Circular dichroism spectroscopy

by Neal Zondlo (1994), edited by Scott Hart (2001)

I. Applications of CD in the Schepartz Lab

A. Background

The most important physical/optical concept in CD is the idea of *circularly polarized light*. In CD, the polarized beam of light can be considered to be composed of right- and left-handed circularly polarized components. The CD instrument itself uses a double monochromator to take a beam of light (UV range) and eliminate stray light. The two monochromators are oriented in different axial directions, which serves to produce linearly polarized light. That's the simple part. The fancy part is called the CD Modulator. The linearly polarized light is passed through a quartz crystal that has been subjected to mechanical stress, producing circular polarization within the crystal. Polarized light that has passed through this crystal is thus modulated to circular polarization.

Now imagine this light passing through an optically active substance. When the light passes through an optically active material, its two components (left- and right-handed circularly polarized light) are absorbed to different degrees. This difference in absorbance of the two forms of light is called *circular dichroism*.

The light that has passed through the optically active substance shows a net effect of being elliptically polarized. Much math is involved in truly understanding this, but if you consider the result of equal portions of left- and right-circularly polarized light as resulting in a circle (no circular dichroism), consider differential amounts of these types of light as producing an ellipse. At the cartoon level, this hopefully makes sense. The *molecular ellipticity* resulting from this phenomenon is represented by the symbol Q (theta).

B. Applications of CD and why we use it

Various protein and peptide secondary structures interact in different and predictable ways with circularly polarized light, and give signature CD spectra. a-Helical, b-sheet, and random coil structures all give signature CD spectra (Figure 1). The absorbances at various minima and maxima of these spectra can be used as diagnostic tools to determine the amount of the pertinent secondary structure present. For instance, as seen in Figure 1, an a-helix has minima at 222 nm and 208 nm. The absorbance, or ellipticity (usually meaning residue ellipticity), at 222 nm is often used as a quantitative measure of helical content when the number is compared to the theoretical ellipticity of a 100% helical peptide.

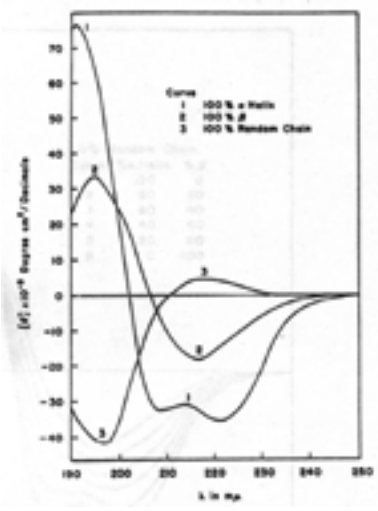

Figure 1. Representation of circular dichroism spectra for various secondary structures. Top curve is helix, middle curve is sheet, bottom curve is coil. Taken from Greenfield and Fasman. For more information of diagnostic spectra for various secondary structures, see: Greenfield & Fasman, *Biochemistry*, 8(10), 4108-4116, 1969.

II. Use of a Circular Dichroism Spectrophotometer

There are instrument-specific procedures for the CD spectrometer that is available to Chemical Biology Laboratory students. Your TA will provide training on the instrument you will be using. No matter what instrument you use, there are some general procedural things to keep in mind. Before you attempt to collect circular dichroism data, be sure you carefully review the sections below and get answers to any questions you have about use of the CD.

A. Questions

1. Where is the CD?

   The location of the instrument designated for use by Chemical Biology students will be provided by your TA.
2. Are there any sign-up procedures?

   Your TA will provide information if any advance scheduling is required.
3. Where can I find general information on CD usage?

   The manual can provide more detailed information on the use of the instrument and its software. Additionally, general information about CD, analysis and interpretation of spectra, sample handling, and the limitations of CD (many) can usually be found with the manual.

B. Start-up procedures

Spectrometers often require up to an hour to warm up and get to the proper temperature. Make sure that this takes place before you prepare your samples and materials to do the experiment so you can avoid wasting time. Your TA will sometimes take care of this in advance, but it is your responsibility to think about it.

C. Checklist of things to bring to the CD room

Collect everything you need before heading out. Things you might need include: CD cell and holder (0.1 or 1.0 cm), tubes with sample(s), DOS-formatted high density disk, lens paper, parafilm (for 0.1 cm cells), ethanol squirt bottle, water squirt bottle, 1% SDS, P200 pipetteman, P200 tips, extra tubes, beaker for tips, buffers, etc., gloves, a pen, and Kimwipes.

1. Sample handling

The specific details of sample handling will vary depending on the exact instrument used. In general, you first need to decide which size cell you want to use, 0.1 or 1.0 cm. This decision is influenced by the concentration of your sample (which affects the absorbance: you need a strong signal, but not too strong to overwhelm the instrument) and the strength of the CD signal. If either of these gets too high, you will see the dynode voltage (dynV) rise, and data acquisition may stop. When handling CD cells, you should wear gloves and use only lens paper to dry or wrap the cells. Use only plastic tips to remove solutions from the cell. For 0.1 and 1.0 cm cells you will use 150-200 µL and 300 µL of sample, respectively. **Be sure to mark down the size of the cell you used!**

During a run you should close the cells with parafilm or the teflon stopper to guard against evaporation. After the run, remove the sample from the cell, wash repeatedly with 1% SDS, water and ethanol solutions, and finally rinse with ethanol and dry using N_2, wiping off excess ethanol with lens paper. A useful diagnostic experiment

to try is to run a spectrum, remove the sample and wash the cell, and then return the sample to the cell to assure that the spectrum remains the same. At the end of the experiment, clean the cell thoroughly, wrap it well in lens paper, and immediately return it to the CD supplies drawer.

2. Use of the software to run experiments

Your TA will train you on the specific software for the CD instrument you'll be using. The software is generally quite user-friendly, but you must think through the details of your experiment in advance. Many CD spectrometers allow you to run experiments in several modes, including CD signal vs. wavelength (**W**), temperature (**T**) for thermal melts, or time (**K**) for kinetics.

Depending on your experiment, you will be able to adjust parameters such as wavelength, temperature, step size, and number of scans. Your TA will help you determine the appropriate settings for your experiment. Examples of parameters that have worked well for b-peptide experiments are as follows:

Temperature	25°C
Path length	2 mm
Averaging time	2 seconds
Bandwidth	2 nm
Peptide Concentration	80, 40, 20, and 10 mM

Collect the relevant data and obtain printouts of the spectra to include in your lab notebook. Be sure to save your data!

3. Shut-down procedures

Just as there are steps to go through when you are starting up the CD, there are also shut-down steps to put the CD into a safe stand-by configuration. You will also need to turn off the power, allow the lamp to cool, and turn off the gas and water supplies. Follow the specific instructions for the CD spectrometer you are using.