

## Continuous Temperature Ramp Experiments with Chirascan Plus

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- This protocol gives step-by-step instructions for recording temperature ramp experiments 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 Dr. Erbse (JSCBB, room C316, phone 303-492-0528, email [erbse@colorado.edu](mailto: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.

### Before You Start Your Temperature Melting Experiment

1. It is a good idea to take a complete spectrum (or as far into the far UV as your buffer allows) of your sample in its native state in the buffer you are going to use for the experiment. This will show if your sample is ok before you start and will allow you to see if enough light hits the detector at the wavelength you are going to use for the ramping experiment.
2. To make sure that you are using a suitable wavelength for the continuous ramping experiment it is a good idea to do a quick step ramping experiment and measure full spectra at several temperatures (e.g. 20, 45, 85 °C ) to check at which wavelengths the change can be monitored best (see stepwise temperature ramping protocol)

### Setting Up the Temperature Probe

1. Switch on the water bath and the temperature controller (under the CD table, upper controller if using the single cuvette Peltier). Make sure the waterbath is full to 1 inch below the rim. Fill it up with 20% Ethanol if necessary.

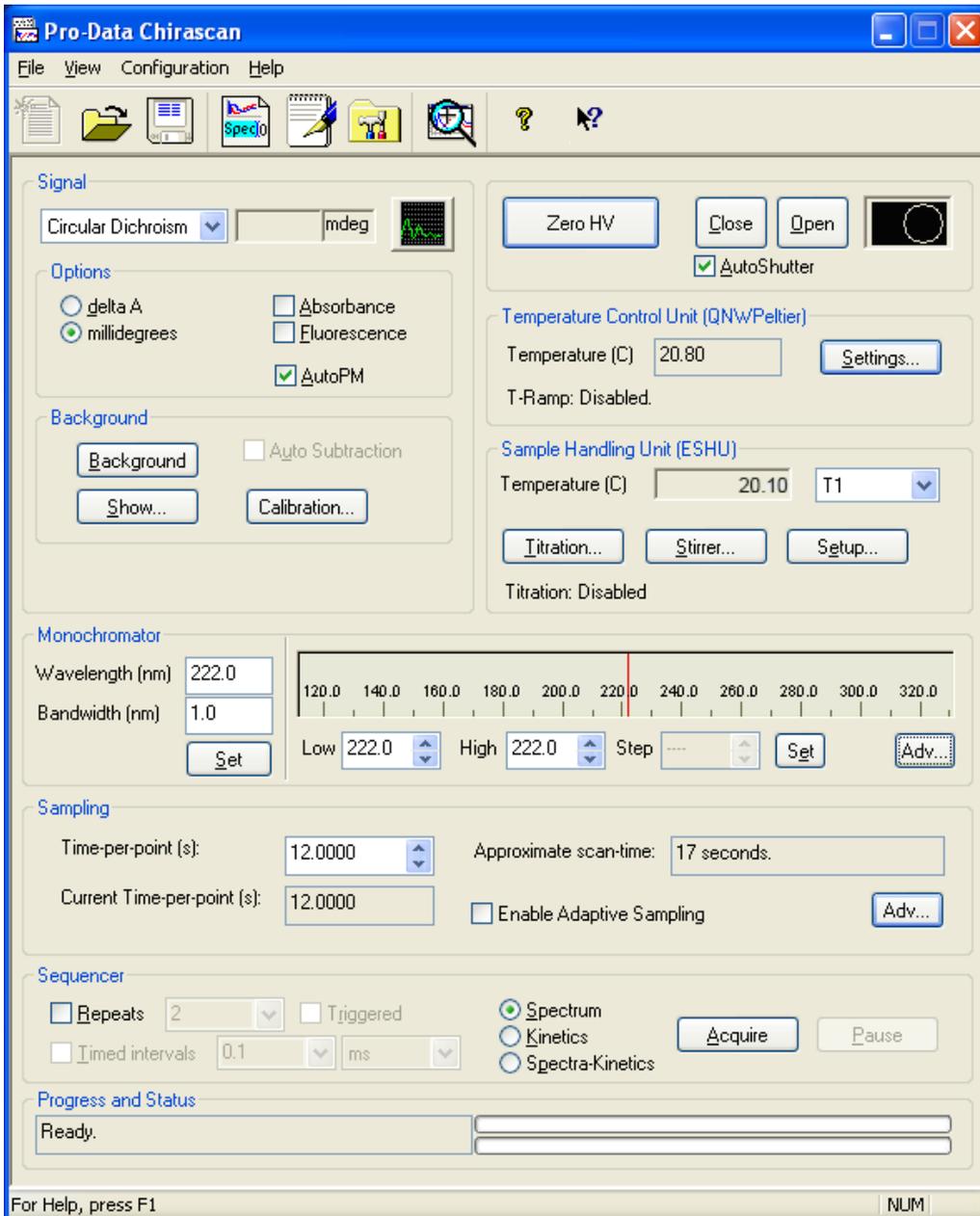
The water bath is the heat sink for the Peltier controlled cuvette holder. It does not control the actual temperature of the cuvette. Leave it at 20 °C.

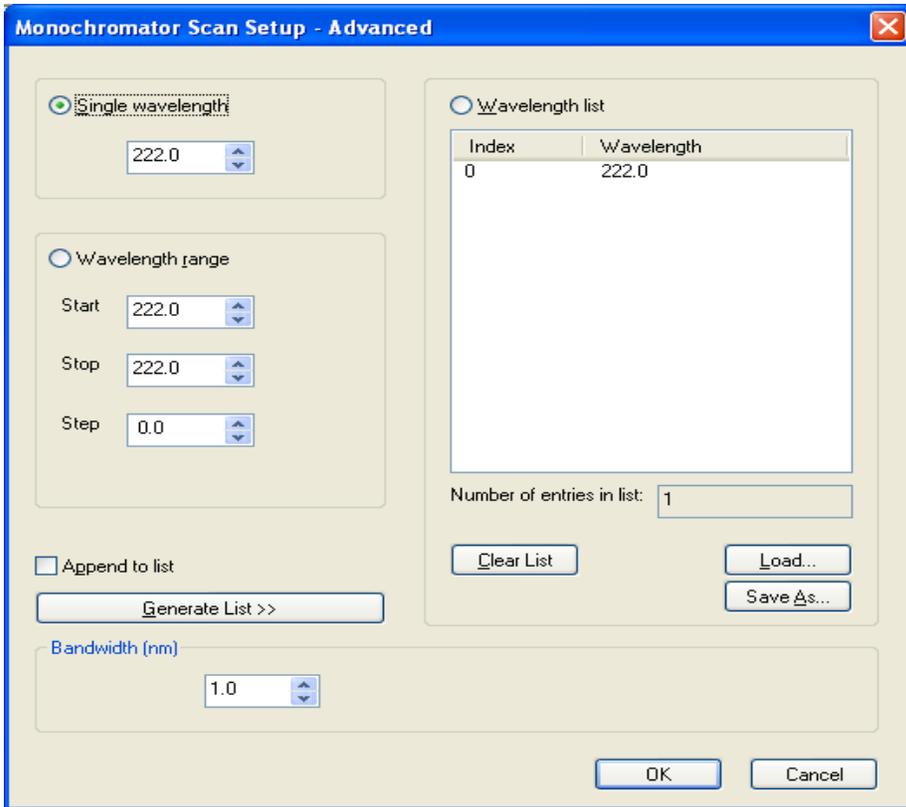
2. Choose the temperature probe according to the cuvette you use.  
0.5 and 1 mm path length cuvettes need the thin probe (0.25 mm)  
4, 5 and 10 mm cuvettes need the thick probe (1 mm)
3. Connect the probe to the T1 socket on the right back corner inside the sample compartment.
4. Carefully remove the sleeve that protects the thin filament
5. Insert the filament into the cuvette in place of the lid.

- 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 Continuous Temperature Ramp at a Single Wavelength

- The "Temperature Control Unit" panel and the "Sample Handling Unit" panel should be activated. If they are grayed out, close the Pro-Data Chirscan program and reopen it. They should now be active.
- In the "Signal" panel chose Circular Dichroism, click the Auto PM function and choose delta A or millidegrees.
- In the "Monochromator" panel chose the bandwidth you want to use. (1 nm is a good starting value).





4. To set your wavelength click "Adv..." in the Monochromator panel. This will open the "Monochromator Scan Set-Up - Advanced" window.

5. Clear the Wavelength list.

6. Activate Single wavelength and type in your wavelength of interest.

7. Check that the Bandwidth is correct.

8. Click OK

9. The window will close and the Monochromator panel of the main window should now look like the one above.

10. Clicking "settings" in the "Temperature Control Unit" panel will open the temperature control window.

- Set the set point to the desired starting temperature.
- Click "Enable temperature ramping".
- Type in desired start and end temperature, step size, Rate (ramping speed per min) and Tolerance.

**Things to consider while choosing these parameters:**

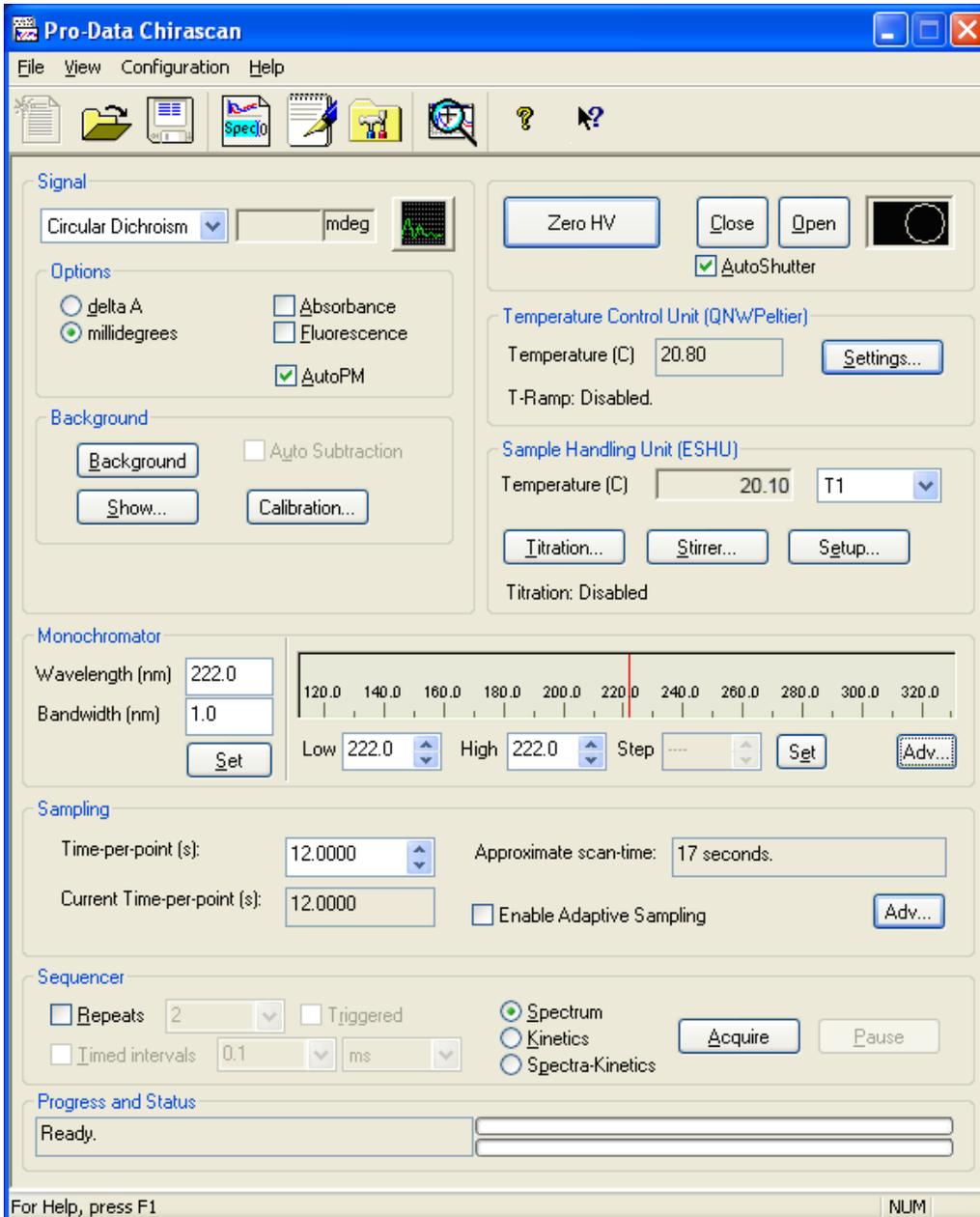
- i. There will always be a lag between the temperature set in the controller and the temperature in the sample. If you scan slower the lag will be somewhat smaller but your measurement will take longer. Try to go 5°C above your desired end temperature if possible. This way, even a 2°C/min scan will reach the desired end temperature.

ii. In an ideal world, you would set the tolerance to exactly half of the step size. For a step size of 0.5°C you would use 0.25°C and therefore start measuring 0.25°C before the actual step and end 0.25°C after the step, during continuous ramping you would collect data during 100% of the time. Unfortunately, that is not a good way to do it. It is recommended to collect data only 80% of the time, since there is some time necessary to write the data and since ramping is not completely linear (it is slowing down before reaching the step in order to not overshoot). Therefore, in our example of 0.5°C steps you should set the tolerance to 0.2°C to only measure during 80% of the time.

iii. The parameters given are a good starting point if you have no prior information about the thermal melting behavior of your sample and if you are using a 0.5 mm path length cuvette with 150 µl sample. Observe the first experiment closely and fine-tune you parameters from there. A slower ramping (1°C/min) and use of a small stir bar is recommended for a 10 mm path length cuvette.

11. 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. Choosing this option, the program will re-plot the curve at the end of the run using the measured temperatures of the sample instead of the set temperature of the controller.

12. Click ok after entering all the parameters



13. The last thing is to enter a suitable Time-per-point in the "Sampling" panel.

14. Time-per-point can be calculated from this formula:

$$= \frac{(2 * \textit{Tolerance})}{\textit{Rate}} * 60\textit{sec}$$

In our example this is:

$$= \frac{(2+0.2)}{2} * 60\textit{sec} = 12\textit{sec}$$

15. You are now ready to run your experiment.