AppliedPhotophysics

# TAKING CIRCULAR DICHROISM ONE STEP FURTHER

## Chirascan spectrometers – more than α-helix and β-sheet

Discover when change is significant

*"Circular dichroism has been used/proposed in 96% of biosimilar applications involving mAbs and other biotherapeutics"*

***Regulatory consideration for characterization of HOS in biotechnology products, M. T. Gutierrez Lugo, Ph. D., OBP/CDER/FDA. 5th International Symposium on HOS of Protein Therapeutics 2016***

*"For us, minor differences detected upstream are as important as proving similarity downstream."*

***Head of a Biosimilars Division***

A CD reminder!

Circular dichroism (CD) is the difference in absorption of left-handed circularly polarized light and right-handed circularly polarized light that occurs when a molecule contains one or more light-absorbing groups (chromophores) that are chiral or in a chiral environment.

# MORE THAN α-HELIX AND β-SHEET

Suitable for studying chiral molecules of all types and sizes, Chirascan™ CD spectrometers are optimized for analysis of biomolecules.

## Deeper understanding – see changes in secondary and tertiary structure

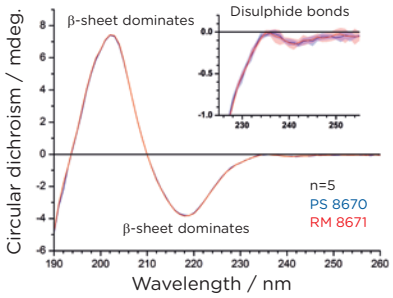
Today, Chirascan systems provide unique insights into changes in secondary and tertiary structure as well as kinetic and thermodynamic information. Thousands of peer-reviewed publications demonstrate the role of Chirascan systems in deepening our understanding of biomolecular characteristics, mechanisms and interactions.

## Make informed decisions – objective, statistically-validated HOS analysis

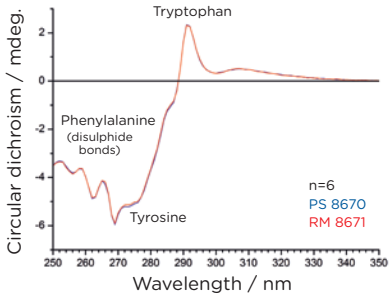
The slightest change in higher order structure (HOS) can significantly impact efficacy and immunogenicity of a biotherapeutic. HOS is one of many critical quality attributes used to define identity, purity, potency and stability when characterizing a complex biopharmaceutical.
The top-of-the-range Chirascan Q100 enables scientists to make informed decisions based on objective, statistically-validated data.

## Comparison of NIST mAb standard with known variant

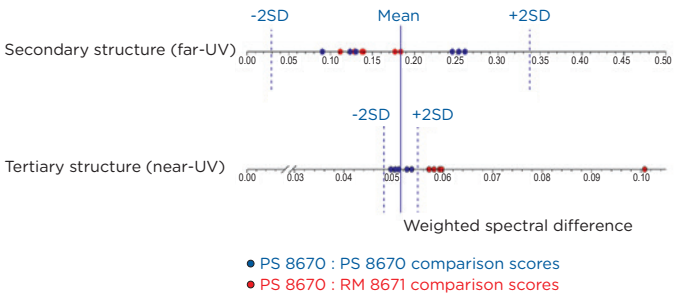
Secondary structure: are spectra superimposable in far-UV?




Tertiary structure: are small differences in near-UV significant?




Differences in secondary structure not significant*
Differences in tertiary structure are significant*




* Tier 2 statistical analysis of data from Chirascan Q100 using 2SD acceptance criteria

# Chirascan™

- Increase productivity with Chirascan 6-cell turret
- Expand capabilities with Chirascan accessories, see page 12-13
- Upgrade to Chirascan V100

## TEMPERATURE-CONTROLLED SAMPLE CHAMBER

- For consistency during analysis
- For thermal denaturation (continuous temperature ramping)

## CHIRASCAN CONTROL

- Ready to run
- Lamp start/shutdown and $N_2$ purge scheduling ensures $O_2$-free conditions

## PHOTOMULTIPLIER DETECTOR


# VERSATILE, HIGH PERFORMANCE RESEARCH SYSTEMS

For more details, request the Chirascan V100 and Chirascan product information sheet or visit www.photophysics.com to download

# Chirascan™ V100

This takes CD analysis to a new level. An avalanche photodiode detector replaces the conventional photo-multiplier to increase sensitivity while optics-based, multiwavelength calibration ensures accuracy.

- Includes software for global thermodynamic analysis of continuous, multiwavelength temperature ramps
- Add dedicated Chirascan accessories to increase productivity or expand capabilities, see page 12

## CUVETTES

- Choice of pathlengths to enable control of sample concentration and absorbance

## AVALANCHE PHOTODIODE DETECTOR

- Higher signal: noise increases sensitivity to enhance data quality
- Faster acquisition of CD spectra in near- and far-UV
- Records CD and absorbance simultaneously for accurate normalization
- Facilitates analysis when sample or time is limited
  - Obtain more datapoints during thermal denaturation
  - Study photolabile samples

## OPTICS-BASED, MULTIWAVELENGTH CALIBRATION

- For CD accuracy at every wavelength
- Eliminates uncertainty and variability of single wavelength chemical calibration

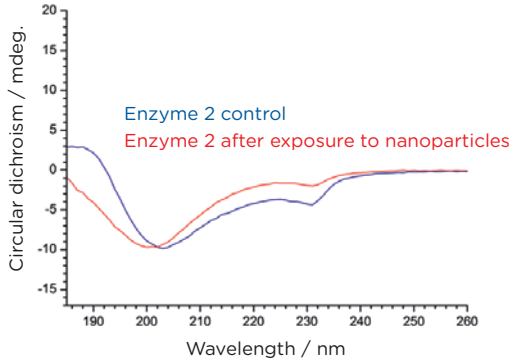
## WHEN SENSITIVITY AND ACCURACY ARE KEY

# Advance your research, publish with confidence

## Detect changes in secondary and tertiary structure

### Effect of transient exposure to nanoparticles on a globular protein

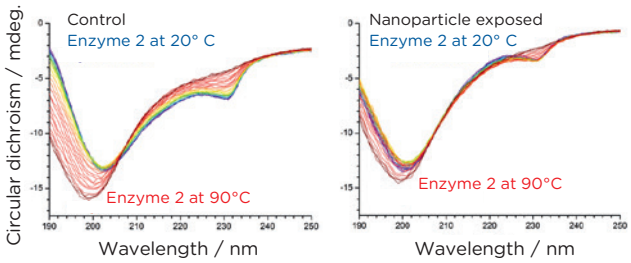
(Data courtesy of leading research university, Germany)




Spectra normalized for protein concentration by simultaneous absorbance measurements, 0.5 mm pathlength, Chirascan™ V100

#### Determine structural effects

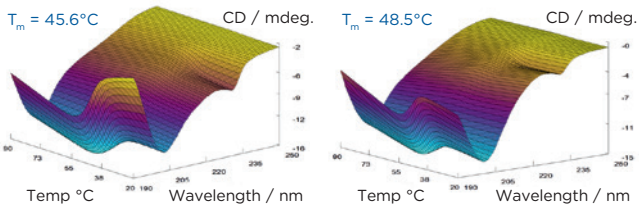
Significant change in far UV CD spectrum of an enzyme indicated perturbation of secondary structure by nanoparticle exposure




Continuous temperature ramp 20°- 90°C, 71 spectra in 71 min, 1°C/min, 0.8 sec per point, 1 nm bandwidth, 1 nm step, Chirascan™ V100

#### Determine effect on protein stability

Thermal denaturation curves showed altered folding profile after nanoparticle exposure. Global fit of multiwavelength data confirmed a 2.9°C change in melting temperature.




Global fit of multiwavelength thermal denaturation data

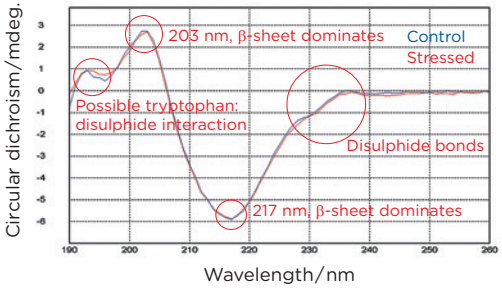
#### Conclusion

Exposure to nanoparticles altered folding of a globular protein which, in turn, enhanced protein stability.

- Deepen understanding of biomolecular characteristics, mechanisms and interactions
- Detect minor differences under native or stressed conditions
- Characterize protein stability
  - Determine response to thermal or chemical changes
  - Determine structural and thermodynamic properties
  - Study folding and unfolding mechanisms

## Comparison of native and stressed mAb

(Data courtesy of biotherapeutic development company)

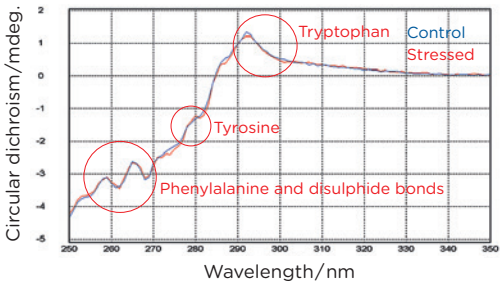



Spectra normalized for protein concentration by simultaneous absorbance measurements, 0.5 mm pathlength, Chirascan™ V100

### Gain insight into secondary structure

Typical beta-sheet structure retained in native and stressed mAb.

Disulphide bond environment unchanged.




Spectra normalized for protein concentration by simultaneous absorbance measurements, 10 mm pathlength, Chirascan™ V100

### Reveal tertiary structure

Environment of aromatic amino acid side-chains appears similar.

### Conclusion

Review of spectra suggests no difference between native and stressed mAb.

Note: A subsequent HOS comparison study using a Chirascan™ Q100 system confirmed that minor differences in tertiary structure were statistically significant (data not shown).

# UNMATCHED PERFORMANCE AND PRODUCTIVITY

## TEMPERATURE-CONTROLLED CONDITIONS

- Sample storage: maintains sample integrity
- Sample chamber: ensures consistent analytical conditions

## AVALANCHE PHOTODIODE DETECTOR

- High signal:noise for highest sensitivity to enhance data quality
- Fast acquisition of CD spectra in near- and far-UV
- Records CD and absorbance simultaneously for accurate normalization
- Facilitates analysis when time or sample is limited


## OPTICS-BASED, MULTIWAVELENGTH CALIBRATION

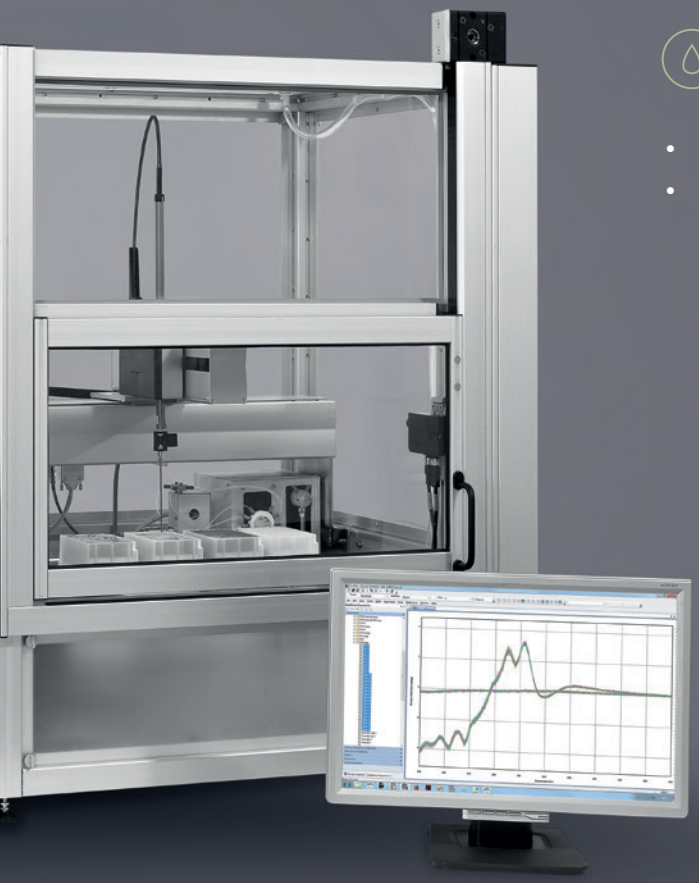
- For CD accuracy at every wavelength
- Eliminates variability and uncertainty of single wavelength chemical calibration

## CHIRASCAN CONTROL

- Ready to run
- Lamp start/shutdown and $N_2$ purge scheduling ensure $O_2$-free conditions
- Recognizes flow cell to select optimal run/wash/dry protocols

## SOFTWARE MODULES

- For statistical analysis of HOS comparisons
- For analysis of multiwavelength, thermal denaturation data (single sample mode)



## REPRODUCIBLE LIQUID HANDLING

- Facilitates statistical analysis
- Eliminates errors in manual handling

## FIXED FLOW CELL

- Eliminates errors of cuvette handling
- Recognized by Chirascan Control – selects correct run/wash/dry protocols
- Choice of pathlength 0.1 mm – 10 mm
- Records sample temperature

# Chirascan™ Q100

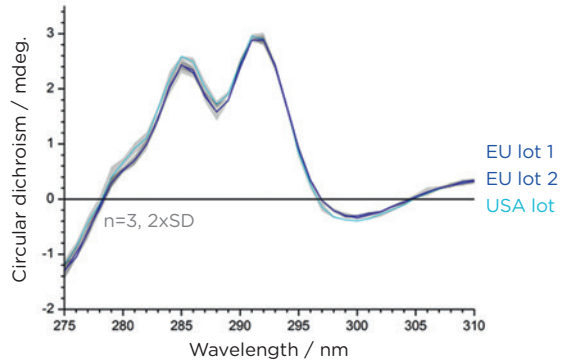
- Save days of operator time
- Fast set-up (30 min), unattended operation
- Run a minimum of 48 buffer-sample pairs in 24 h

For more details, request the Chirascan Q100 product information sheet or visit www.photophysics.com to download

# Make informed decisions, maintain project momentum

## Objective, statistically-validated HOS analysis

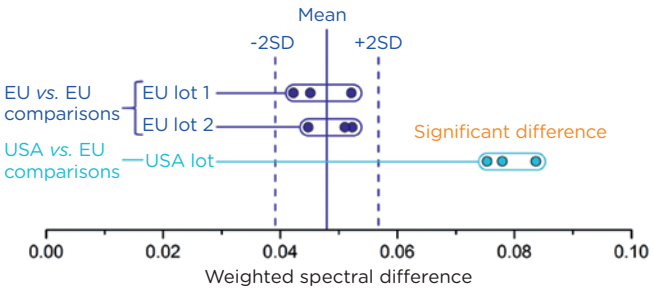
### From QTPP onwards: run effective comparability programs during biosimilar development




Analysis of tertiary structure. Spectra normalized for protein concentration by simultaneous absorbance measurements. Chirascan™ Q100, n=3

#### Determine innovator HOS characteristics

CD analysis of tertiary structure in a highly absorbing chiral formulation buffer suggested differences in tryptophan region of Fab fragments from different innovator lots.




Tier 2 quality range test applied, +/-2SD acceptance criteria Office of Biostatistics and Office of Biotechnology Products, CDER/FDA. Weighted spectral difference method: Dinh, Nikita et al. Anal. Biochem. 464 (2014): 60-62

#### Objective, quantifiable evaluation of differences and similarities

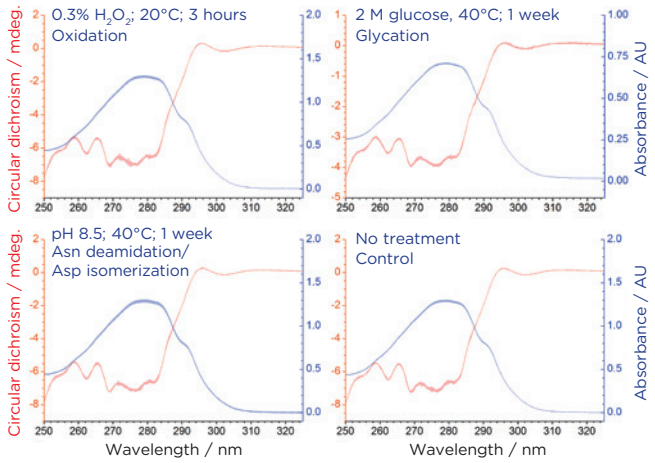
Differences in tertiary structure of innovator lots from different geographical regions were statistically significant.

Subsequent comparison between biosimilar and innovator confirmed statistical significance of minor differences seen in tertiary structure (data not shown).

More details at www.photophysics.com. A novel approach for objective, quantifiable HOS comparisons: a biosimilar case study utilizing circular dichroism (Poster)

- Monitor change throughout biotherapeutic development and scale-up
- Quantify differences and similarities under native or stressed conditions
- Define acceptable range for HOS variability within a control strategy for safety, quality, efficacy and manufacture
- Strengthen totality of evidence for regulatory submissions

## From lead characterization onwards: define characteristics, assess significance of change




Analysis of tertiary structure. Spectra normalized for protein concentration by simultaneous absorbance measurements. Chirascan™ Q100, n=6

### Monitor change under stressed conditions (forced degradation)

High sensitivity CD analysis of $IgG_1$ samples subjected to a range of degradation conditions revealed minor differences in tertiary structure when compared to a control sample.

| Sample | Condition | Expected affect | *p*-value |
|---|---|---|---|
| T1 | 0.3% $H_2O_2$, 20°C, 3 hours | Oxidation | 0.001 |
| T2 | 2 M glucose, 40°C, 1 week | Glycation | 0.001 |
| T3 | pH 8.5, 40°C, 1 week | Asn deamidation/ Asp isomerization | ≤0.001 |
| C | Sample preparation only (dialysis) | No effect (Control) | 0.807 |

*p*-value > 0.05; differences not significant at 2σ confidence interval
*p*-value < 0.05; differences significant at 2σ confidence interval

Weighted spectral difference method: Dinh, Nikita et al. Anal. Biochem. 464 (2014): 60-62

### Quantifiable assessment of changes in tertiary structure

Statistical analysis enabled objective confirmation of spectral results. Degradation conditions have affected local environment of aromatic side chains (no changes were detected in secondary structure – results not shown).

More details at www.photophysics.com. Assessment of statistical significance of minor changes in Higher Order Structure using circular dichroism – a new approach (Poster)

# More than just circular dichroism – dedicated Chirascan accessories


## CCD FLUOROMETER

### Monitor changes in fluorescence

Controlled through Chirascan software, the CCD fluorometer generates emission spectra in seconds providing CD, absorbance and fluorescence data in a single experiment.

## 6-CELL TURRET

### Increase capacity and productivity

Controlled through Chirascan software, the 6-cell turret enables analysis of up to six samples or 6 thermal denaturation data sets per run. Precision Peltier temperature control and magnetic stirring provide optimal conditions for thermal denaturation (temperature ramp) experiments.

## TITRATOR AND pH PROBE

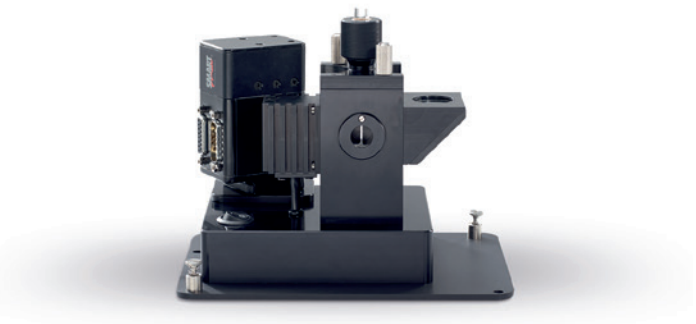
### Monitor concentration- and pH-dependent changes in CD, fluorescence or absorbance

Fully automated and controlled through Chirascan software, this dual syringe titration system systematically varies concentration of solution while maintaining constant volume. The optional pH probe enables measurement of sample pH *in situ*.

## LD COUETTE CELL

### Gain insight into conformation and relative orientation of molecules

The LD Couette Cell enables study of macro-molecular structures and their interactions e.g. DNA-ligand binding or orientation of membrane proteins. These structures are either intrinsically oriented or can be oriented during an experiment.



## STOPPED-FLOW

### Characterize fast reactions, complement CD spectra with kinetic information

Using the world-leading design of the stopped-flow accessory enables study of reactions in solution and determination of rate constants. Changes in CD, absorbance, fluorescence or fluorescence polarization can be monitored as a function of time.

## INTEGRATING SPHERE AND SOLID SAMPLE HOLDER

### See changes in chirality of solid state samples

The integrating sphere contains a large reflecting area to ensure high signal:noise when collecting CD data from highly scattering samples such as solids and powders.

A wheel in the solid sample holder enables rotation of transparent samples such as KBr discs to eliminate birefringence effects.

## ALSO AVAILABLE

A FLUORESCENCE ANISOTROPY DETECTOR to characterize ligand binding events.

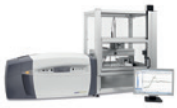
An OPTICAL ROTATORY DISPERSION accessory to characterize chiral molecules at high concentration, where absorption bands are obscured by solvent or buffer salt absorption, or when measuring at wavelengths where a chiral molecule does not necessarily absorb light.

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.

# Select the Chirascan system that meets your needs

| Chirascan systems are delivered ready to run, including flow cells or cuvettes selected for the most common analytical conditions. Installation is followed by a basic training program. | Chirascan™  | Chirascan™ V100  | Chirascan™ Q100  |
|---|---|---|---|
| **Performance // • Good •• Better ••• Best** | | | |
| Secondary and tertiary structure analysis | • | •• | •• |
| HOS comparisons | • | •• | ••• (generate statistically-validated data) |
| Chemical denaturation studies | • | •• | •• |
| Thermal denaturation studies – continuous thermal ramp | • | •• | •• (single sample mode) |
| High signal: noise to maximize sensitivity | • | •• | ••• |
| **Key features** | | | |
| System control and data acquisition software | ✓ | ✓ | ✓ |
| Scheduled start-up/shutdown to ensure oxygen-free conditions: software-controlled lamp and nitrogen-purge | ✓ | ✓ | ✓ |
| Temperature-controlled sample chamber for consistent analytical environment | ✓ | ✓ | ✓ |
| Flow cells for analysis of secondary (0.1 mm pathlength) and tertiary structure (10 mm pathlength) with integrated thermocouples | – | – | ✓ |
| Cell holder including one-piece stoppered cuvettes (0.5 mm and 10 mm pathlengths) and in-sample thermocouples for single sample measurements | ✓ | ✓ | ✓ |
| Liquid handling: elimination of errors associated with manual handling of samples and cuvettes, increased precision | – | – | ✓ (Chirascan Autosampler) |
| 2x Peltier thermostated 96-well plate holders for 'in run' sample storage, 1x 250 mL bottle holder | – | – | ✓ |
| CD and absorbance for normalization | CD and approximate absorbance | CD and absolute absorbance | CD and absolute absorbance |
| Increased productivity | Up to 6 samples or 6 thermal denaturation datasets per run (with Chirascan 6-cell turret) | Up to 6 samples or 6 thermal denaturation datasets per run (with Chirascan 6-cell turret) | Minimum 48 buffer-sample pairs in 24 h |
| Optics-based, multiwavelength calibration for absolute CD accuracy | – | ✓ | ✓ |
| Water circulator for heat dissipation from Peltier units | ✓ | ✓ | ✓ |
| HOS comparison software for statistical analysis of data | – | – | ✓ |
| Modelling software for global analysis of multiwavelength thermal denaturation data | optional | ✓ | ✓ |
| IQ/OQ/PQ | – | optional | optional |
| Chirascan Support Plans | ✓ | ✓ | ✓ |
| Expansion of capabilities with Chirascan accessories (subject to compatibility) | ✓ | ✓ | ✓ (fluorescence only) |

For more information please visit:
**www.photophysics.com**

# TRAINING, SUPPORT AND SERVICE

To ensure every user obtains the highest quality data from their Chirascan system, Applied Photophysics Application Specialists offer basic and advanced training programs for new and experienced users on-site or in our UK and US facilities.

Maintaining optimal performance and receiving timely support when needed are critical for any high performance analytical system to maximize productivity and minimize downtime.

Routine Preventive Maintenance visits, guaranteed response times for support or emergency call outs and coverage of costs for parts, travel and labor are just some of the benefits offered in Chirascan Support Plans. Your Applied Photophysics representative will be happy to assist in selecting a plan best suited to your requirements.

*"Customer service in general is far above my experiences with other companies."*

*"Quick and knowledgeable support when needed."*

*"Staff are very courteous and helpful in providing me with responses in a very timely manner."*

***Extracts from Chirascan™ User Survey, 2016***

AppliedPhotophysics

# About us:

Applied Photophysics is a leading provider of systems and accessories for biophysical characterization. Headquartered in Leatherhead, Surrey, UK, the Company has been established for more than 40 years.

The SX-range of stopped-flow spectrometers, used to monitor changes in absorbance and fluorescence during fast biological and chemical reactions, is acknowledged globally as the gold standard for kinetic studies.

In 2005, the Company introduced the first Chirascan™ system, optimized to use the phenomenon of circular dichroism (CD) to reveal changes in the higher order structure of proteins, specifically their secondary and tertiary structure. Since then, the company has continued to incorporate its knowledge and CD expertise into a range of Chirascan systems and accessories. Today, Chirascan systems are used in cutting-edge research and to support the development of innovator drugs and biosimilars in the biopharmaceutical industry.

# Contact:

**Head office**
Applied Photophysics Limited
21 Mole Business Park
Leatherhead, Surrey
KT22 7BA
United Kingdom
Tel: +44 1372 386 537
info@photophysics.com

**Visit www.photophysics.com for up to date contact details of your local Applied Photophysics representatives.**



CS2000 v.0