Beamline 12: Circular dichroism

Potential Research Fields

Life sciences

- Biological research and drug design
- Plants and crops

Physical sciences

- Advanced materials
  - Functional polymers
  - Micro-electronic and magnetic materials
  - Biomaterials
- Agricultural technology
- Food technology

Introduction

Circular dichroism (CD) is a rapid and widely used technique for measuring the secondary structure (i.e shape and chain folding) of complex molecules. It is used by researchers in the biological, biochemical, chemical, pharmaceutical and crystallographic sciences to examine proteins, peptides, nucleic acids, carbohydrates, biopolymers and small chiral molecules, and to study the interactions of these molecules to form macromolecular and drug complexes.

Advantages of a Synchrotron Source

Laboratory instruments are usually sourced from a laser. Synchrotron radiation significantly extends the capability of a laboratory instrument. It provides high photon fluxes in the vacuum-ultraviolet and ultraviolet (100–300 nm) region. It is also inherently linearly polarised. The high fluxes and extended wavelength range allow the collection of CD data with unprecedented information content. Approximately 70% of pharmaceuticals target membrane proteins, about half of which are G protein coupled receptors. Conventional CD is limited in wavelength range, particularly in the presence of highly scattering and absorbing membrane systems. Protein CD spectra can routinely be measured to a lower wavelength limit of less than 168 nm and these data can be analysed to determine secondary structure content with very high accuracy. Additionally, measurements can be undertaken on compounds such as polysaccharides that do not exhibit CD in the wavelength region accessible to conventional instruments. Low noise spectra can be measured in very short time scales, with minimal sample quantities.

Synchrotron radiation is also an extremely effective source for time-resolved CD. These studies can be extended over a wide wavelength range, permitting the collection of CD spectra at millisecond time resolution, which can be analysed to determine changes in secondary structure in real time. Areas that can be investigated include:

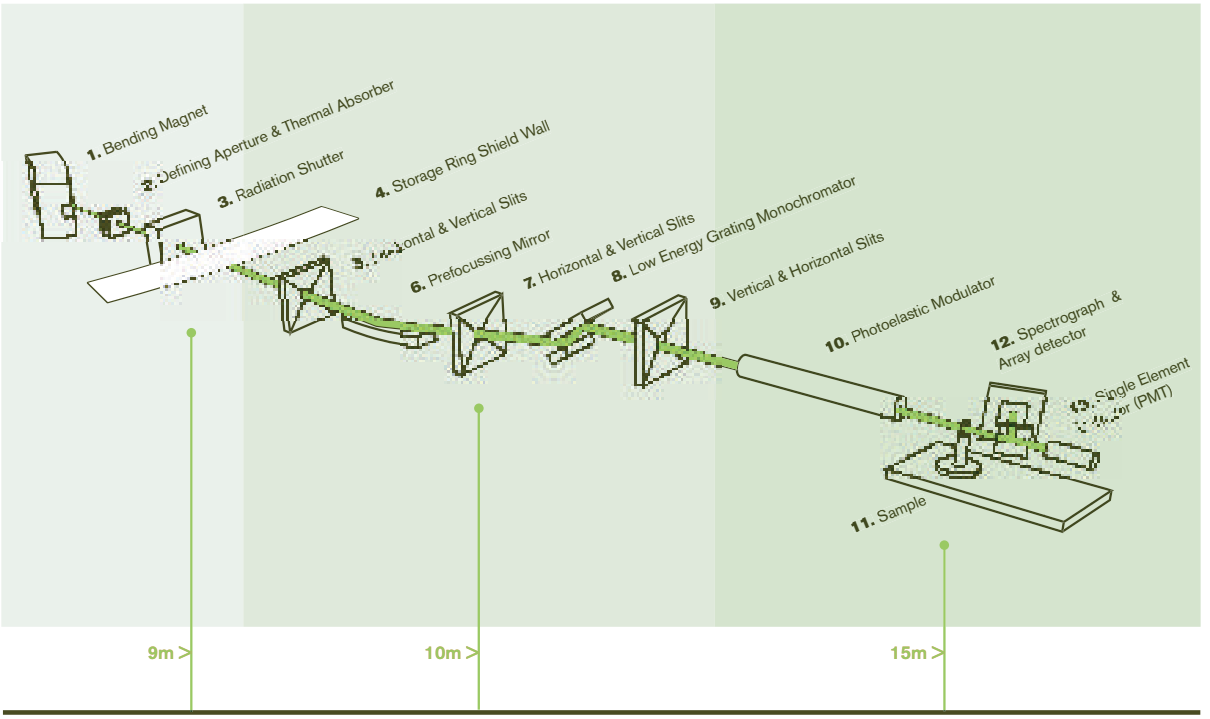
- how proteins fold, misfold and are correctly folded; protein and peptide conformation and folding in or at the surfaces of membranes
- the mutual changes in secondary and tertiary structure that occur when peptides and proteins interact with each other and with other macromolecules
- environmental effects on peptide and protein structure
- the screening of potential drug candidates
- the effects of crystallisation media on protein secondary structure.

The structural, functional and dynamics information that can be obtained by synchrotron radiation based circular dichroism (SRCD) provides information complementary to that produced by techniques such as protein crystallography PX and nuclear magnetic resonance. SRCD is therefore an essential component of any structural biology program.

User Community

SRCD is a very recent development with a rapidly expanding number of applications in a wide range of cutting edge biomedical research. To date Australian CD users have not had the opportunity to access a SRCD beamline.

More than 32 potential users have expressed interest in being able to access the CD beamline. Given the current rate of expansion of biomedical research and structural biology in Australia, and the increased capabilities and



BEAMLINE 12 Circular Dichroism

Figure BL12.1. *Schematic of the proposed beamline for circular dichroism spectroscopy*

application of SRCD compared to conventional instruments, this number could easily reach 100–150 within two years of this beamline opening.

Research Applications

SRCD can provide powerful new information in specific areas.

Protein folding

Rapid conformational changes, for example following dilution of a protein or peptide from denaturant are hard to follow by conventional CD due to the absorbance of the denaturant and the poor signal to noise ratio that results from the need to sample rapidly. These problems can be overcome by SRCD. In addition, data can be acquired to low wavelength even in denaturant (e.g. at 190 nm) providing more detailed resolution of the structural transitions occurring. One recent highlight is the demonstration that the formation of helices is a much slower and more complex process than previously thought[1].

Turbid solutions

CD spectra on highly turbid solutions can be obtained by SRCD, opening the door to studies of membrane proteins, protein aggregates and large protein complexes. One highlight includes CD data on highly turbid solutions of the self-assembly system of clathrin coat protein. This is the first time CD data have been collected on this type of system[2].

Membrane proteins

Membrane proteins are notoriously difficult to study using high resolution structural methods. Nevertheless, detailed information about membrane protein structure has been obtained on a number of membrane proteins[3]. This information has been used as part of the project to construct a CD database for this type of protein, which will allow accurate secondary structural content to be determined and used to aid structure prediction. In addition, conformational changes that occur during membrane protein functions (transporters, receptors and channels) can be determined using SRCD.

Structural genomics

Developing conditions that permit data collection to 158 nm in aqueous solution has extended the secondary structural information content of CD spectra. Accurate data such as this can provide key information to differentiate between alternative models for proteins produced, for example, by structural prediction methods. In addition, SRCD can be used as a rapid screening process for the structural integrity of samples prior to setting up for crystallography[4].

Macromolecular dynamics and function

SRCD can screen for ligands for proteins, and conformational changes occurring upon ligand binding or assembly processes can be assayed in physiologically relevant buffers, and their thermodynamics and kinetics can be determined using SRCD methods. For example,

1 D. Clarke et al. Proc. Nat. Acad. Sci. U.S.A., 1999, 96, 7232-7237.
2 D. Clarke and G. Jones, Biochemistry, 1999, 38, 10457-10462
3 For a review, see B. A. Wallace, Circular Dichroism Spectroscopy and X-ray Crystallography: A Dynamic Duo. CCP4 Newsletter 37:29-30, (1999)
4 Rodi et al, J Mol. Biology 285:197-204 (1999)

CD was used to screen a library of phage-displayed peptides to identify the human apoptotic protein Bcl-2 as a taxol-binding protein. This information will probe proteins in action in real time and complement results emerging from high resolution structural methods.

Physical sciences

Some areas of further interest at the lower energy end of the spectrum might include atmospheric chemistry, electronic spectroscopy of free radicals, and optical metrology of insulating materials.

Beamline Design

The beamline is planned to accommodate a range of experiments including:

- the collection of steady-state CD spectra using a conventional scanning monochromator
- the collection of CD spectra in the sub-millisecond time domain using the energy-dispersive method
- time-resolved CD measurements using stopped-flow, continuous-flow, pressure-jump, and temperature-jump techniques
- the possibility of collecting vibrational CD spectra in the amide-1 region.

Because the beamline is complementary to protein crystallography and would require similar sample preparation facilities, it will be sited close to beamlines 1 and 2.

Also, as for the protein crystallography beamlines, it is expected that there will be a requirement for high throughput and for remote operation so it will be equipped with robotic assistance for automated handling and collection of CD spectra.

Figure BL12.1 shows a schematic representation of the beamline. It is based on beamline CD12 at the SRS (Daresbury) with some modifications. The beamline will be sourced from a bending magnet.

The monochromator mechanism must have the capacity for at least four interchangeable optical elements. As optical elements operating in the VUV–UV region are highly susceptible to contamination, vacuum levels of around 10^{-10} mbar must be maintained in the mirror and monochromator chambers.

Control of beam polarisation is an important consideration for the CD beamline. Ideally, 100% horizontally polarised light is required at the photoelastic modulator. Beamline reflections should be in the 'S' plane for optimal reflection of horizontally polarised radiation, and this is the case for the beamline design shown in figure BL12.1. However, because the polarisation of the synchrotron radiation varies through the vertical plane of the beam, there is also a requirement to select specific regions of the beam to ensure that the optimal polarisation is maintained at the modulator position. This can be achieved by the use of independently adjustable baffles situated before and after the monochromator. These baffles will provide full control of the selected radiation, allowing selection of the optimal combination of polarisation and beam intensity. This method is already being employed at SRS, Daresbury, UK.

End Station

The end station will include:

- a CaF_2 photoelectric modulator
- a linear detector (PMT) for wavelength scan
- an array detector for white light measurements plus spectrograph
- an IR detector for VCD measurements
- laser temperature and pressure jump devices
- stopped flow apparatus.

The set up will be capable of simultaneous detection of absorption and CD spectra.

Two possible detector configurations are shown in figure BL12.1 – a detector system for scanning CD measurements and a configuration for the measurement of energy-dispersive CD.

For monochromatic CD, the photoelastic modulator and sample should be behind the exit slit. For dispersive CD, they should be in front of the exit slit.

The sample stage will provide:

- variable and controlled temperature (for 'thermal melting' curves)
- variable sample-to-detector geometry (for membranes and other scattering samples)
- long, short and variable path-length cells.

Estimates of the performance of this beamline are given below. These figures are intended as a guide to the type of performance that might be expected, and will be confirmed when a detailed design has been completed.

Beamline 12 – Circular Dichroism	
Source	Bending magnet
Energy range	2–10 eV monochromatic radiation; 5–8 eV in white light operation
Resolution $\Delta E/E$	10^{-4}–10^{-3}
Beam size at sample (horizontal × vertical)	3.5 mm × 0.2 mm
Polarisation	70–90% linear, controllable by vertical aperture
Acceptance angle (horizontal × vertical)	35 × 7 mrad