BREAKTHROUGH ADVANCES IN SYNCHROTRON XRPD:

a Powerful Product-Characterization Technique with Extended Applications to Pharmaceuticals

Presented by

Arturo Araque, PhD
Head of Excelsus Consortium

Fabia Gozzo, PhD
Head of Excelsus Structural Solutions

Excelsus

Produced by:
webinar

Quality Assurance

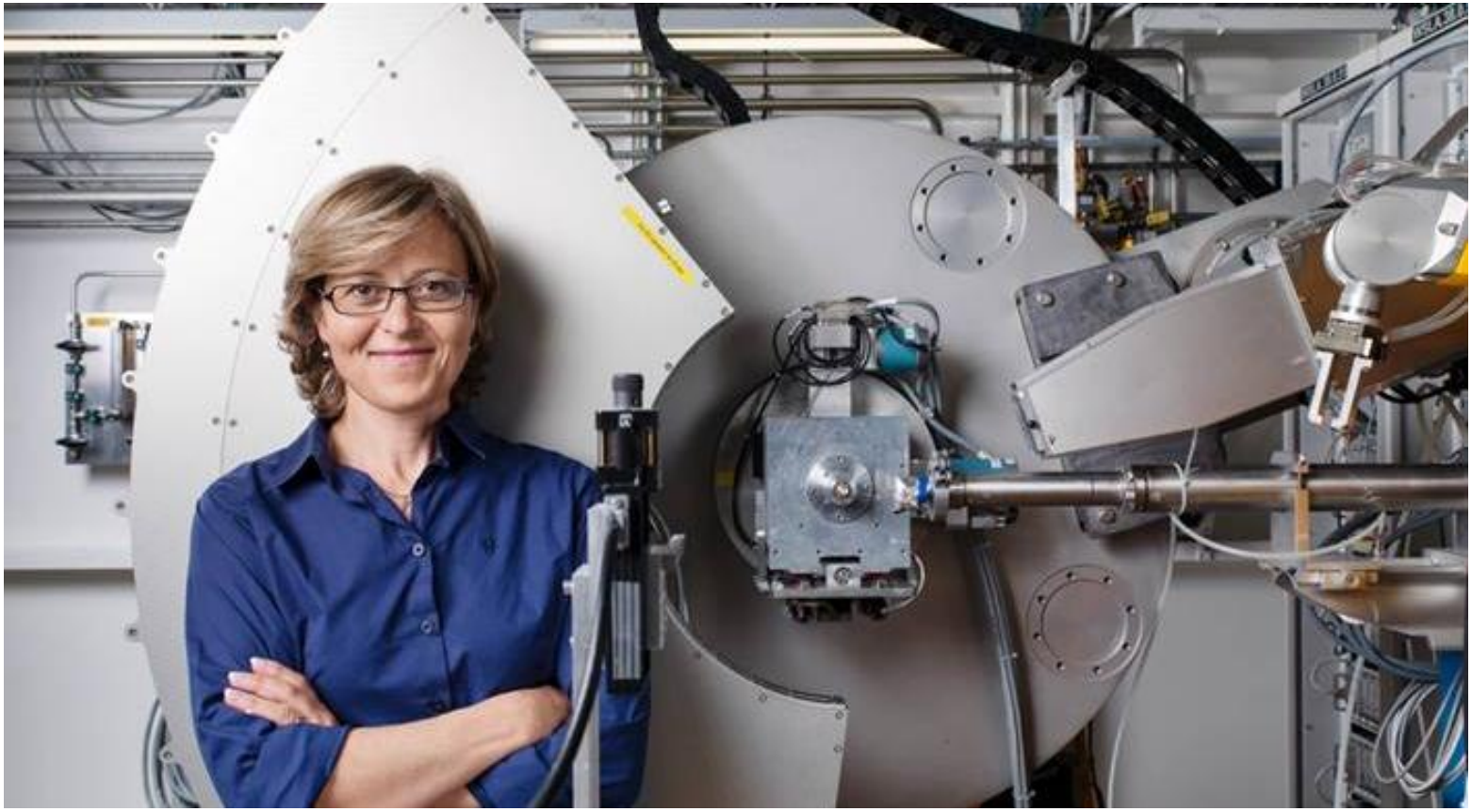
Validation

Commissioning

Qualification

Process Validation

Cleaning Validation



Where in pharmaceutical production?

- **Drug characterization**
- **Product purity**
- **Raw materials control**
- **Counterfeiting prevention**
- **API fingerprinting**
- **Cross-contaminant quantification**

From Research tool to Commercial use

1) **Validate**
2) **Ensure Quality**
3) **Communicate**

From Research tool to Commercial use – Validation

Commissioning

Installation Qualification

Operational Qualification

Performance Qualification

Methods Validation

From Research tool to Commercial use – Quality

Standard Operating Procedures

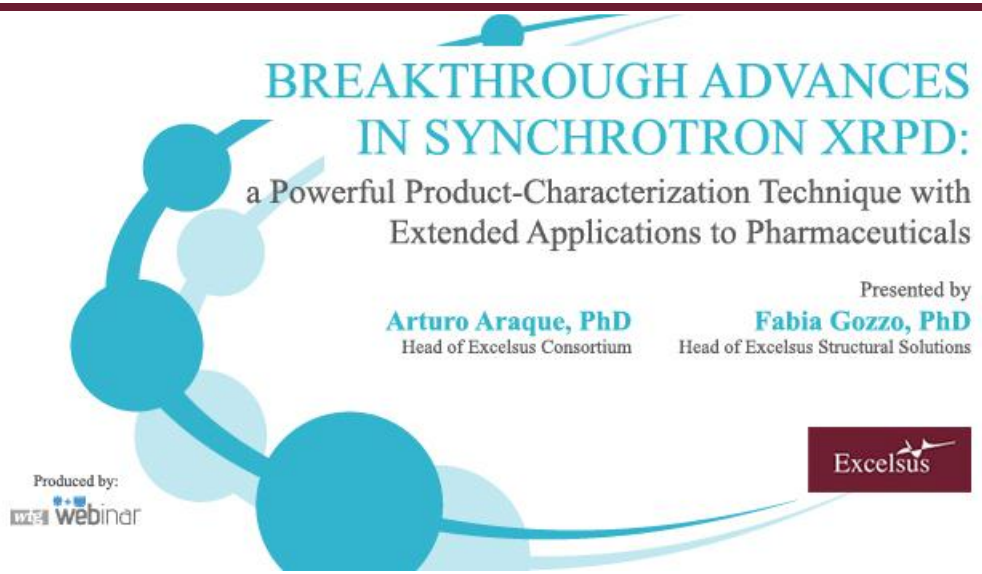
Bill of Materials

Certificate of Analysis

License documentation

From Research tool to Commercial use - Announce

1. **The advantages of using synchrotron over conventional laboratory XRPD**
2. **Technical instrumental advances**
3. **Benefits of this new technology**



Outlook

- Role of structural analysis in the pharmaceutical industry
- X-Ray Powder Diffraction and advantages of synchrotron radiation
- Synchrotron XRPD applications: indexation & structural solutions, radiation damage control, kinetic studies, Quantitative Phase Analysis (L.o.D, L.o.Q)
- Easy and affordable access to state-of-the-art synchrotron XRPD through Excelsus

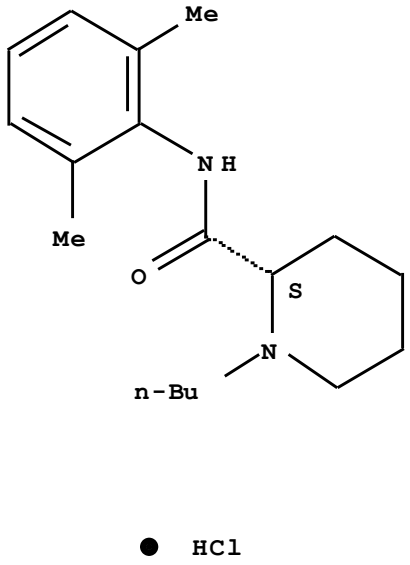
Why is structural analysis of drugs relevant to the pharmaceutical industry?

- Polymorphism of pharmaceuticals and their structure ↔ properties
- Microstructural properties (e.g. influence of stress and strain, particle size and domain)

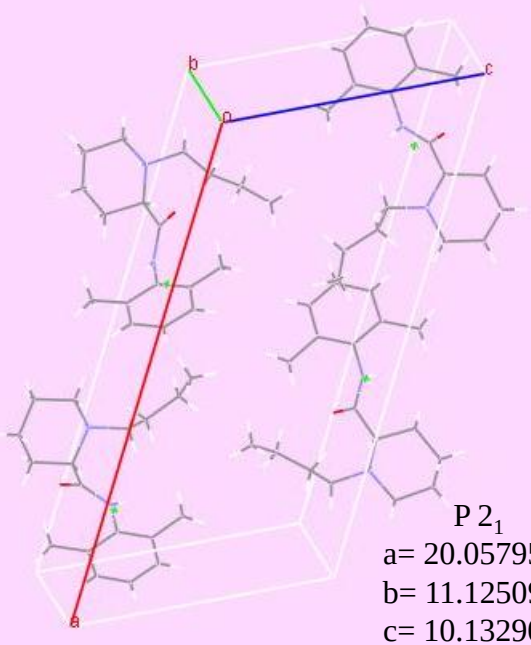
Polymorphism of pharmaceuticals

Polymorphism is the ability of substances to crystallize in solid state phases according to different arrangements or conformations of the molecules in the crystal lattice

Example of
Bupivacaine Hydrochloride

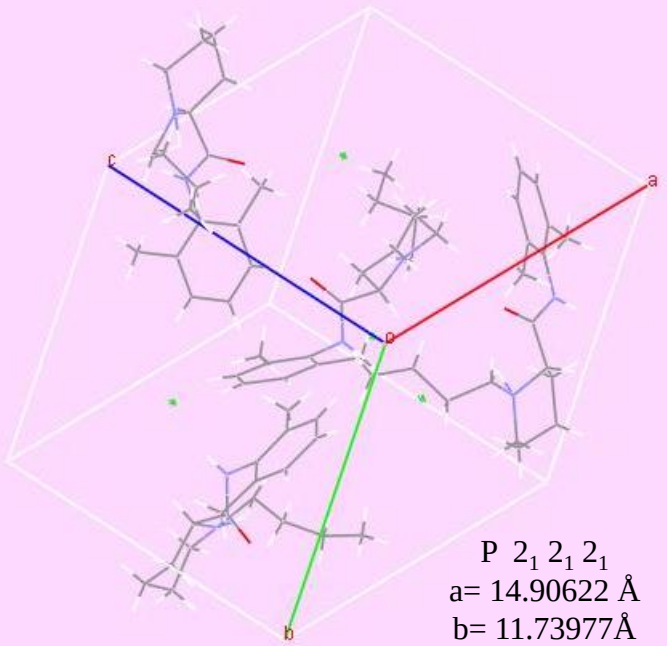


Form B at 112 °C



P 2_1
a= 20.05795 Å
b= 11.12509 Å
c= 10.13290 Å
b= 116.18377 °

Form D at 20 °C



P 2_1 2_1 2_1
a= 14.90622 Å
b= 11.73977Å
c= 11.08386Å

Gozzo, Masciocchi , Griesser, Niederwanger, 2010. Personal Communication

Why is polymorphism such an important property?

Solid-state structure  **Physical and chemical properties**

Characterization and control of pharmaceuticals properties through characterization and control of their solid-state structure

Properties that are influenced by the solid state structure of the substance and, therefore, influenced by polymorphism:

- Solubility
- Pharmacokinetics and pharmacodynamics
- Thermodynamic properties (e.g. stability of drugs) → in-situ non-ambient studies
- Spectroscopic properties
- Mechanical properties (e.g. hardness, compressibility, tableting, tensile strength)

ICH Guideline (Q6A):

Differences in these [polymorphic] forms could, in some cases, ***affect the quality or performance of the new drug products****. In cases where differences exist which have been shown to affect drug product performance, bioavailability or stability, then the appropriate solid state should be specified (3.3.1. New Drug Substance).*

Identification testing should establish the identity of the new drug substance(s) in the new drug product and should be able ***to discriminate between compounds of closely related structure*** *which are likely to be present (3.2.2. New Drug Product)*

New analytical technologies*, and modifications to existing technology, are continually being developed. Such technologies should be used when justified (ICH scope)*

The famous case of Ritonavir*

- Abbott's protease inhibitor Norvir, generically Ritonavir (AIDS treatment)
- Not bioavailable from the solid state → Marketed in 1996 as a oral-liquid and semisolid capsules (both formulations contained ethanol/water based)
- **No crystal form control** performed before distribution on the market because the ICH guidelines stated that "*For a drug product that is a solution, there is little scientific rationale for polymorph control*"
- In Summer 1998, Norvir semisolid capsules supplies were threatened as a result of its spontaneous irrepressible interconversion into a new thermodynamically stable form precipitating out of the semisolid formulated product, due to unexpected supersaturation and nucleation conditions.
- The new form had much lower solubility with greatly reduced bioavailability
- Drug out of market for 1 year until Ritonavir polymorphism was fully understood,
- The metastable form I could then be stabilized with a new gel capsule formulation.

During development, characterization of all possible crystal forms is needed, even in liquid formulations

Ritonavir: an Extraordinary Example of Conformational Polymorphism
Bauer et al, Pharmaceutical Research 18(6). 2001, 859-866

Polymorphic studies play a key role throughout the whole life-cycle of products

Compound selection
- Identification and characterization of individual polymorphic forms
- Selection of desired form based on therapeutic properties

↓

Technical development
- Development of DS and DP manufacturing processes to ensure high and reproducible content of desired polymorphic form via fingerprinting
- Polymorphic studies for impurity detection and stability studies
- Consider crystal engineering (e.g. co-crystallization*) to enhance yield and stability of desired polymorphic form

↓

Commercial production
- Polymorphic characterization to support (1) process validation, (2) comparability studies following process changes, and (3) investigations to assess impact of deviation on product quality

Synchrotron Radiation X-ray Powder Diffraction is a unique and powerful technique for such studies

* Example of carbamazepine: rational co-crystallization with saccharin that prevents the formation of hydrated forms that have strongly reduced solubility!
(see *Organic Crystal Engineering*, Eds. Tiekink, Vittal & Zaworotko, Wiley 2010)

Additional areas where polymorphic studies play a key role

- Fight against **counterfeit drugs**:
  - Trademark infringements
  - Serious attempts to human health → no or wrong API content, hazardous adulterants, substituted and not labeled ingredients

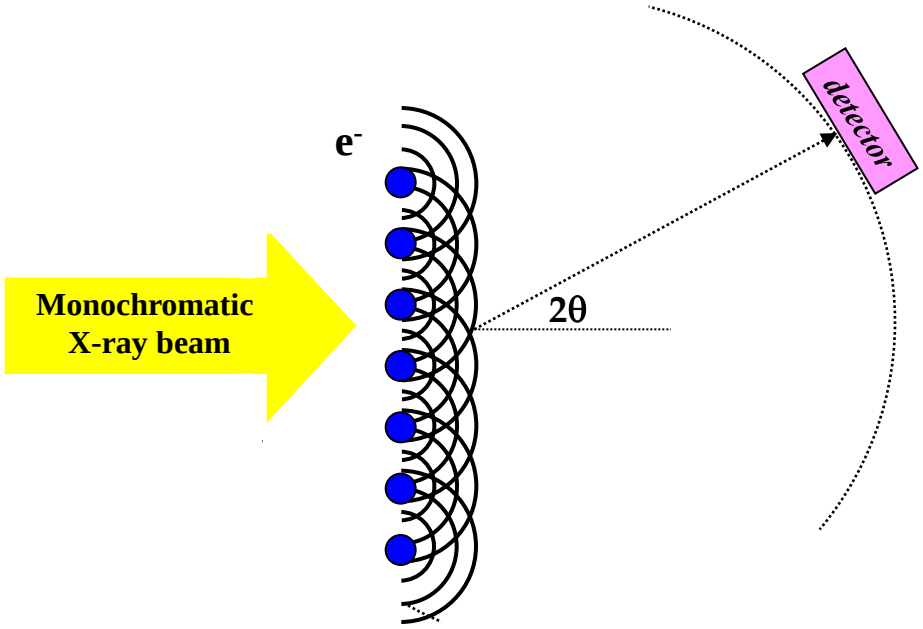
See: "The fight against counterfeit drugs" (http://www.cbsnews.com/video/watch/?id=7359537n)

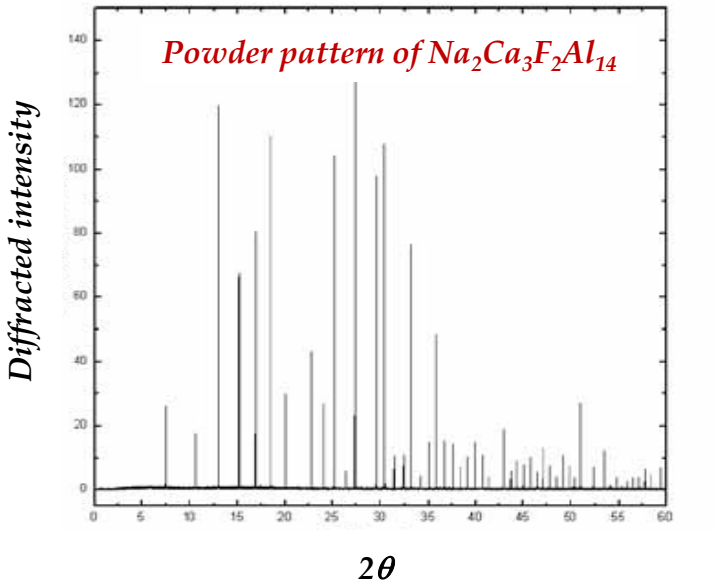
- **Intellectual Property**: → scientific support to litigations involving patent issues

Synchrotron X-Ray Powder Diffraction

(SR-XRPD)

X-Ray diffraction by crystals





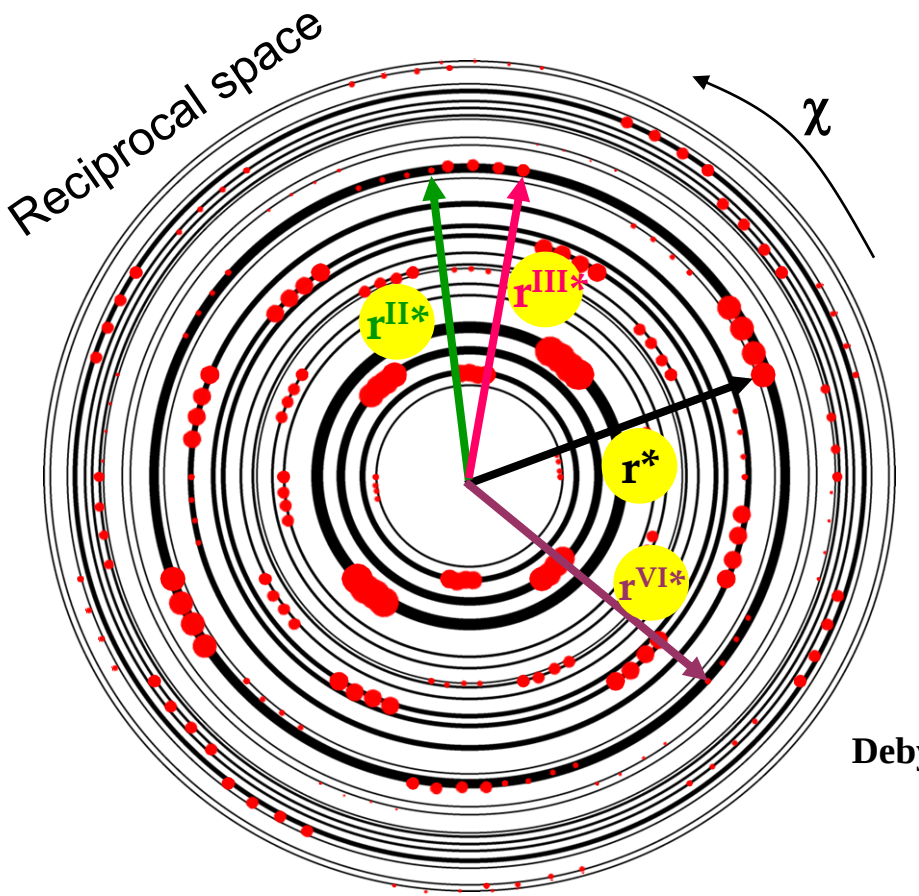
Position of the diffracted peaks → size and dimension of the unit cell

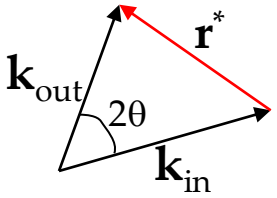
Intensity ratios of the diffracted peaks → type and location of atoms in the unit cell

Full Width at Half Maximum (FWHM) → of the diffracted peaks

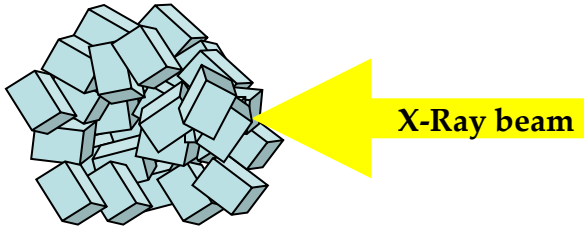
Intrinsic properties of the materials (i.e. microstructural analysis)

X-Ray diffraction from a polycrystalline or a powder sample





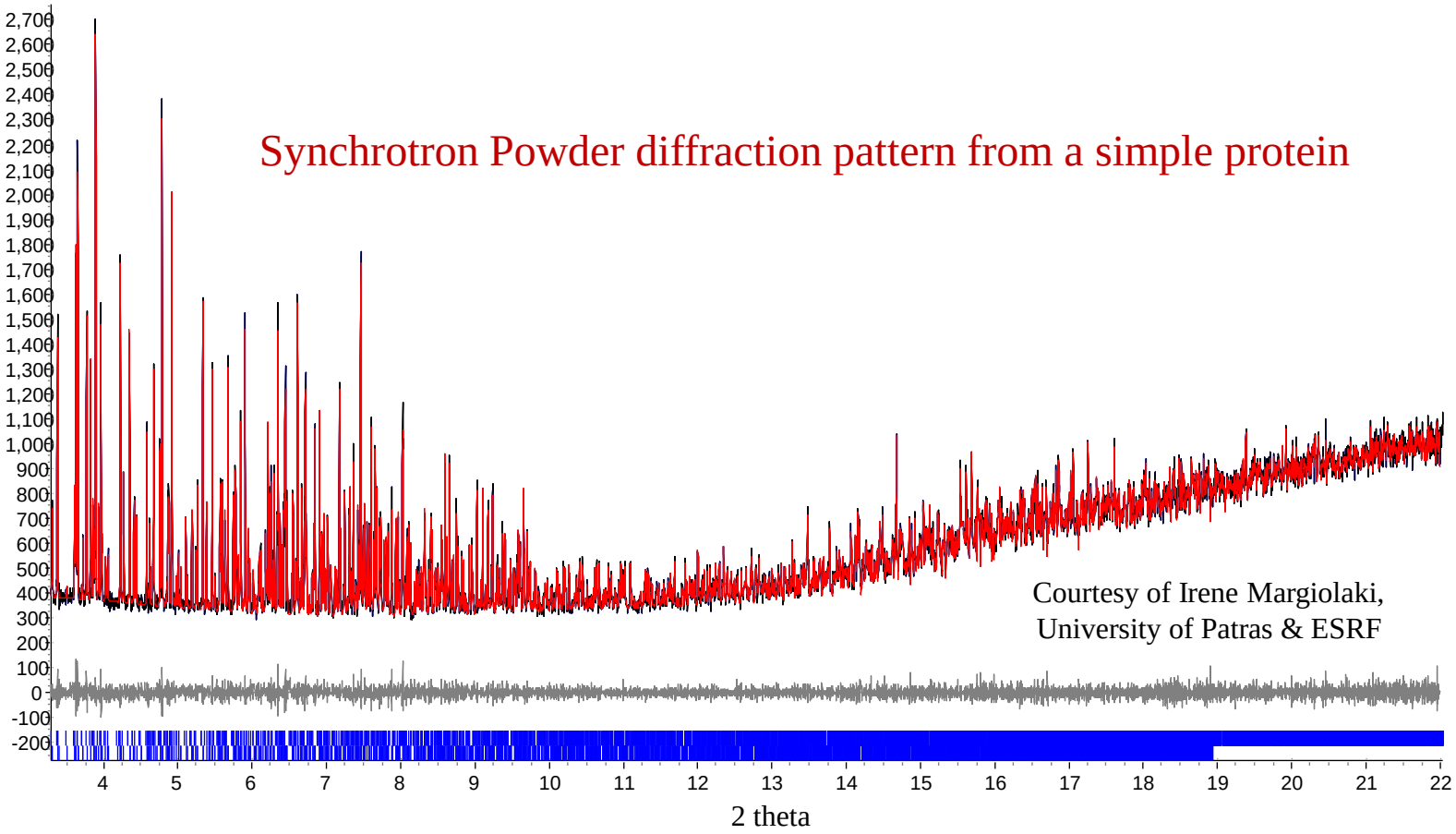
$$|\mathbf{r}^*| = \frac{1}{d_{hkl}}$$



Debye-Scherrer (1916)

In a powder diffraction pattern the 3D of the reciprocal space collapse onto 1D

the information in the orientation space gets lost



Extracting detailed information from a powder pattern requires very high-quality data → the collimation and monochromaticity of a synchrotron X-ray beam allow for a great improvement of the angular resolution of the acquired patterns, whereas the high brilliance of a synchrotron source drastically reduces measurements times allowing the study of the kinetic of transformations in fraction of seconds

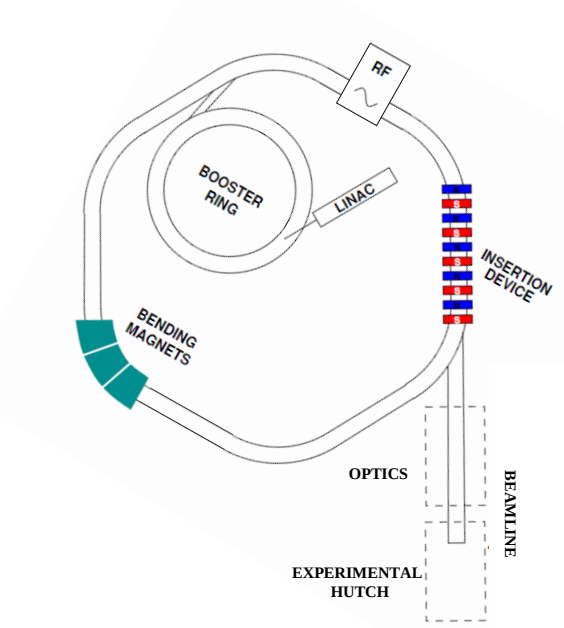
Why powder diffraction?

- Single crystal not always available
- The majority of APIs and excipients in pharmaceutical substances and products are powders (polycrystalline or amorphous powders)
- Quantitative Phase Analysis of mixtures (e.g. drug product)
- In-situ, non-ambient XRD: powders often a better/the only choice
- Radiation damage → XRPD parallel 1D detection
- Total scattering techniques

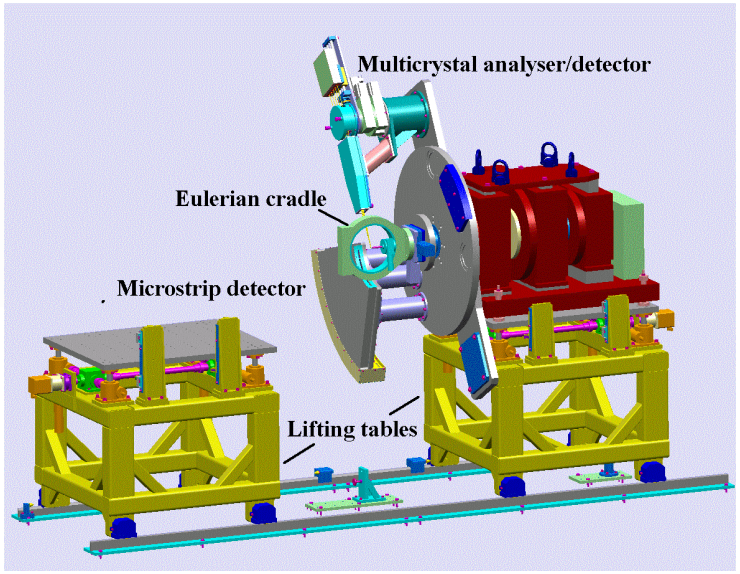
Synchrotron X-Ray Powder Diffraction

What makes it a powerful analytical tool?

Synchrotron Radiation and beamline optics



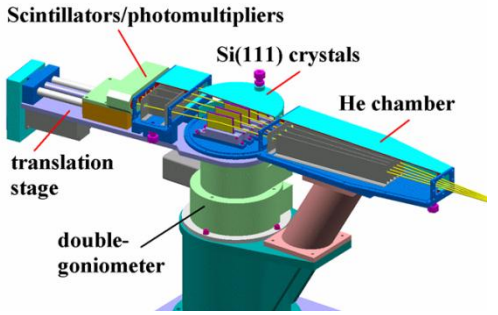
State-of-the-art diffractometers



Swiss Light Source-Materials Science beamline
Powder Diffraction station

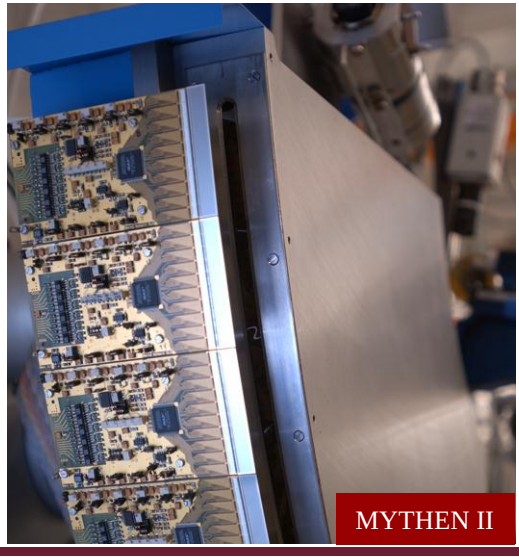
Outstanding detection systems

Hodeau et al, 1998



Multicrystal Analyser

Schmitt et al, 2003,
Bergamaschi, Schmitt et al, 2010



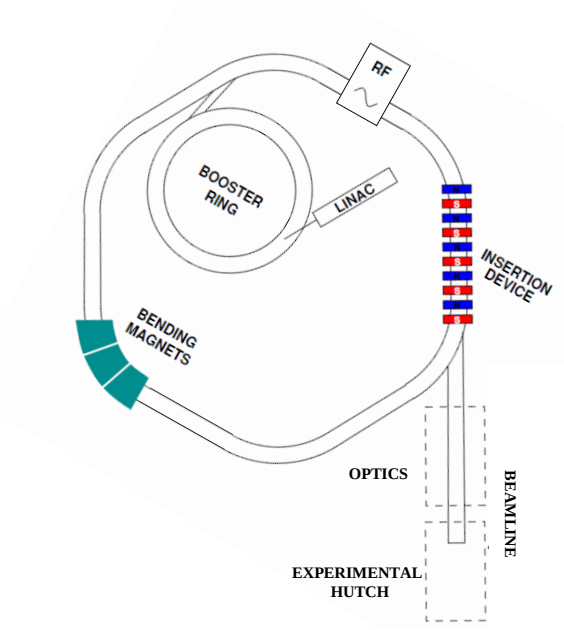
SR Properties:

- High Spectral Brightness: [photons/s/0.1% bandwidth/mm^2/mrad^2] → 10^{12}-10^{15} ph/sec in small beams (μm to mm)
- Tunable photon energy
- Polarization
- Time structure
- Coherence

Resolution: 1 arcsec
Accuracy: ±2 arcsec
Precision: ±1 arcsec

Highest flexibility to accommodate all kinds of sample environments

Synchrotron Radiation and beamline optics



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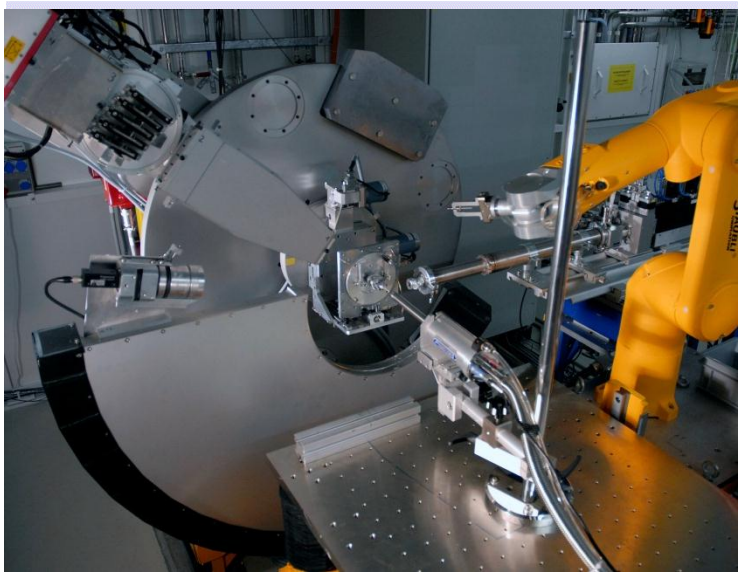

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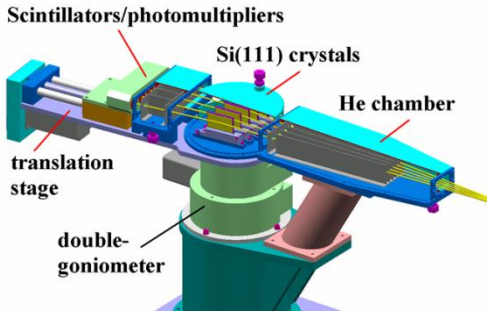
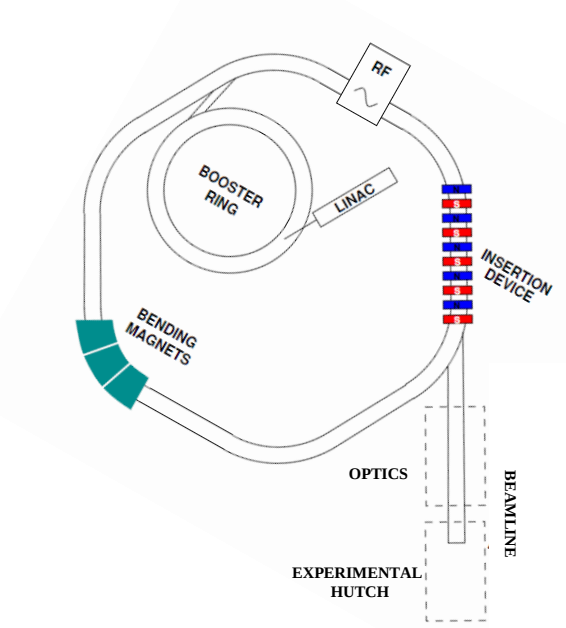


Multicrystal Analyser

- Ultra-high resolution (better than 0.003°) independent of sample dimension
- Angular selection of diffracted beam and all related advantages:
- Fluorescence suppression
- Independence of resolution on sample size and sample positioning
- Independence on transparency effect
- High S/N and S/B
- Long measurements (15 min-several hours)

Synchrotron Radiation and beamline optics



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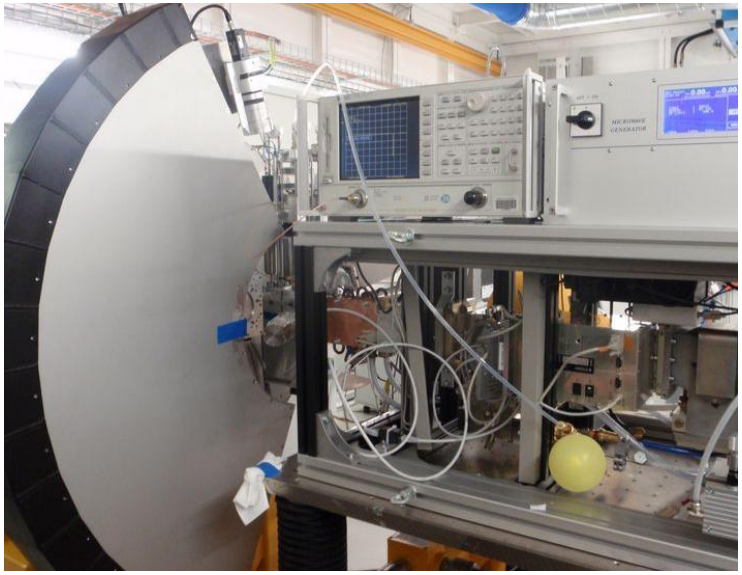


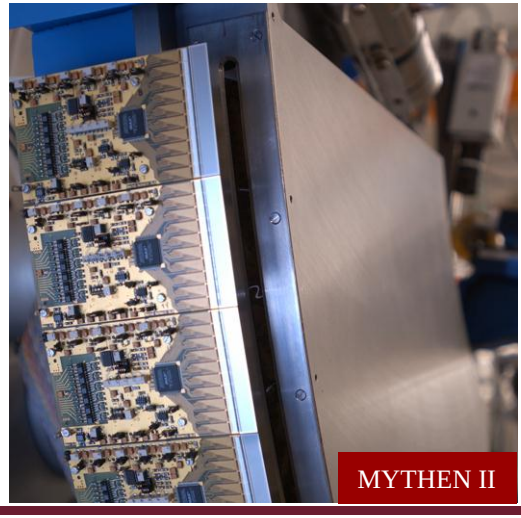
Swiss Light Source-Materials Science beamline
Powder Diffraction station

Outstanding detection systems

- Solid state microstrip modular detector
- 1D position sensitive
- 120º angular coverage
- Single photon counting read out → fluorescence-free bkg
- Large dynamic range (24 bits)
- 0.004º inherent angular resolution
  → FWHM ~0.009º for 0.2 mm capillaries
  ~0.022º for 0.5 mm capillaries
  ~0.044º for 1.0 mm capillaries
- High d-spacing resolution (d_{min}=0.24 Å at 28keV)

Time resolved powder diffraction:

- Average acquisition time 0.5-1s, down to 100msec or nsec (pump-and-probe)
- The acquisition can run 30000 faster than using the single channel crystal analyzer



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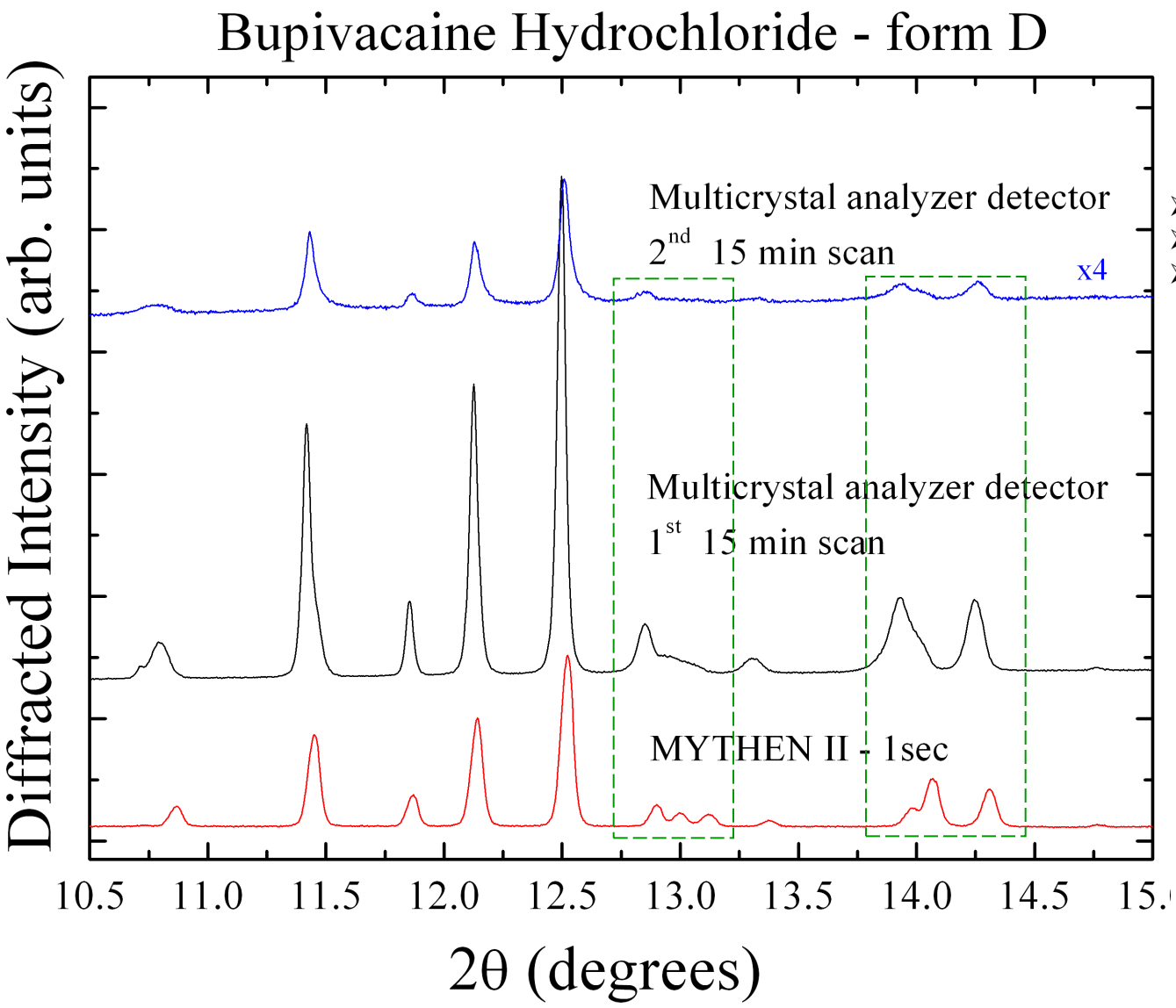


- 1 mm capillary,
- Mythen data at 50% reduced intensity
- No radiation damage up to 3min

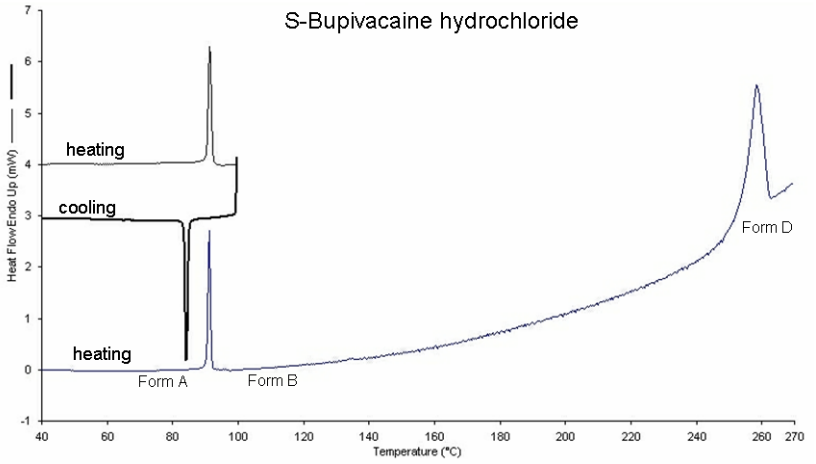
Large counting statistics
in subsec acquisition times →

In-situ kinetic studies of organic compounds!

Gozzo F. , 2008

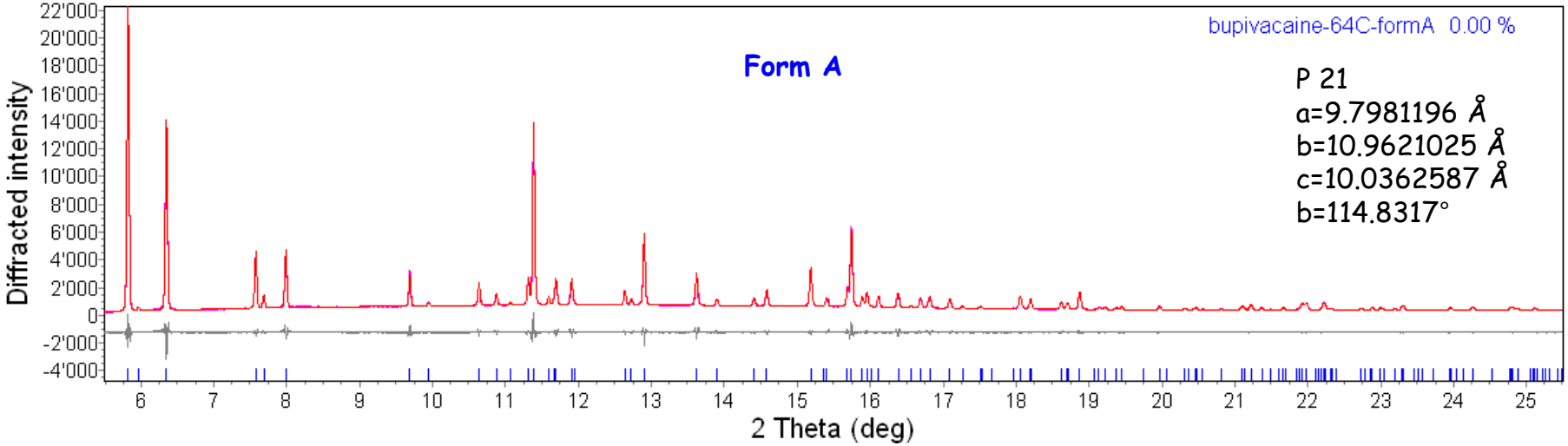
Experimental details:

- MYTHEN I and II
- In-situ measurements in the STOE furnace
- Several cycles to check the reversibility of form B



DSC measurements (Courtesy U. Griesser, University Innsbruck)

In-situ T-dependent powder diffraction patterns



Niederwanger, V., Gozzo, F. & Griesser, U. (2009). J. Pharm. Sci. 98, 1064–1074
Gozzo F. , Masciocchi N., Griesser U. & Niederwanger, V. 2010, private communication

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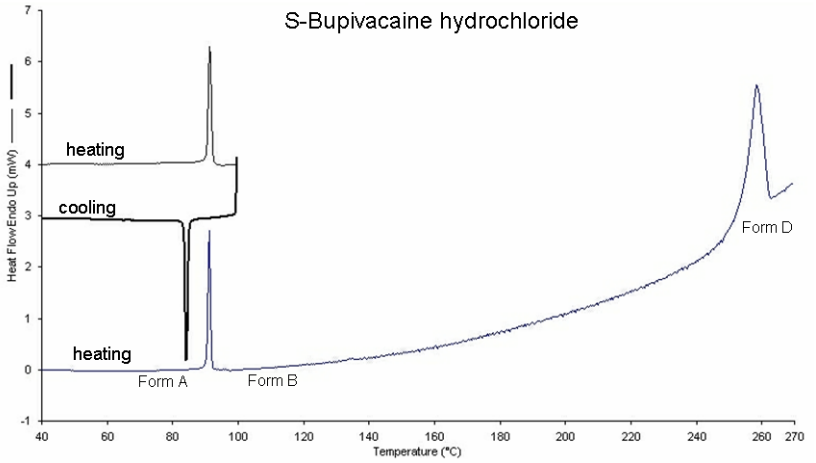

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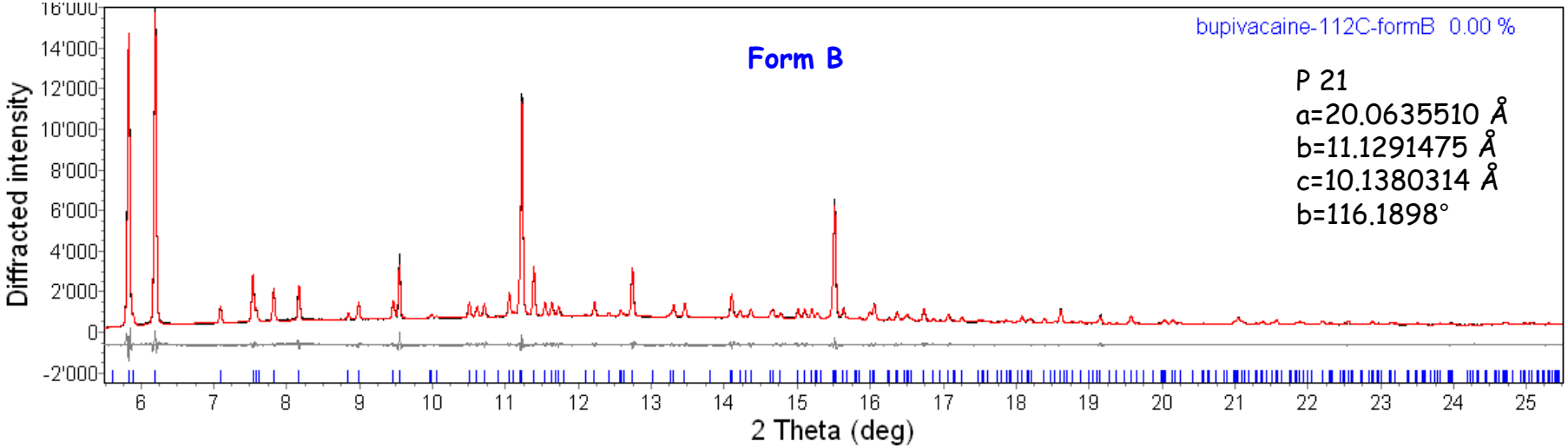

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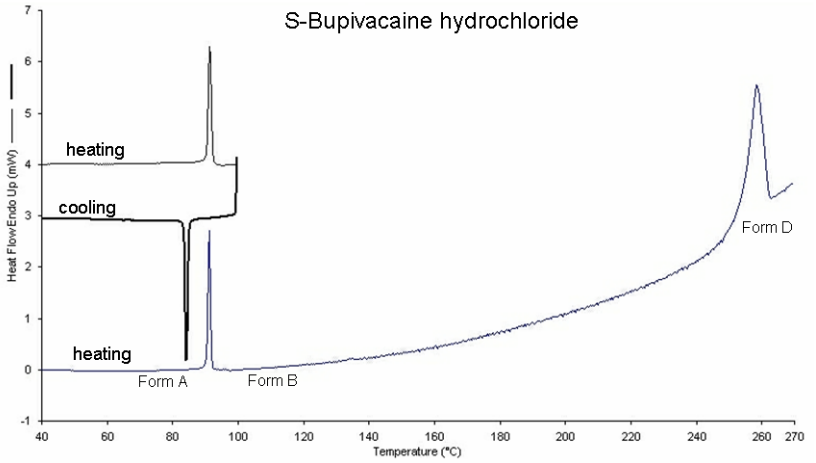

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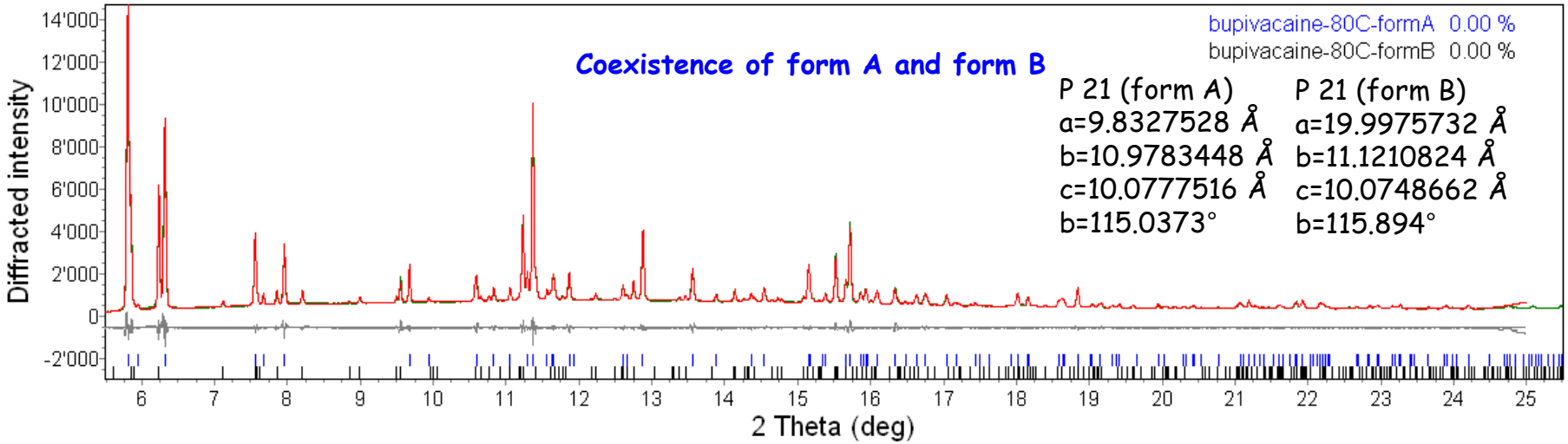

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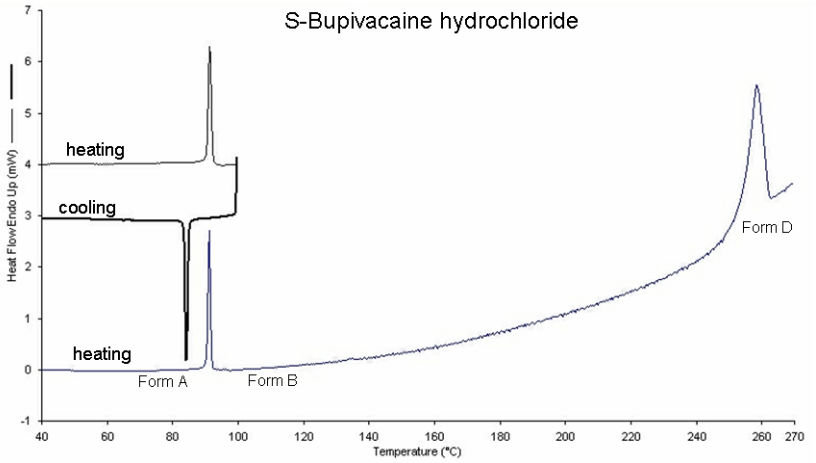

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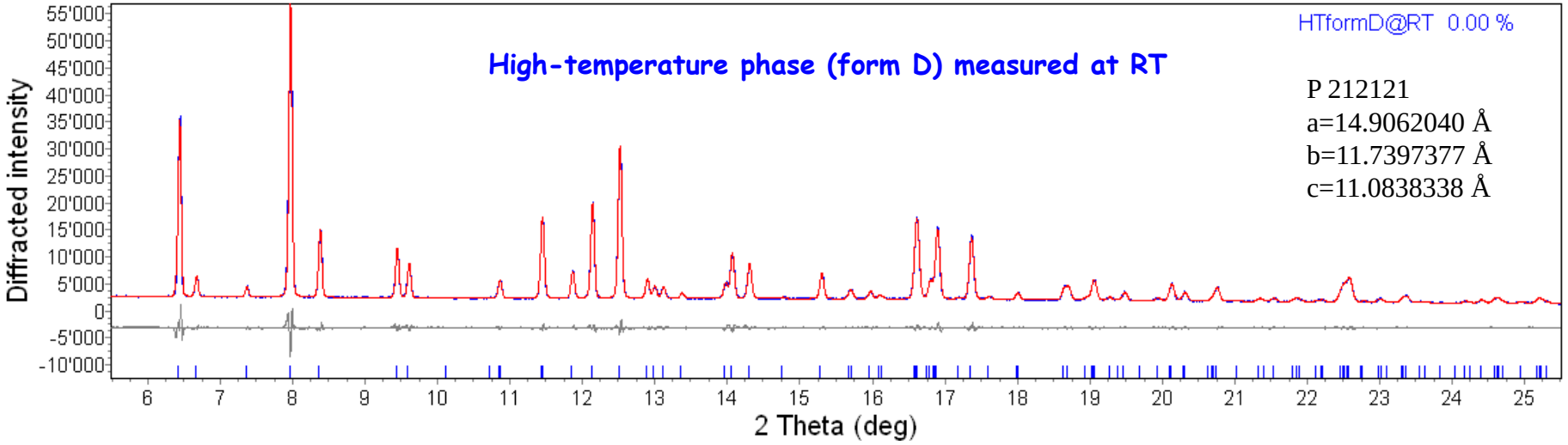

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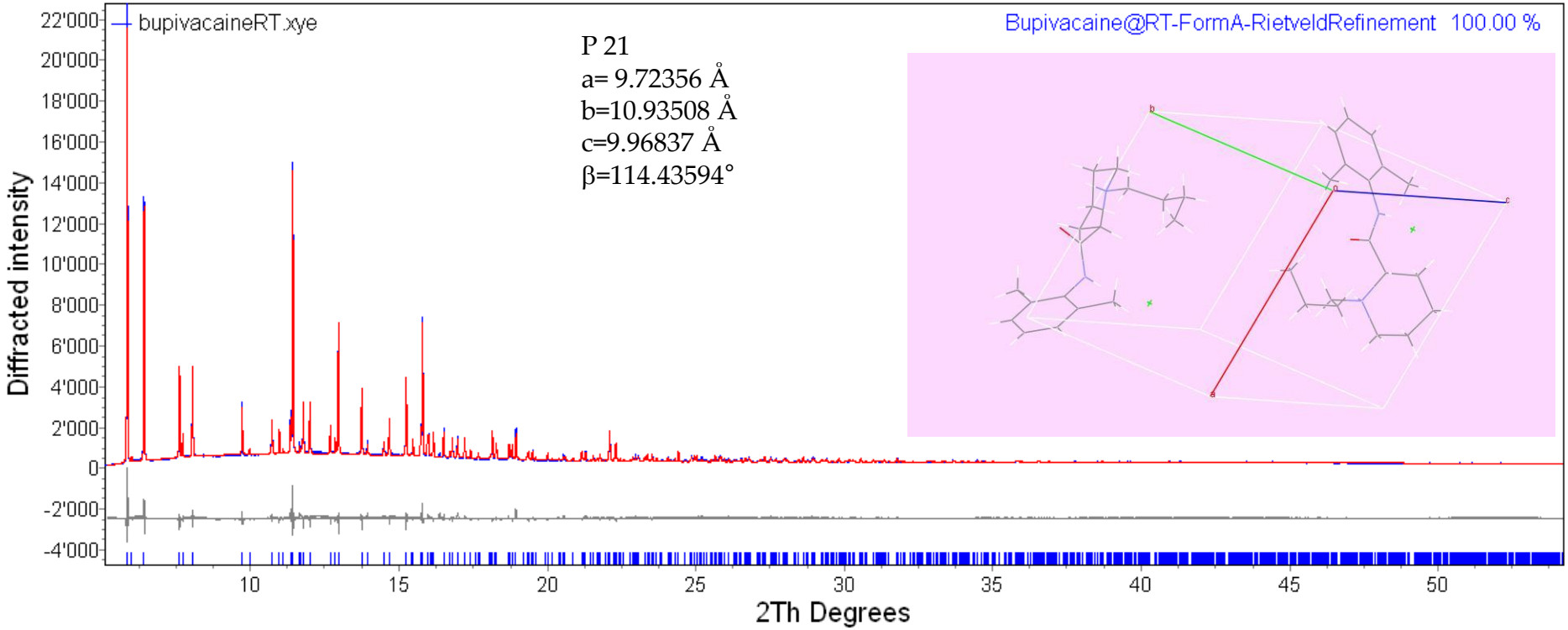
Structural solution with Simulating Annealing and Direct Methods of the RT phase of the Bupivacaine Hydrochloride (Form A)

2 seconds XRPD acquisition time



Details of sa: 2 fragments, Cl free,
7 torsions, 15 DoF

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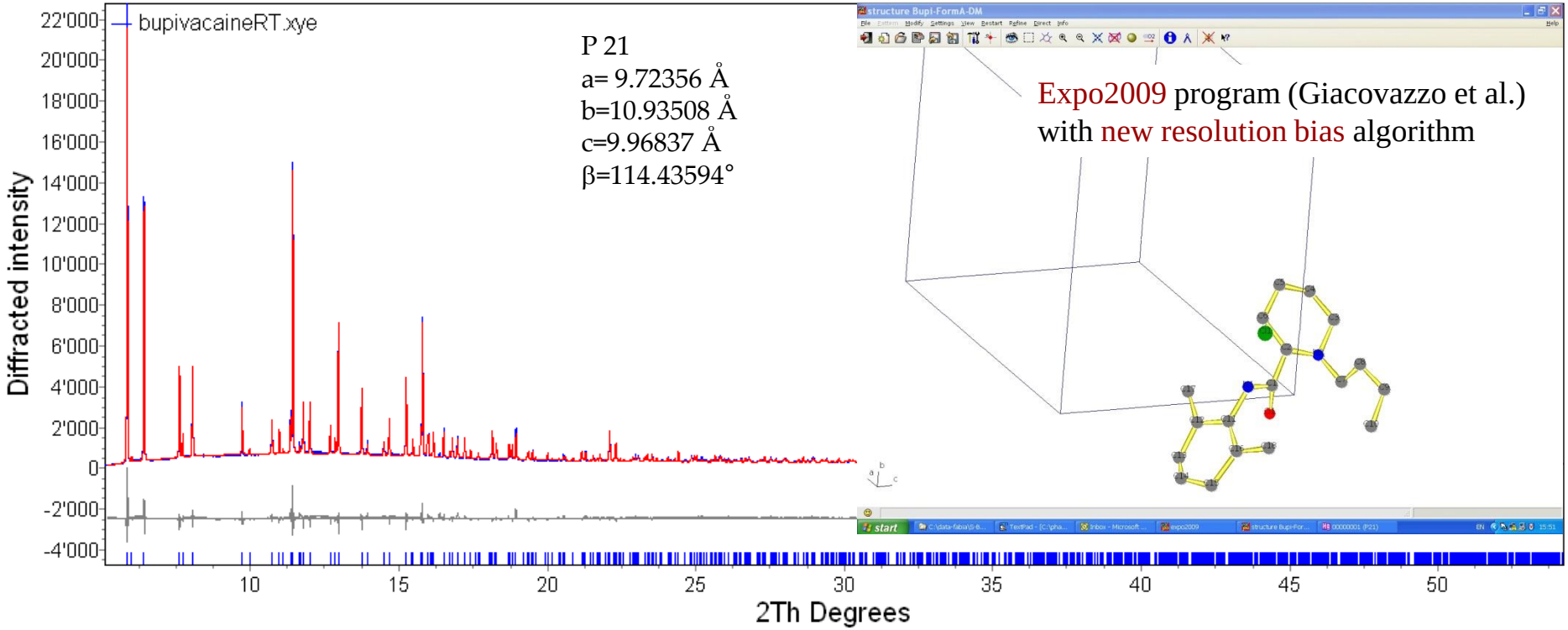

P 21
a= 9.72356 Å
b=10.93508 Å
c=9.96837 Å
β=114.43594°

Expo2009 program (Giacovazzo et al.) with new resolution bias algorithm

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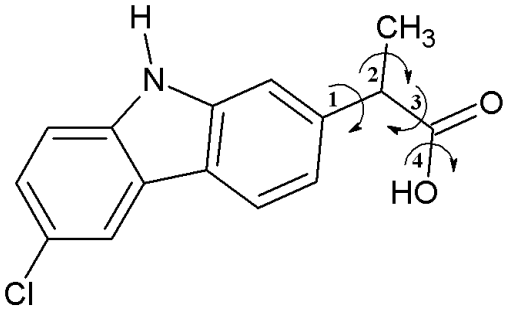
Example of structural solution of a subtle case of polymorphism:

CARPROFEN



Non-steroidal anti-inflammatory drug (NSAIDs)

Identification of two forms:
- Re-crystallization from hot solution of commercial sample in ethanol (*F*orm I)
- High-Temperature Form II as solid-solid transition

Thermal, spectroscopic, lab-XRPD and *ab-initio* SR-XRPD and post-experimental DFT calculations

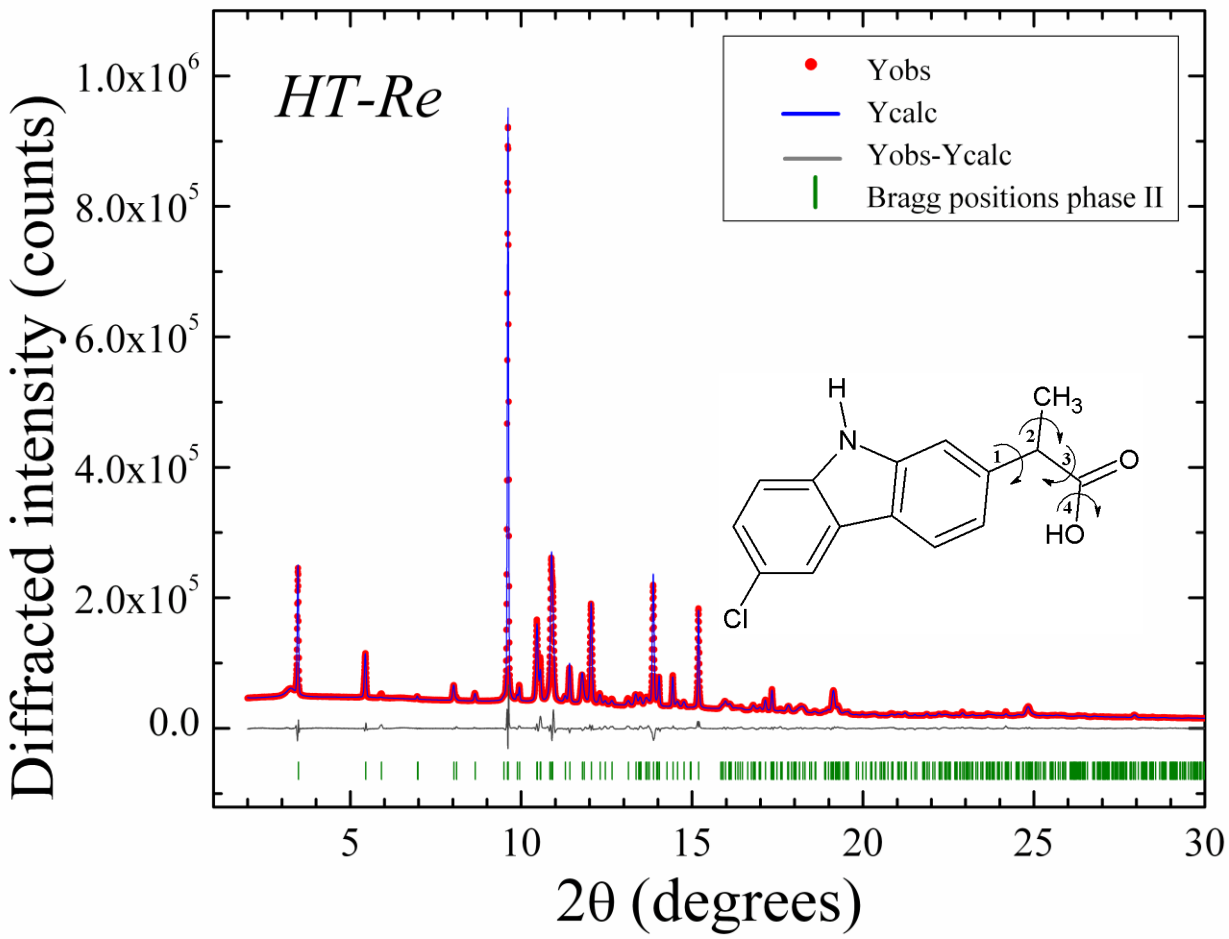
Low-T measurements and high-pressure investigations

Outcome:
Form I → Form II subtle isostructural transformation
Form I and II are conformational polymorphs
Evidence for configurational disorder

SR-XRPD successful in identifying two very close polymorphs that could not be distinguished with laboratory XRPD data

G. Bruni, F. Gozzo et al. J. Pharm. Sci.2011, **100**(6), 2321-2332

10 sec acquisition time with optimized optics,
reduced intensity beam and MYTHEN II

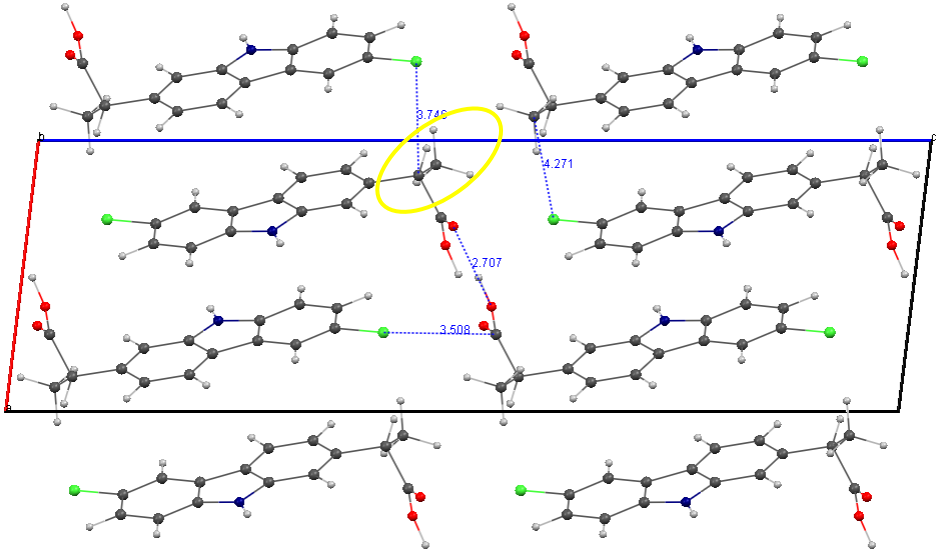


Form I

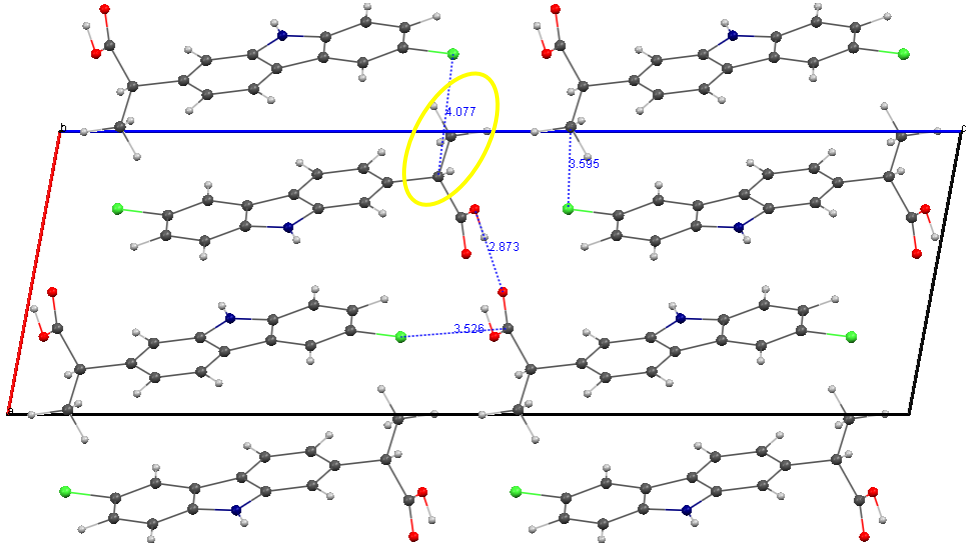
Monoclinic $P2_1/c$
a=8.2855(3)
b=5.7071(3)
c=27.087(1)
β=96.901(5)
Z=4
Rwp(%)=1.77; GoF=2.85

Form II

Monoclinic $P2_1/c$
a=8.5563(3)
b=5.7923(3)
c=26.778(1)
β=100.473(5)
Z=4
Rwp(%)=2.09; GoF=3.26



Form I



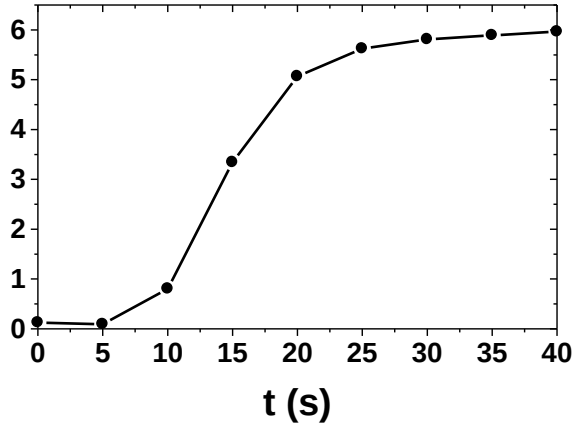
Form II

In-situ dynamic study of the $LaNi_5$ hydrogen absorption process

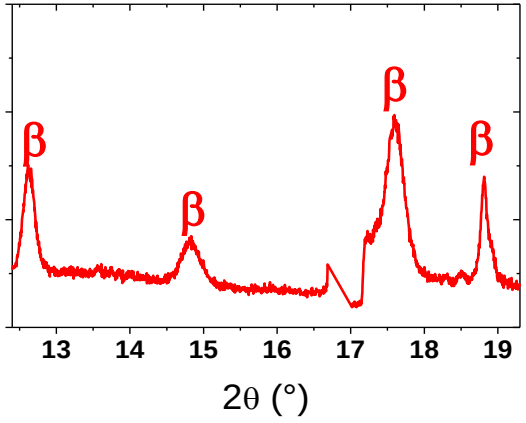
- *In-situ* hydrogen absorption at 15 bar
- *In-situ* desorption by connecting the cell to a vacuum pump
- Continuous measurements using the μstrip detector while the reaction takes place
- Acquisition times between 5 and 20 sec per pattern, depending on the reaction kinetic.

Courtesy of Prof. Cerny, Uni. Geneva - Joubert et al. Acta Materialia, **54** (2006), 713-719

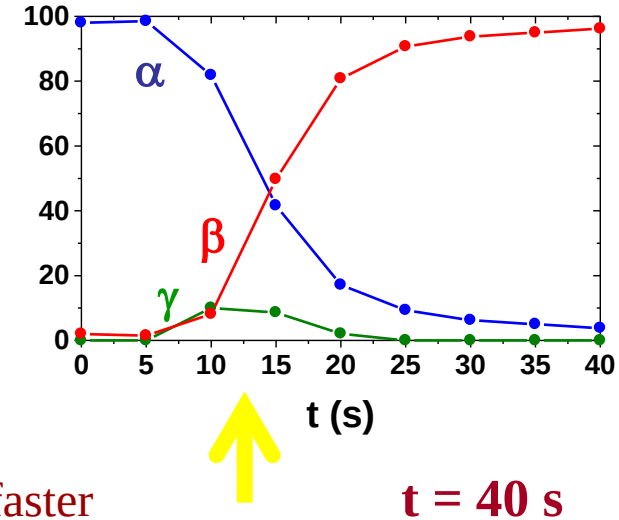
hydrogen uptake



diffraction pattern



phase content



New beamline optics+ Mythen II → 5-10 faster

t = 40 s

Quantitative Phase Analysis (QPA)

QPA refers to the ability of quantitatively state the abundance of the different phases that constitute a mixture.

Why is this relevant?

- Polymorphic purity: detect and quantify unwanted polymorphic forms in both drug substance and drug product
  - Level of Detection (LoD)
  - Level of Quantitation (LoQ)
- Assess the polymorphic composition in drug substance and product
- In formulated materials, the API/excipients relative proportion is paramount and needs to be kept under control
- Degree of Crystallinity in amorphous/crystalline mixtures

QPA analytical methods

Several are the analytical methods used to obtain quantitative phase related information:

- Spectroscopic methods (e.g. Raman and Infrared spectroscopy, Mass spectroscopy, Nuclear Magnetic Resonance spectroscopy)
- Thermal Methods (e.g. Differential Scanning Calorimetry, ThermoGravimetric Analysis)
- Diffraction Methods → XRPD Direct method

More on QPA: *Quantitative Phase Analysis*, Madsen & Scarlett, Chapter II in "Powder Diffraction: Theory and Practice", Dinnebier & Billinge Editors, 2008, The Royal Society of Chemistry, Cambridge, UK
Introduction to X-Ray Powder Diffractometry, Jenkins & Snyder,. Publisher, Wiley 1996
Modern Powder Diffraction, Bish & Post Editors, Reviews in Mineralogy, Vol.20, 1989.
Quantitative phase analysis by combining the Rietveld and the whole-pattern decomposition methods, Giannini, Guagliardi & Millini, J.Appl.Cryst (2002). 35, 481.

Single-peak methods:

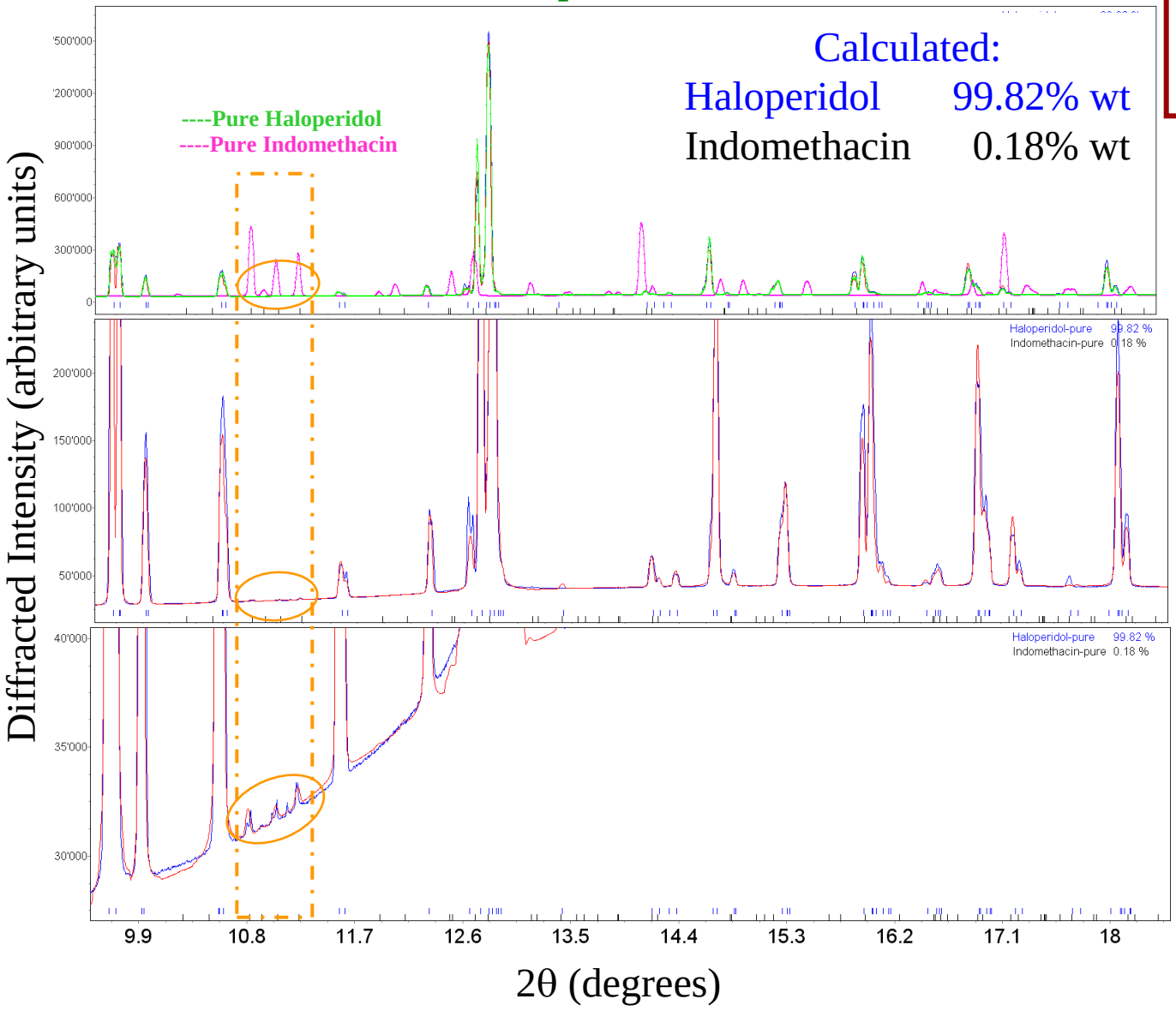
- Intensity ratio $\frac{I_{unknown}}{I_{standard}}$ of one or several diffraction peaks
- No need of structural information but prone to systematic errors caused by preferential orientation (PO) and reflection overlapping

Whole-pattern methods:

- Use of full diffraction patterns
- minimization of systematic errors, peak overlap not a problem
- Preferential Orientation (PO) can be modeled
- Accuracy close to X-Ray fluorescence elemental analysis, with the advantage of being sensitive to structural differences → direct QPA of polymorphs

- Rietveld Method (Rietveld, JAC 1969. 2, 65) → QPA from the structural model
- Partial Or No Known Crystal Structure (PONKCS) Method (Madsen & Scarlett, 2006)
  → needed pure phases (or almost pure) and *ad-hoc* mixtures pure phases+standard in known % wt; PONKCS works well for quantitation of amorphous phase!
- QUANTO/Wilson-plot technique (Giannini, Guagliardi et al, 2002)
  →needed pure phases, cell parameters and SG, but NO NEED of *ad-hoc* mixtures

Standard 99.9% Haloperidol + 0.1% Indomethacin



All synthetic physical mixtures provided by G. Bruni, Uni. Pavia, Italy

The same mixture at 3 different locations on the capillary:

1. Halo/Indom: 99.82%/0.18%
2. Halo/Indom: 99.91%/0.09%
3. Halo/Indom: 99.97%/0.03%

Average: Halo/Indom: 99.9%/0.1%

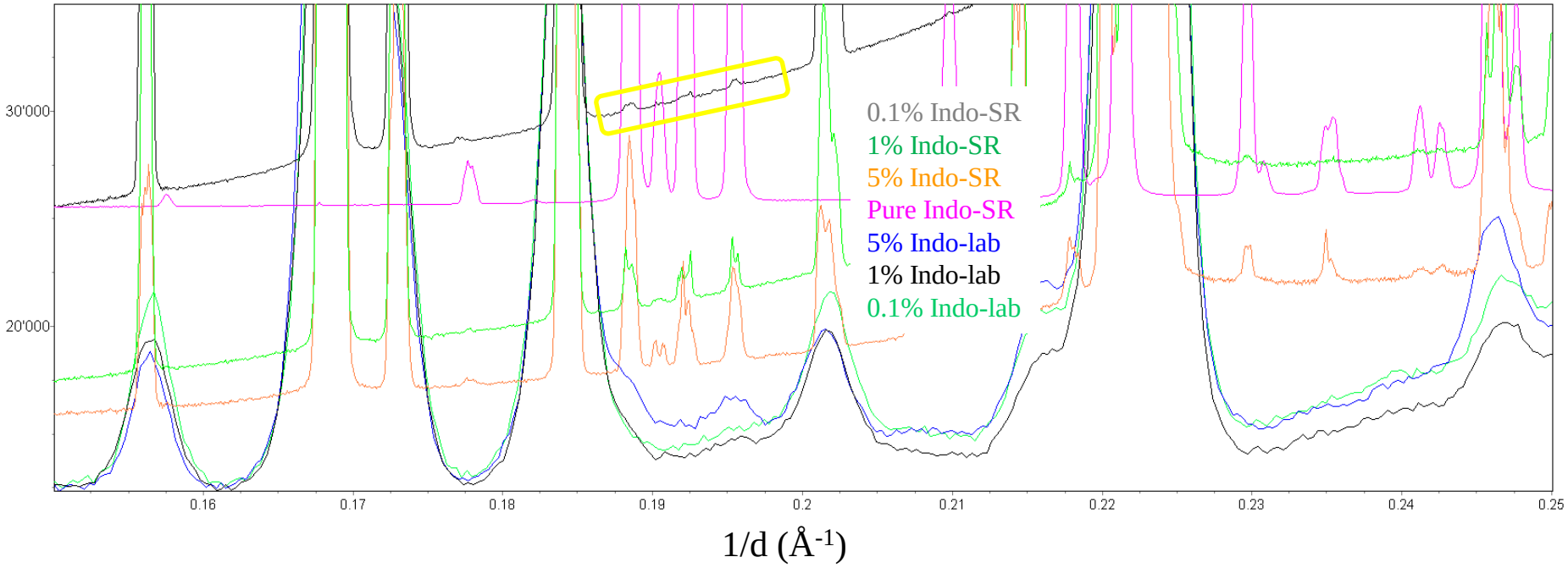
Refinement on merged data:

Halo/Indom: 99.91%/0.09%

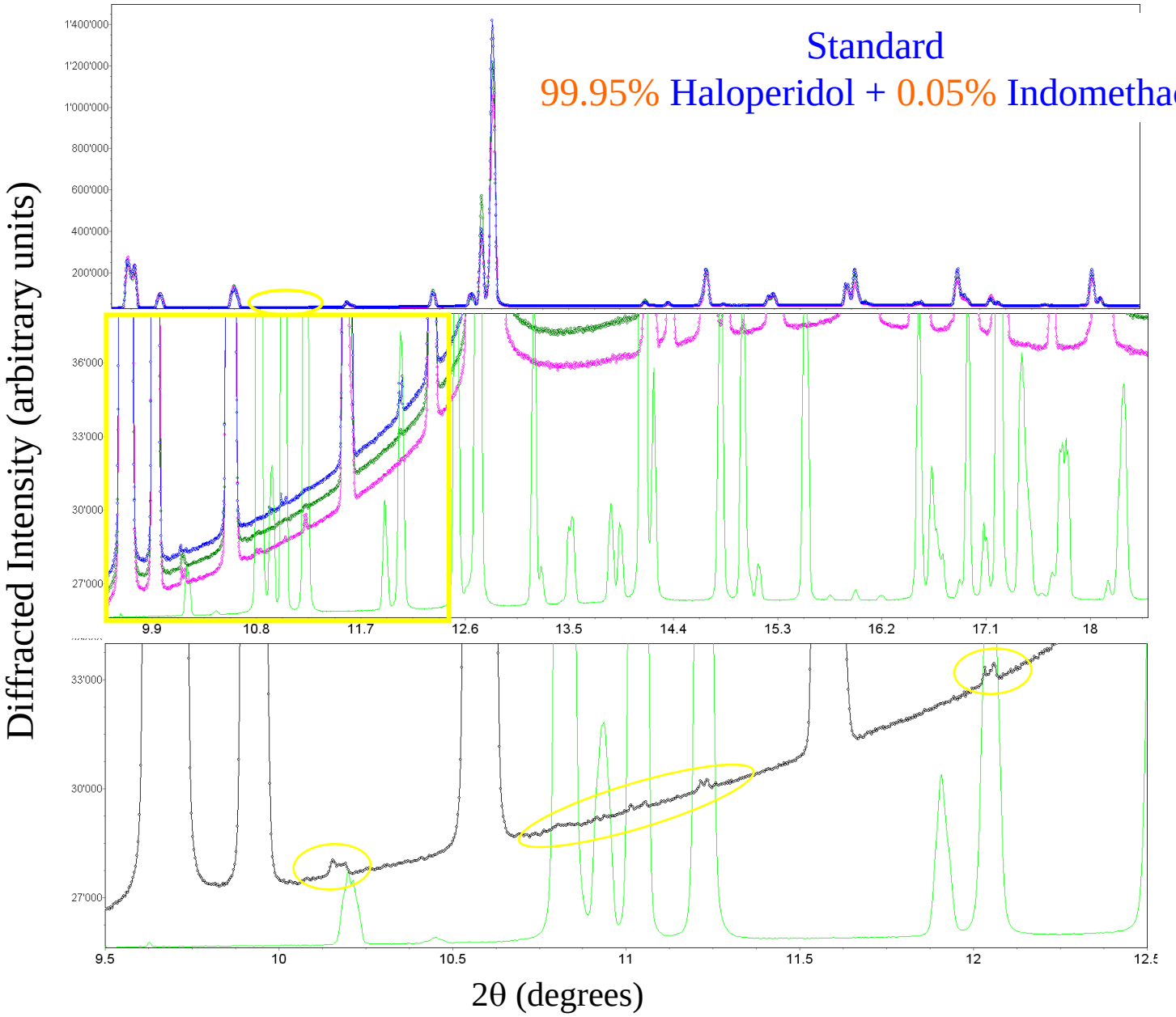
Laboratory XRPD vs SR-XRPD

Laboratory XRPD
1.0 mm capillary; transmission geometry
Fast Inel detector (1024 sec acquisition time)

Lab data
Courtesy Ian Madsen



Lab and SR XRPD data collected on identical synthetic mixtures

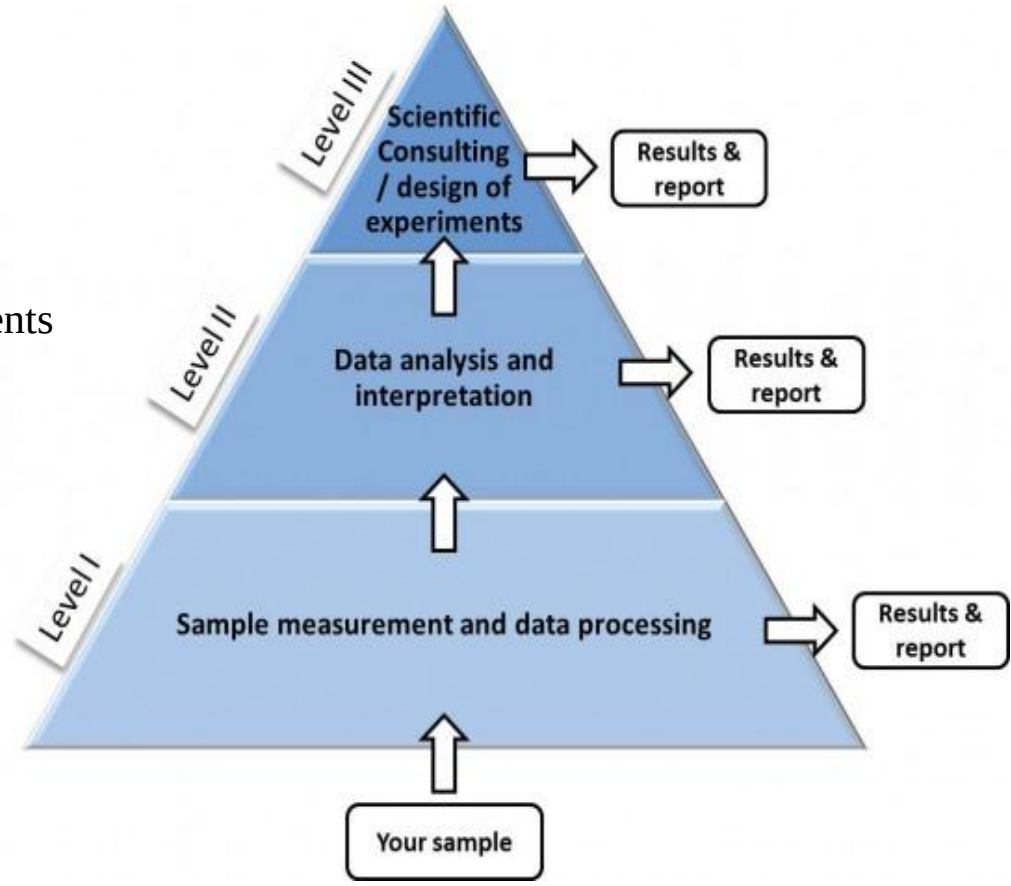


- Polymorphic forms may have a significant impact on the **quality** or **performance** of pharmaceutical products
- A careful characterization of the polymorphism of drug substance and drug product should therefore play a key role throughout the **whole life-cycle** of pharmaceutical products
- Polymorphic studies have also started to play a key role during **patent litigations** and in the fight against **counterfeit drugs**
- **Synchrotron-Radiation Powder Diffraction** has become a unique and very powerful tool for polymorphic studies, such as kinetic analyses, the identification of closely related polymorphic forms and high-sensitivity quantitative phase analyses
- This use is in line with the regulatory expectations (ICH guidelines and FDA guidance) that newly available analytical technologies are used for **continuous improvements** in process understanding and product characterization

Level I:
You internally analyze your synchrotron data.
- we assist you in the choice of the appropriate experimental conditions;
- we prepare the samples
- organize and perform the synchrotron measurements
- deliver the data to you with a written report

Level II:
In addition to Level I services
- we analyze and interpret your SR-XRPD data
- provide a written report

Level III:
- In addition to Level I and II services,
- we provide scientific consulting
- work in close collaboration with you
- assist you in the design of new experiments



Company web site:
www.excels.us

Contact us via email to discuss your specific needs:
fabia.gozzo@excels.us or +32 470 990851
arturo.araque@excels.us or +1 510 5937540

➢**Paul Scherrer Institute Direction** and its **Technology Transfer Department**

➢**Swiss Light Source** and **Materials Science beamline**:
Ch. Quitmann, F. van der Veen
Phil Willmott, Antonio Cervellino, Nicola Casati, Michael Lange, Dominik Meister
Bernd Schmitt, Anna Bergamaschi (PSI detector group)

➢Gianna Bruni (mixtures for QPA scientific project), University of Pavia, Italy

➢Ian Madsen and Nikki Scarlett (QPA scientific project), CSIRO, Victoria, AU

➢Cinzia Giannini & Brunella Aresta (QPA scientific project), CNR, Bari, Italy

➢Roberto Bellotti (QPA scientific project), University of Bari, Italy

➢Irene Margiolaki (powder diffraction of proteins), University of Patras, Greece

➢Scientists and colleagues always open to scientific discussions :
Radovan Cerny, Carmelo Giacovazzo, Angela Altomare, Anna Moliterni, Rosanna Rizzi, Michele Saviano, Thomas Laube, Arnt Kern, Bernd Hinrichsen, Peter Stephens, Thomas Hartmann, Piero Macchi, Norberto Masciocchi, Antonella Guagliardi, Meitian Wang, Martin Fuchs, …