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SAMPLE PREPARATION FOR FAR-UV CD SPECTRA

There are close parallels between CD spectroscopy and ordinary UV/VIS absorbance spectroscopy. CD spectroscopy depends on accurate (see below) measurement of absorbance in the peptide bond (amide) region (230-190 nm), and is subject to the same Beer's law criteria as UV/VIS absorbance spectroscopy. Beer's law is typically regarded as linear (useful) from an absorbance range of 0.1 to 1 absorbance units, works best in the middle of this range—0.3 to 0.7 AU. Absorbance involves comparing the (log of the) intensity of incident light (the unobstructed beam) with that which emerges from the sample. If absorbance is too low (the chromophore is too dilute) the incident light will not find anything to interact with—you cannot see what is not there. If the absorbance is too high (the chromophore is too concentrated), no light will make it out and the absorbance signal will be dominated by noise—the electronics cannot tell the difference between 0.001% versus 0.000001% transmission of the incident light.

CD spectroscopy depends on very small differences between absorbance of leftward and rightward circularly polarized light. Therefore, it is critical that the detection of these absorbances is as accurate as possible. Thus, protein concentration must be in the "linear Beer's law" range in the far-UV (~215 nm). Because the number of peptide chromophores depends on the chain length, optimal sample concentrations are best given in mass density (e.g. mg/ml) rather than number density (e.g. μM). For a 1 cm pathlength (part of Beer's law, remember?) this turns out to be about 30-50 μg/ml (it depends a bit on the how much α-helix there is, which gives stronger CD than β-structure). But there is an additional problem with far-UV CD. Most of the things that are put into samples for protein samples absorb a lot of light in the far-UV. It does no good to carefully optimize the protein concentration for Beer's law, only to have all the light soaked up by the buffer. Troublesome additives in protein samples include buffers, to some extent salts, and especially reducing agents. Here is a partial list and comments:

Buffers: Phosphate is great (especially at concentrations of 25 mM or less). Tris is OK at 25 mM or less. Sulfonate-based ("Goods") buffers such as HEPES, MES, PIPES are less good (a misnomer). Anything with lots of carbonyl functionality like glycine, acetate, citrate, should be avoided.

Salts: Alkali/halide pairs (Na+, K+ with Cl-, F-) are OK at 150 mM. The less polarizable the better!

Additives: Denaturants (urea, guanidine) are terrible (they have carbonyl absorbance). Amino acid stabilizers (Arg, Pro) are similarly terrible. Sugars (sucrose, glycerol) are probably better. EDTA is a disaster at high concentrations (carbonyls) but you can probably get away with trace EDTA (<=mM) if you really need it.

Reducing agents: DTT and BME are a disaster. If you need to protect thiols, use TCEP, and use as little as you can get away with (on the order of stoichiometric with the thiols in your protein).

There are two solutions to the buffer/cosolvent problem. First, switch to buffers/salts that work well, and leave out additives and reductants (other than TCEP if you need). This should always be done. Second, it is typical to decrease the path-length from 1 cm to 1 mm for CD measurement. This decreases by a factor of ten the absorbance from cosolvents/cosolutes. However, it also decreases by a factor of ten the contribution from protein, based on the concentration range given above. Thus, protein sample concentration should be increased by a factor of ten, that is, to 0.3 to 0.5 mg/ml, for CD in a 1 mm cell. The "thin" cells we use with this pathlength take about 0.4 ml of protein, although a little less can be used if needed. If you can, bring 1 ml at 0.5 mg/ml, but if the sample is too precious, bring what you can (within the limits given above). Also, bring extra buffer to dilute if it is needed, and also to measure a blank to subtract.

How to calculate molar residue ellipticity (MRE) from data

The units commonly used to represent CD in the far-UV, MRE, is a bit arcane, and can be confusing. The units are deg•cm^2•(dmol res)$^{-1}$, often multiplied by 10^{-3} to give smaller numbers than, for example, -10,000. When represented in the units above, the measured signal is scaled, divided by concentration (this makes sense, you want to convey an understanding of how much secondary structure you have per molecule), and also divided by the number of residues (to convey a sense that the molecule has a high fraction of helix, for example, rather than an indication that it is a big protein). You also divide by path length, which is typically 1 cm or 0.1 cm. This division accounts for the cm^2 in the numerator, because dividing by concentration would have put volume (cm^3) in the numerator, and dividing by pathlength removes one of these. The dmol is a holdover from god-knows-where. Remember, *deci* means 10^{-1}. And remember, the CD typically gives units of mdeg, not deg. So here is how the conversion goes:

$$MRE = Y\ m\deg \times \frac{1\deg}{10^3 m\deg} \times \frac{liter}{C\ mol} \times \frac{10^3 cm^3}{liter} \times \frac{1}{0.1\ or\ 1\ cm\ path} \times \frac{mol}{10^1 dmol} \times \frac{1}{n_{res}}$$

where Y is the signal you measure, C is your concentration in molar units, and n_{res} is the length in residues of your protein. If your pathlength is 0.1 cm, this translates to

$$MRE = \frac{Y\ m\deg}{C \times n_{res}}$$