INSTRUMENTS for X-ray crystallography and solution scattering experiments

EMBL Grenoble outpost
Instrumentation Group
Diffraction Instrumentation Team, Florent Cipriani
EMBL Basic research in Molecular Biology

- Study the **STRUCTURE** of biological macromolecules
  - Nature and **position** of the ATOMS

- Understand the **function** of the macromolecules
  - Understand the **mechanisms of life**
  - Understand **diseases**
  - Design **drugs**

- **TOOLS** X-rays and Neutrons scattering
Diffraction Instrumentation Team

**Mission:** Develop Instruments and Methods for diffraction experiments

**The TEAM**

Franck Felisaz
Jerome Halbwachs
Raphael Moya
Alexandre Gobbo
Gergely PAPP
Julien Huet
Christophe Landret
Silvia Russi
Florent Cipriani

ESRF → X-Rays

ILL → Neutrons
**Structure determination**

**Two X-ray diffraction techniques**

1 – Macromolecular crystallography

- Atomic resolution (0.8 Å -50 nm)
- Crystallised form
- Size of macromolecules is limited

2 – Small angle scattering (SAXS)

- Sample in solution
- Large macromolecules, assemblies (complexes)
- Kinetics
- Resolution is limited to 10 Å (Up to 500 nm)
Typical Instruments

Crystallography

Small Angle X-ray Scattering

- 25 beamlines equipped in Europe, US, Canada, Australia, Asia

ESRF BM14

ESRF ID14-4
Diffraction Instrumentation TEAM

Our expertise: System engineering

- Precision mechanics
- Optics
- Cryogenics

- Analog/digital Electronics
- Motion control

- Software

\[ \text{Automatic crystal detection & alignment} \]

Bernard Lavault
Instruments for Macromolecular Crystallography

ESRF BM14

MD2 Diffractometer

C3D Crystal Centring

SC3 Sample Changer

EMBL Grenoble
Diffraction Instrumentation Team, cipriani@embl.fr

ESI-2011 May 18th
Macromolecular X-ray crystallography Reminder

Bragg’s reflections in a crystal lattice

\[ \sin \theta = \frac{n\lambda}{2d} \]

X-ray beam

\( \lambda \) (typically 1Å)

Diffraction image

Electron density map

Structure

First crystallize the macromolecules ...

Protein

96 wells plate

Drop dispenser

crystallization solutions

Crystallization farm

EMBL Grenoble
Diffraction Instrumentation Team, cipriani@embl.fr
X-ray crystallography data collection Reminder

Typical Experimental setup

Crystals mounted in a “cryo-loop”

Collecting a diffraction data set
Several hundred images collected during angular Scans
Typical scan: 1 degree in 0.1 to 5 sec
X-ray crystallography data collection Reminder

### Typical Experimental setup

- **X-ray Diffractometer**
- **Cryo-Cooler**
- **Goniometer**
- **OMEGA**
- **X-ray beam**
- **Crystal**
- **Shutter**
- **X-ray detector**

### Crystals mounted in a “cryo-loop”

- 15 μm protein crystals
- 5 to 500 μm

### Data quality

- **Alignment of the crystal with the beam**
- **Precision of the scans**
- **Signal to noise ratio**
- **Radiation damage**

### Collecting a diffraction data set

Several hundred images collected during **angular Scans**

Typical **scan**: 1 degree in 0.1 to 5 sec
Our solution the MD2 diffractometer family

Diffractometer

Control electronics

Control software
Windows (.NET, C++, VB)

C3D Crystal Centring software

Designed for optimal Data Quality and Automation
The MD2 Goniometer

High precision Air bearing goniometer with crystal alignments & centring table

- **Air bearing spindle** *a few nm error motion*
- **Torque motor** driven (no gearbox)
- **Direct encoding** 4.6M pulses/turn

- **XY centring table** 0.2 µm resolution.
- **XYZ Alignment table** 0.2 µm resolution

- **PMAC motion control** (Close loop PID)
  - Angular error <1 mDeg @ 20 Deg/s
  - Shutter synchronisation error <<1 mDeg (scans)
**MD2 Goniometer**

Précision control

Scales
- Green grid: 10 µm
- Red circle: 1 µm diameter
- Screen resolution: 0.25 µm / pixel

Real time video
- Needle with 1 µm hole at tip
- Rotation 45 deg/sec

Observed sphere of confusion ≈ 1 µm
Processing micro-crystals

How to improve the precision of Goniometers?
**Improving the goniometer precision**

- **SOR diameter**
  - 0.9 µm pp
  - 0.25 µm r.m.s

- **Gravity effect on Centering table**
- **2nd+3rd harmonics**
- **20th+23rd harmonics**
- **Poles of the OMEGA motor**
New Goniometer
Set in vertical orientation

- SOC <0.5 \mu m with Kappa
  Crystal down to 2-3 \mu m

2 Glass needle observed with a microscope (125 nm/pixel) + sub pixel interpolation + Image computing

EMBL Grenoble
Diffraction Instrumentation Team, cipriani@embl.fr
**The MD2 – On beam axis video-microscope**

High resolution On Beam Axis video-microscope  
Patented

**Sample viewing**
- Optical axis
- High resolution Camera
- Lighting condenser
- 12µm needle

**beam viewing**
- Beam
- X-ray scintillator
- Real view of the collimated beam

**No parallax error → Perfect alignment with the beam**
The **MD2** micro-diffractometer
to get the best data from the crystals

*BUT... not all the crystals are good...*
Before automation

An ordinary day on a beamline… Screening crystals

Changing a Crystal:

…Opening the hutch
Unmounting the previous
Mounting the new one
Aligning it
Closing the hutch
Starting data collection…
An ordinary day on a beamline! …Screening crystals…

Automatic screening

- 50 samples in 2H30’

…And also thanks to C3D, the automatic crystal centring software developed by Bernard Lavault
Instruments for Small Angle Scattering experiments

- Sample in solution
- Large macromolecules, assemblies (complexes)
- Kinetics
- Resolution is limited to 10 Å (Up to 500 nm)

BioSAXS Sample changer

EMBL Grenoble Diffraction Instrumentation Team, cipriani@embl.fr
**BioSAXS Typical experimental setup**

- **Monochromatic X-ray beam**
- **Exposure cell**
- **Solvant+prot. Solvant**
- **Flight tube**
- **Exposure Cell**
- **All beam path in vacuum**
- **Detector**
- **2D Detector**
- **Model Building**

**X33 beamline** EMBL-HH/Doris *D. Svergun, M. Roessle*
BioSAXS Sample Changer at ID 14-3

Sample Exposure Unit
Sample changer unit
Control electronics
Fluidic rack
GUI

5-200µl

EMBL Grenoble
Diffraction Instrumentation Team, cipriani@embl.fr

ESI-2011 May 18th
A design for low volumes & high speed

Challenging design

- Two separated units
- Pipetting needle is fixed
- Microplates are moved to the samples
BioSAXS Sample Changer – Sample Exposure Unit

Exposure Cell
In Vacuum, T° control

Camera

Ø2 mm quartz capillary
With 10µm wall
Control electronics

- Field bus electronics *EtherCat* (Beckhoff)
- PLC and motion control: *TwinCat* real time layer in a Windows PC
Control software

Design
- Core & GUI Written in **Java**
- Process scripted in **Phyton**
- Image computing **C++/Java**

- Full remote control
  - High and low level control
    - Socket + Libraries
  - **Tango** device server (ESRF)
  - **Tine** device server (EMBL-HH)
Load Sample

Sample arrives in field of view: position control takes over
Flow sample During exposure to X-rays

Optimal use of solution to reduce radiation damage
Clean Needle, tubing and Exposure Capillary

Washing with detergent
Rinsing with water
Drying with dry air
Dry control by image processing
Parking the cleaning station
BioSAXS Sample Changers today

- Two machines installed
  - ESRF ID14-3, routinely used
  - EMBL@PETRA-III, commissioning
- One machine under construction
  for Diamond Light Source
  
  (Maatel/Bruker)
Macromolecular Crystallography \textbf{Crystal harvesting}

Crystallization is automated

MX beamlines are automated

EMBL Grenoble
Diffraction Instrumentation Team, cipriani@embl.fr

ESI-2011 May 18th 32
Crystal Direct

In-plate crystal screening

Harvesting by photo-ablation

The CD plate
Crystal Direct crystal harvesting (Photo ablation)

vapour diffusion experiment

Crystal
**Crystal Direct Harvester prototype**

- Precision mechanics
- Optics (Laser, microscope)
- Robotics & Motion control
- Fluidics (gluing, pico-drops of cryo-protectant)
- Image computing (Laser Auto focus, alignment control)
- Low level automation (PLC)
- Process automation (Java/Phyton/C++)
- Communication (Device server, Databases)

**Again a good mix of physics, mechanics, electronics and software!**
Thank you for your attention!

Franck Felisaz
Jerome Halbwachs
Raphael Moya
Alexandre Gobbo
Gergely PAPP
Julien Huet
Christophe Landret
Silvia Russi
Florent Cipriani