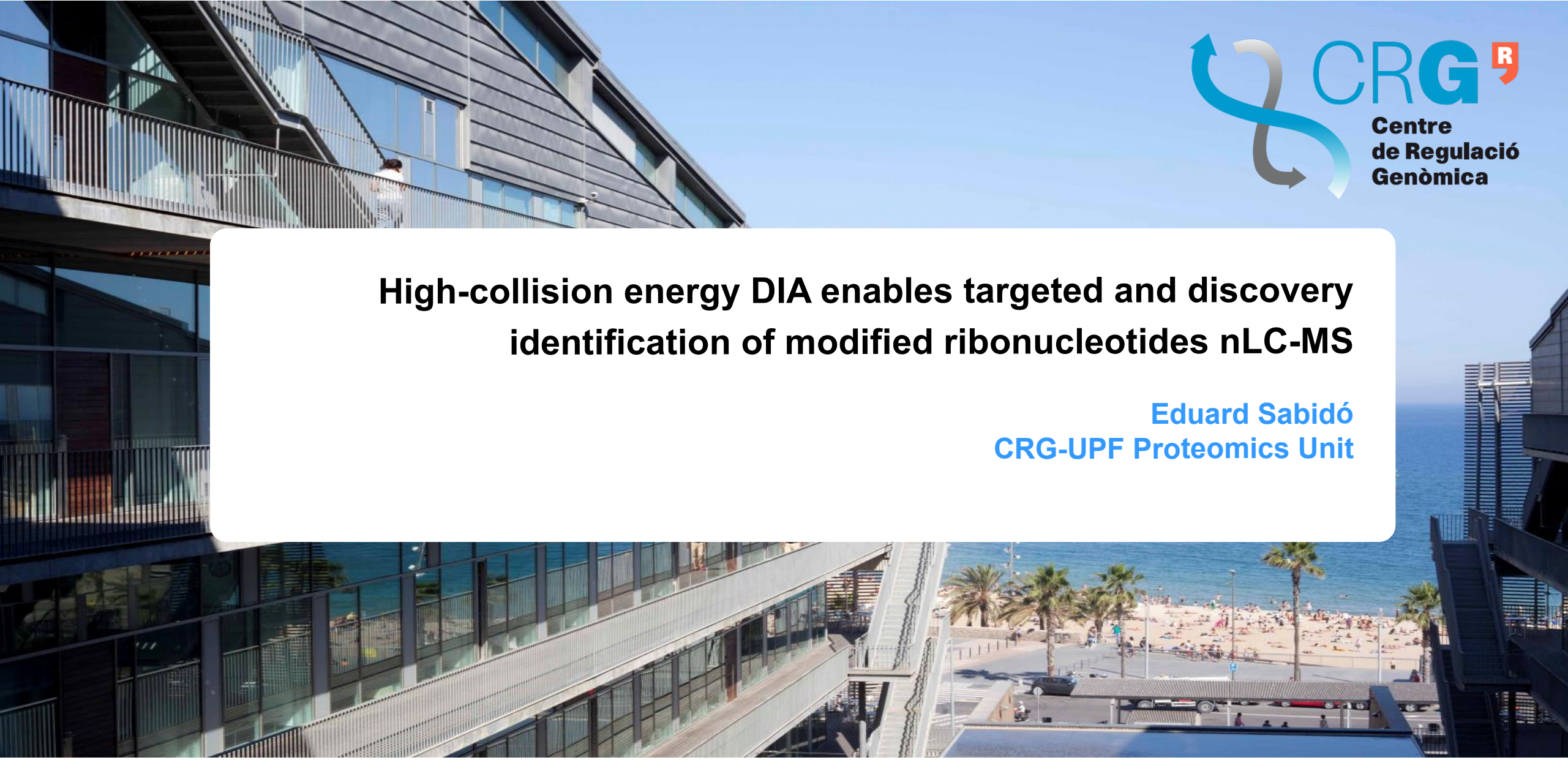


High-collision energy DIA enables targeted and discovery identification of modified ribonucleotides nLC-MS

Eduard Sabidó
CRG-UPF Proteomics Unit

CRG Centre de Regulació Genòmica

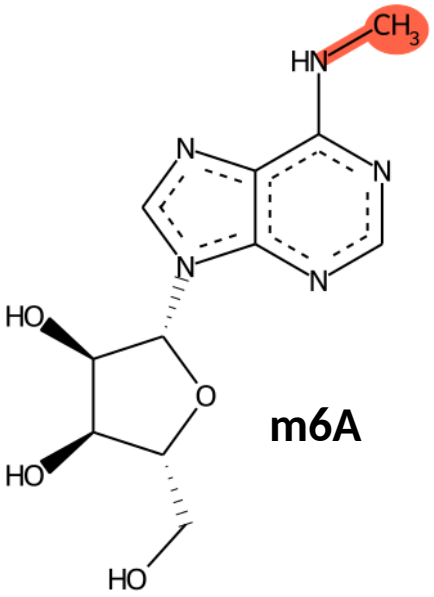
Universitat Pompeu Fabra Barcelona

EPIC-XS

OmicsTech

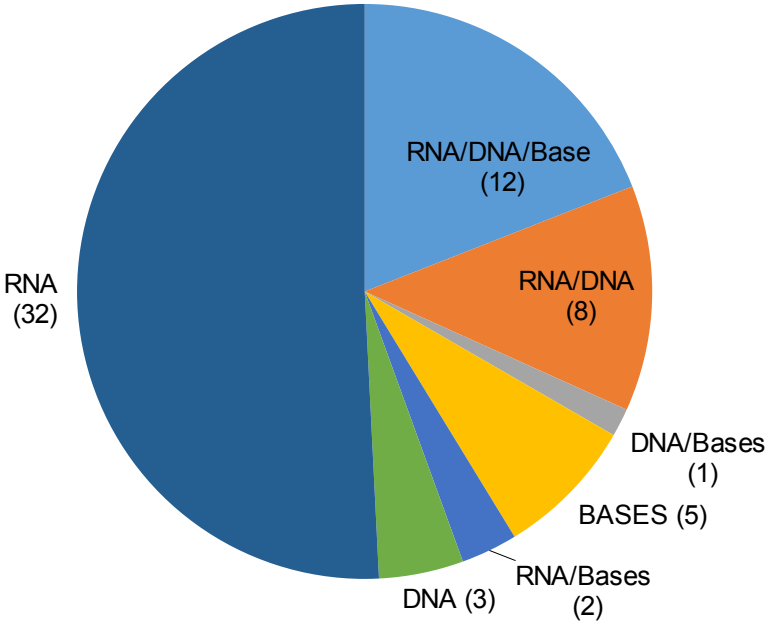
EXCELENCIA SEVERO OCHOA

Quantification of ribonucleoside modifications in a proteomics laboratory



m6A

63 standards



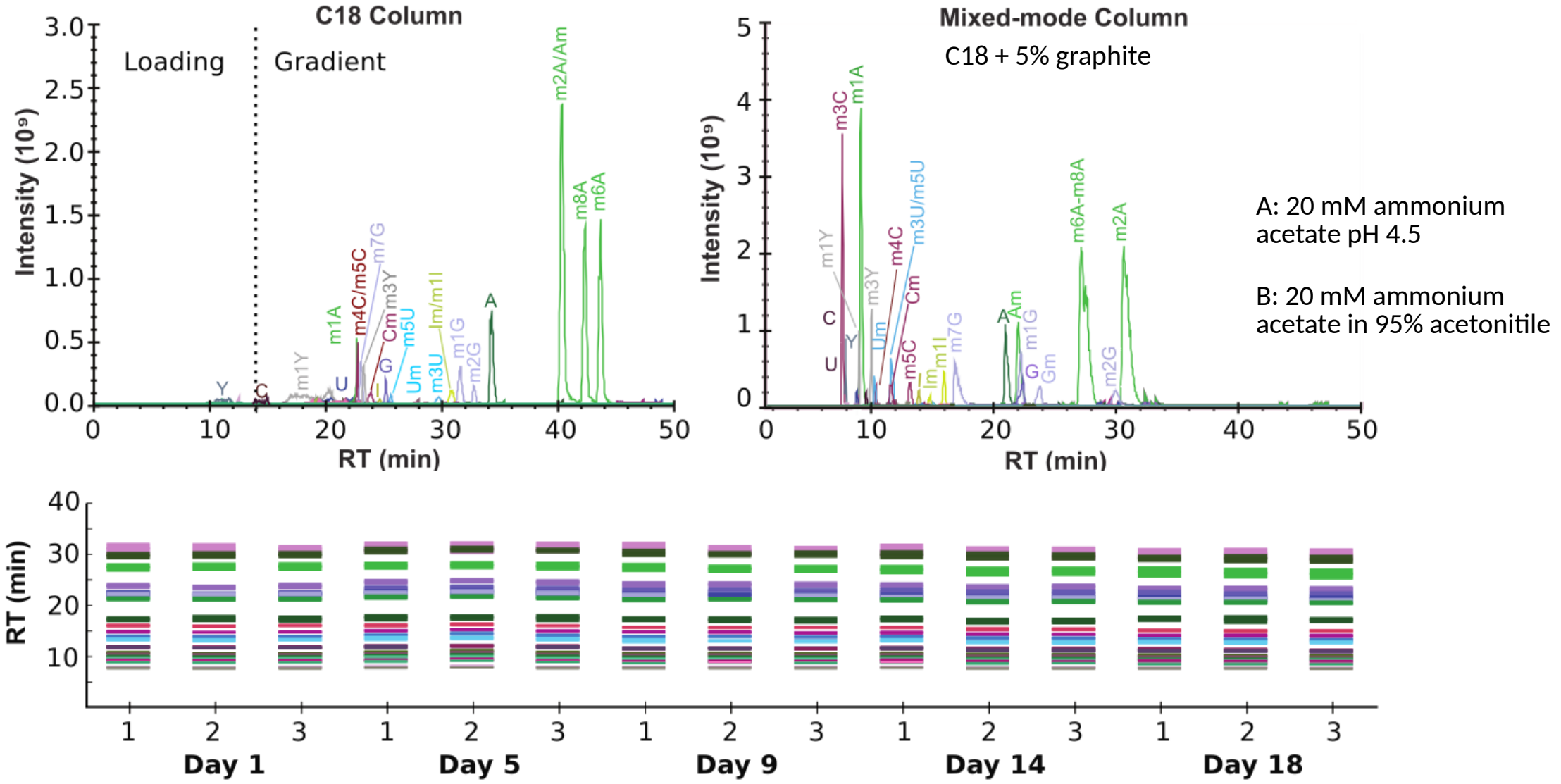
Orbitrap Eclipse

C18 / Mixed Mode nano-flow HPLC

DIA 220-380 m/z with HCD fragmentation
20 windows of 8 Da

Boccaletto, Pietro, et al. "MODOMICS: a database of RNA modification pathways. 2021 update." Nucleic acids research 50.D1 (2022): D231-D235.

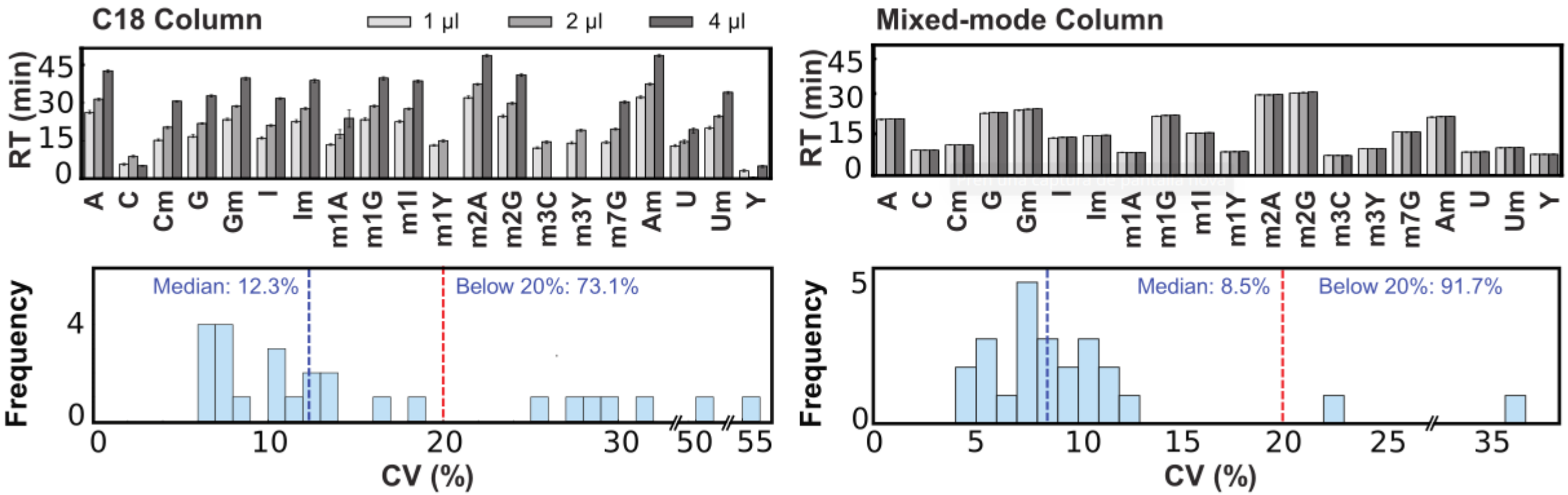
Adaptation of nucleoside analysis to nano-flow liquid chromatography



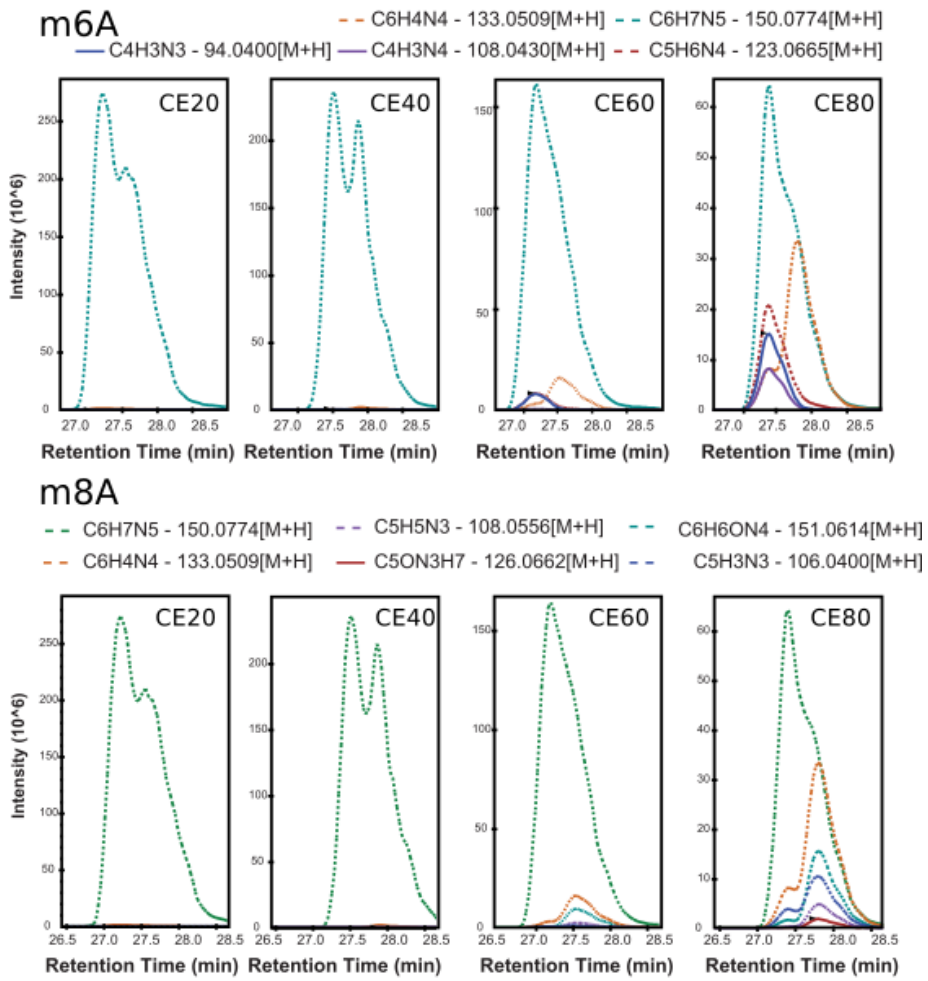
A: 20 mM ammonium acetate pH 4.5

B: 20 mM ammonium acetate in 95% acetonitile

Adaptation of nucleoside analysis to nano-flow liquid chromatography

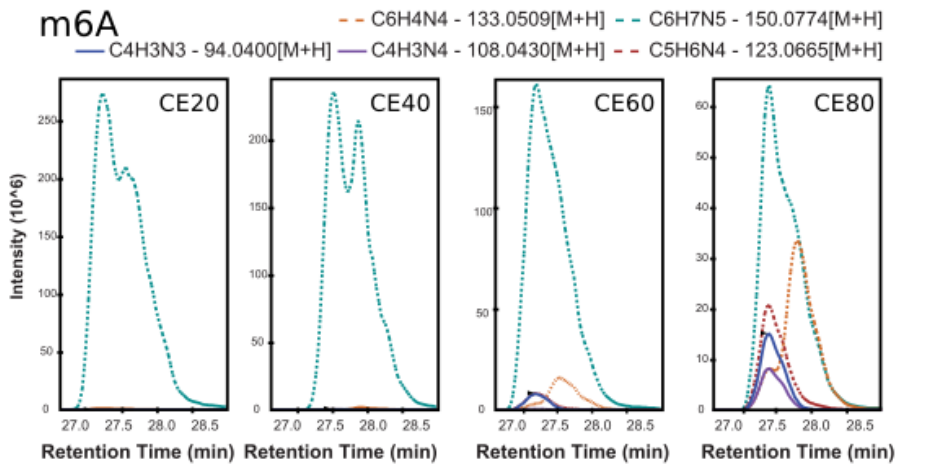


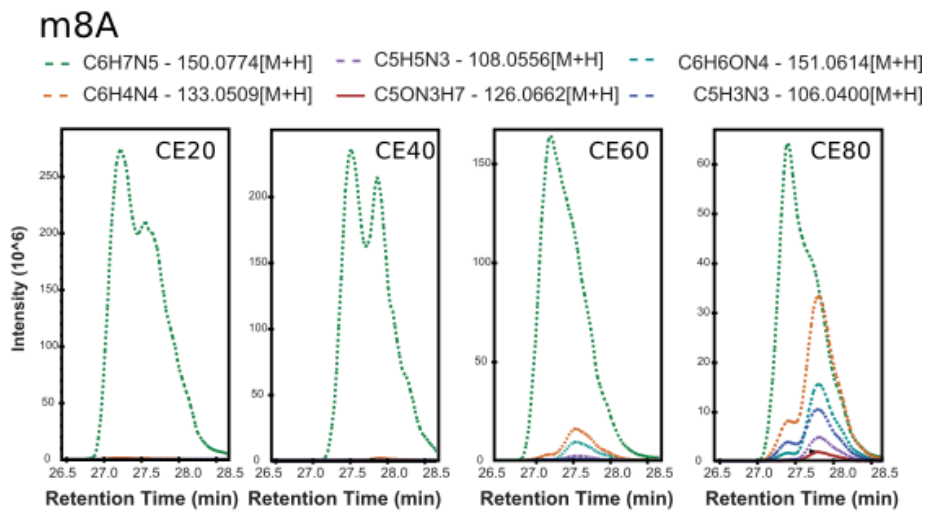
Use of high collision energy to generate information-rich spectra

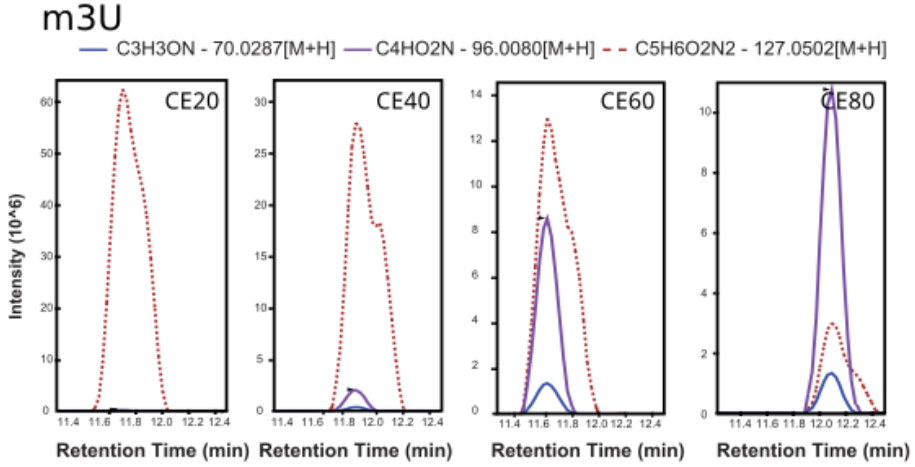


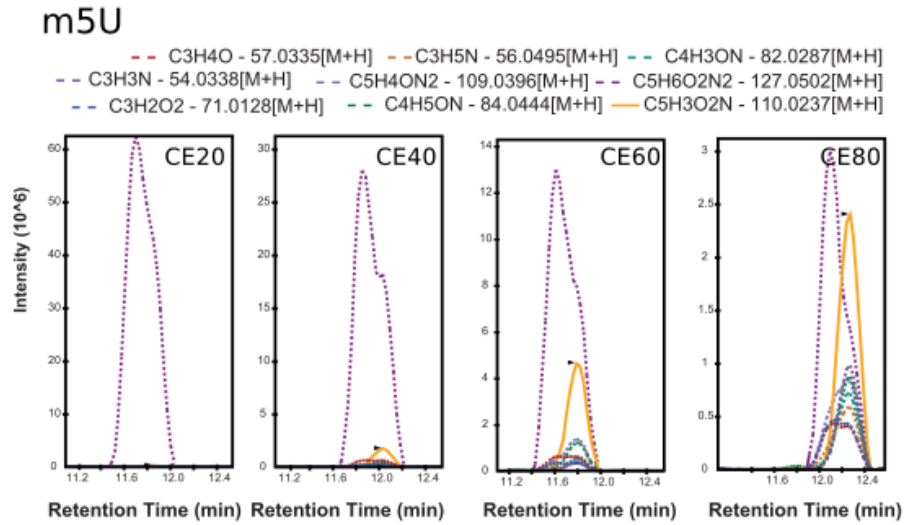
Orbitrap Eclipse DIA 220-380 m/z (HCD) - 20 windows of 8 Da

Use of high collision energy to generate information-rich spectra



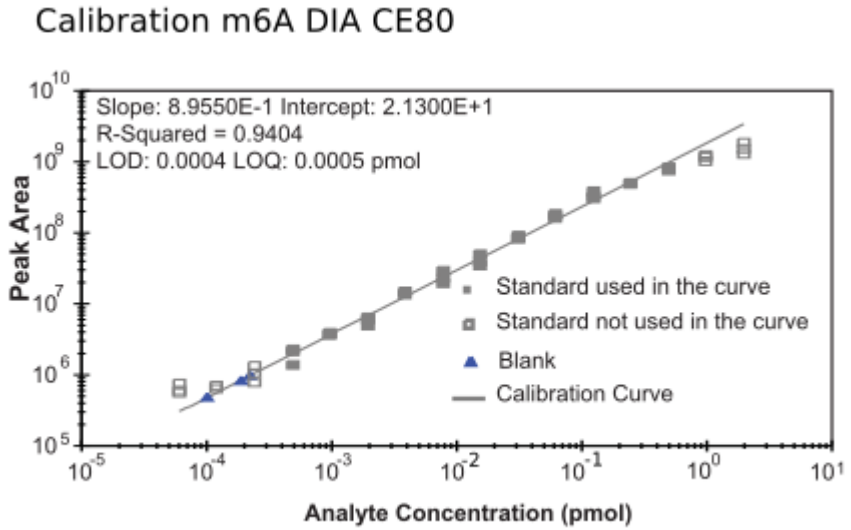


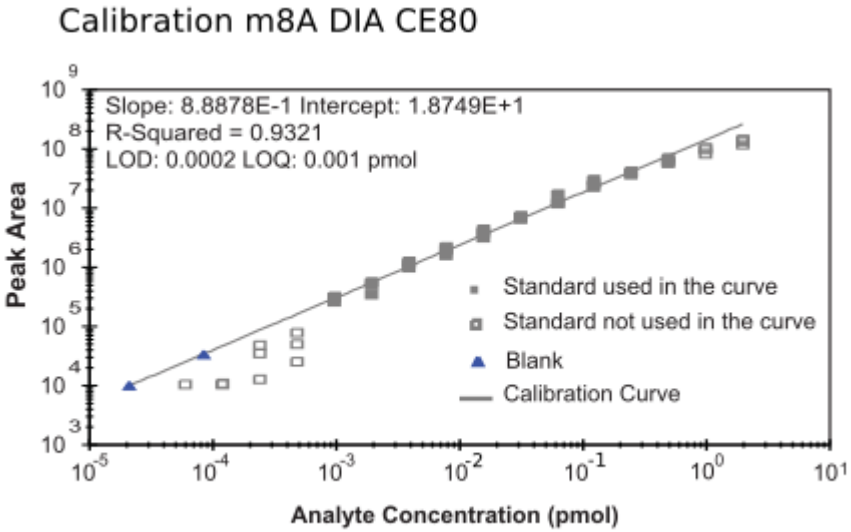


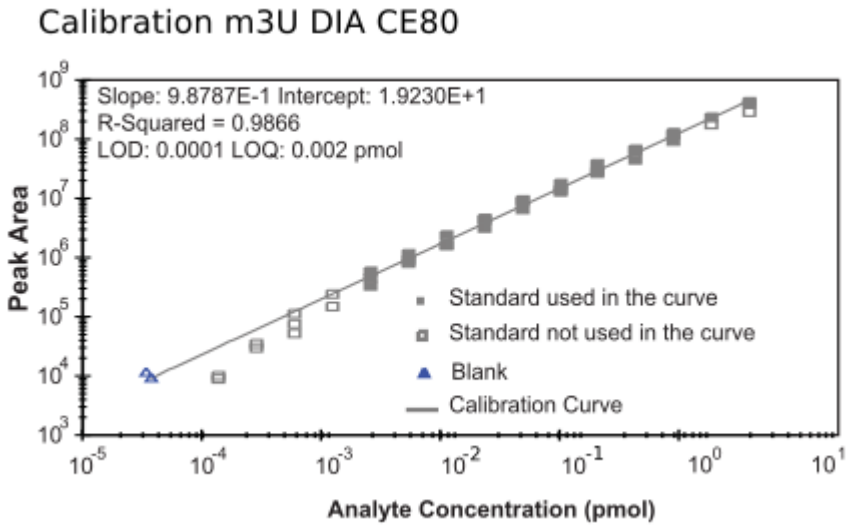


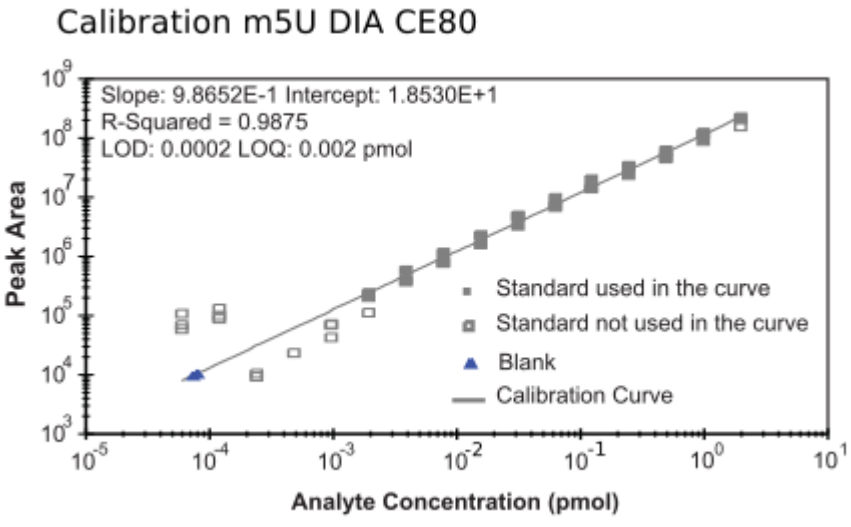
Orbitrap Eclipse DIA 220-380 m/z (HCD) - 20 windows of 8 Da

Use unique fragments for identification and quantification

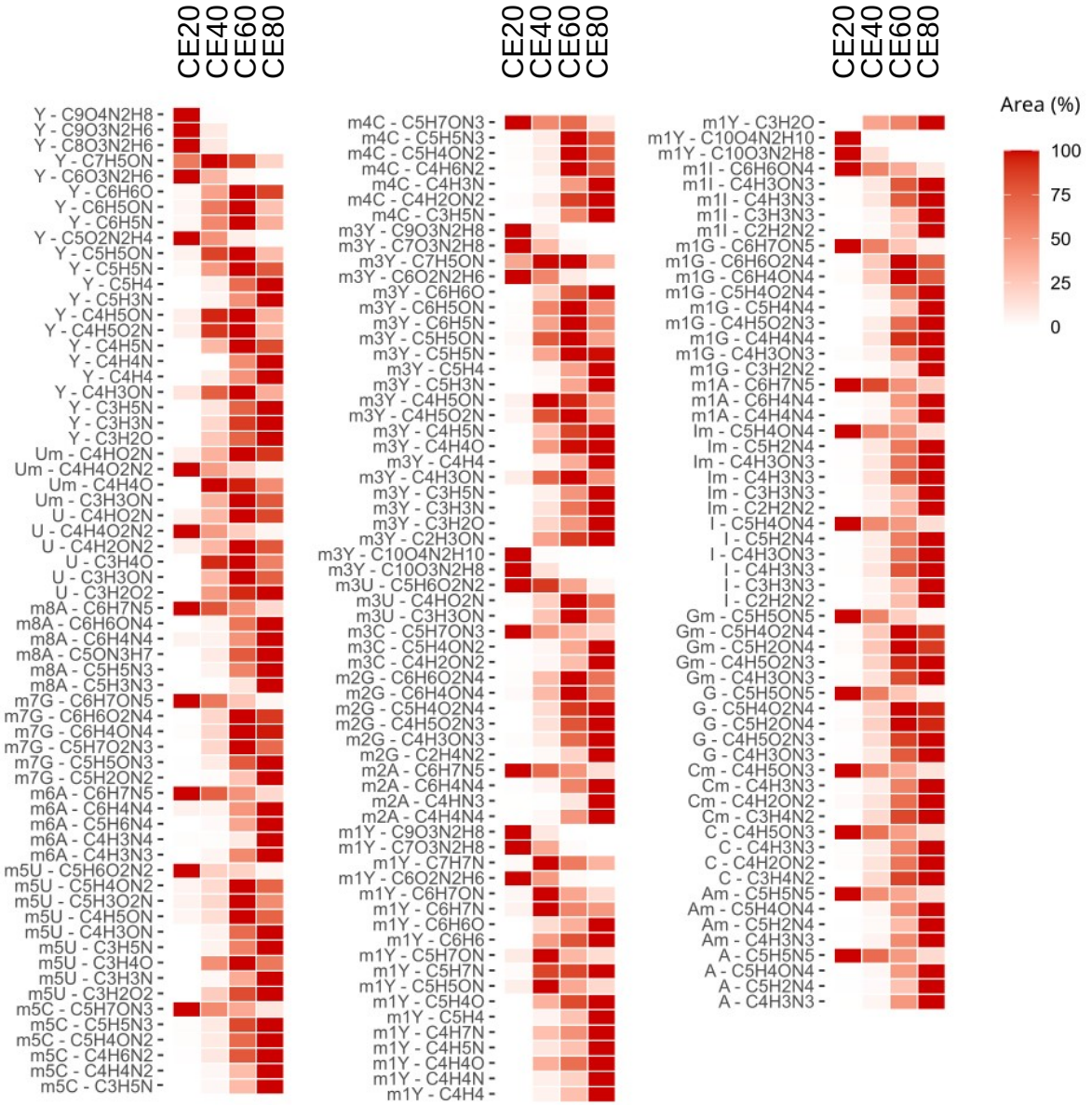




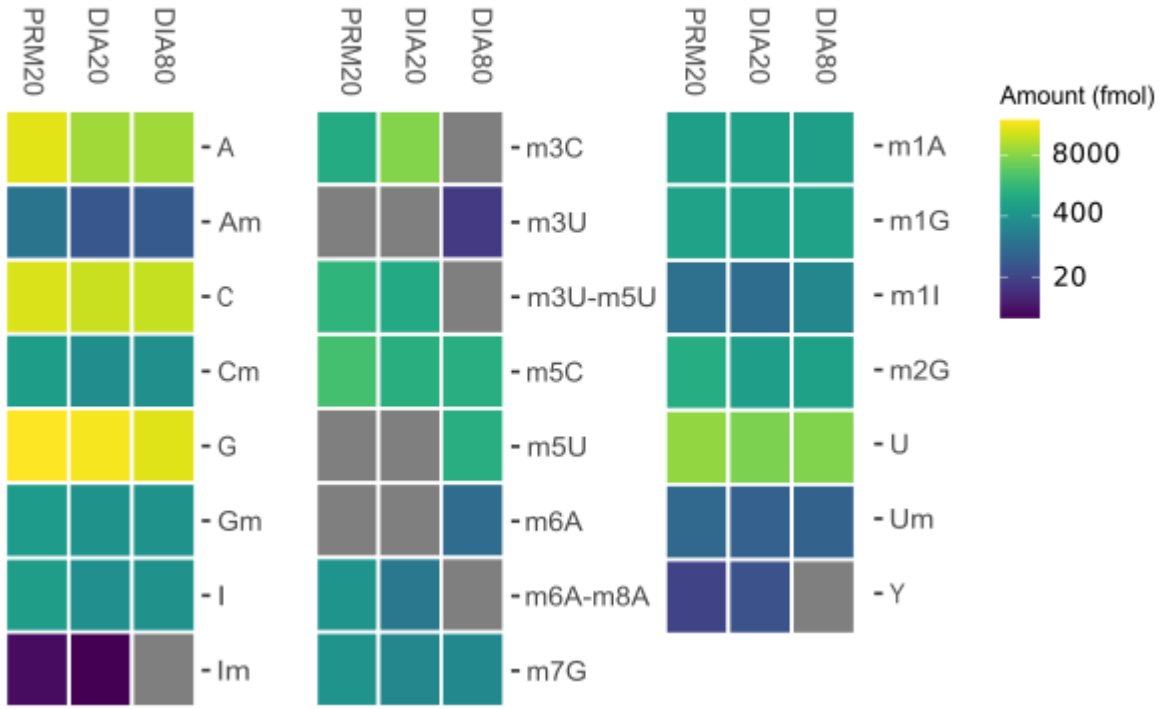


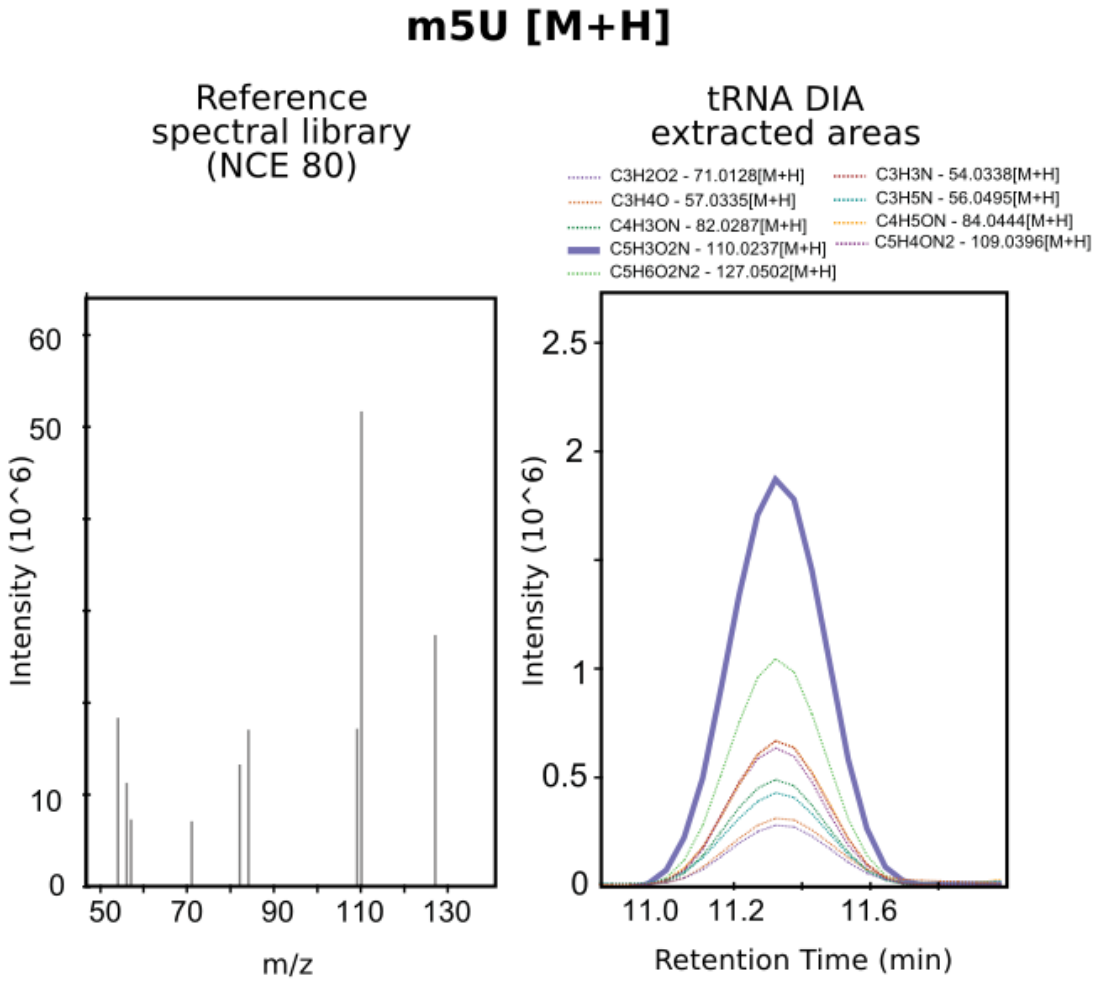


Use of high collision energy to generate information-rich spectra



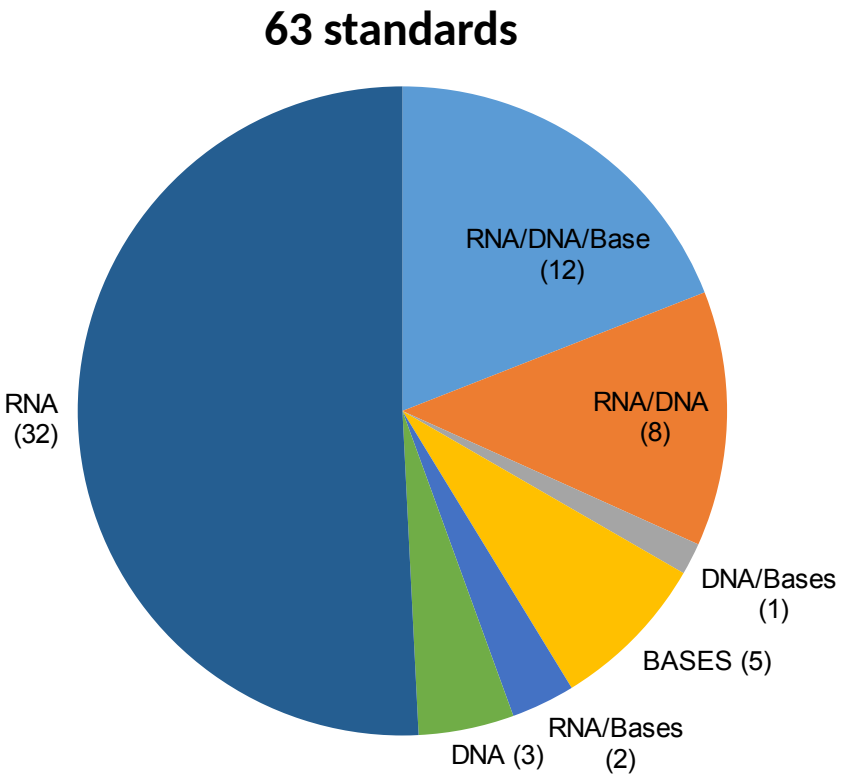
Quantification of tRNA nucleosides from *Saccharomyces cerevisiae*





Espadas G, et al J Chromatogr A. 2022 Feb 22;1665:462803. doi: 10.1016/j.chroma.2022.462803

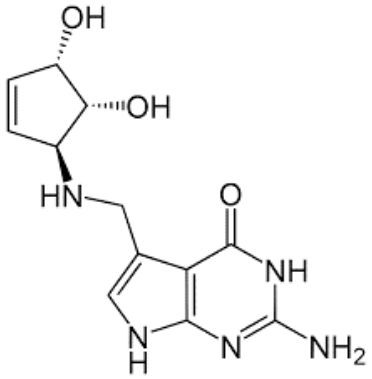
Use of desoxynucleotides and nucleobases as standards for ribonucleosides



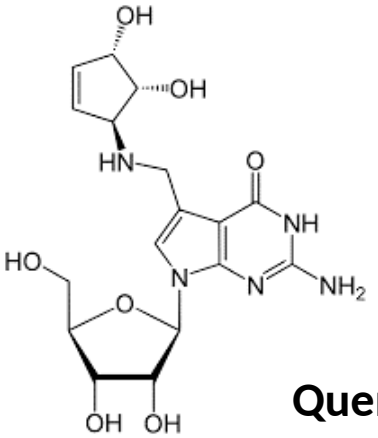
Compound	RNA	DNA	Bases
5fC	✓	✓	✓
A	✓	✓	✓
C	✓	✓	✓
G	✓	✓	✓
hm5C	✓	✓	✓
m2G	✓	✓	✓
m3U	✓	✓	✓
m5C	✓	✓	✓
m5U/T	✓	✓	✓
m6A	✓	✓	✓
m6G	✓	✓	✓
U	✓	✓	✓
m2A	✓	✗	✓
m7G	✓	✗	✓
I		✓	✗
m1G	✓	✓	✗
m3C	✓	✓	✗
Y	✓	✓	✗
8-oxoG	✓	✓	✗
ho5U	✓	✓	✗
m1A	✓	✓	✗
m4C	✓	✓	✗
5caC	✗	✓	✓
m3A	✗	✗	✓
Q	✗	✗	✓
ho5C	✗	✓	✗
e6G	✗	✓	✗
e2T	✗	✓	✗
e7A	✗	✗	✓
e3A	✗	✗	✓
e7G	✗		✓

Compound	RNA	DNA	Bases
Cm	✓	✗	✗
m1Y	✓	✗	✗
m3Y	✓	✗	✗
Um	✓	✗	✗
Am	✓	✗	✗
m8A	✓	✗	✗
Im	✓	✗	✗
m1I	✓	✗	✗
m6Am	✓	✗	✗
m66A	✓	✗	✗
m1Am	✓	✗	✗
m28A	✓	✗	✗
Gm	✓	✗	✗
hm6A	✓	✗	✗
m22G	✓	✗	✗
m27G	✓	✗	✗
mcm5U	✓	✗	✗
m227G	✓	✗	✗
mcm5s2U	✓	✗	✗
42C	✓	✗	✗
t6A	✓	✗	✗
i6A	✓	✗	✗
cm5U	✓	✗	✗
ncm5U	✓	✗	✗
D	✓	✗	✗
acp3U	✓	✗	✗
m5Um	✓	✗	✗
ac4Cm	✓	✗	✗
m44C	✓	✗	✗
m4Cm	✓	✗	✗
m1Gm	✓	✗	✗
m2Gm	✓	✗	✗

Use of desoxyribonucleosides and nucleobases as standards for ribonucleosides

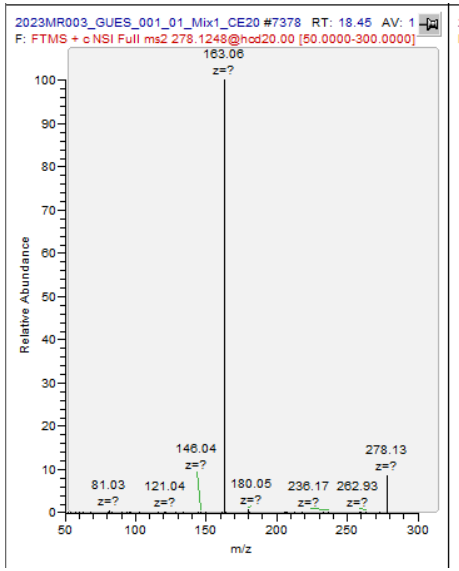


Queuine

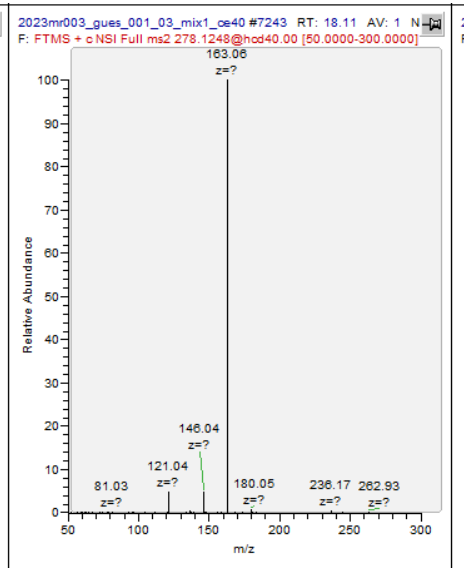


Quenosine

PRM CE20



PRM CE40



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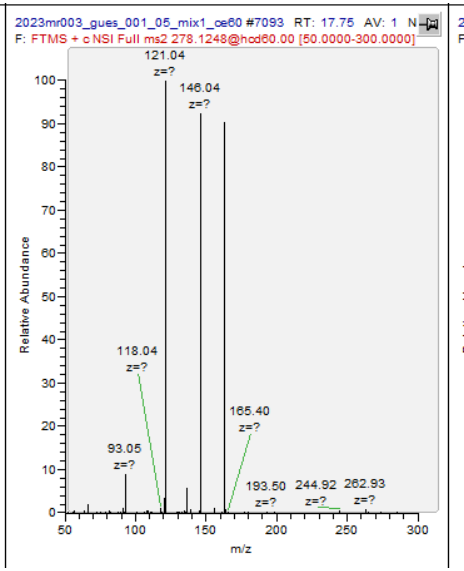

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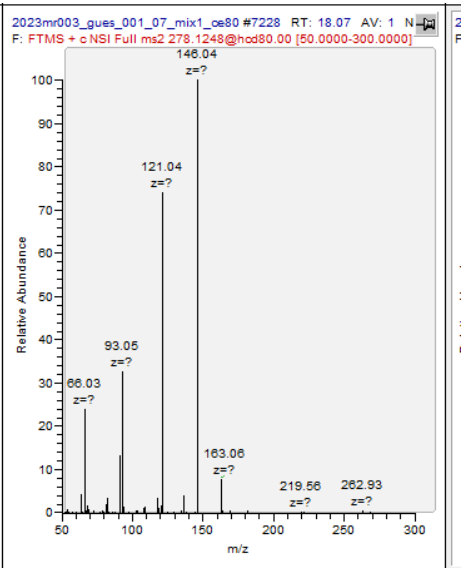
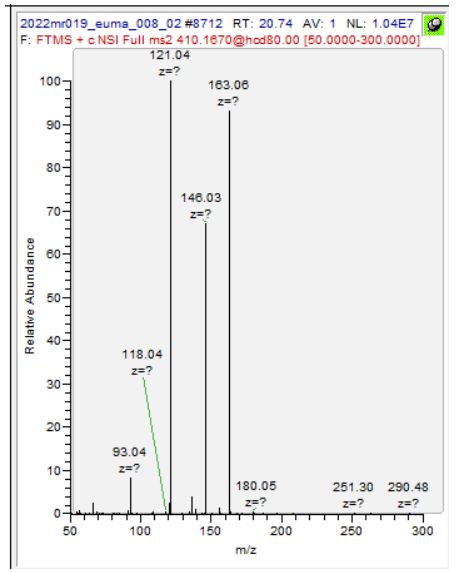


tRNA PRM CE80



Use of desoxynucleotides and nucleobases as standards for ribonucleosides

Information-rich spectral libraries from nucleobases and deoxynucleosides facilitate the identification of ribonucleosides by nLC-MSMS

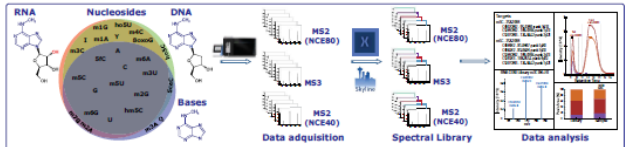
Guadalupe Espadas[1,2]; Eduard Sabidó[1,2]

[1]Center for Genomic Regulation, Barcelona Institute of Science and Technology (BIST), Barcelona, Spain; [2]Universitat Pompeu Fabra, Barcelona, Spain

Introduction

Over 170 post-transcriptional RNA modifications have been described and are common in all kingdoms of life. Liquid chromatography-tandem mass spectrometry is considered the gold standard method for the identification and quantification of these modifications but the analysis is complex due to the presence of positional isomers and complex chemical structures.
In this study, we show that the creation of information-rich spectral libraries using homologues nucleobases and deoxynucleosides facilitates the identification of ribonucleosides for which no commercial standards are available.

Method



Different mixes of commercially available modified and non-modified ribonucleosides (n=22), deoxynucleosides (n=22) and nucleobases (n=17) were prepared.

Mixes were injected in an Orbitrap Eclipse tribrid mass spectrometer with a chromatographic nLC system, 50-cm C18 column and 20 min gradient (A: 0.1% formic acid in water; B: 80% acetonitrile + 0.1% formic acid).

Nucleobases collision energy optimization was performed using a targeted MS2 method with increasing HCD normalized collision energies (NCE), ranging from 20 to 80.

RNA and DNA nucleosides were acquired with targeted high energy MS2-NCE 80 (MS2) and neutral-loss triggered MS3-NCE 40 (MS3).

Xcalibur and Skyline were used to select the fragment ions (relative intensity>10 and ppm<2.5) and create the high energy spectral libraries from RNA/DNA nucleosides and bases.

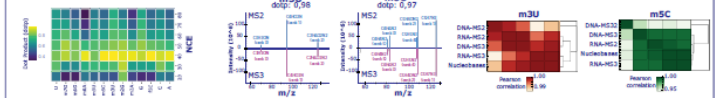
Deoxynucleosides and nucleobases information-rich spectral libraries were used to analyze ribonucleosides data.

Results

1. For all the nucleobases, NCE 40 fragmentation showed the best correlation with the ribonucleosides MS2 library.

2. Same fragmentation pattern was observed in ribonucleoside with high energy MS2 and MS3.

3. High correlation of libraries was observed between nucleobases and RNA/DNA nucleosides, both MS2 and MS3.



4. The high energy spectral libraries from nucleobases and deoxynucleosides enabled the differentation among positional isomers of ribonucleosides.

Conclusions

We demonstrate the utility of using spectral libraries containing information-rich spectra from nucleobases and deoxynucleosides for the identification of ribonucleosides.

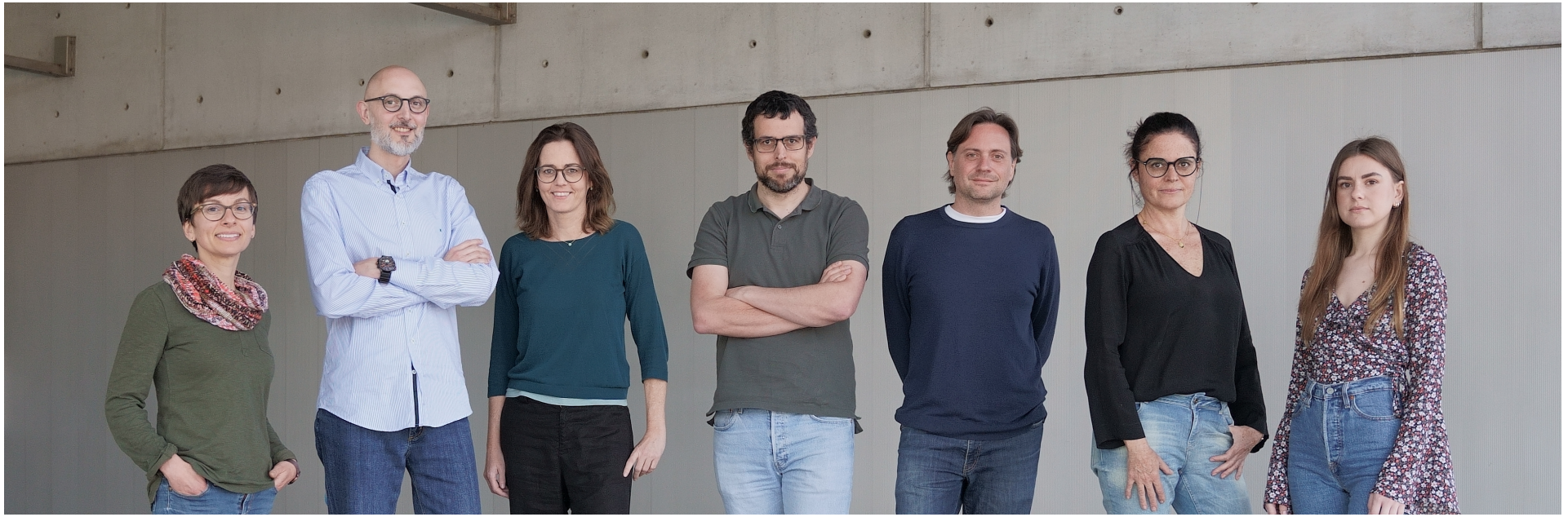
References:
1. Espadas G et al. High-performance nano-flow liquid chromatography column combined with high- and low-collision energy data-independent acquisition enables targeted and discovery identification of modified ribonucleotides by mass spectrometry. Journal of Chromatography A. Volume 1665, 22 (2022).

Guadalupe Espadas

Poster ID Number: 312495

Tuesday June 6: 10:30 - 11:30 am
12:30 - 2:30 pm

Acknowledgments



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