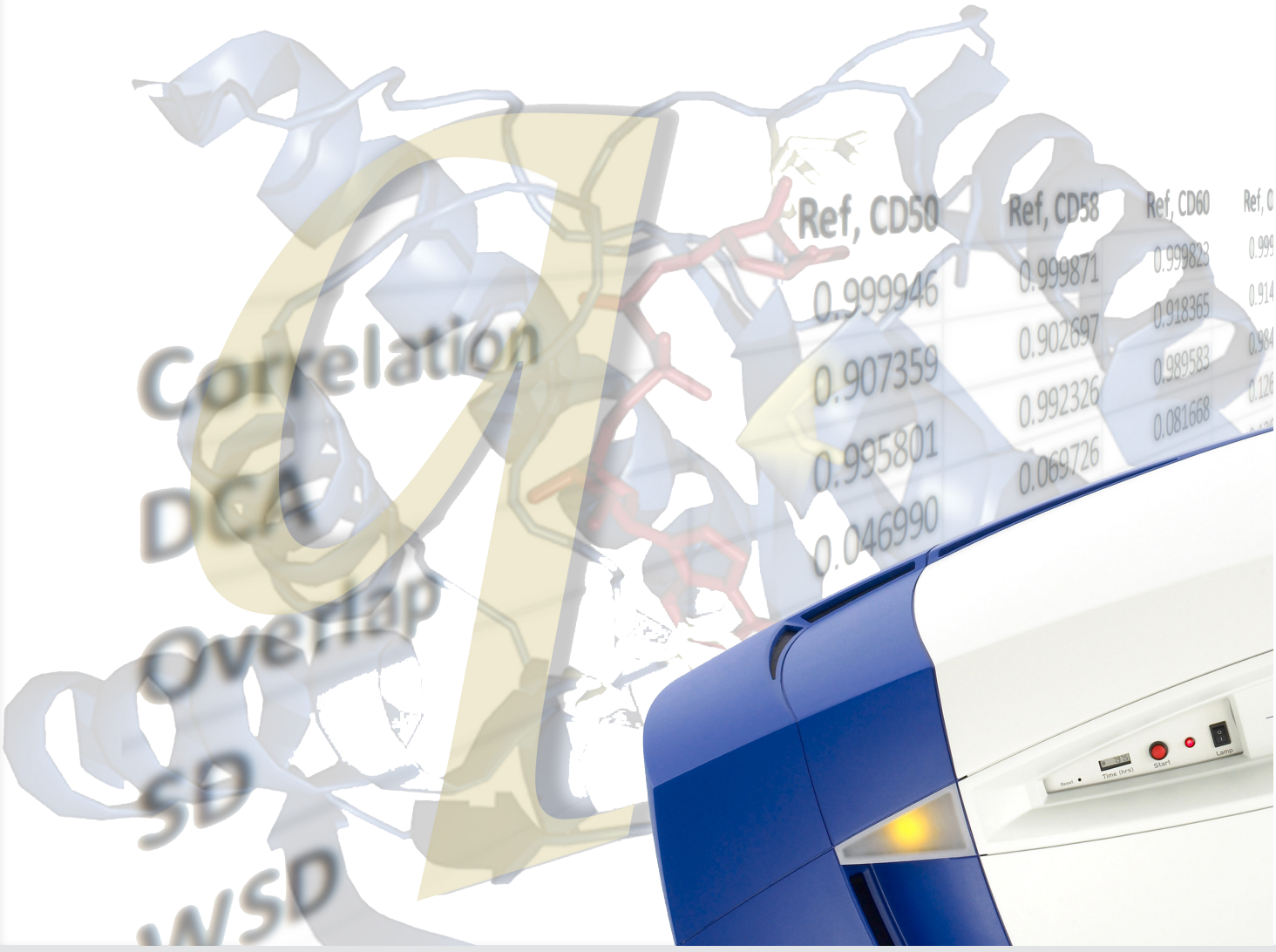
AppliedPhotophysics

More Time for Science

Quantitative Circular Dichroism

Chirascan *q*CD
Chirascan-plus *q*CD

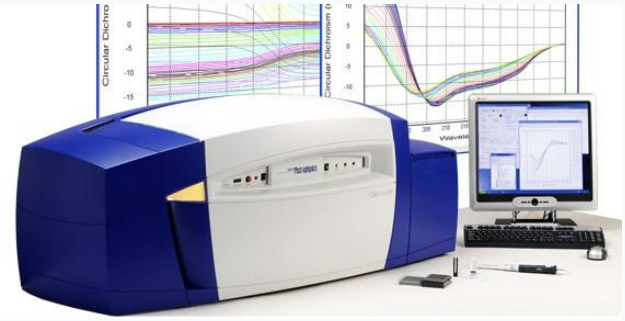


Instrument Overview

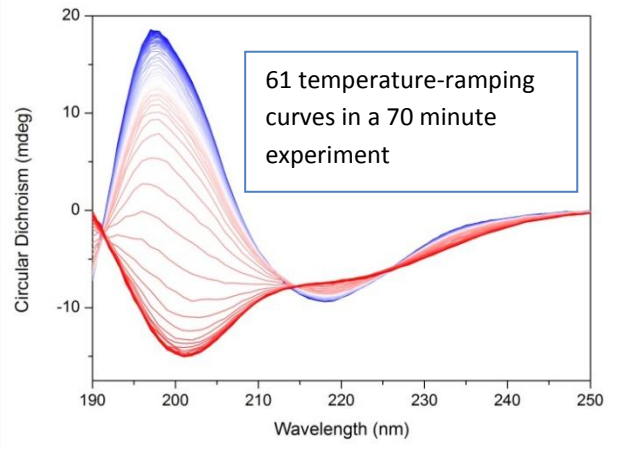
The new *q***CD** series introduces a range of capabilities which increase performance and productivity and which, crucially, make CD spectrometry a truly quantitative technique.

The Chirascan range of CD spectrometers has long been the most sensitive and the most advanced available. We invite you to take a look at how the new *q***CD** series can benefit your research - whether for scientific or pharmaceutical applications.

CHIRASCAN *q*CD

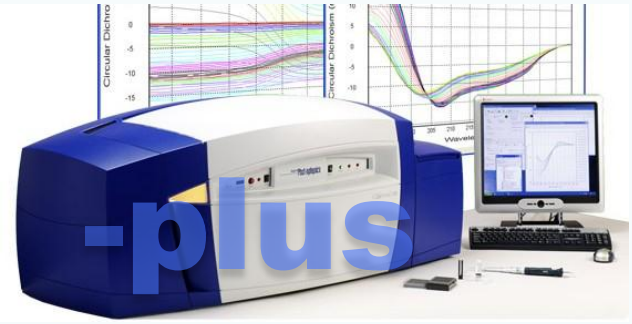


- Quantitative CD measurements. Absolute multi-point CD calibration using **DichOS** optical standard
- Outstanding long-term stability – uniquely specified as a function of temperature
- Highest sensitivity. Equivalent to a synchrotron in the 170 - 260nm wavelength range (detection range 163 – 950nm as standard)
- Multiple temperature-ramping curves in a single experiment - saving time and sample

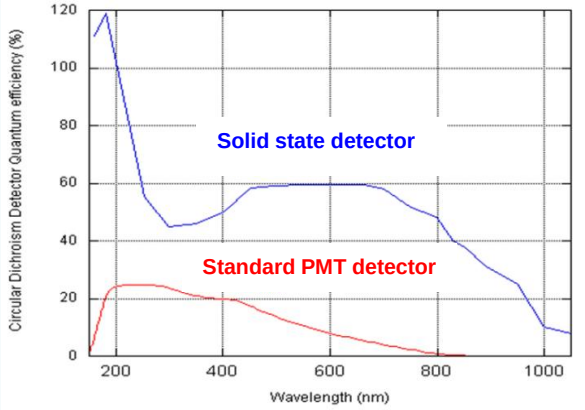


- ProData acquisition, display and analysis software (unlimited seats)
- Large range of unique upgrade options

CHIRASCAN-PLUS *q*CD



- All of the capabilities of Chirascan *q***CD** with <u>even greater sensitivity</u>
- Uses a solid-state detector with automatic variable gain to ensure optimised performance at each wavelength



- Detection range 163 - 1200nm as standard (extendable to 1700nm)
- Accurate simultaneous absorbance with CD
- Straightforward upgrade from Chirascan *q***CD**
- Upgradeable to **Chirascan-auto** *q***CD**

What is *q*CD?

***q*CD redefines the applications of circular dichroism spectroscopy by including novel elements that make CD a truly quantitative technique and able to provide easy-to-understand quantitative results.**

Accuracy: factory calibrated with DichOS

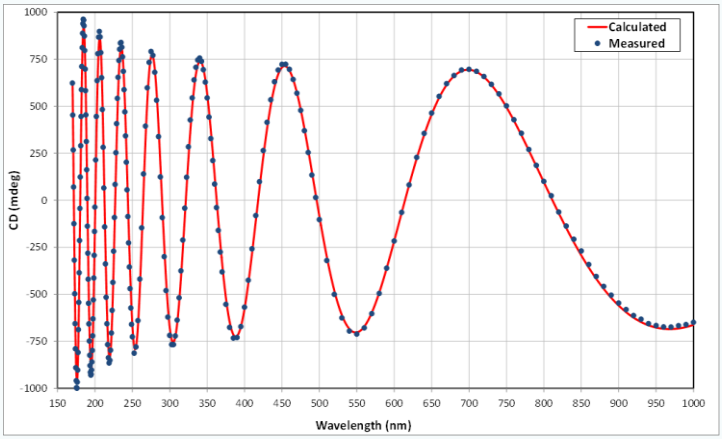
For the first time ever, CD spectrometers can be calibrated using an absolute standard and at multiple wavelengths.

Quantitative calibrated data allows users to properly assess the value of their CD measurements. The need for this was expressed by **Knight et al**[1] (of the **National Physical Laboratory**, UK) who reported that there was ***"…a lack of confidence in the CD technique, arising from an observed lack of comparability in the data obtained by different laboratories, or even different operators"*** and "***we believe that the lack of an absolute reference or measurement traceability in circular dichroism contributes to a lack of confidence in the technique***."

CD spectroscopy is limited if researchers cannot quantify the precision and accuracy of their data. CD measurements with Chirascan ***q*CD** are accurate and so have more value because the user is able to properly address questions such as: is a measured difference in CD significant from my earlier results or from the results of my collaborator's lab? Similarly in the pharmaceutical industry, workers use CD for batch comparison, comparisons over time, and comparison at different manufacturing facilities - and they need to be able to quantify the data.

Inaccurate CD data has more limited value and errors in batch comparison etc. can be costly. Referees and regulatory authorities are increasingly requiring statistically relevant data. In order to achieve this, CD data must be of known accuracy and precision (see next section).

DichOS (**Dich**roism **O**ptical **S**tandard) is a new, non-chemical, multi-point CD calibration standard that eliminates the uncertainties associated with conventional, single-point calibrations. **DichOS** enables measurement of *absolute* CD values and, with **DichOS** calibrated instruments, comparison of CD spectra measured on different instruments or at different times on the same instrument becomes routine. **DichOS** is constructed from optical components whose physical properties and tolerances are precisely characterised. This enables a model CD spectrum to be generated which is used to calibrate the spectrum measured with **DichOS**. A multi-point calibration curve is generated that is accurate to ±1% at all wavelengths.



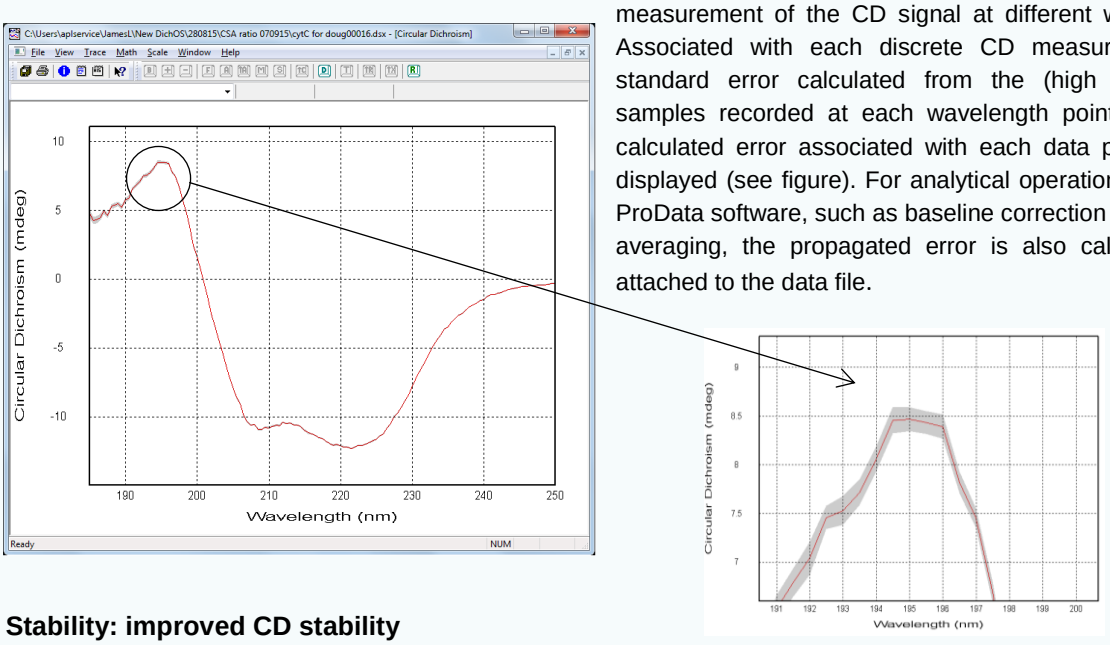
DichOS produces a CD spectrum comprising multiple peaks from the Far-UV to the NIR (see left). The model spectrum (red) can be calculated very precisely and this is compared with the measured spectrum (blue).

1 ***Knight et al International comparability in spectroscopic measurements of protein structure by circular dichroism: CCQM-P59.*** *Metrologia, 2010, 47, (1A), 08022*

What is *q*CD?

Precision: calculated errors are reported with every measurement made

The Chirascan ***q*CD** range of spectrometers always acquires discrete measurements of CD under the experimental conditions used. Filtered, or rolling average, measurements are not used as these are essentially a composite measurement of the CD signal at different wavelengths. Associated with each discrete CD measurement is a standard error calculated from the (high number of) samples recorded at each wavelength point – and the calculated error associated with each data point can be displayed (see figure). For analytical operations within the ProData software, such as baseline correction and spectral averaging, the propagated error is also calculated and attached to the data file.

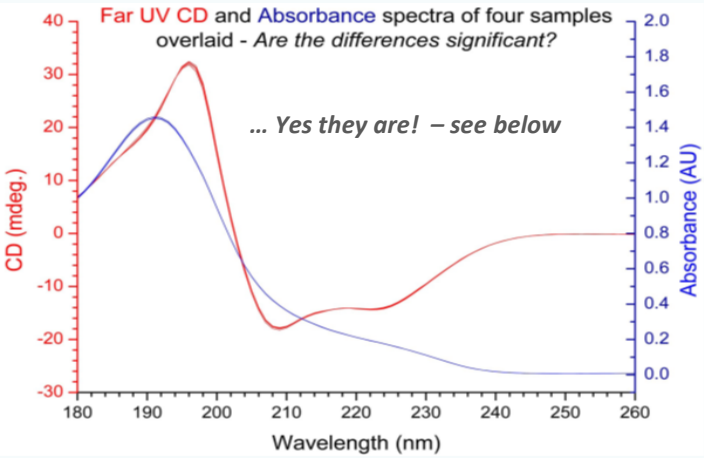


Stability: improved CD stability

A key challenge for any CD spectrometer is to have minimal change in the CD signal as a function of temperature as this is the critical parameter limiting long term stability. ***q*CD** spectrometers have exceptional high stability and especially with respect to changes in temperature. Applied Photophysics is alone in providing CD stability specifications as a function of temperature (≤ 0.01 mdeg/°C in the range 170mm to 650nm).

Analysis: statistical (quantitative) comparison of higher order structures

Comparing and quantifying differences in CD spectra requires measurements that are both accurate and of known precision; requirements that have been advanced by ***q*CD**. The ***q*BiC** Biocomparability Suite is a ***q*CD** software option that enables a quantitative comparison of spectroscopic data by establishing whether differences between similar CD spectra are statistically significant. ***q*BiC** employs a number of approaches for numerically scoring spectrum similarity.



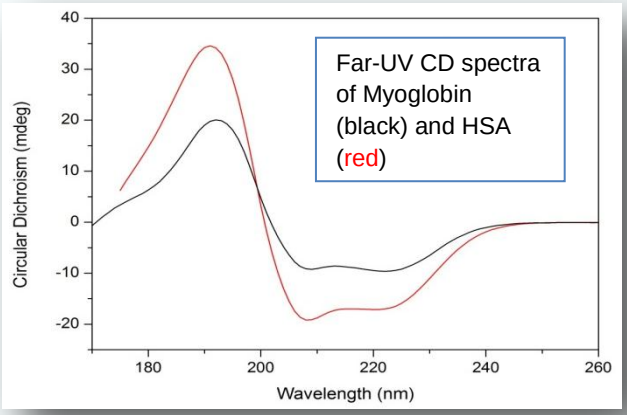
In the example shown, 4 CD spectra are overlaid. They are of insulin and insulin spiked with 2.5%, 5% and 10% Lispro. To the naked eye these spectra appear identical. However statistical analysis using ***q*BiC** shows them to be significantly and quantifiably different. Two statistical methods were employed in the study summarised here. The results are shown in the table below:

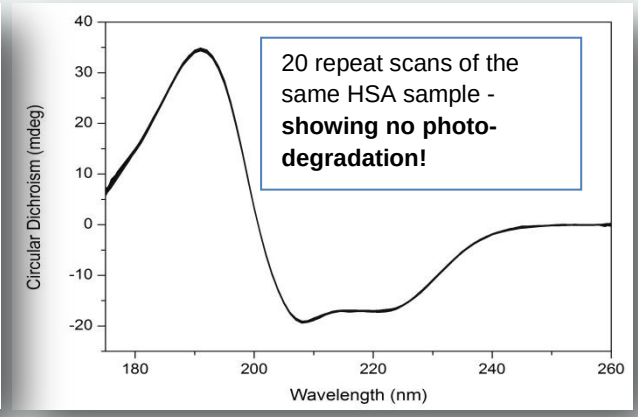
Human Insulin with % Lispro	P value	Z-score
0%	0.464	0.051
2.5%	0.0382	2.892
5%	0.0000428	7.694
10%	2.57E-08	21.04

If $p < 0.05$ = differences are significant at the 2-sigma (95%) confidence level or more.

If $Z > 2$ = differences are significant at the 2-sigma (95%) confidence level or more.

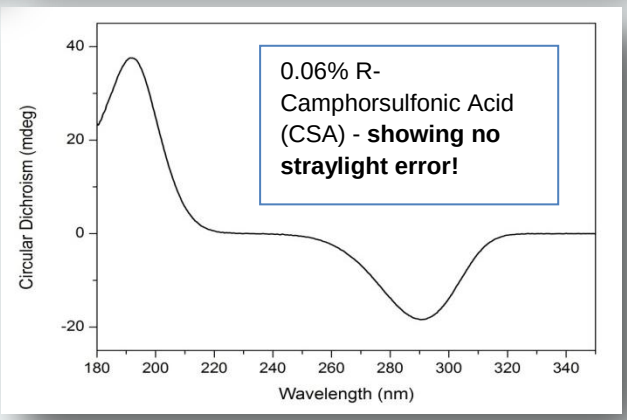
Spectral Performance





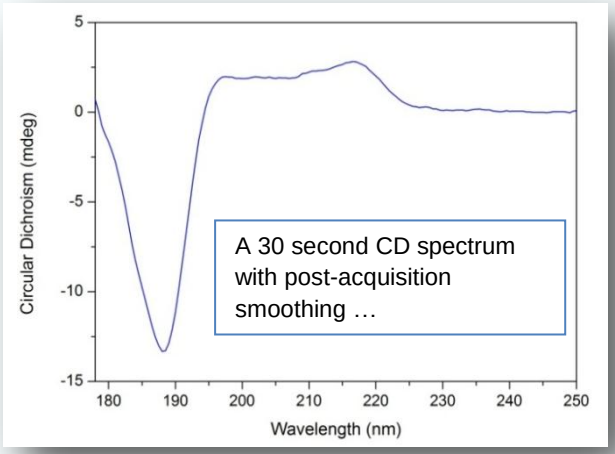
Chirascan *q*CD's unique dual polarising prism design means that it has far higher light throughput than other CD spectrometers, particularly in the Far-UV region. CD spectra in the 170-260nm range are equivalent to what can be measured using a synchrotron beam line – examples of protein spectra measured down to 170nm and 175nm are shown above.

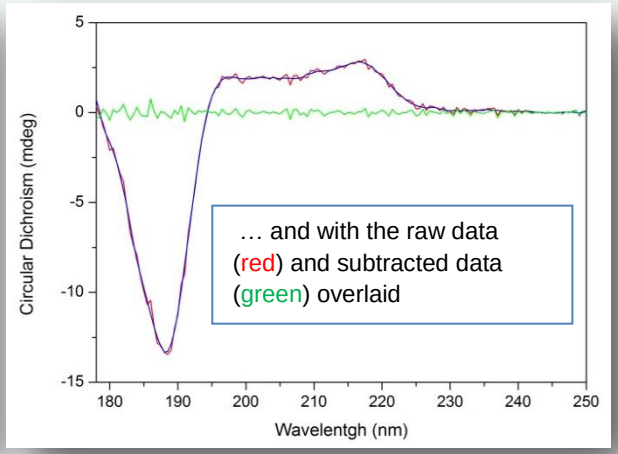
The spectra above-right show that the higher light throughput on Chirascan *q*CD produces better Far-UV performance without causing photo-degradation of the sample. The CSA spectrum (right) is accurate in the Far-UV indicating minimal stray light.



NO Continuous Smoothing!

Each discrete wavelength point on a CD spectrum recorded on Chirascan *q*CD is the actual CD measurement at that wavelength. If required, Savitsky-Golay tools can be used to produce a smoother version of the spectrum - as shown below (left) for a particularly fast scan. This can be useful for cosmetic purposes but it should not be confused with the real CD measurements and it could not be used for statistical spectral comparisons. The figure below (right) shows the smoothed spectrum overlaid on the actual measured spectrum.

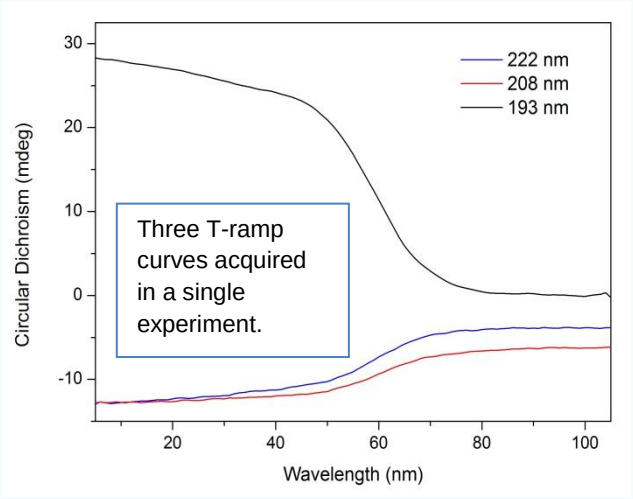




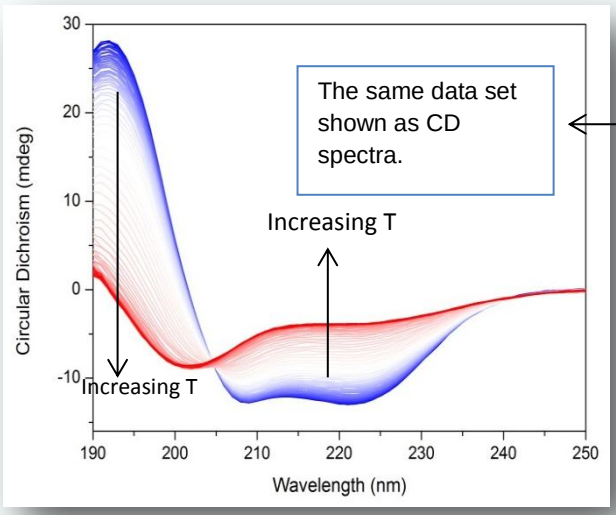
In contrast, a well-known CD manufacturer recommends a scan method where only smoothed CD spectra are recorded. It is not made clear that the recorded spectra have been smoothed using a rolling average and that the data file does not comprise discrete CD measurements at each wavelength. The quality of the actual CD measurement cannot therefore be seen and the user must assume that the spectrum has not been over-smoothed. There is no practical reason for acquiring CD spectra in this way other than to disguise the true quality of the measurement. As shown above, CD spectra can always be smoothed after acquisition in a controlled way such that the user can see the effects of the smoothing process.

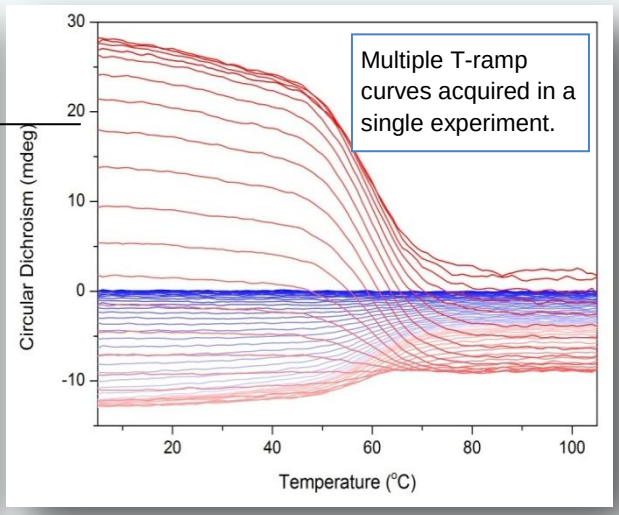
Unique Thermal Ramping Capabilities

In one experiment, temperature ramping curves can be acquired at as many wavelengths as are required. The sample is warmed continuously (typically at 1^0C/min) and the sample temperature is automatically recorded for each data point (typically every 1^0C) at each wavelength using a temperature probe inserted in the sample.



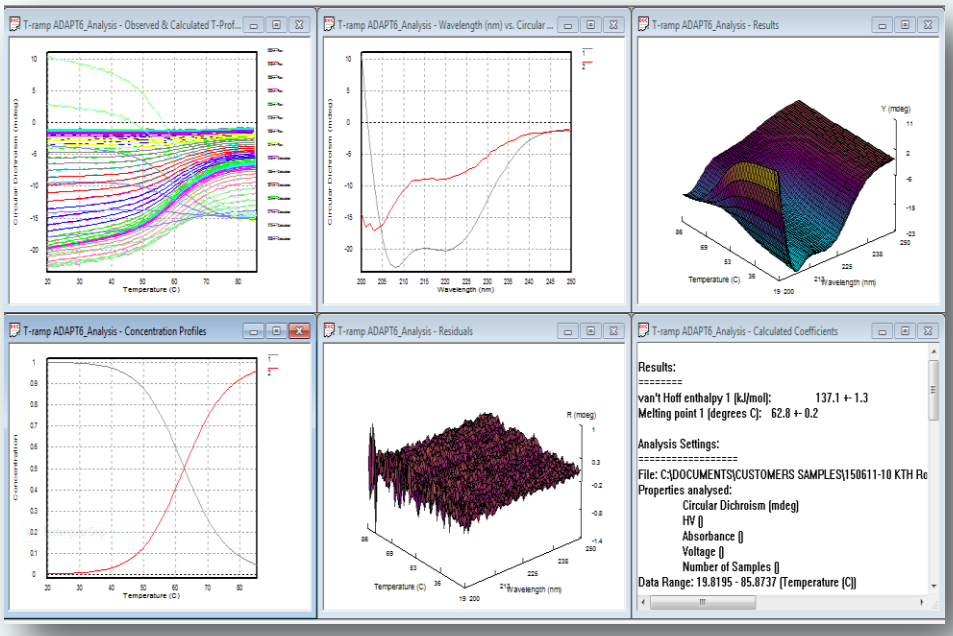
The data below-right shows multiple T-ramp curves acquired in a single experiment. These data can also be viewed as temperature-dependant CD spectra (below left). So, in a single experiment structural as well as robust thermodynamic information is obtained. Absorbance vs. temperature data are acquired simultaneously with CD which also provides information on aggregation onset. Emission spectra can also be acquired in the same experiment with the CCD detector option (see Accessories).





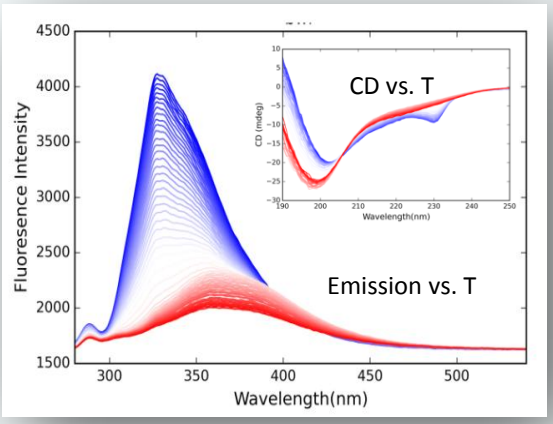
Global Thermodynamic Analysis Software

Global 3 thermodynamic analysis software has been developed specifically for fitting multi-wavelength spectroscopic data measured as a function of temperature. **Global 3** determines mid-point temperatures of transition, Van't Hoff enthalpies, as well as the CD spectra of short-lived transition intermediates and the concentration vs. time profiles of all contributing species in the thermal transition.

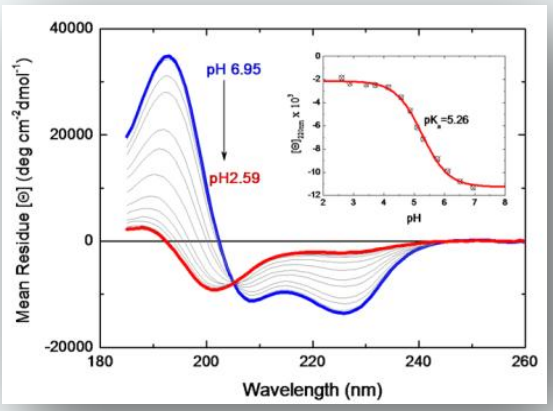


Accessories and Upgrades

- NEW **CCD Emission Spectrometer.** With this accessory CD, absorbance and emission spectra can be acquired in a single ~70 minute thermal denaturation experiment (stability of secondary and tertiary structure monitored in a single experiment).



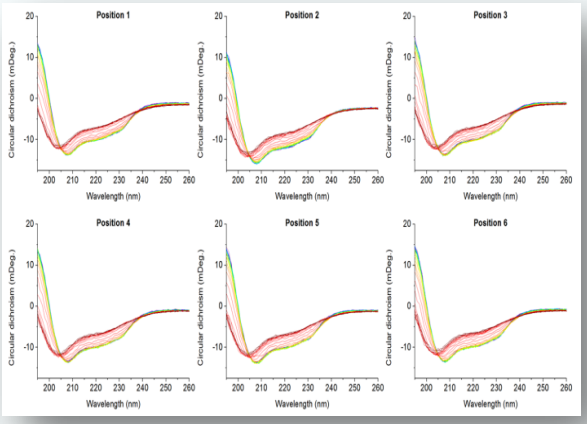
- **Auto-Titrator and pH Meter.** The auto-titrator can also be used with an in-situ pH probe/meter.



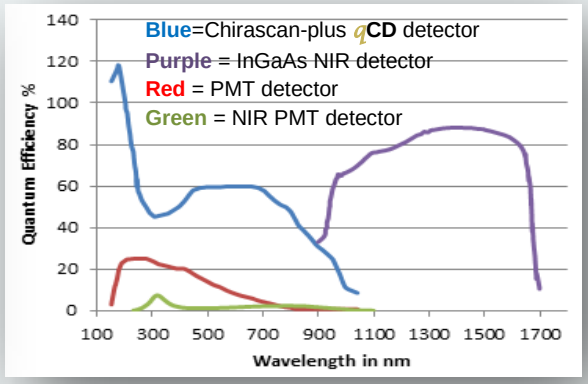
- **Linear Dichroism Couette Cell.** Highest sheer rate and highest homogeneity of sheer (just 11%). A CD cell holder can also be fitted for rapid switching between CD and LD measurements.

- **Chirascan-auto *q*CD**. Upgrade to a fully automated system integrated to an XYZ robot. Transforms productivity and eliminates human error. Contact us for full information on **Chirascan-auto *q*CD.**

- **Six-Cell Holder**. The data below shows 6 thermal denaturation CD spectral datasets acquired in one experiment using the 6-cell holder. For this experiment, the same sample was used in each cuvette. The measured Tm was 72.7^{0}C +/- 0.1^{0}C.



- **NIR Detection to 1700nm.** Just two Chirascan-plus detectors are required for the range 165-1700nm - both use automatic variable gain to ensure optimised performance at each wavelength.



- **Stopped-flow.** Combining the highest sensitivity with the lowest dead-time. The stopped-flow unit is designed specifically for use with Chirascan *q***CD** and the whole system sits on the bench-top. As a further option, the stopped-flow unit can also be used as a separate stand-alone instrument with its own light source, PC etc. Applied Photophysics is the world's leading supplier of stopped-flow instrumentation.

- **Other accessories include** integrating sphere, thin film/KBr disc holder, ORD detection, dedicated fluorescence/anisotropy detector, scanning emission monochromator, magnetic CD, low temperature cryostat, customised cells/cell-holders, **21CFR Part II compliance software, IQOQPQ Validation Service.**

Key Specifications

	Chirascan *q*CD	Chirascan-plus *q*CD
CD calibration & accuracy	Multi-point calibration (**DichOS**) - accuracy to within ±1%	
Light Source	150W air-cooled Xenon arc lamp	
Monochromator	Dual prism, BOTH prisms polarising	
Wavelength range	163nm to 950nm (+ NIR detector option available)	163nm to 1200nm (extendable to 1700nm)
Standard detector	Photomultiplier	Photodiode (with automatic variable gain)
Standard detection modes	CD, absorbance. Also suitable for fluorescence, anisotropy, FDCD, LD	
Detection Channels	5 as standard: CD, absorbance, detector HT, DC voltage and temperature	
Sensitivity. *Typical RMS-noise values with no sample in place for a 1nm Bandwidth, 2 second D.I.T. (with **no smoothing, no rolling averaging**)*	0.07mdeg @ 175nm 0.03mdeg @ 180nm 0.03mdeg @ 185nm 0.03mdeg @ 200nm 0.03mdeg @ 250nm 0.04mdeg @ 500nm 0.09mdeg @ 750nm -	0.04mdeg @ 175nm 0.02mdeg @ 180nm 0.015mdeg @ 185nm 0.02mdeg @ 200nm 0.02mdeg @ 250nm 0.02mdeg @ 500nm 0.03mdeg @ 750nm 0.05mdeg @ 1000nm
CD precision measurement	Calculated error is reported for each wavelength point on the spectrum	
Stray light	< 3ppm at 200nm	
Baseline stability	≤ 0.01 mdeg/°C (170nm to 650nm)	
Temperature ramping	Able to acquire thermal denaturation curves at multiple wavelengths in one continuous temperature-ramping experiment	
Nitrogen purge requirement at 170nm	5 litres/min	
Nitrogen purge & lamp ignition control	Software control of N2 purge and lamp ignition Pre-set /auto start-up of N2 purge and lamp ignition Pre-set switch off of N2 purge and lamp Automatic lamp switch off when N2 flow drops (fail-safe)	

Other Standard Features: Peltier temperature control, external temperature probe, advanced scanning and kinetic acquisition modes, ProData Viewer data display and analysis tools including fitting of thermal ramping curves, kinetic analysis, secondary structure analysis, and unlimited licence to install ProData Viewer on other PCs. USB communications.

A wide range of accessories and upgrades are available including global thermodynamic analysis, CCD emission spectrometer, LD Couette cell, 6-cell autochanger, titration unit, pH meter, stopped-flow unit, thin film & KBr disc holder, integrating sphere, dedicated fluorescence & anisotropy detectors, scanning emission monochromator, cryostat, ORD detection, magnetic CD, customised cells / cell-holders, 21CFR Part II compliance software and IQOQPQ Validation Service.

Free Direct Evaluation – put *q*CD to the test!

We invite researchers to visit our demonstration labs, or to send us samples, to run on the Chirascan and/or Chirascan-plus ***q*CD** spectrometers. We have demonstration labs at our factory in **London, UK** and in **Boston, USA**.

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