 Å



Themis Z (300kV) FEG
Gatan Energy Filter + Ultrascan
Lysozyme

Data within 3 Å

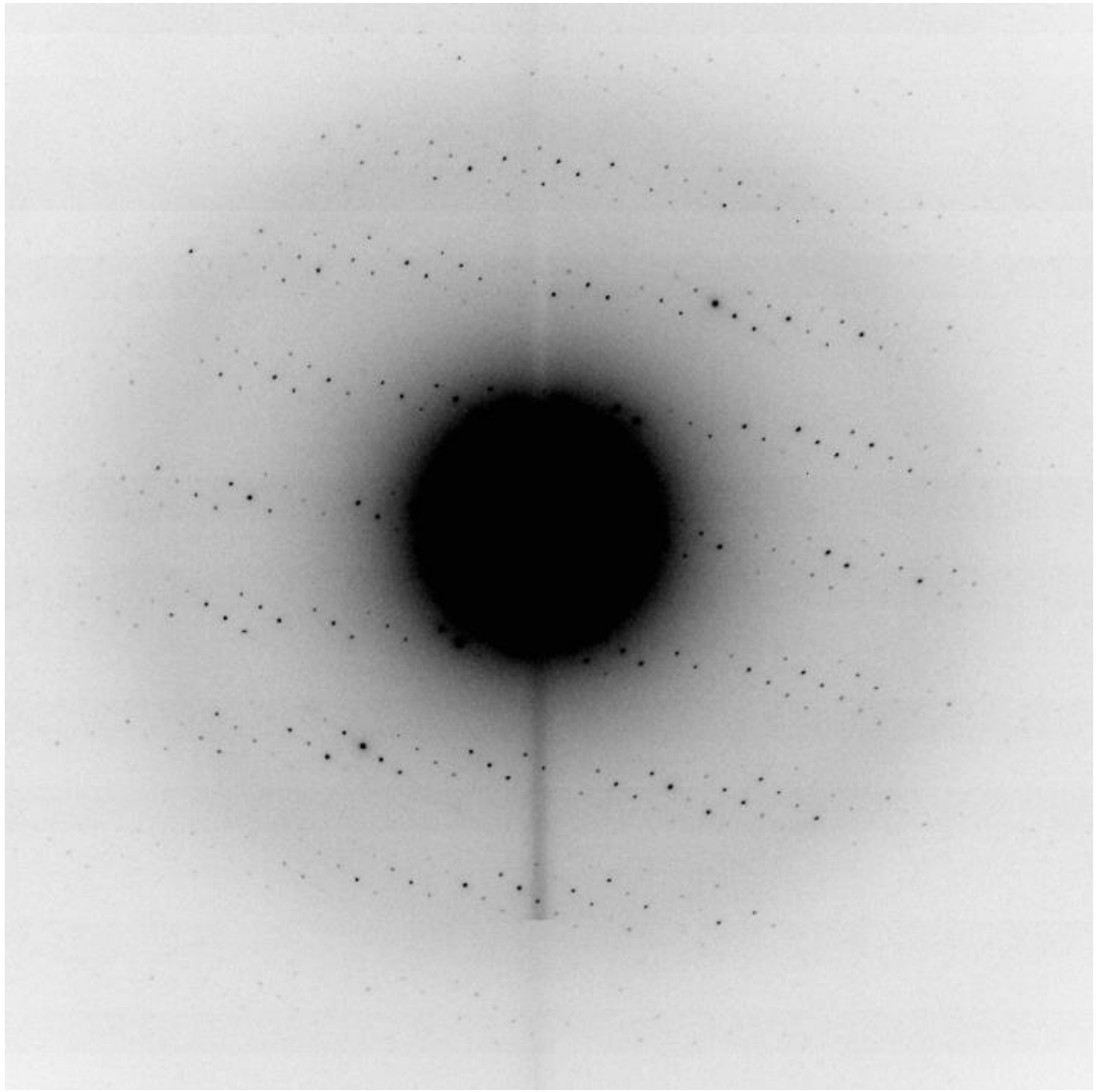
Instrumentation

SciLifeLab

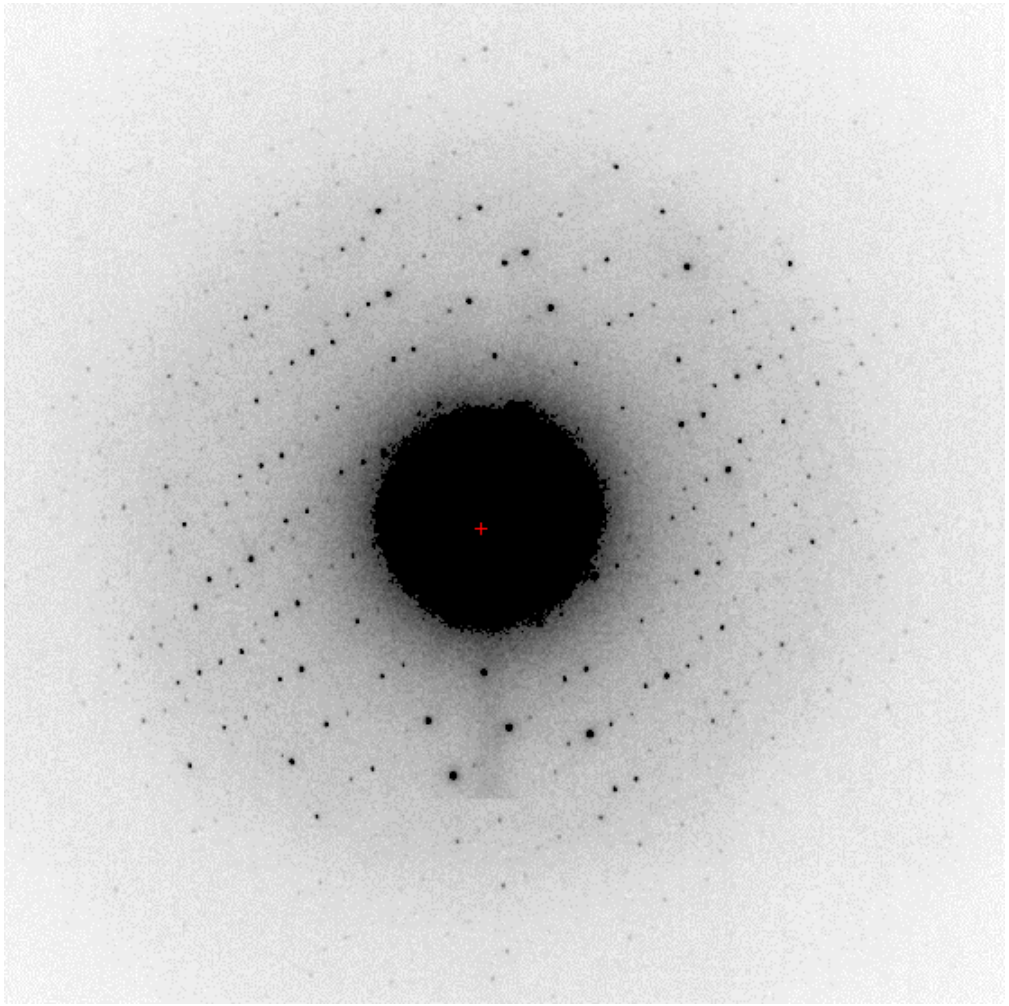
Marta Carroni

Mathieu Coincon

Gerhard Hofer



Talos Arctica + CETA (**high dose**), Arginine Kinase

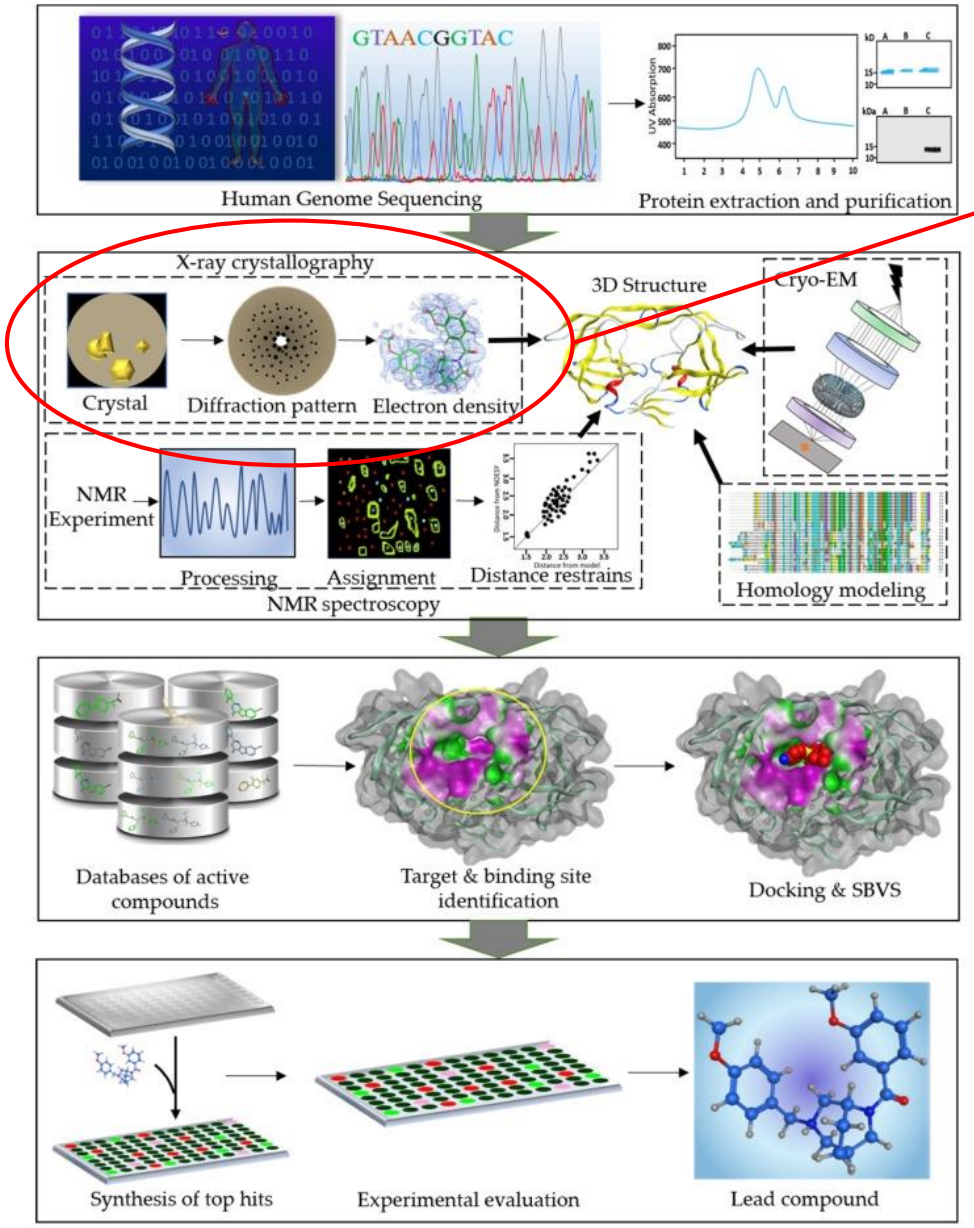


Krios-β + CETA-D (EPU-D), Arginine Kinase

The Stage is Set for

Structure-Based Drug Discovery by 3D ED

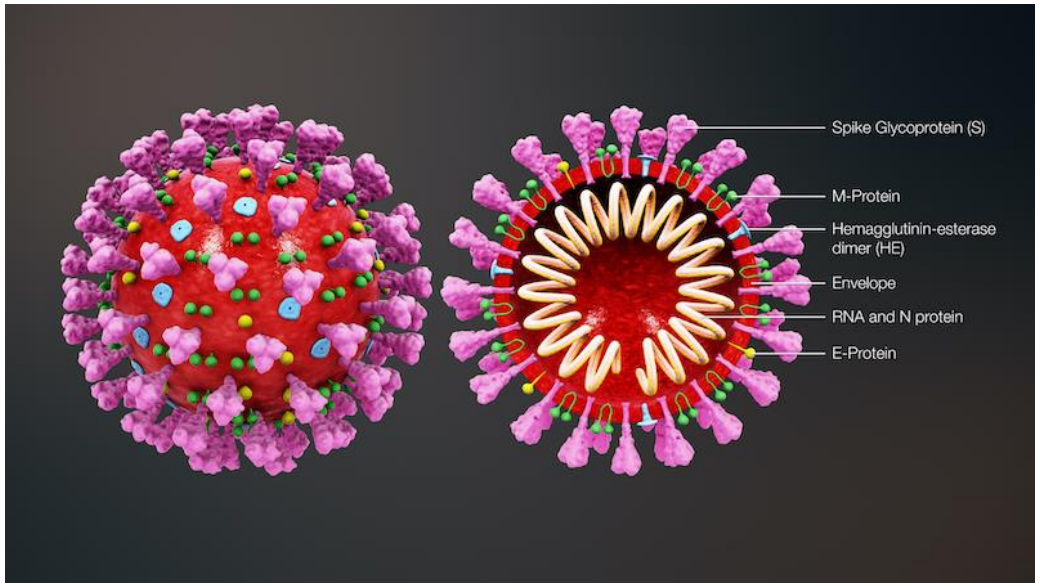
Strucutre-Based Drug Discovery

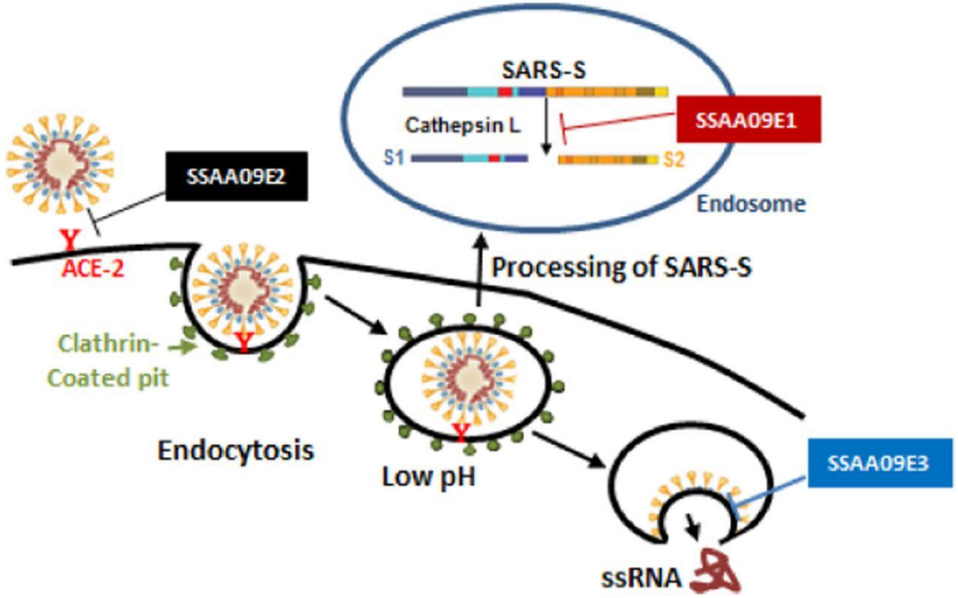


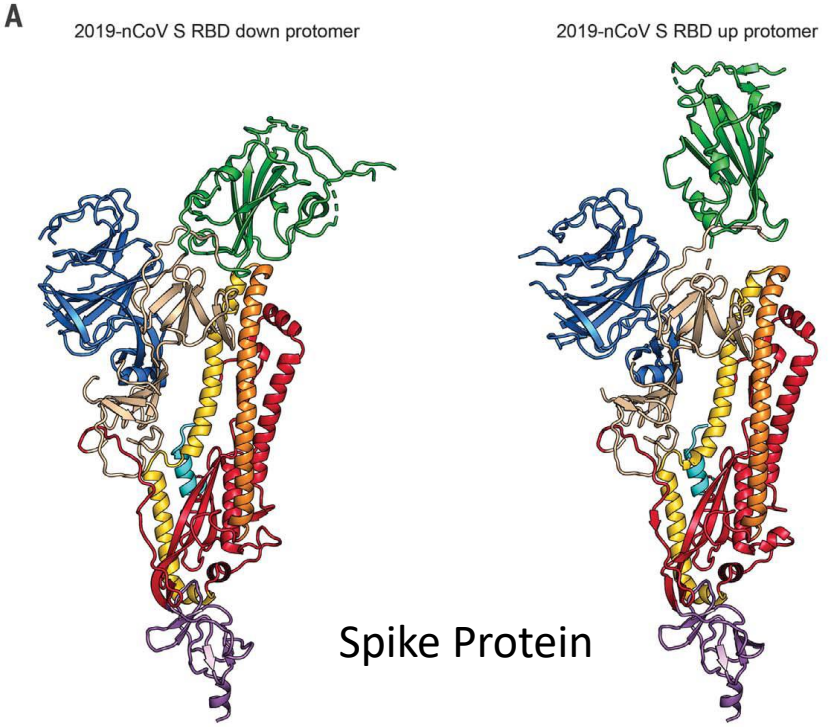
Electron crystallography

- A series of trials (decades)
- Formulation/tableting
- Clinical trials

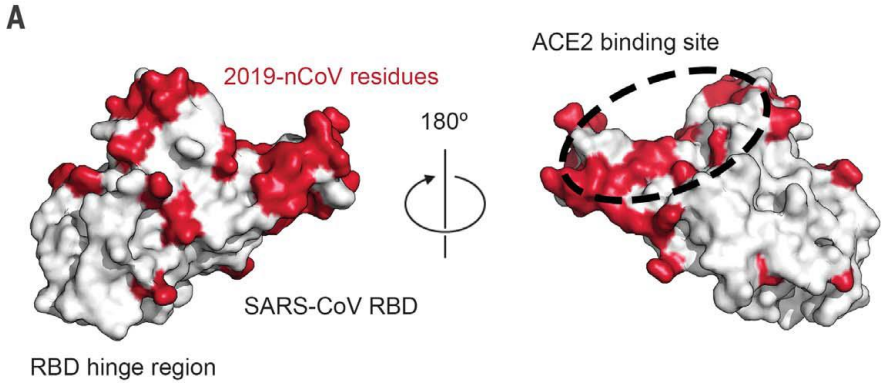
Strucutre-Based Drug Discovery







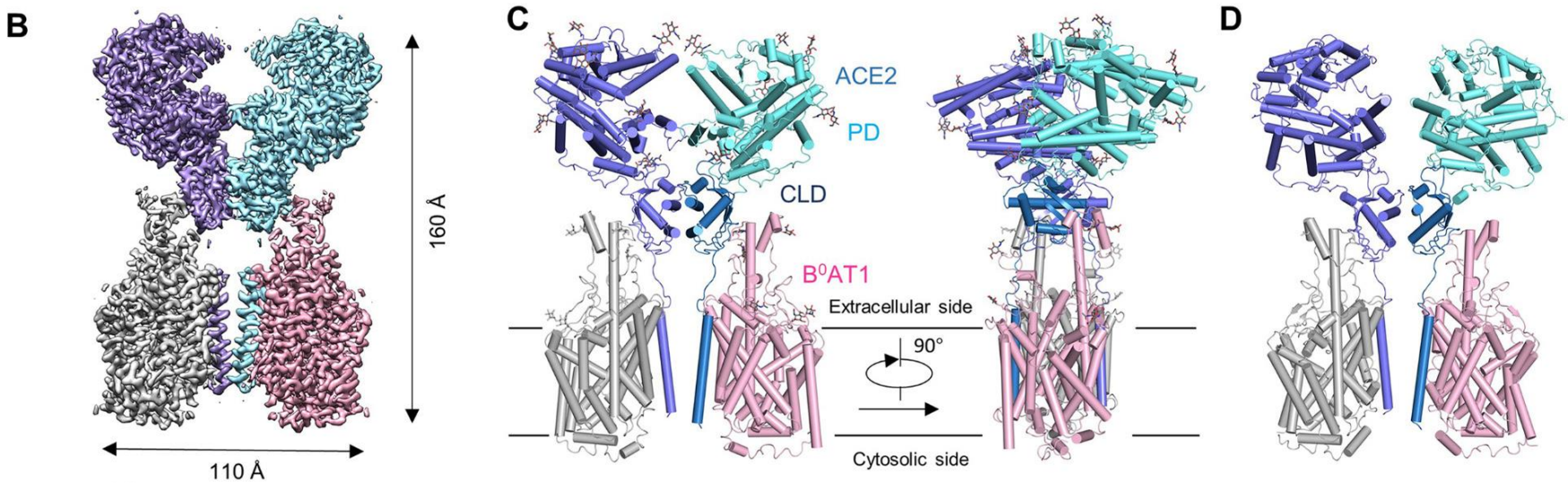
Spike Protein



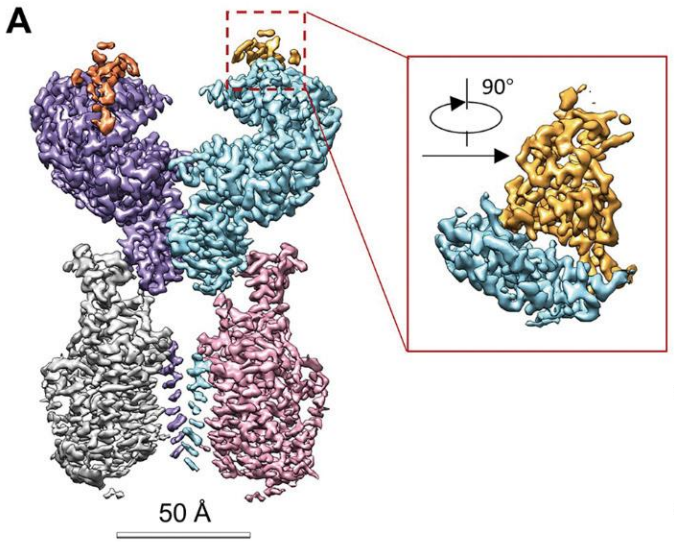
3.5Å Resolution Cryo-EM Strucutre

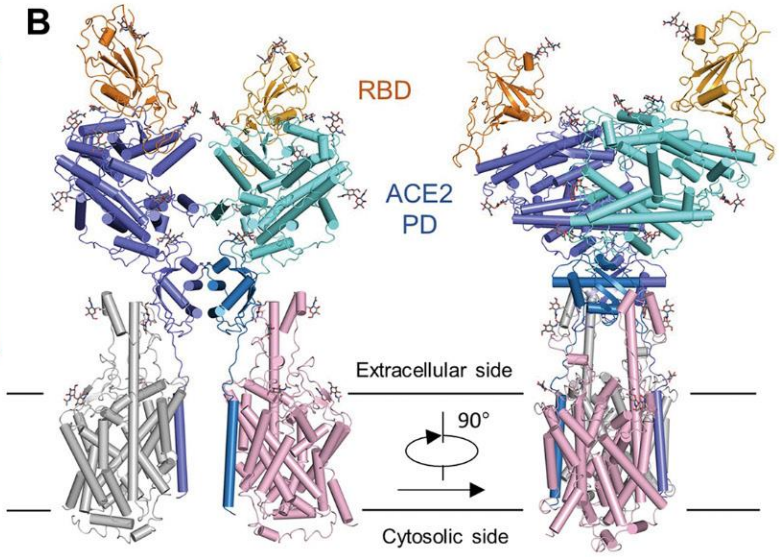
Wrapp et al., *Science* 367, 1260–1263 (**2020**)

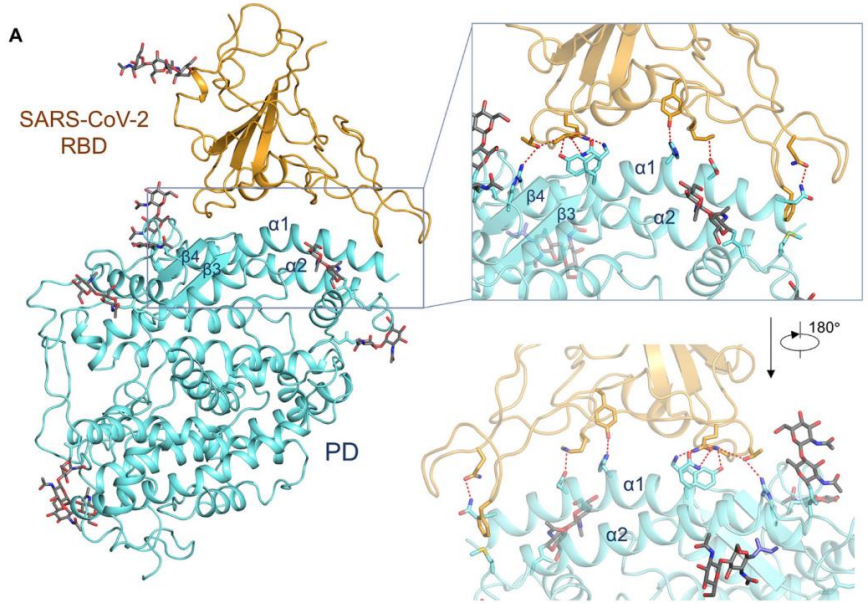
Structure-Based Drug Discovery



2.9 Å Resolution Cryo-EM Strucutre of ACE2





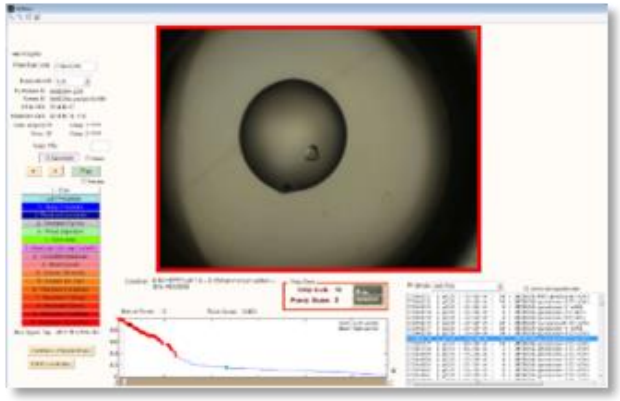


R. Yan et al., *Science* 10.1126/science.abb2762 (**2020**)

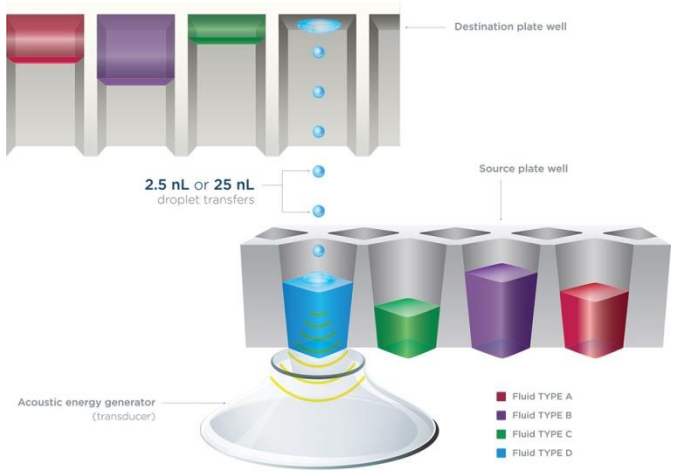
Structure-Based Drug Discovery

Credit: Diamond Light Source

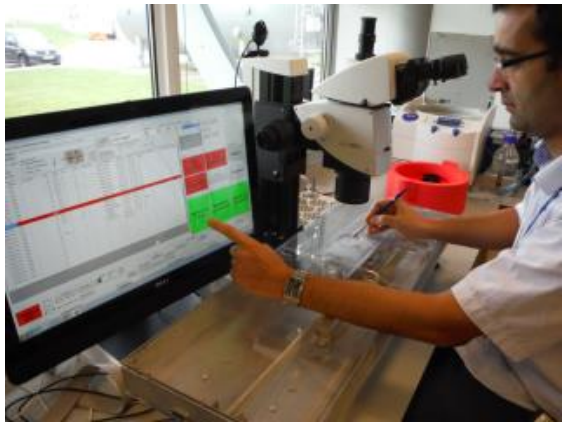
Xchem – Diamond Light Source



Crystal Targeting

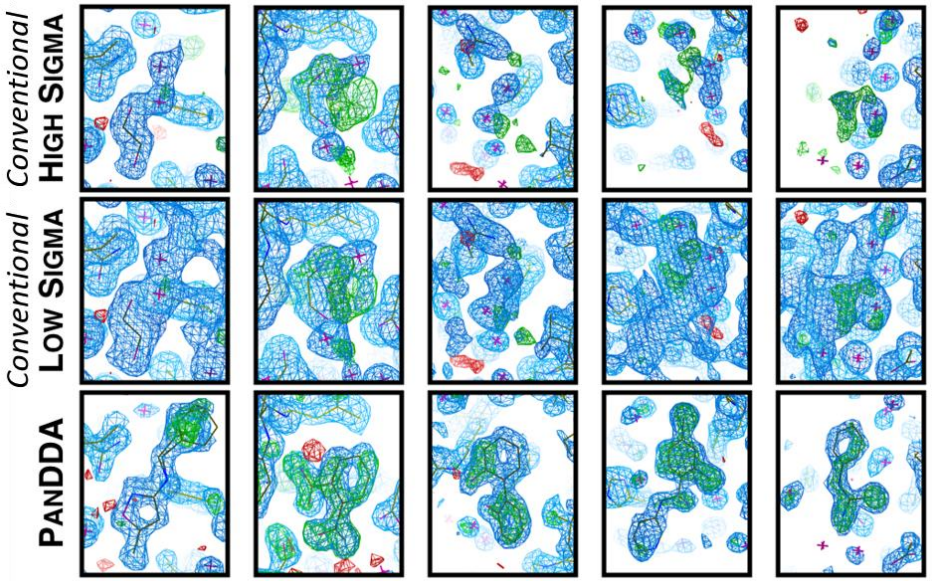


Soaking: ECHO acoustic liquid handler



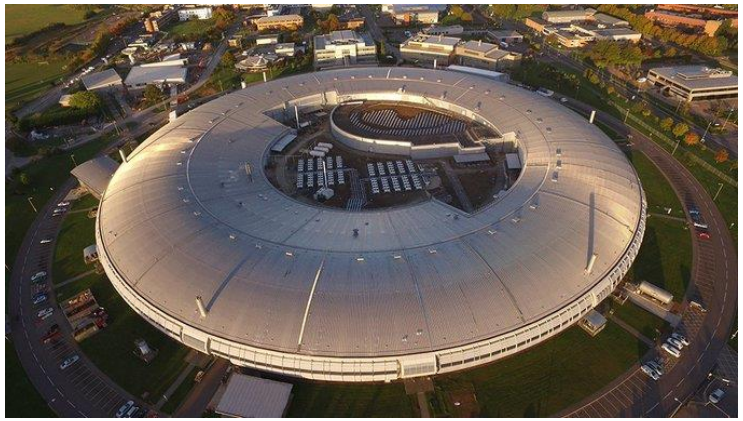
Crystal harvesting: Shifter

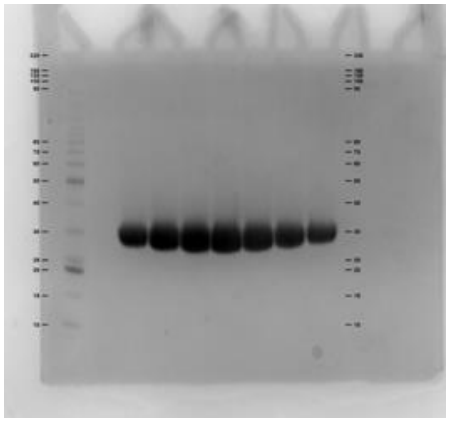
X-ray data collection: beamline I04-1

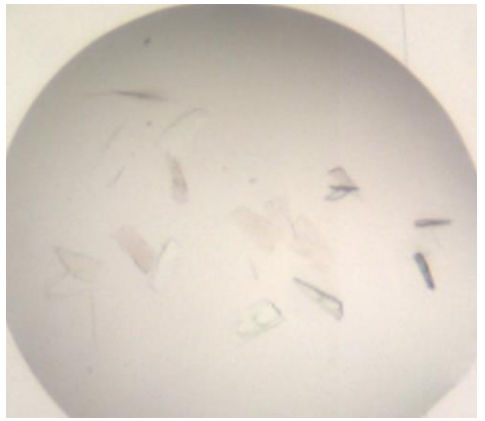


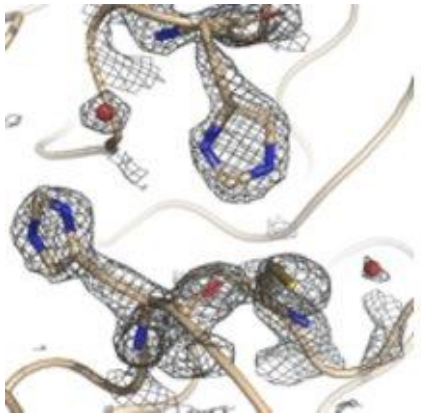
Data analysis and model building

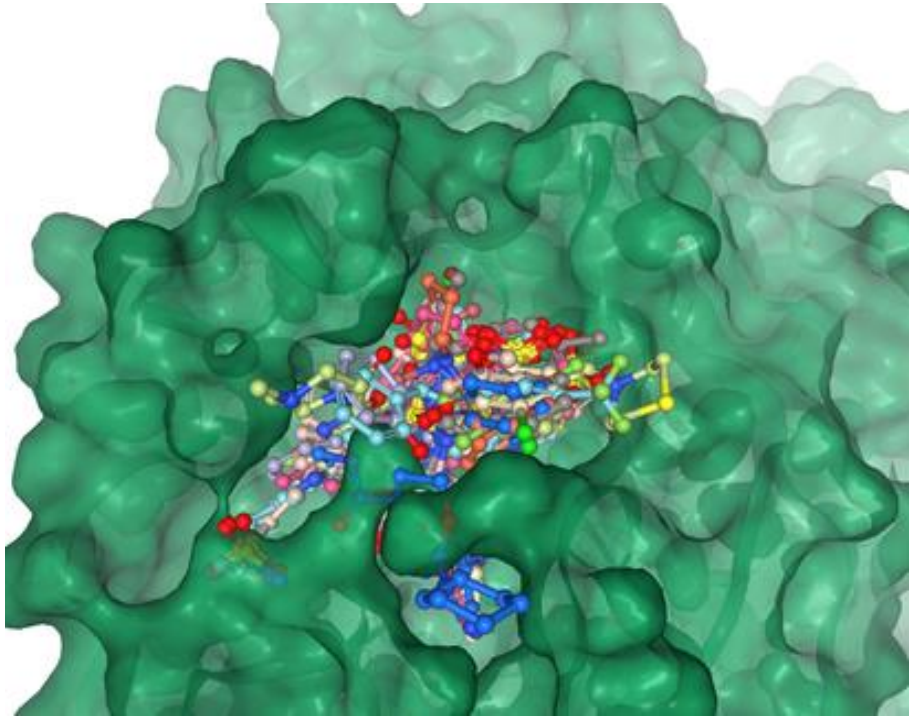
Structure-Based Drug Discovery



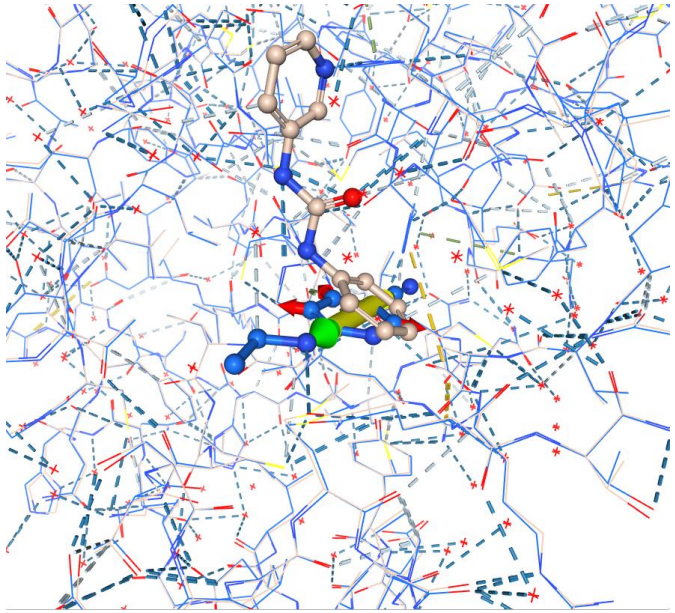


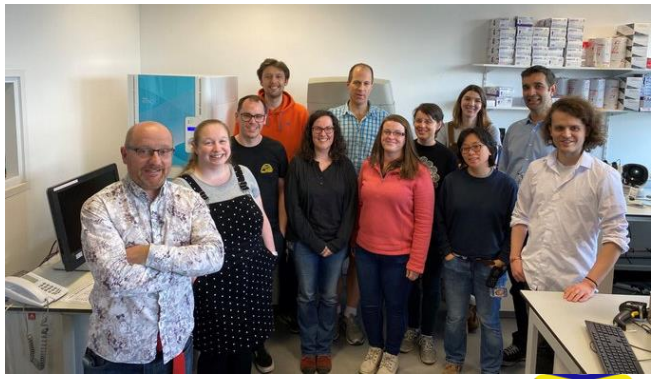






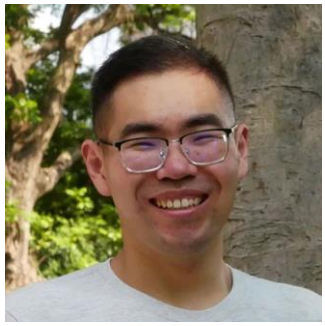
- 19 non-covalent hits in the active site
- 39 covalent hits in the active site
- 2 hits in the dimer interface, one in a calculated hotspot





Credit: Diamond Light Source

Structure-Based Drug Discovery by 3D ED



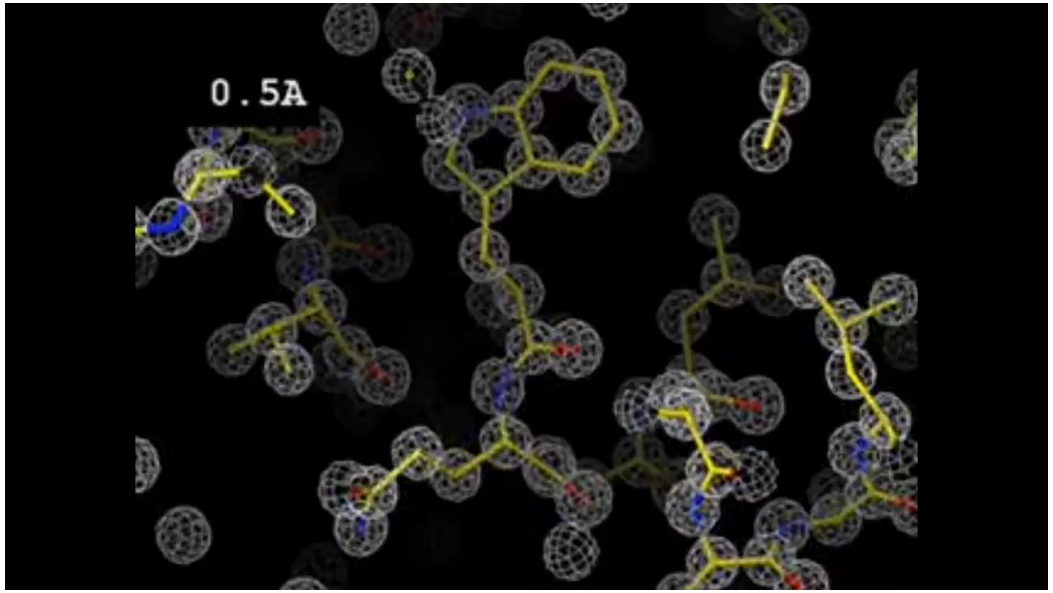
Lei Wang

Motivations:

- Small crystals -> better diffusion
  - Organic solvent
  - Diffusion rate
  - Occupancy
- Availability of TEMs/cryo-TEMs
- Hydrogen and charge

Challenges:

- Data completeness
- Beam damage
- Reliability
- Resolution: 2.5Å -> 2.0Å
- Throughput

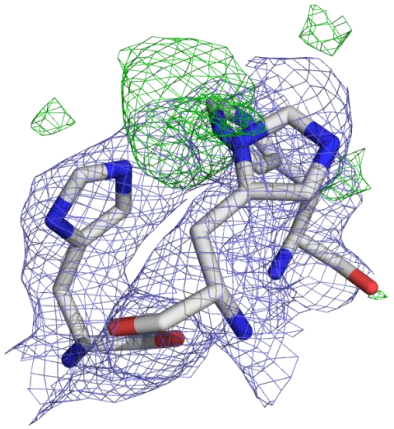


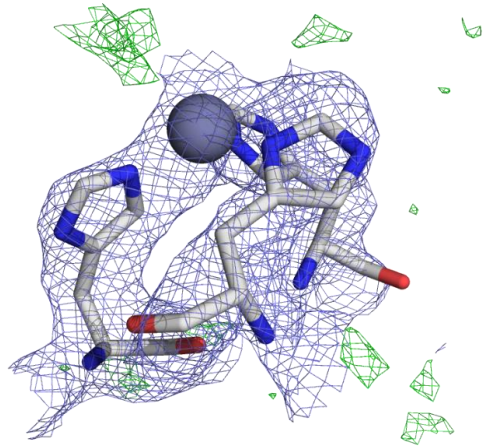
https://www.youtube.com/watch?v=omFOLG5Z5w4

Structure-Based Drug Discovery by 3D ED

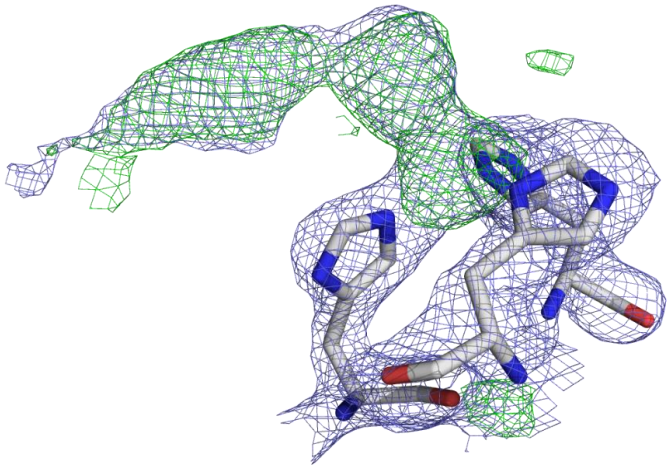
Data collection	
Space group	$P2_1$
Cell dimensions	
a, b, c (Å)	42.11, 41.39, 72.80
α, β, γ (°)	90.00, 104.449, 90.00
Resolution (Å)	35.70-2.30 (2.36-2.30)
R_{merge}	0.388 (0.573)
I/σI	4.06 (1.53)
$CC_{1/2}$	0.947 (0.464)
Completeness (%)	**73.2 (42.5)**
Redundancy	4.95 (1.98)
Refinement	
Resolution (Å)	**35.70-2.30**
No. reflections	14305
R_{work} / R_{free}	0.211/0.256
No. atoms	
Protein	2049
Ligand/ion	18
Water	27
B-factors	
Protein	22.24
Ligand/ion	23.17
Water	15.80
R.m.s. deviations	
Bond lengths (Å)	0.002
Bond angles (°)	0.708

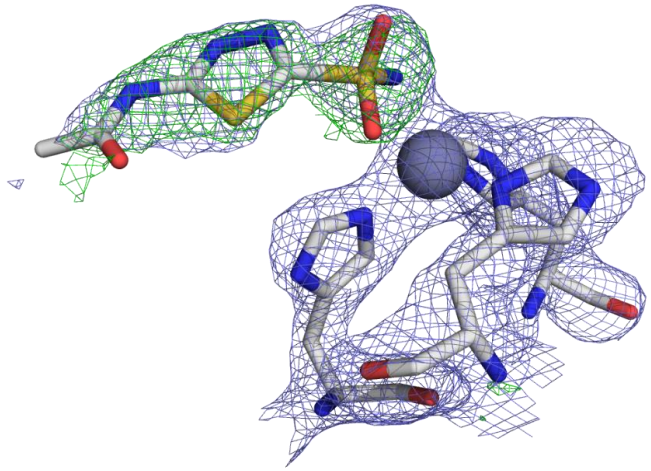
HAC II





HCA II:AZM



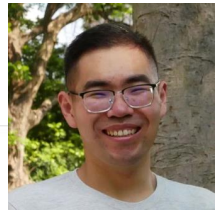


30 min soaking

Data collection + Processing: 2 Days

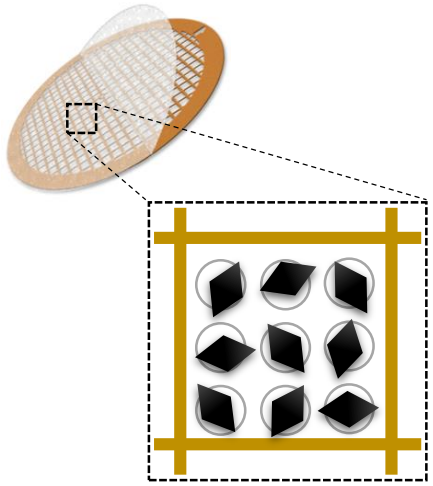
Clabbers *et al. Commun Bio* **2020**

Structure-Based Drug Discovery by 3D ED

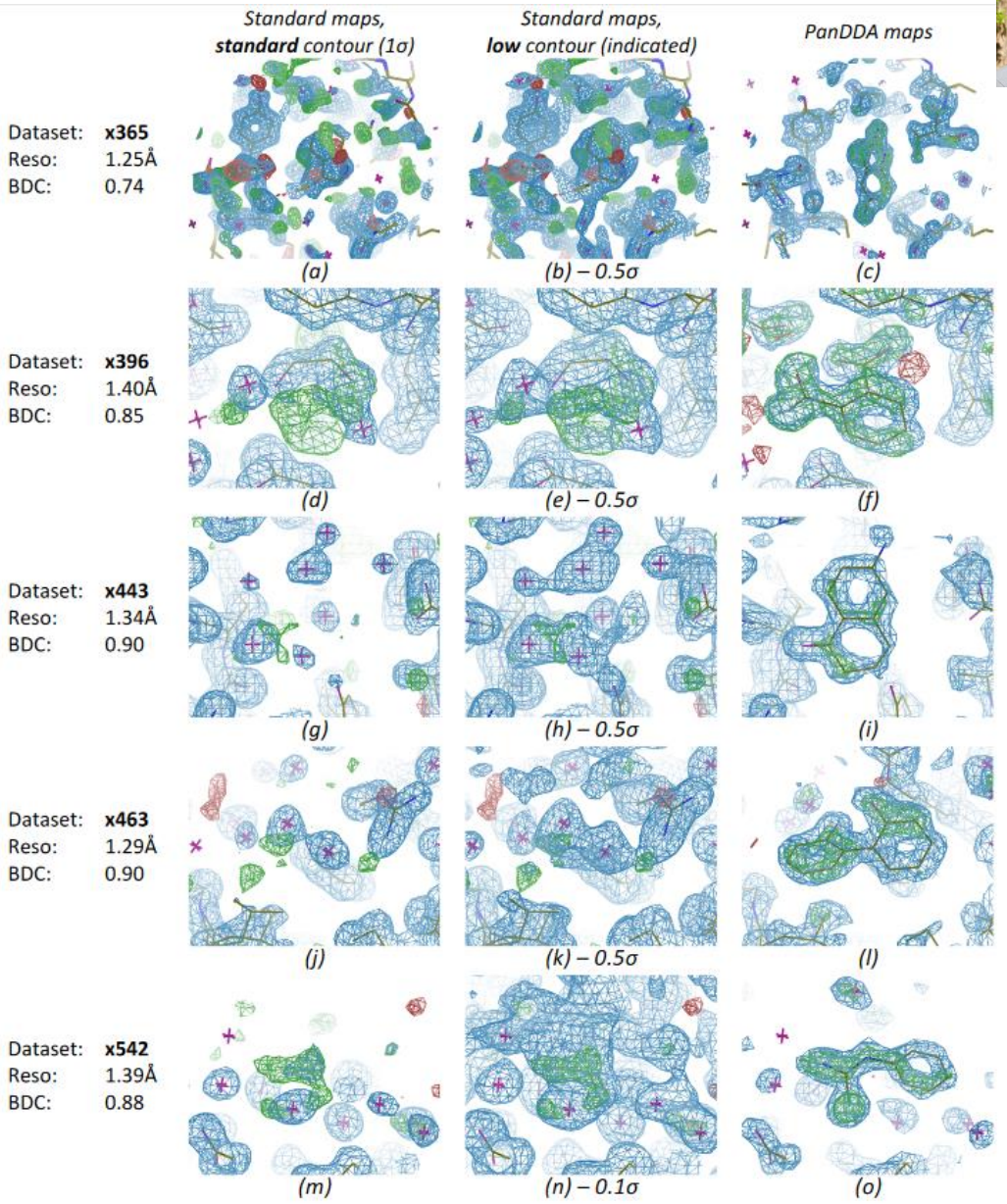


Lei Wang

- High resolution data, better than 2Å
- **On-grid crystallization**
- **Several ligands per grid**



- **Cocktail soaking / Ligand Libraries**
- **High throughput data collection and analysis (ESR 11)**
- Study new drug targets – **Partners**



Pearce *et al. Nat. Commun.* **2017**

Acknowledgements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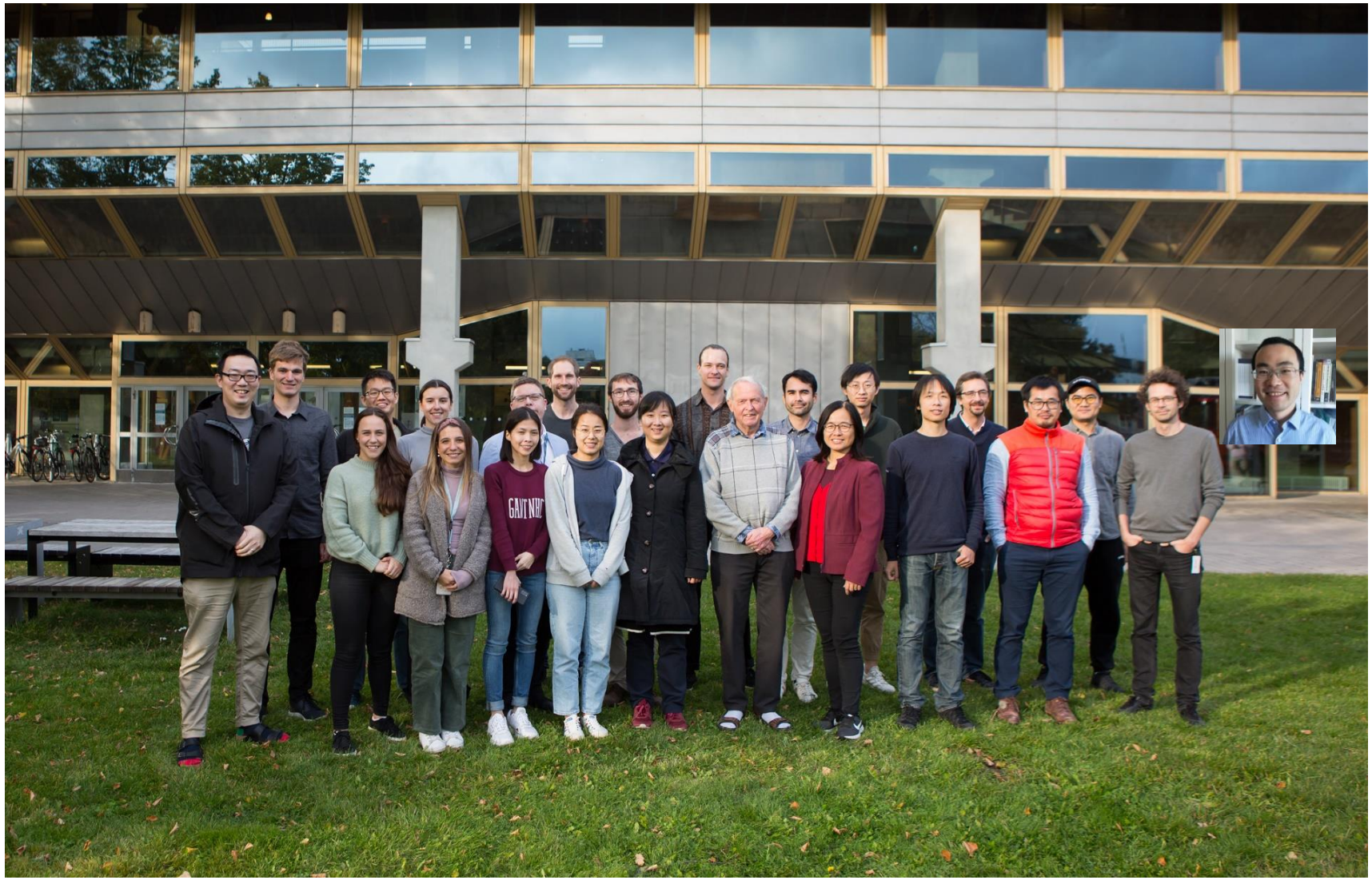

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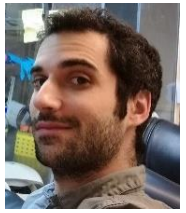

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