Cross Validation and Drug Binding Studies by Synchrotron Radiation Circular Dichroism Spectroscopy (SRCD)

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The principal aim of this study was to undertake cross-validation studies on the new SRCD beamline at the NSRRC for comparison with data we have obtained at SRCD beamlines around the world (Daresbury SRS, Diamond, ISA, NSLS, BESSY2, BSRF). To minimize systematic errors between measurements, we have used the same sample, the same specially made sample cells, and the same data collection procedures we have used at other sites. These studies complement but do not replace the validation studies done by the beamline scientists at the NSRRC.

The first test sample was camphour sulphonic acid, collected at 20° C (temperature is an important parameter for these measurements). We tested both the magnitude of the 290 peak for a sample of known absorbance and the ratio of the 192.5/290 peaks. Often the first of these is not tested, but it is an important parameter. The magnitude measured was 25.3 versus an expected value of 25.1, a very good correspondence, and the ratio was 2.0, which is exactly as expected (and the same as measured on 4 out of 5 SRCD beamlines we tested).

We next checked the wavelength calibration by observing the low wavelength nitrogen absorption peaks (using a narrow bandwidth) and found them to be within 0.2 nm of the expected values (within specifications).

Then we measured the spectra of several proteins which are present in the SP175 reference data set and which we have measured on several beamlines. The myoglobin and lysozyme matched both the canonical spectra in the database and those obtained at ISA, the SRS, and the BSRF. We also measured the beta sheet rich protein subtilisn, which is a "weak signal" standard, and even when the magnitude was very small (~2 mdeg), the spectra were clearly detectable and found to be within acceptable levels of an "ideal spectrum" for this sample.

We then examined the radiation heating parameter of the beamline as this can be a significant problem at high flux density beamlines (such as the SRS and Diamond). No detectable degradation of the sample was seen over the 20 scans measured, an excellent result.

We tested the maximum HT bias that could be used for spectral measurments, by using different combinations of concentration and pathlength and observing the suppression of the 190 nm myoglobin peak. We concluded a conservative maximal value to use would be 600 mv. Using this value, we then tested the low wavelength limit that could be achieved for myoglobin in D_2O in our specially-designed calcium fluoride cells, and estimated this to be ~165 nm, comparable to the limits achievable on the best beamlines elsewhere, and superior to the limits achievable at a number of existing beamlines. In films on calcium fluoride plates (where there was little or no water present) the limit was 144 nm.

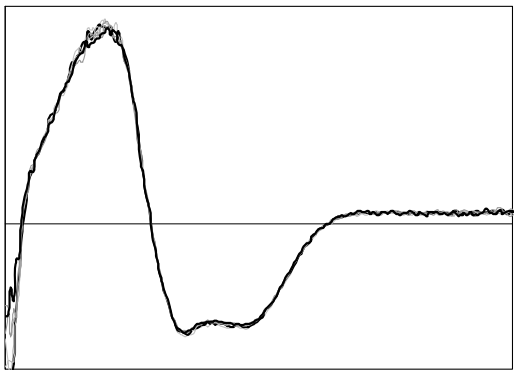

Fig. 1: 20 repeated scans showing no radiation-induced degradtation at the Seya SRCD Beamline

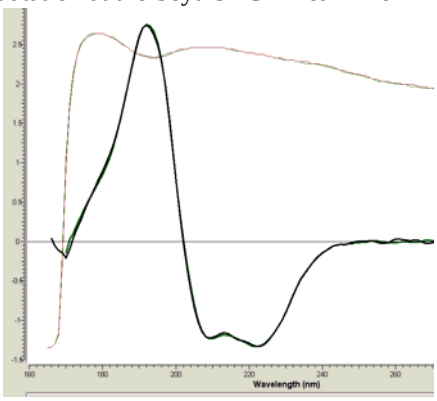

Fig. 2: showing the comparison of the spectra of myoglobin obtained at the NSRRC (grren) and the BSRF (black) beamlines.

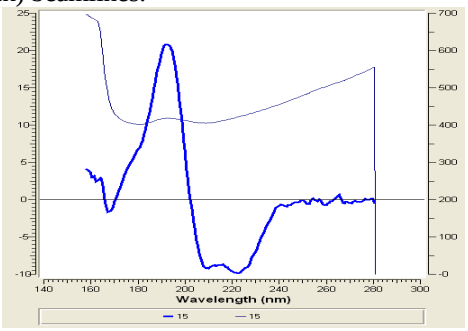

Fig. 3: showing the low wavelength spectrum of myoglobin in D_2O achievable at the Seya beamline in a short pathlength calcium fluoride cell.

Finally, having convinced ourselves of the high quality of the data produced by the beamline, we then measured a number of samples containing the voltage-gated sodium channel with and without ligands (drugs) bound and found a new conformational change associated with binding of a new class of compounds.