

# Applied Photophysics ChirascanPlus Circular Dichroism and Fluorescence Spectrometer

This protocol provides a step-by-step guide for basic CD measurements. It does not replace the required training for each new user

## Reserve Instrument Time

1. Make sure that your PI has provided a speedtype for the purchase of N<sub>2</sub>(l).
2. You must have received training on the **Boulder** ChirascanPlus by Dr. Erbse (JSCBB-C316; 303-492-0528; Annette.Erbse@colorado.edu)
3. Reserve time at the CD Google Calendar. You will receive access to the calendar after your training.
4. At least 2 days prior to your planned experiments, ensure there is more than ¼ tank of N<sub>2</sub>(l).
  - If the tank is low contact Dr. Erbse
  - If your experimental plans change, please adjust your reservation accordingly

## Purging With Nitrogen

It is **imperative** to purge the instrument of oxygen with N<sub>2</sub> gas **before turning on the lamp**. Failure to do so will generate ozone, which will irreversibly damage the optics. This will render the instrument useless, and is extraordinarily expensive to repair.

**If you do not purge the CD properly with nitrogen, you and your lab will permanently lose all privileges on this instrument.**

The length of purge required depends on the recent use of the instrument:

Last use	Purge time
< 3 days	2 hour
< 1 week	4 hours
< 1 month	6 hours
> 1 month	overnight

1. Make sure that there is >¼ tank of N<sub>2</sub> before beginning the purge and that the tank pressure is above 150 psi.
  - To build pressure in the tank, make sure all valves are closed. Open the pressure-building valve (“P.B.” tag; red circle) and monitor the tank pressure using the tank gauge (yellow circle). When the tank is over 150 psi, close the pressure-building valve.
2. Open the nitrogen gas valve on the tank that is connected to the regulator (“Use” tag; green circle).
  - **Do not make any adjustments to the N<sub>2</sub> flow.**
3. The output pressure from the tank should be 50 psi, as marked on the leftmost gauge on the regulator (circled).



4. The flow rates to the instrument should be:

Lamp	1.2 L/min
Monochromator	3 L/min
Sample	1 L/min

5. Check these flow rates on the black box behind and to the left of the CD. The correct rates are marked, as shown above.



Do not make any adjustments to the N<sub>2</sub> flow.

If any of these things are not correct, **get assistance** before moving on to the next step!

## Starting the CD

1. Install the appropriate sample holder for the cuvette that you intend to use.

A Suggestion:

Spectra that will scan below 190 nm should only use 1 and 0.5 mm pathlength cuvettes to minimize solvent absorption (as water absorbs <190 nm).

There are four different single-cell holders, which can be exchanged by unscrewing the two spring-loaded screws on the holders using the small flathead screwdriver that is stored next to the instrument.

2. **After the instrument has been fully purged**, turn on the lamp electronics on the left panel of the instrument by flipping the black rocker switch labeled “lamp”.



**Wait 15 seconds**, then push the red button. The lamp will ignite immediately, and will be indicated by a yellow glow to the left of this panel (as shown above).

3. **Record the starting lamp hours in the logbook.**

4. Turn on the spectrometer by flipping the black rocker switch labeled “system” on the right panel of the instrument.

- As shown here, the green “status” light will be lit, and the “Tx” and “Rx” light will flash red to indicate communication between the instrument & the computer.



Contrary to intuitions, flashing red lights are normal & expected!

5. **If you are using temperature control**, turn on the Quantum Mechanics Temperature Controller. Set the “Target” temperature to the temperature desired for your sample.



- This temperature-control system uses a Peltier device, which requires a heat sink. You must also turn on the water bath. **Do not change the temperature setting, which should remain at 20°C.**
- If the liquid level is more than 1.5 inches below the lid, top it off with 20% ethanol in MilliQ H<sub>2</sub>O.

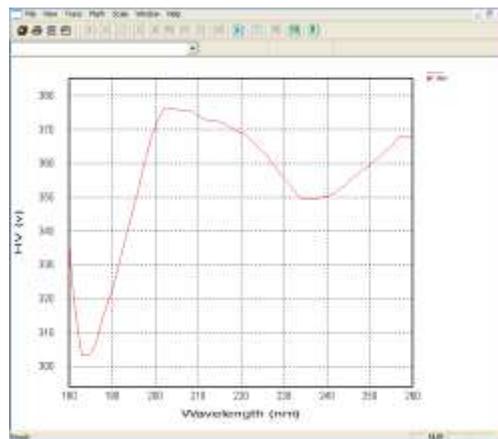


6. Log in to the computer under your lab account.
7. Open the Chirscan ProData software on the desktop.
  - Click on the “View” icon on the toolbar (magnifying glass over a window) to open the Chirscan ProDataViewer. This establishes a connection between the two programs.
  - Within the Viewer, navigate to the folder in which you want to save your data. Right-click on the desired folder and select “set working directory here”. The folder will automatically open, and data will now be saved to this location.
8. Acquire an air baseline immediately before you do something else.
  - Do not wait for the lamp to warm up.
  - Use the following default parameters, regardless of the parameters you will be using for data collection later:

180-260 nm  
0.5 nm step  
0.5 sec/step  
1 scan (no replicates)

*Note: you must click “set” for any parameter changes to be sent to the instrument.*

- Evaluate the quality of the purge by looking at the HV (v) graph. To do this, switch from the CD plot to the HV (v) plot in the viewer by right-clicking on the y-axis label. A drop-down menu will appear. Double-click on “HV (v)” to switch plots. A good purge is indicated by the “tail” of the trace at 180 nm being lower than the peak at 200 nm, as shown below.



- **The voltage at 180 nm ( $HV_{180}$ ) must be lower than the voltage at 200 nm ( $HV_{200}$ )** for you to proceed with data collection. If it is not, it means that  $O_2$  is present and absorbing, and the optics are being damaged. Shut off the lamp immediately.

9. Save the airbase as airbase followed by the date (airbase\_2014\_12\_04).
10. **Record the  $HV_{180}$  and  $HV_{200}$  values in the logbook, and proceed to collecting your data.**

## Acquiring Spectra

*A general overview is presented here; detailed instructions and information can be found in the manual stored on the shelf by the CD. Please read it but DO NOT REMOVE IT.*

*There is limited bench space around the instrument. We suggest you bring a cart for working space.*

1. Let the lamp warm up for 20 min to get the best quality data.
2. Set the wavelength parameters for collection. Edit the wavelength range and wavelength step into the appropriate windows and click “set” to communicate all changed parameter(s) to the spectrometer.
3. Set other parameters: (step, time per step, number of replicates, etc)
4. Acquire a baseline. For a buffer baseline, fill the sample cuvette with your buffer and acquire.

Commonly encountered problems are related to high absorption in the CD spectrum range. Prior to setting up your experiment, you may want to take an absorbance spectrum of both your buffer and a protein sample.

Suggested CD-compatible buffers include phosphate-based buffers and fluoride salts. It is recommended to avoid Tris, most reducing agents, sulfate, and chloride.

Excessive protein absorbance will also reduce data quality. Sample absorbance should stay below 2.0 AU, with ideal signal-to-noise ratios obtained when total sample absorption is between 0.6 and 1.2 AU.

5. Rinse sample cuvette with H<sub>2</sub>O, then 95% EtOH, then dry with house compressed air using the tubing with inline filter on the bench next to the CD. Never use house compressed air without installing a filter!
6. Load your sample into the cuvette and acquire spectrum.
7. When collection is finished, rename the file in the ProData Viewer window.

## Processing and Exporting Data

1. Load baseline and spectrum (or spectra) into ProData Viewer, and average if needed.
2. Subtract baseline.
3. Smooth spectrum (or spectra) if desired.
4. Save the manipulated file. You must save this file – if you close the window without saving, you will lose all of your manipulations.
5. Open the Chirascan ProData Converter using the icon on the desktop.
6. In the Chirascan ProData Viewer, drag and drop the file you want into the ProData Converter.

To save for Microsoft Excel, export as a .csv file.

For export for other graphing programs, export in ASCII (.kin) format.

7. Retrieve your data files with a USB drive. **Do not use a web browser under any circumstances.**

## Shutting Down the Instrument

1. Record and save spectra of the cleaned water filled cuvettes you used to prove you left them clean. (filename: water\_after\_cuvette1.....x).
  - If you have problems with cleaning please see the PDF "cuvette cleaning" or talk to Dr. Erbse.
  - Use the following parameters for the 0.5 and 1 mm path length cuvettes: wavelength range 260 nm(or higher if your signal was at higher wavelengths) to 180 nm, bandwidth 1nm, 1 nm step, 0.5 sec/point.
  - For 10 mm path length cuvettes either measure a dry empty cuvette with the same parameters or measure water with a wavelength range of 260 nm(or higher if your signal was at higher wavelengths) to 200 nm.
2. Check on line if anyone has signed up after you. If the next person starts within 5 hours you should leave the lamp and the purge on.
  - Please send the next person an email that you left the lamp/purge on. In case the samples of that person did not work out they should still come to the CD and switch the lamp and purge off.
3. If the next person starts later than 5 hours after you finished on the same day you can switch of the lamp but leave the purge on. Again let that person know.
4. **Record the end lamp time in the logbook**
5. Close the Chirascan ProData software first, to avoid error messages.
6. Turn off the electronics by flipping the black rocker switch labeled "system".
7. Turn off the lamp by flipping the black rocker switch labeled "lamp" (if nobody is using the instrument in the next 5 hours).
8. Turn off the temperature controller and water bath, if they were used.
9. Turn off the nitrogen flow by closing the nitrogen gas valve on the tank (if nobody is using the instrument after you that day).
10. Shut down the computer.
11. Complete the logbook by recording the fill level of the N2 tank.
12. Return the key.