

## Refeyn TwoMP: CMI Guide to Graphing Mass Photometry Data in GraphPad Prism

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### Export Required Data

1. In DiscoverMP, select the files of interest (control or shift-click). Right-click and batch export. Name the export "Events." This will generate a folder with multiple CSV files.
2. Create a stacked histogram for each sample of interest. Make sure all relevant Gaussian curves and intervals are selected, and that labels do not overlap. Export all histograms.

### Generate a Frequency Analysis

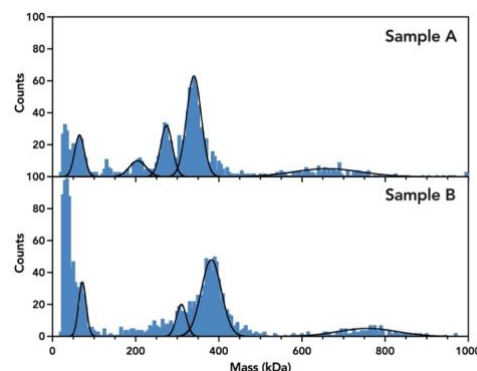
1. Open Prism. Under "NEW TABLE & GRAPH" select "XY". For X, select "Numbers". For Y, select "Enter and plot a single Y value for each point."
2. Open the exported Events files. Select all data in the column labeled "masses\_kDa." Copy and paste the data into the first "Y" column of your Prism data table. Label the column with your sample name. Repeat for all samples of interest.
3. Click the "= Analyze" button. Under "Column analyses," select "Frequency distribution." Select the table and columns you wish to analyze. Click "OK."
4. Parameter Recommendations
  - a. Create: Frequency distribution
  - b. Tabulate: Number of values
  - c. Bin width: 3-6 (recommended: 5)
  - d. Bin Range: Auto
  - e. Click OK.

### Simulate Gaussian Curves

1. Click the "= Analyze" button. Under "Simulate data," select "Simulate XY data." You do not need to select a particular data table. Click OK.
2. Select "Generate a series of X values." Start at X = 0.
  - a. Each value equals the previous value plus 0.1.
  - b. Stop when X equals or exceeds (insert your desired max value for the X-axis).
3. Navigate to the Equation tab. Expand "Gaussian" and select "Gaussian."
4. Navigate to the Parameter values & column titles tab. Specify the number of data sets to simulate (one for each curve/peak). For each data set, generate 1 replicate value.
5. Select a data set to edit. Use the histogram you exported from DiscoverMP to estimate the curve's amplitude (height of the peak center). Input the mean and standard deviation generated by Discover MP. Repeat for each curve.
6. Don't add random error. Click OK.
7. Repeat this step for each sample you would like to graph.

## Graph the Data

1. Add a New Graph. Select the data table with your frequency analyses.
  - a. Create a new graph for each data set (don't put them all in one graph). Set the Y-axis title to "Counts."
  - b. Under "Kind of graph," Show "XY" and select a Points Only graph (this should be the first option on the left).
  - c. Click Create.
2. Double-click the graph area to format and add datasets.
  - a. Deselect "Show symbols." Select "Show bars/spikes/droplines" and change the width to 2 or 3 (this will depend on your bin width. Set border to "None."
  - b. Select the "Data Sets on Graph" tab. Under "Data on graph," click "Add..." and select the table with your simulated Gaussian curves. Select all data sets and plot on the left Y-axis. Click "Add."
  - c. Switch back to the Appearance tab. Select all simulated data sets. Deselect "Show bars/spikes/droplines." Select "Show connecting line/curve." Select a uniform color for the Gaussian curves. Click "OK."
    - i. At this point, you should check the appearance of the curves. Make sure that the amplitude of each curve is appropriate for your data. Adjust if necessary.
3. Double-click any axis to format axes and add intervals.
  - a. Adjust the range of the axes to fit your area of interest.
  - b. To add intervals, navigate to the X-axis tab.
    - i. Under Additional ticks and grid lines, add a line at the minimum and maximum values for each interval range.
    - ii. Under Details, click the settings button.
      1. Select the minimum value for an interval.
      2. Deselect "Show Text" and "Show Tick."
      3. Adjust the Grid Line settings to your desired appearance.
      4. Select "Fill (shade) between this tick and X =" and specify the maximum grid line for that interval. Select a fill color. Select "Under data points."
      5. Click OK.
  - c. Adjust frame, axes, and labels as desired and click OK to apply changes.
4. Add labels for each Gaussian curve and interval using the text box feature.
5. To compare multiple samples, consider stacking graphs vertically rather than overlaying them within the same histogram.



Contact [cmi@hms.harvard.edu](mailto:cmi@hms.harvard.edu) with questions.

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