Circular Dichroism

J-1000 Series Spectrometers

JASCO

Performance
Innovation
Reliability

JASCO has produced the highest quality circular dichroism instrumentation for over 50 years. The J-1000 series is the result of many advances in technology combined with a great deal of customer input. These three new models are adaptable to meet any requirements and can be expanded as applications or budgets evolve.

Chiroptical spectroscopy is one of the most important techniques for the characterization of biomolecules, determination of absolute configuration and stereochemical analysis. Since launching our first spectropolarimeter in 1961, JASCO has designed and built the finest in chiroptical instrumentation.
More than a half-century later, JASCO proudly presents the J-1000 series, our latest circular dichroism spectrometers. Unparalleled optical performance and a variety of measurement modes are combined to make the J-1000 CD spectrometers true state-of-the-art chiroptical spectroscopy workbenches.

Table of Contents

Features of the J-1000 Series

The three models of the J-1000 series offer the most robust feature set available, for applications ranging from education and routine analysis to complex research with strict requirements.

Advanced features of the J-1000 series

- High optical throughput
- Low stray light
- Advanced electronics: Digital Signal Processing (DSP) using Field-Programmable Gate Array (FGPA)
- Simultaneous Multi-Probe (SMP): CD, LD, UV-visible, absorbance, and fluorescence
- Wide dynamic range
- Highly efficient nitrogen purge system: low-volume monochromator designed using a flow simulator
- Integrated Hg lamp for wavelength calibration
- NIST traceable scale calibration standard
- Single USB connection for simplicity and easy PC changes in the future
- Measurement of micro-volume samples
- Three scanning modes: auto, step and continuous

Unique optical features and benefits

- Compact benchtop design
- Air-cooled 150W Xenon lamp
- Highest signal-to-noise ratio
- Collimated sample beam for artifact-free solid sampling and use of external accessories
- Range of precise temperature control accessories
- Automated-titration and stopped-flow
- Spectra Manager™ 2.5 software for control and data analysis
- Spectra Manager™ 2.5 CFR option for 21 CFR Part 11 compliance
- Flexible design allows for field upgrades for different measurement capabilities
- Ability to upgrade accessories as applications and budgets evolve

Versatility for a wide range of applications

- Protein conformational studies
- Protein folding studies
- DNA / RNA interactions
- Enzyme kinetics
- Formulation studies
- Purity testing of optically active substances
- Quantitative analysis of pharmaceuticals
- Natural products chemistry
- Material science
- Rapid kinetics (stopped-flow) CD
- Absorbance and fluorescence studies

Which model is right for you?

J-1100

The basic model, perfectly suited for QA/QC and teaching applications.

J-1500

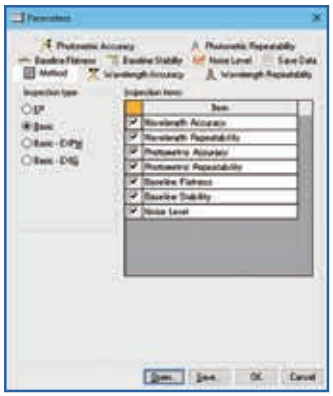
Highest performance with a wide range of accessories to meet complex research demands.

J-1700

UV/Visibile/NIR up to 2500 nm for MCD and specialized applications.

Validation and Data Confidence

Count on the accuracy and repeatability of your data. An integrated validation mode provides users with a list of up to nine instrument performance and calibration tests. Each J-1000 system includes a built-in Hg lamp wavelength calibration source. JASCO also offers the first traceable scale calibration substance (d-10-ammonium camphorsulfonate) for photometric accuracy and repeatability tests.

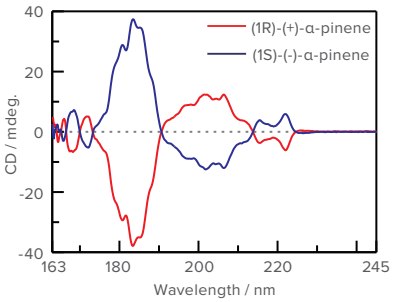


Easy-to-use software to validate instrument performance.

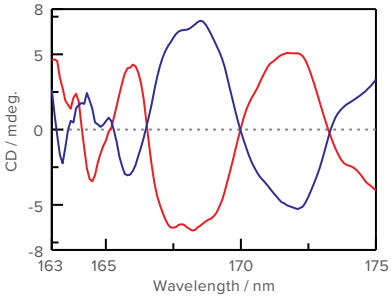
Instrument Performance

Enhanced Vacuum-UV Measurement

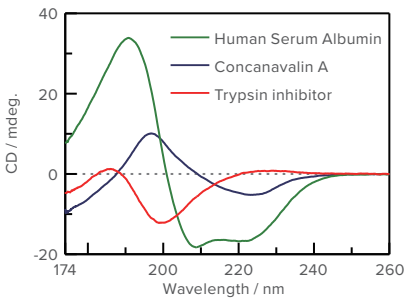
The innovative optical system of the J-1500 permits the measurement of a CD spectrum in the vacuum-UV region down to 163 nm. The vacuum-UV region below 200 nm is of critical importance for biomolecules, particularly in protein secondary structure estimation.



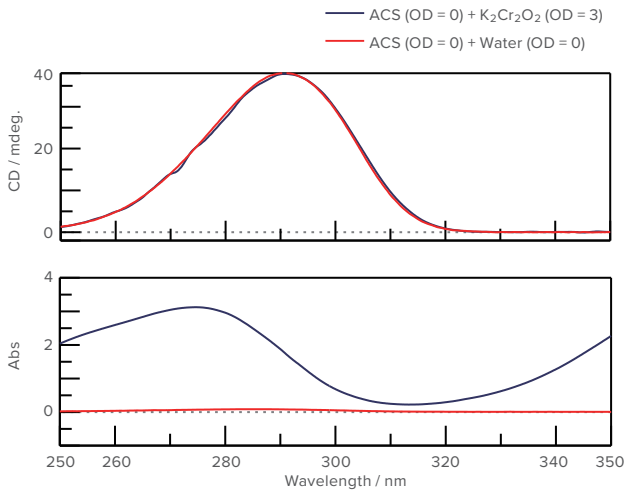
α-pinene enantiomers (gas phase) showing excellent performance in the Far-UV region.



The same α-pinene samples showing similar superior performance in the vacuum-UV region down to 163 nm.



CD spectra of Human Serum Albumin (helix rich), concanavalin A (β-sheet rich) and trypsin inhibitor (random rich) in water with an excellent signal-to-noise ratio down to 174 nm.



CD spectra under conditions with high absorbance

Exceptional Stray-Light Rejection

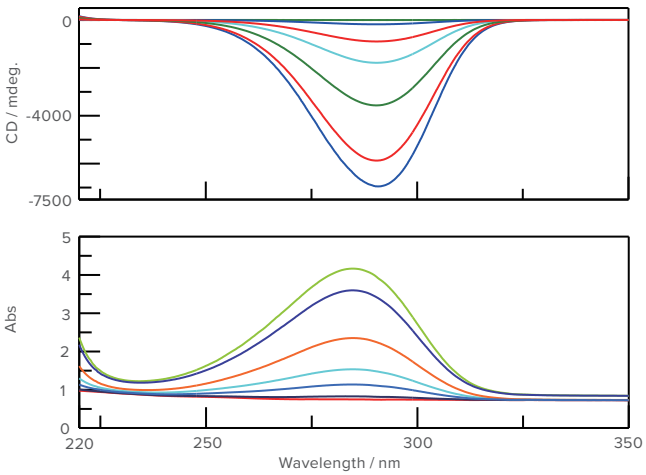
Stray light will result in distortion of the CD spectrum, particularly in the far-UV region where the sample absorbance is high. The dual prism polarizing optical design of the J-1000 series results in stray light lower than 0.0003%, enabling the instruments to obtain high-quality CD data even under conditions with high absorbance.

Rapid Scanning

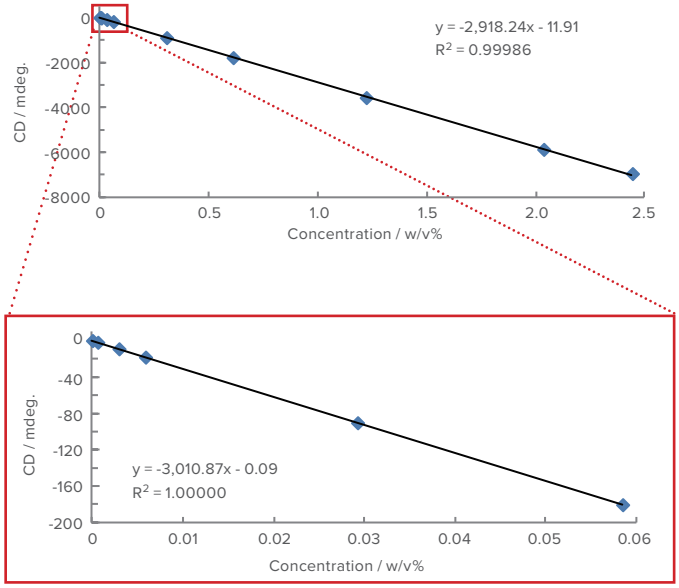
High sensitivity combined with a 10000 nm/min. maximum scan speed allows the J-1500 to measure samples quickly, increasing productivity in your lab. An additional benefit is the minimal time exposure of biological samples to the high-energy UV light, minimizing the risk of sample degradation.

CD Dynamic Range and Linearity

The PMT detectors used in the JASCO system are specially selected for the highest sensitivity and lowest birefringence. The result is superior linearity compared to other types of detectors.



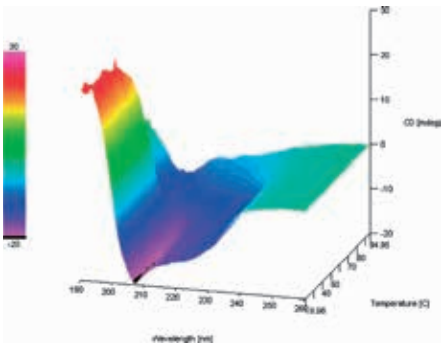
CD spectra of I-10-ACS



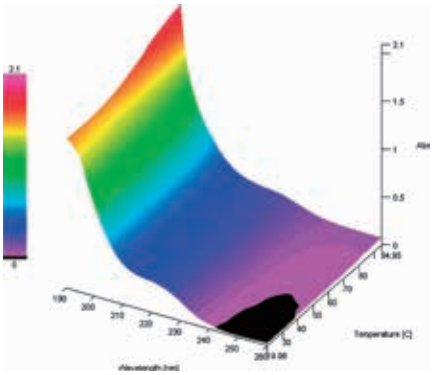
CD linearity (sample: I-10-ACS)

Simultaneous Multi-Probe Measurement

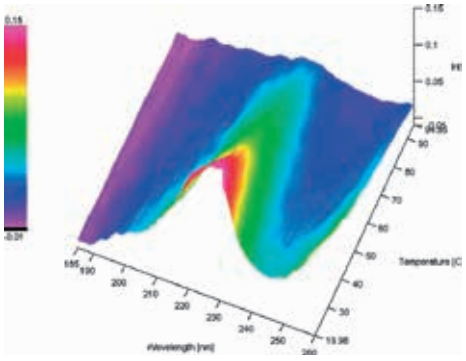
The latest quad-channel lock-in amplifier provides the simultaneous acquisition of up to four data channels including CD, absorbance, linear dichroism (LD), fluorescence, fluorescence-detected CD (FDCD), fluorescence-detected LD (FDLD) and fluorescence anisotropy.



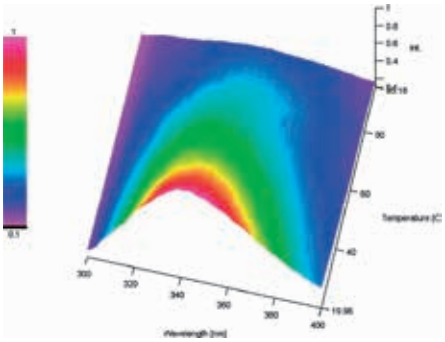
CD



Absorbance



Ex. fluorescence



Em. fluorescence

Multi-probe measurement of Lysozyme showing the simultaneous acquisition of CD, Absorbance, Ex Fluorescence and Em Fluorescence during a thermal-ramping experiment.

Temperature Control

Peltier Thermostatted Single Cell Holders

PTC-510, 514, 517

PTC-517

Three Peltier thermostatted single cell holders are available with a maximum light path length of 10 mm, enabling temperature control using the temperature control program. All three single cell holders feature a temperature setting range of -30 to 130 °C with a measurement probe that can be placed inside or adjacent to the cell and a magnetic stirrer to eliminate thermal gradients.

PTC-517 Single-position Peltier for J-1500 and J-1700 for use with rectangular cells up to 10 mm.

PTC-514 Single-position Peltier for J-1100 for use with rectangular cells up to 10 mm.

PTC-510 Single-position Peltier for J-1500 and J-1700 for use with rectangular and cylindrical cells up to 10 mm.

MPTC-511

Automatic 6-position Peltier Cell Changers

MPTC-511, 513

The MPTC accessories are designed to enable high sample throughput and productivity. The six-position holder for rectangular cells allows automated spectral scans and parallel thermal ramps on up to six samples at a time. Applicable measurement modes include spectral scans, thermal ramping at single or multiple wavelengths and thermal ramping with spectral scans at preset temperatures.

- Temperature setting range of -30 to 130 °C
- Temperature sensors for each cell
- Magnetic stirrers for each cell to eliminate thermal gradients

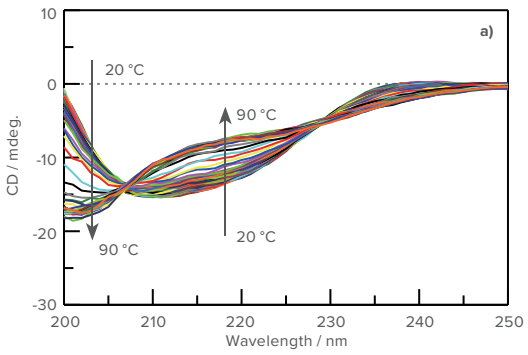
MPTC-513

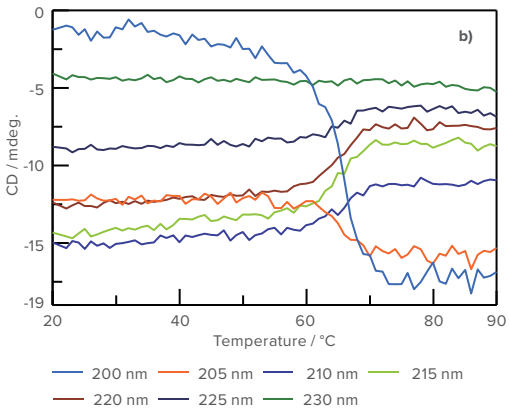
The MPTC-513 system is compatible with optional fluorescence modes including total fluorescence, scanning excitation/emission fluorescence (FMO-522) accessory and fluorescence polarization/anisotropy (see the section on fluorescence on page 16).

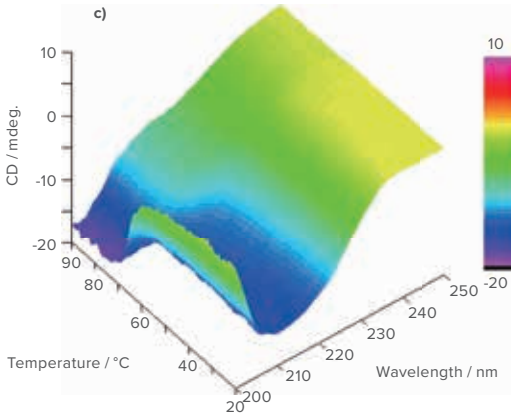
Temperature-Wavelength Scan

Application

Measuring CD spectra while controlling the temperature of a sample provides important information about conformational changes associated with temperature perturbations and is used in the study of biopolymers. The temperature control program measures the thermal denaturation curve (temperature scan) and CD spectra as a function of temperature, according to user-selected parameters. An optional thermal denaturation analysis program determines transition temperatures and thermodynamic parameters, such as ΔH and ΔS, from temperature scans.







Thermal denaturation of Ribonuclease A from 20 to 90 °C

a) CD spectra as a function of wavelength.

b) CD signals at several wavelengths as a function of temperature.

c) 3D view of CD scans as a function of wavelength and temperature.

Other Temperature Control Accessories

HTC-572 | Pressure-resistant high temperature measurement unit

Thermal denaturation measurements at temperatures over 100 °C are made difficult by the boiling point of water. Using the same principle as a pressure cooker, this accessory applies pressure to the sample to increase the boiling point, allowing for higher temperatures. Samples can be heated up to 170 °C by applying up to 1 MPa pressure to the cell chamber.

Capillary Cell Jacket

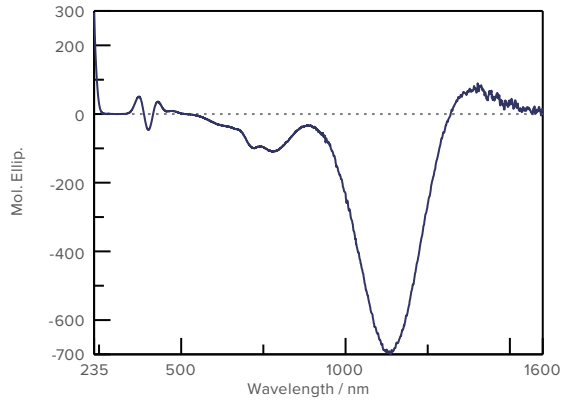
For thermal ramping of microsamples, this accessory allows only 10 µL to be drawn into the capillary cell, which is inserted in a block that is placed in the Peltier cell holder. Accurate T_m measurement of such small sample volumes was not possible in the past.

Extended Wavelength

Extended Wavelength Options

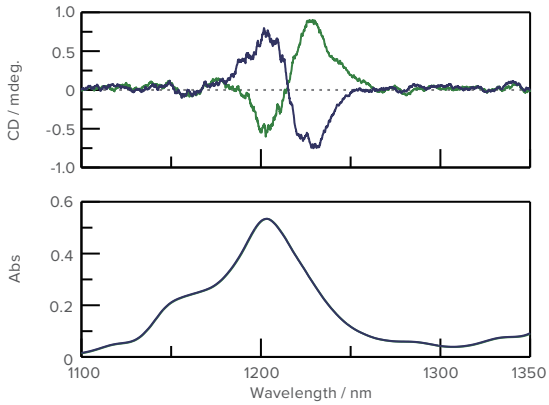
EXPM-531 (PMT), EXIG-542 (InGaAs)

Measurement in the near-infrared (NIR) region is important for samples like colored proteins, including prosthetic groups, transition metal complexes and nanomaterials. The J-1500 with optional detector allows for the measurement of samples in the NIR region. The InGaAs detector kit includes a halogen lamp unit and allows for measurement up to 1600 nm, covering a range where overtone VCD bands are present.



NIR-region CD spectrum

Sample: 0.24 M $NiSO_4$ + 0.36 M KNa-Tartrate
Measurement condition:
235 to 940 nm Xe lamp + PMT
940 to 1600 nm WI lamp + InGaAs



NIR-region CD spectra

Sample: R-(+)-Limonene and S-(-)-Limonene
Measurement condition: WI lamp + InGaAs

Extended Wavelength Accessories

EXPM-531 | Extended wavelength option (PMT)
The EXPM-531 is a red-sensitive PMT detector covering the NIR measurement range to 1250 nm. This has been the traditional method of extending the wavelength range of the CD system into the NIR range. Exchange is quick and easy, and data from the UV/Visible range can be linked with NIR data in the software.

EXPM-531

EXIG-542 | Extended wavelength option (InGaAs)
The EXIG-542 option includes an InGaAs detector combined with a halogen light source that expands the NIR measurement range to 1600 nm.

EXIG-542

Microsampling

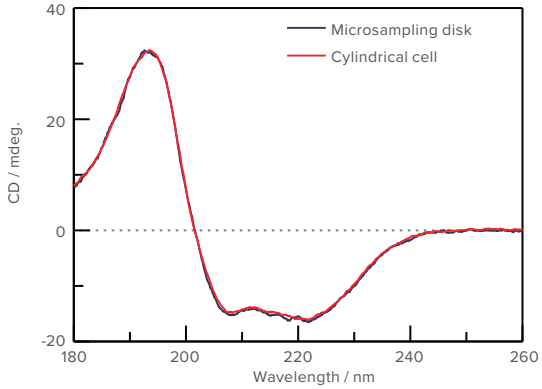
Small quantities of very precious samples have always been a challenge for CD measurement. Conventional micro-cells typically have volumes as low as about 60 µL, but a new breakthrough allows CD measurements for samples with volumes as low as 2 µL.

Microsampling Disk

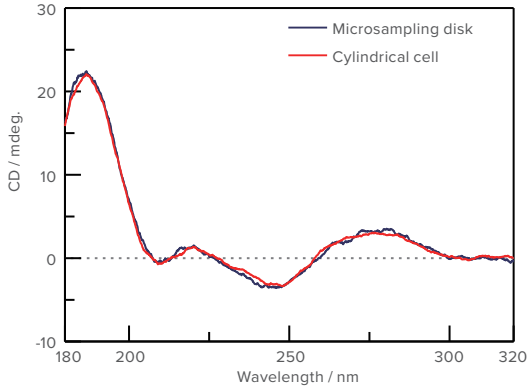
MSD-462

The microsampling disk is designed for measurement of samples as small as 2 µL. High-quality quartz windows with a special surface treatment utilize spacers of 1.0 and 0.2 mm for volumes of 10 and 2 µL, respectively. The sample is applied using a micropipette, and cleaning is quick and easy.

MSD-462



CD Spectra of DNA derived from bovine thymus



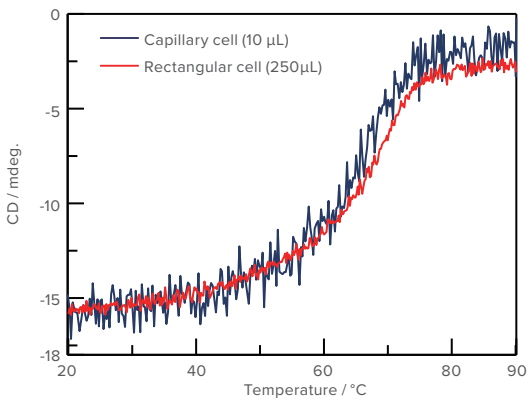
CD Spectra of hemoglobin

Comparison of secondary structural analysis

		Helix (%)	β-Sheet (%)	Turn (%)	Random (%)
Hemoglobin	X-ray	78.0	0.0	6.3	15.7
	Micro sampling disk	73.8	0.0	10.9	15.3
	Cylindrical cell	72.2	0.0	12.0	15.8

Capillary Cell Jacket

For thermal ramping studies of precious samples we also offer the solution by the capillary cell jacket, which allows use of samples as small as 5 µL to be placed in the Peltier cell holder. Melting curves are easily measured, including calculation of T_m, ΔS and ΔH.



T_m measurement of Hemoglobin

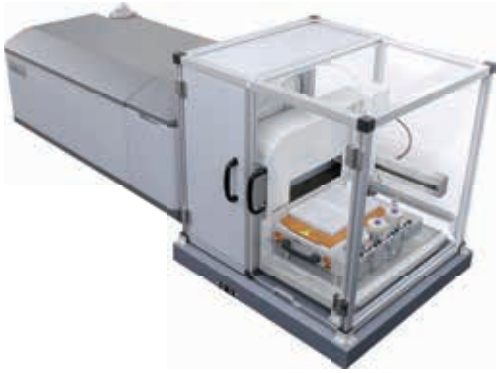
(Wavelength: 222 nm, Temperature: 20 °C to 90 °C)

High-Throughput CD

Autosampling

HTCD Plus

The HTCD allows automated scanning measurements using pre-programmed parameters. The microplate and vial rack can be set to a constant temperature to avoid sample denaturation and evaporation during measurement. An interlock for the sample door also prevents movement of the arm while the door is open. Pre-programmed methods for flushing protein or DNA/RNA samples are included. Flushing methods can also be customized with up to three different flushing solvents and variable drying times to eliminate sample carry-over. The system allows samples to be recovered following measurement, and batch data processing includes secondary structure and comparability analysis.



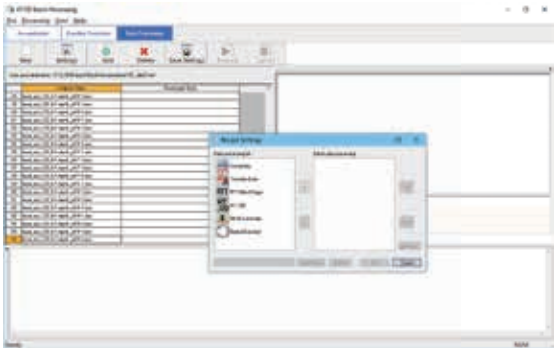
- Fully automated measurement of up to 192 samples (two 96-well microplates) or 120 sample vials
- Pre-registered flush method for protein or DNA/RNA samples can be selected to eliminate sample carry-over
- Retrofit capability to J-1500 CD spectrometer
- Flow monitor function to optimize the sample flow condition
- Capability for performing CD/fluorescence simultaneous measurement (option, see the section on fluorescence on page 16)

Optinal software for HTCD Plus

JWBAT-533 | HTCD Batch processing program

HTCD Batch processing program offers an efficient solution for analysis of multiple samples.

- Data Accumulation - Average multiple scans for a sample
- Baseline Correction- measure solvent independently and then subtract after data collection automatically
- Smoothing – smooth data with several methods
- Data truncation – cut out noisy or unneeded wavelength ranges
- FFT filter – remove any effects of cuvette fringing
- HT to OD conversion – convert the HT voltage channel into absorbance
- Offset correction – correct for baseline offset
- Optical constant – correct for solution concentration and generate molar ellipticity curves



pH and Salt-Induced Denaturation Study of VHH Antibody

Application

Below is an example of the result of a stability evaluation of antibodies (VHH model) by comparing the CD spectra of native and denatured antibodies using statistical analyses. Figure 1 shows the CD spectra of VHH for solutions with different pH values, and Figure 2 shows the qHOS evaluation for VHH structural changes correlated to the pH and NaCl concentration.
The qHOS program can quantify the similarity of CD spectra using a statistical method and can quantitatively evaluate CD spectral changes associated with structural changes in proteins. See page29 for more details on qHOS.

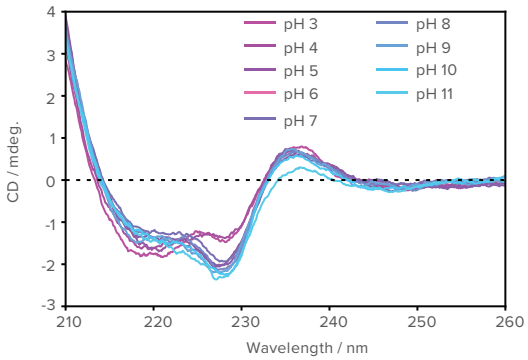


Figure 1. CD Spectra of VHH antibody

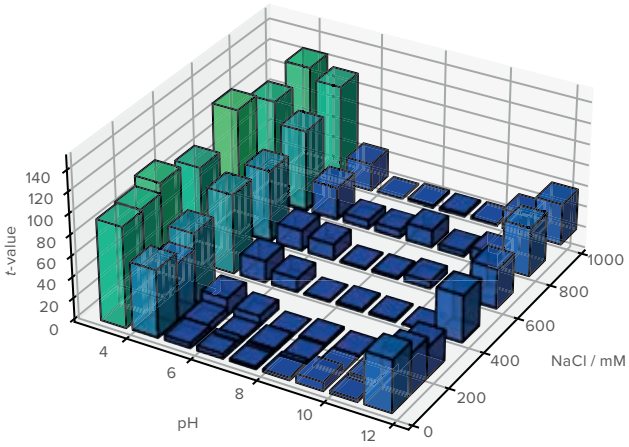


Figure 2. Relationship between pH and NaCl concentration of VHH antibody

Figure 3 shows a plot of the *t*-value obtained by HTCD and T_m obtained by denaturation temperature measurement. The high correlation between the *t*-value and the denaturation temperature suggests that a spectral difference test is a very useful primary screening method before performing a thermal denaturation analysis, which generally requires a great deal of time.

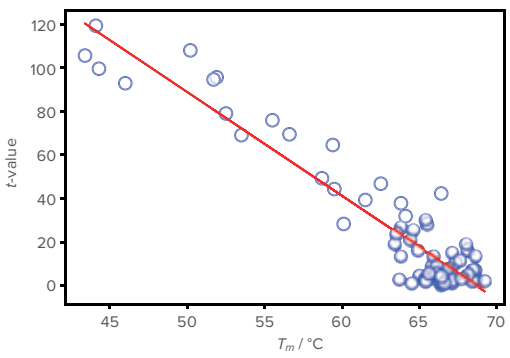


Figure 3. Relation between denaturation temperature and *t*-value

MPTC-513 Automatic 6-position Peltier turret cell changer permits temperature control using the temperature control program and allows for the measurement of a maximum of six samples.

Special thanks for collaboration; Prof. Kouhei Tsumoto, School of Engineering and Institute of Medical Science, The University of Tokyo

Automated Titration

CD spectroscopists have often looked at changes in CD signal due to changes in solvent composition, such as pH and buffer. In addition, studies of binding are often of interest including protein-protein, DNA or ligand interactions. While these studies are often performed manually, the use of automated titration systems has become more popular.

ATS-530

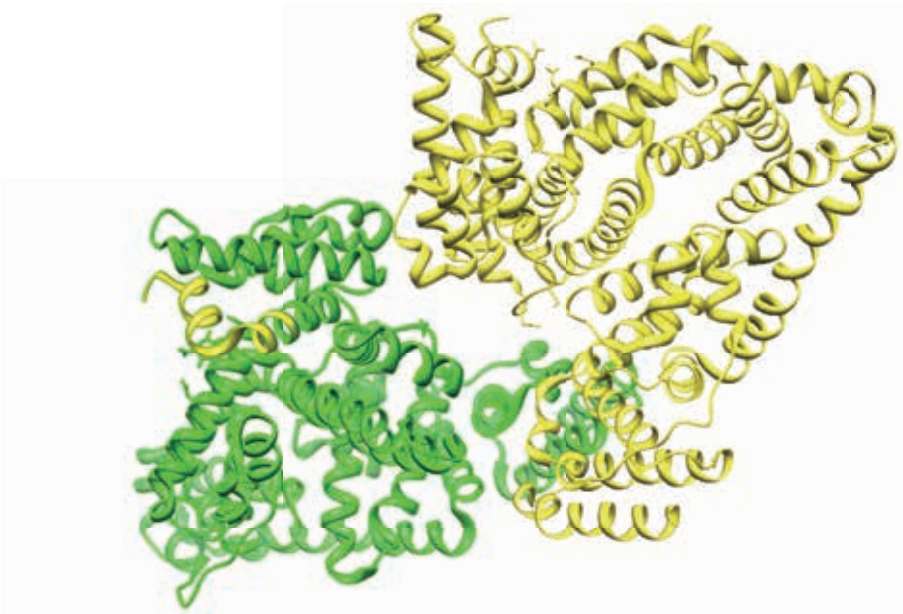
Automatic Titration Unit

ATS-530

The ATS-530 is designed to automatically monitor changes in CD, absorbance and fluorescence as a function of solution pH, chemical denaturant or exogenous ligands in experiments, such as protein denaturation or ligand binding. Dual syringes are employed, each equipped with a valve for automated refilling/flushing during extended runs and for maintaining a constant cell volume. Additionally, the titration measurement program automatically corrects for concentration.

- Automated titration measurement program included
- Optional macro command program available for complex/custom titrations
- Dual syringes (1 mL volume as standard, 1000 steps/syringe)
- Constant cell volume
- Concentration correction
- pH measurement (optional)

Note: A cell holder with stirrer (PTC-510/517 Peltier thermostatted single cell holder) is required for automatic titration measurement.



Secondary Structure Changes of poly-L-glutamate During Sulfuric Acid Titration

Application

pH titration is an example of a typical experiment carried out with the ATS-530 system. Below is an example of the conformational changes of poly-L-glutamate from its native random coil state to an α-Helical state while lowering the pH with dilute sulfuric acid.

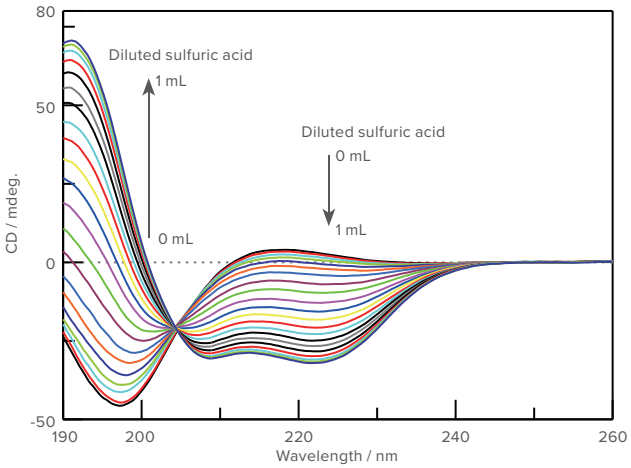
2D-CD Spectra of sodium poly-L-glutamate titrated with diluted sulfuric acid.

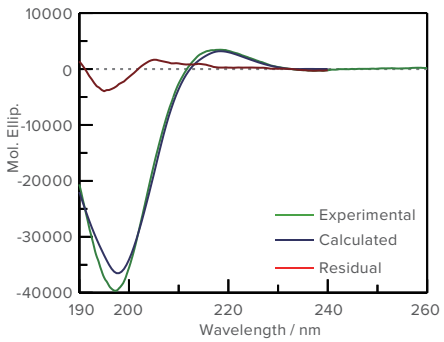
<Measurement parameters>

Starting solution: Aqueous solution of sodium poly-L-glutamate (0.02 mg/mL, 2 mL)

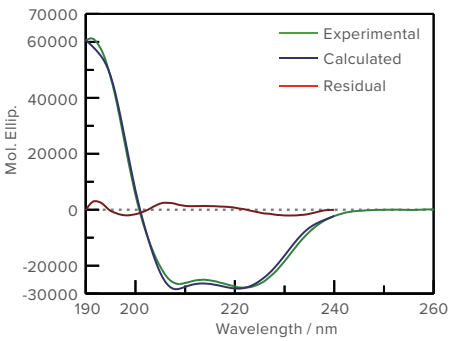
Titrant: Diluted sulfuric acid (10^{-5} N)

Titration step: 50 µL, 20 times (total 1 mL)

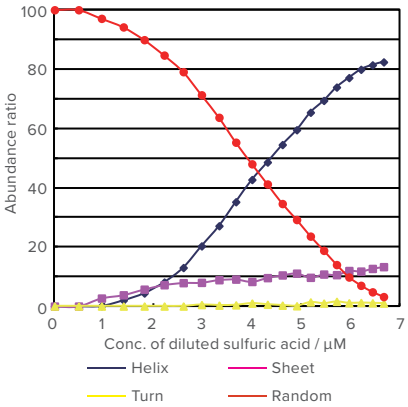




Comparison of experimental and calculated spectrum (before titration).



Comparison of experimental and calculated spectrum (after titration).



Change of SSE abundance ratio of sodium poly-L-glutamate.

Fluorescence

Total Fluorescence and Fluorescence Scanning

FDT-538

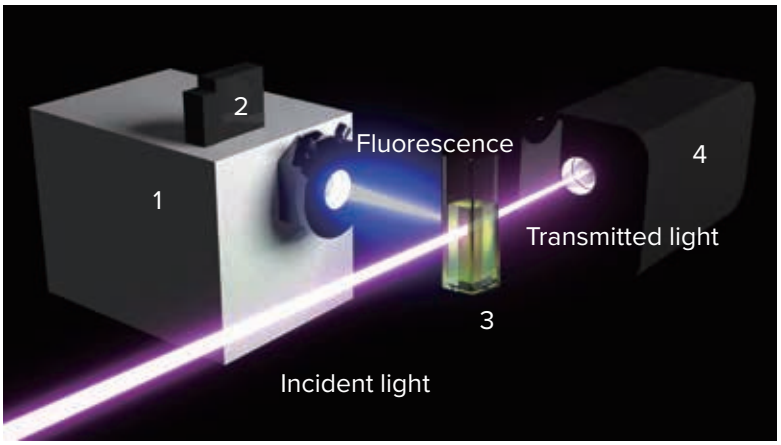
FMO-522, FDT-538

Intrinsic fluorescence can be measured on the J-1500 or J-1700 CD spectrometers, with either the Peltier thermostatted single cell holders (PTC-510 and PTC-517) or automatic 6-position Peltier turret cell changer (MPTC-513). A simple low-cost system for detection of total fluorescence is available using a secondary detector and high-pass filters (FDT-538 and FST-470), allowing the user to select the excitation wavelength while detecting the emission at the wavelengths above the cut-off filter. This allows for simple, yet sensitive, detection of fluorescence changes during titration or thermal ramping experiments.

Alternatively, fluorescence data can be acquired by using the optional scanning emission monochromator (FMO-522) and emission detector (FDT-538). Excitation and fluorescence emission spectra can be scanned by fixing the emission or excitation wavelengths, respectively.

FMO-522

- Fluorescence scanning can be coupled with the titration and thermal ramping capabilities
- With the MPTC-513, CD and fluorescence data can be collected, simultaneously or separately, on up to six samples
- With the HTCD Plus, high-throughput fluorescence scanning is also available.



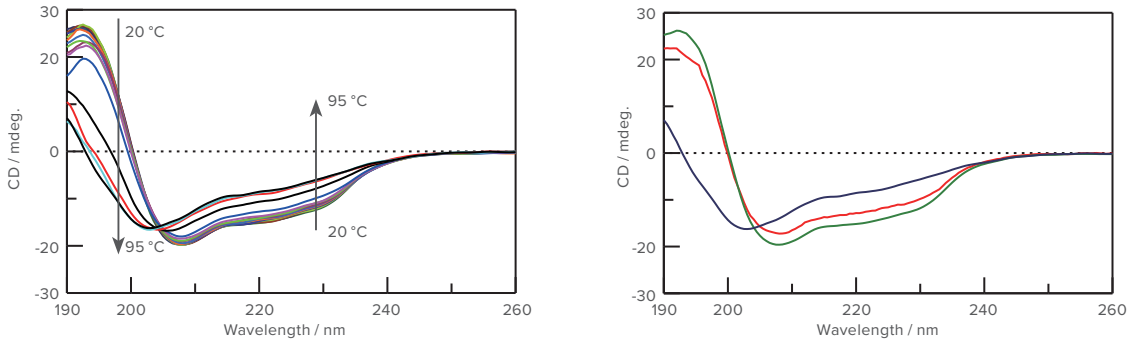
Configuration for emission scanning with FMO-522
(1. FMO-522 monochromator, 2. FDT-538 detector, 3. Sample, 4. CD/UV detector)

Thermal Denaturation of Lysozyme Measured with CD and Fluorescence Spectroscopies

Application

Lysozyme, a globular protein found in the white of a hen's egg, is a model protein used to investigate the denaturation of proteins at high temperature. The secondary structure of lysozyme comprises about 38 % α-helix and 10 % β-sheet.

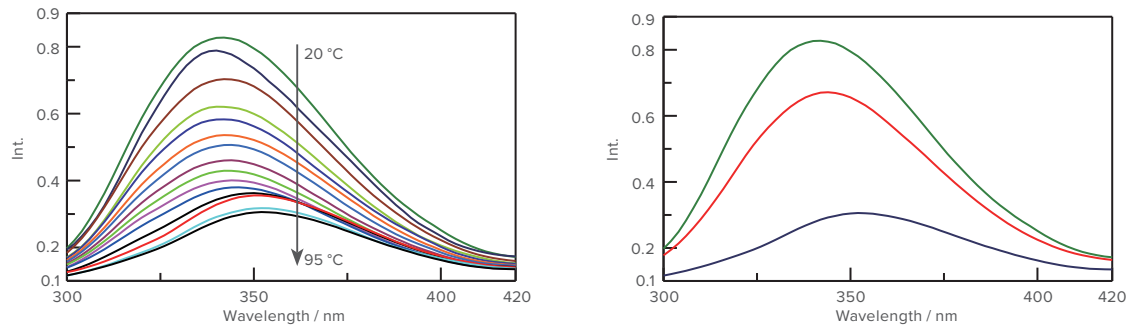
Chicken egg-white lysozyme (1 mg) was dissolved in 15 mL of deionized water. The thermal denaturation of the protein was monitored using the J-1500 CD spectrophotometer equipped with an MPTC-513 automatic 6-position Peltier turret cell changer and an FMO-522 emission monochromator for detection of fluorescence. CD and fluorescence spectra were automatically measured at 5 °C intervals from 20 to 95 °C. After the final measurement at 95 °C, the sample temperature was returned to 20 °C and a final spectrum was collected.



Thermal denaturation measured with CD spectroscopy

Left: As the temperature increases, the intensity of the CD spectra decreases and the minimum at 208 nm blue-shifts to 203 nm.

Right: Upon completion of the melt, the temperature is re-equilibrated at the initial temperature of 20 °C. Comparison of the CD spectra measured at 20 °C before and after the melt demonstrates that while the protein does refold, it does not recover its original structure. (Green: 20 °C initial / Blue: 95 °C / Red: 20 °C final)



Fluorescence data for the thermal denaturation of lysozyme from 20 to 95 °C

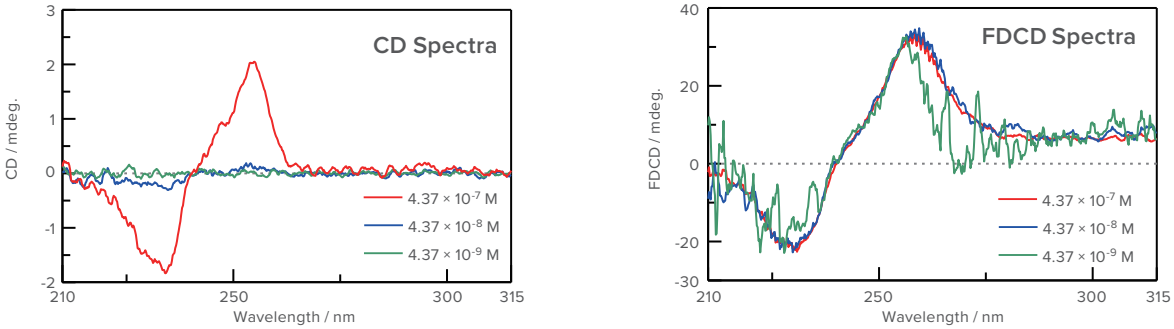
Left: As the protein undergoes thermal denaturation, the fluorescence decreases in intensity and the emission maximum red-shifts from 338 to 347 nm. As with the CD data, the largest shift occurs between 75 and 80 °C.

Right: A comparison of the protein fluorescence spectra measured at 20 °C before and after thermal denaturation supports the CD results, which indicate that the lysozyme structure does not return to its initial native state after denaturation. (Green: 20 °C initial / Blue: 95 °C / Red: 20 °C final)

Fluorescence

Fluorescence-Detected Circular Dichroism (FDCD)

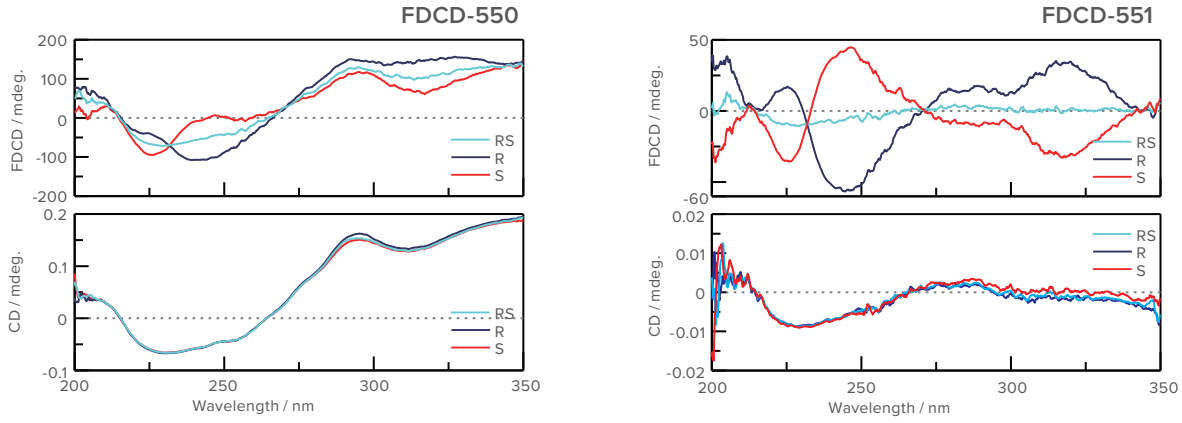
Fluorescence-Detected Circular Dichroism (FDCD) is the measurement of the differential emission intensities from an optically active sample that is excited by left and right circularly polarized light. This method takes advantage of the chiral specificities and the structural sensitivities of CD, combined with the selectivity and sensitivity of enhanced fluorescence detection.
Because FDCD can selectively measure the CD of a specific fluorescent chromophore, it is particularly useful for the study of proteins, which have multiple chromophores. FDCD can be measured with the standard CD detector when paired with the PTC-510, PTC-517 or MPTC-513 cell holder. When samples are free from fluorescence anisotropy, this method is effective because the photoselection artifact is small; however, when the sample has a larger fluorescence anisotropy, the photoselection artifact will distort the FDCD spectrum. The FDCD-551 attachment is specially designed to reduce or eliminate this artifact while greatly enhancing sensitivity due to much more efficient light collection.



Comparison with CD

FDCD offers a significant sensitivity advantage over absorption CD spectroscopy. The graphs illustrate CD and FDCD spectra of the same sample in concentrations varying by 10×. The sample is (1S,2S)-trans-cyclohexanediol bis(6-methoxy-2-naphthoate).

Data courtesy of Dr. Tatsuo Nehira, Faculty of Integrated Arts and Sciences, Hiroshima University, Japan, and Prof. Nina Berova, Department of Chemistry, Columbia University, USA.



Artifact elimination

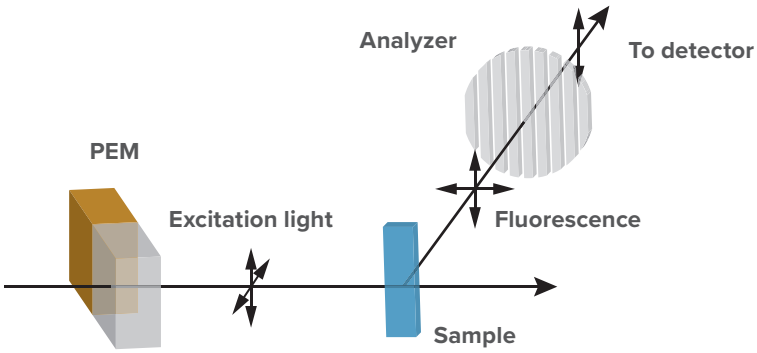
The graph on the left shows photoselection artifacts with conventional FDCD measurement using the FDCD-550.

The graph on the right shows artifact-free FDCD spectra using the FDCD-551 system. The sample is RS-1,1'-Bi-2-Naphthol 0.4 ppm / Glycerin.

Fluorescence Polarization Anisotropy

The J-1500 and J-1700 CD spectrometers use circularly polarized light that is generated by phase modulation. By controlling the amplitude of the phase modulation, it is also possible to measure linear dichroism (LD), which is the differential absorption of light polarized parallel and perpendicular to an orientation direction. Using this same principle, when polarized light interacts with a fluorescent molecule, the resulting fluorescence emission has different intensities along different polarization axes. This fluorescence anisotropy can be measured, as shown in the diagram below, where the polarized light passes first through a polarizer (analyzer) and then a detector that is positioned 90° relative to the excitation beam. The polarizer is oriented so that only the vertical component of light passes to the detector.

FDCD-551



FDCD-550

Additional Fluorescence Accessories

FDCD-550 | FDCD Unit
FDCD measurements are possible with this accessory, which comprises a sample holder for 90° detection, focusing lens, filter holder and a short wavelength cut-off filter. The sample can be maintained at a constant temperature with circulating water. For these measurements, the J-1500 CD detector is repositioned to the emission side.

FDCD-551 | Artifact-free FDCD Unit
The improvement in light collection efficiency and the ability to eliminate artifacts due to fluorescence anisotropy are two advantages of this FDCD unit. Using a unique design that includes a sandwiched, elliptical cylinder mirror with two plane mirrors, all fluorescence light emitted in a circumferential direction from the cell is collected. The result is a highly sensitive, artifact-free FDCD spectrum.

PTC-PLH | Polarizer for PTC-510/517
Adding this polarizer to the emission optics and utilizing the alternating horizontal and vertical polarization allows for the measurement of fluorescence polarization and anisotropy.

FDCD-PLH | Polarizer for FDCD-550
This polarizer can be used with the FDCD-550 system to add fluorescence polarization and anisotropy capability, in addition to FDCD.

Stopped-Flow Series

Stopped-flow measurements involve the rapid mixing of two or more solutions to trigger a chemical reaction, the kinetics of which can be followed by CD, absorbance and fluorescence. All probe methods can be measured on the same instrument when the stopped-flow system is paired with a J-1500 CD spectrometer.

SFS-602

Stopped-Flow Systems

SFS-602

The SFS-600 series is an innovative stopped-flow measurement accessory with a modular design that allows the flow cell unit to be easily installed and removed from the sample compartment without alignment. Two-, three- and four-syringe models are available, offering flexible mixing and upgradeability for quench-flow. For temperature-dependent kinetic experiments, the options are Peltier temperature-controlled syringes. Stepper-motor-driven syringes allow infinitely variable mixing ratios and a mechanical mixer efficiently mixes solutions of different viscosities commonly used in protein folding experiments.

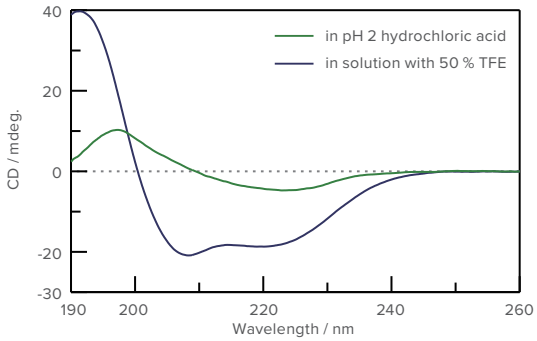
- Applications to studies such as protein folding, substrate binding and enzyme kinetics
- Standard 2 mm cell (optional 0.5, 1 and 10 mm cells)
- Standard 10 mL syringe (optional 1, 2.5 and 5 mL syringes)
- 5 mL/sec. flow rate with 10 mL syringe
- Exact control of flow rate
- Mixing ratio from 1:1 to 1:20
- Dead time: 0.57 msec with a 2 mm cell
- Peltier temperature control (SFS-602T/SFS-603T/SFS-604T) ranges from 5 to 80 °C (with cell) and 5 to 60 °C (with syringe)

Note: The SFS-500 series of stopped-flow accessories is still available for use with the J-1700 CD spectrometer.

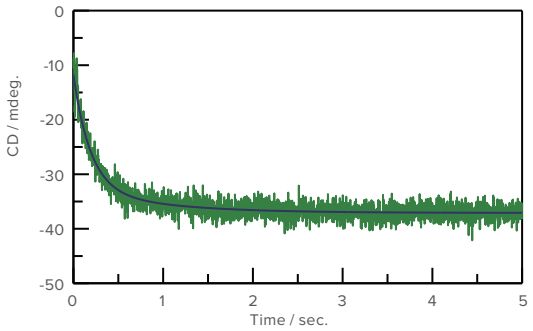
Unfolding of Concanavalin A by Trifluoroethanol

Application

Concanavalin A is a lectin protein derived from the jack bean. In its native state, it is composed of abundant β-sheet structures that change to α-helical form when exposed to Trifluoroethanol (TFE).
The J-1500 and the SFS-602 high-speed stopped-flow system were used to measure the unfolding process of Concanavalin A with TFE.



CD Spectra of Concanavalin A

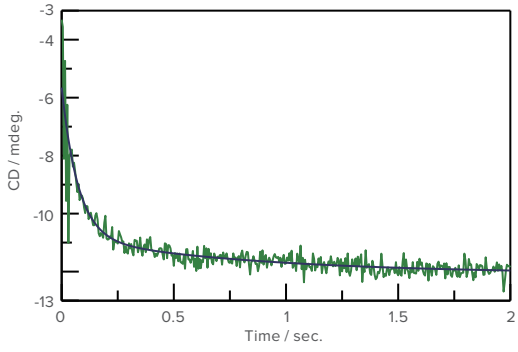


Unfolding process of Concanavalin A in TFE and analysis result

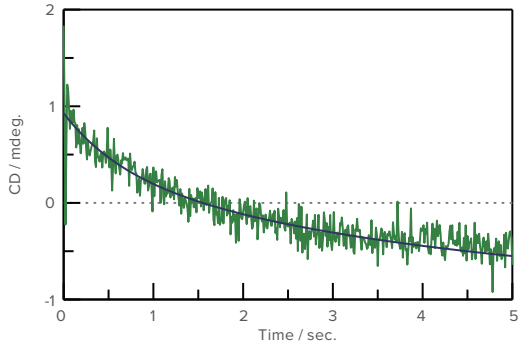
Refolding of Cytochrome C using GuHCl

Application

An aqueous solution of Cytochrome C, which was denatured by Guanidine Hydrocholoride (GuHCl), was prepared. CD stopped-flow measurement was performed using 0.1 M acetic acid buffer/water solution (1:9). The refolding process was observed at both 222 nm for the secondary structure and 289 nm for the environment of the aromatic side chain.



Refolding measurement of Cytochrome C (222 nm)



Refolding measurement of Cytochrome C (289 nm)

HPLC-CD

Using the CD spectrometer as a chiral HPLC detector of CD

HPLC is an effective method for quantitative detection of trace amounts of compounds and for mixture separation/purification. HPLC is also used for analysis of medicinal products and in food science. In these fields, samples often have optical activity allowing the analysis of enantiomeric excess and the separation/purification of the enantiomers. JASCO has been a pioneer in the development of chiral HPLC detection and offers an HPLC flow-cell for J-1000 systems enabling acquisition of high-quality chromatographic data during chiral separations.

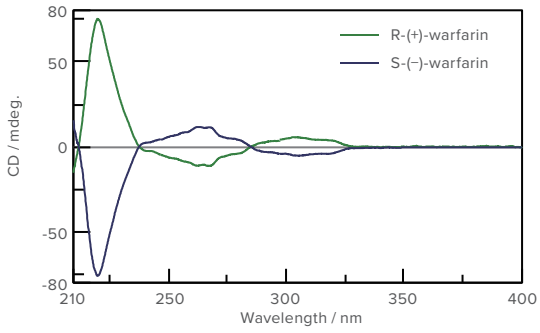
LCCD-420 | Flow cell attachment for LC-CD
The LCCD-420 enables the J-1000 to operate as an HPLC CD detector, and the chromatographic signal can be output into any commercially available Chromatography Data System (CDS).
* JWAOM-514 Analog output function module program is required.

LCCD-420

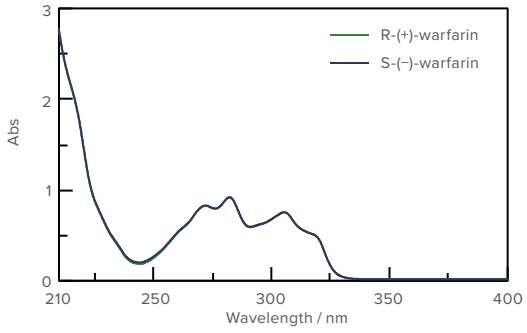
HPLC-CD of Warfarin

Application

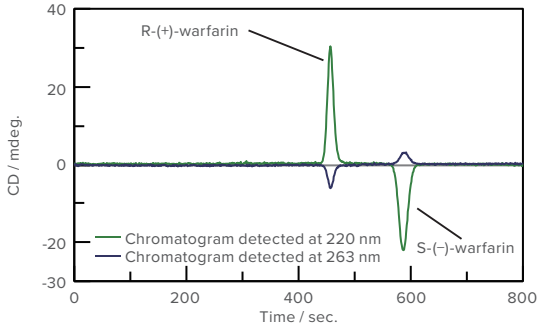
Warfarin was dissolved using a mixture of pH 2.0 aqueous phosphoric acid and acetonitrile. CD measurement was performed using a J-1500 outfitted with the LCCD-420 cell and connected to a conventional HPLC system.



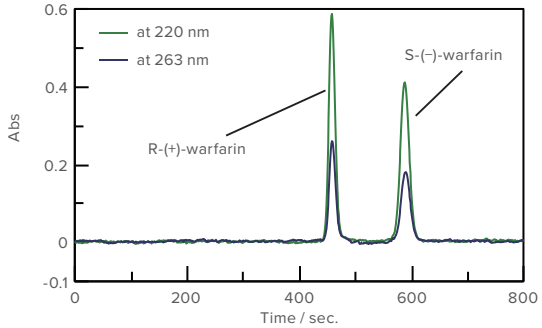
CD spectra of Warfarin



Absorption spectra of Warfarin



CD chromatograms of Warfarin



Absorption chromatograms of Warfarin

Magnetic CD

Magnets and cryostats

Placing the sample in a magnetic field allows measurement of Magnetic Circular Dichroism (MCD) data. This method is a sensitive monitor of structural features that perturb the electronic states of an MCD active chromophore. For protein molecules, MCD allows for more sensitive measurement of a chromophore's local environment. This technique has been generally used to study chromophores with large magnetic moments arising from rotational symmetries (aromatics and porphyrins), unpaired spins (metal complexes) or both (hemes). The MCD signal intensity is proportional to the magnetic field strength, which can be applied using either permanent magnets, electromagnets or super-conducting magnets:

JASCO offers a range of MCD options depending on the field strength and temperature desired, including:

- Permanent magnets with field up to 1.6 tesla at ambient temperature
- Electromagnets with field up to 1.5 tesla and low temperature cryostats
- Superconducting magnets with field of 8 tesla (and more)

MCD-581

MCD-581 | Electromagnet
Measure magnetic hysteresis and MCD spectra in a continuously variable field from -1.5 to 1.5 tesla. This magnet has a pole gap of 15 mm and a pole diameter of 21 mm. A regulated DC power supply unit is used to control the magnetic field strength. The magnet requires cooling water (minimum of 5 L/min.) usually provided by a circulator system. Sample temperature can be maintained with an optional water-jacketed cell holder, which accommodates 10 mm cells. Spacers for 1, 2 and 5 mm cells are included. Optionally low-temperature cryostats (CRYS-582 and CRYL-583) are also available.

PMCD-586 | Permanent Magnet
Measure MCD spectra in a fixed magnetic field of + or -1.6 tesla, where the sign of the field is changed by turning the magnet 180°. With a weight of only 5.9 kg, this accessory can easily be placed in and out of the standard sample compartment. This magnet accommodates 5 mm path length rectangular cells as standard. Optional holders are available for cells with 1 mm or 2 mm path lengths.

PMCD-586

CRYS-582 | Liquid Nitrogen Cryostat for Solid Samples
For solid samples, this cryostat has a temperature range from ambient down to -180 °C. Minimum sample size: 10 × 10 × 0.5 (t) mm, maximum sample size: 15 × 15 × 2 (t) mm

CRYL-583 | Liquid Nitrogen Cryostat for Liquid Samples
For liquid samples, this cryostat has a temperature range from ambient down to -150 °C. Rectangular cells with 5 mm path length can be used.

Note: For both cryostats, temperature control is achieved with the TC-22HK3 Temperature Controller and a vacuum pump is necessary for adequate insulation of the sample holder unit and the liquid nitrogen vessel (2 L capacity).

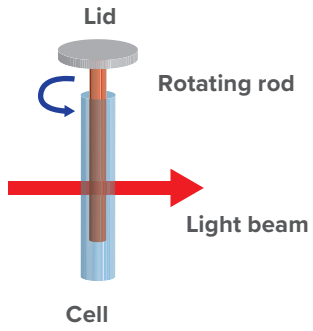
Linear Dichroism

Couette Flow Cell Unit

CFC-573

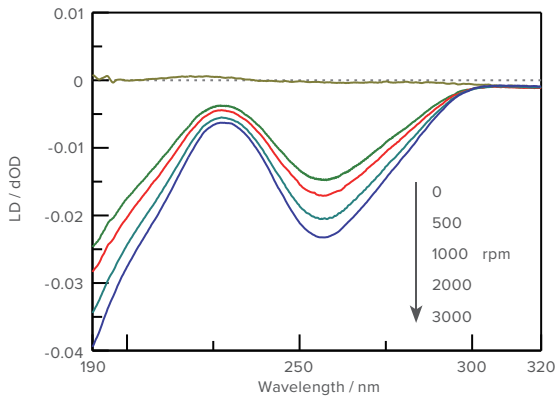
The most versatile approach to orienting macromolecules for LD measurements is the Couette flow system. This system subjects the sample to a constant gradient over the annular gap between an inner quartz cylinder, which is rotating at high speed, and a fixed outer quartz cylinder. The CFC-573 Couette flow cell unit includes a built-in beam condenser, which focuses the beam onto the small LD flow cell to maximize light throughput. The cell unit is easy to install, remove and clean as needed. Features of the Couette flow cell unit include:

- Applications to studies of proteins, DNA and biological or synthetic polymers
- Small (100 µL) sample volume requirements and 0.5 mm path length cell
- Continuously variable spinning speeds up to 5000 rpm
- Temperature control using an external circulator



Schematic of the Couette cell unit

With the sample in the annular gap, flow orientation is achieved by rotating the inner cylinder.



Couette Flow Measurement

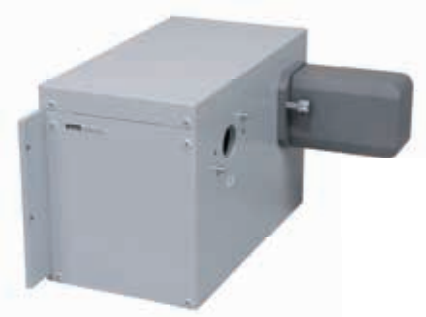
LD spectra of DNA from calf thymus measured at rotation speeds of 500, 1000, 2000 and 3000 rpm. The spectra were measured using a data pitch of 0.2 nm with a scanning speed of 200 nm/min., a data integration time of 0.5 sec. and a 1 nm bandwidth.

ORD and Diffuse Reflectance

Optical Rotatory Dispersion (ORD)

Complimentary and related to CD is the measurement of Optical Rotatory Dispersion (ORD). This technique provides information on chiral molecules even without chromophores such as saccharides. It can be used to measure the chirality of non-absorbing samples and determine the absolute configuration.

JASCO offers two methods of ORD detection: optical null and intensity measurement systems. The intensity measurement method, using a fixed analyzer, is simpler and more economical, while the optical null approach, with its rotating analyzer, is intrinsically more accurate because the measurement is absolute. Because ORD is very sensitive to the effect of strain in the sample cell, window cylindrical cells are strongly recommended for ORD measurements.



ORDM-520

ORDM-520 | ORD unit
This accessory allows detection of ORD over a wide angular range (90°) with very high accuracy using a rotating analyzer. No calibration is required, and switching back and forth from CD to ORD is handled automatically by the PC software. The system uses a separate optical path with a servo-driven analyzer and a dedicated photomultiplier tube.

ORDE-521 | ORD unit
The electrical ORD accessory has an angular range up to 2°. Since the measurement principle is based on an electrical measurement method, the apparatus is simpler and is also able to respond faster to changes in ORD signals.

Diffuse Reflectance CD (DRCD)

Samples that are insoluble or may change conformation when in solution have traditionally been difficult to characterize by CD. Diffuse reflectance, using an integrating sphere, is an effective way to analyze these samples, often dispersed into a finely ground scattering matrix, such as KBr powder. In addition, diffuse transmission measurements of pellets or films are easily accomplished with the same integrating sphere, substantially increasing the collection efficiency for scattering samples and providing a suitable alternative to conventional transmission measurements.

DRCD-574 | DRCD attachment
The DRCD-574 unit utilizes a $BaSO_4$ interior for economical reflectance and transmittance measurements.

DRCD-575 | DRCD attachment
The DRCD-575 offers a Spectralon™ interior as well as N_2 purge port for improved performance at low wavelengths.

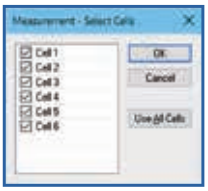
DRCD-575

Spectralon™ is a trademark of Labsphere inc.

Spectra Manager™ Software Suite

Instrument Control

Parameter dialogs allow easy editing of pre-saved parameter files. Data acquired from each instrument is automatically loaded into the analysis program (running in the background) in order to free up the PC and control software to acquire more data. Each instrument driver also has a module to allow for instrument hardware diagnostics and validation.



Sample selection of up to six cells with optional holder.

Flexible Display Features

User-friendly features include overlay printing in colors and patterns, autoscale mode, full control of style and font, plus customized toolbars.

Data Processing and Spectral Analysis

View and process several types of measurement data files (UV/Vis/NIR, FTIR, Fluorescence, etc.) in a single window, using a full range of data manipulation functions. Features include arithmetic operations, derivatives, peak detection and processing, smoothing (several methods), baseline correction, curve fitting and multivariate SSE analysis.

Parameter selection including three scanning modes.

Report Publishing

JASCO canvas allows the user to produce hard-copy layouts of data to meet individual reporting requirements.

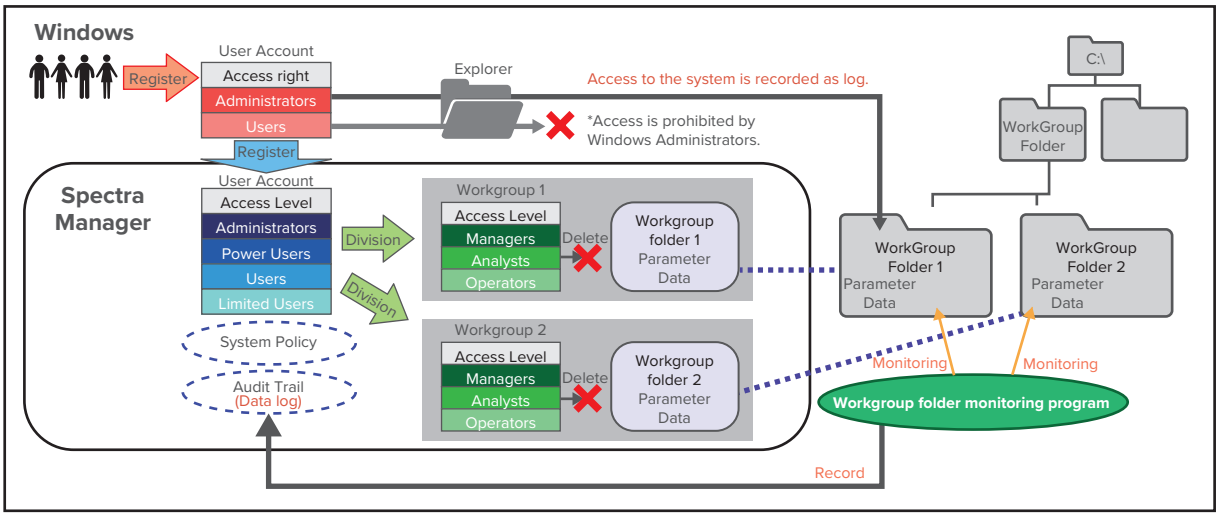
Macro Command Option

This software provides customized programs for a complete range of tasks, including data acquisition, post-run data manipulation and report printing.

Fluorescence parameter selection.

Regulatory Compliance with Spectra Manager™ 2.5 CFR

JASCO's Spectra Manager™ 2.5 CFR is designed and developed under ALCOA+ principles and is a total solution platform to create accurate and complete data.



Overview

Solution for Data Integrity

Enduring Electronic Record

Based on prohibiting the function to delete electronic records and to overwrite saves, while maintaining the function to backup and restore data, electronic records can be saved properly and searched accurately during the data lifecycle.

User Management

Two security categories [Access Level] and [Work Group], allow flexible and independent authorization of users and projects for different applications, instruments and measurement programs.

Audit Trail

Data is categorized and recorded as 3 different records (system log, application log and data log). Each log can be filtered and displayed by recorded date, username, etc., which can then be exported for audit trail review.

User Account Security

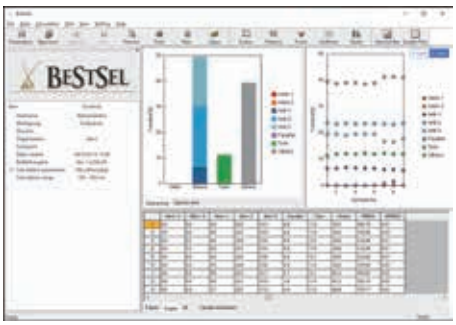
Functions to prevent duplicate accounts, protect passwords, and prevent unauthorized access, administrative authorizations, such as system access and electric signatures, etc., can be managed strictly.

Computerized System Validation

Spectra Manager™ 2.5 CFR is developed and manufactured properly under quality control system adapted ISO 9001, and adapted CSV standard.

Application Software

Spectra Manager BeStSel Program



Recently, Micsonai et al. developed the BeStSel algorithm that can accurately estimate the secondary structure composition from the CD spectrum by taking into account the parallel-antiparallel orientation of the β-strands and the twist of the antiparallel β-sheets. An offline BeStSel software in Spectra Manager Ver. 2.5 (Spectra Manager BeStSel) was jointly developed, and enables seamless analysis in an offline capacity.

This product is applied BeStSel engine (Licensed by Eötvös Loránd University, Hungary, Created by Dr. András Micsonai and Dr. József Kardos).

BeStSel

ELTE EÖTVÖS LORÁND UNIVERSITY

Thermal Denaturation Analysis of Monoclonal Antibody

Application

The advantage of BeStSel is not only its high accuracy in analyzing β-structure-rich-proteins, but also its feature of being able to calculate the fraction of eight secondary structure elements, including three types of twisting in antiparallel β-sheets (Figure 1).
Figure 2 shows the detailed secondary structure change as a function of temperature.

Figure 1. Twisting of β-strand

There is a significant difference between Herceptin® and h-IgG in changes in the distorted α-helix, left-twisted β-strand, relaxed β-strand and right-twisted β-strand. At 30 °C, h-IgG and Herceptin® have a similar secondary structure, but it was found that they have different denaturation mechanisms by using BeStSel. BeStSel is shown to be useful in understanding the detailed mechanisms of structure formation in proteins, including therapeutic antibodies.

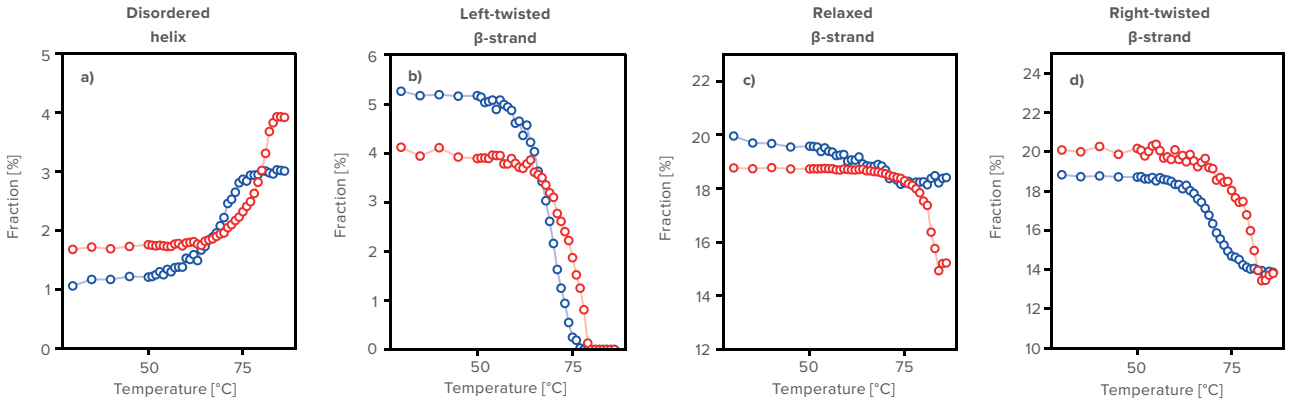


Figure 2. Results of secondary structure prediction using BeStSel

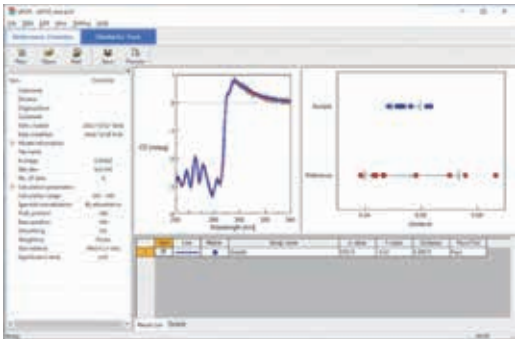
Predicted secondary structure fraction of a) disordered Helix, b) left-twisted, c) relaxed, and d) right-twisted β-strand of Herceptin® (red) and human IgG (blue).

qHOS Program

The qHOS program can statistically determine the significant difference between spectra, considering various error factors. Features of the qHOS program include:

- Statistical similarity evaluation
- Robust evaluation using a noise weighting method
- Student, Welch, TOST *t*-test implementation
- Auto concentration correction
- Orthogonal similarity assessment
- Regulatory compliance with Spectra Manager™ 2.5 CFR

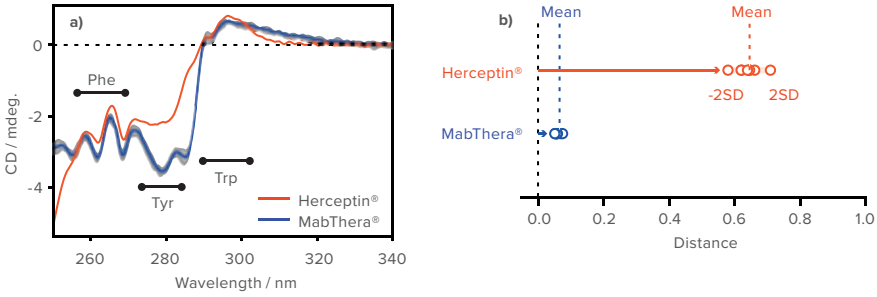
*U.S. patent No.: 12,339,218



Spectral Identity Studies of Therapeutic Antibodies

Application

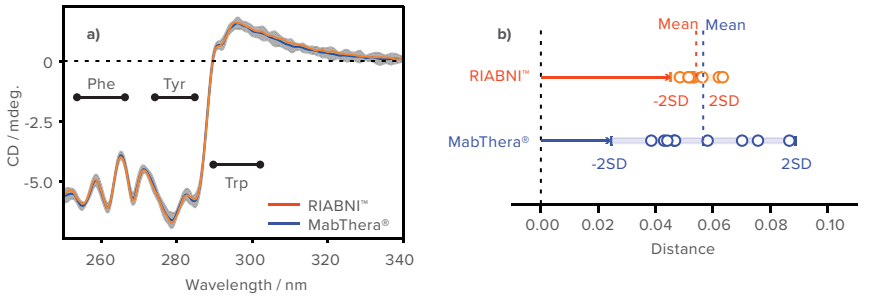
While the tertiary structure of Herceptin® and MabThera®, which are different therapeutic agents, are not identical, the structure of MabThera® and its biosimilar, RIABNI™, are statistically determined to be identical.



Result of *t*-test
***t*-value = 49**
***p*-value = 0.0**
Similarity failed

Results of HOS similarity assessment for different antibody drugs

a) Mean spectra of MabThera® (blue), Herceptin® (red), and standard deviation for MabThera® (gray).
b) Distance and test results for MabThera® (blue) and Herceptin® (red).



Result of *t*-test
***t*-value = 1.098**
***p*-value = 0.15**
Similarity passed

Results of HOS similarity assessment for Rituximab innovator (blue) and biosimilar (red)

a) Mean spectra of MabThera® (blue), RIABNI™ (red), standard deviation for MabThera (gray).
b) Distance and test results for MabThera® (blue) and RIABNI™ (red).

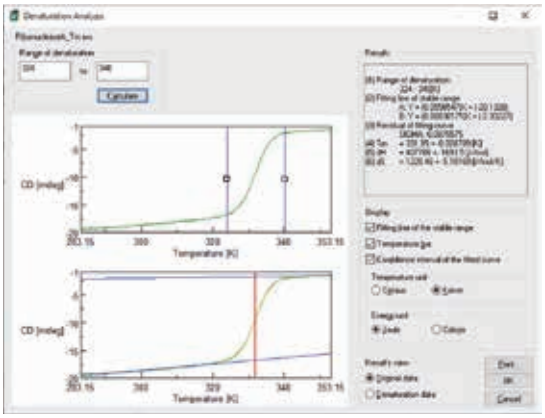
Application Software

Denatured Protein Analysis Program

This program evaluates CD data at various temperatures, enabling the calculation of different thermodynamic parameters (T_m, ΔH, ΔS) for a protein.

$$N \underset{}{\overset{K_D}{\rightleftharpoons}} D$$

$$\ln K_D = -\frac{\Delta H}{R} \cdot \frac{1}{T} + \frac{\Delta S}{R}$$



K_D is the ratio of [N] and [D], and is calculated by using the experimental data, which is the measured CD values θ_i as a function of temperature T_i. This program approximates θ_i by the following equation and calculates the K_D.

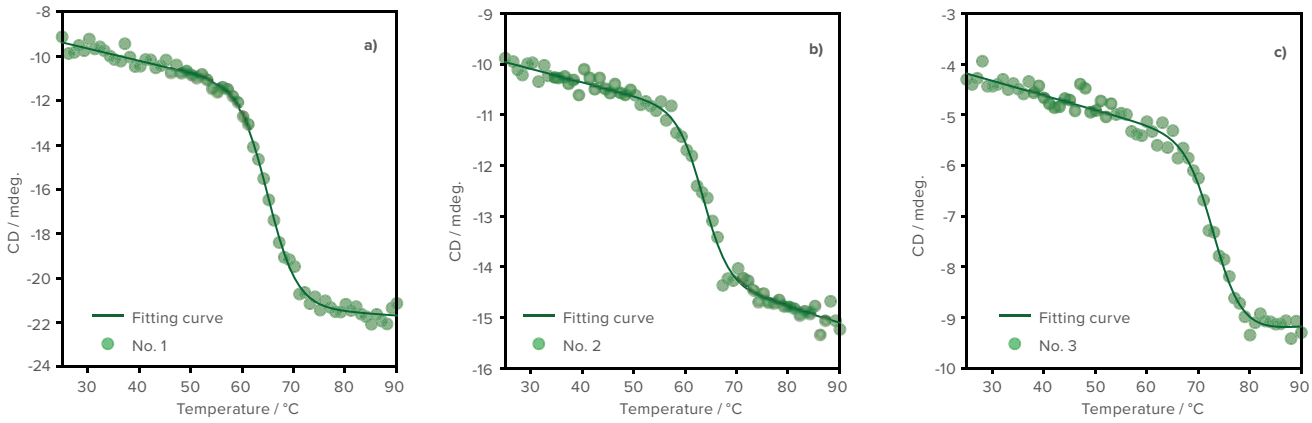
$$\tilde{\theta}(T) = \frac{\theta_N(T) + K_D(T)\theta_N(T)}{1 + K_D(T)}$$

$$\theta_n(T) = \sum_{i=1}^{N} a_{i,n} T^{N-i} \qquad K_D(T) = \sum_{i=1}^{N} A_{i,D} T^{N-i} \qquad N = 2$$

Denaturation Study

Application

The below figure shows the denaturation curve of the CD at 217 nm which reflects changes in β-sheet structure of the VHH antibody. Comparing the denaturation temperatures, the thermal stability of the samples decreases in the following order: No. 3, 1 and 2.

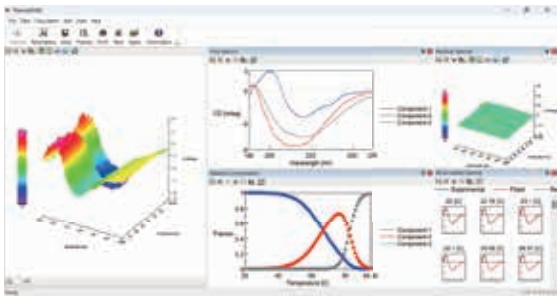


Temperature vs. CD denaturation curves

Anti-SARS-Cov-2 VHH antibody a) No. 1 (0.4 mg/mL), b) No. 2 (0.28 mg/mL), and c) No. 3 (0.2 mg/mL).

ThermaFit3D Program

ThermaFit3D program provides deeper insights into multi-state protein and nucleic acid denaturation by analyzing the entire spectrum, delivering results that single wavelength monitoring cannot achieve.
By applying MCR-ALS* constrained by thermodynamic models to temperature-dependent CD spectra (3D CD spectra), it is possible to obtain spectra of pure components, concentration profiles of each component, and thermodynamic parameters for each phase transition (T_m, ΔH, ΔS). ThermaFit3D automatically provides residual spectra, helping confirm the reliability of every analysis.



$$N \overset{K_1}{\rightleftharpoons} I \overset{K_2}{\rightleftharpoons} U \quad K_i = \exp\left[\frac{\Delta H_i}{R}\left(\frac{1}{T_{m,i}} - \frac{1}{T}\right)\right]$$

* U.S. patent application No.: 18/436,498

Developed through joint research with Ikebukuro Tsugawa Asano Laboratory at Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology. For details of analysis using ThermaFit3D program, please refer to the following paper:

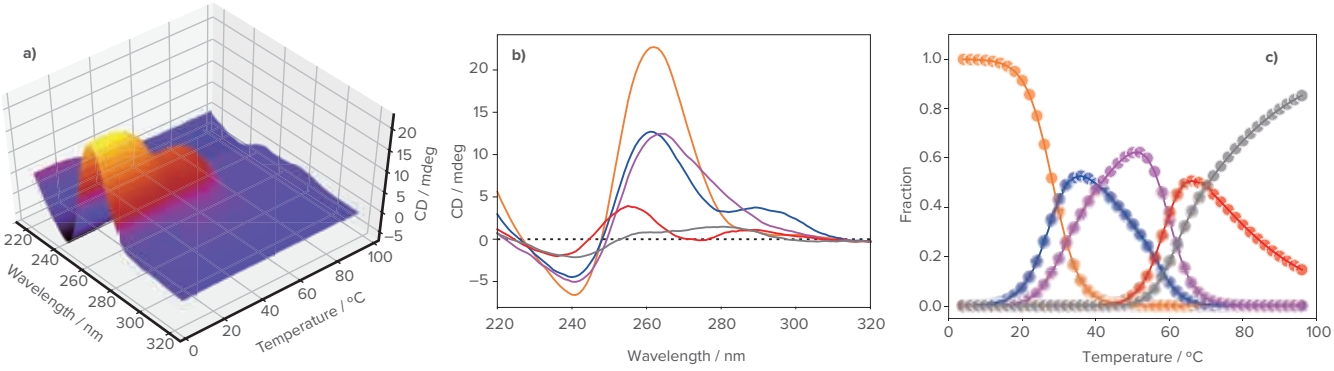
Functional and Structural Analyses of Diverse G-Quadruplex and Non-G-Quadruplex Structures Formed by Guanine-Rich Nucleic Acids: A Study on the Insulin Aptamer. doi:10.1002/smll.202501336.

Deconvolution of Mixed -state structure in Insulin-Binding Aptamer

Application

Beyond canonical double-helical structure, nucleic acids are capable of adopting diverse high-order structures, including triplex and quadruplex. Furthermore, it has been reported that these higher-order structures exist in equilibrium states and dynamically change in response to the surrounding conditions. However, extracting information from complex, mixed samples is not straightforward. ThermaFit3D analyzes CD data to break down complex higher-order structures and reveal their proportions.

Using ThermaFit3D program reveals that the insulin-binding aptamer, previously assumed to adopt a single conformation, actually forms five distinct structures in equilibrium.



Analysis result of mixed structural states in insulin-binding aptamers
a) Temperature-dependent CD Spectra (3D CD Spectra), b) Spectra of pure components , and c) Concentration profiles

JASCO CORPORATION
2967-5, Ishikawa-machi, Hachioji-shi, Tokyo 192-8537 Japan
Tel: +81-42-649-5177 Fax:+81-42-646-4515 Web: www.jasco.co.jp
Japan

JASCO INTERNATIONAL CO., LTD.
11-10, Myojin-cho 1-chome, Hachioji-shi, Tokyo 192-0046, Japan
Tel: +81-42-649-3247 Fax: +81-42-649-3518 Web: www.jascoint.co.jp/english/
Australia, Hong Kong, India, Indonesia, Korea, Malaysia, New Zealand, Pakistan,
Philippines, Russia and CIS countries, Singapore, Taiwan, Thailand, Vietnam

JASCO INCORPORATED
28600 Mary's Court, Easton, Maryland 21601, U.S.A.
Tel: +1-410-822-1220 Fax: +1-410-822-7526 Web: www.jascoinc.com
Argentina, Bolivia, Brazil, Canada, Chile, Colombia, Costa Rica, Guatemala, Mexico,
Paraguay, Peru, Puerto Rico, United States of America, Uruguay, Venezuela

JASCO EUROPE S.R.L.
Via Luigi Cadorna 1, 23894 Cremella (LC), Italy
Tel: +39-039-9215811 Fax: +39-039-9215835 Web: www.jascoeurope.com
JASCO Deutschland www.jasco.de | **JASCO UK** www.jasco.co.uk | **JASCO France** www.jascofrance.fr
JASCO Benelux www.jasco.nl | **JASCO Spain** www.jasco-spain.com
Algeria, Austria, Belgium, Cyprus, Denmark, Egypt, Finland, France, Germany, Greece, Hungary,
Israel, Italy, Jordan, Kuwait, Luxembourg, Morocco, Netherlands, Norway, Poland, Portugal, Romania,
Saudi Arabia, South Africa, Spain, Sweden Switzerland, Tunisia, Turkey,
United Arab Emirates, United Kingdom, Yemen

JASCO CHINA (SHANGHAI) CO., LTD.
Room 603B, Building B, No. 1168 Century Avenue, Pudong New Area, Shanghai, P.R.China
Tel: +86-21-6888-7871 Web: www.jasco-global.com
China

ISO-9001
ISO-14001
CERTIFIED

Products described herein are designed and manufactured by ISO-9001 and ISO-14001 certified JASCO Corporation

6910-2510ENG