Structural Information of ligands with electron diffraction

NYSBC, SEMC and NCCAT MicroED Short Course

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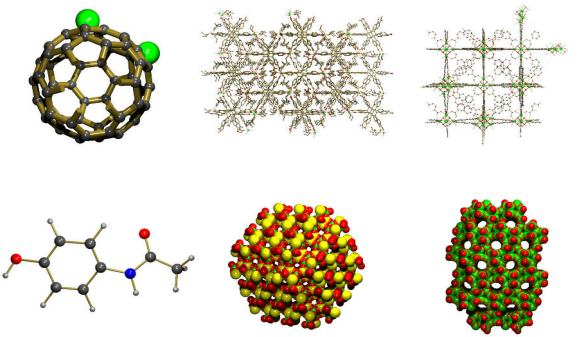
Contents

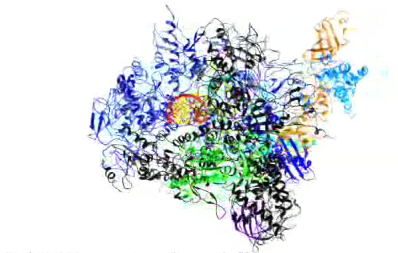
1 Crystal structure

3D models of molecules



- What: position (and ADPs) of atoms in molecules
- Why: to understand chemical and biological properties of compounds, materials, drugs, ...

RNA Polymerase II: Crystal "Snapshots"

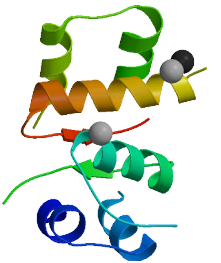


Several structures of RNA Polymerase II in different states of action lead to a concept of the mode of function.

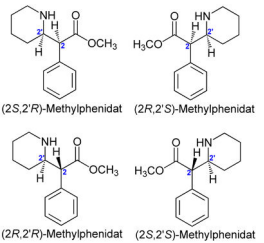
(Brueckner F et al.: "A movie of the RNA polymerase nucleotide addition cycle." Curr Opin Struct Biol 19, 294-299 (2009).) Courtesy P. Cramer, MPI Göttingen

Insulin: Quality Control

- 1982: production of recombinant human insulin (improvement of tolerance compared to bovine insulin)
- recombinant and purified human insulin structurally identical
- structure based point-mutations of insulin improve functionality (*e.g.* rate of release). An extensive list can be found at `https://en.wikipedia.org/wiki/Insulin_analogue`



Small Molecules: Handedness and Purity

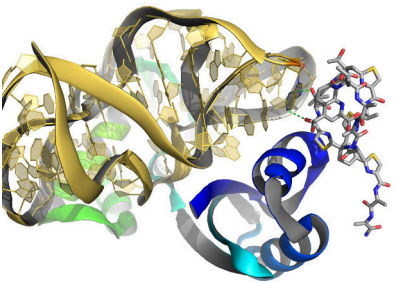


http://de.wikipedia.org/wiki/Methylphenidat

- Methylphenidate (*aka* Ritalin): drug to treat **attention-deficit hyperactivity disorder** (ADHD)
- Contains two stereochemical centres, *i.e.* there are four different forms
- Often only one form has the desired effect, others often contribute to (undesired) side-effects [1]

Crystallography is the only experimental technique to determine absolute chirality.

Structure Guided Drug Design

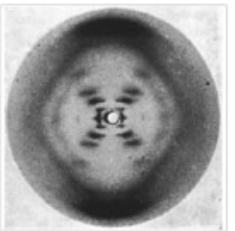


The antibiotic Thiostrepton in contact with its target DNA. Image courtesy Dr. K. Pröpper.

Atomic coordinates for ligand and target enable

- fine-tuning of contact
- fine-tuning of shape: influence mode of function **and** access towards target.

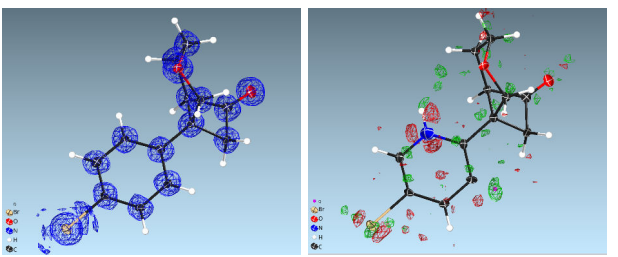
DNA Double Helix



- X-ray image of fibrous, crystalline DNA by R. Franklin, which led her with co-workers and Watson/Crick to the double-helical structure of DNA
- The model is often considered the "birth of modern molecular biology" (Voet & Voet, *Biochemistry* (1995), Wiley & Sons).

The crystallographic map

- A diffraction experiment produces a map within the unit cell of the crystal
- The chemical model is built into the map based on chemical and biological reason
- the difference map indicates discrepancy between the model and the map



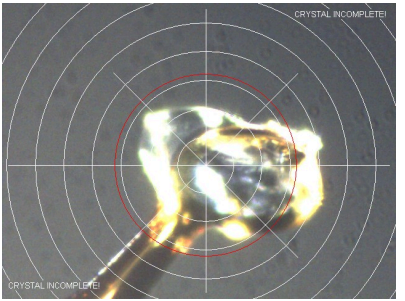
How to get to the structure

Data collection is your last experiment

(Zbigniew Dauter, National Cancer Institute [2])

1. grow crystals: can be the bottleneck
2. collect diffraction data: high availability of synchrotrons and lab sources
3. solving the structure, alias phasing: critical, usually not too difficult
4. model building and refinement: requires biological and crystallographic knowledge
5. validation: very important!!! requires crystallographic knowledge

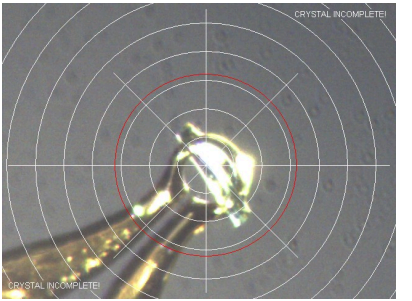
X-ray Crystal gallery



convenient

$250 \times 200 \times 150 \mu m^3$

exposure: $4s/^\circ$

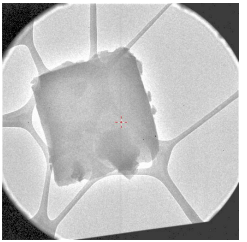


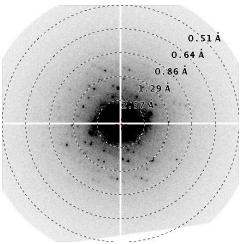
small

$26 \times 40 \times 180 \mu m^3$

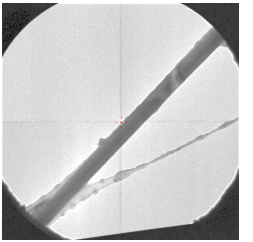
exposure: $32s/^\circ$

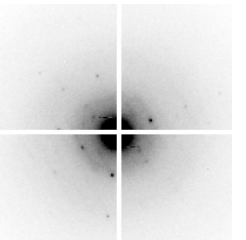
ED Crystal gallery





$1.7 \times 1.7 \times 0.5\mu m^3$
exposure: $2.2s/°$

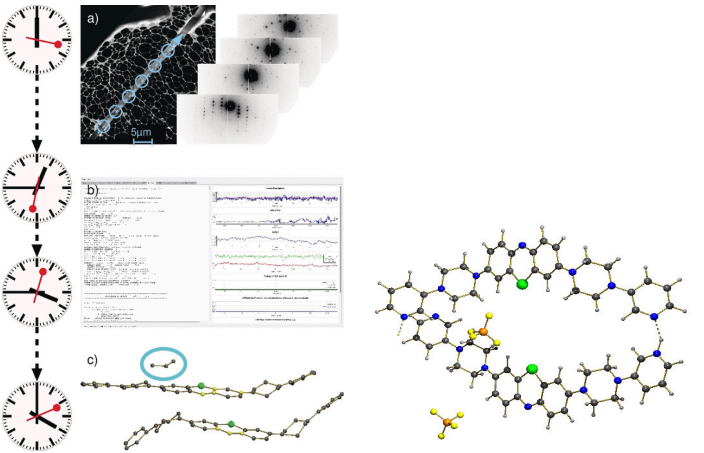




$2.4 \times 0.2 \times 0.2\mu m^3$
exposure: $2.4s/°$

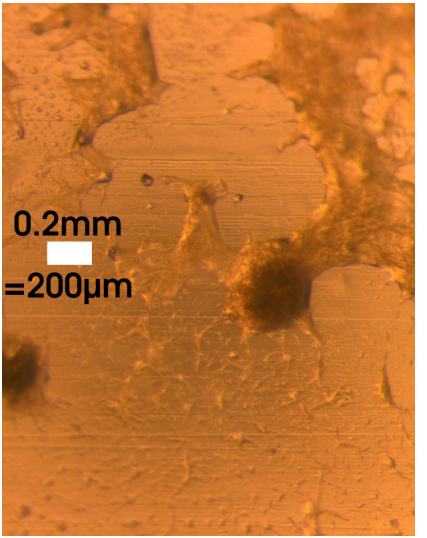
2 Crystallography with electrons instead of X-rays

ED: Stronger than Synchrotron



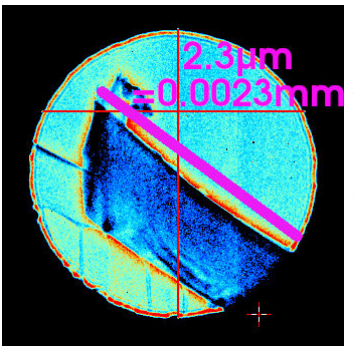
- Structure of a methylene blue derivative (G. Clever, J. H. Holstein, R. Ireni, TU Dortmund)
- Gruene et al (2018), [3]

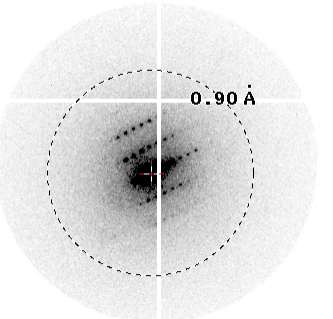
Needles are great for ED



Sample courtesy Jia-Min Chin & Michael Reithofer, photographs courtesy A. Roller

Powerful electron diffraction

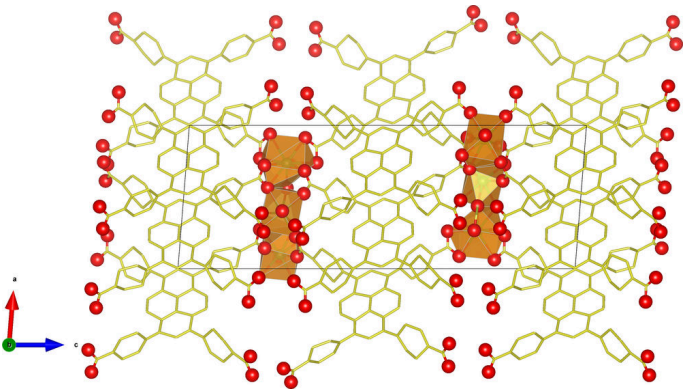




Sample preparation: A. Roller & N. Gajic

At DESY, the strongest X-ray source in the world, this crystal would probably not show any diffraction.

Nd-MOF structure from 5 crystals



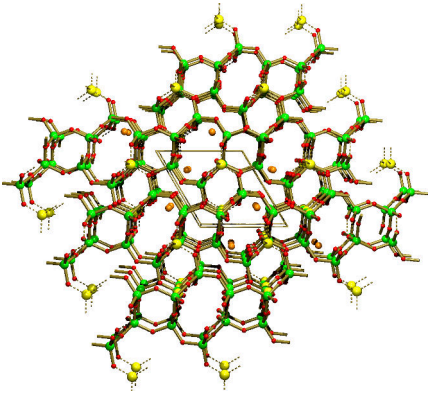
Courtesy Jia Min Chin & Michael Reithofer, unpublished data

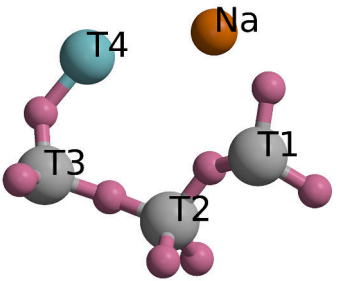
- Room temperature measurement, under vacuum
- R1=33.7 %
- unusual briding of COOH-groups

R1-factor woes

- Crystallographic R1-factor measures fit between model and data
- R1=3%: good, R1=7%: ok'ish, R1=12 %: what's wrong?
- Electron diffraction: R1: 15–25 % ...
- high R-factors in electron diffraction might indicate unreliable structures and suggest that structure interpretation is not very meaningful. But ...:

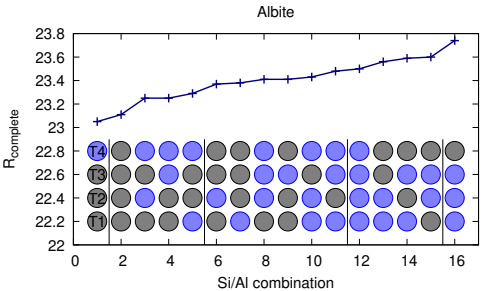
ED tells Aluminum from Silicon

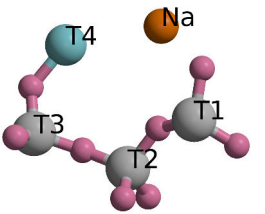




Aluminosilicate Albite contains four T-sites: T1–T3 are Si^{IV}, T4 is Al^{III}, bridged tetrahedrically by oxygen. Na^{+}: counter-ion compensates Al^{III}

ED tells Aluminum from Silicon

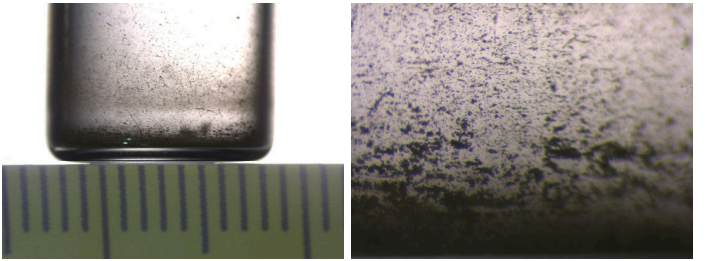




- Each T-site refined as Si or Al: $2^4 = 16$ possibilities
- $R_{complete}$ varies between 23 % and 24 %.
- Unreliable?— No: The $R_{complete}$ increases with number of mis-assigned T-sites [4]

3 Ligand structures with ED

Submicrometer sized crystals

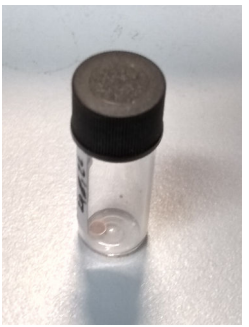


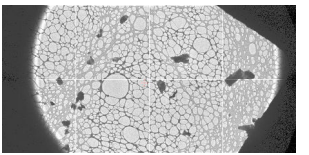
Sample ChWi629, Wittmann et al. [5]

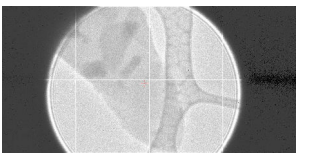
- crystals thickness should be $< 1\ \mu m$
- crystals invisible with light microscope
- crystals should be sufficiently isolated to avoid multiple lattices
- crystals should be sufficiently dense to avoid excessive search duration

Making crystals small

- Often, crystals are still too big for ED.
- Vortex vial with grid



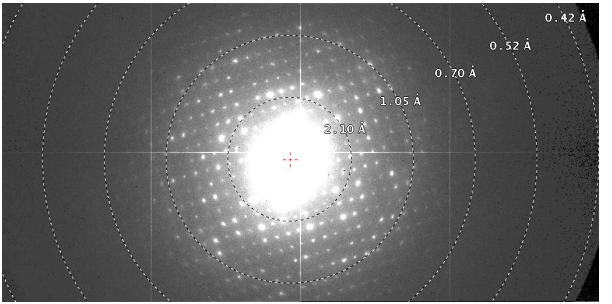




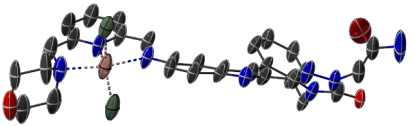
Compatible with dry samples and with suspensions

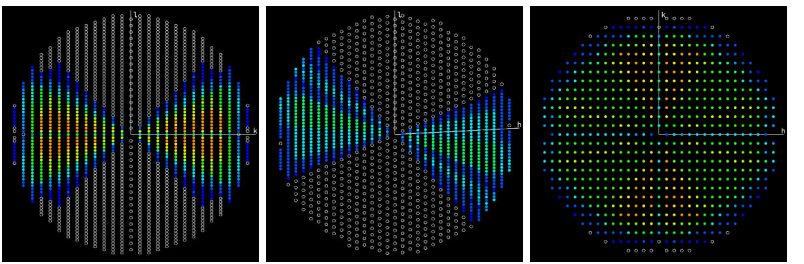
Sonication in “anti-”solvent (water, n-hexane, ...)

Strong diffraction for ChWi629



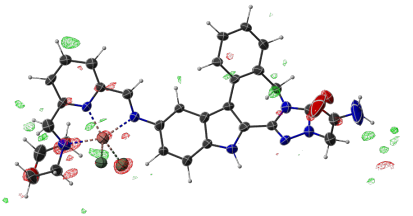
Missing wedge for ChWi629



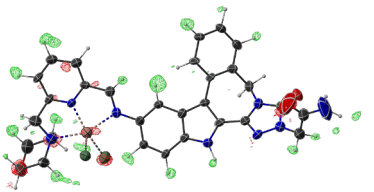


Heat maps of $(0kl)$, $(h0l)$, and $(hk0)$ sections. White: not measured

Details in Structure ChWi629



R1 (all) = 23.91 %
R1 (strong) = 18.91 %
wR2 = 48.23 %



OMIT $H:
R1 (all) = 25.50 %
R1 (strong) = 20.84 %
wR2 = 51.70 %

Summary: Features of Electron diffraction

X-rays	ED
poor interaction with matter, crystal dimensions $\gg 1\mu m$	Crystals can and must be small (thin): $\leq 1\mu m$
light atoms (H, Li) are overwhelmed by the signal of heavy atoms.	H, Li can be detected more easily[6]
Chirality: standard method, but can be tricky	More powerful for determination of absolute chirality [7]
X-rays interact with electrons only: we measure the "electron density map".	differentiation of oxidation states, partial charges [8, 9, 10]

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- Soheil Mahmoudi, Kiyofumi Takaba, Steven van Terwingen, Katarina Stastna, Ronja Schwesig, Alexander Prado-Roller
- Erik Frojdh, Khalil Ferjaoui, Bernd Schmitt, et al. (PSI Detector Group)
- Julian T. C. Wennmacher, Jeroen A. v. Bokhoven, et al. (ETH Zurich)
- and so many more

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