Results from the SNI nanoArgovia project A3EDPI:

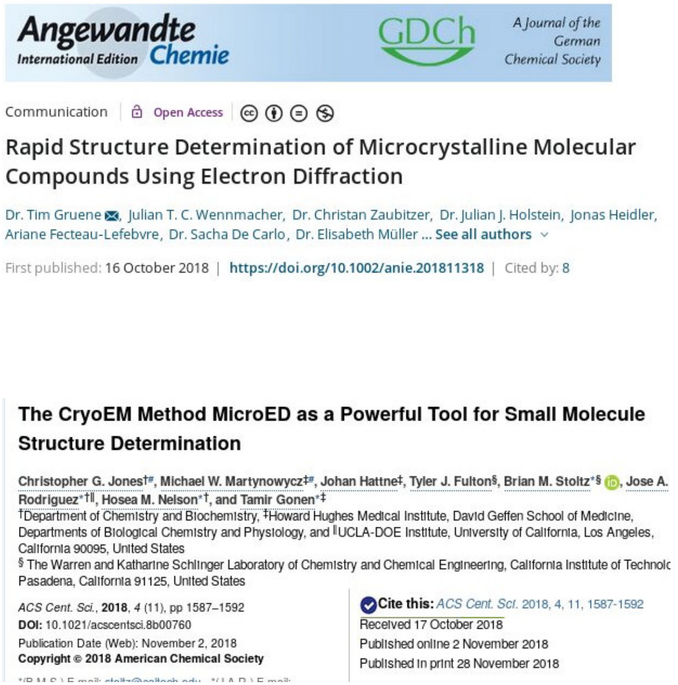
An EIGER X 1M detector turns a Transmission Electron Microscope into an Electron Diffractometer

Netherlands Centre for Electron Nanoscopy (NeCen), 12th June 2019

presented by:
Dr. Tim Grüne
Head of the Centre for X-ray Analytics
Faculty of Chemistry
University of Vienna

tim.gruene@univie.ac.at

Electron Diffraction in the News



Angewandte International Edition Chemie — GDCh — A Journal of the German Chemical Society

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Rapid Structure Determination of Microcrystalline Molecular Compounds Using Electron Diffraction

Dr. Tim Gruene, Julian T. C. Wennmacher, Dr. Christan Zaubitzer, Dr. Julian J. Holstein, Jonas Heidler, Ariane Fecteau-Lefebvre, Dr. Sacha De Carlo, Dr. Elisabeth Müller ... See all authors

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The CryoEM Method MicroED as a Powerful Tool for Small Molecule Structure Determination

Christopher G. Jones[†#], Michael W. Martynowycz[‡#], Johan Hattne[‡], Tyler J. Fulton[§], Brian M. Stoltz[*§], Jose A. Rodriguez[*†∥], Hosea M. Nelson[*†], and Tamir Gonen[*‡]

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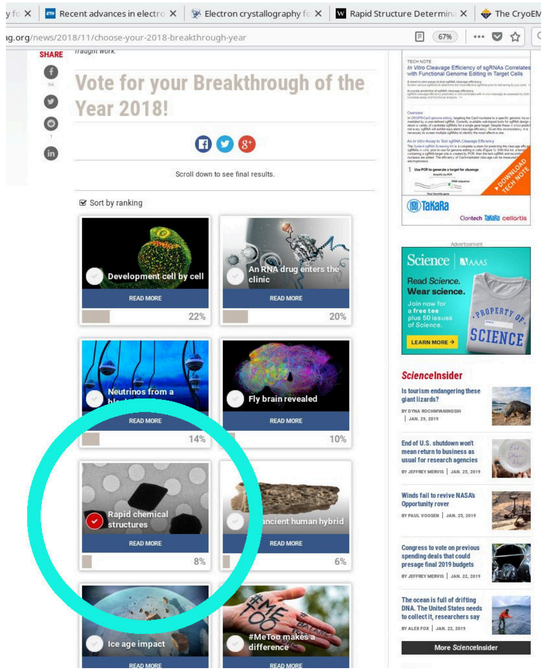
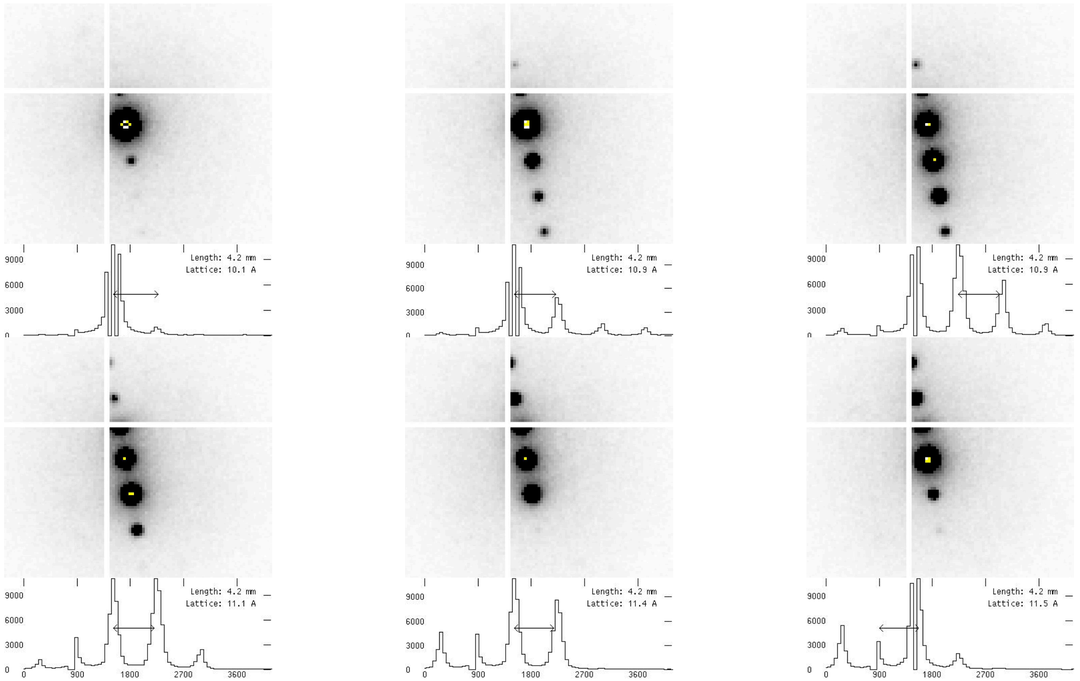


Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

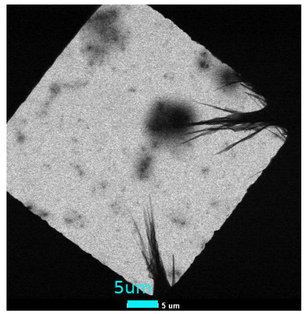
Electron Diffraction is for Small Crystals

Electrons interact more strongly than X-rays

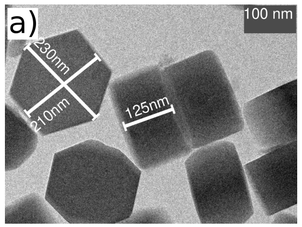


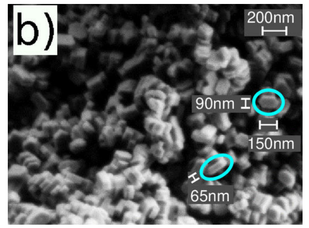
Tinti *et al.*, IUCrJ (2018). 5, 190–199; Data: 10.5281/zenodo.1216026

Powder Crystals are Single Crystals

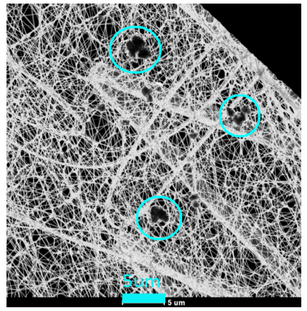


organic compound

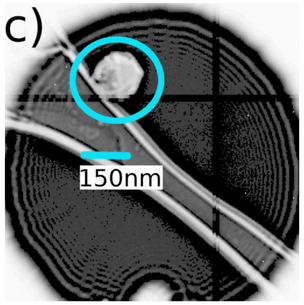


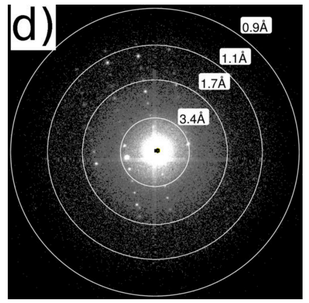


Silicalite–1 / ZSM–5 (Teng Li)



sucrose (ETH coffee bar)

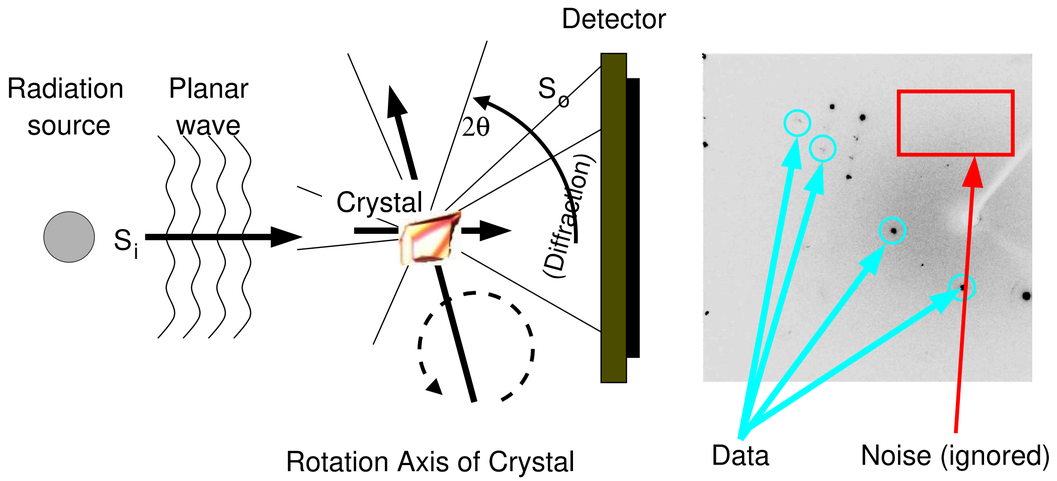




Silicalite–1 / ZSM–5 (Teng Li)

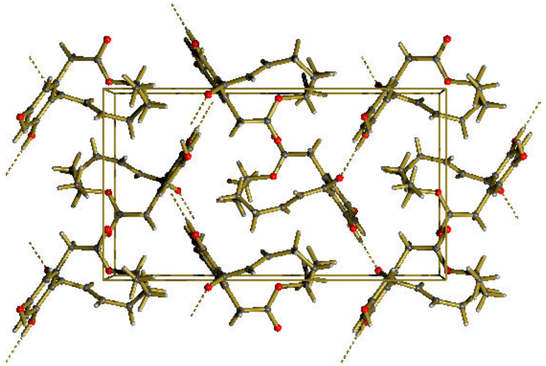
Gruene *et al.*, Chem. EurJ (2018), 24, 2384–2388

Structure Determination by Single Crystal Diffraction

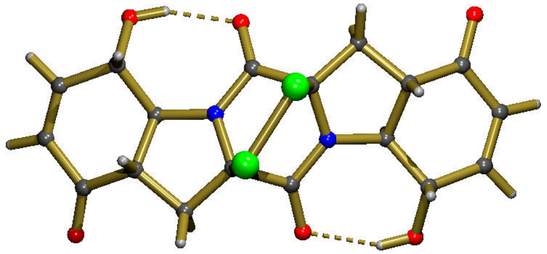


- Diffraction spots: interaction between **wave** and **crystal**
- Experimental result: **Position** and **Intensity** for each spot

Crystal Structure of a Chemical Compound: Determination of 3D atom coordinates



Crystal packing with hydrogen network
Dai *et al.*, Eur. J. Org. Chem. (2010), 6928-6937
CCDC: IRELOH



Intramolecular hydrogen bonding
Deffieux *et al.*, Acta Cryst (1977), **B**33, 1474
CCDC: EPICZA

samples courtesy Novartis (*cf.* Clabbers et al., Acta Cryst. (2019), **A**75, 82–93)

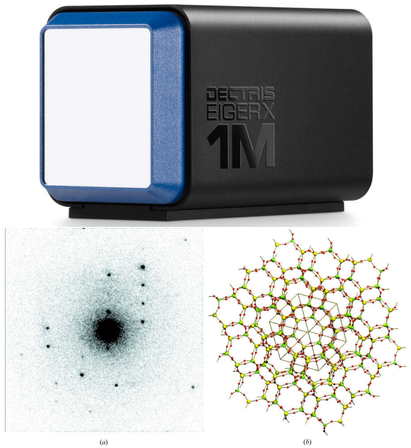
samples courtesy Novartis

Chemistry: Starting point for improvements at atomic level (catalyst efficiency, drug uptake, lacquer brilliance . . .)

How to Turn a TEM into an Electron Diffractometer

J. Heidler *et al.*, “Design Guidelines for an Electron Diffractometer [. . .]”, Acta Cryst. (2019) D75, p. 458–466

EIGER X 1M – made for synchrotrons, suitable for electrons

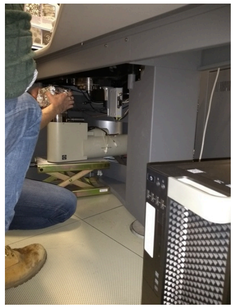


Diffraction pattern and structure for SAPO–34
Tinti *et al.*, IUCrJ (2018). 5, 190–199

- Eiger X 1M designed for X-ray Synchrotron radiation
- 1030x1065 pixel, $75 \times 75\ \mu m^2$
- up to $\nu = 3kHz$ frame rate: data collection at synchrotron speed
- $3\mu s$ dead time: shutterless data collection
- $\leq 200keV$: no radiation damage, no beam stop
- 16 or 32 bit image depth & $2.8 \cdot 10^6 \frac{\text{cts}}{\text{s·pixel}}$: high dynamic range

J. Heidler *et al.*, “Design Guidelines for an Electron Diffractometer [. . .]”, Acta Cryst. (2019) D75, p. 458–466

Installation of the EIGER X 1M in 1/2 day (C. Zaubitzer, ScopeM, ETH)



Removal of the previous camera

Mounting of the EIGER X 1M with adapter flange

Shielding and radiation check

- Final shielding after 1/2 day
- Vacuum OK: next morning
- Return to original state: 1 day
- Gatan camera back with autojustage

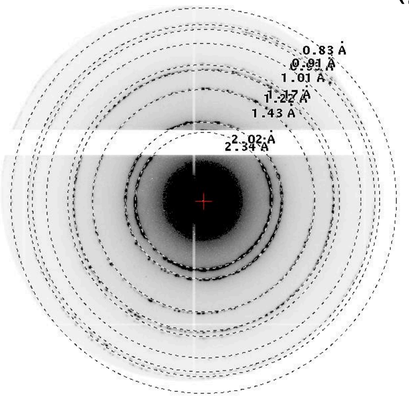
J. Heidler *et al.*, “Design Guidelines for an Electron Diffractometer [. . .]”, Acta Cryst. (2019) D75, p. 458–466

Determination of Experimental Parameters

Detector separated from Instrument: no automated read-out (yet)

- Detector distance (*alias* Camera length)
- Rotation axis
- Direct beam position
- Oscillation width (Rotation per frame)

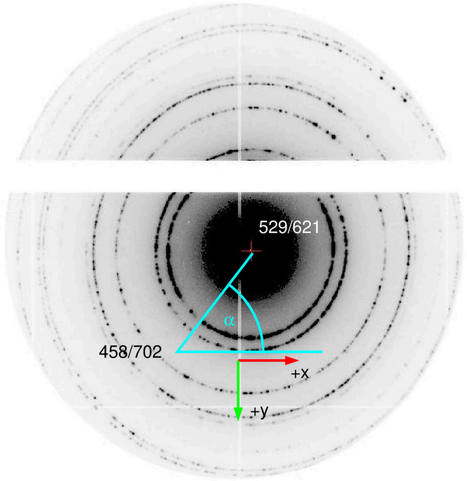
A3EDPI: values can be calibrated, *e.g.* once/month



Hybrid pixel detectors are radiation hard and require no beamstop. This facilitates determination of detector distance, rotation axis, direct beam position

J. Heidler *et al.*, “Design Guidelines for an Electron Diffractometer [. . .]”, Acta Cryst. (2019) D75, p. 458–466

Determination of the Rotation Axis



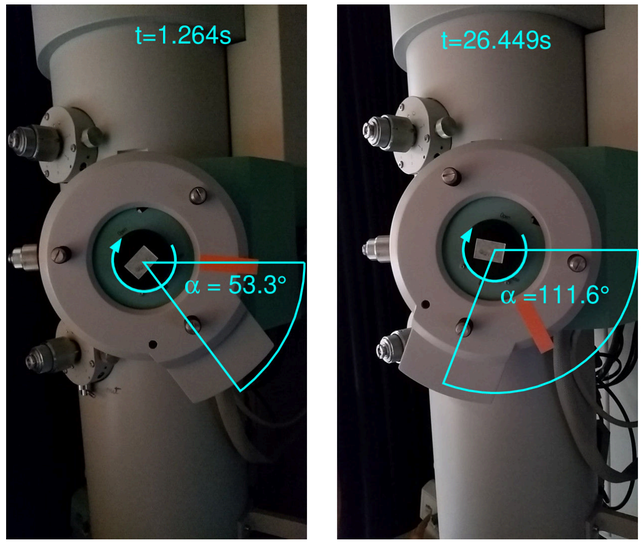
- Rotation axis runs through direct beam and minimum of powder ring
- Rotation axis: region of no spots
- line through 2 points on rotation axis
- $P_1 = 529/621$ and $P_2 = 458/702$
- $\tan(\alpha) = \frac{\Delta Y}{\Delta X} = \frac{702-621}{458-529}$
- `ROTATION_AXIS=` $\cos(\alpha)$; $\sin(\alpha)$; 0
- Large radius of convergence with XDS ($\approx \pm 10°$)

Direction of rotation: from minimal error of spindle axis

`ROTATION_AXIS=` (XDS.INP)	-0.6979	-0.7161	-0.0102	+0.6979	+0.7161	+0.0102
`DEV`$^{\text{N}}$ `OF SPINDLE POS`$^{\text{N}}$ (IDXREF.LP)		$0.37°$			$1.01°$	

J. Heidler *et al.*, “Design Guidelines for an Electron Diffractometer [...]”, Acta Cryst. (2019) D75, p. 458–466

Oscillation Width — manual

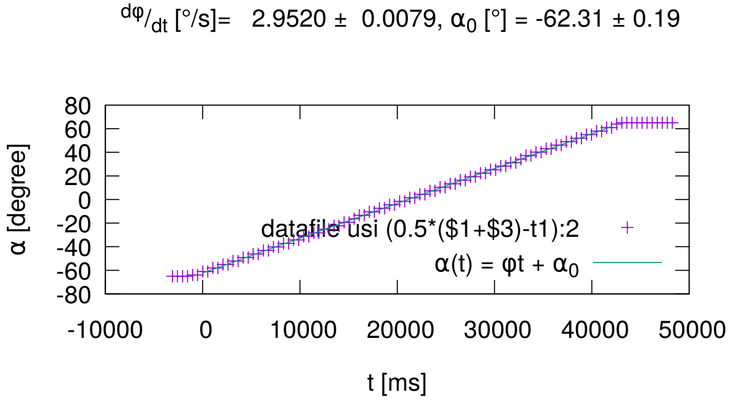


- Used to be most time consuming parameter to be determined
- Step froward at C–CINA: movie during measurements

$$\frac{d\phi}{dt} = \frac{\Delta\alpha}{\Delta t} = \frac{58.15^\circ}{25.185s} = 2.310^\circ/s$$
$$\nu(\text{EIGER}) = 100Hz$$
$$\Rightarrow \Delta\phi = 0.0231^\circ/\text{frame}$$

J. Heidler *et al.*, “Design Guidelines for an Electron Diffractometer [. . .]”, Acta Cryst. (2019) D75, p. 458–466

Oscillation Width — semi-automated



- Probe α angle per 0.5s during experiment
- Fit line to measurements
- fast, reproducible
- Oscillation width $\Delta\phi[^\circ/\text{frame}] = \frac{d\phi}{dt}/\nu(\text{EIGER})$

Acknowledged: Luca Piazza. Dectris Ltd. for initial Digital Micrograph script

J. Heidler *et al.*, “Design Guidelines for an Electron Diffractometer [. . .]”, Acta Cryst. (2019) D75, p. 458–466

Consequence of A3EDPI-Setup: The affordable Electron Diffractometer

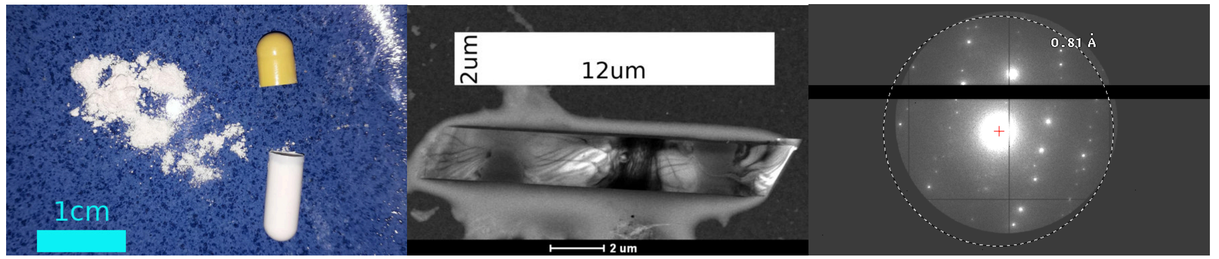
- All parts for a dedicated diffractometer are available
- Pieces need to be assembled
- Electron Microscopes (2-10Mio €): many unnecessary features
- Electron Diffractometer: < 500,000 € including detector

J. Heidler *et al.*, “Design Guidelines for an Electron Diffractometer [. . .]”, Acta Cryst. (2019) D75, p. 458–466

Single Crystal Structure from a Pharmacy Powder

Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

Grippostad®, STADA

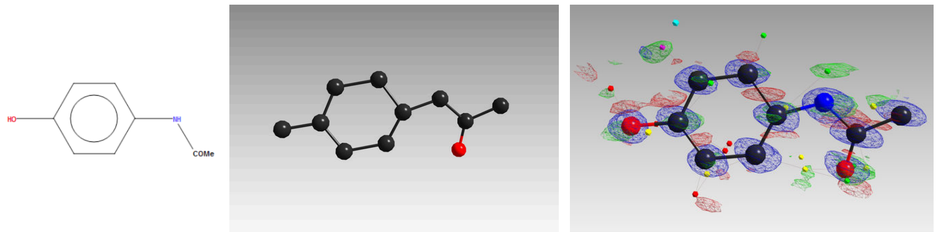


active compounds	non-active compounds
paracetamol	gelatine
ascorbic acid	glycerol tristearate
caffeine	lactose monohydrate
chlorphenamine maleate	quinoline yellow (E104)
	erythrosine (E127)
	titanium dioxide (E171)

Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

Single Crystal Structure from a Pharmacy Powder

1. Exp: $a = 6.9$, $b = 9.4$, $c = 11.6$, $\alpha = 90.6$, $\beta = 98.4$, $\gamma = 89.8$
2. CSD : $a = 7.1\, b = 9.3\, c = 11.7\, \alpha = 90.0\, \beta = 97.7\, \gamma = 90.0$; CSD search: HXACAN04, $P2_1/n$, Paracetamol,
3. Structure solved with $\leq 40\%$ completeness
4. Difference map reveals hydrogen atoms: data sensitivity



"The existence of multiple crystal forms (polymorphs, solvates, hydrates, etc.) is playing an increasingly important role in establishing and protecting intellectual property rights in the pharmaceutical industry"

(Prof. J. Bernstein, ECM-30 (Aug. 2016), MS50-O2)

Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

Consequence of Grippostad: Screening for Polymorphs

- No lower size limit for electron crystallography
- per sample holder: hundreds – thousands of crystals
- combine with automated screening: Cichocka *et al.*, “High-throughput continuous rotation electron diffraction data acquisition via software automation”, J. Appl. Cryst (2018), 51, 1652-1661

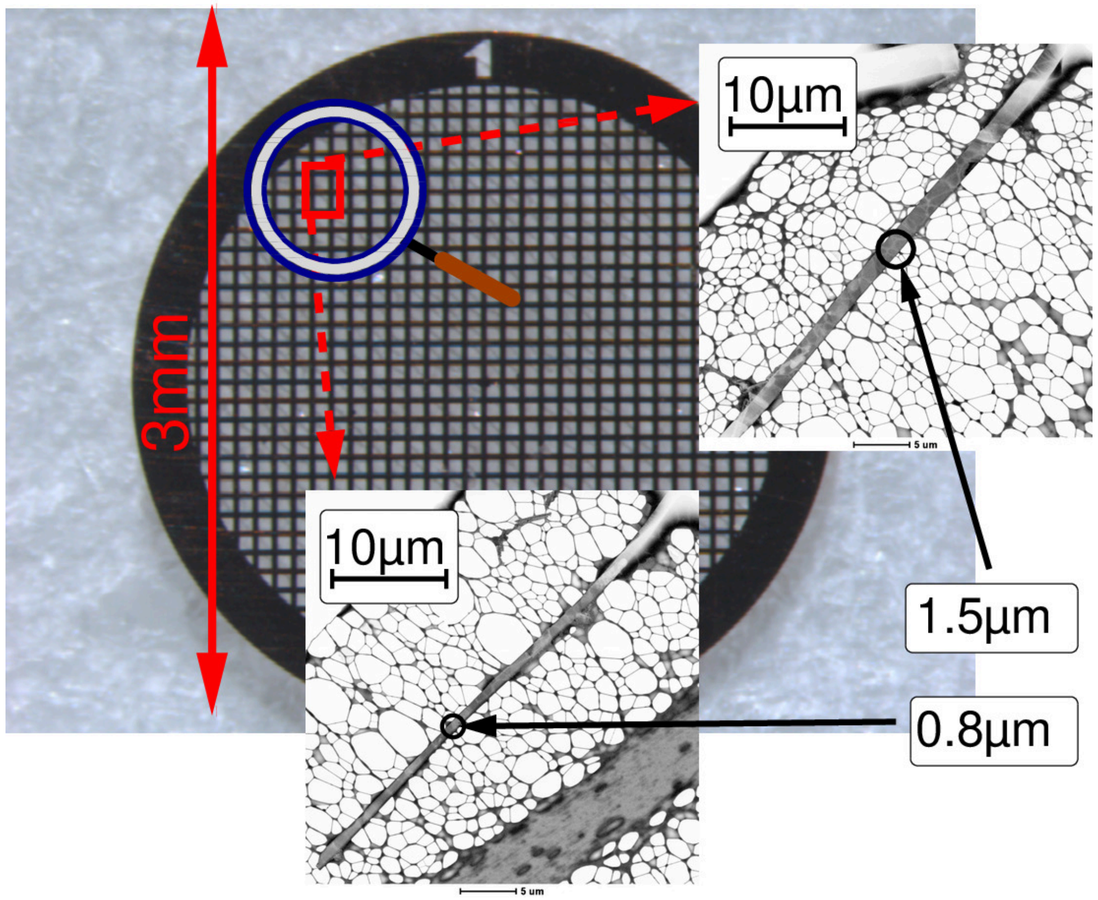
Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

Drug Design: Structure of a New Methylene Blue Derivative $MBBF_4$

Dr. J. Holstein & Prof. G. Clever, TU Dortmund

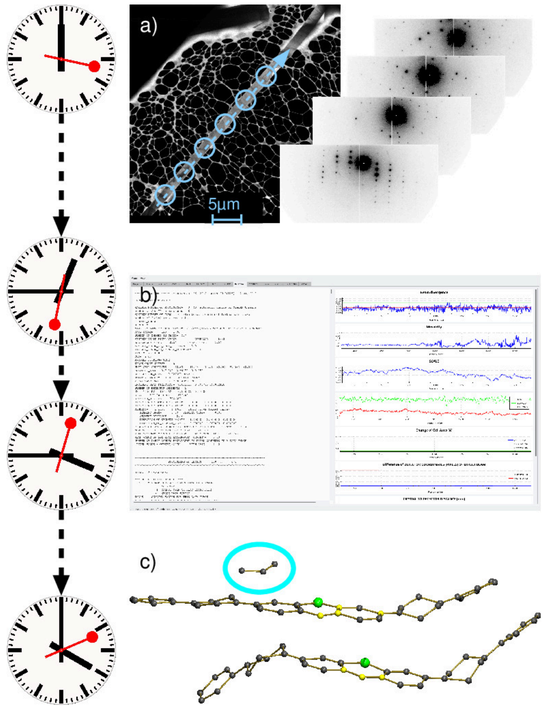
Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

$MBBF_4$-nanoCrystal (Holstein/Clever, TU Dortmund)



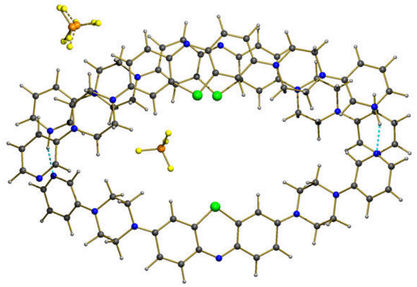
Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

$MBBF_4$ — EIGER and a TEM make a Synchrotron



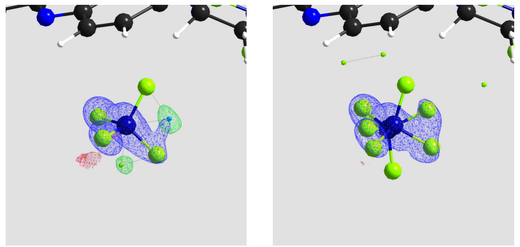
Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

$MBBF_4$ — Data Accuracy (J. Holstein, TU Dortmund)



Structure of $MBBF_4$

- $R1 = 22.7\%(2941 F_o > 4\sigma_F)$
- $R1 = 27.2\%(4832 F_o)$
- GooF = 1.5
- 564 parameters, 1054 restraints



After addition of hydrogen atoms and restraints: Dual conformation of BF_4 becomes visible.

Structure refinement by J. Holstein

Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

Consequence of $MBBF_4$: ED complements XRD

- A dedicated electron diffractometer extends the X-ray diffractometer in every X-ray facility
- Speed of structure determination comparable to X-ray diffractometer
- Reliable Structures from electron diffraction

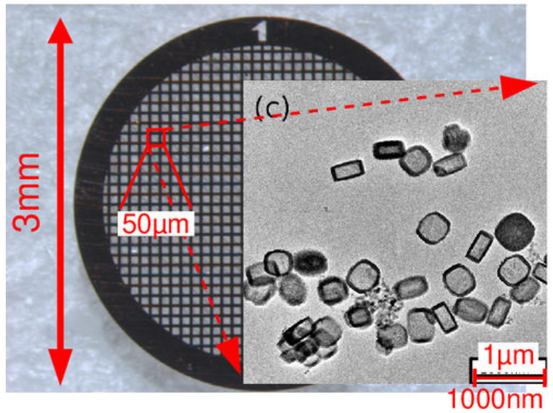
Gruene *et al.*, Angew. Chemie. Int. Ed. (2018), 57, 16313–16317

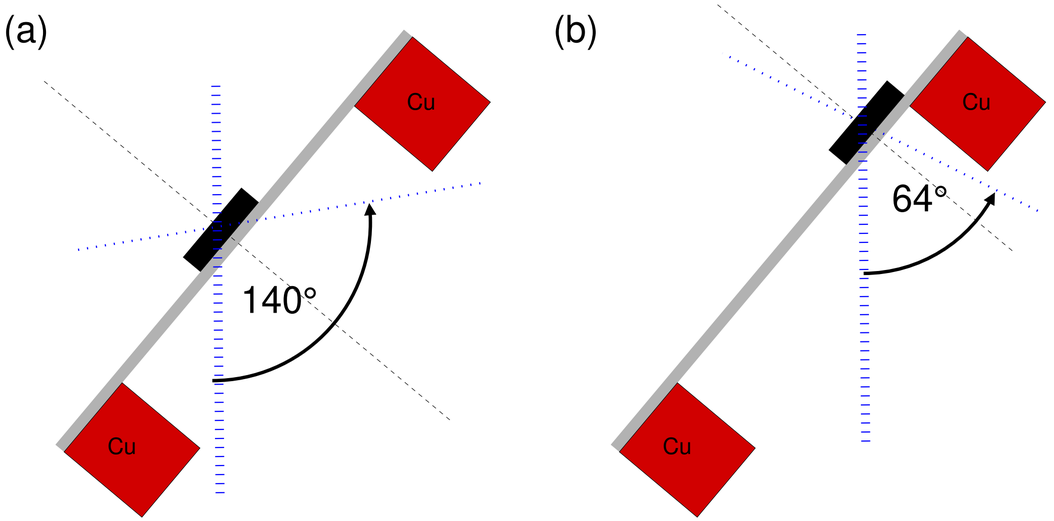
Preferred Crystal Oritentation & the Missing Wedge Problem

(Patent EP 18 202 868)

J. Wennmacher *et al.*, “3D-structured supports create complete data sets for electron crystallography”, under revision

Missing Wedge in Electron Diffraction

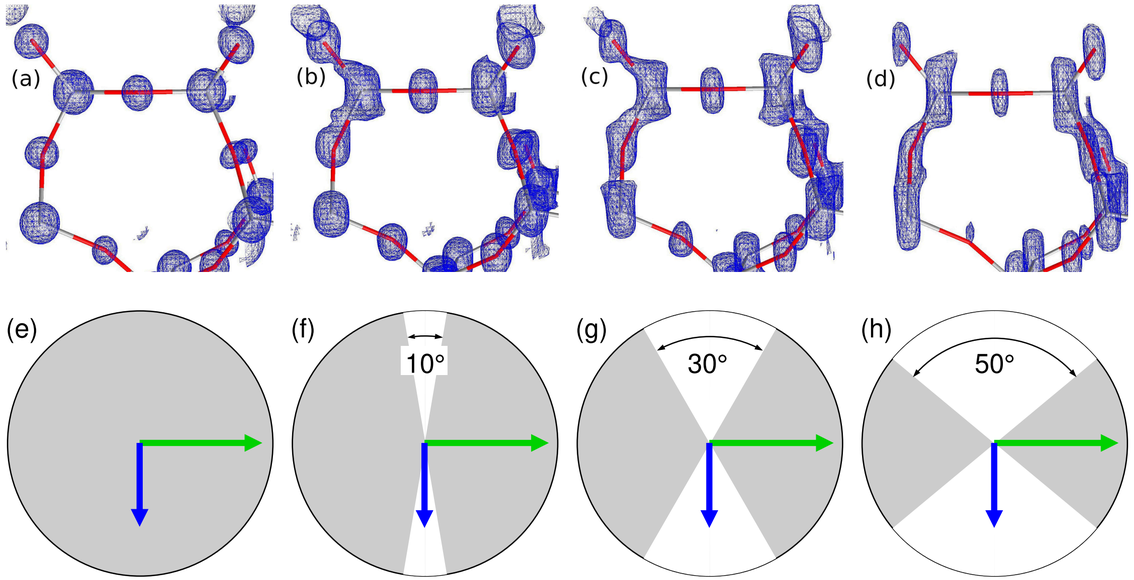




- Crystals very often have a **flat shape**: always the same orientation
- Sample support stabilised by Cu-grid
- Copper grid too thick: intransparent for electrons
- Limited rotation range

Wennmacher *et al.*, “3D-structured supports create complete data sets for electron crystallography” under revision

Effect of Missing Data on Map and Structure

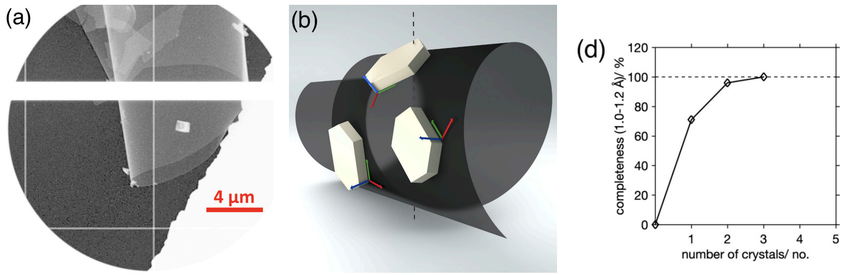


Shearing of experimental map results in unreliable coordinates for structure

Wennmacher *et al.*, “3D-structured supports create complete data sets for electron crystallography” under revision

Complete Data from 3D Structured Grids - Coiled carbon grids

Brush Stroke causes carbon layer to coil

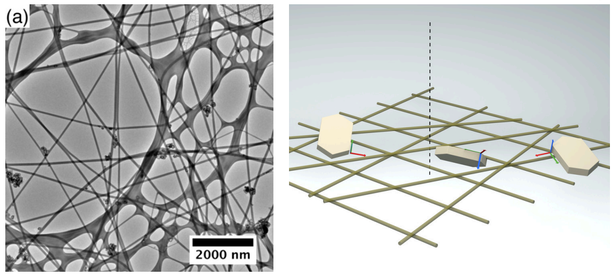


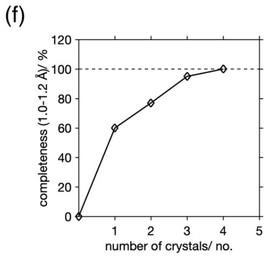
- Visual selection of orientation from carbon curvature
- Complete data from 5’ish crystals

Wennmacher *et al.*, “3D-structured supports create complete data sets for electron crystallography” under revision

Complete Data from 3D Structured Grids - Nylon Fibres

Nylon fibres ($\approx 100nm$ diameter) disturb preferred orientation



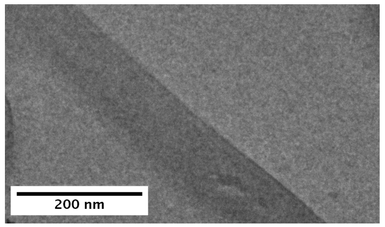


- Orientation less obvious from visual inspection
- possibly more screening required
- Complete data from 5'ish crystals
- Nylon grids adaptable to sample size and shape

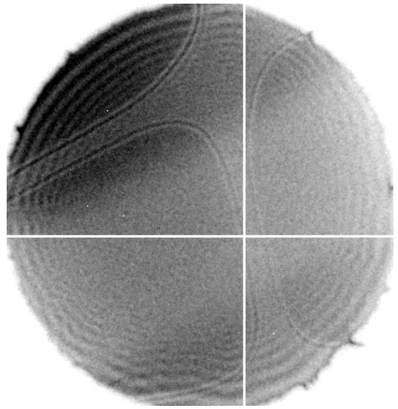
Wennmacher *et al.*, "3D-structured supports create complete data sets for electron crystallography" under revision

Electron Crystallography of Macromolecules

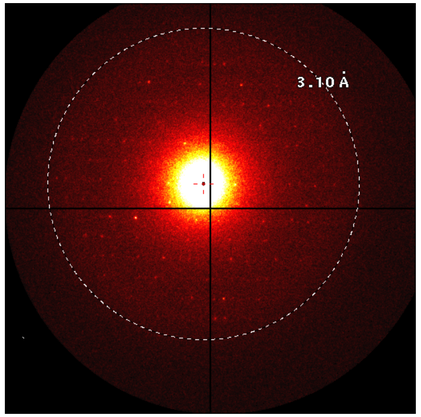
Protein Crystals in the TEM



Lysozyme, ≈ 2.1Å resolution
(Clabbers *et el.* (2017))



Thermolysin:
$\approx 2 \times 1 \times$ very thin μm^3
Solvent reduces contrast
(sample courtesy I. Schlichting)



Thermolysin:
about 3Å resolution
(sample courtesy I. Schlichting)

Comparison of resolution between Electron Diffraction and X-ray diffraction

Some structures from the PDB solved with ED

	e^-		X–ray		resol. ratio
	d_{min}	PDB-ID	d_{min}	PDB-ID	$d(e^-)/d(\text{X-rays})$
Lysozyme	1.80	5K7O	0.94	1IEE	1.9
Lysozyme	2.80	6HU5	0.94	1IEE	1.9
Catalase	3.20	5GKN	1.50	1DGF	2.1
Proteinase K	1.75	5I9S	0.83	2PWA	2.1
Xylanase	2.30	5K7P	0.97	3AKQ	2.3
Thaumatin	2.11	5K7Q	0.90	5X9L	2.3
Trypsin	1.70	5K7R	0.75	4I8H	2.2
Thermolysin	2.50	5K7T	1.12	5JVI	2.2

ED of proteins only reaches half the resolution of X-rays — in contrast to organic and inorganic compounds

High resolution data collection for MX-ED

- X-ray: Test crystals (Thaumatin, Lysozyme, . . .) easily diffract to 1.2–1Å
- Electron: about 2x worse so far
- Idea (I. Schlichting, K. Diederichs, independently): Combine serial crystallography with rotation method
  1. Rotate sample at high dose with short lifetime but maximum resolution, *e.g.* 5° per crystal
  2. Combine data from many crystals for data completness
- Outcome determines whether 3D ED will be useful for Structural Biology

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