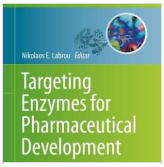

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UV Denaturation of Proteins Monitored by Circular Dichroism in the Far-UV Region (180–260 nm)

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

DOI

dx.doi.org/10.17504/protocols.io.bnxhmfj6

EXTERNAL LINK

https://link.springer.com/protocol/10.1007%2F978-1-0716-0163-1_6

PROTOCOL CITATION

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COLLECTIONS

Enzyme–Ligand Interaction Monitored by Synchrotron Radiation Circular Dichroism

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GUIDELINES

In the far-UV and vacuum-UV regions, the intense photon flux of the highly collimated microbeam of B23 beamline has the potential to cause protein denaturation [5,43,44,45]. UV photo denaturation experiments have been used successfully as a novel assay for the assessment of protein photostability that can be used to determine ligand-binding interactions, in particular for ligands with weak or devoid of any UV chromophores in the far-UV region [5].

For these measurements, samples are loaded into cuvettes following the guidelines mentioned in Subheading 2.1. Repeated continuous scans, usually 20, 30, or in case of stable materials up to 100 are collected from the sample (*see* **Note 8**).

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).

The general protocol is as follows: 2h 30m

1 For a cuvette (either cylindrical or rectangular cell) of 0.02 cm pathlength, **40 µl protein solution** at about

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[M]**0.4 mg/mL** is loaded into the cuvette.

2 Standard data collection parameters in the far-UV region (180–260 nm) include 1 nm increments, 1 s integration time, and 1.2 nm bandwidth. At this point a standard CD measurement can be collected.

3 For the UV denaturation method, repeated continuous measurements are scanned. The number of scans to be collected is dependent on the system under study as the protein sensitive to UV light (photostability) varies from protein to protein. A suggested range is 20–30 scans that correspond to a total time of **01:00:00** – **01:30:00** in order to obtain a good denaturation rate trend within a reasonable time-scale which is often a key consideration especially for Users of the B23 beamline at Diamond Light Source where allocated experimental time is limited. 2h 30m

4 For data analyses see part 3.4 "Processing of Circular Dichroism Data Collected at B23".

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