Organic and Biological Analytical Chemistry Group

LIÈGE université MolSys

CHIMIC CHEMICAL INFORMATION MINING

OBIACHEM Organic and Biological Analytical Chemistry Group

# Lipidomique Sanguine et Métabolomique du Cancer Colorectal par GC×GC-LR/HR-TOFMS

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1ST GREEN ANALYTICAL CHEMISTRY WORKSHOP
5/6 FEBRUARY, 2024 - NOVOTEL PARIS CHARENTON www.tgacworkshop-paris.com
THE GREEN ANALYTICAL CHEMISTRY WORKSHOP

# Acknowledgments

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CHEMICAL INFORMATION MINING

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CHU de Liège

Wallonie recherche SPW

LECO
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THE EXCELLENCE OF SCIENCE

RESTEK

JEOL

SUPELCO Analytical

# Complexes Matrices …







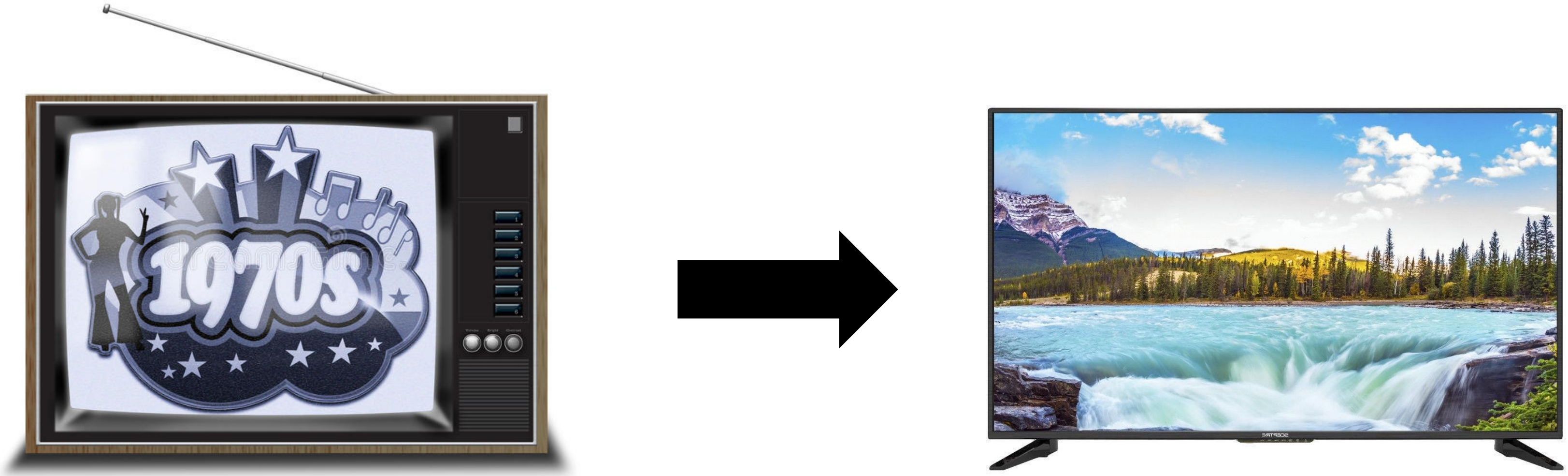






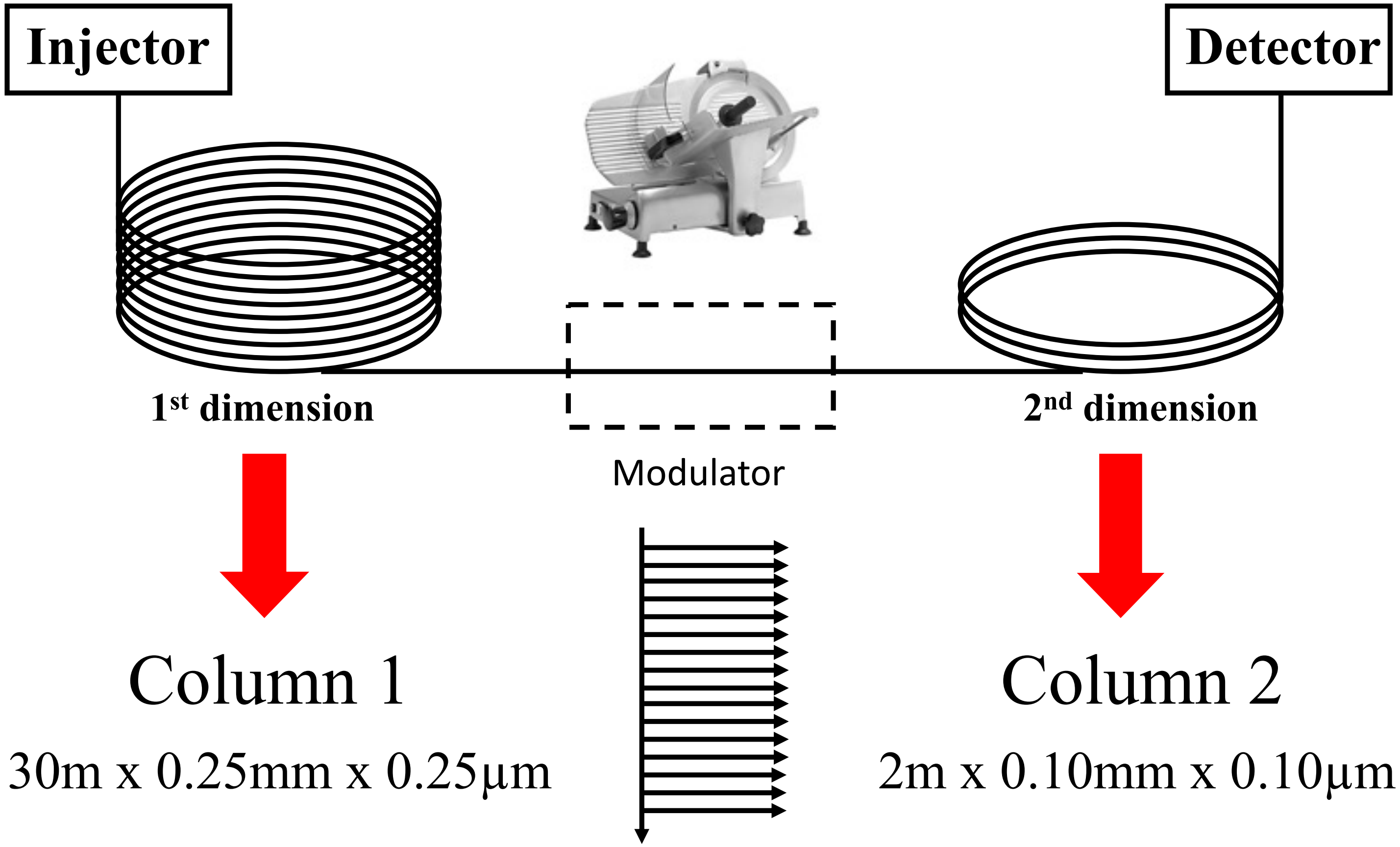


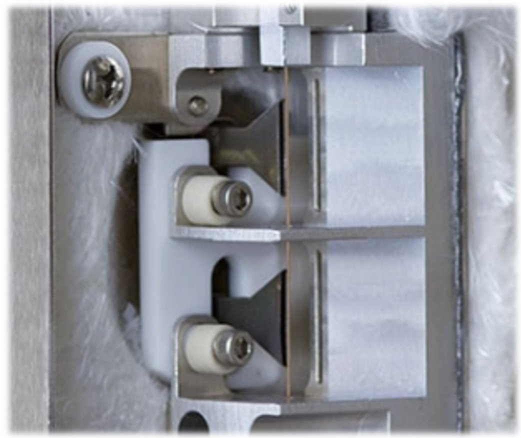


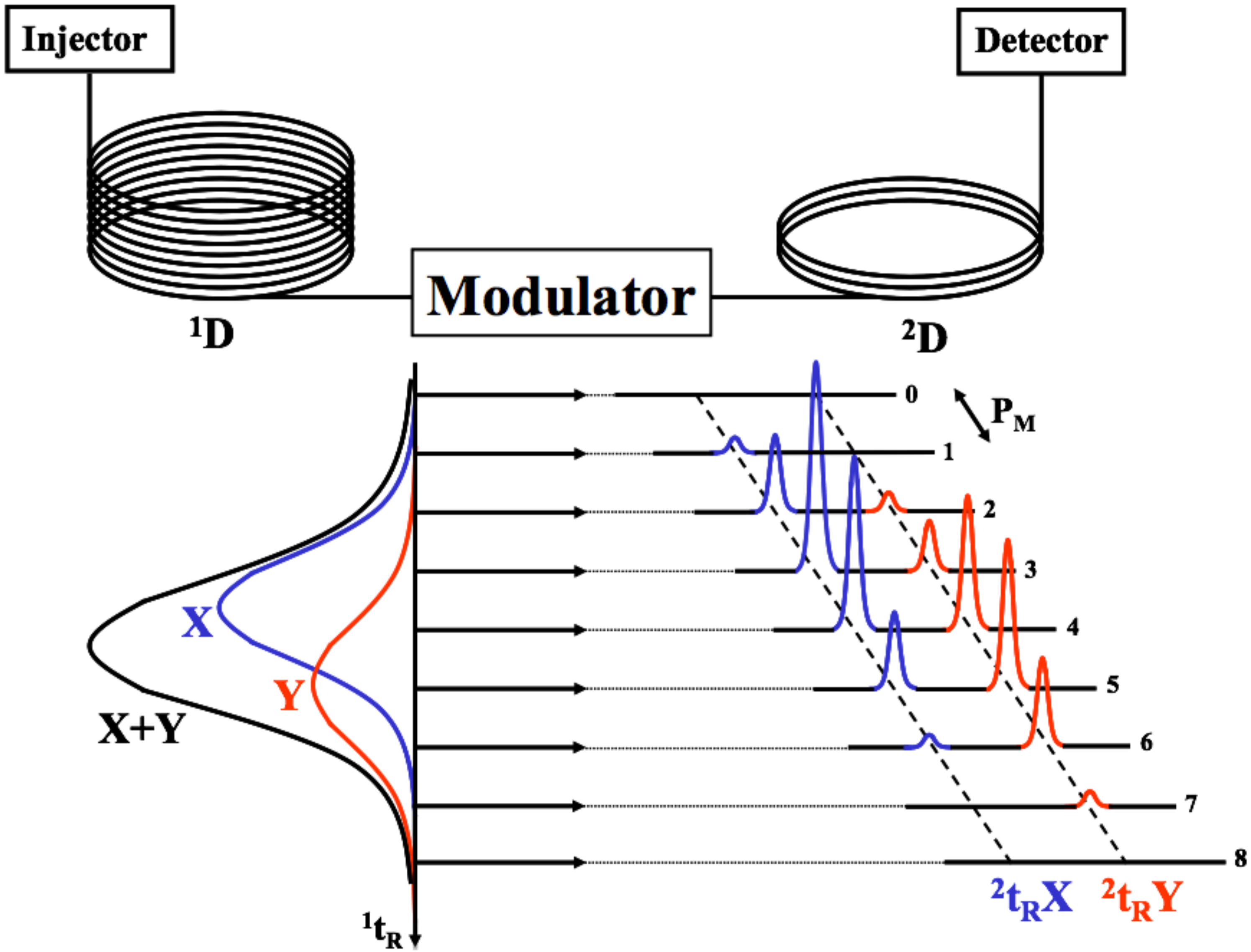



# GC×GC-(HR)TOFMS

# GC×GC-TOFMS Principle





Injector
Detector
Modulator
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2D
PM
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2
3
4
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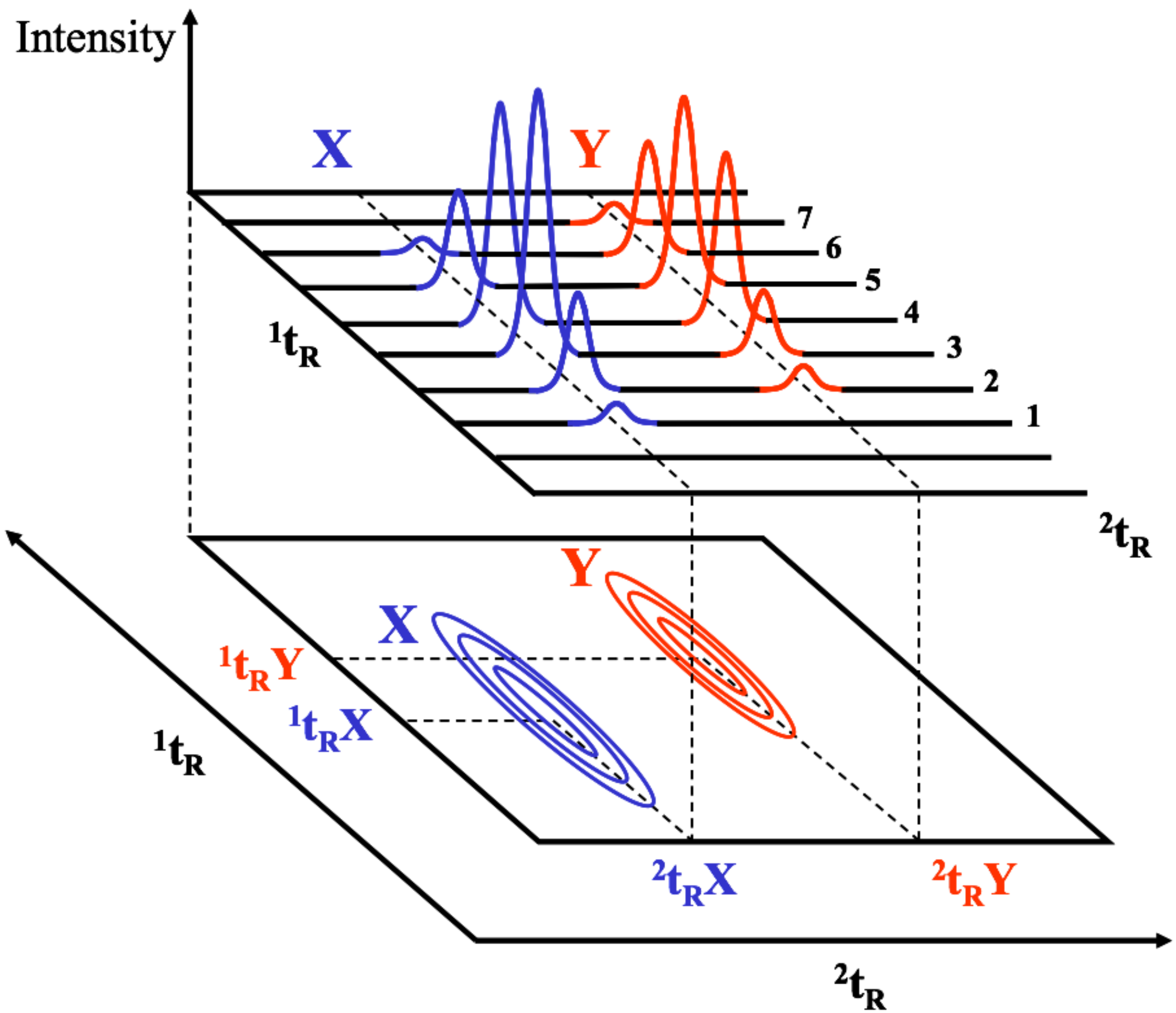
6
7
8
X
Y
X+Y
1tR
2tRX
2tRY


Intensity
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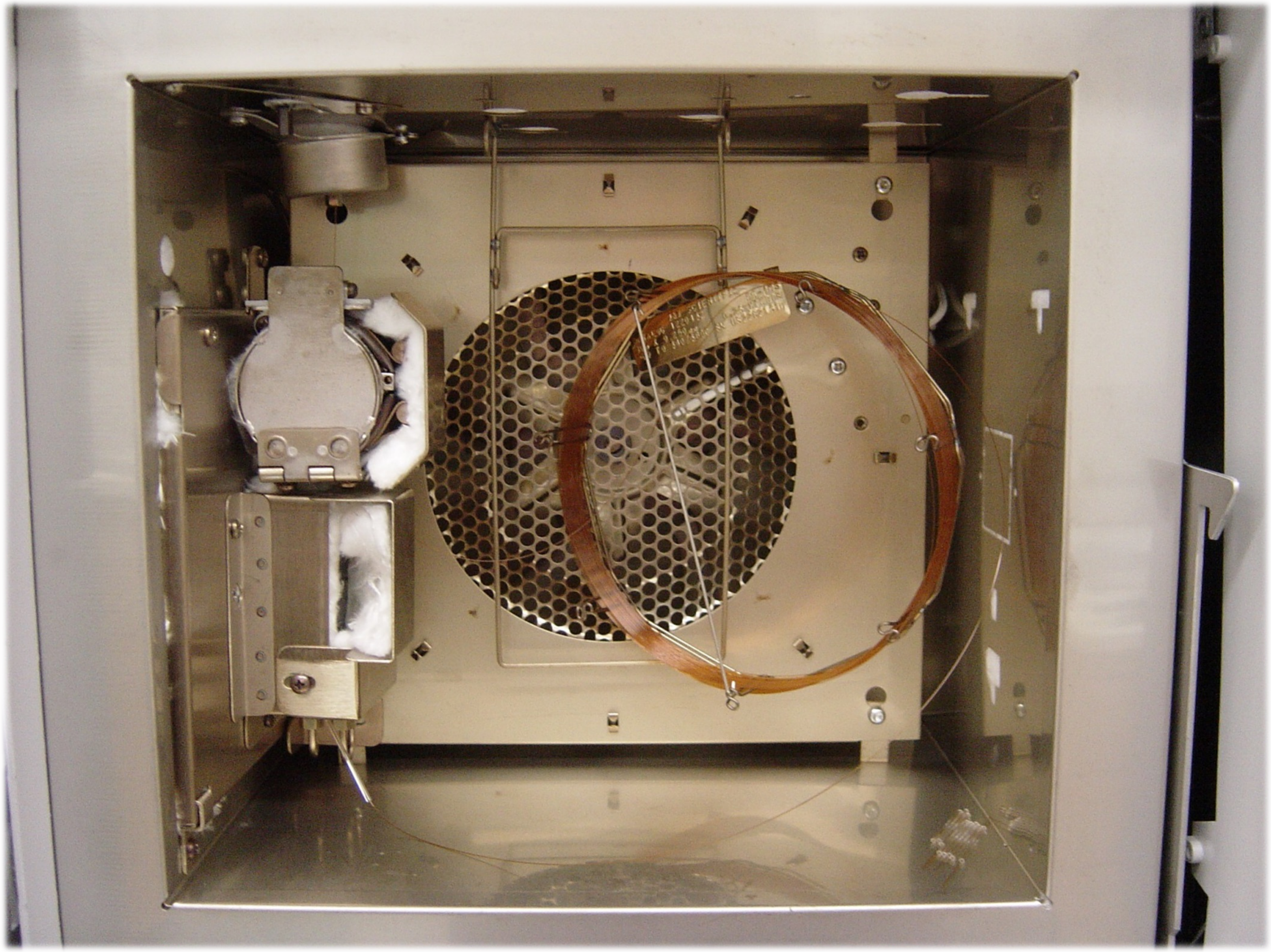
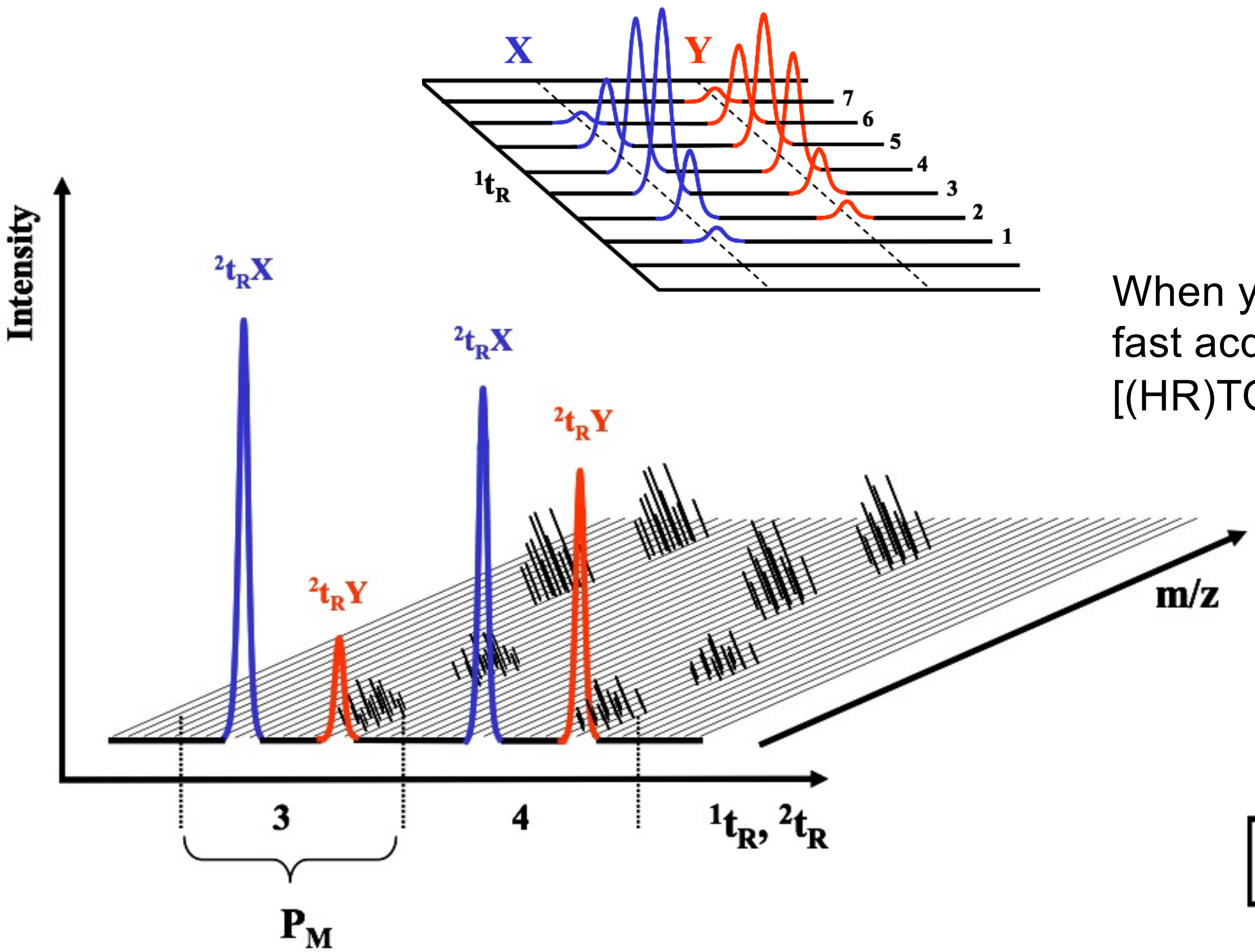
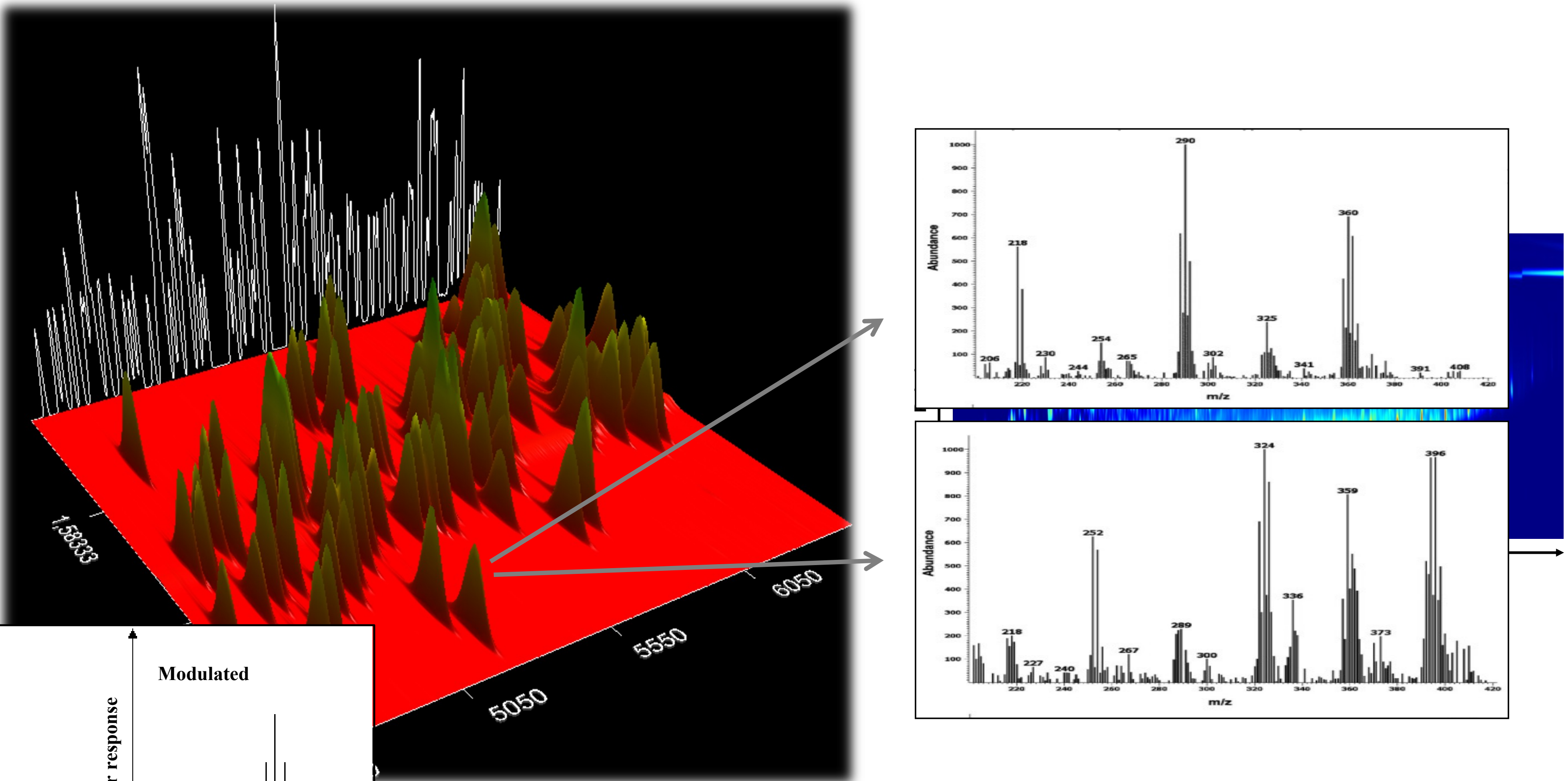
1tRX
1tR
2tRX
2tRY
2tR

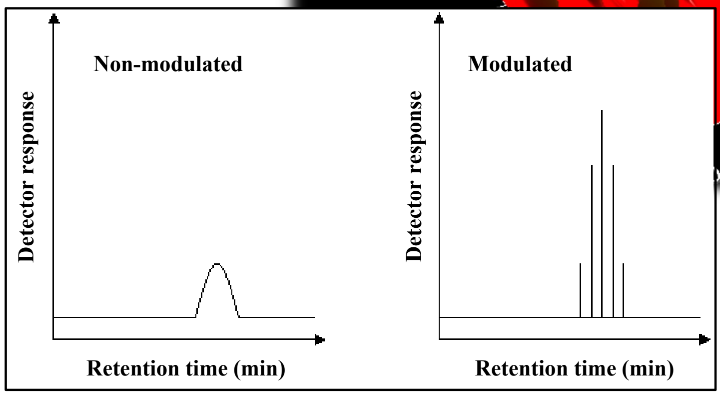


Inside the normal GC oven
-Modulator
-Secondary oven

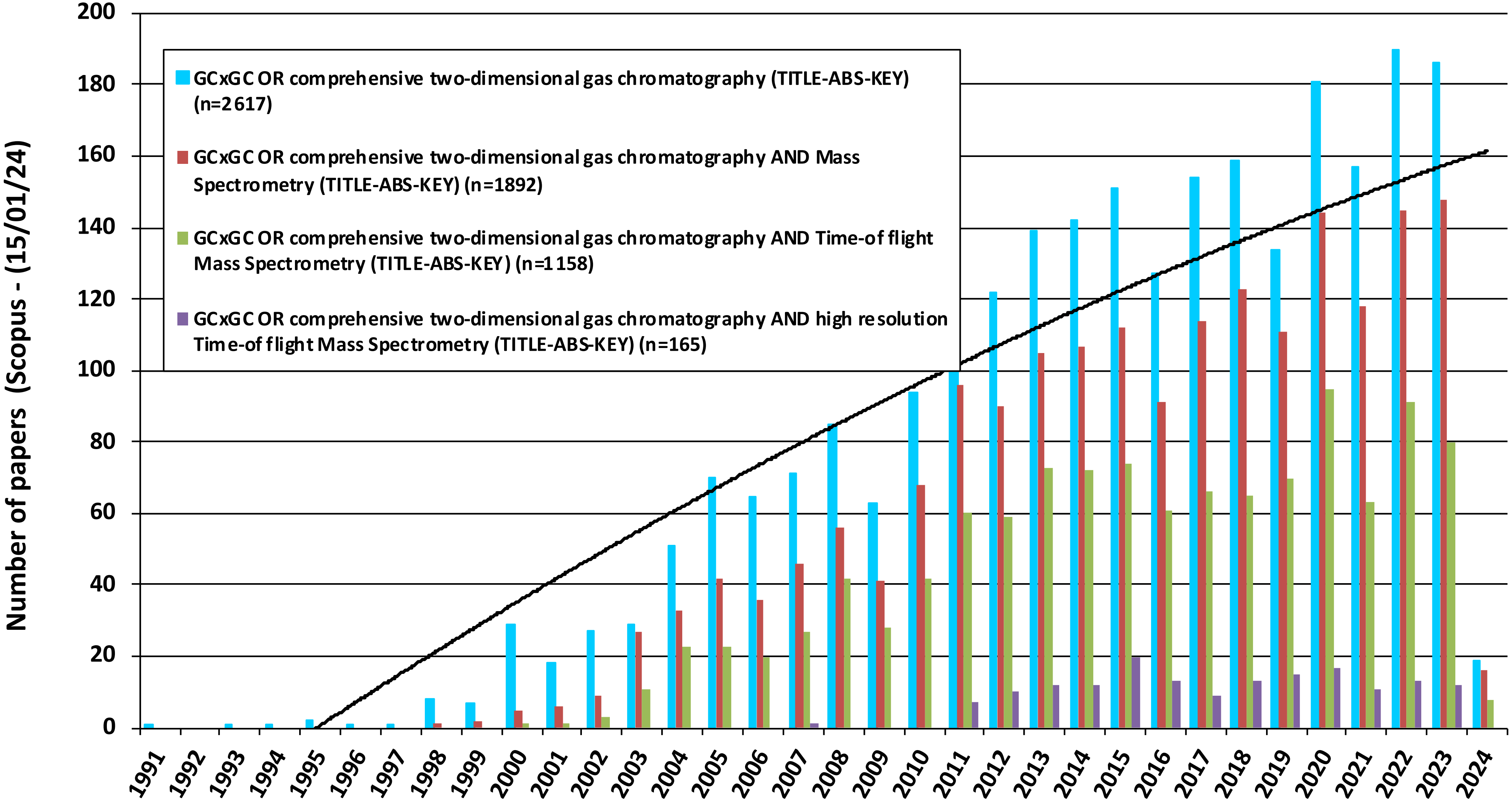



When your detector is a fast acquisition MS [(HR)TOFMS or qMS]




45 min run, 20-200 Hz, 30-400 amu = >1 $10^8$ data points...


GCxGC OR comprehensive two-dimensional gas chromatography (TITLE-ABS-KEY) (n=2617)
GCxGC OR comprehensive two-dimensional gas chromatography AND Mass Spectrometry (TITLE-ABS-KEY) (n=1892)
GCxGC OR comprehensive two-dimensional gas chromatography AND Time-of flight Mass Spectrometry (TITLE-ABS-KEY) (n=1158)
GCxGC OR comprehensive two-dimensional gas chromatography AND high resolution Time-of flight Mass Spectrometry (TITLE-ABS-KEY) (n=165)
Number of papers (Scopus - (15/01/24))
0
20
40
60
80
100
120
140
160
180
200
1991
1992
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1994
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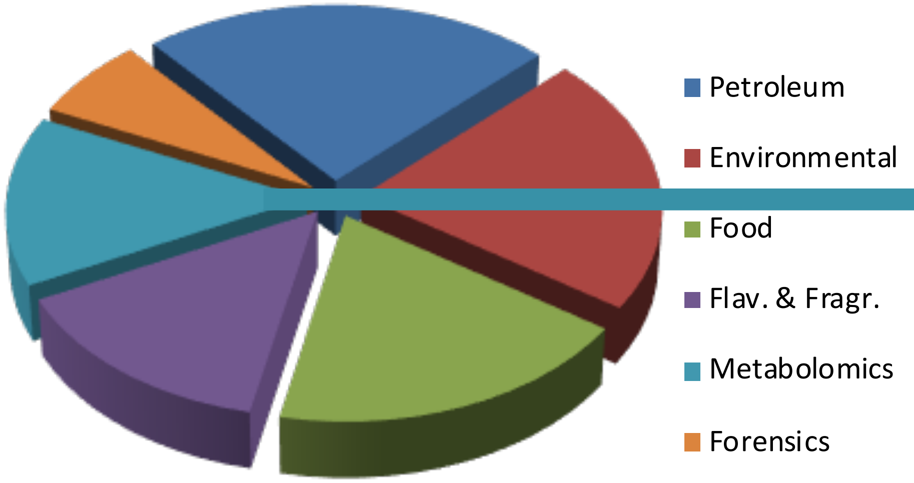
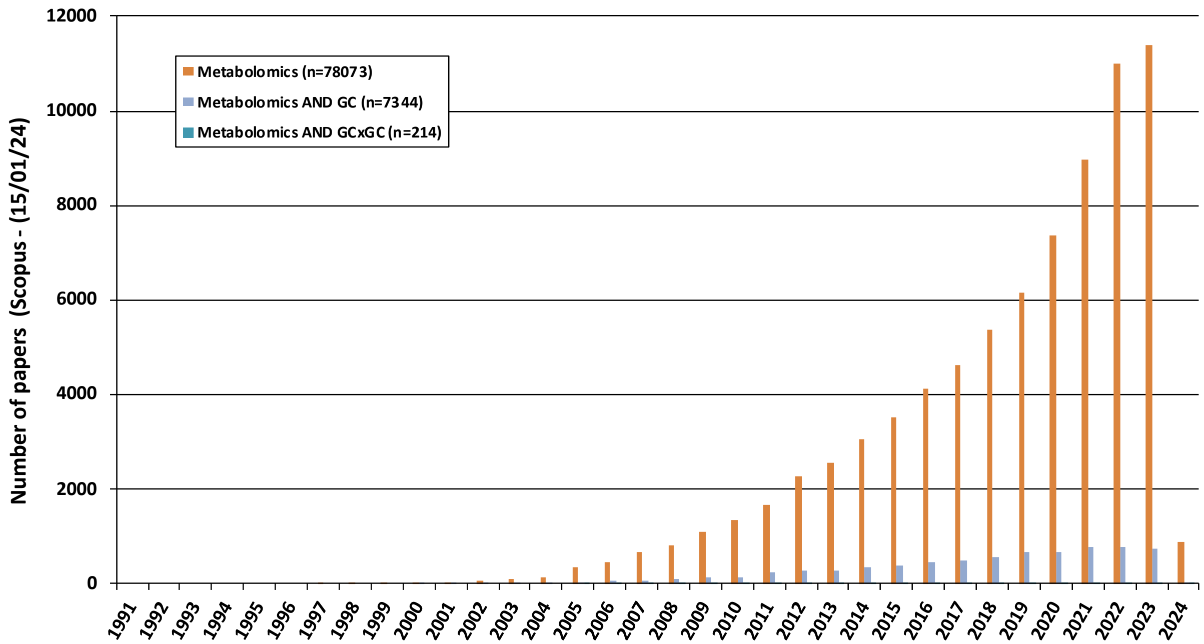
Scopus



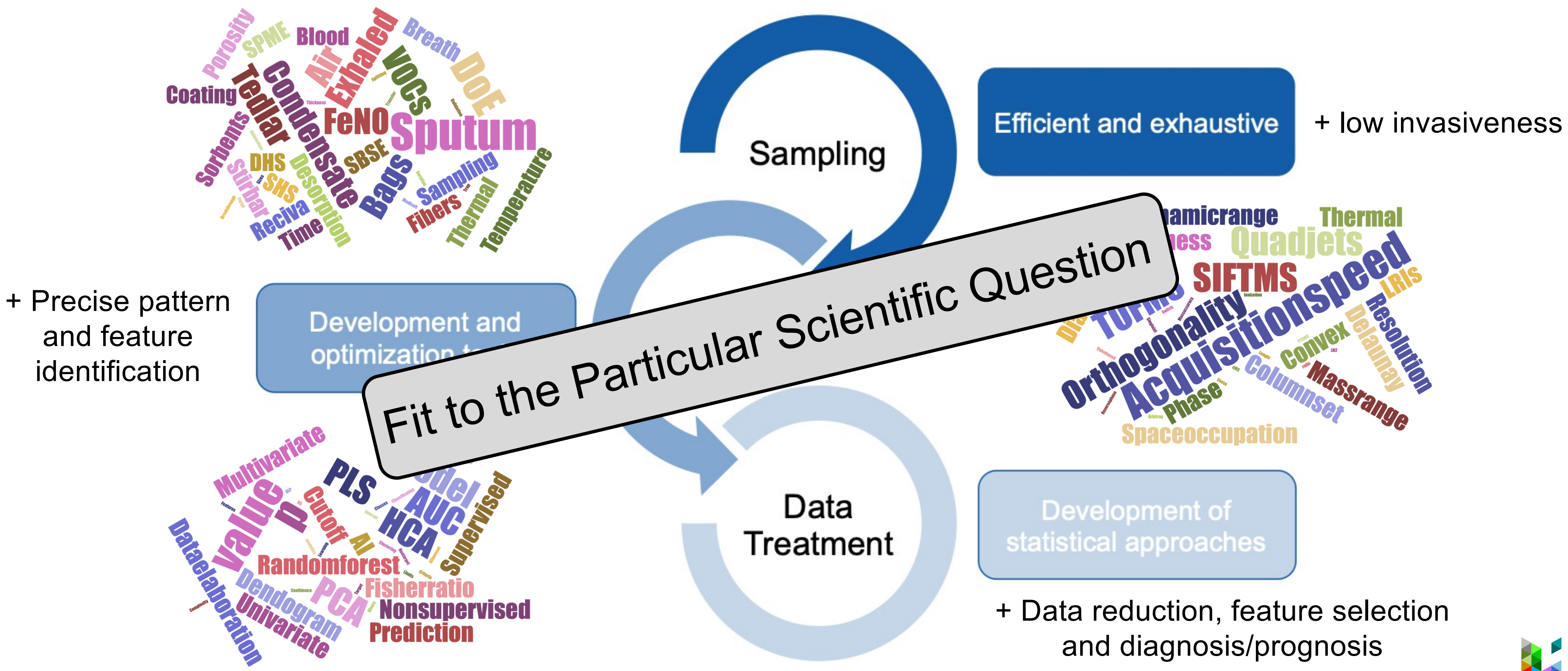



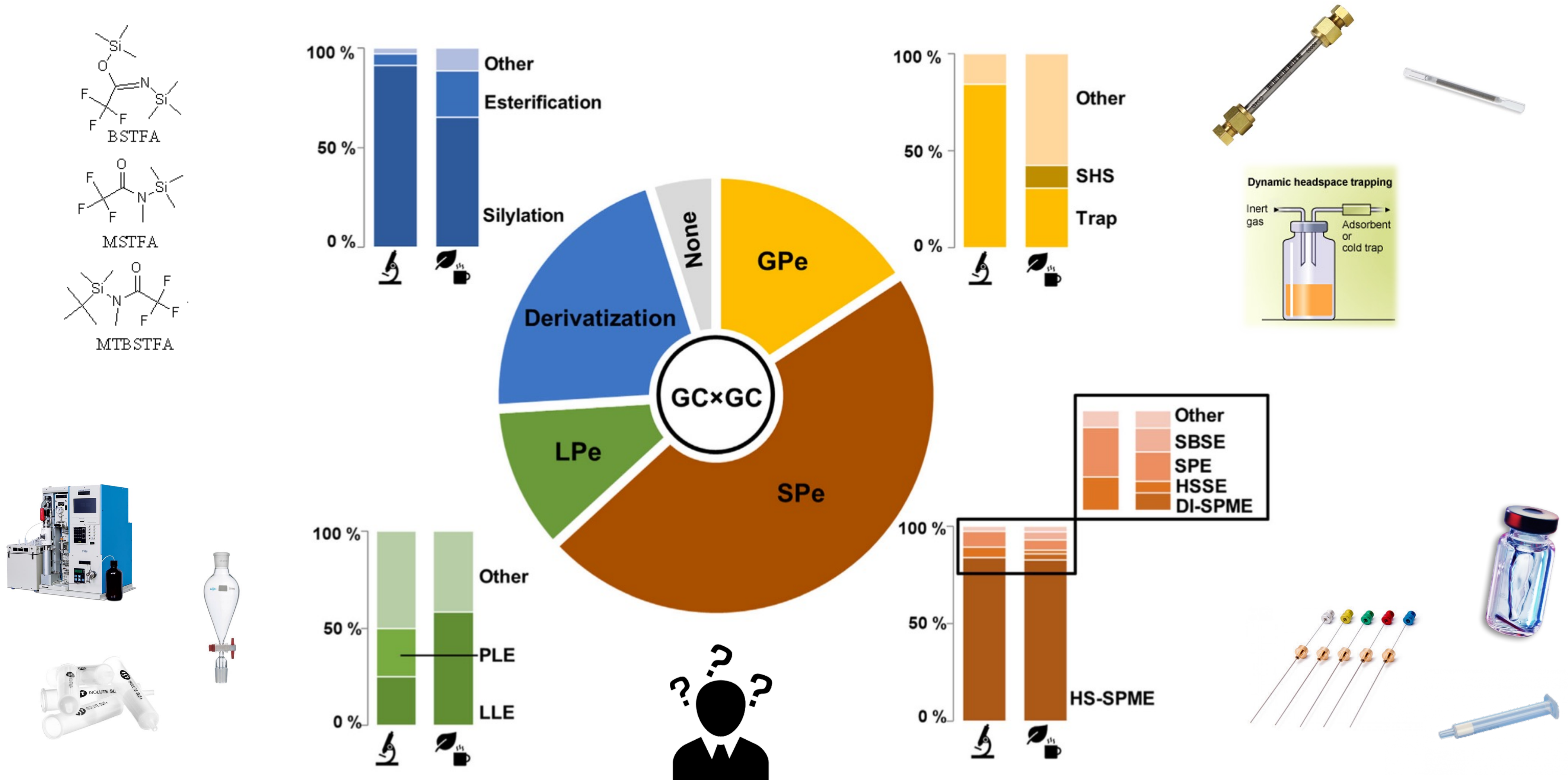

<10 % of metabolomics is done using GC...

<0,3% of metabolomics is done using GCxGC...

Scopus

# The Big Picture – Medical Applications

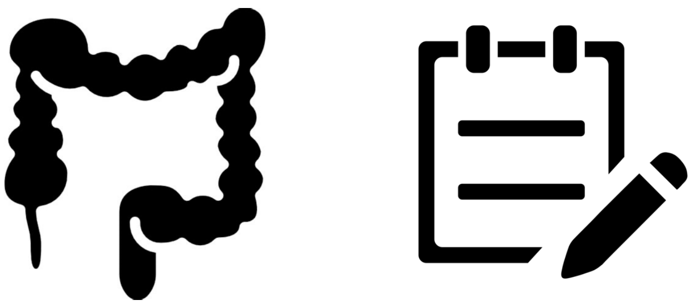





**The role of sample preparation in multidimensional gas chromatographic separations for non-targeted analysis with the focus on recent biomedical, food, and plant applications**

Flavio A. Franchina | Delphine Zanella | Lena M. Dubois | Jean-François Focant

JOURNAL OF SEPARATION SCIENCE



# Colorectal Cancer Screening

# Colorectal Cancer (CRC)

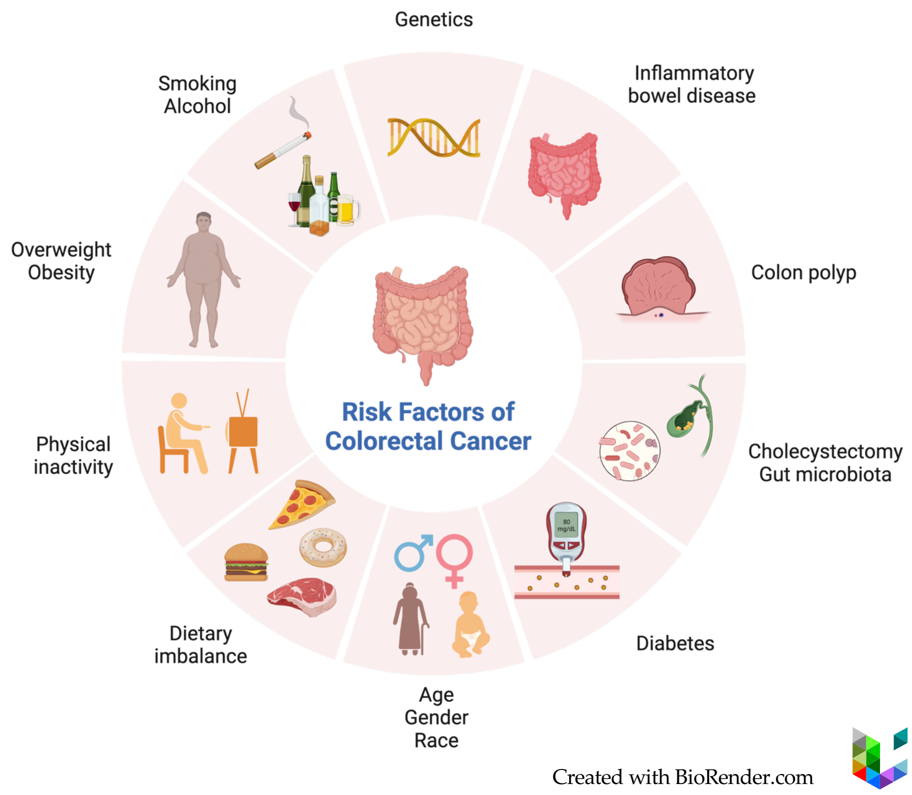
**Estimated New Cases**

| Males | | | Females | | |
|---|---|---|---|---|---|
| Prostate | 288,300 | 29% | Breast | 297,790 | 31% |
| Lung & bronchus | 117,550 | 12% | Lung & bronchus | 120,790 | 13% |
| Colon & rectum | 81,860 | 8% | Colon & rectum | 71,160 | 8% |
| Urinary bladder | 62,420 | 6% | Uterine corpus | 66,200 | 7% |
| Melanoma of the skin | 58,120 | 6% | Melanoma of the skin | 39,490 | 4% |
| Kidney & renal pelvis | 52,360 | 5% | Non-Hodgkin lymphoma | 35,670 | 4% |
| Non-Hodgkin lymphoma | 44,880 | 4% | Thyroid | 31,180 | 3% |
| Oral cavity & pharynx | 39,290 | 4% | Pancreas | 30,920 | 3% |
| Leukemia | 35,670 | 4% | Kidney & renal pelvis | 29,440 | 3% |
| Pancreas | 33,130 | 3% | Leukemia | 23,940 | 3% |
| **All Sites** | **1,010,310** | **100%** | **All Sites** | **948,000** | **100%** |

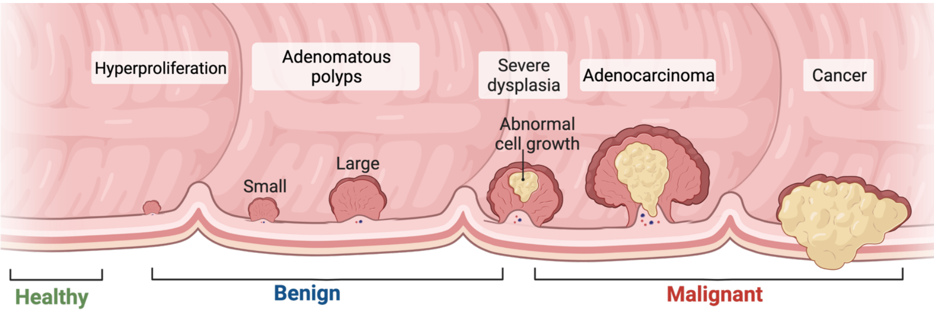
**Estimated Deaths**

| Males | | | Females | | |
|---|---|---|---|---|---|
| Lung & bronchus | 67,160 | 21% | Lung & bronchus | 59,910 | 21% |
| Prostate | 34,700 | 11% | Breast | 43,170 | 15% |
| Colon & rectum | 28,470 | 9% | Colon & rectum | 24,080 | 8% |
| Pancreas | 26,620 | 8% | Pancreas | 23,930 | 8% |
| Liver & intrahepatic bile duct | 19,000 | 6% | Ovary | 13,270 | 5% |
| Leukemia | 13,900 | 4% | Uterine corpus | 13,030 | 5% |
| Esophagus | 12,920 | 4% | Liver & intrahepatic bile duct | 10,380 | 4% |
| Urinary bladder | 12,160 | 4% | Leukemia | 9,810 | 3% |
| Non-Hodgkin lymphoma | 11,780 | 4% | Non-Hodgkin lymphoma | 8,400 | 3% |
| Brain & other nervous system | 11,020 | 3% | Brain & other nervous system | 7,970 | 3% |
| **All Sites** | **322,080** | **100%** | **All Sites** | **287,740** | **100%** |

Cancer Statistics 2023. CA Cancer J Clin 2023



# Classification & Monitoring Methods of CRC

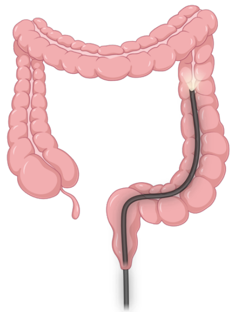



Created with BioRender.com

| | Endoscopy based | Stool based |
|---|---|---|
| Invasive | ✓ | ✗ |
| High sensitivity | ✓ | ✗ |
| Non-invasive | ✗ | ✓ |
| Low sensitivity | ✗ | ✓ |

Screening Methods approved by EU

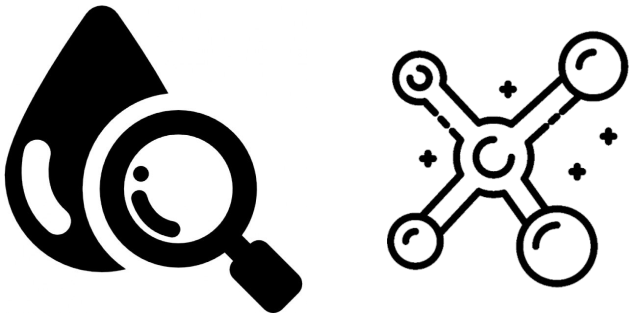
Colonoscopy



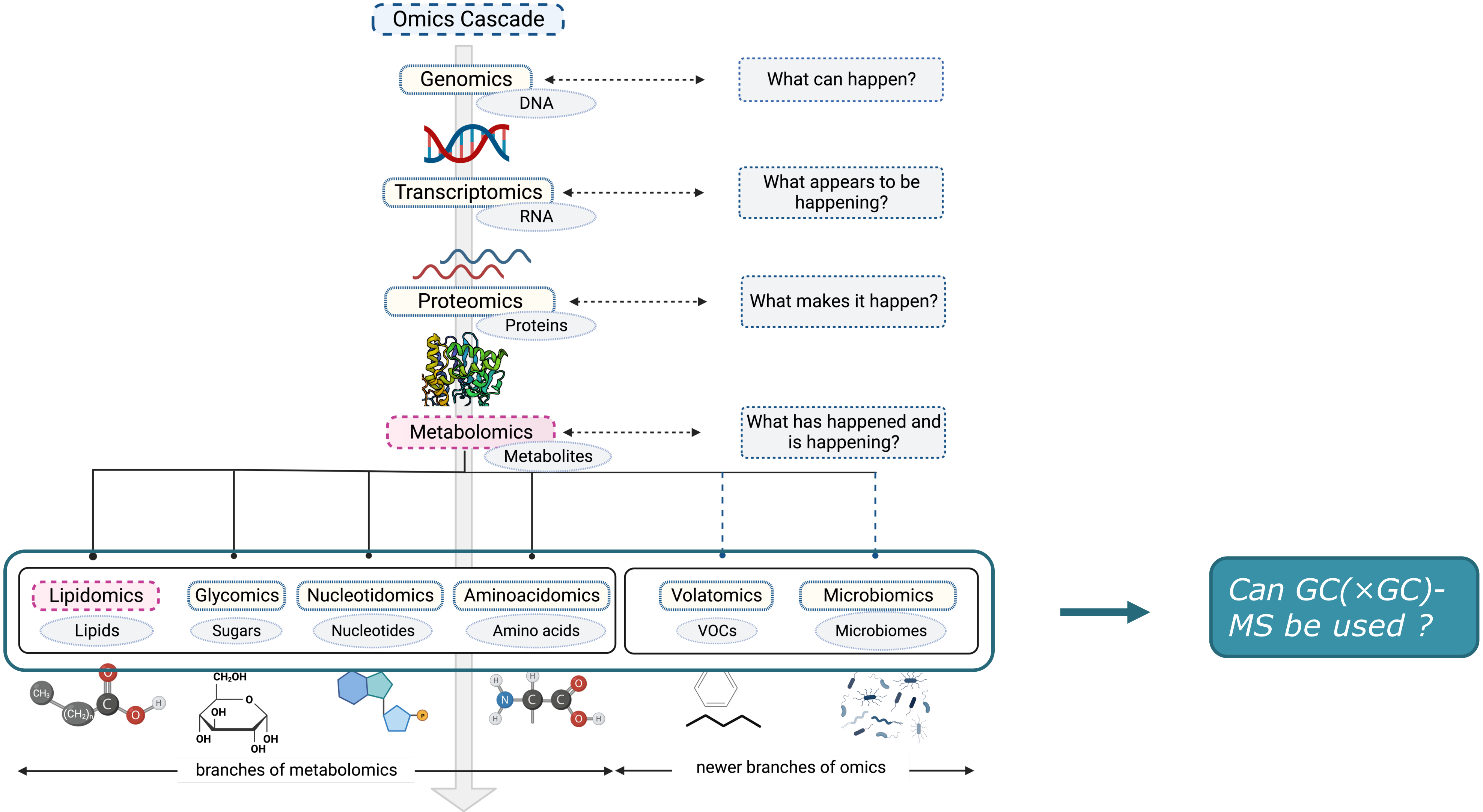
Flexible Sigmoidoscopy

gFOBT
FIT

*There is space for alternative low invasive techniques!*



# Blood Metabolomics & Lipidomics (Colorectal Cancer)


Omics Cascade
Genomics
DNA
What can happen?
Transcriptomics
RNA
What appears to be happening?
Proteomics
Proteins
What makes it happen?
Metabolomics
Metabolites
What has happened and is happening?
Lipidomics
Lipids
Glycomics
Sugars
Nucleotidomics
Nucleotides
Aminoacidomics
Amino acids
Volatomics
VOCs
Microbiomics
Microbiomes
branches of metabolomics
newer branches of omics
Can GC(×GC)-MS be used ?

# Analytical Strategy

Minimally invasive sampling

Global metabolomics

Lipidomics

Analysis

Large scale screening

Identification and annotation

# Sample Categories

**Serum Sample**

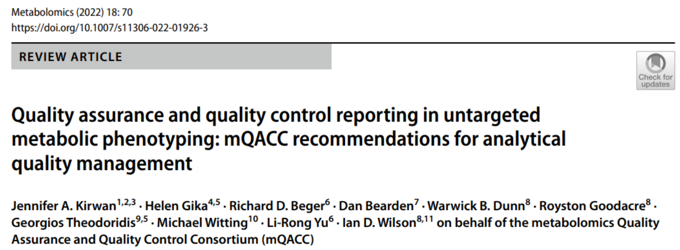
- Adenocarcinoma (n=20)
- Adenoma (n=23)
- Control (n=21)

*How does it translate in analytical count?*

64 samples
9 "bad"
\+
10 baseline QC
19 QCs
\+
6 NIST
SRM1950
\+
6 ID
standards
=
114
injections

***2 methods → 228 injections***

# QA/QC Implementation


Metabolomics (2022) 18: 70
https://doi.org/10.1007/s11306-022-01926-3

REVIEW ARTICLE

Check for updates

**Quality assurance and quality control reporting in untargeted metabolic phenotyping: mQACC recommendations for analytical quality management**

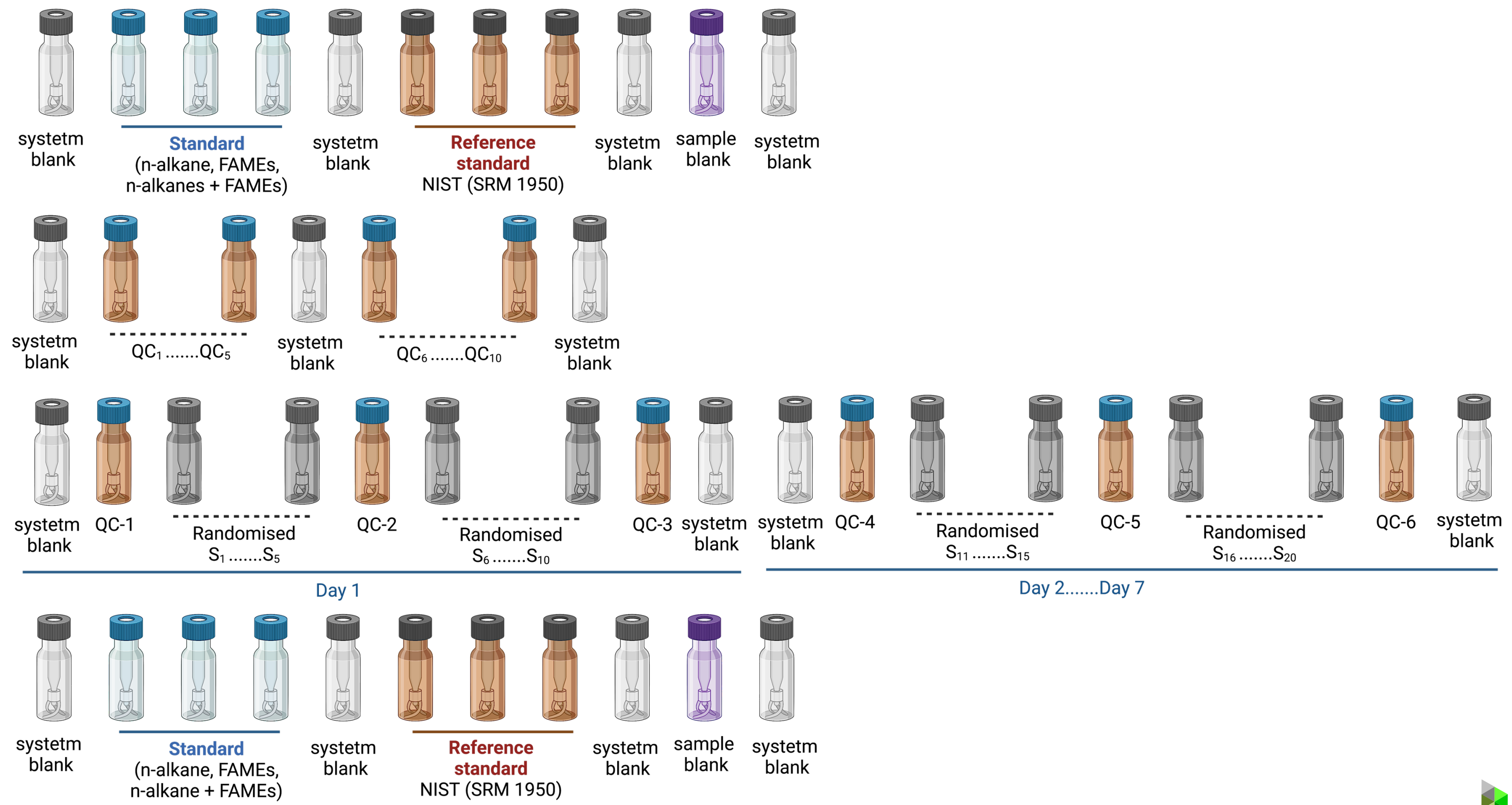
Jennifer A. Kirwan[1,2,3] · Helen Gika[4,5] · Richard D. Beger[6] · Dan Bearden[7] · Warwick B. Dunn[8] · Royston Goodacre[8] · Georgios Theodoridis[9,5] · Michael Witting[10] · Li-Rong Yu[6] · Ian D. Wilson[8,11] on behalf of the metabolomics Quality Assurance and Quality Control Consortium (mQACC)

**Step-1**
Identification assurance, check for carry over

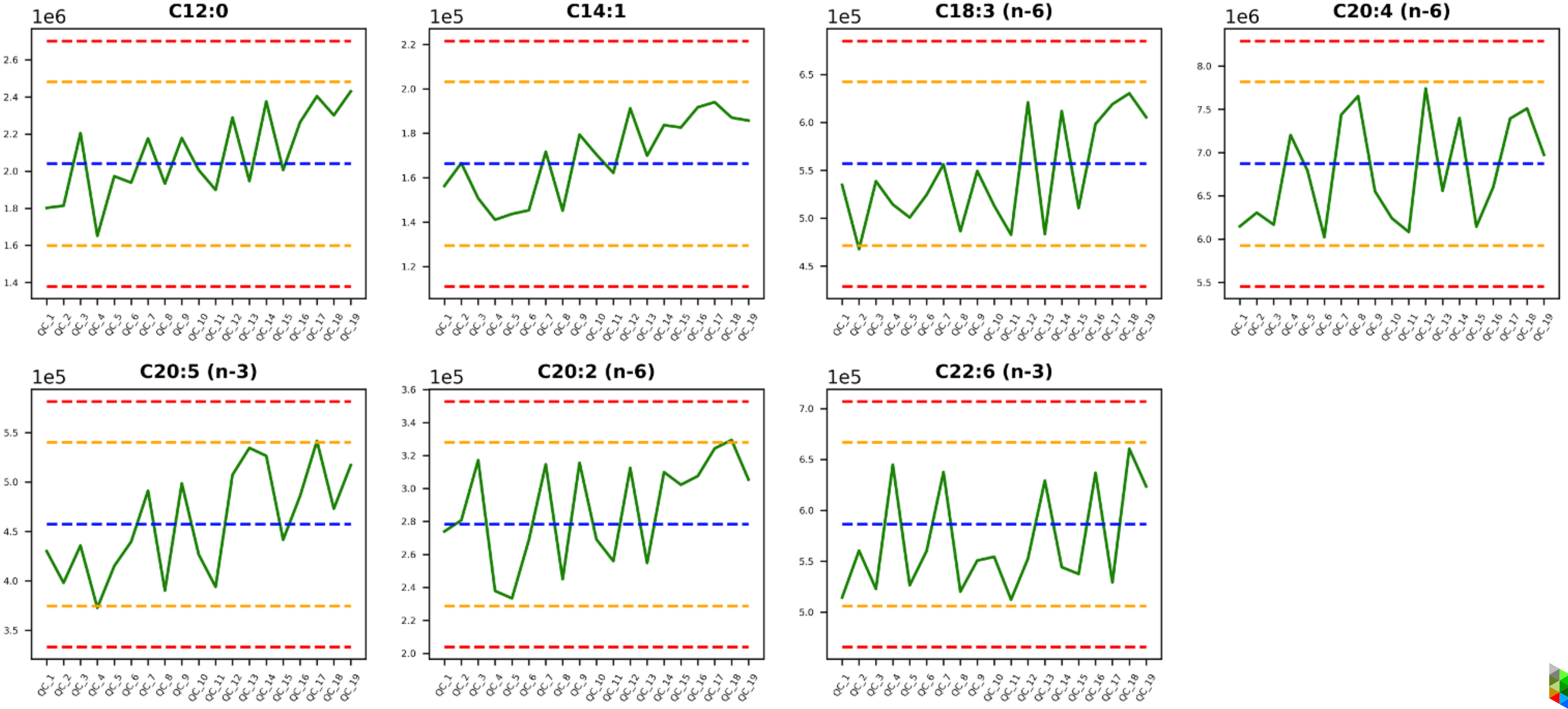
**Step-2**
Baseline for QC Chart

**Step-3**
Randomized sample analysis with QC samples

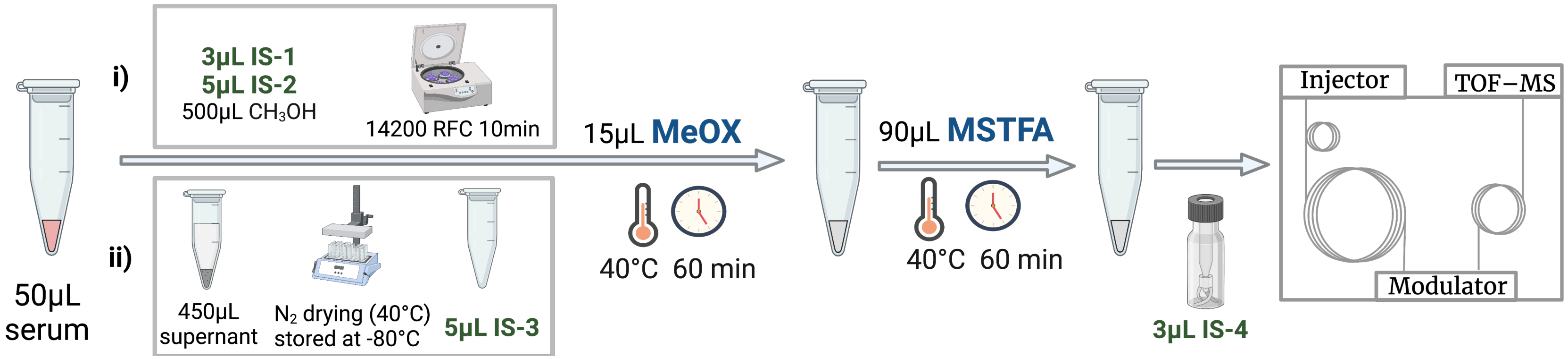
**Step-4**
Repeating step 1 to check the system stability



# QC Charting Lipidomics Pooled Human Plasma



# Sample Preparation Workflow (Metabolomics)





Journal of proteome research
Article
Cite This: *J. Proteome Res.* 2020, 19, 1013−1028
pubs.acs.org/jpr


**Untargeted Serum Metabolic Profiling by Comprehensive Two-Dimensional Gas Chromatography−High-Resolution Time-of-Flight Mass Spectrometry**


Nicolas Di Giovanni,[†] Marie-Alice Meuwis,[‡] Edouard Louis,[‡] and Jean-François Focant*[,†]

[†]Department of Chemistry, Organic and Biological Analytical Chemistry Group, Quartier Agora, University of Liège, Allée du Six Août, B6c, B-4000 Liège (Sart Tilman), Belgium

[‡]GIGA institute, Translational Gastroenterology and CHU de Liège, Hepato-Gastroenterology and Digestive Oncology, Quartier Hôpital, University of Liège, Avenue de l'Hôpital 13, B34-35, B-4000 Liège, Belgium

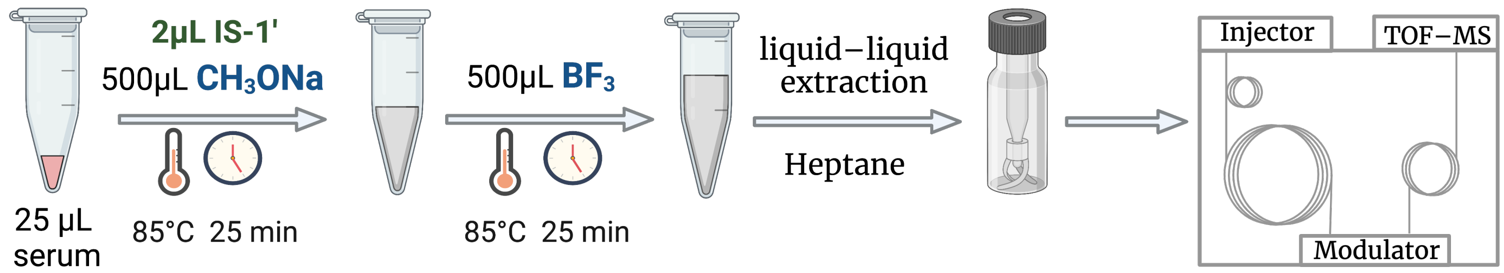

*Supporting Information*

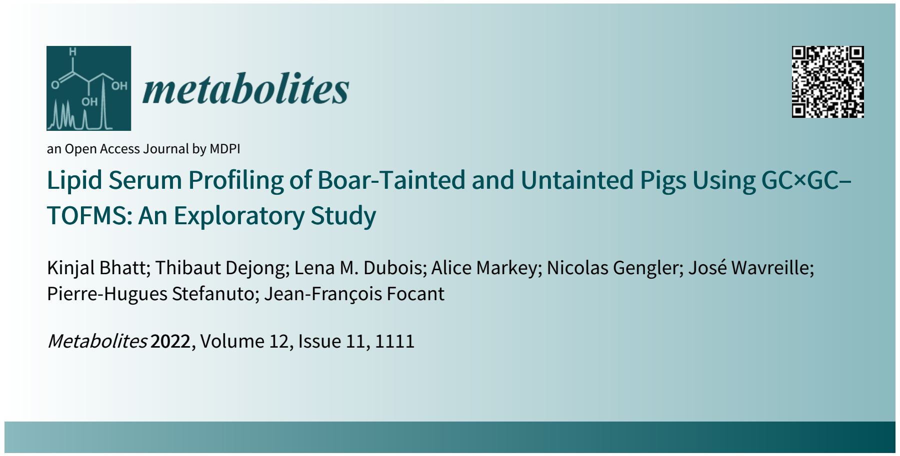
230 analytes (a.a., RCOOH, carboH, FAMEs, nucleosides)

↓

8 selected features

# Sample Preparation Workflow (Lipidomics)





metabolites

an Open Access Journal by MDPI

Lipid Serum Profiling of Boar-Tainted and Untainted Pigs Using GC×GC–TOFMS: An Exploratory Study

Kinjal Bhatt; Thibaut Dejong; Lena M. Dubois; Alice Markey; Nicolas Gengler; José Wavreille; Pierre-Hugues Stefanuto; Jean-François Focant

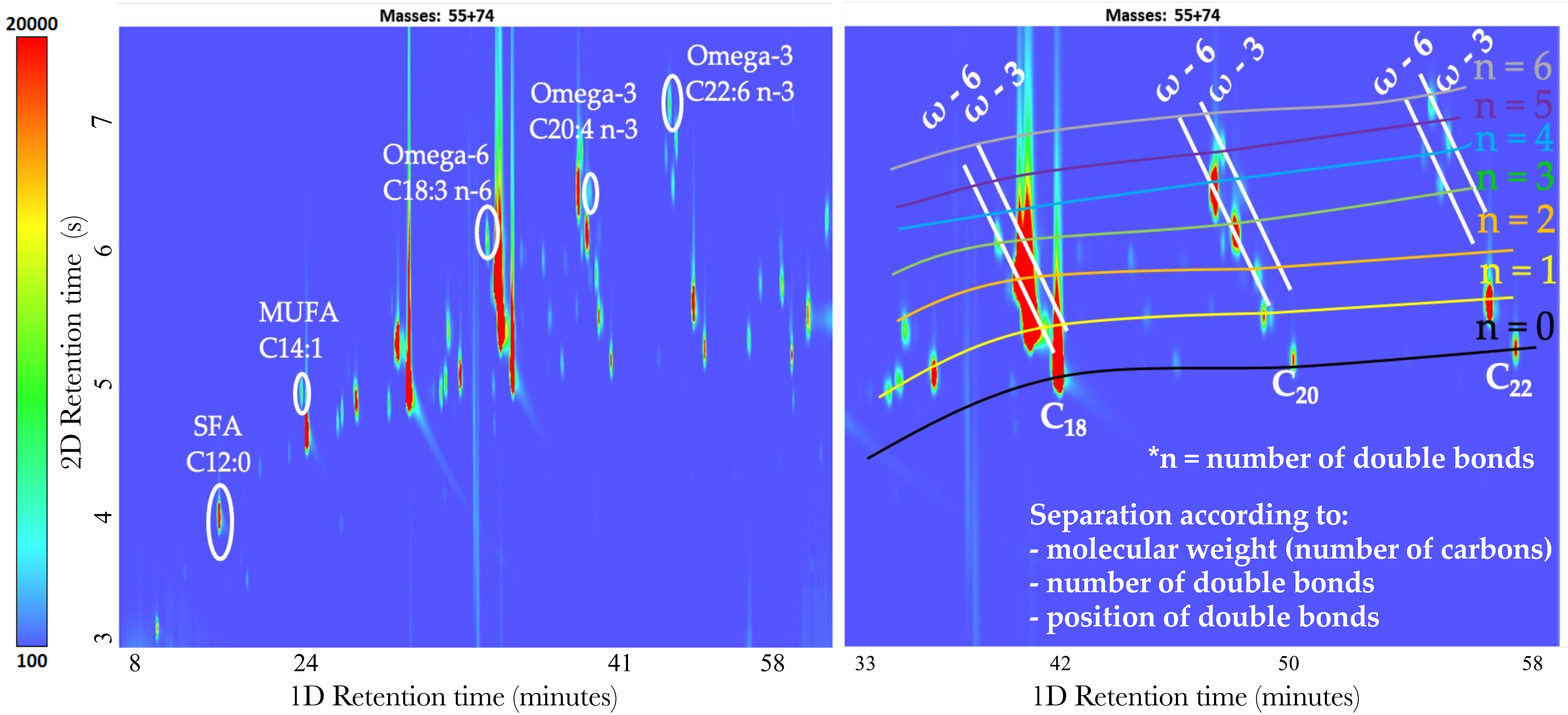
*Metabolites* 2022, Volume 12, Issue 11, 1111

40 analytes (FAMEs)

↓

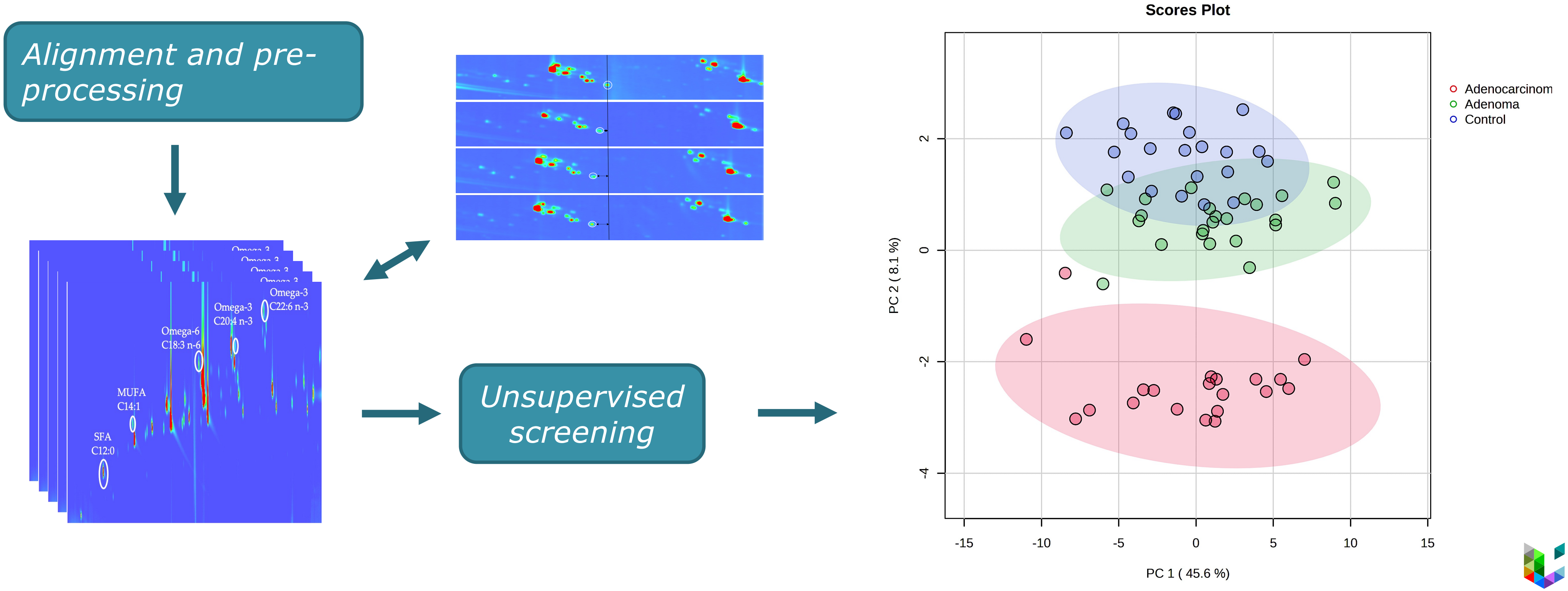
8 selected features

# Structured Separation of FAMEs (NIST SRM 1950)



# Unsupervised Lipidomics PCA

<u>40 analytes </u>(13 SFA, 9 MUFA, 6 PUFA ω-3, 8 PUFA ω-6, 1 PUFA ω-9, 3 cholestadiene isomers)

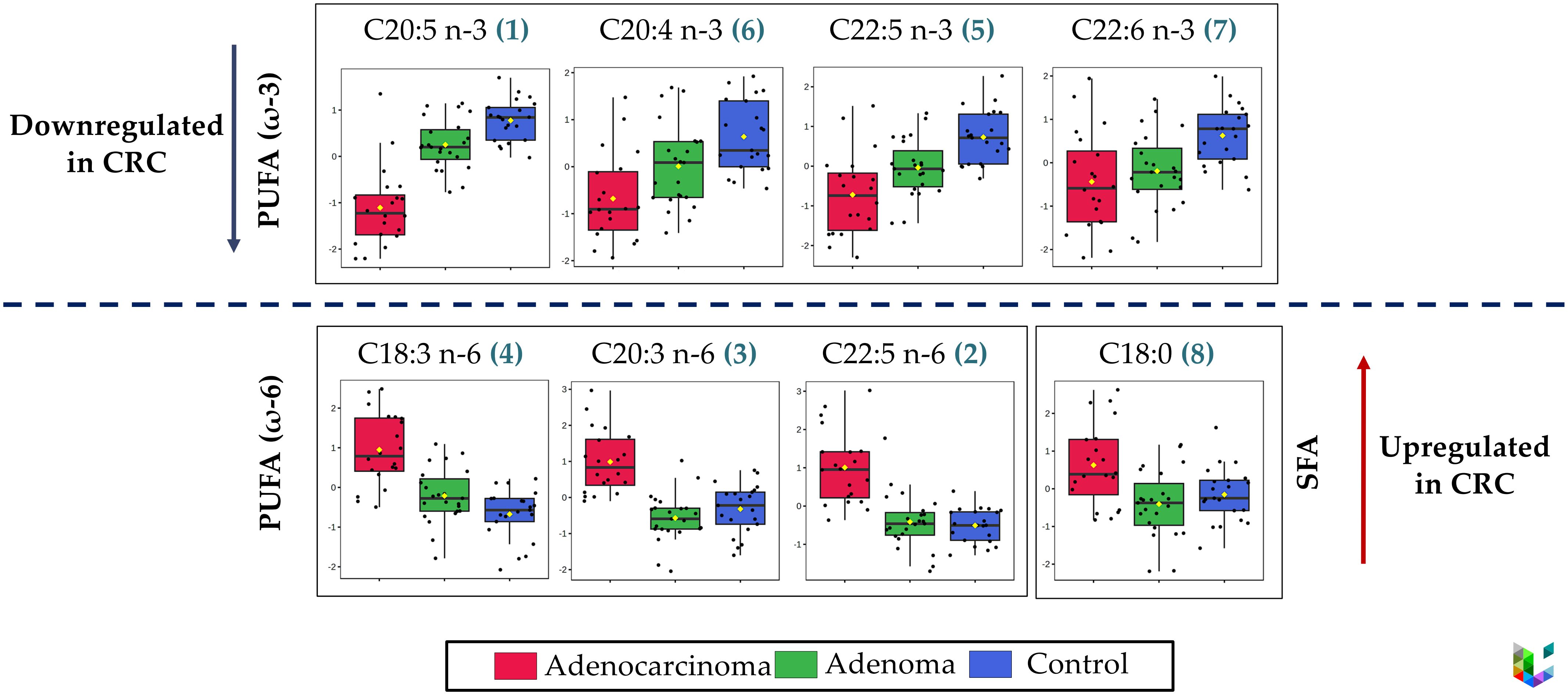


# Feature Selection Lipidomics (PLS-DA, RF)

Top 8 selected features

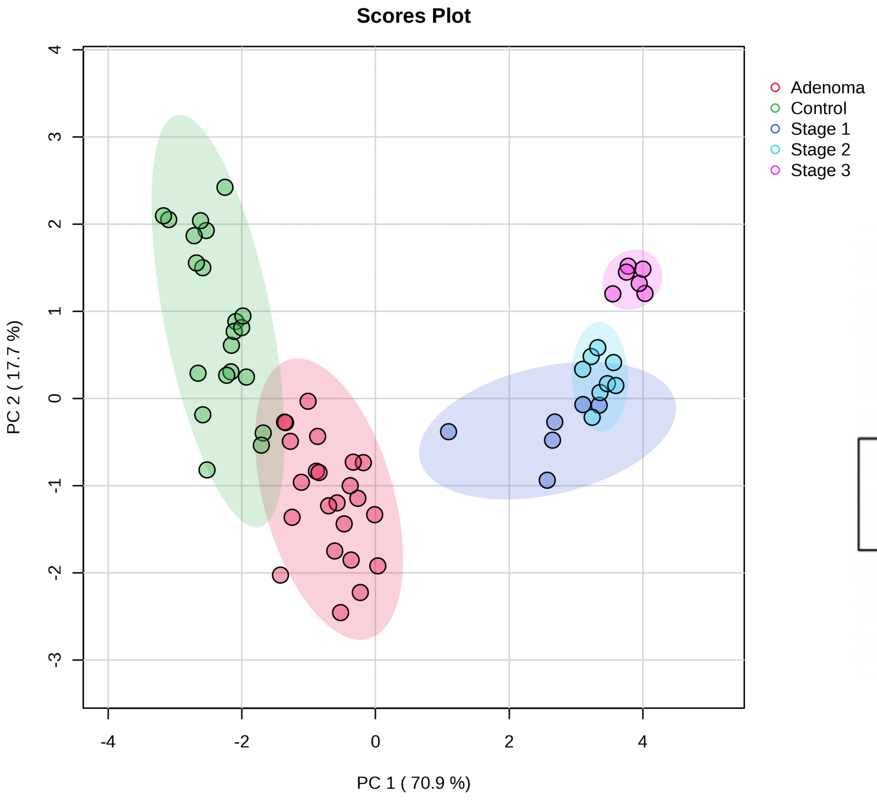
| Potential ID | Class | CAS | Similarity | Reverse | Probability (%) | Δ LRI | Mass accuracy (ppm) | (FDR)<0.05 | VIP Score (>1) | RF, MDA (>0.008) |
|---|---|---|---|---|---|---|---|---|---|---|
| C20:4 n-3 | PUFA (ω-3) | 132712-70-0 | 882 | 875 | 30.1 | 12 | - | $2.6 \times 10^{-4}$ | 1.9847 | 0.019331 |
| **C20:5 n-3*** | PUFA (ω-3) | 2734-47-6 | 890 | 892 | 58.5 | 8 | -1.17 | $1.1 \times 10^{-11}$ | 2.8251 | 0.079084 |
| C22:5 n-3 | PUFA (ω-3) | 108698-02-8 | 851 | 851 | 74.1 | 13 | - | $5.3 \times 10^{-3}$ | 2.1896 | 0.032492 |
| **C22:6 n-3*** | PUFA (ω-3) | 2566-90-7 | 900 | 910 | 72.9 | 16 | -1.01 | $2.0 \times 10^{-3}$ | 1.6093 | 0.019366 |
| **C18:3 n-6*** | PUFA (ω-6) | 16326-32-2 | 875 | 875 | 56.3 | 11 | 0.74 | $9.7 \times 10^{-8}$ | 2.4366 | 0.046362 |
| **C20:3 n-6*** | PUFA (ω-6) | 21061-10-9 | 919 | 907 | 69.7 | 12 | -1.03 | $7.5 \times 10^{-8}$ | 1.9479 | 0.036985 |
| C22:5 n-6 | PUFA (ω-6) | - | 897 | 883 | 28.6 | 18 | - | $7.5 \times 10^{-8}$ | 2.2631 | 0.044829 |
| **C18:0*** | SFA | 112-61-8 | 925 | 955 | 84.3 | 1 | 0.37 | $7.0 \times 10^{-3}$ | 1.1598 | 0.0087911 |

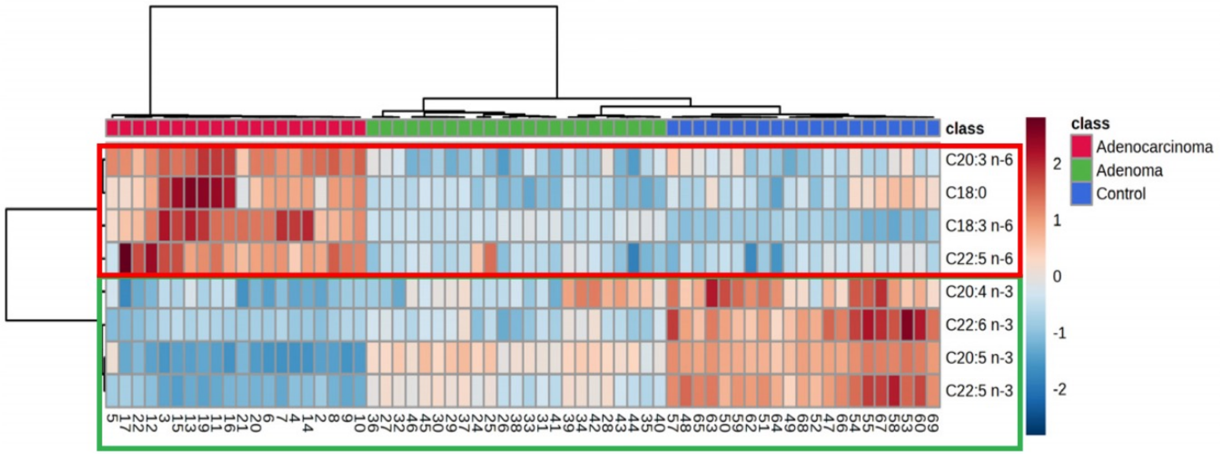
# Feature Selection Lipidomics (PLS-DA, RF)



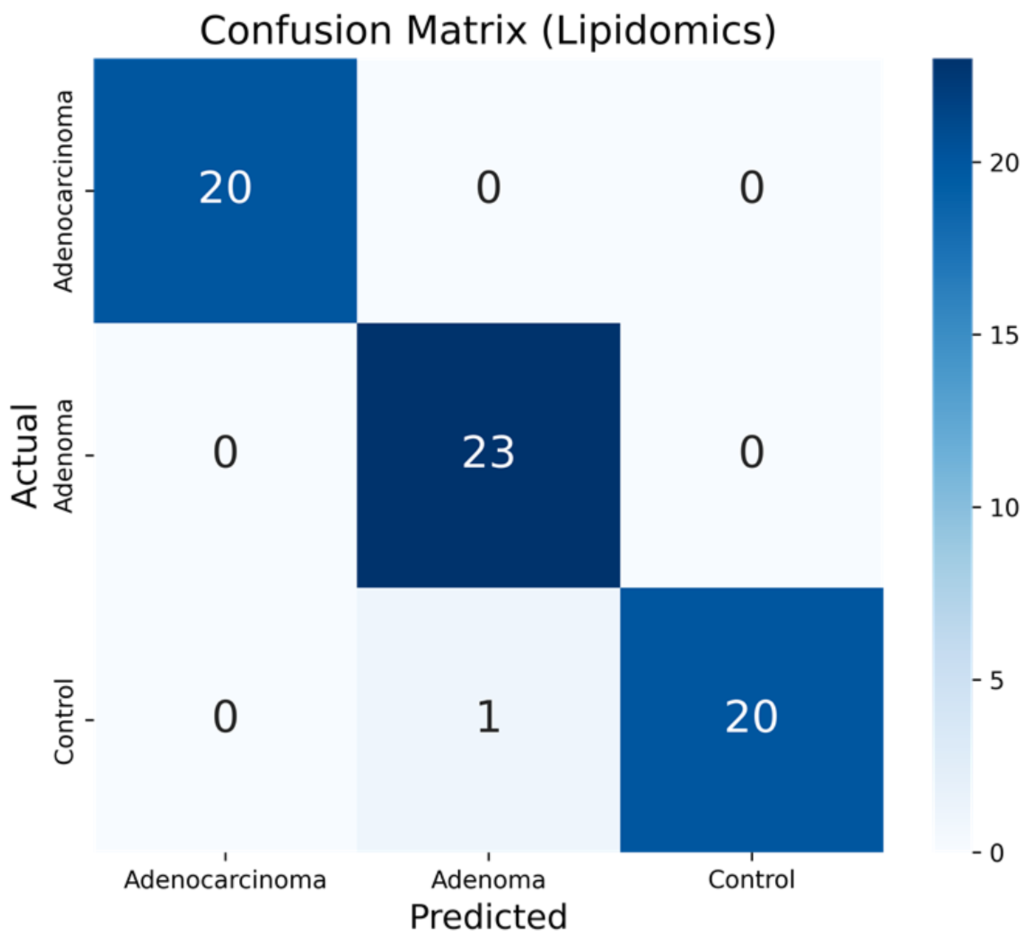
# Feature Selection Lipidomics (PLS-DA, RF) PCA

Top 8 selected features



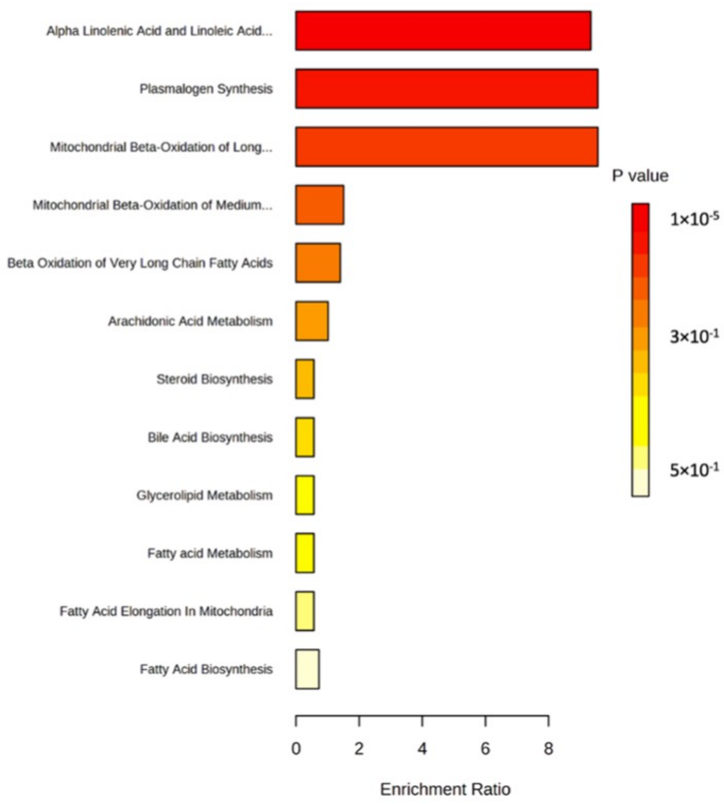
# Random Forest Cross-Validation Lipidomics




Random selection
2/3 data set for building
1/3 data for validation

OOB error 0,015

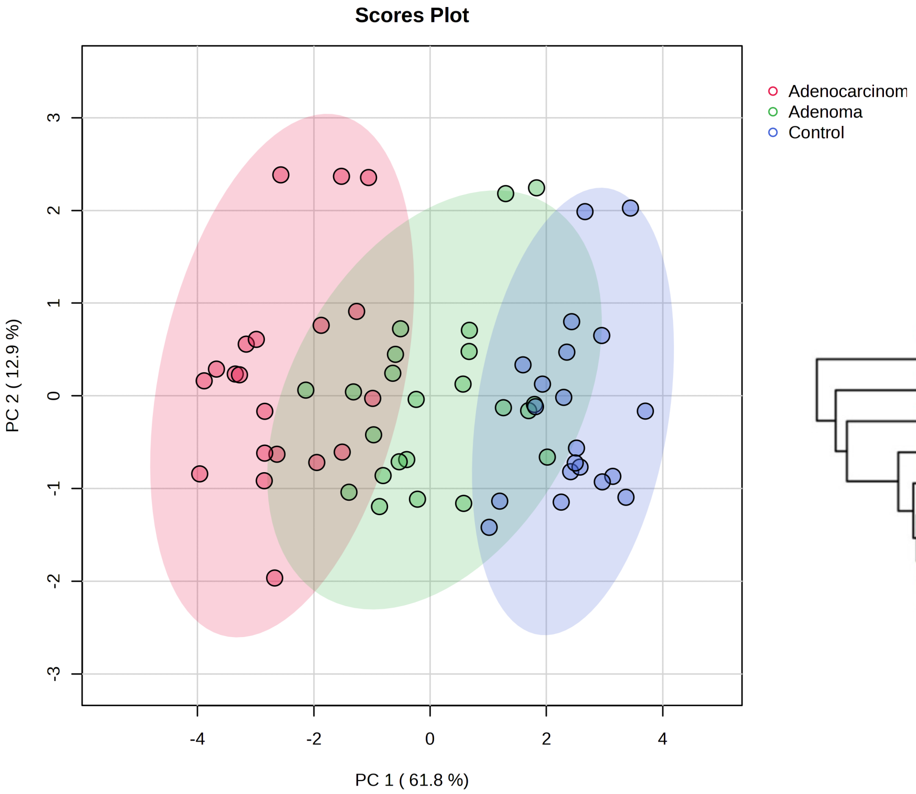
# Quantitative Enrichment Analysis (QEA)

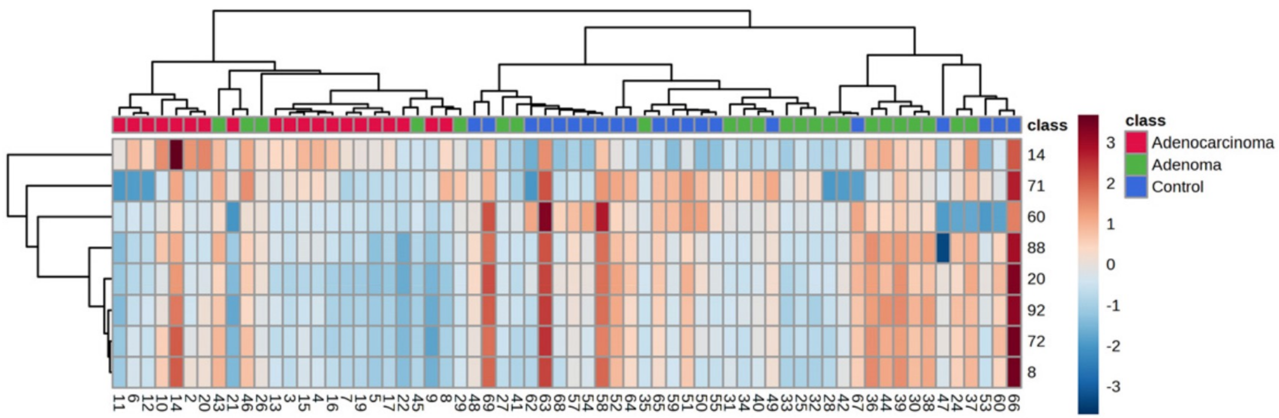



Lipidomics

# Feature Selection Metabolomics (PLS-DA, RF) PCA

Top 105 selected features



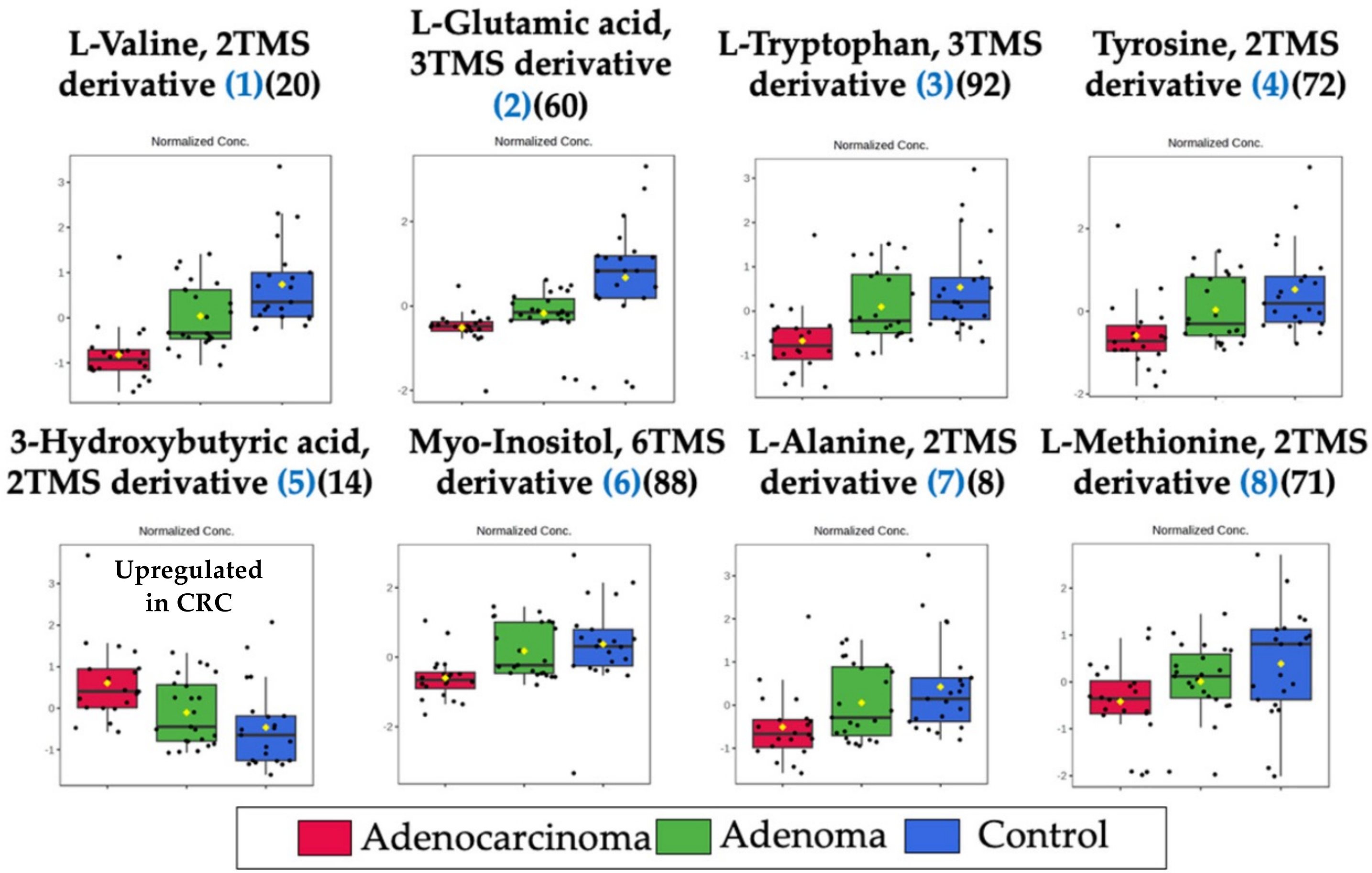



# Feature Selection Metabolomics (PLS-DA, RF)

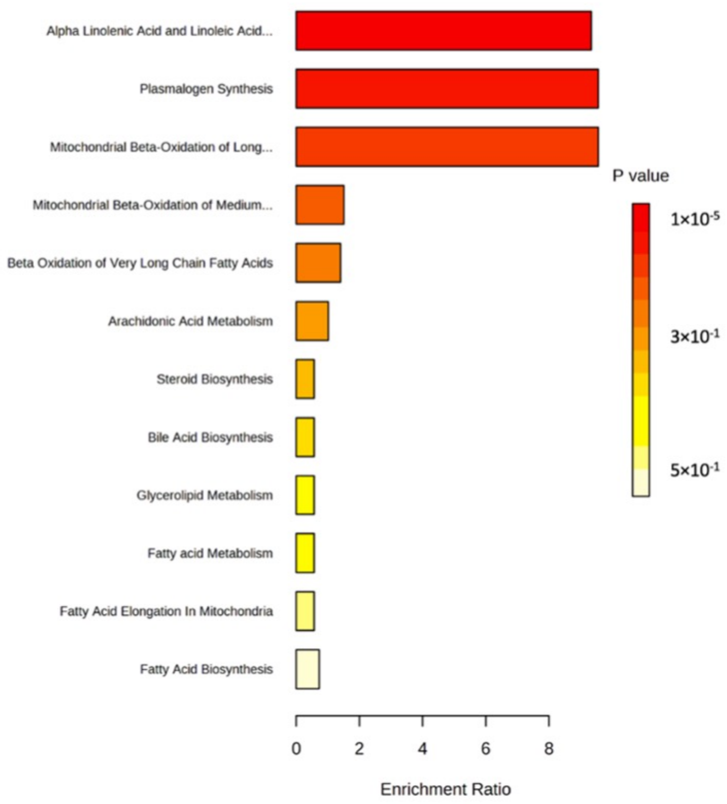
Top 8 features

6 a.a., 1 sugar, 1 acid

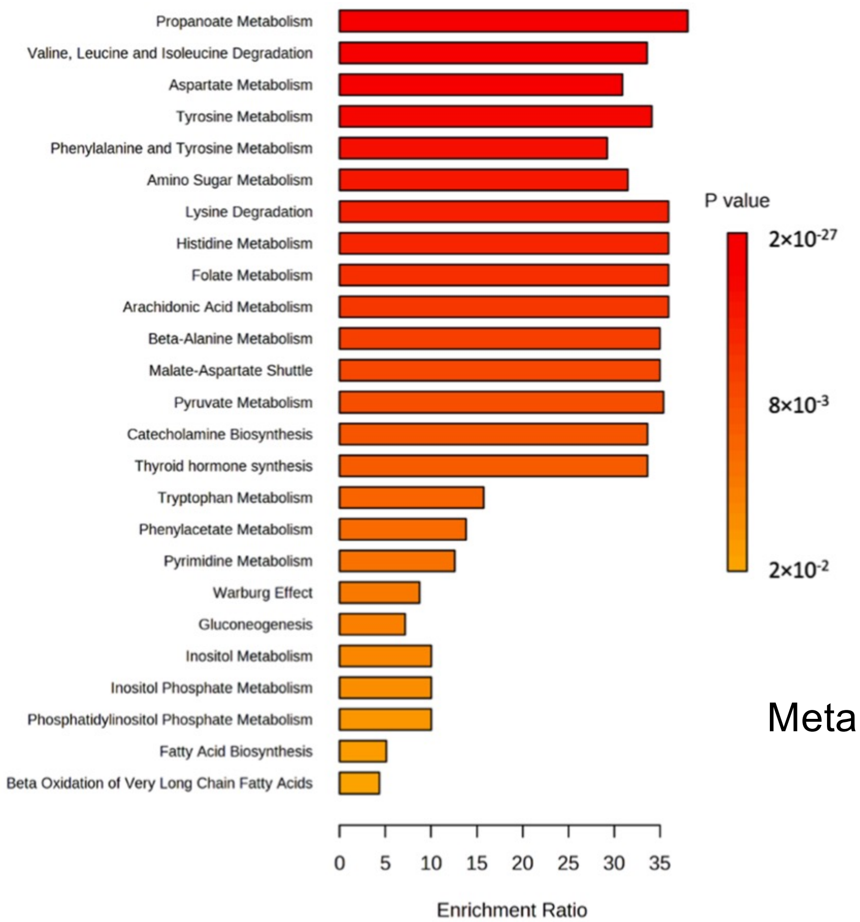
All downregulated in CRC except one



# Quantitative Enrichment Analysis (QEA)




Lipidomics



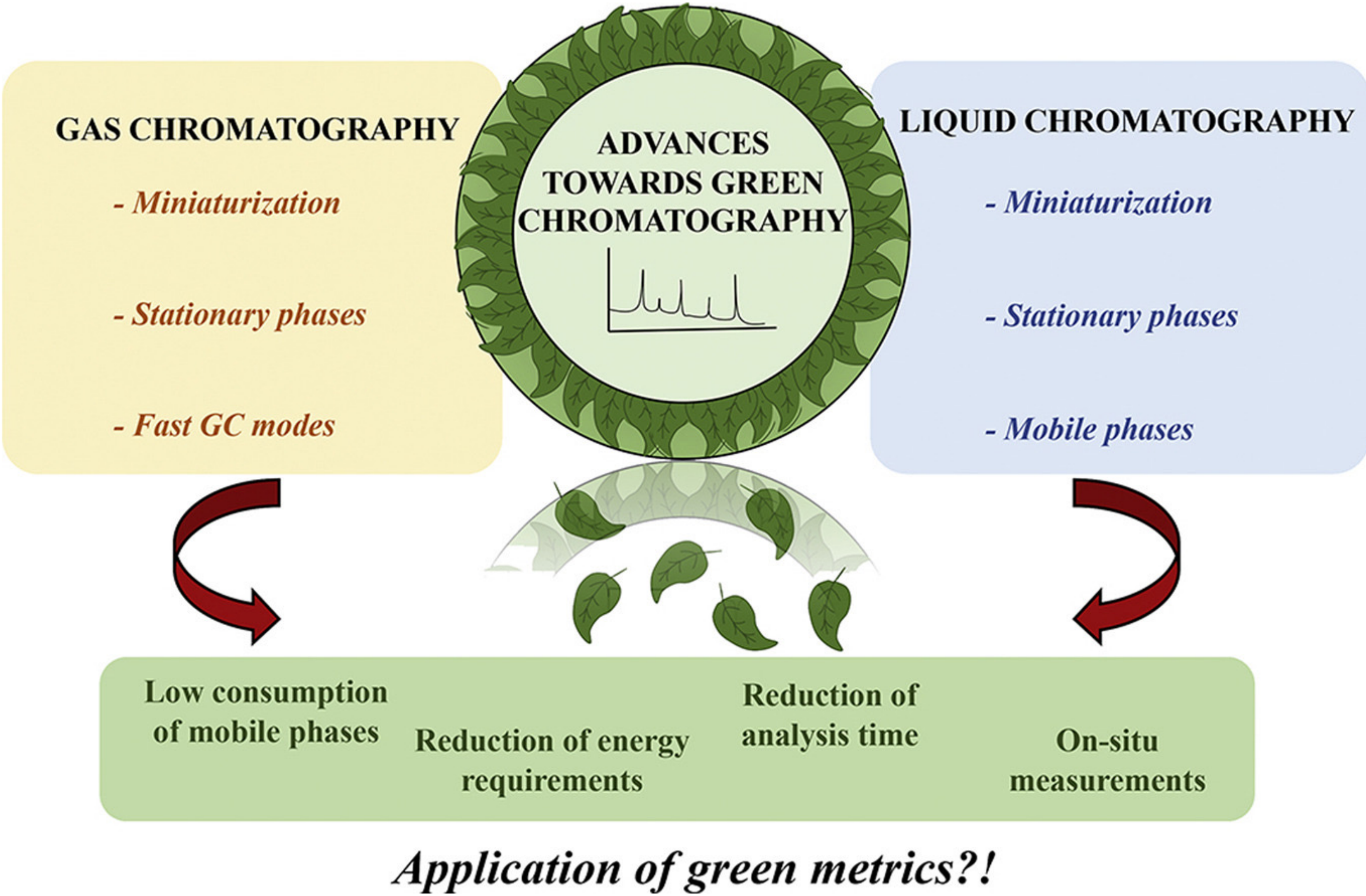

Metabolomics

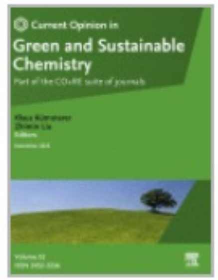
# Take Home Messages: Performances

- GC×GC-(HR)TOFMS can contribute to the study CRC metabolomics/lipidomics
- Complementary QEA can be performed (different pathways)
- Lipidomics gives insights on cancer stages…
- Lipidomics is faster and readily automated
- Lipidomics is more easily transferable to $^{1}$DGC-MS
- Metabolmomics is more comprehensive…

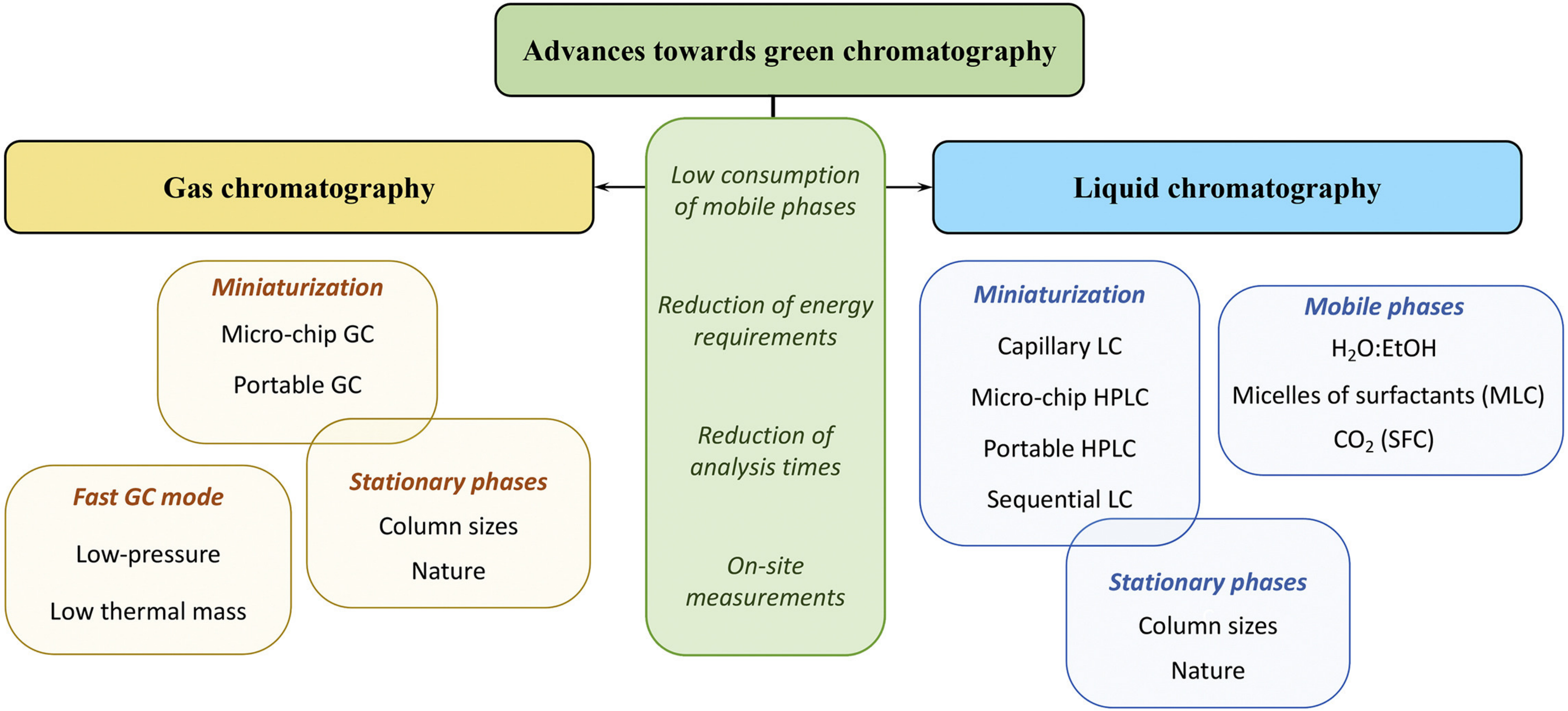
# Take Home Messages: How Green is all this ?

- ‘Quick and dirty’ estimates…
- GC×GC-(HR)TOFMS = MS 1 Kwh + GC 3 Kwh (GCxGC option 0.3 kW) (CF 1.5kW) + Computer 0.5 Kwh = 4.8 Kwh/day (LECO Corp.)
- LC-MS/MS = 5 Kwh/day (my greenlab.org)
- GC-MSD = 8 Kwh/day (Agilent)
- … need for some accurate ‘apple to apple comparison’

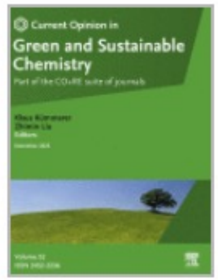



Recent efforts to increase greeness in chromatography, Napolitano-Tabares et al., 2021




Current Opinion in Green and Sustainable Chemistry




Recent efforts to increase greeness in chromatography, Napolitano-Tabares et al., 2021