



**Activation and spectroscopy of mass
and charge selected ions**



Alexandre Giuliani
Synchrotron SOLEIL & INRAE

Motivations

- Profit from the capacity of modern ionization sources to place virtually anything in the gas phase.



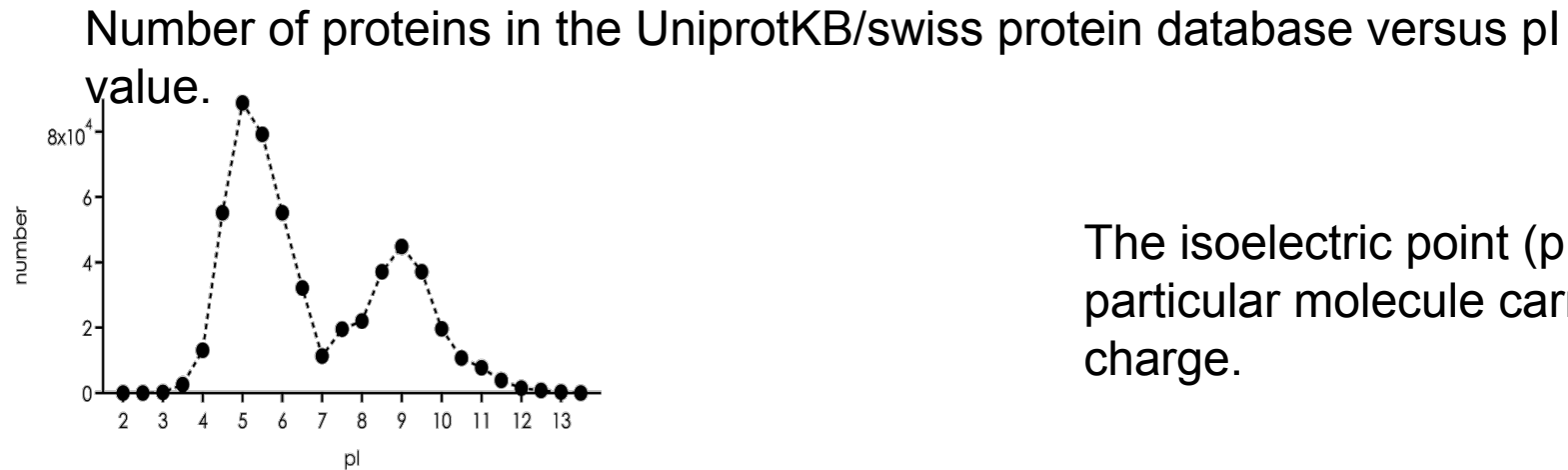
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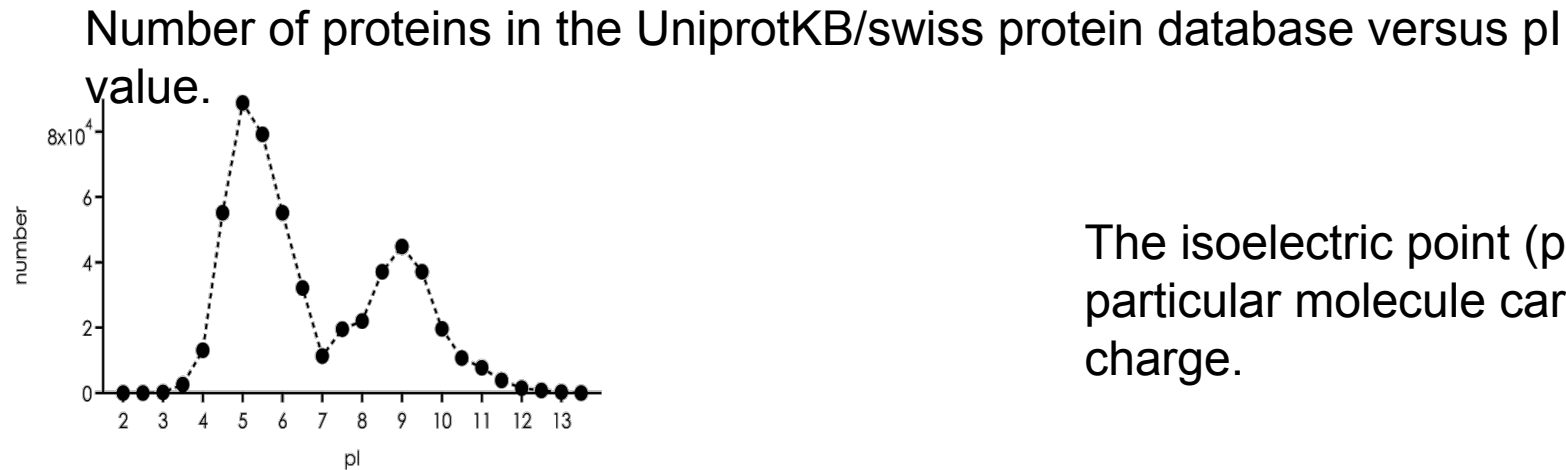


The isoelectric point (pI), is the pH at which a particular molecule carries no net electrical charge.

Figure 1. Number of proteins in the UniProtKB/Swiss-Prot database versus pI value, retrieved with the TagIdent tool (<http://www.expasy.ch/tools/tagident.html>) in June 2010.

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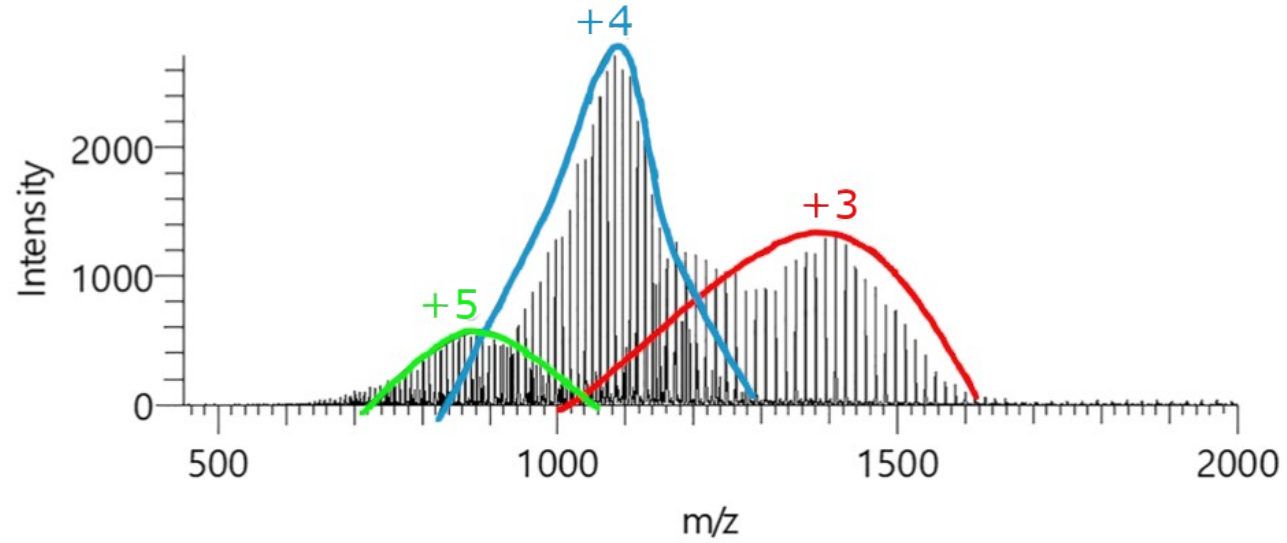
- Most of the proteins are charged in solutions
- Nucleic acids, fatty acids ...

Motivations

- Profit from the capacity of modern ionization sources to place virtually anything in the gas phase.
- Spectroscopy on ions
- Control over the target:
 - mass and charge selected species

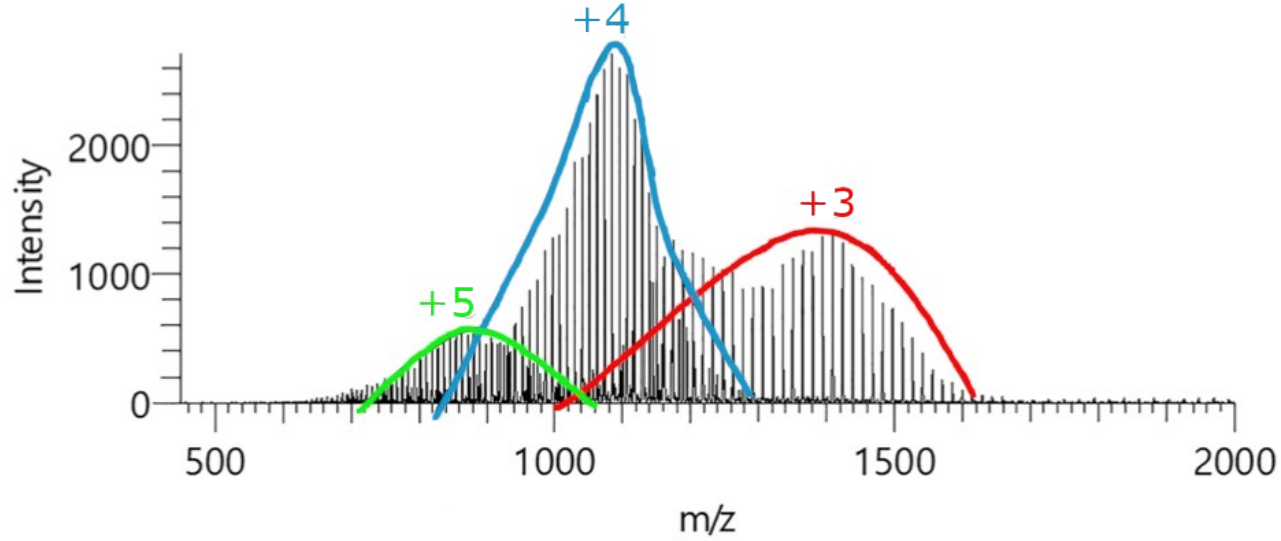


ESI-MS of PEG 4100

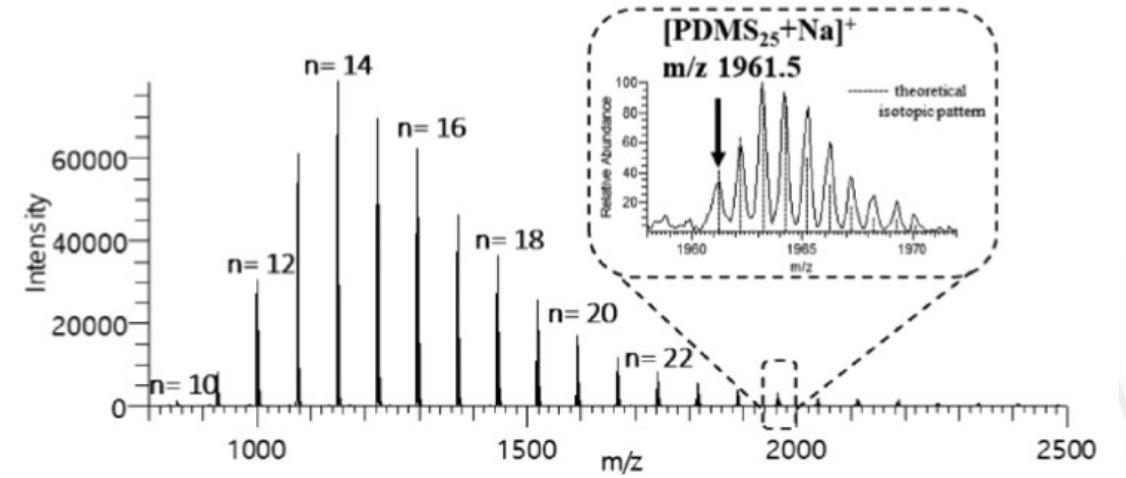


Motivations

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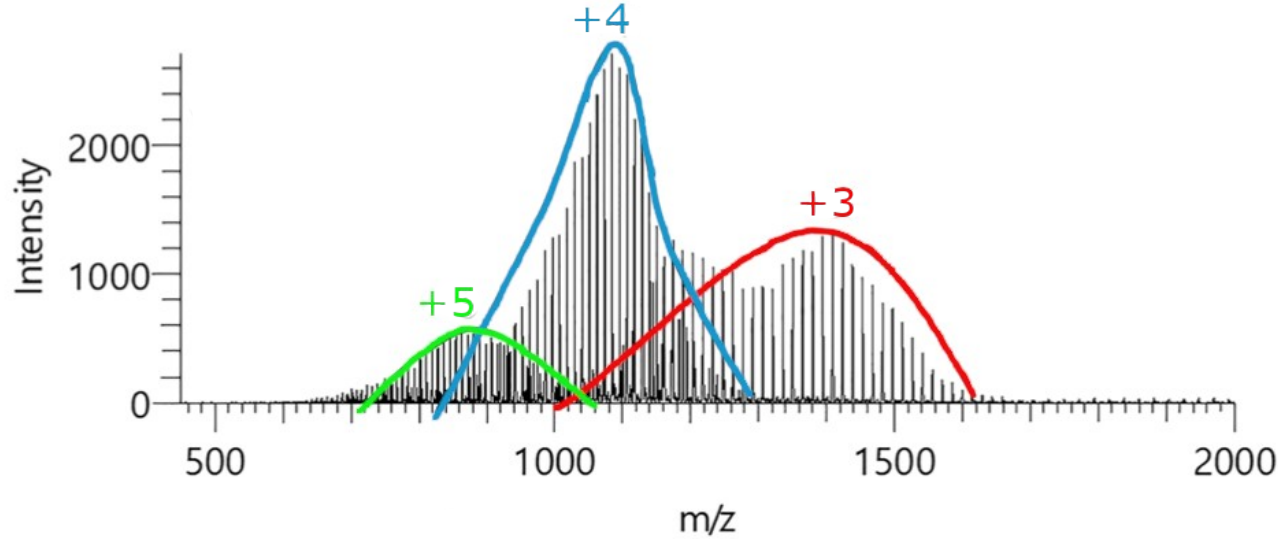


ESI-MS of PDMS. The arrow indicates the monoisotopic ion of $[\text{PDMS}_{25}+\text{Na}]^+$

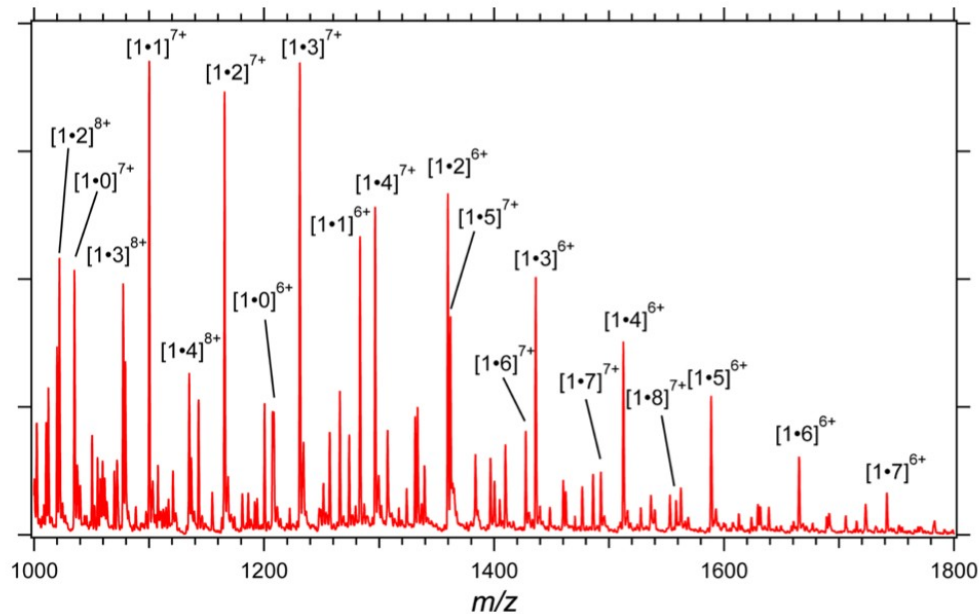
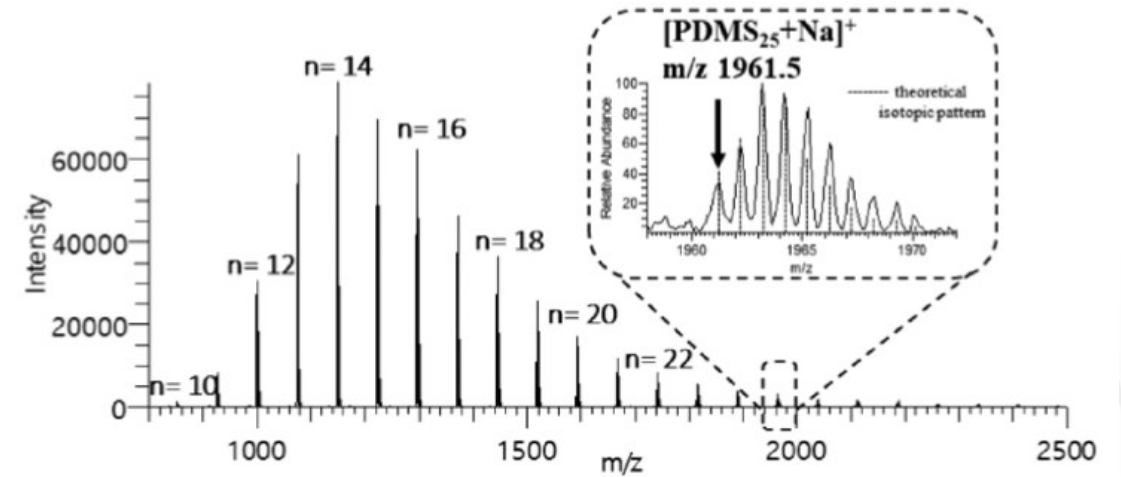


Motivations

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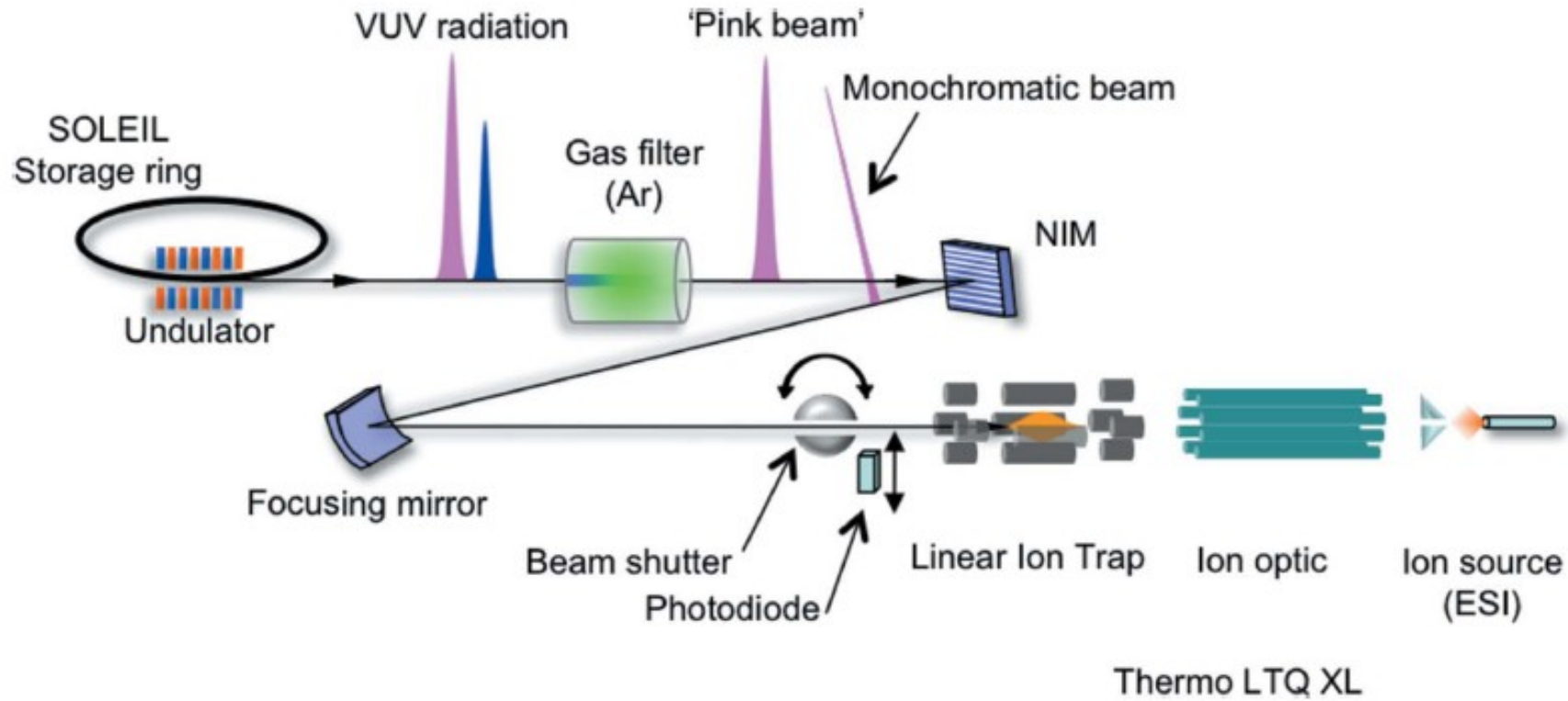


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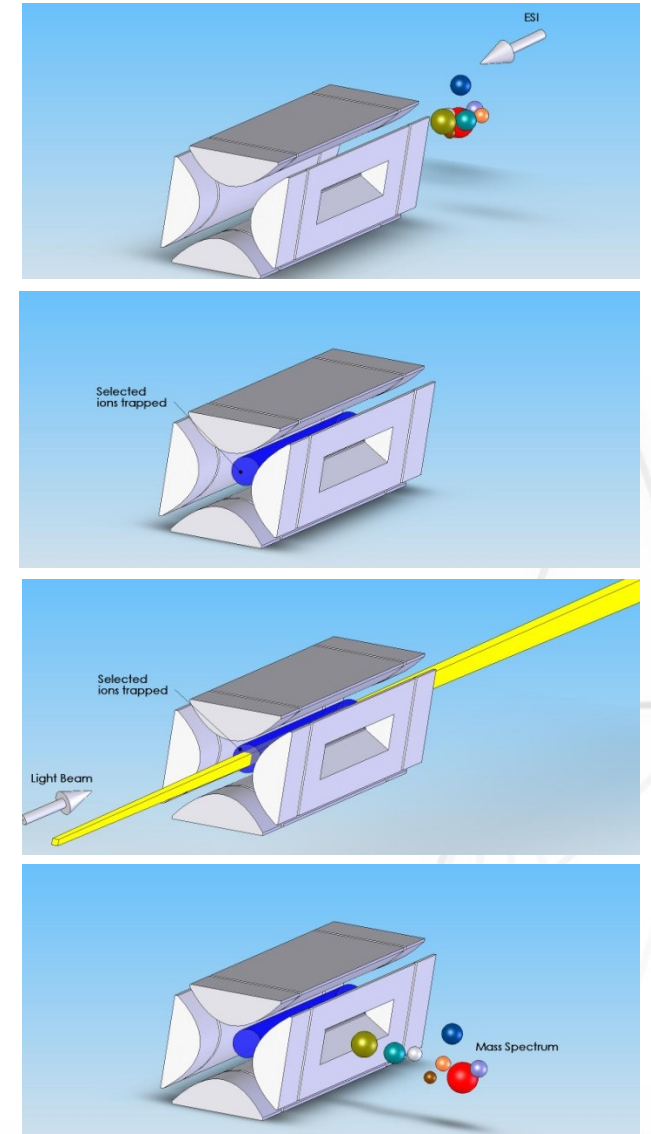
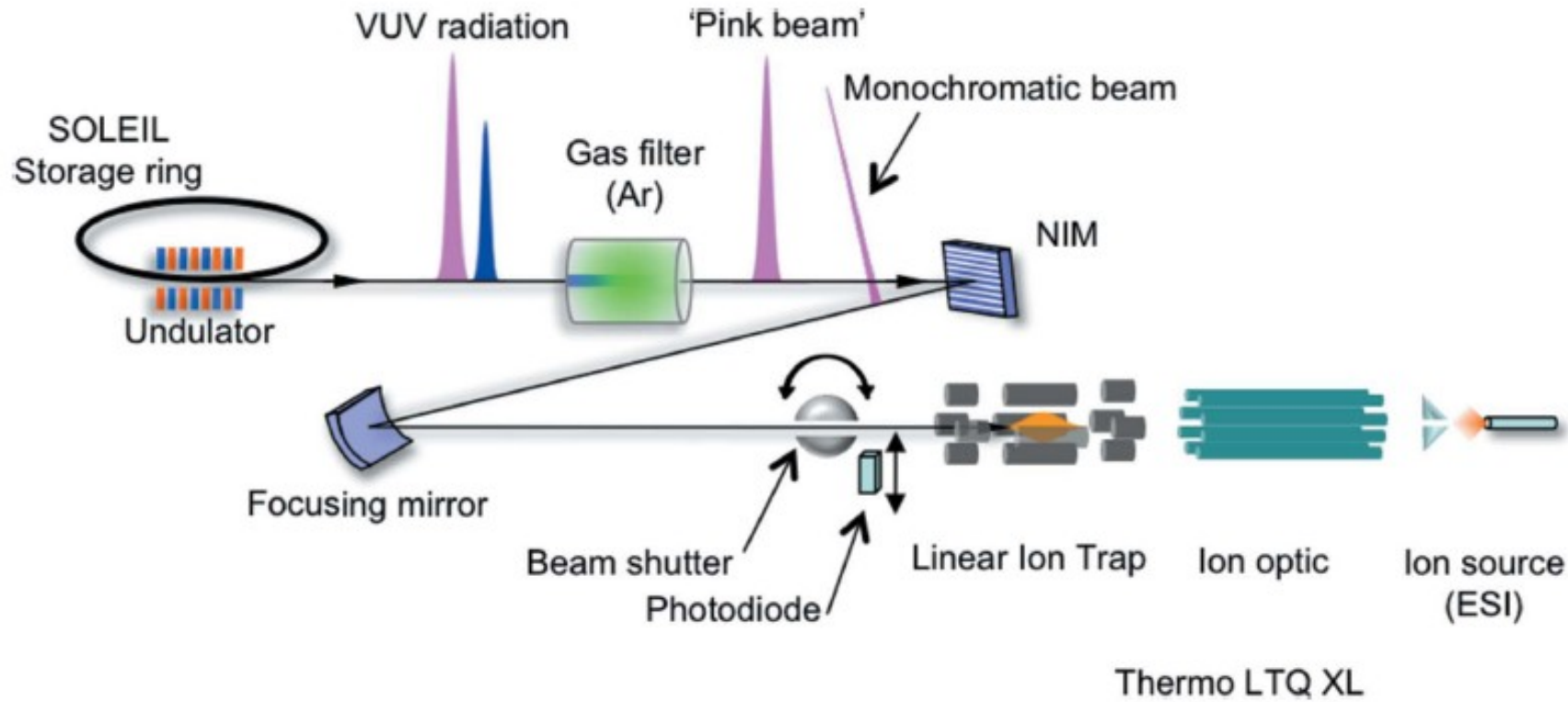


ESI-MS of a protein interacting with its ligand. Mixture of stoichiometries (1.0 à 1.8) and charge states (6+ à 8+).

Experimental setup



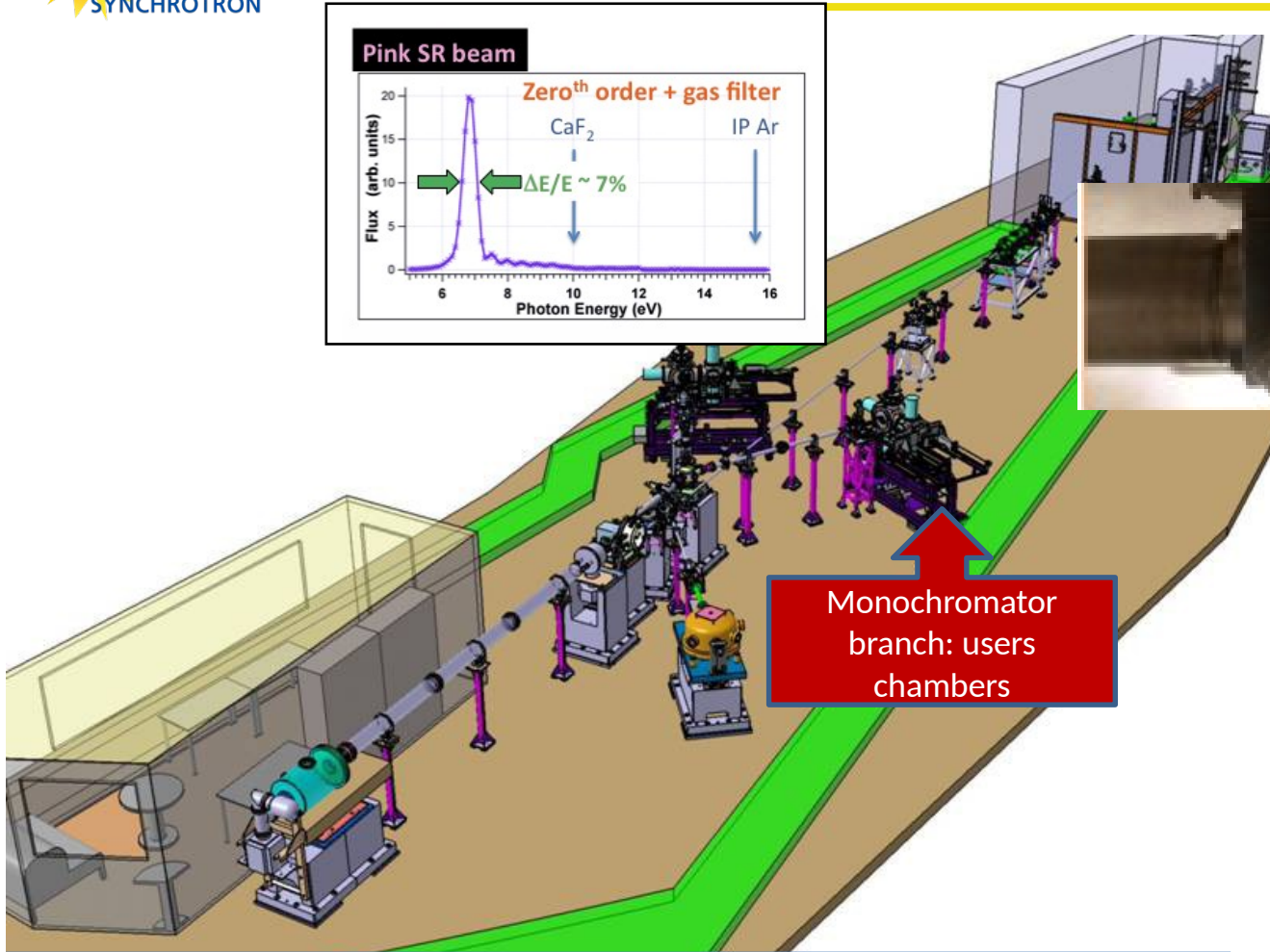
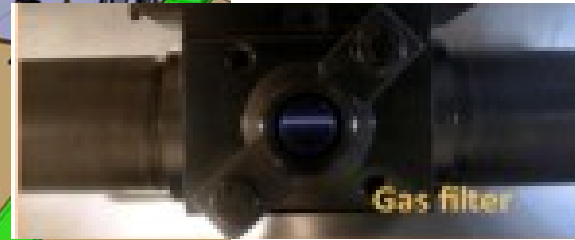
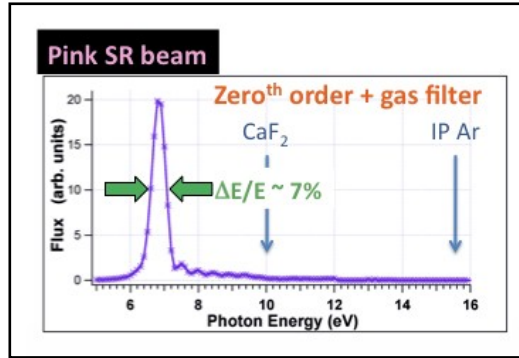
Experimental setup



SRMS2 @ DESIRS

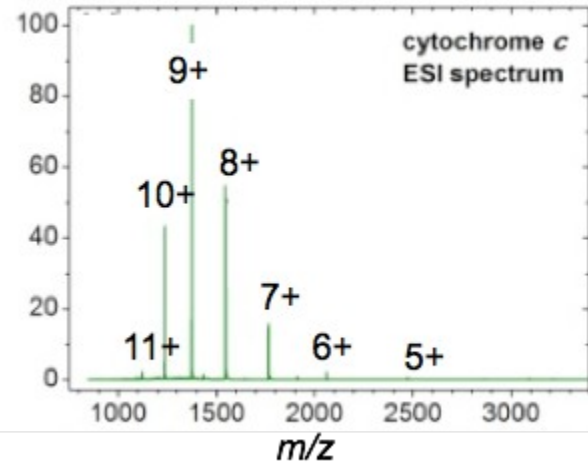
DESIRS beamline, SOLEIL

<http://www.synchrotron-soleil.fr/Recherche/LignesLumiere/DESRS>

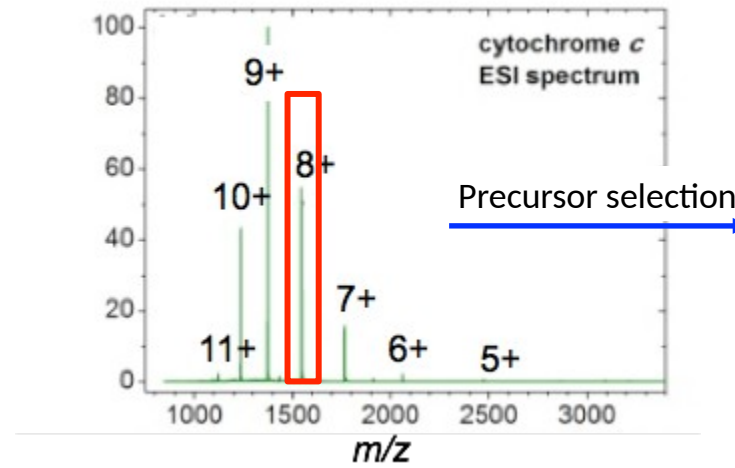


L. Nahon et al., "DESIRS : a state-of-the-art VUV beamline featuring high resolution and variable polarization for spectroscopy and dichroism at SOLEIL " *J. Synchrotron Rad.* **19**, 508-520 (2012)

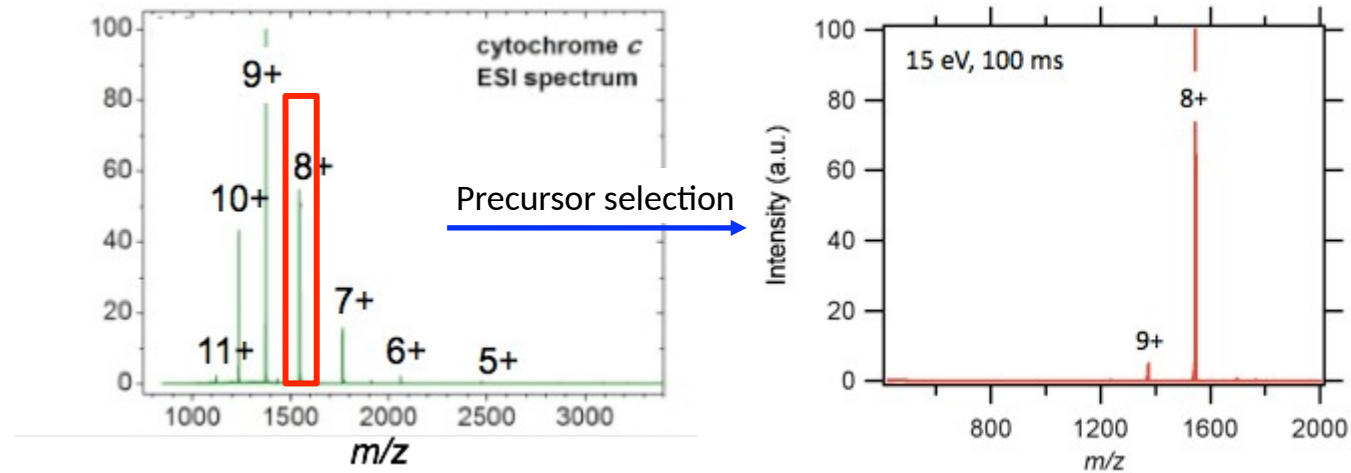
Action spectroscopy



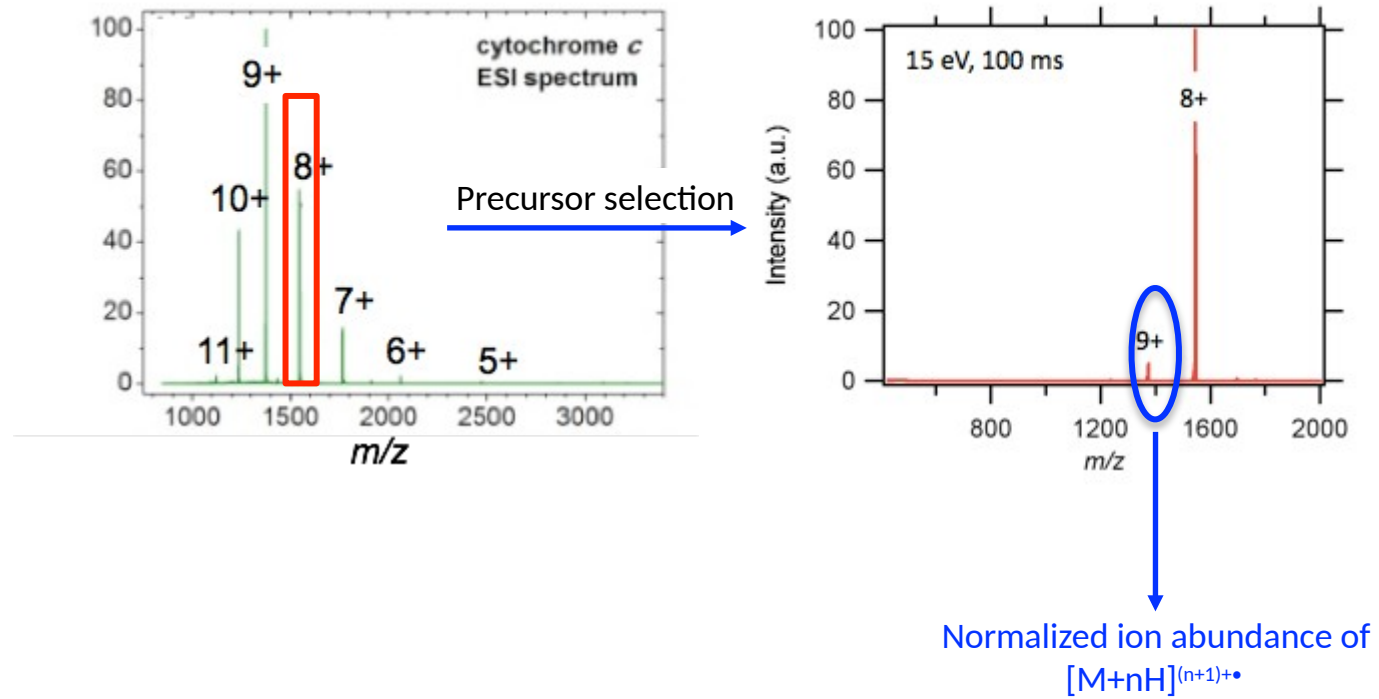
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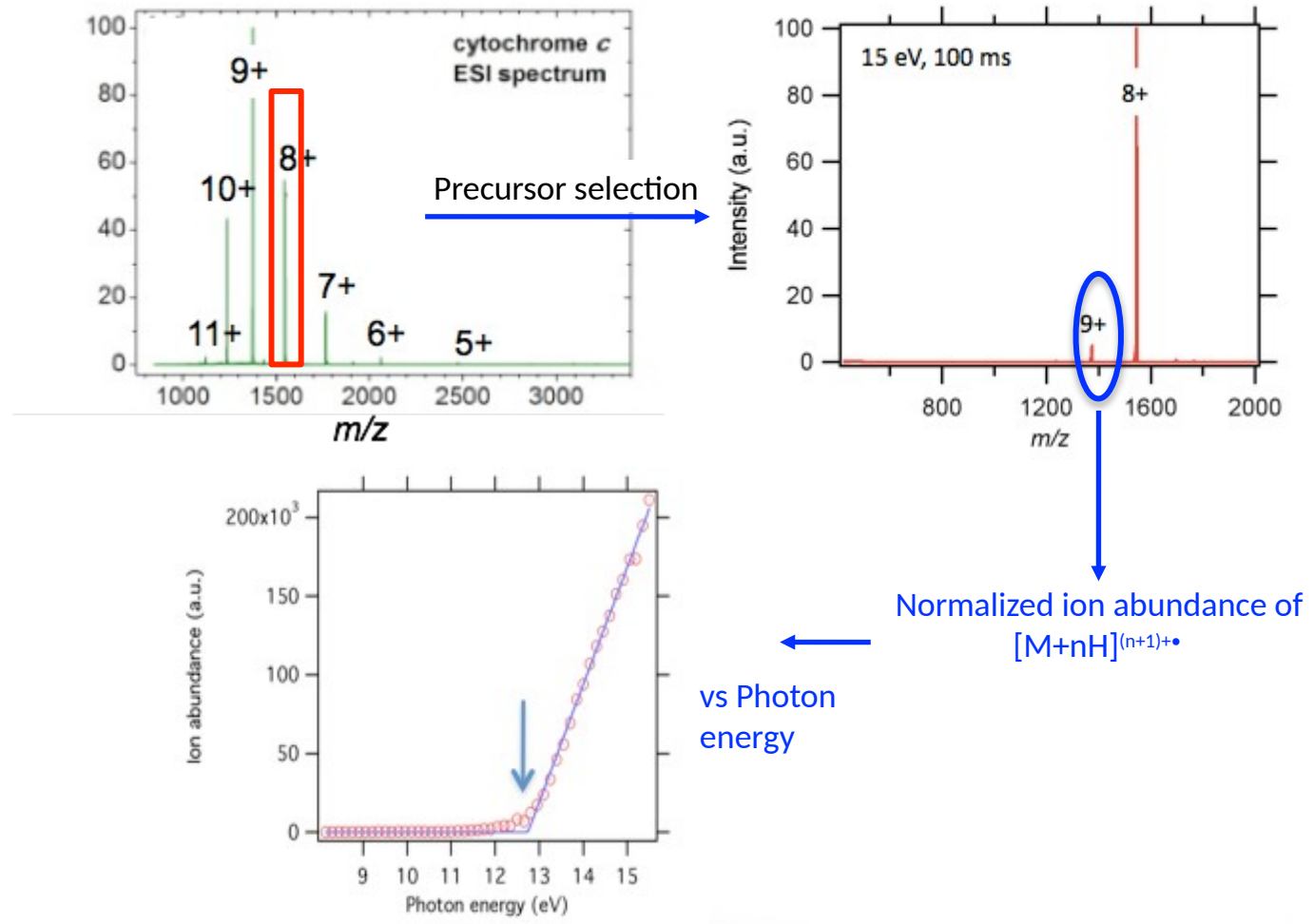
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Outline

- VUV activation of oligosaccharides

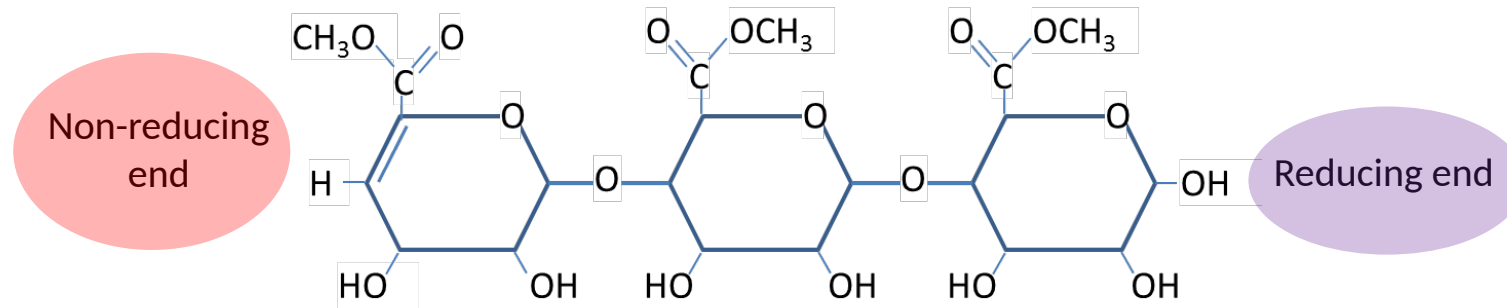
- Serine dimer



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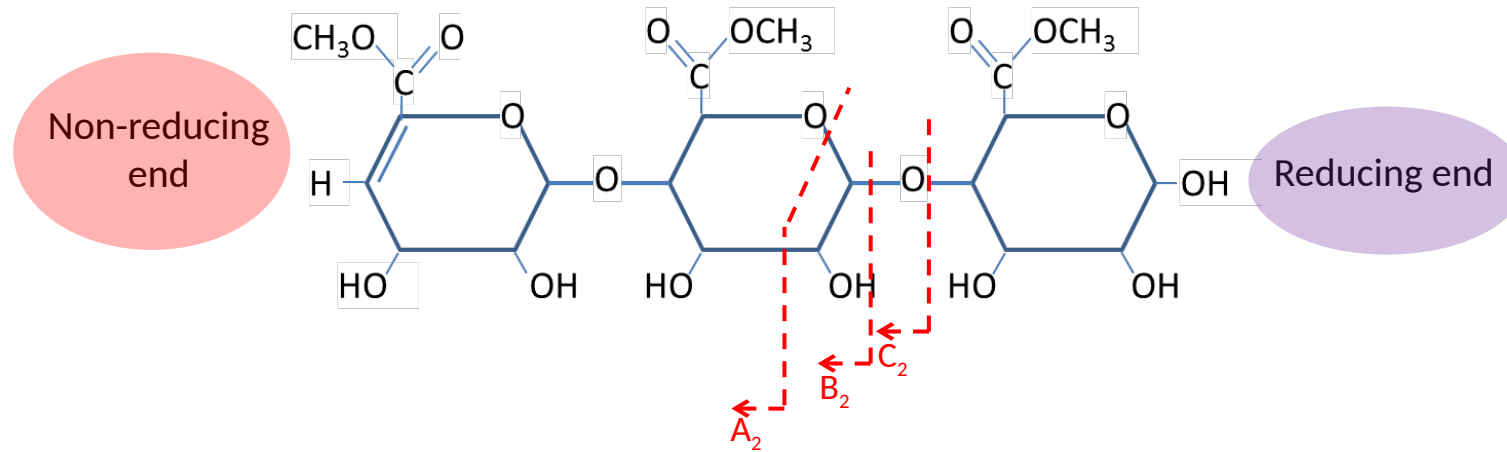


Nomenclature



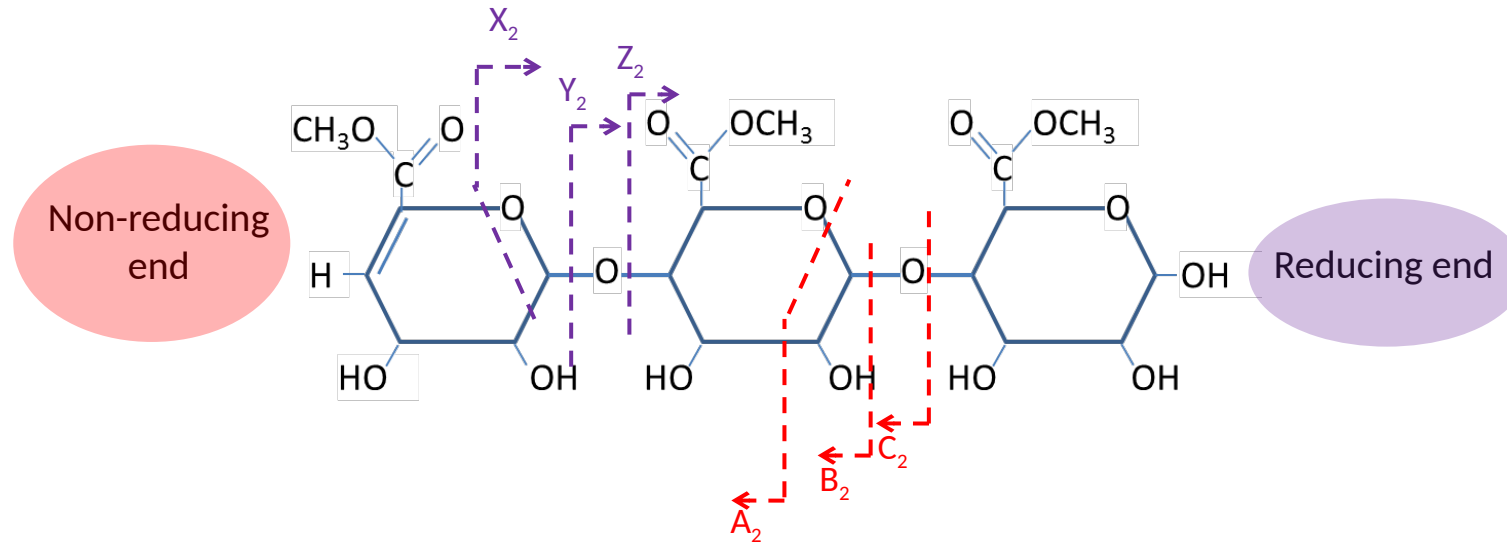
Nomenclature from Domon et Costello (1988)

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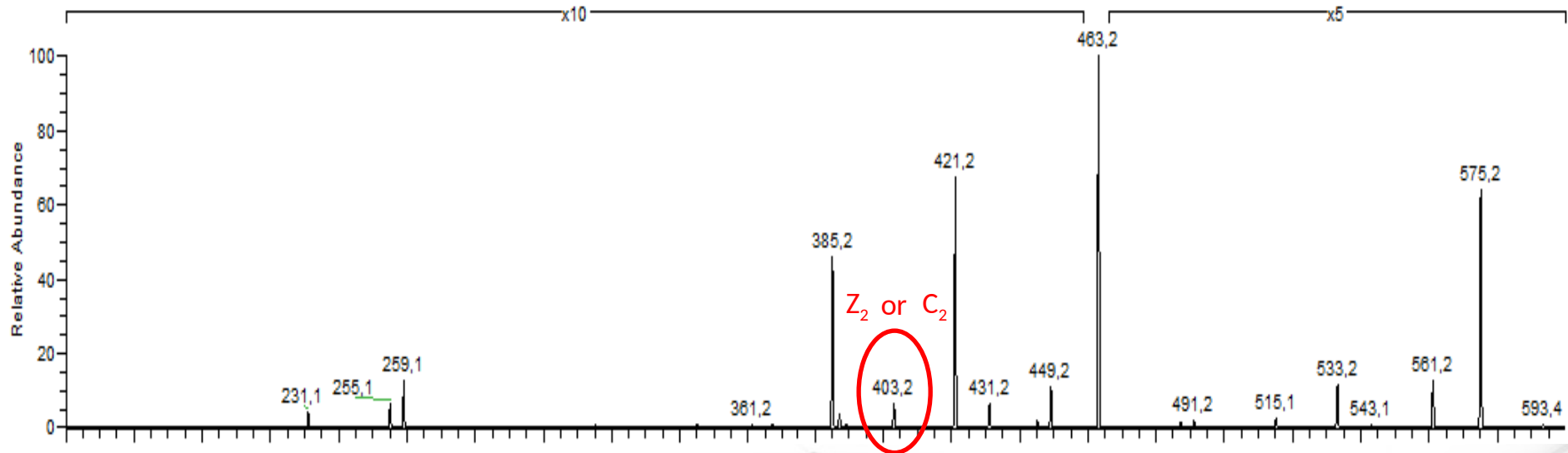
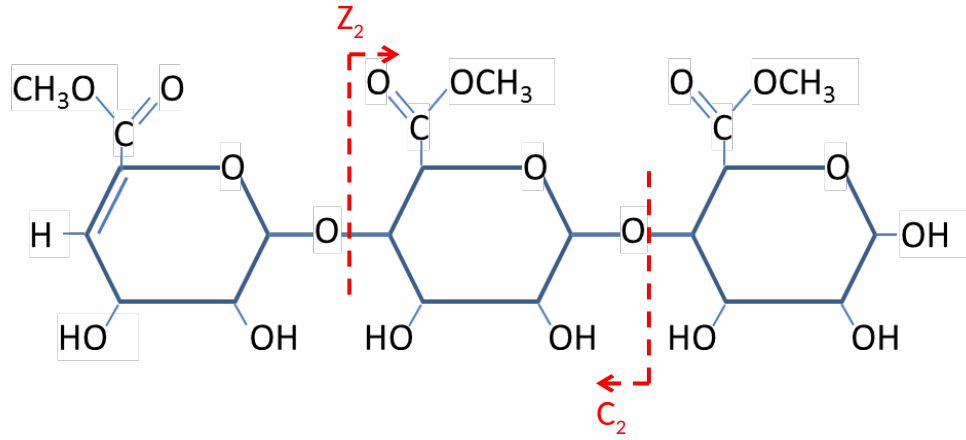
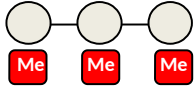
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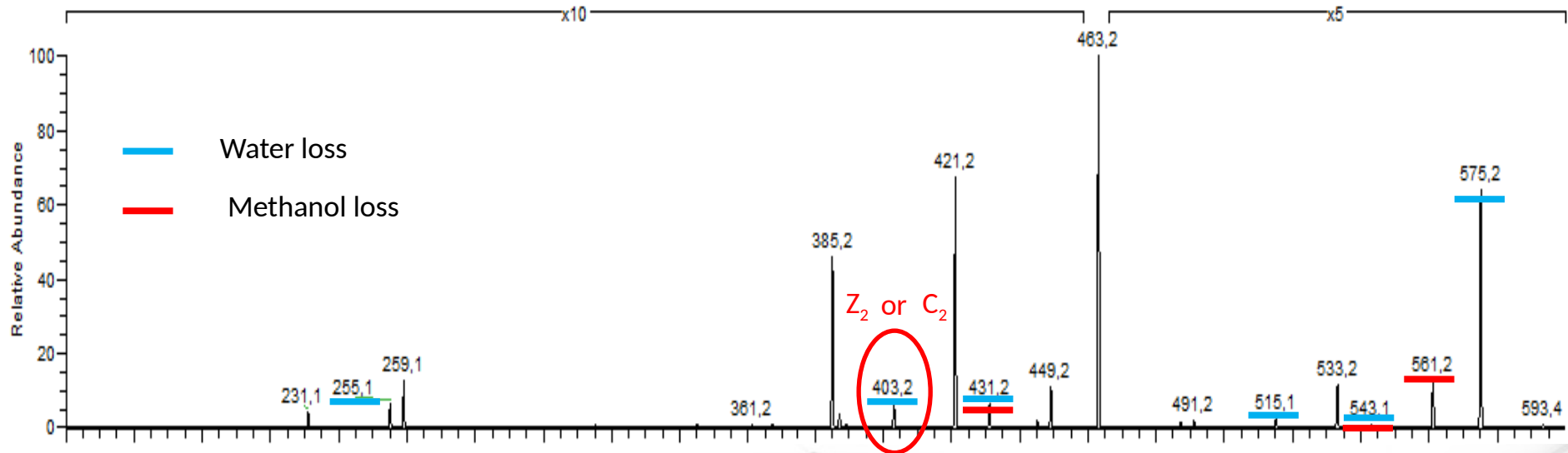
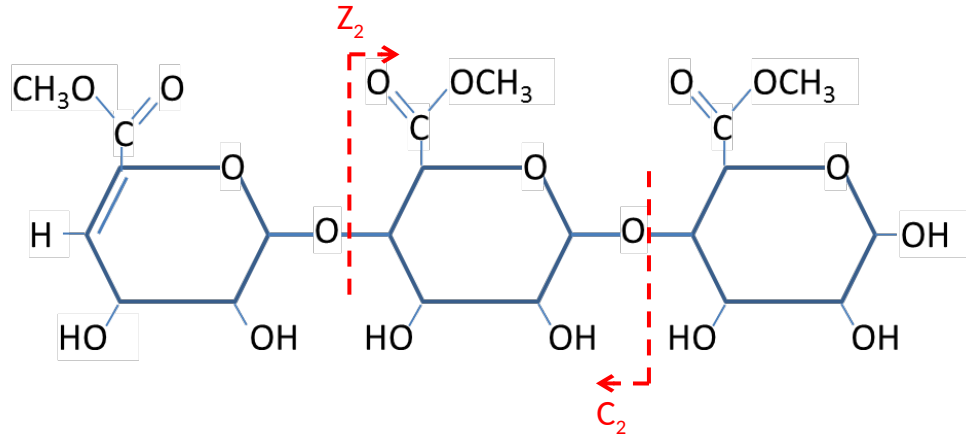
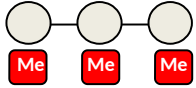
Limitation of collision activation

DP3Me3



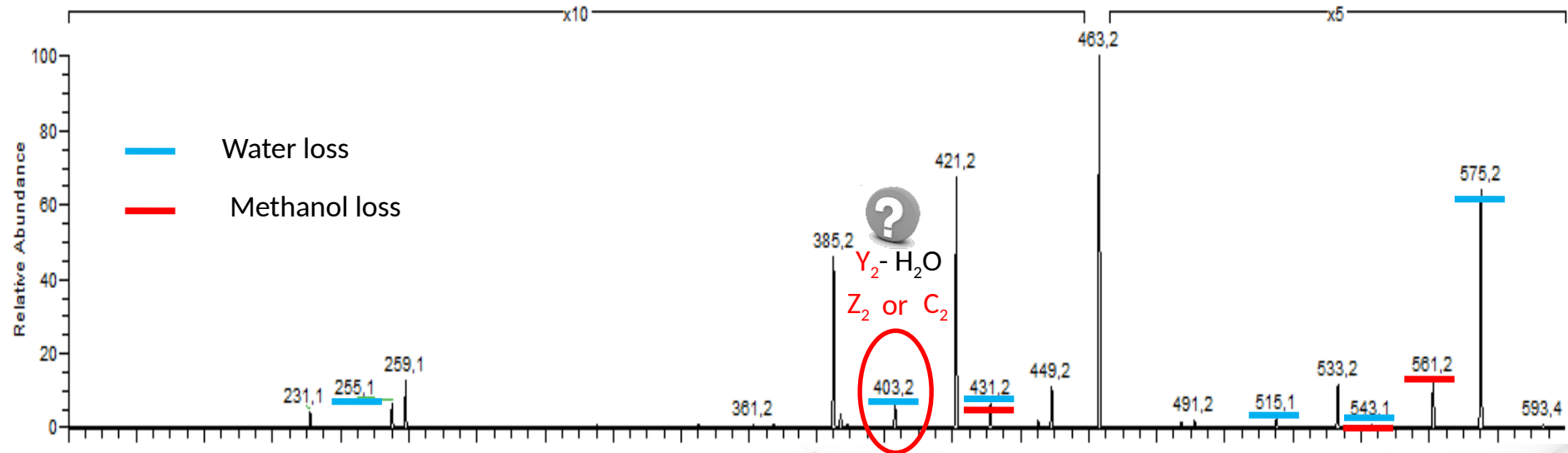
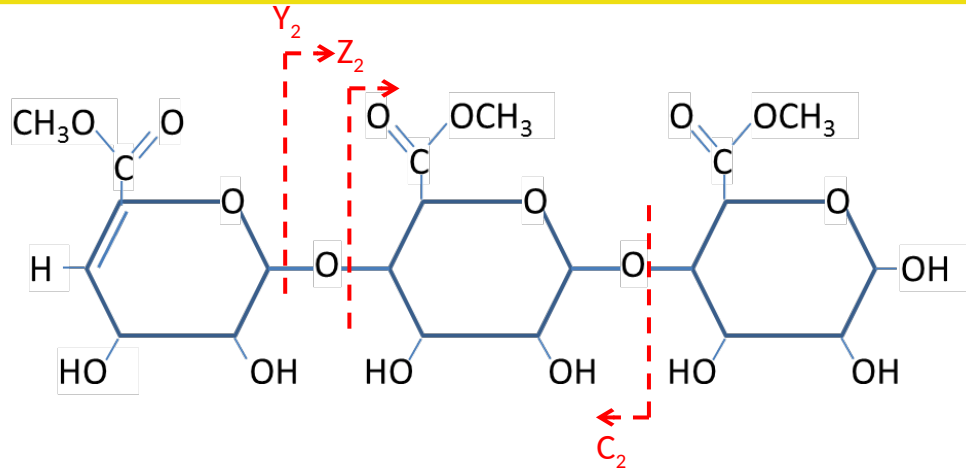
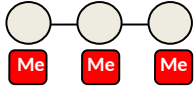
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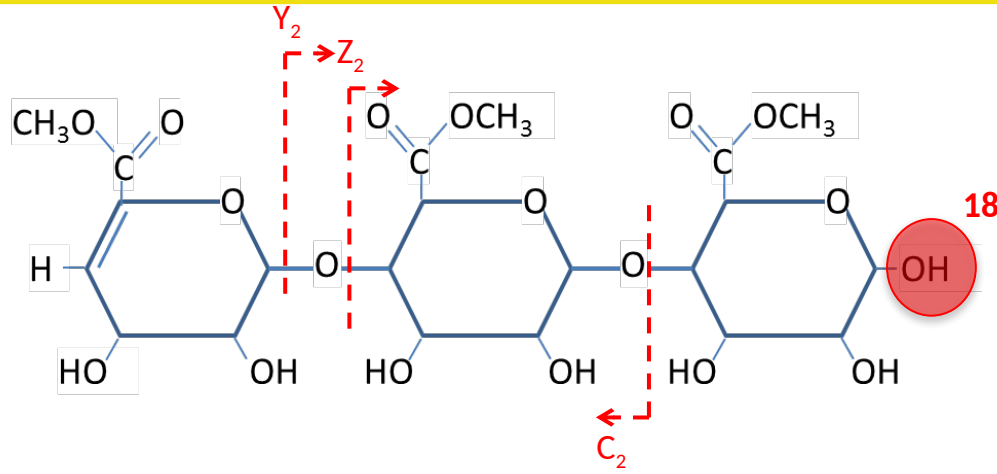
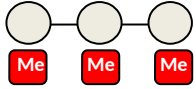
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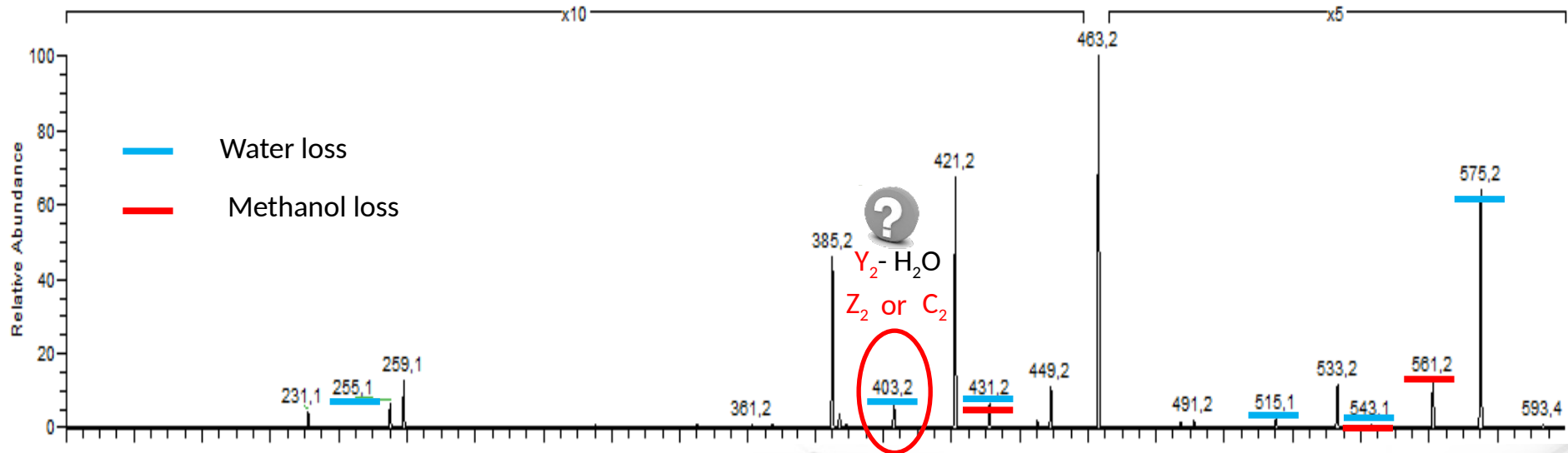


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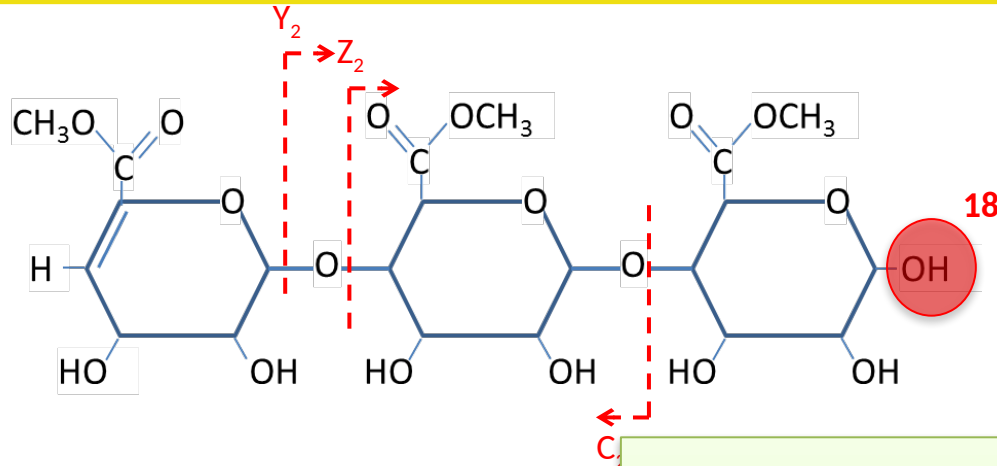
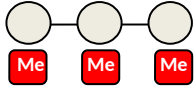


X, Y, Z fragments shift by 2 Da

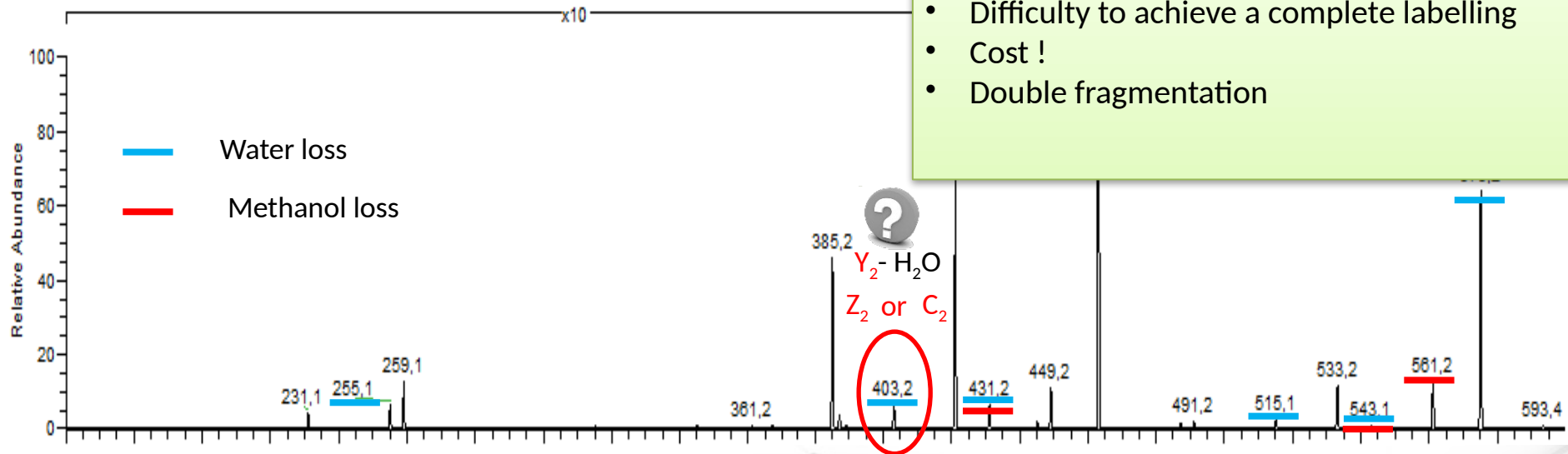


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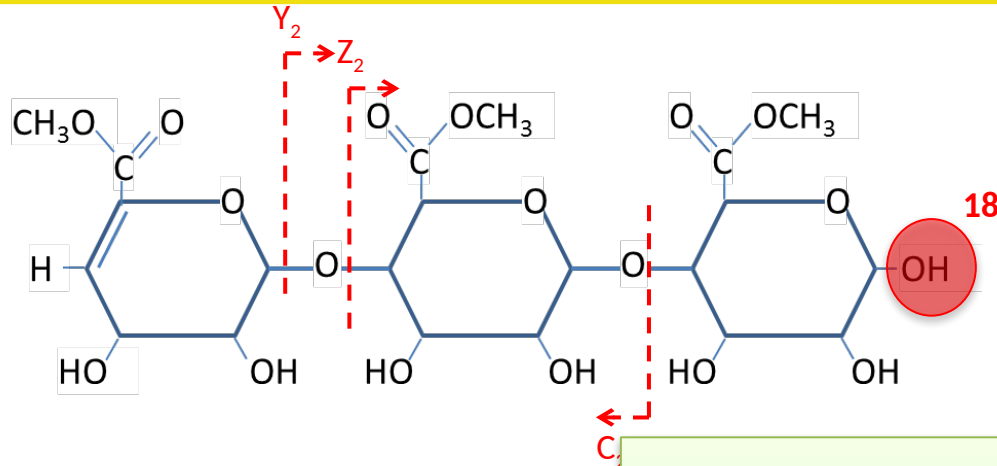
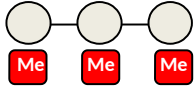
X, Y, Z fragments shift by 2 Da



- Difficulty to achieve a complete labelling
- Cost !
- Double fragmentation

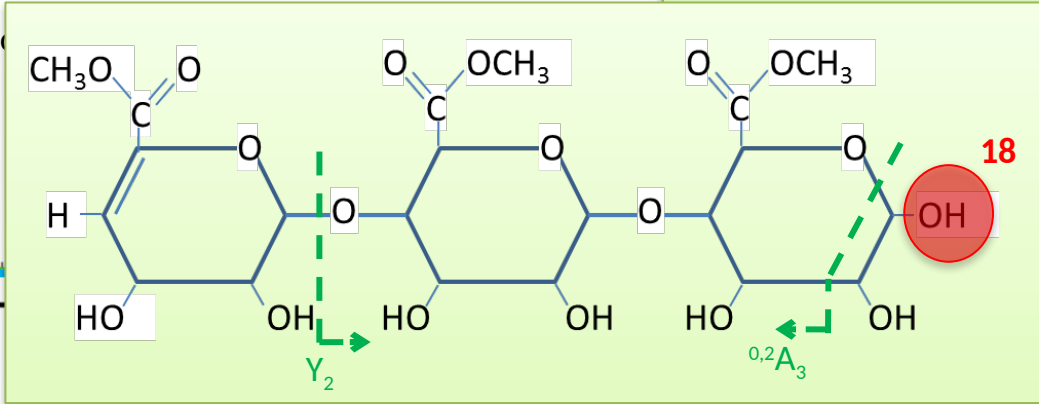
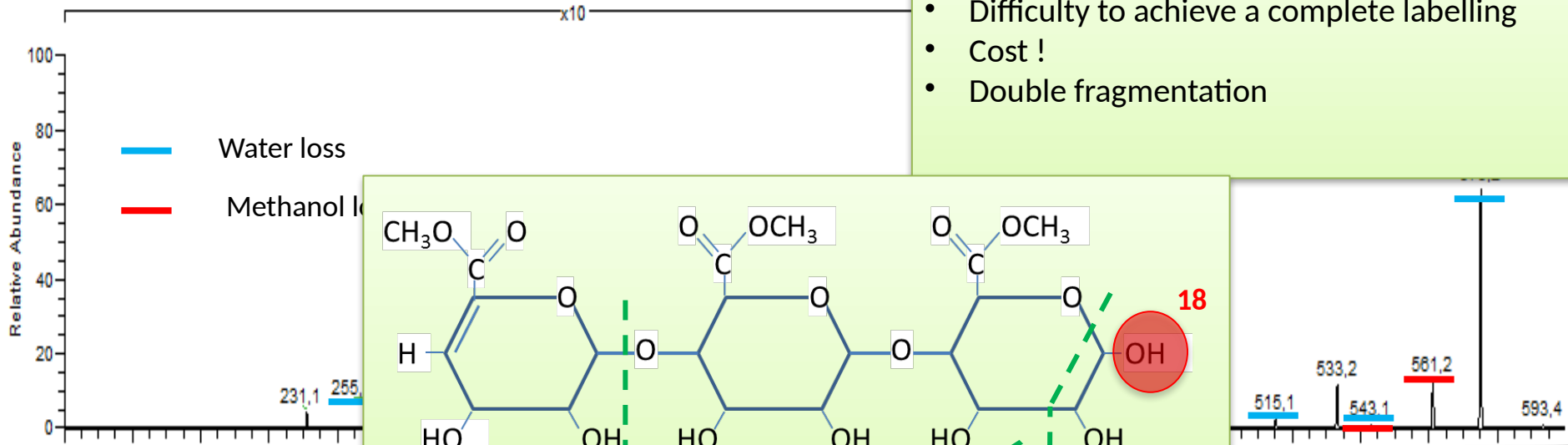
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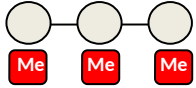
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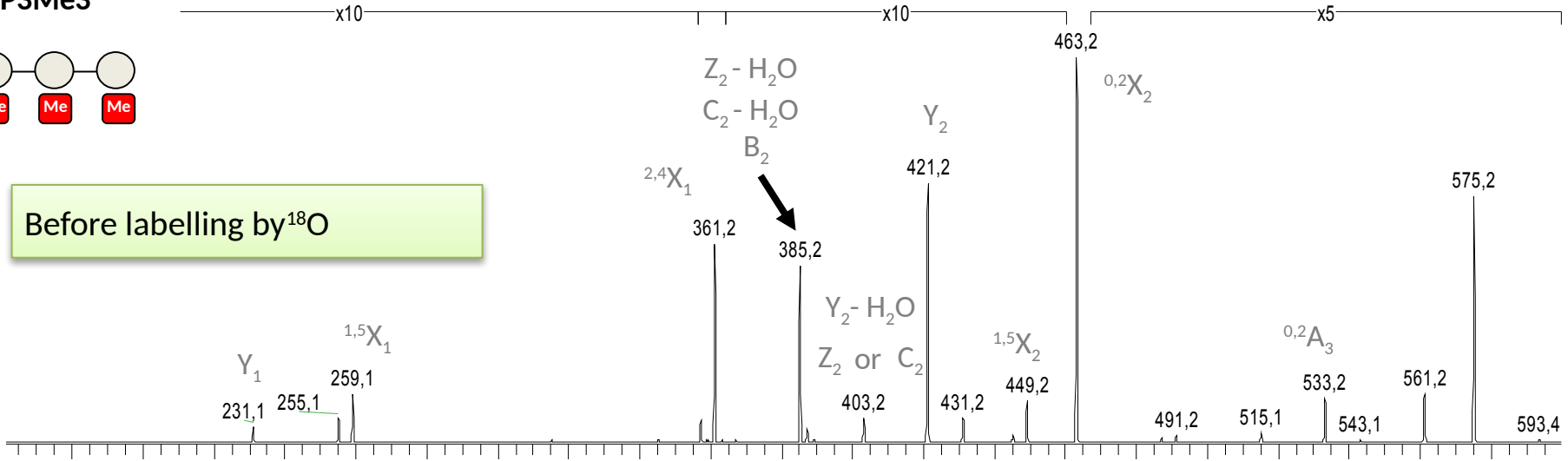


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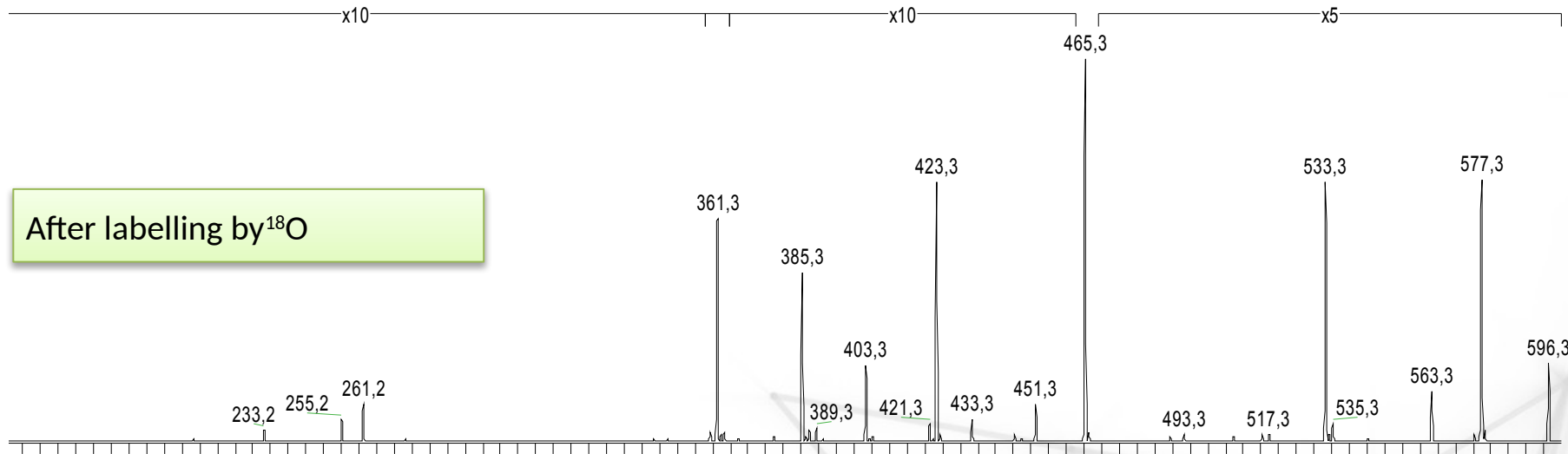
DP3Me3



Before labelling by ^{18}O

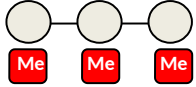


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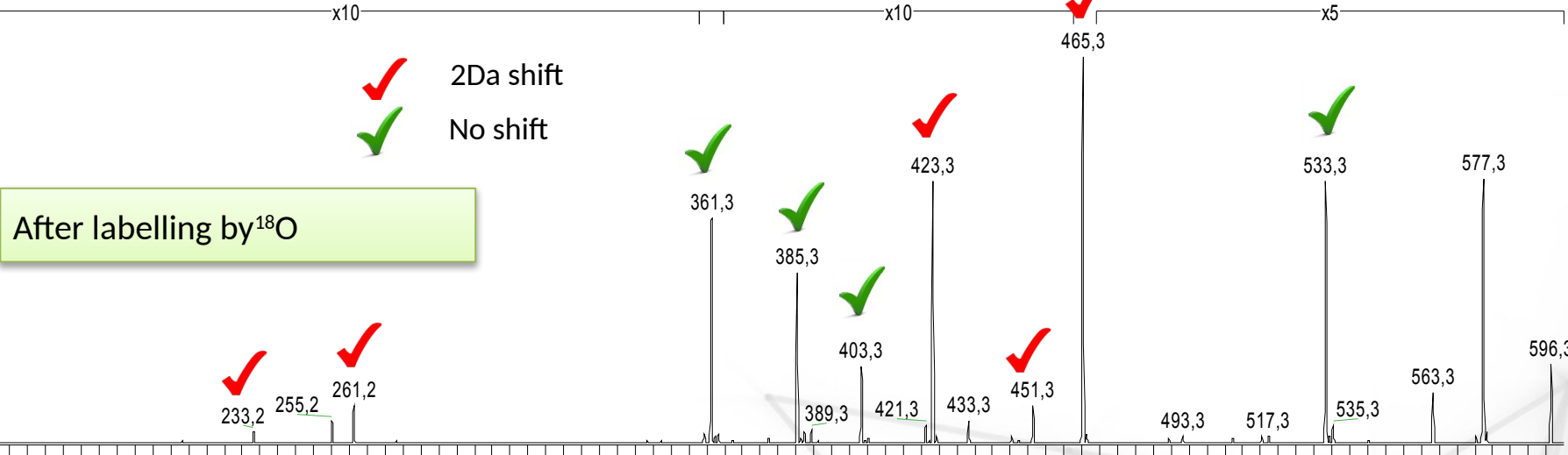
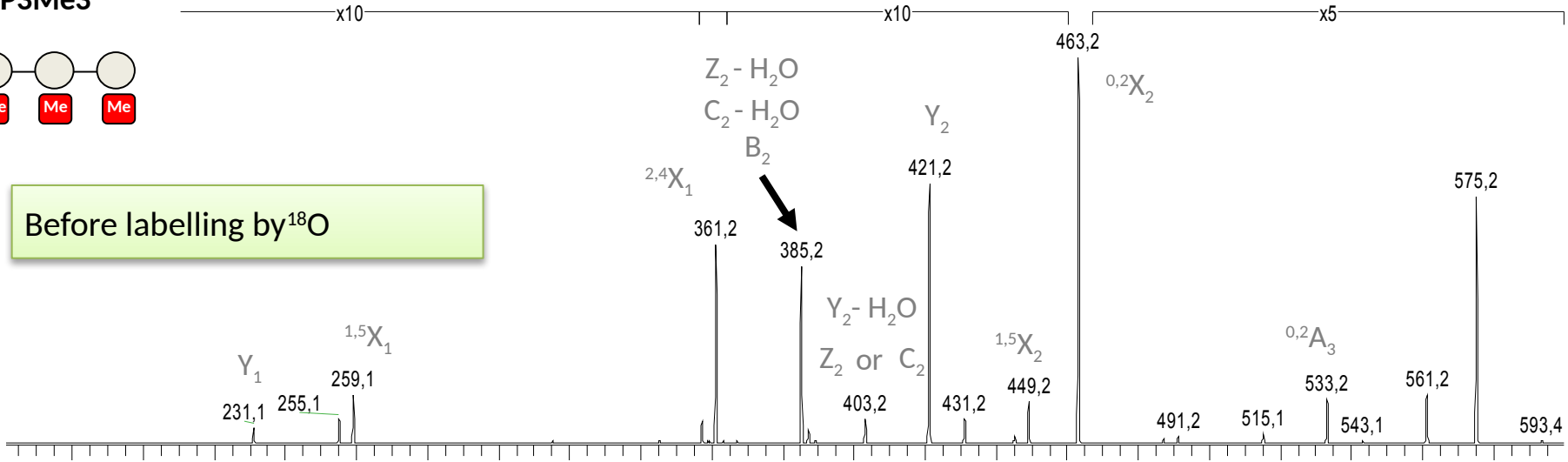


Limitation of collision activation

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Before labelling by ^{18}O

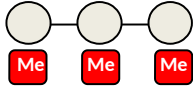


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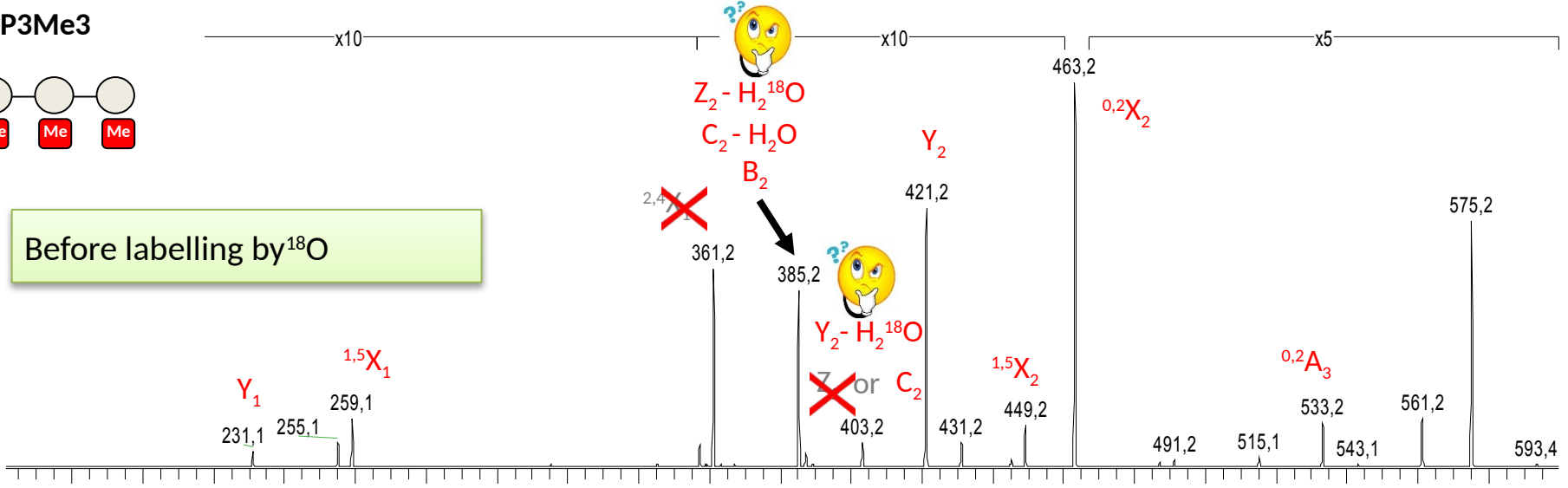
2Da shift
 No shift

Limitation of collision activation

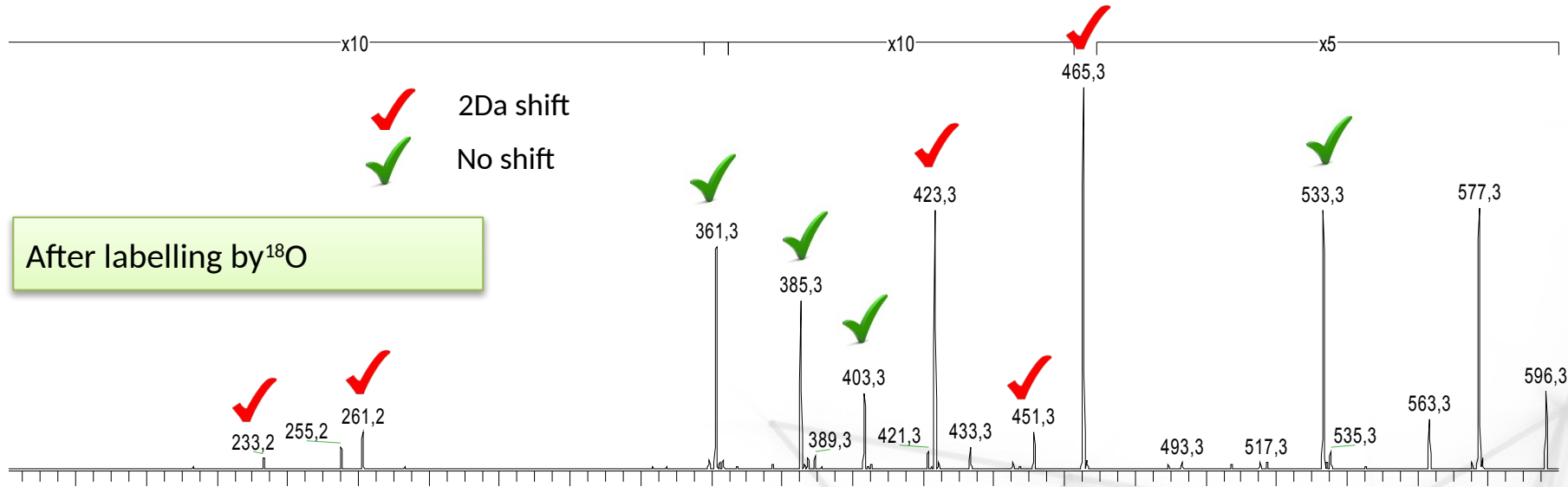
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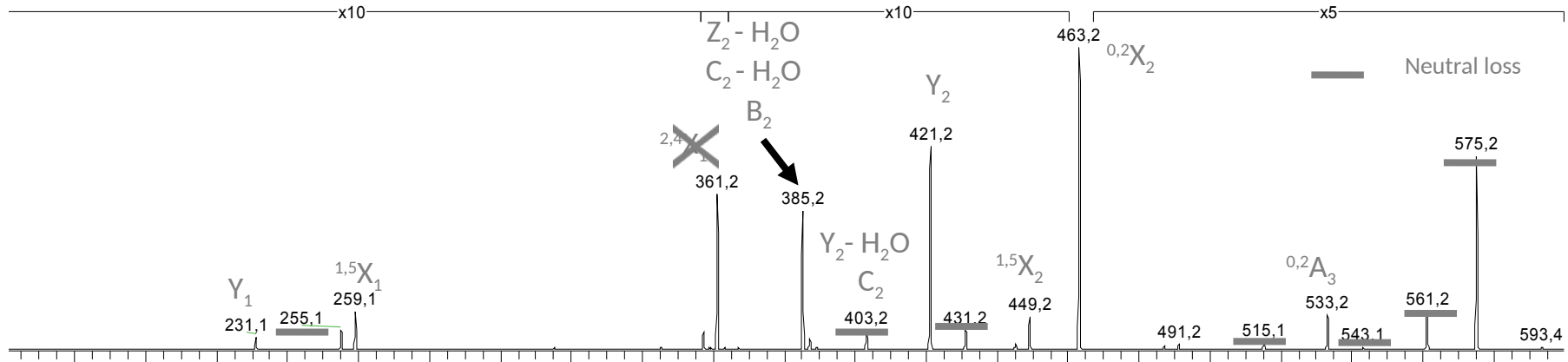
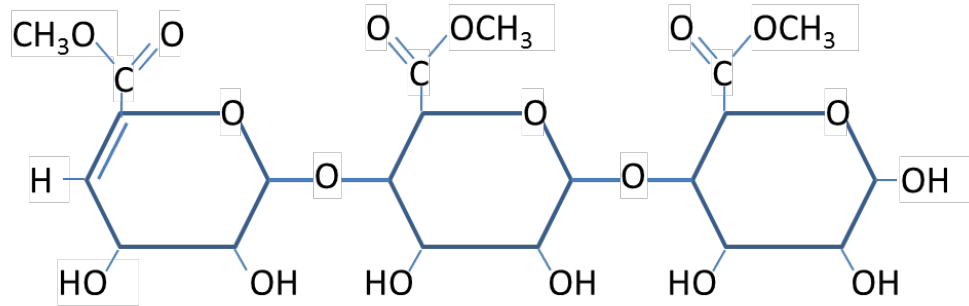
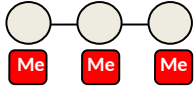


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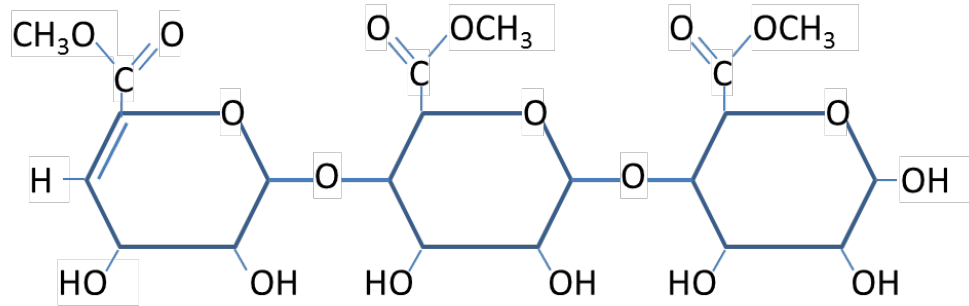
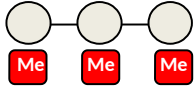
VUV activation vs CID

DP3Me3

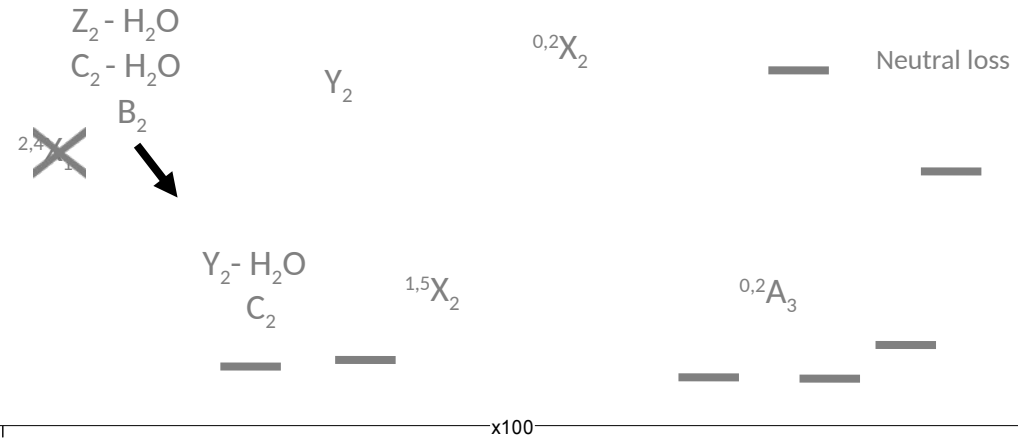


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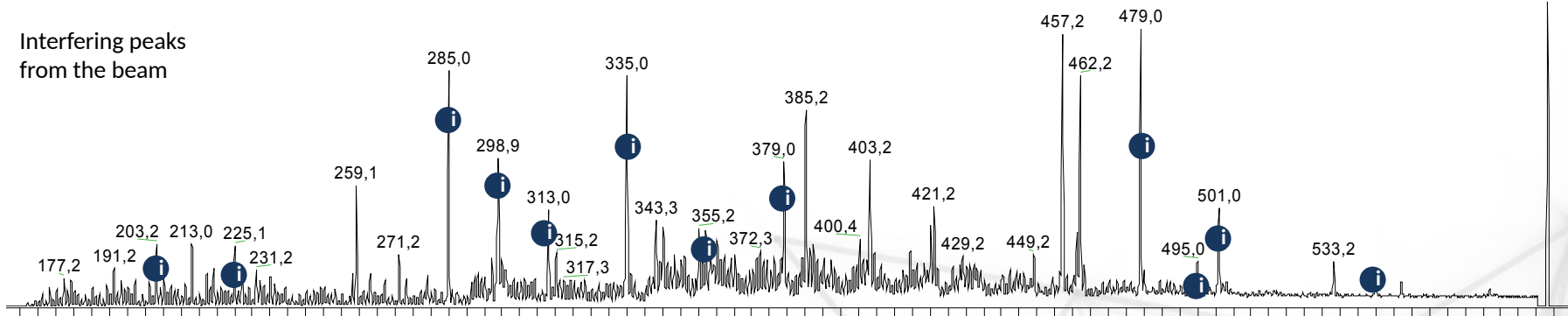


- More intracyclic fragments
- No neutral losses
- No double fragmentation



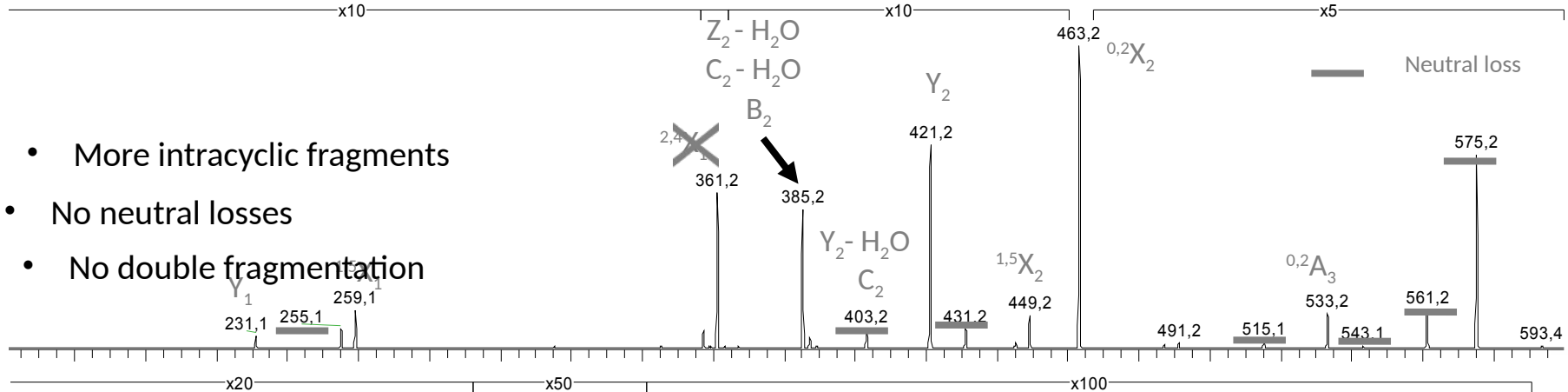
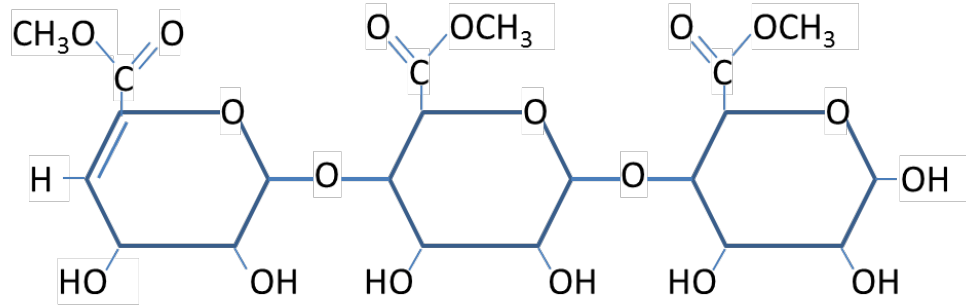
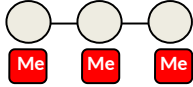
i

Interfering peaks from the beam



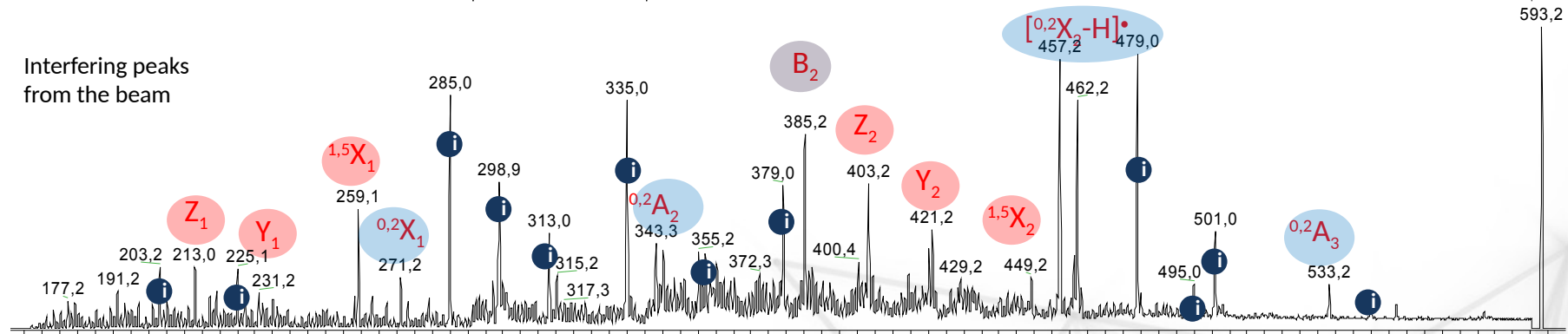
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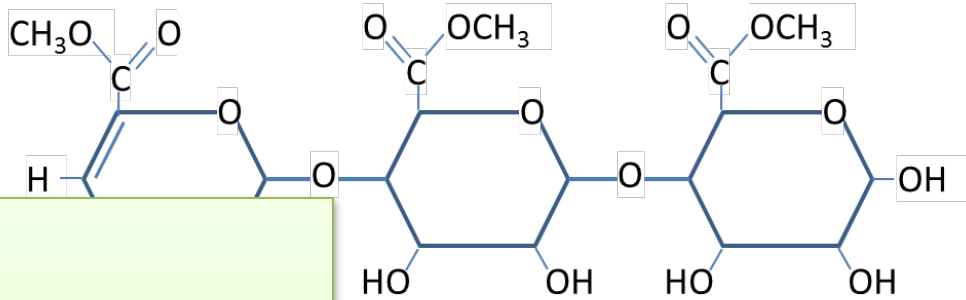
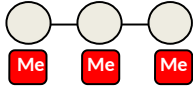
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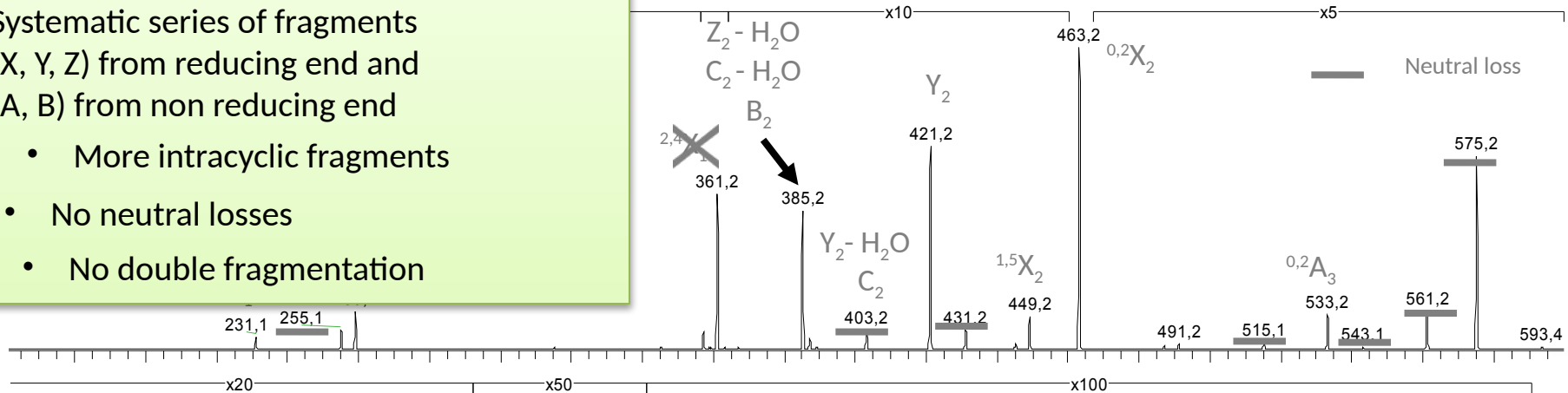
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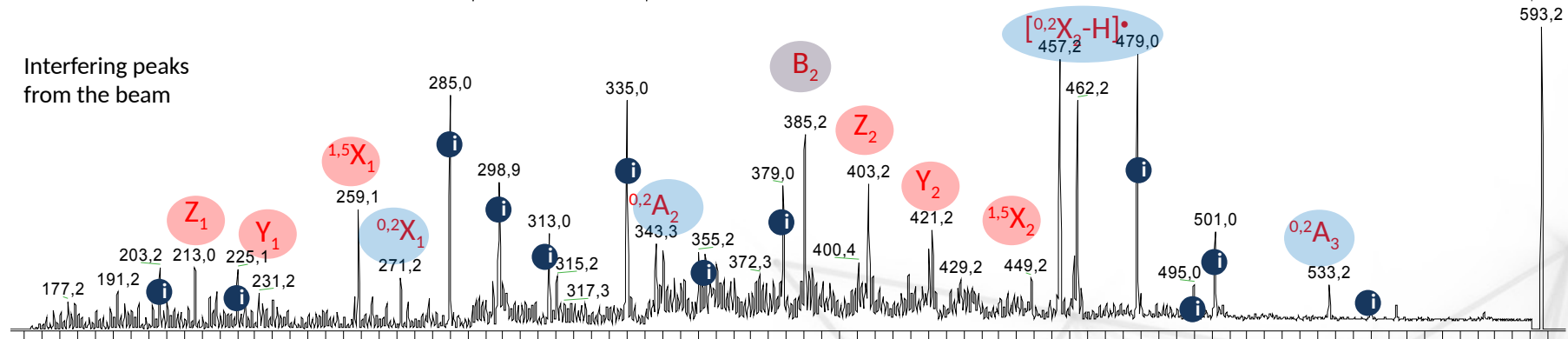


"RULES" :

- Systematic series of fragments (X, Y, Z) from reducing end and (A, B) from non reducing end
 - More intracyclic fragments
 - No neutral losses
 - No double fragmentation

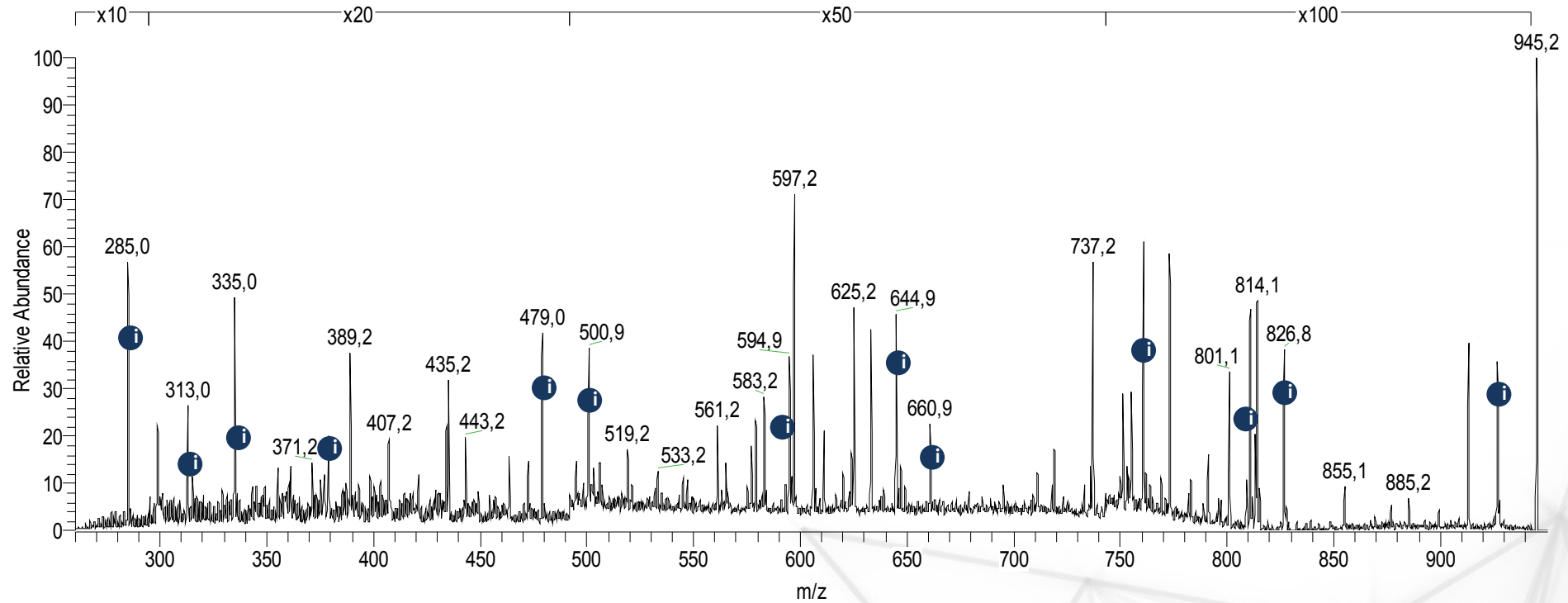
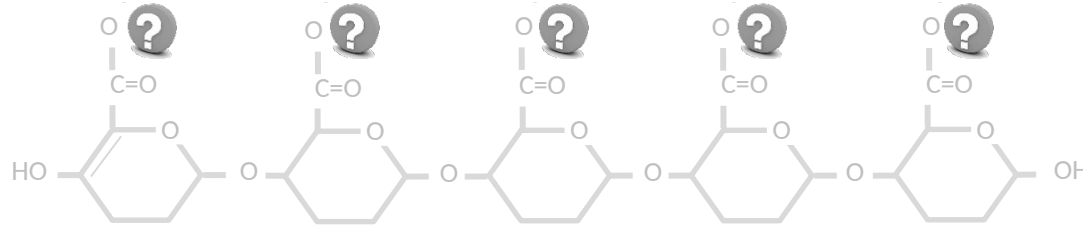
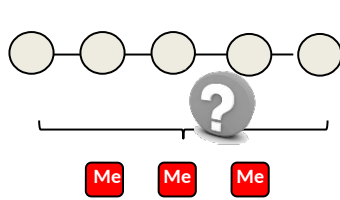


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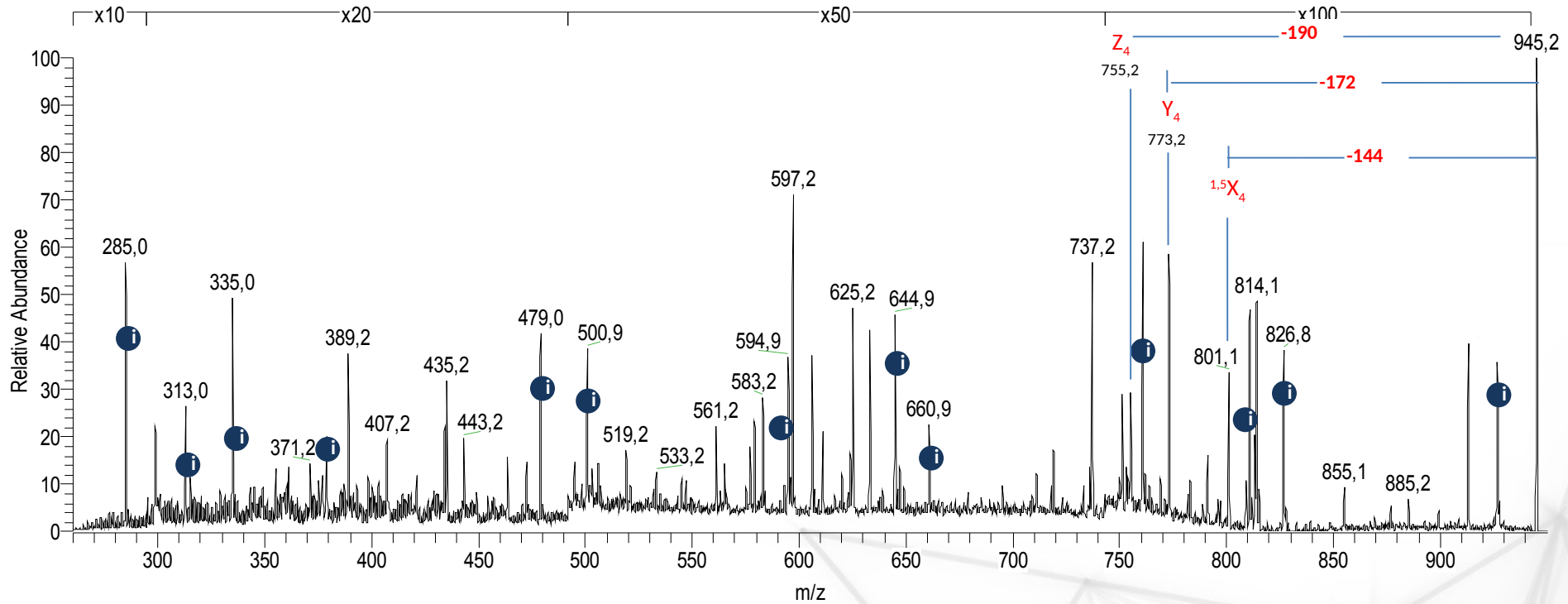
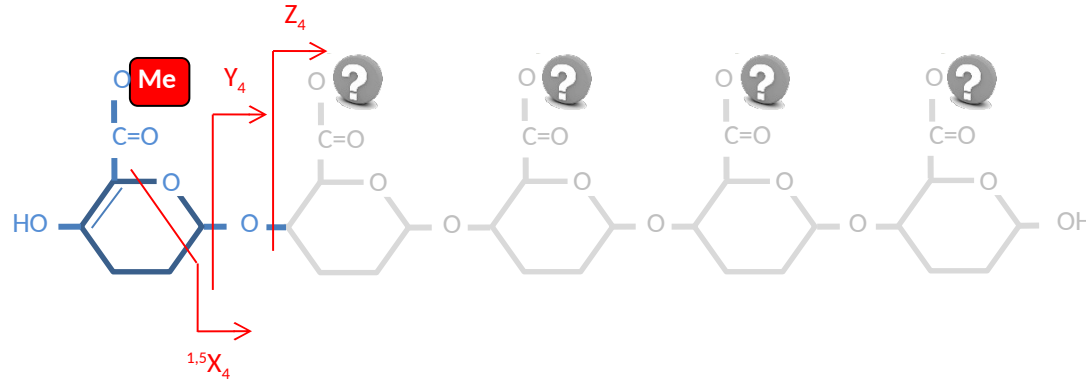
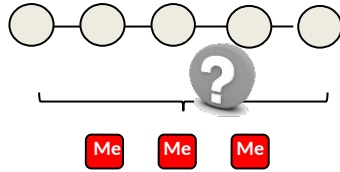
DP5Me3 sequencing

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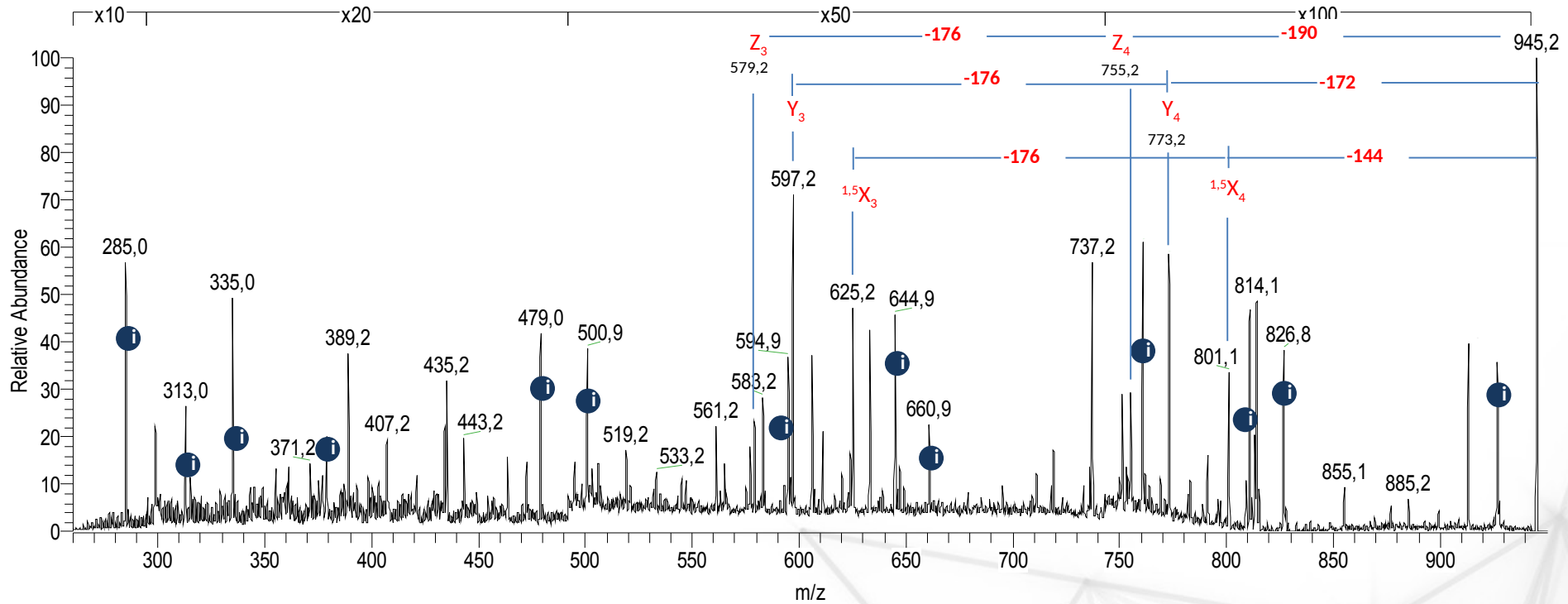
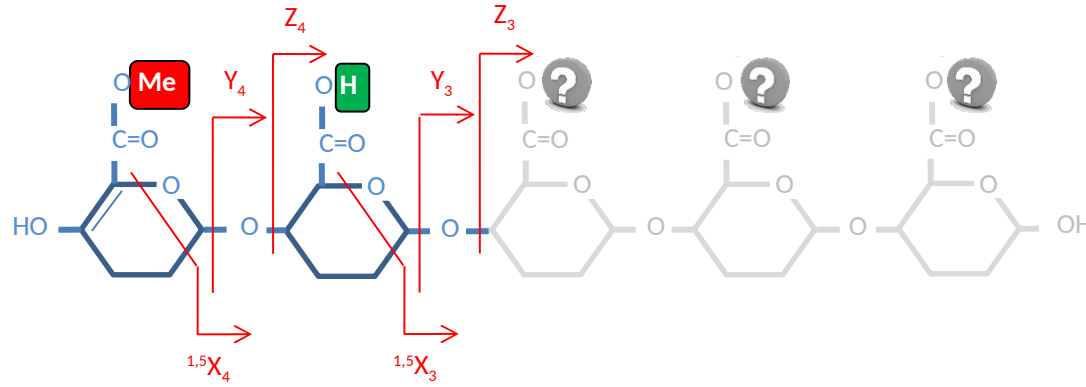
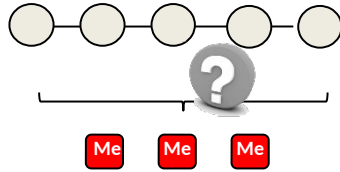
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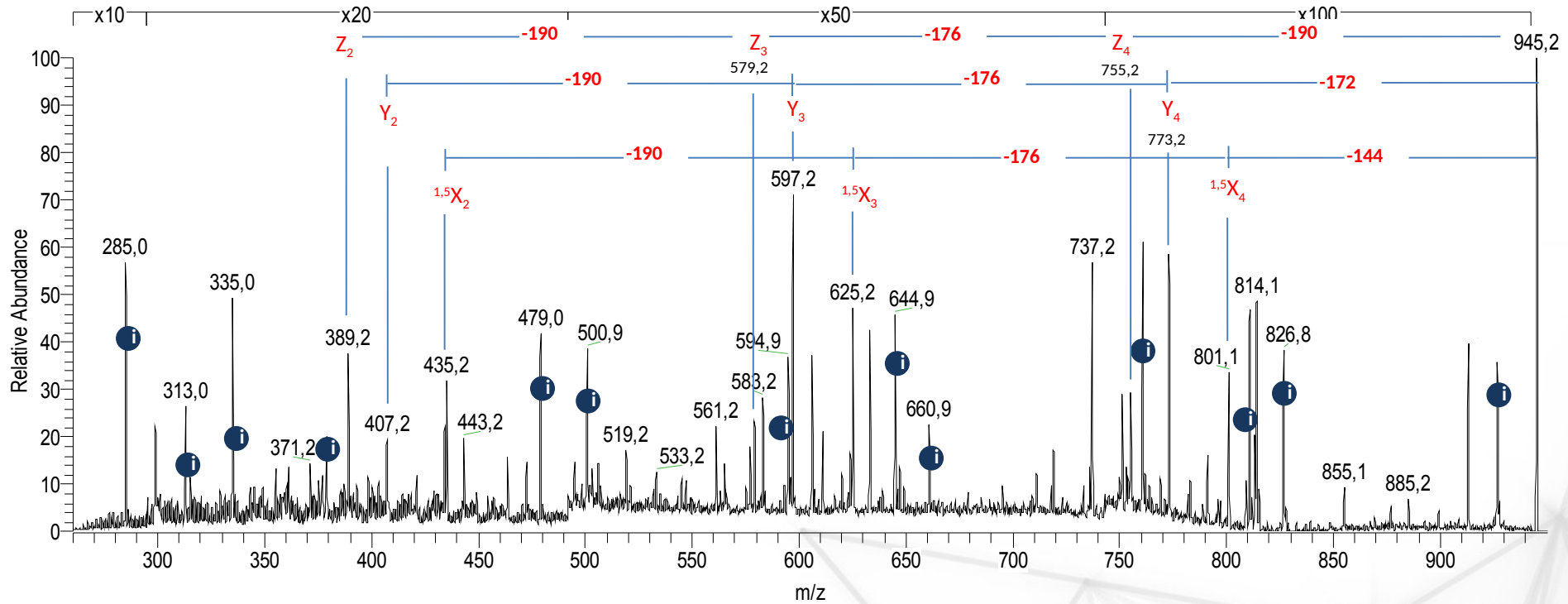
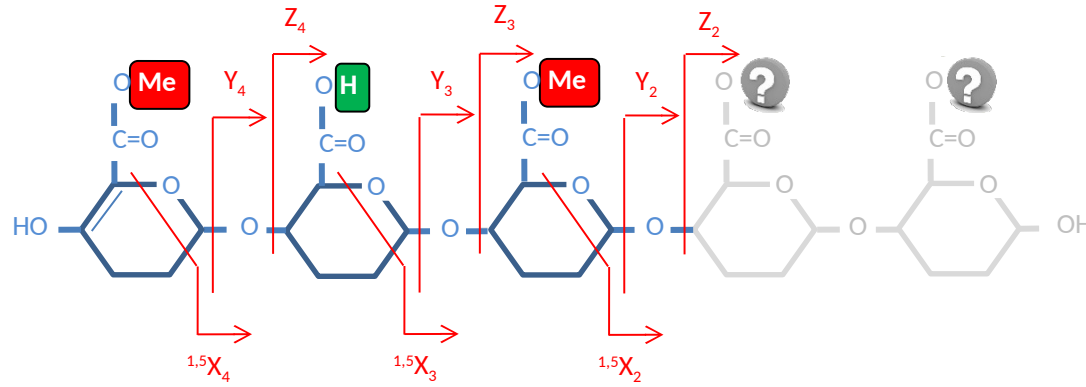
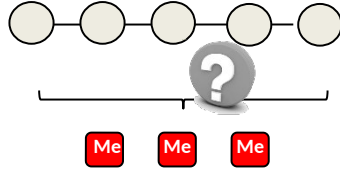
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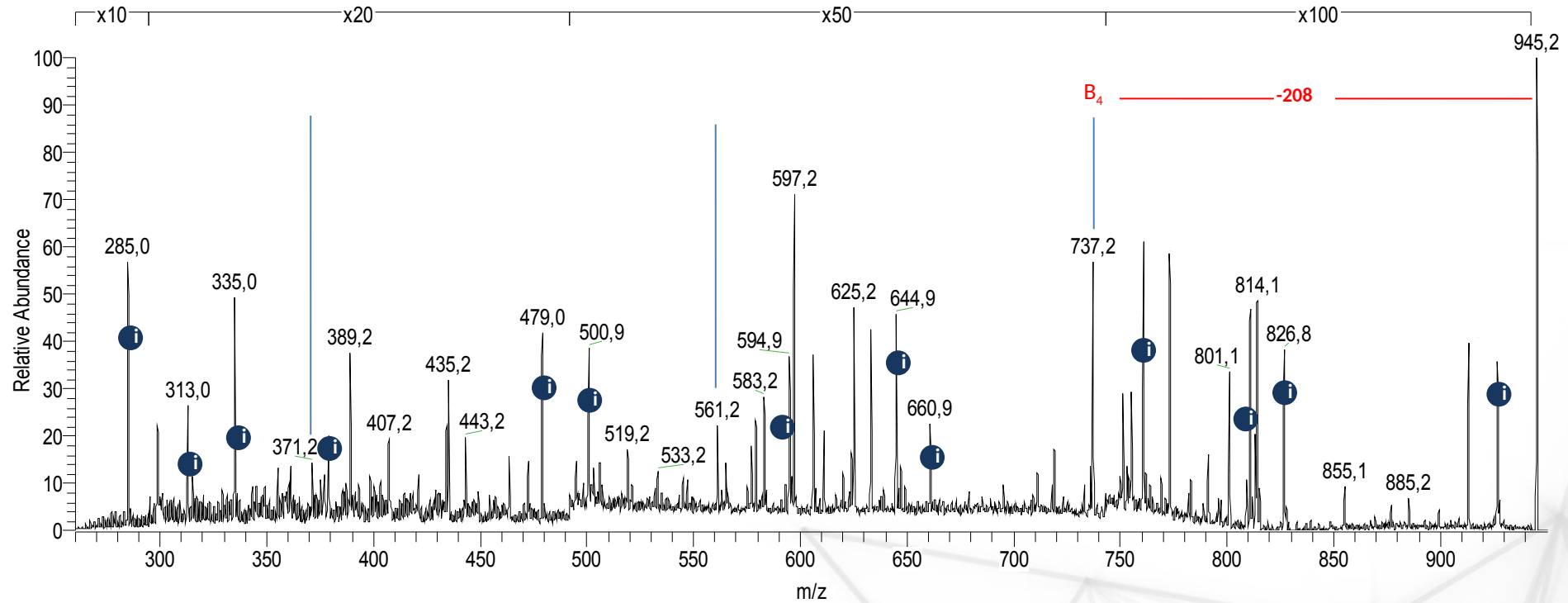
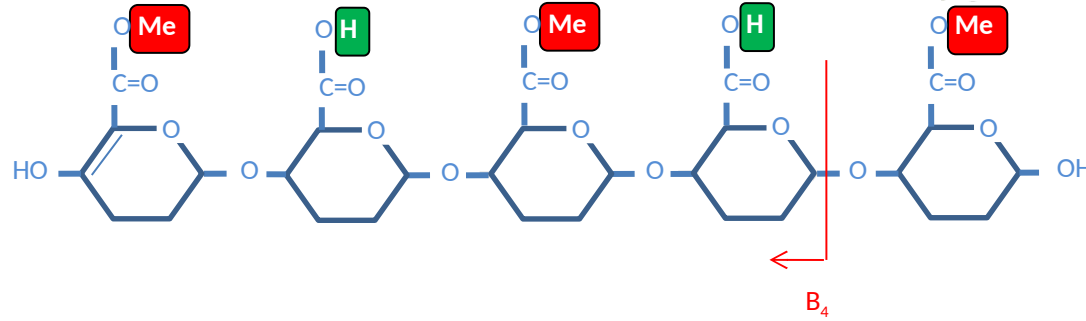
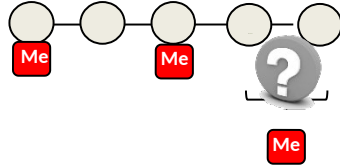
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DP5Me3



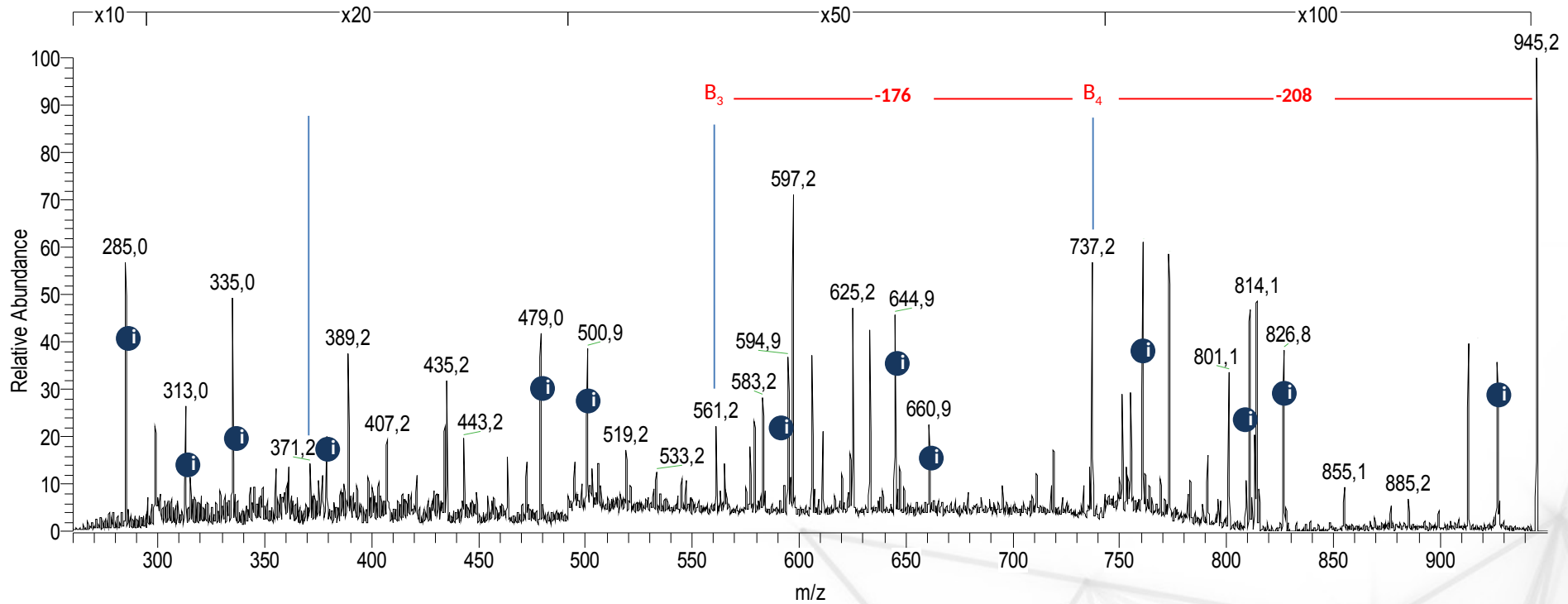
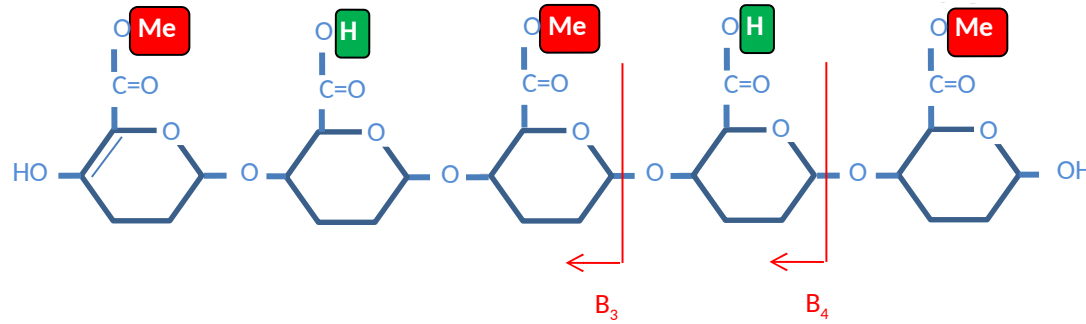
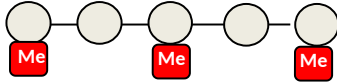
DP5Me3 sequencing

DP5Me3



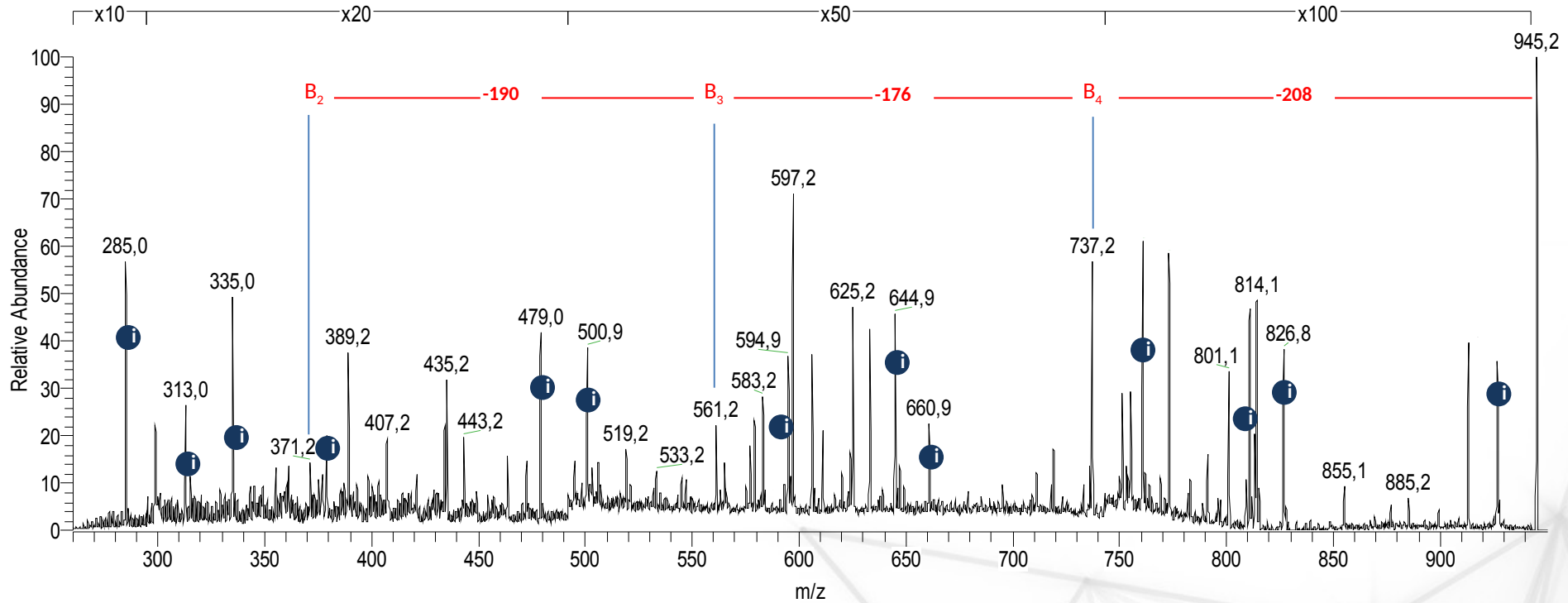
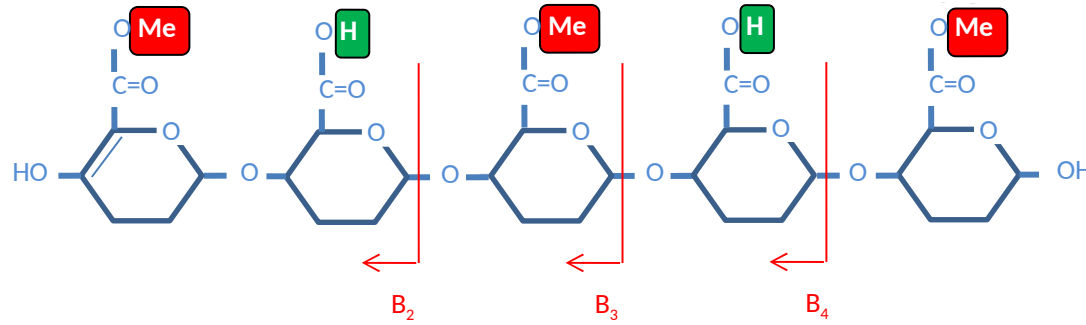
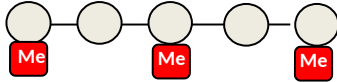
DP5Me3 sequencing

DP5Me3



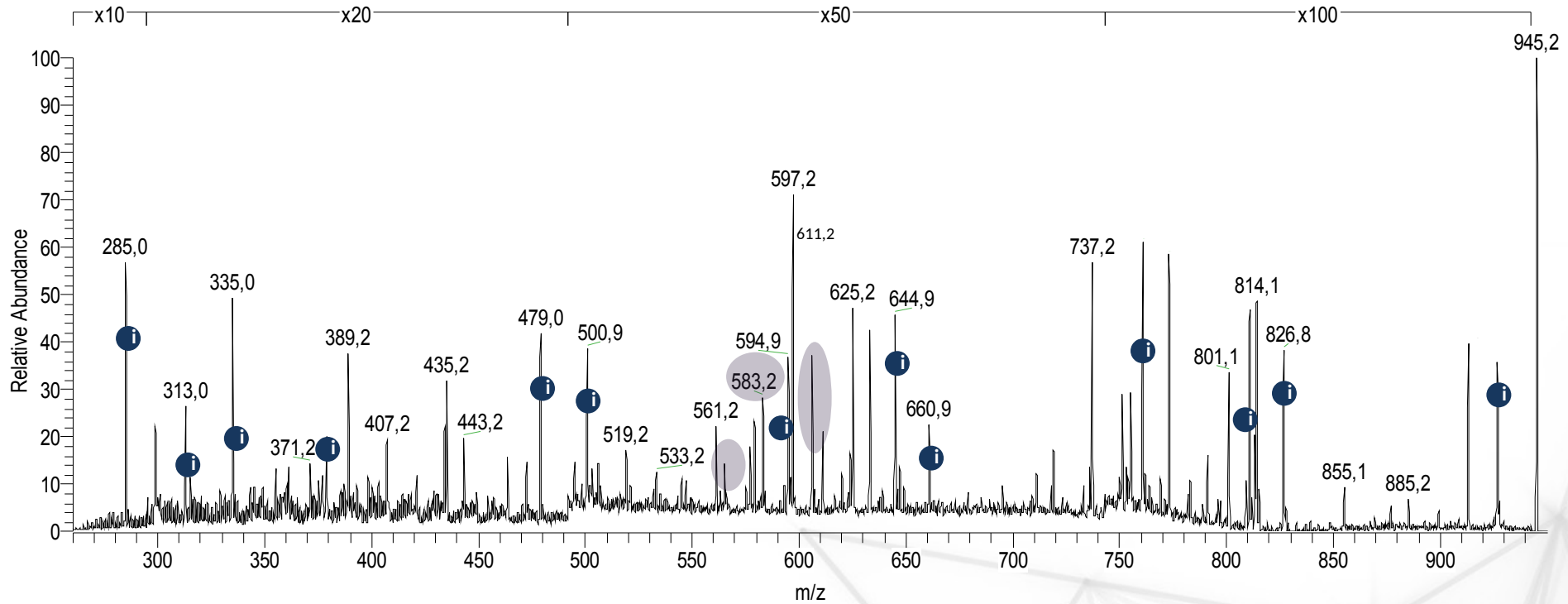
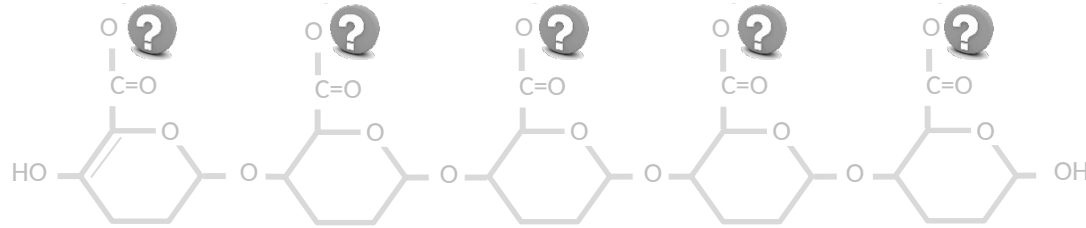
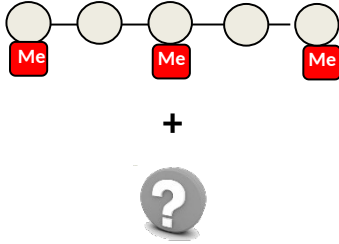
DP5Me3 sequencing

DP5Me3



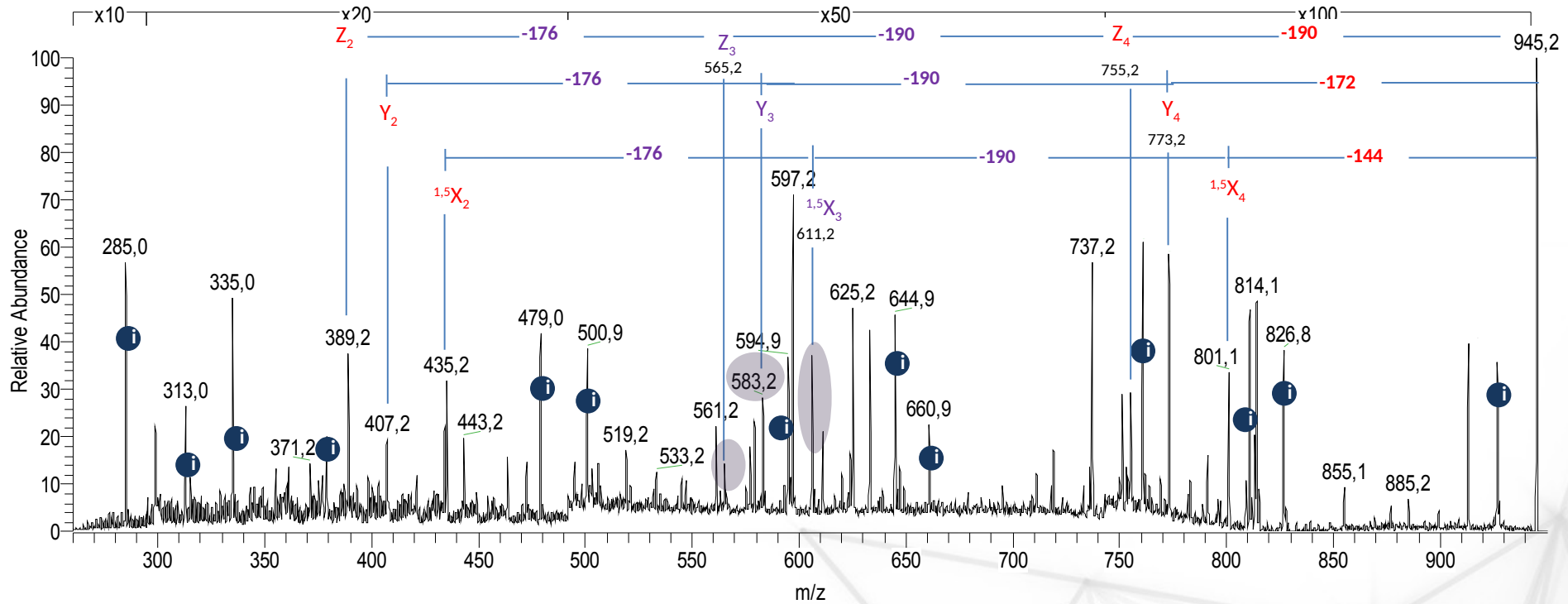
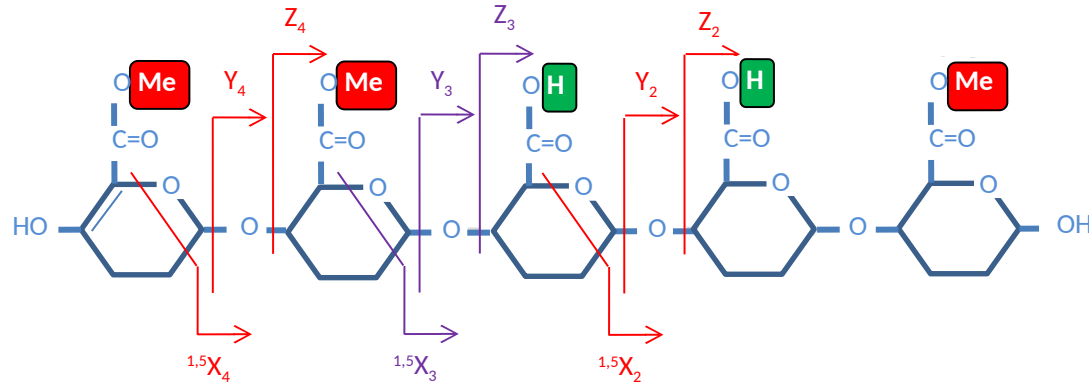
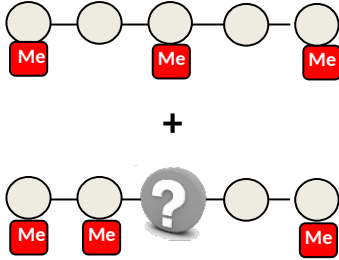
DP5Me3 sequencing

DP5Me3

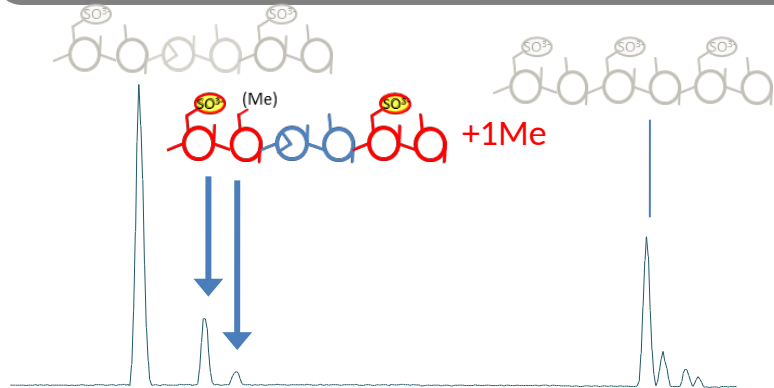


DP5Me3 sequencing

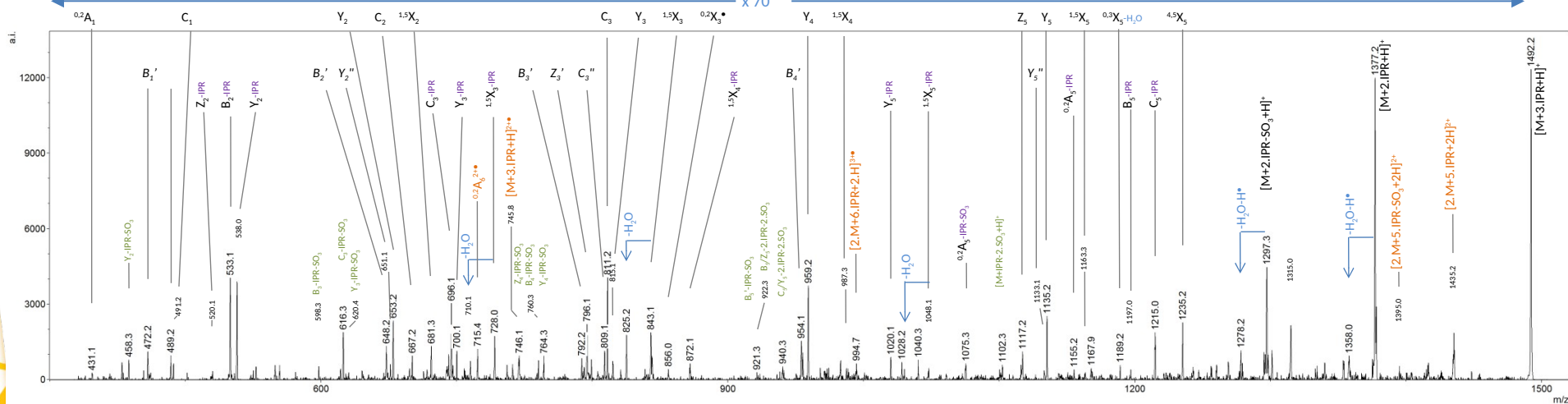
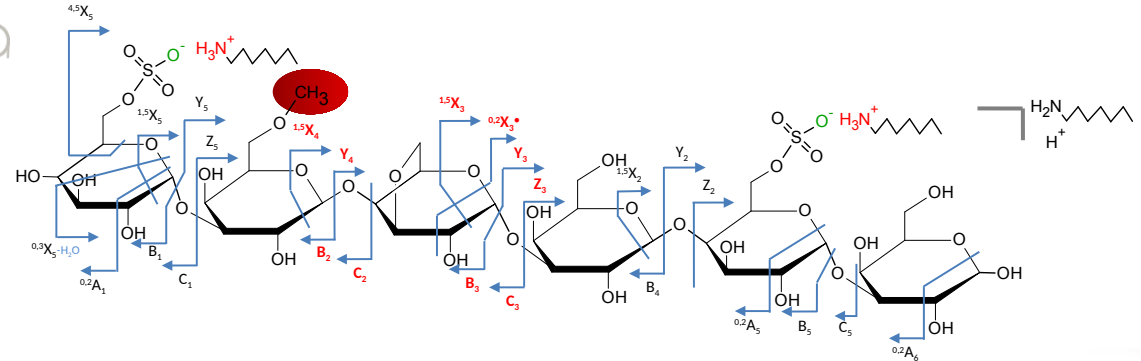
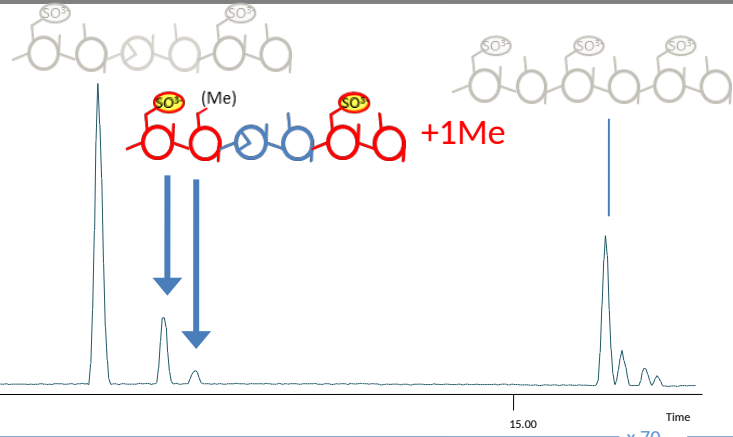
DP5Me3



Liquid chromatography coupling



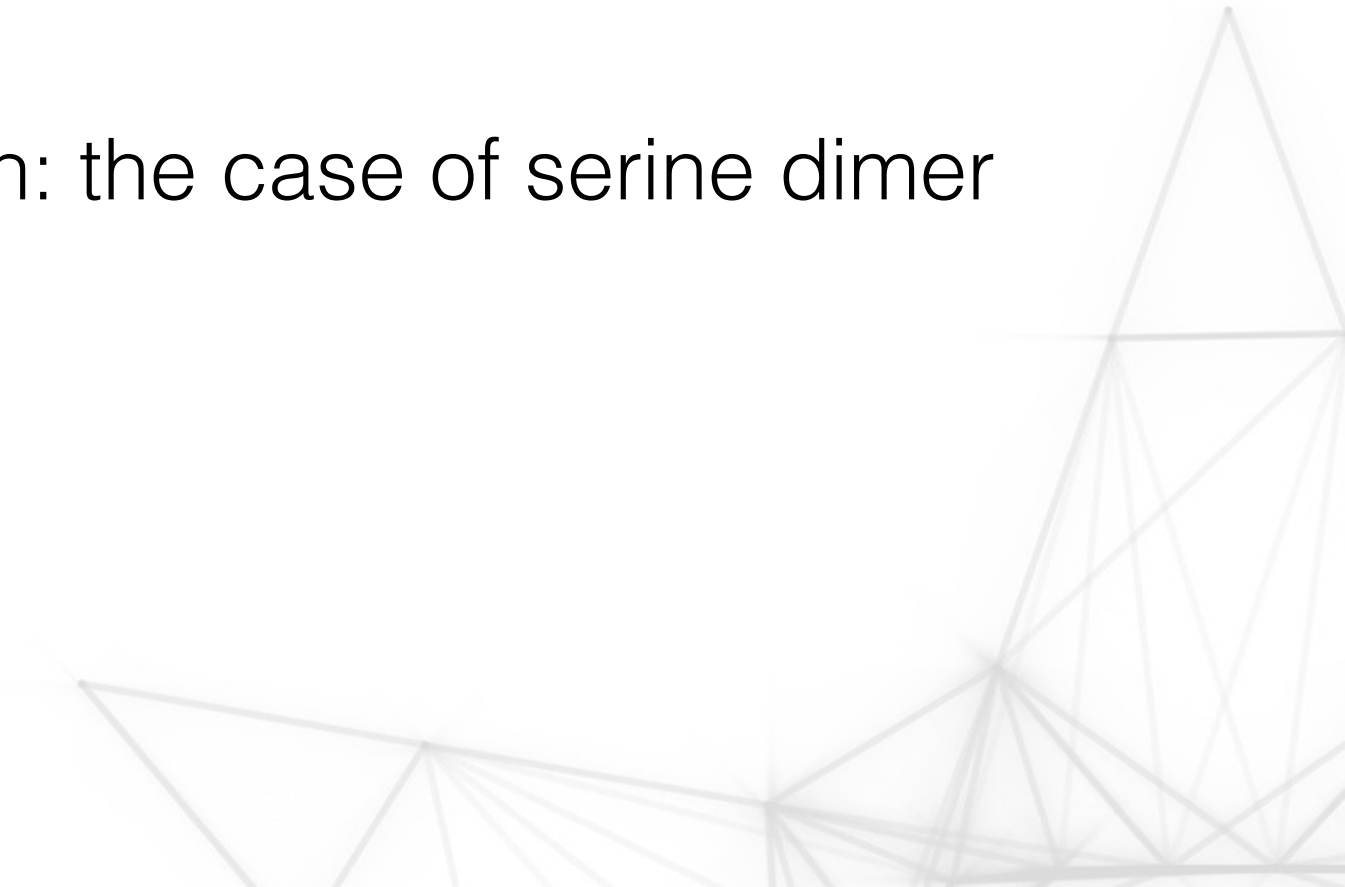
Liquid chromatography coupling



Ropartz, D. et al. Anal. Chem. 2014, 87, 1042–1049, Analytica Chimica Acta 2016, 933, 1–9

Intracluster bond formation

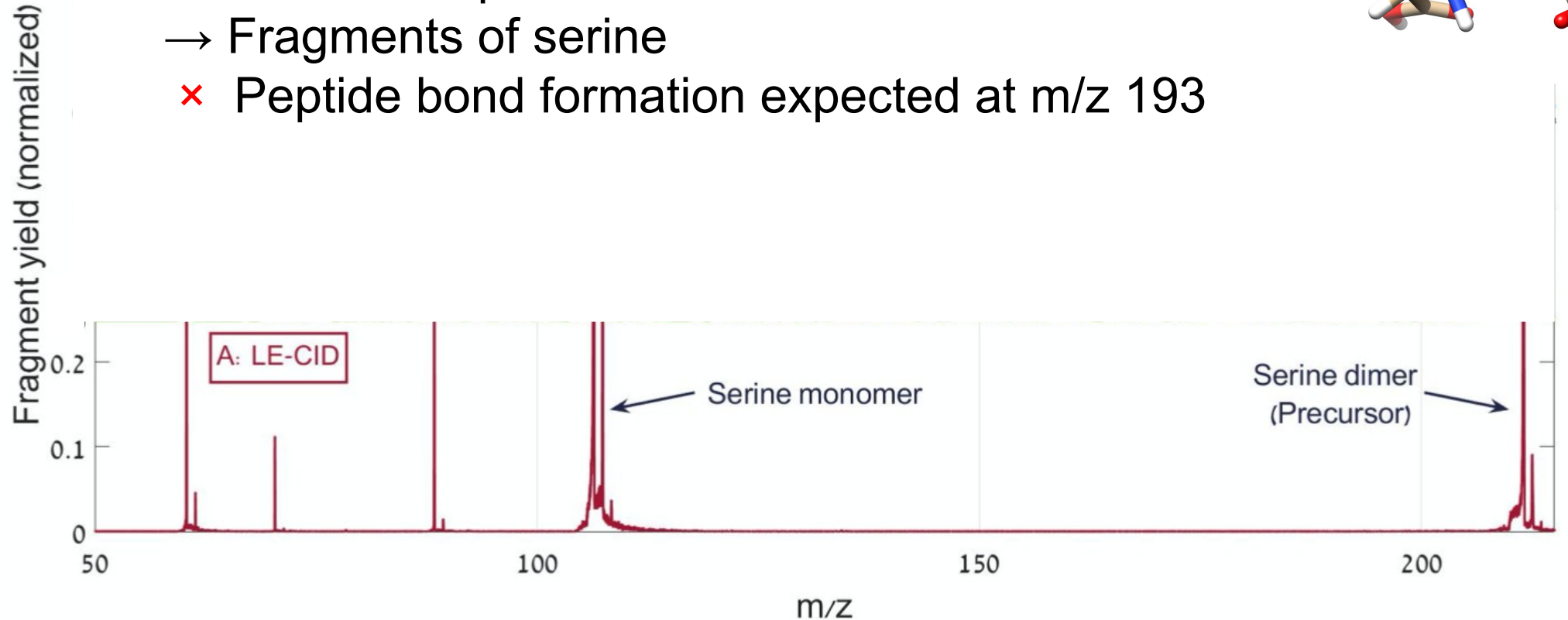
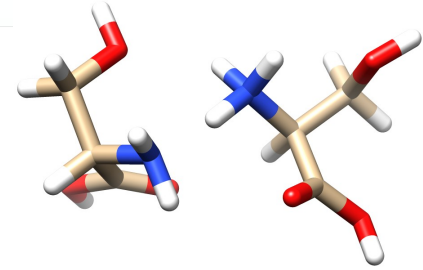
- VUV activation of ligosaccharides
- Intracluster bond formation: the case of serine dimer



LE-CID of protonated serine dimer

$\times 10^{-3}$

- Cluster evaporation
- Fragments of serine
- ✗ Peptide bond formation expected at m/z 193



ICBF region

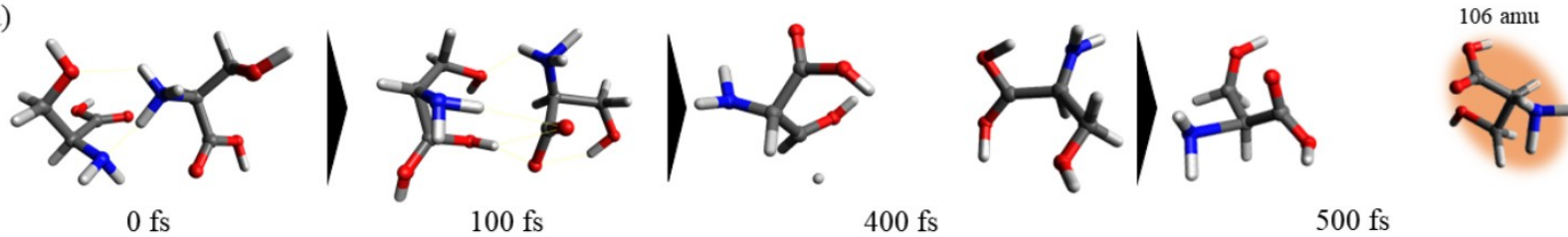
Intracluster bond formation

Intracluster bond formation was never observed using LE-CID.

→ Heating of the cluster leading to statistical products (evaporation more likely than ICBF)

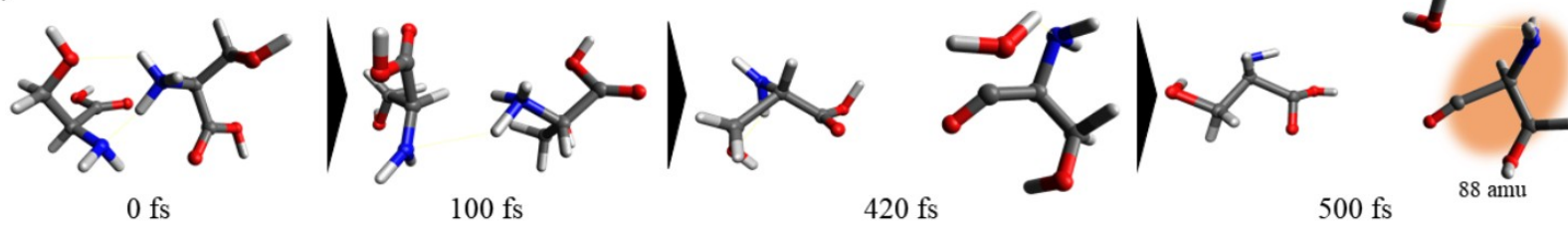
30 eV

a)



Ser+SerH⁺

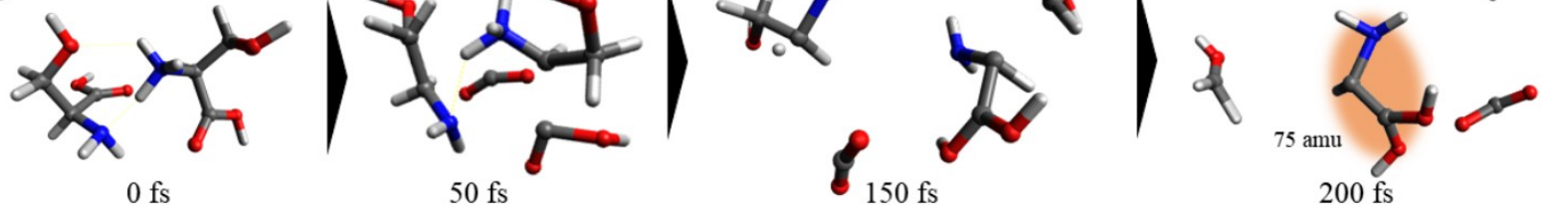
b)



H₂O release

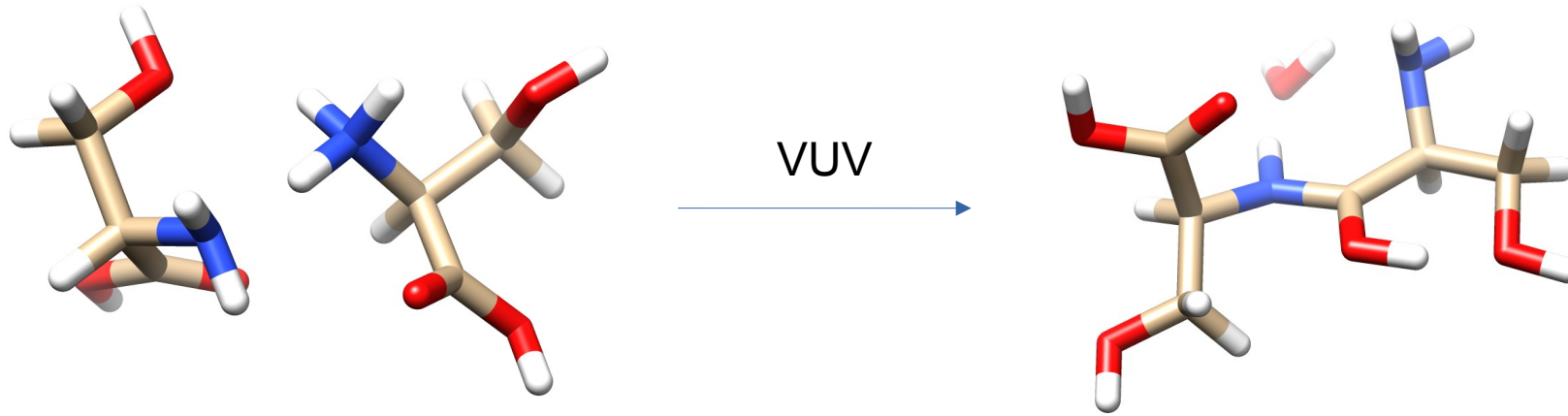
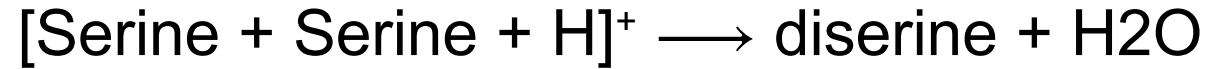
40 eV

c)



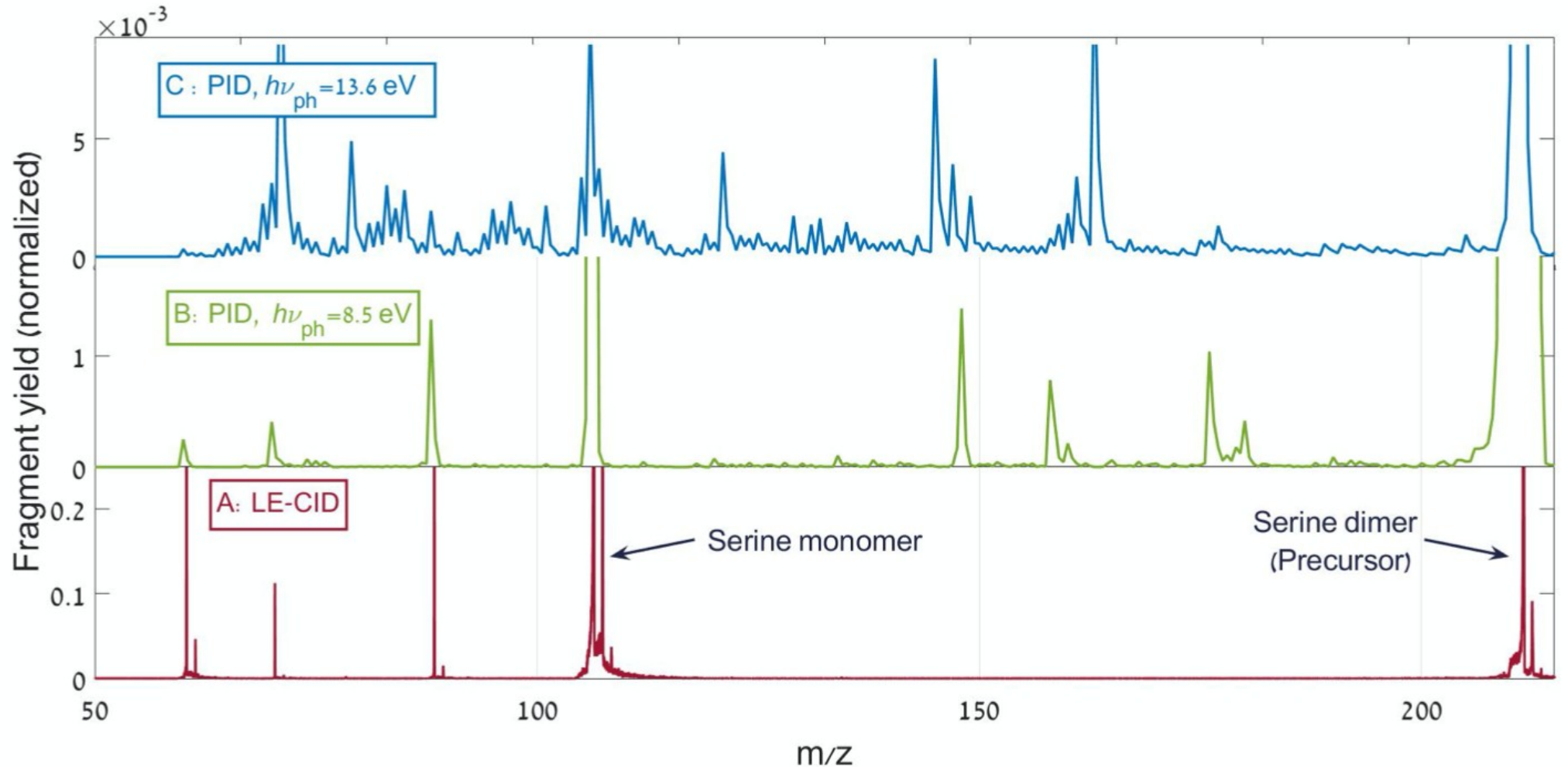
CO₂ release

Intracluster bond formation



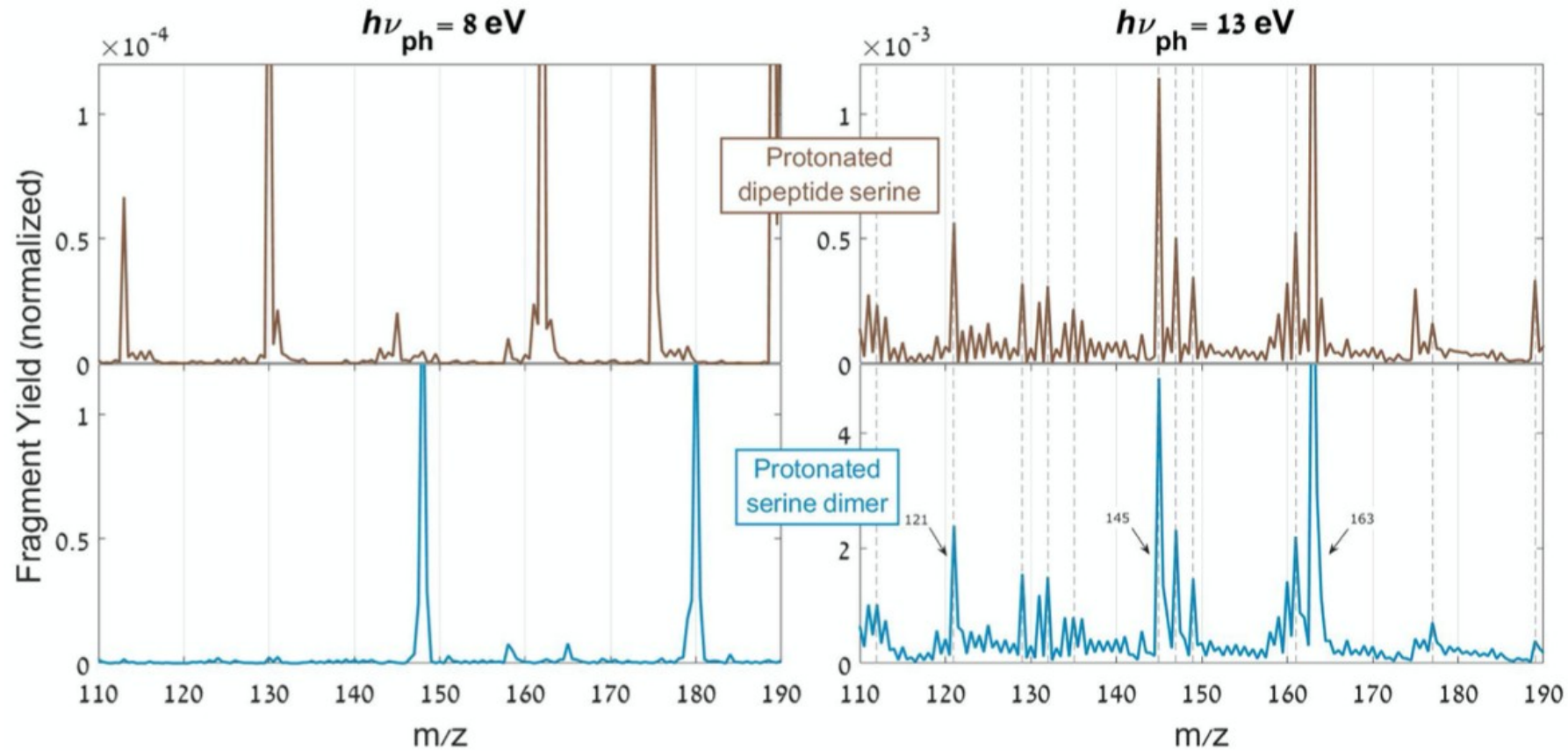
- Excited states dynamics which can possible results in different, non statistical products
→ ICBF has been reported at 157 nm irradiation (JACS 2011, 133, 15834)
- Ability to deposit a well defined amount of energy into the system
- Tunable source : identify the excited states involved

LE-CID versus Photon activation



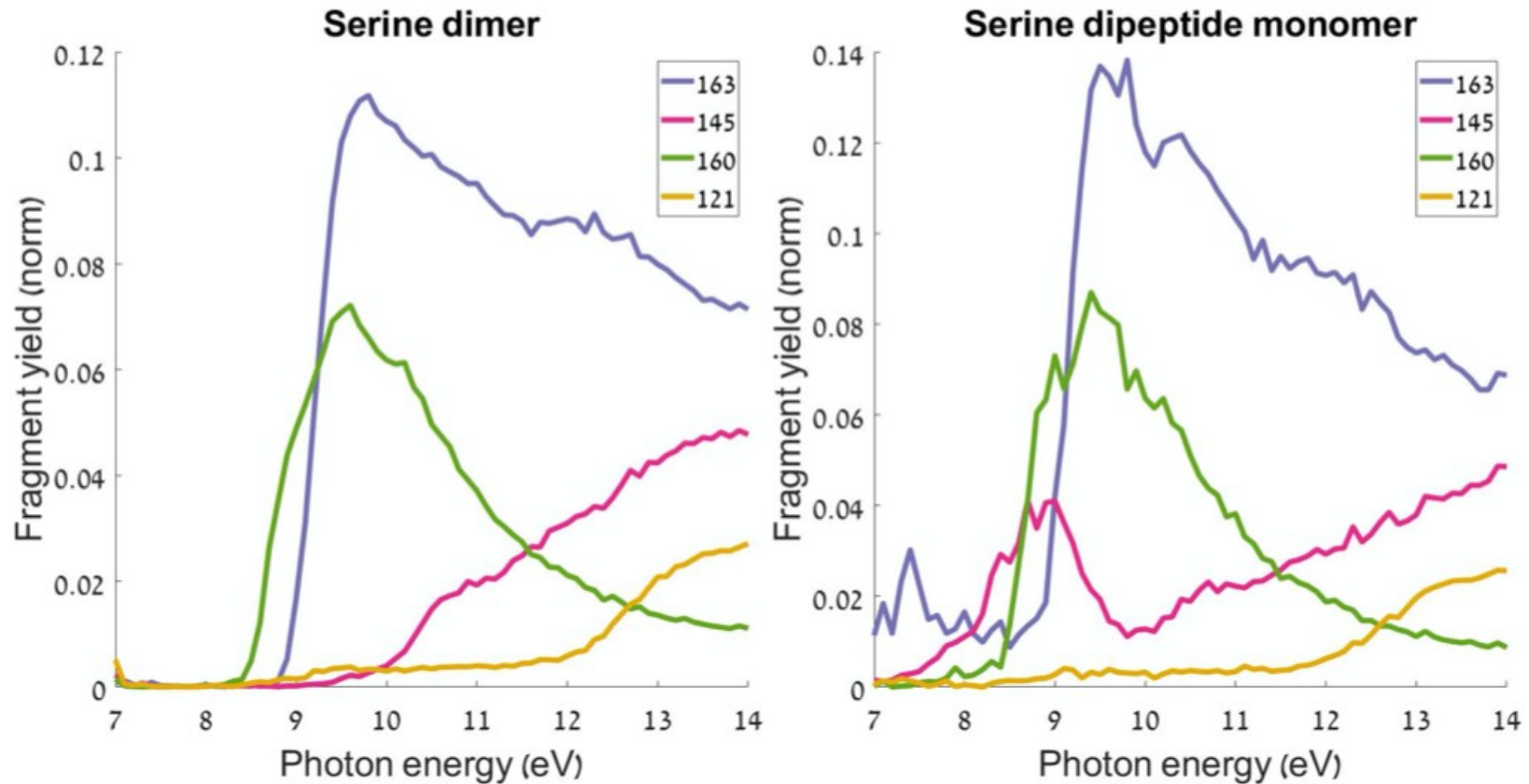
ICBF region

Peptide bond formation



- m/z 193 low abundant (<1%) below 7 eV
- PBF is present at higher energy

Peptide bond formation

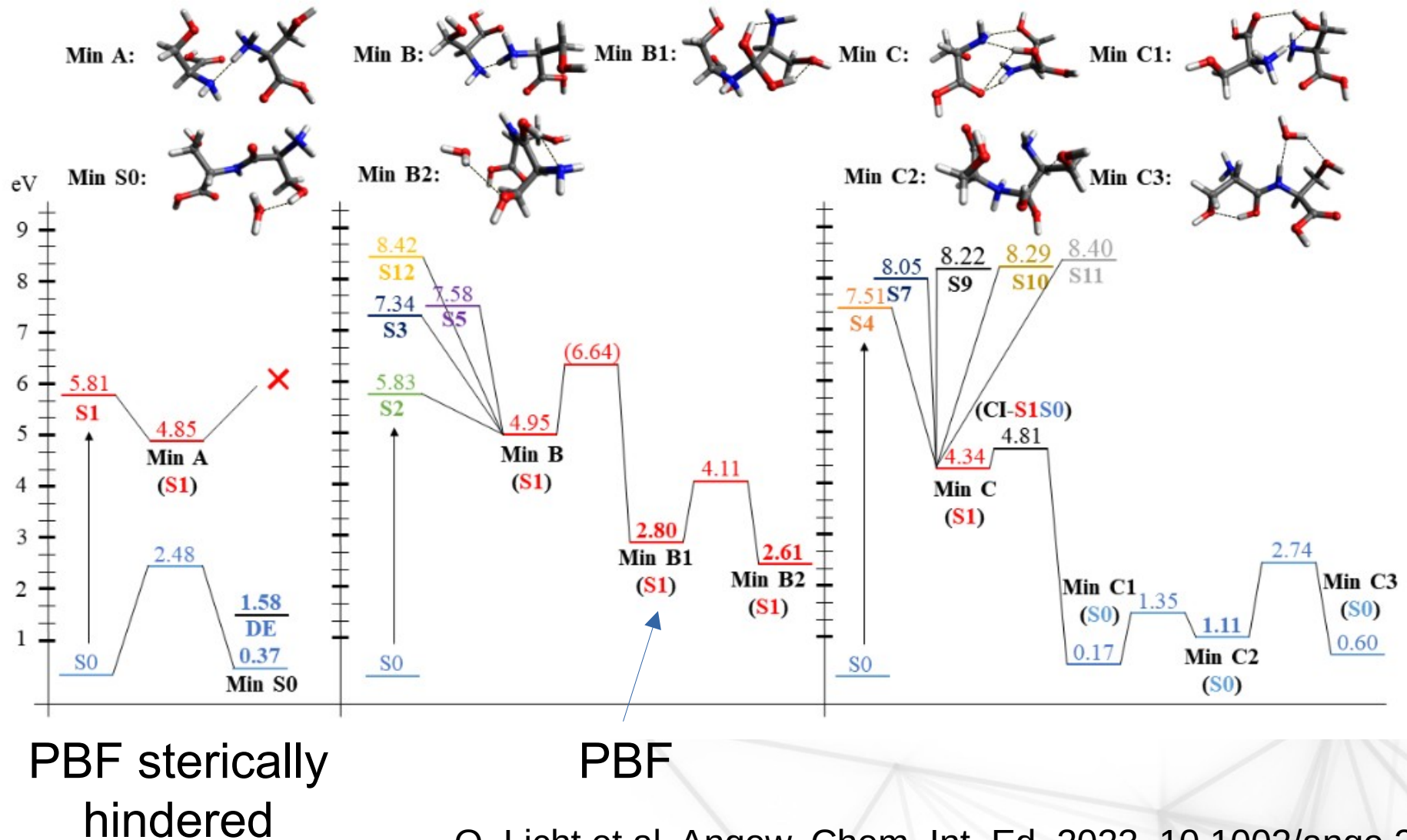


→ PBF is present at higher energy (above 10 eV)

Fragmentation of diserine is a two steps process:

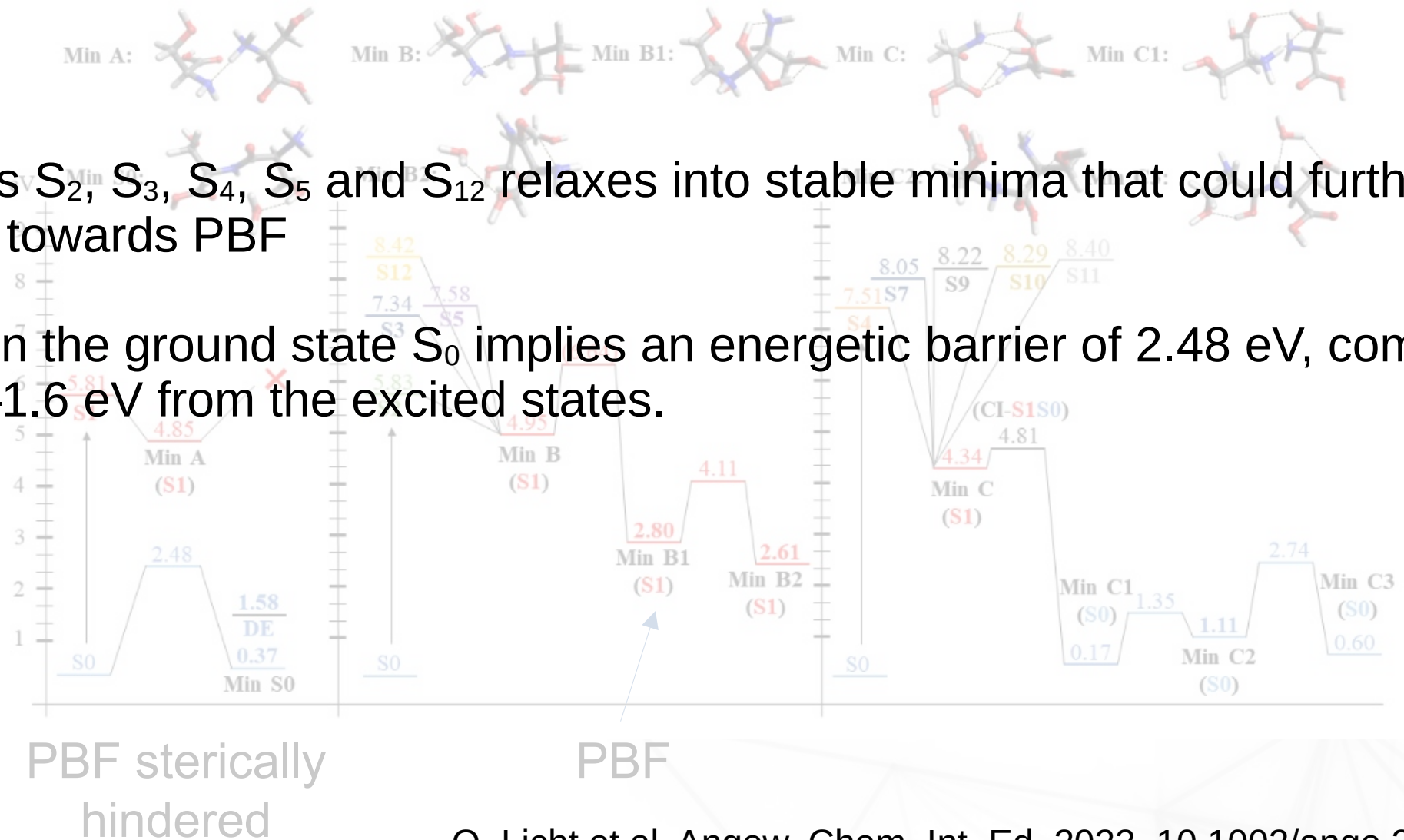
- either PBF followed by fragmentation
- or fragmentation followed by bond formation

Peptide bond formation



Peptide bond formation

- States S_2 , S_3 , S_4 , S_5 and S_{12} relaxes into stable minima that could further evolve towards PBF
- PBF in the ground state S_0 implies an energetic barrier of 2.48 eV, compared to 0.5–1.6 eV from the excited states.



- VUV activation of oligosaccharides
 - Better suited than CID
 - Brilliance of SR makes it compatible with LC
- Intracluster bond formation
 - Evidence for peptide bond within a cluster

Acknowledgements

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Thank you for your attention



