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Set-up and Experimental Parameters of the Protein Crystallography Beamline at the Brazilian National Synchrotron Laboratory

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The Brazilian National Synchrotron Light Laboratory (LNLS) has a dedicated protein crystallography beamline. The optical elements of the beamline include an elastically bent cylindrical mirror and a triangular bent-crystal monochromator, which focus synchrotron radiation at the position of the sample in the vertical and horizontal planes, respectively. The monochromatic radiation is tunable between 2.0 and 1.2 Å with the optimum wavelengths from 1.3 to 1.6 Å, chosen to maximize the photon flux from the bending magnets of the storage ring (1.37 GeV). Diffraction images are recorded on a 345 mm-diameter MarResearch image-plate detector system with on-line readout. The experimental parameters of the beamline, such as the integral monochromatic flux and focus size, have been measured. The size of the beam at the position of the focal point is 0.5 × 0.5 mm^2. The flux density is between 4.4 × 10^{10} and 8 × 10^{10} photons s^{-1} mm^{-2} for wavelengths from 1.28 to 1.6 Å. The energy resolution is sufficient to measure absorption edges of elements between 1.28 and 2 Å. The facility, intended to serve the national and international community, has been commissioned and is available for users.

Keywords: protein crystallography; LNLS; MAD.

1. Introduction

The Laboratório Nacional de Luz Síncrotron (LNLS) is a newly commissioned and unique synchrotron radiation source in Latin America. The electron energy inside the ring was designed to be 1.15 GeV with a bending-magnet magnetic field of 1.37 T, resulting in a critical photon wavelength of 10.0 Å. The magnetic field of the constructed dipoles exceeded the design parameters and reached 1.64 T. This allowed for an increase in the electron energy to 1.37 GeV and reduced the critical photon wavelength to 6.0 Å. The average ring diameter is 28 m. The maximum electron current reached so far is 100.2 mA. The lifetime of the beam at a current of 60 mA is about 7 h, with a strong tendency to increase.

A dedicated protein crystallography beamline has been constructed at the LNLS (Polikarpov *et al.*, 1997). The beamline (Fig. 1) is placed at bending magnet 3 and accepts synchrotron radiation emerging at an angle of 15° to the direction of propagation of the electrons. The X-ray elements of the beamline include an elastically bent mirror, two sets of slits (one before the mirror and one before the monochromator) and a triangular bent-crystal monochromator, a block of pneumatically driven X-ray filters, and a vacuum path between the monochromator vessel and goniometer. Currently, Ni, Co, Cu and Zn filters are installed. The beamline is equipped with a 345 mm-diameter MAR image plate linked to a Pentium-Pro personal computer and a cluster of SGI workstations.

2. Optical elements

2.1. *Mirror*

The mirror was built at the LNLS from a float glass substrate coated with gold. To increase the adhesion of gold on the glass surface, layers of Cr and Cu were initially deposited on the substrate. The thickness of each metal layer is approximately 500 Å. The mirror has a rectangular shape (750 mm long, 100 mm wide and 19 mm high). It is placed on two pairs of supporting metallic spheres lying in the horizontal plane and separated by a distance of 750 mm [for details of the set-up and optical elements, see Polikarpov *et al.* (1997)]. The mirror is elastically bent by pulling the centre of the mirror downwards. The radius of curvature can be varied from 1500 to 500 m, depending on the required distance from the position of the source to the focal point. The reflectivity of the mirror was initially characterized using a conventional Cu $K\alpha$ X-ray source, and for 8 keV photons the critical angle was found to be

equal to 7.8 ± 0.2 mrad (Vincentin, 1995). The reflectivity of the mirror at a glancing angle of 7 mrad is 78%. Further characterization of the mirror was performed directly on the protein crystallography beamline with synchrotron radiation. Reflectivity measurements of the mirror were conducted with monochromatic radiation of wavelength 1.28 Å (Fig. 2). The resulting curve shows that there is no significant decline in reflectivity for this particular wavelength up to an angle of incidence of about 6.5 mrad. The beamline mirror take-off angle has been set at 6 mrad. In practice, this means that the short-wavelength limit of the beamline is about 1.2 Å, since the critical reflectivity angle is approximately linearly proportional to the wavelength of the reflected X-ray radiation. At shorter wavelengths the flux of synchrotron radiation reflected by the mirror is limited by the L_{III}-absorption edge of the gold surface of the mirror (1.04 Å), its lower reflectivity and the decline in the incident synchrotron radiation flux.

2.2. *Monochromator*

The monochromator was designed to produce a highly focused monochromatic beam and to cover the wavelength range from 1.0 to 2.0 Å when using either Si(111) or Ge(111) crystals. The monochromator crystal has a classical triangular shape (Lemmonier *et al.*, 1978) and, after elastic deformation, an almost cylindrical curvature.

The 1.0 mm-thick triangular-shaped crystal (250 mm long and 50 mm at its base) is clamped at its base and the apex rests upon an eccentric cylinder whose rotation controls its elastic bending (Bernardes *et al.*, 1992). The monochromator design permits both rotation of the monochromator crystal around the θ angle and tilting. All these rotations are computer-controlled. The compact housing of the monochromator and the very simple mechanical parts inside it make it ultra-high-vacuum compatible (Bernardes *et al.*, 1992).

The monochromator crystal is placed inside a high-vacuum chamber which is maintained below 10^{-6} mbar (without baking) using a 120 l s^{-1} ion pump. The monochromator chamber is isolated from the mirror chamber (with a pressure below 10^{-8} mbar) by a 200 µm-thick Be window and is fitted with a 150 µm-thick Be exit window.

The monochromator crystal [Si(111)] has been asymmetrically cut at 7.25° and is used in a condensing mode (Helliwell, 1992). The asymmetry angle corresponds to an asymmetry factor $b = 4$ for 1.3 Å radiation and $b = 2.86$ for 1.6 Å radiation. The maximum acceptance angle is 6.7 mrad. With an angle of asymmetry of 7.25° we are able to cover the wavelength range $1.2 \leq \lambda \leq 2.0$ Å in a non-dispersive mode, when the source and the image both lie on the Rowland circle and the energy resolution is essentially limited by the Darwin width of the rocking curve.

3. Beamline characterization

3.1. *Materials and methods*

The geometrical size of the focus, the maximum horizontal divergence of the beam, integrated monochromatic flux at different wavelengths and the energy resolution of the beamline have been measured.

All the measurements were performed in a non-dispersive mode at the maximum energy resolution that we

Figure 1
Photograph of the protein crystallography beamline.

were able to achieve. The intensity of the X-ray flux was detected either with an NaI(Tl) scintillation counter (BICRON, model 1XMP.040B) or with a photodiode (IRD, model AXUV-20HE1) which has almost 100% quantum efficiency between 100 and 12 keV. Since the incident intensity of the synchrotron light was significantly higher than that allowed by the dynamic range of the scintillation detector, it was attenuated by placing an appropriate number of aluminium filters in front of the detector. The number and thickness of filters were chosen to work within the linear region of the scintillation detector response (normally 1000–15000 counts s^{-1}). The total thickness of filters was carefully measured and the absorption of synchrotron radiation was compensated for in the final values, where necessary. Alignment of the beamline was performed with the use of an X-ray-sensitive CCD camera, 'X-ray eye' (Photonic Science).

3.2. *Focus size*

Measurements of the geometrical size of the focus were performed separately for the following wavelengths: 1.284, 1.381, 1.488 and 1.608 Å. The exact wavelength was verified by tuning the monochromator to the *K*-absorption edges of Zn, Cu, Ni and Co, respectively. A block of filters, remotely driven by compressed air, was installed at the beamline for this purpose. Measurements were performed by horizontal or vertical translation of the scintillation detector with a 50 μm linear slit positioned in front of it. For each wavelength the detector was placed at the calculated focus position at which monochromatic radiation had been pre-focused with the use of the 'X-ray eye'. Results of the experiments show that the size of the focus is almost independent of the wavelength. Typical scans of the focus in the horizontal and vertical directions are presented in Figs. 3(*a*) and 3(*b*). They demonstrate that the geometrical size of the beam is ~0.5 × 0.5 mm^2.

3.3. *Flux*

The integrated monochromatic flux at four different wavelengths was measured independently with the photodiode and scintillation counters. In the latter case the incident intensity has been attenuated as described in §3.1. Both types of measurements produced similar results. However, because of the necessity to strongly attenuate the beam with Al filters in the case of the scintillation counter, measurements made with the photodiode appeared to be more reliable.

Results of these measurements are shown in Table 1. The experimentally measured flux density for this beamline is 8.1 × 10^{10} and 7.1 × 10^{10} photons s^{-1} mm^{-2} for incident monochromatic radiation with wavelengths of 1.608 and 1.488 Å, respectively.

3.4. *Energy resolution*

Energy resolution is particularly important in protein crystallography in order to perform multiple anomalous diffraction (MAD) studies. This technique implies measurement of crystallographic diffraction data with wavelengths normally between 0.7 and 2.5 Å, chosen close to the absorption edge of the heavy atoms bound to or embedded in the protein (Hendrickson & Ogata, 1997). The theoretically calculated energy resolution of our monochromator is 3–4 × 10^{-4} between 1.3 and 1.6 Å depending on the wavelength (Matsushita & Hashizume,

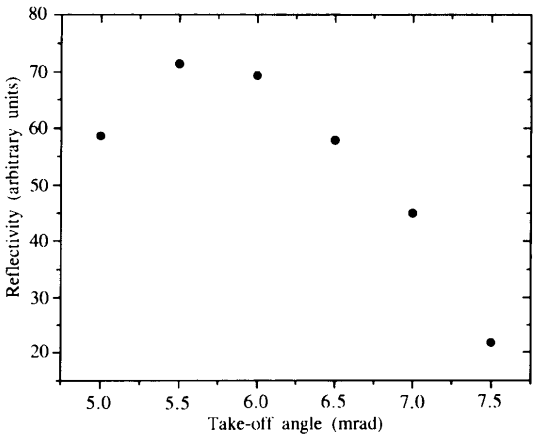

Figure 2
The reflectivity of the elastically bent mirror as a function of angle of incidence of synchrotron light. The wavelength of monochromatic radiation is 1.28 Å.

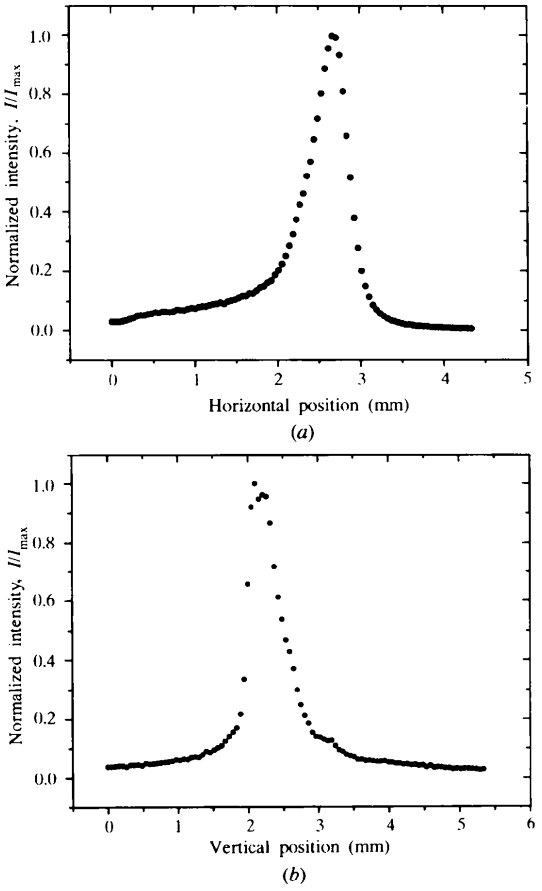

Figure 3
Geometrical profiles of the focus in horizontal (*a*) and vertical directions (*b*). The scans of the profiles have been performed with a 50 μm linear slit placed in front of the scintillation counter.

Table 1
Results of measurements made with the photodiode.

λ (Å)	Current (mA)	Flux (s^{-1})	Flux (at 100 mA) (s^{-1})	Horizontal size (mm)	Vertical size (mm)	Focus (mm^2)	Flux density (mm^{-2})
1.284	60.5	6.659×10^9	1.101×10^{10}	0.5	0.5	0.25	4.403×10^{10}
1.381	48.6	7.431×10^9	1.529×10^{10}	0.5	0.5	0.25	6.116×10^{10}
1.488	54.3	9.652×10^9	1.778×10^{10}	0.5	0.5	0.25	7.110×10^{10}
1.608	51.6	1.043×10^{10}	2.022×10^{10}	0.5	0.5	0.25	8.087×10^{10}

1983). The energy resolution of a real system might be spoiled by imperfections of the monochromator and its bending system, as well as by deviations of the non-dispersive focusing conditions. To demonstrate the feasibility of fine-energy tuning close to absorption edges, we have performed energy scans close to the absorption *K*-edges of Cu and Ni. Experiments have been performed with an NaI(Tl) scintillation detector, placed on a 2θ arm, which is essentially a granite optical table that slides on an air cushion, driven by a remote-controlled stepping motor. The 2θ arm was swung in small angular steps accompanied by a simultaneous rotation of the monochromator around the θ axis. Experimentally measured *K*-absorption edges of Ni and Cu metallic foils are shown in Figs. 4 and 5, respectively. They clearly show all the intrinsic features of the EXAFS spectrum of metallic Ni and Cu, indicating that MAD experiments could potentially be performed on the beamline.

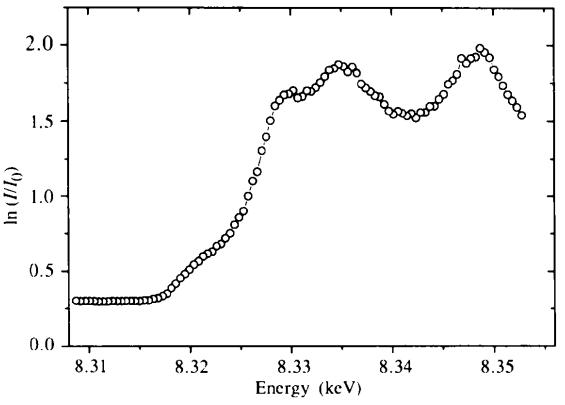

Figure 4
The absorption *K*-edge spectrum of Ni measured at the protein crystallography beamline of LNLS. A metallic Ni foil was used as a sample.

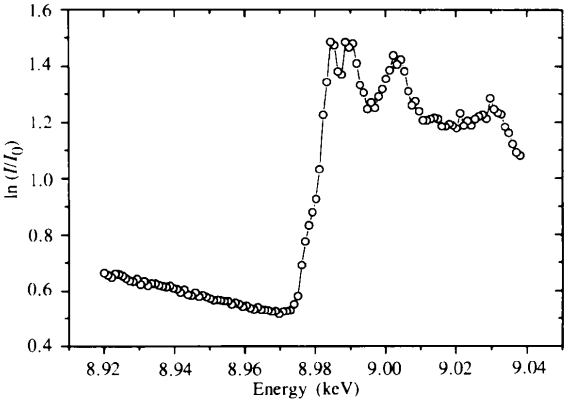

Figure 5
The absorption *K*-edge spectrum of metallic Cu measured at the LNLS protein crystallography beamline.

4. Conclusions

The Brazilian National Synchrotron Light Laboratory has a fully functional dedicated protein crystallography beamline. The beamline set-up includes a biochemical laboratory for protein crystallization, heavy-metal soaks, crystal storage and mounting. Two separate rooms, with stabilized temperatures of 295 and 277 K, are also available. The computing facilities for beamline operation, data collection and evaluation are also part of the beamline. To optimize data collection by external users, full technical support by trained personnel is provided.

The first diffraction data sets have been collected on the beamline from crystals of hen-egg lysozyme and β-lactoglobulin from bovine milk, for characterization purposes, and from the lectin from the camaratu bean (*Cratylia mollis*), the first protein structure studies on the beamline. In this case 61 oscillation photographs were taken with rotations of 1.5° and exposure times of 3 min. A total of 95 560 reflections were measured, of which 27 218 were unique, to a maximum resolution of 1.73 Å. The completeness of this data set is 98.7%; the *R*(merge) is 6.8% and 78% of the reflections have $I/\sigma(I)$ greater than 10. The size of the crystal was about 0.3 × 0.3 × 0.2 mm.

Since the LNLS storage ring is effectively a soft X-ray source, the use of longer wavelengths (1.7–2.5 Å) in MAD experiments exploiting the *L*- and *M*-edges of heavy-metal complexes is planned.

All these facilities are set up to serve the national and international user community. Beam-time allocation is based on the scientific quality of the proposals, as reviewed by a scientific board. Any information concerning beamline usage can be obtained from the address igor@lnls.br.

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