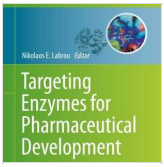

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Low-Volume Titrations for Ligand Binding Monitored by Circular Dichroism

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

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

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COLLECTIONS

Enzyme–Ligand Interaction Monitored by Synchrotron Radiation Circular Dichroism

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GUIDELINES

CD titration experiments enable the determination of the quantitative binding interactions of host-ligand systems. It has been successfully employed for the determination of the dissociation constant K_d for ligand binding to membrane proteins and soluble proteins [6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. This was done by monitoring the CD spectral changes of the protein upon the incremental addition of small but accurate aliquots of ligand corresponding to an increased molar ratio until no more changes were observed.

CD titration experiments conducted in the near-UV region (250–350 nm) have the potential to use a large volume of sample due to the larger pathlength which are often employed to monitor low-concentration solutions. A fused quartz cuvette (Starna) with a window aperture of 2 mm × 2 mm for a 1 cm pathlength requiring only 70 µL to be filled is routinely used at B23 due to the smaller footprint of the synchrotron light source beam that can be conveniently varied from 0.05 to 1 mm in diameter. The larger beamlight of benchtop CD instruments, from about 3 to 8 mm in diameter, makes the use of such a small aperture 1 cm cell very impractical. On the contrary, this cell is routinely used for CD titration with B23 in particular for membrane proteins of which yields are notoriously smaller than most soluble proteins under the same expression and purification conditions. Another advantage of the small aperture 1 cm cell is the fact that the addition of the ligand aliquots and their mixing with the protein can be conducted rather gently reducing the equilibration time when detergents are used to solubilize the membrane proteins. CDApps can also be used to calculate
the absorption of the solution at each titration step point using the molar extinction coefficient for both the protein and the ligand to ensure the total absorption in the cuvette is within the acceptable 0.4–1.5 absorption limits [16] (*see* **Notes 9–11**).

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 Measure the concentration of protein solution at A_{280}. Use this value to calculate the dilution factor for the stock solution for the working solution. The concentration of the protein to be used during the experiment is in part determined with respect to the UV absorbance contribution of the ligand at each titration point which should not be more than 1.5–1.6 (*see* **Notes 9–11**).

2 Aliquots of ligand are added to the host mixture in the cuvette, pipetting up and down to thoroughly combine the components (*see* **Notes 12** and **13**).

3 Mixture incubated for a user-defined period of time (e.g., **00:20:00**) to allow for equilibration to occur (*see* **Note 14**) and ensure the appearance of the solution is homogenous before measuring (*see* **Notes 13**, **15** and **16**). This is particularly important in the presence of detergents as the addition of aliquots affects the vesicle morphology and requires equilibration to obtain reproducible CD baselines. The way to determine the incubation time is to scan repeated consecutive CD spectra until the spectral shape is stable. The incubation time can be calculated as the product of the number of scans multiplied by the time to scan a single spectrum.
20m

4 Measurements are collected for each titration point, after the determined incubation time.

5 CDApps is encouraged for the analysis, using the experimental plan as the template for the analysis as it contains information regarding volumes, concentration, and dilution factors of the mixture components in addition to pathlength(s) used to monitor the mixtures (*see* **Notes 10–12**).

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6 CD spectrum of the Buffer under the same incubation time is measured to be used as baseline to be subtracted from each titration point spectrum, followed by the CD spectrum of the relevant proportion of the maximum-titrate ligand concentration. This is essential if the ligand is chiral. For achiral ligands, this is not necessary as the ligand will be devoid of any CD and the CD spectrum of the baseline will be sufficient for the data processing.

7 Difference in the CD signal at each point is plotted against the respective ligand concentration, fitting with a Hill function for determination of the binding dissociation constant K_d and the stoichiometry of the interaction using a nonlinear regression analysis [46].

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