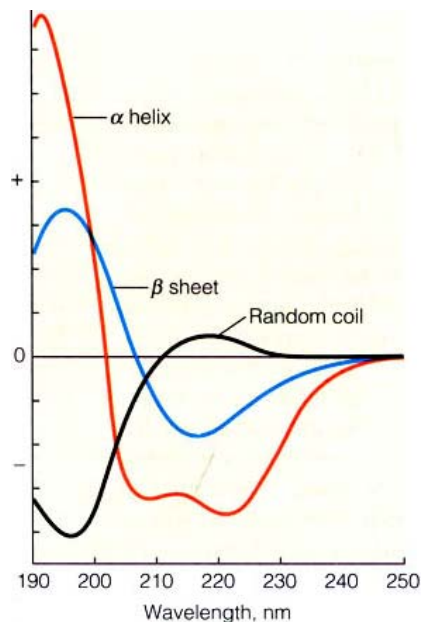


Planning your circular dichroism (CD) experiment

Plan ahead: The instrument requires liquid nitrogen to run. The tank will be empty unless someone has used the instrument lately, and it typically takes 3 business days (or less) to refill the tank. Take this into account when planning your experiments, and contact the CD instrument manager ahead of time.

Experiment types:

- A typical first experiment is a scan in the far-UV range (190-250 nm, see right) to qualitatively examine the secondary structure of a protein. This is best used to confirm that a protein is folded, or check that a mutation did not cause any global structural change compared to wild type.
- Thermal denaturation can be monitored. A single wavelength is chosen (typically 220 nm, detecting both α -helix and β -strand) and sampled at regular intervals while the temperature is increased. This can be used to assay the stability of a protein and/or compare it to the stability of mutants.
- Conformational change induced by a binding partner can be examined. If both partners produce observable CD spectra (protein, DNA, and RNA all do), then care must be taken to perform the appropriate controls. Ideally a split cell should be used, allowing the CD spectrum of each partner alone to be observed additively before they are mixed.



Sample: You will need a cuvette and a syringe to perform the CD experiments; these can be loaned by the Foster Lab for your use. The cuvette must be filled with 300 μ l of sample. The recommended minimum amount of material is \sim 1 mg/ml, although more or less may be appropriate depending on your sample.

Buffer: Many buffers will not interfere with the CD experiment, but high (>50 mM) concentrations of buffer will increase the chances that the instrument will halt acquisition due to excessive dynode voltage. If you are using a relatively concentrated buffer, it may be appropriate to do a buffer-only test run before attempting to collect data using your macromolecule. It is difficult to predict which buffers will cause problems.

Data transfer: Bring a flash drive or 3.5" floppy disk to transfer your data.

Data processing: The instrument stores data in a simple text file that can be manipulated or plotted using a spreadsheet program such as Excel. Choose "Open", then change "Files of type:" to "All Files" and choose your data file. Use the Import Wizard to choose "Delimited" data then specify "Tab" as the delimiter and click "Finish". The header will contain text describing the experiment, including any info you entered in the "Notes" window. The data are found below this; the first column is the wavelength and subsequent columns contain the data. If your experiment contained multiple scans, the rightmost column will contain the averaged value for each wavelength. It is perfectly acceptable to combine and average several datasets from the same sample that were saved in separate data files.

Contact Ian Kleckner with any issues:
kleckner.5@osu.edu, 614-688-8146