New beamline dedicated to solution scattering from biological macromolecules at the ESRF

P Pernot, P Theveneau, T Giraud, R Nogueira Fernandes, D Nurizzo, D. Spruce, J Surr and S McSweeney
ESRF, BP 220, Grenoble, France
E-mail: rejma@esrf.fr

A Round, F Felisaz, L Foedinger, A Gobbo, J Huet, C Villard and F Cipriani
EMBL Grenoble, BP 181, Grenoble, France
E-mail: around@embl.fr

Abstract. The new bio-SAXS beamline (ID14-3 at the ESRF, Grenoble, France) is dedicated exclusively to small-angle scattering experiments of biological macromolecules in solution and has been in user operation since November 2008. Originally a protein crystallography beamline, ID14-3 was refurbished, still as a part of the ESRF Structural Biology group, with the main aim to provide a facility with ‘quick and easy’ access to satisfy rapidly growing demands from crystallographers, biochemists and structural biologists. The beamline allows manual and automatic sample loading/unloading, data collection, processing (conversion of a 2D image to a normalized 1D X-ray scattering profile) and analysis. The users obtain on-line standard data concerning the size (radius of gyration, maximum dimension and volume) and molecular weight of samples which allow on-the fly ab-initio shape reconstruction in order to provide feedback enabling the data collection strategies to be optimized. Automation of sample loading is incorporated on the beamline using a device constructed in collaboration between the EMBL (Grenoble and Hamburg outstations) and the ESRF. Semi/automated data analysis is implemented following the model of the SAXS facility at X33, EMBL Hamburg. This paper describes the bio-SAXS beamline and set-up characteristics together with the examples of user data obtained.

1. Introduction
In January 2008 work began on the conversion of ID14-3 from a beamline dedicated to single-wavelength macromolecular crystallography (MX) experiments to one dedicated to small angle X-ray scattering of solutions of biological macromolecules (bio-SAXS). The new facility is intended to complement already existing SAXS facilities at the ESRF which are highly oversubscribed for a wide variety of experiments in the fields of Biology, Chemistry, Material Science etc. With a rapidly growing interest in bio-SAXS from the ESRF’s MX user community, beam-time on the existing resources was set to become even more sought after. Creating a dedicated to

These authors contributed equally to this work.
bio-SAXS facility alleviates the high rate of over-subscription, improve the throughput of such experiments by removing the need for the station to be optimized for every new user group, which results in more beam-time on the ESRF’s existing SAXS end-stations being available for experiments in other fields. An additional benefit of building this new facility at the ESRF is the creation of collaboration with the already established small angle neutron scattering (SANS) facility at the ILL. Our intention is to allow users, via a single proposal, to access both facilities for appropriate experiments. This will allow the complementary information provided by neutron scattering (i.e. contrast variation) and X-ray scattering to be obtained in a single visit to the Grenoble site.

The conversion of ID14-3 has been undertaken in stages. The first of these allowed testing of the proposed design and proof-of-principle before the major renovation of the experimental hutch undertaken during the summer of 2008. The beamline has been in user operation since November 2008. Automation of sample loading has since been implemented on the beam-line using a device constructed in collaboration between the EMBL (Grenoble and Hamburg outstations) and the ESRF.

Access to the experimental facilities offered at the ESRF bio-SAXS beamline are exclusively through the ESRF MX rolling access system allowing data collection within 6 to 8 weeks of the experiments proposal submission [1]. Feedback from users is positive and the number of proposals for experiments and publications resulting from experiments at ID14-3 beamline are increasing.

2. Beamline and set-up characteristics

Beamline ID14 of the ESRF is an undulator beamline on a high-β section housing four independent stations allowing four different user groups to collect data simultaneously. Three undulators, provide high brilliance at 13.3 keV for ID14-1/2/3 fix energy end-stations, as well as limited tunability on ID14-4. The RMS source size is 402 µm (H) × 7.9 µm (V) and divergence 10.7 µrad (H) × 3.2 µrad (V). The beam size at the sample and at the detector during normal operation are 0.7 mm (H) and (V). ID14-3 was initially dedicated to monochromatic macromolecular crystallography, until its conversion to a fixed energy bio-SAXS end-station. The U23 undulator is a single line undulator and provides the flux for the three fixed-energy stations. The other two undulators, U24.4 and U35 are used only by the tunable end station ID14-4. During the procedure to change energy at station ID14-4 the X-rays flux observed at the other end stations may change, for this reason during movement of the undulators data collection at ID14-3 will pause (at an appropriate point between measurements) and will continue once the undulators have reached the desired position. This procedure is foreseen to be completely automatic so as not to cause disruptions to any of the ID14 stations even during automated or remote access data collection.

The higher energy of this station compared to other fixed energy SAXS stations gives a smaller cone of scatter for the same size of scatterers, meaning the station can record a wider range of momentum transfer (s = 4πSinθ/λ) with a shorter camera length and smaller detector. However, this same effect makes observing the very small angles more difficult requiring the small diameter beamstop to overcome this. ID14-3 has been optimized with the aim of undertaking experiments for macromolecules in solution only. The demand for variable energy solution scattering is low and the hope is that using the standard setup more than 95% of the solution SAXS experiments for the ESRF can be undertaken without modifying the experimental configuration. Much of the remaining 5% could be accommodated with some modifications and it is for this reason the experimental equipment is easily removable to allow users own devices to be installed if needed. However, when appropriate the experiment could be transferred to another of the ESRF SAXS stations if it was agreed it would be more suitable.

2.1. Optical elements

The optical elements of ID14-3 (diamond beam-splitter, Ge (220) monochromator, toroidal mirror) were kept the same as in the original set-up [2]. However, as SAXS experiments require parasitic scattering to be as low as possible new beam conditioning hardware was required. Redesigned attenuators have been installed upstream of the focusing optics in order that any X-rays scattered by the attenuators are rejected by the optics and slits downstream. New beam defining slits just after the mirror (see new elements in green on figure 1) as well as extra guard slits were installed. All hardware for all the beamline is now controlled by ESRF standard controllers (ICEPAPs).
In order to maximize the available space for a long flight path between sample and detector in the experimental hutch, a “mini” hutch (the first of its type at the ESRF) has been built in the ID14 mezzanine, its photo is shown in figure 2, and houses new beam defining slits, experimental shutter and beam monitoring devices. The ‘mini’ hutch is situated about 9 m downstream from the Optics hutch and about 3 m upstream from the sample position in the Experimental hutch (figure 3).

![Figure 1. Optics hutch elements of ID14-3.](image1)

2.2. Set-up description

The experimental hutch of ID14-3 has been completely refitted and now contains a 4 m long marble table which supports the new experimental equipment (figure 3). Two pairs of guard slits are mounted in a slit box prior to the sample exposure unit, which contains the 2 mm diameter quartz capillary (mounted in vacuum) in which the sample is held for measurement. The evacuated flight tube houses a beam-stop with separate (y, z) motor control sitting immediately before the 120 micron thick kapton window behind which is the 2D detector (Pilatus 1M, Dectris) mounted on an independent (y, z) motor stage. The flight tube itself is a modular design for variable length (0.5 to 2.5 m) and is mounted on motorised supports (y-lateral and z-vertical). The sample to detector distance for normal operation is 2.5 m giving a momentum transfer range, \(s (4\pi\sin\theta/\lambda)\) from 0.05 to 5.8 nm\(^{-1}\). The beamline is equipped with fluorescent screens with integral diodes after all beam conditioning elements to aid alignment. However, to prevent parasitic scattering during the data collections all the beam monitoring devices with the exception of the diode mounted in the beamstop (used for normalisation of X-ray intensity) are normally out of the

![Figure 2. ‘Mini’ lead hutch in the ID14 mezzanine.](image2)
beam and are only put into the monitoring position when required (using pneumatic actuators).

Figure 3. Experimental hutch for bio-SAXS at ID14-3. Left: Flight tube exit (with safety screen) and Pilatus 1M detector. Right: Sample changer (with touch screen control) and exposure unit mounted at the flight tube entrance.

2.3. Data acquisition software
A new system named BsxCuBE (BioSAXS Customized Beamline Environment) has been developed to control the experiments at beamline ID14-3, integrating the disparate tools a user requires into a single interface (figure 4). The system has been in operation since summer 2009, with continuing improvements based on feedback from users. This new control system allows good quality data to be obtained even by users with little experience of the technique and has shown promising results: it is capable of a successful control of the data acquisition procedure and users feel comfortable using it. BsxCuBE can display, in near real time 2D scattering images being acquired as well as the processed 1D curves. At this stage, post-processing of 1D curves is also possible, such as averaging, cropping, subtracting or filtering according to the radiation damage. Further post-processing operations are planned for integration into the system.
Figure 4. Snapshots of BxsCuBE software allowing data acquisition, and visualization (using tabs) of 2D data (A) radially integrated 1D curves (B) and control of the sample changer semi (C) and fully automated (D).

BxsCuBE was built using an in-house framework (Bliss Framework 4) which allows creation of sub-windows (Bricks) which are arranged in a main window to form a complete application. The communication with devices is separated into another layer called the control objects which connects to SPEC, ESRF experiment sequencer, and TACO and TANGO, the device server layers. BxsCuBE allows control of different components of the beamline, such as shutters or filters. Ongoing developments of this control software are: i) connection to the Information System for Protein Crystallography Beamlines (ISPyB) to provide automatic logging and sample tracking; ii) automatic batch experiments using the sample changer (basic functionality already available but currently requires sample information to be entered manually); iii) support of a new data storage paradigm called Hierarchical Data Format, following the NeXus convention; iv) integration to display the results of analysis of 1D curves developed by EMBL Hamburg i.e. automatic processing pipeline from 1D data to ab-initio models.

2.4. Sample changer

The sample changer is developed in collaboration between the EMBL (Grenoble and Hamburg outstations) and the ESRF. The robot allows automatic sample loading/unloading, cell cleaning: washing with detergent, rinsing with water and drying with air. The system provides and automated “flow” option, i.e. data collection while flowing the available sample volume through the X-ray beam, thereby mitigating the effects of radiation damage on the data collected. There are two independent thermal controls of sample cell and of sample storage respectively, allowing temperatures in 2-60°C range. Sample storage can contain be optimized with a number of interchangeable configurations. 4 rows of 8 samples in standard PCR tubes (0.2 mL volume) with space for 3 buffers (per row) in eppendorf tubes (1.5 mL volume) or 96 well plates with either shallow or deep wells. The sample changer has place for 3 blocks giving a maximum of 288 separate aliquots for measurement.
On 12th June 2009 the first official users profited from the new sample changer set-up. From this date the robot has been in a constant use and highly appreciated by all visitors, external or in-house. It works very efficiently, reducing the time needed for cleaning and loading new samples from an average of 5 minutes manually to less than 30 seconds increasing throughput. The automated cleaning also improves reliability as the cleaning is more thorough reducing the risk of cross-contamination between samples.

2.5. Sample preparation laboratory

The half of the available bench space in the ID14 sample preparation room was re-equipped for the use of the bio-SAXS users (photo shown in figure 6). A cooled centrifuge is available for use as is a nanodrop spectrophotometer to enable measurements of the sample concentration immediately prior to the experiment. Final sample preparation and characterisation (dilutions, addition of ligands and or altering the concentration of additives) can be done immediately prior to the measurement and new samples prepared following feedback from data analysis.

3. Data examples

As of the end of November 2009 more than 50 external user groups as well as a number of in-house researchers

Figure 5: Left: Sample changer with storage tray in the loading position. Right: Sample Changer software interface.

Figure 6. Bio-SAXS sample preparation bench with the nanodrop spectrophotometer ND1000 and the centrifuge.
have visited ID14-3 for data collection. Papers presenting data collected at ID14-3 have already been published, the first (accepted 3 June 2009) was “Unusual bipartite mode of interaction between the nonsense-mediated decay factors, UPF1 and UPF2” [2]. This paper compared solution scattering data collected at ID14-3 with the theoretical scattering curve calculated from the crystal structure and presented the \textit{ab initio} envelopes calculated from the data showing an elongated shape that accommodates the crystal structures well (see figure 7).

![Graph](image1.png)

**Figure 7.** Left: Data for free UPF1(115-914) and UPF1 in complex with UPF2(1105-1207) (top and bottom respectively) showing experimental Scattering data (experimental data points were removed for clarity) with error bars overlaid with fits from theoretical crystal structure. Right: Ab initio envelopes derived from the small angle X-ray scattering data for UPF1(115-914) (A), (B) and (C) and UPF1(115-914)/UPF2(1105-1207) (D), (E) and (F) with the correspondent crystal structures showing good superimpositions.

Perhaps the clearest example of complimentary SAXS data for structural biology is structure validation, determining the physiologically relevant form of the protein in solution from a number of potential structures obtained from other techniques. An example of this was “The abscisic acid receptor PYR1 in complex with abscisic acid” [4]. The structure of PYR1 was solved using data collected at ID14-4 of the ESRF. However, the PYR1 crystallographic asymmetric unit contained four PYR1 monomers and all biochemistry data from PYR1 suggested that the protein was a dimer and thus the tetrameric form in the asymmetric unit was thought to be an artefact of crystal packing. In order to enable interpretation of the structure with regard to function under physiological conditions SAXS was used to determine which of the possible conformations the protein actually formed in solution. The theoretical scattering curves calculated for possible dimeric ensembles were calculated from the crystal structure and compared to the experimental scattering data (see figure 8). The curve for ensembles A-B (and C-D which is symmetrically identical) produced a good fit to the experimental data demonstrating that ensemble A-B/C-D corresponds to the dimeric form found in solution.
Figure 8. (A) Experimental scattering data (black dots) with error bars calculated from Poisson counting statistics, with fits from ensemble B-D (blue) ($\chi^2=1.15$), A-C (red) ($\chi^2=4.02$) A-B/C-D (green) ($\chi^2=0.72$). (B) Tetrameric configuration from the crystal structure with monomers A,B,C and D labelled (physiologically relevant dimer A-B shown in colour).

4. Conclusions

The new facility is intended to complement already existing SAXS facilities at the ESRF. With the high level of automation for data collection and processing at the bio-SAXS station experiments are easy to undertake, reliable and efficient. An additional benefit of building this new facility at the ESRF is the collaboration with the already established small angle neutron scattering facility at the ILL to allow users, via a single proposal, to access both facilities for appropriate experiments.

Feedback from users is positive, the number of proposals for experiments at ID14-3 beamline is increasing and the already published articles show that the bio-SAXS facility of ID14-3 at the ESRF is already providing results for world leading research.

5. References

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Acknowledgments

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