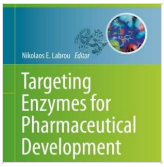

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Standard CD Measurements

Book Chapter

In 1 collection

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ABSTRACT

CD spectroscopy is the essential tool to quickly ascertain in the far-UV region the global conformational changes, the secondary structure content, and protein folding and in the near-UV region the local tertiary structure changes probed by the local environment of the aromatic side chains, prosthetic groups (hemes, flavones, carotenoids), the dihedral angle of disulfide bonds, and the ligand chromophore moieties, the latter occurring as a result of protein–ligand binding interaction. Qualitative and quantitative investigations into ligand-binding interactions in both the far- and near-UV regions using CD spectroscopy provide unique and direct information whether induced conformational changes upon ligand binding occur and of what nature that are unattainable with other techniques such as fluorescence, ITC, SPR, and AUC.

This chapter provides an overview of how to perform circular dichroism (CD) experiments, detailing methods, hints and tips for successful CD measurements. Descriptions of different experimental designs are discussed using CD to investigate ligand-binding interactions. This includes standard qualitative CD measurements conducted in both single-measurement mode and high-throughput 96-well plate mode, CD titrations, and UV protein denaturation assays with and without ligand.

The highly collimated micro-beam available at B23 beamline for synchrotron radiation circular dichroism (SRCD) at Diamond Light Source (DLS) offers many advantages to benchtop instruments. The synchrotron light source is ten times brighter than a standard xenon arc light source of benchtop instruments. The small diameter of the synchrotron beam can be up to 160 times smaller than that of benchtop light beams; this has enabled the use of small aperture cuvette cells and flat capillary tubes reducing substantially the amount of volume sample to be investigated. Methods, hints and tips, and golden rules tomeasure good quality, artifact-free SRCD and CD data will be described in this chapter in particular for the study of protein–ligand interactions and protein photostability.

ATTACHMENTS

Hussain2020_Protocol_EnzymeLigandInteractionMonitor.pdf

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EXTERNAL LINK

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Enzyme–Ligand Interaction Monitored by Synchrotron Radiation Circular Dichroism

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Enzyme–Ligand Interaction Monitored by Synchrotron Radiation Circular Dichroism

GUIDELINES

Standard CD measurements can be performed at the B23 beamline at DLS. Compared to standard benchtop CD instruments, the synchrotron light source is comparatively tenfold brighter due to the higher photon flux in a smaller diameter beam [1]. These characteristics open up new opportunities for CD measurements which are unattainable using conventional CD instruments, some of which will be discussed throughout this chapter. Standard CD measurements can be used to qualitatively assess the conformational property of proteins in terms of secondary structure and local tertiary structural changes occurring upon ligand-binding interactions.

Below will be described the basic experimental setup which can be changed for the sample available, using the principles of the Beer-Lambert law for guidance [35]. For protein solutions of a concentration higher than the standard described previously (about 0.4 mg/mL in 0.02 cm cell pathlength) smaller pathlengths are required, whereas for more dilute solutions larger pathlengths are used [35] (*see* **Notes 5** and **6**).

In the *far-UV region*, protein secondary structure conformational changes as a function of environment (solvent composition and polarity [8, 22], pH [8, 22], ionic strength [8, 22], surfactants [36], pressure (up to 200 MPa) [37], temperature (−170 °C to +350 °C) [38], and ligand interaction [8]) can be promptly monitored. Standard parameters are as follows:

1. A protein solution at concentration of **[M]0.4 mg/mL** , measured using a cuvette of 0.02 cm pathlength. These cuvettes (both cylindrical and rectangular designs) require to be filled at least with **40 µl** of solution.
2. Default parameters for CD measurements in the far-UV region (180–260 nm) using B23 beamline:
(a) 1 nm increments.
(b) 1 s integration.
(c) 1.2 nm bandwidth (bw) corresponding to the monochromator slit width of 0.5 mm (for benchtop CD instruments the recommended bw is 1 nm).
3. The number of scans can be tailored to the sample/experimental design (*see* **Note 6**).

In the *near-UV region*, protein conformation changes with respect to the local environment of aromatic side chains of Trp, Tyr, and Phe residues and disulfide bond of Cys residues as a function of solvent composition and polarity [8], pH [8], ionic strength [8], surfactants [39, 40], pressure (up to 200 MPa) [41], temperature (−170 °C to +350 °C) [42], and ligand [8] interaction can be promptly monitored. Standard parameters are as follows:

1. Protein solution with an absorbance at 280 nm of ~0.8 is desirable (for ligand titration, the recommended absorption range is from 0.4 to 1.6).
2. Data collection occurs in the near-UV region (260–350 nm) using the following parameters:
(a) 1 nm increments.
(b) 1 s integration.
(c) 1.8 nm bandwidth (corresponding to slit width of 1 mm). For benchtop instruments, the recommended bandwidth is 2 nm.
3. The number of scans can be tailored to the sample/experimental design (*see* **Note 6**).

For all types of measurements concerning proteins, clean cuvettes by (*see* **Note 7**):

1. Flushing the cell with deionized water followed by **[M]96 % (v/v) ethanol** .
2. Place the ethanol wet cell in conc. nitric acid in a fume hood, and leave for **00:10:00** .
3. Remove the cell and flush with deionized water to remove the acid.
4. Wash with **[M]96 % (v/v) ethanol** and dry under nitrogen or using a vacuum pump.

MATERIALS TEXT

For materials, please refer to the Guidelines section of the "Enzyme–Ligand Interaction Monitored by Synchrotron Radiation Circular Dichroism" collection.

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

1 See 'Guidelines' section.

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