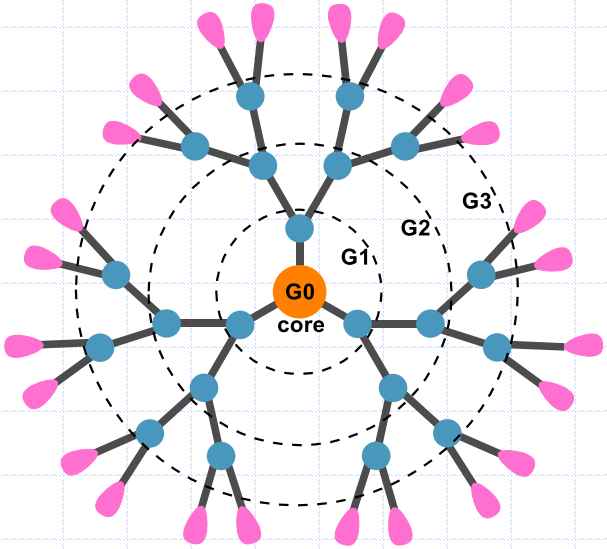
Mass Spectrometry for Nanovectors Characterization



Aura TINTARU

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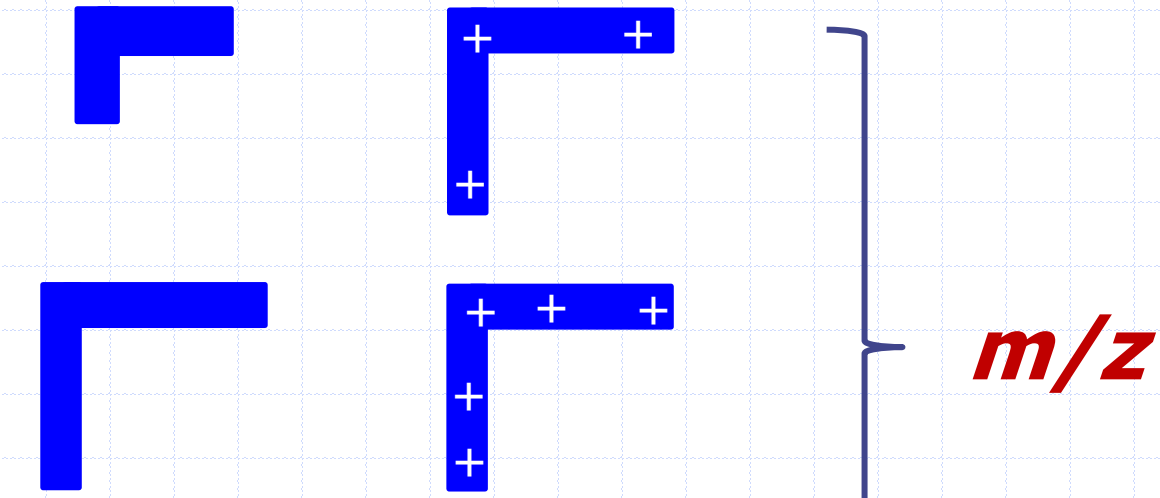
CINaM

Outline

- **Introduction to Mass Spectrometry**
  - Ionization Sources
  - Analyzers
- **Tandem Mass Spectrometry**
- **Ion Mobility Mass Spectrometry**
  - Principles et Definitions
  - Instrumentation
  - Applications
- **Conclusions**

Mass Spectrometry

Distinguish between ionized molecules depending on…



… mass

m

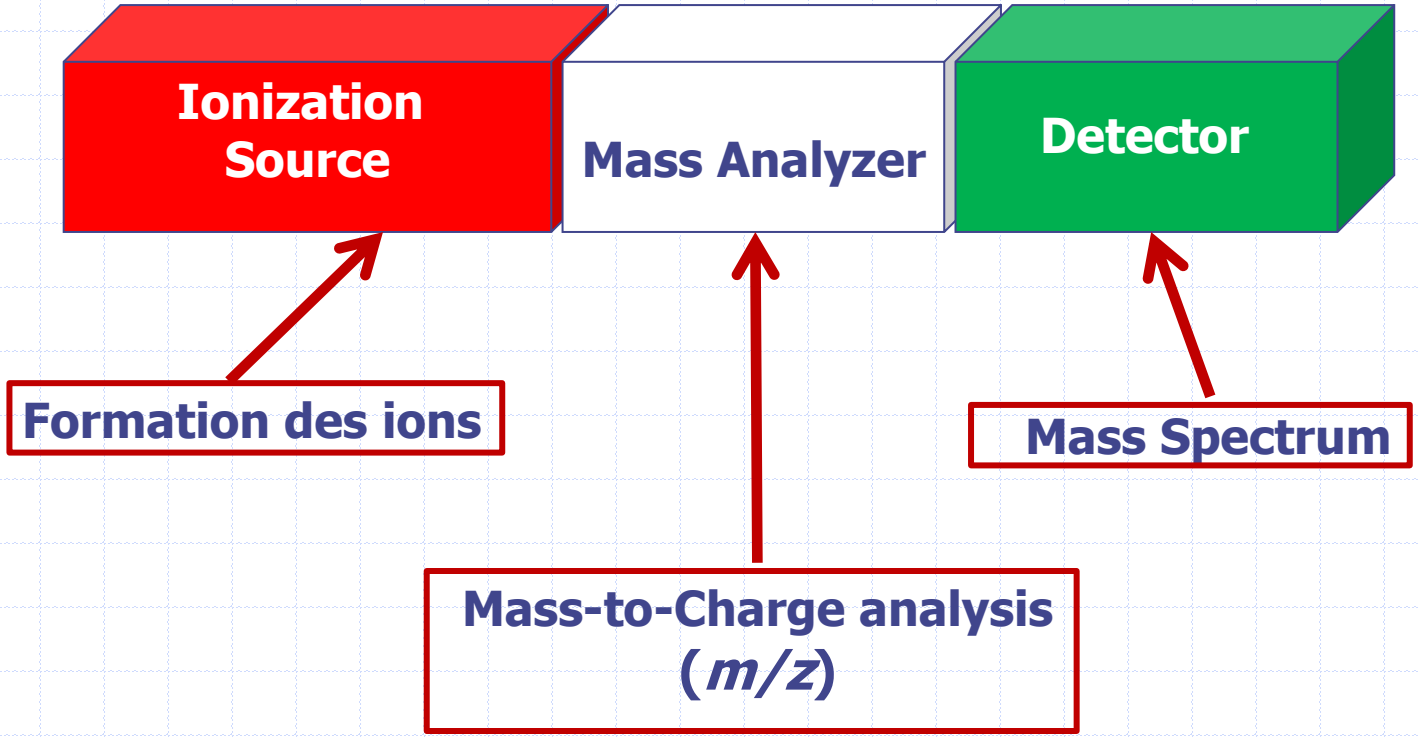
(isotopes)

… charge

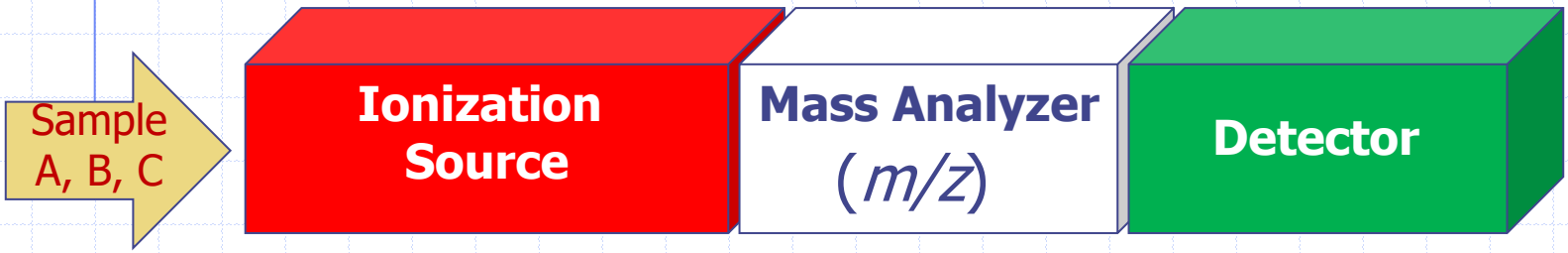
z

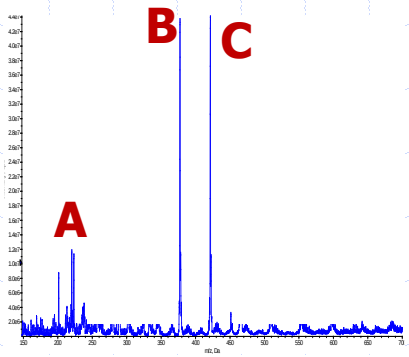
(ions)

Mass Spectrometry



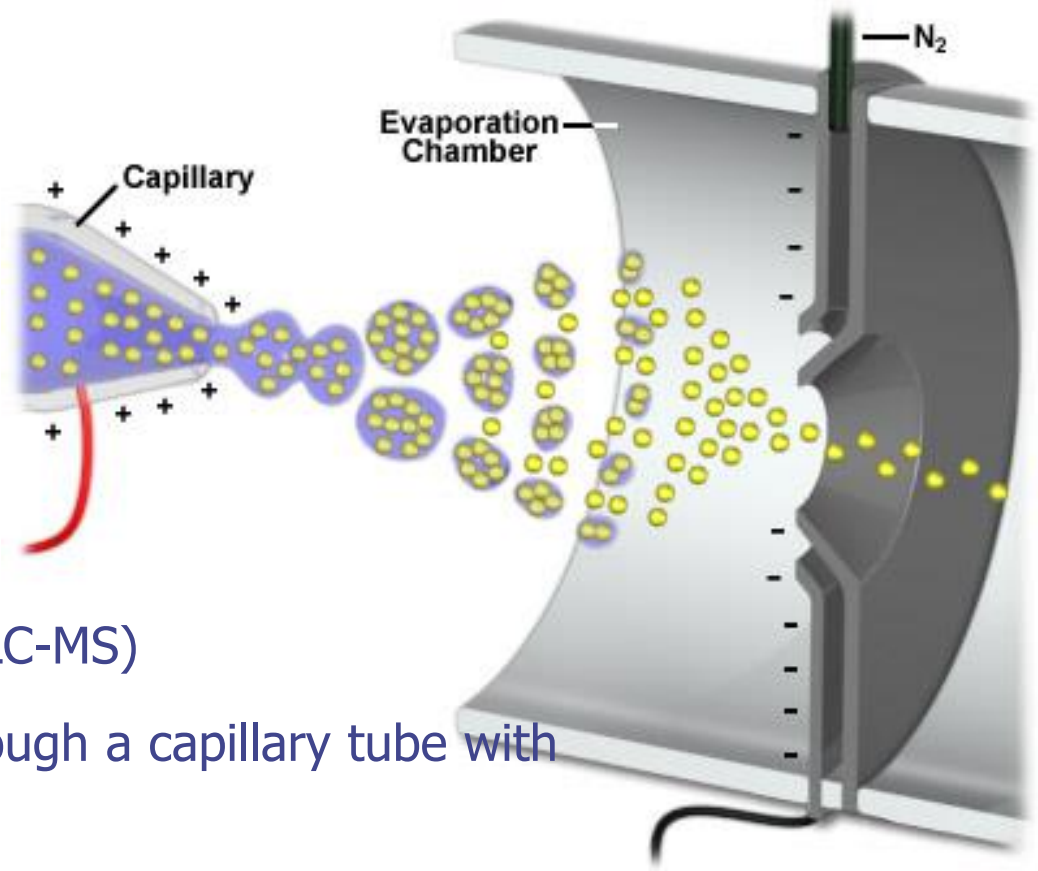
Mass Spectrometry



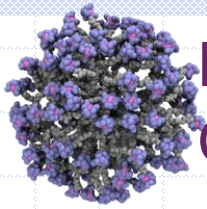


MS spectrum

Electrospray Ionization (ESI)

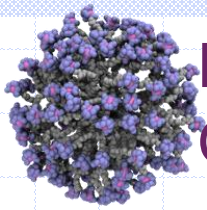


- This is often coupled with LC (LC-MS)
- The solvated analyte is fed through a capillary tube with very high charge differential
- The charged droplet evaporates and ionizes the analyte

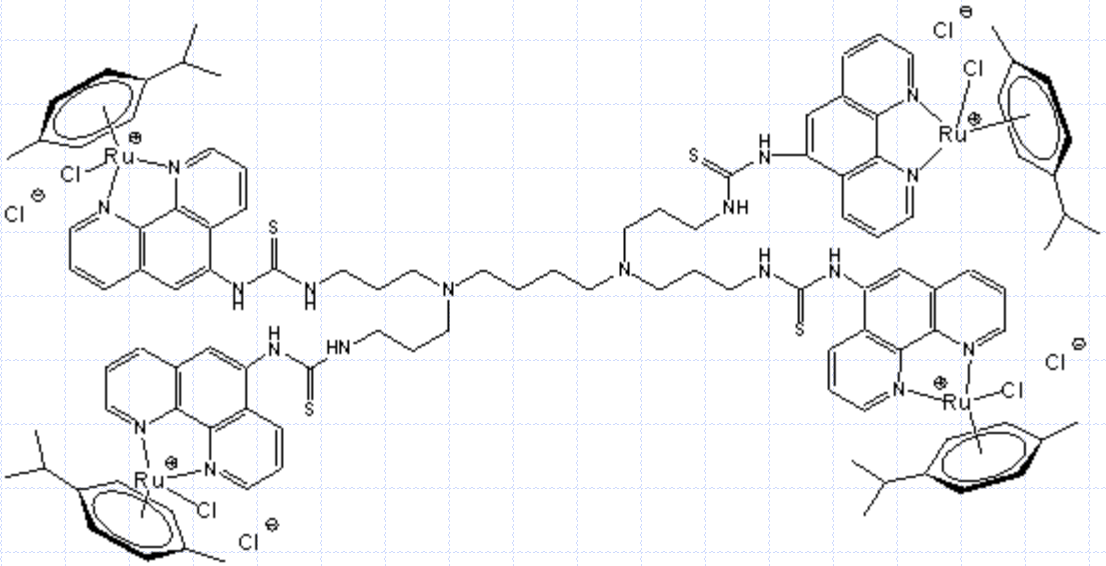


High Resolution Mass Spectrometry

- Fast method to determine the elementary composition (error <5ppm)
- Use of low quantities
- Allows analysis of all types of organic molecules

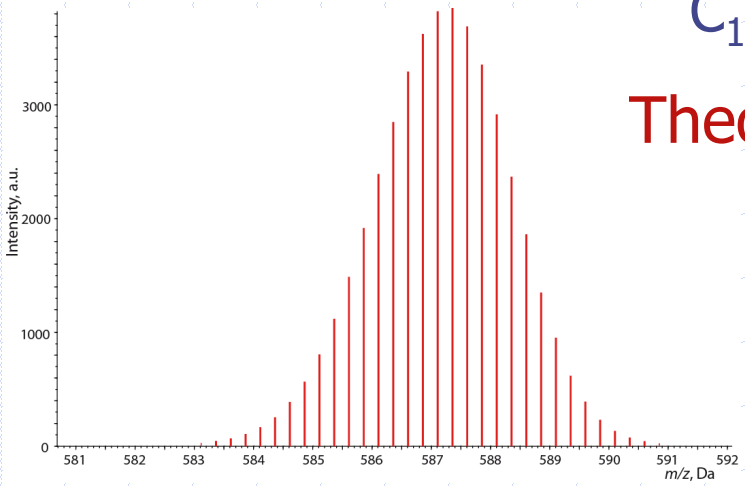


High Resolution Mass Spectrometry

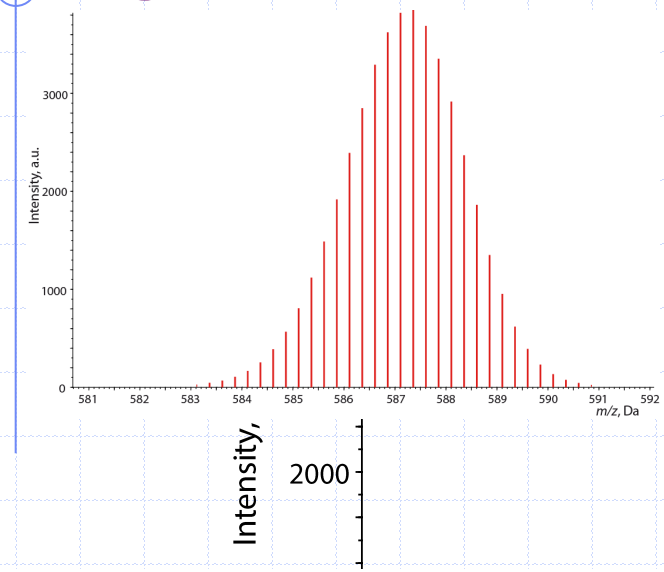


$C_{108}H_{124}N_{18}S_4Ru_4Cl_4^{4+}$

Theoretic: *m/z* 587.1019



High Resolution Mass Spectrometry

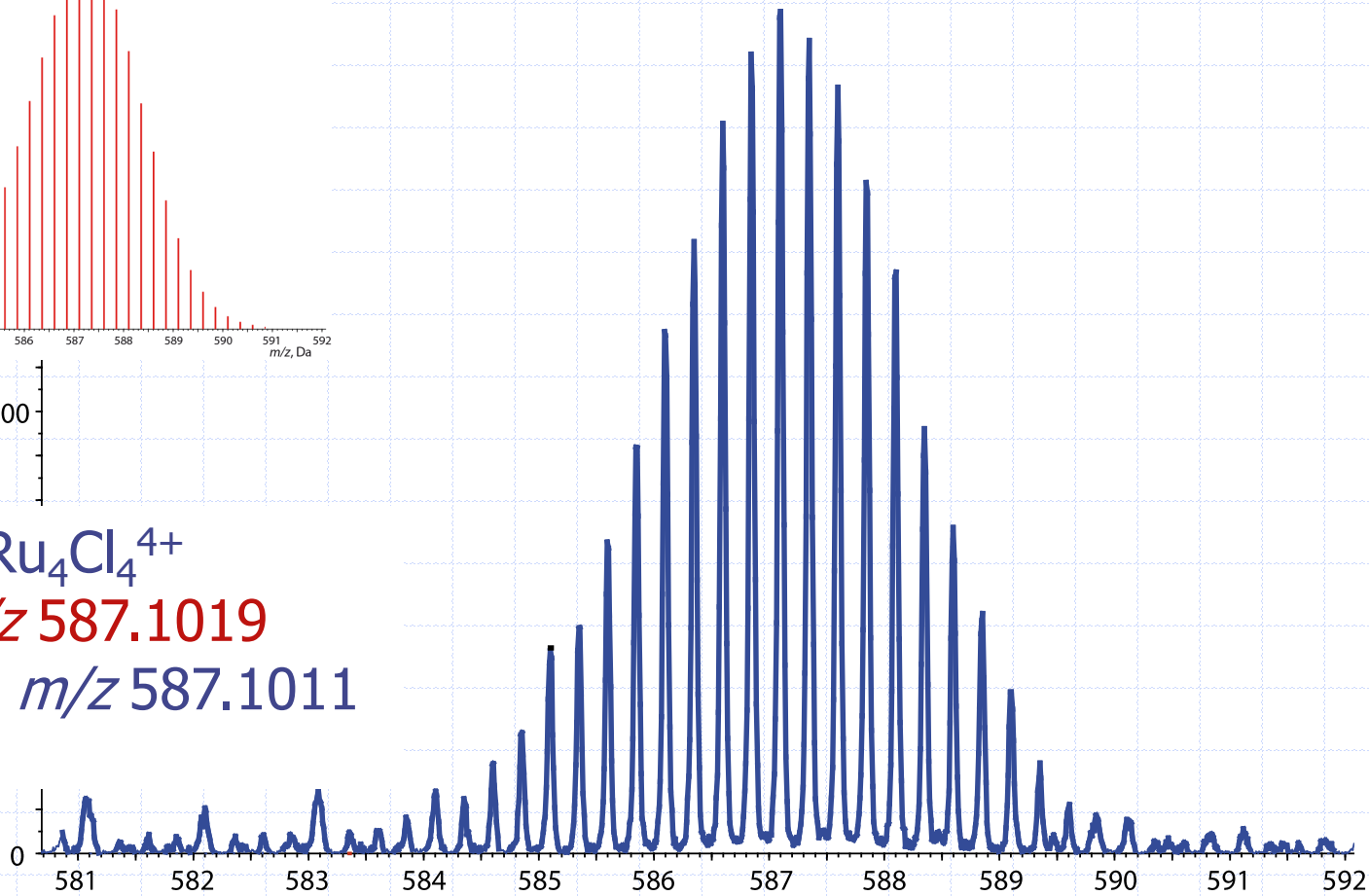


$C_{108}H_{124}N_{18}S_4Ru_4Cl_4^{4+}$

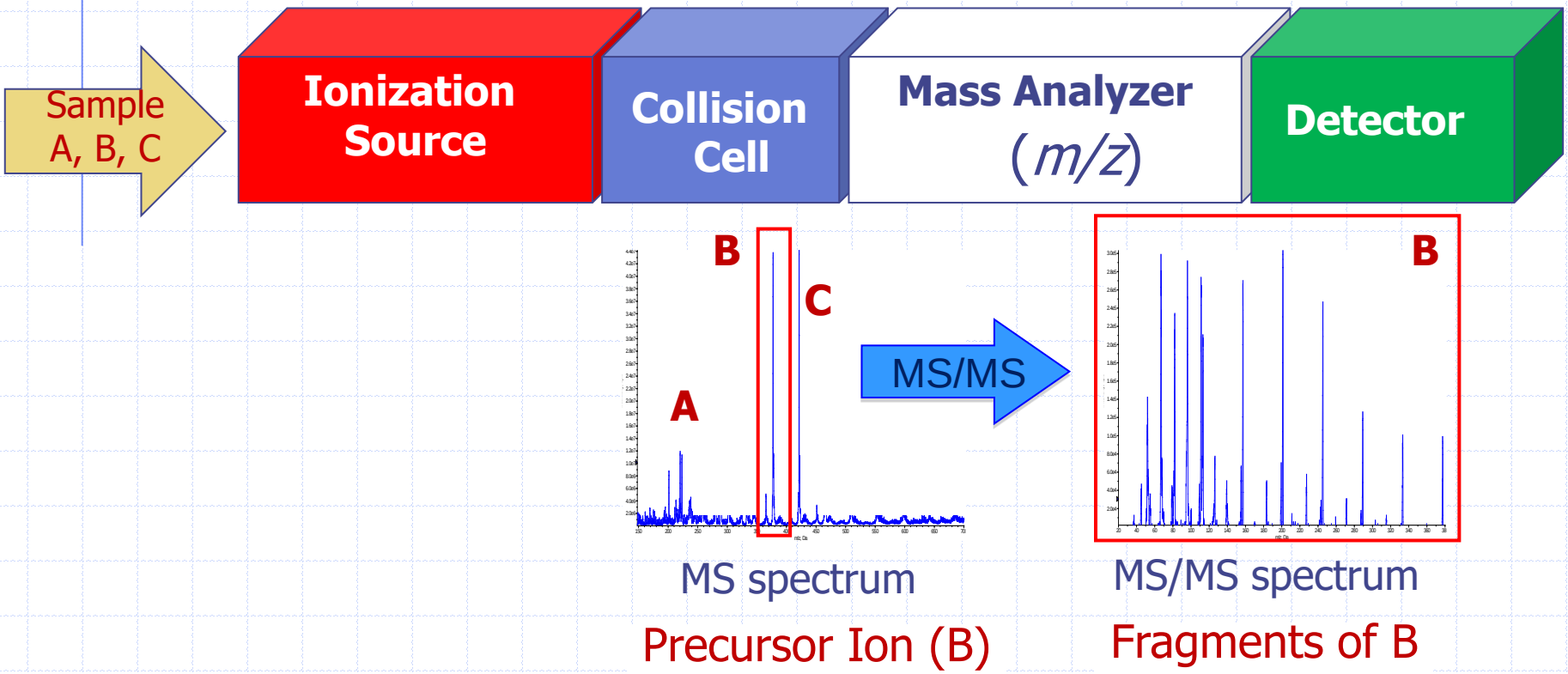
Theoretic: *m/z* 587.1019

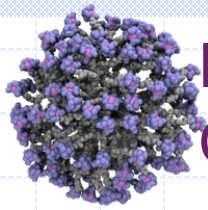
Experimental: *m/z* 587.1011

Error: -1.4 ppm



Tandem Mass Spectrometry

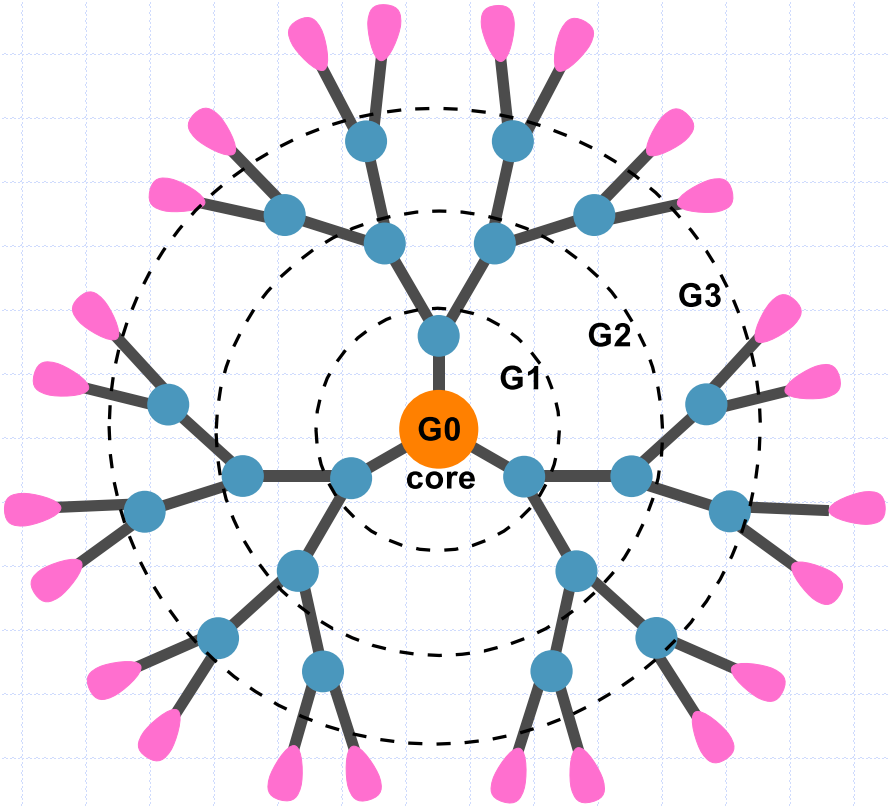




Tandem Mass Spectrometry

- Useful tool for structural characterization
- Appropriate for structural characterization of highly symmetrical molecules: polymers, dendrimers, sugars
- Suitable for structural anomalies screening

Dendrimers as Drug Delivery Systems

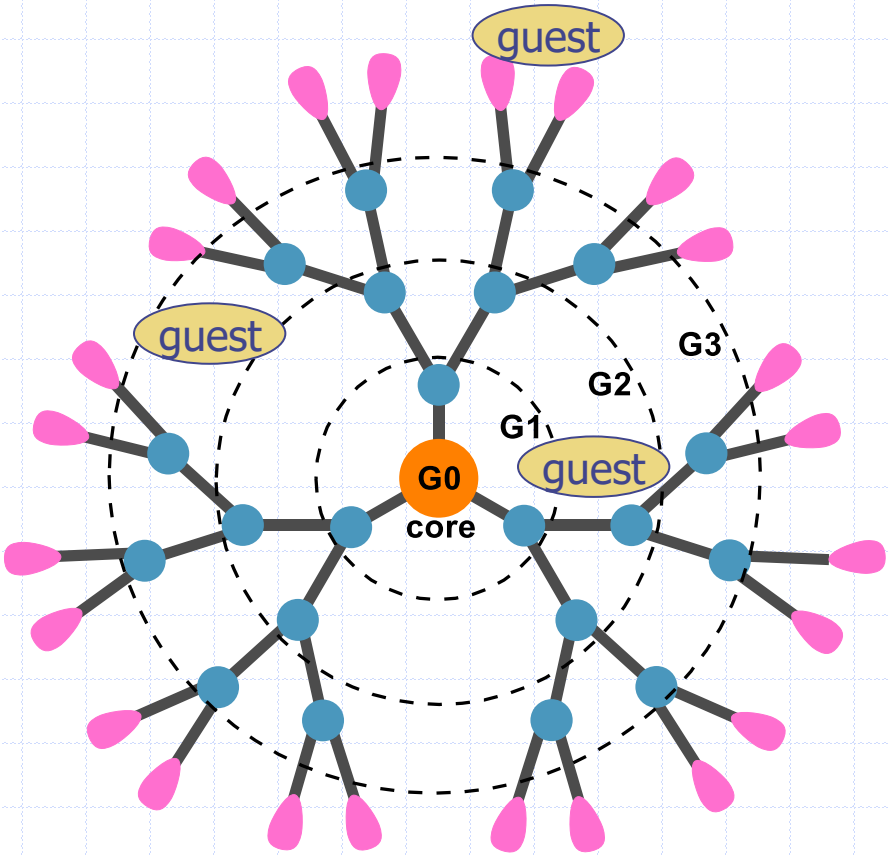


- Highly symetrical structures
- Intrinsic cavities
- Funtionalizated surface

Vectorization agents

Jean J.M. Fréchet and Donald A. Tomalia Dendrimers and Other Dendritic Systems (2001) John Wiley & Sons
George R. Newcome et al. Dendrimers and Dendrons (2002) John Wiley & Sons

Dendrimers as Drug Delivery Systems



- Highly symetrical structures
- Intrinsic cavities
- Funtionalizated surface

Vectorization agents

guest : DNA, RNA, drugs, …

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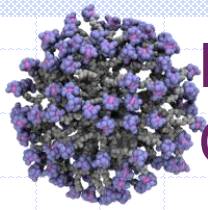
George R. Newcome et al. Dendrimers and Dendrons (2002) John Wiley & Sons

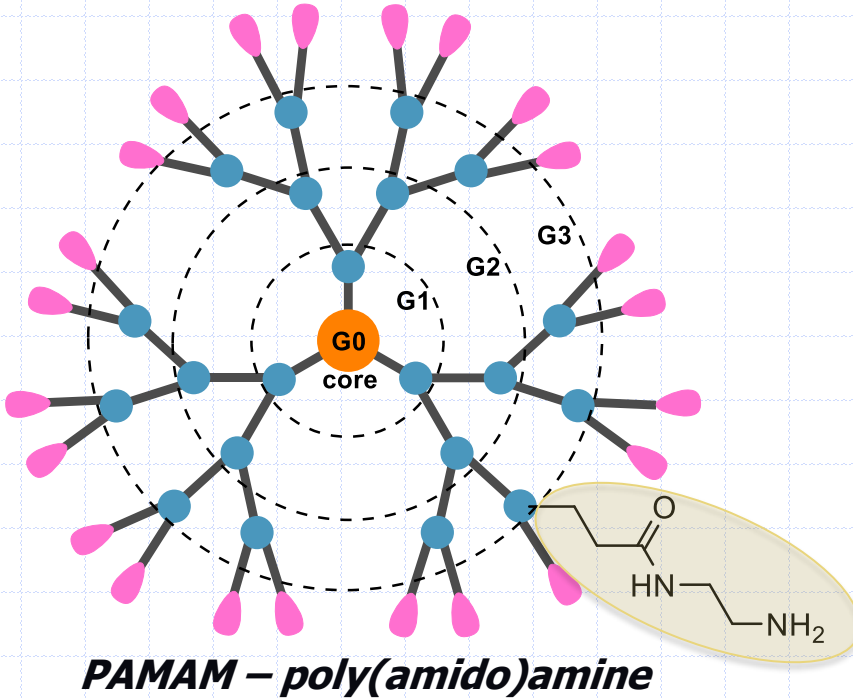


Dendrimers as Drug Delivery Systems

DENDRIMERS

Complex molecules

Original analytical approaches adapted to each specific topic

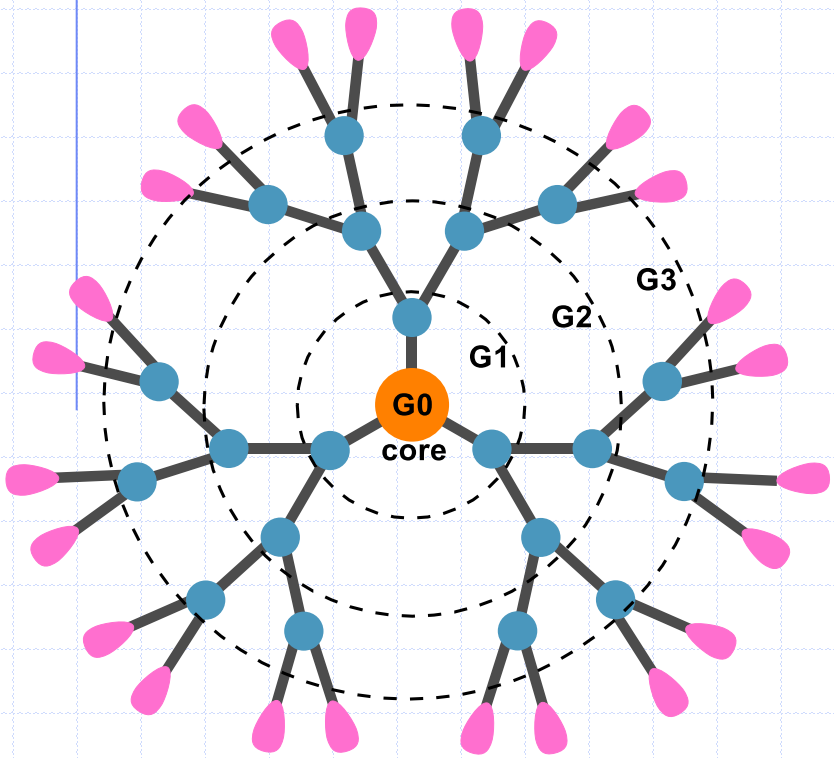


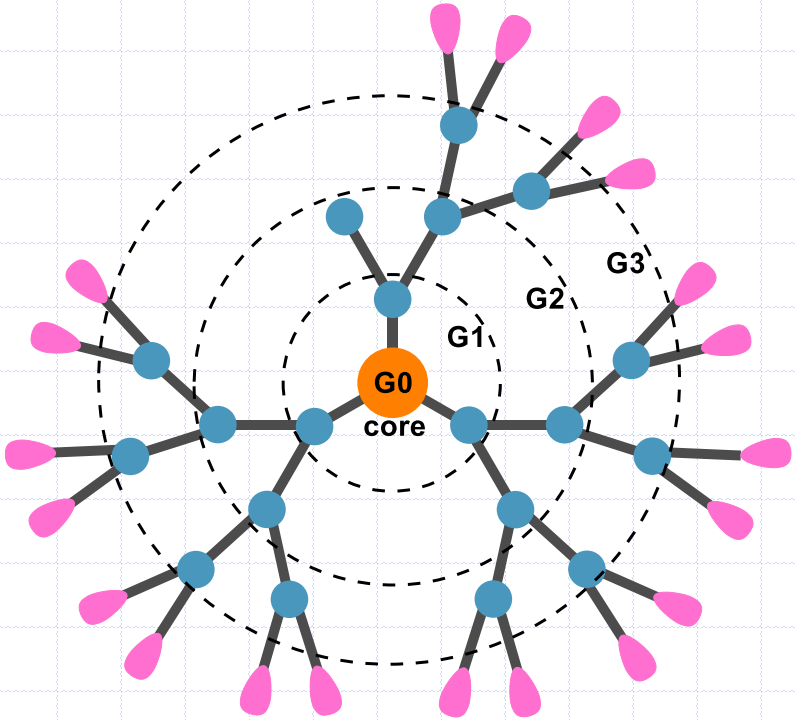
PAMAM – poly(amido)amine

Main goal: Accurate characterization

J.M. Fréchet and D. A. Tomalia : Dendrimers and Other Dendritic Systems (2001) John Wiley & Sons
G. R. Newcome et al: Dendrimers and Dendrons (2002) John Wiley & Sons

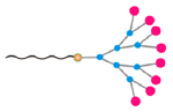
Dendrimers as Drug Delivery Systems

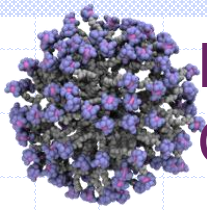




Goal: Detection and characterization of the defective molecules

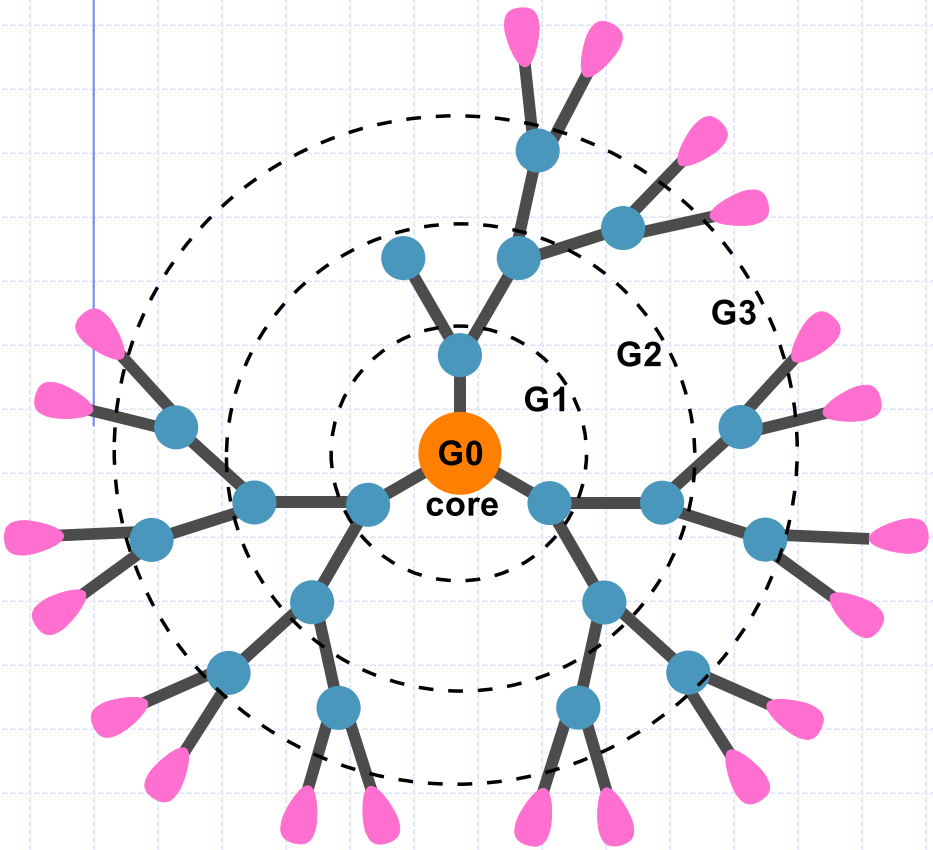
Tintaru et al. Int J Mass Spectrom, 2011, 308, 56.
Tintaru et al. Rapid Commun Mass Spectrom 2010, 16, 2207.

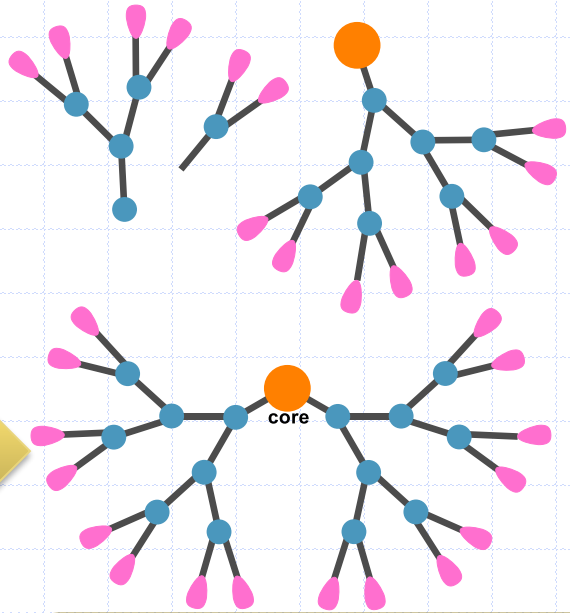


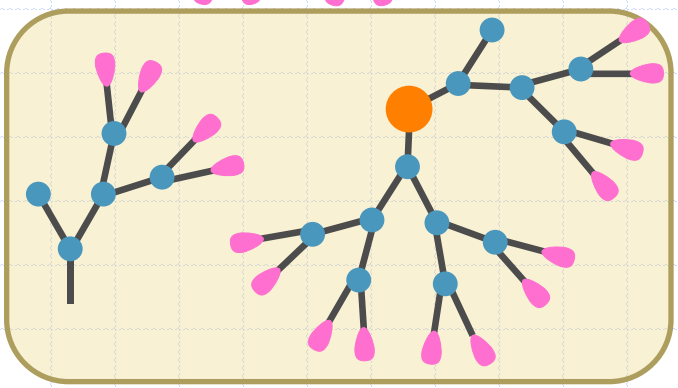


Nano2Clinic
CA17140

Dendrimers as Drug Delivery Systems







MS/MS Study

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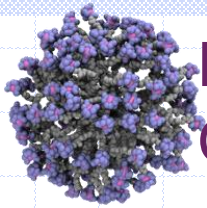
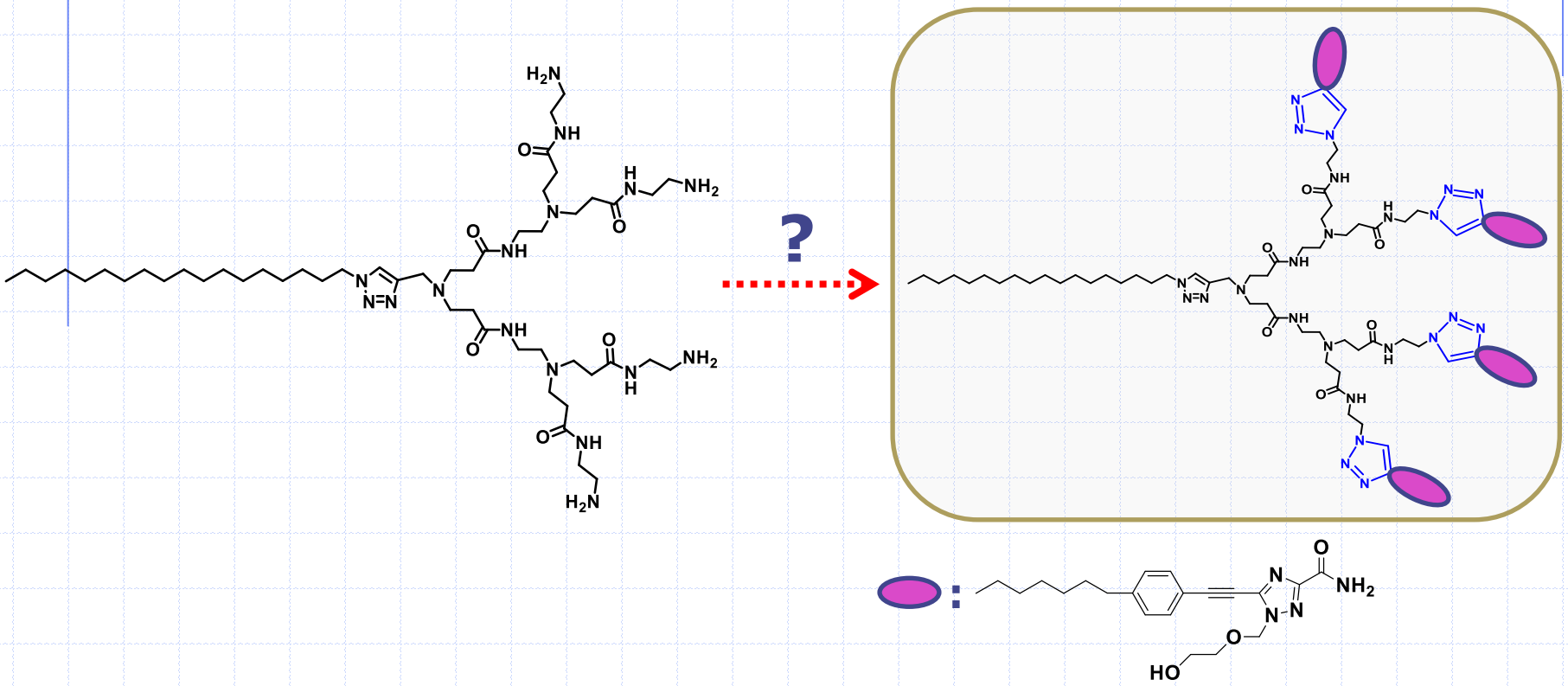


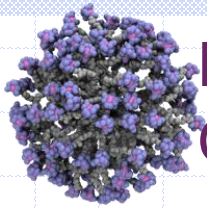
Nano2Clinic
CA17140

MS Study of nanovectors



Complementary approach to non-covalently dendrimer-drug systems

Drug conjugation on the dendrimer arms



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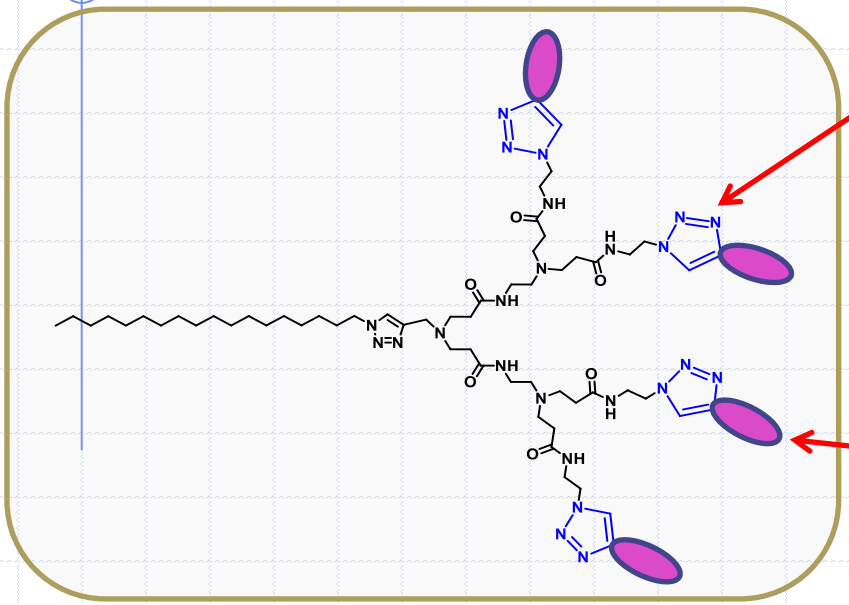
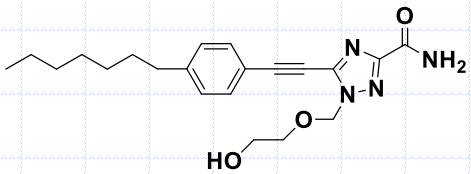


Triazole cycle = linker

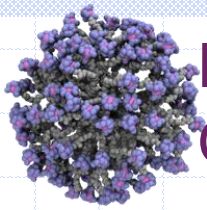
Should play a role in the drug delivery process

Drug



Triazole nucleoside = anticancer activity

- **High drug loading**
- **Can be much easier to control and predict in complex media**
- **Overcome the problem of poor solubility of triazole nucleoside**
- **Preserve the tumor targeting properties of nanosystems**



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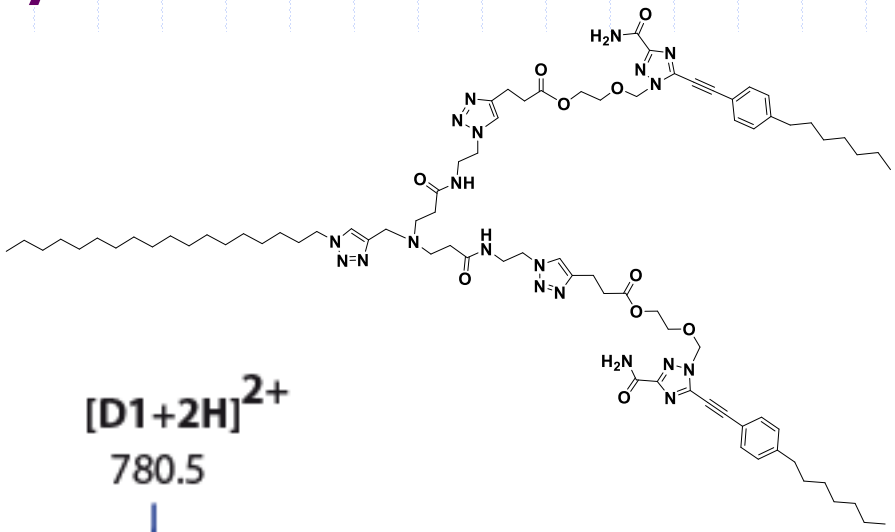

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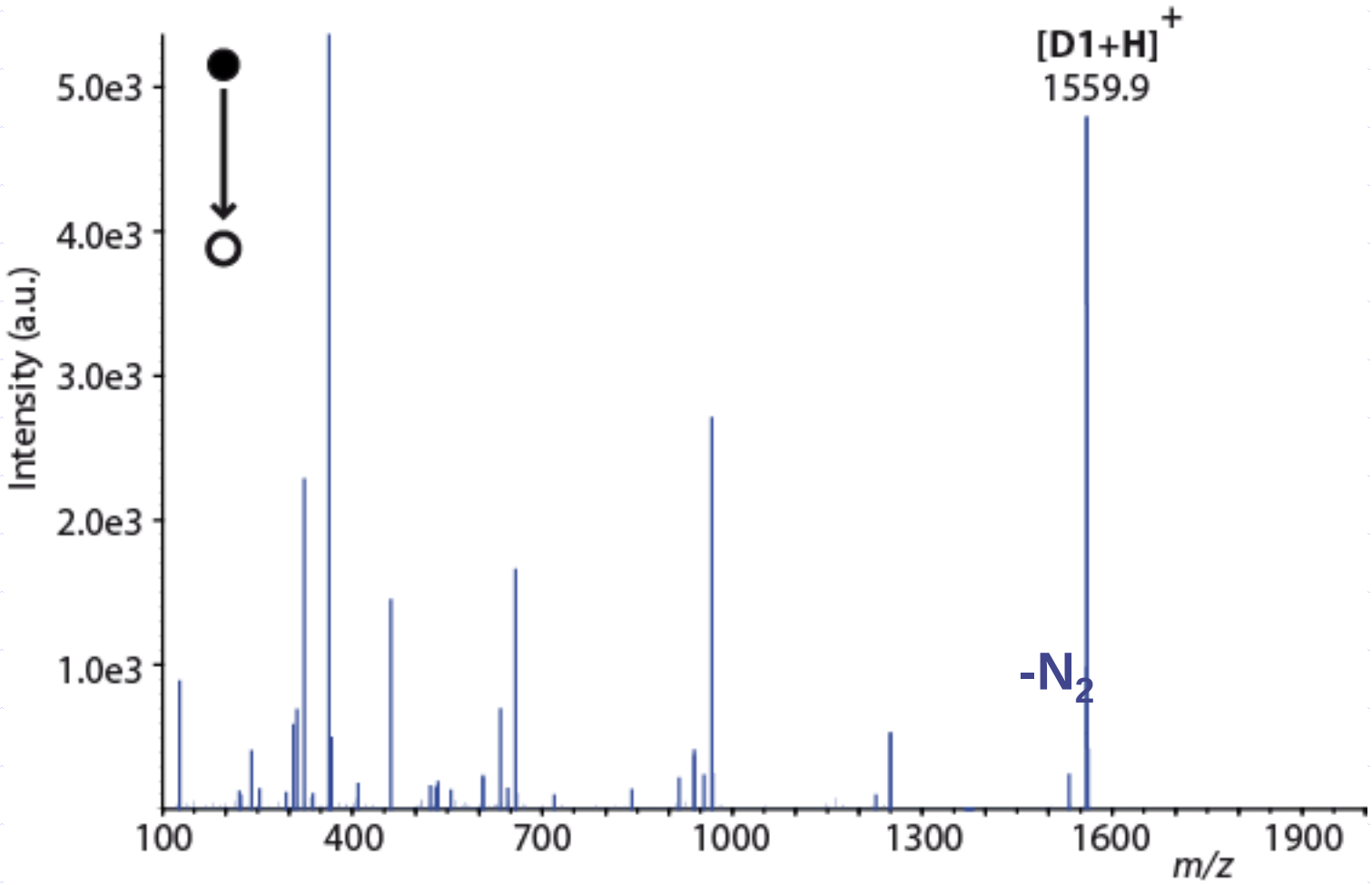

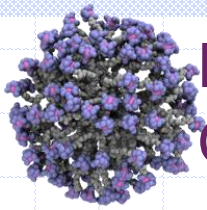

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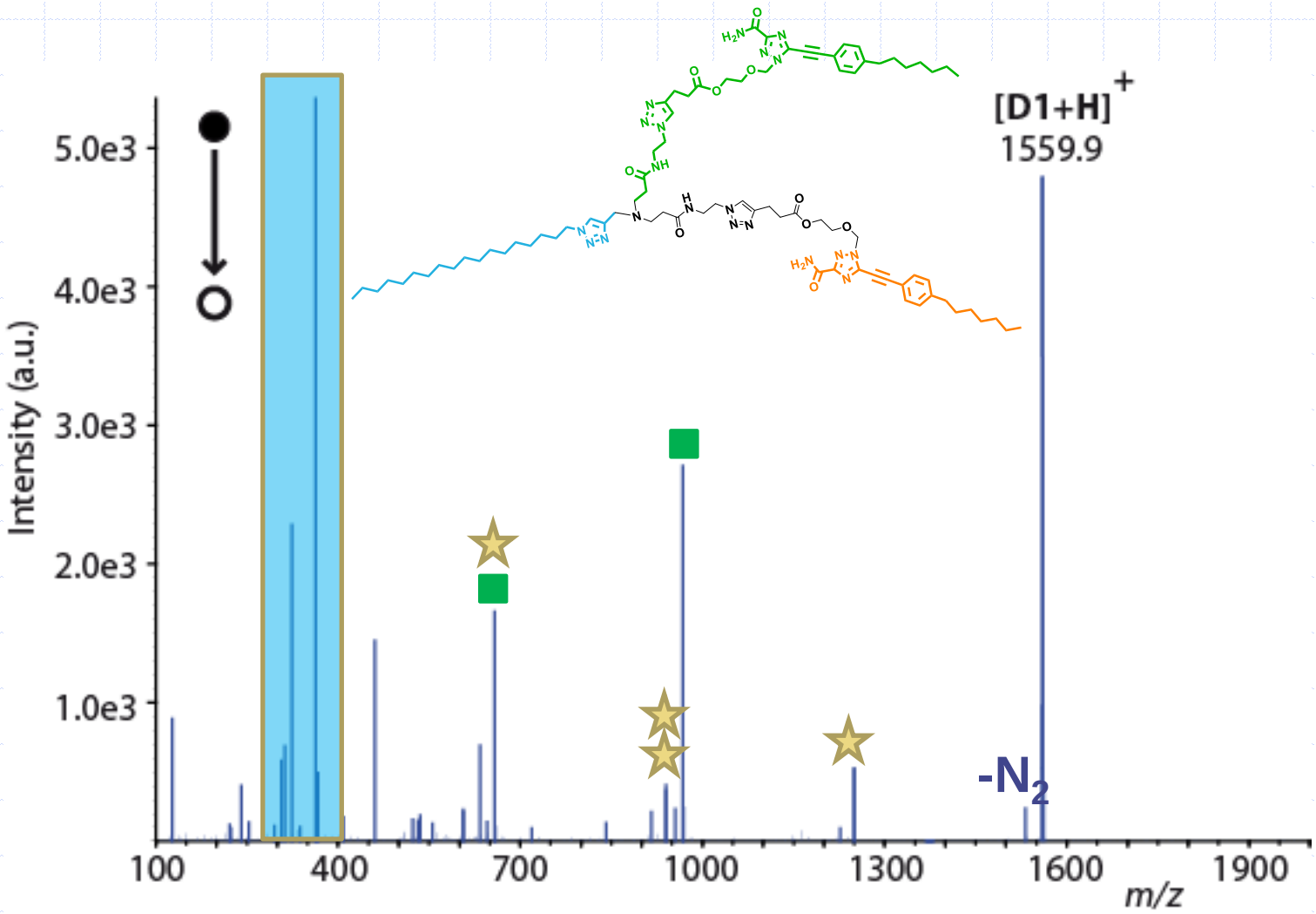

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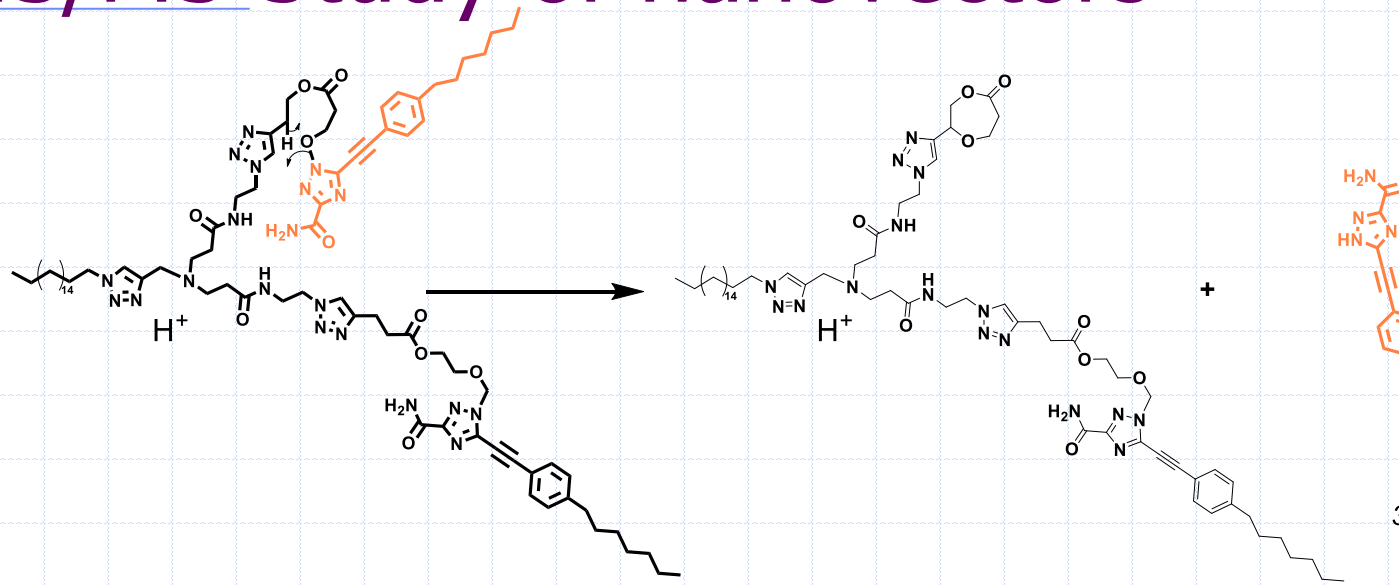
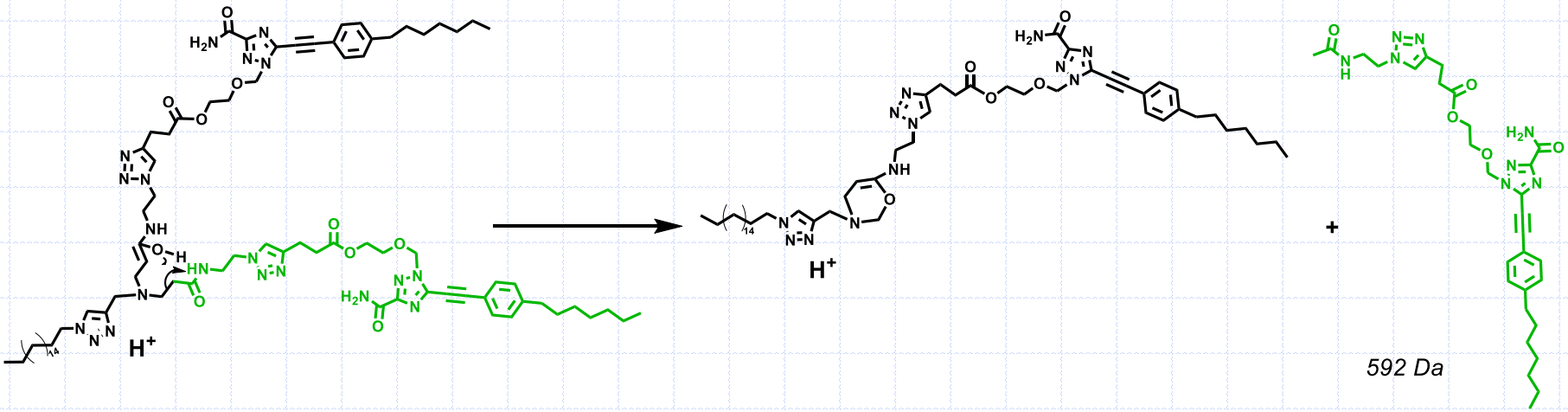
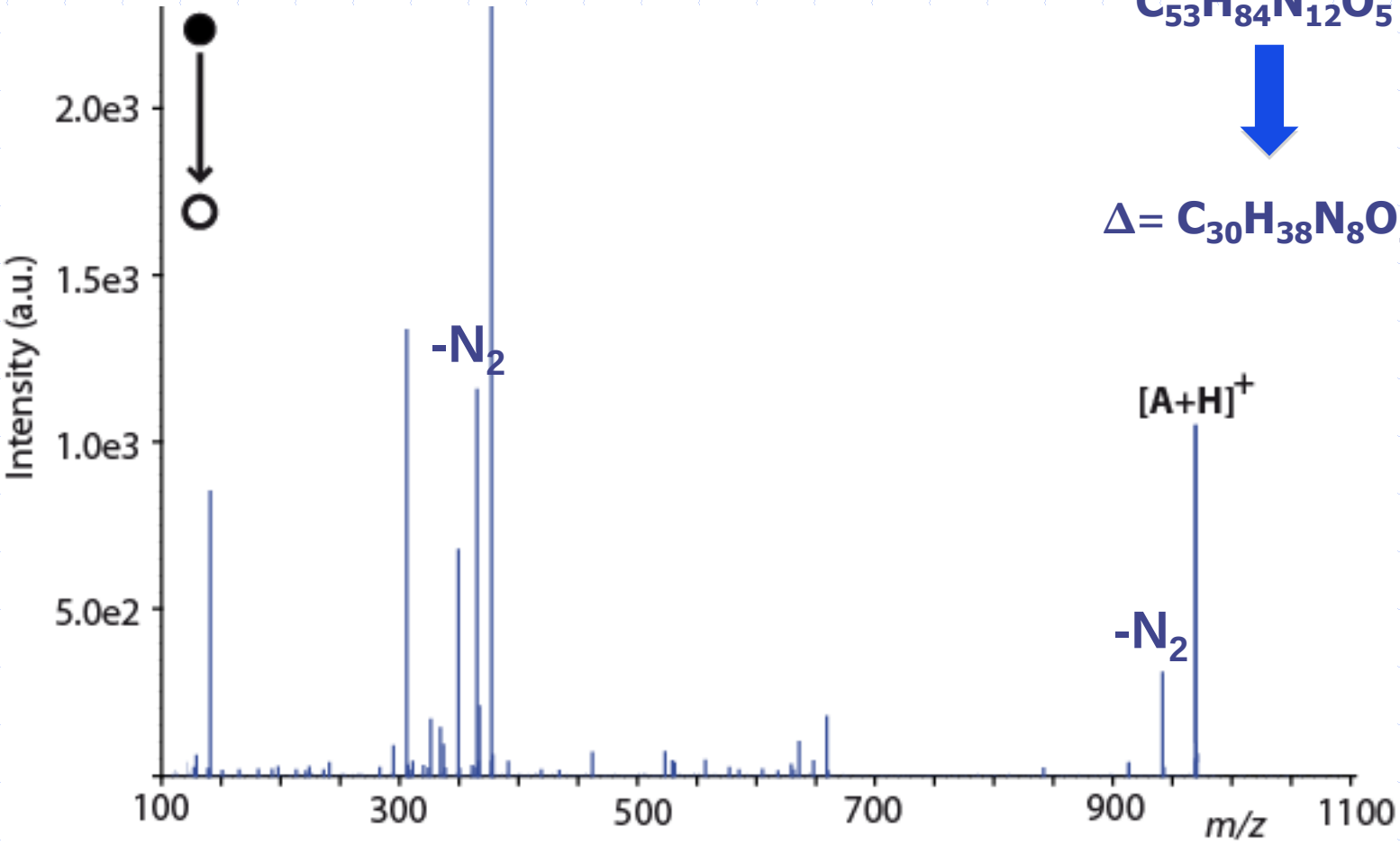




MS/MS Study of impurity A

$C_{53}H_{84}N_{12}O_5$

$\Delta = C_{30}H_{38}N_8O_5$



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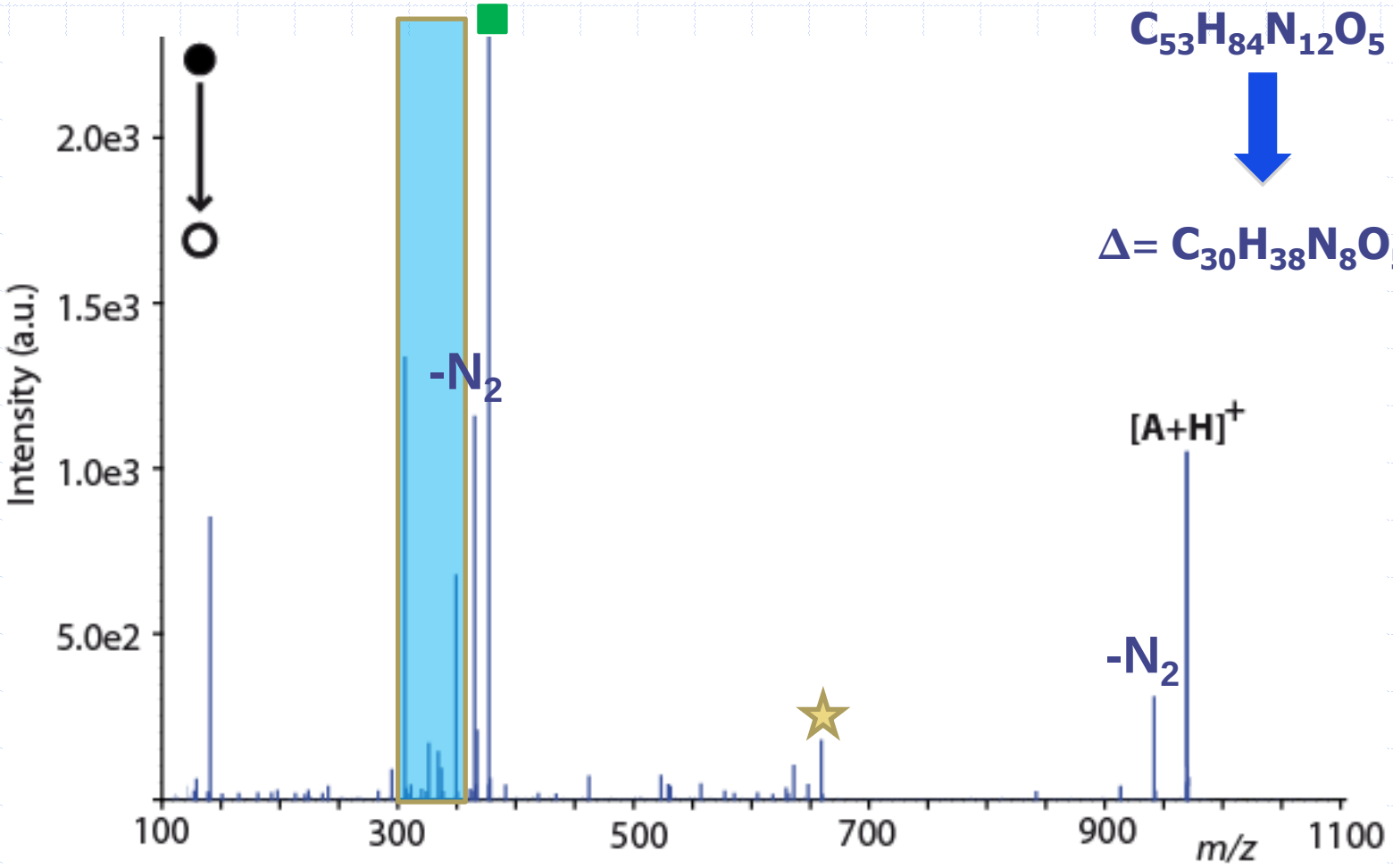

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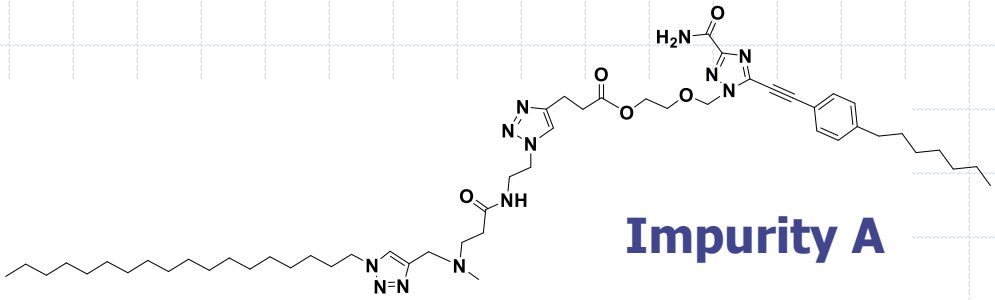

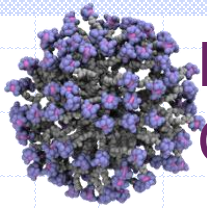

Nano2Clinic
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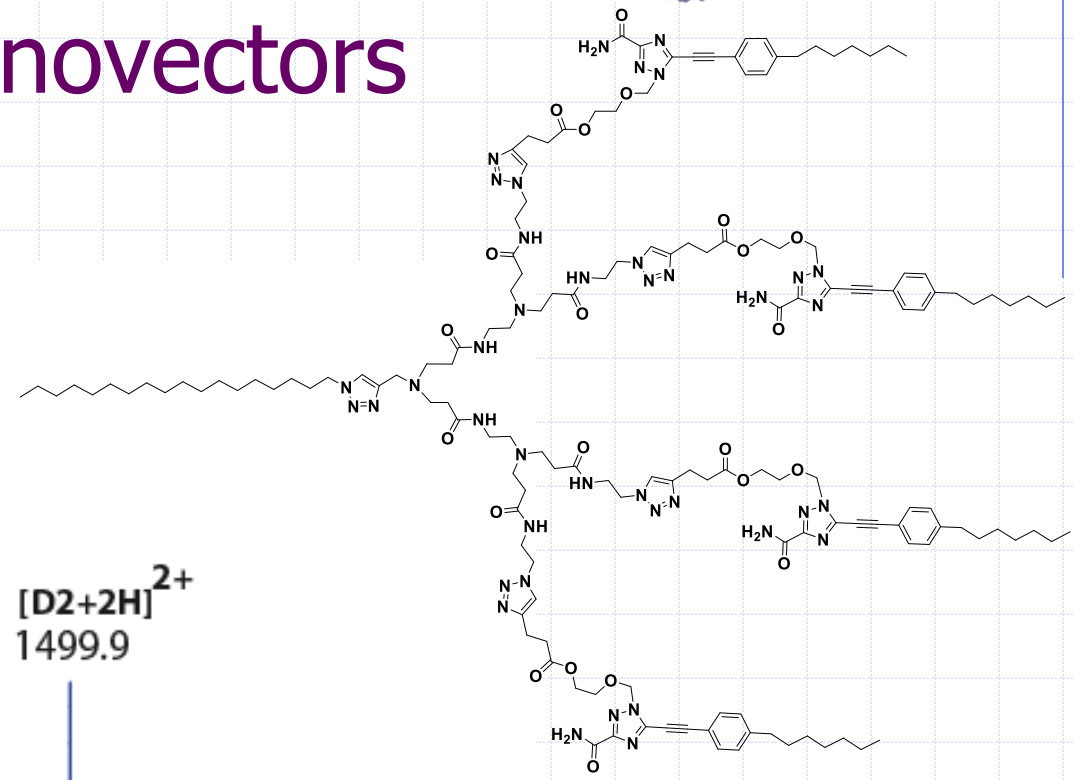
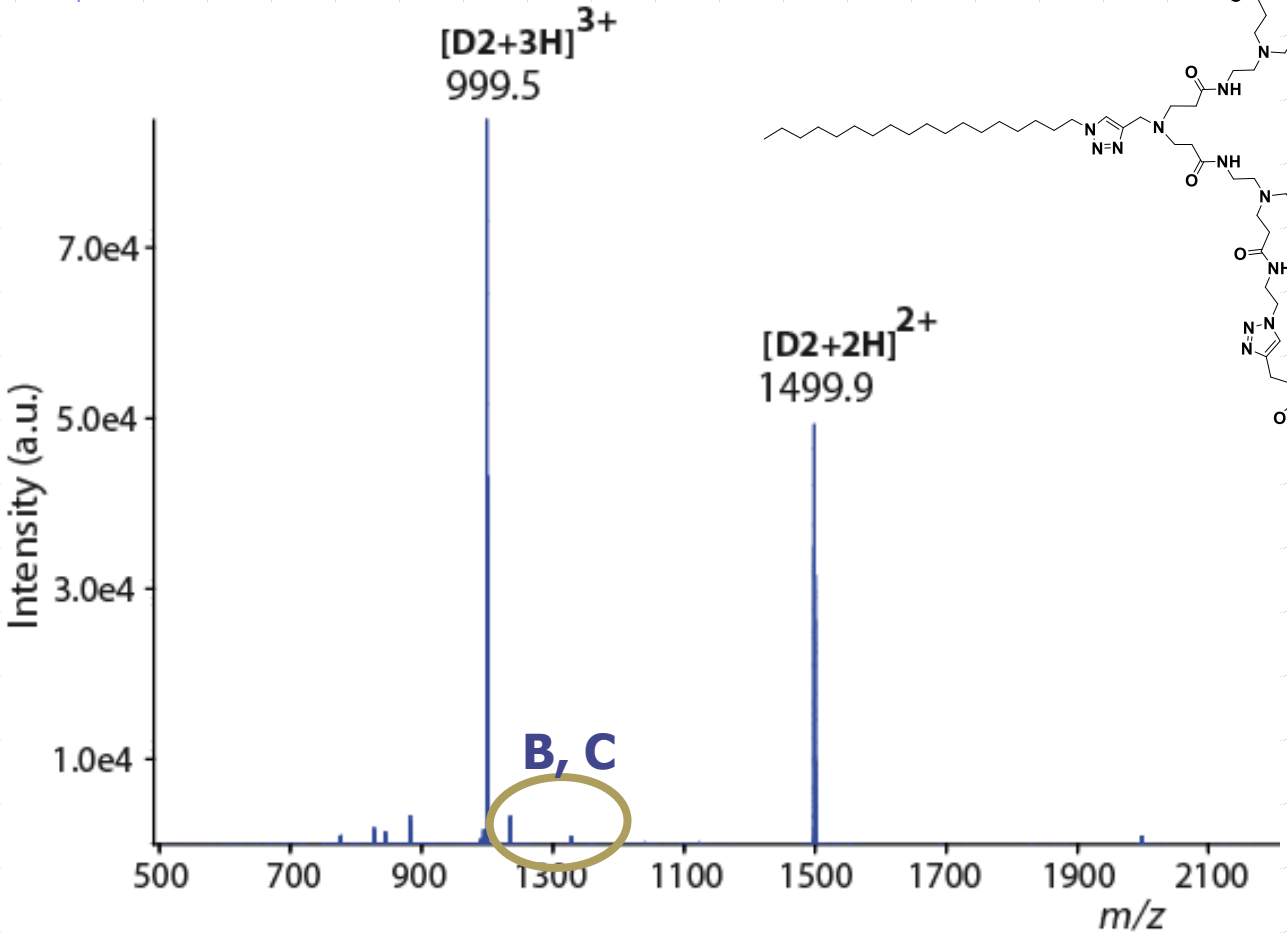
MS Study of nanovectors





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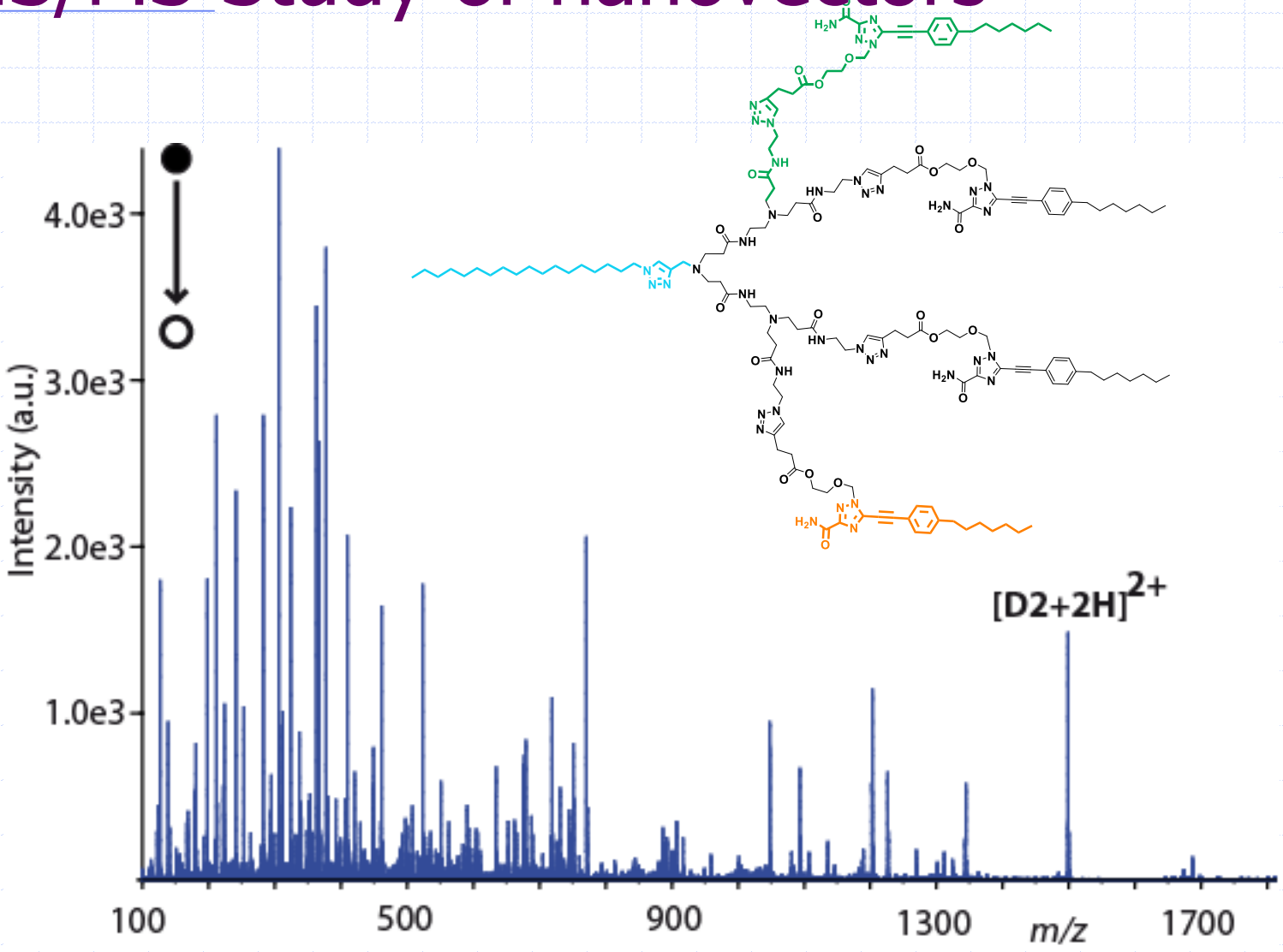

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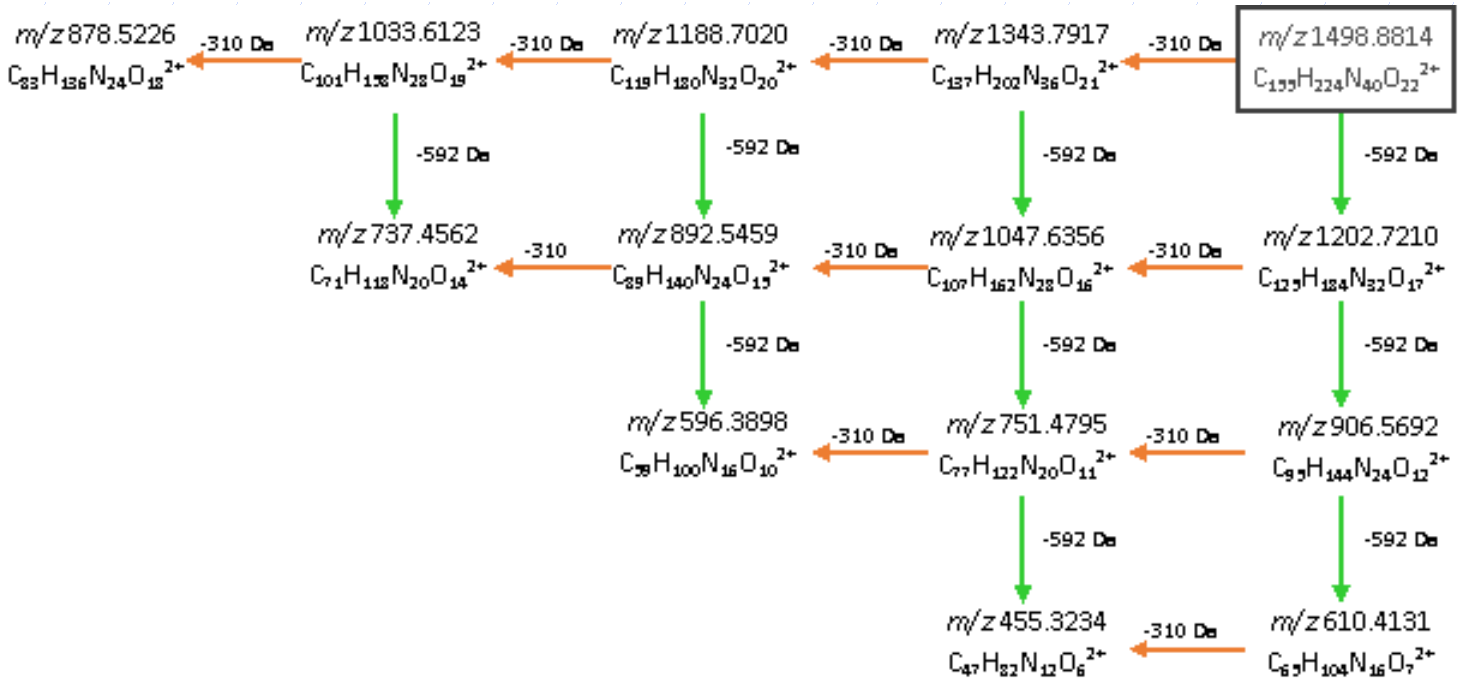
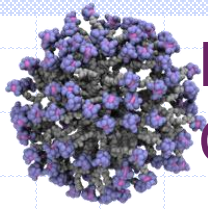


Fragmentation rules → used for the structural characterization of the two impurities B and C



Nano2Clinic
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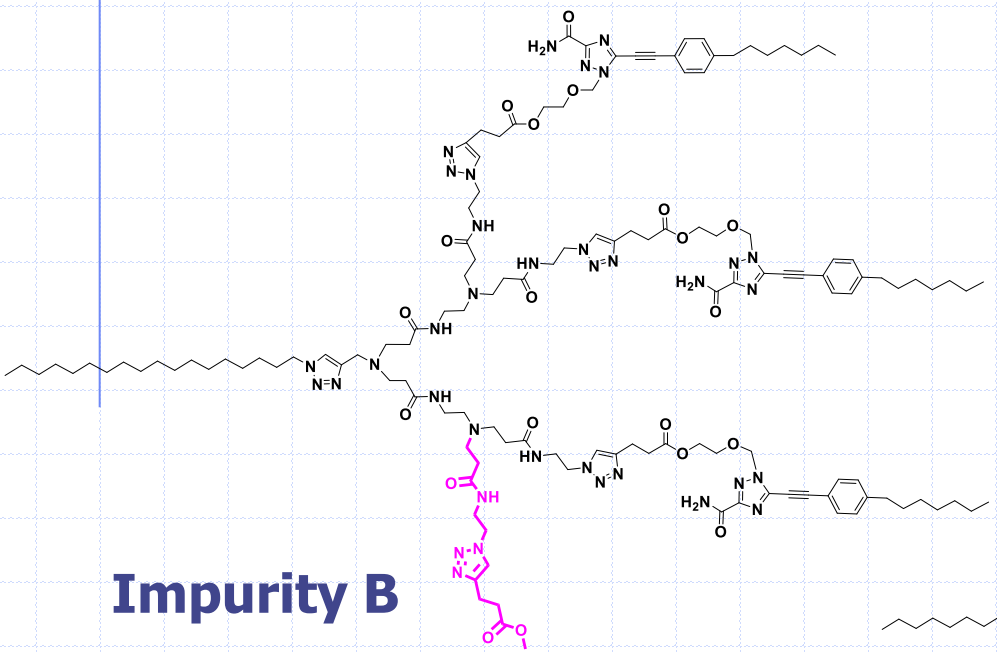
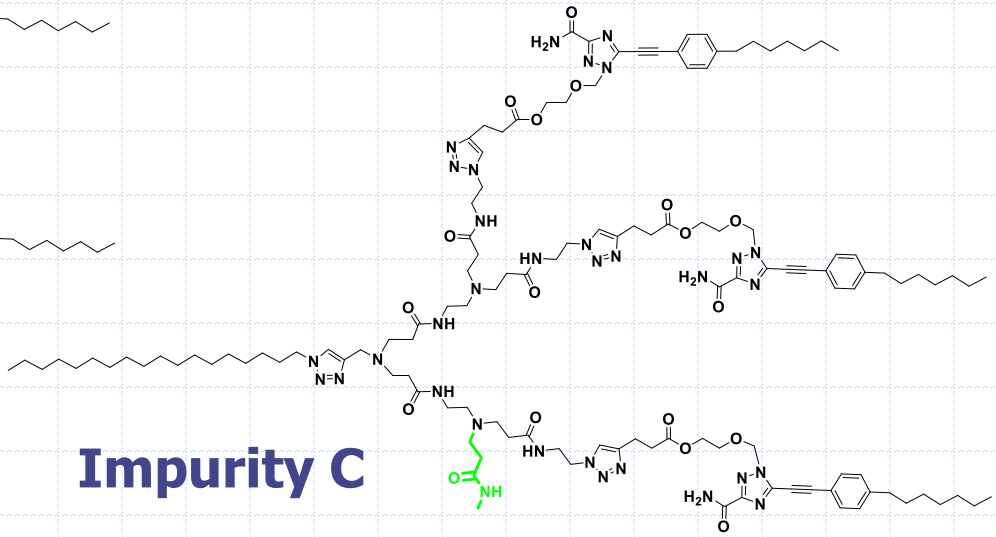
MS/MS Study of nanovectors

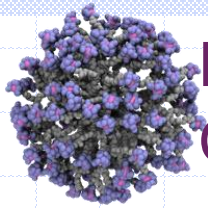


Impurity B



Impurity C

Structure of the “defective” molecules



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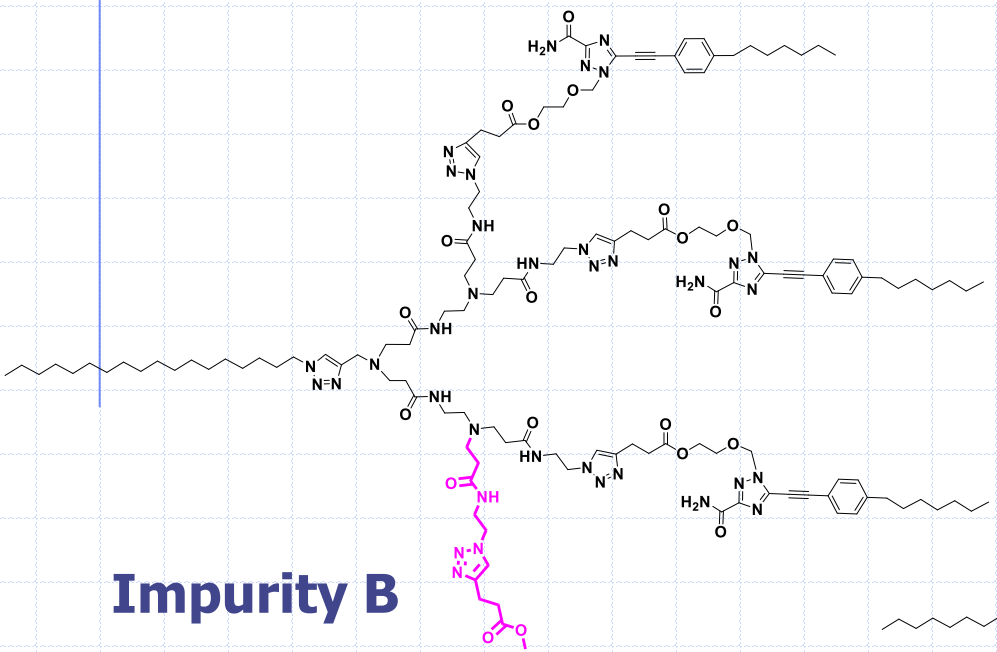
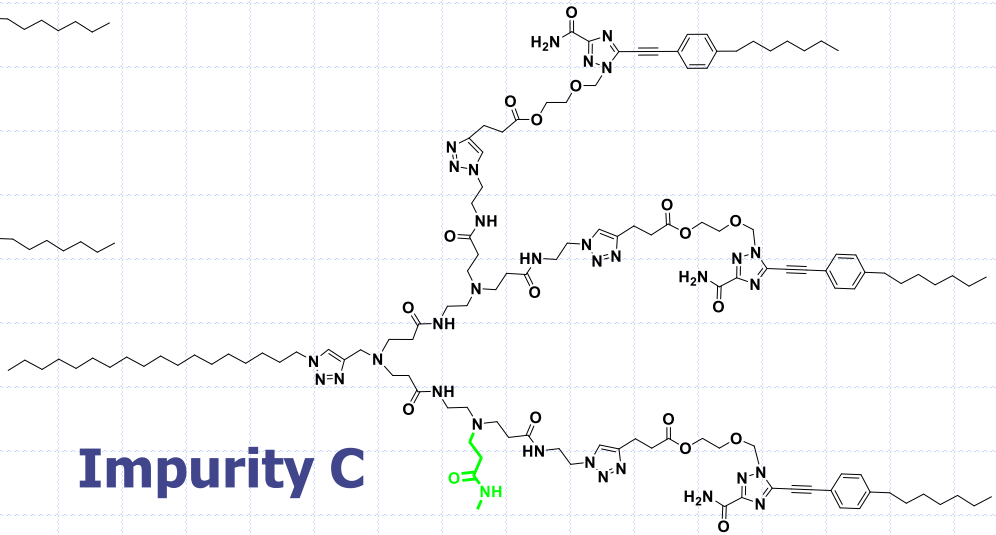
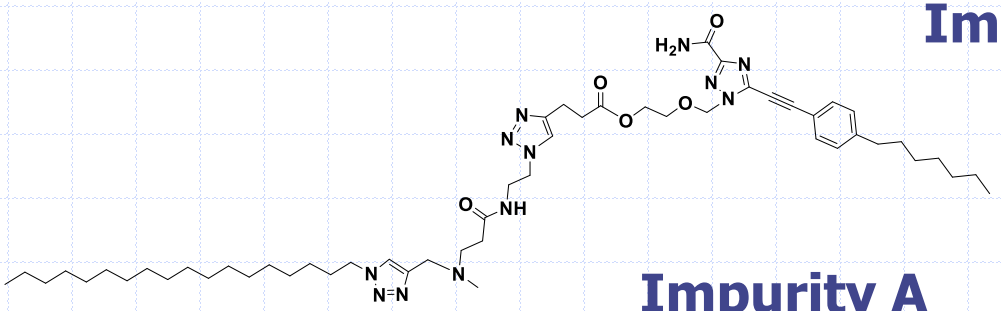
MS/MS Study of nanovectors



Impurity B

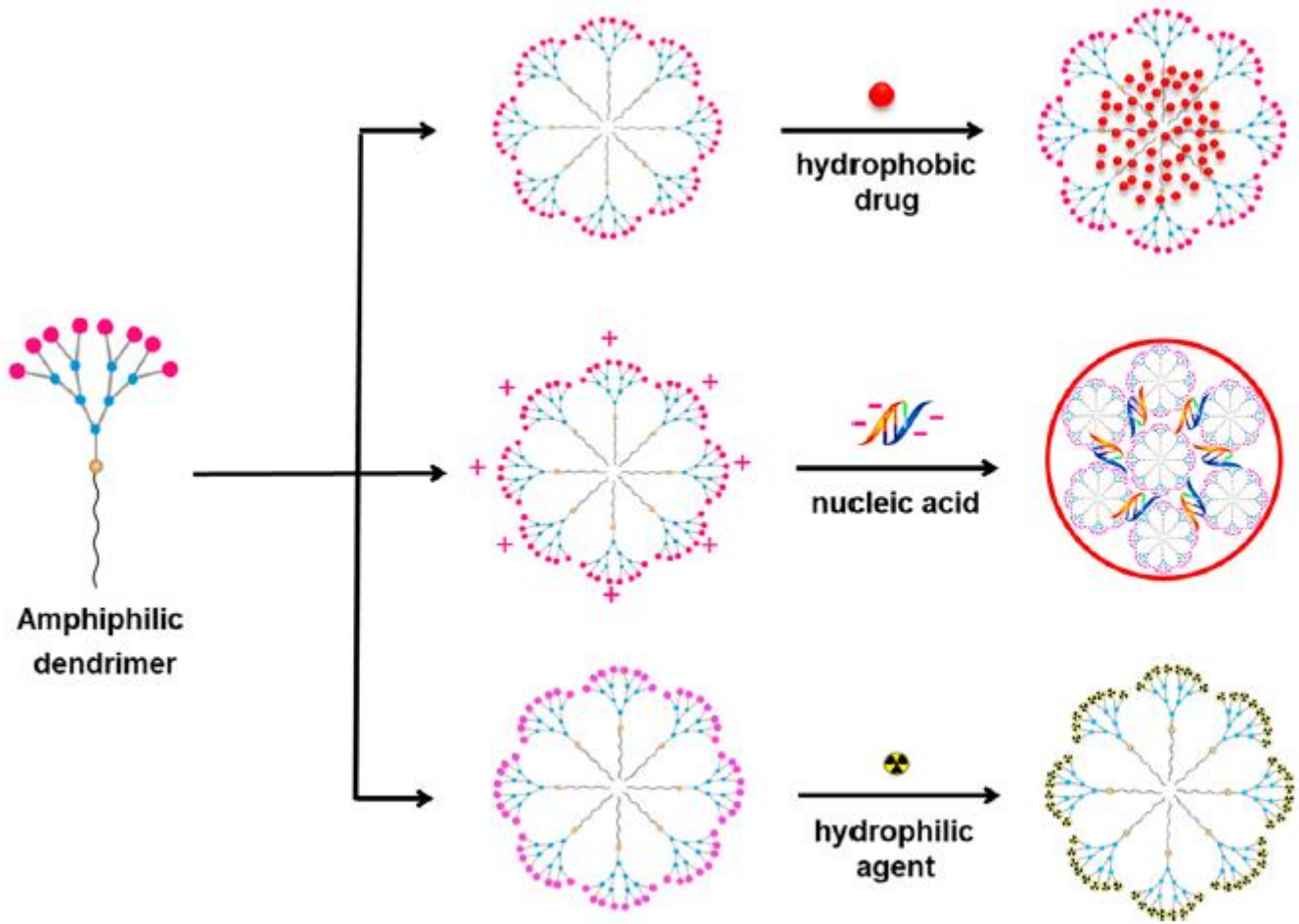


Impurity C



Impurity A

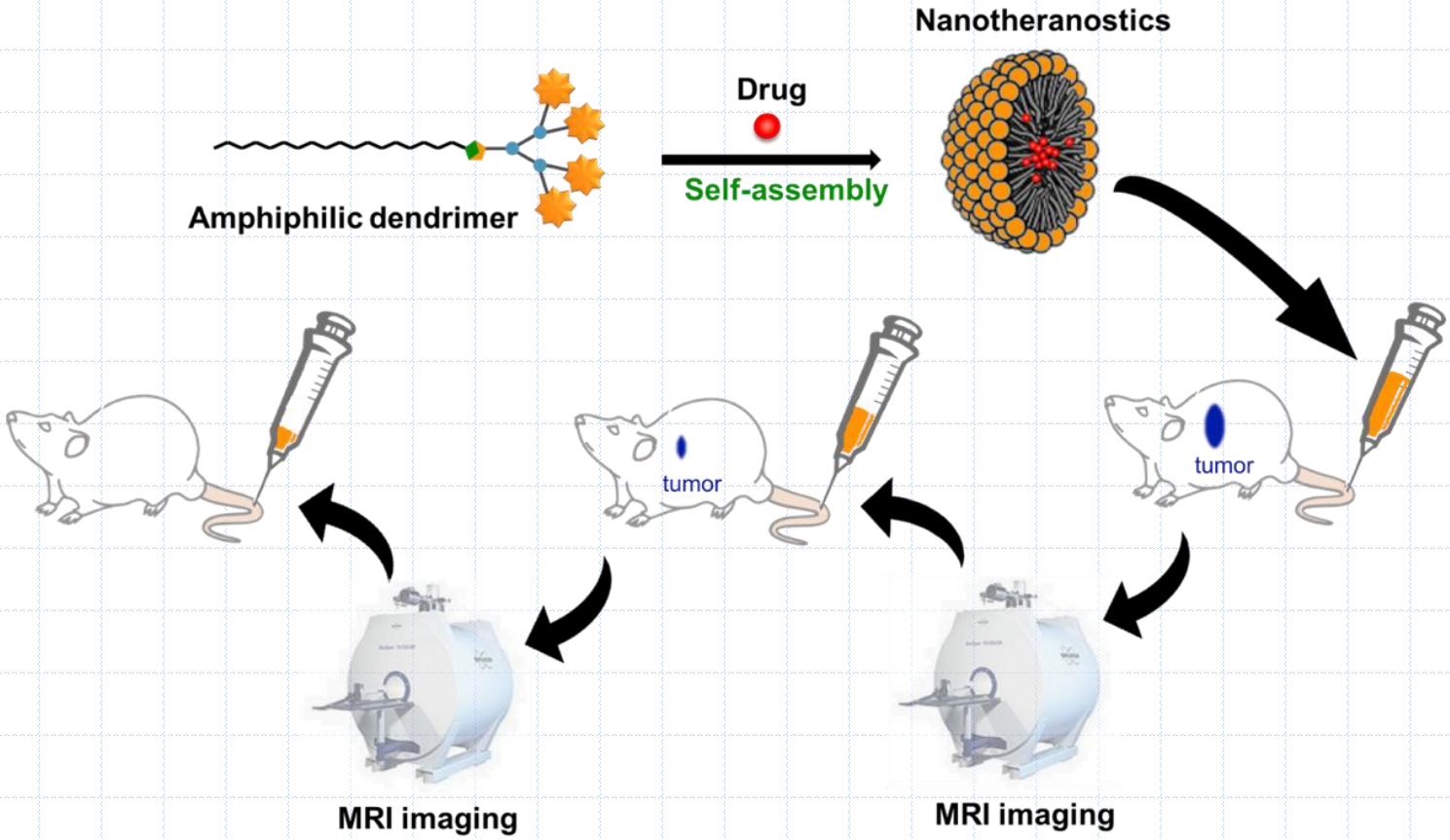
Amphiphilic Dendrimers



Main goal: Highest sample purity required for any for biological application

Lyu Z et al, Acc. Chem. Res. 2020, 53, 12, 2936−2949

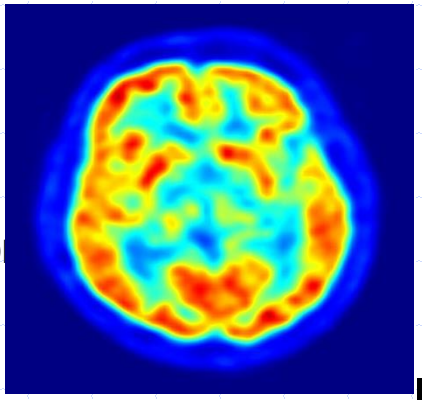
Nanotheranostics



Imaging

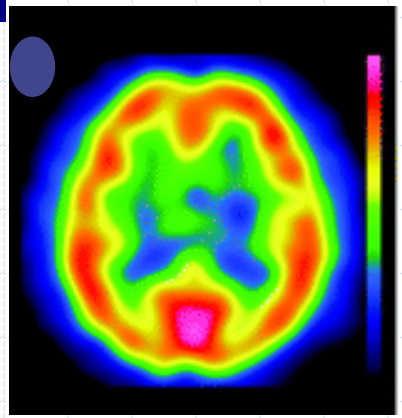
PET

Positron **E**mission **T**omography
Gamma rays emitted indirectly by a positro
emitting radioligand



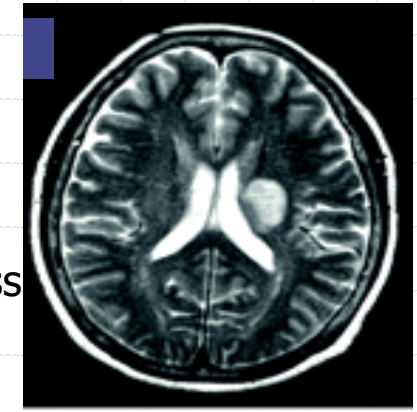
SPECT

Single **P**hoton **E**mission **C**omputed **T**omography
Gamma-emitting radioisotope



MRI

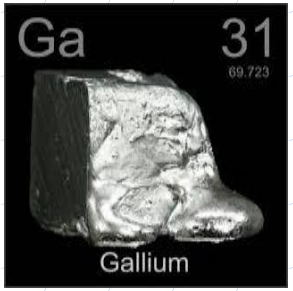
Magnetic **R**esonance **I**maging
Magnetic relaxation of water and fat tiss



Imaging

PET

Positron **E**mission **T**omography
Gamma rays emitted indirectly by a positron-emitting radioligand

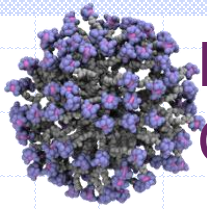


SPECT

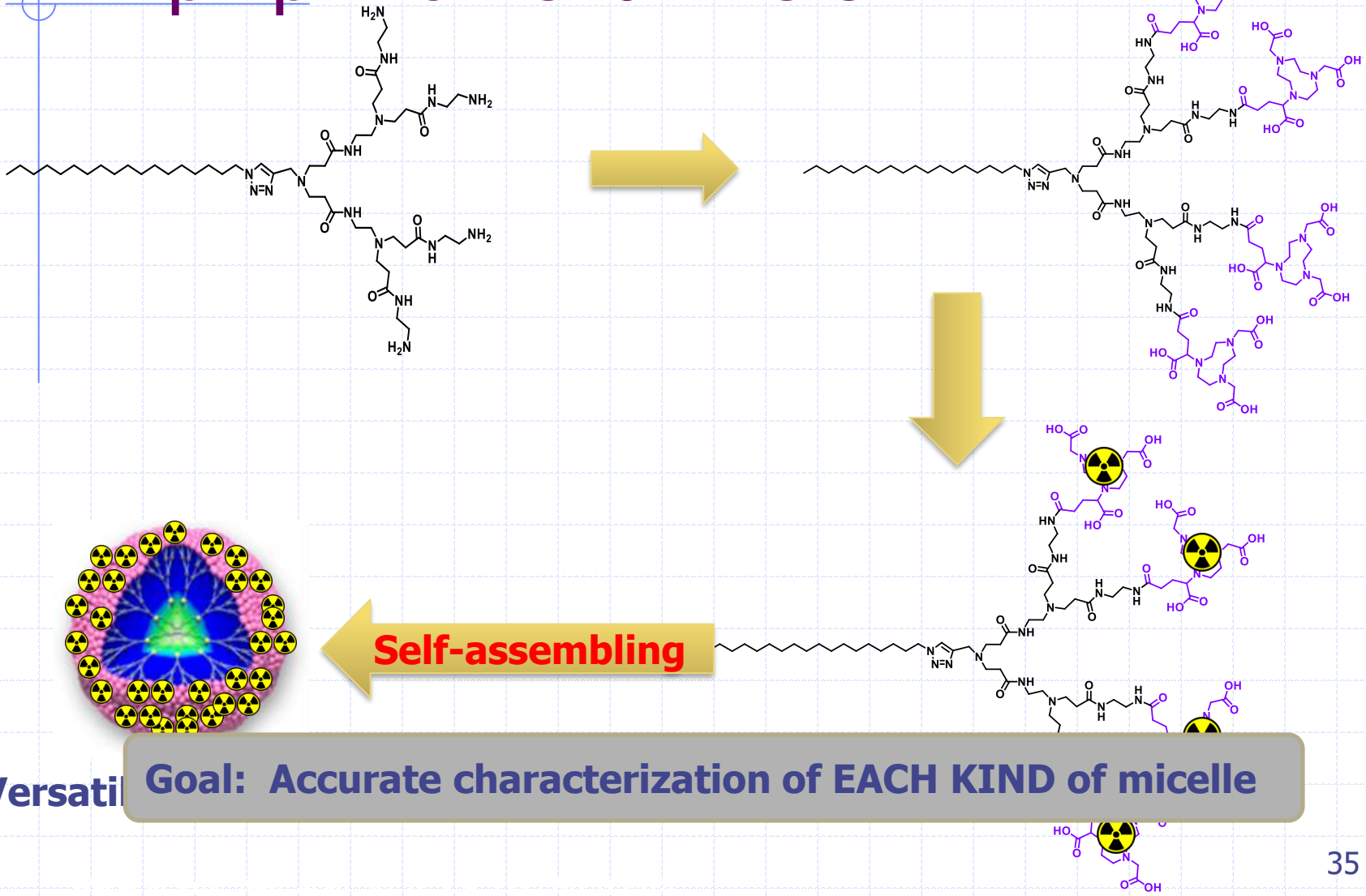
Single **P**hoton **E**mission **C**omputed **T**omography
Gamma-emitting radioisotope

MRI

Magnetic **R**esonance **I**maging
Magnetic relaxation of water and fat tissues



Amphiphilic Dendrimers



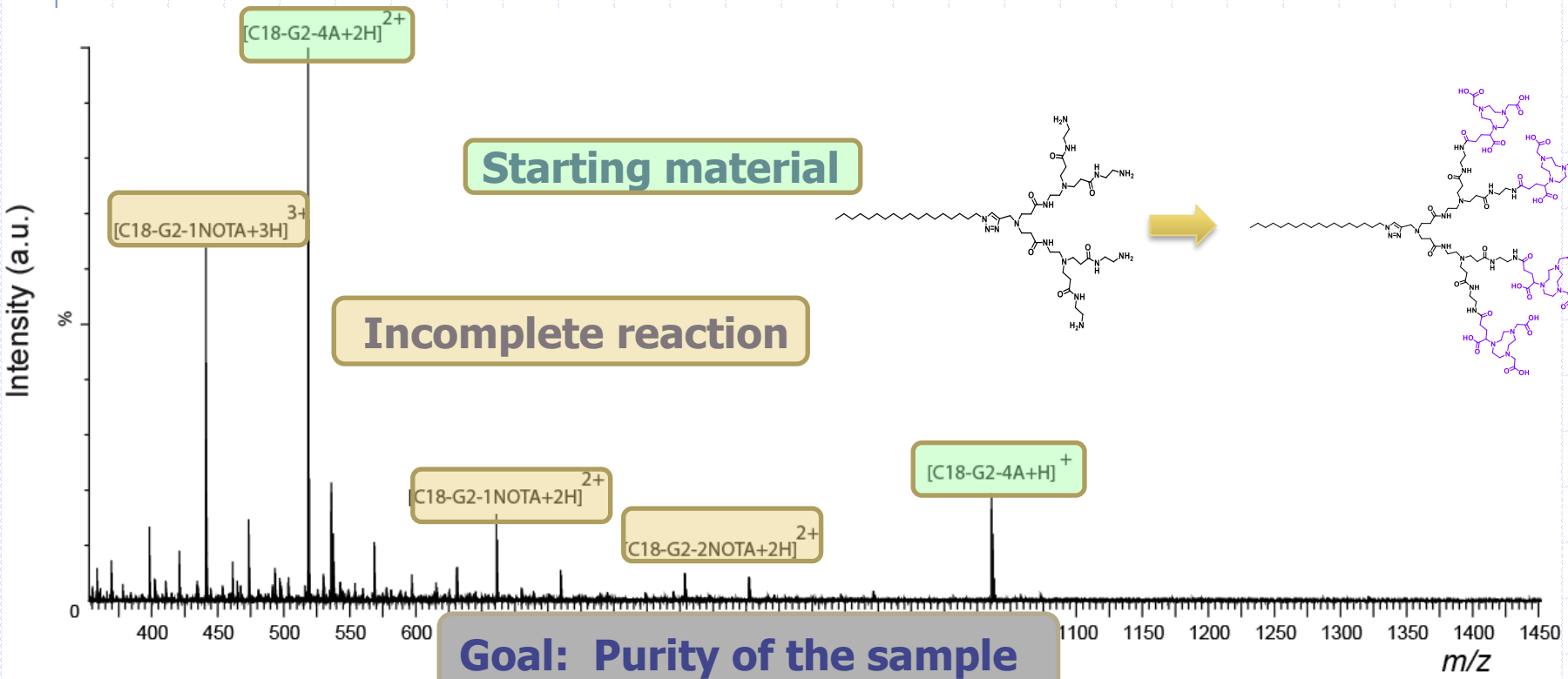
Versati

Goal: Accurate characterization of EACH KIND of micelle

NOTA-Amphiphilic Dendrimers

MS & MS/MS Study

Expected: $C_{111}H_{222}N_{28}O_{34}$



Starting material

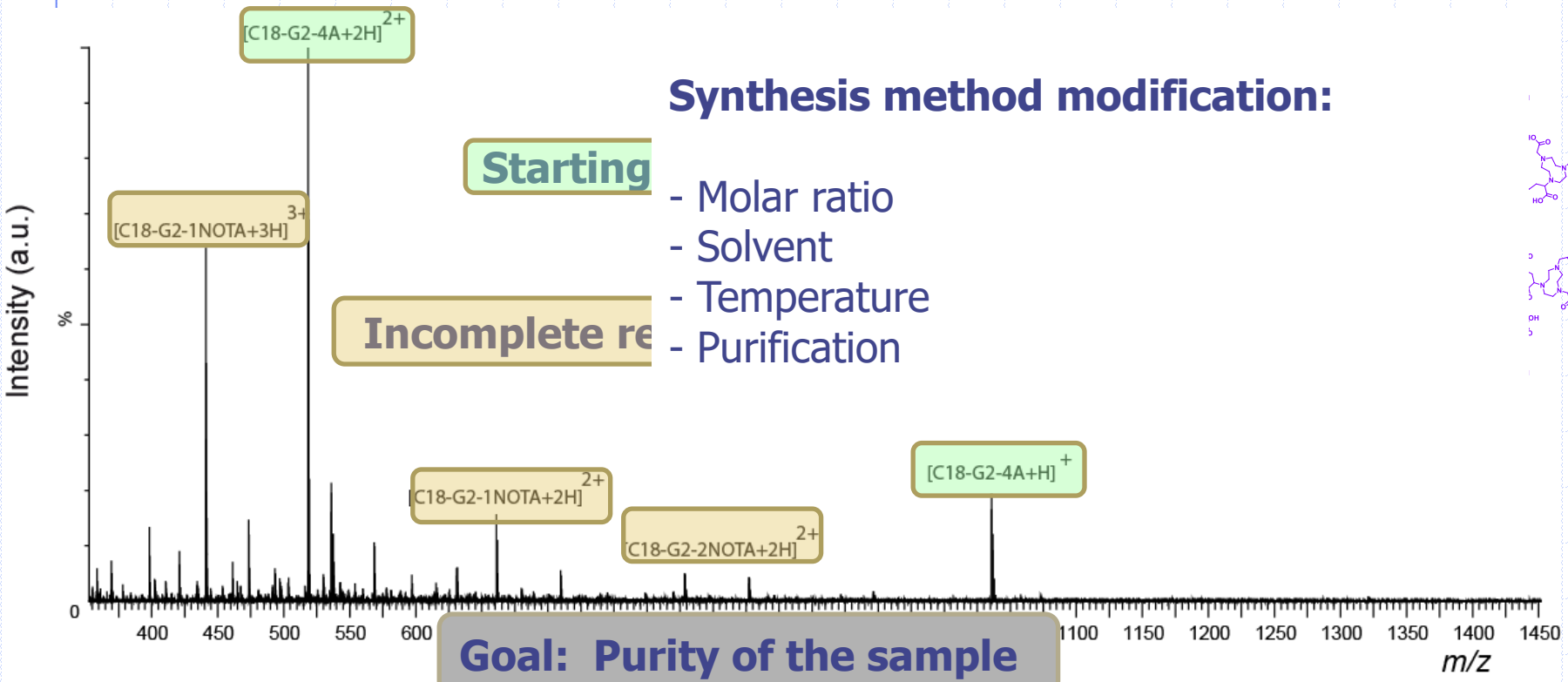
Incomplete reaction

Goal: Purity of the sample

NOTA-Amphiphilic Dendrimers

MS & MS/MS Study

Expected: $C_{111}H_{222}N_{28}O_{34}$



Synthesis method modification:

- Molar ratio
- Solvent
- Temperature
- Purification

Starting

Incomplete re

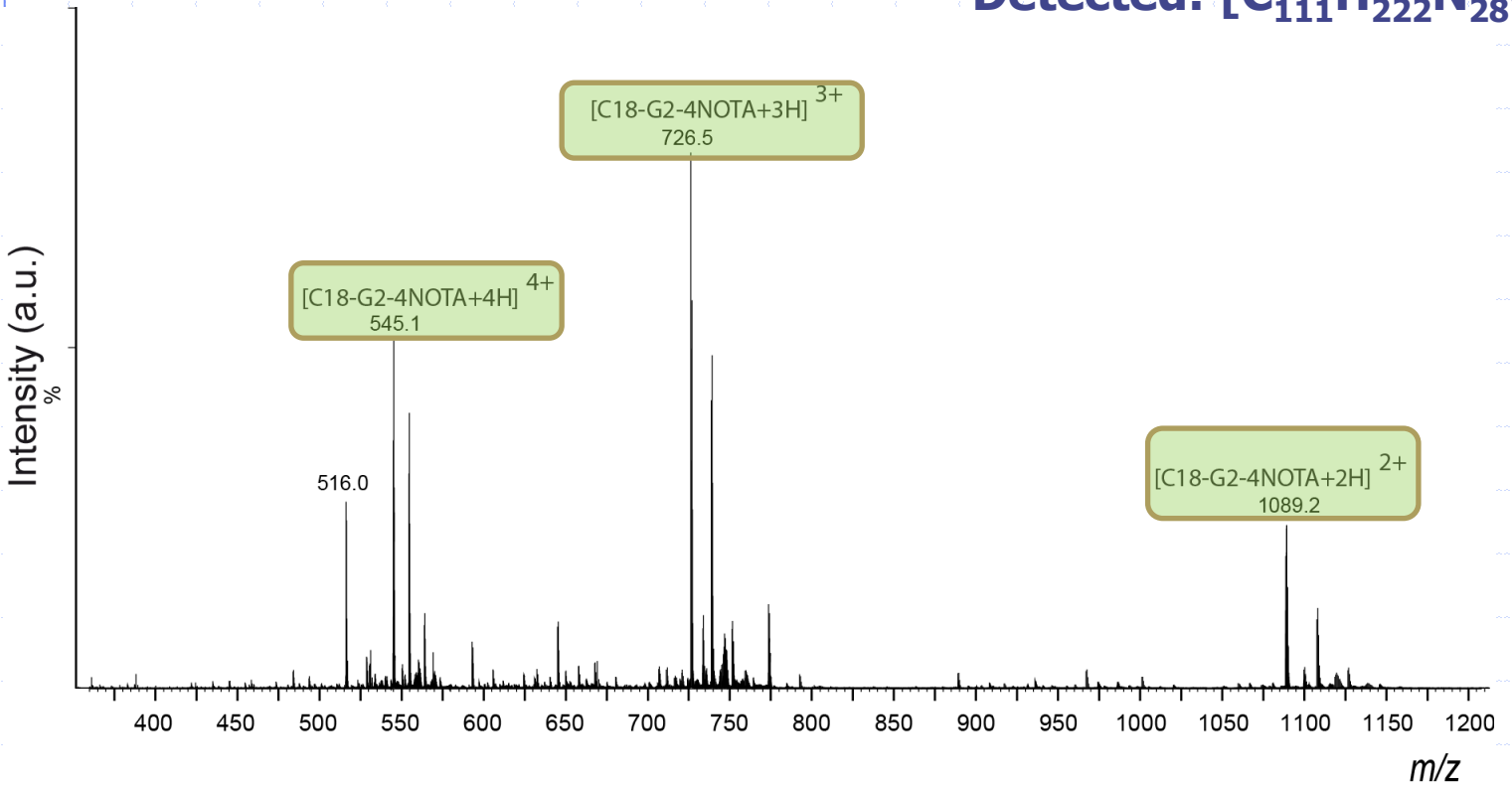
Goal: Purity of the sample

NOTA-Amphiphilic Dendrimers

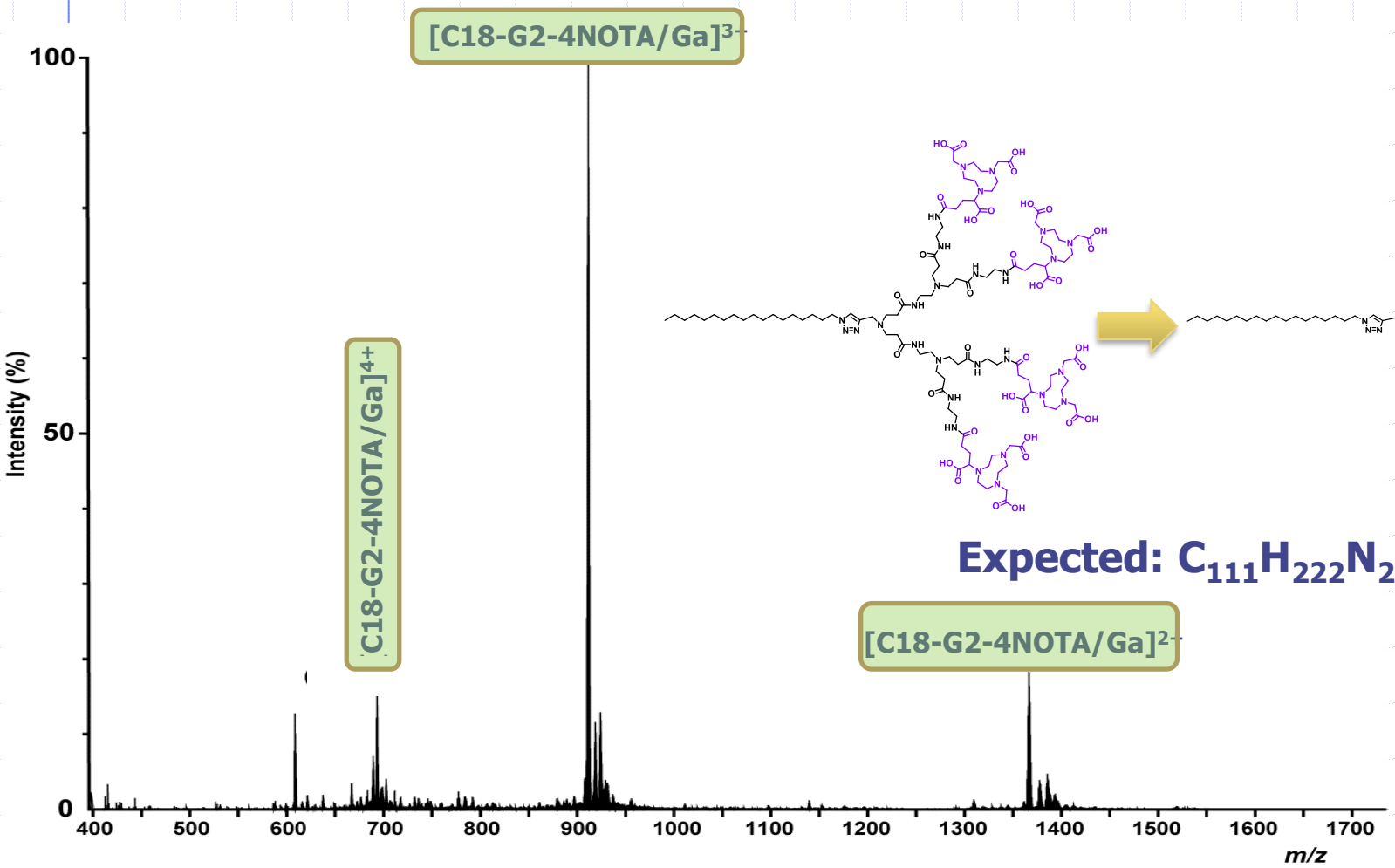
MS & MS/MS Study

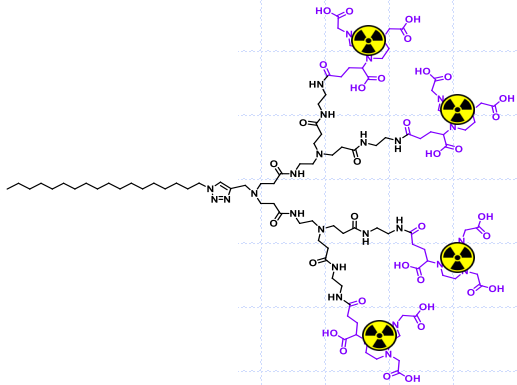
Expected: $C_{111}H_{222}N_{28}O_{34}$

Detected: $[C_{111}H_{222}N_{28}O_{34}+3H]^{3+}$



Ga^{III} -NOTAAmphiphilic Dendrimers

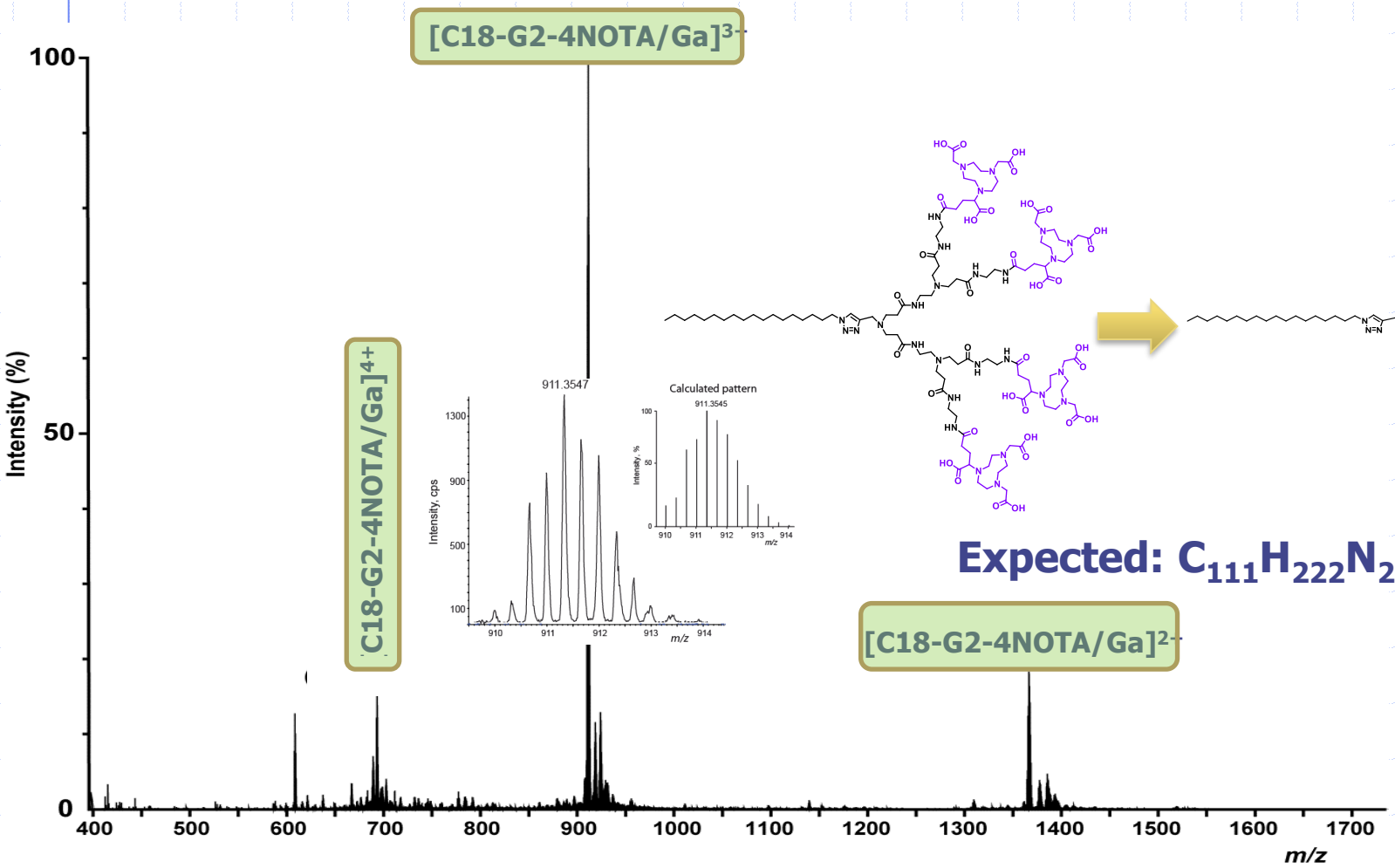


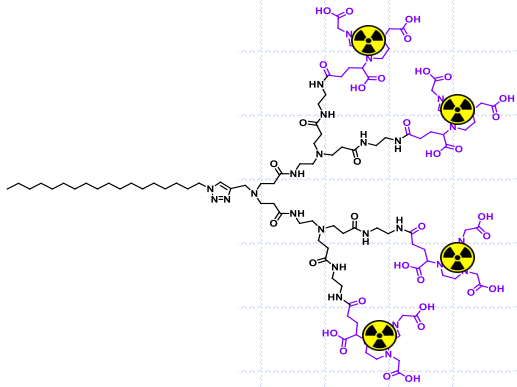


Expected: $C_{111}H_{222}N_{28}O_{34}$ $Ga^{(III)}_4$

Garrigue P et al. PNAS. 2018, 26, 8594–8603

Ga^{III} -NOTAAmphiphilic Dendrimers



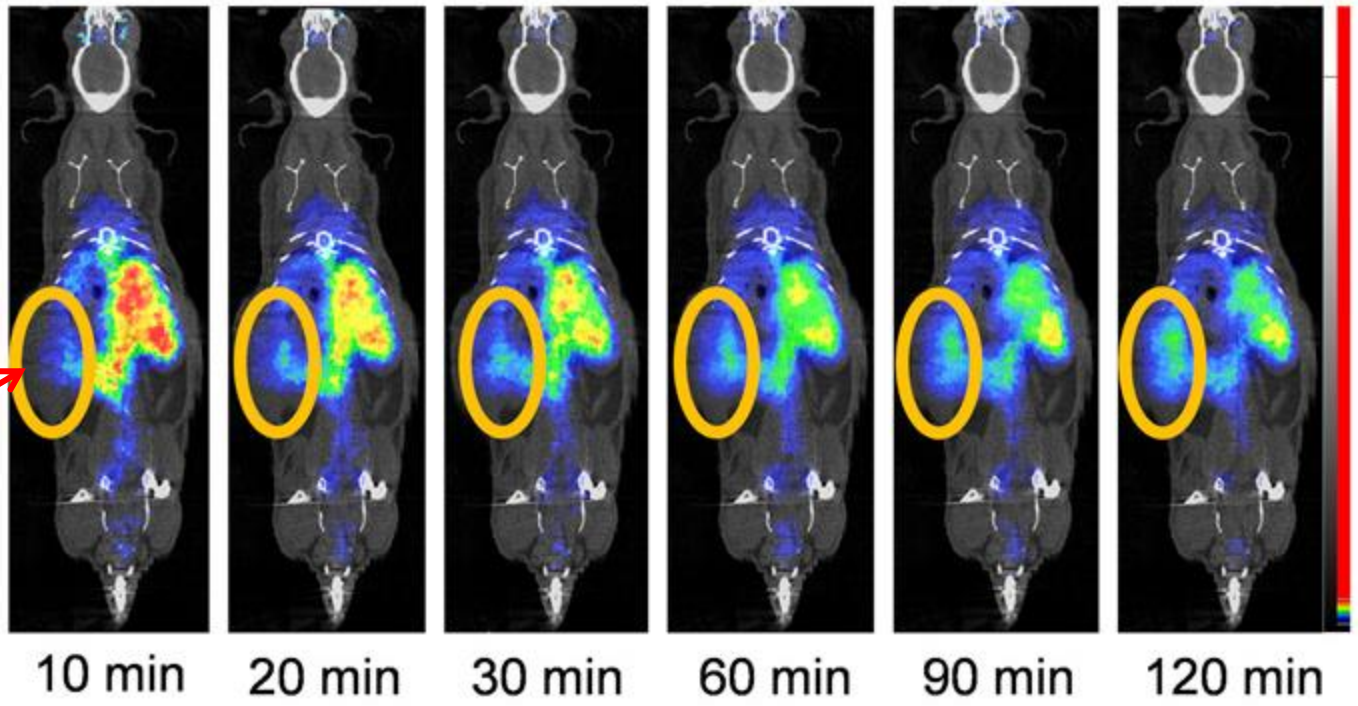


Expected: $C_{111}H_{222}N_{28}O_{34}$ $Ga^{(III)}_4$

Garrigue P et al. PNAS. 2018, 26, 8594–8603

Ga^{III} -NOTAAmphiphilic Dendrimers

PET experiments



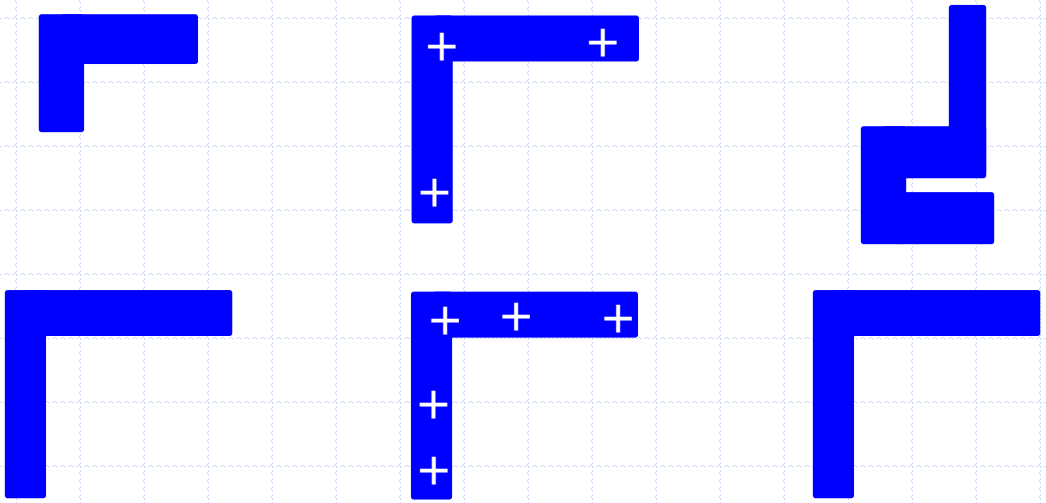
Migration of the dendrimer in the cancerous tissue after 10min

Garrigue P et al. PNAS. 2018, 26, 8594–8603

Ion Mobility: a New Dimension for MS

IMS separates ions based on their mobility through a neutral gas

The mobility of an ionized molecule depends on …



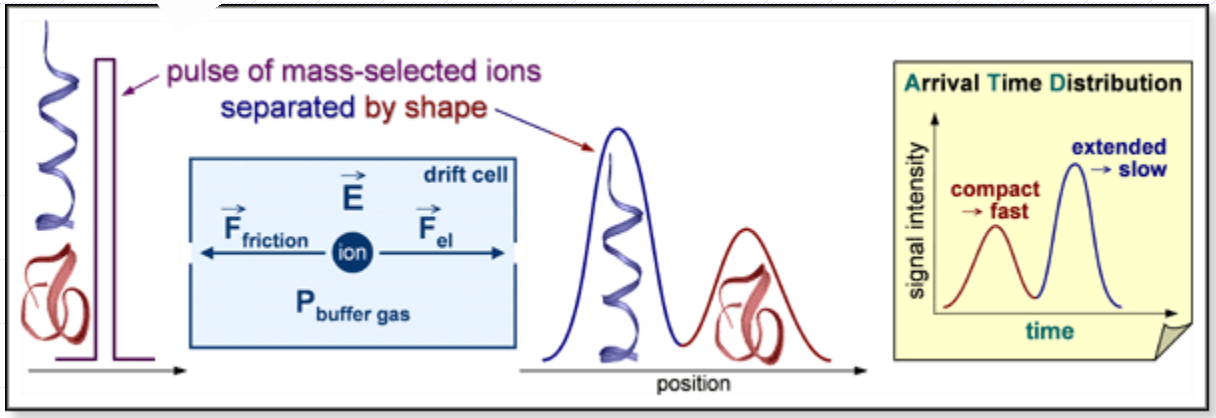
… its size … its charge … its shape

already discriminated in MS

Ion Mobility: Principle

Which will "land" first?

Ion Mobility: Principle

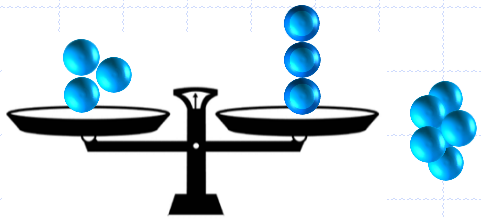


http://bowers.chem.ucsb.edu/theory_analysis/ion-mobility/index.shtml

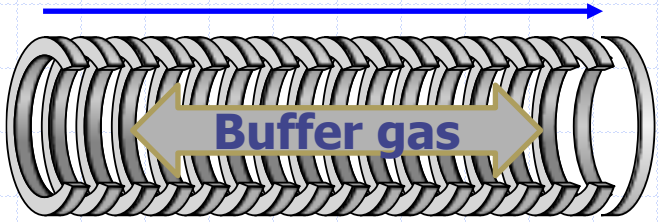
Ion Mobility: Principle

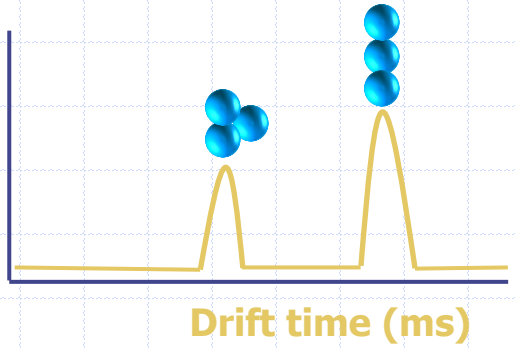
In IMS, ions are separated as a function of their mobility through an inert gas under the effect of an electric field.

Same masses but different conformations



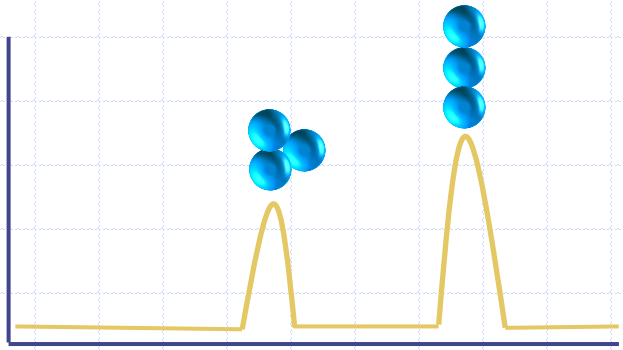
Electric Field





- **Mobility of an ion depends on its WEIGHT, CHARGE STATE & SHAPE**

Ion Mobility: Principle

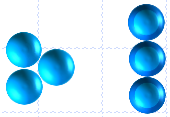


Drift times (ms)

Calibration

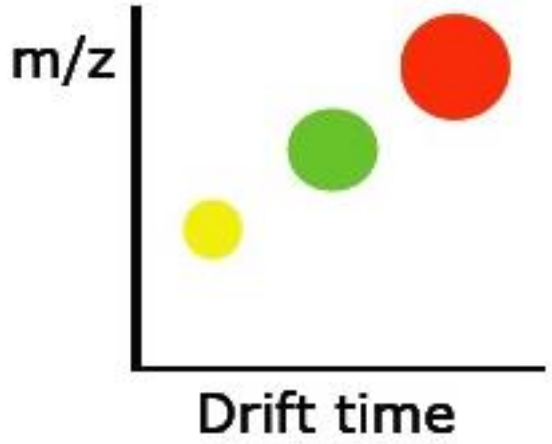
Collision Cross Section (CCS)

Ion Size (Å²)

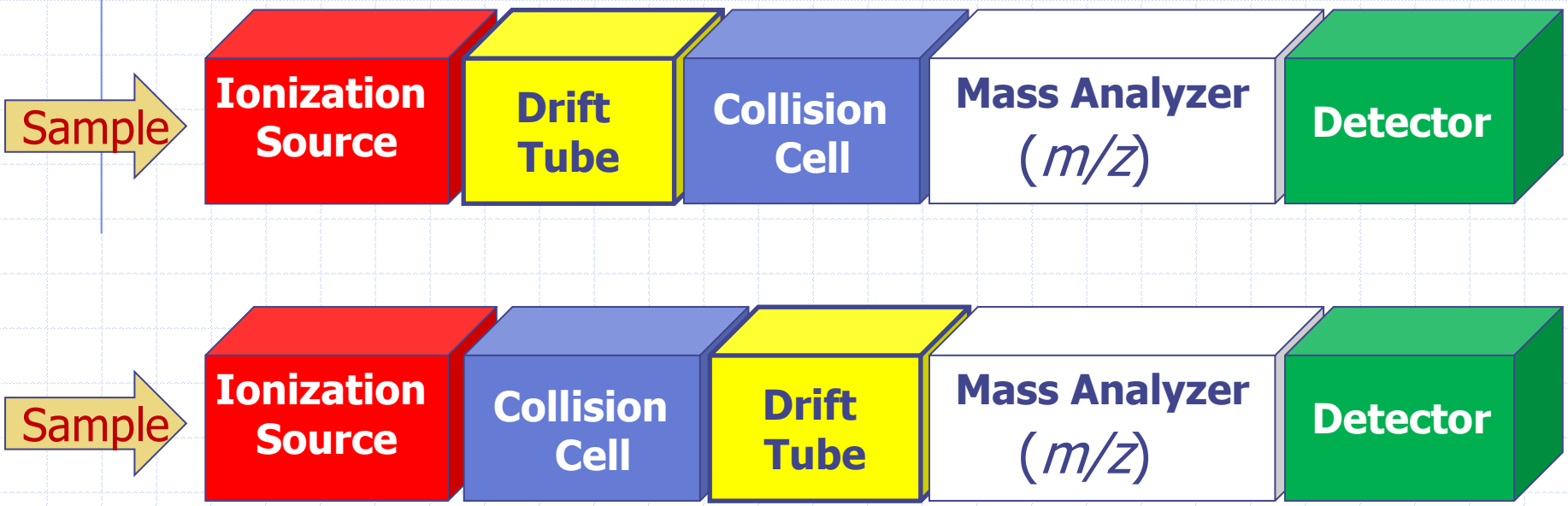


IMS: Gas phase diffusion

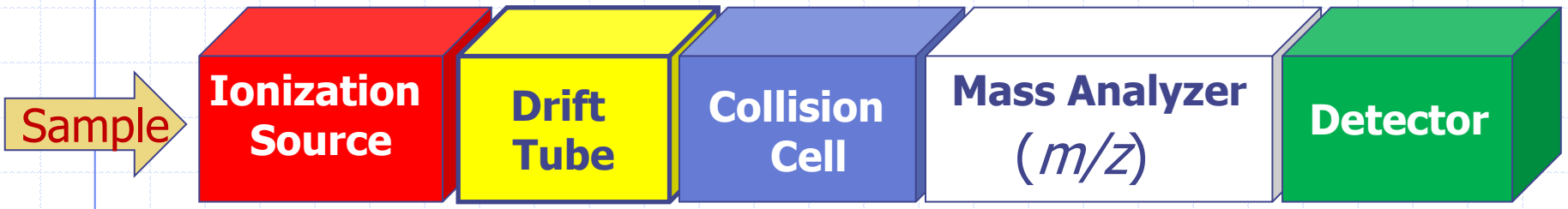
Ion Mobility: Instrumentation

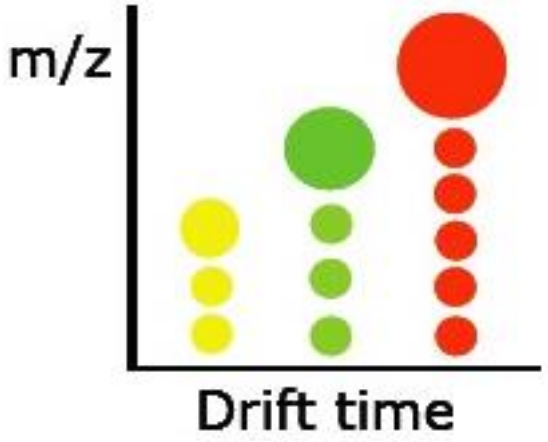


Ion Mobility: Instrumentation

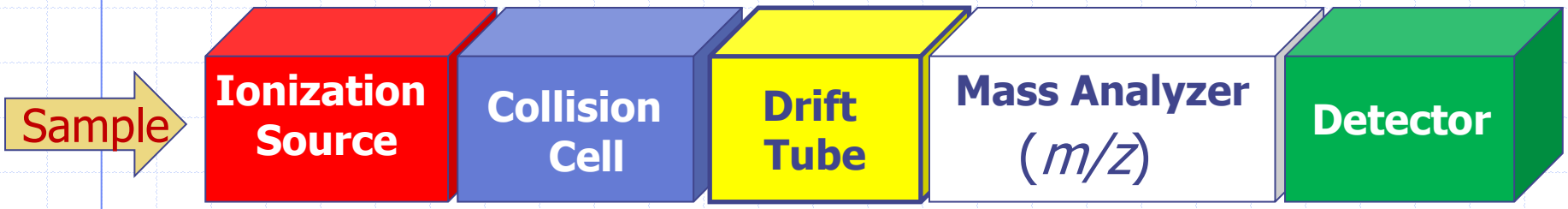


Ion Mobility: Instrumentation

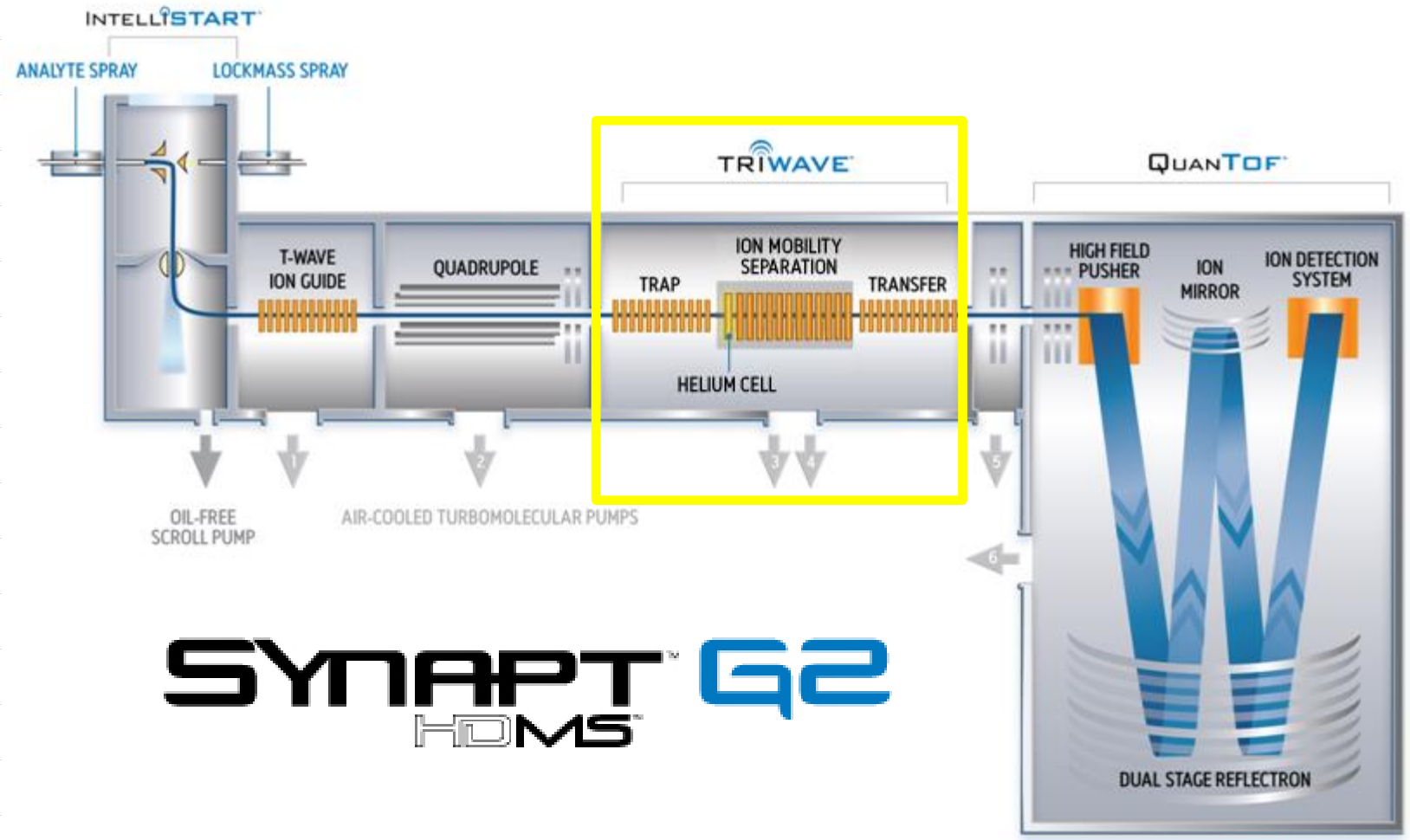




Ion Mobility: Instrumentation

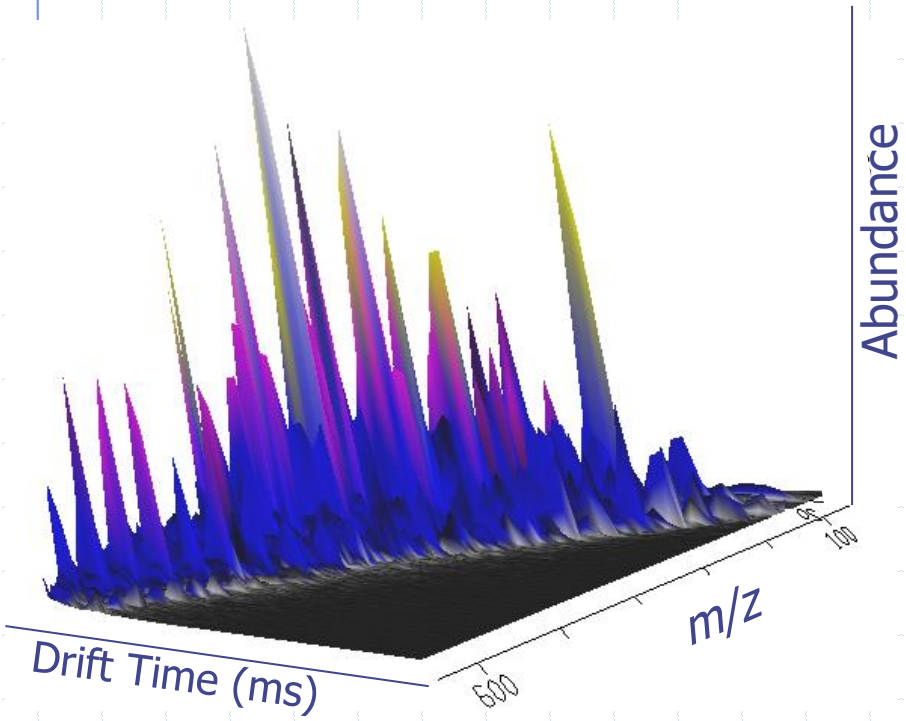


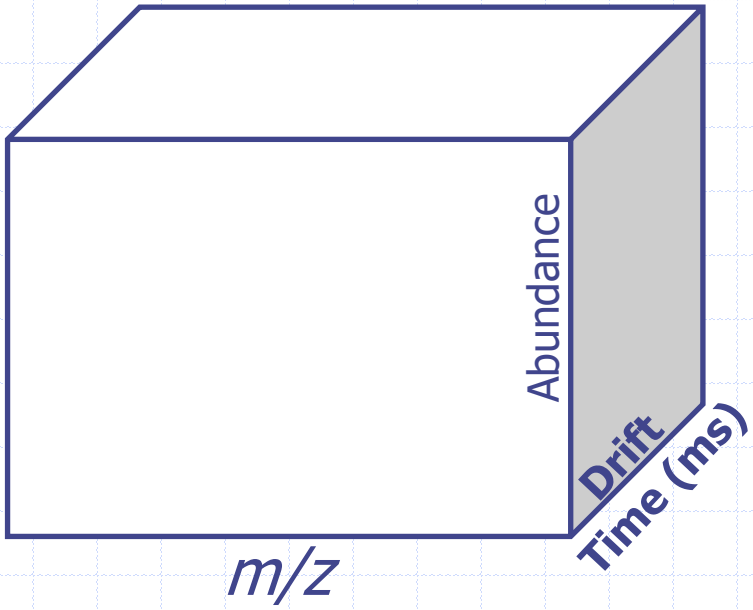
Ion Mobility: Instrumentation



SD Pringle, K Giles, JL Wildgoose, JP Williams, SE Slade, K Thalassinos, RH Bateman, MT Bowers, JH Scrivens *Int. J. Mass Spectrom.* 2007, 261, 1-12

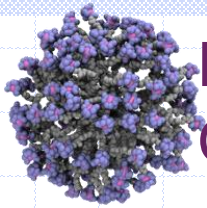
Ion Mobility – Mass Spectrometry





Triple correlation: *m/z*, drift time and abundance

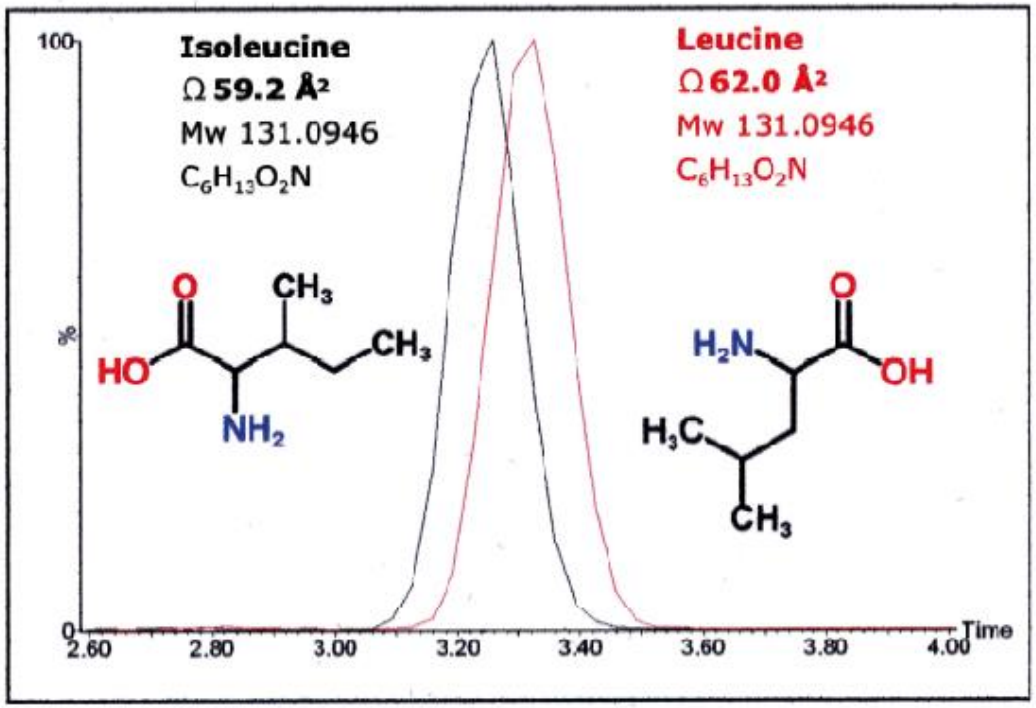
Collision Cross Section (CCS)



Ion Mobility – Mass Spectrometry

- Distinguish between different conformations of the same molecule
- Isomers separation
- Discrimination between isobaric species with different shape

Isomers Separation

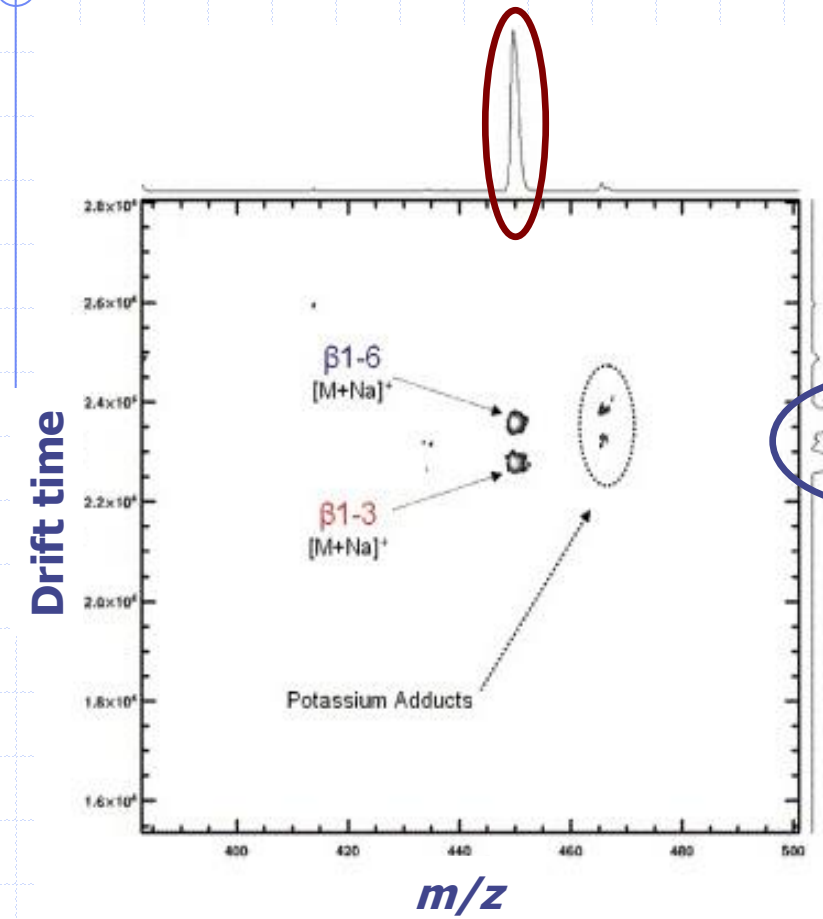


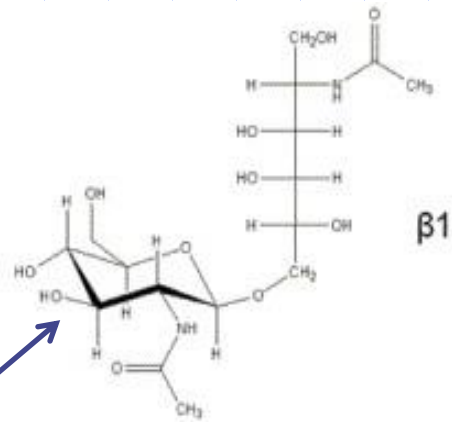
$\Delta\Omega < 3 Å^2$

Isobaric amino acids differentiation

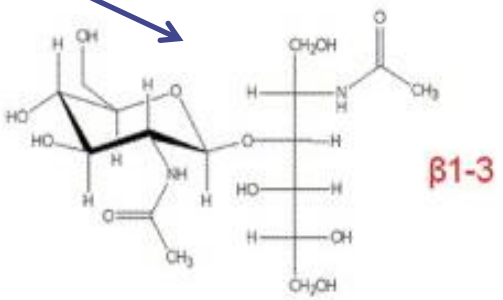
Knapman et al., Int J Mass Spectrom (2010) **298**, 17-23.

Isomers Separation





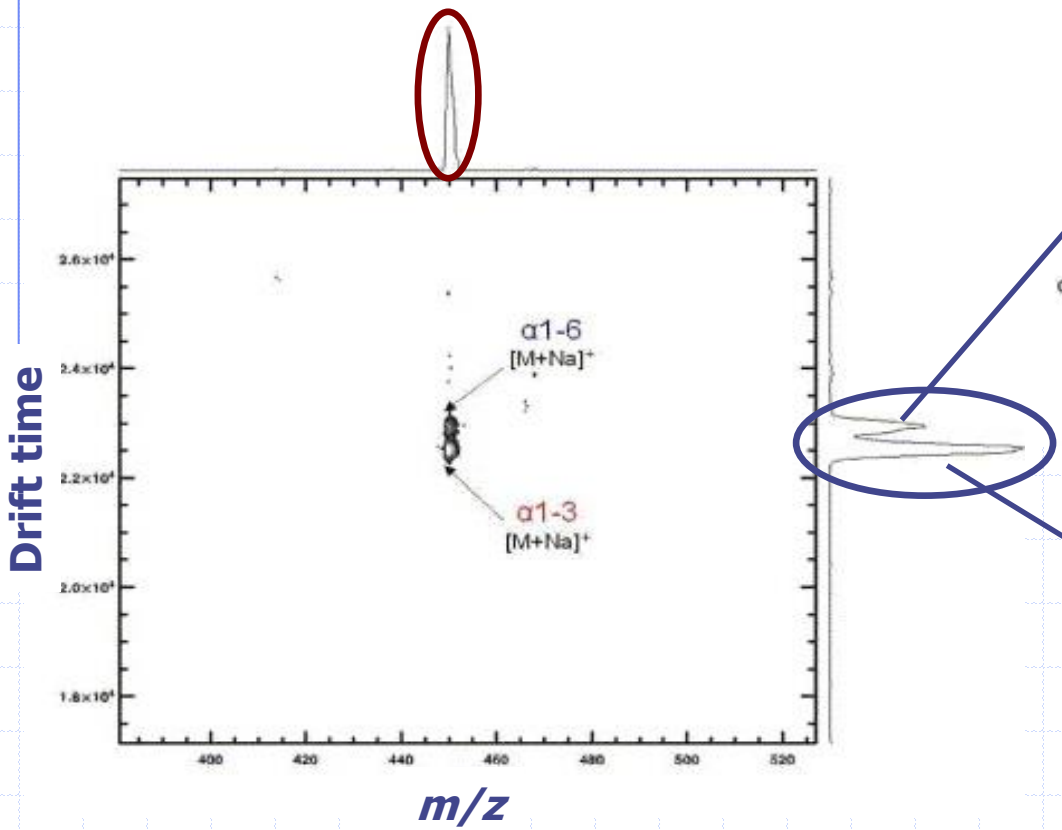
β-D-GlcNAc-(1-6)-D-GalNAc-ol
$C_{16}H_{30}N_2O_{11}$
Exact Mass: 426.18

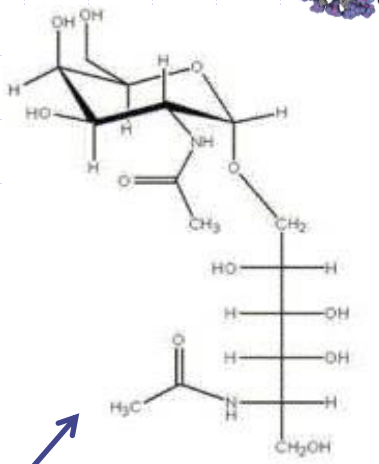


β-D-GlcNAc-(1-3)-D-GalNAc-ol
$C_{16}H_{30}N_2O_{11}$
Exact Mass: 426.18

Clowers BH et al., JASMS (2005) **16**, 660.

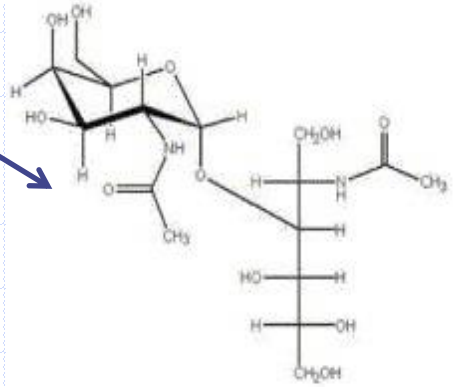
Isomers Separation





α-D-GalNAc-(1-6)-D-GalNAc-ol
$C_{16}H_{30}N_2O_{11}$
Exact Mass: 426.18

α1-6



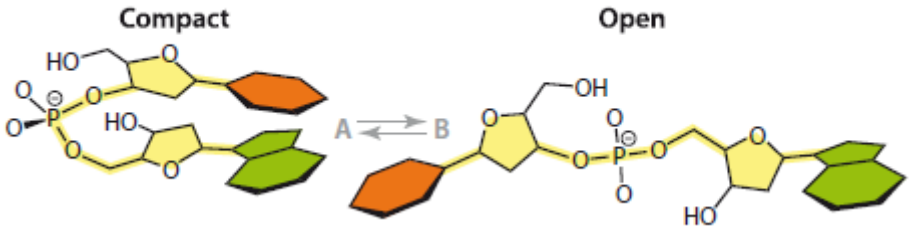
α-D-GalNAc-(1-3)-D-GalNAc-ol
$C_{16}H_{30}N_2O_{11}$
Exact Mass: 426.18

α1-3

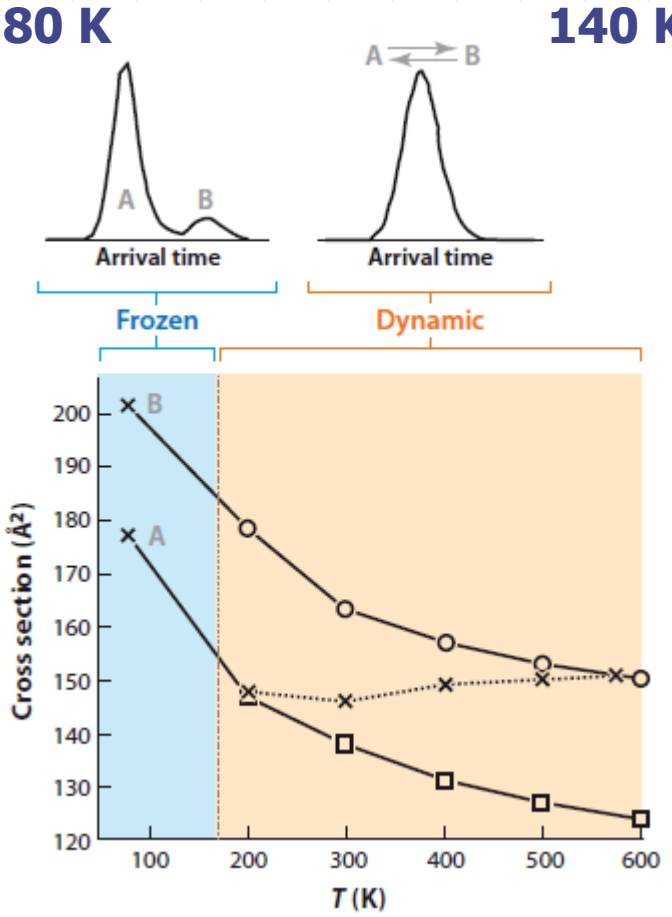
Clowers BH et al., JASMS (2005), **16**, 660.

IMS Applications

Dinucleotides



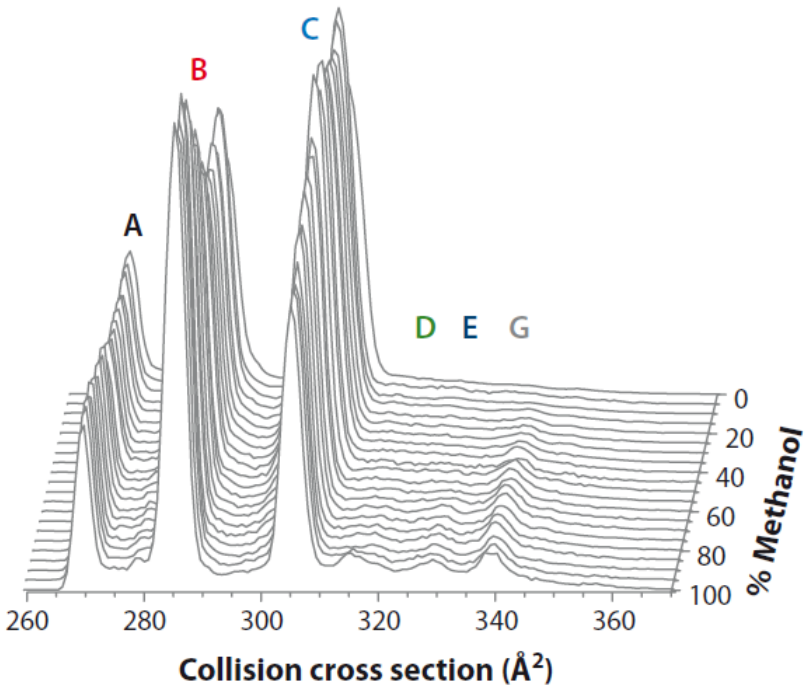
dTA



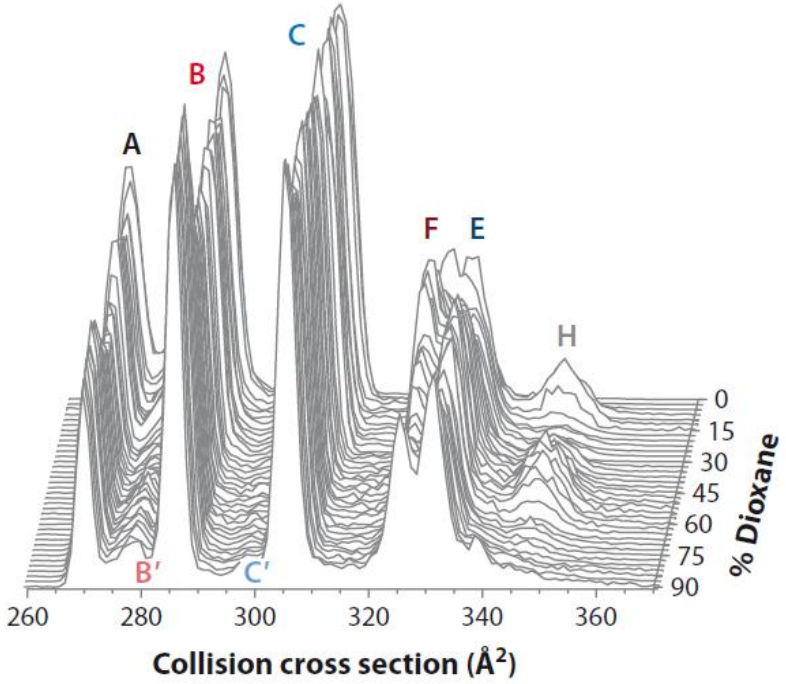
T Wyttenbach, NA Pierson, DE Clemmer, MT Bowers *Annl. Rev. Phys. Chem.* 2014, 65, 175-196

IMS Applications

Peptides: Bradikinin



Water: Methanol



Water: Dioxane

T Wyttenbach, NA Pierson, DE Clemmer, MT Bowers *Annl. Rev. Phys. Chem.* 2014, 65, 175-196

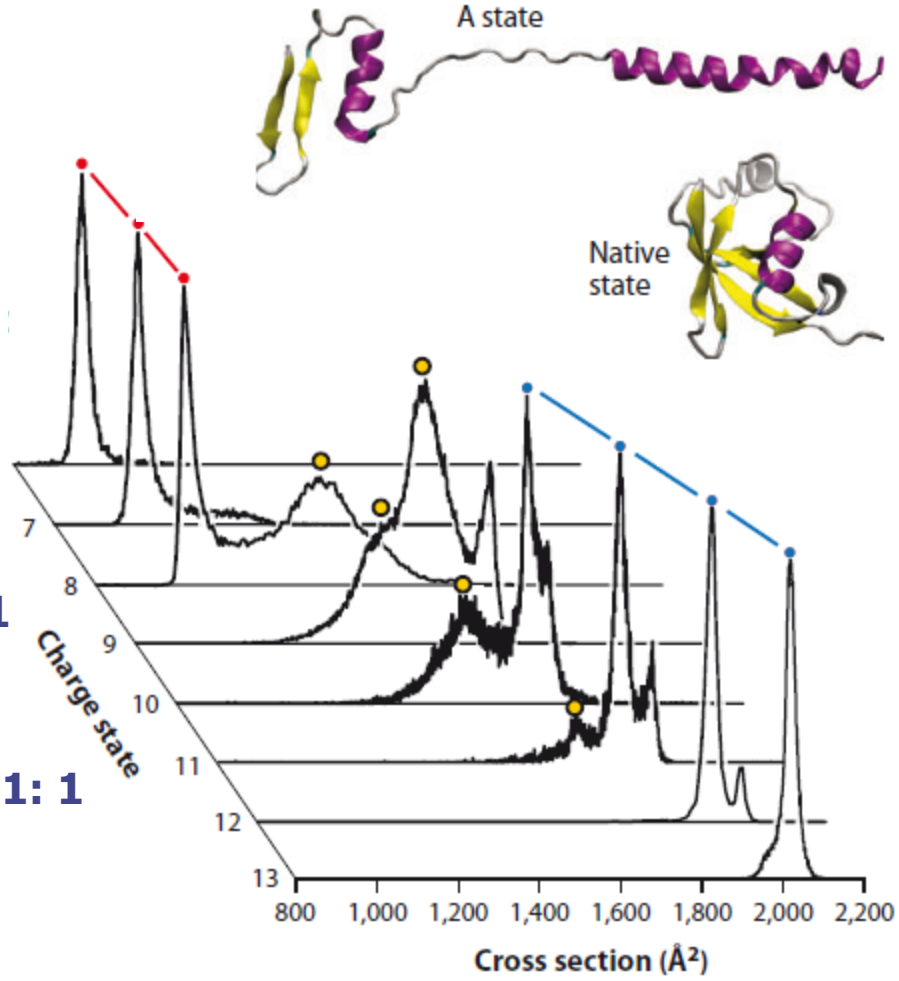
IMS Applications

Proteins: Ubiquitin

Water

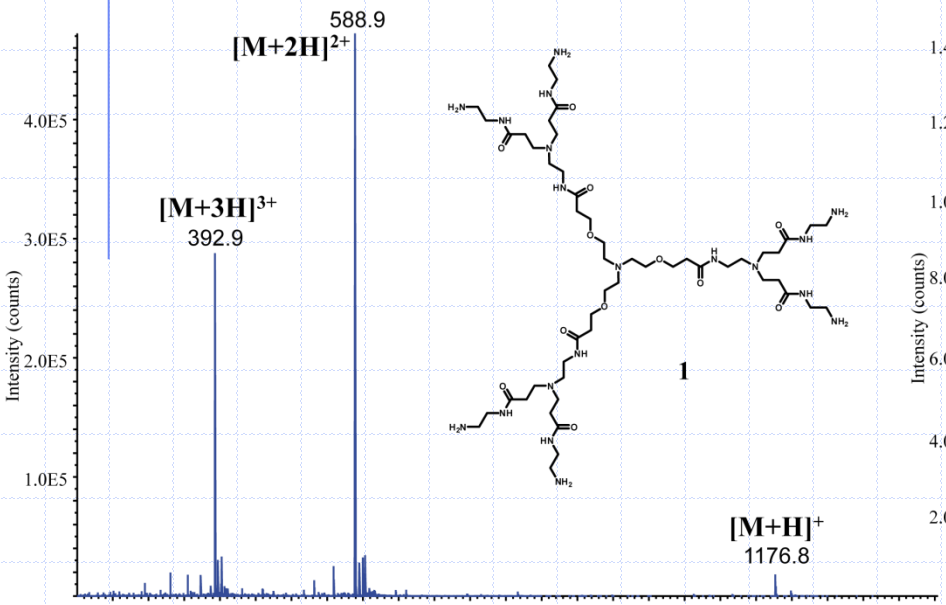
Water: Methanol 1: 1

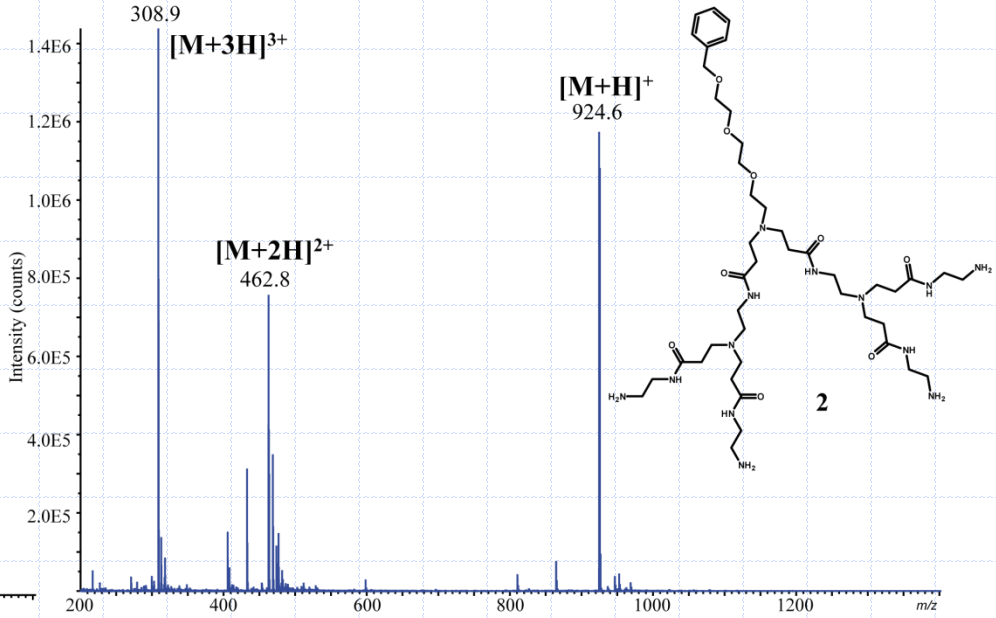
1% acetic acid in Water: Methanol 1: 1



T Wyttenbach, NA Pierson, DE Clemmer, MT Bowers *Annl. Rev. Phys. Chem.* 2014, 65, 175-196

IMS – MS Study of Dendrimers

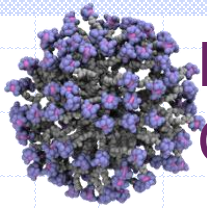




PAMAM
Globular Shape

PAMAM
Fan Shape

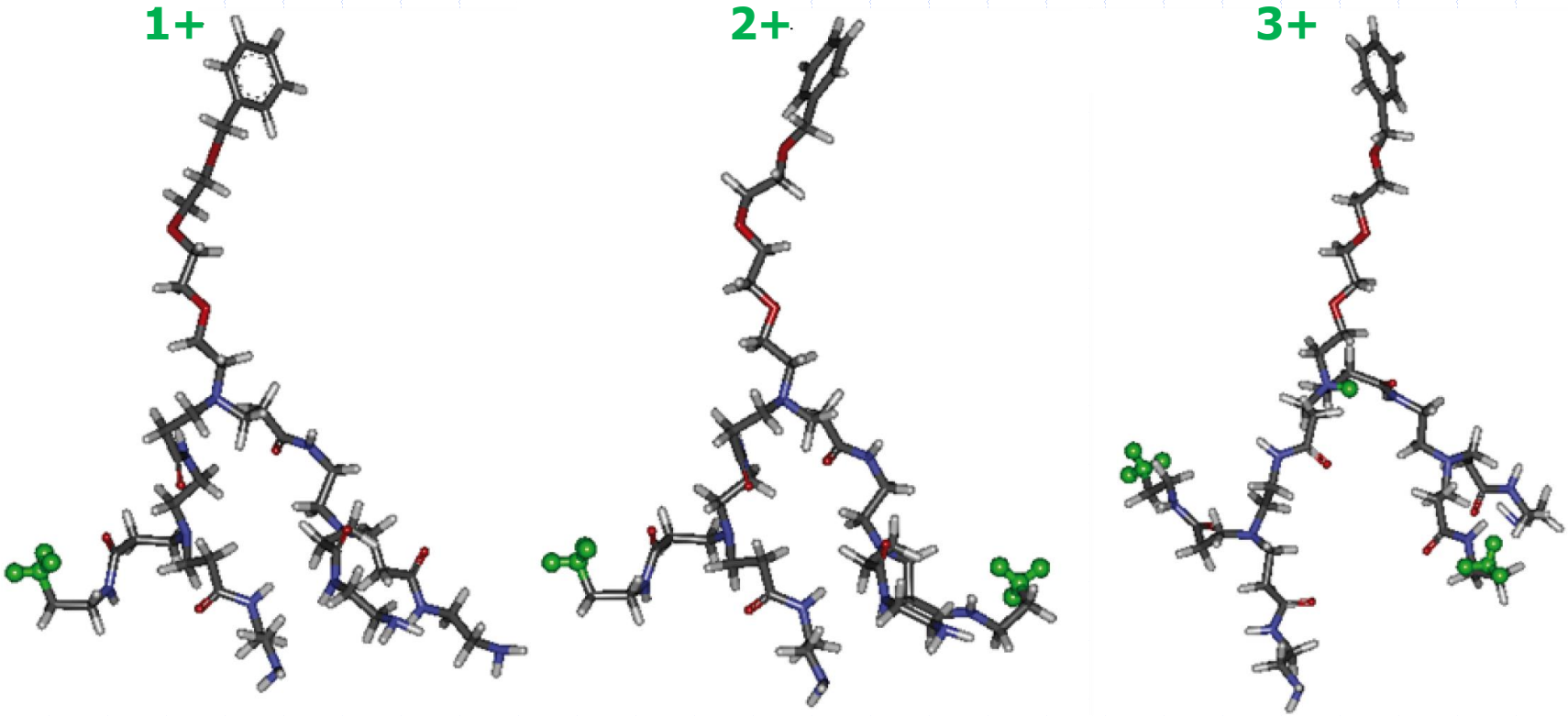
Tintaru et al. *International Journal of Mass Spectrometry* (2013) **354,** 235.



Nano2Clinic
CA17140

IMS – MS Study of Dendrimers

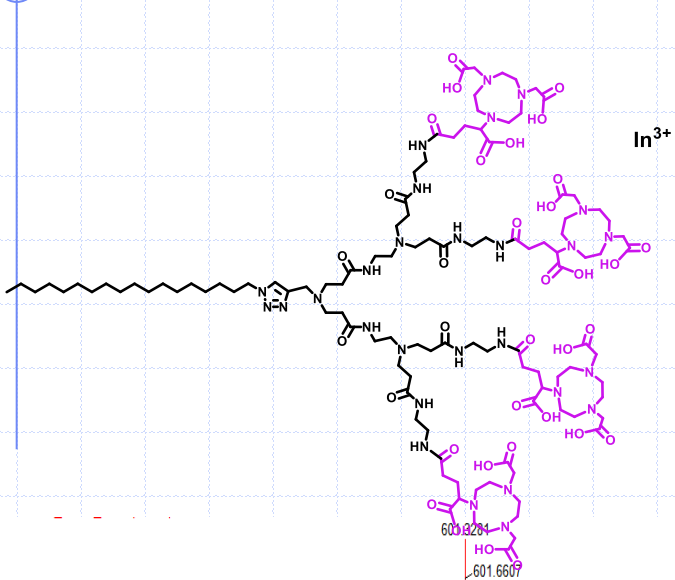
PAMAM Fan Shape

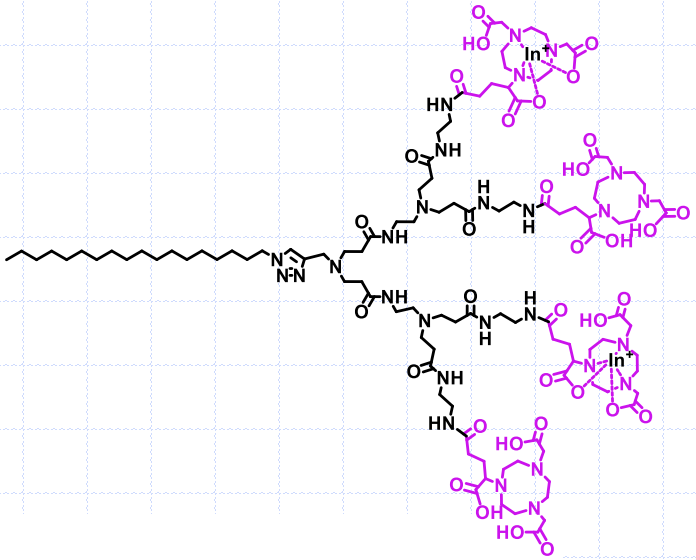


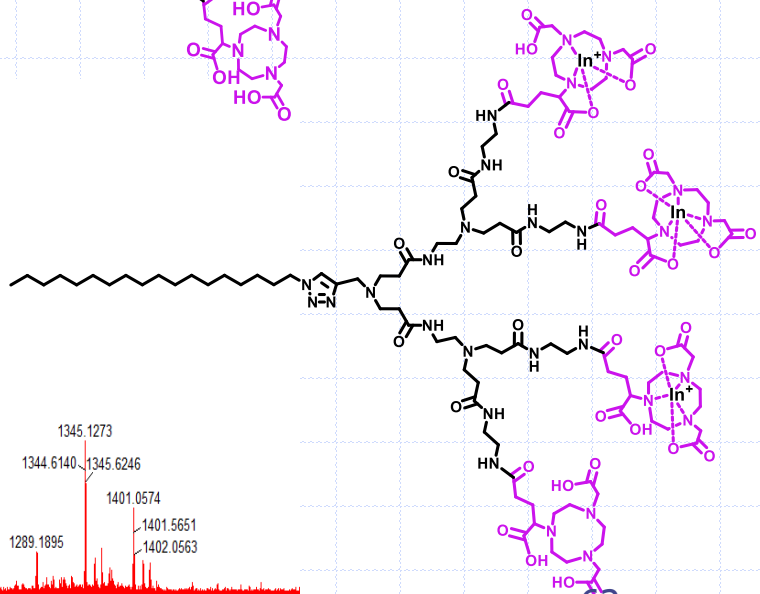
	1+	2+	3+
Theoretical	**221 Å²**	**204 Å²**	**241 Å²**
Experimental	**219 Å²**	**208 Å²**	**229 Å²**

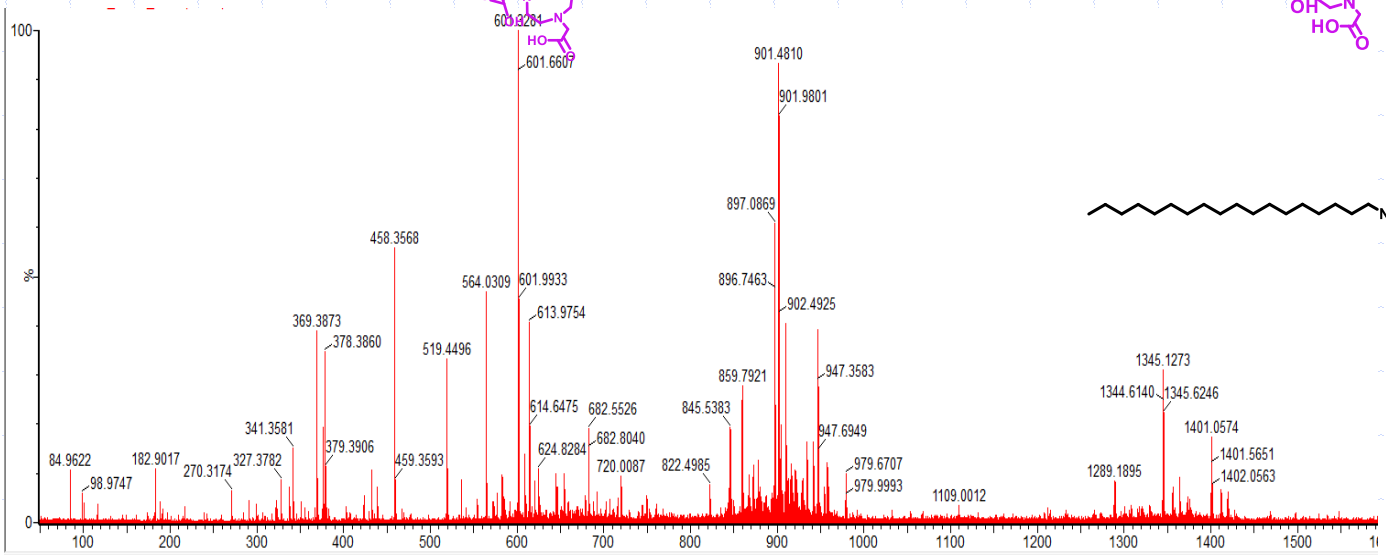
Tintaru et al. *International Journal of Mass Spectrometry* (2013) **354,** 235.

In^{III}-NOTA Amphiphilic Dendrimers



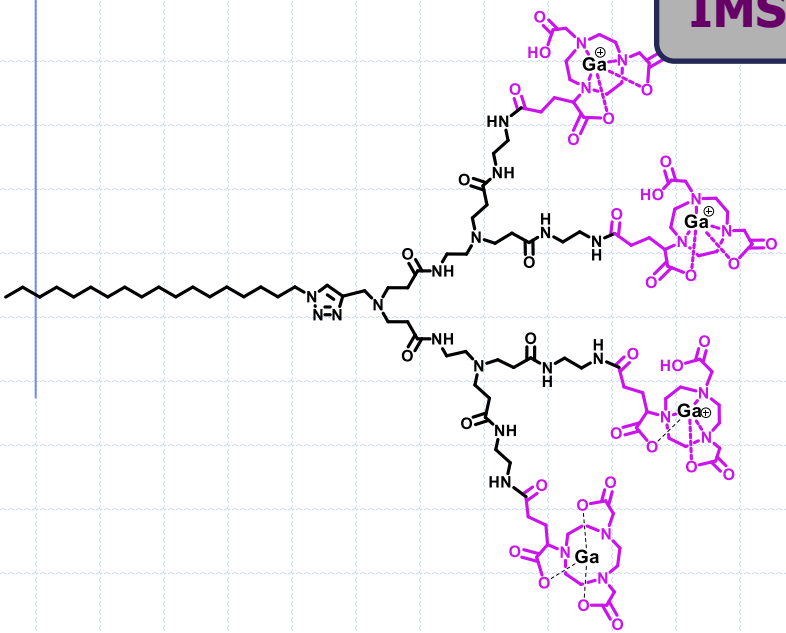


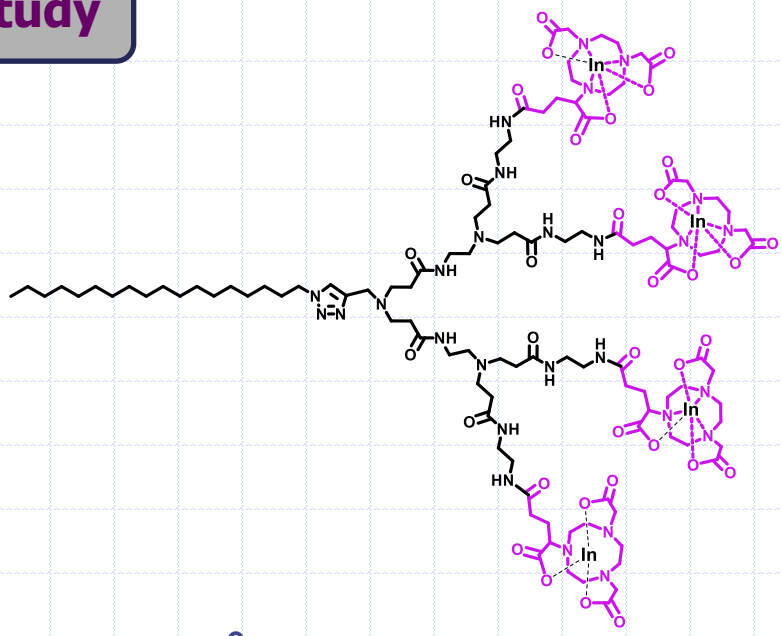




In^{III}-NOTA Amphiphilic Dendrimers

IMS-MS Study



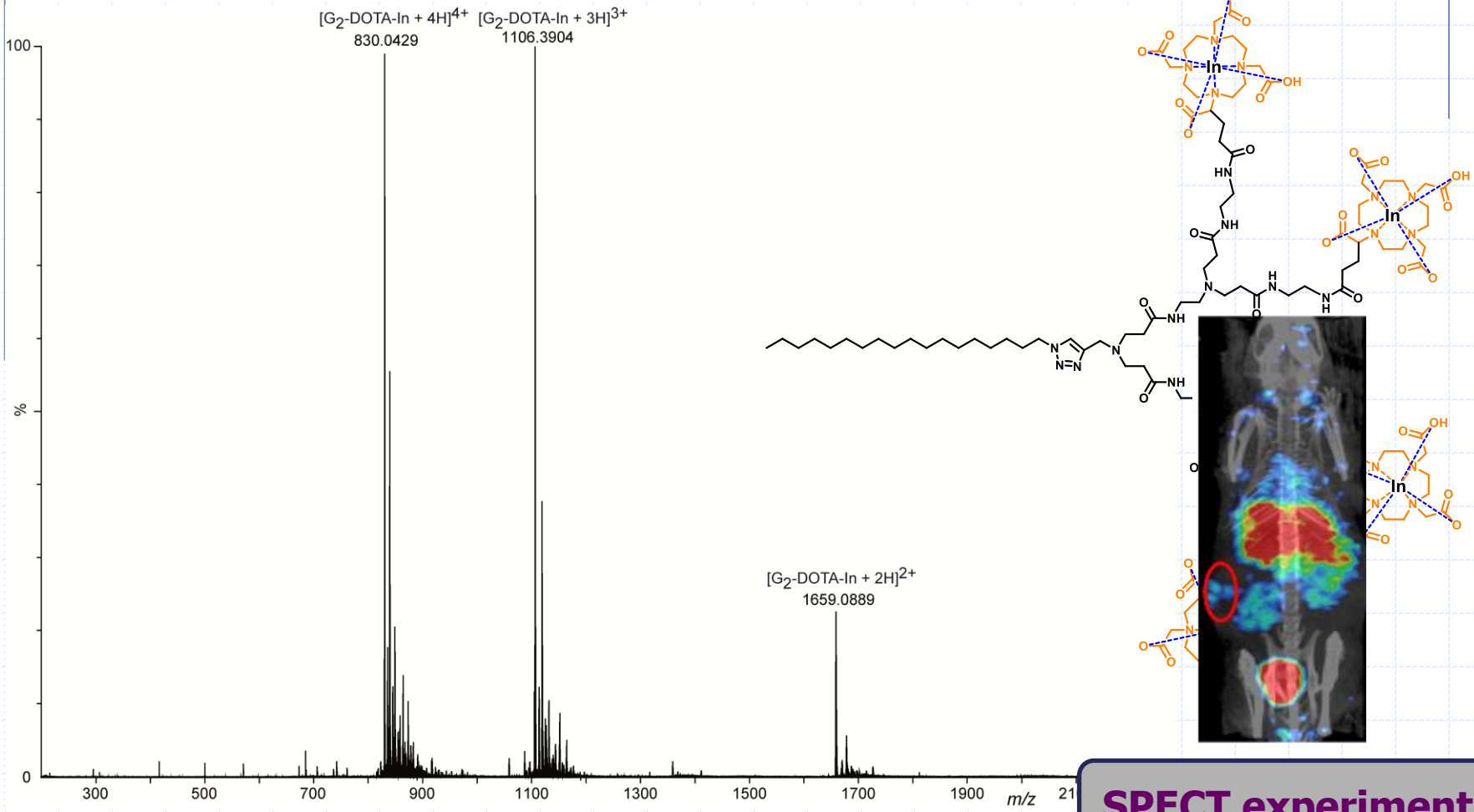


IM-MS → CCS calculation (Å²)

	NOTA	Dendrimer-NOTA
free	149.3	581.2
Ga (III)	154.5	580.7
In (III)	**158.1**	**570.9**

NOTA "cage" folded

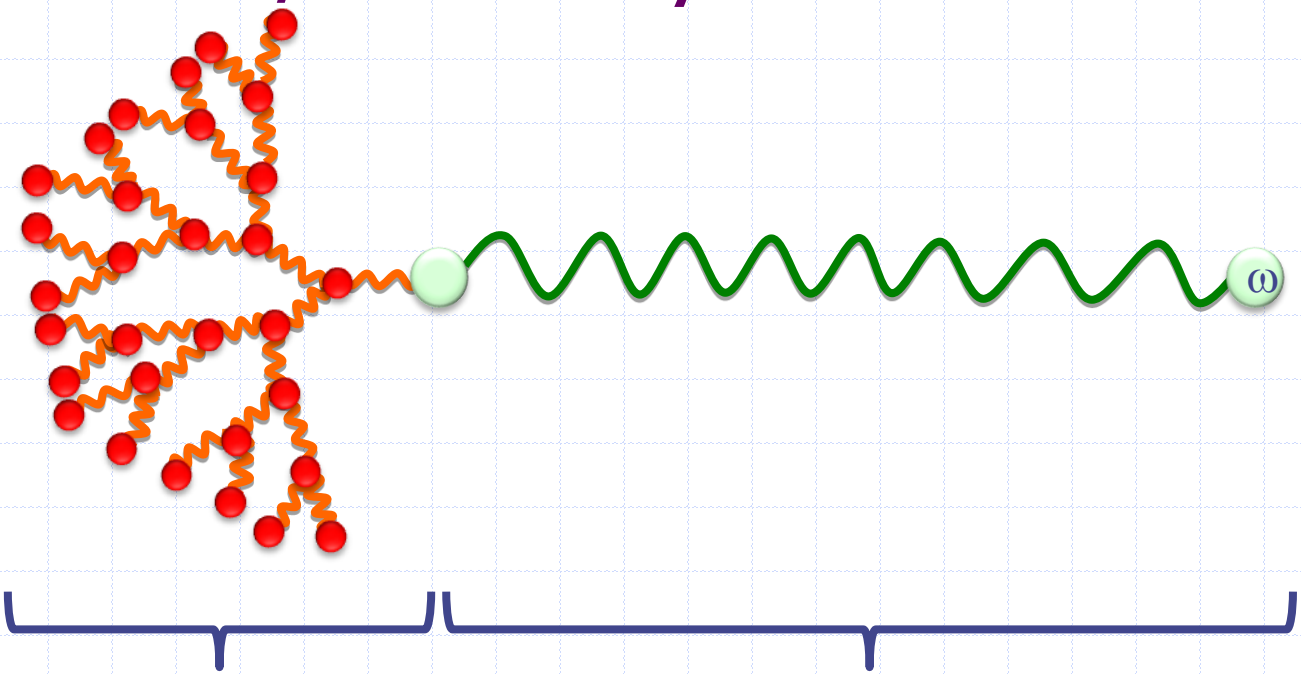
In^{III}-DOTA Amphiphilic Dendrimers



SPECT experiment

Ling D et al. ChemComm 2019, in press

IMS – MS/MS Study of Dendrimers



Hybrids : Dendrimer (PAMAM) + Polymer (PEO)

- Micelles-like compounds → highly medium dependent
- High flexibility
- Larger intrinsic cavities

} novel drug delivery systems

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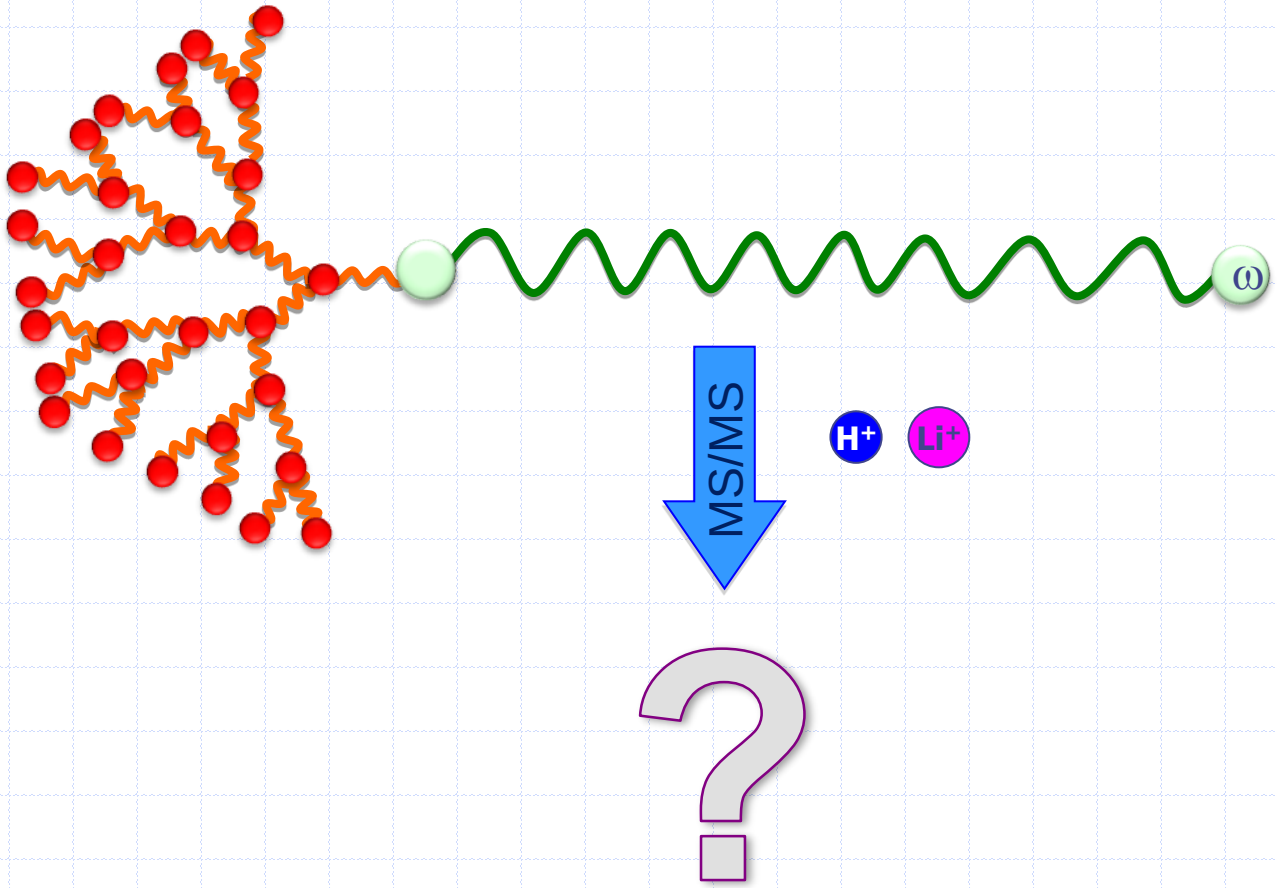
IMS – MS/MS Study of Dendrimers



Dendrimer or polymer MS/MS behavior ?

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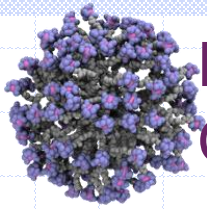

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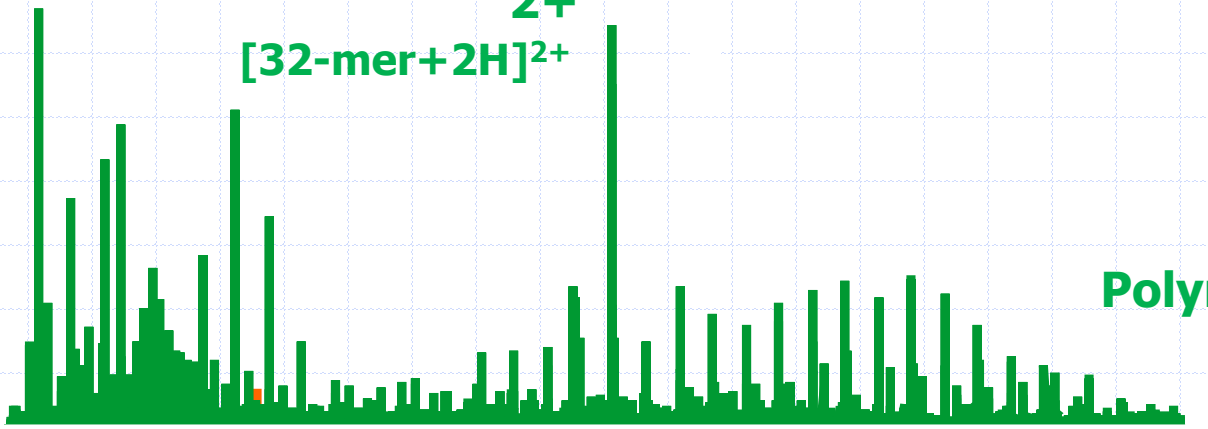
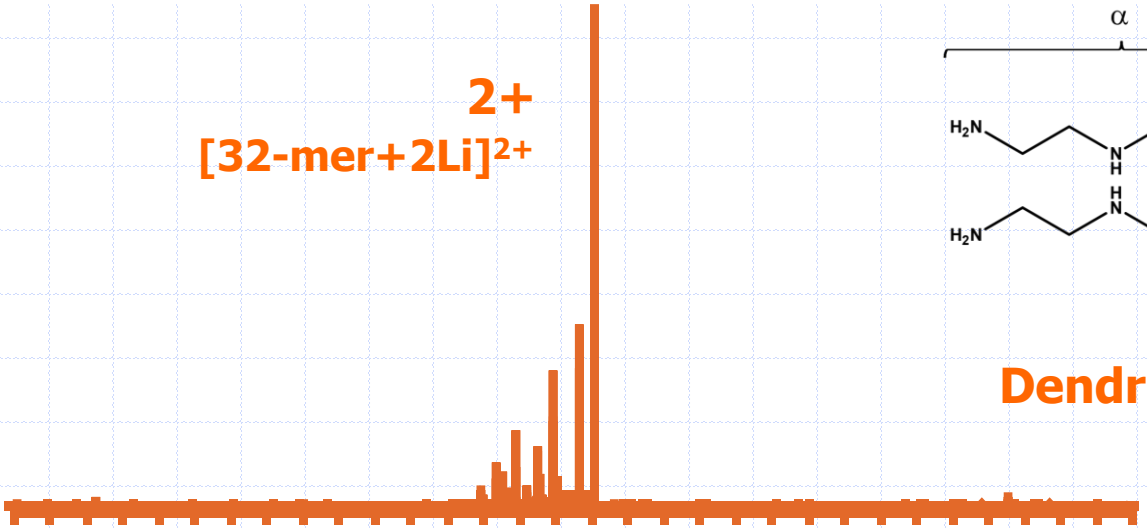
IMS – MS/MS Study of Dendrimers

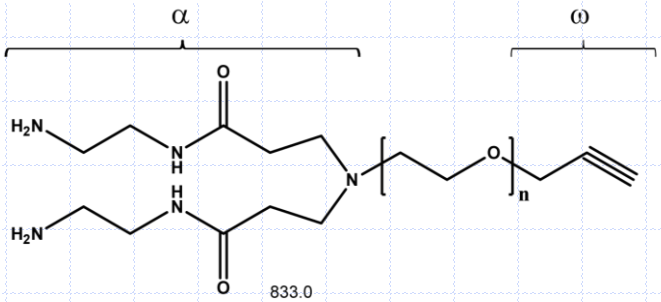
2+
$[32\text{-mer}+2H]^{2+}$



Polymer Fragmentation

2+
$[32\text{-mer}+2Li]^{2+}$





Dendrimer Fragmentation

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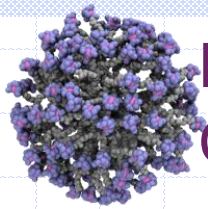

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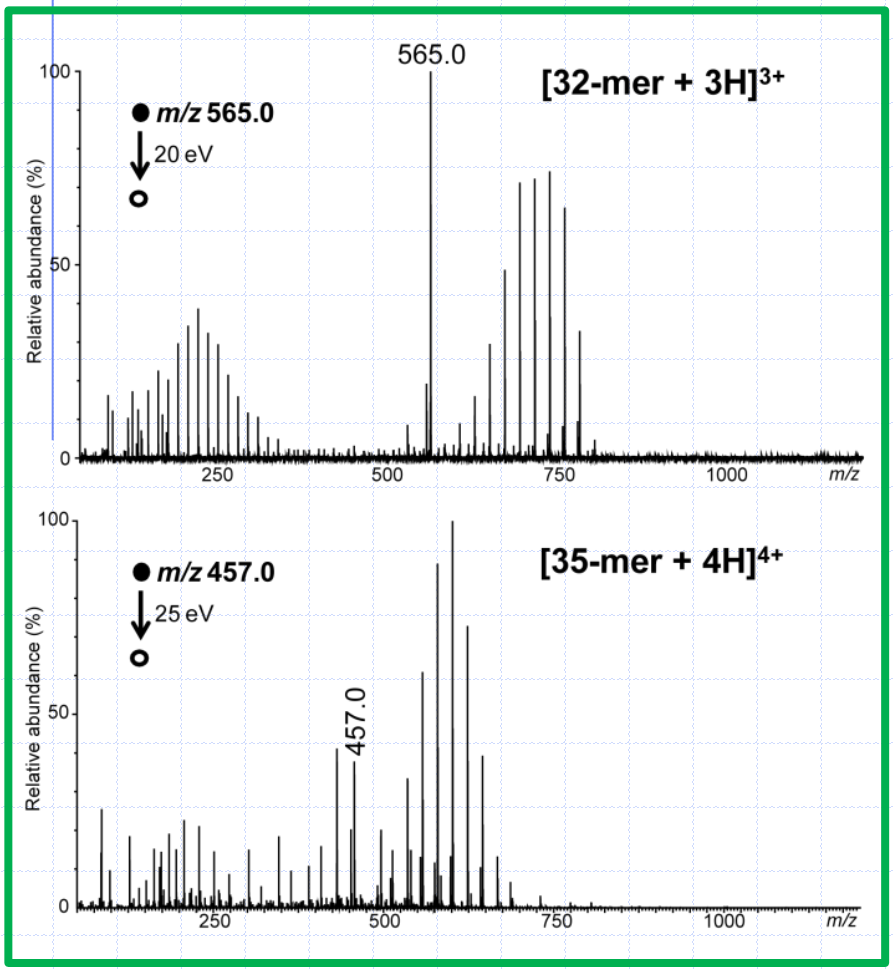
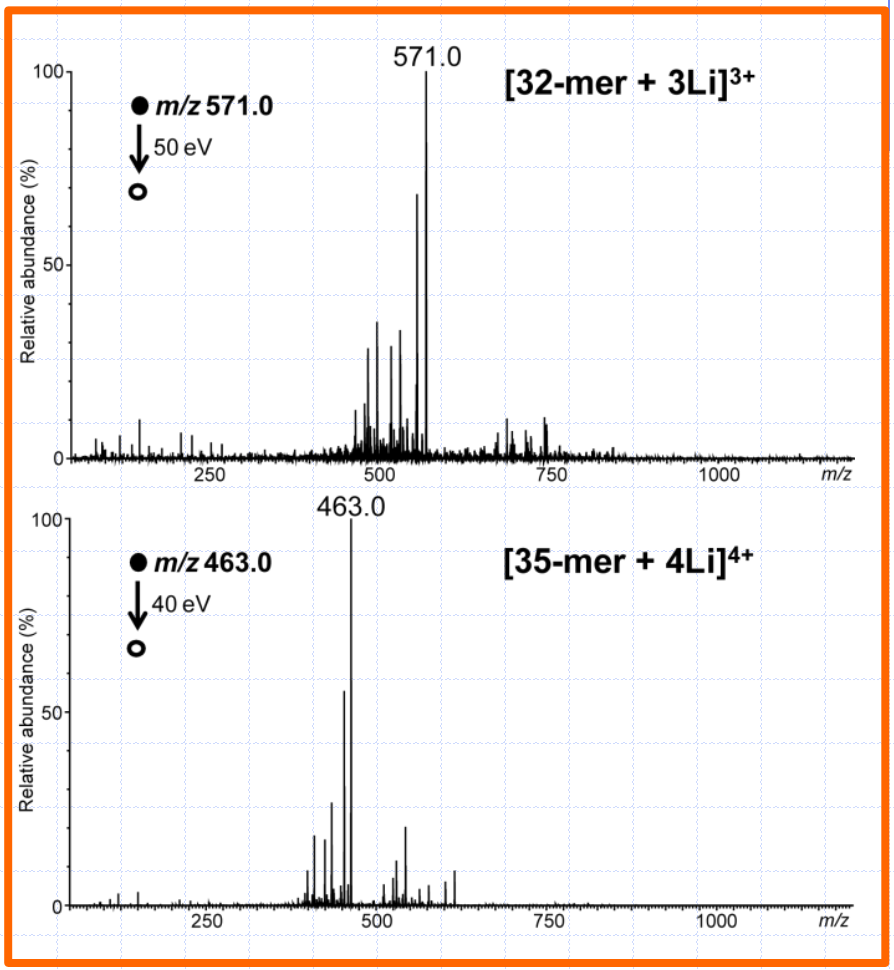
IMS – MS/MS Study of Dendrimers



Polymer Fragmentation



Dendrimer Fragmentation

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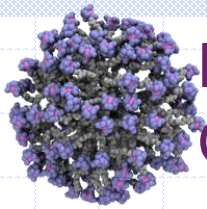

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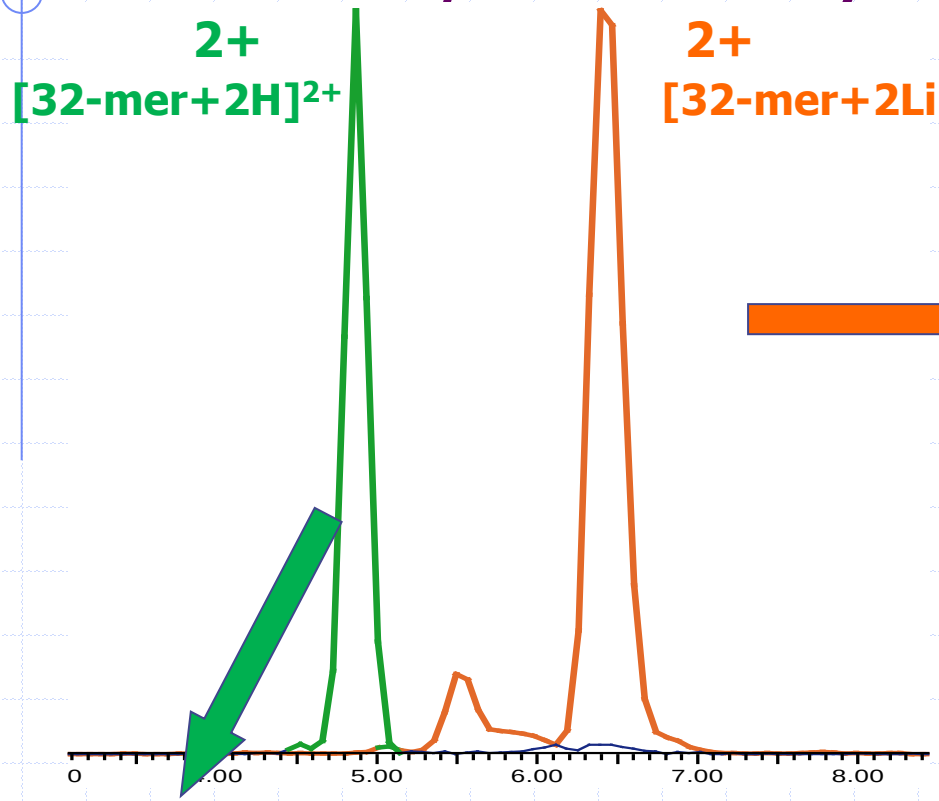
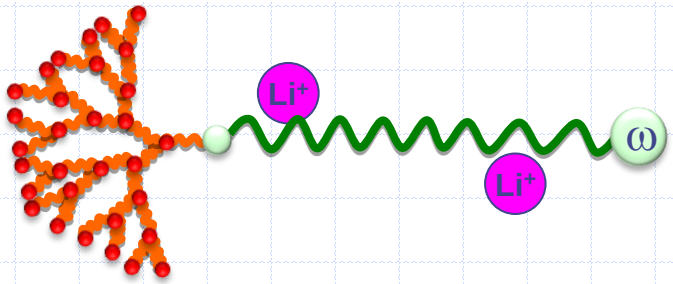
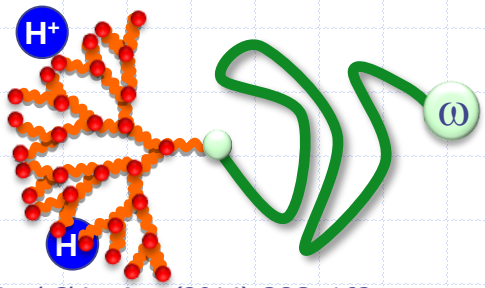
IMS – MS/MS Study of Dendrimers







Experimental

CCS : 441 Å²

CCS : 498 Å²

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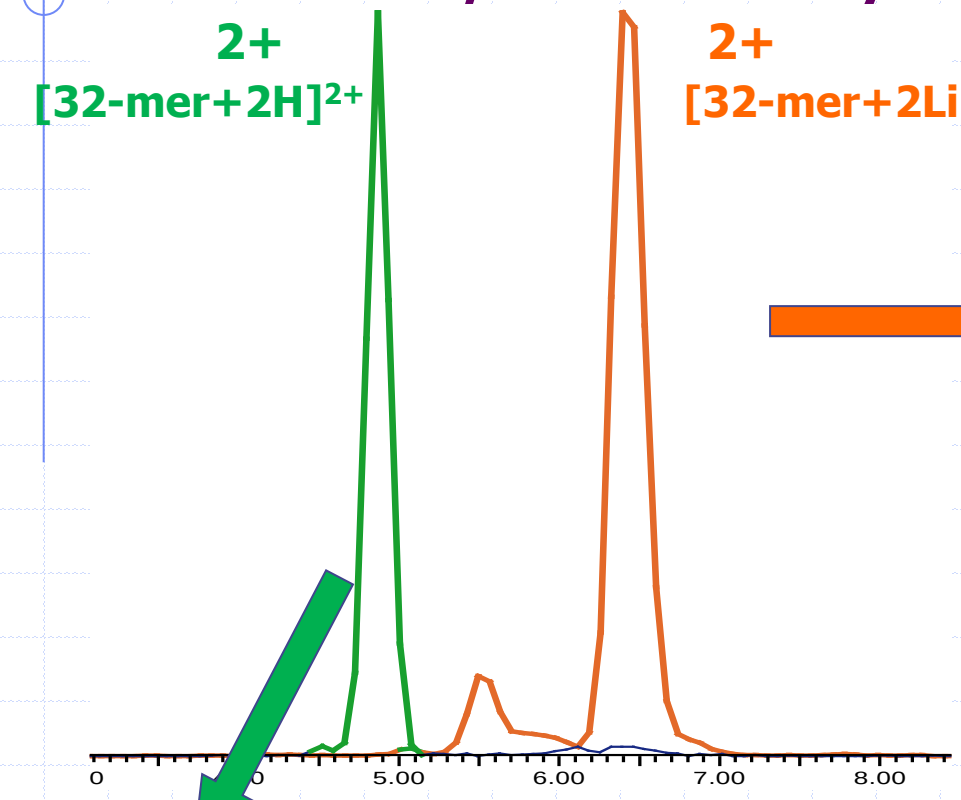
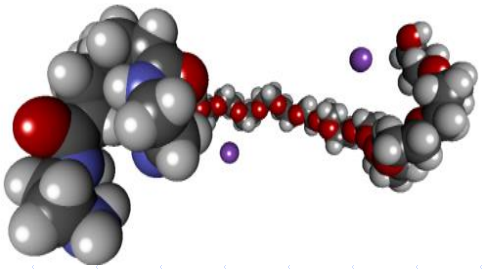
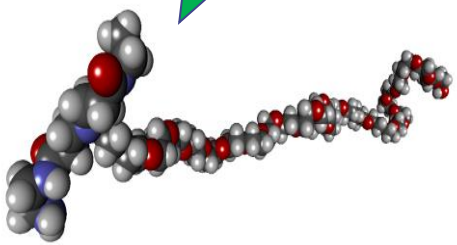
IMS – MS/MS Study of Dendrimers







Theoretical / Experimental

CCS : 445 Å² / 441 Å²

CCS : 515 Å² / 498 Å²

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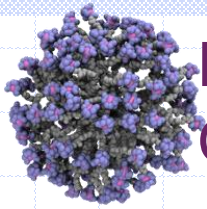
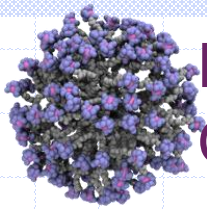


Conclusions

Mass Spectrometry powerful technique:

- **Accurate Mass Measurements**
- **Structural Characterization**
- **Isomers Separation**
- **Differentiation Based on Ions Shape**



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