The Howard Flack Crystallographic Lecture Series – 2022

Flack Lectures 2022

Electron Crystallography

7th – 11th November 2022

Dr. Lukáš Palatinus
FZU, Czech Academy of Sciences

07.11.	07.11.	08.11.	09.11.	10.11.	11.11
ETHZ	PSI	Uni Basel	Uni Bern	EPFL	Uni Geneva

Electron crystallography

Lukas Palatinus

Institute of Physics of the CAS, Prague, Czechia

Electron crystallography

General definition: a scientific field that retrieves crystallographic information by using electrons as a radiation probe

In a stricter sense: crystal structure determination predominantly by means of electron diffraction

Information obtainable (in principle) from crystallographic investigation:

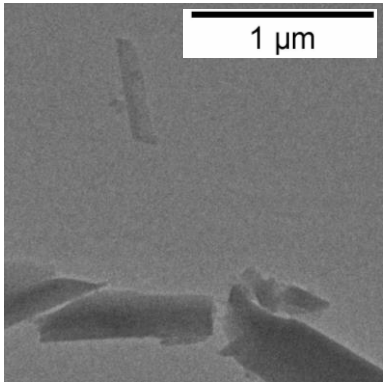
- crystal structure
- chemical composition
- polymorphism
- molecular connectivity
- molecular structure including absolute configuration
- nature of molecular species (salt/co-crystal)
- bonding ...

Electrons interact strongly with atoms

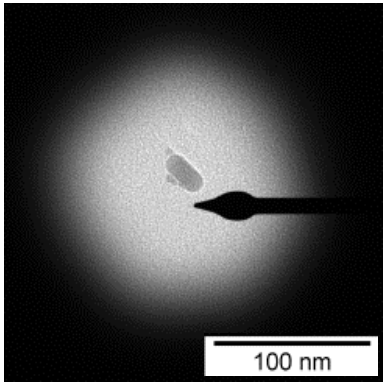
--> possibility to analyze small crystals

--> necessity to deal with multiple scattering

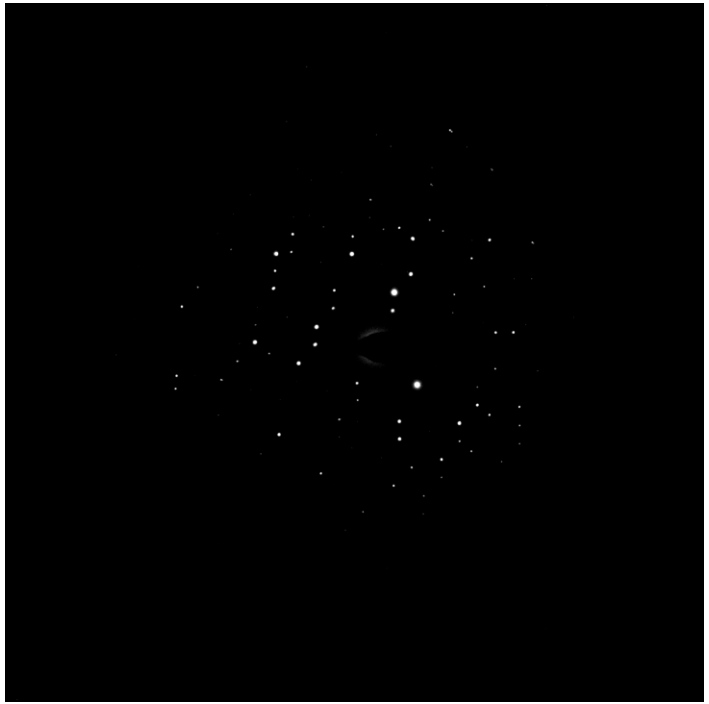
Really small.

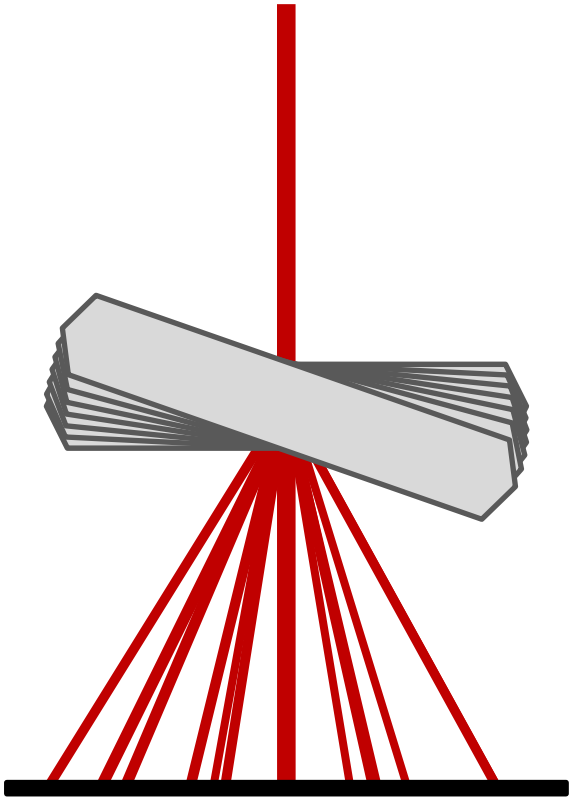


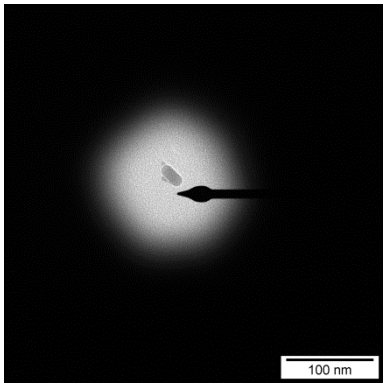
Really really small.

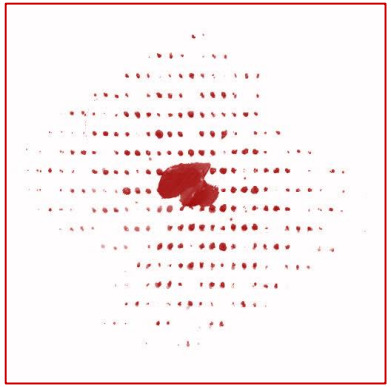


3D electron diffraction (3D ED) – basics









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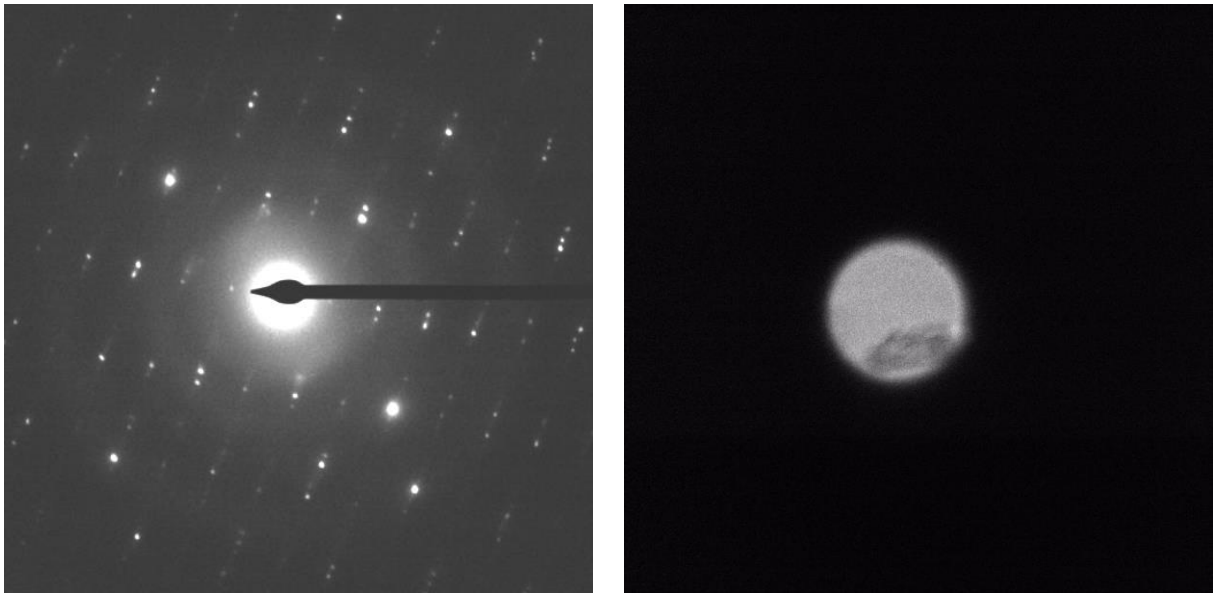

3D ED – 15 years, key milestones

2007: Automated data acquisition of ED data. In this publication, the basics of the method were laid out.
Kolb et al. Ultramicroscopy 107, https://doi.org/10.1016/j.ultramic.2006.10.007

2009: First ab initio structure solution (baryte). Improved intensity integration by using precession electron diffraction. Mugnaioli et al. Ultramicroscopy 109, https://doi.org/10.1016/j.ultramic.2009.01.011

2010: First determination of a previously unknown structure (mineral charoite)
Rozhdestvenskaya et al. Mineral. Mag. 74, https://doi.org/10.1180/minmag.2010.074.1.159

2013: First continuous rotation data collection. First 3D data set of a protein.
Nederlof et al. Acta Cryst. A69, https://doi.org/10.1107/S0907444913009700

2013: First proof-of-principle demonstration of a solution of protein crystal structure. The term MicroED was introduced.
Shi et al. eLife 2, https://doi.org/10.7554/eLife.01345

2015: Dynamical refinement against #3DED data.
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2015: Analysis of charged states of amino acids in protein crystals
Yonekura et al. PNAS 112, https://doi.org/10.1073/pnas.1500724112

2018: Serial ED screening thousands of crystals per hour
Smeets et al. J. Appl. Cryst. 51, https://doi.org/10.1107/S1600576718009500

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3D ED – 15 years, key milestones

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Perspective | Published: 13 July 2021

Establishing electron diffraction in chemical crystallography

Tim Gruene, Julian J. Holstein, Guido H. Clever & Bernhard Keppler

Nature Reviews Chemistry (2021) | Cite this article

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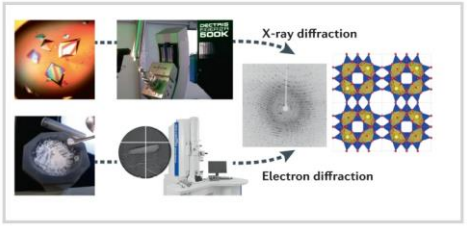
Abstract

The emerging field of 3D electron diffraction (3D ED) opens new opportunities for structure determination from sub-micrometre-sized crystals. Although the foundations of this technology emerged earlier, the past decade has seen developments in cryo-electron microscopy and (X-ray) crystallography that particularly enable the widespread use of 3D ED. This Perspective describes to chemists and chemical crystallographers just how similar electron and X-ray diffraction are and discusses their complementary aspects. We wish to establish 3D ED in the broader chemistry community, such that electron crystallography becomes a common part of the analytical chemistry toolkit. With a suitable instrument at their disposal, every skilled crystallographer can quickly learn to perform structure determinations using 3D ED.



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ACS central science

Outlook

Cite This: ACS Cent. Sci. XXXX, XXX, XXX−XXX

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3D Electron Diffraction: The Nanocrystallography Revolution

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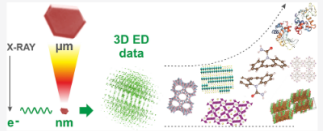
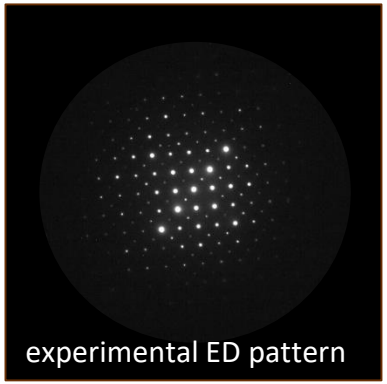
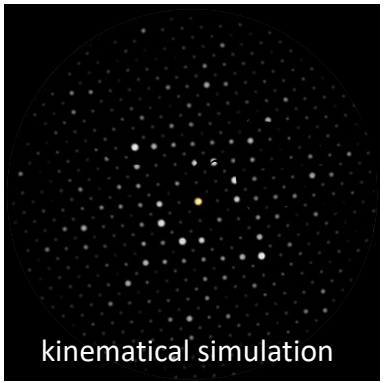
[‡]University of Ulm, Central Facility for Electron Microscopy, Electron Microscopy Group of Materials Science (EMMS), Albert Einstein Allee 11, 89081 Ulm, Germany
[§]Institut für Anorganische Chemie und Analytische Chemie, Johannes Gutenberg-Universität, Duesbergweg 10-14, 55128 Mainz, Germany
[∥]Institut für Angewandte Geowissenschaften, Technische Universität Darmstadt, Schnittspahnstraße 9, 64287 Darmstadt, Germany
[⊥]Department of Structure Analysis, Institute of Physics of the CAS, Na Slovance 2, 182 21 Prague 8, Czechia
[#]CRISMAT, Normandie Université, ENSICAEN, UNICAEN, CNRS UMR 6508, 6 Bd Maréchal Juin, F-14050 Cedex Caen, France
[▽]Inorganic and Structural Chemistry, Department of Materials and Environmental Chemistry, Stockholm University, 106 91 Stockholm, Sweden
[○]Center for Cellular Imaging and NanoAnalytics (C−CINA), Biozentrum, Basel University, Mattenstrasse 26, CH-4058 Basel, Switzerland
[◆]Department of Biology and Chemistry, Paul Scherrer Institut (PSI), CH-5232 Villigen PSI, Switzerland
[¶]Leiden Institute of Biology, Leiden University, Sylviusweg 72, 2333 BE Leiden, The Netherlands

ABSTRACT: Crystallography of nanocrystalline materials has witnessed a true revolution in the past 10 years, thanks to the introduction of protocols for 3D acquisition and analysis of electron diffraction data. This method provides single-crystal data of structure solution and refinement quality, allowing the atomic structure determination of those materials that remained hitherto unknown because of their limited crystallinity. Several experimental protocols exist, which share the common idea of sampling a sequence of diffraction patterns while the crystal is tilted around a noncrystallographic axis, namely, the goniometer axis of the transmission electron microscope sample stage. This Outlook reviews most important 3D electron diffraction applications for different kinds of samples and problematics, related with both materials and life sciences. Structure refinement including dynamical scattering is also briefly discussed.



History of structure analysis by ED = history of fight with multiple scattering


experimental ED pattern


kinematical simulation

Kinematical approximation:

$$I_{\mathbf{h}} \propto |F_{\mathbf{h}}|^2$$

Dynamical theory:

1) Find all reflections that contribute to diffraction

2) Build structure matrix A:

$$a_{ii} = 2KS_{\mathbf{g}_i}, i = 1, N_{beams}$$

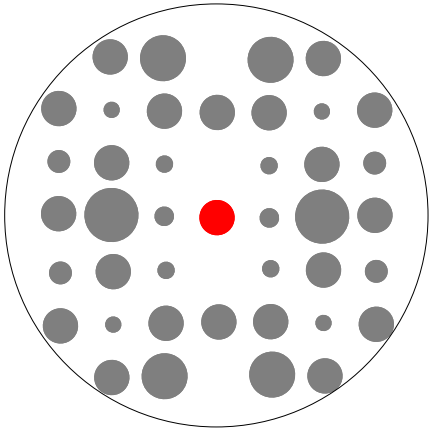
$$a_{ij} = U_{\mathbf{g}_i - \mathbf{g}_j}, i, j = 1, N_{beams}; i \neq j$$

3) Calculate scattering matrix S:

$$\mathbf{S} = \exp\left(\frac{2\pi i t \mathbf{A}}{2K_n}\right)$$

4) Calculate intensities from the first column of **S**:

$$I_{\mathbf{h}_i} = |s_{i1}|^2$$



3D ED structure analysis methods

STRUCTURE SOLUTION

1) Solve structure from 3DED data
2) Use the solution as a final result or refine against other data (typically PXRD)

KINEMATICAL REFINEMENT

1) Solve structure from 3DED data
2) Refine using kinematical approximation, i.e. assuming

$$I_{hkl} \propto F_{hkl}^2$$

+ conceptually simple
+ fast
+ works for all types of 3D ED data
+ available in all refinement programs

- poor approximation
- low accuracy
- high figures of merit

DYNAMICAL REFINEMENT

1) Solve structure from 3DED data
2) Refine using kinematical approximation
3) Refine using dynamical diffraction theory, i.e. accounting for multiple scattering

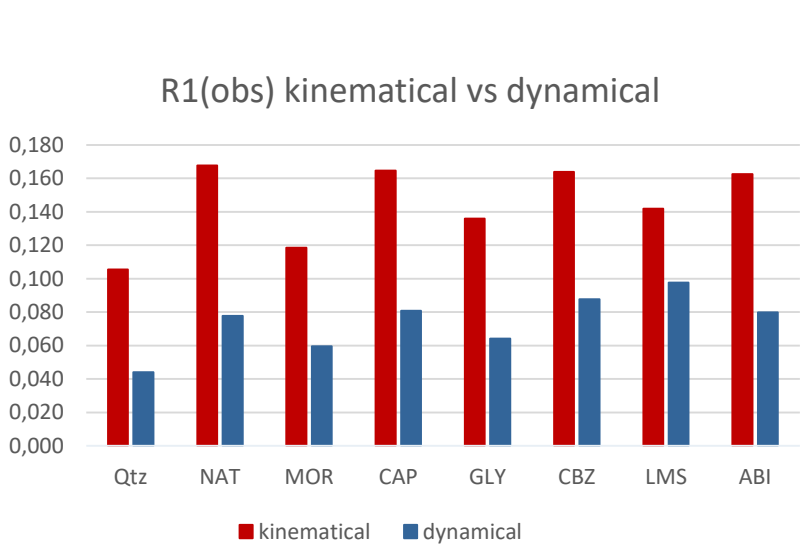
+ more accurate
+ more sensitive to weak signals
+ lower figures of merit

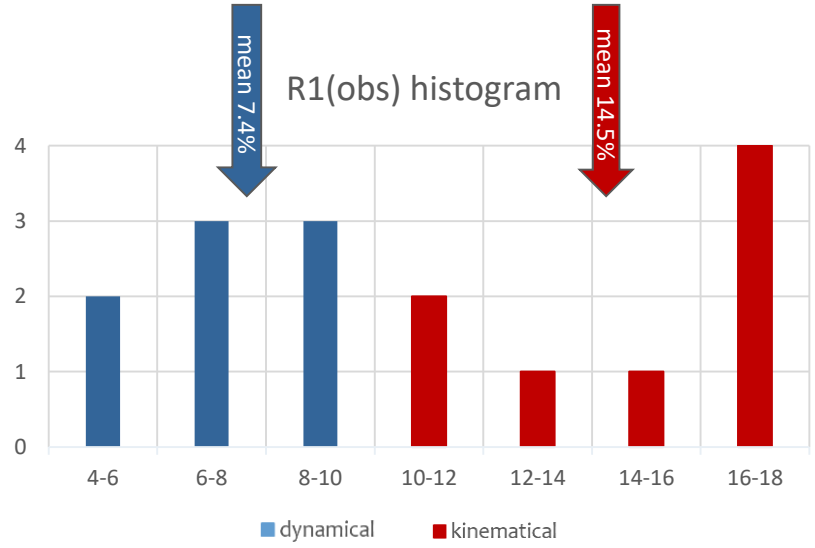
- longer computing time
- requires good data
- works only with PETS2+Jana2020

PETS2

Dynamical refinement – it makes a difference

8 structures carefully refined kinematically and dynamically from the same data



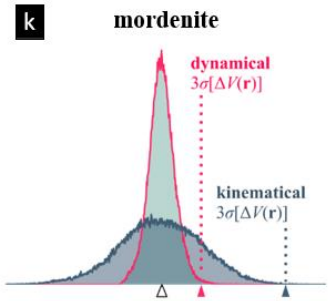


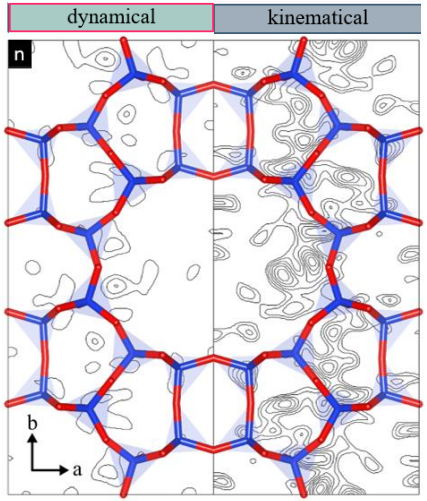
Dynamical: refined against unmerged data, but R-factors calculated on merged data for comparison with the kinematical factors.

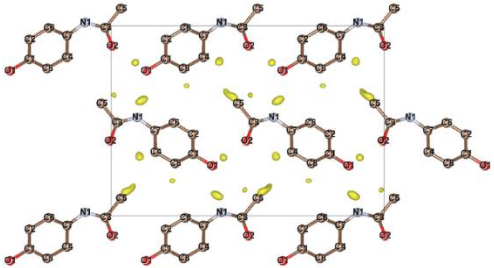
Dynamical refinement – it makes a difference

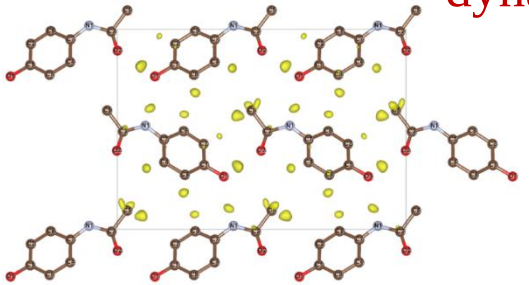
Not only nicer R-values but also:

- ✓ Lower noise in the difference Fourier maps
- ✓ Better sensitivity to weak features like hydrogens
- ✓ More accurate atomic positions
- ✓ More reliable e.s.d.s



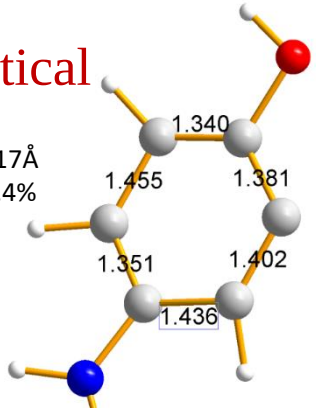






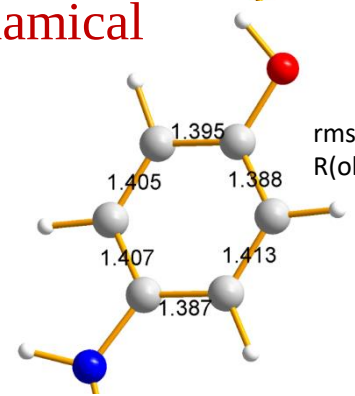
kinematical

rmsd 0.0417Å
R(obs)=19.4%



dynamical

rmsd 0.0098Å
R(obs)= 9.13%



History of structure analysis of molecular crystals by ED = history of fight with multiple scattering AND radiation damage

Electrons are less damaging than x-rays per single elastic event.

(J. P. Abrahams: 1 x-ray photon causes the same damage as 1000 electrons in an organic material)

However,

the probed volume is much smaller in electron diffraction.

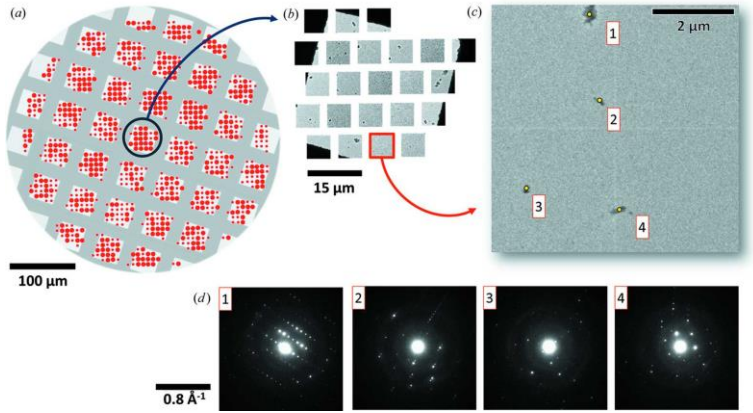
Therefore,

radiation damage is a much bigger issue for electron crystallography!

What is "beam sensitive"? Indicative limiting doses for the loss of crystallinity	
zeolites	>100 e/Å^2
MOFs	5-15 e/Å^2
protein crystals	1-10 e/Å^2
crystals of small organic molecules with hydrogen bonds	0.5-10 e/Å^2
crystals of small organic aliphatic molecules with van der Waals bonds only	0.01-0.5 e/Å^2

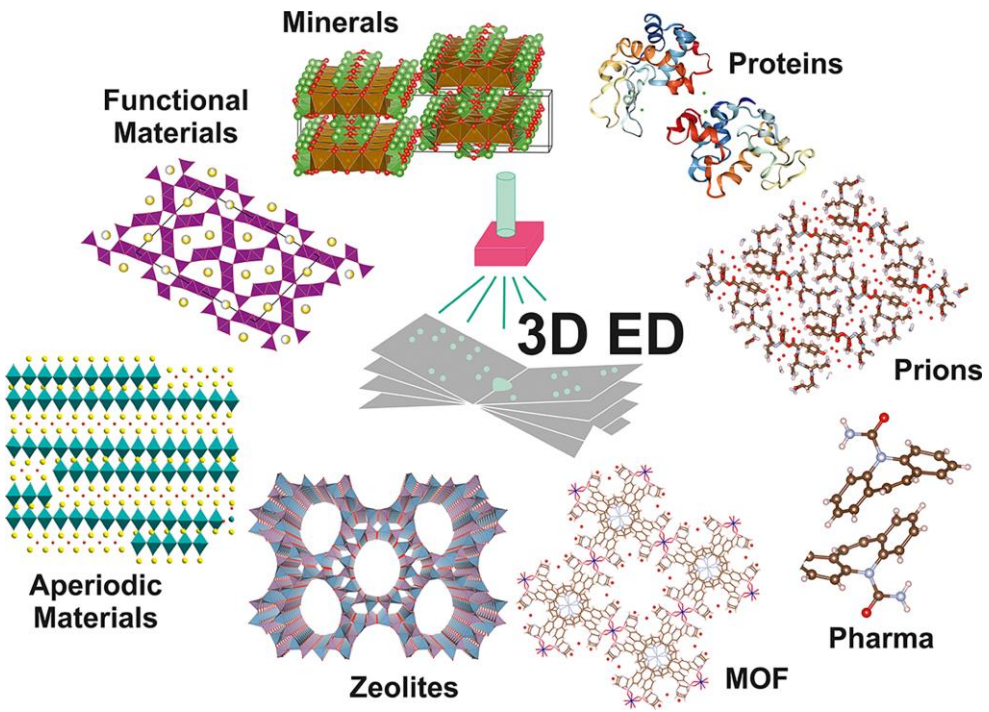
SOLUTIONS:

1) **Use fast data collection with the modern sensitive direct detection cameras**
2) **Collect quickly with continuous rotation**
3) **Collect data on different parts of a large(r) crystal or use serial electron crystallography**



Latychevskaia & Abrahams 2019, Acta Cryst. B 75; Nederlof et al. (2013). Acta Cryst. D 69, Smeets et al. (2018) J. Appl. Cryst. 51, 2018, Bücker et al. (2020), Nat. Comm., 11

Structure analysis from 3D ED – examples

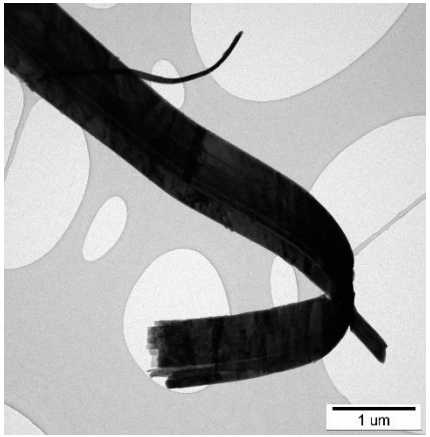


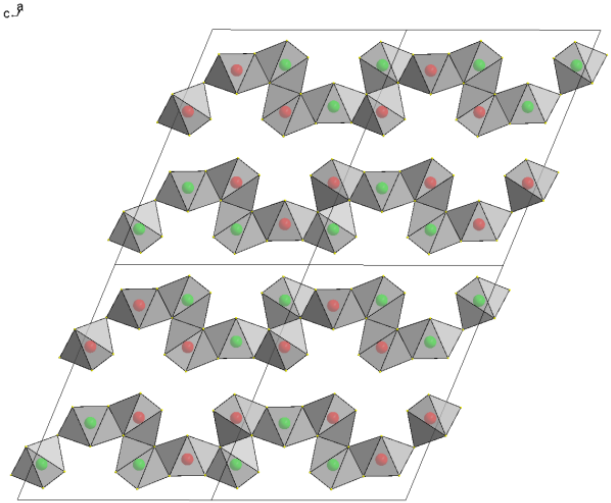
TaS_3

TaS_3 is a thoroughly studied material due to its 1D character with a charge density wave forming at low temperature.

Two polymorphs:

- less common $P2_1/m$
  RT structure known
- more common supposedly $C222_1$
  RT structure unknown





C2/m a=19.72 b=3.42 c=15.58 β=112.75

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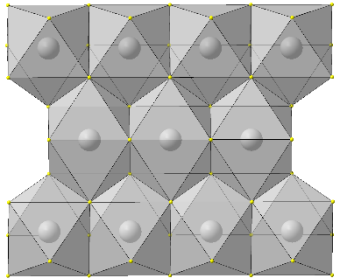
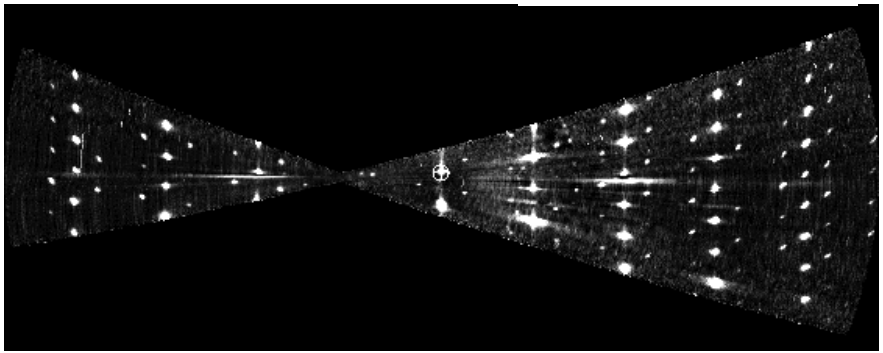
A charge density-wave transformation at 210K.
q=(0.2 0.25 0.125)

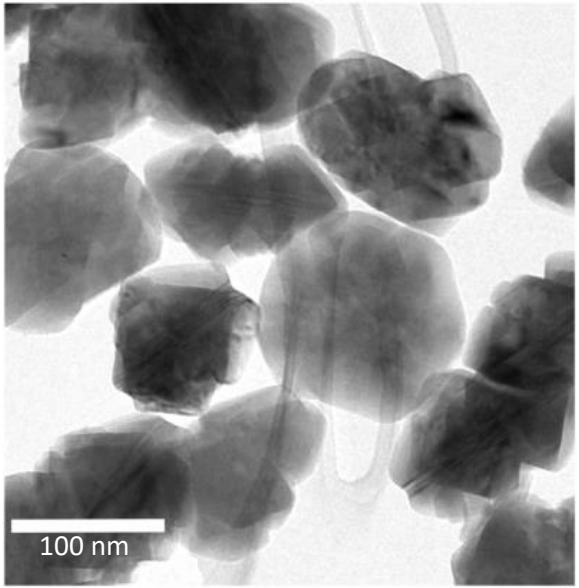




CO_2-loaded chabazite

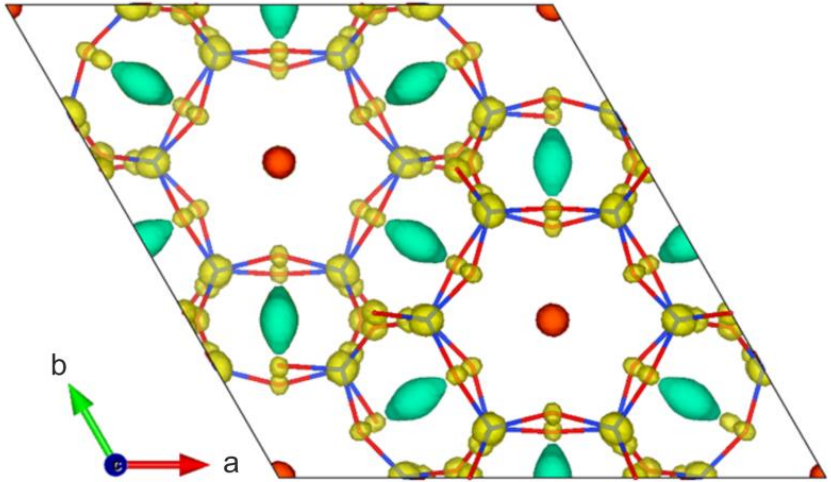
Synthesis of an industrially important zeolite in nanocrystalline form without OSDA.

Extra-framework cations: Na+, K+, Cs+.

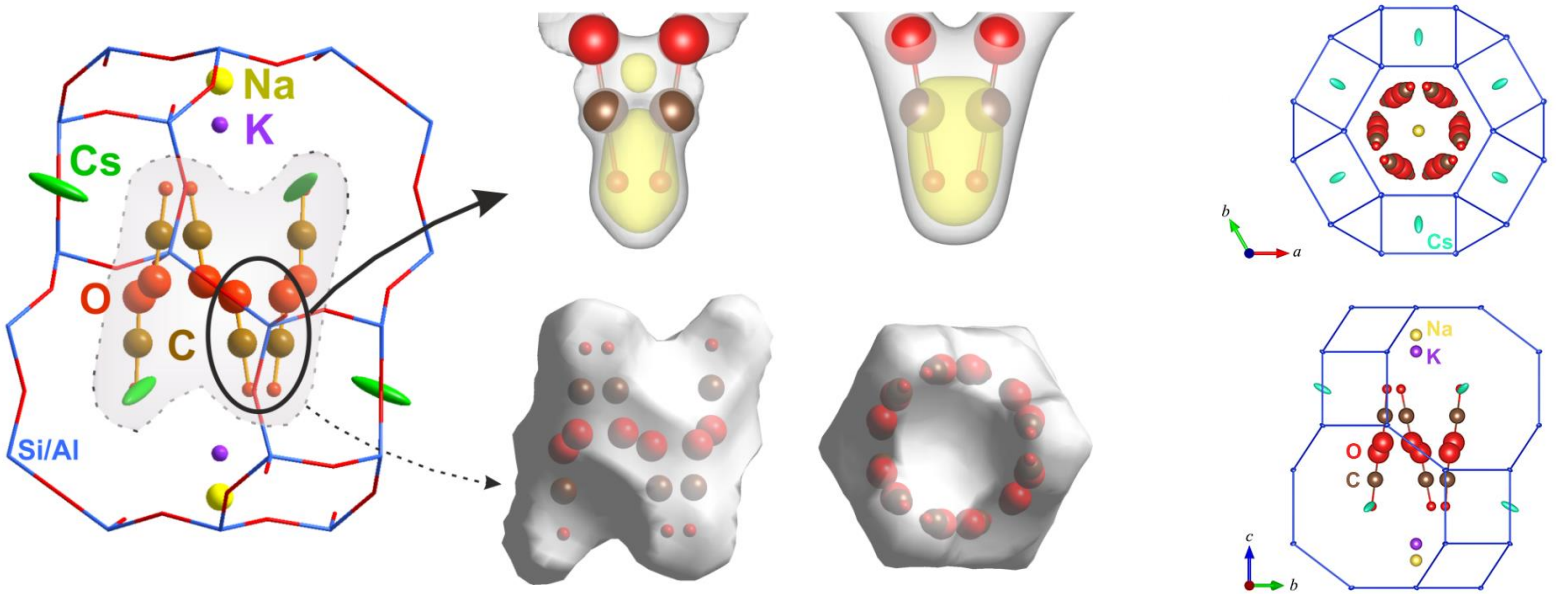


Chabazite has a very good CO_2 adsorption and selectivity towards CH_4.

Crystallographic question:
Can we locate the CO_2 molecules in the chabazite structure?



CO_2-loaded chabazite

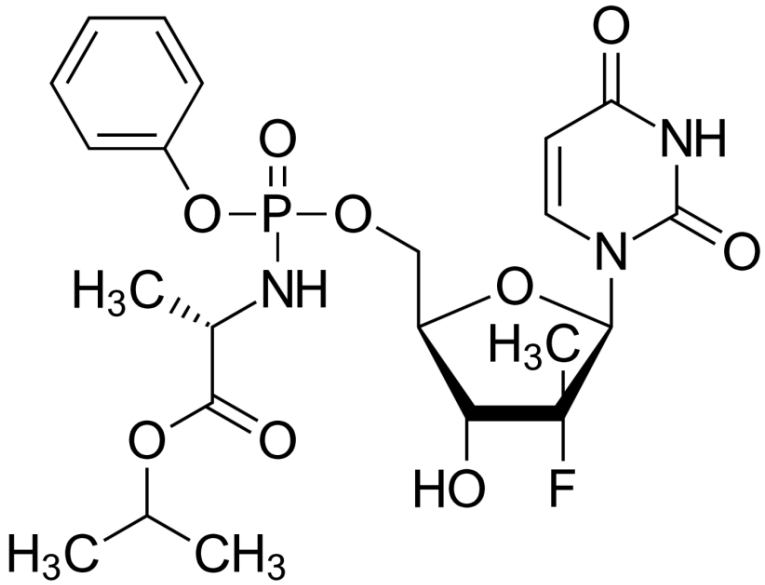


Theoretical maximum adsorption capacity: 9 CO_2 molecules per unit cell
Experimental adsorption capacity: 8 CO_2 molecules per unit cell

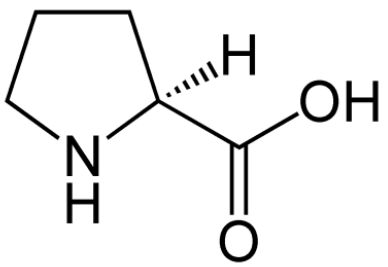
Cationic composition is crucial for the capacity and selectivity of CO_2 adsorption

Sofosbuvir L-prolin

a cocrystal of L-prolin and an anti-hepatitis drug (both chiral)



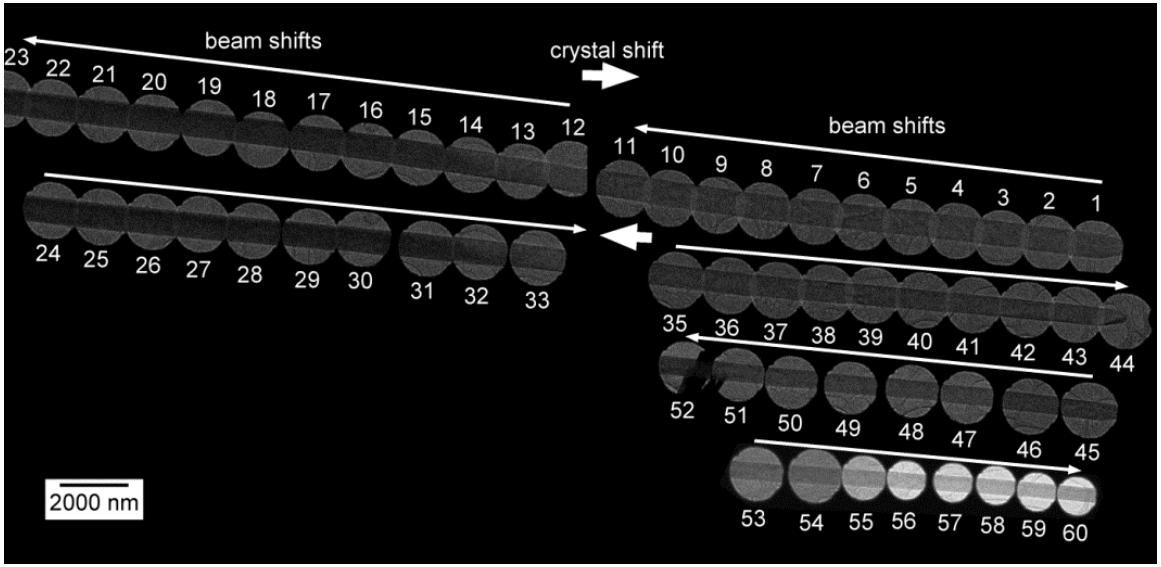
Sofosbuvir – antivirotics

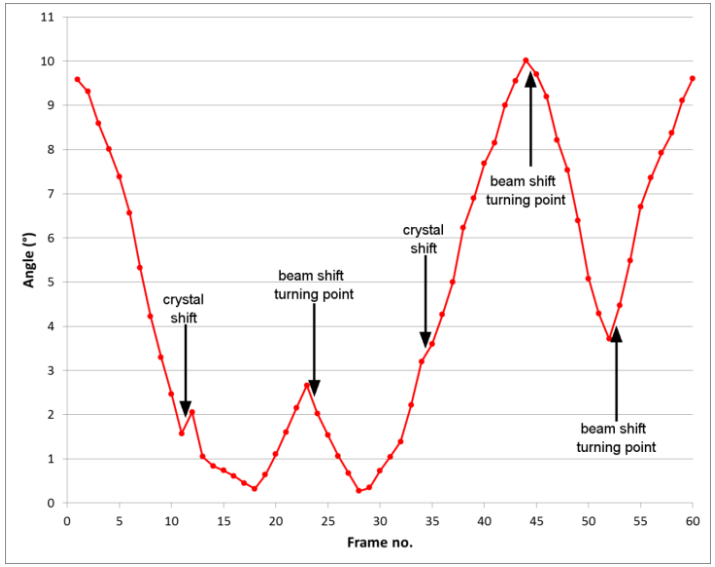


L-proline – amino acid

Sofosbuvir L-prolin

A cocrystal of L-prolin and an anti-hepatitis drug.
Extremely beam-sensitive, most crystals deteriorate after <0.08 e/Å^2.
Crystals form long ribbons.

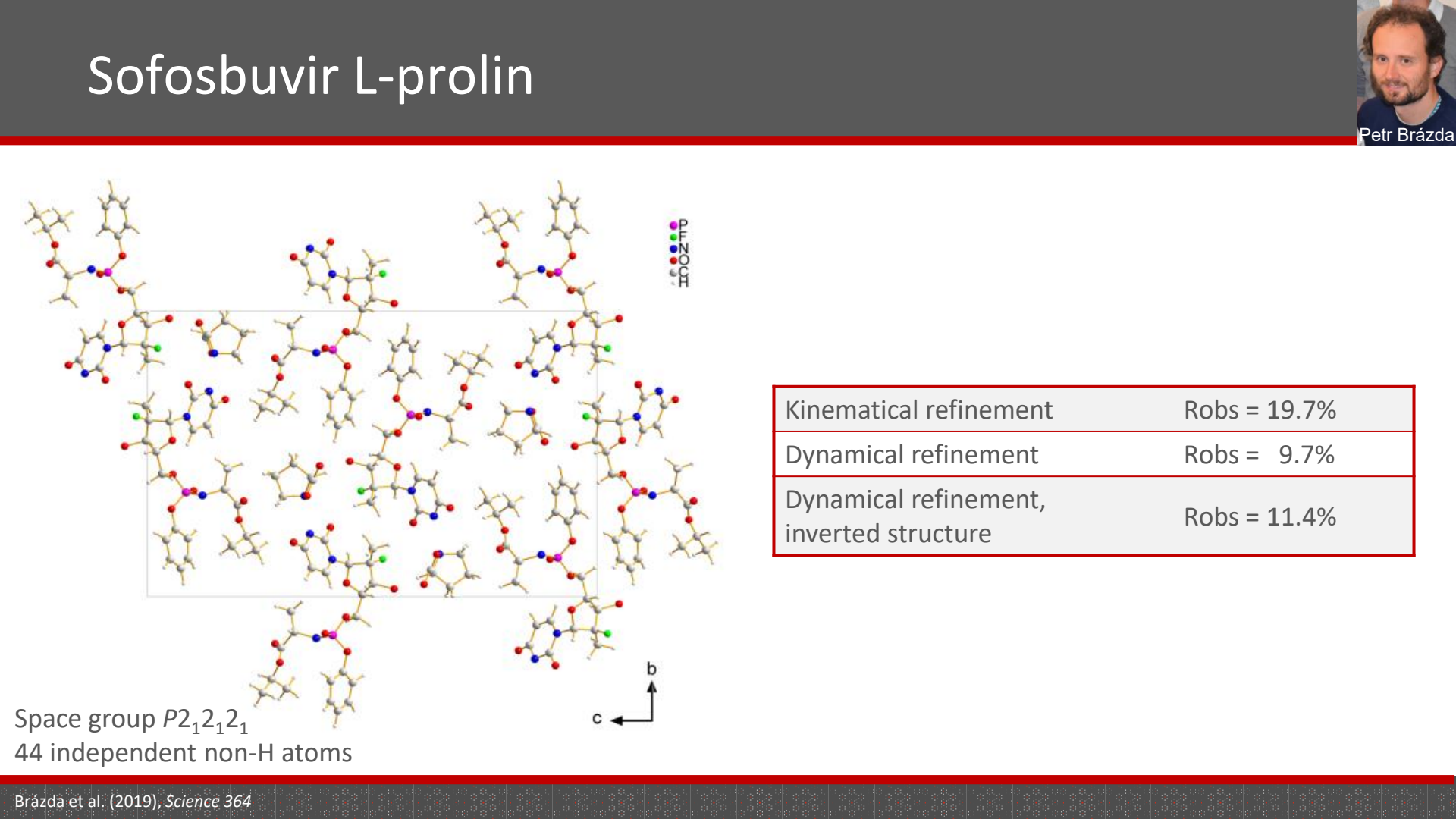




Sofosbuvir L-prolin

A cocrystal of L-prolin and an anti-hepatitis drug.
Extremely beam-sensitive, most crystals deteriorate after <0.08 e/Å^2.
Crystals form long ribbons.

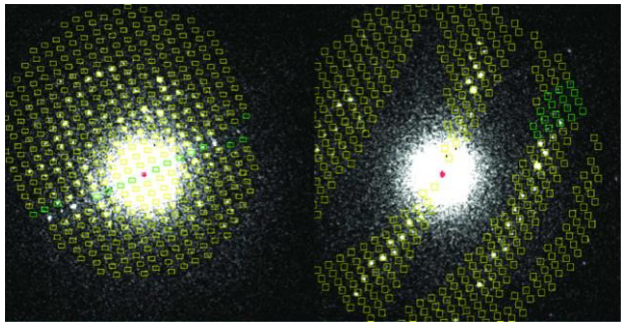
Sofosbuvir L-prolin



Space group $P2_12_12_1$
44 independent non-H atoms

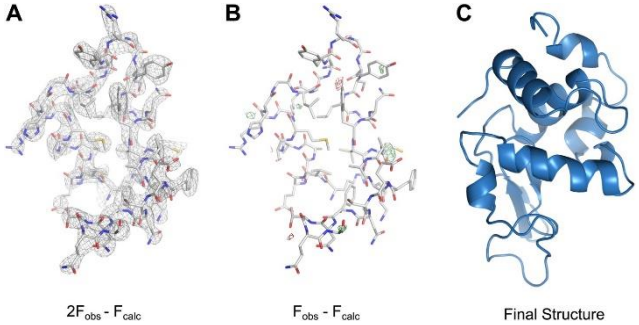
Kinematical refinement	Robs = 19.7%
Dynamical refinement	Robs = 9.7%
Dynamical refinement, inverted structure	Robs = 11.4%

Macromolecular 3DED/MicroED



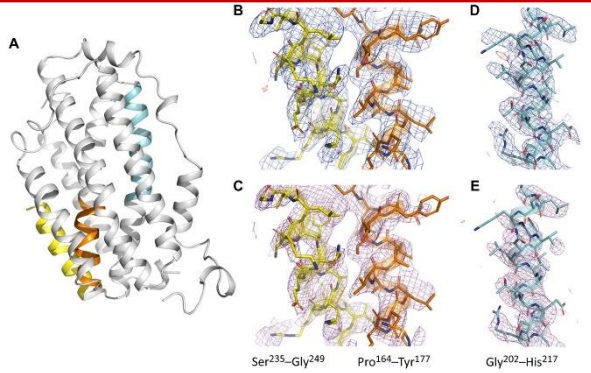
Nederlof et al. (2013), Acta Cryst D, 69

Continuous rotation, only experiment



Shi et al. (2013), eLife 2

First solution (molecular replacement)



Xu et al. (2019), Science Advances 5

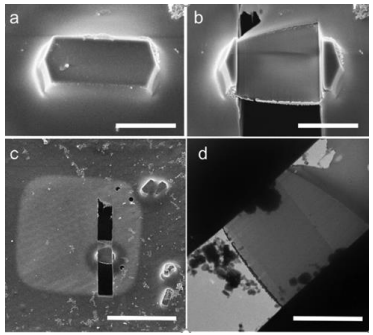
First unknown protein structure (R2-like ligand-binding oxidase, SaR2lox)

38kDa, ~350 residues, resolution 3Å

Duyvestein et al. (2018)

PNAS 115

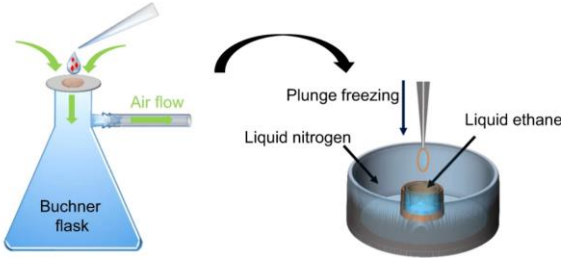
CryoFIB milling



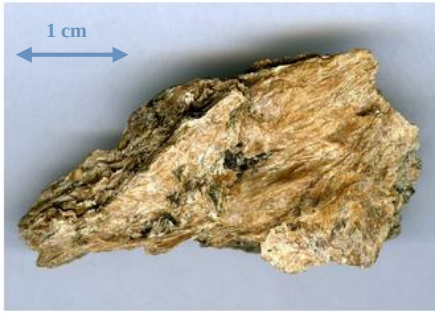
Zhao et al. (2021)

Nat. Comm. 12

Pressure-assisted blotting

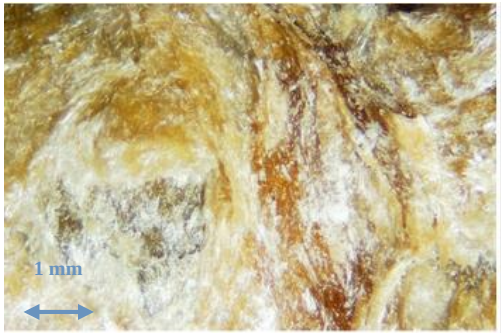


Eveslogite



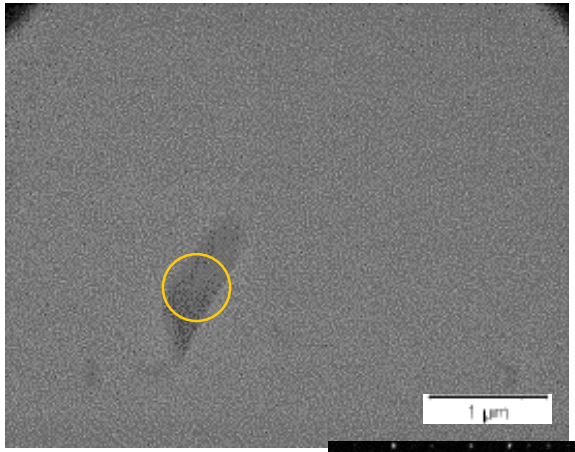
Eveslogite, etc.

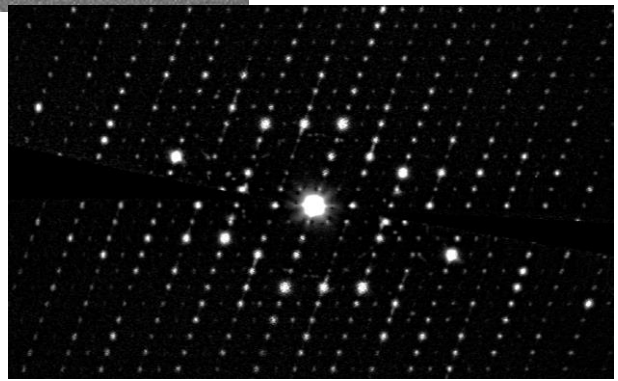
Fersman Gorge, Eveslogchorr Mt, Khibiny Massif, Murmansk Oblast, Russia



Eveslogite

Eveslogchorr Mt, Khibiny Massif, Murmansk Oblast, Russia





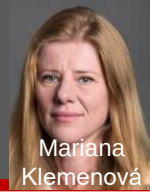
Chemical Properties of Eveslogite

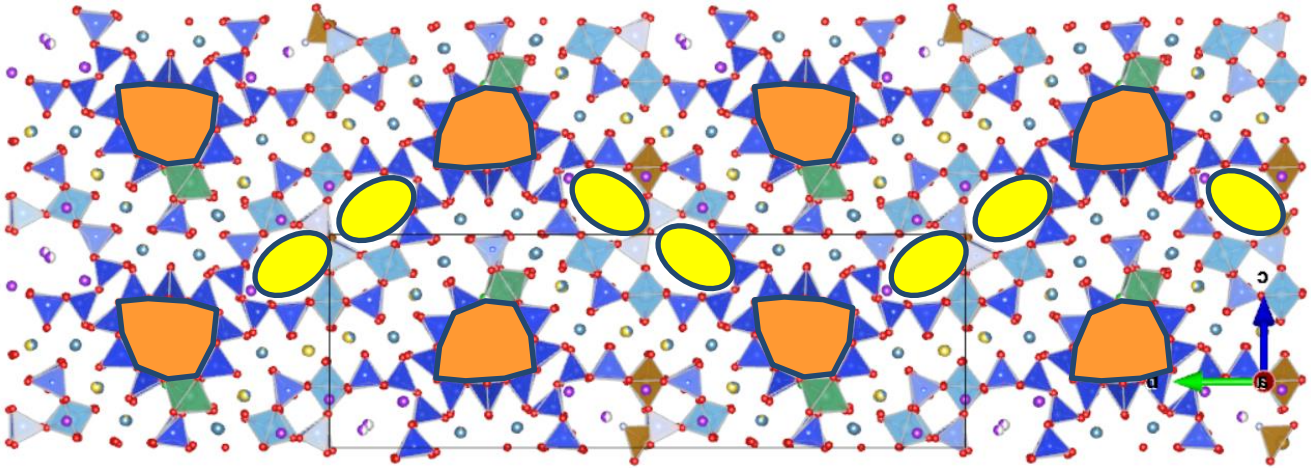
Formula:	$(Na,K,Ca,Sr,Ba)_{48}[(Ti,Nb,Mn,Fe^{2+})_{12}Si_{48}O_{144}(OH)_{12}](F,OH,Cl)_{14}$
IMA Formula:	$(Ca,K,Na,Sr,Ba)_{48}(Ti,Nb,Fe,Mn)_{12}(OH)_{12}Si_{48}O_{144}(OH,F,Cl)_{14}$
Elements listed:	Ba, Ca, Cl, F, Fe, H, K, Mn, Na, Nb, O, Si, Sr, Ti - search for minerals with similar chemistry

mindat.org

E. Götz, U. Kolb (TU Darmstadt), S.V. Krivovichev (University of Saint Petersburg), Marco Schowalter (University of Brehmen)

Eveslogite





space group	$P2_1$
a	14.1898
b	44.7704
c	15.9111
b	109.4677
volume	9530.171

~ 360 atoms in the asymmetric unit

115 842 reflections R_{obs} = 20.10

R_{int} = 17.24 R_{all} = 24.97

Na	
K	
Ca	
Nb	
Ti	
Si	

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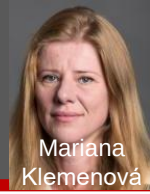
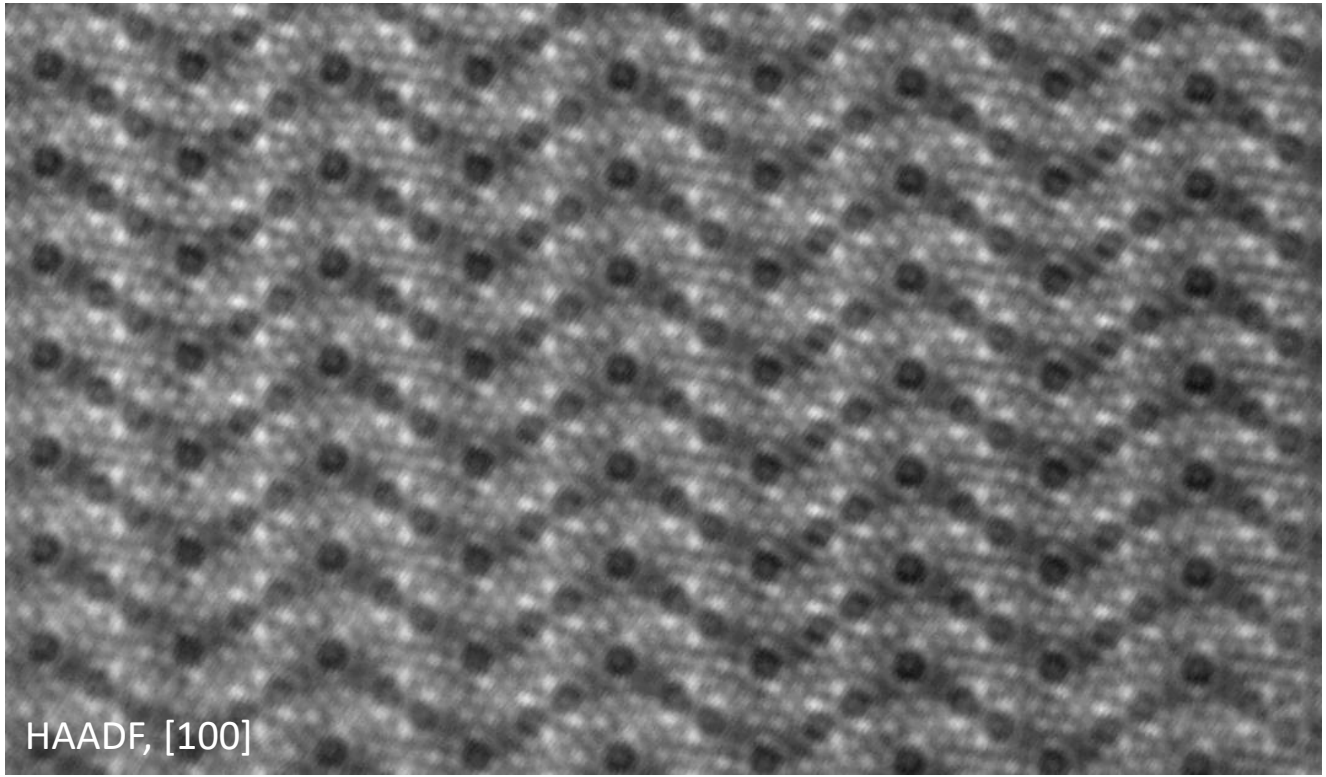
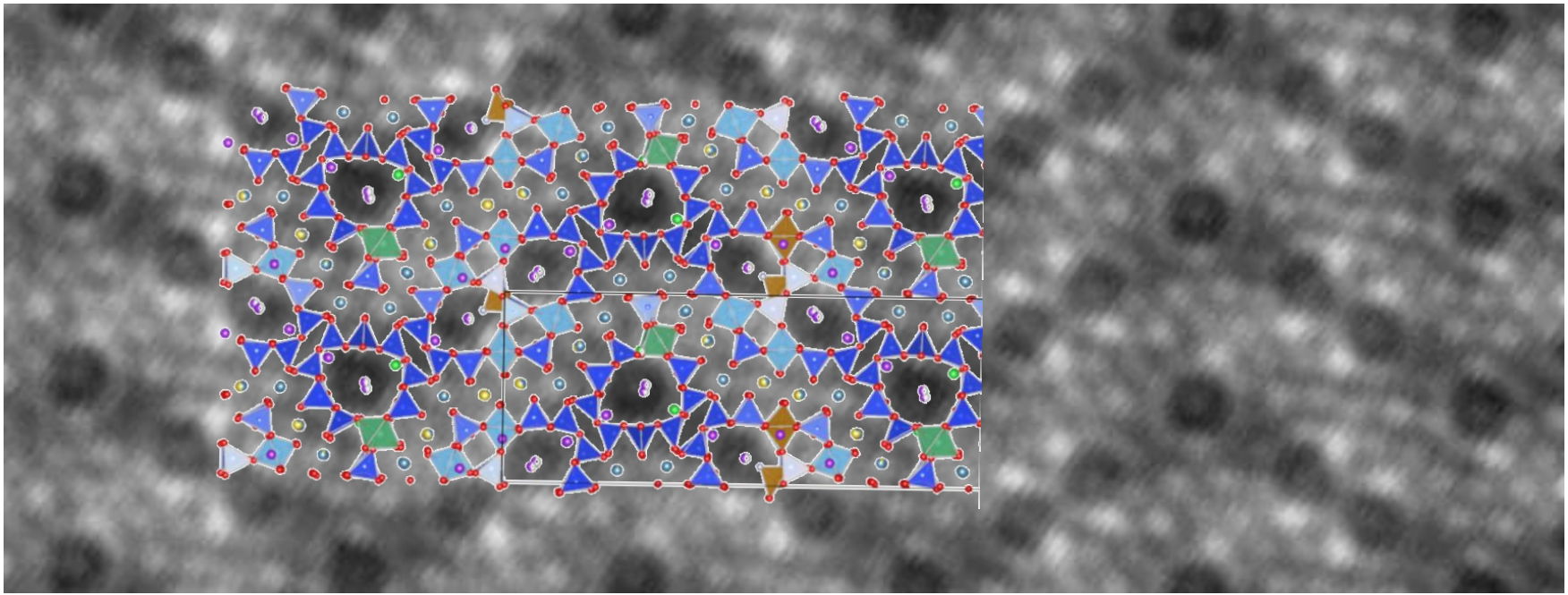
Eveslogite





Eveslogite



Mariana Klemenová

The story of the hydrogen atom

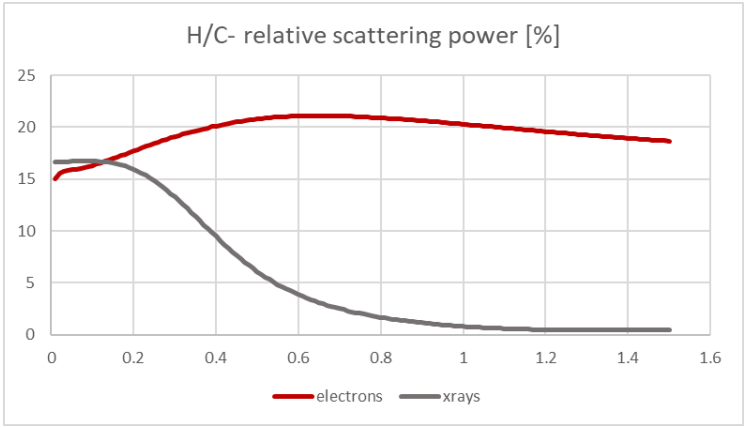
Hydrogens scatter relatively more in electrons than in x-rays.

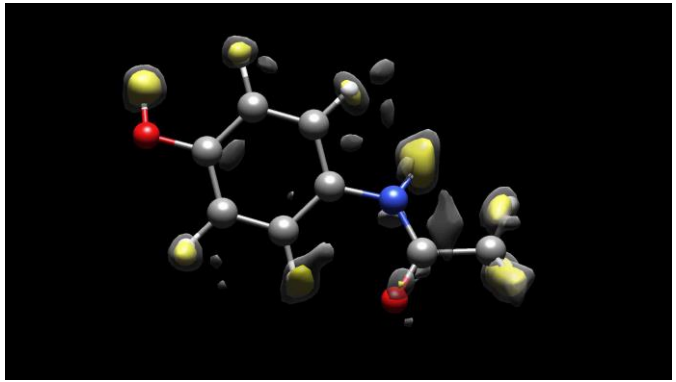
Despite of that for a long time difficult to see.

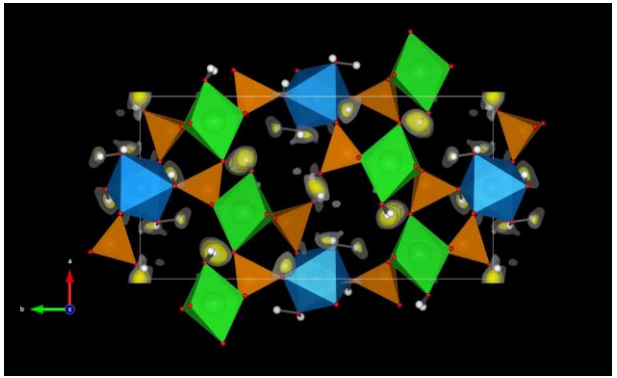
Scattered mentions in literature before 2017 (Vainshtein et al., Palatinus et al., Rodriguez et al.)

The possibility was demonstrated and analyzed in detail in 2017 by Palatinus et al. (2017)

Now almost routine, although not always guaranteed.







Vainshtein et al. in Electron Diffraction Techniques, vol. 1, J. M. Cowley, Ed. (1992); Palatinus et al. (2015), Acta Cryst. B; Rodriguez et al. (2015), Nature 525; Palatinus et al. (2017), Science 355

The story of the hydrogen atom

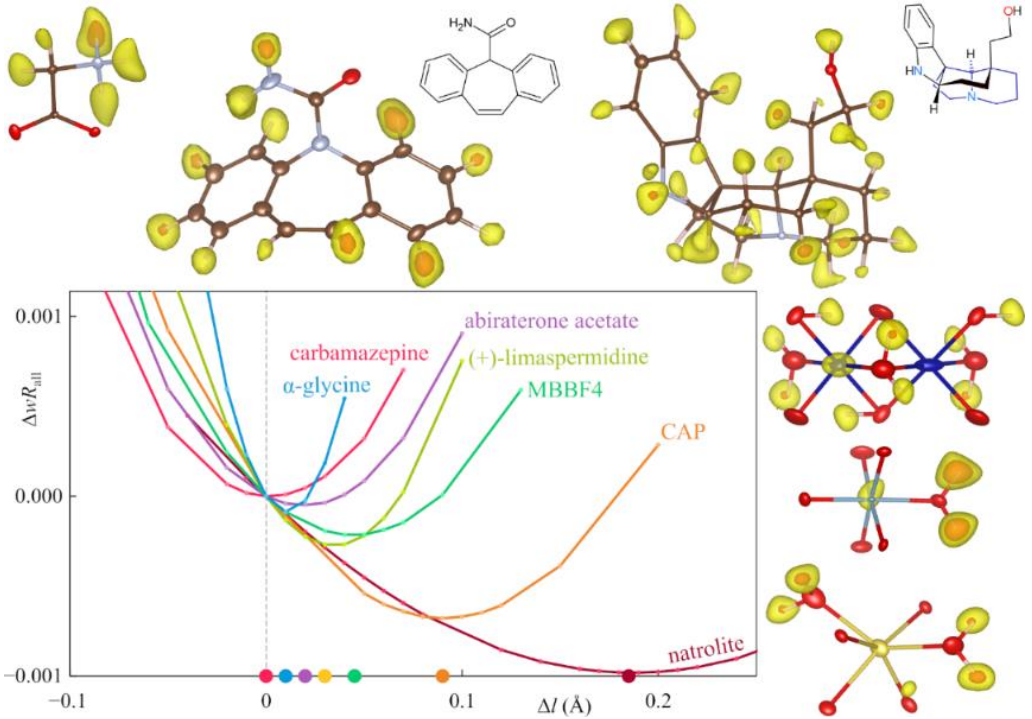
Together with observation of hydrogens, observations also pointed to longer C-H, O-H and N-H distances.

Probably the first to notice was in the paper by Palatinus et al. (2017) (distances on average longer by 0.1Å!). However, we were not confident enough to claim it as an observation and discuss it.

Later refinements clearly showed this as a trend. Confirmed also by theoretical calculations (e.g. Gruza et al.)

Observed also in SPA (Nakane et al. 2020)

We analyzed carefully C-H and O-H distances in several compounds. The results confirm the trend, but do not agree quantitatively with the theoretical predictions made by Nakane et al.



Palatinus et al (2017), Science 355; Gruza et al. (2020), Acta Cryst. A 76, Nakane et al. (2020) Nature 587, Klar et al. (2022), Nat. Chem., accepted

cocrystal empagliflozin/L-prolin

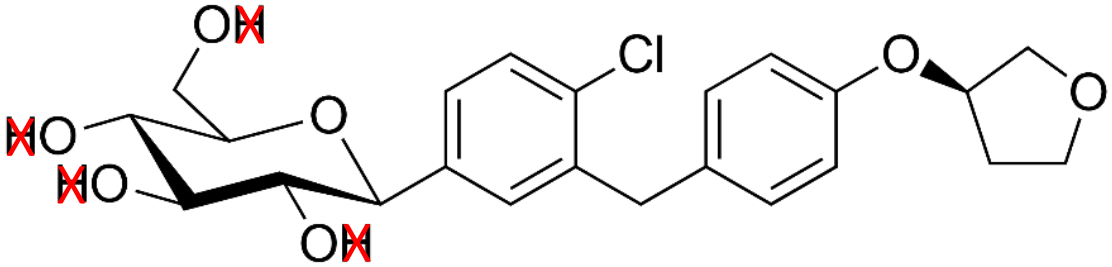
Monoclinic $P2_1$

Unit cell volume 1350 Å^3

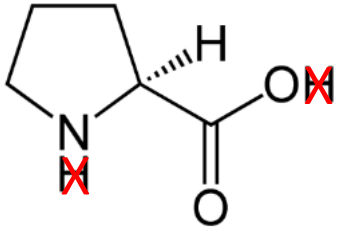
38 independent non-H atoms

Solved *ab initio* (Sir2014), refined in Jana2006/Dyngo

Data from 13 crystals (332 frames, completeness 71%)



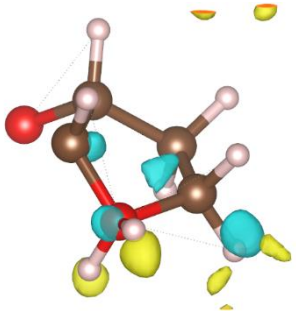
Empagliflozin
Treatment of diabetes type II

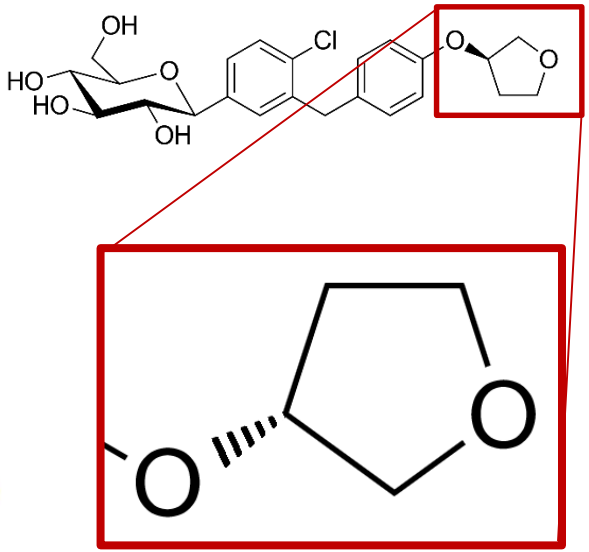


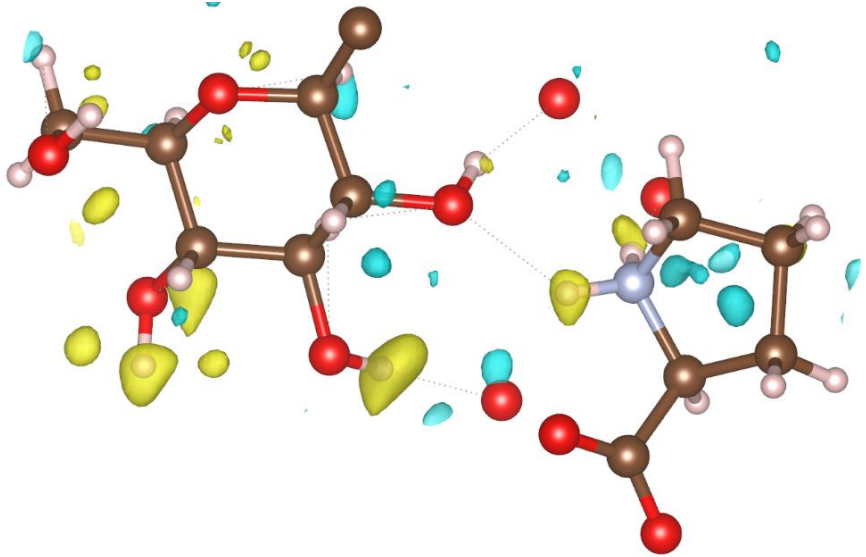
L-proline
Amino acid

cocrystal empagliflozin/L-prolin

Localization of hydrogens







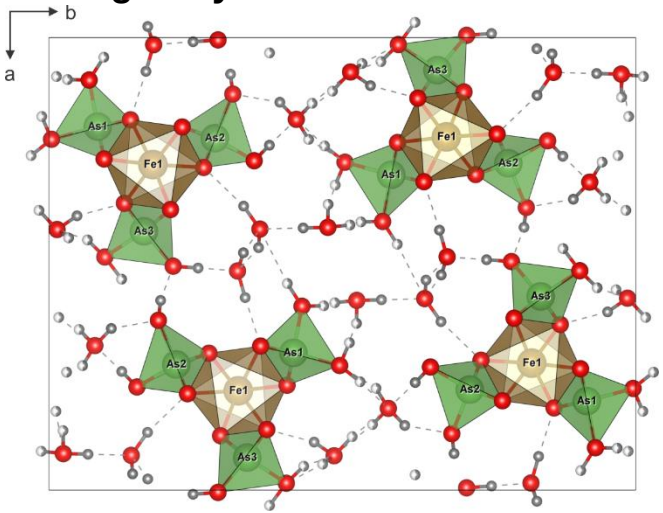
Dynamical refinement

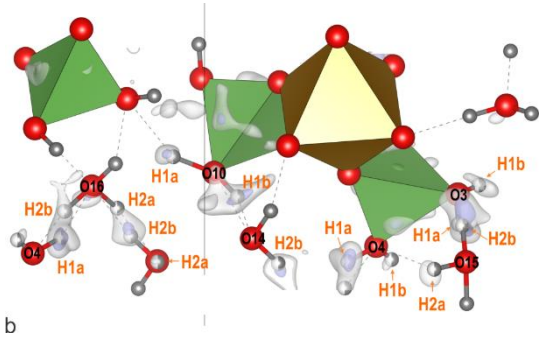
https://en.wikipedia.org/wiki/Empagliflozin

Hydrogens next to heavy atoms

Hydrogen disorder in kaatialaite Fe[AsO2(OH)2] 5H2O

The structure of synthetic kaatialaite known (Boudjada & Guitel, 1981) but the hydrogen sites remained **undetected from X-ray single-crystal data**.





Map after dynamical refinement of the structure including the non-disordered hydrogen

22 independent hydrogen positions, out of them 12 disordered. R(obs)=9.90%

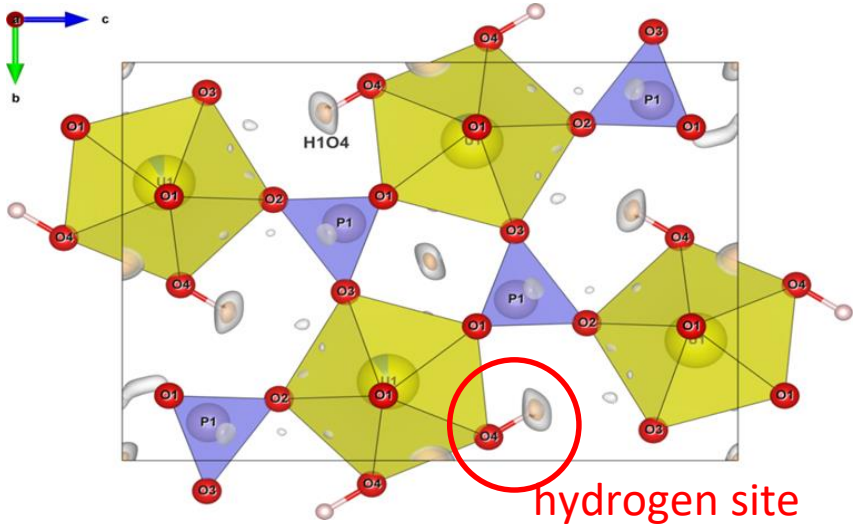
Hydrogens next to heavy atoms

Vyacheslavite is a secondary mineral – a product of a back-reduction of U(VI) to U(IV) in the supergene enrichment areas of the oxidation zones of U deposits.

Why 3D ED? grows only as nano crystals. very common for alteration products.

Hydrogen among heavy atoms in Ca-vyacheslavite

$Ca_xU_{1-x}(OH)_{1-2x}(H_2O)_{2x}$



mR(obs)/mwR(obs) 3.91%/4.72%

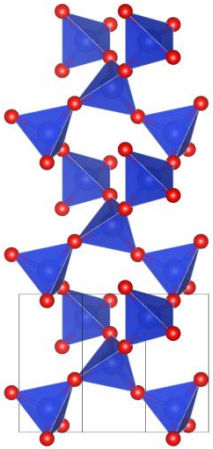
Apparent distance between O4 and H1O4 = 1.17 A

Steciuk, G. et al. (2019). RSC Adv. 9; Steciuk, G. et al. (2021). American Mineralogist, DOI: 10.2138/am-2021-7875

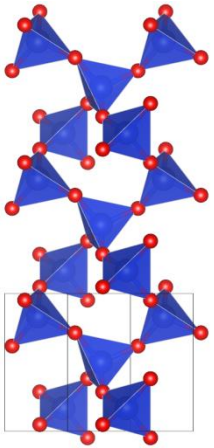
Absolute structure and absolute configuration

Absolute structure is a specification of the orientation of a non-centrosymmetric crystal structure under the operation of inversion (Online dictionary of Crystallography)

A non-centrosymmetric crystal may or may not be composed of chiral species.

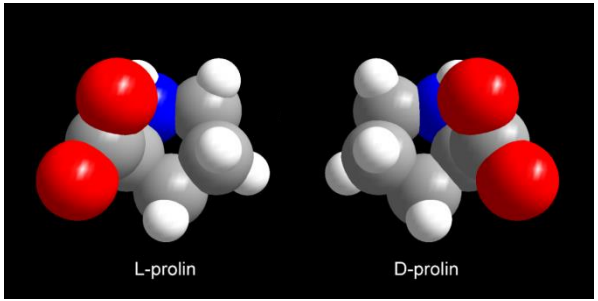


left quartz ($P3_121$)



right quartz ($P3_221$)

Absolute configuration is a specification of the spatial arrangement of atoms in a molecule containing *chiral centers.* Such molecules are not superimposable onto their mirror images. Different absolute configuration may mean (and often means) different biological function of the molecule.



Absolute configuration of molecules is most reliably and most often determined by determining the **absolute structure** of crystals containing the molecule.

Absolute structure and absolute configuration

Breaking Friedel's law

Friedel's law: In kinematical approximation, opposite structure factors have equal amplitudes: $|F_{-h}| = |F_h|$
Consequence: It is impossible to determine absolute structure from kinematical diffracted intensities

X-rays:

Resonant scattering shifts the phase of scattered photons from atoms -> Friedel's law does not hold exactly.

Strength of resonant scattering **depends on the** degree of non-centrosymmetricity, on the wavelength and **atomic number**.

Light atoms have very low resonant scattering -> difficulties in determination of absolute structure of organic species.

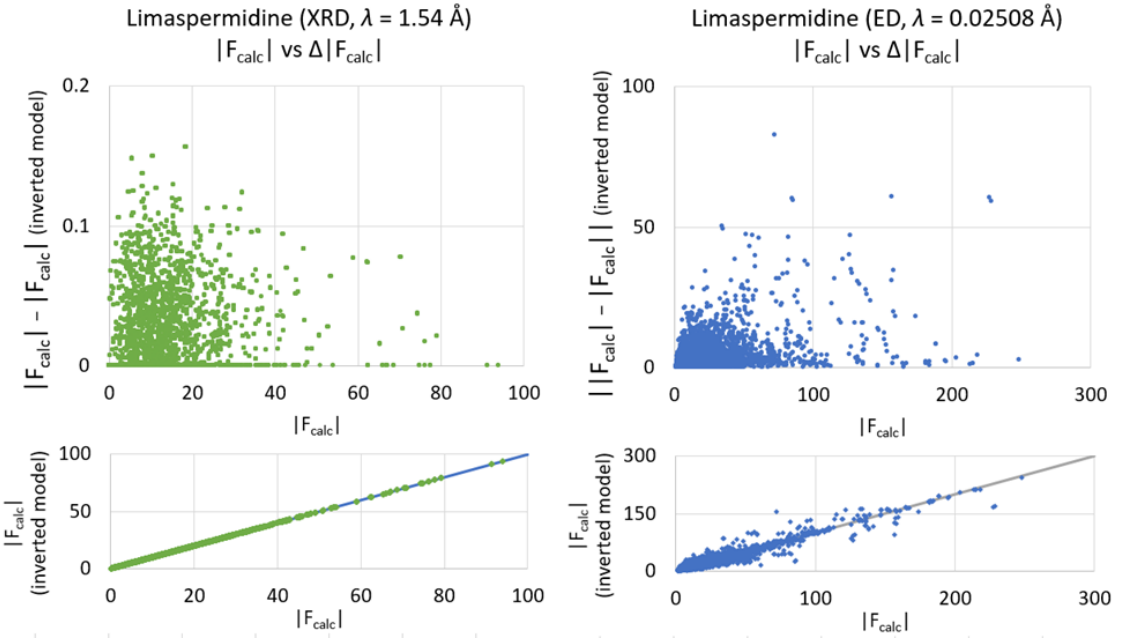
Electrons:

Electron diffraction is dynamical = coherent interference of more than one diffracted beam -> Friedel's law does not hold. In three-beam approximation:

$$I_h - I_{-h} \propto F_h F_g F_{h-g} \sin\varphi$$

where φ is the sum of structure factor phases.

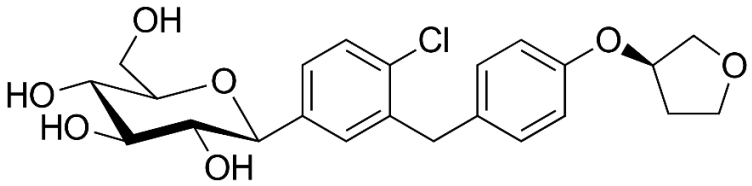
Strength of the breaking of Friedel's law **depends only on the degree of non-centrosymmetricity** (deviation of $\sin\varphi$ from 0), not on the atomic number. Absolute structure is equally easily determined for light and heavy atoms.



Absolute structure and absolute configuration

cocrystal empagliflozin/L-prolin

10 datasets refined
The fit clearly shows the correct absolute structure

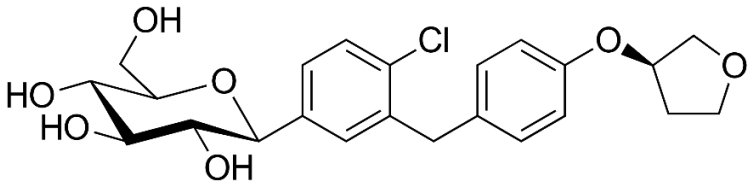


	wRall /%	wRall inv /%	Δ /p.p.	Confidence level (σ)
1	10.6	12.4	1.8	**4.1**
2	11.1	11.9	0.9	**1.2**
3	8.7	11.0	2.3	**5.2**
4	10.0	11.7	1.6	**4.9**
5	9.6	12.4	2.7	**9.6**
6	10.2	11.8	1.6	**4.7**
7	9.4	11.8	2.3	**4.4**
8	9.8	12.0	2.3	**7.8**
9	10.1	11.7	1.7	**5.3**
10	8.8	10.3	1.6	**4.3**
all	9.7	12.1	2.3	**17.2**

Absolute structure and absolute configuration

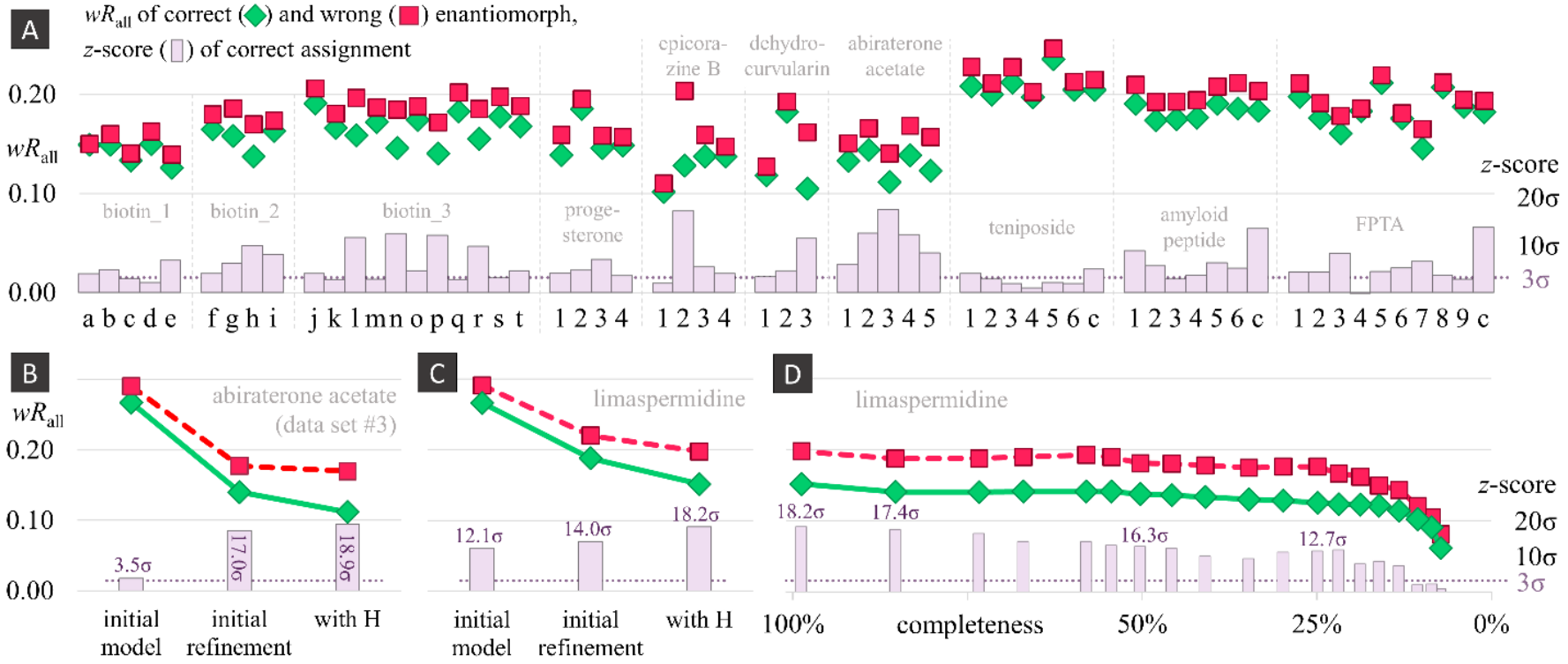
cocrystal empagliflozin/L-prolin

10 datasets refined
The fit clearly shows the correct absolute structure



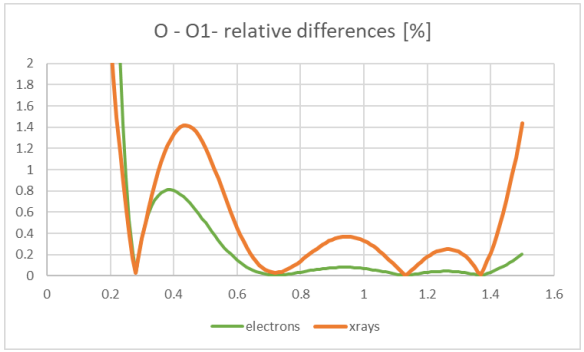
	wRall /%	wRall inv /%	Δ /p.p.	Confidence level (σ)	Flack x	Flack s.u.
1	10.6	12.4	1.8	**4.1**	0.25	0.04
2	11.1	11.9	0.9	**1.2**	0.33	0.03
3	8.7	11.0	2.3	**5.2**	0.25	0.02
4	10.0	11.7	1.6	**4.9**	0.29	0.06
5	9.6	12.4	2.7	**9.6**	0.23	0.02
6	10.2	11.8	1.6	**4.7**	0.29	0.02
7	9.4	11.8	2.3	**4.4**	0.31	0.03
8	9.8	12.0	2.3	**7.8**	0.26	0.02
9	10.1	11.7	1.7	**5.3**	0.36	0.03
10	8.8	10.3	1.6	**4.3**	0.32	0.04
all	9.7	12.1	2.3	**17.2**	0.29	0.03

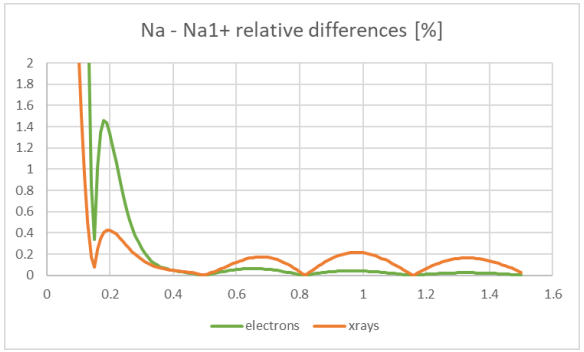
Absolute structure and absolute configuration

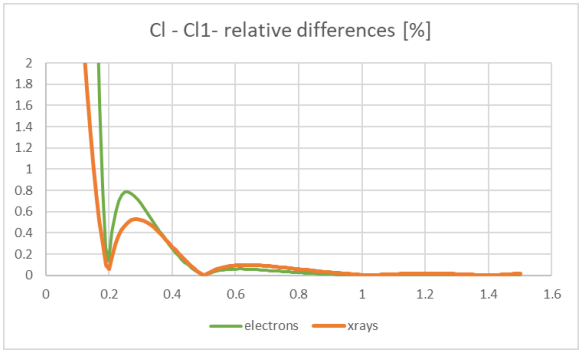


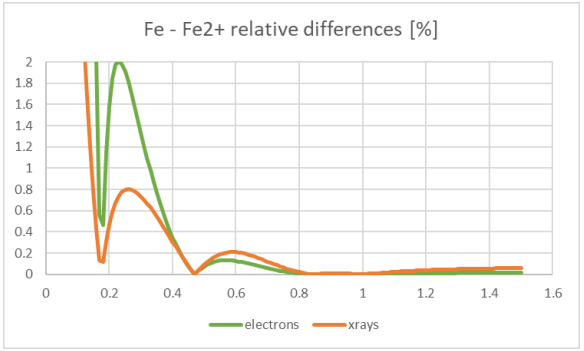
Beyond IAM – charge density from 3D ED?

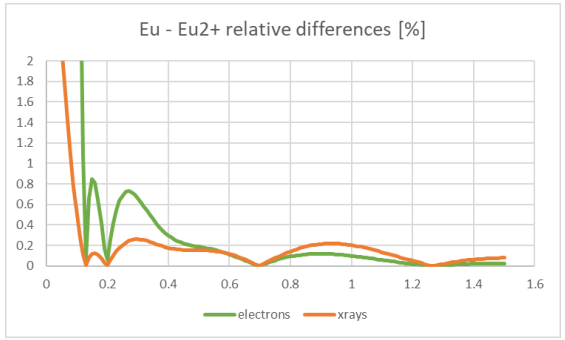
How does the signal strength of bonding effect compare between x-ray and electron diffraction?







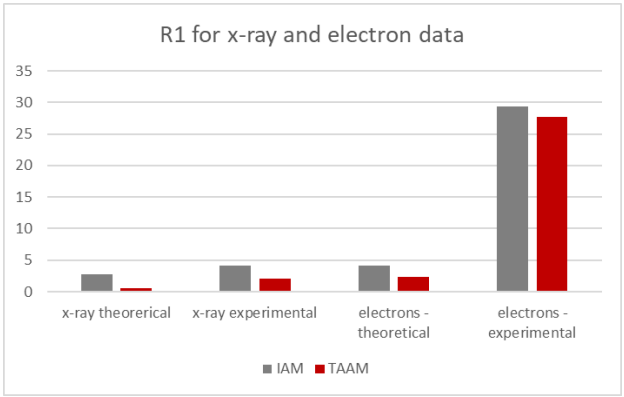


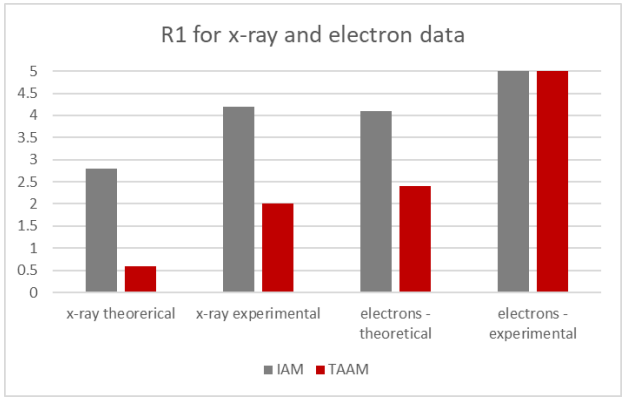


Beyond IAM – transferrable aspherical atom model

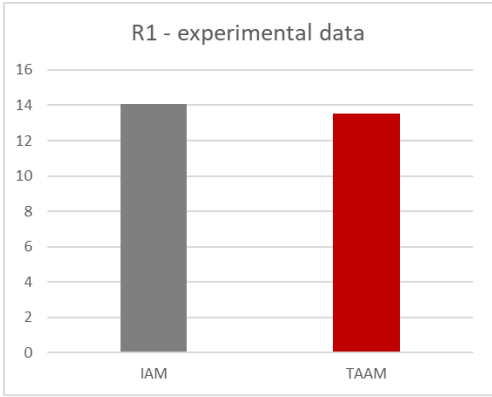
- Use external information to derive the non-spherical scattering factors of bonded atoms
- Use these scattering factors in the refinement against 3D ED data
- Available in MoPro, Olex2, Jana2020

So far tested on simulated as well as experimental data:



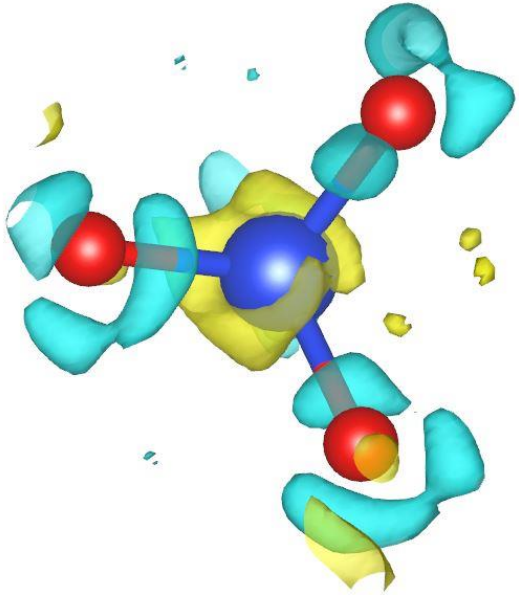


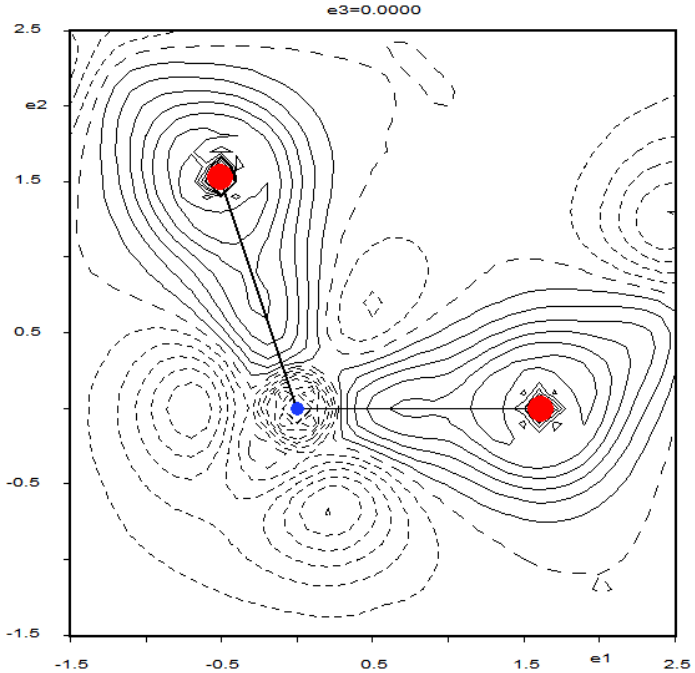
carbamazepine

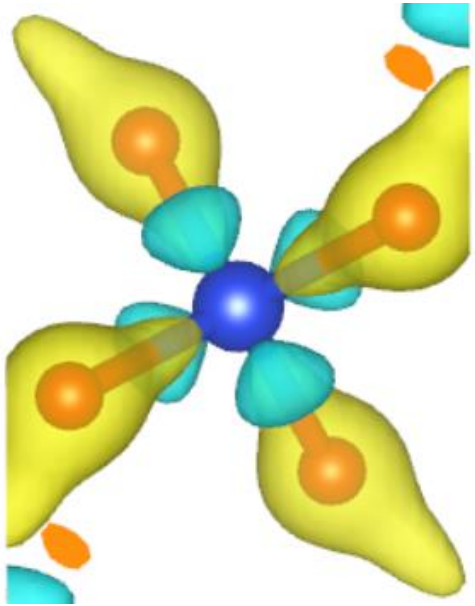


β-glycine

Quartz – multipole refinement



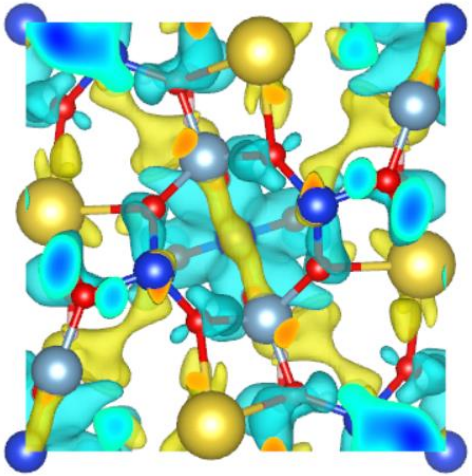




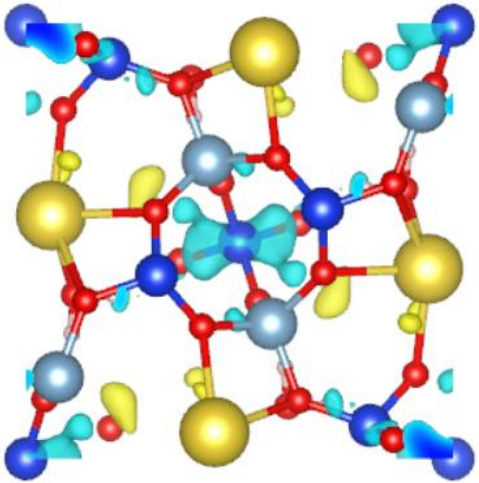
IAM difference potential
mR(all)=2.71%
R(all)=4.60%

Multipole refinement static deformation map
mR(all)=2.14%
R(all)=3.38%

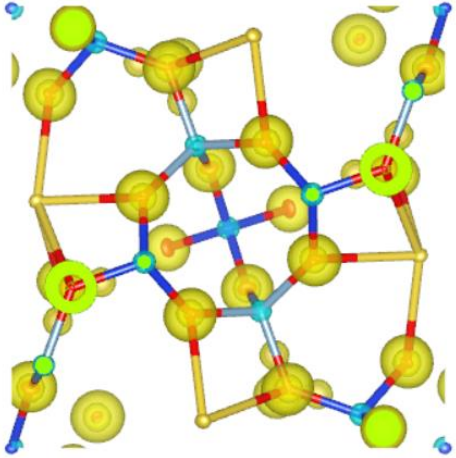
Natrolite – kappa refinement



IAM difference potential
mR(all)=4.79%
R(all)=6.04%

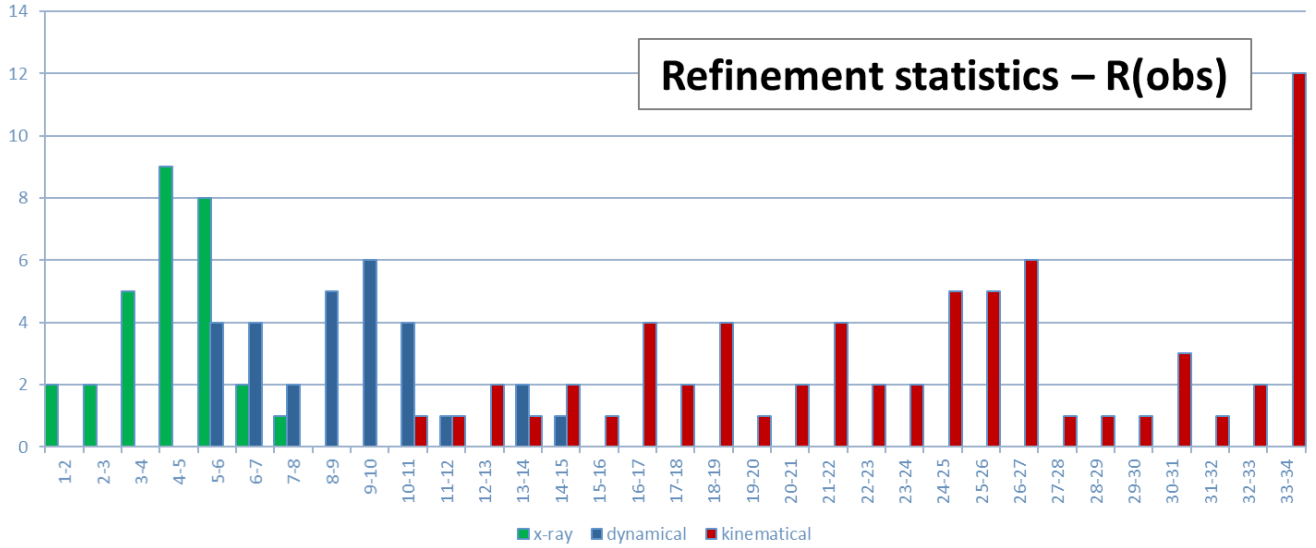


Kappa refinement difference potential
mR(all)=4.20%
R(all)=5.48%



Kappa refinement
static deformation map

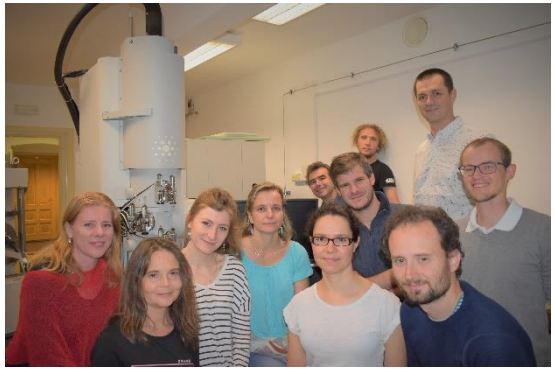
The R-factor gap in electron crystallography



Despite all the progress, we are still not doing as well as we could.
The mismatch is not only against x-ray data but also against expectation statistics.

The most likely reason for the remaining discrepancy is the unaccounted-for crystal imperfections

Acknowledgment



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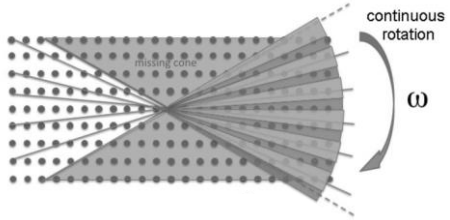
Conclusions and (bright) prospects

A lot has been done in electron crystallography, but the story continues:



- ✓ Dealing with dynamical effects: solved to some extent
- ✓ Dealing with beam damage: solved to a large extent
- ✓ Closing the R-factor gap: remains to be solved
- ✓ Bringing the method to the users: Being solved right now with more accessible methods and instruments