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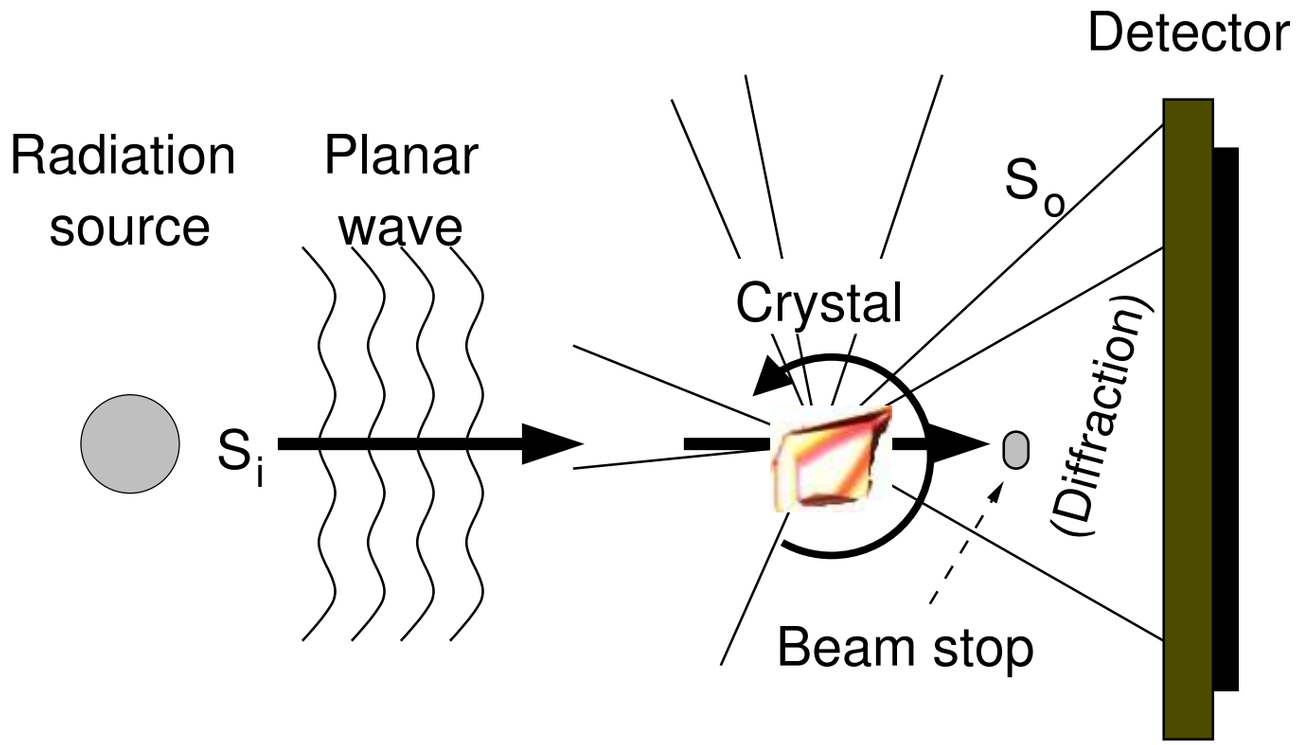
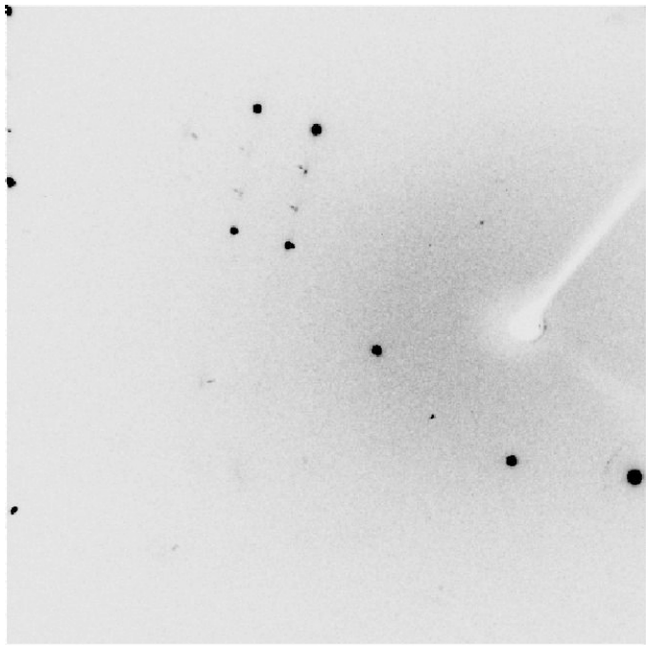
# Macromolecular Crystallography with Nanocrystals based on Electron Diffraction

CCP4 Study Weekend
10th January 2017

# 1 - Electron Diffraction

# Structure Determination by Single Crystal Diffraction



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

Laue equations:

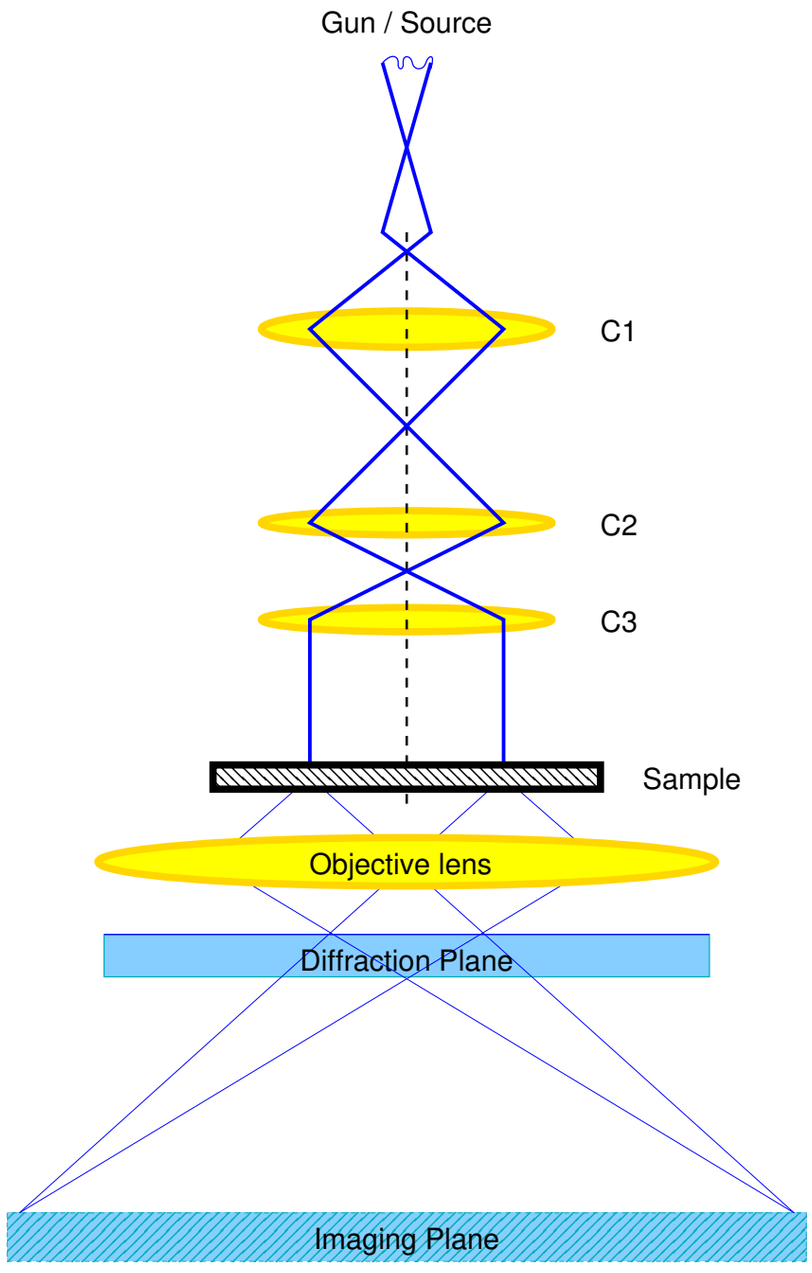
$$(\vec{S}_o - \vec{S}_i) \cdot \vec{a}^* = h$$
$$(\vec{S}_o - \vec{S}_i) \cdot \vec{b}^* = k$$
$$(\vec{S}_o - \vec{S}_i) \cdot \vec{c}^* = l$$

Independent of radiation type: $X$, $e^-$, $n$

# Electrons as Radiation Source

- wave–particle dualism (*cf* de Broglie wavelength)
- typical electron energy: 100–300keV (200keV = 0.02508Å)
- suitable for, but also **require** small samples: 100keV: $< 100nm$, 200keV: $< 300 - 500nm$
- electrons interact with charge: map = electrostatic potential inside crystal
- strong interaction: multiple scattering events do occur
- require high vacuum throughout ($10^{-8}$mbar)

# The Lens System

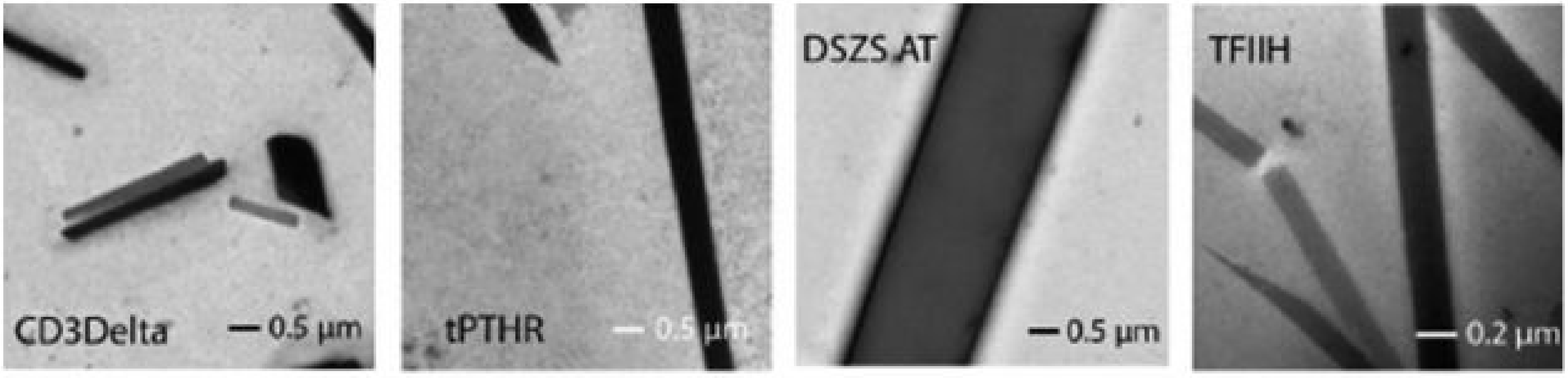



- Lenses C1–C3 shape beam
- Crystallography: Parallel beam
- Objective lens: sets effective detector distance to back-focal plane = diffraction mode
- C3 not present in all microscopes

**Lenses cause distortions.**

# 2 - Motivation

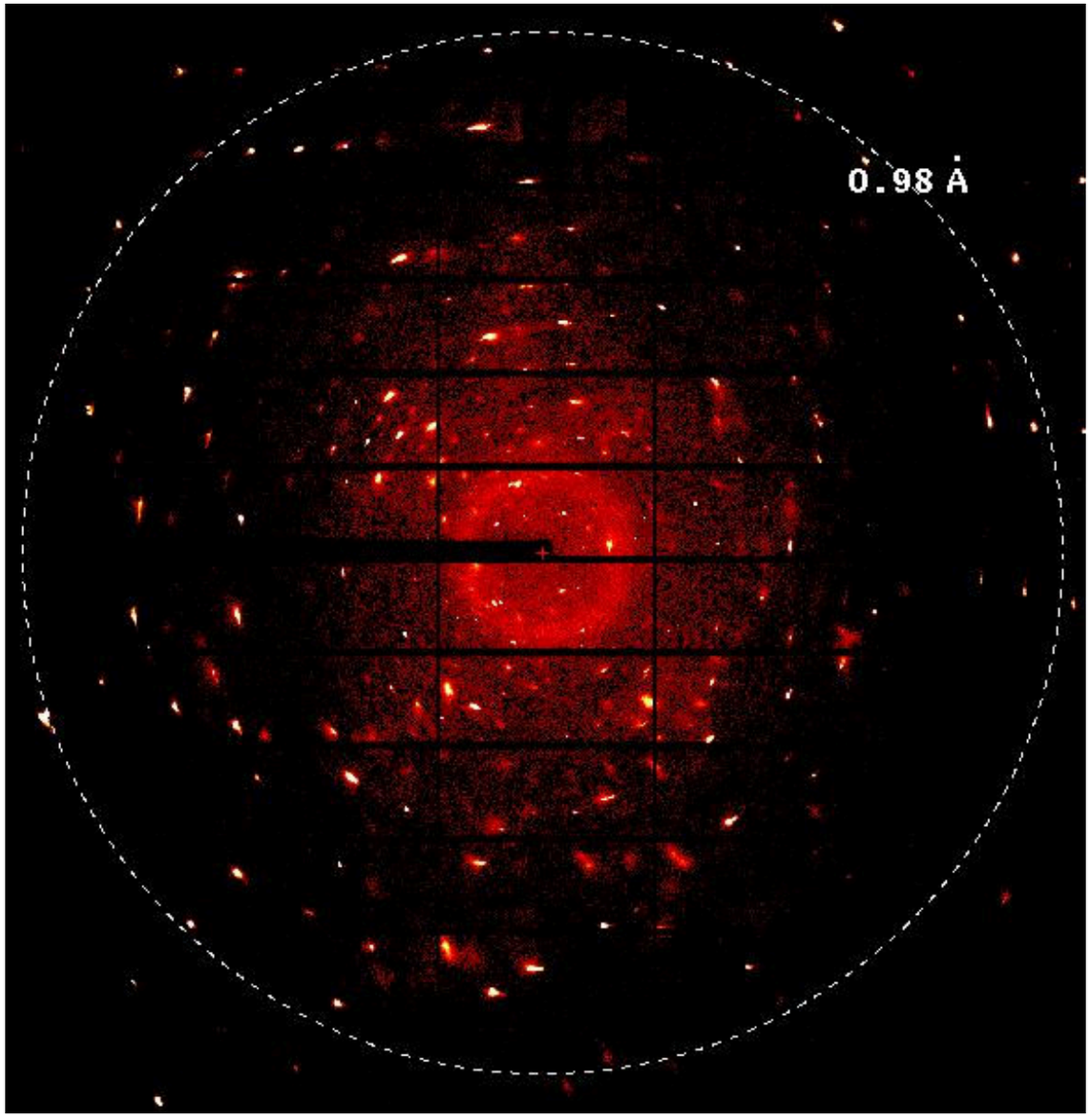
# “Failed” Crystallisation Drops viewed through TEM

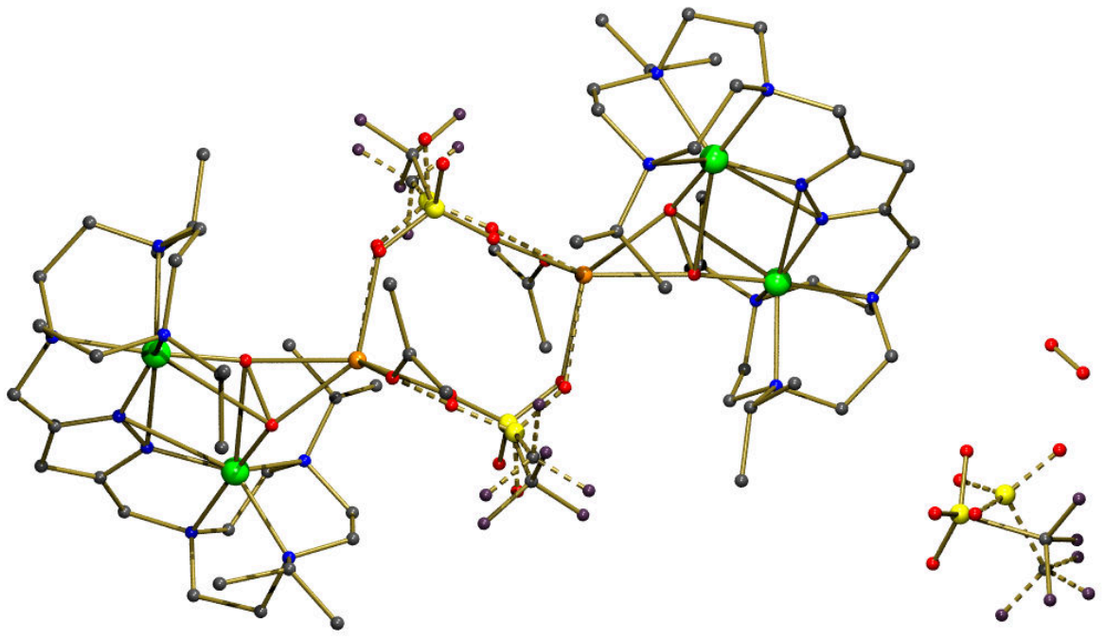



Stevenson,. . . , Calero, PNAS (2014) 111, 8470–8475 / Calero, . . . , Snell, Acta Cryst (2014) F70, 993–1008

*“Our work [. . . ] shows that [nanocrystals] are commonly observed in crystallization drops [. . . ]”*

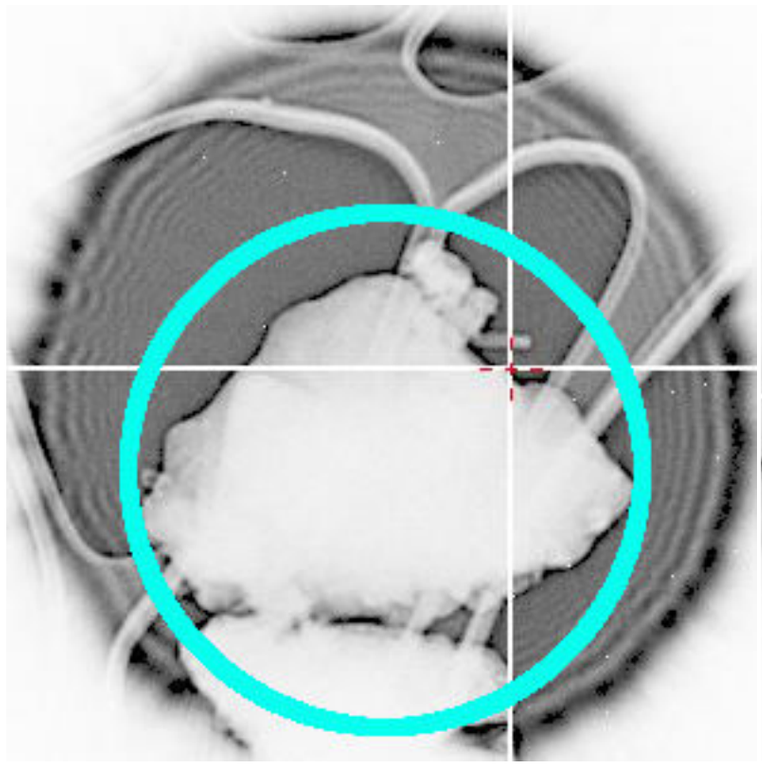
# Crystalline Disorder — a Matter of Size?



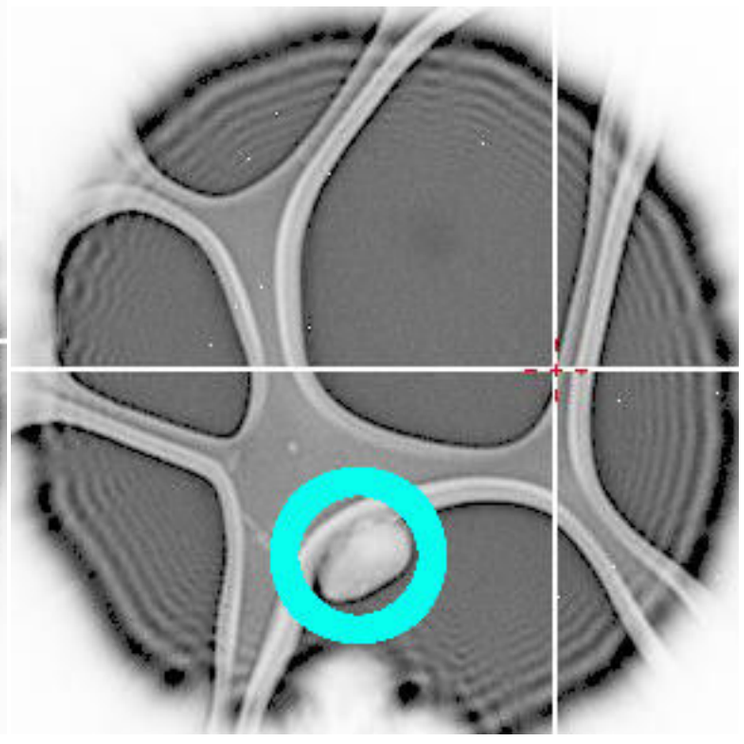



K Dalle, T Gruene, S Dechert, S Demeshko, and F Meyer, *A weakly coupled biologically relevant* $Cu_2^{II}(\mu-\eta^1:\eta^1-O_2)$ *cis-peroxo adduct that binds side-on to additional metal ions* JACS (2014), 136, 462–46

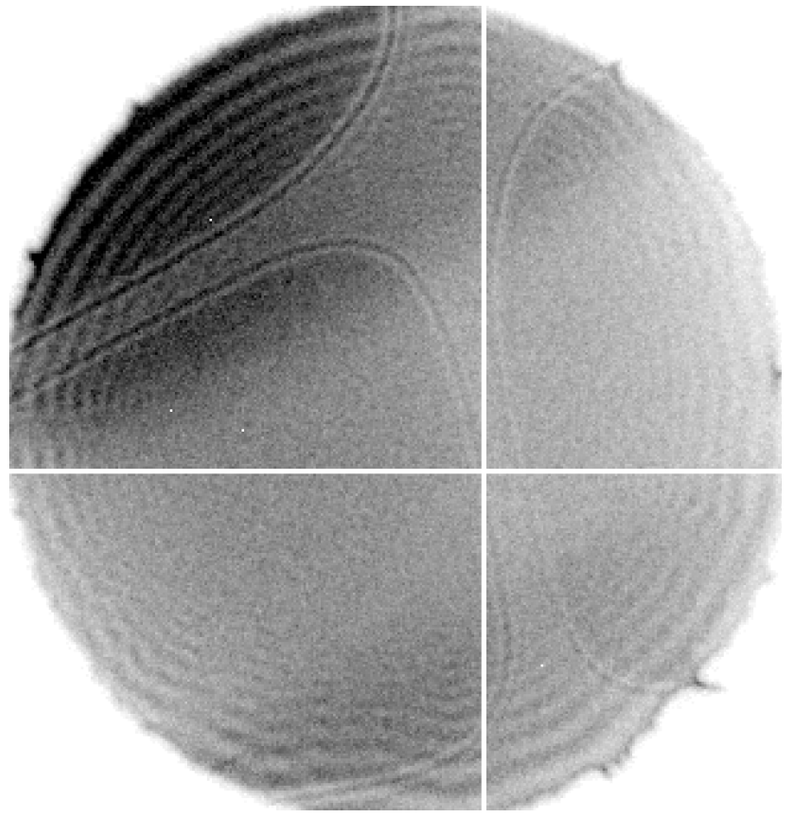
# Nanocrystals



**Novartis I**:
$\varnothing = 1,700nm = 1.7\mu m$



**Novartis II**:
$\varnothing = 500nm = 0.5\mu m$

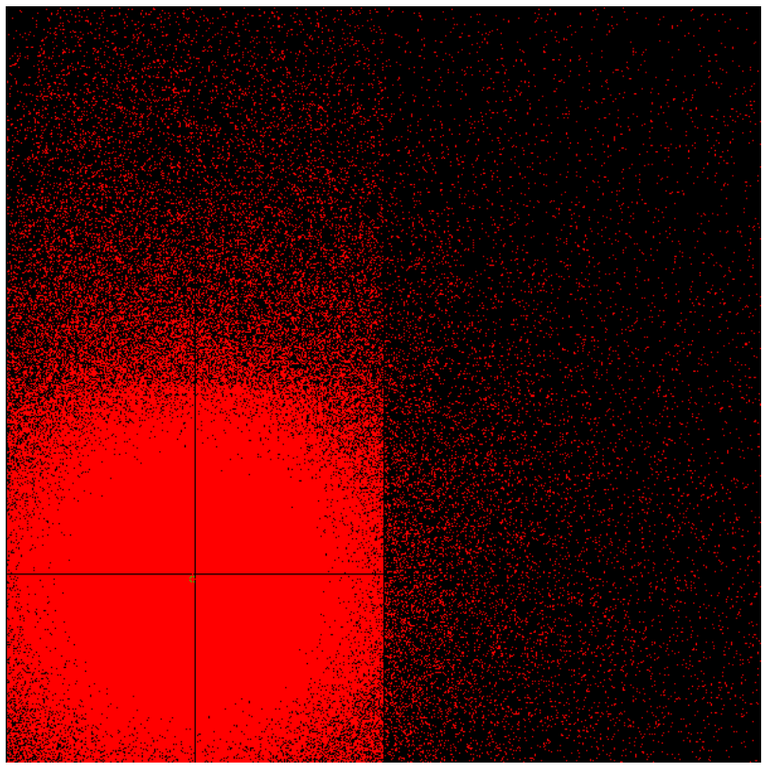


**Thermolysin:**
$\approx 2 \times 1 \times$ very thin $\mu m^3$
Solvent reduces contrast

# 3 - Electron Diffraction Instrumentation

# Medipix / Timepix Detector Family

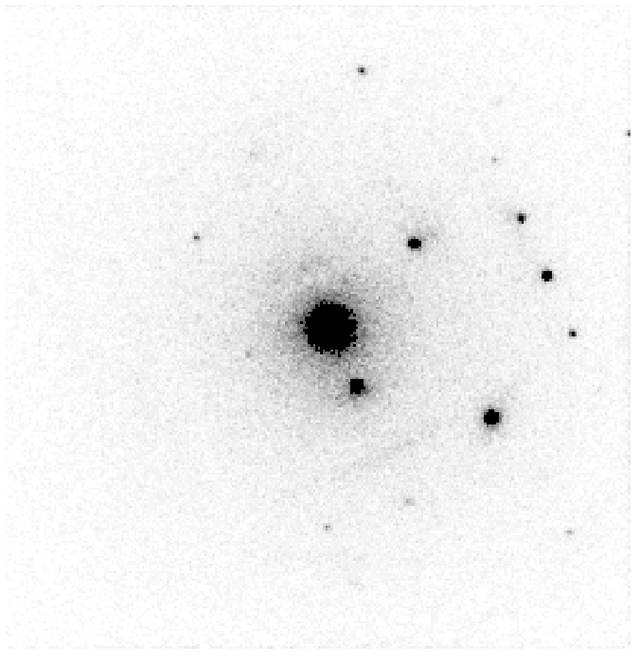
- first hybrid pixel detector for electrons (*cf.* Pilatus / Eiger)
- no read–out noise
- high dynamic range
- fast read–out: non–stop sample rotation (“shutterless data collection”)
- 512x512 and 1024x1024 pixel cameras installed in Basel (and Pisa (Prof. Mauro Gemmi) and Stockholm (Prof. Sven Hovmöller))



Diffraction image from a MFI type zeolite:
black = 0 counts
red $\geq$ 1 (carbon scatter + crystal signal) count

# Eiger Chip

- Developed at PSI
- 256x256 pixel test chip with 200keV instrument
- pilot for improving phosphor to higher energies $\geq$ 300 keV
- higher read–out (up to 8kHz), much lower dead time
- Next: Jungfrau and Mönch with $Si$, $GaAs$, or $CdTe$



Electron diffraction (from an inorganic compound) on a 256x256 Eiger chip

# The Rotation Method

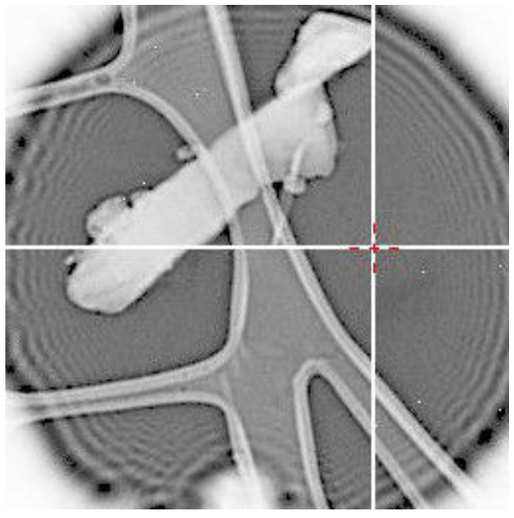
- Material Science: diffraction from oriented crystals
- Rotation Method: random orientation
- Standard (“Universal”) data collection mode for organic and macromolecular crystallography
- First (?) applications in electron crystallography:
  - Prof. Ute Kolb, Mainz — AD3DT, step motion ($\approx$ 2011)
  - Dr. Wei Wan, Stockholm — RED, beam precession + sample rotation ($\approx$ 2013)
  - Prof. Jan Pieter Abrahams — first diffraction pattern from 3D protein crystals (2011)
  - Dr. Tamir Gonen — first 3D crystal structure in PDB (3J4G, 2013)
- Currently: No connection between goniometer and detector: “manual” rotation leads to very inaccurate oscillation width
- Benefit from well advance integration/ scaling programs (XDS, DIALS, SAINT, evalCCD)

# 4 - Example Structures

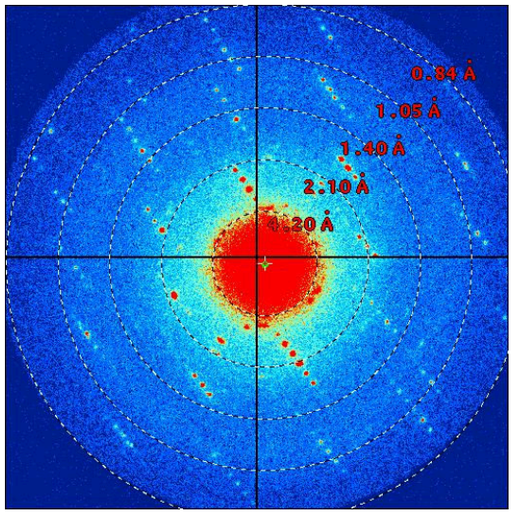
# Pharmaceutical I: Visualisation of Hydrogen Atoms

H–atom positions can be refined against electron diffraction data

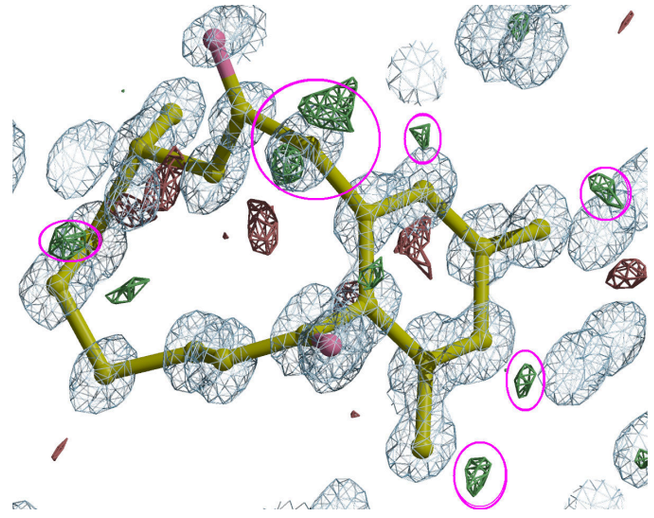
CCDC: IRELOH, Dai et al., Eur. J. Org. Chem (2010), 6928-6937



- Field of view: $3\mu m$
- Crystal: $1.6\mu m \times 400nm$



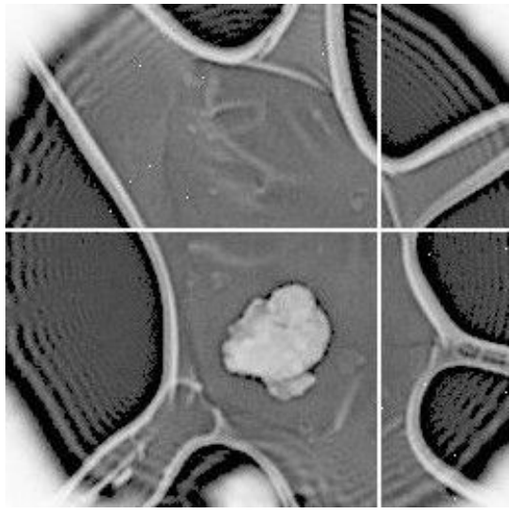

- $d_{\text{min}} < 0.8$Å
- $I/\sigma_I(0.91 - 0.81\text{Å}) : 1.8$
- $P2_12_12_1$: 85% completeness with 3 crystals
- a=8.06Å b=10.00Å c=17.73Å



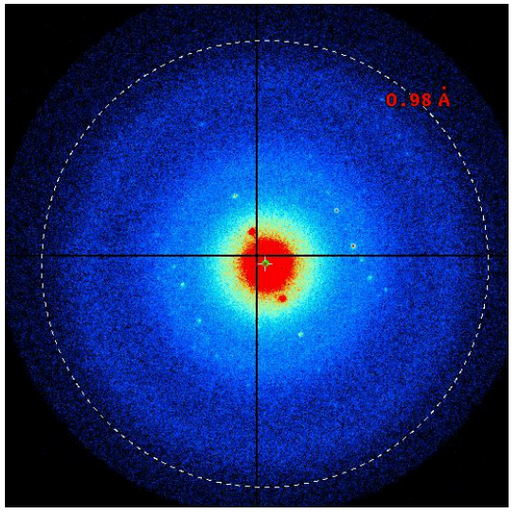
- **Refinement** of hydrogen atom positions with mild restraints (SADI))
- 1334 reflections, 195 parameters, 156 restraints (RIGU)
- $R1 = 15.5\%, R_{\text{complete}} = 18.5\%$

# Pharmaceutical II: Differentiation of Atom Types

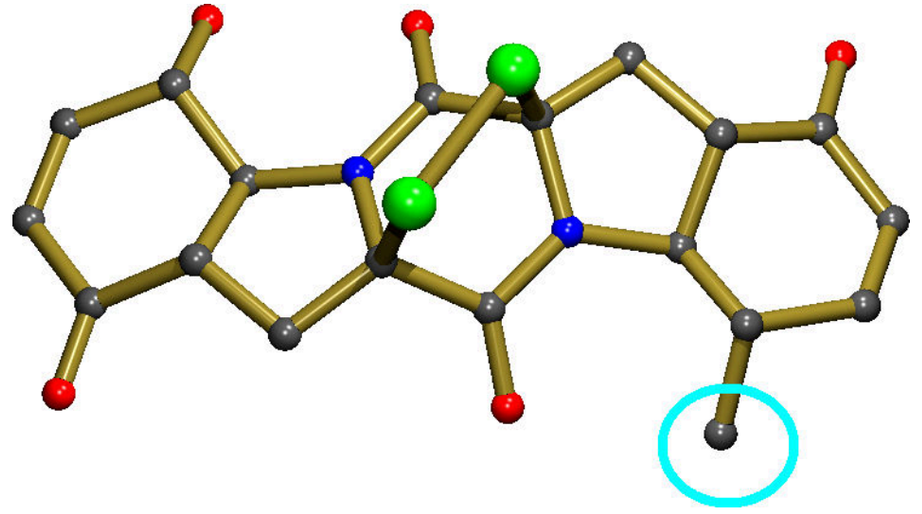
Data quality: recognition of atom types, C *vs.* O *vs.* N *etc.* (CCDC: EPICZA)



- Field of view: $3\mu m$
- Crystal: $400nm$ diameter

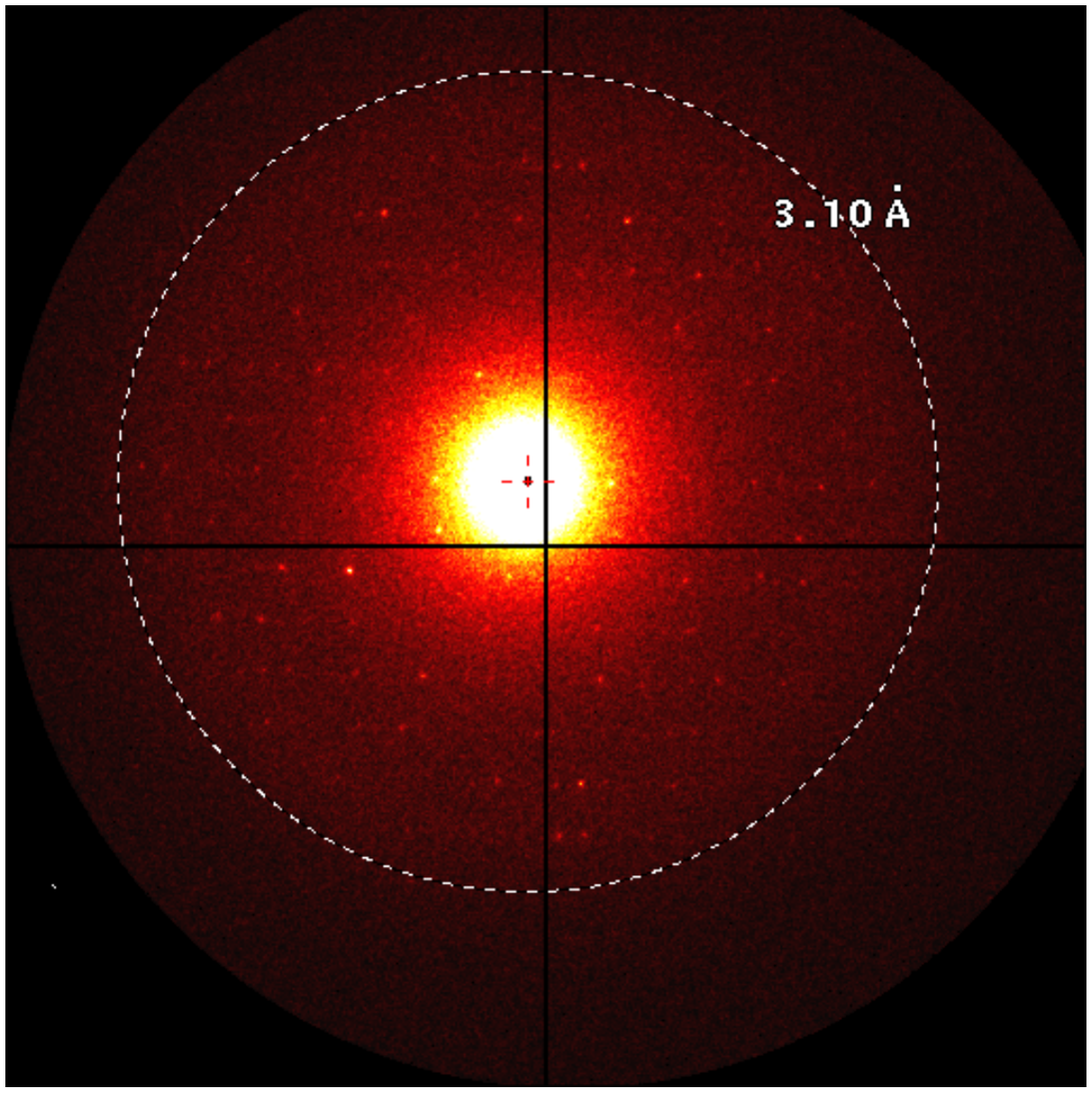



- $d_{\text{min}} = 0.80$Å
- $I/\sigma_I(0.90 - 0.80\text{Å}) : 2.5$
- $P2_12_12_1$: 92% completeness with 6 crystals ($d_{\text{min}} > 0.84$Å : 96%)

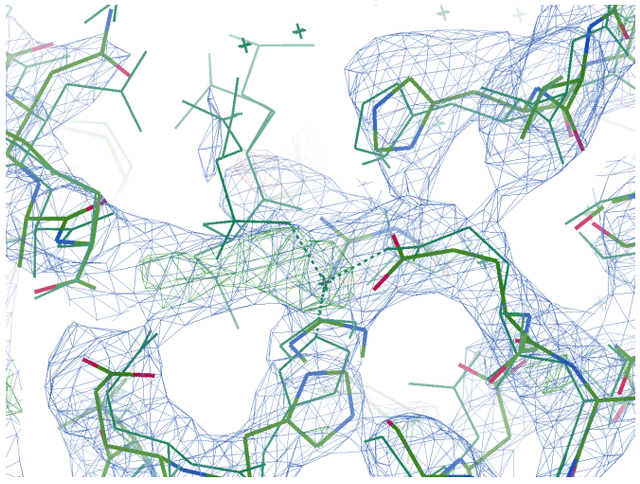


- Direct methods: only 1 wrong atom type
- Visualisation of hydrogen atoms
- 1806 refl., 258 param., 267 restraints
- $R1 = 18.5\%, R_{\text{complete}} = 21.9\%$

# Thermolysin (sample courtesy Ilme Schlichting)



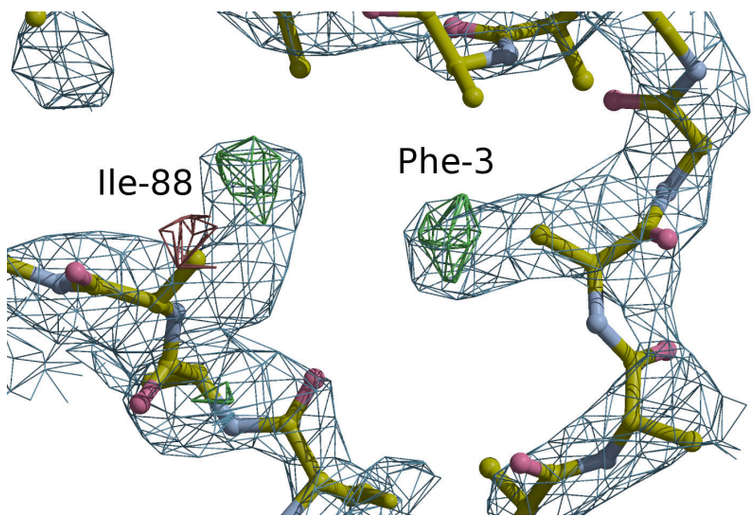

- Spacegroup $P6_122$
- Unit Cell 94.3 94.3 130.4 $90^\circ$ $90^\circ$ $120^\circ$
- $d_{\text{min}} = 3.5$Å
- 72.4% completeness
- MR with 3DNZ poly Alanine: TFZ=26.4, LLG=433
- Buccaneer: side chain extension 315/316
- Refmac5: R1/“Rfree” = 28.0% / 29.9% (4N5P w/o water)



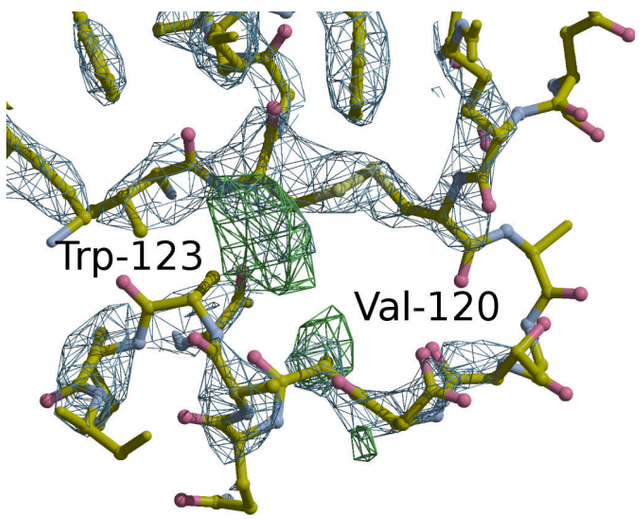
# Lysozyme

| | Single crystal | Merged data |
|---|---|---|
| **Data integration** | | |
| Space group | $P2_12_12$ | |
| Unit cell dimensions | | |
| a, b, c (Å) | 104.56, 68.05, 32.05 | |
| α, β, γ (°) | 90.0, 90.0, 90.0 | |
| Number of crystals | 1 | 6 |
| Resolution (Å) | 32.05-2.50 (2.57-2.50) | 57.04-2.50 (2.57-2.50) |
| $R_{merge}$ (%) | 31.7 (107.3) | 35.7 (113.2) |
| I/σI | 2.92 (1.10) | 2.87 (1.10) |
| Completeness (%) | 41.0 (40.5) | 69.0 (51.3) |
| Reflections | 9518 (817) | 25148 (1373) |
| Unique reflections | 3445 (236) | 5808 (299) |
| Redundancy | 2.76 (3.46) | 4.33 (4.59) |
| | | |
| **Refinement** | | |
| R1 (%) | 25.90 | 23.54 |
| $R_{complete}$ (%) [4] | 32.49 | 27.21 |
| <B> (Å$^2$) | 33.08 | 36.49 |
| RmsZ bonds | 0.779 | 0.765 |
| RmsZ angles | 0.974 | 0.911 |

1. MR (Phaser) from poly Ala **monomer** determines space group $P2_12_12$ (TFZ=19.8, LLG=335.3)
2. Side chain completion with Buccaneer all except 27 atoms
3. Refinement with refmac5




After MR: difference density for bulky side chains




Refined map guides model completion

# 5 - Electron Crystallogaphy in CCP4

1. Data processing: DIALS (with D. Waterman)
2. Scaling: Aimless
3. MR: Phaser / Molrep
4. Autobuilding: Buccaneer
5. Refinement: Refmac5
   - `SOURCE ELECTRON MB`
   - `MAPC FREE EXLCUDE`
6. Model Building: Coot

# 6 - Summary: Electron Crystallography for non–Material Scientists

| Sample Prep | Instrumentation | Proessing | Analysis |
|---|---|---|---|
| + from Powder<br>- from Solution<br>- Data sets / day | ++ Detector*<br>- Rot$^{n}$ Axis*<br>- Lenses<br>- Crystal Orient$^{n}$* | + Integration<br>- Param. Stability<br>+/- Scaling | ++ Direct Methods<br>+ Molec. Repl.<br>+ Refinement<br>- Potential Repr. |
| * Current project at LBR / PSI | | | |

# 7 - Acknowledgements


- Prof. J. P. Abrahams, Dr. E. van Genderen, M. Clabbers, Dr. T. Blum, C. Borsa, J. Heidler (group members at PSI / C-CINA, Basel)
- Novartis (Compounds)
- Dr. I. Nederlof, ASI (Medipix / Timepix)
- Dr. B. Schmitt, PSI Detector group
- Prof. K. Diederichs (XDS)
- Dr. W. Kabsch (XDS)
- Dr. D. Waterman (DIALS)
- Dr. Ilme Schlichting (samples of Thermolysin, Thaumatin, Lysozyme)
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- Prof. S. Hovmöller, University Stockholem