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Journal of Physics: Conference Series **425** (2013) 012001 doi:10.1088/1742-6596/425/1/012001

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Abstract. Automation and advances in technology are the key elements in addressing the steadily increasing complexity of Macromolecular Crystallography (MX) experiments. Much of this complexity is due to the inter- and intra-crystal heterogeneity in diffraction quality often observed for crystals of multi-component macromolecular assemblies or membrane proteins. Such heterogeneity makes high-throughput sample evaluation an important and necessary tool for increasing the chances of a successful structure determination. The introduction at the ESRF of automatic sample changers in 2005 dramatically increased the number of samples that were tested for diffraction quality. This "first generation" of automation, coupled with advances in software aimed at optimising data collection strategies in MX, resulted in a three-fold increase in the number of crystal structures elucidated per year using data collected at the ESRF. In addition, sample evaluation can be further complemented using small angle scattering experiments on the newly constructed bioSAXS facility on BM29 and the micro-spectroscopy facility (ID29S). The construction of a second generation of automated facilities on the MASSIF (Massively Automated Sample Screening Integrated Facility) beam lines will build on these advances and should provide a paradigm shift in how MX experiments are carried out which will benefit the entire Structural Biology community.

1. Introduction

The current Macromolecular Crystallography (MX) beam lines at the European Synchrotron Radiation Facility (ESRF) are an integral, and highly successful, part of its publically available beam line portfolio (table 1). The first dedicated MX beam line, exploiting a bending magnet source and with an energy tunability from 7 to 17 keV, was constructed in the slot 14 (BM14) [1]. Soon after, in 1998, three fixed-energy (~13.2 keV) and one tunable (9 – 15 keV) end-stations were opened to users on the sector ID14 [2,3]. An independent, tunable, high photon flux beam line (6 – 20 keV) was opened on the ID29 sector in 2001 [4] and in 2006, one tunable (6 – 20 keV) and one micro-focus beam line were put into user operation on sector 23 [5,6]. Here, we briefly describe the status of the renewal, as part of the ESRF Upgrade program, of the ESRF's ID14 end-stations on sector ID30. This new facility named MASSIF (Massively Automated Sample Screening Integrated Facility) is due to be on-line in mid-2013 and will provide the European Structural Biology User Community with a unique suite of end-stations able to effectively evaluate the diffraction properties of macromolecular crystals and thus allow for a more rational choice of experiment strategy and beam line for data collection. The relocation of a large part of the ESRF's Structural Biology Facilities to sector ID30 has created a 'Structural Biology Village' comprising facilities on ID30, BM29 and ID29 (figure 1a). Two new facilities (BM29 and ID29S) that will also form part of this 'village' will also be briefly described.

2. MASSIF

At the heart of the current upgrade programme for Structural Biology at the ESRF is the construction of MASSIF (figure 1b; table 1). MASSIF will comprise the three fixed energy beam lines, will provide a state-of-the-art facility for sample evaluation and ranking and will provide a hub from which pre-characterised samples will be distributed to the most suitable facility for complete data collection

(figure 2). Two stations (MASSIF-1, MASSIF-2) will provide a stable X-ray beam 100 μm in diameter at the sample position. The third station (MASSIF-3) will be a micro-beam facility producing a beam 10 μm in diameter. All three beam lines are characterized by low beam divergence, a high photon flux and a fixed energy chosen to be slightly above the selenium K-edge. Automation will allow each of the stations to screen the diffraction characteristics of up to 1000 crystals per day. All three end-stations will be equipped with high capacity sample changing robotics and MASSIF-1 will, in addition, have the capability to screen crystals in a variety of mounts such as crystallisation plates and micro-fluidic chips. A tunable beam line ID30B from an independent canted undulator source will also be constructed. In contrast to the existing ID23-1 [5] and ID29 [4] which are located on low-β sections and thus have a comparatively high horizontal divergence, the high-β, low divergence characteristics of the ID30B source will allow the possibility to straightforwardly tailor beam sizes to between 20 and 200 μm in diameter over its entire accessible energy range (6 – 20 keV). First user operation of the MASSIF stations is expected in 2nd half 2013, that of ID30B a year later.

Table 1. Current and future portfolio of Structural Biology beam lines at the ESRF. Beam size values (HxV) correspond to the diameter of the beam at the sample position; those in brackets for the ID23-1 and ID29 beam lines are referring to apertures used to tailor the beam size. The flux given for the MASSIF and ID30B beam lines are calculated values.

Beam line	Beam size [μm]	Energy [keV]	Flux [ph/sec]
MASSIF-1/-2	100	12.8	10^{13}
MASSIF-3	10	12.8	10^{13}
BM29	700 & 100	7-15	$3x10^{12}$
ID14-4	100	9.6-14.5	10^{12}
ID30B	20-200	6-20	10^{13}
ID23-1	30 (10/20)	6-20	10^{12}
ID23-2	5x7	14.2	$4x10^{11}$
ID29	50x30 (10/20/30)	6-20	10^{13}

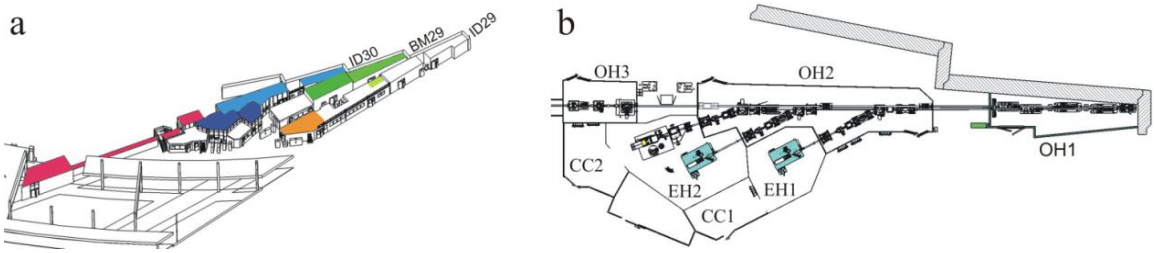

Figure 1. Structural Biology beam lines at the sector 30. (a) shows a top view of the Structural Biology Village with the location for the optical micro-spectroscopy facility (ID29S) shown in orange, the bioSAXS facility on BM29 in green, the three MASSIF beam lines in blue and ID30B in red. (b) shows a general layout of MASSIF (OH, Optics Hutch; EH, Experimental Hutch; CC, Control Cabin). OH2 will contain all optical elements for MASSIF and OH3 all for ID30B.

3. The BM29 BioSAXS Facility

As part of the construction of a "Structural Biology Village' the ESRF's bioSAXS facility (figure2), originally housed in hutch 3 of ID14 [7], has been re-built on the bending magnet source BM29. This has enabled both synergies with ID29 and the future MASSIF beam lines and the extension of the functionality available for BioSAXS at the ESRF. This functionality now includes data collection in an energy range between 7 and 15 keV, thus increasing the accessible q-range and providing more

flexibility for experiment optimization. Using a newly designed multi-layer monochromator with a bandpass of $1.3x10^{-2}$, the intensity at the sample position has been increased by more than a factor of 10 compared to that available on the ID14-3 facility and the use of a torodial mirror (focal point at the detector plane) means that background scattering has been significantly reduced. The BM29 sample environment includes a liquid-handling sample changer and, taken together with the beam characteristics of BM29, this allows for high-throughput, low noise data collections. User operation of BM29 began in June 2012. Further work will now focus on improving the experimental environment of BM29 with the incorporation of an online HPLC device, extension of automation, quality control and feedback for users. The integration of experiment control with the ISPyB database [8] will allow real-time storage and retrieval of experiment parameters, raw and processed data and the results obtained from these.

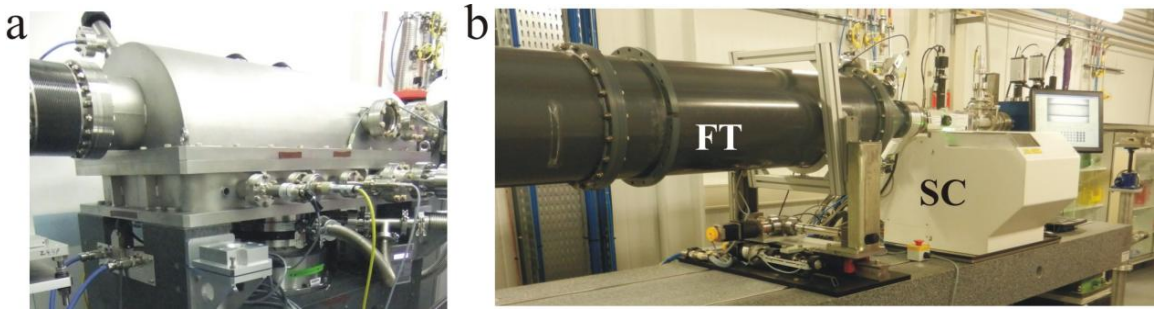

Figure 2. The bioSAXS facility on BM29. (a) shows the vessel of the multilayer monochromator in the optics hutch and (b) the experimental setup with FT being the flight tube and SC the automatic sample changer.

4.The ID29S cryobench facility

The 'Structural Biology Village' also houses the ESRF's *in crystallo* optical micro-spectroscopy facility (‘cryobench’) [9,10], which has been relocated to a dedicated laboratory on ID29. This facility, renamed ID29S-cryobench (S standing for ‘spectroscopy’), allows the on- and offline analysis of macromolecular crystals using UV/Vis, fluorescence or Raman spectroscopy to characterize the functional state of a protein in its crystalline state, the monitoring of X-ray induced processes or the execution of elaborate kinetic crystallography experiments [11].

5. Future Perspective

The construction of both the MASSIF facility for high-throughput sample evaluation and of ID30B should be seen in a context with the evolution of the current ESRF MX beam lines located on ID23 and ID29 (figure 3). As described in [4], future plans see ID29 being specialised for the collection of diffraction data at lower energies (> 5 keV) and with the possibility to micro-focus the beam down to sizes of around 5 μm in diameter covering the whole energy range. In contrast, modifications to the current ID23-1 end-station, which currently has a beam size at the sample position of around 30x30 μm^2, will provide the possibility to enlarge the beam such that beam sizes up to 150 μm in diameter will be available. The second end-station on the ID23 sector, ID23-2, which is currently highly oversubscribed, is a fixed energy, micro-focus beam line with a beam size of about 5x 7 μm^2. Plans for this end-station foresee a decrease in the beam size to approximately 1 x 1 μm^2. MX facilities at the ESRF will thus, in the very near future, include high throughput screening end-stations (MASSIF), tunable data collection facilities with a wide energy range and variable beam size (ID23-1, ID29, ID30B) and a facility with a 1 μm focal spot. Taken together these resources will provide an enhanced platform for MX at the ESRF backed up by access to facilities (BM29, ID29S) for experiments using techniques complementary to MX. However, for these beam-lines to operate to their full potential, reliable beam delivery in conjunction with a high degree of automation is essential. For these reasons the Upgrade Programme for the ESRF's Structural Biology beam-lines also includes on-going and continuous efforts to develop improved software for beam line control (MxCuBE) [12] as well as experiment planning, execution and tracking (EDNA [13]; BEST [14]; ISPyB [8]).

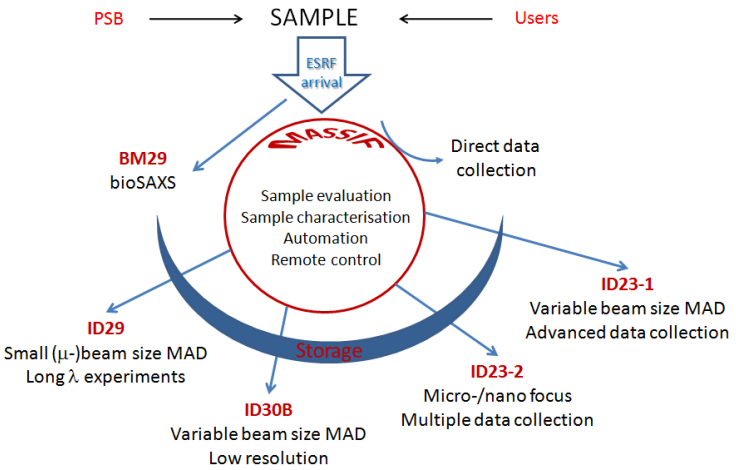

Figure 3. Evolution of the Structural Biology beam lines at the ESRF. The three MASSIF beam lines present the central hub for all incoming samples to the MX beam lines at the ESRF. After careful evaluation, diffraction data collection of these samples will either take place on one of the three MASSIF end-stations or on the ESRF MX beam-line best adapted to the experimental aim.

Acknowledgements

We would like to acknowledge the financial and scientific support provided by the management, scientists, engineers and technicians of the European Synchrotron Radiation Facility and the European Molecular Biology Laboratory, Outstation Grenoble.

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