



# Macromolecular Powder Diffraction

**Irene Margiolaki**  
University of Patras, Greece  
& Hellenic Crystallographic Association  
[www.hecra.gr](http://www.hecra.gr)

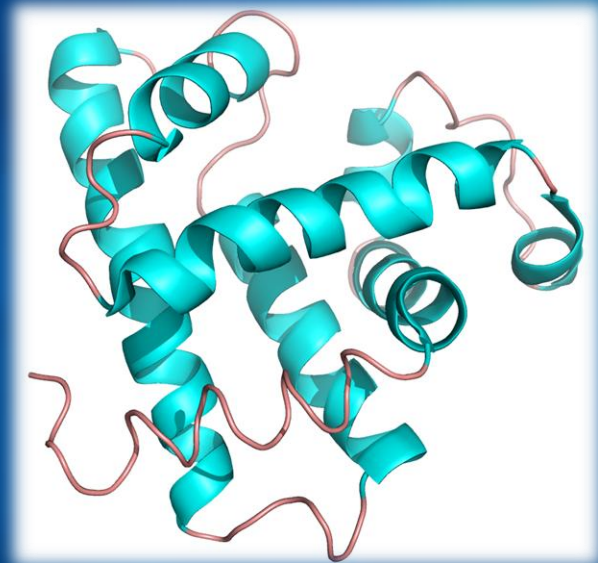
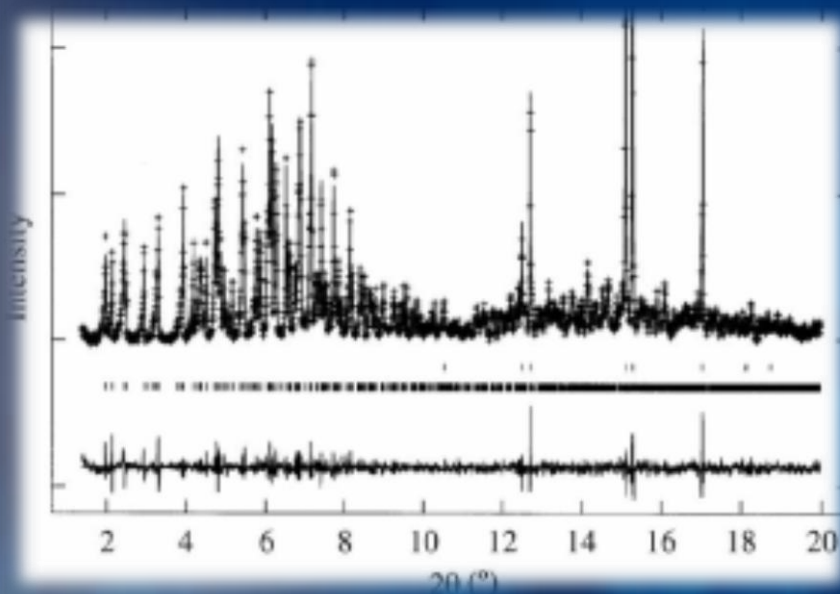
# X-RAY POWDER DIFFRACTION



Can we extract any structural information from a protein polycrystalline sample?



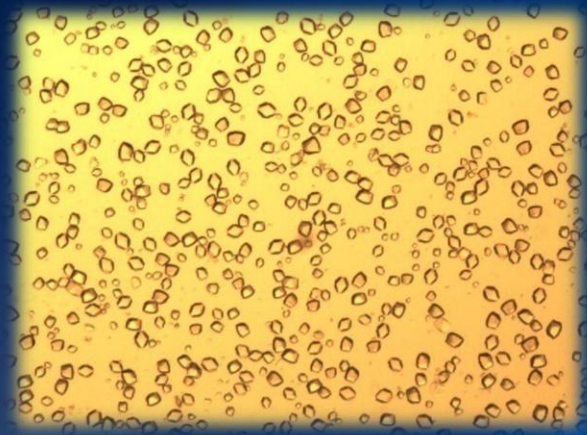
## The first protein structure refinement using powder diffraction data: Whale Metmyoglobin



R. B. Von Dreele  
J. Appl. Cryst. (1999). 32, 1084-1089

- Sample Preparation
  - Data Collection
  - Data Analysis
  - Case Studies
- Future & Concluding Remarks

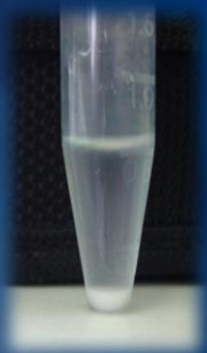
# POWDER CRYSTALLINE SAMPLES



hundreds of microcrystals



Glass capillary tube containing  
white protein crystalline precipitate  
in brass sample holder



# GOOD VS BAD PRECIPITATES



UPPSALA  
UNIVERSITET

## Protein Crystallization

Home

Calendar

Publications

Presentations

Teaching

Crystallization Tutorials

Professional Activities

Links

Me

Contact

### Tutorial 2.

#### Precipitates

One of the most difficult things for beginners is to recognize promising precipitates and distinguish them from precipitates that are not worth pursuing. Except for drops where the protein is denatured, precipitate can be regarded as a positive drop result. This is because precipitate occurs in a state of supersaturation. Crystals can only grow from supersaturated states, and thus they can grow from precipitates.

How do I tell a "good" precipitate from a "bad" precipitate?

#### "Good" Precipitate

Non-amorphous precipitate

Precipitate shows **birefringence**

Precipitate redissolves if given the right conditions

#### "Bad" Precipitate

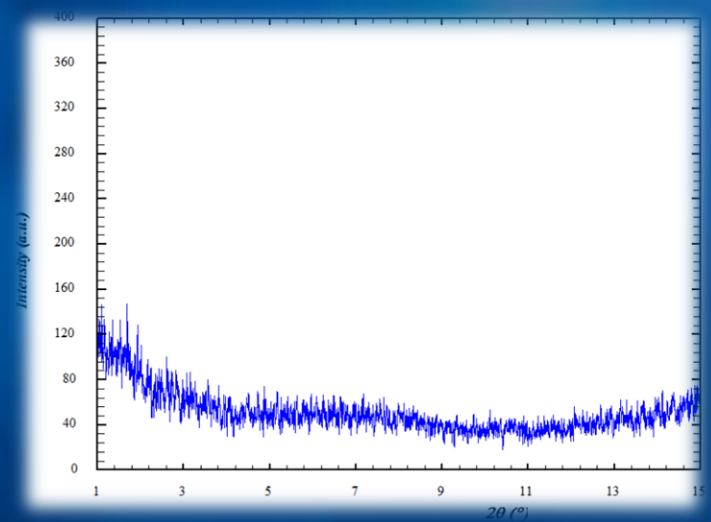
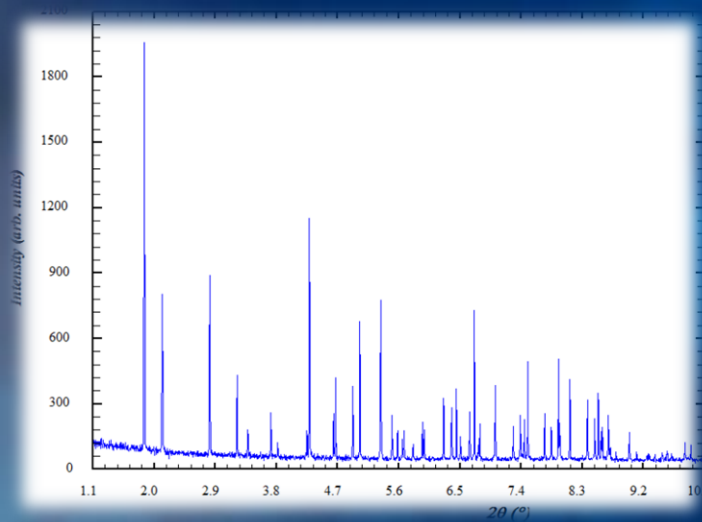
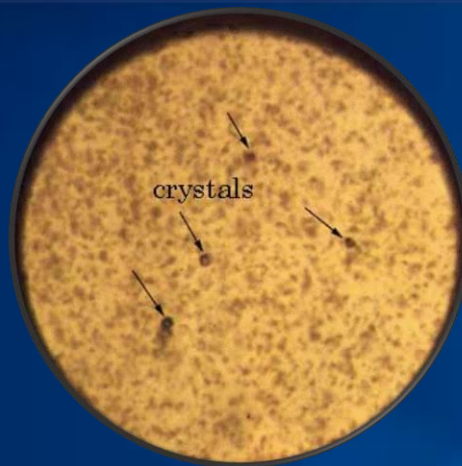
Characteristic brownish tinge to the precipitate

Skins on the drop

Precipitate do not redissolve upon dilution



# GOOD VS BAD PRECIPITATES



- Data Collection



1999 - present  
ESRF- Grenoble, France



New collaborations with other synchrotrons



FRANCE



SOLEIL est le centre de rayonnement synchrotron français,  
à la fois grand instrument pluridisciplinaire  
et laboratoire de recherche



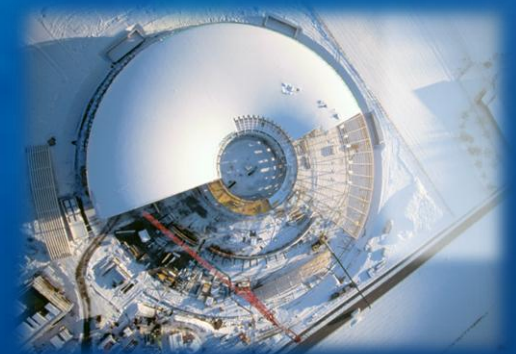
<http://www.synchrotron-soleil.fr/>

JAPAN



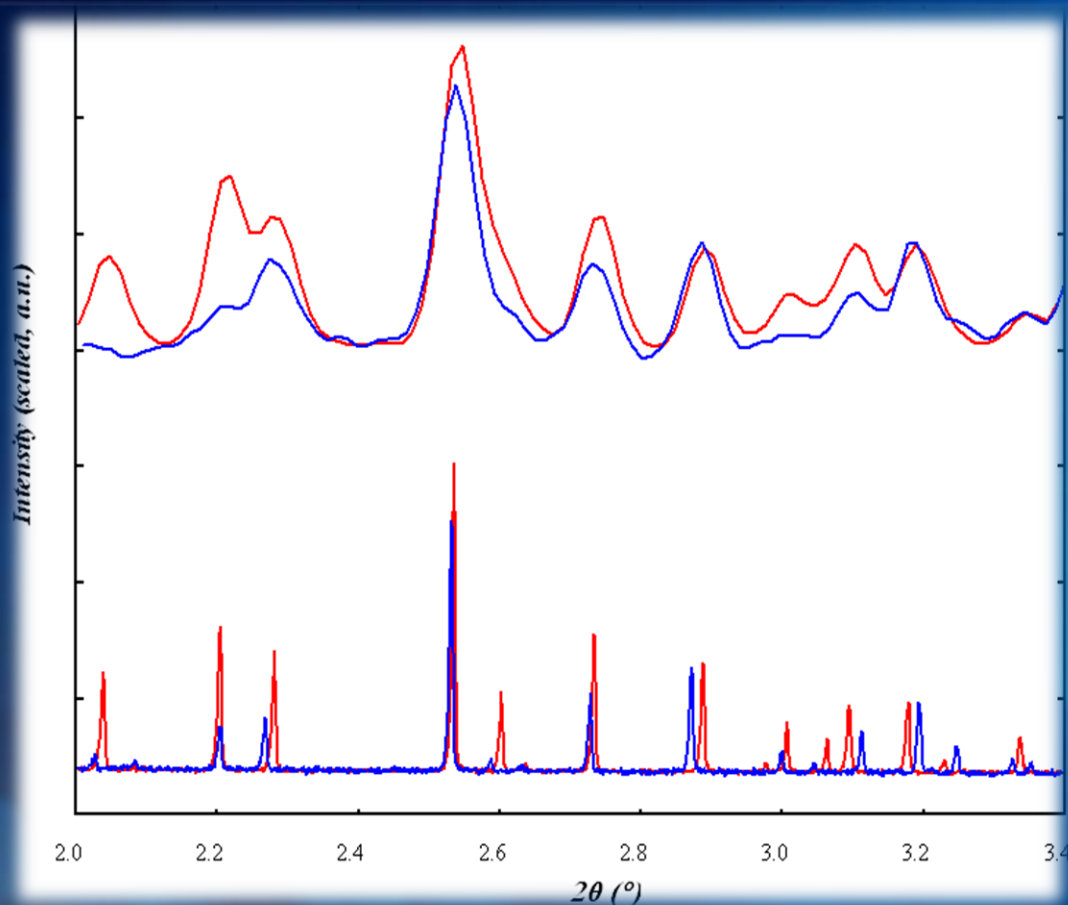
<http://www.spring8.or.jp/en/>

SWITZERLAND

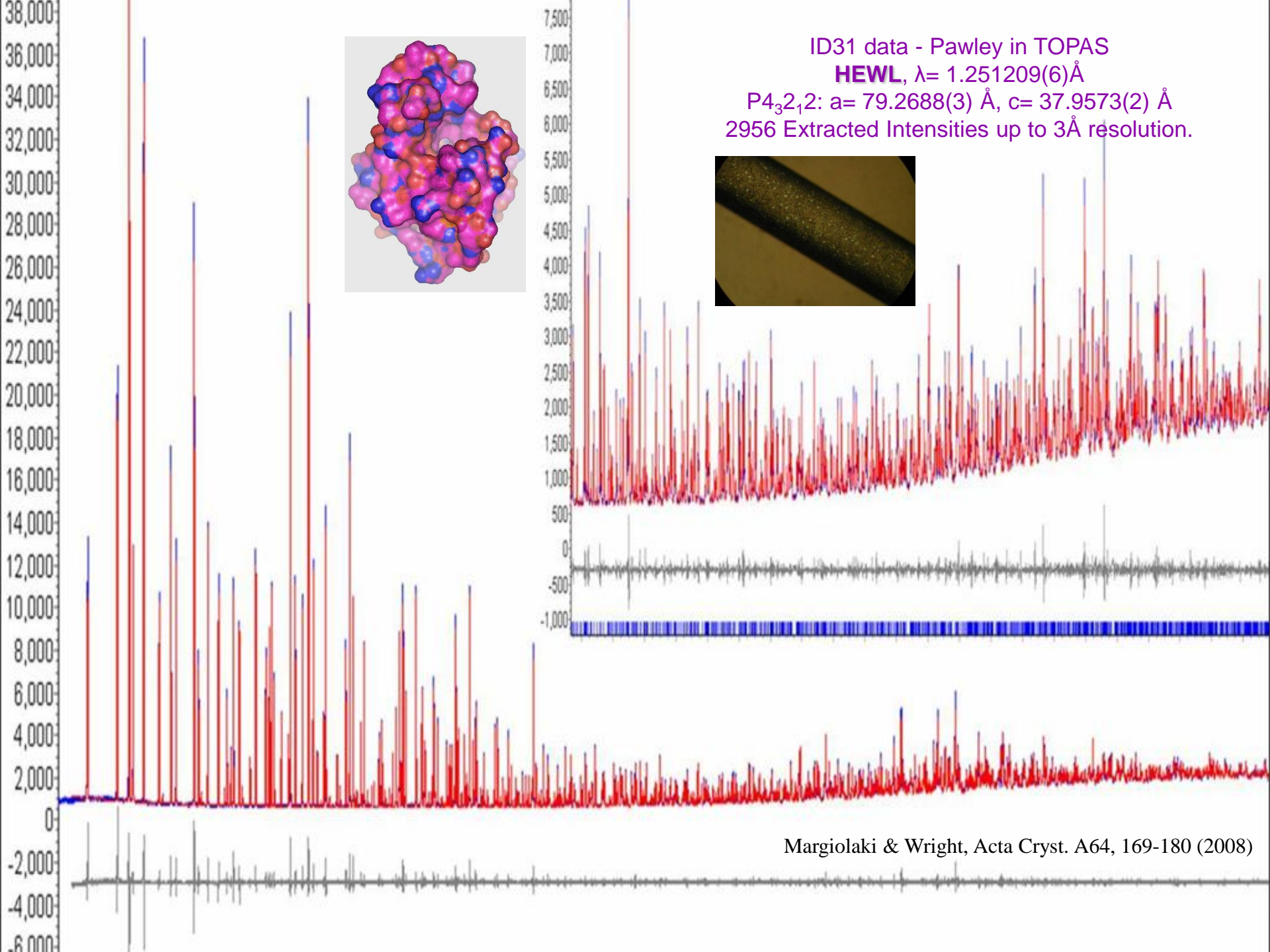


<http://www.psi.ch/sls/>

# AREA DETECTORS VS HIGH RESOLUTION



*Low-angle region of powder diffraction patterns from lysozyme samples. The lower and upper patterns correspond to analyser crystal (ID31) and area detector (BM01A) data respectively. In these experiments, the intensities of certain low angle reflections are modulated by varying the mean electron density of the solvent surrounding the protein molecules in the crystal lattice via variations in the solvent composition. The red and blue spectra correspond respectively to samples soaked in low and high electron-dense solvents.*





# LAB DIFFRACTOMETER X' PERT PRO BY PANALYTICAL

From nanomaterials  
to bulk samples and proteins

X'Pert PRO system handles a wide  
range of applications. It is especially  
suitable for :

- **thin film** analysis applications such  
as **rocking curve** analysis and  
**reciprocal space mapping**,
  - **reflectometry**
- **thin film phase analysis**
  - **residual stress** and
  - **texture analysis**



# X' PERT PRO PIXCEL: A NEW GENERATION'S DETECTOR

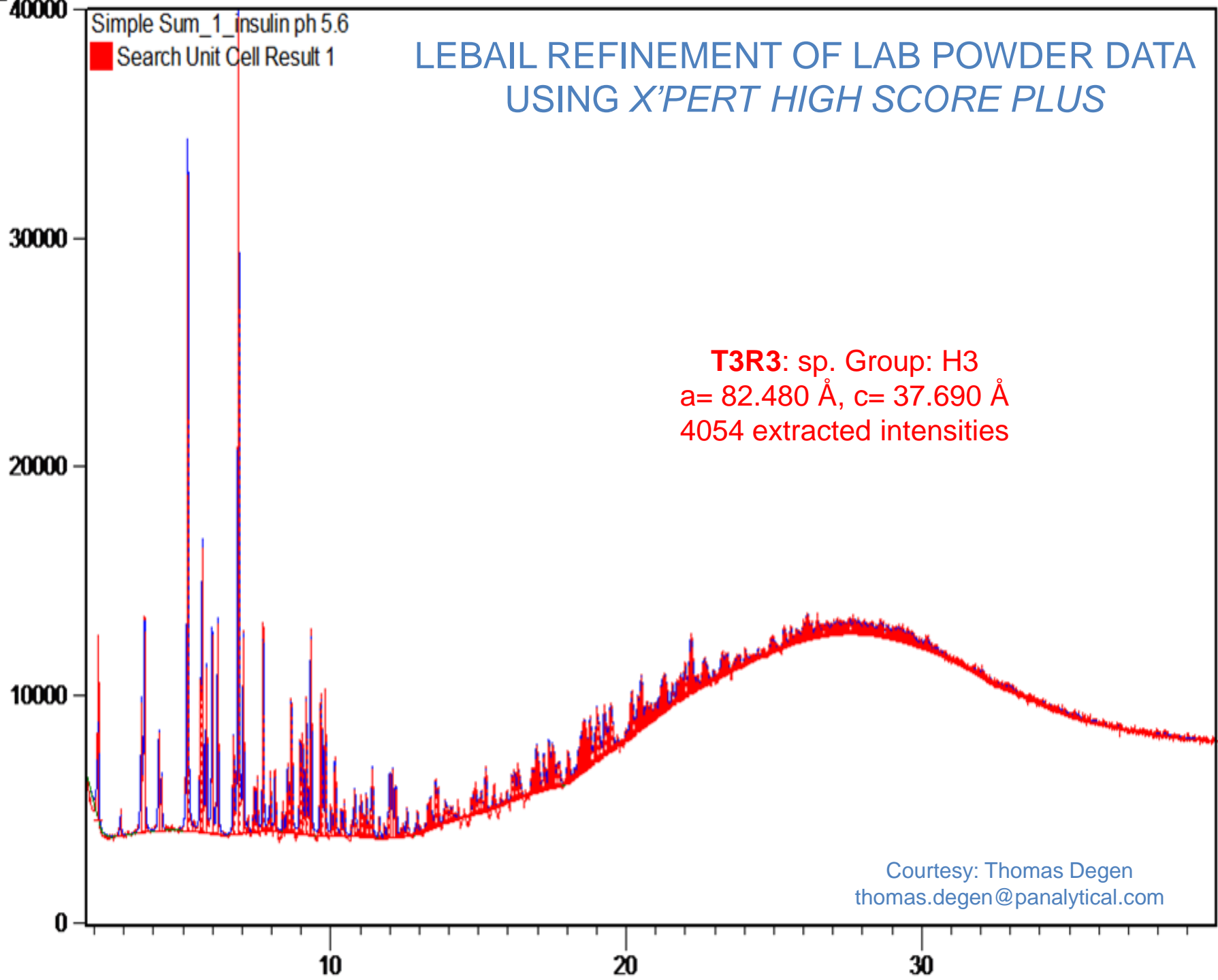
This detector is a member of the Medipix photon counting pixel detector family.



PIXcel is the result of a collaboration with CERN, one of the world's foremost particle physics laboratories and other research institutes as part of the Medipix2 project.



# LEBAIL REFINEMENT OF LAB POWDER DATA USING *X'PERT HIGH SCORE PLUS*





- Data Analysis

## SOFTWARE USED

### Powder Diffraction & CCP14

Fit2D  
DASH  
TOPAS  
FULLPROF  
GSAS

### Single Crystal & CCP4

CCP4 software package  
MOLREP  
PHASER  
CNS  
REFMAC  
PHOENIX  
WINCOOT  
PYMOL  
CHIMERA

### Home made

PRODD  
SFCHECK  
(modified version)  
Short routines in PYTHON  
Pycluster  
ID31sum

### Other useful software

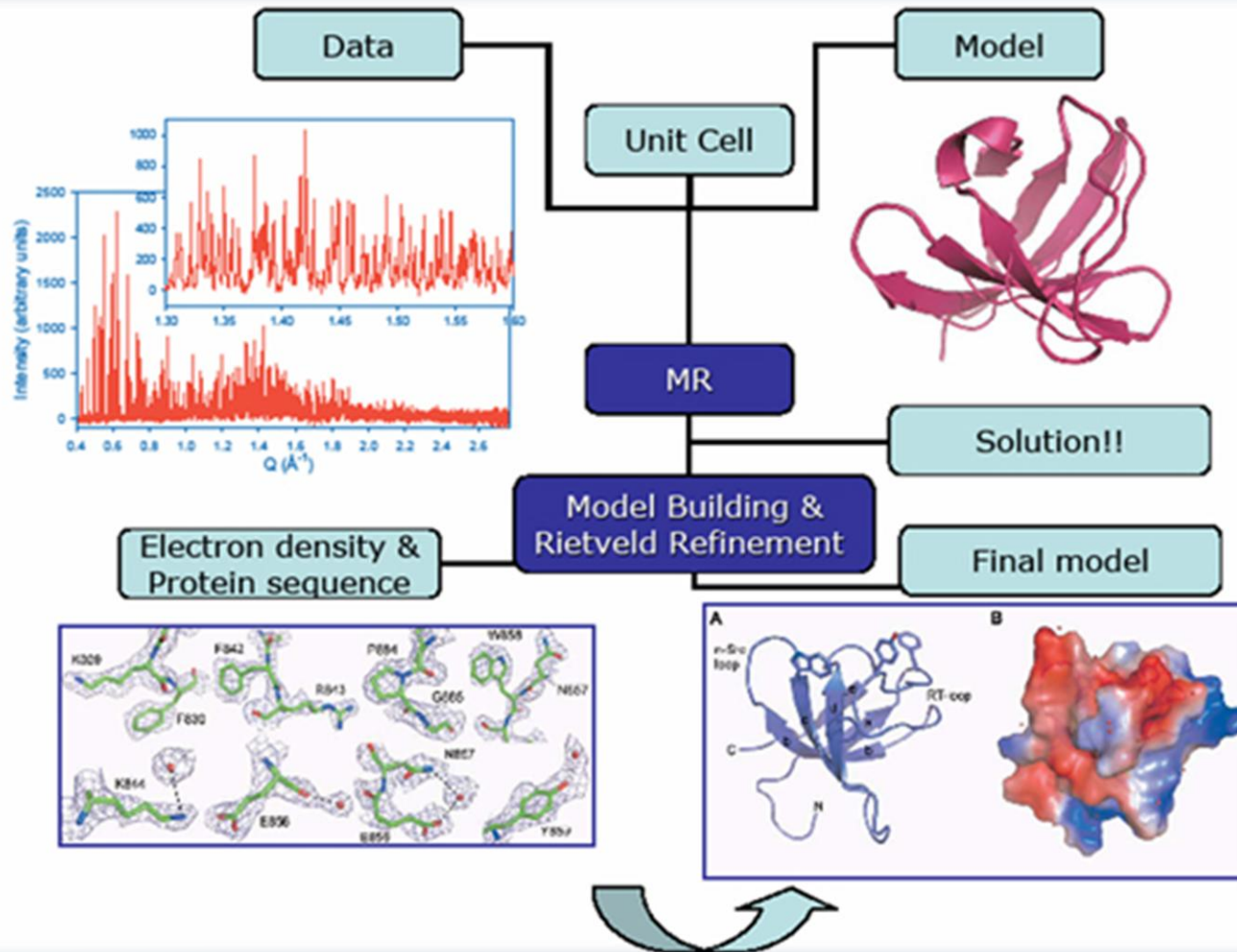
El nemo server  
<http://www.igs.cnrs-mrs.fr/elnemo/>

- Case Studies

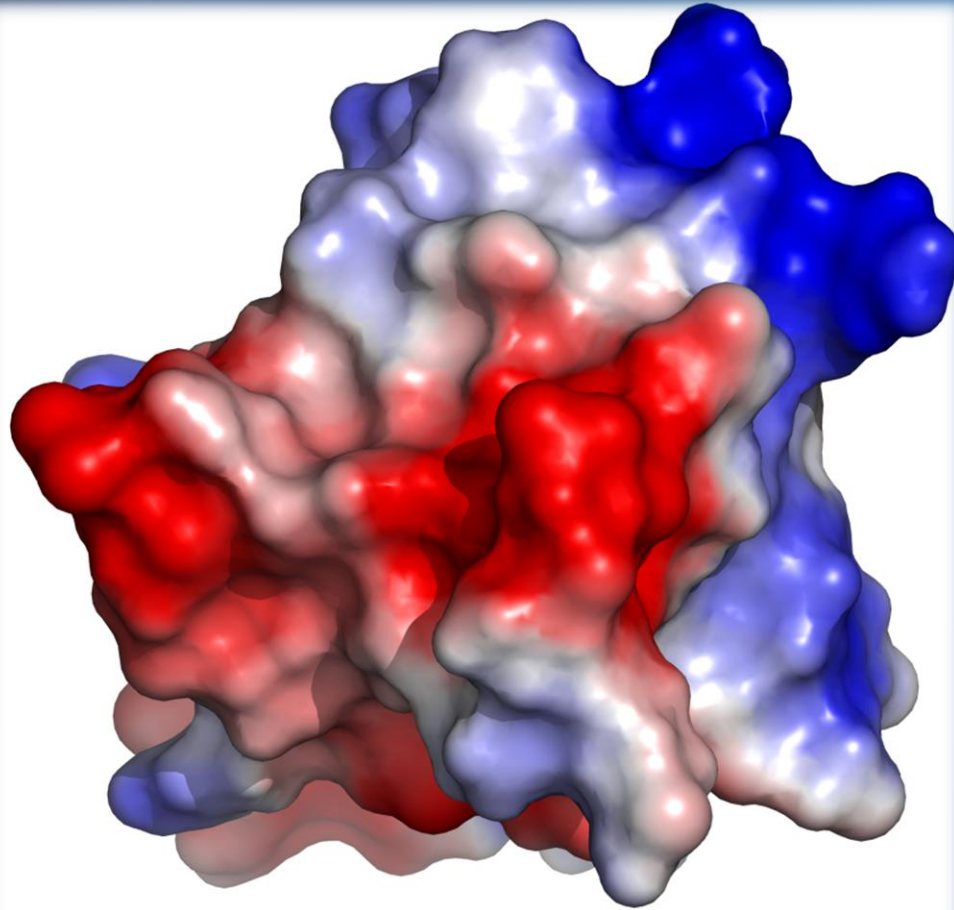


# AN UNKNOWN PROTEIN STRUCTURE SOLVED FROM POWDERS

## The Second SH3 domain of Ponsin



## The Second SH3 domain of Ponsin



<http://www.esrf.eu/news/general-old/general-2007/powder/>

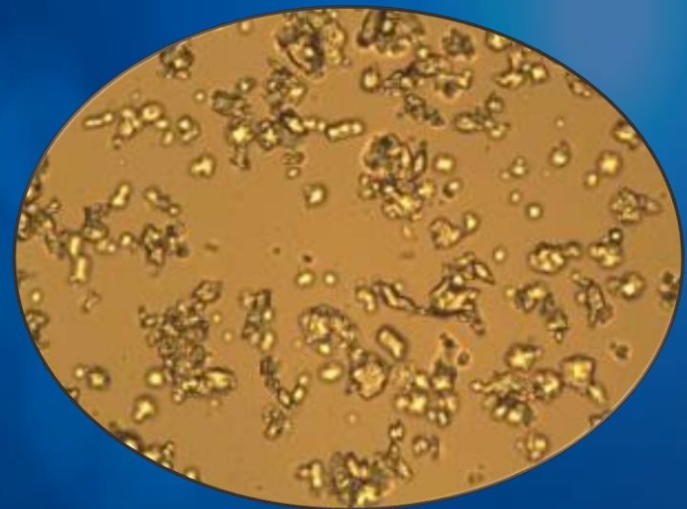
Novo Nordisk, Copenhagen



Research Collaborators  
Gerd Schluckebier & Mathias Norrman



# THE CASE OF HUMAN INSULIN

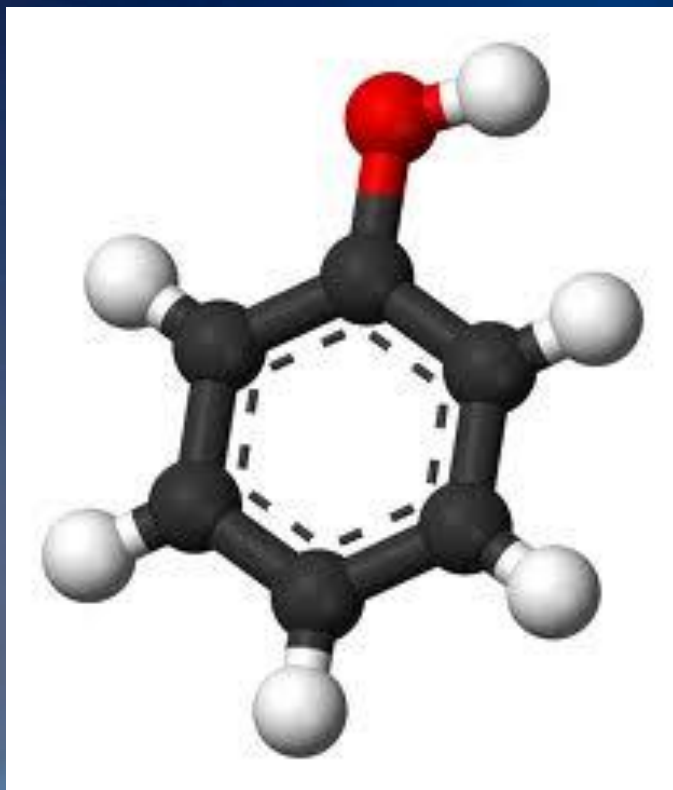


## MICROCRYSTALLINE INSULIN

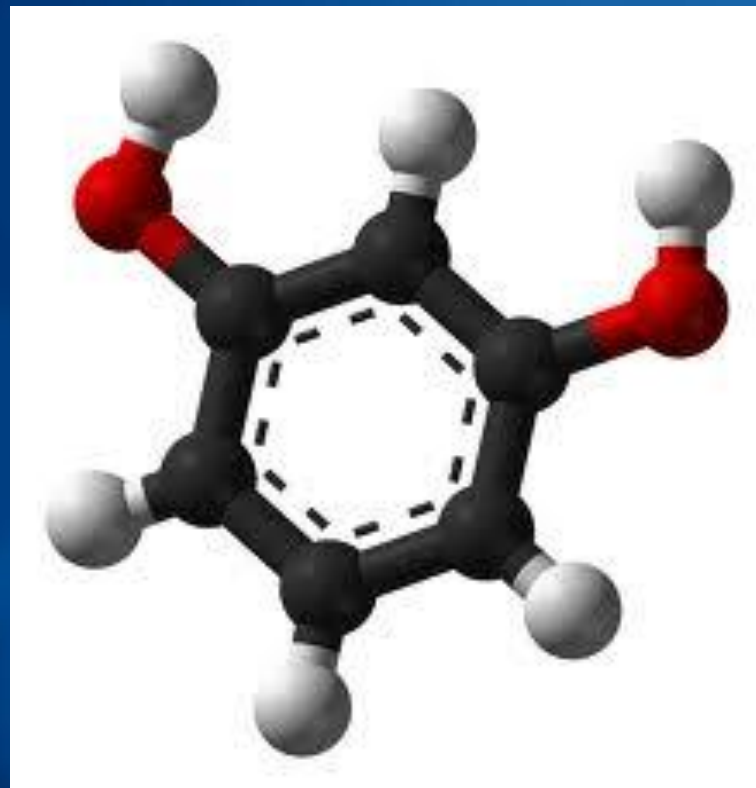
- Several polymorphs exist depending on pH of crystallization and concentration of additives.
- Great interest in finding new forms of potentially therapeutic applications.
- Study microcrystalline insulin crystallized as a function of pH (4 – 8.9) and with phenol – based additives.
- 3 forms already known with structures (from single crystals) in space groups  $C222_1$ ,  $C2$  and  $P2_1$ .

# HUMAN INSULIN – LIGAND COMPLEXES

## CRYSTALLINE PRECIPITATES



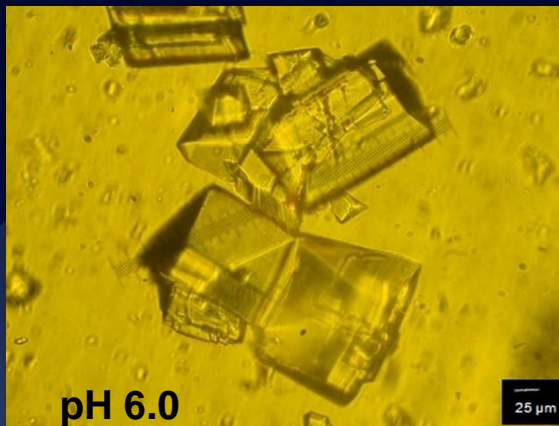
PHENOL



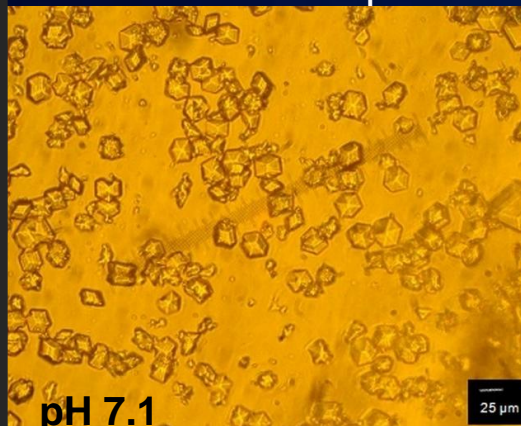
RESORCINOL



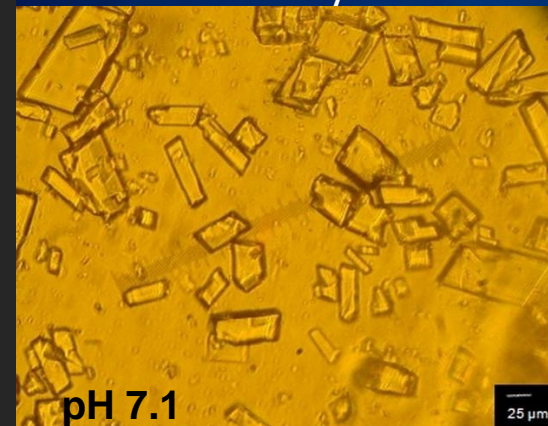
M-cresol



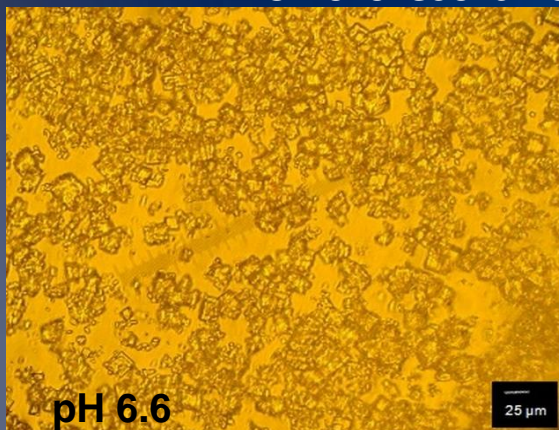
4-Nitrophenol



4-Ethylresorcinol



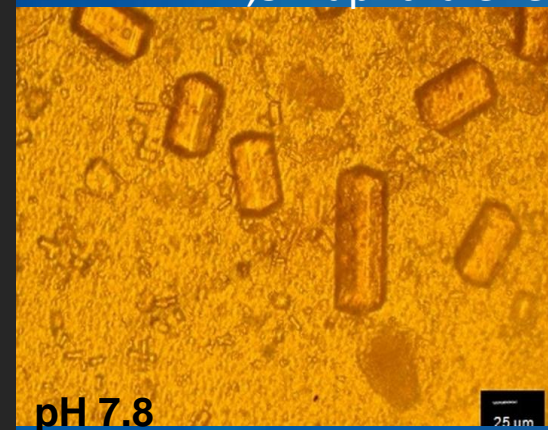
4-Chlororesorcinol



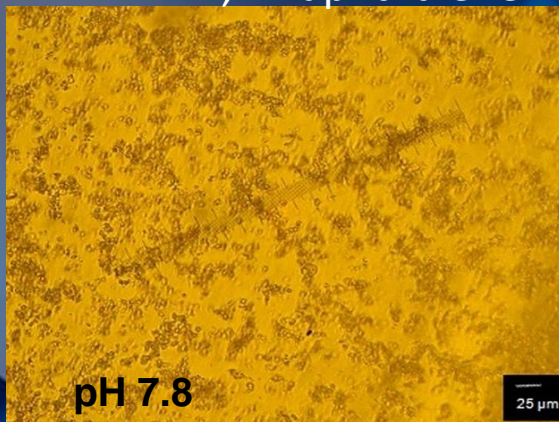
4-Bromoresorcinol



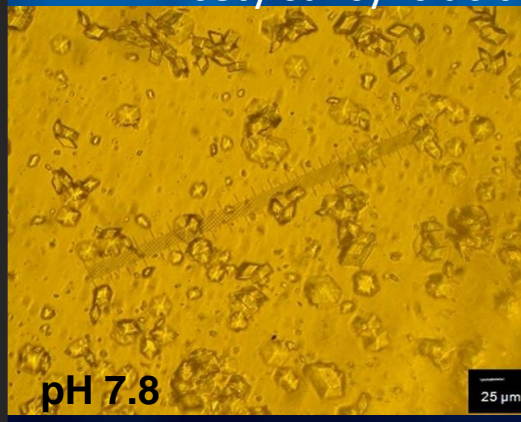
1,3 Naphthalene



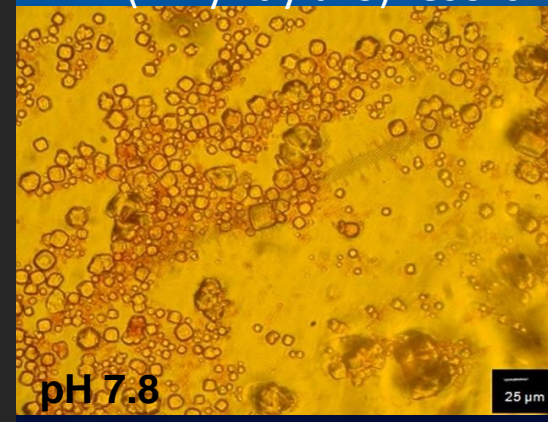
2,7 Naphthalene



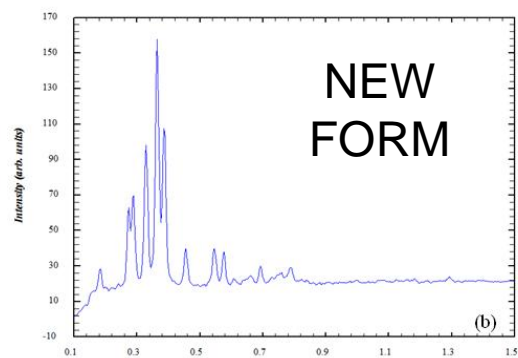
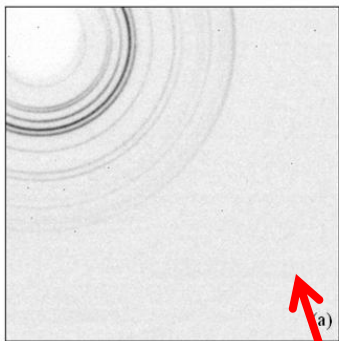
Acetylsalicylic acid



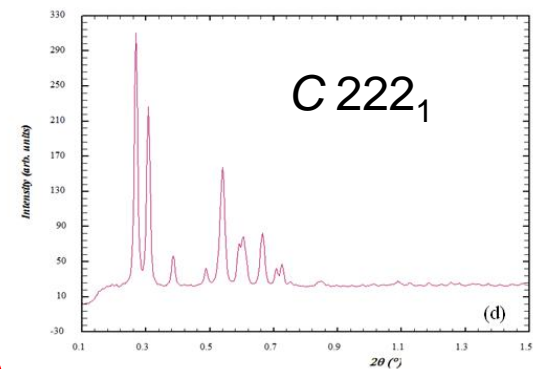
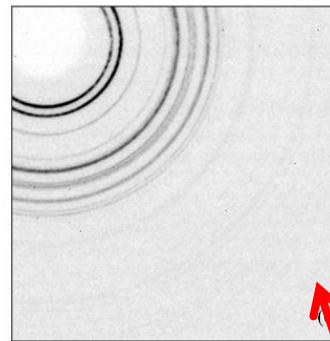
4(2-Pyridylazo)Resorcinol







NEW  
FORM

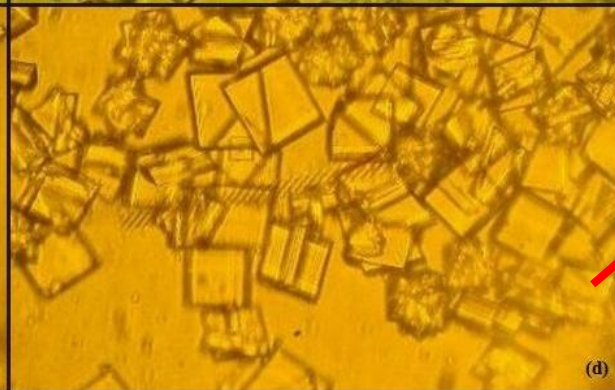
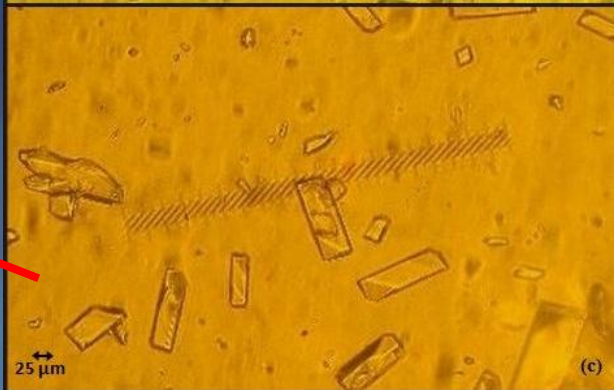


C 222<sub>1</sub>

pH<5.6

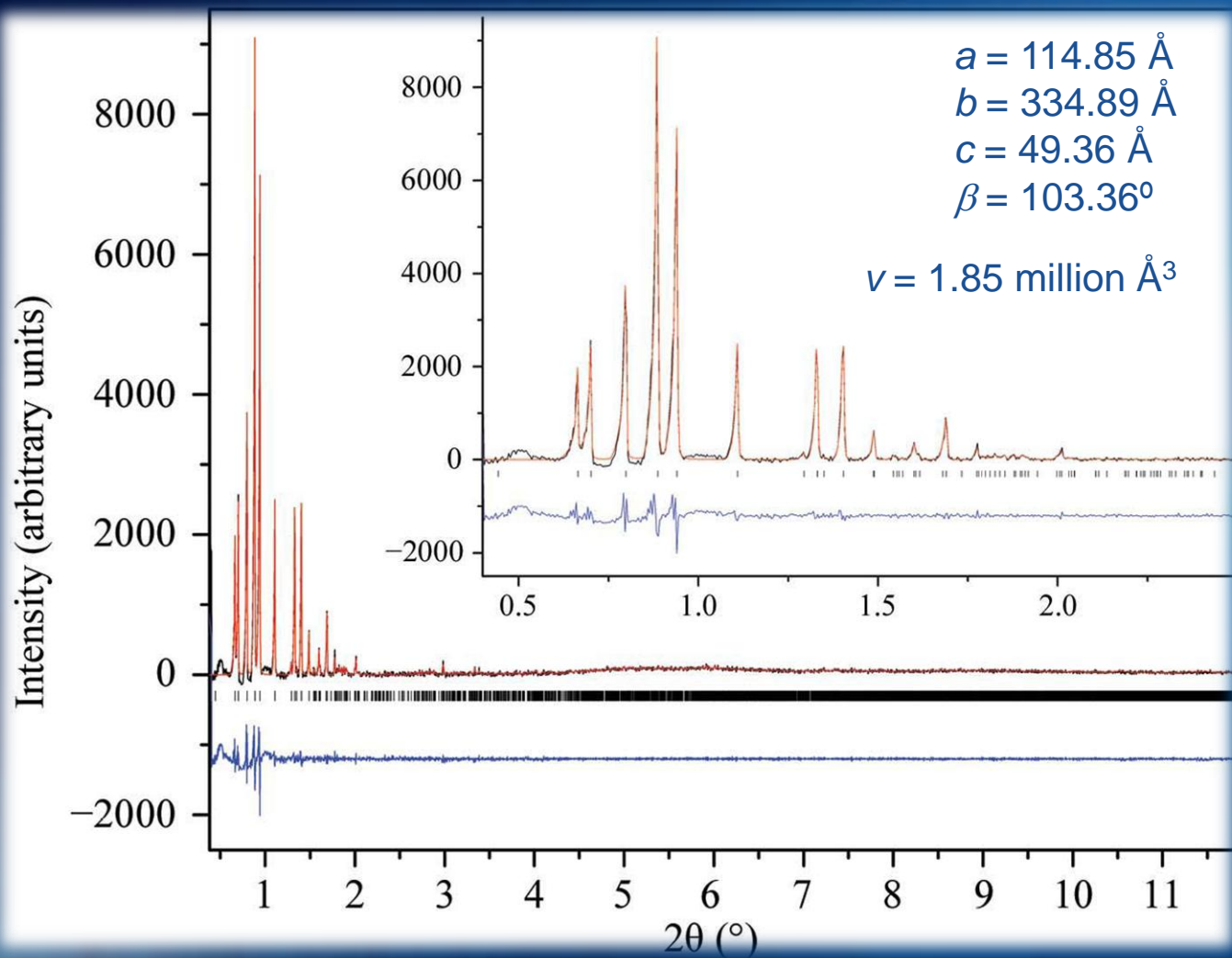


C2

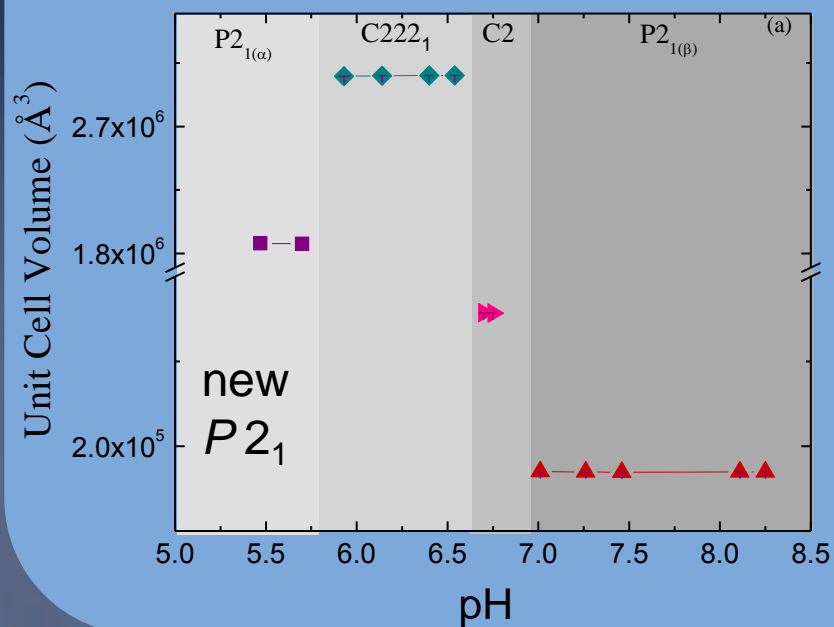


P2<sub>1</sub>

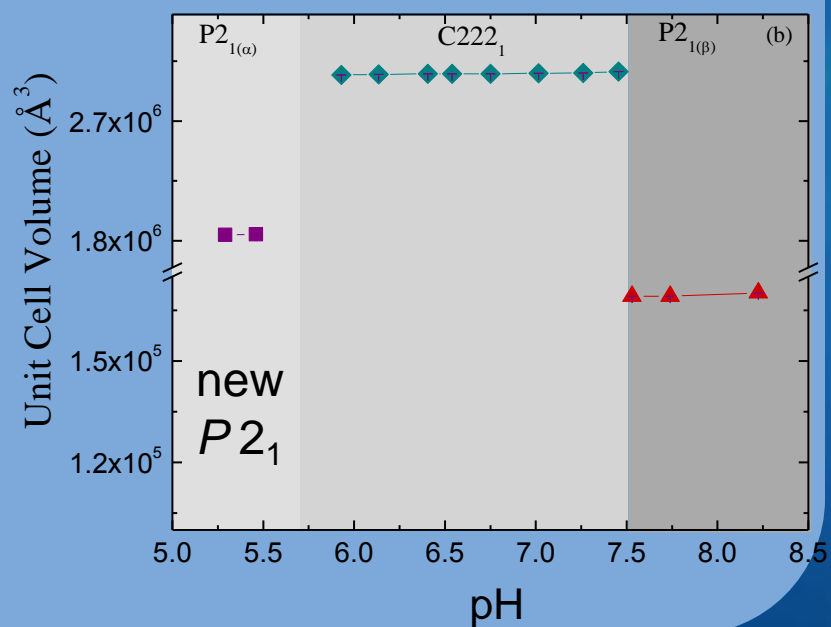
# NEW POLYMORPH pH 5.18 + resorcinol



## phenol

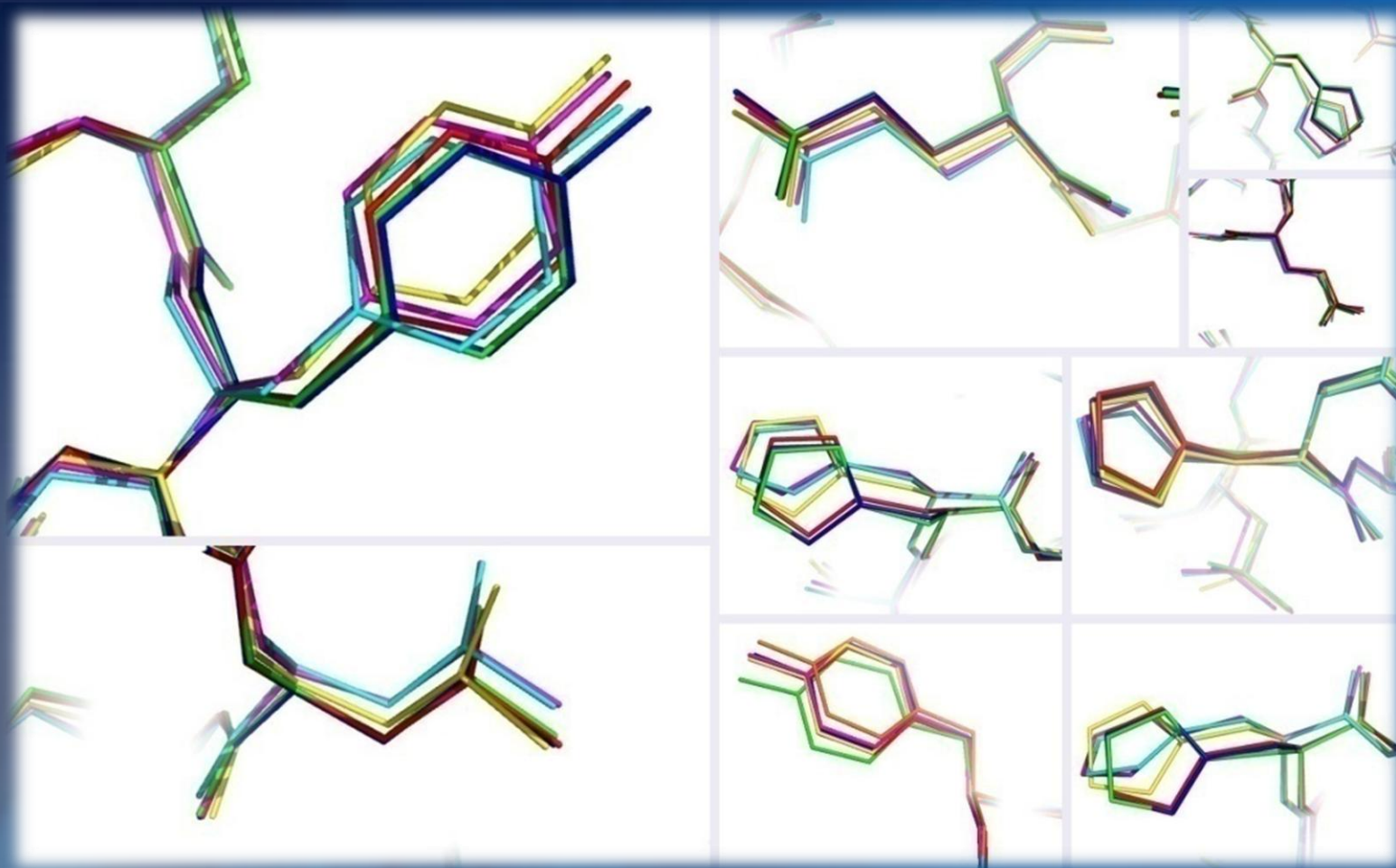


## resorcinol



# ENHANCED STRUCTURE REFINEMENTS

## Rigid body description of amino acids



Margiolaki et al.  
**Acta Cryst. (2013). D69, 978–990**



## AFMB - Marseille

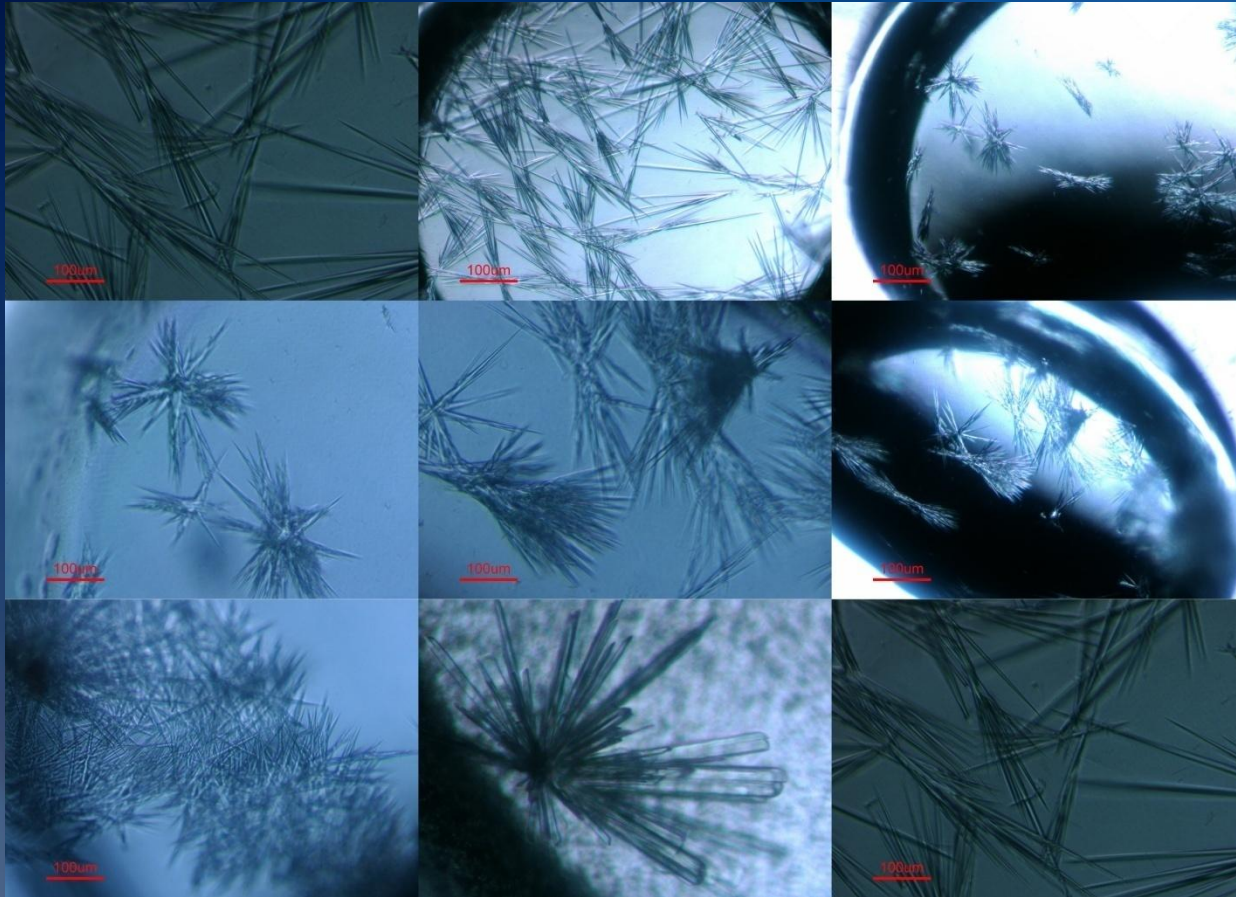


Research Collaborators  
Bruno Canard, Nicolas Papageorgiou & Bruno Coutard

# **The First Case of a Virus Protein:**

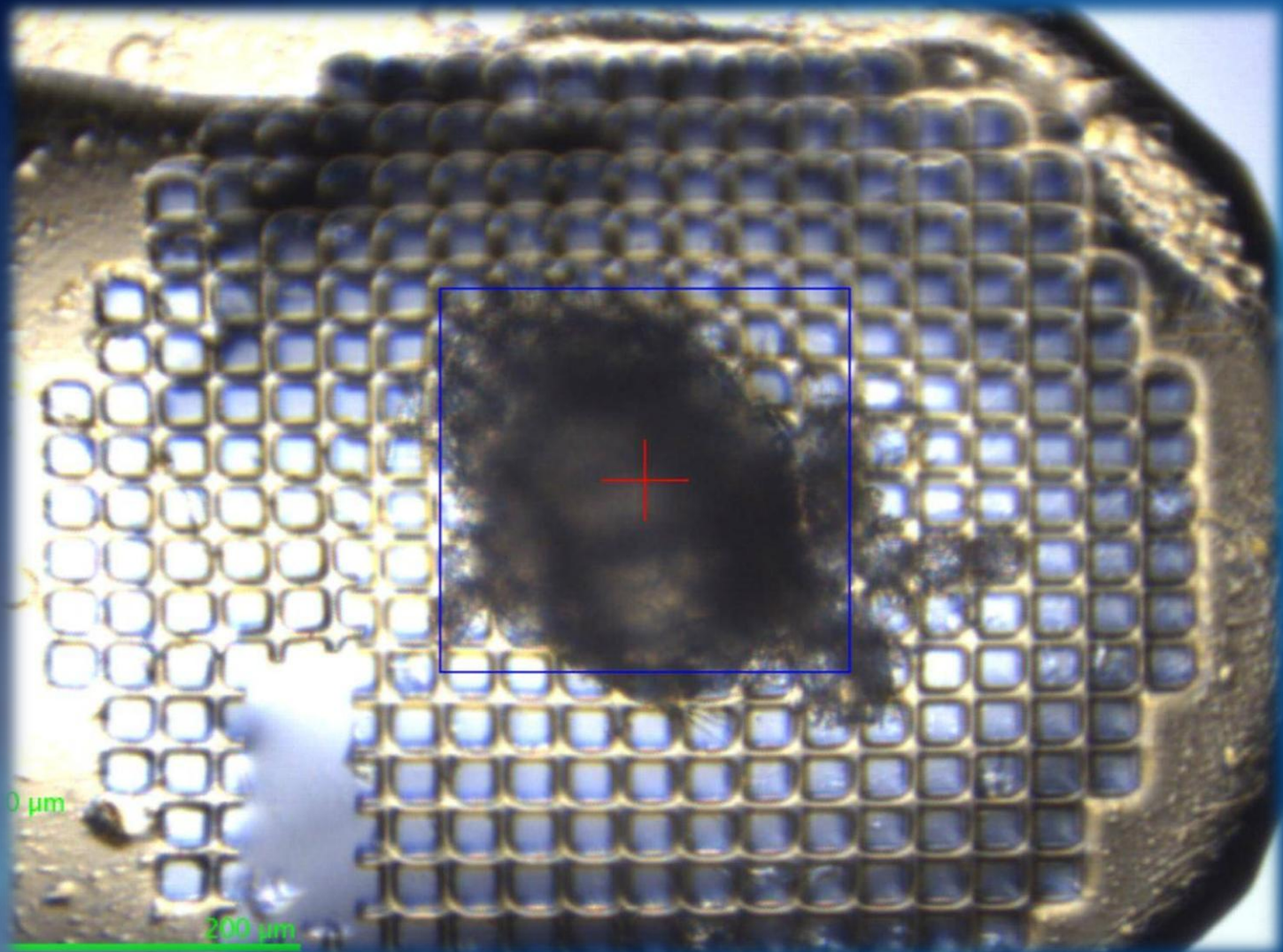
**nsP3 macro domain of the Mayaro virus  
(MAYV)**

# “SEA URCHIN” CRYSTALS OF MAYV

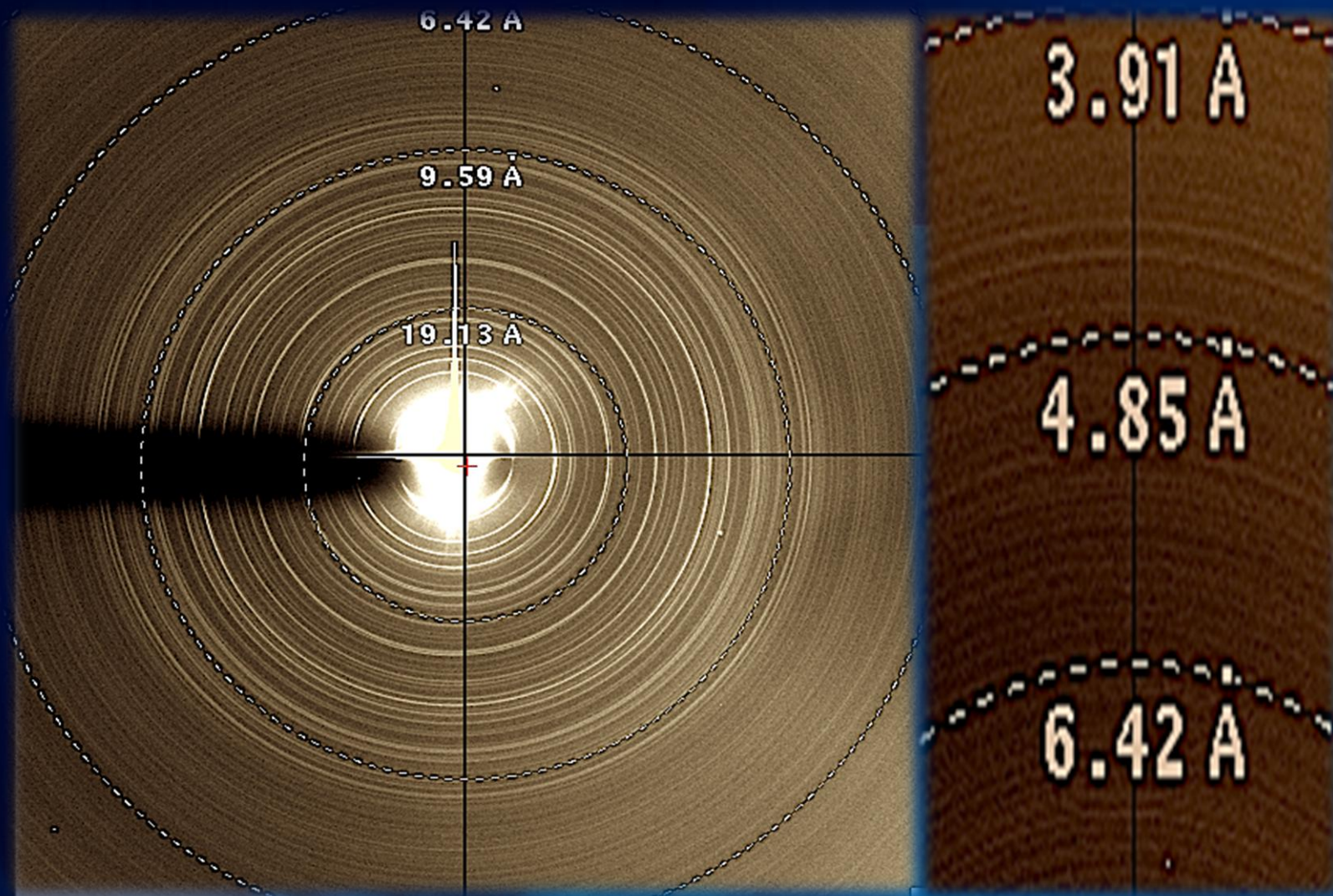




## MAYV IN A GRID





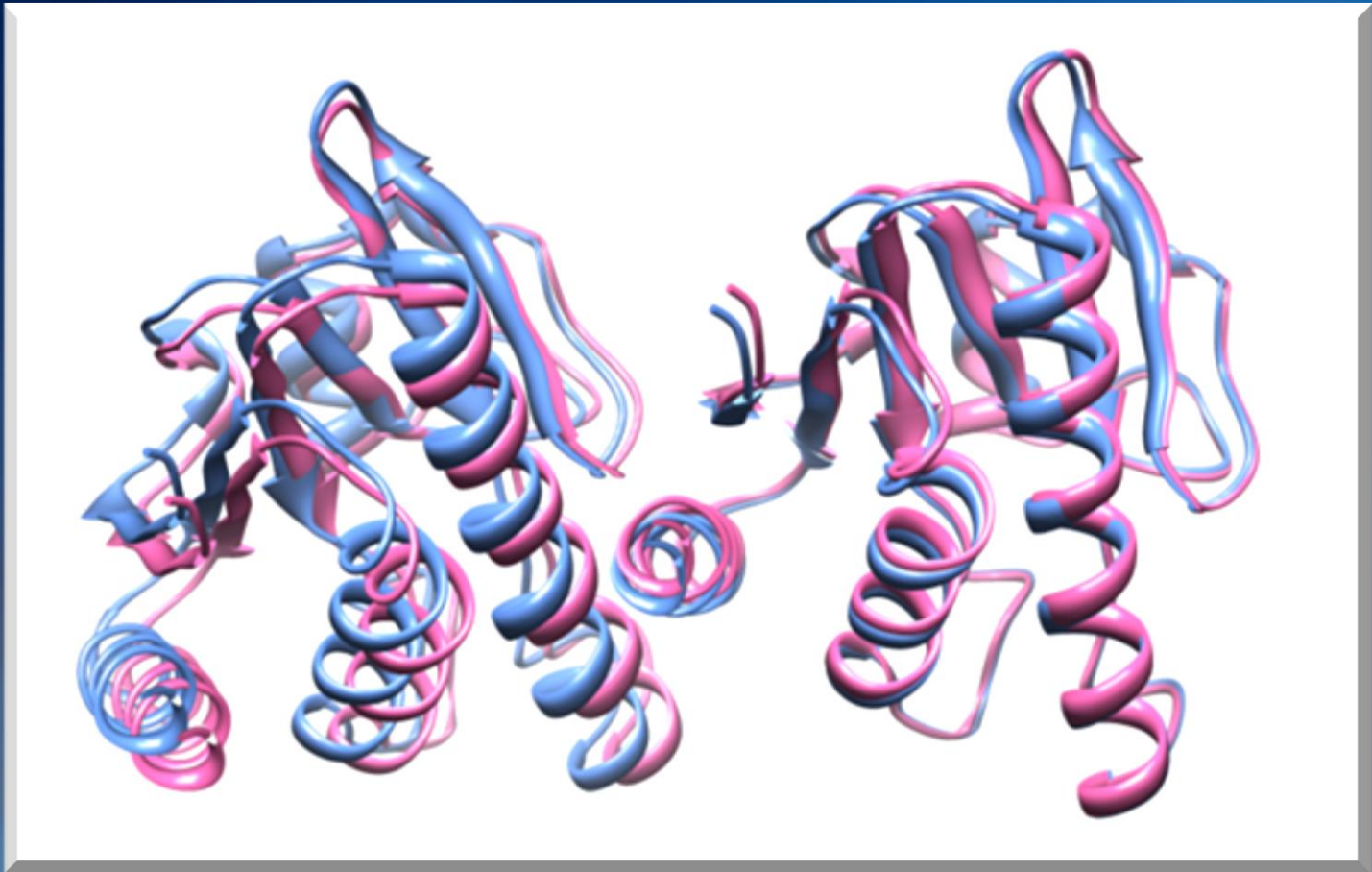


**ID14- 2, area detector**  
 **$\lambda=0.9934 \text{ \AA}$**

**Matthew Bowler, Yves Watier,  
Nicolas Papageorgiou**



## PRELIMINARY MODEL AFTER MR SOLUTION AND RB REFINEMENT





- Future & Concluding Remarks

- **CRYSTAL SCREENING**

Polymorph Identification &  
Ligand Binding

Crystal size and morphology  
(XFEL)

Phase Mapping



- **COMBINED USE WITH**

XFEL measurements on nano-crystalline precipitates

Electron Diffraction on single nano-crystals

- Spence, Weierstall, Chapman , Rep. Prog. Phys. 75, 102601, 2012  
Barty, Küpper, Chapman , Annual Review of Physical Chemistry 01/2013

- *Three-dimensional electron crystallography of protein microcrystals*  
Shi et al., 2013, eLIFE

## CONCLUDING REMARKS

- Protein samples are often easily obtained as microcrystalline precipitates
- Ideal method for Crystal Screening – Phase Identification
- Structure solution and refinement are possible but time consuming
- Good quality data are routinely collected using synchrotrons or modern lab sources
- Combined use with XFEL and ED measurements

# ACKNOWLEDGMENTS

## X-RAY DIFFRACTION

### ESRF, Grenoble

Andy Fitch  
Jon Wright  
Yves Watier  
The ID31 team

#### Former members :

Lucy Saunders  
Ines Collings  
Sotonye Dagogo  
Lisa Knight  
Mark Jenner  
Sebastian Basso

### Department of Biology

P. P. Das  
S. Fili  
F. Karavasili  
A. Valmas  
K. Magioun  
D. Lahana

#### Former members:

A. E. Giannopoulou  
M. Kalatha  
E. Kotsiliti

## COLLABORATORS

CINaM, Marseille

Marion Giffard  
Françoise Bonneté

EMBL, Hamburg

Nikos Pinotsis  
Matthias Wilmanns

EPFL, Lausanne

Marc Schiltz  
Celine Besnard

University of  
Geneva

Radovan Cerny

AFMB, Marseille

Bruno Canard  
Nicolas Papageorgiou  
Bruno Coutard  
Violaine Lantéz

APS, Chicago

Bob Von Dreele

Soleil, Paris

Gavin Fox

University of Manchester

John Helliwell

University of Amsterdam

Henk Schenk

Bruker, Germany

Diederik Ellerbroek,  
Cees Baas  
Patrick Romijn  
Robbert Jan Brandenburg

Nanomegas

Stavros Nicolopoulos

Novo Nordisk, Copenhagen

Gerd Schluckebier  
Mathias Norrman

Sanofi Aventis, Montpellier

Mohamed El Hajji  
Bertrand Castro

University of Patras

G. Spyroulias

PaNalytical, Netherlands

Detlef Beckers  
Thomas Degen  
Celeste Reiss  
Stjepan Prugovecki  
Martijn Fransen



## IYCr - EARLY ACTIONS IN GREECE

- **International Workshop 1: Fundamentals of Crystallography**  
1-7 April 2013 (Tutor: Carmelo Giacovazzo)
- **International Workshop 2: Powder & Electron Crystallography**  
8-12 July 2013  
**Patras Greece**

Lectures available from:  
<http://crystallographypatras.wordpress.com/>



IUCr Commission on Electron Crystallography

## IYCr - ACTIONS IN GREECE

- Current Trends in Structural Biology & 7th meeting of the Hellenic Crystallographic Association, September 19th-21st, 2014, FORTH/IMBB, Heraklion, Crete, Greece
- Workshop on “Strategic pipeline planning: from sample preparation to 3D structure determination with bio SAXS and other biophysical techniques” co-organised with National Hellenic Research Foundation. April 5 - 10 - 2014 National Hellenic Research Foundation in Athens, Greece.
- Lectures/presentations on crystallography and its impact in science and applications for students of secondary education. Agricultural University of Athens, NCSR “Demokritos”, University of Patras and others.
- Competitions for secondary education children, (i) a crystal-growing competition; (ii) competition on output inspired by Crystallography, e.g. photographic, video etc. Details will be given in the website of HeCrA ([www.hecra.gr](http://www.hecra.gr)).

## FUNDING

### IAEA

Coordinated Research Project (CRP) on  
“Utilisation of accelerator-based real-time  
and in-situ methods in investigation of  
materials for energy applications”

2012-2015

CRP code: F12024

### EU & University of Patras

FP7: SEE-DRUG

PI: George Spyroulias

<http://www.seedrug.upatras.gr/>

2012-2015

### UNESCO & L'OREAL Foundations

International

Fellowship for Women in Science

2010-2012

### Karatheodoris Foundation &

EAKE (UPATRAS)

2010-2013

### Nanomegas

Stavros Nicolopoulos

2012-Present