Introduction

Circular Dichroism (CD) spectroscopy is a fast and easy photometric method used to assess the stability and structure of proteins and nucleic acids.

Features of the Jasco J-1500 Spectropolarimeter:

Wide wavelength measurement range

- Standard detector measures 163-1600nm, covering the entire far-UV, visible light, and near-infrared region spectra.

Low sample concentration needed

- 0.05 - 2.0mg/mL, depending on cuvette

Six-position cell holder with Peltier system

- Spectral scans and single- /multi-wavelength thermal ramps of up to 6 cells simultaneously
- Peltier temperature system allows for a wide range of temperatures (-30 to 130˚C)
- Magnetic stirrers for each cell eliminate thermal gradients

Fast run time

- High sensitivity and a 10,000 nm-per-minute maximum scan speed increases productivity and minimizes sample degradation by high energy UV light.

Microsampling disk

- Allows for sample volumes as low as 2µL.

Dual polarizing prism optical design

- Stray light distorts CD spectra, so the dual polarizing prism is designed to reject all but 0.0003% of stray light .

Simultaneous multi-probe measurements

- All six cells can undergo simultaneous acquisition of both CD and linear dichroism (LD) spectral scans or thermal ramps.

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Produced by James Magnus Jorgensen

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Structural and Biophysical Core Facility

Jasco J-1500 Spectropolarimeter

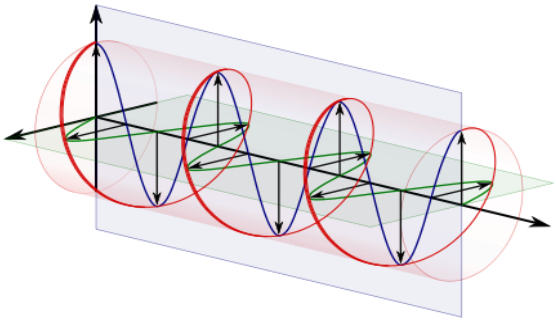
Circular Dichroism Spectroscopy

- Protein secondary structure determination
- Assess protein stability with variable wavelength thermodenaturation assays
- Detect ligand-induced conformational changes in proteins
- Compare structural properties of wild-type and mutant proteins
- Analyze the structure and stability of nucleic acids

CD Spectroscopy — BSA Analysis

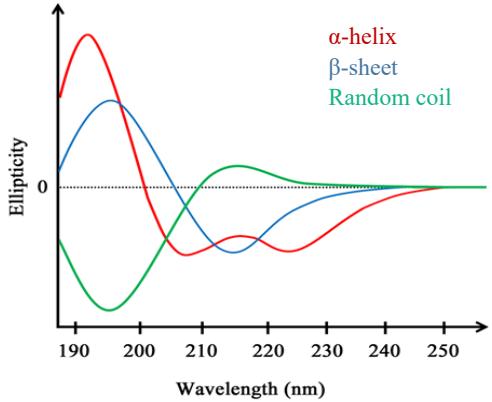
Theoretical Background

CD measures the difference in absorption of left-handed (LCPL) and right-handed circularly polarized light (RCPL).



The spectropolarimeter xenon lamp generates white light, which is made monochromatic by quartz prisms, then linearly polarized by crystal prisms. Light becomes circularly polarized by a quarter-wave plate, which works to de-synchronize one linear component of the beam by one quarter wave. Depending on the orientation of the quarter-wave plate, the circularly polarized light will be either LCPL or RCPL.

A chiral chromophore will absorb each type of CPL to a different extent. Output data is detected by a photomultiplier tube (PMT). The secondary structure of the molecule will influence the shape of the resulting CD spectrum, and therefore denaturation will decrease the ellipticity of the measurement.



An α-helix has two distinct minima at 208nm and 222nm, and a maximum at 192nm. A β-sheet has a minimum at 218nm and a maximum at 198nm.

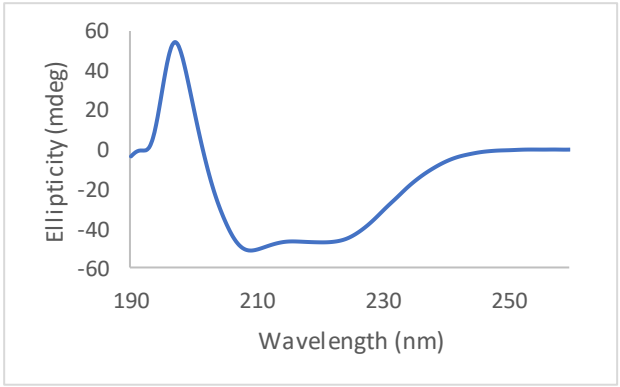
BSA CD Spectrum Analysis

Protein Sample: 0.25mg/mL BSA (Bovine Serum Albumin)
Buffers: 1x PBS
Scanning Mode: Continuous
Response Time: 1 second
Bandwidth: 1nm

The 3 following spectra and corresponding specifications are common examples of CD experiments. The data pitch is the measurement increment of the scan. The accumulation number represents how many replicate scans are measured and averaged to give the final spectrum.

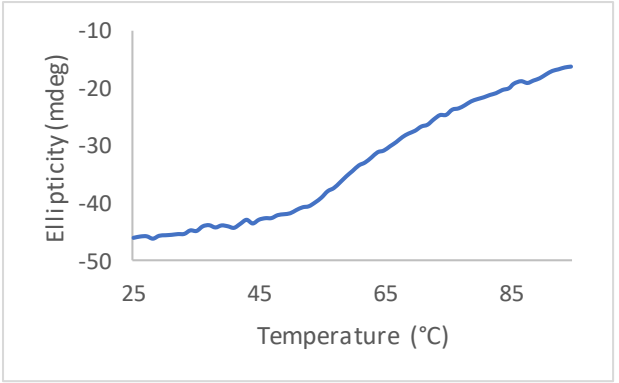
The BSA concentration used was 0.25mg/mL , so a strong signal would be achieved. If sample concentration is too high, then a higher voltage is needed during the scan. A high voltage results in increased noise of the low-wavelength data. A low scanning speed and large number of accumulations will increase the resolution of a spectrum.

1) Full Spectrum Wavelength Scan of BSA
Temperature: 20˚C
Scanning Speed: 50nm/min
Data Pitch: 0.5nm
Accumulations: 3 (Averaged)



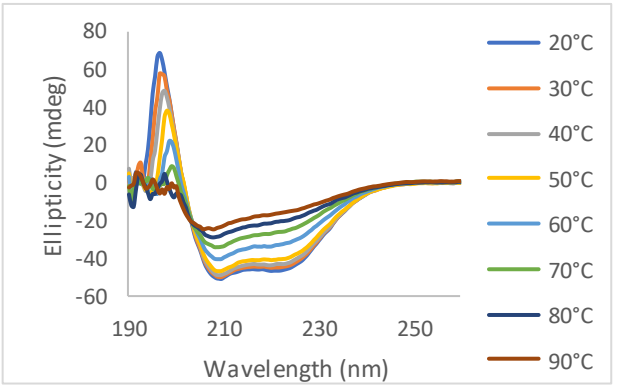
The standard absorbance range for a wavelength scan of a protein is 190-260nm. The minima at 208nm and 222nm, and the maximum at ~195nm are suggestive of a helical structure for BSA (see the reference α-helix curve to the left).

2) Thermal Melt of BSA at 222nm
Temperature Range: 25 - 95 ˚C
Scanning Speed: 1˚C/min
Data Pitch: 0.2˚C



Single-wavelength CD measurements across a wide temperature range can give information on the stability of the protein. The chosen wavelength should reflect the shape of the full spectrum wavelength scan (Scan 1). The thermal melt above suggests denaturation starting at ~55˚C.

3) Full Spectrum Thermo-denaturation
Temperatures: 20 - 90˚C in 10˚C increments
Scanning Speed: 100nm/min
Data Pitch: 0.5nm
Accumulations: 3 (Averaged)



A step-wise temperature gradient of full wavelength scans provides another assessment of structural stability. Denaturation can be seen to start at ~60˚C.