Circular Dichroism (CD) - Applied Photophysics Q100/autosampler

In a nutshell: Fast determination of the secondary structure of proteins and nucleic acids in solution. Conformational stability measurements.

Services: Biophysics Facility offers CD as an open-access instrument. First-time users must complete a short training session before gaining access to the instrument reservation calendar.

Location: Building 50, room 3123

Description: CD spectroscopy measures the difference in the sample absorption of the left and right circularly polarized light. For the randomly oriented samples, such as isotropic solutions, this difference will be observed for the chiral solute molecules. For example, the D- and L-tryptophans have mirror image CD spectra. Since the absorption difference is very small ($\Delta A = 10^{-3} – 10^{-5}$), these measurements require a specialized instrument known as the CD spectrometer or spectropolarimeter. The protein CD spectrum is usually divided into the “near” and “far” UV regions. The aromatic amino acids - phenylalanine, tyrosine, tryptophan - make distinctive contributions to the near UV, also described as the aromatic region (250-300 nm). The far UV (wavelengths shorter than 250 nm) is dominated by the transitions of the protein backbone polypeptide chain, which has permitted identification of the spectral characteristics of different secondary structures.

The Q100 spectrometer is equipped with an autosampler. The very-short pathlength (0.1 mm) autosampler flow cell can be used to minimize buffer contributions to the sample absorbance. Unlike manual CD cells, the autosampler’s flow cell does not have to be repositioned between measurements. This helps with the detection of the very small CD signal changes induced by ligand binding, site-directed mutations or by the buffer conditions. The autosampler can also be used to perform very precise chemical unfolding studies. A denaturant concentration series can be conveniently prepared using our pipetting robot and loaded directly into the autosampler well-plate. This way the number of the unfolding isotherm data points can be increased while minimizing sample consumption and saving multiple hours of the operator’s time.

Typical applications:

- Characterization of the secondary structure of proteins, peptides and nucleic acids
- Detection of the interaction-induced conformational changes in proteins and nucleic acids
- Determination of the effect of mutations on the protein structure
- Study of the conformational stability of macromolecules at varying temperature, pH or denaturant concentrations:
    - Multiwavelength thermal unfolding measurements can be performed to simultaneously obtain the unfolding temperatures as well as the structural information on the intermediate and unfolded states from the 3D unfolding data.
    - Precise multi-point chemical unfolding studies can be performed automatically using the pipetting robot and Q100 autosampler

Basic instrument specifications:

- spectral range: from 163 nm to 1150 nm
- Autosampler format: 96-well plates, deep-well-plates standard
- Sample temperature control: Peltier controllers for the autosampler flow cells and for the manual cell holder; Peltier-cooled autosampler well-plate holder
- Available autosampler flow-cells:
    - 0.1 mm pathlength; minimum sample volume 100 µL, 200 µL recommended
    - 1 mm pathlength; minimum sample volume 200 µL
    - 10 mm pathlength; minimum sample volume 900 µL

  The 0.1 mm cell is recommended for far-UV and the 10 mm for the near-UV range
- Available manual loading cells: 0.5 mm, 1 mm and 10 mm
  Manual cells are required for thermal unfolding studies

Sample requirements and recommended buffers: For the far-UV measurements the choice of buffer is very important. The preferred buffers are either acetate or the 10 mM phosphate. PBS is usually acceptable, but the relatively high NaCl and KCl concentrations in PBS might prevent measurements at wavelengths shorter than 200 nm. Tris is also acceptable, but the pH should be adjusted with phosphoric acid rather than HCl, since chloride ions have a strong far-UV absorbance. For the same reason, either Na_2SO_4 or NaF should be used instead of the NaCl. Use of imidazole, MOPS, and PIPES should be avoided. DTT and EDTA can be used only at a low, sub-millimolar concentrations.

Always measure the absorbance spectrum of a sample and the absorbance spectrum of a buffer using a scanning spectrophotometer and water as a reference. Perform absorbance measurements in the wavelength range desired for the CD scans and compare with the OD recommendations (see below).

The autosampler is highly recommended for spectrum measurements, but should not be used for thermal scans. An instrument cleaning charge will be levied if the sample precipitates in the flow cell.

The recommended measurement sequence is: buffer-sample-buffer-sample-buffer, etc. for both the manual and automated measurements. To calculate the total time of the autosampler experiment add the scan time and the cell cleaning time for each buffer and sample acquisition. For the above three buffer and two sample sequence example, assuming spectral measurements in the 200-260 nm range, 1 nm step, 1 s accumulation, and 3 averages, the total experiment time will be approximately 50 minutes (3 min. scans and 7 min. cell washing times multiplied by 5 samples).

Minimum sample amount: The optimum CD signal to noise ratio requires a sample with an approximately 0.9 OD. The optical density should be collected at the wavelength range and pathlength used for the CD scans, and measured against water to account for both the buffer and the sample absorbance. Good results can be obtained for samples in the 0.4 OD to 1.6 OD range, and sample OD should never exceed 2. For the Far-UV CD spectra of protein samples the typical concentration required to measure using the 1 mm pathlength cell is approximately 0.1 to 0.2 mg/mL.

Consumables: We stock the deep-well autosampler plates. Quartz cells for CD measurements are provided by core.