Sample Preparation for CD Measurements

Volume requirements:
For the 1 mm path-length cuvette, 400μL is required to ensure beam coverage. Spin down or filter samples to remove particulates.

Cuvettes:
1 mm (~450 μL) and 2 mm (~900 μL) path-length cuvettes may be borrowed from the facility (they are kept in the drawer by the instrument). These are manufactured from strain-free QS quartz and have good baseline performance.

Cleaning:
Please ensure the cuvettes are returned scrupulously clean. Protein aggregates frequently remain on the inner surface after simple rinsing, particularly after thermal melting experiments. Soaking for 30 mins-24 hours in a solution of 2% Hellmanex is usually sufficient to remove them. Rinse the cells thoroughly (5-10 times) with mQ water after soaking in the cleaning agent. The final 2-3 rinses should be with 70% EtOH to ensure no smearing on the quartz surfaces.

Sample concentration:
The signal-to-noise of a CD measurement is greatest at 0.87 OD. The profile is relatively broad so deviations are acceptable. However, it is always desirable to have an absorption level in the range 0.5-1.0 OD at the wavelength and path-length of interest. As a guide, this equates to approximately 0.1 - 0.2 mg/mL for an α-helical protein when using the 1 mm path-length cuvette.

Buffer considerations:
It is usually desirable to record wavelength experiments over a wide nm range, and at least 190-240 nm is required if you intend to deconvolute[1] the secondary-structure content of your sample using e.g. Dichroweb. The ideal situation is for the sample molecules and nothing else to be the active chromophores. Unfortunately, in the UV and particularly the far UV (< 250 nm), many buffer components absorb light, reducing the amount of light available for the measurement and adding nothing to the CD signal. When taken to the extreme there is so little light passing through the sample that CD measurements are impossible.

Table 1 lists the absorbances of many commonly-used salts and buffers. In particular it should be noted that chloride ions are strongly absorbing in the far UV and should be removed by buffer exchange (by e.g. PD10, Centricon, dialysis). ***Buffers must therefore be non-HCl based, and NaCl must be avoided if at all possible***. Most proteins are stable in an equivalent concentration of fluoride, and high-purity NaF may be used up to a concentration of 150 mM, although < 50 mM will give superior results. 10 mM potassium phosphate is a good choice of buffer for most work. Low concentrations of perchlorate, TRIS, sodium phosphate and borate are also

[1] If you are analysing the data by deconvolution you must determine the protein concentration accurately. This is best done with the sample that you used for the CD at the end of the measurements, using a spectrophotometer.

reasonably transparent, but do not adjust the pH with HCl. Other agents such as DTT, BME, or EDTA can be present at low concentrations (≤ 1mM). Detergents such as SDS, CHAPS and octylglucoside are reasonably transparent, but avoid Triton. No imidazole! If in doubt, run a UV wavelength scan on a spectrophotometer first; the OD should be <1.0 over the nm range of interest.

Additional information:
Bring several mL of spare buffer for baseline scans, rinsing the cuvette between experiments and for dilutions. A P200 Gilson and a gel-loading tip are suitable for loading and rinsing the cuvette.

Table 1: Absorbance of Various Salt and Buffer Substances in the Far-UV Region

Compound	pH	No Absorbance Above	Absorbance of a 10 mM solution in a 1.0 mm Cuvette at:			
			210 nm	200 nm	190 nm	180 nm
$NaClO_4$		170 nm	0	0	0	0
NaF, KF		170 nm	0	0	0	0
Boric Acid		180 nm	0	0	0	0
NaCl		205 nm	0	0.02	>0.5	>0.5
Na_2HPO_4		210 nm	0	0.05	0.3	>0.5
NaH_2PO_4		195 nm	0	0	0.01	0.15
Na Acetate		220 nm	0.03	0.17	>0.5	>0.5
Glycine		220 nm	0.03	0.1	>0.5	>0.5
Diethylamine		240 nm	0.4	>0.5	>0.5	>0.5
NaOH	pH 12	230 nm	>0.5	>2	>2	>2
Boric Acid, NaOH	pH 9.1	200 nm	0	0	0.09	0.3
Tricine	pH 8.5	230 nm	0.22	0.44	>0.5	>0.5
TRIS	pH 8.0	220 nm	0.02	0.13	0.24	>0.5
HEPES	pH 7.5	230 nm	0.37	0.5	>0.5	>0.5
PIPES	pH 7.0	230 nm	0.2	0.49	0.29	>0.5
MOPS	pH 7.0	230 nm	0.1	0.34	0.28	>0.5
MES	pH 6.0	230 nm	0.07	0.29	0.29	>0.5
Cacodylate	pH 6.0	210 nm	0.01	0.20	0.22	

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