Measuring Temperature Transitions Using the DynaPro NanoStar

Summary

Dynamic and static light scattering are powerful techniques for studying macromolecules or colloids which undergo a thermal transition. If a molecule changes conformation or aggregates with temperature then a change in its size and molar mass can be determined from dynamic and static light scattering measurements using the DynaPro NanoStar. The DynaPro NanoStar has a dynamic light scattering detector as well as an independent static light scattering detector, both of which measure at an angle of 90°. This technical note describes how to perform a temperature dependence analysis in DYNAMICS software version 7.1.8 or higher.

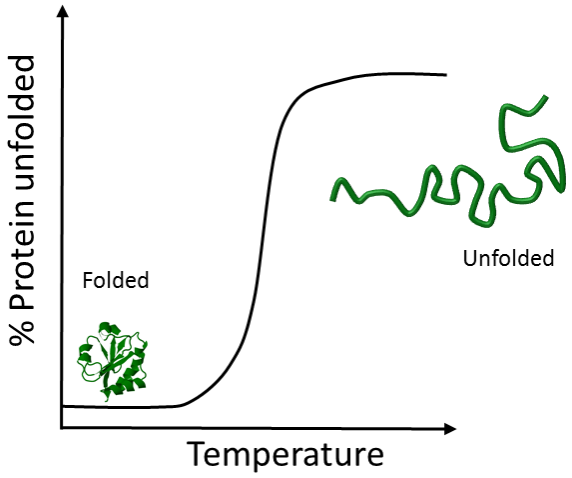


Figure 1: Schematic of protein unfolding with temperature. (The structure of Thioredoxin is used as an example here of a folded structure, PDB: 1XOA)

Related Technical Notes and References

M1400	DYNAMICS User's Guide
M3300	DynaPro NanoStar User's Guide
TN7500	DynaPro NanoStar Quick Start Guide
TN9000	Quartz Cuvette Cleaning Protocol

Contents

Background

Macromolecules from different classes exhibit thermal transitions, such as the unfolding of proteins, phase separation of temperature-responsive polymers, or micellization of block copolymers. If these macromolecules or colloids change conformation or aggregate with temperature, then a change in their size and molar mass can be measured using dynamic and static light scattering (DLS/SLS). Most proteins unfold as temperature is increased, resulting in the unraveling of their compact "globular" structure and the increase of the measured hydrodynamic radius (Figure 1). The molar mass does not change if the protein does not aggregate upon unfolding. However, proteins that do aggregate increase in molar mass as well as increase in hydrodynamic radius. Additionally, polymers such as poly(N-isopropylacrylamide) aggregate when heated, resulting in an increase in the measured molar mass and hydrodynamic radius.

An increase in size can be monitored with DLS. The hydrodynamic radius, R_h, is determined from the translational diffusion coefficient (D_t) of a macromolecule in solution. D_t can be determined from DLS measurements performed using the DynaPro NanoStar. DLS signals are based on the fluctuations in the measured intensities of the light. A smaller macromolecule diffuses faster and will result in more rapid fluctuations compared to larger macromolecules.

The Stokes-Einstein equation relates the hydrodynamic radius and the translational diffusion coefficient as follows:

$$R_h = \frac{k_B T}{6\pi\eta D_t}$$

where k_B is the Boltzmann constant, T is temperature, and η is viscosity.

An increase in molar mass can be monitored with SLS. The DynaPro NanoStar is capable of performing SLS experiments at a 90° angle, from which the molar mass (M) of a macromolecule can be determined. For dilute solutions with negligible second virial coefficient, the Zimm equation relates the light scattering intensity to the molar mass as follows:

$$\frac{K^* c}{R(\theta)} = \frac{1}{M}$$

where K^* is an optical constant calculated by the DYNAMICS software, c is the mass concentration, and $R(\theta)$ is the Rayleigh ratio (i.e., the normalized SLS intensity).

Dynamic light scattering and static light scattering measurements as a function of temperature often are not sufficient to allow knowledge of protein melting temperature (temperature at which half of the protein molecules are unfolded). Proteins or macromolecules that aggregate upon unfolding do not reach an equilibrium state and therefore prevent the determination of a midpoint. However, such measurements may robustly estimate an onset temperature for the combined unfolding/aggregation event, T_{onset}.

The focus of this note is on the thermal ramping capability of the DynaPro NanoStar and how to utilize this functionality to determine the thermal behavior of a macromolecule from plots of R_h and molar mass versus temperature. The thermal ramping method described here collects acquisitions while the sample is being continuously heated, i.e., the temperature will vary while measurements are being collected. Due to the typically short measurement times of less than 30 seconds, temperature variations within a single measurement can be neglected.

Data presented here as an example are from bovine serum albumin (BSA) monomer in phosphate buffered saline (~ 66 ˚C melting temperature) and lysozyme in pH 4 acetate buffer (~ 75 ˚C melting temperature).

Tools and Supplies needed

- Clean Quartz cuvette

  A disposable cuvette may be used if only DLS measurements are collected and the measured temperature range is below 80 ˚C.
- 1 % to 2 % solution of Tergazyme, Hellmanex, or Liquinox for cleaning cuvettes
- 100% ethanol
- Nanopure water
- Filtered nitrogen line for cuvette drying
- Syringe tip filters with a pore size of 0.02 µm (such as Whatman Anotop) for filtering solvent

- Syringe tip filters with appropriate pore size for filtering sample (e.g., 0.02 µm for most globular proteins)
- Syringe (1 mL is suitable)
- Clean microcentrifuge tubes
- Pipettor and gel-loading pipette tips
- Toluene (for cuvette calibration)
- Solvent or buffer (0.02 µm filtered) that will be used for the measurements
- Sample at a suitable concentration, typically 0.2-5 mg/mL
- Paraffin or silicone oil to prevent evaporation of the sample (optional)

Experimental Procedure

Calibrate Cuvette and Measure Solvent Offset

Note: If you are only collecting DLS data or using a disposable cuvette, proceed to the Sample Preparation section. If using a quartz cuvette, clean the cuvette thoroughly with Hellmanex or other cleaning agents as described in the *Quartz Cuvette Cleaning Protocol* Technical Note (TN9000).

1) Dispense filtered toluene into the clean, dry quartz cuvette using a gel-loading pipette tip or syringe with 0.02 µm filter and needle. Use at least the minimum sample amount required for your cuvette (1.25 µL for the 1 µL cuvette, 50 µL for the 45 µL cuvette).

Note: Always filter solvents through 0.02 µm pore size filters. Dispense the first few drops from the filter directly to waste before filtering a sample or solvent into a clean container, such as a microcentrifuge tube. More information about calibrating cuvettes and solvent offsets can be found in Chapter 4 of the *DYNAMICS User's Guide* in the "Managing and Calibrating Cuvettes" section.

2) Calibrate the cuvette at 25 ˚C as described in the *DYNAMICS User's Guide*.

3) Rinse the toluene out of the cuvette with ethanol or isopropanol. Then, wash the cuvette with detergent (e.g., Tergazyme or Liquinox) as described in the cuvette cleaning technical note (TN9000).

Note: Do not use 70 % alcohol as it is not miscible with toluene.

4) Dispense filtered solvent into the clean, dry cuvette.

5) Measure the solvent offset at four temperatures covering the temperature range scanned in this experiment as described in the *DYNAMICS User's Guide*:

   a. Measure the solvent offset at the **lowest temperature** in the temperature range measured.
   b. Measure the solvent offset at **two additional temperatures** within the temperature range measured.
   c. Measure the solvent offset at the **highest temperature** in the temperature range measured.

Sample Preparation

1) Prepare a sample at the appropriate concentration, typically 0.2 mg/mL to 5 mg/mL for proteins.

2) Filter this sample through the smallest pore size possible using a syringe tip filter. A 0.02 µm filter is suitable for many proteins.

3) Load the sample into a clean, dry cuvette using a gel-loading pipette tip.

Note: To prevent evaporation, either dispense a drop of paraffin or silicone oil on the sample or overfill the cuvette such that sample evaporation is negligible.

Measuring Temperature Transitions

1) Open DYNAMICS, open a new experiment and connect to your instrument. Alternatively, an experiment can be generated from a Preset, such as *NanoStar 2 – Thermal Scan 1 C Delta.pst* by selecting **File → Open Preset.**

2) Go to **Parameters → Instrument**, and make sure the space next to "*DLS only*" is set to "**False**" to measure the signal from DLS and SLS simultaneously (Figure 2).

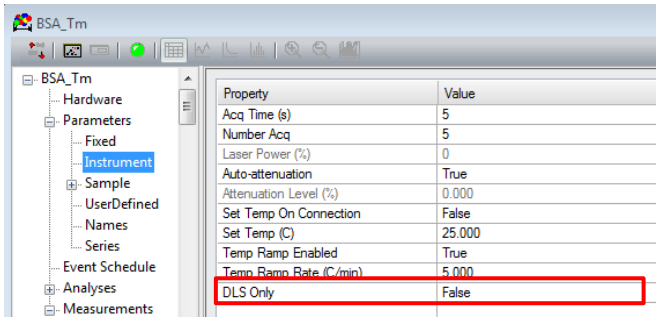


Figure 2: In order to determine molar mass, the static light scattering detector must be enabled. By default, SLS is enabled, but it is good practice to check in the instrument parameters to make sure that "False" is selected under "DLS Only".

3) Set up a thermal ramping experiment in the **Event Schedule** similar to that shown in Figure 3. If using a preset, modify the Event Schedule to resemble that shown in Figure 4. Use the Event Schedule to set the **desired starting temperature** (˚C), **temperature ramp rate** (˚C/min), **end temperature** (˚C), **the acquisition time** (s), and **number of acquisitions per measurement**. Generally, an acquisition time of 5 s and 5 acquisitions per measurement are sufficient. More information can be found in Chapter 5 of the *DYNAMICS User's Guide*.

Note: Make sure to use the "**Set temperature (C), don't wait**" step as that will allow DYNAMICS to move on to the next step in the Event Schedule without waiting for temperature equilibration at the set temperature. Also, it is recommended to add another "**Set temperature (C), don't wait**" step at the end of the **Event Schedule** to return the instrument to room temperature.

4) Click on the field next to "**Save data as**" and name your data file.

5) Go to **Tools → Calculations → Ramp Rate** to determine the run time, the temperature interval between measurements and the number of loops DYNAMICS will perform during the experiment. (Figure 4). Enter 1 for **Total Number of Wells**.

	Command	Value
	Auto-attenuation enable	
	Temperature ramping enable	
	Set acquisition time (secs)	5
	Set temperature(C)	25
	Set temperature ramp rate(C/min)	1
	Set temperature(C), don't wait	85.5
	Do until Temp(C) >	85
	Collect acquisitions	5
	Label meas as current sample temperature	
	Save data as	BSATm.exp
	Loop	
	Set temperature(C), don't wait	25
	Auto-attenuation disable	
	Set laser power (%)	0

Figure 3: Setting up an Event Schedule to perform temperature ramping in DYNAMICS. The Event Schedule contains a cool down step at the end to return the instrument to room temperature.

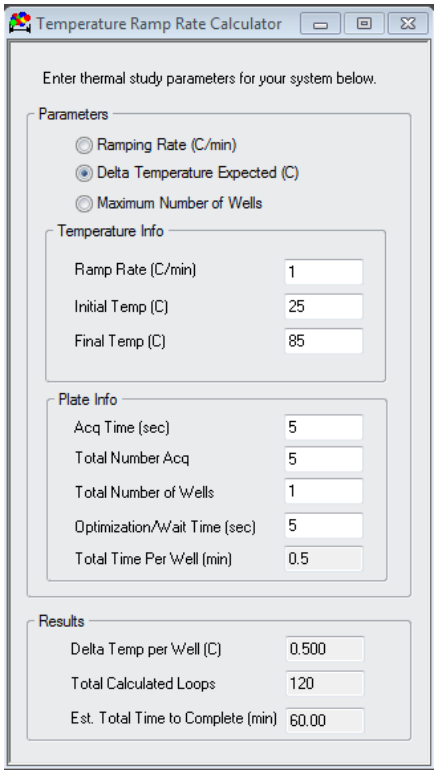


Figure 4: Temperature Ramp Rate Calculator helps determine run time, delta temperature and number of loops as well as wait time if desired.

Note: A "**Wait (min)**" step can be inserted after each measurement in the Event Schedule to increase the temperature interval at which data is collected. This is especially helpful when slow ramp rates are used, as it reduces the number of data points. The wait time and its effect on the temperature interval can be determined in the temperature ramp rate calculator (Figure 4).

6) Place the cuvette into the NanoStar sample chamber.

7) Examine the autocorrelation curve on the front panel to verify that the sample and cuvette are both particle-free before starting your Event Schedule. If necessary, clean the cuvette and reload the sample before making the measurement. For more details on interpreting autocorrelation data, please refer to the chapter on "Data Interpretation" in the *DYNAMICS User's Guide*.

8) Click the green button in the DYNAMICS software to start collecting data.

9) Observe the autocorrelation curve in DYNAMICS by clicking on the **Correlation Graph** button, shown in Figure 5.

Note: It is critical to clean the cuvette properly after each experiment to minimize error, as DLS is highly sensitive to small amounts of large particles. Between experiments, rinse the inside and outside of the Quartz cuvette thoroughly with detergent (e.g., Tergazyme or Liquinox), rinse with DI water, and finish with an ethanol rinse. Carefully dry the cuvette with a stream of filtered air or nitrogen. Please refer to our cuvette cleaning technical note for further information (TN9000).

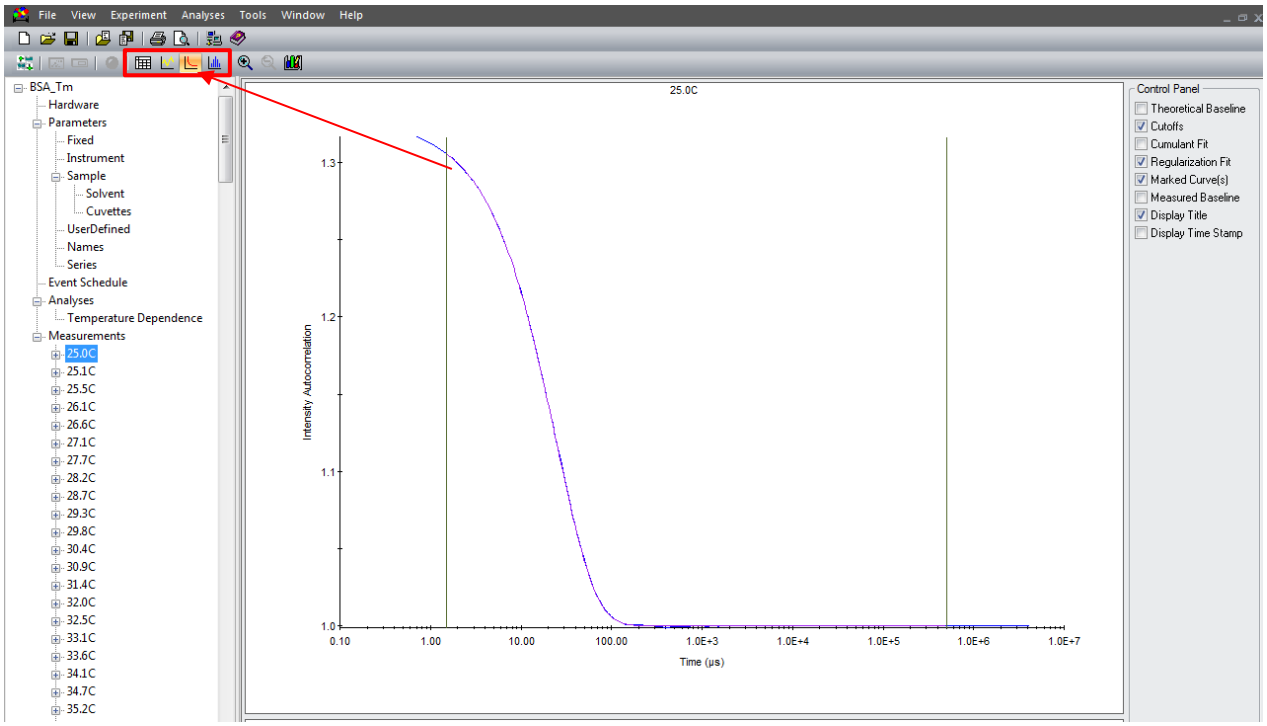


Figure 5: As you perform multiple measurements, verify that your samples are particle contaminant-free by checking the autocorrelation curves. The curves shown above are of filtered BSA monomer and display a monomodal decay, indicative of good sample preparation.

Data Analysis

1) After all the data has been collected, review the results in the **Datalog Grid** and **Correlation Graph**. If necessary, use the **Data Filter** by clicking on the **Datalog Grid** and right clicking anywhere on the graph and selecting **Data Filter** (Figure 6).

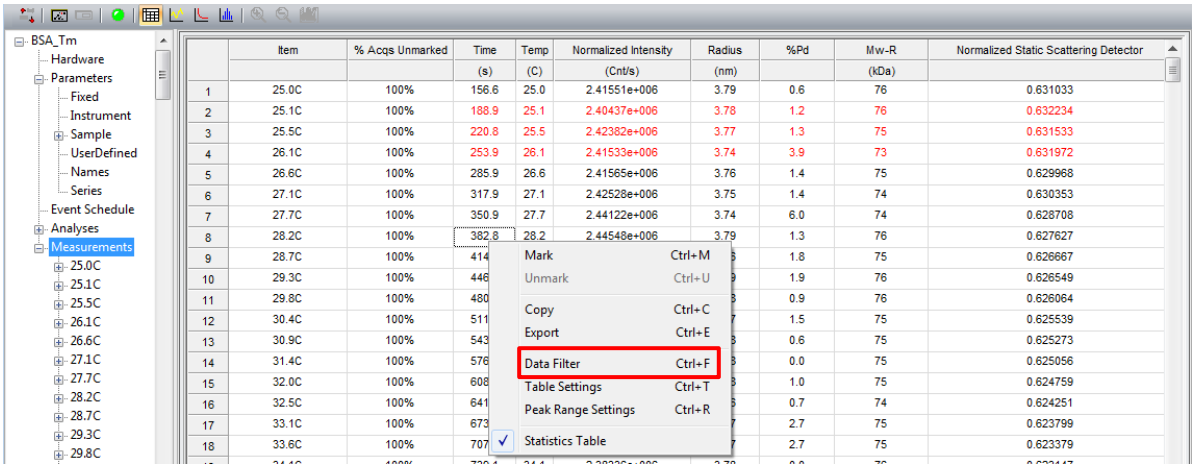


Figure 6: DYNAMICS gives the user the option to view either filtered or unfiltered data depending on the settings of the Data Filter. The Data Filter menu can be accessed by right clicking on the Datalog Grid.

2) Deselect the filter parameters (Figure 7). You can then view any measurement that was filtered out. It is recommended to use the **Minimum Amplitude**, **Maximum Amplitude** and **Baseline Limit** criteria only to filter out measurements using the default values of 0, 1, and 0.01, respectively. Filtering using SOS criteria may filter measurements that are multimodal as the *Cumulants* fit will fail to provide a good fit of the autocorrelation function.

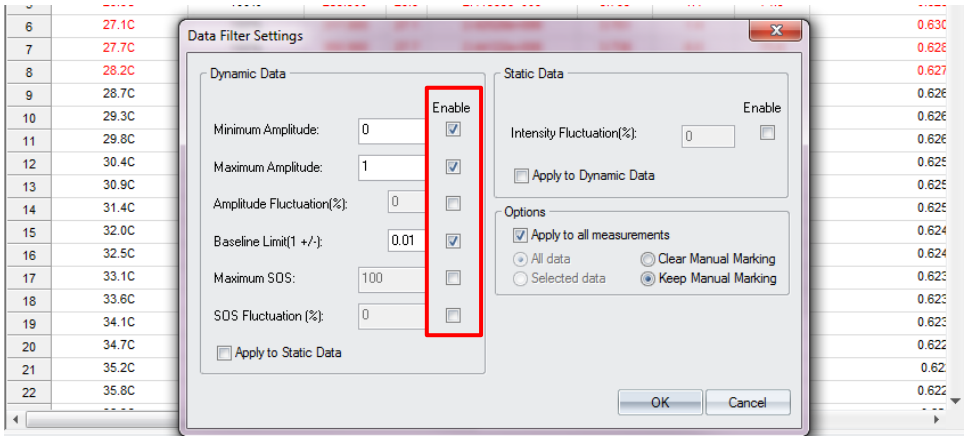


Figure 7: You can de-select the checked boxes to view any data that may have been filtered out.

3) If no **Data Filter Settings** are enabled, manually mark any measurements that display particle contamination. Additionally, mark measurements that you would like to exclude from the analysis.

4) If multiple samples were measured and saved in the same DYNAMICS file, different sample names can be assigned from the **Sample** menu. Click **Add** to name the sample and then click **Assign** to choose the measurements corresponding to that sample (Figure 8). Remember to select the cuvette for which the offsets were measured and the concentration of the sample for SLS analysis.

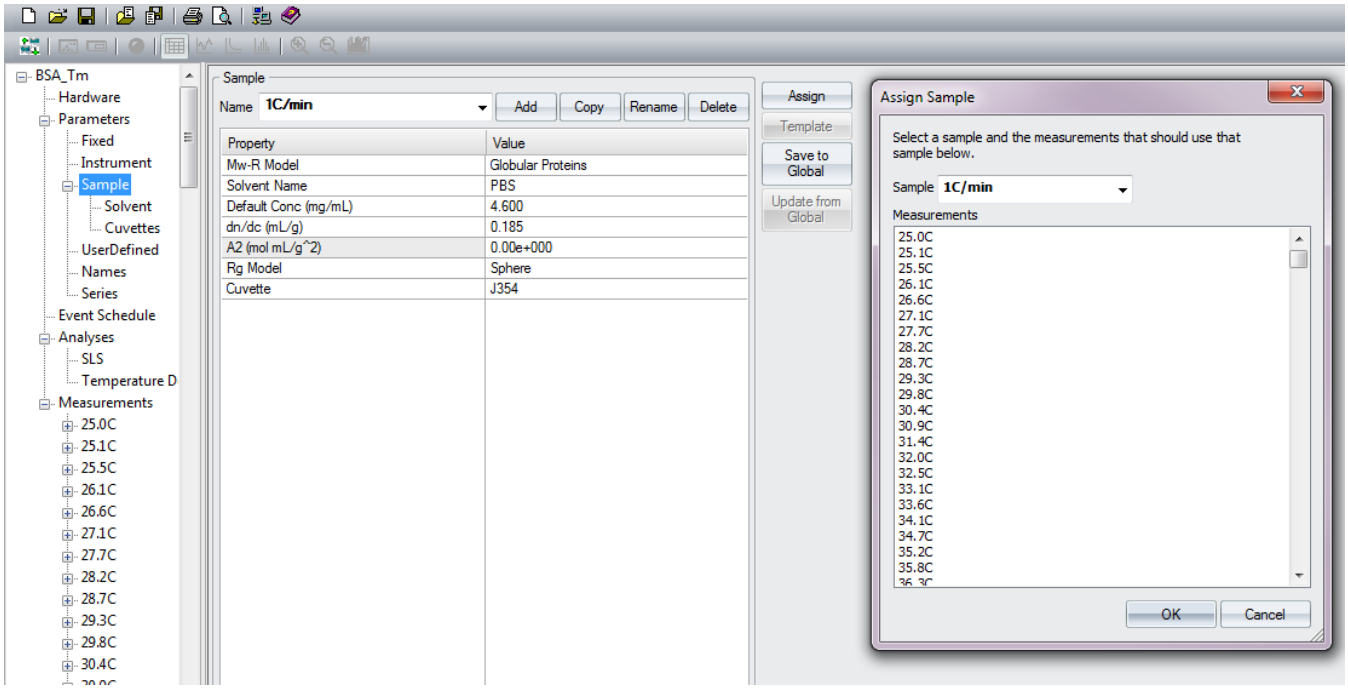


Figure 8: Assigning Sample names to measurements.

Temperature Dependence Analysis

DYNAMICS offers various analysis fits from which to determine the transition temperature and depending on the thermal behavior of the sample, one or more of these fits might be appropriate.

a. ***Onset Analysis*** is appropriate when the radius increases with temperature without ever reaching a constant value, such as samples that form large aggregates. The transition temperature reported is the onset temperature, i.e., the temperature at which the transition begins. For proteins, this typically signifies the initial unfolding of the protein before the unfolded chains aggregate.

b. ***Sigmoid Analysis*** is appropriate when the sample reaches an equilibrium radius with temperature. Examples where this analysis is suitable are oligomerization, micelle formation or molecule unfolding/folding. The transition temperature reported is the midpoint of the transition.

c. ***Linear Intersection Analysis*** is similar to Onset Analysis. Here, the user can manually select and adjust linear ranges below and above the transition temperature by moving two sets of thresholds lines. The transition temperature reported is the intersection of the two linear fits. Linear analysis is typically used if the Onset analysis does not converge within the desired temperature range.

d. ***Polynomial Analysis*** uses a polynomial function to fit the data. This analysis is generally not used for temperature analysis.

Analysis Procedure

1) Go to the **Analyses** menu and choose **Temperature Dependence**. Clicking on this will make the "**Temperature Dependence**" analysis appear as a subsection under the **Analyses** menu (Figure 9). This analysis plots the change in R_h obtained from the *Cumulants* fit versus temperature.

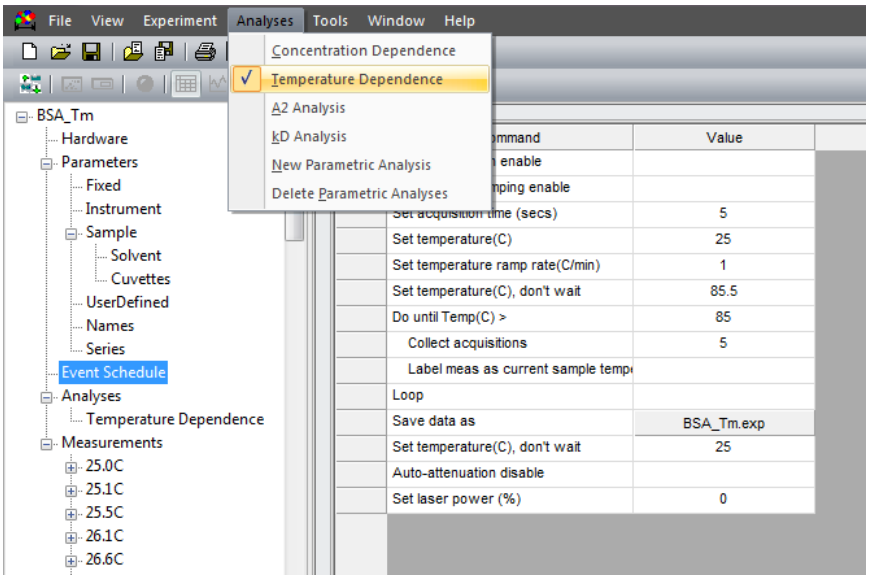


Figure 9: Activating the Temperature Dependence Analysis in the experiment tree.

2) In the ***Temperature Dependence*** window, select your **Sample** from the **Measurement Data** window on the right. Select the desired **Analysis Fit** from the pull down menu as shown in Figure 10. For this example, Onset analysis is used to determine T_{onset}. You can find each sample's analysis fit type, onset temperature, and radius at the onset temperature displayed in a table below the graph as shown in Figure 11. Each analysis fit is detailed below.

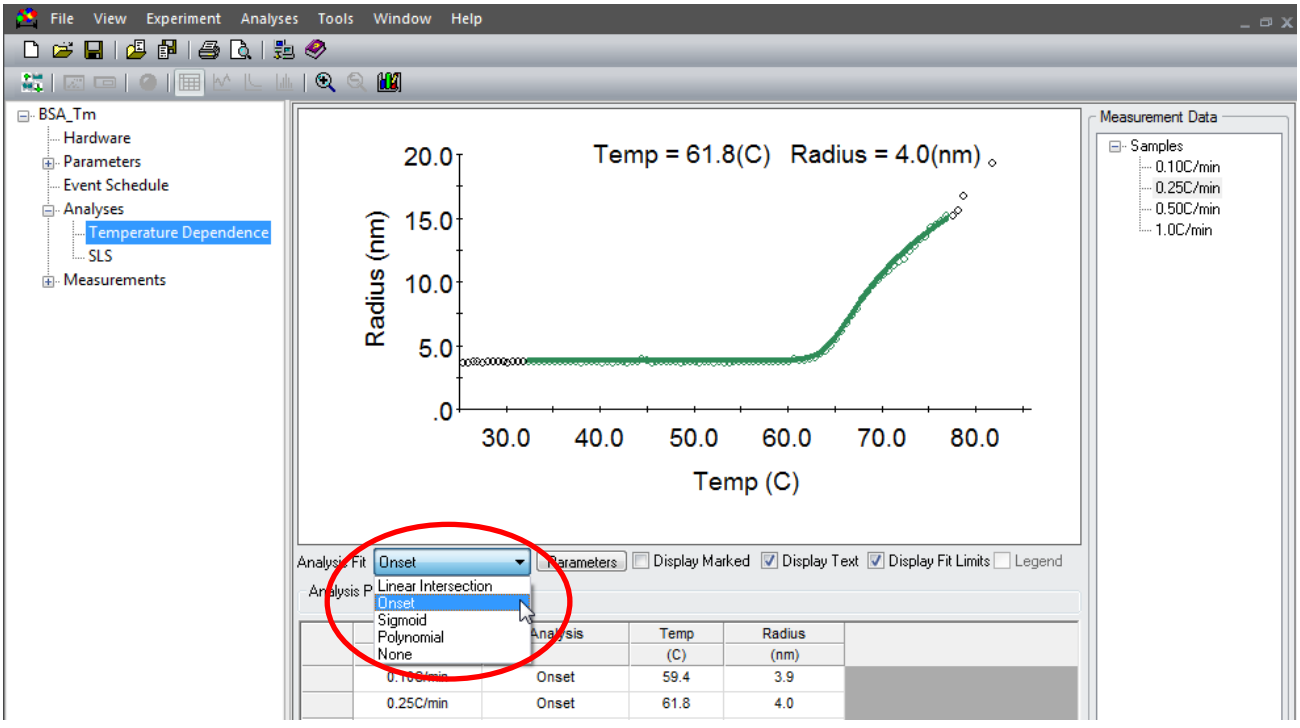


Figure 10: Selecting an Analysis fit in the Temperature Dependence analysis window.

Onset Analysis

Click the **Parameters** button next to the **Analysis fit** pull down menu to show the **Analysis Parameters**. Uncheck **Use Experiment Defaults** if the default parameters are not suitable. The **Threshold Percentage** determines the percentage above the baseline (measurements before transition) to be used as the onset point. The range of temperature to include in the curve fitting can be set in the **Below Threshold** and **Above Threshold** fields. If **Zero Slope** is unchecked an overall slope to the analysis is allowed. An example of this analysis for a BSA sample which unfolds and aggregates is shown in Figure 11.

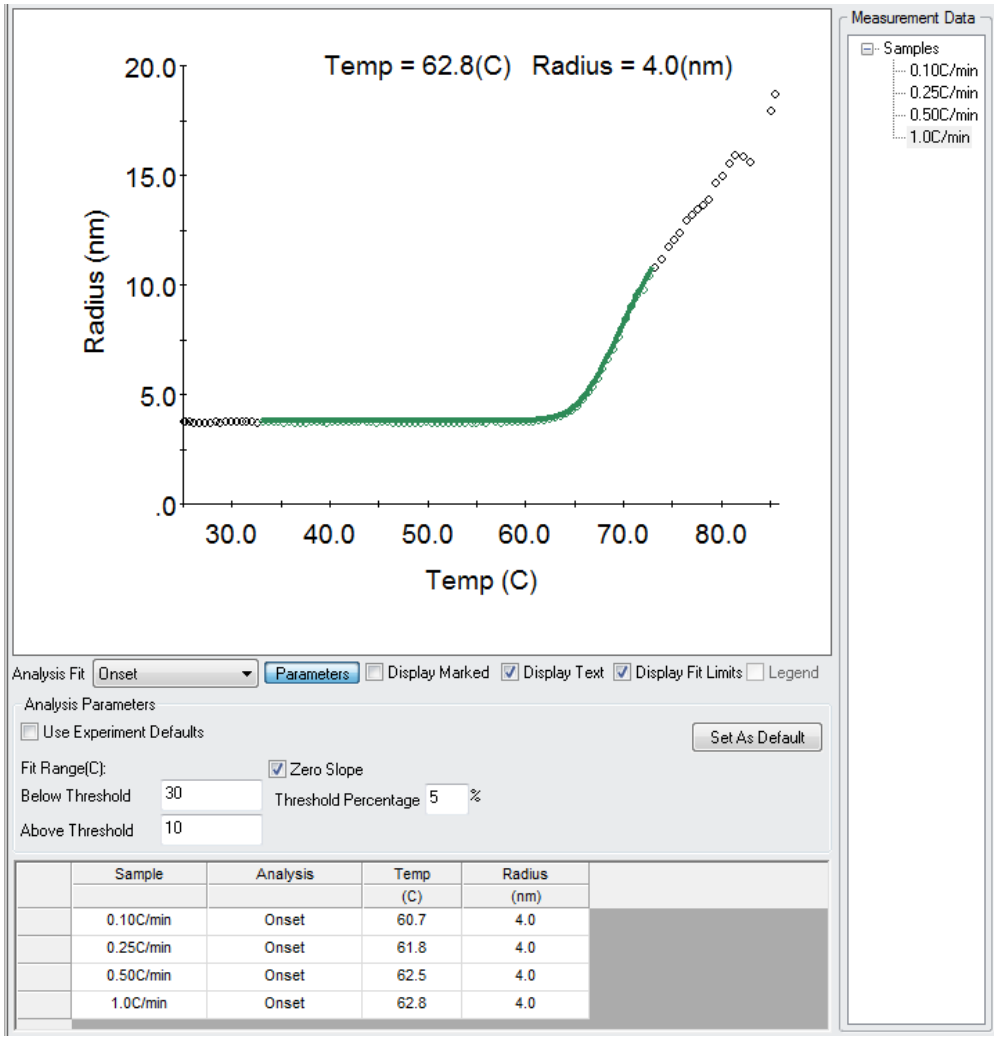


*Figure 11: Using the **Onset** analysis fit to determine T_{onset} of bovine serum albumin in phosphate buffered saline measured using a ramp rate of 1˚C/min. Please see text below for detailed description.*

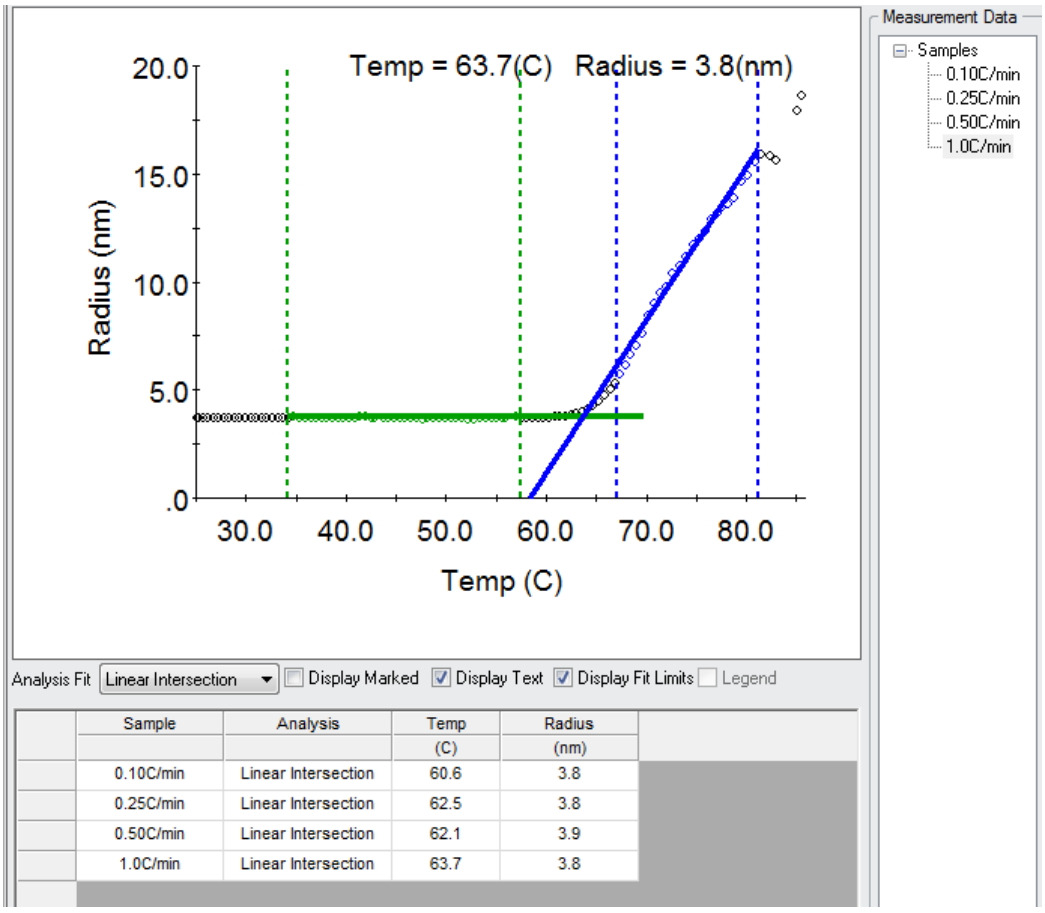
Analysis Parameters are changed from the default so that a range of 30 ˚C below threshold and 10 ˚C above threshold (~33 ˚C – 73 ˚C) is chosen for the curve fitting. The **Zero Slope** box is checked as no change in radius below the onset temperature is expected; the radius of BSA is 3.8 nm below the onset temperature. The green line indicates the fit curve and the dots are the hydrodynamic radii determined from the *Cumulants* fit. The reported T_{onset} for this analysis is the temperature at which the transition begins.

In this experiment, four samples are analyzed; all of which are BSA but are heated at different ramp rates. In Figure 11, the 1 ˚C/min sample is selected from the right panel to be viewed but T_{onset} for all samples is shown in the table underneath graph.

Note: During a temperature ramping experiment, the temperature of the sample cell lags the read head temperature. Because of this lag, the read head temperature used in the calculation is higher than the actual sample temperature. Based on the Stokes-Einstein equation, if the temperature used in the calculation is higher than the sample temperature, the calculated viscosity will be too low and the reported size will be correspondingly too high. The current NanoStar firmware incorporates a correction of the temperature lag using thermal modeling. This correction predicts the actual sample temperature based on the history of the measured read head temperature. This allows you to run faster experiments and obtain more accurate results from a thermal ramping experiment. Using the quartz cuvette for these measurements provides the most accurate results. The recommended maximum ramp rate is 5 °C/min.

Linear Intersection Analysis

Drag the green vertical lines to set the temperature range to fit below the onset point and the blue vertical lines to set the temperature range to fit above the onset point. An example of this analysis for a BSA sample which unfolds and aggregates is shown in Figure 12.



	Sample	Analysis	Temp (C)	Radius (nm)
	0.10C/min	Linear Intersection	60.6	3.8
	0.25C/min	Linear Intersection	62.5	3.8
	0.50C/min	Linear Intersection	62.1	3.9
	1.0C/min	Linear Intersection	63.7	3.8

*Figure 12: Using the **Linear Intersection** analysis fit to determine T_{onset} of bovine serum albumin in phosphate buffered saline measured using a ramp rate of 1 ˚C/min. Please see text below for detailed description.*

By dragging the vertical lines, two temperature regions are selected for linear fitting: green (below onset temperature) and blue (above onset temperature). The onset temperature is the temperature at which the two lines intersect. The dots are the hydrodynamic radii determined from the *Cumulants* fit. In this experiment, four

samples are analyzed all of which are BSA but are heated at different ramp rates. In Figure 12, the 1 ˚C/min sample is selected from the right panel to be viewed but T_{onset} for all samples is shown in the table underneath graph.

Sigmoid Analysis:

Click the **Parameters** button next to the **Analysis fit** pull down menu to show the **Analysis Parameters**. Uncheck **Use Experiment Defaults** if the default parameters are not suitable. The range of temperature below and above the threshold to include in the curve fitting can be set in the **Below Threshold** and **Above Threshold** fields. If **Zero Slope** is unchecked an overall slope to the analysis is allowed. An example of this analysis for a lysozyme sample which unfolds but does not aggregate (in pH 4 acetate buffer) is shown in Figure 13.

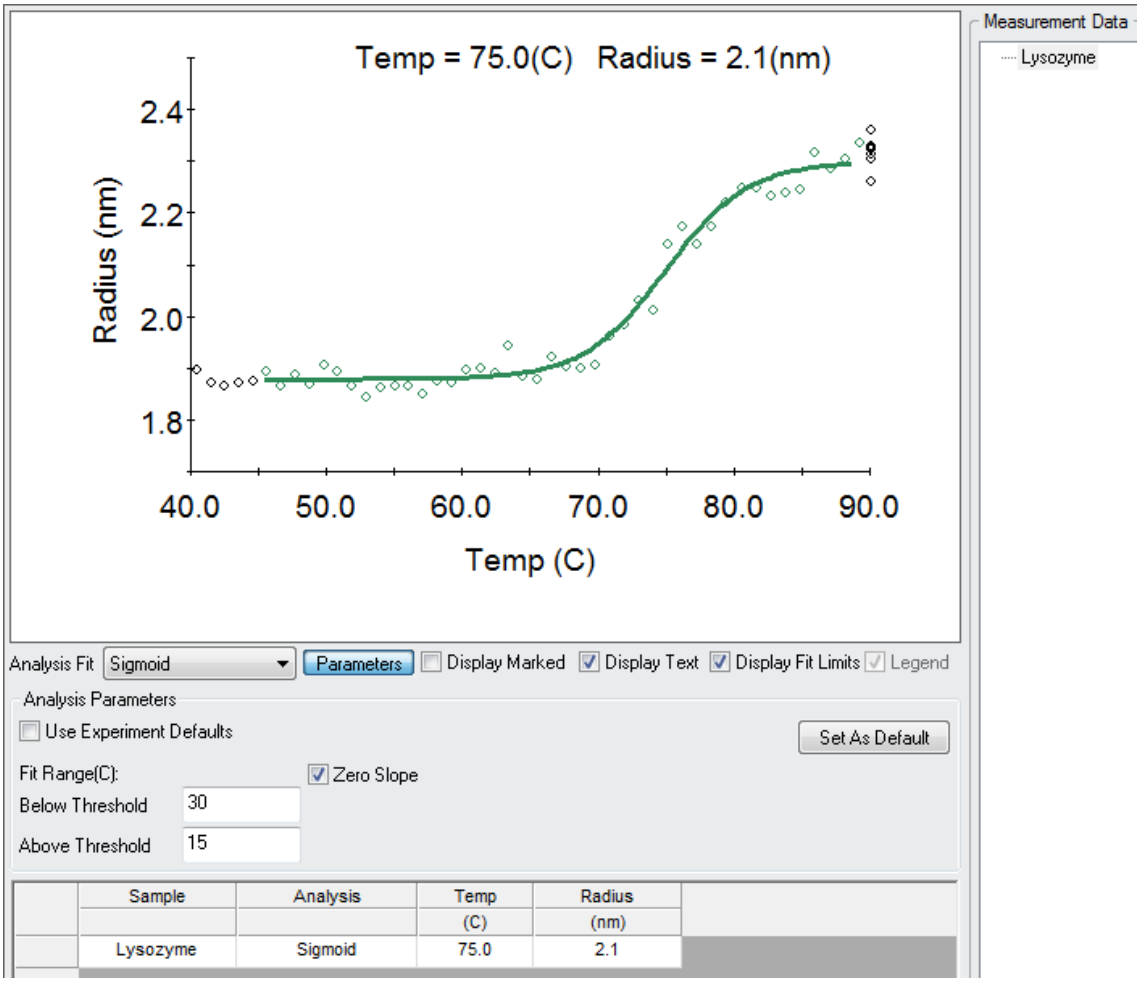
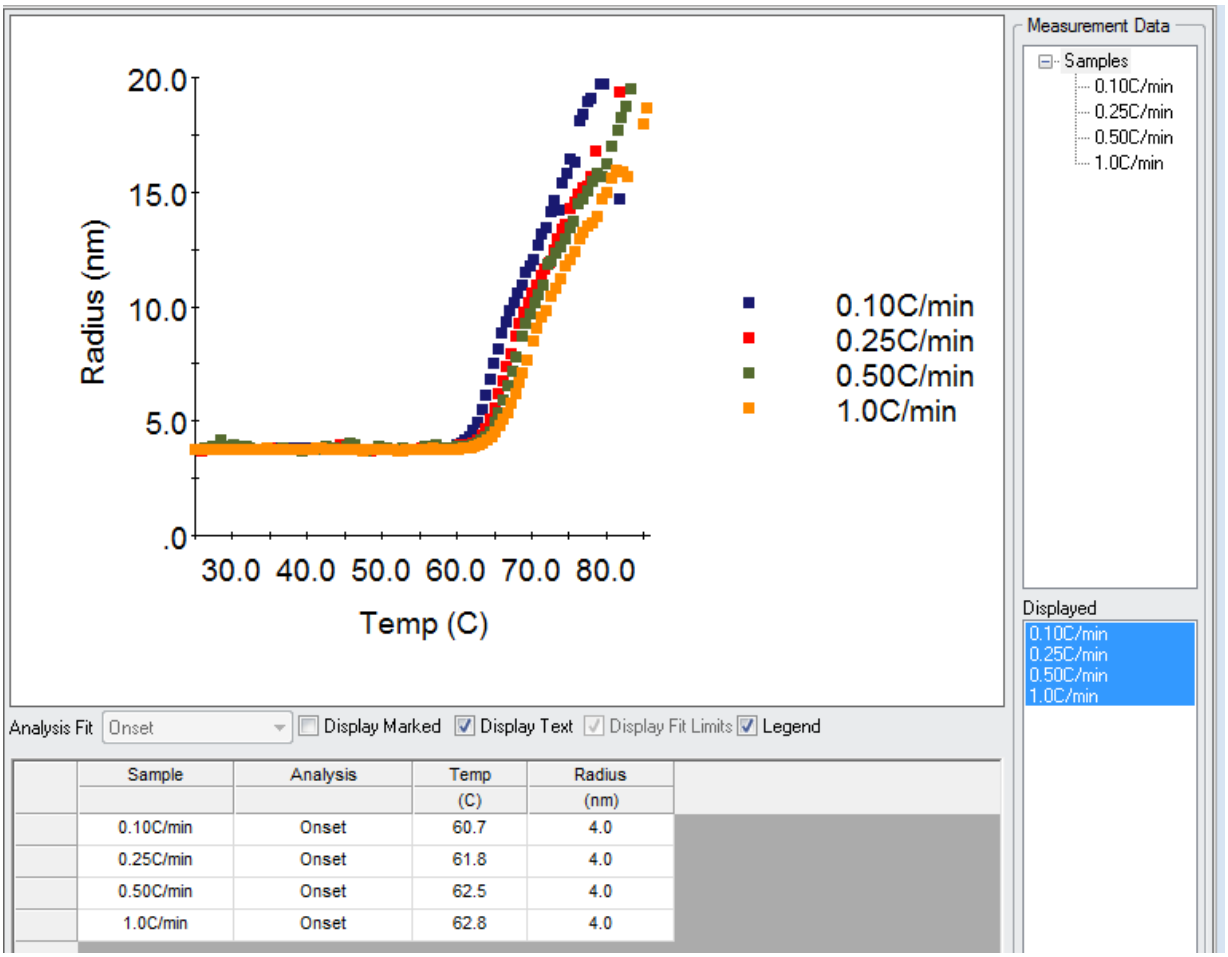


*Figure 13: Using the **Sigmoid** analysis fit to determine the melting temperature of Lysozyme in pH 4 acetate buffer measured using a ramp rate of 0.25 ˚C/min. Lysozyme is expected to unfold but not aggregate under these conditions which correlates with the observed increase in R_h corresponding to the unfolding followed by an observed equilibrium R_h corresponding to the unfolded state. Please see text below for detailed description.*

Analysis Parameters are changed from the default so that a range of 30 ˚C below threshold and 15 ˚C above threshold (~ 45 ˚C – 90 ˚C) is chosen for the curve fitting. The Zero Slope box is checked as no change in radius below the onset temperature is expected; the radius of Lysozyme is 1.9 nm below the onset temperature. The green line indicates the fit curve and the dots are the hydrodynamic radii determined from the *Cumulants* fit. The reported temperature ("**Temp**") in the table below is the temperature at the midpoint of the transition.

Comparing Samples

To overlay temperature dependence curves of all samples, click on the **Samples** title in the right **Measurement Data** panel and a **Displayed** panel will appear in the right bottom panel as shown in Figure 14. By default all samples will be displayed. You can select a subset of samples to display by clicking on the sample names while holding down the Ctrl key.



Sample	Analysis	Temp (C)	Radius (nm)
0.10C/min	Onset	60.7	4.0
0.25C/min	Onset	61.8	4.0
0.50C/min	Onset	62.5	4.0
1.0C/min	Onset	62.8	4.0

Figure 14: Displaying the Temperature dependence plot of R_h versus temperature of multiple samples. Bovine serum albumin in phosphate buffered saline was analyzed using four different heating ramp rates.

Parametric Analysis

DYNAMICS allows the user to setup a **Parametric Analysis** that generates an XY-plot of any one parameter versus any other parameter. This section will describe how to setup a Parametric Analysis to analyze the change in molar mass measured by static light scattering (Mw-S) as a function of temperature. However, by simply choosing different parameters for the X-axis and Y-axis the effect of any parameter on another can be obtained by following these steps.

1) To analyze the temperature dependence of the molar mass, go to the **Analyses** menu and choose **New Parametric Analysis**. Name the analysis and add *Temp* as the X-axis variable (under the Instrument section) and the molar mass, *Mw-S*, as the Y-axis variable (under the Static Light Scattering section) as shown in Figure 15. Clicking **OK** will make the new analysis appear as a subsection under the **Analyses** menu. The plot of the Mw-S analysis will have units of Temperature (˚C) on the X-axis and [R(θ)/(K*c)] (Da) on the Y-axis.

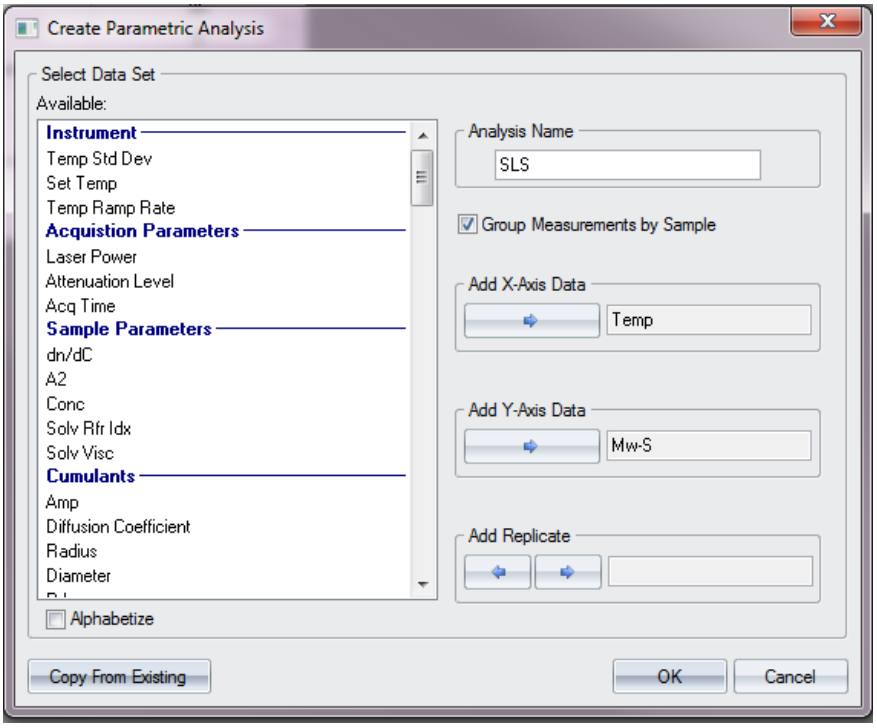


Figure 15: Creating New Parametric Analysis to display change in molar mass with temperature as calculated from SLS measurements. A Parametric Analysis can be set up for any two parameters chosen in this dialog box.

2) Click on the new parameteric analysis under the **Analyses** node. A pop-up window will appear notifying that by default, 15 % of the total data range is included in the analysis (Figure 16).

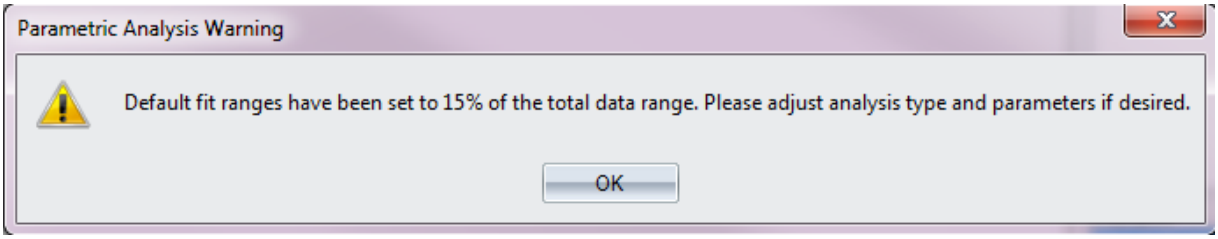


Figure 16: Pop-up when adding new Parametric Analysis warning that by default 15 % of the total data range is included in the analysis.

3) Select your sample from the **Measurement Data** window on the right. Select the desired analysis fit from the pull down menu. DYNAMICS offers various analyses fits to determine the transition temperature and depending on the thermal behavior of the sample one or more of these fits might be appropriate. Refer to the Temperature Dependence section for more information about each type of analysis fit and the adjustable fit parameters. An example of an **Onset** analysis fit of Mw-S versus temperature is shown in Figure 17 for a BSA sample which unfolds and aggregates.

4) Analysis Parameters for the example in Figure 17 are changed from the default so that a range of 30 ˚C below threshold and 10 ˚C above threshold (~ 33 ˚C to 73 ˚C) is chosen for the curve fitting. The reported T_{onset} for this analysis is the temperature at which the transition begins. In this experiment, four samples are analyzed, all of which are BSA but are heated at different ramp rate. In Figure 17, the 1 ˚C/min sample is selected from the right panel to be viewed but T_{onset} for all samples is shown in the table underneath the graph. You can find each sample's onset temperature, analysis fit type, and the Mw-S at the onset temperature displayed in a table below the graph.

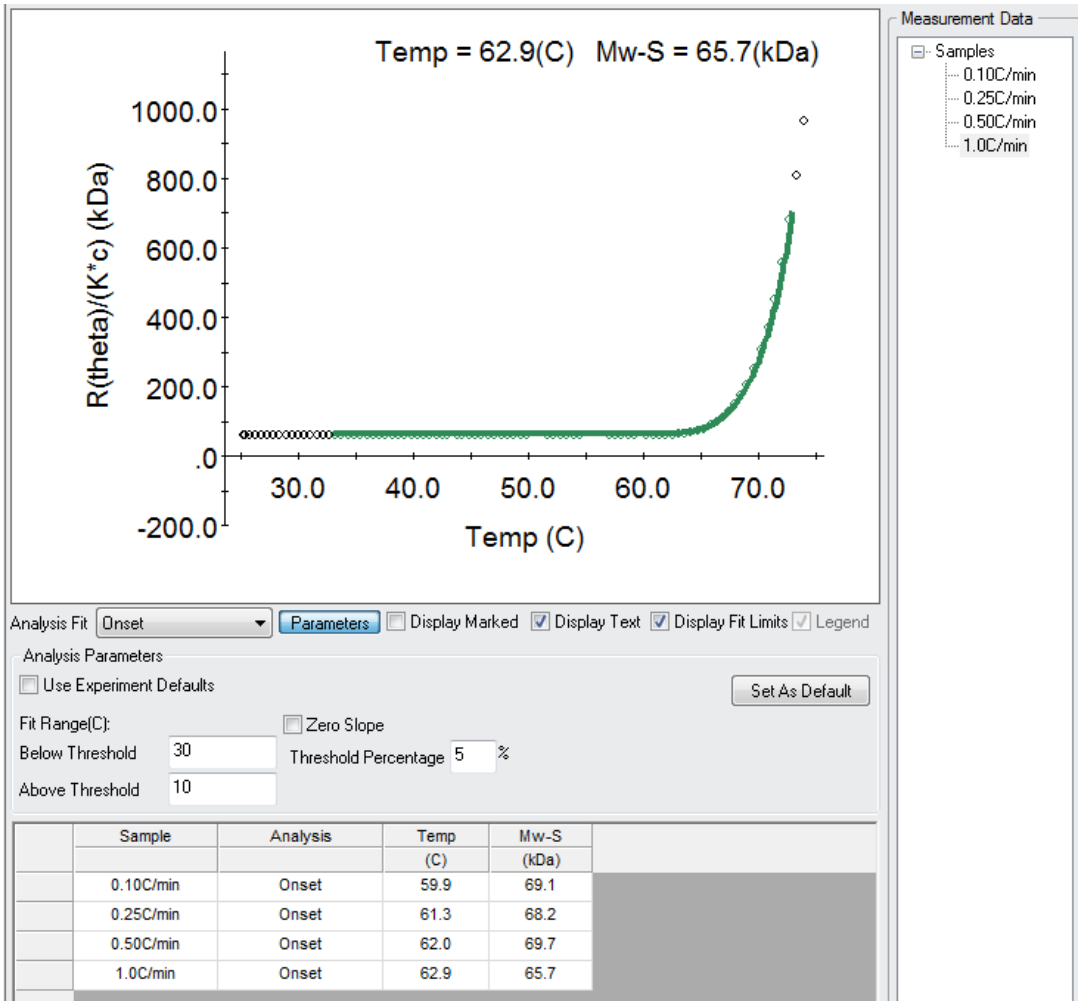


*Figure 17: Using **Onset** analysis fit to determine T_{onset} of aggregation of bovine serum albumin in phosphate buffered saline measured using a ramp rate of 1 ˚C/min. T_{onset} is determined from the analysis of the change in Mw-S [R(θ)/(K*c)] (kDa) with temperature as measured by SLS.*

5) To overlay temperature dependence curves of all samples as shown in Figure 18, click on the **Samples** title in the right **Measurement Data** panel and a **Displayed** panel will appear in the right bottom panel. By default all samples will be displayed. You can select a subset of samples to display by clicking on the sample names while holding down the Ctrl key.

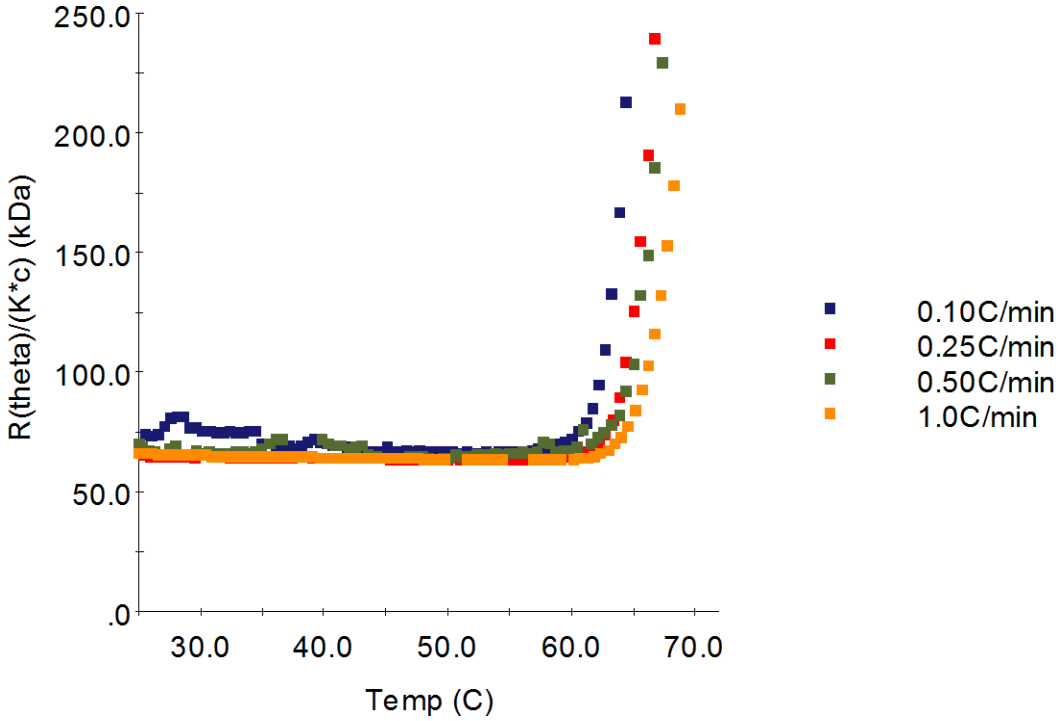


Figure 18: Displaying the parametric plot of Mw-S versus temperature for multiple samples. Bovine serum albumin in phosphate buffered saline was analyzed at four different heating ramp rates.

Comparing DLS and SLS Data

Compare the analysis of R_h versus temperature to that of Mw-S versus temperature. Molecules that unfold or change conformation but do not aggregate will show an increase in R_h but not in Mw-S (such as lysozyme in pH 4 acetate buffer, Figure 19). Molecules that aggregate with an increase in temperature subsequent to unfolding or due to micellization for example will exhibit an increase in both parameters. Protein that unfold and aggregate might exhibit a lag between an increase in R_h and Mw-S with an increase in temperature as the protein unfolds first (increasing its size) and then subsequently aggregates (increasing its size and molar mass). R_h and Mw-S versus temperature plots can be overlaid in the **Datalog Graph** view.

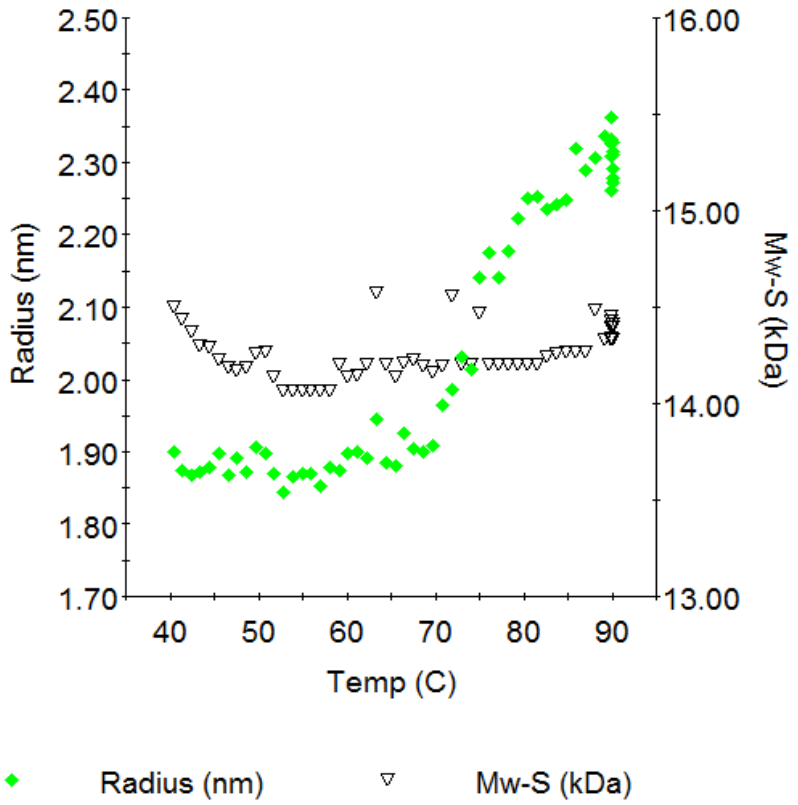


Figure 19: Thermal behavior of Lysozyme as studied by change in hydrodynamic radius (measured by DLS) and molar mass (SLS) with temperature. A sample of 1 mg/mL in pH 4 acetate buffer was heated at a rate of 0.25 ˚C/min. Lysozyme unfolds but does not aggregate upon unfolding as the radius increases (green diamonds) but the molar mass remains unchanged (black triangles).

Conclusion

By following this protocol, the user will be able to analyze the thermal behavior of molecules that exhibit a change in conformation or aggregate with an increase in temperature using the DynaPro NanoStar. DLS measures the change in size associated with these transitions while SLS measures the change in molar mass. DYNAMICS provides several methods to determine a thermal transition temperature using the different analysis fits available (*Onset*, *Sigmoid*, and *Linear Intersection*). DYNAMICS also offers the flexibility to add a new parametric analysis to determine the effect of any one parameter on the other and fit those curves using one of the analysis fits above.

Using both DLS and SLS allows the user to distinguish aggregation of a macromolecule where both size and molar mass increase from a conformational change where only the size of the molecule changes but the molar mass remains constant.