



EU FT-ICR MS

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Center for Molecular Biosciences Innsbruck

**Michael Palasser, Maria A. van Agthoven,
Kathrin Breuker**

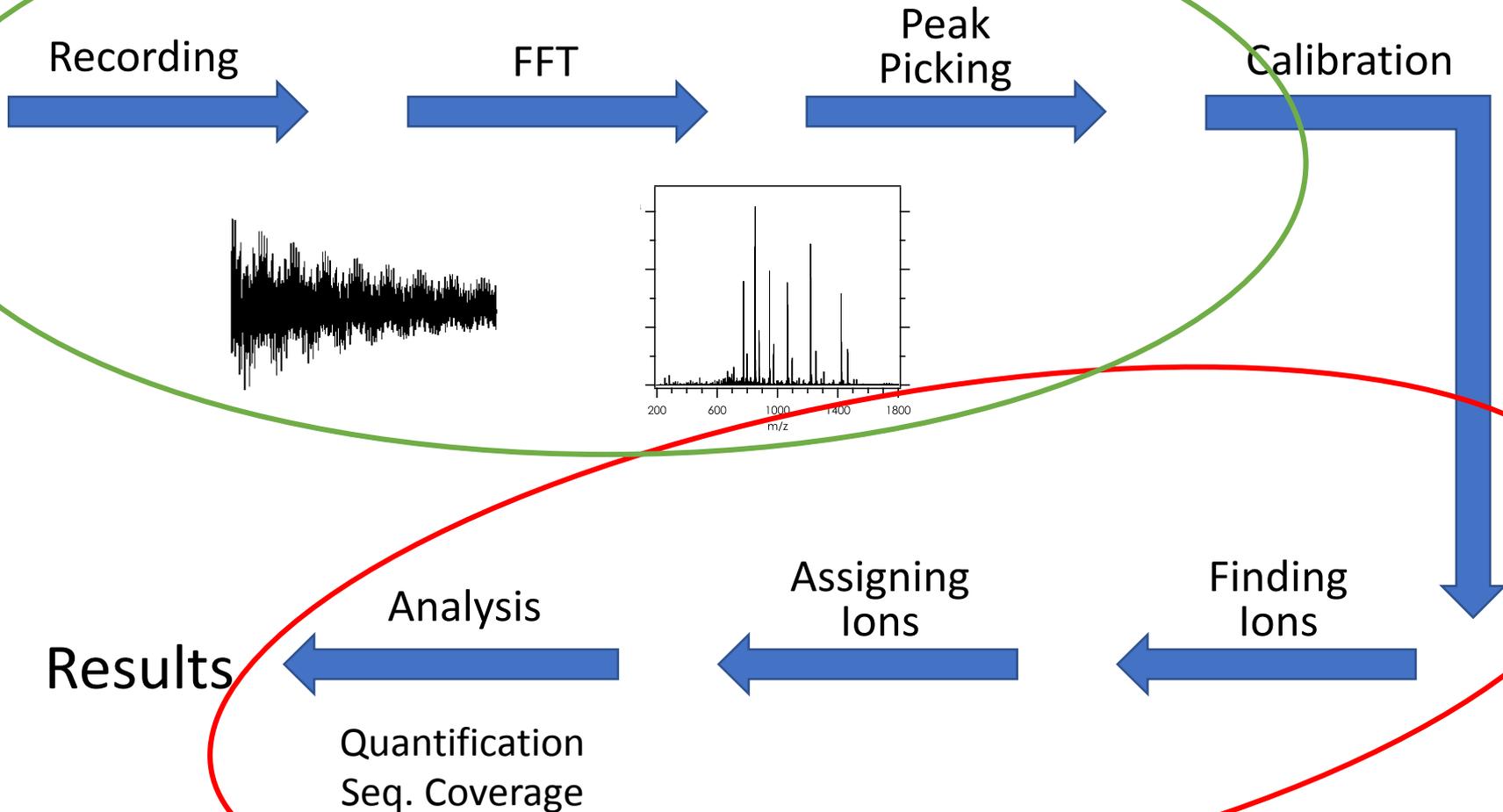
Institute of Organic Chemistry and
Center for Molecular Biosciences Innsbruck (CMBI)
University of Innsbruck, Austria

FAST MS:



An open-source software for automated and
quantitative analysis of top-down mass spectra

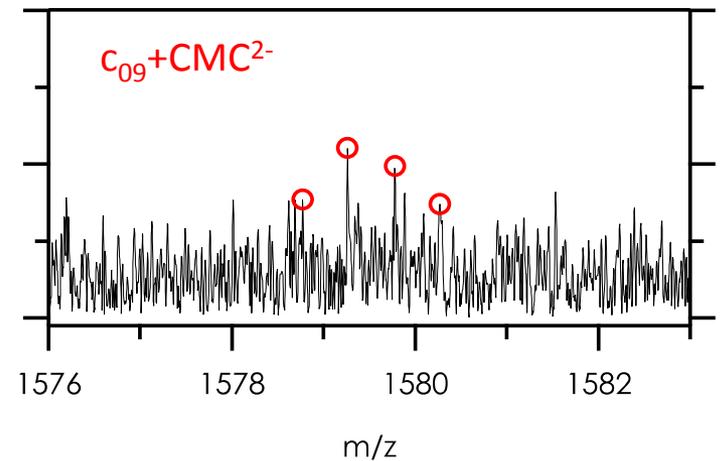
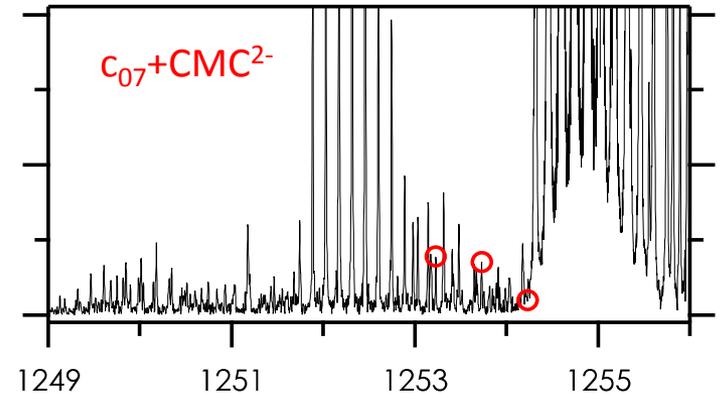
Data Processing and Analysis in FTMS



Data Analysis ?

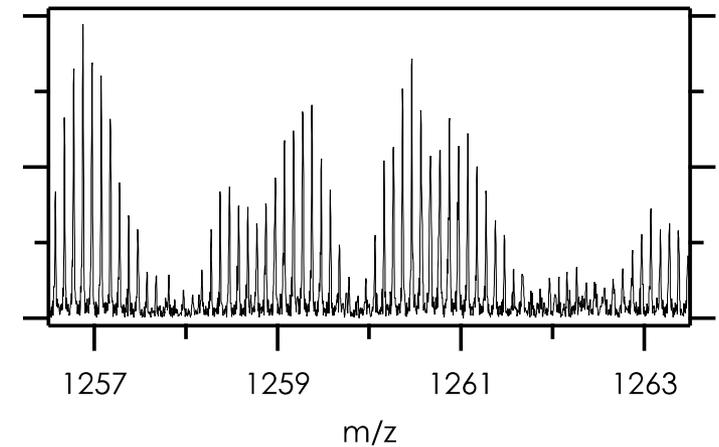
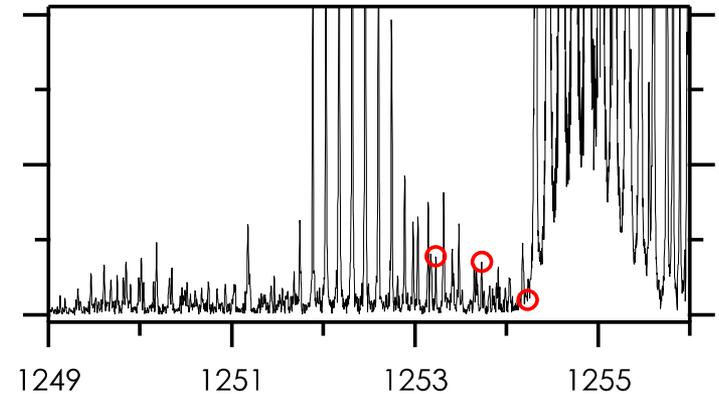
SNAP?

- Great for de-novo sequencing
- Misses many ions → Problem for:
 - Characterisation of PTMs, ligand binding sites, ...
 - Spectra with low S/N
 - Larger molecules



Data Analysis ?

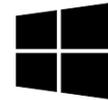
- SNAP?
- Other software tools?
 - just for proteins
- Finding ions manually?
 - Quantification
 - Reproducibility?
 - Very tedious



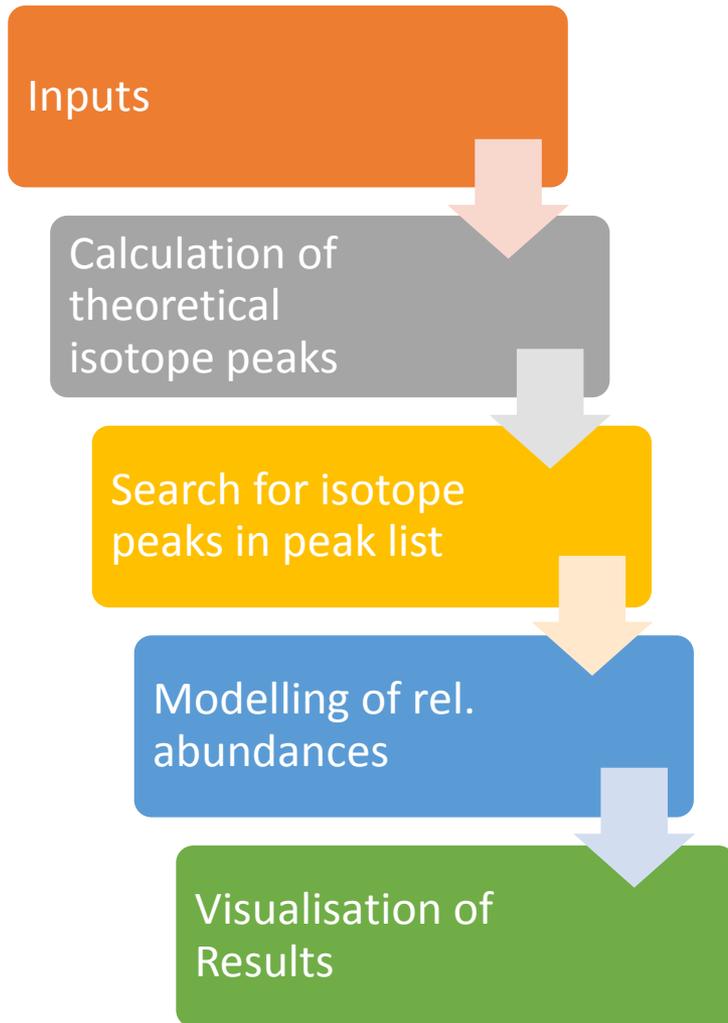


Free **A**nalysis **S**oftware for **T**op-down **M**ass **S**pectrometry

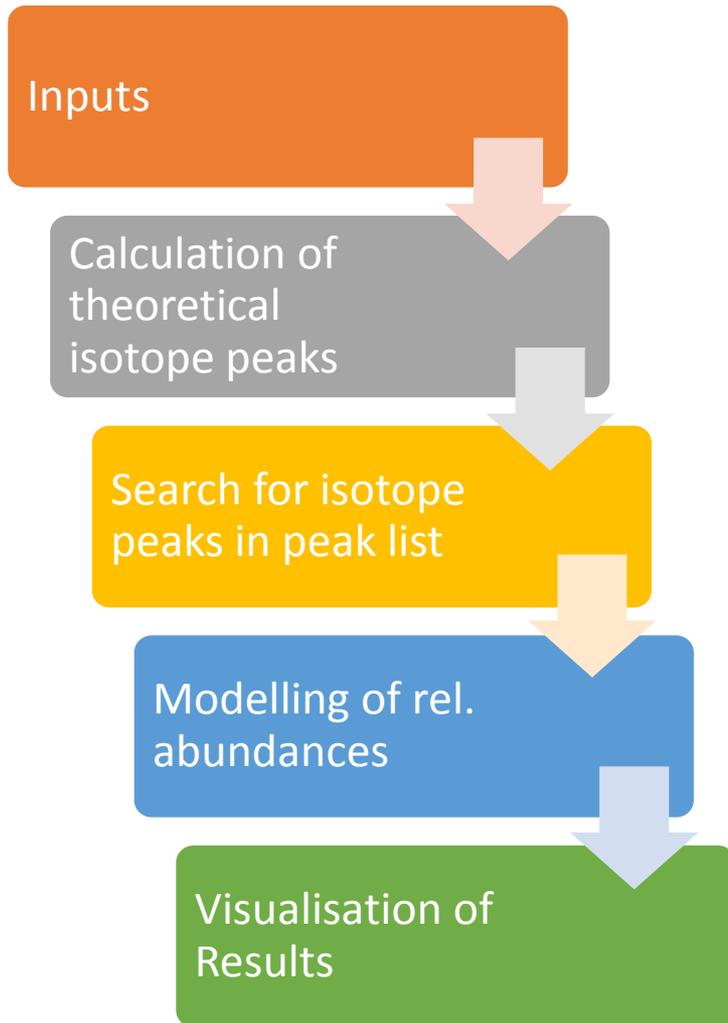
- Written in Python (open-source)
- Cross platform
- Full GUI support
- For analysis of top-down and mass spectra of intact ions (known sequence)
- RNA, DNA, proteins, ...



FAST MS: Top-Down MS

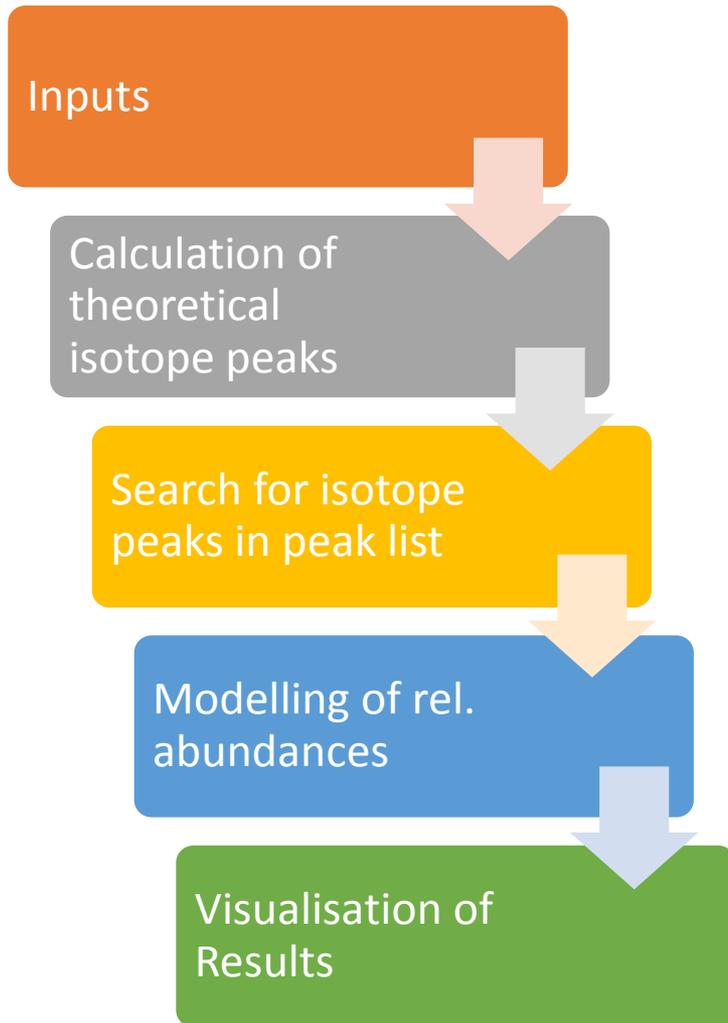


FAST MS: Top-Down MS

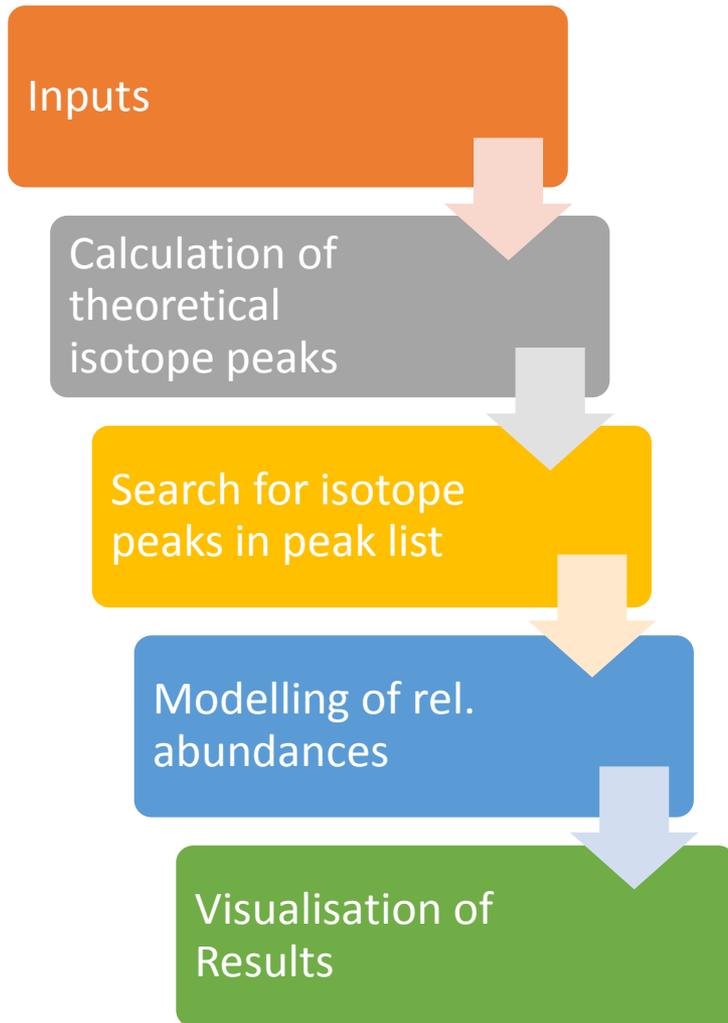


- Peak list
- Sequence
- Charge of precursor
- Modifications, ligands, ...

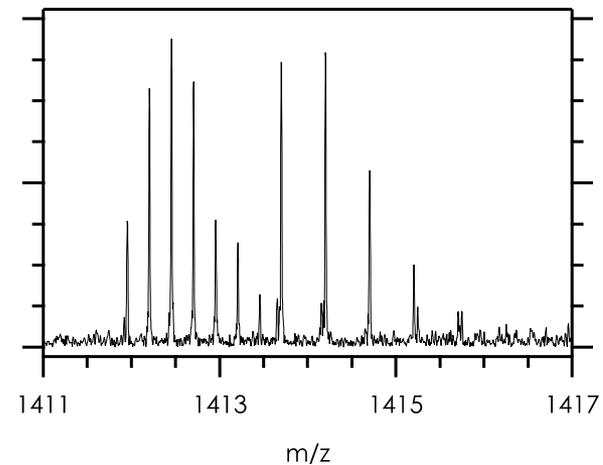
FAST MS: Top-Down MS



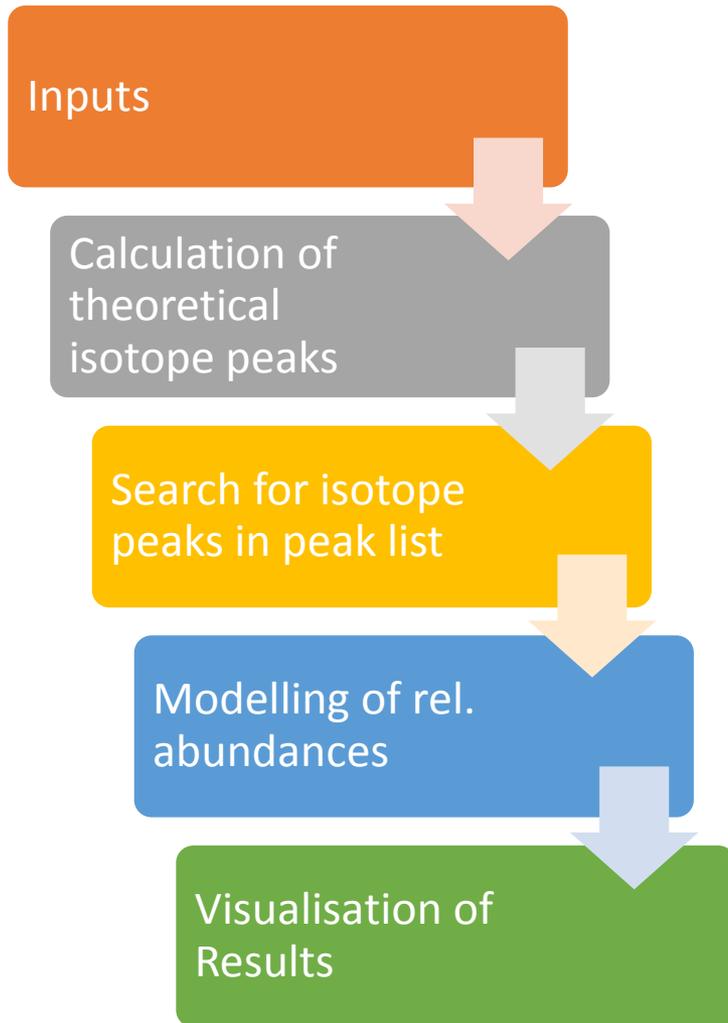
FAST MS: Top-Down MS



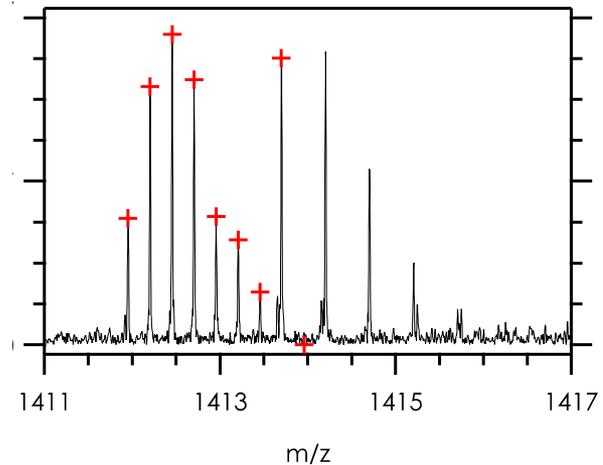
Charge state estimation



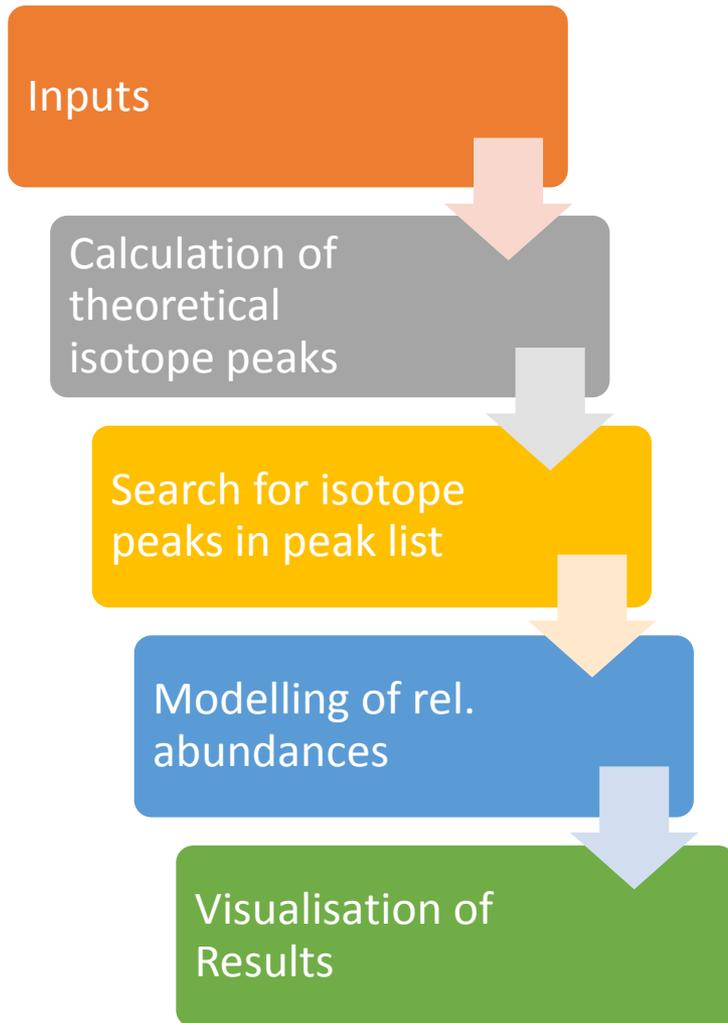
FAST MS: Top-Down MS



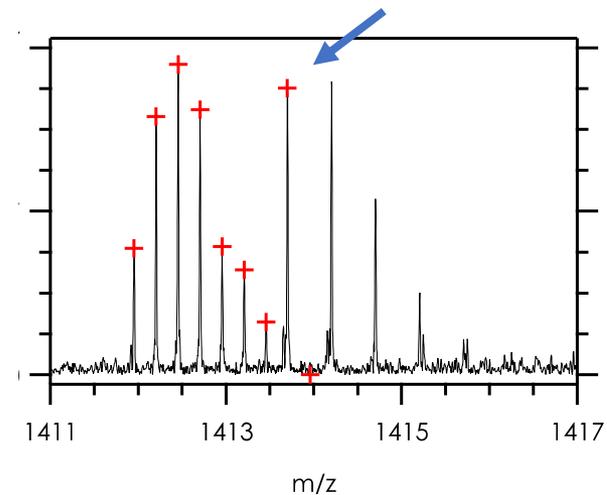
Charge state estimation



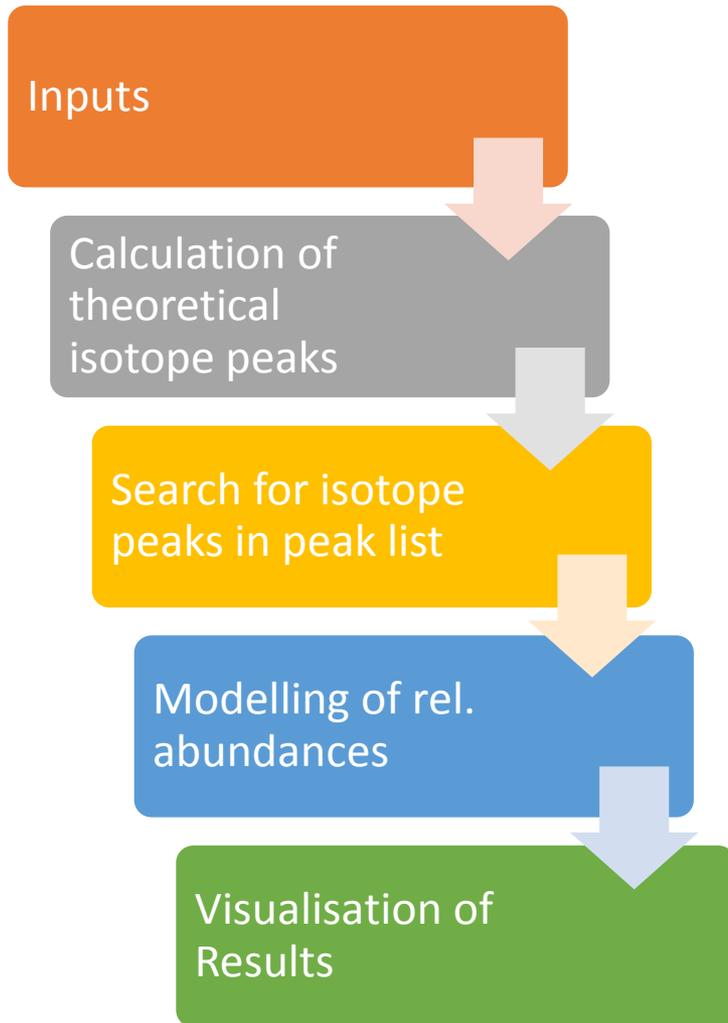
FAST MS: Top-Down MS



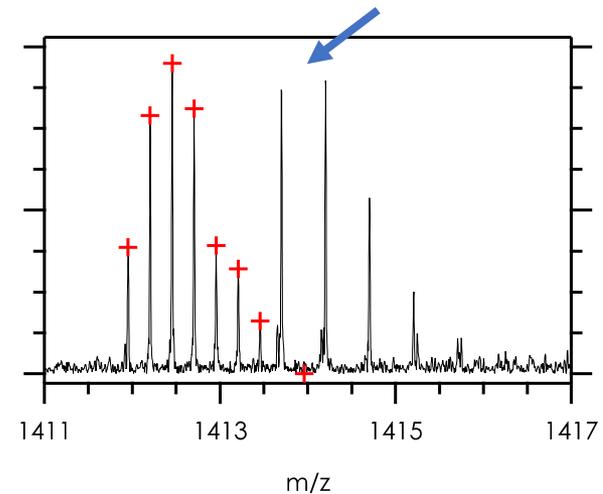
- Least-square fit
- Outlier detection



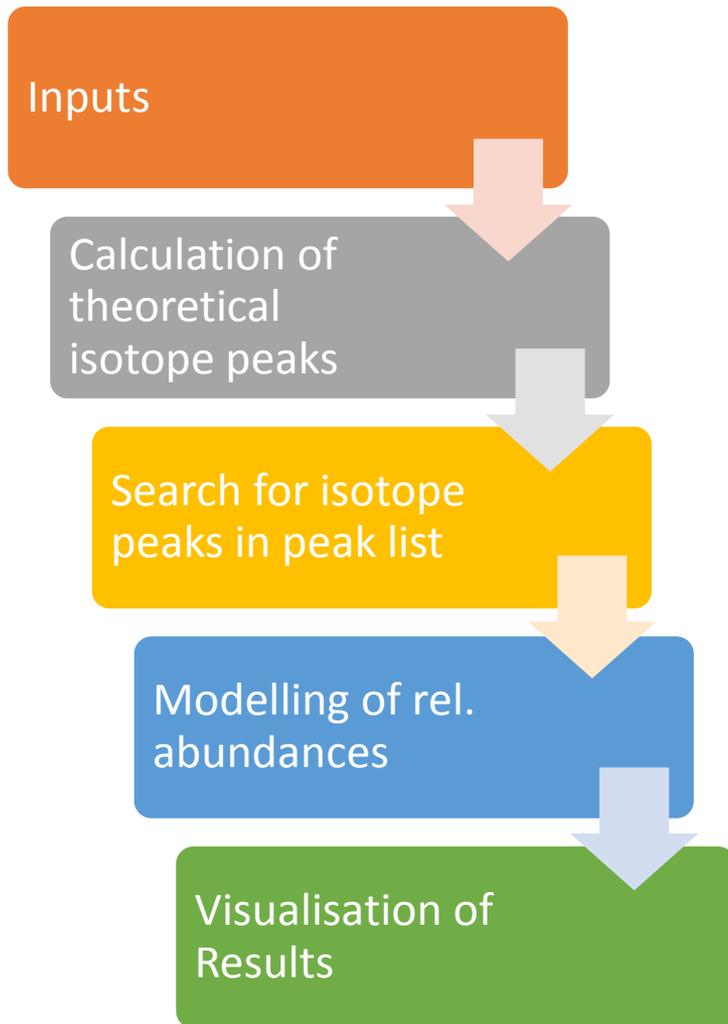
FAST MS: Top-Down MS



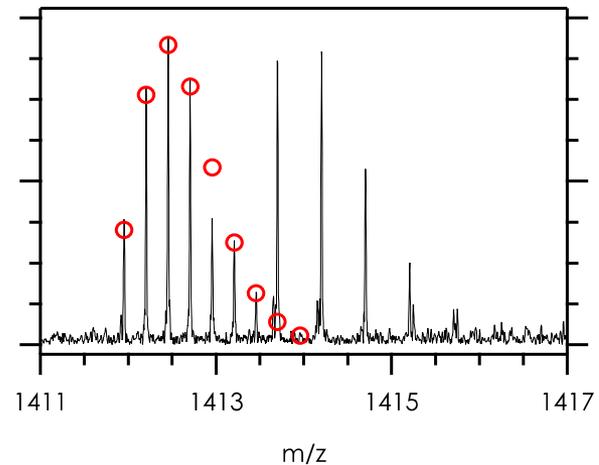
- Least-square fit
- Outlier detection



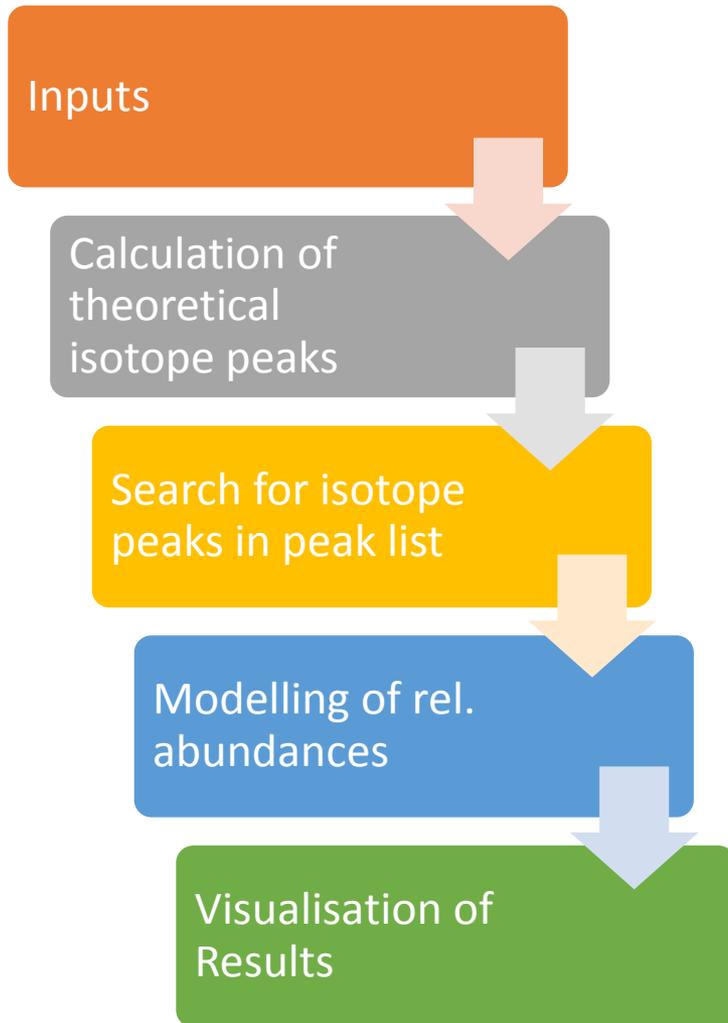
FAST MS: Top-Down MS



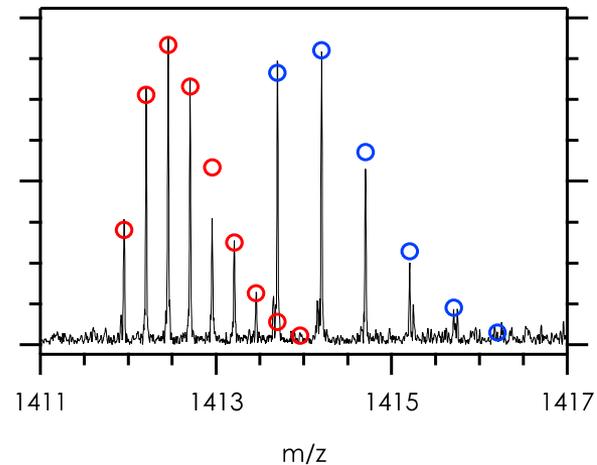
- Least-square fit
- Outlier detection
- Evaluation



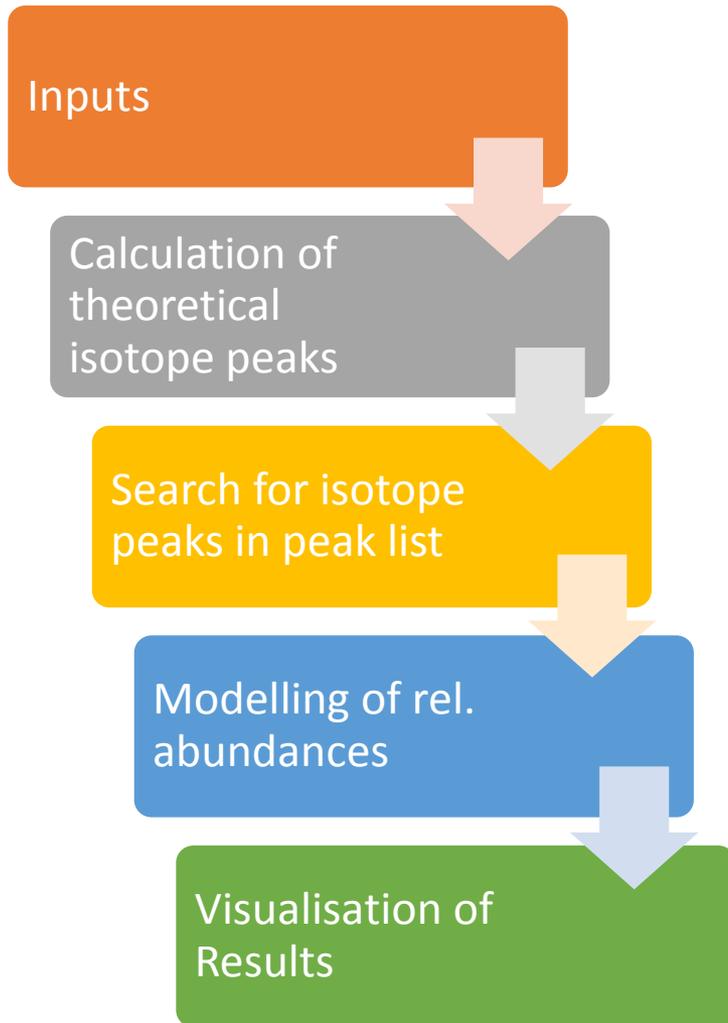
FAST MS: Top-Down MS



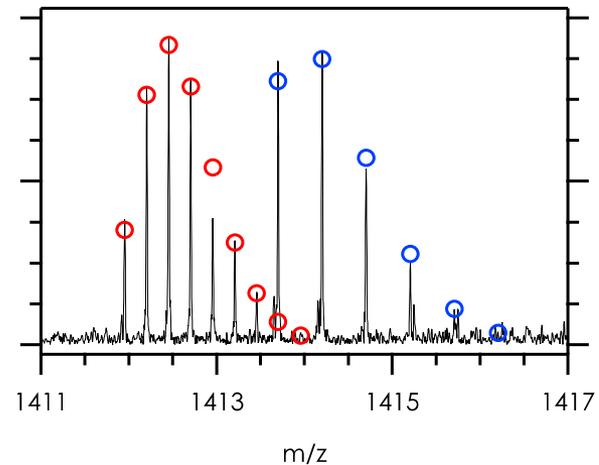
- Least-square fit
- Outlier detection
- Evaluation
- Overlapping ions



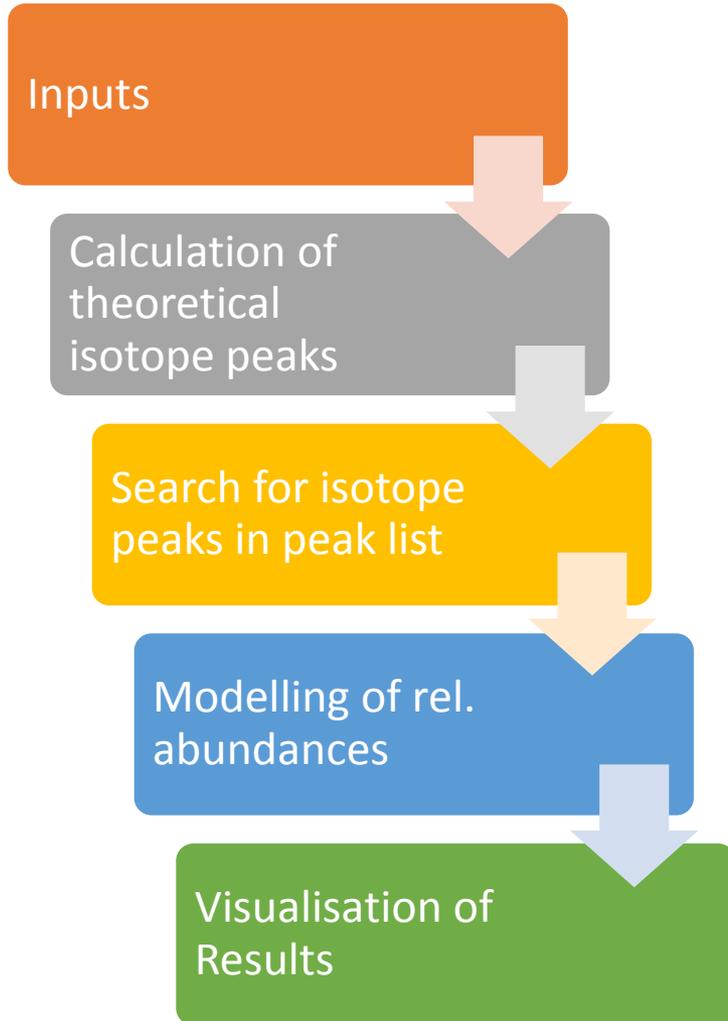
FAST MS: Top-Down MS



- Least-square fit
- Outlier detection
- Evaluation
- Overlapping ions



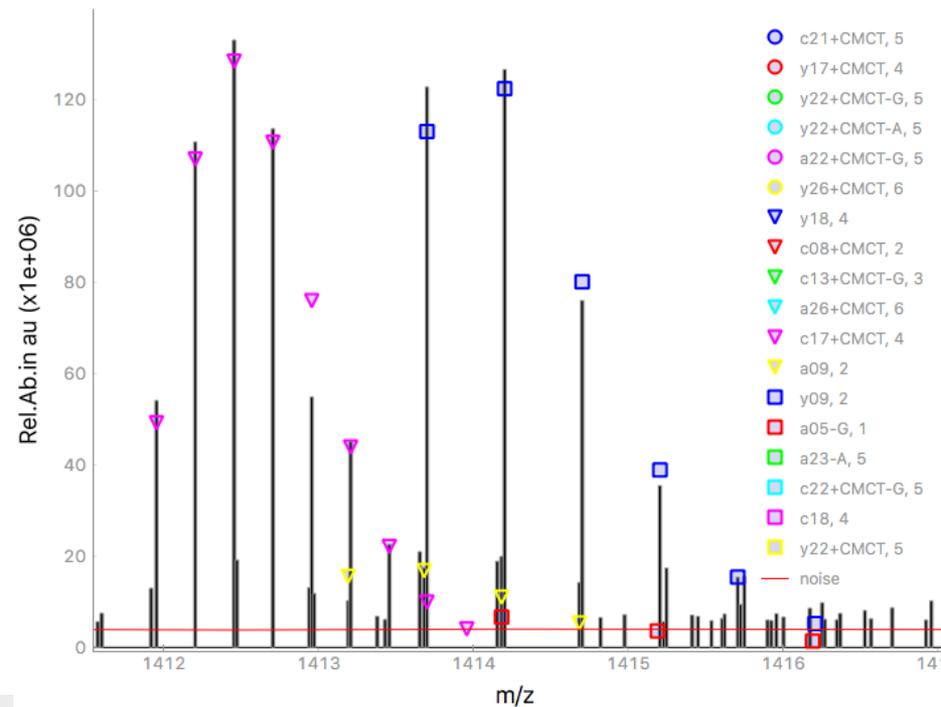
FAST MS: Top-Down MS



Observed Ions Deleted Ions									
m/z	z	intensity	fragment	error /ppm	S/N	quality	score	comment	
914.16164	1	7763389	a03	-0.99	2.5	0.29	0.7		
763.11379	1	43808966	a03-G	0.85	14.6	0.24	1.8		
1565.23223	1	7275260	a05	-1.95	1.9	0.45	4.8		
1414.18217	1	11628606	a05-G	-2.62	2.9	0.19	0.2	ov:[a09_2],	
706.58974	2	34709391	a05-G	0.63	12.1	0.13	0.3		
934.63618	2	15960984	a06	1.64	5.0	0.17	0.3		
984.71228	2	5858788	a06+CMCT-G	2.49	1.8	0.58	19.8		
859.11055	2	5534207	a06-G	0.71	1.9	0.47	5.0		
1413.18317	2	48738704	a09	-0.36	12.4	0.19	1.0	ov:[a05-G_1],	
1463.25892	2	17669133	a09+CMCT-G	0.05	4.7	0.32	2.3		
1337.65939	2	45546680	a09-G	0.32	11.8	0.15	0.5		
1043.79320	3	88247501	a10	-1.84	26.1	0.36	19.2		
1145.47591	3	23219522	a11	0.32	6.3	0.32	3.2		
1229.20865	3	13235564	a11+CMCT	-0.13	3.2	0.19	0.3	ov:[c18-G_4],high,1.07,,	
1247.15644	3	46558657	a12	0.37	11.4	0.18	0.9		
935.11818	4	8851251	a12	3.23	2.7	0.55	19.3		

FAST MS: Visualisation of Results

- Manual editing, deleting, restoring, adding of ions possible
- Visualisation of spectrum
- Protocol
- Analysis tools

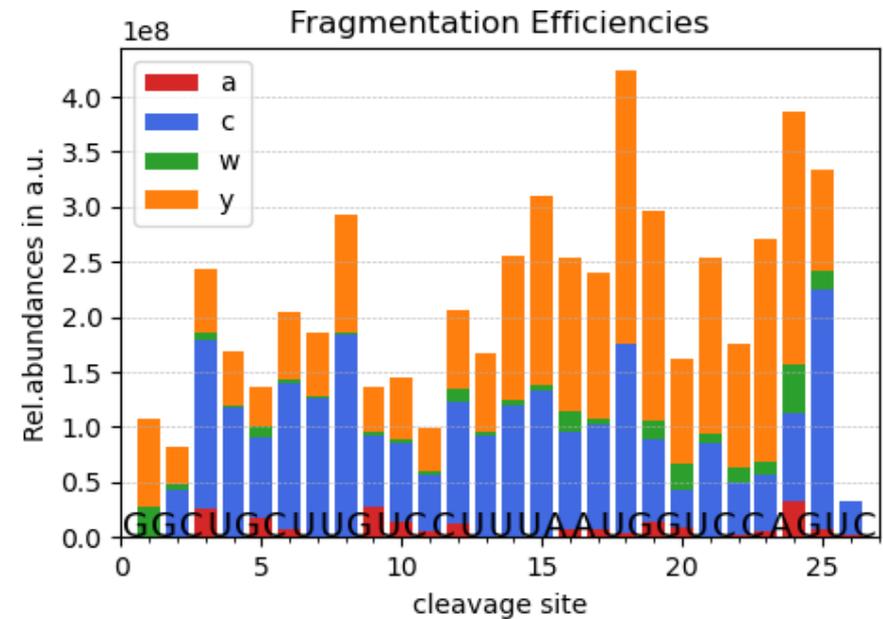


Observed Ions		Deleted Ions								
m/z	z	intensity	fragment	error /ppm	S/N	quality	score	comment		
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984.71228	2	5858788	a06+CMCT-G	2.49	1.8	0.58	19.8			
859.11055	2	5534207	a06-G	0.71	1.9	0.47	5.0			
1413.18317	2	48738704	a09	-0.36	12.4	0.19	1.0	ov: [a05-G_1],		

FAST MS: Visualisation of Results

Analysis:

- Fragmentation efficiencies



type	rel.proportion
neoRibo	0.762
a	0.009
c	0.105
w	0.011
y	0.114

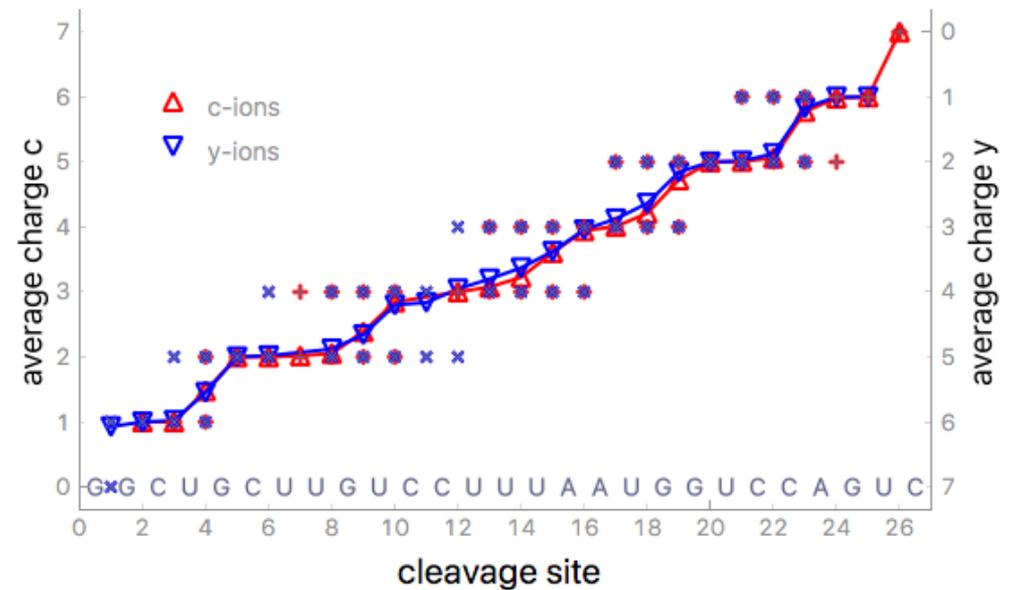
sequ. (f)	cleav.side (f)	a	c	w	y	cleav.side (b)	sequ. (b)
G	1	0	0	26941802	80804496	26	G
G	2	0	42693262	4713574	34853025	25	C
C	3	25786178	153220090	6959211	58460113	24	U
U	4	0	118445190	1750776	49280631	23	G
G	5	18129281	72844497	9657250	35673648	22	C
C	6	6838495	132896376	3127108	62327208	21	U
U	7	0	125863821	2016989	57450035	20	U
U	8	0	104632005	706125	107665000	19	C

FAST MS: Visualisation of Results

Analysis:

- Fragmentation efficiencies
- Charge distributions

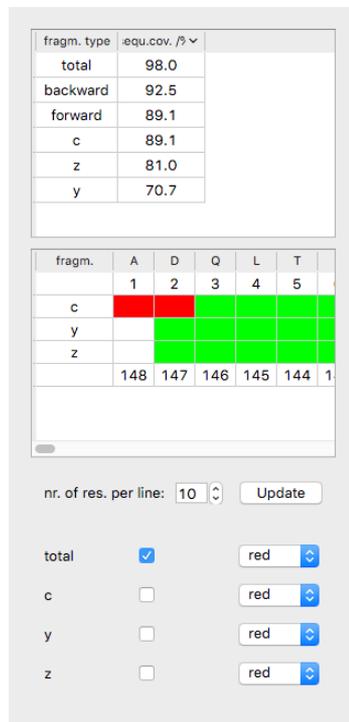
sequ. (f) ▾	cleav.side (f)	c	y	cleav.side (b)	sequ. (b)
G	1		6.1	26	G
G	2	1.0	6.0	25	C
C	3	1.0	6.0	24	U
U	4	1.5	5.5	23	G
G	5	2.0	5.0	22	C
C	6	2.0	5.0	21	U
U	7	2.0	5.0	20	U
U	8	2.1	4.9	19	G
G	9	2.4	4.7	18	U
U	10	2.8	4.2	17	C
C	11		4.2	16	C
C	12	3.0	4.0	15	U
U	13	3.1	3.8	14	U
U	14	3.2	3.6	13	U
U	15	3.6	3.4	12	A
A	16	3.9	3.0	11	A
A	17	4.0	2.9	10	U



FAST MS: Visualisation of Results

Analysis:

- Fragmentation efficiencies
- Charge distributions
- Sequence coverage



```
1   A L D L Q L T E E Q I A
11  L E L F K E A F S L F D
21  L K L D L G L D L G L T L I L T L T L K
31  L E L L G L T L V L M L R L S L L G
41  L Q L N L P L T L E L A L E L L L Q L D
51  L M L I L N L E L V L D L A L D L G L N
61  L G L T L I L D L F L P L E L F L L T
71  L M L M L A L R L K L M L K L D L T L D
81  L S L E L E L E L I L R L E L A L F L R
91  L V L F L D L K L D L G L N L G L Y L I
101 L S L A L A L E L L L R L H L V L M L T
111 L N L L G L E L K L L L T L D L E L E
121 L V L D L E L M L I L R L E L A L D L I
131 L D L G L D L G L Q L V L N L Y L E L E
141 L F L V L Q L M L M L T L A L K
```

FAST MS: Visualisation of Results

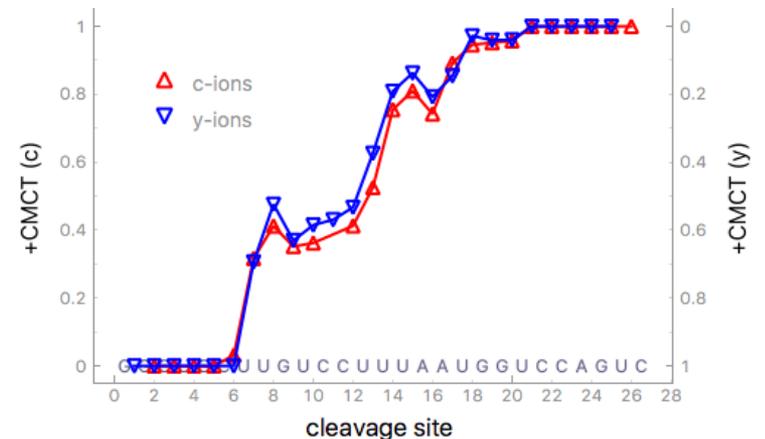
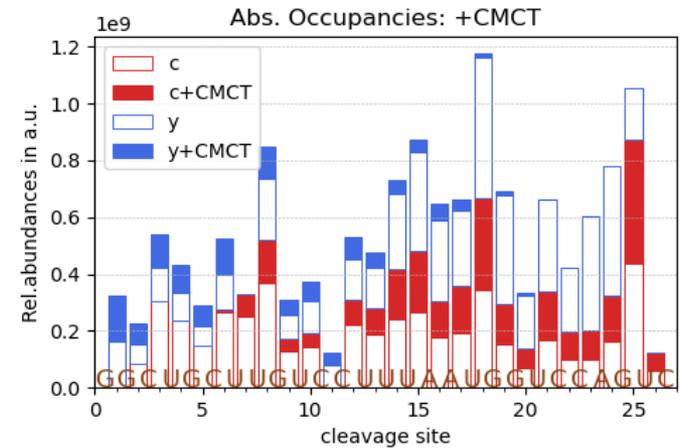
Analysis:

- Fragmentation efficiencies
- Charge distributions
- Sequence coverage
- Locating & quantification of modifications / ligands

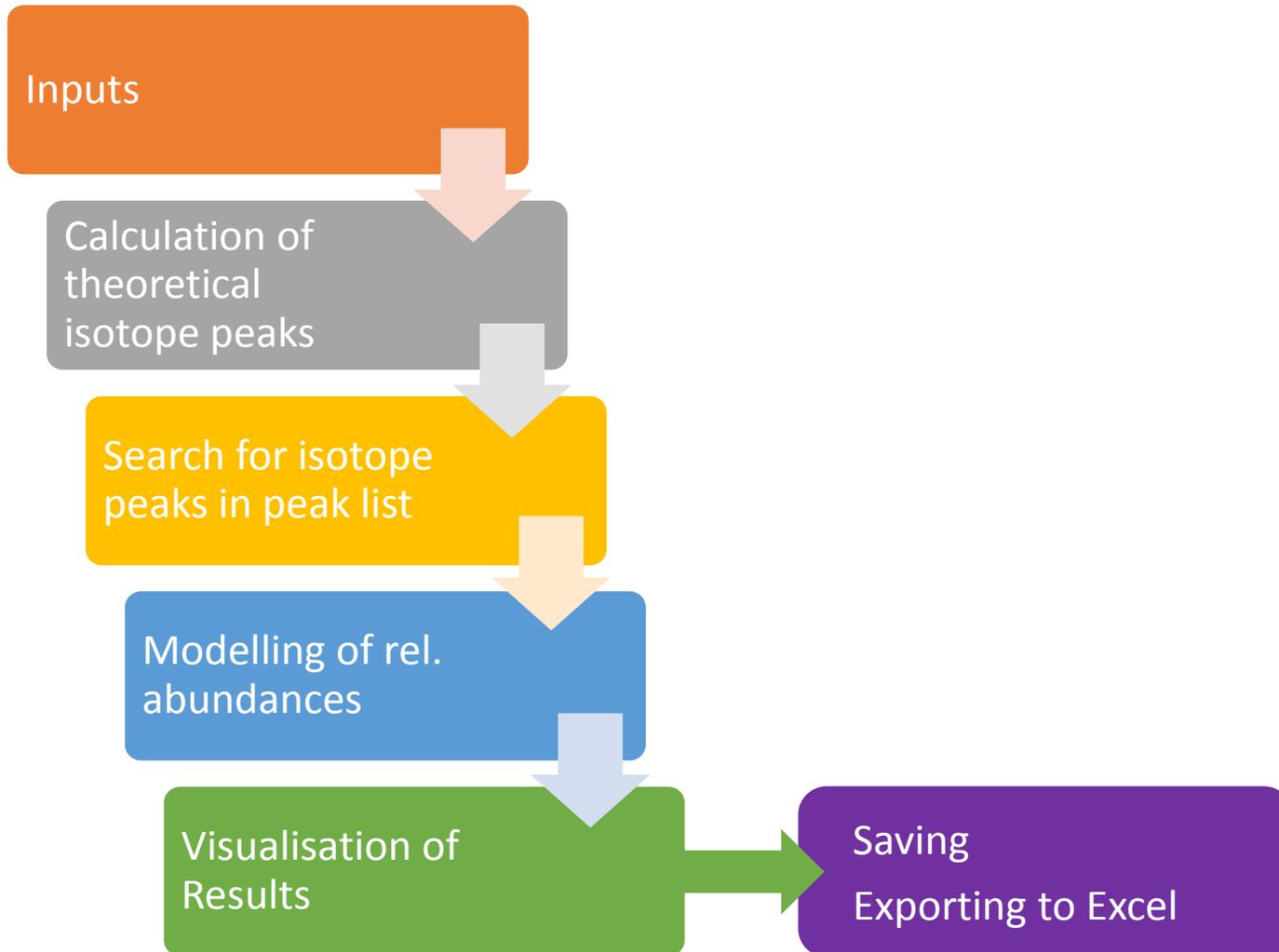
prec. mod. loss: 0.6 %

sequ. (f) ▾	cleav.side (f)	c	y	cleav.side (b)	sequ. (b)
G	1		1.000	26	G
G	2	0.000	1.000	25	C
C	3	0.000	1.000	24	U
U	4	0.000	1.000	23	G
G	5	0.000	1.000	22	C
C	6	0.031	1.000	21	U
U	7	0.317	0.694	20	U

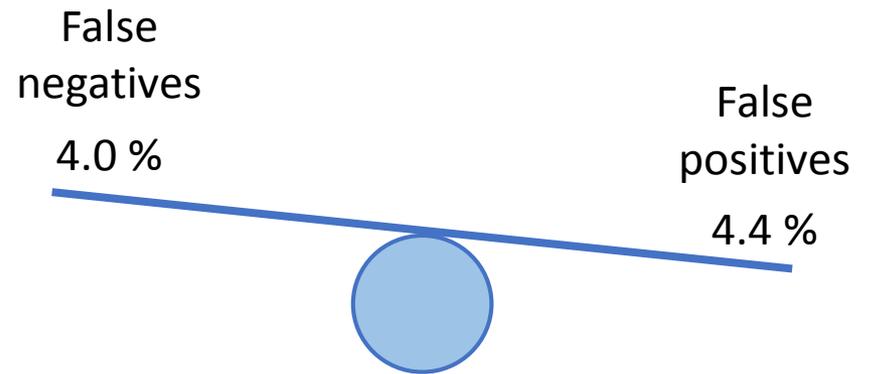
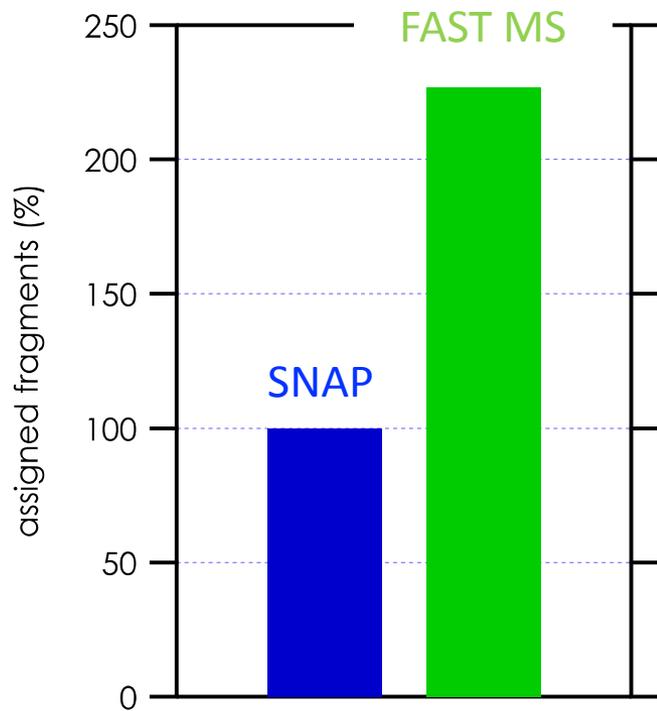
sequ. (f) ▾	cleav.side (f)	c	c+CMCT	y	y+CMCT
G	1	0.0	0.0	161608991.6	161608991.6
G	2	85386524.0	0.0	69706050.5	69706050.5
C	3	306440180.0	0.0	116920226.8	116920226.8
U	4	236890380.5	0.0	98561261.7	98561261.7
G	5	145688994.5	0.0	71347295.2	71347295.2



FAST MS: Top-Down MS



FAST MS: Evaluation



Other Tools

- Analyse ESI spectra with intact ions
 - Ion Assignment and Analysis
 - Autocalibration
- Tool to model isotope patterns
- Tool to compare ion lists of different spectra

.TGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRGG Mode: Protein

Charge: 8 Electrons: 1

Exact Calculate Model

Fragmentation: Protein_ECD
Fragment: Prec+e
Modif. Pattern: -
Modification:
Nr. of Mod.: 0

m/z	z	intensity	name	quality	formula	neutral mass	av.mass
1 1071.08534	8	1000	Prec+e	0.00	C378H629N105O118S1	8559.6167	8572.8109

Peaks:

	m/z	int. (spectrum)	int. (calc.)	used
1	1071.08534	0	8	<input checked="" type="checkbox"/>
2	1071.21070	0	35	<input checked="" type="checkbox"/>
3	1071.33605	0	82	<input checked="" type="checkbox"/>
4	1071.46140	0	133	<input checked="" type="checkbox"/>
5	1071.58673	0	165	<input checked="" type="checkbox"/>
6	1071.71206	0	167	<input checked="" type="checkbox"/>
7	1071.83739	0	143	<input checked="" type="checkbox"/>
8	1071.96271	0	107	<input checked="" type="checkbox"/>
9	1072.08802	0	71	<input checked="" type="checkbox"/>
10	1072.21334	0	43	<input checked="" type="checkbox"/>
11	1072.33865	0	24	<input checked="" type="checkbox"/>
12	1072.46396	0	12	<input checked="" type="checkbox"/>
13	1072.58926	0	6	<input checked="" type="checkbox"/>

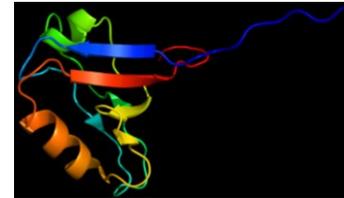
Conclusion



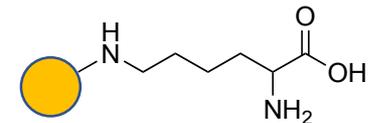
- Robust:
 - Beta-Version(s) User-tested for > 1 year
 - Unit-tested

- Universal:

- All kinds of polymers
- Isotopically depleted/enriched molecules
- User-defined building blocks



^{12}C ^{13}C



dissociation methods

- Positive and negative spray mode - +
- 2D MS

EDD UVPD
CAD IRMPD ECD
EDD

- User-friendly

Outlook

- Coming soon: Autocalibration for top-down mass spectra
- ASMS 2021:
Utilizing the power of mass spectrometry for chemical probing of RNA



Acknowledgements



EU FT-ICR MS



Kathrin Breuker
Maria van Agthoven
Sarah Heel



FWF

Der Wissenschaftsfonds.



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 731077.