Polyacrylamide analysis by ECD

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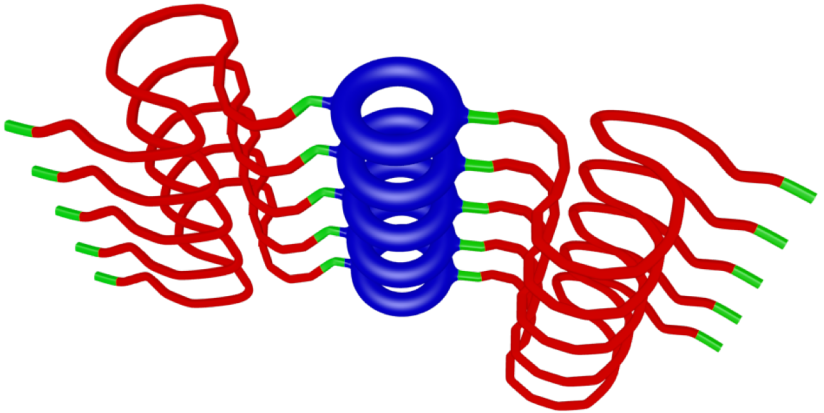
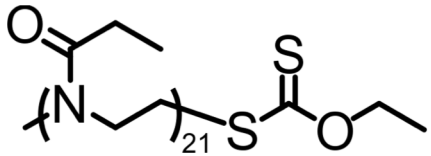
Biocompatible polymers for conjugation

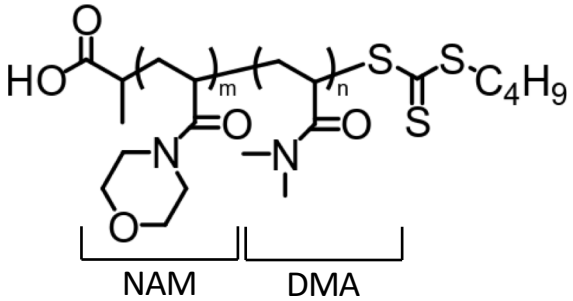
- Self-assembly peptide polymer conjugates
- A central cyclic peptide structure lacks biocompatible solubility and transport
- Polymer conjugation allows solvation and addition of targeting linkers
- Poly(oxazolines) and poly(acrylamides) are seeing more interest over poly(ethylene gylcol) (PEG)



Poly(oxazoline)



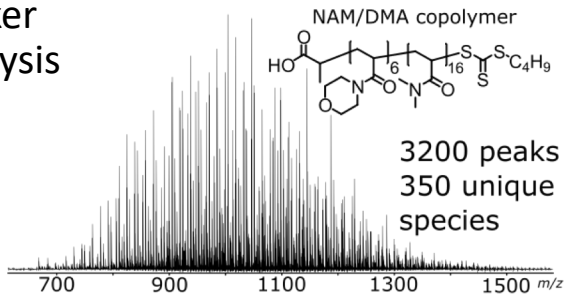
Poly(acrylamide)

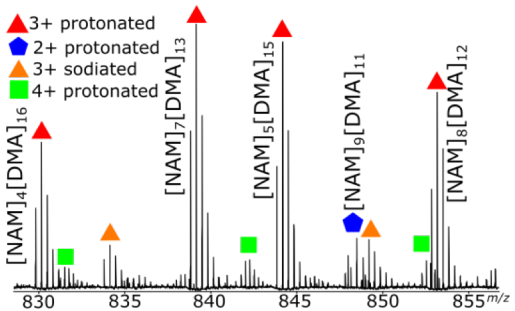


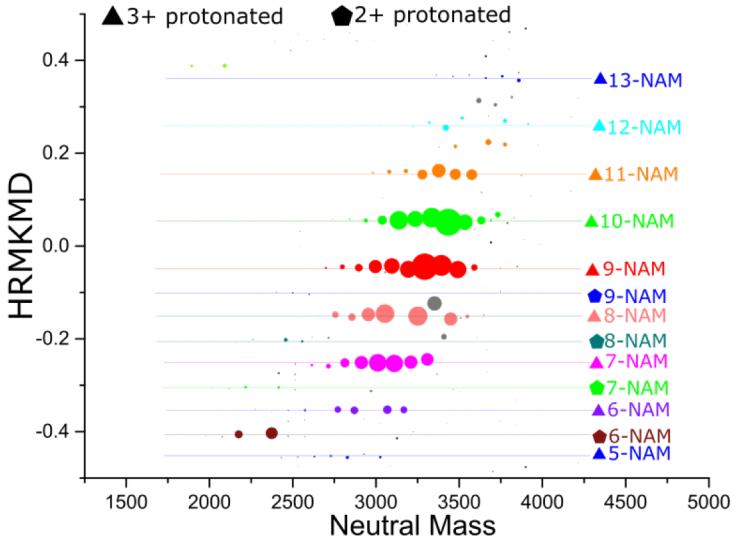
M. Danial *et al*., *Nature Comm.,* **2013**, 4, 2780

Biocompatible polymers MS analysis

- Nano-Electrospray ionisation and Bruker SolariX FT-ICR is used for polymer analysis
- Water soluble
- High resolution and mass accuracy achieves comprehensive analysis of copolymers < 5000 Da
- Use of modified Kendrick mass defect analysis







Further polymer Kendrick mass defect studies:

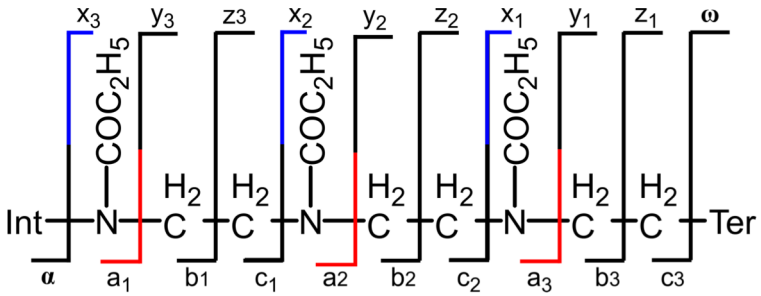
T. Fouquet, H. Sato, *Rapid Commun. Mass Spectrom.*, **2016**, 30 (11), 1361

T. Fouquet, R. B. Cody, H. Sato, *J. Mass Spectrom.*, **2017**, 52 (9), 618

T. Fouquet *et al*, J. Am. Soc. Mass Spectrom., **2018**, 29 (8), 1611

Biocompatible polymers MS and MS/MS analysis

- Sequence analysis of poly(oxazolines) and end groups
- Electron capture dissociation achieved sequence and end group coverage
- Radical dissociation less affected by weak bonds



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Coupling Electron Capture Dissociation and the Modified Kendrick Mass Defect for Sequencing of a Poly(2-ethyl-2-oxazoline) Polymer

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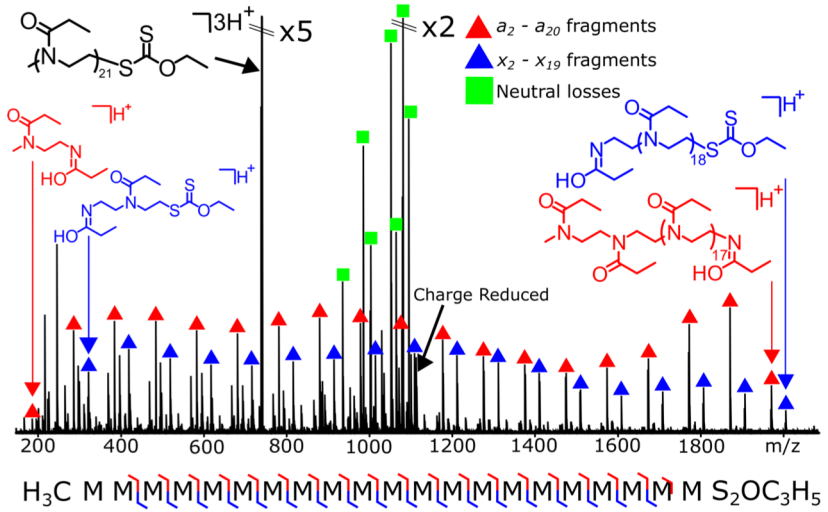
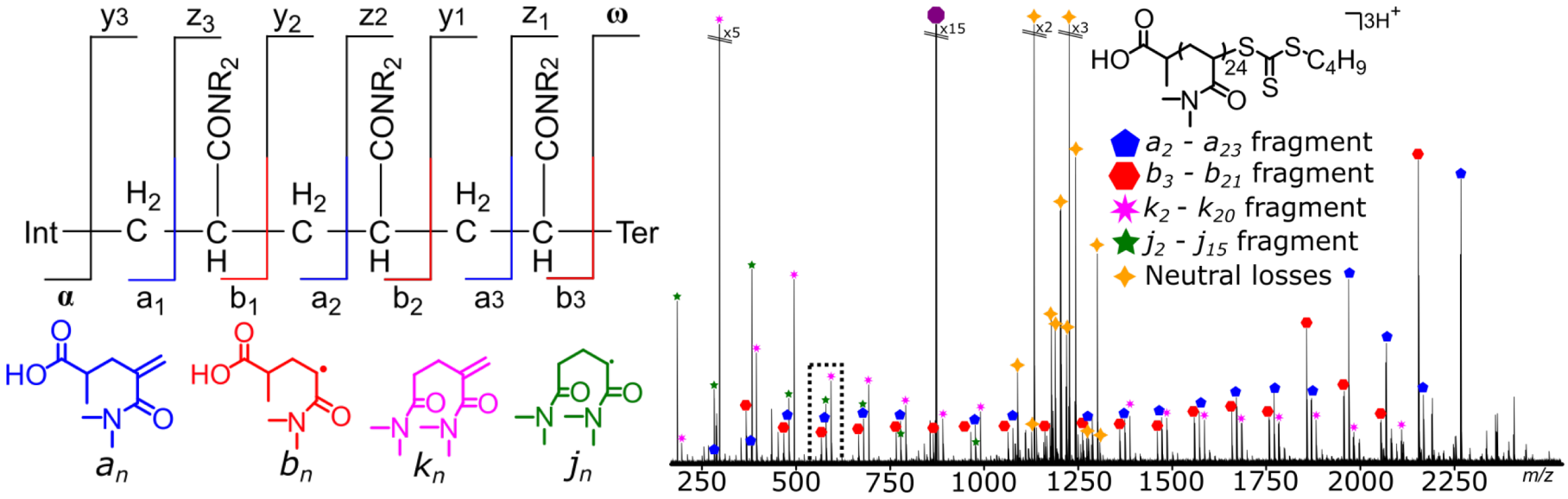


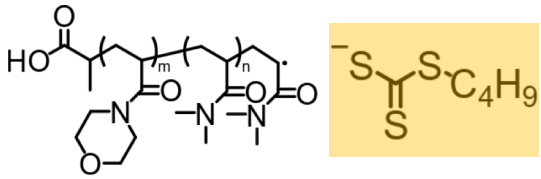
Acrylamide tandem MS analysis with ECD

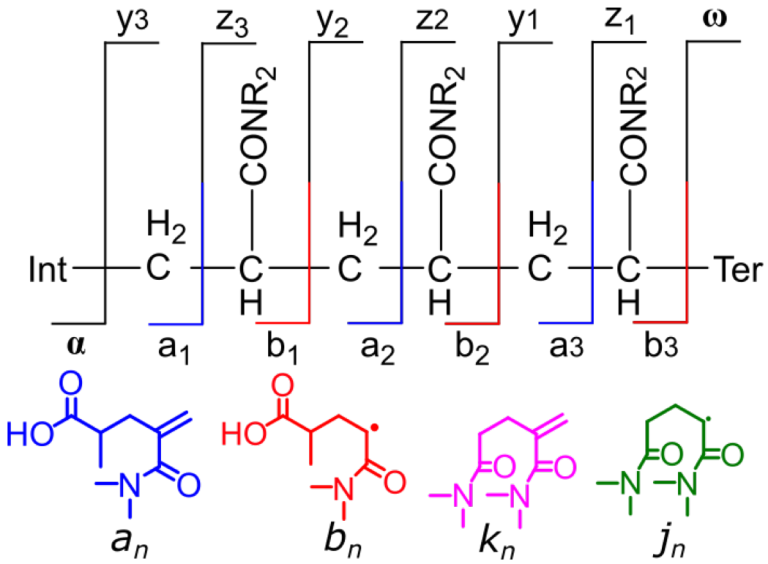
- Acrylamide fragmentation by ECD shows significant fragmentation coverage
- Fragmentation occurs along the carbon backbone suggesting the fragmentation isn't occurring by the usual ECD mechanism

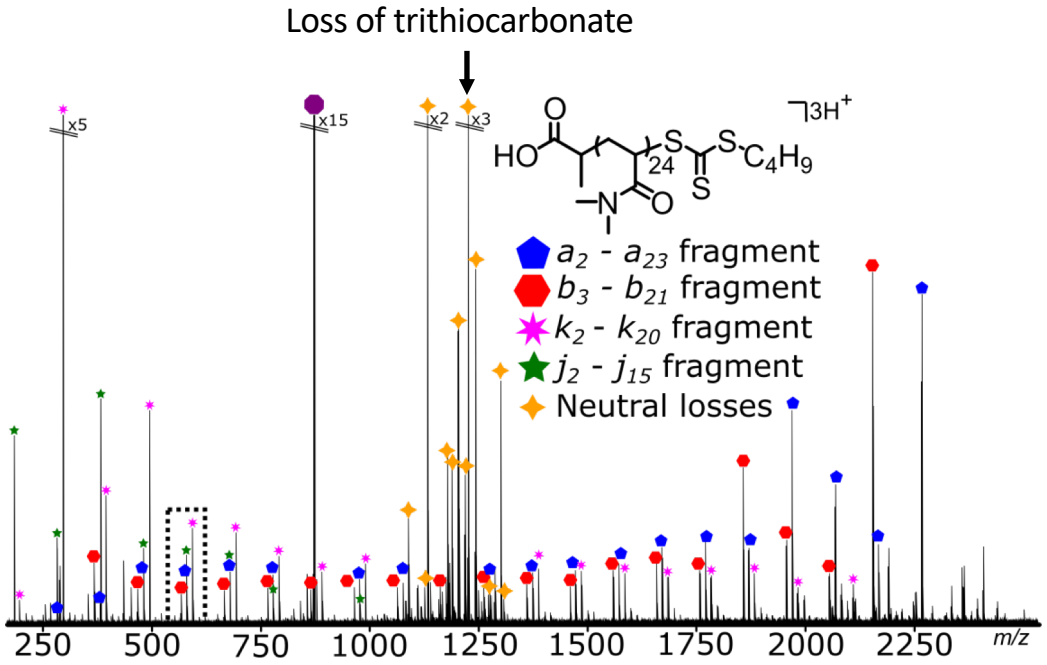


Acrylamide tandem MS analysis with ECD

- Largest neutral loss peak is loss of trithiocarbonate
- No sequence fragments contain the trithiocarbonate RAFT agent

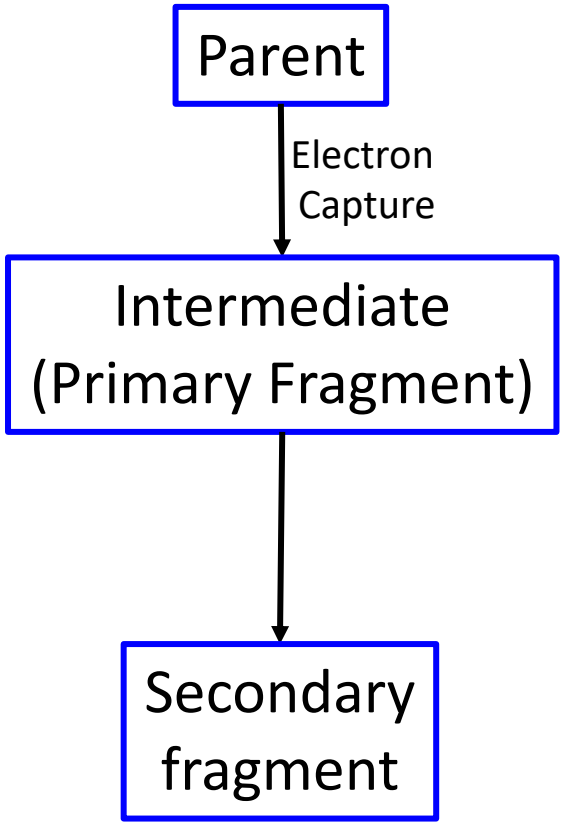


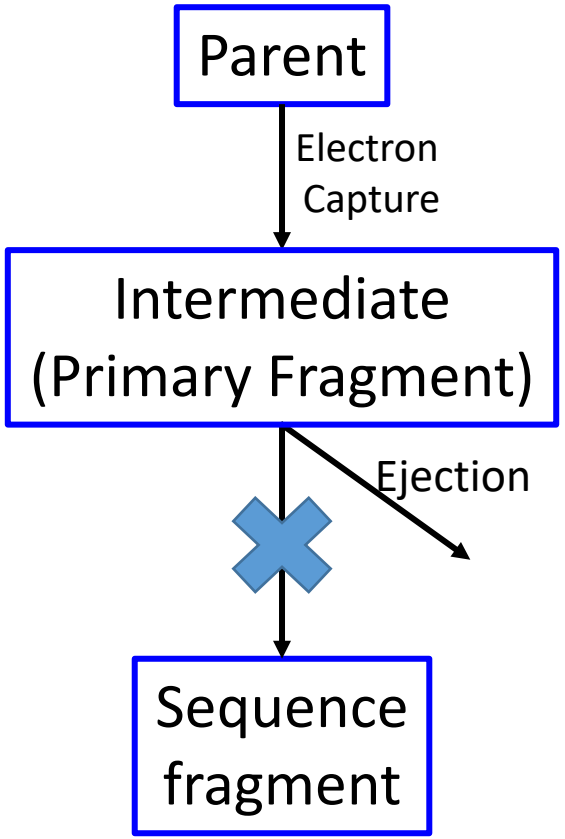




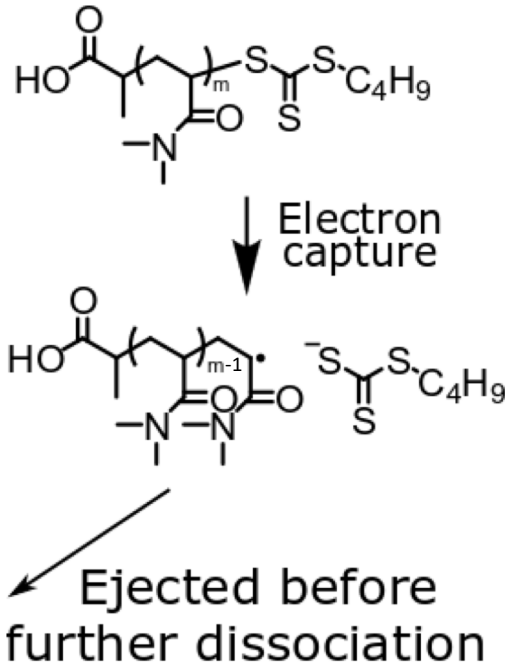
ECD MS/MS – Double resonance – ion ejection experiments

- How much does the trithiocarbonate loss affect the fragmentation?
- We can test this using double resonance.
- Removal of fragment before it is able to dissociate further.
- Shows intermediates or parent ions to other fragments:

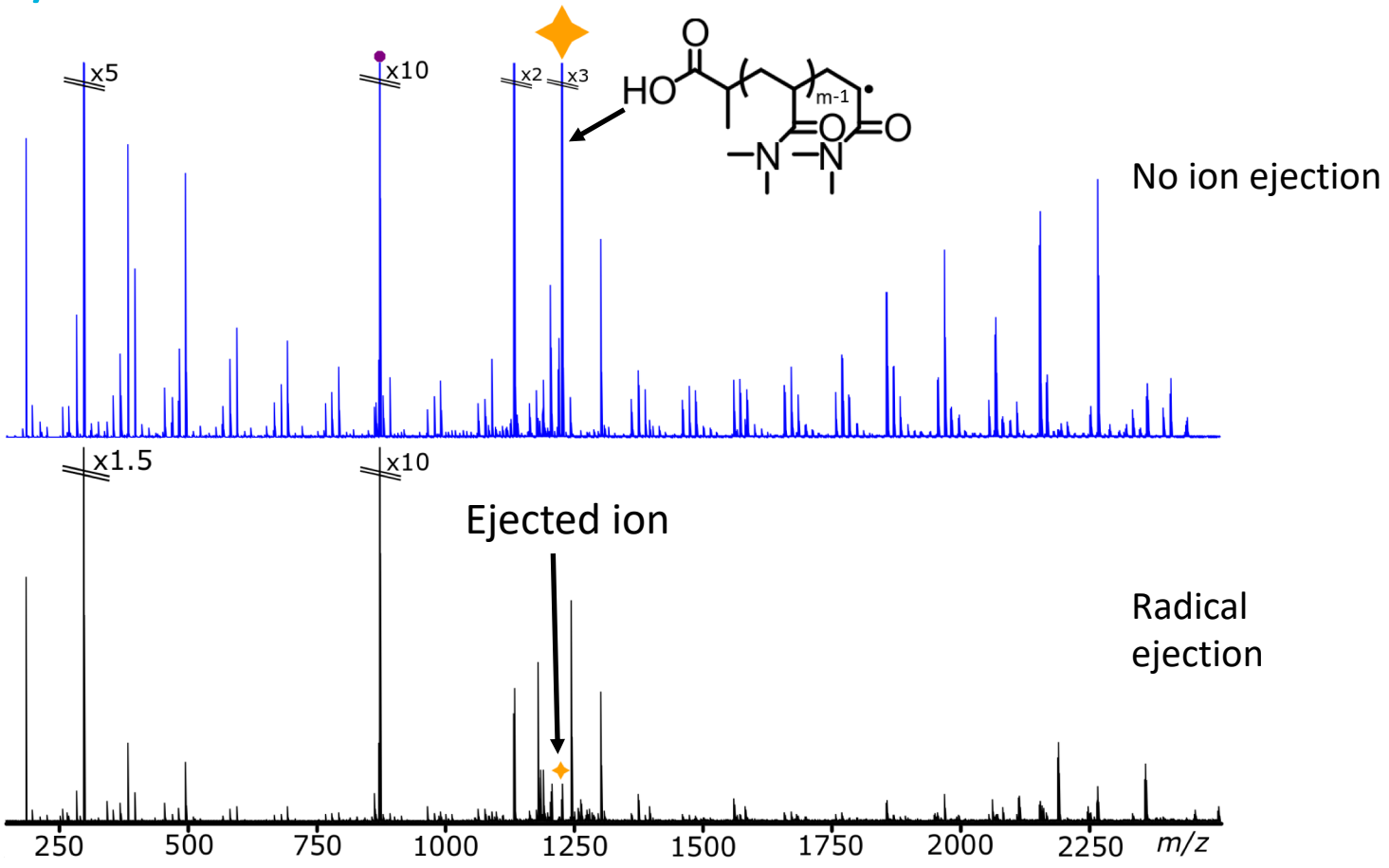




ECD MS/MS – Double resonance

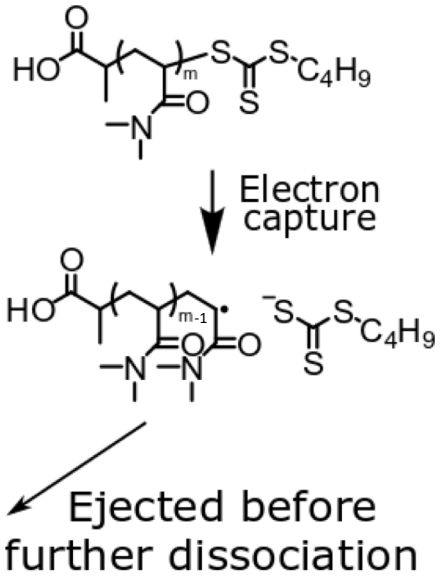


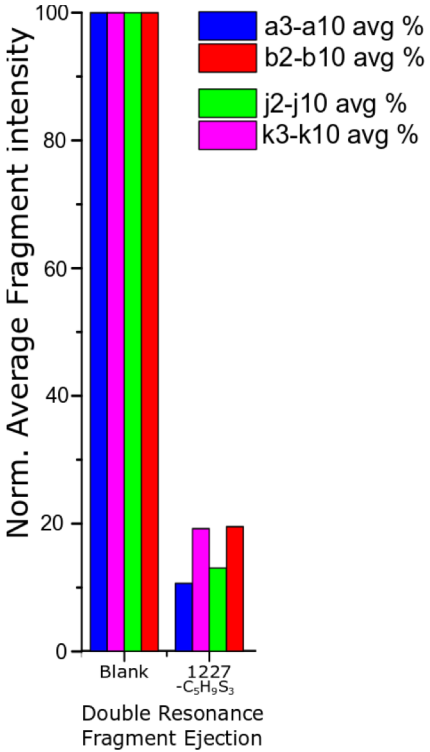
ECD MS/MS – Double resonance



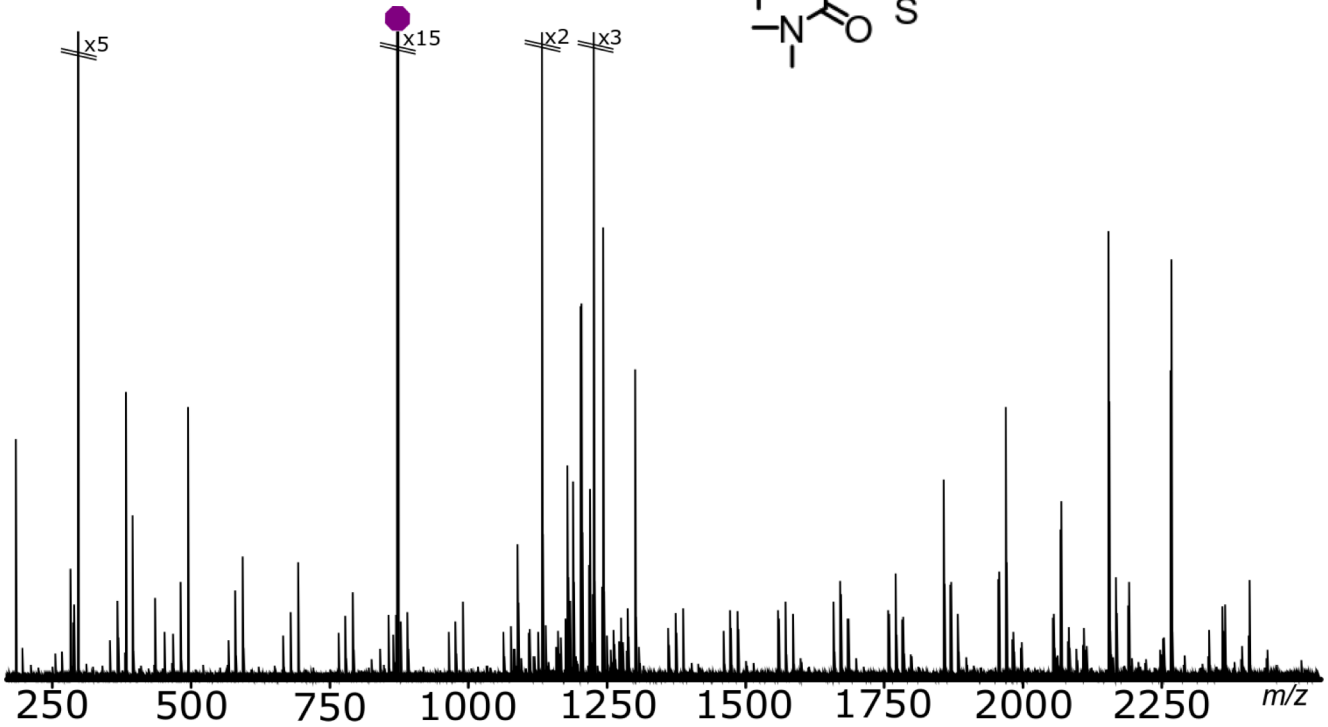
ECD MS/MS – Double resonance

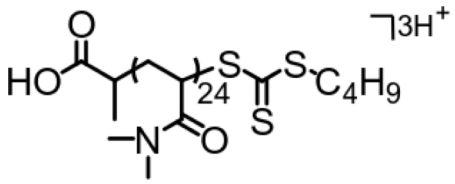
- Trithiocarbonate loss forms polymer radical
- Polymer radical is removed before further dissociation can occur
- Large reduction in sequence fragment intensity with removal of remaining radical

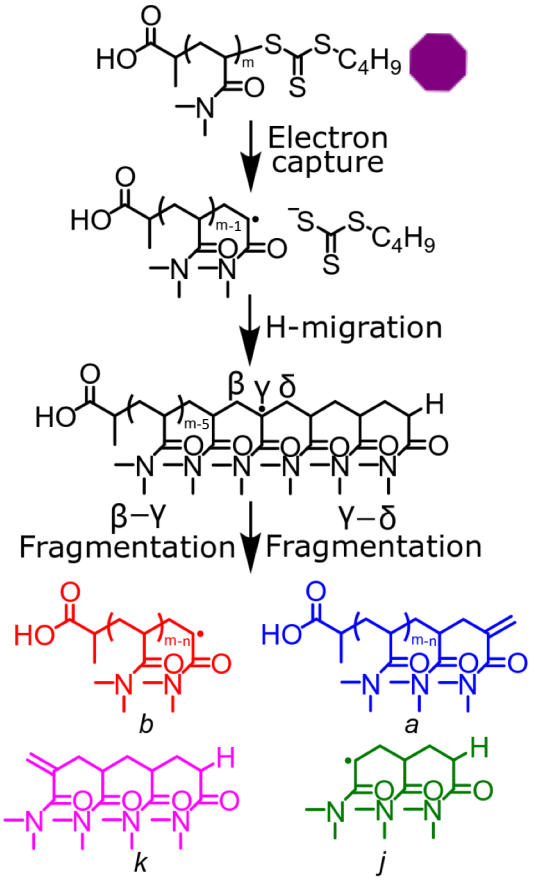




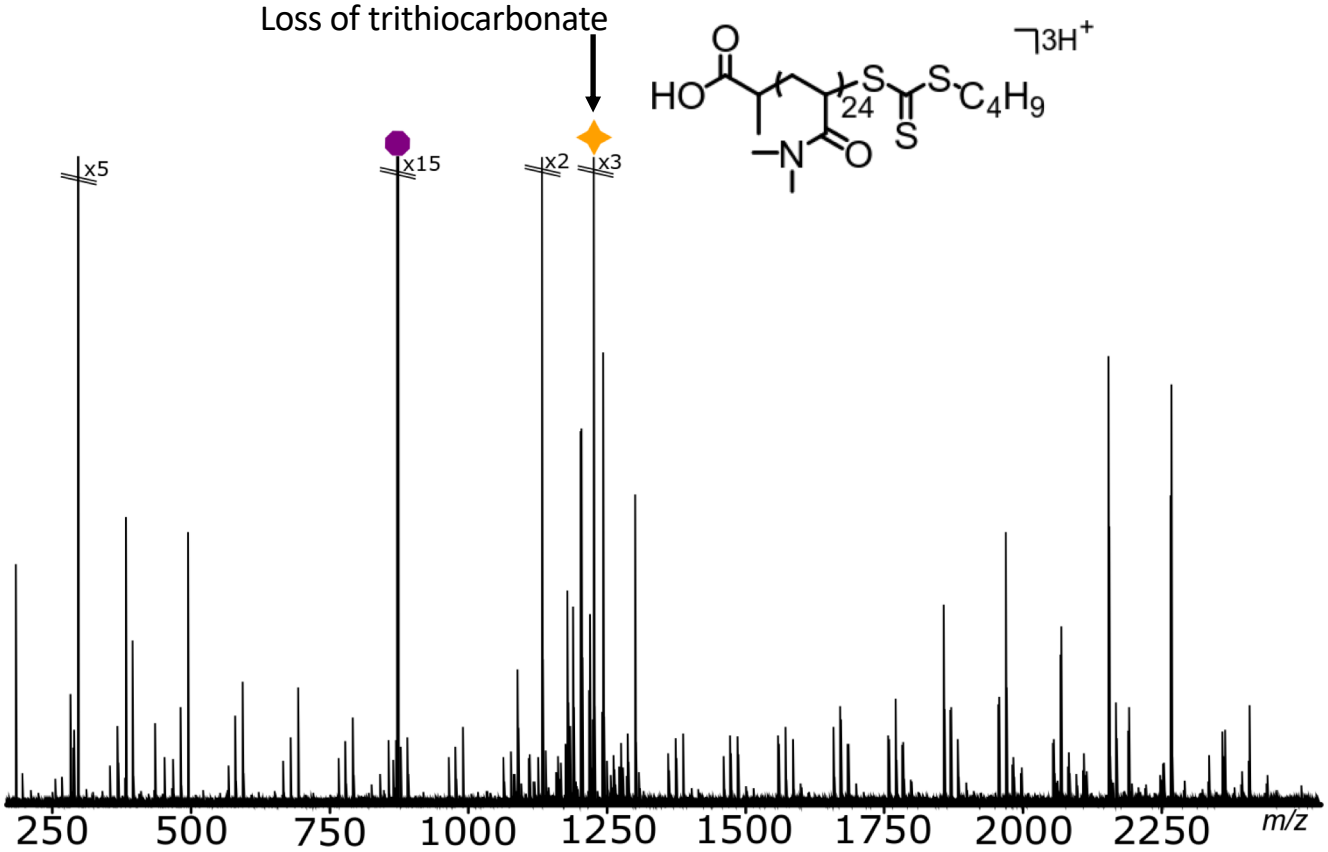
Trithiocarbonate end group DMA homopolymer

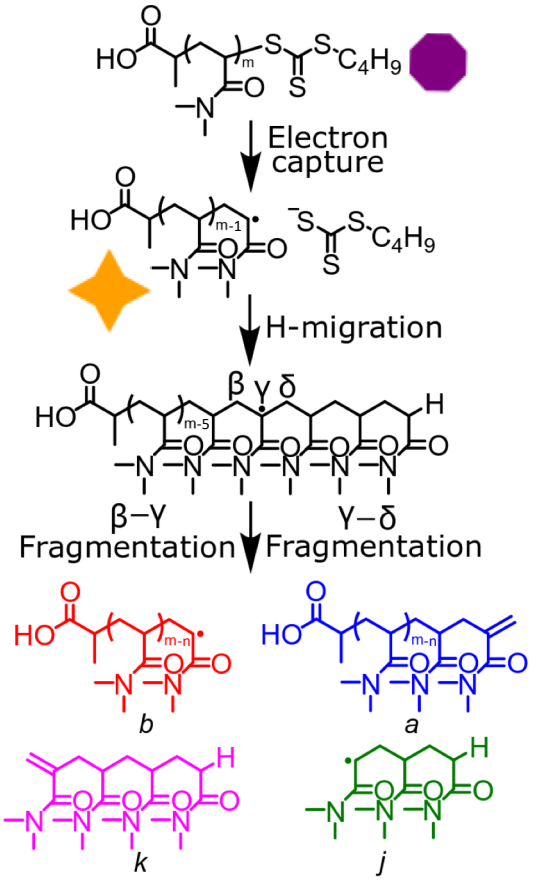




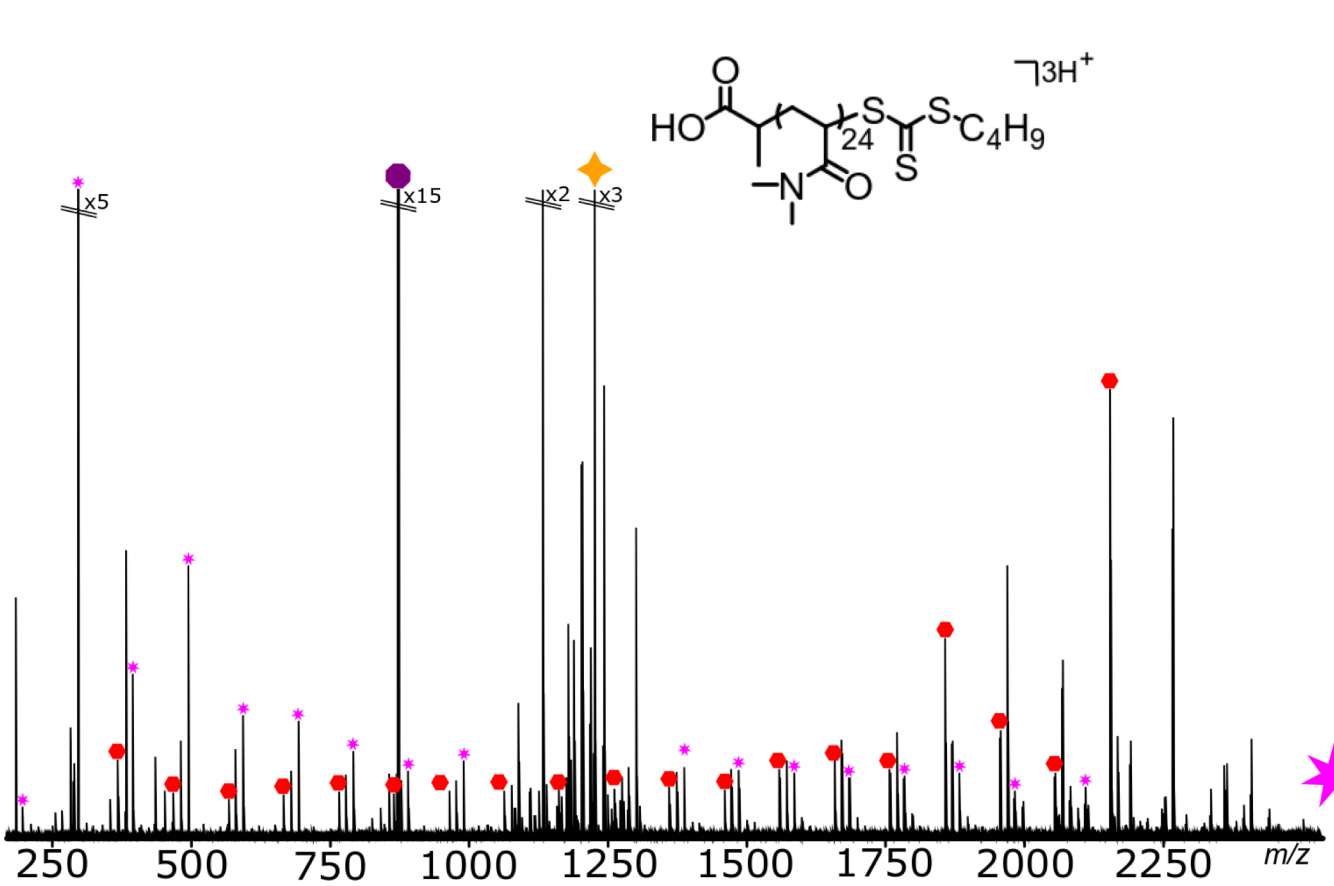


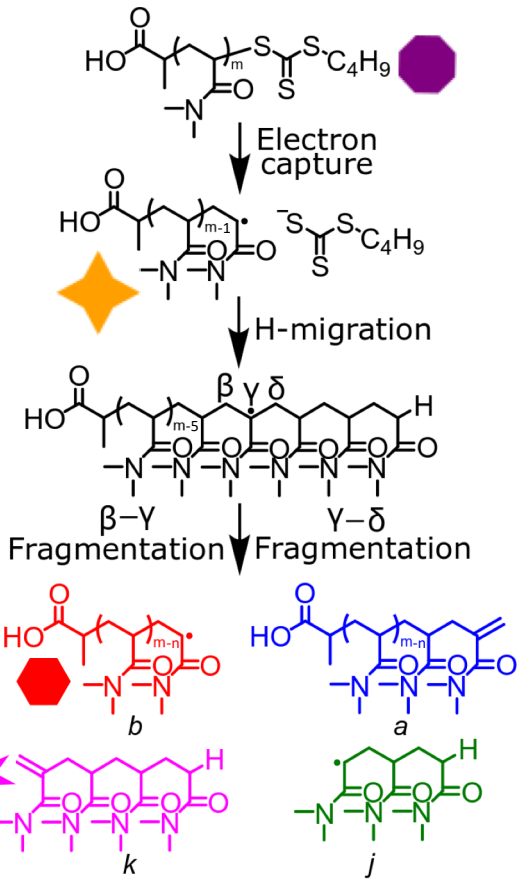
Trithiocarbonate end group DMA homopolymer





Trithiocarbonate end group DMA homopolymer





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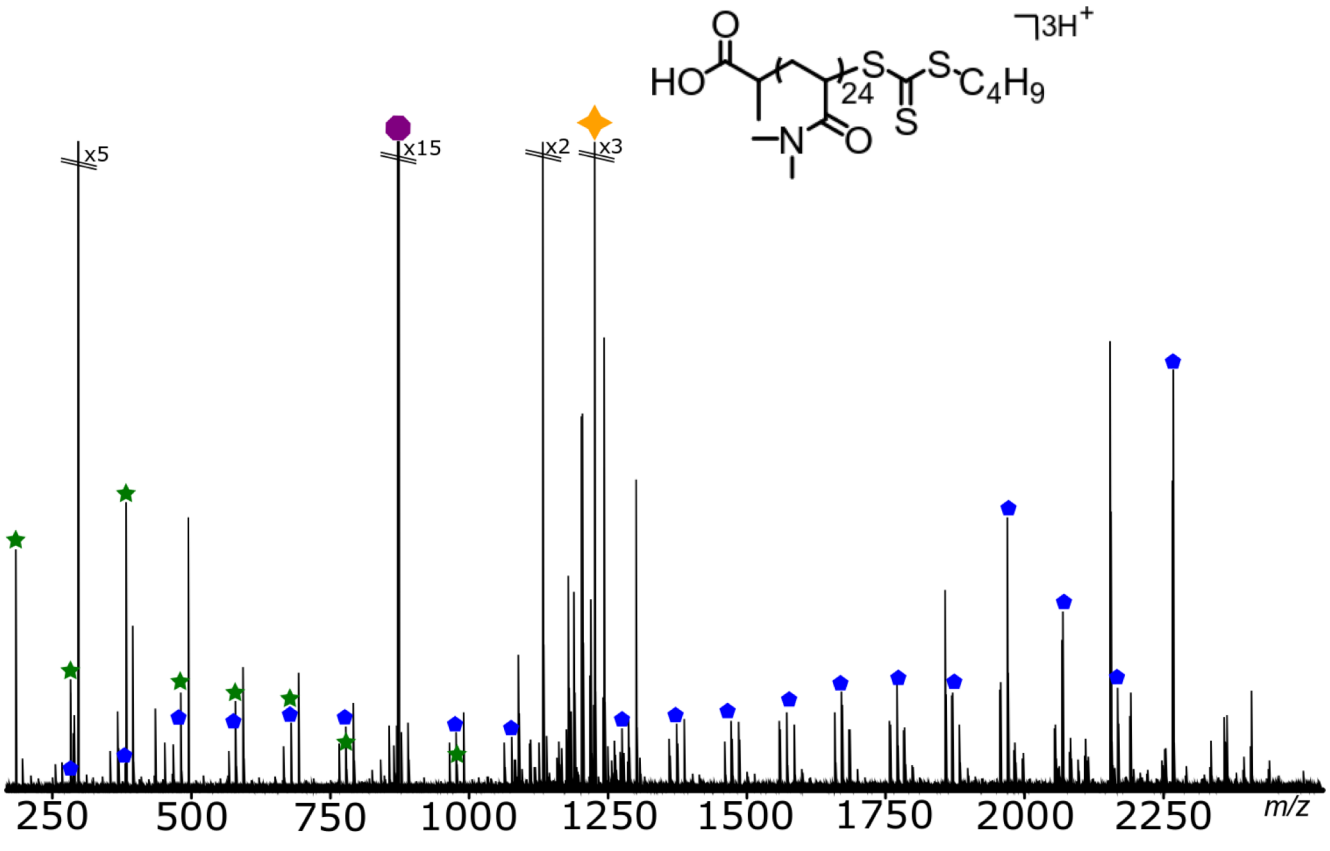

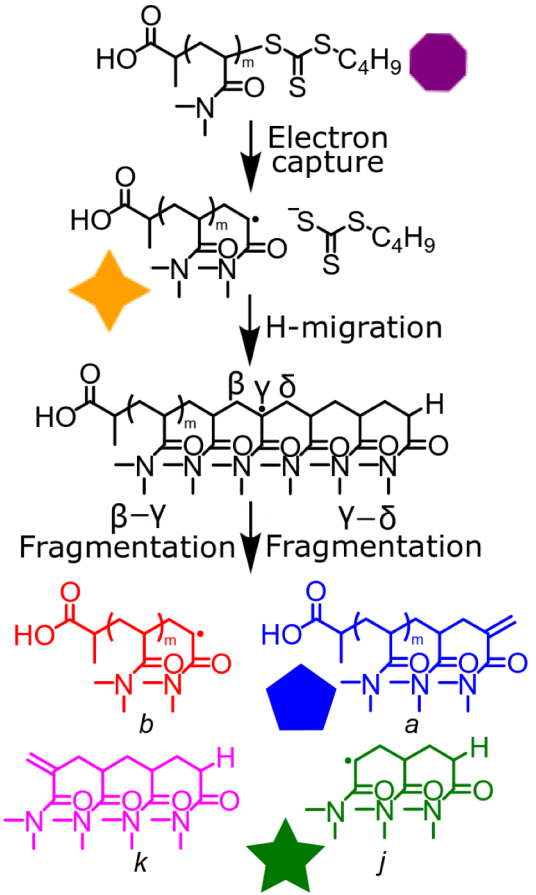

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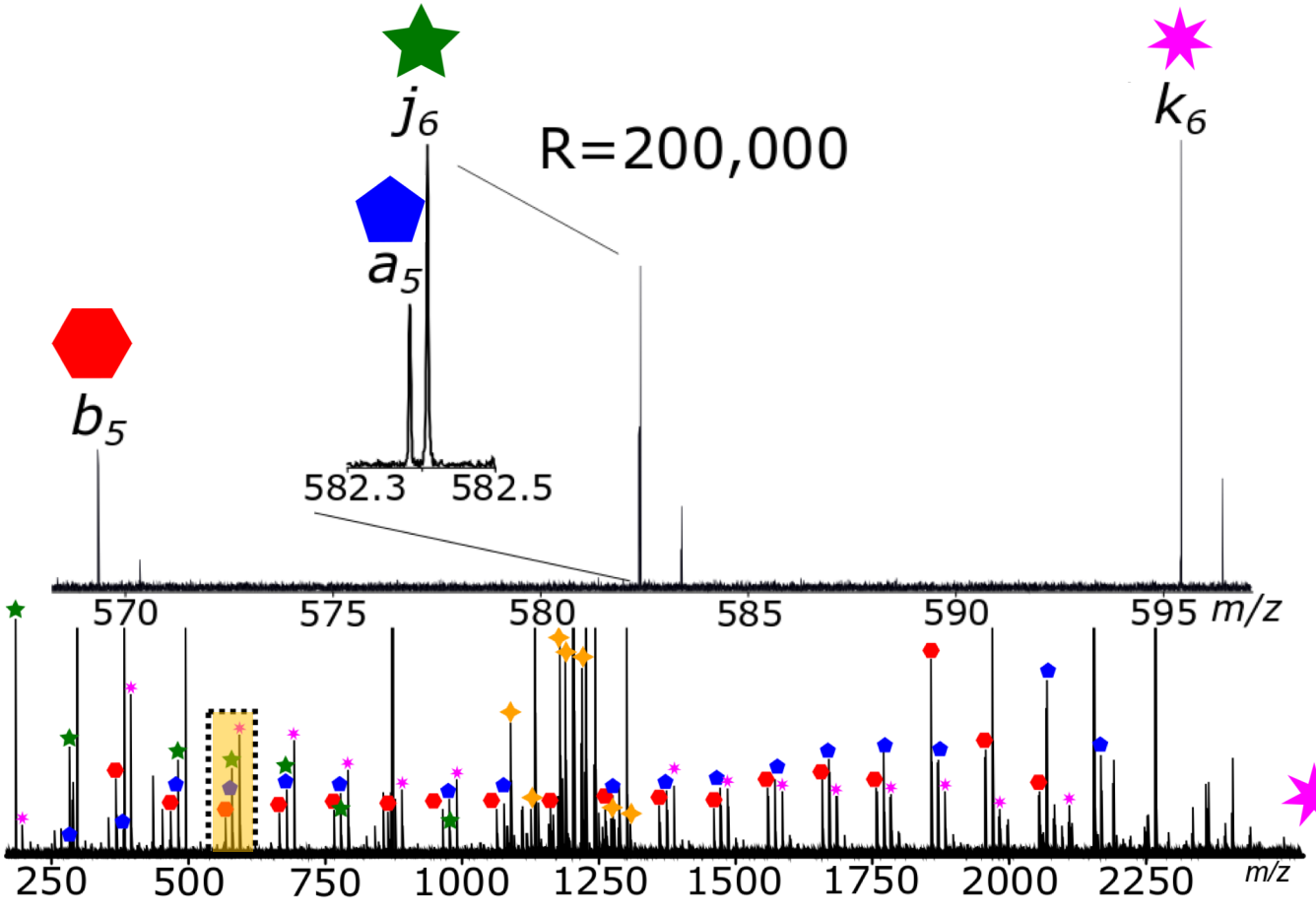
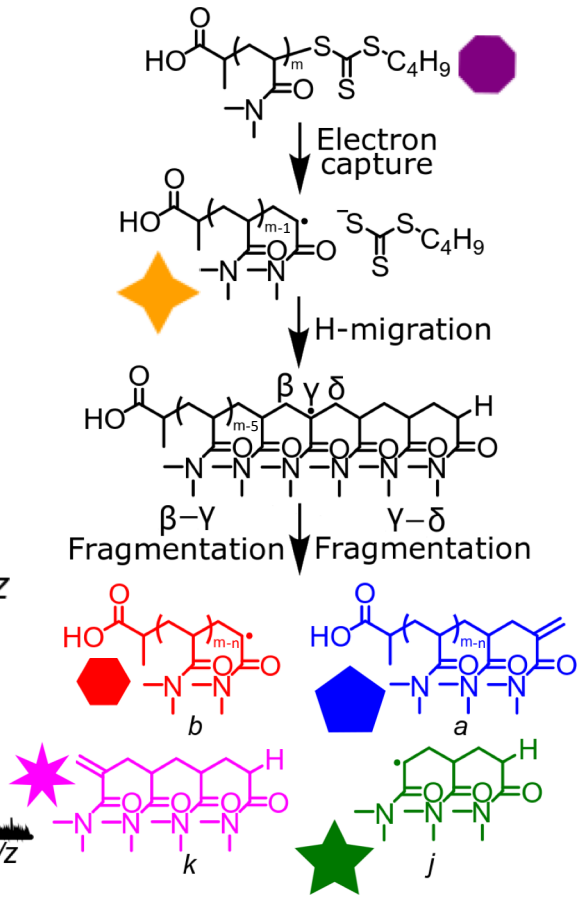
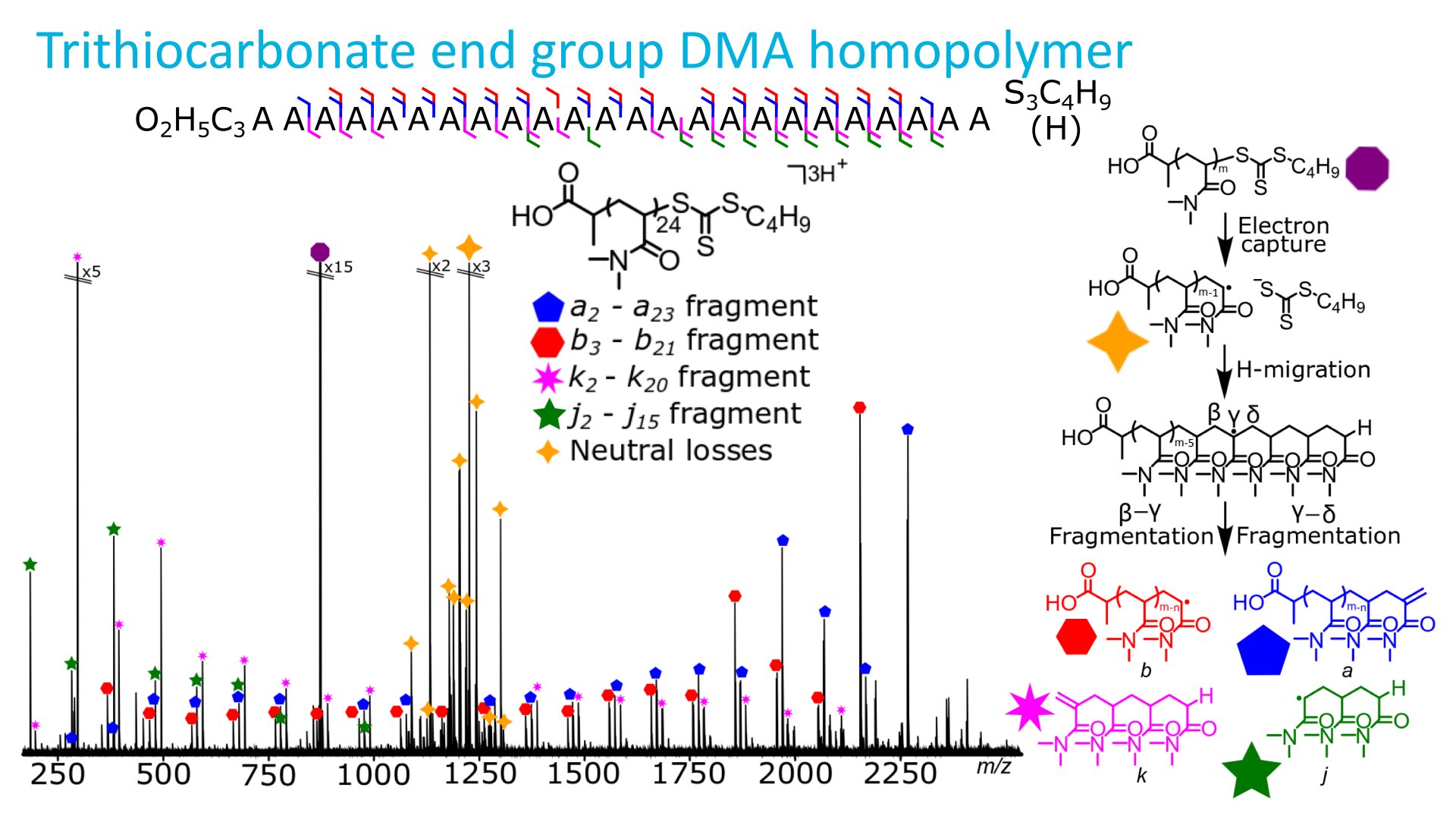




Trithiocarbonate end group DMA homopolymer



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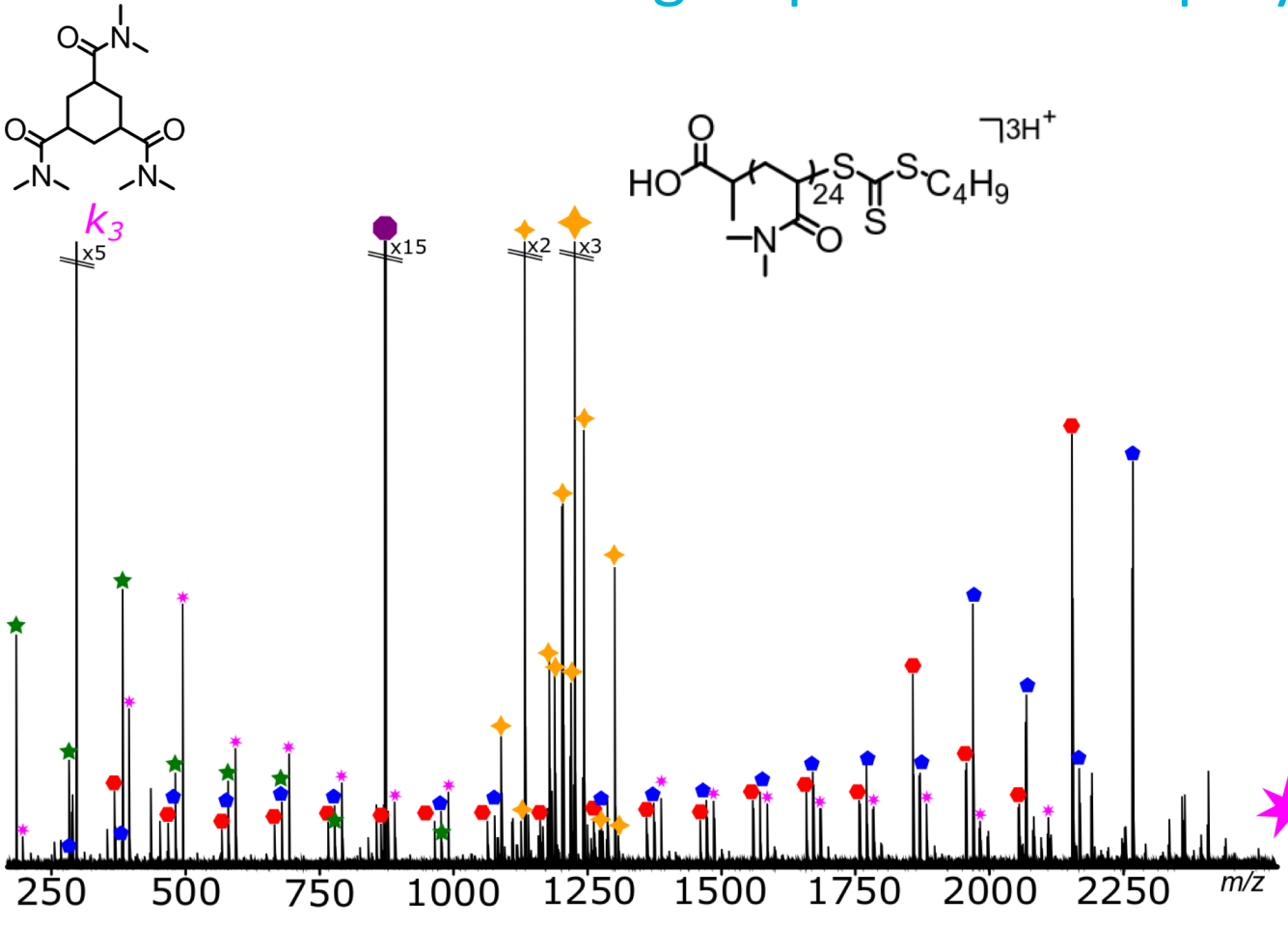

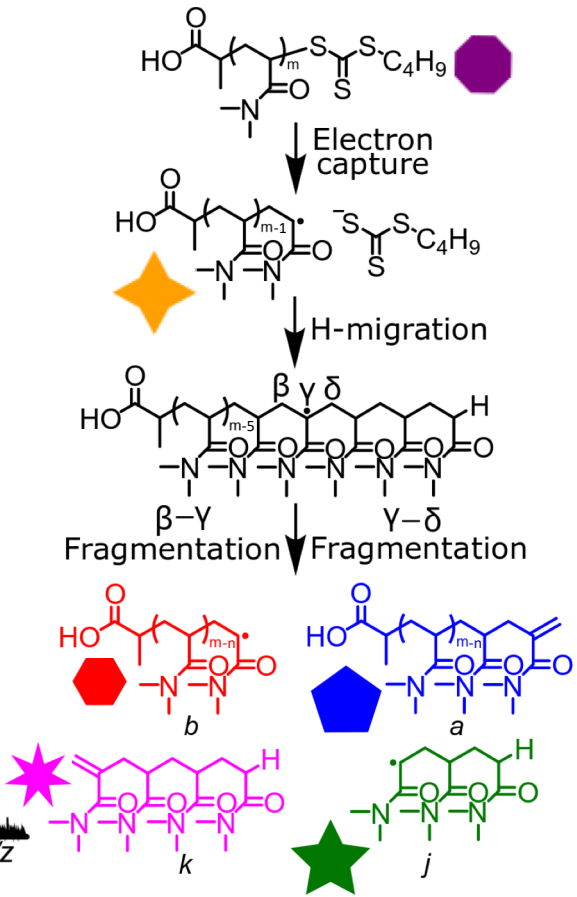

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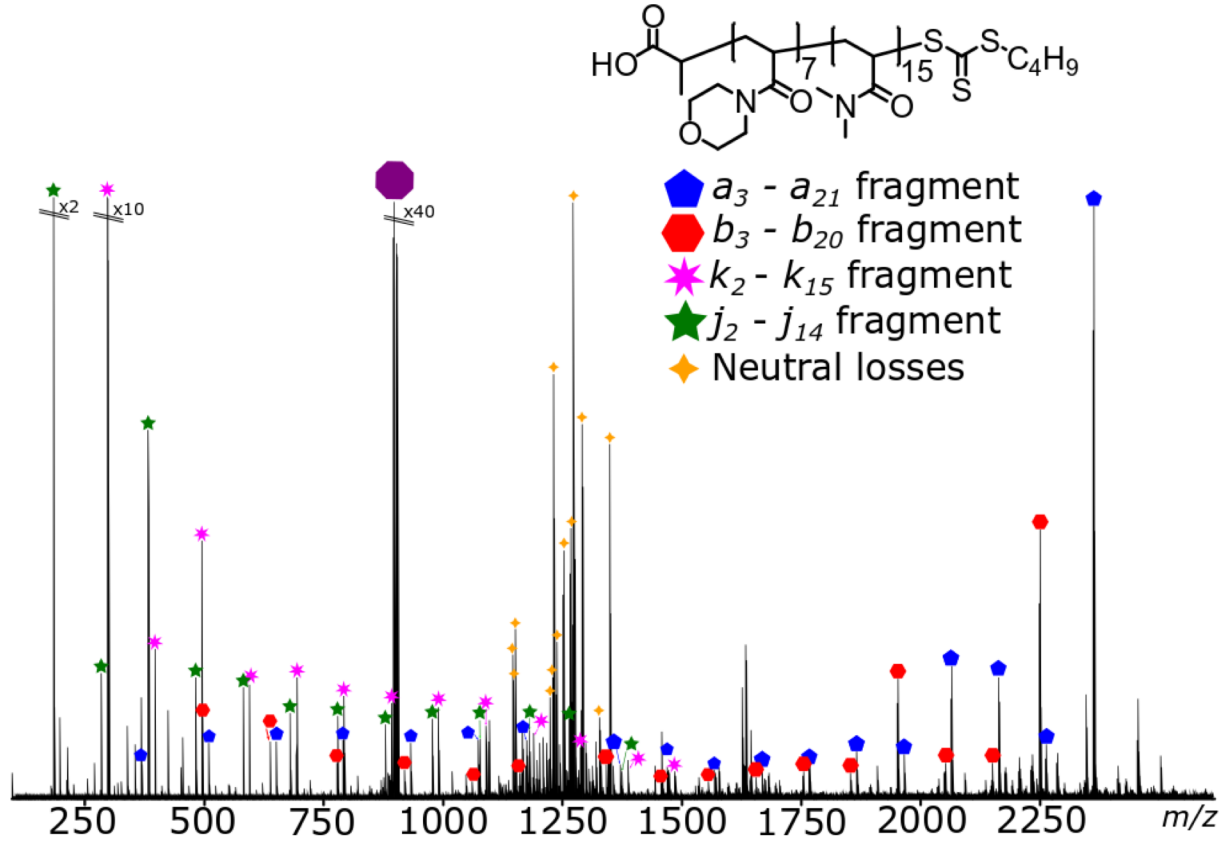

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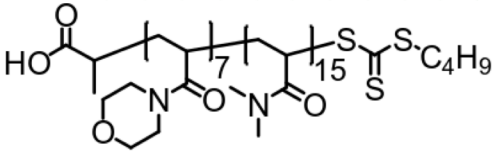

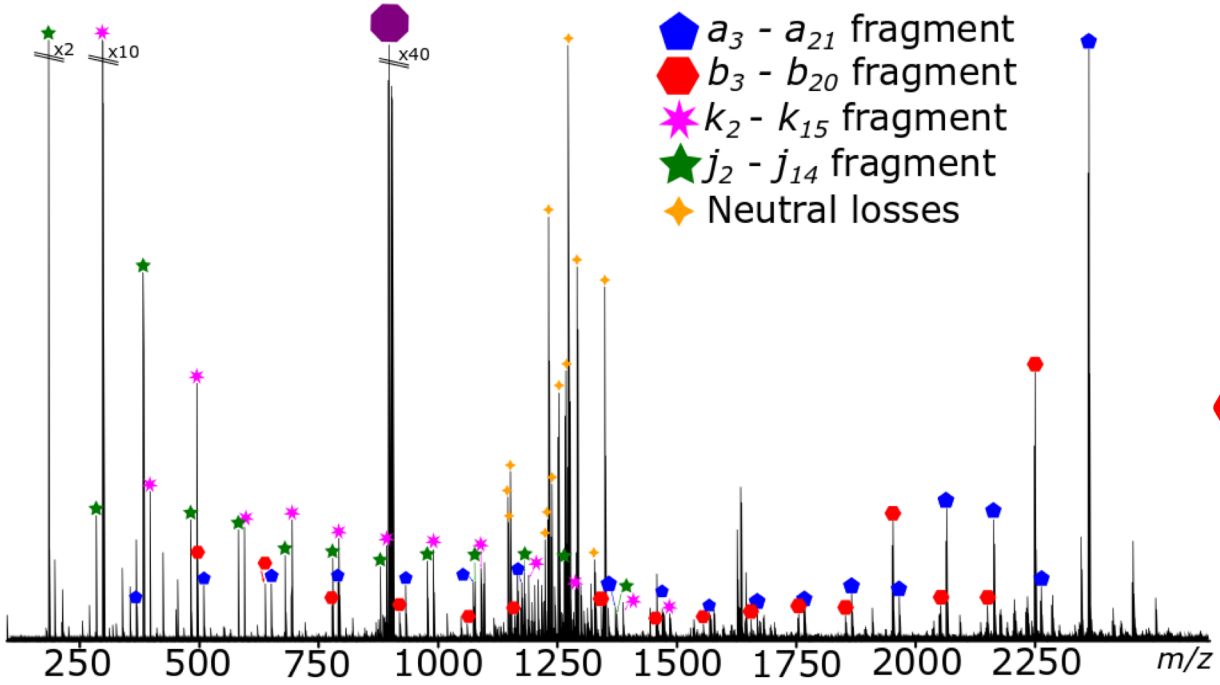

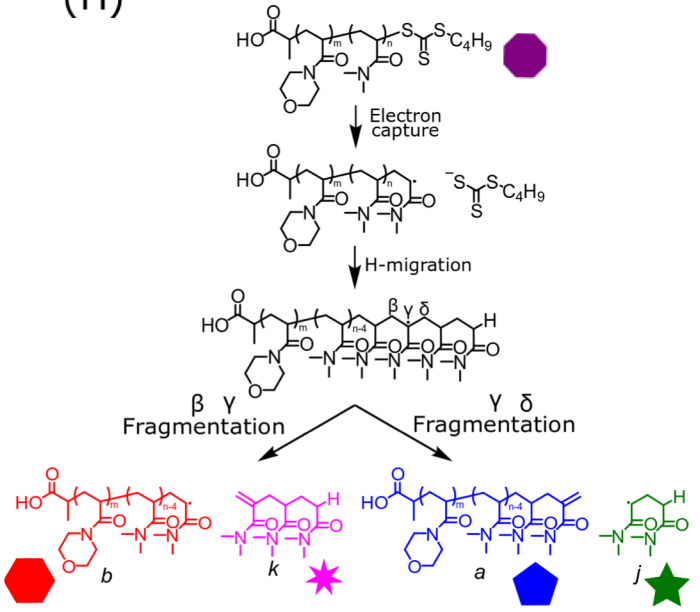

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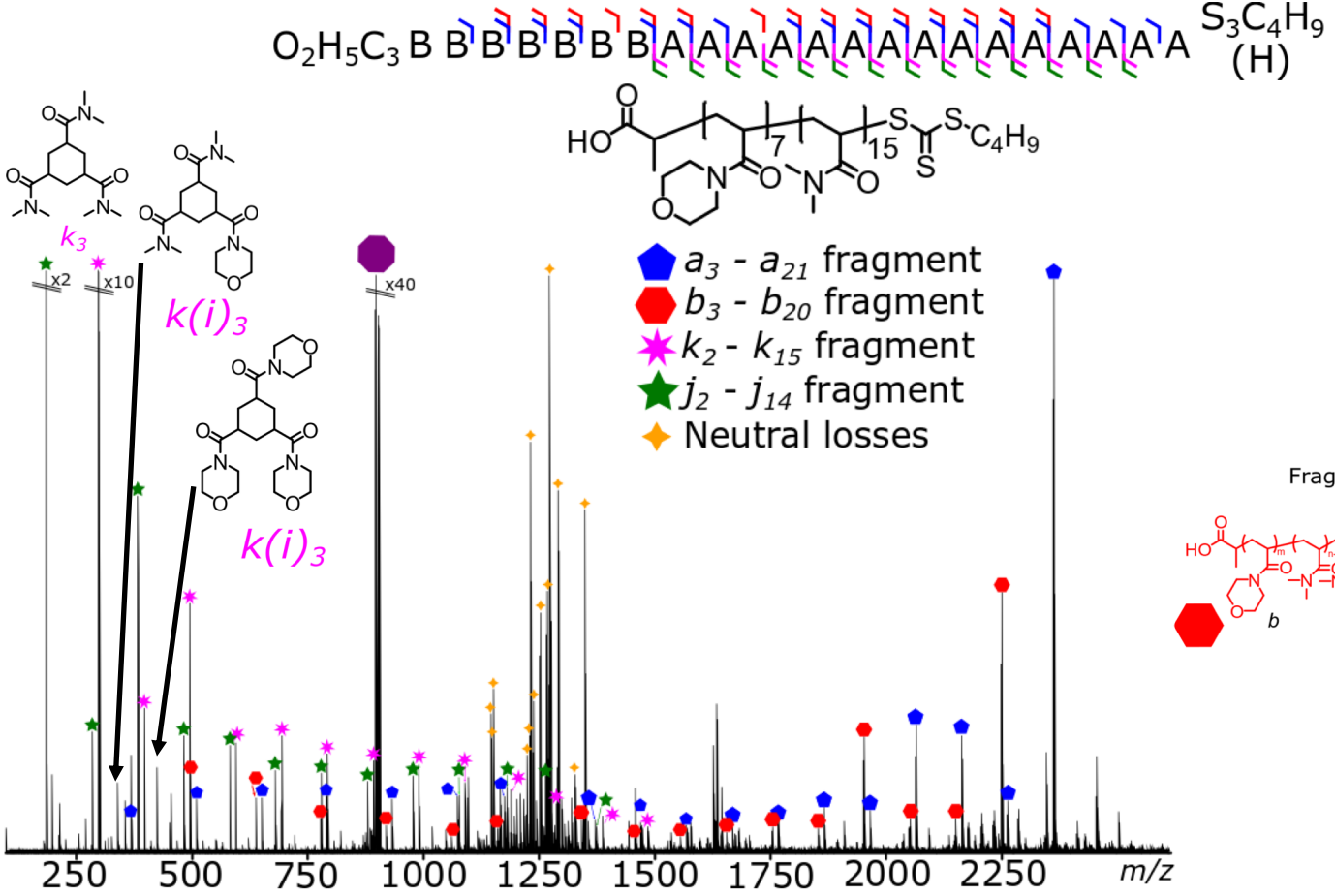
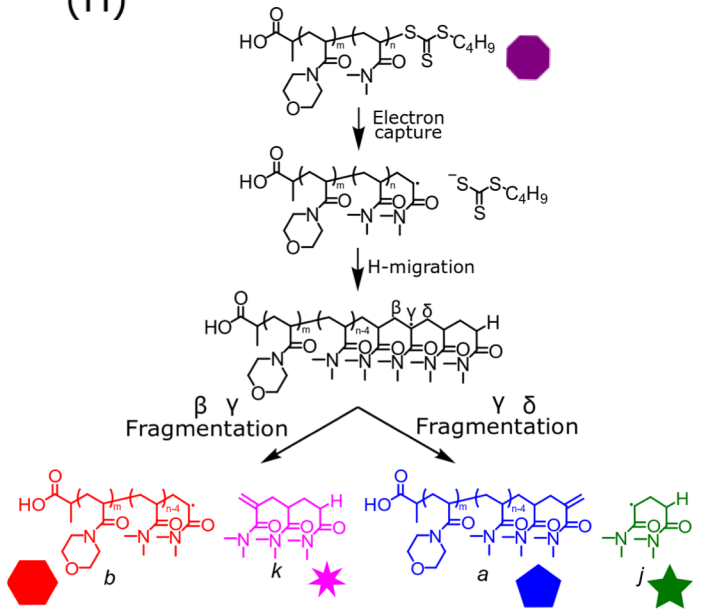
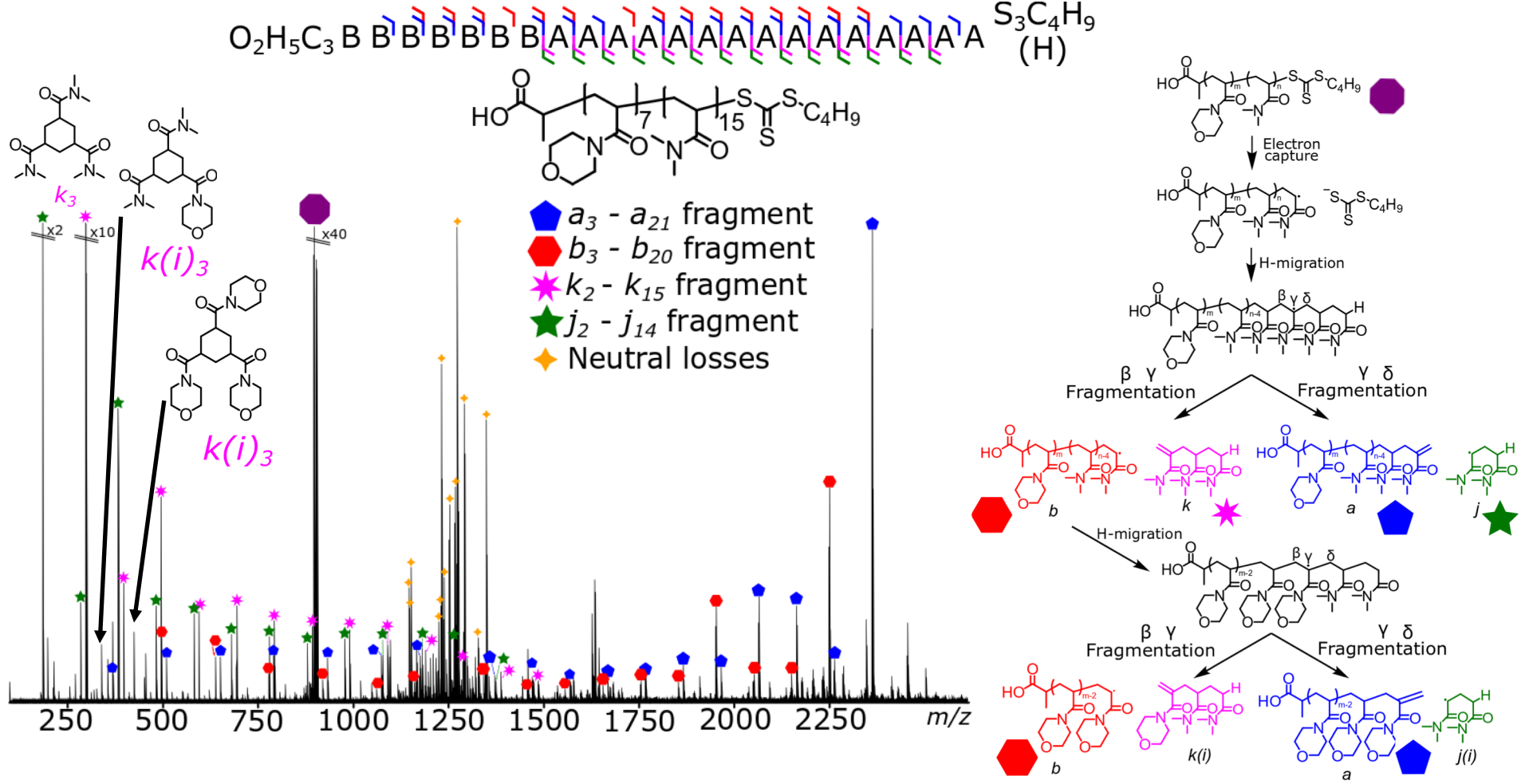




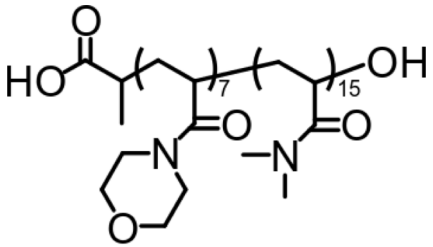
Trithiocarbonate end group DMA homopolymer

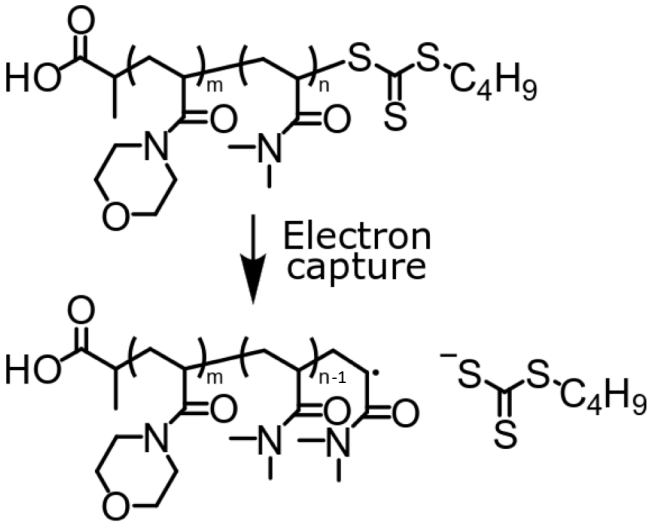


Replacing the trithiocarbonate

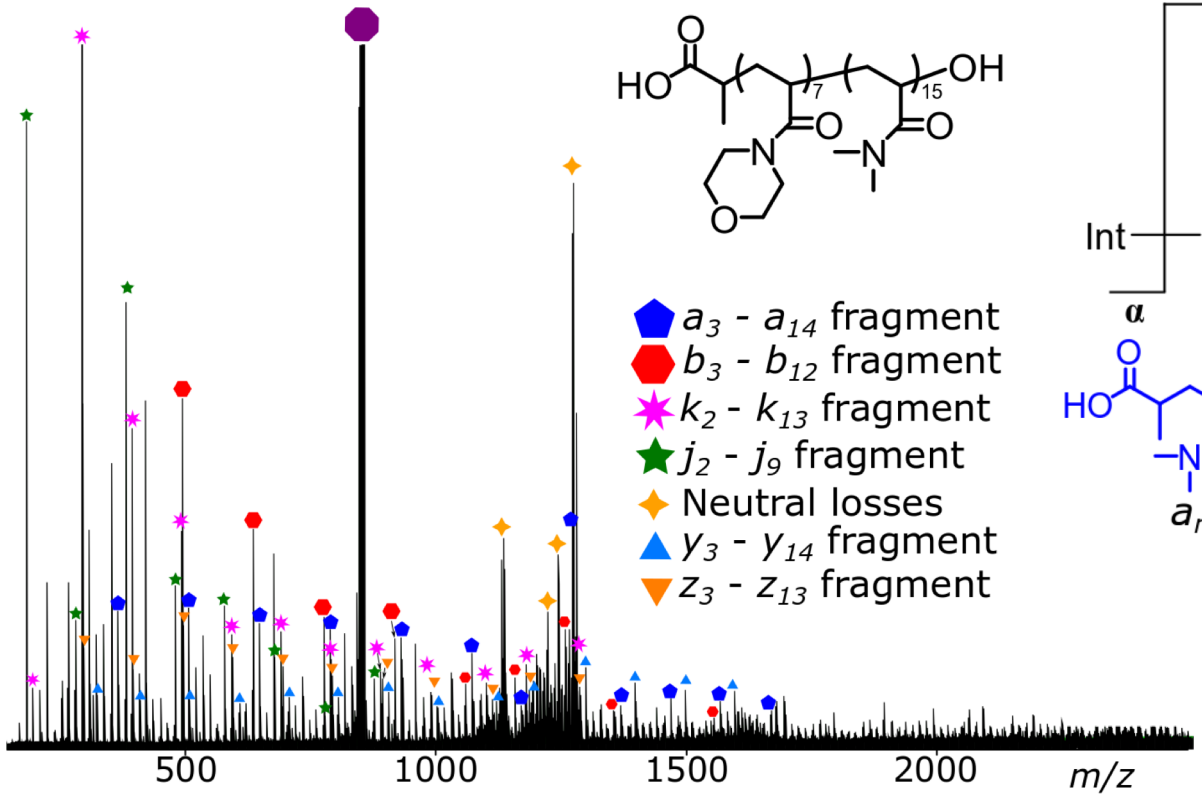
- Does fragmentation occur without the presence of a trithiocarbonate?

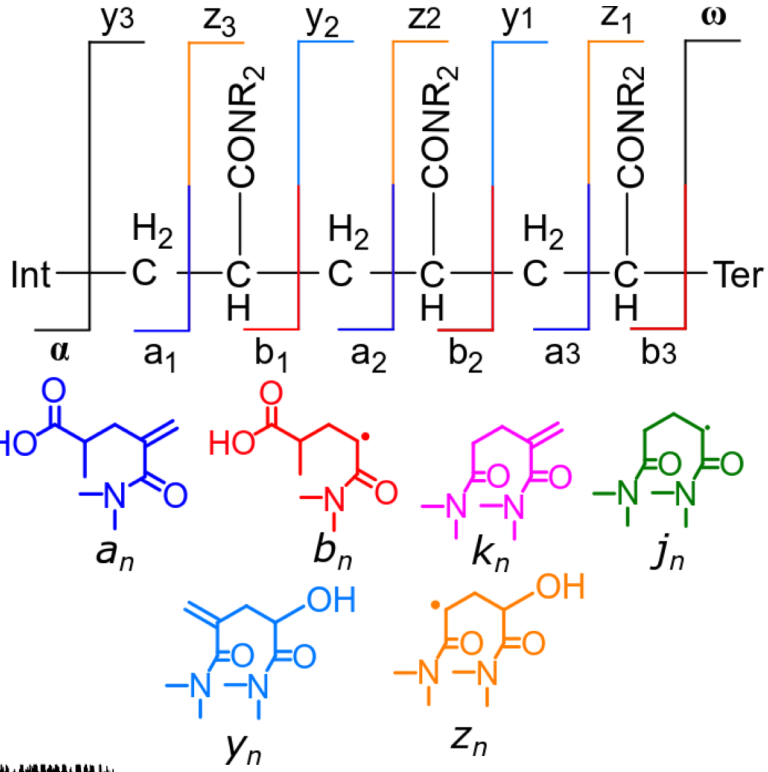
- Replacing the trithiocarbonate with a hydroxyl end group may show different fragmentation properties



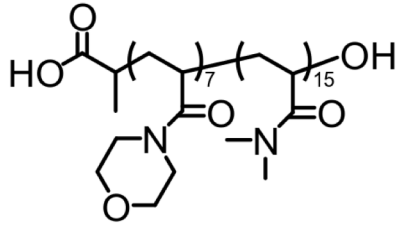


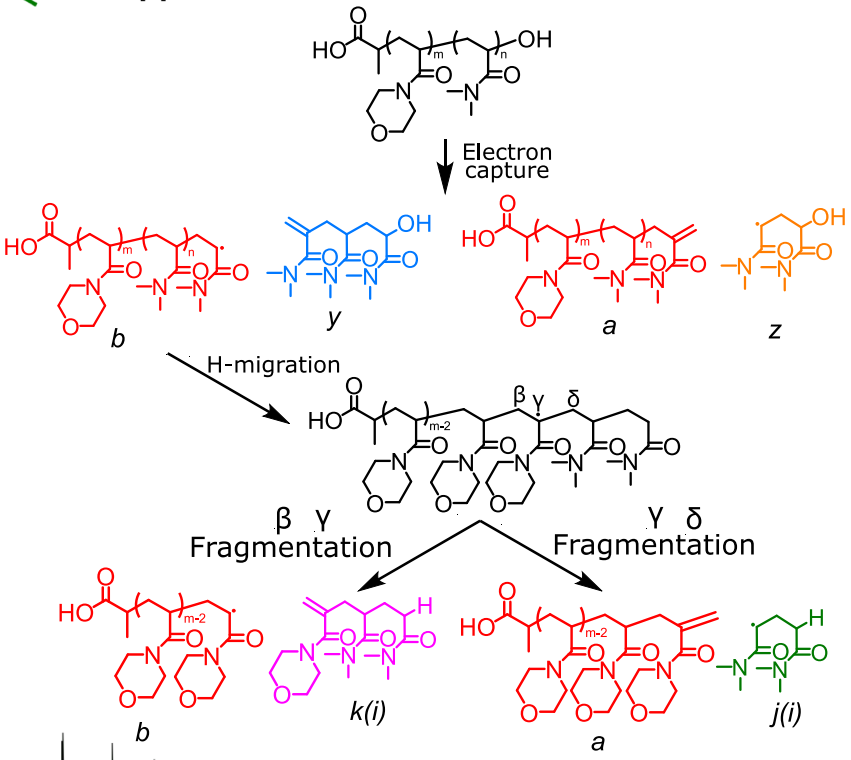
Hydroxyl end group DMA/NAM block copolymer





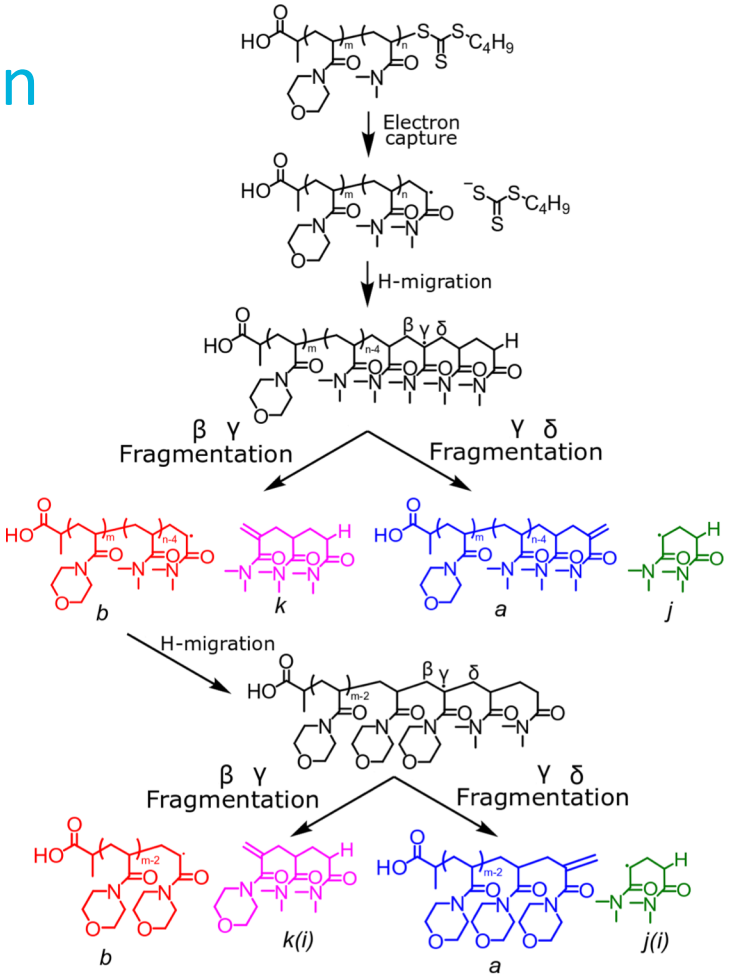
Hydroxyl end group DMA/NAM block copolymer





Summary of acrylamide dissociation

- Acrylamide ECD fragmentation can be used to sequence poly(acrylamide) block copolymers
- Multiple fragmentation events occur during the ECD process
- Presence of internal fragments can be used for assistance in sequence elucidation.

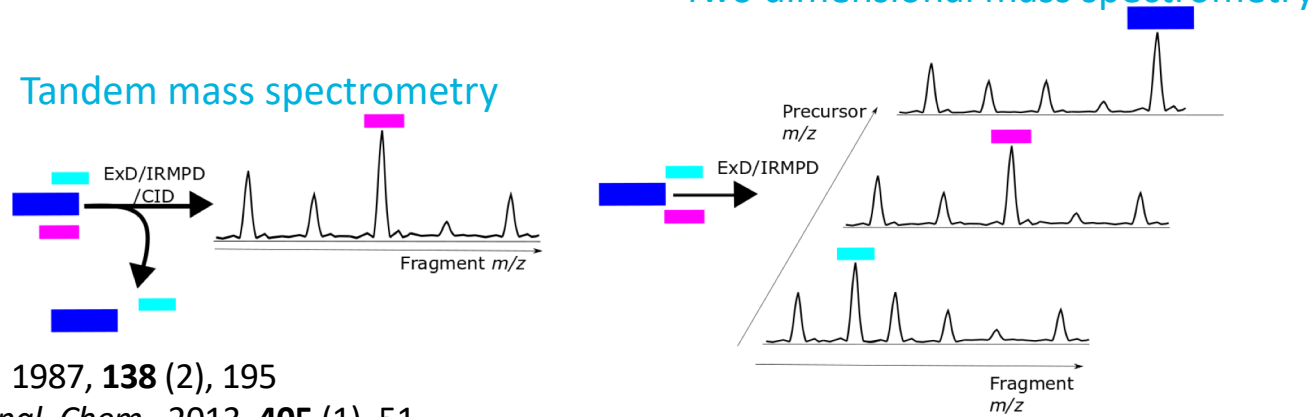


Two dimensional mass spectrometry

- A polymer sample is naturally disperse.
- Fragmentation measurement across the whole dispersity allows chemical and sequence determination of the whole polymer.
- 2D MS correlates fragments with precursors across the entire dispersity being measured – in one experiment.

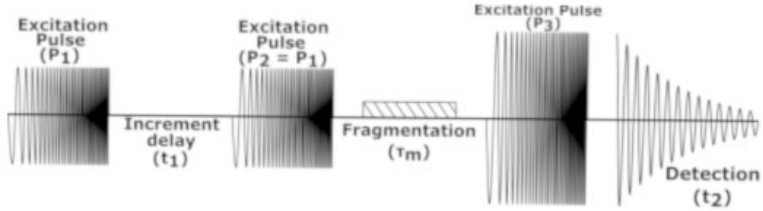
Two dimensional mass spectrometry

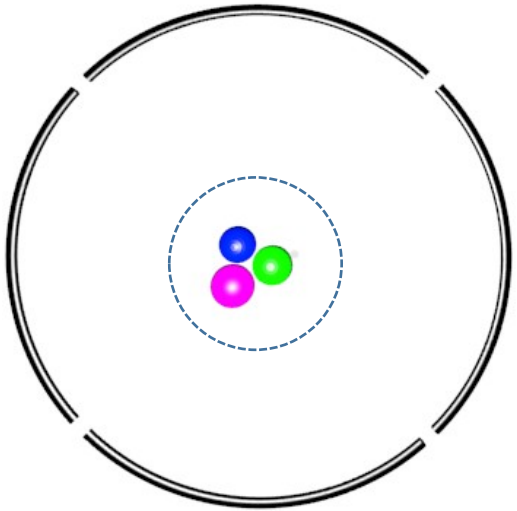
Tandem mass spectrometry



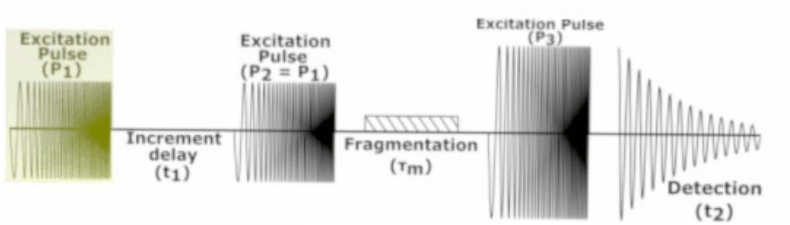
Pfandler P. *et al.*, *Chem. Phys. Lett.*, 1987, **138** (2), 195
van Agthoven, M. *et al., Anal. Bioanal. Chem.,* 2013, **405** (1), 51.

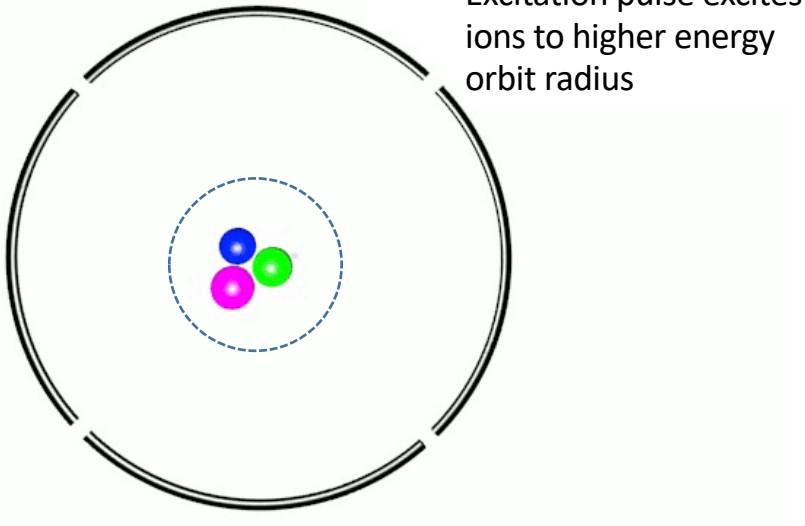
Two dimensional mass spectrometry





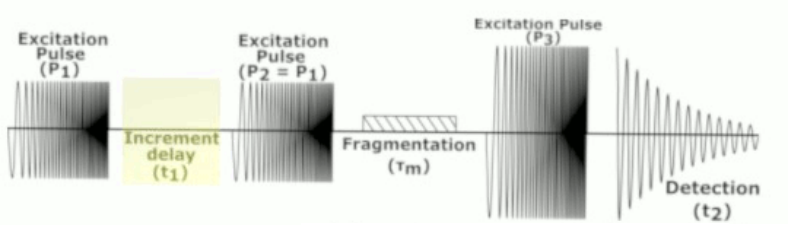
Two dimensional mass spectrometry

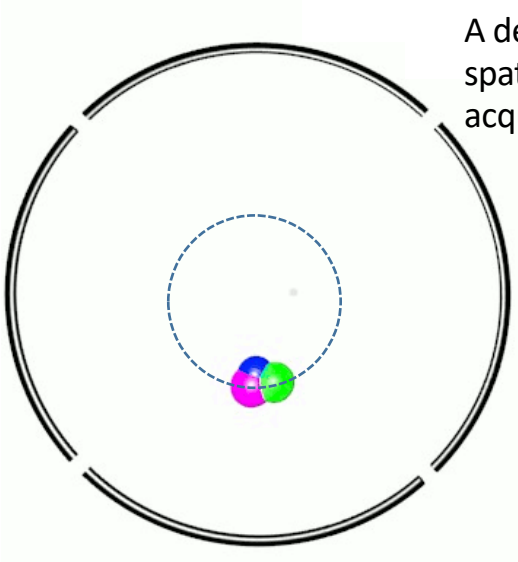




Excitation pulse excites ions to higher energy orbit radius

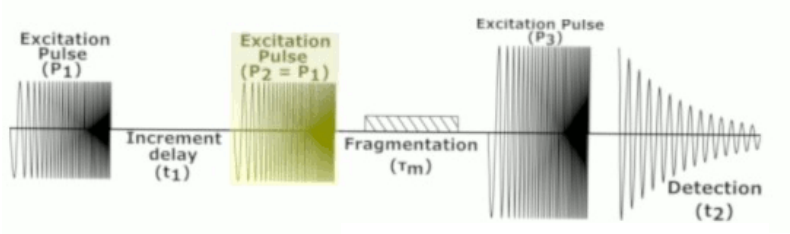
Two dimensional mass spectrometry



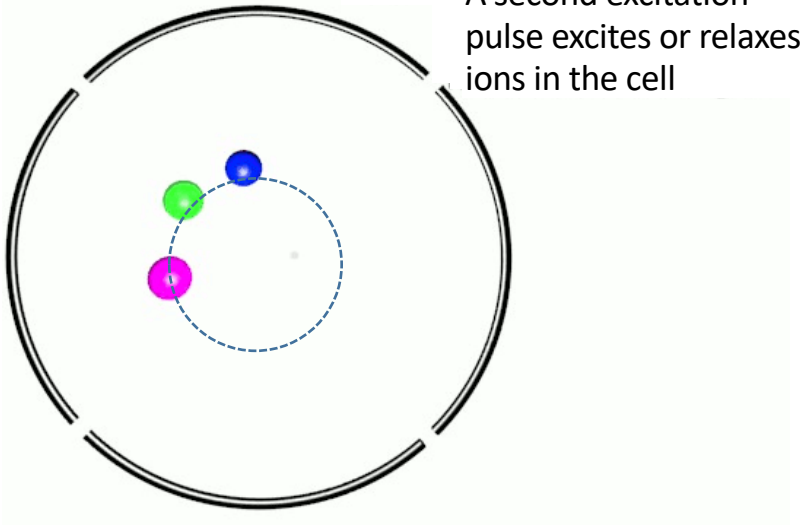


A delay allows ions to spatially resolve and acquire phase

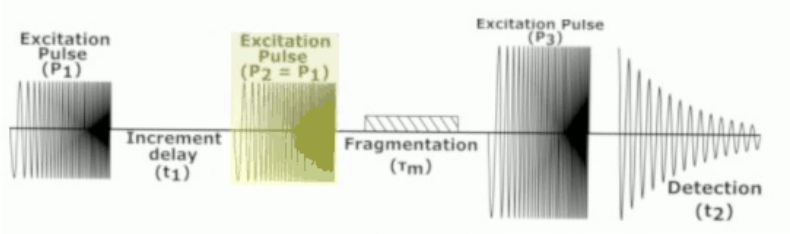
Two dimensional mass spectrometry

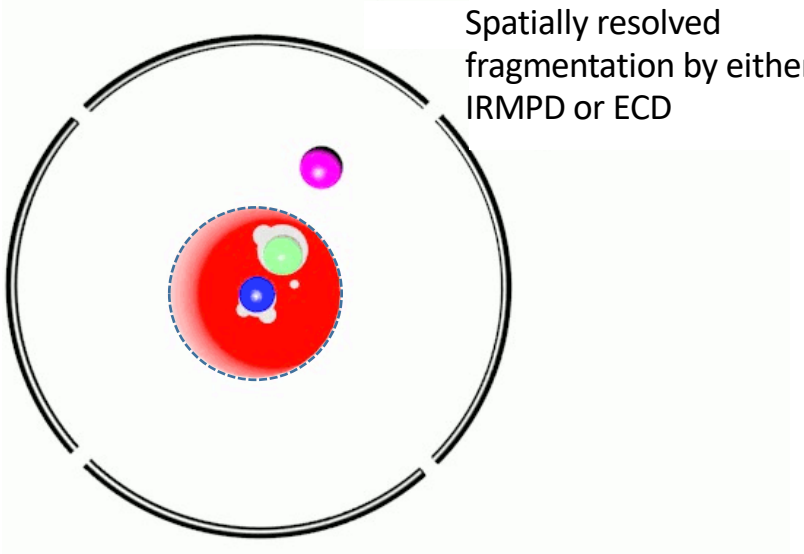


A second excitation pulse excites or relaxes ions in the cell



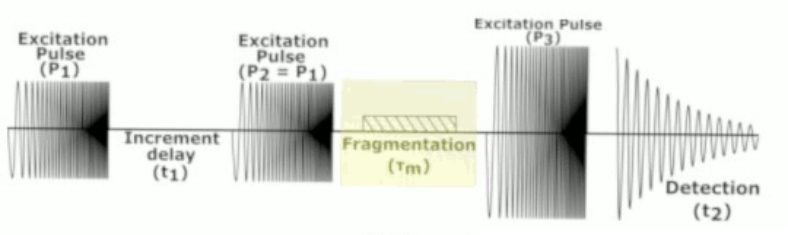
Two dimensional mass spectrometry



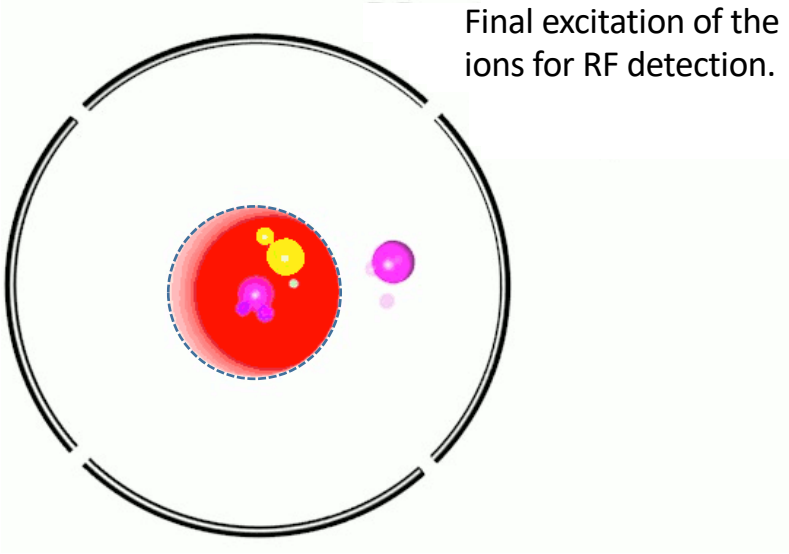


Spatially resolved fragmentation by either IRMPD or ECD

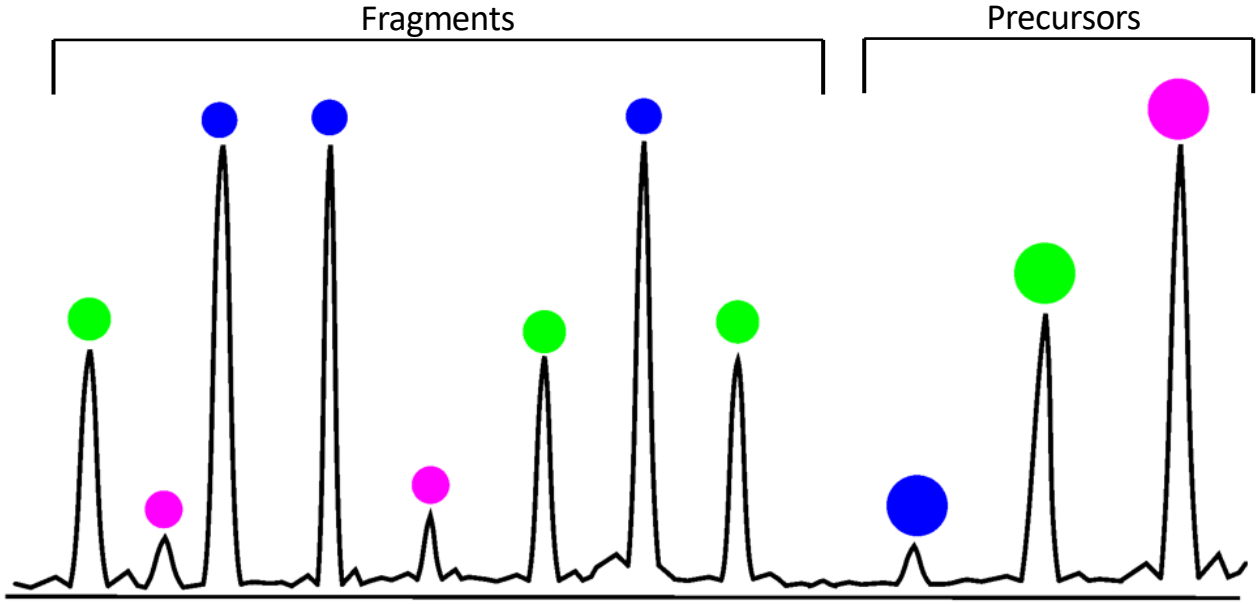
Two dimensional mass spectrometry



Final excitation of the ions for RF detection.

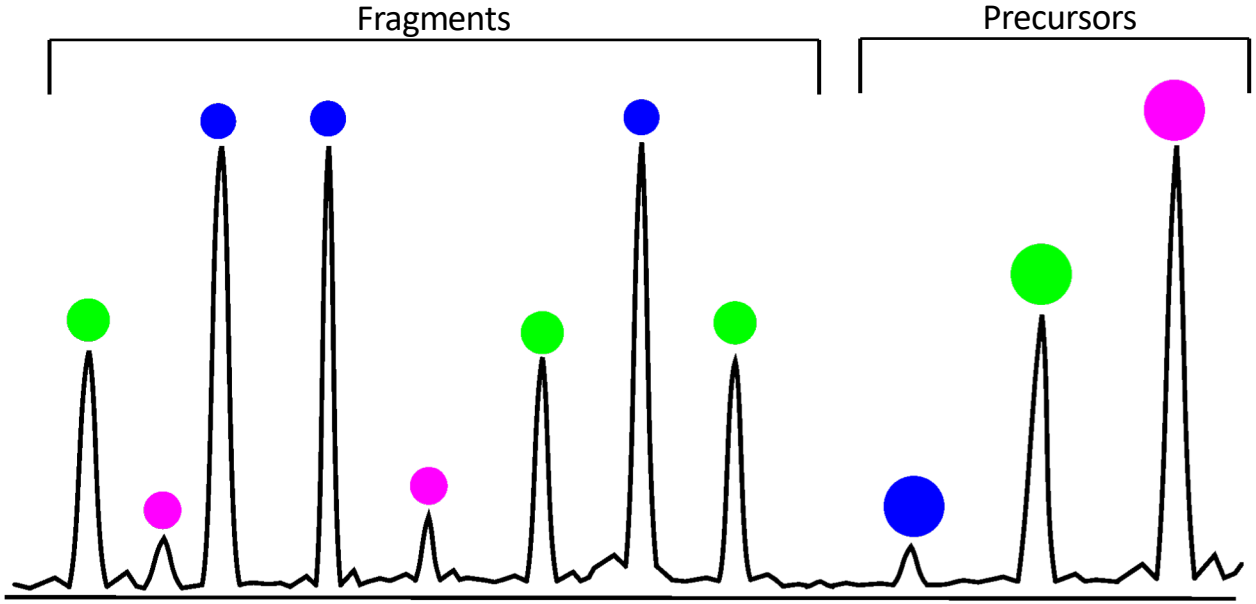


Two dimensional mass spectrometry



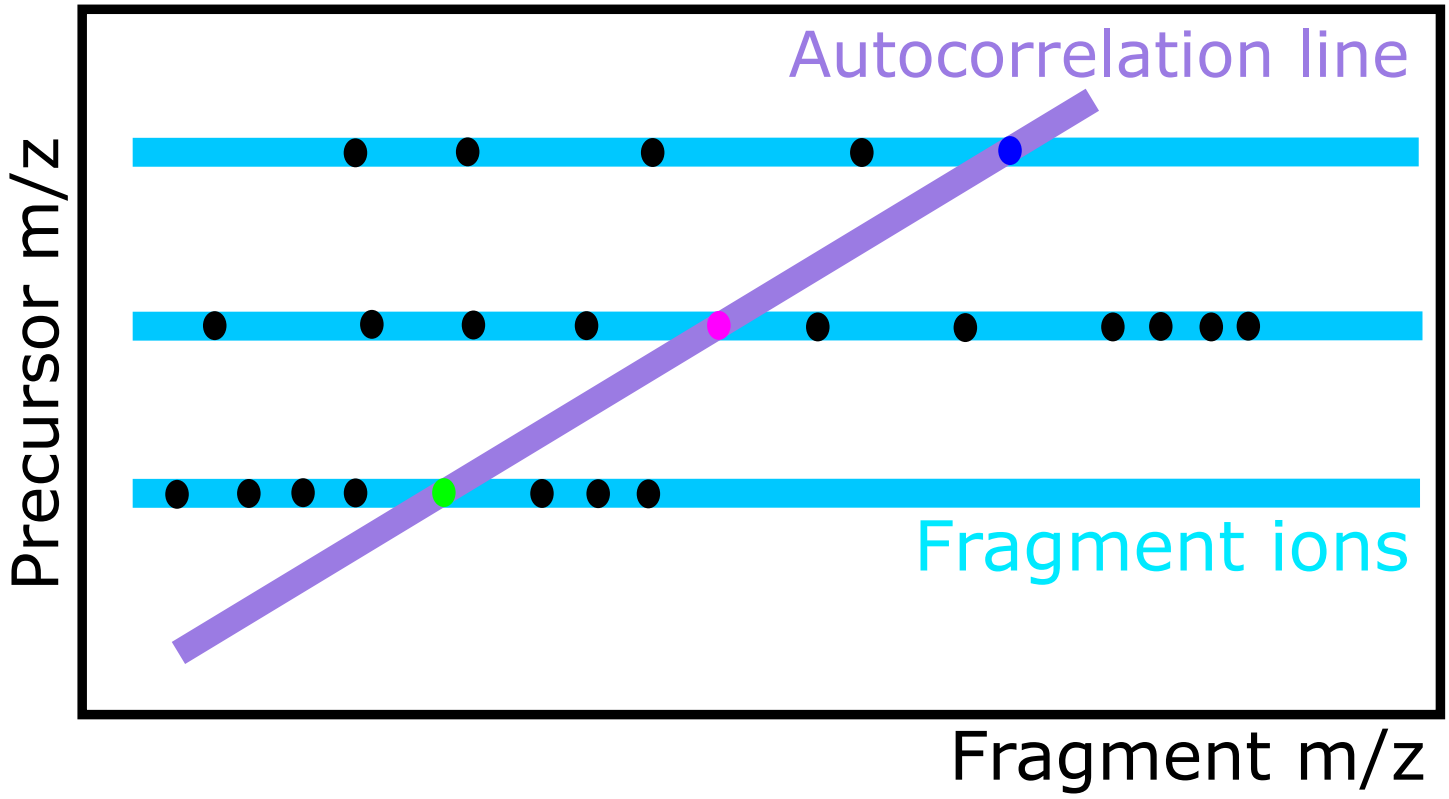
Increment delay produces varying fragment and precursor intensities
Fragment and precursor intensities vary with same frequency

Two dimensional mass spectrometry

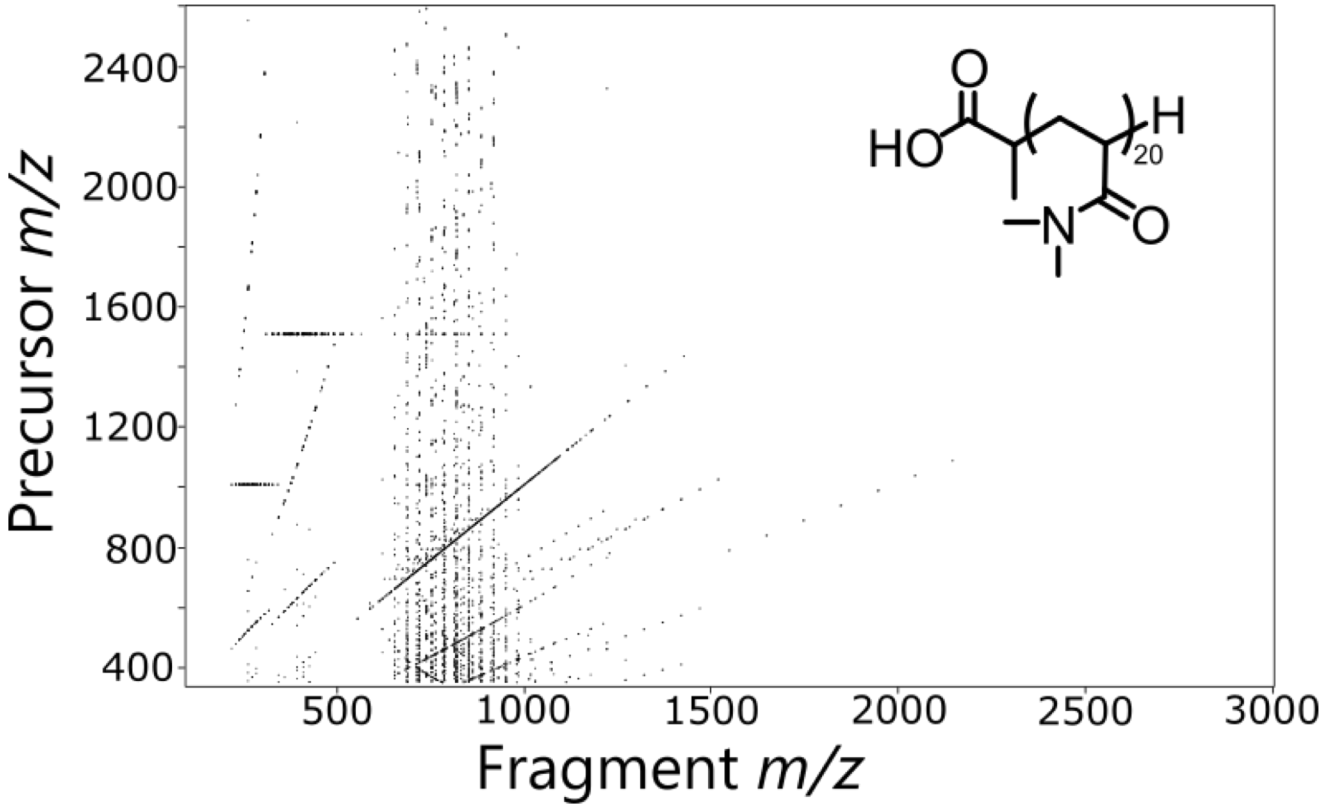


Increment delay produces varying fragment and precursor intensities
Fragment and precursor intensities vary with same frequency

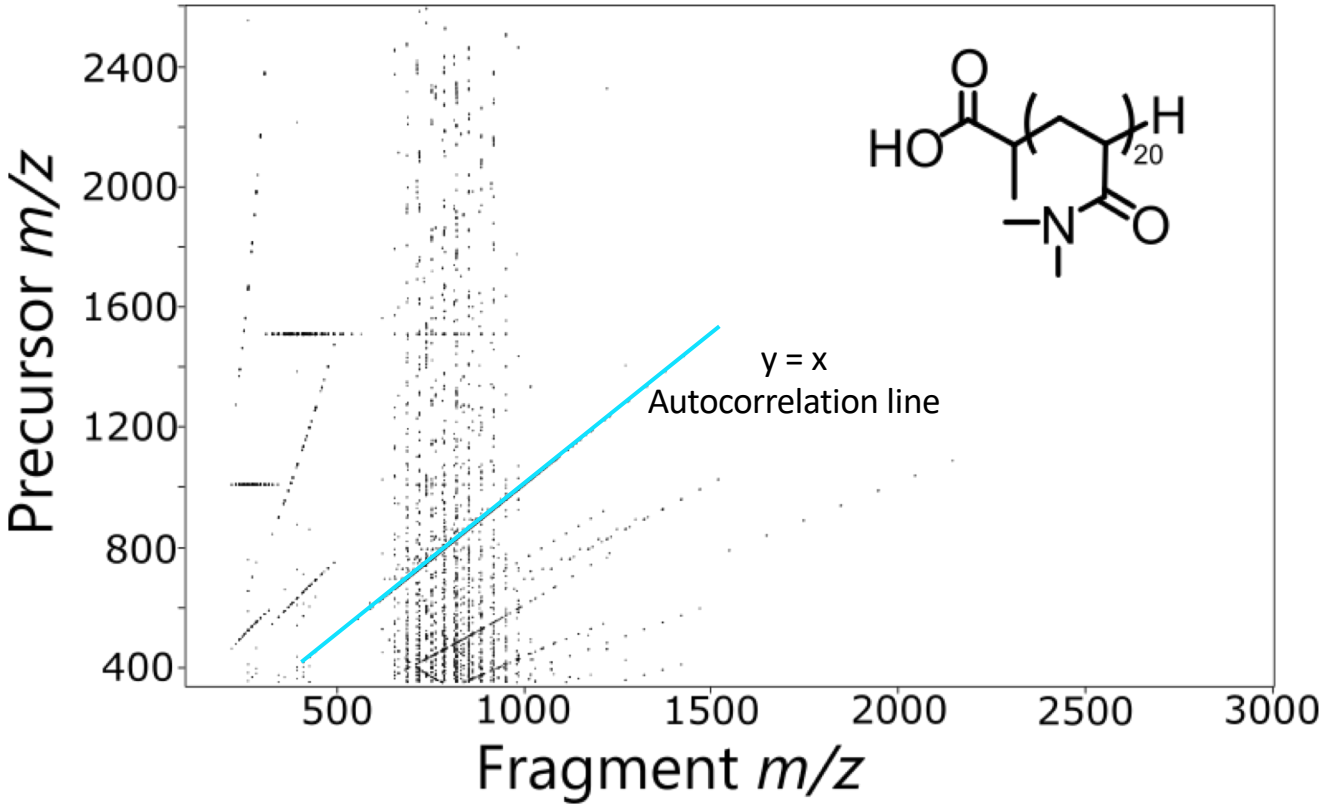
2D MS of acrylamide species



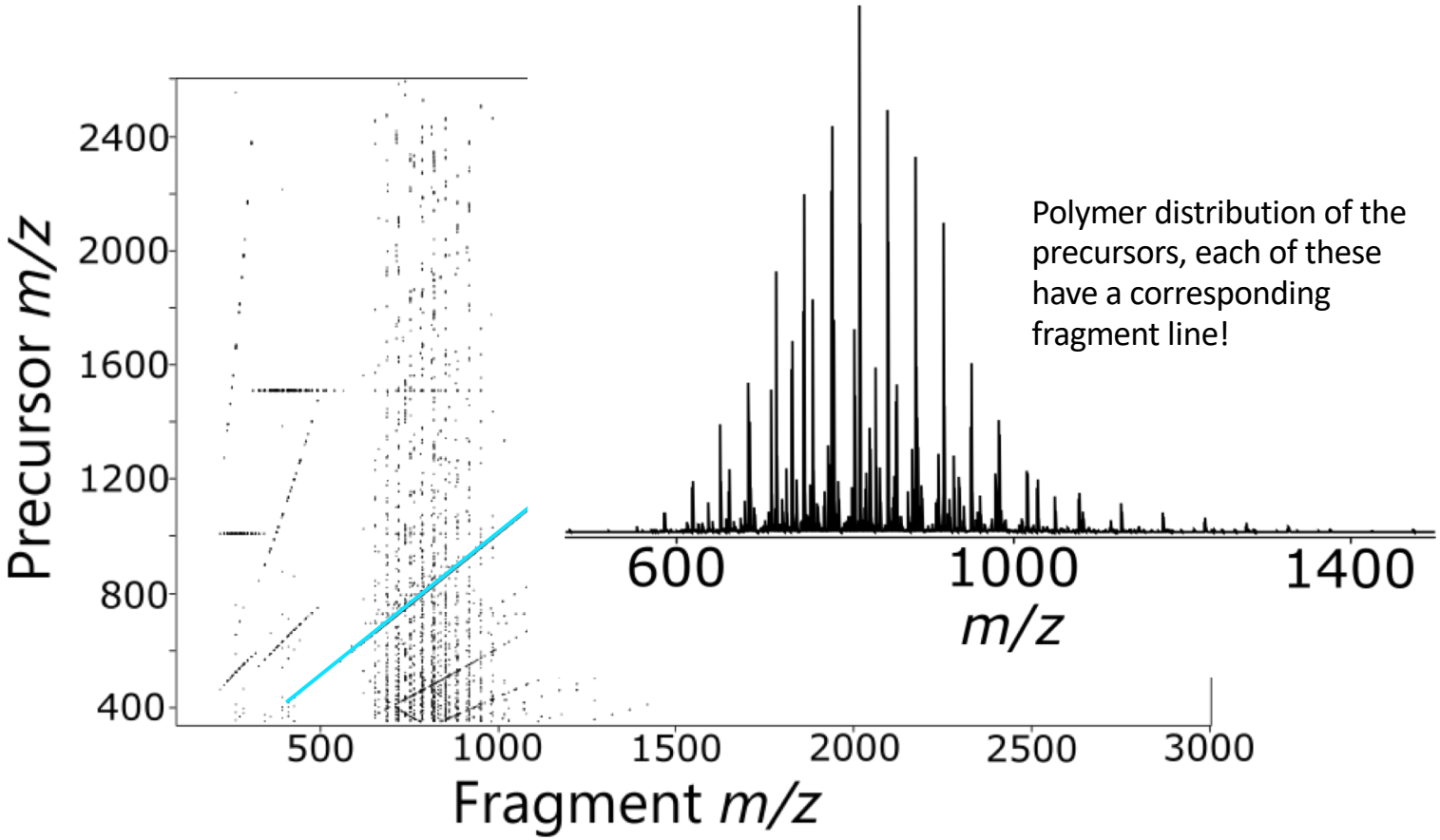
2D MS of acrylamide species



2D MS of acrylamide species

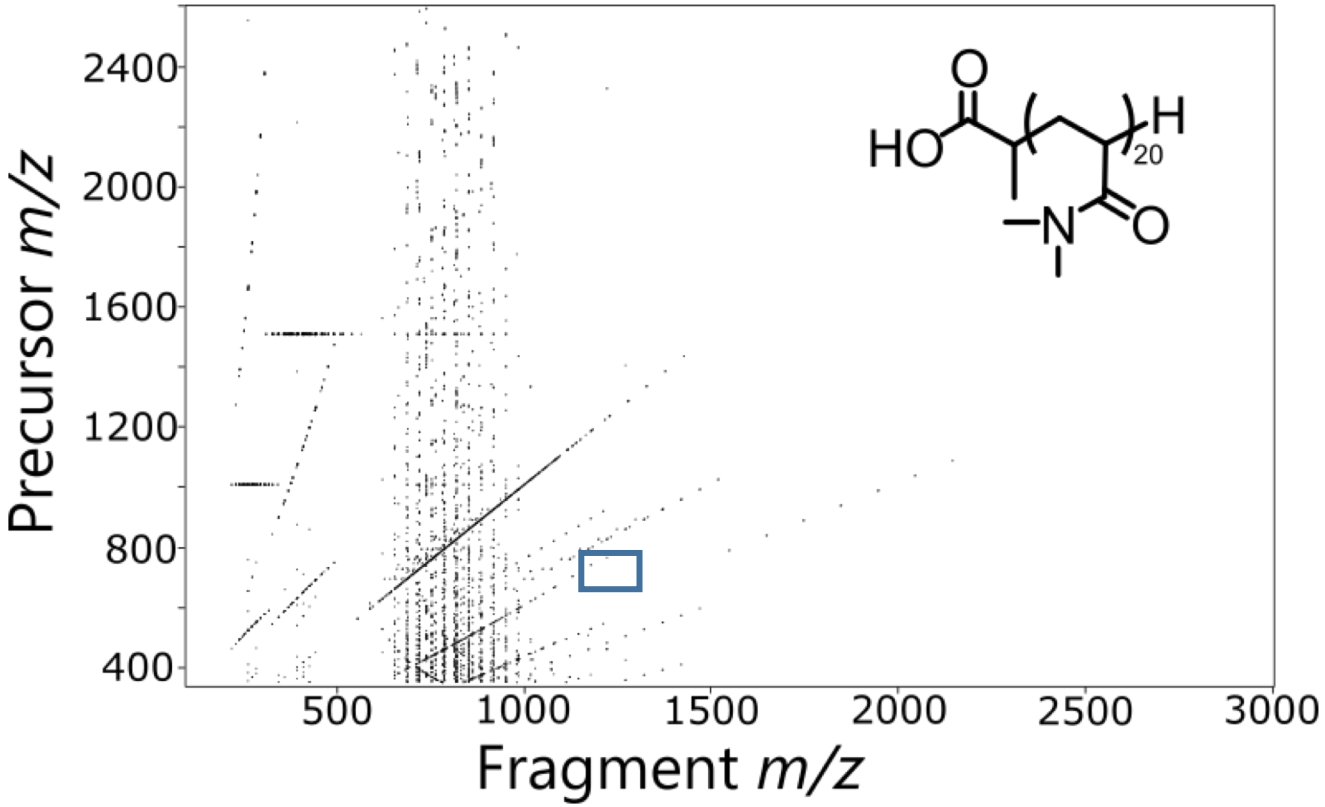


2D MS of acrylamide species

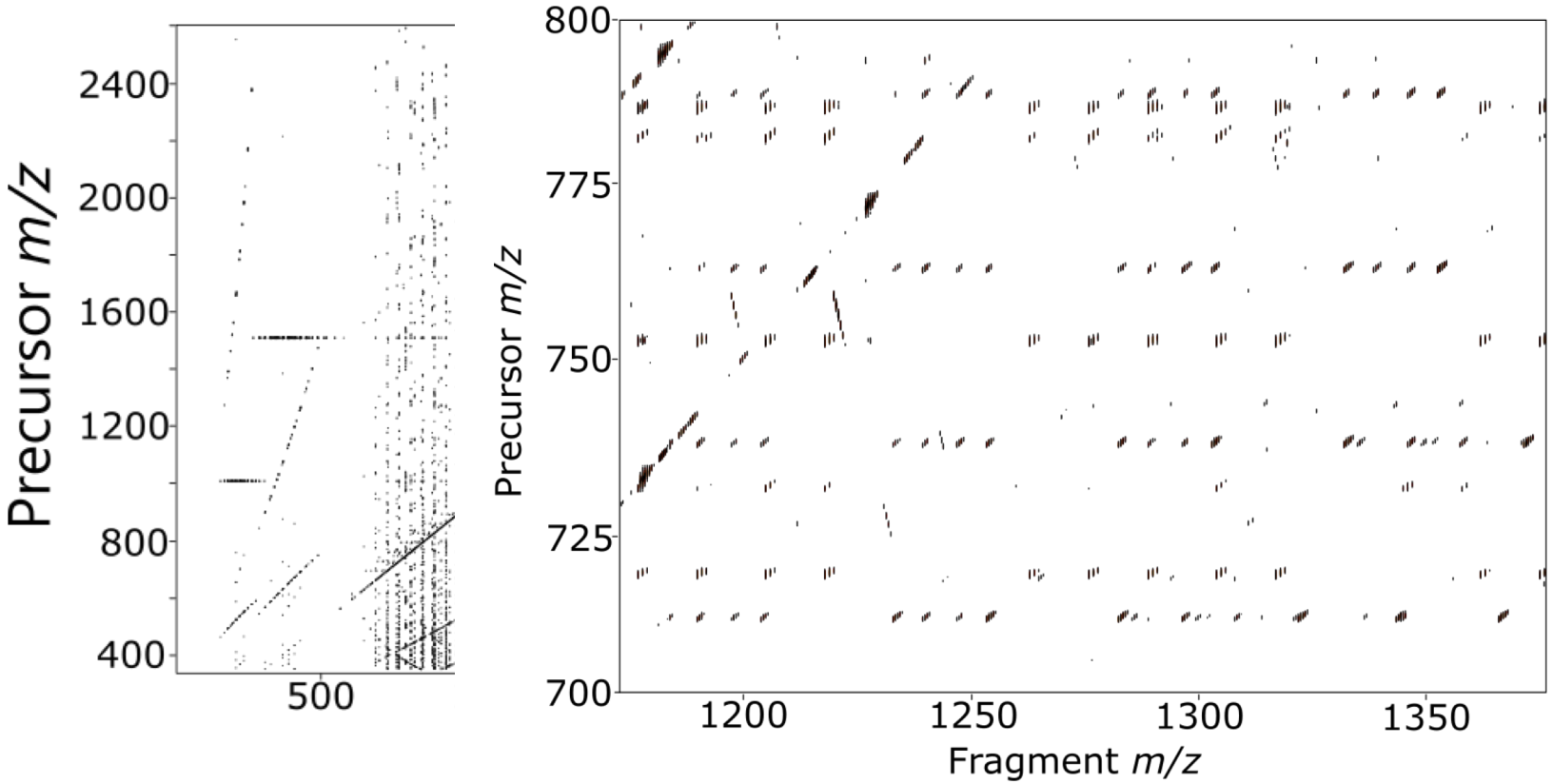


Polymer distribution of the precursors, each of these have a corresponding fragment line!

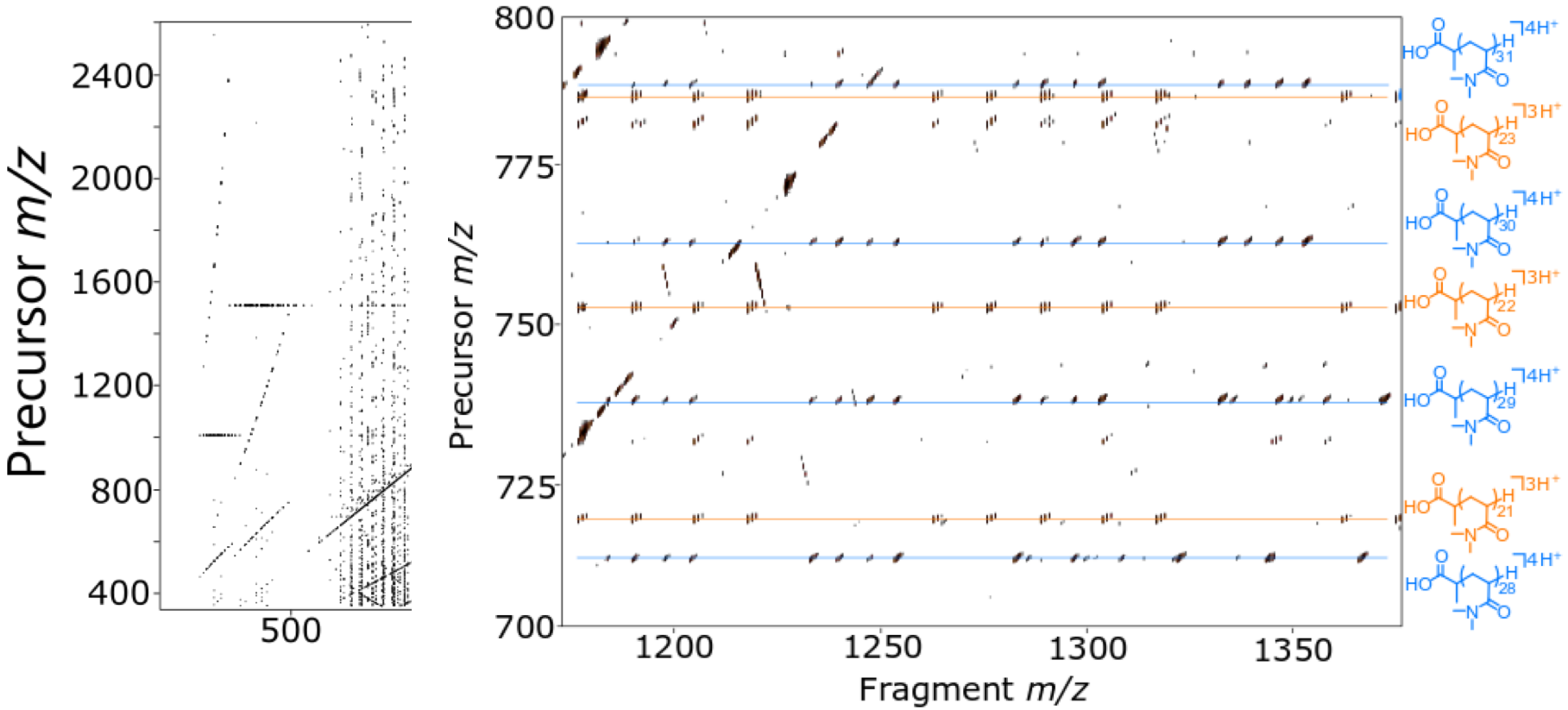
2D MS of acrylamide species



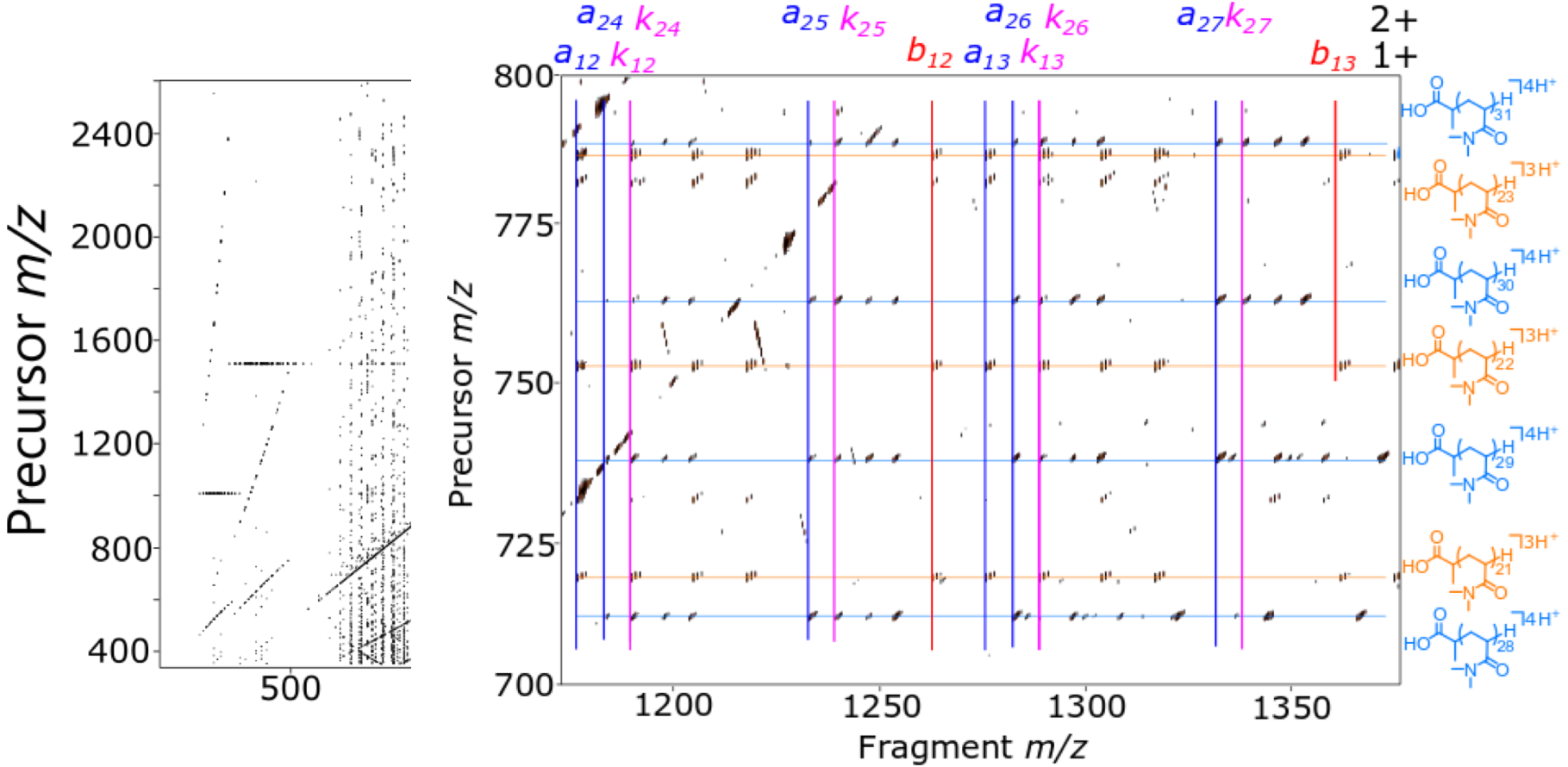
2D MS of acrylamide species



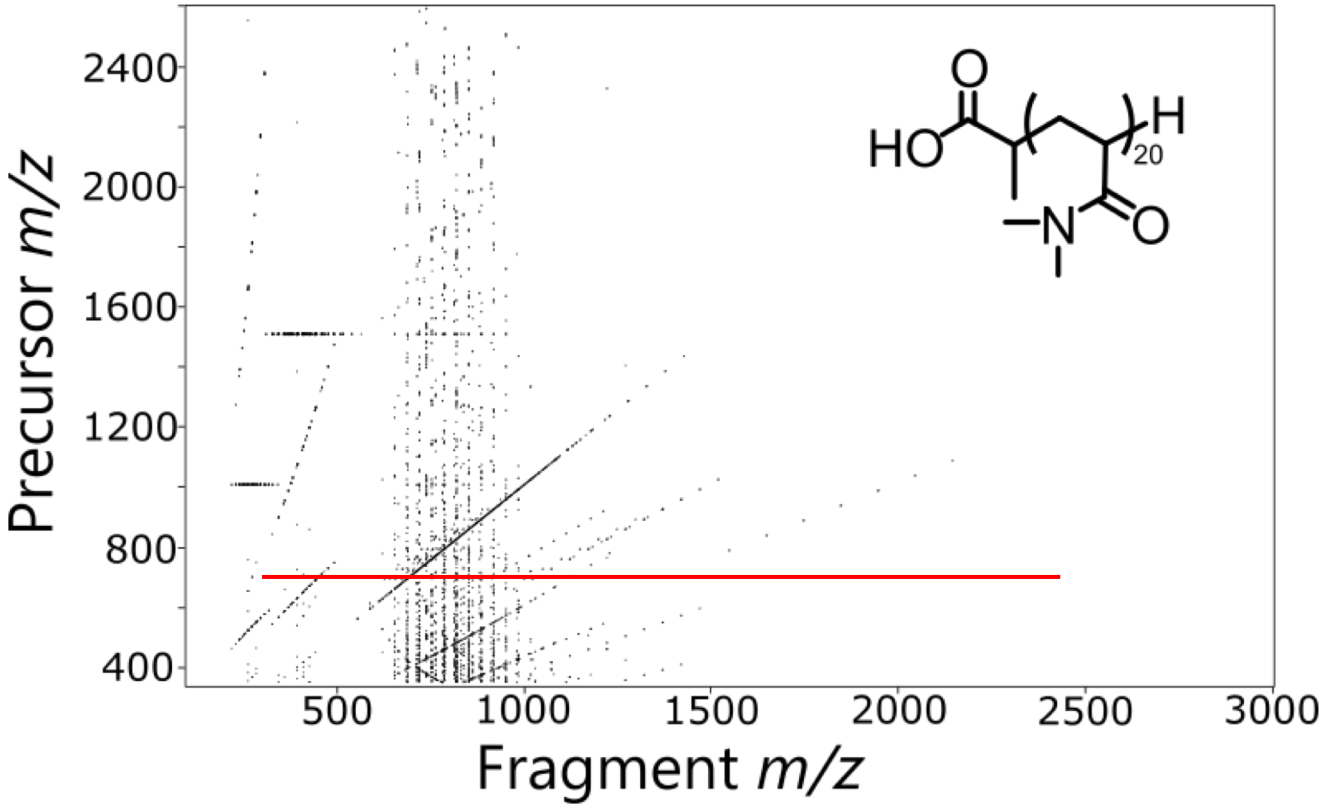
2D MS of acrylamide species



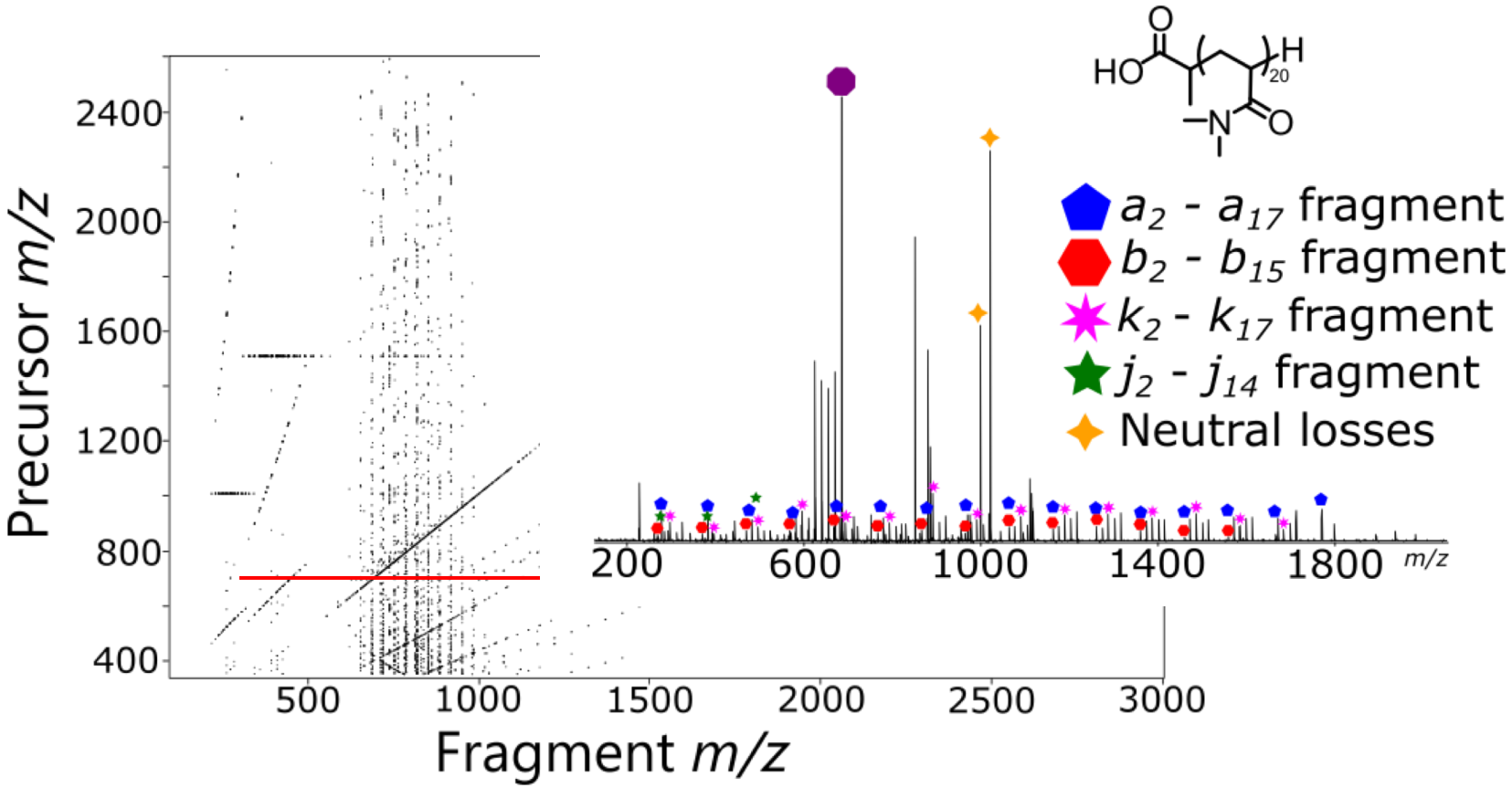
2D MS of acrylamide species



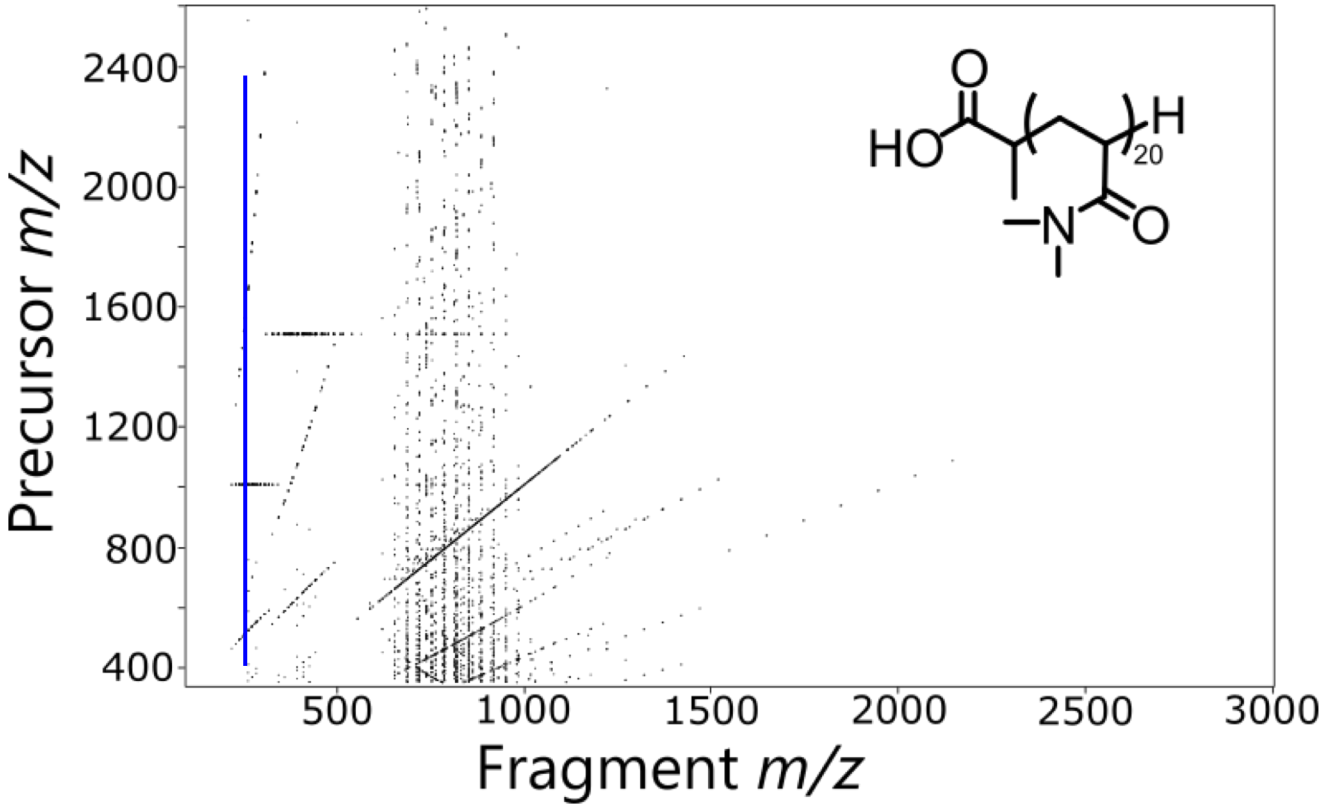
2D MS of acrylamide species



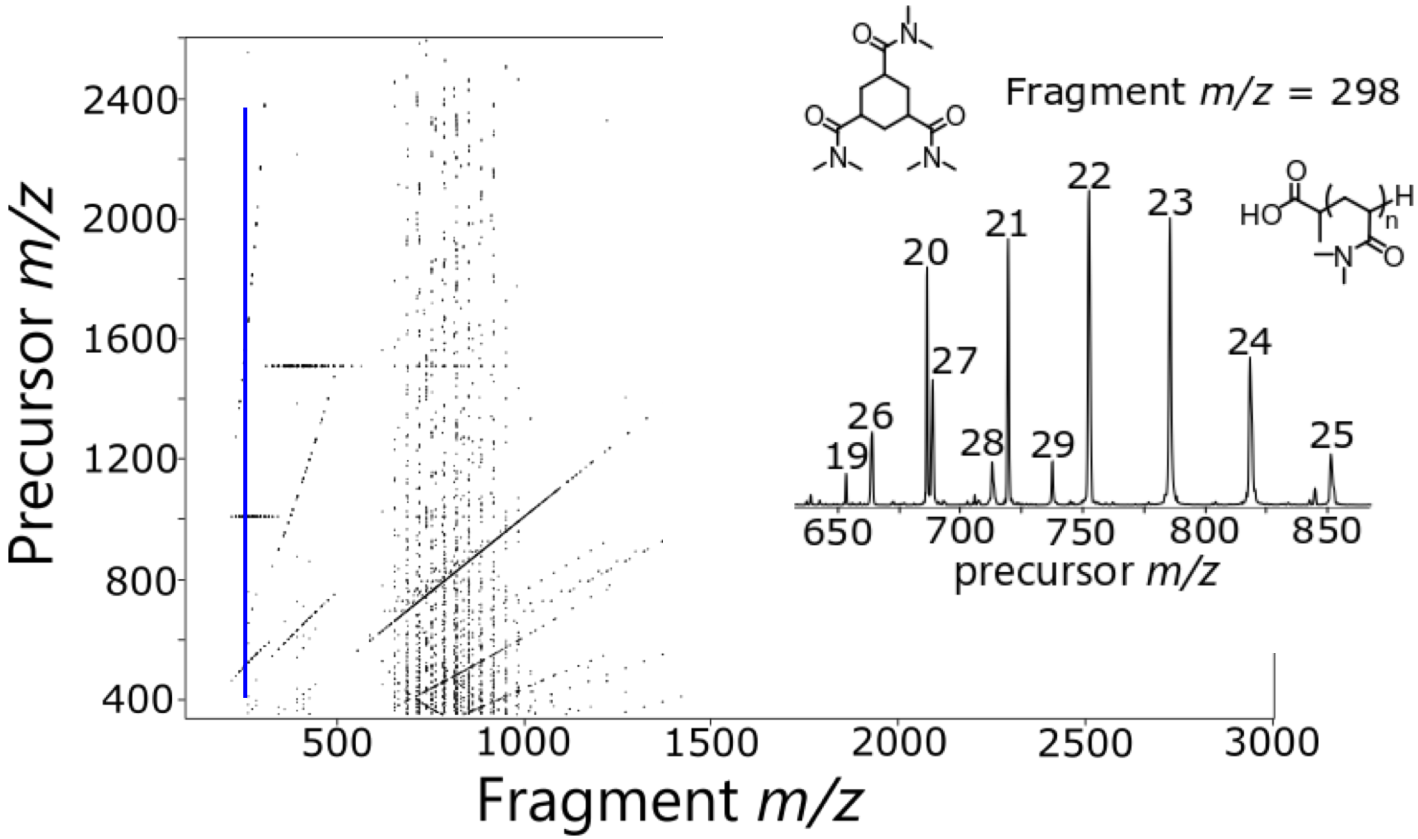
2D MS of acrylamide species



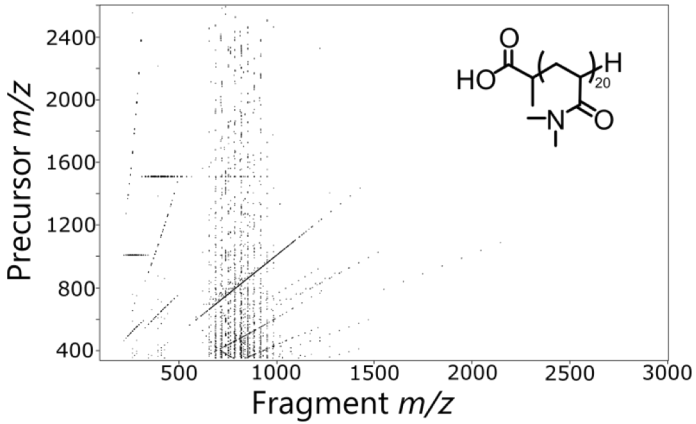
2D MS of acrylamide species

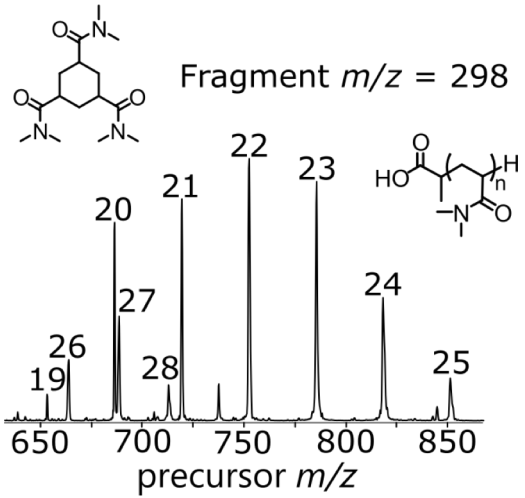


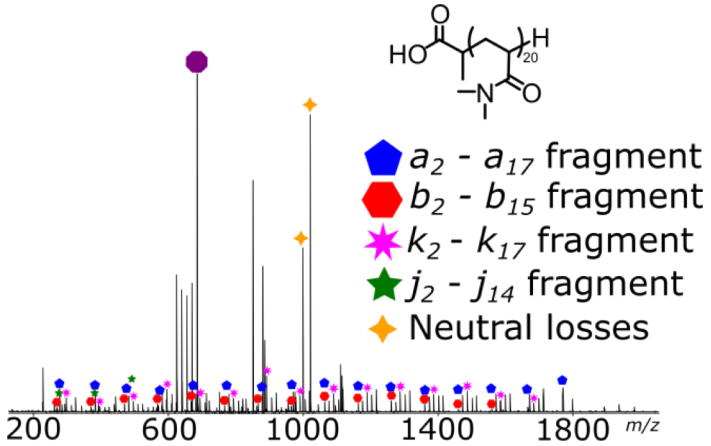
2D MS of acrylamide species

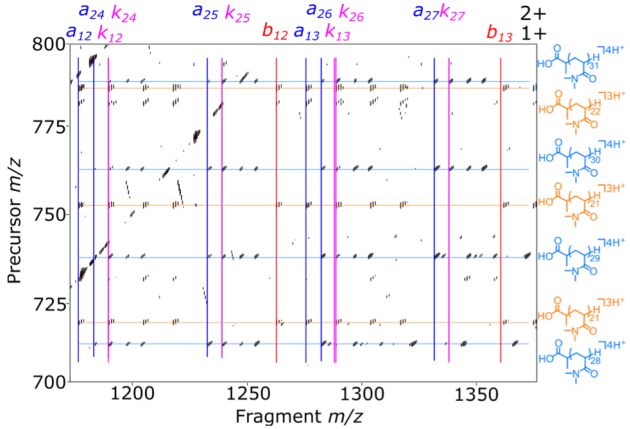


2D MS of acrylamide species



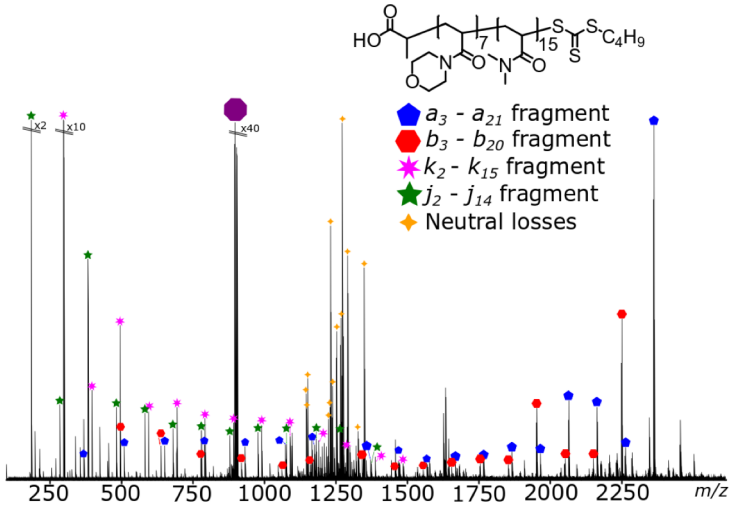


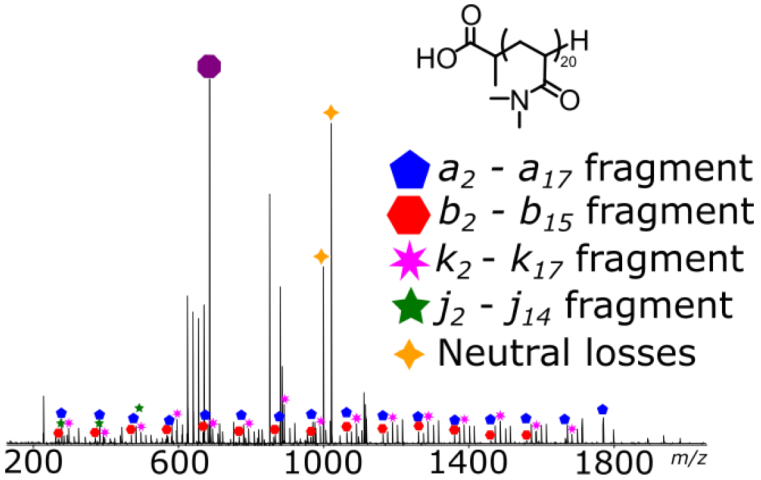




Summary

- Electron capture dissociation of acrylamide polymers can offer complete sequence and end group coverage.
- Acrylamide polymers show a free radical cascade producing sequence fragments
- 2D MS can produce sequence coverage of multiple precursors in one experiment.
- 2D MS will be expanded into the analysis of co-polymer dispersity's.





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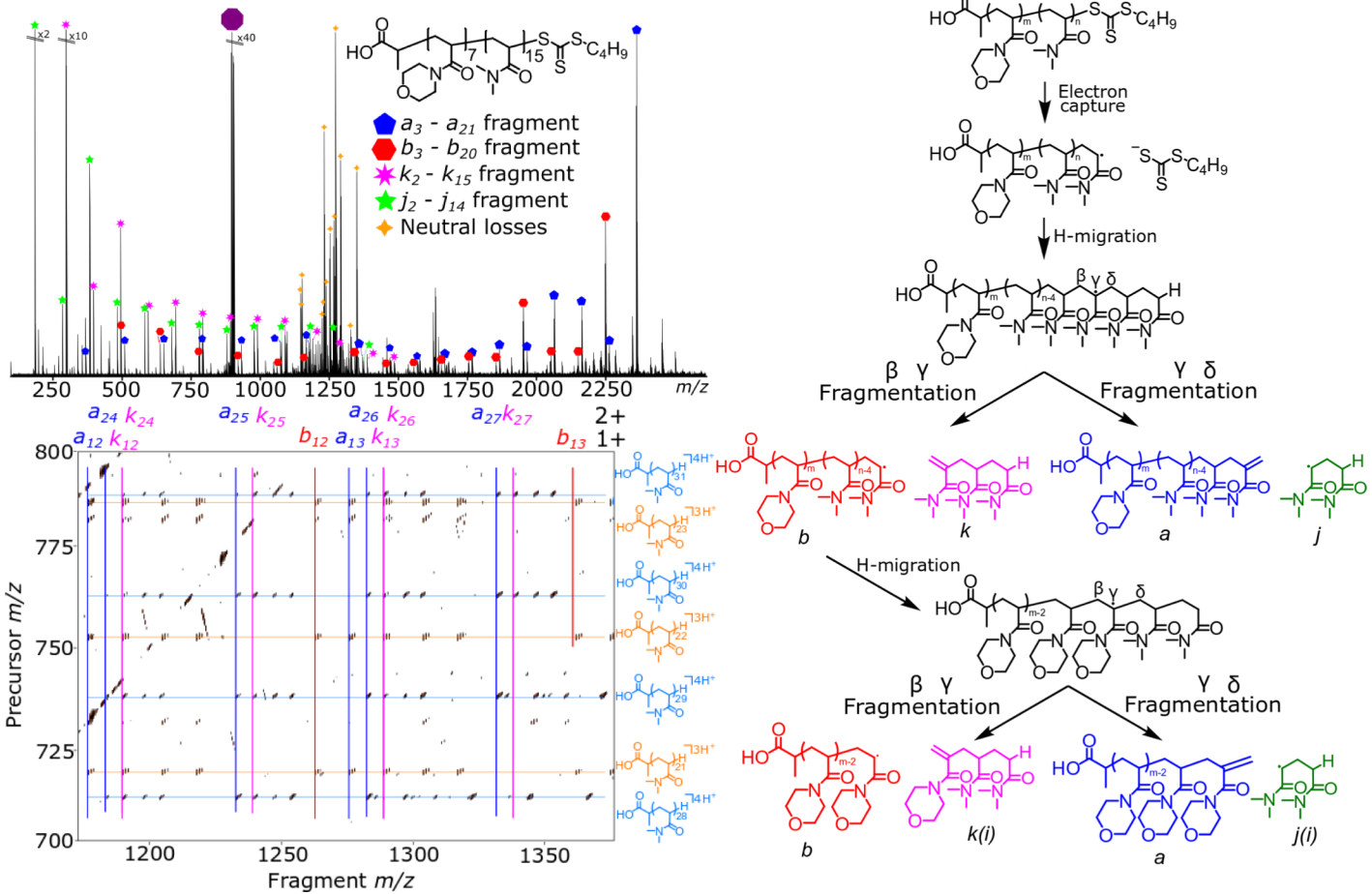
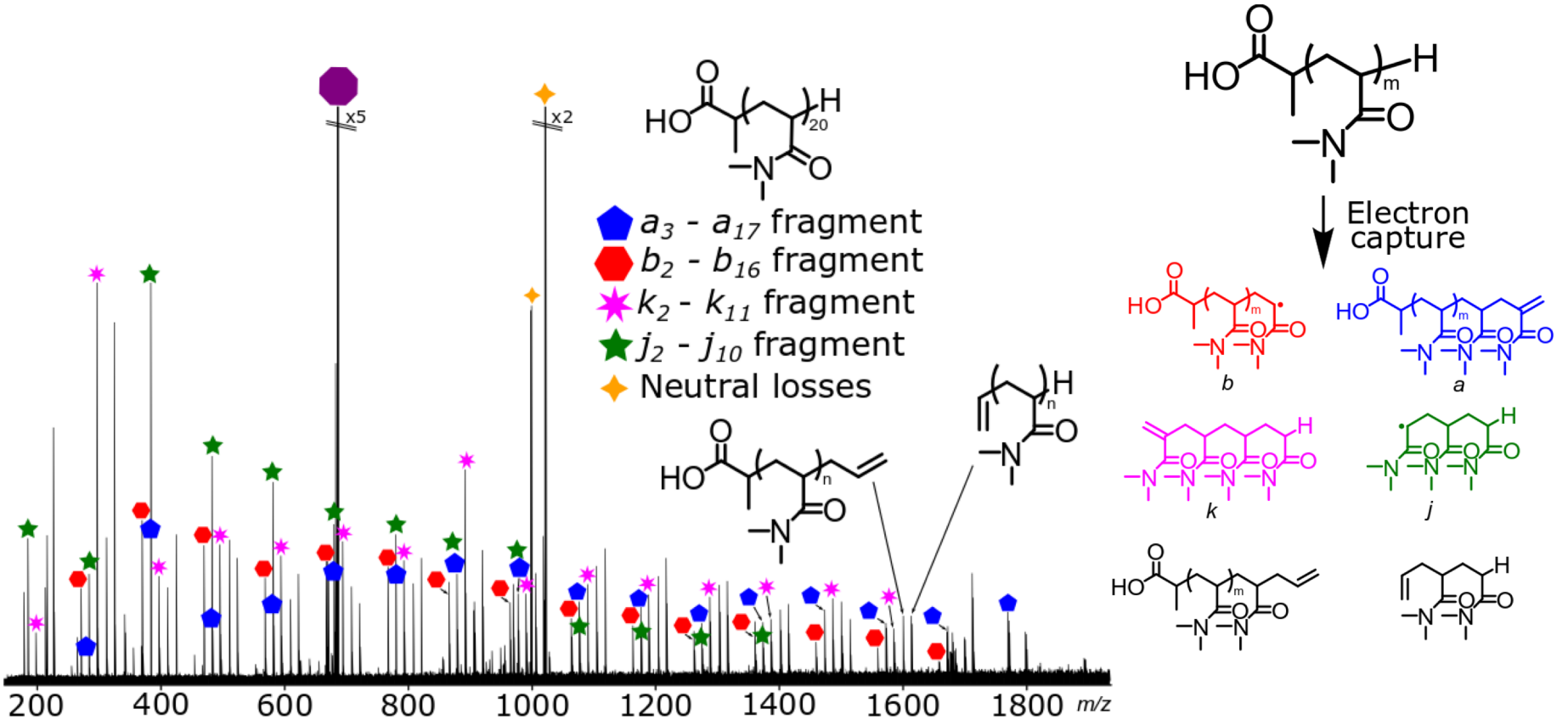
Thank you!


x2
x10
x40
a_3 - a_{21} fragment
b_3 - b_{20} fragment
k_2 - k_{15} fragment
j_2 - j_{14} fragment
Neutral losses
250 500 750 1000 1250 1500 1750 2000 2250 m/z
a_{24} k_{24}
a_{12} k_{12}
a_{25} k_{25}
a_{26} k_{26}
b_{12} a_{13} k_{13}
a_{27} k_{27}
b_{13}
2+
1+
Precursor m/z
800
775
750
725
700
1200 1250 1300 1350
Fragment m/z
Electron capture
H-migration
β γ δ
β γ Fragmentation
γ δ Fragmentation
b
k
a
j
H-migration
β γ Fragmentation
γ δ Fragmentation
b
k(i)
a
j(i)

Hydrogenated end group DMA/NAM block copolymer



Hydrogenated end group DMA/NAM block copolymer