## **AppliedPhotophysics**

## CHIRASCAN AND CHIRASCAN V100 VERSATILE RESEARCH SYSTEMS



## HIGH PERFORMANCE, READY TO RUN

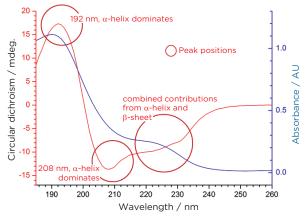
- Determine structural and thermodynamic properties
  - Gain insight and detect changes in secondary and tertiary structure
  - Determine response to thermal or chemical changes
  - Study folding and unfolding mechanisms
- Achieve highest sensitivity and accuracy
- Generate highest quality data
- Optimize sample concentration and absorbance
- Expand capabilities with dedicated Chirascan accessories

Discover when change is significant

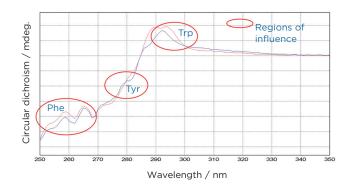
### DETERMINE STRUCTURAL AND THERMODYNAMIC PROPERTIES

# Gain insight and detect changes in secondary and tertiary structure

Secondary structure: far-UV spectrum of a globular protein



Simultaneous acquisition of CD and absorbance spectra, 0.5 mm pathlength, Chirascan V100. Courtesy of leading research university, Germany Tertiary structure: near-UV spectra of two monoclonal antibodies



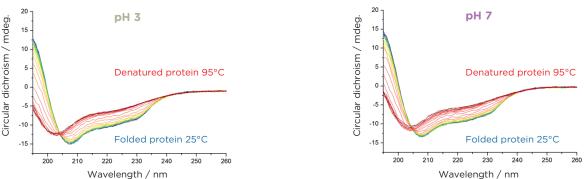
Differences between near-UV spectra due to slight changes in orientation of aromatic moieties, Chirascan V100, 10 mm pathlength

# Determine thermodynamic properties – continuous thermal ramping

- Monitor at each wavelength
- Typical run: 70 spectra in 70 min, 1°C/min
- Record temperature directly thermocouple in sample
- Derive melting points and enthalpies for multiple thermal transitions
- Associate change in structure with each thermal transition

рН	Melting temperature (°C)	van't Hoff enthalpy (kJ/mol)
pH 2	55.4	354
pH 3	69.4	385
pH 4	75.8	380
pH 5	76.9	400
pH 6	74.2	423
pH 7	72.7	367

Six datasets analyzed using Chirascan global thermodynamic analysis



Two of six denaturation datasets acquired at pH 2-7, lysozyme, Chirascan 6-cell turret, Chirascan V100, raw data, no baseline adjustment, no smoothing, 0.5 mm pathlength

#### Effect of pH on thermal denaturation

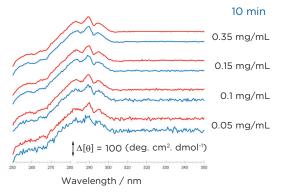
## ACHIEVE HIGHEST SENSITIVITY AND ACCURACY

Since their introduction in 2005, Chirascan<sup>™</sup> systems have continued to feature in thousands of peer-reviewed publications covering a wide range of research areas. Chirascan V100 now offers the increased sensitivity and accuracy preferred for CD analysis of biomolecules.

- Avalanche photodiode detector enhances sensitivity
- · Increased signal:noise compared to conventional photomultiplier
- Accurate normalization from simultaneous measurement of absorbance and CD

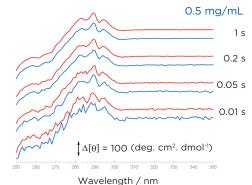
Increased sensitivity when sample is limited

Chirascan V100 (avalanche photodiode detector) Chirascan (photomultiplier)



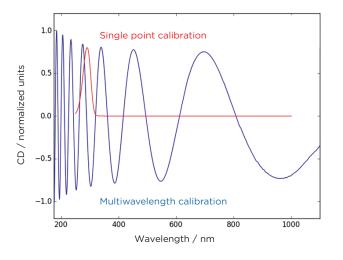
Tertiary structure of lysozyme – raw data, no smoothing, 10 min. baseline / 10 min. sampling, n=3 scans, 0.5 nm step, 10 mm pathlength, spectra offset for clarity Increased sensitivity: faster measurements for thermal studies or photolabile samples

Chirascan V100 (avalanche photodiode detector) Chirascan (photomultiplier)



Tertiary structure of lysozyme – raw data, no smoothing, baseline corrected, n=3 scans, 1 nm step, 10 mm pathlength, spectra offset for clarity

- Accurate CD values across entire wavelength range
- Overcome challenges of chemical calibration
- Optics-based, multiwavelength calibration



Conventional chemical calibration methods require considerable skill in preparation. Standards, such as camphor-10-sulfonic acid (CSA), are unstable, photolabile and hygroscopic. In addition, single wavelength calibration (290.5 nm) assumes the same linear response at all wavelengths.

The optics-based, multiwavelength calibration method used in Chirascan V100 overcomes these challenges. The correct calibration is applied to every wavelength to yield accurate CD values.

## **READY TO RUN - GENERATE HIGHEST QUALITY DATA**

Chirascan systems are supplied with features and accessories required for acquisition of high quality CD data - from built-in temperature control during analysis to cuvettes for the most common analytical conditions.\* A basic training program follows installation to familiarize users new to Chirascan.

#### AVALANCHE PHOTODIODE DETECTOR (CHIRASCAN V100)

• Highest sensitivity (high signal: noise)

PHOTOMULTIPLIER DETECTOR (CHIRASCAN)

#### PHOTOELASTIC MODULATOR

• Converts horizontally polarized light to circularly polarized light. Alternates between left- and right-handed circular polarized light

#### MONOCHROMATOR

- Produces horizontally, linearly polarized monochromatic light
- Two polarizing prisms maximize light throughput

#### AIR-COOLED XENON LAMP

- Software-controlled
- Up-time recorded

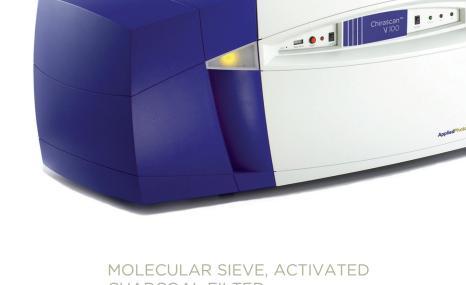
#### ACTIVE NITROGEN MANAGEMENT SYSTEM

- Regulates purge gas consumption
- Software-controlled

#### MOLECULAR SIEVE, ACTIVATED CHARCOAL FILTER

• Removes common gas impurities

\* Your local Applied Photophysics representative can supply specific details of components supplied for your region.





#### TEMPERATURE-CONTROLLED SAMPLE CHAMBER

- Consistent analytical conditions
- Continuous temperature ramps
- Temperature measured directly in sample

#### OPTICS-BASED, MULTIWAVE-LENGTH CALIBRATION

• For CD accuracy (Chirascan V100)

#### CUVETTES AND HOLDERS

• Selected for far- and near-UV CD analysis of biomolecules



#### WATER CIRCULATOR

• Dissipates heat from sample chamber Peltier

## CONTROL AND ANALYSIS SOFTWARE



#### CHIRASCAN CONTROL

- Easily define run parameters and store routine protocols
- Saves time with scheduled start-up/ shutdown of lamp and N<sub>2</sub> supply
- Fail-safe lamp switch-off if N<sub>2</sub> flow drops
- Ensures O<sub>2</sub>-free conditions with N<sub>2</sub> purge

## GLOBAL THERMODYNAMIC ANALYSIS

• Derive melting points and enthalpies from multiwavelength, thermal denaturation experiments

## OPTIMIZE SAMPLE CONCENTRATION AND ABSORBANCE: CUVETTES AND HOLDERS

Selecting a suitable cuvette with optimal pathlength is critical to acquisition of highest quality data. Cuvettes for Chirascan systems are manufactured from far-UV quartz to enable analysis of secondary structure. The range of cuvettes and compatible holders provides full flexibility when optimizing sample concentration and absorbance.

For secondary structure (far-UV) analysis				
0.5 mm 175 μL one-piece stoppered cuvette Adaptor for 0.5 mm and 1 mm one-piece cuvettes	Supplied with Chirascan and Chirascan V100 Not suitable for fluorescence			
1.0 mm 350 μL one-piece stoppered cuvette	Not suitable for fluorescence; requires adaptor			
2.0 mm 700 μL one-piece stoppered cuvette Spacer for 2 mm pathlength cuvette	Not suitable for fluorescence			
2.0 mm 400 μL one-piece stoppered cuvette for manual systems	Simultaneous measurement of secondary structure by CD and tertiary structure by fluorescence. No adaptor needed. Not compatible with Chirascan 6-cell turret.			
4.0 mm 1400 $\mu L$ one-piece stoppered cuvette	Simultaneous measurement of secondary structure by CD and tertiary structure by fluorescence. No adaptor needed.			
0.1 mm 160 μL cylindrical cell, Holder for cylindrical cells	Useful for highly absorbing/chiral buffers. Scan further into far-UV at fixed pathlength. Not suitable for fluorescence.			
For tertiary structure (near-UV) analysis				
10 mm 3500 μL one-piece stoppered cuvette	Supplied with Chirascan and Chirascan V100 Suitable for fluorescence, certified free from strain birefringence.			
10 mm 600 μL one-piece stoppered cuvette Holder for 10 mm 600 μL cuvettes	Suitable for fluorescence; reduced volume			
5.0 mm 1750 μL one-piece stoppered cuvette Spacer for 5 mm pathlength cuvettes	Not suitable for fluorescence.			

For secondary structure analysis with rapid cell cleaning. Scan further into the far-UV Not suitable for fluorescence or for use with chiral buffers			
Adaptor for demountable/slide cells			
0.01 mm 3 μL demountable/slide cell			
0.1 mm 30 μL demountable/slide cell			
0.2 mm 60 μL demountable/slide cell			
0.5 mm 150 μL demountable/slide cell			

## EXPAND CAPABILITIES WITH DEDICATED CHIRASCAN ACCESSORIES

#### CCD FLUOROMETER

Monitor changes in fluorescence



#### TITRATOR AND PH PROBE

Monitor concentration- and pH-dependent changes in CD, fluorescence or absorbance



#### 6-CELL TURRET

Increase capacity and productivity



#### LD COUETTE CELL

Use linear dichroism to gain insight into conformation and orientation of molecular structures



### STOPPED-FLOW

Characterize fast reactions, complement CD spectra with kinetic information



#### INTEGRATING SPHERE AND SOLID SAMPLE HOLDER

See changes in chirality of solid state samples



#### ALSO AVAILABLE

A fluorescence anisotropy detector to characterize ligand binding events. An optical rotatory despersion accessory to characterize chiral molecules. A magnetic CD accessory for study of molecules such as metalloproteins. A near IR extension kit to expand scanning range of a Chirascan V100 to 1700 nm.

Please contact your Applied Photophysics representative to discuss requirements. and system compatibility.

## PRODUCT SPECIFICATIONS

Performance characteristics					
Spectral information	CD, absorbance as standard, Fluorescence and other detection modes available				
Isothermal analysis, typical measuring time	Full spectrum < 2 min				
Isothermal analysis, typical sample consumption	Secondary structure (far-UV), 0.5 mm pathlength, cell width 9 mm: mAb 0.06 mg Tertiary structure (near-UV), 10 mm pathlength, cell width 10 mm: mAb 2.8 mg Tertiary structure (near-UV), 10 mm pathlength, cell width 4 mm: mAb 0.5 mg				
Thermal denaturation (thermal ramping)	Full spectrum per 1°C, continuous ramp rate 1°C/min				
Technical specifications	Chirascan V100	Chirascan			
Light source	150W air-cooled Xenon arc lamp				
Monochromator	Two polarizing prisms to maximize light throughput				
Detection	Avalanche photodiode	Photomultiplier			
Wavelength range Note: using quartz prisms within monochromator limit measurements to wavelengths > 163 nm	163 nm to 1150 nm Typical wavelength range for biomolecule analysis 180 nm to 350 nm	163 nm to 900 nm Typical wavelength range for biomolecule analysis 180 nm to 350 nm			
Wavelength resolution	±0.1 nm				
CD calibration	Optics-based, multiwavelength Accuracy ±1% determined across wavelength range (selected wavelengths)	Chemical-based, single point			
Measurement error on absolute absorbance	< 0.01 AU (simultaneous measurement of CD and absorbance signals)	< 0.1 AU			
Bandwidth	160 nm: up to 2 nm 180 nm: up to 4 nm 200 nm: up to 7.5 nm 240 nm: up to 16 nm				
Bandwidth precision	±0.1 nm at 267 nm				
Stray light	< 3 ppm at 200 nm				
Typical Root Mean Square (RMS) noise values, no sample in place, 1 nm bandwidth, 2 s digital integration time – no smoothing, no rolling average	0.03 mdeg at 185 nm 0.03 mdeg at 250 nm 0.03 mdeg at 500 nm	0.045 mdeg at 185 nm 0.045 mdeg at 250 nm 0.055 mdeg at 500 nm			
Baseline stability (16 h drift test)	< 0.4 mdeg	< 0.5 mdeg			
Sample temperature during analysis, coolant at 15°C or above	Hardware tolerance: -20°C to +105°C Typical range for biomolecule analysis: 4°C to 95°C				
Data handling and storage					
PC operating system	Microsoft® Windows® 7 Professional, 64 bit				
Data storage and export	Storage in proprietary format, exportable as .csv				
Compliance					
Electrical safety and other regulatory requirements	Standard: IEC/EN 61010-1:2010.Standard: IEC/EN 61010-1:2010. USA National Registered Testing Laboratory (NRTL) under OHSA Federal code 29 CFR 1910.7. Canada. Approval agency TUV-SUD. Standard: UL 61010 1:2012, CAN/CSA C22. No. 61010-1:2012 EU Restriction of Hazardous Substances Directive (ROHS) 2011/65/EU Standard: EN 50581:2012 (Cat 9 Monitoring and control instruments) EU electromagnetic compatibility directive (EMC) 2004/108/EC Standard: IEC/EN 61326-1:2013 (EMC Class A Group 1)				
Physical and environmental specifications					
Instrument weight and dimensions (WxDxH)	60 kg, 150 x 55 x 60 cm				
Operating conditions: temperature	20 to 25°C controlled to within 1.5°C				
Operating conditions: humidity	20 to 80 % non-condensing				
Nitrogen requirement (flow rate, pressure, purity)	> 5 L per min, > 4 bar, > 99.998%				
Electrical requirements (Voltage, Frequency, Power) 100 to 240 VAC, 50/60 Hz, UPS rated to ≥1500 VA					

#### Ordering information

To order Chirascan systems or accessories, please contact your local Applied Photophysics representative to discuss your specific requirements or submit your enquiry online at www.photophysics.com.

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