Stepped Temperature Ramping Experiments with Chirascan Plus

This protocol gives step-by-step instructions for a stepped temperature ramping experiment with the Chirascan-plus CD spectrometer, assuming you have been trained on the instrument by Dr. Erbse. It **does not** replace the **training.** If you need **to schedule training**, please **contact Annette Erbse** (JSCBB, room C316, phone 303-492-0528, email erbse@colorado.edu).

This protocol assumes that you know the startup process for the CD and are able to acquire basic CD spectra (see basic CD protocol). Please follow the startup procedure and record an airbase spectrum for the records as described in the basic CD protocol.

Setting Up the Temperature Probe

1. Switch on the water bath and the temperature controller (under the CD table). Make sure it is filled to 1 inch below the rim. Top it up with 20% Ethanol if necessary. The water bath is the heat sink for the Peltier controlled cuvette holder. It does not control the actual temperature of the cuvette. Leave it at 20 ^{O}C.

2. Choose the temperature probe according to the cuvette you use.

3. 0.5 and 1 mm path length cuvettes need the thin probe (0.25 mm)

4. 4, 5 and 10 mm cuvettes need the thick probe (1 mm)

5. Connect the probe to the T1 socket on the right back corner inside the sample compartment.

6. Carefully remove the sleeve that protects the thin filament.

7. Insert the filament into the cuvette in place of the lid.

8. If you don't have a cuvette in place (during cleaning and loading) carefully place the temperature probe in the sample compartment so that the filament is hanging free and does not get bent.

Configuring a Stepped Temperature Ramping Experiment
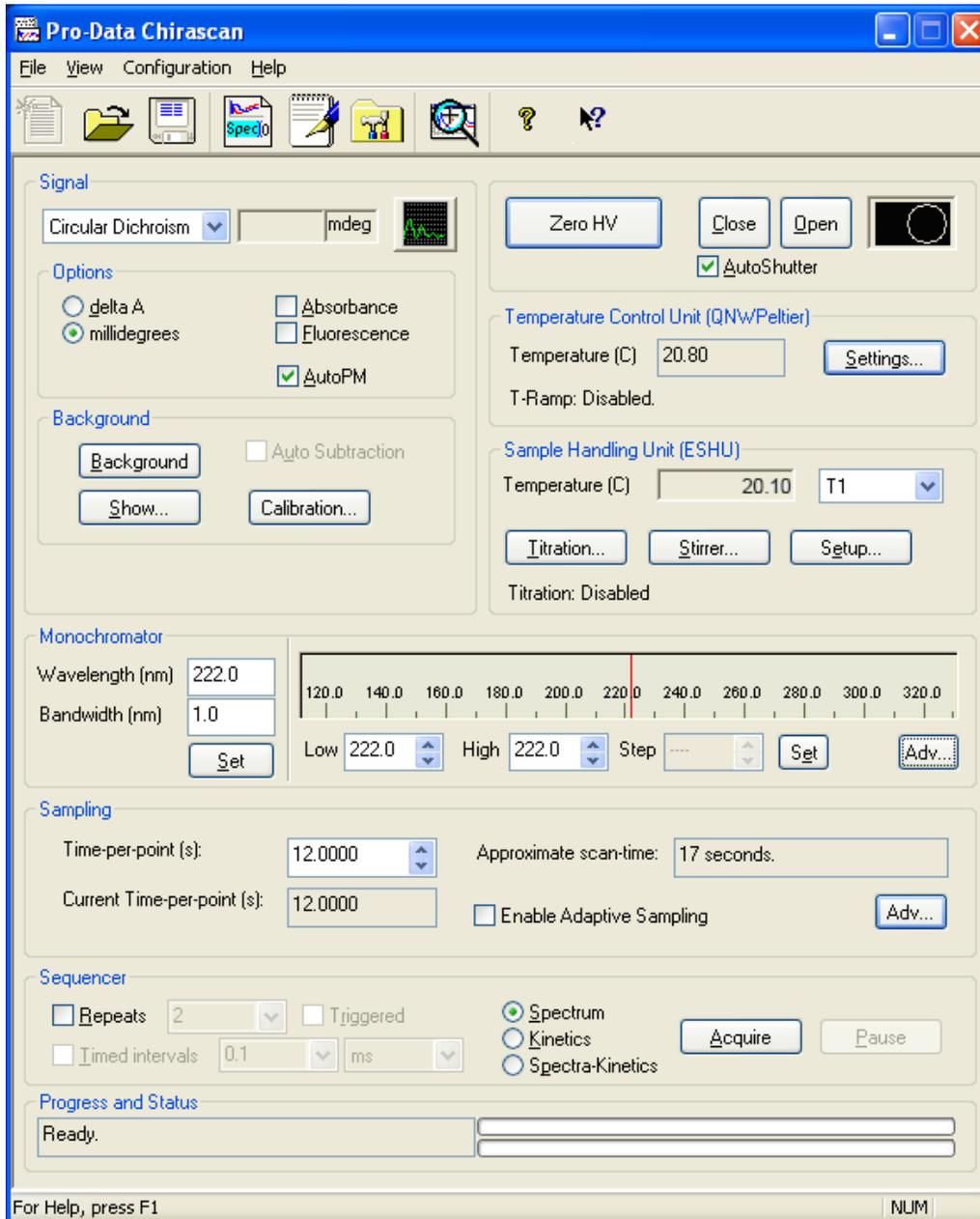
1. The "Temperature control Unit" panel and the "Sample Handling Unit" panel should be activated. If they are grayed out, close the Pro-Data Chirascan program and reopen it. They should now be active.

2. In the "Signal" panel choose Circular Dichroism, click the Auto PM function and choose delta A or millidegrees.

3. In the "Monochromator" panel choose the bandwidth you want to use. (1 nm is a good starting value).

4. Choose your wavelength range and step size (e.g. Low: 180 nm; High: 260 nm, Step: 1 nm).

5. In the "Sampling" panel choose the Time-per-point (e.g. 0.5 sec).

6. In the "Sampling" panel choose the Time-per-point (e.g. 0.5 sec). In the "Sequencer" choose if and how many repeats you want to measure at each temperature.

7. Clicking "settings" in the "Temperature Control Unit" panel of the main window will open the temperature control window.

8. Set the set point to the desired starting temperature.

9. Click "Enable temperature ramping".

10. Type in desired start and end temperature and step size. You do not have to type in a rate or a tolerance.

Temperature Control

Set Point (C)
20.0   Set

☑ Enable temperature ramping

Temperature range

Start (C)	Stop (C)	Step (C)	Rate (C/min)
4	96	0.5	2

☐ Return ramp   Tolerance (C) 0.20

Stepped temperature ramping

☐ Enable stepped ramping   Setting time (s) 30.0

Select appropriate probe for temperature measurements:

◉ SHU (T1..T4)
◯ Temperature Controller
☑ Over-write temperature set-points with measured temperatures

End-of-ramp behaviour

☑ At end of temperature ramp goto:
◯ Start temperature
◯ Stop temperature
◉ Specified temperature: 20.0
☐ Take a final measurement.

OK   Cancel

11.

In the "Stepped temperature ramping" panel choose "Enable stepped ramping" type in the desired setting time. Choose a setting time that will allow the sample to equilibrate at the desired temperature. You should do a dummy run with water to test out how long the setting time has to be for your cuvette. 120 sec are sufficient for the 0.5 mm cuvettes if filled with 150 µl.

12.

Notice that the "Rate" (C/min) and "Tolerance" in the "Enable temperature ramping" are grayed out now and that "Return ramp" is activated. If you choose "Return ramp" the instrument will ramp down the temperature in the same steps as up and collect spectra at each point.

13.

In the "Select appropriate probe for temperature measurements" panel select SHU(T1....T4) and "Over-write temperature set points with measured temperatures" if you use the temperature probe. The program will now re-plot the curve <u>at the end of the run</u> using the measured temperatures of the sample instead of the set temperature of the controller.

14. In the "End-of-ramp behavior" panel you can choose to what temperature the instrument should go after the run is finished.

15. Click OK after you have entered everything. You are now ready to start your run.