

Thermal Melt Instructions for Chirascan CD Spectropolarimeter

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These instructions are intended as a reminder of the basic settings and parameters for the Chirascan and not as a substitute for training. Please do contact me if you have any questions.

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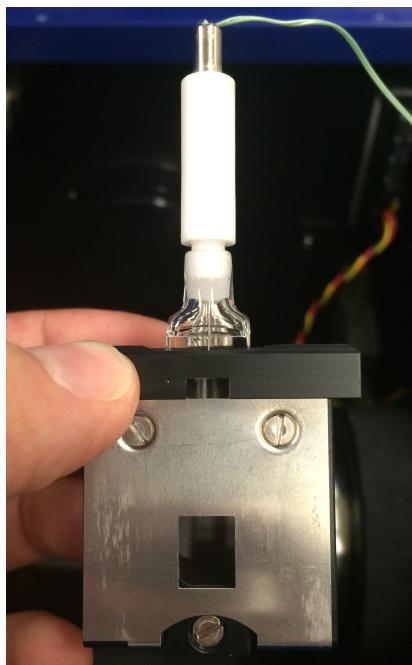
Locate the temperature probe (a) in the sample compartment (attached to the instrument with a green wire). Remove the black cover over the thin metal probe (b), and **gently** clean the metal tip with water, then ethanol.

Locate the 1 mm pathlength cell holder (c), either in the sample compartment or in the drawers beneath the instrument. The side with the polished metal plate and the hole for the cell should face the lamp.

(d)



(e)



To avoid artifacts from sample out-gassing, it is advisable to degas the sample under vacuum with stirring for ~ 5 mins before measurement. You can use the degassing station of the ITCs for this. Alternatively, extensively degas the buffer before diluting your sample.

After degassing your sample, place 200 μ l of your protein solution into a 1 mm pathlength Quartz-Suprasil (QS) cuvette.

Place the temperature probe into the cell (d). The probe also acts as a lid for the cell. Place the cell and temperature probe into the cell holder (e). Make a note of the orientation of the label on the cell (for best reproducibility, use the same orientation for replicate measurements).

The cell should be sufficiently full that the so that the temperature probe sits in the solution and the meniscus of the solution is not visible through the mask of the cell holder.

(f)



(g)



Place the cell holder, cell and probe into the hole in the Peltier jacket (f) in the sample compartment.

The side of the cell holder with the polished metal plate and the hole for the cell should face the lamp (g). There is no need to screw the cell holder into place.

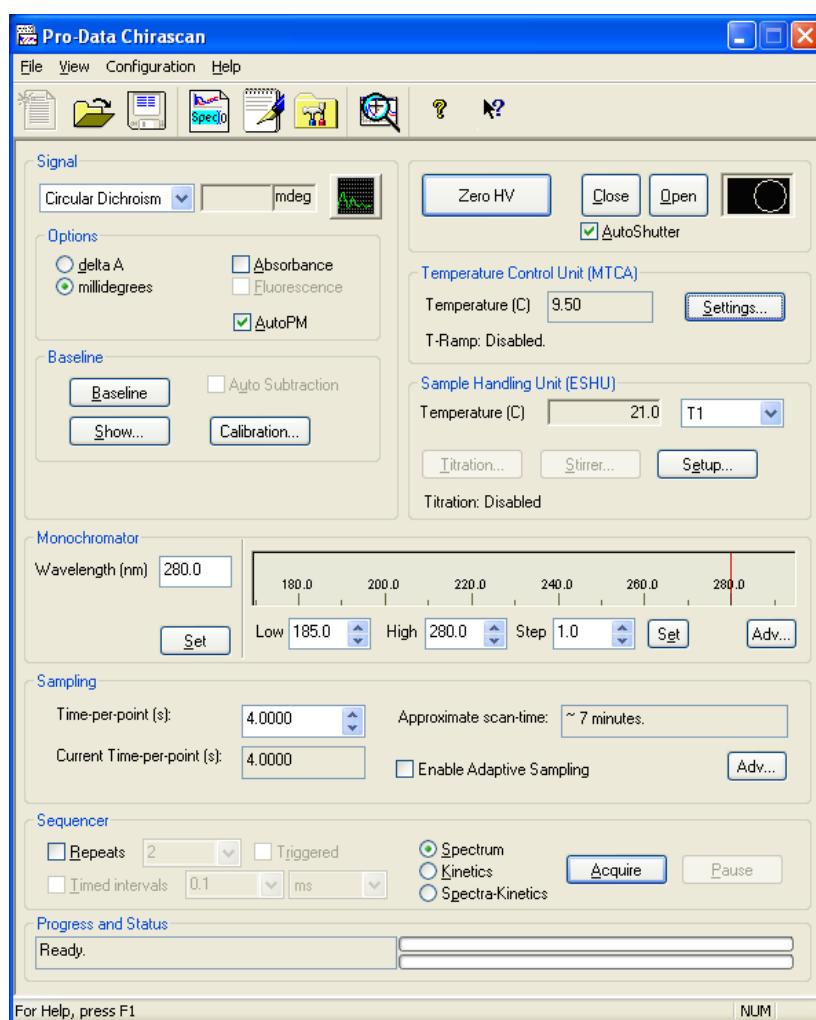
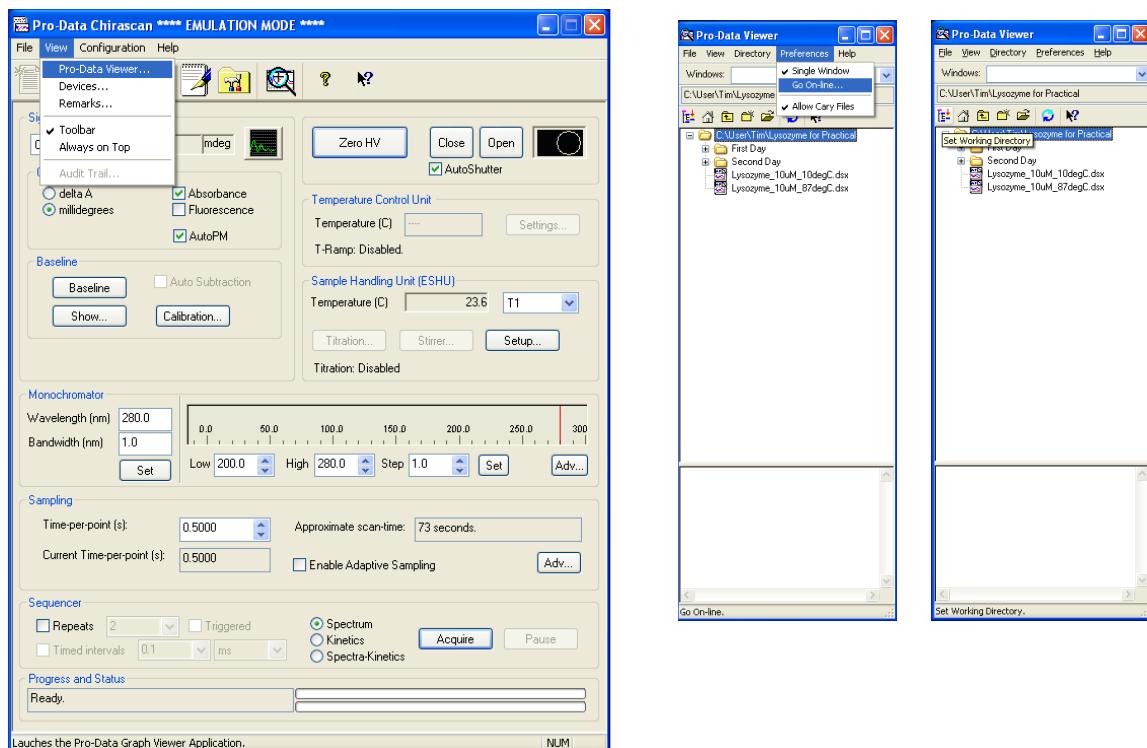
You can now shut the sample compartment door and begin to set up your experiment.



The peltier controller is the device shown above. There is no need to change settings directly on this device, all of the settings are controlled via the software. The red light on the front panel will flash when the peltier is active (i.e. it has been instructed to control the temperature by the software).

The water bath (shown at left) supplies water to the peltier heat-exchanger. It should be left set at a constant temperature of 20 degC. The actual temperature of the water bath will vary +/- 2 degC. There is no need to change settings on the water bath.

Open the Chirascan Software, then select View/Pro-Data Viewer from the menus. This will start the viewer software. Select “Go On-line” from the Instruments menu in the viewer software so that data is shown during acquisition. Choose your directory in the viewer software and click the “set working directory” button.



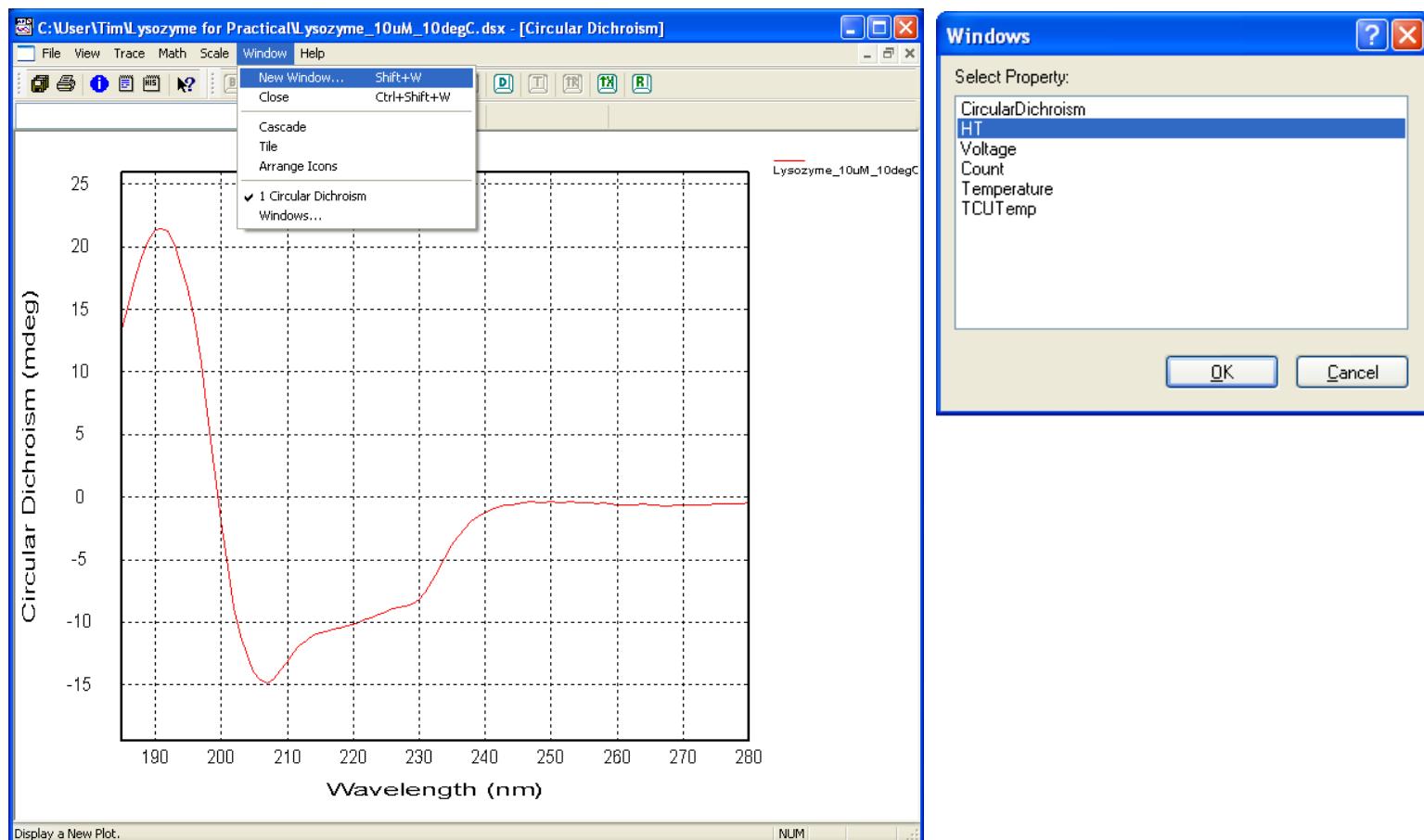
The main panel is shown here configured to acquire a spectrum. The controls should mostly be self-explanatory.

Use autoshutter for spectra acquisition, but for thermal melts when data acquisition is near-continuous over a long time period, de-select autoshutter and manually open the shutter (“Open” button) before the melt starts.

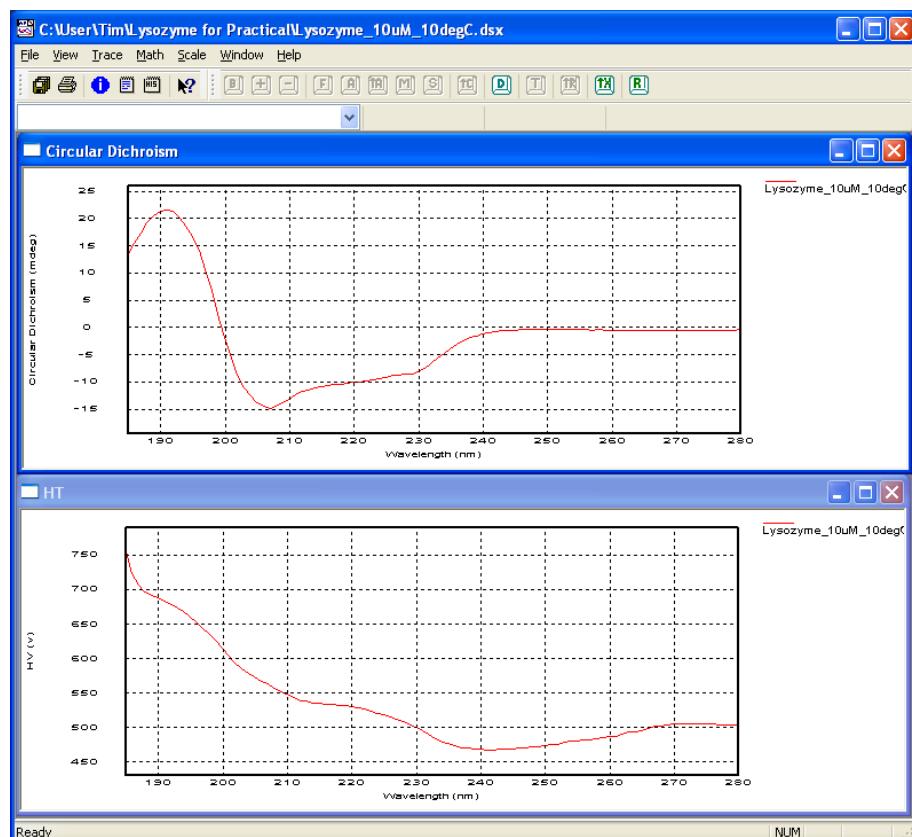
The temperature of the sample is set by pressing the “Settings” button in the Temperature Control Unit Section. The temperature measured by the probe is shown in the Sample Handling Unit Section.

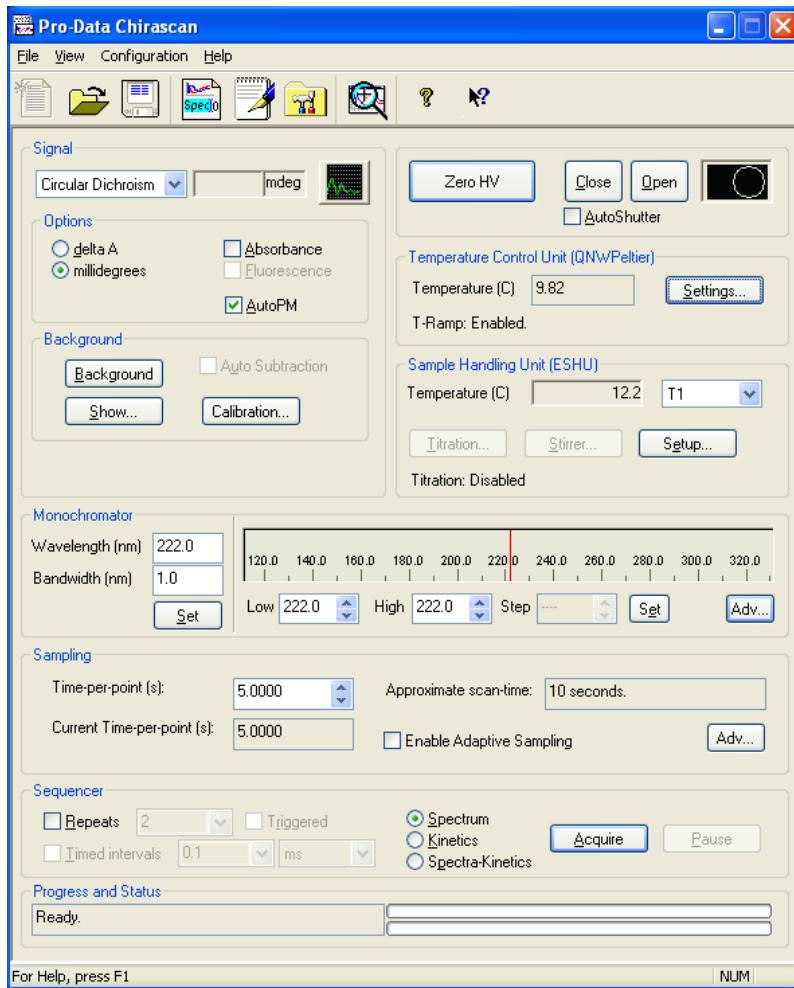
The Monochromator Section settings control the wavelength range for acquisition. When making changes to the values, be sure to press “Set” afterwards to retain the changes. More complicated wavelength selections are possible by adjusting the settings accessed by the “Adv.” button.

During or after data collection, select “New Window” in the viewer software, the HT from the list of properties. This will display (simulated) HT voltage which is a measure of the detector gain setting, and is related to the photometric absorbance of your sample at a given wavelength. If the HT voltage exceeds 950 V, very little light is transmitted by your sample and so the CD measurement will not be accurate and should not be trusted.



The image below shows a CD spectrum (10 μ M HEWL, 100 mM phosphate, pH 2.5) with the corresponding HT voltage data. All of this data is saved in the spectrum file (.dsx) and can be accessed at a later date. If you process the data in the viewer software, be sure to save all data, rather than just saving the selected spectrum. The latter will lose all of the associated data.





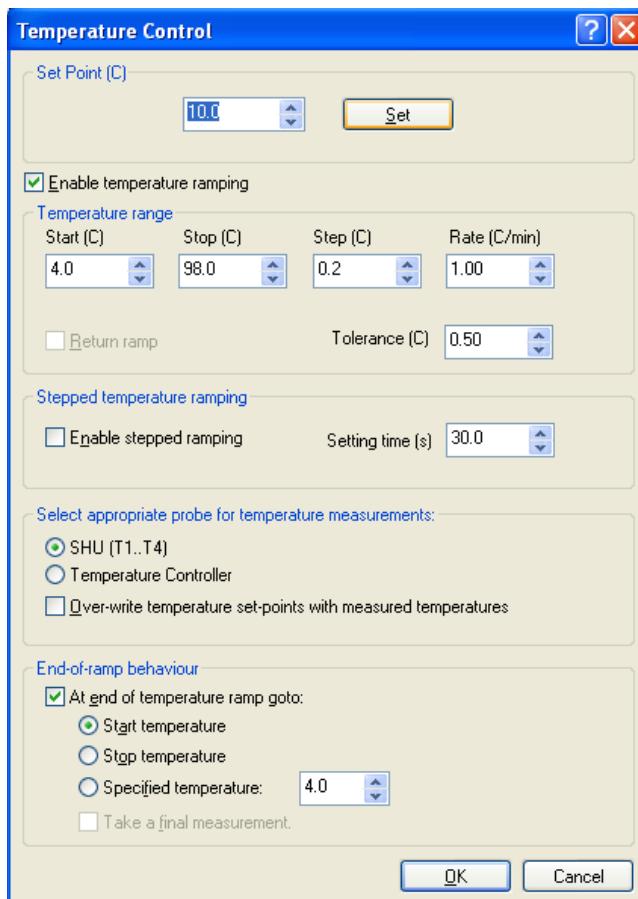
The main panel is shown here configured to acquire a thermal melt.

De-select autoshutter and manually open the shutter (“Open” button) before the melt starts.

The Temperature value reported in the Temperature Control Unit section is the temperature of the probe inside the cuvette. The current temperature and thermal melt parameters are accessed and set by the “Settings” button in this section (see the next section on this page).

You can acquire data at a single wavelength (shown here, 222 nm), or at multiple wavelengths (shown on the next page).

Make sure that the total approximate scan time (shown in the Sampling section) is short enough to be measured in the time available at a given temperature point. i.e. if you are scanning the temperature at 1 degC per minute, and taking measurements at 0.2 degC intervals, you will have a maximum of 12 seconds per interval in the temperature axis. Therefore your measurements will have to take less than 12 seconds. Adjust the number of wavelengths and the time-per-point to fit your required temperature interval. The settings shown work well and facilitate data fitting. One can always get better signal-to-noise by smoothing data in the temperature axis after collection.



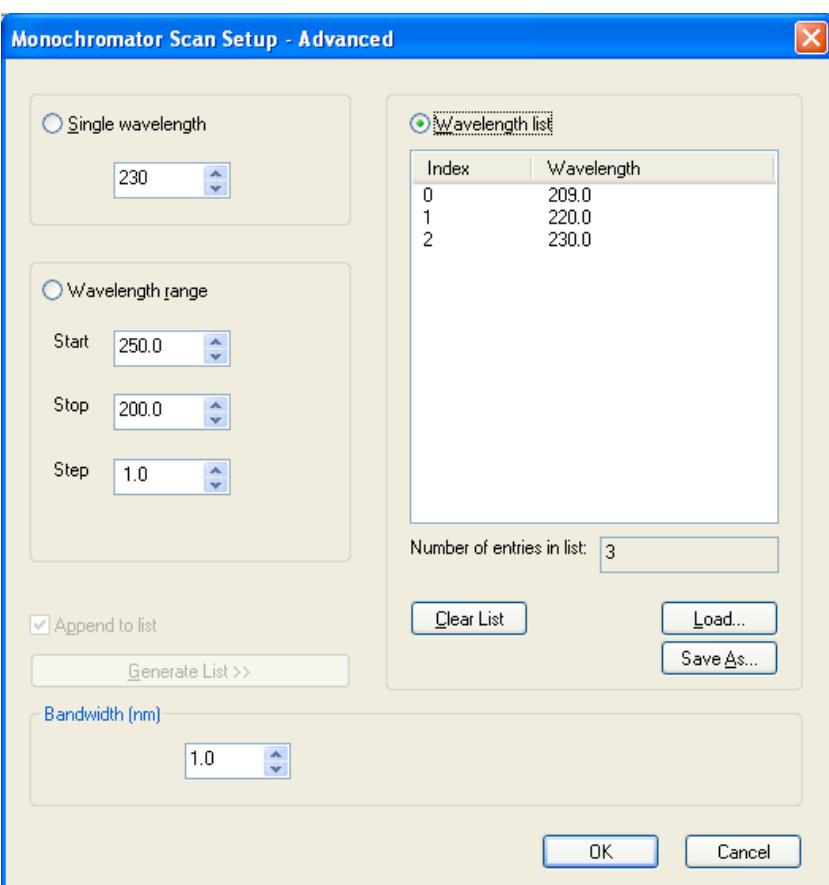
The Set Point is the current Peltier set temperature. To change it so that your sample equilibrates at the start temperature for your denaturation experiment, enter the required temperature and press “set” (n.b. for the experiment settings shown opposite, it should have been changed to 4 degC to equilibrate the sample).

The settings shown opposite produce a continuous temperature ramp where the measured sample temperature increases linearly from 4 degC to 92 degC.

The actual sample temperature in the cuvette is measured by the SHU (Sample Handling Unit) temperature probe. To ensure that this is recorded, you should select the SHU probe for temperature measurements.

The measured sample temperature lags behind the set temperature at high temperature. When analysing thermal denaturation data, the data should always be re-plotted with the SHU probe temperature on the x-axis for maximum temperature accuracy. This is covered on page 6 of this guide. The lag between measured and set temperature can be reduced, and a higher final temperature obtained, by using a slower ramp-rate (e.g. 0.5 C/min).

Stepped ramping is more thermodynamically accurate for proteins where the native-denatured equilibrium is attained slowly, as a longer equilibration time can be set at each temperature. It is also the better option to obtain complete spectra at a range of temperatures.

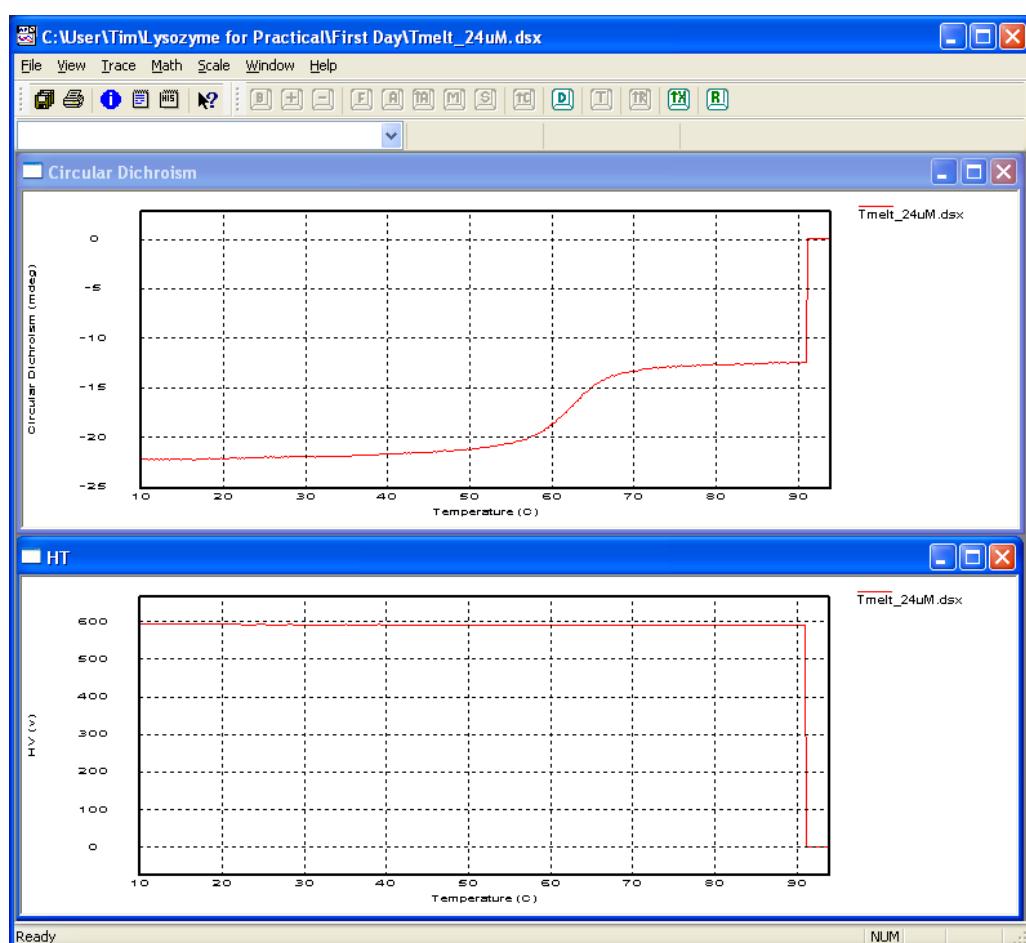


The advanced Monochromator Scan Setup window (accessible from the Adv. button in the Monochromator Section) allows different options for data collection.

You can set a single wavelength, a wavelength range for a spectrum, or a list of single wavelengths at which measurements will be made. The last of these can be useful for monitoring thermal denaturation of large and in-particular multi-domain proteins where thermal denaturation of different domains may be asynchronous, which in turn may be reflected by differences in the melting curves for different secondary structure elements.

First clear the current list using the “Clear List” button. Then choose a single wavelength in the box below the “Single Wavelength” button. Then tick “Append to list” and click “Generate List”. Enter another wavelength and click “Generate List” again, and so on.

You should see the list of wavelengths looks something like the example opposite. Tick “Wavelength List” to measure at those wavelengths. Be sure to adjust the time-per-point in the Sampling Section of the main panel to account for the greater time needed to measure at multiple wavelengths.



Here is an example of thermal denaturation data during collection, with windows showing the CD signal and the HT voltage signal. Note that during collection, points on the temperature axis where data has not yet been collected are assigned a zero value by the software, hence the odd-looking step function in the data at high-temperature.

If you find that your CD signal is suddenly very noisy in the region of the thermal denaturation transition, or just afterwards, and is accompanied by a peak in the HT voltage (sometimes followed by a loss of CD signal), this is indicative of aggregation of your sample. The aggregate material blocks the light-path, leading to the high HT signal and noisy CD signal. Then the material can settle to the bottom of the cuvette, resulting in a loss of CD signal.



To convert files to .csv for use in other graphing software, open the APL Pro-Data Converter application. Drag and drop files from the Windows Explorer (file manager) onto the window shown and a converted file will appear in the same directory as the original file.

Circular Dichroism

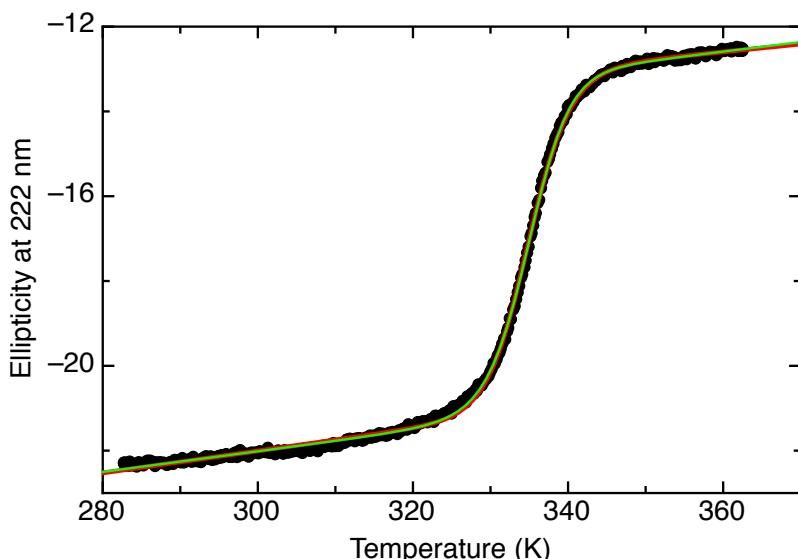
Temperature	Wavelength
222	
10	-22.2883
10.2	-22.3124

Temperature	Wavelength
222	
10	9.56339
10.2	9.61395

Import the data into Excel, or a graphing program. Near the top of the file you will find the heading "Circular Dichroism". This section tabulates the temperature controller set-point (**not the sample temperature**) in the first column and the CD signal (ellipticity) in the second column (more columns for more wavelengths).

Further down the file, near the bottom, you can find the heading "Temperature" which tabulates the temperature controller set-point (first column) and the measured sample-probe temperature (second column). For correct temperature calibration, you should plot the sample-probe temperature as the x-axis and your CD data as the y-axis.

Here is the plotted and fitted thermal denaturation for HEWL in 100 mM phosphate, pH 2.5. The temperature axis has been converted to Kelvin so that the data can readily be fitted to a two-state denaturation model. The thermodynamic parameters derived from this kind of data compare well with those derived from differential scanning calorimetry, in the absence of oligomerisation and aggregation.



Fitted to Two-State Model with $dCp=0$

Iterations: 11

Chi squared = 2.7954

Parameters:

T	=	x	Standard deviations:
δH	=	83.5465	$\delta \delta H$ = 0.7885
T_m	=	334.9233	δT_m = 3.3419e-2
$N_{intercep}$	=	-29.7925	$\delta N_{intercep}$ = 0.1391
N_{slope}	=	2.5953e-2	δN_{slope} = 4.5833e-4
$D_{intercep}$	=	-19.2106	$\delta D_{intercep}$ = 0.5441
D_{slope}	=	1.8378e-2	δD_{slope} = 1.5339e-3