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INTERACTIONS

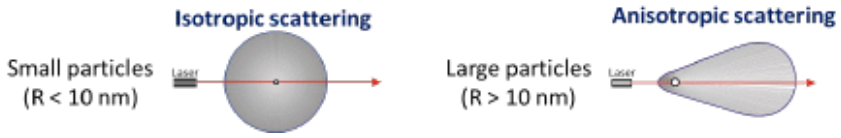
Wyatt DynaPro Plate Reader III: CMI Getting Started Guide to Differential Light Scattering with Static Light Scattering

Sample Preparation | Getting Started | Data Collection | Data Analysis | Shutdown

Introduction

Static Light Scattering (SLS) measures the weight-averaged molar mass calculated from the intensity of scattered light, $I(\theta)$, which is proportional to molar mass, M, concentration, c, and square of the refractive index increment, $(dn/dc)^2$. SLS also accounts for the angular dependence of light scattering, $P(\theta)$ allowing for accurate calculation of molecular weight (Mw) for larger samples with anisotropic scattering. For samples with a radius smaller than 10 nm, $P(\theta) = 1$ at all angles. For larger samples, scattering intensity diminishes as the angle increases due to intramolecular interactions.

$$I(\theta) \propto M\, c\, (dn/dc)^2\, P(\theta)$$



Source: Wyatt Technology: Technical Note TN7004

Instrument Overview

The CMI has the DynaPro Plate Reader III instrument from Wyatt Technologies, which measures dynamic and static light scattering at high throughput for many sample types using standard microplates.

DLS can be used to monitor sample quality during any (or all) stages of a protein purification project, or after storage. DLS with SLS provides an assessment of Mw, hydrodynamic radius (Rh), particle monodispersity, and aggregation state and can be used to monitor sample quality during any (or all) stages of a protein purification project or after storage. When using SLS, samples should be relatively pure and monodisperse.

Applications

- Rh and Mw of a wide range of particles
- Protein stability from thermal denaturation.
- Aggregation analysis of samples, including chemical compounds.

Key Features

- Measure Mw from 1 to 1000 kDa
- Measure Rh from 0.5 nm to 1000 nm
- Sensitivity for size as low as 12.5 µg/mL IgG, 0.125 mg/mL Lysozyme
- Measure in 96-1536 well microplates
- Temperature ramps from 20 to 85 C (option for low temp, 4 C, with nitrogen gas)
- Compatible with a range of buffers

Required Supplies

- Compatible plate, such as:
    - Aurora Microplates 384-well plate (part no. ABM2-10100A)
    - Greiner BioOne 384 well Sensoplate (part no. 781 892)
    - Greiner BioOne 96 well Sensoplate (part no. 655 892)
- Molecular weight standard, such as Wyatt Lot-certified Dextran 40 (part no. 900114)

Sample Preparation

Assay Buffers

- SLS requires solvent offsets. Solvents should be filtered, especially those prone to aggregation, such as in DMSO.
- Small molecule aggregation analysis should be done at working concentrations in and working solvents (e.g., 5% DMSO).

Samples

- SLS is more sensitive to aggregates than DLS.
    - Molecular weight from SLS (Mw-S) is based on an average of all molecules, and will not be accurate for polydisperse samples.
    - Samples should be filtered or centrifuged (6000g, 10-30 min) prior to loading the clear bottom plate to remove large aggregates and precipitates.
- The minimum amount of sample required depends on the size of the molecule, as large molecules scatter more light than small molecules.
    - For most protein samples, concentrations of 0.2 mg/ml or higher are recommended.
    - As little as 0.125 mg/ml of lysozyme (~14 kDa) or 12.5 µg/ml of IgG (150 kDa) can be detected.
- When working with membrane proteins, note that empty detergent micelles will not resolve from protein-filled micelles (except when empty micelle varies in size by 5-10X)
    - Avoid excess detergent by purifying membrane proteins by affinity and/or size-exclusion chromatography and avoid concentrating after purification.

Getting Started

Resources

Additional resources are available at the instrument, including:

- Wyatt DynaPro Plate Reader III User Guide and Dynamics 7.9 User Guide, for additional information about data collection and analysis.
- Wyatt Technical Note TN7004, Performing Static Light Scattering Measurements with DynaPro Plate Reader III, which includes information on measuring A_2 with SLS.

Experimental Design Tips

- The measurement acquisition time and number of acquisitions needed will depend on the sample concentration and size. Try several options to find the best conditions for your samples.
- Static Light Scattering (SLS) experiments are most reliable for monodisperse samples.
    - Even a small number of aggregates will shift the weight-averaged mass significantly.
    - A SEC-MALS experiment allows for more accurate mass measurements than SLS alone, especially for polydisperse samples or samples with any amount of aggregate.
- Because concentration is used to calculate Mw, accurate concentration measurements are critical.
- The most accurate determination of molar mass is achieved by measuring a concentration series at constant temperature.

General Care and Maintenance

- The DynaPro Plate Reader instrument will generally be powered on, with the laser off.
- If you restart the instrument, allow 30 min to equilibrate the temperature.

Startup

1. Book time on the PPMS calendar before you start.
2. Log in to the computer using your PPMS credentials (eCommons ID and password).
3. Open Dynamics software for DynaPro Plate Reader III data collection and analysis.
4. Load microplate with samples.
    a. Add the recommended minimum volume according to plate guidelines to each well.
    b. Each individual plate *must* be calibrated for SLS before data collection. The best way to do this is with a concentration series of 40 kDa dextran:
        i) Fill at least two wells with filtered water.
        ii) Fill one well per concentration of 40 kDa dextran, ranging from 2 mg/mL to 10 mg/mL in 1 mg/mL increments.
    c. Spin the plate after loading to minimize air bubbles.
    d. Open the door on the DynaPro using the button on instrument LED panel or in Dynamics.
    e. Insert plate in the instrument with A1 position toward the front of the instrument.
        i) DO NOT leave plastic plate covers on the plate while in the instrument.

Data Collection

Plate Calibration

1. Start a new experiment in Dynamics software.
    a. File→New OR File→Open Preset
2. Expand Parameters and select plate.
3. Click "New" to define a new plate.
    a. Give the plate a unique identifier. Please include your initials if you hope to use the plate for repeated experiments.
    b. Select the manufacturer, number of wells, and well plate name and click "OK".
4. After selecting the plate, click "calibrate".

   a. Under Method, select "instrument" and enter the information from the Certificate of Analysis for your weight standard.
   b. Click "Select wells" to bring up the calibration standard table. Enter the concentration of the standard (in mg/mL) in the individual wells, starting with "0" for the filtered water. Click "OK".
   c. Under attenuation level, select Auto-attenuation. This will save calibration constants for multiple attenuation levels, allowing for more flexibility in data collection.
   d. Click "OK" in the plate calibration dialog to begin the calibration process. The process may take up to 30 minutes.
   e. It is recommended that you stay near the plate reader during this time. Calibration is occasionally unsuccessful, and in this case, you may need to restart. If this happens more than three times, consider redoing the concentration series.
5. Upon completion, you will see Plate Calibration Data.
   a. The software will tell you if any wells are excluded. This does not negatively affect the calibration data.
   b. The despiking filter can be used to select how many of the individual values from this well are to be included in averaging. Generally, the default is acceptable.
   c. By clicking "OK", the calibration constant is saved with this plate.
6. **If you wish to re-use this plate in future DLS/SLS experiments, click "Save to Global" in the Plate settings.** This will allow you to select your plate when setting up an SLS experiment without recalibrating.

Experiment Design

1. Click "Experiment Designer" in the side panel.
   a. If you do not see "experiment designer" in the side panel, right-click on "Event Schedule" and switch to Experiment Designer.
   b. Click "Edit Experiment."
   c. Select an experiment type:
      i. SLS has a 5% accuracy rate at fixed temperatures, and 10% with variable temperature measurements.
         1. Fixed temperature, for standard dynamic light scattering measurements.
         2. Continuous Temperature Ramp, for constant temperature ramp rate (best for very small number of samples)
         3. Discrete Temperature Increment, for experiments in which all samples are measured at a defined temperature before ramping to the next (generally best for thermal stability measurements, especially with multiple samples).
   d. For static light scattering, choose "Yes."
      i. Select the previously calibrated plate.
   e. Click "Next" to proceed.
2. Click on "Select Wells" or on the plate image.
   a. Select by clicking on the individual wells, or click and drag to mark a rectangular group of wells for measurement. Include both wells containing samples and those with solvent only.
   b. Click "Input Template" to access the Plate Templates dialog.

i. Choose "Solvent Name" in the Value dropdown menu and choose the correct solvent for each well.
ii. Choose "Concentration" from the Value dropdown menu and add the concentration (in mg/ml) for each well. Leave solvent-only wells blank or enter "0."
iii. Click "OK" to continue.

c. In the Static Light Scattering panel, click "Solvent Offsets" to access the "Indicate Pure Solvents" dialog.
i. Choose the solvent in the dropdown menu, then click to designate wells of pure solvent. Include two wells of pure solvent for each experiment.
ii. Click "OK" to save the selection.
iii. The Dynamics software will automatically calculate and apply solvent offsets to your experiment.

3. Click "Properties."
a. Enable Auto-attenuation: Yes
b. Acquire an image of each well: Yes
c. DLS Acquisition Time(s): 5-20 (use longer acquisition times for low concentration and large proteins)
d. Number of DLS Acquisitions: 5-20
e. Label Measurements
i. Click [...] to add measurement labels.
ii. Recommended minimal labeling: Well, TimeStamp
f. Set temperature
g. Click next to proceed.
4. Review
a. View experimental summary.
b. Save as Preset (optional) to save parameters as a template.
c. Click Finish when experiment editing is complete.
5. Start Experiment
a. Click Connect to Instrument (plug icon, top left) if not already connected.
b. Click on green start button to begin acquisition.
c. You will be prompted to name your experiment. Data will save automatically with your experiment file as it is collected.
d. You may collect additional data in the same file by re-running the same experiment or editing the experimental parameters and running again.

Data Analysis

Data can be examined in various ways:

1. Datalog Grid
2. Datalog Graph
3. Correlation Graph
4. Regularization Graph
5. Spectral View

1. Datalog Grid
    a. Displays summary data table of measurements.
    b. Selecting data to display:
        i. Select individual measurements in the Left Side Pane will show data from all acquisitions.
        ii. Select "Measurements" to show a summary table of all data.
    c. The default settings for the Datalog grid do not include the SLS results, so you will need to add these to the display:
        i. Right-click anywhere in the table and select "Table Settings."
        ii. Select Mw-S, Concentration, dn/dc, and Sample Concentration. Sample Concentration and dn/dc are used to calculate Mw-S and can be manipulated after the experiment has been performed.
    d. To Filter Data:
        i. Right-click the table and select Data Filter to omit data by criteria.
        ii. Right-click on an acquisition or measurement and select "Mark" to exclude from analysis.
2. Datalog Graph
    a. Displays experimental data in graphical form.
    b. Simple graphs of data can be created for reports.
3. Correlation Graph
    a. Displays the intensity auto-correlation function (ACF), the raw dynamic light scattering data.
    b. Displays fit for both cumulants (a single species fit) and regularization (multi-species fit).
    c. Correlation data should be examined carefully to assess data quality.
        i. See Assessing Data Quality below for tips on evaluating data.
4. Regularization Graph
    a. Displays the calculated hydrodynamic radius distribution using regularization fit of the autocorrelation function (Regularization is a multi-modal fit).
    b. Select measurement(s) to be displayed.
    c. Lower panel displays peak results.
        i. Radius (nm)
        ii. %PD, polydispersity
            1. PD < 15% is considered a narrow peak and monodisperse.
            2. PD > 30% is considered a broad peak and polydisperse.
    d. Mw-R is mass calculated from Radius and shape model.
    e. %Intensity is size distribution as a function of the measured scattered light intensity.
    f. %Mass is the size distribution in terms of the mass of the particles.
        i. Note: these peaks are distinct from the user-defined Ranges in the Datalog Grid, which are an arbitrary binning of the data.
5. Spectral View
    a. Graphical view of the data in a well-plate format.
    b. Used to quickly screen large sample sets.
    c. Use Sequence to toggle different measurements taken in the same well.
6. Exporting

a. Right-click on tables or graphs to export.
b. Export Values or Graph to the clipboard or to a file.

Assessing Data Quality

1. Examine the Correlation Graph to assess data quality (see dls_evaluation_criteria.pdf for examples).
2. Proceed with analysis if:
    a. Correlation function shows a single decay.
    b. Correlation function shows multiple decays that are resolved in the evaluation time window.
3. Use Caution when analyzing if:
    a. Correlation function shows premature termination (noisy at long times). i) Increase acquisition time.
    b. Correlation shows a foot at long delay times indicating particle number fluctuations.
        i. Decrease acquisition time.
        ii. Increase number of acquisitions.
        iii. Use data filtering.
    c. Correlation function is not smooth, indicating a weak signal.
        i. Increase acquisition time.
        ii. Increase number of acquisitions.
        iii. Increase laser power (if auto-attenuation is disabled).
        iv. Increase concentration.
4. Do not analyze data if:
    a. Correlation function shows evidence of large particles.
        i. Centrifuge or filter sample.
    b. Correlation function shows no evidence of particles (looks like solvent).

Shutdown

1. Remove your plate from the instrument.
    a. Plates can be reused.
    b. Mark used wells (black marker on the clear bottom works well).
    c. Cover your plate and store in a clean, safe place for future use. (Remember: Avoid Dust!)
2. Close the door using the button on instrument LED panel or in Dynamics.
3. **Turn off the DynaPro III laser.**
4. Quit Dynamics Software.
5. Log off from PPMS! Rates are based on booked and real-time usage.

Book time and Report Problems through the PPMS system: https://ppms.us/hms-cmi

Contact cmi@hms.harvard.edu with questions

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