CD spectrophotometer Jasco J-815

Circular dichroism spectroscopy (CD)

Circular dichroism spectroscopy measures differences in the absorption of left and right-handed polarized light that arise from the structural asymmetry (presence of chiral atoms in the molecule). It has a wide range of applications in many different fields. Most notably, CD spectroscopy is used to **investigate the secondary structure** of proteins. α-helices, β-strands and random coils can be identified in the far UV region where they give rise to a characteristic shape and magnitude of the spectrum.
The absorption, dipole orientation and the nature of the surrounding of some amino acids (phenylalanine, tyrosine, tryptophan and cysteine) affects the signals obtained in the near UV spectrum, so it provides information on the **tertiary structure** of the protein.
The instrument offers a range of measurement modes and techniques: circular dichroism (CD), fluorescence, total fluorescence and stopped-flow CD, fluorescence and absorbance.

CD spectroscopy can be used for:

- determination of the protein folding
- characterization of protein´s secondary and tertiary structure
- detection of changes in the structure upon mutagenesis
- detection of changes in the conformation of a protein upon protein-ligand or protein-protein interaction
- study of conformational stability of proteins (influence of pH, temperature, denaturants, buffer composition, addition of stabilizers)

Fluorescence measurement can be used for:

- study of the protein folding
- characterization of protein-ligand or protein-protein interaction
- determination of the protein concentration

Technical specifications:

Instrument: **CD spectrometer J-815** (Jasco)

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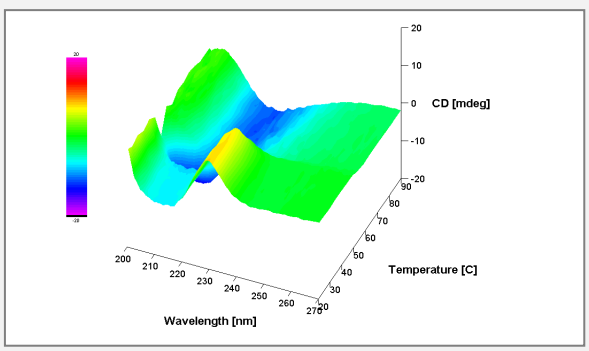

Secondary structure shift in CD-temperature 3D graph

Features:

- light source: 150 W air-cooled **Xe lamp**
- wavelength range: 163-900 nm
- simultaneous acquisition of circular dichroism and fluorescence data

Accessories:

- Peltier temperature control (accessible temperature range -10 – 110 °C)
- Scanning emission monochromator for fluorescence
- Bio-Logic: SFM-20, two channel stopped-flow setup
- Various cuvettes with path lengths from 0.1-10 mm (contact CF staff for actual status)

Data collection:

- **Wavelength scanning**
  - continuous scan: running average method offering high speed measurements
  - step scan: discrete wavelengths and response time to optimize signals
  - auto-scan: based on step scan but offering a range of response times to speed up
  - data accumulation
- **Time scan**
  - fixed wavelength time scan for chemical denaturation and stopped-flow experiments
- **Temperature scan**
  - fixed wavelength for CD versus temperature thermal ramping
  - pre-set temperatures with equilibration times for spectral scanning
  - 3-dimensional display of CD versus wavelength vs temperature or time

Operational mode:

CD spectroscopy measurements are performed by the users themselves. New users are obliged to attend special training that can be ordered as a service.

Data processing:

JASCO´s Spectra Manager (possibility to train people in data processing); freely accessible web applications (e.g. K2D2, K2D3) may be used for protein secondary structure analysis.

Sample requirements – importance of sample preparation

- Far-UV CD spectra (secondary structure measurement at 190-230 nm) require typically between **180 µl – 2000 µl** of **~ 0.1 – 0.2 mg/ml protein solution** (sample amount depends on the cuvette used). Many common buffer components (Hepes, MES, Tris, NaCl, citrate, etc.) absorb at wavelengths bellow 220 nm. Even more compounds absorb bellow 200 nm.
- **Use the lowest possible concentrations of your buffer or discuss its composition with the CF responsible person.** For example, 10 mM phosphate is suitable, NaF is better than NaCl, borate is better than Tris/HCl, etc. – see the table.
- **Near-UV CD spectra** (protein tertiary structure at 250-300 nm) requires typically **500 µl of >1 mg/ml** protein solution. At higher concentration, smaller volume may be sufficient.
- **Substances not optimal for CD** include DTT, β-ME, DMSO, EDTA, imidazole, Triton X-100 – try to avoid them. SDS and < 20% glycerol should be compatible with the CD measurement.
- **Fluorescence** spectra acquisition requires typically 500 µl of protein solution. Concentration of the sample depends on the amount and character of fluorophore present (Trp, GFP, fluorescent dyes)

Approximate wavelength cutoffs [nm] of various buffers / solvents for 1 mm cell

Buffer / solvent	Lower wavelength cutoff
dD_2O	175
dH_2O	180
10mM Na-phosphate	182
50mM NaF	<185
150mM $NaClO_4$	<185
10mM K-phosphate, 100mM KF	185
100mM Na-phosphate	190
150mM $(NH_4)_2SO_4$	190
100mM NaCl	195
50mM Na-borate	195
Ethanol (100%)	195
PBS	200
100mM Tris-HCl	200
100mM MES	205
50mM Na-acetate	205
4M guanidine-HCl	210
4M urea	210
100mM PIPES	215
100mM ammonium citrate	220
150mM $NaNO_3$	245
DMSO (100%)	252

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Instrument location

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