Journal of
Synchrotron
Radiation

ISSN 0909-0495

Received 5 August 2011
Accepted 22 September 2011

Circular dichroism beamline B23 at the Diamond Light Source

Rohanah Hussain,* Tamás Jávorfi and Giuliano Siligardi*

Diamond Light Source, Chilton, Didcot, Oxfordshire OX11 0DE, UK. E-mail: rohanah.hussain@diamond.ac.uk, giuliano.siligardi@diamond.ac.uk

Synchrotron radiation circular dichroism (SRCD) is a well established technique in structural biology. The first UV-VIS beamline, dedicated to circular dichroism, at Diamond Light Source Ltd, a third-generation synchrotron facility in south Oxfordshire, UK, has recently become operational and it is now available for the user community. Herein the main characteristics of the B23 SRCD beamline, the ancillary facilities available for users, and some of the recent advances achieved are summarized.

Keywords: synchrotron radiation circular dichroism (SRCD); vacuum-ultraviolet (VUV); secondary structure; protein; denaturation; capillary.

1. Introduction

Circular dichroism (CD) is a versatile spectroscopic technique which is often used to obtain low-resolution structural information about a wide variety of chiral materials in solution such as small molecules, proteins and (bio)polymers (Fasman, 1996; Berova *et al.*, 2000). Unfortunately the usefulness of conventional CD instruments is limited by several factors: (i) the low photon flux, especially in the far-UV region which gives limited use in sample stability and binding differentiation studies (Hussain *et al.*, 2012); (ii) a larger beam spot size at the sample position, making it impossible to perform measurements in small-pathlength microcapillary cells unless a focusing lens is applied after the beam polarizer that can potentially introduce artifacts; and (iii) a divergent light beam that does not allow measurement in long-pathlength and, at the same time, low-volume (<800 µl) cells. The development of the B23 SRCD beamline at Diamond, making use of the very intense light of the 3 GeV synchrotron source, can help us overcome these limitations. The beamline has been designed to produce a highly collimated beam which not only extends the utility of the technique down to the vacuum-UV (VUV; <190 nm) region (Sutherland *et al.*, 1980) but also allows measurements in very low volume capillary cells without the need of additional optical elements. This feature can also be exploited by measuring very dilute samples in long-pathlength cells which require less than a millilitre of sample. Contrary to B23, most of the other SRCD beamlines around the world, in order to reduce stray light, employ solar-blind photomultiplier tube (PMT) detectors, which reduce the available wavelength range to about 140–320 nm, excluding a good part of the near-UV (and the visible) regions. At B23, because of the double-grating subtractive monochromators, stray light is greatly reduced making it possible to reach a much wider spectral range (see Table 1 for details). In most SRCD beamlines the beam spot size at the sample position is also considerably larger than that of B23, making the photon flux density significantly poorer (see Table 2).

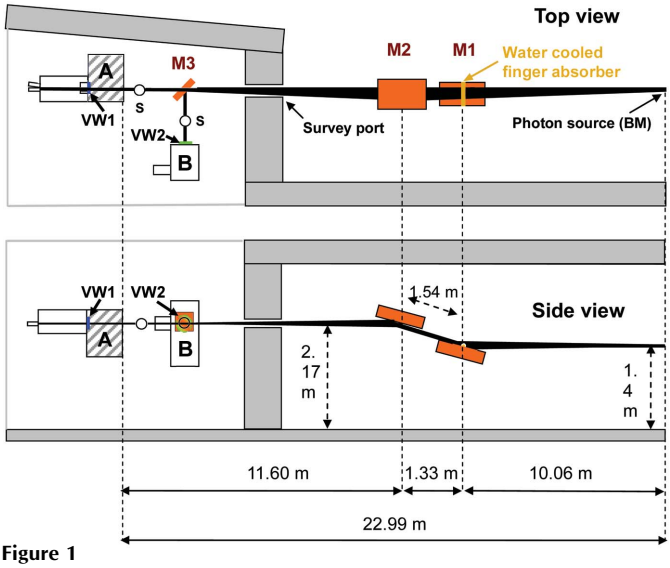

Figure 1
Schematic optical layout of the B23 front-end and experimental end-stations. M1, plane mirror with thin copper finger absorber; M2, toroidal focusing mirror; VW1: LiF or CaF_2 vacuum windows (blue online); VW2: HEM sapphire or Suprasil quartz vacuum windows (green online); M3, plane mirror; S, shutter; A, UHV monochromator CD module for films and solids; B, purged N_2 gas monochromator CD module for solutions.

2. Beamline overview

The B23 SRCD beamline is based on one of the 1.4 T dipole bending magnets (BMs) of the synchrotron storage ring (Hussain *et al.*, 2008). The light emerging from the BM has an aperture of 9 mrad (vertical) × 20 mrad (horizontal). The beamline can be divided into two parts: the front-end, inside the storage ring tunnel, and the experimental hutch, outside the shielding wall (Fig. 1). The front-end contains the beamline optics: two silicon mirrors which reflect and also focus the light onto the slit of a monochromator in the experimental hutch. The first mirror (M1), a water-cooled plane mirror, oriented at a 15° grazing angle, reflects the light up onto a toroidal focusing mirror (M2), oriented at a −15° grazing angle. M1 is further protected by a water-cooled copper finger (8 mm diameter × 23 cm long), which

Table 1
Beamline B23 parameters.

The divergence of the beam was analysed by measuring the spot size (using a Beamage beam profile camera made by Gentec-EO, Canada) at different distances from the exit slit of the monochromator. The photon flux was determined by a calibrated radiometer device (UDT Instruments, USA).

Beamline parameters	Module A	Module B
Wavelength range	155–500 nm; upgrade to reach 140 nm is expected by December 2011	165–650 nm
Image size at the sample position (20 cm from exit slit) (slit width: 0.5 mm)†	0.7 × 0.8 mm	0.7 × 0.8 mm
Beam divergence	1° (horizontal) × 0.5° (vertical)	1° (horizontal) × 0.5° (vertical)
Photon flux measured at the sample position [photons s^{-1} (0.1% bandwidth)$^{-1}$] at 6.2 eV (200 nm) normalized for 300 mA ring current	0.9×10^{12}	3.2×10^{12}

† With lens before the Rochon prism.

Table 2
Comparison of beamline parameters of various SRCD beamlines.

Apart from the B23 parameters, the parameters from the other beamlines are taken from Table 1 of Miles *et al.* (2008). The B23 parameters in parentheses are obtained using different slit width sizes.

Parameters	4B8, BSRF Beijing	CD1, ISA, Aarhus	U11, NSLS, Brookhaven	CD12, SRS, UK	B23-B, Diamond
Slit width size (mm)	0.8	0.4	1.0	4.4	0.5 (0.2–1.0)
Horizontal beam size at slit (mm)	40	5	5	10	0.6
Spectral bandwidth (nm)	1.0	0.6	0.32	1.0	1.1 (0.5–2.5)
Scan speed (nm min^{-1})	12	22	15	16	40 (10–100)
Maximum flux at 200 nm ($\times 10^{11}$ photons s^{-1})	0.6	4.5	30	>250	75
Spot size at sample (mm^2)	6	12	4	25	0.5 (0.25–4)
Flux density at slit size used ($\times 10^{11}$ photons s^{-1} mm^{-2})	0.1	0.37	7.5	20	150 (15–300)

absorbs the hard X-ray component of the synchrotron radiation. The distance between the source and M2 as well as that of M2 and the entrance slit of module A (or that of module B) is 11.6 m.

In the experimental area there are two CD spectrographs (module A and module B) and a small silicon plane mirror (M3), the position of which determines the amount of light entering into each of the modules. The components of both CD modules are the same: a $MgCl_2$ Rochon polarizing prism followed by a photo-elastic modulator (PEM) made of CaF_2 for module A and fused silica for module B, sample chamber and two PMT detectors (Electrontubes, UK, model 9407B with MgF_2 window for module A and model 9924QB for module B). Both modules can operate either in a single or double beam mode. The Rochon prism splits the incident light into two linearly polarized beams that are both modulated by the PEM to generate the left and right circularly polarized light at 50 kHz frequency. With both modules the CD is measured by the direct subtracting method, which was developed by On-Line Instruments Systems (Olis, USA). This method is based on the measurement of the intensities for each circular polarization (left and right) component by using fast analogue-to-digital converters. The data are then processed by a computer and the CD is calculated as the difference between the measurement for the left and right circularly polarized light. Contrary to the conventional approach, which is based on a lock-in amplifier system and still applied in many bench-top instruments and other synchrotron beamlines, the direct subtraction method means that the result is absolute so there is no need for calibrating a proportionality constant, and even large CD signals are measured correctly (Johnson, 1996). The two CD modules target slightly different applications: module A, having an ultra-high-vacuum (UHV) monochromator (RMP srl, Italy) with the first grating made of silicon and the second made of MgF_2-coated aluminium (1200 lines mm^{-1}, JY Horiba) and a CaF_2 window behind the exit slit, is designed to measure solid samples (films) down to about 140 nm, while module B, with a monochromator employing MgF_2-coated aluminium gratings, under nitrogen gas atmosphere and a sapphire vacuum window in front of its entrance slit, is suitable for liquid samples down to 165 nm. The choice for the different vacuum window materials was based on their UV transparency and resistance to UV damage. For module A, the vacuum window made of CaF_2 is transparent down to 120 nm but is susceptible to colour centre formation. As the window is after the exit slit of the UHV monochromator, the monochromator filters out the wavelengths below 140 nm protecting the CaF_2 window from UV damage (colour centre formation). For module B, the vacuum window made of sapphire with crystal orientation along the *c*-axis (0001) to substantially minimize the crystal birefringence, and UV transparent down to 160 nm, is much less susceptible to colour centres formation induced by the synchrotron light beam than any other material tested (LiF, MgF_2, CaF_2, SrF_2, BaF_2 and Suprasil fused silica) and can withstand the synchrotron 'white' beam for longer. To increase the window life time the sapphire window is attached to an UHV *XY* stage coupled with a shutter that is opened only during measurements. When the window irradiated area becomes too dark, and hence less transparent (see Fig. 2), a clear area can be selected. In this manner the window is expected to last up to 2.5–3 years of beamline operation.

3. Ancillary facilities

The control and data acquisition of both modules is achieved by using the Olis *SpectralWorks* program suite. The advantage of this program is that it also contains modules for various data processing tasks such as secondary structure determination or analysis of kinetic traces based on different reaction schemes. It also allows the integration of a script file for sample auto-measurements such as variable temperature and kinetics assay measurements. The program also controls the external shutters in the vacuum pipe: the shutters are opened during data collection and closed when the measurement is finished, hence protecting the gratings and vacuum windows from UV damage.

A simple data converter with graphical user interface, developed in-house, is also available for the users to bring the collected data files into the format suitable for off-site data processing. The sample preparation area is mainly in the beamline experimental cabin and also in a peripheral laboratory which are equipped with a UV-VIS nanophotometer, pH meters, microbalances, Zeiss microscope, sonicator bath, concentrator and 15000 r.p.m. centrifuge for Eppendorf-type vials. Other requirements may be accommodated using other peripheral laboratories.

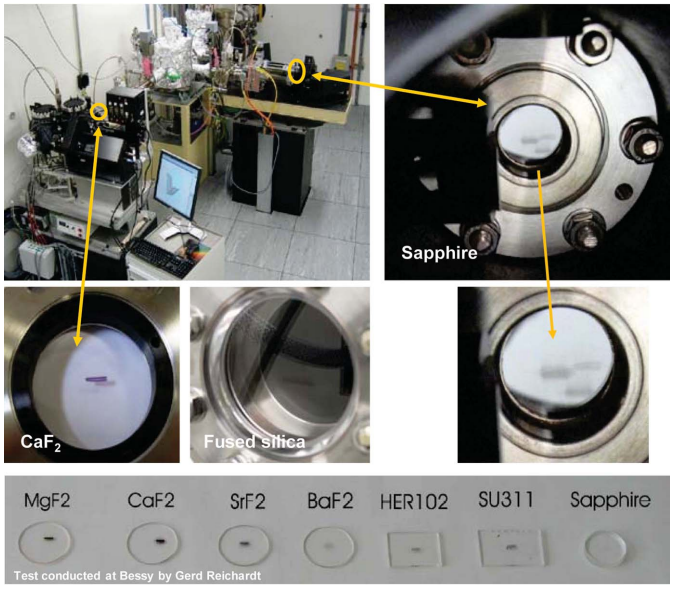

Figure 2
The material of the vacuum windows was selected based on two criteria: transmittance and susceptibility to the high-intensity UV radiation. The experiments conducted at BESSY, Germany, show different degrees of radiation damage (colour centre formation) in cases of various commonly used window materials [MgF_2, CaF_2, SrF_2, BaF_2, Suprasil quartz HER102 and SU311, and sapphire with crystal orientation along the *c*-axis (0001) to substantially minimize its birefringence] which were exposed to the same level of light intensity (DIP 3.1B beamline; G. Reichardt, personal communication). During the commissioning of module A, a vacuum window was placed before the entrance slit to allow the alignments of the optical elements inside the UHV monochromator. The CaF_2 window was damaged within an hour of irradiation whereas the fused silica, although less transparent than CaF_2, lasted longer. This is because the footprint was different as the CaF_2 window flange (CF40) was closer to the entrance slit than that of the fused quartz flange (CF100). The sapphire window for module B shows three irradiated areas with different amounts of colour centre formation (right side of the figure). After about six months of use a clear area is selected by moving the UHV *XY* stage to which the vacuum window is attached. This device is a key and novel technical feature of B23. For both modules, before the monochromator, a shutter (Fig. 1) is operating in the open position only during measurements, avoiding unnecessary irradiation and hence damage of the optic elements (vacuum windows and monochromator's gratings and mirrors).

4. Facility access

The User Office is the first point of contact for all users. There are two calls for proposals per year, one with a deadline of 1 April and the second of 1 October. Access to B23 is available *via* direct access or programme access, for long-term research project proposals. Users can contact the beamline staff for initial discussion [http://www.diamond.ac.uk/Home/ForUsers.html] on the suitability of projects for submission. Proposals will be peer-reviewed by an external panel and all applicants notified accordingly of the outcome of the proposal review.

5. Highlights

Previously we have reported the ability of exploiting the microbeam by measuring CD spectra of protein in a capillary cell of 0.018 mm pathlength and 2 mm width (Jávorfi *et al.*, 2010; Siligardi *et al.*, 2010). To extend further the capability and functionality of the B23 collimated microbeam, attempts were made to measure low-volume diluted samples of nanomolar concentration. This was achieved by designing a 10 cm capillary cell with volume requirement of just under 800 µl. Fig. 3 shows a spectrum of 10 n*M* essentially fatty acid free human serum albumin (HSAff) using a 10 cm pathlength cell

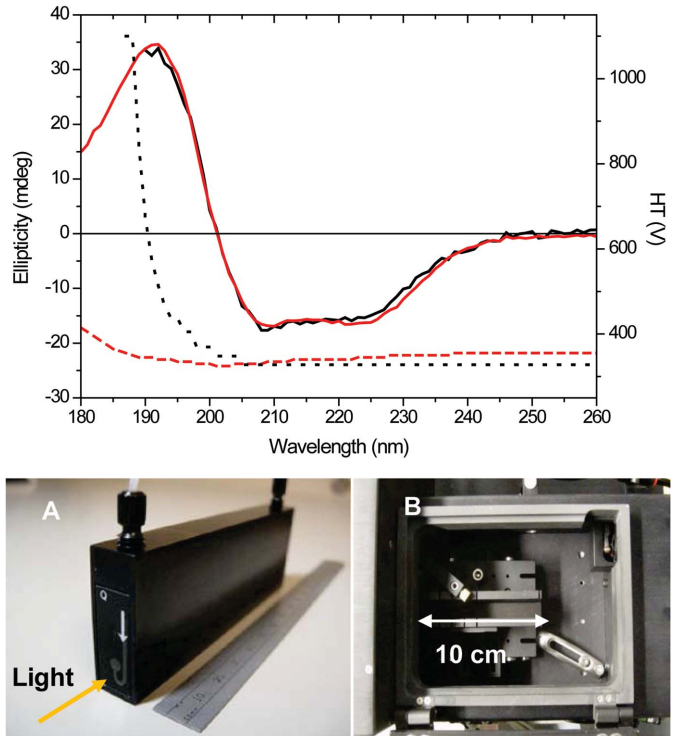

Figure 3
Different concentrations of HSAff in water measured on module B with a standard 0.02 cm and 10 cm pathlength × 3 mm-diameter microcell (spectra unsmoothed, water baseline subtracted). Black line: 10 µ*M* solution in standard cylindrical cell with 0.02 cm pathlength. Grey line (red online): 10 n*M* solution in a microcell with 10 cm pathlength. CD and high-tension (HT) data are shown by the solid and dashed lines, respectively. Spectra were normalized at 210 nm. Lower figure: (*A*) Photograph of the 10 cm micro-flow cell (Starna). (*B*) Open door of the sample house compartment with cell holder. The 3 mm-diameter cylindrical sample cavity of the flow cell is designed to accommodate about 0.7 ml of sample.

compared with that obtained with a 10 µ*M* sample in a 0.02 cm-pathlength cylindrical cell. This cannot be achieved with existing bench-top instruments owing to the larger (in diameter) and more divergent beam. This could be especially important as in many cases obtaining suitable samples in sufficiently large quantities is either very labour intensive or dreadfully expensive.

6. Discussion and conclusions

In summary, the B23 end-stations have the capability to perform measurements from 165 to 650 nm for module B with variable beam spot size (0.25 mm^2 to 4 mm^2), depending on user requirements, or 155 nm to 500 nm for module A, which is more suitable for films and solid samples. A temperature-controlled sample environment is available on module B with the capability to run scripts for automated measurements. In the upgrade pipeline, we have a stopped-flow attachment for kinetic analysis and a high-throughput screening system based on a vertical sample chamber for 96 well plates that are in the commissioning phase.

References

Berova, N., Nakanishi, K. & Woody, R. W. (2000). *Circular Dichroism Principles and Applications*, 2nd ed. New York: Wiley.
Fasman, G. D. (1996). *Circular Dichroism and Conformational Analysis of Biomolecules.* New York: Plenum.
Hussain, R., Jávorfi, T. & Siligardi, G. (2012). *Comprehensive Chirality*, edited by G. Tranter. The Netherlands: Elsevier. In the press.

Hussain, R., Krumpa, N., Strachan, J., Clarke, D., Wagner, U., Macdonald, B., Reading, D., Cobb, T., Gillingham, I., Price, A., Smith, M. & Siligardi, G. (2008). *Adv. Synchrotron Rad.* **1**, 265–270.
Jávorfi, T., Hussain, R., Myatt, D. & Siligardi, G. (2010). *Chirality*, **22**(Suppl. 1), E149–E153.
Johnson, W. C. Jr (1996). In *Circular Dichroism and Conformational Analysis of Biomolecules*, edited by G. D. Fasman. New York: Plenum.
Miles, A. J., Janes, R. W., Brown, A., Clarke, D. T., Sutherland, J. C., Tao, Y., Wallace, B. A. & Hoffmann, S. V. (2008). *J. Synchrotron Rad.* **15**, 420–422.
Siligardi, G., Hussain, R., Myatt, D. & Jávorfi, T. (2010). *Diamond Light Source Proc.* **1**(Issue SRMS-7), e104.
Sutherland, J. C., Desmond, E. J. & Takacs, P. Z. (1980). *Nucl. Instrum. Methods*, **172**, 195–199.