Sample Submission Guideline for

AppliedPhotophysics ChiraScan-plus qCD Spectrometer

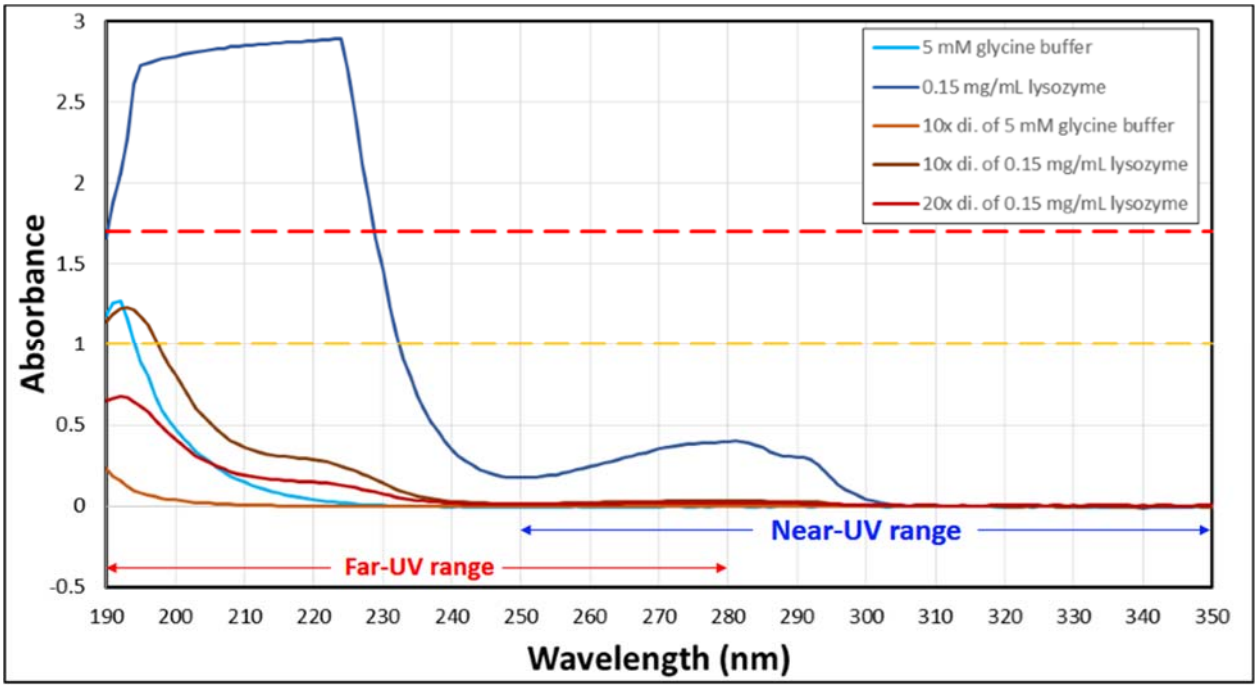
I. Sample Submission

1. Apply an account on the Biophysics Core Facility (BCF) reservation system.
2. File sample submission form on-line under the instrument, "ChiraScan-plus qCD". Please note that there is a set-up fee associated with your first sample. Up to 5 samples in the same buffer as your first sample apply discount prices. On the other hand, a sample in different buffer compositions require optimization and buffer scans. Therefore, it is considered "first sample".
3. After confirming the charges and experimental time with Miss Yu, Jin-Hsuan, samples and buffers may be submitted to IBC 402. Tel: 27855696 x4024; e-mail: bcf@gate.sinica.edu.tw
4. BCF may not compensate for your sample loss or data loss under any circumstances (hardware or software failure, operator error, or others). Without written permission from Academia Sinica, the user shall not claim, announce, or mislead the public into interpreting that the results of this testing is in any way related to the commercial development of the user. In addition, the user shall not in any form (including but not restricted to commercial marketing, for example advertisements, either online or offline, product packaging, catalogs, investment information etc,) use the title, logo, name, trademark or symbols that are that of Academia Sinica or similar to that of the facility, that gives the false impression of a commercial collaboration.
5. All experimental results are for research only.

II. Sample preparation

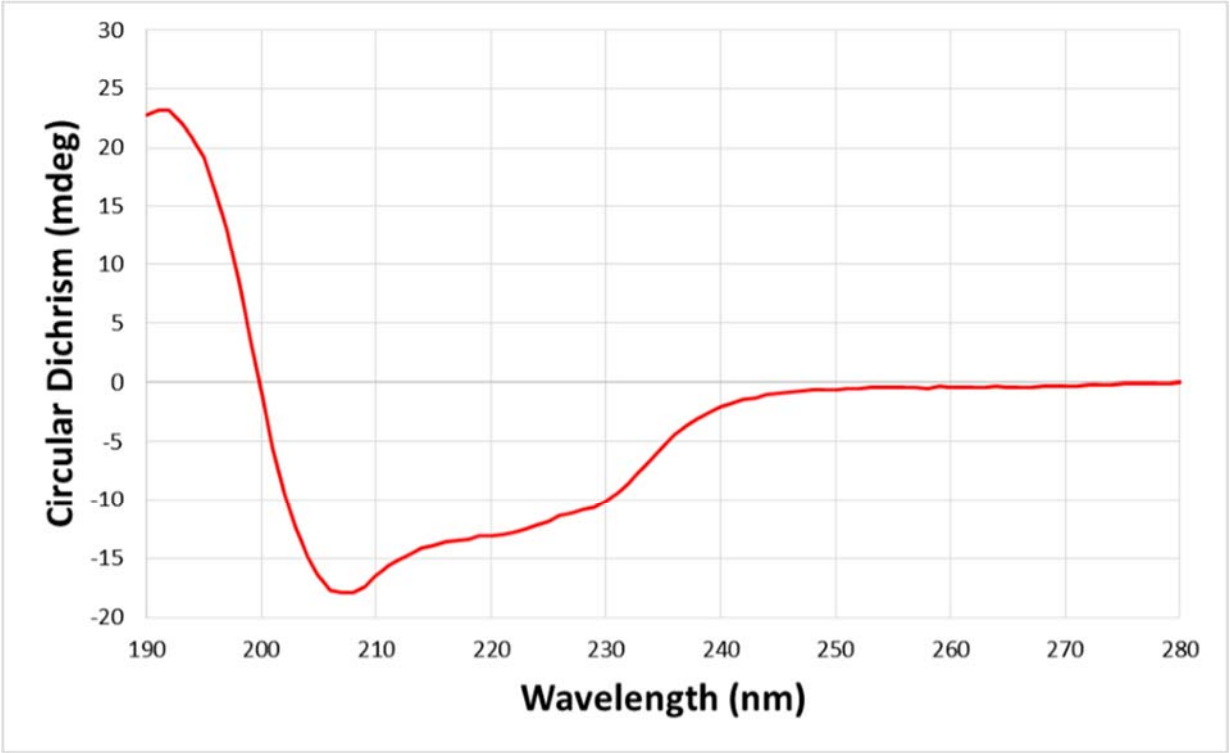
1. Sample volume is **200-300 µl** depending on the concentrations and the pathlength of CD cells. In addition, please provide (**5.0** + **1.0 x number of samples**) ml buffer for measuring baselines and cleaning purpose.
2. For far-UV (260nm to 190nm) protein measurements, we need about **0.2-0.5 mg/ml** when a cell pathlength of 1 mm is used. If a 0.1mm pathlength is requested for high buffer background subtraction, 2-5mg/ml is required. The optimal sample concentration depends on the absorbance of your sample and the buffer. For example, if you want your CD spectrum acquired to 190nm, the absorbance of your sample (+ buffer) at 190 nm, using water as the blank, should be kept below **1.7** (**better about 0.9**) and buffer absorbance below **1.0** (**the lower the better!**) at the chosen pathlength. BCF will adjust the wavelength range according to instrument HV (HV < 800 Volts) if necessary.

   The figure below shows UV/VIS spectra of 0.15mg/mL lysozyme sample in 5mM glycine buffer, measured in a cuvette of 1cm pathlength. Because 20-fold dilution of this sample has an absorbance of 0.67 at peak position, 192nm, it is expected to reach 1.34 OD when a cuvette of 0.1cm pathlength is used. Therefore, it is safe to say that we will get a reasonable CD spectrum scanned down to 190nm with 0.1cm pathlength. For accurate UV/Vis measurements, please check the dynamic range of your UV/Vis spectrometer. For example, when we compare the spectra of 10-fold and 20-fold dilution samples measured on our spectrometer, we notice the absorbance at 190nm is not in the linear range!



Chris Jao 2022/01/01

The figure below shows a CD spectrum of 0.15mg/mL lysozyme in 5mM glycine buffer, measured in a cuvette of 0.1cm pathlength.



3. For near-UV (320nm to 250nm) measurements, please prepare **500 µl** of **1-2 mg/ml** protein to fill a cell of 10 mm pathlength for optimal CD signal.
4. Please spin your samples at 12,000 g in a 0.22uM Ultrafree-MC filter for 5 minutes to remove possible precipitations.
5. Samples submitted to BCF should be non-hazardous, non-toxic and nonpathogenic. No radioactive or microbial samples are allowed.
6. For more detail description of CD sample preparation, such as buffer selection, please refer to review article, **How to study proteins by circular dichroism**, Biochim Biophys Acta. 2005 Aug 10;1751(2):119-39.
7. Please note that BCF will collect extra charges if your sample precipitates during an experiment.

III. During Experiments

1. Follow the start-up and shutdown protocols for start-up tests.
2. Measure CD spectrum and determine wavelength range using your first sample.
3. Perform background measurements using air (without cuvette).
4. Measure CD and absorbance spectra of your samples and buffers.
5. Average triplicate measurements and subtract buffer signals.
6. Convert data to ascii or Excel spreadsheet formats.

IV. After Experiments

1. Because the short pathlength of 1mm cuvette, we will not recover your sample.
2. Raw data will be sent via BCF reservation system.
3. Please note that data analysis is the responsibility of users. BCF currently only provides buffer-subtracted CD data and its absorbance at 220nm (if available).

V. Softwares for protein CD data analysis

1. **CDNN** for protein secondary structure prediction.
2. **qBIC** and **Global 3 for** spectra comparison and global analysis.
3. **DichroWeb** http://dichroweb.cryst.bbk.ac.uk/html/process.shtml

VI. Acknowledgement

Please acknowledge us if research supported and/or data generated by this instrument results in publications. For example, “We acknowledge CD data collected by [operator] in the Biophysics Core Facility funded by Academia Sinica Core Facility and Innovative Instrument Project (AS-CFII-111-201).”