Establishment of a quality control mixture for benchmarking LC-MS based dereplication protocols in NP research

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Prof. Jean-Luc Wolfender

Section des sciences pharmaceutiques
University of Geneva - University of Lausanne
Phytochemistry and bioactive natural products

He is currently developing innovative MS- and NMR-based **metabolomics strategies** in the frame of projects related to phytochemistry, microbial interactions and phytotherapy. He is specialised in the *de novo* structure identification of biomarkers at the microgram scale and is using a miniaturised approach that combines activity-based HPLC profiling and high content information bioassays such as those involving zebrafish.

Overview of a bioassay-guided natural product drug discovery process

Source materials (plant extracts)

Bioassay

Active extract(s)

Fractionation

Chromatographic fractions

Bioassay

Active fraction(s)

Isolation, Purification

Isolated compound(s)

Identification by Spectroscopic techniques e.x. NMR, MS, UV, IR

Identified compound(s)

Bioassay

Active compound(s)

Identified bioactive compound(s)

Issue of classical method

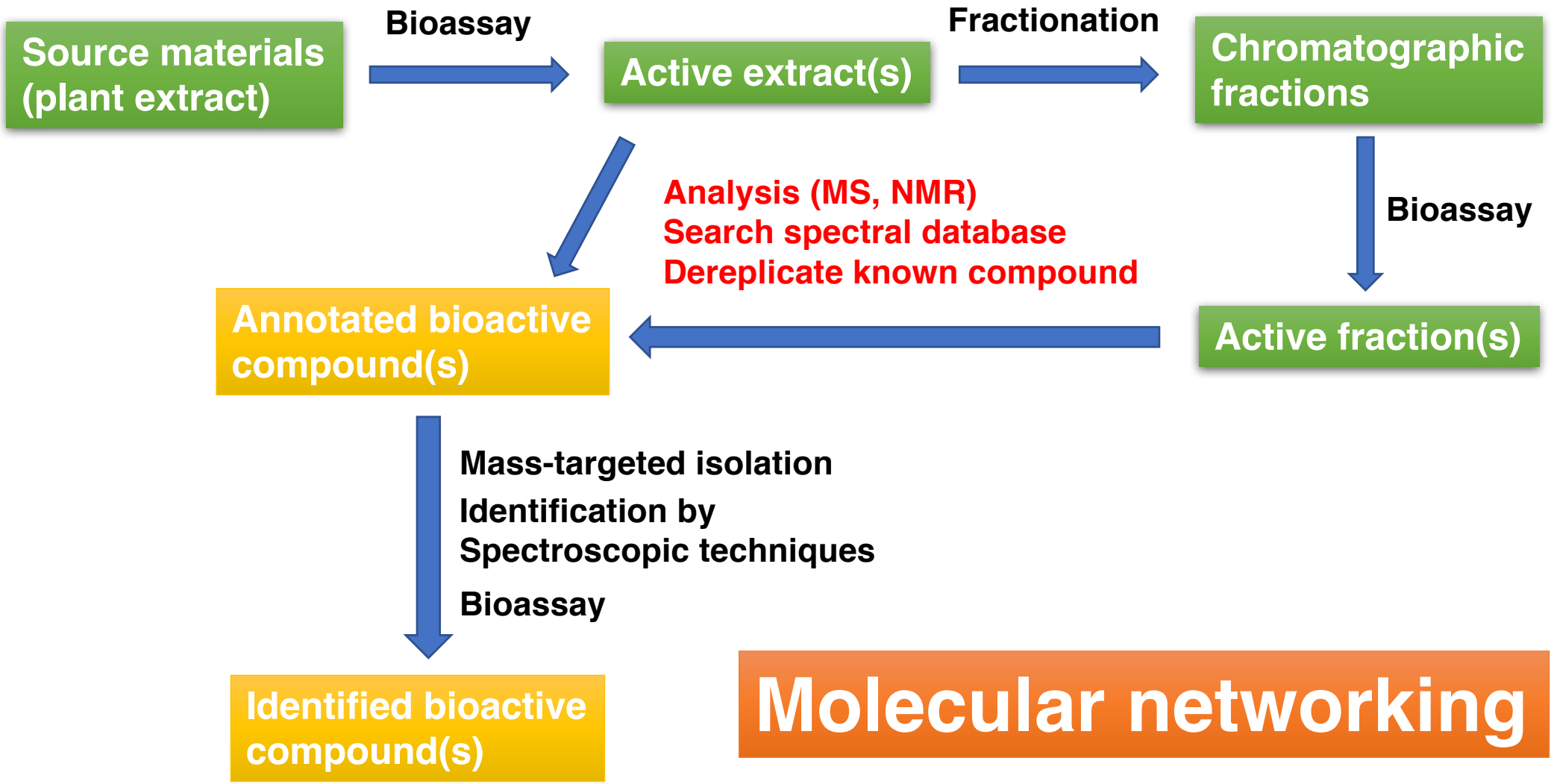
Can not secure sufficient material
Lose activity
Isolation of known compound

Metabolomics

Dereplication

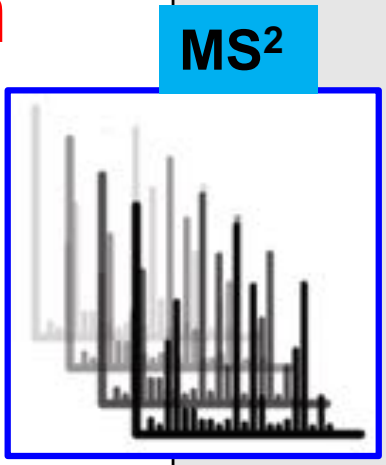
- **The early identification of known compounds without time-consuming manual structure elucidation**
- **Spotting unknowns of interest could also enter in the definition of dereplication**

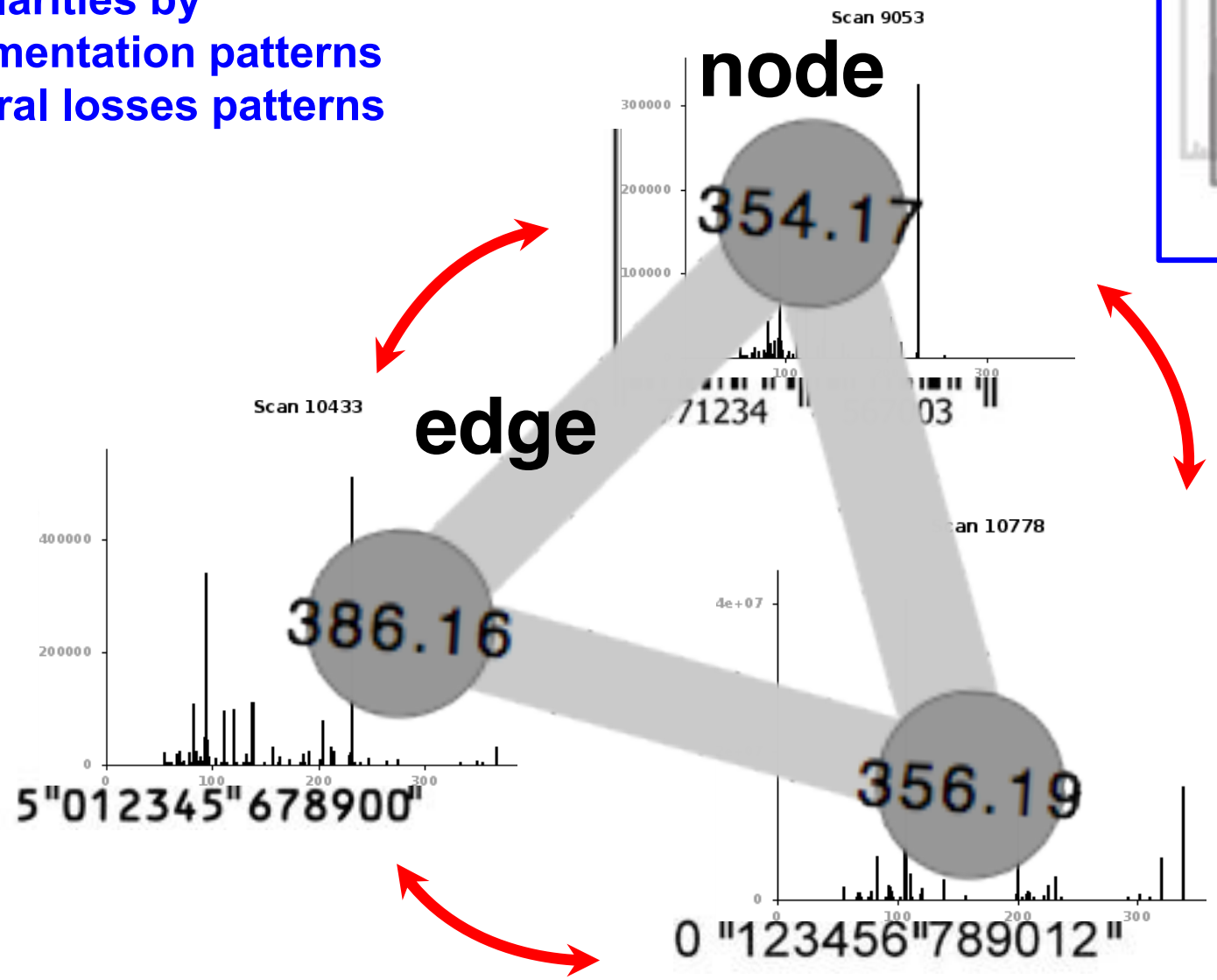
Overview of a mass-guided natural product drug discovery process



MS/MS network for interpretation

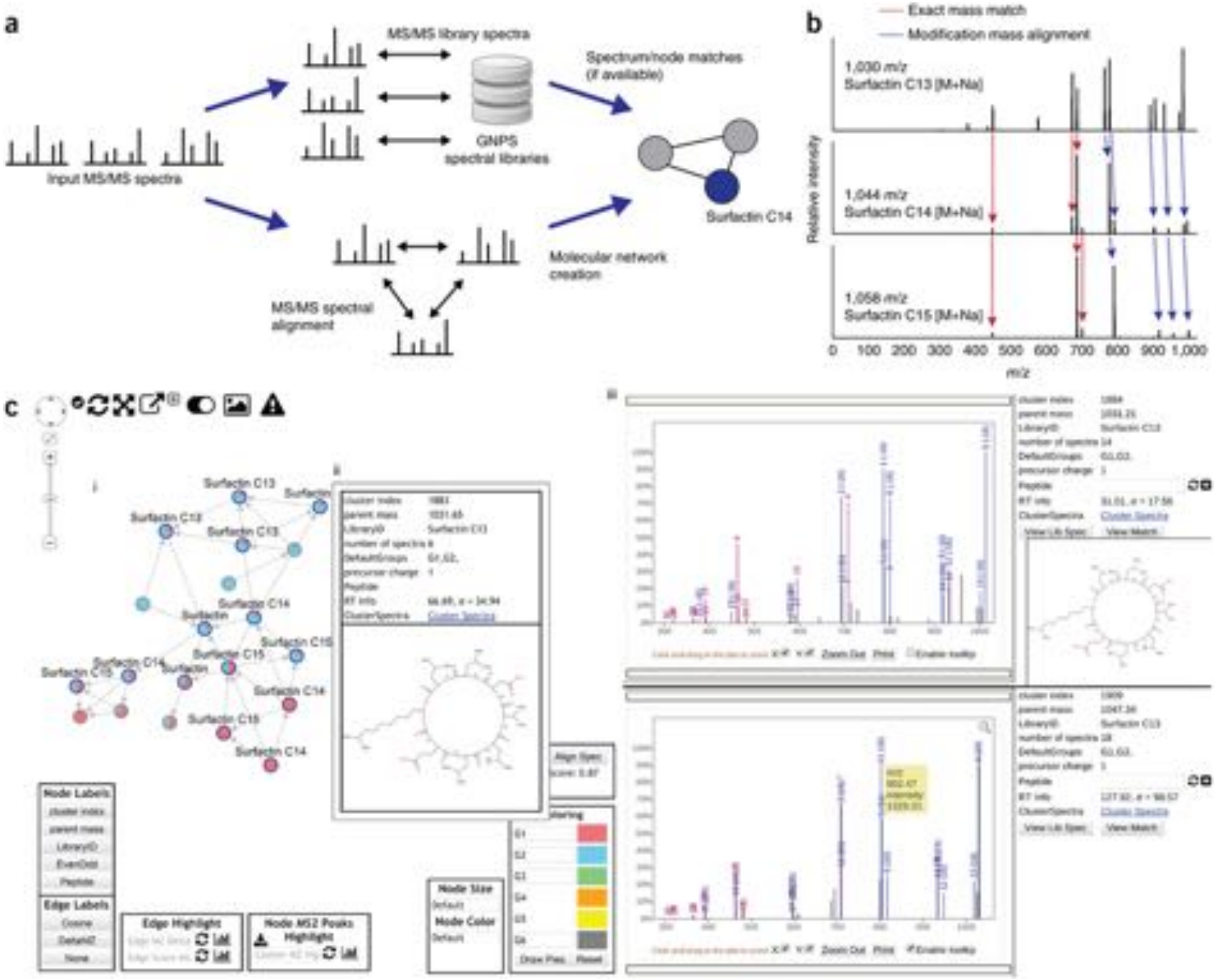
Similarities by
fragmentation patterns
neutral losses patterns



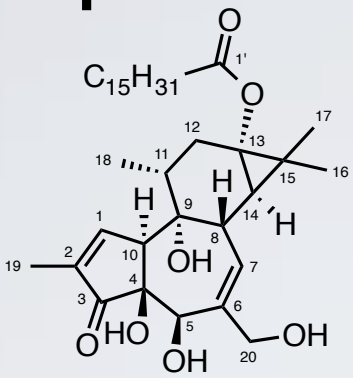


Wang, M. et al. *Nature Biotech.*, 34, 828-837, 2016.

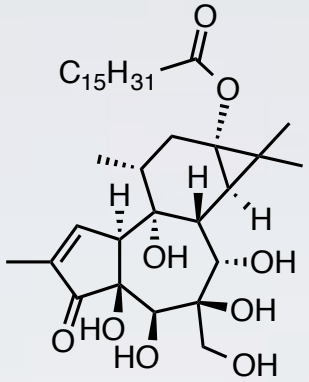
https://gnps.ucsd.edu/



Bio-chemical input



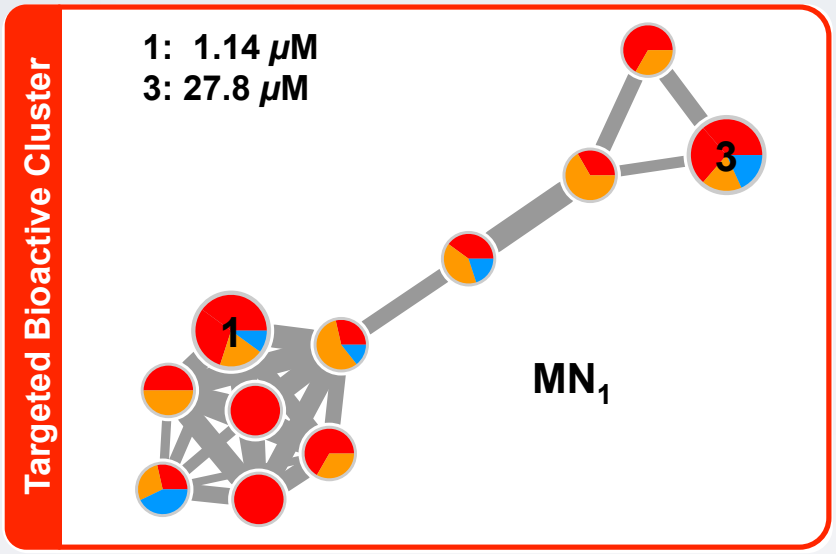
1

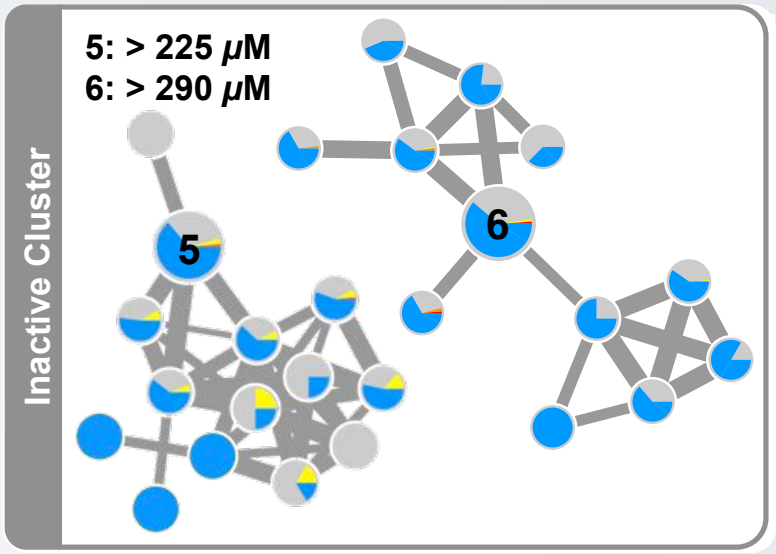


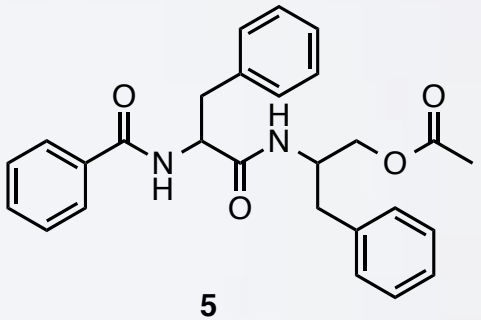
3

Extracts IC_{50}

- 0 - 5 μg.mL^{-1}
- 5 - 20 μg.mL^{-1}
- 20 - 50 μg.mL^{-1}
- > 50 μg.mL^{-1}
- n.d.







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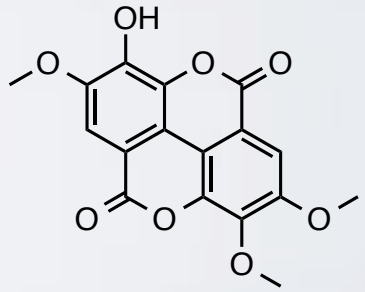


6

Number of natural products (NPs)

- existing ~unknown
- known ~300000
- with associated MSMS data ~25000

Bérdy, J. *J. Antibiot*. **2005**, 58, 1–26.

Number of natural products (NPs)

Dereplication

CFM-ID
Competitive Fragmentation Modeling for Metabolite Identification

MAGMa

MetFrag

SIRIUS

Generation of the **ISDB** (*in-silico* database)

Universal **N**atural **P**roducts **D**atabase

北京大学 PEKING UNIVERSITY

250000 entries

170000 SMILES

TXT

CSV

340 files of 500 SMILES

Baobab login node

HPC cluster Baobab

Baobab is a High Performance Computing (HPC) cluster available to all UNIGE's researchers.

Baobab

~2400 compute cores
(Intel Sandy Bridge @ 2.20GHz)
10TB of RAM

cfm-id

12 hours

metadata

CSV

UNPD-ISDB

> 170 000 *in silico*
MS/MS spectra

Generation of the **ISDB** (*in-silico* database)

263446 entries

221771 SMILES + metadata

455 files of 500 SMILES

Baobab login node

HPC cluster Baobab

Baobab is a High Performance Computing (HPC) cluster available to all UNIGE's researchers.

Baobab

~2400 compute cores
(Intel Sandy Bridge @ 2.20GHz)
10TB of RAM

cfm-id

12 hours

metadata

DNP-ISDB

> 220 000 *in silico*
MS/MS spectra

Number of natural products (NPs)

Dereplication

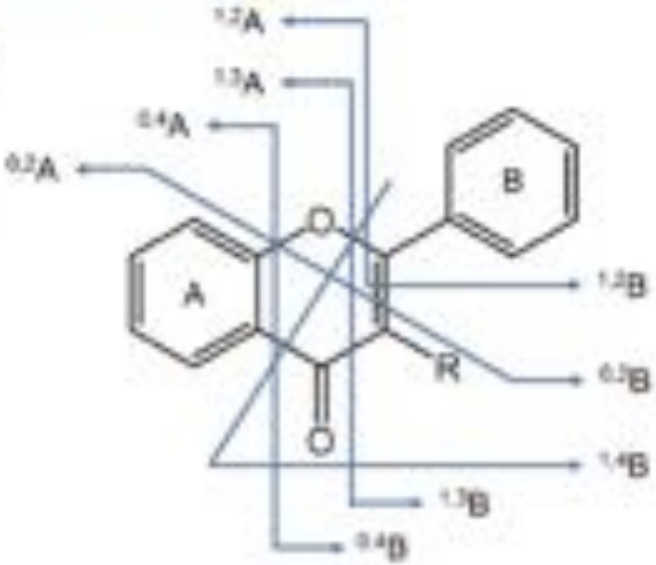
- existing ~unknown
- known ~300000
- with associated MSMS data ~25000

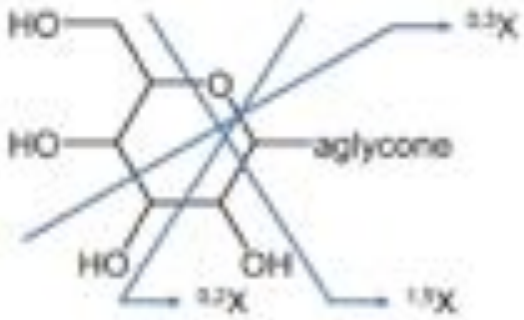
ISDB

Pierre-Marie Allard *et al., Anal. Chem.*, **2016**, *88*, 3317–3323.

Fragmentation of flavonoids

- typical fragmentation of flavonoids and flavonoid-glycosides

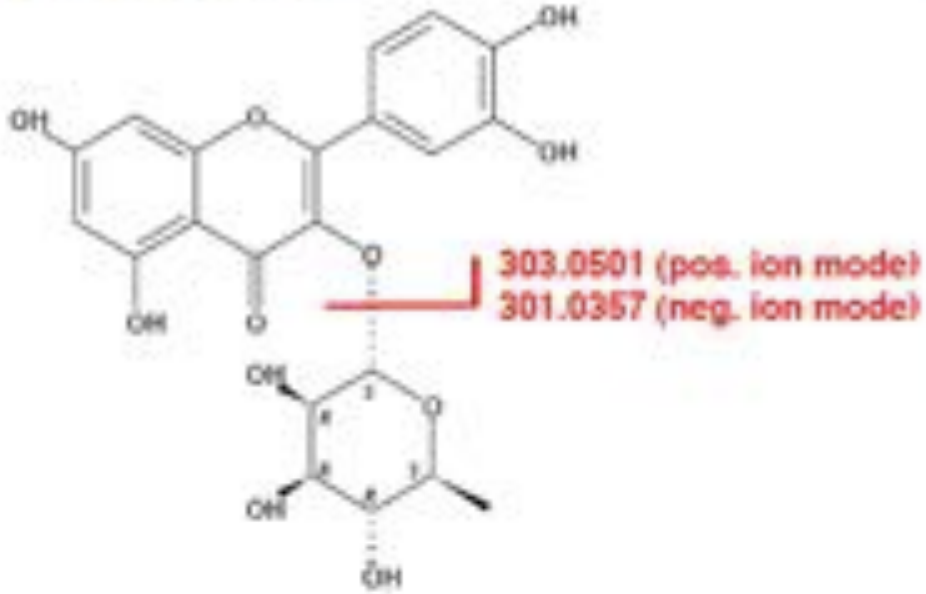




$^{0,2}X$: $[M\pm H-120]^+$
$^{0,3}X$: $[M\pm H-90]^+$
$^{1,5}X$: $[M\pm H-134]^+$

$^{0,2}X$: $[M\pm H-90]^+$
$^{0,3}X$: $[M\pm H-60]^+$
$^{1,5}X$: $[M\pm H-104]^+$

- Quercetrin



Quercetin

Collaboration with Pierre Fabre laboratories in the frame of their Open Innovation program

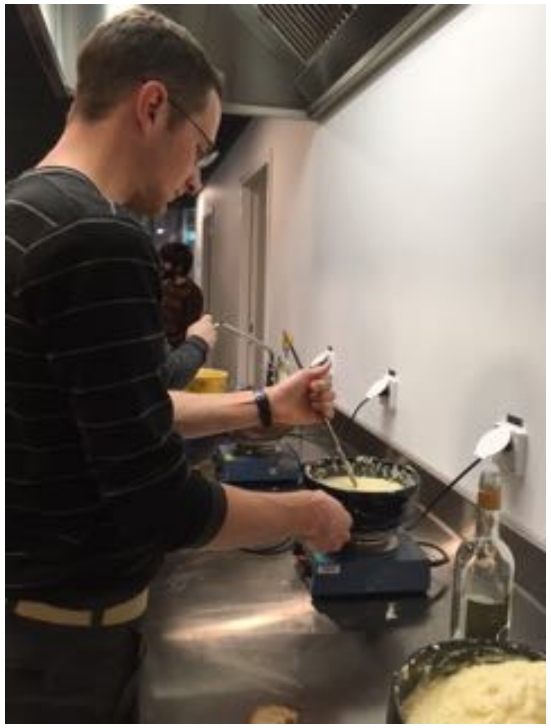
NATURE OPEN LIBRARY
AN OPEN INNOVATION INITIATIVE BY PIERRE FABRE

http://nature-open-library-pierre-fabre.force.com/index

Objective:

- establish a molecular cartography of the PF library in order to prioritize high added values molecules (specific structures or bioactivities)

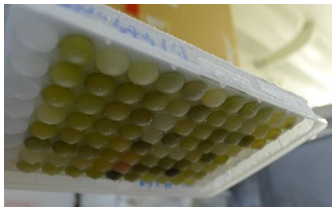
- shift from classical bioguided fractionation to mass-directed approaches



Methodology

Thermo Fisher
Orbitrap Q Exactive
Focus Hybrid MS

Waters
ACQUITY UPLC I-Class
PLUS



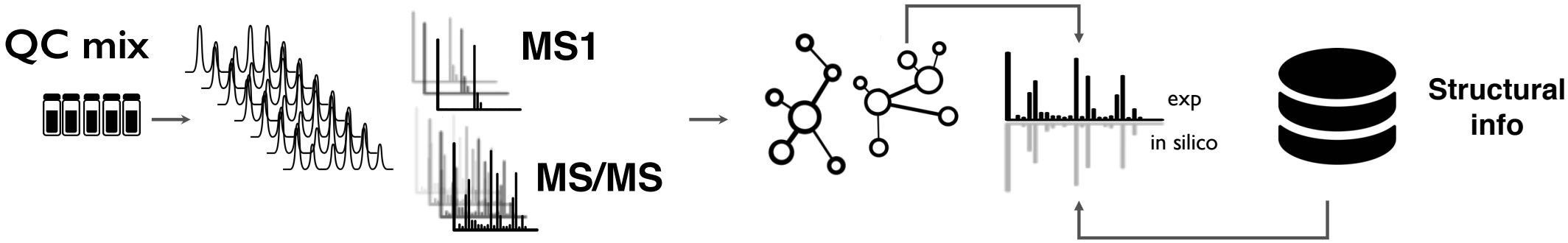
ANALYSIS

METABOLITE
PROFILING

Quality Control (QC)

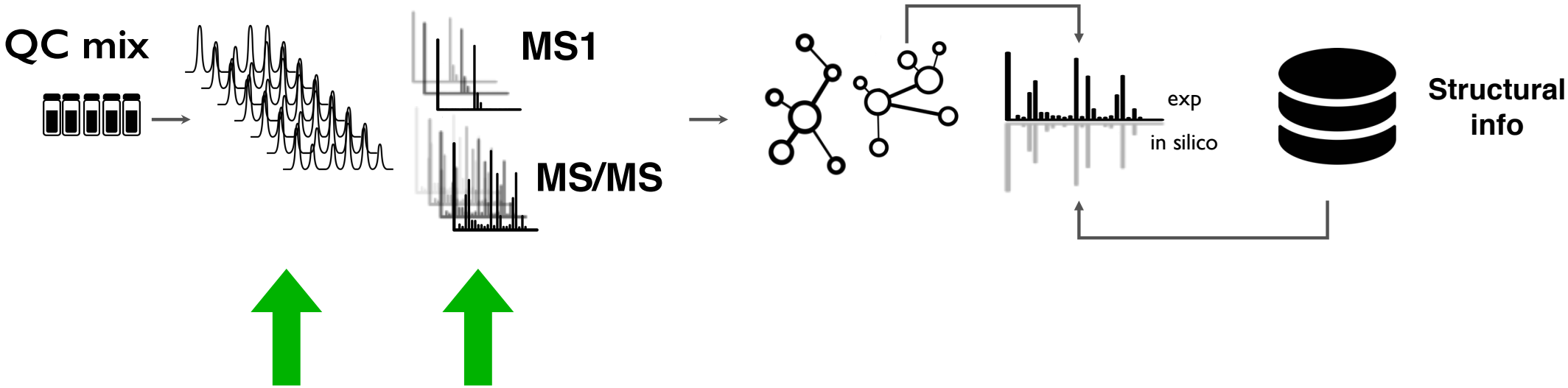
The QC allow the user to evaluate and benchmark their LC and LC-MS chromatography system before analysis of critical analytes.

Objective:
Establish a universal QC mix for the quality evaluation of NPs profiling



Objective:
Establish a universal QC mix for the quality evaluation of NPs profiling

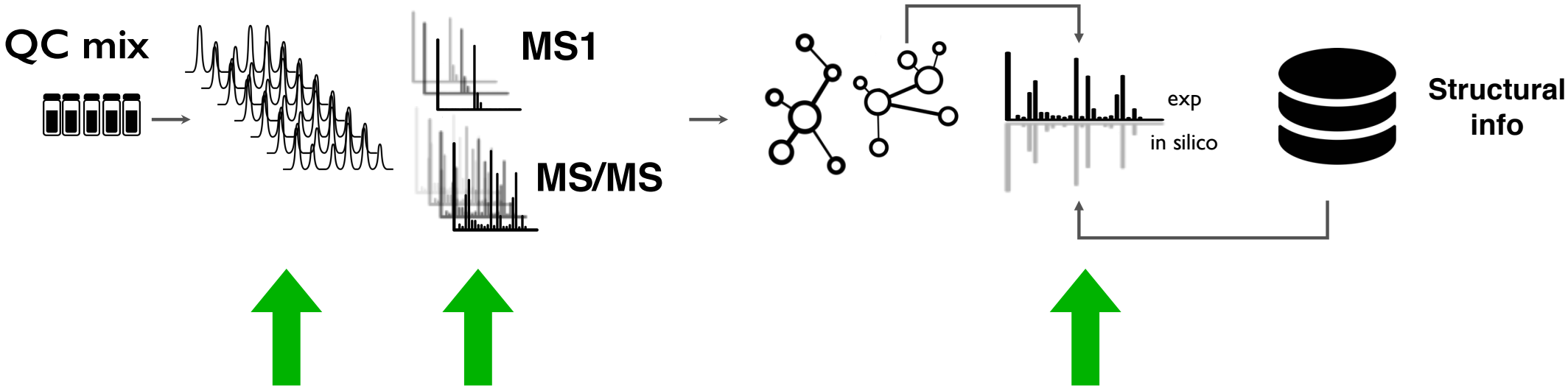
- at the analytical level (chromatography and MS acquisition)



Objective:
Establish a universal QC mix for the quality evaluation of NPs profiling

- at the analytical level (chromatography and MS acquisition)

- at the data treatment and analysis level (metabolite identification)



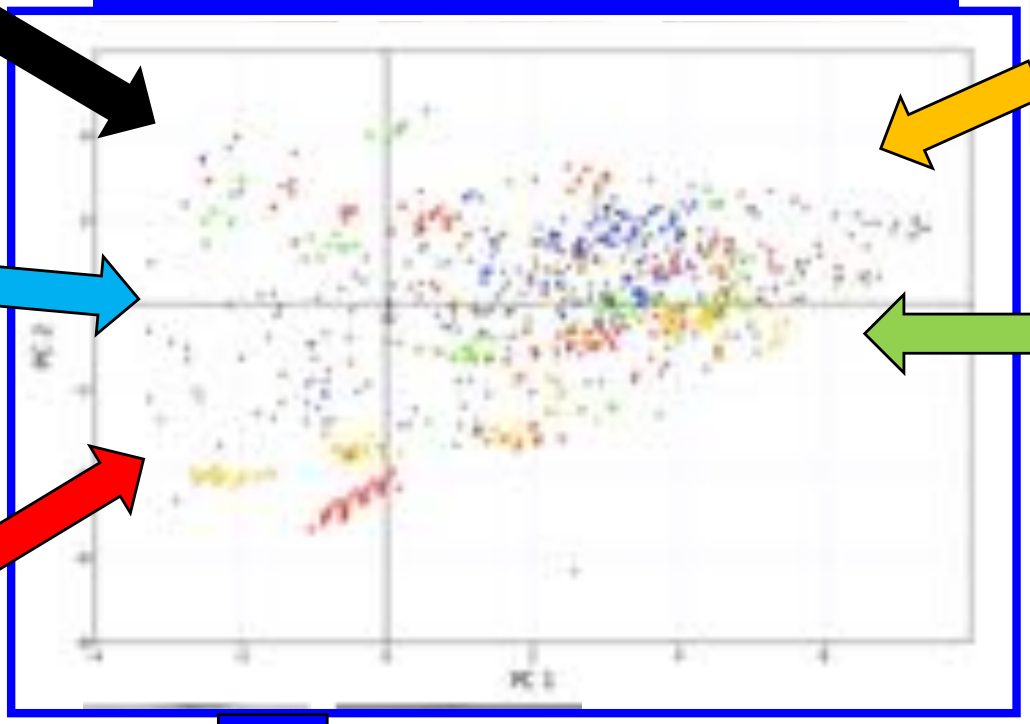
Chemical space of secondary metabolites of the QC-mix of 5 herbs

Salvia (1133 NPs)

Cinchona (86 NPs)

Arnica (146 NPs)

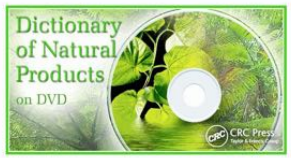
Extended chemical space

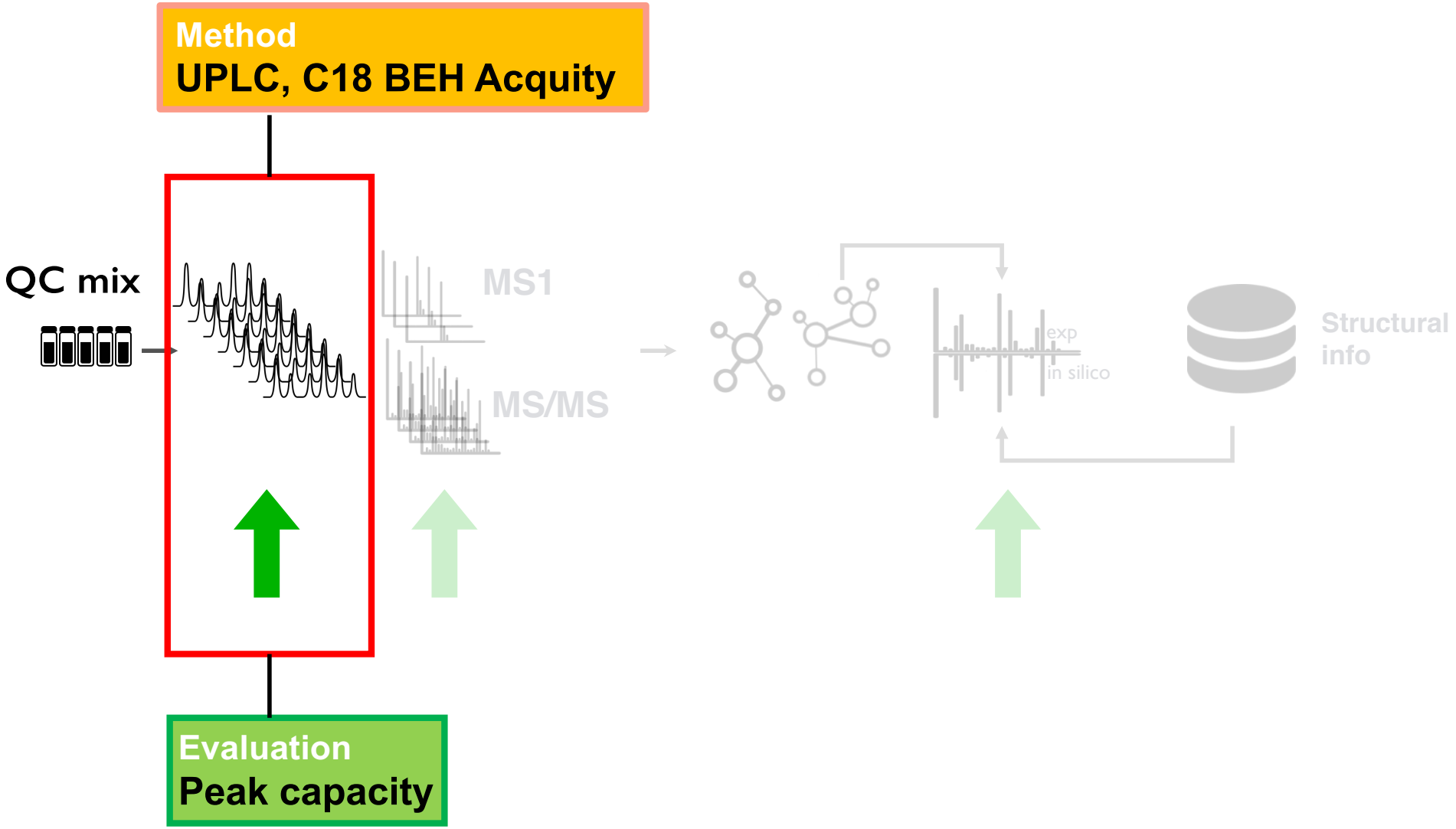


(Ginseng) (457 NPs)

Ginkgo (81 NPs)

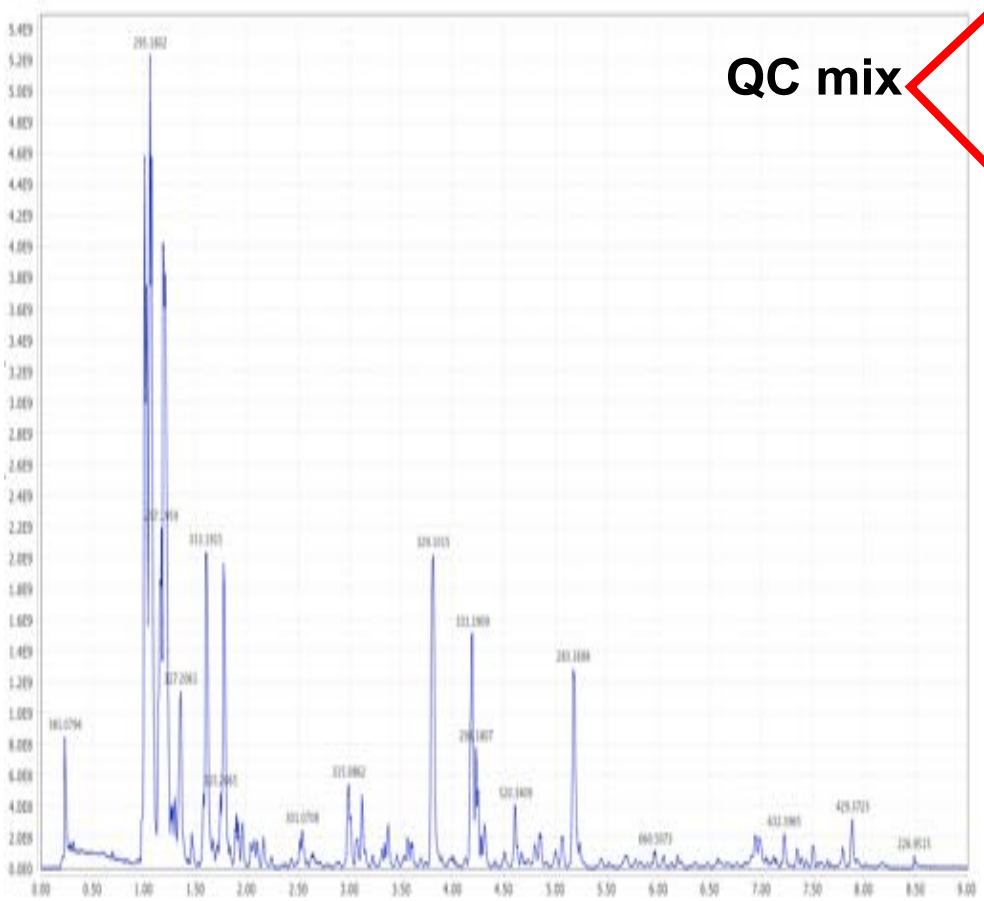
1903 NPs reported in DNP



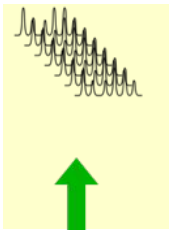

Method
UPLC, C18 BEH Acquity
QC mix
MS1
MS/MS
exp
in silico
Structural
info
Evaluation
Peak capacity

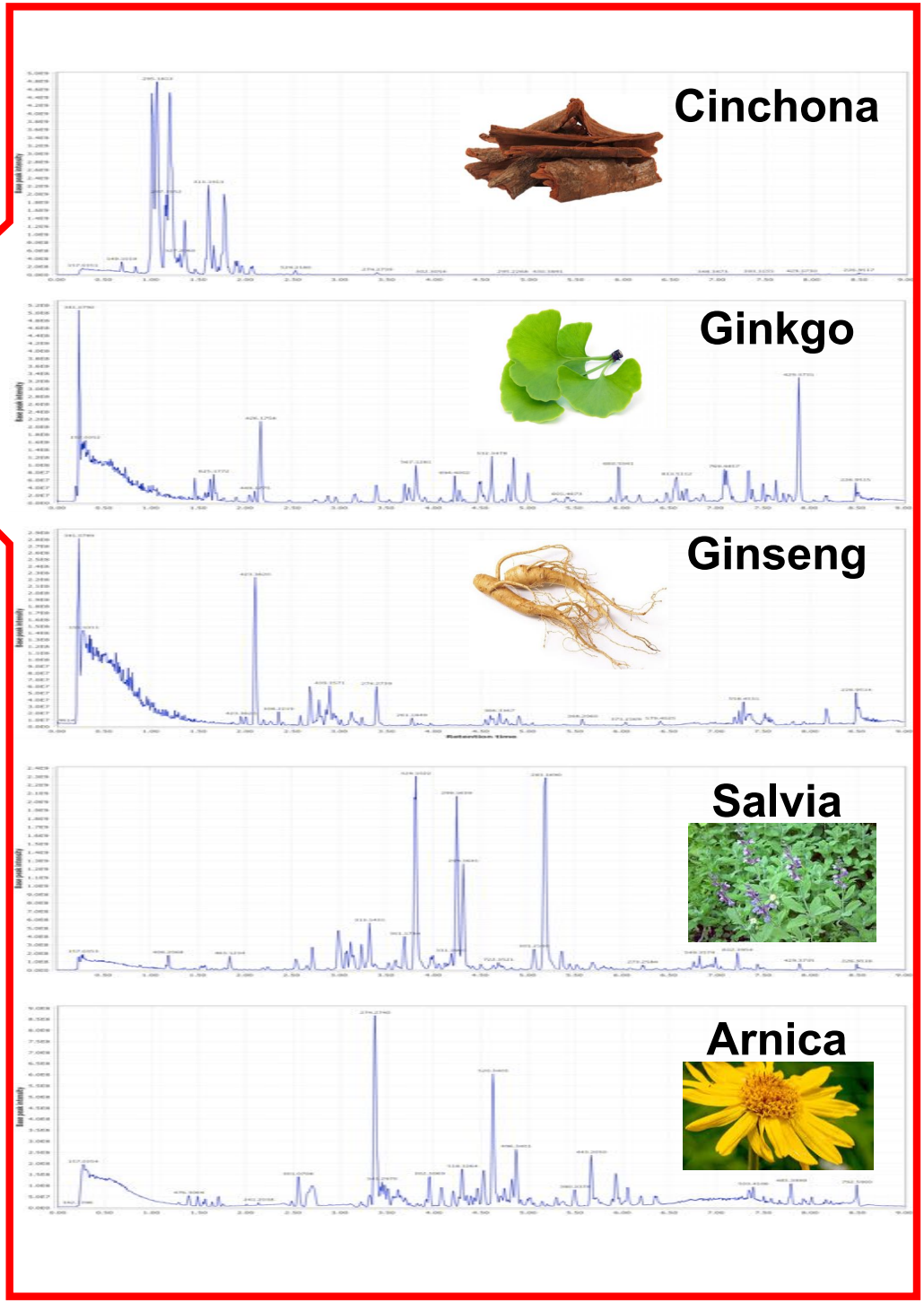
QC mix?

UHPLC-HRMS profiling of the QC mix and 5 herbs



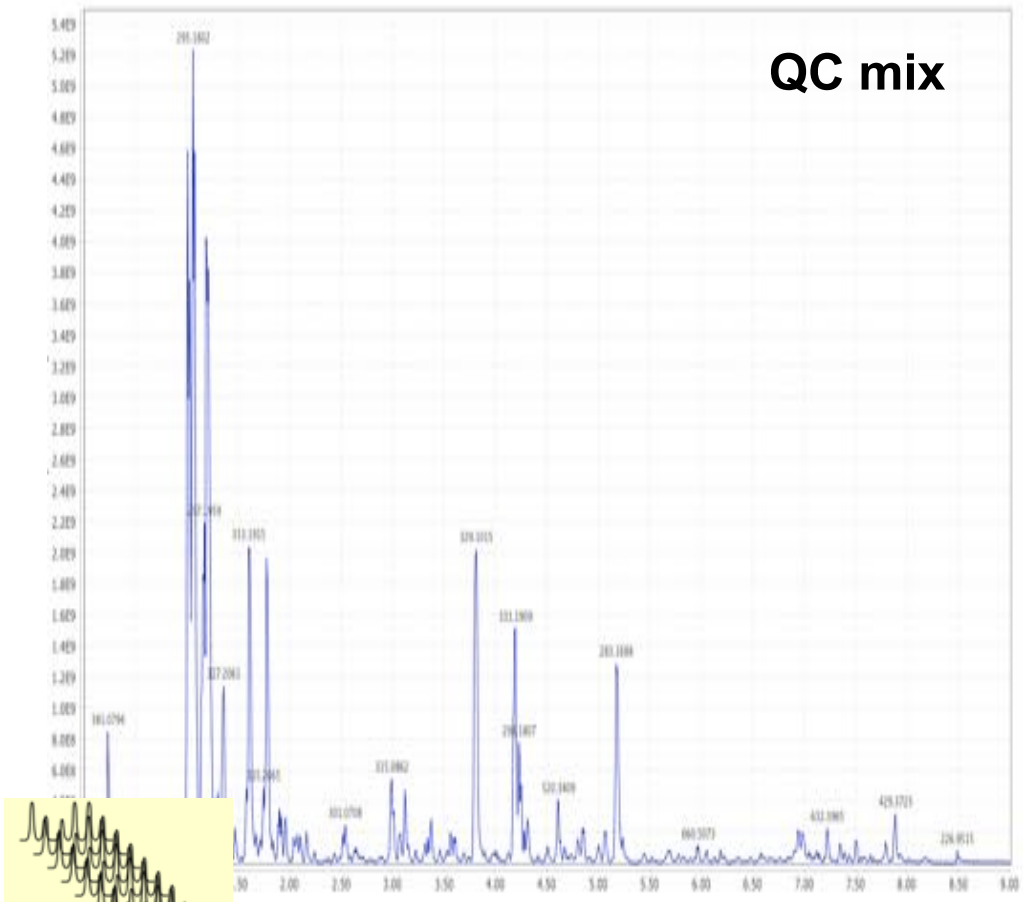
QC mix

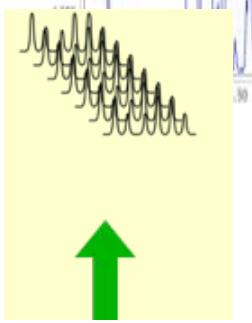


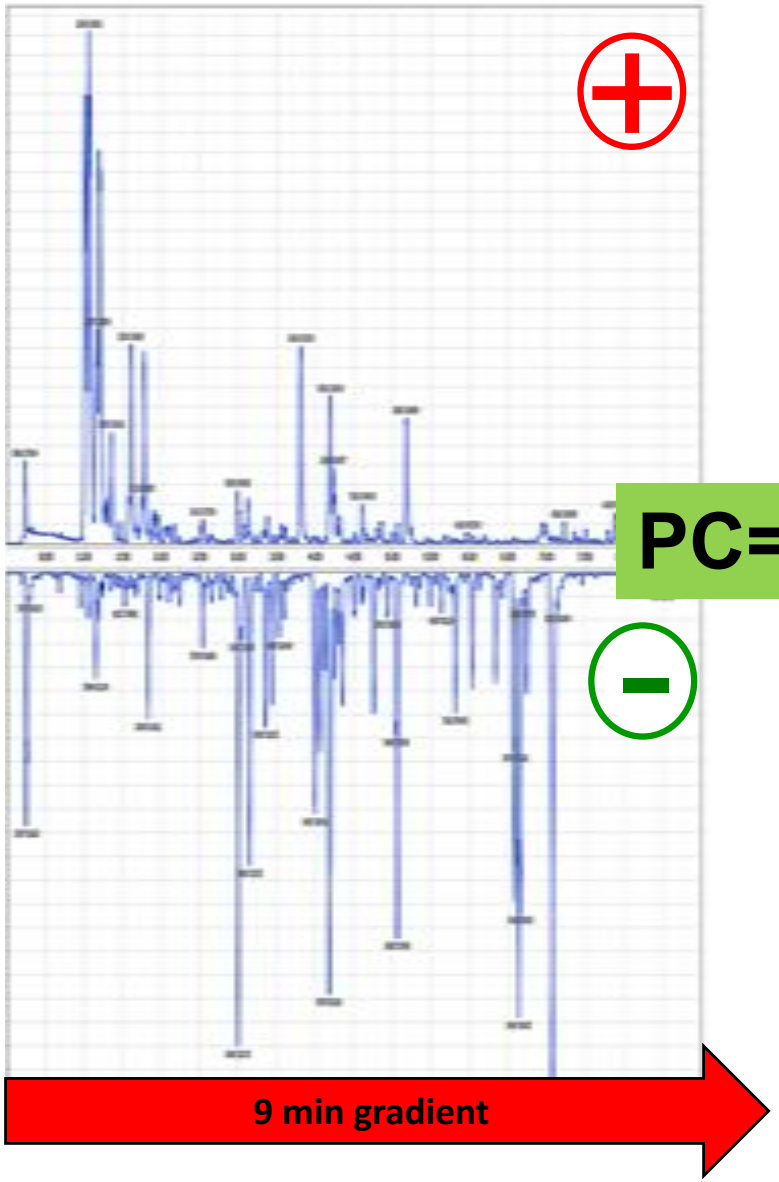


UHPLC-HRMS profiling of the QC mix of 5 herbs

Assess peak capacity



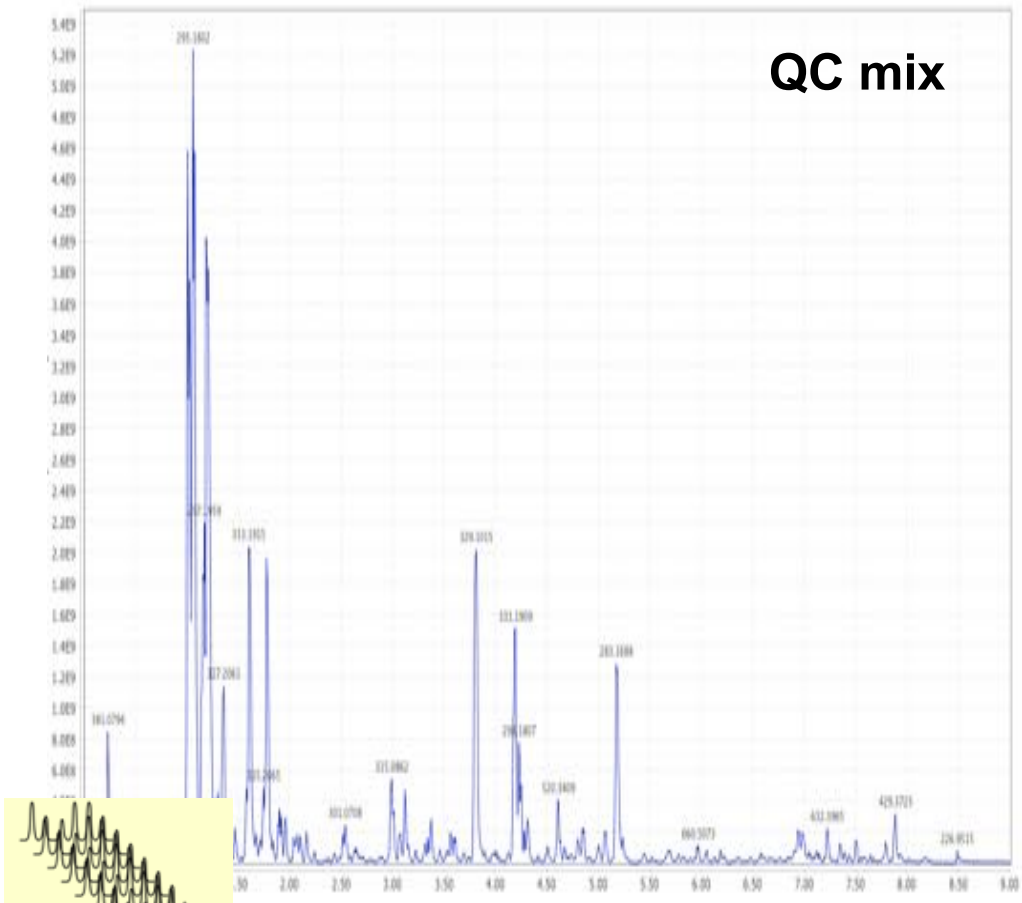


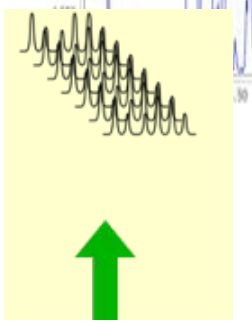


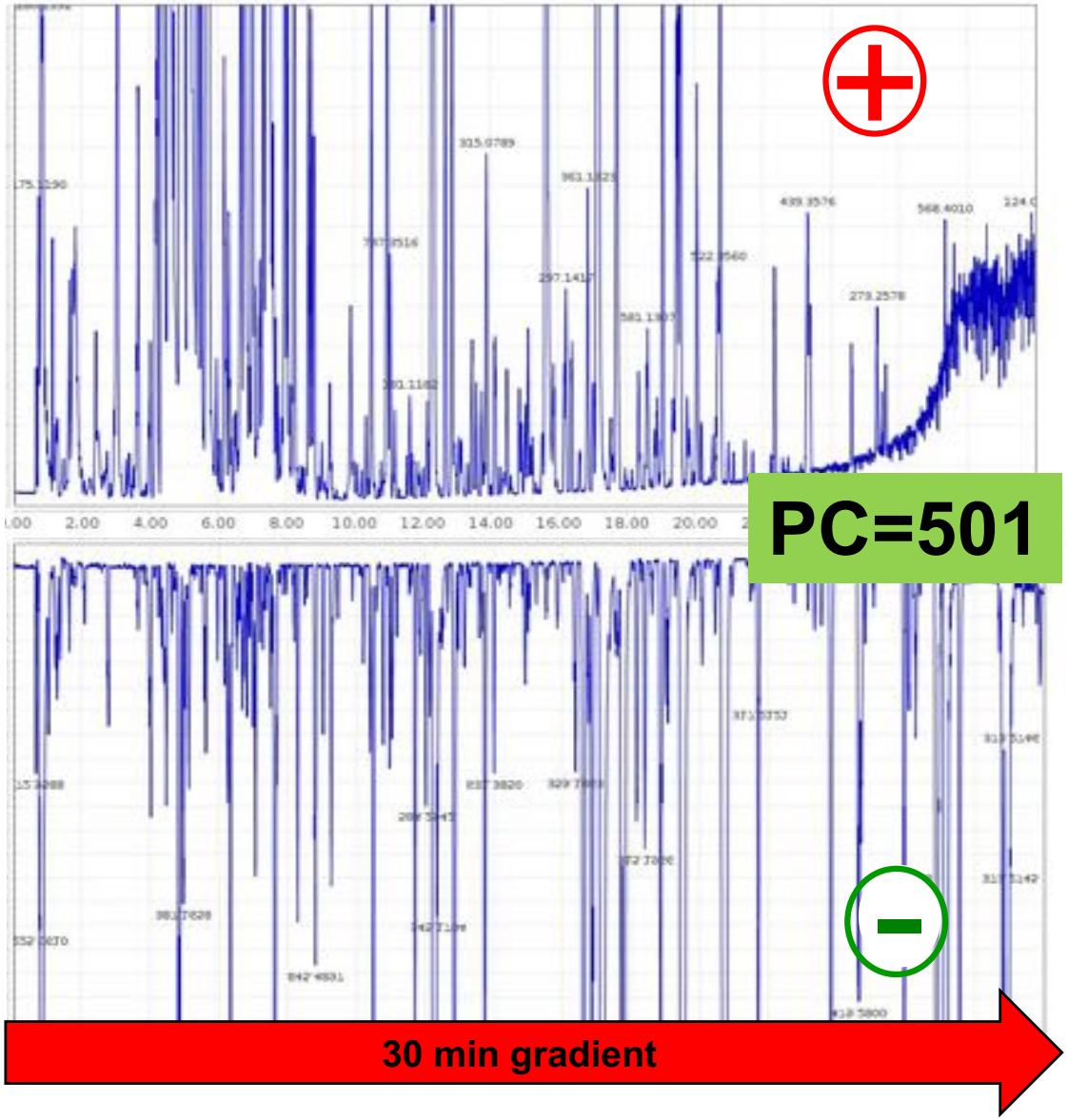
2 x 50 mm, C18 BEH Acquity column, 9 min

UHPLC-HRMS profiling of the QC mix of 5 herbs

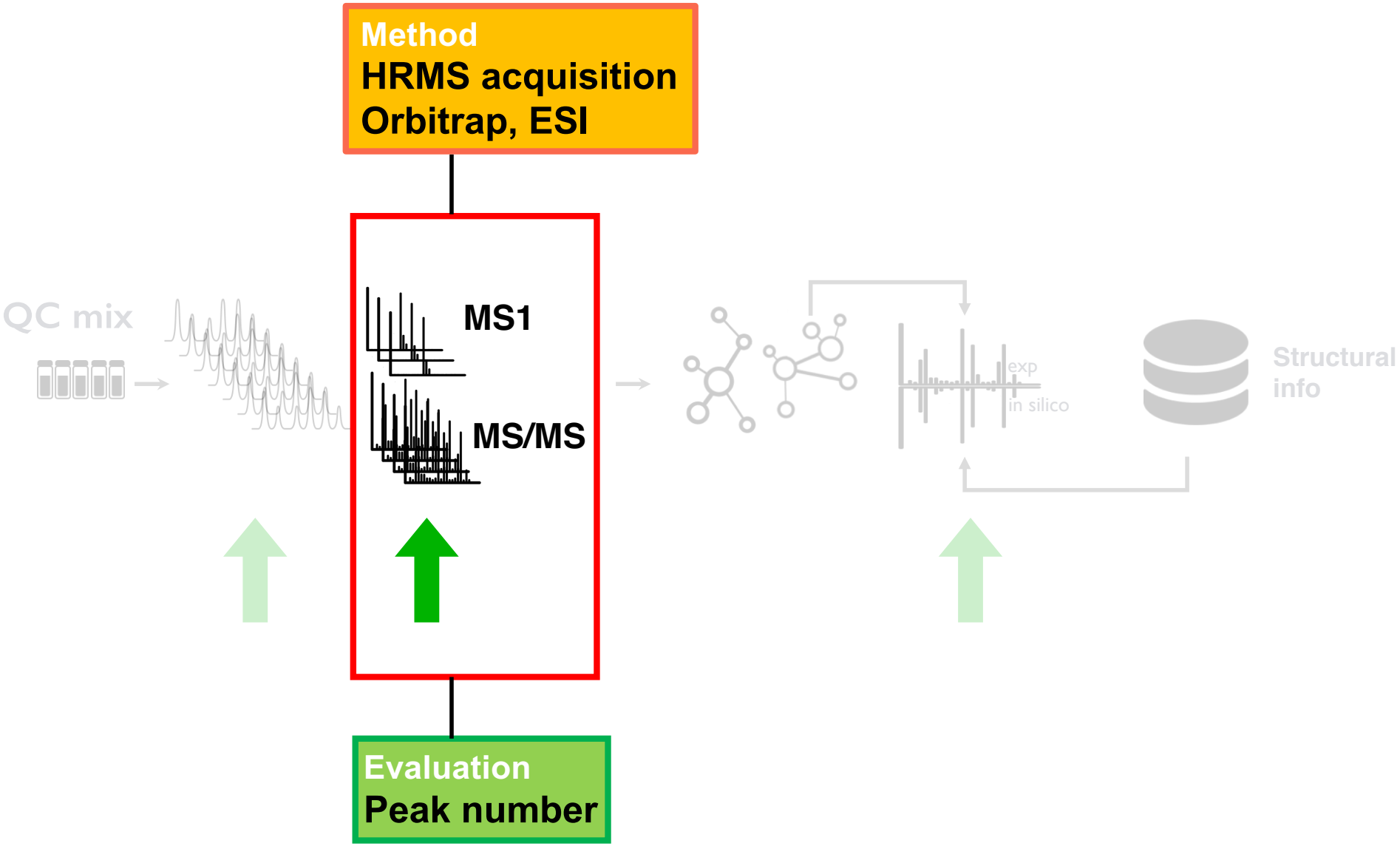
Assess peak capacity





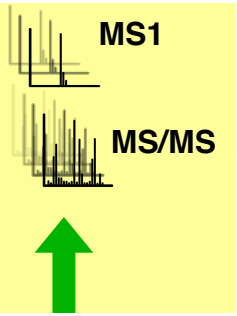


2 x 150 mm, C18 BEH Acquity column, 30 min

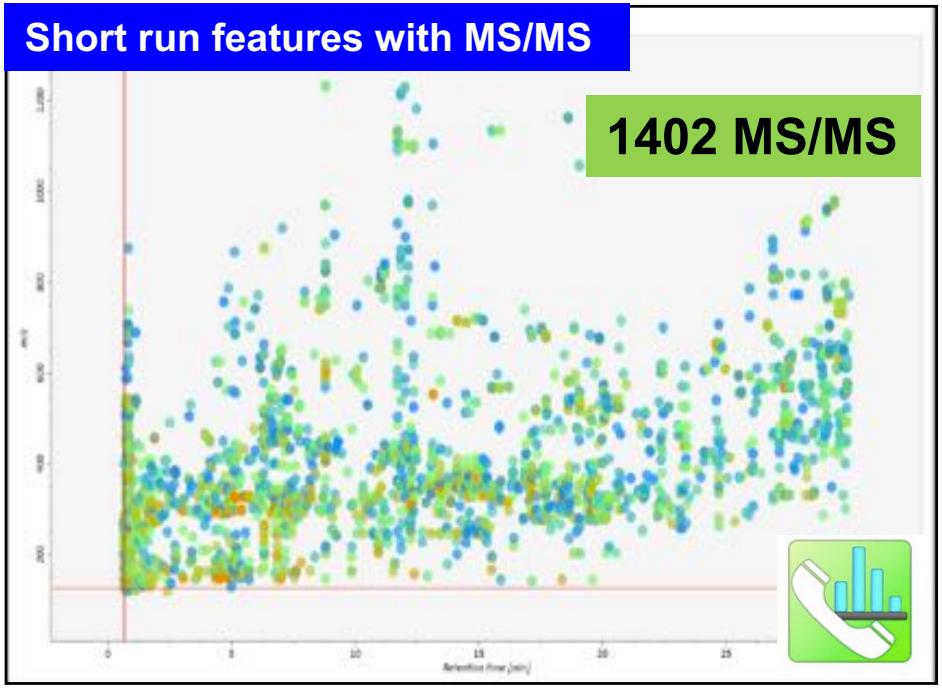

Method
HRMS acquisition
Orbitrap, ESI
QC mix
MS1
MS/MS
exp
in silico
Structural
info
Evaluation
Peak number

MS/MS ion map of the QC mix of 5 herbs

Assess peak number

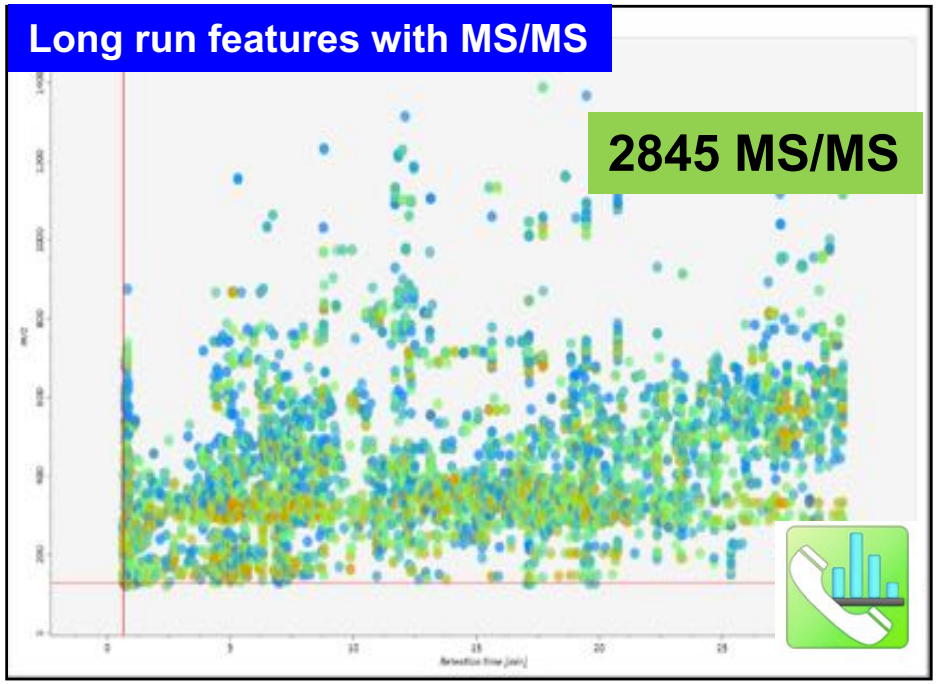


Short run features with MS/MS



2 x 50 mm, C18 BEH Acquity column, 9 min

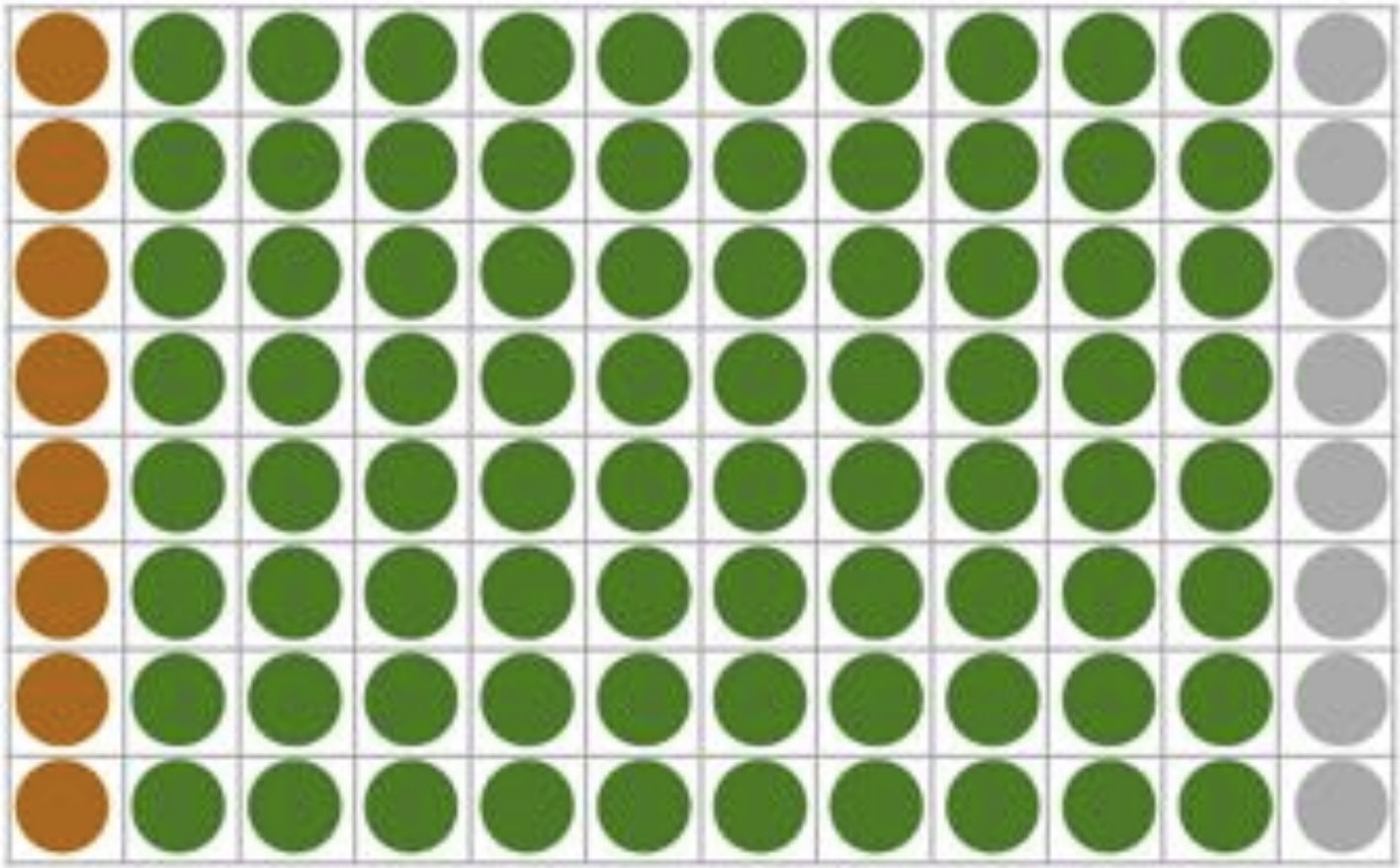
Long run features with MS/MS



2 x 150 mm, C18 BEH Acquity column, 30 min

Plate plan

QCmix samples blank



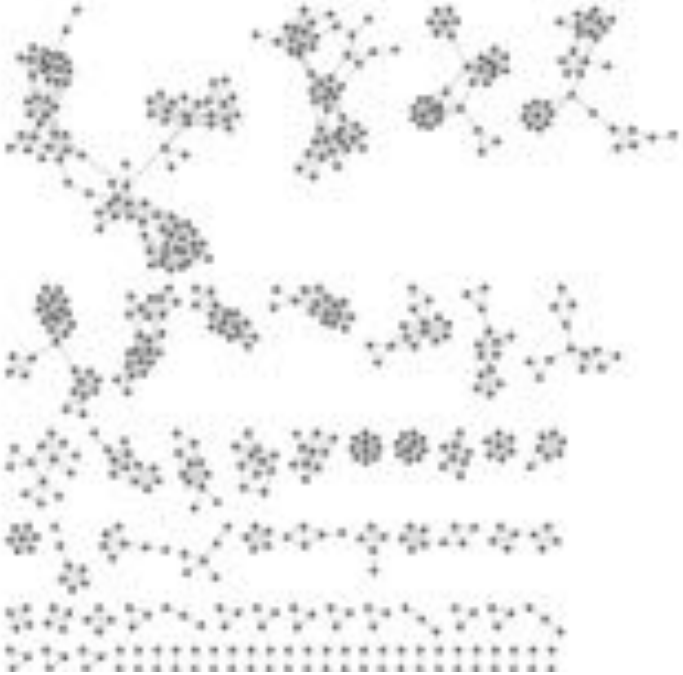
Analyzed so far:
480 extracts (6 plates containing 80 extracts)

Visualization in Cytoscape

Cytoscape

http://cytoscape.org/index.html

python



2 new things in the MN annotations :
- exact mass and RT info by MzMine pretreatment
- new script gives all additional metadata of annotation

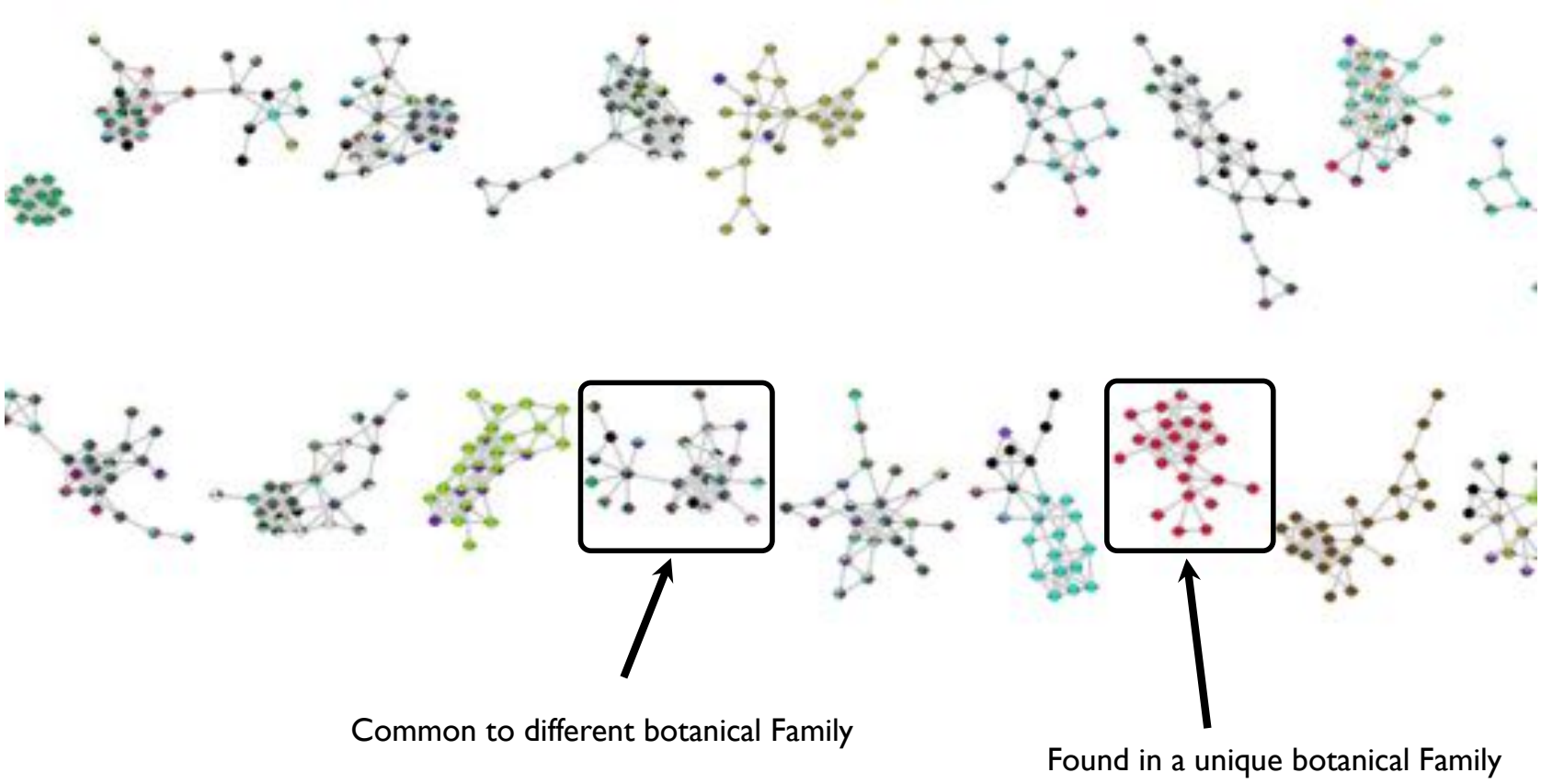
b0149cfbadb74b7e913be915c7423630.pairsinfo

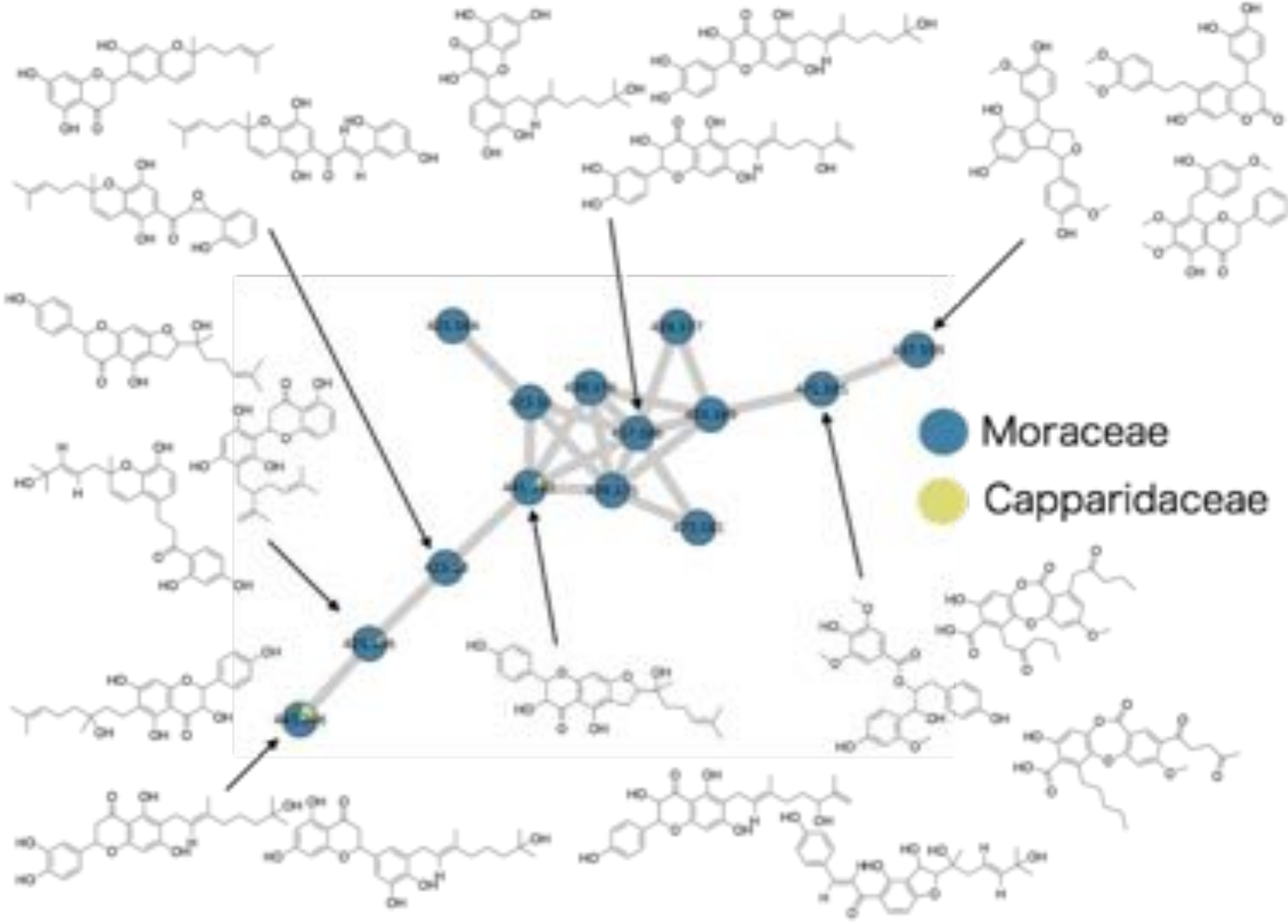
Table Panel

SMILES_DNP	DNP_IDs	Molecular_Formula_DNP	Compound_Types_DNP	Biological_Source_DNP	Molecule_Name_DNP
[CCCCCCCCCCCC...	[PQF41-A, OYY...	[C34H67NO5, C34H67NO5, C3...	[VA7200 ZQ7020, VA7200 ZQ...	[Constit. of the radix of Angel...	[2-Amino-7-octadecene-1...

Molecule_Name_DNP	Biological_Use_DNP	Spectral_Score_DNP	IS_libname	IS_match_mzerror	SMILES_DNP_QC	DN
Amino-7-octadecene-1...	[]	[0.300686, 0.289631, 0.28...	[New_DNP_full_p...	[0, 0, 0]	[]	[]

Addition of a taxonomical layout according to the group mapping. Done at the Family level

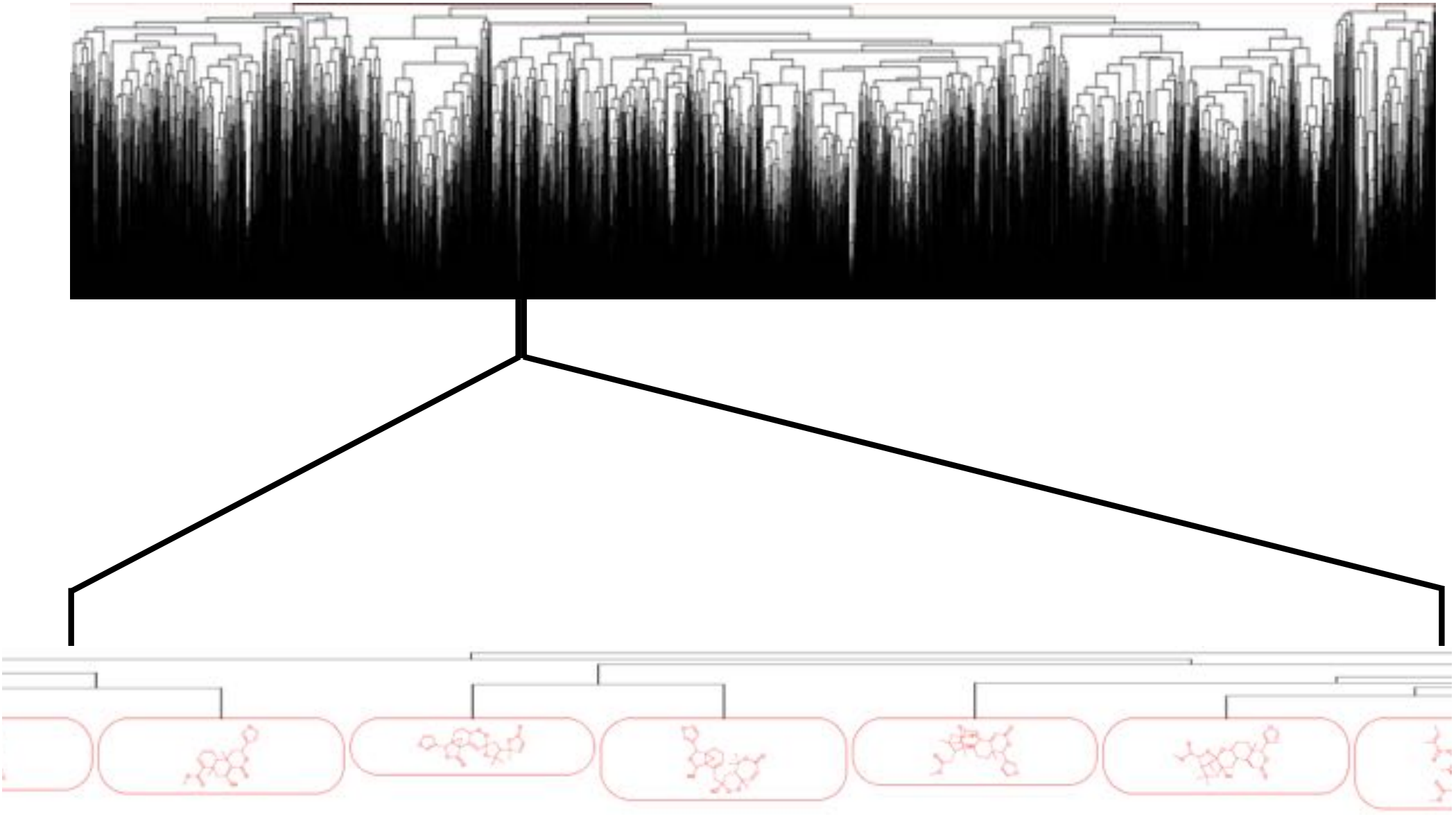



Moraceae
Capparidaceae

The 7500 structures were loaded in Scaffold Hunter for structural organisation and queries.

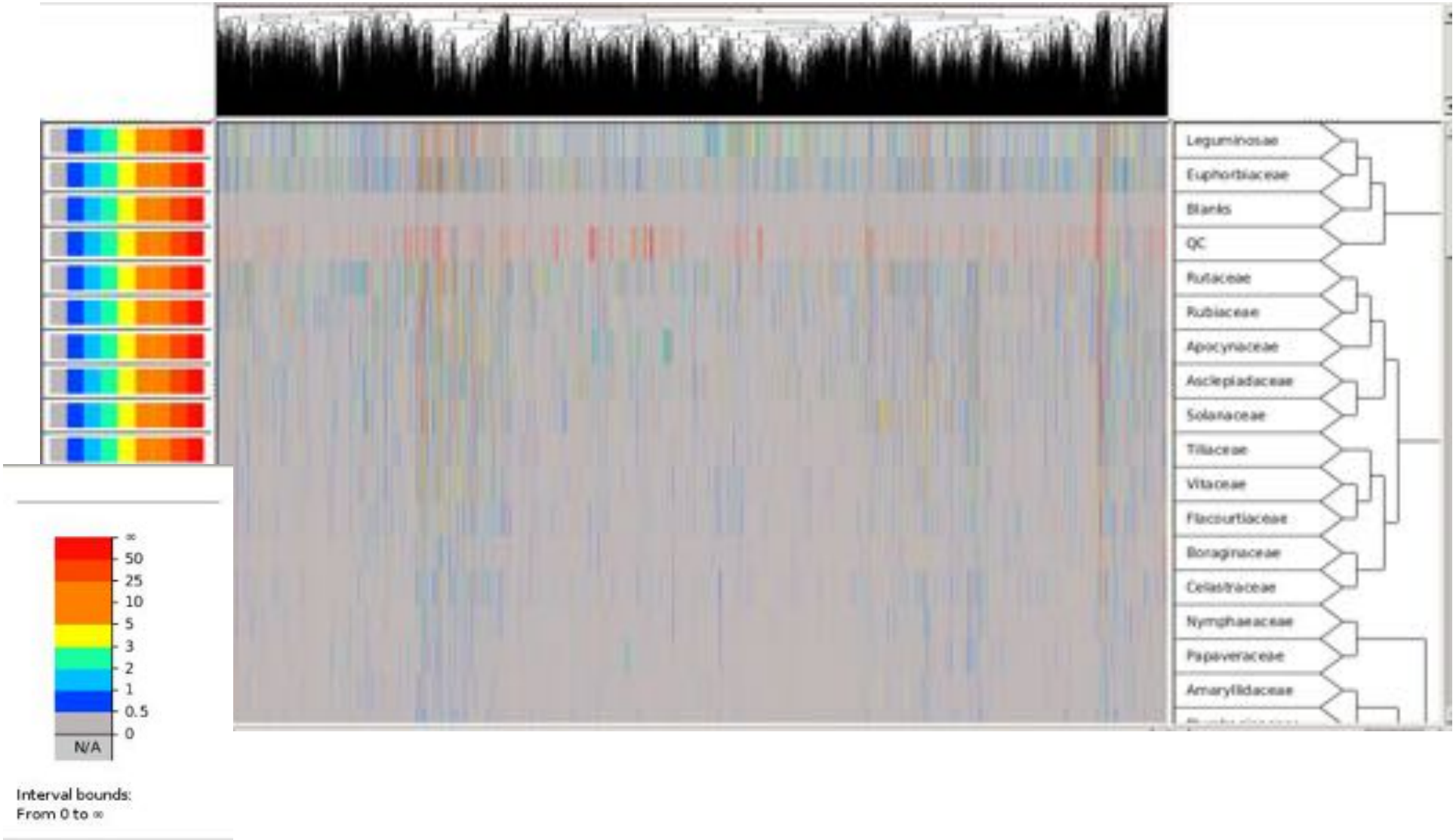
http://scaffoldhunter.sourceforge.net/

Till Schafer *et al.*, *J. Cheminfo*, **2017**, 9.

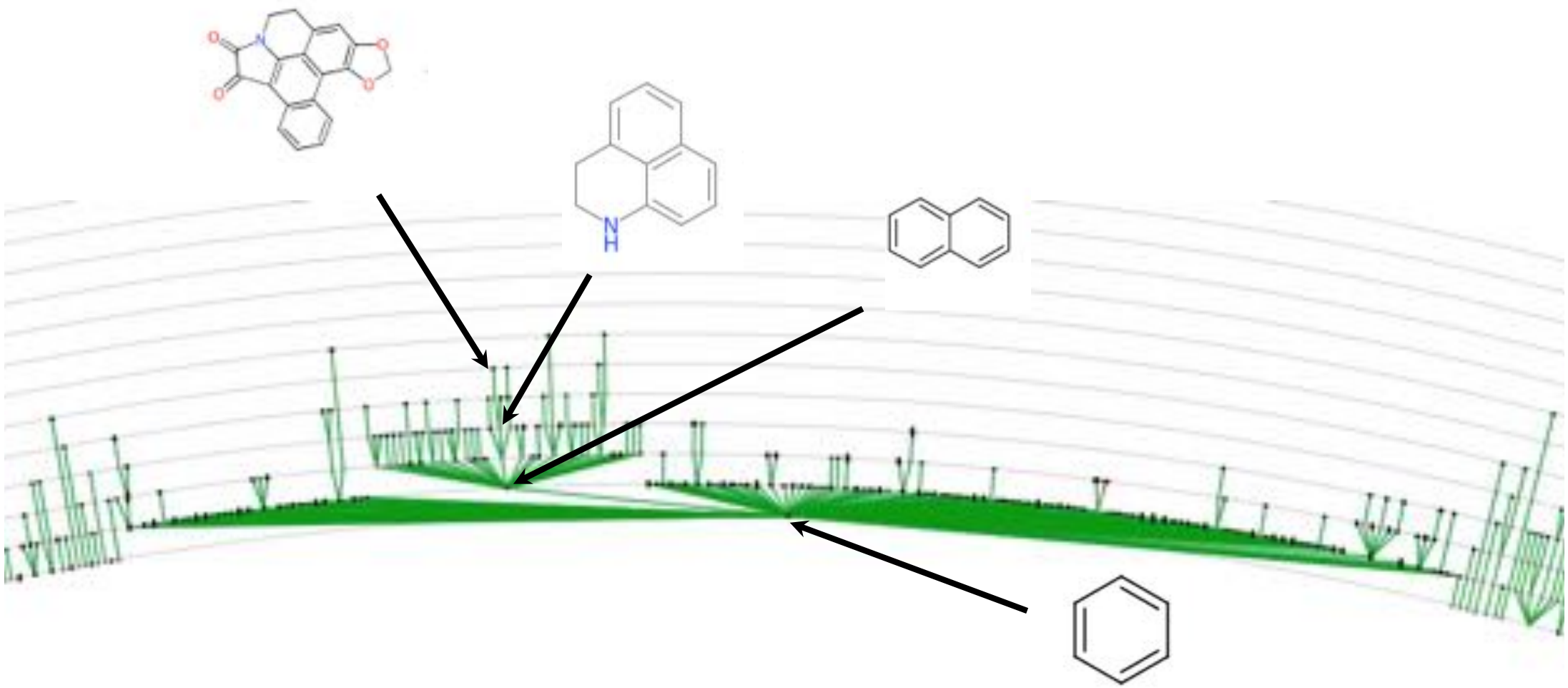


Tanimoto clustering

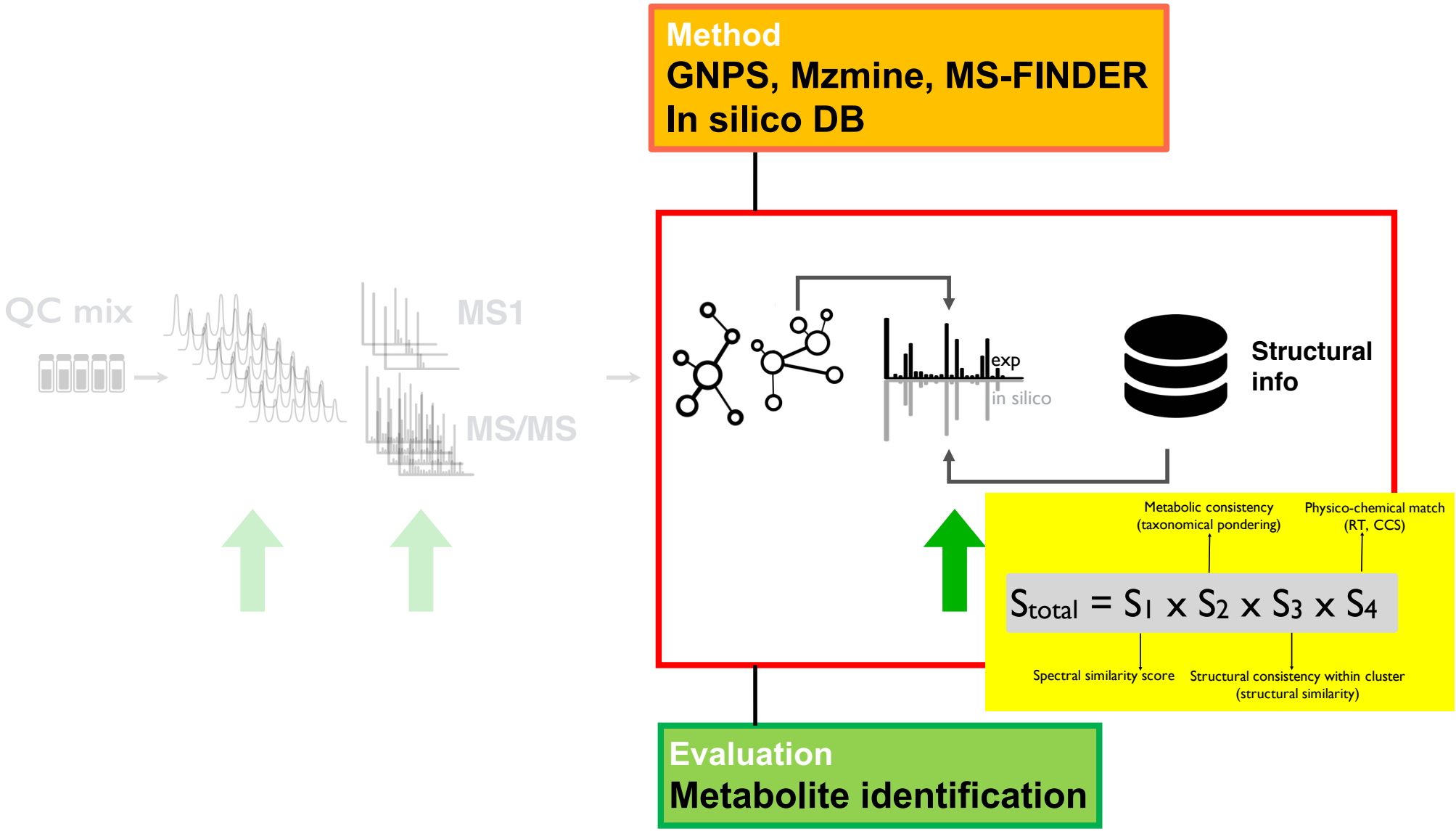
Heat mapping



Scaffold view of the 7500 compounds

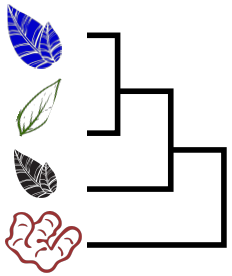


Useful for finding common structure and interesting material based on pharmacophore


QC mix
MS1
MS/MS
Method
GNPS, Mzmine, MS-FINDER
In silico DB
exp
in silico
Structural info
Metabolic consistency (taxonomical pondering)
Physico-chemical match (RT, CCS)
Stotal = S1 x S2 x S3 x S4
Spectral similarity score
Structural consistency within cluster (structural similarity)
Evaluation
Metabolite identification

Development of hypothesis metascore

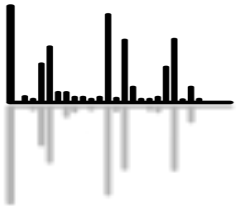
Metabolic consistency
(taxonomical pondering)



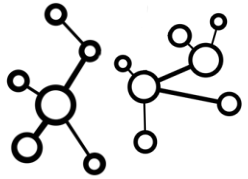
Physico-chemical match
(RT, CCS)

$$S_{total} = S_1 \times S_2 \times S_3 \times S_4$$

Spectral similarity
score



Structural consistency
within cluster
(structural similarity)



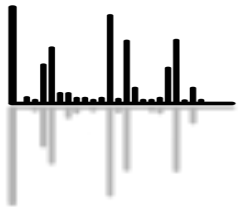
Development of hypothesis metascore

Metabolic consistency
(taxonomical pondering)

Physico-chemical match
(RT, CCS)

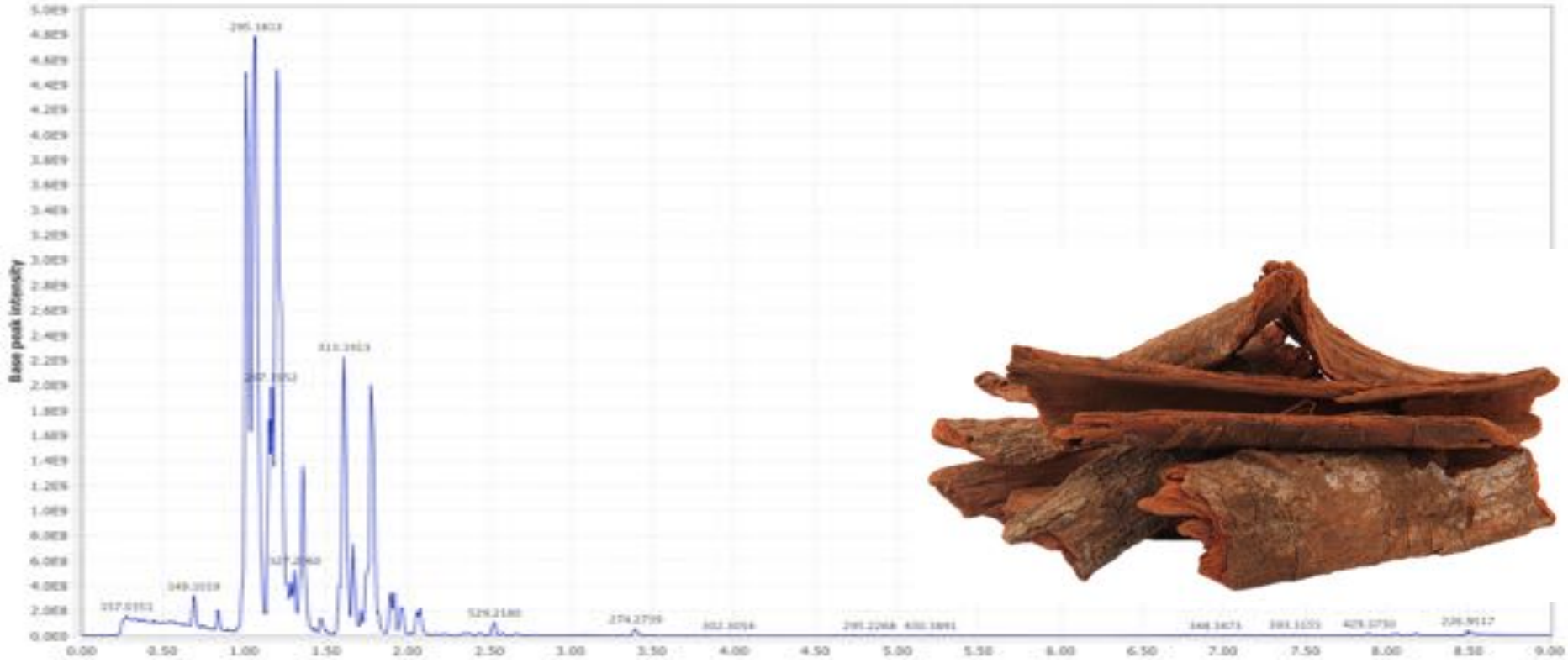
$$S_{total} = S_1 \times S_2 \times S_3 \times S_4$$

Spectral similarity
score



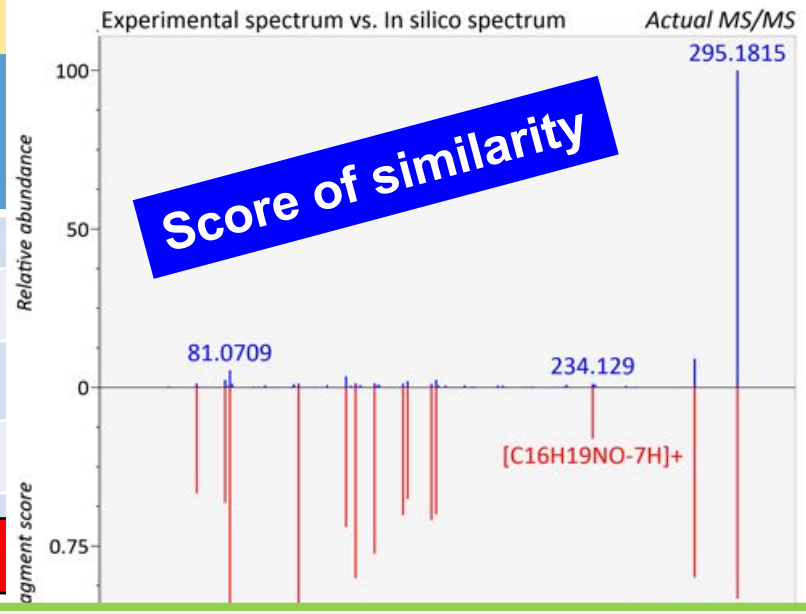
Structural consistency within cluster
(structural similarity)

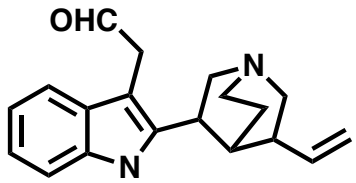
Cinchona pubescens



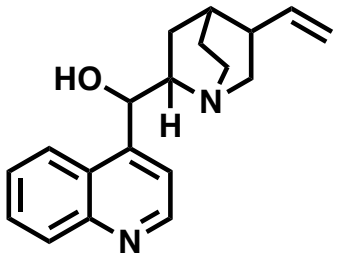
Dereplication results of *C. pubescens* with score of spectral similarity

No	m/z	MS2 Top5 hit structure in MS-Finder	MS2 Score
1	295.18134	OGY41	7.4705
		GPF00	7.088
		CFP87,80	6.9084
		OTG15	6.6168
		CFB99	6.5119

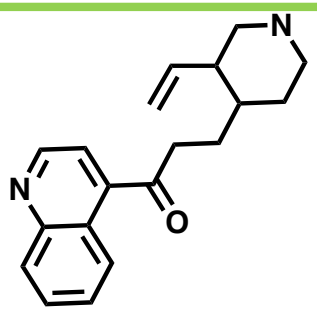




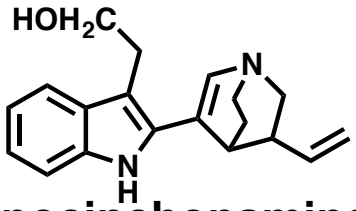
JYL65



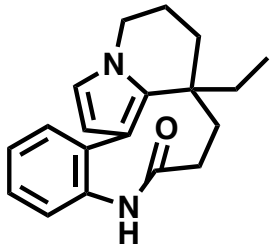
CFP80, CFP87



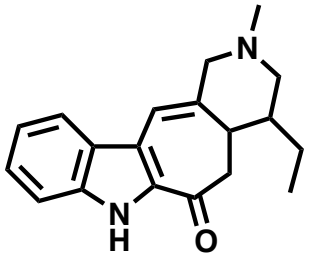
CFB99



apocinchonamine (OGY41)



rhazinilam (GPF00)

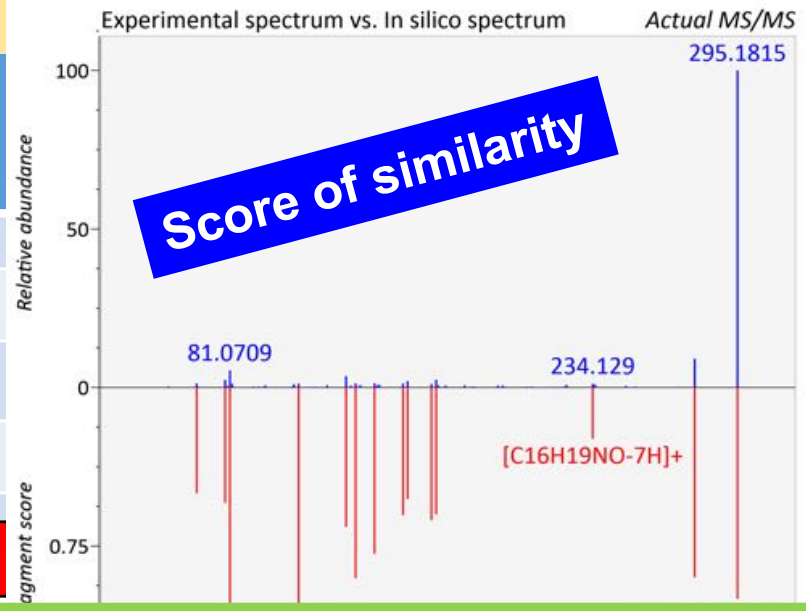


6,16-dihydro-20-episilicine (OTG15)

$S_{total} = S_1$

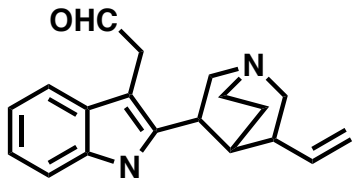
Dereplication results of *C. pubescens* with score of spectral similarity

No	m/z
1	295.18134

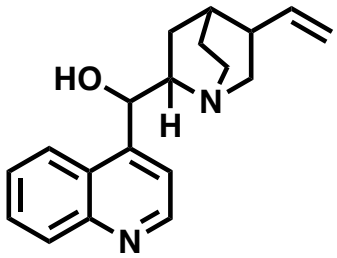


	MS2	
	Top5 hit structure in MS-Finder	S_1
1	OGY41	7.4705
2	GPF00	7.088
3	CFP87,80	6.9084
4	OTG15	6.6168
5	CFB99	6.5119

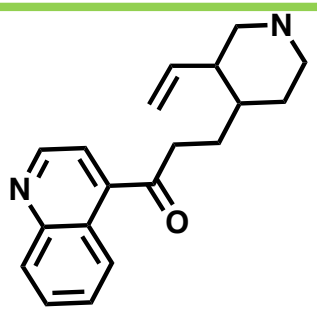
MS/MS scoring whole DB



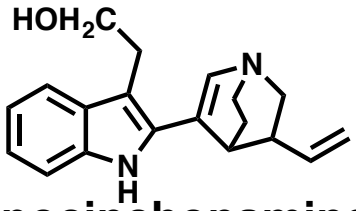
JYL65



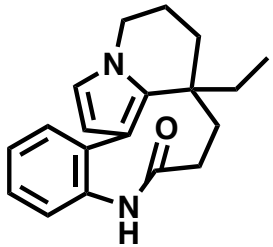
CFP80, CFP87



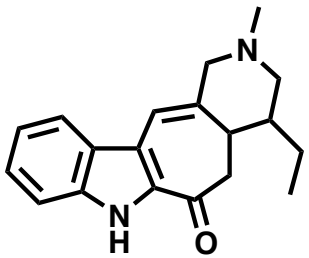
CFB99



apocinchonamine (OGY41)



rhazinilam (GPF00)



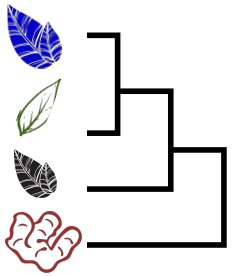
6,16-dihydro-20-episilicine (OTG15)

CGJ39: No structure information

$S_{total} = S_1$

Development of hypothesis metascore

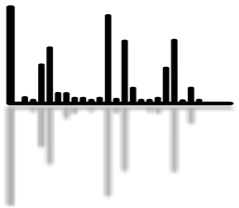
Metabolic consistency
(taxonomical pondering)



Physico-chemical match
(RT, CCS)

$$S_{total} = S_1 \times S_2 \times S_3 \times S_4$$

Spectral similarity
score

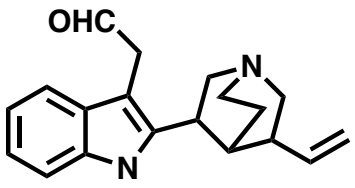


Structural consistency within cluster
(structural similarity)

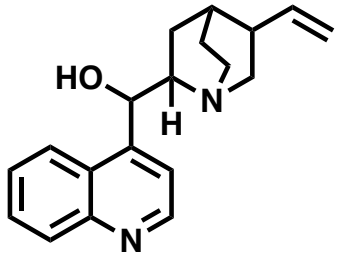
Dereplication results of *C. pubescens* with Metascore

No	MZmine	Top5 hit structure in MS_Finder	S_1		genus	point*
1	CGJ39	OGY41	7.471	1	No IF	1
C19H22N2O	CFP80 JYL65	GPF00	7.088	2	another family	1
		CFP87,80	6.908	3	C. officinalis and all Cinchona spp.	1.6
	CFP87	OTG15	6.617	4	another family	1
	CFB99	CFB99	6.512	5	C. succirubra (pubescens)	1.6

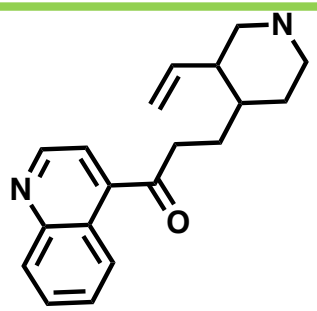
1.6 point: same sp
1.4 point: same genus
1.2 point: same family
1.0 point: another family and No IF



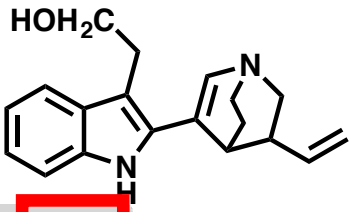
JYL65



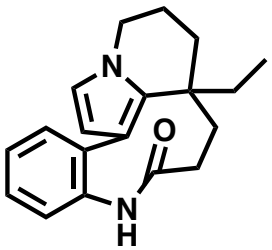
CFP80, CFP87



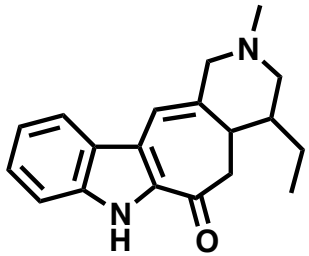
CFB99



GY41



GPF00



OTG15

CGJ39: No structure information

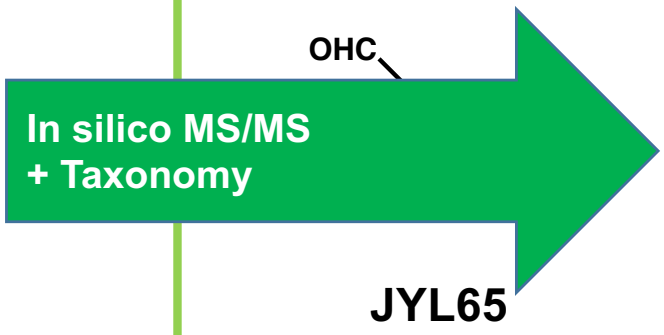
$$S_{total} = S_1 \times S_2$$

Dereplication results of *C. pubescens* with Metascore

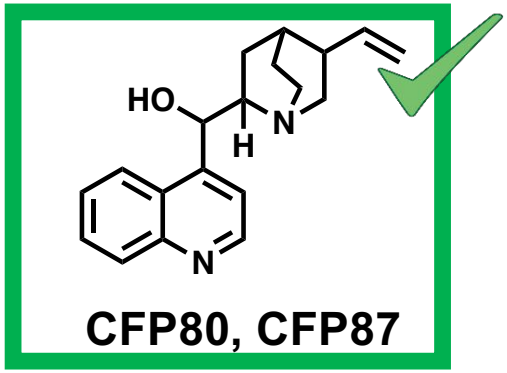
No	MZmine	Top5 hit structure in MS_Finder	S_1		genus	point*	Top5 hit structure in MS_Finder	Metascore Score_taxonomicaly	
1	CGJ39	OGY41	7.471	1	No IF	1	OGY41	7.471	3
2	CFP80	GPF00	7.088	2	another family	1	GPF00	7.088	4
3	JYL65	CFP87,80	6.908	3	C. officinalis and all Cinchona spp.	1.6	CFP87,80	11.053	1
4	CFP87	OTG15	6.617	4	another family	1	OTG15	6.617	5
5	CFB99	CFB99	6.512	5	uccirubra (pubescens)	1.6	CFB99	10.419	2

C19H22N2O

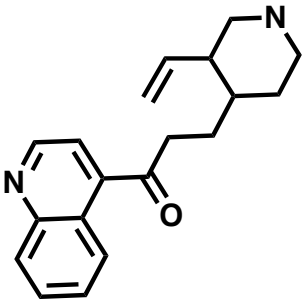
*1.6 point: same sp, 1.4 point: same genus, 1.2 point: same family, 1.0 point: another family and No IF



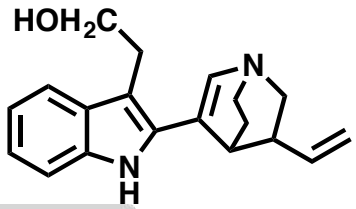
JYL65



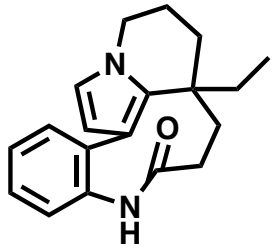
CFP80, CFP87



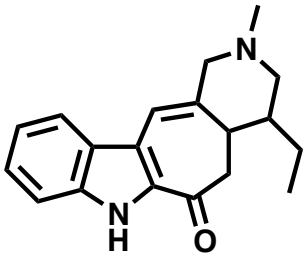
CFB99



GY41



GPF00

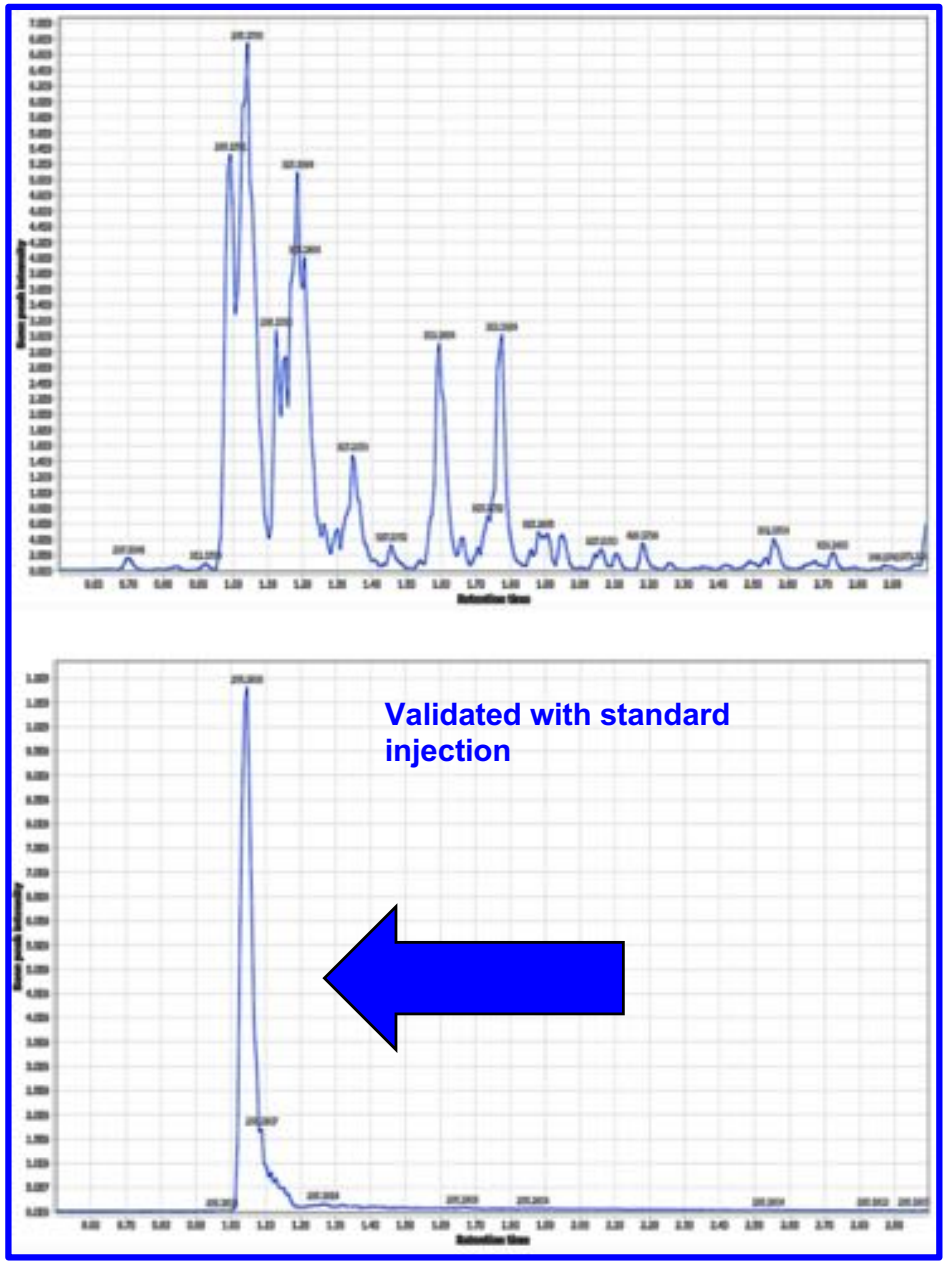


OTG15

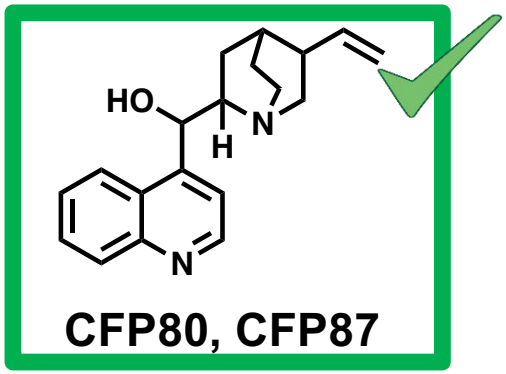
CGJ39: No structure information

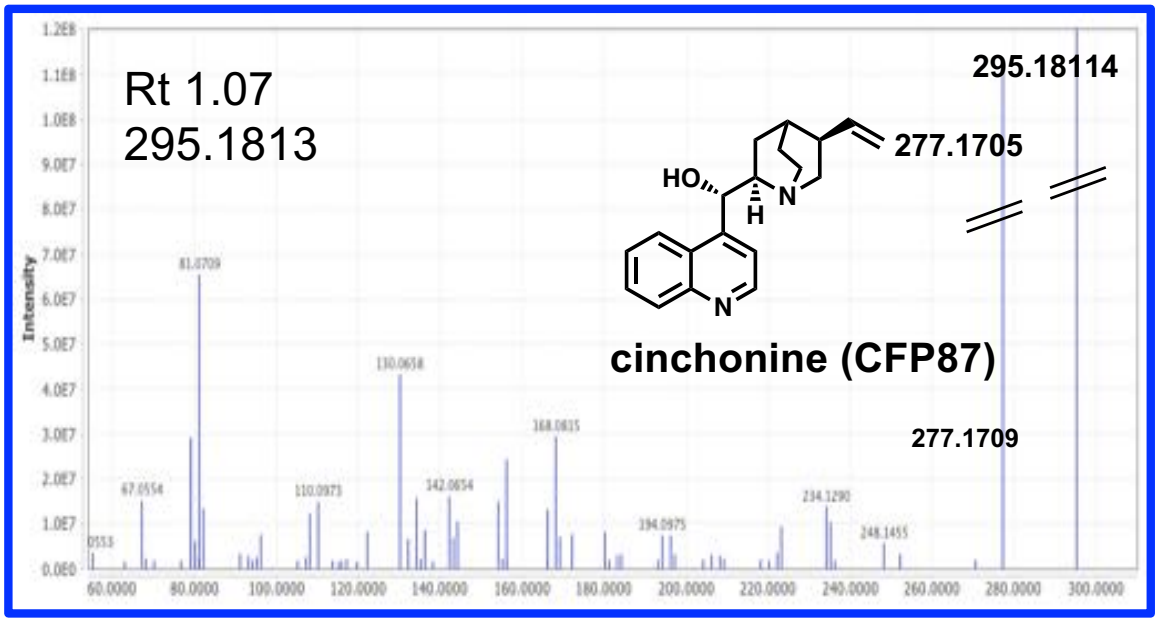
$$S_{total} = S_1 \times S_2$$

Validated with standard injection

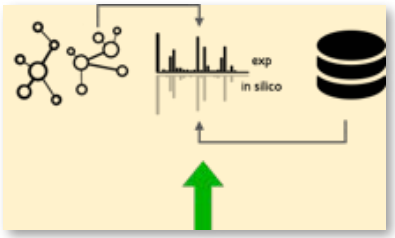


In silico MS/MS
+ Taxonomy

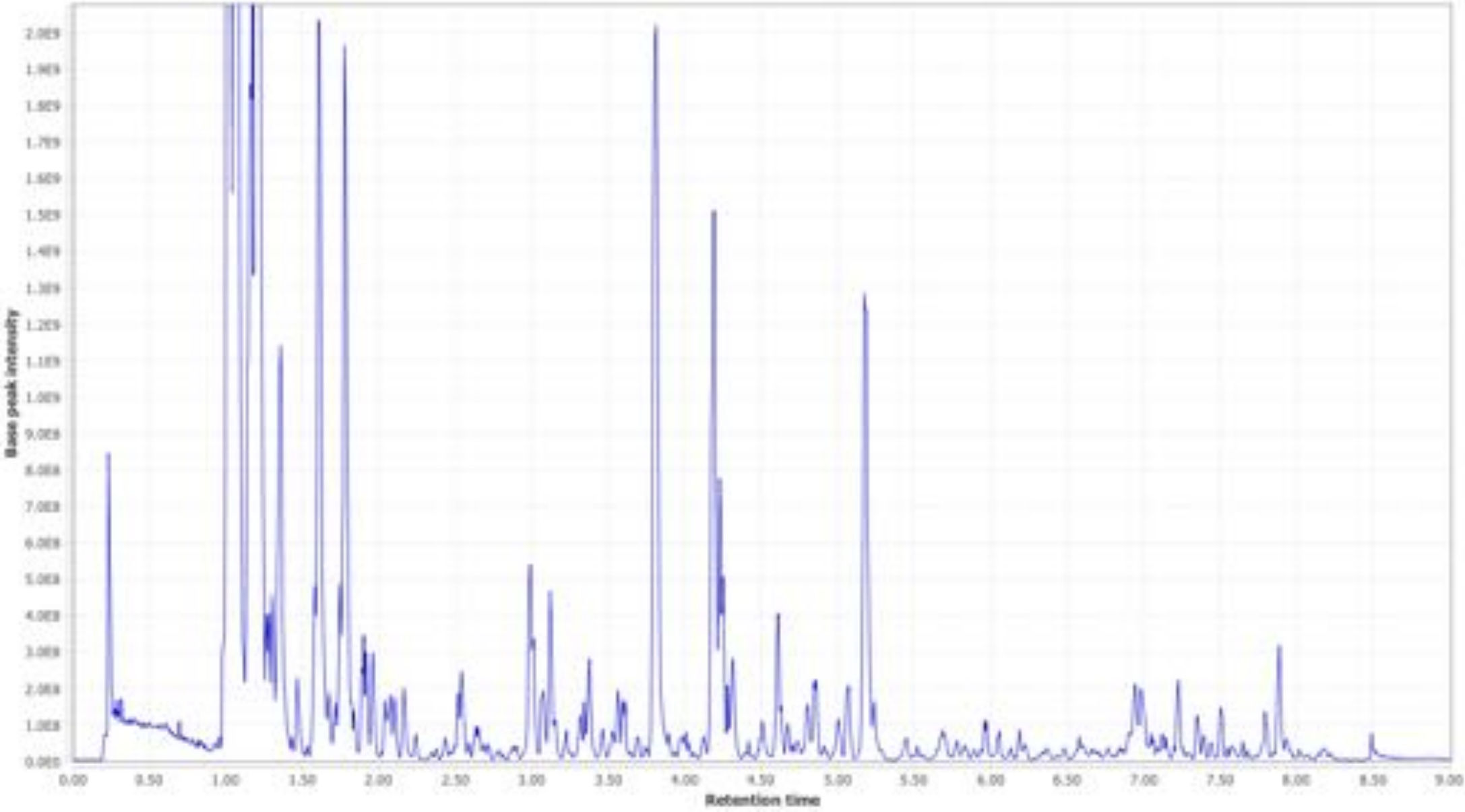




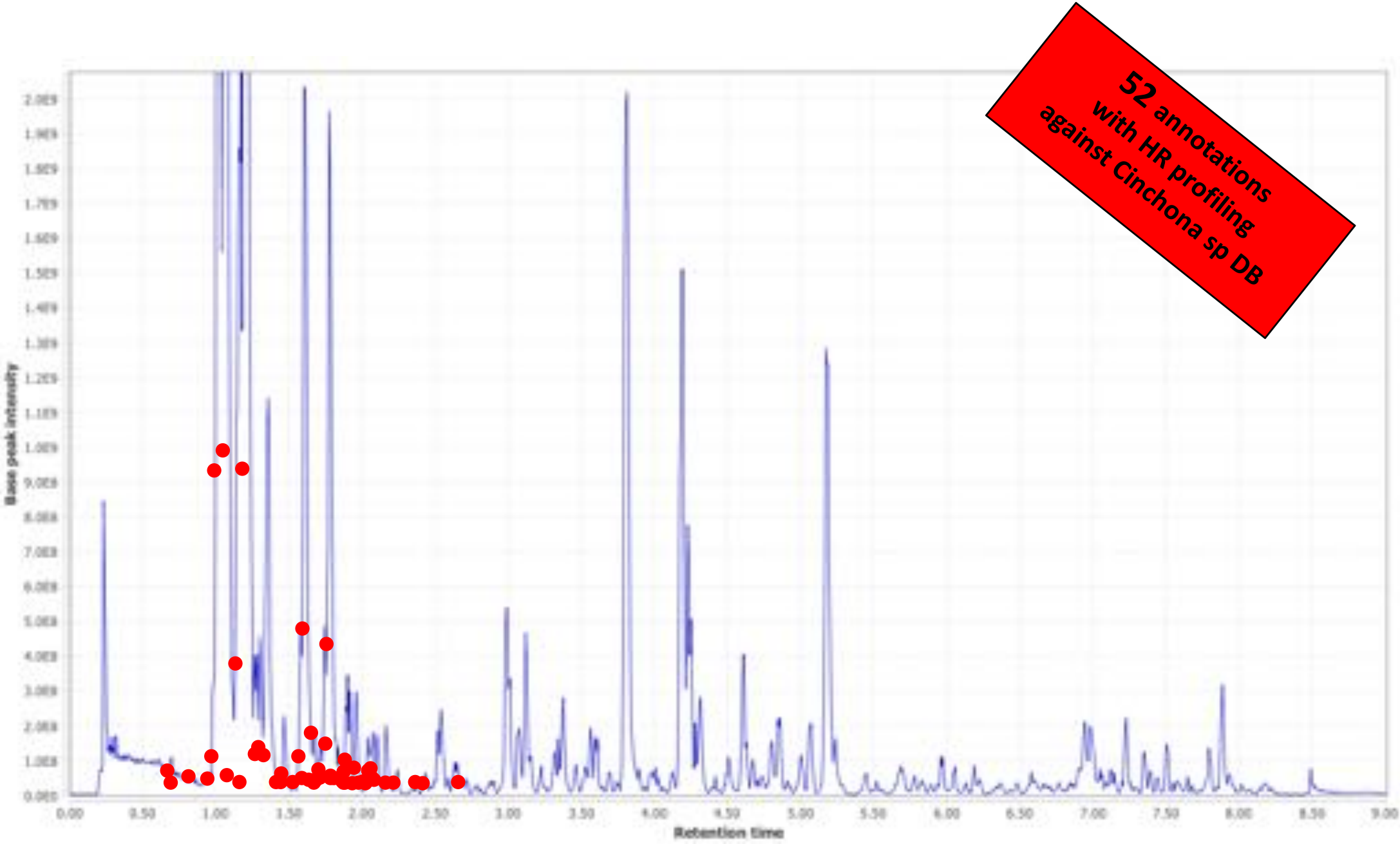
$$S_{total} = S_1 \times S_2$$



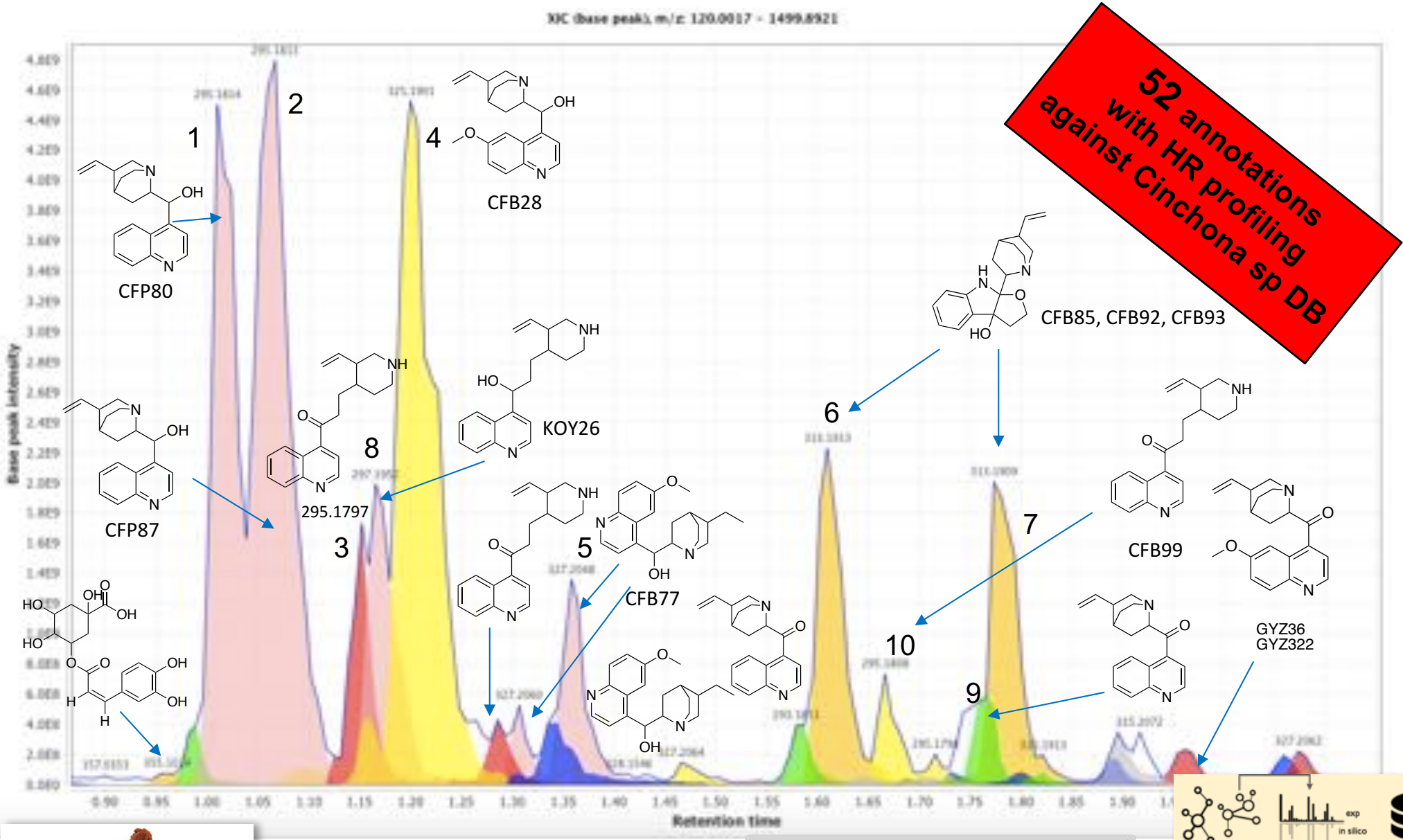
UHPLC-HRMS profiling of the QC mix of 5 herbs



UHPLC-HRMS profiling of the QC mix of 5 herbs



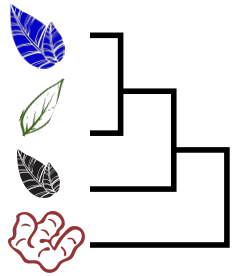
Alkaloids annotated from *C. pubescens*



$$S_{total} = S_1 \times S_2$$

Development of hypothesis metascore

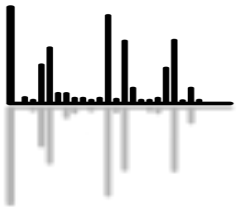
Metabolic consistency
(taxonomical pondering)



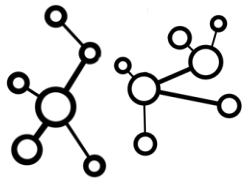
Physico-chemical match
(RT, CCS)

$$S_{total} = S_1 \times S_2 \times S_3 \times S_4$$

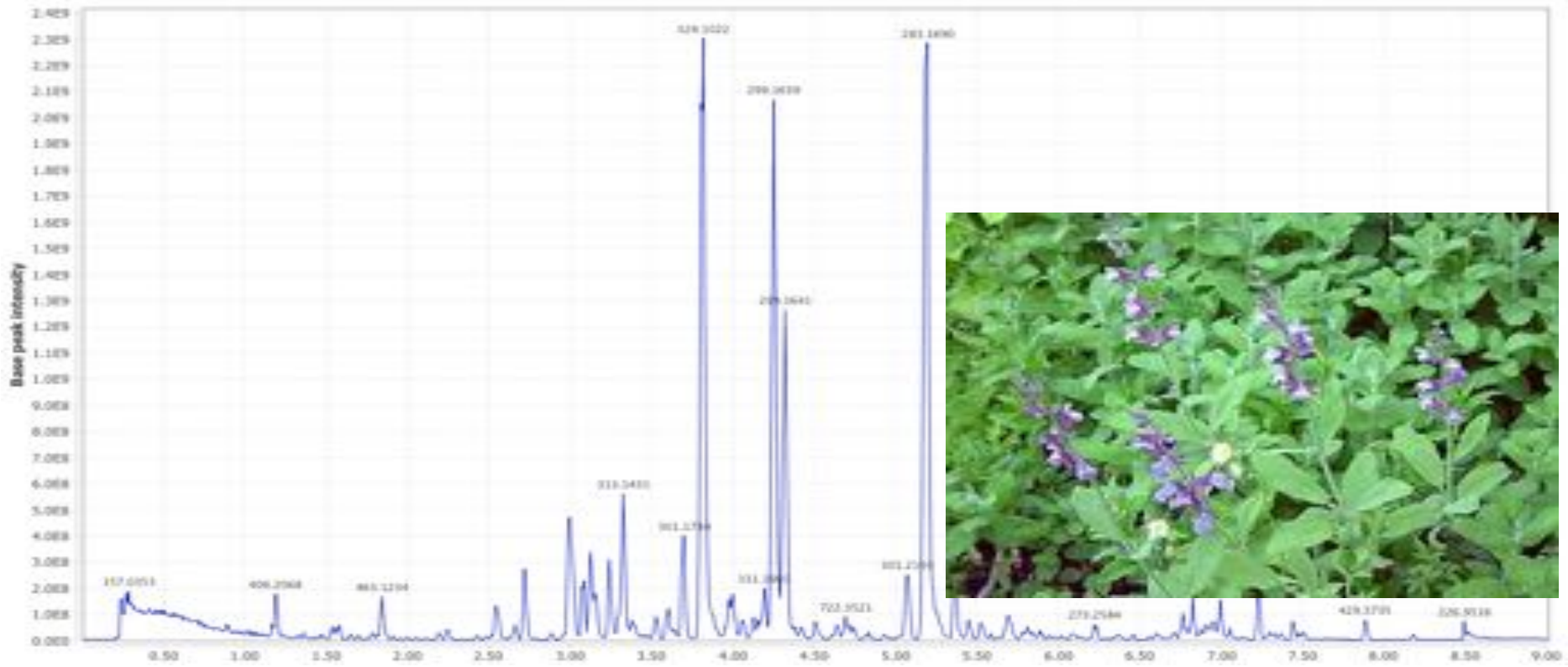
Spectral similarity
score



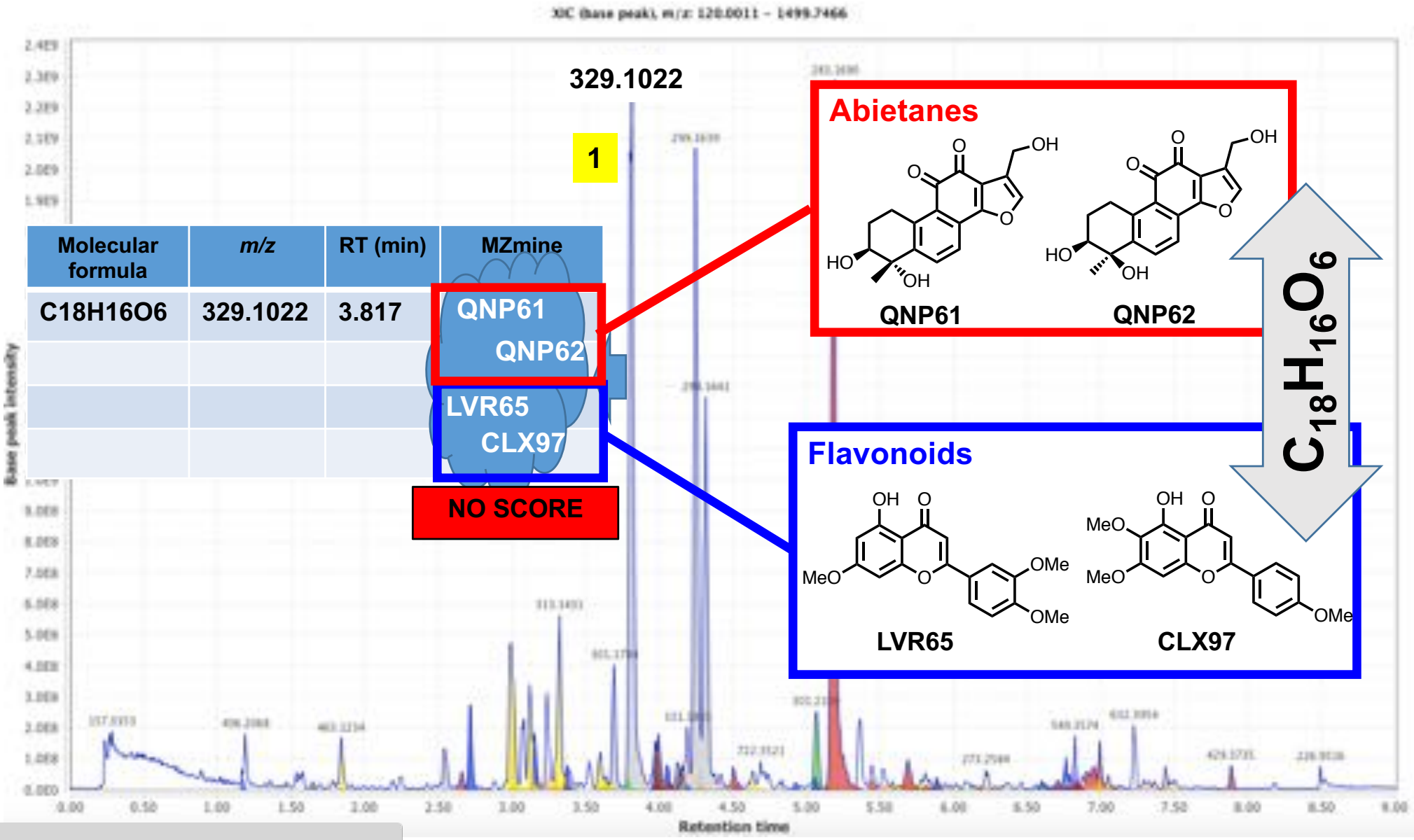
Structural consistency
within cluster
(structural similarity)



Salvia officinalis



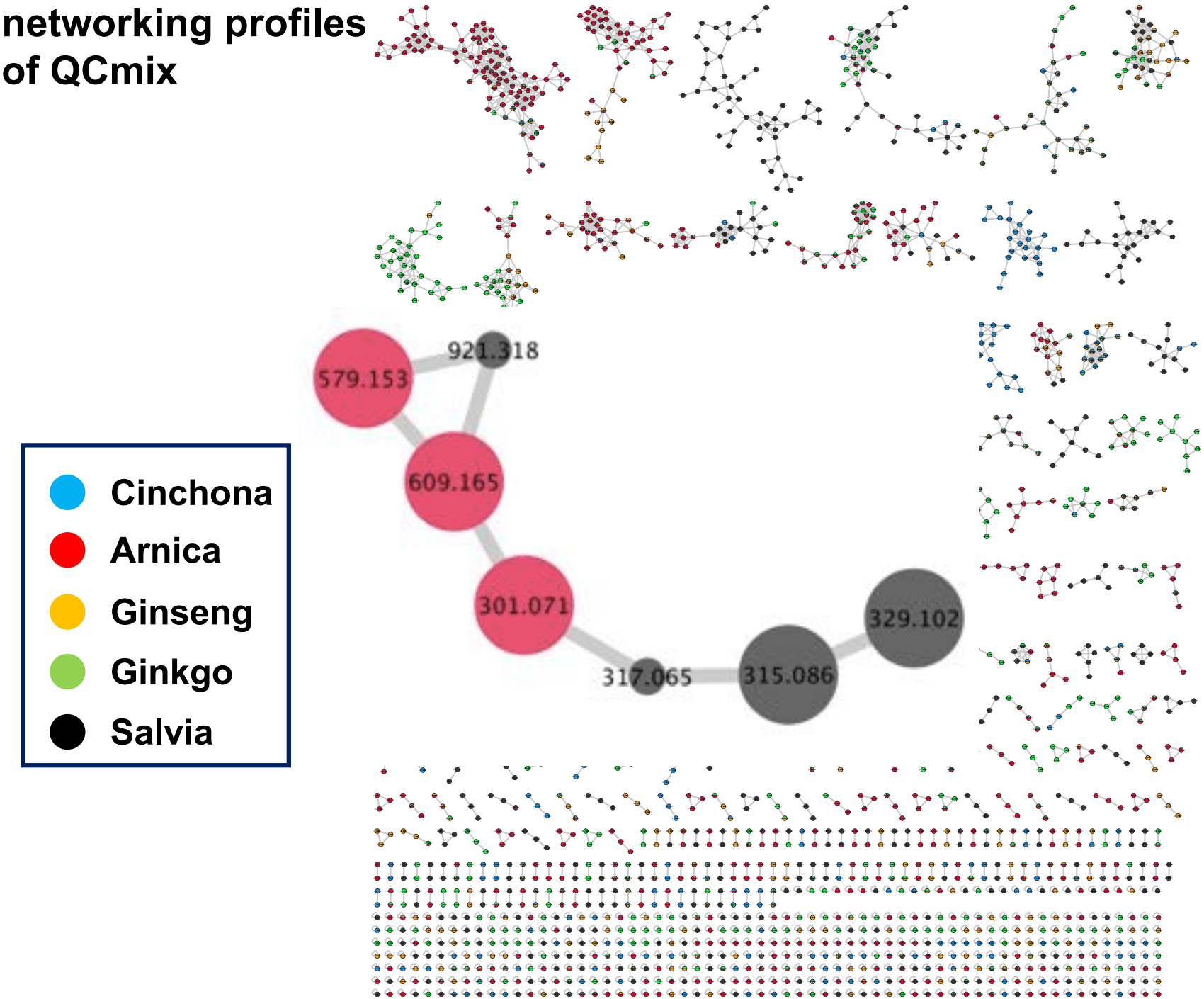
HRMS-ESI (+) of the MeOH extract of *S. officinalis* with annotation restricted to *Salvia spp*.



Molecular formula	*m/z*	RT (min)	MZmine
C18H16O6	329.1022	3.817	QNP61 QNP62 LVR65 CLX97

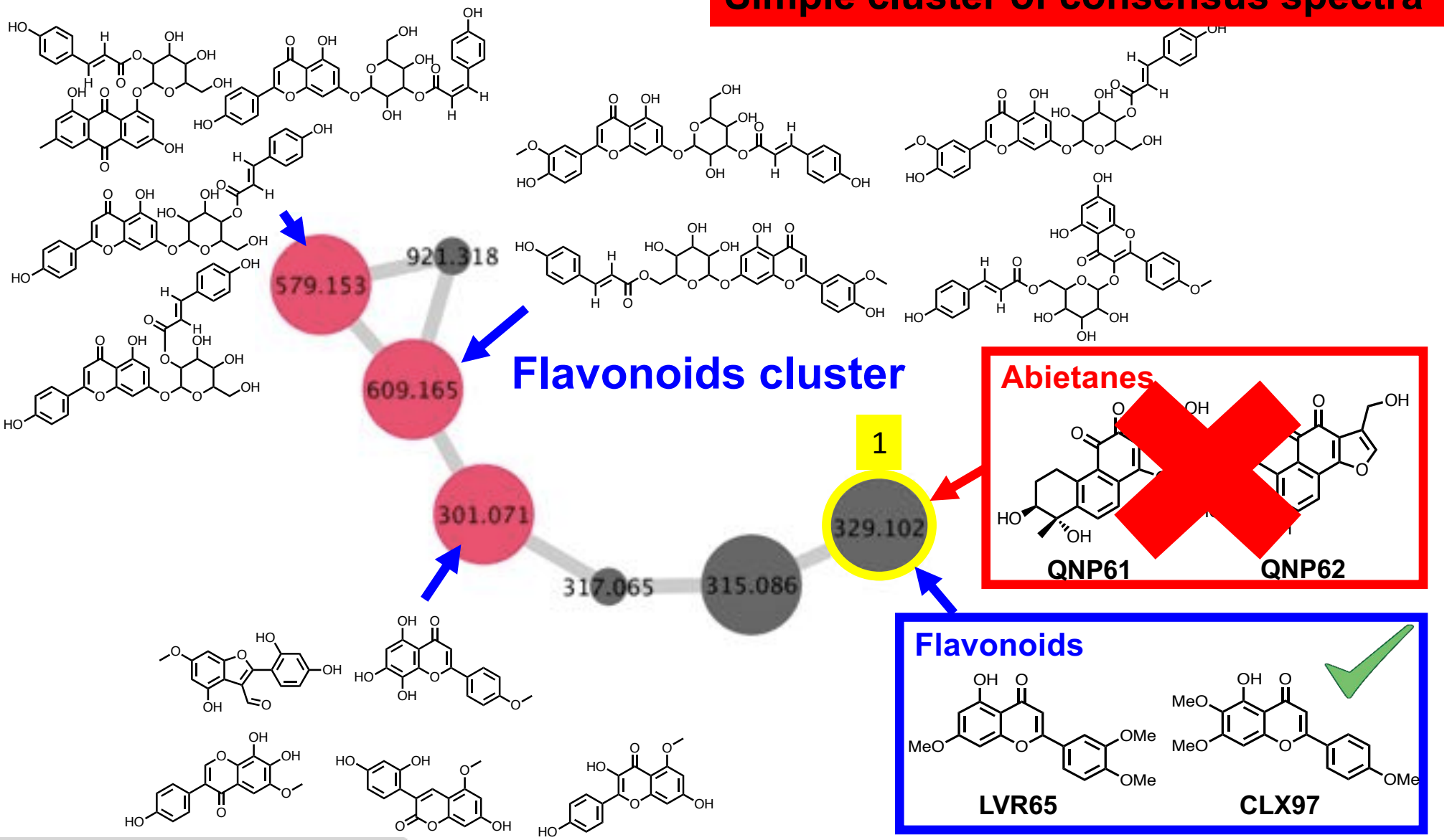
$$S_{total} = S_1 \times S_2 \times S_3$$

Molecular networking profiles of QCmix



Molecular networking profiles of *S. officinalis*

Simple cluster of consensus spectra



Flavonoids cluster

Abietanes

QNP61 **QNP62**

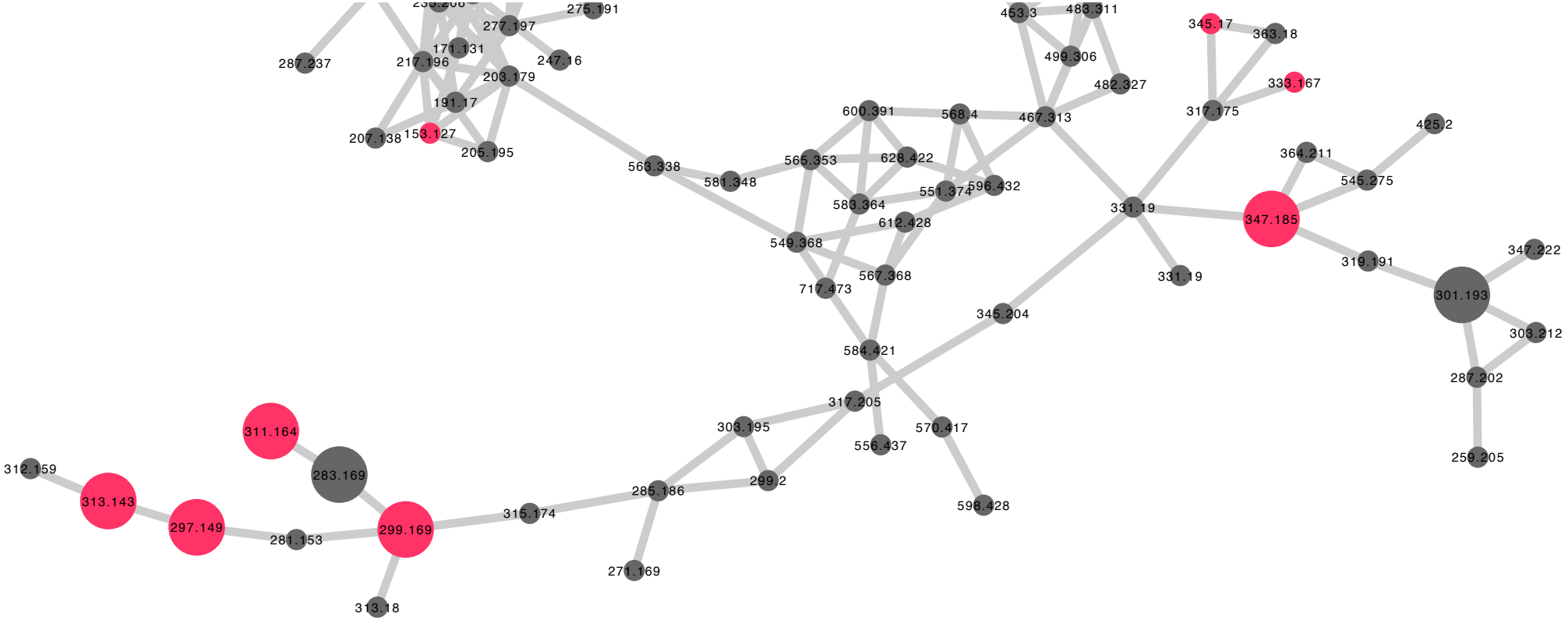
Flavonoids

LVR65 **CLX97**

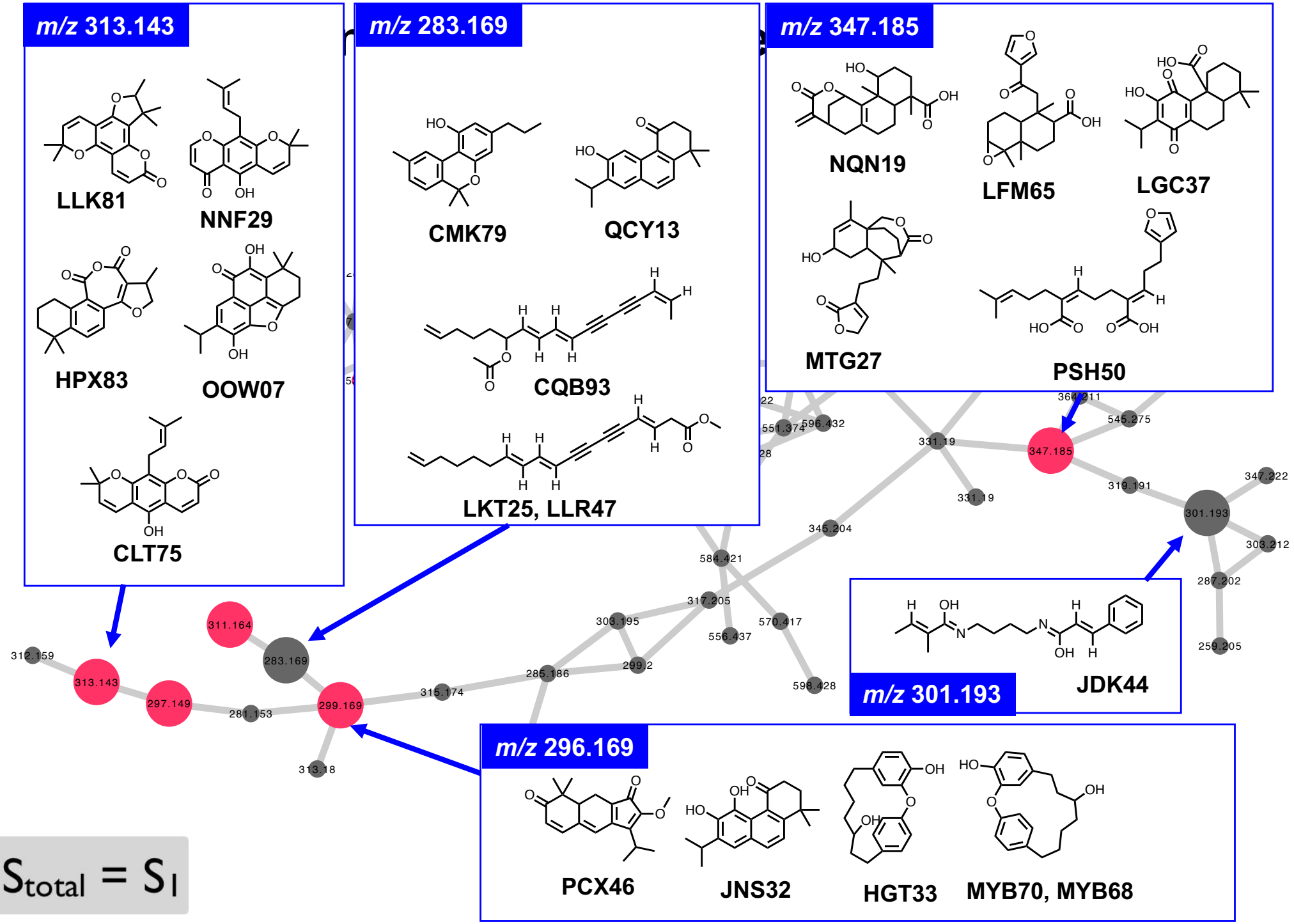
$S_{total} = S_1 \times S_2 \times S_3$

Manual comparison of structure types

Molecular networking profiles of *S. officinalis*



$$S_{total} = S_1 \times S_2 \times S_3$$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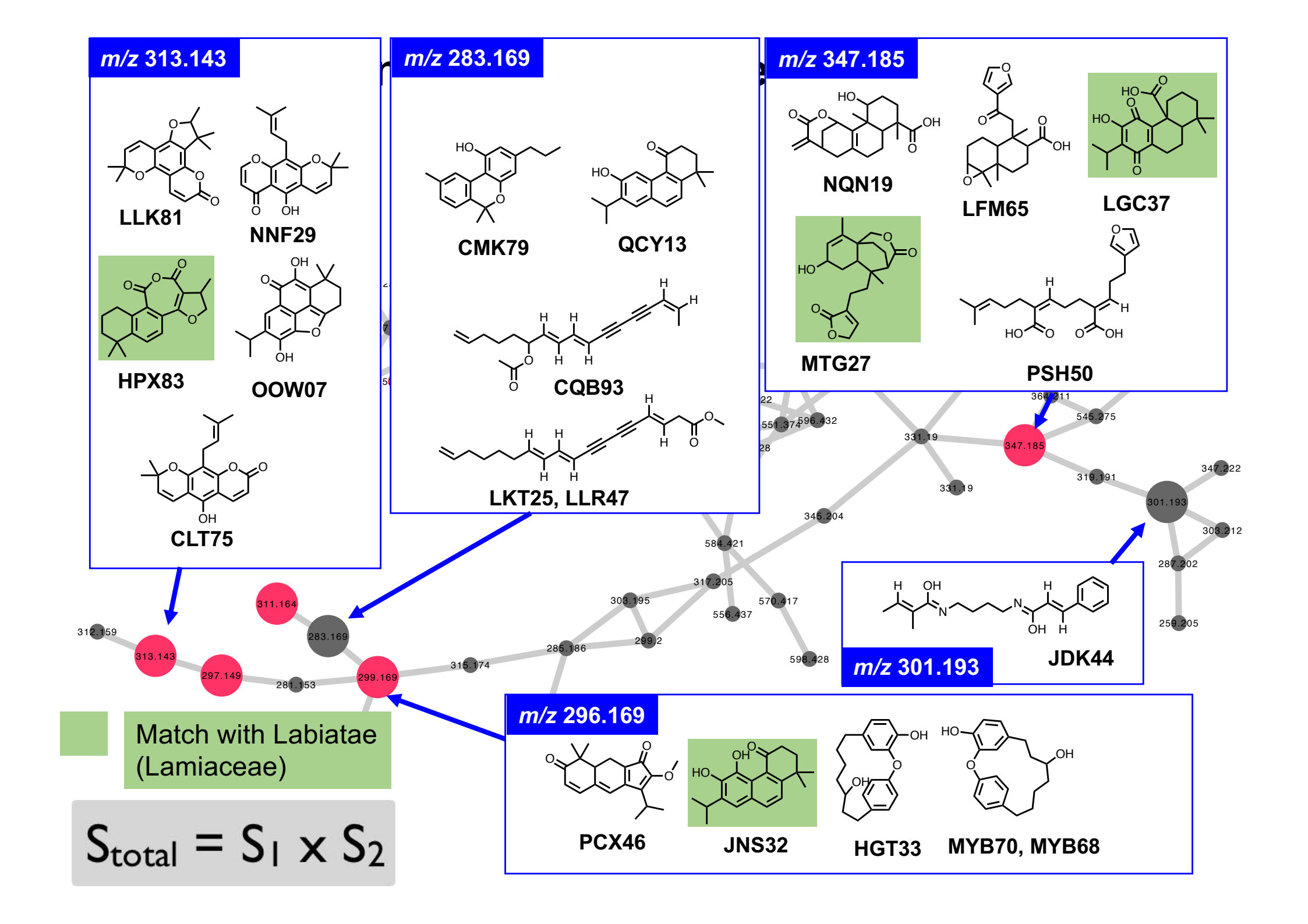
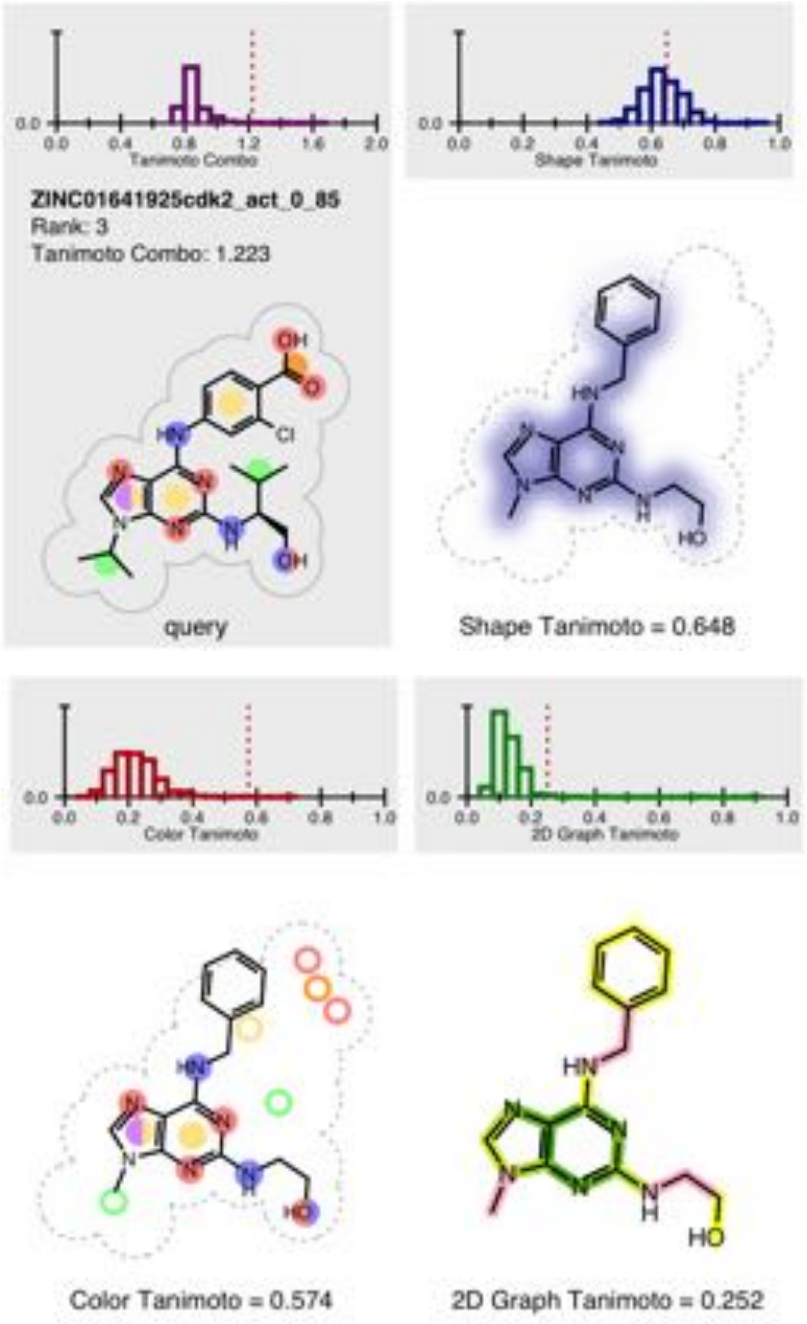
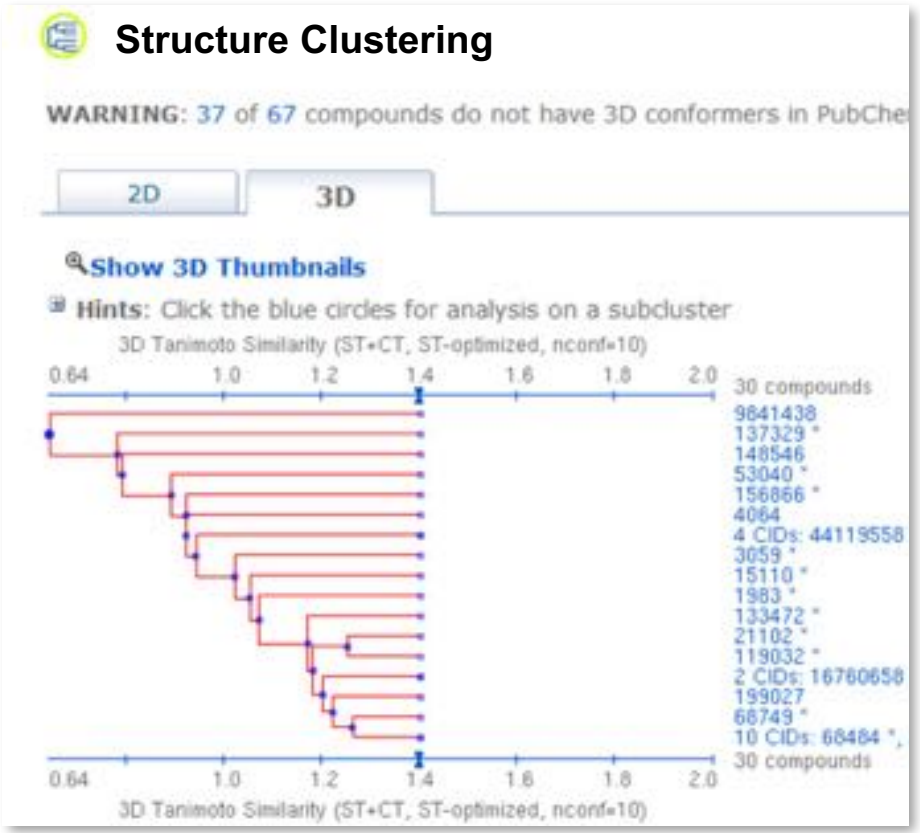
LLK81
NNF29
HPX83
OOW07
CLT75
m/z 283.169
CMK79
QCY13
CQB93
LKT25, LLR47
m/z 347.185
NQN19
LFM65
LGC37
MTG27
PSH50
m/z 301.193
JDK44
m/z 296.169
PCX46
JNS32
HGT33
MYB70, MYB68
Stotal = S1


m/z 313.143
LLK81
NNF29
HPX83
OOW07
CLT75
m/z 283.169
CMK79
QCY13
CQB93
LKT25, LLR47
m/z 347.185
NQN19
LFM65
LGC37
MTG27
PSH50
m/z 301.193
JDK44
m/z 296.169
PCX46
JNS32
HGT33
MYB70, MYB68
Match with Labiatae (Lamiaceae)
$S_{total} = S_1 \times S_2$

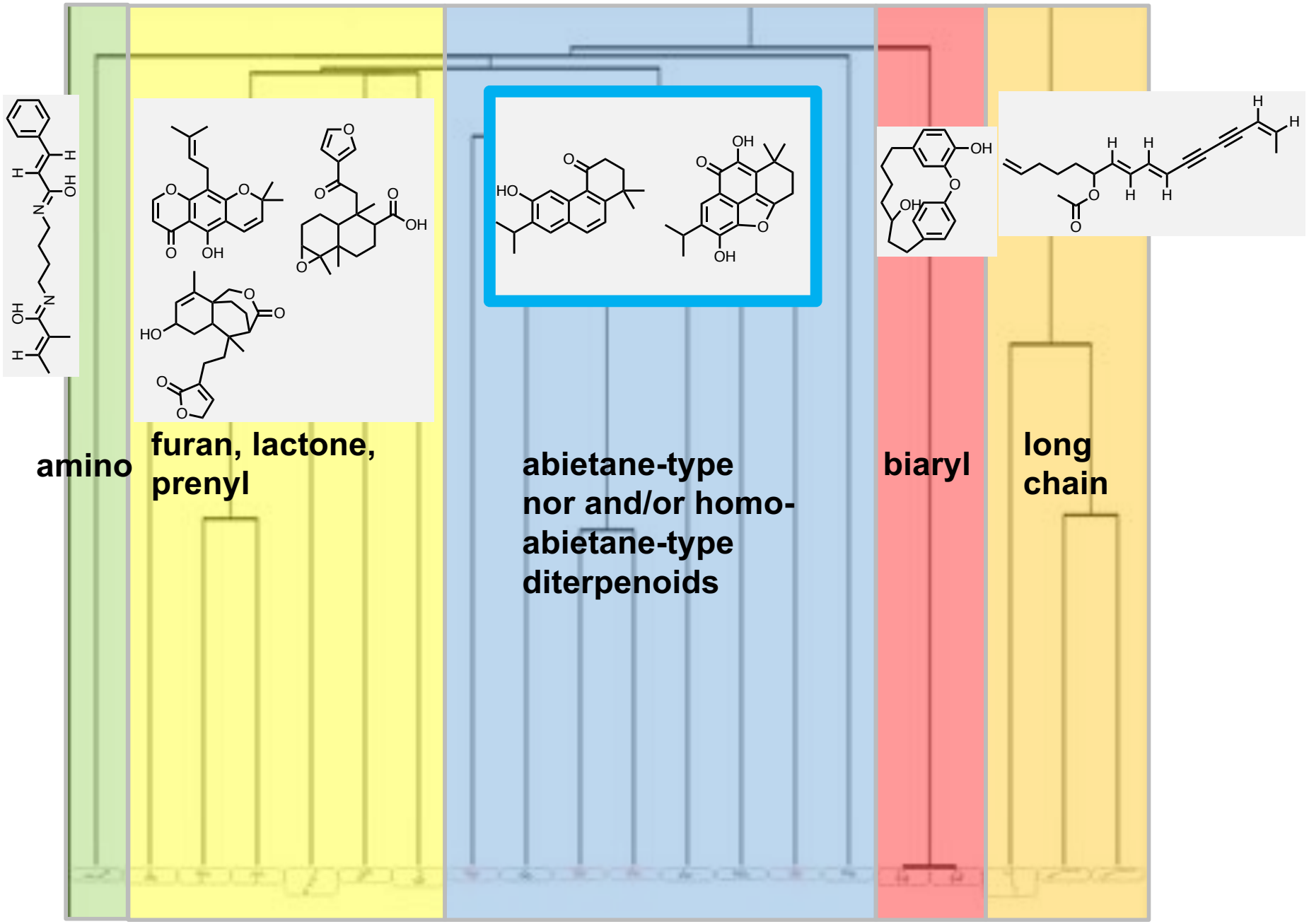
Structural consistency within clusters using Tanimoto scores



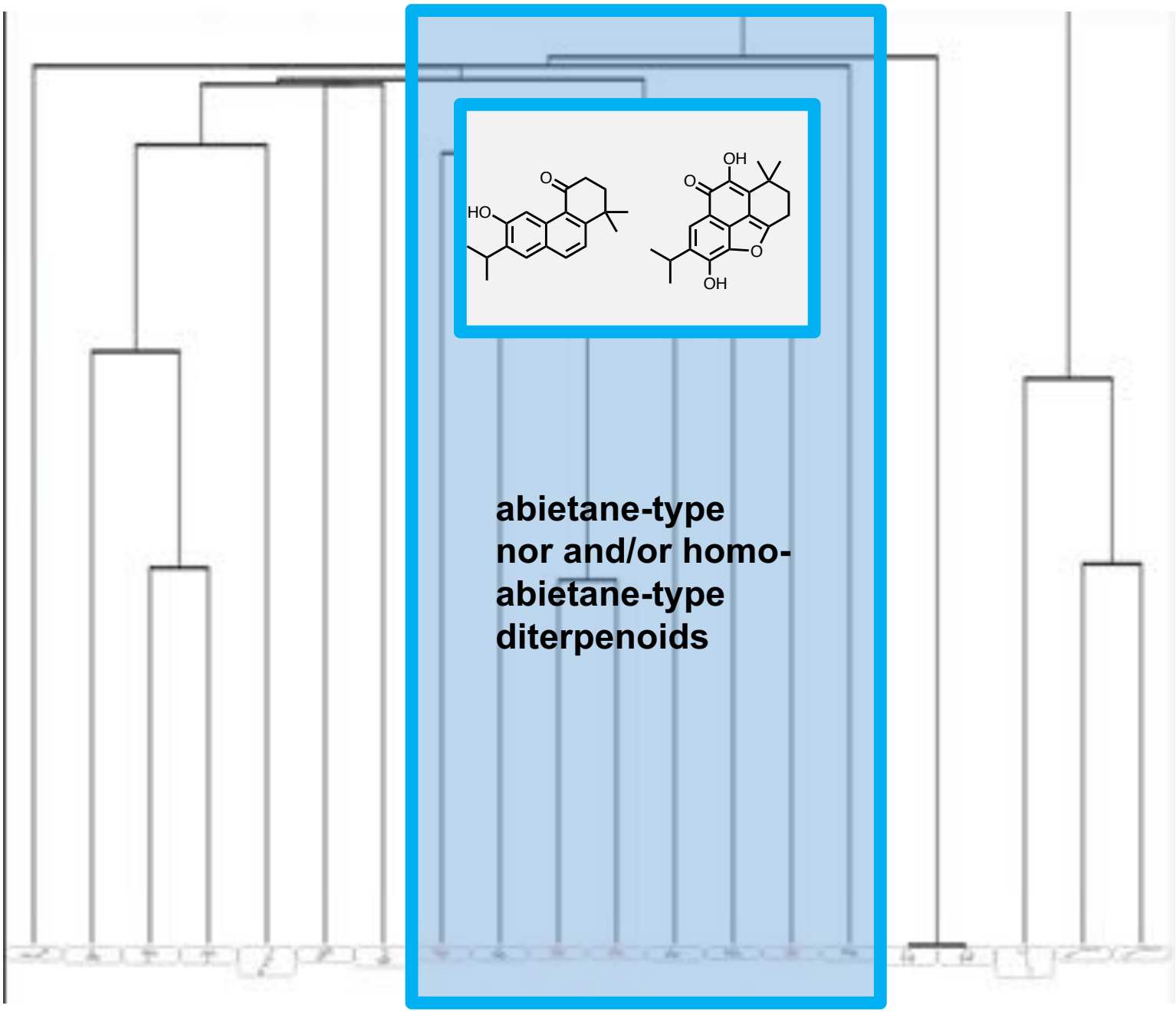


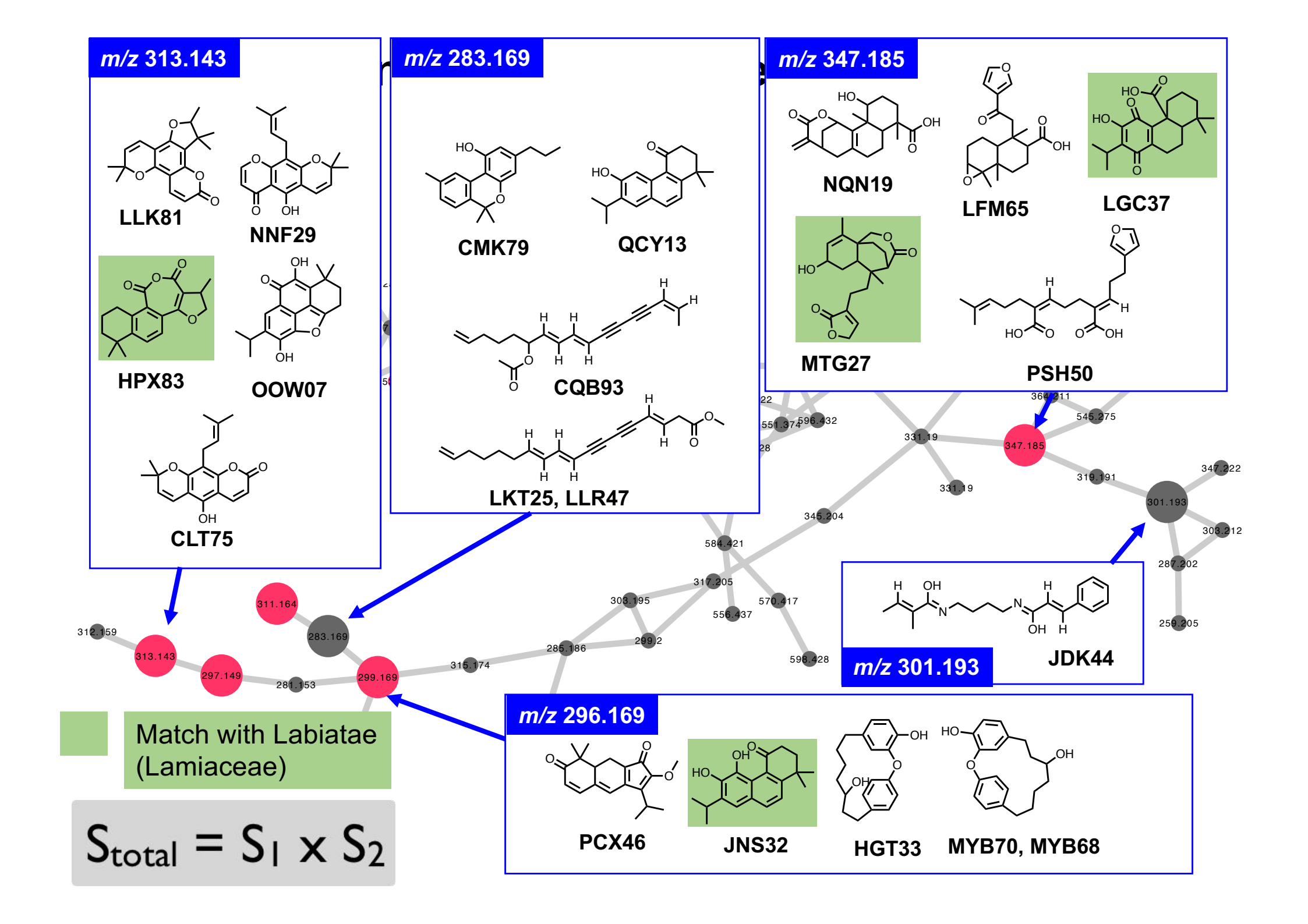
Assigning similarity of structures by substructure search

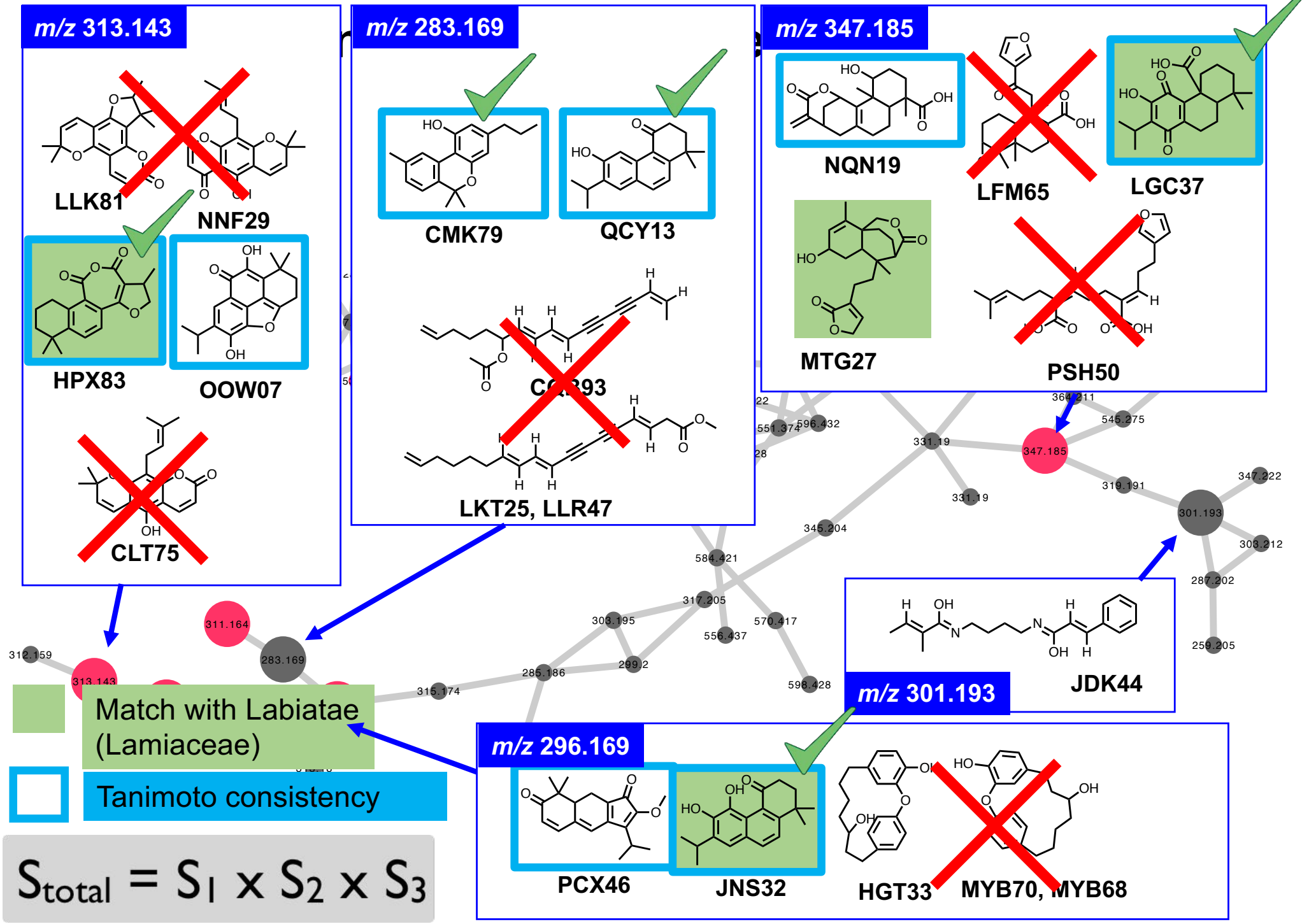
Tanimoto dendrogram of a MN *Salvia* cluster



Tanimoto dendrogram of a MN *Salvia* cluster




m/z 313.143
LLK81
NNF29
HPX83
OOW07
CLT75
m/z 283.169
CMK79
QCY13
CQB93
LKT25, LLR47
m/z 347.185
NQN19
LFM65
LGC37
MTG27
PSH50
m/z 301.193
JDK44
m/z 296.169
PCX46
JNS32
HGT33
MYB70, MYB68
Match with Labiatae (Lamiaceae)
$S_{total} = S_1 \times S_2$


m/z 313.143
LLK81
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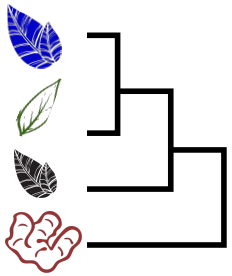
HPX83
OOW07
CLT75
m/z 283.169
CMK79
QCY13
LKT25, LLR47
m/z 347.185
NQN19
LFM65
LGC37
MTG27
PSH50
JDK44
m/z 301.193
m/z 296.169
PCX46
JNS32
HGT33
MYB70, MYB68
Match with Labiatae (Lamiaceae)
Tanimoto consistency
$S_{total} = S_1 \times S_2 \times S_3$

Development of hypothesis metascore

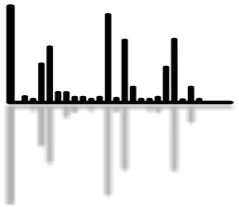
Metabolic consistency
(taxonomical pondering)



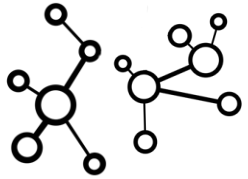
Physico-chemical match
(RT, CCS)

$$S_{total} = S_1 \times S_2 \times S_3 \times S_4$$

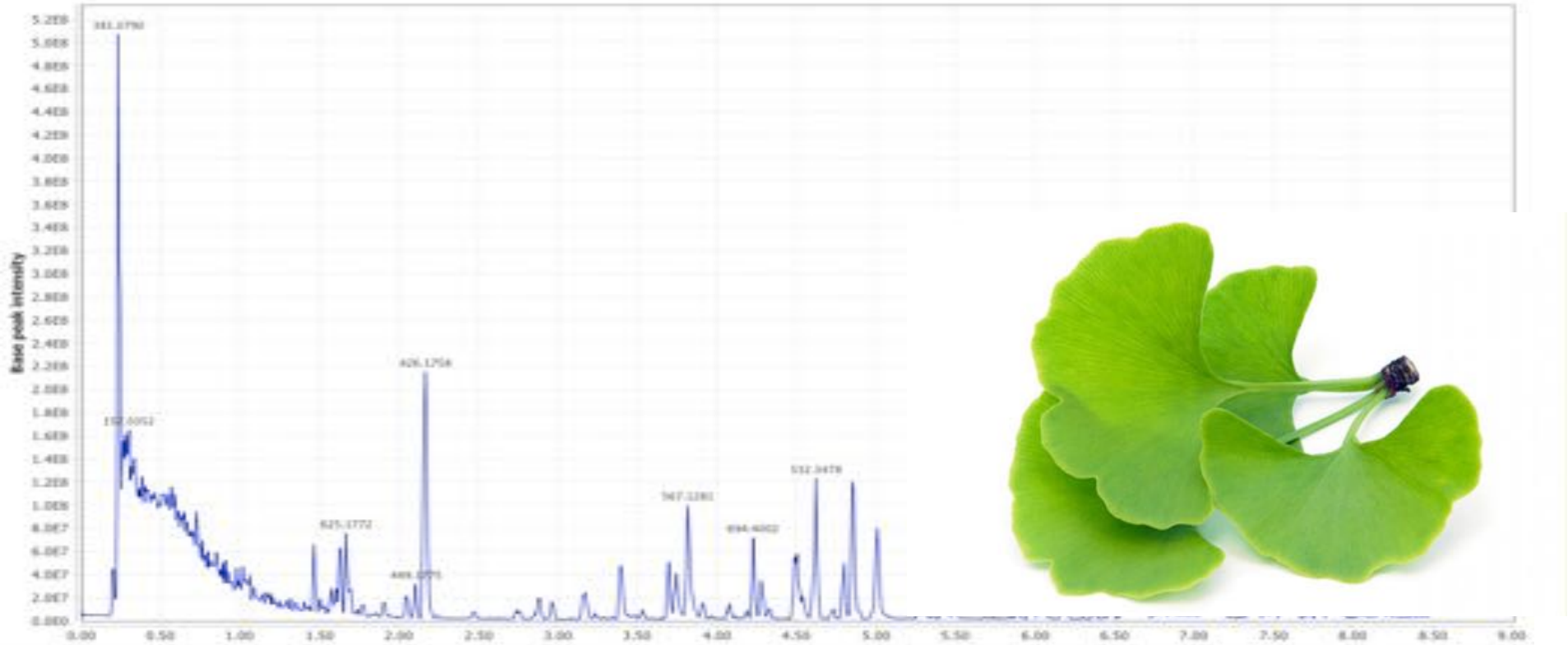
Spectral similarity
score



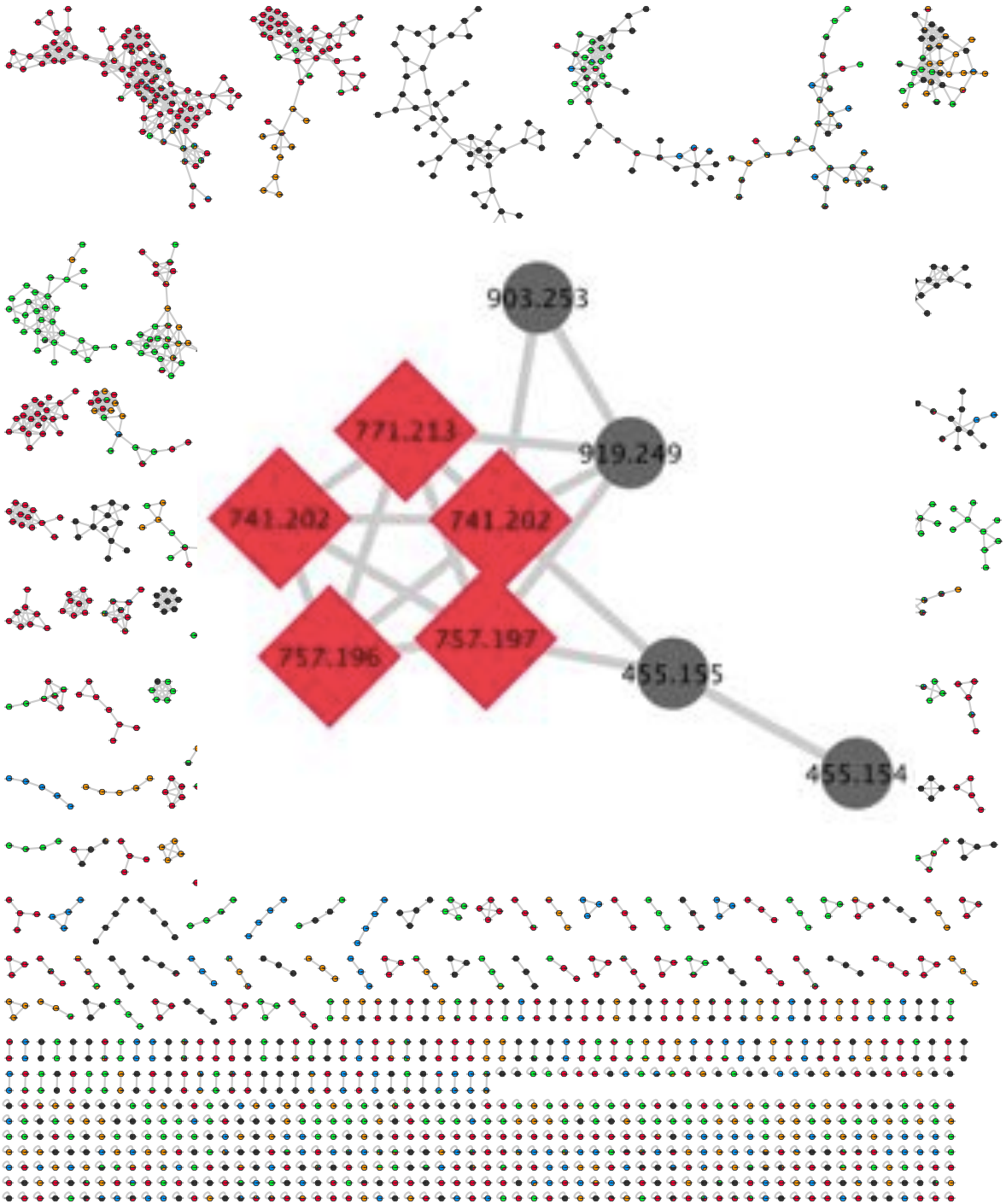
Structural consistency
within cluster
(structural similarity)



Ginkgo biloba

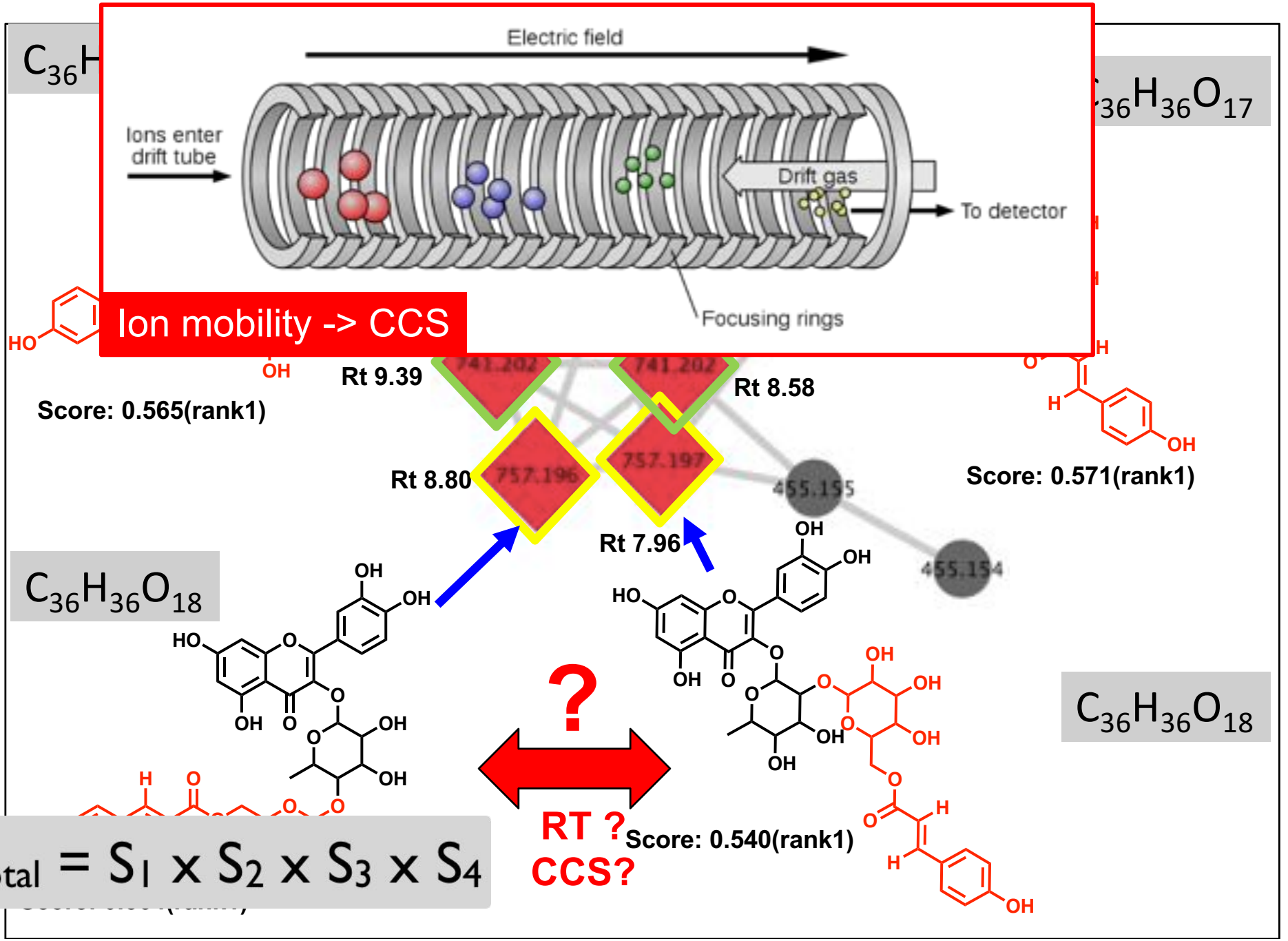


Molecular networking profiles of QCmix

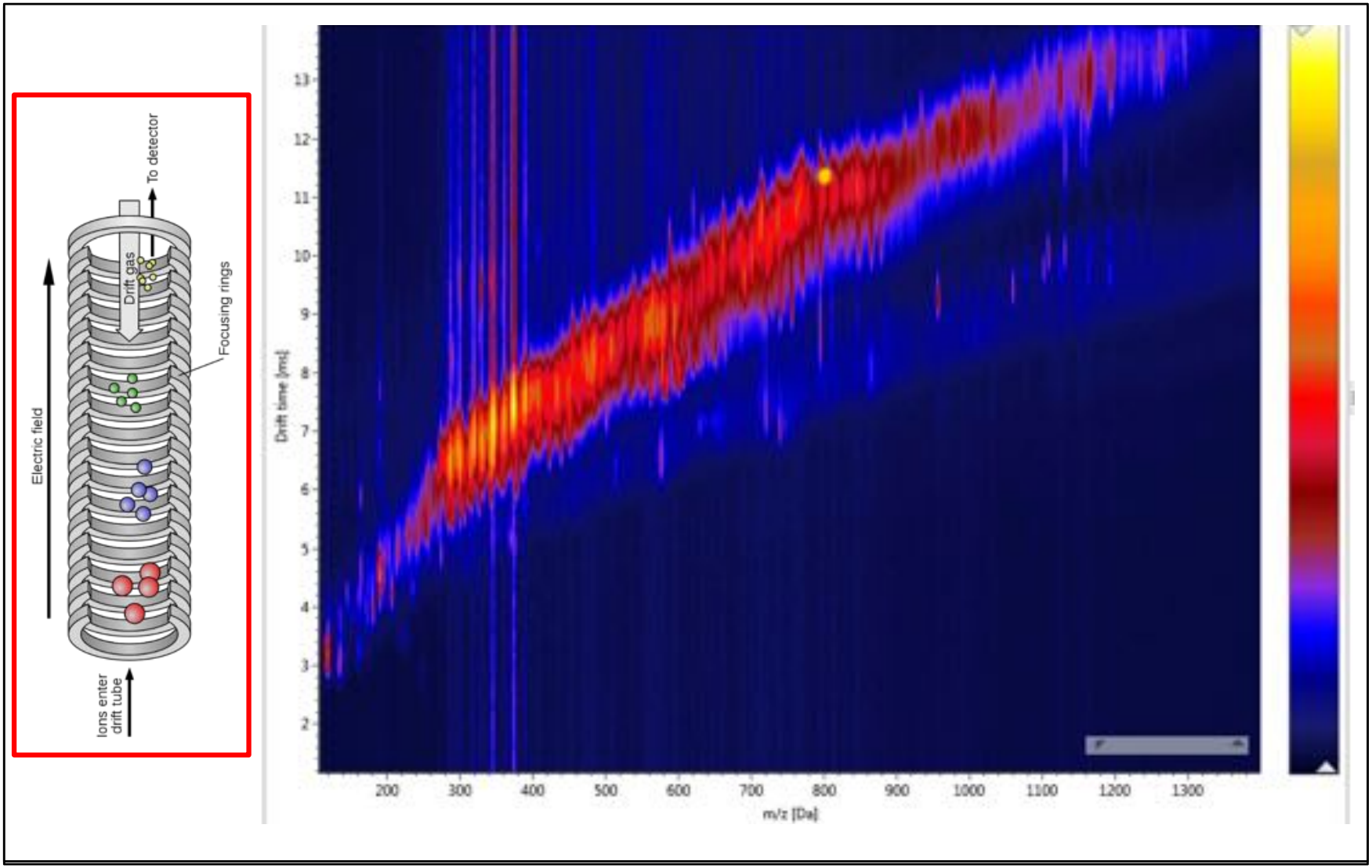


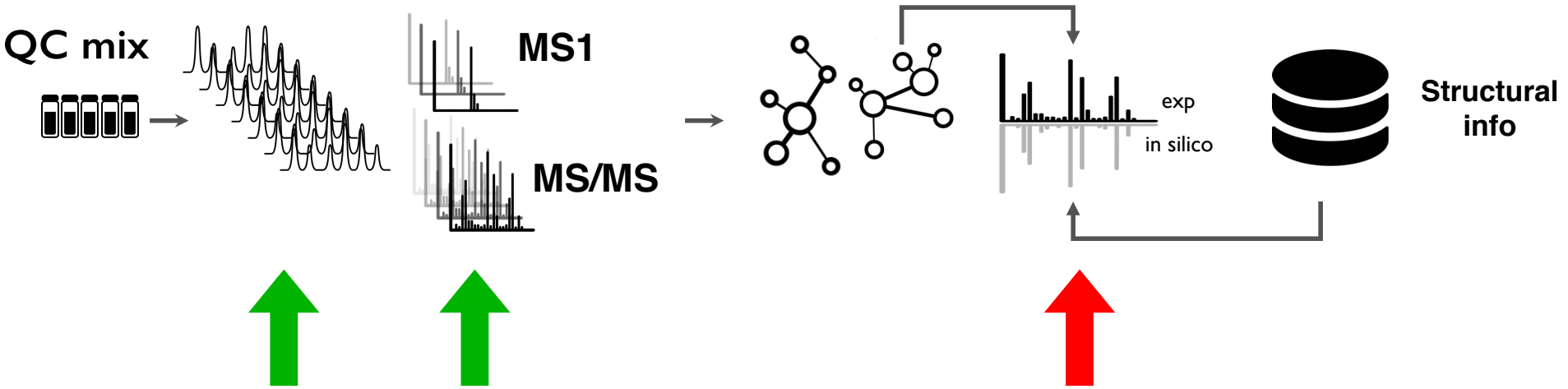
- Cinchona
- Arnica
- Ginseng
- Ginkgo
- Salvia

Molecular network of the MeOH extracts of *Ginkgo biloba*



Ion mobility for IDA HRMS/MS





Metabolic consistency (taxonomical pondering)

Physico-chemical match (RT, CCS)

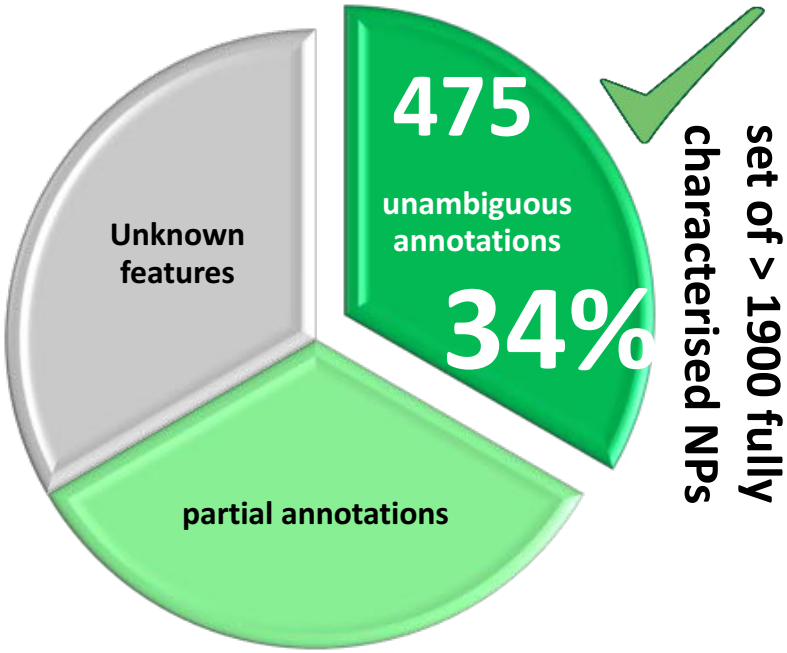
$$S_{total} = S_1 \times S_2 \times S_3 \times S_4$$

Spectral similarity score

Structural consistency within cluster (structural similarity)

Goal

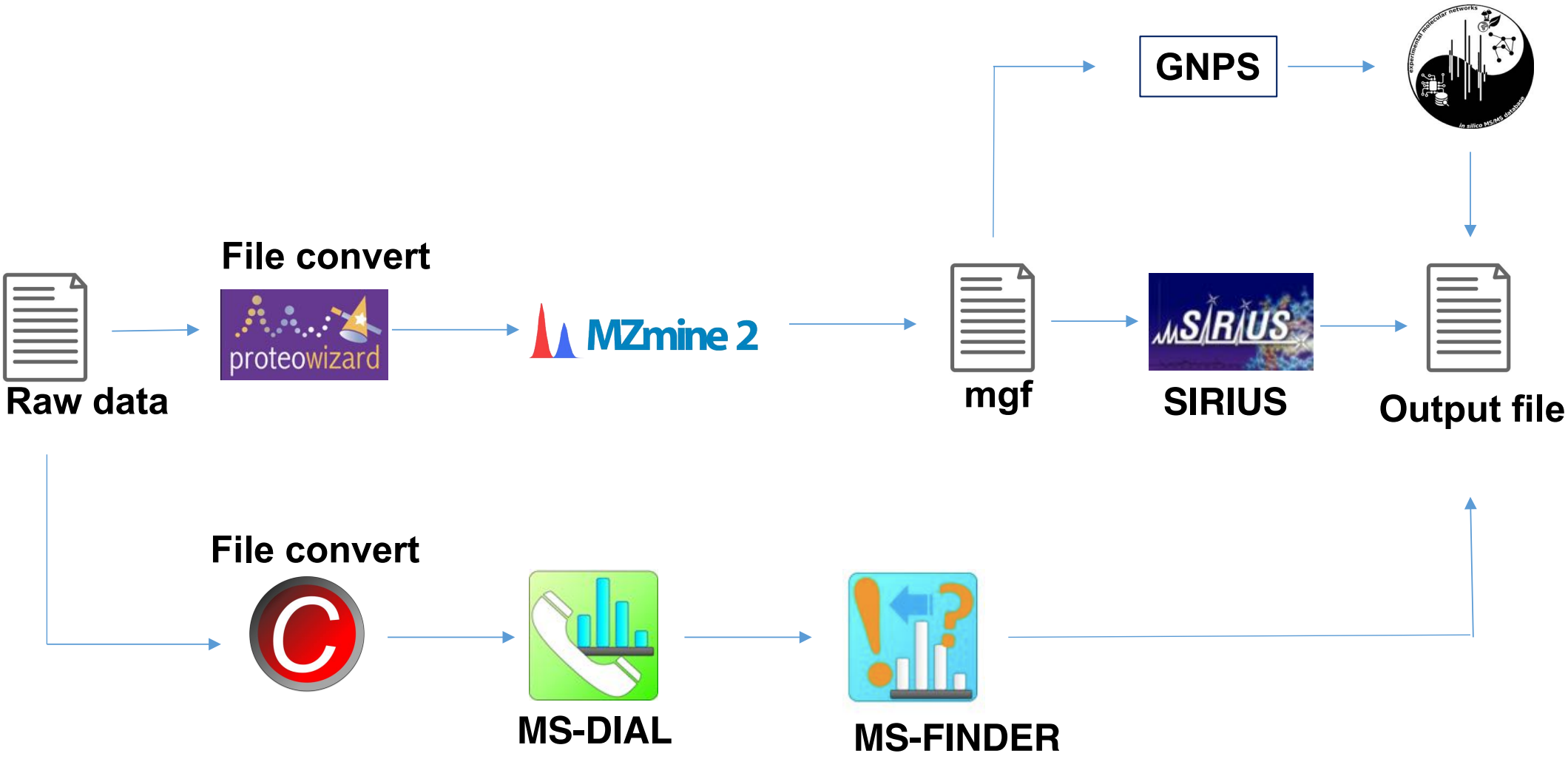
Assessment of metabolite ID capacity of given platform with a given dereplication workflow



To do:

- ☐ appropriate pondering
- ☐ confirm annotation by standard analysis
- ☐ implement the metascore system automatically

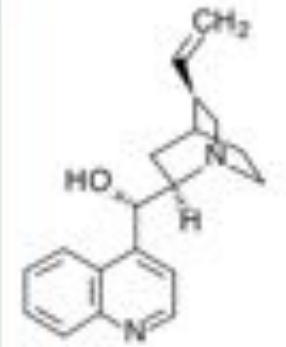
Work flow


GNPS
File convert
proteowizard
MZmine 2
Raw data
mgf
SIRIUS
Output file
File convert
MS-DIAL
MS-FINDER

Output file

KEGG COMPOUND: C06528

Help

Entry	C06528 Compound
Name	Cinchonine; (9S)-Cinchonan-9-ol
Formula	C19H22N2O
Exact mass	294.1732
Mol weight	294.3908
Structure	

SMILES

O[C@H]([C@@H]1C[C@H]2[C@H](C[N@@]1CC2)C=C)C3=CC=NC4=CC=CC=C34

InChI

InChI=1S/C19H22N2O/c1-2-13-12-21-10-8-14(13)11-18(21)19(22)16-7-9-20-17-6-4-3-5-15(16)17/h2-7,9,13-14,18-19,22H,1,8,10-12H2/t13-,14-,18-,19-/m0/s1

InChIKey

KMPWYEUPVWOPIM-LSOMNZGLSA-N

Output file

SMILES
- PlantCyc(Plant)
- ChEBI
- UNPD(NPs)
- NANPDB(NPs)
- KNApSAcK(NPs)
- DNP

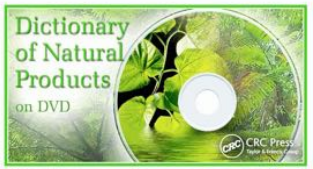
CRC code — DNP

Short InChIKey — PubChem (biological db)

Conversion

Short InChIKey

-export information
-search in MetaData (DNP)



Annotated results file

Work on Excel ← **limitation**

MS-Finder rank new	MS-Finder (pos)	InChIKey	score_S1	Biological_Source	Compound_Types	point_sp	point_genus	point_family	total_point	score_S2
1	HDC47-U	YXHVCZZLWZYHSA	8.1314	Constit. of Ginkgo b	DA7300 DB2340 DG0040 VA	0.2	0.2	0.2	1.6	13.0102
	KZV56-S	KEQVPNIOSICVBG-	7.8132	Constit. of Juniperus	VS5550 ZN2000	0	0	0	1	7.8132
	KGG90-Y	DEPBIQJKIVZYES-Z	7.7299	Constit. of Cladiella	VS6440 ZT1000	0	0	0	1	7.7299
	GZG27-V	UEOBFNCQTNUCC	7.7261	Constit. of Ozoroa r	DA7300 DG0040 VA7300 VG	0	0	0	1	7.7261
	NRL91-E	SVXPDLNSXIXYEA-	7.7234	Constit. of Myrosper	VS5800 ZQ3840	0	0	0	1	7.7234

Output file

SMILES
- PlantCyc(Plant)
- ChEBI
- UNPD(NPs)
- NANPDB(NPs)
- KNApSAcK(NPs)
- DNP

CRC code — DNP

Short InChIKey — PubChem (biological db)

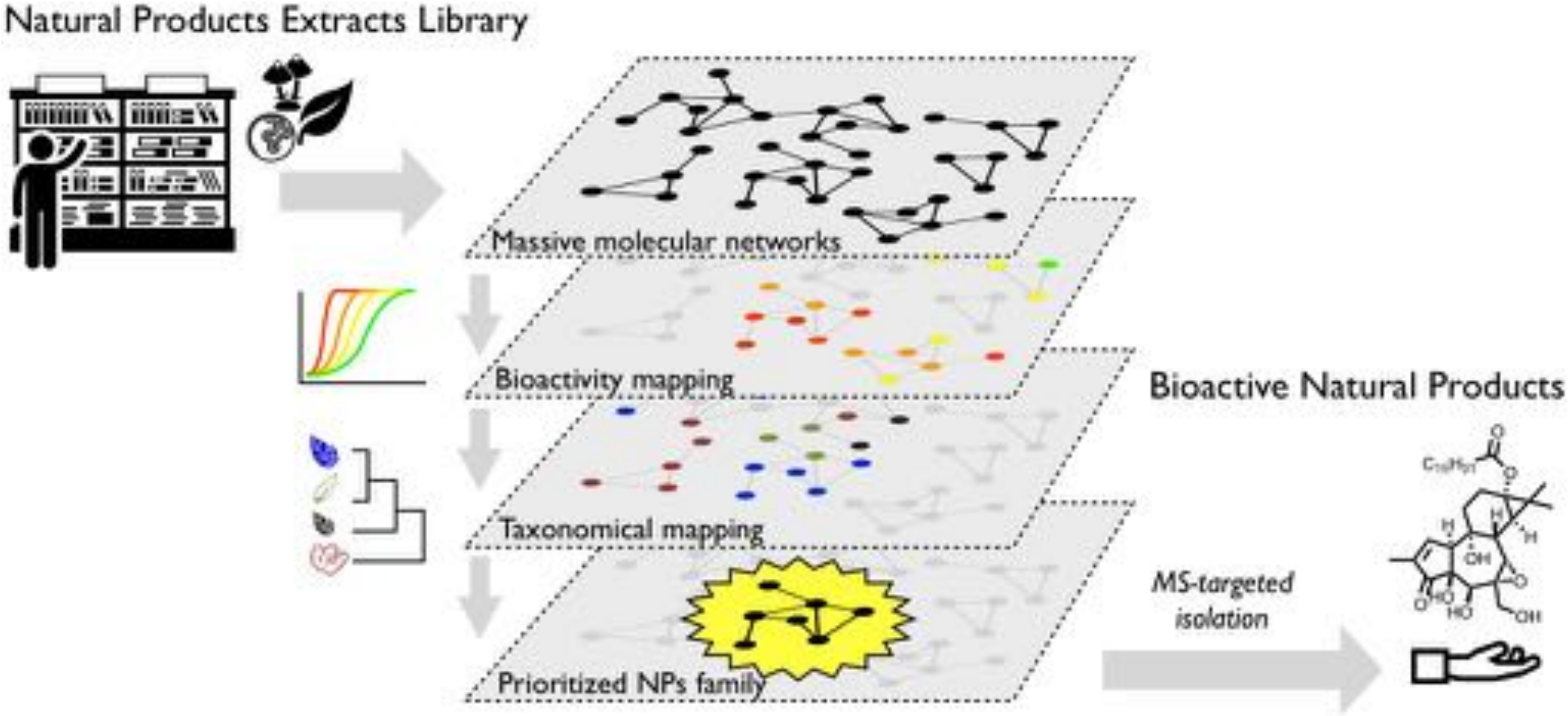
Conversion

R Studio

Short InChIKey → csv → InstantJchem → Annotated results file

...	CdId	Structure	Mol Weight	Formula	File path	File name	Title	MS1 count	MSMS count	PRECURSO	PRECURSO	Rank	Structure rank	Total score	Databases	Ontology	Short InChIKey
1	1		328.50	C22H3...	C:User...	Peak I...	Unkno...	752	54	329.25	[M+H]+	1	C0001...	6.93	ECMDB...	NA	PHENP...
2	2		328.50	C22H3...	C:User...	Peak I...	Unkno...	752	54	329.25	[M+H]+	2	O~15~...	6.90	ChEBI...	NA	QGNJR...
3	3		328.50	C22H3...	C:User...	Peak I...	Unkno...	752	54	329.25	[M+H]+	3	C0001...	6.85	ECMDB...	NA	DVQKS...
4	4		328.50	C22H3...	C:User...	Peak I...	Unkno...	752	54	329.25	[M+H]+	4	UNPD3...	6.76	ECMDB...	NA	CGCM...
5	5		328.50	C22H3...	C:User...	Peak I...	Unkno...	752	54	329.25	[M+H]+	5	HMDB...	6.74	BMDB...	NA	MBMB...

Bioactive Natural Products prioritization pipeline



Florent O., Pierre-Marie A. et al., *ACS Chem. Biol.* **2017**, 2644-2651.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Schwyz
Glarus
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Interlaken
Lausanne
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Brig
Sion
Geneva
Locarno
Martigny
Lugano


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Broken Chair
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COLLADON
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メゾン・ロワイヤル
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Patek Philippe Museum
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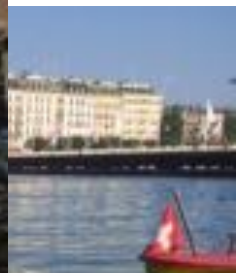
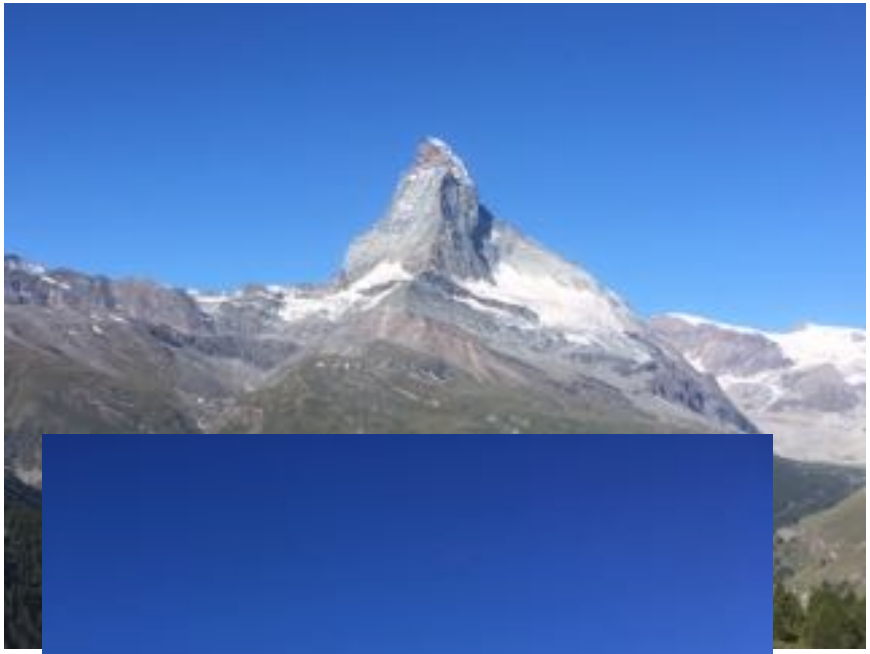
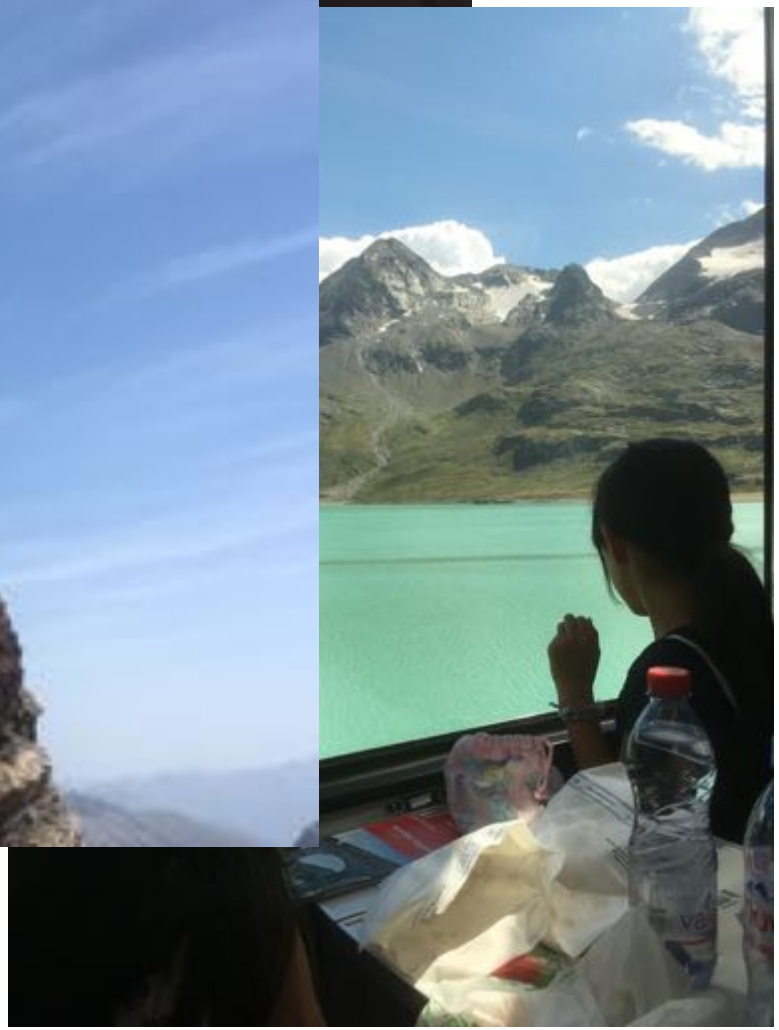
オーギュスタン
AUGUSTINS
ボワッソナ
BOISSONNAS
アカシア
ACACIAS
シャンペル
CHAMPEL
Google



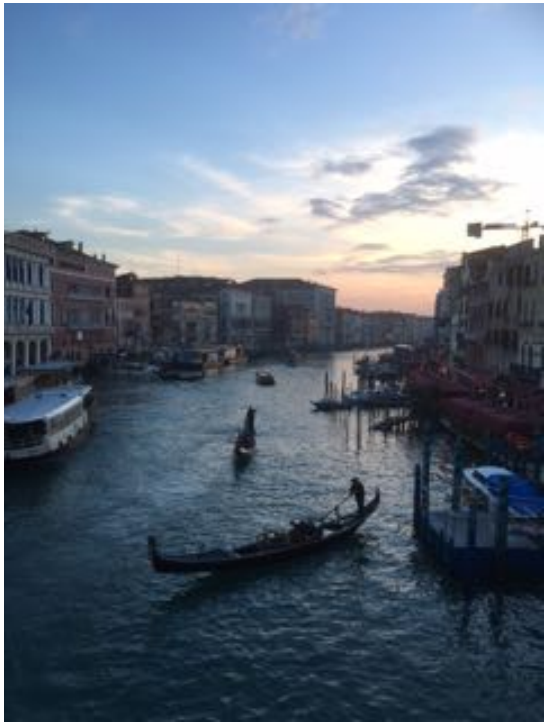


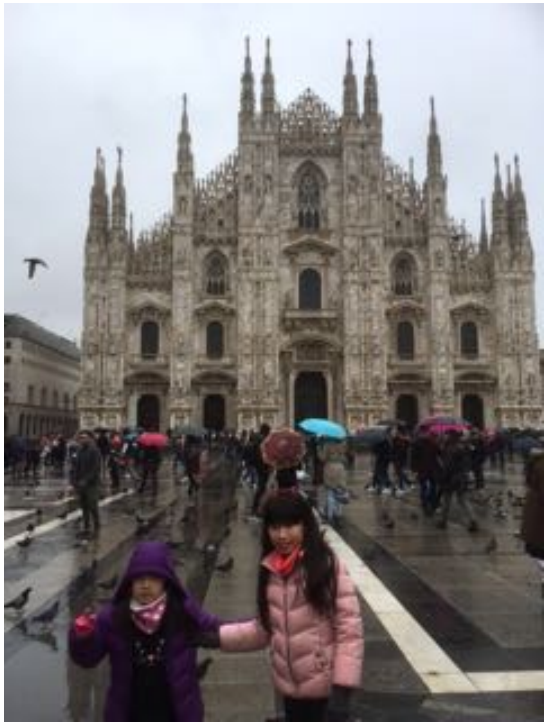

GLACIER




Apotheke und Drogerie
Scheidegger


HERBARIUM
PHARMAZIE





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Prof. Yoshiyasu Fukuyama
Prof. Yoshinori Asakawa
Dr. Kenichi Harada
Dr. Masato Murasaki

Mrs Corinne Galland (Welcome center)
My friends and my family

公益財団法人　山田科学振興財団

公益財団法人　永井記念薬学国際交流財団

The best friends @ Jean-Luc's lab

Merci beaucoup!