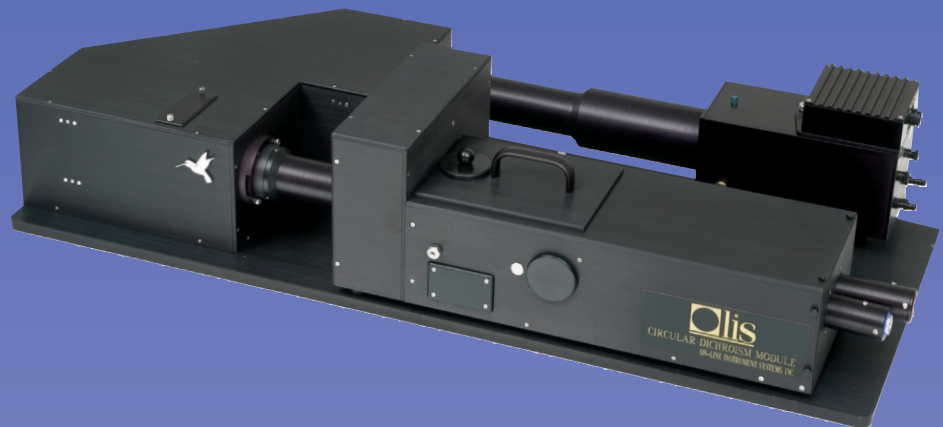


# A Practical Guide to Using the Olis CD



## Doing CD the Olis Way...

Circular dichroism (CD) is defined as the differential absorbance of left circularly polarized light (LCPL) and right circularly polarized light (RCPL):  
 $CD = Abs(LCPL) - Abs(RCPL)$ .

The sample should have an absorbance value of 1 Au and be chiral for CD to be a useful technique.

The three common secondary structure motifs (alpha-helix, beta sheet, and random coil) exhibit distinctive CD spectra in the far-ultraviolet region (170-260 nm).

CD is commonly used in denaturation experiments in which the CD signal of protein is monitored while the protein is perturbed in some fashion (e.g., increasing temperature or chemical denaturant).

Choice of cuvette and buffer are also key to making useful CD readings, as inappropriate selection of each can greatly limit readings in the far UV region.

This guide gives practical advice in using Olis CD spectrophotometers to their best advantage. Most of the sample preparation information is valid for single beam, Olis-CD, or others.

# The Basics of Circular Dichroism

**To be “CD active,” a molecule must be structurally asymmetric and exhibit absorbance.**

**Proteins are CD active (all amino acids except glycine contain a chiral carbon, thus are asymmetrical), and the resulting CD signals are sensitive to protein secondary and tertiary structure.**

- Asymmetry can result from chiral molecules such as the peptide backbone of proteins, a non-chiral molecule covalently attached to a chiral molecule (aromatic amino acid side chains), or a non-chiral molecule in an asymmetric environment (e.g., a chromophore bound to a protein).
- Increased relative absorption of left polarized light results in a positive CD signal, while a negative CD signal is the result of right polarized light being more highly absorbed.
- Three common secondary structure motifs (alpha-helix, beta sheet, and random coil) exhibit distinctive CD spectra in the far-ultraviolet region (170-260 nm; Fig 1).<sup>1</sup>
- Using CD spectra, secondary structure of proteins can be estimated using a variety of computer algorithms.
- The near ultraviolet region (320-260 nm) provides a fingerprint of the tertiary structure of proteins.
- Asymmetric environments of aromatic amino acids, which are sensitive to protein conformation, provide the basis of the near-UV CD signal.
- Changes in CD signal reflect changes in the protein structure.
- Information about protein stability or folding intermediates can be obtained.
- In addition to the ultraviolet region, structural information from the visible region can be obtained as well in proteins containing chromophores (e.g., hemes).

<sup>1</sup> Copeland, R.A., Methods for Protein Analysis: A Practical Guide, 1994.

# CD Instrumentation

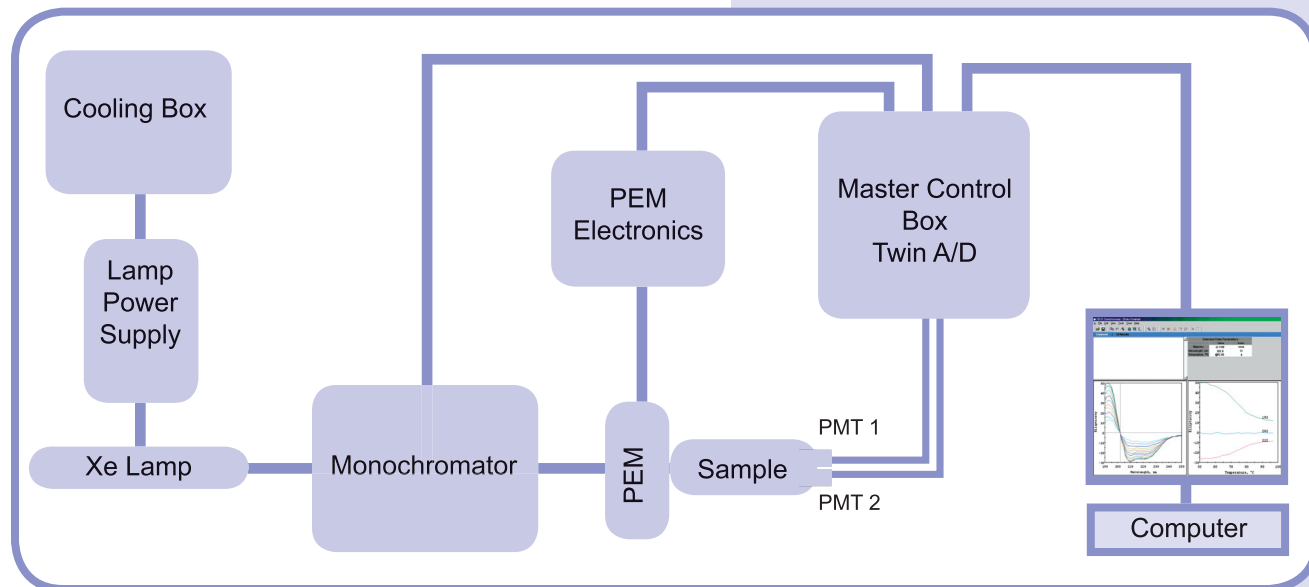
## Lamp, Power Supply, Coolant

To produce the requisite UV light, an ozone producing 150-watt xenon arc lamp is used. A continuous flow of nitrogen gas is required to remove oxygen from the housing (see Nitrogen Purging, page 8). The lamp housing used in Olis CDs maintains a cool lamp temperature with continuous flow of water from the Olis cooling box. The cooling box providing the flow contains a sensor, which cuts power to the lamp in the event of water flow disruption, thus providing assurance against overheating while also extending the useful life of the lamp.

When starting the lamp, the electronics and computer should be off to prevent possible damage due to power surges. In addition, the lamp should not be restarted when hot; wait at least 15-20 minutes with the cooling box on before restarting a xenon arc. A lamp can last 1000 hours of run time with proper care albeit losing UV light intensity as the lamp ages. The recommended lamp coolant is commercial anti-freeze, although water may be substituted. Change the coolant regularly, every 3-12 months depending on use. The 150 W lamp is run with a constant current of 7.5 A. This is best monitored by setting the display to present amperes.

**When starting the lamp, the electronics and computer should be off to prevent possible damage due to power surges.**

A schematic of an Olis CD instrument

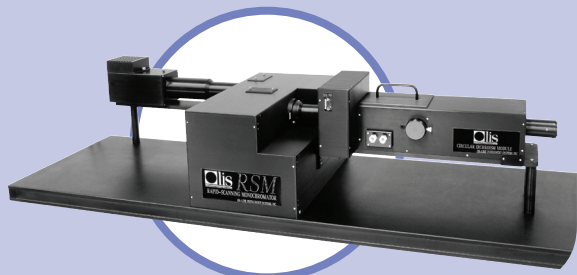
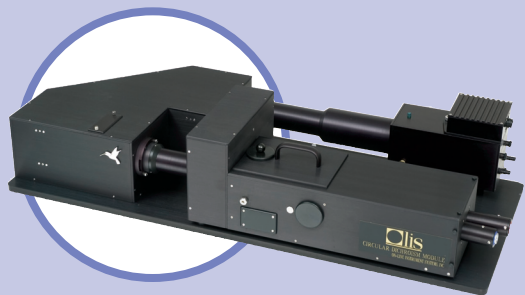


# CD Instrumentation

## Monochromator

The function of the monochromator is to select the wavelength(s) of light for the measurement. The output wavelength is determined by the rotation of two gratings (in a double grating monochromator) or rotation of a prism/grating (in a DSM 17, for instance). At a given wavelength, the slit width will determine the bandwidth (wavelength distribution of the output) and the intensity. Thus, relatively wide slits give low wavelength resolution and high intensity, and thus, high signal to noise ratio. Conversely, narrow slits give sharp resolution and relatively lesser signal to noise.

There are three Olis CD instruments—models 20, 17, and 1000—which use three different monochromators. In the DSM 20, a compact, double grating monochromator is used to give a spectral output of 170-700 nm. In the DSM 17, a Cary 17 monochromator is used with a spectral output of 184 - 2600 nm. The DSM 2000 uses an Olis RSM monochromator with a spectral range of 167-1700 nm. As an F/4.4 monochromator, the model 1000 provides the highest throughput of light of the three. An additional difference among the monochromators is that the DSM 17 has variable slit widths during data collection, while the DSM 20 and DSM 2000 have fixed slits during scanning. The DSM 2000 also possesses the possibility to rapid scan (up to 62 scans/sec), which is best used in the spectral ranges above 400 nm.



# CD Instrumentation

## Photo Elastic Modulator (PEM)

The PEM is a photoelastic device which acts as a variable retardance modulation and transforms linearly polarized light alternately to left and right circularly polarized light at a rate of 50 kHz. In the

Olis dual beam CD, both beams produced by the polarizer pass through the PEM; since the beams are orthogonally polarized, they emerge from the PEM modulated 180° out of phase.

## Master control box and Computer

The power supplies and drivers are contained in a single master control box. The master control box also contains control boards that provide for motor movements and data collection. The computer, or MCB, contains a twin 14-bit analog to digital (A/D) converter board that digitizes the analog signals from both beams simultaneously. Communication between the master control box and the computer occurs through the Com Port 1. Any automated temperature control occurs through additional com ports.



# CD Instrumentation

## Sample Chamber

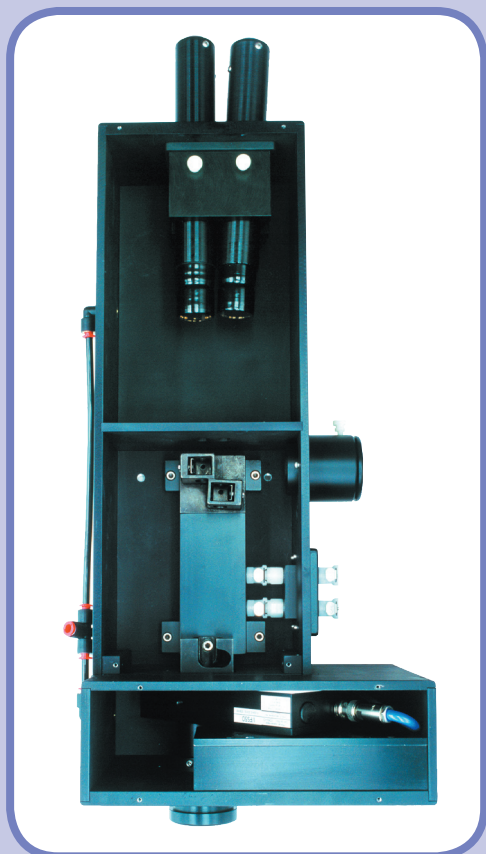
The sample chamber contains the cuvette holder through which the polarized light beams pass simultaneously. Typically, cylindrical cells with a very short pathlength (see Sample Concentration Effects on page 9) are used. Either a Peltier controller or a water bath, under computer control, determines the temperature of the cuvette holder.

Two photodetectors are used to monitor the two light beams of the Olis CD. Typically, the photodetectors are photomultipliers, which provide adjustable gain (via high voltage control), low noise, and utility over a wide wavelength range.

Note: Care must be taken not to expose the PMTs to room light when a high voltage is applied to them. Permanent damage to the phototube may result.

The sample chamber contains the cuvette holders for the sample. The typical CD cuvette is cylindrical, although rectangular cuvettes can be used. Temperature regulation is achieved by either a water jacketed cuvette holder (a) or a Peltier controlled holder (b). For CD the sample must be in a position where both beams travel through the sample, which is achieved at the far left of the sample chamber (3a). The cell holder is capable of holding either a rectangular or cylindrical cell. The rectangular cell holders on the right side of the sample chamber allow the sample to be placed in only one beam (c), which is required for absorbance measurements. In addition these cuvette holders are used for fluorescence measurements, as a detector can be placed in the port perpendicular to the light beam (d). For the use with an automated titrator, ports are provided for both tubing and a fluid reservoir.

If CD is measured using only one of the two rectangular cuvette holders the resulting CD signal will be one half of the true signal.



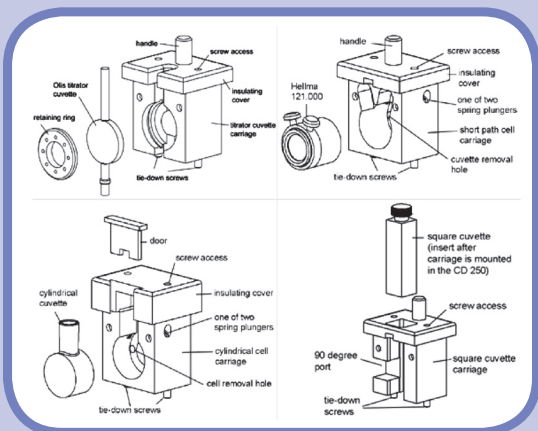
a



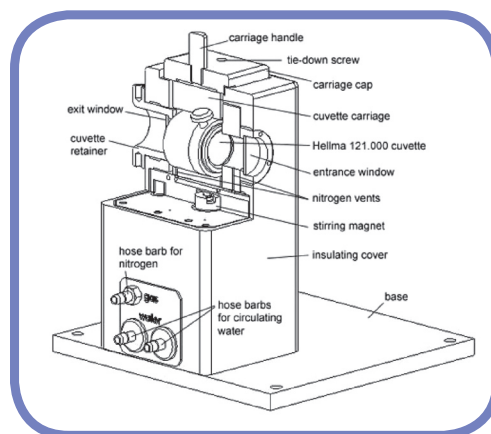
b



c



d



# Nitrogen Purging

## Nitrogen Purging

The function of purging the CD instrument with nitrogen is to remove oxygen from the lamp housing, monochromator, and the sample chamber. The presence of oxygen is detrimental for two reasons. When deep ultraviolet light strikes oxygen, ozone is produced. Ozone causes degradation of optics and can cause respiratory problems. The second reason for removing oxygen is that oxygen absorbs deep UV light, thus reducing the light available for the measurement.

To prevent ozone production, the lamp and monochromator are purged with at least 5 L/min of nitrogen (each) when the lamp is on. When measuring CD below 200 nm, increase the flow to the lamp housing, monochromator, and sample chamber<sup>1</sup>. When CD data are required as deep into the UV as possible. Increase the nitrogen flow to 15 L/min each for the lamp housing and sample chamber and 25 L/min for the monochromator. After ten minutes or so the flow can be reduced to conserve nitrogen.



# Units of CD Measurement

## Units of CD Measurement

CD is reported in units of absorbance or ellipticity. Each of these can be normalized for molar concentration of the sample. The most direct data from the Olis CD instrument is absorbance (Abs(L)- Abs(R)). This value is often reported in milliabsorbance units (mA), which are a thousandth of an absorbance unit.

CD data are commonly reported as ellipticity ( $\theta$ ), which is related to absorbance by a factor of 32.98 ( $\theta = 32.98 \Delta\text{Abs}$ ). Ellipticity is usually reported in millidegrees (mdeg or  $m^\circ$ ), which are a thousandth of a degree.

Molar ellipticity ( $[\theta]$ ) is CD corrected for concentration. The units of molar ellipticity are historical ( $\text{deg}\cdot\text{cm}^2/\text{dmol}$ ). Conversion from molar extinction (absorbance corrected for concentration) to molar ellipticity uses a factor of 3298 ( $[\theta] = 3298\Delta\epsilon$ ). To calculate molar ellipticity, the sample concentration (g/L), cell pathlength (cm), and the molecular weight (g/mol) must be known.

If the sample is a protein, the mean residual weight (average molecular weight of the amino acids it contains) is used in place of the molecular weight, essentially treating the protein as a solution of amino acids.

| From<br>↓<br>To → | Absorbance <sup>1</sup>  | Milliabsorbance <sup>2</sup> | Molar Extinction <sup>3</sup> | Degrees <sup>4</sup>    | Millidegrees <sup>5</sup> | Molar Ellipticity <sup>6</sup> |
|-------------------|--------------------------|------------------------------|-------------------------------|-------------------------|---------------------------|--------------------------------|
| (A)               | A                        | A*1000                       | A*M/(C*L)                     | A*32.98                 | A*32980                   | A*M*3298/(L*C)                 |
| (mA)              | mA/1000                  | mA                           | A*M/(C*L*1000)                | mA*0.03298              | mA*32.98                  | mA*M*3.298/(L*C)               |
| ( $\epsilon$ )    | $\epsilon^*C*L/M$        | $\epsilon^*C*L*1000/M$       | $\epsilon$                    | $\epsilon^*C*L*32.98/M$ | $\epsilon^*C*L*32980/M$   | $\epsilon^*3298$               |
| ( $^\circ$ )      | $^\circ/32.98$           | $^\circ/0.03298$             | $^\circ*M/(C*L*32.98)$        | $^\circ$                | $^\circ*1000$             | $^\circ*M*100/(L*C)$           |
| ( $m^\circ$ )     | $m^\circ/32980$          | $m^\circ/32.98$              | $m^\circ*M/(C*L*32980)$       | $m^\circ/1000$          | $m^\circ$                 | $m^\circ*M/(10*L*C)$           |
| [ $\theta$ ]      | $[\theta]^*C*L/(3298*M)$ | $[\theta]^*C*L/(3.298*M)$    | $[\theta]/3298$               | $[\theta]^*C*L/(100*M)$ | $[\theta]^*C*L*10/M$      | [ $\theta$ ]                   |

<sup>1</sup>Units are Absorbance (Abs)

<sup>2</sup>Units are milliabsorbance (mAbs)

<sup>3</sup>Units are  $A^*L/\text{mol}\cdot\text{cm}$

<sup>4</sup>Units are degrees ( $^\circ$ )

<sup>5</sup>Units are millidegrees ( $m^\circ$ )

<sup>6</sup>Units are  $\text{deg}\cdot\text{cm}^2/\text{dmol}$

C is concentration in g/L

M is average molecular weight (g/mol)

L is path length of cell (cm)



# Sample Concentration Effects

CD signals obey Beer's law – CD intensity is proportional to the concentration of the active species – so it is tempting to increase the concentration of the sample to improve the signal to noise ratio. This strategy is not always useful, as the signal to noise is a function of the signal strength and the overall light intensity passing through the sample to the detectors. Since absorbance must occur at the CD active wavelengths, increasing the concentration also increases the overall absorbance, thus reducing the amount of light reaching the detectors. This necessitates the need for higher PMT high volts, which, in turn, increases the noise. The relationship between sample absorbance and signal to noise ratio is illustrated in Figure 3<sup>1</sup>.

**There is an optimum absorbance to use (Abs = 0.89). For a 1 mm pathlength cell, this absorbance is achieved with a protein concentration of about 0.1-0.3 mg/mL.**

The optimal protein concentration is a function of the pathlength of the cuvette. Figure 4 shows a plot of the protein concentration required to produce an absorbance of 0.5<sup>2</sup>. This is lower than the optimal 0.9 to account for absorbing buffer components. This plot indicates an optimal protein concentration of approximately 0.1 mg/mL for a protein solution, if the absorbance due to the buffer itself is minimized.

**In the Olis dual-beam CD, no concentration calibration is required because the CD signal is directly digitally derived from the two photodetectors signals.**

Figure 3

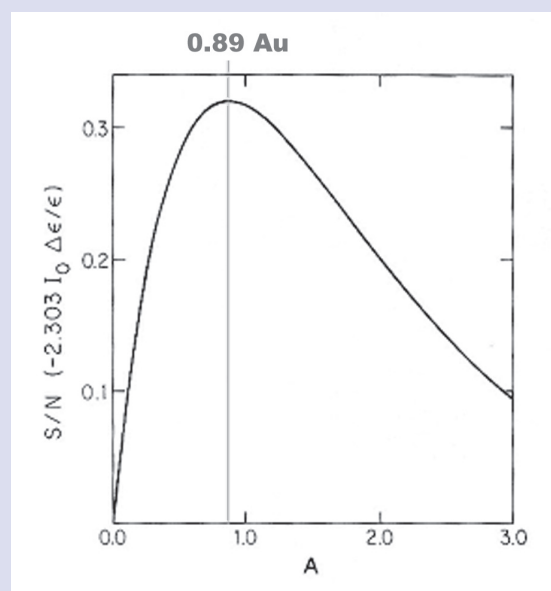
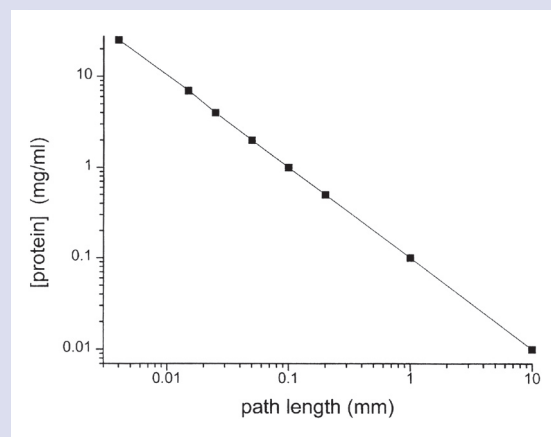


Figure 4



<sup>1</sup> Johnson C. W. (1996) in Circular Dichroism and the Conformational Analysis of Biomolecules. Fasman G.D., Editor. Plenum Press, New York pp 635-352

<sup>2</sup> Sutherland, J.C. (1996) in Circular Dichroism and the Conformational Analysis of Biomolecules. Fasman G.D., Editor. Plenum Press, New York pp 599-633

# Cutoff Wavelengths For Common Solvents and Buffers

In addition to the sample, buffers and oxygen also absorb UV light. Oxygen can be removed by purging the instrument with nitrogen. Buffer components (especially those that contain carboxylates) that have a high absorbance below 200 nm must be avoided (e.g. Tris, dithiothreitol, imidazole, histidine, chloride, etc.). This table shows the “cutoff wavelength” or wavelength in which Abs=1 for a 1 mm cuvette for common solvents and buffer components<sup>1</sup>. Cuvettes with short pathlengths are better, since the amount of buffer the light travels through is minimized. The disadvantage of short pathlength cuvettes is the higher concentration of protein which must be used (increased 10-fold relative to a 1 cm cell). Ideally, the buffer concentration is low ( $\leq 10$  mM).

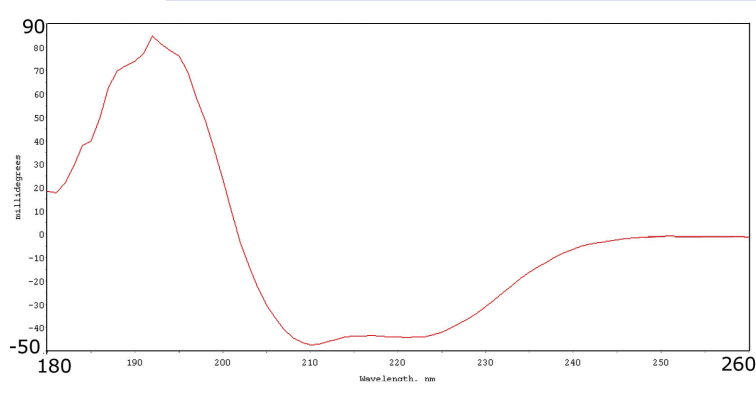
| SOLVENT SYSTEM   | CUT OFF (nm) for 1mm PATHLENGTH |
|--|---------------------------------|
| Water  | <185                            |
| Trifluoroethanol                                       | <185                            |
| Hexafluoroisopropanol                                  | <185                            |
| Acetonitrile   | 185                             |
| Methanol   | 195                             |
| Ethanol  | 196                             |
| 2-Propanol   | 196                             |
| Cyclohexane  | <185                            |
| Dimethylsulfoxide                                      | 251                             |
| Dioxane  | 232                             |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 0.15 M | 191                             |
| NaCl 0.15 M  | 196                             |
| NaClO <sub>4</sub> 0.15 M                              | <185                            |
| NaNO <sub>3</sub> 0.15 M                               | 245                             |
| Phosphate 100 mM                                       | <185                            |
| Tris 100 mM  | 195                             |
| Pipes 100 mM   | 215                             |
| Mes 100 mM   | 205                             |
| GdnHCl 4 M   | 210                             |
| Urea 4 M   | 210                             |

<sup>1</sup> Martin, S. R. Proteins Labfax (Protein, N.C., ed.), Bios Scientific Publishers, Oxford, 1996.

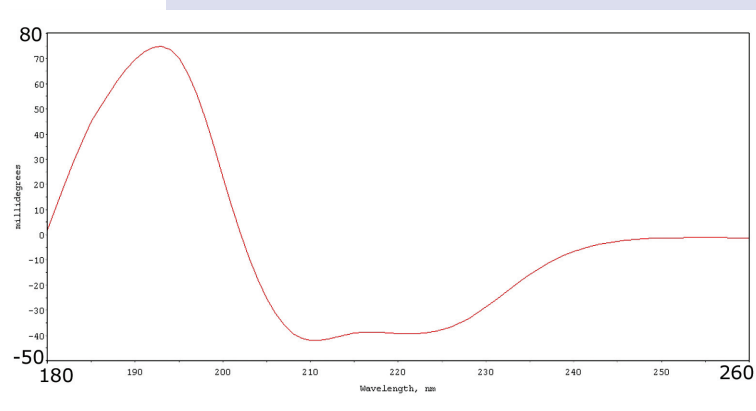
# Choosing Acquisition Mode

CD data can be collected as a function of wavelength (scan) or time (assay). To collect a scan, the wavelength range and spacing, number of data points, and the integration mode must first be selected with one point per nm' after '260-190 nm'. Note that choosing an exact wavelength range is essential in order to apply a secondary structure analysis (see Secondary Structure Analysis of Proteins for details; 260-190 nm is the standard for application of most algorithm.

Two integration modes are available. The first is a constant integration time mode, in which the time spent collecting data is constant and user defined for each data point. Because light levels often vary enormously across a spectral range in a CD scan, a second integration time mode is available in which the integration time is a function of the PMT detector high volts (thus, it is a function of the intensity of light). At regions of the spectrum in which light levels are high, a short integration time is used. When light levels are low, a longer time is spent collecting data. The integration time increases as an exponential function that can be adjusted by the user. The goal of this function mode is to achieve a reasonably constant signal/noise ratio throughout the spectrum.



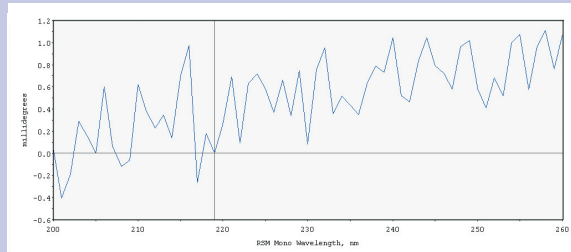
**Figure 5a: Fixed Time/Datum**  
Note insufficient time for best S/N below 200 nm



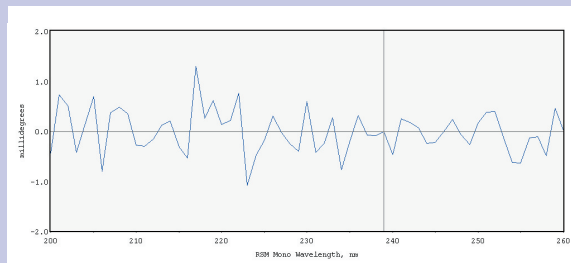
**Figure 5b: Variable Time/Datum**  
Equal S/N along entire range

# Checking Baselines

## 1 minute uncorrected baseline scan



## 1 minute corrected baseline scan



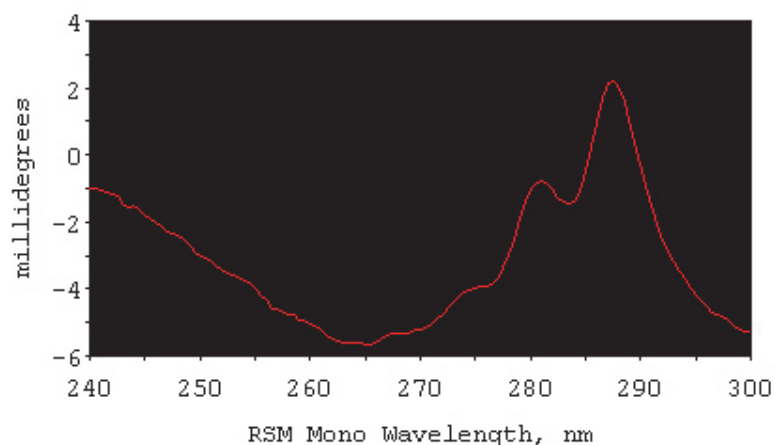
## Collecting to Use Secondary Structure Determination Fits

To collect a baseline, place a cuvette containing all components except the sample (e.g. for a protein the baseline will be the cuvette containing buffer only). It is important to include the cuvette in the baseline because birefringence of the cuvette can cause an apparent CD signal. It is also beneficial to place the cuvette in the same orientation it will be during the actual measurement. Once a scan is collected, it must be assigned as the baseline. The baseline will be subtracted from all subsequent data until this baseline is deselected. In the assay mode, data is collected as a function of time. The total time of the assay as well as the number of points can be selected. In addition, a baseline value of the cuvette and buffer can be subtracted from the data.

# Collecting Magnetic CD

Magnetic CD (MCD) is collected in an identical manner to conventional CD except that the sample is placed in a strong magnetic field. A small portable 1.4 Tesla magnet is available as an accessory for all Olis CD instruments. Typically, an MCD spectrum is collected in each orientation of the magnet. Subtracting the spectrum collected in the South direction from that of the North orientation results in a spectrum equal to twice the MCD spectrum. Each spectrum is a sum of the MCD, CD, and the baseline. Because the MCD signal is the only one that reverses direction upon reversing the magnet, the CD and baseline are subtracted out in this process.

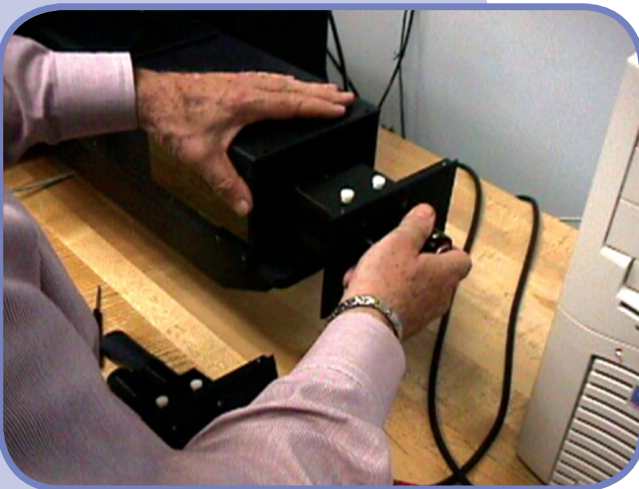
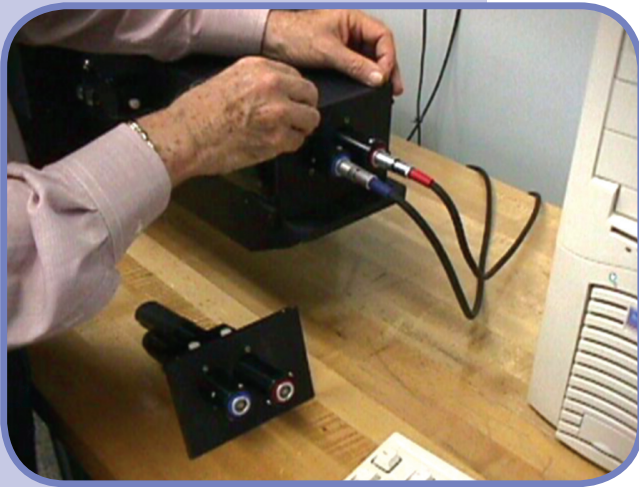
MCD is distinct from CD in that molecules do not have to be chiral to exhibit an MCD signal. Only absorbance at the wavelength of interest. In addition, the MCD signal is proportional to the strength of the magnetic field. MCD is sensitive to electronic states and is most commonly used in the studies of heme and metalloproteins.



The result of subtracting digitally the reverse MCD data from the forward MCD data in order to cancel the normal CD and produce the 2MCD spectrum.

## Optional Detectors for CD

In addition to the standard ultraviolet sensitive photomultiplier tubes (PMTs) used in CD, red sensitive PMTs or Indium Gallium Arsenide (InGaAs) array detectors are available as well. These extend the range of CD collection to 850 nm in the case of the PMTs and 1700 nm for the InGaAs arrays. Changing detectors is quite facile and involves replacing a detector plate and clicking on a check box in the software. The red sensitive PMTs operate identically to the UV sensitive ones, while the InGaAs detectors do not use the PMT HV. Operation of the CD instrument is almost identical with these detectors active.



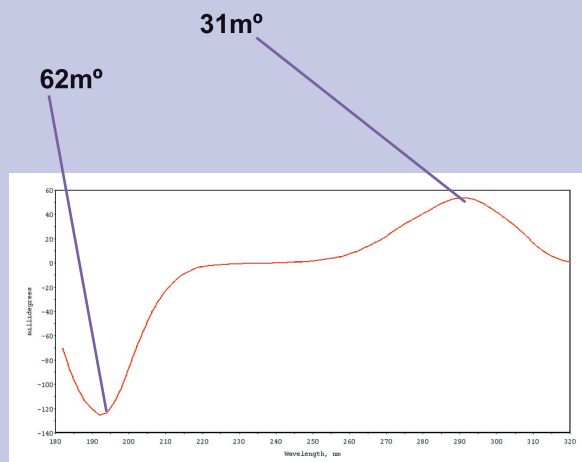
# Standards

Single beam CD instruments require calibration with a CD standard. Olis DSM CDs do not require this correction factor. However, it is still prudent to run a known standard occasionally to confirm that the instrument is functioning properly.

The most common standard used is (+)-10-camphorsulfonic acid (CSA). At a concentration of 1mg/mL and a 1mm pathlength, two peaks are observed. A positive peak at 290.5 nm exhibits an intensity of 31 mdeg (2.36 milliabsorbance) and a negative intensity of 62 mdeg (5.0 milliabsorbance) at 192.5 nm.

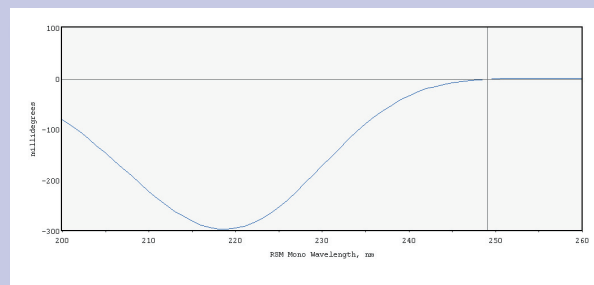
Another popular standard is (S)-(+)-pantolactone, which gives a negative CD peak in a region used for protein secondary structure. A 1 mg/mL aqueous solution of pantolactone will exhibit a CD signal of -122 millidegrees at 220 nm. An example of this spectrum is shown in Figure 6B.

Finally nickel tartarate is often used as a standard for CD in the near-infrared region. A 1:1 mixture of 0.36 M sodium tartarate and 0.24 M nickel (II) sulfate will yield a CD signal of -85 millidegrees at 1100 nm when a 1 mm path length cuvette is used. The mixture must be freshly prepared as the CD signal decays with time. This sample also has characteristic peaks at 370, 400 and 425nm. A sample spectrum is shown in third graph.

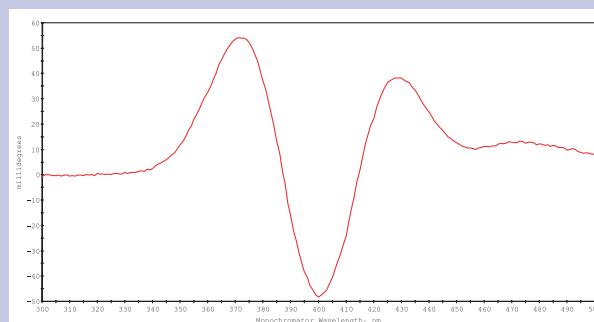


**31m° = .94 mA.U.**

**1 minute pantolactone,  
1 cm pathlength, 247 mg/ L**



**62m° = 1.9 mA.U.**



# Thermal and Chemical Denaturations of Proteins

One of the most useful applications of CD in the study of proteins is monitoring protein denaturations, which can be initiated either thermally or chemically. In the experiment, CD data are collected as a function of temperature or denaturant concentration. Data can be collected at a single wavelength, resulting in 2D denaturation curves, in which CD signal is recorded versus temperature or denaturant concentration. These curves are fit by known denaturation models to give information about protein structure and stability.

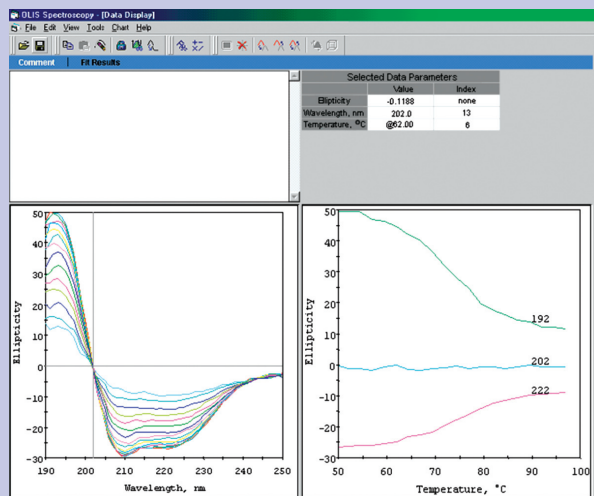
Better, CD spectra can be collected as a function of temperature or denaturant, resulting in a three-dimensional data set with the axes being wavelength, temperature or concentration, and CD intensity. The advantage of this is that data from many wavelengths are included in the subsequent fitting procedures. This 3D data set should be passed through singular value decomposition (SVD) to determine the number of species involved in denaturation and to remove the noise so that the data can be better fitted to unfolding mechanisms to obtain thermodynamic information.

The thermal and chemical denaturation experiments can be automated using Olis accessories such as a titrator, water bath, or Peltier cell holder. The titrator is used to automatically add specified volumes of denaturant. Data collection is automatically initiated after each addition and mixing cycle. Titrators are available in two, three, or four syringes. Sample temperatures can be changed using either a software controlled water bath or Peltier cell. Both are programmed by the software, but the Peltier temperature changes much more quickly. Temperatures or volumes to be added are 'programmed' using an Olis script file. Script files are text files which can be edited directly or using the Olis script file editor located in the Utilities folder of the Olis GlobalWorks folder.

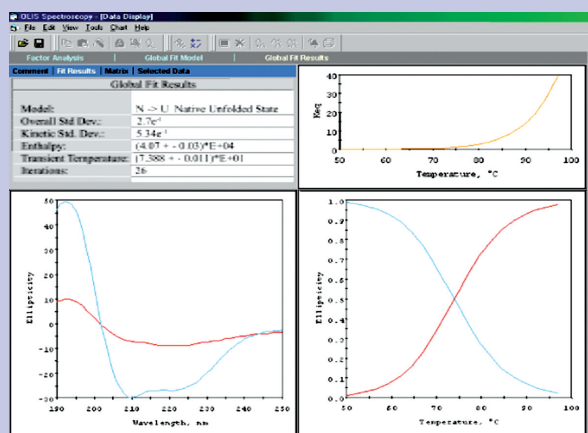
## Thermal and Chemical Denaturations of Proteins

The thermal and chemical denaturation experiments can be automated using Olis accessories such as a titrator, water bath, or Peltier cell holder. The titrator is used to automatically add specified volumes of denaturant. Data collection is automatically initiated after each addition and mixing cycle. Titrators are available in two, three, or four syringes. Sample temperatures can be changed using either a software controlled water bath or Peltier cell. Both are programmed by the software, but the Peltier temperature changes much more quickly. Temperatures or volumes to be added are 'programmed' using an Olis script file. Script files are text files which can be edited directly or using the Olis script file editor located in the Utilities folder of the Olis GlobalWorks folder.

## Raw Data



## Fitted Results





# Circular Dichroism Protein Secondary Structure Fits

Globalworks supports the use of four commonly used secondary structure fitting algorithms. Far-UV Circular dichroism spectra of proteins and peptides are extremely sensitive to protein secondary structure. Many empirical methods have been developed to use far-UV circular dichroism spectra to quantify protein secondary structure. These algorithms were not developed by Olis, and we have reproduced them faithfully from their original sources. There are several reviews available in the literature which compares these fits [1-4]. All of these fits are available under the 'Fits' menu of Globalworks. Note that the CD Units for SELCON3, CONTINLL, CDSSTR, and CLUSTER must be molar extinction.

**Compton:** The Compton fit, which originates from the laboratory of Curtis Johnson [5], is the original secondary structure fit provided with the Olis software. This rather simple approach uses inverse spectra derived using singular value decomposition (SVD) from CD spectral of proteins with known X-ray crystal structures. The fraction of each component is obtained from a dot product of the inverse spectrum with the CD spectrum of the unknown protein. This algorithm differs from the others in that it requires data spaced at one point every two nm, must start at 260 nm, and end at 184 nm – 178 nm.

**SELCON3:** Developed in the laboratory of R. W. Woody [6], SELCON incorporates the self-consistent method in which successive iterations achieve an rms noise of 0.0025 to predict secondary structure. This method is termed self consistent for this reason.

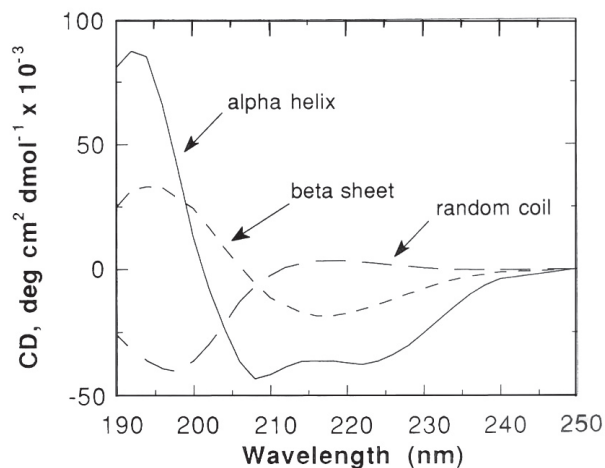
The algorithm first assigns an initial guess at the composition using the SVD method of Hennessey and Johnson [7]. Then, in the second step, the initial starting compositions are iterated using the SVD calculation until a convergent solution is obtained.

The results are then constrained to the sum of compositions being close to 1 and no composition less than -0.05. Finally, the results are further constrained by the helix limit theorem as described by Hennessey and Johnson [7].

**CONTINLL:** The CONTIN algorithm was originally developed by Provencher and Glockner [8]. In this algorithm, ridge regression [9] is used to fit a linear combination of spectra of known composition to match a spectrum of unknown composition.

CONTIN3 uses this algorithm, with a locally linearized model [10]. In this model, basis set proteins are screened for those with the lowest rms deviation from the spectrum of unknown composition. Thus, only spectra which closely match the unknown spectrum are used.

**CDSSTR:** CDSSTR is from the laboratory of Robert Woody [11]. This algorithm uses all



<sup>1</sup> Compton, L.A. and Johnson, C.W., Analytical Biochemistry. 155-167, 1986.

<sup>2</sup> <http://www2.umdj.edu/cdrwjweb>, <http://www.cryst.bbk.ac.uk/cdweb/html/home.html>, <http://akilonia.cib.csic.es/~pablo/K2D/>

<sup>3</sup> Greenfield N.J., Analytical Biochemistry. 235, 1-10, 1996. Sreerama, N., and Woody, R.W., Analytical Biochemistry. 282, 243-251, 2000.

<sup>4</sup> Morrow, J.A., Segall, M.L., Lund-Katz, S., Phillips, M.C., Knapp, M., Rupp, B. and Weigraber, K.H. Biochemistry. 39, 11657-11666, 2000.

# Circular Dichroism Protein Secondary Structure Fits cont...

## References:

1. Johnson, W. C. (1988) *Annu. Rev. Biophys. Chem.* 17, 145-166.
2. Greenfield, N. J. (1996) *Anal. Biochem.* 235,1-10.
3. Venyaminov, S. Y. and Yang, J. T. (1996) in *Circular Dichroism and the Conformational Analysis of Biomolecules*, Fasman, G. D. ed., Plenum Press, New York, pp. 69-107.
4. Woody, R. W. (1995) *Meth. Enzymol.* 246, 34-71.
5. Compton, L. A. and Johnson, W. C., Jr. (1986) *Anal. Biochem.* 155, 155-167.
6. Sreerama, N. and Woody, R. W. (1993) *Anal. Biochem.* 209, 32-44.
7. Hennessey, J. P. and Johnson, W. C. (1981) *Biochemistry* 20, 1085-1094.
8. Provencher, S. W. and Glockner, J. (1981) *Biochemistry* 20, 33-37.
9. Tikhonov, A. N. and Arsenin, V. Y. (1974) *Methods of Solving Ill-Posed Problems [In Russian]*, Nauka, Moscow.
10. van Stokkum, I. H. M., Spoelder, H. J. W., Bloemendal, M., van Grondelle, R., and Groen, F. C. A. (1990) 191, 110-118.
11. Sreerama, N. and Woody, R. W. (2000) *Anal. Biochem.* 282, 252-260.
12. Sreerama, N., Venyaminov, S. Y., and Woody, R. W. (2001) *Anal. Biochem.* 299, 271.

possible combinations of a fixed number of proteins in the reference set. This method is generally the most accurate of the algorithms in Globalworks, but can take a long time to perform all the necessary calculations.

**Cluster:** The cluster program was developed by Sreerama et. al. [12]. This approach is to create a basis set of proteins of similar tertiary structure, and is intended to improve the secondary structure fit by more closely matching the basis sets to the protein of interest.

## Basis sets

All of the secondary structure fits require a basis set, which contains CD spectra of proteins of known secondary structure. Secondary structures of these model proteins were obtained from X-ray crystal structures.

## Secondary Structure Fit Results

The results of a protein secondary structure fit are presented as fractional composition of secondary structure motifs. These include alpha-helix, beta-strand, turns, proline turns, and unordered segments (260 nm to 320 nm). The list of motifs included in the results will depend on the basis set used to produce the fit. The typical standard deviation for this fit is 0.06. The sum of these compositions should be close to 1.0 for a good secondary structure fit. A graphical display of the fit shows how closely the calculated spectrum represents the data. A residual plot, which displays the difference between the calculated spectrum and the original data, is also displayed with the results.

Using protein secondary structure fits in Globalworks  
To fit a CD spectrum using one of the above fits, open the desired spectrum and click on the data to select the curve. It will turn yellow and a cursor will appear. Select the desired fit from the list of 'Secondary Structure Fits' in the 'Fits' menu. A screen will appear showing the ten available basis sets to use. Choose the appropriate basis set for the analysis, and click on 'Apply' to initiate the fit. The original (red) and calculated (blue) spectra are shown on the results page along with the resulting compositions and residuals. The typical standard deviation for these types of fits is about 0.06.

If the data are recorded in molar ellipticity, the alpha-helical content can be estimated from the molar ellipticity at 222 nm by Equation 1<sup>4</sup>:

$$\% \text{ alpha-helix} = (-[\theta]_{222 \text{ nm}} + 3000)/39000$$

Changes in protein tertiary structure can be observed using CD. The wavelength range most sensitive to tertiary structure changes is the near UV region of the spectrum. CD signals in this region originate from aromatic side chains, and unlike the relatively predictable far-UV, signals in this region are more sensitive to tertiary conformational changes, which alter the environment of the aromatic side chains. This is in contrast to the far-UV in which changes in secondary structure are required to change the CD signal originating from the peptide backbone. For proteins containing chromophores, such as heme proteins, the chromophore can act as a tertiary structure probe. For example, the heme in hemoglobin only exhibits a CD signal only when in an asymmetric environment (i.e. bound to a folded protein). Thus the CD signal will act as a probe of protein tertiary structure.

# Tutorial on Fitting Thermal Unfolding Data with Singular Value Decomposition

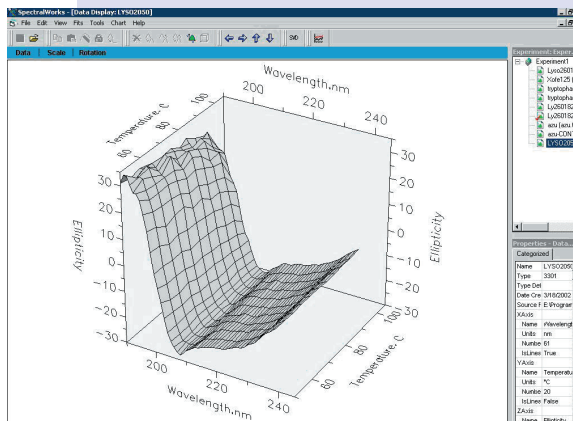
Global fitting can be used to fit spectra collected as function of temperature or concentration. In this case the fitting is quite similar to the kinetic fits. In fact, the SVD steps are identical.

A. The following is an example of a series of circular dichroism spectra of the protein hen egg white lysozyme recorded at increasing temperatures.

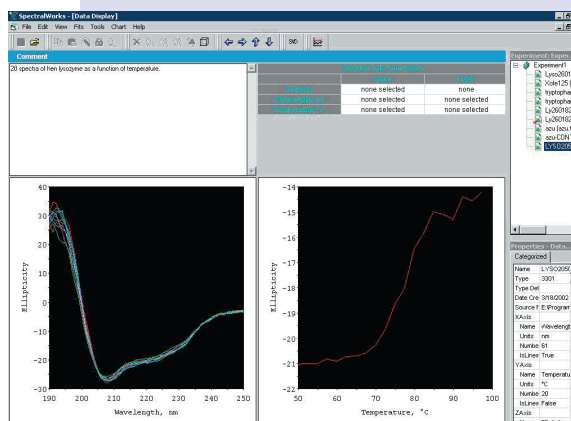
B. The same data can be shown in split screen mode.

C. Choose 'SVD' from the 'Fits' menu or click on the 'SVD' button on the toolbar to initiate the SVD process.

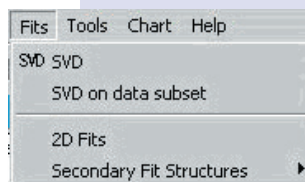
D. The resulting SVD eigenvectors are displayed as shown. Note that only the first two eigenvectors appear to be significant. This is indicated by the non random look of the thermal data in the second eigenvector compared to that of the third eigenvector. Also contributing to this observation are the weight percent values of the eigenvectors which are plotted on the small graph. Note that a sharp deviation occurs between eigenvectors two and three.



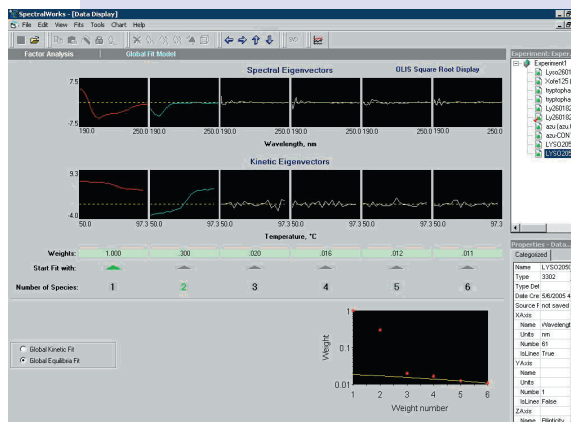
A.



B.



C.



D.

# SVD tutorial on thermal fitting with SVD cont...

E. Click on the button labeled two to fit two eigenvectors. A list of equilibrium models will appear. In this case a simple native to unfolding model ( $N \leftrightarrow U$ ) is chosen. Click on 'Fit Data.'

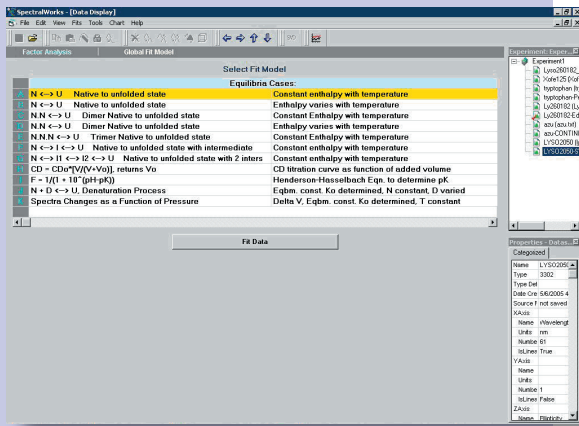
F. A message box will appear prompting for starting values. Typical starting values are given in the example. Click 'Accept' to begin the fit.

G. The results page presents the fitted parameters in the upper left panel. These include enthalpy and melting temperatures. Also included are the standard deviations for the fit, which is the standard deviation of the residuals, and the overall standard deviation, which includes the spectral data as well. The graph on the lower left contains the calculated spectra for all species involved in the fit. In this case the blue spectrum is the folded lysozyme and the red represents the unfolded species. Their contributions at each temperature are plotted on the lower right graph. Also included are the calculated total contribution (purple) and the experimental data (green). Finally, the residuals (the difference between the calculated and experimental total contributions) are plotted in the upper right hand graph.

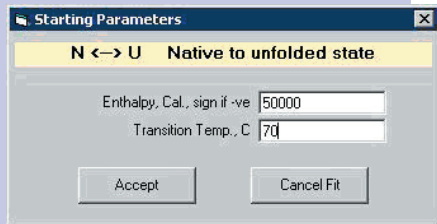
H. The fit results can be saved by choosing 'Save Dataset...' from the 'File' menu.

Any of the traces can be exported by following the instructions in the 'Export a dataset' section.

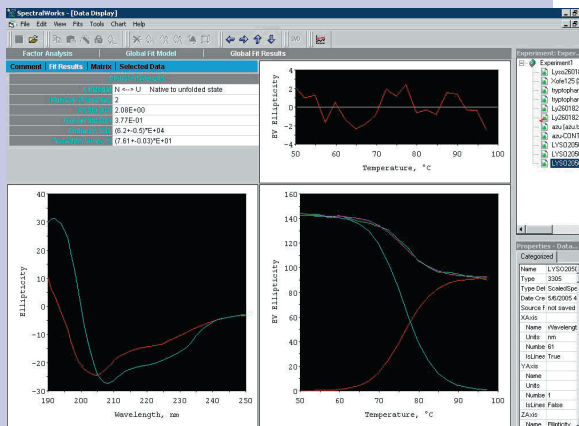
E.



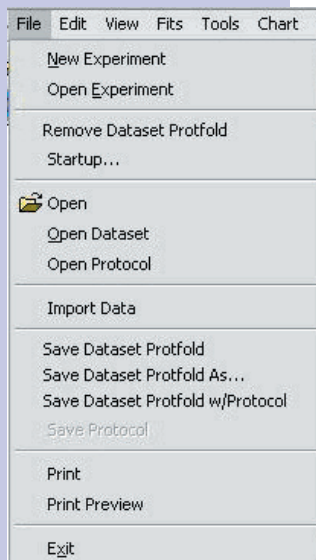
F.



G.



H.

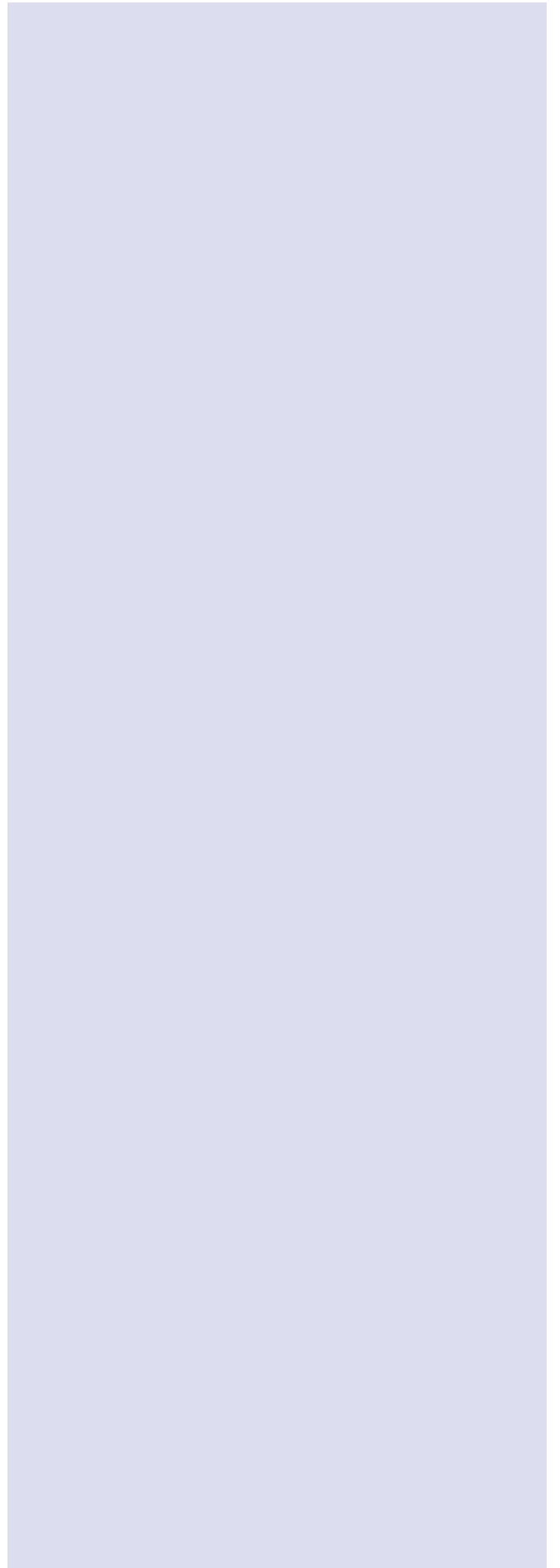


# Troubleshooting Guide

| SYMPTOM                                   | POSSIBLE PROBLEM  | SOLUTION   |
|---|---|--|
| Lamp difficult to light                   | <ul style="list-style-type: none"> <li>• Old lamp (1,000 hours)</li> <li>• Current control on power supply</li> </ul>                       | <ul style="list-style-type: none"> <li>• Change lamp</li> <li>• Increase power supply current</li> </ul>   |
| Lamp shuts off repeatedly                 | Bubble in cooling box fluid   | Use “burper bottle” to remove air from system  |
| PEM Error                                 | Hinds PEM unit is not powered or connected to C4 on A/D board (computer)  | Turn Hinds unit on and ensure connections are secure   |
| PMT HV Increases to Maximum (1100 V)      | PMT detectors seeing no light   | <ul style="list-style-type: none"> <li>• Ensure that lamp is on</li> <li>• Slits are open</li> <li>• No inappropriate filters are in place</li> <li>• The sample is not opaque at the current wavelength</li> </ul>  |
| Very high PMT HV in far UV                | <ul style="list-style-type: none"> <li>• UV absorbing buffer components</li> <li>• Old lamp</li> <li>• Too much oxygen in system</li> </ul> | <ul style="list-style-type: none"> <li>• Remove UV-absorbing components</li> <li>• Replace lamp</li> <li>• Increase nitrogen purging</li> </ul>  |
| Irreproducible signal intensities         | <ul style="list-style-type: none"> <li>• Sample sticking to cuvette</li> <li>• Sample degraded by measurement beam</li> </ul>               | <ul style="list-style-type: none"> <li>• Clean cuvette</li> <li>• Increase concentration</li> <li>• Change buffer components to avoid sticking</li> </ul><br><ul style="list-style-type: none"> <li>• Reduce slit width</li> <li>• Reduce measurement time</li> <li>• Increase sample concentration</li> </ul> |
| CD intensity not correct (or upside down) | <ul style="list-style-type: none"> <li>• Phase adjust incorrect</li> <li>• PMT cables reversed</li> </ul>                                   | <ul style="list-style-type: none"> <li>• Run phase adjust test.</li> <li>• Plug in cables correctly</li> </ul>   |

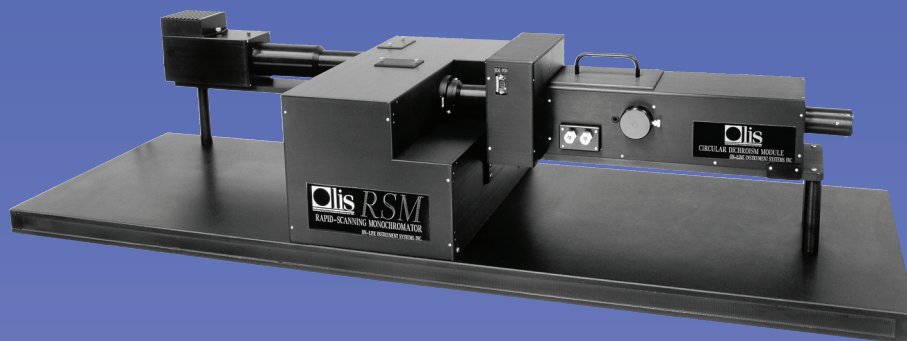
## Notes

# Notes



# The Olis DSM 1000 CD Spectrophotometer

Front cover, Olis DSM 20 CD.



**For more information on this and other Olis products:**

Visit [www.olisweb.com](http://www.olisweb.com)

Write [sales@olisweb.com](mailto:sales@olisweb.com)

Call **1-800-852-3504** in the US & Canada  
**1-706-353-6547** worldwide