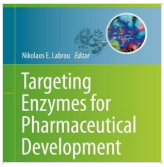

Sep 02, 2021

Processing of Circular Dichroism Data Collected at B23

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

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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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null, Circular dichroism, Ligand binding, Titration, Binding constant, UV denaturation, Protein stability, Data processing

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Enzyme–Ligand Interaction Monitored by Synchrotron Radiation Circular Dichroism

GUIDELINES

Data is processed using CDApps available at the B23 beamline for use on-site and as a USB downloadable copy for use off-site (found at Computer > Software (W:) > cd_programs > CD Apps > USB).

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).

Loading CDApps for Processing of Circular Dichroism Data Collected at B23

1 Below are the initial steps required to load CDApps software:

Open the CDApps software by:

(a) On-site—Computer > Software (W:) > cd_programs > CD Apps > CD Apps.exe. Double click the CD Apps icon.
(b) Off-site—double-clicking the CD Apps icon.

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The following options page enables the User to select the type of analysis appropriate for the data set.

(a) **CD Measurement (including Automation)**—analysis options available include 1 to *n* spectra, titration, UV denaturation, thermal melt using both the Quantum Peltier (+5 °C to +95 °C) and Linkam MDS 600 (–150 °C to +350 °C) controllers and 96-well format.

(b) **CD Titration**.

The analysis using both of these methods shall be discussed. For all types of analyses, the most important bits of information for subsequent analyses including conversion of units and secondary structure estimations (SSE) are concentration, pathlength, concentration and molecular weight and Δε (the average amino acid molecular weight). Step 1 includes a Step case.
"CD Measurement"
"CD Titration"

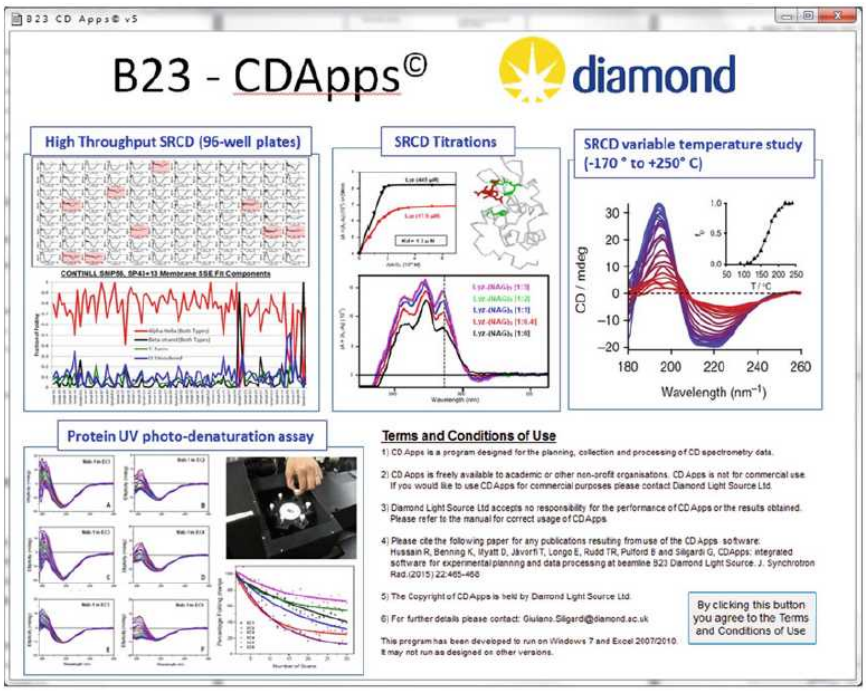
Analysis

step case

"CD Measurement"

Analysis Using the "CD Measurement (Including Automation)" Option

2 Select the "CD Measurement (including Automation)" (highlighted in Fig. 1).



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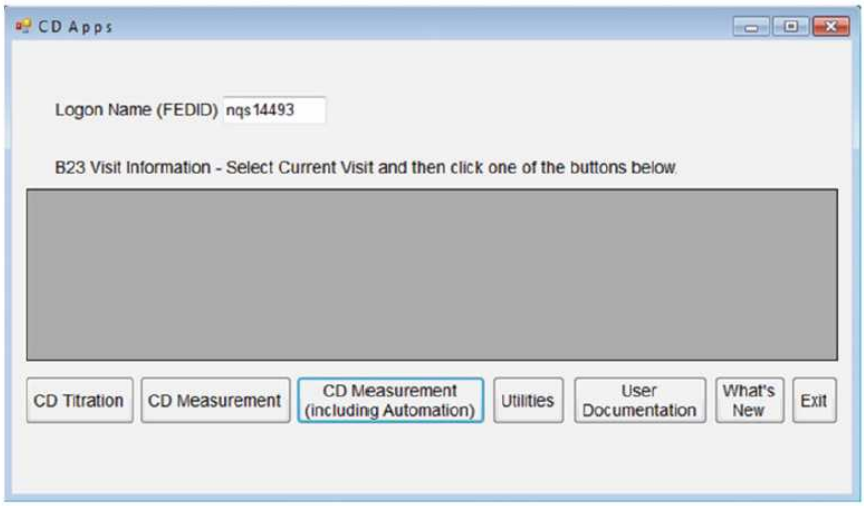

Fig. 1 (Top) Terms and conditions page which appears upon opening. (Bottom) Initial options page which opens upon loading of the program

3 Click on the "Sample Description" section of the sample line to be completed and enter details, e.g., name of the sample (Fig. 2).

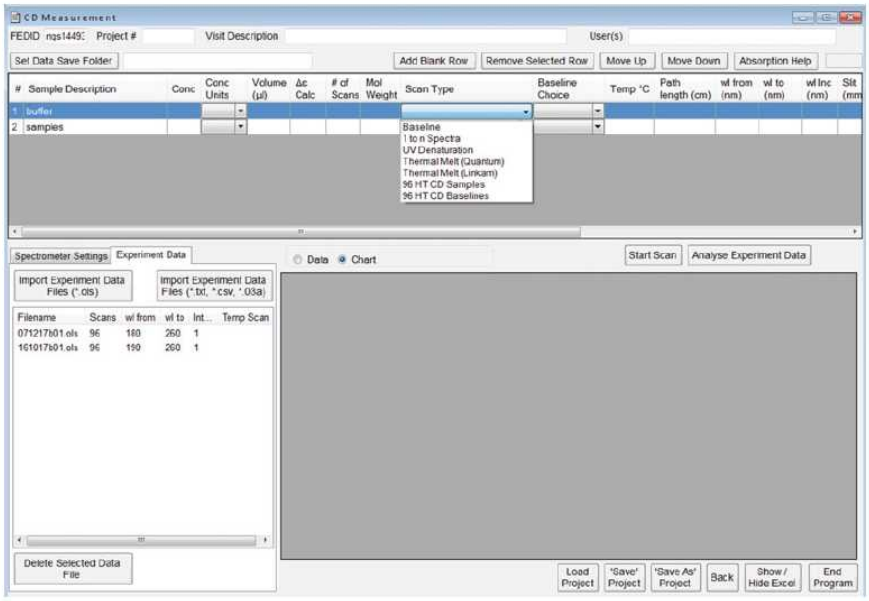

Fig. 2 Opening screen for CDApps. From here all of the experimental details including data type and experimental parameters are added ready for the analysis. From here the initial information is input before progressing through the analysis steps by pressing "Analyse Experiment Data"

4 To add experimental data to the spreadsheet:

Select the "Experiment Data" tab, selecting the appropriate button for the type of file to be uploaded (.ols or .csv/. txt).

Generally, files collected at the beamline are saved as .ols files. Files collected using both the benchtop Chirascan instrument and the beamline modules can be converted into .csv format.

Attach the appropriate data file to the sample line by clicking on the file to highlight (line becomes blue), then drag and drop the file across to the sample line.

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Experiment details (number of scans, wavelength ranges, wavelength increment) are automatically completed for the attached file.

5 Complete the remaining empty experiment details (concentration, concentration units (mg/mL or µM), volume (µL), Δε calculated, molecular weight (Da), temperature (°C), pathlength (cm), slit (mm), and integration time(s)).

6 New rows can be created by selecting “Add blank row” and repeating **steps 4–6**. If all of the details of a row are to be replicated for additional rows, this can easily be done by a single right-click and selecting the “replicate” option. Appropriate data can then be attached using **step 5**.

Note: For any type of analysis, the correct scan type needs to be selected and appropriate baseline allocated. Baseline is assigned to background or buffer measurements, which will be subtracted from all other measurements selected in the group during the analysis. Depending on the analysis selected, the software will subtract the baseline differently.
For *1 to n* Spectra multiple entries should be used during the analysis, one per sample measured which can each contain multiple scans per loaded file. The 1 to *n* Spectra option creates an average scan for each loaded file and subtracts the baseline from each sample. *UV Denaturation analyses* subtract the average spectra of the baseline from every individual scan in the file.

7 From here, a range of analyses are available for different types of data sets. The type of analysis is selected using the “Scan type” drop-down menu for each sample line (*see* **Note 17**). Each analysis option will be discussed below.
Step 7 includes a Step case.
1 to n Analysis
UV Denaturation Analysis
Thermal Melt Analysis
96-Well Analysis

step case

1 to n Analysis

Multiple samples can be analyzed by completing the required number of sample lines as detailed in part 3.3 "Low-Volume
Titrations for Ligand Binding Monitored by Circular Dichroism", step 2, and highlighting the desired number of samples before selecting the “Analyse Experimental Data.”

8 Complete the workbook with the appropriate number of sample lines and data.

Ensure that the data files to be analyzed are within the same wavelength range and increments. For analyses in the far-UV region (180–260 nm) where secondary structure estimations (SSE) are desired as part of the analysis, endure that loaded files are collected in 1 nm increments.

Select “1 to *n* Spectra” option from drop-down menu for “Scan type.”

9 Highlight (blue) samples to be analyzed by clicking on the desired sample line and click “Analyse Experimental Data.”

10 Select the zeroing region for the data which will be used to off-set all data sets. This is often in a region where no CD signal is observed. Click “Graph Data.”

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11 Graphs will appear in the Excel Workbook, detailing the different units for CD presentation (Δε, Δ*A*).

Note: Only after the zeroing has been performed can SSE be calculated. A new tab will appear under the zeroing section (Fig. 3b, c).

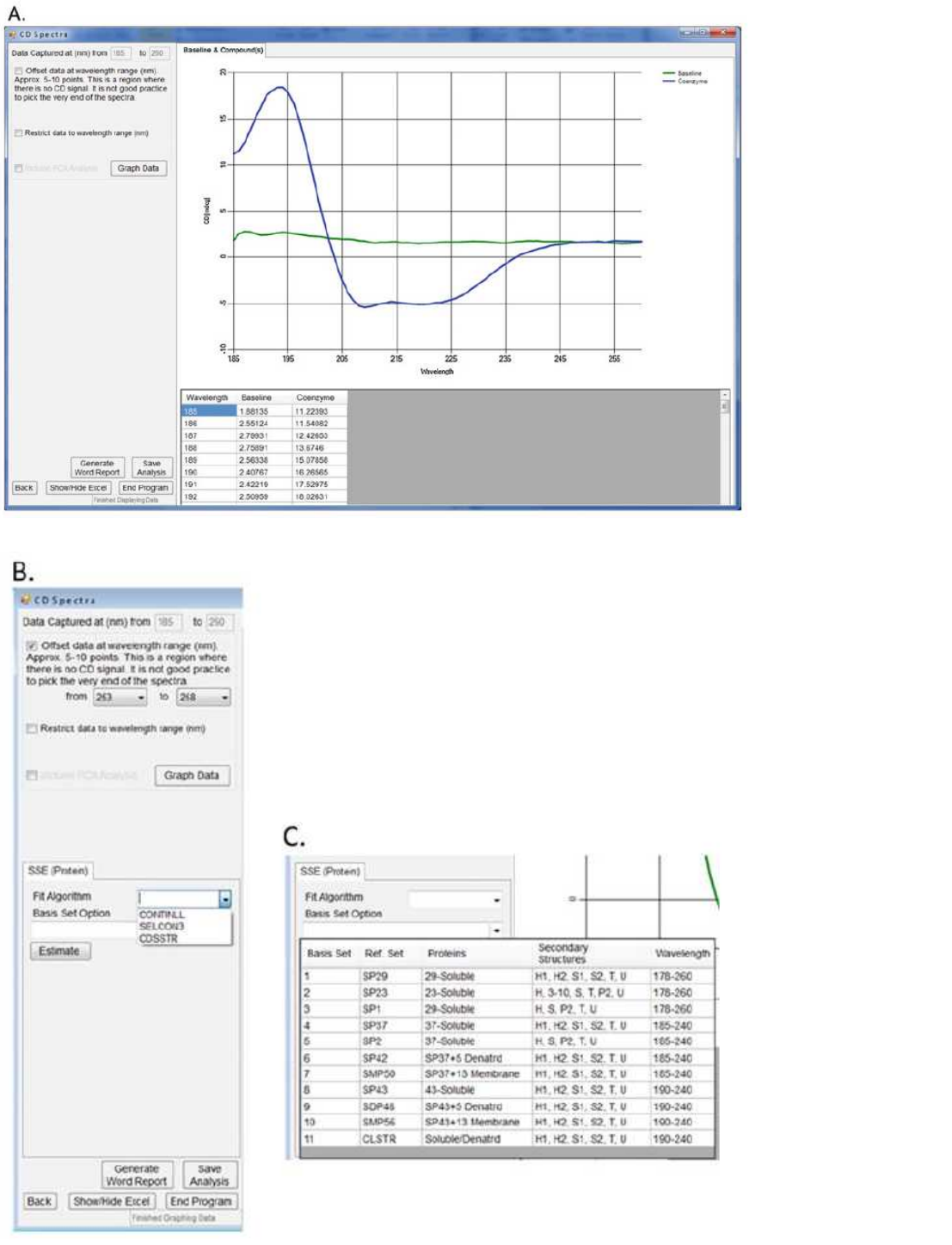

Fig. 3 (a) Analysis page of CDApps after pressing "Analyse Experimental Data" button for "1 to *n*" function. (b) After setting the data offset a SSE tab appears from which the appropriate algorithm and (c) reference dataset is selected

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12 For SSE, select the appropriate algorithm (CONTINLL [19–21], CDSSTR, or SECLON3 [20]) and database (SP29, SP23, SP1, SP37, SP2, SP42, SMP50, SP43, SDP48, SMP56, CLSTR).

13 Data will be output into an Excel Workbook containing graphical and numerical outputs. Graphs can be used as they are by copying, or numerical outputs can be used for further analysis and redesigning of graphs for data presentation.

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