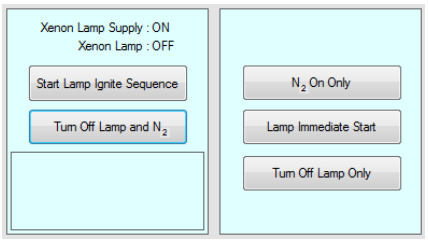
Chirascan plus qCD Start-up/Shut down Protocol Simplified

STOP This protocol is for qualified users operating Chirascan plus qCD in IBMS N133 only. Dr. Jao accepts no responsibility for actions taken as a result of using this protocol. Reading the manufacturer's handbooks is highly recommended.

Start-up procedure:

1. **Turn on the nitrogen supply on the liquid nitrogen tank**. The pressure regulator on the wall shows 4 bars and it should remain at 4 bars all the time. **Do not adjust it.**

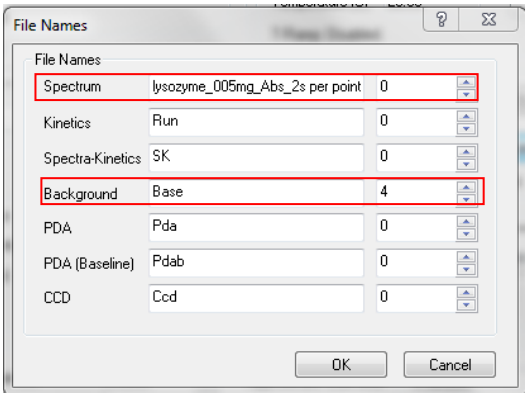
2. The gas safety shut-off valve locates at the right-hand side of bench and turn it to the "on" position (12 o'clock position). It is on when the lever is in line with the pipe.
3. **Turn on the chiller (CW-3000)** on the floor, **the temperature control unit** on the shelf, **the system power of spectrometer** located on the right hand side of the front panel and finally **the computer and monitor.**
4. Log on to Windows using **Chirascan User**.
5. Start Pro-Data Chirascan by double-click the **Chirascan** icon on the desktop.
6. Start the **Active Nitrogen Monitoring System** (ANMS) by clicking ANMS icon.



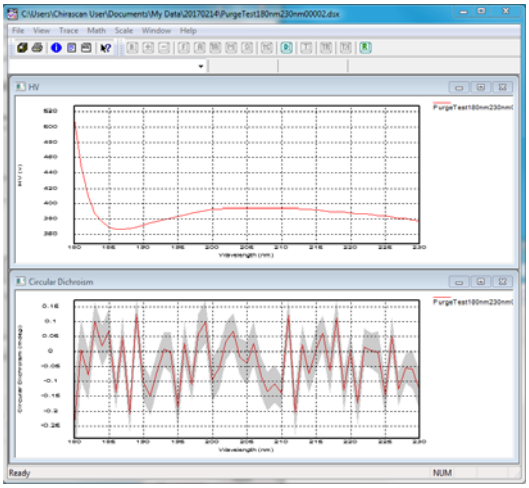
7. The instrument needs to be purged with nitrogen prior to ignite the lamp:
   ※ If the instrument has not been used for less than 7 days, click **N2 On Only** button firsts to purge nitrogen for **4 hours**. Check regulator on the wall. After 4 hours, click **Turn Off Lamp and N2** button then start the lamp by clicking **Start Lamp Ignite Sequence**. The program will not start lamp ignition process without turning off the N2 first.
   ※ If the instrument is constantly used within 1 days, **Start Lamp Ignite Sequence** is sufficient. It takes 20 minutes to turn on the lamp.
   ※ If the instrument has not been used for more than one week, an overnight purge is necessary.
8. Jot down the lamp hour on the log book when the lamp is on.
9. Start the Pro-Data Viewer in the **View** pulldown of Chirascan program. Create a folder, right click the folder and click "**Set Working Directory Here**".

10. Set temperature to 20 ℃.

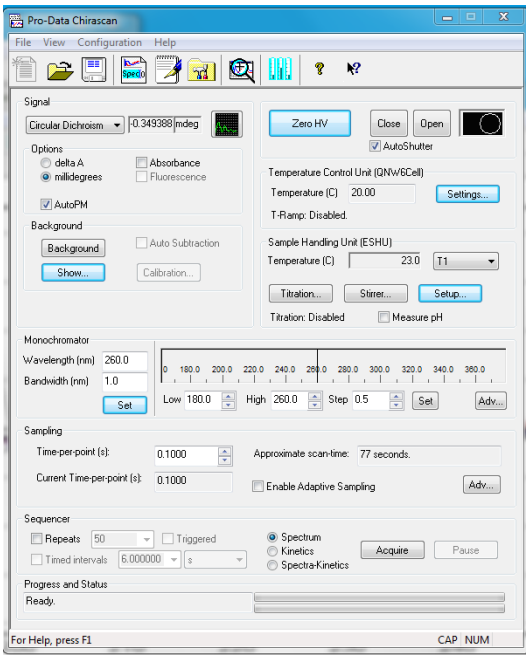
11. To start purge test, find *StartUp* directory in *My Data* folder and drag the file, **1_Purge Test HV180 to HV210.dsx**, to the Pro-Data Chirascan window to copy the experimental parameters. After changing the filename by clicking SpecID icon , click **Acquire** to start experiment. Once the acquisition is done, in the Data Display window, choose **Window: New Window…** and select HV result. Calculate the ratio of the HV reading at the 180nm to that at 210nm. This number should not exceed 1.5. Bigger number than 1.5 indicates too much oxygen. Further purge N2 is needed.



12. To start background acquisition and wavelength calibration, drag and drop the file, **2_background Air Check 468nm.dsx**, to the Pro-Data Chirascan window and click **Background**. Open the HV window and check the Troughs at 468-469nm.



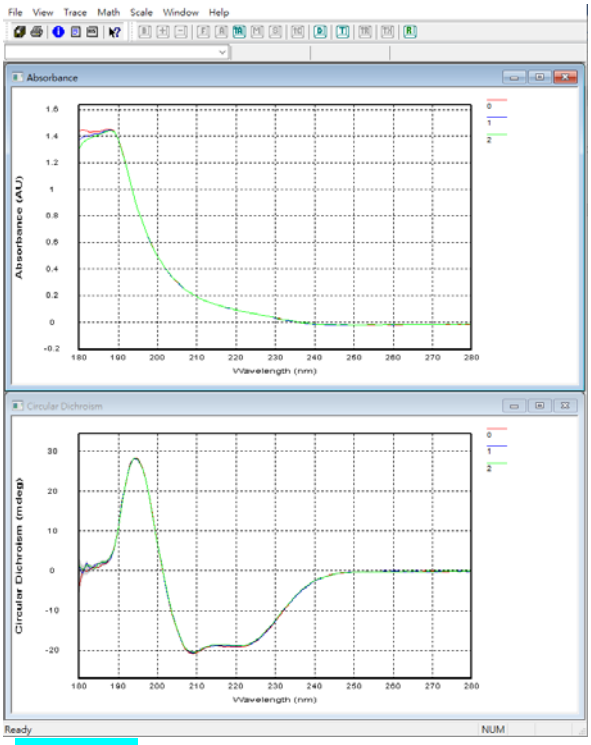
13. To start a CSA test, drag and drop the file, **3_CSA CD peak height ratio.dsx**, to the Pro-Data Chirascan window, insert CSA standard sample (in drawer) at cell 1 position and click **Acquire** to start the experiment. Check the CD intensity at 192.5 and 290nm. The ratio should be around 2.
14. The instrument is now ready.
15. Please refer to Chirascan User's Manual for experimental setup of solvent baseline, sample and absorbance measurements.



Sample measurement Setup (Far-UV CD):

16. Find the Startup folder and drag the file **4_Air Background_FarUV.dsx** to Chirascan window. Changing the filename in background block and click **Background** to obtain background signal of empty cell.

17. Loading the 1-mm-pathlength cuvette with 200µL buffer. Drag the file **5_Buffer CD with Abs_FarUV.dsx** to Chirascan window and change file name in spectrum block. Insert the cuvette at cell 1 position and click **Acquire** to start the experiment.



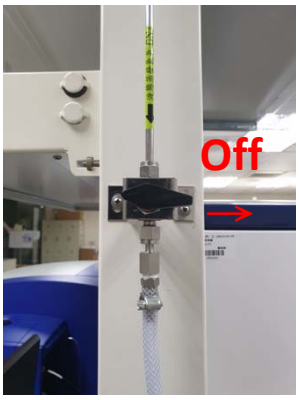
18. Discard buffer and clean cuvette thoroughly using ddH2O. Air-blow dry the cuvette and load 200µL protein sample. Insert the cuvette at the same cell position for buffer and drag the file **6_Sample CD with Abs_FarUV.dsx** to Chirascan window. Make sure to change file name in spectrum block. click **Acquire** to start the experiment.
19. You can display absorbance data by choosing **Window: New Window…** and select Absorbance result.

Steps for cleaning cuvette (example for 1 mm cuvette):

20. Remove sample in the cuvette by pipetting.
21. Add 300µL solvent into 1 mm cuvette, dissolve any remaining sample.
22. Discard the solution and add 300µL 1% Hellmanex II into cuvette and let stand for 10 minutes.
23. Rinse with sufficient deionized water to flush out residual of detergent.
24. Dry and remove dust with filtered dry compressed air. Once the cuvette is dried keep it closed to prevent dust.
25. Check the exterior surface of the cuvette windows, and if there is any smudges or fingerprints, clean softly with the Lens cleaning paper.

Shut down procedure:

26. Jot down the lamp hour on the log book.
27. In the ANMS window, click on Turn Off Lamp and N2 to turn off lamp and N2 supply.
28. Close ANMS and Chirascan program.
29. Turn off the spectrometer power located on the right-hand side of the front panel.
30. Turn off temperature control unit.
31. Return the gas safety shut-off valve at the right-hand side of bench and turn it to the "off" position It is off when the lever is at a right angle to the pipe.
32. Turn off chiller under the bench.
33. Turn off the air supply on the N2 tank.
34. Turn off PC.
35. File the log book.



Data Process:

36. Please refer to Chirascan Series User Manual for CD spectra process and CDNN program for protein secondary structure analysis.