



Binding

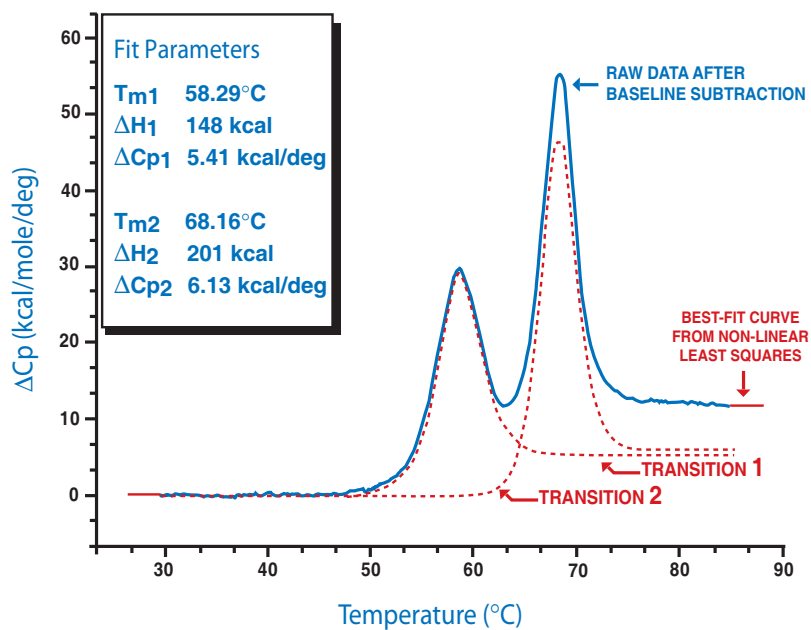
Stability

Kinetics

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# DSC Data Analysis in Origin<sup>®</sup> Tutorial Guide

Using Origin<sup>®</sup> scientific plotting software to analyze calorimetric data  
from all MicroCal Isothermal Titration Calorimeters



MAU120010 REV C



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**Introduction to DSC Data Analysis**

MicroCal Origin is a general purpose, scientific and technical data analysis and plotting tool. In addition, Origin can carry add-on routines to solve specific problems. Analyzing *Differential Scanning Calorimetric* data from the MicroCal MC-2, MCS or VP-DSC instruments is one such specific application.

This version of Origin includes routines designed to analyze DSC data. Most of the DSC routines are located in the Peak and DSC (Differential Scanning Calorimeter) menus in the Origin menu display bar. A number of routines are implemented as buttons in plot windows. This tutorial will show you how to use all of the DSC routines. When working through this tutorial it is recommended that you do not save changes to the original files, but use a different filename so that the next person may follow this tutorial.

Lesson 1 provides an overview of the entire DSC data analysis and fitting process. Please work through this lesson first. The subsequent lessons each look in more detail at particular aspects of DSC data analysis, and may be read in whatever order you see fit.

If you are unfamiliar with the basic operation of Origin, you may find it helpful to read through OriginLab's *Origin - Getting Started Manual* (particularly the introductory chapter). Note that this DSC tutorial contains information about Origin only in so far as it applies to DSC data analysis.

If you have questions or comments, we would like to hear from you. Technical support and customer service can be reached at the following numbers:

Toll-Free in North America: 800.633.3115

Telephone: 413.586.7720

Fax: 413.586.0149

Email: [info@microcalorimetry.com](mailto:info@microcalorimetry.com)

Web Page: [www.microcalorimetry.com](http://www.microcalorimetry.com)



## Getting Started

In this chapter we describe how to install Origin on your hard drive, how to configure Origin to include the DSC add-on routines, and how to start Origin.

### System Requirements

Origin version 7 requires the following minimum system configuration:

- Microsoft Windows® 95 or later or Windows NT® version 4.0 or later.
- 133 MHz or higher Pentium compatible CPU.
- 64 megabytes (MB) of RAM
- CD-ROM drive.
- 50 MB of free hard disk space.
- Internet Explorer version 4.0 or later (we recommend version 5.0 or later). Internet Explorer need not be your default browser, but it must be installed for viewing Origin's compiled HTML Help.

### Installing Origin - Single User License

**Note: We recommend that when installing Origin you do not accept the default directory, but choose the folder C:\Origin70\**

To install a new copy of Origin or to upgrade an existing copy, insert the Origin 7 CD into your CD-ROM. A window opens with a number of options, including installing Origin. Click the link to install Origin. If the CD does not start automatically, browse the CD and run ORIGINCD.EXE directly.

The Setup program prompts you to type in your Origin serial number and license key. These numbers are located inside your registration card in the Origin product package.

***Please refer to the 'Origin : Getting Started Booklet' for further information***

*(When choosing a Destination directory (or folder) name to place Origin, make sure this name or any other name in the path does not include a space, otherwise Origin will not operate properly)*

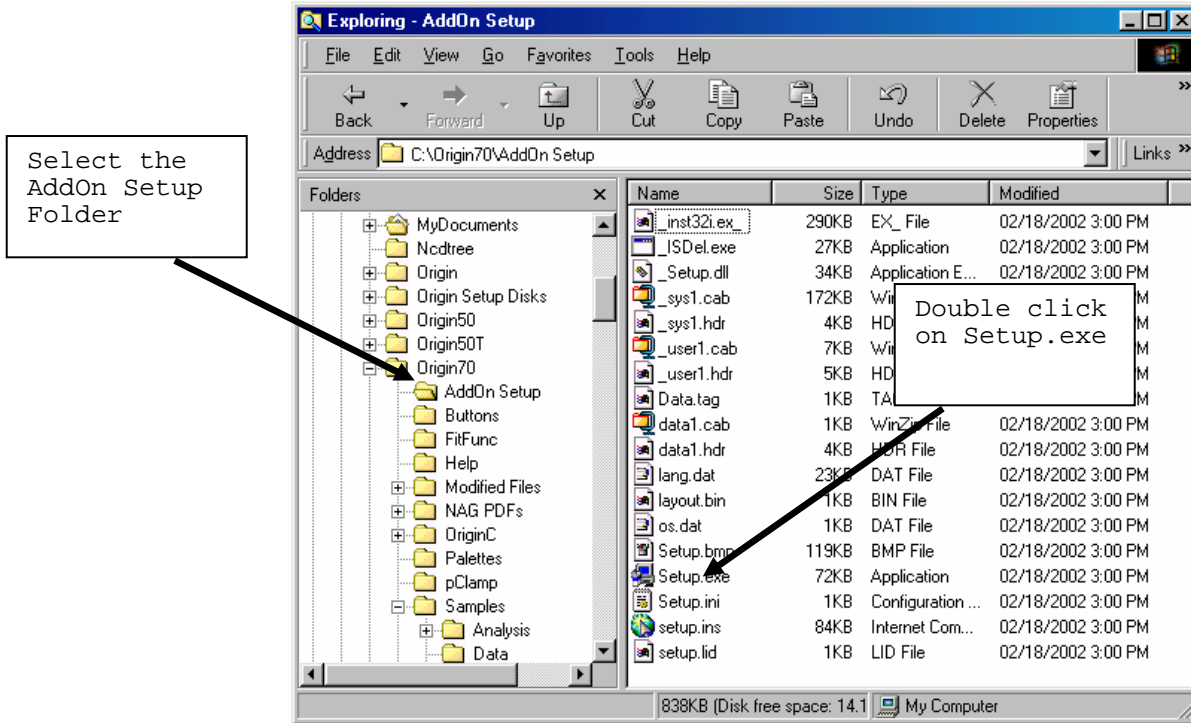
After installation is complete you will see the Origin70 program folder with the program icons. If you wish to create a shortcut desktop icon you may do the following. Right click the MicroCal LLC DSC icon and select copy from the drop down menu, then right click anywhere on the desktop and select paste to install a desktop icon for Origin DSC.

### Installing Origin for DSC Custom Disk

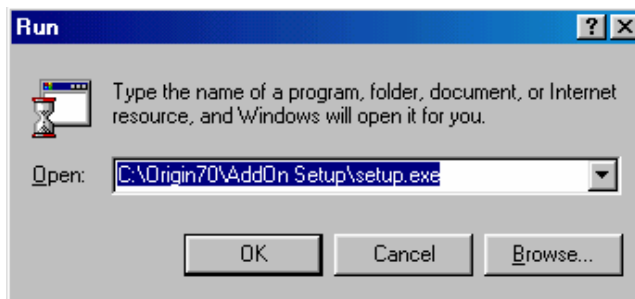
After installing Origin you will need to install the Custom disk for the DSC routines (if you have purchased other options, the relevant Autosampler, PPC and ITC routines will be included on this disk).

- Insert the Custom Disk CD into your CD-ROM

- Using Windows Explorer, navigate to the **AddOn Setup** sub folder of the **Origin 7.0** folder, and open the **Setup.exe** application file by double clicking on the filename.



*Note: you may also launch the setup up program by selecting **Run** from the **Start Menus** then entering **c:\Origin70\AddOn Setup\Setup.exe** into the text box and clicking **OK**.*



- Follow the instructions on screen.





## Registering with OriginLab

OriginLab™ Corporation., a separate company from MicroCal™, LLC, produces and supports the Origin software package. MicroCal™, LLC produces and supports the calorimetric fitting routines imbedded in the Origin for DSC and Origin for ITC packages. MicroCal, LLC will provide technical support for all aspects of the software without registration. OriginLab will not provide technical support for the calorimetric fitting routines, but if the copy is registered, will provide standard technical support for the general purpose routines of the program.

Upon receipt of Origin, please fill out and return the registration form included with your package to OriginLab. You may also register at any time by contacting the Customer Support Department at OriginLab.

## Starting Origin

To start Origin, double-click on the Origin 7.0 program icon on the Desk Top. Alternatively, click Start, then point to **Programs**. Point to the MicroCal Origin70 folder then click on the MicroCal LLC DSC program icon from the submenu.

## Menu Levels

This DSC version of Origin comes with a minimum of three distinct menu configuration options, or **menu levels** (there are other levels available if you purchased the optional PPC software, the Autosampler DSC software or the ITC software). Each menu level has its own distinct menu commands. After Origin has opened you may change a menu level option under the **Format:Menu** command option.

The seven menu levels are:

**General Full Menus** - Select this option to run Origin in the generic, non-instrument mode. This menu level contains no instrument-specific routines, but does contain many general data analysis and graphics routines that you may find useful for other applications.

**DSC Data Analysis** - Select this option to run Origin in a configuration that includes the instrument-specific DSC data analysis routines.


**Auto DSC Data Analysis** - Select this option to run Origin in a configuration that includes the instrument specific autosampler with DSC data analysis routines for handling multiple data files.

**PPC Data Analysis** - Select this option to run Origin in a configuration that includes the instrument-specific PPC data analysis routines. Note that this menu level is available only if you purchased the optional PPC attachment for the DSC.

**ITC Data Analysis** - Select this option to run Origin in a configuration that includes the instrument-specific ITC data analysis routines. Note that this menu level is available only if you purchased the optional ITC software module.

**Auto ITC Data Analysis** - Select this option to run Origin in a configuration that includes the instrument specific autosampler with ITC data analysis routines for handling multiple data files.. Note that this menu level is available only if you purchased the optional AutoITC software module.

**Short Menus** - Select this option to run Origin with menus that are an abbreviated version of the General Full Menus configuration. Note that you cannot switch to a new menu level if there is a maximized plot window or worksheet in the current project. A warning prompt will appear if you try to switch levels while a window is maximized. If this happens, simply click on the window

Restore button.  You will then be able to switch levels.

## Simultaneously Running DSC and ITC Configurations

If you purchased the DSC, ITC, Autosampler and PPC software modules, the installation program will have automatically created icons in the MicroCal OriginLab program group for the relevant software. This allows you to run the configurations simultaneously. The most likely reason to do this would be if you have the MicroCal DSC with the PPC or Autosampler attachment and the MicroCal ITC microcalorimeters, and you intend to run them on the same computer.

Double-click on any icon to run that configuration.

## View Mode

Each Origin plot window can be viewed in any of four different *view modes*: Print View, Page View, Window View, and Draft View.

**Print View** is a true WYSIWYG (What You See is What You Get) view mode. This view mode displays a page that corresponds exactly to the page from your hard copy device. Exact font placement and size is guaranteed, at some sacrifice to screen appearance, since the printer driver fonts must be scaled to fit their positions on the page (this will not harm the appearance of true vector fonts). This is a slow process, and screen refresh speed suffers as a result. Thus, reserve the Print View mode for previewing your work prior to printing.

Origin automatically changes to Print View mode when graphics are exported to another application and when printing. The view mode automatically returns to the selected view mode after the operation is complete.

**Page View** provides faster screen updating than Print View, but does not guarantee exact text placement on the screen unless you are using typeface scaling software (such as Adobe Type Manager). Use Page View mode until your application is ready for printing or copying to another application. Change to Print View mode to check object placement before exporting, copying, or printing.

**Window View** expands the page to fill up the entire graph window. Labels, buttons, or other objects in a graph window that reside in the gray area of the page are not visible in Window View mode.

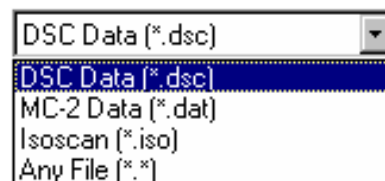
**Draft View** has the fastest screen update of the four view modes. In Draft View, the page automatically sizes to fill the graph window. This is a convenient mode to use when you are primarily interested in looking at on-screen data.

Note that view mode will not affect your print-outs. Only on-screen display is affected.

## Note About Data Import

MicroCal offers four versions of the DSC instrument (the MC-2, the MCS, VP-DSC and VP-CAPILLARY DSC). All together, there are five different data collection software packages available for use with these instruments - 1) a DOS-based and 2) a Windows-based package for the MC-2, 3) a Windows-based package for the MCS 4) a Windows-based version for the VP-DSC and 5) a Windows based version for the VP-CAPILLARY DSC.

This version of Origin, with the optional autosampler module will accept data files from any MicroCal DSC instrument, and from any of the five versions of the data collection software. To import a data file generated by the MC-2 DOS-based data collection software, you click on the **Read Data...** button in the **RawDSC** plot window and select **MC-2 data (\*.dat)** from the List Files of type box. To import a data file generated by the Windows-based data collection software (either from the MC-2, the MCS or the VP-DSC), you click on the **Read Data...** button in the **RawDSC** plot window and select **DSC Data (\*.dsc)** from the List Files of type box. PLEASE NOTE: Data



file names should not begin with a number, nor should they contain any hyphens, periods or spaces.

Once a data file is called into Origin, all further operations on the data are identical, regardless of the original source of the data. Note that, in this tutorial, all data were generated by a Windows based data collection software, and so we will be using only the **DSC data** file opening procedure. If your own data files are generated with DOS data collection programs, you must open them via the **MC-2** procedure. Refer to Lesson 1 for more information about data import.

An "isothermal" mode of collecting experimental data is available for the VP-DSC. This mode may be used when it is desirable to use the DSC to monitor a process at a constant temperature for varying periods of time, such as when determining kinetics of a chemical reaction at several different temperatures (e.g., estimates of shelf life). For this mode data is plotted Vs time rather than temperature. Selecting **Isoscan (\*.iso)** will read in data that has been collected in the VP-DSC "isothermal" mode and will plot the data (mcal/min) Vs time (seconds).

### Analyzing Previous Versions of Origin (\*.ORG) Documents and Projects (\*.OPJ)

*Shortcut: Saved Origin documents or projects (\*.org or \*.opj) may be opened from explorer by double-clicking on the file name.*

To open a previous version of an Origin document (project), select **File:Open**. This menu command opens the Open dialog box. Select **Old version (\*.ORG)** from the List Files of type: drop-down list. Select the desired file from the list box and click Open to close the dialog box and open the document. You may then make formatting changes and print the graph. If you wish to analyze the previous version of the document you must update the document to version 7.0.

To update a previous version of Origin Document (project), select **File:Update to Origin 7.0 Interface**. All templates will be updated to new templates compatible with version 7.0. You may then analyze the data with version 7.0. Please note: when you update the Origin templates to Origin 7.0 most of the text labeling on the graphs will be lost, including the fitting parameters. If you want to save the old fitting parameters text, you must copy the text before you update to Origin 7.0. To copy the fitting parameter text (or any other text) right-click anywhere in the text box and select copy. After you update to Origin 7.0 you may right-click in any of the 7.0 templates and select paste.

## Lesson 1: Basic DSC Data Analysis

This first lesson presents an overview of basic DSC data analysis procedures. You will learn how to start Origin; read a DSC data file into Origin; open multiple DSC data files; subtract reference data from sample data; normalize data by concentration; create and subtract baseline data; and use Origin's curve fitting models to fit a curve to your data. We will also briefly discuss instrument calibration.

After working through this lesson you will know all of the basic procedures involved in analyzing DSC data. Subsequent chapters look at specific procedural elements in more detail.

### Starting Origin



Before starting this lesson, you should have Origin up and running. If you are in Windows:

- From the Desk Top, double-click on the **MicrCal LLC DSC** icon. Or alternatively select **Start : Programs : OriginLab : MicroCal LLC DSC**.

When you start Origin, the program automatically opens the **RawDSC** plot window. Note that this window contains several buttons. These buttons let you execute certain DSC routines.

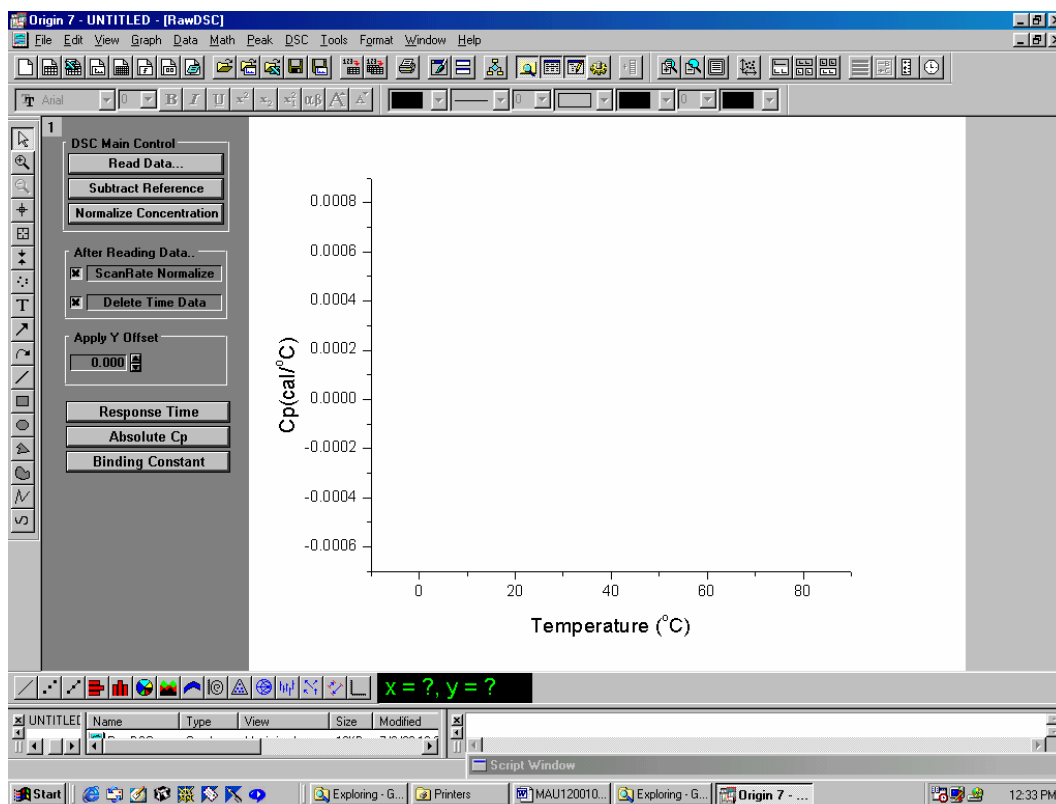
### Reading DSC Data

Before we proceed, you should understand the difference in Origin between a *data file* and a *project (or document for versions of Origin previous to 4.0)*. A *data file* contains your experimental data. In order to plot the data, you must read the data file into an Origin project. A *project (or document)* will contain both the experimental data and any plots you have made from the data.

To save a project, select the **File:Save Project** or **File:SaveProject As** command. The project saves as a .OPJ file in Origin 4.0 or higher. To open a project, use the **File:Open** command.

To import a data file you will use the buttons that are attached to the **RawDSC** plot window. The **RawDSC** plot window contains nine buttons, four of which are used for data import. The buttons are described below:

<b>BUTTON NAME</b>	<b>FUNCTION</b>
<b>Read Data...</b>	Reads a data file into the project. Use this button if the data were generated by MicroCal's data collection software.
<b>Subtract Reference</b>	After data files have been imported into Origin, use this button to subtract reference data from the sample data.
<b>Normalize Concentration</b>	Before you proceed to fit the data, you will need to normalize the result by concentration. Normalizing the data divides by the number of moles of the sample substance in the cell, to convert from cal/degree to cal/mole/degree. Concentration normalization is <i>always</i> carried out after subtracting the reference data.
<b>Scan Rate Normalization</b>	If this option is selected when you open a data file, Origin converts from the experimental data units of <i>mcal/minute</i> to units of <i>cal/degree</i> . You should always leave this box checked <i>except</i> when you are checking calibration of the instrument. Click on this checkbox to select or de-select this option. If the box is <u>not</u> checked, then unnormalized raw data will read in as <i>mcal/minute</i>
<b>Delete Time Data</b>	In raw data files, separate columns are included for Y axis, temperature, and time for each data point. When data are being Scan Rate Normalized, the time and temperature data are used to determine scan rate whereupon the time data is discarded and only the normalized Y axis readings and temperature data are imported into the project. The operator has the option of importing or not importing the time data ( <i>_time</i> extension) into the project as active data, though the time data is not plotted automatically.
<b>Apply Y Offset</b>	This is useful for offsetting repetitive scans, so they do not overlap on the Y axis. Click once on the number 0.000 in the offset text box to open the offset dialog box. Enter the offset you wish in the +/-Step Size box ( <i>enter the offset in mcal, even though the data are plotted in cal</i> ) and click OK. Click on either scroll arrow so the proper positive or negative Y offset for the next data set appears in the offset field. For each successive data set, the offset will advance automatically by the same increment.
<b>Response Time VP-DSC</b>	This is used for removing small amounts of peak broadening due to using a feed back mode which has a response time too slow to resolve a peak adequately. Refer to pages 88 - 91 for more information.
<b>Absolute Cp VP-DSC</b>	This is used to calculate Absolute heat capacity from raw DSC data. Refer to pages 54 - 57 for more information.
<b>Binding Constant</b>	Clicking this button will open dialog boxes to allow the user to input the parameters used to calculate binding constants from DSC data.
<b>Calculate Delta G(T)</b>	Using three experimental parameters of a protein scan, midpoint temperature ( $T_M$ ), enthalpy change at $T_M$ ( $\Delta H(T_M)$ ) and heat capacity change at $T_M$ ( $\Delta C_p$ ), the Gibbs free energy ( $\Delta G(T)$ ) may be obtained. at temperature (T).

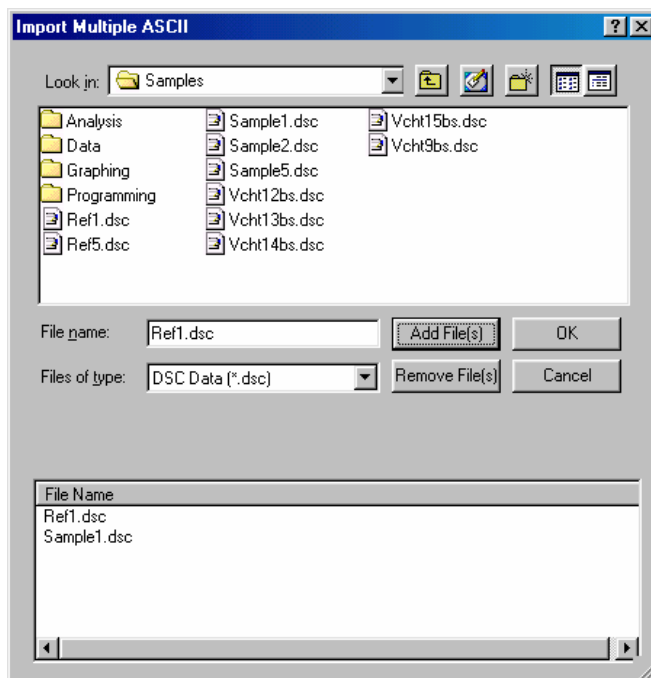


*The RawDSC window, which contains buttons that are used to import DSC data.*

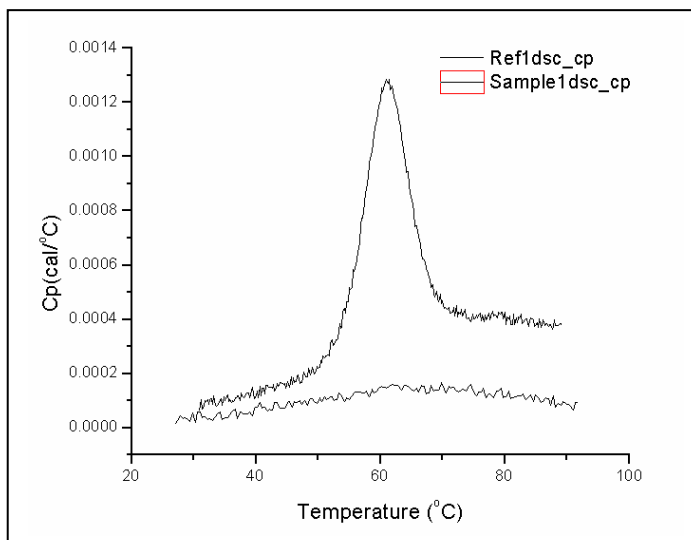
Two DSC data files (**SAMPLE1.DSC** and **REF1.DSC**) are included for your use with this lesson. **SAMPLE1** contains sample DSC data; **REF1** contains the associated reference data. Both are located in the **[Samples]** sub-folder of the **[Origin70]** folder. These files were obtained on the MC-2 DSC instrument using the Windows data collection software. Files from MCS or VP-DSC data collection software packages are similar, but have different header information.

To open the **SAMPLE1.DSC** sample data file and **REF1.DSC** reference data file

- Click on the **Read Data** button in the **RawDSC** plot window.  
The **Import Multiple ASCII** dialog box appears.



- Navigate to the Samples folder from the Look in: drop down list box. Note: You may configure Origin to always look in the Samples folder (or your own folder) by selecting **Files:Set Default Folder..** and entering the path in the text box.
- To add the file **Sample1.dsc** to the lower list box, double-click on the file **Sample1.dsc** in the "File Name" list box, alternatively you may click on the file and click on the Add File(s) button. Note: When assigning filenames to raw data, no hyphens/dashes or spaces may be used in the filename, the names should not begin with a number nor should the letter e be followed by a number.
- Repeat the above procedure for the file **Ref1.dsc**.
- Click **OK** to plot all files in the lower list box into the RAWDSC plot window.





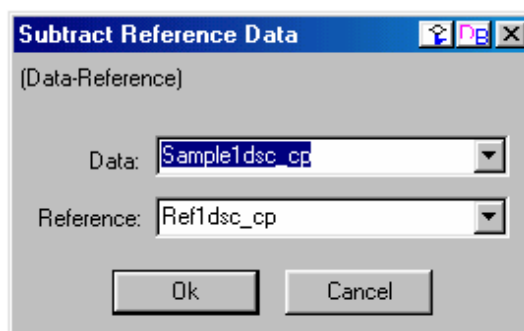
You can read any number of data files into the same **RawDSC** window. Note that when multiple data plots appear in the same window, you can set the *active* data plot by clicking on the *plot type icons* next to the filename in the legend. A box around the line/symbol type indicates the currently active data plot. Editing, fitting, and other operations can only be carried out on the active plot.

## Subtracting Reference Data

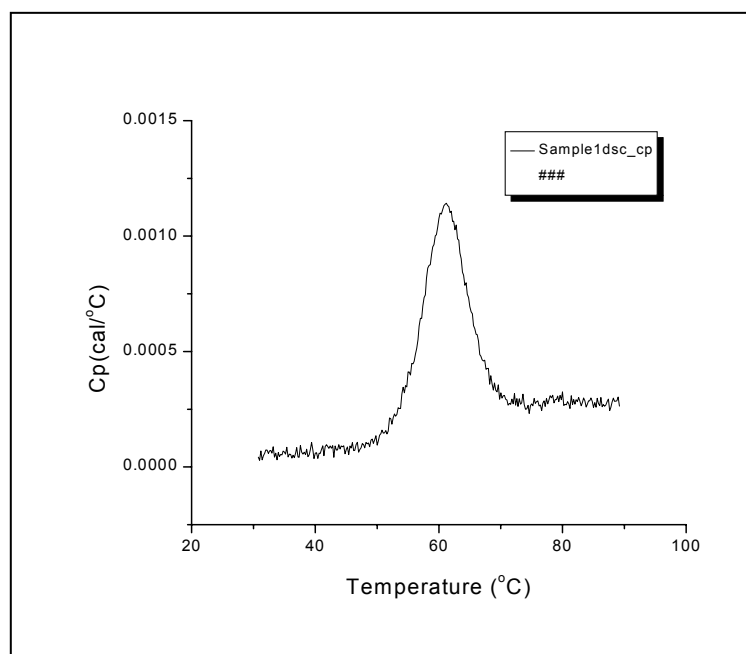
In most experiments you will have obtained both sample reaction heat data and reference data, and will need to subtract the reference data from the sample data.

To subtract the REF1 reference data from the SAMPLE1 sample data

- Click on the **Subtract Reference** button in the **RawDSC** window. The **Subtract Reference Data** dialog box opens. The first file opened, in this case **Sample1.DSC**, will appear in both the **Data** and **Reference** drop down list box. Note that the data set listed in the Reference box will be subtracted from the data set listed in the Data box.
- Select **Sample1dsc\_cp** from the Data drop down list. **Sample1dsc\_cp** becomes highlighted and will be entered as the Data.
- Select **Ref1dsc\_cp** from the Reference drop down list. **Ref1dsc\_cp** becomes highlighted and will be entered as the Reference.



- Click **OK**. Every point in **Ref1dsc\_cp** is subtracted from the corresponding point in **Sample1dsc\_cp**. The result is plotted as **Sample1dsc\_cp** in the **RawDSC** plot window.

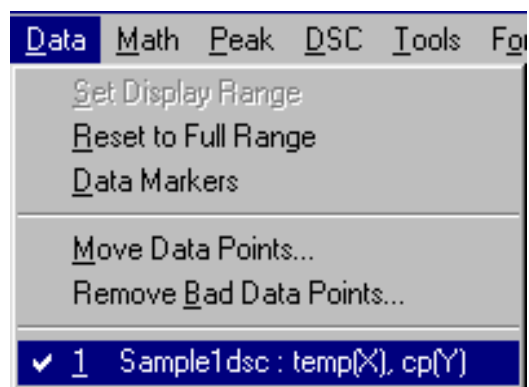


## Viewing Worksheet Data

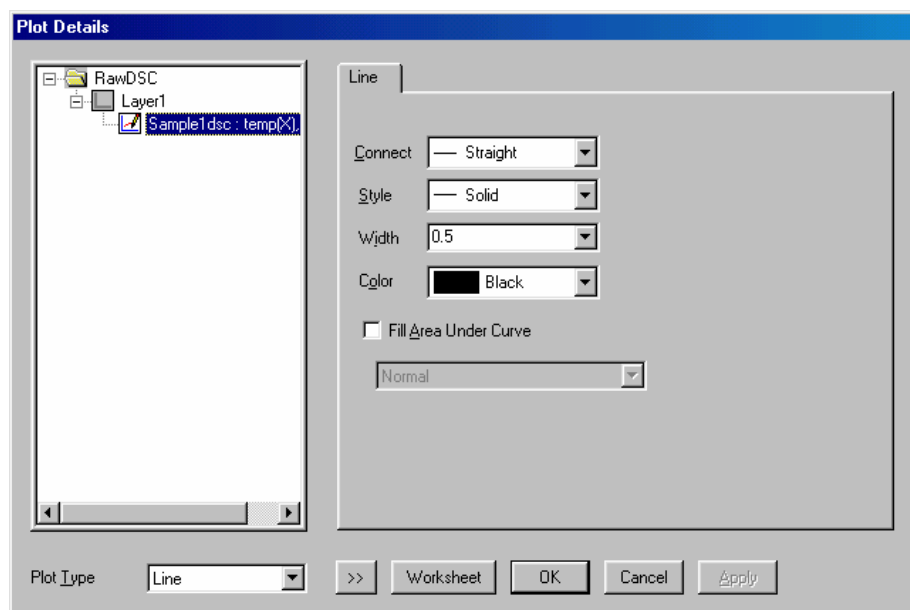
The series of values from which Origin creates a data plot is called a **data set**. Each data set is contained in a unique worksheet column. The data set is named after its worksheet and worksheet column, separated by an underscore. When you opened the **Sample1.DSC** data file, the data were placed in a worksheet named **Sample1dsc**, with the **Y** data in a column named **cp**. Thus the **Sample1dsc** data set is called **Sample1dsc\_cp**.

To open the Sample1dsc worksheet

- Select **Sample1dsc** from the data list in the **Data** menu.



The **Plot Details** dialog box opens. (Note that you can also open this dialog box by double-clicking on the data plotted in the RawDSC plot window).



- Click on the **Worksheet** button.  
The **Sample1dsc** worksheet opens.

	temp(X)	cp(Y)
1	30.79999	4.2636E-5
2	31	2.89146E-5
3	31.20001	6.99336E-5
4	31.40002	3.04161E-5
5	31.60004	4.9134E-5
6	31.80005	5.97788E-5
7	32.00006	7.35419E-5

## Normalizing the Data

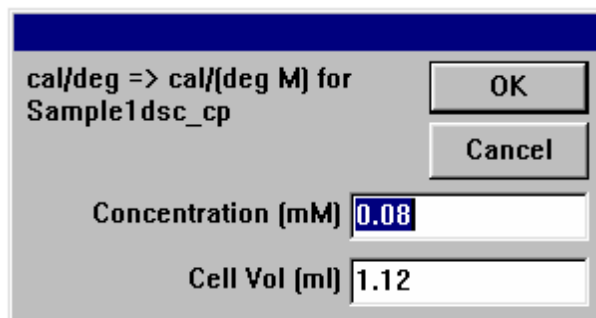
Before you proceed to fit (deconvolute) the data, you will need to normalize the result by concentration. Normalizing the data divides by the number of moles of the sample

substance in the cell, to convert from cal/degree to cal/mole/degree. Concentration normalization is *always* carried out after subtracting the reference data.

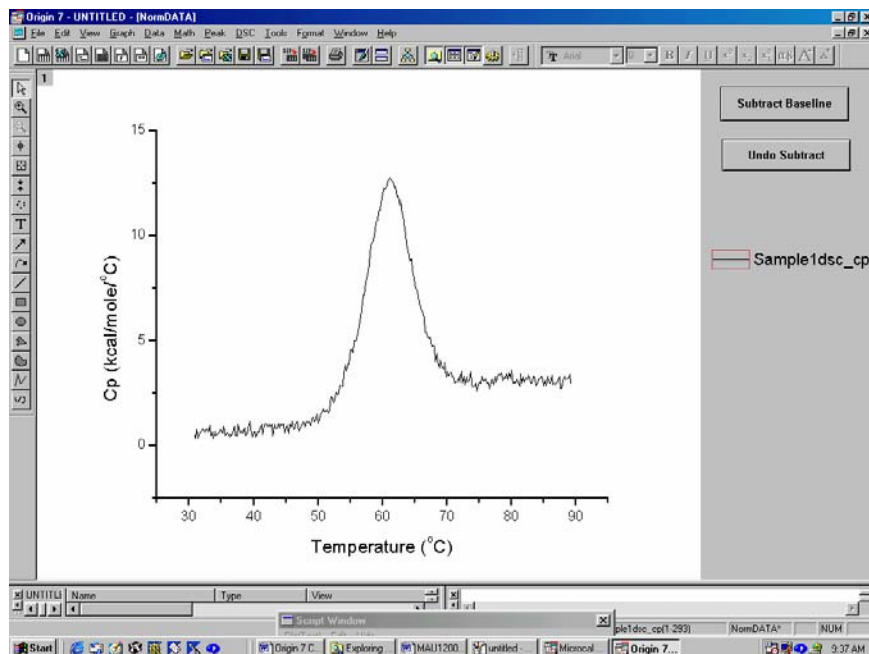
To normalize Sample1dsc by concentration

*To quickly switch between Origin windows, press and hold down the Ctrl key while pressing the Tab key.*

- Click on the **RawDSC** window to make it active, or select **RawDSC** from the **Window** menu.
- Click on the **Normalize Concentration** button. A dialog box opens.
- Enter the sample concentration in mM, and the cell volume in ml. For this example, enter **0.08** into the **Concentration mM** text box (**Sample1dsc** was at a concentration of 80  $\mu$ M), and **1.12** in the **Cell Vol (ml)** text box. (The concentration will automatically appear if you originally entered it into the header of the raw data file. For the VP-DSC, Cell volume is set in the **VPViewer.ini** (**CELL1.CON** for the MCS, and **CALDSC.dat** for the MC-2) file in the folder containing the data acquisition program and is stored in the header information of each data file.)



- Click **OK**. The **Sample1dsc\_cp** data plot is removed from the **RawDSC** plot window. **RawDSC** closes, and a new plot window, **NormDATA**, opens. The normalized data are plotted as **Sample1dsc\_cp** in the **NormDATA** plot window.



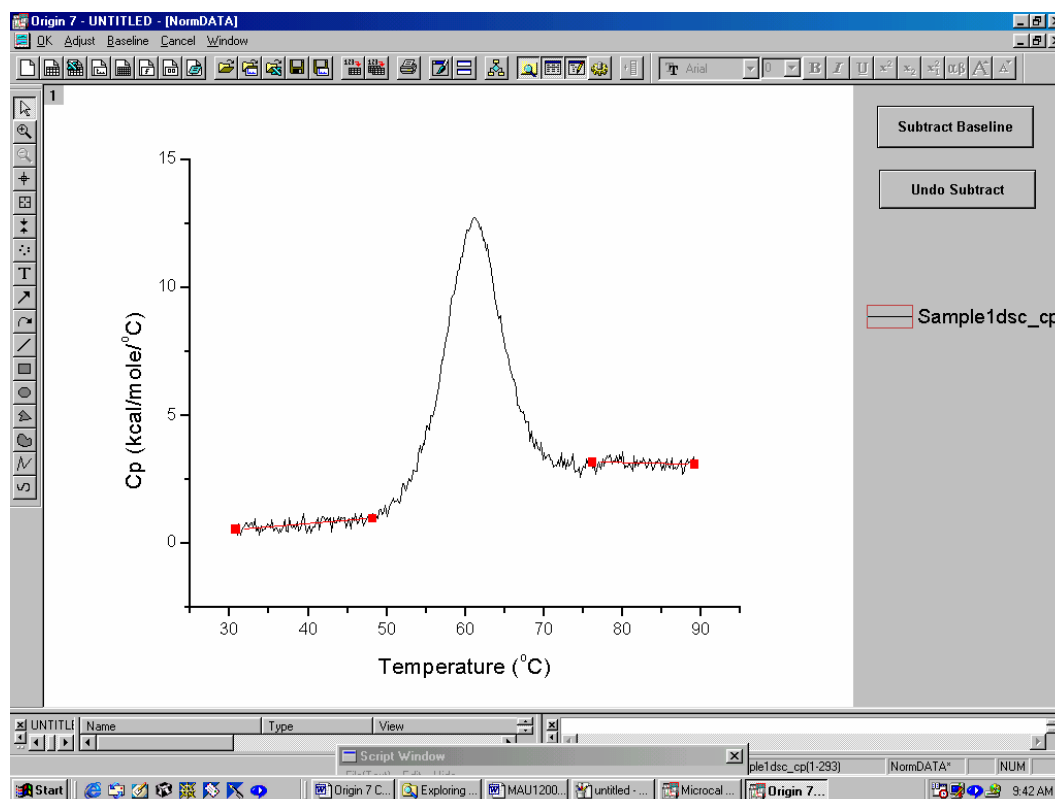
Note that NormDATA contains two buttons, which are used in the subtract baseline procedure.

## Creating a Baseline

The final step before deconvolution is to start the baseline session and plot a baseline for your data. You will then decide whether or not to subtract the baseline and, based on that decision, choose from one of three curve-fitting models.

To start the baseline session

- Choose the **Start Baseline Session** command from the **Peak** menu. Based on the normalized data, Origin calculates and plots in red the left and right linear line segments from which to determine the baseline. A new menu bar appears, containing the baseline session command menus.

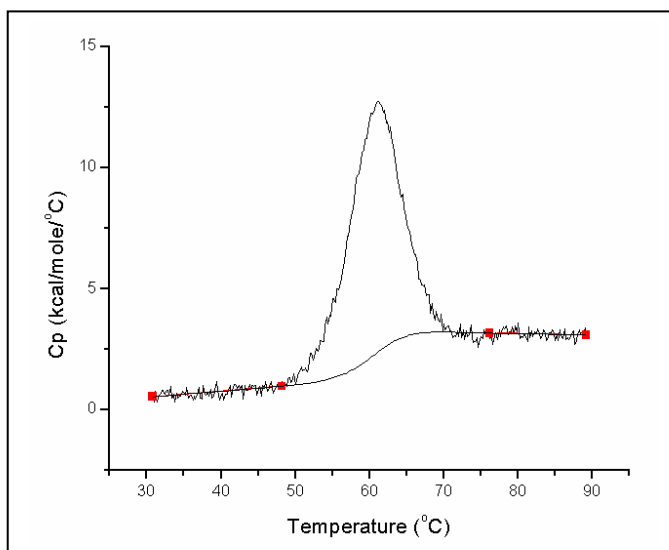


Origin lets you adjust the left and right linear segments, and provides several options for connecting the segments to create the baseline. For this example we will simply accept the

default segments as plotted, and connect them using a **progress** baseline. For greater detail see Lesson 4, **Baseline Determinations**.

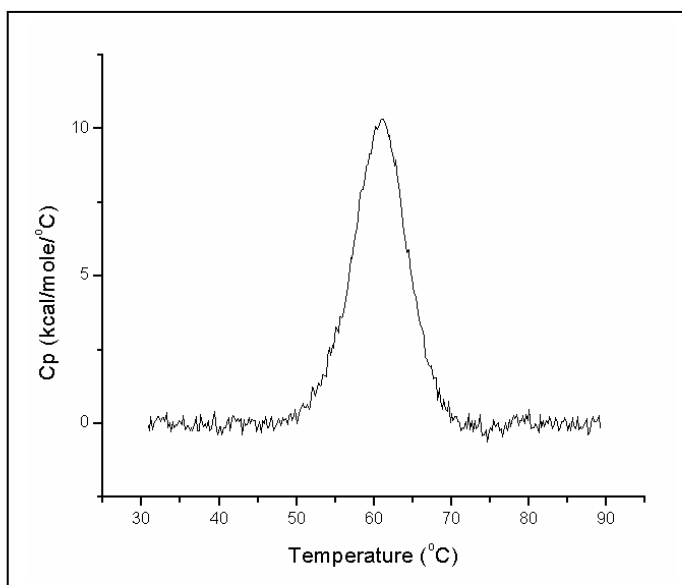
To plot the baseline

- Select **Progress Baseline** from the **Baseline** menu.  
Origin calculates and plots the progress baseline.



The progress baseline is created such that each point of the baseline in the peak region reflects the extent of progress of the reaction. For a single transition such as this, the area between the progress baseline and the data approximates the total heat change due to the transition.

- Click **OK** in the menu bar to exit the baseline session.  
Origin asks if you want to subtract the baseline data.
- Click **YES**.  
Origin subtracts the baseline data from the normalized data, then exits the baseline session.



## Fitting the Data

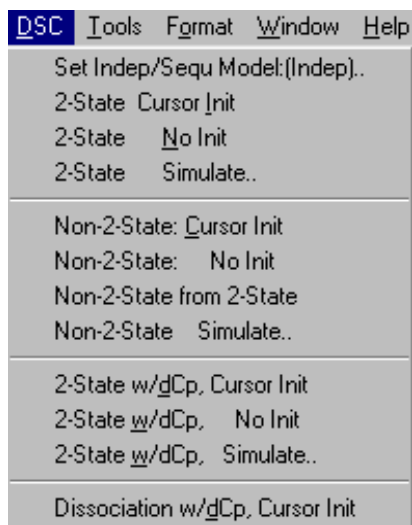
Origin provides four models with which to fit a curve to your data. All four models use the Levenberg/Marquardt (LM) non-linear least-square method. In general, you will want to get the best possible fit using the simplest model that fits the data. Curve fitting is discussed in greater detail in Lesson 5. This section will serve to acquaint you with the basic procedure.

The four curve-fitting models are located in the **DSC** menu. They are:

Model	Parameters
Model 1: 2 State	$T_m, H$
Model 2: Non 2 State	$T_m, H, H_v$
Model 3: 2 State w/dCp	$T_m, H, C_p, BL0, BL1$
Model 4: Dissoc w/dCp	$T_m, H, C_p, BLO, BL1, n$

All models except model 4 offer three menu-selection options (model 4 offers only a **Cursor Init** option):

- 1) **Cursor Init** -allows cursor initialization of each transition ( $T_m$ 's).
- 2) **No Init** - accepts the previously defined parameters, if any.
- 3) **Simulate..** - allows you to create a transition by entering user-defined parameters.



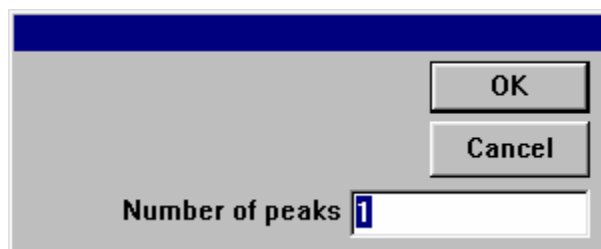
We will be using only the **Cursor Init** options in this lesson.

In the previous section you subtracted a baseline from your normalized data. Models 1 and 2 require you to subtract the baseline before fitting, while model 3 requires that you NOT subtract the baseline. Model 1 is the simplest model, so let's try fitting to this model first.

To fit a curve with the 2-state model (model 1)

---

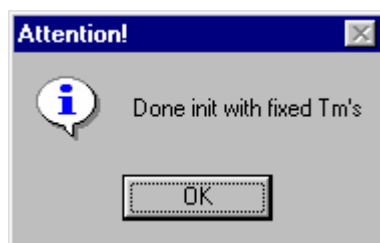
- Select **2-State Cursor Init** from the **DSC** menu.  
Origin integrates the **Sample1** data, then opens a dialog box asking you to enter the number of transitions.
- You will fit this data with a single  $T_m$ , so enter a **1** and click **OK**.



- Double-click to set the  $T_m$  for this peak. Or click once, use the arrow keys to position the cursor, then press RETURN. Set  $T_m$  equal to about  $X = 61$ .

Origin initializes the fitting parameters, displays the parameters and  $\chi^2$  in the **Fitting Session** dialog box, and draws an initial fit curve.

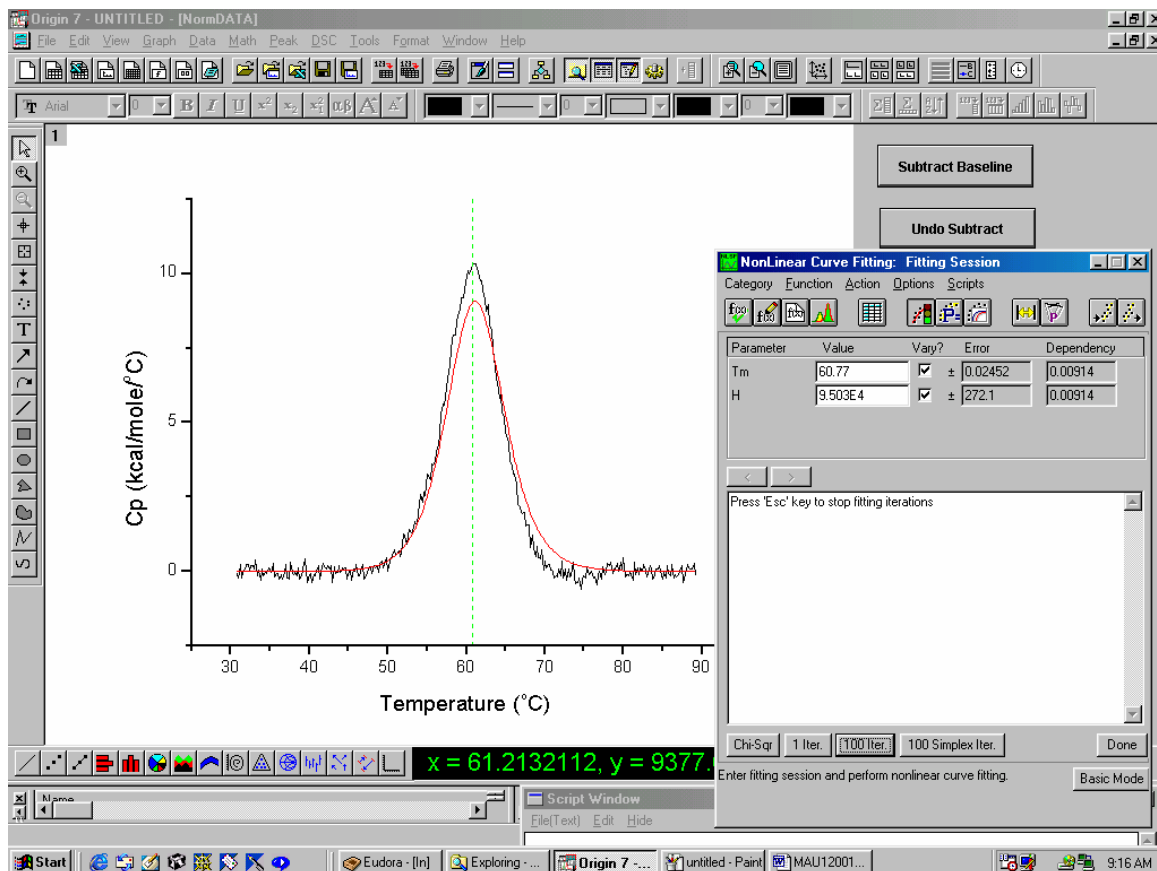
- When initialization is complete, the Attention! dialog box appears with the message **"Done init with fixed  $T_m$ 's"**.  
Click **OK**.





## The Fitting Session

You are now in the Advanced Mode of the **Fitting Session** dialog box. Note that a new window displays the fitting button commands. Please refer to the Origin User's Manual or press the F1 key for Online help for more information about the fitting procedures.



### Chi-Sqr

Click the Chi-Sqr button to display the reduced  $\chi^2$  value for the current parameter values. The reduced  $\chi^2$  value is displayed in the view box at the bottom of the dialog box. This feature allows the user to change the values of the parameters (i.e.  $T_m$ ,  $\Delta H$ ) to see an update of the fit using the new parameters. This option can also be used for a quick simulation of curves.

### 1 Iter.

Click this button to perform one iteration of the Levenberg-Marquardt (LM) routine. At the end of the iteration the fit curve, fitting parameters, and  $\chi^2$  are updated.

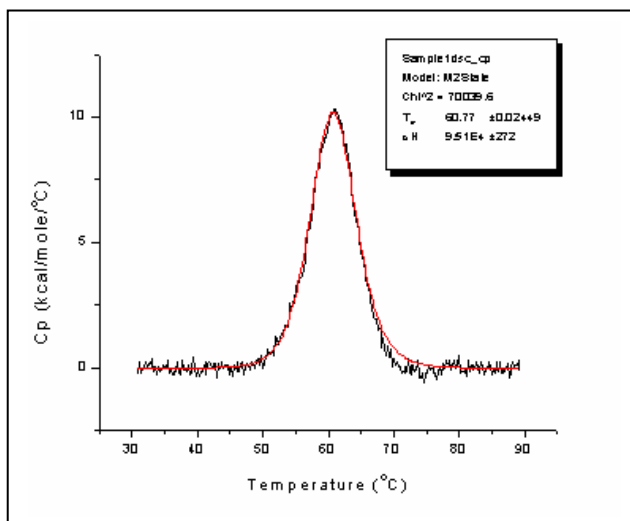
### 100 Iter.

Same as above, but performs up to 100 LM iterations. The number of Iterations can be changed in the Advanced Mode fitting sessions window (if you are in the Basic Mode, click on the **More..** button) and selecting **Options : Control**. Then change the **Max Number of Iterations** from the drop down list box.

### 100 Simplex Iter.

Click this button to perform up to 100 Simplex iterations. Normally, you will not need to do this because the Simplex method is much less robust and reliable than the LM method. However, if the LM method for a set of data behaves poorly, try the Simplex method.

- Basic...** Click this button to switch from the Advanced Mode of the curve fitting dialog box to the Basic Mode which contains its own menu bar, but with fewer options for fitting procedures.
- Done!** Exits the Fitting Session dialog box, returns to the NormData window, the fit curve is plotted and the fitting results are plotted in the Results text window and to a text box in the Normdata window.
- Use the **1 Iter.** & **100 Iter.** buttons to control the iteration of the fitting cycles, as described above. Watch the fitting parameters and Chi<sup>2</sup> update in the **Fitting Session** dialog box. When the Chi<sup>2</sup> value no longer reduces, you may click the **Done** button to exit the session.



To fit a curve with the non 2-state model (model 2)

- Select **Non 2-State Cursor Init** from the **DSC** menu.
- Repeat the steps you followed above when fitting with the 2-state model. The fitting procedure is identical for the two models.
- Click **Done!** when finished. A fit curve is generated, and the fitting results copy to the parameter text box in the **Normdata Window**. Note the difference in parameter values between the two models.

Data:	Sample1dsc_cp	
Model:	MN2State	
Chi <sup>2</sup> /DoF =	5.897E4	
T <sub>m</sub>	60.79	±0.022
ΔH	9.180E4	±503
ΔH <sub>v</sub>	9.947E4	±675

To fit a curve with the 2-state w/dCp model (model 3)

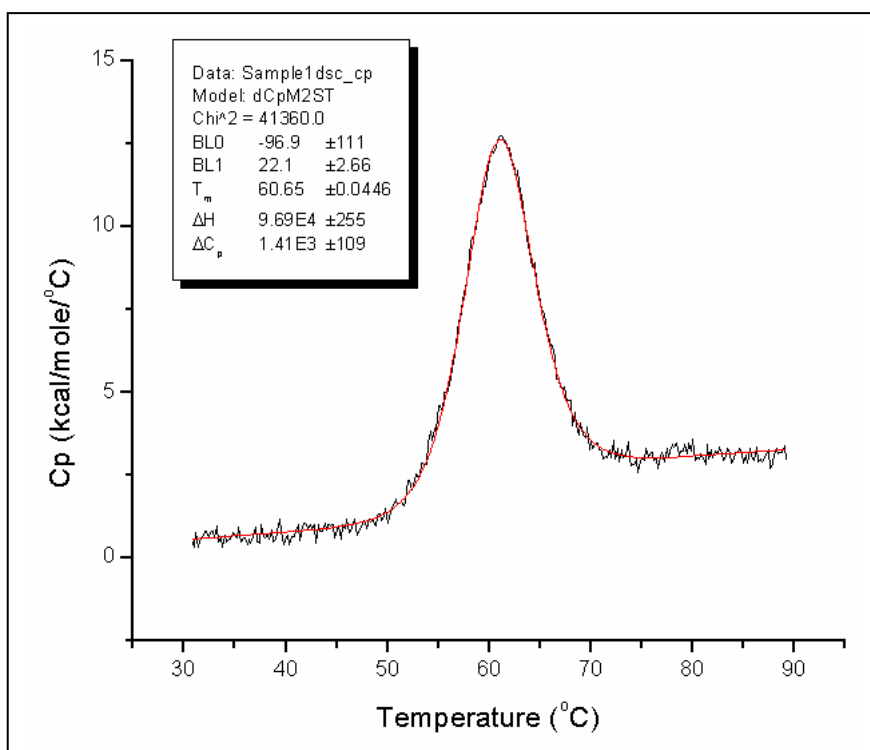
Model 3 is designed for use with data having a non-zero ΔCp. Model 3 requires that a baseline be associated with the data, so first we need to retrieve the baseline we subtracted previously. To do so:

- Select **Simple Math** from the **Math** menu.  
The **Math** dialog box opens.

- Select **Sample1dsc\_cp**, then click on the upper => button.  
**Sample1dsc\_cp** copies to the **Y1** text box.
- Select **SAMPLE1DSC\_CP.BASE** (this is the original baseline data set), then click on the lower => button.  
**SAMPLE1DSC\_CP.BASE** copies to the **Y2** text box.
- Click in the **Operator:** text box, and type a "+" in the operator text box.
- Click **OK**.  
The original Sample1 data is restored, since the baseline which was subtracted earlier has now been added back.
- Now that you have retrieved the baseline data, go ahead and fit a curve using model 3. Select **2 State w/dCp Cursor Init** from the **DSC** menu. Then follow the same steps as outlined for the previous two models. Click on **Done** to exit the fitting session.

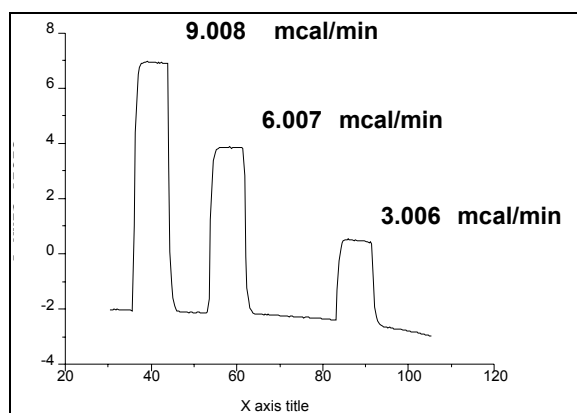
To format the fitting parameters text

- Right-click on the text in the plot window and select **Properties...** from the drop down menu.  
The **Text Control** dialog box opens.
- Change the text style (for example, change the font to **Times New Roman**, and point size to **16**).  
Note that to ensure a true What-You-See-Is-What-You-Get text display on screen, you should select the **Print View** mode from the **View** menu. View mode does not affect output so, for the sake of screen refresh speed, it is usually adequate to use **Page View**, **Window View**, or **Draft View**, all of which are faster.
- Click **OK**.



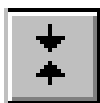
## Calibration

We will close this lesson with a brief note about instrument calibration. Every two or three months you will want to calibrate the DSC instrument against the Y axis scale in Origin. To do this you will fill both cells with water, then key in heat pulses to produce a **calibration file** (as described in your DSC instrument manual). You then open the calibration file into Origin, and check that the heat pulses match Origin's Y axis scale readings (+/- 1%). Note that the measured peak heights of 9.008, 6.007, and 3.006 indicated on the figure below agree very closely with experimentally entered pulses of 9, 6, and 3 mcal/minute. Pulse heights can be readily measured using the data reader tool from the Toolbox. One way to do this is to take a reading immediately before the pulse and immediately after the pulse, average these two readings, and then subtract the average from a third reading taken in the middle of the pulse.



When you open a calibration file you must remember **not to normalize on scan rate**. This means you should remove the checkmark from the **Scan Rate Normalization** checkbox in the **RawDSC** plot window before you read in the data file. If you neglect to do this and leave the scan rate normalization on, your calibration results will be wrong. Also note that when unnormalized DSC data are read into Origin, the Y axis units are *mcal/min* rather than *cal/deg*, which is obtained when data are normalized.

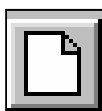
You now know how to perform basic data analysis of DSC files using Origin. The following lessons in this tutorial each look at a specific aspect of DSC data analysis in detail. You may work through these lessons in any order.

**Lesson 2: Using the Data Selector Tool**

The **Data Selector** is a toolbar tool that is used to select a segment of plotted data. Subsequent math, editing, or fitting operations will be carried out on the selected segment only.

In this lesson you will learn how to select a plot segment, delete the segment, set the data plot display range, and reset the display range. A sample DSC data file, **SAMPLE2.DSC**, has been included for your use with this lesson.

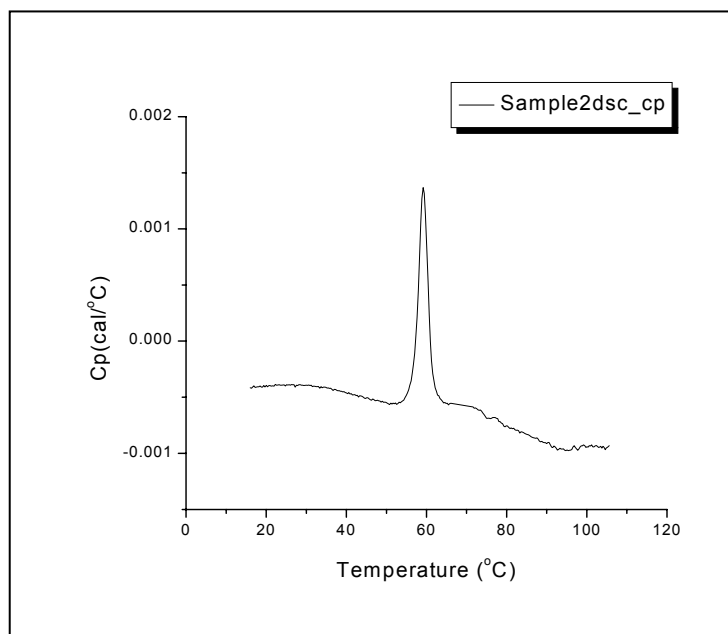
To open the SAMPLE2.DAT data file




*Shortcut:*  
Click the **New Project** button on the Standard toolbar.

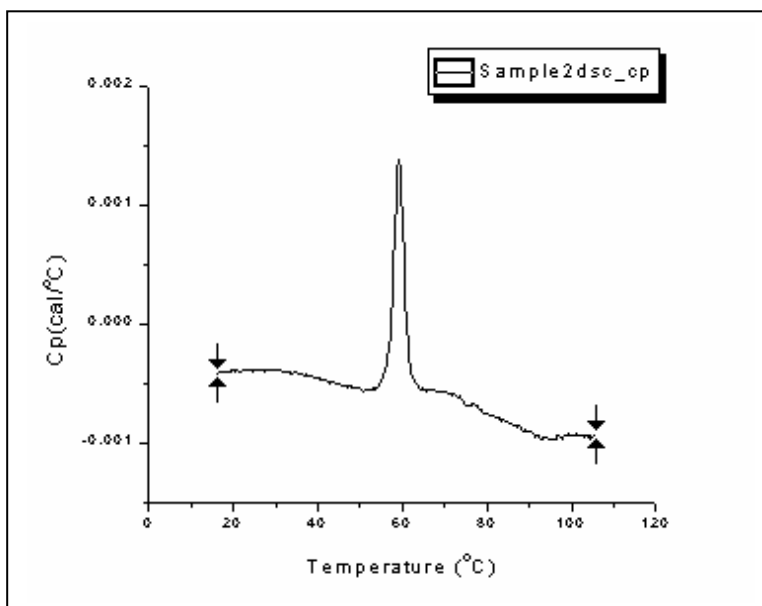
- Select **File:New:Project** to open a new project.
- Click on the **Read Data** button in the **RawDSC** window. The **Import Multiple ASCII** dialog box opens.
- Double-click on **Sample2.dsc** in the **Files** list box. **Sample2.dsc** is located in the **[Samples]** sub-folder of the **[Origin70]** folder. The file name will be added to the lower list box.
- Click **OK**.

The **Sample2.dsc** file opens into the **RawDSC** plot window.

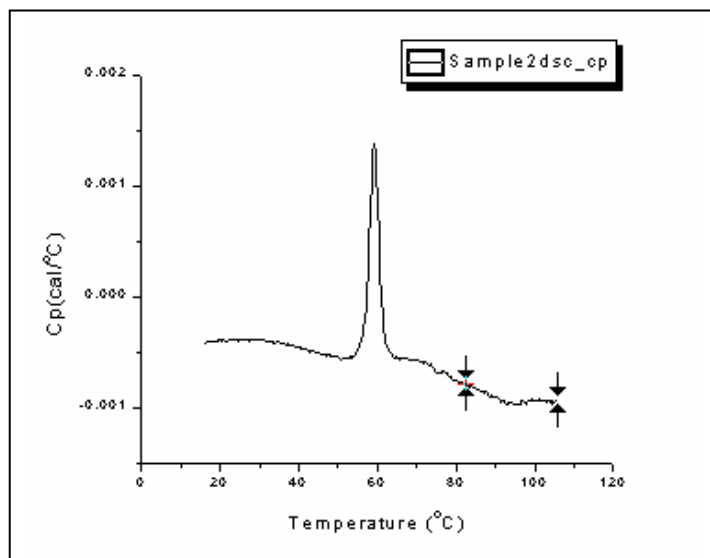


To select a segment of plotted data

- Click on the **Data Selector tool**  in the toolbox.  
The **Data Selector** becomes highlighted.  
The pointer turns into a cross-hair.  
Two **data markers** appear, one at either end of the **Sample2dsc\_cp** data plot in the **RawDSC1** plot window.



- Position the cross-hair on the left data marker, hold down the mouse button, and drag the marker to the right, approximately to the position shown below. Or use the **arrow keys** to move the marker: use the **Left** or **Right** arrow keys to select a marker; use **Control + Left** or **Right** to move the selected marker (hold down the **Control+Shift** key to increase the speed).

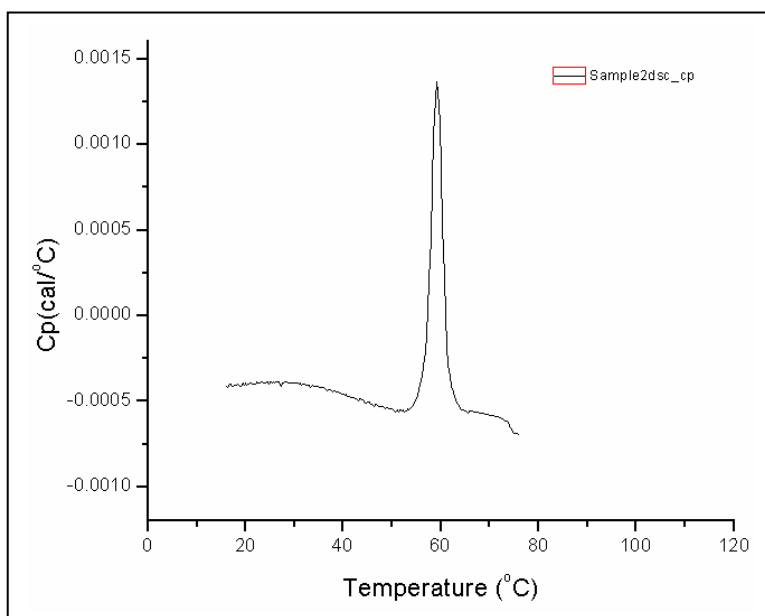


- To set the markers, double-click in the plot window, press **Return** (pressing the **escape** key with your left index finger is equivalent to pressing Return and this allows you to keep your right hand on the mouse), or click on any toolbar tool. The markers are set, and the **Pointer tool** is re-selected (unless you selected a different tool).

Any math, editing, or fitting operation now performed will affect only the selected plot segment. For example, you could delete the segment.

#### To delete the selected plot segment

- Choose **Cut** or **Clear** from the **Edit** menu, or press the **Delete** key. The selected plot segment is deleted from the plot window. The selected values are also deleted from the **SAMPLE2** worksheet. The data markers are removed. You may select **Edit:Undo** to replace the data.

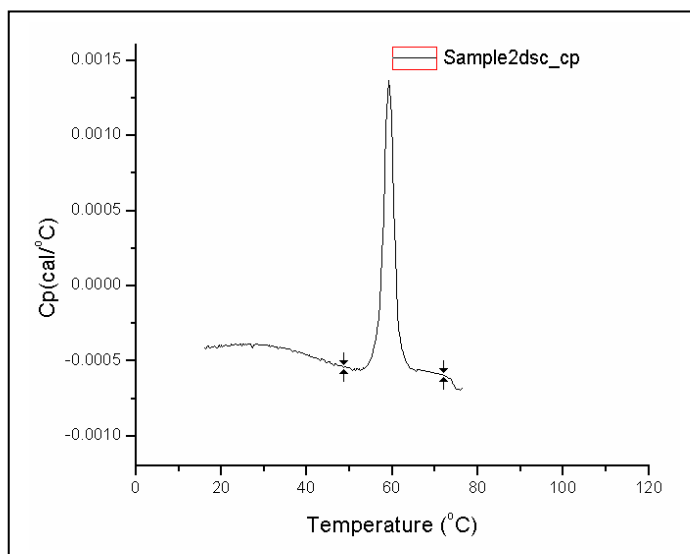


Data markers are only meant to be displayed temporarily. They will disappear as soon as another data set or plot window becomes active. To select a segment of data for a longer period of time, use the **Set Display Range** command.

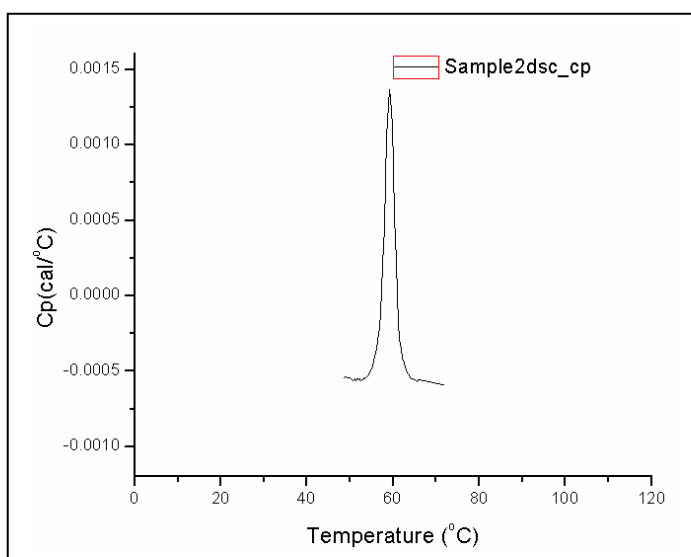
Say that you would like to display or manipulate a central range of data in the **SAMPLE2** data plot, but you do not want to delete any of the data. This is an ideal use for the **Set Display Range** command.

#### To set data plot display range

- Click on the **Data Selector** tool, click on the right data marker, and position it as shown below. Then click on the left data marker, and position it as shown:



- Double-click or press **Return** to set the markers.
- Choose **Set Display Range** from the **Data** menu.  
The data outside the selected range are hidden from view.



You may expand your data to fill the graph by selecting **Graph: Rescale to Show All**. All math, editing, and fitting operations will be done on the display range only.

The data outside the display range are not deleted; they are merely hidden. Use the **Reset to Full Range** command to bring the hidden data back into view.

To reset display to full range

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- Choose **Reset to Full Range** from the **Data** menu.  
Origin resets the data markers so as to display the entire curve.



## Lesson 3: Integration

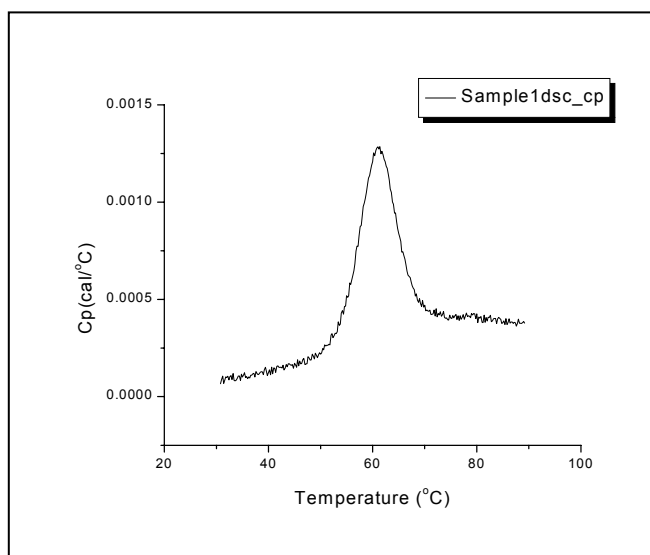
If you have created a baseline for a DSC data plot, you can select a range of data to integrate. Integration yields the following information about the data: integration range, temperature range, area, thermal midpoint, and width of curve in degrees C at half height. In this lesson you will use the **Data Selector** tool to set the integration range, integrate the data, copy the integration results to the plot window, and plot the integration data.

### Setting Range and Integrating the Data

As you saw in Lesson 2, all math, editing, and fitting operations are performed on the currently selected plot segment or on the current display range. This is true for integration as well. The first step in integration is to use the **Data Selector** to set the **integration range**. If you do not set the integration range, the entire display range will be integrated.

Before beginning this lesson, open a new project:

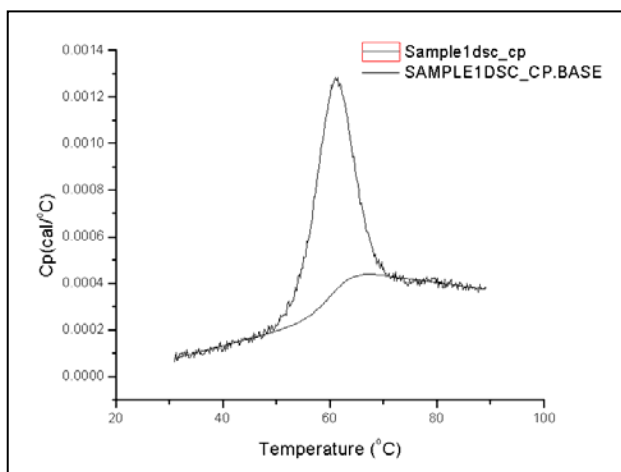
- Select the **File:New:Project** (or click on the New Project button) menu item.
- Click on the **Read Data** button in the **RawDSC1** plot window. The **Import Multiple ASCII** dialog box opens.
- Double-click on **Sample1.dsc** (located in the **[Samples]** sub-folder of the **[Origin70]** folder).
- Click **OK** to plot the files from the lower list box. **SAMPLE1.DSC** opens into the **RawDSC** plot window.




Before integrating, you must plot a baseline for the data. You will then choose to either integrate from the baseline, or subtract the baseline and integrate from zero (see Lesson 4 for more about baseline determinations).

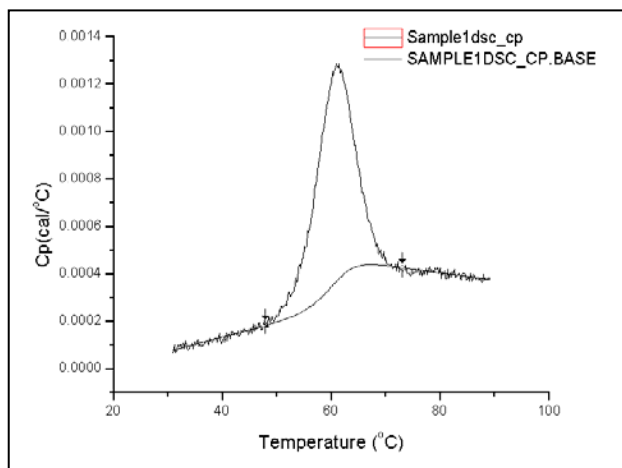
## Plotting a baseline

- Select **Start Baseline Session** from the **Peak** menu.  
Origin starts the baseline session, and plots two red linear baseline segments.
- Select **Progress Baseline** from the **Baseline** menu.  
Origin connects the linear segments with a progress baseline.
- Select **OK** from the menu bar.  
Origin asks if you want to subtract the baseline. Click **No**.  
Origin exits the baseline session.



## To set the integration range

- Click on the **Data Selector** tool  in the toolbox.  
Two data markers appear on the **SAMPLE1** curve (note that these appear on **SAMPLE1**, and not on the baseline curve, because **SAMPLE1** is currently set as the active data plot in the **Data List**, under the **Data** menu).
- Using the mouse or the arrow keys, position the data markers approximately as shown below. (If you are not sure how to position the markers, refer to Lesson 2 in this tutorial.)




- Double-click or press **Return** to set the markers.  
The area between the markers is the range that will be integrated.

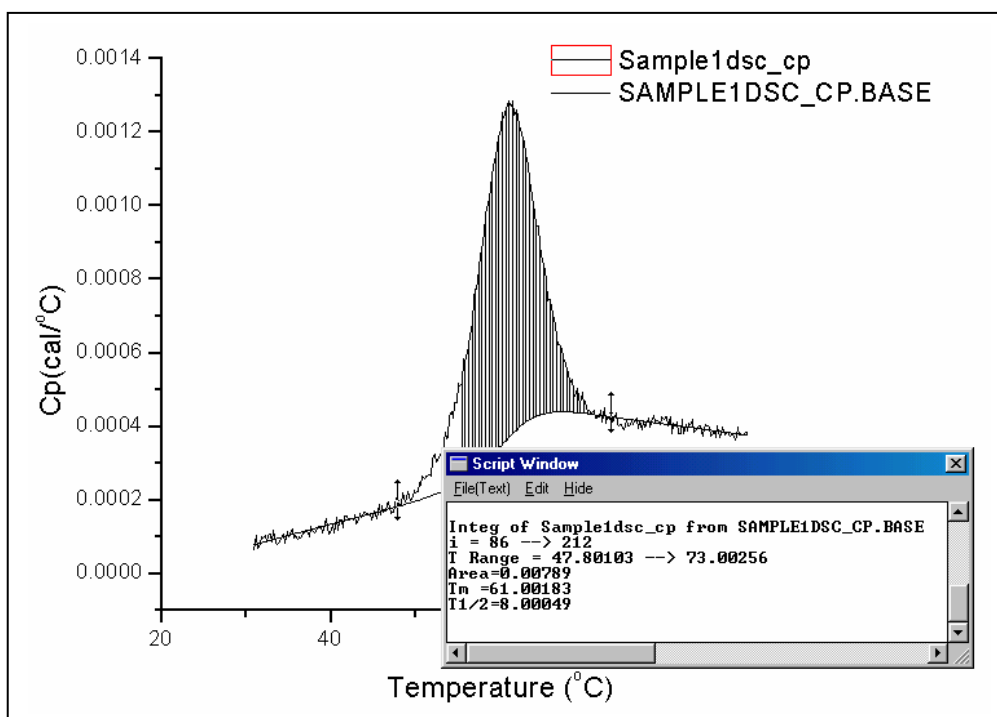
To integrate the selected range

- Since you have created a baseline but have not subtracted it from the data, integrate by choosing **Integrate from Baseline** from the **Peak** Menu.

If you had subtracted the baseline from the data, you would integrate by choosing **Integrate from 0** from the **Peak** menu.

The area between the two data markers is integrated. The integration results copy to the **Script Window**.

**Note:** The Script Window may hard to find. It may be located at the bottom edge of the work area with just the title bar showing or be hidden. You may show/hide the Script Window by either holding down the **Alt** key and pressing the number **3** key (not on the key pad) or by clicking the Script Window button  located on the standard toolbar.



If you wish, you can reset the markers and try again. Each time you integrate, the results are pasted to the **Script Window**. To clear the integration shading from the plot window, select **Refresh** from the **Window** menu.

The procedure that copies the integration results to the Script Window is a macro called **EndInteg**. **EndInteg** is defined in the **DSC.CNF** file, and can be redefined to customize the output to suit your needs. For more information see the **LabTalk Reference Manual**, available separately from MicroCal.

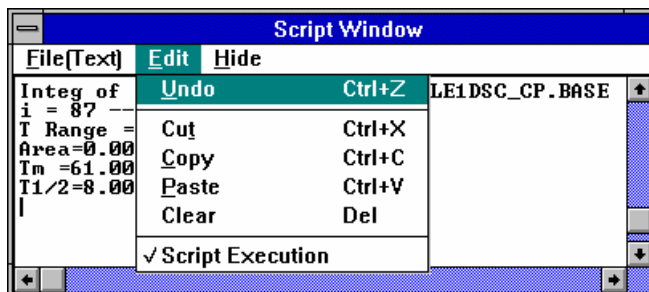
## Displaying the Integration Results

The Script Window is a full-featured text editor. Use it to edit the integration text, save the text to a file, or copy and paste the text with the clipboard.

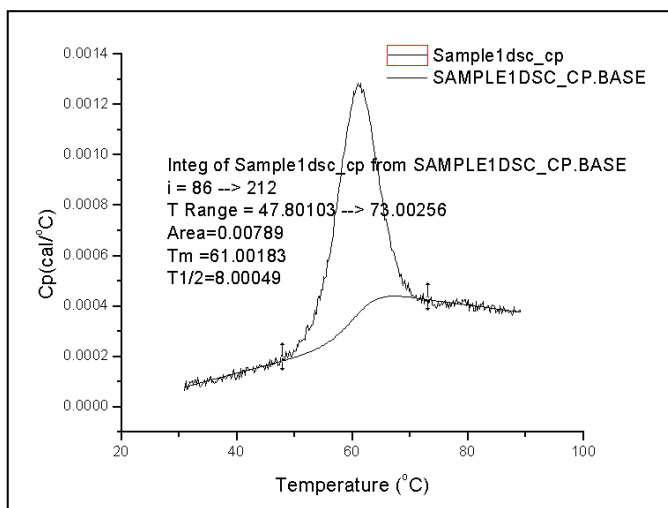
To copy the integration results to the plot window

- Click and drag with the mouse to highlight the integration results in the **Script Window**.
- Choose **Copy** from the Script Window **Edit** menu.  
The text is copied to the clipboard.

*Alternatively, to copy and paste, you may right-click on the highlighted text, select copy from the menu, then right-click where you want to position the text label and select paste.*



- Choose **Edit:Paste** from the menu bar along the top of the Origin project window.  
The integration text is pasted to the **RawDSC1** plot window (the **active window**).



The text as pasted is too large and placed poorly. Let's reformat and reposition the text, and add a text border.

To change the text style

- **Right-click** on the integration results text in the plot window and select **properties** from the drop down menu.  
The **Text Control** dialog box opens. From here you can edit the text, set font style, color, and point size, rotate the text, and add a border.

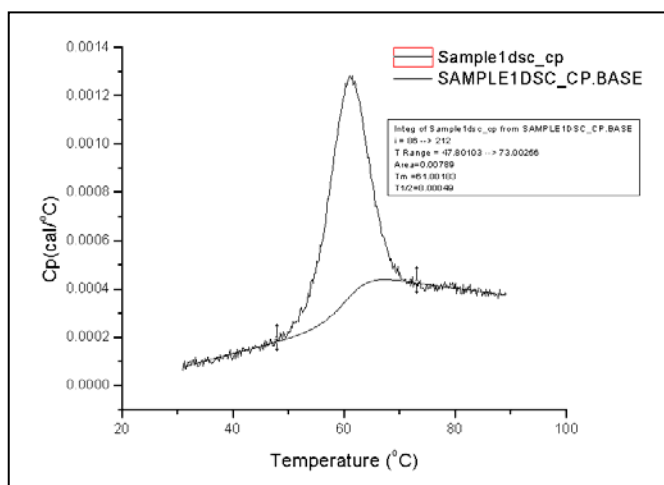
Note that the **Text Control** dialog box is divided into two display boxes all text entries and formatting is done in the upper text box, the lower text box displays the text as it will actually appear (WYSIWYG) in the graph window.

- Type **12** into the center points text box.
- Select **Black Line** from the **Background** drop down list box.  
Note that if you select none for the Background the box may not be erased till you perform a screen refresh (select **Window:Refresh**).
- Click **OK**.  
The **Text Control** dialog box closes, and the integration results text updates to show your changes.
- **Click and drag** on the integration results text to place the text in the upper right corner of the plot window.

To see the graph exactly as it will appear when printed, switch to the **Print View** view mode. View mode is chosen from the **View** menu.

To change the view mode

- Click once on the **View** menu.  
The **View** menu commands appear. Notice that **Page View** is checkmarked, showing it to be the selected view mode. Page View uses the screen driver to display font information.
- Select **Print View** from the menu.  
**Print View** is now the selected view mode. Print View gets font information directly from the printer driver, and is a true WYSIWYG view mode.  
The plot window text is now sized and positioned correctly.



- Before proceeding with this lesson, re-select the **View:Page View** command.

For more about view mode see Chapter 12: Plotting: Customizing the Page Display in the Origin User's Manual or select the Index tab from the **Help:Origin** menu option and enter View modes into the upper text box.

To remove the data markers

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- Click **Data Markers** from the **Data** menu (to remove the check mark).  
Origin hides the data markers.

## Plotting the Integration Area Data

The integration area data is saved (as a function of X, which is temperature for calorimetric data) into a data set named **\_integ\_area**. The leading underscore signifies that **\_integ\_area** is a temporary data set which will be deleted whenever memory is running low. To access the worksheet of **\_integ\_area** data, or use it for any other purpose, you should first copy **\_integ\_area** to a permanent data set.

To copy the integration area data to a new data set

---

- Click on the **Edit** menu in the **Script Window** menu bar. Note that the **Script Execution** command is checkmarked, which means you can currently execute **LabTalk** scripts directly from the Script Window.
- On a new line, type the following script exactly as it appears. Be sure to include the single space before **\_integ\_area** and also before **my\_test**: *Please note that the data set name must consist of two parts separated by the underscore character. The first part will be the worksheet name (in this case the worksheet will be named my) the second part will be the column name of the data set.*

```
copy _integ_area my_test
```

- Press **Return**.  
Origin should append a semicolon ";" to the text you just entered. This indicates that the script was executed. For more information about the **Lab-Talk** scripting language, please refer to the **LabTalk** manual or select the online **Help:Programming:LabTalk Reference** menu option.

**My\_test** is now an Origin data set that can be plotted and manipulated like any other data set.

To plot the **my\_test** integration area data

---



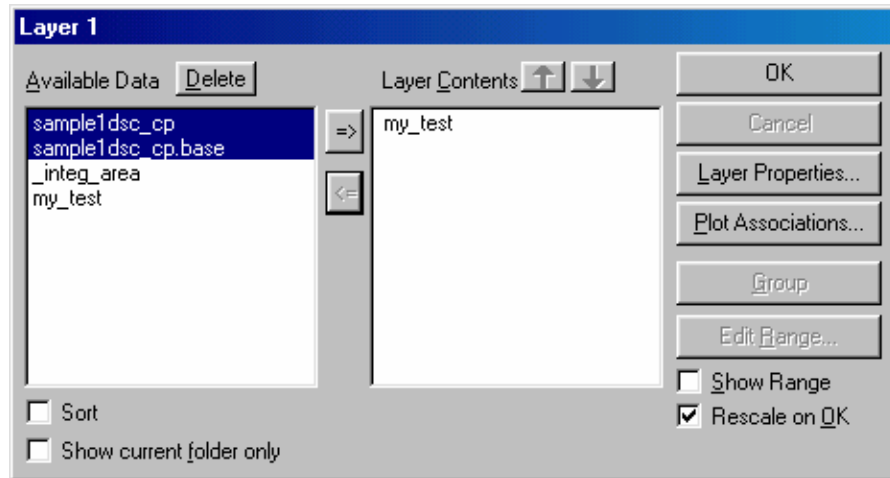
*Shortcut:  
Click on the  
New Graph  
button*

- Select **File:New:Graph** to open a new plot window.

The **Graph1** plot window opens.

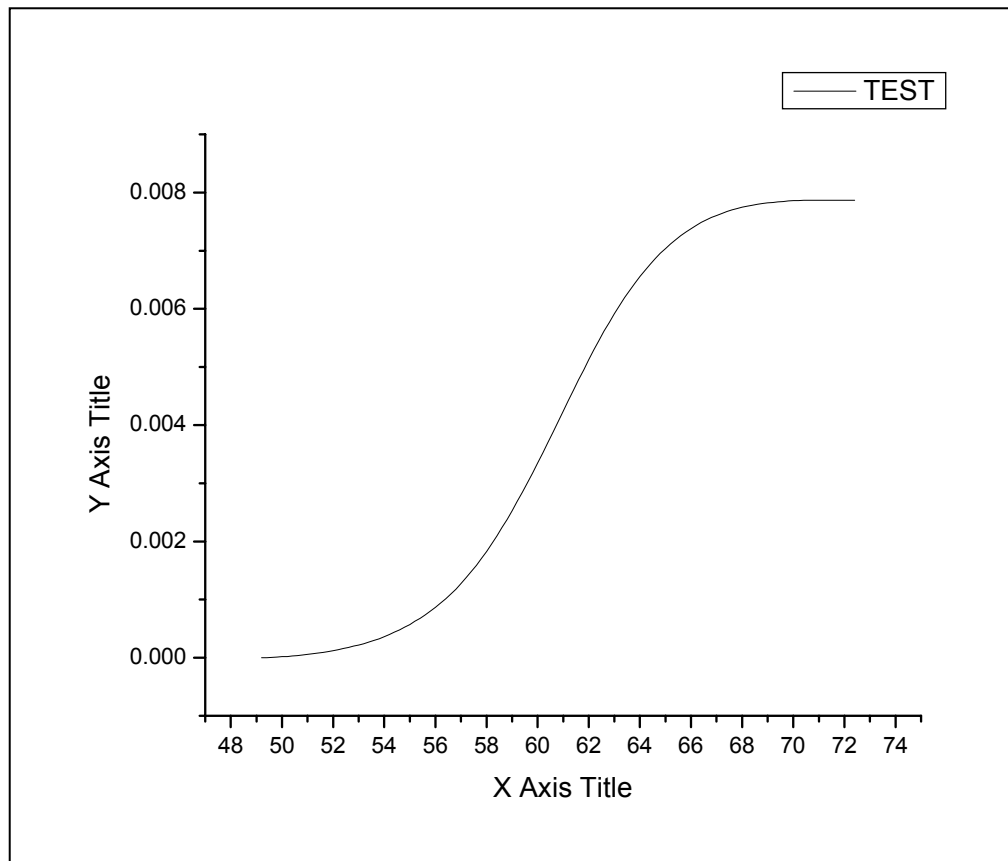


- Double-click on the layer icon in the **Graph1** plot window.  
The **Layer Control** dialog box opens.
- Click on **my\_test** in the **Available Data** list, then click on the => button.  
**My\_test** appears in the **Layer Contents** list. All data sets in the **Layer Contents** list will plot in the active layer.



- Click **OK**.

The **Layer Control** dialog box closes, and **my\_test** plots as a line graph in the **Graph1** plot window.



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## Lesson 4: Baseline Determinations

Origin creates a baseline for the reaction heat data by establishing a left linear segment and a right linear segment that are considered to be the "baseline region". Several methods can be used to connect these two segments to form the baseline. In this lesson you will learn how to create, format, subtract, and open the related worksheet for a baseline. You will also learn about the various baseline creation options that are available.

Baseline commands are located under the **Peak** menu.

### Starting the Baseline Session

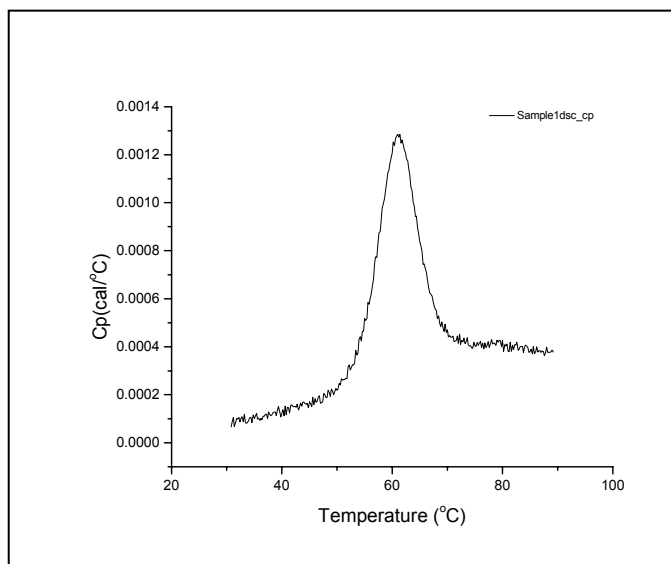
When you are ready to create a baseline for your data, you must start the **baseline session**. In this lesson we will create a baseline for the **SAMPLE1** DSC data file.

To open the SAMPLE1 data file

- If you are continuing from a previous lesson, first select **File:New:Project** (or click on the New Project icon) to open a new Origin project.
- Click on the **Read Data** button in the **RawDSC** plot window. The **Import Multiple ASCII** dialog box opens.
- Double-click on **Sample1.dsc** in the **Files** list. **SAMPLE1.DSC** is located in the **[Samples]** sub-folder of the **[Origin70]** folder. The path and file name will be added to the lower list box.
- Click **OK**.

*Shortcut: You can program Origin to always Look in: the Samples folder (or any other folder) by selecting **File:Set Default Folder** and entering a new path in the text box*

**SAMPLE1** opens into the **RawDSC** plot window.

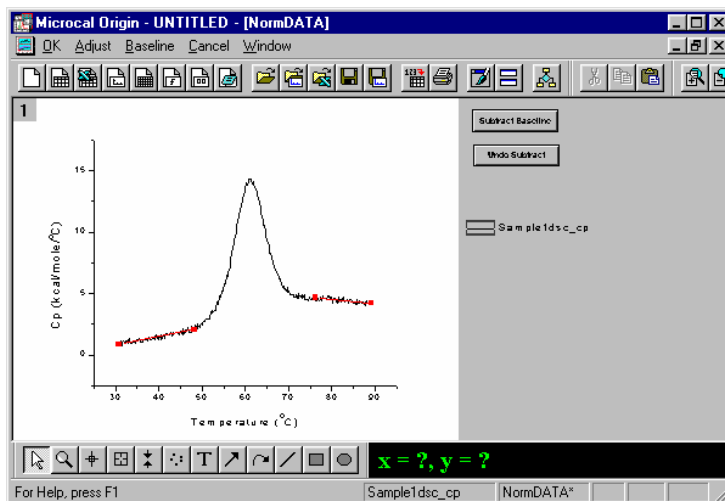


Before creating the baseline, you would normally subtract reference data, and then normalize the data on concentration. For the sake of brevity, we will bypass those steps here. They are discussed in detail in Lesson 1, **Basic DSC Data Analysis**.

To start the Baseline Session

---

- Choose Start Baseline Session from the Peak menu. Origin enters the baseline session, and automatically analyzes the data to determine the linear segments to be drawn on either side of the peak



Note that a new command menu bar now appears at the top of the project. These command menus provide complete control over the baseline determination process, as follows:

- OK** Accepts the current baseline, provides an opportunity to subtract this baseline from the data curve, and then exits the baseline session.
- Adjust** Allows the user to adjust the two linear segments.
- Baseline** Choose one of five options for creating a baseline, or draw a baseline manually.
- Cancel** Exits the baseline session unconditionally. The current baseline, if any, is deleted.

If you are having difficulty seeing the linear segments, you can maximize the plot window for a better view.

To maximize the RawDSC plot window

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- Click on the Maximize button,  at the upper-right corner of the window. The window zooms to full size.

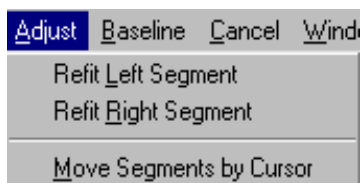
## User Adjustment of Linear Segments

If you are not satisfied with the two automatically determined linear segments, use the **Adjust** menu to change the segments as you see fit. The **Adjust** menu provides two options: refit the segments, or move the segments by cursor.

To refit the linear segments

---

- Choose Refit Right Segment from the Adjust menu.



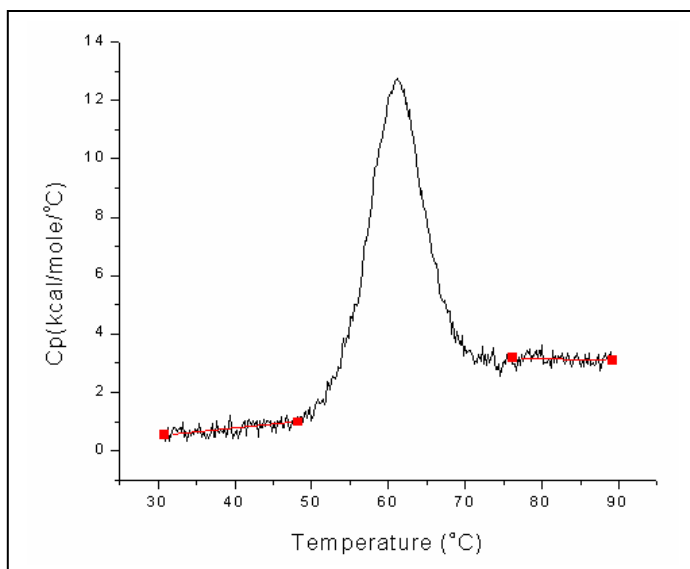
- Click the cross-hair on or near the data you want as the first point of the linear segment. A red cross appears on that point.
- Use the left and right arrow keys to move the red cross, until you have selected the point you want.
- Press **Return**. This sets the selected point as the first point of the linear segment.
- Repeat the previous three steps to select the last point of the linear segment. Origin now generates a new linear segment by fitting the data between the two points to a straight line.

Now for the left linear segment. Instead of using the refit command, lets adjust the left segment by cursor.

To move linear segments by cursor

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- Select **Move Segments by Cursor** from the **Adjust** menu.
- Select and move one of the left segment end points by either:
  - Clicking on one of the terminal black squares and dragging the mouse, or
  - Selecting an end point with the **LEFT** or **RIGHT arrow keys**, then using the **UP** and **DOWN arrow keys** to move the point up or down. Use the **CONTROL + LEFT** or **CONTROL + RIGHT arrow keys** to move the point left or right.
- Repeat the previous step for the other segment end point.
- When you are satisfied with the placement of the left linear segment, double-click in the plot window, or press the **Return** key.



## Choosing a Baseline Option

Once you have set the linear segments, you are ready to select one of the six options for creating a baseline. You will find the baseline options in the **Baseline** menu.

To select an option for creating the baseline

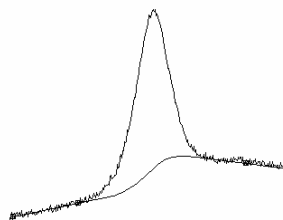
---

- Click on the **Baseline** menu option.
- Choose a baseline option from the menu.
- When you are satisfied with the baseline, click on the **OK** command in the menu bar to exit the baseline session. Origin gives you the opportunity to subtract the baseline, then returns you to the **RawDSC** window (or, if you had normalized the data, the **NormDATA** plot window).

The six baseline options are described in detail below.

### Progress Baseline

In this option, the baseline in the peak region is created such that each point reflects the extent of progress of the reaction. That is, the placement of a baseline point at any temperature in the transition region (i.e., relative to the left and right baseline extrapolated to the same temperature) is determined by the fraction of the total area that has been completed at that temperature. The progress baseline for the **SAMPLE1** data is shown below:



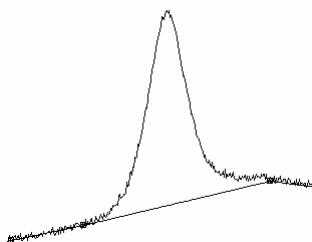
For a single transition such as this, the calculated baseline then approximates the molar heat capacity for the mixture of the states as they exist at any temperature in the transition region. The area between this baseline and the data then approximates the total heat change due to the transition.

Note that only the slope and intercept of the linear segments are used in the calculation for the progress baseline. The actual end points of the linear segments are not used.

During the calculation of a progress baseline, Origin integrates over the entire display range of the active data set. If you prefer, you can restrict the region of integration to just the peak area. After adjusting the low and high temperature linear baseline segments, use the **Data Selector** tool (described in Lesson 2) to set the region where integration should be carried out. If you now select the **Baseline:Progress Baseline** command, integration will be restricted to the region that lies between the two data markers.

#### **Linear Connect**

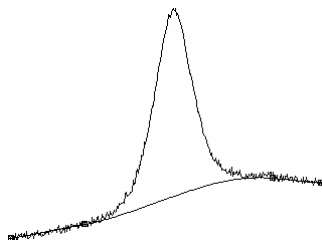
This baseline is formed by connecting the two linear segments with a straight line, as shown below:



Whereas the progress baseline uses only the linear segment slope and intercept to form the baseline, linear connect uses only the position of the two end points.

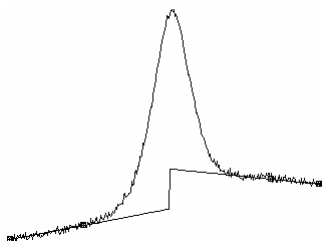
### **Cubic Connect**

Similar to Linear Connect, except the connection is a cubic polynomial. The end points are used to create the baseline, as with Linear Connect, but with Cubic Connect the slope is used also:



### **Step Baseline**

There are two step baseline options. **Step at Peak** is based on a linear connect baseline, and places the step directly under the peak. **Step at Half Area** places the step at the position of half the integrated area:



### **Move Baseline by Cursor**

This option lets you manually adjust the baseline by specifying the position of each of the baseline data points.

To use the **Move Baseline by Cursor** option, first create a baseline by selecting one of the baseline creation commands described above. Next, select **Move Baseline by Cursor** from the **Baseline** menu. Origin places twelve data points along the baseline you just created. To move the data points either click and drag with the mouse, or use the left and right arrow keys to select a point, and the up and down arrow keys to move the selected point (points can only move vertically).

When you are satisfied with the baseline, double-click or press **Return**. A spline connection option is automatically applied to the new baseline. You can remove the spline connection by changing the baseline's plot display options (see page 42, below).

## Cursor Draw Baseline

**Cursor Draw Baseline** is another method for creating a baseline. The **Cursor Draw Baseline** method does not require you to enter the baseline session, set linear segments, or select a baseline option. In this method, you simply draw the baseline directly in the plot window.

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### To use the Cursor Draw Baseline option

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- If you have not yet exited the baseline session, do so now by clicking **OK** or **Cancel** in the baseline session menu bar.
- Select **Cursor Draw Baseline** from the **Peak** menu in the standard menu display bar. The cursor changes to a small cross-hair.
- Double-click with the mouse to place each baseline point. Each point connects to the next with a straight line. The XY coordinates of the point appear in the **Data Display Tool**. You can place any number of points you wish.
- You can also use the arrow keys to place points. Click once with the mouse to place a cross-hair on the screen. Use the **UP**, **DOWN**, **LEFT**, and **RIGHT arrow keys** to move the cross-hair. Press **RETURN** to set the position of a point.
- When you are satisfied, click on the **Pointer** tool in the toolbox to set the baseline.

Once you have created a baseline, you can use the **Adjust Baseline** option to fine-tune the default baseline.

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### To adjust the baseline

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- Select **Adjust Baseline** from the **Peak** menu. The pointer becomes a cross-hair.
- To adjust a baseline point, click and drag with the mouse. Or use the arrow keys (select a point with **LEFT** or **RIGHT arrows**; move up and down with the **UP** or **DOWN arrows**; move left and right with **CONTROL + LEFT** or **CONTROL + RIGHT arrows**).
- When you are satisfied, double-click on a data point or press **Return** to set the baseline.

The baseline points in **Cursor Draw Baseline** and **Move Segments by Cursor** connect with a straight line by default. Connect type (and other display options) can be changed by double-clicking on a point in the baseline and selecting new options in the **Plot Details** dialog box.

To change connect type for a baseline

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- Double-click on the baseline.  
The **Plot Details** dialog box opens.
- Select a connection option (e.g., spline, straight, step) from the **Connect** drop down list box.
- Click **OK**.

Each time you create a baseline, Origin names the baseline data set and appends a column to the active dataset worksheet for the baseline data. The baseline data set is given the name *NAME.BASE* (*NAME.DRBASE* in the case of **Cursor Draw Baseline**) where *NAME* is the name of the data set for which you created the baseline. To see the baseline data, open the associated worksheet.

To open the worksheet containing baseline data

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*Shortcut to the Worksheet: Right-click anywhere on the data trace and select Open Worksheet from the drop down menu.*

- Double-click on the baseline to open the **Plot Details** dialog box, or select the baseline name from the **Data** menu, then select **Plot** from the **Format** menu to open the **Plot Type** dialog box.
- Click on the **Worksheet** button.  
The baseline worksheet opens with the baseline data set named CP.BASE(Y).

To subtract the baseline from the DSC data

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- Select **Subtract Baseline** from the **Peak menu**.  
The baseline is subtracted.



## Lesson 5: Curve Fitting

In Lesson 1 we discussed Origin's basic curve fitting procedures. Here we will look at the curve fitting process in greater detail. In the first section below, we will make some general observations about the curve fitting models used in Origin. Following that, we will work through two fitting examples to illustrate some of the flexibility of the curve fitting process.

### General Comment

Origin provides four models with which to fit a curve to your data. All four models use the Levenberg-Marquardt non-linear least-square method, but differ in the number of parameters involved, as shown below:

Model	Parameters
Model 1: 2-State with zero DCp	$T_m, H$
Model 2: Non-2-State with zero DCp	$T_m, H, H_v$
Model 3: 2-State with non-zero DCp	$T_m, H, C_p, BL0, BL1$
Model 4: Dissoc with non-zero DCp	$T_m, H, C_p, BL0, BL1, n$

$T_m$  is the thermal midpoint of a transition;  $H$  is the calorimetric heat change ( $\Delta H$ );  $H_v$  is the van't Hoff heat change ( $\Delta H_v$ );  $C_p$  is the  $\Delta C_p$  for each transition; BL0 and BL1 define the slope and intercept of the low-temperature baseline segment; and  $n$  is the number of sub-units

All of the models except model 4 can be used to fit to one or more transitions. In the case of multiple transitions, each transition has its own complete parameter set; e.g., if model 1 is used to fit two overlapping transitions there will be two independent parameter sets [ $T_{m1}, H_1$ ] and [ $T_{m2}, H_2$ ]. These specify the thermal midpoint ( $T_m$ ) and the heat change ( $\Delta H$ ) at the  $T_m$  for each transition. The BL0 and BL1 parameters are an exception to this rule. These parameters appear only once in the model; they are not repetitive for each transition.

While all four models use a calorimetric heat change ( $\Delta H$ ), only the non-2-state model (model 2) has a van't Hoff heat change ( $\Delta H_v$ ). The calorimetric heat  $H$  is determined only by the area under a transition peak, while the van't Hoff heat  $H_v$  is determined only by the shape of the transition peak. The sharper the transition, the larger is  $H_v$ , and vice versa. The relationship between  $H$  and  $H_v$  can sometimes provide insights not accessible from  $H$  alone. For example, if a protein is composed of two identical domains which unfold independently with the same  $T_m$  and  $H$ , then the ratio of  $H/H_v$  will be 2.0, while it would be 1.0 if the protein had only a single domain. If, on the other hand, the protein dimerized and the dimer underwent only a single coupled transition then the  $H/H_v$  ratio would be 0.5. It is clear from this that the calorimetric heat  $H$  refers to *heat change per mole* while  $H_v$  is *heat change per unfolding unit* (called the *cooperative unit*). Thus the ratio  $H/H_v$  can, in simple cases, be thought of as *the number of cooperative units per mole*.

**(Note:** It is important to realize that the ratio of calorimetric to van't Hoff heat depends on concentration normalization, since calorimetric heat is always expressed on a *per mole*

basis. Thus for the protein dimer example considered above, the ratio will be 0.5 if concentration normalization is carried out on a *per mole of monomer* basis while it will be 1.0 if concentration is entered in terms of *moles of dimer* present.)

In model 1, it is possible to prescribe that overlapping transitions are either independent or sequential in nature. For example, if two structural domains are interacting strongly, it is possible that their transitions will be coupled in a sequential manner, whereas the independent model might better describe two transitions which are completely uncoupled from one another. In practice, this choice is often not critical since the sequential and independent models lead to virtually identical results whenever the  $T_m$ 's of two transitions are separated by a couple of degrees or more. To switch between, select the **DSC:Set Indep/Sequ Model** command and answer the prompt that appears.

Origin provides the option to float all parameters during the fitting procedure, or to assign some parameters as fixed and float others. In fixing a parameter, it may be given an invariant numerical value.

The mathematical derivations for each model are included in the appendix. In general, the objective of most investigators is to use the simplest model (e.g., fewest floating parameters) which provides a good fit of the data. Thus, if data are satisfactorily described by the two-state model using two transitions, this is to be preferred over a two-state model with three transitions or a non-two-state model with two transitions. It is usually only when a given model is unable to fit the data that complexity should be increased by adding more fitting parameters.

## Fitting Example 1

To better understand the flexibility of Origin's curve fitting process, work through the following example. The example uses data from a protein of structure ABA, i.e., two identical and non-interacting A domains and a single B domain. Head-to-head covalently-linked dimers can readily form ABA type of structures. By comparison of results from different fitting models, structures of this type can be recognized from DSC data.

If you are continuing from the previous lesson, first select **File:New:Project** (or click on the New Project button) to open a new project.

### Step 1: Prepare the data

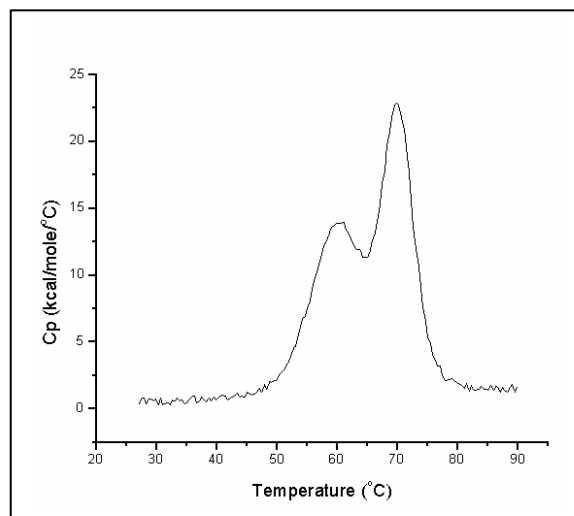
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The first step in any fitting procedure is to open the DSC data file and reference data file, then prepare the data by subtracting reference data and normalizing the result on concentration. We have dealt with these data preparation procedures in Lesson 1 of this tutorial. Here we will briefly review the steps involved:

- 1) Click on the **Read Data** button in the **RawDSC** window, and open the **SAMPLE5.DSC** and **REF5.DSC** data files (located in the **[Origin70][Samples]** folder).
- 3) Click on the **Subtract Reference** button. The **Subtract Reference Data** dialog box opens.
- 4) Select **Sample5dsc\_cp** for the Data drop down list box entry.
- 5) Select **Ref5dsc\_cp** for the Reference drop down list box entry.
- 6) Click **OK** to subtract **Ref5dsc\_cp** from **Sample5dsc\_cp**.
- 7) Click on the **Concentration Normalization** button.

8) Enter **0.074 mM** and **1.22 ml** in the dialog box, and click **OK**.

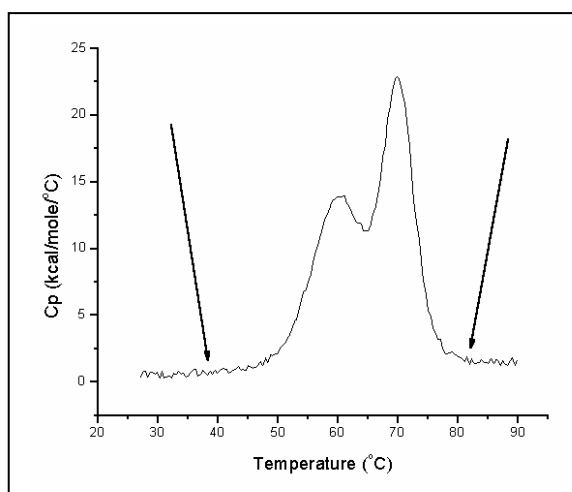
After completing these eight steps, your screen will show the normalized **SAMPLE5** data plotted in the **NormDATA** plot window.



It is important to remember that data must always be normalized on concentration before doing curve fitting to any of the fitting models.

#### Step 2: Plot a baseline

As we have discussed elsewhere in this tutorial, the next step is to plot a baseline for the data. Note that the **SAMPLE5** plot shows flat baseline segments on either side of the transition, with a  $\Delta C_p$  in between. The transition itself actually starts and ends at about the positions shown by the arrows below:



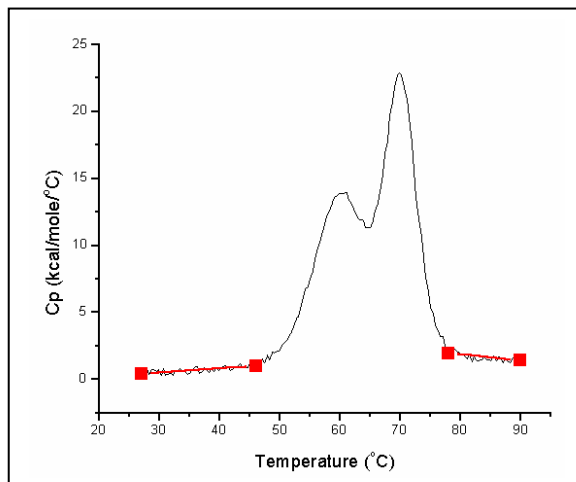
To create a good progress baseline for this data, you may wish to manually adjust the linear baseline segments so that they are parallel to each other, and coincident with the baseline data. Origin does not use the position of the endpoints of the linear segments to calculate the progress baseline, only the slope and offset. You are thus free to create

linear segments of any length, which makes it much easier to align the segments parallel to each other.

To create the baseline:

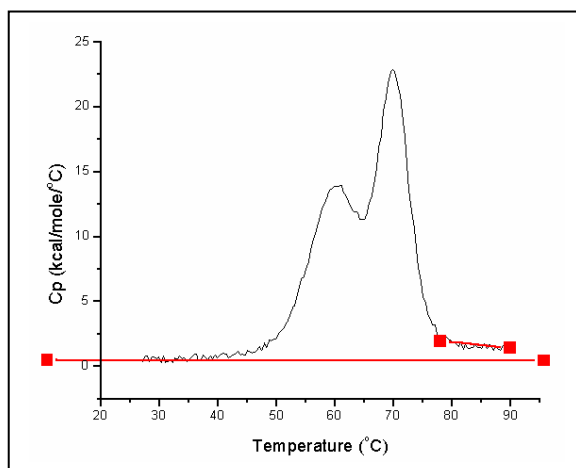
1) Select **Peak:Start Baseline Session...**

Origin calculates and draws the two linear baseline segments, faintly visible in the figure below:

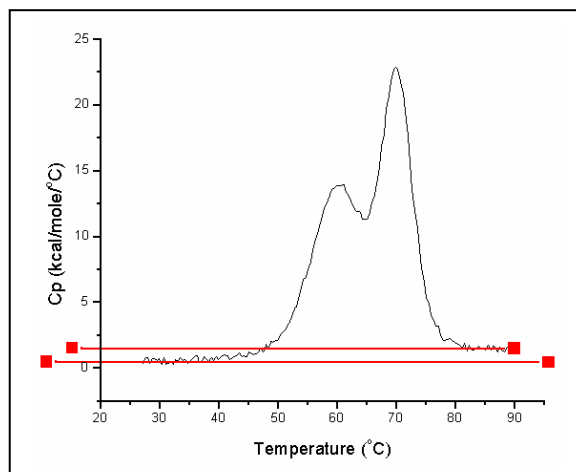


2) Select **Adjust:Move Segments by Cursor.**

3) Beginning with the left linear segment, click and drag on first the left and then the right endpoint until the graph looks approximately like the figure below (you can also use the arrow keys to move an endpoint. Use the **Up** and **Down** arrows to move up or down, **Control+Left** and **Control+Right** arrows to move left and right, and **Right** and **Left** arrows to select a new endpoint):

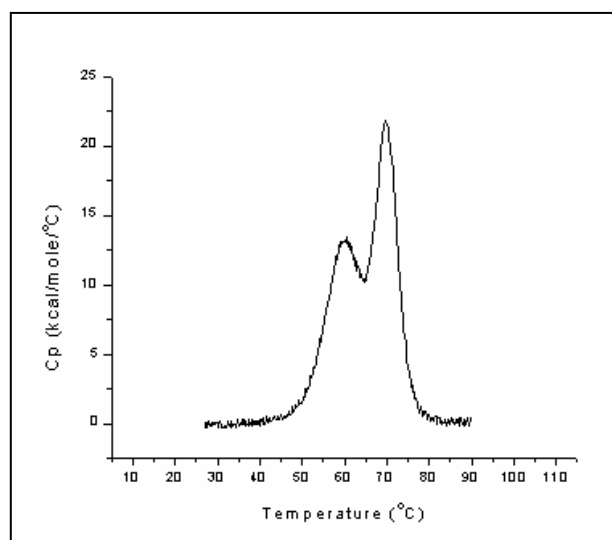


4) Now use the cursor to adjust the right linear segment as follows:



- 5) Select **Baseline:Progress Baseline**.  
Origin plots a progress baseline.
- 6) Select **OK** from the menu bar.
- 7) Click **Yes** in the dialog box to subtract the progress baseline.

Your graph should now look like this:



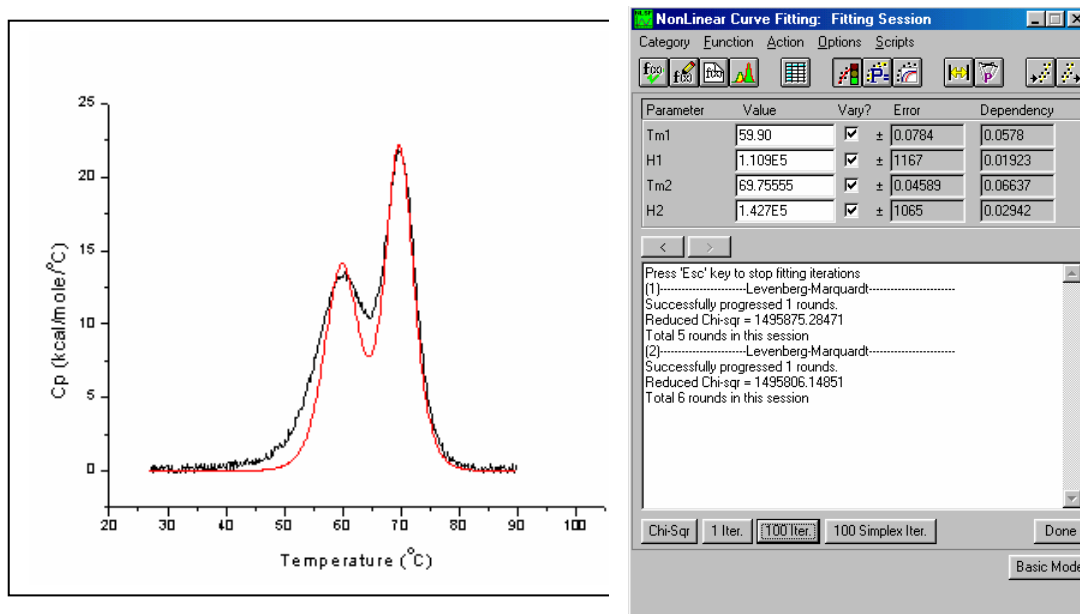
### Step 3: Fit to the least complex model

Generally, you will want to use the simplest model (e.g., with the fewest floating parameters) that provides a good fit to the data. You can then add complexity if necessary. Begin the fitting session by using model 1 (2-state with zero  $\Delta C_p$ ) to fit the data. To do so:

- 1) Select **2-State Cursor Init** from the **DSC** menu.  
This starts the fitting session, with model 1 selected. The **Cursor Init** option allows you to initialize the parameters, rather than accept any existing parameters.

- 2) Origin prompts you to enter the number of transitions. The simplest fit would be to two transitions, so enter a **2** and click **OK**.
- 3) Double-click on or near each peak to set a  $T_m$  (thermal midpoint) for each transition.

As soon as you set the  $T_m$ 's, Origin initializes the fitting parameters (in this case  $T_{m1}$ ,  $H_1$ ,  $T_{m2}$ ,  $H_2$ ), displays the parameters in the **Fitting Session** dialog box, and draws an initial fit curve. An **Attention!** dialog box notifies you when initialization is complete. Click **OK** to proceed with the fitting.




- 4) The **1 Iter.** and **100 Iter.** commands let you control the iteration of the fitting cycles. Click on the **100 Iter.** button a couple times. Origin iterates the fitting cycle until the change in  $\chi^2$  between two successive iterations is less than a pre-set value (this value is determined by the value set in the Tolerance Text box of the Control Parameters dialog box, MicroCal has set the default value to be  $10^{-5}$ ). Select **100 Iter.** once or twice more. At the end of each fitting cycle Origin updates the  $\chi^2$  and fitting parameters values and draws a new fit curve.

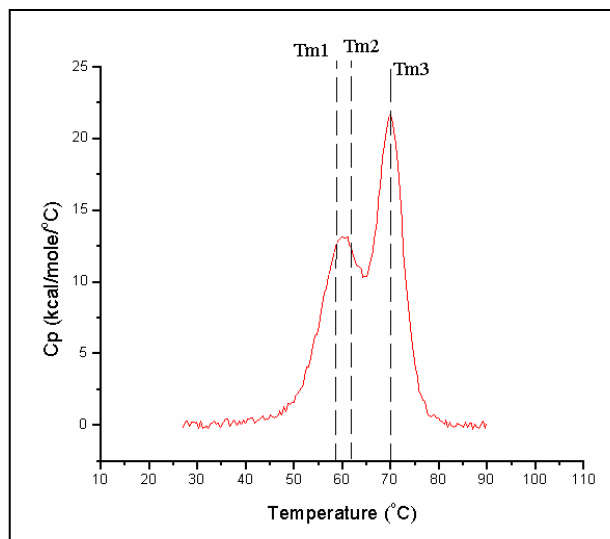
By now you should have a pretty good fit to the 70 degree peak, but the fit to the 60 degree peak is not so satisfying. At this point you can begin to think about improving the fit by adding complexity to the model.

#### Step 4: Add complexity

You did not get a good fit using model 1 with two 2-state transitions. While the high peak fit pretty well, the low peak did not. This could mean that the low peak is not really a single peak, but two smaller, overlapping peaks that combine and obscure each other. To test this hypothesis, see if you can get a better fit using model 1 and three transitions:

- 1) Click on the Close button  located in the upper right corner of the Fitting Session dialog box to abort the current fit.
- 2) Select **2-State Cursor Init** from the **DSC** menu.
- 3) Type **3** in the "**Number of peaks**" dialog box, and click **OK**.

4) Double-click to set each of the three Tm's, one for each transition. Place the first Tm just to the left of the low peak, the second just to the right, and center the third on the high peak.



Upon setting the third Tm Origin calculates all three sets of fitting parameters (six parameters in all, two for each transition) and displays the initial fit curve.

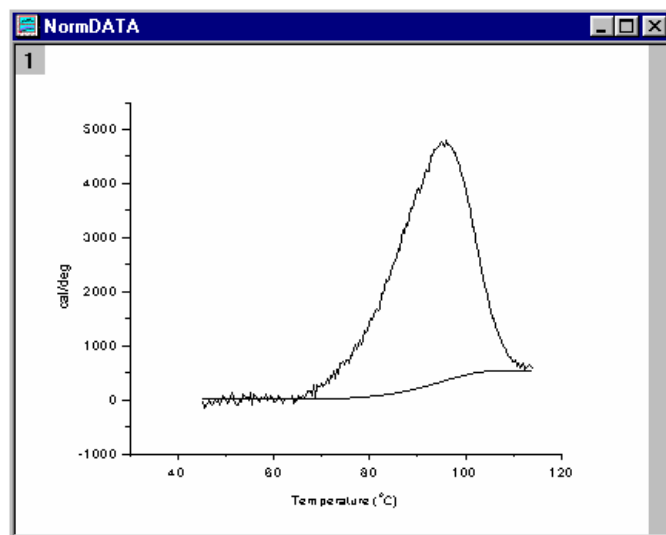
5) As before, select **100 Iter.** from the menu bar to fit the curve to the data. After several fitting cycles, you should get a pretty good fit, implying that the three-transition model describes this data better than the two-transition model.

## Fitting Example 2

For multisubunit proteins, it often happens that the subunits dissociate simultaneously with the unfolding of the protein, i.e.,  $A_n = nA$ , where  $n$  is the number of subunits. When this happens, the shape of the DSC peak is broadened relative to unfolding of the same protein where no dissociation occurs (e.g., covalently linked subunits). The mathematics for curve fitting to systems which undergo simultaneous unfolding and dissociation are included in the Appendix.

You may wish to try fitting the data set in the Origin project **Dissoc.OPJ**, contained in the [Origin70][Samples] folder. The data was obtained on a helical polypeptide thought to contain two identical chains linked together in a coiled-coil state. These data have already been normalized on concentration and a progress baseline has been created, so curve fitting may begin immediately. (Note that whenever using the dissociation model, the concentration used for data normalization should be that for the associated low-temperature state, not that for the dissociated high-temperature state.)

1) To open **Dissoc.OPJ**, click **Done** to exit the fitting session, then select **File:Open**, navigate to the [Samples] sub-folder of the [Origin70] folder, then double-click on the file name in the **Files** list. **Dissoc.OPJ** opens to show the **lv2p0.cp** data set with its associated baseline plotted in the **NormDATA** window:



2) Select fitting model 4, **Dissociation w/dCp, Cursor init** from the **DSC** menu.

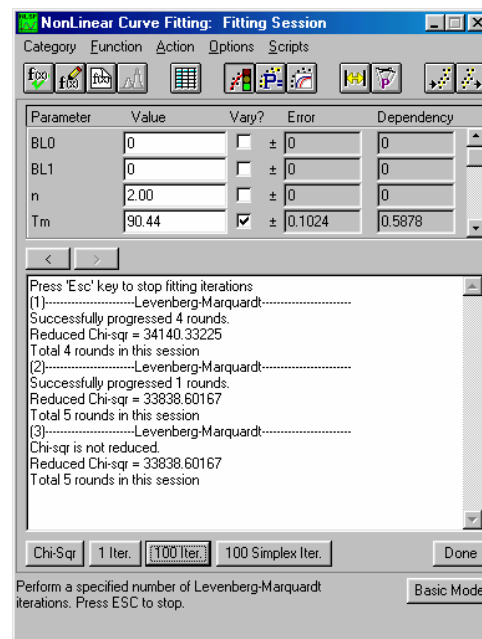
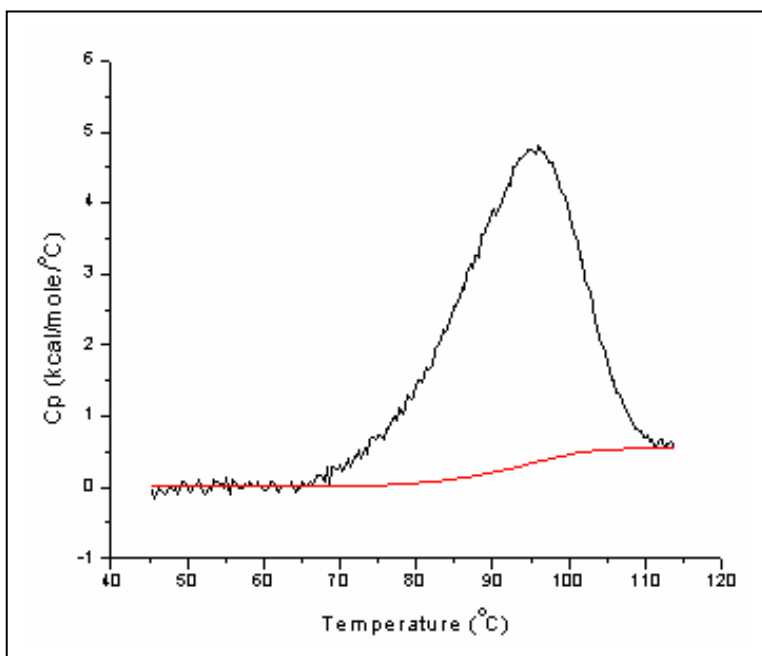
3) Step 2 may have caused an error message to appear, warning you that no baseline is selected for this data. If this happened, you need to let Origin know that you want to use the baseline supplied with this data, which is named lv2p0\_cp.base. To do this, select the **Peak : Cursor Pick Baseline** command, then double-click on the plotted baseline data. When you have finished, again select **Dissociation w/dCp, Cursor init** from the **DSC** menu.

4) Select **2** as the **Number of Subunits**.

Note that the number of subunits **n** and the concentration **Ct** appear in the **Fitting Function** dialog box as non-floating parameters.

5) Assign value of **0** to the **BL0** and **BL1** parameters and remove the checks from each **Vary?** Box so the low temperature baseline is not allowed to float. Click on **100 Iter** a couple of times. You will note that the overall fit curve is fairly good.





6) Now, change the value of **n** from **2.0** to **1.0**. Click on **10 Iter** several times. Note that the new fit curve is much worse than the original fit curve ( $\chi^2$  is ca. Five times larger) and that the experimental data curve is now substantially broader than the fit curve. This means the data are most consistent with the idea that dissociation into subunits occurs simultaneously with unfolding for this polypeptide.

7) Change the value for **n** from **1.0** to **4.0** and fit the data again. It is again obvious that the fit is not as good as the original fit using an **n** value of **2.0**, which suggests that the polypeptide is more likely to contain two dissociable subunits rather than four.

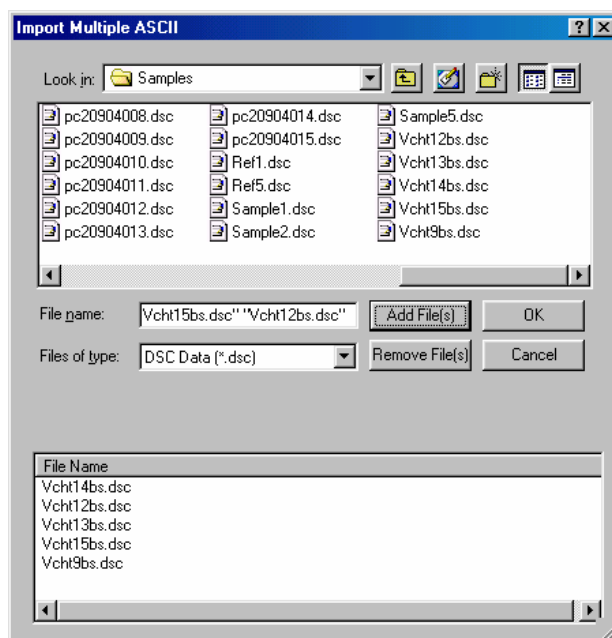
### Fitting Example 3

The **Absolute Cp** button will facilitate calculation of Absolute heat capacities for proteins (this is discussed in more detail in Appedix IV, starting on page 100. To illustrate the function of this button there are 5 raw data files of chymotrypsinogen solutions at five different concentrations covering a 10-fold range from 0.258 to 2.58 mg/ml.

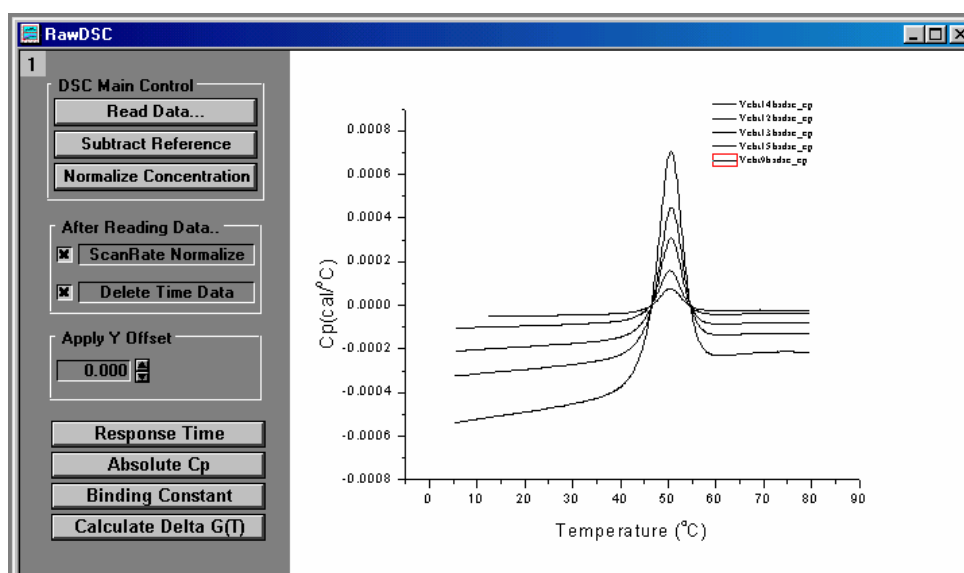
#### Step 1: Input the data

The 5 raw data files used for this example are Vcht9bs.dsc, Vcht15bs.dsc, Vcht12bs.dsc, Vcht13bs.dsc and Vcht14bs.dsc. The concentration of the sample in each scan is .258, .516, 1.03, 1.55 and 2.58 mg/ml, respectively. The samples were run on a VP-DSC at 60 deg/hr using the passive response mode for each scan. A buffer-buffer reference trace has already been subtracted from each data scan.

1) Click on the Read data button in the RawDSC window, navigate to the C:\Origin70\Samples folder and open the **Vcht9bs.dsc, Vcht12bs.dsc, Vcht13bs.dsc, Vcht14bs.dsc and Vcht15bs.dsc** data files.



Your Origin graph will look like below.



Notice the square around the line type of the last file opened (in this case data set Vcht9bs.dsc) in the legend of the graph. This indicates it is the active data set. When you click on the Absolute Cp button the routine will convert this data set.

Step 2: Converting raw data to absolute heat capacity values.

1) Click on the Absolute Cp button.

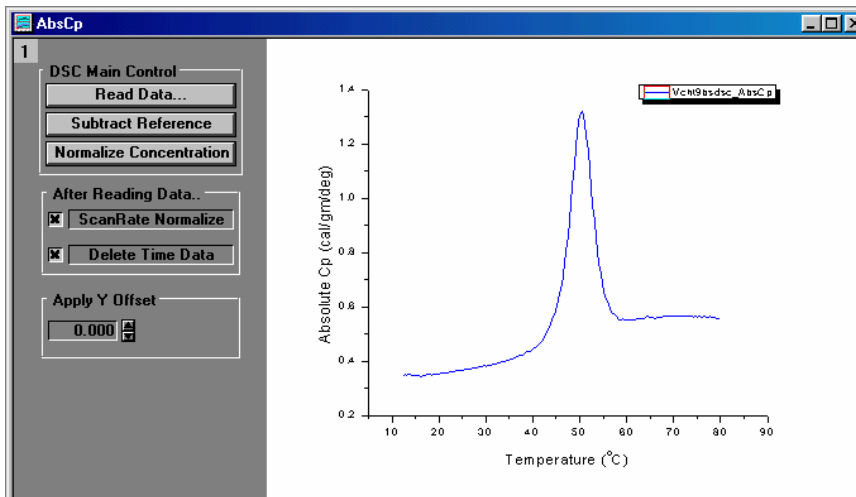
**Absolute Cp**

The Absolute Cp dialog box will open with the following default parameters.

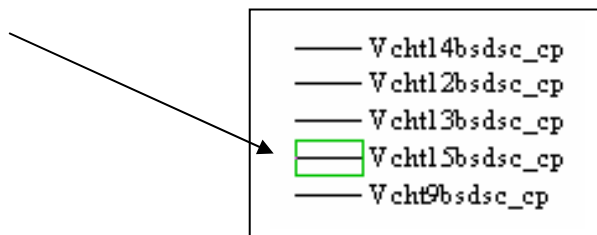
2) For this example all the default parameters are correct<sup>1</sup>, Click **OK**.

<sup>1</sup> Literature value for the partial specific volume of chymotrypsinogen is .717 ml/gm, and the coefficient of volume expansion is  $7 \times 10^{-4} \text{ deg}^{-1}$ .

A new window named AbsCp will be created. With the converted data plotted as shown below

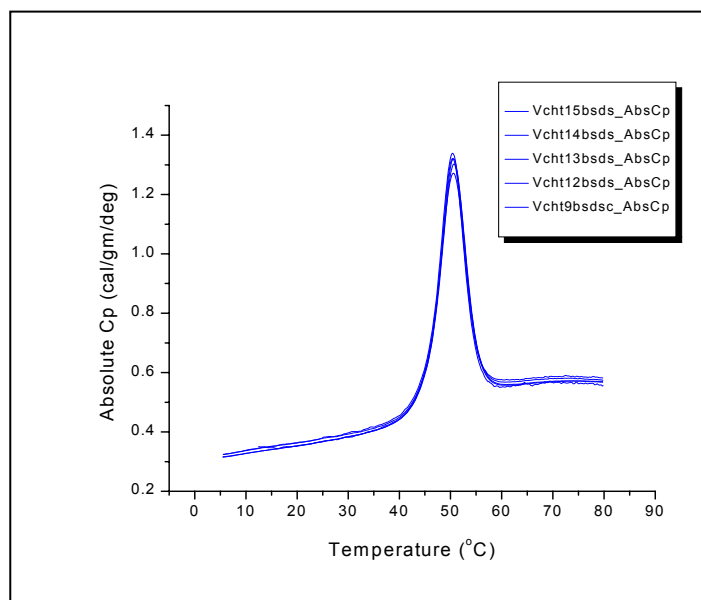


- 3) Now hold down the Ctrl key and press the tab key once to switch back to the RawDSC window.
- 4) Click on the next data set in the legend to make it the active data set.

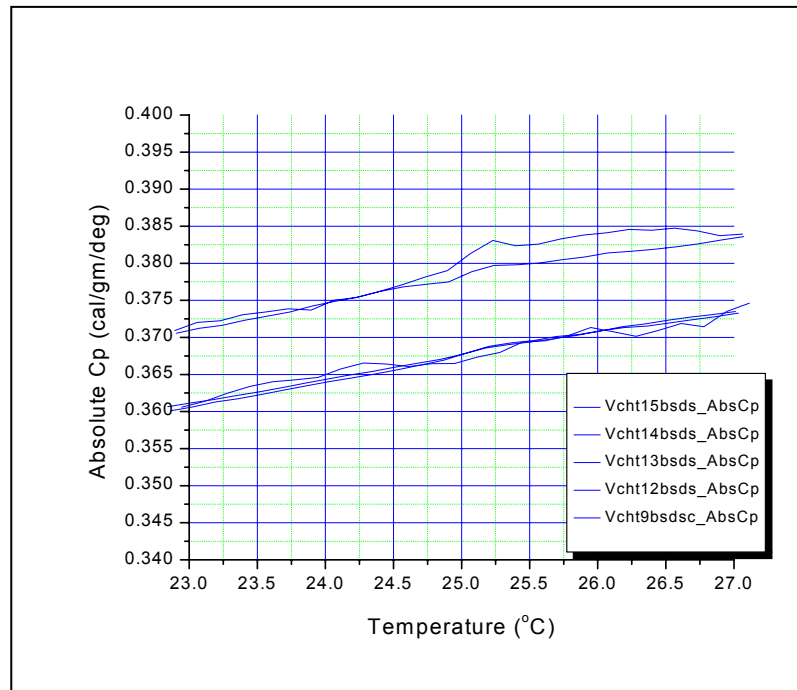


- 5) Click on the **Absolute Cp** button again.

Repeat steps 1 – 4 for each data set till you have completed the data conversion for all 5 data sets. Your graph should now look like below.



You may wish to expand the view around 25 deg and add some grid lines.



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## Lesson 6: Autosampler Data (Optional Accessory)

The Autosampler is an optional accessory for use with the Capillary DSC to allow multiple unattended scans. There are two pharmaceutical applications where multiple scans may be required. The first is optimizing solution conditions for protein formulation stability, where a number of solution variables (pH, salts, antibacterial agents, sugars, etc.) are examined to find the appropriate blend of additive which maximize protein stability by increasing its melting temperature ( $T_m$ ) (Remmele, R. L., Nightlinger, N. S., Srinivasan, S., & Gombotz, W. R. (1998), *Pharm. Res.* **15**, 200-208.). The second application is determining binding constants of protein target molecules to small ligands or other proteins, where  $T_m$  shift resulting from addition of a potential drug candidate allows calculation of binding constant to target protein (Brandts, J. F. & Lin, L. -N. (1990) *Biochemistry* **29**, 6927-6940.) The DSC can determine the  $T_m$ s for different drug target complexes at varying concentrations, thereby allowing determination of individual binding constants.

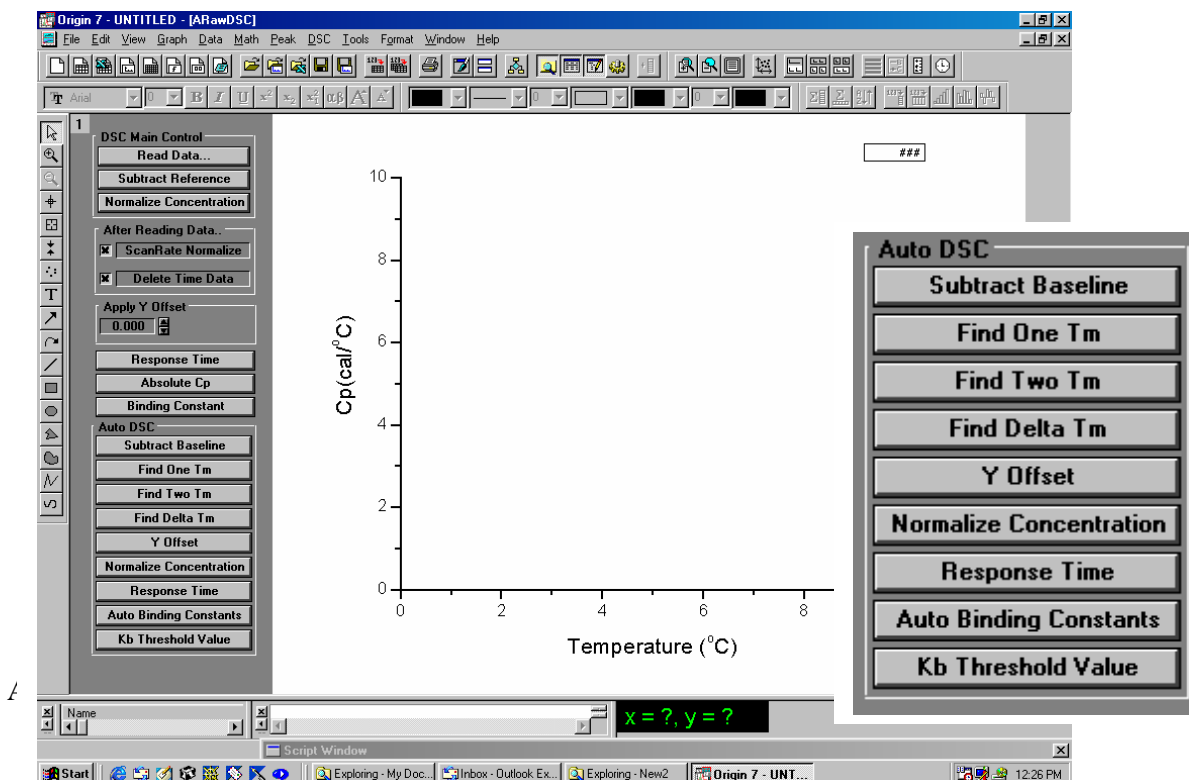
### Launching the DSC Autosampler Data Session



Before starting this lesson, you should have Origin up and running. The routines for Auto DSC files are located in a separate window (named ARawDSC) from the standard DSC routines. In addition to the Auto DSC routines, this window (also known as a menu level) contains all the functions of the regular DSC data analysis.

- To launch the Auto DSC menus, double-click on the **MicroCal LLC Autosampler** icon on the Desk Top. Or alternatively select **Start : Programs : OriginLab : MicroCal LLC Auto DSC**

When you start Origin, the program automatically opens the **ARawDSC** graph window. Note that this window contains several buttons in addition to the regular DSC routines. These buttons let you execute certain multiple data file manipulations.



The buttons displayed in the gray area to the left of the Origin graph and described below are exclusive to the ARawDSC template and allow easy manipulation of the multiple Auto DSC files. Examples of the use of each button are presented in the following pages.

<b>Subtract Baseline</b>	Allows you to select a baseline to be used to subtract from all other scans plotted in the active layer.
<b>Find One Tm</b>	Will determine a single Tm for all scans plotted in the active layer
<b>Find Two Tm</b>	Will determine two Tm's for all scans plotted in the active layer
<b>Find Delta Tm</b>	This button will allow you to select a scan to use as a reference Tm. The program will then calculate the temperature difference between the reference Tm and the Tm's of all other scans.
<b>Y Offset</b>	When this option is selected you will be prompted to enter a value to offset each scan that is plotted in the active layer. This may be useful when scans are overlapping and you want to view individual curves.
<b>Normalize Concentration</b>	Normalizing the data divides the data by the number of moles of the sample substance in the cell, to convert the data from cal/degree to cal/mole/degree. When you click this button all scans will be normalized by the concentration (mmoles/l) for each sample.
<b>Response Time</b>	This is used for removing small amounts of peak broadening due to using a feedback mode which has a response time too slow to resolve a peak adequately. When you click on this button all scans plotted in the active layer will be "corrected" for their response time. The original data will be deleted. For more information about response time, refer to the <b>Response Time – VP-DSC</b> section starting on page 88
<b>Auto Binding Constants</b>	This button provides an automated method to calculate ligand-protein binding constants for the multiple scans plotted in the active layer.
<b>Kb Threshold Value</b>	When this button is selected you will be prompted to enter a "Threshold value" for Kb. All binding constants that exceed this value will be highlighted in the summary table.

---

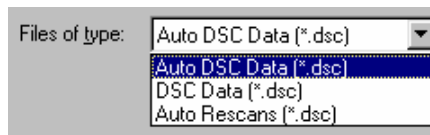
#### To input the data

---

Raw data files from the autosampler are typically a series of data files of the form

FamilyName001.DSC, FamilyName002.DSC, FamilyName003.DSC, etc.. The family name may have up to 9 alphanumeric characters, VPViewer™

adds the sequential 3 digit number, therefore there may be a maximum of 12 characters for the filename. To open the file series click on the **Read Data...** button and the **Import Multiple ASCII** dialog box will open. Select the **Auto DSC Data (\*.dsc)** as the **Files of type:**, the upper File Name list box will list only the first file of the series (\*001.DSC), but when selected for import all files with the same family name (except for rescans, see below) will be read in until the end of the series is reached.





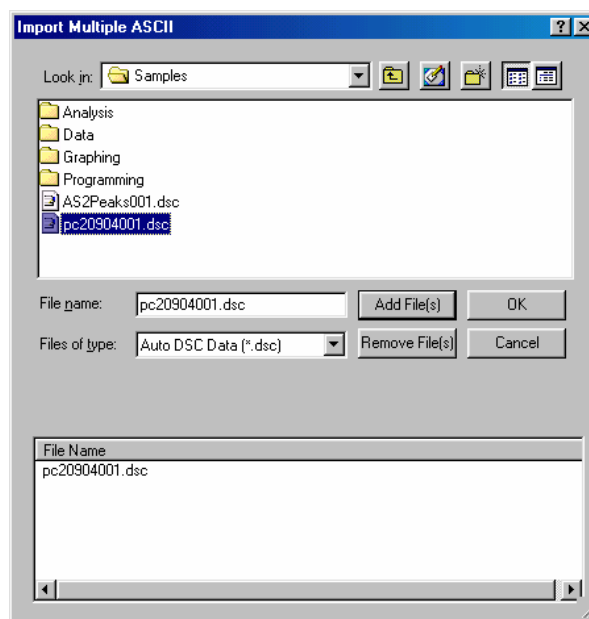
Alternatively, you may open individual files or a selection of files similar to the normal DSC method. When you select the **Files of type:** to be **DSC Data (\*.dsc)**, then all files with the 'DSC' extension will be listed in the upper list box. You may then manually select the files to add to the lower list box and read into Origin.

A third option will allow you to read in all data files with the same family name, including rescans. ( Note: Scans that were designated as rescans in VPViewer, when the experiment was run, had a sequential alphabet character (i.e. a, b, c, etc.) appended after the scan number.) When you select the **Files of type:** to be **Auto Rescans (\*.dsc)**, then select the first file of the series (\*001.DSC) for file import, all files of the series will be read into Origin and plotted.

To open serial Auto DSC (\*001.DSC) files

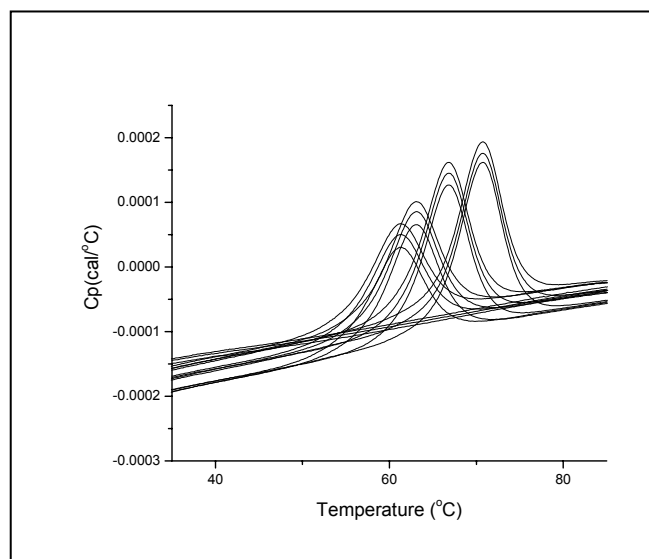
- Click on the **Read Data..** button.  
The **Import Multiple ASCII** dialog box opens. Select **Auto DSC Data (\*.dsc)** as the **Files of type:**. Only DSC files that have an 001 before the extension dot will be listed.
- Navigate to the C:\Origin70\Samples folder, then select **PC20904001.DSC** from the files list.  
**Please Note:** Raw data file names should not begin with a number, nor should they contain any hyphens, periods or spaces.

**Note:** You may select a default folder for Origin to “Look in” for a data file by selecting **File : Set Default Folder...** and entering the default path (e.g. for this tutorial you may wish to choose the path to be **C:\Origin70\Samples**



- Select the **PC20904001.DSC** file and click **Add File(s)**.
- Click **OK**.

The PC20904001 family of files is then read in sequentially (i.e. PC20904001, PC20904002, PC20904003, etc.) and plotted in the graph. Use the magnifying glass tool to rescale the graph around the peaks. After rescaling your graph should look similar to what is shown.

**Subtract Baseline**

### To Subtract a Baseline

You may select a baseline to subtract from all scans plotted in the active layer by one of three methods, Manually Select, File Name or Tray and Well label. Each method is described below.

- Click the **Subtract Baseline** button.  
The Select Method dialog box will open.

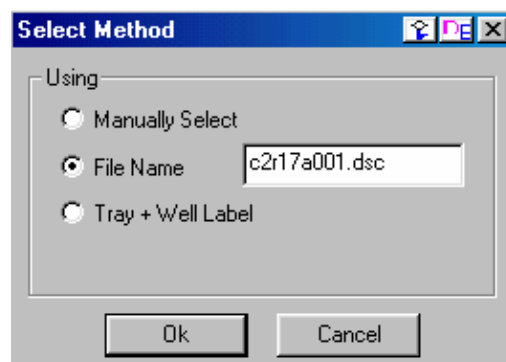
#### Manually Select

When you select **Manually Select** and click **OK** the cursor will change into a cross hair. You may then double click (or single click and press enter) on any trace, this trace will then be subtracted from all other traces that are plotted in the active layer.



#### File Name

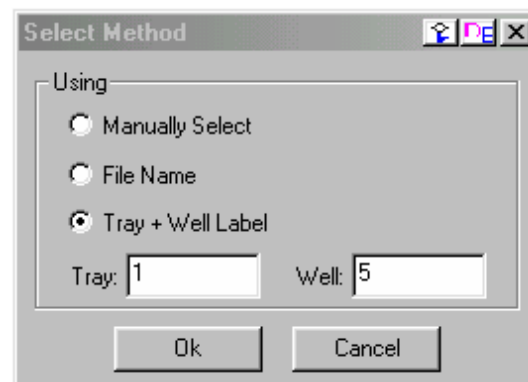
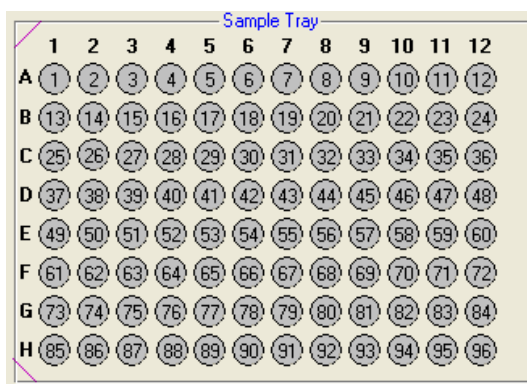
When you select **File Name**, a text box will pop up and you may enter the filename of a file (the file must have been read into Origin) to be used to subtract from all other traces that are plotted in the active layer. *Note:* When entering the filename you must include the '.dsc' file extension.



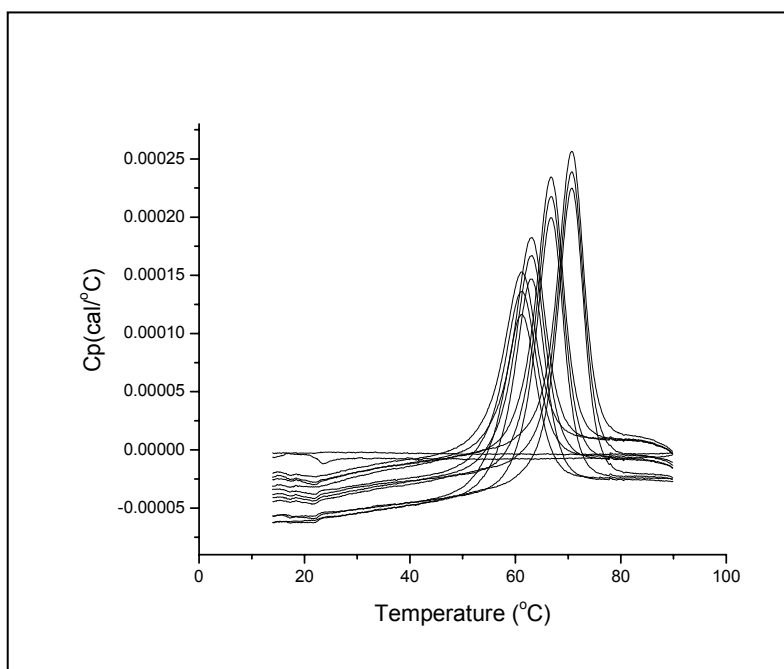
### Tray + Well Label

When you select **Tray + Well Label** two text boxes will appear, one will allow you to enter the tray number, the second will allow you to enter the well designation. The sample that was in that tray and well will be then used to subtract from all other traces that are plotted in the active layer.

*Note: Tray designations must be numbers and not start with zero (0), the Well designation was designated when the experiment was set up in VPViewer (and stored in the data file header) and may be the number of the well (e.g. 1,2,3, etc.) or the matrix number (e.g. A1, A2, etc).*



- Select **Manually Select** and click **OK**.  
The cursor will change to a cross-hair.
- Double click on one of the baselines (i.e PC20904001, PC20904002 or PC20904003).  
The selected baseline will be subtracted from all scans plotted in the active layer, your results should look similar to what is shown below.  
*Note: There may be a slight Tm shift when using different baselines for subtractions, for this example the PC20904003.dsc dataset was selected.*



**Response Time**

### To apply the **Response Time** “correction”

---

Due to the very fast scan rates available (up 250 deg/hr) for the Capillary DSC and the finite response time of the instrument, there may be some  $T_m$  shifting and/or peak broadening. A Response Time button is available that when activated will apply a response time correction for all scans plotted in the active layer. This functions similar to the Response Time button available for the standard DSC scans (see pages 88 - 91). After you apply the time constant the traces will change color from black to blue. The original data will be removed from memory.

- Click the **Response Time** button. (Note: make sure to click the **Response Time** button located in the Auto DSC group of buttons and not the button associated with the standard DSC) The traces will change in color from black to blue (indicating the Response time “correction” has been applied) and the original data has been removed from Origin.  
*Note: This action may take a minute or two, if there are many datasets plotted.*



### To **Normalize Concentration**

---

When you click the **Normalize Concentration** button, the data is divided by the number of moles of the sample substance in the cell, to convert from cal/degree to cal/mole/degree. Concentration normalization is always carried out after subtracting the reference data.

The concentrations (mM) of the proteins are usually entered in VPViewer when setting up the experiment and stored in the data file header. You may verify the concentration values in the summary table (**Conc. 1**) and correct them in the table, if necessary.

- To view the summary table click the **Window** menu item and select **MicroCal VP-DSC Autosampler Scans Summary Table** from the Windows list. The summary table will be displayed, similar to what is shown below (i.e. without the  $T_m$  entries), you may edit any of the entries for Conc. 1.
- Select **Window:ARawDSC** menu item then click the **Normalize Concentration** button, from the Auto DSC group of buttons. All data scans will be normalize on the protein concentration (conc. 1), the y axis scale will change to kcal/mole/°C and the data will be replotted.

*Note:* Normalization on Concentration is applied to all data files, not just the data plotted in the active layer. For baselines and other scans where the value for Concentration 1 may have been entered as zero Origin will insert a one (1) for the value to prevent division by zero (0).



### To **Find One Tm**

---

- Click the **Find One Tm** button  
The program will find one  $T_m$  for all scans plotted in the active layer, then display a summary, as shown in the table below. *Note: If a scan was not plotted in the active layer (in this case the baseline, pc20904003.DSC was subtracted and automatically removed from the active layer) then a  $T_m$  will not be listed. The program will try to find a  $T_m$  for baselines, if they are plotted in the active layer, as can be seen in the first and second scans of the table below.*

Filename	Tray #	Well #	Conc. 1	Conc. 2	Tm1	Comments
pc20904001.DSC	1	1	1	0	17.72	Baseline
pc20904002.DSC	1	3	1	0	23.08	Baseline
pc20904003.DSC	1	5	1	0	-	Baseline
pc20904004.DSC	1	7	0.09	10	61	Rnase only - no ligand
pc20904005.DSC	1	9	0.09	10	62.7	Rnase + Phosphate
pc20904006.DSC	1	11	0.09	10	66.57	RNase + 3'CMP
pc20904007.DSC	1	13	0.09	10	70.3	RNase + 2'CMP
pc20904008.DSC	1	15	0.09	10	60.97	Rnase only - no ligand
pc20904009.DSC	1	17	0.09	10	62.63	Rnase + Phosphate
pc20904010.DSC	1	19	0.09	10	66.5	RNase + 3'CMP
pc20904011.DSC	1	21	0.09	10	70.36	RNase + 2'CMP
pc20904012.DSC	1	23	0.09	10	60.93	Rnase only - no ligand
pc20904013.DSC	1	25	0.09	10	62.75	Rnase + Phosphate
pc20904014.DSC	1	27	0.09	10	66.54	RNase + 3'CMP
pc20904015.DSC	1	29	0.09	10	70.35	RNase + 2'CMP

## Conc. 1 & Conc. 2

*Conc. 1* is the concentration (mM) of the protein in the cell. *Conc. 2* is the concentration (mM) of the ligand in the cell. These values are usually entered into the data files when setting up the experiments, but may be edited in the table.

- Select the menu item **Windows:ARawDSC** to return to the **ARawDSC** window.

At this point you may wish to save your project for use later in calculating binding constants.

### Find Delta Tm

#### To Find Delta Tm

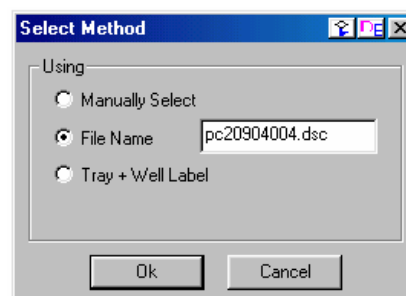
You may select a scan to represent as a reference Tm, to calculate the shift of Tm's for all other scans that are plotted in the active layer.

- Click the **Find Delta Tm** button.  
The Select Method dialog box opens.
- Click the **File Name** option and enter the name pc20904004.dsc (this is one of the protein only scans) and click **Ok**.

*Note: you must enter the .dsc extension.*

The summary table will open listing the filename, Tray #, Well #, Concentration 1, Concentration 2, the Tm, the Delta Tm and any comments that might have been included in the data file.

Filename	Tray #	Well #	Conc. 1	Conc. 2	Tm1	DTm	Comments
pc20904001.DSC	1	1	1	0	17.72	-43.28	Baseline
pc20904002.DSC	1	3	1	0	23.08	-37.92	Baseline
pc20904003.DSC	1	5	1	0	-	-	Baseline
pc20904004.DSC	1	7	0.09	10	61	0	Rnase only - no ligand
pc20904005.DSC	1	9	0.09	10	62.7	1.7	Rnase + Phosphate
pc20904006.DSC	1	11	0.09	10	66.57	5.57	RNase + 3'CMP
pc20904007.DSC	1	13	0.09	10	70.3	9.3	RNase + 2'CMP
pc20904008.DSC	1	15	0.09	10	60.97	-0.03	Rnase only - no ligand
pc20904009.DSC	1	17	0.09	10	62.63	1.63	Rnase + Phosphate
pc20904010.DSC	1	19	0.09	10	66.5	5.5	RNase + 3'CMP
pc20904011.DSC	1	21	0.09	10	70.36	9.36	RNase + 2'CMP
pc20904012.DSC	1	23	0.09	10	60.93	-0.07	Rnase only - no ligand
pc20904013.DSC	1	25	0.09	10	62.75	1.75	Rnase + Phosphate
pc20904014.DSC	1	27	0.09	10	66.54	5.54	RNase + 3'CMP
pc20904015.DSC	1	29	0.09	10	70.35	9.35	RNase + 2'CMP



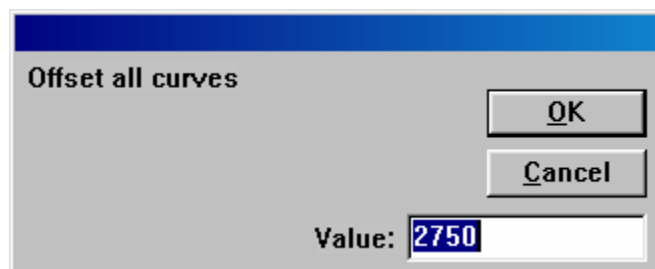
---

**To apply a Y Offset**

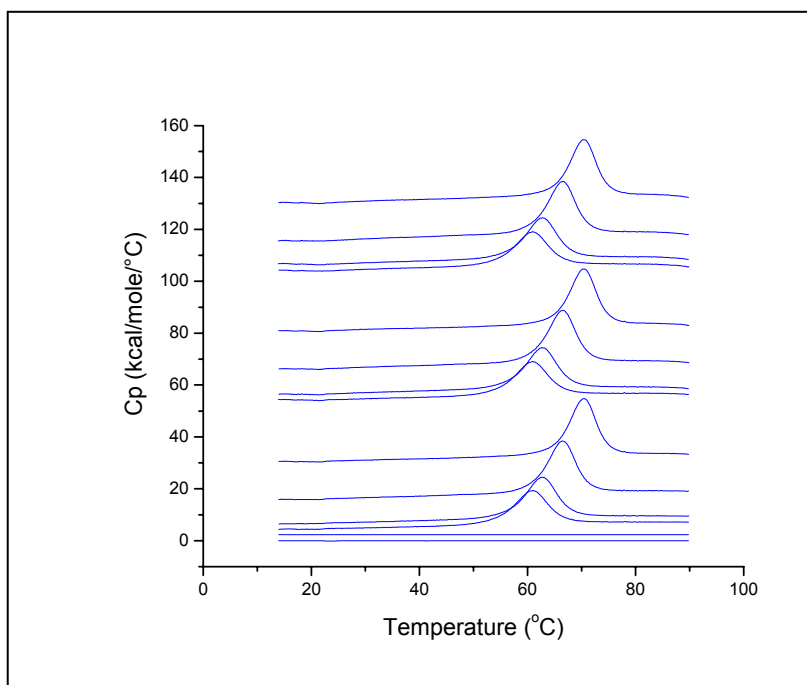
---

Many times when reading in multiple files the curves will overlap each other making it difficult to view the individual curves. You may use the **Y Offset** button to offset each curve, so that at the peak, the closest point of the next curve is offset by the amount entered.

- If you are not continuing from the previous example, read in a series of scans (pc20904001.DSC) as described above.
- Click the Y Offset button and the following dialog box will pop up.



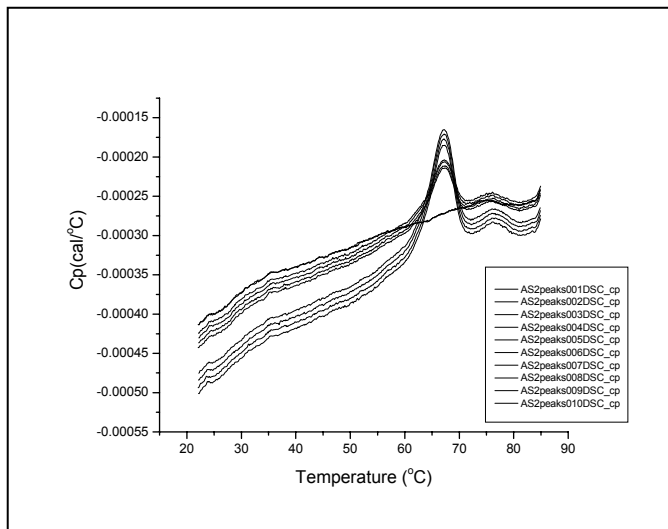
- You may select the default value or enter a different value and click **OK**. Each curve will be offset so that the closest point of each adjacent curve will be equal to the amount entered, as shown below.



Find Two Tm

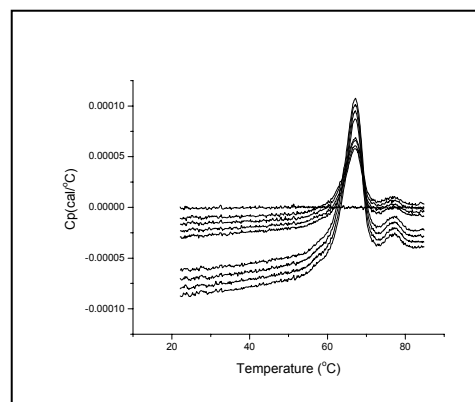
**To Find Two Tm**

- Select **File:New:Project** to open a new Origin project,
- Click on the **Read Data..** button.  
The **Import Multiple ASCII** dialog box opens, with the Auto DSC Data (\*.dsc) selected as the Files of type. Only DSC files that have an 001 before the extension dot will be listed.
- Navigate to the **C:\Origin70\Samples** folder, then select AS2Peaks001.DSC to add the data file series to the lower list box and click **OK**. The AS2Peaks family of data files will open and your graph should look as shown.



**To Subtract a Baseline** (Tray + Well Label method)

- Click the **Subtract Baseline** button.  
The Select Method dialog box will open
- Select the **Tray + Well Label** option and enter the number **1** for the tray and **A11** for the well matrix number.  
The baseline (AS2Peaks006.dsc) will be subtracted from all scans that are currently plotted .



As described in the previous example, you may select the **Response Time** and **Normalize Concentration** buttons.  
*Note:* The **Find Delta Tm** does not operate for two Tm's.

- Click the Find Two Tm button  
The following table will open listing the filename, Tray #, Well #, Concentration 1, Concentration 2, Tm1, Tm2 and any comments that might have been included in the data file.

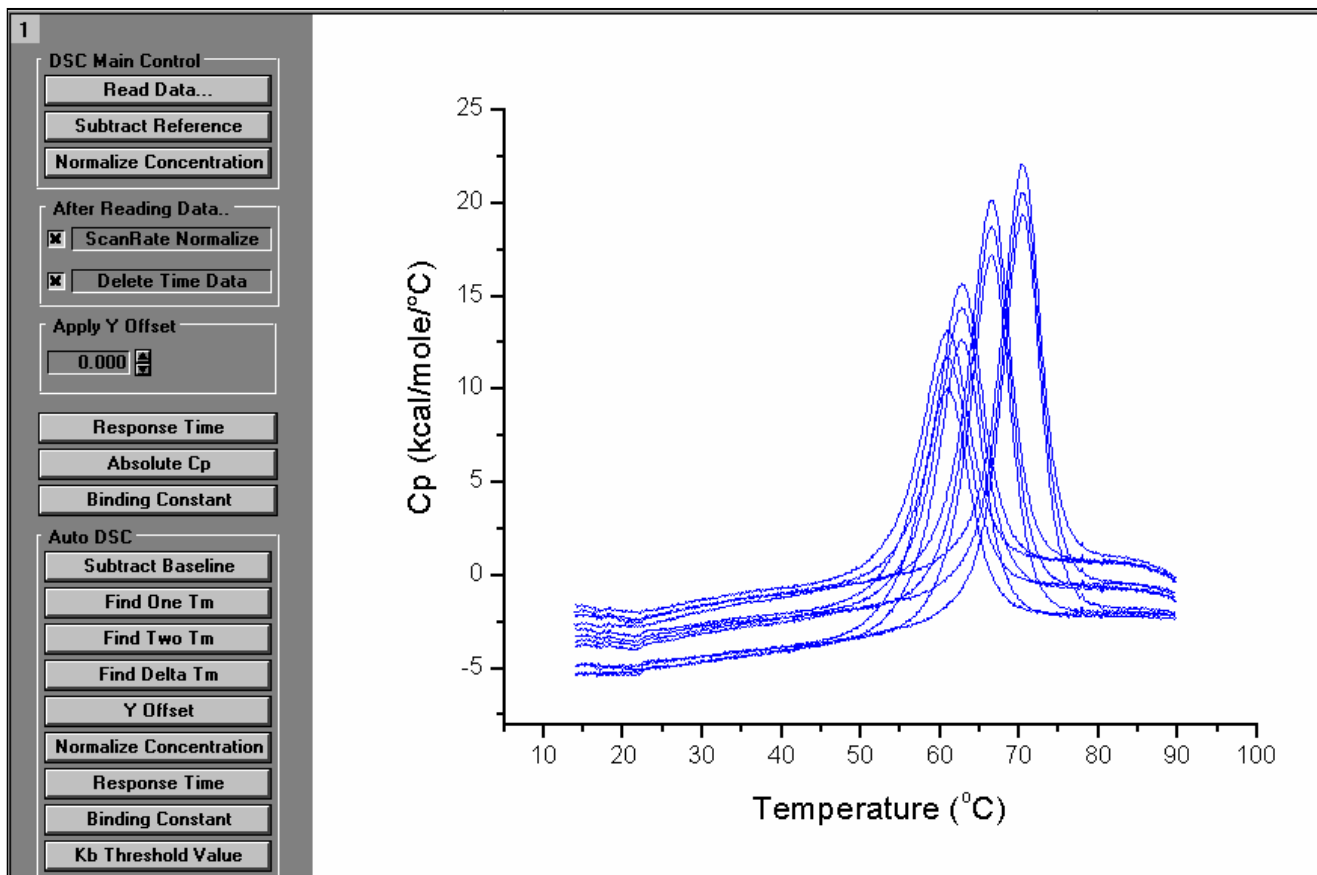
Filename	Tray #	Well #	Conc. 1	Conc. 2	Tm1	Tm2	Comments
AS2Peaks001.DSC	1	A1	0.72	0	66.88	76.6	
AS2Peaks002.DSC	1	A3	0.72	0	66.84	75.91	
AS2Peaks003.DSC	1	A5	0.72	0	66.84	77.45	
AS2Peaks004.DSC	1	A7	0.72	0	66.95	76.67	
AS2Peaks005.DSC	1	A9	1	0	98	44	Baseline
AS2Peaks006.DSC	1	A11	1	0	-	-	Baseline
AS2Peaks007.DSC	1	A13	1.44	0	66.84	76.46	
AS2Peaks008.DSC	1	A15	1.44	0	66.95	76.57	
AS2Peaks009.DSC	1	A17	1.44	0	66.95	76.57	
AS2Peaks010.DSC	1	A19	1.44	0	66.95	76.67	

## Ligand-Protein Binding Constants from Autosampler DSC Data

Data obtained from DSC scans may be used to calculate binding constants for protein-ligand or protein-protein interactions ( for further information refer to page 101 of the Appendix). The addition of a ligand into a protein solution acts to shift the transition midpoint to higher temperatures, if that ligand binds to the low-temperature native form of the protein. The more strongly the ligand is bound, the greater the increase in  $T_m$ . Accurate estimates of binding constant  $K_B$  may be obtained from DSC data in the presence and absence of ligand. The parameters required to calculate  $K_B$  are the protein concentration, ligand concentration, the heat ( $\Delta H^\circ$ ) and the heat capacity ( $\Delta C_p$ ) change in the absence of ligand, and the temperature midpoint of the transition in the presence ( $T_m$ ) and absence ( $T_o$ ) of Ligand.

The project **BindingConstants.OPJ** is available in the **C:\Origin70\Samples** directory for the following example to determine binding constants from DSC data. For the following example we will first determine the  $\Delta C_p$  then  $\Delta H$  for the protein with no ligand. The  $T_m$ 's for both the bound and unbound protein can be read from the previously determined values (refer to the table on page 65).

- Launch a new instance of Origin or select **File>New:Project** to open a new Origin project.
- Select **File:Open** navigate to the **C:\Origin\Samples** directory and select **BindingConstants.OPJ** and click **Open**.  
Your project should look similar to what is shown below. This data has had a baseline subtracted, has been “corrected” for instrument response time, has been normalized on concentration and the  $T_m$ 's have been determined.

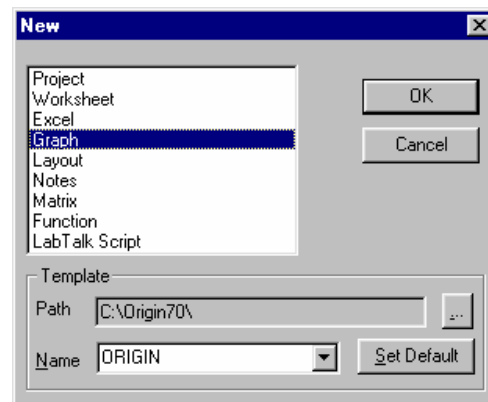




- Select **File : New : Graph** and click **OK**.  
A new graph will be created.

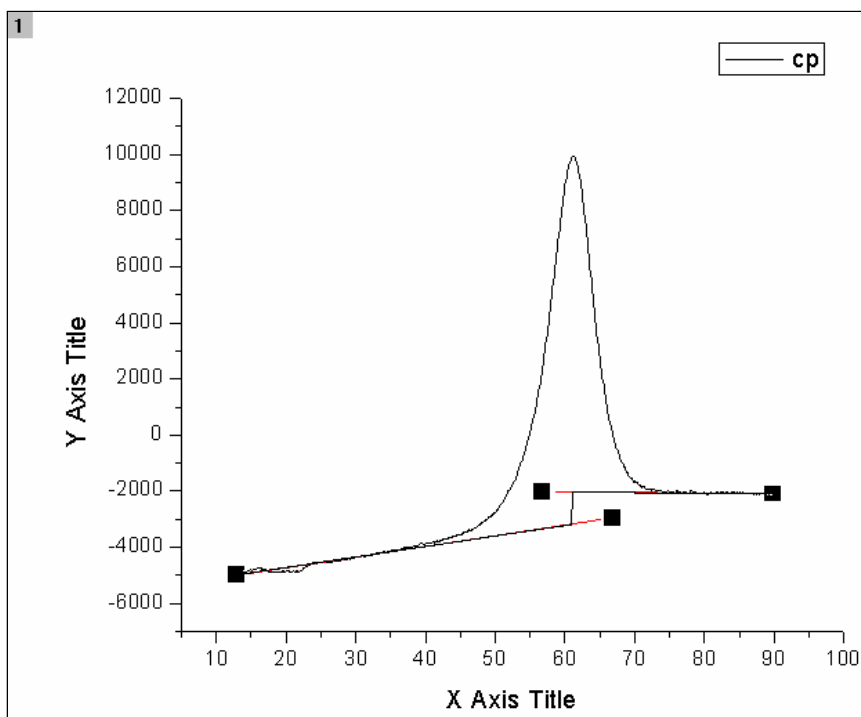
- Double click on the Layer 1 icon **1** located in the upper left hand corner of the new graph.

- Click the **pc20904004dsc\_cp** dataset from the Available Data list, to highlight it, then click the right pointing arrow, => , to place this dataset in the Layer Contents list box and click **OK**.



#### To determine $\Delta C_p$

- Select the menu item **Peak:Start Baseline Session..**  
Origin calculates and plots in red the left and right linear line segments from which to determine the baseline. A new menu bar appears containing the baseline session menus.
- Select the menu item **Adjust:Move Segments by Cursor.**  
The cursor will change to a cross-hair icon.
- Adjust the low temperature and high temperature segments to approximately what is shown below, then double click an endpoint or press enter to set the baseline segments. (See Lesson 4: Baseline Determinations for greater detail)
- Select **Step at Peak** from the **Baseline** menu.  
The Step at Peak baseline will provide a quick and easy method to determine the  $\Delta C_p$ . At this point your graph should look similar to what is shown below.



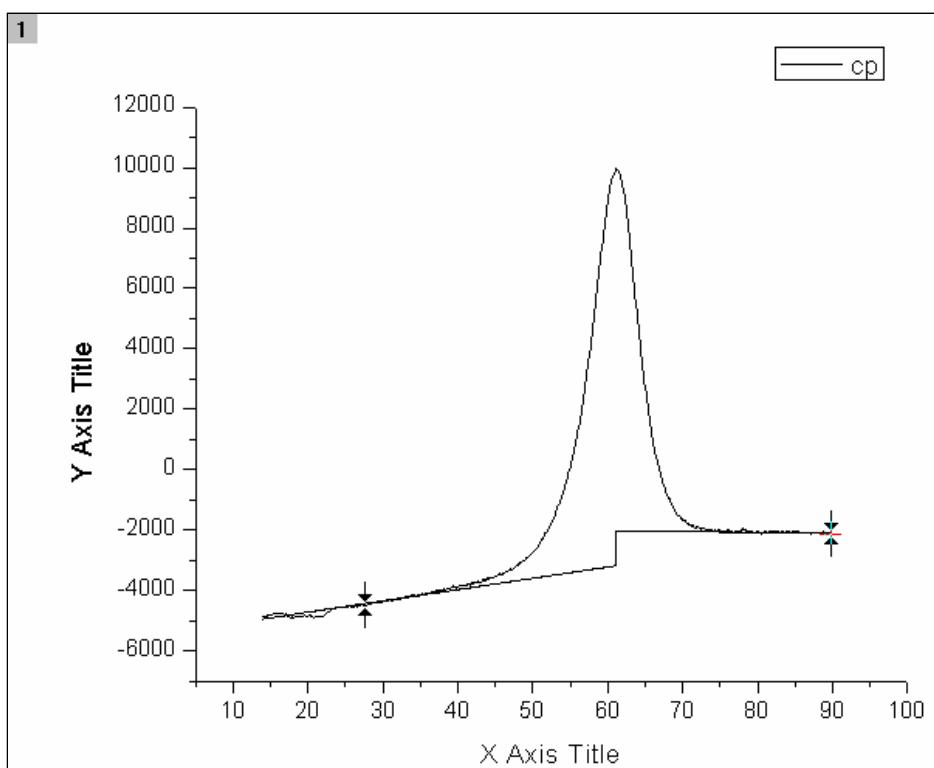
- Click **OK.**, then click **NO** when asked **Subtract baseline?**
- Select the Data Reader tool from the tools toolbar then use the left and right arrow keys to read the difference of baseline y values at the step at the peak, for this example (determined by where the baselines were set), there was measured to be a high value of -2030 and a low value of -3160 at the step, to determine the  $\Delta C_p$  to be approximately **1100 cal/deg/mole**.  
*Note:* Your  $\Delta C_p$  value will be slightly different depending on where you placed the lower and higher temperature baseline segments during the baseline session.

Data Reader  
Tool

To determine  $\Delta H$  at  $T_0$  by calculating the area under the curve.

Data Selector  
Tool

- Select the **Data Selector** tool and using the mouse or arrow keys position the left data marker to the approximate position, as shown below. This will eliminate any effect of the aberration located at the beginning of the scan.



- To set the markers, press enter or double-click on one of the markers.
- Select **Integrate from Baseline** from the **Peak** menu.  
The area of the peak between the markers will be determined and printed in the Script Window. For this example the area was found to be equal to approximately 110,000 cal/mole. Your value may be different, depending on where you placed the lower and higher temperature baseline segments during the baseline session.

*Note:* The script window may be hidden near the bottom edge of the workspace, if the Script Window is not open, you may open it by clicking on the Script Window button located on the standard tool bar.

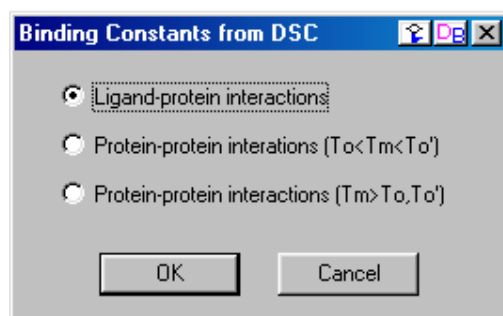


To calculate a single DSC ligand-protein binding constant from autosampler data

In the following example we will calculate the binding constant for a ligand to a protein then extrapolate the binding constant to a different temperature. From the previous example we determined  $T_o$  (61°C),  $\Delta H^\circ$  at  $T_o$  (110,000 cal/mole) and  $\Delta C_p$  (1,100 cal/mole/deg) for the protein only.

For the following example we will use the values from the pc20904006.DSC row of the summary table on page 65. From the table we can see that  $T_m = 66.57$  °C, the Protein Concentration = .00009 moles/liter and the Ligand Concentration = .010 moles/liter. From previous information we know the stoichiometry,  $n=1$ . Knowing the heat of binding  $\Delta H_B$  and  $\Delta C_P$  independently, we will extrapolate the  $K_B$  value to another temperature  $T_F$ .

- Return to the ARawDSC window.
- Click the **Binding Constant** button, from the standard DSC group of buttons. The **Binding Constants from DSC** dialog box will pop up.



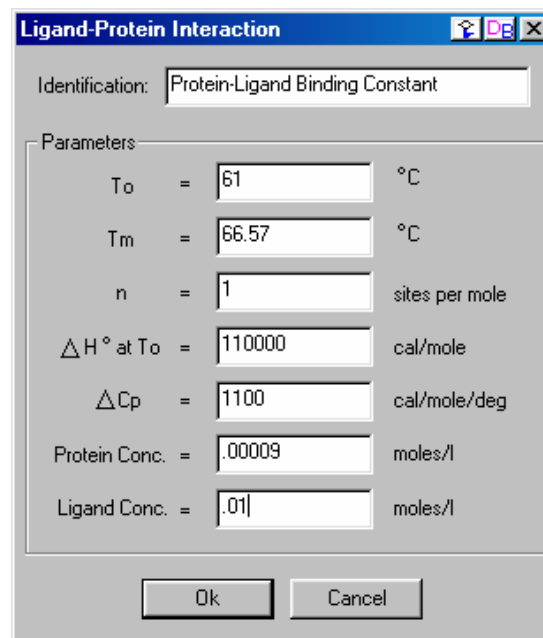
- Select the Ligand-protein interactions radio button and click **OK**.

Select the Ligand-protein

The **Ligand-Protein Interaction** dialog box will open up.

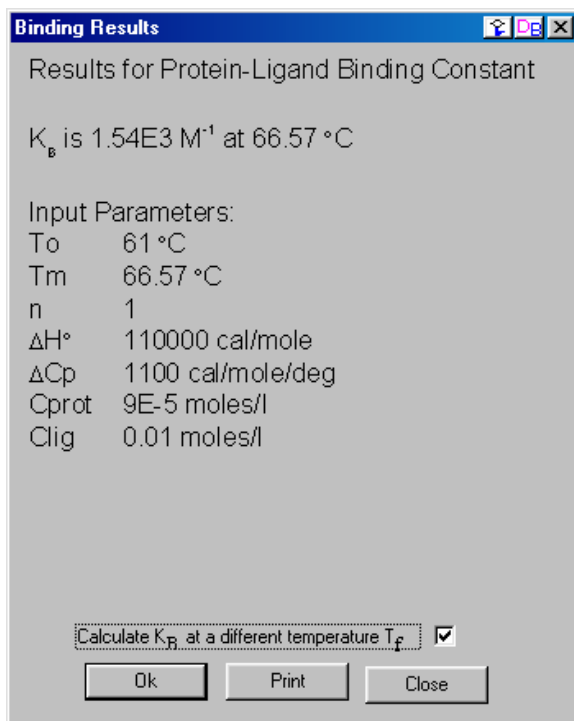
- Enter the following values in to the Ligand-Protein Interaction dialog box, as determined in the preceding pages.

$T_o = 61$  °C  
 $T_m = 66.57$  °C  
 $n = 1$   
 $\Delta H^\circ$  at  $T_o = 110000$  cal/mole  
 $\Delta C_p = 1100$  cal/mole/deg  
 Protein Conc. = .00009 moles/l  
 Ligand Conc. = .01 moles/l



- Click **OK**

The binding constant at  $T_m$  will be calculated and the Binding Results dialog box will open listing the entered parameters and the calculated  $K_B$ .



You may print the results and/or extrapolate the K<sub>B</sub> to a different temperature.

To extrapolate the binding constant to a different temperature.

When extrapolating the binding constant to a different temperature you must determine  $\Delta H_B$  and  $\Delta C_{pB}$  from an ITC experiment or other independent information.

- Click the **Calculate  $K_B$  at a different temperature  $T_F$**  check box to insert a checkmark, then click **OK**.
- Enter the following values in the parameter text boxes.

$T_f = 25\text{ }^\circ\text{C}$   
 $\Delta H_B$  at  $T_f = -15000\text{ cal/mole}$   
 $\Delta C_{pB} = -1000\text{ cal/mole/deg}$

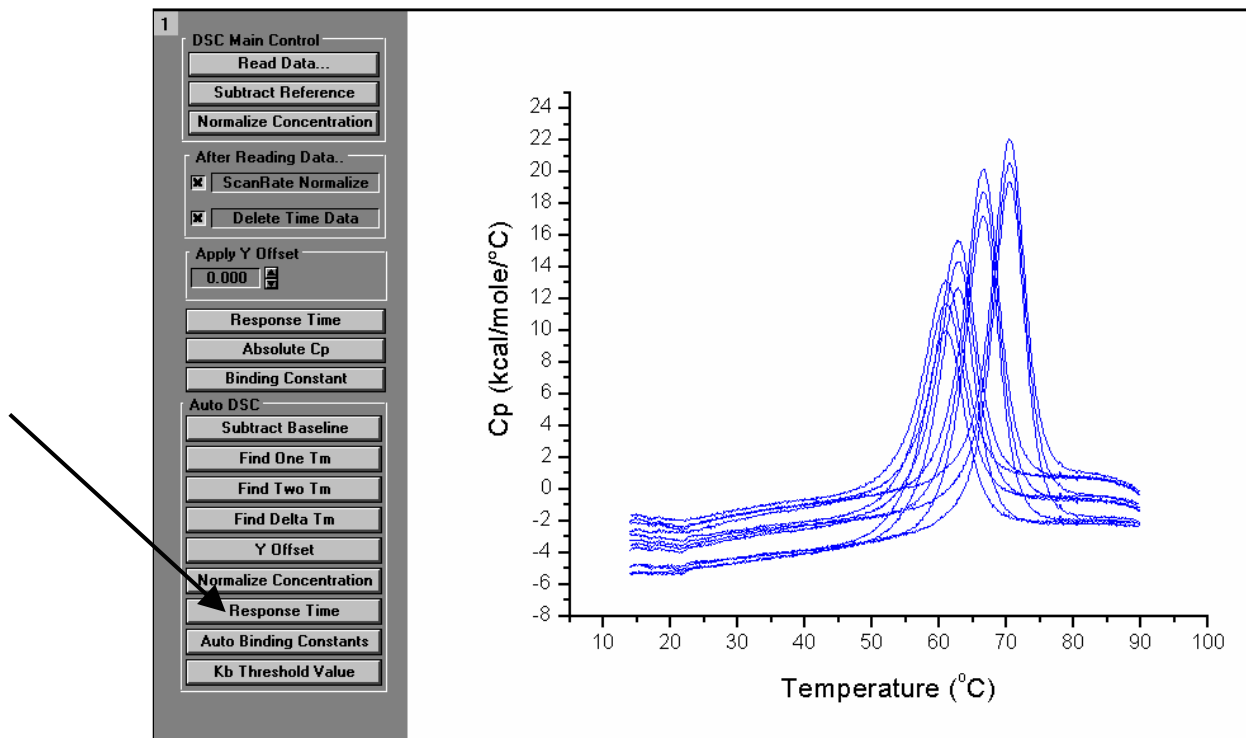
- Click **OK**.

The binding constant at  $T_f$  will be calculated and the following dialog box will open listing the entered parameters and the extrapolated  $K_B$ . You may print the results or click **OK** or **Close** to close the dialog box.

## Ligand-Protein Binding Constants from Multiple Samples

- Launch a new instance of Origin.
- Select **File:Open** navigate to the **C:\Origin\Samples** directory, select the project **BindingConstants.OPJ** and click **Open**.

Your project should look similar to what is shown below. This data has had a baseline subtracted, has been “corrected” for instrument response time, has been normalized on concentration and the  $T_m$  has been determined.



- Click the **Auto Binding Constants** button from the Auto DSC button group.  
The DSC Ligand-Protein Binding Constants dialog box will open

- Enter the following values, as determined in the preceding pages.

$$T_o = 61 \text{ } ^\circ\text{C}$$

$$n = 1.0$$

$$\Delta H^\circ \text{ at } T_o = 110000 \text{ cal/mole}$$

$$\Delta C_p = 1100 \text{ cal/mole/deg}$$

- Click **OK**.  
The binding constants are calculated for all data sets that are plotted in the active layer and listed in the summary table.

Filename	Tray #	Well #	Conc. 1	Conc. 2	Tm1	Kb	Comments
pc20904001.DSC	1	1	1	0	–	–	Baseline
pc20904002.DSC	1	3	1	0	–	–	Baseline
pc20904003.DSC	1	5	1	0	–	–	Baseline
pc20904004.DSC	1	7	0.09	10	61	0	Rnase only - no ligand
pc20904005.DSC	1	9	0.09	10	62.7	134	Rnase + Phosphate
pc20904006.DSC	1	11	0.09	10	66.57	1540	RNase + 3'CMP
pc20904007.DSC	1	13	0.09	10	70.3	10900	RNase + 2'CMP
pc20904008.DSC	1	15	0.09	10	60.97	0	Rnase only - no ligand
pc20904009.DSC	1	17	0.09	10	62.63	126	Rnase + Phosphate
pc20904010.DSC	1	19	0.09	10	66.5	1480	RNase + 3'CMP
pc20904011.DSC	1	21	0.09	10	70.36	11200	RNase + 2'CMP
pc20904012.DSC	1	23	0.09	10	60.93	0	Rnase only - no ligand
pc20904013.DSC	1	25	0.09	10	62.75	139	Rnase + Phosphate
pc20904014.DSC	1	27	0.09	10	66.54	1510	RNase + 3'CMP
pc20904015.DSC	1	29	0.09	10	70.35	11200	RNase + 2'CMP

### K<sub>B</sub> Threshold Value

To find K<sub>B</sub> values that exceed a “K<sub>B</sub> Threshold Value”

You may easily find binding constants that exceed a designated value by entering a “threshold value” for K<sub>B</sub>. All values in the summary table that exceed the designated value will be highlighted.

- Return to the ARawDSC window by selecting **Window : ARawDSC**.
- Click the **K<sub>B</sub> Threshold Value** button located in the Auto DSC button group.

The following dialog box opens.

- Enter a threshold value (e.g. 1500) and click **OK**.

The Summary Table will become the active window and cells in the table of all K<sub>B</sub> values that exceed the entered value will be highlighted.

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## Lesson 7: Other Useful Details

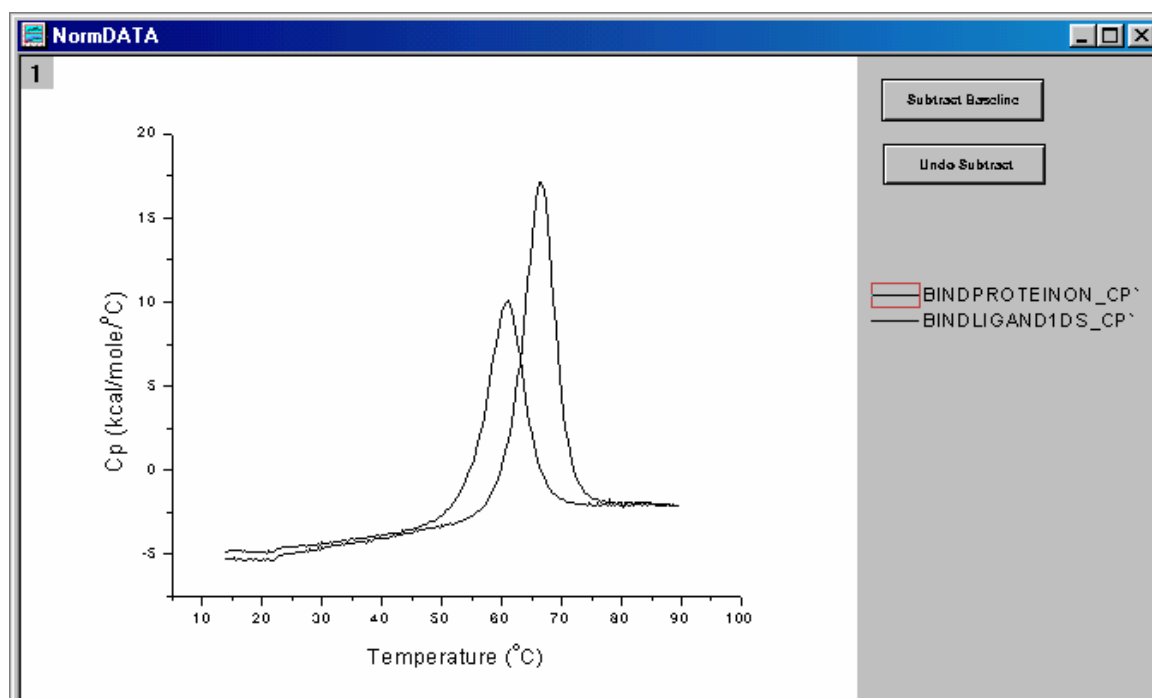
### Ligand-Protein Binding Constants from Standard DSC Data

Data obtained from standard DSC scans may be used to calculate binding constants for protein-ligand or protein-protein interactions (as discussed on page 101 of the Appendix). The addition of a ligand into a protein solution acts to shift the transition midpoint to higher temperatures, if that ligand binds to the low-temperature native form of the protein. The more strongly the ligand is bound, the greater the increase in  $T_m$ . Accurate estimates of binding constant  $K_B$  may be obtained from DSC data in the presence and absence of ligand. The parameters required to calculate  $K_B$  are the protein concentration, ligand concentration, the heat ( $\Delta H^\circ$ ) and the heat capacity ( $\Delta C_p$ ) change in the absence of ligand, and the temperature midpoint of the transition in the presence ( $T_m$ ) and absence ( $T_o$ ) of Ligand.

The equations and parameters required to calculate binding constants for the standard DSC are the same as used with autosampler DSC data. The methods used are a little different; ( $\Delta C_p$  and  $\Delta H^\circ$ ) are determined by the same method (see below and pages 69 - 70) for both autosampler and standard DSC data. The peak temperatures ( $T_o$  and  $T_m$ ) are determined automatically by autosampler routines, but require some user interaction (see page 80) for standard DSC data.

- Launch a new instance of Origin or select **File:New:Project** to open a new Origin project,
- Select **File:Open** navigate to the **C:\Origin70\Samples** directory and select **Binding Data from standard DSC.OPJ** and click **Open**.

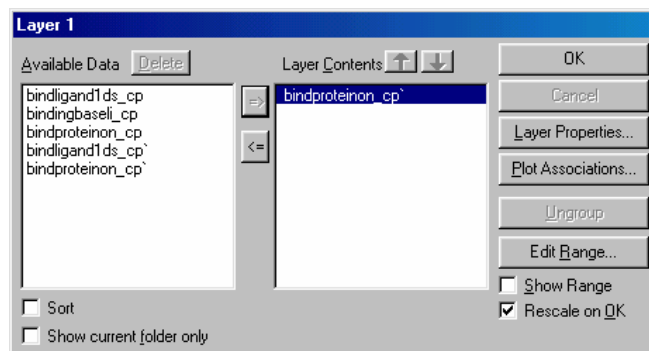
This project contains 3 data files, one with the protein in the absence of ligand, one with the protein in the presence of ligand 1, and another with the protein in the presence of ligand2. The data has had a baseline subtracted, "corrected" for the response time of the instrument and has been normalized on concentration.



- Select **File : New : Graph** and click **OK**.  
A new graph will become the active graph in Origin.
- Double click on the Layer 1 icon located in the upper left hand corner of the new graph.

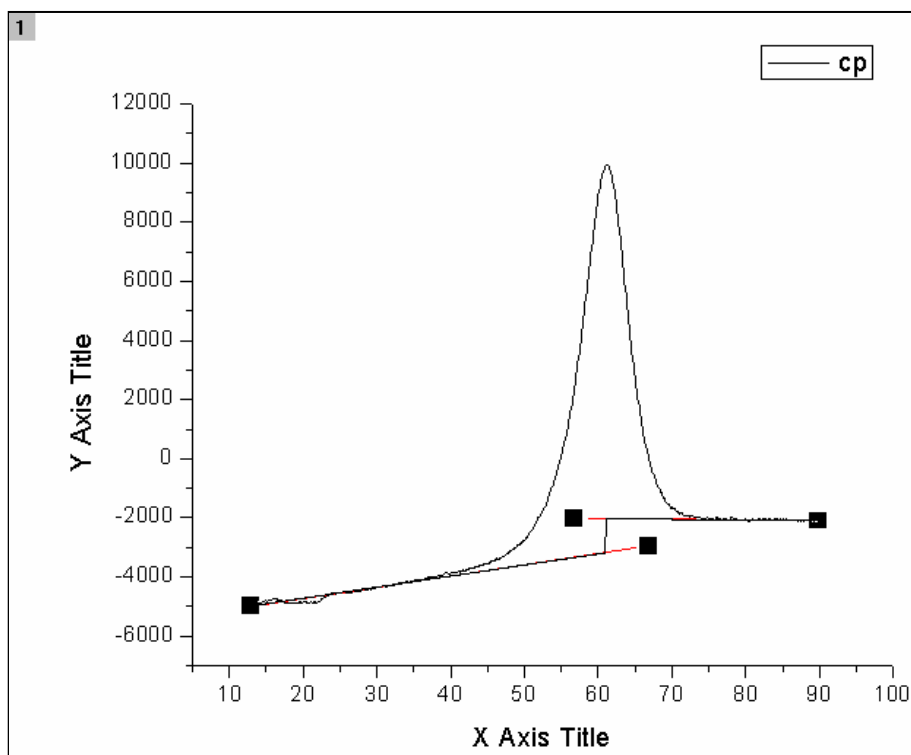
- Click the **bindproteinon\_cp'** dataset from the Available Data list, to highlight it, then click the right pointing arrow, => , to place this dataset in the Layer Contents list box and click **OK**.

*Note:* The apostrophe ' at the end of the data set name indicates that this data has been "corrected" for the response time of the instrument.



#### To determine $\Delta C_p$

- Select the menu item **Peak : Start Baseline Session..**  
Origin calculates and plots in red the left and right linear line segments from which to determine the baseline. A new menu bar appears containing the baseline session menus.
- Select the menu item **Adjust : Move Segments by Cursor**, adjust the low temperature and high temperature segments, then double click an endpoint or press enter to set the baseline segments. (See Lesson 4: Baseline Determinations for greater detail)
- Select **Step at Peak** from the **Baseline** menu.  
The **Step at Peak** baseline will provide a quick and easy method to determine the  $\Delta C_p$ . At this point your graph should look similar to what is shown below.



- Click **OK.**, then click **NO** when asked **Subtract baseline?**
- Select the **Data Reader** tool, then use the left and right arrow keys to read the difference of baseline y values at the step of the peak, for this example (determined by where the baselines were set), there was measured to be a high value of -1960 and a low value of -3040 at the step, for a  $\Delta C_p$  to be approximately **1100 cal/deg/mole**.



Data Reader  
Tool

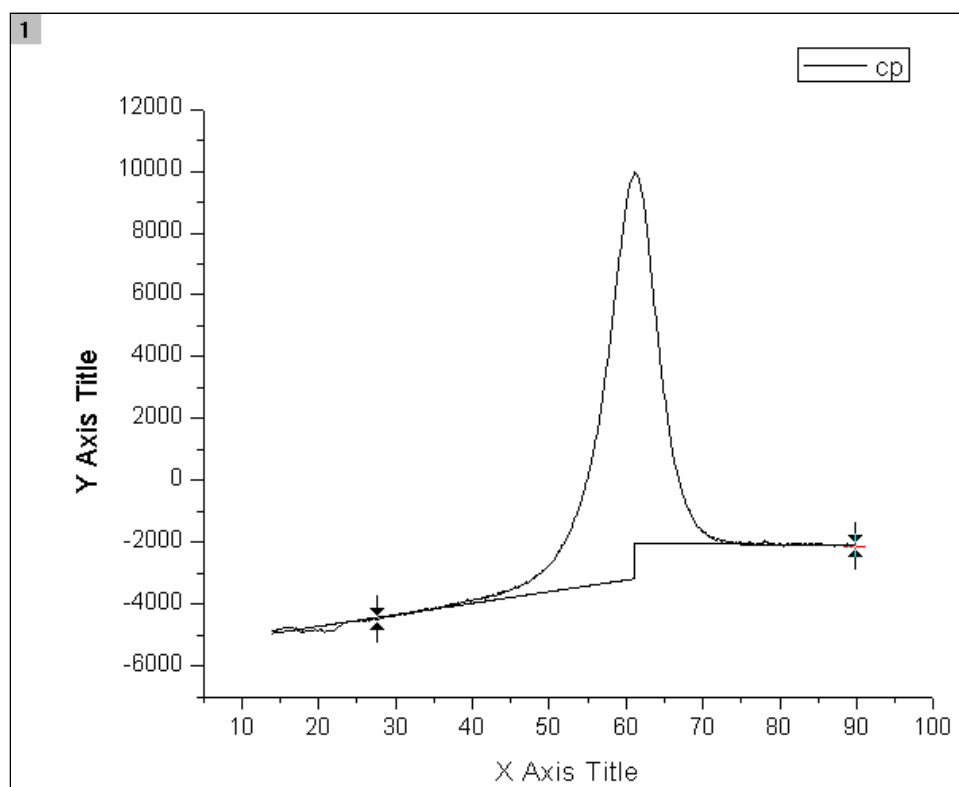
*Note:* Your  $\Delta C_p$  value may be different, depending on where you placed the lower and higher temperature baseline segments during the baseline session.

To determine  $\Delta H$  by calculating the area under the curve.



Data Selector  
Tool

- Select the **Data Selector** tool and using the mouse or arrow keys position the left data marker to the approximate position, as shown below. This will eliminate the effect of the aberration located at the beginning of the scan.



- To set the markers, press enter or double-click on one of the markers.
  - Select the **Peak : Integrate from Baseline** menu item. The area of the peak between the markers will be determined and listed in the Script Window. For this example the area was found to be equal to approximately **110,000 cal/mole**. Your value may be different depending on where you placed the lower and higher temperature baseline segments during the baseline session.
- Note:* The script window may be hidden near the bottom edge of the workspace, if the Script Window is not open, you may open it by clicking on the Script Window button



---

To find the peak temperature

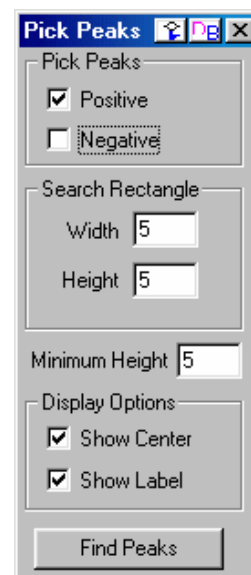
---

- Return to the **NormDATA** window (select **Window : NormDATA**).
- Select the **Tools : Pick Peaks** menu item.  
The Pick Peaks dialog box will open. Enter the options that are shown.

The Pick Peaks tool operates on the active data plot in the graph window. (Refer to Origin's on-line help for more information about the Pick Peaks tool.)

*Shortcut:* You may make a data set, the active data set, by clicking on the plot type icon in the legend.

- Make the **bindproteinon\_cp**' data set the active data set by selecting **Data** and clicking on the **BINDPROTEINON: temp(x), cp'(y)** menu item to place a check mark next to the name.
- Click the **Find Peaks** button on the Pick Peaks dialog box. Origin will place a cross at the peak and print the temperature.  
The peak temperature ( $T_0$ ) should be ca. **61.0°C** for the protein only scan.
- Make the data set **bindligand1d\_cp**' the active data set, then click the **Find Peaks** button.  
The peak temperature should be ca. **66.6°C** for the protein with ligand 1
- Close the **Pick Peaks** dialog box.

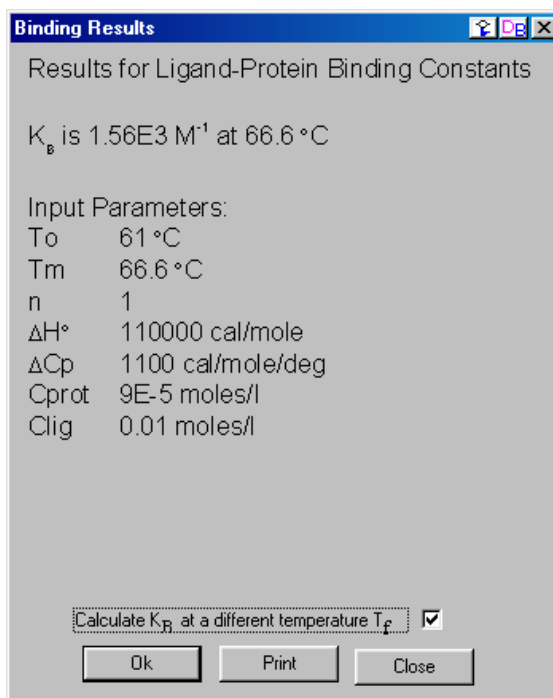
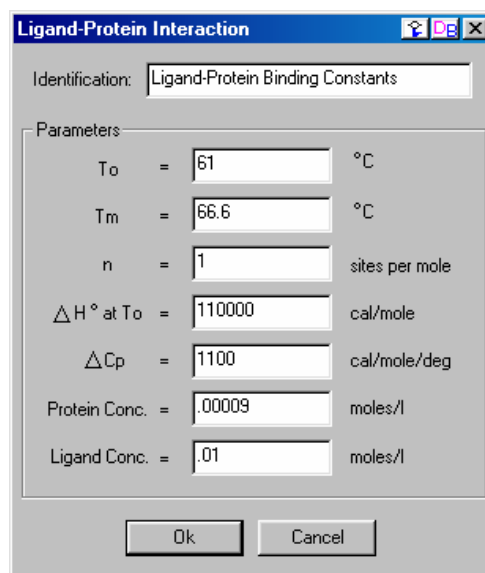
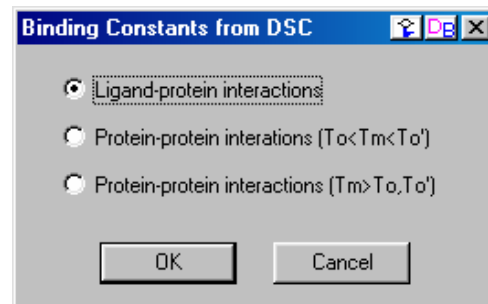


To calculate the ligand-protein binding constant

- Return to the **RawDSC** window (i.e. select the menu item **Window:RawDSC**)
- Click the **Binding Constant** button and the **Binding Constants from DSC** dialog box will open up.
- Click the **Ligand-protein interactions** radio button then click **OK**.  
The Ligand-protein interactions dialog box will open up, as shown below.
- Enter the following values, as determined in the preceding pages (pages 78 - 80).

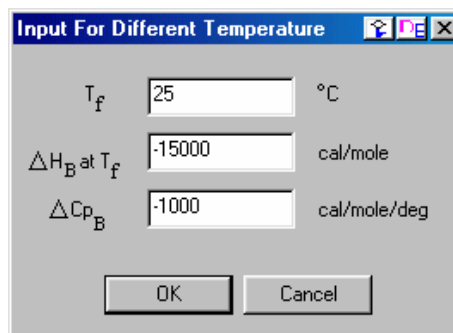
$T_o = 61\text{ }^\circ\text{C}$   
 $T_m = 66.6\text{ }^\circ\text{C}$   
 $n = 1$   
 $\Delta H^\circ \text{ at } T_o = 110000\text{ cal/mole}$   
 $\Delta C_p = 1100\text{ cal/mole/deg}$   
 Protein Conc. = **.00009 moles/l**  
 Ligand Conc. = **.010 moles/l**

- Click **OK**  
The binding constant at  $T_m$  will be calculated and the Binding Results dialog box will open listing the entered parameters and the calculated  $K_B$ . You may print the results and/or calculate the  $K_B$  at a different temperature.



To extrapolate the binding constant to a different temperature.

- Click the **Calculate  $K_B$  at a different temperature  $T_f$**  check box to insert a checkmark, then click **OK**. A new dialog box will open up allowing you to enter the parameter information to extrapolate the  $K_B$  value to another temperature. Choose the File pc20904007.DSC to use for extrapolating the binding constant to a different temperature. Repeat the method described on the previous pages to determine the  $\Delta H$  and the  $\Delta C_p$  at the different temperature  $T_f$ .



The dialog box titled "Input For Different Temperature" contains three input fields with units to their right. The first field is labeled  $T_f$  and contains the value 25, with the unit °C. The second field is labeled  $\Delta H_B$  at  $T_f$  and contains the value -15000, with the unit cal/mole. The third field is labeled  $\Delta C_{p_B}$  and contains the value -1000, with the unit cal/mole/deg. At the bottom of the dialog box are two buttons: "OK" and "Cancel".

- Enter the following values in the parameter text boxes.

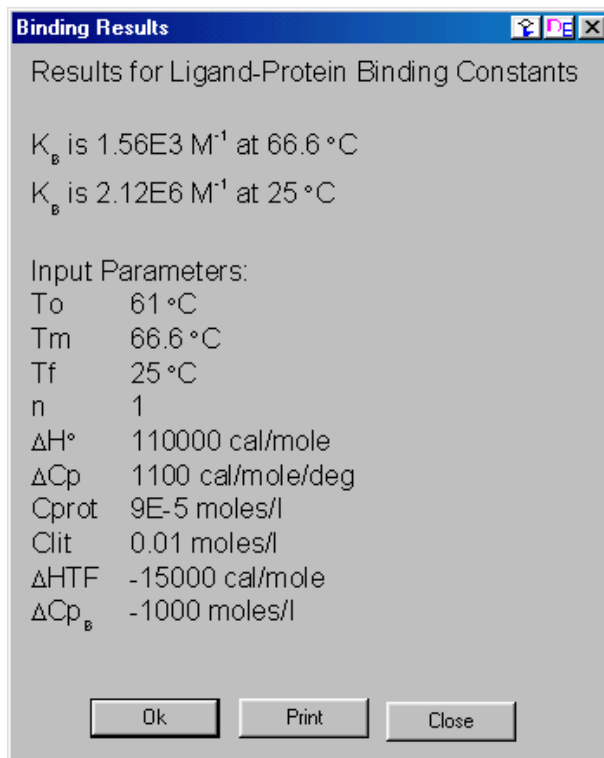
$T_f = 25$  °C

$H_B$  at  $T_f = -15000$  cal/mole/deg

$\Delta C_{p_B} = -1000$  cal/mole

- Click **OK**.

The binding constant at  $T_f$  will be calculated and the following dialog box will open listing the entered parameters and the extrapolated  $K_B$ . You may print the results.



The dialog box titled "Binding Results" displays the following information:

Results for Ligand-Protein Binding Constants

$K_B$  is 1.56E3  $M^{-1}$  at 66.6 °C  
 $K_B$  is 2.12E6  $M^{-1}$  at 25 °C

Input Parameters:

$T_o$	61 °C
$T_m$	66.6 °C
$T_f$	25 °C
$n$	1
$\Delta H^\circ$	110000 cal/mole
$\Delta C_p$	1100 cal/mole/deg
$C_{prot}$	9E-5 moles/l
$C_{lit}$	0.01 moles/l
$\Delta H_{TF}$	-15000 cal/mole
$\Delta C_{p_B}$	-1000 moles/l

At the bottom of the dialog box are three buttons: "Ok", "Print", and "Close".

## Protein-Protein Binding Constants from Standard DSC Data

When estimating the binding constant for protein-protein interactions, the approach is a little different than for ligand-protein interactions, since both of the proteins P and P' will undergo their own thermal unfolding transition with transition temperatures  $T_o$  and  $T_o'$ . When P and P' are mixed together in the same solution, then a separate transition will be observed for the complex PP' with transition midpoint  $T_M$ . If  $T_M$  occurs at a temperature between  $T_o$  and  $T_o'$ , then the regular model for ligand-protein is used to calculate the binding constant.

If  $T_M$  occurs at a temperature higher than both  $T_o$  and  $T_o'$ , then a *different model* is used which takes into account the temperature shift in both transitions. Please refer to the Section VI of the Appendix for more information about protein-protein binding constants.

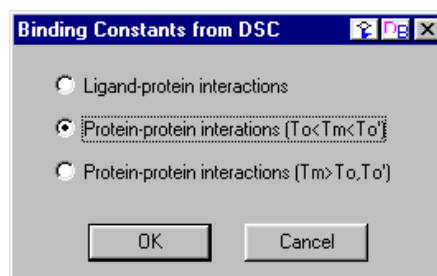
The procedure for measuring binding constants of protein-protein interactions is similar to that of ligand-protein interactions, as described in the previous pages. Separate DSC experiments of each protein alone must be performed to determine the respective values of  $T_o$ ,  $\Delta H^\circ$ , and  $\Delta C_p$  and experiments on the complex of the two proteins are performed to measure the  $T_M$  values.

### Protein-Protein Binding Constants from Standard DSC Data – $T_o < T_M < T_o'$

- Launch a new instance of Origin for DSC

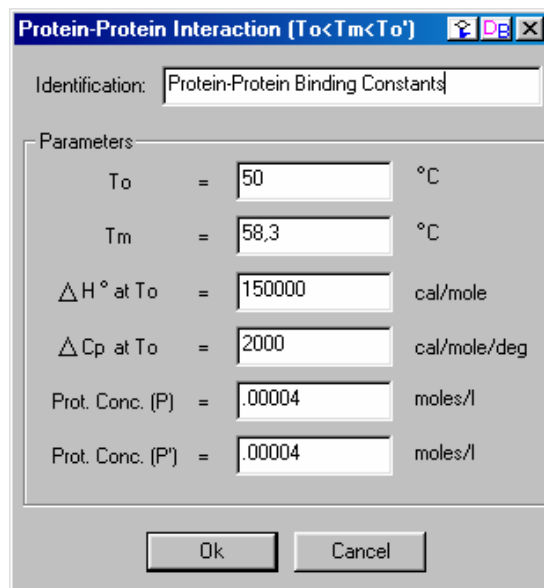
*Note:* Normally, you would calculate the parameters  $\Delta C_p$ ,  $\Delta H^\circ$ ,  $T_o$  for each protein and the  $T_M$  for the complex. Since the method of these calculations has been described on pages 78 - 80, we will simply provide the parameters below.

- Click the **Binding Constant** button and the **Binding Constants from DSC** dialog box will open up.
- Click the **Protein-protein interactions (To<Tm<To')** radio button then click **OK**. The Protein-protein interactions dialog box will open up, as shown below.

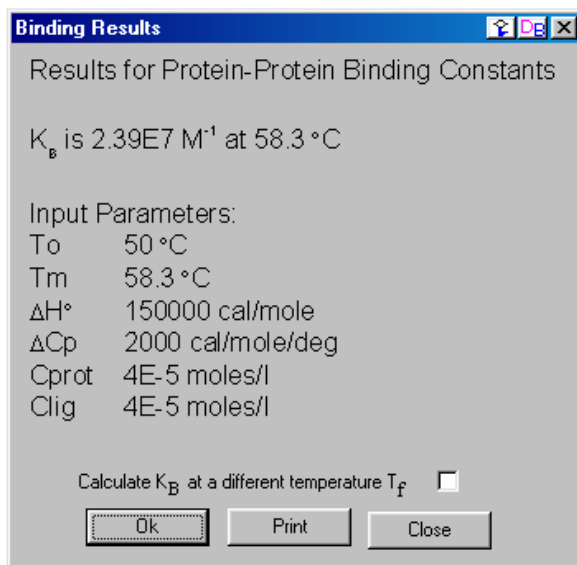


- Enter the following values:

$T_o = 50 \text{ }^\circ\text{C}$   
 $T_M = 58.3 \text{ }^\circ\text{C}$   
 $\Delta H^\circ \text{ at } T_o = 150000 \text{ cal/mole}$   
 $\Delta C_p \text{ at } T_o = 2000 \text{ cal/mole/deg}$   
 Protein Conc. (P) = .00004 moles/l  
 Protein Conc. (P') = .00004 moles/l



- Click **OK**.  
The binding constant at  $T_M$  will be calculated and the **Binding Results** dialog box will open listing the entered parameters and the calculated  $K_B$ . You may print the results and/or calculate the  $K_B$  at a different temperature



### Protein-Protein Binding Constants – $T_M > T_o, T_o'$

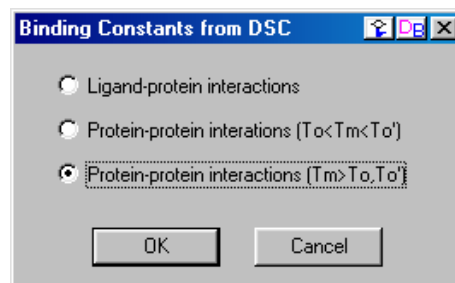
If  $T_M$  occurs at a temperature higher than both  $T_o$  and  $T_o'$ , then a *different model* is used which takes into account the temperature shift in both transitions.

To calculate the binding constant at  $T_M$ ,  $K_B(T_M)$ , when  $T_M > T_o, T_o'$ ,

- Launch a new instance of Origin for DSC

*Note:* Normally, you would calculate the parameters  $\Delta C_p$ ,  $\Delta H^\circ$ ,  $T_o$  for each protein and the  $T_M$  for the complex. Since the method of these calculations has been described on pages 78 - 80, we will simply provide the parameters below.

- Click the **Binding Constant** button and the DSC **Binding Constants from DSC** dialog box will open up.



- Click the **Protein-protein interactions ( $T_M > T_o, T_o'$ )** radio button then click **OK**.  
The Protein-protein interactions dialog box will open up, as shown below.
- Enter the following values.



$T_o = 50\text{ }^\circ\text{C}$   
 $\Delta H^\circ \text{ at } T_o = 150000\text{ cal/mole}$   
 $\Delta C_p \text{ at } T_o = 2000\text{ cal/mole/deg}$   
 Protein Conc. (P) = .0001 moles/l  
 $T_o' = 65\text{ }^\circ\text{C}$   
 $\Delta H^\circ \text{ at } T_o' = 150000\text{ cal/mole}$   
 $\Delta C_p \text{ at } T_o' = 2000\text{ cal/mole/deg}$   
 Protein Conc. (P') = .0001 moles/l  
 $T_M = 68.4\text{ }^\circ\text{C}$

- Click OK.  
 The binding constant at  $T_m$  will be calculated and the **Binding Results** dialog box will open listing the entered parameters and the calculated  $K_B$ . You may print the results and/or calculate the  $K_B$  at a different temperature

To extrapolate the binding constant to a different temperature.

When extrapolating the binding constant to a different temperature you must determine  $\Delta H_B$  and  $\Delta C_{pB}$  from an ITC experiment or other independent information.

- Click the **Calculate  $K_B$  at a different temperature  $T_f$**  check box to insert a checkmark, then click **OK**. A new dialog box will open up allowing you to enter the parameter information to extrapolate the  $K_B$  value to another temperature.

- Enter the following values in the parameter text boxes.

$T_f = 25\text{ }^\circ\text{C}$

$H_B$  at  $T_f = -15000\text{ cal/mole}$

$\Delta C_{pB} = -1000\text{ cal/mole/deg}$

- Click **OK**.

The binding constant at  $T_f$  will be calculated and the following dialog box will open listing the entered parameters and the extrapolated  $K_B$ . You may print the results.

## Calculate $\Delta G$ at temperature T

From analysis of a DSC scan on a protein, three experimental parameters for the unfolding transition are obtained. These include midpoint =  $T_M$ , Enthalpy change at  $T_M = \Delta H(T_M)$  and Heat capacity change at  $T_M = \Delta C_p$

Using these three experimental parameters, the Gibbs free energy change  $\Delta G$  may then be obtained at any temperature T. The routine assumes a reversible two-state transition and also assumes  $\Delta C_p$  is independent of temperature.

To calculate  $\Delta G$  at temperature T

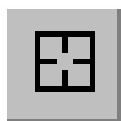
- Click the **Calculate Delta G(T)** button.  
The calculate Delta G(T) dialog box opens.
- Enter the following values for  $T_M$ ,  $\Delta H(T_M)$  and  $\Delta C_p$ .

$$T_M = 60 \text{ }^\circ\text{C}$$

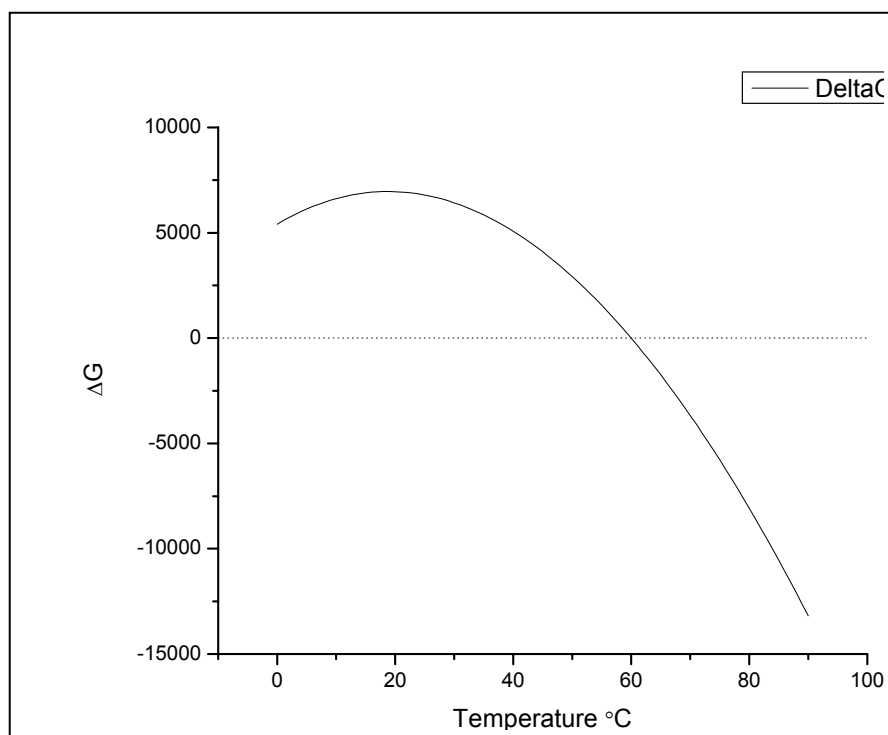
$$\Delta H(T_M) = 110000 \text{ cal/mole}$$

$$\Delta C_p = 2500 \text{ cal/mole/deg}$$

- Click **OK**.  
A new window opens plotting  $\Delta G$  from 0 to 90  $^\circ\text{C}$ , at 1 degree intervals. A dashed line is plotted at  $y=0$  and crosses the  $\Delta G$  curve at the temperature,  $T_M$ .



Use the Data Reader tool to read the value of  $\Delta G$  at any integer temperature from 0 to 90  $^\circ\text{C}$ .



- You may use the **Data Reader** tool to read the value of  $\Delta G$  at any integer temperature from 0 to 90  $^\circ\text{C}$ . For example at 25  $^\circ\text{C}$ ,  $\Delta G$  is ca. 6840.

## Chi-square (chi<sup>2</sup>) Minimization

The aim of the fitting procedure is to find those values of the parameters which best describe the data. The standard way of defining the best fit is to choose the parameters so that the sum of the squares of the deviations of the theoretical curve(s) from the experimental points for a range of independent variables is at a minimum. For the ITC models, where there is no weighting, the theoretical models can be represented by:

$$y = f(x; p_1, p_2, p_3, \dots)$$

where:

$p_i$  = the fitting parameters

the expression for  $\chi^2$  simplifies to :

$$\chi^2 = \frac{1}{n^{eff} - p} \sum [y_i - f(x_i; p_1, p_2, \dots)]^2$$

where:

$n^{eff}$  = the total number of experimental points used in the fitting

$p$  = total number of adjustable parameters

$y_i$  = experimental data points

$f(x; p_1, p_2, p_3, \dots)$  = fitting function

Note: the difference  $d = n^{eff} - p$  is usually referred to as the number of degrees of freedom.

The above equation states that the Chi squared value of the fit is equal to the sum of the squares of the deviations of the theoretical curve(s) from the experimental points divided by the number of degrees of freedom. Since there is no weighting, it can be seen that the calculated values are dependent on the magnitude of the scale and the number of data points. After fitting this value is reported as Chi<sup>2</sup>/DoF.

## Response Time – VP-DSC

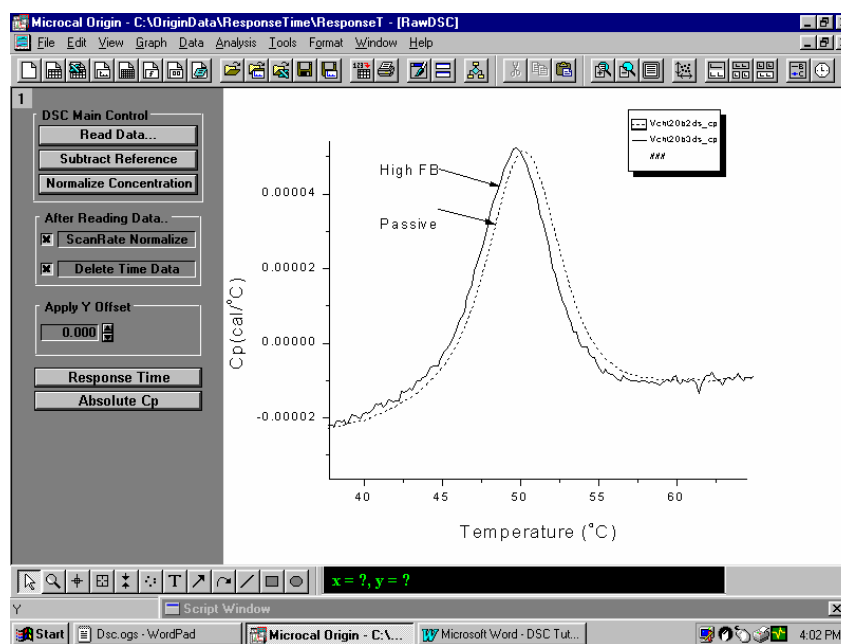
The VP-DSC is the first calorimeter to have operator-selectable response time. It is made possible by passage of the amplified  $\Delta T$  signal (temperature difference signal between the two cells) into the computer for digital processing before the feedback voltage is returned to provide feedback power to the sample cell. This allows software control of the gain (the ratio of power applied to the sample cell relative to the magnitude of the  $\Delta T$  signal) which in turn controls the response time of the instrument to heat effects occurring in the cells. The passive mode with zero gain is best to use for studying systems having very slow transients (broad transition peaks, slow scan rates, or isothermal studies) since the baseline is quieter and more stable due to the absence of feedback noise. For systems with fast transients (sharp thermal transitions and/or fast scan rates), shorter response times must be used to avoid distorting the shape of the

thermogram even though this will result in lower sensitivity. In the current version of VPViewer, there are four response time selections in the experiment set-up window No-Gain (Passive), Low-Gain, Mid-Gain and High-Gain, with respective relaxation time constants of 40.9, 18.0, 10.4 and 5.6 seconds. The respective designations in the header of the data files are 0 (Passive), .5 (Low-Gain), .75 (Mid-Gain) and 1 (High-Gain).

Some protein transitions, as well as those of polynucleotides, are fairly sharp on the temperature axis so that detectable peak broadening may result when using the passive mode at usual scan rates. In such a case, faster response times or slower scan rates may be used to improve peak resolution in real time. However, Origin contains a *Response Time* button, which when activated quickly removes small amounts of response-time broadening for any of the four response times. This post-run capability sometimes avoids the necessity of introducing extraneous noise associated with faster response times, as well as allowing the operator to quickly determine if peak resolution is satisfactory for any data set. Whenever peak broadening is severe, however, this post-run correction will not be completely accurate.

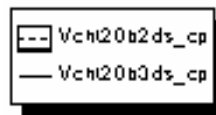
To illustrate the functionality of the *Response Time* button we have included the Origin project **ResponseT.opj** in the C:\Origin70\Samples directory. This Origin project contains the results of two successive experiments run on a sample at 60 deg/hr. The first experiment was scanned using the Passive FeedBack mode, the scan was repeated using the High-Gain Mode. The data was scanrate normalized and a baseline has been subtracted from each trace.

- Select **File:Open**.
- Navigate to the [Origin70][Samples] directory , double –click the project name **ResponseT.opj**. The project will open as shown below.



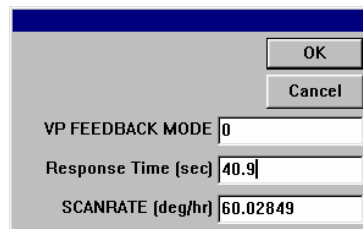
You may see from the that the data collected in the passive mode (dashed line) has a slightly higher  $T_m$  and is slightly broadened from the data collected at the High-Gain FeedBack mode (fastest response time).

- Make sure the data set Vcht20b2ds\_cp is the active data set (box in legend is around the dashed line type of the Vcht20b2ds\_cp data set).

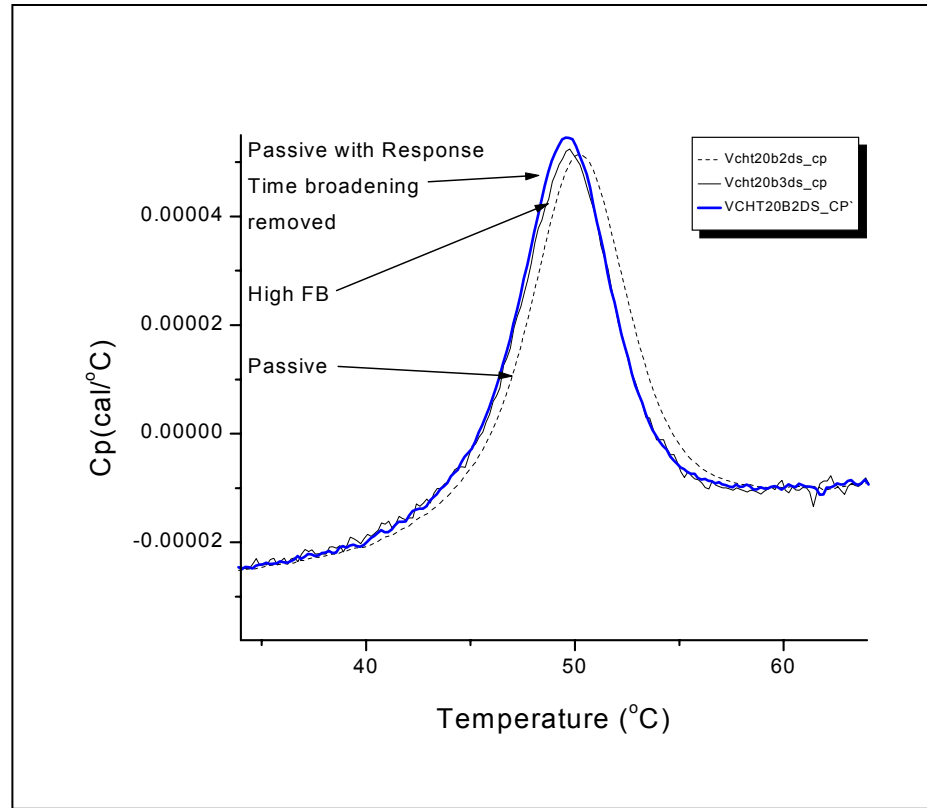


- Click on the *Response Time* button. The Response Time dialog box will open.

- Enter 0 for the FeedBack Mode (No Gain) and 40.9 for the Relaxation Response Time. The scanrate will be automatically entered. Please Note: Normally the FeedBack Mode and Relaxation Response Time will be read in from your experimental data file.



- Click **OK**. A new curve will be generated and plotted as a blue line (shown below as a wider line). This is the modified curve, for the passive mode, that removed the small amount of response-time broadening. The smaller peak of the repeated scan (using High-Gain FeedBack) is due to some non-reversibility of the sample.



## Line Types for Fit Curves

You may select a line type to plot your data or fit curves from the **Plot Details** dialog box. The Plot Details dialog box is available by double-clicking on the data plot, right-clicking on the data plot and selecting Plot Details from the shortcut menu or selecting the desired data plot from the Data menu data list and selecting Format:Plot.

### The Line Group

Select the desired line connection from the associated drop-down list. The line connection type affects interpolation results. The default line type for fit curves is Straight line. The most common methods of connecting the fit curve data points are straight, spline or B-spline:

**Straight:** A straight line is displayed between data points. This type of line connection will not give a smooth representation of the fit curve if there are few data points.

**Spline:** This option generates a cubic spline connection. To use the connection, the X values must be discrete and increasing. Furthermore, the number of data points cannot exceed 900 (if the data set exceeds this number, the operation will fail). Since curvature information is held in memory, the spline resolution remains the same regardless of page magnification. The SplineStep variable in the ORIGIN.INI file controls the spline calculation increment. It is expressed in units of .1 point. This is

usually the most satisfactory representation of the fit curve, but may exhibit an excursion from the actual fit curve if there is a sharp corner in the data.

**B-Spline:** The B-spline curve can be described by parametric equations. Unlike spline curves, which pass through the original data points, the B-spline curve winds around the original data points without passing through them. Thus this curve may not produce a satisfactory representation of the fit curve. For a complete discussion of the B-spline connection, see the Origin User's Manual.

## Inserting an Origin graph into Microsoft® Word

There are two ways to include your Origin graph into Word (or other applications), you may *import* your graph into Word or you may *share* your graph with Word. When you *import* your graph, Word will display the graph as an object and it cannot be edited by Origin tools (although it may be resized or reposition in the Word document). When you *share* your graph, Word displays the graph as an object which can be edited by Origin, or linked to Origin and updated when the Origin graph changes.

Please refer to the Origin manual for more information about *Creating a Graphic Presentation*.

### Importing your graph into Word

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- Create your graph in Origin and when you are satisfied with its appearance, select **Edit:Copy page**.
- Open your Word document and click at the location where you want the graph to be located.
- Select **Edit:Paste Special**.
- Select Origin Graph Object from the As list box.
- Select the **Paste** radio button.
- Click **OK**.

### Linking your graph into Word

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- You must first create your graph in Origin and then save it as part of an Origin project (\*.OPJ).
- Open the saved Origin project (if it is not already opened) that includes the desired graph window.
- Make the desired graph window active, select **Edit:Copy Page**.
- Open your Word document and click at the location where you want to insert the graph.



- Select **Edit:Paste Special**.
- Select Origin Graph Object from the **As:** list box
- Select the **Paste Link** radio button.
- Click **OK**.

After your Origin graph is linked to Word you may return to the original Origin graph and make changes to the graph. These changes can be reflected in the Word document by:

- Select **Edit:Update Client** from the Origin menu (to make immediate changes to the Word document graph).

*(Shortcut: You may quickly start Origin and load the linked graph by simply double-clicking on the graph while in Word. Origin will be started with the original document loaded, the changes can be made and by selecting **Edit:Update Client**, the changes will be transferred to the Word document.)*



## Appendix: Equations Used to Deconvolute DSC Data

### I. Independent Transitions

It will be assumed here that a protein (or other macromolecule) is composed of a number of structural domains A, B, C, ..., each of which is involved independently in a transition between the folded and unfolded forms ( $A = A'$ ,  $B = B'$ , ...). Equilibrium constants will be expressed as fractions ( $K_A = f_{A'}/f_A$ ,  $K_B = f_{B'}/f_B$ , ...) with the usual designation for the actual (i.e., calorimetric) molar enthalpy changes ( $\Delta H_A$ ,  $\Delta H_B$ , ...). The total molar enthalpy of the system H will be

$$H = H_N + f_{A'}\Delta H + f_{B'}\Delta H_B + \dots \quad (1)$$

The first term for the totally native form where all domains are in their folded state,  $H_N$ , must be included since all  $\Delta H$  values are measured relative to the folded form. The total molar heat capacity of the system  $C_p$  is the temperature derivative of the enthalpy in eq 1, so

$$C_p = C_{pN} + \left[ f_{A'}\Delta C_{pA} + \Delta H_A \left( \frac{\partial f_{A'}}{\partial T} \right) \right] + \dots \quad (2)$$

where  $C_{pN}$  is the molar heat capacity of the totally folded state,  $\Delta C_{pA}$  is the change in heat capacity for unfolding the A domain, and where the term in brackets will be repeated for each domain involved in unfolding. Remembering that  $f_A = 1 - f_{A'}$ , then the fractional concentration of the primed species can be readily expressed in terms of the corresponding equilibrium constant

$$f_{A'} = \frac{K_A}{1 + K_A} \quad (3)$$

which can then be differentiated to give

$$\left( \frac{\partial f_{A'}}{\partial T} \right) = \left( \frac{K_A}{(1 + K_A)^2} \right) \left( \frac{\partial \ln K_A}{\partial T} \right) \quad (4)$$

The derivative on the right of eq 4 is known from elementary thermodynamics

$$\left( \frac{\partial \ln K_A}{\partial T} \right) = \frac{\Delta H_A^*}{RT^2} \quad (5)$$

where  $\Delta H_A^*$  is the so-called van't Hoff heat change for the reaction which corresponds to the heat change for the cooperative unit which actually participates in the reaction. Substituting from eq's 3-5 into eq 2 then gives

$$C_p = C_{pN} + \left[ \frac{K_A \Delta C_{pA}}{1 + K_A} + \frac{K_A \Delta H_A^* \Delta H_A}{(1 + K_A)^2 RT^2} \right] + \dots \quad (6)$$

Eq 6 is perfectly general at this point and can be applied to either two-state or non-two-state transitions so long as all parameters are evaluated at the same temperature T.

#### Model for Independent Two-State Transitions including $\Delta C_p$ Effects.

If we assume each transition is two-state, then all of the above van't Hoff  $\Delta H^*$  values become equal to the calorimetric  $\Delta H$  values. If we further assume that  $C_{pN}$  can be expressed as a linear function of temperature ( $C_{pN} = B_0 + B_1 T$ ), then eq 6 becomes

$$C_p(T) = B_0 + B_1 T + \left[ \frac{K_A(T) \Delta C_{pA}}{1 + K_A(T)} + \frac{K_A(T) \Delta H_A(T)^2}{(1 + K_A(T))^2 RT^2} \right] + \dots \quad (7)$$

where the temperature-dependent parameters  $C_p(T)$ ,  $K_A(T)$ , and  $\Delta H_A(T)$  have been indicated. We can express  $\Delta H_A(T)$  in terms of its temperature-independent value at the midpoint  $T_{mA}$  and the heat capacity change for the transition  $\Delta C_{pA}$ , i.e.,

$$\Delta H_A(T) = \Delta H_{mA} + \Delta C_{pA} (T - T_{mA}) \quad (8)$$

and then integrate eq 5 from  $T_{mA}$  where  $K_A(T)$  is unity to an unspecified temperature T

$$K_A(T) = \exp \left\{ \frac{-\Delta H_{mA}}{RT} \left( 1 - \frac{T}{T_{mA}} \right) - \frac{\Delta C_{pA}}{RT} \left( T - T_{mA} - T \ln \frac{T}{T_{mA}} \right) \right\} \quad (9)$$

Now, eq 7 (using substitutions from eq's 8 and 9 for each transition A, B, ...) may be used to calculate the value of the system heat capacity  $C_p(T)$  at any temperature T once values of the temperature-independent fitting parameters  $B_0$ ,  $B_1$ ,  $T_{mA}$ ,  $\Delta H_{mA}$ ,  $\Delta C_{pA}$ ,  $T_{mB}$ ,  $\Delta H_{mB}$ ,  $\Delta C_{pB}$ , ... are given. To begin curve-fitting with Origin, the operator indicates the number of transitions needed to fit the experimental DSC heat capacity curve,  $C_p(T)_{\text{exp}}$ , and then initializes all of the  $T_m$  values so that Origin can provide guesses for each of the other fitting parameters. Knowing these, it then calculates  $C_p(T)$  from eq's 7-9 and compares these values with  $C_p(T)_{\text{exp}}$ . Using Marquardt methods based on non-linear-least-squares, the guesses for each parameter are then improved, the calculations carried out again, and this iterative process continued until there is no further improvement in the fit of the calculated  $C_p(T)$  to the experimental  $C_p(T)_{\text{exp}}$  as indicated by a minimum value of  $\chi^2$ .

#### Model for Independent Two-State Transitions excluding $\Delta C_p$ Effects.

When all heat capacity changes  $\Delta C_{pA}$ ,  $\Delta C_{pB}$ , ... are assumed to be zero, certain simplifications can be made in eq's 7-9. Before curve-fitting begins, the operator subtracts a baseline from the data which effectively sets  $C_{pN}$  equal to zero at all

temperatures so that B0 and B1 are no longer used as fitting parameters. Incorporating this, setting all heat capacity changes to zero, and recognizing that all  $\Delta H_A(T)$  for each transition may be replaced by the temperature-independent heat  $\Delta H_{mA}$  (i.e., heat changes must be temperature-independent if there is no change in heat capacity), then eq's 7-9 simplify to

$$C_p(T) = \frac{K_A(T)\Delta H_A(T)^2}{(1+K_A(T))^2 RT^2} + \dots \quad (10)$$

$$K_A(T) = \exp\left\{\frac{-\Delta H_{mA}}{RT} \left(1 - \frac{T}{T_{mA}}\right)\right\} \quad (11)$$

where again the right-hand term in eq 10 must be repeated for each transition and where eq 11 must be evaluated for each transition. In this case the fitting parameters are  $T_{mA}$ ,  $\Delta H_{mA}$ ,  $T_{mB}$ ,  $\Delta H_{mB}$ , ..., but otherwise the curve-fitting procedure is the same as described above.

### Model for Independent Non-two-state Transitions.

Because of the extra fitting parameters required for the non-two-state vs two-state model, this model is only applied to transitions with no  $\Delta C_p$ . Before curve-fitting with this model, a progress baseline must be subtracted from the experimental data to remove the  $\Delta C_p$  effects if they are present (this also sets  $C_{pN}$  to zero at all temperatures). To treat non-two-state transitions, the appropriate place to begin is eq 6 which still includes both calorimetric and van't Hoff heat changes. Indicating the temperature-dependent parameters, this can be rewritten as

$$Cp(T) = \frac{K_A(T)\Delta H_{mA}^* \Delta H_{mA}}{(1+K_A(T))^2 RT^2} + \dots \quad (12)$$

In this case, the equilibrium constants will be calculated as in eq 11 with the important exception that the van't Hoff heat, rather than the calorimetric, must be used since now the two will be different, i.e.,

$$K_A(T) = \exp\left\{\frac{-\Delta H_{mA}^*}{RT} \left(1 - \frac{T}{T_{mA}}\right)\right\} \quad (13)$$

Eq's 12-13 are then used for curve-fitting in the usual way and the parameter set will be  $T_{mA}$ ,  $\Delta H_{mA}$ ,  $\Delta H_{mA}^*$ ,  $T_{mB}$ ,  $\Delta H_{mB}$ ,  $\Delta H_{mB}^*$  ...

## II. Sequential Transitions

Sequential transitions may occur in proteins (or other macromolecules) when the stability of each domain A, B, C, ... is dependent on whether other domains are folded or unfolded. The sequential model imposes a precise order in which domains must unfold for every molecule in solution, i.e.,

$$P_0 \underset{\Delta H_1}{=} P_1 \underset{\Delta H_2}{=} P_2 \underset{\Delta H_3}{=} P_3 \underset{\Delta H_4}{=} P_4 = \dots \quad (14)$$

where each step in the reaction scheme involves the unfolding of one of the domains in the appropriate sequence (i.e., step 1 is always the A domain, step 2 always the B domain, etc.). The total molar heat content of the system (relative to a value of zero being assigned to the  $P_0$  state) will be

$$H = f_0 \cdot 0 + f_1 \Delta H_1 + f_2 (\Delta H_1 + \Delta H_2) + f_3 (\Delta H_1 + \Delta H_2 + \Delta H_3) + \dots \quad (15)$$

where the  $f$ 's indicate the fractional concentration of each state and the  $\Delta H$ 's are the molar enthalpy changes for each step. Using the  $K$  values, all of the  $f$  values can be related to  $f_0$ , so that

$$\begin{aligned} 1 &= f_0 + f_1 + f_2 + f_3 + \dots \\ 1 &= f_0 (1 + K_1 + K_1 K_2 + K_1 K_2 K_3 + \dots) \\ f_0 &= \frac{1}{Q}; f_1 = \frac{K_1}{Q}; f_2 = \frac{K_1 K_2}{Q}; \dots \end{aligned} \quad (16)$$

where

$$Q = 1 + K_1 + K_1 K_2 + K_1 K_2 K_3 + \dots \quad (17)$$

Eq's 16 may be substituted into eq 15, which may then be differentiated with respect to temperature to obtain the system heat capacity. Since  $\Delta H$  values are treated as temperature-independent in Origin's sequential model, most of the derivatives involve using the relation

$$\left( \frac{\delta(K_1 K_2 K_3 \dots)}{\delta T} \right) = (K_1 K_2 K_3 \dots) \left( \frac{\Delta H_1 + \Delta H_2 + \Delta H_3 + \dots}{RT^2} \right) \quad (18)$$

The final expression for the heat capacity of the system then becomes

$$\begin{aligned} C_p(T) &= \left( K_1(T) \frac{\Delta H_1^2}{RT^2 Q} + K_1(T) K_2(T) \frac{(\Delta H_1 + \Delta H_2)^2}{RT^2 Q} + \dots \right) - (K_1(T) \Delta H_1 + K_1(T) K_2(T) (\Delta H_1 + \Delta H_2) + \dots) \\ &\quad \left( K_1(T) \frac{\Delta H_1}{RT^2 Q^2} + K_1(T) K_2(T) \frac{(\Delta H_1 + \Delta H_2)}{RT^2 Q^2} + \dots \right) \end{aligned} \quad (19)$$

where the temperature-dependent quantities have been indicated in the usual way. The equilibrium constants can be evaluated in terms of the temperature-independent  $\Delta H$  parameters from

$$K_1(T) = \exp\left(\frac{-\Delta H_1}{R} \left[ \frac{1}{T} - \frac{1}{T_{m1}} \right]\right)$$

$$K_2(T) = \exp\left(\frac{-\Delta H_2}{R} \left[ \frac{1}{T} - \frac{1}{T_{m2}} \right]\right) \quad (20)$$

Using eq's 19-20 and fitting parameters  $K_1$ ,  $\Delta H_1$ ,  $K_2$ ,  $\Delta H_2$ , ..., the fitting procedure is carried out as described above for the models involving independent transitions.

### III. Single Two-State Transition with Subunit Dissociation

It sometimes happens with biological macromolecules having subunits that subunit dissociation occurs simultaneously with thermal unfolding, i.e.,

$$A_n = nA \quad K = \frac{[A]^n}{[A_n]} \quad (21)$$

where  $n$  is the number of dissociable subunits and where the brackets signify molar concentrations of species. If  $f$  is the fraction of macromolecule in the  $A$  state and  $1-f$  the fraction in the  $A_n$  state, then

$$1-f = \frac{[A_n]}{C_t} \quad f = \frac{[A]}{nC_t} \quad (22)$$

where  $C_t$  is the total bulk molar concentration (expressed as  $n$ -mer equivalents). The equilibrium constant, using eq's 21-22, then becomes

$$K = \frac{f^n}{1-f} n^n C_t^{n-1} \quad (23)$$

Using eq 23 and the expressions

$$\frac{\delta \ln K(T)}{\delta \frac{1}{T}} = \frac{-\Delta H_A(T)}{R}$$

$$\Delta H(T) = \Delta H_{mA} + \Delta C_{pA}(T - T_{mA}) \quad (24)$$

and integrating from  $T_{mA}$  (where  $f=0.5$ ) to  $T$  then gives

$$K(T) = 0.5^{n-1} n^n C_t^{n-1} \exp\left\{ \frac{-\Delta H_{mA}}{RT} \left(1 - \frac{T}{T_{mA}}\right) - \frac{\Delta C_{pA}}{RT} \left(T - T_{mA} - T \ln \frac{T}{T_{mA}}\right) \right\} \quad (25)$$

Once  $K(T)$  is known then  $f(T)$  may be solved from eq 23 using numerical methods and the excess heat capacity calculated from the equation

$$C_p(T) = B_0 + B_1 T + f(T)\Delta C_p + \frac{\Delta H_A(T)}{RT^2} \left( \frac{1-f(T)}{1-n + \frac{n}{f(T)}} \right) \quad (26)$$

The above equations are general for any value of  $n$  so long as the bulk concentration  $C_t$  is expressed as  $n$ -mer equivalents. For systems which *associate* when unfolding occurs i.e.,

$$A = \frac{1}{j} A_j \quad (27)$$

the same equations are valid, providing the bulk concentration  $C_t$  is expressed as monomer equivalents and that  $n$  in the above equations is equated to  $\frac{1}{j}$ .

#### IV. Absolute Heat Capacity of Proteins

The equation used to calculate Absolute Heat Capacity of Proteins is as follows:

$$\Delta C_p = g_o \rho(t) V_o (1 + .00002t) [C_p^P(t) - \nu(1 + \alpha t) C_p^W(t)] \quad (28)$$

Where  $\Delta C_p$  (cal/deg) is the sample-buffer baseline minus the buffer-buffer baseline,  $g_o$  is the concentration of protein (gm/ml) in the solution,  $\rho(t)$  is the relative density of water (stored in Origin as a polynomial in  $t$ ),  $V_o$  is the nominal volume (ml) of the sample cell,  $t$  is the temperature in °C,  $C_p^P(t)$  is the absolute heat capacity (cal/deg/gm) of the protein in solution,  $\nu$  is the partial specific volume of the protein (ml/gm),  $\alpha$  is the coefficient of thermal expansion of the protein, and  $C_p^W(t)$  is the unit-volume heat capacity of water (cal/deg/ml).  $C_p^W(t)$  is calculated from literature data on the specific heat and density of water as a function of temperature, by fitting to a polynomial in temperature. The thermal coefficient of cubical expansion of tantalum is .00002.

The above equation is solved for  $C_p^P(t)$  from the experimental data file  $\Delta C_p$ , using the stored polynomial expressions for  $C_p^W(t)$  and  $\rho(t)$  as well as the operator-input parameters  $g_o$ ,  $\nu$ ,  $\alpha$ , and  $V_o$ .

This procedure assumes the relative density and the unit-volume heat capacity  $C_p^W$  of the buffer solution can be replaced by the corresponding properties of pure water. This should be a very good approximation at electrolyte concentrations commonly used in buffer solutions, but would be less accurate at very high electrolyte concentrations (>1M) or in the presence of high concentrations of organic additives. Also, the value obtained for  $C_p^P$  depends strongly on the partial specific volume of protein, so it is desirable to have an experimental value of  $\nu$  for the protein in question at a single temperature, at the least. The coefficient of thermal expansion is typically small ( $\sim 10^{-3} - 10^{-4}$ ) and exerts only a small effect on the  $C_p^P$  value over a moderate temperature range.



## V. Calculating Protein-Ligand Binding Constant from DSC Data

Ligands frequently bind much stronger to the native, folded form of a protein than they do to the thermally unfolded form. The treatment below assumes no binding to the unfolded form. This being the case, the thermal midpoint of the unfolding transition will necessarily occur at a higher temperature,  $T_M$ , when the ligand is present in solution than when the ligand is absent,  $T_0$ . For reversible transitions, the equation relating the shift in midpoint to the binding constant  $K_B$  has been derived earlier (Brandts, J. F. & Lin, L. -N. (1990) *Biochemistry* **29**, 6927-6940.) and is

$$K_B(T_M) = \frac{\exp\left\{\frac{-\Delta H^0}{nR}\left(\frac{1}{T_M} - \frac{1}{T_0}\right) + \frac{\Delta C_P}{nR}\left(\ln\frac{T_M}{T_0} + \frac{T_0}{T_M} - 1\right)\right\} - 1}{[L]_{T_M}} \quad (29)$$

where  $K_B(T_M)$  is the binding constant at  $T_M$ ,  $\Delta H^0$  and  $\Delta C_P$  are the enthalpy and heat capacity change for the transition at  $T_0$ ,  $[L]_{T_M}$  is the concentration of *unbound* ligand at  $T_M$ , and  $n$  is the number of binding sites on the protein. Transition temperatures are expressed in °K. The above equation is most useful when  $T_M$  and  $T_0$  are well-separated in temperature. A very large  $K_B$  insures there will be large separation in midpoint no matter what the ligand concentration, while measuring  $T_M$  in the presence of high ligand concentration will produce large separation even for weak or moderate binding. In the former case involving large binding constants, addition of less-than-saturating ligand concentration results in separate transition peaks for the liganded and unliganded proteins so that  $T_0$  and  $T_M$  may be obtained in the same experiment.

The value of free ligand concentration at the midpoint  $T_M$  can be calculated from the equations

$$\begin{aligned} [L]_{T_m} &= L_{tot} - \frac{nP_{tot}}{2} && (L_{tot} \geq nP_{tot}) \\ [L]_{T_m} &= \frac{nP_{tot}}{2} && (L_{tot} \leq nP_{tot}) \end{aligned} \quad (30)$$

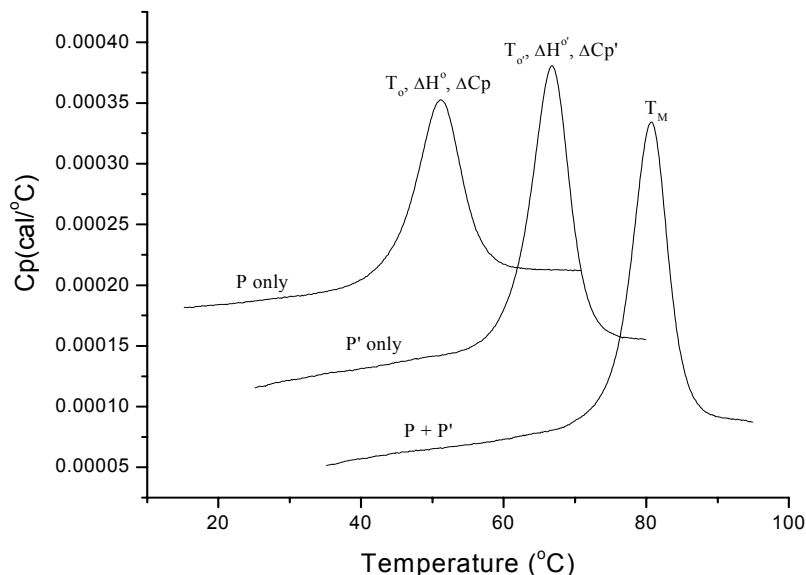
where  $P_{tot}$  and  $L_{tot}$  are the total concentrations of protein and ligand, respectively, in the solution. While ligand release will occur at  $T_M$  and contribute to the total experimental heat of unfolding, it is generally not possible to obtain a reliable estimate of this since the heat from ligand release usually constitutes such a small fraction of the total heat of unfolding.

However, if one knows the heat of binding independently (e.g., from ITC experiments) it then becomes possible to extrapolate the  $K_B$  value, obtained from equations (29) and (30) at  $T_M$ , to another temperature  $T_F$  using the equation (Brandts, J. F. & Lin, L. -N. (1990) *Biochemistry* **29**, 6927-6940)

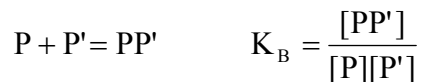
$$K_B(T_F) = K_B(T_M) \exp\left[\frac{-\Delta H_B}{R}\left(\frac{1}{T_F} - \frac{1}{T_M}\right) + \frac{\Delta C_P}{R}\left(\ln\frac{T_F}{T_M} + 1 - \frac{T_F}{T_M}\right)\right] \quad (31)$$

where  $\Delta H_B$  and  $\Delta C_P$  are the heat and heat capacity of ligand binding, respectively, at  $T_F$ .

## VI. Calculating Protein-Protein Binding Constant from DSC Data



When estimating the binding constant for protein-protein interactions,



the approach is a little different than for ligand-protein interactions, since both of the proteins P and P' will undergo their own thermal unfolding transition. This is illustrated in the above Figure where  $T_o$ ,  $\Delta H^o$ , and  $\Delta C_p$  refer to transition parameters for P alone and  $T_o'$ ,  $\Delta H^{o'}$ , and  $\Delta C_{p'}$  are the corresponding parameters for P' alone.

When P and P' are mixed together in the same solution, then a separate transition will be observed for the complex PP' with transition midpoint  $T_M$ . A second transition might also be seen at either  $T_o$  or  $T_o'$  depending on whether P or P' is in excess (i.e. in autosampler routine the program will have to determine the largest peak). If  $T_M$  occurs at a temperature between  $T_o$  and  $T_o'$  then the regular model for ligand-protein may be used to calculate the binding constant.

If  $T_M$  occurs at a temperature higher than both  $T_o$  and  $T_o'$  then a *different model* must be used which takes into account the temperature shift in both transitions. In this latter case, the binding constant at  $T_M$ ,  $K_B(T_M)$ , can be obtained from the following equation

$$K_B(T_M) = \frac{\exp\left(\frac{-\Delta H^o}{R} \left[\frac{1}{T_M} - \frac{1}{T_o}\right] + \frac{\Delta C_p}{R} \left[\ln \frac{T_M}{T_o} + \frac{T_o}{T_M} - 1\right] - \frac{\Delta H^{o'}}{R} \left[\frac{1}{T_M} - \frac{1}{T_o'}\right] + \frac{\Delta C_{p'}}{R} \left[\ln \frac{T_M}{T_o'} + \frac{T_o'}{T_M} - 1\right]\right)}{K_{eq}} \quad (32)$$

where

$$K_{eq} = P_{tot} - \frac{P'_{tot}}{2} \quad \text{when } P_{tot} \geq P'_{tot}$$

$$K_{eq} = P'_{tot} - \frac{P_{tot}}{2} \quad \text{when } P_{tot} \leq P'_{tot}$$
(33)

## VII. Using DSC data to calculate $\Delta G$ at temperature $T$ by extrapolating from the midpoint $T_M$

From analysis of a DSC scan on a protein, three experimental parameters for the unfolding transition are obtained. These include

$$\begin{aligned} \text{midpoint} &= T_M \\ \text{Enthalpy change at } T_M &= \Delta H(T_M) \\ \text{Heat capacity change at } T_M &= \Delta C_p \end{aligned}$$

Using these three experimental parameters, the Gibbs free energy change  $\Delta G$  may then be obtained at any temperature  $T$  as indicated below. The derivation assumes a reversible two-state transition and also assumes  $\Delta C_p$  is independent of temperature.

Derivation:

Starting from well-known thermodynamic relation

$$\left( \frac{\partial \frac{\Delta G}{T}}{\partial \frac{1}{T}} \right)_P = \Delta H$$

and transforming to an integral equation gives

$$\int_{T_M}^T d\left(\frac{\Delta G}{T}\right) = \int_{T_M}^T \Delta H d\left(\frac{1}{T}\right) = \int_{T_M}^T [\Delta H(T_M) + \Delta C_p(T - T_M)] d\left(\frac{1}{T}\right)$$

Integrating the above equation from  $T_M$  (where  $\Delta G=0$ ) to another temperature  $T$  then gives

$$\frac{\Delta G}{T} = \Delta H(T_M) \left( \frac{1}{T} - \frac{1}{T_M} \right) + \Delta C_p \left( \ln \frac{T_M}{T} - \frac{T_M}{T} + 1 \right)$$

Once the temperature  $T$  is assigned, all other parameters in the above equation are known except  $\Delta G$  which can then be calculated.



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