H2020-MSCA ITN
Grant n. 956099

Stockholm University

Serial 3DED

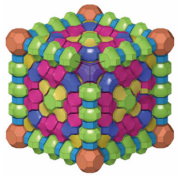
Xiaodong Zou, Department of Materials and Environmenal Chemistry,
Stockholm University

Nobel Prize lectures in Physics and Chemistry are held here every year, on December 8

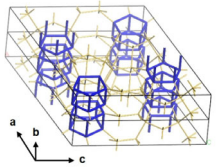
Electron Crystallography Research in My Lab

Materials

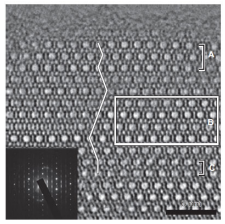
Zeolite



Nature **2015**

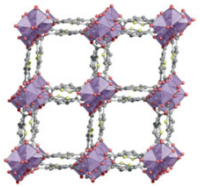


Angew Chem **2020**

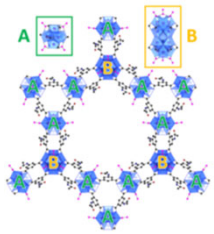


Nat Chem **2012**

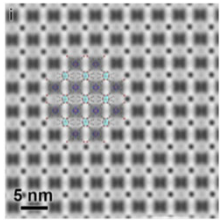
Metal-Organic Framework (MOF)



Nat Comm **2019**

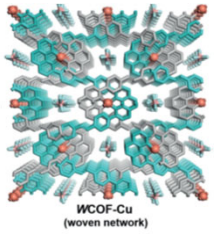


JACS **2020**

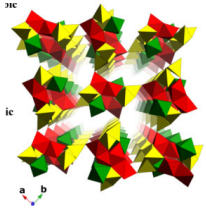


JACS **2020**

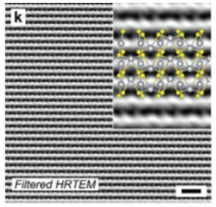
Other Materials



Angew Chem **2020**



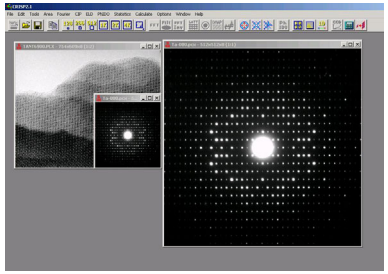
Cryst. Growth Des. **2014**



Adv. Mater. **2021**

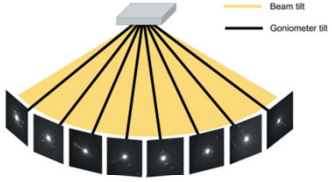
Method Development

Crystallographic Image Processing

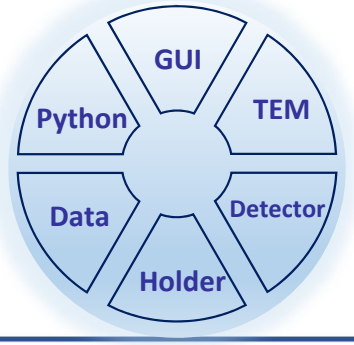


CRISP
QFocus
STEM
iDPC-STEM

3D ED (RED, cRED, SerialED, SerialRED)



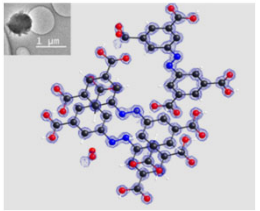
ELD, Trice
CrystDiff
REDc
REDp
Instamatic
InsteaDMatic

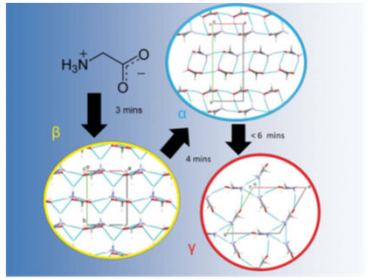


Instamatic

Biomolecules

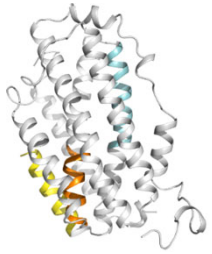
Small Molecules & Pharmaceuticals



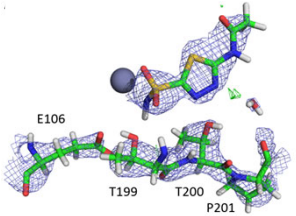


IUCrJ **2020**

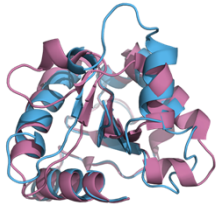
Protein



Sci. Adv. **2019**



Commun Bio **2020**



Nat Comm. **2021**

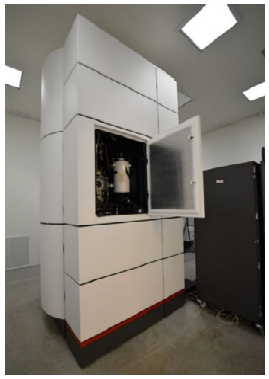
Nat. Comm.. **2021**

Electron Microscopy Centre (EMC) at MMK, SU

TEMs

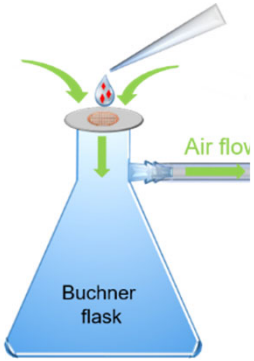
JEOL 2100LaB6 & 2100F

FEI Themis Z



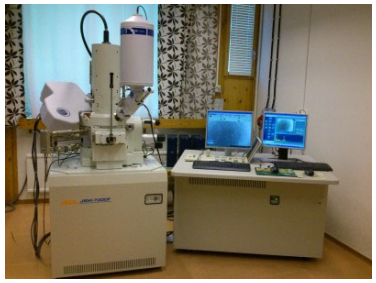
JEOL Cross-section polisher

Vitrobot



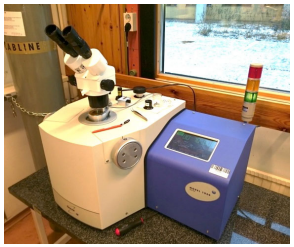
Preassis

SEMs



JEOL JSM7000F & 7401F

Hitachi TM3000



Fischione TEM Mill 1050

Ultramicrotome for sectioning

A large collection of holders:
Heating, cooling, cryo-transfer, high tilt, double tilt

Two TEMs at SciLifeLab

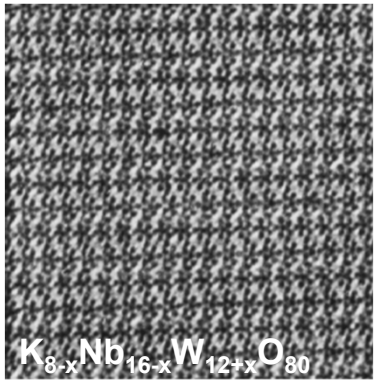
Titan Krios G3i with a Ceta-D camera and a Gatan K3 BioQuantum detector with EPU-D

Talos Arctica with Falcon III, Gatan K2 & Ceta

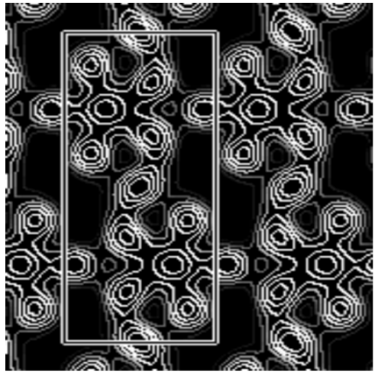
1982- : Electron crystallography on 3D inorganic crystals

Sven Hovmöller

From HRTEM image

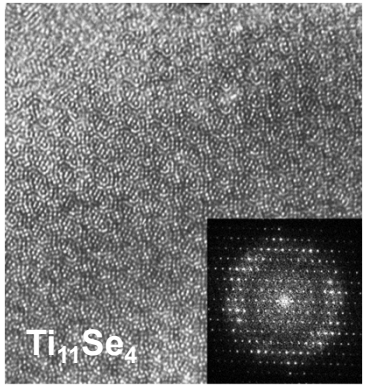


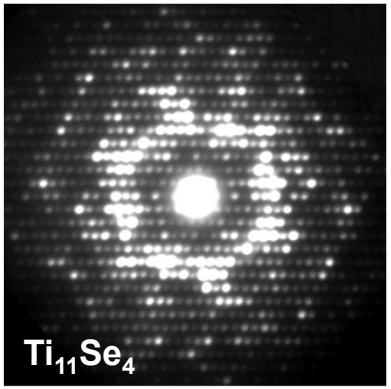
Resolution 2.5 Å



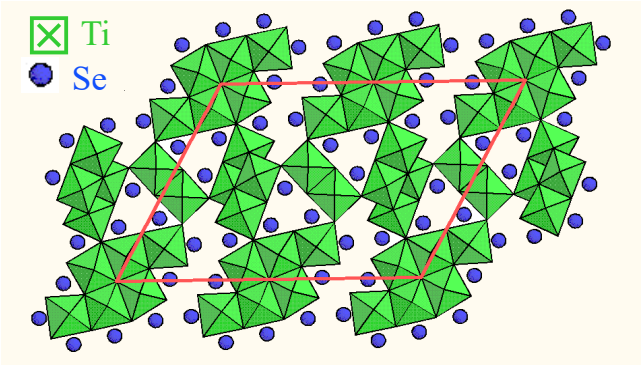
Hovmöller et al. *Nature*, 1984

Combining HRTEM image and ED pattern



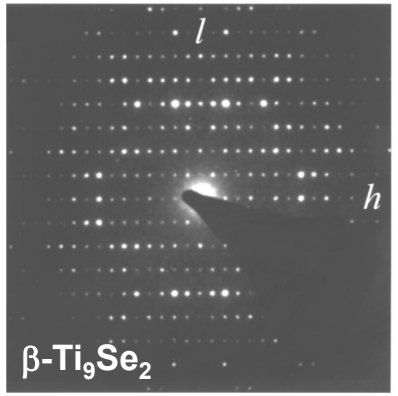


2.0 Å → 0.75 Å (*R*1 = 0.147)

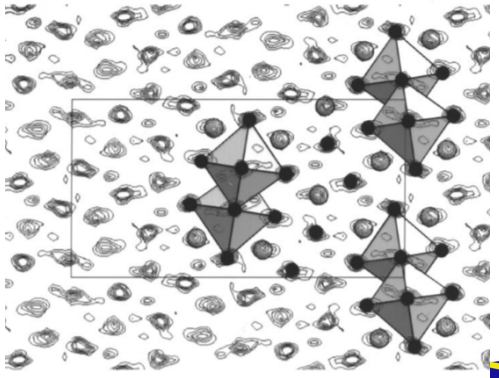


Weirich et al. Nature 1996

From ED pattern



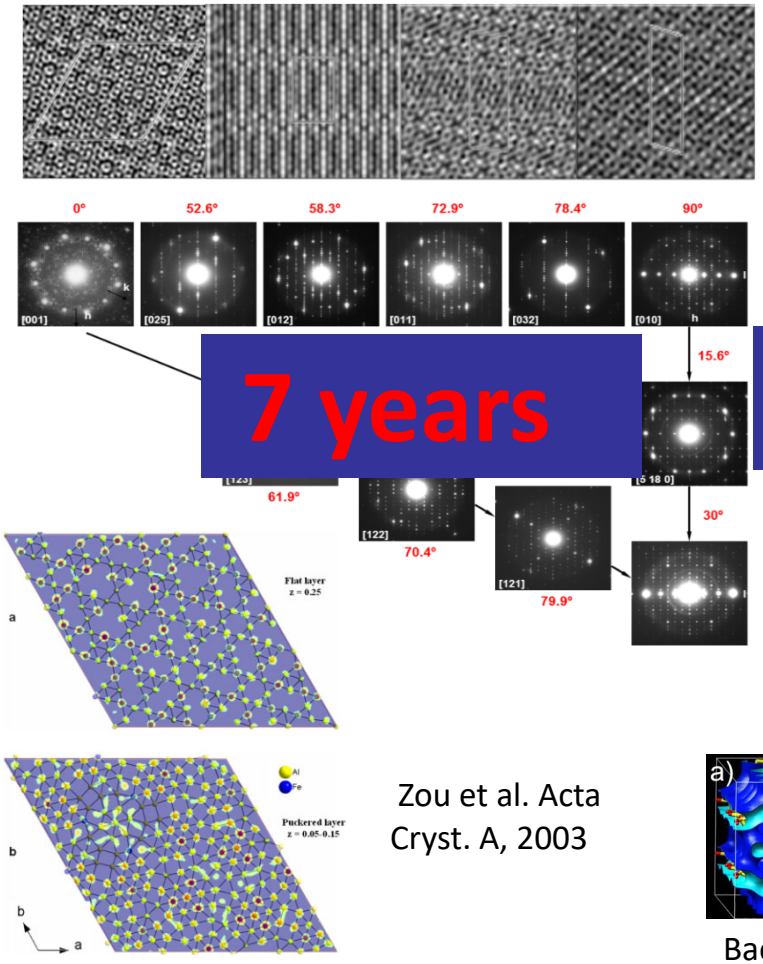
0.77 Å (*R*1 = 0.239)



Weirich *et al. Acta Cryst. A*, 2000

Atomic structures from HRTEM images along several projections

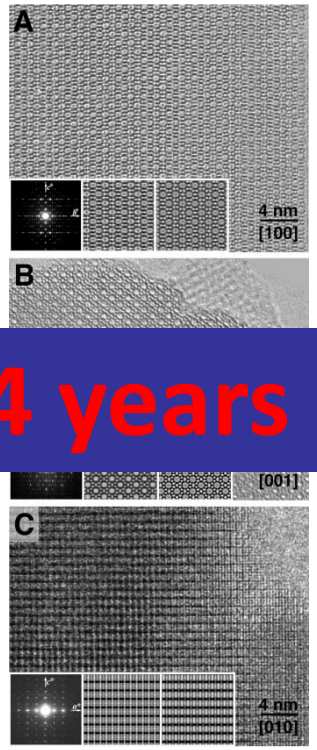
ν-AlFeCr 13 projections



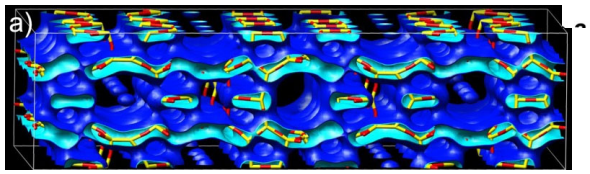
7 years

Zou et al. Acta Cryst. A, 2003

Zeolite: 3 projections

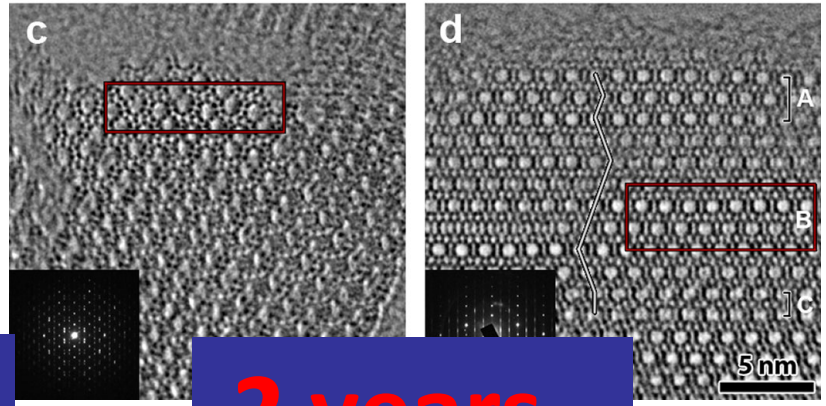


4 years

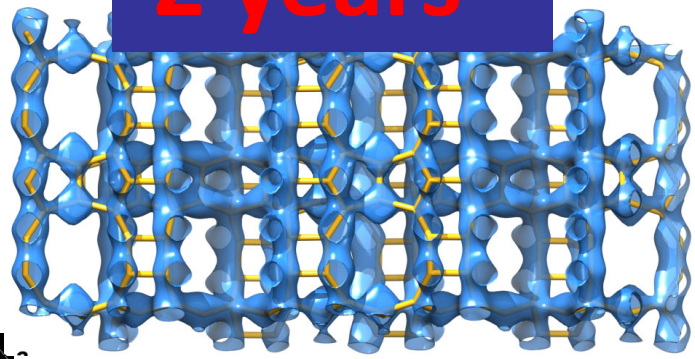


Baerlocher et al. Science, 2007

Zeolite: 2 projections + nanosize



2 years



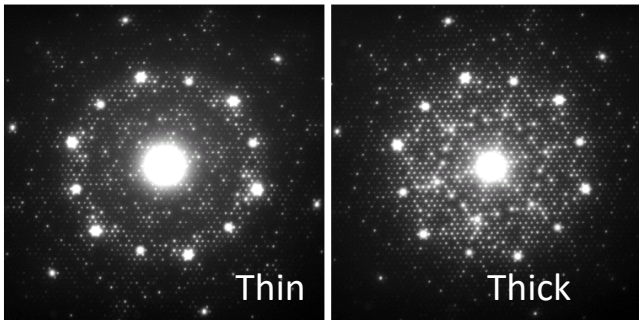
Willhammar et al. Nat Chem, 2012

Stockholm University

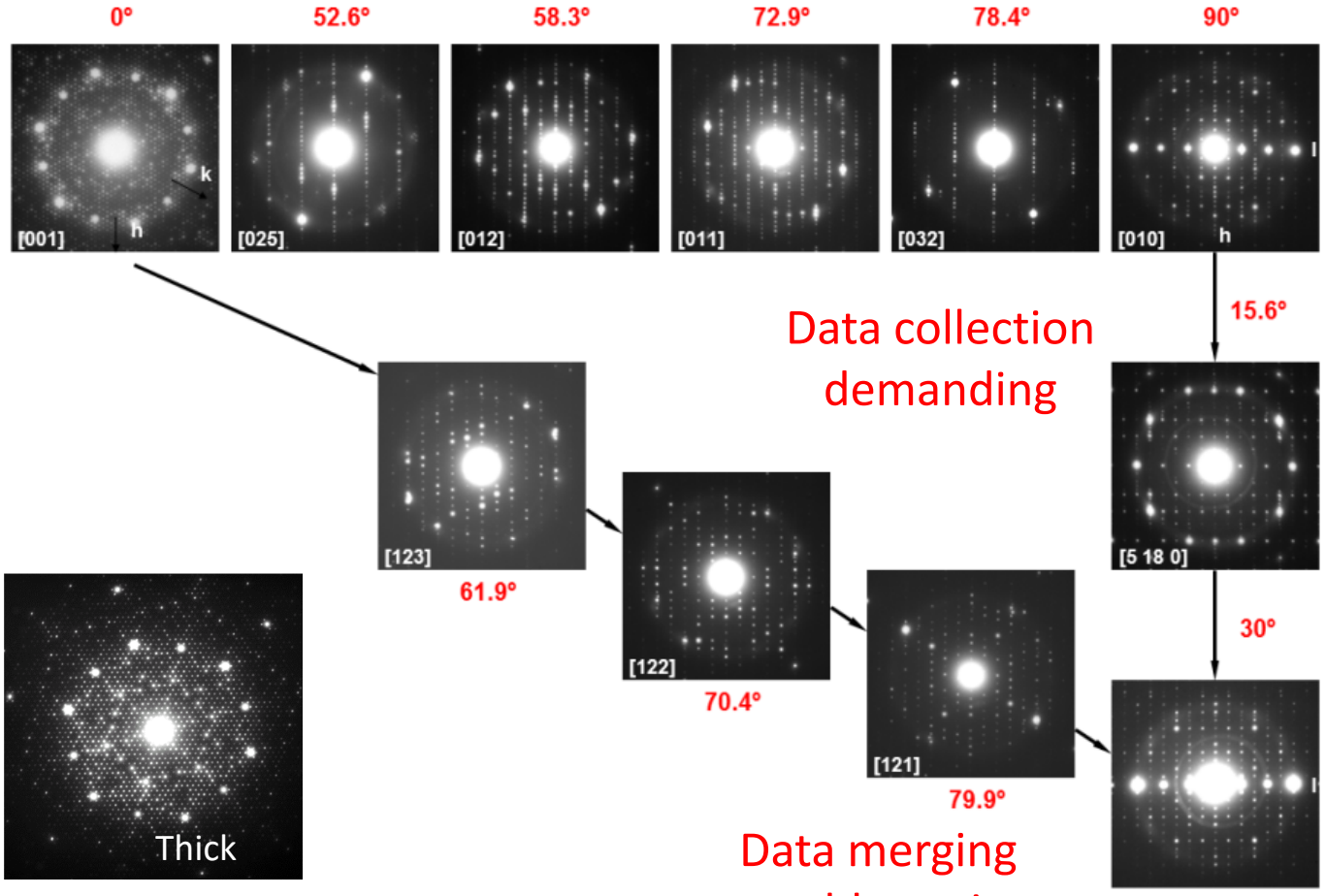
3D electron diffraction from zone axes

- Sub-Å resolution
- 3D data
- Low dose

Not affected by
- Spherical aberration
- CTF/Defocus
- Sample drift



Dynamical scattering



Data collection demanding

Data merging problematic

Many groups developed 3D electron diffraction ED data

Precession Diffraction Tomography (ADT/PEDT)

FEI Tecnai
STEM
Stepwise goniometer tilt
e^- beam precession


Ute Kolb


Tatiana Gorelik

Ultramicroscopy 2007
Ultramicroscopy 2008
Ultramicroscopy 2009

Rotation Electron Diffraction (RED)

JEOL JEM2100
TEM
Stepwise goniometer tilt
e^- beam tilt


Sven Hovmöller


Daliang Zhang


Wei Wan

Patent, WO/2008/060237 A1, 2008
Z. Kristallogr. 2010
J. App. Crystallogr. 2013

Microcrystal electron diffraction on proteins (MicroED)

FEI Tecnai
TEM
Stepwise & continuous goniometer tilt


Tamir Gonen


Dan Shi


Brent Nannenga

eLife, 2013, *Nat Methods,* 2014


Jan Pieter Abrahams


Chikashi Toyoshima

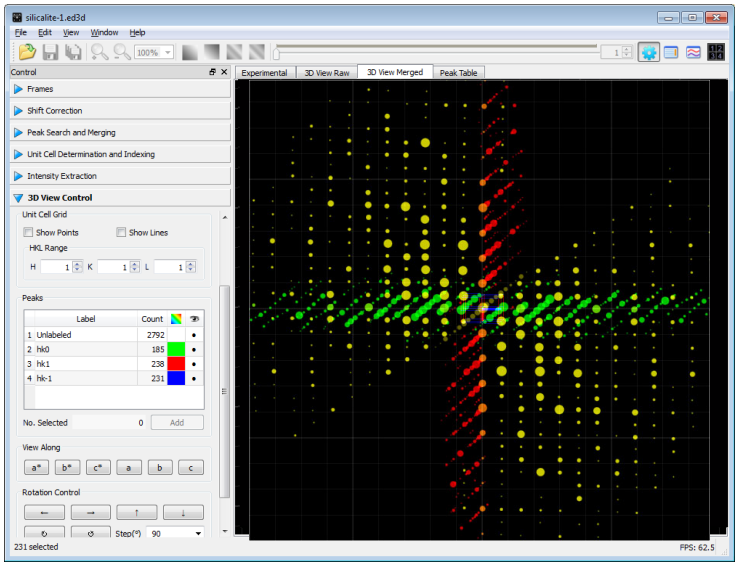

Koji Yonekura

Acta Cryst. D, 2013

PNAS, 2015

Data processing and structural analysis

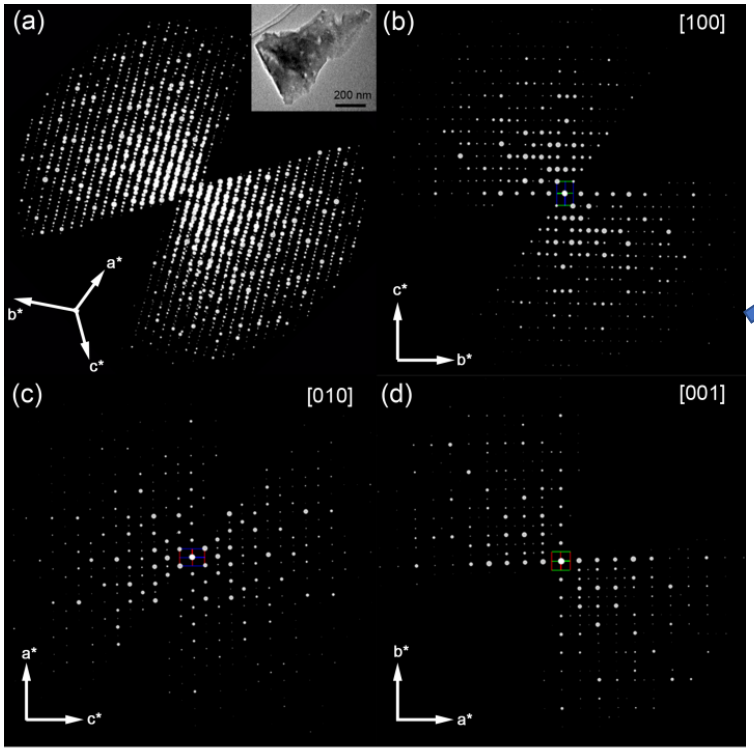
Data processing: REDp, XDS, Dials



Unit cell determination
Space group determination
Indexing the diffraction spots
Extract ED intensities

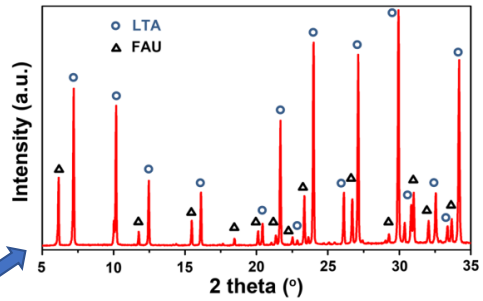
Wan et al. *J. App. Crystallogr.* 46 (2013) 1863
Su et al. *Micro- Mesoporous Mater*. 189 (2014) 115

Space group determination

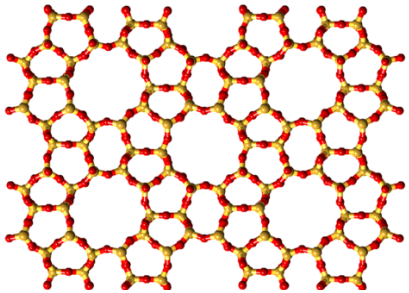


Space group can be determined from the systematic absences

Phase identification



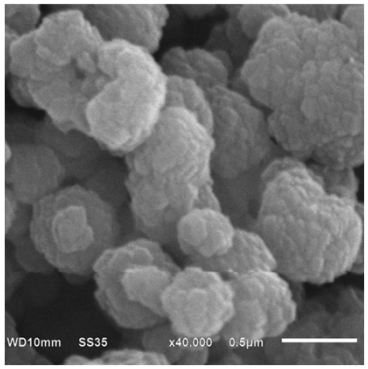
Structure determination



Structure solution:
SHELXS/T, SIR
Structure refinement:
SHELXL, JANA

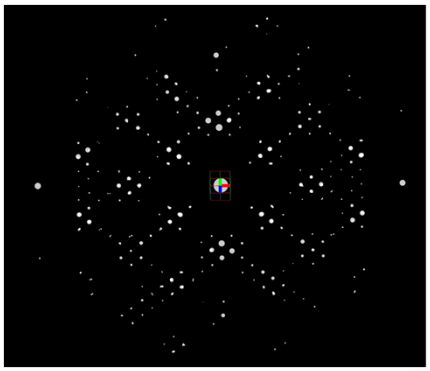
Zeolite: from structure to new discovery

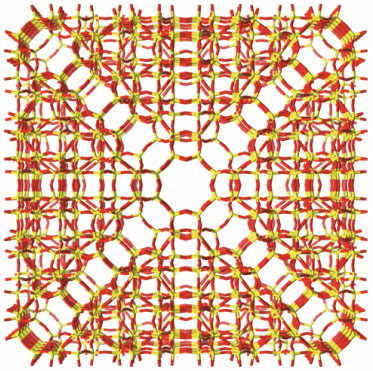
First reported in 1981 by Mobil



Doherty et al., US patent, **1981**

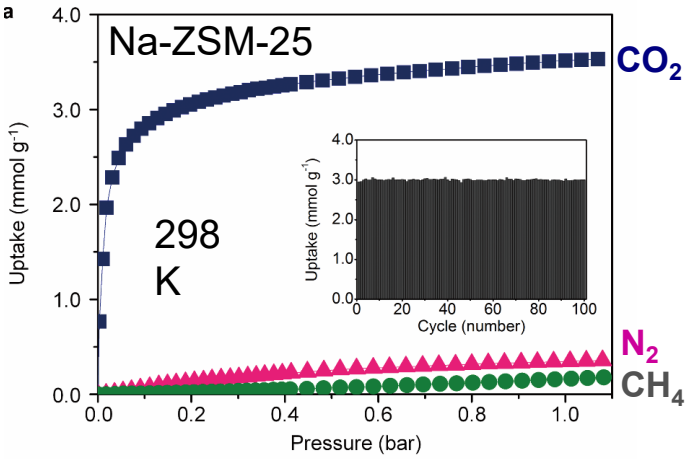
Structure determination

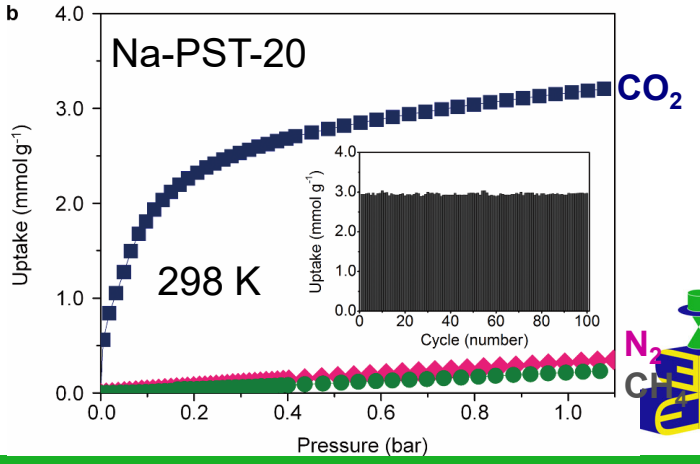




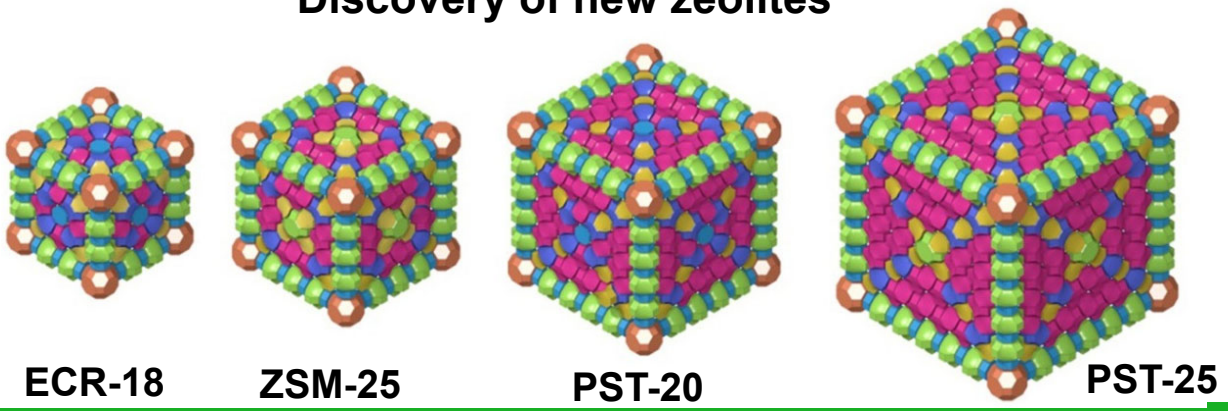
Guo et al. *Nature* **2015**

CO_2 uptake property





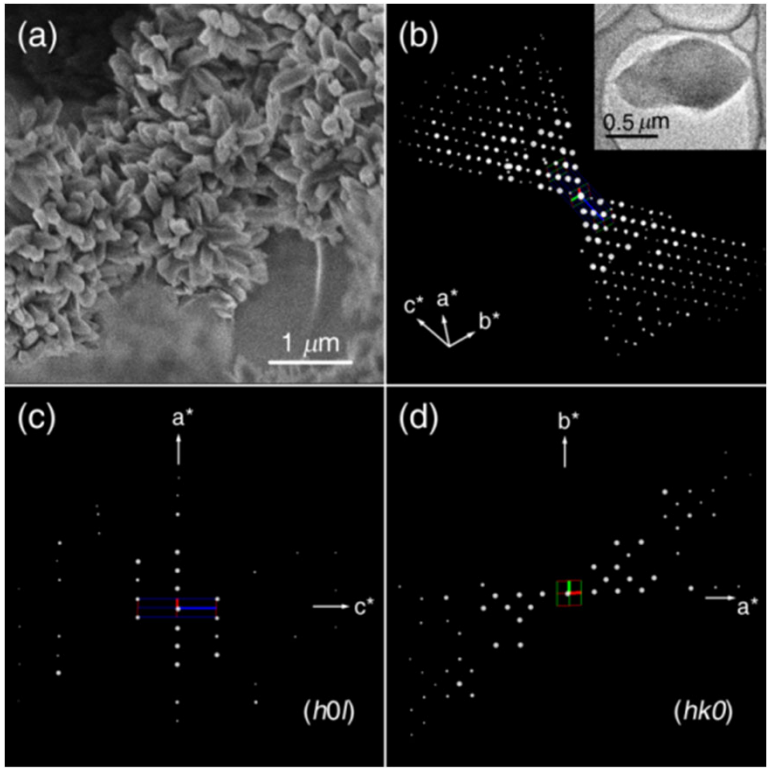
Discovery of new zeolites



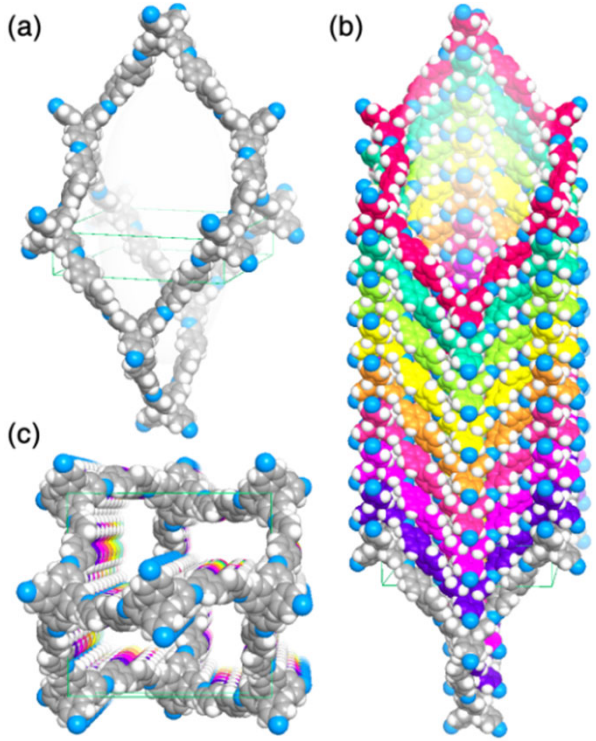
ECR-18 **ZSM-25** **PST-20** **PST-25**

First single crystal COF structure determined from RED data

RED data with 1.5 Å resolution



I-42*d*, *a* = 30.17 Å, *c* = 7.28 Å



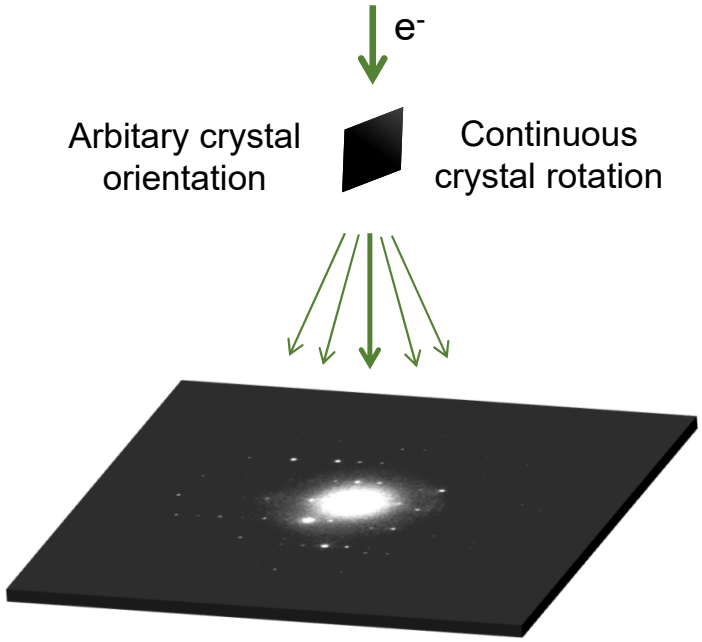
9-fold interwoven diamond net

The structure was solved using simulated annealing parallel tempering algorithm using FOX. All non-H atoms were refined using SHELXL (*R*1 = 0.31).

Zhang, Su, Zou, Yaghi et al. *J. Am. Chem. Soc.* **2013,** 135, 16336

Fast data collection and crystal tracking during continuous crystal rotation

Continuous rotation electron diffraction (cRED)

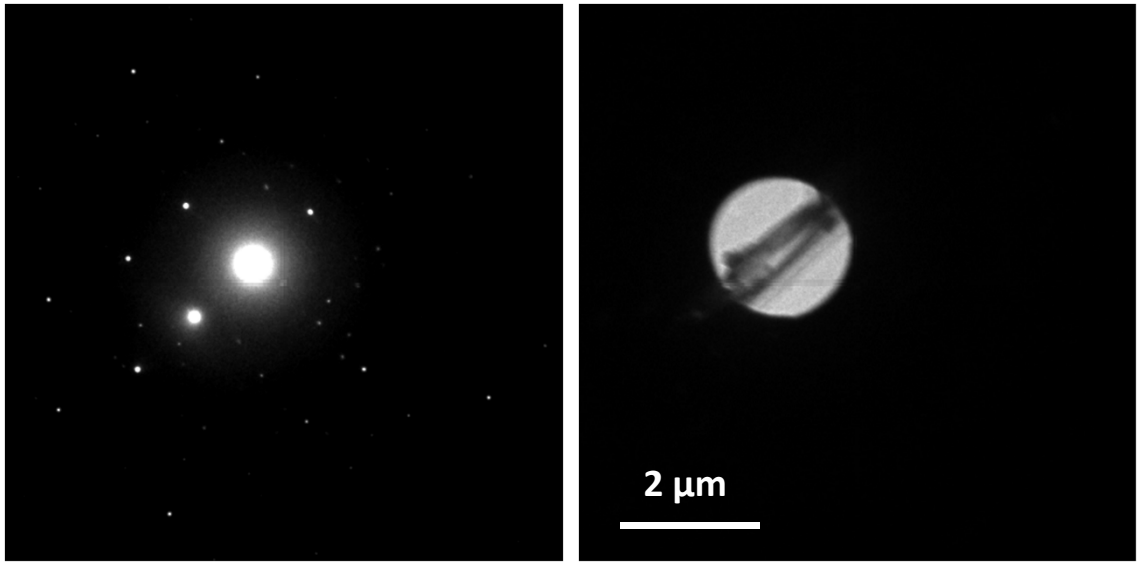


Fast detector in 'movie' mode

Nederlof et al. *Acta Cystography D*, **2013**
Nannenga et al. *Nature Methods*, **2014**
Gemmi et al. *J. Applied Crystallogr.*, **2015**

cRED data acquisition by software ***Instamatic***:

- crystal tracking by defocusing every 10th diffraction pattern



- Rotation range: : -44.0 to 47.4° (91.4°)
- Rotation speed: 0.76°/s
- Exposure time 0.5 s/frame

Cichocka *et al. J. Appl. Cryst.*, **2018**, *51*, 1652-1661

Magdalena Cichocka

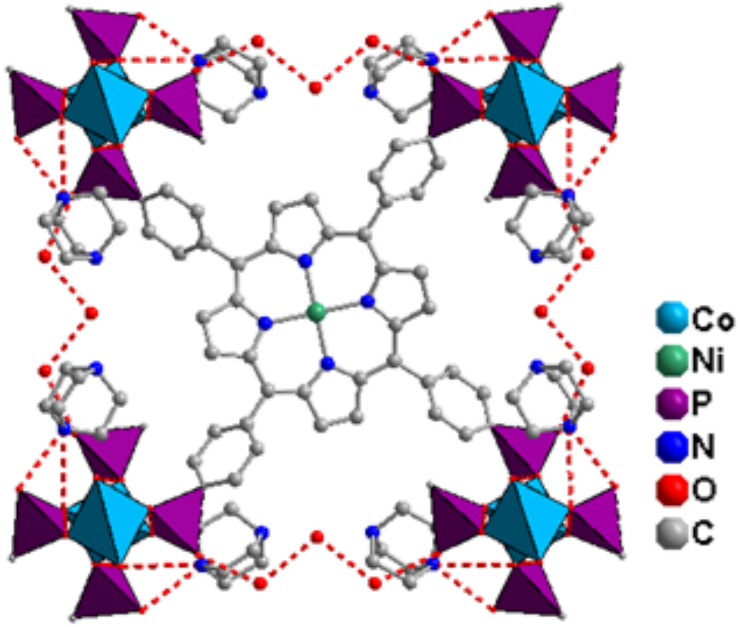
Bin Wang

Robustness of structure determination by cRED

Bin Wang

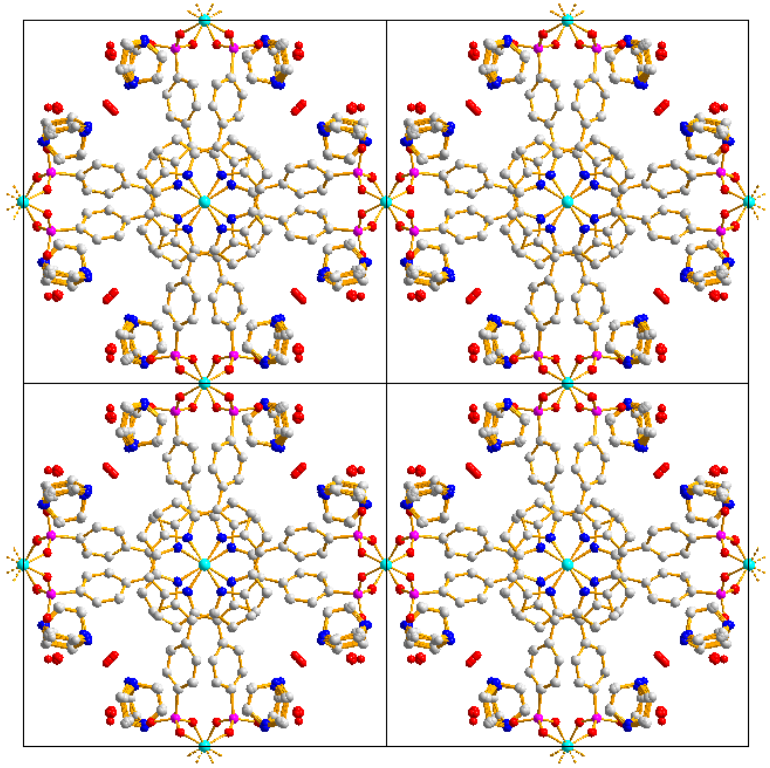
CAU-36 (Co): $[Co_2(Ni\text{-}H_4TPPP)]\cdot 2DABCO\cdot 6H_2O$

8 datasets collected at 96 K, tilt range: ~ 100°
Crystal size: ~ 500 nm, time/dataset: ~ 3 min



P-4*c*2, a = 21.98, b = 8.96 Å

8 structural models superimposed



Average deviations: Framework (18) 0.03(2) Å, DABCO (8) 0.09(6) Å, water (2) 0.12(11) Å

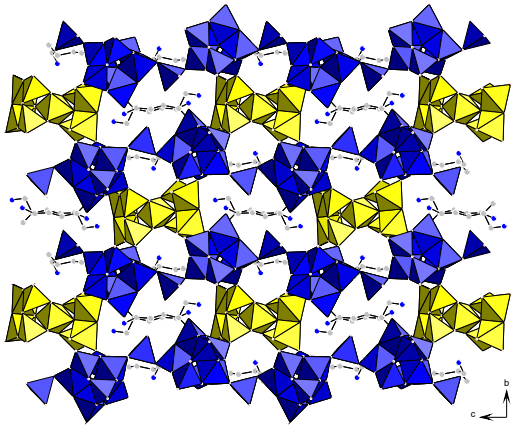
Wang, Rhauderwiek, Inge, Stock, Zou et al. *Chem. Eur. J.* **2018,** *66*, 17429

Stockholm University

Can accurate atomic positions of guest moelcules be determined from cRED data?

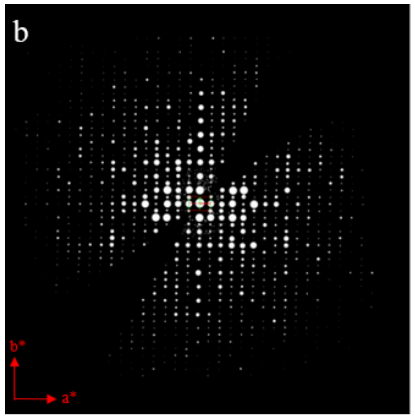
Meng Ge

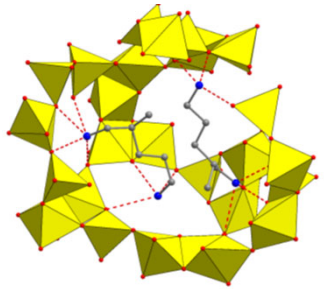
Open-framework germanate SU-8 determined by SC-XRD



$P2_1/c$

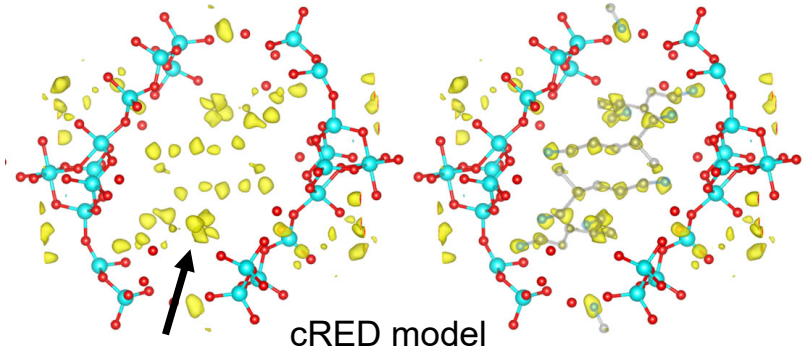
a = 12.169(2) Å
b = 19.442(4) Å
c =19.289(4) Å
β = 92.45(3)º



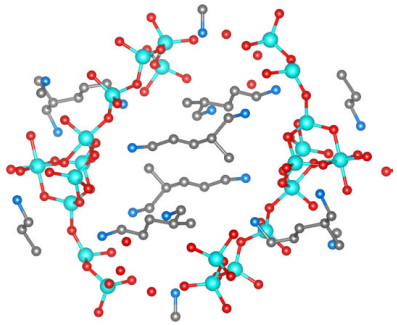


Strong H-bonding between OSDAs and framework

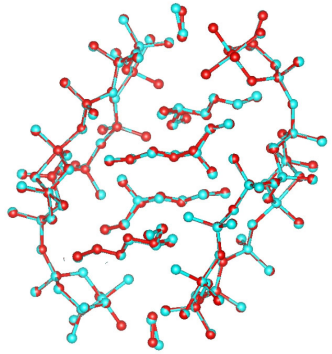
Atomic positions of OSDAs in the difference map



cRED model



SC-XRD model



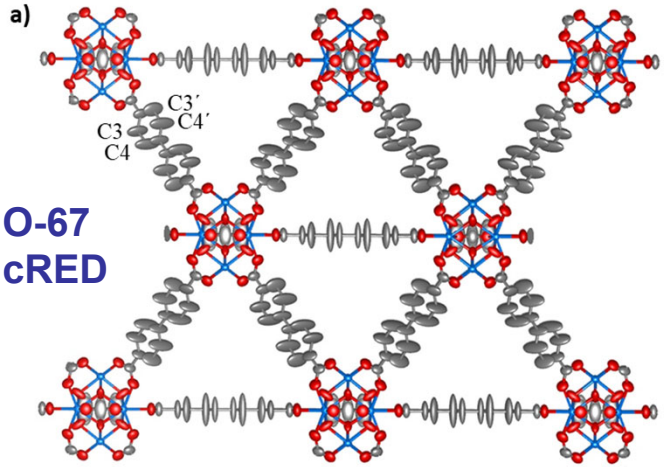
Comparison of SC-XRD and cRED models

Christensen, Zou et al. *J. Am. Chem. Soc.* **2006**, *128*, 14238. Meng, Huang, Zou et al. ***unpublished***

Probing molecular motions and disorders

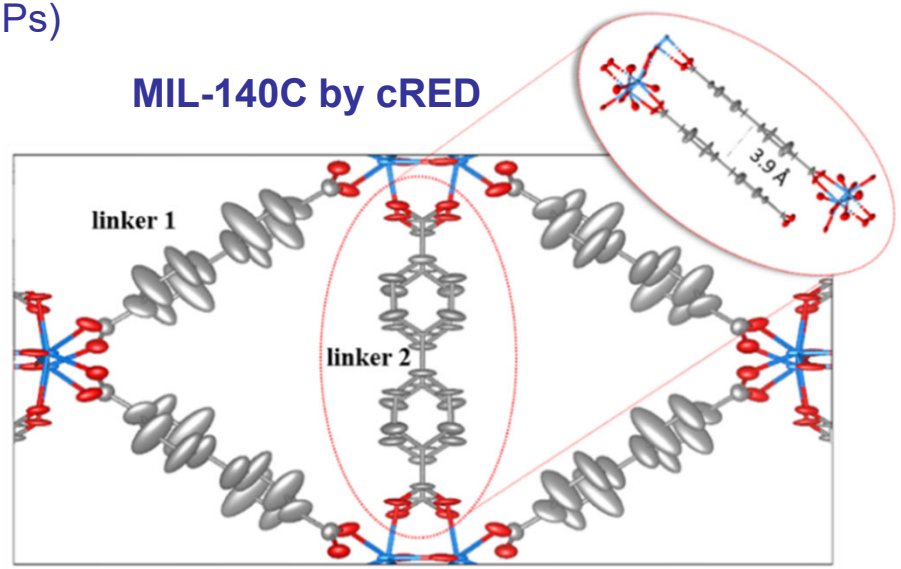
Laura Samperisi

Molecular motions/disorders can be identified by anisotropic atomic displacement parameters (ADPs)



UiO-67 by cRED

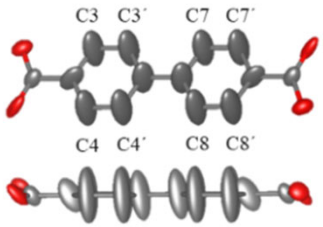
MIL-140C by cRED



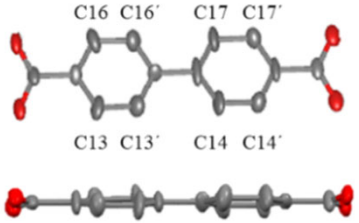
Comparison of SC-XRD and cRED models

Dark: SC-XRD; Light: cRED

Linker 1



Linker 2

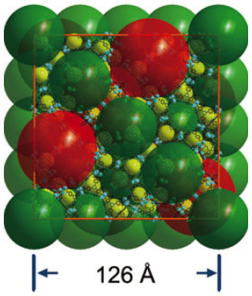


Linker 1 exhibits larger motions than linker 2

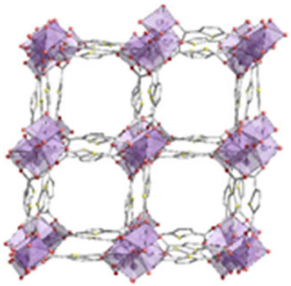
Samperisi, Jaworski, Kaur, Lillerud, Zou, Huang, *J. Am. Chem. Soc.* **2021**, 143, 43, 17947

> 200 structures determined using cRED data

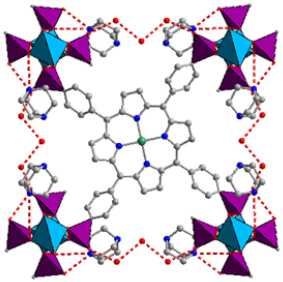
Metal-organic frameworks



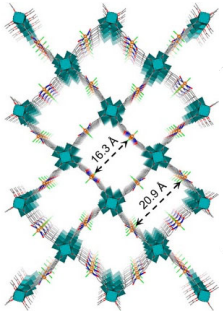
PCN-333
Nat. Commun.
2015



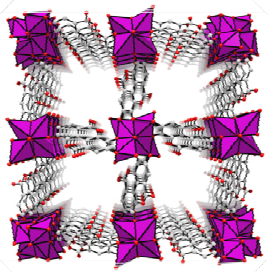
CAU-23
Nat. Commun.
2019



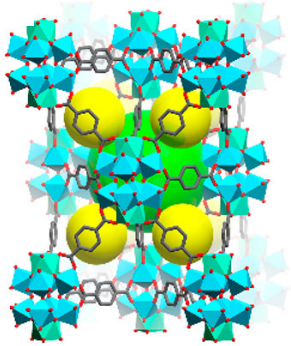
CAU-36 (Co)
Chem. Eur. J.
2019



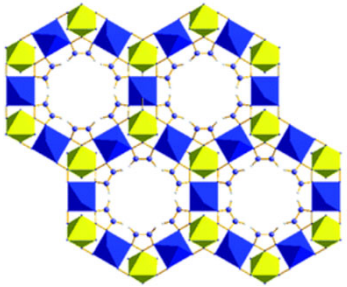
UU-100
JACS **2019**



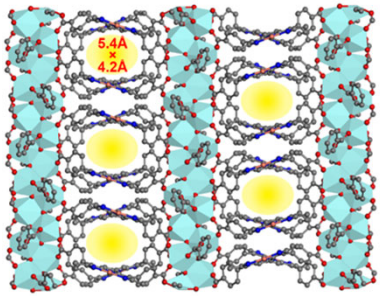
SU-101
JACS, **2020**



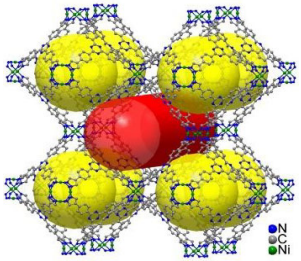
PCN-415
ACS Cent. Sci.
2018



Cu-kag
J. Mater. Chem. C
2019

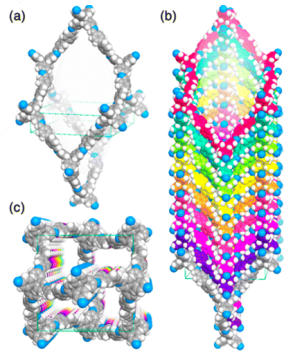


PCN-226
JACS **2020**

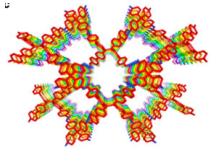


BUT-33
JACS **2020**

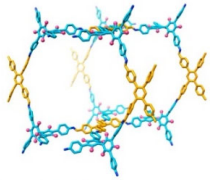
Covalent organic frameworks



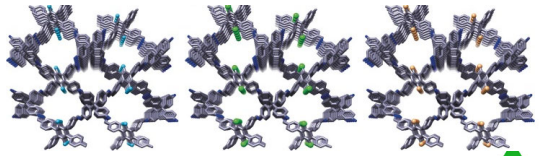
COF-320
JACS
2013



3D-TPE-COF
Nat. Commun.
2018

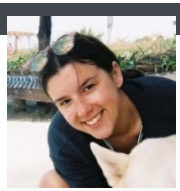


3D-BMTA-COF
JACS, **2020**



3D-TPB-COF-R, R=H, Me, F
Angew. Chem. Int. Ed. **2019**

Discover new polymorphs of pharmaceuticals



Molly Lightowler

Sun Yat-sen University

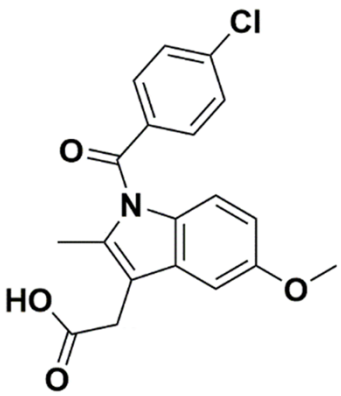
Associate Prof. Ming Lu

Ms. Shuting Li

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

1974

Indomethacin



Melt-crystallization →

↓

Form-δ

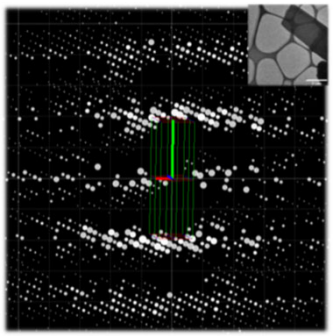
↑

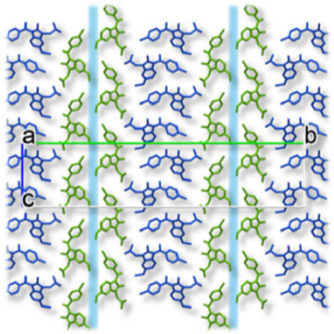
Solution-crystallization →

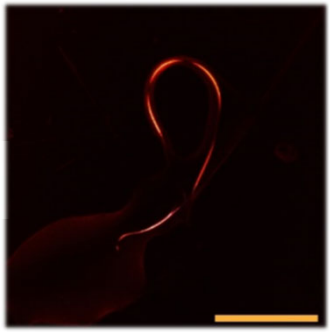
2021

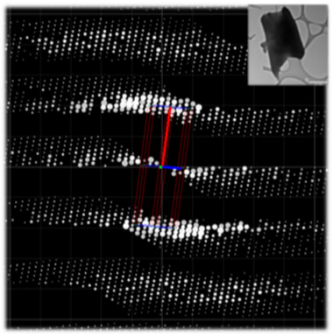
New!

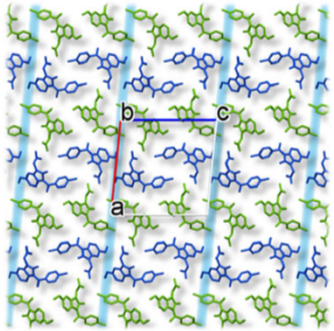
Form-θ (Melt δ)









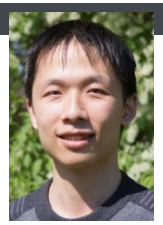


Form-δ (Solution δ)

I. Andrusenko, Gemmi et al. *Int. J. Pharm.* **2021**, *608*, 121067.

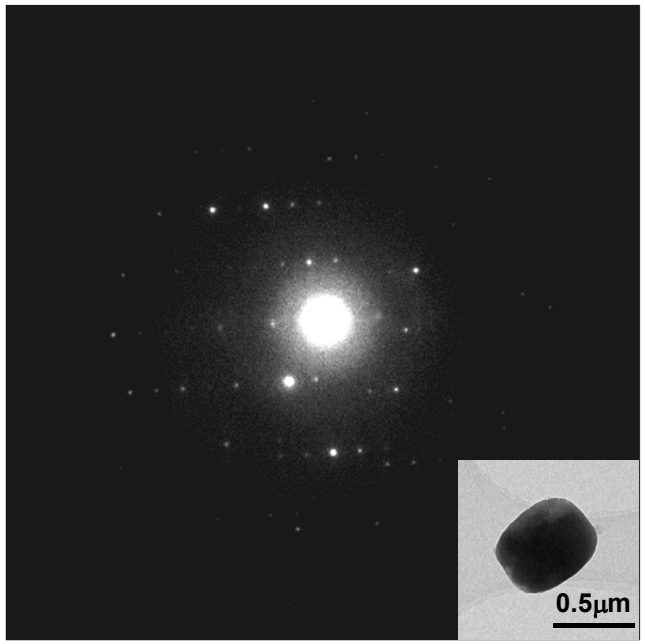
M. Lightowler et al. *ChemRxiv* **2021**. https://doi.org/10.33774/chemrxiv-2021-s9fhf

PCN-415: a photoactive MOF built from mixed metal $Ti_8Zr_2O_{12}(COO)_{16}$ cluster



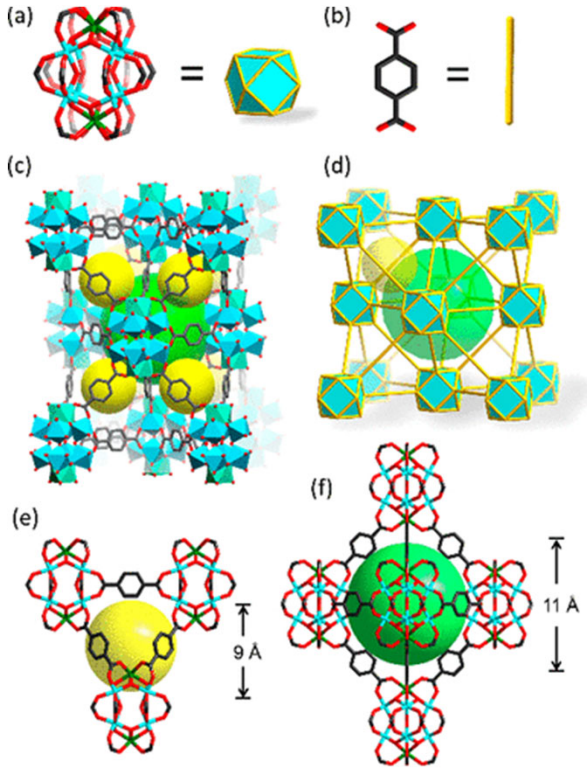
Zhehao Huang

Fast data collection
Low electron dose



Resolution 0.82 Å
Total data collection time: **17 s**

$Ti_8Zr_2O_{12}(COO)_{16}$ Cluster



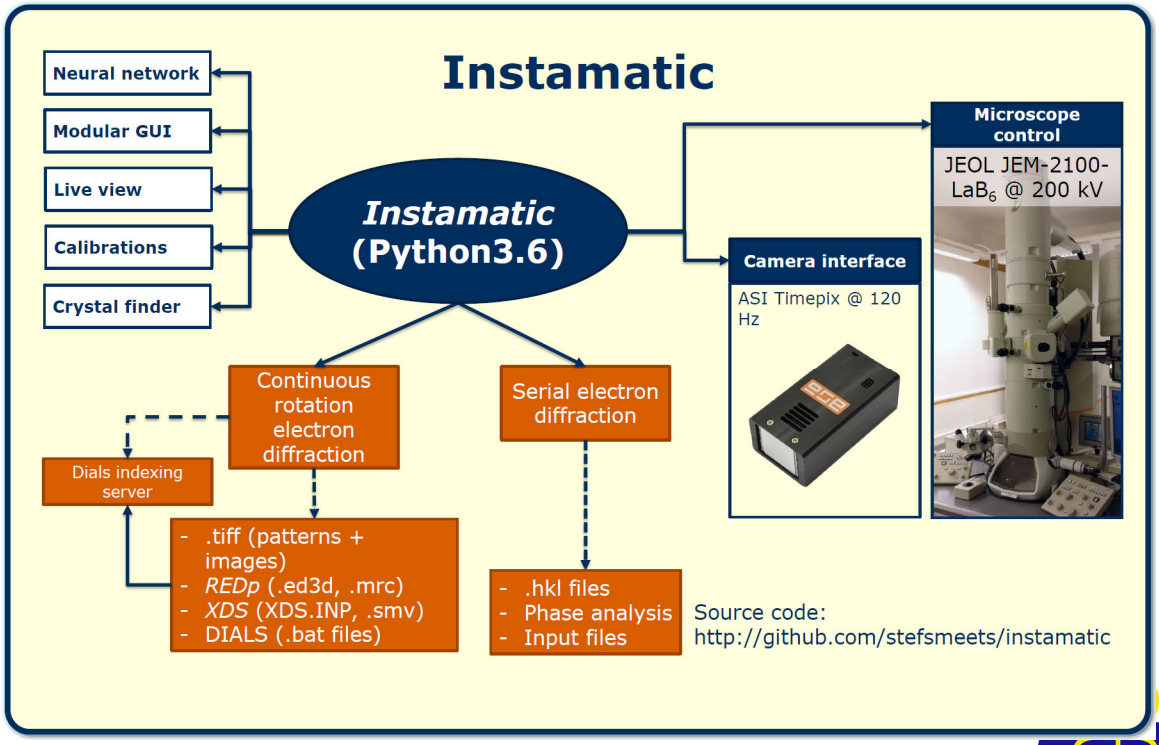
PCN-415	
Data Processing	
Software	**XDS**
Wavelength (Å)	0.0251
Space group	*I* 4/mmm
a, b, c (Å)	14.7, 14.7, 27.4
α, β, γ (°)	90, 90, 90
Resolution (Å)	0.82
R_{sym}	0.26
I / Sigma I	4.52
Completeness (%)	94.5
Redundancy	6.2
Structure Solution and Refinement	
Software	**Shelx**
Scattering factors	Electrons
R(Int)	0.2256
R1	0.2022

All **Zr**, **Ti**, **O** and **C** atoms were found by **SHELXS**.
Kinematic refinement was applied using **SHELXL**.

Yuan et al. ACS Cent. Sci. **2018**, *4*, 105

Automated data collection, data processing and structure solution pipeline

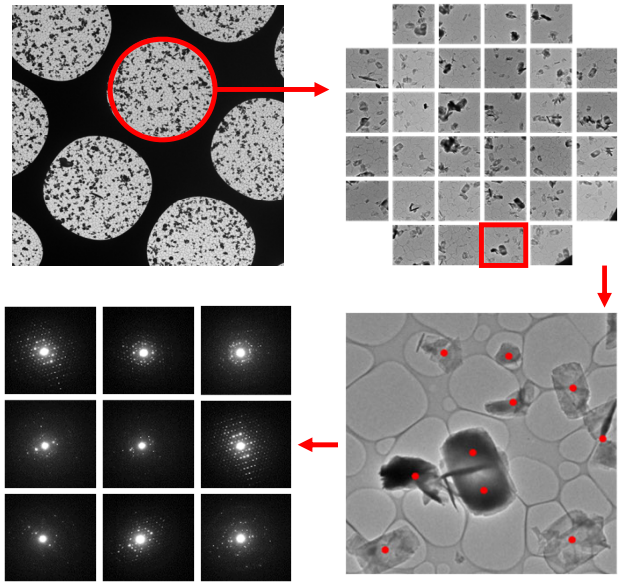
- Crystal screening
- Selection of suitable crystals using machine learning
- Data collection assisted by auto-crystal tracking
- Data processing pipeline integrated with existing software for X-ray diffraction
- Online structure solution using SHELXT
- **SerialED & SerialRED**



Smeets et al., *J. Appl. Cryst.* **2018,** 51, 1262
Cichocka et al., *J. Appl. Cryst.* **2018**, *51,* 1652
Wang et al. *IUCrJ*, **2019**, *6*, 854

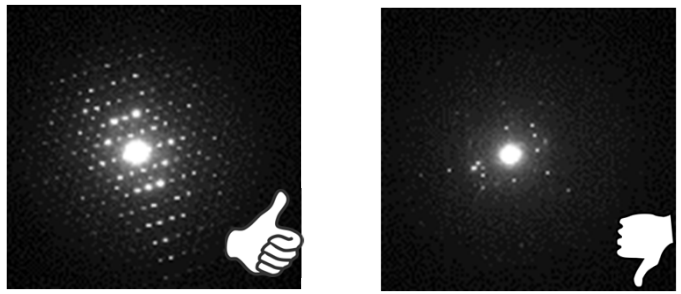
Towards high-throughput structure determination and phase analysis

Serial ED



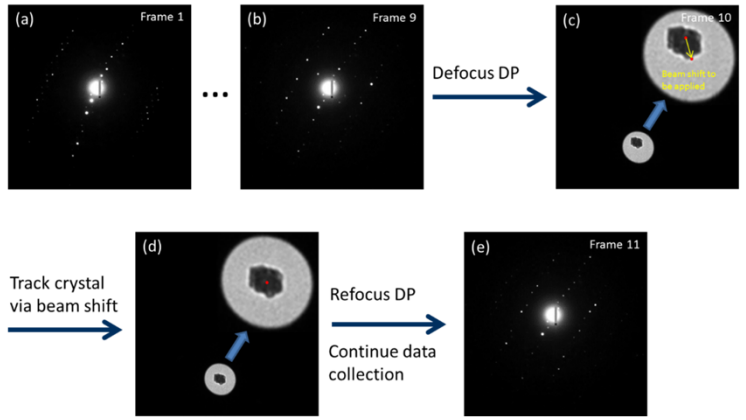
Smeets, Zou, Wan, *J. Appl. Crystallogr.* **2018,** *51*, 1262.

Machine learning for selecting "good" crystals



Cichocka, Ångström, Wang, Zou, Smeets, *J. Appl. Crystallogr.* **2018,** *51,* 1652.

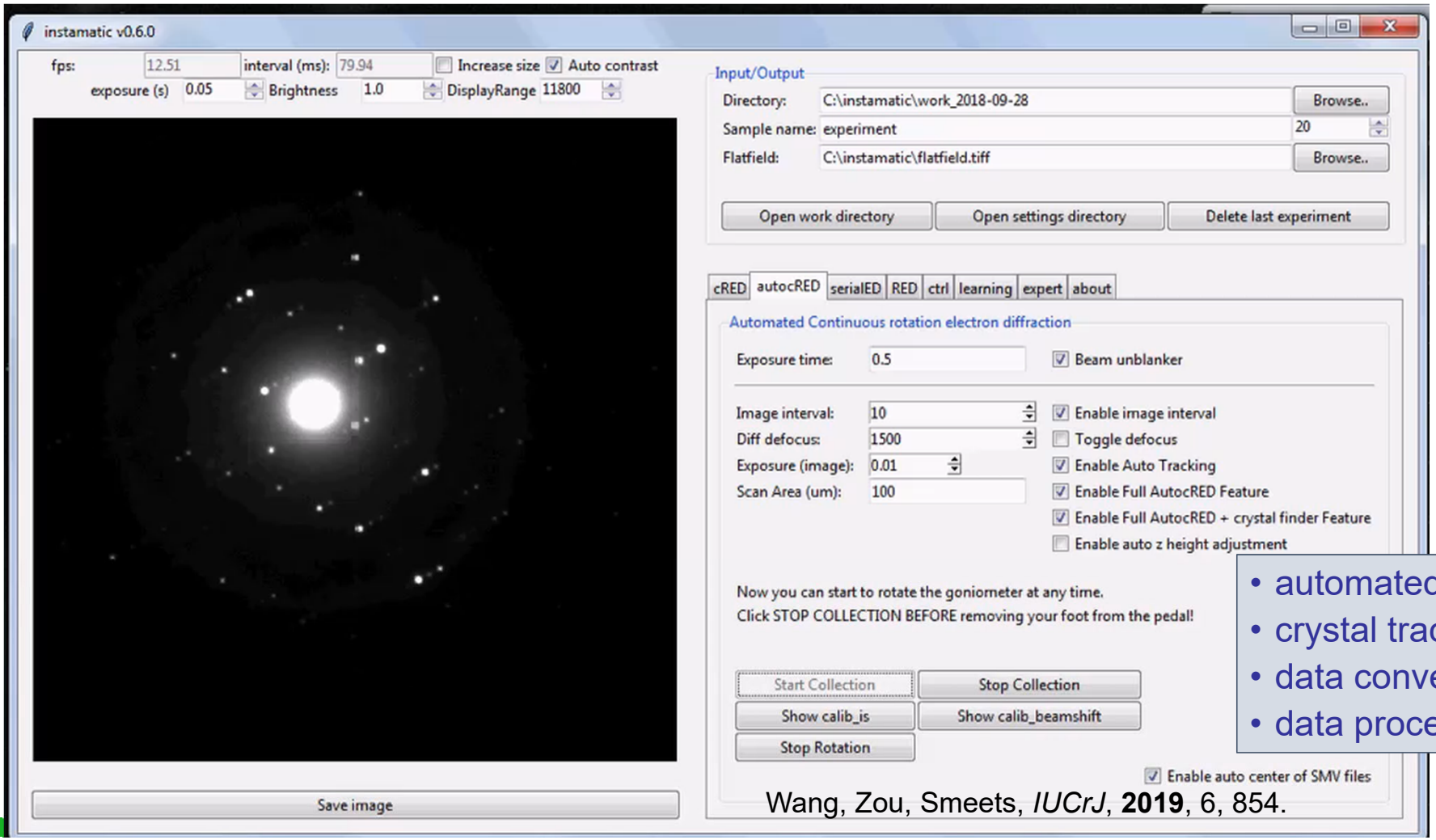
SerialRED

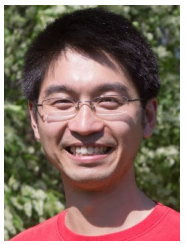


Wang, Zou, Smeets, *IUCrJ,* **2019**, *6*, 1.

Automated data processing and structure solution pipeline

SerialRED: automated cRED data collection





Bin Wang

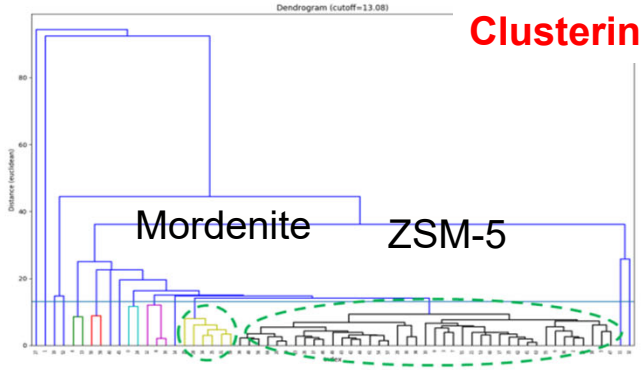
- automated crystal finding
- crystal tracking
- data conversion
- data processing

Wang, Zou, Smeets, *IUCrJ*, **2019**, 6, 854.

Edtools: Hierarchical clustering for high throughput phase analysis and data merging

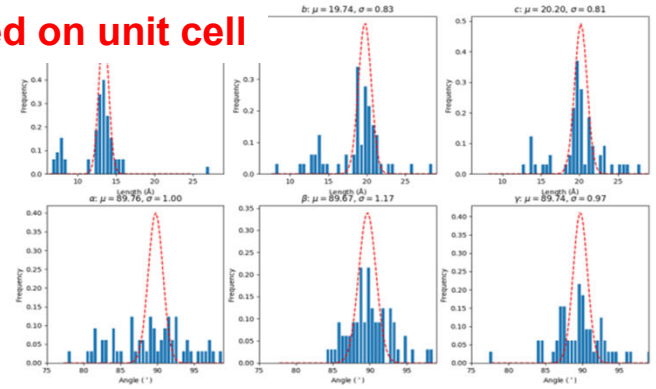
Stef Smeets

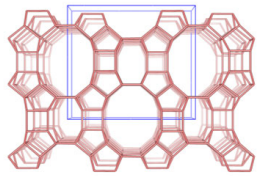
(a)



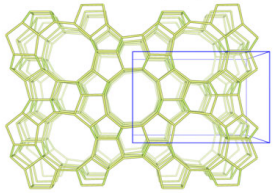
Clustering based on unit cell

(b)

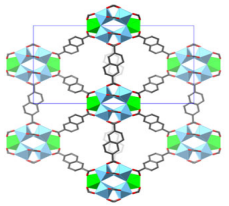




Mordenite (001)



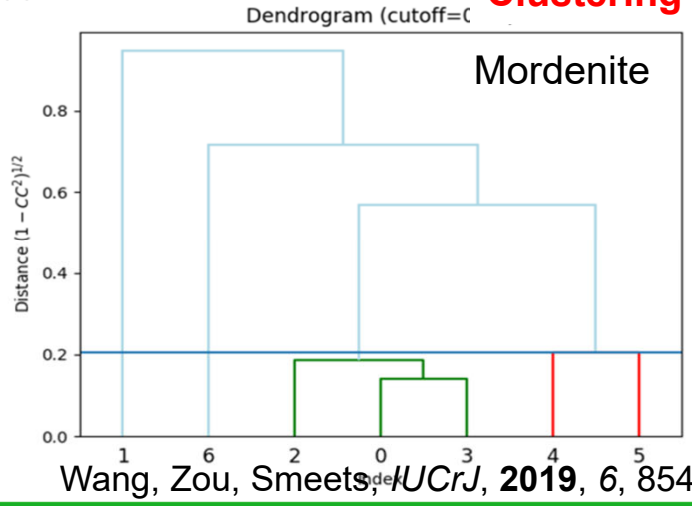
ZSM-5 (010)

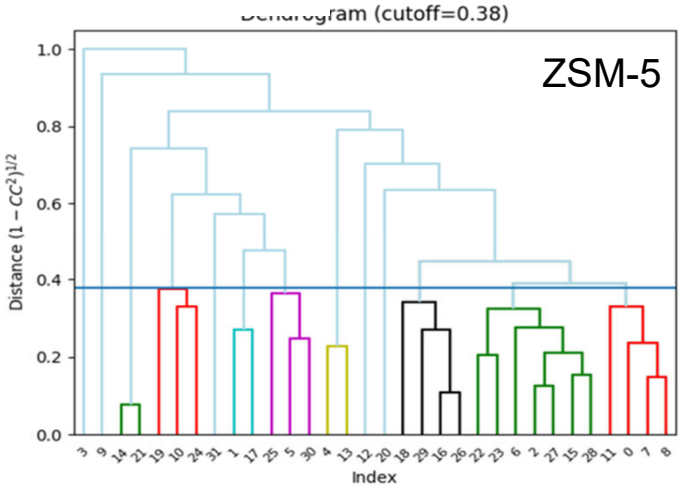


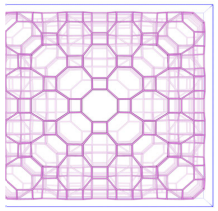
PCN-416 (100)

(d)

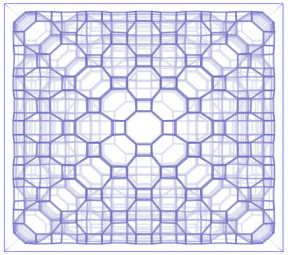
Clustering based on intensities







ZSM-25 (100)



PST-20 (100)

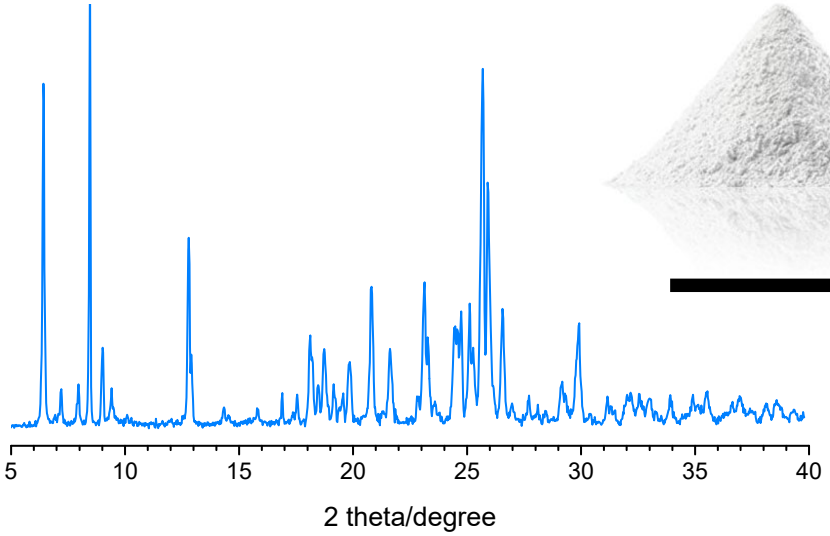
Structures solved using SerialRED

Wang, Zou, Smeets, *IUCrJ*, **2019**, *6*, 854

SerialRED: high-throughput phase analysis of polycrystalline samples

Yi Luo

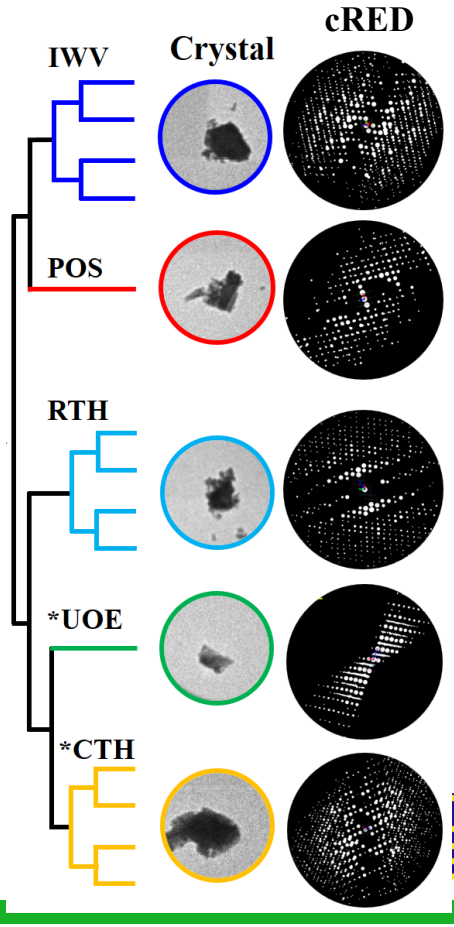
High-throughput synthesis screening combining multiple T elements (Si, Al, B, and Ge)



cRED datasets from 340 crystals collected in 6 hours

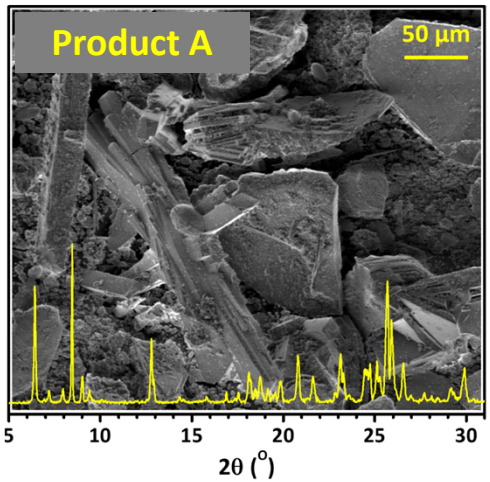
Five different zeolites found:
Major phases: **RTH, IWV, *CTH**
Minor phases: **POS, *UOE**

Hierarchical cluster analysis



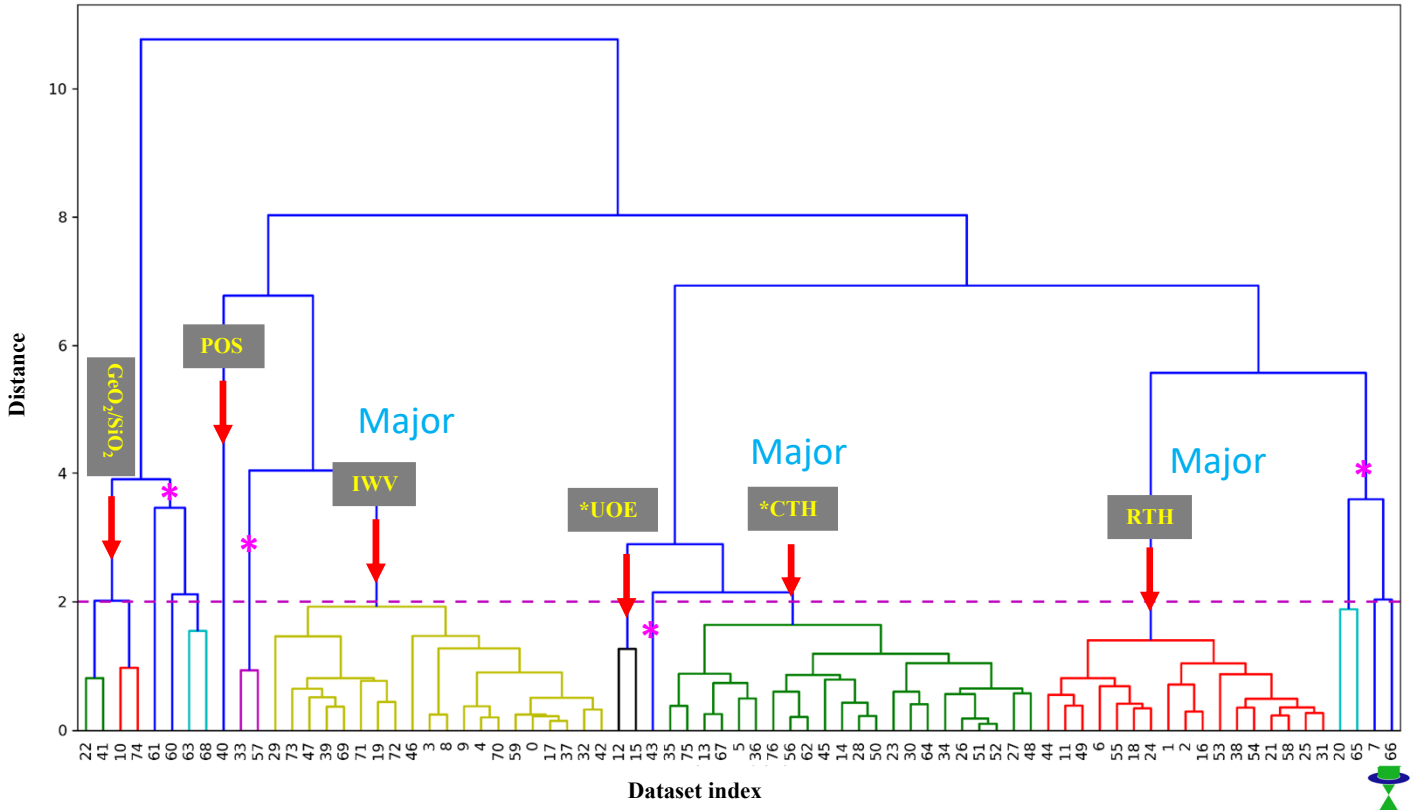
Y. Luo, B. Wang, S. Smeets, J. Sun, W. Yang, X. Zou, *ChemRxiv,* preprint, **2021,** DOI:10.33774/chemrxiv-2021-34v44

SerialRED: high-throughput phase analysis of polycrystalline samples



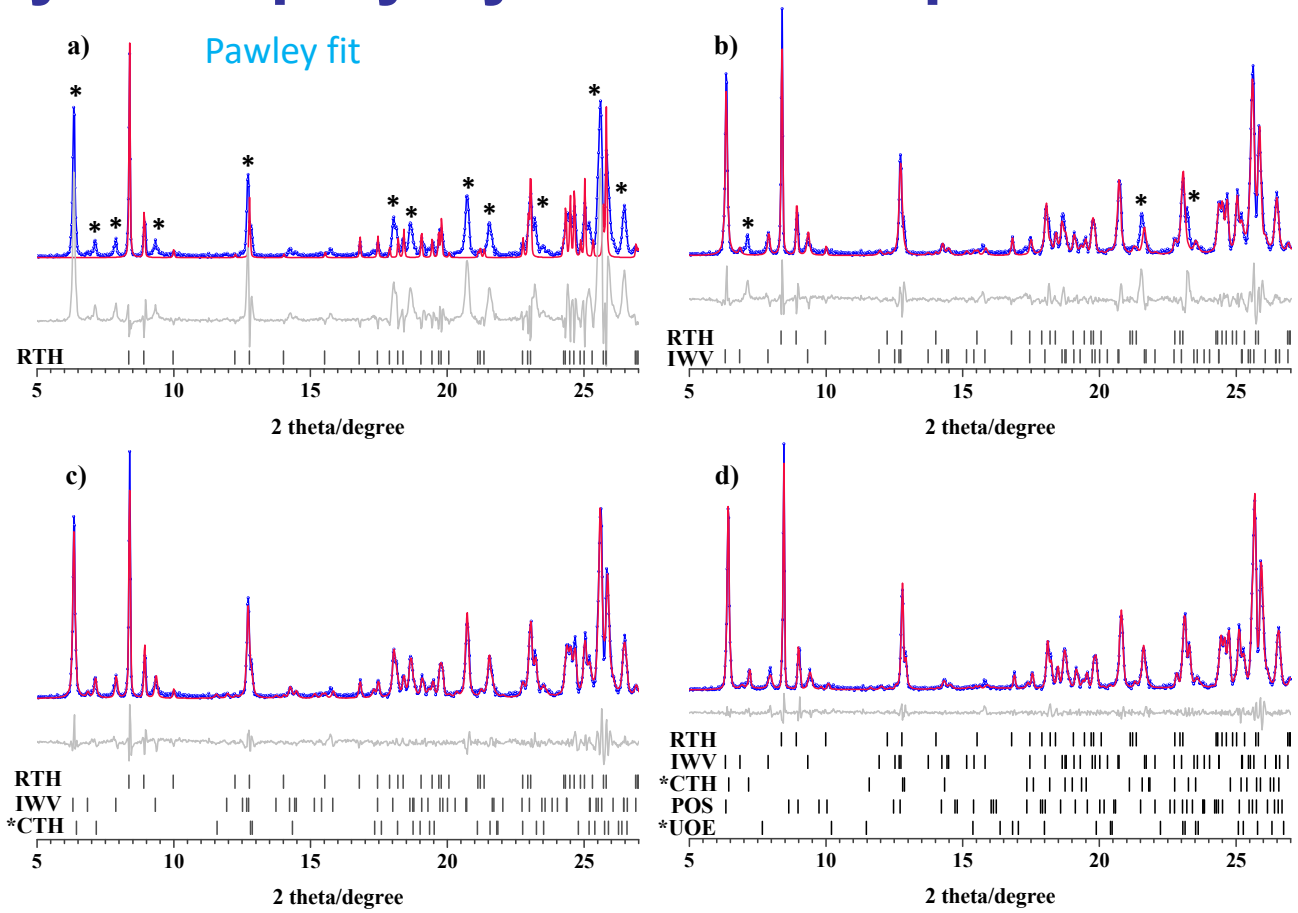
- Mixture
- Needle- and plate-like crystals

Bi-phase mixture?



Five distinct zeolite phases in one batch, the most complex mixture in zeolite chemistry

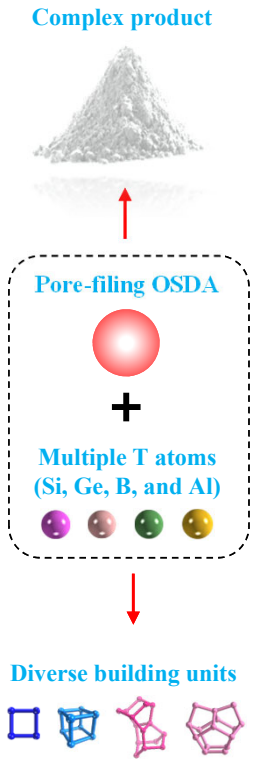
SerialRED: high-throughput phase analysis of polycrystalline samples



- RTH, IWV and *CTH are the major phases
- The contents of POS and *UOE are too low to be dectected by PXRD
- POS, *UOE, and RTH share the same morphology
- IWV and *CTH have similar unit cells

Complex synthesis system of polycrystalline materials (zeolites)

OSDA/Si=**0.6**, HF/Si=**0.6**, H_2O/Si=**10**				
		Si/Ge		
		15	**10**	**5**
(Si+Ge)/T^{III} =∞		**TON**	**TON+POS**	**POS**
(Si+Ge)/Al	**100**	**NON**	**Amor. +Den.+*UOE**	**Amor.+Den.+*UOE**
	20	**Amor.**	**Amor. +RTH+IWV+*CTH**	**RTH+*UOE+POS+IWV+*CTH**
	15	**Amor.+Den.+*UOE+IWV**	**Den.+RTH+*UOE+POS+IWV+*CTH**	**RTH+*UOE+POS+IWV+*CTH**
	10	**RTH**	**RTH+*UOE+POS+IWV+*CTH**	**RTH+IWV+*CTH**
	5	**Amor.**	**Amor.**	**RTH**
(Si+Ge)/B	**100**	**NON**	**Amor.**	**Amor.+POS**
	20	**Amor.+SFE**	**SFE**	**SFE+TON**
	15	**Amor.+SFE**	**SFE**	**SFE+TON**
	10	**SFE**	**SFE**	**SFE**
	5	**SFE**	**SFE**	**SFE**



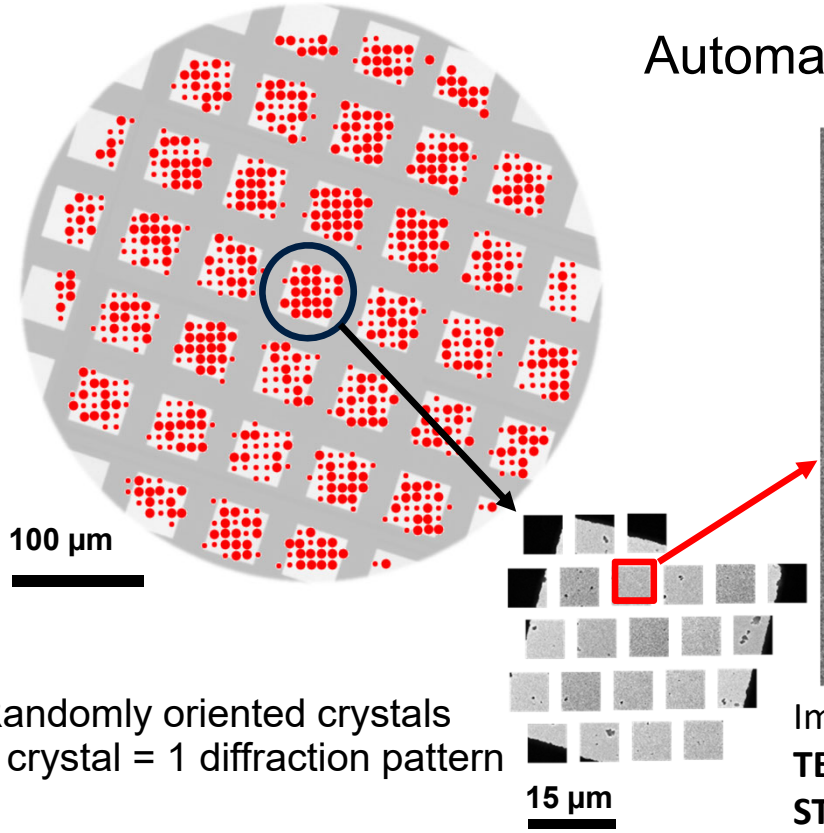
Y. Luo, B. Wang, S. Smeets, J. Sun, W. Yang, X. Zou, *ChemRxiv,* preprint, **2021,** DOI:10.33774/chemrxiv-2021-34v44

SerialED: automated high-throughput ED data collection

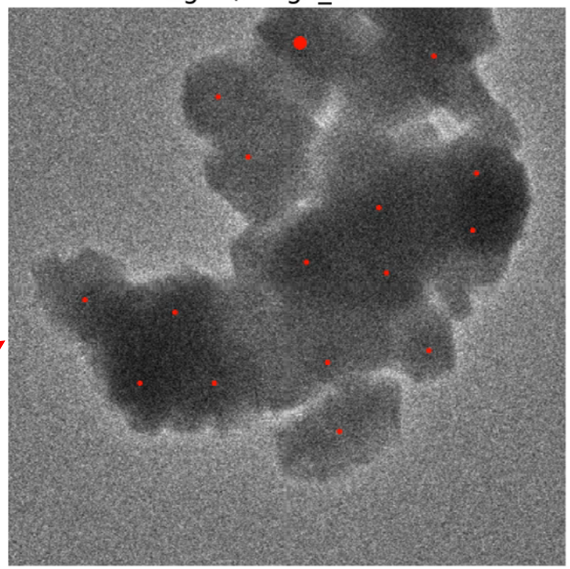
Stef Smeets

Stockholm University

Automatically collect diffraction data on > 3500 crystals/h



images\image_0000.h5



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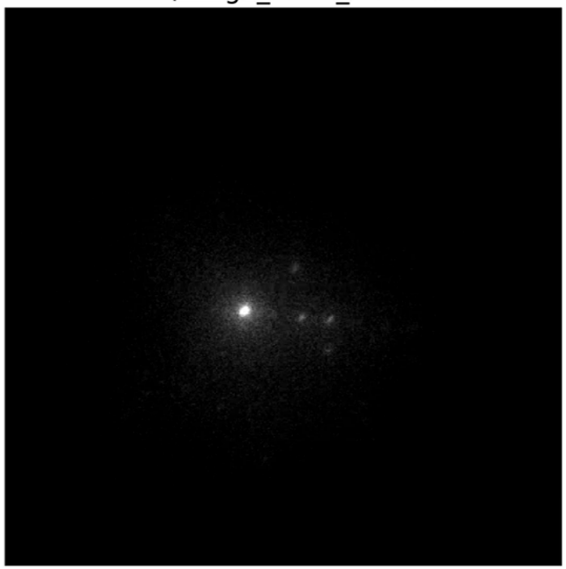


Randomly oriented crystals
1 crystal = 1 diffraction pattern

Implementations:

TEM Map/Small Molecules: Smeets *et al.*, *J. Appl. Cryst.* 51 (2018): 1262.

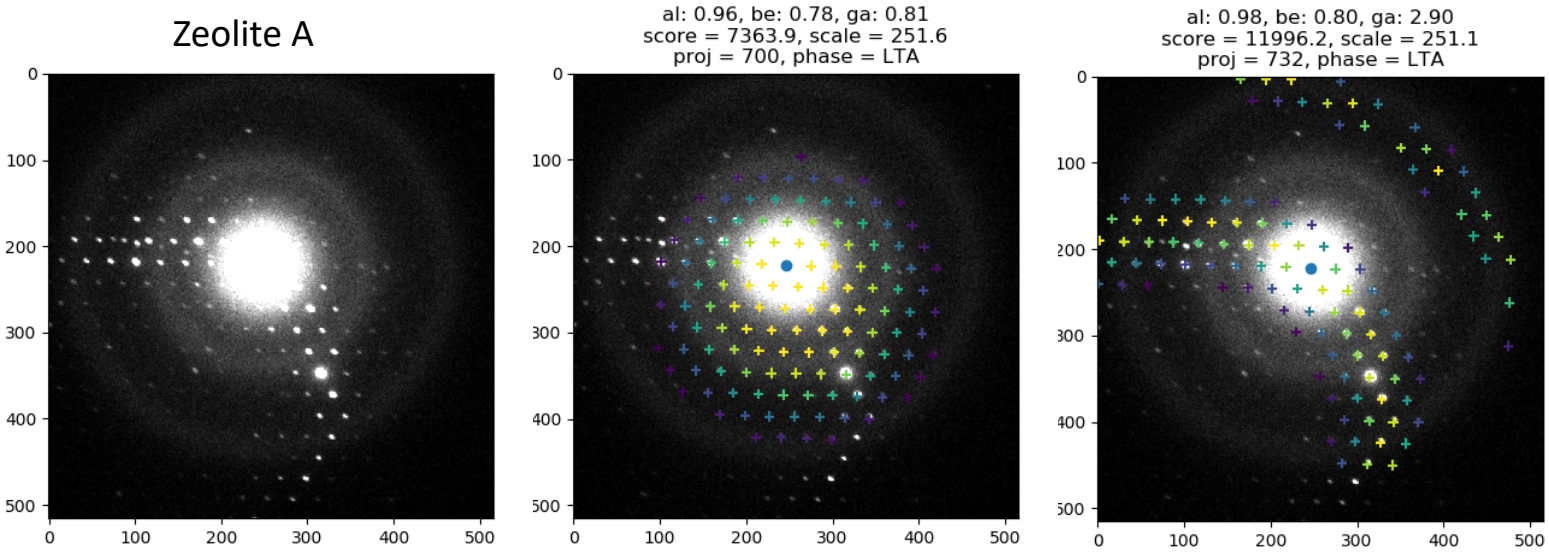
STEM Map/Proteins: Bücker *et al.*, *Nat. Comm.* 11 (2020): 996.

Source code: www.github.com/stefsmeets/instamatic

Structure determination using SerialED data

Orientation determination & reflection indexation

- Forward projection model using known lattice parameters
- Generate pattern library of all possible orientations (~1.5 Million in $P1$)
- Match best orientation and index data



Smeets, Zou, Wan, *J. Appl. Cryst*. 51, 1262-1273.

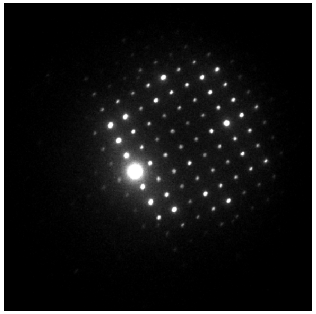
Source code: www.github.com/stefsmeets/problematic

Courtesy Stef Smeets

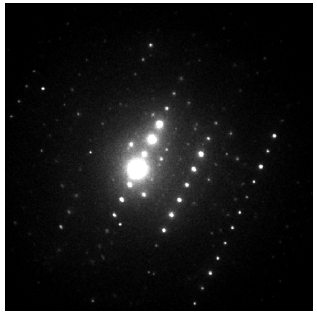
Automatic crystal selection using machine learning

Jonas Ångström

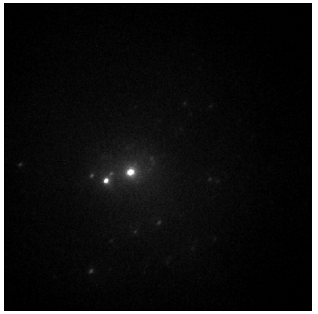
- A deep convoluted neural network (CNN) was used to distinguish between *good* and *bad* diffraction patterns.
- ~78.000 diffraction patterns were used to train the CNN.
- Each diffraction pattern is passed through the CNN, a prediction score between 0.0 and 1.0 is given (any value >0.5 corresponds to a *good* quality diffraction pattern).



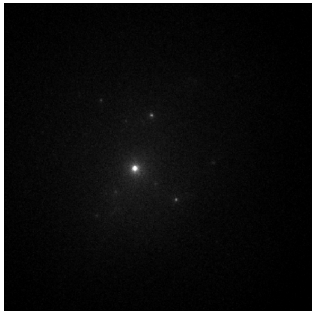
Prediction: 1.0



Prediction: 1.0



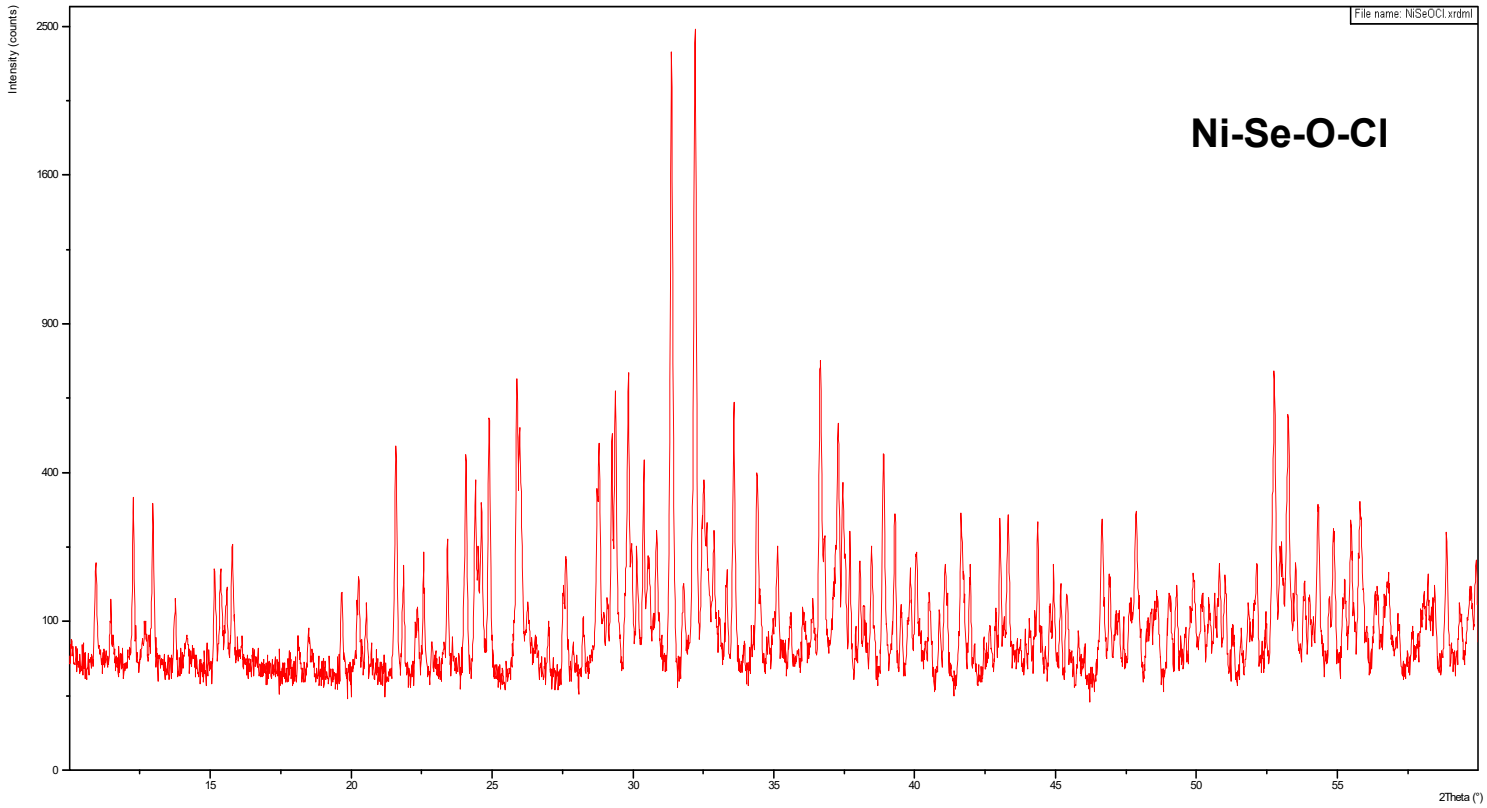
Prediction: 0.26



Prediction: 0.25

Cichocka, Ångström, Wang, Zou, Smeets, *J. Appl. Cryst.* **2018**, *51,* 1652

A multi-phasic powder sample in the Ni-Se-Cl-O system

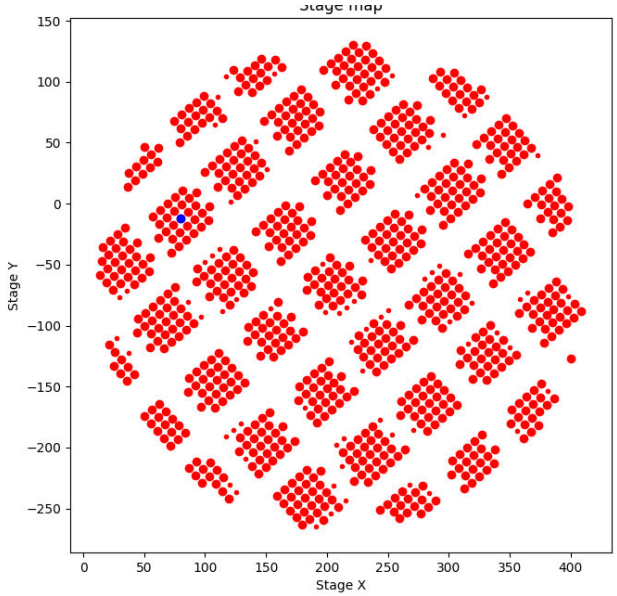


Yun, Wan, Rabbani, Su, Hovmöller, Johnsson and Zou, J. Appl. Crystallogr. **2014**, *47*, 2048

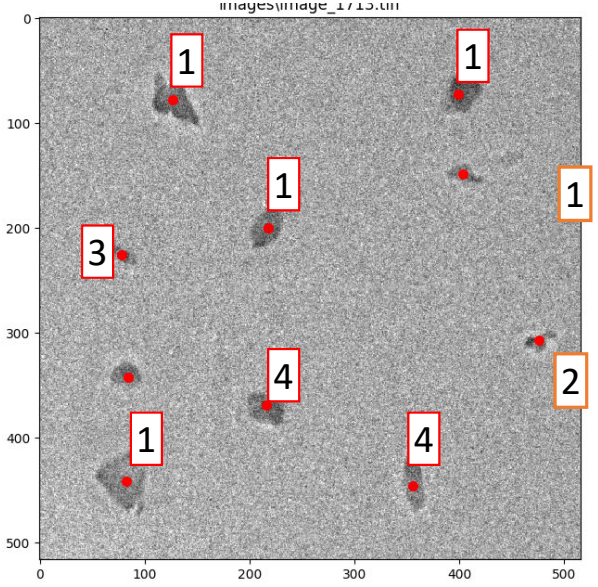
SerialED: phase analysis and orientation mapping
(Search for four phases in Ni-Se-O-Cl system*)

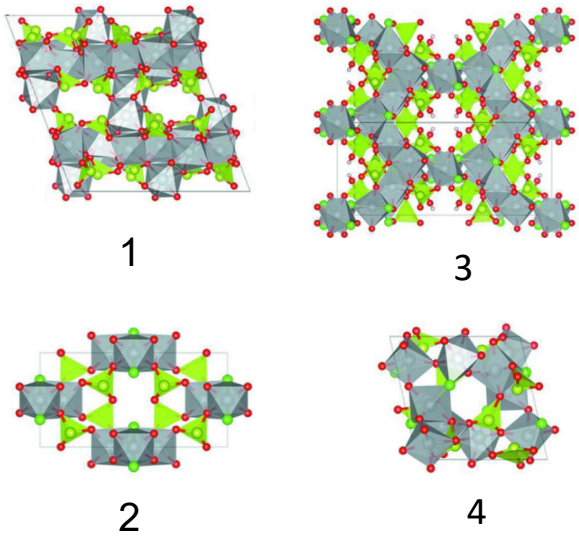
Stockholm University

Stage map



Identify crystals and orientation





Total scan area: 400 x 400 μm^2
No. images: 925 images
No. ED frames: 6171
Total data collection time: ~90 min

Courtesy Stef Smeets

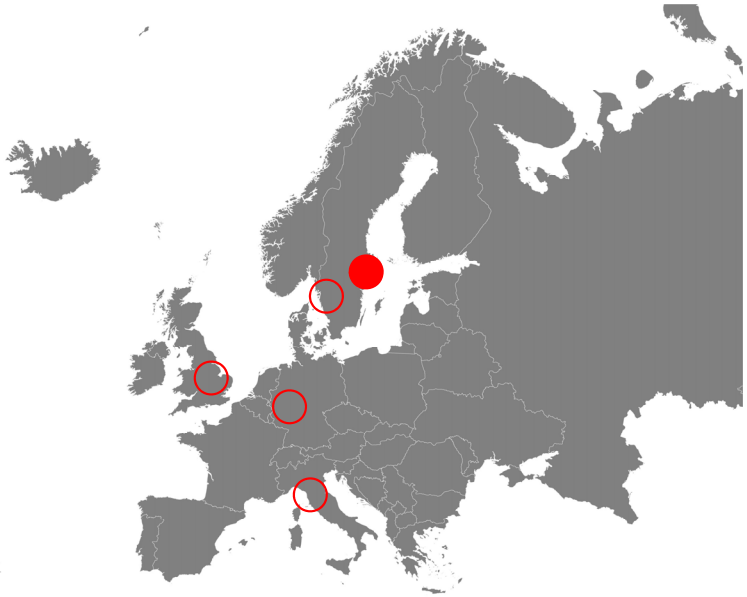
Searched phases:
1: $NiSeO_3$
2: $Ni_3Se_4O_{10}Cl_2$
3: $Ni_5Se_6O_{14}(OH)_2Cl_4$
4: $Ni_4Se_4O_{12}Cl_2$

Unpublished

*Yun et al., 2014, *J. Appl. Crystallogr.*, 47, 2048

ESR 11 – Overview

- Development of high-throughput serial (rotation) electron diffraction and its application for phase and structural analysis of pharmaceutics and metal-organic frameworks (MOFs)
  - To establish automation strategies and develop software for high-throughput serial (rotation) electron diffraction (SerialED/RED) data collection
  - To establish high-throughput protocols for data processing
  - To apply machine learning and clustering algorithms for phase analysis and structure determination of pharmaceutical compounds
  - To develop MOFs as new “crystalline sponges” for structural analysis of natural products and molecules with tiny (nano-/pico gram) quantity



Blank map made by Vemaps.com

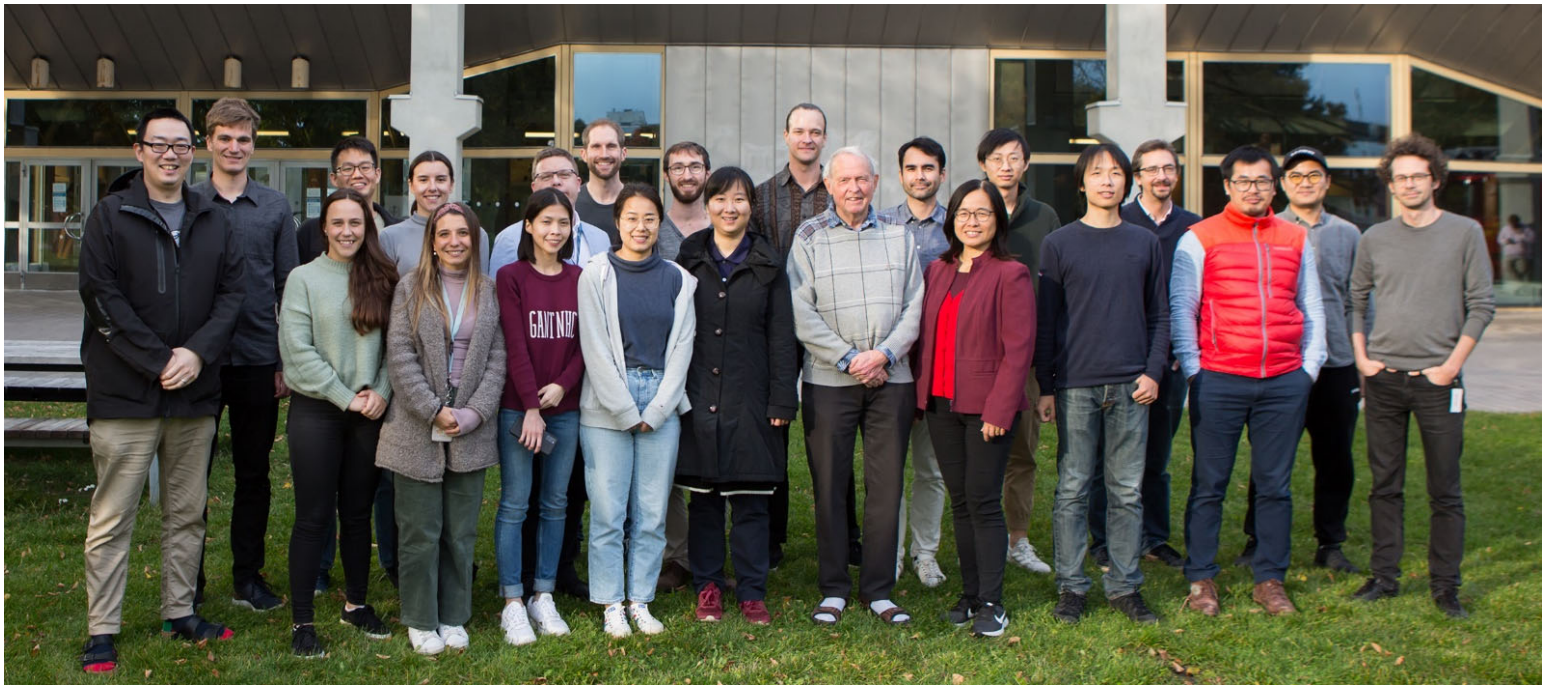
Supervisor: Xiaodong Zou

Planned Secondments:

- E. Mugnaioli, **IIT**
- U. Kolb, **JGU**
- D. Waterman, **STFC**
- S. Norberg, **AstraZeneca**

Stockholm University

Acknowledgements



Former members: Magdalena Cichocka, Peng Guo, Stef Smeets, Junliang Sun, Jie Su, Wei Wan, Bin Wang, Daliang Zhang, Max Clabber, Jingjng Zhao

Collaborators: Norbert Stock (Univ. of Kiel), Hong-cai Zhou (Texas A&M Univ.), Lynne McCusker & Christian Baerlocher (ETH Zurich), Avelino Corma (ITQ), Suk-Bong Hong (POSTECH), Simon Parson (Univ. Edinburgh), Min Liu (Sun Yat-sen Univ.), Weimin Yang (SRIPT, SINOPEC), Karl-Petter Lillerud (Univ. Oslo)

Free software available at

https://www.mmk.su.se/zou/electron-crystallography-software

Postdoc positions available

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xzou@mmk.su.se