Macromolecular Crystallography (MX)

The technology at a glance

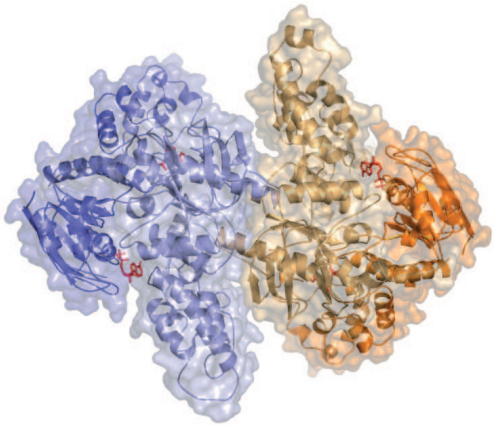
The MX beamlines allow scientists to investigate the fundamental structures of biological macromolecules, which are at the root of all life processes. Each of these molecules is made up of one or more proteins which are responsible for functions as diverse as energy storage, signalling, cell division 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)

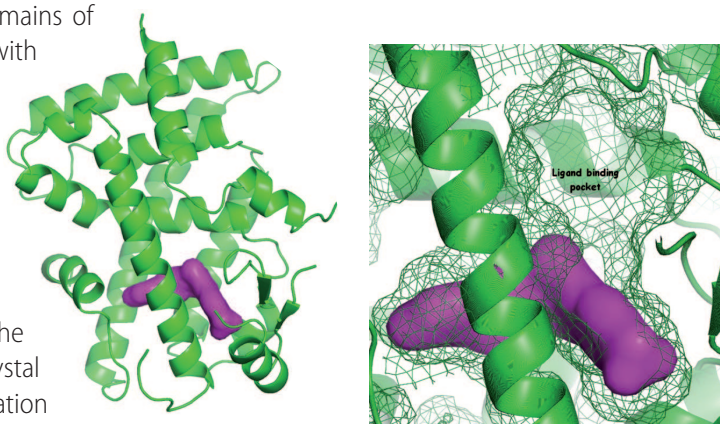
"We are especially delighted with the sample changer, which has literally changed our lives. Instead of coming onto the beamline every few minutes to change a sample, we can press a button and watch hundreds of results arrive."
- Sanofi-Aventis (France)

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.

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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 (μm^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 μm^2 and 200 x 200 μm^2. **Spot size at sample can be varied between 30 x 30 μm^2 and 200 x 80 μm^2.
***Spot sizes at sample: 50 x 30 μm^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

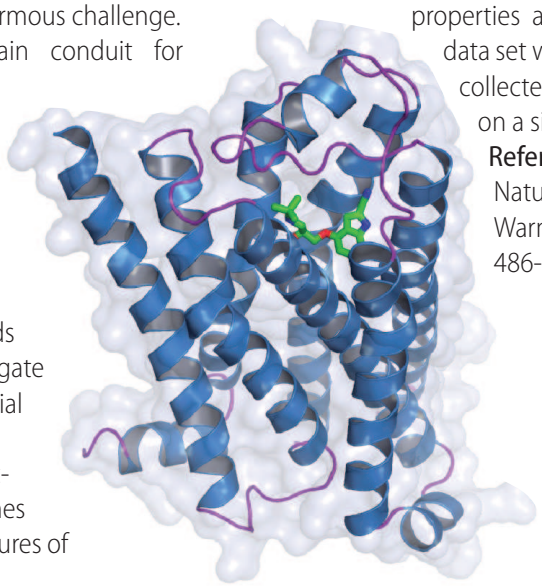
The challenge: G-Protein Coupled Receptors (GPCRs) are typically unstable proteins and thus very difficult to purify and crystallise. The fragile nature of the resulting crystals make data collection an enormous challenge.

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.