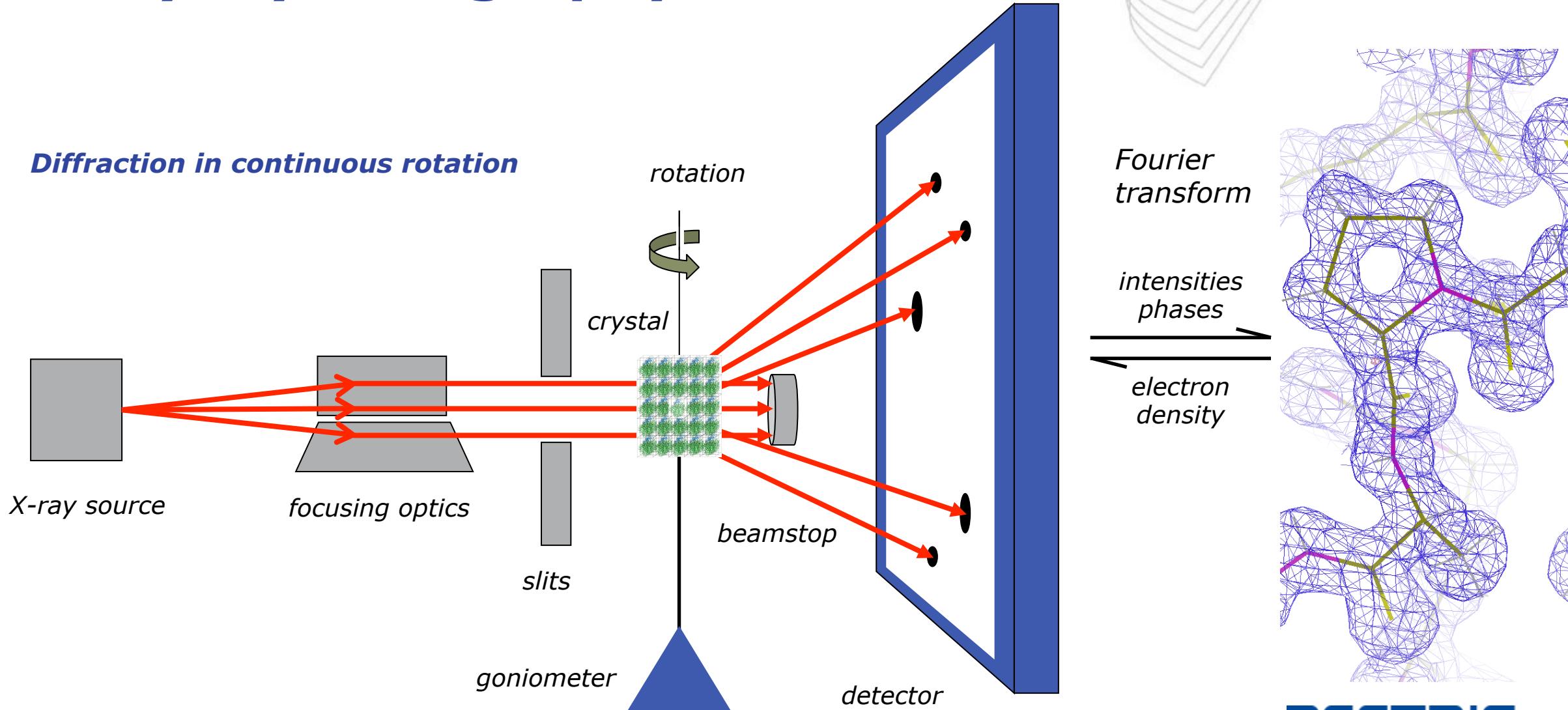




Micro-electron diffraction

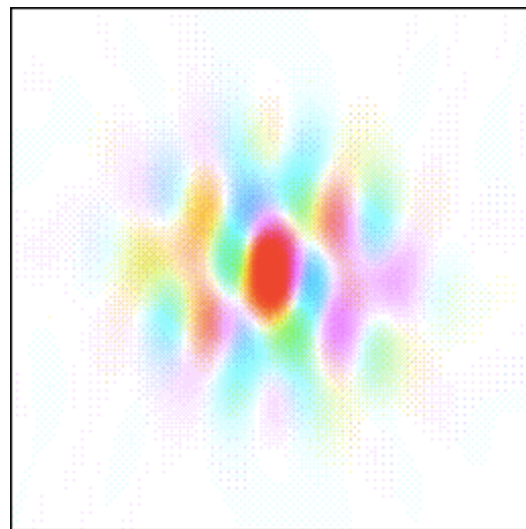
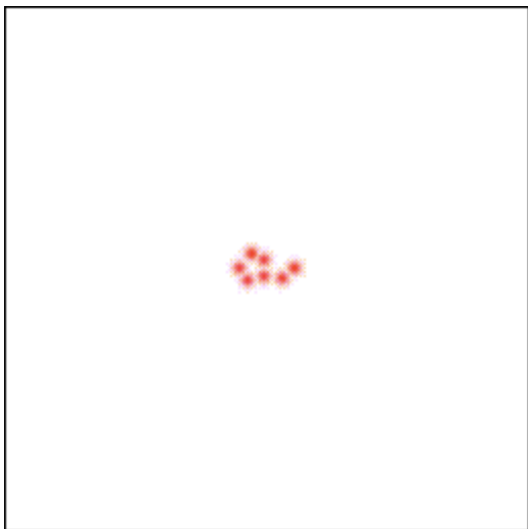
***Andreas Förster, Application Scientist Crystallography
EMBO Cryo-EM Course, Birkbeck, 5 Sep 2019***

X-ray crystallography

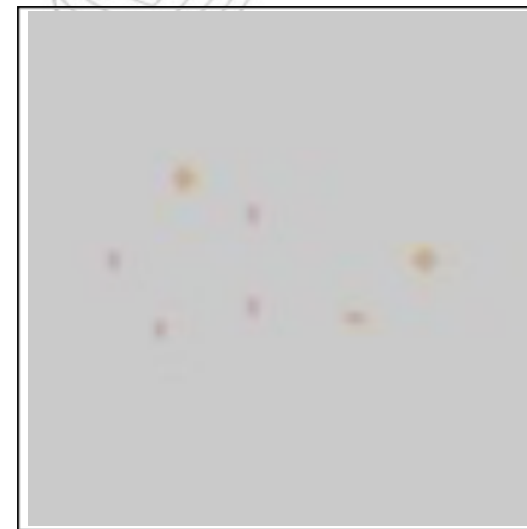


Imaging and diffraction

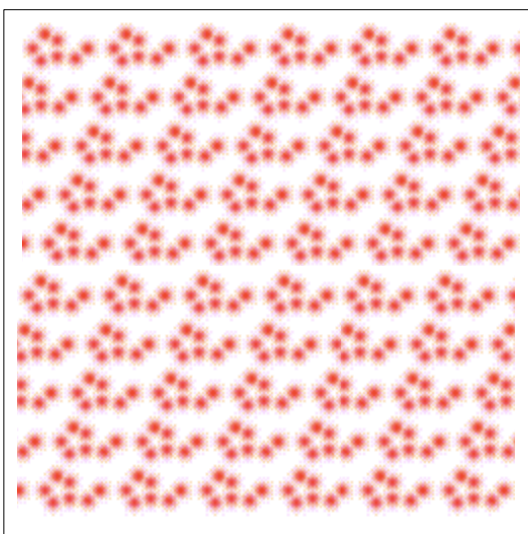
Object



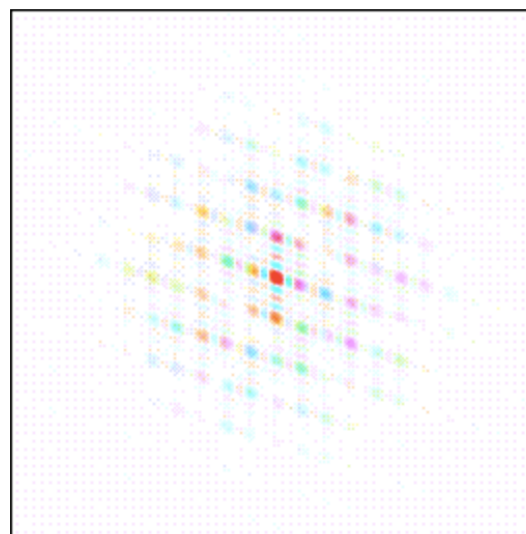
Objective
lens



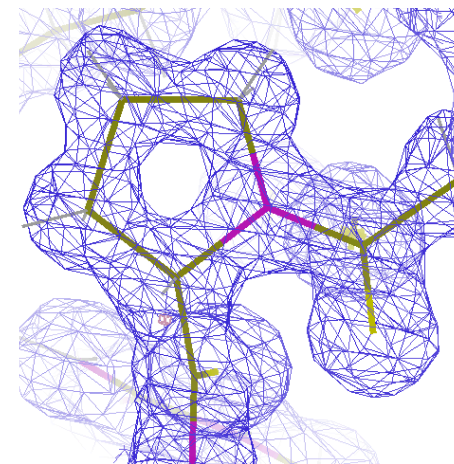
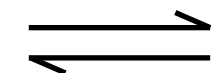
Crystal



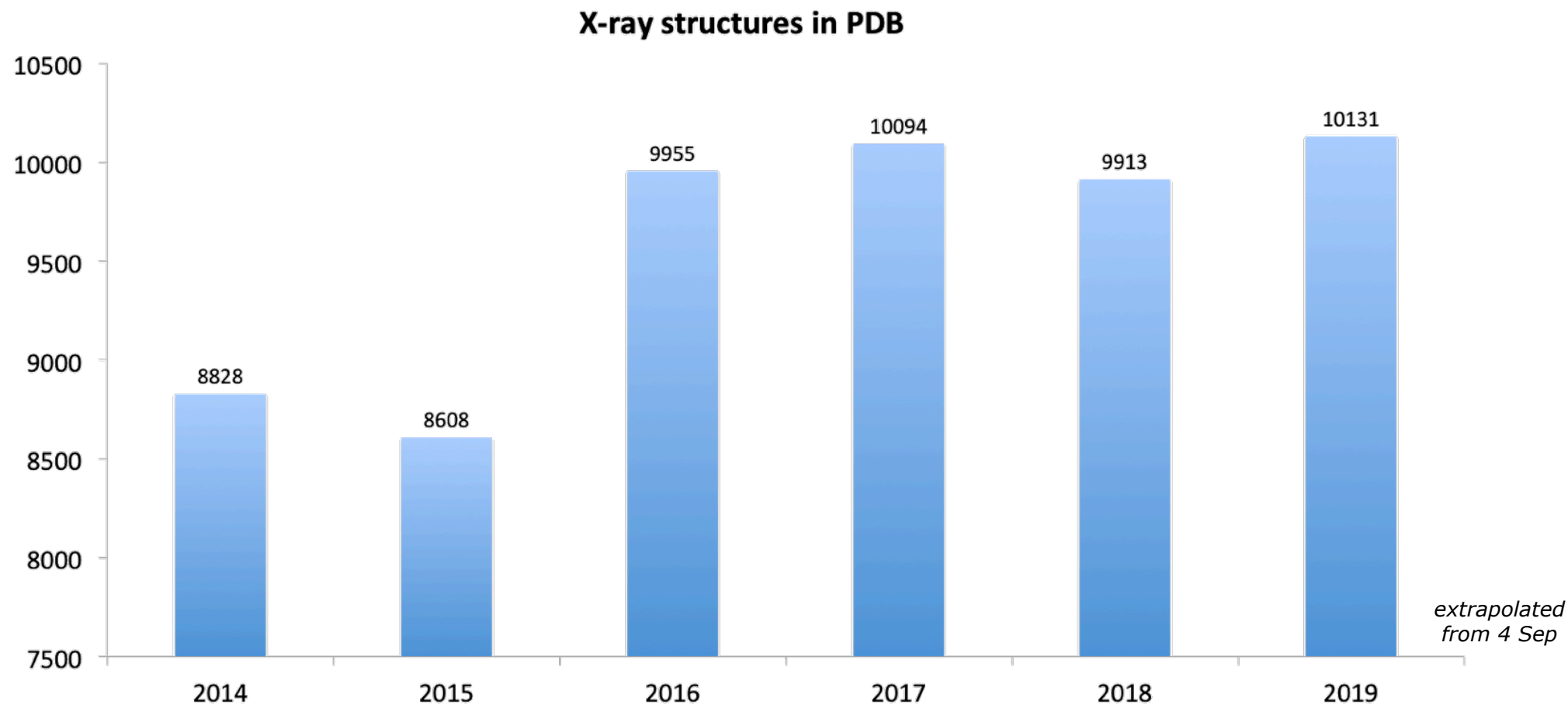
Diffraction



Fourier
transform



Crystallography is going strong



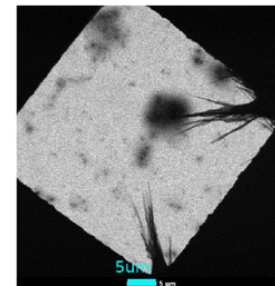
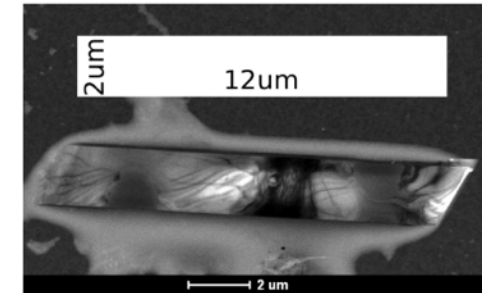
No crystals? Use powders!

Powders are microcrystals

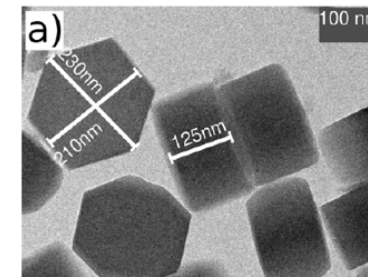
- Too small for X-ray crystallography.
- Visible under TEM.

Electrons as radiation source

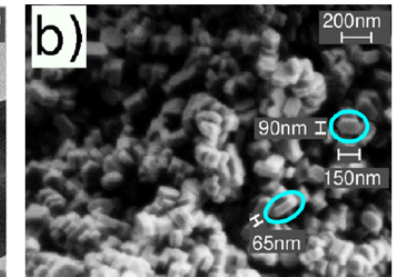
- Much stronger interaction with matter than X-rays.
- Visualisation of hydrogens.
- Coulomb potential to visualize ions, charged amino acids (Yonekura, JAC, 2016)
- Much less damaging than X-rays.



organic compound
(Grippostad®)



Silicalite-1



ZSM-5

Grüne (2018), Chem Eur J

EIGER and QUADRO for microED

EIGER (since 2015)

- Can detect electrons.
- Used to solve structures.

QUADRO and CRISTALLINA

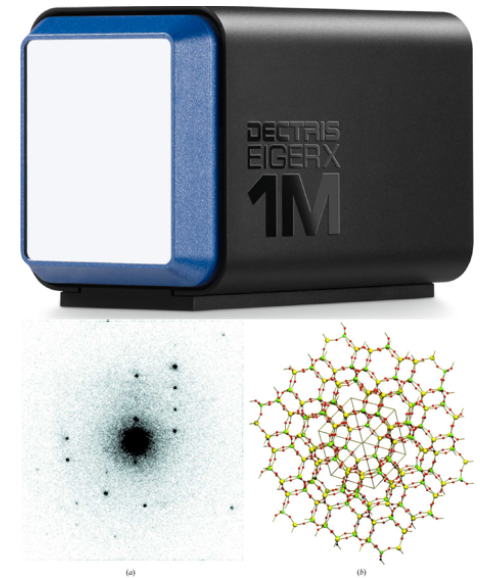
- Based on EIGER2.
- Up to 10^7 electrons per pixel and second.
- Continuous rotation (<100 ns dead time)
- Up to 18 kHz in ROI.
- Radiation-hard, no beam stop required.
- Available with Si (for up to 200 keV) or CdTe (for up to 300 keV) sensors.



QUADRO (250k pixels)

CRISTALLINA (1M pixels)

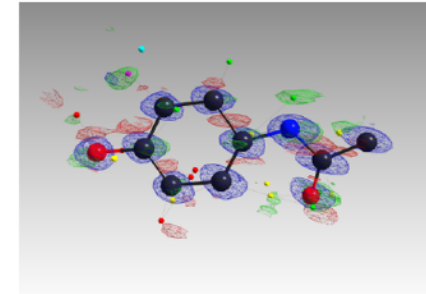
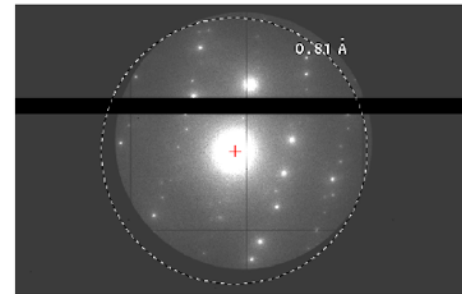
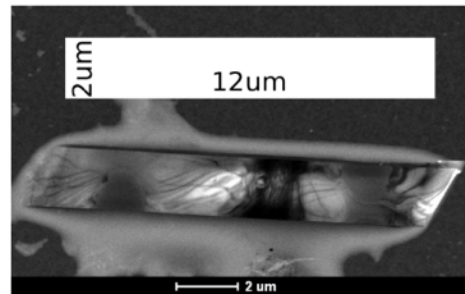
EIGER



Tinti (2018), IUCr J

Crystal structure from the drug

Grippostad[®], STADA



active compounds non-active compounds

paracetamol (US: acetaminophen)

ascorbic acid

caffeine

chlorphenamine maleate

gelatine

glycerol tristearate

lactose monohydrate

quinoline yellow (E104)

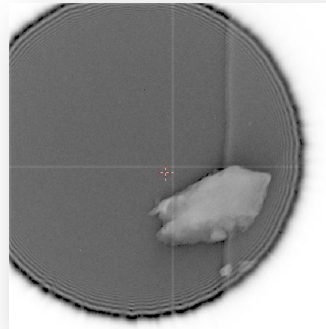
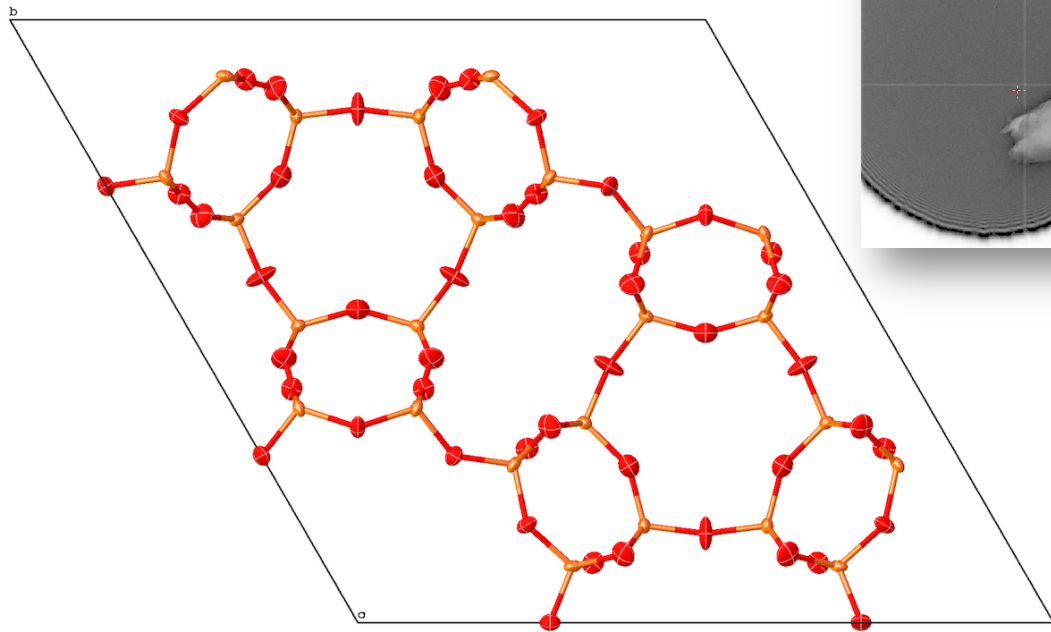
erythrosine (E127)

titanium dioxide (E171)

EIGER X 1M on Tecnai F30

Grüne (2018), *Angew Chem Int Ed*

Rapid structure determination of a zeolite

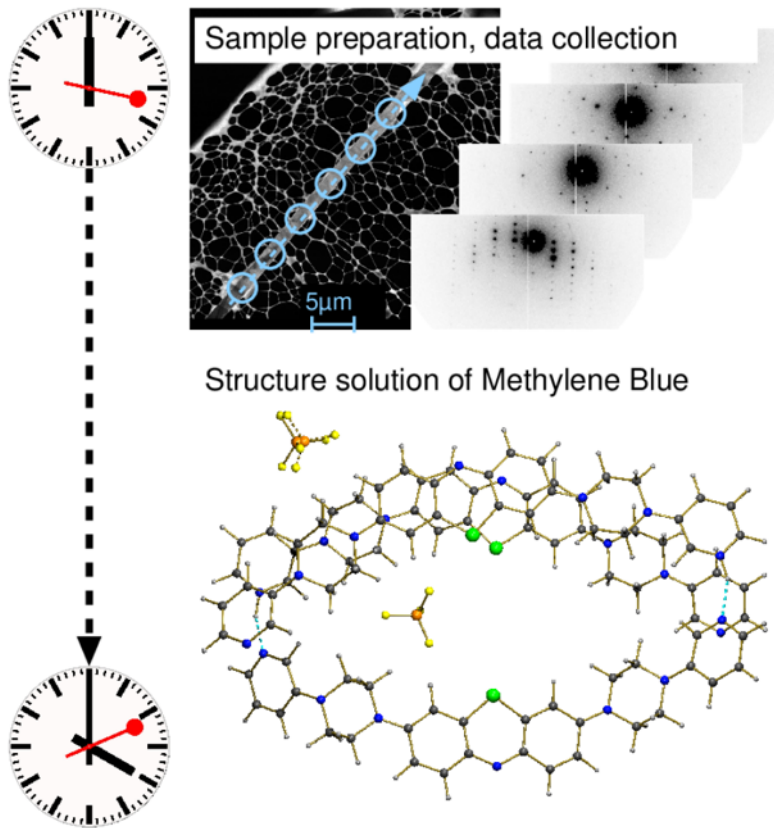


- 110° at $0.015^\circ/\text{img}$ at 100 img/s .
- $SG = P6_3/mmc$
- Resolution 0.65 \AA
- $I/\sigma(I) = 3.9$ (highest shell)
- $R_{int} = 24\%$
- Completeness = 99.9%

QUADRO on Tecnai F20

Wennmacher, submitted

Rapid structure determination



Application example MBBF_4

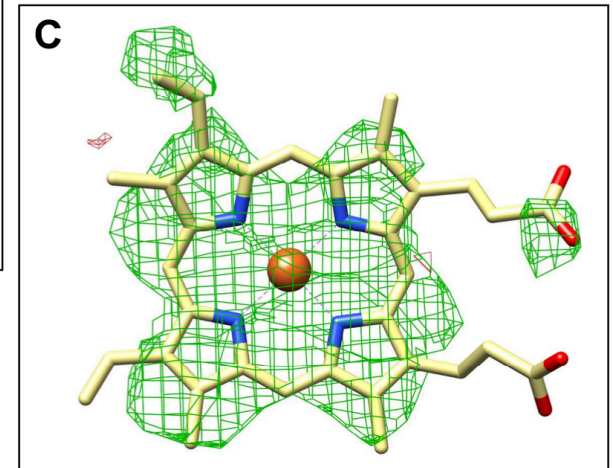
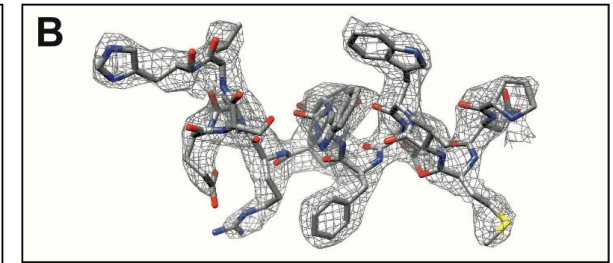
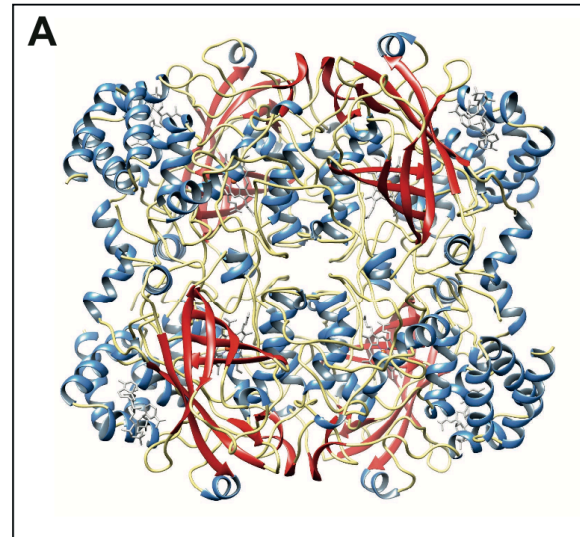
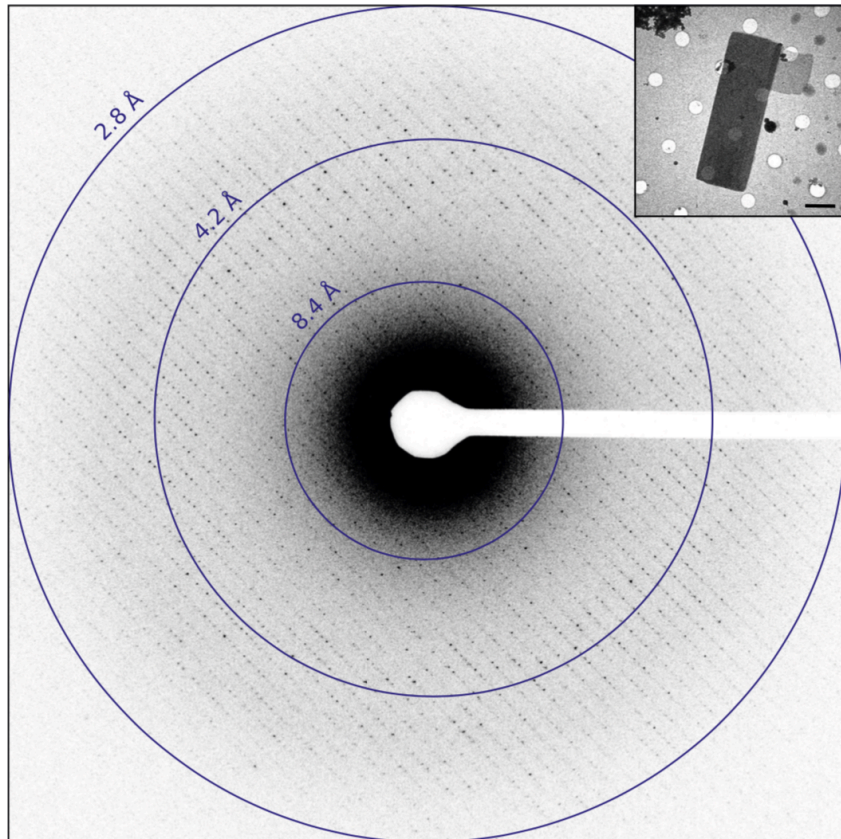
- Methylene Blue derivative with BF_4 .

MicroED as a complement/upgrade to X-ray facilities

- Proper calibration of instrument.
- Setup of processing from known parameters.
- Overlapping datasets for completeness.
- From flask to refined structure in a few hours.

Grüne (2018), Angew Chem Int Ed

Protein structure determination

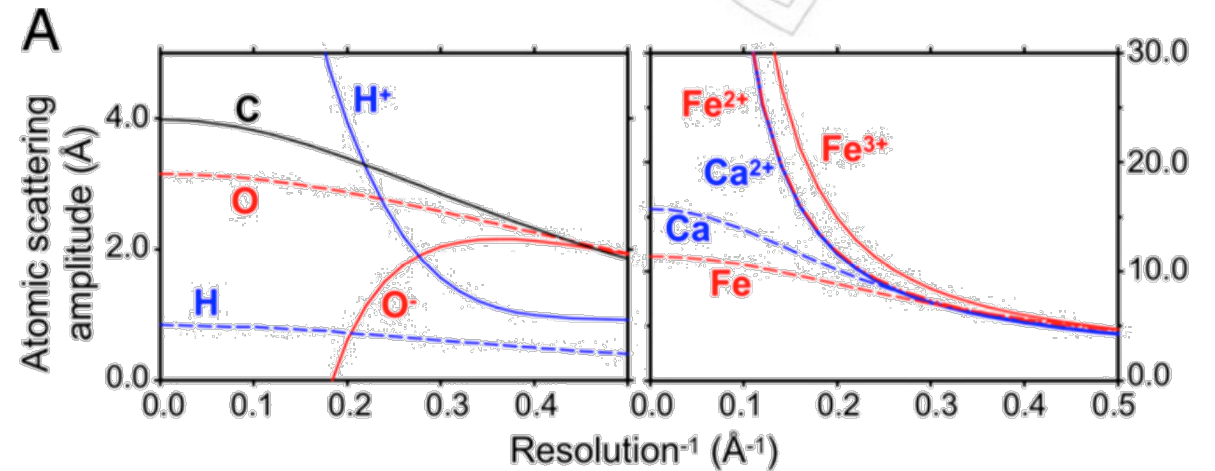
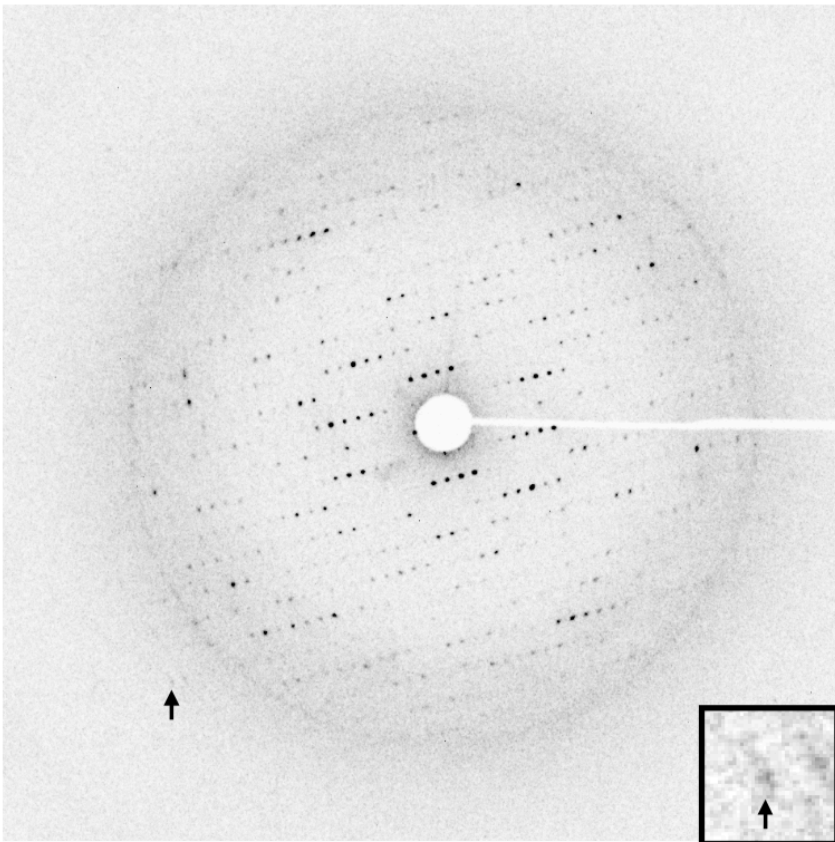


Structure of catalase

- *Tightly spaced spots.*
- *Data to 3.2 Å.*
- *Missing density for charged residues*

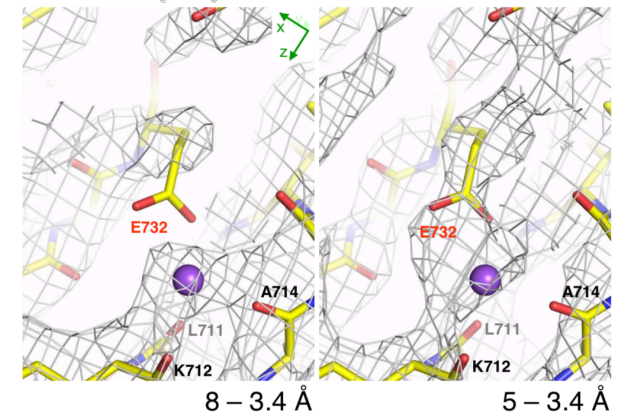
Nannenga (2014), eLife

Protein structure determination



Structure of Ca²⁺-ATPase

- *Tightly spaced spots*
- *Data to 3.4 Å.*
- *Information on charged species*

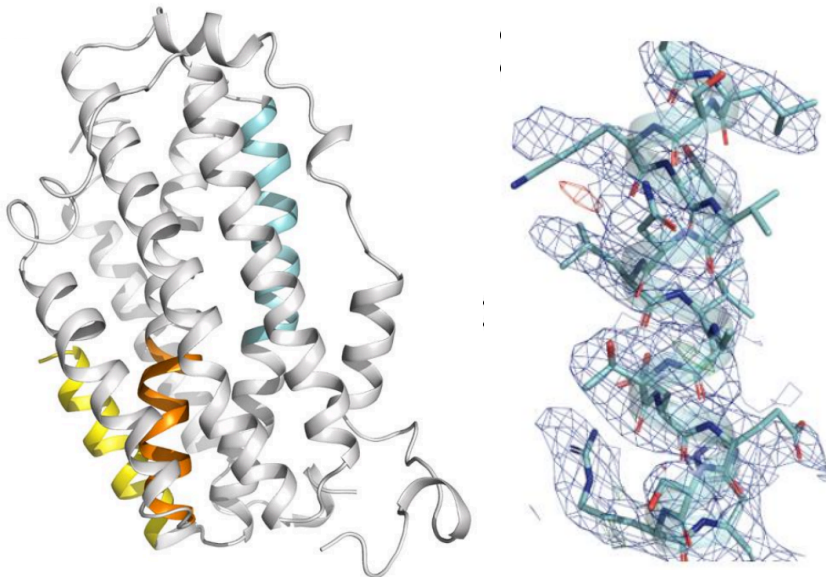


Yonekura (2015), PNAS

Good structures and bad numbers

First novel protein structure

– R2-like ligand-binding oxidase.



Data collection

Wavelength (Å) 0.02508
Resolution range 29.00-3.00 (3.08-3.00)
Space group $P2_12_12$
Unit cell dimensions

a, b, c (Å) 63.31, 108.93, 48.17

α, β, γ (°) 90.00, 90.00, 90.00
Total reflections 144428 (2254)
Unique reflections 4452 (264)
Multiplicity 32.4 (8.5)
Completeness (%) 62.8 (52.9)
Mean $I/\sigma(I)$ 6.12 (0.75)
Wilson B-factor 47.93
 R_{merge} 0.553 (3.776)
 R_{meas} 0.561 (3.995)
 $CC_{1/2}$ 0.981 (0.597)
 CC^* 0.995 (0.860)

Refinement

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)
Number of non-hydrogen atoms 2243
 macromolecules 2241
 ligands 2
 solvent -
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

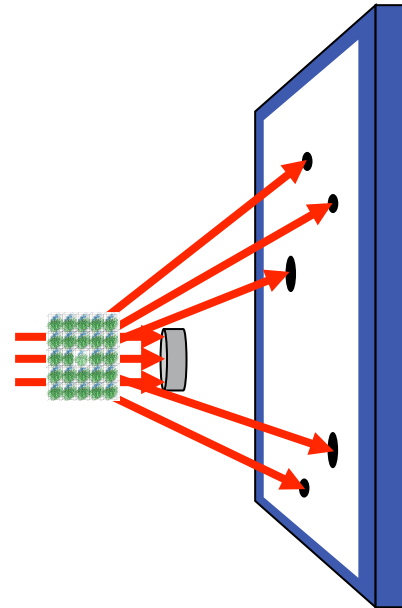
Xu (2019), bioRxiv

Dynamical scattering

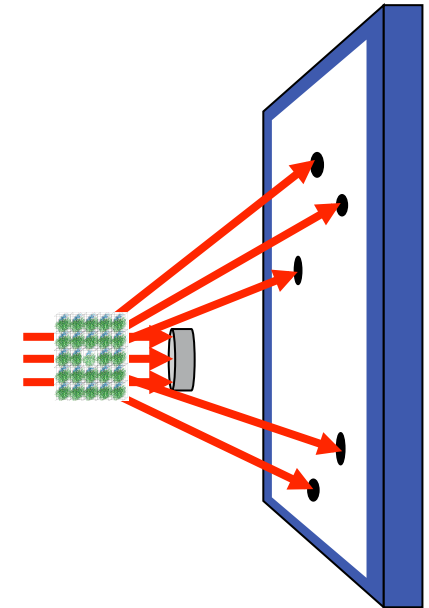
Spot intensities change

- Overestimation of weak reflections.
- Underestimation of strong reflections.
- Dependent on crystal size, orientation and energy.

Kinematic diffraction



Dynamical diffraction



Dynamical scattering

Spot intensities change

- Overestimation of weak reflections.
- Underestimation of strong reflections.
- Dependent on crystal size, orientation and energy.

Breaks processing and refinement

Data collection

R_{merge}

R_{meas}

0.553 (3.776)

0.561 (3.995)

Refinement

R_{work}

R_{free}^*

0.3179 (0.4299)

0.3347 (0.4623)

Xu (2019), bioRxiv

Dynamical scattering

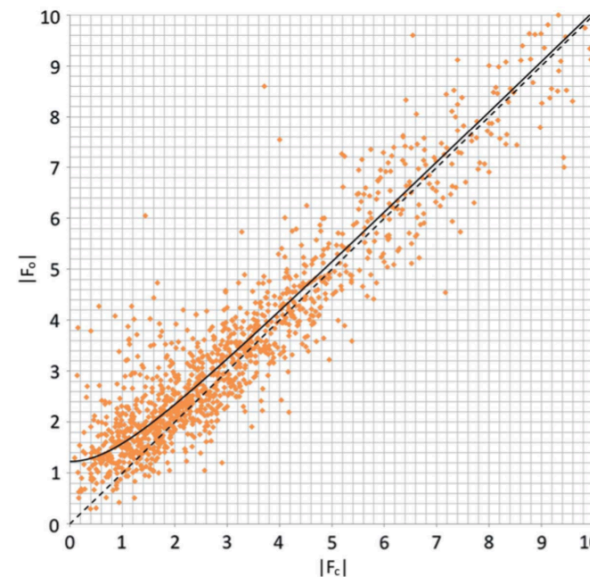
Spot intensities change

- Overestimation of weak reflections.
- Underestimation of strong reflections.
- Dependent on crystal size, orientation and energy.

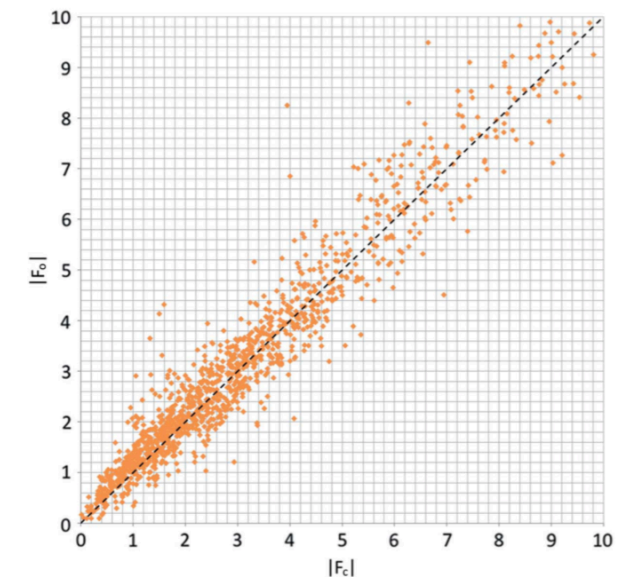
Breaks processing and refinement

- Reduced by reorienting crystals.
- Reduced algorithmically.
- Dynamical refinement.

Original amplitudes



Corrected amplitudes



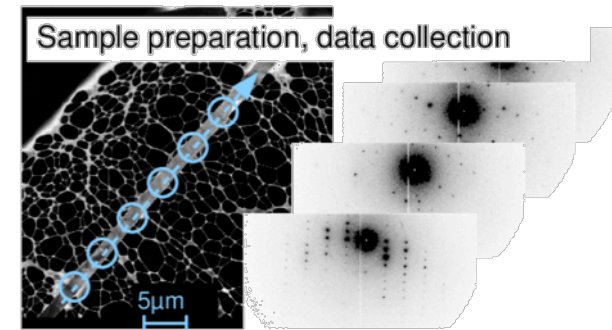
Clabbers (2019), Acta Cryst A

Challenges in microED

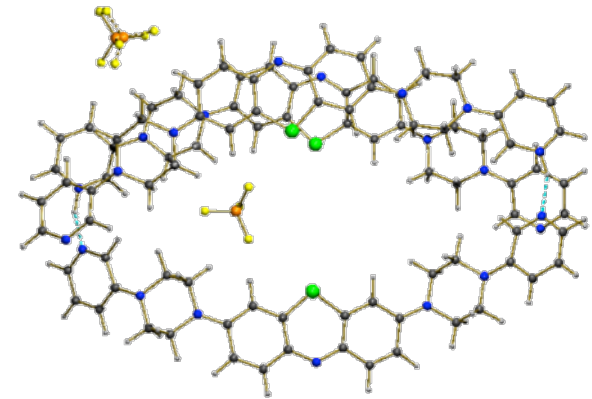
Solutions to common problems:

- *Radiation damage.*

***Collect and merge
multiple partial datasets***



Structure solution of Methylene Blue



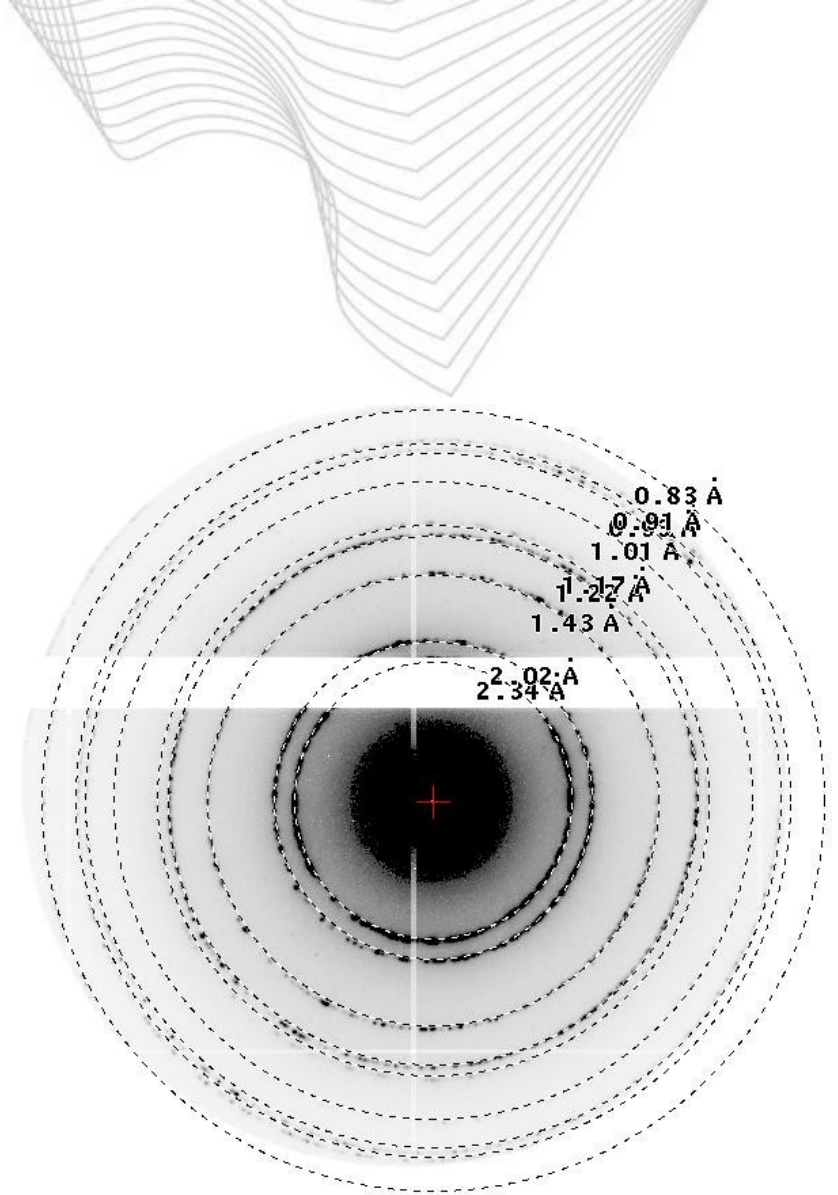
Grüne (2018), Angew Chem Int Ed

Challenges in microED

Solutions to common problems:

- *Radiation damage.*
- *Determining the detector distance.*

***Aluminum powder for
instrument calibration***



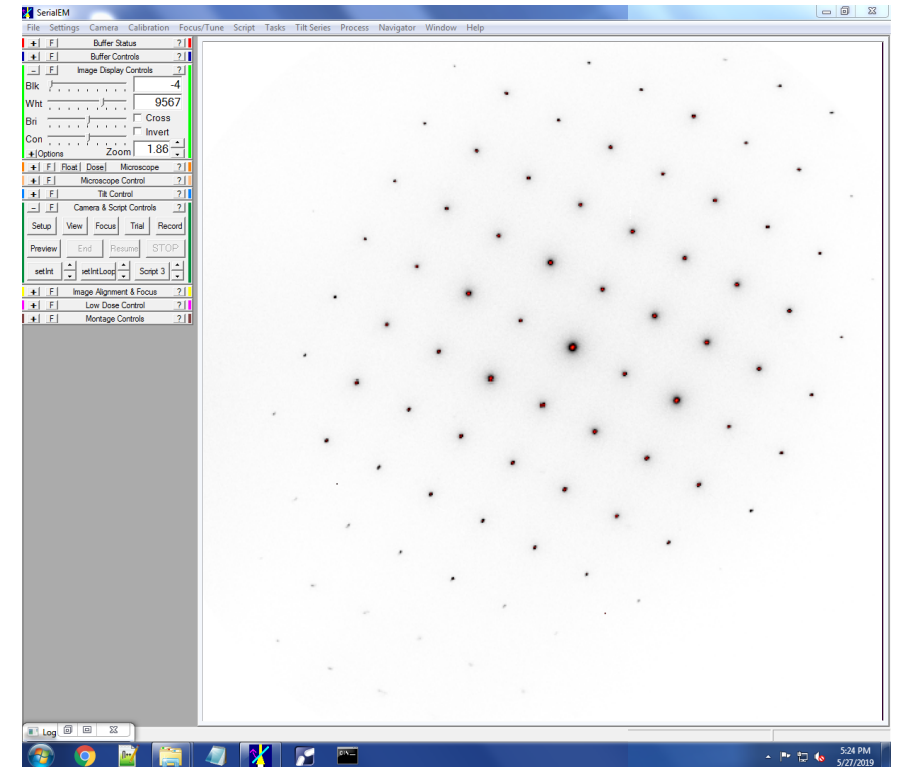
Heidler (2019), Acta Cryst D

Challenges in microED

Solutions to common problems:

- *Radiation damage.*
- *Determining the detector distance.*
- *User-friendly data acquisition.*

***SerialEM integration
(SerialED workflows)***

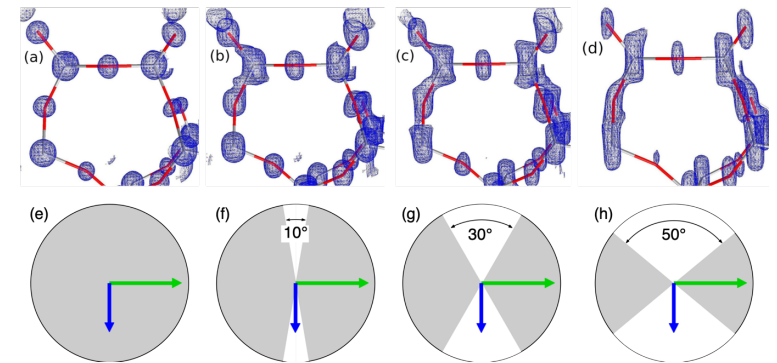
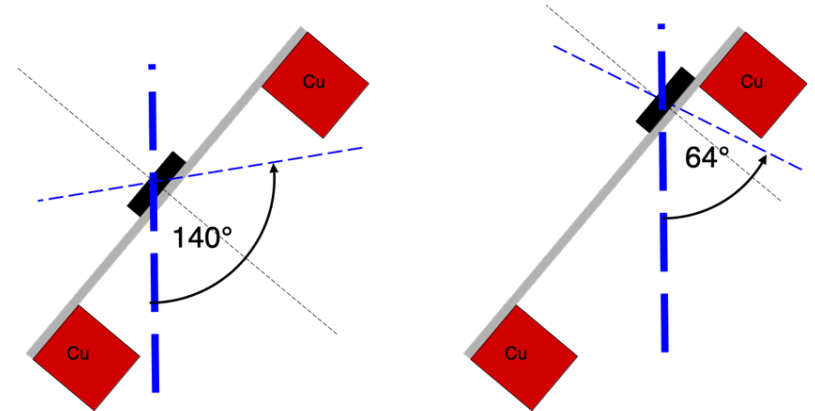


SerialEM and Gonen lab

Challenges in microED

Solutions to common problems:

- Radiation damage.
- Determining the detector distance.
- User-friendly data acquisition.
- Incomplete data.



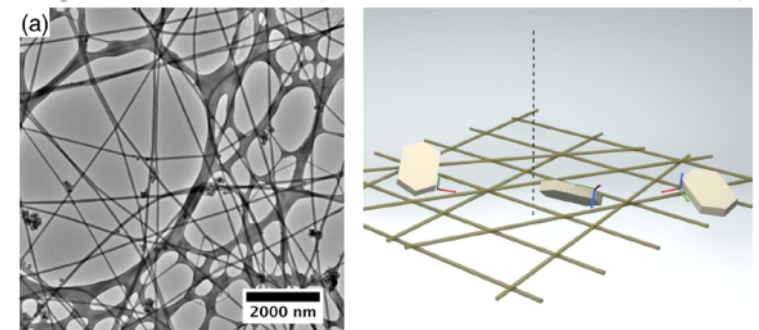
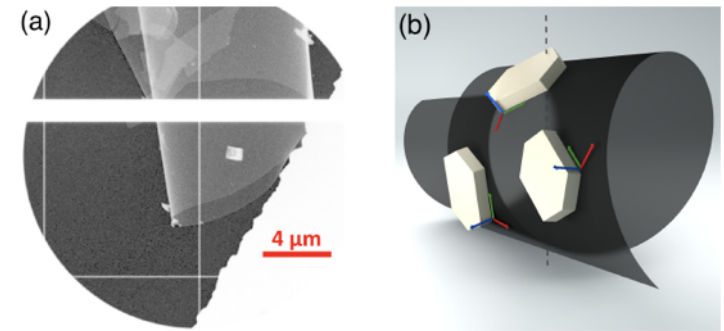
Wennmacher (2019), Nat Commun

Challenges in microED

Solutions to common problems:

- *Radiation damage.*
- *Determining the detector distance.*
- *User-friendly data acquisition.*
- *Incomplete data.*

***Force crystals into
random orientations***



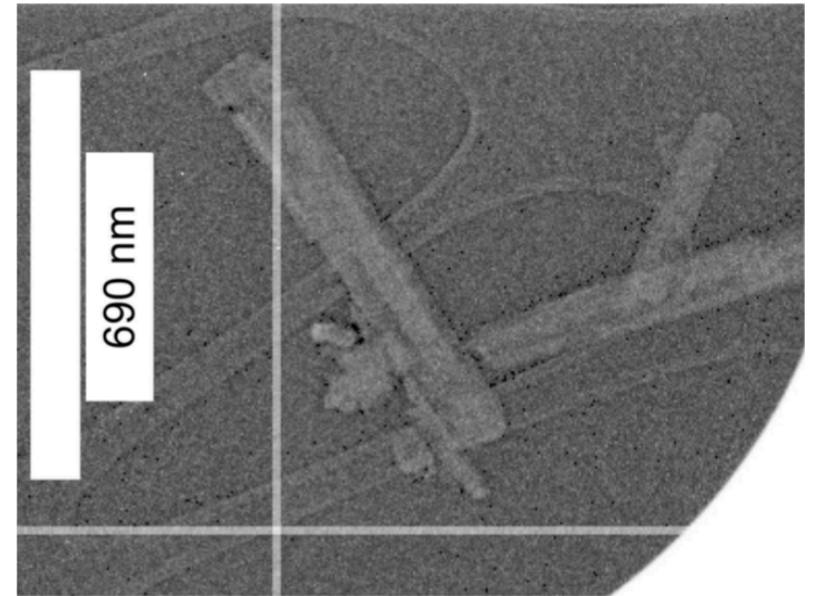
Wennmacher (2019), Nat Commun

Challenges in microED

Solutions to common problems:

- *Radiation damage.*
- *Determining the detector distance.*
- *User-friendly data acquisition.*
- *Incomplete data.*
- *Identification of crystals.*

***Low-magnification,
low-dose imaging mode.***



Heidler (2019), Acta Cryst D

Challenges in microED

Solutions to common problems:

- *Radiation damage.*
- *Determining the detector distance.*
- *User-friendly data acquisition.*
- *Incomplete data.*
- *Identification of crystals.*



***Low-dose raster scan
with fast detector in
diffraction or imaging mode.***

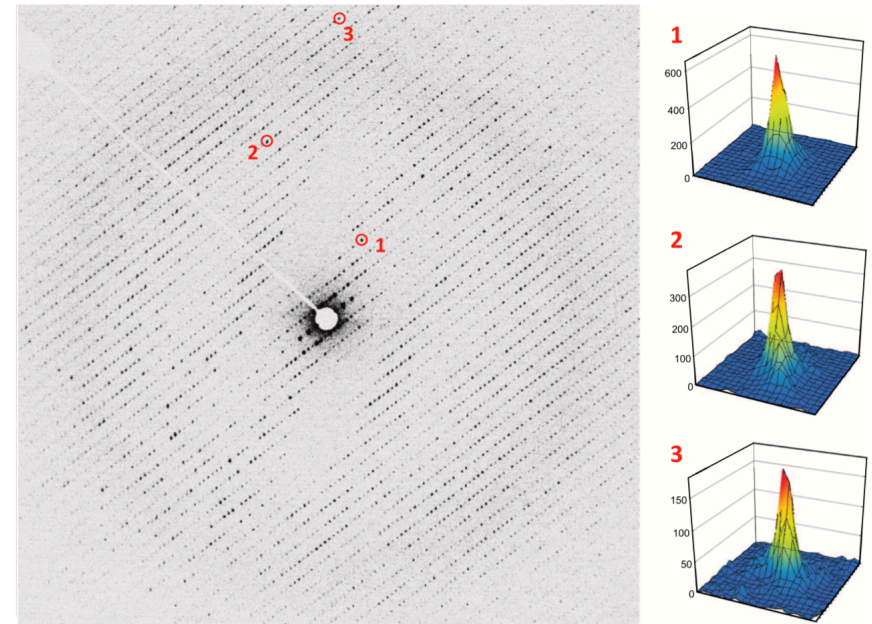
de la Cruz (2019), Ultramicroscopy

Challenges in microED

Solutions to common problems:

- Radiation damage.
- Determining the detector distance.
- User-friendly data acquisition.
- Incomplete data.
- Identification of crystals.
- Inelastic scattering.

**Energy filter to
reduce background**



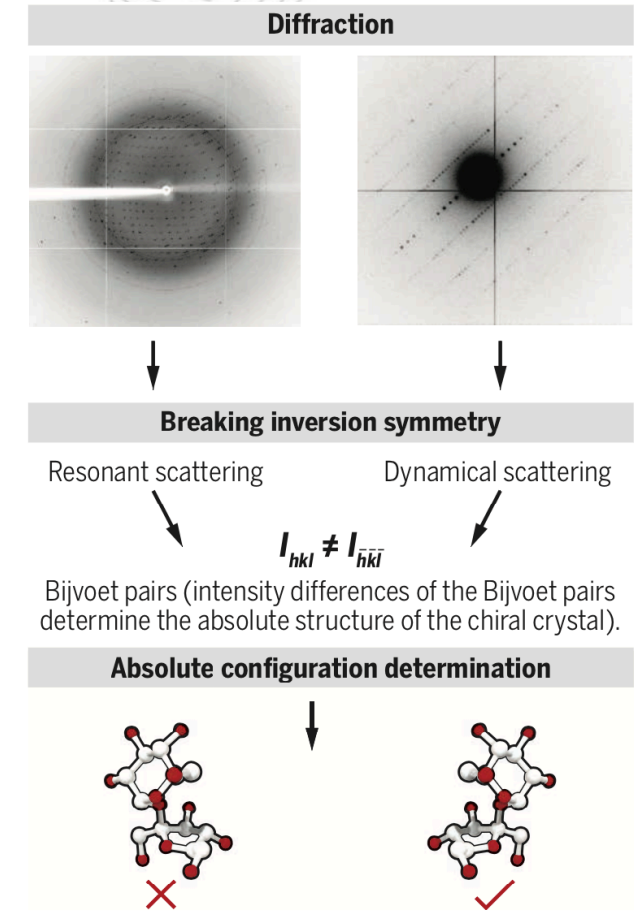
Yonekura (2019), *J Struct Biol*

Challenges in microED

Solutions to common problems:

- Radiation damage.
- Determining the detector distance.
- User-friendly data acquisition.
- Incomplete data.
- Identification of crystals.
- Inelastic scattering.
- Dynamical diffraction.

**Use like anomalous signal
to solve absolute structure**



Brazda (2019), *Science*
Xu (2019), *Science*

Quo vadis electron diffraction?

Chemical crystallography – fast and cheap

- Structures from powders.
- Absolute structures possible?
- Simple hardware.

Protein crystallography from microcrystals

- Alternative to SSX and XFELs.
- Cheap hardware.

Hardware and software questions to be addressed



Conclusion

Electron diffraction is becoming routine

- Any microscope will do. Dedicated instruments cannot be bought yet.
- Better goniometer + automation + faster, more sensitive detector.
- QUADRO and CRISTALLINA bring tangible benefits:
 - Speed, dynamic range, sensitivity, radiation hardness.
- Data processing algorithms remain to be improved.

Acknowledgments

- Tim Grüne (University of Vienna, Austria).
- Julian Wennmacher (Paul Scherrer Institute, Switzerland).



Further reading

Useful papers for quiet hours

- Nannenga and Gonen (2019). The cryo-EM method microcrystal electron diffraction (MicroED). [*Nature Methods*. 16:369.](#)
- Heidler et al. (2019). Design guidelines for an electron diffractometer for structural chemistry and structural biology. [*Acta Cryst D*. 75:458.](#)
- Clabbers et al. (2018). Electron diffraction data processing with DIALS. [*Acta Cryst D*. 74:506.](#)
- Grüne et al. (2018). Rapid Structure Determination of Microcrystalline Molecular Compounds Using Electron Diffraction. [*Angew Chem Int Ed*. 57:16313.](#)
- Brázda et al. (2019) Electron diffraction determines molecular absolute configuration in a pharmaceutical nanocrystal. [*Science*. 364:667.](#)
- Yonekura et al. (2015). Electron crystallography of ultrathin 3D protein crystals: Atomic model with charges. [*PNAS* 112:3368.](#)
- Latychevskaia and Abrahams (2019). Inelastic scattering and solvent scattering reduce dynamical diffraction in biological crystals. [*Acta Cryst B*. 75:523.](#)

***Thank you for
your attention!***

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