

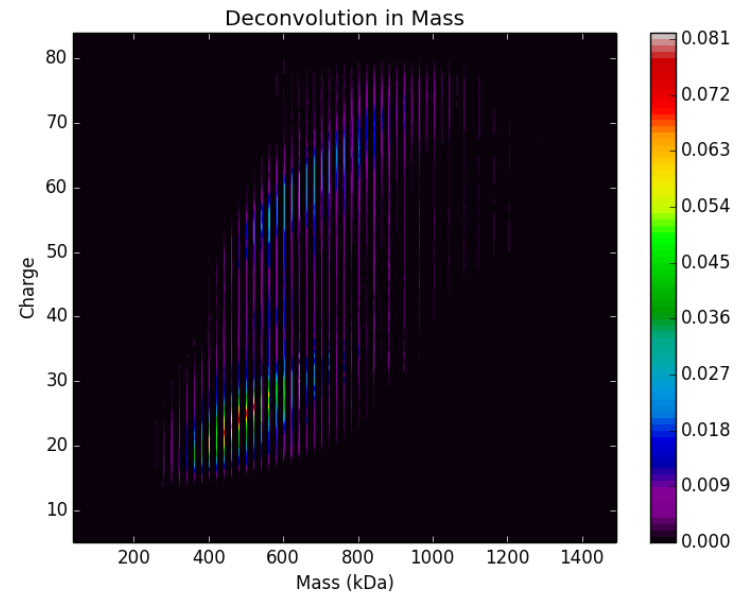
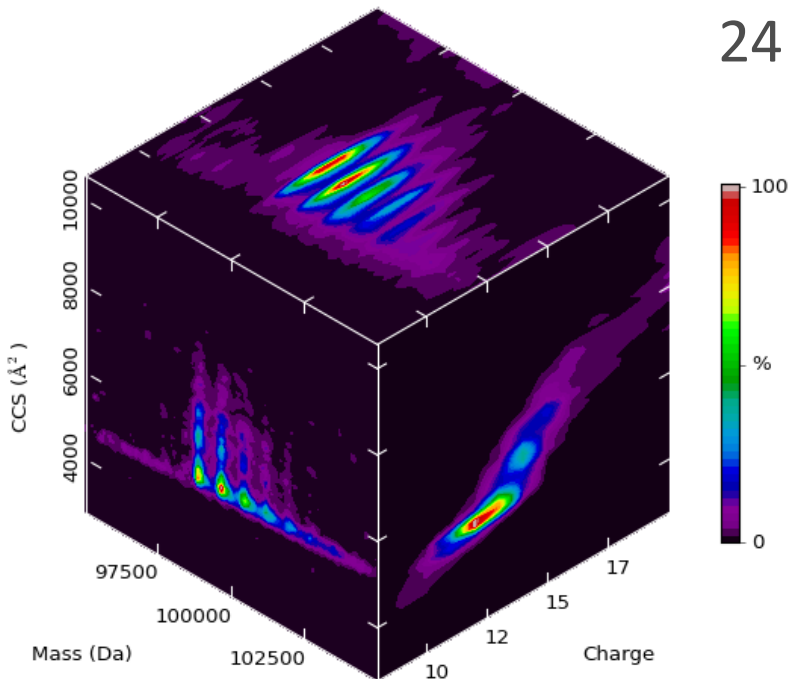
# What can UniDec do for You?

## A UniDec Tutorial

Michael Marty

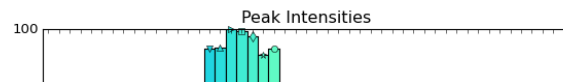
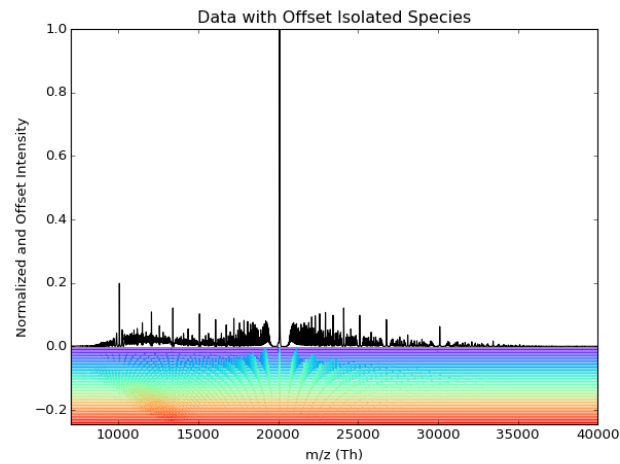
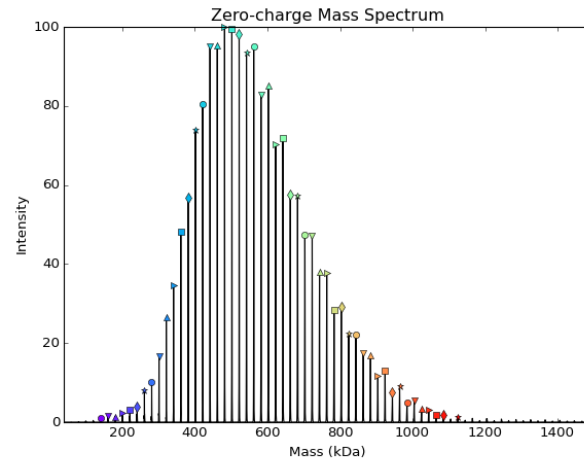
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24 March 2015



# Outline

- Overview
- Basic Controls
- Useful Tools
- Some Tips



Mass (Da)	Intensity	Area	Name
139815.0	1.05057...		A
159915.0	1.47380...		B
179915.0	1.40471...		C
200015.0	2.23806...		D
221015.0	3.07173...		E
241115.0	3.96168...		F
261215.0	7.97889...		G
281315.0	10.2648...		H
301415.0	16.6783...		I
321415.0	26.4453...		J
341515.0	34.7187...		K
361615.0	48.2429...		L
381715.0	56.8435...		M
401715.0	73.8371...		N
421815.0	80.4820...		O
441915.0	95.0164...		P
462015.0	95.4040...		Q
482115.0	100.0		R
502215.0	99.4811...		S
522215.0	98.1826...		T
542315.0	93.3859...		U
562415.0	95.1427...		V
582515.0	82.8428...		W
602615.0	85.0466...		X
622815.0	70.4101...		Y
642815.0	71.8189...		Z
663115.0	57.5120...		A2
683215.0	57.2034...		B2
703315.0	47.3238...		C2
723415.0	47.1965...		D2
743615.0	38.0195...		E2
763615.0	37.8020...		F2
783715.0	28.4978...		G2
803715.0	29.1811...		H2
823915.0	22.3432...		I2
843915.0	22.0337...		J2
864115.0	17.3746...		K2
884115.0	16.7984...		L2
904315.0	11.6450...		M2
924215.0	12.9356...		N2
944415.0	7.65843...		O2
964415.0	9.11882...		P2
984615.0	5.02037...		Q2
1004515.0	5.43658...		R2
1024815.0	3.26135...		S2
1044715.0	3.20506...		T2
1064915.0	1.75823...		U2
1084715.0	1.90388...		V2
1124815.0	1.33017...		W2

Data Manipulation

m/z Range: 7000.0 to 40000.0 Th

Subtract Curved: 100

Gaussian Smoothing: 0.0

Bin Every: 5

Process Data

Additional Data Processing Parameters

Intensity Threshold: 0.0

Adduct Mass (Da): 1.0

Acceleration Voltage (kV): 0

Linear m/z (Constant  $\Delta m/z$ )

UniDec Parameters

Additional Filters/Restrains

Charge Smooth Width: 1

Mass Difference (Da): 40170

Mass Smooth Width: 1.0

Maximum # of Iterations: 50

Isotope Mode  Manual Mode

Mass List Window: 1000 Da

Native Charge Offset Range

-100 to 100

Peak Selection and Plotting

Peak Detection Range (Da): 10000.0

Peak Detection Threshold: 0.01

Peak Normalization

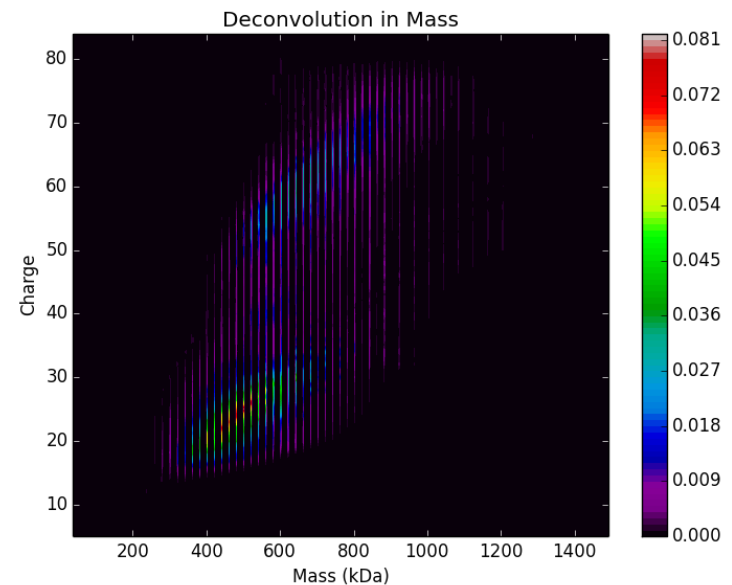
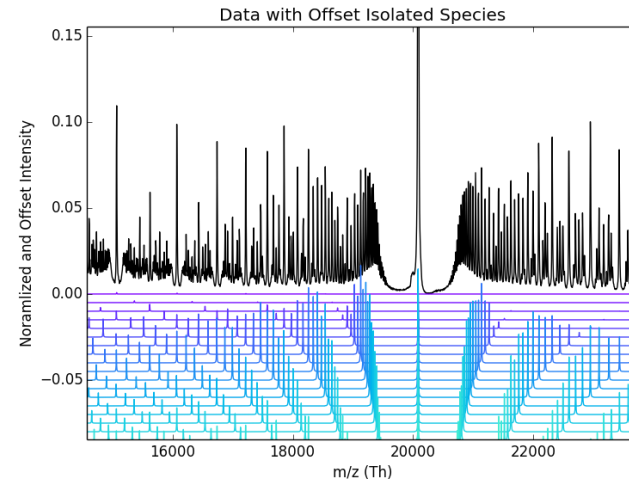
None  Max  Total

Peak Detection Plot Peaks

Additional Plotting Parameters

# Why UniDec?

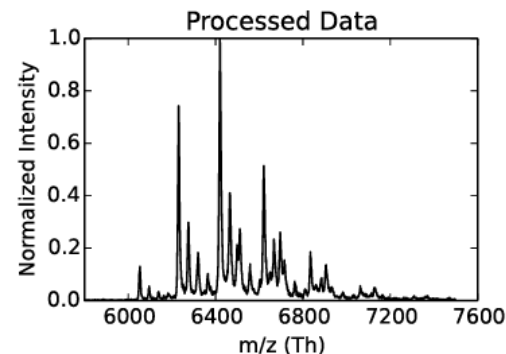
- It's *Fast!*
- To Extract Intensities
- To Understand Complex Spectra
- To Visualize Charge Dimensions
- To Interpret Ion Mobility Data



# How Does UniDec Work?

## 1. Inputs

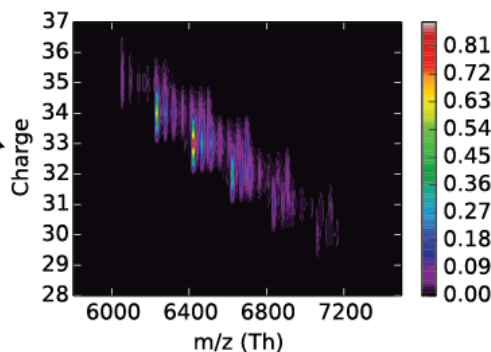
- Data
- Peak Shape
- Optional Restraints:
  - Mass, Charge, CCS



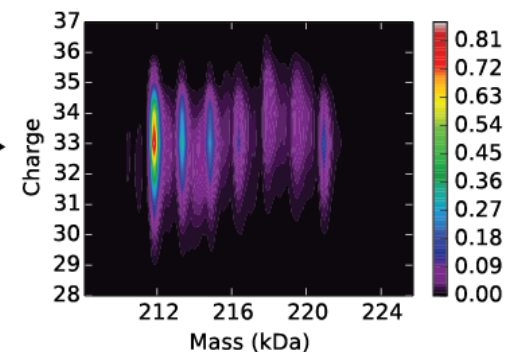
## 2. Iterative Bayesian Deconvolution

- Smooth Charge, Oligomer, or CCS:  $f^t = S_{z,m,ccs}(f^t)$
- Deconvolution:  $f^{t+1} = f^t \frac{h}{f^t * g}$

## 3. Add in Peak Width



## 4. Transform Into Mass



See Marty et Al. Anal. Chem. 2015, DOI: 10.1021/acs.analchem.5b00140  
<http://pubs.acs.org/doi/abs/10.1021/acs.analchem.5b00140>

# The Layout

The image shows a screenshot of the UniDec software interface. The window title is "UniDec" and the menu bar includes "File", "Tools", "Advanced", and "Experimental". The main area is a large plot with a table header at the top: "Mass (Da)", "Intensity", "Area", and "Name". The plot area is currently empty. On the right side, there is a vertical panel of controls and parameters. The labels and arrows in the image point to the following components:

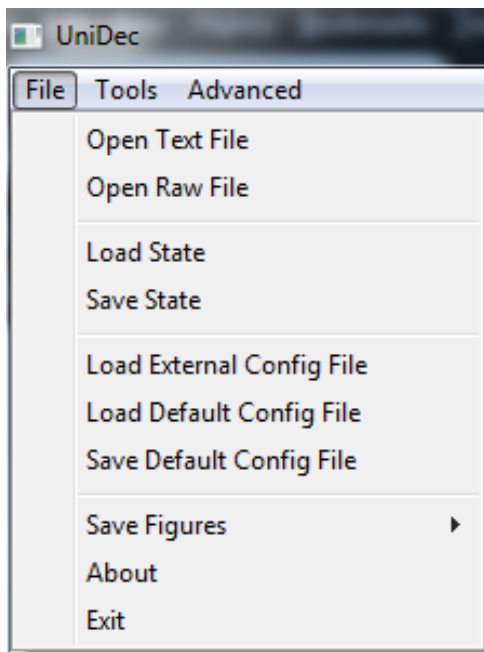
- Menus:** Points to the menu bar at the top left.
- Plots:** Points to the large central plot area.
- Peak Info:** Points to the table header at the top of the plot area.
- Controls:** Points to the right-hand panel containing various parameter settings and buttons.
- Input and Output Information:** Points to the status bar at the bottom of the window.

The right-hand panel contains the following sections and controls:

- Data Manipulation:** Includes fields for "m/z Range" (with "to" and "Th" indicators), "Subtract Minimum" (set to 0.0), "Gaussian Smoothing" (set to 0.0), and "Bin Every" (set to 1.0). A "Process Data" button is located below these fields.
- Additional Data Processing Parameters:** A section header.
- UniDec Parameters:** A section header.
- Charge Range:** Set to 1 to 100.
- Mass Range:** Set to 100.0 to 5000000.0 Da.
- Sample Mass Every (Da):** Set to 100.0.
- Peak FWHM (Th):** Set to 20.0.
- Peak Shape Function:** Radio buttons for "Gaussian" (selected), "Lorentzian", and "Split G/L".
- Run UniDec:** A button.
- Additional Filters/Restrains:** A section header.
- Peak Selection and Plotting:** A section header.
- Peak Detection Range (Da):** Set to 500.0.
- Peak Detection Threshold:** Set to 0.1.
- Peak Normalization:** Radio buttons for "None", "Max" (selected), and "Total".
- Peak Detection:** A button.
- Plot Peaks:** A button.
- Additional Plotting Parameters:** A section header.

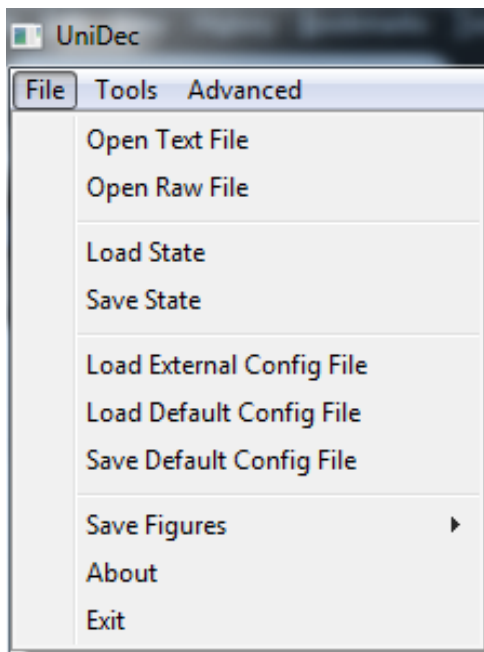
The status bar at the bottom right of the window displays "Loaded Default".

# Loading Data



- Load data from File menu
- Open Text File: May be either <x y> text file or <x y z> for IM-MS
  - Interface will automatically adjust for either MS or IM-MS data
- Open Raw File: Will convert .raw to text file and then open that file.
  - Needs “MassLynxRaw.dll” for MS and “cdt.dll” for IM-MS.
  - Water’s will not allow us to distribute these files, but they can be obtained from Waters online at:
    - [http://www.waters.com/waters/supportList.htm?cid=511442&locale=en\\_GB&filter=documenttype|DWNL&locale=en\\_GB](http://www.waters.com/waters/supportList.htm?cid=511442&locale=en_GB&filter=documenttype|DWNL&locale=en_GB)
    - cdt.dll: Ion Mobility-Enabled MassLynx Raw Data Reader Interface Library, Support Number: DWNL134825112
      - Note: This is also often installed in C:\\DriftScope\\lib.
    - MassLynxRaw.dll (32-bit): MassLynx Raw Data Reader Interface Library, Support Number: DWNL134815627
- Opening a file will create a folder next to the .txt file or in the .Raw folder called <filename>\_unidecfiles
- All useful outputs, inputs, and figures (File > Save Figures) are stored here

# Loading Data



- Save State will zip the `_unidecfiles` folder. Load State will unzip it and load everything back into the program
  - Note: This will overwrite the current configuration.
- Load External: load a `_conf.dat` from a previous run
  - Look for these in the `_unidecfiles` folder
- Saves the current configuration as the default. Will load on opening the program on hitting the Load Default button.
  - Note: The lists in the Mass and Oligomer Tools as well as Manual Assignments are not saved.
- Advanced > Reset Factory Default will return all settings to the program defaults

# Processing Data

- Limit  $m/z$  range
- Baseline subtraction
  - Minimum: Subtracts a flat line at the minimum if  $n \neq 0$
  - Line: Takes the first  $n$  and last  $n$  data points, plots a line between the averages, and subtracts the line
  - Curved: See Morgner, Robinson. Anal. Chem., 2012, 84 (6).
    - Smaller  $n$  is a more drastic. Larger  $n$  is less drastic.
- Smoothing
  - Convolves raw data with a Gaussian of width  $n$  in units of data point number prior to binning.
- Bin Size
  - Sets how much the data will be linearized or compressed
  - Compression improves speed of algorithm and helps to smooth noisy data.

Data Processing

m/z Range: 7000.0 to 40000.0 Th

Subtract Minimum 0.0

Gaussian Smoothing: 0.0

Bin Every: 1.0

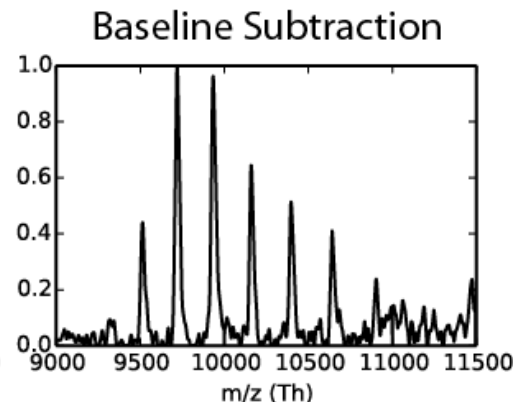
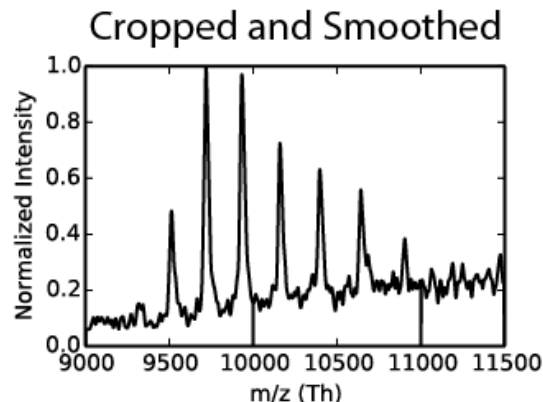
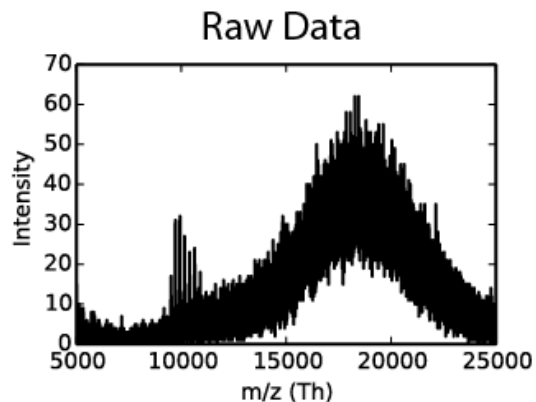
Process Data

Subtract Curved

Subtract Minimum

Subtract Line

Subtract Curved





# Advanced Processing Data

- Intensity Threshold: Disallows  $m/z$  values where spectrum falls below this threshold

- Adduct Mass: Sets the mass of the electrospray adduct

$$-\frac{m}{z} = \frac{m_{\text{species}} + m_{\text{adduct}} * z}{z}$$

- Acceleration Voltage: Sets the ToF voltage in order to correct for detector efficiency.

- Choose how to bin and compress the data

- Linear  $m/z$

- $m/z$  axis is linear (500,501,...)
- Bin Every sets spacing between bins
- Set Linear Interpolated if you want to oversample the data (i.e. data is originally 500, 501,... but you want 500.1, 500.2,...)

- Linear resolution

- $m/z$  axis is nonlinear such that the difference between data points is proportional to the  $m/z$  value (500,501,...,1000,1002,...)
- Bin Every sets the spacing at the minimum  $m/z$  value (1 in example)
- Note: the peak width will also scale with  $m/z$ . Here the peak width is defined as the peak width at the minimum  $m/z$  value
- As above, Interpolation can be used to oversample

- Nonlinear

- $m/z$  axis depends strictly on the density of the raw data
- Bin Every allows  $n$  points to be averaged together, yielding the mean  $m/z$  and intensity of each group

Additional Data Processing Parameters ▼

Intensity Threshold:

Adduct Mass (Da):

Acceleration Voltage (kV):

Linear  $m/z$  (Constant  $\Delta m/z$ ) ▼

Linear  $m/z$  (Constant  $\Delta m/z$ ) ▼

Linear  $m/z$  (Constant  $\Delta m/z$ )

Linear resolution (Constant  $(m/z)/(\Delta m/z)$ )

Nonlinear

Linear Interpolated

Linear Resolution Interpolated

# IM-MS Data Processing

- Many parameters similar to MS
- Set Arrival Time range
- Baseline subtraction and smoothing allow incorporation of arrival time dimension, although this is often unnecessary
- Binning is always performed with linear m/z
- Pusher Interval allows conversion from bin number to arrival time.
  - $AT = Bin * Pusher / 1000$
- Compress when converting
  - IM-MS data is often very memory intensive
  - Selecting this option compresses the data when it is converted before it gets imported and plotted as raw data
  - Try this if you are getting memory errors with IM-MS data

The screenshot displays the 'Data Processing' window of a software application. It features several input fields and a 'Process Data' button. The parameters are organized into two sections: 'Data Processing' and 'Additional Data Processing Parameters'.

**Data Processing Section:**

- m/z Range: [ ] to [ ] Th
- Arrival Time Range: [ ] to [ ] ms
- Subtract Minimum: [Subtract Minimum] dropdown, [0.0] m/z
- [0] A.T.
- Gaussian Smoothing: [0.0] m/z
- [0] A.T.
- Bin Every: [1.0]
- [Process Data] button

**Additional Data Processing Parameters Section:**

- Pusher Interval (μs): [0]
- Intensity Threshold: [0.0]
- Adduct Mass (Da): [1.0]
- Acceleration Voltage (kV): [0.0]
- Compress when converting to .txt

# IM-MS Parameters

- Linear Cell

$$\Omega = \frac{(18\pi)^{\frac{1}{2}}}{16} \frac{ze}{(k_b T)^{\frac{1}{2}}} \frac{1}{N} \frac{t_d V p_0}{L^2 p} \frac{T}{T_0} \sqrt{\frac{1}{\mu}}$$

- V: Voltage across cell
- p: Pressure in cell (Torr)
- T: Temperature of cell
- Mass of buffer gas (He is default)
- Instrumental dead time
  - $t_d = t_a - t_o$
- L: Drift cell length in meters

- Travelling Wave

- $t'_d = t_d - \left( \frac{EDC}{1000} * \sqrt{\frac{m}{z}} \right)$
- $\Omega' = \exp(CP1 * \ln(t'_d) + CP2)$
- $\Omega = \Omega' * z * \sqrt{1/\mu}$

Ion Mobility Parameters ▾

Linear Cell  Travelling Wave

Voltage (V):

Pressure (Torr):

Temperature (°C):

Gas Mass (Da):

Dead Time (t<sub>0</sub> in ms):

Drift Cell Length (m)

Ion Mobility Parameters ▾

Linear Cell  Travelling Wave

Calibration Parameter 1:

Calibration Parameter 2:

EDC Parameter:

Gas Mass (Da):

# UniDec Controls

- Allowed Charge Range
- Allowed Mass Range
- Linear resolution of the zero-charge mass spectrum
- Peak full width half max for the three peak shape functions
  - Split G/L is a Gaussian on the low  $m/z$  side and Lorentzian on the high  $m/z$  side to give a long tail at higher  $m/z$
  - Try fitting a peak using Tools > Peak Width Tool
- Run UniDec
  - Click to run, import, and plot the result
  - Error shows up along the bottom bar

UniDec Parameters

Charge Range: 1 to 100

Mass Range: 100.0 to 5000000.0 Da

Sample Mass Every (Da): 100.0

Peak FWHM (Th): 20.0

Peak Shape Function

Gaussian  Lorentzian  Split G/L

Run UniDec

# Advanced UniDec Controls

- Charge Smooth
  - Width of charge smooth filter
  - Almost always set to 1 unless using Mass Smooth or Isotope Mode instead, in which case it may be turned off if desired using 0
- Mass Smooth
  - Set mass difference and smooth width
  - Mass difference will incorporate neighboring species of known mass, such as neighboring oligomers, to help determine the charge
  - Best to use a width of 1 for on and 0 for off
- Number of Iterations
  - 100 seems to work well for nearly everything
- Isotope Mode
  - Uses Averagine model to project isotopic distributions, determine the charge state, and return the monoisotopic masses
- Manual Mode
  - Set list in Tools > Manual Assignment
  - Forces m/z values within a defined window to be a defined charge
- Mass List
  - Set list in Tools > Oligomer and Mass Tools
  - Turn on with the check box
  - Set window size in the box in units of Da
  - Masses will be limited to values in the list +/- the window size
- Native Charge Offset Ranges
  - Limits charges to a windowed offset from the predicted native charge
  - $Z_{native} = 0.0467 * m^{0.533}$
  - See Experimental > Plot Native Charge-Mass for additional tools
  - Useful for eliminating extremely high or low charge states in complex samples

Additional Filters/Restrains ▼

Charge Smooth Width: 1.0

Mass Difference (Da): 0.0

Mass Smooth Width: 0.0

Maximum # of Iterations: 100

Isotope Mode  Manual Mode

Mass List Window: 0 Da

Native Charge Offset Range

-100.0 to 100.0

# IM-MS Controls

- Allowed CCS Range
- Resolution of CCS sampling
- Peak full width half max arrival time dimension
  - Note: the peak is assumed to be Gaussian in arrival time and set to the user-defined peak shape function in m/z
- CCS Smooth Width
  - Similar to the charge and mass smooth filters
  - Uses the CCS difference defined above as the resolution of CCS sampling
  - Usually 0 but can be increased to account for unfolded charge states
- Native CCS Offset Range
  - Similar to Native Charge Offset
    - $\Omega_{native\_He} = 4.06739 * m^{0.629424}$
    - $\Omega_{native\_N_2} = 5.33311 * m^{0.613072}$

UniDec Parameters

Charge Range: 1 to 100

Mass Range: 100.0 to 5000000.0 Da

CCS Range: 100 to 25000 Å<sup>2</sup>

Sample Mass Every (Da): 100.0

Sample CCS Every (Å<sup>2</sup>): 100

Peak FWHM (Th): 20.0

Peak FWHM (ms): 0.2

Peak Shape Function

Gaussian  Lorentzian  Split G/L

Run UniDec

Additional Filters/Restrains

Charge Smooth Width: 1.0

Mass Difference (Da): 0.0

Mass Smooth Width: 0.0

CCS Smooth Width: 0

Maximum # of Iterations: 100

Mass List Window: 0 Da

Native Charge Offset Range

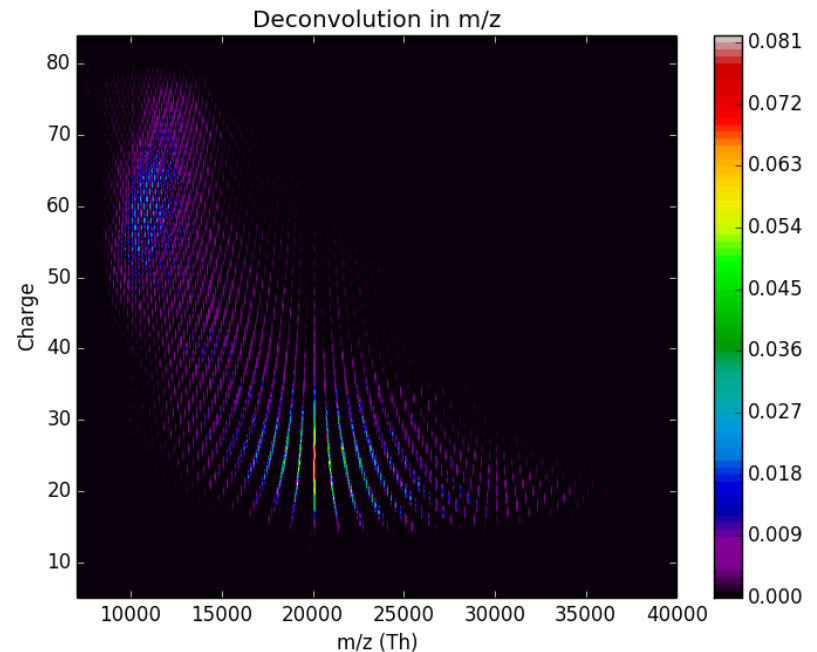
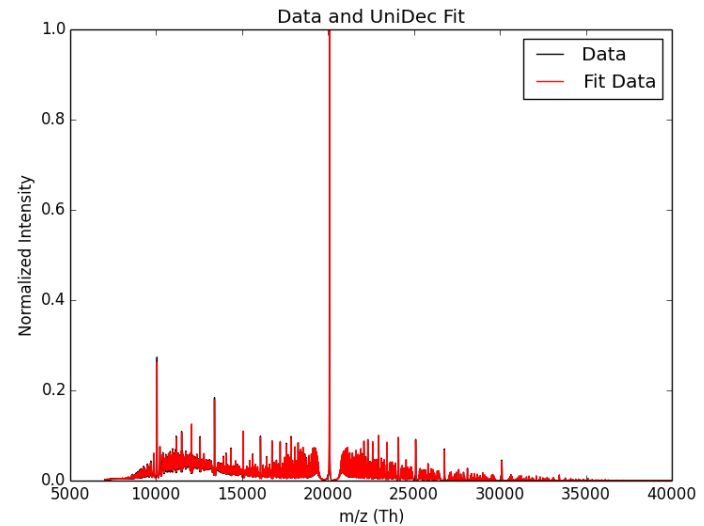
-100.0 to 100.0

Native CCS Offset Range

-20000 to 20000 Å<sup>2</sup>

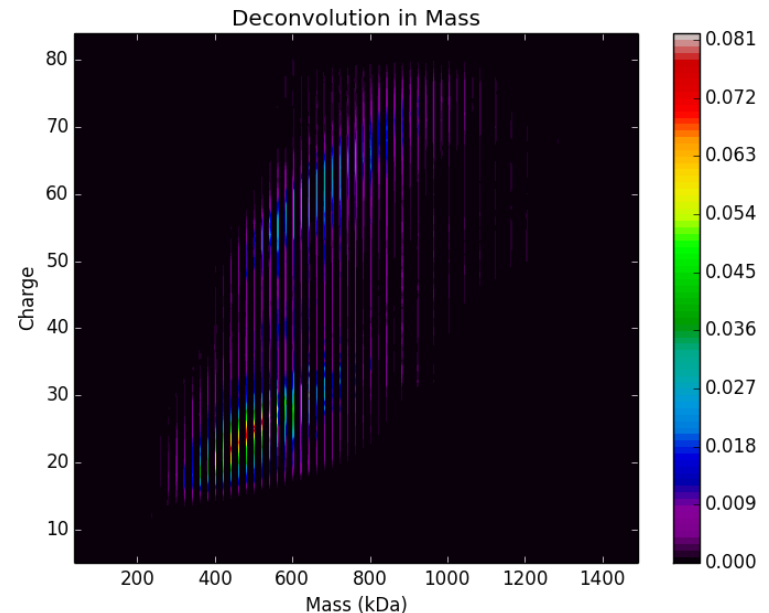
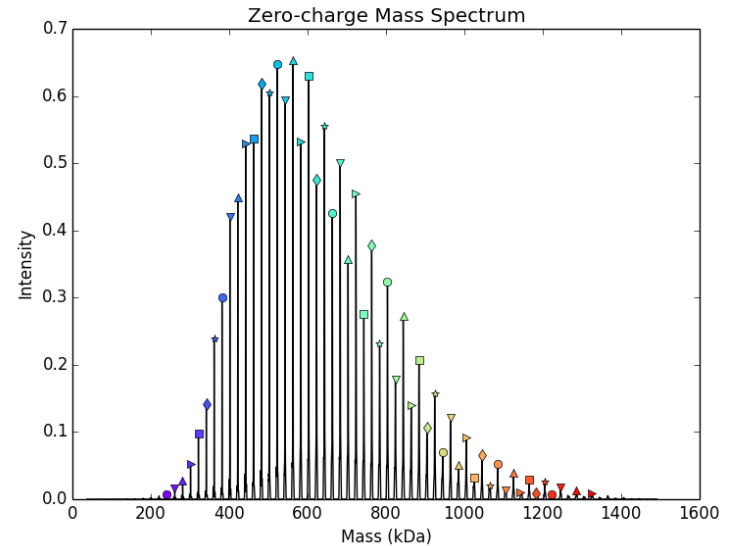
# Plotting the Results

- Plots are interactive
  - Click and drag to zoom
  - Click to unzoom
- Figure 1: Fit to the experimental data
  - Tip: If you zoom into a region and right click, you can zero the intensity in that region
  - Useful if there are contaminants dominating the spectrum
- Figure 3:  $m/z$  vs. charge



# Plotting the Results

- Figure 2: Zero-charge mass spectrum
  - Peaks are selected from this spectrum (see below)
  - Color and shapes match peak info list
  - Tip: Right clicking this plot gives integration results
    - If zoomed out and peaks are selected, it will integrate the peaks based on the specified windows
    - Right click spits out integral of zoomed region
    - If there is a single peak in the zoomed region, it will assign the integral to that peak and update the peak window
- Figure 5: Mass vs. charge





# Peak Detection and Plotting

- Peak Detection picks from the zero-charge mass spectrum
  - Must be maximum within Peak Detection Window
  - Must be above proportional Peak Detection Threshold
- Normalization allows peaks to be normalized to the maximum or normalized to the sum of all peaks
- Peak Information
  - Shows symbol, Mass, Peak Height, Peak Area, and Name of each peak
  - The name can be assigned using Tools > Oligomer and Mass Tools
  - Colors and symbols correspond to Figures 2, 4, and 6
  - Can be sorted by mass, intensity, or area
  - Right click gives options related to Figure 4 and the list:
    - Delete peak from Figure 4
    - Isolate peak in Figure 4
    - Repopulate the list with all
    - Label Charge States in Figure 4
    - Manually select that peak's color
  - The overall color function for peaks can be set in the Additional Plotting Parameters

Peak Selection and Plotting

Peak Detection Range (Da): 500.0

Peak Detection Threshold: 0.1

Peak Normalization

None  Max  Total

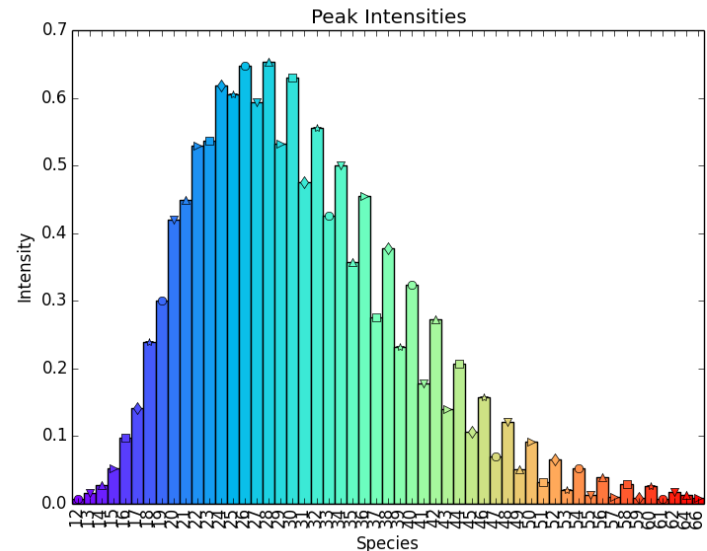
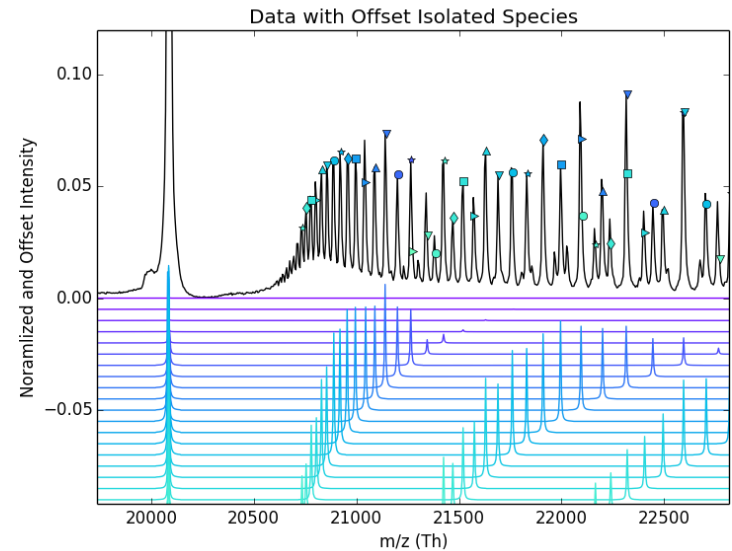
Peak Detection Plot Peaks

	Mass (Da)	Intensity	Area	Name
○	211788.0	100.0	100.0	A
▽	213288.0	37.9195...	42.9...	B
△	21			
▷	21			
□	21			
◇	21			
☆	22			

- Delete
- Isolate
- Repopulate
- Label Charge States
- Color Select

# Peak Detection and Plotting

- Figure 4: Individual peaks
  - Adds peak markers for species above Plot Marker Threshold
  - Plots Individual Species button plots extracted species offset by the Species Separation
- Figure 6: Bar chart of peak intensities
  - Names are assigned in Tools > Oligomer and Mass Tools



# Advanced Plotting Parameters

- Adjust 2D plots
  - Color map
  - Discrete Plot: Changes from continuous plot to discrete
- Publication Mode cleans up the plots and axes
- Select the deconvolved raw output vs. the reconvolved output that matches the data
- Marker Threshold and Species Separation specify parameters for Figure 4 (see above)
- Set integration range: Will integrate from peak-min to peak+max
- Plot Composite sums all of the curves in Figure 4 and plots it as a blue composite spectrum

Additional Plotting Parameters ▼

2D Color Map:

Peaks Color Map:

Discrete Plot  Publication Mode

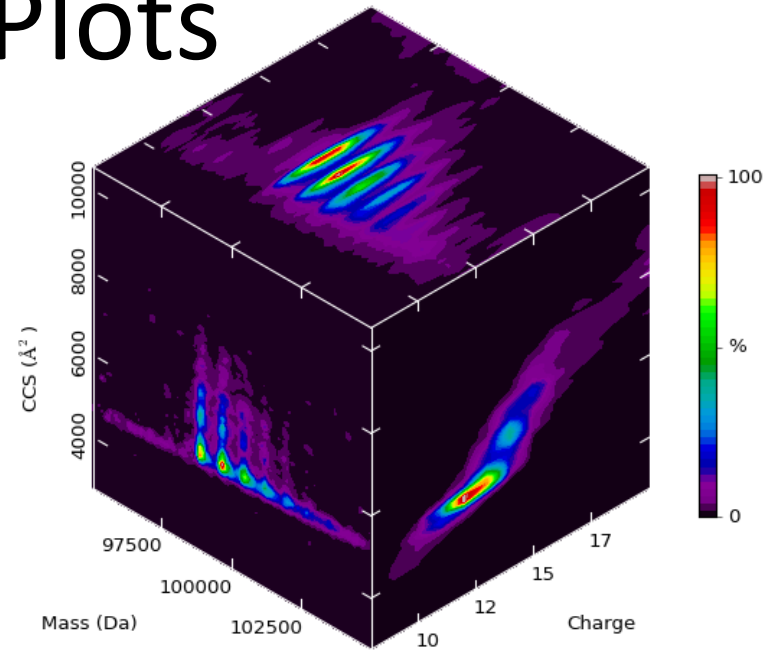
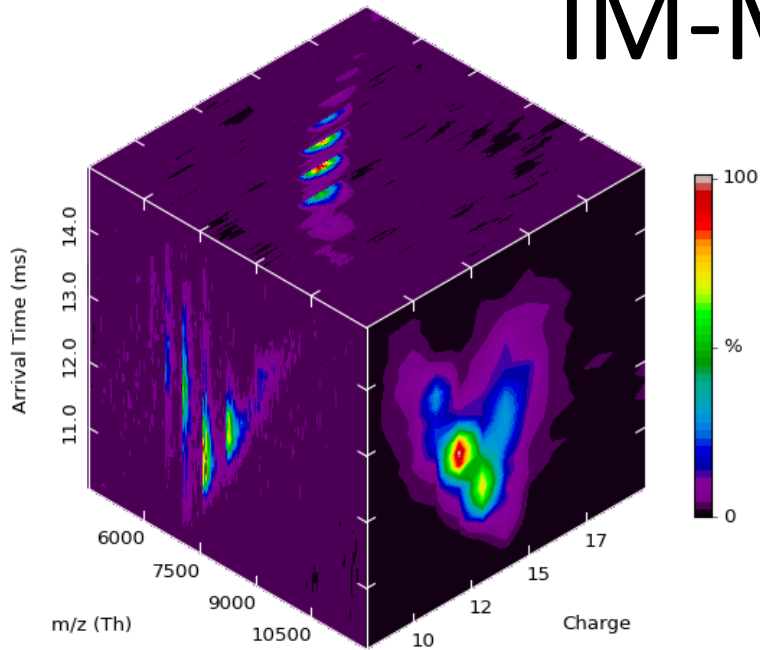
Reconvolved  Raw Results

Marker Threshold:

Species Separation:

Integration Range  
 to  Da

# IM-MS Plots



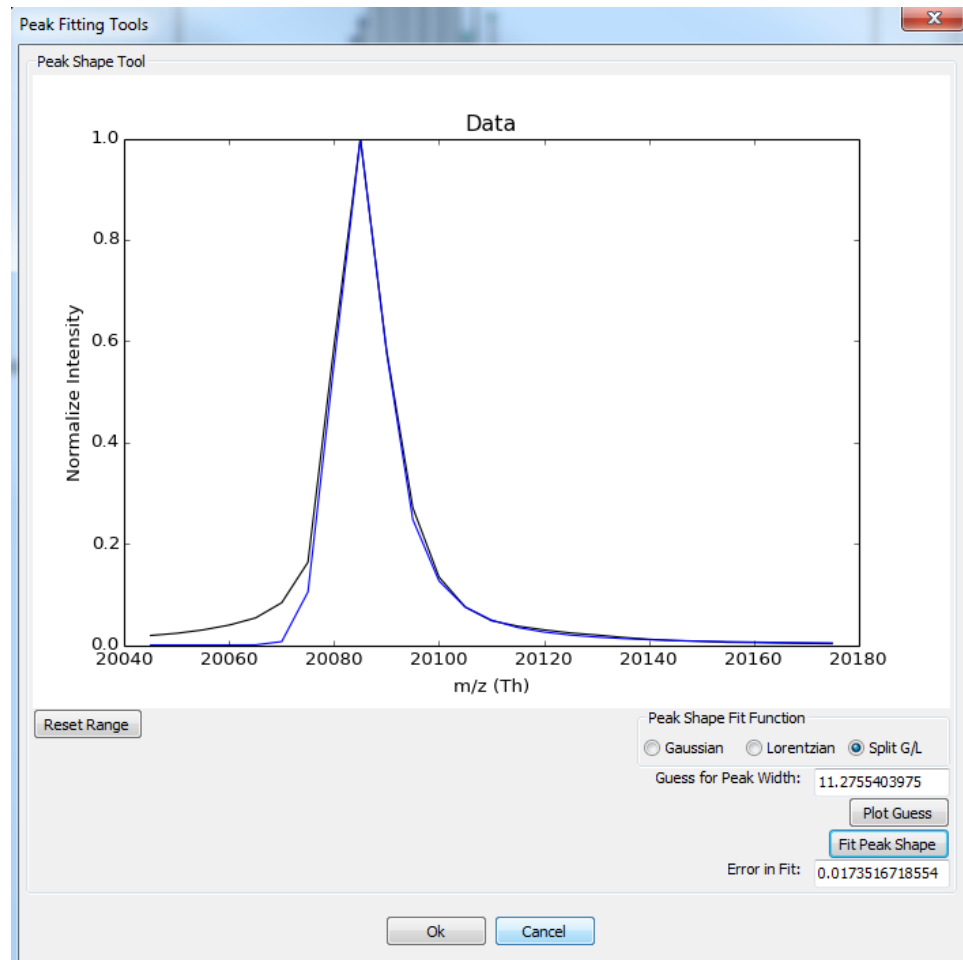
- IM-MS Plots are 1D or 2D projections of two 3D matrices
  - m/z vs. Arrival Time vs. Charge
  - Mass vs. CCS vs. Charge
- These cubes show projections on each face

# Useful Tools



# Peak Width Tool

- Zoom into an isolated peak in the processed data
- Reset Range returns to the full data
- Select peak shape function
- A guess may be entered and plotted
- Fitting the peak shape will update the guess and print out an error
- Ok will send guess and function selection to main control panel, Cancel will leave them alone
- IM-MS
  - Fit in 1D MS to get m/z peak width
  - Flip m/z and AT to extract arrival time trace at the center of the m/z peak
  - Fit arrival time distribution to get Arrival Time peak width



# Oligomer and Mass Tools

Limit Masses To Select Values

Set the Mass List for Limited UniDec

Populate from Peak List

Import from File

Populate from Isolated Oligomers

Populate from All Possible Oligomers

Manual Add Species

Clear List

Mass List

Mass (Da)

20085.0

40170.0

60255.0

80340.0

100425.0

120510.0

140595.0

160680.0

180765.0

200850.0

220935.0

241020.0

261105.0

281190.0

301275.0

321360.0

341445.0

361530.0

381615.0

Oligomer Maker

Import from File

Add Oligomer Species

Clear Oligomer List

Oligomer List

Base Offset (Da)	Monomer Mass (Da)	Min # of Oligo...	Max # of Oligo...	Name
0.0	20085.0	0	100	

Match Peaks to Oligomers

Match to Isolated Oligomers

Matched to Mixed Oligomers

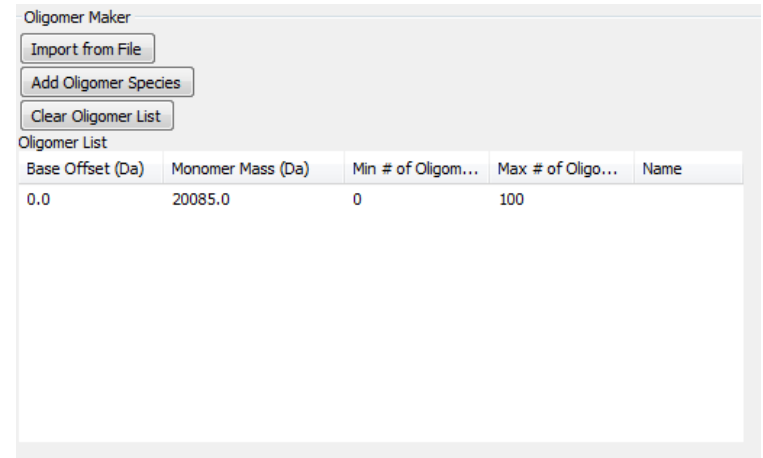
Peak Mass (Da)	Match	Error	Name
1004325.0	1004250.0	75.0	50
1024325.0	1024335.0	-10.0	51
1044425.0	1044420.0	5.0	52
1064425.0	1064505.0	-80.0	53
1084525.0	1084590.0	-65.0	54
1104625.0	1104675.0	-50.0	55
1124725.0	1124760.0	-35.0	56
1144725.0	1144845.0	-120.0	57
1164825.0	1164930.0	-105.0	58

Ok Cancel

Tools to limit masses, determine oligomeric states, and restrain the algorithm

# Oligomer and Mass Tools

- Oligomer Maker
  - Central platform for defining oligomeric species
  - Each row represents a separate subunit species
  - Define optional Base Offset, Monomer Mass, and range
  - Names are used for subunit number and may be blank
  - Add rows or clear list with buttons, delete specific elements with right click
  - Can Import from File usually with `_ofile.dat` suffix



Base Offset (Da)	Monomer Mass (Da)	Min # of Oligom...	Max # of Oligo...	Name
0.0	20085.0	0	100	

$$Mass[n] = Base + n * Monomer$$

For integers  $n$  from Min # of Oligomers to  
Max # of Oligomers



# Oligomer and Mass Tools

- Limit UniDec to masses in this list
  - Can import from the current peak list or from a file, often \_mfile.dat suffix
  - Manually add and clear species with buttons and delete with right click
  - Populate from Oligomer Maker using either isolated rows (see equation on previous slide) or by taking any possible combination from all rows

Set the Mass List for Limited UniDec

Populate from Peak List

Import from File

Populate from Isolated Oligomers

Populate from All Possible Oligomers

Manual Add Species

Clear List

Mass List

Mass (Da)

20085.0

40170.0

60255.0

80340.0

100425.0

120510.0

140595.0

160680.0

180765.0

200850.0

220935.0

241020.0

261105.0

281190.0

301275.0

321360.0

341445.0

361530.0

381615.0

401700.0

421785.0

441870.0

Match Peaks to Oligomers

Match to Isolated Oligomers

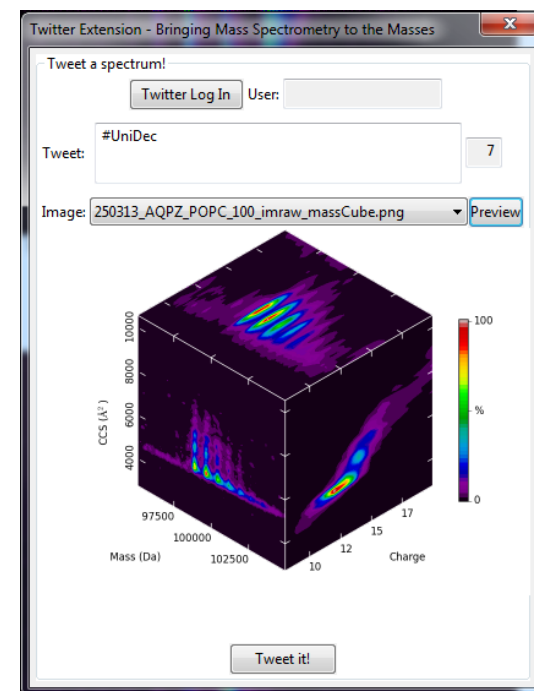
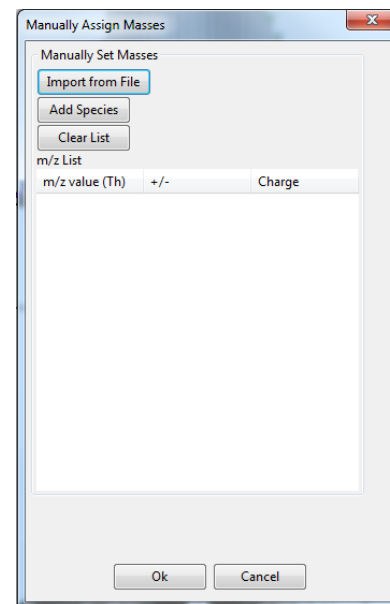
Matched to Mixed Oligomers

Peak Mass (Da)	Match	Error	Name
1004515.0	1004250.0	265.0	50
1025115.0	1024335.0	780.0	51
1044515.0	1044420.0	95.0	52
1065415.0	1064505.0	910.0	53
1084615.0	1084590.0	25.0	54
1105615.0	1104675.0	940.0	55
1124815.0	1124760.0	55.0	56
1143415.0	1144845.0	-1430.0	57
1164915.0	1164930.0	-15.0	58
1183415.0	1185015.0	-1600.0	59
.....	.....	...	..

- Match Peak to Oligomers
  - May be matched to isolated rows or any possible combination of rows from Oligomer Maker
  - Shows the measured Peak Mass, the nearest match, the difference between the mass and the match, and the number of subunits + the name of each oligomer

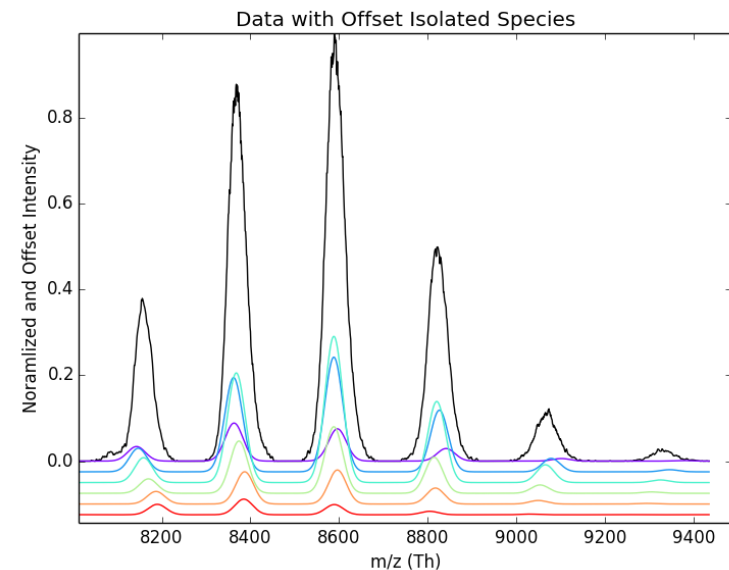
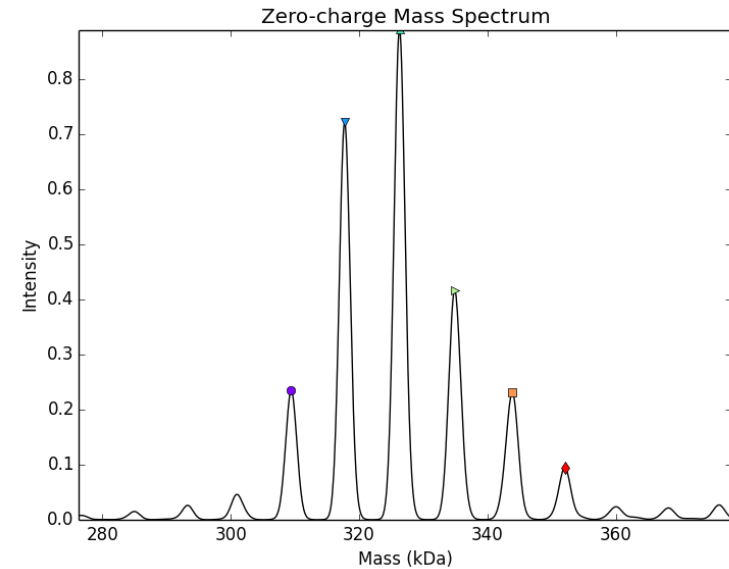
# Other Useful Tools

- **Batch Process**
  - Batch Process: Automatically applies the current settings to every selected text file, runs the program, and saves the state
  - Batch Process – Unfixed Data Ranges: Runs in batch with all of the same settings except the m/z and arrival time ranges. The m/z and arrival time ranges can be set independently ahead of time for each file.
  - Batch process Raw to Text: Automatically converts each Raw file selected into a .txt file
    - It will convert to 1D when in 1D mode and both 1D and 2D if in IM mode
- **Export Peaks' Charge Data**
  - Exports a 2D grid of peak vs. charge state for downstream analysis
- **The type of transformation from m/z to mass is selected under Advanced**
  - Interpolation is best for dense mass compared to sparse m/z
  - Integration is best for sparse mass and dense m/z
- **Manually Assign Masses**
  - For a given m/z value +/- some window, all of the intensity will be assigned to a defined charge state
  - Pretty much essential for MS/MS experiments
  - May be imported from file, often with suffix \_manualfile.dat
  - Make sure to check box on the Additional Filters/Restrains to tell UniDec to include manual assignments
- **File > Save Figures > Generate PDF Report**
  - Needs to be able to run the command “pdflatex” from the command line
  - For windows, install MikTeX and select install packages automatically from installation options
- **IM Tool**
  - Simulate peak locations in m/z vs. arrival time for specific mass and CCS values
  - For uncalibrated T-wave data, this is a way to guess at the parameters
- **Twitter Extension:**
  - Tweet your spectrum!
  - “Bringing Mass Spectrometry to the Masses”
  - Be a mass spectrometry social media pioneer



# Some Tips

- Start general and gradually add in restraints
- Watch for main sources of error:
  - Harmonics at double or half mass
    - Typically small
  - Satellites at plus or minus one charge state
    - Obvious from Figure 4
    - No unambiguous peaks
  - Noise/background
    - Often a smear at high charge
- Background subtraction can help
- Better data is always the best solution



# Thanks For Using UniDec!



Please let me know if you have questions, suggestions, or find any bugs