# Macromolecular Crystallography (MX)

## The technology at a glance

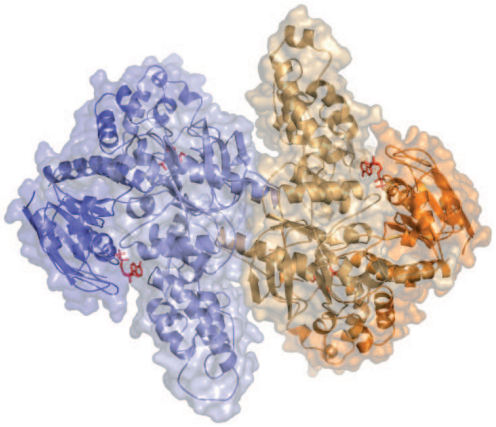
**The MX beamlines allow scientists to investigate the fundamental structures of biological macromolecules, which are at the root of all life processes. Each of these molecules is made up of one or more proteins which are responsible for functions as diverse as energy storage, signalling, cell division and muscle contraction, amongst many others. MX also allows scientists to understand how chemicals interact with these proteins and thus design more rational and structure-based drugs.**

## The added value of the ESRF MX facilities

The ESRF is recognised as the world leader in MX. Its six MX beamlines, which are all equipped with automated and robotised sample handling, can process and analyse a great number of samples quickly and efficiently. Also, the ESRF has the world's first micro-focus beamline dedicated to MX, which means that scientists use a high-intensity microbeam with consistent performance characteristics. This is essential since macromolecules are difficult to prepare for diffraction (up to 1000 crystals may have to be screened to obtain a useful dataset) and, to be efficient, all samples must be exposed to a beam that matches the crystal size.

> "You're working in the dark without a molecular structure. Seeing it turns the light on."
>
> - Matthew Bowler, Scientist in charge of the ESRF MX beamline ID14-2

The development of structural biology worldwide, driven by advances in the field of biomolecular crystallisation, has generated greater demand for beam time from industry. To respond to this need, the ESRF has set up a data collection service, MXpress, allowing users to send their frozen samples directly to the facility.



The experiment is usually carried out within a few days of receiving the samples. Whilst experiments are being carried out, the collected data are available to users in real time. The final report is sent rapidly to the client once the experiment is complete. This procedure means industrial personnel no longer have to travel to the ESRF and they benefit from fast sample processing by experienced on-site staff.

Users may also collect their data in Remote Access mode, from their home laboratory.

## Fields of application

**Pharmaceutical companies**, such as AstraZeneca (UK and Sweden), Sanofi-Aventis (France and Germany)

and GlaxoSmithKline (UK) regularly use the ESRF MX beamlines to gain insight into the structure of target proteins and small molecules. Pharmaceutical companies are the ESRF's longest-running industry customers, having regularly worked with the facility since the mid-Nineties.

"We are really enthusiastic about the ESRF since our scientists like to collect on their own samples and they can do that at the ESRF. The ESRF is easy to use, we are comfortable with the procedures now and of course there is the quality of the beamlines."
- AstraZeneca (UK)

Having used the ESRF for a long time now, we know what to expect, which is an advantage, everyone is highly efficient and helpful, it is easy to work with the ESRF staff and the data quality is excellent.
- AstraZeneca (Swe)

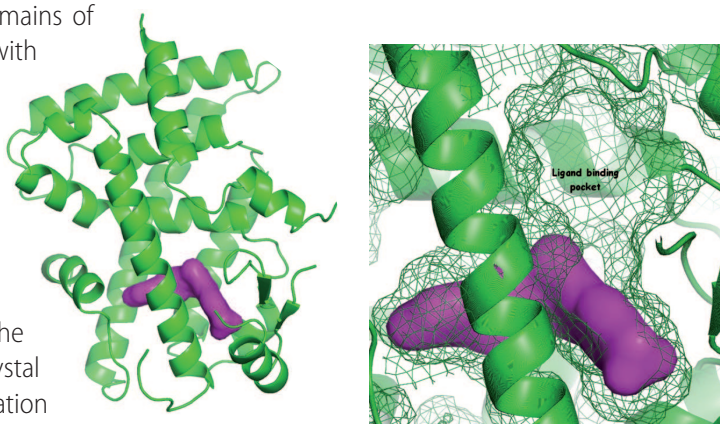
"We are especially delighted with the sample changer, which has literally changed our lives. Instead of coming onto the beamline every few minutes to change a sample, we can press a button and watch hundreds of results arrive."
- Sanofi-Aventis (France)

CASE STUDY

# Sanofi-Aventis used the MXpress service for data collection on PPARδ crystals to understand how better to control diabetes.

**The challenge:** To find an agonist which would stimulate the body's reaction to insulin in sick patients.
**Background:** Increased activity of PPARδ, which regulates the response to insulin, is considered a positive effect for type-2 diabetes. The crystal structures of the ligand binding domains of the PPARδ receptor in complex with agonist molecules were studied to find an agonist which could be used to stimulate a patient's reaction to insulin.
**Results:** Crystal structures with designed agonists showed a high degree of plasticity of the agonist binding pocket – a key discovery for the chemists developing drugs. The crystal structure analyses allowed optimisation of ligand properties to produce agonists which activated PPARδ with a high specificity. Aided by these crystallographic studies Sanofi-Aventis has a new drug in clinical trials.

**How did the synchrotron help?** PPARδ crystals grow slowly, diffract weakly and are particularly sensitive to radiation damage. The MXpress team used their expertise to collect good quality X-ray diffraction data that allowed crystal structure solution.




Left: Cartoon representation of the PPARδ ligand binding domain structure, with agonist in shown magenta.
Right: Close-up of the binding pocket (mesh representation) showing that the ligand occupies only part of the pocket, but with a good surface fit.

# Macromolecular Crystallography (MX)

| Beamline name | ID14-1 | ID14-2 | ID14-4 | ID23-1 | ID23-2 | ID29 |
|---|---|---|---|---|---|---|
| Website | http://www.esrf.eu/UsersAndScience/Experiments/MX | | | | | |
| Operational | Y | Y | Y | Y | Y | Y |
| Operation schedule | http://www.esrf.eu/Accelerators/Operation/Schedules | | | | | |
| Time available (general use) | 25% | 25% | 25% | 25% | 25% | 25% |
| Ring current (mA) | 90-200mA | 90-200mA | 90-200mA | 90-200mA | 90-200mA | 90-200mA |
| Spot Size at sample ($\mu m^2$) | 100 x 100* | 100 x 100* | 100 x 80** | 50 x 30 | 5 x 7 | 50 x 30*** |
| Flux @ 200mA (ph/s) | $5.8x10^{10}$ | $1.3x10^{11}$ | $1.8x10^{12}$ | $1.5x10^{12}$ | $4.0x10^{11}$ | $1.0x10^{13}$ |
| Flux density @ 200mA (ph/s/$mm^2$) | $5.8x10^{12}$ | $1.3x10^{13}$ | $2.2x10^{14}$ | $1.0x10^{15}$ | $1.1x10^{16}$ | $6.7x10^{15}$ |
| Microfocus experiments | | | | | Y | |
| Microbeam experiments | | | | | | Y |
| Wavelength min (Å) | 0.934 | 0.934 | 0.9 | 0.6 | 0.873 | 0.6 |
| Wavelength max (Å) | 0.934 | 0.934 | 1.3 | 2.1 | 0.873 | 2.1 |
| Maximum resolution (Å) | 1.0 | 1.0 | 0.9 | 0.6 | 0.9 | 0.6 |
| Detector | ADSC-Q4r | ADSC-Q210 | ADSC-Q315r | ADSC-Q315r | Mar 225 | ADSC-Q315r |
| Sample changer | Y | Y | Y | Y | Y | Y |
| MAD data collection | | | Y | Y | | Y |
| Fluorescence detector | Y | Y | Y | Y | Y | Y |
| Helical data collection | | | | Y | Y | Y |
| 'Line/Mesh' scans (crystal location) | | | | (Y) | Y | (Y) |
| Automatic crystal annealing | Y | Y | Y | Y | Y | Y |
| X-ray emission analysis (XRF) | Y | Y | Y | Y | Y | Y |
| Automatic loop centering | Y | Y | Y | Y | Y | Y |
| Remote Access | Y | Y | Y | Y | Y | Y |
| Automatic crystal characterisation | Y | Y | Y | Y | Y | Y |
| MXpress data collection | Y | Y | Y | Y | Y | Y |
| †Crystal dehumidification | | Y | | | | |
| †On-line microspectrophotometer | Y | Y | | | | |
| Room temperature collection | Y | Y | | | | |
| Lab facilities | Y | Y | Y | Y | Y | Y |

*Spot size at sample can be varied between 30 x 30 $\mu m^2$ and 200 x 200 $\mu m^2$. **Spot size at sample can be varied between 30 x 30 $\mu m^2$ and 200 x 80 $\mu m^2$.
***Spot sizes at sample: 50 x 30 $\mu m^2$ ; 30 µm, 15 µm, or 10 µm diameter. †Requires 2 weeks advance notice

CASE STUDY

# Schering-Plough used the ESRF MXpress service to determine the important characteristics of the progesterone-receptor binding site.

**The challenge:** To obtain a crystal structure of the mifepristone-progesterone receptor complex to better understand the specificity of the receptor's ligand binding domain.

**Background:** Mifepristone is a clinically-used antiprogestin. Whilst it is known that mifepristone exerts its clinical effect by binding to the ligand binding domain of the progesterone receptor, there was no structural information concerning this interaction. Mifepristone also binds to two other receptors, which could have undesirable effects.

**Results:** A high-resolution crystal structure showed that mifepristone was able to bind to the receptor in the conformation expected for an agonist. Prior to these studies, it was predicted that steric hinderance would preclude this. These studies have extended knowledge on the structural form of the ligand binding domain of the progesterone receptor thus permitting the design of more specific antiprogestin drugs for future clinical use.

**How did the synchrotron help?** The crystal structure was solved to 1.95Å resolution, following data collection using the ESRF MXpress service.

**Reference:** Raaijmakers et al. J. Biol. Chem. 284 (2009), 19572-19579.

Mifepristone (ball and stick representation) viewed within the progesterone receptor ligand binding domain (worms representation).

CASE STUDY

# MRC Cambridge used ESRF microfocus beamlines to determine the crystal structures of two β-adrenergic receptors.

**Beamlines:** ID23-2, ID13

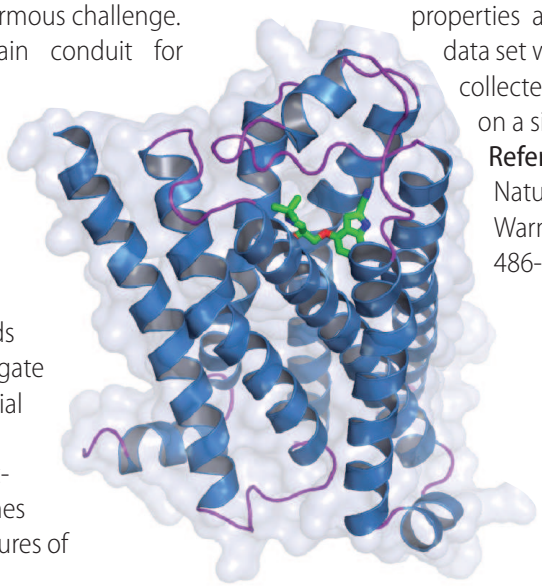
**The challenge:** G-Protein Coupled Receptors (GPCRs) are typically unstable proteins and thus very difficult to purify and crystallise. The fragile nature of the resulting crystals make data collection an enormous challenge.

**Background:** GPCRs are the main conduit for transmembrane signal transduction in response to hormones and neurotransmitters. Many hundreds of drugs targeting GPCRs are currently in development. Crystal structures of GPCRs are crucial in the understanding of how hormone binding (adrenalin in this case) leads to signal transduction and to investigate the mode of binding of potential therapeutic drugs to GPCRs.

**Results:** Single-crystal X-ray diffraction data collected at ESRF beamlines led to the first reported crystal structures of members of this GPCR family.

**How did the synchrotron help?** Small, poor quality crystals needed a highly automated, high-intensity microfocus beam to obtain the best data. Over a thousand crystals were screened for diffraction properties and the final high resolution data set was obtained by merging data collected at several different points on a single crystal.

**Reference:** Rasmussen et al. Nature 450 (2007), 383-387; Warne et al. Nature **454** (2008), 486-492 .



Crystal structure of a β-adrenergic receptor with bound ligand.

# Crystal Dehydration

## A novel device for hydration control of macromolecular crystals is available on ESRF beamlines.

**The diffraction properties of crystals can often be improved by controlled dehydration. The EMBL and ESRF have developed, and now operate as standard, a novel device for controlling crystal hydration while mounted on a standard macromolecular crystallography beamline. This allows the fine-tuning of the dehydration protocol and enhances the possibility to fully characterise a given system, thus increasing the chances of finding a suitable dehydration protocol. In addition, the ease and simplicity of its use makes these experiments feasible within a reasonable time.**

Among the different effects that may be observed during dehydration are space group changes, unit cell shrinkage, mosaic spread changes, spot profile improvement and an increase in diffraction resolution limits.

The device delivers an air stream of precise relative humidity that can be used to alter the amount of water in macromolecular crystals and is rapidly installed. Samples are mounted on mesh loops, and the progress of dehydration can be monitored both optically and by the acquisition of diffraction images. Once the optimal hydra-tion level is obtained, cryo-cooling is easy to achieve by hand or by using a sample changer.

## Practical Details

### What do I need ?

Several unfrozen crystals from approximately the same crystallisation condition which you do not mind sacrificing

- Mother liquor solutions
- Mesh loops mounted on SPINE bases
- ESRF pucks filled with empty SPINE standard vials

### How long does it take ?

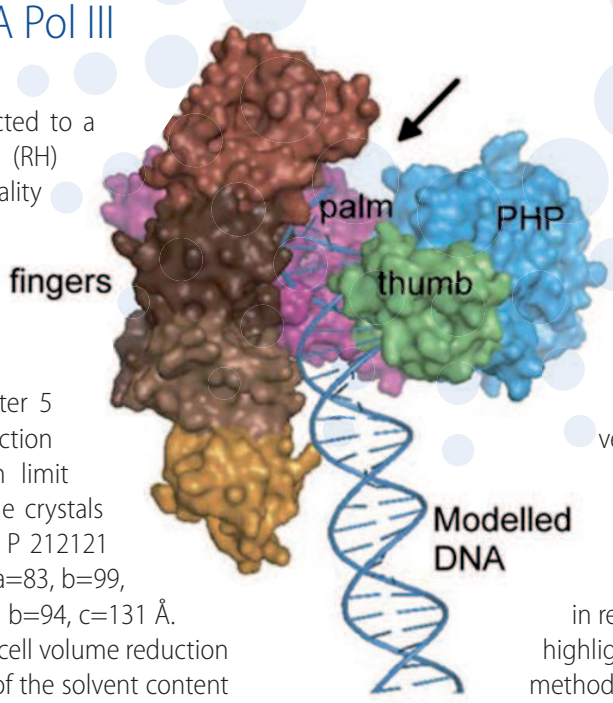
- A quick test can be made to see if your crystal system is susceptible to changes in dehydration within a few hours.
- Dehydration protocols vary considerably between different crystal systems so refining a dehydration protocol can be time-consuming work and requires several crystals. It usually takes 24 hours to explore and refine a dehydration protocol.
- Once a protocol is found, crystals can be conditioned rapidly and stored for data collection on a different beamline.

### Will it work ?

- In 10-20% of cases, dehydration results in an increase in the observed diffraction limit.
- If your crystal has a high solvent content and/or low symmetry, dehydration may improve it.
- If you have observed variability in cell dimensions after cryo-cooling, this may indicate that your system can be easily changed by dehydration.

CASE STUDY

## Escherichia coli DNA Pol III



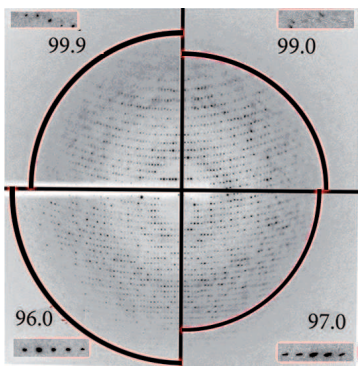

When these crystals are subjected to a descending Relative Humidity (RH) gradient, a decrease in the quality of the diffraction is observed at around 96% RH, such that it is not possible to index the diffraction pattern until the RH reaches 89%. Here, the diffraction starts to recover. After 5 to 10 minutes at 88% RH, diffraction is restored and the resolution limit increases by 1Å to ca 2.8 Å. The crystals undergo a transition; the initial P 212121 unit cell with dimensions of ca a=83, b=99, c=144 Å, is reduced to ca a=83, b=94, c=131 Å. This corresponds to a 14% total cell volume reduction and an approximate reduction of the solvent content from 57% (v/v) to 50%.

These crystals have been previously reported to increase their diffraction limits when dehydrated using the FMS (Lamers et al., 2006, Sanchez-Weatherby et al. 2009). Despite the fact that the two devices work in a very different manner, these crystals undergo the same transition. The changes happen at almost identical RH values and the increase in resolution is comparable. This highlights the robust nature of the method and its reproducibility.

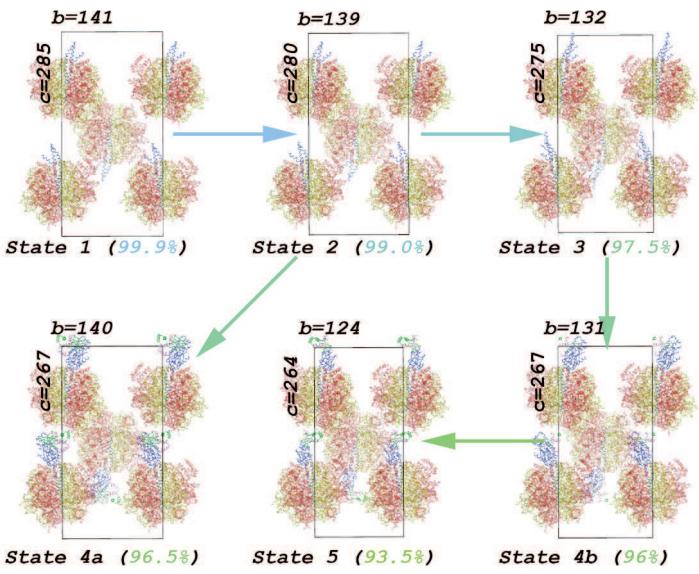
CASE STUDY

## Bovine $F_1$-ATPase

Crystals of bovine F1-ATPase demonstrate more complex behaviour when subjected to controlled dehydration. These crystals have previously been characterised when dehydrated using the FMS (Bowler et al., 2006b, a, 2007); however, the ease and simplicity of the new device, coupled with the much greater time resolution that a synchrotron beamline offers, has allowed a much more detailed study of the changes undergone by these crystals upon dehydration. This system has furthered our understanding of the process of crystal dehydration and has helped in defining the general principles involved.




**Changes in X-ray diffraction of $F_1$-ATPase crystals during dehydration.** Each quadrant shows the different resolution limits of each dehydration state: 3 Å, 3.8 Å, 4 Å and 2.5 Å . The inserts show a magnified view of the same area on the detector, demonstrating the improvement in Bragg peak profile after dehydration.




**The structures of the different transition states of $F_1$-ATPase.** Crystal packing of $F_1$-ATPase crystals at each dehydration state. The different unit cells are shown viewed along the a axis. The asymmetric unit and symmetry related particles are shown as Cα traces coloured by subunit (α- red, β- yellow, γ- blue, δ- magenta and ε- green). Arrows indicate the different paths that crystals can follow.

# Small-angle X-ray scattering of biological samples (BioSAXS)

## The technology at a glance

Small-angle X-ray scattering (SAXS) is a highly effective tool for determining low-resolution molecular envelopes of macromolecules (from a few kDa to 1MDa) in solutions. This powerful technique allows the study of macromolecules (proteins, nucleic acids, carbohydrates) and their complexes in solution whilst not requiring crystallisation. In addition, medium to large protein conformational changes can be monitored over a wide range of conditions.

## What information can I obtain?

### From SAXS data alone

- Monodispersity and behaviour of a macromolecule in solution (useful during optimisation of crystallisation conditions or protein stabilisation studies)
- Dimension of the particles, and hence oligomeric state
- Shape of a non-crystallisable protein (low-resolution phases obtained)
- Shape of a macromolecular assembly

### From SAXS data coupled with crystallographic data

- Confirmation of the quaternary structure of a protein or a multiprotein complex
- Elucidation of the shape of a domain that is not present - or visible - in the crystal structure
- Monitoring of structural changes and domain movements upon ligand binding or complex formation.

## The added value of the ESRF BioSAXS beamline

### Easy to use, automated and requiring small amounts of sample

The dedicated ESRF BioSAXS beamline is simple and efficient to use. Its automatic liquid handler (sample changer) automates the entire cycle of sample loading, unloading and sample cell cleaning. Very small amounts of sample (typically 50 µl at 10 mg/ml) are sufficient to perform a complete experiment. The simple point-and-click data collection interface is linked to an automated data analysis pipeline which handles all preliminary data treatment. Our dedicated scientists offer support and help for experiments and data analysis.

## Practical Details

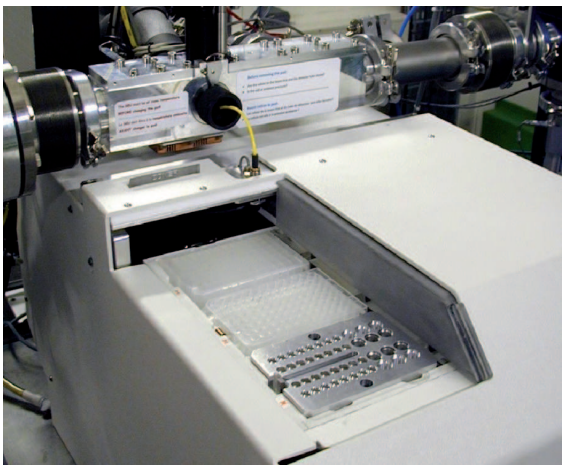
### What do I need?

- Sample in solution which is mono-dispersive (no aggregation)
- 3 different dilutions of the sample with known concentrations (from 1 to 10 mg/ml)
- Buffer solution that matches exactly that of the samples

### How long will it take?

Studying a single sample takes about 1 hour of beam time including preliminary data analysis* (initial beamline calibration by our scientists is not included) .

**This includes data collection from 3 different concentrations of sample, verification of monodispersity, determination of radius of gyration and molecular weight of the particle.*



Close-up view of ID14-3 sample changer with the sample storage compartment open

### How do I access the technique?

Two modes of access are currently available: standard beam time access, with and without scientific assistance, and an express mail-in data collection service.

**Beamline technical specifications**

| | |
|---|---|
| Q-range | 0.05 - 5 $nm^{-1}$ |
| Sample cell | Quartz capillary |
| Volume of exposed sample | Typical 10-30 µl |
| Exposure time | Typically 10 seconds |
| Sample changer | PCR, eppendorf tubes and 96-well plates |
| Sample temperature range | 2-60 ºC |

CASE STUDY

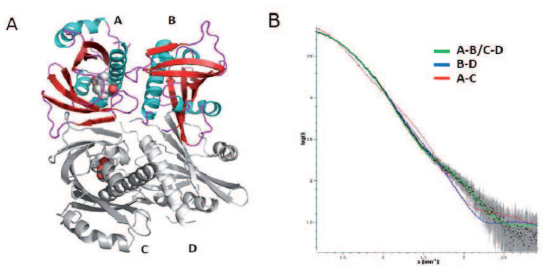
# The intracellular receptor PYR1 from *A. Thaliana*.

**The challenge:** To identify the biologically active dimer of PYR1 present in solution and its relation to the crystal structure which had shown a tetrameric arrangement.

**Background:** The plant hormone abscisic acid (ABA) has a central role in the adaptive response to desiccation stress. In this study, the authors [1] determined the crystal structure of the *A. thaliana* PYR1 protein in complex with ABA. The refined crystallographic model of PYR1 contains four monomers in the asymmetric unit as shown in panel A. However, size-exclusion chromatography combined with multi-angle laser light scattering (MALLS) demonstrates that PYR1 is a dimer in solution.

**Results:** Small angle X-ray scattering (SAXS) data were collected at the ID14-3 BioSAXS beamline on PYR1 samples. The three putative dimers A-C, B-D and A-B were fitted into the SAXS data (panel B) showing a good fit only for the A-B dimer, confirming the latter as the biologically relevant dimer of the PYR1 protein.

**How did the synchrotron help?** Small angle X-ray scattering (SAXS) data collected at the ID14-3 BioSAXS beamline were essential to elucidate which is the biologically relevant dimer in solution.




[1] ] Santiago J, Dupeux F, Round A, Antoni R, Park SY, Jamin M, Cutler SR, Rodriguez PL, Márquez JA. *"The abscisic acid receptor PYR1 in complex with abscisic acid"*, Nature 462, 665-668.

# X-ray Absorption Spectroscopy

## The technology at a glance

**X-ray absorption spectroscopy techniques provide information on the atomic organisation and chemical bonding around an absorbing atom in whatever medium it is embedded, i.e. solids and liquids. There are essentially two types of absorption spectroscopy: X-ray Absorption Fine Structure (EXAFS) and X-ray Absorption Near Edge Structure (XANES). Both techniques are element-selective, which means that scientists and researchers can study and characterise elements in their "working state" within compounds.**

## The added value of the ESRF absorption spectroscopy facilities

The ESRF offers a suite of three beamlines for absorption spectroscopy, each optimised for different applications, thus enabling research on a wide range of materials to detect very diverse factors under various conditions.

• ID24 is ideal for fast chemical processes and can be used for rapid micrometre-resolution *in situ* mapping of heterogeneous samples. Currently, the core activity of ID24 is to investigate catalysts in operation in subsecond sequences and to produce relatively high-resolution mapping. It is also well suited for studies at extreme pressure levels and within high magnetic fields in pulsed mode.

> "Absorption spectroscopy allows us to know not only what a molecule does, but above all why. It helps us find underlying principles and changes in molecular activity *in situ*."
>
> - Mark Newton and Pieter Glätzel, beamline scientists

• ID26 is a high-brilliance X-ray spectroscopy beamline for absorption studies on samples with low levels of concentration, such as highly-diluted trace elements or toxic chemicals. Detailed information on the electronic structure can be obtained by employing a high-energy resolution setup. It is equipped for different sample environments to perform *in situ* studies. It can also be adapted to a variety of user experimental stations. Fast scanning software allows efficient data collection.

• BM29 is the general purpose X-ray absorption spectroscopy beamline at the ESRF. It offers conventional X-ray absorption spectroscopy to perform experiments which do not require the specialist characteristics of other ESRF X-ray absorption instruments. It offers a large-energy range bending magnet which provides high-quality data based on the quick scanning of spectrums. It offers a good balance between quality of data and speed of utilisation.

Recently, BM29 undertook micro XAS measurements, producing quality of data that surpasses other similar beamlines worldwide.

## Fields of application

**Environmental science:** trace elements, including identification of toxic concentrations of heavy metals, analysis of air pollution filters, effluent separation techniques, etc.
**Earth and planetary science:** geological studies, mapping, mineral characterisation.
**Medicine and pharmacology:** cancer cell research, observation of underlying activities and molecular activity of active ingredients and formulations *in situ*.
**Automobile industry:** study of key components, including fuel cells and catalytic converters.
**Oil industry:** analysis of trace elements in petroleum and petrochemical products.

"We come to the ESRF since they offer unique techniques and are the only synchrotron facility able to build us a dedicated sample cell for *in situ* measurement."
- Toyota Motor Europe nv/sa (Belgium)

**Corporate clients include Toyota, BASF, Total, Johnson-Matthey, IFP (Institut Français du Pétrole – French Petroleum Institute), Daihatsu**

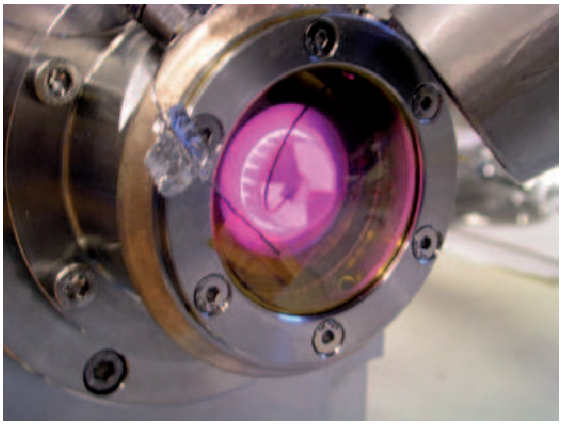
CASE STUDY

# Toyota studied exhaust catalysts using X-ray techniques to examine catalyst surface structure and the chemical reactions taking place.

**The challenge:** To study the noble metal components of a working vehicle exhaust catalyst under *in situ* conditions and in real time.

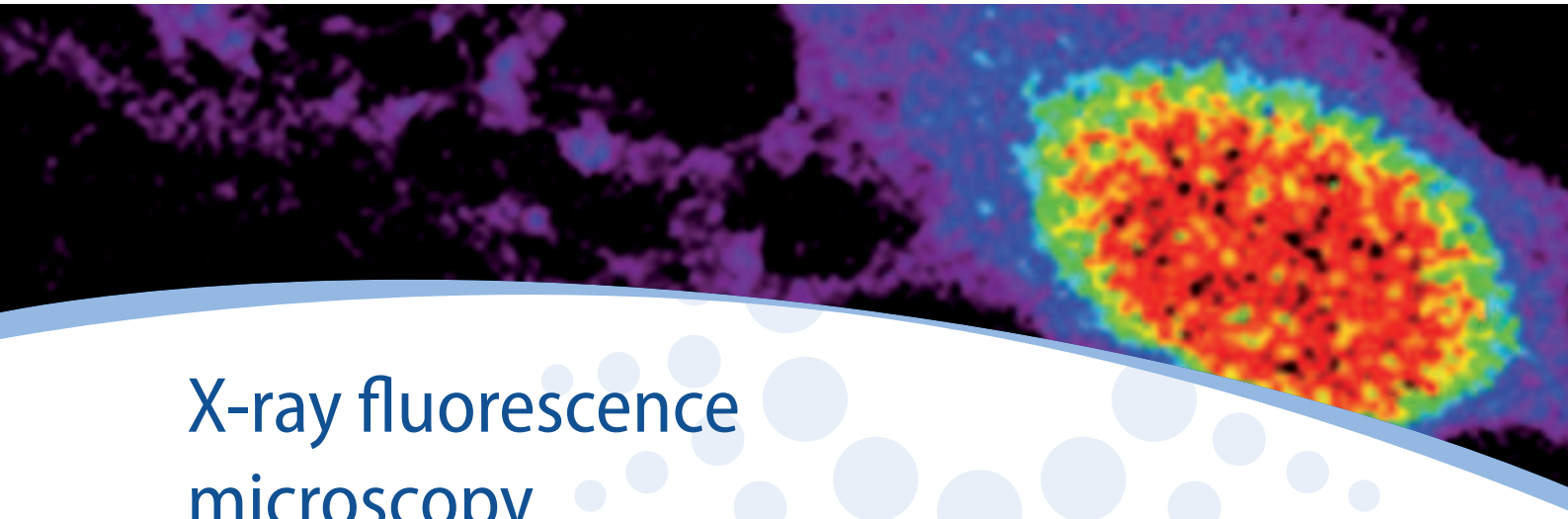
**Background:** A supported metal catalyst is responsible for much of the oxidation of CO and unburned hydrocarbons in car exhaust gases. These catalysts lose efficiency as they age, due in particular to the sintering of the supported noble metals used in the catalysts. Toyota wanted to study the sintering process in detail to improve the catalysts.

**Results:** Data analysis led to the discovery of an unexpected phenomenon: efficient oxidative redispersion of Pt nanoparticles on some metal oxide supports during quick redox cycling. This redispersion process led to a tangible potential for incorporation into "on board" methodology for extending vehicle catalyst lifetime through curtailing or reversing the effects of metal sintering during operation.



Heating a catalyst sample in the *in situ* cell for time-resolved XAFS.

**How did the synchrotron help?** Using a purpose-built cell, energy-dispersive X-ray absorption scattering was used to study the local environment and electronic structure of the Pt metallic active site. The experiment was done at working exhaust conditions (high temperature and fluctuating oxidative and reductive gas compositions), while *in situ* TEM was used to study the catalyst surface. Infrared/EXAFS experiments were also used to study other major components of the catalytic system, Rh and Pd. Spectra were recorded on a millisecond timescale.



# X-ray fluorescence microscopy

## The technology at a glance

**X-ray fluorescence microscopy and microspectroscopy use very fine high-quality beams, focused on extremely small areas within heterogeneous materials. For example, irradiation of trace elements within hard or soft substances enables scientists to probe deeply and isolate minute quantities of substances within a large volume. This enables new investigations, such as access to elements of major interest in the biological and material sciences, identification of heavy metals and trace element mapping, with very little preparation needed for the materials being used.**

## The added value of the ESRF X-ray fluorescence microscopy facilities

The ESRF has a suite of four beamlines (ID13, ID18F, ID21, ID22) fully dedicated to microscopy and microspectroscopy techniques. In particular, ID22 is a versatile hard X-ray microprobe focused on X-ray fluorescence, absorption and diffraction on the micrometre scale. The three beamlines enable a variety of different approaches to be combined, including fluorescence tomography, XANES imaging, holography and phase-contrast microtomography with micrometre resolution. Their potential for detecting and mapping trace elements, quantitative fluorescence analysis, chemical state specificity and structural probing is ideal for a wide range of industrial applications.

> "How precise are our probing techniques for finding trace elements? You could pour a glass of wine into an Olympic swimming pool and we could tell you if it was Bordeaux or Burgundy."
>
> - Jean Susini, Head of Instrumentation Support and Development Division

## Fields of application

**Environmental science:** trace elements, including identification of toxic concentrations of heavy metals, analysis of air pollution filters, etc.

**Earth and planetary science:** analysis of minute samples to study bulk morphology, internal structures, crystallography and trace composition, used for example to study a cometary grain collected by the NASA Stardust mission.

**Microelectronics:** identification of metallic microcontamination levels in microprocessors and integrated circuits, improvement of silicon wafers.

**Cosmetics:** in-depth knowledge of the interaction between cosmetic substances and living organisms.
**Cultural heritage and archaeology:** study of art masterpieces (Grünewald Triptych, Van Gogh painting) and stone samples (Pompeii).
**Oil industry:** analysis of trace elements in petroleum and petrochemical products.

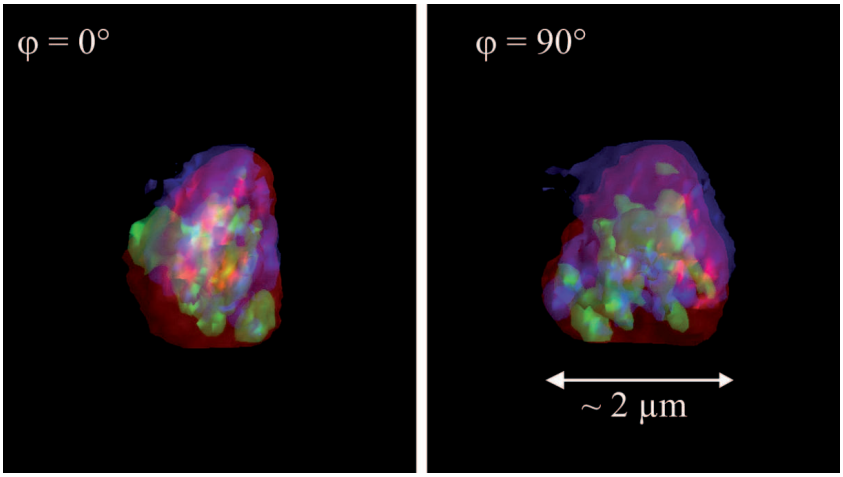
"Our main motivation for coming to the ESRF is the guarantee that we will find exactly what we expect. The beamline is always set up according to our needs and we always reach our objective."
- Phi-Axis (a small service company)

Corporate clients include CEA, Lafarge, L'Oréal, Saint-Gobain

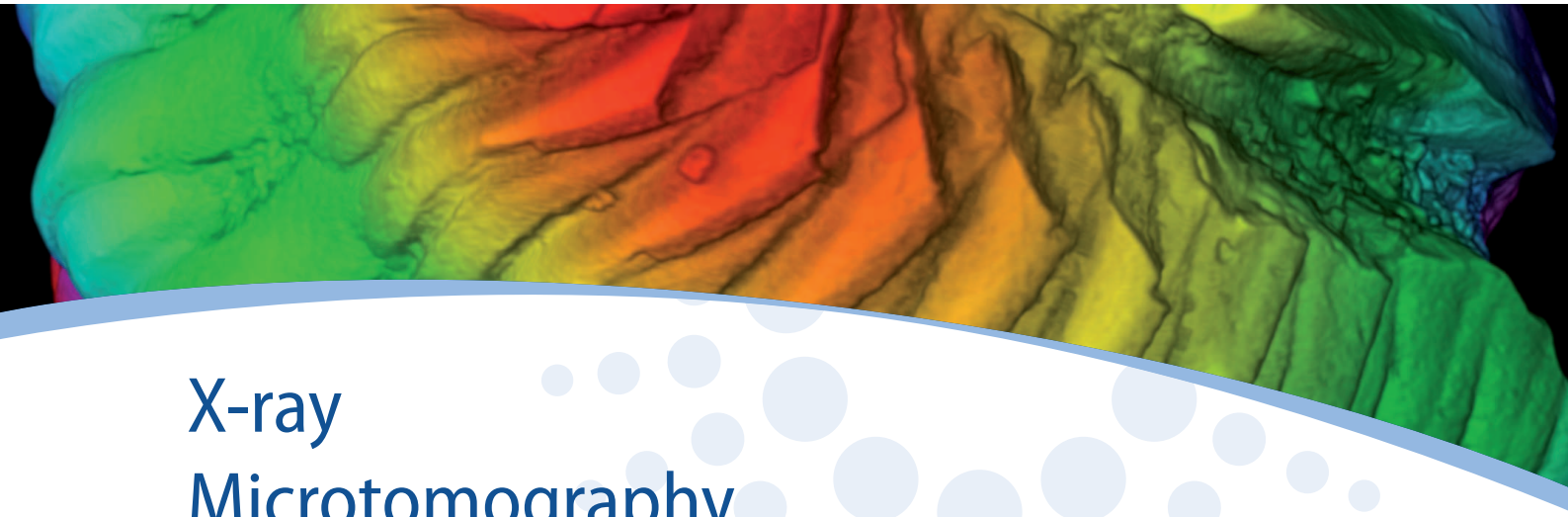
CASE STUDY

# X-ray fluorescence nanotomography on cometary matter from Comet 81P/Wild2 returned by the NASA Stardust mission.

**The challenge:** To determine the 3D distribution of main and trace elements in a cometary dust particle embedded in an aerogel matrix.
**Background:** The NASA Stardust mission captured and returned extremely precious cometary dust particles to Earth. The particles were captured in space using an aerogel matrix to stop and trap the dust particles. The terminal particles captured are around 2 microns in size.
**Results:** Fully 3D, non-destructive, trace-level elemental imaging was made on a dust particle showing the distribution of elements from calcium to selenium at sub-micron (200nm) spatial resolution.
**How did the synchrotron help?** X-rays are an element-selective, non-destructive probe. The very fine X-ray beams available on beamline ID13 (the ESRF pilot project for further nano-focus X-ray beams), enabled nanotomographic images of the minute cometary dust particles to be recorded and transformed into the full 3D reconstruction.
**Reference:** Silversmit et al. Anal. Chem. (2009). In press.




The reconstructed comet dust particle with colours showing the distribution of different elements (red: iron; green: chromium; blue: selenium).



# X-ray Microtomography

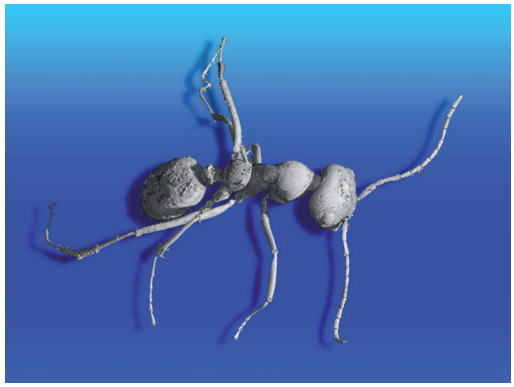
## The technology at a glance

**X-ray microtomography extends the capacities of X-ray imaging to produce pictures of ultra-high resolution and contrast. Using the same principles as medical scanners, coupled to synchrotron radiation and phase-contrast imaging, scientists can produce 2D and 3D representations with micrometre resolution. Often, samples can be studied where classical techniques do not provide any useful image at all.**

## The added value of the ESRF X-ray microtomography system

The ESRF X-ray microtomography beamlines offer state-of-the-art technology generating an extremely bright light source and high spatial resolution below 1 micron, as well as the ability to follow phenomena as they develop, therefore enabling the production of dynamic 3D images. Microtomography is one of the major imaging techniques at the ESRF, and has been used for industrial applications for over a decade. The ESRF therefore has at its disposal a unique know-how in the use of the different aspects of microtomography, allowing us to reply rapidly and efficiently to requests from industry, and so obtain optimum results. Furthermore, the ESRF provides a unique sample environment, with temperature ranges from -60° to 1600°C, as well as tension, compression and fatigue stress devices. ESRF staff are dedicated to producing the highest quality images in response to user specifications.

> "X-ray microtomography has the magnifying power to find the proverbial needle in a haystack the size of a building."
>
> - Elodie Boller, Engineer in charge of industrial experiments on ID19



## Fields of application

**X-ray microtomography offers vast possibilities and some quite unexpected applications:**

**Polymers:** structure of fibres, polyurethane, polystyrene foams (opened/closed cells).

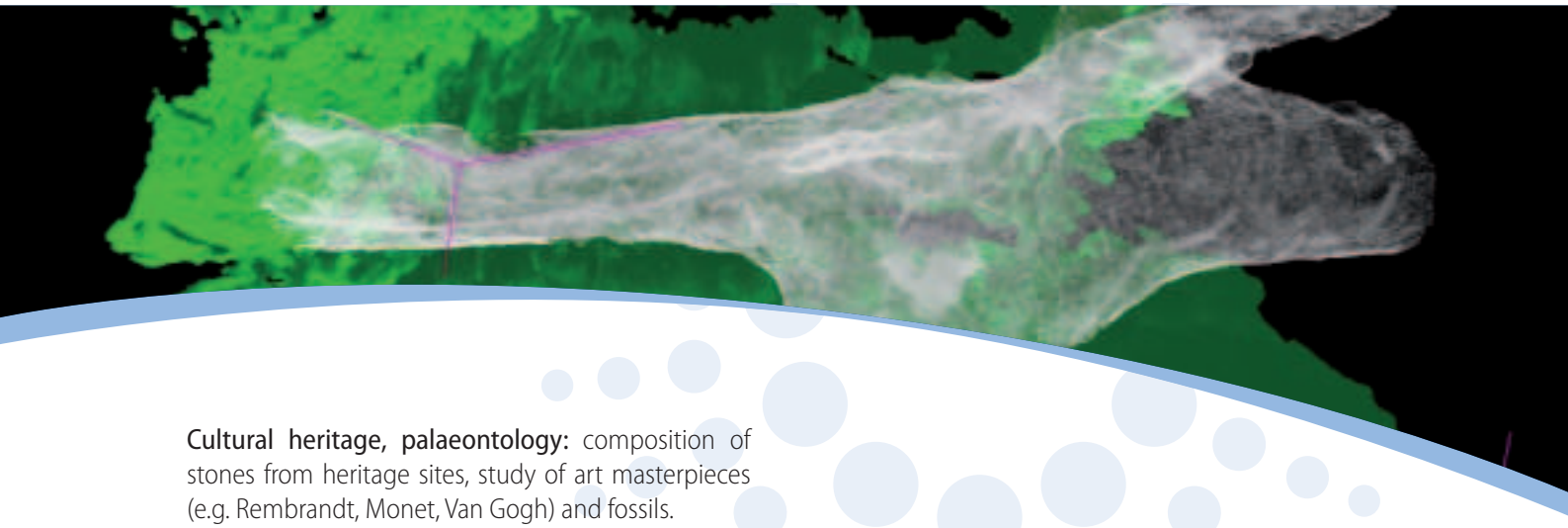
**Mining, oil:** permeability and determination of microstructure of rocks, hardening of cement.

**Cosmetics:** lipsticks, structure of hair, shaving foams.

**Food:** cooking conditions of bread, structure of chocolate mousse, seeds, sugar, salt, fruit, ice-cream, etc.

**Processing:** structural and process validation, quality control support.

**Environment:** structure of snow, soil analysis.



**Cultural heritage, palaeontology:** composition of stones from heritage sites, study of art masterpieces (e.g. Rembrandt, Monet, Van Gogh) and fossils.

"The quality of the experiments at the ESRF is way above my expectations – *fantastique!* I really appreciate not only the scientific results, but also the contact with the staff and their very high level of expertise. I fully recommend the ESRF as a state-of-the-art facility."
- Rhodia (France)

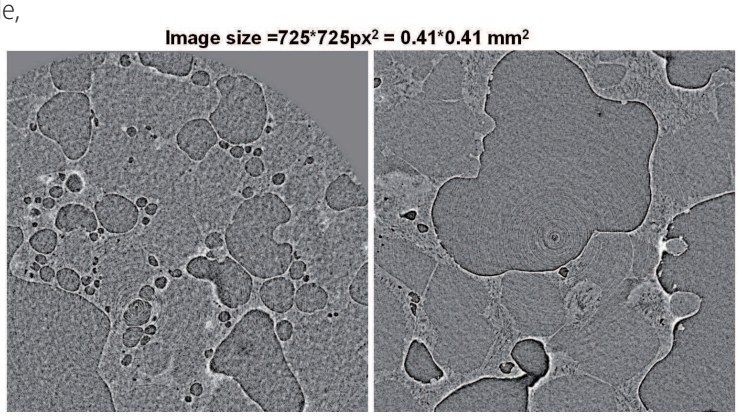
"What I appreciate most when using the ESRF facility is the easy working relationship we have with their staff. Things always go very smoothly. We get some pretty serious work done, but in a very pleasant and friendly environment. Even though we're a small company, we get first-class treatment at the ESRF."
- ERM SARL (France - 14 employees)

**Corporate clients include CEA, AREVA, Lafarge, L'Oréal, Rhodia, Schneider Electric, Unilever**

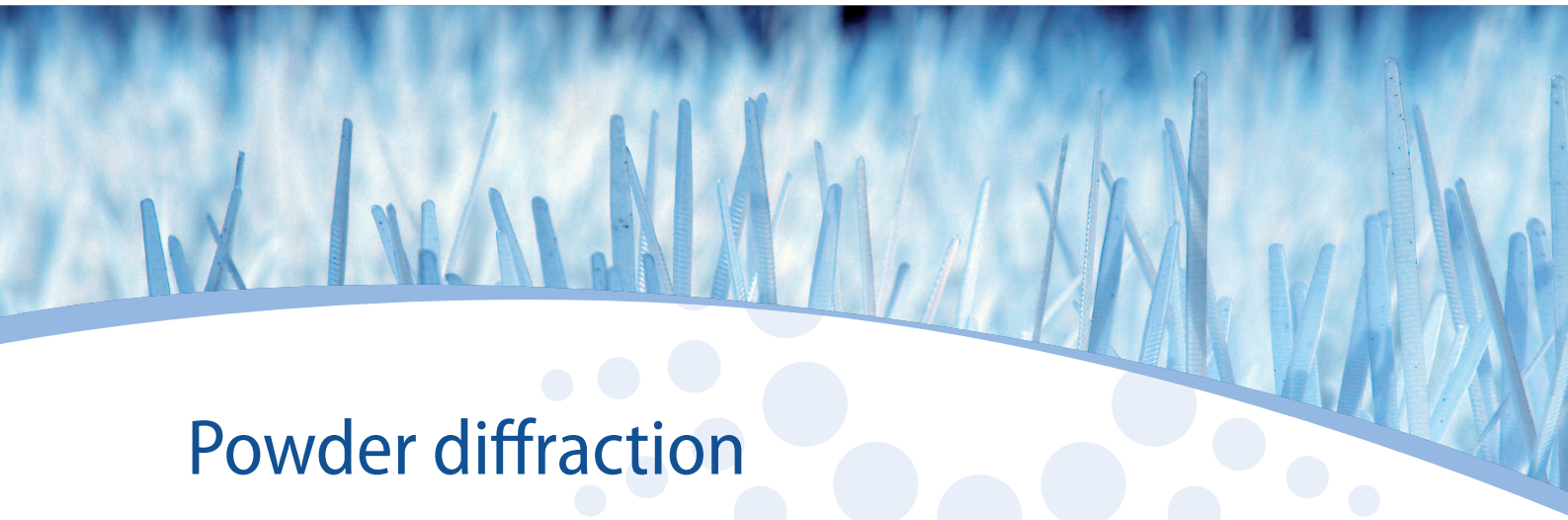
CASE STUDY

# Unilever used high-resolution tomography imaging to characterise the microstructure of ice-cream.

**The challenge:** To understand how the microstructure of ice-cream changes after temperature abuse.
**Background:** The microstructure of ice crystals and air bubbles is critical to ice-cream's quality and sensory properties. Ice cream is of course inherently unstable, and temperature increases during transport, storage or even on exit from the customer's freezer can all play a factor in destroying the microstructure of small ice crystals, leading to recrystallisation and a coarser structure.
**Results:** Using the X-ray tomography setup of ID19 at low temperature, the microscopic structure of the ice-cream became very clear, showing the change in structure on several samples previously prepared.
**How did the synchrotron help?** The Unilever research was a challenge for the ID19 team, requiring a specialised *in situ* sample environment and highly-reduced acquisition times. The ID19 intensity, its detector system and the low temperature device compatible with high resolution (sample rotation over 180°) led to the high-quality 3D images with a voxel size of just 0.56 micron.




Comparison between fresh (left) ice-cream and "temperature-abused" ice cream (right), clearly showing the large ice crystals and air bubbles after the temperature cycling.



# Powder diffraction

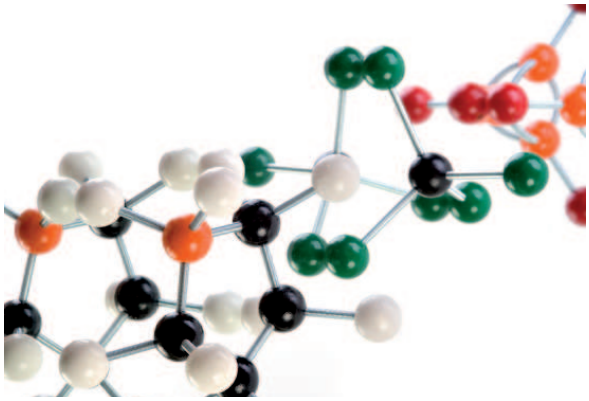
## The technology at a glance

Many bulk solids are composed of microcrystals, which are hard to study by standard single-crystal techniques. Powder diffraction makes it possible to analyse the structures of such materials under a wide range of conditions, e.g. while heating or cooling, or under different atmospheric conditions. The positions, intensities and shapes of the peaks in the powder diffraction pattern reveal information about the microscopic structure and strain state of a sample, and can also be used to identify which substances are present in a (possibly complex) mixture. Such information is crucial for understanding the properties and behaviour of materials.

## The added value of the ESRF powder diffraction facilities

At the ESRF, the synchrotron X-ray powder diffraction beamline delivers very accurate, high-resolution data, with the choice of a wide range of wavelengths, which can be used to investigate complex and highly absorbing materials. Furthermore, an automatic sample changer means that a series of samples can be quickly and efficiently studied. Finally, users have the possibility of coming to the ESRF and carrying out the experiment themselves with the support of the beamline staff or, for a small number of samples, can benefit from the facility's mail-in service, thus eliminating the need to travel and be present when the measurements are carried out.

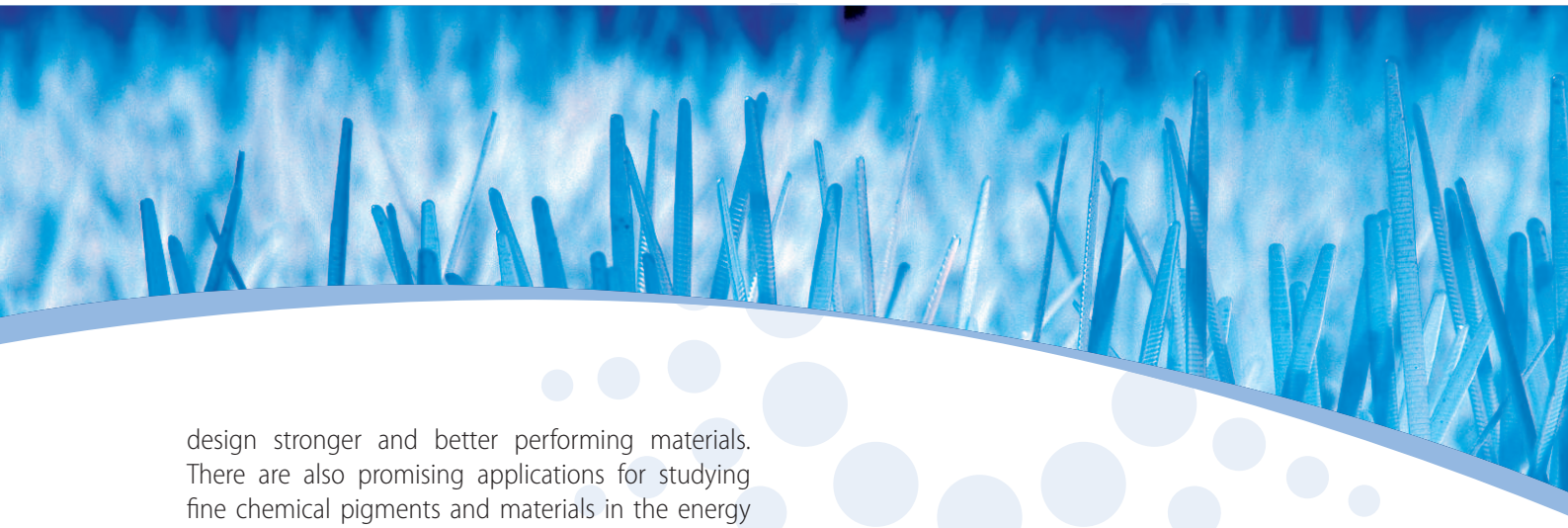
> "We can see things in the samples that people haven't even imagined were there."
>
> - Andy Fitch, Scientist in charge of ID31, the ESRF powder diffraction beamline



## Fields of application

**Pharmaceutical companies** regularly use ESRF powder diffraction techniques to characterise pharmacologically-active components and other ingredients in formulations e.g. to investigate polymorphism and hence enable them to comply with regulatory requirements and to protect their intellectual property.

**Steelmakers, aerospace companies, car manufacturers and other metallurgy-based industries** use the facility to examine alloy structures, carry out stress and fatigue tests and thereby help



design stronger and better performing materials. There are also promising applications for studying fine chemical pigments and materials in the energy sector, such as for batteries, hydrogen storage and superconductors, as well as in nano-technology and other related materials.

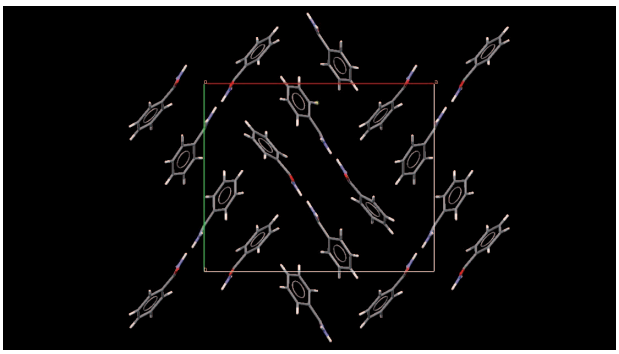
"ID31 gives me quality data that I can get nowhere else – *incomparable* ! "
- Sanofi-Aventis (Montpellier, France)

"When we come to the ESRF, we are always satisfied with the efficiency of data collection, but above all we really appreciate the great technical support we get here. When we can't come to the ESRF, we ship samples to them and we know they will run tests thoroughly for us. Last, but not least, the ESRF respects our intellectual property. Companies are really at home here and free to develop their technologies."
- a formulation scientist from a "Big Pharma" company, USA

CASE STUDY

# Powder diffraction reveals the crystal structure of the metastable polymorph of benzamide.

**The challenge:** To obtain a crystal structure of metastable benzamide, unsolved for over 170 years.
**Background:** Understanding the influences of structural, thermodynamic and kinetic factors that control crystallisation processes is important for fields such as pharmaceuticals, health care, optoelectronics and speciality chemicals.
A metastable transient Form II of benzamide has been known since 1832, but the crystal structure proved intractable until very recently.
**Results:** The crystal structure was solved from high-resolution powder diffraction data from ID31. Numerous powder diffraction patterns were collected to monitor Form II in the sample, which also contained Form I. The structure showed that the polymorphism arises from the disorder of one of the independent benzamide molecules in the unit cell and highlights the delicate balance between kinetics and thermodynamics in the appearance of polymorphs.
**How did the synchrotron help?** Rapidly collected high-resolution powder diffraction patterns were the key to solving this structure, together with the *in situ* kinetic and temperature control of the sample. The very high angular resolution of the ID31 diffractometer allowed high-quality data to be collected with good separation of the powder peaks, which led to the final detailed crystal structure.
**Reference:** Blagden et al. Crystal Growth & Design **5** (2005), 2218-2224.



Crystal structure of Form II of benzamide showing the noncentric molecular dimer determined from the ID31 powder data.

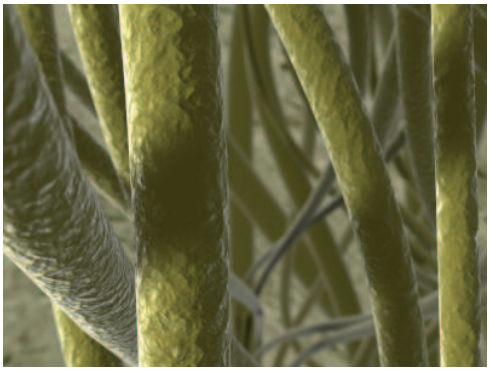
# Small- and wide-angle X-ray scattering (SAXS and WAXS)

## The technology at a glance

**Small- and wide-angle X-ray scattering (SAXS and WAXS) use the high brilliance of an undulator source to study condensed matter samples in liquid or solid form. It offers sub-micrometre spatial resolution and deep penetration into materials, such as colloids, polymers, surfactant membranes and proteins, even when these are opaque or turbid. SAXS and WAXS can be combined with other techniques, such as rheology and light scattering, to provide better understanding of sample behaviour on short time scales (sub-milliseconds).**

## The added value of the ESRF SAXS/WAXS facilities

The ESRF offers a range of SAXS and WAXS beamlines and combined techniques, thus making it possible to examine different types of specimens with different detail. Also, ESRF beamlines, and in particular ID02, offer higher levels of brightness which means that weaker signals can be treated efficiently. In terms of industrial applications, the ESRF is particularly experienced in studying real product behaviour and has the expertise to set up different sample environments and *in situ* processes that simulate industrial processing conditions. It provides its commercial users not only with raw data, but offers a collaborative work environment geared to producing applicable results.



> “Our techniques allow researchers to see, in very real-life conditions, how products are structured and how they interact.”
>
> - Narayanan Theyencheri, Scientist in charge of ID02, the main ESRF SAXS and WAXS beamline

## Fields of application

**Medical research organisations** use SAXS and WAXS – one example is Fermiscan (Australia) who are currently using the diffraction technique to examine an experimental early-warning system for detecting breast cancer using hair samples, as a complementary technique to mammography. They have entered Phase III of their research in Australia in what could become a breakthrough technique for women's health worldwide.

**Cosmetic firms** have studied the diffraction of hair to design improved conditioners.

**Pharmaceutical companies** use SAXS and WAXS to study active ingredients and formulas, and their behaviour under different conditions.

**Home product manufacturers** examine, for example,

detergent efficiency in order to design products that work more efficiently at lower temperatures and use less water.
Several other industrial companies use the facility, from plastics and polymer producers to manufacturers of Kevlar bulletproof jackets.

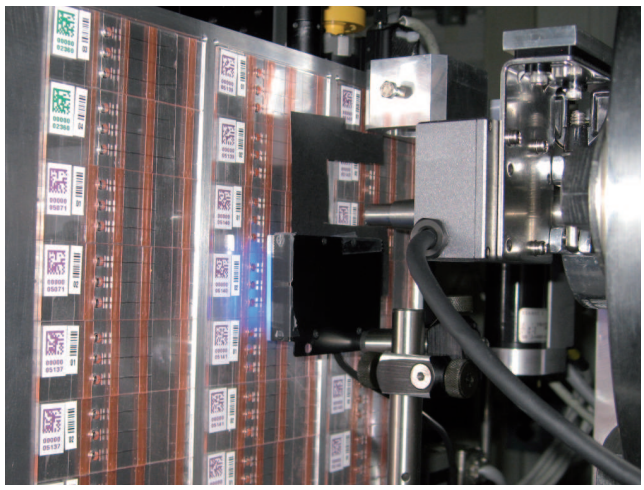
"What we appreciate the most at the ESRF is that it's a high-tech, high-profile centre where people practise science at its highest level."
- Procter & Gamble (UK)

"ID02 is the best beamline we have ever used and potentially the best available for our work. The ESRF staff are easy to deal with, they are friendly, communicative and supportive of what we're trying to achieve. The ESRF is truly the European centre of excellence. Through working at the ESRF we have also been able to make important contacts who have helped us in our research."
- Fermiscan (Australia)

CASE STUDY

# Clinical trials of an innovative screening test for breast cancer using SAXS analysis of hair performed by Fermiscan Pty Ltd (Australia).

**The challenge:** To confirm the use of SAXS as a determinant for breast cancer.
**Background:** A 2008 study on synchrotron SAXS analysis of hair confirmed an earlier observation that a correlation exists between an altered SAXS pattern of hair and the presence of disease. SAXS patterns of hair from women with breast cancer contain a feature appearing as a ring at 4.76±0.07 nm.
**Results:** Since that time Fermiscan has conducted several trials. In Australia a trial with 2000 patients yielded a sensitivity (i.e. an ability to detect breast cancer) of 74% in women under 70 years of age and a negative predictive value of 99.5%. A recent trial with the National Health Service of Italy yielded a sensitivity of 83% and a specificity (i.e. an ability to accurately detect the absence of cancer) of 76%. Trials have been made in France and Italy.
**How did the synchrotron help?** An automated sample processing system was developed and installed on ESRF beamline ID02 which allowed the analysis of 72 samples without intervention. The high brilliance of ID02 meant that sample analysis took only a few seconds. Developments of the system with the beamline staff further enhanced the capability of the sample processing system.



The dedicated scanning setup for Fermiscan on ESRF beamline ID02.